# The effect of cultivar, nutrient solution concentration and season on the yield and quality of NFT produced lettuce (*Lactuca sativa* L.)

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# Abstract

Two series of experiments were carried out to examine the effect of nutrient solution concentration on nutrient uptake, growth and quality of Nutrient Film Technique grown lettuce at the Plant Growth Unit, Massey University. In the first study, the influence of nutrient solution concentration, ranging from 0.5 to 3.5 mS $cm^{-1}$ and growing season, on plant nutrient uptake, growth, yield, market quality and nutritional quality of three lettuce cultivars was examined. The second study researched approaches to controlling tipburn incidence of lettuce by investigating the effect of day/night nutrient solution concentration combinations and extra calcium at 100 mg Ca $l^{-1}$ at night with the butterhead lettuce cultivar Cortina. Here the plants were exposed to a tipburn inducing treatment of 30 $^0$C for 4 days.

The results from these studies revealed that generally there were not large variations in nitrogen and phosphorus concentrations of the leaves across nutrient solution concentrations. Leaf potassium concentration increased with increasing nutrient solution concentration up to 2.5 mS $cm^{-1}$. As leaf potassium increased in concentration with increasing nutrient solution concentration, this increase mediated decreases in calcium and magnesium concentrations of the leaves. Leaf nitrogen and potassium concentrations were greater than in the root, the reverse was true for phosphorus, while calcium and magnesium levels did not differ greatly. Nitrogen and phosphorus concentrations increased from the outer to inner leaves, while potassium, calcium and magnesium decreased.

Shoot fresh weight and dry weight increased up to 1.5 mS $cm^{-1}$ with increases in nutrient solution concentration. At higher nutrient solution concentrations dry weight levelled off, while fresh weight levelled off or decreased slowly depending on the level of stress imposed by the season. Thus fresh weight was more sensitive to stress at high nutrient solution concentrations than dry weight. With both seasons and cultivars, the order of the initial RGR was the same order as for final shoot dry weights, with the initial NAR being the important component of the initial RGR. Apart from the autumn crop, where no tipburn occurred, tipburn incidence increased with increasing nutrient solution concentration with the level of incidence increasing as environment stress

increased. Shelf life increased with increasing nutrient solution concentration, but the level of increase was not great enough to be of commercial significant.

Season, nutrient solution concentration and cultivar all affected the nutritive value. The affect depended on the nutritive quality attribute under consideration. The nutritive values obtained in this study were in the ranges reported by other workers. The summer crop had the highest ascorbic acid concentration. Where ascorbic acid concentrations were high, such as in summer or with the cultivar Impuls, then ascorbic acid concentrations decreased with increases in nutrient solution concentration. The only difference in dietary fibre occurred with the butterhead cultivar Cortina, which had the lowest dietary fibre concentration of the three cultivars. Nitrate concentration increased with nutrient solution concentration, was highest in autumn and winter, while differences between cultivars depended on the season. The nitrate concentration of lettuce produced at nutrient solution concentrations up to 1.5 mS $cm^{-1}$ were within the permissible levels reported overseas. There were no treatment effects on protein concentration despite some reports in the literature of the effects of nitrogen level on protein content. At the lower nutrient solution concentrations, the spring and summer crops tended to have the highest soluble sugar concentrations. Generally soluble sugar concentrations decreased within increasing nutrient solution concentration up to 2.5 mS $cm^{-1}$ and then levelled off.

When 0.5 mS $cm^{-1}$ nutrient solution concentration was used alternately with 1.5 mS $cm^{-1}$ during day and night, the nitrogen and potassium concentration of the leaves increased and the increases in potassium mediated decreases in calcium and magnesium concentrations of the leaves. These effects were more marked when 1.5 mS $cm^{-1}$ was maintained during the day and 0.5 mS $cm^{-1}$ during the night. Nutrient concentration of the innermost leaves was not affected by different nutrient solution concentration at night. Lowering the nutrient solution concentration at night to 0.5 mS $cm^{-1}$, when the nutrient solution during the day was maintained at 1.5 mS $cm^{-1}$, tended to give higher shoot fresh and dry weights, and reduced tipburn percentage. However, under extremely stressful conditions, tipburn affected almost every plant. Under these conditions 0.5 mS $cm^{-1}$ at night still had an effect, as the number of tipburn leaves per plant and the tipburn index was reduced. Root pressure was considered to provide the benefits from the 0.5 mS $cm^{-1}$ nutrient solution concentration at night.

Extra calcium either alone or in combination with other nutrients enhanced nitrogen and phosphorus concentration of the outer leaves and reduced potassium, calcium and magnesium concentration of the innermost leaves after tipburn induction. Thus extra calcium at night increased fresh and dry weight after tipburn induction and so increased tipburn incidence.

The important commercial outcomes of this research are as follows. The optimum nutrient solution concentration at which to grow a range of lettuce cultivars across all seasons is 1.5 mS $cm^{-1}$. At this nutrient solution concentration yield will be satisfactory and the level of tipburn will be minimised. At this nutrient solution concentration the nitrate concentrations were within the permissible levels reported overseas. The growers can also benefit from lowering the nutrient solution concentration at night to 0.5 mS $cm^{-1}$, as this treatment will increase fresh weight and reduce tipburn.

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# Chapter 1

## General Introduction

An increase in the consumption of fruit and vegetable food products is recommended by health organisations to improve public health (Mackerras, 1995; Wiseman et al., 1997). The risk of death by many non-hormone-dependent cancers can be reduced approximately twofold in subjects who consume relatively high amount of fruits and vegetables (Block et al., 1992). National Cancer Institute (NCI) and U.S. Department of Agriculture (USDA) guidelines are similar, with recommendations for eating at least five fruits and vegetables daily (Levine et al., 1996). Despite consumer interest in health-related issues changing nutrition habits remain difficult. Nevertheless, consumers expect food products to be healthy, as well as tasty and functional. Such an expectation requires food products to meet the functional, nutritional and health needs of the consumer (Verschuren, 1997). The challenge for the horticultural industry is to use science and technology in an attempt to meet these expectations. The research reported in this thesis attempted to do this using lettuce and the potential that the nutrient film technique system of hydroponics offer in managing the root environment.

Lettuce is the world's most popular salad crop and is typically grown for the fresh market (Care, 1991). Lettuce is one of the most important vegetables grown in North America, and is an important crop of temperate climate production systems in many regions of the world. The crop's tolerance of cool temperatures and low light has made it a popular greenhouse crop in Europe (Wien, 1997), particularly during winter and early spring (Winsor & Adams, 1987). In the USA, lettuce is the third in importance among vegetables after potatoes and tomatoes (Wien, 1997). Lettuce provides some of the minerals, vitamins and fibre needed in the diet, and a significant amount of edible biomass as it sustains a high level of photosynthetic activity during growth (Knight & Mitchell 1983; Wheeler et al. 1994).

The Nutrient Film Technique (NFT) has the potential of providing a standardised and easily controlled root environment with greatly simplified crop management (Morgan et al. 1980a). With soilless culture systems, a better control of crop production (quantity and quality) is possible than with soil-based systems. In recirculating systems, water and fertiliser uses are more efficient, and environmental pollution can be reduced (Heinen et al., 1991). In such systems the lettuce plants may be marketed with a proportion of the root system intact so extending their postharvest life (Morgan & Tan, 1983).

There has been a significant amount of research examining the role of nutrient solution concentration on the yield and quality of hydroponically produced lettuce. However, few seasonal comparisons have been made and at times the nutrient solution concentrations used has covered a limited range. The response of different lettuce types has also not been widely considered. In the present study a broad-based approach was taken. Thus, there was a series of experiments, which covered the four seasons, a wide range of nutrient solution concentrations and a number of distinctly different lettuce types. The effect of all these factors on growth, nutrient uptake, yield and quality of lettuce was examined. Quality assessment included tipburn incidence, shelf life and nutritional value.

Consumers are becoming discriminating buyers with a high expectation for quality. Concern for how different growing conditions influence nutritional quality, especially the content of nitrate and vitamins exist. For this reason the effect of growing season, nutrient solution concentration on concentration of ascorbic acid, dietary fibre, nitrate, protein and soluble sugar of three lettuce types was studied.

Tipburn is perhaps the major problem concerning lettuce quality, particularly in production systems where the crop is encouraged to have a high growth rate. In this study the role of different day/night nutrient solution concentrations on tipburn was examined as well as approaches to charging the inner leaves by supply extra calcium at night.

The response of the crops grown to the treatments applied was interpreted in terms of practical outcomes. The need to produce high yield along with high quality. An objective which at times may require compromises.

# Chapter 2

# Literature Review

## 2.1 Introduction

Lettuce is the world's most popular salad plant and is usually grown for the fresh market (Care, 1991). Lettuce is one of the most popular vegetables grown in North America, and forms an important part of temperate climate production systems in Europe and other regions. The crop's tolerance for cool growing conditions and low light has made it a popular glasshouse crop in Europe (Wien, 1997), particularly during winter and early spring (Winsor & Adams, 1987). Lettuce is one of several candidate species under study for use in controlled life support systems proposed for human life support in space (Wheeler et al.1994).

In the USA, lettuce is the third in importance among vegetables after potatoes and tomatoes (Wien, 1997). In the USA, more than 82,500 ha of crisphead lettuce was planted in 1994. Total production was more than 3.0 million metric tons with a crop value of more than $800 million. Leaf and butterhead lettuces were grown on 15,400 ha, producing 360,158 metric tons valued at $178 million. Romaine lettuce was grown on 10,000 ha, producing 290,127 metric tons valued at $110 million (Davis et al., 1997).

Modern lettuce cultivars can be grouped into four or five types, according to plant form and predominant use. The crisphead type (or iceberg) forms firm, closed heads with a crisp texture. The butterhead type forms loose, open heads, and has soft leaves easily damaged in handling. Leaf lettuce also has soft leaves that may be long or broad; rounded, spatulate, or lobed; frilled or smooth margined. Cos or romaine lettuce has erect, elongated leaves that form into a loose, loaf-shaped head. Stem lettuce is grown for its thickened, parenchymatous stem (Davis et al., 1997; Wien, 1997).

## 2.2 Factors affecting growth and productivity of lettuce

### 2.2.1 Seedling

The yield of a plant is the product of its initial weight and its relative growth rate (Scaife & Jones, 1976). Vigorous seedlings are a prerequisite to a successful crop, especially in lettuce where the length of the period as a containerised transplant can comprise up to 30% of the cropping time (Kratky & Mishima, 1981).

Nicola and Cantliffe (1996) reported that the smaller cell sizes (1.9 and 10.9 $cm^3$) saved medium and space in the greenhouse and increased the root growth ratio, but it reduced plant growth compared to the bigger cells (19.3 and 39.7 $cm^3$). Gruda and Schnitzler (1997) reported that the microbial nitrogen fixation that occurs in some organic substrates may result in poor seedling growth. Lettuce seedlings grown in impregnated wood fibre with slow release nitrogen fertiliser, had greater leaf area, fresh weight, dry weight and net photosynthetic rate than in peat or unimpregnated wood fibre.

Morgan et al. (1980a) found that the most satisfactory nutrient solution concentration for lettuce seedlings was in the range between 0.6 to 1.1 mS $cm^{-1}$. Kratky & Mishima (1981) suggested that high nitrogen in combination with high phosphorus increased shoot and root dry weight, leaf area and shoot: root ratio and predisposed the transplants to recover from transplanting shock and such seedlings resumed growth sooner, resulting in earlier yields. Karchi et al. (1992) argued that a high phosphorus concentration, with appropriate nitrogen level, will enhance and extend the period of root growth, will provide moderate leaf development while might better overcome transplanting shock and as a result growth will resume sooner and result in better yield and quality.

Wurr et al. (1987) concluded that plants raised at ambient temperatures and transplanted before the end of May in the UK, produced heavier heads and matured later than those plants raised at higher temperatures under glass. They also suggested that the influence of transplant age on head weight was much smaller than that of raising conditions, while there were inconsistent effects on the time of crop maturity.

Wurr and Fellows (1986) recommended that the use of younger transplants may minimise variation in head weight within a lettuce crop.

### 2.2.2 Temperature

Temperature is the main factor determining the growth rate of lettuce during seedling emergence and the early growth period (Bierhuizen et al., 1973; Scaife, 1973; Gray & Morris, 1978). Wurr and Fellows (1984) reported that there was a significant association between head weight and the mean temperature up to 42 days from emergence. They also suggested that temperatures during the early growth of drilled crops affected leaf growth and thus head formation and so head weight.

The growth rate of lettuce depends on the temperature of the growing point, and this organ is located close to the soil surface (Wien, 1997). Thus some researches have suggested that soil temperature can be more closely related with lettuce growth rate than air temperature (Scaife, 1973; Wurr et al., 1981). There is, for example, a close relationship between plant growth and root zone temperatures in the NFT system (Takano, 1988). Root temperature affects many plant functions in lettuce such as respiration, water absorption, water movement, transpiration and ion uptake, all of which directly affect plant growth (Ikeda & Osawa, 1984). Researchers have shown that headed lettuce can be grown successfully in the tropics by lowering root temperatures (Lee & Cheong, 1996; Jie & Kong, 1998a, 1998b; Thompson et al., 1998). Increased nutrient solution temperatures have also stimulated the growth of lettuce grown in NFT during spring (Mongeau & Stewart, 1984) and winter (Moorby & Graves, 1980; Van Der Boon & Steenhuizen, 1986; Van Der Boon et al., 1988, 1990; Kanaan & Economakis, 1992). Mongeau and Stewart (1984) concluded that the benificial effects of solution temperature were most evident when the air temperature were limiting to production.

On the other hand, Maaswinkel and Welles (1987) have reported that night air temperatures had a greater positive effect on head formation of iceberg lettuce rather than soil temperature. Hicklenton and Wolynetz (1987) also reported that the overall effect of root temperature on plant size was minor.

Temperature also affects head quality. Zink and Yamaguchi (1962) reported that lettuce of good quality was produced with a mean temperature in the range of 51 to 67 $^0$F (10.6-19.4 $^0$C) during head formation.

Wurr and Fellows (1984) reported that low mean temperatures (< 12$^0$C) were associated with lower head weights of the bolting resistant cultivar 'Ithaca', but higher head weights of winter cultivar 'Saladin'. The opposite was true for mean temperatures greater than 16 $^0$C. The different responses may be because the two varieties were bred in different places for different conditions. Maaswinkel and Welles (1987) reported that the formation of open heads in iceberg lettuce was related to conditions where a low temperature in the root environment during the period until head formation was followed by higher temperatures thereafter. Maintaining relatively high temperatures (higher than 14 $^0$C) in the root environment throughout the growing period reduced the risk of the formation of open heads considerably.

Hicklenton and Wolynetz (1987) have reported that there were no significant interaction effects between day and night temperature. An increase of day temperature from 12 $^0$C to 19.5 $^0$C increased fresh and dry leaf weight and leaf area at final harvest, but increasing night temperature from 5 $^0$C to 14 $^0$C had little effect. Knavel (1981) reported that the greatest yield was received from lettuce grown at a night temperature of 18 $^0$C compared to 7 $^0$C and 13 $^0$C, when day temperature was maintained at 20 $^0$C. On the other hand, Glenn (1984) reported that daytime air temperatures was moderately positively correlated with growth, whereas night temperature was weakly negatively correlated. The greenhouse temperature in his experiments were set at 14 $^0$C night and 25 $^0$C day, but the actual temperatures normally exceeded the thermostat settings.

### 2.2.3 Light

Light is generally the most important variable in heated greenhouses that depend on natural radiation as the sole source of illumination (Klapwijk, 1979). Natural radiation levels are potentially rate limiting for lettuce growth anywhere in the world (Glenn, 1984). Radiation rather than temperature is a more important factor affecting growth at the latter stage of growth, after self shading of leaf and hearting occur in lettuce

(Bierhuizen et al., 1973; Gray & Morris, 1978). Wurr et al. (1987) found highly significant positive correlation between head weight at maturity and total solar radiation in the period 7 and 10 days before 50% hearting. Thus the relative growth rate of lettuce is positively correlated with solar radiation at hearting and post-hearting (Wurr & Fellows, 1991).

Sanchez et al. (1989) reported that the light saturation point of crisphead lettuce for photosynthesis during the heading stage could reach 800 $\mu mol\ s^{-1}\ m^{-2}$ and the maximum net $CO_2$ assimilation rate for lettuce decreased as the irradiance at which the plants were grown decreased. The water uptake of lettuce is also related to the radiation levels (Voogt, 1988).

Growth of Romaine lettuce was found to be better under full sunlight, while leaf lettuce was better in reduced light. This was due to the upright growth habit of Romaine lettuce protecting the growing point of the plant while the growing point of leaf lettuce is exposed (Foley, 1965). Sanchez et al. (1989) also demonstrated the importance of light as continuous shading from thinning to harvest reduced crop growth approximately in direct proportion to the reduction in irradiance. Similarly Maaswinkel and Welles (1987) found that the application of a thermal screen resulted in a reduction of the head weight, probably due to continuous shading.

The use of supplementary light has been beneficial. Thus Schlagnhaufer et al. (1987) found that fresh weight of 'Grand Rapids' and 'Etus' increased up to 770% and 241% respectively under continuous supplementary lighting. Similarly, Gaudreau et al. (1994) found the impact of continuous supplementary lighting of 100 $\mu mol\ m^{-2}\ s^{-1}$ was more pronounced during those months when natural light levels were low; it increased biomass accumulation ($\leq$270%), head firmness and tipburn incidence with shorter production cycles ($\leq$30%) than under natural light. The most beneficial light source for fresh and dry weight of lettuce at equal photosynthetic photon flux (PPF) is high pressure sodium lamp compared to other sources (Koontz et al., 1987; Ito, 1989). Ito (1989) found the optimum PPF density was found to be 280 $\mu E\ m^{-2}\ sec^{-1}$ in case of 14 hours photoperiod.

### 2.2.4 The interaction of temperature and radiation

The interaction of temperature and radiation is the most important factor influencing lettuce growth (Glenn, 1984; Economakis, 1990). The best predictor of growth was suggested by Glenn (1984) to be the product of day temperature and the log of radiation while Bensink (1971) found that lettuce responds more favourably to increased temperature at high, but not at low irradiation.

Wurr et al. (1992) found that denser heads were associated with low temperatures during the period up to and around hearting, while less dense heads were primarily associated with higher temperatures in the period up to hearting and high levels of solar radiation in periods well after hearting. Larger heads were associated with low temperature up to and around hearting, with high temperatures in the immediate post-hearting period and with large amplitudes of temperature change and high levels of solar radiation around hearting.

### 2.2.5 Carbon dioxide

Mitchell et al. (1997) reported that carbon dioxide enrichment enhanced leaf number of lettuce, but increased leaf dry weight only at high photosynthetic photon flux. Ito (1989) found that a carbon dioxide concentration of 600 ppm gave the highest growth rate and quality when grown under fluorescent lamps. Further increases in carbon dioxide concentration resulted in a slightly reduction of fresh weight although dry weight ratio and chlorophyll content increased. Capom (1989) found that the rate of emergence and expansion of leaves and the growth of young lettuce plants increased in high carbon dioxide (1200 μmol $mol^{-1}$) compared to low carbon dioxide (380 μmol $mol^{-1}$). The benefit of carbon dioxide enrichment to the growth of lettuce was attributed to an increased rate of photosynthesis per unit of leaf area.

Knight and Mitchell (1988) reported that enriching carbon dioxide to 46 mmol $m^{-3}$ at moderate photosynthetic photon flux (PPF) resulted in a 40% increase in leaf fresh weight and a 75% increase in leaf dry weight relative to that at 16 mmol $m^{-3}$ carbon dioxide. Elevating PPF at high $CO_2$ further increased shoot leaf weight. Relative

growth rate was enhanced until 15 days after seeding (9 days of treatment), after which there no longer were treatment difference. They suggested that RuBP carboxylase levels increased as lettuce adapted to higher levels of carbon dioxide.

### 2.2.6 Effect of salinity on lettuce growth

Lettuce is considered moderately sensitive to salt (Davis et al., 1997). Growth inhibition occurrs when plants were grown in high concentrations using hydroponics culture (Care, 1991; Nonami et al., 1992). Salinity or osmotic stress reduces plant growth by water deficit (Behboudian et al., 1986; Mengel & Kirkby, 1987; Nonami et al., 1992), toxicity of ions (Walker et al., 1983; Yeo et al., 1985; Mengel & Kirkby, 1987) or ionic imbalances (Walker, 1986; Mengel & Kirkby, 1987; Martinez et al., 1996).

Rate of photosynthesis are usually lower in osmotic stressed plants (Longstreth & Nobel, 1979; Yeo et al., 1985; Munns & Termaat, 1986; Lawlor, 1995) and De Pascale and Barbieri (1995) found that salinity reduced gas exchange rates and stomatal conductance of lettuce.

Despite research such as where Munns & Termaat (1986) concluded that there is no evidence for a causal relationship between ion concentration and photosynthesis. Growth reductions from salt stress is due to effects in the growing tissue rather than on photosynthesis (Dalton & Poss, 1990; Li et al., 1991; Longnecker, 1994). Thus decreases in leaf elongation are due to direct effects on cell expansion rather than to a reduced supply in carbohydrates (Longnecker, 1994). Gallardo et al. (1996) reported that leaf water potential and photosynthesis in mature lettuce did not show differences between irrigation treatments ranging from field capacity to 55% below field capacity. They also suggested that small differences in water supply lead to non-detectable differences in instantaneously measured physiological parameters. This effect was also reported in tomato where the effects of salt stress (NaCl) are less readily detectable in physiological parameters than in leaf and stem dry weight (Caro et al., 1991).

When growth is inhibited at low water potential, the growth-induced water potential is decreased resulting in cessation of water flow from the xylem to the enlarging cells

(Nonami & Boyer, 1989) and subsequently cell wall extensibility is decreased (Nonami & Boyer, 1990). When cell elongation occurs, water needs to be absorbed by the elongating cells and thus a water potential gradient inevitably exists between the elongating cell and its surroundings. Such a water potential gradient is called the growth-induced water potential (Nonami & Boyer, 1993).

## 2.3 Nutrient Film Technique (NFT) of lettuce

### 2.3.1 Introduction

Growing plants in the nutrient film hydroponic technique, NFT, began at the Glasshouse Crops Research Institute in 1966 (Cooper, 1975). In 1973 Dr Cooper from Littlehampton, UK, popularlized the practical guidelines for NFT (Benoit & Ceustermans, 1995). NFT is a true hydroponic system with bare roots in the nutrient solution, with only sufficient substrate to hold the seed for germination and handling of the small plants (Vestergaard, 1988).

With soilless culture systems, better control of crop production (quantity and quality) may be possible than with soil-based systems. In recirculating systems, water and fertiliser use is more efficient, and environmental pollution is reduced (Heinen et al., 1991). The NFT system has become a very useful research technique for experimentation in plant nutrition. The ability to control the root environment has led to practices such as solution heating, variation in solution concentration and intermittent flow to control crop growth. The minimal use of water and nutrients has made it highly desirable in arid climates. This minimal use of materials and the high level of automation that is possible enables a rapid turn around in cropping (Burrage, 1992). The heating the nutrient solution control is both simple and cheap because it can be done at one point in the system (Graves, 1986). Moreover, the plants may be marketed with part of the root system intact as an added sales attraction (Morgan & Tan, 1983).

The accumulation of toxic trace metals in crops is receiving a great deal of attention. In lettuce the correlation of concentration in leaf tissue and soil solution concentration was positive for zinc, cadmium and copper whereas there was a negative correlation for

lead (Gerritse et al., 1983). The heavy metals, lead, nickel, zinc, chromium and cadmium, which are known to be highly dangerous to humans, are presented in 2 to 10 times higher in plants grown in soil than in hydroponics (Massantini et al., 1988). Researches have shown that cadmium concentration increased with increasing level of external cadmium supply (Singh et al., 1988; Florijn et al., 1991). Florijn et al. (1991) reported that fresh and dry weights of lettuce were not affected by cadmium levels in the nutrient solution up to 0.1 mg $l^{-1}$, whereas Singh et al. (1988) reported that fresh and dry weights decreased with increasing cadmium concentration up to 50 mg Cd $kg^{-1}$ in the soil.

The foliar ascorbic acid content of lettuce and spinach grown in hydroponics is greatly increased by soaking the roots in a sodium ascorbate solution at concentrations ranging from 1000 - 2000 mg $l^{-1}$ for 12 - 16 hours (Inoue et al., 1995) and the leaves still maintained the ascorbic acid introduced for 7 days at 4 $^{0}$C (Inoue et al., 1998). It is expected that the production of hydroponically grown ascorbic acid rich leaf vegetables is feasible in practice as ascorbic acid can be cheaply produced from glucose on a commercial scale (Inoue et al., 1998).

It is possible that organic compounds with potentially phytotoxic effects, like phenolic acid, resulting from root exudation or plant tissue degradation, may accumulate in recirculation systems (Jensen et al., 1994). Lettuce production can be raised 25% to 35% if the system is low in total organic carbon and at the same time kept sterile (Vestergaard, 1988). Jensen et al. (1994) reported that the shoot dry weight of lettuce decreased when 100 µM or higher concentration of ferulic acid was added in the nutrient solution. The concentration of phosphorus, potassium, calcium and magnesium in the plant were strongly reduced at 1000 µM ferulic acid.

The main disadvantages of the NFT system were originally thought to be the risk of pump failure and the spread of diseases, but neither of these has proved to be serious. The need for a higher level of management expertise may be the main inhibitory factor in its development (Burrage, 1992).

## 2.3.2 Source of nitrogen

Nitrogen is the only macronutrient, which may be present as both an anion or a cation. Plant roots will release $H^+$ if cations are taken up more rapidly than anions, and roots will release $HCO_3^-$ and $OH^-$ if anions are taken up more rapidly than cations (Willumsen, 1984). Spinu et al. (1998) stated that plant roots release an $OH^-$ ion for every nitrate ion ($NO_3^-$) that is taken up to maintain charge neutrality within the plant. The supply of ammonium nitrogen can be an important factor controlling the pH of recirculating solutions (Willumsen, 1984, 1985) and the use of high ammonium treatments result in rapid decrease in solution pH (Johnson & Moore, 1983).

The average fresh weight of lettuce increased with increasing ammonium content in the nutrient solution up to 12% (Johnson & Moore, 1983), 17% (Knight & Mitchell, 1983), 30% (Ikeda & Osawa, 1984) and 20% (Van Der Boon et al., 1988, 1990). However, high concentrations of ammonium adversely affect lettuce crops (Grogan & Zink, 1956; Ikeda & Osawa, 1984; Van Der Boon et al., 1988). Toxicity of ammonium to vegetables is influenced by the ratio of nitrate to ammonium and the ammonium concentration in the nutrient solution (Ikeda & Osawa, 1984). Nevertheless, Van Der Boon et al. (1990) reported that increasing the ammonium concentration to 50 or 80% of the total nitrogen concentration before harvest (14 d in winter and 8 d in summer) did not affect head weight or dry matter percentage, but decreased the nitrate concentration of the product.

The consumption of nitrate nitrogen, potassium and calcium has been reported to decrease with increasing ammonium nitrogen supply (Ikeda & Osawa, 1984; Willumsen, 1984;) while the phosphorus concentration in leaves of plants supplied with both nitrate and ammonium was higher than that with nitrate alone (Ikeda & Osawa, 1984)

Lettuce grown under different sources of nitrogen have different leaf colours. Leaves are light green in nitrate only, green in nitrate + ammonium and dark green in high ammonium concentrations (Ikeda & Osawa, 1984; Van Der Boon et al., 1990), while the effect of nitrogen form in the solution on the leaf shape and root growth of lettuce is

unclear (Ikeda & Osawa, 1984). Willumsen (1984) also reported that the quantity of final yield was not affected by ratio of ammonium to nitrate.

### 2.3.3 pH

The pH of a solution is a property that is inherent to its composition. The pH of an aqueous solution is determined by the initial concentration of acids and bases. In the case of nutrient solutions, this is dihydrogen phosphate ($H_2PO_4^-$), bicarbonate ($HCO_3^-$) and/or ammonium ($NH_4^+$) (Derijck & Schrevens, 1997). Plant growth and development are greatly influenced by alterations of pH in the root environment (Islam et al., 1980). Symptoms associated with changes in uptake, transport and function of mineral ions frequently occur at inappropriate pH conditions in the rhizosphere, especially in the soilless media which lack the buffering capacity of soil (Zieslin, 1994).

The pH of the nutrient solution is modified by the plants as they take up nutrients. Willumsen (1984) stated that plant roots will release $H^+$ if cations are taken up more rapidly than anions, and roots will release $HCO_3^-$ and $OH^-$ if anions are taken up more rapidly than cations. Spinu et al. (1998) stated that plant roots release an $OH^-$ ion for every nitrate ion ($NO_3^-$) that is taken up to maintain charge neutrality within the plant. This results in a continuous increase of nutrient solution pH, which must be counteracted by frequent addition of acid.

In field grown lettuce, maximum yield was obtained at pH 6, while pH 7.2 markedly depressed the yield of hearted lettuce (Adams & Winsor, 1984). Heavy liming to pH 6.9 reduced the yield of lettuce, but had no effect on quality (Adams et al., 1978b). In NFT lettuce, where the pH ranged between 5.0 – 6.5, there was no effect on tipburn incidence of lettuce and the effect on fresh weight was minimal (Bres & Weston, 1992).

The pH of the nutrient solution recommended for NFT lettuce growing in Belgium is between 5.8 and 6.2 (Benoit & Ceustermans, 1989) and 5.5 - 6.5 in the UK (Burrage, 1992). This is to ensure that phosphates remain in the more soluble dihydrogen form and the iron chelate remains associated (Burrage, 1992).

Bres and Weston (1992) reported that solution pH ranging between 5.0 – 6.5 generally did not effect the concentration of total nitrogen and nitrate in lettuce tissue. Increasing the pH increased potassium concentration and result in an increased proportion of potassium compared with magnesium and calcium. Although the influence of solution pH on phosphorus, calcium and magnesium concentrations was significant, nutrient accumulation differences were not reflected in lettuce fresh weight differences.

Willumsen (1984) suggested that owing to the importance of the stability of the pH only 5-10 percent of nitrogen should be present as ammonium. If tap water with a considerable concentration of $HCO_3^-$ is used for the water culture, then the pH should be controlled by regular additions of a diluted solution of $HNO_3$ and $H_3PO_4$ (with a ratio of nitrogen to phosphorus equivalent to the nitrogen and phosphorus requirement of the plants). A combination of phosphoric acid and nitric acid at a ratio of three to one (by volume) is commonly used (Care, 1991).

### 2.3.4 Nutrient solution concentration

Electrical conductivity (EC) values can not be used as measure of nutrient levels for managing the culture solution, owing to the ionic imbalance and depletion caused by rapid uptake of $NO_3^-$ and $K^+$ from the nutrient solution (Takano, 1988). However, Ho & Adams (1995) stated that the most practical method for adjusting the nutrient supply in relation to demand in hydroponic systems is by measuring the total ionic concentration of the solution as the electrical concentration (EC) in the root zone.

Klaring et al. (1997) stated that the variation in nutrient solution concentration causes changes in the ratio of nutrient uptake and water uptake. Nutrient and water uptakes are affected by growth stage and environmental conditions, while the ratio of nutrient uptake and water uptake depends on environmental conditions. With increasing mean daily temperature the concentration of nutrient solution should be lowered.

A range of nutrient solution concentrations have been used to grow lettuce. Satisfactory growth and yield of lettuce cultivar 'Ravel' was obtained with nutrient solution concentrations up to 5.5 mS $cm^{-1}$, in an expanded clay substrate system during January and April in Ireland, with an optimum of about 2 mS $cm^{-1}$ (Morgan et al., 1980b).

Willumsen (1984) stated that a nutrient solution concentration of anions and cations between 5 and 10 me $l^{-1}$ (0.5-1.0 mS $cm^{-1}$) gave the highest yield with a reduced risk of tipburn for the butterhead lettuce, cultivar 'Ostinata', grown in water culture at Virum, Denmark.

Van Der Boon et al. (1988) stated that increasing the nutrient solution concentration from 1.27 to 3.00 mS $cm^{-1}$, in summer and spring, did not affect fresh weight of the head when no $NH_4$ was present in the nutrient solution. The presence of 20% $NH_4$-N decreased head fresh weight in the spring crop, but not in the summer crop. Increasing nutrient solution concentration and air temperature both resulted in an increased growth rate of the winter crop. Tipburn was aggravated by high nutrient solution concentrations and was alleviated when the solution was heated.

Economakis (1990) found that the interaction of temperature and radiation was the most important factor influencing growth and the overall effect of solution concentration on shoot fresh weight was minor. It was suggested that lettuce could be grown over a wide range of solution concentrations without tipburn incidence, but nutrient solution concentrations within the 2.0-3.0 mS $cm^{-1}$ range will give more satisfactory results.

Care (1991) reported that the optimum nutrient solution concentration and flow rate was dependent on cultivar. Nutrient solution concentration between 1.2 and 2.0 mS $cm^{-1}$, depending on season, and a flow rate of 2 l $min^{-1}$ appeared to be optimum to grow a range of cultivars.

Huett (1994) investigated the effect of nutrient solution concentration between 0.4 and 3.6 dS $m^{-1}$ (mS $cm^{-1}$), on yield of head lettuce cultivar 'Coolguard' during Aug-Sep, cultivar 'Fame' during Feb-Mar and cultivar 'Red Mignonette' during Nov-Dec, grown in gravel culture in New South Wales, Australia. He reported that lettuce grown at an EC of 0.4 $dSm^{-1}$ was nitrogen and potassium deficient. The highest fresh weight of head and/or leaf of mature heading and non-heading lettuce was obtained from the 1.6 dS $m^{-1}$ concentration.

### 2.3.5 Cation ratio

Different ratios of potassium, calcium, and magnesium maintained in NFT solution had no influence on lettuce tipburn development (Willumsen, 1984) and did not result in any significant yield differences (Steiner, 1980; Willumsen, 1984). The consumption and uptake of nitrate, ammonium, phosphorus, sulphur and potassium were almost the same for all cation ratios examined. Only the uptake of calcium and magnesium revealed significant differences (Willumsen, 1984).

Voogt (1988) claimed that with a sufficient supply of potassium, the potassium: calcium ratio in the recirculating nutrient solution is of minor importance and that the ratios in which potassium and calcium are added may vary between 0.8 and 5.8 for lettuce in circulating water.

Increasing potassium: calcium ratio increased lettuce shoot fresh weight (Huett, 1994) and slightly increased tipburn incidence (Voogt, 1988). Huett (1994) reported that increasing the potassium: calcium ratio from 1.00: 3.50 to 3.50: 1.00 increased the fresh weight of the leaf and shoot at week 6, but had no effect at week 4 after transplanting.

Table 2.1 summarises the range of major nutrient concentrations used or found to be satisfactory by a range of researchers. Nitrogen concentration ranged from 28-210 mg $l^{-1}$, with 100-200 mg $l^{-1}$ being a typical concentration. Phosphorus concentration ranged from 6.5-62 mg $l^{-1}$, with 30 mg $l^{-1}$ being a typical concentration. Potassium concentration ranged from 56-332 mg $l^{-1}$, with concentration higher than 200 mg $l^{-1}$ being a typical concentration. Calcium concentration ranged from 24.2-200 mg $l^{-1}$, with 100-200 mg $l^{-1}$ being a typical concentration, while magnesium concentration ranged from 5.7-63 mg $l^{-1}$, with 20-30 mg $l^{-1}$ being a typical concentration.

**Table 2.1 Concentration of nutrient ions (mg $l^{-1}$) used by various researchers for hydroponic lettuce growing**

| sources | $NO_3$-N | $NH_4$-N | P | K | Ca | Mg |
|---|---|---|---|---|---|---|
| Perez Melian et al., 1977 | 168 | - | 31 | 273 | 180 | 28.6 |
| Hammer et al., 1978 | 105 | - | 15.5 | 117 | 100 | 24.3 |
| Cooper, 1979 | 200 | - | 60 | 300 | 170 | 50 |
| Johnson & Moore, 1983 | 166 | | 45 | 240 | 136 | 20 |
| Knight & Mitchell, 1983 | 210 | - | 30.98 | 234.6 | 200 | 48.6 |
| Os & Kuiken, 1984 | 133 | 3.5 | 30.98 | 195 | 90 | 18 |
| Prince & Koontz, 1984 | 205.8 | - | 48 | 210 | 200 | - |
| Suzuki et al., 1984 | 84 | - | 45 | 156 | 40 | 12 |
| Willumsen, 1984 | 44.8-226.8 | 11.2-57.4 | 6.2-37.2 | 27.3-128.7 | 32- 164 | 12- 60 |
| Tregidga et al., 1986*-start | 208 | - | 62 | 332 | 168 | 49 |
| Tregidga et al., 1986*-topping up | 98 | - | - | 142 | 67 | 32 |
| Francois, 1988 | 28 | 7 | 15.5 | 58.5 | 80 | 24 |
| Voogt, 1988 | 133 | 7 | 30 | 156-292 | 54- 120 | 9-18 |
| Economakis, 1990 | 118 | - | 26 | 236 | 123 | 46 |
| Heinen et al., 1991 | 140 | - | 20.65 | 205.4 | 94 | 19 |
| Bres & Weston, 1992 | 140 | | 60 | 150-225 | 150 | 40 |
| Kanaan & Economakis, 1992 | 159 | - | 36 | 320 | 167 | 63 |
| Kratky, 1993 | 93 | - | 33 | 108 | 110 | 18 |
| Gaudreau et al., 1994 | 119 | - | 24.1 | 113 | 116 | 29.1 |
| Jang et al., 1994 | 210 | - | 31 | 59 | 200 | 48 |
| Jensen et al., 1994 | 52.15 | 4.38 | 15.5 | 107.2 | 45 | 6 |
| Huett, 1994 | 35.85 | 0.2 | 6.5 | 56.3 | 24.2 | 5.7 |
| Steingrobe& Schenk, 1994 | 28 | - | 45 | 59 | 140 | 24 |

* Nutrient solution for tomato growing

## 2.3.6 Dissolved oxygen level in the nutrient solution

In NFT gasses move to and from the roots mainly by a mass flow of gas dissolved in the moving solution. In principle, aeration of the roots in NFT should be at least as effective as in well drained border soil (Jackson et al., 1984). The amount of dissolved oxygen in the nutrient solution depends on the nutrient flow rate, the length and width of the gully, the microbial population, the temperature and the amount of root (Gislerod & Kempton, 1983).

Goto et al. (1996) reported that there was no significant differences in fresh weight, shoot and root dry weights of lettuce grown in floating system where the dissolved oxygen concentration ranged from 2.1 (25% of saturated at 24 $^0$C) to 16.8 (200%) mg $l^{-1}$. The super saturated dissolved oxygen using pure, pressurized, oxygen showed no positive biological effect. They concluded that the critical dissolved oxygen concentration for vigorous lettuce growth was considered to be lower than 2.1 mg $l^{-1}$. Wees and Stewart (1987) also concluded that dissolved oxygen was not limiting to lettuce growth. On the other hand, Jang et al. (1994) reported that the highest dry matter accumulation of lettuce was achieved where the dissolved oxygen concentration ranged from 6-7 ppm compared to 3.9 and 7.9 ppm. An excess of aeration decreased dry matter product. The growth response of the lettuce root to aeration was more sensitive than that of leaf and there was a positive relationship between root growth and aeration.

Variation in flow rate has not been shown to influence plant growth appreciably (Prince & Koontz, 1984). Care (1991) found that a flow rate of the nutrient solution between 1-2 l $min^{-1}$ showed no difference in lettuce growth, but when the flow rate was increased to 12 l $min^{-1}$ the lettuce growth was negatively affected.

The steeper the slope, the greater the flow and hence the more oxygen there is in the nutrient solution, and the less chance of stagnation and infection. The slope of the gullies for butterhead lettuce in Belgium is between 1.5 to 2 % (Benoit & Ceustermans, 1989), while the slope of the gully for lettuce growing was set at 1: 100 by Johnson and Moore (1983).

Wees and Stewart (1987) reported that the dissolved oxygen decreased with increasing temperature of the nutrient solution and the stage of plant growth, due to the increased root respiration. Jackson (1980) had earlier stated that at higher temperatures less oxygen was dissolved in the nutrient solution. A rise from 20 $^0$C to 30 $^0$C depressed the oxygen content of air-saturated water from 9.62 mg $l^{-1}$ to 7.8 mg $l^{-1}$. Higher temperature also increased respiratory demand for oxygen by the roots, this approximately doubled for each 10 $^0$C rise in temperature up to about 30 $^0$C.

## 2.4 Growth analysis

Growth analysis using frequent destructive harvests leads to a good understanding of the effect of environmental conditions on growth during the entire period from transplanting until harvest. A quantitative analysis of growth, applied for plants with a long period of growth and with various ontogenetic stages, is complex, but gives valuable information with the applied mathematical approach (Van Holsteijn, 1980). Simple empirical models can provide useful information and predictions, particularly if they are based on biological meaningful parameter (Richards, 1959).

Relative growth rate (RGR) is defined as the increase in plant material per unit of material per unit of time. It is equivalent to the slope of the plot of the logarithms of weight against time (Hunt, 1978). RGR is important because yield of a plant is the product of its initial weight and its relative growth rate (Scaife & Jones, 1976). In plants, the relative growth rate may be partitioned into two components:

1. Net assimilation rate (NAR) and

2. Leaf area ratio (LAR).

LAR may be partitioned into a further 2 components:

1. Specific leaf area (SLA) and

2. Leaf weight ratio (LWR)

Net assimilation rate (NAR) is the net gain in weight per unit of leaf area (Hunt, 1982). It may be regarded as a measure of net photosynthetic rate (Diputado, 1989).

Leaf area ratio (LAR) is the ratio of total leaf area to whole plant dry weight. It represents the ratio of photosynthesizing to respiring material within the plant (Hunt, 1982). It is a measure of the balance of payments between 'income' and 'expenditure' because it deals with the potentially photosynthesizing and the potentially respiring components of the plants (Hunt, 1990).

Specific leaf area (SLA) is an index of the leafiness of the leaf. It is a measure of density or of relative thinness, because it deals with leaf areas in relation to their dry weight (Hunt, 1990).

Leaf weight ratio (LWR) is an index of the leafiness of the plant on a dry weight basis. It is a measure of the productive investment of the plant, because it deals with the relative expenditure on potentially photosynthesizing organs (Hunt, 1990).

In lettuce, the use of an environmental time scale results in a better fit than the fit of the data with the normal time scale (Nichols, 1971; Aikman & Scaife, 1993; Tei *et al.*, 1996b). The accumulation of total plant dry matter by lettuce from the emergence until final harvest can be described by a logistic relationship (Lee, 1974; Heinen et al., 1991; Heinen & Moolenbroek, 1995; Heinen, 1997). However, Tei et al. (1996a) stated that the best fit for lettuce was obtained by the Gompertz function compared with logistic or expolinear functions of Scaife and Jones (1976). Scaife and Jones (1976) reported that the logistic curve included a considerable apparent linear phase on either sides of its inflexion point. Lettuce, for commercial purposes, is normally harvested about the time when the inflexion point is reached, so the final linear phase is not seen.

Growth is normally exponential for lettuce plants in a constant environment for about half the life of the crop (Cox et al., 1976; Scaife & Jones, 1976) after which relative growth rate decreases as plant weight increases (Cox et al., 1976). Lee (1974) found that the trend for RGR, NAR and LAR for lettuce grown in sand culture was to decrease during the growing period. Mutual and self shading diminish the growth of lettuce, and their effect becomes evident at a later stage for the dry weight increase than for the soil cover rate (Van Holsteijn, 1980; Tei et al., 1996b).

Tei et al. (1996b) reported that lettuce showed very high ability in light interception and growth during the early growth stages but, throughout the growth cycle, it showed the

lowest radiation use efficiency, compared to onion and red beet, due to mutual shading of the leaves within the plant canopy and to high respiration cost of production and maintenance of the leaves.

Watson (1952) reviewed crop growth studies and concluded that variation in yield from the application of manure and seasonal effects were mainly due to their effects on leaf area, rather than NAR. Brouwer and Huyskes (1968) reported that the faster growth of one cultivar of lettuce could be ascribed solely to a better exposition of its leaves to light as a result of a larger leaf area. Lee (1974) concluded that the bigger size of lettuce plant was mainly due to the different rate of leaf area production. On the other hand, the differences in RGR between fertiliser treatments were reported to be associated with differences in NAR in carrot (Austin, 1963), two populations of orchard grass (Eagles, 1967) and lettuce (Nichols, 1971). Jang et al. (1994) also concluded that the differences in RGR of lettuce by aeration rate of the nutrient solution were attributed to differences in the net assimilation rate.

## 2.5 Mineral nutrient

### 2.5.1 Introduction

Nutrient uptake is mainly an active process, if necessary against a gradient in concentration and the plant has to spend energy in order to take up nutrient. Uptake rate is usually described by uptake isotherms giving the uptake rate as a function of external concentration and to an important degree governed by plant demand, which in its turn depends on plant age, and meteorological conditions (radiation, temperature) (Willigen & Heinen, 1998). It is assumed that the plant roots can regulate the uptake in a wide concentration range for an adequate nutrition of the different tissues. The nutrient absorption curves of field crops deviate from those under constant conditions because external factors such as light, temperature and relative humidity vary during cultivation (Van Goor et al., 1988).

Tissue analysis data can show large differences in elemental concentration among experiments conducted by the same investigator as well as by different investigators.

These differences, however, may have no observable effect on the vegetative growth of lettuce (Berry et al., 1981). Thus lettuce do not respond to changes in tissue elemental content as long as the concentration remains within an adequate range (Perez Melian et al., 1977; Hammer et al., 1978; Berry et al., 1981).

In lettuce, total nutrient uptake detected from changes in nutrient solution conductivity during the day and night is similar (Care, 1991; Huett, 1994). Heinen et al. (1991) found that the relative cumulative uptake of nitrogen, potassium, B, Zn and Cl (and to a lesser extent of calcium, magnesium and S) has the same shape as the relative growth rate. The uptake of phosphorus was relatively small as plants grew older, while the uptake of Fe, Mn and Na was relatively larger.

Table 2.2 summarises the concentration of major nutrients concentration in lettuce. The data covers soil and greenhouse crops and different cultivars, plant age and sampling techniques. Comparison of the data within table should be made by keeping these differences in mind.

**Table 2.2 Concentration of nitrogen, phosphorus, potassium, calcium and magnesium in % dry weight of lettuce.**

| | Nitrogen | Phosphorus | Potassium | Calcium | Magnesium |
|---|---|---|---|---|---|
| **Zink & Yamaguchi, 1962** | | | | | |
| shoot | 3.59 | 0.54 | 5.25 | 1.24 | 0.43 |
| root | 2.22 | 0.58 | 4.46 | 0.46 | 0.17 |
| range | 3.10 - 6.65 | 0.34 - 0.79 | 4.57 - 9.44 | 0.91 - 1.60 | 0.32 - 0.84 |
| **Haworth & cleaver, 1967** – shoot | 4.41 - 5.25 | 0.34 - 0.66 | 3.44 - 8.50 | 1.00 - 1.31 | 0.33 - 0.68 |
| **Knavel, 1974** | | | | | |
| youngest leaves | 4.78 - 5.46 | 0.66 - 0.68 | 4.39 - 4.45 | 0.26 - 0.33 | 0.28 - 0.31 |
| oldest leaves | 3.40 - 3.54 | 0.38 - 0.44 | 4.57 - 5.57 | 1.35 - 1.72 | 0.51 - 0.52 |
| average head | 4.44 - 4.99 | 0.60 - 0.62 | 4.48 - 4.68 | 0.54 - 0.68 | 0.34 -0.36 |
| **Temple-Smith & Menary, 1977** | | | | | |
| shoot | | 0.06 - 0.74 | | | |
| root | | 0.13 - 0.69 | | | |
| **Greenwood et al., 1980a, b, c** – leaf | 1.90 - 2.61 | 0.28-0.44 | 1.58-4.04 | | |
| **Berry et al., 1981**-leaf | | 0.57 | 11.60 | 1.39 | 0.32 |
| **Knavel, 1981** - whole plant | 4.61 - 6.20 | 0.75 - 0.92 | 9.10 - 10.43 | 1.23 - 1.60 | 0.55 - 0.84 |
| **Costigan, 1984** –whole plant | | | | | |
| 21 days after sowing | 4.8 - 5.8 | 0.50 - 1.25 | | | |
| 36 days after sowing | 4.7 - 6.1 | 0.5 - 1.13 | 4.9 - 8.0 | | |
| experiment 2 – 30 days (-NH4) | 4.6 - 4.8 | 0.82 - 0.95 | 6.0 - 8.7 | 1.4 - 1.9 | 0.42 - 0.56 |
| (+NH4) | 5.0 - 5.2 | 0.98 - 1.41 | 5.4 - 8.2 | 1.4 - 2.1 | 0.58 - 0.73 |
| **Costigan, 1985** –whole plant | | | | | |
| ≈2 weeks after sowing | 3.9 – 5.5 | 0.31 - 0.95 | 3.8 – 8.7 | 0.14 – 0.45 | 1.1 – 1.9 |
| **Sanchez et al., 1988** -oldest sound leaf | | | | | |
| eight leaf stage | 4.78 | 0.44 | 5.97 | 1.79 | 0.43 |
| maturity | 3.68 | 0.44 | 6.67 | 2.27 | 0.42 |
| **Sanchez et al., 1990** oldest sound leaf | 3.34 - 3.84 | 0.23 - 0.36 | 9.5 - 11.3 | | |
| **Bres & Weston, 1992** -leaf | | | | | |
| Butter crunch | 5.32 | 0.87 | 7.26 | 1.16 | 0.30 |
| Summer Bibb | 5.69 | 1.03 | 7.67 | 1.58 | 0.42 |
| Butter crunch | 5.72 | 1.00 | 8.10 | 1.47 | 0.41 |
| Summer Bibb | 6.14 | 1.41 | 7.93 | 1.89 | 0.53 |
| **De Kreij et al., 1992**-leaf | 4.2-5.6 | 0.62-0.77 | 7.81-13.67 | 0.8-1.2 | 0.24-0.73 |
| **Jang et al., 1994**-eight leaf stage | 3.39 - 3.54 | 1.62 - 1.75 | 8.70 - 10.03 | 1.95 - 2.18 | 0.45 - 0.48 |
| **Drews et al., 1997**-head | 3.23 ± 0.41 | 0.66 ± 0.09 | 4.4 ± 0.76 | 0.66 ± 0.13 | 0.23 ± 0.10 |

## 2.5.2 Nitrogen

Nitrogen and potassium are the dominant nutrients taken up by the plant, and the head was the major sink for lettuce. Huett and Dettmann (1992) found the highest ratios of nutrients in head to whole plant for lettuce were recorded when the 11 M $m^{-3}$ nitrogen level were applied, whereas the lowest ratios were recorded for the 2 and 36 M $m^{-3}$ nitrogen level. Nitrogen and potassium were remobilized from outer leaves and stem of lettuce to the head over the last week of the growth period.

Huett and Dettmann (1992) also reported that nitrogen uptake rate of lettuce reached a peak 6 weeks after transplanting and then declined rapidly, whereas Van Goor et al. (1988) found the rate of nitrogen uptake (daily uptake per plant) by lettuce increases rapidly (partly exponentially) during the growing period and was constant at the end. Over 70% of the above ground dry weight, which accumulates during the last few weeks of the crop cycle, is accompanied by the maximum uptake rate of nitrogen (Zink & Yamaguchi, 1962; Jackson et al., 1994), phosphorus and potassium (Zink & Yamaguchi, 1962).

The yield of lettuce first increases and then either remains the same or declines with further increases of nitrogen fertiliser (Goodall et al., 1955; Greenwood et al., 1980a). Greenwood et al. (1980a) achieved the optimum yield in the field with 170 kg N $ha^{-1}$. In peat, Adams et al. (1978a) found that the yield and proportion of hearted lettuce increased with applied nitrogen until the peat contained 60 mg N $l^{-1}$. There was little response to nitrogen over the range 60 – 250 mg N $l^{-1}$. Rapid growth with minimum risk of tipburn was achieved by Willumsen (1985) when the nitrogen concentration in the solution was between 60 and 130 ppm. Knavel (1981) reported that the ratio of root to shoot decreased with increasing nitrogen and potassium levels at both 21 and 13 $^{0}$C.

The rate of nitrogen uptake and the nitrogen content of the leaves is influenced by temperature (Winsor & Adams, 1987). Generally, higher levels of nitrogen are found in plants grown in cooler soil than in those grown in warmer and most of the nitrogen is found in the youngest leaves (Knavel, 1974). The rate of uptake both of ammonium and nitrate increases with temperature, though the response is greater with nitrate (Frota & Tucker, 1972).

The nitrogen concentration is highest when the plants are young and generally decreases with age (Zink & Yamaguchi, 1962; Knavel, 1981). Nitrogen concentration is also highest in young leaves and lowest in the outer leaves (Knavel, 1974). The nitrogen content corresponding to maximum yield reported by various workers was 1.92 % N in the midribs of the wrapper leaves and about 5.1 % N in the laminae (Bishop et al., 1973), while in the leaves it was 4.1% N (Perez Melian et al. 1977), 5% N (Adams et al., 1978a) and 5.4 - 5.7 % N (Knavel, 1981).

The relationship between the concentration of nitrogen in the leaf tissue and yield is not particularly sensitive and is of limited use as a diagnostic parameter for lettuce (Perez Melian et al., 1977; Greenwood et al., 1980a; Sanchez et al., 1988). For example, Greenwood et al. (1980a) reported that a difference of 0.1% nitrogen in the plant material was associated with a 10% increase in yield of a range of crops which included lettuce.

Nitrogen deficiency decreases the growth and delays the hearting of lettuce (Perez Melian et al., 1977; Adams et al., 1978a; Winsor & Adams, 1987) and such plants often have well developed root systems (Winsor & Adams, 1987). Deficient plants remain small and pale green whilst the older leaves became yellow and the roots wither. With a severe deficiency the plants are stunted, no heart is formed and the leaves may develop a purple or brown flush (Winsor & Adams, 1987). Perez Melian et al. (1977) reported that in mature plants 3% nitrogen and 3.6% nitrogen were associated with severe and mild deficiency respectively. Adams et al. (1978a) found that nitrogen deficiency depressed the proportion of marketable lettuce by 50% and 29% in autumn and winter crops, but only 3% in spring. High levels of nitrogen increase the ratio of foliage to root dry weights, while total dry matter is unaffected and these changes are associated with a considerable increase in the % N in the root dry matter (Greenwood et al., 1980a).

### 2.5.3 Phosphorus

The uptake of phosphorus continues to increase over the last 2 weeks of the growth period (Huett & Dettmann, 1992). Temple-Smith and Menary (1977) reported that the maximum plant yield of lettuce occurred at a mean rate of phosphate absorption of 8.4 μM phosphate per gram (0.026 μg $g^{-1}$) root fresh weight per day.

Costigan (1984) found that the percent phosphorus in the plants at 36 days from sowing could account for 60% of the variation in plant dry weight whereas % N could account for only 12% (regression analysis). Leaf analysis suggests that phosphorus is often the most limiting nutrient. Thus Sanchez et al. (1990) concluded that in nearly every case where a fertiliser deficit exists the leaf tissue phosphorus concentration was below the critical level of 0.43 %. The critical level is defined as the nutrient concentration in the tissue associated with a 10% reduction in yield (Ulrich, 1952). These responses to phosphorus are most likely related to the availability of phosphorus in the soil in which the research was carried out (Costigan & Heaviside, 1988).

With soil grown lettuce, the phosphorus content of the leaves increases with the increasing level of phosphorus applied (Grant Lipp & Goodall, 1958; Greenwood et al., 1980b; Adams & Winsor, 1984), and decreases with increasing age of the plant (Grant Lipp & Goodall, 1958; Zink & Yamaguchi, 1962). The younger leaves contain more phosphorus than the older leaves (Grant Lipp & Goodall, 1958; Knavel, 1974). The younger leaves contain nearly twice as much as the older leaf laminae, while the midribs contain even less than the latter (Grant Lipp & Goodall, 1958).

Lettuce seedlings can grow at the expense of phosphorus stored in the seed during the first 11 days (Grant Lipp & Goodall, 1958). However, as the growth curves for the various phosphorus treatments used by Scaife and Smith (1973) began to separate at about 4 days from emergence, it is apparent lettuce seedlings are sensitive to lack of phosphorus. The critical concentrations of phosphorus in lettuce seedlings were 780, 600, 580 ppm soluble $PO_4$-P on a dry weight basis for the conductive, lamina and root tissue respectively in the study of Berry (1971) and 0.44% at the six to eight leaf stage in the study of Sanchez et al. (1988). Adams and Winsor (1984) suggest that the yield of lettuce is closely related to the phosphorus content of the leaves up to value of about

0.6% phosphorus. Scaife and Smith (1973) conclude similarly that the optimum plant phosphorus concentration is 0.6% for lettuce. The phosphorus percentage in the dry matter of lettuce, it is suggested, could be useful for detecting deficiencies (Perez Melian et al., 1977; Greenwood et al., 1980b).

Phosphorus deficient plants are stunted with purple coloured leaves. The plants fail to form a heart and the older leaves die prematurely (Perez Melian et al., 1977; Adams & Winsor, 1984).

