INVESTIGATION OF RELATIONSHIPS THROUGH WHICH BIODYNAMIC GROWING PRACTICES AFFECT PLANT GROWTH AND NUTRIENT COMPOSITION

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ABSTRACT

More research attention has been paid to development of indicators of soil quality in relation to environmental sustainability than to food quality. Challenges in measuring and showing relationships between soil quality, food quality and human health are discussed. Comparative and long-term studies have shown that organic and biodynamic farming methods and use of composts and manures favourably affect soil quality, enhancing organic matter content and soil organism activity. However, measured effects on food quality have been lacking or inconsistent. Antioxidants, nitrates, trace elements, protein quality and ratios between element concentrations can be measured in assessing food quality. Many of these factors vary considerably with growing conditions and soil management techniques. Effects of factors such as water, light, soil type, variety and nutrient supply on lettuce growth, lettuce nutrient requirements, and recent research into effects of light on plant signalling and nitrogen metabolism are reviewed and discussed.

Recent research into factors influencing food quality has focussed on integration of growth and differentiation forces into "vital quality" food. The biodynamic field-spray preparations 500 and 501 are used to balance effects of growth or "shade" forces from humus and fertilisers with the differentiating or "light' forces on plants. Literature indicates that the preparation 501 (silica-spray) appears to increase plant nutrient assimilation and production of more complex organic acids. Similar effects have been found for silica compounds applied to soil or nutrient solution.

The main objective of the experimental work conducted for this thesis was to investigate whether relationships exist between soil management techniques and application of biodynamic sprays and plant product quality.

Transplanted lettuces (cv. Canasta) were grown in a factorial designed field trial on Te Puke Series sandy loam with six treatments: control, soluble fertilisers (DAPCAN) and compost, each with, or without, biodynamic field-sprays 500 (twice) and 501 (3 times). High variability between plants within treatments and small differences between treatment means for most parameters measured prevented many statistically significant differences or relationships being found.

Compost amendments appeared to enhance water and nutrient uptake during a dry season. However compost application at a rate to provide equivalent nitrogen to the soluble fertilisers resulted in high leaf concentrations of nitrates and potassium and low DM% and concentrations of sugars, antioxidants, calcium and magnesium. Plants in treatments given compost had highest yields; highest N, P and K concentrations at 28 days from transplanting (DAT); and highest K at 48 DAT. Plants in treatments given soluble fertilisers had highest Ca, Mg, Fe, Zn, and Cu concentrations and greater Ca: P and K: Ca + Mg ratios at 48 DAT.

Application of biodynamic field-sprays appeared to have different effects on the plants in plots, depending upon whether they received compost or not. Plants in treatments given field-sprays but no compost had generally small head weight, greater dry matter % and root: shoot ratios at 28 DAT, and highest crude protein and Ferric reducing ability of plasma (FRAP) antioxidant concentration at 48 DAT. Plants in the biodynamic treatment, given compost and field-sprays, had highest P uptake between 28 and 48 DAT and highest fresh-weight at 48 DAT.

Measurements of nitrate and sugar contents of leaf cell sap and amino acid concentrations in leaves yielded few, or no, significant differences between treatment means. Microbial activity measured by soil respiration ex situ at 28 DAT was highest in composted plots and lowest in sprayed plots. Measurement of AM fungi colonisation of roots gave inconclusive results. In a sensory evaluation, no significant differences in taste, bitterness, sweetness and preference ranking were found between lettuces from the different treatments.

A greenhouse pot trial was undertaken to study the effects of the biodynamic silica spray in more detail. Lettuce transplants (cv. Cos Little Gem) were grown in the same soil and biodynamic compost as were used in the first trial and preparation 500 applied. Half the plants were sprayed 3 times with preparation 501. Measurements before and after the last spray time yielded insignificant differences in light absorption at most wavelengths, net photosynthesis and nitrate, sugar and amino acid concentration in leaves. Silica sprayed plants had higher rates of transpiration and stomatal conductance and higher estimated light absorption of blue and near infrared wavelengths 2½ hours after spraying.

Mainly inconclusive effects of treatments were due partly to the large natural plant to plant (within replication) variation. It was concluded that organic and biodynamic management of lettuces may result in some favourable quality attributes compared to soluble fertilisers but not necessarily all. Results are likely to be specific to particular climatic and soil conditions.

It is recommended that further trials be carried out to evaluate influences of biodynamic practices on vegetable food quality in controlled, well-replicated conditions, to improve likelihood of showing statistical differences between treatments. Such trials are needed in a variety of soil, climatic and management conditions, to better understand how different conditions and their interactions affect food quality parameters. Relationships between biodynamic preparation application, soil biota populations and activity, plant metabolism and food product quality, particularly nitrogen assimilation into complex molecules such as essential amino acids, should be explored.

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1 INTRODUCTION

Claims that human health is linked to subtle differences in food quality rather than just quantity of particular food types and nutrients have been hard to substantiate through research because of the many variable factors involved. Links between health and dietary components have been established, for example McCullough et al. (2002) found significant association of diet scores with relative risk of major chronic disease. Consumption of fruit and vegetables and food content of components such as essential amino acids, vitamins and trace elements have been linked to health (e.g. Reeds, 1988, Ames, 2001).

Claims that organically grown food is higher quality and better for health have not been consistently demonstrated by research. There is much variation in soil and climatic conditions and in what is deemed to be "organic" in comparative research trials, so it is not surprising that such variable results have been obtained. Anecdotal evidence that less medication and veterinary attention is needed for cattle raised in an organic system suggests that the same could be true for human health.

Organic farmers stress the importance of developing a living soil with good humic content and a diverse, dynamic soil food web for healthy crop and animal production. Links between humus quality and diversity, quantity and activity of soil biota and crop production have been demonstrated (Mäder et al., 2002). Biodynamic farmers consider that not only the soil, but the whole solar system and beyond affect crop growth and seek to balance processes that come from above and below the plant by management practices that include applying the biodynamic preparations.

This thesis addresses the questions:

- How can soil quality and food quality be defined and measured?
- How do organic systems, different composts, soluble fertilisers and biodynamic preparations affect plant growth and nutrient content?
- How does the horn silica spray affect light absorption, water uptake, photosynthesis and nitrate assimilation?

- What parameters might be used as indicators that soil and plants will provide high quality food?

The aim of the literature review and two trials was to investigate what factors are involved in these questions and evaluate these factors under trial conditions. It is recognised that this is a very wide subject for a Master of Science thesis and most of it could not be covered in much depth. However, the trials undertaken enabled learning how to design and carry out trials and to analyse data obtained and the problems involved in such comparative trials, and have provided indications of what further research could be useful.

The literature review discusses:

- how soil quality and food quality can be defined and measured,
- the nature of organic and biodynamic systems;
- measurement of soil and food quality parameters.
- research comparing organic and conventional systems and factors involved;
- effects of organic systems on soil quality and soil organisms;
- possible relationships between soil and soil biota parameters and plant nutrient uptake and assimilation
- the biodynamic preparations and silica and their effects on food quality;
- factors affecting lettuce physiology and nutrient content including light, nutrient uptake, nitrogen metabolism and interactions with soil organisms.

Methods, results and discussion relating to two trials are then reported. Firstly, lettuces were grown in a field trial comparing effects of compost and soluble fertiliser amendments with and without the biodynamic field-sprays. Growth, yields, nutrient contents, taste, life activity and self-decomposition were measured. Secondly, lettuces were grown in pots of soil and compost in a greenhouse with and without the biodynamic horn-silica spray. Leaf nitrate and soluble solids content, photosynthesis, stomatal resistance, water uptake, spectral reflectance and life activity were measured before and after application of the spray. Results are discussed in relation to possible plant signalling and metabolic processes.

2 LITERATURE REVEW

2.1 Relationships between soil health and quality, food quality and human health

Relationships between soil health and quality and human health were reviewed by Doran et al. (1996). They defined soil quality as:

> *"the capacity of soil to function, within ecosystem and land-use boundaries, to sustain biological productivity, maintain environmental quality, and promote plant, animal and human health. "*

They commented that it is easier to see direct effects on health from contaminated soil or anti-quality factors in food, such as nitrates contaminating food and drinking water, but harder to establish positive effects from organically grown food. The failure to link food quality to actual soil health conditions, regardless of method of production, would continue to impede an informed discussion on the relationship between soil health and human nutrition. They recommended a set of soil quality indicators, recognizing soil as a living community, which develops resilience and stability over time.

2.1.1 Indicators of soil health and quality

Doran and Safley (1997) stated that the terms "soil quality" and "soil health" can be used interchangeably, and defined soil health in almost the same words as that for soil quality above. They discussed the development of a "soil health index" which could be used in a similar way to medical examinations to assess human health. Indicators should, among other things, describe the major ecological processes in soil, relate to major ecosystem functions such as carbon (C) and nitrogen (N) cycling and be usable by as many people as possible. Pankhurst et al. (1997) reviewed the use of biological indicators of soil health, including soil microbial biomass activity, soil enzyme activities, soil microflora and macrofauna and plant root pathogens. Such indicators can be used to complement the physical and chemical measures already widely used.

Most research appears to have concentrated on sustainability and environmental quality aspects of soil health rather than ability to provide for animal and human health. Albrecht (1975) discussed the higher quality of food produced in the dryer prairie soils

of the USA than from the wetter, leached soils of the Eastern States. He developed indicators of soil health such as cation exchange. These measures have been developed further e.g. the Total Exchangeable Cations measure by Brookside Laboratories in the USA, to provide practical soil management advice for farmers.

Reganold et al. (1993) measured a large number of soil parameters in comparing the potential for mitigation of detrimental effects of conventional and biodynamic farms on the environment in New Zealand. The measures included cation exchange capacity, earthworm numbers bulk density, penetrability and topsoil thickness. They also compared financial viability of the farms. The abundance of fluorescent pseudomonad bacteria was measured as a biological indicator of soil quality by van Steensel (1995). Pfeiffer (1983) discussed how humus quality and amount and earthworm and bacteria activity determine soil fertility. Sarapatka (2000) found negative correlations between quality of humus (humic: fulvic acid ratio) and available P content. However he found correlations between soil phosphatase activity and content of organic C and total N and discussed how enzyme activity is an indicator of soil quality.

Goh et al. (1999) evaluated many potential soil quality indicators in organic, conventional and integrated orchard fruit production systems. They found that the only soil sensitive indicators capable of distinguishing significant differences between different orchard management systems were microbial biomass C, microbial biomass C: total carbon and hot-water extractable carbohydrate: hot-water extractable C. This indicates that measurement involving the activity of soil biota may be useful indicators of soil quality and perhaps plant nutrition.

Sparling and Schipper (2002) reported on measurement of soil quality at 222 sites in New Zealand in order to report on national and regional environmental performance, raise community awareness about soil quality, set land management guidelines and develop policies. They found that total C, total N, mineralisable N, pH, Olsen P, bulk density and macroporosity explained 87% of the total variability. The study provides information about how these parameters vary with different kinds of land use.

Measurements that distinguish between different types of soil organism such as Biolog^{TM} and microbial fatty acid methyl esters (FAMES) assays can indicate microbial

species diversity. The Biolog™ measure also provides some indication of the major food of microbes. Schutter et al. (2001) used these methods to compare effects of season, soil type and winter cover-cropping or winter fallowing on soil microbial communities in vegetable cropping systems. They found that season had the biggest influence and system of management the least influence on microbial communities. Fungi and protozoa were most plentiful in the spring, whereas bacteria were dominant in the summer and autumn. They considered that the high spring numbers of fungi could have related to greater moisture and plant residue content, and that tillage could have subsequently reduced the fungal content. They found the Biolog™ substrate diversity measurements were more sensitive to different management systems, being more diverse in the cover-cropped treatment. They said the differences in Biolog™ substrate use patterns suggested utilization of different substrates by the communities. Those under soils that had been cover-cropped utilized more sugars and amino acids, whereas those in fallow soils utilized more carboxylic acids. As carboxylic acids are more likely to come from plant root exudates than from decomposing plant residues, the researchers thought it likely that the communities in soils that had been cover-cropped were more able to utilise decomposing residues.

Long-term trials such as the K-experiment in Sweden and the Swiss DOC trial (discussed in Sections 2.2 and 2.3) have enabled monitoring of both crop quality and soil parameters under different fertilization and pest management treatments. However soils and overall management systems are the same for all treatments.

2.1.2 Food quality, nutrition and human health

Nutrition and health

Grimme (2000) listed food quality criteria, which make up a "health value" as:

- the classical content of nutrients in total diets (nutritional value);
- the hygienic and toxicological criteria (food safety),
- the physiological factors of food (sensory and intestinal quality); and
- the environmental quality of food production (sustainable farming).

Relationships between nutrition and human health are difficult to establish. Most focus has been on food safety, and effects of particular nutrients and diets on disease risk.

McCullough et al. (2002) found that an Alternate Healthy Eating Index (AHEI) score of information collected by dietary questionnaires was significantly associated with relative risk of cardiovascular disease and other chronic disease and mortality.

Relationships between particular nutrients and health have been established. For example, zinc is one of the many nutrients that has been found to affect immune response: even small deficiencies can result in immunological defects (Wellinghausen, 2001). Zinc is required for development of the immune system and cognitive performance (Shinjini and Sunita, 2001). Lack of selenium has been linked to several kinds of cancer, heart disease and other chronic and life threatening conditions (Gupta and Gupta, 2000). A deficiency of any of the vitamins and micronutrients: folic acid, Vitamin B12, Vitamin B6, niacin, Vitamin C, Vitamin E, iron, or zinc, mimics radiation damage to DNA by causing single- and double- strand breaks, oxidative lesions, or both, which is a likely cause of increased cancer risk (Ames, 2001).

A shortage of particular essential amino acids in the diet has been shown to affect health, for example, a link between a low lysine: arginine ratio in protein fed to rats with atherosclerosis (Rajamohan and Kurup, 1990). Reeds (1988) discusses the importance of glycine in nucleic acid synthesis. For humans to remain healthy they require a sufficient supply from their diet of the essential (indispensable) amino acids for protein synthesis, as their carbon chains cannot be synthesized in the body (Stipanuk, 2000). These essential amino acids are: threonine, valine, leucine, isoleucine, phenylalanine, methionine, lysine, histidine and tryptophan. Cysteine and tyrosine are semi-essential, as they can be synthesised from other amino acids but this is irreversible.

Young and Borgonha (2000) discuss methods of scoring protein according to its provision of essential amino acids and the importance of its source (plant or animal), bioavilability to humans and requirements by different age-groups into account. This makes the Massachusetts Institute of Technology (MIT) amino acid requirement pattern preferable to the FAO/WHO/UNU (1985) protein digestibility-corrected amino acid score method, which is based on requirements for children of preschool age and digestibility prediction by the rat faecal-balance method for predicting protein quality.

Vegetables and health

Links have been established between consumption of fruit and vegetables and beneficial health effects, particularly on reduction of cardio-vascular disease (Liu *et al.,* 2000), reduction of cancer risk (Ames et al., 1995), and less well established links with respiratory problems and diabetes. One response to such findings by the food industry and nutritionists has been to isolate the minerals and vitamins that appear to be involved and try to increase consumption of them by supplements and food fortification. Some nutritionists, however, point to adverse effects of consumption of vitamins in isolation e.g. the increase in deaths from lung cancer in patients given high doses of b-carotene as an anti-oxidant (Albanes, 1999). The difficulties of achieving a healthy balance of nutrients for each individual with differing metabolism and circumstances (e.g. children, pregnant women, older people, diabetics), all of which affect nutrient requirements and bioavailability, can be illustrated by the problems with iron supplementation, such as excessive intake by individuals with haemochromotosis and increased uptake when consumed with vitamin C (Pippard, 1995, Ward, 1995).

Some nutritionists advocate increased consumption of fruit and vegetables rather than pills and food fortification (Jacobs and Murtaugh, 2000). A large proportion of the world's population depends on vegetables to supply much of the minerals and vitamins required for health, in particular vegetarians and those who find meat too expensive. Measurements of vegetarians' body levels of antioxidants show that a vegetarian diet maintains higher antioxidant vitamin status (vitamin C, vitamin E, beta- carotene) but variable antioxidant trace element status as compared with an omnivorous diet (Rauma and Mykkanen, 2000).

However, there is evidence that the nutrient content of fruit and vegetables has declined over time. In a comparison of mineral content of a range of fruit and vegetables as recorded in UK nutrient composition tables in 1936 and 1986, significant decreases in a number of elements, were evident (Meyer, 1995).

Table 1 Geometric mean ratio of change in mineral content and dry matter of 20 vegetables and 20 fruits over a 50 year period (from Meyer, 1995)

Element (Mineral)	Vegetable ratio, GM+	Fruit ratio, GM
Calcium	0.81 (0.014)++	1.00 (0.96)
Magnesium	0.65 (0.000)	0.89 (0.016)
Iron	0.78 (0.088)	0.68 (0.002)
Copper	0.19 (0.000)	0.64 (0.006)
Sodium	0.57 (0.013)	0.90 (0.56)
Potassium	0.86 (0.090)	0.80 (0.000)
Phosphorus	0.94 (0.49)	0.99 (0.90)
Dry Matter	0.97 (0.53)	0.91 (0.023)

\+ Geometric mean: the antilogarithm of the mean of the logarithm of the ratio of 1986 to 1936 values
++ Probability from t-test

The table shows that there were significant reductions in the levels of calcium, magnesium, copper and sodium in vegetables and of magnesium, iron, copper and potassium in fruits over the 50 year period. The actual values should be treated as a rough guide, as analyses for food composition tables are carried out on a fresh weight rather than dry weight basis. However, the difference between the dry matter content of the two sets of vegetables analysed was too small to be statistically significant. Also, it is the fresh weight of vegetables that is purchased and eaten, and therefore of nutritional relevance. The author points out some of the problems of comparing data compiled at different times by different people. The analytical methods were not always the same and the methods of sampling could have been different. Nevertheless, the results raise some interesting questions as to what could have caused the differences. The likely biggest contributor to this decline is the emphasis in plant breeding on breeding plants for high growth rates and yields rather than nutrient composition. Growing systems could also contribute to differences in nutrient contents.

Food quality and growing systems

Frossard et al. (2000) reviewed possibilities for increasing the content and bioavailability of iron, zinc and calcium in edible parts of staple crops as a way to combat mineral deficiencies in human populations. This could be achieved by increasing the total level of Fe, Zn and Ca in the plant foods, while at the same time increasing the concentration of compounds which promote their uptake (e.g. ascorbic

acid), and/or by decreasing the concentration of compounds which inhibit their absorption (e.g. phytic acid or phenolic compounds). They concluded that the content of Zn and Ca in grains and fruits in some cases can be increased through soil and/or foliar applications of Zn and Ca fertilisers, but plant breeding and genetic engineering techniques have the greatest potential to increase Fe and Zn content in grains, roots and tubers.

Welch (1997) discussed how agricultural systems could be modified in some developing nations in ways that would provide adequate dietary amounts of pro-vitamin A carotenoids from plant food sources, particularly for infants and children of low-income families. The main ways highlighted were growing more vitamin A rich crops and plant breeding. Welch concluded that the world community should strive to find food-based system approaches to eliminating vitamin A deficiency by modifying agricultural systems in ways that will not only maximize food production, but provide quality food.

Organic, biological and biodynamic systems are claimed to be more sustainable and provide higher quality food than soluble fertiliser based systems. Research comparing these systems has yielded variable results.

2.2 Organic Farming Systems and Soil Quality

2.2.1 What is an organic farming system?

"Organic farming, which includes the terms biodynamic, ecological and biological, is an approach to farming that seeks to create an integrated, sustainable and humane agricultural system. Organic farming relies primarily on locally, or farm derived, renewable resources and the management of biological processes for crop, livestock and human nutrition and for protection from pests and diseases. The same principles apply to organic horticulture and aquaculture." (MAF, 1990)

This definition incorporates a concept of regional or agro-ecosystem sustainability, which includes the importance of forests and water supplies, as well as stressing the importance of the farm as a unique individual unit and production of high-quality food and integration with the local community. Organic certification standards are based on such definitions but also clearly define what materials can be imported to the farm and how they should be treated. For example, cow manure brought in from a non-organic farm should be put through a composting process before being used. "Natural" amendments, such as rock phosphate, are brought in to restore mineral balance, then the soil organic matter and mineral content of the soil is maintained by regular incorporation of decomposed plant and animal wastes. Organic farming systems and how certification standards relate to them is discussed by Wright (2002). MacRae et al. (1990) argued that to achieve an environmentally sustainable system involves redesign of the farming system rather than just substituting some inputs and practices for others.

The concept of a whole farm organism as an agro-ecosystem is discussed by Raupp (2000). The organic system relies on biological processes of soil organisms to make nutrients available to crops, rather than providing soluble nutrients. Pest and disease management is by strengthening plant health and cell walls, build up of secondary metabolites in the plant and biological pest controls. Soil conditions are developed to favour flourishing, diverse soil organism communities, which then cycle nutrients. Pankhurst et al. (1997) discussed the importance of improved management of soil biota in sustainable farming systems. A good organic farmer is farming the soil organism community, which then provides for healthy crops and animals.

Understanding and managing the processes which lead to nutrient mineralisation synchronised to crop growth needs has been studied by researchers such as Carpenter-Boggs et al. (2000) and Pakrou and Dillon (2000). There is also growing recognition that an organic system can only produce good crops if the balance of minerals in the soil is suited to the soil biota and crops wanted. The biological system of farming is based on the Albrecht system of balancing soil minerals, particularly cations, to nurture soil life and produce healthy plants and animals (Zimmer, 2000).

The main ways organic systems may differ from conventional farming are:

- An organic system aims to replenish nutrients through mainly biological processes rather than application of soluble mineral fertilisers.
- Increased diversity of soil biota, plant and animal varieties and farm enterprise in an organic system rather than reduction in diversity and monocultures.
- Adaptation of an organic farm to the particular soil and climatic conditions rather than standardization of conventional farms.

Waldon et al. (1998) wrote that attempts to modify soils to some general quality standards may be misplaced because each soil ecosystem has unique properties and system balances. They compared carbon, nitrogen and phosphorus balances over an extended time under organic and conventional management systems and a native soil ecosystem. They found that the organic system had a high production of healthy plants in spite of having a lower nitrogen and phosphorus content, suggesting that mineral nutrients are used in a more efficient manner under an organic system.

2.2.2 How do organic farming systems affect soil and soil organism properties?

Reganold et al. (1993) compared paired conventional and biodynamic farms in New Zealand. They reported that the biodynamic farms in most enterprises had soils of higher biological and physical quality, significantly greater organic matter content and microbial activity, more earthworms, better soil structure, lower bulk density, easier penetrability and thicker topsoil. These results could be due to greater plant and animal matter recycling in an organic system. Murata and Goh (1997) found that total C and N decreased with increasing period of cropping on a biodynamic farm but the reverse

occurred under pasture. This increase under pasture was attributed to greater biological N_2 fixation and the return of plant residues and excreta from grazing animals. Whereas organic matter inputs may be greater under pasture than cropping, the increase in soil organic matter under pasture is often due to the slower organic matter decomposition rates in uncultivated soils compared to cultivated soils (Jenkinson, 1980).

Fliessbach and Mäder (2000) found higher microbial biomass C and N in soils that had been under organic management compared to soluble fertiliser[1] systems for about 30 years. All systems included rotation of cropping and pasture and differed only in fertilisation and plant protection methods. The ratios of microbial C and N to total and light fraction, soil organic matter C and N were also higher, indicating enhanced decomposition activity. Mäder et al. (2002) reported further results from analyses of this long term experiment. Nutrient input (N, P, K) was 34 to 51% lower in the organic compared to the soluble fertiliser systems, whereas mean crop yield was only 20% lower over a period of 21 years. In soils of the organic systems, dehydrogenase, protease, and phosphatase activities were higher than in the soluble fertiliser systems, possibly indicating a higher overall microbial activity and a higher capacity to cleave protein and organic phosphorus (Increased enzyme activity alone may result from increased enzyme synthesis rather than an increased microbial population.). Phosphorus flux through the microbial biomass was faster in the organic soils, and more phosphorus was bound in the microbial biomass, rather than being dissolved in the soil solution.

The recycling of plant and animal matter is dependent on soil organisms. Soil food web studies (e.g. Hendrix et al., 1986, Ingham et al., 1985) illustrate the roles of the large diversity of soil organisms in nutrient cycling. In the long-term European crop rotation trial, biomass and abundance of earthworms were higher by a factor of 1.3 to 3.2 in the organic compared to soluble fertiliser plots (Mäder et al. 2002). Average activity densities of scarabids, staphylinids, and spiders, which they considered sensitive indicators of soil fertility, were almost twice as high in the organic compared to mineral plots. Soil aggregate stability was 10 to 60% higher in the organic than in the mineral plots and there were positive correlations between aggregate stability and microbial and earthworm biomass.

[1] Some researchers refer to "mineral" or "conventional" systems or fertilisers. In this thesis, all will be referred to as "soluble fertiliser" systems.

Soil aggregate stability, drought stress tolerance and nutrient availability have also been found to be positively related to the density of AM fungi colonisation (Tarafdar and Rao, 2002; Marschner, 2002). Mäder et al. (2000) found that root length colonized by mycorrhizae in organic farming systems was 40% higher than in soluble fertiliser systems. However, there was less difference in plots provided with high levels of nutrients under both systems. Brechelt (1990) also found that the positive effect of VA mycorrhiza on plant yield decreased with increasing amounts of organic manure due to better growth of the non-mycorrhizal plants.

Microbial diversity and other soil parameters were found to be further enhanced by application of the biodynamic preparations in the european long-term trials (Mäder, 2002). Carpenter-Boggs et al. (2000) found that plots receiving the biodynamic field sprays had significantly higher amounts of soil C mineralised in 10 days (MinC) than non-sprayed plots in one out of 2 years measured, but addition of the biodynamic compost preparations to compost did not significantly affect soil biotic parameters, even though earlier tests showed discernible changes in biodynamic compost chemical and microbial parameters (Carpenter-Boggs et al., 1999).

Some likely reasons for variation in research results on effects of organic amendments and biodynamic preparations on soil and soil organism parameters are: length of time from change to an organic system; and variations in contents, manufacture methods and quality of inputs. Woods End Research Laboratory (2000) has developed quality tests for composts and biodynamic preparations. Brinton (1997) discussed results of testing samples of biodynamic preparation 500 made in different ways and in different countries.

The research discussed has shown that links have been found between organic systems and soil health, but that it depends on actual practices used and what other systems are used for comparison. Possible links between organic systems and product quality have been harder to establish.

2.2.3 How do organic farming systems affect food quality?

Reviews of trials comparing food from organic and conventional farming systems

Numerous studies have attempted to show differences in the nutritional content of conventionally and organically grown fruit and vegetables. Woese et al. (1997) reviewed over 150 of such investigations, and concluded that most studies either contradicted each other, or did not show any major differences in analyses apart from far higher nitrate content in vegetables produced with soluble fertilisers. Worthington (2001) reviewed the same studies. She then took 41 studies for which there was adequate data and used the Wilcoxon signed-rank test to identify significant differences in nutrient content. She concluded that the organically grown crops contained significantly more vitamin C, iron, magnesium and phosphorus.

A difficulty of this kind of amalgamation of results is that the experiments would have been carried out on such different bases. Some were field trials, others pot trials and others surveys. A large range of factors would have varied between trials, including whether or not growing periods for the different treatments were the same and whether nutrient content was measured on a dry weight basis. Worthington stated that the actual sizes of differences in nutritional content were not reliable figures, but they indicated the direction of differences. She also pointed out that Woese et al. (1997) found that half of the studies indicated no difference in vitamin C content between organic and soluble fertiliser treatments and half higher vitamin C content in the organic vegetables, and that this leads to the conclusion that on average vitamin C content in organic vegetables is higher.

Some other difficulties identified by Woese et al. (1997) were that the studies were hard to evaluate as they used different methodology – some compared the whole system of growing over a number of years, while many compared different treatments of various organic and soluble fertilisers. Some were surveys of food marketed from the different systems, using a variety of methods of sampling. Bourn (1994) pointed out that factors such as soil type, climate, planting and harvest date affect nutritional value irrespective of farming system, which complicates interpretation of the studies. Plant variety, soil type and climate often appear to cause more variation than growing system. Bourn and Prescott (2002) reviewed previous comparisons and found little evidence that organic

and conventional foods differ in composition (with the possible exception of nitrates) and that findings for sensory properties are inconsistent.

Heaton (2002) also reviewed organic food production comparative studies. He assessed them for validity using the following criteria:

- Only data from certified organic produce and farms are considered valid;
- Agricultural practices in experimental trials must reflect typical practices within the respective methods of agriculture
- Only comparisons of nutrients on a fresh-weight basis are included (this enables relevance to consumers and inclusion of effects of higher water contents in some products);
- Studies must include relevant quality comparisons ie they should compare a variety of essential nutritional components that could have an impact on health.

From 99 studies found, only 29 fitted these criteria. Of these, slightly more showed higher trends for dry matter and/or minerals and vitamin C in organically grown fruit and vegetables than reported no difference. Heaton recommended more primary research to quantify differences in nutritional content and to further investigate under controlled conditions the link between organic food consumption and human health.

Measurement of product nutrient contents

Warman and Havard (1997, 1998) conducted three-year comparative field trials of several treatments, growing carrots, cabbages, sweet corn and potatoes. They adjusted compost and soluble fertiliser composition and application rates to provide as far as possible a similar nitrogen supply. Although there was considerable variation in content of various minerals and vitamins between different treatments in some years, overall there was very little difference because there was greater variation from different climatic conditions in different years. Similar results were obtained by Nilsson (1979), who compared the effects of organic and soluble fertilisers at normal and half levels of application on yield, storage ability and chemical composition of carrots, cabbages and leeks. The main result was the reduced yield of cabbages and leeks which received half rates of fertiliser of either kind. He found very little difference in yield, storage ability or composition of vegetables grown by either method.

Many researchers have found lower levels of nitrates in organically grown leafy vegetables. A field trial comparing organic and soluble fertilisation of lettuces found that similar yields and nutrient content resulted from applying all treatments on a N-equivalent basis (Lairon et al., 1984). The main difference was much lower nitrate content in the lettuces grown with organic fertiliser. In this trial, nitrogen was supplied in 3 different ways – by ammonium nitrate, sodium nitrate and castor-oil seed cake, each at 2 rates, 120 kg N/ha and 200 kg N/ha.. Little difference between treatments at each rate was found for yield, dry matter content and mineral content. There was significant difference in nitrate content, which increased in the order seed cake – ammonium nitrate- sodium nitrate. This could be because when all the nitrogen is supplied as nitrate, and it is in high concentration around the roots, most has to be transported to the leaves before reduction and assimilation into amino acids (Marschner, 2002) and the leaf cells would have insufficient capacity to utilize all this nitrate. Nitrogen as ammonium is more quickly incorporated into amino acids in the roots, and providing both nitrate and ammonium is less likely to cause problems of cation-anion imbalance for the plant.

Lieblein (1993) compared quality and yield of carrots from composted manure and soluble fertiliser field treatments. He concluded that the organically grown carrots were more often of higher quality, mainly due to slower release of nutrients from composted manure.