### 2.5.4 Potassium

Zink and Yamaguchi (1962) concluded that the shape of the nitrogen, phosphorus, potassium, calcium, magnesium, and sodium uptake curves was very similar to that for dry matter production. Over 70% of the nutrient uptake of the crop was absorbed during the twenty-one days preceding first harvest.

Huett and Dettmann (1992) reported that the potassium uptake rate of lettuce reached a peak 6 weeks after transplanting and then declined rapidly. The potassium: nitrogen ratio of lettuce was about 2:1, while nitrogen and potassium were remobilized from the outer leaves and stem of lettuce to the head over the last week of the growth period.

Van Goor et al. (1988) however reports changes in nutrient ratios in the plant over time. Thus potassium: nitrogen ratio in lettuce decreases strongly during growth from about 1.3 to about 0.50. Also the calcium: potassium ratio decreases strongly from about 0.5 to 0.05. The low calcium: potassium ratio at the end of the growing period may be due to low evaporation after the onset of head formation, especially in the younger leaves.

In soil grown crops the uptake of potassium by the crop increases in a 'diminishing returns' manner with increasing levels of potassium fertiliser (Greenwood et al., 1980c). Thus in the research of Berry and Carey (1971) the yield of lettuce increased with increasing potassium supply to 2 mM $l^{-1}$, with no further increases in yield with higher rates of potassium supplied. Potassium provided at 150 mg $l^{-1}$ in the nutrient solution was found to be sufficient for NFT lettuce production (Bres & Weston, 1992). Effects of potassium fertiliser on lettuce quality are generally small, while the

percentage potassium at harvest is a good indicator of the extent to which crop growth is restricted by lack of potassium (Greenwood et al., 1980c).

Zink and Yamaguchi (1962) concluded that the potassium content fluctuated throughout the growth of the crop, and no general trend was observed. Barta and Tibbitts (1991) report that the potassium concentrations were highest at the leaf apex and decreased towards the base and also decreased from the midrib to the margin. The critical concentrations of potassium in lettuce tissue are reported as 2, 1.8, and 1.3% in the conductive, lamina and root tissue respectively (Berry & Carey, 1971), 15-22 mM $l^{-1}$ in petiole sap from young expanding leaves (Burns, 1986) and 5.6% at the six to eight leaf stage (Sanchez et al., 1988). Knavel (1981) reported that relatively high concentrations of potassium (9-10%) in leaf dry matter were associated with good growth. Potassium supplies must be sufficient to produce leaf concentrations of about 9-10%, whether the plants are grown under cool or under warm soil conditions. Knavel, (1974) has earlier reported that the cation potassium was highest in the oldest leaves.

Fertiliser applications have marked effects on the mineral composition of the plants, especially on the potassium, phosphorus and magnesium contents. Any treatment that increases the potassium content of the plant tends to reduce the magnesium content and vice versa (Haworth & Cleaver, 1967). Diagnosis of malnutrition may be based on leaf analysis for potassium deficiency, but not for an excess of potassium (Perez Melian et al., 1977).

The potassium deficiency symptoms are stunted growth, yellowing of the leaf-edges of the oldest leaves, which turn necrotic later on (Voogt, 1988). With a severe deficiency, yellow spots develop near the tips of the older leaves. These spots then spread and coalesce, and may eventually become brown (Winsor & Adams, 1987).

## 2.5.5 Calcium

### 2.5.5.1 Introduction

Calcium is the fifth most abundant element in the earth's crust (Shear, 1975b), accounting for about 3.5% of its composition (Havlin et al., 1999). Because of the importance of calcium in lettuce production, due to its involvement in tipburn, it is discussed in more detail in this review than the other nutrients.

### 2.5.5.2 Physiology

The role of calcium in plants includes involvement in cell integrity, membrane function, ionic balance, cell elongation and secondary messenger transduction (Poovaiah & Reddy, 1987). The physiological role of calcium in plants is summarised in Table 2.3.

One of the most well known functions of calcium in plant nutrition is its role in membrane stability and the maintenance of cell integrity (Epstein, 1972; Battey, 1990) with at least 60% of the total calcium in plants being associated with the cell wall (Poovaiah, 1993). Calcium has long been known to have a binding role in the complex of polysaccharides and proteins forming the cell wall. Cell cohesion is typically attributed to calcium pectate of the middle lamella laid down during cell division (Hanson, 1984). Cell separation during abscission involves acidification and loss of calcium, with the pectins of the middle lamella glatinizing as they progress from Ca-pectate to pectic acid and then to methylated pectin (Sexton & Roberts, 1982). Calcium retards abscission and its removal accelerates it (Poovaiah & Leopold, 1973a).

Fruit and vegetable tissues are well known for physiological disorders that result from calcium deficiency (Simon, 1978; Bangerth, 1979; Battey, 1990). Calcium deficiency weakens walls to the point of osmotic rupture, while high calcium in fruit tissue is associated with low respiration and low solute leakage, suggestive of an equal importance of apoplastic calcium in membrane integrity (Simon, 1978).

**Table 2.3 Calcium related physiological processes in plants**

| Physiological process | Reference |
|---|---|
| Plant growth hormone action | Leopold, 1977 |
| Auxin-induced elongation | Cleland & Rayle, 1977 |
| Auxin binding | Poovaiah & Leopold, 1976a |
| Auxin transport | Dela Fuente & Leopold, 1973;<br>Brown & Ho, 1993 |
| Auxin- mediated H secretion | Cohen & Nadler, 1976 |
| Cytokinin-induced bud formation | Saunders & Hepler, 1982 |
| Abscission | Poovaiah & Leopold, 1973a<br>Poovaiah & Rasmussen, 1973a<br>Poovaiah & Rasmussen, 1973b |
| Senescence and ripening | Poovaiah & Leopold, 1973b; Ferguson, 1984 |
| **Secretory processes** | |
| alpha-Amylase secretion | Jones & Jakobsen, 1983<br>Jones et al., 1986<br>Moll & Jones, 1982 |
| Polysaccharide secretion | Morris & Northcote, 1977 |
| Peroxidase secretion | Sticher et al., 1981 |
| **Tropism** | |
| Gravitropism | Lee et al., 1983a<br>Lee et al., 1983b<br>Lee et al., 1984 |
| spore germination | Wayne & Hepler, 1985 |
| Cytoplasmic streaming in Nitella | Hayama et al., 1979 |
| Membrane depolarisation of Nitella cells | Weisenseel & Ruppert, 1977 |
| Phototactic behavior | Schmidt & Eckert, 1976 |
| Cell division | Hepler, 1985; Hepler, 1986 |
| Osmoregulation | Kauss, 1981; Kauss, 1983 |
| Mimisa leaf movement | Toriyama & Jaffe, 1972 |
| Pollen tip growth | Reiss & Herth, 1979 |
| Tuberization | Balamani et al., 1986 |
| **Membrane structure and function** | |
| Ultrastructural membrane alteration | Moore & Bracker, 1976 |
| Membrane damage and leakiness | Poovaiah & Leopold, 1976b |
| Betacyanin synthesis | Elliot, 1983 |
| Cation transport | Epstein, 1961;<br>Schulte-Baukloh & Fromm, 1993 |
| Transmembrane calcium fluxes | Dieter & Marme, 1980 |
| Wound-induced nuclear migration | Schnepf & Volkmann, 1974 |
| Guard cell swelling | Hetherington et al., 1986 |
| Cell polarity | Robinson & Jaffe, 1975<br>Robinson & Cone, 1980 |
| Ripening | Poovaiah, 1979 |
| Regulation of phloem translocation | Schulte-Baukloh & Fromm, 1993 |
| Physiological disorders | Bangerth, 1979; Hopfinger & Poovaiah, 1979 |
| Secondary messenger transduction | Poovaiah & Reddy, 1993 |

Source: Adapted from Poovaiah & Reddy, 1987

Calcium binds the phospholipid molecules into the membranes. These phospholipids influence the size of pores in the membrane and thereby influence the selective permeability of the membranes (Ferguson & Drobak, 1988). External calcium tightens membranes, reducing passive ion fluxes making the membranes more hydrophobic (Hanson, 1984).

Apart from the well recognized functions of calcium, new dimensions are being added to its list of functions. Calcium's role as secondary messenger where intracellular calcium distribution plays a critical role in cell function is an example (Poovaiah, 1988). It is suggested that calcium acts as a second messenger in the response of plant tissues to external signals (Hepler & Wayne, 1985; Ferguson & Drobak, 1988). Environmental signals such as light (Roux et al., 1986; Roblin et al., 1989), gravity (Roux & Serlin, 1987; Gehering et al., 1990), salt stress (Pardo et al., 1998) and plant growth regulators such as ABA (Tester, 1990; Gehering et al., 1990), $GA_3$ (Gilroy & Jones, 1993), IAA (Felle, 1988) and cytokinin (Saunder & Hepler, 1981) increase the cytoplasmic $Ca^{2+}$ concentration by activating calcium channels in the membrane of calcium pools (Marschner, 1995).

Viets (1944) was one of the first workers to demonstrate the promotive effect of external calcium ions ($Ca^{2+}$) on the uptake of other ions. The results from solution studies suggest that the uptake rate of both ammonium and nitrate nitrogen by plants may be increased by increasing the concentration of calcium ion in the rhizosphere (Morgan et al., 1972; Fenn et al., 1987; Scherer et al., 1987; Bailey, 1992). Hanson (1984) stated that the promotion of anion uptake by calcium ion can be attributed largely to atomic shielding of the negative charge at the plasma membrane surface.

The uptake of anions is by symport (or cotransport) with $H^+$ expelled by the $H^+$-ATPase (Hanson, 1984). Promotion of anion uptake by calcium can be attributed largely to cationic shielding of the repulsive negative charge at the plasma membrane surface. The negative charges of the cell wall are implicated. The Viets effect is not calcium specific, and very likely one component of it lies in neutralization of anion-repulsive negative charges. Transport of anions into cells is against the electrochemical gradient due to the negative transmembrane electrical potential, and some form of energy-linked carrier (e.g. a $H^+$/anion symporter) is required. (Franklin, 1970).

In general terms, there are two characteristics of cation interaction at the membrane surface; the screening reaction of cations attracted into the adjacent aqueous layer, and the formation of cation-acid group complexes. The screening reaction is nonspecific for calcium, and can account for some of the Viets effect by facilitating anion uptake (Hanson, 1984).

### 2.5.5.3 Calcium uptake and transport within plant

Calcium uptake is mainly by mass flow as the ion (Barber et al. 1963; Clarkson, 1988). Uptake is passive and follows the influx of water (Kirkby & Pilbeam, 1984). Both calcium uptake and transport are associated with the exchange sites (Van de Geijn & Petit, 1979) and are independent of metabolism (Drew & Biddulph, 1971; Atkinson, 1991).

The rate of calcium uptake of apple tree from the soil is dependent on the total volume of the root system, root density, the periodicity of both root growth and activity in relation to tree demand, and the distribution of the root system in the soil (Himelrick & McDuffie, 1983). Furthermore, calcium uptake is dependent on external concentration and transpiration (Clarkson, 1988).

After reaching the root surface, calcium moves across the root cortex either by diffusion, by displacement exchange in the free space, or by a combination of these processes (Bangerth, 1979). The uptake and transport of calcium is restricted to an area just behind the root tips in which the cell walls of the endodermis are still unsuberized (Ferguson & Clarkson, 1975). Thus the movement of calcium from the cortex into the steel is restricted to the apoplastic or free space pathway which is only accessible in non suberized young roots (Russel & Clarkson, 1976). Once in the steel, calcium ions may enter the xylem vessels through active secretion by xylem parenchyma cells (Biddulph, 1967) or by passive leakage (Bowling, 1973).

Calcium translocation is very slow, typically in 2 phases: (1) a reversible exchange phase and (2) an irreversible accumulation phase (Biddulph et al., 1961; Bell & Biddulph, 1963). The transport of calcium preferrencially occurs in the xylem of plants (Biddulph et al., 1961; Tibbitts, 1979) as ion exchange on the plasma membrane and

deposition reactions with conductive tissue (Shear & Faust, 1970; Van de Geijn & Petit, 1979). The xylem cylinder operates as an exchange column (Biddulph et al., 1961; Clarkson, 1984), the bound calcium in the xylem tissue can be exchanged by free $Ca^{2+}$ and other cation species and moves upward in the transpirational stream by a series of exchange reactions (Van de Geijn & Petit, 1979; Clarkson, 1984).

The mobility of calcium in the xylem is promoted by the presence of other divalent cations, such as magnesium, which are also adsorbed on exchanged sites (Himelrick & McDuffie, 1983). The presence of the uncharged or negative charged Ca-complexes in the xylem result in the saturation of the apoplastic electronegative complex (Singh & Jacobson, 1979), thereby increasing calcium transport (Vang-Petersen, 1980).

Chelated calcium is more readily translocated than free calcium (Ferguson & Bollard, 1976; Van de Geijn & Petit, 1979). High concentrations of chelating compounds or malic or citric acids may also promote calcium movement in plants (Shear & Faust, 1970; Ferguson & Bollard, 1976) and as much as 50% of the calcium in xylem sap may be complexed with citric and malic acid (Bradfield, 1975).

Removal of calcium ion from exchange sites to a more immobile state may occur through the utilisation of ions by actively metabolising cells of meristematic regions, and the deposition of ions in the crystal systems of the oxalate type (Bell & Biddulph, 1963). Young leaves tend to have greater metabolic activity than older leaves and therefore calcium preferentially accumulates in young leaves (Shear & Faust, 1970). Competition between sinks is intensified when calcium in the xylem is low and transpiration is high. When the calcium ion is abundant in the sap the distribution of the ion will be closely related to the intensity of transpiration and calcium will move in relative large amounts to older leaves. In plants with large heads of enclosed leaves excessive transpiration by the outer leaves diverts calcium from meristems (Clarkson, 1984).

In lettuce the role of transpiration in calcium transport is very apparent. Thus the average calcium concentrations in the outer leaves was always higher than in the inner leaves (Misaghi & Grogan 1978a; Collier & Huntington, 1983; Barta & Tibbitts, 1991). Similarly the concentration at the periphery of the outer leaves was always greater than

in the midrib, whereas for the inner leaves the converse was true (Collier & Huntington 1983; Barta & Tibbitts, 1991).

### 2.5.5.4 Calcium remobilisation and transport in phloem

Apical meristems and young growing fruits with rapid cell division have a high demand for calcium and depend on a continuous supply (Vang-Petersen, 1980). Once calcium is deposited in the leaves and fruit of plants, it often becomes highly immobile (Addiscott, 1974, Himelrick & McDuffie, 1983). It is transported in only very small concentrations in the phloem (Bangerth, 1979; Wiersum, 1979). Thus plants are not able to utilise calcium from older leaves for the growth of meristematic tissue, even when calcium deficiency symptoms are observed in the growing tips (Loneragan & Snowball, 1969). A consequence of this is the calcium requirement of enlarging organs may not be met by the xylem transport even when large amount of this ion are applied to the plant (Kirkby, 1979).

### 2.5.5.5 Factors affecting calcium uptake and transport

#### 2.5.5.5.1 Genetic factor

The calcium content of plant is to a large extent genetically controlled (Mengel & Kirkby, 1987; Havlin et al., 1999) and is little affected by the calcium supply in the root medium, provided that the calcium availability is adequate for normal plant growth (Mengel & Kirkby, 1987). Maximum growth rates of monocotyledons are obtained at a much lower calcium concentration than for dicotyledons (Loneragan & Snowball, 1969). The reason for this higher demand of dicotyledons for calcium is causally connected with the higher cation exchange capacity of the roots as well as in the other plant parts (Mengel & Kirkby, 1987).

### 2.5.5.5.2 Hormonal factor

There is experimental evidence that IAA plays an important role in calcium transport (Banuelos et al., 1987). Auxin promotes the acropetal efflux of calcium from Helianthus hypocotyl segments and the process is inhibited by the auxin transport inhibitor TIBA (De Guzman & DeLa Fuente, 1984). Opposite movement of calcium and auxin occur in both gravistimulated shoots and roots (Hepler & Wayne, 1985). Inhibitors of auxin transport prevent movement of calcium to the slower growing side and consequently inhibit gravitropic curvature (Lee et al., 1984). Bangerth (1979) suggests that the basipetal IAA transport forces calcium to be translocated acropetally.

Brown and Ho (1993) reported that they found no evidence that the basipetal IAA movement is essential to the concurrent uptake and transport of calcium within tomato fruit from cultivars with differing susceptibilities to blossom end rot (BER) nor IAA efflux to be consistently reduced by growth conditions such as shading or high salinity which induce BER. They also suggested that the calcium status of detached tomato fruit may be important to the basipetal transport of IAA and calcium may regulate the movement of IAA rather than vice versa.

### 2.5.5.5.3 Salinity

Increasing salinity reduced total calcium accumulation of tomato fruit, but had little effect on their dry weight, resulting in a progressive decline in the calcium concentration (% in the dry matter). In contrast, salinity had little effect on total calcium accumulation by the leaves but reduced their dry weight, so increasing the calcium concentration (Adams, 1990). Salinization of the NFT solution with either NaCl or major nutrients (K, calcium and $NO_3$-N) had a similar effect on calcium accumulation, suggesting a common osmotic effect on calcium transport (Adams & Ho, 1990). Salinization of the NFT solution decreased the calcium content (%) of tomato fruit (Adams & Ho, 1990; Adams, 1992; Brown & Ho, 1993) and sweet pepper (Tadesse, 1997) due to an osmotic effect on calcium transport. This response was most marked at night, when more of the newly absorbed calcium moved into the fruit rather

than during the day (Adams & Ho, 1990). The effect of high salinity in decreasing calcium uptake has been widely reported in lettuce (Cresswell, 1991; Huett, 1994).

### 2.5.5.5.4 Nitrogen

Nitrogen supplied to roots as nitrate ($NO_3$) not only supplies an easily absorbed companion anion for calcium, but since it must be reduced in the roots before transport, it does not increase the level of competitive organic cations in the translocation stream (Shear, 1980). Furthermore, the leaves, petioles, stems and roots of tomato plants grown with nitrogen supplied as nitrate had higher organic acid contents than those supplied with nitrogen as ammonium (Kirkby & Mengel, 1967). Therefore nitrogen supplied as nitrate promotes calcium uptake and translocation.

On the contrary, nitrogen available as ammonium ions impairs the calcium status of plant (Wilcox et al., 1973). The effectiveness of $NH_4$ > potassium > magnesium > Na in depressing calcium uptake in tomatoes has been observed (Geraldson, 1971). Ammonium ions are absorbed preferrentially over nitrate and actually inhibit nitrate uptake by blocking nitrate reductase activity in the roots (Shear, 1980; Faust, 1986). Ammonium ions may also inhibit water uptake (Quebedeaux & Ozbun, 1973). Furthermore, ammonium ions reduce the soil pH (Shear & Faust, 1971) which increases the availability of metallic ions that reduce calcium uptake (Clarkson & Sanderson, 1971).

### 2.5.5.5.5 Phosphorus

The steady uptake of calcium may be furthered by increasing the availability of phosphate (Jakobsen, 1979). At elevated levels calcium will react with inorganic phosphate forming an insoluble precipitate (Hepler & Wayne, 1985). It has been suggested that living organisms evolved a method for removing calcium from the cell, lowering its concentration to 0.1 μM, at which point its reaction with inorganic phosphate would be insignificant (Kretsinger, 1977).

### 2.5.5.5.6 Cations

It is the general rule that increasing the supply of one cation species results in lowering the concentration of other cation species. This relationship is called 'cation antaginism' (Mengel & Kirkby, 1987). Thus the absolute concentration of calcium in the soil solution is less important in controlling calcium uptake than is the relationship of calcium to the total salt concentration and its proportionate concentration to that of other ions in solution (Shear, 1975a). Potassium (Smith & Wallace, 1956; Geraldson, 1971), ammonium (Battey, 1990) and magnesium (Mason, 1964; Haworth & Cleaver, 1967; Geraldson, 1971; Willumsen, 1984; Winsor & Adams, 1987) are antagonistic to calcium and retard its uptake. The cations Rb and Al also inhibit calcium uptake (Yamada, 1975).

Uptake rate depends on the concentration of the individual cation species in the nutrient solution and also on the uptake mechanism. Potassium which is taken up by the cell rapidly either actively or by facilitated diffusion competes strongly in cation uptake (Mengel & Kirkby, 1987).

Singh and Jacobson (1979) have reported that previous absorption of calcium or other cations such as potassium, resulted in the saturation of the apoplastic electronegative complex, thereby increasing calcium transport (Vang-Petersen, 1980).

### 2.5.5.5.7 Relative humidity (RH)

As calcium uptake is dependent on transpiration (Stebbins & Dewey, 1972; Clarkson, 1988) any climatic factors that influence transpiration affects calcium uptake. Generally, as relative humidity increases, transpiration and calcium uptake by leaves (Barta & Tibbitts, 1986; Adams & Holder, 1992; Adams & Hand, 1993) and fruit (Banuelos et al., 1985; Cline & Hanson, 1992) decrease. However, high relative humidity at night favour the transport of calcium into low transpiring organs, due to root pressure development in lettuce (Collier & Wurr, 1981; Collier & Tibbitts, 1984), cabbage (Palzkill & Tibbitts, 1977), chinese cabbage (Van Berkel, 1988) and strawberry (Bradfield & Guttridge, 1979; Guttridge et al., 1981; Choi et al., 1997).

### 2.5.5.5.8 Temperature

Calcium uptake is mainly governed by root activity, which increases as temperature rises, and decreases as nutrient solution concentration rises (Clover, 1991). Lettuce grown at lower temperatures contained less calcium than those grown at higher temperatures (Knavel, 1974; Ikeda & Osawa, 1984). Higher air temperatures at night (7-18 $^{0}$C) also increased calcium concentration of the leaves (Knavel, 1981). The rate of water uptake is a positive function of root temperature as is the rate of nutrient uptake (Moorby & Graves, 1980). Also as low temperatures restrict root development, it may affect calcium uptake since calcium is largely restricted to uptake via the root tips (Kirkby & Pilbeam, 1984). However, Chang et al. (1968) suggested that calcium was immobilised in the stem of tobacco at the higher temperature.

### 2.5.5.5.9 Root pressure

Absorption of water occurs along gradients of decreasing water potential from the soil or other root medium to the root xylem. This gradient is produced differently in slowly and rapidly transpiring plants. This results in two absorption mechanisms: osmotic absorption in slowly transpiring plants where roots acts as osmometers, and passive absorption in rapidly transpiring plants where water is pulled in by the decreased pressure or tension produced in the xylem sap. Osmotic absorption is responsible for root pressure, guttation, and most of the exudation of sap that occurs from wound in the stem. Root pressure has been attributed to secretion of water into the root xylem, electroosmosis and osmosis (Kramer, 1983).

Root pressure develops because water continues to move into the root, developing a hydrostatic pressure that forces water and calcium through the plant (Palzkill & Tibbitts, 1977). Root pressure occurs under conditions where transpiration is not functioning, and can be encouraged by increasing night time relative humidity, and by reducing the resistance to water movement into the plant (Bradfield & Guttridge, 1984; Wein, 1997). Maintaining adequate soil water, and having a low night time osmotic pressure of the soil solution are two ways in which water uptake can be maximised (Wien, 1997).

Root pressure is believed to be a simple osmotic process, caused by accumulation of sufficient solutes in the xylem to lower the water potential to the xylem sap below that of the substrate. The reduction of root pressure caused by insufficient aeration, low temperature, and respiration inhibitors is attributed to reduction in salt accumulation in the root xylem and to changes in root permeability, rather than to inhibition of any nonosmotic water transport mechanism (Kramer, 1983).

Positive root pressure at night promotes transport of calcium into tissues or organs that have restricted transpiration (Bradfield & Guttridge, 1984). When day time and night time concentration of nutrient were different, only the concentration given at night affected the concentration of calcium in the distal wall tissue of tomato (Bradfield & Guttridge, 1984) and emerging leaves of strawberry (Guttridge et al., 1981). Adding extra calcium to the nutrient solution increased the calcium concentration in the proximal, but not in the middle or distal, segments of the fruit (Bradfield & Guttridge, 1984).

### 2.5.6 Magnesium

Huett and Dettmann (1992) report that the nitrogen uptake rate of lettuce reached a peak 6 weeks after transplanting and then declined rapidly. The potassium, calcium and magnesium uptake rate followed a similar trend. The magnesium concentration is highest in the oldest leaves (Knavel, 1974). Chemical analyses of plants have shown a trend for magnesium to decrease as plants approach market maturity (Zink & Yamaguchi, 1962).

The magnesium content of lettuce plants is depressed by increasing levels of potassium (Winsor & Adams, 1987) and any treatment that increases the potassium content of the plant tends to reduce the magnesium content and vice versa (Haworth & Cleaver, 1967).

Sanchez et al. (1988) found no consistent trends in the concentrations of magnesium in the lettuce leaf tissue at the six to eight leaf stage and at maturity as affected by nitrogen, phosphorus and potassium fertilisation, while Francois (1988) reports calcium and magnesium concentrations in healthy leaf tissue and necrotic tissue of leaf margins decreased significantly with increasing soil boron concentration.

The growth rate is greatly reduced and hearting is prevented by magnesium deficiency. Interveinal and marginal yellowing develop on the leaves, and the margins of the oldest leaves eventually become scorched (Winsor & Adams, 1987).

## 2.6 Tipburn - a physiological disorder

### 2.6.1 Importance and symptoms

Tipburn is the most important physiological disorder in lettuce and cause substantial economic loss. Occurrence and intensity varies considerably from year to year and even within a growing period (Brumm & Schenk, 1993). Tipburn of lettuce is a serious problem that can lead to poor appearance (Collier & Tibbitts, 1984; Brumm & Schenk, 1993; Nagata & Stratton, 1994), increased pre-and post-harvest rots, and ultimately the reduction in the quality and grade of lettuce (Nagata and Stratton, 1994).

In greenhouses and growth chambers, tipburn may develop on young plants when enlarging leaves begin to bend inward, partially enclosing the young leaves around the growing point. Under field conditions, tipburn usually develops somewhat later in plant development when the head is well formed and close to maturity. Whenever tipburn develops, it appears as collapsed areas on the young leaves which rapidly become necrotic so that further development is restricted (Collier& Tibbitts, 1982). Its most severe form is characterized by a necrotic breakdown of the marginal tissue of the leaves within the head. Internal browning or blackening may occur and secondary pathological infection then gives rise to internal breakdown of the tissue (Cox & McKee, 1976), new leaf development may be completely inhibited and at times the terminal growing point is killed (Tibbitts & Rao, 1968).

Termohlen and Hoeven (1966) classified different types of symptoms as dry tipburn, normal tipburn, veinal tipburn and latex tipburn. However, it is agreed that these symptoms are all expressions of a common cause because the different symptoms are frequently found on the same plant (Cox et al., 1976; Collier & Tibbitts, 1982).

There are stages of lettuce plant growth where tipburn development is especially critical. Thus Stratton and Nagata (1993) report that susceptible plants at the 38 day-old stage of growth were in first stages of leaf cupping or enclosure and were growing very rapidly. At this point, leaf expansion may be too rapid to allow lettuce to transport adequate calcium to the expanding leaf margin. The age of first leaf cupping or enclosure is therefore critical to tipburn development.

Tipburn occurs in the inner leaves; it develops on only those leaves and those parts of the leaves which expanded most rapidly (Misaghi & Grogan 1978a; Collier & Huntington, 1983; Battey, 1990; Barta & Tibbitts, 1991). The youngest leaves which contain the lowest calcium concentrations are the most susceptible to tipburn (Thibodeau & Minotti, 1969). For the inner leaves, leaf breadth increases more rapidly than leaf length, and that the increase in breadth is greatest for the leaves on which tipburn develops most frequently. Tibbitts and Rao (1968) reported that tipburned leaves had width-length ratios greater than 1.0 (leaves wider than long), while tipburn was absent in leaves with width-length ratios less than 1.0.

In mature detached plants subjected to tipburn inducing temperatures, symptoms occurr on central leaves that exhibited appreciable growth during the temperature treatment, while middle and outer leaves, which grow only slightly, are usually symptomless (Misaghi & Grogan 1978a)

## 2.6.2 Causes of tipburn

Tipburn development appears to depend on the supply of calcium relative to the rate of leaf growth (Collier & Huntington, 1983; Barta & Tibbitts, 1991). A direct correlation between growth rate or head size and tipburn development has also been suggested (Cox et al., 1976; Misaghi & Grogan 1978a; Brumm & Schenk, 1993). Thus growing conditions reported as causing tipburn may do so by affecting growth rate (Cox et al., 1976). Several factors lead to the conclusion that loss of membrane integrity is the probably the initial stage in injury development (Collier & Tibbitts, 1982). The spontaneous release of latex near the margins of the leaf has been found to precede the development of tipburn. The release of latex is closely related to plant growth rate and

maturation. Possibly the released latex disrupts the vascular tissue which causes the collapse and necrosis of the leaf (Tibbitts et al., 1965).

Misaghi and Grogan (1978b) suggested that tipburn development is a manifestation of a localized calcium deficiency resulting from chelation of calcium by organic acids and other metabolites that are increased in plants during exposure to elevated temperature. Biological activities that are influenced by calcium nutrition and might contribute to tipburn development include membrane permeability, structural abnormalities, selective ion transport, increase in surface potential of membranes, protection against heavy metal toxicity, influencing the activity of several enzymes, maintenance of the plasma and vacuolar membranes, membrane integrity and formation of mitochondria (Misaghi & Grogan, 1978b)

Tipburn is a disorder recognised as associated with localised inadequacy of calcium in the leaf tissue (Shear, 1975b; Misaghi & Grogan, 1978b; Barta & Tibbitts, 1986) even when there is an adequate supply of calcium to the roots (Kirkby, 1979; Collier & Tibbitts, 1984; De Kreij, 1995). Calcium concentrations were significantly lower in enclosed leaves that exhibited tipburn symptoms than in exposed leaves that did not exhibit tipburn (Barta & Tibbitts, 1991). The lowest level of calcium were found in areas with tipburn (Ashkar & Ries, 1971; Collier & Wurr, 1981; Barta & Tibbitts, 1986, 1991). Magnesium and potassium levels increased acropetally and were generally highest in areas with tipburn (Barta & Tibbitts, 1986). The critical concentration for tipburn development in lettuce is 0.4 mg Ca $g^{-1}$ DW (Barta & Tibbitts, 1991).

A recent review by Saure (1998) has suggested that tipburn is a stress related disorder. External factors may cause stress and mild stresses below a damaging level, may reduce the risk of tipburn incidence by increasing stress tolerance.

## 2.6.3 Factors affecting tipburn development and severity

### 2.6.3.1 Introduction

As tipburn is a physiological disorder, which develops due to an inadequate calcium level for rapid growth of the inner leaves, factors inhibiting calcium uptake and translocation to the inner leaves when associated with factors enhancing growth, affect tipburn development and severity.

### 2.6.3.2 Genetic factor

Variation between cultivars in their susceptibility to tipburn has been demonstrated (Cox & McKee, 1976; Misaghi & Grogan 1978a; Bres & Weston, 1992; Gaudreau et al., 1994). Misaghi and Grogan (1978a) reported that the concentration of soluble and total calcium in mature head of tolerant cultivars was consistently greater than susceptible cultivars.

### 2.6.3.3 Salinity

The incidence of tipburn tends to be more serious in recirculating water systems than in soil grown crops (Voogt, 1988) and is reduced as the nutrient solution concentration is reduced (Willumsen, 1984;Van Der Boon et al., 1988; Huett, 1994). The incidence of tipburn also increased at higher soil salinity levels with both lettuce and endive (De Pascale & Barbieri, 1995). Root pressure in relation to transpiration rate, both at night and in day time, may have an important influence on calcium distribution and tipburn development. It was found that a high concentration of nutrients in the root zone reduced root pressure and may induce tipburn (Willumsen, 1984).

Burrage and Varley (1980) reported that water use was linearly related to incident radiation and did not differ significantly between plants grown in different nutrient concentration. However different nutrient concentrations significantly affected the percentage leaf dry matter and susceptibility to marginal tipburn. There was a

significantly higher incidence of marginal tipburn in the lower solution concentration (1.5 mmhos) with a graduation of occurrence from the lowest to the highest concentration (1.5, 2.4,2.9 and 3.5 mmhos).

De Pascale and Barbieri (1995) stated that the incidence of tipburn in lettuce increased at the higher soil salinity levels (NaCl 1%). The net photosynthesis rate, transpiration rate and stomatal conductance were not affected by the treatment 57 days after transplanting, whereas there was a significant reduction of these physiological parameters in the high-salinity treatment at harvest.

Sonneveld and Van den Ende (1975) grew lettuce in containers irrigated with various salts. The yield reduction were higher as the salt concentration became higher. The incidence of tipburn was decreased by calcium chloride, but sodium chloride, potassium chloride, magnesium chloride, sodium nitrate, sodium sulphate and in particular sodium bicarbonate, increased the disorder. Sonneveld and Mook (1983) confirmed the result of Sonneveld and Van den Ende (1975) and added that the calcium uptake was affected most strongly by the application of sodium bicarbonate.

### 2.6.3.4 Nitrogen

In field grown lettuce, tipburn increased with nitrogen supply. This was related to an increase in head size and decreasing root: shoot ratio (Brumm & Schenk, 1993). Steenhuizen (1988) reported that in spring, the incidence of tipburn was higher in crops grown in nutrient solutions with a high nitrogen level than in those with a low nitrogen level, and it was lower in heated than unheated solutions.

### 2.6.3.5 Calcium in the solution

Higher calcium levels in the root environment does not prevent calcium deficiency (Bradfield & Guttridge, 1984; Schlagnhaufer et al., 1987; De Kreij, 1995), while adding extra calcium to the nutrient solution increased the calcium concentration in the proximal, but not the in the middle or distal, segments of tomato fruit (Bradfield & Guttridge, 1984).

### 2.6.3.6 Cations

The severity of the tipburn symptoms decreases with decreasing potassium: calcium ratio (Voogt, 1988; Huett, 1994). On the contrary, Benoit and Ceustermans (1989) reported that a potassium: calcium ratio of 1.1 (4.4 mM K, 4 mM Ca), applied over the whole growing period, caused tipburn, while no tipburn occurred at a ratio of 2.3 (11 mM K, 4.75 mM Ca). Bres and Weston (1992) reported that neither potassium concentration (150 and 225 mg $l^{-1}$) nor pH level (5.0 to 6.5) consistently affected tipburn incident.

Voogt (1988) grew lettuce in an NFT system using K/Ca ratio ranging between 0.8 and 5.8 and conclude that growth was not affected by K/Ca ratios. The severity of tipburn symptoms increased slightly with increasing K/Ca ratios and the uptake of magnesium was depressed by increasing the potassium: calcium ratio.

### 2.6.3.7 Above ground factors of the environment

#### 2.6.3.7.1 Introduction

Tipburn incidence in NFT-produced lettuce appears to be primarily affected by environmental conditions maintained during greenhouse growth (Bres & Weston, 1992). Catalytic radiant heating rather than air-heated system (Gaudreau et al., 1994), increasing air temperatures (Misaghi & Grogan 1978a; Huett, 1994), increasing carbon dioxide concentrations from 300 to 1500 ppm (Tibbitts & Read, 1976) and increasing relative humidity (Read, 1972) gave higher biomass production and more serious tipburn development, while heating the nutrient solution decreased tipburn incidence in spring NFT lettuce (Steenhuizen, 1988).

Barta and Tibbitts (1991) compared crisphead lettuce grown under controlled-environment and field conditions. They reported that deficient calcium concentrations were present in areas of leaf tissue developing tipburn symptoms and that concentrations were significantly higher in similar areas of other leaves that had no

symptom. The amount of calcium in plants that develop tipburn in controlled environments were lower than in field grown plants that did not develop tipburn. The reduced levels of calcium in plants grown in controlled environment was associated with faster developmental rates than in field grown plants.

Read and Tibbitts (1970) reported that the incidence of tipburn was accelerated both by increasing carbon dioxide concentration and by increasing light intensity. Acceleration of tipburn appeared to be a consequence of an increased growth rate.

### 2.6.3.7.2 Relative humidity

Bottenberg and Tibbitts (1968) grew lettuce in different relative humidities and light period. Both leaf size and rate of leaf development were approximately 25% greater under continuous 90% relative humidity than under continuous 50% relative humidity. Tipburn incidence was accelerated with increased humidity. There was no acceleration of tipburn by the abrupt light-dark changes in the humid environment. Read (1972) reached a similar conclusion. Collier and Tibbitts (1984) found that decreasing relative humidity during the light period increased calcium concentration of the lettuce plant, and growth was retarded and the onset of tipburn delayed.

Collier and Wurr (1981) stated that tipburn incidence in lettuce was positively correlated with the total evaporation from an open water surface during the seven days prior to maturity, but unrelated to head weight at maturity. As evaporation and therefore transpirational stress increase due to environmental conditions, then under these conditions it is likely that most of the movement of calcium in the xylem is towards the outer photosynthesizing leaves and that inner leaves receive calcium are largely helped by root pressure induced flow. Tipburn developed when growth of the inner leaves exceed a critical value because they do not get sufficient calcium.

However, high relative humidities at night, so that a root pressure flow can develop, can be used to control tipburn of lettuce (Collier & Tibbitts, 1984; Goto & Takakura, 1990), chinese cabbage (Van Berkel, 1988) and cabbage (Palzkill et al., 1976).

### 2.6.3.7.3 Temperature

Increasing air temperatures (Misaghi & Grogan 1978a; Huett, 1994) and increasing root temperature (Collier & Tibbitts, 1984) has given higher biomass production and more serious tipburn development in lettuce.

Calcium uptake is mainly governed by root activity which increases as temperature rises (Clover, 1991). Van Der Boon et al. (1988) concluded that an increase in nutrient solution temperature at night to 17 $^0$C in spring, stimulated growth rate by 20% and tipburn was alleviated. On the other hand, Collier and Tibbitts (1984) reported that root temperature of 23.5 $^0$C, compared with 15.0 $^0$C, slightly increased calcium concentration, but induced earlier tipburn development due to a greater dry weight.

Misaghi and Grogan (1978a) concluded that temperature was an important influence in tipburn development, while relative humidity only slightly influenced tipburn development in mature detached heads. Both tipburn severity and percentage of tipburned plants increased in direct proportion with time of exposure to 30 $^0$C. The effect of temperature on tipburn development was apparently cumulative, the cumulative temperature (sum of hourly temperatures above 24 $^0$C during the exposure period) required for tipburn induction in 50% of the heads was fairly similar in treatments of constant or alternating temperatures. Temperatures inside heads in the field can be 6 $^0$C higher than ambient during most of the daylight hours and this may be the reason why tipburn has occurred at times when ambient temperatures have not exceeded 24$^0$ C.

### 2.6.3.7.4 Light

Tipburn severity increases with increasing light intensities (Tibbitts & Rao, 1968; Ashkar & Ries, 1971; Gaudreau et al., 1994) and/or extended light duration (Tibbitts & Rao, 1968; Gaudreau et al., 1994). A reduction in light intensity resulted in decreased RGR and delayed tipburn incidence (Cox et al., 1976). The highest tipburn ratings were associated with treatments involving the higher light levels and abrupt changes in environmental conditions (cloudy days alternating with sunny days, for example),

which may explain the high incidence of tipburn observed during Jan - Mar crop in Canada (Gaudreau et al., 1994).

The effects of light in inducing tipburn appear to be an indirect effect of an increase in photosynthetic activity with resultant increases both in the growth rate and the dry matter accumulation of the plants (Tibbitts & Rao, 1968; Koontz & Prince, 1986; Ikeda et al., 1988; Gaudreau et al., 1994).

### 2.6.3.7.5 Carbon dioxide concentration

Increasing carbon dioxide concentrations to 1500 ppm (Tibbits & Read, 1976) or 1200 $\mu M\ mol^{-1}$ (Caporn, 1989) gave higher biomass production and more serious tipburn development. Ciolkosz et al. (1998) suggested that increased carbon dioxide concentrations increase tipburn in lettuce unless the growth rate is slowed by other means.

### 2.6.3.8 Hormonal factors

One of the causal agents in the development of lettuce tipburn might be the presence of supra-optimal levels of the auxin IAA (Crisp et al., 1976). Collier et al. (1979) suggested that high levels of auxin can arise because the enzyme IAA oxidase can be inactivated by chlorogenic acid, and they found that the lettuce cultivar most susceptible to tipburn had indeed the highest concentration of this polyphenol.

## 2.6.4 Effect of calcium spray

Corgan and Cotter (1971) evaluated the effects of thirteen different foliar sprays and 1 soil treatment on tipburn of 'Great Lake 659' head lettuce. Succinic acid-2,2-dimethylhydrazide (SADH) (5000 ppm), N-6 benzyladenine (BA) (25 ppm), simazine (2000 ppm) and 2-chloroethylphosphonic acid (ethephon)(100 ppm) plus $CaCl_2$ (2500 ppm) each reduced tipburn. Triiodobenzoic acid (TIBA)(50 ppm) and phenylmercuric acetate (PMA)(100 ppm) each increased tipburn, while gibberellic acid (GA)(50 ppm)

plus BA (25 ppm), $CaCl_2$ (2500 ppm), urea (2500 ppm), calcium chelate (100 ppm) and ethylenediaminetetraacetic acid (EDTA)(1 lb $acre^{-1}$) plus $CaCl_2$ (100 lb $acre^{-1}$) soil treatment each had no significant effect on tipburn injury. $CaCl_2$ sprayed alone had no effect on tipburn, but when applied with ethephon, tipburn was reduced. Failure of $CaCl_2$ spray to correct tipburn may be related to lack of translocation of calcium to the inner leaves.

Misaghi et al. (1981) applied different amounts of calcium chloride and calcium nitrate to the soil or to head lettuce in field plots throughout Arizona and California during 1976 and 1980. Calcium chloride and calcium nitrate were added to the soil either before or after planting at rates of 112, 224 or 448 kg $ha^{-1}$. Plants were sprayed with aqueous solution of calcium chloride (4.7 g $l^{-1}$) and calcium nitrate (7.1 g $l^{-1}$) at a rate of 940 l $ha^{-1}$ about 4, 6, 8 and 10 weeks after planting. They concluded that in all field trials, soil and foliar application of calcium nitrate and foliar application of calcium chloride at different levels did not significantly change tissue calcium and severity of tipburn.

Thibodeau and Minotti (1969) sprayed $Ca(NO_3)_2$ (0.4 M) compared to distilled water at the 10 leaf stage and every other following night by using a small atomizer. Once the plants began to head it was necessary to gently open the heads to allow spray contact with heart leaves. They reported that foliar sprays of $Ca(NO_3)_2$ completely controlled lettuce tipburn under conditions where plants sprayed with distilled water were severely affected. The $Ca(NO_3)_2$ spraying increased both the soluble and insoluble calcium content of the heart leaves, which were the most susceptible to injury and the first to be affected. The spray also increased the soluble calcium of inner and middle leaves. The calcium sprays were equally effective in controlling tipburn whether applied at night or in the morning. They also concluded that practical control of tipburn for field grown head lettuce by the use of calcium sprays seems remote due to the relative immobility of calcium and the inaccessibility of the susceptible immature leaves.

### 2.6.5 Approaches to tipburn control

A tolerant cultivar should be used to significantly reduce tipburn damage in lettuce (Collier & Tibbitts, 1982; Bres & Weston, 1992). Environmental control of greenhouse temperatures, light intensity and duration, and relative humidity should be practiced (Collier & Tibbitts, 1982, 1984). This will allow plant growth rate to be controlled and may also allow more calcium to susceptible leaves.

Cresswell (1991) stated that replacing nutrient solution (EC 2 dS $m^{-1}$) by tap water (EC 0.19 dS $m^{-1}$) and calcium nitrate solutions containing 100 or 200 mg Ca $l^{-1}$ (EC 0.08 and 1.45 dS $m^{-1}$) during night time decreased tipburn incidence, but did not affect the fresh weight of mature lettuce. This effect was associated with an increase in the concentration of calcium in new leaves, except in the water treatment. The tissue analysis procedure used was based on a sample of whole leaves. It is possible that tipburn was reduced in lettuce receiving water at night because calcium was more effectively translocated to the margins of the enclosed leaves.

Goto and Takakura (1992) reported that all day air supply to inner leaves prevented tipburn completely without loosing a rapid growth rate, presumably by allowing these inner leaves to transpire effectively and so receive more calcium. Vertical air flowing at high humidity increases yield and suppress tipburn incidence in a plant factory due to promotion of gas exchange (Shibata et al., 1995). A 3 h day/night cycle as well as high RH (> 90%) in the dark period reduced tipburn in the plant factory (Goto & Takakura, 1990)

## 2.7 Nutritional value of lettuce

### 2.7.1 Introduction

The relationships between diet and health are becoming more and more substantial (Verschuren, 1997). Health organizations actively endorse increasing the consumption of fruits and vegetables to improve public health (Mackerras, 1995; Wiseman et al.,

1997). Despite consumer interest in health-related issues changing nutrition habits remain difficult. Nevertheless, consumers expect food products to be healthy, as well as tasty and functional. Such products would provide the consumer with food products tailored to their functional, nutritional and health needs (Verschuren, 1997).

Today, diet alone is suggested to account for almost one-third of cancer deaths in Western populations, and along with tobacco, is amongst the most modifiable environmental factors which affect human health (Verschuren, 1997).

The risk of death by many non-hormone-dependent cancers can be reduced approximately twofold in subjects who consume relatively high amount of fruits and vegetables (Block et al., 1992). U.S. Department of Agriculture (USDA) and National Cancer Institute (NCI) guidelines are similar, with recommendations for eating at least five fruits and vegetables daily (Levine et al., 1996).

Food composition books provide a general indication of typical vitamin contents of vegetables. However such information must be treated with caution as the vitamin content of food can vary significantly depending on variety, growing conditions, stage of maturity, regional difference, and seasons of the year (Vanderslice & Higgs, 1991). Another reason for the vast differences could be a result of different accuracy of the methods used in analysing the vitamin. Different analysis methods give different results for the same sample of vegetable (Tee & Lim 1991).

Table 2.4 summarises the concentration of ascorbic acid, dietary fibre, nitrate, protein and soluble sugar of lettuce on both a fresh and dry weight basis. The data covers soil and greenhouse crops, different cultivars and analysis method. Comparison of the data within table should be made by keeping these differences in mind.

**Table 2.4 Concentration of ascorbic acid, dietary fibre, nitrate, protein and soluble sugar of lettuce on fresh weight and dry weight basis.**

| | ascorbic acid | dietary fibre | nitrate | protein | soluble sugar |
|---|---|---|---|---|---|
| **Fresh weight basis** | **mg $100g^{-1}$** | **g $100g^{-1}$** | **mg $100g^{-1}$** | **g $100g^{-1}$** | **g $100g^{-1}$** |
| **Franke & Lawrenz, 1980** | | | | 0.81 | |
| **Lairon et al. 1984** | | | | 0.78 - 1.02 | |
| **Vestergaard, 1988** | 10 | | | 1.5 | |
| **Albrecht 1993** | | | | | |
| Butter crunch | 22.37 ± 2.09 | | | | |
| Red sails | 19.71 ± 3.01 | | | | |
| Red salad Bowl | 23.66 ± 6.65 | | | | |
| **Sorensen et al. 1994** | 5.15 - 6.48 | | 33 - 77.4 | 0.61 - 0.71 | glucose 1.05 - 116 fructose 1.31 - 1.44 |
| **Waycott and Ryder 1994** | | | | | |
| Salinas | 3.6 | 1 | | 0.8 | |
| Mini-Green | 3.2 | 1.2 | | 1.2 | |
| Valmaine | 10.7 | 1.6 | | 1.5 | |
| **Poulsen et al.1995** | 48-59.5 | | | | glucose 1.05 -1.25 |
| **Drews et al. 1996** | 8 – 16 | | 165 - 333 | | 0.7 - 0.9 |
| **Burlingham & Milligan, 1997** | | | | | |
| inner leaves | 12 | 0.7 | | 1.1 | 0.4 |
| outer leaves | 12 | 0.7 | | 1.1 | 0.4 |
| lettuce | 12 | 0.7 | | 1.1 | 0.4 |
| **Davis et al., 1997** | | | | | |
| Crisphead lettuce | 6 | 0.5 | | | |
| Butterhead lettuce | 8 | 0.5 | | | |
| Leaf lettuce | 18 | 0.7 | | | |
| Romaine lettuce | 18 | 0.7 | | | |
| **Dry weight basis** | **mg $g^{-1}$** | **mg $g^{-1}$** | **mg $g^{-1}$** | **%** | **mg $g^{-1}$** |
| **Frota & Tucker 1972** | | | 0.6 - 6.85 | | |
| **Franke & Lawrenz, 1980** | | | | 21.32 | |
| **Subramanya et al. 1980** | | | 10.4-27 | | |
| **Grimstad, 1984** | 1.53-3.02 | | | | |
| **Brunsgaard et al. 1994** | | | | 16.0 - 20.1 | |
| **Wheeler et al., 1994** | | 111 ± 1 | | 30.0 ± 0.8 | |
| | | 94 ± 2 | | 27.2 ± 0.2 | |

## 2.7.2 Ascorbic acid (vitamin C)

The association between low intake of fresh fruits and vegetables and the risk of epithelial cancers is probably the most consistent finding in epidermiologic studies of neoplastic disease. It is well accepted that fruits and vegetables are the main source of antioxidant nutrients, especially, vitamins C, E, and carotenoids (Correa, 1995). Diets with high vitamin C content from fruits and vegetables are associated with lower cancer risk, especially for cancer of the oral cavity, oesophagus, stomach, colon and lung (Byers & Guerrero, 1995). In contrast, Levine et al. (1996) stated that consumption of vitamin C as a supplement had no effect on development of colorectal adenoma and stomach cancer.

There is evidence that vitamin C could be preventing cancer by inhibition of nitrosamine (a carcinogen) formation, preventing activation of carcinogens, enhancing detoxification of carcinogens, enhancing the immune response, and by inhibition of the promotion phase (Machlin, 1995).

The content of vitamin C shows seasonal variations (Grimstad, 1984, Evers, 1994; Drews et al., 1995; Levine et al., 1996). Vitamin C increases with increasing radiation (Shinohara & Suzuki, 1981; Grimstad, 1984; Primak, 1985). In contrast, Drews et al. (1995) stated that ascorbic acid generally decreased with increasing levels of solar radiation and the influence of radiation on the vitamin C contents was modified by head weight because vitamin C decreased with enlarging head weight.

Primak (1985) reported that a reduction of light intensity from 35000 to 6000 lux reduced lettuce dry matter content from 7.23 to 4.39 % and ascorbic acid content from 16.54 to 9.86 mg $100g^{-1}$. Fresh weight and leaf number of butterhead lettuce decrease with increasing shade level, the leaves became narrower, and ascorbic acid content decreased. The content of ascorbic acid was higher in the day time and lower during the night. This tendency is more marked on a sunny than on a cloudy day (Shinohara & Suzuki, 1981). Grimstad (1984) reported that plants irradiated with fluorescent tubes had the lowest vitamin C content and highest with high pressure sodium lamps. He suggested that this was due to the higher infrared radiation ($\lambda > 750$ nm) of the high

pressure sodium lamp, not the photosynthetically active radiation (PAR), because the fluorescent tubes had the highest PAR.

Vitamin C concentrations decrease with progression of growth stage and increase in head size (Shen et al., 1992; Sorensen et al., 1994; Drews et al., 1995, 1996, 1997). Between start and end of head formation stage, the concentration of vitamin C in greenhouse lettuce decreases by 51% (Drews et al., 1996). This is due to the different compositions of outer, middle and inner leaves and to their changing ratio during heading. Middle and inner leaves have lower vitamin C concentrations than outer leaves (Shen et al., 1992; Drews et al., 1995, 1997) and the content decreases gradually from outer to innermost head leaves (Shen et al., 1992). Cultivar differences also have a marked effect on vitamin C content (Albrecht, 1993; Evers, 1994; Sorensen et al., 1994).

The fresh weight of lettuce was greatly reduced when the plants were grown at ¼ concentrations of the standard nutrient solution, compared with ½ concentrations or more, but the ascorbic acid content increased (Shinohara et al., 1978; Shinohara & Suzuki, 1988). Positive relations were found between the ascorbic acid and the sugar content in both lettuce and garland chrysanthemum (Shinohara et al., 1978).

Optimal nitrogen levels had a small effect, but excess nitrogen decreased vitamin C content in the study of Evers (1994) while Sorensen et al. (1994) found that the vitamin C content decreased from 64.8 to 51.5 mg $kg^{-1}$ with increased nitrogen supply from 50 to 200 kg N $ha^{-1}$ in the field.

### 2.7.3 Dietary fibre

Shen et al. (1992) reported that during head expansion the crude fibre content increased gradually, and that the crude fibre content in the inside and outside head leaves was less than in the other leaves. Total dietary fibre content is approximately 25% of DM and this value is not much influenced by the different times of planting, rates of N applied or time of harvest (Brunsgaard et al., 1994).

## 2.7.4 Nitrate

The content of nitrate in lettuce shows seasonal variation with nitrate decreasing with increasing radiation (Gysi et al. 1985; Evers, 1994; Drews et al. 1995; Primak, 1985). Drews et al., (1995) found that a close connection could be established between sugar and nitrate concentration. High sugar content correlated with low nitrate content.

In lettuce, nitrate concentrations decrease with the progression of growth stage and increase in head size. This is due to the different compositions of the outer, middle and inner leaves and to changes therein during heading. Middle and inner leaves have lower nitrate concentrations than outer leaves (Drews et al., 1995, 1997). Drews et al. (1996) found that nitrate concentration decreased from 3330 to 1650 mg $kg^{-1}$ FW between the start of head formation and the head firmness stage with greenhouse cultivars and by 35% with field cultivars. Nitrate concentration in lettuces can be minimised by harvesting when head formation is complete.

Fresh weight and leaf number of butterhead lettuce decrease with increasing shade level, the leaves became narrower, ascorbic acid and sugar contents decreases, and nitrate levels tended to increase. The contents of ascorbic acid, sugars and chlorophyll are higher in the daytime than during the night, while nitrate content increased in the night. These tendencies were more marked on a sunny than on a cloudy day (Shinohara & Suzuki, 1981).

Differences exist in nitrate accumulation between cultivars (Gysi et al., 1985: Evers, 1994). Drews et al. (1996) reported that nitrate concentrations were lower in field cultivars than in greenhouse cultivars.

Evers (1994) reports that increasing N fertiliser application, genotype, low light intensity, low temperature and drought increase the nitrate content of vegetables, but levels are low in vegetables grown in the Nordic countries, except in lettuce grown under glass with supplementary lighting during the dark winter months. These conclusions are similar to those of other researchers.

### 2.7.5 Sugar

The content of sugar shows seasonal variations. The content of sugar increased with increasing radiation (Grimstad, 1984; Blom-Zandstra & Lampe, 1985; Drews et al., 1995). The influence of radiation on the sugar content is modified by head weight because sugar increases with enlarging head weight. A close connection has been established between sugar and nitrate concentration. High sugar content correlates with low nitrate content (Drews et al., 1995). Research has shown that fresh weight and leaf number of butterhead lettuce decreases with increasing shade level, the leaves became narrower and sugars contents decrease. The content of sugars is higher in the day time and lower during the night (Shinohara & Suzuki, 1981; Forney & Austin, 1988). This tendency is more marked on a sunny than on a cloudy day (Shinohara & Suzuki, 1981).

Forney and Austin (1988) found that the sucrose in the cap leaf of crisphead lettuce increased from the morning to the afternoon and was translocated to the actively growing leaves in the afternoon. Sucrose was converted to glucose and fructose after it was translocated to the head leaves. Lettuce harvested near sunrise contained more glucose and fructose than in the afternoon suggesting the translocation of assimilates continued through the night. Bolin and Huxsoll (1991) reported that the percentage of the three major sugars found in iceberg lettuce were glucose 47%, fructose 42% and sucrose 11%.

Blom-Zandstra & Lampe (1985) concluded that increased light intensity caused a distinct shift from nitrate accumulation in the plant sap towards accumulation of sugar (mainly glucose) and organic acid (mainly malate).

Drews et al. (1996) found that the concentration of reducing sugars increased by 44% between start and end of head formation stage. The reducing sugar concentration increased with progression of growth stage and increase in head size. This was due to the different compositions of outer, middle and inner leaves and to their changing ratio during heading (Drews et al., 1995, 1997). The effect of fertiliser application on sugar content is small (Evers, 1994).

### 2.7.6 Protein

Lairon et al. (1984) found that the protein concentration in lettuce, by determination of the protein amino acids, did not show any significant change either with mineral or organic nitrogen fertiliser or with the application rate used. The protein content of lettuce ranges between 0.78 – 1.02% fresh matter. In contrast, Brunsgaard et al. (1994) reported that the protein content (N × 6.25) increased progressively from 16.00 to 19.88 % with nitrogen fertiliser levels from 50 to 200 kg N $ha^{-1}$. Early harvests as well as early planting also increased the protein content compared to late harvest and late planting. The protein content (N × 6.25) determined represents crude protein and thus contains a non protein nitrogen fraction, including nitrate.

## 2.8 Nitrate accumulation in vegetables

Nitrate accumulation in vegetables is of concern because of the potential conversion to nitrite after uptake (Maynard & Barker, 1979; Van Der Boon et al., 1990). Despite this the risks of consuming nitrate as a natural substance in vegetables has not been evaluated (Kreij, 1994). Nitrate itself has no toxic effect on human or animal metabolism, but nitrite may be harmful (Mengel, 1979; Addiscott et al., 1991). Nitrite can cause methaemoglobinaemia especially in infants, and being a precursor of nitrosamines, which are carcinogenic (Shuval & Gruener, 1977; Addiscott et al., 1991)

Methaemoglobinaemia or `blue-baby' syndrome can occur when children, less than about 1 year old, consume too much nitrate. Microbes in the stomach reduce nitrate into nitrite, which reacts with the haemoglobin in the bloodstream. Oxyhemoglobin, which contains iron in the ferrous form, becomes methaemoglobin, which contain iron in the ferric form. As a result the oxygen-carrying capacity of the blood is lessened (Shuval & Gruener, 1977). Very young children are susceptible because their stomachs are not acid enough to inhibit the nitrate reducing bacteria and the foetal haemoglobin, which has a greater affinity for nitrate than normal, persists for a while in the blood steam. However, this `blue-baby' syndrome is extremely rare and only about 1060 cases have been reported. In most cases, the death were associated with water from privately dug well (Addiscott et al., 1991).

In human nutrition about 90% of the nitrates come from vegetables (Pavlovic et al., 1998). According to FAO regulations, the maximum daily dose of nitrates should not exceed 5 mg $kg^{-1}$ of human body (Szwonek, 1986). The Dutch government has set maximum permissible nitrate levels for endive, spinach and lettuce. For winter-grown lettuce, the value is 4500 mg $kg^{-1}$ fresh weight and is 3500 mg $kg^{-1}$ for summer grown lettuce (Benoit & Ceustermans, 1989; Van Der Boon et al., 1990). The European standards from 1/1/98 under Belgian and Dutch climatological conditions is 3,500 ppm $NO_3$ per kg fresh head lettuce weight from 1/11 to 30/4 and 2,500 from 1/5 to 31/10 (Benoit & Ceustermans, 1995; McCall & Willumsen, 1998).

Nitrate accumulation in leaves decreases during daytime and increases during the early part of the night (Steingrover et al., 1986; Carrasco & Burrage, 1992a; Scaife & Schloemer, 1994). Nitrate accumulation within the leaf tissue showed a single peak during the night period in the research of Carrasco and Burrage (1992a, 1992b). This was due to the increased uptake by the roots during the initial hours of darkness and its transport directly into the leaf blade vacuole (Steingrover et al., 1986), and the increased activity of nitrate reductase enzyme after sunrise (Carrasco & Burrage, 1992b).

In the light, nitrate concentration fall at a rate, which is dependent on the light intensity. Scaife and Schloemer (1994) reported that the rate of nitrate reduction increased with light up to an intensity of 320 μmol $m^{-2}$ $s^{-1}$.

The nitrate concentration in vegetables varies between plant parts. In most cases, the highest concentration is found in petioles (Maynard & Barker, 1979; Pavlovic et al., 1998) with lower concentration in roots, leaf lamina, fruit or grain, and flower parts respectively (Maynard & Barker, 1972, 1979). Nitrate concentration in plants also varies with age. Initially the concentration increases, whereupon it decreases again (Blom-Zandstra & Eenink, 1986).

Besides being a nitrogen source for the synthesis of amino acids (McCall & Willumsen, 1998) nitrate has a role as an intracellular osmoticum (Smirnoff & Stewart, 1985; McCall & Willumsen, 1998). Vacuoles are a major storage pool for nitrate (Martinoia et al., 1981; Granstedt & Huffaker, 1982; Steingrover et al., 1986). They contained

58% of the total cellular nitrate in green barley (Granstedt & Huffaker, 1982), while Martinoia et al. (1981) reported that barley leaf vacuoles contained 99% of the cellular nitrate. Nitrate replaces organic compounds such as carbohydrates and organic acids in the cell sap during conditions that are unflavorable for photosynthesis. The decrease of nitrate uptake is due to a reduction in demand for nitrate because of the availability of organic solutes and carbohydrates in the leaves (Blom-Zandstra & Lampe, 1985; Steingrover et al., 1986). Nitrate concentration is inversely related to the concentration of organic acids and sugars (Blom-Zandstra et al., 1988). The negative correlations are very close between sugars and nitrate contents ($r^2 = 0.99$) and photosynthetic rate and nitrate content ($r^2 = 0.79$) (Behr & Wiebe, 1992).

Nitrate accumulated in mature leaves is assimilated into organic nitrogen (Smirnoff & Stewart, 1985). Most of the cellular nitrate is stored in the vacuole, while only nitrate in the cytoplasmic pool and in the xylem flow is readily available for reduction (Shaner & Boyer, 1976). About 25% of the energy of photosynthesis is consumed in driving nitrate assimilation (Solomonson & Barber, 1990).

# Chapter 3

# Effect Of Nutrient Solution Concentration On Growth, Nutrient Uptake, Yield And Quality Of Lettuce Grown Over Four Seasons

## 3.1 Introduction

Water is essential for the existence of life. The kinds and amounts of vegetation occurring on various parts of the earth's surface depend more on the quantity of water available than on any other single environmental factor (Kramer, 1983). Water is an important structural component in plants. Water is a solvent for many substances such as inorganic salts, sugars and organic anions. It is the medium in which all biochemical reactions take place. It is also essential for the translocation and distribution of nutrients and metabolites throughout the entire plant (Mengel & Kirkby, 1987).

A difference in water potential is the driving force for water movement. Water moves from a higher to a lower water potential (Salisbury & Ross, 1992). Plants generally have a high tissue water potential, whereas the atmosphere usually has a relatively low water potential (Hickley & Braatne, 1994). Similarly, the water potential of the soil solution is usually much higher than the water potential of the plants. Thus a large energy gradient exists within the soil-plant atmosphere continuum. This gradient is the driving force that causes the translocation of water from the soil solution through the plant to the atmosphere (Mengel & Kirkby, 1987).

The nutrient solution used in an NFT system is comparable with the soil solution. The difference in water potential between the nutrient solution and the plant is the driving force of water movement from the solution into the plant. Increasing the nutrient solution concentration decreases the water potential of nutrient solution, therefore the gradient

between the water potential of the nutrient solution and the plant decreases. This results in less water movement up the plant, thus a lower plant water status.

As the nutrient solution concentration increases from a low level, plant growth and thus yield is promoted via a nutritional effect. At higher concentrations however, growth is increasingly restricted by reduced water uptake. This is because as dissolved salt concentrations increase, it makes it increasingly more difficult for water and nutrients to move through root membranes and into the plant (Volkmar et al., 1998). The positive effect of fertilisation can therefore vanish under high salt stress (Imas & Feigin, 1995). Such responses have been widely reported in hydroponic systems with crops such as lettuce (Willumsen, 1984; Huett 1994), cucumber (Ho & Adams, 1994), tomatoes (Ehert & Ho, 1986; Ismail & Ahmad, 1997; Schwarz & Kuchenbuch, 1997) and pepper (Tadesse, 1997). Yield quality trades off also occur. Thus with tomatoes at high nutrient solution concentrations yield will be reduced, but the flavour of the fruit improves (Ho & Adams, 1995; Mpelasoka, 1996; Ismail & Ahmad, 1997; Schwarz & Kuchenbuch, 1997), while with lettuce a high concentration may favour good yields, but also increases the risk of tipburn (Willumsen, 1984; Huett 1994).

A range of nutrient solution concentrations have been used to grow lettuce. Experiments with lettuce seedlings, cultivar 'Plevanos', indicated that nutrient solution concentration in the range 600 – 1100 μS gave the best results during the propagation stage (Morgan et al., 1980a; Morgan & Tan, 1983). Satisfactory growth and yield of lettuce cultivar 'Ravel' was obtained with nutrient solution concentrations up to 5.5 mS $cm^{-1}$, in an expanded clay substrate system during January and April in Ireland, with an optimum of about 2 mS $cm^{-1}$ (Morgan et al., 1980b). Willumsen (1984) stated that a nutrient solution concentration of anions and cations between 5 and 10 me $l^{-1}$ (0.5-1.0 mS $cm^{-1}$) gave the highest yield with a reduced risk of tipburn for butterhead lettuce, cultivar 'Ostinata', grown in water culture at Virum, Denmark.

Van Der Boon et al. (1988) stated that increasing the nutrient solution concentration from 1.27 to 3.00 mS $cm^{-1}$, in summer and spring, did not affect fresh weight of the head when no $NH_4$ was present in the nutrient solution. The presence of 20% $NH_4$-N decreased head

fresh weight in the spring crop, but not in the summer crop. Increasing nutrient solution concentration and air temperature both resulted in increased growth rate of the winter crop. Tipburn was aggravated by high nutrient solution concentrations and was alleviated when the solution was heated.

Economakis (1990) investigated the effect of nutrient solution concentrations between 1.5 and 5.0 mS $cm^{-1}$ on the butterhead and cos lettuce cultivars 'Bellona' and 'Paris Cos Island' grown in NFT during Nov-Dec and Apr-May in Greece. He found that the interaction of temperature and radiation was the most important factor influencing growth and the overall effect of solution concentration on shoot fresh weight was minor. It was suggested that lettuce could be grown over a wide range of solution concentrations without tipburn incidence, but nutrient solution concentrations within the 2.0-3.0 mS $cm^{-1}$ range will give more satisfactory results.

Huett (1994) investigated the effect of nutrient solution concentration between 0.4 and 3.6 dS $m^{-1}$ (mS $cm^{-1}$), on yield of head lettuce cultivar 'Coolguard' during Aug-Sep, cultivar 'Fame' during Feb-Mar and cultivar 'Red Mignonette' during Nov-Dec, grown in gravel culture in New South Wales, Australia. He found that lettuce grown at an EC of 0.4 $dSm^{-1}$ was nitrogen and potassium deficient. The highest fresh weight of head and/or leaf of mature heading and non-heading lettuce was obtained from the 1.6 $dSm^{-1}$ concentration. Maintenance of the nutrient solution concentration at about 1.6 $dSm^{-1}$ minimised changes in nutrient solution composition over time.

There has been a significant amount of research examining the role of nutrient solution concentration on the yield and quality of hydroponically produced lettuce. However, few seasonal comparisons have been made and at times the nutrient solution concentrations used has covered a limited range. The response of different lettuce types has also not been widely considered. In the present study a broad-based approach was taken. The objective was to examine the effect of all these factors on growth, nutrient uptake, yield and quality of lettuce. Thus, there was a series of experiments, which covered the four seasons, a wide range of nutrient solution concentrations and a number of distinctly different lettuce types.

## 3.2 Materials and methods

### 3.2.1 Propagation and greenhouse environment

The experiments were conducted in a greenhouse at the Plant Growth Unit (PGU), Massey University. Pelletted seeds were sown in 45 ml single cells, which allowed the roots to grow unimpeded out of the side of the cell. These individual cells were placed in a cell tray for support. The propagation media was sieved peat with agricultural lime added at 3 kg $m^3$ for the summer experiment and fine grade vermiculite for the autumn, winter and spring experiments (Plate 1). The trays were placed on a heated bench at 25 $^0$C for 5 days. After 5 days, the heat was turned off.

After emergence the seedlings were shifted onto a mesh bench and fed once a day with a nutrient solution of 0.5 mS $cm^{-1}$ concentration (3.2.3). The seedlings remained on the mesh benches until they were planted into the NFT channels (Plate 2). The NFT channels were made from white 50 × 100 mm plastic down pipe with holes drilled, at the appropriate plant spacing, into which the cells holding the plants were placed. Glasshouse temperatures were maintained at 10°C via the heating system with fan ventilation operating at 20°C.

### 3.2.2 Treatments, experimental design and glasshouse layout

There were four seasonal, four nutrient solution concentrations and three cultivar treatments. The seasonal treatments were summer, autumn, winter and spring. There were 7 successional harvests to allow for growth analysis and nutrient uptake determinations. Yield was determined at the final harvest.

Within each season a Randomized Complete Block (RCB) design was used with the four nutrient solution concentrations and the three cultivars combined factorially to give 12 plots with a guard plant at the end of each plot. There were four blocks. Each plot

contained plants that were selected in a systematic manner for the growth analysis study (3.2.4.1). The details of the sowing, planting and harvesting dates for the four seasonal crops and the number of plants per harvest (growth analysis) are presented in Table 3.1. The seedlings were planted out into the NFT channels earlier than is commercially practised in New Zealand. This was so that the treatments could be applied from an early stage in the life of the crop.

The four nutrient solution concentrations were 0.5, 1.5, 2.5 and 3.5 mS $cm^{-1}$. These concentrations were achieved by adding to tap water all the major and minor nutrients (3.2.3) in equal proportions until the required concentration was achieved. The concentration of the tap water was 0.2 mS $cm^{-1}$. The three cultivars grown were 'Cortina' (butterhead), 'Lollo Bionda' (green Lollo Rosso) and 'Impuls' (red Lollo Rosso) (Plate 3).

The NFT channels were supported on 6 benches in a 6 × 15 m glasshouse. The benches were 50 cm apart and each bench contained 8 channels 4.5 m long and spaced 25 cm apart. There was thus 48 channels running across the width of the glasshouse (Plate 4). A channel was an experimental plot (4 blocks × 4 nutrient solution concentrations × 3 cultivars). Each channel had 41 planting holes spaced 10 cm apart. The fall down the length of the channels was 1:100 and the flow rate per channel was approximately 1.0 litre per minute. There were four nutrient holding tanks, one for each of the four nutrient solution concentrations. Each tank held 80 litres and its exposed surfaces were painted white to prevent heat build in the tank from solar radiation.

**Table 3.1 Date of sowing, transplanting, harvesting and number of plants per plot per harvest for growth analysis and plant nutrient determinations and heat unit calculations for each harvest.**

| | Summer crop | Autumn crop | Winter crop | Spring crop |
|---|---|---|---|---|
| Sowing date | 20/11/95 | 28/03/96 | 30/05/96 | 28/07/96 |
| Transplant date | 1/12/95 | 11/04/96 | 13/06/96 | 11/08/96 |
| First harvest | 2/12/95 | 11/04/96 | 23/06/96 | 18/08/96 |
| Plant number | 10 | 10 | 7 | 7 |
| Heat unit | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Second harvest | 7/12/95 | 18/04/96 | 2/07/96 | 24/08/96 |
| Plant number | 5 | 8 | 5 | 5 |
| Heat unit | 114.16 (5) | 152.77 (7) | 132.12 (9) | 113.95 (6) |
| Third harvest | 12/12/95 | 25/04/96 | 11/07/96 | 30/08/96 |
| Plant number | 4 | 5 | 4 | 4 |
| Heat unit | 222.10 (10) | 282.92 (14) | 237.25 (18) | 223.26 (12) |
| Fourth harvest | 17/12/95 | 2/05/96 | 20/07/96 | 6/09/96 |
| Plant number | 3 | 4 | 3 | 3 |
| Heat unit | 330.11 (15) | 405.69 (21) | 348.93 (27) | 332.21 (18) |
| Fifth harvest | 22/12/95 | 9/05/96 | 29/07/96 | 12/09/96 |
| Plant number | 2 | 2 | 2 | 2 |
| Heat unit | 442.17 (20) | 523.08 (28) | 470.18 (36) | 439.11 (24) |
| Sixth harvest | 28/12/95 | 16/05/96 | 7/08/96 | 18/09/96 |
| Plant number | 2 | 2 | 2 | 2 |
| Heat unit | 565.45 (26) | 635.41 (35) | 598.04 (45) | 545.55 (30) |
| Final harvest | 3/01/96 | 23/05/96 | 16/08/96 | 24/09/96 |
| Plant number | 2 | 2 | 2 | 2 |
| Heat unit | 693.68 (32) | 742.53 (42) | 712.54 (54) | 659.79 (36) |

( ) = days after 0 heat unit

a)

b)

**Plate 1 Propagation stage a) peat – Experiment 1 Summer crop b) vermiculite-all subsequent crops**


**Plate 2 Establishment stage – Experiment 1**

a)


1 2 3

b)


**Plate 3 a) Cultivars of lettuce in the Experiment: Cortina (1), Lollo Bionda (2) and Impuls (3) b) Tipburn incidence of cultivar Cortina in Experiment 1 - winter crop**

a)


b)

**Plate 4 Experiment 1: General view of winter crop a) at harvest 3 and b) before final harvest**

### 3.2.3 Nutrient solution

The nutrient solution formulation used was modified from a New Zealand Ministry of Agriculture and Fishery (MAF) recommendation for a NFT tomato-starting solution (Tregidga et al., 1986) to have the potassium: calcium ratio approximately 1: 1. This was to provide more calcium to the nutrient solution as Huett (1994) reported that the calcium concentration in the youngest fully expanded leaves increased with decreasing potassium: calcium ratio in the nutrient solution, the ratio ranged between 1.00: 3.50 to 3.50: 1.00. Two stock solutions were prepared and stored in separate containers. The stock solutions were as follows:

Stock solution A. 15 litres consists of:

| | | | |
|---|---|---|---|
| Calcium nitrate | $Ca(NO_3)_2 .4 H_2O$ | 2606 | gm |
| Chelated iron | FeNa EDTA | 157.76 | gm |

Stock solution B: 15 litres consists of:

| | | | |
|---|---|---|---|
| Potassium nitrate | $KNO_3$ | 677.2 | gm |
| Magnesium sulphate | $MgSO_4. 7H_2O$ | 993.2 | gm |
| Potassium phosphate | $KH_2PO_4$ | 544.0 | gm |
| Manganous sulphate | $MnSO_4. 2H_2O$ | 12.308 | gm |
| Boric acid | $H_3BO_3$ | 3.428 | gm |
| Copper sulphate | $CuSo_4 . 5H_2O$ | 0.55 | gm |
| Ammonium molybdate | $(NH_4)_6 M_7O_{24}. 4H_2O$ | 0.184 | gm |
| Zinc sulphate | $ZnSO_4 .7H_2O$ | 0.616 | gm |

The solutions in the four nutrient tanks were adjusted manually on a daily basis. Equal amounts of both solutions were added to raise the conductivity up to what was required for a particular treatment. The pH was maintained at 5.5 to 6.5. Potassium hydroxide (KOH) and Phosphoric acid ($H_3PO_4$) were used to raise and lower the pH respectively. The nutrient solutions were dumped weekly to avoid imbalances in nutrients occurring.

### 3.2.4 Data collection and analysis

#### 3.2.4.1 Growth analysis

Growth analysis within each crop consisted of 7 successional harvest. The number of plants sampled per plot per harvest is as described in Table 3.1. Alternate plants were sampled during the first 4 harvests. This resulted in plants being at a 20 cm spacing as their leaf area began to expand. During the last 3 harvests only plants that had plants at 20 cm on either side of them were harvested. The exception was the summer crop. Here there were fewer plants left as they had suffered from the stress at the high nutrient solution concentrations after transplanting.

The root was separated from the shoot at the base of the cotyledons and washed thoroughly. The shoot was divided into stem and leaves, and the leaf area was measured using a Leaf Area Meter Model Li-Cor 3100. The plant parts (leaves, stem and root) were oven dried at 70 $^0$ C for at least 24 hours and their dry weights were recorded.

Based on the significance of the components of the polynomial model (linear, quadratic and cubic) the quadratic model was selected. After $\log_e$ transformation, the leaf area, leaf dry weight and total dry weight per plant were fitted using a quadratic model with a heat unit time scale above a base temperature of 0 $^0$C (equation 3.1 – 3.3) and compared to the normal time scale (day). The heat unit time scale above a base temperature of 0 $^0$C was selected because it gave a better fit than the normal time scale. The environmental time scale has also been reported to give better fit than the normal time scale by other researchers (Nichols, 1971; Aikman & Scaife, 1993; Tei et al., 1996b).

From the equations, relative growth rate (RGR) (equation 3.4), net assimilation rate (NAR) (equation 3.6), leaf area ratio (LAR) (equation 3.5), specific leaf area (SLA) (equation 3.7) and leaf weight ratio (LWR) (equation 3.8) were calculated according to the methods described by Hunt (1990) as follows:

$$\log_e W = a + b \times t + c \times t^2 \quad [3.1]$$

$$\log_e A = a' + b' \times t + c' \times t^2 \quad [3.2]$$

$$\log_e W_L = a'' + b'' \times t + c'' \times t^2 \quad [3.3]$$

$$RGR = b + 2 \times c \times t \quad [3.4]$$

The following were derived from the predicted value of the above equations:

$$LAR = \exp \times (\log_e A - \log_e W) \quad [3.5]$$

$$NAR = \frac{RGR}{LAR} \quad [3.6]$$

$$SLA = \exp \times (\log_e A - \log_e W_L) \quad [3.7]$$

$$LWR = \exp \times (\log_e W_L - \log_e W) \quad [3.8]$$

Where:

W is the plant dry weight at times (t)

A is the leaf area at times (t)

$W_L$ is the leaf dry weight at times (t)

a, b, c, $a'$, $b'$, $c'$, $a''$, $b''$, and $c''$ are constants.

### 3.2.4.2 Plant nutrient determination

The oven dried plant material, from each of the growth analysis harvests, were kept for plant nutrient determinations. Leaves, stem and root from every block were combined together for the first six harvests. At the final harvest leaves and roots from each block were kept separate. With stems, there was not enough plant material for determination on a block basis.

Potassium, calcium and magnesium were assessed by Atomic Absorption Spectroscopy (AAS) following digestion in nitric acid (Technicon, 1973). Samples were put in digestion tubes, digested with 4 ml of concentrated nitric acid (70% $HNO_3$), and covered with funnels to stimulate refluxing on a heating block maintained at a temperature of 150 $^0$C until clear (8-10 hours). After the solution had cleared, the funnels were removed and the remaining liquid was boiled dry by raising the temperature to 250 $^0$C for 3 hours.

The digestion samples were diluted to a volume of 50 ml with a stock solution made up of strontium nitrate (Sr $(NO_3)_2$) 1,000 ppm, caesium chloride (CSCl) 1,000 ppm, 0.2M hydrochloric acid (HCl) and deionized water.

Total nitrogen and phosphorus were determined by colorimetric autoanalysis following the Kjeldahl digestion technique (Twine and Williams, 1971). Samples were put in digestion tubes and 4 ml of Kjeldahl digest solution (25 g selenium powder and 2.5 l of concentrated $H_2SO_4$) was added. The samples were digested at 350 $^0$C until clear (4-5 hours). After the solution had cleared the digest was made up to 50 ml using deionized water.

The following standard solutions were prepared for determination of minerals with an atomic absorption spectrophotometer (AASP): calcium (Ca) 2, 4, 6, 8, and 10 ppm; magnesium (Mg) 0.2, 0.4, 0.6, 0.8, and 10 ppm; potassium (K) 3, 6, 9, 12, and 15 ppm. These standards were made from stock solutions of 1000 ppm calcium, magnesium and potassium.

The readings from the AAS expressed in ppm were used to determine tissue mineral concentration using the following formula:

$$Y = \frac{a \times b \times (c)}{10 \times d} \qquad [3.9]$$

Where:

Y = Concentration of the mineral (% DW)

a = volume after digestion (50 ml)

b = AAS reading (ppm)

(c) = this variable is the dilution rate applicable for determination of calcium, magnesium and potassium in the plant and was also applicable if the tissue is the leaf.

d = weight of sample used for digestion

### 3.2.4.3 Head fresh weight

Shoot fresh weight at final harvest was recorded from 10 plants per plot.

### 3.2.4.4 Shoot and total plant dry weight

Head and total plant dry weights at final harvest were recorded from 2 plants per plot, which was the sample for growth analysis determination.

### 3.2.4.5 Shoot dry matter percentage

Shoot dry matter percentages were calculated from the 2 plants per plot that were sampled for growth analysis determination. The summer crop was excluded from the analysis of variance because the shoot fresh weight determinations were for different plants from those sampled for growth analysis.

### 3.2.4.6 Incidence of tipburn

Tipburn incidence was assessed from 10 plants per plot at final harvest.

### 3.2.4.7 Shelf life

After the fresh weight determinations 3 heads from each plot were packed in 550 × 380 × 220 mm. plastic produce containers. They were kept in a controlled temperature room at 25 $^{0}$C 75% RH for the summer crop and 10 $^{0}$C 80% RH for the autumn, winter and spring crops. The fresh weight of 3 heads were measured every day to determine fresh weight loss. The fresh weight loss percentage was calculated for each plot. The relationships of fresh weight loss percentage with number of days from harvest, from day 1 onwards were linear. The data from day 1 onwards were fitted to a linear regression and the day to 10 % fresh weight loss was calculated for each plot. The 10% fresh weight loss was selected as the symptoms of water loss become objectionable when vegetables have lost between 5% and 10% of their fresh weight (Ryall & Lipton, 1979).

### 3.2.4.8 Statistical procedure and analysis

The design used for evaluating growth analysis attributes, plant nutrient concentration at final harvest, yield and tipburn incidence was a Randomized Complete Block (RCB) with 4 × 3 factorial arrangement (nutrient solution concentraton × cultivar) with 4 replications over 4 seasons except for shoot dry matter percentage where only 3 seasons were analysed (3.2.4.5). Tipburn was expressed as the percentage of plants which showed symptoms. Data were subjected to analysis of variance following the Statistical Analysis System (SAS) General Linear Model (GLM) procedure (SAS Institute Inc., 1989). Data were checked as to whether they conformed to the assumption of ANOVA. The percentage data was converted by arcsine transformation for analysis.

As the data for plant nutrition concentrations was not replicated from the start to harvest 5, statistical analysis was made possible by using cultivars as replicates. The design used for evaluating plant nutrient solution concentration was a Randomized Complete Block (RCB) with 4 concentrations with 3 replications (cultivars) over 4 seasons.

## 3.3 Results

### 3.3.1 Growth analysis

The results of the analysis of variance carried out on the effects of the treatments on the growth analysis attributes are presented in Table 3.2. For all attributes the crop × concentration and crop × cultivar interactions were significant. These interactions are presented in Figure 3.1 - Figure 3.15 and the main conclusions based on these interactions is presented in Table 3.3. For each attribute, the first figure presents the main treatment effects, due to the interactions the data in these figures are not discussed. They are presented as background information.

**Table 3.2 Significance level of RGR, NAR, LAR, SLA and LWR of 3 lettuce cultivars grown in different concentrations over 4 seasons**

| heat unit | 0 | 100 | 200 | 300 | 400 | 500 | 600 | 700 |
|---|---|---|---|---|---|---|---|---|
| **Relative Growth Rate (RGR)** | | | | | | | | |
| Crop | **** | **** | **** | **** | **** | **** | **** | **** |
| Concentration (conc) | **** | **** | **** | **** | **** | **** | **** | **** |
| Cultivar (cv) | **** | **** | **** | **** | **** | **** | **** | **** |
| Crop × conc | **** | **** | **** | **** | **** | **** | **** | **** |
| Crop × cv | **** | **** | **** | **** | **** | **** | **** | **** |
| Conc × cv | ns | ns | ns | ns | ns | ns | ns | ns |
| Crop × conc × cv | ns | ns | * | ns | ns | ns | ns | ns |
| **Net Assimilation Rate (NAR)** | | | | | | | | |
| Crop | **** | **** | **** | **** | **** | **** | **** | **** |
| Concentration (conc) | *** | **** | **** | **** | **** | **** | **** | **** |
| Cultivar (cv) | **** | **** | ** | *** | **** | **** | **** | **** |
| Crop × conc | **** | **** | **** | **** | **** | **** | **** | **** |
| Crop × cv | **** | **** | **** | **** | **** | **** | **** | **** |
| Conc × cv | ns | ns | * | ** | *** | *** | ns | ns |
| Crop × conc × cv | ns | ns | ns | ns | ns | ns | ns | ns |
| **Leaf Area Ratio (LAR)** | | | | | | | | |
| Crop | **** | **** | **** | **** | **** | **** | **** | **** |
| Concentration (conc) | ns | ns | ns | ns | ns | ns | **** | **** |
| Cultivar (cv) | **** | **** | **** | **** | **** | **** | **** | **** |
| Crop × conc | ns | * | * | ** | **** | **** | **** | **** |
| Crop × cv | **** | **** | **** | ** | ** | ns | **** | **** |
| Conc × cv | ns | ns | ns | ns | * | * | ns | ns |
| Crop × conc × cv | ns | ns | ns | ns | ns | ns | ns | ns |
| **Specific Leaf Area (SLA)** | | | | | | | | |
| Crop | **** | **** | **** | **** | **** | **** | **** | **** |
| Concentration (conc) | ns | ns | * | * | * | ns | ** | **** |
| Cultivar (cv) | **** | **** | **** | **** | **** | **** | **** | **** |
| Crop × conc | * | ** | *** | **** | **** | **** | **** | **** |
| Crop × cv | **** | **** | **** | **** | *** | ns | **** | **** |
| Conc × cv | ns | ns | ** | ** | ** | ** | ns | ns |
| Crop × conc × cv | ns | ns | ns | ns | ns | ns | ns | ns |
| **Leaf Weight Ratio (LWR)** | | | | | | | | |
| Crop | *** | *** | **** | **** | ** | ** | **** | **** |
| Concentration (conc) | ns | ns | ** | **** | **** | **** | **** | **** |
| Cultivar (cv) | **** | ns | *** | **** | **** | **** | **** | ns |
| Crop × conc | ns | ns | *** | **** | **** | **** | **** | **** |
| Crop × cv | **** | **** | **** | **** | **** | **** | **** | ** |
| Conc × cv | ns | ns | ns | ns | ns | ns | ns | ns |
| Crop × conc × cv | ns | ns | ns | ns | ns | ns | ns | ns |

ns,*, **, ***, **** = non significant, significant at $P \leq 0.05, 0.01, 0.001$ and 0.0001 respectively

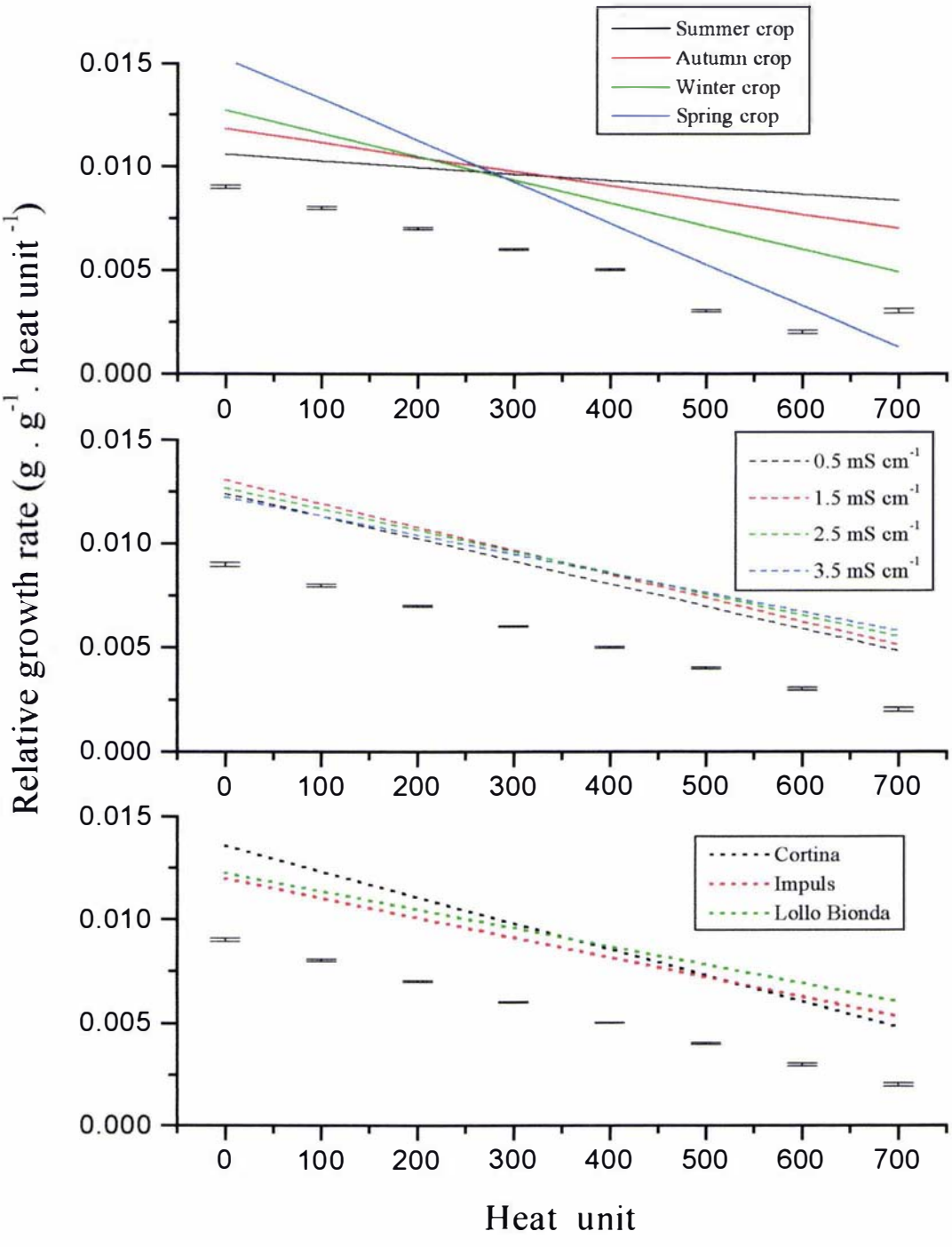


**Figure 3.1** **Effect of crop, nutrient solution concentration and cultivar on relative growth rate of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the main effect at that stage.**

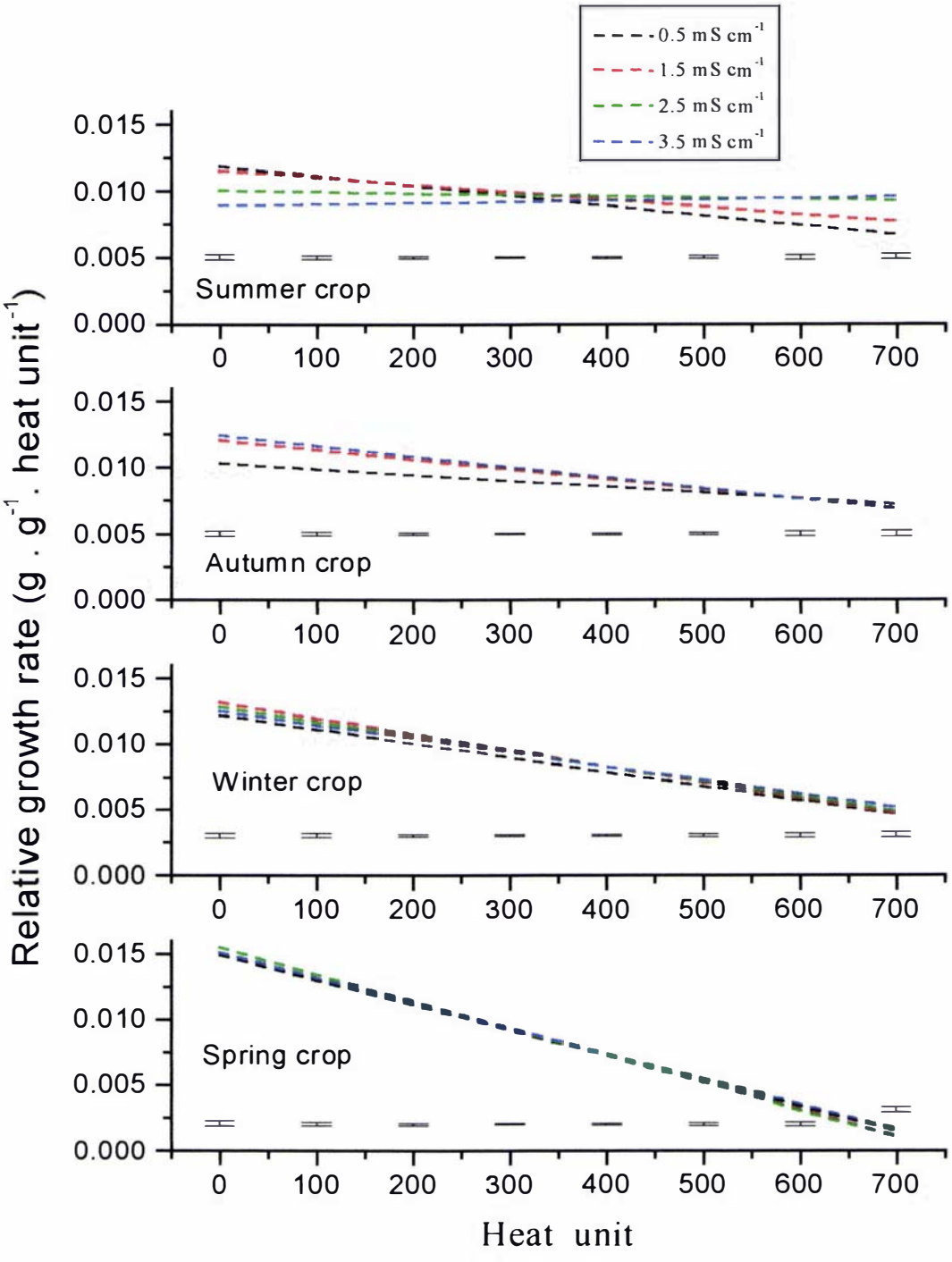


**Figure 3.2 Interaction between crop and concentration on relative growth rate (mean of 3 cultivars) of lettuce grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction effect at that stage.**

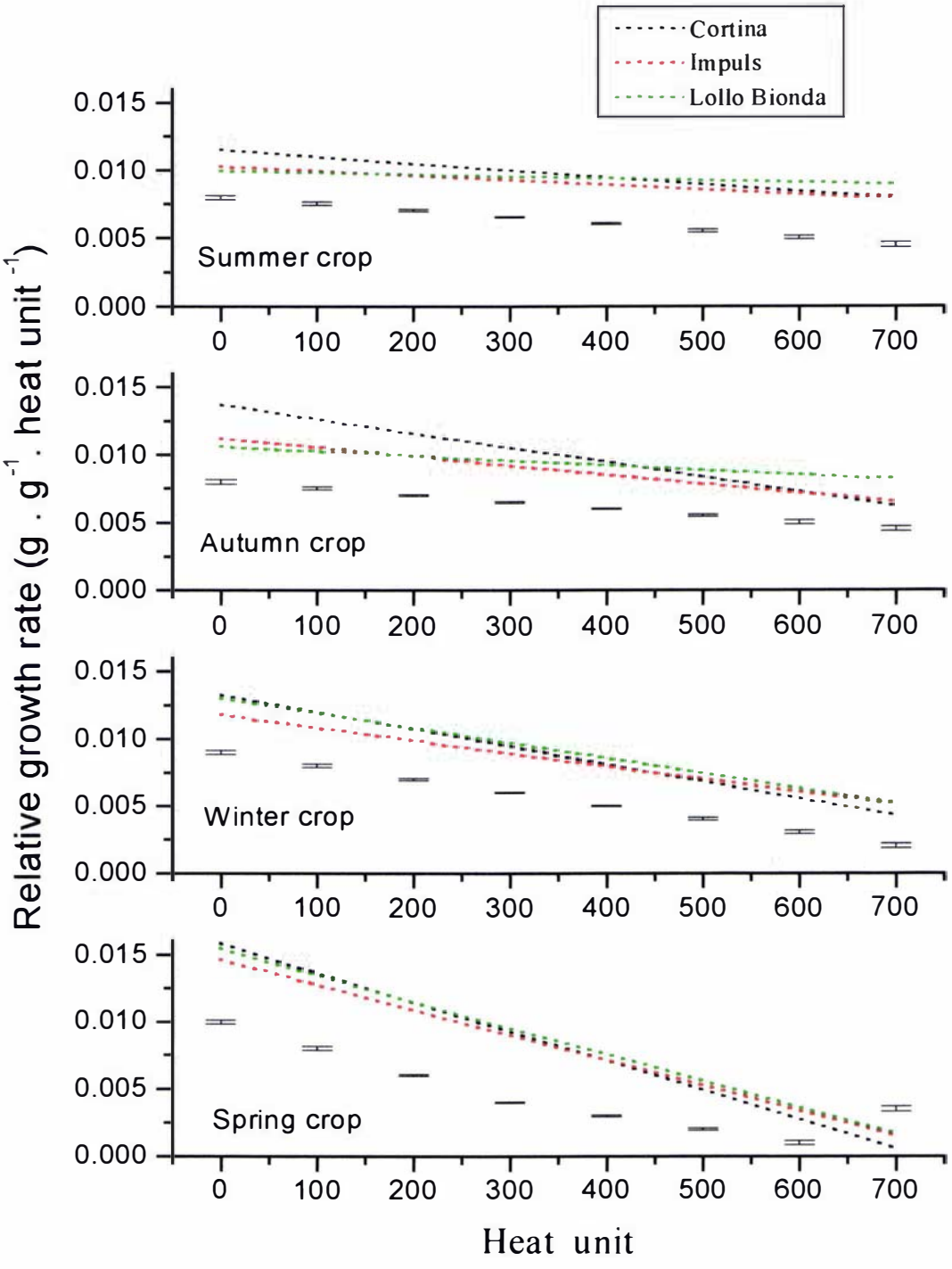


**Figure 3.3** **Interaction between crop and cultivar on relative growth rate of 3 lettuce cultivars (mean of 4 nutrient solution concentrations) over 4 seasons. The bars represent the standard error of the interaction effect at that stage.**

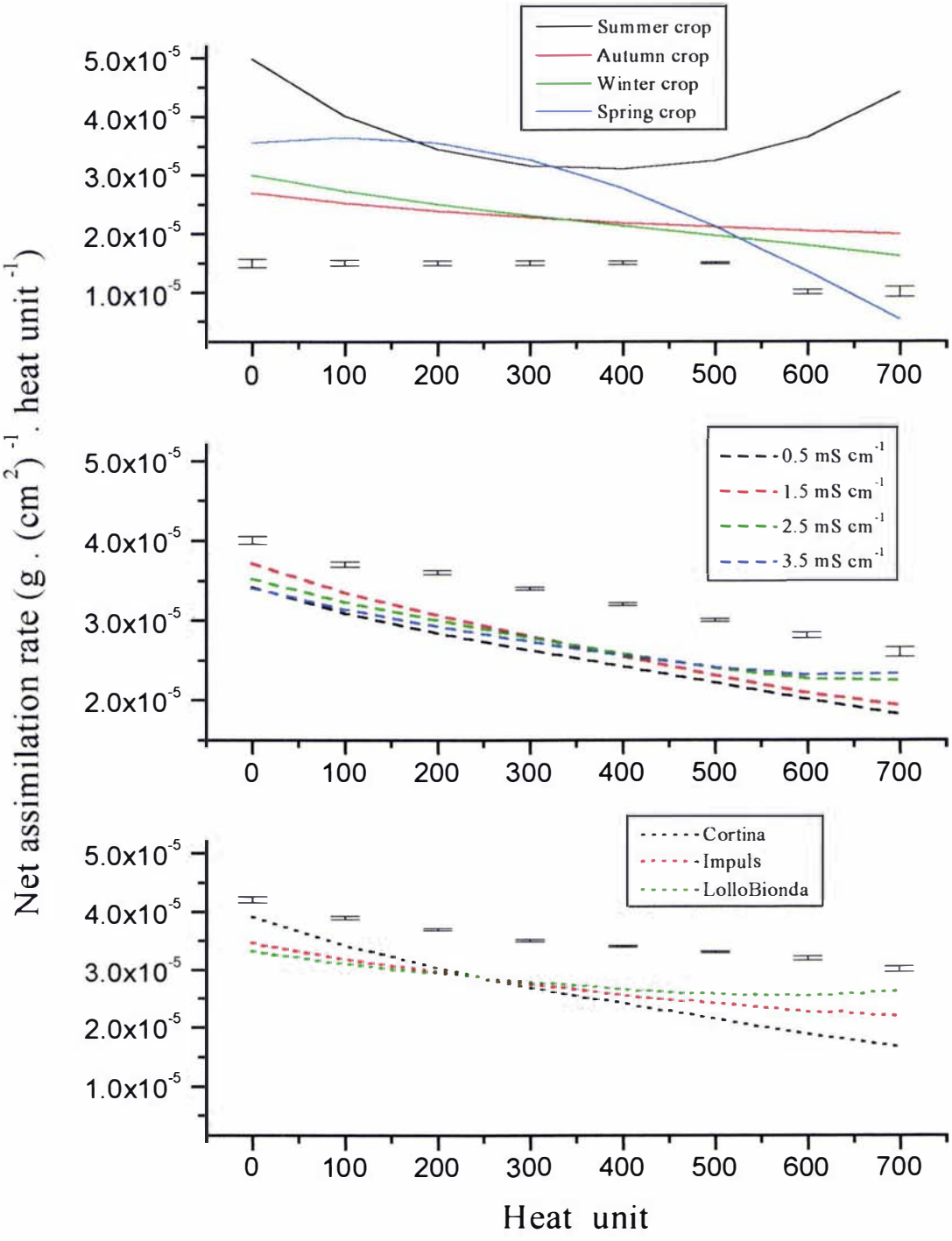


**Figure 3.4 Effect of crop, nutrient solution concentration and cultivar on net assimilation rate of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons.The bars represent the standard error of the main effect at that stage.**

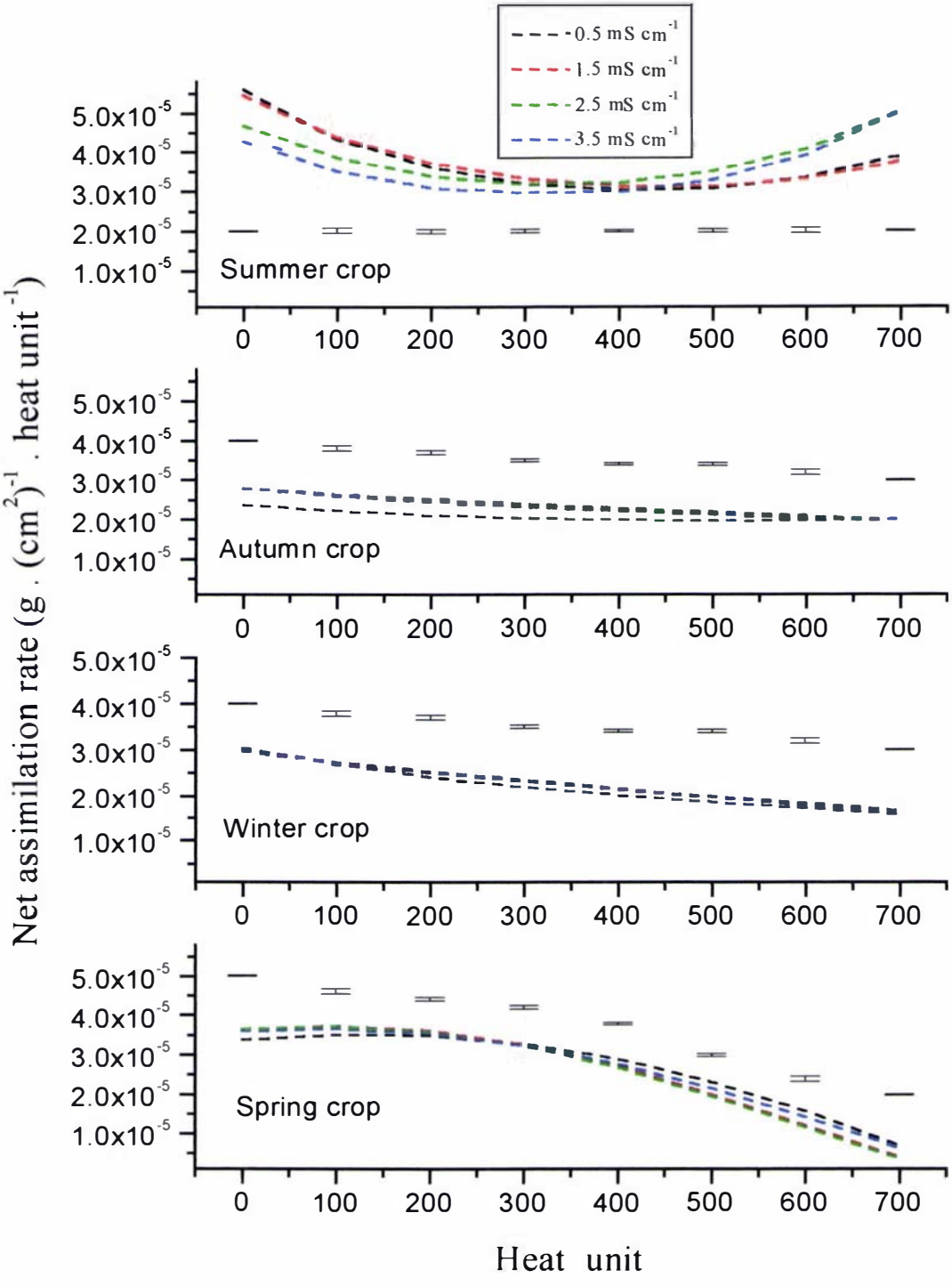


**Figure 3.5 Interaction between crop and concentration on net assimilation rate (mean of 3 cultivars) of lettuce grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction effect at that stage.**

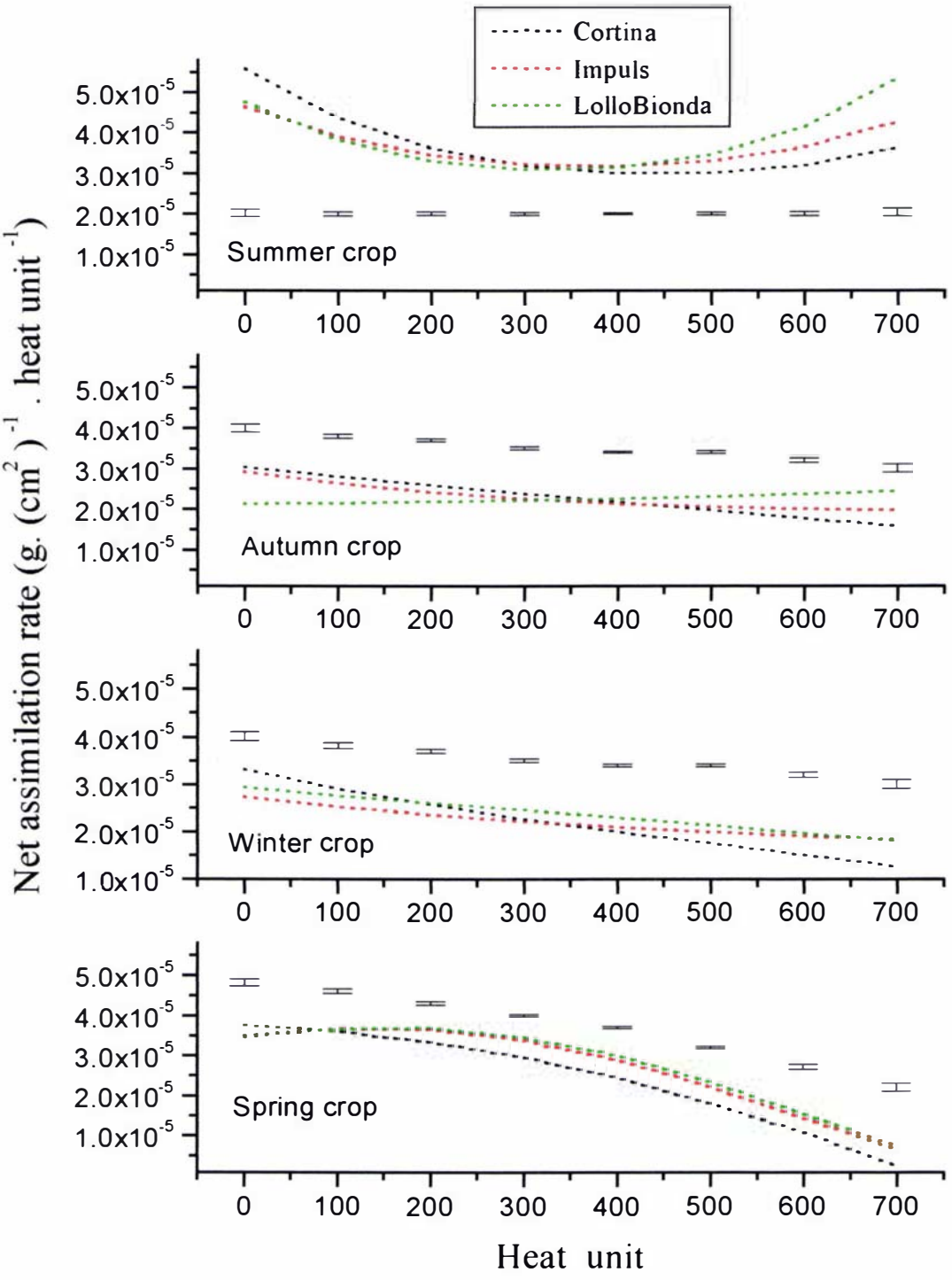


**Figure 3.6 Interaction between crop and cultivar on net assimilation rate of 3 lettuce cultivars (mean of 4 nutrient solution concentrations) over 4 seasons. The bars represent the standard error of the interaction effect at that stage.**

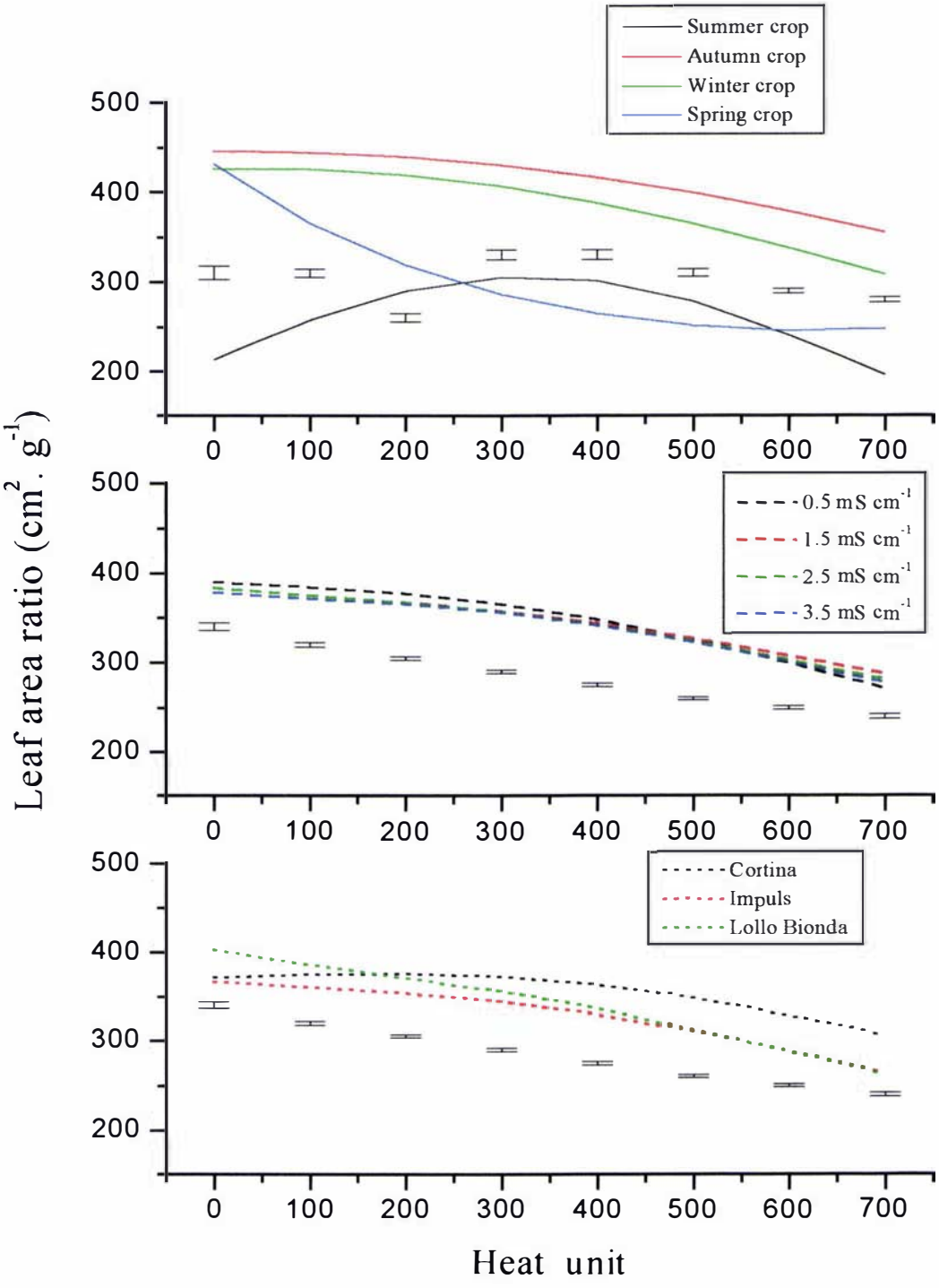


**Figure 3.7 Effect of crop, nutrient solution concentration and cultivar on leaf area ratio of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the main effect at that stage.**