Long-term trials

A major problem with fertiliser treatment comparisons is that they are generally short-term, whereas it takes many years to establish and stabilize an organic system. Mäder et al. (1992) analysed beetroot grown in a long-term field trial in Switzerland, comparing bio-dynamic, bio-organic and conventional farming systems incorporating soluble fertilisers (DOC trial). They found the biological systems more efficient – as input of N and P was 60% lower, but yields were 75% of those from the conventional system. There was little difference in mineral composition of the beetroot.

Granstedt and Kjellenberg (1996) compared effects on wheat plants of conventional treatments with organic, with and without the biodynamic preparations in several long-term experiments. The first, the "K- experiment" ran from 1958 to 1990, and included

8 fertilisation treatments, each with a four-fold crop rotation, but there were no replications and it was considered to be a fertilisation experiment, rather than a comparison between different farming systems. The next, "UJ-experiments" were carried out from 1971-76 in 2 locations, using a split-split-plot design with 3 replications. NPK content of fertilisers\manures was slightly lower for the organic than for the conventional treatments. They studied chemical/biological, physiological and morphological quality parameters of the plants and plant products and concluded that the organic fertilising systems resulted in a higher organization of the plants, similar to the effect of more light exposure. This effect was shown by lower content of free amino acids and better storage properties, which were further enhanced in the treatment using biodynamic preparations.

In all the experiments, there was greater variation in mean values between years than between treatments. Overall yields were similar for organic and conventional treatments, being greater for organic in years with higher precipitation which favoured mineralisation of the N in the manure. Crops from the organic treatments tended to have a higher dry matter content and higher protein quality, as measured by an index of the essential amino acids and relative free amino acid content. The report does not specify whether the crops from the different treatments were harvested at the same time. If they took longer to mature this would account for some of the differences measured. Raupp (1996) reviewed these results and concluded that plant concentrations of nitrate, K, Mg, Ca, P, S, Cl, Mn, Zn and Se, and food quality indices are meaningful parameters of food quality.

Secondary metabolites

Brandt and Molgaard (2001) postulated that the main benefit of organic products could be their secondary metabolite content. Secondary metabolites were defined as seemingly less essential or non-essential by-products which usually occur only in special, differentiated cells and are not necessary for the cells themselves but may be useful for the plant as a whole. They are often defence-related plant compounds which were thought to have no apparent physiological role as a human nutrient, but recently beneficial effects have been attributed to some of these compounds. Brandt and Molgaard discussed theories such as the carbon: nitrogen balance and growth/differentiation theories in which products, such as most secondary metabolites,

are favoured by a fairly high carbon: nitrogen ratio in the plant. They suggested that the presence of secondary metabolites that reduce protein assimilation in human digestion could be a health benefit in diets too high in protein content. Many secondary metabolites are known to have other health benefits such as antioxidant and free radical reduction actions.

Image forming techniques and vital quality

Many European researchers have moved away from measuring nutrient contents to trying to demonstrate differences in nutritional quality by other means. A key concept investigated is the "vitality" of the food. Weibel et al. (2000) measured fruit "vitality quality" by image-forming techniques (crystallization of apple juice in copper chloride, and chromatography in silver nitrate and iron sulphate) in apples harvested from 5 pairs of organic and integrated fruit production fruit farms in Switzerland. Image-forming quality was 60% higher in organic apples. The image-forming method distinguished correctly (100%) between samples and was closely in line with technical quality.

In a study of apple quality, Bloksma et al. (2000) developed a coherent "vital quality" concept by classifying plant growth and food quality parameters into three processes – growth, differentiation and integration. The authors postulated that good food quality requires a balance between the growth processes which result in high yield and the differentiation processes which lead to fruit and seed production and formation of secondary metabolites. The balance is achieved in a dynamic integration of these processes, resulting in coherent products, for example apples being tasty, juicy, crisp, sweet, acidic and aromatic.

Sensory tests

Haglund et al. (1999) grew carrots with soluble fertilisers and ecologically and found that growing system and variety had an impact on sensory quality. A consistent trend for two consecutive years was that carrots grown with soluble fertilisers scored higher for carrot-taste, while ecologically grown carrots scored higher for bitter taste (likely related to stress). In one out of two years, carrots grown with soluble fertilisers had a sweeter taste and were crunchier, while ecologically grown carrots were harder and had a more pronounced aftertaste.

Some difficulties with sensory tests are that they depend on what the taster is used to and on their ability to distinguish different tastes. These problems can be partly overcome using trained panels. Villeneuve (1997) reported that carrots grown in different soil types tasted by a panel of tasters revealed clear differences in the organoleptic appeal of carrots grown at the Mont-Saint-Michel as compared with those grown in Val-de-Saire, Nantes and the Landes region of France. They said that other factors such as the year, the cultivar, the fertilisation regime and the time of harvest could interfere with the effects of the soil. (Higher K uptake would increase sugar content).

Animal feeding tests

Feeding preference tests can also be conducted, for example Mäder et al. (1992) found rats had greatest preference for organically grown beetroot over beet grown biodynamically or with soluble fertilisers. Knorr and Vogtmann (1983) reviewed experiments to compare the effects of organic and soluble fertiliser grown feed on animals. They cited experiments by Schiller (1971) in which higher rates of sterility were recorded in cows on soluble fertilised pasture and by Gotteschewsi (1975) in which higher numbers of rabbits born alive to does fed on an organic diet were recorded. Knorr and Vogtmann also cited a trial by McCarrison (1926) in which pigeons fed on a basal ration of grain lost less weight when the grain had been grown using cattle manure than when soluble fertiliser was used. Such trials are of little value unless they can establish cause and effect.

Delayed luminescence

Delayed luminescence is increasingly being used in Europe and Japan to test food products for quality and human tissue for evidence of disease. The tests use principles of quantum optics. Popp et al. (1994) describe experimental results of measuring delayed luminescence of plants and other living systems. They have measured how the biophoton emission relaxes back to its resting state after excitation by white light. This appears to vary according to the coherence of the electromagnetic field within and between cells. Popp (1999) discusses how biophotons are an ultraweak emission by all living cells and particularly DNA. He describes coherency as the ability of waves to overlap to form a communicating field. Bajpai (2000) discussed how photons play a crucial role in both information transfer as well as in chemical reactions. Muths (2001)

reports on how tumour tissue can no longer communicate coherently via biophotons and on research by Popp in which free range eggs showed a significantly higher capacity to store light than eggs from battery caged chickens. Potatoes treated with artificial fertilisers were found to emit more irregular biophotons than organically grown potatoes. Triglia et al. (1998) used delayed luminescence as an indicator of tomato quality.

2.3 The Biodynamic Preparations and Silica

Biodynamic farmers and growers use an organic system that emphasizes the uniqueness of each farm unit. In addition to the usual organic practices, biodynamic farmers use the biodynamic preparations to stimulate activity in the soil and organic matter, to improve the decomposition process in the compost heap and to bring more light and warmth into plants to assist cell differentiation and flower, fruit and seed formation. There has been considerable research on the effects of these preparations but much has only been published in German. In New Zealand there is only anecdotal evidence for their effects. Summaries of research have been made by Goldstein (1990), Koepf (1993) and Koppenol (2002).

2.3.1 The biodynamic preparations

The biodynamic preparations have been developed from recommendations made in 1924 by Rudolf Steiner (1993). Procter (1995) has provided detailed descriptions of how they are made and applied. They consist of:

- The field-sprays, horn-manure (500) and horn-silica (501), are made by burying horns filled with cow manure during the winter, or with finely-ground quartz during the summer, respectively. Only small quantities are needed – about 25 g /37 L /ha (500) and 1 g / 37 L / ha (501). A third preparation has recently been added: horn-clay, alluded to by Steiner (1993) to mediate between the 500 and 501. The horn-manure is sprayed on the soil or pasture in spring and/or autumn to activate soil life. The horn-silica is sprayed up in the air over plants to

"increase light activity" in the plants.

- The compost preparations 502 – 507, which are each made by decomposing particular plant parts in specific animal parts buried in soil. About 1g of each of these is placed separately in a compost heap or liquid fertiliser to bring order to the decomposition processes.

- Equisetum (508), which is made up as a homeopathic solution to spray on plants for pest and disease control.

Some research into the effects of individual preparations (e.g. Kolisko, 1978, Pfeiffer, 1983, Remer, 1995) enables understanding of their function and how specific preparations can be used for specific purposes. However, it is generally recommended that all the preparations be applied each year to the land at appropriate times because they were developed to be used together bring about balance and harmony (Biodynamic Association, 2002). Raupp (1996) wrote that both preparations need to be applied and that their principle effect is to regulate plant growth – either one alone leads to unbalanced plant growth, particularly if no compost or farmyard manure containing the compost preparations has first been applied to the soil. Under poor growing conditions, preparations 500 and 501 generally increase growth and yields, but under very good growing conditions they may depress growth (Raupp, 1996).

2.3.2 Effects of the biodynamic preparations on quantity and quality of crops

Experiments with preparations 500 and 501 on a range of crops have generally resulted in yield changes of –9 to +30% (Goldstein, 1990). Research into effects of the biodynamic preparations on quantity and quality of crops was reviewed by Koepf et al. (1993). Experiments by Pettersson (1979) showed increased crude protein and nitrate contents in autumn compared to spring, Composted manure produced better quality food products than uncomposted or mineral fertiliser. Pettersson developed a food quality index composed of scores which were higher for lower crude protein content; higher true: crude protein, less darkening of tissue, better crystallization pattern and less growth of pathogenic organisms. He found that food quality indices were improved after applying preparation 500 to the land, but only if there was more than 300 mm rain

during the growing season: quality indices were negatively affected by preparation 500 if rain was less than 300 mm. The interaction of preparation 501 with moisture was similar. Application of 501 in morning and at early or middle plant growth stages had better effect than treatment in the afternoon and at later growth stages. Statistical significance of these results was not published.

Koepf (1993) quotes results published by Wistinghausen who found considerable differences in free amino acids and nitrate content of carrots grown with soluble fertilisers or with biodynamic compost and sprays (Table 2.3.1).

Table 2.3.1 Influence of Soluble fertiliser (NPK) and Biodynamic management on the free amino acid and nitrate concentration in carrots (Koepf, 1993)

	NPK	Biodynamic compost & sprays
Free amino acids +	481	379
Nitrate (mg/kg fresh material)	202.2	95.7

+ no units specified

Many studies have found that organic and biodynamically grown vegetables contain significantly lower levels of nitrates than those grown with soluble fertilisers. For example, Mäder et al. (1993) analysed results from long-term field-trial comparisons between organic, biodynamic and soluble fertiliser treatments in Sweden, Germany and Switzerland. These trials have shown consistent differences in food quality parameters from the different systems. Pettersson (1978) found higher quantities of essential amino acids, particularly methionine, higher dry matter and lower crude protein content of potatoes from the biodynamic treatment in the Swedish UJ experiment 1971 – 1979 (Table 2.3.2).

Table 2.3.2 Pure protein as % of crude protein in potatoes grown by biodynamic method or with mineral fertilisers (Granstedt and Kjellenberg, 1996)

	Biodynamic	Mineral fertilisers
	Pure protein as % of crude protein	
Jaerna	62.1	60.2
Ultuna	63.0	60.0

Experiments 1971-79 in Sweden by Pettersson (1982)

These differences were small (<5%) but had weak statistical significance ($p<0.1$). Pettersson considered the pure protein: crude protein ratio a good measure of food quality.

Soil and soil biota analyses have shown considerable differences between treatments in the long-term trials in Europe, as discussed in Section 2.2. Although it appears likely that differences in food quality are related to these differences, it is difficult to show correlations or causal relationships because of the many factors involved.

Comparison of several types of New Zealand biodynamic farms with their conventional neighbours by Reganold et al. (1993) revealed that the biodynamic farms in most enterprises had soils with significantly greater organic matter content and microbial activity, more earthworms, better soil structure, lower bulk density, easier penetrability and thicker topsoil. However it was not possible to say whether this was solely due to application of biodynamic preparations.

One likely reason for variation in results of research on the biodynamic preparations is variations in quality of the preparations, which can vary considerably in quality according to original materials, method of making and of storage. The quality characteristics of horns used to make preparations 500 and 501 can vary depending on the age and health of the cows they come from and how many times they have been used. Brinton, (1997) compared the quality of preparations made in "good" and "poor" quality horns with raw manure. In a good horn the N% increases slightly, whereas in the poor horn it decreases. In a good horn pH and C: N decrease to about 5.6 and 13 respectively, whereas in a bad horn they are much higher.

Effects of the compost preparations

Application of biodynamic preparations has also been shown to affect soil biota activity and plant growth processes. Several researchers have investigated the effects of the biodynamic compost preparations. Carpenter-Boggs et al. (1999) compared composts made with and without the preparations. The biodynamic-treated composts maintained an average 3.4 deg. C higher temperature over 8 weeks, suggesting more thermophilic

microbial activity. Final samples, taken when active composting slowed and the piles entered a ripening stage, showed a 10% lower rate of CO_2 respiration, a larger ratio of dehydrogenase enzyme activity to CO_2 production and 65% more nitrate in biodynamic than control piles. However a further trial by Carpenter-Boggs et al. (2000) found no significant difference between biodynamic and non-biodynamic composts in soil microbial biomass, respiration, dehydrogenase activity, quantity of soil carbon mineralised in 10 days (Min C), earthworm population, and metabolic quotient of respiration per unit biomass by the second year of study.

Effects of the field spray preparations 500 and 501

Koepf (1993) reported on trials by Tegethoff (1987) who recorded reduced leaf surface area, reduced stomatal openings and lower transpiration rates in bush beans after applying preparation 501. Applying 501 did not affect photosynthesis rate, but increased chlorophyll content, and diameter of roots. Preparations 500 and 501 together increased the number of stomata on the lower side of beetroot leaves at low light intensity and increased the specific density of leaves.

Koenig (1988) carried out comprehensive trials with preparations 500 and 501 and found that the preparations when applied several times together (500 in the evening, 501 in the morning) increased relative leaf surface in upper, younger leaves, reduced overall leaf area, increased net CO_2 assimilation, and reduced stomatal oscillations. Application of 501 only, decreased CO_2 assimilation. No effects on soil respiration were found. However Carpenter-Boggs et al. (2000) found that plots receiving the biodynamic field sprays had significantly higher mineralisable C than non-sprayed plots in one out of 2 years measured, when mineralisable C, basal respiration and substrate induced respiration were measured on the same set of soil samples.

2.3.3 Light and dark effects: preparations 500 and 501 and the calcium and silica processes

The growth processes enhanced by the field-sprays 500 and 501 were first researched in the 1920s by Kolisko and Kolisko (1978). They designed trials to demonstrate the "calcium" and "silica" processes described by Steiner (1993). They described the effects of the silica, or light process, as though the plant had been grown in high light

intensity, and the calcium or life process as though the plant grew in the shade. Granstedt and Kjellenberg (1996) described the effects seen in long-term trials of the preparations in similar terms.

It is instructive to examine how proponents of biodynamic husbandry describe the processes involved in the beneficial actions of biodynamic management including the application of preparations. Koepf et al. (1976) describe experiments by Klett that compared the effects of full daylight, half shade and deep shade, organic and mineral fertiliser, with and without preparation 501 (Table 2.3.3).

Table 2.3.3 Effects of light and shade conditions on quality of food products (From Koepf, 1993)

	Contrasting factors	
Factors producing effects	Light intensity, duration	Shade
	Dry, warmth	Moisture, cool, humid
	Balanced composts	Crude animal manures, N fertilisers
	Sandy soil	Rich humus soil
	Preparation 501 and 500	Preparation 500 only
	Contrasting effects of above factors	
Plant form	Deep, less divided roots	Shallow, branching roots
	Short internodes	Long internodes
	Small, thick, short leaves	Large, thin, elongated leaves
Plant development	Premature ripening	Ripening delayed
	Reproductive process enhanced	Vegetative process enhanced
	Leaf metamorphosis enhanced	Leaf metamorphosis delayed
Pests	Mainly insect pests	Mainly pathogenic fungi
Product keeping quality	Long shelf life	Short shelf life
Product composition	High dry matter content	Low dry matter content
	Low crude protein content	High crude protein content
	High true protein:crude protein	Low true protein:crude protein
	Lower nitrate, amides, free amino acids content	Higher nitrate, amides, free amino acids content
	Higher disaccharides	Lower disaccharides
	Lower monosaccharides	Higher monosaccharides
	High vitamin C content	High vitamin A content
	Rich fragrance, taste	Poor fragrance, taste

Effects of full light on spinach plants included increased root length and vitamin C content, decreased crude protein and nitrate content and increased true protein:crude protein. Organic composts and preparation 501 gave similar effects to full light; mineral fertiliser gave similar effects to shade. Bloksma et al. (2001) used a similar approach to investigate parameters affecting apple quality. They divided parameters into those that promote growth and those that promote differentiation. In general, the parameters that promote growth correspond to the "calcium" or shade factors and those that promote differentiation the silica or "light" factors.

Lievegoed (1951) discussed how the calcium and silica and other processes referred to by Steiner (1993) are expressed as observable effects of the sun, moon and planets working on the earth, plants, animals and humans. They influence the life processes (which could also be visualised as forces or activities) "behind" the physical body. The cosmic silica process takes light and warmth from the outer planets down into the siliceous rocks underground, enhanced by the summer sun. The calcium process is the effect of the inner planets in the air moisture above the soil, which is "sucked down" and held by lime in the soil. Atkinson (2002) has developed this approach further and discusses the processes behind the use of each preparation. In Atkinson's view (Figure 2.3.1), the influence of these processes within the plant is more complex.

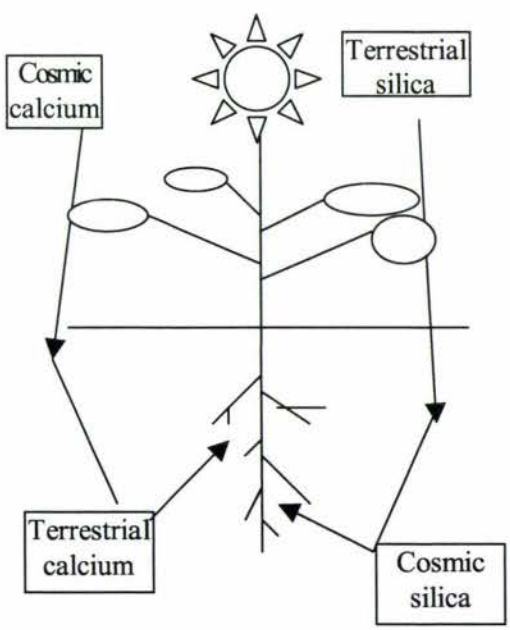


Fig. 2.3.1 The calcium and silica processes described by Steiner (1993) as they affect a growing plant. Adapted from Atkinson (2002). Atkinson draws the calcium and silica processes as 2 contiguous cycles.

According to Atkinson, the cosmic silica process works up from the soil into the plant, assisted by clay, drawing nutrients up the plant – particularly nitrates and phosphate anions. But this is counteracted by a secondary terrestrial silica process working straight into the plant in the light and warmth received from above which tends to make the plant more compact into a flat rosette form, and later to flower and seed. The terrestrial calcium process flows up from the lime in the soil, bringing and mobilising cations, particularly calcium and magnesium, into the plant. In the cosmic calcium process, carbon dioxide is drawn down into the plant by humus and water in the soil. The calcium processes enable reduction of nutrients; releasing oxygen, e.g. photosynthesis, growth, cell division and expansion and formation of sugars, whereas the silica processes e.g. respiration, involve oxidation, release of hydrogen and acidification, differentiation of cells and formation of protein.

Atkinson describes how applying the preparation 500 enhances the calcium processes, while the preparation 501 enhances the silica processes. If any one or more of these processes is stronger or weaker than the rest the plant shows signs of stress and growth is impeded. Lovell (2003) writes about how the third field-spray, horn clay increases the effects of 500 and 501. Horn manure increases root exudates of sugars, improving the plant's relationship with the nitrogen fixing microbes such as *Azotobacter* spp. and the protozoa that eat them and supply the plant with nitrogen as amino acids. The horn clay improves symbiosis with mycorrhyzal fungi that take up calcium, magnesium, phosphorus, etc.

Laboratory analysis of nutrients, compounds and organisms provides a snapshot view, but like any picture, it is understood that behind the compound tested is a flux associated with dynamic nutrient and energy cycling processes. As has been stated in biodynamic preparations research, "The question becomes, how can we see the relationship between the static representation on the one hand and the original wholeness of the process on the other" (Brinton, 1997).

It is likely that the biodynamic preparations affect the processes of cell signalling, hormone and enzyme activity described in Section 2.4.

2.3.4 Effects of Silica and preparation 501 on plant metabolism and nutrient content

Silica

The biodynamic preparation 501 is applied to crops at the rate of 1 g in 37 L water/ha. This is a very low application rate per hectare compared to studies in which silica has been applied to test the effects of silica as an essential element.

The question of whether silica is essential for plant growth has been debated for a long time. Remer (1995) discussed how there had been a decline in silica content and increase in potassium content of grasses and grains in Europe since the last century, having a considerable effect on animal health through decline in roughage quality. He also discusses the effect of silica in soils. Marschner (2002) discussed increased fruit yield in strawberries, soybean, tomato and cucumber attributed by researchers to silica (some in culture solution with 100 ppm SiO_2 and cucumber in alluvial soil with 700 or 1400 kg SiO_2 /ha/year). Re-examination revealed this effect was due to the silica increasing physiological availability of zinc in plants grown with high P and low Zn that were suffering effects of zinc deficiency induced phosphorus toxicity. However, Marschner reported that other research showed how silica assisted incorporation of inorganic phosphate into ATP, ADP and sugar phosphates in sugarcane.

Silica has been applied to crops in a variety of forms to the soil and as an aerial spray. Improved yields of sugar cane and rice have been recorded from calcium silicate applied to the soil (Berthelsen et al., 2001). Foliar application of potassium silicate twice a month at 603 mL/100 m^2 and 1205 mL/100 m^2 stimulated antioxidant superoxide dismutase activity and increased photosynthetic capacity and chlorophyll content in bentgrass, especially under a high fertiliser regime (Schmidt et al.,1999). Agarie et al. (1998) found that a reduction in rate of transpiration of rice leaves by application of silica 100 ppm silica in the nutrient solution was mainly attributable to reduction in the rate of transpiration through stomatal pores.

Yanishevskaya and Yagodin (2000) found that a silica compound (at unspecified rate of application) used either to pre-treat the seeds, to apply to the soil, or to spray mangold (chard), daikon (Japanese radish), and lettuce plants improved yield and vitamin C

content. Zaimenko (1998) found that silicon compounds made from polycondensate boiler waste (94-96% CH3 SiOCl3) applied at the rate of 4g per 1 litre of "Voloknisty" substrate had positive effects on the intensity of respiration processes, the activity of oxido-reductive enzymes and the accumulation of carbohydrates and free amino acids in leaves. Some of the considerable differences in free amino acid content (ug/mg dried leaves) are shown in Table 2.3.4.

Table 2.3.4 Effects of a silicon compound made from polycondensate boiler waste, applied at the rate of 4 g/L substrate, on amino acid composition (ug/mg dried leaves) of 2 Arum plant varieties (Zaimenko, 1998).

Plant	**Aglaonema**		**Anthurium**	
Amino acid	**Control**	**Silicon prep**	**Control**	**Silicon prep**
		ug/mg dried leaves		
Glutamine	9.9	27.4	33.0	57.4
Proline	4.0	9.7	2.1	11.3
Alanine	2.5	5.1	31.6	49.7
Glycine	4.4	10.3	3.9	8.8
Histidine	13.3	27.6	4.9	11.1
Arginine	3.7	5.1	0.9	5.8
Lysine	2.3	3.5	-	-
Methionine	1.0	1.2	-	-

Watanabe et al. (2001) investigated amino acids composition and sucrose concentration in leaf extracts and phloem sap of rice plants grown in nutrient solutions with and without silicon (at 100 ppm SiO_2). The proportion of asparagine was significantly lower in leaves of plants grown without silica, and in phloem sap, and glutamine was significantly higher in plants without silica. (The lower glutamine content contrasts with the other research results.) They suggested that nitrogen assimilation enzymes, asparagine synthesase and/or glutamine-glutamate synthase activities were altered by silica nutrition. Total amino acid concentration was also considerably higher in plants grown with silica. There was no significant difference between sucrose concentration in leaf extracts of plants grown with or without silica, indicating that photosynthetic activity per unit leaf area was not affected by different concentrations of silica. However, sucrose concentration was higher in phloem sap of plants grown without silica than those grown with silica, indicating that loading and/or unloading of sucrose into phloem could be affected by silica nutrition.

Preparation 501 (horn-silica)

The preparation 501 is applied as a very dilute concentration of colloidal silicon dioxide as a spray above the plant. Although it appears to have similar effects to silica salts applied to the soil, it may work in different ways due both to the form of application and its properties arising from being buried in a cowhorn in the soil during the summer. The main effects of the preparation 501 are on the quality of products – in which it is claimed to enhance flowering, ripening and flavour (Pettersson, 1978). Different effects of preparation 501 result from application at different plant growth stages – it reportedly does not have one specific effect but acts on whatever physiological processes are happening when the plant is sprayed (Klett, 1968).

Fritz et al. (1997) investigated the effects of application of composted manure, 100 or 50% light conditions and 3 early or 3 late applications of the biodynamic silica preparation on dwarf beans and lettuce grown on a biodynamic farm. Experiments on both species showed an inhibiting effect of early silica treatment on young vegetative growth, whereas late silica application caused a delay in plant senescence. In controlled experiments with dwarf beans grown in pots containing several different soils (loess, loamy sand, clay loam and meadow loam, all previously organically farmed, germination rate was significantly increased by silica treatment to the mother crop. There were no significant differences between silica treatments when bean pod yield was relatively high but pod yield was increased by silica application in a year when its yield was lower. There was a significant improvement of post-harvest quality of lettuce in shaded plants given silica compared with untreated plants, but in plants grown without shading, storage quality was reduced in the silica-treated plants. The authors considered that there are similarities between the effects of silica and gibberellin.

Remer (1995) trialled a homoeopathic D7 solution of preparation 501 on Savoy cabbages and recorded the following levels of some amino acids (Table 2.3.5) (statistical analysis not provided).

Table 2.3.5 Effect on concentrations of some amino acids of weekly spraying homoeopathic D7 solution of preparation 501 on to Savoy cabbages (Remer, 1995)

	Treated	Untreated
	mg/L	
Aspartic acid	120	80
Glutamic acid	200	150
Alanine	50	25

Remer concluded that it appeared that the horn- silica preparation promotes the formation of the enzymes responsible for acid metabolism.

Pfeiffer (1984) discussed the importance of the "light process" in plants and for human nutrition and its effect on ability to metabolise proteins and the relative quantities of each amino acid making up the proteins. He found that biodynamic sprays increased relative proportions particularly of lysine, tryptophan and arginine in grains. Other studies have found that horn-silica appears to increase content of the amino acids containing sulphur such as methionine and to lesser extent, cysteine. For example, Stolz et al. (2000) compared amino acid content of beans grown hydroponically and biodynamically. Methionine + cysteine content was slightly higher in the biodynamically grown beans. There were higher levels of most other amino acids in the biodynamic beans and their chemical score (FAO, 1973) was 76.01% as compared to 73.39% for the hydroponic beans. However, amino acid requirements have been revised downwards since 1973 (Young and Borgonha, 2000).

2.3.5 Silica and light

Spraying the horn-silica preparation in the air above a plant could affect its metabolism by changing the:

- quality of light – polarization, quantity or relative quantities of particular wavelengths which affects phytochrome and possibly other cell signalling,
- quantity of light reaching plant surface.

The question of if and how the silica spray affects the light reaching the plant relates to the properties of silica used in a number of areas. Perhaps silica may modify the leaf surface. Phoenix et al. (1999) studied the silification of cyanobacteria in hotsprings and

observed that the silica builds up outside the bacterial sheath, being particles too large to be able to pass through the sheath and increases the alkalinity inside the sheath matrix, which is favourable for photosynthesis. However, in the case of the silica spray, the silica is present in such low dilution that it is unlikely to have any effect beyond that of wetting the leaf surface. An effect on the properties of the light itself is more likely.

Silica aerogels are used in industry because of their Rayleigh scattering effects, which causes the reddening of transmitted light (Hunt and Ayers, 2003) – this could trigger plant responses as plants have been shown to respond to changes in the relative amount of red wavelength in the light they receive (Section 2.4.1).

Silica also exhibits the Raman amplification or atomic polarisation effect (Cisternino and Sordo, 2003). Light is composed of an electric field and a magnetic field oriented perpendicular to each other. When light travels through a material, the electric or magnetic field may interact with the constituent atoms of that material, shifting the electron cloud so it is no longer concentric with the nucleus. In ionic and partially ionic materials like silica, polarization of one ion may induce an opposite polarization in the neighbouring ion. Bjorn (1976) discussed the assymetry of most organic compounds, which are only able to react with other compounds that are orientated the same way. Levitt et al. (1999), writing about the large masses of cyanobacteria on oceans and the earth, said that dyssymmetry is an essential property of life. The integration and synchronisation of biological systems in space and time is related to the dynamic organisation of metabolic reactions (Aon and Cortassa, 1997). They discussed how the spatio-temporal organisation of a cell's cytostructure affects how it responds to a chemical, mechanical or electrical stimulus, its regulation of gene expression and developmental path.

This concept of the integrity of the dynamic cytoplasmic organisation could relate to results of picture-making by capillary dynamolysis and to biophoton emission discussed in Section 2.2.3.

Possibly the role of silica in increasing "light" effects in the plant, and the integration of this process with the growth process enhanced by other biodynamic preparations, affects the dynamic spatio-temporal organisation of plant cells. Such effects may be

involved in some of the apparent changes in plant metabolism from silica application discussed above or may not be measurable by chemical analysis. Possibly any health effects from consuming plant products, such as the light energy released when digesting proteins discussed by Pfeiffer (1958), is more related to their dynamic organisational properties than to their chemical constituents.

In conclusion, the horn-manure and horn-silica preparations are used to balance the calcium (growth and shade) and silica (light and differentiation) processes, which likely affect plant signalling through ion, hormone and enzyme production and activity. Silica compounds applied to soil appears to have a direct effect on plant metabolism. This effect cannot be a silica nutrition effect, at least when the preparation 501 is applied, as it contains a very low concentration of silica.

The silica preparation may change quantity and ratios of different wavelengths absorbed by plants which could trigger signals affecting stomatal opening, plant hormones, redox and phosphorylation reactions, respiration and DNA transcription which could affect e.g. nitrate reductase activity in plant leaves or nutrient transport. Alternatively, some of these effects could occur as the result of assimilation of small concentrations of silica by plant leaves. Application of such sprays could result in more assimilation of nitrates into amino acids and protein, a higher true protein: crude protein and more essential amino acids. Effects of the silica preparation on both the properties of light received by plant leaves, and on concentrations of various nitrogenous compounds in plant leaves may be measurable.

2.4 Factors affecting lettuce physiology and food quality

A large number of factors affect lettuce growth and nutrient quality, often having greater effects than, and interacting with, management practice.

2.4.1 Climatic factors

Water supply

Quantity and frequency of rainfall, irrigation and soil water holding capacity all affect the quantity of water available for plant uptake. An adequate water supply is particularly important for a leaf crop such as lettuces. Wein (1997) wrote that top growth is more affected than roots by low water supply as generally, when resources are limited, the part nearest their source are relatively stimulated. Berg et al. (2001) found that temporal variability in water supply from once a week watering had an adverse effect on lettuce production, (even with high volume of water which resulted in soil water content similar to that from applying the same volume in more frequent smaller volumes so there appeared to be no more run-off, leaching or evaporation). They considered this was because plant growth shows a decelerating response to resource availability, so variability in water supply will decrease plant growth even when the average supply is the same. However, soil type, soil water holding capacity and evapo-transpiration rates were not reported, so it is not known how applicable these results would be to other conditions.

Low water supply results in reduced uptake of nutrients, and decreased activity of N assimilatory enzymes (Dubey and Pessarakli, 2002). A water deficit results in plant physiological adaptations which include reduced photosynthesis, changed assimilate partitioning, increase in reactive oxygen species and quantitative and qualitative changes in the synthesis of proteins (Artlip and Wisniewski, 2002). Protein changes, which include reduction in polyribosome stability and changes in transcription, were found to be related to abscissic acid production.

Temperature

Average, maximum and minimum air temperatures affect rate of growth. The rate of plant respiration has been found to approximately double for each 10°C increase (Moss,

1984). Optimum temperatures for lettuce growth are 7 - 24°C with average of 18°C (Lorenz and Maynard, 1980). However, lettuce growth is more affected by soil temperature than air temperature. Heating the soil to 18°C reduced the time for butterhead lettuce to mature by 14-17 days compared to lettuces in unheated soil when minimum air temperature reduced to 7°C (Boxall, 1971).

Light

Intensity and wavelengths of sunlight, day length, and leaf shading by other leaves all affect what light is actually received by plant leaves. A fully exposed leaf of a C_3 plant such as lettuce reaches maximum photosynthetic response to sunlight at about ¼ of midday sunlight intensity; however leaves that are partially shaded require a greater light intensity (Moss, 1984). Increased light interception increases average lettuce daily fresh weight gain, which results in increased harvest weight and reduced number of days to harvest (Wein, 1997).

Light also affects leaf shape, which affects head formation: low light results in long, narrow leaves, while increased light results in broader, shorter leaves, and high temperatures accentuate both of these tendencies (Wien, 1997) The increase in far red wavelength at the end of the day favours enhanced stem growth and reduced root growth. This fits with reports by Senger (1987) that red light results in photosynthetic responses in higher plants similar to plants grown in shade conditions. However, under pure red light, the chloroplast thylakoid membranes become disorganised and photosynthetic capacity decreases (Kowallik, 1987).

Many plant reactions to light such as germination and leaf expansion are via non-photosynthetic receptors such as phytochrome. Hart (1988) described how phytochrome responds to red and far-red wave-lengths. Garcia-Martinez and Gil (2002) discussed research on how red and far-red light effects on lettuce growth appears to be via phytochrome which inhibits stem elongation, either by a decrease in the content of active gibberellins or a reduction of gibberellin sensitivity of the stem, or both effects. This appears to contradict effects of red light on photosynthesis discussed in the previous paragraph.

Smith (1982) discussed how changes in the red:far-red ratio

$$\frac{\text{(photon fluence rate in 10nm band centered on 660nm}}{\text{photon fluence rate in 10nm band centred on 730nm)}}$$

appears to be a shade index which triggers growth responses in plants. This ratio remains fairly constant during full daylight over a range of weather conditions and locations, but is reduced at twilight or when plants are shaded. Sleeman and Dudley (2001) found that plants that grew under high R: FR showed higher rates of photosynthesis and stomatal conductance and higher water-use efficiency relative to plants under comparatively lower R: FR.

Less is known about the blue-light receptors which regulate responses such as inhibition of stem growth, stomatal opening, pigment synthesis, protein synthesis and enzyme activation and possible activation of auxins and/or gibberellin (Hart, 1988). Blue light has been found to increase carbohydrate degradation in plants, resulting in lower concentration of carbohydrates and higher concentration of proteins in plant cells (Kowallik, 1987). This could relate to a rapid increase in respiration and oxygen uptake, and a slower, longer effect on enzyme capacity.

Solar UV-A and UV-B radiation have been shown to have inhibitory effects on lettuce growth and to increase anthocyanins in a red-pigmented lettuce cultivar (Krizek *et al.*1998). UV-B radiation was found to be more important than UV-A radiation for flavonoid induction. Tosserams et al. (2001) grew Plantago plants under different levels of UV-B radiation at low and high nutrient supply. They found that increased levels of UV-B reduced biomass production under non-limiting nutrient conditions only. Plants at low nutrient supply produced 50% less biomass than high nutrient-supply plants, while net photosynthesis was decreased by only 12%. At higher UV-B levels the plants accumulated more carbohydrates. Tosserams et al. concluded that the increased accumulation of carbon in nutrient-stressed plants, may lead to a reduction of UV-B induced damage because of increased foliar UV-B absorbance by enhanced accumulation of phenolic compounds and leaf thickening.

Effects of light intensity and duration on ascorbic acid content of crops were reviewed by Somers and Beeson (1948). They quoted research which showed up to 800% higher ascorbic acid content with increasing light intensity.

Light has been shown to affect nitrogen assimilation through effects on nitrate reductase, the tricarboxylic acid cycle and amino acid synthesis, but the mechanism by which this occurs is still debated (see more detailed discussion in Section 2.4.5). Increased photosynthetic activity from increased light results in higher carbon assimilation also and this enables more root exudation to increase uptake of more nutrients from the soil. Rao et al. (2001) found that increased light intensity increased organic acid production, which induced rhizosphere acidification by plant roots and increased solubilisation of P from calcium phytate.

2.4.2 Soil type

Soil structure affects mineral uptake by plants, through effects on both intensity and quantity of nutrient ions in soil solution (Marschner, 2002). A soil with low bulk density is well aerated and does not impede root elongation, but there is less root contact with the soil matrix and therefore lower nutrient uptake per unit length of root.

There appear to have been few comparisons of lettuce quality grown in different soil types, probably because of the large number of variables between different soils in the field, and because more lettuce is now grown hydroponically. Gianquinto (1996) reported on a long-term experiment on fertiliser application on 3 soil types (clay, peaty-clay and sandy) in Italy. Crop quality, as measured by head weight, uniformity, shape, DM and crude protein content was higher, but nitrate content also higher, for lettuces grown on clay and peaty-clay soils than on sandy soil, even when large quantities of nutrients were applied to the sandy soil. There was less response to fertilisers on the clay soils.

It is likely that differences between soil type and structure would have a large influence on the availability of nutrients and nutritional quality of crops particularly when grown under organic systems, which rely on the soil system itself rather than added inorganic nutrients for crop growth. Physical and chemical differences in the same soil type such as compaction induced by tillage, or water-logging from excess irrigation can therefore be responsible for much spatial variability in lettuce growth.

In a preliminary investigation (for this thesis) of the differences in lettuce growth and nutrient content of lettuces grown in pots in a sandy loam or silt-clay loam with 2 levels of added compost or fertiliser application, the lettuces grown in the silt-clay loam had longer, narrower, darker leaves and larger size then those grown on the sandy soil.

Many lettuces are not grown in soil and there are considerable differences between average nutrient contents of field-grown and hydroponic lettuces in the New Zealand Food Composition Tables (Ather et al., 2001). The large number of different factors involved make direct comparison trials difficult. However, Siomos et al. (2001) grew lettuces hydroponically or in the soil of an unheated glasshouse. They reported that plants grown in the soil exhibited excellent visual quality and were free from any defect and tipburn symptoms. The hydroponically grown plants developed some tipburn symptoms, especially when grown in perlite. Hydroponically grown plants had lower dry matter, chlorophyll, Mg, Fe, and Mn contents and had higher titratable acidity and nitrate, N, P contents than those grown in soil culture.

2.4.3 Lettuce type and variety

Table 2.4.1 shows the considerable differences in average nutrient contents between different lettuce types (and presumably the growth media management) recorded in the USDA nutrient database (1998). For comparison, average values and ranges recorded by Souci et al. (1994) and mean values recorded in the New Zealand Food Composition Tables (Athar et al., 2001) for field- grown and hydroponic lettuce are included (as well as results from the current research). Particularly notable are the much higher content of P, K and protein in the *Romaine* type compared to the *Iceberg* type, and the high concentration of Ca and Fe in the looseleaf type compared to the *Iceberg* type. However, as the *Iceberg* type is generally grown in the field and the looseleaf type generally in hydroponic solution in glasshouses, some of these differences could be due to different cultural conditions.

Efficiency of nutrient uptake has been shown to vary between crop varieties. Tei et al. (1999) compared nitrogen uptake by Canasta and Audran varieties and found that although the estimated fertiliser-N rate to obtain maximum fresh and dry weights was about 155 kg N/ha in both cultivars, the estimated N uptake at that fertiliser rate was

about 145 kg/ha in cv. Canasta and 131 kg/ha in cv. Audran, whereas N uptake with no N fertiliser was 63 kg/ha in Audran and 58 kg/ha in Canasta. These results indicate that different varieties may be best suited to different soil nutrient levels.

2.4.4 Nutrient supply

The range of nutrient contents found in lettuces recorded by Souci et al. (1994) and shown in Table 2.4.1 would be partly due to varietal differences but differences in nutrient supply would be a major factor. Nutrient uptake is important not only for growth and yield, but also for nutrient content of the crop. Because of the large variation in nutrient uptake by different varieties in different soils and nutrient supply conditions, it is not possible to prescribe ideal nutrient levels in lettuce. As lettuces are one of the main vegetables consumed, their level of nutrients, particularly of minerals and vitamins is important, but more is not necessarily better. For a leafy vegetable, high dry matter content may not be desirable, as it could be tough to eat. The balance of nutrients and how they are assimilated into compounds such as proteins and sugars is more important.

Nitrogen

Nitrogen supply is generally considered to have the greatest effect on growth. As with water supply, low N or P reduces the top growth more than root growth (Wein, 1997). Greenwood et al. (1980) produced standard growth curves to show the critical concentrations of the minimum levels of nitrogen, phosphorus and potassium required for maximum yields. For lettuce they found that mean optimum nitrogen level to provide for critical nutrient requirement for field-grown lettuce was 170 kgN/ha. Actual requirements vary with conditions such as soil type, previous soil management and climate. The effects of varying levels of nitrogen on cabbage and carrot nutrient contents were demonstrated by Sorenson (1999).

Greenwood et al. (1980) showed that levels of nitrogen higher than the critical rate can be uneconomic as uptake rate is reduced. A high soil nitrogen level can depress uptake of other nutrients. Somers and Beeson (1948) discussed the effect of high nitrogen levels on reducing calcium uptake by plants and in reducing iron uptake when applied as nitrate. (Koepf, 1993) discussed how high nitrogen level produces rampant, watery

Table 2.4.1 Trial lettuce mineral content compared to standard tables. Nutrient content per 100 gm fresh weight leaves

	Water	Protein	K	Ca	Mg	Na	Fe	Zn	Cu	P	Se	Cell sap nitrate
	%	g/100g freshwt	mg/100g freshwt								µg/100g freshwt	
Trial												mg/L
Control		1.4	410	27	11					22	<0.05	633
Control + sprays		1.5	424	31	11					21	<0.05	433
DAPCAN		1.3	360	33	11		1.1	0.65	0.10	18	<0.05	700
DAPCAN + sprays		1.5	423	34	12		1.5	0.74	0.11	19.24	<0.05	600
Organic		1.3	401	27	10		1.1	0.63	0.08	16	<0.05	500
Biodynamic		1.4	431	32	11		1.0	0.60	0.08	20	<0.05	533
NZ Food Tables+												
Field	95	1.1	245	36		14	1	0.2			0.3	
hydroponic	95	1.9	280	61		13	1	0.2			12.5	
Souci et al ++												mg/100g
Average			224	37		10	1.1	0.22	0.05	33	2	262
range			23 - 661	17-51		5-14	0.5 - 2	0.16–0.35	0.03–0.08	19 - 57	1 - 4	23 - 661
USDA*												
iceberg	95.9	1.01	158	19	9	9	0.5	0.22	0.03	20	0.2	
looseleaf	94.0	1.30	264	68	11	9	1.4	0.29	0.04	25	0.2	
romaine	94.9	1.62	290	36	6	8	1.1	0.25	0.04	45	0.2	
butterhead	95.6	1.29	257	32	13	5	0.3	0.17	0.02	23	0.2	
Lairon et al **									µg/g			µg/g
Organic fert	92.9 – 93.4		481-504	88-91.5	25.3-27.1		3.5-3.7		13.5 – 15.0	4.2-4.7		271-319
Mineral fert	93 – 93.4		450-505	73.5-82.5	25-27.5		3 -3.2		16.1 – 16.2	3.4-4.5		534-991

Standard tables: + NZ Food Composition Tables, 2001 Crop and Food Research, Palmerston North
++ Souci, S.W. et al, 1994 Food Composition and Nutrition Tabels. Medpharm Scientific Publishers Stuttgart
* USDA Nutrient Database for Standard Reference, Release 12 (March 1998)
Research trial ** Lairon D et al. 1984. Organic and mineral nitrogen fertilised butterhead lettuce.

growth, similar to plants grown in the shade, and accumulation of leaf nitrates in plant leaves.

Most annual plants, including lettuces, yield best when they take up nitrogen partly as nitrate and partly as ammonium (Marschner, 2002). The relative amounts taken up and their efficiency of utilization depend considerably on climatic and soil conditions. An acid soil favours ammonium uptake (Marschner, 2002) as nitrification may be inhibited in an acid soil. Ammonium uptake is less depressed at low temperatures than nitrate (Mengel and Kirkby, 1987). Ammonium has to be incorporated into amino acids and amides in the roots, to avoid toxicity and excess proton production, whereas in lettuces nitrate is mainly transported to the leaves, where it is reduced to ammonium and assimilated into amino acids. When ammonium is assimilated in the roots, energy has to be supplied by oxidative phosphorylation, which requires carbohydrates supplied by the phloem and oxygen consumption. At high root temperature and low potassium supply this can be a problem, leading to poor plant growth (Marschner, 2002). Some nitrogen is also taken up in the form of amino acids (Scheller, 1996) Assimilation of nitrates is discussed in the next Section, 2.4.5.

The form of nitrogen supply can be regulated by what fertiliser is applied, but in an organic system it depends largely on soil conditions, and whether nitrogen-fixing microbes such as *Azotobacter* spp. and nitrifying microbes such as *Nitrosomonas* and *Nitrobacter* (Russell, 1988) are favoured. Nitrification is favoured by increasing soil temperature, aeration and moisture. Soil fungi also contribute to nitrification, which may involve superoxide free radicals produced during degradation of lignin. These then generate hydroxyl radicals that may drive the nitrification process (Killham, 1994). The form of nitrogen taken up may influence product quality. Simonne et al. (2001) compared lettuces grown with the same quantity of nitrogen from calcium nitrate, potassium nitrate, or ammonium nitrate. They found no significant difference in yields, but sensory testing by panelists found that crunchiness of calcium nitrate-fed plants was significantly higher than that of the other treatments.

Phosphorus

Low phosphorus supply can also limit plant growth. Phosphorus is important for energy storage, particularly in ATP, and is also needed for nucleic acids and phospholipids for membrane nutrient transport and in chloroplasts (Mengel and Kirkby, 1987). Ladebusch and Melzer (1999) found that lack of P had a higher effect on lettuce yield than any on other vegetable crop. In a 40 year trial, in sandy loam soil, no provision of P fertiliser reduced lettuce yield by 60% compared to providing optimum P level, whereas yield reduction was less than 20% for most other crops (including Brassicas, spinach, and most root vegetables). Low P uptake can be because of low P content of the soil; high soil P adsorption rate leading to unavailability of P; small root area; low root temperature; and water shortage, which reduces diffusion of P to roots (Scaife and Barnes, 1977).

Terry and Rao (1991) discussed how low P mainly affects growth of new leaves and leaf area, reducing the area for, rather than the rate of photosynthesis. Low P also reduces the rate of carbon export from the leaf, either by reducing ATP energy for loading sugars into the phloem, or by reducing the sinks for carbohydrates to flow to. Low P appears to change the carbon metabolism towards higher concentrations of starch and sucrose and low levels of sugar phosphates such as ribulose phosphate. The activity of phosphatase enzymes in roots and shoots are increased, to increase the availability of phosphate for photosynthesis. Rao et al. (2001) demonstrated how rhizosphere acidification and ability to solubilise P from calcium phytate occurred in light, but not dark conditions. Pendias and Pendias (1992) describe how high P intake by plants reduces the uptake of zinc.

Potassium

Potassium is important for maintaining turgor of stomata cells, for intake of CO_2 and therefore the rate of photosynthesis, and for transport of nutrients in the plant (Mengel and Kirkby, 1987). Potassium was shown to be less crucial for lettuce yield than P in the trial by Ladebusch and Melzer (1999) reported in the section on *phosphorus:* providing no K reduced yield of lettuces by about 30% compared to optimum K. However, potassium has a large effect on plant metabolism and thus nutrient quality.

High potassium uptake reduces uptake of other cations, particularly of calcium and magnesium. Low K or Mg reduces root growth more than leaf growth Wein (1997) Albrecht (1975) recommended cation balancing for optimum soil fertility and crop and animal health, using a formula for soil cation exchange (CEC) % base saturation ratios of about 65-75% Ca, 10-15% Mg, 2-5% K, 0.5-3% Na and 10-15% H. For sandy soils with low CEC, the formula is modified slightly, to about 60% Ca, 20% Mg and 6-8% K.

Potassium is also important for activating a large number of enzymes. Low potassium levels increase sugar and soluble amino compounds and nitrate levels and decrease levels of starch and protein (Marschner, 2002). Sidlauskas (2000) found that increased potassium content in spring oilseed rape plants was related to increased protein and fat yields. Marschner (2002) discussed how K seems to be needed for both synthesis and activation of nitrate reductase and for protein synthesis.

Calcium and magnesium

Calcium is required for cell division and elongation and is important for stabilizing cell membranes and maintaining selective permeability (Mengel and Kirkby, 1987). They describe how polypeptides such as calmodulin bind with calcium enabling phosphorylation of enzyme proteins in the cytoplasm. Calcium is therefore involved in uptake of other ions and acts as a chemical messenger. Calcium is particularly important for lettuces. Wein (1997) discussed the problem of tip burn in lettuces, caused by a calcium deficiency in young, rapidly developing leaves. Calcium deficiency also results in reduction in protein synthesis, elevated levels of free oxygen radicals and increased respiration rate to replace leaking respiratory substrates through permeable membranes (Marschner, 2002).

Magnesium is particularly important as a constituent of the chlorophyll molecule, as a cofactor in phosphorylation enzymes and particularly in activating the Rubisco enzyme (Mengel and Kirkby 1987). Insufficient magnesium reduces the true protein: crude protein ratio as it is necessary for synthesis of RNA (Marschner, 2002). Low magnesium uptake also results in accumulation of starch and high leaf dry matter content. Marschner writes

that this is probably due to reduced phloem loading of sucrose, as transport of carbohydrate to roots is reduced, resulting in a higher shoot: root ratio.

Calcium and magnesium are important for regulating enzyme activity in humans, particularly those involved in muscle function.

Iron, zinc and copper

Iron is involved in many redox reactions in the plant because of the ease with which it is oxidized or reduced and forms complexes with organic ligands (Marschner 2002). It is involved in chlorophyll production, and in activating nitrite reductase. Iron can be taken up either as Fe^{2+} or as a chelate (Mengel and Kirkby, 1982). Terry and Rao (1991) studied the effects of iron deficiency on photosynthesis and concluded that it affects mainly electron transport rather than light absorption, although deficiency also reduces thylakoid membranes in chloroplasts and the production of chlorophyll and carotene.

Lairon et al. (1984) found higher iron content in lettuces in organic treatment (0.35 – 0.37 mg/g fresh weight) compared to those grown with mineral fertiliser (3.0 – 3.2 mg/g). Many researchers have found higher uptake of iron and zinc when more organic matter is present for formation of phytosiderophores. Remer (1995) found higher iron content of plants treated with a homoeopathic silica spray.

Lettuces generally acquire iron through "Strategy I" exudation of organic acids to assist dissolution of ferric hydroxide to Fe^{2+} that can be taken up (Jones et al., 1996). They produced a model to predict the rate of ferric hydroxide dissolution, which is rapid in soils of pH $\leq$ 6.8. Chen and Stevenson (1986) discuss experiments which showed the importance of chelation of micronutrients such as Cu, Zn, Mn and particularly Fe. For example Jalali and Takkar (1979) showed that the uptake of Fe, Zn and Cu by rice was directly correlated to the soil organic matter content. Chen and Stevenson list several reasons why inconsistent results have been obtained by different researchers: plant uptake mechanism may vary with the plant and its physiological age; uptake is affected by the concentration of the soluble organic materials, the concentration of the micronutrients and

the stability constant of the metal-organic matter complex, and uptake is reduced by the presence of insoluble humin, which may contain groups that fix the micronutrient, preventing plant uptake.

Zinc is also bound to soil organic complexes, some soluble and some insoluble (Mengel and Kirkby, 1982). Plant uptake may be active or passive. Zinc is involved in many enzyme reactions, either as a component or as a catalyst, and is particularly important in DNA replication and regulation of gene expression (Marschner, 2002). Zinc forms tetrahedral complexes in polypeptide chains and is therefore needed for synthesis of proteins from amino acids. Free amino acids have been found to accumulate in plants deficient in zinc. Zinc is also involved in carbon metabolism, and deficiency results in accumulation of sugar and starch levels. Zinc deficiency also interferes with auxin metabolism, resulting in stunted leaf growth. Zinc is required for membrane integrity, reducing permeability and protecting against oxidative damage (Marschner, 2002).

Zinc interacts with other minerals. High uptake of zinc particularly reduces the uptake of iron and also of copper (Pendias, 1992). High phosphorus uptake can reduce zinc uptake, or it may be the case that low zinc increases root membrane permeability, enabling higher P uptake (Marschner, 2002).

Copper is also involved in electron transport and in enzyme activity. For example it combines with zinc in superoxide dismutase, which detoxifies superoxide radicals (Marschner, 2002). Copper is a constituent of ascorbate oxidase, which oxidizes ascorbic acid to dehydroascorbic acid, and in polyphenol oxidase, which is involved in lignin synthesis and in initiating flowering and maturation of plants. A further important role of copper is in plastocyanin, which is involved in electron transport in photosynthesis. Soluble carbohydrate concentrations are therefore reduced in copper deficient plants.

Copper may be deficient as it is not very mobile in soil or in the plant, and is less available in humus rich soils because it is held tightly to organic cation exchange sites (Mengel and Kirkby, 1982). Copper has been found to be transported in plant xylem sap by amino acids such as glutamine, histidine, asparagine and nicotianamine (Liao et al., 2000).

Average nutrient uptake

The nutrient content of leaves varies considerably with the factors listed above and also with age and stage of development of the lettuce. Sunlarp (1999) listed results of a various lettuce leaf analyses in which % dry weight of nitrogen varied between 1.9% to 6.14%, phosphorus 0.06 – 1.41% and potassium 1.58 – 13.62%. Differences in nutrient supply, light intensity, temperature, lettuce type and the lettuce leaves being at different stages of development could all have contributed to these differences. Some current recommendations for sampling lettuce leaves for nutrient sufficiency are shown in Table 2.4.2.

Table 2.4.2 Recommended nutrient sufficiency concentrations in lettuce leaves

Source	Sampling time	Plant part	N	P	K	Ca	Mg	Zn	Fe
					%			ppm	
HortResearch	Heads ½ size	Wrapper leaf	2.5-4.0	0.40-0.60	6.0-8.0	1.4-2.0	0.50-0.70		
Clemson Univ. Ext. Serv.			2.50-4.00	0.20-1.50	3.50-8.00	1.30-4.00	0.30-0.80	100-800	18-150

Hort Research (1995), Clemson University Extension Service, (1996).

For human nutrition, nutrients are measured in the appropriate fresh edible parts.

The New Zealand Food Composition Tables (Athar, 2001) lists average nutrient levels per 100g wet wt fresh inner and outer lettuce leaves (Table 2.4.1).

2.4.5 Nutrient metabolism

The factors discussed earlier in this section and the relationships between them all affect how all the nutrients taken in by the plant through uptake from the soil and through stomata are metabolized and built into plant structures.

Carbon and nitrogen metabolism and nitrate reduction

The interaction of carbon and nitrogen metabolism is a key factor in plant nutrient assimilation. A large number of different enzymes are involved in this metabolism. Reduction of nitrates to ammonia is the first step towards protein formation. The ability of a plant to process all the nitrates it takes up has considerable implications for the health and safety of consumers.

Lettuces may accumulate large quantities of nitrates in their leaves, which give the leaves a bitter taste and can be nutritionally toxic. Nitrates can be harmful to human health when reduced to nitrites either before or after ingestion (Maynard et al., 1976). If nitrite ions enter the blood, haemoglobin may be oxidized to methaemoglobin which does not transport oxygen, resulting in cyanosis and death if 70% or more of haemoglobin is oxidized.

Maynard et al. (1976) also discussed factors that affect nitrate content in lettuces. They quoted results of a trial by Minotti, that nitrate levels were higher: in a summer crop than a spring crop, - after fertilisation with sodium nitrate compared to ammonium sulphate, - for higher quantities of fertiliser applied and when harvested before maturity (except for high fertiliser rates when nitrate content increased to maturity). Levels measured varied between 0.1 – 1.59 NO_3-N as % of dry weight lettuce heads. Nitrate concentration decreases with growth as heads form and the proportion of middle and inner leaves containing lower nitrate increase (Drews et al. 1997).

In further research by Minotti quoted by Maynard et al. (1976), nitrate levels varied in different lettuce cultivars and showed large diurnal fluctuations, being highest at sunrise and lowest at sunset. These diurnal fluctuations have been shown to relate partly to light intensity and partly to air and soil temperatures. Low light levels and low temperatures reduce nitrate assimilation, which explains why nitrate levels are highest in the morning after darkness and lower air and soil temperatures (Scaife et al. 1994).

Matt et al. (2001) investigated this diurnal process in wild-type tobacco. The first step in nitrate assimilation is reduction to ammonia by the enzyme, nitrate reductase (NR). Matt et

al. found that in leaves, NR activity resulting from the relevant gene transcription increased three-fold during the first half of the light period and declined during the second half. Nitrate decreased during the day and recovered at night, ammonium, glutamine, glycine and serine increased during the day and decreased at night, and 2-oxoglutarate increased three-fold after illumination and decreased during the last part of the light period. During the first part of the light, the rate of nitrate assimilation was about two-fold higher than the rate of nitrate uptake, and also exceeded the rate at which reduced nitrogen is metabolized. The resulting decrease of leaf nitrate and accumulation of nitrogen in intermediates of ammonium metabolism and photorespiration represented about 40 and 15%, respectively, of the total nitrate that entered the plant in 24 hours. Later in the diurnal cycle as NR activity declined, this imbalance was reversed.

Blom-Zandstra et al. (1988) showed how nitrate accumulation in cell sap reduced and glucose and organic acids increased with increasing light intensity. They also found that reduction in light intensity led to higher fresh weight production per mmol N absorbed in one lettuce cultivar but not in another and concluded that partitioning of C and N between dry matter and cell sap varies in cultivars. Nitrate reduction in the leaf also involves production of organic acids such as malic and oxalic acid to maintain anion and pH balance (Marschner, 2002).

Muller et al. (2001) found that malate accumulated to high levels in tobacco leaves during the light period and decreased during the night. They also measured levels of glutamine, which is produced from ammonia, and which results from the tricarboxylic acid cycle and reacts with glutamate in the pathway to amino acid and protein synthesis. The level of 2-oxoglutarate increased by 40% at the beginning and decreased towards the end of the light period. The glutamine: 2-oxoglutarate ratio was steady during the first part of the light period and increased markedly during the second part of the light period. They concluded that malate inhibits NR transcription and activity, but 2-oxoglutarate only slightly increases NR expression.

Matt et al. (2002) challenged the traditional view that low nitrogen uptake always results in high C: N ratio and lower amino acid and protein levels. They reviewed research that found that high nitrate concentrations produce signals that induce transcription of genes that promote uptake and assimilation of nitrate and change carbon metabolism in favour of synthesis of amino acid precursor organic acids. These acid anions balance the negative charge lost as nitrate is assimilated into near neutral amino acids. In addition, sugar production in the leaf induces genes promoting nitrate transporters, nitrate reductase, cystosolic glutamine synthetase and enzymes involved in synthesizing organic acids. This effect of sugars can lead to simultaneous increase or decrease of carbon and nitrogen metabolites. They found that activity of the enzyme ribulose bisphosphate carboxylase/oxygenase (rubisco) involved in assimilation of CO_2 was reduced by growing tobacco with nitrate only compared to ammonium nitrate, particularly at high levels of N supply, resulting in accumulation of more nitrate and less amino acids, whereas nitrate reductase activity was higher in nitrate than ammonium nitrate grown plants and reduced as N supply and/or light decreased. The result was that inhibition of photosynthesis can lead to an inhibition of nitrate assimilation and decrease of amino acid levels, or there can be a shift in the balance between rate of growth and nitrate supply leading to an increase of amino acid levels. Changes in amino acid: sugar ratios were correlated with a reduction of both carbon and nitrogen rich secondary metabolites.

Lillo and Appenroth (2001) reviewed research on nitrate reductase activity and concluded that there may be several mechanisms, including a small effect of far-red light via phytochrome on nitrate reductase gene expression and a larger effect of photosynthesis on the supply of carbon precursors for synthesis of amino acids (e.g. glutamate, via the glutamine synthetase/glutamate synthase cycle), glucose and sugar phosphates; and signaling by calcium ion flux. White light has been shown to promote activation (dephosphorylation) of nitrate reductase, possibly through concentration of sugar phosphates in leaves, and/or by activity of protein phosphatases that appear to be regulated by redox control.

Lillo and Appenroth (2001) provide an illustration of how various factors affect post translational regulation of nitrate reductase activity. Activity is regulated by phosphorylation in the dark, and dephosphorylation in the light.

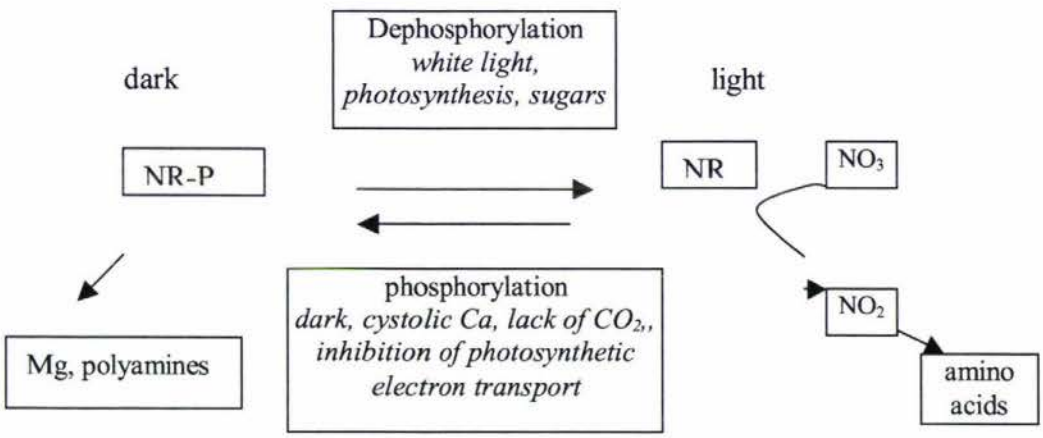


Fig. 2.4.1 Regulation of nitrate reductase activity adapted from diagram by Lillo and Appenroth (2001)

Redinbaugh et al. (1996) investigated the effects of inhibiting NR dephosphorylation with okadaic acid and demonstrated that protein dephosphorylation is necessary for the design and production of new protein using the information contained in plant DNA. Camacho-Cristobal et al. (2002) found that phosphate increased NR activity of tobacco leaf extracts in vitro. The role of iron in ferrodoxin in nitrite reduction was discussed in the trace element section above.