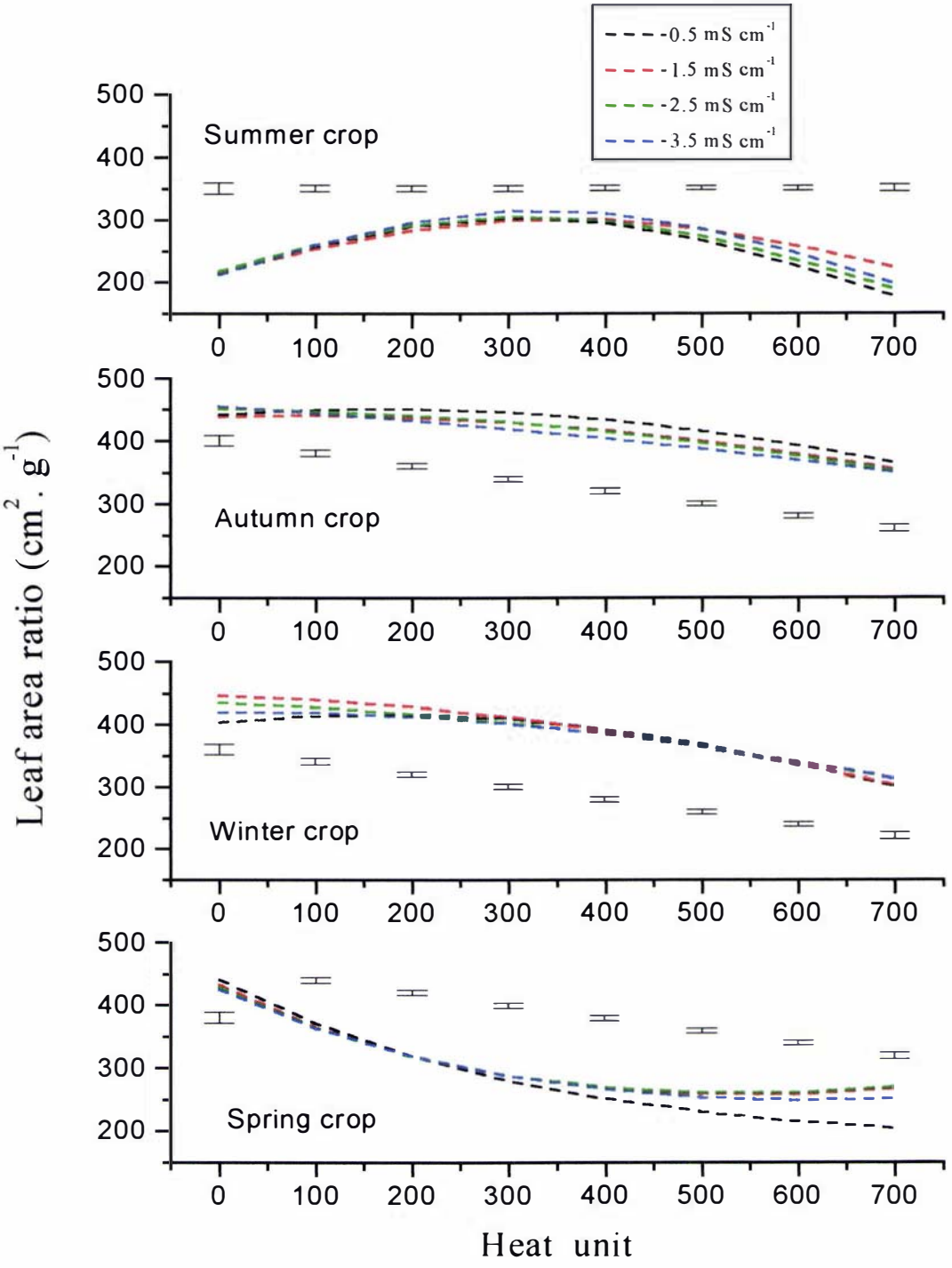


**Figure 3.8 Interaction between crop and concentration on leaf area ratio (mean of 3 cultivars) of lettuce grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error bar of the interaction effect at that stage.**

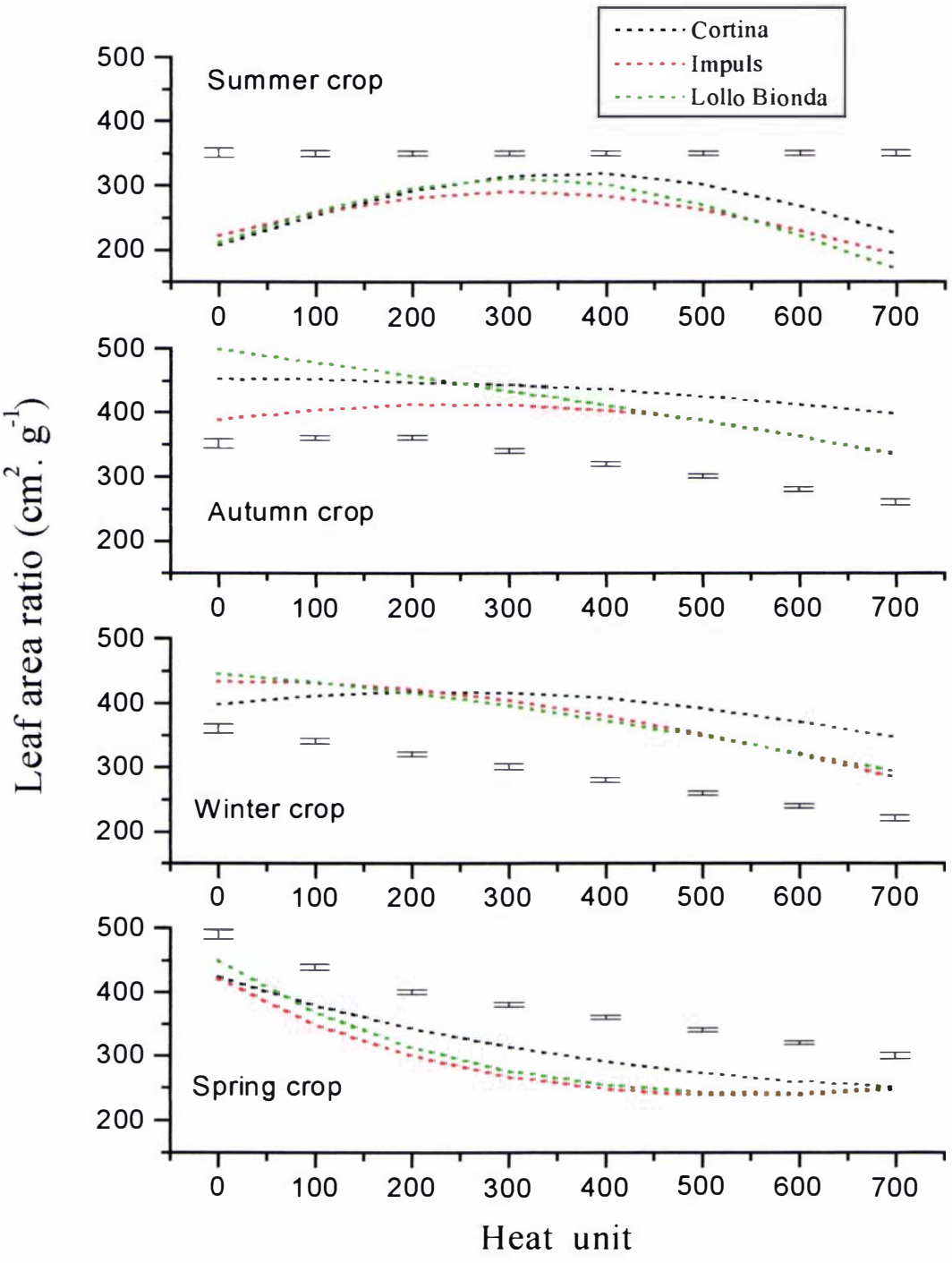


**Figure 3.9** **Interaction between crop and cultivar on leaf area ratio of 3 lettuce cultivars (mean of 4 nutrient solution concentrations) over 4 seasons. The bars represent the standard error of the interaction effect at that stage.**

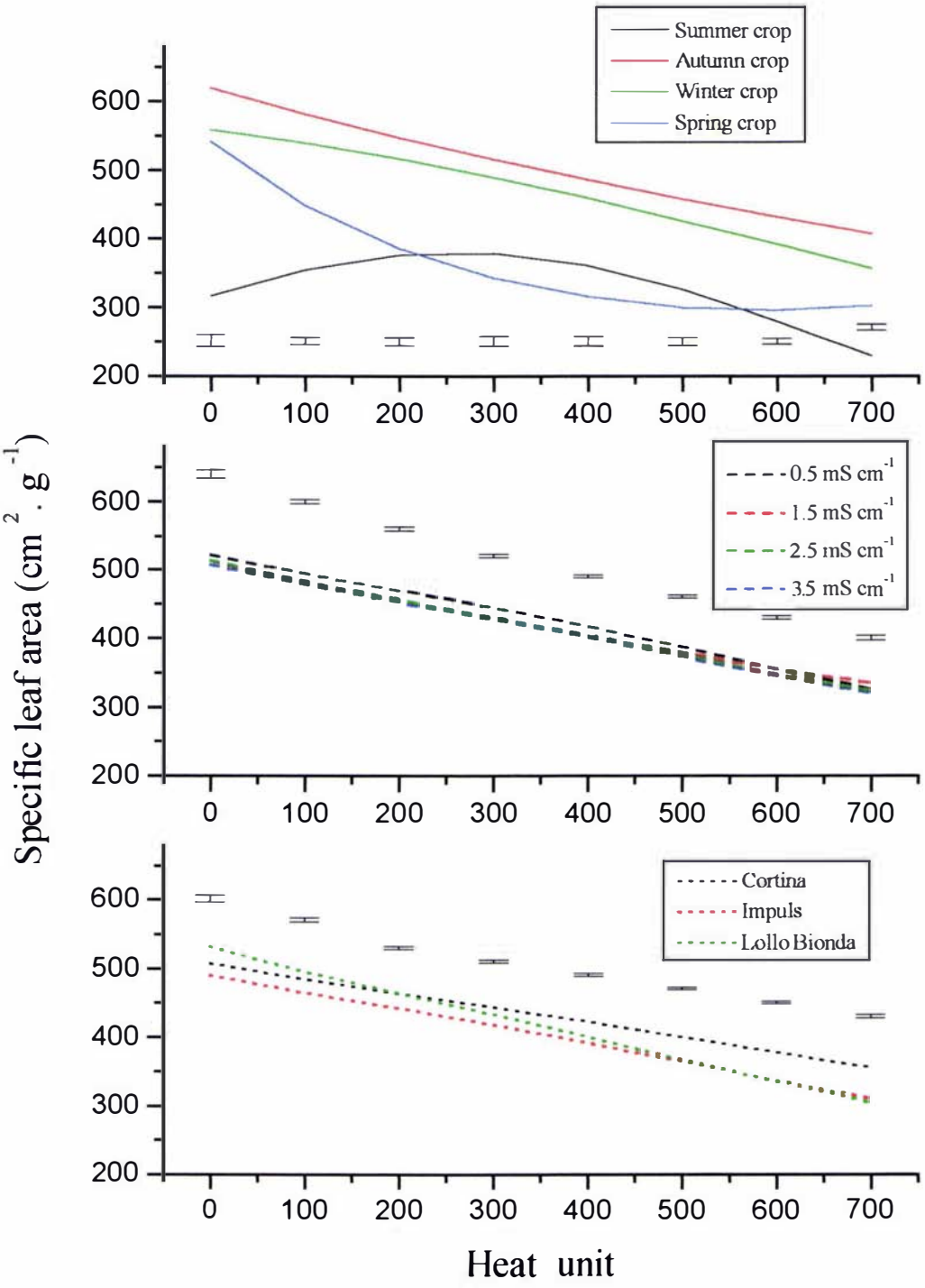


**Figure 3.10** **Effect of crop, nutrient solution concentration and cultivar on specific leaf area of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the main effect at that stage.**

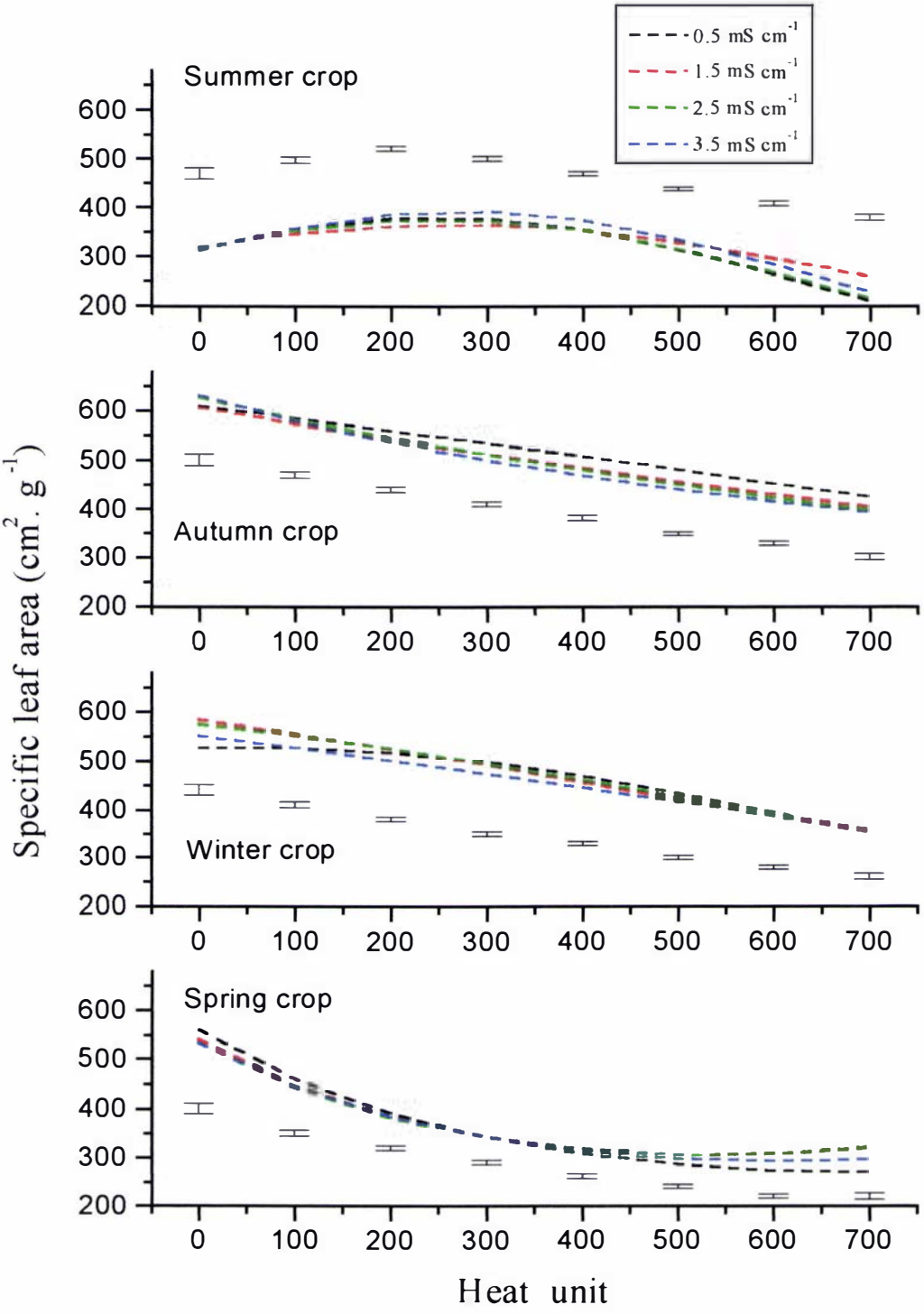


**Figure 3.11** **Interaction between crop and concentration on specific leaf area (mean of 3 cultivars) of lettuce grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction effect at that stage.**

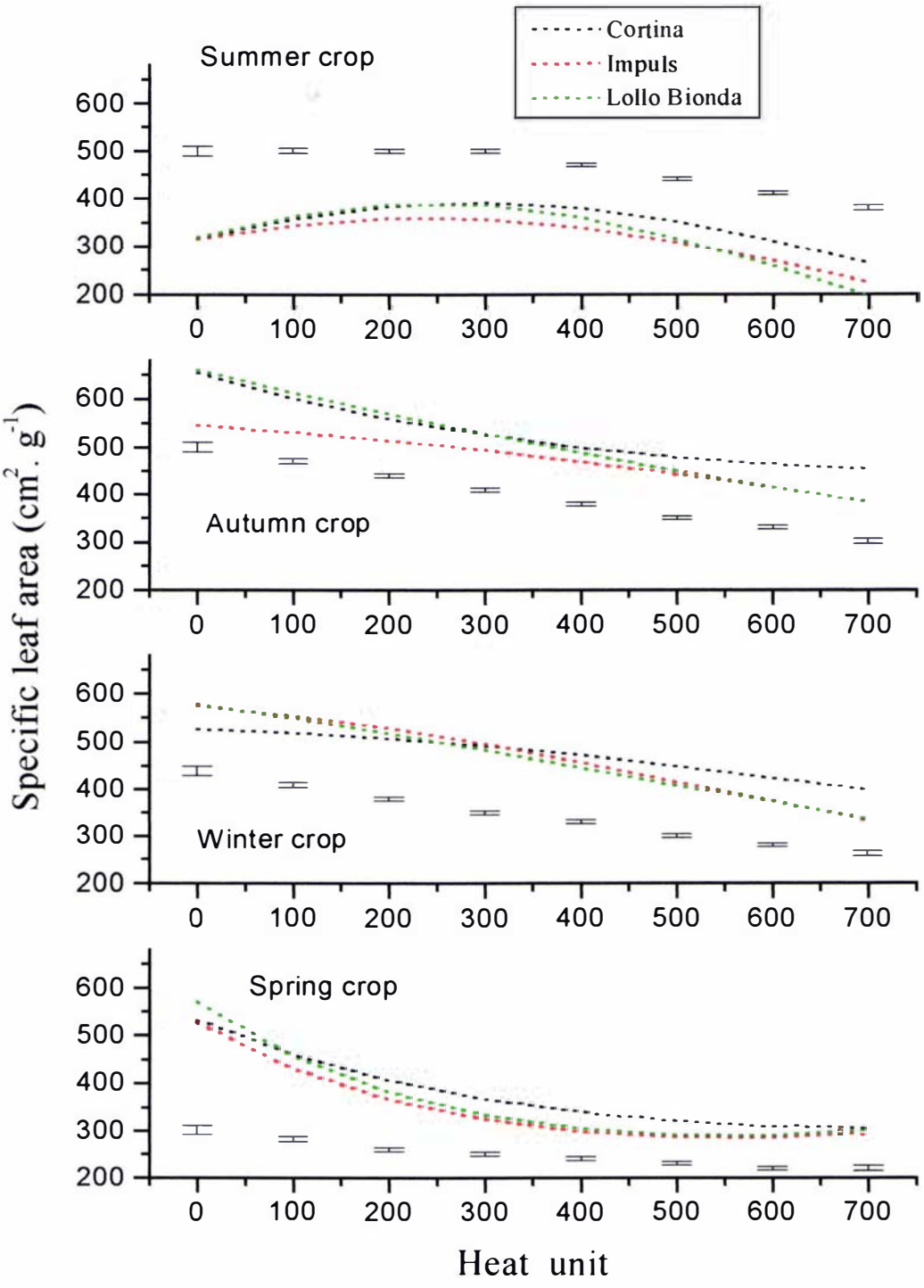


**Figure 3.12 Interaction between crop and cultivar on specific leaf area of 3 lettuce cultivars (mean of 4 nutrient solution concentrations) over 4 seasons. The bars represent the standard error of the interaction effect at that stage.**

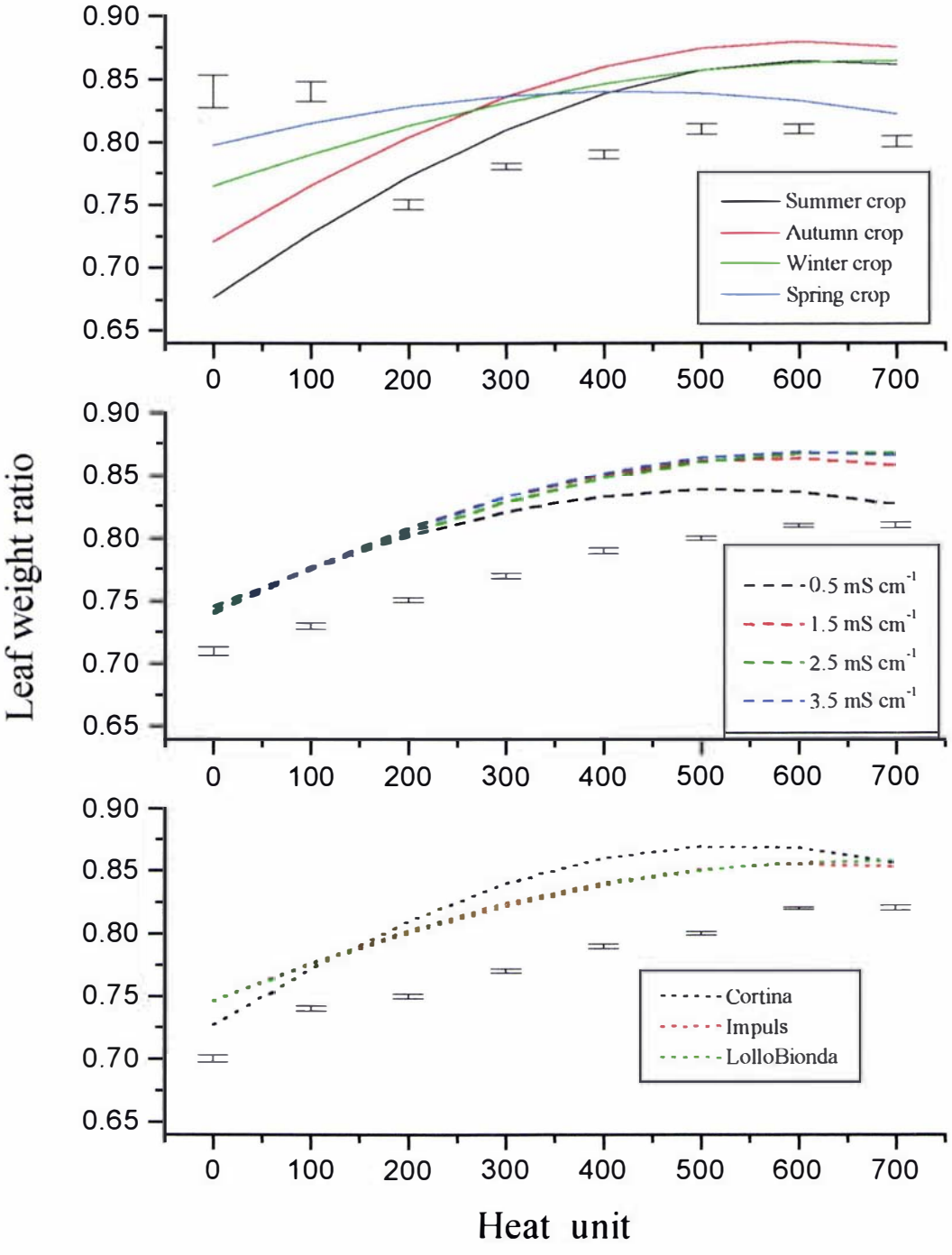


**Figure 3.13 Effect of crop, nutrient solution concentration and cultivar on leaf weight ratio of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error bar of the main effect at that stage.**

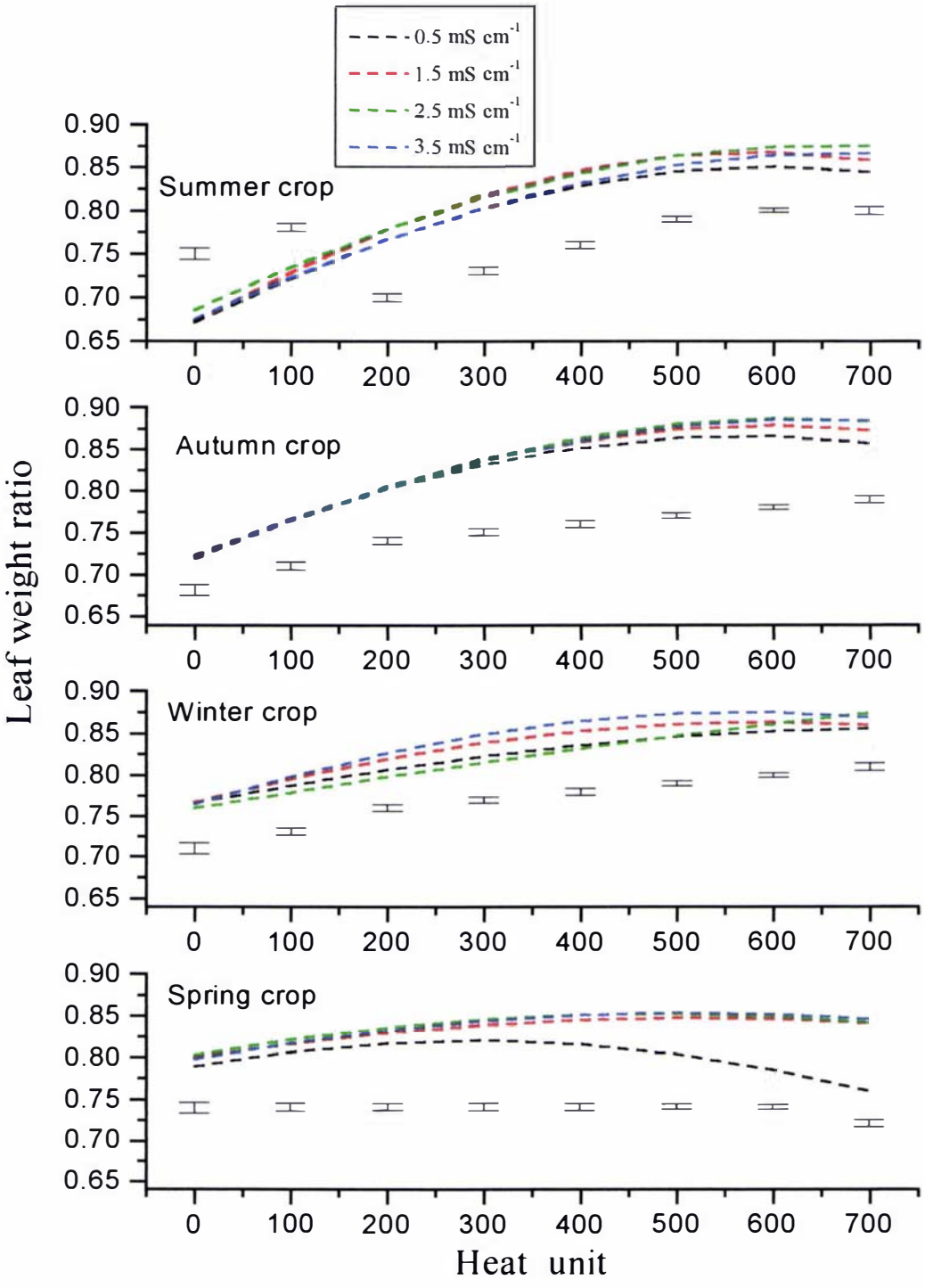


**Figure 3.14 Interaction between crop and concentration on leaf weight ratio (mean of 3 cultivars) of lettuce grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction effect at that stage.**

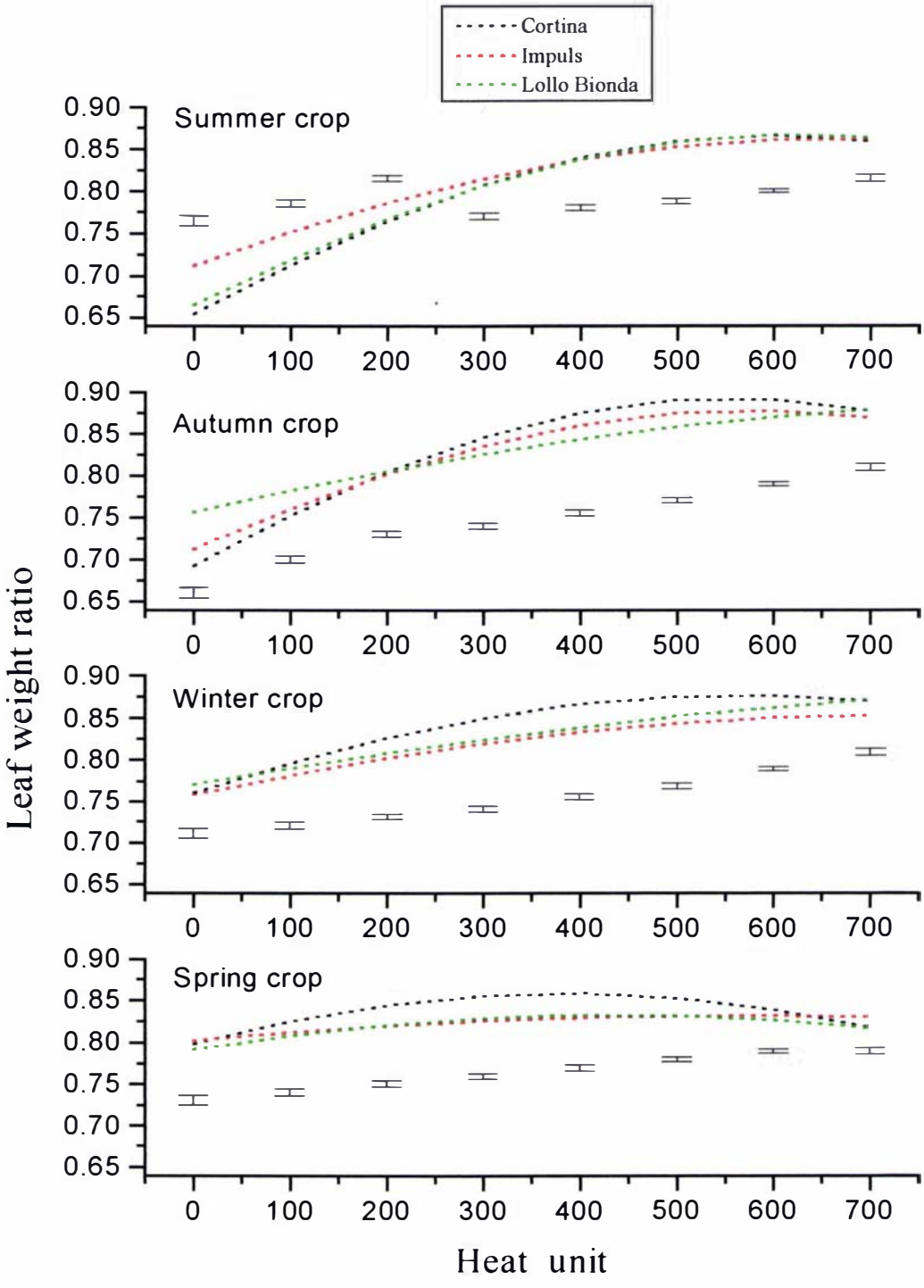


**Figure 3.15** **Interaction between crop and cultivar on leaf weight ratio of 3 lettuce cultivars (mean of 4 nutrient solution concentrations) over 4 seasons. The bars represent the standard error of the interaction effect at that stage.**

**Table 3.3 Main conclusions based on the treatment effects on the growth analysis attributes.**

| Season | RGR | NAR | LAR | SLA | LWR |
|---|---|---|---|---|---|
| All | RGR decreased with time except with 3.5 mS $cm^{-1}$ summer crop. | NAR decreased with time except with summer crop and Lollo Bionda in autumn. | LAR decreased with time except with summer crop and Cortina in winter and Impuls in autumn | SLA decreased with time except with summer crop. | LWR increased with time except with 0.5 mS $cm^{-1}$ spring crop. |
| **Interaction between crop × concentration** | | | | | |
| | (Figure 3.2) | (Figure 3.5) | (Figure 3.8) | (Figure 3.11) | (Figure 3.14) |
| Summer crop | Initial RGR in the following order: 0.5, 1.5 >2.5 >3.5. Final RGR in the following order: 3.5,2.5 >1.5>0.5. | Initial NAR in order: 0.5, 1.5 >2.5 >3.5. Final NAR in order; 3.5,2.5 >0.5, 1.5. NAR decreased and then increased. | Final LAR in order: 1.5 >3.5, 2.5, 0.5. Lowest LAR of all crops apart from spring mid season. LAR increased and then decreased. | Final SLA in order: 1.5 >3.5, 2.5, 0.5. Lowest SLA of all crops apart from spring mid season. SLA increased and then decreased. | Initial LWR in order: 2.5 > 3.5, 1.5, 0.5. Final LWR in order: 2.5 >3.5, 1.5>0.5. |
| Autumn crop | 0.5 mS $cm^{-1}$ low initial RGR. | 0.5 mS $cm^{-1}$ low initial NAR. | 0.5 mS $cm^{-1}$ highest mid to final LAR. | 0.5 mS $cm^{-1}$ highest mid to final SLA. | Final LWR in order: 3.5, 2.5 >1.5 >0.5. |
| Winter crop | Initial RGR in order: 1.5 >2.5, 3.5>0.5. | | Initial LAR in order: 1.5 >2.5 >3.5 >0.5. LAR decreased slowly at first. | Initial SLA in order: 1.5, 2.5 >3.5 >0.5. | 2.5 mS $cm^{-1}$ low initial LWR. Final LWR in order: 2.5, 3.5>1.5, 0.5. |
| Spring crop | 2.5 mS $cm^{-1}$ highest initial and lowest final RGR | NAR decreased slowly at first. 0.5 mS $cm^{-1}$ low initial NAR. Final NAR in order: 0.5, 3.5 > 1.5, 2.5 | LAR decreased slowly mid to late season. Final LAR in order: 2.5, 1.5 >3.5 >0.5. | SLA decreased slowly mid to late season. 0.5 mS $cm^{-1}$ high initial SLA. Final SLA in order: 2.5, 1.5 >3.5 >0.5. | 0.5 mS $cm^{-1}$ lowest LWR throughout and decreased from mid season. |
| All | Generally the higher the initial RGR the greater the rate of decline. Initial RGR in order: spring > winter > autumn, summer. Final RGR in order: summer, autumn >winter >spring. | Initial NAR in order: summer > spring > winter > autumn. Final NAR in order: summer >autumn >winter >spring. | Initial LAR in order: autumn, spring, winter > summer. Final LAR in order: autumn >winter > spring, summer. | Initial SLA in order: autumn > winter, spring > summer. Final SLA in order: autumn > winter >spring, summer. | Initial LWR in order: spring > winter >autumn >summer. |

**Table 3.3 (Continued) Interaction between crop × cultivar.**

| Season | RGR (Figure 3.3) | NAR (Figure 3.6) | LAR (Figure 3.9) | SLA (Figure 3.12) | LWR (Figure 3.15) |
|---|---|---|---|---|---|
| All | Crop effect on initial and final RGR as for crop × concentration. | Crop effect on initial and final NAR as for crop × concentration. | Crop effect on initial and final LAR as for crop × concentration. | Crop effect on initial and final SLA as for crop × concentration. | Crop effect on initial LWR as for crop × concentration. |
| All [1] | Initial RGR in the following order: Cortina (W, Sp) ≥ Lollo Bionda (S, Sp) ≥ Impuls. | Initial NAR in the following order: Cortina > Lollo Bionda (S, Sp) ≥ Impuls, except that Impuls > Lollo Bionda in autumn. | Initial LAR in the following order: Lollo Bionda (S) ≥ Impuls, Cortina.<br>In autumn Cortina > Impuls.<br>In winter Impuls > Cortina. | Initial SLA in the following order: Lollo Bionda (S, A, W) ≥ Impuls, Cortina.<br>In autumn Cortina > Impuls.<br>In winter Impuls > Cortina. | Initial LWR in summer: Impuls > Lollo Bionda, Cortina.<br>In autumn Lollo Bionda > Impuls > Cortina. |
| All [1] | Final RGR in the following order: Lollo Bionda (W, Sp) ≥ Impuls (S, A)> Cortina | Final NAR in the following order: Lollo Bionda (W, Sp)≥ Impuls > Cortina. | Final LAR in the following order: Cortina (Sp) ≥ Lollo Bionda, Impuls.<br>In summer Impuls > Lollo Bionda. | Final SLA in the following order: Cortina (Sp) > Lollo Bionda, Impuls.<br>In summer Impuls > Lollo Bionda. | In winter final LWR: Cortina, Lollo Bionda > Impuls. |

[1] ( ) except in season where S = summer, A = autumn, W = winter and Sp = spring for at least one of the following cultivars.

**Summary of conclusions in Table 3.3:**

i. Generally RGR, NAR, LAR and SLA decreased with time while LWR increased.

ii. Initial RGR were in the order spring > winter > autumn, summer, which was, apart from summer, the order of the initial NAR.

iii. With the summer crop the initial RGR were in order 0.5, 1.5 > 2.5 > 3.5 mS $cm^{-1}$, which was similar to the order of NAR.

iv. Final RGR were in the order summer, autumn > winter > spring, which was similar to the order for final NAR

v. The initial RGR in autumn for 0.5 mS $cm^{-1}$ was low due to a low NAR as the LAR was high.

vi. The final LAR of 0.5 mS $cm^{-1}$ spring crop was lower than other concentrations which was similar to the LWR.

vii. The initial RGR for the three cultivars were in order Cortina ≥ Lollo Bionda ≥ Impuls. There was a similar order for initial NAR with Lollo Bionda having the highest LAR.

viii. The final RGR were in the order Lollo Bionda ≥ Impuls ≥ Cortina, which was similar to the order of final NAR with Cortina having the highest final LAR.

### 3.3.2 Nutrient uptake of the whole plant during cropping.

The nutrient concentrations for all the nutrients at the first determination (harvests) were ignored as this harvest was taken at transplanting and the plants had not been affected by the treatments. Thus nutrient concentrations at harvest 1 are not presented.

The nutrient concentration was affected by crop and concentration. There were significant interactions between crop and concentration on plant nutrient concentrations of nitrogen, phosphorus, potassium, calcium and magnesium.

**Table 3.4 Levels of significance of nitrogen, phosphorus, potassium, calcium and magnesium for the interaction between crop and concentration for each harvest**

| | Nitrogen | Phosphorus | Potassium | Calcium | Magnesium |
|---|---|---|---|---|---|
| Harvest 2 | 0.001 | 0.01 | ns | 0.05 | 0.001 |
| Harvest 3 | ns | 0.0001 | ns | ns | 0.001 |
| Harvest 4 | ns | 0.001 | 0.01 | ns | 0.0001 |
| Harvest 5 | 0.0001 | 0.0001 | ns | ns | 0.001 |
| Harvest 6 | 0.0001 | 0.0001 | 0.01 | 0.001 | 0.0001 |
| Harvest 7 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.001 |

ns, 0.05, 0.01, 0.001, 0.0001 = non significant, significant at 0.05, 0.01, 0.001 and 0.0001 respectively

For nitrogen concentration (Figure 3.16) there was a trend with all crops to decrease with plant growth and this was marked with the spring crop. With the spring and summer crops, as the crop aged, the nitrogen concentration became lower for 0.5 mS $cm^{-1}$ than for the other concentrations. Generally for these two crops the plant nitrogen concentrations were lower than for autumn and winter crops.

Phosphorus concentration remained fairly constant throughout for the winter and spring crops and for the summer and autumn crops from harvest 2 - 3 onwards (Figure 3.17). Phosphorus concentrations for 0.5 mS $cm^{-1}$ were lower than the other concentrations, apart from in spring, where it increased late in the season. The latter result was in contrast to nitrogen concentration, which decreased with plant growth. The increase in phosphorus concentration with the spring crop was due to the amount of phosphoric acid that this treatment required to adjust the pH rise that occurred. Generally autumn and winter crops maintained higher plant phosphorus concentrations throughout cropping than summer and spring crops.

Potassium concentrations with 2.5 and 3.5 mS $cm^{-1}$ were steady or slightly increased with plant growth depending on the season (Figure 3.18). This trend also applied to 1.5 mS $cm^{-1}$ in autumn and winter. For 1.5 mS $cm^{-1}$ in summer and spring and 0.5 mS $cm^{-1}$ in all seasons, the potassium concentration decreased with plant growth late in the season.

With the summer crop, the calcium concentration started off high otherwise the levels were generally steady (Figure 3.19). Late in the season 0.5 and 1.5 mS $cm^{-1}$ were high, apart from with 0.5 mS $cm^{-1}$ in spring.

Magnesium concentrations at 0.5 and 1.5 mS $cm^{-1}$ increased late in the season. There was a greater variation in magnesium concentration with nutrient solution concentration late in the season than with calcium (Figure 3.20).

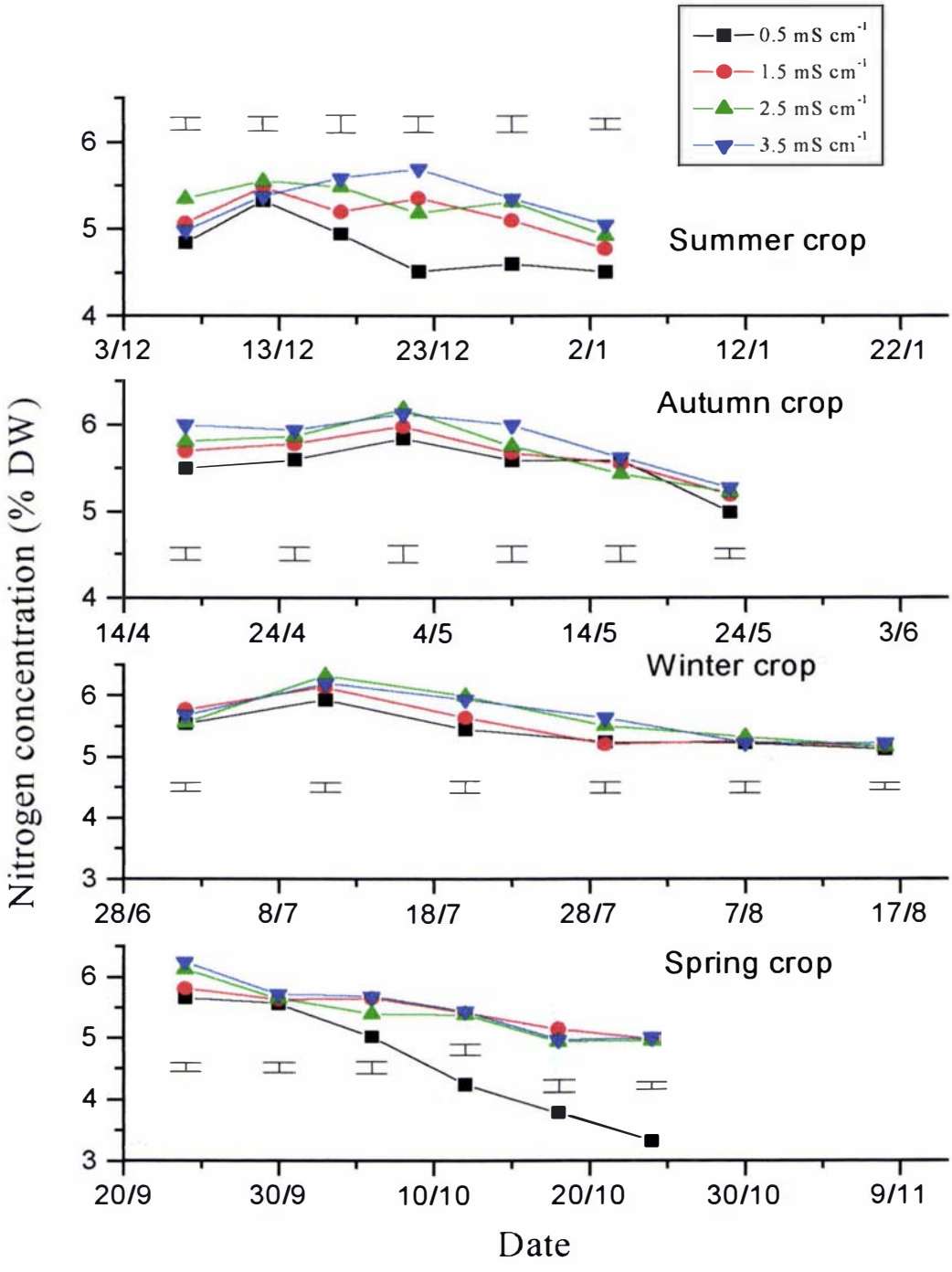


**Figure 3.16** **Nitrogen concentration (% dry weight) of whole lettuce plant during cropping. The bars represent the standard error of the interaction between crop and concentration at that stage.**

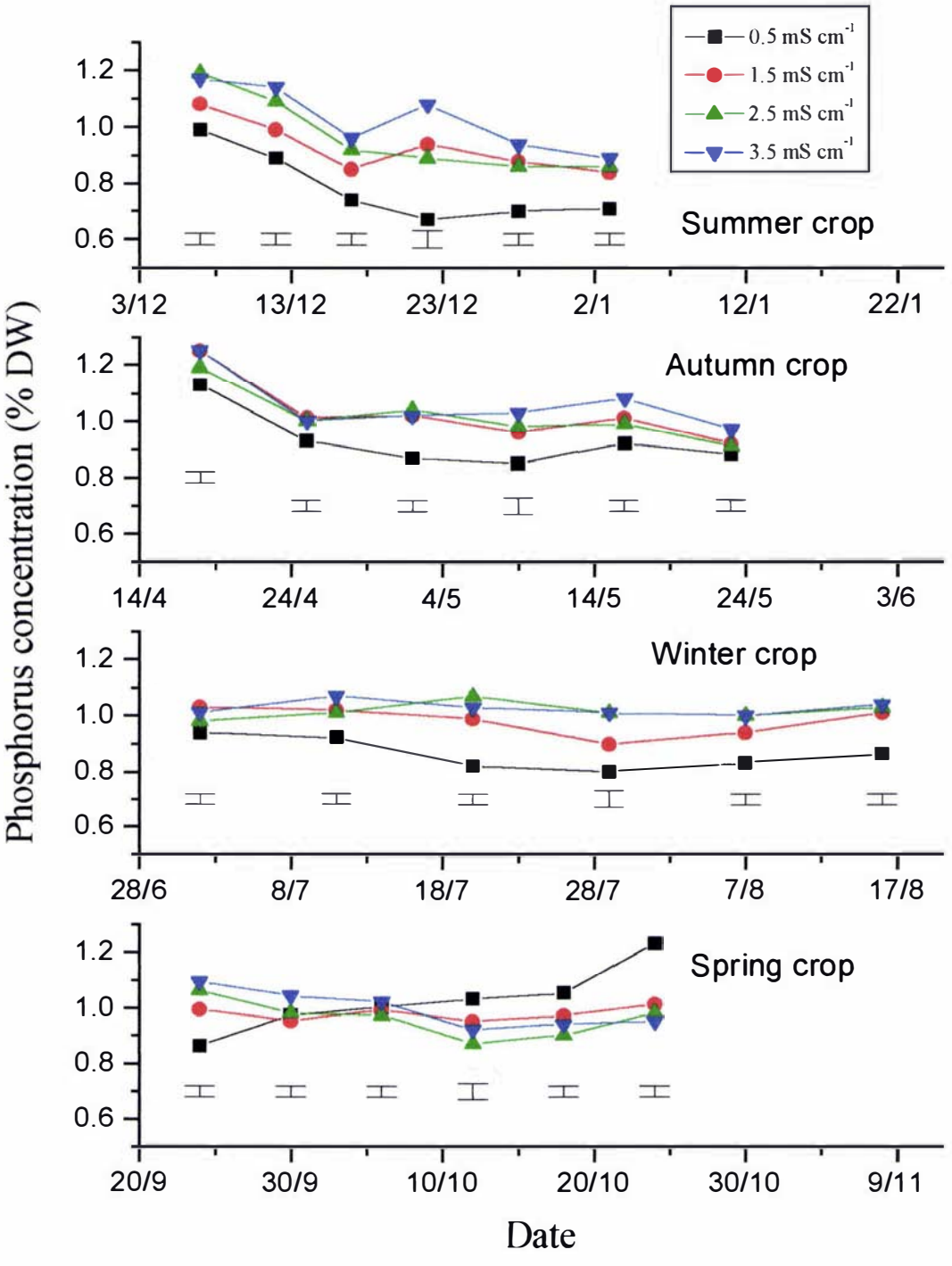


**Figure 3.17** **Phosphorus concentration (% dry weight) of whole lettuce plant during cropping. The bars represent the standard error of the interaction between crop and concentration at that stage.**

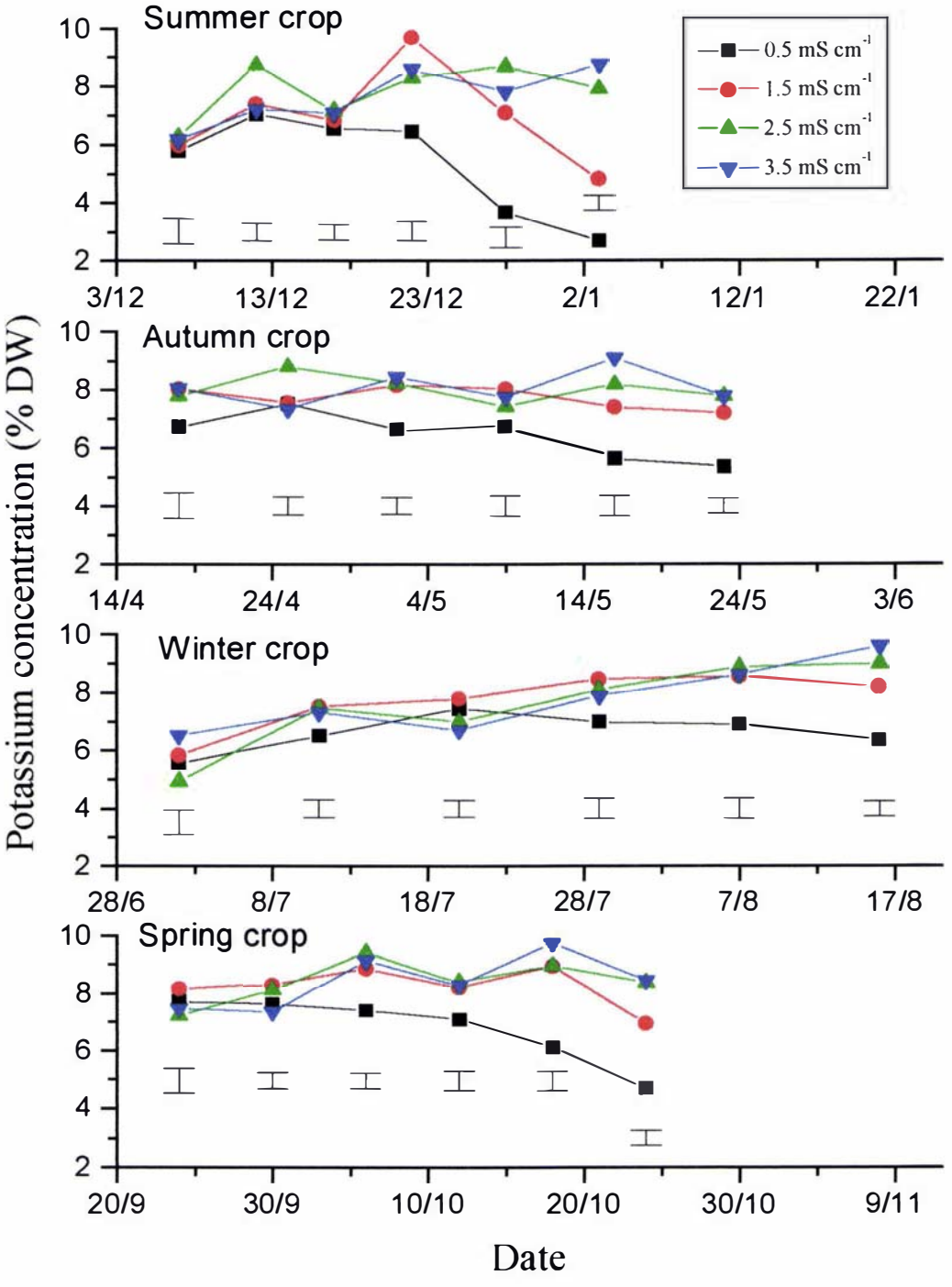


**Figure 3.18** **Potassium concentration (% dry weight) of whole lettuce plant during cropping. The bars represent the standard error of the interaction between crop and concentration at that stage.**

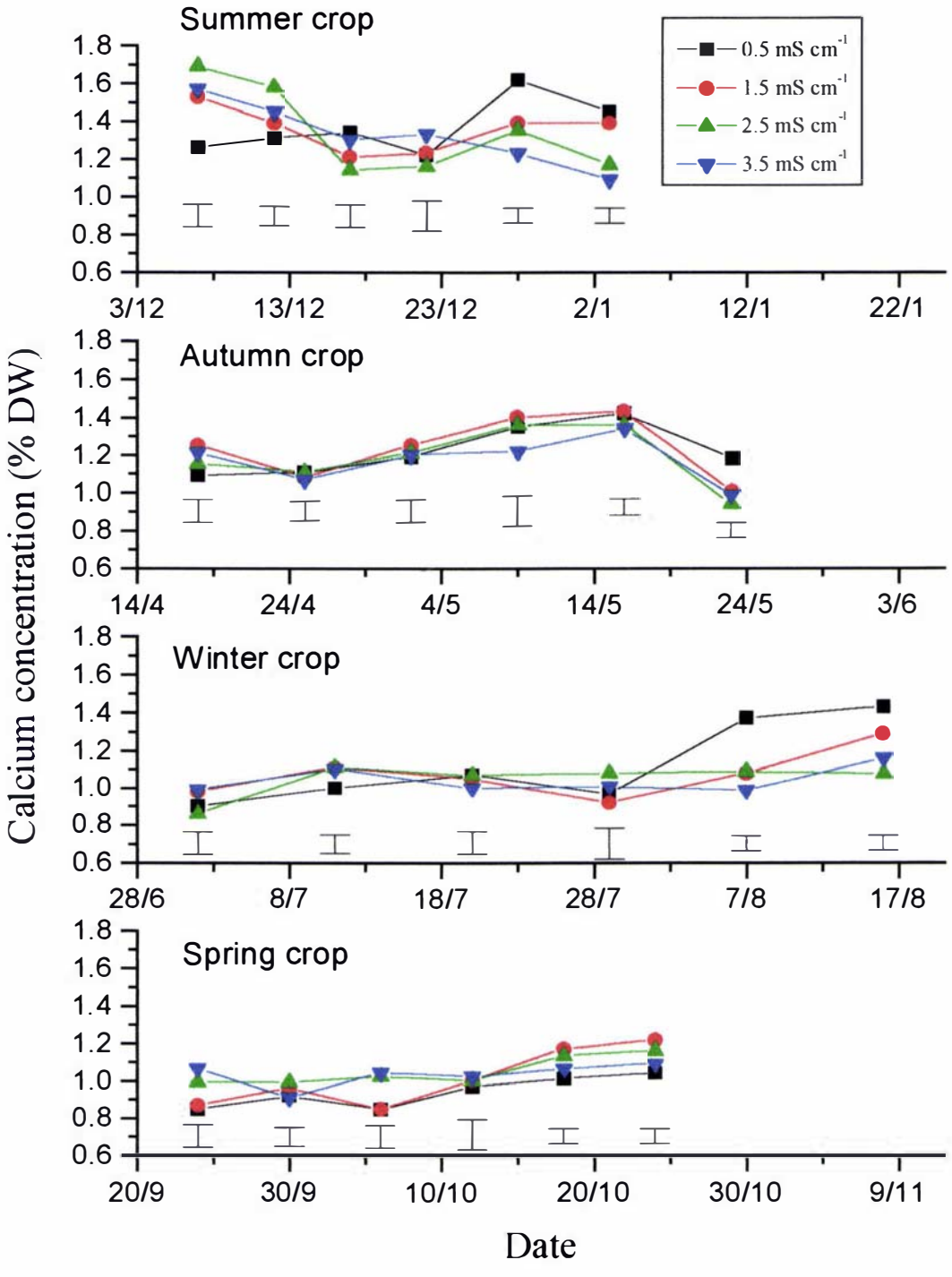


**Figure 3.19** **Calcium concentration (% dry weight) of whole lettuce plant during cropping. The bars represent the standard error of the interaction between crop and concentration at that stage.**

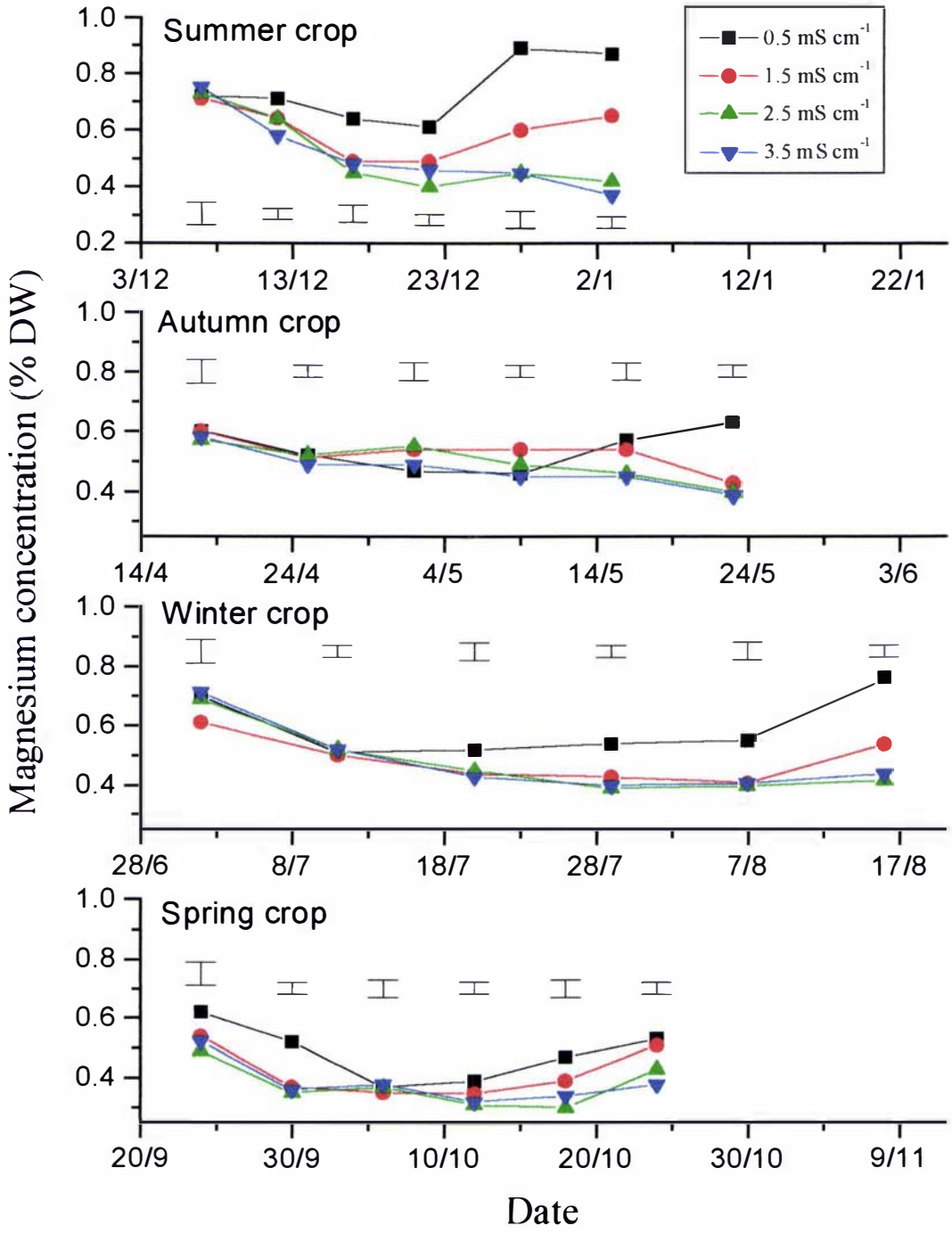


**Figure 3.20** **Magnesium concentration (% dry weight) of whole lettuce plant during cropping. The bars represent the standard error of the interaction between crop and concentration at that stage.**

### 3.3.3 Leaf and root nutrient concentrations at final harvest

#### 3.3.3.1 Results of analysis

Both root and leaf nutrient concentrations were affected by season (crop), nutrient solution concentration and cultivar. There were interactions between crop × concentration ($P \leq 0.0001$ for each nutrient for both leaf and root) and crop × cultivar ($P \leq 0.0001$ for each nutrient for both leaf and root, except for nitrogen for root where $P \leq .01$ for both root and leaf nutrient concentrations). The interactions between crop × concentration and crop × cultivar for leaf and root nutrient concentrations are presented in Figure 3.21 and Figure 3.22 respectively.