This brief sketch of some current research on factors affecting nitrate reductase activity indicate that the ability of a plant to manufacture protein from the nitrogen it takes up depends on the interaction of a large number of factors including:

- form of nitrogen taken up – nitrate, ammonium or amino acid
- rate of photosynthesis and quantity of sugars and organic acids accumulating
- effects of quantity and quality of light received on nitrate reductase activity
- interactions with phosphorus, and calcium in the cell, which affect nitrate reductase phosphorylation and dephosphorylation.

Plant metabolism therefore depends on the interaction of the quantity and quality of light from above and the quantity and type of nitrogen and other nutrients from below the plant. The effects of these interactions give rise to varying plant form, growth and nutrient composition. In biodynamic theory the light effects are thought of as the "cosmic" (coming from above) process, while the nutrient stream coming up the plant from below can be thought of as the "terrestrial or earthly" process. These processes were discussed in relation to the "silica" and "calcium" processes introduced by Steiner (1993) discussed in Section 2.3.

Secondary metabolites

Variations in carbon and nitrogen metabolism result in different quantities and types of secondary metabolites (by-products which usually occur only in differentiated cells, see Section 2.2.1). This was the focus of the research by Matt et al. (2002), to find what type of metabolism favoured production of metabolites affecting tobacco quality. Secondary metabolites in plants often provide protection against pests and diseases, and may have beneficial or harmful health effects when consumed in food. Of particular interest at present are antioxidant metabolites in food, which provide protection from cardiovascular disease, cancer etc (Liu et al., 2000). High light intensity, and particularly UV light, have been found to increase antioxidant content in lettuce leaves (Krizek et al., 1998) as discussed in section 2.4.1.

Growing method has been found to affect secondary metabolite contents. Ren et al. (2001) found that the antioxidative activity shown by organic vegetables was 120% times higher than that shown by conventionally grown vegetables in the case of spinach, and 20-50% higher in the case of Welsh onion, Chinese cabbage and Qing-gen-cai. Antimutagenic activity was also often higher. Concentration of several flavonoids - quercitrin, caffeic acid and baicalein in juices of the organically grown vegetables was 1.3-10.4 times higher than in conventional vegetables.

Phytohormones and cell signaling

Effects of the external factors discussed in preceeding sections on lettuce nutrient assimilation and metabolism may either be direct effects or be transmitted to other parts of the plant by phytohormones or other chemicals, such as cystolic calcium, that act as signals. Marschner (2002) discusses how cystolic calcium acts as a second messenger, transducting signals such as from light, as cystolic free calcium is in very low concentration. High concentrations in adjacent cell compartments can flow through plasma membrane channels in response to stimuli, increasing the level of cystolic calcium.

Environmental conditions interact with nutrient supply, affecting relative growth and differentiation of plant parts through activation of plant hormones. However, the older concept of clear effects by each hormone (e.g. apical dominance, cell expansion and vertical growth by auxin (IAA)) has largely been repudiated by research. Gaspar et al. (2002) wrote that it could now hardly be claimed that a single hormone is responsible for one growth or development process. Different plant hormones interact synergistically or antagonistically, hormonal controls act in a developmental and tissue-dependant manner and distinct cell types respond differentially to various signals. Hormone receptors may control hormone levels and the level of any one hormone affects the levels of the others by affecting their biosynthesis, degradation, conjugation or transport.

Gibberellins promote vertical cell elongation and division, whereas cytokinins produced by roots and also present in organic matter have been found to stimulate cell division, side buds and branching, as does nitrogen, whereas low light intensity, or far-red light, reduces branch growth (Wein, 1997). Cytokinins may also be involved in changes of root to shoot ratio at different levels of nitrogen supply. Low light intensity favours plant tops rather than roots. Cytokinins are important in root to shoot signaling and appear to be linked to the mass flow of sugars. Cytokinins and sucrose differentially regulate the transcription of a protein kinase WPK4, which is likely to be involved in regulation of carbon metabolism through protein phosphorylation (Van der Werf and Nagel, 1996) Cytokinins also increase nitrate reductase activity (Marschner, 2002)

However, there are many different forms of cytokinins, which may elicit different physiological changes in the shoot. Small quantities promote cell division but larger quantities may inhibit root development and it is hard to detect their production and flow as they may originate from the plant roots or bacteria in the rhizosphere or even intracellular bacteria in the roots (Emery and Atkins, 2002). Holland (1997) proposed that plants are incapable of manufacturing their own cytokinins and that all are produced by microbial symbionts which occur all over and in all plant organs.

Research results often seem contradictory. For example, Macisaac and Sawhney (1990) found that kinetin, a cytokinin, inhibited formation of lettuce lateral roots, whereas naphthalene acetic acid, an auxin, stimulated their induction. According to Gaspar et al. (2002) root growth is stimulated by low concentrations of auxins and inhibited by high concentrations, and auxin-cytokinin interactions are complex. However, Emery and Atkins (2002), writing in the same book, stated that root induction, initiation and growth require a high auxin to cytokinin ratio. Tanimoto (2002) discussed conflicting effects of externally applied gibberellic acid on root growth, probably caused because auxin, ethylene and cytokinins inhibit root elongation, so their effects may override gibberellic acid effects.

Under water stress, plant roots synthesise abscisic acid, which is transported to the leaves where it initiates closure of stomata and limits stomatal conductance and transpiration (Wilkinson and Davies, 1997). They reported that a drought induced alkalinization of the xylem stream – even mild soil drying, enables a greater proportion of root-sourced ABA signal to penetrate to sites of action within the leaf. It appears that abscisic acid activates slow anion and Ca^{2+} channels, enabling Cl^- and malate efflux and Ca^{2+} influx, which de-polarize the cell membrane, leading to K^+ efflux from guard cells and closure of the stomata (Ward and Schroeder, 1997).

Ethylene, which is also produced under water stress, inhibits leaf growth and inhibits development of bacterial and fungal symbioses (Lynch and Brown, 1997). Ethylene also widens cells, increases cellular respiration, sugar synthesis from starch and ripening (Marschner, 2002).

The variation in effects of different phytohormones at different concentrations and on different plants at different growth stages and their interactions with each other makes it difficult to assess whether they are causes or effects of any particular process. However, research reviewed in this section has shown that even quite small changes in environmental conditions such as water, light levels and phytohormones produced by soil organisms can induce changes in carbon and nitrogen metabolism in plants via cell signaling and changes in hormone activity. These changes can therefore affect nutrient concentrations found in plant products.

2.4.6 Interactions between nutrient uptake, humus and soil biota

In organic systems, plant nutrient supply is through nutrient cycling and interactions of the plant with soil humic material and soil biota. What effects of humus and soil biota on plant growth and interactions have been observed?

Nutrient cycling

Nutrient cycling and soil organisms and processes involved are discussed by Killham (1994). Ingham (1985) and Hendrix (1986) researched aspects of the soil food web in which a large variety of soil organisms, including microbes, fungi and larger organisms take part. Protozoa are particularly important in feeding on microorganisms and releasing the nutrients they contain. Ingham (1998) describes how protozoa and nematodes influence what microbial species predominate by preferring more palatable and non-toxic species and reducing the viability of spores that are eaten. They may accelerate detritus decomposition rate by consuming more of the early than later successional microbe species and by releasing waste products that stimulate decomposition. An optimal balance is likely to be reached in a mature soil ecosystem. Ingham has shown how nematodes increase N availability and plant growth rates by 25 – 75% above that obtained when only bacteria are present in the soil. Larger organisms such as earthworms and arthropods mix and help to decompose organic matter (Killham, 1994).

Nutrient availability from microbial activity and plant root exudates

Mäder et al. (2002) showed that soil organism activity affects plant nutrient uptake. For nutrients such as phosphorus, more could be made available to plants from soil organism biomass than from soil solution. Vancura (1988) reports work by Moghimi et al. (1978) which showed that root exudates and microbial metabolites together dissolved calcium phosphates and observations by Scheffer et al. (1967) that phenolic compound exudates assisted release of phosphorus from some minerals.

A key factor in organic crop production is quantity and timing of nutrient mineralisation (the process through which soil organisms and plant root exudates release nutrients in soluble form from soil organic matter and mineral compounds). Koopmans and Bokhurst (2000) carried out regular monitoring of N fertilisation on 38 farms in the Netherlands and developed a model that identifies sources of N mineralisation and simulates levels of inorganic N in the soil. However, many factors affect whether nutrients are mineralised or immobilised in microbial biomass, and too much mineralisation may result in nutrient leaching or reduction in microbial activity. Hodge et al. (2000) investigated competition between soil microbes and grass roots for nitrogen. Maloney et al. (1997) found that bacteria numbers on lettuce roots were significantly negatively correlated with nitrate concentration.

Nutrient mineralisation appears not to be the only, or necessarily the optimum way of nutrient transfer between microbes and plants. Jones and Darrah (1994) demonstrated that maize roots could release amino acids by passive diffusion from their roots and the roots could re-capture free amino-acids by an active transport mechanism. A computer simulation model was used to estimate that when inorganic-N concentrations in soil were limiting ($\leq$ 0.1 µmoles cm $^{(-3)}$ soil), the uptake of amino-N accounted for up to 90% the total N taken up by the roots. In situations where fertiliser inputs are high and levels of organic matter in soil are low, the contribution of amino-N might still be expected to be up to 30% of the total N taken up by the root system. Yamagata and Ae (1999) found that upland rice takes up nitrogen preferentially in the organic form rather than as nitrate. They

demonstrated that upland rice took up nitrogen before mineralisation from N-15-labelled rice bran, and suggested the mechanism was endocytosis.

It appears that plants take up amino acids mainly when soluble N-forms are scarce in the soil. Uptake of N as amino acids could have considerable implications for nutritional quality of plants. Lovell (2003) considers that when plants take up nitrogen as amino acids the DM% of cells is increased and food quality enhanced. Scheller (2000) discussed how amino acid metabolism in the soil provides amino acids for microbe growth and increases the availability of P. He also describes how plants take up indol and metabolize it to tryptophan. Tryptophan is an essential amino acid often lacking in human diets.

Interactions between plants and soil organisms are difficult to measure because any method is likely to alter the intimate connection between them. However it should be possible to link particular soil communities with enhanced crop growth. Schutter et al. (2001) used Biolog plates to compare soil communities under different soil treatments. They found that microbial communities utilizing carboxylic acids could be indicative of utilization of more root exudates rather than decomposing organic material. However as composition of root exudates has been found to vary with form and concentration of nutrients available to plants, it could be misleading to generalize in this way.

Phytohormone producton by soil biota

Greater nutrient efficiency could also result from the effects of phytohormones produced by soil biota. For example, Asghar et al (2002) found that some strains of rhizobacteria grown in vitro in the presence of L-tryptophan produced auxins. When Brassica seeds were inoculated with those strains of rhizobacteria they significantly increased plant size and yield as compared to strains producing less auxins. They hypothesized that plant root exudation of L-tryptophan could stimulate rhizobacteria to produce auxins which are taken up by the plants and increase plant growth. AM fungi may also synthesise auxins. Ludwig-Muller et al. (1997) found that young roots of maize colonized with *Glomus intraradices* had a higher content of IBA (a slow release source for indole acetic acid, IAA) than non-infected control roots, but total IAA content of the roots was lower. In older roots, total

IBA content was slightly lower in AM infected roots than in controls, but activity of IBA synthesase was threefold higher in infected roots than in control roots. It was not clear whether the IBA was synthesised by the host plants or by the AM fungus.

Effects of humus on plant physiology

Albiach et al. (2000) found that different types and quantities of organic amendments favoured different types and levels of microbial enzyme activities in a long-term field experiment. Figliolia et al. (1994) found that application of humic acids had variable effects on plant yields, depending on amounts applied and whether or not mineral fertilisers were also applied, and also resulted in increases of leaf area index of treated plants. Hoang and Boehme (2001) compared the effects of several forms of humic substance on the growth of water spinach. They found that all treatments increased growth, but there was most growth in the fulvic acid treatment. The quality of organic matter amendments is therefore an important factor. Sarapatka (2000) studied the effects of the content of organic substances on enzyme activities in the soil, including effects of different types of humus on phosphorus availability. He found the humic:fulvic acid ratio was negatively correlated with phosphorus availability, probably because smaller molecular weight molecules would be more easily decomposed by phosphatase enzymes.

Humic substances appear to increase the ATPase enzyme activity and the intake of nitrate ions through plant cell membranes by plasma membrane proton pumps. An increase in nitrate uptake was observed by Pinton et al. (1999) when water extractable humic substances were present. Nardi et al. (1999) found that the growth, alpha -amylase and invertase activity were affected by humic fractions and by gibberellic acid, indicating that humic matter had a gibberellin-like activity. The electrophoretic patterns of esterase was influenced in a similar way by humic fractions and by indoleacetic acid, which also suggested that the humic fractions exhibited an auxin-like activity. The auxin- and the gibberellin-like activities were related to a high content of phenolic and carboxylic groups. They attributed the biological activity of the humic substances to their chemical structure and to their functional groups, which could interact with hormone-binding proteins in the membrane.

AM fungi and nutrient uptake

Arbuscular mycorrhizal (AM) fungi have also been shown to contribute to plant nutrient uptake and to affect plant metabolism. P uptake from less soluble sources (Bolan, 1991), and also N, Zn and Cu uptake is increased. Uptake of K, Ca and Mg is enhanced in acidic soils (Clark and Zeto, 2002).

Effects of AM fungi on nitrogen uptake vary with form of nitrogen and fungal species. Azcon et al. (1996) found that *Glomus fasciculatum* increased uptake of nitrate, nitrate reductase activity and protein content of lettuce compared with uncolonised, P fertilized plants under reduced moisture conditions, but there was no difference in N uptake between treatments when N was applied as ammonium. Innoculation with *G. mosseae* did not increase nitrate uptake but increased photosynthetic activity. In further trials, maximum uptake was recorded at an N concentration of 84mgN/kg soil for *G. mosseae* colonized plants and 168mgN/kg for *G. fasciculatum* colonized plants. At 252mgN/kg, uptake was reduced. Azcon et al. commented that their results suggested that better N utilization was not due to better P nutrition of mycorrhizal plants but to direct fungal effect.

AM fungi inhabitation in plant roots is increased by lower N and P status. Alloush et al. (2000) showed how addition of P fertiliser to acidic soil reduced AM fungi colonisation of roots and uptake of P in acidic soil; whereas phosphorus rock and innoculation with AM fungi increased shoot contents of P, S, Ca and Mg. Addition of any fertiliser or organic matter decreased Fe and Cu uptake and organic matter addition reduced Ca uptake. Graves (2002) found that dry weight biomass of maize seedling shoot tissues was negatively correlated with increased soil-fungal inhabitation or intercellular and intra-cellular fungus and there was less root growth in permanent pasture compared to new pasture and in maize grown under winter light conditions. He hypothesised that in these circumstances mycorrhizae were a drain on plant carbon.

Interactions between AM fungi and microbial activity also occur. Brown and Carr (1984) studied the interactions between *Azotobacter chroococcum* and AM fungi and found that

inoculation of the roots of lettuce seedlings with *Glomus mosseae, G. caledonium* and *A. chroococcum* produced larger plants than either inoculum alone although there was no association between the fungi and *Azotobacter. Azotobacter* did not influence the level of fungal infection in the roots, but numbers of *Azotobacter* on the root systems were decreased in the presence of the fungi.

From analysis of mycorrhizal colonization of lettuces grown by farmers in California, related to a number of factors, Miller and Jackson (1998) found that use of pesticides, inorganic fertilisers and deep tillage practices decreased the amount of infection. Crop rotations predominated by AM hosts increased the probability of infection. Total soil phosphorus showed a closer relationship to AM colonization than Olsen available phosphorous. Higher total soil carbon percentages tended to correlate to an increase in root infection levels. There was a greater incidence of AM fungi in lettuce in 'organic' fields.

AM fungi effects on plant metabolism

AM fungi have been found to increase the transpiration, photosynthesis and respiration rates of host plants, enabling more carbon to be accessed and metabolized (Sharma and Johri (2002). An increase in net carbon assimilation in mycorrhizal plants was related to both an increase in specific leaf area and enhanced photosynthesis even though colonization did not affect plant dry mass. Subramanian and Charest (1995) found that AM plants had higher total and reducing sugar concentrations than non-AM plants and an increase in protein content after drought.

It appears that carbon is transported to the AM fungi as glucose, and the fungi synthesise more complex sugars such as trehalose, glycogen and mannitol. Nutrients are stored by the fungi as lipids and phospholipids (Gaspar, 2002). AM fungi can also take up substantial quantities of soil NH_4^+ and NO_3^- nitrogen, some of which is translocated to the plants. Some is incorporated into amino acids such as aspargine, aspartate, alanine and glutamine (Sharma and Johri, 2002). These authors also discuss research indicating that AM fungi can increase plant zinc uptake, possibly through changing the rate and composition of root exudates, favouring acids which form complexes with soil zinc which diffuse more readily

in soil solution. Changes in root exudates including exudation of growth promoting hormones also enable interactions with other soil organisms including actinomycetes, N fixing and P solublising bacteria, resulting in enhanced plant nutrient uptake (Calvet et al., 2002).

Quantity and population composition of AM fungi associated with crops could possibly affect plant product quality through influencing form and structure of nutrients assimilated in some of the ways reviewed.

AM fungi and water stress

Leinhos and Bergmann. (1995) found that inoculation with AM fungi reduced the effect of drought on lettuce growth, but the protein pattern of crude extract from lettuce leaves was affected by drought stress and this effect was still present in inoculated plants. Higher transpiration rates, levels of leaf conductance, and proline, N, and P contents were found to be related to maintenance of plant growth under water stress conditions by mycorrhizal fungi (Ruiz Lozano et al., 1995). Different fungus species appeared to induce different degrees of osmotic adjustment as shown by differences in proline accumulation in leaves, transpiration, stomatal conductance and P and K acquisition.

Berg et al. (2001) investigated interaction of AM fungi with variability in water supply. Innoculated lettuces given low water supply once a week had increased root/shoot ratios and decreased tissue concentrations of N and P. The same quantity of water given in small daily doses had less negative effects. Inoculated plants had higher photosynthesis rates than non-inoculated plants when water supply was less variable, but lower photosynthesis rates than non-inoculated plants for the more variable water supply. They concluded that lettuce plants may have a fairly low dependence on mycorrhiza, and high mycorrhizal populations may immobilize more nutrients rather than make them available for plants, or the mycorrhiza species inoculated could have been less beneficial to lettuces than native species.

2.5 Indicator tests for soil and compost characterisation and plant nutrient composition

2.5.1 Soil characterisation

The suitability of a soil to provide for a particular crop does not depend only on the content of nutrients and physical characteristics as determined by laboratory analysis. Under an organic system it is important that the soil is functioning properly as a balanced living habitat for a wide range of soil organisms which interact with root exudates from the crops and are essential for providing nutrients (Scheller, 1996). However, standard laboratory analysis of total N, C and P, Olsen P, P retention, sulphate, CEC, exchangeable cations, pH and bulk density as described by Blakemore et al. (1987), provide an indication of likely nutrient deficiencies or toxicities and what amendments are needed. Estimated carbon to nitrogen and phosphorus ratios and mineralisable nitrogen tests provide some indication of likely nutrient cycling by soil biota. Some of these tests are further discussed in the following sections.

Carbon

Total soil carbon content determined by a LECO induction furnace (Searle, 1967) can be used to estimate soil organic matter content and ratios with other nutrients. The content and availability of carbon varies considerably with soil type and land-use history. For pasture soils on yellow-brown loam soils at 3 sites in the Waikato region, total C% varied between 8.4 and 12.39% (Ghani et al., 1996). They reported that total C and N values were higher under sheep-beef than under dairy pasture and there was also some variation according to season of the year.

Distinguishing between the different forms of carbon gives an indication of how well the soil might provide for crop growth and composition. About 0.27-4.8% of the total soil C and 0.5 – 15.3% of total soil N is in the living microbial biomass (Tate, 1987). Sparling (1997) discussed how the carbon quotient, microbial C/total C can give an indication of soil health. For a silty clay loam at Kairanga under pasture, he determined this quotient at 2.97, but it fell to 1.5 after 13 years maize cropping.

Goh et al. (1999) found that microbial biomass C and its ratio with total C and the ratio of hot-water extractable C to hot-water extractable carbohydrate were sensitive indicators capable of distinguishing significant differences between different orchard management systems. Mäder et al. (2002) measured the soil microbial carbon, activity and diversity and traced the fate of 14C-labeled plant material in soil under different management systems in the long-term DOC trial. Under controlled conditions, the more diverse microbial community of the biodynamic soil decomposed more 14C-labeled plant material than that of the conventional soils, and in the field, undecomposed plant material decayed more completely in organic systems. They concluded that microbial communities with an increased diversity in organic soils transform carbon from organic debris into biomass at lower energy costs, building up a higher microbial biomass. Gunapala and Scow (1998) found an increase in the proportion of C in the microbial biomass under an organic system compared to a conventional system and wrote that although for conventional systems it can reduce N availability, they found it contributed to increased N availability for plant uptake.

Characterisation of the various forms of non-microbial C can also provide an indication of soil fertility. Some would be in partially decomposed plant and animal material but most is in relatively stable humic compounds. Apfelthaler (1992) reviewed humus research and discussed a quality coefficient, based on the determination of optical densities of soluble low molecular weight substances and of well humified dark coloured substances of high molecular weight. Fliessbach and Mäder (2000) fractionated soil organic matter according to size density and found that the light fraction (mainly fresh organic material) of particulate organic matter was decomposed more quickly in organic systems, and that the ratio of microbial biomass to light fraction material could be used as an indicator of the quality of recently added organic material.

Nitrogen

Total N can be determined by LECO furnace (Kowalenko, 2001) or by Kjeldahl digestion (McKenzie and Wallace 1954) then measured by Technicon auto-analyser (Twine and Williams, 1971, Technicon 1976). Both methods of measurement should give similar results. The nitrogen, as for carbon, is partly in humus, part in partially decomposed organic matter and partly in the soil biota.

C:N ratios

Carbon: nitrogen ratios can be calculated from total C and total N measurements. However, to interpret what this C: N indicates for plant growth, requires more measurement of the various fractions as discussed above. Fliessbach and Mäder (2000) found that in soils given different long-term treatments in the Swiss DOC trial, sizes of microbial biomass C and N were highest but the microbial C:N ratio was lowest for the biodynamic treatment, whereas there was little difference for organic C:N ratios. There were also differences in C: N ratios for different density fractions of the soil organic matter. Gunapala and Scow (1998) found significantly lower C: N for organic compared to conventionally managed soil and concluded that this is indicative of a habitat where bacteria may be more important than fungi for decomposition in an organic compared to soluble fertiliser system.

Nitrogen mineralisation

Mineralisation occurs when organic debris and soil organisms are decomposed, releasing ammonium ions from cell proteins and other nitrogenous organic compounds. This can occur when soil organisms with a low carbon: nitrogen ratio, such as most bacteria, are consumed by other soil organisms which have a lower requirement for nitrogen. Net mineralization can also occur when aerobic organisms are replaced by anaerobic organisms in anaerobic incubations (Waring and Bremner (1964). The ammonium ions may then be immobilised by being taken up by soil organisms or nitrified to nitrite and then nitrate by nitrifying bacteria. However, as nitrifying bacteria are obligate aerobes (Paul and Clark, 1989), there is unlikely to be much nitrification under anaerobic incubation. Waring and Bremner (1964) pointed out that no nitrate or nitrite would be produced under laboratory anaerobic incubation. They found a close relationship between the quantity of ammonium nitrogen produced by this method and the quantity of ammonium+ nitrate +nitrite produced in aerobic incubation for cultivated Queensland Black Earth soils. Myrold (1987) found a good correlation between the quantity of nitrogen produced by this anaerobic incubation and the quantity of nitrogen produced after chloroform fumigation, indicating that the nitrogen produced by anaerobic incubation was almost all from the microbial biomass.

For estimating the amount of easily mineralised organic N in soils, aerobic laboratory incubations are an alternative method to anaerobic incubations, although Sparling (1997) wrote that there is sometimes less microbial immobilisation of N under anaerobic than aerobic incubation. Hart et al. (1994) wrote that different soils will show maximal rates of net N mineralisation under different sets of environmental conditions because of the diversity of soil organic N substrates and microbial populations involved. They pointed out that incubation of intact soil cores gives a better result than a mixed soil sample. Wang et al. (2001) compared net N production under aerobic and anaerobic conditions for 20 soil samples incubated for 14 days at 30°C and found that gross N mineralisation was not always higher and immobilisation was greater under aerobic than under waterlogged conditions. Significant amounts of N were lost from some soils during the 2 weeks of incubation. Craighead and Clark (1989) described how Ravensdown Fertiliser Cooperative changed to using the anaerobic incubation from the aerobic method to assess nitrogen status because the results from aerobic incubation could not be correlated to yields.

Tate (2000) wrote that collection, preparation and laboratory incubation of soil samples can considerably alter the conditions for mineralisation from that happening in the field. Mineralisation kinetics can vary considerably with variations in temperature and moisture, and there is also much spatial variation. He quoted research by Goovaerts and Chiang (1993), who estimated that 24 – 37 samples are needed to estimate nitrogen mineralisation potential of a 1600 m^2 plot with a precision of 0.1. Van Steensel (2002), quoting a Dutch publication by Bloksma (1996), wrote that net mineralisation occurs under relatively warm aerated, alternately wet and dry, relatively alkaline conditions, whereas net immobilisation occurs in cold, compacted, wet, or very dry acidic conditions with little plant root activity.

When crops are growing there is a symbiosis between plants and microorganisms: the plants provide carbonaceous nutrients to the microorganisms in root exudates, enabling bacteria such as Rhizobia to multiply and fix nitrogen from the air. Other biota secrete hydrolytic enzymes which can release more nutrients from the soil humus and organic debris. Hodge et al. (2000) found that there was higher N utilization and/or mineralisation where roots were present than by soil organisms alone. Waldon et al. (1998) found that an organic system had a high production of healthy plants in spite of having a lower nitrogen

and phosphorus content, indicating that mineral nutrients are used in a more efficient manner under an organic system. Reddy et al. (1986) point out that in well-drained mineral soils where aerobic respiration predominates, soil organic matter decomposition can potentially release about 10-20% of its N as inorganic N, but this reduces considerably under anaerobic conditions.

Continual growth and decay of microorganisms and predation by larger soil organisms such as protozoa and nematodes provides for a continual release of nitrogenous substances available for plant uptake (Ingham, 1998). Ingham has shown how nematodes increase N availability and plant growth rate by 25 – 75% above that obtained when only bacteria are present in the soil, as nematodes have a C: N ratio of 10:1. However, Ferris et al. (1997) measured the C:N of several cultured nematode species and recorded ratios of between 5.16 and 6.83 but estimated that bacteria-feeding nematodes could still make a considerable contribution to mineralised N, up to 1.01ugN/g soil/day in rhizosphere soil. This varies with soil temperature, increasing from 5g/ha/month in April, to 90g/ha/month in June (Northern Hemisphere). There is also considerable variation as new soil organic matter decomposes, with the nematode population and its contribution to N mineralisation growing at first, then decreasing again after about 60 days. There is a huge variety of different types and species of soil organism that could greatly affect nutrient dynamics.

Net N immobilisation is more likely if the soil is fungal rather than bacteria dominated. Chen and Ferris (1999) measured nitrogen mineralisation in columns of sand containing various combinations of fungi and fungal-feeding nematodes, and found low levels of mineralised N in columns with fungi alone, particularly at high C:N, but higher N mineralisation when nematodes were included. However, as observed above, the trial soil was more likely to be predominantly bacterial. Soil pore size can also make considerable difference to N mineralisation (Hassink et al, 1993). They found that N mineralisation was higher in coarse-textured than fine-textured soils, due to higher grazing pressure by nematodes and flagellates.

Phosphorus

Total soil P (TP) is measured by Kjeldahl digestion and Technicon Auto- analyzer (McKenzie and Wallace 1954, Twine and Williams, 1971, Technicon 1976). Much of the

P measured as TP is unavailable for plant uptake being present as occluded forms (P within Al and Fe hydrous oxides) of inorganic P and recalcitrant humic–P. (Russell, 1988)

Various soil tests have been developed to measure as far as possible the labile P that would actually be available for plant uptake. In practice this is hard to do because of the variability of this amount according to type of soil, temperature, water content, pH, availability of other nutrients, cultivation carried out and type and variety of crop grown. The Olsen P test (Olsen et al., 1954) is most used in New Zealand and has been calibrated against pasture yield. However, this test may not be best for soils derived from volcanic ash that have high P retention (Saunders et al. 1964). Helyar and Price (1999) used data from Holford to estimate the quantity of isotopically exchangeable P extracted by various tests. For a soil with strong buffering capacity (400-550 mgP sorbed/kg soil) /mgP/L) the Olsen P test extracted 20% of the P. Barlow (2001) tested soil P levels on over 30 kiwifruit orchards in the Bay of Plenty by Olsen P, Mehlich 2 and Bray 2 tests and compared results with results of kiwifruit leaf phosphorus levels and found that the 2 latter tests were better predictors of leaf phosphorus concentration than the Olsen P test. The Resin P test (Saggar et al., 1990) could be a better indicator of available P. Saggar et al. (1999) compared the Olsen, Colwell and Resin tests for New Zealand pasture soils treated with TSP and phosphate rock. They found the resin test extracted 2 – 3 times more P than the Olsen test and was a better yield predictor than the Olsen test for soils fertilised with phosphate rock.

Possibly the Olsen P test is less helpful for soils containing mainly organic P, which is likely in this case as Mullen (1998) wrote that organic P may be 93% of total P in sandy soil. Paul and Clark (1989) wrote that microorganisms have a higher requirement for P than plants, and that much of the organic P may be in complex stable compounds. Parfitt et al. (1989) pointed out that immobilisation of phosphate in the biomass can contribute to the loss of availability of phosphate in soils. Oberson et al. (1993) found no relationship between Olsen P levels and acid soil phosphatase activity and concluded that the larger biomass and higher enzyme activity in biological systems provide an increased P mineralisation potential. In permanent pastures, a significant source of P may be its net mineralisation from the microbial biomass and organic residues (Peverill et al 1999 citing Blair et al. 1977).