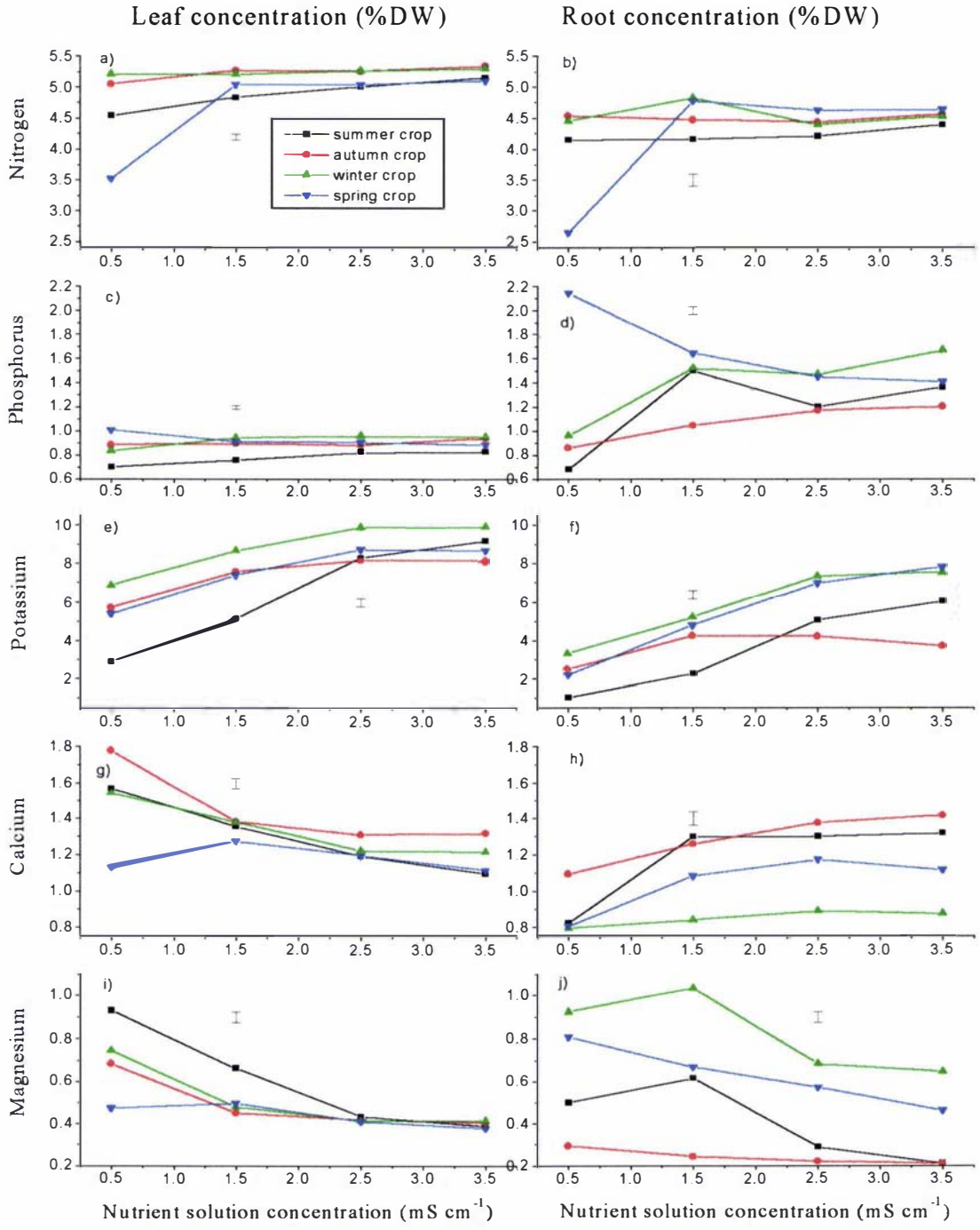


**Figure 3.21 Interaction between crop and concentration on nutrient concentration of leaf and root at final harvest of lettuce grown in different nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction between crop and concentration.**

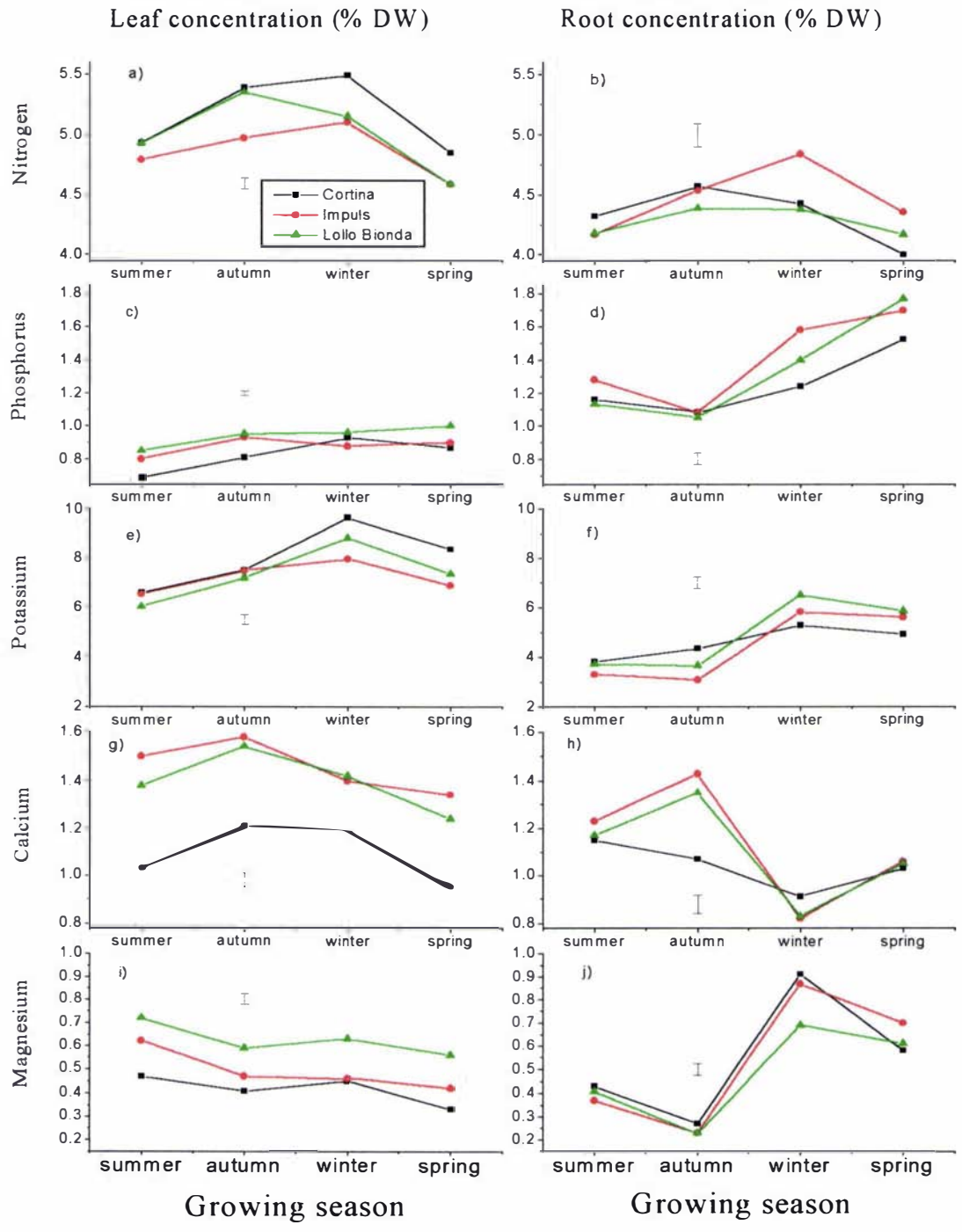


**Figure 3.22 Interaction between crop and cultivar on nutrient concentration of leaf and root at final harvest of lettuce grown in different nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction between crop and cultivar.**

### 3.3.3.2 Nitrogen

Leaf nitrogen concentrations were greater than in the root (Figure 3.21a and b). Apart from the spring crop at 0.5 mS $cm^{-1}$, the variation in nitrogen concentration of the leaves and roots across nutrient solution concentrations within seasons was not great. The nitrogen concentration in the leaves and roots of the spring crop at 0.5 mS $cm^{-1}$ was much lower than that of the other concentrations. The trend for the leaf nitrogen concentration was to increase, particularly with the summer crop, with increasing nutrient solution concentration.

In leaves, the summer and spring crops had the lower nitrogen concentrations particularly at 0.5 mS $cm^{-1}$ nutrient solution concentration. In the root, the summer crop had a lower nitrogen concentration than other crops.

For the interaction between crop and cultivar (Figure 3.22a and b) in the leaf Cortina had the highest nitrogen concentration, but not significant different from Lollo Bionda in the summer and autumn crops. Impuls had the lowest nitrogen concentration, but not different from Lollo Bionda in the winter and spring crops. In the root, Impuls had the highest nitrogen concentration in the winter and spring crops.

### 3.3.3.3 Phosphorus

Root phosphorus concentrations were greater than in the leaves (Figure 3.21c and d). Apart from the summer crop at 0.5 mS $cm^{-1}$, the variation in phosphorus concentration of the leaves across nutrient solution concentrations within seasons was not great. The trend for the leaf phosphorus concentration of the summer crop was to increase with increasing nutrient solution concentration.

The variation in phosphorus concentration of the roots across nutrient solution concentrations within seasons was greater than in the leaves. Apart from the spring crop at 0.5 mS $cm^{-1}$, root phosphorus concentrations were lowest at 0.5 mS $cm^{-1}$.

For the interaction between crop and cultivar (Figure 3.22c and d) in the leaf Lollo Bionda had the highest phosphorus concentration, but not different from Impuls in the summer and autumn crops and Cortina in winter. Cortina had the lowest phosphorus concentration in the summer and autumn crops. In the root, Impuls had the highest phosphorus concentration in the summer and winter crops. Cortina had the lowest phosphorus concentration in the winter and spring crops. In the autumn crop, there were no differences in phosphorus concentration.

### 3.3.3.4 Potassium

Leaf potassium concentrations were greater than in the root (Figure 3.21e and f). Potassium concentrations of the leaves increased with increasing nutrient solution concentration up to 2.5 mS $cm^{-1}$ and were fairly stable after that. Potassium concentration of the winter crop was higher than in the other seasons, while the summer crop at 0.5 and 1.5 mS $cm^{-1}$ was lower than the other crops.

Potassium concentration of the root increased with increasing nutrient solution concentration in the summer, winter and spring crops. In the autumn crop, potassium concentration of the root increased with nutrient solution concentration up to 1.5 mS $cm^{-1}$. At the higher nutrient solution concentrations the winter and spring crops had the higher root potassium concentrations.

For the interaction between crop and cultivar (Figure 3.22e and f) in the leaf, there was no difference in potassium concentration in the summer and autumn crops. In the winter and spring crops the potassium concentration in the leaf was highest in Cortina and lowest in Impuls. In the root, Cortina had the highest potassium concentration in the autumn crop and lowest in the winter and spring crops. Lollo Bionda had the highest potassium concentration in the winter crop and Impuls had the lowest in the summer and autumn crops.

### 3.3.3.5 Calcium

Leaf calcium concentrations were greater than the root at 0.5 mS $cm^{-1}$ and then became similar for all but the winter crop (Figure 3.21g and h). Apart from the spring crop at 0.5 mS $cm^{-1}$, the calcium concentration of the leaves decreased with increasing nutrient solution concentrations up to 1.5 mS $cm^{-1}$ and then continued to decrease at a lower rate after that. Leaf calcium concentration of the spring crop at 0.5 mS $cm^{-1}$ was lower than for the other seasons and the autumn crop had the highest leaf calcium concentration.

Generally root calcium concentration increased with increasing nutrient solution concentration up to 1.5 mS $cm^{-1}$. The exception was in winter where the calcium concentration remained at a low level. The summer and autumn crops had, apart from 0.5 mS $cm^{-1}$ in summer, the highest root calcium concentrations.

For the interaction between crop and cultivar (Figure 3.22g and h) in the leaf, Cortina had the lowest calcium concentration in every crop. Impuls had the highest calcium concentration in the summer and spring crops. In the root, Cortina had the lowest calcium concentration in the autumn crop.

### 3.3.3.6 Magnesium

Leaf and root magnesium concentrations were in the same range, but variation in magnesium concentration of the roots across seasons was greater than in the leaves. Leaf magnesium concentrations decreased from 0.5 mS $cm^{-1}$ to 1.5 mS $cm^{-1}$ with autumn and winter crops and to 2.5 mS $cm^{-1}$ in the summer crop. The exception was in the spring crop, where the magnesium remained low (Figure 3.21i and j).

Generally root magnesium concentrations decreased with increasing nutrient solution concentrations. With the autumn crop the root magnesium concentration remained low. The magnesium concentration of 1.5 mS $cm^{-1}$ in the summer and winter crops was higher than for 0.5 mS $cm^{-1}$. The winter crop had the highest magnesium concentration followed by the spring crop.

For the interaction between crop and cultivar (Figure 3.22i and j) in the leaf, Lollo Bionda had the highest magnesium concentration in every crop. Cortina had the lowest magnesium concentration apart from in winter. In the root, in the winter crop, Lollo Bionda had the lowest magnesium concentration. The winter and spring crops had the highest root magnesium concentration.

### 3.3.4 Shoot fresh weight

Season (crop), nutrient solution concentration and cultivar affected shoot fresh weight at final harvest. There were significant interactions between crop × concentration ($P \leq 0.0001$), crop × cultivar ($P \leq 0.0001$), concentration × cultivar ($P \leq 0.001$) and crop × concentration × cultivar ($P \leq 0.05$). The result of the latter interaction only is considered here.

All crops increased in fresh weight up to 1.5 mS $cm^{-1}$, apart from the cultivar Impuls in winter (Figure 3.23). This crop did not show a significant response to nutrient solution concentration. With the summer crop Cortina maintained this fresh weight through to 2.5 mS $cm^{-1}$ and then decreased, whereas the fresh weight of the other two cultivars decreased from 1.5 mS $cm^{-1}$. The fresh weight of the autumn and winter (apart from Impuls) crops levelled off at 1.5 mS $cm^{-1}$. The spring crop decreased slowly from 1.5 mS $cm^{-1}$ apart from Lollo Bionda, which levelled off.

At 1.5 mS $cm^{-1}$ the order of fresh weights was spring > winter > summer > autumn, apart from with the cultivar Impuls where summer > winter.

Impuls maintained the lowest fresh weight throughout and Cortina the highest, apart from in summer and spring, when with a number of comparisons with Lollo Bionda the fresh weights were similar.

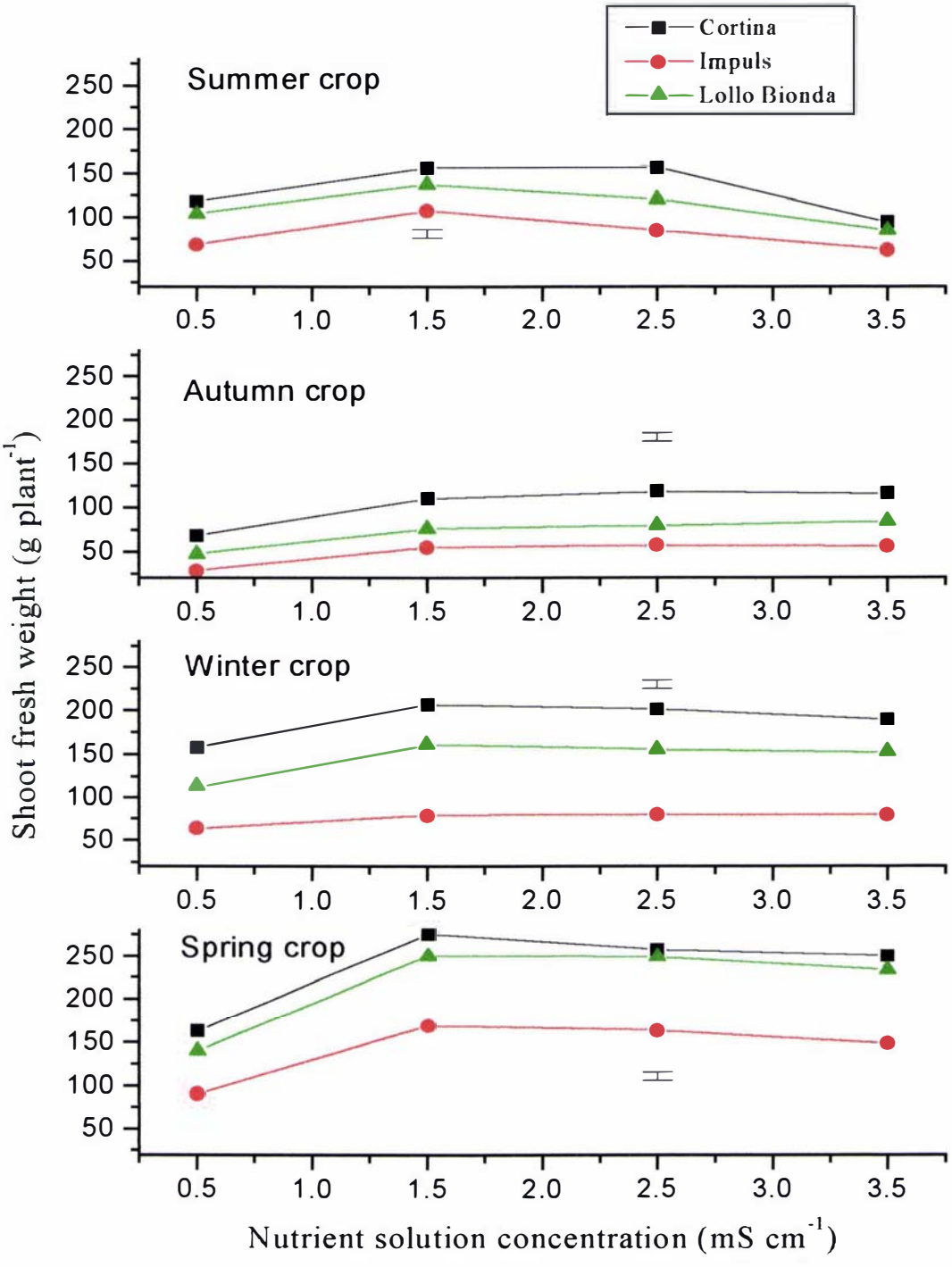


**Figure 3.23** **Shoot fresh weight (grams per plant) of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction effect.**

### 3.3.5 Shoot dry weight

Season (crop), nutrient solution concentration and cultivar affected shoot dry weight at final harvest. There were significant interactions between crop × concentration ($P \leq 0.0001$) and crop × cultivar ($P \leq 0.0001$).

For the interaction between crop and concentration, shoot dry weight increased to 1.5 mS $cm^{-1}$ and then levelled off, apart from the summer crop, which decreased from 2.5 mS $cm^{-1}$ (Figure 3.24a). For seasons, the order of dry weights was spring > summer, winter > autumn.

For the interaction between crop and cultivar, the order of shoot dry weights was spring > winter > summer > autumn, apart from the cultivar Impuls where summer > winter (Figure 3.24b). In all seasons the order of shoot dry weights for cultivars was Cortina > Lollo Bionda > Impuls.

### 3.3.6 Total plant dry weight

Season (crop), nutrient solution concentration and cultivar affected total plant dry weight at final harvest. There were significant interactions between crop × concentration ($P \leq 0.0001$) and crop × cultivar ($P \leq 0.0001$).

For the interaction between crop and concentration, total plant dry weight increased to 1.5 mS $cm^{-1}$ and then levelled off, apart from the summer crop, which decreased from 2.5 mS $cm^{-1}$ (Figure 3.25a).

For the interaction between crop and cultivar, the order of total plant dry weights was spring > winter > summer > autumn, apart from the cultivar Impuls where summer > winter (Figure 3.25b). In all seasons the order of total plant dry weights for cultivars was Cortina > Lollo Bionda > Impuls.

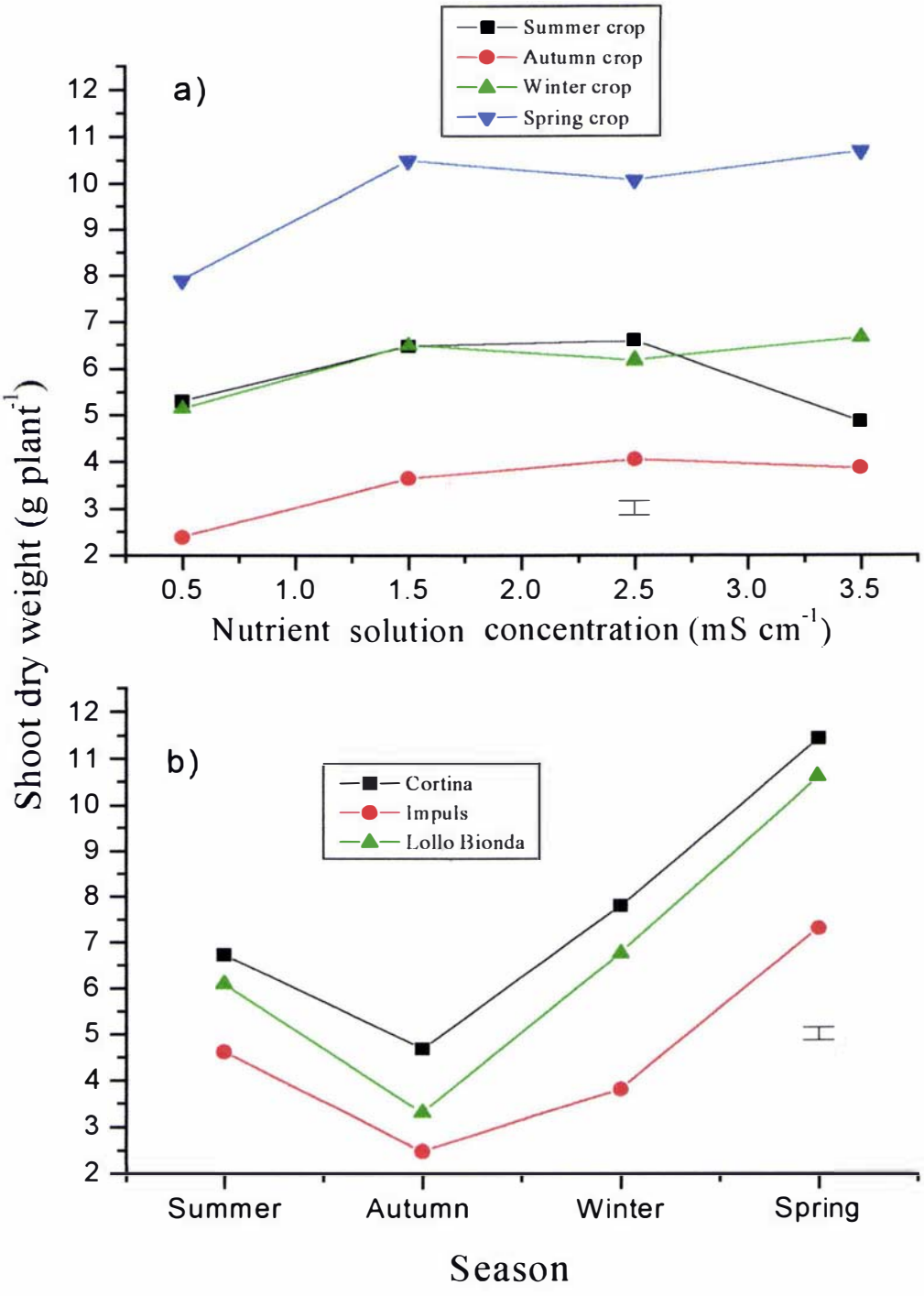


**Figure 3.24 Interaction effects between crop × concentration (a) and crop × cultivar (b) on shoot dry weight of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction effect.**

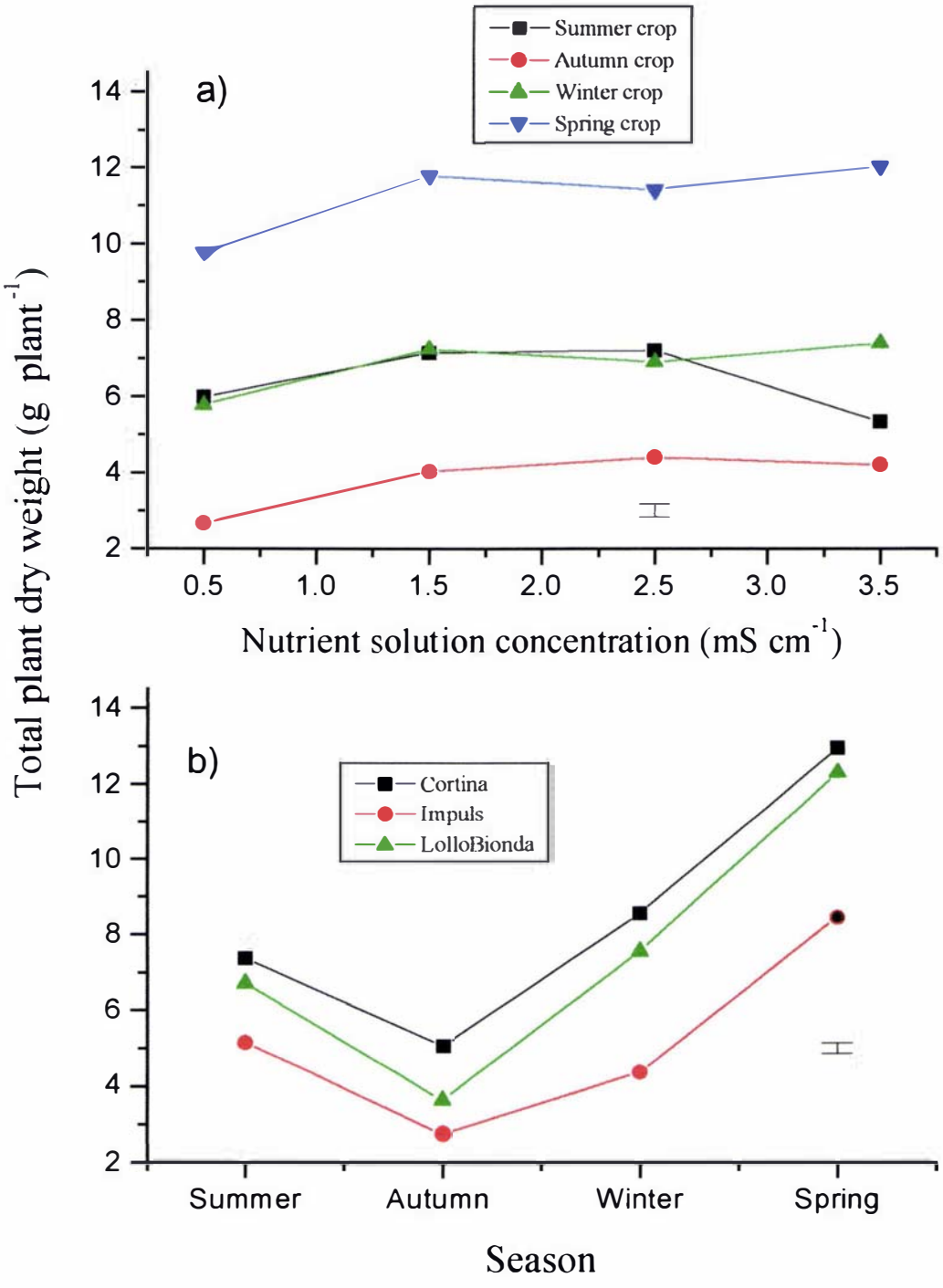


**Figure 3.25 Interaction effects between crop × concentration (a) and crop × cultivar (b) on total plant dry weight of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction effect.**

### 3.3.7 Shoot dry matter percentage

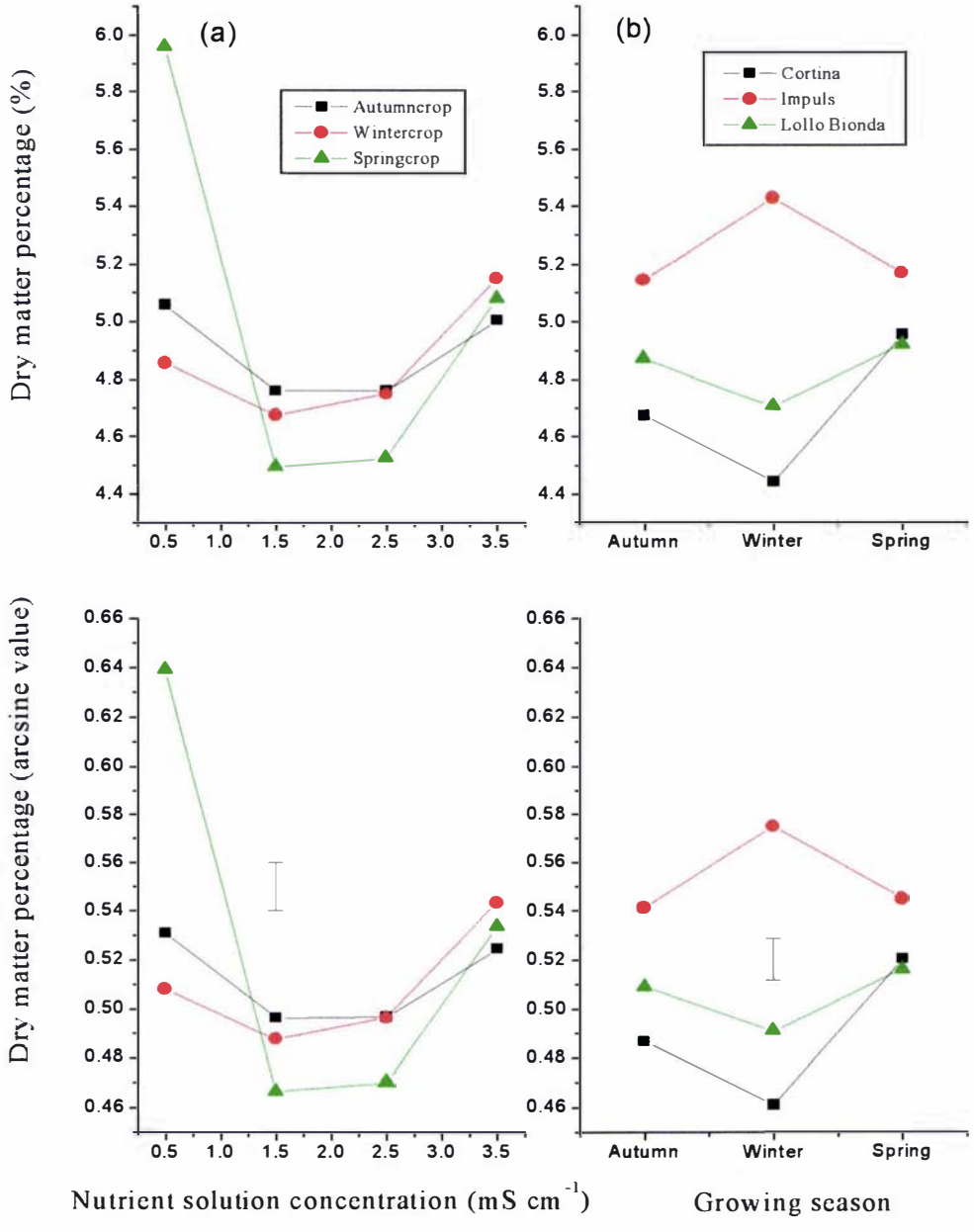


**Figure 3.26 Interaction effects between crop × concentration (a) and crop × cultivar (b) on shoot dry matter percentage (%) and its arcsine value of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 3 seasons. The bars represent the standard error of the interaction.**

Nutrient solution concentration and cultivar affected shoot dry matter percentage. There were significant interactions between crop × concentration ($P \leq 0.0001$) and crop × cultivar ($P \leq 0.0001$).

Generally shoot dry matter percentages decreased to 1.5 mS $cm^{-1}$ and remained constant to 2.5 mS $cm^{-1}$ and then increased to 3.5 mS $cm^{-1}$ (Figure 3.26a). At 1.5 and 2.5 mS $cm^{-1}$, the spring crop had the lowest dry matter percentage, but had the highest dry matter percentage at 0.5 mS $cm^{-1}$, much higher than other crops.

For the interaction between crop and cultivar, the shoot dry matter percentage of Impuls was the highest and Cortina the lowest, except for the spring crop where the shoot dry matter percentage of Cortina and Lollo Bionda were similar (Figure 3.26b).

### 3.3.8 Incidence of tipburn

The tipburn percentage data were analysed within each season due to the heterogeneity of variance between crops. There were interaction effects between concentration × cultivar for the summer ($P \leq 0.01$), winter ($P \leq 0.01$) and autumn($P \leq 0.0001$) crops.

No tipburn occurred in the autumn crop or with the cultivar Impuls (Figure 3.27and Figure 3.28 for arcsine value). Tipburn increased with increasing nutrient solution concentration, except with the cultivar Lollo Bionda in summer crop, where it decreased after 2.5 mS $cm^{-1}$ and with the spring crop where tipburn increased markedly as the concentration increased from 0.5 to 1.5 mS $cm^{-1}$. On a cultivar basis Lollo Bionda had a greater incidence of tipburn at 2.5 mS $cm^{-1}$ in summer, while Cortina had the greater incidence of tipburn in winter at 2.5 and 3.5 mS $cm^{-1}$.

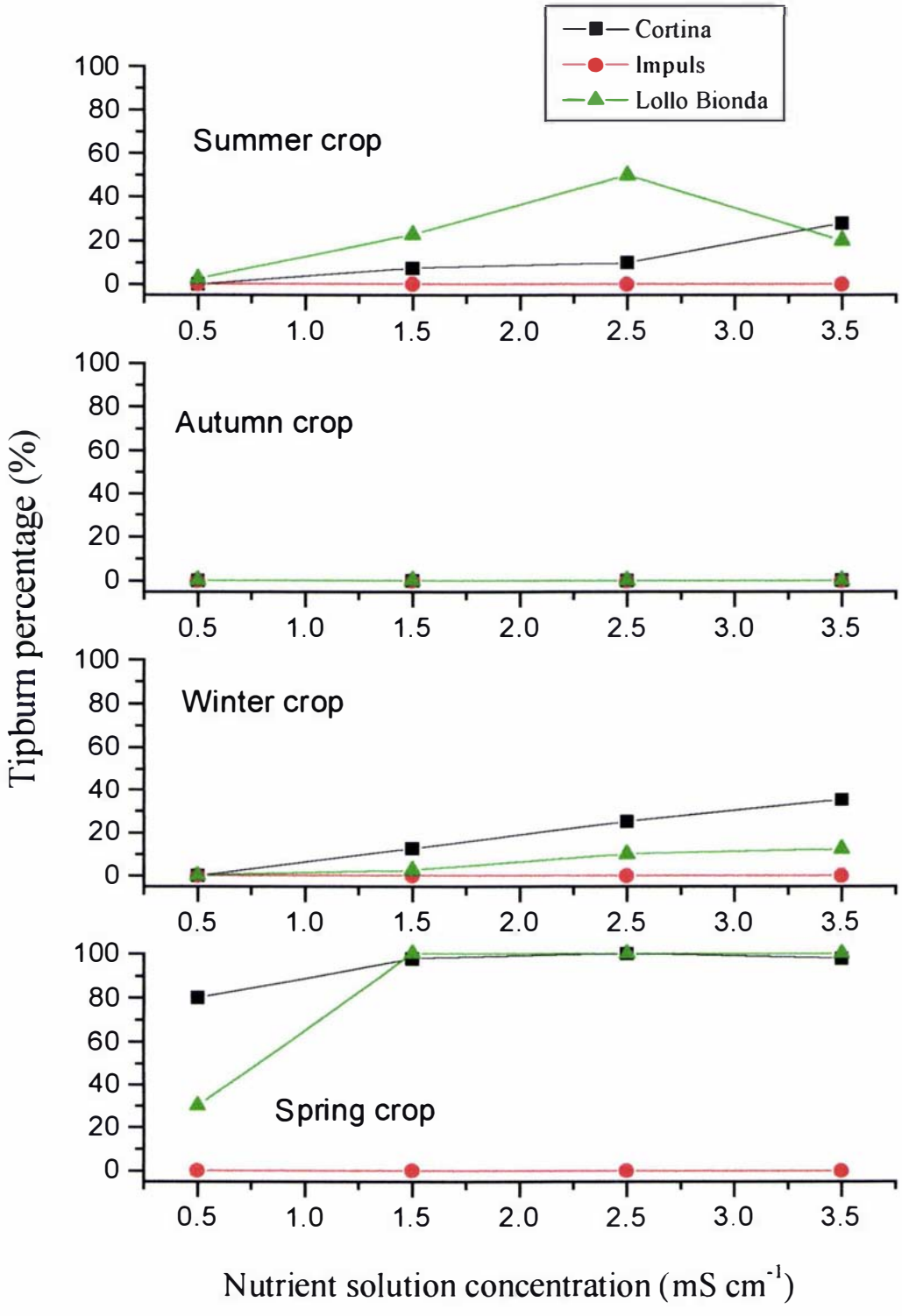


**Figure 3.27** **Tipburn percentage (%) of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons.**

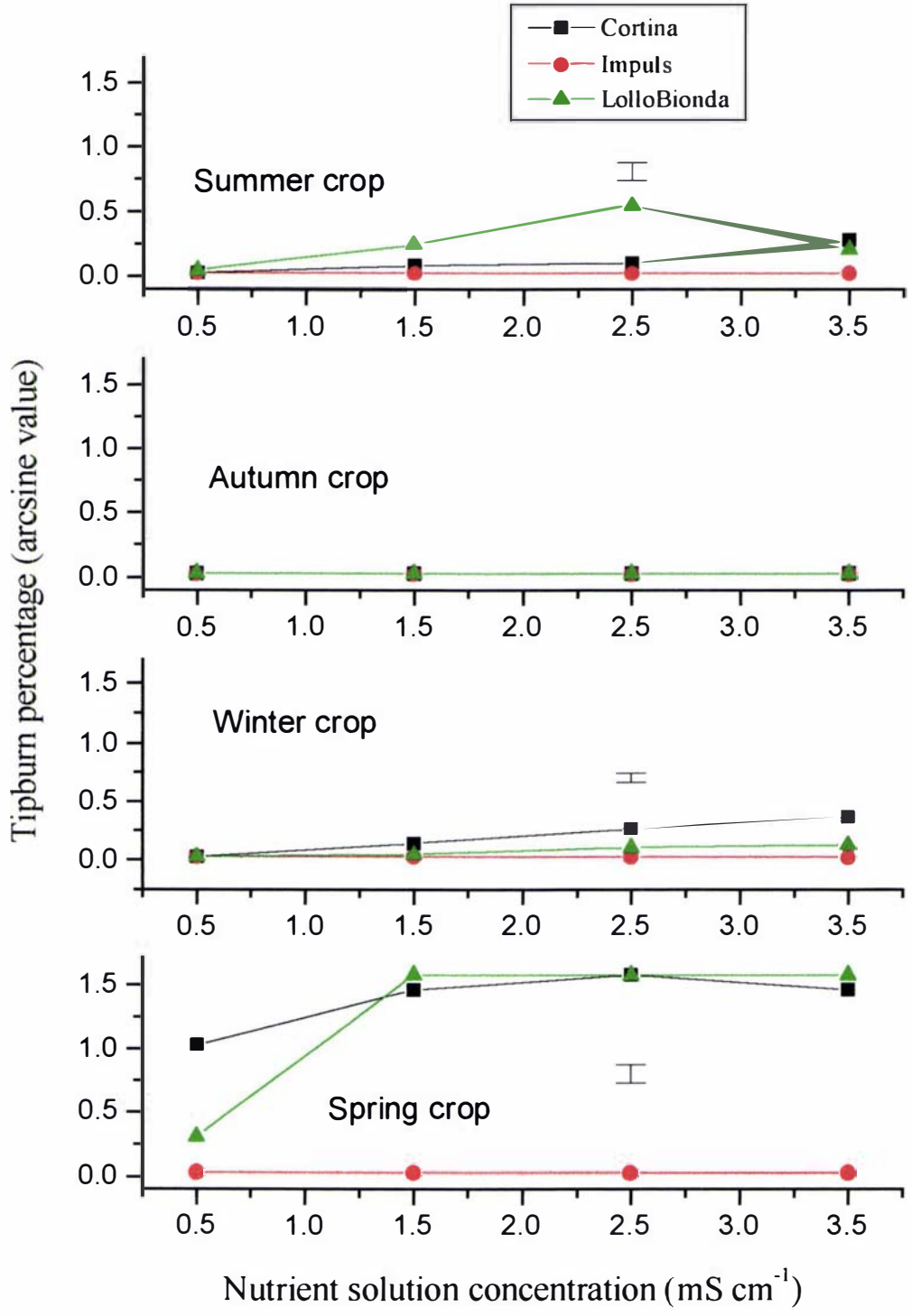


**Figure 3.28** **Arcsine value of tipburn percentage of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction.**

### 3.3.9 Shelf life

**Table 3.5 Predicted day to 10 % fresh weight loss as affected by nutrient solution concentration and cultivar within each growing season.**

| | Season[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration | Summer | | Autumn | | Winter | | Spring | |
| | (days) | | | | | | | |
| 0.5 mS $cm^{-1}$ | 2.28 | ns | 4.04 | b | 5.65 | d | 2.67 | c |
| 1.5 mS $cm^{-1}$ | 2.73 | | 5.64 | a | 6.37 | c | 4.14 | b |
| 2.5 mS $cm^{-1}$ | 2.51 | | 5.61 | a | 6.98 | b | 4.28 | b |
| 3.5 mS $cm^{-1}$ | 2.27 | | 5.73 | a | 7.55 | a | 4.58 | a |
| Cultivar | Summer | | Autumn | | Winter | | Spring | |
| | (days) | | | | | | | |
| Cortina | 3.50 | a | 7.88 | a | 9.90 | a | 5.50 | a |
| Impuls | 1.73 | c | 3.45 | c | 4.62 | c | 2.84 | c |
| Lollo Bionda | 2.12 | b | 4.44 | b | 5.39 | b | 3.40 | b |

[1] Means within the same column sharing the same letter are not significantly different by LSD at $P \leq 0.05$

The predicted days to 10 % fresh weight loss within each season was affected by nutrient solution concentration and cultivar, except for the summer crop where there were no significant concentration effects (Table 3.5).

For the autumn, winter and spring crops 0.5 mS $cm^{-1}$ had the lowest and 3.5 mS $cm^{-1}$ had the highest number of predicted days to 10 % fresh weight loss. In the winter crop the number of predicted days to 10 % fresh weight loss increased with each increase in nutrient solution concentration.

For the cultivar effect, Cortina had the highest number of predicted days to 10 % fresh weight loss followed by Lollo Bionda and Impuls respectively.

## 3.4 Discussion

### 3.4.1 Growth analysis

Nichols (1971) found that the use of an environmental time scale resulted in a better fit than the fit of the data with the normal time scale. Aikman and Scaife (1993) similarly concluded that under conditions of varying temperature, plant growth processes are frequently better described by thermal time than physical time, while Tei et al. (1996b) found that the use of day degrees instead of time improved the fit and give a better estimate of lettuce growth. In the present study the use of heat units also provided a better fit for the data than the normal time scale.

The accumulation of total plant dry matter by lettuce from the emergence until final harvest can be described by a logistic relationship (Lee, 1974; Heinen et al., 1991; Heinen & Moolenbroek, 1995; Heinen, 1997). However, Tei et al. (1996a) stated that the best fit for lettuce was obtained by the Gompertz function compared with the logistic or expolinear functions of Scaife and Jones (1976). Scaife and Jones (1976) reported that the logistic curve included a considerable apparent linear phase on either sides of its inflexion point. Lettuce, for commercial purposes, is normally harvested about the time when the inflexion point is reached, so the final linear phase is not seen. This study found that the accumulation of leaf dry matter, total plant dry matter and leaf area satisfactorily fitted a quadratic pattern when using natural log transformations of the data and heat units time scale above a base temperature of 0 $^{0}$C (3.2.4.1).

RGR, NAR and LAR in this study all decreased with growing period, except for the summer crop. Lee (1974) also found that the trend for RGR, NAR and LAR for lettuce in sand culture was to decrease during the growing period. Mutual shading of leaves and changes in the partitioning of dry matter as plants age provide the explanation of these responses. Mutual and self shading diminish the growth of lettuce, and their effect becomes evident at a later stage for the dry weight increase than for the soil cover rate

(Van Holsteijn, 1980). Watson et al. (1966) concluded that the decrease in NAR with age was mainly due to a decrease in the rate of photosynthesis per unit leaf area, due either to an increase in mutual shading reducing the efficiency of the leaves or by changes in internal factors. One of these internal factors may increase the proportion of non-photosynthetic tissue relative to the leaf area, which is indicated by a falling LAR.

Tei et al. (1996b) also commented on the importance of mutual shading of leaves during the latter stages of growth of a lettuce crop. Thus lettuce showed a very high ability in light interception and growth during the early growth stages, but throughout the growth cycle, it showed the lowest radiation use efficiency, compared to onion and red beet, due to mutual shading of the leaves within the plant canopy and to a high respiration cost in the production and maintenance of the leaves.

The initial RGR were in the order spring > winter > autumn, summer. Spring was high because of the high NAR and summer was low because of the low LAR. This order was, with the exception of the summer crop, related to effects on NAR rather than LAR. The order of initial NAR for the four seasons (summer > spring > winter > autumn) were in the approximate order of the amount of solar radiation received.

The initial RGR for the summer crop decreased as the nutrient solution concentration increased. With this crop, as the application of the nutrient solution treatments did not allow for a period of adjustment, the plants at the higher nutrient solution concentrations experienced a growth check. This check appeared to be effect NAR rather than LAR, which was low for all nutrient solution concentrations. The low LAR of the summer crop was possibly due to the heat stress after transplanting as the temperature inside the greenhouse reached 38 $^{0}$C the day after transplanting. Over the next few weeks LAR increased from this low level before following the expected decreased through to the final harvest. These changes in LAR brought about the observed responsein NAR. A decrease and then an increase.

The order of the initial RGR for the spring > winter > autumn crops was in the same order as for shoot fresh weight (3.3.4) and shoot dry weight (3.3.5), with the initial NAR being the important component of RGR. The final RGR for these three crops were in the reverse

order. The summer crop did not fit this pattern because of the early check to growth it received, which affected LAR and thus NAR. As a consequence NAR increased late and the final RGR of the summer crop was higher than the other crops, which allowed the summer crop to recover somewhat in terms of shoot dry weight.

The low initial RGR for 0.5 mS $cm^{-1}$ autumn crop was due to a low NAR as the LAR was high. For the 0.5 mS $cm^{-1}$ autumn crop leaf tissue concentration were low for all major nutrients (nitrogen, phosphorus and potassium (3.3.2)). Lee (1974) found that the applications of phosphate resulted in increases in NAR and RGR of lettuce, but these increases persisted only during the first stage of growth.

Apart from the summer crop and the 0.5 mS $cm^{-1}$ autumn crop, marked effects of nutrient solution concentration on RGR and its components were not observed. Where effects were obtained 1.5 and 2.5 mS $cm^{-1}$ nutrient solution concentrations were associated with positive responses. Thus 1.5 mS $cm^{-1}$ for the summer and spring crops was associated with a high final LAR and 2.5 mS $cm^{-1}$ for the spring crop had a high initial and low final RGR. This latter response was as for NAR. The initial RGR for the winter crop were in the order 1.5 > 2.5 > 3.5 > 0.5, which was the same order as for LAR. This reflected the situation where low radiation levels reduced the importance of NAR. Brouwer (1973) has also reported that at low light intensities the effect of LAR is most important to differences in RGR, whereas at the higher light intensities RGR increases mainly as a consequence of increasing NAR.

The relative contribution that the components of RGR make to RGR differs with the crop and the environmental conditions. Watson (1952) reviewed crop growth studies and concluded that variation in yield from the application of manure and seasonal effects were mainly due to their effects on leaf area, rather than NAR. On the other hand, the differences in RGR between fertiliser treatments were reported to be associated with differences in NAR in carrot (Austin, 1963), two populations of orchard grass (Eagles, 1967) and lettuce (Nichols, 1971). Jang et al. (1994) also concluded that the differences in RGR of lettuce by aeration rate of the nutrient solution were attributed to differences in the

net assimilation rate. Like in the present study the latter researcher concluded that with lettuce the effects on RGR are more likely to be due to NAR than LAR.

The initial RGR for the three cultivars were in the order Cortina ≥ Lollo Bionda ≥ Impuls. There was a similar order for NAR with Lollo Bionda having the highest LAR. The final RGR were in the order Lollo Bionda ≥ Impuls ≥ Cortina, which was also a similar order for NAR with Cortina having the highest final LAR. Thus, comparisons of the initial and final RGR of the three cultivars suggests that variation in RGR between cultivars appeared to be closely related to difference in NAR. LAR did not contributed regularly to the variation in RGR between cultivars. Again a high initial RGR and low final RGR was associated with a high final shoot dry weight (3.3.5) with the initial NAR being the important attribute. Cortina, a butterhead type had the higher initial NAR because of the way it presented it leaves to the available solar radiation, while the lowest NAR was obtained with the red leafed cultivar Impuls. Brouwer and Huyskes (1968) attributed the faster growth of one lettuce cultivar over another cultivar in controlled environment to a better exposition of its leaves to light as a result of a larger leaf area.

Environmental studies have shown that variation in LAR is dependent on variation in SLA rather than LWR (Blackman, 1956; Evan & Hughes, 1961). This study also found that SLA contributed more to LAR than LWR.

The LAR of 0.5 mS $cm^{-1}$ spring crop late in the season was much lower than the other concentrations. This was due to the composition of nutrient solution, as this treatment required large amounts of phosphoric acid to adjust the pH rise that occurred. The nutrient solution concentration as measured by conductivity was maintained at about 0.5 mS $cm^{-1}$ after pH adjustment. This resulted in a low level of nutrients in the nutrient solution. The results of plant nutrient analysis for nitrogen and phosphorus confirmed this assumption (3.4.2).

The most significant conclusion of the growth analysis study is that with both seasons and cultivars the order of the initial RGR was the same order as for final shoot dry weights with the initial NAR being the important component of the initial RGR.

### 3.4.2 Nutrient concentration of the whole plant during cropping

The 0.5 mS $cm^{-1}$ spring crop had lower nitrogen and higher phosphorus concentrations than other crops (3.3.2). This was due to the amount of phosphoric acid that this treatment required to adjust the pH rise that occurred. Spinu et al. (1998) stated that plant roots release an $OH^-$ ion for every nitrate ion ($NO_3^-$) that is taken up to maintain charge neutrality within the plant. This results in a continuous increase of nutrient solution pH, which must be counteracted by frequent additions of acid. Thus the amount of nutrient stock solution added to the 0.5 mS $cm^{-1}$ of the spring crop, was less than for the other crops. The 0.5 mS $cm^{-1}$ summer crop also had a low leaf nitrogen concentration. Contributing factors could have been the high final RGR of this crop and the low level of nitrogen in this nutrient solution. That the 0.5 mS $cm^{-1}$ feed was generally low in the major nutrients is apparent also with phosphorus and potassium for all four crops, with the exception of the spring crop for phosphorus.

The general trend for nitrogen concentration was to decrease with plant growth. This trend was consistent with the reports of other researchers (Zink & Yamaguchi, 1962; Knavel, 1981). Apart from the spring and summer crops at 0.5 mS $cm^{-1}$ the nitrogen concentrations were similar as that reported for the whole plant by Knavel (1981).

Phosphorus concentrations were constant over much of the growth period, which is different from soil grown lettuce where phosphorus concentration decreased with plant age (Grant Lipp & Goodall, 1958; Zink & Yamaguchi, 1962). These responses to phosphorus could most likely be related to availability of phosphorus in the soil in which the research was carried out (Costigan & Heaviside, 1988). Apart from the summer crop at 0.5 mS $cm^{-1}$ the phosphorus concentrations were within the range of that reported for the whole plant by Knavel (1981).

In general, for both nitrogen and phosphorus, the autumn and winter crops maintained higher nitrogen and phosphorus concentrations in their plant tissue than the spring and summer crops.

Potassium concentrations increased slowly with plant growth in the high nutrient solution concentrations and decreased in the lower nutrient solution concentrations, especially in the summer and spring crops. The decrease with the 1.5 mS $cm^{-1}$ nutrient solution was much less than for 0.5 mS $cm^{-1}$. Compared to nitrogen and phosphorus there was more variation in potassium levels, within a season, in the latter growth period. Zink and Yamaguchi (1962) reported that the potassium content fluctuated throughout the growth of the crop, and no general trend was observed. Apart from with 2.5 and 3.5 mS $cm^{-1}$, the plant potassium concentrations were below the range reported by Knavel (1981). This might suggest that in the latter part of the season the 1.5 mS $cm^{-1}$ nutrient solution may have been on the low side for potassium.

The calcium and magnesium concentrations late in the season showed a response that was the reverse of potassium, with magnesium appearing to be more affected than calcium. Thus the 0.5 and 1.5 mS $cm^{-1}$ solution concentrations had the highest plant calcium and magnesium concentrations. It is the general rule that increasing the supply of one cation species results in lowering the concentration of other cation species. This relationship is called 'cation antagonism' (Mengel & Kirkby, 1987). Uptake rate depends on the concentration of the individual cation species in the nutrient solution and also on the uptake mechanism. Potassium which is taken up by the cell rapidly, either actively or by facilitated diffusion, competes strongly in cation uptake (Mengel & Kirkby, 1987).

Furthermore, the absolute concentration of calcium in the soil solution is less important in controlling calcium uptake than is the relationship of calcium to the total salt concentration and its proportionate concentration to that of other ions in solution (Shear, 1975b). Potassium (Smith & Wallace, 1956; Geraldson, 1971) and magnesium (Mason, 1964; Geraldson, 1971; Willumsen, 1984) are antagonistic to calcium and retard its uptake. The lower levels of potassium in the 0.5 and 1.5 mS $cm^{-1}$ solution concentrations would account for the increases in calcium and magnesium concentrations in the plant.

Calcium levels were below, while magnesium levels covered a wider range than those reported for whole plants by Knavel (1981).

The autumn crop showed less variation in nutrient concentration with nutrient solution concentration for all the nutrients examined. This may be because this crop grew into poorer weather and experienced less stressful growing conditions. There were no marked variations in nutrient levels, apart from that due to nutrient solution concentration effects, during the growing season for any of the nutrient studies. This observation is supported by the data (redrawn) from Heinen et al. (1991) for nitrogen, phosphorus, potassium, calcium and magnesium, for potassium by Burns and Hutsby (1986) and for nitrogen, potassium and calcium by Zink and Yamaguchi (1962).

### 3.4.3 Leaf and root nutrient concentrations at final harvest

#### 3.4.3.1 Nitrogen

Leaf nitrogen concentrations were greater than in the root (3.3.3.2). This was consistent with the work of Zink and Yamaguchi (1962).

The nitrogen concentration in the leaves and roots of the spring crop at 0.5 mS $cm^{-1}$ was much lower than those of the other concentrations. This response was discussed previously (3.4.2). The spring crop at 0.5 mS $cm^{-1}$ aside, the variation in nitrogen concentration of the leaves and roots across nutrient solution concentrations was not great. Apart from this treatment the nitrogen concentration were similar to those reported for leaves of healthy plants by other researchers (Haworth & Cleaver, 1967; Knavel, 1974; Sanchez et al., 1988; Wheeler et al., 1994; Drews et al., 1997).

The rate of nitrogen uptake and the nitrogen content of the leaves are influenced by temperature (Winsor & Adam, 1987). Generally higher levels of nitrogen were found in plants grown in cooler soil than in those grown in warmer (Knavel, 1974). This study obtained similar response as the leaf nitrogen concentration of the summer and spring crops were lower than for the autumn and winter crops. A similar conclusion was reached for whole plant nitrogen and phosphorus concentrations during the season (3.4.2).

In the leaf, Cortina had the highest nitrogen concentration, but not significant different from Lollo Bionda in the summer and autumn crops. Impuls had the lowest nitrogen concentration but not different from Lollo Bionda in the winter and spring crops. In the root, Impuls had the highest nitrogen concentration in winter and spring crops. Wurr and Fellows (1984) have suggested that the different responses of two lettuce cultivars to temperature was because the two varieties were bred in different places for different conditions, while Schwarz and Kuchenbuch (1997) have stated that differences between cultivars are often connected to environmental factors such as climatic conditions or nutrient supplied. The distinctly different characteristics of the three cultivars grown in the present study could also have affected nutrient uptake. Thus Cortina was a butterhead type with significant shading of the inner leaves in the latter part of cropping, while Lollo Bionda was a green leafed type and Impuls a red leafed type.

### 3.4.3.2 Phosphorus

Root phosphorus concentrations were greater than in the leaves (3.3.3.3). This was consistent with the results of Zink and Yamaguchi (1962) and Temple-Smith and Menary (1977).

The phosphorus concentration in the leaves and roots of spring crop at 0.5 mS $cm^{-1}$ was higher than of the other seasons. This was due to the amount of phosphoric acid that this treatment required to adjust the pH rise that occurred. The response of the root was more marked than the shoots and it would appear therefore that it was in this tissue that most of the excess phosphorus remained. Apart from this treatment, the phosphorus concentrations were similar to those reported for leaves of healthy plants by other researchers (Haworth & Cleaver, 1967; Knavel, 1974; Berry et al., 1981; Costigan, 1984; Sanchez et al., 1988; Wheeler et al., 1994; Drews et al., 1997) and above the critical level of 0.43 % as reported by Sanchez et al. (1990).

Apart from spring crop at 0.5 mS $cm^{-1}$, the variation in phosphorus concentration of the leaves across nutrient solution concentrations within seasons was not great.

With soil grown crops, the phosphorus concentration of the leaves increased with the level of phosphorus applied (Grant Lipp & Goodall, 1958; Greenwood et al., 1980b; Adams & Winsor, 1984). In the present study this only occurred with the winter and summer crops.

In the leaf, Lollo Bionda had the highest phosphorus concentration, but not always different from the other cultivars. In the root, Impuls had the highest phosphorus concentration in the summer and winter crops. Although differences occurred between cultivars in the phosphorus concentration of shoot and root tissues no clear patterns or trends were apparent.

### 3.4.3.3 Potassium

Leaf potassium concentrations were greater than in the root (3.3.3.4). This was consistent with the report of Zink and Yamaguchi (1962).

Potassium concentrations of the leaves increased with nutrient solution concentration up to 2.5 mS $cm^{-1}$, apart from the summer crop, where the leaf potassium concentration increased to 3.5 mS $cm^{-1}$. Greenwood et al. (1980c) reported that the uptake of potassium by lettuce crops increases in a 'diminishing returns' manner with increasing levels of potassium fertilizer and the percentage potassium at harvest was a good indicator of the extent to which crop growth was restricted by lack of potassium. Apart from in autumn, the potassium concentration of the root tissue also increased with increasing nutrient solution concentration.

Apart from 0.5 mS $cm^{-1}$ and the summer crop at 1.5 mS $cm^{-1}$, the potassium concentration were similar to those reported for leaves of healthy plants by other researchers (Haworth & Cleaver, 1967; Knavel, 1974; Costigan, 1984; Sanchez et al., 1988; Bres & Weston, 1992; Drews et al., 1997) and above the critical level of 5.60 % as reported by Sanchez et al. (1990).

As shoot fresh weight (3.3.4) and dry weight (3.3.5) increases did not occur above 1.5 mS $cm^{-1}$, then this suggests that above 1.5 mS $cm^{-1}$ luxury consumption of potassium took place in both shoot and root tissues and that the potassium nutrition of the 1.5 mS $cm^{-1}$ nutrient solution concentration was in fact adequate. If the 1.5 mS $cm^{-1}$ nutrient concentration provided sufficient or approaching sufficiency of potassium for growth, then the fall in potassium concentration of the whole plant late in the season as outlined in Figure 3.18, may represent a decline over time from what were luxury levels of potassium, rather than a lack of potassium. The winter crop had a high potassium concentration and the summer crop a low concentration in both shoot and root tissues.

In the winter and spring crops the potassium concentration in the leaf was highest in Cortina and lowest in Impuls. Thus with Cortina in the winter and spring crops the potassium and nitrogen uptakes were high. Furthermore the relative position of the cultivars, in terms of potassium uptake, varied depending on season, and as with nitrogen and phosphorus, no pattern was apparent.

### 3.4.3.4 Calcium

Leaf calcium concentrations were greater than the root at 0.5 mS $cm^{-1}$ and then became similar (3.3.3.5). Zink and Yamaguchi (1962) reported that leaf calcium concentrations were greater than the root.

Apart from spring crop at 0.5 mS $cm^{-1}$, the calcium concentration of the leaves decreased with increasing nutrient solution concentrations up to 1.5 mS $cm^{-1}$ and then continued to decrease at a lower rate after that. The effect of high nutrient solution concentration in decreasing calcium uptake has been reported in lettuce (Cresswell, 1991; Huett, 1994), tomato (Ho & Adams, 1989b) and sweet pepper (Tadesse, 1997). This would at least in part be a response to high potassium as discussed in 3.4.2. On the other hand the calcium concentration in the root increased with increasing nutrient solution concentration.

The calcium concentration were similar to those reported for leaves of healthy plants by other researchers (Zink & Yamaguchi, 1962; Haworth & Cleaver, 1967; Knavel, 1974; Costigan, 1984; Bres & Weston, 1992; Drews et al., 1997).

The leaf calcium concentration of the spring crop at 0.5 mS $cm^{-1}$ was lower than the other nutrient solution concentrations and seasons. This was possibly due to the unbalanced nutrient concentration in the nutrient solution due to the amount of phosphoric acid that this treatment required to adjust the pH rise that occurred. The autumn crop, which suffered least from tipburn (Figure 3.27), had the highest calcium concentration in both the shoot and root.

In the leaf, Cortina had the lowest calcium concentration in every crop. This may be because as a heading type root pressure at night was the main calcium uptake mechanism for non-transpiring leaves of lettuce (Thibodeau & Minotti, 1969; Collier & Wurr, 1981; Cresswell, 1991; Wein, 1997). These leaves would have therefore suffered more in comparison to the inner leaves of non heading lettuce in term of calcium uptake. Impuls had the highest calcium concentration in the summer and spring crops. In the root Cortina had the lowest calcium concentration in the autumn crop. This response was reverse of that with the potassium concentration (3.3.3.4).

### 3.4.3.5 Magnesium

Leaf and root magnesium concentrations were in the same range, but the variation in magnesium concentration of the roots across seasons was greater than in the leaves (3.3.3.6). Zink and Yamaguchi (1962) reported that leaf magnesium concentrations were greater than the root.

Apart from the spring crop, where the magnesium concentration was low, the leaf magnesium concentration decreased with increasing nutrient solution concentration up to 1.5 or 2.5 mS $cm^{-1}$, depending on season. This levelling off coincided with the levelling off of the increase in potassium concentration. The mechanism involved would be as described for calcium in 3.4.3.4. Haworth and Cleaver (1967) reported that any treatments that

increased the potassium content of the plants tended to reduce the magnesium content and vice versa. At the higher nutrient solution concentrations, leaf magnesium levels were in the range reported by other workers (Costigan, 1984; Bres & Weston, 1992). Root magnesium concentration decreased with increasing nutrient solution concentration. It was the only nutrient to do this in the root.

There was evidence of cation antagonism between calcium and magnesium as calcium concentration were high in autumn for both the leaves and roots, while magnesium concentration were low over the same period.

In the leaf, Lollo Bionda had the highest magnesium concentration in every crop, while Cortina had the lowest magnesium concentration. In the root, in the winter crop, Lollo Bionda had the lowest magnesium concentration. This response was the reverse of that with potassium concentration (3.3.3.4). This result is further evidence of cation antagonism between potassium and both calcium and magnesium.

### 3.4.3.6 Summary on nutrient concentrations

Leaf nitrogen and potassium concentrations were greater than in the root, the reverse was true of phosphorus, while calcium and magnesium levels did not differ greatly. The concentration of nutrients in the leaves were, apart from a few exception, all within the range reported by other researchers.

Generally there were not large variations in nitrogen, which decreased over time (Figure 3.16), and phosphorus concentrations across nutrient solution concentrations. At 0.5 mS $cm^{-1}$ the nutrient solution did not appear to meet the nitrogen requirements of the spring and summer crops (Figure 3.16 and Figure 3.21 a) and phosphorus requirement of the autumn, winter and summer crops (Figure 3.17). The phosphoric acid required to adjust the pH of the spring crop modified the response of this crop to phosphorus.

Leaf potassium concentration increased with increasing nutrient solution concentration up to 2.5 mS $cm^{-1}$ (Figure 3.21e). It is suggested that the initial increase increased yield, but at the higher nutrient solution concentrations, luxury consumption probably operated. In designing the nutrient solution used in this study, a high potassium feed was avoided to maintain higher levels of other cations in the plant tissue. As the yield response was only at the lower nutrient solution concentrations (3.4.4), this suggests that the potassium level selected was probably a good compromise. As nitrogen and phosphorus concentrations, did in some seasons, increase from 0.5 to 1.5 mS $cm^{-1}$ nutrient solution concentration, as did potassium, the weight of evidence is that at 0.5 mS $cm^{-1}$ nutrient solution concentration yield was limited by nitrogen, phosphorus and potassium. Particularly with nitrogen and phosphorus, this response was influence by season. The concentration of these two nutrients were higher in the plant for both the autumn and winter crops. Leaf nitrogen, phosphorus and potassium concentrations were related to yield because of their lower concentrations at 0.5 mS $cm^{-1}$, but as these concentrations were often within range reported for 'normal' crops the usefulness of this information would be limited.

Potassium was the dominant cation in this study. As it increased in concentration with increasing nutrient solution concentration, this increase mediated changes in calcium and magnesium levels. Thus calcium and magnesium levels decreased with increasing nutrient solution concentration and it is suggested that this related to the ability of potassium ions to be taken up faster than calcium and magnesium ions so that as the potassium concentration increased with increasing nutrient solution concentration, calcium and magnesium concentrations decreased in the plant tissue.

The spring 0.5 mS $cm^{-1}$ crop behaved differently from the other seasons and it was suggested that some of these differences were related to pH changes in the nutrient solution. Root nitrogen concentrations did not vary with nutrient solution concentration, while the other ions, apart from magnesium, increased to varying extents with increases in nutrient solution concentration. Root magnesium concentration decreased with increasing nutrient solution concentration.

### 3.4.4 Shoot fresh weight

The ranking in shoot fresh weight at final harvest for the different seasons, apart from cultivar Impuls in winter, was spring > winter > summer > autumn (3.3.4). Care needs to taken when interpreting these rankings as the final harvests did not necessarily coincide with maximum horticultural yield. The final harvest was determined by the needs of growth analysis rather than the need to coincide with horticultural maturity. During the 3 weeks preceding the final harvest of lettuce, more than 70 % of fresh weight (Kratky & Mishima, 1981) and dry weight (Gallardo et al., 1996) are accumulated so that small differences in harvest time could account for significant differences in fresh weight. The lower ranking of the summer crop could also relate to the check this crop received at establishment as shown by its low initial RGR (3.3.1).

As the nutrient solution concentration increased from 0.5 to 1.5 mS $cm^{-1}$ shoot fresh weight increased and then levelled off for the autumn and winter crops, but where the plants were stressed, such as in spring and summer, presumably due to high temperatures, then shoot fresh weight was reduced at the higher nutrient solution concentrations. Thus shoot fresh weight decreased slowly from 1.5 mS $cm^{-1}$ in spring, apart from with Lollo Bionda, while in summer shoot fresh weight decreased through to 3.5 mS $cm^{-1}$ with Lollo Bionda and Impuls, while with Cortina it decreased from 2.5 mS $cm^{-1}$.

The increase in shoot fresh weight above 0.5 mS $cm^{-1}$ could be explained in terms of a response to nutrients. The data on plant nutrient concentrations would support this, as in most instances over the four seasons, 0.5 mS $cm^{-1}$ maintained a lower concentration of nitrogen, phosphorus and potassium throughout the season (3.3.2).

Shoot fresh weights over the nutrient solution concentration range 1.5 – 3.5 mS $cm^{-1}$ in the autumn and winter crops were not different. This result was consistent with the results from winter crops of Morgan et al. (1980b) and Economakis (1990). Thus Morgan et al. (1980 b) reported that lettuce cultivar 'Ravel' could be grown satisfactory with nutrient solution concentrations up to 5.5 mS $cm^{-1}$ in an expanded clay substrate system during January and April in Ireland, with an optimum of about 2 mS $cm^{-1}$. Economakis (1990)

suggested that butterhead and cos lettuce cultivars, 'Bellona' and 'Paris cos island', could be grown in solution concentrations between 1.5 and 5.0 mS $cm^{-1}$ during Nov-Dec and April-May in Greece, but nutrient solution concentrations within the range 2.0-3.0 mS $cm^{-1}$ gave more satisfactory results.

The decrease in shoot fresh weight at the higher nutrient solution concentrations in summer and spring it is proposed was due to stress. Thus Imas & Feigin (1995) found that the reduction in dry matter production of sweet corn due to salt stress was steeper in spring than in autumn. The summer crop was the most stressed and the reduction in initial RGR that this stress produced at the higher nutrient solution concentrations can be seen in Figure 3.29. Figure 3.29 also details that there was a relationship between initial RGR and fresh weight.

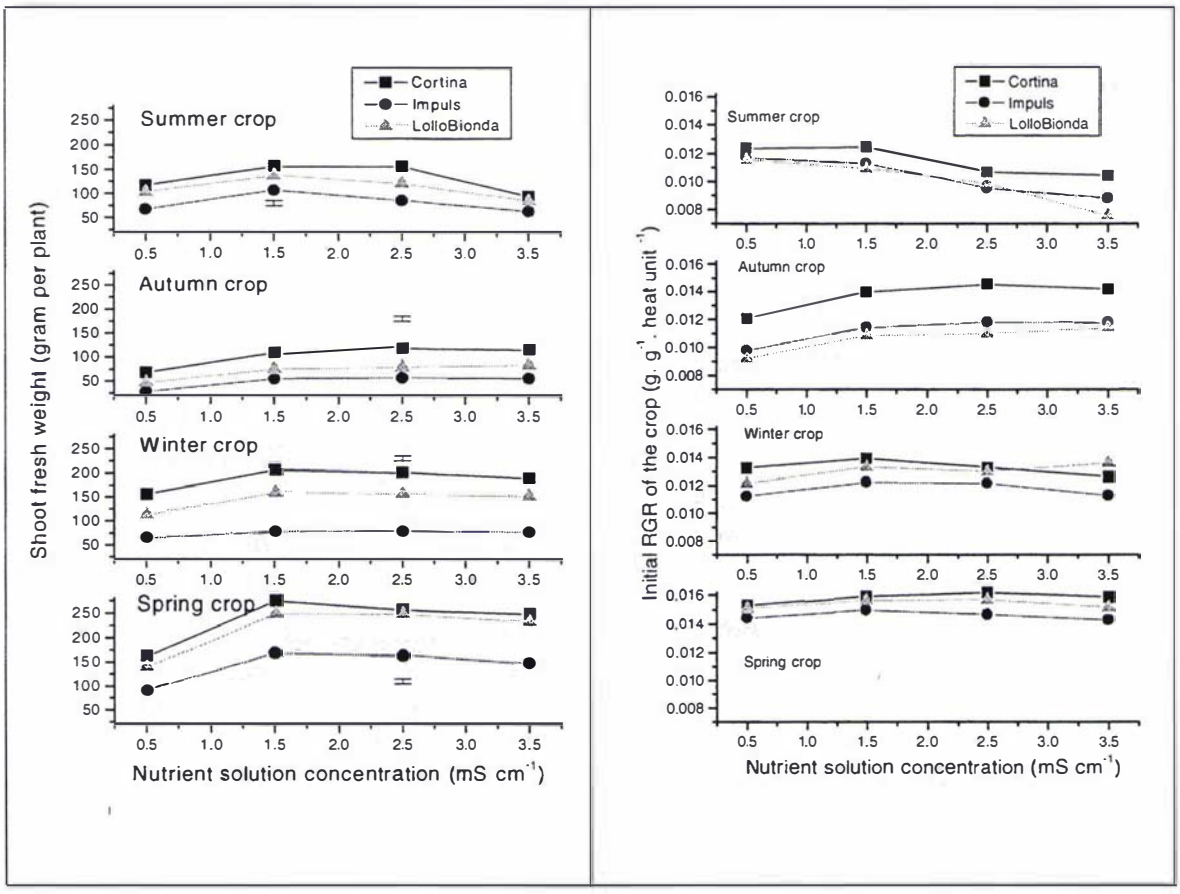


**Figure 3.29 Comparison of shoot fresh weight (g) at final harvest and initial RGR (g. $g^{-1}$ heat unit) of 3 lettuce cultivars grown in 4 nutrient solution concentrations over 4 seasons.**

It is often observed that yield is reduced uniformly with decreasing osmotic potential of the nutrient solution (Terry et al., 1983; Dalton & Poss, 1990; Alarcon et al., 1994). The growth reduction from salt stress is due to an effect on the growing tissue rather than an effect on photosynthesis (Terry et al., 1983; Longnecker, 1994). Al-Harbi (1994) reported that the effect of high salinity was greater during the day than during the night, which indicates that salinity acts through its effect on water uptake by the plant. Decreases in leaf elongation are due to direct effects on cell expansion rather than to a reduced supply in carbohydrates (Longnecker, 1994).

The growth inhibition that occurrs under salt stress conditions is caused first by a decrease of the growth-induced water potential (Nonami et al., 1995), which exists in actively growing tissue to sustain cell enlargement (Nonami & Boyer, 1993). The volume expansion during growth requires continuous water uptake from the water source and a distinctive water potential gradient exists between enlarging cells and the xylem (Nonami & Boyer, 1993). At low water potential, the growth- induced water potential is decreased, resulting in a cessation of water flow from the xylem to the enlarging cells (Nonami & Boyer, 1989). Volkmar et al. (1998) suggested that the slower growth of salt stressed plants over long periods may be attributed to something other than reduced cell turgor. Munns and Termaat (1986) suggested that saline salts induce the roots to send a growth regulator-like chemical signal to the shoot that leads to shoot growth inhibition.

Across the four seasons the order of fresh weight for the three cultivars were Cortina > Lollo Bionda > Impuls. Differences between cultivars are often related to environmental factors such as climatic conditions or nutrient supplied (Schwarz & Kuchenbuch, 1997). In this study it is considered however it was the growth characteristics of the cultivars which affected fresh weight more than the role of environmental factors. Thus Cortina presented leaves in a flat rosette pattern early on to effectively intercept radiation and so achieve a high initial RGR. The red pigmented Impuls maintained a low LAR throughout much of the cropping period (Figure 3.9) and this together with its low initial NAR (Figure 3.6) would account for its low final fresh weight. In winter it showed little response to increases in nutrient solution concentration (Figure 3.23).

One must conclude that 1.5 mS $cm^{-1}$ was the most satisfactory nutrient solution concentration to use across all seasons. Higher concentrations will reduce yields when an environmental stress such as high temperatures occur and also high concentrations increased the risk of tipburn (3.3.8). Huett (1994) also concluded that the highest fresh weight of head and/or leaf of mature heading and non-heading lettuce was consistently produced at an EC of 1.6 dS $m^{-1}$ in experiments in Australia. He also suggested that the maintenance of the nutrient solution concentration at about 1.6 dS $m^{-1}$ will minimise changes in nutrient composition over time, and that the timing of daily adjustments to EC is not critical because nutrient concentrations are high, nutrient uptake during the day and night is similar and that the small changes over a few hours will not affect lettuce growth.

### 3.4.5 Shoot dry weight

The ranking for shoot dry weight was spring > summer, winter > autumn (3.3.5). These rankings for shoot dry weight for season across all nutrient solution concentrations was similar to that for shoot fresh weight, except that for shoot dry weight a difference between the summer and winter crops only occurred at 3.5 mS $cm^{-1}$, where summer had the lower shoot dry weight. On a similar basis the rankings for seasons were as for shoot fresh weight.

As with fresh weight, all shoot dry weights increased to 1.5 mS $cm^{-1}$, but with dry weights they then levelled off with the only decrease occurring with the summer crop at 3.5 mS $cm^{-1}$. Thus fresh weight was far more susceptible to temperature stress, which was proposed as the reason for decreasing shoot fresh weights at the higher nutrient solution concentrations with the spring and summer crops. Two of the seasons that showed no changes in shoot dry weight after 1.5 mS $cm^{-1}$, autumn and winter, also showed less variation in shoot dry matter percentage across the nutrient solution concentrations (3.3.7). Gallardo et al. (1996), working with lettuce, have also reported that shoot fresh weight was more markedly affected than shoot dry weight by a reduction in water supply. Schmidhalter and Oertli, (1990), have also reported the same response in carrot by decreasing matrix potentials which reduced water potential.

The ranking of cultivars was as for shoot fresh weight except that in all instances Cortina had a greater shoot dry weight than Lollo Bionda.

### 3.4.6 Total plant dry weight

The effect of nutrient solution concentration on total plant dry weight (3.3.6) could be explained as for shoot dry weight (3.4.5).

### 3.4.7 Shoot dry matter percentage

In all cases shoot dry matter percentage decreased to 1.5 mS $cm^{-1}$ and then had increased by 3.5 mS $cm^{-1}$ (3.3.7). It appeared, therefore, that from 0.5 to 1.5 mS $cm^{-1}$ as shoot fresh weight increased (3.4.4) so shoot dry matter percentage decreased, while at 3.5 mS $cm^{-1}$ water uptake was constrained and so was fresh weight, while dry weight was not reduced and thus the dry matter percentage increased. The effect of high nutrient solution concentration in increasing dry matter percentage of lettuce crops is reported in the literature (Burrage & Varley, 1980; Economakis 1990). Soil salinity also increased dry matter percentage of lettuce cultivars 'Bix' and 'Ariane R2' (De Pascale & Barbieri, 1995). Mengel (1979) has suggested that the plants growing in arid areas need to build up a higher concentration of osmotically active solutes in their vacuoles in order to achieve a low osmotic potential which retains the water in their tissues.

The shoot dry matter percentage was in the range 4.5 – 6.5% which is similar to that reported in the literature. Thus the average dry matter percentage for field lettuce were 6.56 – 7.00% (Lairon et al., 1984), in NFT lettuce were 2.7 – 5.7% (Burrage & Varley, 1980), 3.9 % (Heinen et al., 1991), and 5.5 % (Heinen, 1994) and 3.5 % in lettuce grown in sand beds (Heinen & Moolenbroek, 1995).

### 3.4.8 Incidence of tipburn

The highest incidence of tipburn occurred in the spring where the weather was improving and where there were some days when lack of cloud provided for periods of significantly increased temperature and solar radiation levels (3.3.8). Conversely there was no tipburn in autumn, which was the season where the weather was deteriorating. Although temperature was highest over summer, the weather was more uniform over this period.

In the seasons where tipburn occurred, the level of tipburn apart from with Lollo Bionda at 3.5 mS $cm^{-1}$ in summer, increased with increasing nutrient solution concentration. In spring, the level of tipburn incidence did not increase above 1.5 mS $cm^{-1}$ as at this concentration 100% incidence had already occurred.

The two green leafed cultivars, which had the higher initial RGR (Table 3.3), suffered from tipburn, while the red leafed cultivar Impuls did not. It is surprising that in summer Lollo Bionda had a higher incidence of tipburn than the butterhead cultivar Cortina.

Tipburn development appears to depend on the supply of calcium relative to the rate of leaf growth (Collier & Huntington, 1983; Barta & Tibbitts, 1991). Tipburn incidence is favoured by high growth rates and the inability of the plant to match this growth with an adequate supply of calcium to the inner leaves (Wien, 1997). This could explain the differences between the incidence of tipburn between seasons. Furthermore, the calcium content of the tobacco stem increased as temperature increased, suggesting that calcium was more immobilized at higher temperatures (Chang et al., 1968).

The effect of high nutrient solution concentration in increasing the incidence of tipburn in NFT lettuce has been widely reported (Willumsen, 1984; Van Der Boon et al., 1988; Huett, 1994). High soil salinity also increased the incidence of tipburn in lettuce and endive (De Pascale & Barbieri, 1995) and chinese cabbage (Van Berkel, 1988). The effect of root zone salinity on blossom end rot is also reported in tomato (Ho & Adams, 1989a; Brown & Ho, 1993).

The greater susceptibility of plants grown in high nutrient solution concentrations to tipburn may be attributed to a low uptake rate of calcium and reduced translocation (De Pascale & Barbieri, 1995). The highest leaf tissue calcium concentrations for both head and non-heading lettuce were recorded over an EC range of 0.4 – 1.0 dS $m^{-1}$ (Huett, 1994). This study also found that the calcium concentration of the leaves decreased with increasing nutrient solution concentrations up to 1.5 mS $cm^{-1}$ and continued to decrease at a lower rate after that (3.3.3.5). The lowest concentration was with the spring crop, which also had the highest incidence of tipburn.

### 3.4.9 Shelf life

Apart from the summer crop, the time to 10% fresh weight loss increased with increasing nutrient solution concentration (3.3.9). Although this increase did not always occur over the full range of nutrient solution concentrations, 3.5 mS $cm^{-1}$ always had a higher number of days to 10% weight loss than 0.5 mS $cm^{-1}$.

The summer crop was kept at 25 $^{0}$C 75% RH, so the weight loss rate of this crop was higher than the other crops where they were kept at 10 $^{0}$C 80% RH. This may explain why there were no significant effect of nutrient solution concentration on days to 10% fresh weight loss for the summer crop. The rate of water loss was too fast. Ryall and Lipton (1979) have stated that the postharvest water loss increased with increasing temperature because the amount of moisture the air can hold before it is saturated rises and water also has a greater tendency to evaporate as its temperature rises.

One might expect LAR to effect the rate of water loss and leaf dry matter percentage to effect the proportion of fresh weight that is water and therefore what is available to be lost by transpiration. Thus a high LAR and a low leaf dry matter percentage are two plant attributes that one might expect to decrease the number of days to 10% weight loss.

Cortina had the highest and Impuls had the lowest number of days to 10% weight loss. In this case Cortina had the highest LAR and the lowest shoot dry matter percentage, two attributes, which as has been suggested above, would decrease the number of days to 10% weight loss. Cortina on the other hand was a heading type so that this characteristic would have reduced water loss from the inner leaves and would have had a major influence on how this cultivar performed. Ryall and Lipton (1979) stated that the structure and condition of a vegetable strongly influence the rate of water loss. Leaf lettuce, with all leaves exposed, will wilt more rapidly than head lettuce where the exposed surface is relatively small.

Other factors affecting water loss are physiological age and cuticle layer thickness of the leaves. Poulsen et al. (1994) reported that weight loss during storage of crisphead lettuce was greater for the young plants than for older plants and effects of plant weight and surface to volume ratio were not found. They also suggested that differences in wax layer thickness could be involved. Bengston et al. (1978) concluded that the cuticle layer of oat leaves grown with adequate water is thinner than on leaves subjected to water deficits, while Crisosto et al. (1994) reported that peach fruit grown under deficit irrigation had a thicker cuticle and lower water loss than from excess or optimum irrigation. Shriveling symptoms were visible when weight loss exceeded 10% of the initial fresh weight. These latter reports provide an explanation as to why increases in nutrient solution concentration increased shelf life.

The results of the present study suggest that high nutrient solution concentrations extend the time to 10% fresh weight loss, but the data in Table 3.5 suggests that the differences in real time are not great. Also visual assessments of improved shelf life were not made and this is an important aspect in terms of a consumer buying or not buying a product.

### 3.4.10 Summary of treatment effects on growth, tipburn incidence and shelf life

Fresh weight increased up to 1.5 mS $cm^{-1}$ with increases in nutrient solution concentration and then levelled off or decreased slowly depending on the level of stress imposed by the season. Dry weight also increased up to 1.5 mS $cm^{-1}$ with increases in nutrient solution concentration and then levelled off. Fresh weight was thus affected by stress at the higher nutrient solution concentrations, but dry weight was not.

As tipburn increased with increasing nutrient solution concentration (apart from in autumn where no tipburn occurred), with the level of incidence increasing as environment stress increased, then 1.5 mS $cm^{-1}$ should be regarded as the optimum nutrient solution concentration at which to grow a range of lettuce cultivars across all seasons. At this nutrient solution concentration yield will be satisfactory and the level of tipburn will be minimised.

Shelf life increased with increasing nutrient solution concentration, but the level of increase was not great enough to be of commercial significant.

Cultivar differences were related to their plant characteristics. The butterhead cultivar Cortina was the most responsive to increases in nutrient solution concentration above 0.5 mS $cm^{-1}$ and to improvements in environmental conditions, whereas the red leafed cultivar Impuls was the least responsive. Cortina performed well, as early on it had a flat rosette of leaves which was efficient in intercepting radiation.

# Chapter 4

## Effect Of Nutrient Solution Concentration On Nutritional Quality Of Lettuce Grown Over Four Seasons

## 4.1 Introduction

Today, poor diet is suggested to account for almost one-third of cancer deaths in Western populations, and along with tobacco, is amongst the most modifiable environmental factors which affects human health. The relationships between diet and health are becoming more and more substantial (Verschuren, 1997). The risk of death by many non-hormone-dependent cancers can be reduced approximately twofold in subjects who consume relatively high amount of fruits and vegetables (Block et al., 1992).

Health organisations actively endorse increasing consumption of fruits and vegetables to improve public health (Mackerras, 1995; Wiseman et al., 1997). Vegetables and fruits contain a large number of components that could, by a variety of mechanisms, explain these cancer-protective effects such as vitamin C, pro-vitamin A and dietary fibre (Hertog et al., 1997). The association between low intake of fresh fruits and vegetables and the risk of epithelial cancers is probably the most consistent finding in epidermiologic studies of neoplastic disease. It is well accepted that fruits and vegetables are the main source of antioxidant nutrients, especially, vitamins C, E, and carotenoids (Correa, 1995). Extensive studies have related the dietary intake of fruit and vegetables to reduced strokes and heart attacks (Yalpani, 1997).

Despite consumer interest in health-related issues nutritional habits remain difficult to change. Nevertheless, consumers expect food products to be healthy, as well as tasty and functional. Thus new developments should provide the consumer with food products tailored to their functional, nutritional and health needs (Verschuren, 1997).

Ascorbic acid (vitamin C) is the main water-soluble antioxidant in plasma and is found in many fruits and vegetables (Leake, 1997). The most important compound possessing

vitamin C is L-ascorbic acid (AA) which is the enolic form of 3-keto-L-gulofuranolactone. The enediol group on the carbons atoms 2 and 3 of L-ascorbic acid is readily oxidised to a diketo group, resulting in L-dehydroascorbic acid, which retains full vitamin C activity (Chogugudza, 1995).

Ascorbic acid is a good reducing agent because it loses electrons easily (Levine et al., 1996). Diets with high vitamin C content from fruits and vegetables are associated with lower cancer risk, especially for cancers of the oral cavity, esophagus, stomach, colon and lung (Byers & Guerrero, 1995). There is evidence that vitamin C could be preventing cancer by inhibiting nitrosamine formation (a carcinogen), preventing the activation of carcinogens, enhancing detoxification of carcinogens, enhancing the immune response, and by the inhibition of the promotion phase (Machlin, 1995).

Dietary fibre is defined as all the polysaccharides and lignin in the diet that are not digested by the endogenous secretions of the human digestive tract (Selvendran et al., 1989). It may also be defined as non-starch polysaccharide (NSP) to allow rapid estimation of total, soluble, and insoluble dietary fibre in plant foods (Englyst & Cummings, 1988). In practice, both definitions encompass essentially the same heterogeneous mixture of plant components (Gallaher & Schneeman, 1996).

The interest in fibre as an important component of the diet has remained high as a result of epidemiological associations of a high fibre intake with a lower incidence of certain chronic disorders, such as cardiovascular disease and large bowel cancer (Gallaher & Schneeman, 1996). The Ministry of Health in the UK suggest that intakes of 18 g/day of nonstarch polysaccharides are needed in a healthful diet (Cummings & Englyst, 1995).

Fruit and vegetables also contain dietary fibre which helps in the proper functioning of the human digestive system (Chogugudza, 1995). In lettuce, during head expansion crude fibre content increases gradually. Crude fibre content in the inside and the outside head leaves was less than in the other leaves (Shen et al., 1992). Total dietary fibre content was approximately 25% of DM and this value was not much influenced by the different times of planting, rates of N applied or time of harvest (Brunsgaard et al., 1994).

Nitrate accumulation in vegetables is of concern because of the potential conversion to nitrite after uptake (Maynard & Barker, 1979; Van Der Boon et al., 1990). Although the risks of consuming nitrate as a natural substance in vegetables has not been fully evaluated (Kreij, 1994). Nitrite can cause methaemoglobinaemia especially in infants and is a precursor of nitrosamines which are carcinogenic (Addiscott et al., 1991)

In human nutrition about 90% of the nitrates come from vegetables (Pavlovic et al., 1998). The Dutch government has set maximum permissible nitrate levels for endive, spinach and lettuce. For winter-grown lettuce, the value was 4500 mg nitrate per kg fresh weight and 3500 mg/kg for summer grown lettuce (Benoit & Ceustermans, 1989; Van Der Boon et al., 1990). The European standards (1/1/98) under Belgian and Dutch climatological conditions is 3,500 ppm $NO_3$ per kg fresh head lettuce weight from 1/11 to 30/4 and 2,500 from 1/5 to 31/10 (Benoit & Ceustermans, 1995; McCall & Willumsen, 1998).

Environmental difference between seasons affects the content of ascorbic acid (Grimstad, 1984; Evers, 1994; Drews et al., 1995; Levine et al., 1996), beta-carotene (Drews et al., 1995), nitrate (Evers, 1994; Drews et al., 1995) and sugar (Drews et al., 1995) in lettuce. Beta-carotene, vitamin C and nitrate decreased, while the content of sugar increased with progression of growth stage and increase in head size (Drews et al., 1995). This was due to the different compositions of outer, middle and inner leaves and to their changing ratio during heading. Middle and inner leaves had lower nitrate, vitamin C and beta-carotene concentrations, but higher reducing sugar concentration than outer leaves (Drews et al., 1995).

The purpose of this study was to investigate the effect of nutrient solution concentration on the ascorbic acid, dietary fibre, nitrate, protein and soluble sugar concentration of three lettuce cultivars grown in NFT during four seasons of the year.

## 4.2 Materials and methods

The experiment discussed in Chapter 3 provided the plant material for this study. The production system used and treatments applied are as detailed in 3.2. At final harvest, after fresh weight recording, 3 lettuce plants (shoots only) per plot were sampled for nutritive value determinations. The leaves were detached on an every other leaf basis.

They were frozen immediately in sealable plastic bags and then freeze-dried 24 hours later. The freeze-dried materials were ground at room temperature and kept at -10 $^{0}$C prior to determination of ascorbic acid, dietary fibre, nitrate, protein and soluble sugar.

### 4.2.1 Ascorbic acid determination

Many methods that estimate the ascorbic acid content in foods use the reducing power of the vitamin 2,6-dichloroindophenol. This method is popular because it is convenient and gives a rapid estimation of ascorbic acid with simple equipment (Chogugudza, 1995). The method used for ascorbic acid determination was the 2,6-dichlorophenolindophenol spectrometric method after extraction with xylene, following the procedure of ISO 6557/2 (Anon, 1984).

### 4.2.2 Dietary fibre determination

The AOAC-enzymatic-gravimetric methods of Prosky et al. (1985) has been approved as the legal or recommended procedure for food analysis in at least ten countries, including the USA, the Nordic Countries, West Germany and Switzerland (Prosky et al., 1988). These methods, however, are time-consuming, difficult for untrained staff and provide more information than is necessary. They are unsuitable for quality control where a robust, reproducible and rapid method is needed to produce a single total value (Faulks & Timms, 1985).

An improved and simplified procedure (gas chromatography and colorimetric) of dietary fibre determination as non-starch polysaccharides (NSP), is now recommended as the official method in the United Kingdom (Englyst & Cummings, 1988). The colorimetric method is suitable for routine analysis of virtually all food products and the results obtained for NSP by gas-liquid chromatography and colorimetry are very similar (Englyst & Hudson, 1987). Results using the methods of component analysis correlate well with the AOAC Total Dietary Fibre procedure, but are generally lower (Gallaher & Schneeman, 1996). Furthermore, data for non-starch polysaccharides have been collected for a wide range of foods (Englyst et al., 1988; Englyst & Cummings, 1988) including lettuce in New Zealand (Burlingham & Milligan, 1997).

The freeze-dried materials were prepared and determined for dietary fibre following the colorimetric procedure of Englyst and Cummings (1988).

### 4.2.3 Nitrate determination

Bedwell et al. (1995) compared four nitrate analysis methods, diphenylamine spot plate, spectrophotometric, nitrate selective electrode and high-performance liquid chromatographic method. They reported that the spectrophotometric and nitrate selective electrode indicated similar percent recoveries, which were close to 100%, and the variation in the nitrate selective electrode method was lower than the other methods ($P < 0.05$). Thus the nitrate selective electrode method was more accurate and precise than the other methods.

Freeze dried materials were prepared following the procedure of Bedwell et al. (1995) except that activated charcoal was not used and the nitrate concentration was determined by a nitrate selective electrode (Orion Cat 9307 BN) attached to an Orion meter model SA 720.

### 4.2.4 Protein determination

Kjeldahl analysis and subsequent estimation of protein by multiplying total N by 6.25 is the most frequently used procedure. However, the Kjeldahl method does not distinguish between protein N, free amino acid N or other nitrogenous compounds (Khanizadeh et al., 1995). The conversion factor of 6.25 is inexact and results in overestimation of total protein in most plant tissue (Khanizadeh et al., 1992).

In this study, freeze dried materials were digested by petroleum spirit following the procedure of Olley et al. (1996) and protein determination was performed using Bio Rad Protein assay kit II (Coomassie Brilliant Blue dye binding method) following the procedure of Bradford (1976).

### 4.2.5 Soluble sugar determination

Freeze dried materials were extracted with methanol and determination of soluble sugar was performed following the procedure of Haslemore and Roughan (1976).

### 4.2.6 Statistical procedure and analysis

A Randomized complete block (RCB) with 4 × 3 factorial arrangement with 4 replications over 4 seasons was used for evaluating human nutritional quality of the plants at final harvest. Data were subjected to analysis of variance following the SAS General Linear Model (GLM) procedure (SAS Institute Inc., 1989).

## 4.3 Results

### 4.3.1 Ascorbic acid

Season (crop), nutrient solution concentration and cultivar affected ascorbic acid concentration at final harvest. There were significant interactions between crop × concentration ($P \leq 0.05$), crop × cultivar ($P \leq 0.001$) and concentration × cultivar ($P \leq 0.01$).

In the summer crop, ascorbic levels were highest and decreased with increasing nutrient concentration (Figure 4.1a). The ascorbic acid concentration was markedly lower for the other seasons and was not affected by nutrient concentration. The spring crop had a higher ascorbic acid concentration than the autumn and winter crops.

With the summer crop there were no significant differences between cultivars while Impuls had the highest ascorbic acid concentration for the other seasons (Figure 4.1b). The ascorbic acid concentration of Cortina and Lollo Bionda were similar The order of ascorbic acid concentrations across the seasons was summer > spring ≥ autumn, winter.

Impuls also had the highest ascorbic acid for every nutrient concentrations and decreased with increasing nutrient concentration (Figure 4.1c). The ascorbic acid concentration of Cortina and Lollo Bionda were similar at all nutrient concentrations.

### 4.3.2 Dietary fibre

Only cultivar affected dietary fibre concentration at final harvest. Lollo Bionda and Impuls had the highest dietary fibre concentration, significantly higher than Cortina (Table 4.1).

**Table 4.1 Dietary fibre concentration (mg $g^{-1}$ dry weight) (mean of 4 concentrations over 4 seasons).**

| Cultivar | Dietary fibre (mg $g^{-1}$ DW) | |
|---|---|---|
| Cortina | 283.54 | b |
| Impuls | 316.79 | a |
| Lollo Bionda | 320.11 | a |

Means sharing the same letter are not significantly different by t-test at $P \leq 0.05$

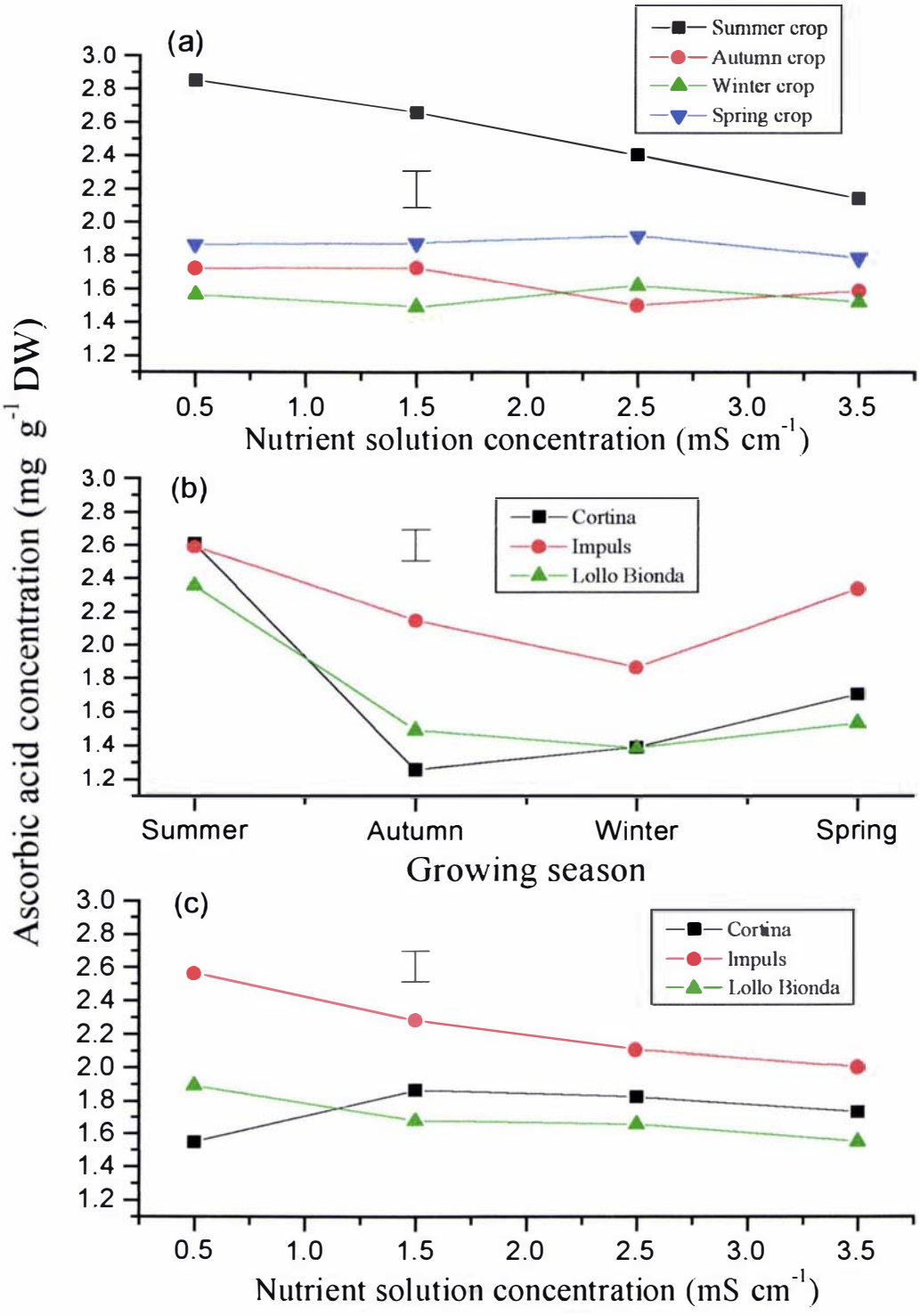


**Figure 4.1** **Interaction effects between crop × concentration (a), crop × cultivar (b) and concentration × cultivar (c) on ascorbic acid concentration (mg $g^{-1}$ DW) of 3 cultivars of lettuce grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction effects.**

### 4.3.3 Nitrate

Season (crop), nutrient solution concentration and cultivar affected nitrate concentration at final harvest. There were significant interactions between crop × concentration ($P \leq 0.0001$), crop × cultivar ($P \leq 0.0001$) and concentration × cultivar ($P \leq 0.05$).

Nitrate concentration increased with increasing nutrient concentration (Figure 4.2a). The nitrate concentration for seasons was in the order autumn > winter ≥ spring, summer. The 0.5 mS $cm^{-1}$ spring crop had the lowest nitrate concentration, much lower than the other treatments.

Cortina and Lollo Bionda had the highest nitrate concentration in winter and spring, while with the summer crop Impuls had the highest nitrate concentration (Figure 4.2b). Apart from Impuls in winter, autumn and winter had the highest nitrate concentrations.

At 0.5 mS $cm^{-1}$ there was no significant difference between cultivars, while at the higher nutrient solution concentrations Cortina had the highest nitrate concentration (Figure 4.2c). Nitrate concentration increased with increasing nutrient concentration.

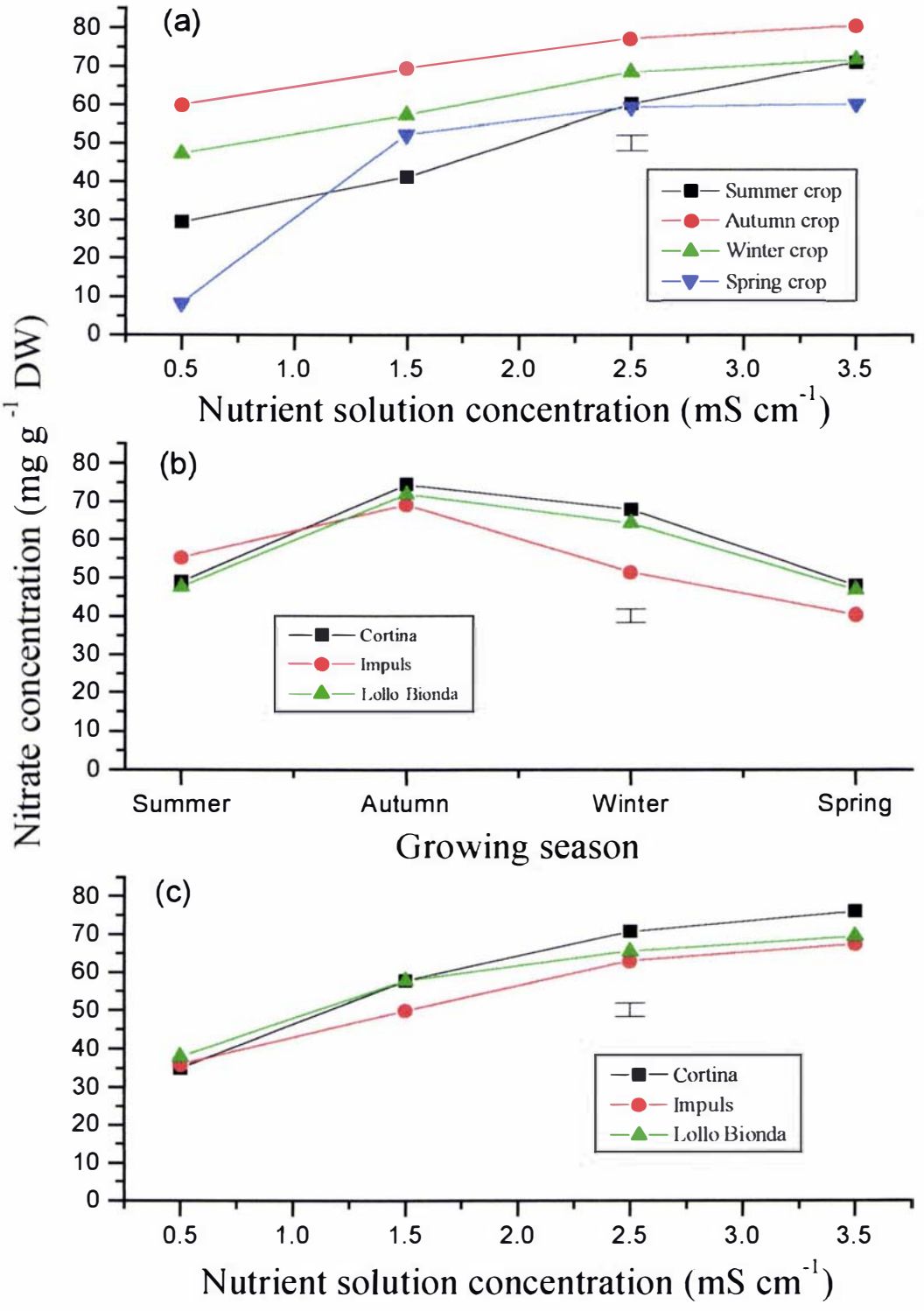


**Figure 4.2 Interaction effects between crop × concentration (a), crop × cultivar (b) and concentration × cultivar (c) on nitrate concentration (mg $g^{-1}$ DW) of 3 cultivars of lettuce grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction effects.**

### 4.3.4 Protein

There was no effect of season, nutrient concentration or cultivar on protein concentration. The average protein concentration of the plants was 142.25 mg $g^{-1}$ dry weight.

### 4.3.5 Soluble sugar

Nutrient solution concentration and cultivar affected soluble sugar concentration at final harvest. There were significant interactions between crop × concentration ($P \leq 0.0001$) and crop × concentration × cultivar ($P \leq 0.01$). The result of the latter interaction only is considered here.

Generally soluble sugar concentrations decreased with increasing nutrient concentration to either 1.5 or 2.5 mS $cm^{-1}$ and then levelled off (Figure 4.3). This fall was more noticeable with the summer and spring crops. For Cortina in summer and Lollo Bionda in winter, 1.5 mS $cm^{-1}$ had a higher soluble sugar concentration than 0.5 mS $cm^{-1}$. Over the range of 0.5 and 2.5 mS $cm^{-1}$, summer followed by spring tended to have the highest soluble sugar concentrations.

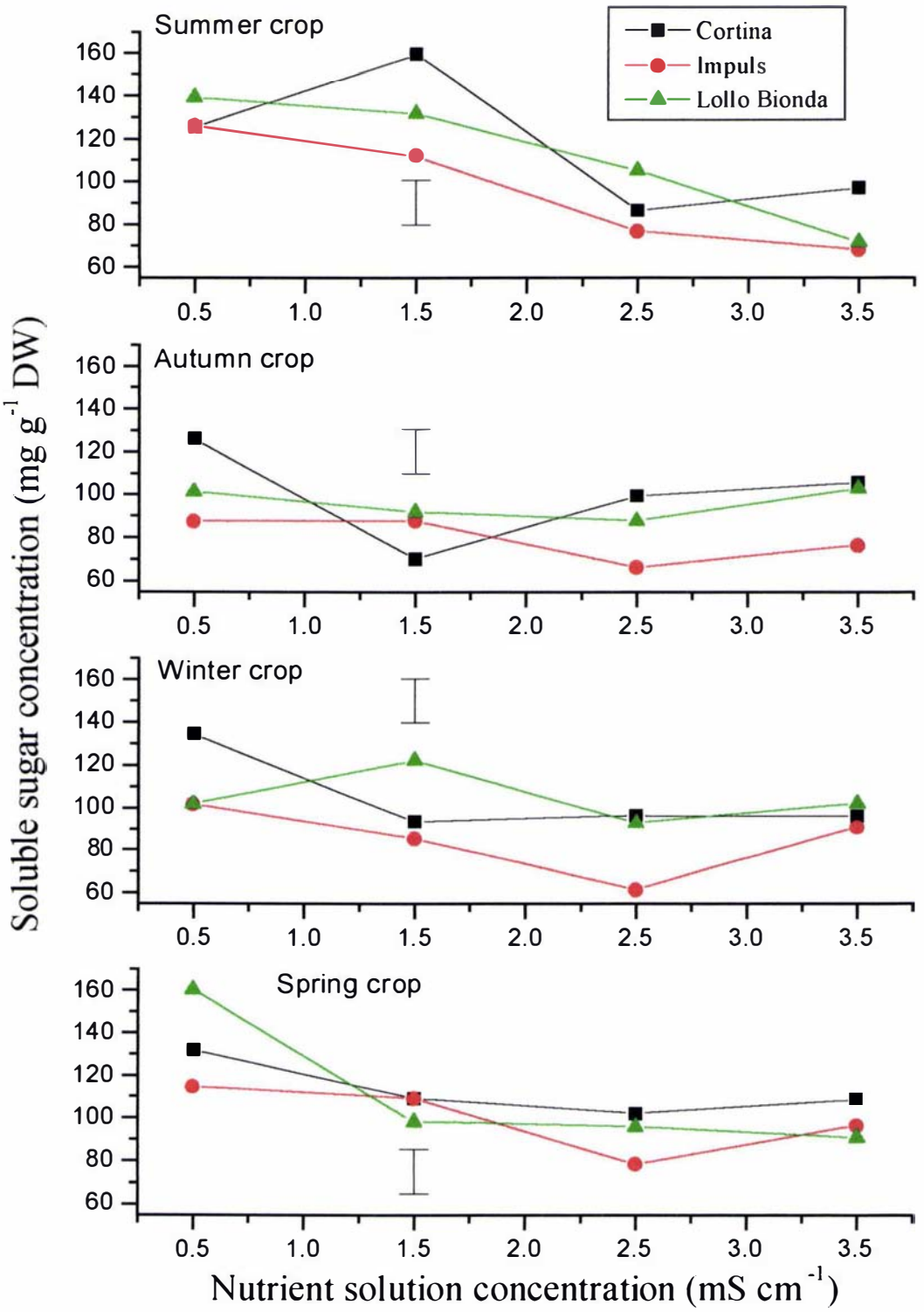


**Figure 4.3** **Soluble sugar concentration (mg $g^{-1}$ dry weight) of 3 cultivars lettuce grown in 4 nutrient solution concentrations over 4 seasons. The bars represent the standard error of the interaction effect.**

## 4.4 Discussion

### 4.4.1 Ascorbic acid

The ascorbic acid concentration was in the order summer > spring ≥ autumn, winter. The seasonal effect on the difference in ascorbic acid concentration of lettuce has been reported in the literature (Grimstad, 1984, Evers, 1994; Drews et al., 1995). The higher ascorbic concentration in summer and spring suggest a relationship with solar radiation. This relationship has also been widely reported in the literature (Shinohara & Suzuki, 1981; Grimstad, 1984; Primak, 1985).

Ascorbic acid concentration decreased with increasing nutrient concentration in summer. Shinohara et al. (1978) reported that the ascorbic acid content increased when the plants were grown at ¼ rates of the standard nutrient solution, compared with ½ rates or more. As ascorbic acid concentration has been reported to decreased with progression of growth stage and increase in head size (Sorensen et al., 1994, Drews et al., 1995, 1996, 1997). These differences could be due to the different physiological ages at final harvest. Differences in physiological age could have been due to stress at the higher nutrient solution concentrations. Thus mild water deficit stress was reported to increase earliness by decreasing the time to flowering in wheat (Angus & Moncur, 1977) as did salt stress (Maas & Grieve, 1990; Grieve et al., 1994). Ebel et al. (1993) suggested that regulated deficit irrigation may advance fruit maturity and alter quality at harvest of apple.

Alternately, the increasing nutrient supply at the higher nutrient solution concentrations in summer, may have accounted for the response, as excess rates of nitrogen application have also been found to decreased ascorbic acid content in lettuce (Evers, 1994; Sorensen et al., 1994).

Where ascorbic acid concentration were high, such as in summer and with the cultivar Impuls, then ascorbic acid concentration was sensitive to increasing nutrient solution concentration. Impuls was the most responsive cultivar. It had the highest ascorbic acid concentration in all seasons but summer, and decreased markedly with increases in nutrient solution concentration. Differences in ascorbic acid concentrations between

cultivars have been previously reported by other researchers (Albrecht, 1993; Evers, 1994; Sorensen et al., 1994).