The phosphate retention test is a measure of the ability of the soil to remove phosphorus rapidly from solution (Blakemore et al. 1987), but net mineralisation or immobilisation would not be taken into account in that test either. This could lead to an underestimation of the likely availability of P to plants under an organic system, particularly in undisturbed soil, where AM fungi could contribute up to 80% of plant P uptake (Li et al. 1991). Mullen (1998) pointed out that the biomass contains 20% or more of the organic P in some grassland soils, and the quantity of P mineralised or immobilised depends on the C: P ratio. He estimated that C: P should be less than 200:1 for net mineralisation

Sarapatka (2000) studied the effects of the content of organic substances on enzyme activities in the soil, including effects of different types of humus on phosphorus availability. He found the humic:fulvic acid ratio was negatively correlated with phosphorus availability, probably because smaller molecular weight molecules in the fulvic fraction would be more easily decomposed by phosphatase enzymes.

Sulphur

A potassium phosphate extraction of soil (Watkinson and Kear, 1996), followed by S determination by the method of Johnson and Nishita (1952) as modified and described by Blakemore et al. (1987), extracts mainly the inorganic sulphate which is adsorbed on clay minerals and also sulphides, sulphites and very labile forms of organic sulphur. According to Germida (1998) 75 – >90% of the soil sulphur is in organic forms, particularly as sulphate esters, which are the most labile form, and also as carbon-bonded amino acids. Soil organisms mineralise the carbon-bonded S biologically when oxidising the carbon for energy. Net mineralisation is likely when C: S is less than 200. The non-carbon-bonded S eg the esters, is mineralised biochemically by sulphatase enzymes released externally by soil biota, particularly by Thiobacillus spp. and fungi. Lewis (1999) points out that sandy loams would not contain much adsorbed sulphate and that mineralisation of the organic sulphur depends on soil temperature and moisture. Highest sulphate levels are likely to be recorded in spring and summer when it has not been very wet.

Soil S mineralisation can be measured, generally by open incubation and leaching. However, Pamidi et al. (2001) compared three different methods, (open incubation, pots with and without plants) and found that periodic leaching of soil in the open incubation system does not simulate crop removal of S or provide a means of predicting plant S. Leaching removed more S, mainly in the first 4 weeks, whereas mineralisation extended over a longer time in the pots, and was greater in pots with plants than those without, indicating the inter-reaction of plants and soil biota.

CEC and Cations

The Cation Exchange Capacity (CEC) is a measure of the quantity of cations that a soil can hold when a buffered or unbuffered salt solution is leached through the soil (Blakemore et al. 1987). Negatively charged cation exchange sites are provided by the soil clay and organic matter. The CEC on soil organic matter is pH dependent rising with pH to a maximum associated with maximum dissociation of carboxylic acid functional groups. An organic system aims to maintain high soil organic matter levels to obtain continuous release of nutrients by mineralisation. Provided pH is near neutral released cations will be held on the ample CEC ready for plant uptake. Nutrient cations will have greater protection against leaching in soils with high organic matter.

The total exchangeable bases (TEB) provides an indication of balance between the cations, the %TEB levels are not the same as base saturation ratios calculated under the Albrecht system as TEB does not include hydrogen. Albrecht (1975) recommended cation balancing for optimum soil fertility and crop and animal health, using a formula of base saturation ratios of about 65-75% Ca, 10-15% Mg, 2-5% K, 0.5-3% Na and 10-15% H For sandy soils with low CEC, the formula is modified slightly, to about 60% Ca, 20% Mg and 6-8% K. The US Brookside laboratory works with this concept and measures ammonium acetate extractable total exchangeable cations (TEC) which includes the four bases plus hydrogen.

Soil organism activity

Soil organisms and AM fungi could be stimulated by compost and also by plant root exudates, which would be increased by increased photosynthesis. Carbon dioxide production is a measure of respiration of all aerobic organisms in the soil. Measurement in

the field prevents changes from disturbance of the soil, but these changes can be minimized by careful handling of the soil, immediate transfer to the laboratory, and keeping the soil at similar temperature and moisture content as in the field. A laboratory method is described by Pramer and Schmidt (1965). Measures of enzyme production and of soil organism diversity would be helpful but were beyond the scope of this research.

2.5.2 Compost characterisation

The same tests can be carried out on composts as for soils, but nutrient composition is of less importance than the stability, maturity, aeration, soil organism and humic contents of the compost. These characteristics depend on the type and quality of materials used and method and conditions of compost making.

Temperature, aeration

In hot composting the heaps are turned frequently to increase aeration and generate several high temperature levels peaks, and this is the method recommended for organic growers by Ingham (2001). Such composts are generally ready for use more quickly than cold composts, which do not reach such high temperatures. A disadvantage of the hot composting method is that the high temperature can kill macro-organisms such as earthworms. Biodynamic heaps are often matured for a year or more and only turned once or twice during that time, whereas in commercial composting frequent turning is generally the rule to avoid anaerobic conditions and speed up decomposition with high temperatures. Anaerobic conditions can favour microbes which do not result in healthy soil conditions when incorporated in the soil (Ingham, 2001). Epstein (1997) described how temperatures over 45°C increase decomposition rate, and favour thermophilic species, which are mainly bacteria, whereas under 45°C mesophilic organisms predominate which include more fungi. Size of compost also affects temperature.

Stability, maturity and organic matter content and quality

The US Woods End Research Laboratory carries out routine comprehensive tests on compost and has published a guide to interpreting compost tests (2000), that describes a large number of tests and what they indicate. Epstein (1997) discusses research and tests for compost stability and maturity. Respiration (carbon dioxide evolution) tests are

commonly used to indicate how far the decomposition process has proceeded and the stability of the compost. Nappi et al. (1992) reviewed parameters for evaluating compost quality and concluded that biological assays and methods for evaluating the quality and quantity of the organic matter are particularly important. They compared many different composts and found large variability in results according to the quality of starting materials, inert materials present, management of the composting process and maturation length.

Mineralisable nitrogen

Most of the published research results on nutrient levels in compost and organic amendments are from municipal waste, biosolids and farmyard manure, which have different nutrient contents, and generally have lower C: N content than a compost made from predominantly plant material. Epstein (1997) reviewed research on nitrogen mineralisation in composts, finding that biosolids composts had a high rate of N mineralisation during the first 3 weeks of decomposition, and mature compost generally had a rate of N mineralisation of 7-10% over the first 20-35 weeks, a higher rate than for fresh compost. Heller (1999) incubated 5 different composts over 149 days at constant optimal temperature and humidity and found < 32.5 kg mineralised N/ha and that between 0.7% and 6.9% of the total organic nitrogen was nitrified, depending on the properties of the composts, but could not find any correlation between any set of analytical data and nitrification capability.

Much of the research on N mineralisation has been done after incorporation of the compost into soil. Navarro et al. (1992) carried out aerobic and anaerobic incubation of soil and soil mixed with fresh and mature compost and measured the various forms of nitrogen extracted by water, by KCl and by Kjeldahl digestion at weekly intervals. They found that the ammonium nitrogen declined to low levels by 3 weeks for all treatments and extraction methods. The nitrate nitrogen increased during this time for the soil only treatment, but for the composted soils, nitrate nitrogen did not start to increase until after 3 weeks, whereas the Kjeldahl nitrogen level remained fairly constant for all treatments, so there must have been net immobilisation until then. The carbon: nitrogen ratios of their composts were 20.1 (fresh) and 18.1 (mature). They concluded that N mineralisation rate was lower the lower

the initial concentration of organic N, the more thorough and lengthy the treatment/stabilization method, and the more recalcitrant the carbonaceous materials.

2.5.3 Tests of plant nutrient composition

Leaf sap nitrate content

Leaf nitrate content depends on amount and rate of nitrate uptake and rate of assimilation into amino acids. Rate of assimilation could be affected by the silica spray. A rough field measurement of leaf sap nitrates can be obtained using Merck nitrate (Mer 110020.0001) test strips. The strips are dipped into the sap sample (or diluted sap sample), and then the amount of colour developed in one minute is measured against a colour scale. More accurate laboratory analysis of leaf nitrate can be done by extraction from dried leaf material by acetic acid (Prasad and Spiers, 1984) or by KCl extraction from leaf sap (Westerman, 1990) followed by analysis by Technicon Auto-analyser.

Leaf sap soluble solids

Brix level in cell sap depends on rate of photosynthesis and production of sugars and other solids and rate of conversion to insoluble solids e.g. starch and/or transport to the rest of the plant. This could be affected by the silica spray.

The hand-held Brix refractometer provides a field method of estimating percentage by weight of soluble solids in cell sap. Most of the soluble solids are generally sugars, but other solids, including proteins are also included. The "fuzziness" of the reading is considered to be an indication of protein content (Harrill, 1998). He provided standards for Brix readings of lettuce leaves (Table 2.5.1):

Table 2.5.1 Brix standards for lettuce

Brix reading	4	6	8	10
Lettuce quality	poor	average	good	excellent

Mineral Composition

Total N and P of dried leaf matter can be analysed in a similar way as described for soils in Section 2.5.1, by Kjeldahl digestion (McKenzie and Wallace, 1954), followed by analysis (Technicon, 1976; Twine and Williams, 1971). Cation and trace element analysis (e.g.

potassium, magnesium, calcium, iron, zinc, copper and selenium) can be done by nitric acid digestion (Westerman, 1990) followed by atomic absorption spectrophotometry.

Amino acids

Concentrations of most amino acids can be determined in dried leaf material using the method described by Fierabracci (1991).

Antioxidants

Antioxidants are secondary metabolites which could be increased by the silica spray. Many researchers have measured one or more vitamins in plant material, particularly vitamin C, as indicators of nutrient content. Some properties making accurate measurement of vitamins difficult, are their instability, as soon as plant material is removed from the plant, and the various forms they can assume. One of the most important health-related properties of vitamin C, and also of other plant constituents such as vitamin E and flavonoids is their antioxidising ability. Reactive oxygen species, or "free radicals", generated during cell metabolism, can destroy cell membranes. Cell health therefore depends on its ability to prevent generation of, remove or inactivate these free radicals. Antioxidant substances can inactivate free radicals by reacting with them, and reducing them, so that they do not affect cell integrity. The Ferric Reducing Ability of Plasma (FRAP) assay (Benzie and Strain, 1996) measures antioxidant power by measuring the ability of the test material, when mixed with standard human or bovine plasma, to reduce ferric-tripyridyltriazine, producing a measurable colour change.

Taste and other sensory qualities

Taste is an important measure of quality. Two aspects that particularly affect taste are bitterness, largely determined by nitrate content, and sweetness, which depends on soluble solids content, which are mainly soluble sugars.

Problems of variation in sensitivity to taste differences between tasters can be reduced by training prior to the test (ISO, 1994).

2.6 Conclusions from literature review and research questions

2.6.1 Literature Review Conclusions

1. Soil health indicators developed from a sustainable environmental perspective are relevant, but do not indicate whether a soil and its management will produce high quality food. Soil organism diversity, enzyme activity and humus quality may be indicators relevant to food quality.

2. Food quality indicators such as quantity of individual minerals and vitamins are relevant but are in-directly linked to consumer health. Nutrient ratios indicate how nutrients are assimilated into sugars, amino acids and proteins. Dynamic measures, e.g. antioxidant activity, enzyme activity and biophoton activity are indicators worth further research but are difficult to measure.

3. Links between nutrition and health have been found but it is difficult to show links between particular soils, management systems and diet components.

4. Organic systems have been found to positively affect soil quality indicators such as bulk density, organic matter content, microbial biomass activity and diversity, soil enzymes and soil aggregate stability. Such systems rely on soil biota activity and nutrient cycling. Little is known about the influence of soil, management and soil biota activity and diversity on the form of nutrients available to plants e.g. percentage of N supplied as amino acids and chelated trace elements and any possible effects on plant metabolism and product quality.

5. Links between organic systems and food quality are not clear. Nitrate contents of leaves are generally lower and higher concentrations of some minerals and vitamins have been found under particular weather and soil conditions but there are no consistent consequences of organic production.

6. Variability of factors such as soil parameters, weather, site history and the organic system used make statistically significant differences difficult to show.

7. Biodynamic systems also use biodynamic preparations which have been shown to positively affect soil and compost parameters.

8. The concept of using the horn-manure and horn-silica preparations is to balance the calcium (growth and shade) and silica (light and differentiation) processes which likely affect plant signalling through ion, hormone and enzyme production and activity. The silica preparation may change quantity and ratios of different wavelengths absorbed by plants which could trigger signals affecting stomatal opening, redox and phosphorylation reactions, respiration and DNA transcription, which could affect e.g. nitrate reductase activity in plant leaves. Application of such sprays could result in more assimilation of nitrates into amino acids and protein, a higher true protein: crude protein and more essential amino acids. Such effects may be measurable.

9. Lettuce plants have particular requirements for water, light, nutrients which have to be met whatever system is used. Different soils and treatments have been shown to affect nutrient contents. Different forms of nutrient uptake, e.g. N as NO_3^-, NH_4^+ or amino acids, varying particular light wavelengths such as blue and red light, and interactions with soil organisms and AM fungi have been shown to affect nutrient metabolism, plant hormones, cell signalling and DNA transcription. Nutrient concentrations found in lettuces are very variable.

10. Characterisation of soil mineral content, soil organism activity and plant nutrient content could provide useful indicators of plant nutritional value.

2.6.2 Some issues to consider when researching organic systems

Many previous trials comparing organic and conventional products compared particular soil amendments rather than whole systems. A whole organic system is difficult to research as it relies on developing soil structure, fertility and the soil food web to provide

for crop production over many years. Site history, crop rotation and interactions with adjacent land all affect current crop growth. Further considerations are:

- High variability in what is taken to be "organic" production and type and quality of organic amendments used;
- Biodynamic systems can be more variable from quality of the biodynamic preparations used, and timing and frequency of their application;
- Previous crop and amount of soil cultivation affect mycorrhizal activity;
- Effects of different management systems can be masked by greater effects of other factors such as weather conditions, soil type, structure, and nutrient content, light intensity and day-length, crop variety.

Variations in such factors between trials would account for much of the variability in results of growing method comparisons. These factors can generally be kept more constant in greenhouse trials, but then how applicable are these results to field conditions? Many of these problems can be overcome by long-term trials that by definition require large time and funding commitments. A trial set up to hold most factors constant enables short-term study of effects of particular treatments.

2.6.3 Research questions

Before the literature review was undertaken, the author intended to investigate interactions between application of biodynamic sprays, soil organism populations and activity, plant metabolism and food quality. The literature review convinced the author that the extensive research programme required to investigate possible relationships would be too large for a Masters' project. The research section of this thesis therefore focused on conducting a field trial to determine:

- How do organic systems, different composts, soluble fertilisers and biodynamic preparations affect plant growth and nutrient content?
- How does the horn silica spray affect nitrate assimilation?

A greenhouse trial then evaluated possible effects of the silica spray on light absorption, water uptake, photosynthesis and nitrate assimilation under more controlled conditions.

3 FIELD TRIAL METHOD

Choice of field-trial treatments

Treatments were chosen to evaluate effects of composts, soluble fertilisers and biodynamic preparations on growth and nutrient assimilation, and to use the biodynamic fieldsprays to provide fairly extreme differences in growth (shade) and differentiation (light) in order to obtain statistically significant differences.

Choice of trial plant

Lettuces were chosen as the trial plant even though they are not a major source of nutrients in the human diet. The reasons lettuces were chosen are:

- Short growing time, suitable for short time available,
- Uniform plants could be chosen at transplanting time,
- Fairly low requirements for shelter and water, suitable for exposed site and low irrigation availability,
- One of the main vegetables consumed by most people.

3.1 Compost preparation

Over a period of 6 weeks, initiated on 15.4.02, two compost heaps were built from the following proportions of material, mixed in small batches (final volume 3 m^3):

Table 3.1 Compost composition before decomposition

Material	Proportion	Material	Proportion
Grass mowings	35%	Dry tree leaves	8%
Cow dung	25%	Mature compost	2%
Weeds (mainly thistles)	20%	Lime	200g
Hay	10%		

Mature compost was included to "seed" decomposer microorganisms. Sufficient material had been added to the two heaps by 29.5.02.

Samples (1 g) of each of the biodynamic compost preparations 502 to 506 inclusive (see Procter, 1995 for description of these preparations) were inserted into one of the heaps (biodynamic compost) and 10 mL of the preparation 507 was stirred in 1.5 L rainwater

and spread over the top of the same heap. The same volume of rainwater was spread over the other heap (organic compost). The heaps were covered with a thin layer of soil and hay to make them partially waterproof. Both heaps were turned on 9.6.02 and again on 17.8.02 to increase aeration and speed up decomposition. A second set of compost preparations was inserted into the biodynamic heap on 27.8.02 and both heaps were turned on 12.9.02 and again on 23.9.02. The heaps were watered and covered with black polythene during the last few weeks to prevent them drying out.

Plate 3.1 Organic and biodynamic compost heaps

3.2 Compost stability, toxicity and life activity tests

Compost sampling technique

To obtain homogenous compost samples, at the first 2 sampling times on 19.8.02 and 9.9.02, six 2.5 cm diameter by 15 cm depth cores were taken from different parts of the heap and mixed. At the third sampling time, on 23.9.02, each heap was divided into 8, remixed then divided again into 8. Sub-samples were taken from each eighth, mixed and separated into 2 samples for testing.

Table 3.2 Dates of compost and soil tests

Date	**19.8.02**	**9.9.02**	**15.9.02**	**23.9.02**
Days from completion of heaps	82	103	109	117
Compost Respiration		✔		✔
Compost Phytotoxicity			✔	
Compost dynamolysis			✔	✔
Soil and compost elemental analysis, pH, CEC, bulk density	✔			
Soil and compost Kjeldahl and Mineralisable N				✔

Microbial respiration activity test for stability

At 82 days from completion of the compost heaps, a respiration test was carried out, using the following method. Two 30 g samples of each compost were weighed out and placed in preserving jars. A beaker containing 20 mL of 0.5 M KOH was placed inside each preserving jar and the lid placed on tightly. The jars were incubated, the first time at 30° C, the second time in a cupboard at room temperature (approximately 25° C) to more closely simulate field conditions. After 3 – 4 days, 4 mL of $BaCl_2$ were added to 8 mL of the KOH to precipitate $BaCO_3$. The residual hydroxide was titrated against 0.2 M HCl, using phenolphthalein as the end point indicator. The quantity of CO_2 in the KOH was then calculated from the volume of titre used. The resulting CO_2 evolution figures were compared with an index published by Epstein (1997). The second respiration test on the 9.9.02 indicated a low rate of microbial activity, probably mainly because the heaps had dried out too much and some organic matter remained undecomposed, so the heaps were turned and watered with equal volumes of water twice, to speed up decomposition.

Phytotoxicity test

On 8.9.02 a maturity check was carried out by a phytotoxicity test (Epstein, 1997). Cress seeds were sown in 5cm deep samples of each compost in 10 x 15cm plastic containers, moistened with rainwater. The containers were placed in a shady place near the compost heaps and remoistened as necessary. On 15.9.02, seven days later, the appearance of the seedlings was examined when they were about 50mm high.

Capillary dynamolysis test

On 15.9.02, 23.9.02, 26.10.02 and 21.11.02 the 2 composts were tested for life activity by capillary dynamolysis, following the method of Brinton (1983). Compost samples were taken as described in section 3.1.2. Alkali soluble organic matter was extracted from 10 g sub-samples of compost in 100 mL 0.25 N sodium hydroxide over 12 hours, stirring occasionally, then allowing solids to settle.

Pieces of Whatman No.1 filter paper were prepared by making small holes in the centre, marking radial distances of 35 mm and 50 mm, and pushing rolled 20 mm* 20 mm

square filter paper wicks through the central holes. The filter paper was placed over a 12 cm diameter Petri dish, in the centre of which was a small bottle lid containing 0.5% silver nitrate solution, so that the wick was in the lid. The wick and paper were removed when the silver nitrate solution had been eluted into the paper as far as the first, 35mm mark. The paper was then dried at room temperature in subdued light. The humic extract was then placed in a bottle lid and allowed to elute into the filter paper through the wick, as for the silver nitrate solution, until it reached the second, 50 mm mark. This was done in a humid chamber. The paper was then removed and left to dry at room temperature in subdued light.

3.3 Soil and compost laboratory analysis

Soil sampling and preparation

On 19.8.02, samples of soil from each trial block and from each compost were taken for laboratory analysis. The soil was sampled by taking three 2.5 cm diameter x 15 cm deep cores at random sites in each of the 4 blocks and thoroughly mixing all the cores together. Compost samples were taken from each heap as described in section 3.1.2. The soil and compost samples were then air-dried at 40°C to constant weight and sieved through a 2mm sieve.

On 23.9.02, further soil and compost samples were taken. This time the soil from each block was sampled separately by taking and mixing five 25 cm cores from each block. A different sampling method was also used for the composts as described in section 3.2. Two sub-samples of each sample were taken and used in their moist state for a mineralisable nitrogen test as described below. The rest of each sample was passed through a 2 mm sieve, air-dried for over 24 hours to constant weight at 50°C, then ring-ground for 1 minute, ready to be sampled for total N and P analysis.

Laboratory soil and compost tests

Samples of soil and compost taken on 19.8.02 were analysed for total N and C by LECO FP2000 dry combustion furnace, similar to that described by Kowalenko (2001). The LECO FP2000 uses dry combustion at 1050 °C, with infrared determination for C and thermal conductivity detection for N. Sample weights of 100-300 mg were used.

Sub-samples of the same soil and compost samples taken on 19.8.02 were also used for the following methods of analysis, all described by Blakemore et al. (1987), developed from methods as referenced:

Olsen P: (Olsen et al., 1954)

Exchangeable cations and CEC: (Brown, 1943)

pH: in water (Blakemore et al., 1987)

bulk density.

Kjeldahl digestion for total N and P analysis (McKenzie and Wallace, 1954), followed by analysis by Technicon Auto-analyser, and a phosphate retention test (Saunders 1965) were conducted on sub-samples of the soil and compost sampled on 23.9.02. For each extraction and analysis two Wageningen standard soil samples and 2 digest blanks (acid only) were also included and the results checked against the known quantities in those samples.

Soil and compost mineralisable N test

The soil and compost samples taken on 23.9.02 were tested for mineralisable N by the anaerobic incubation method of Waring and Bremner (1964). The wet soil and composts were passed through a 2 mm sieve and mixed well. Two sub-samples (5 g) of each soil and compost sample were weighed into beakers then dried in an oven at 105°C to constant weight. Fresh and oven-dried weights were used to calculate the soil and compost moisture contents. Paired sub-samples (5 g) of each soil and compost sample were weighed into 50mL centrifuge tubes. To one set of samples 30 mL 2M KCl was added, and the tubes shaken for 1 hour, centrifuged at 8000 rpm for 3 minutes then filtered through Whatman's 41 paper into vials. The vials were frozen for later analysis. To the other set of samples, 20 mL of deionised water was added and the tubes incubated at 35-40°C for 2 weeks before extraction with 10 mL 3M KCl as above. Ammonium and nitrate-N concentrations in the KCl-filtrates were determined using a Technicon II auto-analyser (Twine and Williams, 1971; Technicon, 1976).

Calculation of N content of soil and composts and how much compost and fertiliser to apply

Results from the soil mineralisable N test and the compost LECO total nitrogen measurements were used to estimate how much compost and fertilisers to apply to the appropriate trial plots. The calculations are shown in section 4.1.2. Diammonium phosphate and calcium ammonium nitrate were used to supply the calculated required nitrogen and phosphorus after consultation with the fertiliser supplier. No potassium was applied because the soil already contained more than the recommended level.

3.4 Trial design and treatments

A factorial design was chosen for the trial to effectively provide more replicates of each of the main factors under investigation and increase the precision of estimation of variance (σ^2) and the likelihood of obtaining a significant difference between means. A factorial design also enables interactions between the variables to be seen investigated. Mead (1988) provided a formula for calculating the number of replications required in order to have a good chance of showing statistically significant differences between treatments at the 5% level. Assuming equal replication of treatments,

$\sqrt{(2\sigma^2 / n)} \leq (d) / 3$

where replication per treatment, n = N (no. of units) / t (no. of treatments)
d = standard error of difference between 2 treatment means ($\mu_1 - \mu_2$)

The likely variance, σ^2 and the differences between means have to be estimated in order to estimate n. However, few similar trials have been carried out and they do not provide sufficient statistical information. If the likely true difference between population means is low, possibly below 10%, there is a high probability that the treatment difference between means measured will be even lower than the population difference, and will not be significant at the 5% level.

An experiment by Warman and Havard (1997) compared organic and conventional systems of growing cabbages and carrots in the field over 3 years. They did not find many statistically significant differences. Most of the differences in mean mineral content

were less than 10%, with standard deviations for each mean value under 4 g/kg dry-weight for Ca, Mg and S in the cabbages, and between 2 – 4 mg/kg dry-weight for most of the micronutrient values. A comparison of organic and mineral nitrogen fertilisation on nutritive contents of butterhead lettuce found about 16% difference in iron content and over 100% difference in nitrate content (Lairon et al., 1984). About 20-100% differences were reported from earlier evaluations of amino acid content with and without the biodynamic silica spray with no record of statistical analysis. Comparison of leaf amino acid content of rice plants with and without silica in the nutrient solution found 65% difference (Watanabe *et al*, 2001). None of these researchers reported variance in their trials.

From the research discussed above, it is hard to predict likely differences, but a coefficient of variation (σ^2) in the range of 10 – 15 and differences (d) of the order of over 15% appears possible.

Then:

$$\sqrt{(2\sigma^2/n)} \leq (d)/3 \text{ is equivalent to } 2\sigma^2(3^2/d^2) \leq n$$

$$2*10^2(3^2/15^2)$$

$$n \text{ is then} \geq 8$$

To compare 3 soil amendment treatments, each with and without biodynamic fieldsprays, gives 6 treatments. If each treatment is replicated 4 times, n is 8 for the comparison between soil amendments, and 12 for comparing sprays and no sprays. which means that differences between spray and amendment treatments greater than 15% of the mean value are likely to be detected as significant at the 5% level.

Table 3.3 Trial treatment design and treatments codes

Spray application	**No amendments**	**Chemical fertilisers**	**Compost**
No sprays	Control (Ctrl)	DAPCAN* (DC)	Organic (Org)
Plot Nos.	1d,2c,3f,4c	1a,2d,3d,4b	1f,2e,3e,4a
Sprays	Control + sprays (C+sp)	DAPCAN + sprays (DC+sp)	Biodynamic (Bd)
Plot Nos.	1b,2f,3b,4d	1c,2b,3a,4e	1e,2a,3c,4f

*Diammonium phosphate and calcium ammonium nitrate

3.5 Field-site plot preparation

During August 2002 most plants and kikuyu grass roots were removed from 4 blocks in a permanent pasture on volcanic sandy loam soil that had been organically managed for at least 15 years. The site had been grazed by dairy cows from a neighbouring non-organic farm for the past few years. The blocks were lightly forked over and covered with a grass-mowing mulch to reduce moisture loss from the soil. Twenty four plots 1.4 m*1.4 m (6 plots per block) were marked with pegs. Plots in each block were adjacent except for 20cm paths between each. A balance had to be drawn between having plots as close as possible to reduce variations in soil composition and keeping plots far enough apart to avoid spread of spray influence. Opinion on distance of spray influence varies, but Bloksma (2002) considers that it extends up to 1m from area sprayed.

Soil uniformity test

On 13.9.02, mulch was removed from the plots, and the soil raked and sown with mustard seed. The seed was sown as evenly as possible by weighing out an equal quantity for each plot, then dividing each portion into 4 and hand-sowing - twice east-west and twice north-south.

On 4-5.10.02, when mustard plants were about 10-15cm high, they were harvested from an area 69x76 cm in the centre of each plot, using a 2cm high quadrant and hand shears. The harvested plant fresh weight was determined for each of the 24 plots. The un-harvested areas of each plot were then mown to the same height.

Plate 3.2 Mustard harvest

Lime application

As initial soil tests indicated that the soil pH was 5.7, which is lower than the 6.3 – 7.3 reported desired level for lettuces (Hort Research, 1995), and the exchangeable calcium level of the soil was at the lower end of the recommended range, a dressing of 0.1 kg lime/m^2 was applied to all plots on 7.10.02. This quantity was less than the quantity required to raise pH to an ideal level for the reasons discussed in section 4.1

Randomisation of trial plots

Treatments were allocated to the plots in each of 4 blocks using random number tables.

Incorporation of composts and fertiliser

On 19.10.02 biodynamic and organic composts were applied to plots 1e, 2a, 3c, 4f and 1f, 2e, 3e, 4a, respectively, at the rate of 16.3 kg/plot, to provide 150 kgN/m2 – the recommended N application rate required by lettuces (HortResearch, 1995) as calculated from soil and compost N contents, described in section 4.1.2.

On 20.10.02 the soil on all plots was lightly turned with a fork. On 23.10.02 the fertiliser treatment plots 1a,2d,3d,4b,1c,2b,3a,4e received dressings of 3.75g diammonium phosphate (20%P, 18%N) and 79g calcium ammonium nitrate (27%N), to supply equivalent quantities of nitrogen and phosphorus, and roughly equivalent calcium, to the compost treatments. The soil on all plots was lightly forked again.

3.6 Lettuce seedling preparation and planting

On 21.9.02 the lettuces (Lactuca sativa L. cultivar Canasta) were sown. On 7-8.10.02 seedlings were pricked out into large trays containing half soil and half compost. They were given 2 sprays of a liquid seaweed and vermicast mixture to improve growth.

On 25-26.10.02 the lettuce plants were planted out, ensuring that good plants of as similar size as possible were planted in the centre of each plot, as these would be used for sampling. Spacing was 23 cm apart within and between rows. All centre plants had 3-4 true leaves at planting. Outside row plants had 2-5 leaves.

Plate 3.3 Planting lettuces

3.7 Spraying of biodynamic field-sprays

On the evening of 26.10.02, during a descending phase of the moon, the horn-manure preparation (500) was applied to the plots 1b,2f,3b,4d,1c,2b,3a,4e,1e,2a,3c,4f, that were to receive field-sprays. Twenty-five grams of the preparation (obtained from the Bio Dynamic Association) was stirred in about 12 L warm rainwater for 1 hour then applied to the plot soil surfaces in large drops with a brush (method described by Procter 1996)

The horn-silica preparation (501) was applied to the plots that received field-sprays on the morning of 30.10.02, when the moon was ascending and in the constellation of Cancer. One gram of the preparation was stirred in 10 litres of warm rainwater for 1 hour, in the same way as for the horn-manure preparation. It was then sprayed up in the air over the plots, as fine droplets, as much as was possible without allowing it to spread to neighbouring plots. This spray was repeated on 8.11.02, when the moon was in the constellation of Scorpio, as recommended by Thun (2001). Thun recommended a series of 3 sprays at approximately 10 days intervals, when the moon is in each of the constellations appropriate to the particular crop plant. Lettuce is a leafy vegetable, which according to Thun (2001) is most influenced by the constellations of Cancer, Pisces and Scorpio. The spray was not repeated a third time because the weather was so

sunny and dry and the plants in 2 of the 3 treatments receiving field-sprays were showing stress effects of excess light (effects described in Section 4.2).

On 2.12.02, shortly before final harvest, a further field-spray of horn- manure was applied to the appropriate plots and on 3.12.02 horn silica was sprayed.

3.8 Weather records and Irrigation

Weather

The following weather records were kept for each day of the trial:

- Rainfall – from a rain-gauge near to the trial plots.
- Maximum and minimum temperature using a thermometer hung in shade.
- Evaporation, by weighing a 17cm square ice-cream container of water each day.
- Windspeed and direction: initially from a weather station in Te Puke and later from the newspaper.
- Sunshine hours: from the newspaper, taken at Tauranga airport.