### 4.4.2 Dietary fibre

There were no effects of season or nutrient concentration on dietary fibre concentration. Brunsgaard et al. (1994) found that total dietary fibre was approximately 25% of dry matter and was not much influenced by the different times of planting, rate of N applied or time of harvest. The cultivar Cortina, a butterhead type, had less dietary fibre than the other two cultivars. This is perhaps to be expected, as it was the fastest growing (3.3.1) and had the lowest dry matter percentage of the three cultivars (3.3.7).

### 4.4.3 Nitrate

The nitrate concentration for season was in the order autumn > winter > spring, summer. The seasonal effect on the difference in nitrate concentration of lettuce has been reported in the literature (Evers, 1994; Drews et al., 1995). The lower nitrate concentration in summer and spring suggest a negative relationship with solar radiation. This relationship has also been widely reported in the literature (Shinohara & Suzuki, 1981; Primak, 1985; Evers, 1994; Drews et al., 1995). Blom-Zandstra & Lampe (1985) concluded that increased light intensity caused a distinct shift from nitrate accumulation in the plant sap towards accumulation of sugar (mainly glucose) and organic acid (mainly malate).

This study found that nitrate concentration increased with increasing nutrient concentration. The effect of increasing the nutrient solution concentration on increasing the nitrate content of lettuce has been widely reported in the literature (Willumsen, 1984; Primak, 1985; Van Der Boon et al., 1988; Evers, 1994; Sorensen et al., 1994). The increasing supply of nitrate in the nutrient solution would be an important factor here. Maynard and Barker (1979) have stated that water stress enhanced nitrate accumulation in vegetables. Assimilation of nitrate in water stressed plant may be restricted by lowered nitrate reductase activity, or by reduced availability of photosynthetic reducing equivalents.

The 0.5 mS $cm^{-1}$ spring crop had the lowest nitrate concentration, much lower than the other treatments. This was due to the amount of phosphoric acid that this treatment required to adjust the pH rise that occurred (3.4.3), which then lowered the nitrate concentration in the nutrient solution.

Cortina and Lollo Bionda had the highest nitrate concentration in winter and spring, while with the summer crop Impuls had the highest nitrate concentration. The differences in nitrate concentration between cultivars of lettuce have been widely reported (Blom-Zandstra & Eenink, 1986; Reinink & Eenink, 1988; Behr & Wiebe, 1992).

Nitrate accumulation in vegetable crops is of concern because of the potential conversion to nitrite after uptake which can cause methaemoglobinaemia in infants and because it is a precursor of nitrosamines, which are carcinogenic (Addiscott et al., 1991). The European standards for nitrate concentration under Belgian and Dutch climatological conditions(from 1/1/98) is 3,500 ppm $NO_3$ per kg fresh head lettuce weight from 1/11 to 30/4 and 2,500 from 1/5 to 31/10 (Benoit & Ceustermans, 1995). After conversion from a dry weight (Figure 4.2) to a fresh weight basis (3.3.7) the range of nitrate values for the present investigation was 124 – 397 mg/100g with the highest values for the autumn crop when grown at a nutrient solution concentration of 3.5 mS $cm^{-1}$. The nitrate concentrations of lettuce produced at nutrient solution concentrations up to 1.5 mS $cm^{-1}$ are within the European standard.

### 4.4.4 Protein

There was no effect of season, nutrient concentration or cultivar on protein concentration. Lairon et al. (1984) reported that the protein concentration in lettuce, by determination of the protein amino acids, did not show any significant change either with mineral or organic nitrogen fertiliser or with the application rate used. On the other hand, Brunsgaard et al. (1994) reported that the protein content (N × 6.25) of crisphead lettuce increased progressively from 16.00 to 19.88 % with nitrogen fertiliser levels from 50 to 200 kg N $ha^{-1}$. Early harvest as well as early planting also increased the protein content compared to late harvest and late planting.

### 4.4.5 Soluble sugar

Over the range of 0.5 and 2.5 mS $cm^{-1}$, spring and summer tended to have the highest soluble sugar concentrations whereas autumn and winter had the lowest. The higher levels of sugar in spring and summer would be related to the higher levels of solar radiation at that time of the year. The relationship between light intensities and soluble sugar has been documented for lettuce (Shinohara & Suzuki 1981; Grimstad, 1984; Blom-Zandstra & Lampe, 1985; Drews et al., 1995).

For Cortina in summer and Lollo Bionda in winter, the 1.5 mS $cm^{-1}$ had a higher soluble sugar concentration than 0.5 mS $cm^{-1}$. Longnecker (1994) stated that the primary effect of nitrogen and phosphorus deficiency is the limitation of sink growth, with feedback effects on photosynthesis because of decreased requirement for assimilate.

Generally soluble sugar concentrations decreased with increasing nutrient concentration to 2.5 mS $cm^{-1}$ and then levelled off. The nutrient solution concentration effect is likely be an indirect effect on photosynthesis, possibly via stomata closure. A negative relationship between sugar and nitrate concentration in lettuce has been reported by a number of researchers (Shinohara & Suzuki, 1981; Drews et al., 1995). Blom-Zandstra and Lampe (1985) concluded that increased light intensity cause a distinct shift from nitrate accumulation in the plant sap towards accumulation of sugar (mainly glucose) and organic acid (mainly malate).

### 4.4.6 Nutritive value comparisons

The concentrations of ascorbic acid, dietary fibre, nitrate, protein and soluble sugar reported by other sources have been reported in Table 2.4. The results of the present study have been reported on the basis of mg $g^{-1}$ dry weight. The shoot dry matter percentages have been presented in 3.3.7 with the overall average of 4.92%. The comparisons show that the nutritive values obtained in the present study are in similar ranges to that reported in other studies.

### 4.4.7 Summary

i. The summer crop had the highest ascorbic acid concentration. Where ascorbic acid concentrations were high, such as in summer or with the cultivar Impuls, then ascorbic acid concentrations decreased with increases in nutrient solution concentration.

ii. The only difference in dietary fibre occurred with the butterhead cultivar Cortina, which had the lowest dietary fibre concentration of the three cultivars. As this was a heading type, it had more enclosed succulent leaves.

iii. Nitrate concentration increased with nutrient solution concentration, was highest in autumn and winter, while differences between cultivars depended on the season. The nitrate concentrations of lettuce produced at nutrient solution concentration up to 1.5 mS $cm^{-1}$ were within the permissible levels reported overseas.

iv. There were no treatment effects on protein concentration despite some reports in the literature of the effects of nitrogen level on protein content.

v. At the lower nutrient solution concentrations, the spring and summer crops tended to have the highest soluble sugar concentrations. Generally soluble sugar concentrations decreased within increasing nutrient solution concentrations up to 2.5 $mScm^{-1}$ and then levelled off.

vi. Season, nutrient solution concentration and cultivar all affected the nutritive value. The affect depended on the nutritive quality attribute under consideration.

vii. The nutritive values obtained in this study were in the ranges reported by other workers.

# Chapter 5

# The Effect Of Day/Night Nutrient Solution Concentration Combinations And Extra Calcium At Night On Nutrient Uptake, Yield And Tipburn Incidence

## 5.1 Introduction

Tipburn of lettuce is a serious problem that can lead to poor appearance (Collier & Tibbitts, 1984; Brumm & Schenk, 1993; Nagata & Stratton, 1994), increased pre-and post-harvest rots, and ultimately the reduction in the quality and grade of lettuce (Nagata & Stratton, 1994). It is the most important physiological disorder in lettuce and can cause substantial economic loss. Occurrence and intensity varies considerably from year to year and even within a growing period (Brumm & Schenk, 1993).

Tipburn occurs mainly in the inner leaves. At the time of occurrence it develops on those leaves and those parts of the leaves which are expanding most rapidly (Misaghi & Grogan, 1978a; Collier & Huntington, 1983; Barta & Tibbitts, 1991), while middle and outer leaves, which are growing only slightly, are usually symptomless (Misaghi & Grogan 1978a).

It is a disorder recognised as associated with localised inadequacy of calcium in the leaf tissue (Shear, 1975a; Misaghi & Grogan, 1978a; Barta & Tibbitts, 1991) even when there is an adequate supply of calcium to the roots (Kirkby, 1979; Collier & Tibbitts, 1984). The lowest level of calcium is found in areas with tipburn (Ashkar & Ries, 1971; Collier & Wurr, 1981; Barta & Tibbitts, 1987, 1991), while magnesium and potassium levels increased acropetally and are generally highest in areas with tipburn (Barta and Tibbitts, 1987). Barta & Tibbitts (1991) suggested that the injured areas of tipburned leaves had calcium concentrations lower than 0.4 mg $g^{-1}$ dry weight and magnesium concentrations were negatively correlated with calcium concentration.

A direct correlation between growth rate or head size and tipburn development has been suggested (Cox et al., 1976; Misaghi & Grogan 1978a; Brumm & Schenk, 1993). Cox et

al. (1976) concluded that growing conditions causing tipburn may do so by affecting growth rate.

Thus, tipburn development appears to depend on the supply of calcium relative to the rate of leaf growth (Collier & Huntington, 1983; Barta & Tibbitts, 1991). Tipburn incidence is favoured by high growth rates and the inability of the plant to match this growth with an adequate calcium to the inner leaves (Wien, 1997). Several factors lead to the conclusion that loss of membrane integrity is probably the initial stage in injury development (Collier & Tibbitts, 1982). The spontaneous release of latex near the margins of the leaf has been found to precede the development of tipburn. The release of latex was closely related to plant growth rate and maturation. Possibly the released latex disrupts the vascular tissue which causes the collapse and necrosis of the leaf (Tibbitts et al., 1965).

There are stages in lettuce growth where plants become particularly sensitive to tipburn. Stratton and Nagata (1993) found that at the 38 day-old stage of growth, plants were in the first stages of leaf cupping or enclosure and were growing very rapidly. At this point, leaf expansion may be too rapid to allow lettuce to transport adequate calcium to the expanding leaf margin and the plants are at risk of developing tipburn. The age of first leaf cupping or enclosure is therefore critical to tipburn development.

Increasing the concentration of the NFT solution decreased the calcium concentration (%) of tomato fruit (Adams & Ho, 1990; Brown & Ho, 1993) and sweet pepper (Tadesse, 1997) due to an osmotic effect on calcium uptake. This response was most marked at night, when under normal conditions, more of the newly absorbed calcium moves into the fruit than during the day (Adams & Ho, 1990). High nutrient solution concentration also increases the incidence of tipburn in lettuce (Willumsen, 1984; Huett, 1994) and Huett (1994) suggested a concentration of 1.5 mS $cm^{-1}$ produced the best yield and that a reduction to 1.0 mS $cm^{-1}$ reduced yield slightly and substantially reduced tipburn. He was critical of the industry practice in Australia of often growing lettuce at 2.0 to 2.5 mS $cm^{-1}$.

High relative humidities at night favour the transport of calcium into low transpiring organs, due to root pressure development, of lettuce (Collier & Wurr, 1981), cabbage

(Palzkill & Tibbitts, 1977), strawberry (Bradfield & Guttridge, 1979; Guttridge et al., 1981) and tomato (Bradfield & Guttridge, 1984; Clover, 1991).

Root pressure develops because water continues to move into the root, developing a hydrostatic pressure that forces water and calcium through the plant (Palzkill & Tibbitts, 1977). Root pressure occurs under conditions where transpiration is low or not functioning, and can be encouraged by increasing night time relative humidity, and by reducing the resistance to water movement into the plant (Bradfield & Guttridge, 1984; Wein, 1997). Maintaining adequate soil water, and having a low night time osmotic pressure of the soil solution are two ways in which water uptake can be maximised (Wien, 1997).

Root pressure is believed to be a simple osmotic process, caused by the accumulation of sufficient solutes in the xylem to lower the water potential of the xylem sap below that of the substrate. The reduction of root pressure caused by insufficient aeration, low temperature, and respiration inhibitors is attributed to a reduction in salt accumulation in the root xylem and to changes in root permeability, rather than to inhibition of any nonosmotic water transport mechanism (Kramer, 1983).

Positive root pressure at night promotes transport of calcium into tissues or organs that have restricted transpiration (Bradfield & Guttridge, 1984). When day time and night time concentrations of nutrients were different, only the concentration provided at night affected the concentration of calcium in the distal wall tissue of tomato (Bradfield & Guttridge, 1984) and emerging leaves of strawberry (Guttridge et al., 1981). Adding extra calcium to the nutrient solution increased the calcium concentration in the proximal, but not in the middle or distal, segments of the tomato fruit (Bradfield & Guttridge, 1984).

Cresswell (1991) examined the effect of lowering the nutrient solution concentration at night to reduce tipburn. Calcium nitrate treatments and tap water were compared with a control nutrient solution, which had a concentration of 2.0 mS $cm^{-1}$. All treatments reduced the incidence of tipburn and there was no difference between the tap water and the calcium treatments. None of the treatments reduced the fresh weight of lettuce. A criticism of this work is that the control concentration was high and likely to favour tipburn so that any treatment at night would reduce tipburn providing that it was at a

lower concentration. The response of the tap water treatment supports this view. Whether differences would be obtained at a more commercially acceptable concentration of 1.5 mS $cm^{-1}$ (Huett, 1994) was not clear from this research of Cresswell.

The research outlined above, with a number of crops, shows the value of lowering the concentration at night to allow root pressure to operate and so distribute calcium more uniformly around the plant. The potential to use root pressure in lettuce to reduce tipburn has however not been fully investigated. Such an approach should take into account the goal of the commercial grower to balance the need to maintain growth and yield with the need to reduce tipburn.

The following experiments grew plants during the day at a maximum concentration of 1.5 mS $cm^{-1}$, which the seasonal study (3.3.4) and the work of Huett (1994) showed produced good yields. The treatments included a range of concentrations at night, using both complete nutrient solutions and extra calcium. The concentrations of some of these treatments, were such, that it was expected that root pressure would operate and so enhance the supply of calcium to the inner leaves.

The objectives of the research were:

1.1 To investigate the effect of different day/night nutrient solution concentration combinations on nutrient uptake, yield and tipburn incidence.

1.2 To examine approaches to charging the inner leaves with calcium, so that when the plants were exposed to tipburn inducing conditions, tipburn incidence was eliminated or greatly reduced without significant yield loss.

## 5.2 Materials and Methods

### 5.2.1 Propagation and greenhouse environment

The experiments were conducted in a 6 × 16.5 m greenhouse at the Plant Growth Unit (PGU), Massey University. Pelletted seeds of the butterhead lettuce cultivar Cortina were sown in 45 ml single cells, which allowed the roots to grow unimpeded out of the cell. These individual cells were placed in a cell tray for support. The propagation media for the first experiment was sieved peat with agricultural lime added at 3 kg $m^3$ and the in second experiment was fine grade vermiculite. The trays were placed on a heated bench at 25 $^0$C for 5 days. After 5 days, the heat was turned off. A nutrient solution of concentration 0.5 mS $cm^{-1}$ and pH 6.0 was applied to the seedlings once a day. The nutrient solution used was as described in 3.2.3. The young plants were transplanted into and grown on in 5 x 10 cm PVC channels 4.5 m long, spaced 25 cm apart. Each channel had 21 holes spaced 20 cm apart in which the plants were grown (3.2.2).

The greenhouse temperature was maintained at 10 $^0$C via the heating system and ventilated by fans when the temperature reached 25 $^0$C.

### 5.2.2 Experiment 2.1: The effect of different day/night nutrient solution concentration combinations on nutrient uptake, yield and tipburn incidence

#### 5.2.2.1 Treatments and experimental design

Experiment 2.1 was conducted during January - February 1996. The seeds were sown on December 26, 1995. The seedlings were transplanted into the channels on January 7, 1996. The treatments commenced on January 9, 2 days after transplanting and the final harvest was on February 8.

Complete nutrient solutions of concentration 0.5 and 1.5 mS $cm^{-1}$ were combined factorially as day and night treatments to provide four treatment combinations as follows:

1. Day 0.5 Night 0.5 mS $cm^{-1}$
2. Day 0.5 Night 1.5 mS $cm^{-1}$
3. Day 1.5 Night 0.5 mS $cm^{-1}$
4. Day 1.5 Night 1.5 mS $cm^{-1}$

The experimental plot was a 4.5 m long channel. The channels were placed 25 cm apart. The slope of the channels was 1:80 with the flow rate of the nutrient solution at 1.6 l $min^{-1}$.

There were four 100 l nutrient solution tanks, two at 0.5 and two at 1.5 mS $cm^{-1}$. The 0.5/1.5 and 1.5/0.5 day/night combinations shared two tanks crossing over twice every 24 hours. The 0.5/0.5 and 1.5/1.5 day/night combinations had their own separate tanks. The nutrient solution concentration between day and night were changed by switching over the submerged pumps. Day and night concentrations were imposed manually at approximately 0800h and 2000h each day. The concentration and pH of the nutrient solutions were adjusted 10 minutes after switching the submerged pumps. Nutrient solutions were dumped and replaced each week.

A Randomized Complete Block (RCB) design with 2 × 2 factorial arrangement with four replications was used. A block consisted of 4 channels with a channel allocated to each of the four treatments. There were 20 plants in each channel (plot). The blocks shared the same nutrient solution tank due to space constraints.

### 5.2.2.2 Data collection and analysis

The fresh weights were determined for 10 heads per plot at final harvest, while the dry weight of the shoot and root was determined from 2 plants per plot at final harvest after

the samples were dried in an oven at 70 $^0$C for 48 hours. Tipburn incidence was determined by assessing the tipburn percentage from 10 plants at final harvest.

The plant materials that was used to determined the dry weight was kept for determination of plant nutrition concentrations. Potassium, calcium and magnesium were determined by Atomic Absorption Spectroscopy (AAS) following digestion in nitric acid (Technicon, 1973). Total nitrogen and phosphorus were determined by colorimetric autoanalysis following the Kjeldahl digestion technique (Twine and Williams, 1971). See 3.2.4.2 for full details.

Data were subjected to analysis of variance following the Statistical Analysis System (SAS) General Linear Model (GLM) procedure (SAS Institute Inc., 1989). Data were checked as to whether they conformed to the assumption of ANOVA. Percentage data were arcsine transformed.

## 5.2.3 Experiment 2.2: Approaches to charging the inner leaves with calcium

### 5.2.3.1 Treatments and experimental design

Experiment 2.2 was conducted during March - May 1997. The seed was sown on March 7, 1997 and the seedlings were transplanted into the NFT channels on April 4. The different treatment solutions were applied from transplanting.

The treatments were applied at night as then transpiration would be minimal and root pressure could operate. During the daytime the nutrient solution was maintained at 1.5 mS cm$^{-1}$, pH 6.0 ± 0.5. At night three nutrient solution concentrations, 0.2 (tap water only), 0.5 and 1.5 mS cm$^{-1}$, were combined factorially with two calcium levels using calcium chloride ($CaCl_2$) at 0 and 100 mg Ca l$^{-1}$.

A chloride ion concentration of more than 10 me l$^{-1}$ may damage susceptible plant species. Lettuce can tolerate chloride to 2-3 times this level (Marschner, 1995). The

chloride ion concentration of the 100 mg Ca $l^{-1}$ series was only 4.99 me $l^{-1}$ so that this level was well within the acceptable range.

The 6 treatment combinations were as follows:

| **Ca 0 series** | **Ca 100 series** |
|---|---|
| 1. Tap water | 4. Tap water + calcium 100 $mgl^{-1}$ |
| 2. Nutrient solution at 0.5 mS $cm^{-1}$ | 5. Nutrient solution at 0.5 mS $cm^{-1}$+ Ca100 $mgl^{-1}$ |
| 3. Nutrient solution at 1.5 mS $cm^{-1}$ | 6. Nutrient solution at 1.5 mS $cm^{-1}$+ Ca 100 $mgl^{-1}$ |

From April 21 to April 24, the temperature inside the greenhouse was maintained at $30^0$C to induce tipburn, otherwise greenhouse temperatures were maintained as detailed in 5.2.1.

There were six 70 l nutrient solution tanks, one for each treatment combination. During the day all the treatments used the same nutrient solution. The nutrient solution concentration between day and night were changed by switching over the submerged pumps. Day and night concentrations were imposed manually at approximately 0730h and 1930h each day. The NFT channels were flushed with tap water and with nutrient solutions for ten minutes each, before changing the nutrient solution to avoid solution contamination. The nutrient solutions were made up each day to full volume to replace the volumes used to flush out the channels. The nutrient solutions were dumped and replaced fortnightly as about half of their contents was dumped daily.

The experimental design used was a Randomised Complete Block (RCB) design with a $2 \times 3$ factorial arrangement with four replications. A block consisted of 6 channels with a channel allocated to each of the 6 treatments. There were 18 plants in each channel (plot) with planting holes 20 cm apart. The channels were placed 25 cm apart (Plate 5a). The blocks shared the same nutrient solution tanks due to space constraints. The slope of the channels was 1:80 with the flow rate of the nutrient solution at 0.8 l $min^{-1}$.

a)

b)

c)


**Plate 5 Experiment 2.2 a) General view, b) Final harvest (Ca0 series on left, Ca100 series on right) and c) Ranking system for tipburn severity**

### 5.2.3.2 Data collection and analysis

Three plants from each channel were sampled the day before (April 20) and the day after (April 25) the 30$^0$C tipburn inducing heat treatment was applied. Leaves from each plant were divided into groups of five leaves, with the last group including the innermost leaves that were very small (<1 cm.). There were four and five groups of leaves before and after tipburn induction respectively. This was done to follow the growth of the inner and outer groups of leaves and to allow their nutrient uptake to be determined. Dry weight was recorded from each leaf group after samples were dried in an oven at 70 $^0$C for at least 48 hours. There was also a final harvest on May 2 when the fresh weight was determined from 10 heads per channel (Plate 5b).

Tipburn incidence was assessed from the 10 plants at final harvest as follows:

1. Number of plants per plot with tipburn incident. This was expressed as a percentage of the number of plants with tipburn

2. Number of leaves affected per plant.

3. Ranking the appearance of the tipburn affected leaves by averaging the affected leaves per plant as follows to provide a tipburn score (Plate 5c):

   1 = tipburn symptoms just started, leaf tip curl with brown colour

   2 = leaf tip burn less than 2 cm wide at leaf margin

   3 = leaf tip burn between 2 to 5 cm wide at leaf margin

   4 = leaf tip burn between 5 to 8 cm wide at leaf margin

   5 = leaf tip burn more than 8 cm wide at leaf margin

4. Tipburn index was calculated from number of leaves with tipburn per plant × the tipburn score

Leaf nutrient concentrations were determined for the individual groups of leaves for the harvests on 20 and 25 April. It was based on the sample of leaves that had been oven dried. Potassium, calcium and magnesium were determined by Atomic Absorption Spectroscopy (AAS) following digestion in nitric acid (Technicon, 1973). Total nitrogen and phosphorus were determined by colorimetric autoanalysis following the Kjeldahl digestion technique (Twine and Williams, 1971). See 3.2.4.2 for full details.

The data were analysed using the Statistical Analysis System (SAS) General Linear Model (GLM) procedure (SAS Institute Inc., 1989). Data were checked whether they conformed to the assumption of ANOVA. Percentage data were arcsine transformed.

## 5.3 Results

### 5.3.1 Experiment 2.1

#### 5.3.1.1 Tissue nutrient concentrations

##### 5.3.1.1.1 Leaf nutrient concentrations

There were significant interactions between day and night time nutrient solution concentrations for total leaf nutrient concentrations of nitrogen, potassium, calcium and magnesium (Table 5.1a-e). The 1.5/0.5 mS $cm^{-1}$ day/night treatment had the highest leaf nitrogen concentration and the 0.5/0.5 mS $cm^{-1}$ treatment had the lowest, while leaf phosphorus concentrations were not significantly different.

A daytime concentration of 1.5 mS $cm^{-1}$ gave significantly greater potassium concentration than 0.5 mS $cm^{-1}$. For the 0.5 mS $cm^{-1}$ daytime concentration, 0.5 mS $cm^{-1}$ at night had a lower leaf potassium concentration than 1.5 mS $cm^{-1}$.

The 0.5/0.5 and 1.5/1.5 mS $cm^{-1}$ treatments had a greater leaf calcium concentration than the 1.5/0.5 mS $cm^{-1}$ treatment, while the leaf magnesium concentration for 0.5 mS

$cm^{-1}$ during the day was greater than for 1.5 mS $cm^{-1}$. With 0.5 mS $cm^{-1}$ during the day series, 0.5 at night had a greater leaf magnesium concentration than 1.5 mS $cm^{-1}$.

### 5.3.1.1.2 Root nutrient concentrations

A daytime nutrient solution concentration of 1.5 mS $cm^{-1}$ had a higher root nitrogen concentration than 0.5 mS $cm^{-1}$, while the response was reversed at night (Table 5.2a). There was no effect of the concentration treatments on root phosphorus concentration (Table 5.2b).

There were significant interactions between day and night time nutrient solution concentrations for concentration of potassium, calcium and magnesium in the roots (Table 5.2c - e). The 1.5/0.5 mS $cm^{-1}$ day/night treatment had the highest potassium concentration and the 0.5/0.5 treatment had the lowest. The 1.5/1.5 mS $cm^{-1}$ day/night treatment had the highest root calcium concentration and the 0.5/1.5 treatment the lowest. The 1.5/1.5 mS $cm^{-1}$ day/night treatment had the highest root magnesium concentration.

**Table 5.1 Nitrogen, phosphorus, potassium, calcium and magnesium concentrations (% dry weight) of leaf at final harvest of lettuce cultivar Cortina grown under different day night nutrient solution combinations.**

| | | Night concentration | | Mean |
|---|---|---|---|---|
| | Day concentration | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| **a) Leaf nitrogen** | 0.5 mS $cm^{-1}$ | 4.585[1] c | 4.723 b | 4.654 |
| | 1.5 mS $cm^{-1}$ | 5.018 a | 4.762 b | 4.890 |
| | Mean | 4.801 | 4.743 | |
| | Day concentration (D) | **** | | |
| | Night concentration (N) | ns | | |
| | D × N | *** | | |
| **b) Leaf phosphorus** | 0.5 mS $cm^{-1}$ | 0.608 | 0.628 | 0.618 |
| | 1.5 mS $cm^{-1}$ | 0.581 | 0.591 | 0.586 |
| | Mean | 0.594 | 0.609 | |
| | Day concentration (D) | ns | | |
| | Night concentration (N) | ns | | |
| | D × N | ns | | |
| **c) Leaf potassium** | 0.5 mS $cm^{-1}$ | 3.484[1] c | 5.728 b | 4.606 |
| | 1.5 mS $cm^{-1}$ | 6.898 a | 6.959 a | 6.928 |
| | Mean | 5.191 | 6.343 | |
| | Day concentration (D) | **** | | |
| | Night concentration (N) | ** | | |
| | D × N | ** | | |
| **d) Leaf calcium** | 0.5 mS $cm^{-1}$ | 1.651[1] a | 1.396 ab | 1.523 |
| | 1.5 mS $cm^{-1}$ | 1.198 b | 1.549 a | 1.374 |
| | Mean | 1.425 | 1.472 | |
| | Day concentration (D) | ns | | |
| | Night concentration (N) | ns | | |
| | D × N | ** | | |
| **e) Leaf magnesium** | 0.5 mS $cm^{-1}$ | 0.663[1] a | 0.479 b | 0.571 |
| | 1.5 mS $cm^{-1}$ | 0.386 c | 0.397 c | 0.392 |
| | Mean | 0.525 | 0.438 | |
| | Day concentration (D) | **** | | |
| | Night concentration (N) | *** | | |
| | D × N | **** | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within the table sharing the same letter are not significantly different by t-test at $P \leq 0.05$

**Table 5.2 Nitrogen, phosphorus, potassium, calcium and magnesium concentrations (% dry weight) of root at final harvest of lettuce cultivar Cortina grown under different day night nutrient solution combinations.**

| | | Night concentration | | Mean[1] |
|---|---|---|---|---|
| | Day concentration | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| **a) Root nitrogen** | 0.5 mS cm$^{-1}$ | 4.274 | 4.153 | 4.214 b |
| | 1.5 mS cm$^{-1}$ | 4.550 | 4.313 | 4.431 a |
| | Mean[1] | 4.412 a | 4.233 b | |
| | Day concentration (D) | ** | | |
| | Night concentration (N) | * | | |
| | D × N | ns | | |
| **b) Root phosphorus** | 0.5 mS cm$^{-1}$ | 0.583 | 0.563 | 0.573 |
| | 1.5 mS cm$^{-1}$ | 0.569 | 0.601 | 0.585 |
| | Mean | 0.576 | 0.582 | |
| | Day concentration (D) | ns | | |
| | Night concentration (N) | ns | | |
| | D × N | ns | | |
| **c) Root potassium** | 0.5 mS cm$^{-1}$ | 0.785[2] c | 2.776 b | 1.781 |
| | 1.5 mS cm$^{-1}$ | 4.278 a | 2.910 b | 3.594 |
| | Mean | 2.532 | 2.843 | |
| | Day concentration (D) | **** | | |
| | Night concentration (N) | * | | |
| | D × N | **** | | |
| **d) Root calcium** | 0.5 mS cm$^{-1}$ | 0.867[2] bc | 0.858 c | 0.862 |
| | 1.5 mS cm$^{-1}$ | 0.946 b | 1.169 a | 1.057 |
| | Mean | 0.906 | 1.013 | |
| | Day concentration (D) | **** | | |
| | Night concentration (N) | ** | | |
| | D × N | ** | | |
| **e) Root magnesium** | 0.5 mS cm$^{-1}$ | 0.297[2] b | 0.280 b | 0.289 |
| | 1.5 mS cm$^{-1}$ | 0.275 b | 0.415 a | 0.345 |
| | Mean | 0.286 | 0.348 | |
| | Day concentration (D) | **** | | |
| | Night concentration (N) | **** | | |
| | D × N | **** | | |

*, **, ***, **** and ns = significant F test at P ≤ 0.05, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD (P ≤ 0.05)

[2] Means within the table sharing the same letter are not significantly different by t-test at P ≤ 0.05

### 5.3.1.2 Head fresh weight

There was a significant interaction between day and night time nutrient solution concentration for head fresh weight (Table 5.3a). The highest head fresh weight was obtained where a concentration of 1.5 mS $cm^{-1}$ was applied during the day. Where a 0.5 mS $cm^{-1}$ concentration was applied during the day, yield was increased by applying 1.5 mS $cm^{-1}$ at night.

### 5.3.1.3 Head dry weight

Only daytime nutrient solution concentration affected head plant dry weight (Table 5.3 b). The highest head dry weight was obtained where a concentration of 1.5 mS $cm^{-1}$ was applied during the day. There was a trend for head dry weight to increase with increasing nutrient solution concentration at night where 0.5 mS $cm^{-1}$ was applied during the day.

### 5.3.1.4 Total plant dry weight

Only daytime nutrient solution concentration affected total plant dry weight (Table 5.3 c). The highest dry weight was obtained where a concentration of 1.5 mS $cm^{-1}$ was applied during the day.

**Table 5.3 Head fresh weight, head dry weight and total plant dry weight of lettuce cultivar Cortina grown under different day night nutrient solution combinations**

| **a) Head fresh weight (g plant$^{-1}$)** | Night concentration | | Mean[1] |
|---|---|---|---|
| Day concentration | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| 0.5 mS cm$^{-1}$ | 160.34[2]c | 202.29 b | 181.32 |
| 1.5 mS cm$^{-1}$ | 242.99 a | 236.11 a | 239.55 |
| Mean | 201.66 | 219.20 | |
| Day concentration (D) | **** | | |
| Night concentration (N) | ** | | |
| D × N | *** | | |
| **b) Head dry weight (g plant$^{-1}$)** | Night concentration | | |
| Day concentration | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| 0.5 mS cm$^{-1}$ | 8.03 | 9.05 | 8.54 b |
| 1.5 mS cm$^{-1}$ | 10.03 | 10.63 | 10.33 a |
| Mean | 9.03 | 9.84 | |
| Day concentration (D) | * | | |
| Night concentration (N) | ns | | |
| D × N | ns | | |
| **c) Total plant dry weight (g plant$^{-1}$)** | Night concentration | | |
| Day concentration | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| 0.5 mS cm$^{-1}$ | 9.04 | 10.05 | 9.54 b |
| 1.5 mS cm$^{-1}$ | 11.04 | 12.03 | 11.53 a |
| Mean | 10.04 | 11.04 | |
| Day concentration (D) | * | | |
| Night concentration (N) | ns | | |
| D × N | ns | | |

*, **, ***, **** and ns = significant F test at P ≤ 0.05, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within column sharing the same letter are not significantly different at LSD (P ≤ 0.05)

[2] Means within the table sharing the same letter are not significantly different by t-test at P ≤ 0.05

### 5.3.1.5 Tipburn incidence

There was a significant interaction between day and night time nutrient solution concentration for tipburn percentage (Table 5.4). Lettuce grown in 1.5 mS $cm^{-1}$ solution at night had the highest tipburn incidence. The 0.5/0.5 mS $cm^{-1}$ day/night combination had the lowest tipburn incidence. Analysis was performed on arcsine transformed data.

**Table 5.4 Tipburn percentage (%) of lettuce cultivar Cortina grown under different day night nutrient solution combinations**

| **Tipburn percentage (%)** | Night concentration | | Mean |
|---|---|---|---|
| Day concentration | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| 0.5 mS $cm^{-1}$ | 2.50[1]c | 57.50 a | 30.00 |
| 1.5 mS $cm^{-1}$ | 22.50 b | 50.00 a | 36.25 |
| Mean | 12.50 | 53.75 | |
| Day concentration (D) | ns | | |
| Night concentration (N) | **** | | |
| D × N | * | | |

*, **, ***, **** and ns = significant F test at P ≤ 0.05, 0.01, 0.001 or 0.0001 levels or non significant respectively

[1] Means within the table sharing the same letter are not significantly different by t-test at P ≤ 0.05

## 5.3.2 Experiment 2.2

### 5.3.2.1 Nutrient concentration of different groups of leaves

#### 5.3.2.1.1 Nitrogen concentration

Before tipburn induction, extra calcium increased the nitrogen concentration in leaves 1-15, while nutrient solution concentration affected the nitrogen concentration of leaves 6-15, where tap water had the lowest nitrogen concentration (Table 5.5).

After tipburn induction, there were interaction effects between calcium level and nutrient solution concentration for nitrogen concentration of leaves 1-10 (Table 5.6). For leaves 1-5 the Ca0/ tap water treatment had the lowest nitrogen concentration. For leaves 6-10 extra calcium increased leaf nitrogen concentration with tap water only, while nutrient solution concentration increased the nitrogen concentration within the Ca0 series only. Here 1.5 mS $cm^{-1}$ had the highest leaf nitrogen concentration. Nutrient solution concentration affected the nitrogen concentration of leaves 11-20. For leaves 11-15, 0.5 mS $cm^{-1}$ had a lower nitrogen concentration than 1.5 mS $cm^{-1}$, while for leaves 16-20 1.5 mS $cm^{-1}$ had the highest nitrogen concentration.

Both before and after induction extra calcium increased the total leaf nitrogen concentration, while before induction, nutrient solution concentrations of 0.5 and 1.5 mS $cm^{-1}$ had the higher total leaf nitrogen concentrations and after induction 1.5 mS $cm^{-1}$ had the highest total leaf nitrogen concentration (Table 5.7). Both before and after induction, the leaf nitrogen concentration increased from the outer to the inner leaves.

#### 5.3.2.1.2 Phosphorus concentration

Before induction, there was an interaction between calcium level and nutrient solution concentration for phosphorus concentration of leaves 1-5 (Table 5.8). Extra calcium

increased the phosphorus concentration with tap water and 1.5 mS $cm^{-1}$. 0.5 mS $cm^{-1}$ had the highest phosphorus concentration for the Ca0 series, while the phosphorus concentration increased with increasing nutrient solution concentration for the Ca100 series. Extra calcium increased the phosphorus concentration in leaves 6-10, while increasing the nutrient solution concentration above that of tap water increased the phosphorus concentration in leaves 6-15.

After induction there was an interaction between calcium level and nutrient solution concentration for phosphorus concentration of leaves 1-5 (Table 5.9). Here the Ca0/tap water combination treatment had the lowest phosphorus concentration. An increase in nutrient solution concentration above that of tap water increased the phosphorus concentration in leaves 6-10.

There was an interaction between calcium level and nutrient solution concentration for total leaf phosphorus concentration before induction (Table 5.10). Extra calcium increased the phosphorus concentration with both tap water and 1.5 mS $cm^{-1}$, while an increase in nutrient solution concentration above that of tap water increased total leaf phosphorus concentration in both the Ca0 and Ca100 series. After induction there was a similar response to an increase nutrient solution concentration. Thus an increase in nutrient solution concentration above that of tap water increased the total leaf phosphorus concentration. Both before and after induction the leaf phosphorus concentration increased from the outer to the inner leaves.

### 5.3.2.1.3 Potassium concentration

Before induction there were no effects of extra calcium on leaf potassium concentration (Table 5.11). An increase in nutrient solution concentration above that of tap water increased the potassium concentration of leaves 11-15.

After induction there was an interaction between calcium level and nutrient solution concentration for potassium concentration of leaves 11-15 (Table 5.12). Here with tap water extra calcium decreased leaf potassium concentration. Extra calcium also decreased the potassium concentration of leaves 21-inner.

For total leaf potassium concentration before induction, 0.5 mS $cm^{-1}$ had a higher potassium concentration than tap water (Table 5.13). Both before and after induction the leaf potassium concentration decreased from the outer to the inner leaves.

### 5.3.2.1.4 Calcium concentration

Before induction there were no significant treatment effects on leaf calcium concentration (Table 5.14).

After induction extra calcium decreased the calcium concentration of leaves 21-inner (Table 5.15).

There were no significant treatment effects on total leaf calcium concentration either before or after induction (Table 5.16). Both before and after induction the leaf calcium concentration decreased from the outer to the inner leaves.

### 5.3.2.1.5 Magnesium concentration

Before induction there were no significant treatment effects on leaf magnesium concentration (Table 5.17).

After induction extra calcium decreased the magnesium concentration of leaves 21-inner (Table 5.18).

Total leaf magnesium concentration before induction of 0.5 mS $cm^{-1}$ was less than that of tap water (Table 5.19a). There were no significant treatment effects on total leaf magnesium concentration after induction (Table 5.19b). Both before and after induction the leaf magnesium concentration decreased from the outer to the inner leaves.

**Table 5.5 Nitrogen concentration (% dry weight) before tipburn induction of different leaf groups of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **Nitrogen concentration before induction** | | | | |
|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean[1] |
| Calcium level | tap water | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| Ca0 | 4.015 | 4.276 | 4.162 | 4.151 b |
| Ca100 | 4.457 | 4.553 | 4.383 | 4.464 a |
| Mean | 4.236 | 4.415 | 4.273 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ** | | | |
| S × C | ns | | | |
| **b) Leaves 6-10** | | | | |
| Ca0 | 4.956 | 5.272 | 5.150 | 5.126 b |
| Ca100 | 5.045 | 5.496 | 5.400 | 5.314 a |
| Mean[1] | 5.000 b | 5.384 a | 5.275 a | |
| Solution concentration (S) | ** | | | |
| Calcium level (C) | * | | | |
| S × C | ns | | | |
| **c) Leaves 11-15** | | | | |
| Ca0 | 5.904 | 6.124 | 6.099 | 6.042 b |
| Ca100 | 5.930 | 6.376 | 6.329 | 6.212 a |
| Mean[1] | 5.917 b | 6.250 a | 6.214 a | |
| Solution concentration (S) | ** | | | |
| Calcium level (C) | * | | | |
| S × C | ns | | | |
| **d) Leaves 16-inner** | | | | |
| Ca0 | 7.675 | 7.422 | 7.309 | 7.469 |
| Ca100 | 7.282 | 7.479 | 7.521 | 7.427 |
| Mean | 7.479 | 7.450 | 7.415 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or columns sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

**Table 5.6 Nitrogen concentration (% dry weight) after tipburn induction of different leaf groups of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| Nitrogen concentration after induction | | | | |
|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 4.242[2] b | 4.758 a | 4.826 a | 4.609 |
| Ca100 | 4.731 a | 4.899 a | 4.819 a | 4.816 |
| Mean | 4.486 | 4.828 | 4.822 | |
| Solution concentration (S) | ** | | | |
| Calcium level (C) | ** | | | |
| S × C | * | | | |
| **b) Leaves 6-10** | | | | |
| Ca0 | 5.351[2] c | 5.538 bc | 5.824 a | 5.571 |
| Ca100 | 5.757 ab | 5.723 ab | 5.728 ab | 5.736 |
| Mean | 5.554 | 5.630 | 5.776 | |
| Solution concentration (S) | * | | | |
| Calcium level (C) | * | | | |
| S × C | * | | | |
| **c) Leaves 11-15** | | | | |
| Ca0 | 5.925 | 5.513 | 6.488 | 5.975 |
| Ca100 | 6.111 | 6.091 | 6.375 | 6.192 |
| Mean[1] | 6.018 ab | 5.802 b | 6.432 a | |
| Solution concentration (S) | * | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **d) Leaves 16-20** | | | | |
| Ca0 | 6.501 | 6.647 | 6.932 | 6.693 |
| Ca100 | 6.514 | 6.546 | 6.703 | 6.588 |
| Mean[1] | 6.507 b | 6.597 b | 6.818 a | |
| Solution concentration (S) | ** | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **e) Leaves 21-inner** | | | | |
| Ca0 | 7.700 | 7.364 | 7.713 | 7.593 |
| Ca100 | 7.366 | 7.443 | 7.733 | 7.514 |
| Mean | 7.533 | 7.404 | 7.723 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

[2] Means within the table sharing the same letter are not significantly different by t-test at $P \leq 0.05$

**Table 5.7 Nitrogen concentration (% dry weight) of total leaf of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **a) Nitrogen concentration total leaf before induction** | | | | |
|---|---|---|---|---|
| | Solution concentration at night | | | Mean [1] |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 5.162 | 5.558 | 5.437 | 5.386 b |
| Ca100 | 5.345 | 5.608 | 5.652 | 5.535 a |
| Mean[1] | 5.253 b | 5.583 a | 5.545 a | |
| Solution concentration (S) | **** | | | |
| Calcium level (C) | **** | | | |
| S × C | ns | | | |
| **b) Nitrogen concentration total leaf after induction** | | | | |
| Ca0 | 5.664 | 5.667 | 5.944 | 5.758 b |
| Ca100 | 5.823 | 5.924 | 5.983 | 5.910 a |
| Mean[1] | 5.743 b | 5.795 b | 5.964 a | |
| Solution concentration (S) | *** | | | |
| Calcium level (C) | *** | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

**Table 5.8 Phosphorus concentration (% dry weight) before tipburn induction of different leaf groups of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **Phosphorus concentration before induction** | | | | |
|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean[1] |
| Calcium level | tap water | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| Ca0 | 0.405[2] d | 5.82 b | 5.41 c | 5.09 |
| Ca100 | 0.529 c | 5.93 b | 6.47 a | 5.90 |
| Mean | 0.467 | 5.88 | 5.94 | |
| Solution concentration (S) | **** | | | |
| Calcium level (C) | **** | | | |
| S × C | **** | | | |
| **b) Leaves 6-10** | | | | |
| Ca0 | 0.649 | 0.7.54 | 0.732 | 0.712 b |
| Ca100 | 0.711 | 0.803 | 0.773 | 0.763 a |
| Mean[1] | 0.680 b | 0.778 a | 0.753 a | |
| Solution concentration (S) | *** | | | |
| Calcium level (C) | ** | | | |
| S × C | ns | | | |
| **c) Leaves 11-15** | | | | |
| Ca0 | 0.881 | 0.945 | 0.958 | 0.928 |
| Ca100 | 0.912 | 0.994 | 0.963 | 0.956 |
| Mean[1] | 0.896 b | 0.969 a | 0.960 a | |
| Solution concentration (S) | ** | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **d) Leaves 16-inner** | | | | |
| Ca0 | 1.197 | 1.180 | 1.171 | 1.183 |
| Ca100 | 1.184 | 1.202 | 1.200 | 1.195 |
| Mean | 1.190 | 1.191 | 1.185 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

[2] Means within the table sharing the same letter are not significantly different by t-test at $P \leq 0.05$

**Table 5.9 Phosphorus concentration (% dry weight) after tipburn induction of different leaf groups of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **Phosphorus concentration after induction** | | | | |
|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 0.531[2] b | 0.788 a | 0.731 a | 0.683 |
| Ca100 | 0.741 a | 0.759 a | 0.793 a | 0.765 |
| Mean | 0.636 | 0.774 | 0.762 | |
| Solution concentration (S) | ** | | | |
| Calcium level (C) | * | | | |
| S × C | * | | | |
| **b) Leaves 6-10** | | | | |
| Ca0 | 0.923 | 1.079 | 1.100 | 1.034 |
| Ca100 | 1.054 | 1.104 | 1.092 | 1.083 |
| Mean[1] | 0.988 b | 1.092 a | 1.096 a | |
| Solution concentration (S) | ** | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **c) Leaves 11-15** | | | | |
| Ca0 | 1.226 | 1.293 | 1.326 | 1.282 |
| Ca100 | 1.218 | 1.294 | 1.328 | 1.280 |
| Mean | 1.222 | 1.293 | 1.327 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **d) Leaves 16-20** | | | | |
| Ca0 | 1.197 | 1.236 | 1.259 | 1.231 |
| Ca100 | 1.196 | 1.246 | 1.270 | 1.237 |
| Mean | 1.196 | 1.241 | 1.265 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **e) Leaves 21-inner** | | | | |
| Ca0 | 1.239 | 1.320 | 1.298 | 1.286 |
| Ca100 | 1.237 | 1.253 | 1.283 | 1.258 |
| Mean | 1.238 | 1.287 | 1.290 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

[2] Means within the table sharing the same letter are not significantly different by t-test at $P \leq 0.05$

**Table 5.10 Phosphorus concentration (% dry weight) of total leaf of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| a) Phosphorus concentration total leaf before tipburn induction | | | | |
|---|---|---|---|---|
| | Solution concentration at night | | | Mean |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 0.692[2] d | 0.817 ab | 0.801 b | 0.770 |
| Ca100 | 0.769 c | 0.824 ab | 0.837 a | 0.810 |
| Mean | 0.730 | 0.820 | 0.819 | |
| Solution concentration (S) | **** | | | |
| Calcium level (C) | **** | | | |
| S × C | ** | | | |
| **b) Phosphorus concentration total leaf after tipburn induction** | | | | |
| Ca0 | 1.007 | 1.120 | 1.092 | 1.073 |
| Ca100 | 1.061 | 1.121 | 1.119 | 1.101 |
| Mean[1] | 1.034 b | 1.121 a | 1.106 a | |
| Solution concentration (S) | * | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

[2] Means within the table sharing the same letter are not significantly different by t-test at $P \leq 0.05$

**Table 5.11 Potassium concentration (% dry weight) before tipburn induction of different leaf groups of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **Potassium concentration before induction** | | | | |
|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 11.587 | 10.684 | 10.722 | 10.997 |
| Ca100 | 11.762 | 11.930 | 11.467 | 11.720 |
| Mean | 11.674 | 11.307 | 11.095 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **b) Leaves 6-10** | | | | |
| Ca0 | 8.736 | 10.082 | 9.479 | 9.432 |
| Ca100 | 9.328 | 9.456 | 9.660 | 9.481 |
| Mean | 9.032 | 9.769 | 9.569 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **c) Leaves 11-15** | | | | |
| Ca0 | 6.487 | 7.474 | 7.735 | 7.232 |
| Ca100 | 6.870 | 7.170 | 6.969 | 7.003 |
| Mean[1] | 6.678 b | 7.322 a | 7.352 a | |
| Solution concentration (S) | * | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **d) Leaves 16-inner** | | | | |
| Ca0 | 5.332 | 5.922 | 5.678 | 5.644 |
| Ca100 | 5.670 | 5.465 | 5.769 | 5.635 |
| Mean | 5.501 | 5.694 | 5.724 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

**Table 5.12 Potassium concentration (% dry weight) after tipburn induction of different leaf groups of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night**

| **Potassium concentration after induction** | | | | | |
|---|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean[1] | |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | | |
| Ca0 | 12.739 | 13.799 | 12.349 | 12.962 | |
| Ca100 | 14.141 | 14.017 | 12.736 | 13.631 | |
| Mean | 13.440 | 13.908 | 12.542 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **b) Leaves 6-10** | | | | | |
| Ca0 | 11.892 | 13.799 | 13.120 | 12.937 | |
| Ca100 | 12.422 | 12.529 | 13.066 | 12.672 | |
| Mean | 12.157 | 13.164 | 13.093 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **c) Leaves 11-15** | | | | | |
| Ca0 | 10.797[2] a | 10.491 a | 9.257 ab | 10.182 | |
| Ca100 | 8.680 b | 10.348 ab | 10.404 ab | 9.811 | |
| Mean | 9.738 | 10.420 | 9.830 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | * | | | | |
| **d) Leaves 16-20** | | | | | |
| Ca0 | 6.518 | 6.263 | 6.362 | 6.381 | |
| Ca100 | 6.417 | 6.488 | 6.462 | 6.456 | |
| Mean | 6.467 | 6.375 | 6.412 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **e) Leaves 21-inner** | | | | | |
| Ca0 | 5.574 | 5.758 | 5.697 | 5.677 | a |
| Ca100 | 5.181 | 5.248 | 5.183 | 5.204 | b |
| Mean | 5.378 | 5.503 | 5.440 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | * | | | | |
| S × C | ns | | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

[2] Means within the table sharing the same letter are not significantly different by t-test at $P \leq 0.05$

**Table 5.13 Potassium concentration (% dry weight) of total leaf of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **a) Potassium concentration total leaf before tipburn induction** | | | | |
|---|---|---|---|---|
| | Solution concentration at night | | | Mean |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 8.526 | 9.098 | 8.817 | 8.814 |
| Ca100 | 8.787 | 9.237 | 8.888 | 8.971 |
| Mean[1] | 8.656 b | 9.168 a | 8.852 ab | |
| Solution concentration (S) | * | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **b) Potassium concentration total leaf after tipburn induction** | | | | |
| Ca0 | 10.492 | 11.453 | 10.883 | 10.943 |
| Ca100 | 10.515 | 10.752 | 10.677 | 10.648 |
| Mean | 10.504 | 11.103 | 10.780 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

**Table 5.14 Calcium concentration (% dry weight) before tipburn induction of different leaf groups of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **Calcium concentration before induction** | | | | |
|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 1.242 | 1.204 | 1.270 | 1.239 |
| Ca100 | 1.385 | 1.274 | 1.406 | 1.355 |
| Mean | 1.314 | 1.239 | 1.338 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **b) Leaves 6-10** | | | | |
| Ca0 | 0.762 | 0.772 | 0.826 | 0.787 |
| Ca100 | 0.780 | 0.704 | 0.814 | 0.766 |
| Mean | 0.771 | 0.738 | 0.820 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **c) Leaves 11-15** | | | | |
| Ca0 | 0.408 | 0.446 | 0.484 | 0.446 |
| Ca100 | 0.429 | 0.406 | 0.422 | 0.419 |
| Mean | 0.419 | 0.426 | 0.453 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **d) Leaves 16-inner** | | | | |
| Ca0 | 0.131 | 0.169 | 0.157 | 0.152 |
| Ca100 | 0.135 | 0.135 | 0.150 | 0.140 |
| Mean | 0.133 | 0.152 | 0.153 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

**Table 5.15 Calcium concentration (% dry weight) after tipburn induction of different leaf groups of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **Calcium concentration after induction** | | | | | |
|---|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean[1] | |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | | |
| Ca0 | 1.858 | 1.880 | 1.723 | 1.820 | |
| Ca100 | 1.742 | 1.909 | 1.864 | 1.838 | |
| Mean | 1.800 | 1.895 | 1.793 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **b) Leaves 6-10** | | | | | |
| Ca0 | 1.491 | 1.389 | 1.286 | 1.388 | |
| Ca100 | 1.361 | 1.488 | 1.427 | 1.425 | |
| Mean | 1.426 | 1.438 | 1.356 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **c) Leaves 11-15** | | | | | |
| Ca0 | 0.995 | 0.852 | 0.802 | 0.883 | |
| Ca100 | 0.797 | 0.864 | 0.858 | 0.840 | |
| Mean | 0.896 | 0.858 | 0.830 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **d) Leaves 16-20** | | | | | |
| Ca0 | 0.479 | 0.356 | 0.364 | 0.399 | |
| Ca100 | 0.329 | 0.355 | 0.374 | 0.353 | |
| Mean | 0.404 | 0.355 | 0.369 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **e) Leaves 21-inner** | | | | | |
| Ca0 | 0.232 | 0.285 | 0.227 | 0.248 | a |
| Ca100 | 0.203 | 0.220 | 0.198 | 0.207 | b |
| Mean | 0.218 | 0.252 | 0.213 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | * | | | | |
| S × C | ns | | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

**Table 5.16 Calcium concentration (% dry weight) of total leaf of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **a) Calcium concentration total leaf before induction** | | | | |
|---|---|---|---|---|
| | Solution concentration at night | | | Mean |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 0.728 | 0.686 | 0.741 | 0.718 |
| Ca100 | 0.741 | 0.724 | 0.752 | 0.739 |
| Mean | 0.735 | 0.705 | 0.746 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **b) Calcium concentration total leaf after induction** | | | | |
| Ca0 | 1.171 | 1.152 | 1.120 | 1.148 |
| Ca100 | 1.081 | 1.131 | 1.133 | 1.115 |
| Mean | 1.126 | 1.141 | 1.127 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

**Table 5.17 Magnesium concentration (% dry weight) before tipburn induction of different leaf groups of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| Magnesium concentration before tipburn induction | | | | |
|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 0.513 | 0.512 | 0.547 | 0.524 |
| Ca100 | 0.541 | 0.456 | 0.621 | 0.539 |
| Mean | 0.527 | 0.484 | 0.584 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **b) Leaves 6-10** | | | | |
| Ca0 | 0.278 | 0.261 | 0.236 | 0.258 |
| Ca100 | 0.291 | 0.227 | 0.271 | 0.263 |
| Mean | 0.285 | 0.244 | 0.253 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **c) Leaves 11-15** | | | | |
| Ca0 | 0.169 | 0.176 | 0.181 | 0.175 |
| Ca100 | 0.169 | 0.181 | 0.176 | 0.175 |
| Mean | 0.169 | 0.179 | 0.179 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **d) Leaves 16-inner** | | | | |
| Ca0 | 0.153 | 0.158 | 0.146 | 0.152 |
| Ca100 | 0.155 | 0.147 | 0.159 | 0.154 |
| Mean | 0.154 | 0.152 | 0.152 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

**Table 5.18 Magnesium concentration (% dry weight) after tipburn induction of different leaf groups of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **Magnesium concentration after tipburn induction** | | | | | |
|---|---|---|---|---|---|
| **a) leaves 1-5** | Solution concentration at night | | | Mean[1] | |
| Calcium level | Tap water | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | | |
| Ca0 | 0.575 | 0.525 | 0.448 | 0.516 | |
| Ca100 | 0.503 | 0.505 | 0.508 | 0.505 | |
| Mean | 0.539 | 0.515 | 0.478 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **b) Leaves 6-10** | | | | | |
| Ca0 | 0.367 | 0.353 | 0.316 | 0.345 | |
| Ca100 | 0.349 | 0.354 | 0.332 | 0.345 | |
| Mean | 0.358 | 0.353 | 0.324 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **c) Leaves 11-15** | | | | | |
| Ca0 | 0.294 | 0.257 | 0.227 | 0.259 | |
| Ca100 | 0.239 | 0.258 | 0.242 | 0.246 | |
| Mean | 0.266 | 0.257 | 0.235 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **d) Leaves 16-20** | | | | | |
| Ca0 | 0.210 | 0.183 | 0.193 | 0.195 | |
| Ca100 | 0.176 | 0.184 | 0.183 | 0.181 | |
| Mean | 0.193 | 0.183 | 0.188 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | ns | | | | |
| S × C | ns | | | | |
| **e) Leaves 21-inner** | | | | | |
| Ca0 | 0.185 | 0.202 | 0.173 | 0.187 | a |
| Ca100 | 0.178 | 0.171 | 0.174 | 0.174 | b |
| Mean | 0.181 | 0.187 | 0.173 | | |
| Solution concentration (S) | ns | | | | |
| Calcium level (C) | * | | | | |
| S × C | ns | | | | |

*, **, ***, **** and ns = significant F test at P ≤ 0.05, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within column sharing the same letter are not significantly different at LSD (P ≤ 0.05)

**Table 5.19 Magnesium concentration (% dry weight) of total leaf of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **a) Magnesium concentration total leaf before tipburn induction** | | | | |
|---|---|---|---|---|
| | Solution concentration at night | | | Mean |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 0.288 | 0.259 | 0.257 | 0.268 |
| Ca100 | 0.290 | 0.260 | 0.292 | 0.281 |
| Mean[1] | 0.289 a | 0.259 b | 0.275 ab | |
| Solution concentration (S) | * | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **b) Magnesium concentration total leaf after tipburn induction** | | | | |
| Ca0 | 0.344 | 0.331 | 0.303 | 0.326 |
| Ca100 | 0.319 | 0.317 | 0.314 | 0.317 |
| Mean | 0.331 | 0.324 | 0.309 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within row sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

### 5.3.2.2 Head fresh weight

There was an interaction between calcium level and nutrient solution concentration for head fresh weight before tipburn induction (Table 5.20a). Extra calcium (Ca100) increased head fresh weight except at 0.5 mS $cm^{-1}$, where there was no difference. Within the Ca0 series 0.5 mS $cm^{-1}$ increased fresh weight more than 1.5 mS $cm^{-1}$ above that of tap water, while for the Ca100 series fresh weight increased as the solution concentration increased above that of tap water, but there was no difference between the 0.5 and 1.5 mS $cm^{-1}$ treatments.