The windspeed recordings from the Te Puke weather station were discarded after realizing that they were generally much lower than those experienced on the trial site, and those recorded for Tauranga and Te Puke in the newspaper. The trial site was exposed to most wind directions, whereas the Te Puke site must have been well sheltered.

Irrigation

Irrigation was applied about once a week, when the soil dried out and plants started to show signs of water stress. The first time water was applied (on 31 October) 250 mLs per plant per plant was given to each plant using a measuring jug. This method was not found to be satisfactory as the water tended to run off rather than soaking in the dry soil, even when the water was applied in 3 smaller doses. Subsequent irrigation was done by estimating the time taken for a particular volume of water to flow from the hosepipe using a calibrated bucket, then spraying a plot as evenly as possible for this length of time. The volume of water required was calculated by taking ten 15 cm deep soil cores from sites distributed over all the blocks, weighing them, air drying, reweighing and

calculating the depth of water per surface area then reweighing a week later. This calculation was according to the equation:

Irrigation water at time (t) =

(mm water in soil (0-15 cm) at field capacity – mm water in soil (0-15 cm) at time t.

3.9 Calendar of Events

The timing of trial preparation and measurement dates are shown in Tables 3.4 and 3.5.

Table 3.4 Trial preparation and treatment dates

Activity	Date	Days from planting
Compost		
Started building	15.4.02	
Finished, added compost preps	29.5.02	
Turned	9.6.02, 17.8.02, 12.9.02, 18.9.02	
More compost preps added	27.8.02	
Plot preparation		
Prepared plots	10-30.8.02	
Sowed mustard	13.9.02	
Harvested mustard	4 -5.10.02	-20-21
Lime dressing	7.10.02	-18
added compost,	19.10.02	-6
forked over plots	20.10.02	-5
added fertiliser	23.10.02	-2
Lettuce preparation		
Sown	21.9.02	-34
pricked out	7-8.10.02	-17-18
Sprayed with seaweed, vermicast	8.10.02, 10.02	
Planted	24-25.02	0
Fieldspray application		
Horn-manure (500)	26.10.02, 2.12.02	1, 38
Horn-silica (501)	30.10.02, 8.11.02, 3.12.02	5, 9, 39

Table 3.5 Calendar of Trial measurement dates

Date	30 O	2 N	9 N	11 N	12 N	13 N	15 N	22 N	30 N	1 D	3 D	4 D	5 D	9 D	10 D	11 D	12 D
Days from planting	5	8	15	17	18	19	21	28	36	37	39	40	41	45	46	47	48
Visual observations	✔	✔	✔			✔	✔		✔						✔		
Weigh plants						✔		✔								✔	✔
Root profiles					✔										✔		
Root lengths						✔		✔								✔	✔
Leaf length								✔									
Leaf number						✔		✔									
Leaf area				✔						✔				✔			
Soil sampled								✔									✔
Soil respiration								✔									
Leaf sap nitrate			✔				✔				✔	✔	✔	✔			
Leaf sap Brix											✔	✔	✔	✔			
Leaf sap dynamolysis													✔	✔			
Sensory														✔			
Mycorrhizae																	✔
Self disintegration																	✔

3.10 Visual observations

The lettuces in each plot were assessed visually each week from planting and scored on vitality, colour, size. Leaf numbers per plant were counted at the earlier observations and any leaf damage was recorded. Observations of leaves, roots and soils were also recorded at weighing and sap sampling times. In addition the roots of harvested plants were examined.

3.11 Plant growth measurement

Plant weights and lengths

On 13.11.02, 22.11.02 and 11-12.12.02, whole plants were harvested, roots washed, the roots cut from the shoots and the fresh weight of each recorded. Root length was

measured with a ruler and number of leaves counted. On 22.11.02 the length of the longest leaf of each plant was measured.

The roots and shoots were placed separately in paper bags for drying and subsequent re-weighing and analysis. At the first harvest, on 13.11.02, the leaves were air-dried on racks outside before being put into paper bags. However, even on a relatively wind-free day in a sheltered place, some leaves blew from one sample to another. At the subsequent harvests, on 22.11.02 and 11-12.12.02, the leaves were placed straight into paper bags and taken to the laboratory for oven drying. Leaves and roots were dried at 70°C to constant weight.

Root profiles

On 12.11.02 an assessment of the extent of the plant root systems was made by excavating soil 2.5 cm from the leaf edge of a plant in each plot, clearing soil away from around exposed roots, then placing a 15 cm x 15 cm piece of plastic square mesh netting against the exposed soil face. The number of roots covered by the mesh was counted. On 10.12.02 this process was repeated, and in addition, the numbers in each quarter of the whole square were recorded separately.

Canopy cover

On 11.11.02, 1.12.02 and 9.12.02, an assessment of total canopy cover was made by photographing the central plants in each plot and including a lid of measured diameter in each photo. The diameters of the largest and the smallest plants in each photo were measured and actual diameter and hence canopy area of the plants calculated by reference to the lid size.

3.12 Plant preparation for final analyses

At the last harvest on 11.12.02, two plants from each plot were harvested and roots washed, cut from heads and weighed. After recording the fresh weight of heads, they were stored cool in plastic bags until they could be processed and dried in the laboratory. A third plant from each plot was harvested on 12.12.02 and transported with its

surrounding soil to the laboratory in plastic containers. The soil material from around the roots of this plant was used to study rhizosphere respiration and mycorrhizal infection.

Soil sampling and root sampling for mycorrhizal colonisation

At the laboratory, samples of soil from the rhizosphere of the plants still in soil were taken for mycorrizal observation, then roots were washed. Root washing took some time as soil was adhering very closely to them, and it was attempted to retain as much as possible of the roots with as little as possible of soil still attached. Samples of fine roots were taken (2 samples per plant) and put in vials of water, and later in ethyl alcohol, for later examination of mycorrhizal colonisation.

Leaf sampling and drying

On 12.12.02 leaves and roots of the 3 final harvest plants were weighed and measured as for previous harvests. The leaves of lettuce heads were then removed one by one. Many had to be washed in deionised water to remove soil. They then were dried by carefully patting with paper towels. Every alternate leaf was put in a weighed plastic bag and weighed and put in a deep-freeze to freeze as quickly as possible for later antioxidant analysis. The other alternate leaves were put in weighed paper bags, then weighed again and oven-dried. The roots were placed in separate paper bags and dried. As this whole process took some time, plants were kept as hydrated as possible by watering the soil with equal quantities of deionised water. Plants were processed in order of block so that no particular treatment was favoured by earlier processing.

3.13 Sap Nitrate and Brix measurements

Sap Nitrates

On 9, 15.11.02 and 3, 4, 5, 9.12.02, lettuce leaf sap was tested for nitrate concentration using Merck nitrate (Mer 110020.0001) test strips. One to two leaves (youngest mature leaves) were taken from each of 2 plants per plot, washed in rainwater, and water shaken off. They were cut up finely with a knife, then mashed with a pestle and mortar and placed in a square of thin plastic, cut from a plastic bag. The plastic was gathered up tightly and a few pinholes made in it, then squeezed. The juice was collected in a small medicine measure and the quantity checked with a small calibrated pipette. The juice

was then diluted 1:20 with distilled water. A Merck strip was dipped briefly in the solution, excess shaken off, then the colour was read against the colour code provided by Merck after one minute. Samples of leaves and diluted sap were collected and frozen for possible later laboratory analysis.

Test plants were marked and the same plants used from each plot each time. Several test plants were needed to enable several sampling over a short time – 2 from the central 9 plants and 2 from the inner guard row for each plot. For most measurement times, only plants from 2 blocks were tested because leaf NO_3 concentrations are sensitive to solar radiation and the time lapse between the first and last plants to be tested could have made considerable difference to the results. In addition, for the initial tests it would have meant removing so much material from the test plants that their subsequent growth would have been affected

Timing of measurements to look for silica-spray and diurnal effects on sap nitrate content

On 9.11.02 the first cell sap nitrate measurements were made, just before the second spraying of horn-silica spray on sprayed plots, to provide a baseline to compare subsequent results with. Plants were tested again a week later to look for possible effects of the spray. At these testing times it was difficult to obtain sufficient sap for accurate measurement because leaves were still small and plants fairly dry. At these two early testing times, leaves were collected over a period of about 2 hours, from 3 plots at a time to ensure they were all fresh when tested.

On 3, 4 and 5.12.02 leaf cell sap nitrates were measured at different times of the day to look for effects of the third horn-silica spray on nitrate concentration, and diurnal effects. At these measurement times, leaves were collected in order of block so that they were all picked at the same time.

On 9.12.02 final cell sap nitrate measurements were made, six days after the third horn-silica spraying. Undiluted sap from plants from each plot was collected in vials and frozen for later laboratory analysis.

Brix Measurements

On 3.12.02 onwards, at the same time as nitrate content measurements, leaf cell sap was tested for soluble solids content using a Brix refractometer. Two drops of the undiluted sap were placed on the screen and the scale underneath read by holding up to the light. Brix measurements could not be made at earlier nitrate testing times because leaves were too small and there was insufficient sap.

3.14 Capillary dynamolysis

On 5 and 9.12.02, 5 mL lettuce leaf sap was diluted with 5 mL distilled water, and eluted into filter paper. The filter paper was prepared in the same way and the atmosphere kept humid as described for compost testing in section 3.2.

3.15 Soil sampling

On 7.10.02, soil samples were taken from each plot by taking five 25 cm diameter, 15cm deep cores randomly over each plot. The samples were air-dried and saved for possible analysis. On 22.11.02, further samples were taken again by shaking off the rhizosphere soil from harvested plant roots. Some of each sample was used fresh in a soil respiration test and the rest was air-dried for future analysis.

3.16 Leaf nutrient content analysis

The oven-dried leaves of plants harvested on 22.11.02 and 11-12.12.02 were dried at 70°C to constant weight then ground up and the following tests carried out on the dry plant material:

Kjeldahl digestion for total N and P analysis (McKenzie and Wallace, 1954) followed by analysis by Technicon Auto-analyser (Technicon, 1976; Twine and Williams, 1971)
Nitric acid digestion for cation and trace element analysis (Westerman, 1990).
Acetic acid extraction for nitrate analysis (Prasad and Spiers, 1984).

The freeze-dried leaves from the third plant of each harvest were also weighed and ground and the powder tested for antioxidants by the Ferric Reducing Ability of Plasma (FRAP) assay (Benzie and Strain, 1996).

3.17 AM fungi colonisation assessment

Preparation, staining and slide mounting of root samples

On 12.12.02 the amalgamated 2 samples of finer roots from one plant per plot were stored in 90% ethyl alcohol solution in the cool room. They were they taken out, washed with deionised water and stained following the procedure used by Turner, 2001, (modified from Koske and Gemma, 1989). To first clear dark coloured, phenolic compounds, the roots were placed in a test tube containing 15 mL of a 2.5% aqueous solution of KOH, then heated in a water bath kept at 80 - 90°C for 45 minutes, rinsed again and transferred to 20 mL of 1% HCl solution. They were kept in the dark in this solution for 24 hours.

An acidic glycerol solution was made by mixing 400 mL glycerol with 360 mL water and 40 mL of 1% HCl. A staining solution was made by placing 0.2g of Trypan blue powder in 20mL of water, bringing it to boiling point, and filtering through a Whatmans 41 filter paper into 400mL of the acidic glycerol solution. The samples were placed in test tubes containing 15 mL of the staining solution and kept in a water bath at 85 - 95°C for 30 minutes. The samples were then placed in 15 mL of acidic glycerol solution, in the dark, for 2-3 days to remove excess stain. Pieces of root 1-2cm long were then taken out carefully, teased out and placed in straight lines on slides and covered by cover slips (Brundrett et al., 1996) and examined for mycorrhizal hyphae, arbuscles and vesicles. About 12 cm of root was placed on each slide. Two slides were made and assessed for each root sample. The roots were handled using fine forceps, but it was found difficult not to damage them. The method recommended by Graves (2002) – using a pipette to manipulate roots in solution was not found to be any better.

Microscopic examination of samples

When mycorrhizal fungi had been identified and distinguished from other types of fungal hyphae, a visual estimate of the amount of infection was carried out at x 200

magnification, using a cross-hair eye-piece, according to the method recommended by Brundrett et al. (1996). Only sections of root that were intact and stained properly (ie the central xylem vessels were still present and had taken up the stain) were used. As in most samples, some root pieces were not suitable for assessment, the best intact and stained approximately 8cm of length was selected for estimation. The microscope view was moved along the root, approximately every 10 mm, attempting to make a random selection of crosshair position each time, then a recording was made at each intersect of whether it passed through root only, hyphae, hyphae and arbuscles or hyphae and vesicle. This was repeated at least 60 times for each slide, except when there was insufficient qualifying material on the slide.

Guidelines used for distinguishing mycorrhizal fungal hyphae from other fungal hyphae (Rilllig, 2002):

- Mycorrhizal hyphae are bendy and knobby, they do not grow in straight lines
- Mycorrhizae are stained blue, not brown
- Mycorrhizal hyphae do not have regular cell divisions
- Mycorrhizal hyphae generally have dichotomous branching only, not multiple branching
- Vesicles are darkly stained
- An arbuscle or vesicle like structure is only likely to be so if there is a hypha associated with it.

3.18 Sensory evaluation

On 6.12.02 a preliminary questionnaire was designed and tested on 4 assessors. First the assessors were asked to taste the lettuce samples and answer all the questions on the questionnaire form. A group discussion was then held with these assessors to discuss possible changes and additions to make descriptor terms as appropriate as possible, following some of the official recommendations for sensory analysis (International Standards Organisation, 1994). The questionnaire was amended as appropriate to a final version (Appendix 2). On 9.12.02, samples of young mature leaves were taken from lettuces of each treatment from 2 blocks and tested in a sensory evaluation by 10 volunteers. Before the evaluation the purpose and details of the trial were explained to

the volunteers and they were given extreme bitter and sweet tasting leaves to indicate what was being looked for. Half of the volunteers were given the samples to test in reverse order.

3.19 Self disintegration test

On 12.12.02 three leaves (1 outer, 1 middle and 1 inner) from one lettuce from each plot were washed, dried, weighed and put in an unsealed plastic bag in a closed drawer at room temperature. On 20.12.02 and 6.1.03 the leaves were re-weighed and scored for decay. Moisture loss was calculated.

3.20 Statistical analysis

Results were analysed for variance by SAS. Treatment means were compared by t- test and by Tukey's multiple comparison procedure. Residuals were examined for normality and constant variance. Where necessary, if any failed to satisfy those tests the data was transformed. Compost treatment data were not included in the grouped spray v no spray analysis because different composts were used for organic and biodynamic treatments. The grouped spray and no spray data were analysed using means of Control and DAPCAN treatments only.

4 FIELD TRIAL RESULTS AND DISCUSSION

4.1. Soil and Compost Analyses

4.1.1 Soil analysis

The soil and composts used for the trial were tested in order to characterize their quality, plant nutrient contents and suitability for growing lettuces, and to determine how much nitrogen should be supplied in composts and fertiliser.

Soil Type

The interpretation of soil analysis requires an understanding of the soil type and parent material. The soil used for the trial was a Te Puke Series sandy loam, classed as Typic Orthic Allophanic (Plate 4.1.1). General characteristics of such soils (Molloy, 1998) are low bulk density, high water holding capacity, high P retention, high organic matter content (carbon), with high C: N ratio, no significant trace element deficiencies, and highly porous free draining B and C horizons.

Plate 4.1.1 Soil profile near trial site

Laboratory analysis results compared with lettuce requirements and MAF Quicktest averages

Results of chemical analysis of a single bulked soil sample composed of five 25 mm diameter soil cores (0-15 cm depth), taken on 19.8.02 (9½ weeks before lettuces were planted), are shown in Table 4.1.1 and 4.1.2.

Table 4.1.1 Initial analysis of Te Puke sandy loam topsoil at the trial-site and recommended levels for lettuces

Test	Trial-site	HortResearch recommended levels+	Weir and Cresswell++
Total N% (Leco)	0.47*		
Total C %(Leco)	5.29		
pH	5.7	6.3-7.3	
Olsen P (µgP/g air-dry)	50.5	46 – 55	
Sulphate (µgS/g air-dry)	18.4		
Soil bulk density (g/cm^3)	0.81		0.6 – 1.0
CEC (me/100g air-dry)	20		12 - 25
Potassium (me/100g air-dry)	1.57	0.8 – 1.0	0.6 – 1.0
Calcium (me/100g air-dry)	6.4		6.0 – 12.0
Magnesium (me/100g air-dry)	1.67		1.0 – 3.0
Sodium (me/100g air-dry)	0.09		0 – 0.5

* Subsequent analysis reassessed this level as about 0.64% - see Table 4.1.3 and comments
+ HortResearch 1995. Fertiliser Recommendations for Horticultural Crops. http://www.hortnet.co.nz/publications/guides/fertmanual/fertinf.htm
++ Weir, R.G.and Cresswell, G.C. 1995. Plant Nutrient Disorders 3. Vegetable crops. Inkata Press

Table 4.1.2 Soil analysis results compared with MAF Quicktest results from a kiwifruit orchard survey of similar soil types.

	pH	P µgP/cm^3	K	Mg	Ca
Trial area	5.7	50.5	20	31	6
Oropi, Awakeri and Paengaroa series*	6.1(5.2-7.5)	39 (3-181)	7 (1-27)	16 (4-52)	7 (2-18)

* Bay of Plenty Kaharoa ash soils from Summary of 1079 MAF Quicktest results (mean and range) received at Ruakura Research Centre from Nov. 1981 to May 1984 in Smith GS, Asher CJ, Clark CJ. Kiwifruit Nutrition diagnosis of nutritional disorders. HortResearch Publication HortNet

Comparison with average MAF Quicktest for the same soil type (Table 4.1.2) shows that phosphorus and magnesium levels were slightly higher than average, potassium much higher than average and pH and calcium slightly lower than average. These differences could partly reflect the difference between soil test levels under kiwifruit orchards and under the pasture recently present at the trial site.

Comparison of results with levels recommended for lettuces by HortResearch (1995) and Weir and Cresswell (1995) reveals that the pH was below recommended level, but all other results were within the recommended range. These results are discussed in more detail later in this section. The soil test nutrient levels only provide a rough guide as to suitability of the soil for growing lettuces, particularly under an organic system in which the speed and nature of nutrient cycling by soil organisms has a large influence on nutrient provision to the crop (Scheller, 1996).

Total Nitrogen content

Results of analysis of soil samples from each block (2 samples/block) prepared for the trial, taken on 23.9.02 (1 month before planting) for total nitrogen and phosphorus by Kjeldahl digestion (McKenzie and Wallace, 1954) and Technicon Auto-analyser are shown in Table 4.1.3.

Table 4.1.3 Total nitrogen in Te Puke sandy loam topsoil, at the trial-site, measured by Kjeldahl digestion and Autoanalyser and by LECO combustion

	Kjeldahl		LECO	
Sample	N%	Mean	N%	Mean
Block 1 a	**0.73**		**0.61**	
b	**0.77**	**0.75**	**0.62**	**0.62**
Block 2 a	**0.61**		**0.61**	
b	**0.63**	**0.62**	**0.65**	**0.63**
Block 3 a	**0.61**		**0.64**	
b	**0.66**	**0.64**	**0.64**	**0.64**
Block 4 a	**0.67**		**0.65**	
b	**0.68**	**0.67**	**0.68**	**0.67**

The total nitrogen content of soil samples as measured after Kjeldahl digestion (0.62 – 0.75%) (Table 4.1.3) was considerably higher than that earlier measured by the LECO method (0.47%) (Table 4.1.1). According to most researchers, the methods should have given similar results. For example, Kowalenko (2001) found that LECO dry combustion measurements of a variety of soil samples were slightly lower but proportional to Kjeldahl measurements of total nitrogen. Further analysis by LECO combustion was therefore undertaken of samples of soil taken on 7.10.02, 18 days before transplanting lettuce plants. This time results were similar to those for Kjeldahl digestion, except for

Block 1 (Table 4.1.3). Reasons for the difference between earlier and later LECO results are likely to be that the soil samples were not well mixed prior to the initial analysis (Table 4.1.1). For the second analysis (Table 4.1.3) more field replicate cores were taken and they were well mixed prior to analysis.

Total Carbon content

The total carbon, 5.29% (Table 4.1.1) to 7.1% (Table 4.1.4), is higher than the 4.03% in Te Puke sandy loam soil from a Te Puke kiwifruit orchard found by Sparling and Rijkse (1998). However, higher carbon content under dairy pasture compared to a kiwifruit orchard is not surprising. Using the usual carbon to organic matter conversion factor of 1.72, gives a soil organic matter content of 9.1 – 12.2%, although this is only a rough guide as appropriate conversion factors for different soils vary between 1.3 and 2.0 (Baldcock and Skjemstad, 1999). The soil organic matter level is appropriate for soil that has been under permanent pasture, and higher than it would be for a soil that has been under cropping for some years, which is usually the case in commercial lettuce production. Cotching et al. (1979) found that the organic C declined by 40% over 8 years of continuous maize cropping on a similar soil at Horotiu.

The carbon measured by dry combustion in the LECO induction furnace would be virtually all organic carbon. Determination of relative proportions of carbon in stable humus, the microbial biomass and in partially decomposed material and low molecular weight compounds, could have been helpful in assessing the suitability of the soil to produce well under an organic system, as discussed in the literature review, section 2.5, but was beyond the scope of this research. There would be a high content of grass roots and living biota from the recently removed pasture cover. A further consideration is that this level of organic matter could provide more plant available nutrients than in some other soils because of its allophanic clay content. Microbial growth has been shown to be more efficient in the presence of allophanic clays (Paul, 1984).

The total carbon content was used to determine ratios with other elements, which provides an indication of whether nutrients will be made available for crop growth by the microbial biomass or whether they will be immobilised.

Carbon to nitrogen ratio

Soil samples taken 18 days before lettuce planting, from each block used in the trial, had C:N ratios ranging between 10.0 – 10.6 as measured by LECO combustion (Table 4.1.4). These estimates can be compared with the C: N ratio for some Waikato pasture soils measured by Ghani et al. (1996), which varied from 9.99 – 10.9 and for a Te Puke kiwifruit orchard measured by Sparling and Rijkse (1998) of 12. Although the Te Puke sampling site was a similar soil closer to my trial site, there would be considerably different management effects between a kiwifruit orchard and dairy cow pasture. According to the Landcare SINDI guide (2002) a C: N of 10 is ample for plant demands. However, the rate of nitrogen mineralisation at this C: N ratio could be suited more to long-term permanent pasture rather than short-term crop demands.

Table 4.1.4 Carbon (C) and nitrogen (N) content and C: N ratio of Te Puke sandy loam topsoil from each trial block as measured by LECO combustion.

Sample	Carbon %	Nitrogen %	C:N
1a	6.4	0.61	10.5
1b	6.41	0.62	10.3
1b	6.39	0.61	10.5
2a	6.67	0.65	10.3
2b	6.72	0.64	10.5
3a	6.48	0.64	10.1
3b	6.53	0.65	10.0
4a	7.09	0.68	10.4
4a	7.06	0.68	10.4
4b	7.09	0.67	10.6

The C: N of 10 – 10.6 in the trial soil would indicate a slightly fungi dominant microbial biomass, which is less suited to lettuce production than a bacterial dominated biomass with lower C:N according to Ingham (2001). She asserts that a low C:N generally indicates more bacteria than fungi, and the ratio of fungi to bacteria for healthy growth of leafy vegetable crops (such as lettuce) should be 0.5 – 1.0. However, the relative numbers of bacteria and fungi also depends on the soil pH and the nature of the soil organic matter, as bacteria require a higher soil pH and more easily decomposable material than do fungi (Baath, 1998, Grayston et al., 2001). A further consideration is that the ideal C: N and the relative numbers of bacteria and fungi could be different under organic and soluble fertiliser systems. Gunapala and Scow (1998) found significantly lower C:N for organic compared to conventionally managed soil and

concluded that this is indicative of a situation where fungi would be less active relative to bacteria and that bacteria may be more important than fungi in organic compared to conventional management.

Mineralisable nitrogen

Results of a mineralisable nitrogen test (method of Keeney and Bremner, 1966), conducted to enable estimation of how much nitrogen was likely to be available to the lettuces during the trial, and how much extra nitrogen should be applied, are shown in Table 4.1.5.

Table 4.1.5 Mineralisable nitrogen in the Te Puke sandy loam topsoil at trial-site

	Moisture content %	Unincubated NH_4	Unincubated NO_3 µgN/g dry soil	Incubated NH_4	Incubated NO_3	Unincubated NO_3 µgN/g wet soil
Soil block 1	25.95	8.62	49.65	24.81	<0.58	12.88
Soil block 2	22.93	17.41	197.04	17.63	2.00	45.18
Soil block 3	23.09	14.15	143.02	34.98	<0.58	33.02
Soil block 4	21.9	15.99	103.36	14.31	1.86	22.64

The lower quantity of nitrogen found in the incubated soil indicates that there was net immobilisation of nitrogen during the incubation. The high nitrate values in the initial samples indicate that considerable mineralisation had occurred in the field soil prior to air drying. The subsequent lower nitrate and ammonium concentrations in the incubated samples suggested that immobilisation of N and perhaps denitrification occurred during incubation (Roper and Ophel-Keller, 1997). The results suggest that further decomposition of residues in the field soil may net immobilise nitrogen in early stages of the trial. Possibly the presence of undecomposed roots from the recently cleared pasture could have led to net immobilisation of N in the trial soils. However, Paul and Clark (1989) wrote that net mineralisation should occur when the C: N of added crop residues is less than 25:1. Although some bacteria have a C: N of 4:1, some fungi have a C: N of 15:1 and can therefore liberate nitrogen from substrates with higher C: N. Microbial C is generally only 1.5-3% of the soil organic matter (Sparling, 1997).

Despite the lack of net mineralisation of N during the anaerobic incubation, the overall soil C: N ratio of 7.9 – 11.25 indicates that net mineralisation should occur under field cropping conditions. Soil organic matter is dominantly stable humic materials with a

small amount of more recently added organic material, with a wider C: N ratio. The latter material would have a large effect on microbial growth rates and the short term N dynamics.

As discussed in Section 2.5, a series of complex interrelated microbial and plant processes influence whether net mineralisation occurs under field conditions. Many of these processes are not represented in a laboratory anaerobic mineralisation test. This discussion is resumed in Section 4.5.1 which discusses N uptake by the lettuce crop.

Phosphorus content and availability

Table 4.1.6 Total Phosphorus[1] content of Te Puke sandy loam topsoil at trial site

Sample	P%	Mean	% P retention
block1 a	0.24		
b	0.24	0.24	56
block 2 a	0.20		
b	0.20	0.2	60
block 3 a	0.28		
b	0.22	0.25	57
block 4 a	0.46		
b	0.47	0.47	55

[1] Determined after Kjeldahl digest

Comparison of the total P (0.23% or 2300 µg P/g soil, mean of blocks 1 – 3, Table 4.1.6) with the Olsen P results of 50.5 µg/g in the first, composite, soil sample, shows that less than 0.25% of the total P was recovered by the Olsen P test and therefore likely to be available for plant uptake. Possibly about half of this Olsen extractable P could have been from the soil biomass which was killed by drying the soil for testing. Sparling et al. (1985) found that up to 74% of the P measured by the Olsen P test could be from the microbial biomass, particularly in soils with more than 2% organic C and less than 20 µg/g Olsen P. The P is likely to be predominantly in organic form as the soil is sandy (Mullen, 1998). However the availability for plant uptake of this organic P would depend on the C: P ratio and the amount and type of soil biological activity (see discussion in Section 2.5). As the trial soil appears to have a C: P of 23:1 and net immobilisation generally occurs at a C: P of >200:1, net immobilisation is very unlikely.

The inorganic P component measured by the Olsen P test is the readily exchangeable fraction. The medium phosphate retention test results (55 – 60%) indicate that most inorganic P added would be rapidly adsorbed by the trial soil (Blakemore et al.., 1987). HortResearch recommendations take the soil's phosphate retention value into account when stating the target Olsen P level for optimum lettuce growth. The trial soil Olsen-P result of 50.5 µg/g, considered with the phosphate retention estimate of 57%, is within the Olsen P range 46-55 µg/g adequate for lettuce grown on soils of medium phosphate retention as recommended by HortResearch (1995). However, the availability to plants of the inorganic P also partly depends on the pH and soil biological activity and plant root exudates (see discussion in Section 2.5). The P availability to the plants also depends on the amount and type of colonisation by AM fungi, which can contribute up to 80% of plant P uptake (Li et al., 1991).

A further consideration is that the Olsen P test may not be the best test of phosphorus availability for the trial soil type, as discussed in Section 2.5.1, in relation to Barlow's (2001) study of kiwifruit orchards in the Bay of Plenty. However, the Olsen P test is still the most used P availability test in New Zealand, and from the results obtained and the other considerations discussed, it could be reasonably anticipated that an Olsen –P test of 50 µg/g soil suggests that lettuce growth at the trial site would not be limited by P availability.

Sulphate

The level of inorganic S of 18 µg/g extracted was likely to be adequate, based on recommendations of 5-10 µg/g soil for pastures (Peverill, 1999). However the quantity of sulphur likely to become available during the lettuce growing period would mainly depend on net mineralisation of S from the 75-90% of total soil S in the organic fraction (Germida, 1998). As for the other organically bound elements, and as discussed in Section 2.5 of the literature review, this availability would depend on the composition of the organic fraction, the C: S ratio, and the activity of the soil biota. HortResearch (1995) reported that sulphur deficiency is rare in New Zealand.

Bulk density

The soil bulk density of 0.81 g/cm^3 is low, but higher than the 0.57 -0.66 g/cm^3 for some soils derived from volcanic ash (McLaren and Cameron, 1996) and on the border between loose and adequate according to the Landcare SINDI guide (2000). The most similar soil described in Soil Bureau Bulletin 26 (1968), the Waiteti loamy sand near Mamaku, had a dry bulk density of 0.66 g/cm^3. This low bulk density indicates that the soil has a high porosity, enabling good aeration, and high drainage. As the soil is a sandy loam it has a large number of large pores which enable free drainage rather than water retention, but water retention would be increased by the allophanic clay and organic matter content. The low bulk density also would give the soil low thermal conductance, particularly when dry, which would have made the soil slow to warm up below the surface in the spring when the trial was conducted. On the other hand the dark colour of the soil would enable it to absorb more heat energy (McLaren and Cameron, 1996).

pH

The soil pH of 5.7 indicates a slightly acid –normal soil, according to the Landcare SINDI guide. The pH would be slightly more favourable to fungi than to bacteria (Ingham, 2001). Bacteria generally prefer a pH of over 7, although even in an acid soil there are likely to be higher pH microsites, particularly in mucilages on root surfaces. Baath (1998) showed how bacteria have lower growth rates in low pH soils. A fairly low pH is likely to increase adsorption and unavailability of phosphorus and molybdenum. The pH was slightly lower than the level recommended for lettuces by HortResearch (1995).

CEC and Cations

The Cation Exchange Capacity of 20 me/100 g soil is fairly high for a sandy soil. Exchange sites would be provided by the allophanic clay and the soil organic matter. As a biological system depends to a large extent on continuous release of nutrients by soil organisms, the ability of the soil to hold inorganic ions is less important than if soluble fertilisers are applied. The estimated base saturation, used in the Albrecht system as discussed in Section 2.5.1 of the literature review is shown in Table 4.1.7.

Table 4.1.7 Cation concentrations and base saturation of the Te Puke sandy loam topsoil at the trial-site.

	me/100g air-dry	% TEB	Estimated % base saturation	Albrecht recommended % base saturation
Potassium	1.57	16.1	7.85	6-8
Calcium	6.4	65.8	32	60
Magnesium	1.67	17.2	8.35	20
Sodium	0.09	0.9	0.45	0.5 - 3.0
Hydrogen (est.)	10.27+		51.35	10 - 15
CEC	20			

+ It is assumed that aluminium and microelements are negligible, which may not be the case

The relative levels of cations for the trial soil indicate that potassium is slightly high, and calcium and magnesium are lower than ideal. The apparent high content of hydrogen ions is a result of doing the ammonium acetate extraction at a pH of 7. This results in more free hydrogen ions dissociating from exchange sites than would be the case at the soil pH of 5.7. The % base saturation of calcium and magnesium would therefore be higher than estimated above, but would still be lower than the Albrecht recommended values. Albrecht (1979) wrote that the quantity of calcium available for plant uptake is more important than the pH level. The high potassium level would also reduce plant uptake of calcium and magnesium because the potassium ion is monovalent and more mobile than divalent calcium and magnesium. However if the calcium level is low, potassium uptake may be less through the Viets effect (Mengel and Kirkby, 1987). If the calcium level outside the outer root cell membrane is low, there may be high efflux rates of potassium ions, but this is more likely to be a problem under anaerobic conditions.

As the calcium level and pH were at the lower end of the recommended ranges, lime (calcium carbonate) was added to the trial soil at the rate of 0.1 kg/m2. This was only one tenth of the quantity required to raise pH by 1 unit (McLaren and Cameron, 1996) and one sixth of the quantity recommended by Woods End Research Laboratory (2002) for a soil with a CEC of 12-20, and total base saturation of 40-50. However, adding a large quantity of lime shortly before the compost was applied could have volatilized compost N. Dolomite would have also provided magnesium but takes longer to be broken down.

From the results discussed above, it appears that low pH and calcium and magnesium supply could be the main deficiencies for optimum lettuce growth at the trial site, whilst soil P and S availability may be adequate.

Nitrogen will be supplied partly from the soil and partly from the composts and manures that will be applied as treatments. An attempt was made to supply nitrogen at the rate of 150kg/ha recommended for lettuces by HortResearch (1995). This rate is slightly less than the critical concentration of 168kg/ha found by Greenwood *et al.* (1980) to be the least quantity of nitrogen required to provide for maximum growth rate of lettuces. However, this was based on nitrogen fertiliser application, rather than relying on continuous supply of nutrients from the mineralisation of soil organic matter by soil organisms. Also it is expected that there would be variations in N demand according to lettuce variety, soil type and climatic conditions, as discussed in the literature review, section 2.4.2.

Trial site variability - Mustard yields from pre-trial cover crop

In order to assess the variability of nutrient supplying power of the Te Puke sandy loam topsoil at the trial-site the site was initially cropped with mustard. The mustard yields also provide an indication of the variability of nutrient status between plots. The yields of mustard grown in all plots before the lettuces were planted are shown in Table 4.1.8.

Table 4.1.8 Mustard yields (g) from a 69 x 76 cm area in centre of each trial plot

	Plot	Weight (g)	Plot	Weight (g)	Plot	Weight (g)	Plot	Weight (g)
Block	1		2		3		4	
	a	952	a	840	a	728	a	868
	b	896	b	1289	b	1036	b	980
	c	868	c	1401	c	1261	c	476
	d	784	d	1232	d	1120	d	224
	e	896	e	1148	e	896	e	840
	f	588	f	952	f	952	f	308
mean		831		1144		999		616

The results show considerable variability. The average yield per block did not relate well to total nitrogen content of the soil as determined by Kjeldahl digestion or to pre-incubation nitrate-N. Later analysis of the mustard yields in conjunction with lettuce

yields showed very slight negative relationship, indicating that the mustard may have removed some available nitrogen from the soil, but the variation in mustard yields explained little of the lettuce yield variability due to plots and blocks in the statistical analysis. These results indicate the variability of soil conditions and nutrient availability within a small area, and the need for sufficient replication of trial treatments, as discussed by Johnstone and Sinclair (1991). A contributing factor to the high variability was that the trial area was previously an occasional camp-site for dairy cows.

4.1.2 Compost analysis

Composting temperature and aeration

The method of compost making was the cold composting rather than hot composting method. Although the compost did heat up when first made and again a little after each turning, it did not quite reach the temperature level (65°C) recommended for commercial composting. In hot composting the heaps are turned frequently to increase aeration and generate several high temperature levels peaks, and this is the method recommended for organic growers by Ingham (2001). Such composts are generally ready for use more quickly. A disadvantage of the hot composting method is that the high temperature can kill macro-organisms such as earthworms.

The compost heaps were turned more frequently than biodynamic composts often are, in order to increase aeration and speed up decomposition. Biodynamic heaps are often matured for a year or more and only turned once or twice during that time, whereas in commercial composting frequent turning is generally the rule to avoid anaerobic conditions and speed up decomposition with high temperatures. Anaerobic conditions can favour microbes which do not result in healthy soil conditions when incorporated in the soil (Ingham, 2001).

Epstein (1997) described how temperatures over 45°C increase decomposition rate, and favour thermophilic species, which are mainly bacteria, whereas under 45°C mesophilic organisms predominate, including more fungi. Size of compost heap also affects temperature. The small size of the trial compost heaps (about $3m^3$ at making, $1m^3$ when used) would have prevented the heaps reaching higher temperatures and slowed down maturing, as the recommended size is at least $5m^2$ (Procter, 1995). The temperature of

the two composts was not compared. Carpenter-Boggs et al. (1999) found that a biodynamic compost maintained an average 3.4°C higher temperature than a corresponding organic compost throughout the eight-week active composting period, suggesting more thermophilic microbial activity and/or faster development of compost with biodynamic treatment.

Compost respiration

The results of two respiration (carbon dioxide evolution) tests, to test for compost stability, are shown in Table 4.2.1.

Table 4.1.9 Respiration and moisture content of organic and biodynamic composts at 9.9.02 and 23.9.02, 40 and 26 days respectively before applying to trial plots.

Date samples taken	9.9.02		23.9.02	
	Respiration mg CO_2/day/g dry compost	Moisture content %	Respiration mg CO_2/day/g dry compost	Moisture content %
Biodynamic compost				
sample 1	1.98	62.92	0.77	55.1
sample 2	1.80	66.15	0.85	57.3
Organic compost				
sample 1	1.22	61.19	0.71	53.8
sample 2	1.32	62.46	0.61	54.9

The results show a reduction in respiration rates as the composts became more stable, but they were all very stable composts, according to E&A Consultants Inc.1994, quoted in Epstein (1997) who rated any respiration rate under 2 mg CO_2/day/g compost as very stable. The biodynamic compost had higher respiration rates than the organic compost, which contrasts with the findings by Carpenter-Boggs et al. (1999). They compared biodynamic and organic composts and found that compost treated with biodynamic compost preparations respired CO_2 at a 10% lower rate and had a larger ratio of dehydrogenase enzyme activity to CO_2 production than control piles at the final sampling, taken when active composting slowed and the piles entered a ripening stage. However the biodynamic compost may stimulate more biota activity when added to soil. Carpenter-Boggs et al. (2000) found slight (non-significant) differences (30.9 and 28.4 μL CO_2g soil/h.) in soils to which biodynamic and non-biodynamic compost had been added.

The decreasing moisture content of the composts was consistent with recommendations by Woods End Research Laboratory (2000), that composts still very biologically active require a higher moisture content, up to 65%, than more mature composts.

Compost Phytotoxicity

The germination test carried out on compost samples taken from the 2 composts on 15.9.02 resulted in a good germination rate and seedlings grew normally on both composts, indicating mature composts. One unexpected observation was the difference in appearance of the seedlings grown on each compost.

Plate 4.1.2 Results of compost phytotoxicity test

The seedlings grown on the biodynamic compost had blue/green small leaves, whereas those grown on the organic compost had more yellow-green, larger spreading leaves. This is an indication that the organic compost contained less available nitrogen than the biodynamic compost.

Analysis of compost samples taken at the same time as the soil tests showed higher levels of pH, CEC and all nutrients as shown in Table 4.2.2.

Laboratory analysis

The two composts were sampled for laboratory analysis at the same times (19.8.02 and 23.9.02), and the same tests performed, as for the soils. The results are shown in Tables 4.2.2 and 4.2.3.

Table 4.1.10 Compost laboratory analysis of compost sampled on 19.8.02

Test	Biodynamic compost	Organic compost
Total N% (LECO)[1]	1.07	1.45
Total C %(LECO)[1]	12.26	16.34
pH	6.8	6.8
Olsen P (µgP/g air-dry)	291.4	295.2
Sulphate (µgS/g air-dry)	223.8	n.d.
Potassium (me/100g air-dry)	14.82	13.57
Calcium (me/100g air-dry)	23.2	26.1
Magnesium (me/100g air-dry)	13.35	13.16
Sodium (me/100g air-dry)	1.51	1.7
CEC (me/100g air-dry)	53	55
Compost density g/mL	0.59	0.5

[1] on an air-dry basis
n.d. = not determined

Table 4.1.11 Total nitrogen and phosphorus in composts sampled on 23.9.02 measured by Kjeldahl digestion and Autoanalyser

Sample		N%	mean	P%	mean
Biodynamic[1]	a	1.73		0.46	
	b	1.73	1.73	0.46	0.46
Organic[1]	a	1.59		0.40	
	b	1.58	1.59	0.41	0.4

[1] on an air-dry basis

Total Nitrogen and phosphorus content

As the biodynamic compost had slightly higher moisture content, its total N and P contents would have been slightly lower relative to the organic compost than as shown on a dry weight basis.

It would not be meaningful to compare the nutrient levels with other analyses because they depend on the material that was used. Composts have variable nutrient content and each batch requires separate chemical analysis. Variation in amendment materials used and likely high variability in nutrient content and biological activity is one reason why such variable results have been obtained in researching nutrient content of organically grown crops. Composts used in Europe are often made from farmyard manure which has high nitrogen content, and commercial biosolids compost would also have higher nitrogen content than composts of predominantly plant materials.

Total Kjeldahl nitrogen and phosphorus levels were slightly higher in the biodynamic as compared to the organic compost, but generally the levels were very similar as would be expected from composts made of the same materials. The biodynamic preparations are

intended to improve breakdown conditions and result in less nutrient losses. The higher nutrient level developed in the biodynamic compost is consistent with findings by Carpenter-Boggs et al. (1999) that biodynamic-treated composts contained 65% more nitrate than control piles made from the same dairy manure and wood-shaving bedding.

C: N ratio

The C: N ratio is one of the main indicators used to assess maturity and suitability for purpose of composts. The LECO data (Table 4.2.2) show a C: N ratio of 11.5, 11.3 for the 2 composts. This can be compared to recommendations by Mathur (1991) that stable compost should have a C/N ratio of about 10, as in humus, and by Woods End Research Laboratory (2000) that it can be up to 17. According to Follett et al. (1981) the ratio indicates that the composts could have been predominantly fungal as fungi have a C: N of 10:1 whereas for bacteria this ratio is 5:1. However, as the compost would contain a huge range of species of organisms of varying C: N ratios it seems difficult to estimate what species predominate unless the C: N is very high or very low. Species balance would also depend on other factors such as temperature, moisture content, pH and the degree of decomposition of materials.

The C: N ratio can also be used to assess compost maturity. Different ratios of C: N and maturity are used for different purposes. Vegetable crops require composts with fairly low C: N and of medium maturity, as nutrients become less readily available as the compost matures.

Nutrient availability

As discussed in the sections on soil nutrients, nutrients would become available as they are released by soil biota. Quantity and rate of release depend on environmental conditions and the relative quantity of carbon to that nutrient. Woods End Research Laboratory (2000) provides a rough guide to nutrient availability of 20-75% for N, 85% for K and Na and 50% for P, Ca and Mg. The trial composts would be likely to have a greater effect on plant growth than indicated by their nutrient content, due to positive effects on soil structure and from their content of humic and fulvic acids, phytohormones and enzymes, and this would vary according to the humic contents, as discussed in section 2.2.3 of the literature review. For example, Piccolo et al. (1992) found that the low molecular weight fractions of humic extracts enhanced biological activity of plant

systems: the lower the molecular size the higher the auxin-like activity. Humus containing high contents of both acidic low molecular fractions and carboxyl groups most enhanced plant nitrate uptake.

pH

pH levels of 6.8 were in the middle of the range of 6.0 – 7.5 recommended by Woods End Research Laboratory (2000) They point out that there is more nitrogen lost by ammonia volatisation at a high pH.

Mineralisable nitrogen

Table 4.1.12 Mineralisable nitrogen in composts used for trial

Sample		Moisture content	Unincubated NH4	Unincubated NO3	Incubated NH4	Incubated NO3
				ugN/g soil		
Biodynamic	a	61.46	24.16	97.72	4.79	<0.58
	b	60.14	8.93	99.97	4.04	<0.58
Organic	a	54.54	10.12	109.68	nr	nr
	b	54.54	14.14	111.67	4.12	<0.58

The mineralisable nitrogen results show that nitrogen levels after incubation were much lower than those before incubation for all compost samples, indicating that there was net immobilisation of nitrogen by microorganisms during incubation under the anaerobic conditions of the test and biological immaturity. It is possible that an aerobic incubation might have given a different result. However, Bezdicek and Fauci (1997) compared aerobic and anaerobic nitrogen mineralisation and hot KCl extraction methods and found that although in soil the methods were highly correlated, none of them worked well for compost. Woods End Research Laboratory (2000) reported that their research had shown variation of 20 -75% of nitrogen release from total N content of similar manures applied to the same soil. They also recommend that for advanced composts most nitrogen should be in nitrate form. Comparison with other research on composts is difficult because of the variability in contents and method variables such as the amount of aeration, moisture content and composting time. Research is discussed in section 2.5 of the literature review.

Calculation of N content of soil and composts and how much compost and fertiliser to apply

As so little soil N was mineralised in the test incubation (Table 4.1.5), the unincubated nitrate N content was used to estimate N availability for lettuce growth. The average calculated nitrate N content of 28.4 μg N/g of wet soil would supply much less than the 150-160 kg/ha fertiliser N recommended by HortResearch (1995). To supply the equivalent of 150 kg soluble N as compost. Mahimairangi (1998) measured the N use efficiency of sulpho- and phospho- chicken manure composts ranging from 12 – 60% for a maize crop. The trial composts were very different from chicken manure composts and N release from similar manures applied to the same soil may vary according to climatic and management conditions from 20% to 75% (Woods End Research Laboratory, 2000). However, in the absence of other data it was assumed that the composts would release 25% of their total N during a 50 day lettuce growth period.

Quantities of compost to apply to the appropriate trial plots, to provide 150 kg N/ha were calculated as follows:

1 Compost N required = 150/0.25 = 600 kg N/ha.

2 Average compost is 1.26 %N (dry wt .basis) = 600/0.0126 = 47,619 kg compost/ha.

3 A trial plot 1.4*1.4m (1.96m^2) requires 476000/10000 * 1.96 = 9.3 kg/plot

4 The compost moisture content was 43% (ie 57% dry matter)

5 Therefore 93/0.57 = 16.3 kg compost is required per plot.

The quantity of phosphorus and nitrogen fertiliser as diammonium phosphate (DAP) and calcium ammonium nitrate (CAN) to provide equivalent quantities of nitrogen and phosphorus to the appropriate trial plots was then calculated as follows:

1 64% of compost total P was extractable in Olsen reagent (Tables 4.2.2 and 4.2.3)

2 16.3 kg wet compost at 0.58% dry matter contains 293 ugP/g dry matter available P = 16.3*293*0.58 * 30% plant availability =832 mg P

3 To supply 832mgP/plot requires 832*100/20 =4.2g DAP as DAP contains 20% P.

4 4.2g DAP contains 0.75 g N (18%), so extra N to be supplied from CAN=(12.7*1.4*1.4)-7.5 =25g/plot

5. As the CAN used contained 26%N, the quantity of CAN required/plot = 25*100/2 =68g

No potassium was applied as this was already oversupplied in the soil.

Subsequent plant growth, particularly the growth of lettuces in the unfertilized control plots indicated that more soil N was likely to have been mineralised than estimated.

Capillary dynamolysis pictures

Brinton (1983) described the capillary dynamolysis method to assess the amount and "coherency" of life in the composts. Chromatograms developed using this method are shown in Plates 4.1.3 -4.1.6.

Plate 4.1.3 Chromatogram of biodynamic compost prepared from NaOH extract on 26.10.02, 21 weeks after completion of compost and 6 days after compost was applied to trial plots

Plate 4.1.4. Chromatogram of organic compost (made from same materials as biodynamic compost, but without the biodynamic preparations) prepared from NaOH extract on 26.10.02, 21 weeks after completion of compost and 6 days after compost was applied to trial plots.

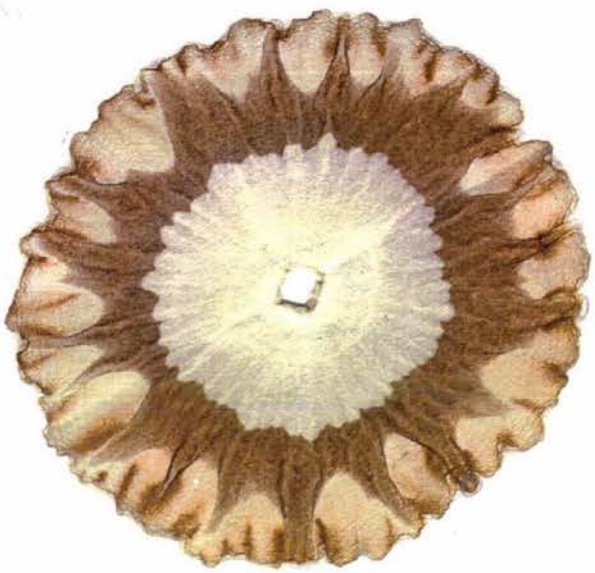


Plate 4.1.5 Chromatogram of biodynamic compost prepared from NaOH extract on 21.11.02 , 25 weeks after completion of compost

Plate 4.1.6 Chromatogram of organic compost prepared from NaOH extract on 21.11.02 , 25 weeks after completion of compost

Carter (2003), an experienced user of the method, described greater dynamic movement, order and life in the 25 weeks samples as compared to the 21 weeks samples, with rays penetrating right out in the more mature sample. The later chromatograms in the current study also appear to show a more complex structure. However, this could partly be due to improved methodology at the later date. The more complex rays or "finger patterns" (Plates 4.1.5, 4.1.6) can also signify mature composts containing well mineralised

material, a variety of soluble salts and low molecular weight organic molecules that have different reactions with the silver nitrate embedded in the filter paper, from a chemical viewpoint. From an organisational viewpoint, regularity of patterning could be important.

The chromatograms and their "rays" can be compared with a chromatogram made from mature biodynamic compost by Gerrard (2001) (Plate 4.1.7) and chromatograms by Pfeiffer (1984) (Plate 4.1.8).

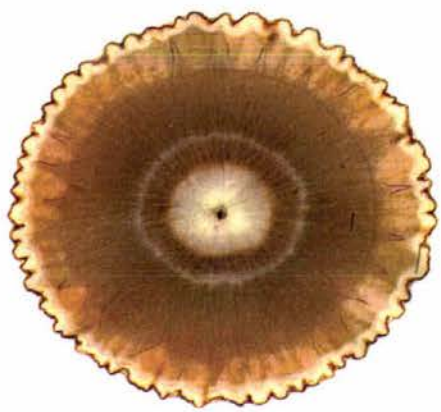


Plate 4.1.7 Chromatogram made from mature biodynamic compost (Gerrard, 2001)

The chromatogram in Plate 4.1.7 contains deep coloration from humic substances. The colour provided by organic matter compared to the grey colour of a poorly made city garbage containing mainly ashes and dust and little organic wastes is shown in Plate 4.1.8. (Pfeiffer, 1984). The next chromatogram shows the effect of adding the biodynamic preparations. Pfeiffer describes how the violet/grey colour in the chromatograms indicates the degree of mineralisation of the compost. The outer, light brown edge zone in #8 (Plate 4.1.8) indicates good humus formation. The chromatogram in #9 he describes as an "almost ideal humus formation". This appears more similar to the biodynamic compost chromatogram shown in Plate 4.1.5, although the latter has more of a mineralised inner zone, according to Pfeiffer's interpretation of the violet/grey colour.

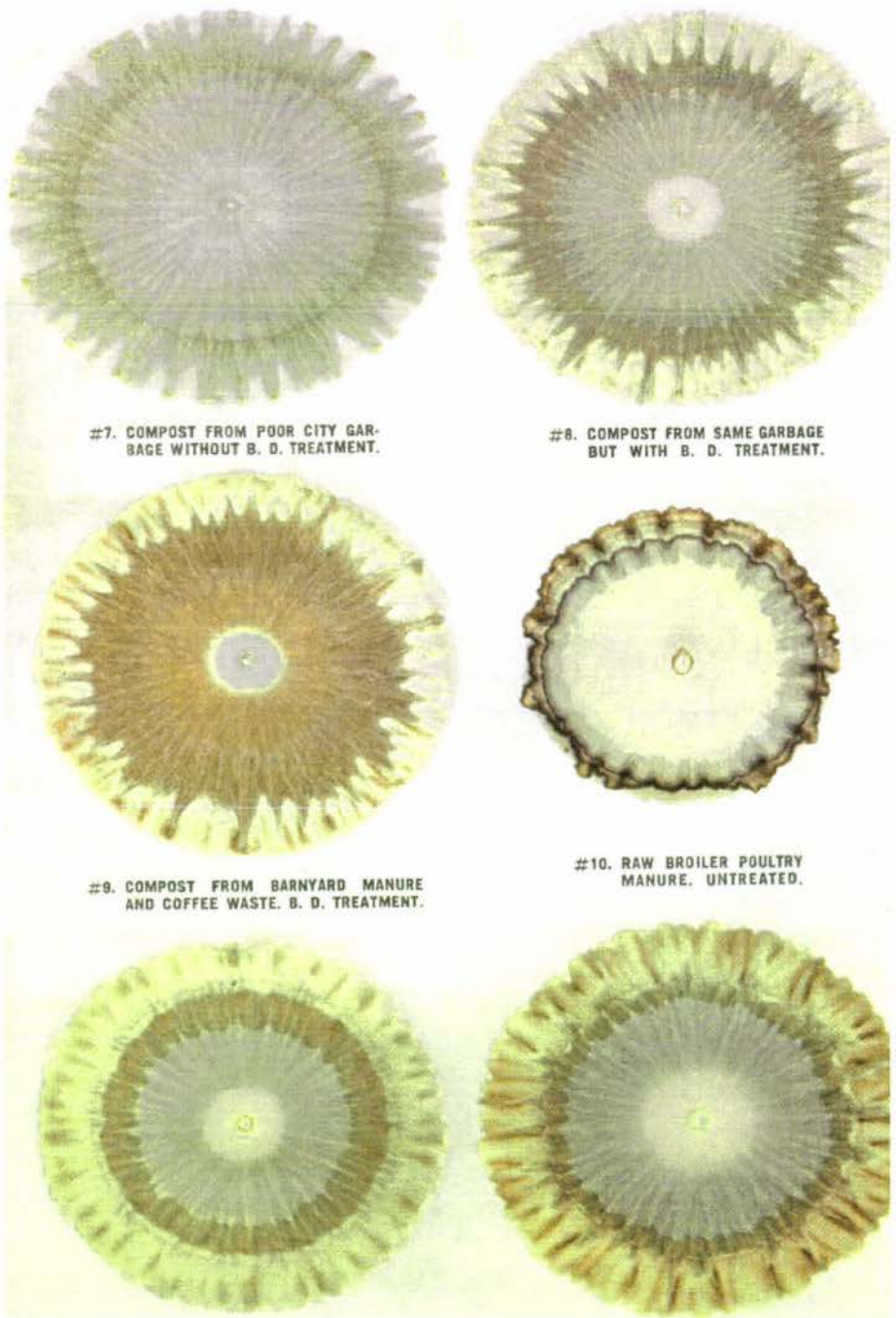


Plate 4.1.8 Chromatograms of composts made with BD compost starter (containing biodynamic preparations) by Pfeiffer (1984). The bottom 2 composts are from the poultry manure shown in #10, the first more mature than the second.

4.2. Observations of plant growth

When planted the lettuce seedlings had on average 3 leaves, varying from 2 to 5. The smaller and larger plants were planted in the outside rows of plots, to ensure that the centre plants were as even in size as possible.

Observations of the centre plants in each plot are summarised by treatment in Tables 4.2.1 – 4.2.6. Observational scores were fairly variable between plots of the same treatment: a definite treatment score indicates that at least 3 of the 4 plots had that score.

Table 4.2.1 Plant observations on 30.10.02, 5 days after transplanting

Treatment	Vitality	Leaf colour	Form	Texture
Ctrl	1	G	U	
C+sp	2	V pv	V	
DC	1-2	Y G rv	S	h
DC+sp	1-2	V rv	U	h
Org	1-2	V rv	U	
Bd	1	B, R, rv	U	s

Key: Vitality: 1 = most vitality, 3 =least vitality
Leaf Colour: G = predominantly green, R = predominantly red, V = variable, Y = yellow-green, B = green and red brightly contrasted, V = variable. pv = pale veins, rv = red veins
Form: U = upright, S = spreading, V = variable, c = compact, H = starting to heart, E =even
Texture: h = hard, cold , s= soft, warm, f=firm

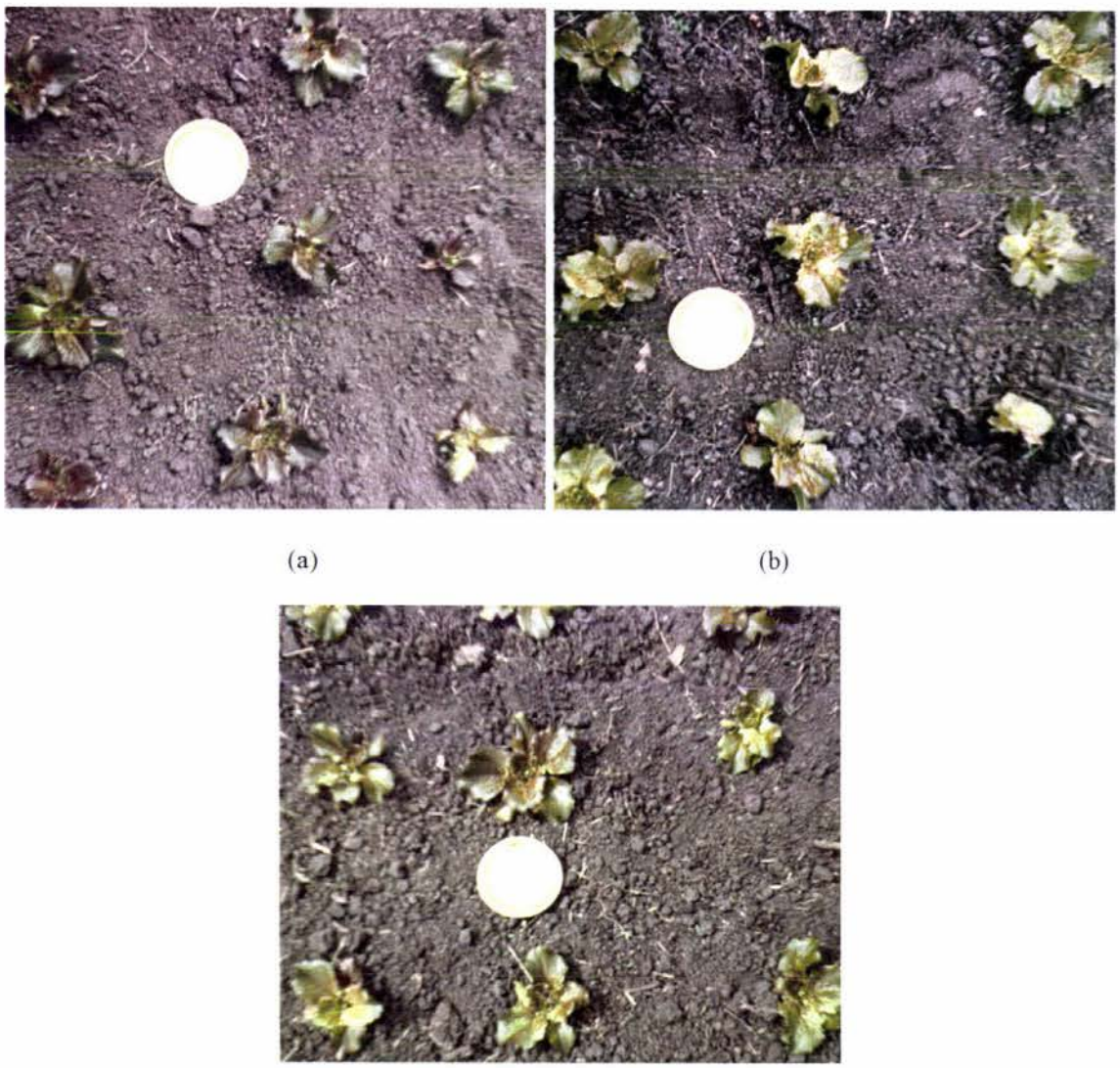
Vitality

Vitality was an assessment of how vigorous and healthy the plants in a plot appeared. The vitality of plants in all treatments deteriorated between 30 October and 9 November (Tables 4.2.1 and 4.2.3) as water stress became more evident. Plants in the Control and Biodynamic treatments appeared to have most vitality initially, but as plots dried out plants in the Control, Control + sprays and DAPCAN + sprays treatments appeared most stressed.

Colour

The varying leaf colour was one of the most striking features evident. There was considerable difference in red coloration of the leaves and their veins. The plants in composted or fertilized plots initially had greener leaves, with a few red blotches and redder veins. In the unfertilized plots, the plant leaves developed a more diffuse, duller

red colour (Plate 4.2.1. (a)). Later, (Table 4.2.4) this coloration was most evident in the control +sprays, and DAPCAN with and without sprays treatment plants, while the organic, biodynamic and control plants were greener. Some plants, particularly in the biodynamic, and initially in the DAPCAN treatments, had particularly intense contrasting green and red coloration.



(a) (b)

(c)

Plate 4.2.1 Lettuces on 11.11.02, 17 days from transplanting. (a) Control+sprays, (b) Organic (c) DAPCAN treatments. The disc was 7cm in diameter.

Form and Texture

Most of the plants developed a fairly upright form, but initially plants in the DAPCAN, and latterly plants in the organic treatments had a more spreading form (Tables 4.2.1, 4.2.4). All plants were fairly regular in growth, but some plots, particularly the Biodynamic, showed more regularity of growth both within the plants and between

plants in the plot. Those showing least even, regular growth were in DAPCAN treatments. Some increase in upright form in sprayed plots was observed after the second silica spray. Plants in the Control + sprays and DAPCAN + sprays treatments became very compacted and flat with hard feeling leaves between 5 and 36 days from transplanting (DAT) (Tables 4.2.1, 4.2.5, Plate 4.2.1a). This hardness was different from the firm feel of leaves later in the trial. In contrast, plants in the Organic and Biodynamic treatments felt softer and warmer.