For the harvest after induction (Table 5.20b) and the final harvest (Table 5.20c) both extra calcium and nutrient solution concentration at night affected fresh weight. Plants grown in the Ca100 series had a greater fresh weight than those in the Ca0 series, while as the solution concentration increased above that of tap water fresh weight increased. There was however no significant difference in fresh weight between the 0.5 and 1.5 mS $cm^{-1}$ treatments.

### 5.3.2.3 Total leaf dry weight

There was an interaction between calcium level and nutrient solution concentration for total leaf dry weight before tipburn induction (Table 5.21a). Extra calcium increased the total leaf weight of the plants grown in tap water only. As the solution concentration increased, within both the Ca0 and the Ca100 series, above that of tap water total leaf dry weight increased. There was no difference however between the 0.5 and 1.5 mS $cm^{-1}$ treatments, apart from Ca0-1.5 mS $cm^{-1}$ and Ca100-0.5 mS $cm^{-1}$ where the latter had the greater leaf dry weight. Extra calcium increased the total leaf dry weight after induction (Table 5.21b).

**Table 5.20 Fresh weight before, after tipburn induction and at final harvest of lettuce cultivar Cortina grown under different nutrient solution concentration and calcium level at night.**

| **a) Fresh weight before induction (g plant$^{-1}$)** | | | | |
|---|---|---|---|---|
| | Solution concentration at night | | | Mean[1] |
| Calcium level | Tap water | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| Ca0 | 35.06[2] c | 53.87 a | 46.37 b | 45.10 |
| Ca100 | 47.17 b | 54.04 a | 53.50 a | 51.57 |
| Mean | 41.12 | 53.95 | 49.93 | |
| Solution concentration (S) | **** | | | |
| Calcium level (C) | ** | | | |
| S × C | * | | | |
| **b) Fresh weight after induction (g plant$^{-1}$)** | | | | |
| Ca0 | 78.57 | 100.68 | 86.13 | 88.46 b |
| Ca100 | 90.53 | 102.66 | 102.84 | 98.68 a |
| Mean[1] | 84.55 b | 101.67 a | 94.48 a | |
| Solution concentration (S) | ** | | | |
| Calcium level (C) | ** | | | |
| S × C | ns | | | |
| **c) Fresh weight at final harvest (g plant$^{-1}$)** | | | | |
| Ca0 | 119.88 | 158.38 | 149.10 | 142.45 b |
| Ca100 | 136.78 | 158.17 | 159.78 | 151.58 a |
| Mean[1] | 128.33 b | 158.27 a | 154.44 a | |
| Solution concentration (S) | **** | | | |
| Calcium level (C) | * | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at P ≤ 0.05, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD (P ≤ 0.05)

[2] Means within the table sharing the same letter are not significantly different by t-test at P ≤ 0.05

**Table 5.21 Dry weight of total leaf before and after tipburn induction of lettuce cultivar Cortina grown under different nutrient solution concentrations and calcium levels at night.**

| **Total leaf dry weight before induction (g plant$^{-1}$)** | | | | |
|---|---|---|---|---|
| | Solution concentration at night | | | Mean[1] |
| Calcium level | tap water | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| Ca0 | 3.39[2] d | 4.76 ab | 4.34 bc | 4.16 |
| Ca100 | 4.33 c | 4.84 a | 4.77 ab | 4.65 |
| Mean | 3.86 | 4.80 | 4.55 | |
| Solution concentration (S) | **** | | | |
| Calcium level (C) | *** | | | |
| S × C | * | | | |
| **Total leaf dry weight after induction (g plant$^{-1}$)** | | | | |
| Ca0 | 6.17 | 6.87 | 7.05 | 6.70 b |
| Ca100 | 7.33 | 7.84 | 7.38 | 7.51 a |
| Mean | 6.75 | 7.35 | 7.22 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | * | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at P ≤ 0.05, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within column sharing the same letter are not significantly different at LSD (P ≤ 0.05)

[2] Means within the table sharing the same letter are not significantly different by t-test at P ≤ 0.05

## 5.3.2.4 Tipburn incidence

There were no significant differences in tipburn percentage (Table 5.22). This analysis was performed on arcsine transformed data. Extra calcium increased the number of leaves with tipburn per plant, the tipburn score and the tipburn index. Nutrient solution concentration affected the number of tipburn leaves per plant and the tipburn index. In both cases the 0.5 mS cm$^{-1}$ treatment decreased the incidence of tipburn.

**Table 5.22 Tipburn percentage, number of leaves with tipburn per plant, tipburn score and tipburn index of lettuce cultivar Cortina under different nutrient solution concentrations and calcium levels at night.**

| **Tipburn percentage (%)** | | | | |
|---|---|---|---|---|
| | Solution concentration at night | | | Mean[1] |
| Calcium level | tap water | 0.5 mS $cm^{-1}$ | 1.5 mS $cm^{-1}$ | |
| Ca0 | 100.00 | 96.43 | 100.00 | 98.81 |
| Ca100 | 100.00 | 96.43 | 100.00 | 98.81 |
| Mean | 100.00 | 96.43 | 100.00 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **Number of leaves per plant with tipburn** | | | | |
| Ca0 | 6.96 | 4.71 | 6.21 | 5.96 b |
| Ca100 | 8.54 | 6.32 | 10.36 | 8.40 a |
| Mean[1] | 7.75 a | 5.52 b | 8.29 a | |
| Solution concentration (S) | * | | | |
| Calcium level (C) | ** | | | |
| S × C | ns | | | |
| **Tipburn score** | | | | |
| Ca0 | 1.54 | 1.36 | 1.36 | 1.42 b |
| Ca100 | 1.61 | 1.46 | 1.89 | 1.65 a |
| Mean | 1.57 | 1.41 | 1.63 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | * | | | |
| S × C | ns | | | |
| **Tipburn index** | | | | |
| Ca0 | 10.78 | 6.49 | 8.54 | 8.60 b |
| Ca100 | 14.21 | 9.57 | 19.46 | 14.41 a |
| Mean[1] | 12.50 a | 8.03 b | 14.00 a | |
| Solution concentration (S) | * | | | |
| Calcium level (C) | ** | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

### 5.3.2.5 Dry weight and relative growth rate of different leaf groups

Before induction the only significant treatment effect on the dry weight of the different leaf groups was with leaves 6-10 (Table 5.23). Here extra calcium increased leaf dry weight, while tap water had a lower dry weight than the 0.5 and 1.5 mS $cm^{-1}$ treatments.

After induction there was an interaction between calcium level and nutrient solution concentration for dry weight of leaves 1-5 (Table 5.24a). Extra calcium increased leaf dry weight only with tap water. For the Ca0 series, leaf dry weight increased with solution concentration, while there were no differences within the Ca100 series. Extra calcium increased the leaf dry weight of leaves 16-inner (Table 5.24d-e).

There were no significant treatment effects on the RGR of the various leaf groups or for total leaf during induction (Table 5.25). There was a trend however, with both calcium series for the RGR to be higher for the inner leaves (Figure 5.1). There was a trend for tap water to have the greatest growth rate during tipburn induction period (Table 5.25).

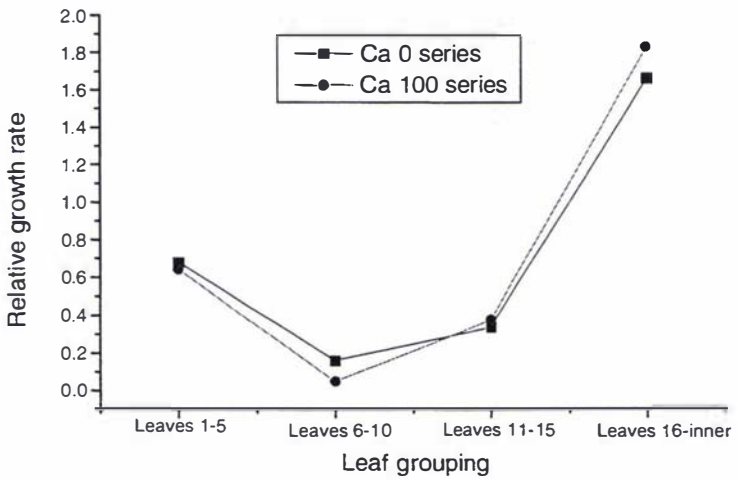


**Figure 5.1 Relative growth rate of different leaf groups of lettuce grown in two levels of calcium concentration at night.**

**Table 5.23 Dry weight before tipburn induction of different groups of leaves of lettuce cultivar Cortina under different nutrient solution concentrations and calcium levels at night.**

| Dry weight before induction (g plant$^{-1}$) | | | | |
|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean[1] |
| Calcium level | tap water | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| Ca0 | 0.66 | 0.81 | 0.73 | 0.73 |
| Ca100 | 0.76 | 1.07 | 0.82 | 0.88 |
| Mean | 0.71 | 0.94 | 0.77 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **b) Leaves 6-10** | | | | |
| Ca0 | 1.62 | 2.02 | 1.90 | 1.85 b |
| Ca100 | 2.00 | 2.32 | 2.13 | 2.15 a |
| Mean[1] | 1.81 b | 2.17 a | 2.01 a | |
| Solution concentration (S) | ** | | | |
| Calcium level (C) | *** | | | |
| S × C | ns | | | |
| **c) Leaves 11-15** | | | | |
| Ca0 | 0.95 | 1.52 | 1.42 | 1.30 |
| Ca100 | 1.29 | 1.20 | 1.55 | 1.35 |
| Mean | 1.12 | 1.36 | 1.49 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **d) Leaves 16-inner** | | | | |
| Ca0 | 0.16 | 0.41 | 0.29 | 0.28 |
| Ca100 | 0.28 | 0.26 | 0.27 | 0.27 |
| Mean | 0.22 | 0.33 | 0.28 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within rows or column sharing the same letter are not significantly different at LSD ($P \leq 0.05$)

**Table 5.24 Dry weight after tipburn induction of different groups of leaves of lettuce cultivar Cortina under different nutrient solution concentrations and calcium levels at night.**

| **Dry weight after induction (g plant$^{-1}$)** | | | | |
|---|---|---|---|---|
| **a) Leaves 1-5** | Solution concentration at night | | | Mean [1] |
| Calcium level | tap water | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| Ca0 | 1.02[2] c | 1.48 b | 1.79 a | 1.43 |
| Ca100 | 1.74 ab | 1.51 b | 1.69 ab | 1.65 |
| Mean | 1.38 | 1.50 | 1.74 | |
| Solution concentration (S) | ** | | | |
| Calcium level (C) | * | | | |
| S × C | *** | | | |
| **b) Leaves 6-10** | | | | |
| Ca0 | 2.06 | 2.17 | 2.35 | 2.19 |
| Ca100 | 2.14 | 2.38 | 2.25 | 2.25 |
| Mean | 2.10 | 2.27 | 2.30 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **c) Leaves 11-15** | | | | |
| Ca0 | 1.73 | 1.79 | 1.76 | 1.76 |
| Ca100 | 1.99 | 2.19 | 1.69 | 1.96 |
| Mean | 1.86 | 1.99 | 1.72 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **d) Leaves 16-20** | | | | |
| Ca0 | 0.95 | 0.95 | 0.84 | 0.91 b |
| Ca100 | 0.98 | 1.13 | 1.12 | 1.08 a |
| Mean | 0.97 | 1.04 | 0.98 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | * | | | |
| S × C | ns | | | |
| **e) Leaves 21-inner** | | | | |
| Ca0 | 0.41 | 0.47 | 0.32 | 0.40 b |
| Ca100 | 0.48 | 0.63 | 0.63 | 0.58 a |
| Mean | 0.45 | 0.55 | 0.47 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | *** | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at P ≤ 0.05, 0.01, 0.001 or 0.0001 levels and non significant respectively

[1] Means within column sharing the same letter are not significantly different at LSD (P ≤ 0.05)

[2] Means within the table sharing the same letter are not significantly different by t-test at P ≤ 0.05

**Table 5.25 Relative growth rate (RGR) during tipburn induction period of different groups of leaves of lettuce cultivar Cortina under different nutrient solution concentrations and calcium levels at night.**

| **RGR during induction (g g$^{-1}$)** | | | | |
|---|---|---|---|---|
| **Leaves 1-5** | Solution concentration at night | | | Mean |
| Calcium level | tap water | 0.5 mS cm$^{-1}$ | 1.5 mS cm$^{-1}$ | |
| Ca0 | 0.42 | 0.68 | 0.96 | 0.68 |
| Ca100 | 0.81 | 0.36 | 0.75 | 0.64 |
| Mean | 0.62 | 0.52 | 0.85 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **Leaves 6-10** | | | | |
| Ca0 | 0.23 | 0.05 | 0.21 | 0.16 |
| Ca100 | 0.06 | 0.02 | 0.05 | 0.05 |
| Mean | 0.15 | 0.04 | 0.13 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **Leaves 11-15** | | | | |
| Ca0 | 0.60 | 0.20 | 0.23 | 0.34 |
| Ca100 | 0.43 | 0.61 | 0.10 | 0.38 |
| Mean | 0.52 | 0.41 | 0.16 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **Leaves 16-inner** | | | | |
| Ca0 | 2.16 | 1.40 | 1.43 | 1.66 |
| Ca100 | 1.65 | 1.94 | 1.90 | 1.83 |
| Mean | 1.91 | 1.67 | 1.66 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |
| **Total leaf** | | | | |
| Ca0 | 0.59 | 0.36 | 0.49 | 0.48 |
| Ca100 | 0.53 | 0.48 | 0.44 | 0.48 |
| Mean | 0.56 | 0.42 | 0.46 | |
| Solution concentration (S) | ns | | | |
| Calcium level (C) | ns | | | |
| S × C | ns | | | |

*, **, ***, **** and ns = significant F test at $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels and non significant respectively

## 5.4 Discussion

### 5.4.1 Experiment 2.1

#### 5.4.1.1 Tissue nutrient concentrations

##### 5.4.1.1.1 Leaf tissue

Leaf nitrogen concentration was lowest with the 0.5/0.5 mS $cm^{-1}$ combination, which is not an unexpected result as this was a low feeding programme (5.3.1.1.1). Leaf nitrogen concentration was increased by the 1.5 mS $cm^{-1}$ treatment at night or during the day where 0.5 mS $cm^{-1}$ had been applied during the alternative 12 hour period. In both cases a response to an increase above a less than adequate supply of nitrogen. The 1.5/0.5 mS $cm^{-1}$ combination had the highest nitrogen concentration. This could be explained in term of a root pressure effect supplying more nitrogen to the inner leaves. Similarly Choi et al.(1997) reported that high humidity at night, which will favour root pressure, increased the nitrogen concentration of both outer and inner leaves of strawberry.

Root pressure occurs under conditions where transpiration in not functioning or is at a very low level, and can be encouraged by increasing night time relative humidity, and by reducing the resistance to water movement into the plant (Bradfield & Guttridge, 1984; Wien, 1997). Thus maintaining adequate soil water, and having a low night time osmotic pressure of the soil solution are two ways in which water uptake by root pressure can be maximised (Wien, 1997). As 0.5 mS $cm^{-1}$ was of a lower concentration than 1.5 mS $cm^{-1}$ then more nitrogen would have been taken up by the 1.5/0.5 mS $cm^{-1}$ treatment via root pressure.

Phosphorus concentration was unaffected by the treatments. Potassium concentrations were affected by the treatments and increased in concentration in an order that could be

explained by the concentrations in the nutrient solutions. Luxury consumption may have been involved as reported in 3.4.3.3. Vacuolar storage during the active growth period has been termed ' luxury consumption', it enables plants to accumulate nutrients during pulses of nutrient availability to support growth when nutrient uptake is insufficient (Chapin, 1988). When grown under a similar nutrient regime, low-nutrient adapted plants exhibit greater luxury consumption than high-nutrient adapted plants (Stulen et al., 1981; Chapin, 1988).

With calcium, although the 0.5/0.5 mS $cm^{-1}$ treatment had the highest leaf concentration it was only significantly different from the 1.5/0.5 mS $cm^{-1}$ combination. This could be explained in terms of competitive effect of potassium on calcium uptake (Smith & Wallace, 1956; Geraldson, 1971). The other leaf calcium concentration trends were in the order expected, apart from the 1.5/1.5 mS $cm^{-1}$ day/night treatment. That is, apart from this treatment, leaf calcium concentrations decreased with increasing potassium concentration in the nutrient solution. No explanation can be offered for the high leaf calcium concentration for the 1.5/1.5 mS $cm^{-1}$ day/night treatment.

Contrasting explanations have been used above for the nitrogen and potassium responses. In the case of nitrogen it was suggested that root pressure had a role in determining leaf nitrogen concentration, whereas with potassium it was suggested that it was a simple response to nutrient solution concentration. Luxury absorption was also a factor here. Root pressure it has also been argued is a means of distributing nutrients, particularly calcium, which is not redistributed via the phloem, more effectively to the inner leaves. An alternative explanation for the nitrogen response is that root pressure improved calcium distribution to the inner leaves, which accounted for the increased fresh weight of this treatment and the high nitrogen concentration was associated with this increased growth.

Magnesium leaf concentrations demonstrated the competitive effect of potassium on the uptake of this nutrient (Mason, 1964; Haworth & Cleaver, 1967; Geraldson, 1971; Willumsen, 1984; Winsor & Adams, 1987). Thus the highest concentration for magnesium occurred with the 0.5/0.5 mS $cm^{-1}$ combination and the lowest with 1.5 mS $cm^{-1}$ during the day.

### 5.4.1.1.2 Root tissue

Root nitrogen concentration increased with an increase in concentration during the day and with a decrease in concentration at night (5.3.1.1.2). The day effect could be explained in terms of a nutrient solution concentration effect and the night effect as a response to root pressure in the shoot.

As with the leaf there were no treatment effects on root phosphorus concentration. Root potassium concentrations are hard to explain. The 0.5/0.5 mS $cm^{-1}$ was very low while 1.5/0.5 mS $cm^{-1}$ was very high. The high 1.5/0.5 mS $cm^{-1}$ could be a root pressure effect and the increase on 0.5/0.5 mS $cm^{-1}$ a response from a very low to satisfactory levels.

Root calcium and magnesium concentrations did not appear to be greatly effected by nutrient solution potassium concentration as the highest concentrations occurred with the 1.5/1.5 mS $cm^{-1}$ treatment.

### 5.4.1.2 Fresh weight

Where 0.5 mS $cm^{-1}$ was applied during the day head fresh weight at maturity increased with 1.5 mS $cm^{-1}$ at night (5.3.1.2). It is suggested that this was a response to a more adequate supply of nutrients. The increases in leaf nitrogen and potassium concentrations that were obtained with the 1.5 mS $cm^{-1}$ plants support this conclusion. It is suggested where nutrient supply during the day is low then root pressure is not of significance as in that case all leaves require nutrients.

Where 1.5 mS $cm^{-1}$ was applied during the day, then the response of treatments 0.5 and 1.5 mS $cm^{-1}$ from the Ca0 series of Experiment 2.2 should be considered at the same time as they are an identical set of treatments to 1.5/1.5 and 1.5/0.5 mS $cm^{-1}$. In both instances it is proposed that root pressure was active. The results of both Experiments are considered therefore when the results of the treatments of Experiment 2.2 on fresh weight are discussed (5.4.2.2). Another explanation is that lowering of concentration at night allowed more water to be taken up by the plants and so their fresh weight

increased. Alternatively both processes may have operated, as they are both components of root pressure.

### 5.4.1.3 Dry weight

Head and total plant dry weight increased with increasing nutrient solution concentration during the day (5.3.1.3 and 5.3.1.4) and this was accompanied by increases in leaf nitrogen (only 0.5 mS $cm^{-1}$ at night significant) and potassium concentrations (5.3.1.1.1). A straight forward response to an increase in nutrient supply.

Where 0.5 mS $cm^{-1}$ was applied during the day, dry weight did not increase with 1.5 mS $cm^{-1}$ at night, although the trend was in this direction for both head dry weight and total dry weight. The increased leaf nitrogen and potassium concentrations would have supported such growth.

### 5.4.1.4 Tipburn

Where 0.5 mS $cm^{-1}$ was applied at night, the tipburn percentage was reduced (5.3.1.5). The lowest level of tipburn occurred where 0.5 mS $cm^{-1}$ was applied both during the day and at night, but this was associated with a very low fresh weight yield. The level of tipburn where 1.5 mS $cm^{-1}$ was applied during the night was not affected by the concentration during the day. This implies that a high concentration at night is a major contributing factor in tipburn occurrence and that root pressure at night is important in tipburn control. In support of this proposal is the observation that the 1.5/1.5 mS $cm^{-1}$ treatment did not increase growth compared to 1.5/0.5 mS $cm^{-1}$, but it significantly increased tipburn. The effect of high potassium, which occurs with high nutrient solution concentrations, in reducing calcium uptake is also a component in tipburn incidence. No data is available from this experiment on the calcium concentration of the inner leaves.

## 5.4.2 Experiment 2.2

### 5.4.2.1 Leaf nutrient concentrations on a leaf group and total leaf basis

#### 5.4.2.1.1 Nutrient concentration from the outer to the inner leaves

The concentration of nitrogen and phosphorus increased from the outer to the inner leaves whereas the concentration of potassium, calcium and magnesium decreased from the outer leaves to the inner leaves (5.3.2.1). The same pattern of nutrient element concentration has been reported (Knavel, 1974).

#### 5.4.2.1.2 Nitrogen

An examination of the responses of individual groups of leaves provides the detail of which leaves were responding to the treatments. Extra calcium increased the leaf nitrogen concentration of leaves 1-15 before induction, while after induction this only occurred with tap water for leaves 1-10. On a total leaf basis extra calcium increased leaf nitrogen concentration both before and after induction (5.3.2.1.1). Thus extra calcium increased leaf nitrogen concentration of a greater range of leaves and across all nutrient solution concentration before induction compared to after induction. This suggests that the extra calcium treatment was less effective in terms of increasing nitrogen uptake during the tipburn induction period, presumably because the nutrient solution treatments, particularly 1.5 mS $cm^{-1}$ were more able to meet the nitrogen demands of the plants.

The promotional effect of calcium on nitrate and anion uptake is documented in the literature. Thus calcium was found to enhance nitrogen uptake in perennial ryegrass (Bailey, 1992), wheat (Minotti et al., 1968), rice (Krasaesindhu & Sims, 1972), ryegrass (Morgan et al., 1972) and bean (Fenn et al., 1987). Bailey (1992) reported that

where calcium was present in the rhizosphere at relatively high concentrations then it had specific effects on root membrane structure and/or function, which facilitated more rapid rates of $NH_4$ and $NO_3$ absorption by root tissue.

Viets (1944) was one of the first workers to demonstrate the promotive effect of external calcium ion ($Ca^{2+}$) on the uptake of other ions. Hanson (1984) stated that the promotion of anion uptake by $Ca^{2+}$ can be attributed largely to atomic shielding of the negative charge at the plasma membrane surface. Thus in the present study it is likely that the extra calcium promoted the uptake of anions as it increased the nitrogen and phosphorus concentrations in the leaf tissue.

Increases in nutrient solution concentration above that of tap water increased leaf nitrogen concentration for leaves 6-15 and for total leaves before induction. After induction increases in nutrient solution concentration provided increases in leaf nitrogen concentration for the Ca0 series for leaves 1-10, for both series for leaves 11-20 (leaves 11-15; tap water not different from 1.5 mS $cm^{-1}$) and for total leaf. In most instances this was with an increase to 1.5 mS $cm^{-1}$. The increase in leaf nitrogen concentration due to increases nutrient solution concentration was a response to an increased supply of nitrogen at night. Root pressure would have contributed to this increased nitrogen uptake. This result appeared to contrast with the results of Experiment 2.1, where leaf nitrogen concentration decreased with an increase in nutrient solution concentration at night from 1.5/0.5 to 1.5/1.5 mS $cm^{-1}$ (Table 5.1 a). Examination of Table 5.7 a shows that a similar trend (Ca 0 series 0.5 versus 1.5 mS $cm^{-1}$) was however apparent in the data of Experiment 2.2.

### 5.4.2.1.3 Phosphorus

An examination of the responses of individual groups of leaves provides the detail of which leaves were responding to the treatments. Before induction extra calcium increased the leaf phosphorus concentration of leaves 1-10 (not leaves 1-5 at 0.5 mS $cm^{-1}$) and of total leaf (for tap water and 1.5 mS $cm^{-1}$), while after induction increases only occurred with tap water for leaves 1-5 (5.3.2.1.2). The latter response (tap water) also occurred with respect to leaf nitrogen concentration. Thus extra calcium increased

leaf phosphorus concentration of a greater range of leaves before induction than after induction. This suggests that the extra calcium treatment was less effective in terms of phosphorus uptake during the tipburn induction period. A similar response occurred with respect to leaf nitrogen concentration

Calcium is found to enhance phosphorus uptake by screening electronegative charges on the roots (Franklin, 1970; Robson et al., 1970). Thus similar process operate to explain the increased phosphorus uptake as for nitrogen.

Increases in nutrient solution concentration above that of tap water increased leaf phosphorus concentration for leaves 1-15 before induction. After induction, increases in nutrient solution concentration above tap water provided increases in leaf phosphorus concentration for the Ca0 series for leaves 1-5 and for both series for leaves 6-10. On a total leaf basis, increases in nutrient solution concentration increased leaf phosphorus concentration above that of tap water both before and after induction.

Thus increases in nutrient solution concentration above that of tap water increased leaf phosphorus concentration for a greater number of leaves before induction than after induction and in both instances 0.5 mS $cm^{-1}$ at night was adequate for growth. This increase in leaf phosphorus concentration due to increases in nutrient solution concentration above that of tap water was a response to an increased supply of phosphorus at night, with root pressure providing a mechanism for some of this increased uptake.

### 5.4.2.1.4 Potassium

An examination of the responses of individual groups of leaves provides the detail of which leaves were responding to the treatments. There was no effect of the treatments on the outer leaves (1-10). Before induction, extra calcium had no effect, while increases in nutrient solution concentration above that of tap water increased leaf potassium concentration for leaves 11-15 and of total leaf. After induction extra calcium decreased leaf potassium concentration of the tap water treatment for leaves 11-15 and all nutrient solution concentrations for leaves 21-inner, while increases in

nutrient solution concentration had no effect on leaf potassium concentration (5.3.2.1.3).

Thus, there was no effect on leaf potassium concentration before induction by extra calcium and after induction by nutrient solution concentration. Leaf potassium concentration was therefore affected much less by the treatments than leaf nitrogen and phosphorus concentration. The outer leaves were not affected, perhaps an indication that these leaves, the oldest leaves on the plant, had in the past been more than adequately supplied with potassium during the day. Before induction it was nutrient solution concentration that had an effect. Based on the response of leaves 11-15 this was a response to an increased supply of potassium at night above that of tap water and 0.5 mS $cm^{-1}$ appeared to be adequate. As with nitrogen and phosphorus root pressure is likely have provided a mechanism for some of this increased uptake.

In Experiment 2.1, there was no significant increase in leaf potassium concentration with a nutrient solution concentration increase at night from 1.5/0.5 to 1.5/1.5 mS $cm^{-1}$, although the trend was for it to increase (Table 5.1c). Across the full range of treatments in Experiment 2.1, it was concluded that leaf potassium concentrations increased with increasing nutrient solution concentration (5.4.1.1.1). At the high end, leaf potassium concentrations approached 7%. In the seasonal study, it was proposed that luxury uptake of potassium occurred up to a nutrient solution concentration of 2.5 mS $cm^{-1}$ (3.4.3.3). There was no evidence of luxury uptake in Experiment 2.2. This may have been however because the leaf potassium concentrations were already high at 8-9 plus % (Table 5.13). These concentrations were, in fact, similar to the levels at which the potassium concentrations levelled off in the seasonal experiment (Figure 3.21).

After induction, it was extra calcium that had an effect, and here extra calcium brought about a decrease in leaf potassium concentration, mainly of the inner leaves. A possible explanation is that during tipburn induction, the extra calcium reduced the uptake of the competing cation potassium under circumstances where the high temperatures encouraged the rapid growth of the inner leaves. As a result, the leaf potassium concentration of these leaves fell.

### 5.4.2.1.5 Calcium

An examination of the responses of individual groups of leaves provides the detail of which leaves were responding to the treatments. There was no effect of the treatments on leaf calcium concentration before induction for individual groups of leaves or with respect to total leaf before or after induction (5.3.2.1.4).

The increasing potassium concentration that was part of the increases in nutrient solution concentration did not decrease leaf calcium concentration either before or after induction. This was not so in the seasonal study (3.4.3.4), or in Experiment 2.1 where an increase in nutrient solution concentration at night from 1.5/0.5 to 1.5/1.5 mS $cm^{-1}$ (Table 5.1d) brought about a decrease in leaf calcium concentration. The lack of such a response in Experiment 2.2 may have been because as leaf potassium concentrations had levelled off at the higher nutrient solution concentrations, leaf calcium concentrations were not affected. Certainly in the seasonal study as leaf potassium levels levelled off then the decrease in leaf calcium and magnesium concentrations that were occurring slowed down or levelled off.

After induction extra calcium reduced the leaf calcium concentration for leaves 21-inner. In this case the inability of the plant to redistribute calcium in the phloem (Marschner, 1995) would have contributed to this effect.

Thus extra calcium failed to charge the inner leaves with calcium via root pressure. This was the main objective of applying this treatment. The possible explanation is that because of the increased uptake of the nitrogen and phosphorus brought about by extra calcium, growth increased which then increased the demand for calcium which the plant could not supply and so diluted the calcium concentration of the inner leaves.

### 5.4.2.1.6 Magnesium

An examination of the responses of individual groups of leaves provides the detail of which leaves were responding to the treatments. Before induction the only treatment effect was on a total leaf basis where an increase in nutrient solution concentration

from tap water to 0.5 mS $cm^{-1}$ decreased leaf magnesium concentration (5.3.2.1.5). The opposite response was obtained with potassium. Thus this could be explained in term of competition between these two cations (Haworth & Cleaver, 1967; Winsor & Adams, 1987). A similar response was obtained in the seasonal study (3.4.3.5) and in Experiment 2.1 (5.4.1.1.1).

After induction extra calcium decreased leaf magnesium concentration for leaves 21-inner. This response was obtained with all three cations, potassium, calcium and magnesium and could be explained as was suggested for calcium (5.4.2.1.5) that the extra calcium increased anion uptake and so growth and thus the concentration of these three cation decreased in leaves that were actively growing. With potassium and magnesium the increased calcium concentration would have also reduced the uptake of these elements due to cation antagonism.

### 5.4.2.1.7 Summary: Treatment effects on leaf nutrient concentrations – leaf group and total leaf basis

This section summarises in general terms, the conclusions that can be made with respect to the treatment effects on leaf nutrient concentrations. In broad terms leaf nutrient concentrations of nitrogen and phosphorus responded similarly to the treatments as did potassium, calcium and magnesium.

**General - nutrients**

i. Nitrogen and phosphorus increased in concentration from the outer to inner leaves, while potassium, calcium, and magnesium decreased in concentration from the outer to inner leaves

ii. Nitrogen and phosphorus concentrations of the innermost group of leaves was not affected by treatments, not so for potassium, calcium, and magnesium.

iii. Extra calcium and increased nutrient solution concentration both before and after induction affected nitrogen and phosphorus leaf nutrient concentrations. Leaf nitrogen concentration was affected more than phosphorus.

iv. Nutrient solution concentration affected potassium and magnesium before induction and extra calcium affected calcium, potassium and magnesium after induction.

v. Responses to an increased nutrient solution concentration were due to an increased supply of nutrients at night, with root pressure providing a mechanism for some of this uptake.

vi. The extra calcium affected nutrient uptake differently depending on whether an anion or cation was under consideration. Thus nitrogen and phosphorus uptake were increased via the calcium providing shielding of the negative charge at the plasma membrane and cation uptake was reduced (potassium and magnesium) by cation antagonism and due to increased uptake of nitrogen in particular increasing growth.

**Detail – nutrients**

i. Extra calcium increased leaf nutrient concentration for a greater number of leaves before induction than after induction for both nitrogen and phosphorus. The number of leaves affected in each case was greater for nitrogen than phosphorus. Extra calcium increased leaf nutrient concentration of total leaves for nitrogen both before and after induction, but with phosphorus increases were only obtained before induction. Thus, extra calcium affected nitrogen leaf concentration more often than phosphorus.

ii. The increase in leaf nitrogen concentration due to increases in nutrient solution concentration was a response to an increased supply of nitrogen.

iii. The increase in leaf phosphorus concentration, due to increases in nutrient solution concentration, was a response to an increased supply of phosphorus. The

response covered a greater number of leaves before induction than after induction and in both instances 0.5 mS $cm^{-1}$ at night was adequate for growth.

iv. Potassium, calcium, and magnesium. The outer leaves were not affected by the treatments, perhaps an indication that these leaves, the oldest leaves on the plant, had in the past been more than adequately supplied with potassium during the day. Extra calcium reduced the concentration of all the cations (potassium, calcium, and magnesium) in leaves 21-inner after induction. This is the group of leaves that grew most actively during the four day tipburn induction period. Leaf potassium, magnesium and calcium concentrations were effected much less by the treatments than leaf nitrogen and phosphorus concentration.

v. There was no effect of nutrient solution concentration on leaf calcium concentration.

vi. With potassium and magnesium, nutrient solution concentration was only effective before induction. They responded differently.

### 5.4.2.2 Fresh weight

With fresh weight before induction, there was an interaction between calcium level and nutrient solution concentration (5.3.2.2). The feature of this interaction was that with the Ca0 series, 0.5 mS $cm^{-1}$ had a greater fresh weight than 1.5 mS $cm^{-1}$. After induction and at final harvest there were no such significant interactions, although similar trends in the data were apparent. A similar trend was also apparent for fresh weight for 1.5/1.5 versus 1.5/0.5 mS $cm^{-1}$ in Experiment 2.1 (5.3.1.2) and in the data of Cresswell (1991), when the fresh weight of the whole plants for the control (169 g; 2 mS $cm^{-1}$) is compared with the 100 calcium treatment (186g; 0.8 mS $cm^{-1}$). Based on the above evidence, it is proposed that in the present experiment, that the plants responded similarly at all three fresh weight determinations. Thus, the issues discussed and conclusions reached for fresh weight before induction are considered to also apply after induction and at final harvest and also to the fresh weight results of Experiment 2.1 (5.4.1.2).

The greater fresh weight of 0.5 mS $cm^{-1}$ was due to the root pressure component of this treatment being more important than at 1.5 mS $cm^{-1}$. This is because root pressure is related to the difference in osmotic pressure between the external solution and the internal sap (Weatherley, 1982). The only direct evidence of a root pressure was for phosphorus for leaves 1-5 before induction, where the leaf phosphorus concentration was greater in the Ca0 series for 0.5 mS $cm^{-1}$ than for 1.5 mS $cm^{-1}$ (5.4.2.1.3). There was a similar trend when the phosphorus concentration before induction for total leaf for these two treatments was compared (Table 5.10). In Experiment 2.1 the evidence came from leaf nitrogen, where the concentration of the 0.5 mS $cm^{-1}$ treatment at night was greater than for 1.5 mS $cm^{-1}$ (5.4.1.1.1).

The extra calcium of the Ca100 series increased plant fresh weight above that of the Ca0 series before induction for the tap water and 1.5 mS $cm^{-1}$ treatments, but not 0.5 mS $cm^{-1}$. In the latter case it is suggested that root pressure was less active due to the higher concentration (1.0 mS $cm^{-1}$) and the response to root pressure was replaced with a response to extra calcium which maintained fresh weight at a similar level. A similar trend was apparent for fresh weight after induction and at final harvest.

Increases in fresh weight in response to the extra calcium were associated with increased total leaf nitrogen and phosphorus (P: before induction, tap water and 1.5 mS $cm^{-1}$ only) concentrations (5.4.2.1.2 and 5.4.2.1.3). These increases in nitrogen and phosphorus were associated with higher leaf nutrient concentrations in the outer to middle leaves. As has already been discussed (5.4.2.1.2 and 5.4.2.1.3) calcium can enhance the uptake of anions.

Increases in fresh weight in response to increases in nutrient solution concentration above that of tap water were associated with increases in nitrogen concentration of total leaf and of the middle group of leaves 6-15 before induction and with increases in nutrient solution concentration above 0.5 mS $cm^{-1}$ for total leaf after induction (5.3.2.1.1). Similar increases in phosphorus levels (5.3.2.1.2) ~~were obtained and~~ for potassium before induction (5.3.2.1.3). The potassium response was therefore similar, but not as positive as that of nitrogen and phosphorus. In all these cases the improved nutrient supply at night, something versus nothing, would have assisted nutrient uptake and growth.

There were thus, three distinctly different responses effecting fresh weight. They were:

i. A root pressure effect, of particular importance with the Ca0 0.5 mS $cm^{-1}$ plants. The real issue with root pressure may not be the quantity of nutrients that are taken up, but rather that it provides additional nutrients to the inner leaves. Thus, with 0.5 mS $cm^{-1}$ at night, growth increased because root pressure supplied more nitrogen and other nutrients to the inner leaves which were the leaves that had the potential to grow.

ii. A response to an increased supply of nutrients at night, of particular importance as the concentration increased above that of tap water for Ca0 1.5 mS $cm^{-1}$ and for Ca100 0.5 and 1.5 mS $cm^{-1}$ plants.

iii. A response to extra calcium, which enhanced growth due to increased anion uptake.

### 5.4.2.3 Dry weight

Extra calcium increased total leaf dry weight before induction with tap water only (5.3.2.3). In the Ca0 series the 1.5 mS $cm^{-1}$ had a lower, but not significantly lower dry weight than 0.5 mS $cm^{-1}$. It is suggested that despite this difference not being significant, that the data can be interpreted as for fresh weight before induction (5.4.2.2). Similarly the only increase in dry weight that occurred with increasing concentration was for increases above that of tap water. This suggests that some extra nutrients at night were required to support growth. The extra calcium with tap water had the same effect by increasing anion uptake. Before induction it was leaves 6-10 that showed significant treatment responses (5.3.2.5). Here extra calcium increased dry weight, as did increases in concentration above that of tap water. This group of leaves would have completed their period of active growth

After induction the extra calcium of the Ca100 series increased growth at all nutrient solution concentration levels. This growth response was marked with the inner leaves (5.3.2.5). This was associated with increased nitrogen concentrations of total leaves and

leaves 1-10 (tap water only) (5.3.2.1.1) and in phosphorus concentration of leaves 1-5 (tap water only) (5.3.2.1.2). On the other hand potassium, calcium and magnesium concentrations for leaves 21 to inner all decreased (Table 5.12, Table 5.15 and Table 5.18 respectively). These changes in nutrient concentration in the leaves reflect where the growth was taking place. The outer leaves which were growing less, increased in nutrient concentration, whereas the inner leaves, which were growing, did not. The stimulating effect of extra calcium, it is sugggested, was due to its effect on anion uptake. This increased anion uptake could have taken place during the day or night.

There were no treatment effects on RGR during the induction period, but as shown in Figure 5.1, RGR were higher with the outer (1-5) and inner leaves (16-inner). The highest RGR was, as to be expected, in the leaves 16-inner. The after induction treatment effects were obtained in these outer and inner leaves. As the data on RGR shows, these are the leaves where growth was most active during tipburn induction and therefore explains why this is where the treatments after induction were more effective.

### 5.4.2.4 Comments on both fresh and dry weights

#### 5.4.2.4.1 Before induction

This period covered the full growing period up until the application of the tipburn inducing treatment, a period of 17 days. During the day the plants were adequately supplied with nutrients. The treatments were only applied at night. The response in terms of increased growth to extra calcium and increasing concentration mainly involved tap water. It was suggested that this was a response to extra nutrients at night.

The growth improvement of Ca0 0.5 mS $cm^{-1}$ was explained as a root pressure effect. Root pressure improved growth as it provided a more adequate supply of nutrients to the inner actively growing leaves. Here, the middle group of leaves, which had recently competed their rapid growth phase, were the most affected. Some nutrients at night were obviously need to improve growth

### 5.4.2.4.2 After induction

This period covered the full growing cycle up until the application of the tipburn inducing treatment plus the four days of the tipburn inducing treatment. The difference in response from before induction was due to the four day induction period, a time when the plants were exposed to 30°C both day and night. Growth increases were particularly due to extra calcium where the effect was more marked than before induction. This was with the outer and inner leaves, which had the higher growth rates over the induction period. The extra calcium enhanced nutrient uptake and so improved growth.

### 5.4.2.5 Tipburn

Tipburn incidence was assessed at final harvest. Extra calcium increased tipburn, while the 0.5 mS $cm^{-1}$ treatment had less tipburn than the other concentrations with both the Ca0 and Ca100 series. The extra calcium (Ca100) series increased tipburn because nitrogen, and phosphorus uptake was increased and thus growth, particularly of the inner leaves, was enhanced by calcium. Increases in the incidence of the level of tipburn with increases in growth rate under tipburn inducing conditions is well documented in the literature (Cox et al., 1976; Misaghi & Grogan 1978a; Brumm & Schenk, 1993). Root pressure can be used to explain, as it was in Experiment 2.1 with the 1.5/0.5 mS $cm^{-1}$ treatment, the lower incidence of tipburn with Ca0 0.5 mS $cm^{-1}$. This is supported by the high concentration (but not significant) of calcium of the Ca0 0.5 mS $cm^{-1}$ treatment in leaves 21- inner after induction (Table 5.15). It is the leaves in this group which suffered from tipburn. Why then did extra calcium with tap water not reduce tipburn as a result of increased calcium uptake due to root pressure. This increased calcium uptake clearly did not occur (Table 5.14d and Table 5.15e). One possibility is the report that calcium transport in the xylem is promoted by the presence of other cations (Himelrick & McDuffie 1983). Singh and Jacobson (1979) have reported that previous absorption of calcium or other cations such as potassium, resulted in the saturation of the apoplastic electronegative complex, thereby increasing calcium transport (Vang-Petersen, 1980). With this particular treatment in the present investigation, calcium was the only cation present. If this is so then this would also explain

why Ca100 0.5 mS $cm^{-1}$ plants had less tipburn, because although the combined concentration of the extra calcium and 0.5 mS $cm^{-1}$ nutrient solution (1.0 mS $cm^{-1}$) would have reduced the effectiveness of root pressure, the presence of the other cations may have enhanced calcium movement in the xylem. Tipburn incidence was high with Ca100 1.5 mS $cm^{-1}$ because of the combined effects of a high concentration (2.0 mS $cm^{-1}$) and the growth promoted by the extra calcium treatment.

### 5.4.2.6 Practical implications

i. Low night nutrient solution concentrations will increase leaf fresh weight and reduce tipburn. This, it is proposed, is due to root pressure increasing calcium supply to the inner leaves, which then reduces tipburn. The reduction in tipburn may also account for the increase in fresh weight, as there would be less damaged to the leaf margin of the leaves, which as result can now grow more.

ii. Calcium at night, on its own, will not reduce tipburn, even at low concentrations, because it enhances the uptake of nitrogen in particular, and so encourages leaf growth. Calcium may need the presence of other cation(s) to increase calcium uptake and transport to the inner leaves, as the presence of other cations favour the release of calcium into the xylem.

iii. If the latter proposition is correct, then a night feed relatively high in calcium, but balanced by the presence of other cations, and providing the nutrient solution still maintains a low concentration, may reduce tipburn.

# Chapter 6

# General Conclusion

This chapter presents an overall conclusion based on the results from the earlier chapters and also outlines possible future research.

## 6.1 Nutrient uptake

Leaf nitrogen and potassium concentrations were greater than in the root, the reverse was true of phosphorus, while calcium and magnesium levels did not differ greatly. Nitrogen and phosphorus increased in concentration from the outer to inner leaves, while potassium, calcium, and magnesium decreased in concentration from the outer to inner leaves.

Generally there were no large variations in nitrogen and phosphorus concentrations of the leaves across nutrient solution concentrations, which ranged between 0.5 – 3.5 mS $cm^{-1}$. Leaf potassium concentration increased with increasing nutrient solution concentration up to 2.5 mS $cm^{-1}$. As leaf potassium increased in concentration with increasing nutrient solution concentration this increase mediated decreases in calcium and magnesium concentrations of the leaves (Figure 3.21). The concentration of nutrients in the leaves were, apart from a few exceptions, all within the range reported by other researchers.

At the lowest nutrient solution concentration in this study, 0.5 mS $cm^{-1}$, the nutrient solution did not appear to meet the nitrogen requirements of the spring and summer crops (Figure 3.16) and phosphorus requirements of the autumn, winter and summer crops (Figure 3.17). The phosphoric acid required to adjust the pH of the spring crop modified the response of this crop to phosphorus.

When 0.5 mS $cm^{-1}$ was used alternately with 1.5 mS $cm^{-1}$ during day and night, the nitrogen and potassium of the leaves increased with 1.5 mS $cm^{-1}$ and the increases in

potassium mediated decreases in calcium and magnesium concentrations of the leaves. These increases in leaf nitrogen and potassium were more marked when 1.5 mS $cm^{-1}$ was maintained during daytime and 0.5 mS $cm^{-1}$ during the night (Table 5.1). This was explained in terms of a nutrient concentration effect during the daytime and a root pressure at night more adequately supplying nutrients to the leaves.

Both extra calcium and increases in nutrient solution concentration at night, above that of tap water, increased leaf nutrient concentration of total leaves for nitrogen both before and after tipburn induction, but with phosphorus increases were only obtained before induction. Nitrogen and phosphorus concentrations of the innermost group of leaves were not affected by the treatments. Increasing the nutrient solution concentration above that of tap water at night, increased potassium and decreased magnesium of total leaves before tipburn induction.

Extra calcium on its own at night affected nutrient uptake differently depending on whether an anion or cation was under consideration. Nitrogen and phosphorus uptake were increased (Table 5.7 and Table 5.10) via, it was proposed, the calcium providing shielding of the negative charge at the plasma membrane. Extra calcium at night had no effect on concentration of cations (potassium, calcium and magnesium) of total leaves, but reduced the concentration of all the cations (potassium, calcium, and magnesium) in leaves 21-inner after the plants had been exposed to high temperature/tipburn inducing conditions (Table 5.12, Table 5.15 and Table 5.18). Cation uptake, where it was affected, was reduced by cation antagonism, and increased growth, in particular, was due to increased uptake of nitrogen.

## 6.2 Growth

Shoot fresh weight and dry weight increased up to 1.5 mS $cm^{-1}$ with increases in nutrient solution concentration in all 4 seasons. Shoot fresh weight and dry weight over the nutrient solution concentrations range 1.5 – 3.5 mS $cm^{-1}$ in the autumn and winter crops were not different (Figure 3.23 and Figure 3.24). The decrease in shoot fresh weight at the higher

nutrient solution concentrations in summer and spring it is proposed was due to stress. At higher nutrient solution concentrations dry weight levelled off but fresh weight levelled off or decreased slowly depending on the level of stress imposed by the season. Thus fresh weight was more sensitive to stress at high nutrient solution concentrations than dry weight.

Generally RGR, NAR, LAR and SLA decreased with growing period while LWR increased. With both seasons and cultivars the order of the initial RGR was the same order as for final shoot dry weights with the initial NAR being the important component of the initial RGR. The initial RGR were in the order spring > winter > autumn, summer, while the initial RGR for the three cultivars were in order Cortina ≥ Lollo Bionda ≥ Impuls (Table 3.3).

Different day/night nutrient solution concentration combinations were examined using 1.5 mS $cm^{-1}$ as the high and 0.5 mS $cm^{-1}$ as the low level. Evidence from 2 separate experiments suggest that 1.5/ 0.5 mS $cm^{-1}$ day/night nutrient solution concentration gave the highest shoot fresh and dry weights (not always different from 1.5/1.5 mS $cm^{-1}$ day/night) (Table 5.3, Table 5.20 and Table 5.21). This was explained in terms of both a nutrient concentration effect during daytime and a root pressure effect at night more adequately supplying nutrients to the leaves.

Another option examined was to supply extra calcium at night either alone or in combination with other nutrients. The extra calcium improved growth by increasing the uptake of anions (Table 5.5 – Table 5.10). Thus nitrogen and phosphorus uptake was increase and so was growth (Table 5.20 and Table 5.21).

## 6.3 Tipburn incidence

Here the effect of season, cultivar, nutrient solution concentration during the growing period, day night nutrient solution concentration combinations and extra calcium at night on tipburn incidence was examined.

Apart from the autumn crop, where no tipburn occurred, tipburn incidence increased with increasing nutrient solution concentration. On a cultivar basis Lollo Bionda had a greater incidence of tipburn at 2.5 mS $cm^{-1}$ in summer, while Cortina had the greater incidence of tipburn at 2.5 and 3.5 mS $cm^{-1}$ in winter (Figure 3.27).

Tipburn percentage was reduced when the nutrient solution concentration at night was lowered to 0.5 mS $cm^{-1}$, when the day time nutrient solution was maintained at 1.5 mS $cm^{-1}$ under natural condition (Table 5.4). This effect was explained in term of root pressure at night more adequately supply calcium to the innermost leaves. When plants were under extreme stress (30 $^{0}C$ tipburn induction), neither extra calcium nor different nutrient solution concentration at night affected tipburn percentage. However, extra calcium increased tipburn severity because extra calcium increased growth due to an increase in nitrogen uptake. The 0.5 mS $cm^{-1}$ nutrient solution concentration at night decreased the number of tipburn leaves per plant and the tipburn index (Table 5.22).

## 6.4 Shelf life

Shelf life increased with increasing nutrient solution concentration (Table 3.5), but the level of increase was not great enough to be of commercial significant.

## 6.5 Season

Seasonal differences were related to the degree of stress on the plants. In the summer and spring crops, shoot fresh weight and dry weight increased up to 1.5 mS $cm^{-1}$ with increases in nutrient solution concentration and then decreased after that, while in the autumn and winter crops shoot fresh weight and dry weight over the nutrient solution concentrations range 1.5 – 3.5 mS $cm^{-1}$ were not different.

In the summer, plants were growing under stressful conditions for tipburn. The plants were often under a level of stress, but this level of stress did not vary as markedly as in spring.

In the spring crop, plants were growing into improving environmental conditions with exposure to short period of relatively high stress. This can explain why tipburn incidence in spring was higher than in summer. In the autumn and winter crops, plants were growing into deteriorating conditions and so there was no or less tipburn incidence.

## 6.6 Cultivars

Cultivar differences were related to their plant characteristics. The butterhead cultivar Cortina was the most responsive to increases in nutrient solution concentration above 0.5 mS $cm^{-1}$ and to improvements in environmental conditions, whereas the red leafed cultivar Impuls was the least responsive. Cortina performed well, as early on it had a flat rosette of leaves which was efficient in intercepting radiation.

## 6.7 Nutritional value

Season, nutrient solution concentration and cultivar all affected nutritive value. The response depended on the nutritive quality attribute under consideration. The nutritive values obtained in this study were in the ranges reported by other workers. Where ascorbic acid concentrations were high, such as in summer or with the cultivar Impuls, then ascorbic acid concentrations decreased with increasing nutrient solution concentration (Figure 4.1).

There were no effects of season or nutrient concentration on diary fibre concentration. The only difference in dietary fibre occurred with the butterhead cultivar Cortina, which had the lowest dietary fibre concentration of the three cultivars (Table 4.1).

Nitrate concentration increased with nutrient solution concentration, was highest in autumn and winter, while differences between cultivars depended on the season. The nitrate concentrations of lettuce produced at nutrient solution concentrations up to 1.5 mS $cm^{-1}$ were within the permissible levels reported overseas (Figure 4.2).

There were no treatment effects on protein concentration despite some reports in the literature of the effects of nitrogen level on protein content.

Generally soluble sugar concentrations decreased within increasing nutrient solution concentrations up to 2.5 mS $cm^{-1}$ and then levelled off. At the lower nutrient solution concentrations, the spring and summer crops tended to have the highest soluble sugar concentrations (Figure 4.3).

## 6.8 Practical outcomes

i. Fresh weight increased up to 1.5 mS $cm^{-1}$ with increases in nutrient solution concentration and then levelled off or decreased slowly depending on the level of stress imposed by the season. Tipburn incidence increased with increasing nutrient solution concentration (apart from in autumn where no tipburn occurred), with the level of incidence increasing as environmental stress increased. Thus 1.5 mS $cm^{-1}$ should be regarded as the optimum nutrient solution concentration, from the grower's point of view, at which to grow a range of lettuce cultivars across all seasons. At this nutrient solution concentration yield will be satisfactory and the level of tipburn will be minimised.

ii. The concentration of ascorbic acid in summer and soluble sugar decreased while dietary fibre and protein remained unaffected, and nitrate increased with increasing nutrient solution concentration. The nitrate concentrations of lettuce produced at nutrient solution concentration up to 1.5 mS $cm^{-1}$ were within the permissible levels reported overseas. Thus again 1.5 mS $cm^{-1}$ should be regarded as the optimum nutrient solution concentration, from the consumer's point of view, at which to grow a range of lettuce cultivars across all seasons.

iii. Low nutrient solution concentrations at night will increase leaf fresh weight and reduce tipburn. This, it is proposed, is due to root pressure increasing calcium supply to the inner leaves, which then reduces tipburn. The reduction in tipburn may also

account for the increase in fresh weight, as there is now less damage to the margin of the leaves, which as result can now grow more.

iv. Calcium at night, on its own, will not reduce tipburn, even at low concentrations, because it enhances the uptake of nitrogen in particular, and so encourages leaf growth. Calcium may need the presence of other cation(s) to increase calcium uptake and transport to the inner leaves, as the presence of other cations favour the release of calcium into the xylem.

## 6.9 Comments on future work

In the present research on the effect of nutrient solution concentration at night and in other similar studies it is assumed that root pressure is active. The role of root pressure in distributing calcium to low transpiring organs has been widely reported (Palzkill & Tibbitts, 1977; Collier & Wurr, 1981; Guttridge et al., 1981; Bradfield & Guttridge, 1984; Clover, 1991). However, more physiological and quantitative analysis of root pressure is required in lettuce.

Sonneveld and Van den ende (1975) suggested that the ion relationship of the soil solution played a major part in the incidence of tipburn of lettuce. Willumsen et al. (1996) also suggested that the relationship between ion activity ratios of potassium with magnesium and calcium in the root medium ($a_k / \sqrt{a_{Ca}+a_{Mg}}$ and $a_{Mg}/a_{Ca}$) might be more important than salinity *per se* in inducing blossom-end rot of tomato. Tadesse (1997) also suggested that there was possibly a relationship between low calcium: potassium and $a_k / \sqrt{a_{Ca}+a_{Mg}}$ ratios with the incidence of blossom-end rot in sweet pepper. This relationship should be explored in future research with respect to lettuce.

However, Willumsen et al. (1996) concluded that the activity ratios and the salinity are not the only factors controlling the incidence of blossom end rot in tomato. Cultivar susceptibility, xylem tissue development within the fruit, the rate of fruit enlargement and

environmental factors are other important factors. Similarly the importance of the aerial environment on tipburn incidence of lettuce needs further investigation.

A recent review by Saure (1998) suggested that tipburn is a stress related disorder. External factors may cause stress and mild stresses below a damaging level may reduce the risk of tipburn incidence by increasing stress tolerance. Thus, in the present research the evidence of tipburn was less in summer, where the plants regularly experienced stress compared to in spring where stress levels fluctuated markedly. Options for maintaining a low level of stress and/or reducing severe stress from solar radiation and air temperature effects during the spring and summer may require re-examination.

Misaghi and Grogan (1978b) reported that both tipburn severity and percentage of tipburned plants increased in direct proportion with increase in time of exposure to 30 $^{0}$C. Misaghi and Grogan (1978a) also found that organic acids such as citrate, isocitrate, succinate, fumarate and malate increased substantially in central head leaves of heads subjected to 30 $^{0}$C for 5 days compared with those kept at 5 $^{0}$C. Pyruvate, which serves as fuel for the tricarboxylic acid cycle, was decreased. The concentration of some soluble amino acids such as valine, isoleucine, leucine, tyrosine and phenylalanine in plants with tipburn increased to more than 300% that of control plants. They also suggest that tipburn is a manifestation of a localised calcium deficiency that results from chelation of calcium by organic acids and other metabolites that increased during exposure to elevated temperature. Gallaher (1975) suggested that in plants, calcium serves to reduce the toxic effects of oxalic acid like in animals when the acid is ingested. Plants produce large quantities of oxalic acid when under environmental stresses and this could result in calcium deficiency especially when calcium supply is limited. The above researchers have all emphasised apparent aspects of biological changes that take place, or might take place, in plant tissue affected by tipburn. For example, the relationship between forms of calcium and tipburn incidence. Thus research is required to more fully understand the biological changes that take place in tipburned lettuce leaves. Similarly more detailed examination of the effect of tipburn on leaf tissue remains to be undertaken. Advances in knowledge in these areas may provide new insights to possible control options.

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