Most plants only started to heart just before the final harvest at 47 DAT. Some lettuces in the DAPCAN treatment were the first to heart (Table 4.2.5). Later in the trial, the plants in the Biodynamic treatment were found to have the longest leaves, whereas most leaves were characteristically rounded. At the final harvest, it was very noticeable that plants generally from treatments that had received the fieldsprays had a spirally twisted form.

Table 4.2.2 Plant observations on 2.11.02 , 8 days after transplanting

Treatment	Size	Vitality	Leaf colour	Form	Bird damage
Ctrl	V	1-2	R	V	1-3
C+sp	3	2-3	V	U	1-3
DC	3	2-3	G B	S	3
DC+sp	2	2	R	S	3
Org	2-3	2-3	G	V	2-3
Bd	2	1-2	V B	V	1-3

Additional symbols:
Size: 1 = large, 3 = small, V = variable
Bird damage: 1 = > ½ outside 2-4 leaves missing, 2 = ¼ - ½ outside 2-4 leaves missing, 3 = < ¼ outside 2-4 leaves missing.

Bird, pest and disease damage

Birds were a problem particularly on blocks 2 and 3 (Tables 4.2.2, 4.2.3). They were mostly scratching up the soil and plants looking for worms, but as the soil dried out and the seedlings became more stressed the birds started to eat the leaves. The worst affected plants at first seemed to be the smallest, least vigorous and possibly most water stressed plants, particularly in the control plots. It is interesting to note that in the 4 Biodynamic plots, those in blocks 1 and 3 received top score for vitality and had lowest scores for bird damage, and those in blocks 2 and 4 received bottom score for vitality and highest scores for bird damage. However, between 2 and 9 November the birds

seemed to eat more of the faster growing, bigger plants, particularly in the Organic and DAPCAN treatments (Table 4.2.3). A few plants were scratched out, and were replaced as necessary- these were mainly outside row plants.

Regression analysis to look for a relationship between scores for bird damage and lettuce weights failed to find any relationship.

No other pest or disease damage was seen on any plant in the whole trial except that one plant harvested from one of the Biodynamic plots had deformed growth similar to clubroot on its roots. A few ladybirds were evident at one point during the trial, indicating that there could have been a few aphids for them to eat, but none were seen.

Table 4.2.3 Plant observations on 9.11.02 , 15 days after transplanting

Treatment	Vitality	Leaf colour	Form	Bird damage
Ctrl	3	V pv	U	2-3
C+sp	3	R pv	U	2-3
DC	variable	R rv	S	2
DC+sp	variable	V rv	U	2-3
Org	variable	G pv	S	1-2
Bd	variable	B rv	U	2-3

(a) (b)

Plate 4.2.2 Lettuces on 21.11.02, 27 days from transplanting. (a) Biodynamic (b) Control+sprays treatments

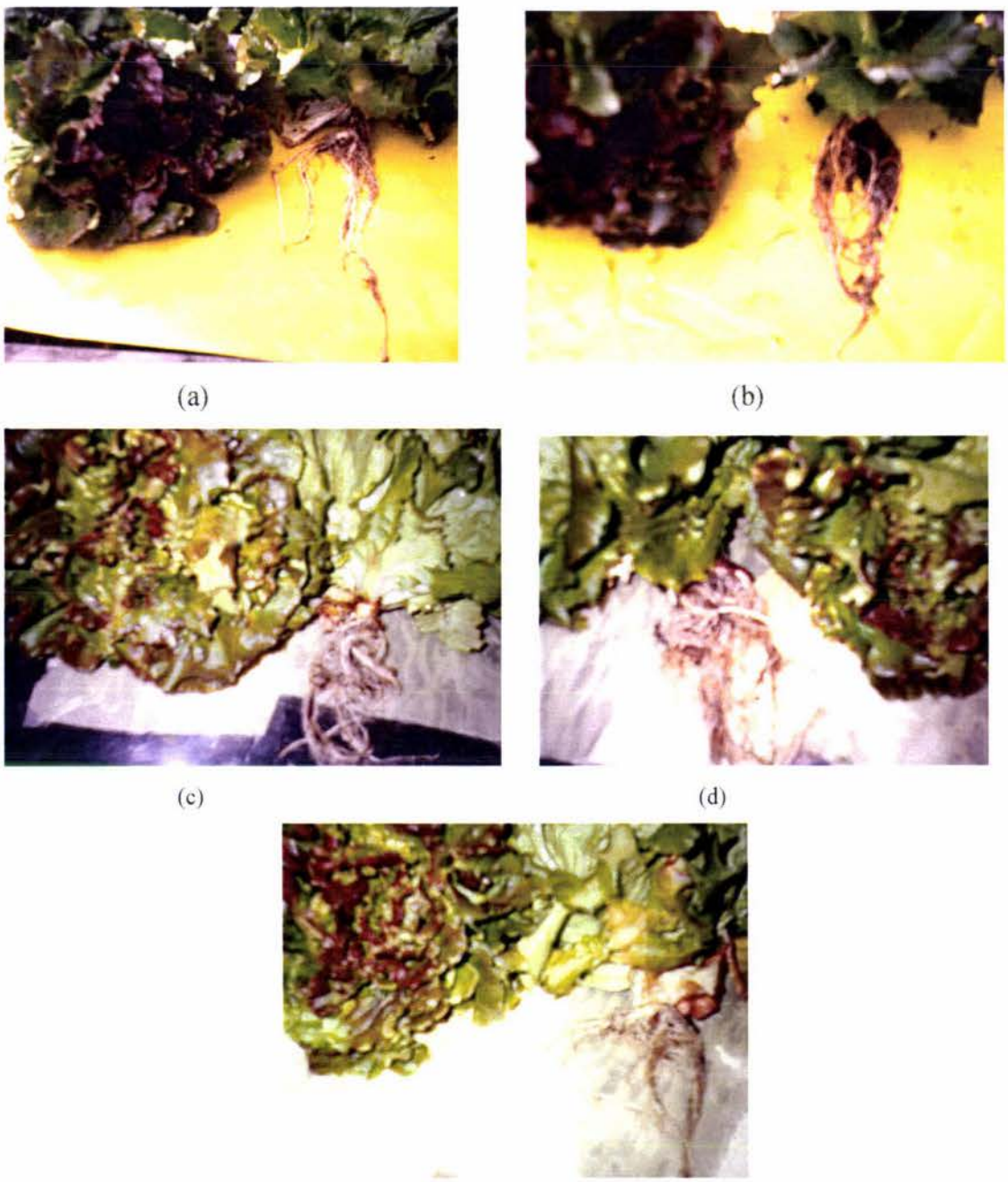
Table 4.2.4 Plant observations on 15.11.02, 21 days after transplanting

Treatment	Vitality	Leaf colour	Form	Roots	Soil
Ctrl	1	V			C
C+sp	1-2	V	C		D
DC	1-2	R		L, B	D
DC+sp	2	R	C	B	D
Org	1	G	S	L	W
Bd	1	G	U		C,W

Additional symbols:
Roots: L = much lateral spreading, Th = thick mainroot, B: = breakable Tw = twisted
Soil: C = crumb structure, D = dry, W = damp

Roots

When harvesting plants on 15 November, the roots of plants mainly in the organic and soluble fertiliser plots had noticeably the most lateral spread, and roots in the soluble fertiliser plots were easily broken (Table 4.2.4). At later harvests there was more difference between roots from different treatments (Plate 4.2.3). When root washing, the roots from composted plots were harder to wash because the roots had grown around compost particles and the soil adhered more to the roots. This could have been an effect of mucilage exuded by roots and soil organisms.



(a) (b)

(c) (d)

(e)

Plate 4.2.3 Lettuces at final harvest (a) DAPCAN+spray (b) Control+spray (c) Organic (d) Biodynamic (e) Control treatments.

Soil

At the first plant harvest, the soil was quite dry, but noticeably damper in most of the DAPCAN, Organic and Biodynamic plots (Table 4.2.4). At the final harvest there was a similar difference except that the DAPCAN plots were now drier and the control plots damper (Table 4.2.6). The soil dampness appeared to relate to the degree of ground

covered by the lettuce plants. In most of the Biodynamic and Control plots the soil appeared to have more of a crumb structure.

It had been hoped to count earthworms around roots in each plot, as there were a lot when the plots were initially prepared. However, very few were seen in any plot during the trial, presumably because the soil was too dry for them.

Table 4.2.5 Plant observations on 30.11.02 , 36 days after transplanting

Treatment	Vitality	Leaf colour	Form	Texture
Ctrl	1-3	V	E	
C+sp	2-3	G	C	h
DC	2-3	R/G	E c H	h
DC+sp	2-3	R	V	h
Org	1	B	U E	s
Bd	1	G/Y	U E	s f

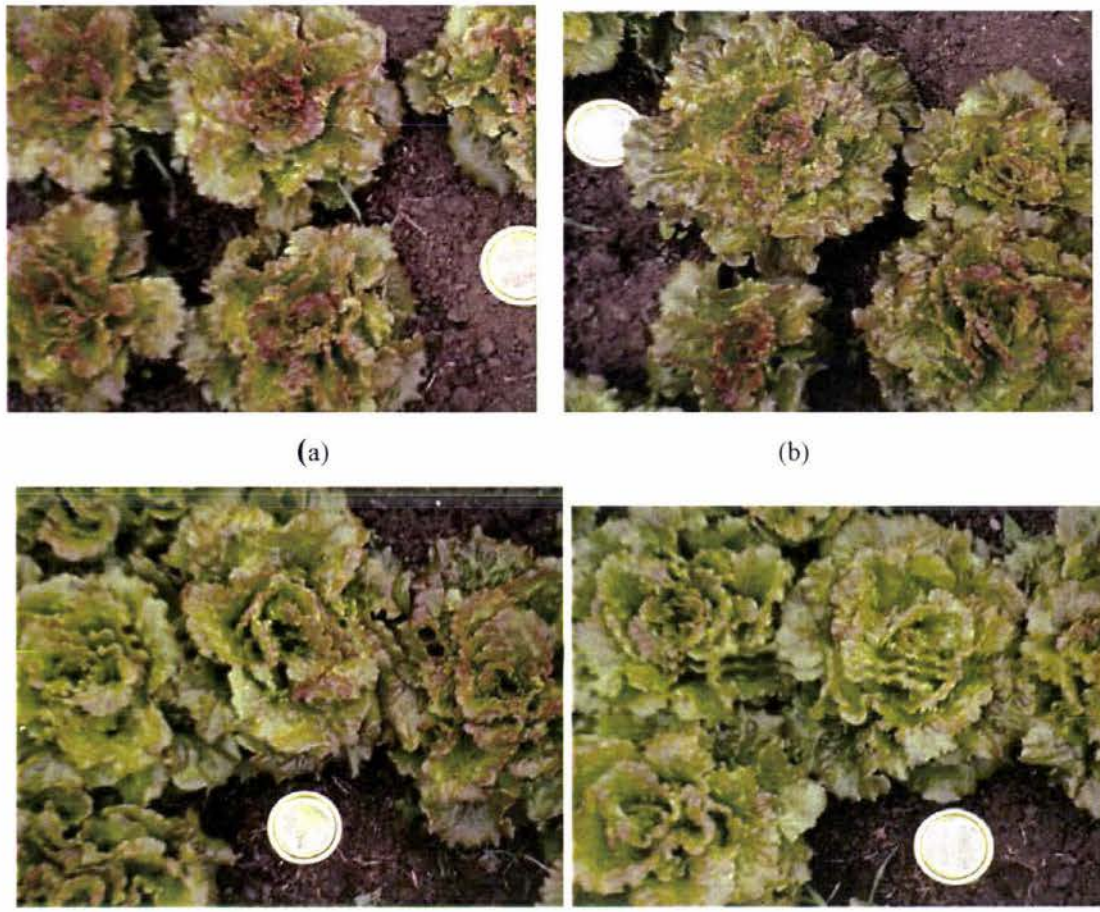
(a) (b)

Plate 4.2.4 Lettuces on 1.12.02, 37 days from transplanting. (a) DAPCAN (b) Control+sprays (Disc was 7cm in diameter).

Table 4.2.6 Plant observations on 11.12.02 , 47 days after transplanting

Treatment	Leaf colour	Leaf texture	Roots	Soil
Ctrl	V	s		W, C
C+sp	V	h	L	D
DC	R	h		D
DC+sp	R	h		D
Org	G	s	L	W
Bd	G	s	T	W, C

Much of the observable differences between plants from different treatments had gone by final harvest on 11.12.02 (Plate 4.2.5).



(a) (b)

(c) (d)

Plate 4.2.5 Lettuces on 9.12.02, 45 DAT. (a) Control (b) DAPCAN+spray (c) Biodynamic (d) Organic (Disc was 7cm in diameter).

4.3 Weather and Irrigation Records

Temperature, rainfall and irrigation records are shown in Figure 4.3.1. The temperature fell to below 5°C overnight on 3 occasions during the first 25 days from transplanting (DAT) (25.10.02) (Figure 4.3.1). There was little rainfall between 7 and 30 DAT, and irrigation was applied fairly regularly throughout the growing period. There was also strong, gusty wind and for most of the trial period (data not shown), which would have increased evapo-transpiration levels.

The quantity of water to apply by irrigation was calculated using the method described in Section 3.8. Calculation results are shown in Table 4.3.1

Table 4.3.1 Estimated volumetric depth of water in trial soil prior to irrigation events and irrigation volumes applied

Days from transplanting	Wet soil weight	Dry soil weight	Weight water	θv	Water/15cm depth	Water applied
	g				*mm*	L/m^2
8	440	340	100	0.17	26.3	
13	365	310	55	0.10	14.5	12
14	430	335	95	0.17	25.0	
20	305	255	50	0.09	13.2	12
21	445	340	105	0.18	27.6	
26	300	265	35	0.06	9.2	20

Soil water deficit for the trial period was calculated using data provided by the Meteorological Service for Tauranga airport (about 15 km from the trial site) and a pasture production model (Figure 4.3.2) (Moir et al, 2000). The graph shows that there was approximately 60 – 80 mm of soil water deficit at the time of transplanting lettuces (25.10.02), which was only partially reduced to approximately 20 – 30mm by irrigation and rainfall. Soil water deficit for the period of the trial was managed between 30 – 50mm by measuring soil moisture content and applying irrigation (Section 3.8).

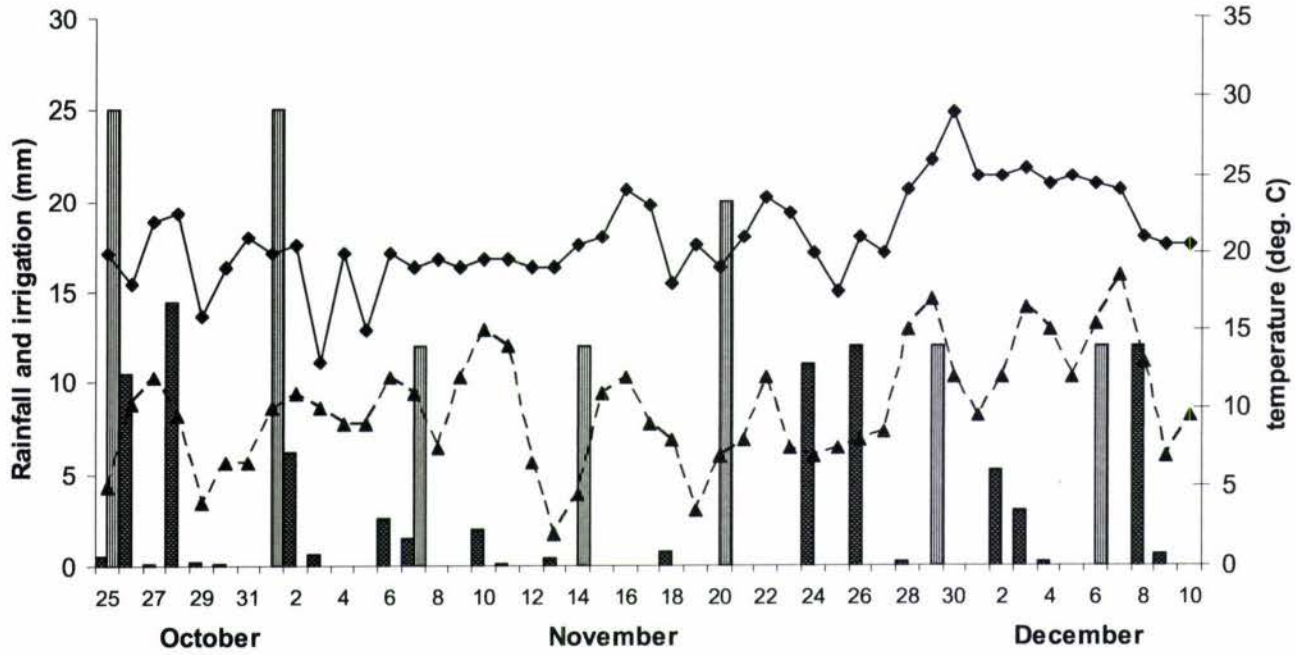


Fig. 4.3.1 Maximum and minimum temperature and rainfall recorded for each day of the trial period close to trial plots, and water provided by irrigation.

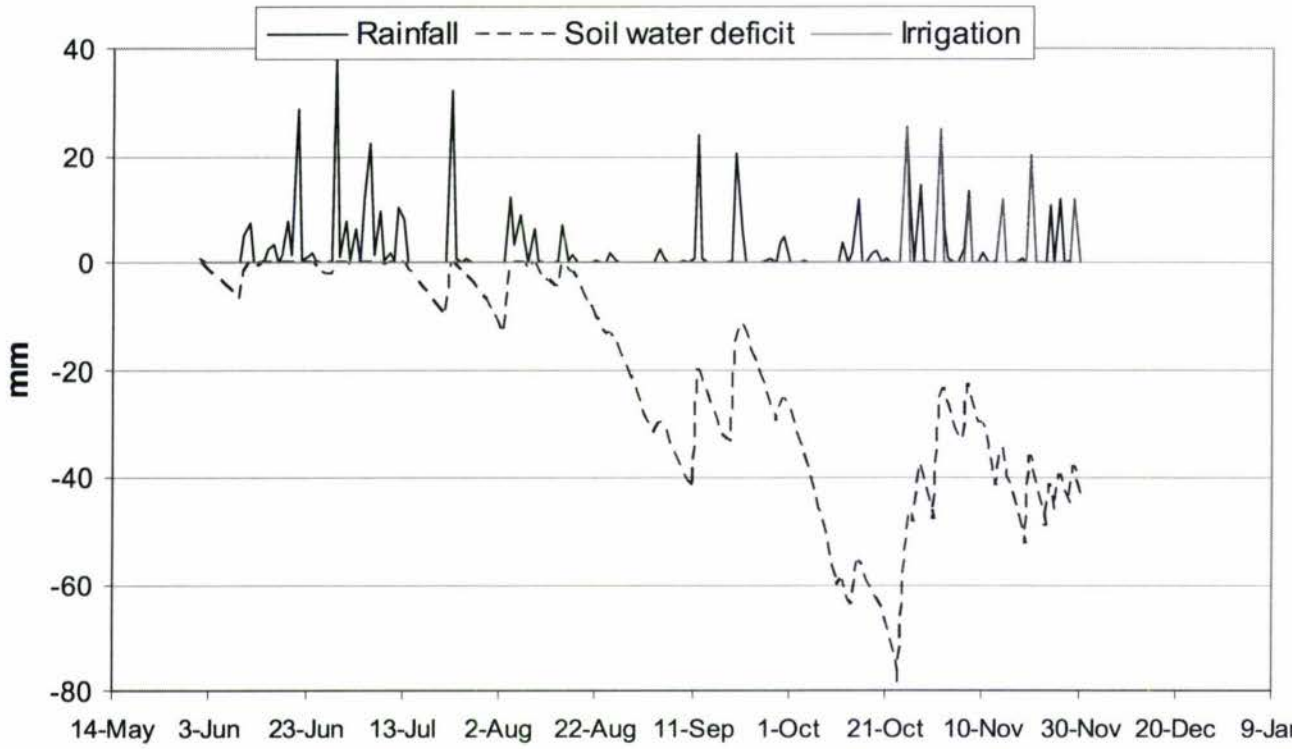


Fig. 4.3.2 Soil water deficit calculated for before and during the trial period using actual rainfall and irrigation data and data recorded by the Meteorological Service at Tauranga Airport (approx. 15 km from trial site) using a pasture production model (Moir et al., 2000).

4.4. Growth measurements and analysis and dry matter content

4.4.1 Plant Growth as shown by fresh weight and size of shoots and roots

Plant growth was slow in all treatments for the first 30 days from transplanting (DAT) (Figure 4.4.1) because dry, windy conditions and insufficient weekly irrigation resulted in dry soil. In the fifth week there was a good rainfall, which resulted in rapid growth of all plants.

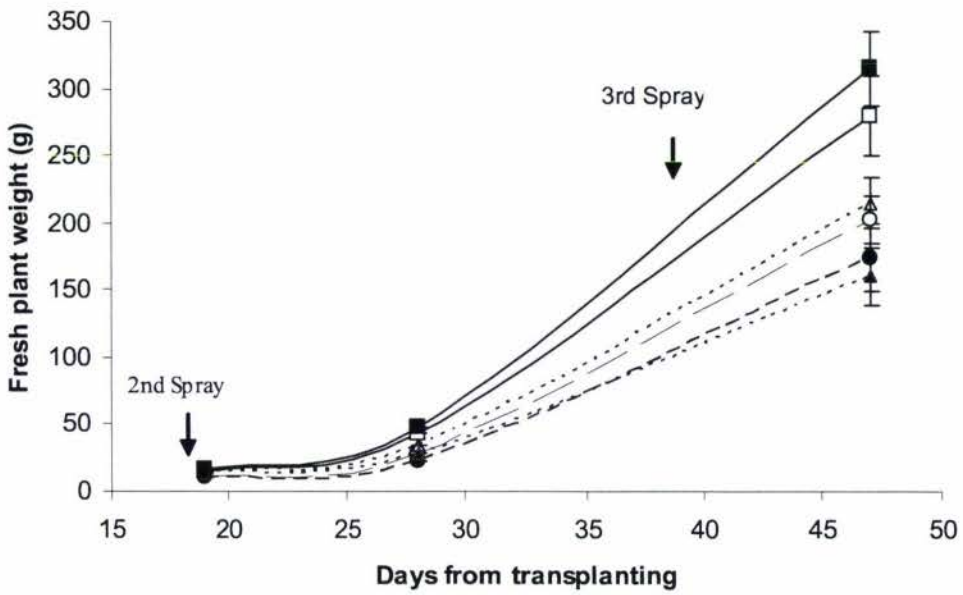


Fig. 4.4.1 Whole plant fresh weight at 3 harvest times – mean of 8 plants/treatment at 19 and 28 days after transplanting (DAT) and 12 plants/treatment at 47 DAT. Treatments were○ = Ctrl, ● = C+sp, △ = DC, ▲ = DC+sp, □ = Org, ■ = Bd. Treatment codes are shown in Table 3.3. Times for the 2nd and 3rd silica spray are shown by arrows. Bars show standard error of means.

At the first harvest, at 19 days after transplanting (DAT), the plants in composted plots had highest weights, followed by those in the DAPCAN treatment (Figure 4.4.1 and Table 4.4.1). At the second and third harvests at 28 and 47 DAT respectively, the compost treatment plants had significantly higher fresh weights than other treatments. At the third harvest, lettuces from the Control + sprays and DAPCAN + sprays treatments had significantly lower fresh weights than those from the Biodynamic treatment.

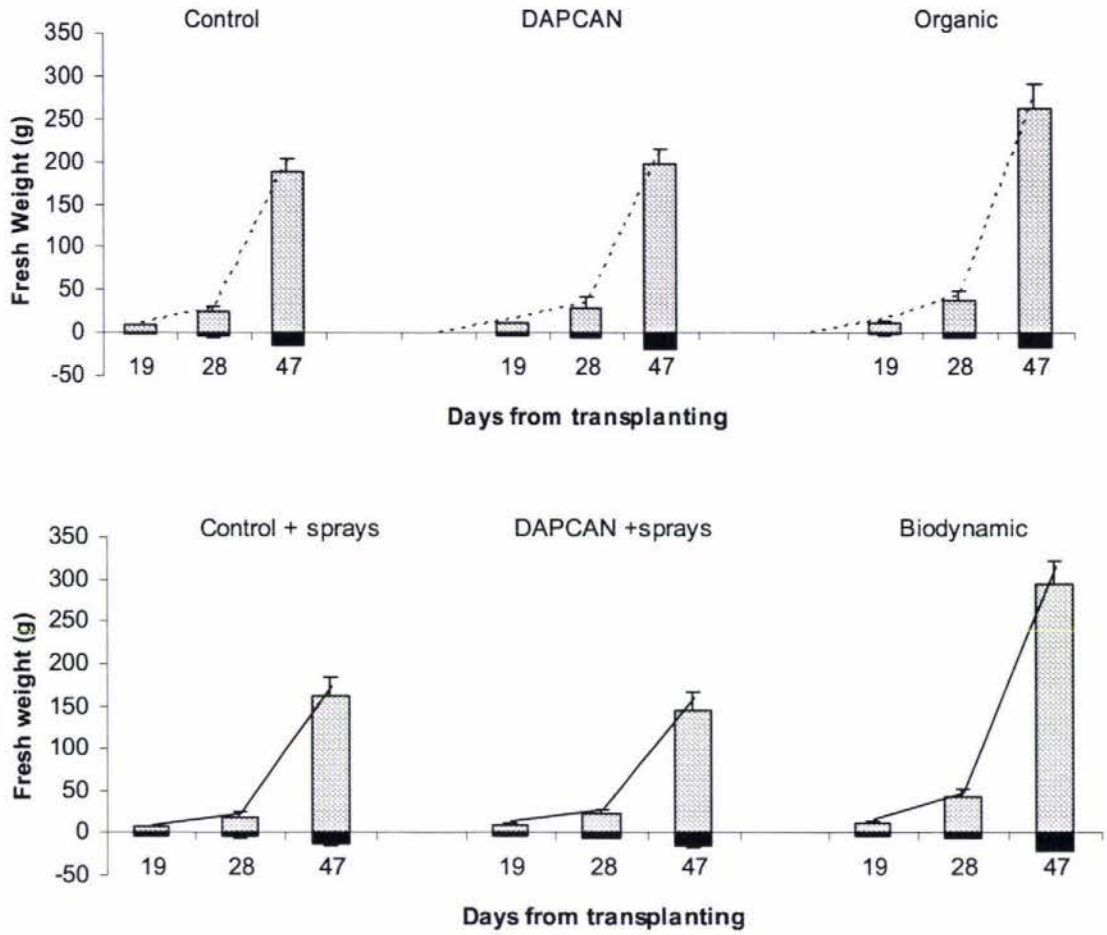
Table 4.4.1 Fresh plant weights and root weight : shoot weight ratios at 28 and 47 days after transplanting (DAT). Mean of 8 plants/treatment at 19, 28 DAT and 12 plants/treatment at 47 DAT

Treatment	Mean plant fresh weight (g)		Root: shoot ratio	
	28 DAT	47 DAT	28 DAT	47 DAT
Ctrl	28.94 cd	202.75 bc	0.186 ab	0.082 ab
C + sp	22.25 d	174.19 c	0.206 a	0.085 ab
DC	36.75 bc	214.92 bc	0.191 ab	0.098 a
DC + sp	27.38 cd	160.23 c	0.230 a	0.109 a
Org	43.75 ab	279.37 ab	0.146 bc	0.068 b
Bd	47.44 a	315.08 a	0.133 c	0.072 b
Fertiliser				
None	25.59 b	188.47 b	0.196 a	0.084 b
DAPCAN	32.06 b	187.57 b	0.210 a	0.103 a
Compost	45.59 a	297.22 a	0.139 b	0.070 b
Spray*				
No sprays	32.84 a	208.83 a	0.188 a	0.093 a
Sprays	24.81 a	167.21 a	0.220 a	0.100 a

Letters indicate differences between means by t-test at 5% level. Means with the same letter are not significantly different.
* Means of control and DAPCAN treatments only

All results shown in Section 4 tables were analysed as described in Section 3.20. Very few differences were significant by Tukey test at 5% level. Letters shown in the results tables indicate differences at 5% level by t-test. It is recognized that this does not provide a definitive measure of statistically significant differences. Throughout the results shown in Section 4, large variability between plants within treatments (and interactions between the 2 variable management factors, fertiliser amendments and biodynamic field sprays) may have prevented many significant differences being shown. Compost treatment data were not included in the grouped spray v no spray analysis because different composts were used for organic and biodynamic treatments.

Mean treatment differences between root and shoot fresh weights are shown in Figures 4.4.2 and 4.4.3.



Figs. 4.4.2, 4.4.3 Mean fresh weights of shoots (above line) and roots (below line) of lettuce plants in each treatment at 19, 28 and 47 days from transplanting. Bars show standard error of mean plant fresh weights.

Root: shoot ratios

The ratios of root fresh weight to shoot fresh weight at each harvest time (Table 4.4.1, Figure 4.4.4) show that the Organic and Biodynamic treatment plants had significantly lower ratios and the Control + sprays and DAPCAN + sprays treatments significantly higher ratios, indicating that the former plants grew more efficiently than the latter.

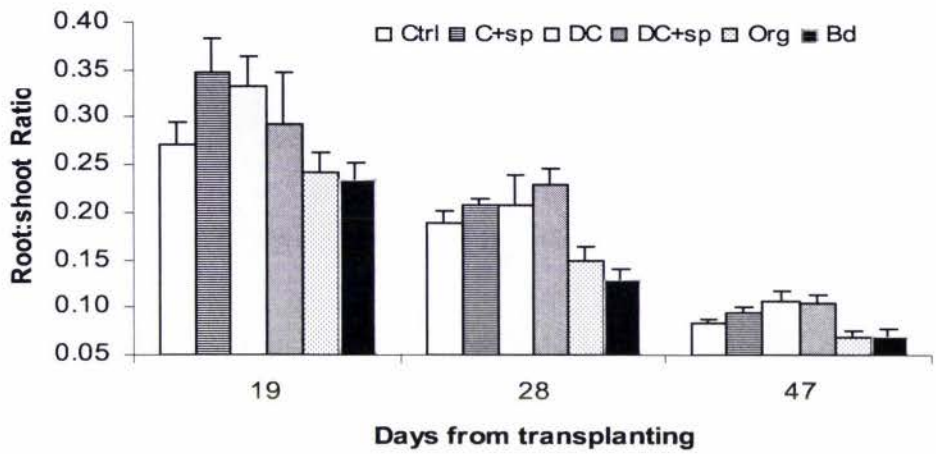


Fig. 4.4.4 Ratio of root wet weight (g) to shoot wet weight (g). Mean of 4 plants/treatment at 19 DAT,8 plants/treatment at 28 DAT and 12 plants per treatment at 47 DAT. Bars show standard error of means. Treatment codes are shown in Table 3.3

Canopy cover

Canopy cover (effective leaf area), measured by taking photos from above, shows significant differences between treatments (Figure 4.4.5, Table 4.4.2).

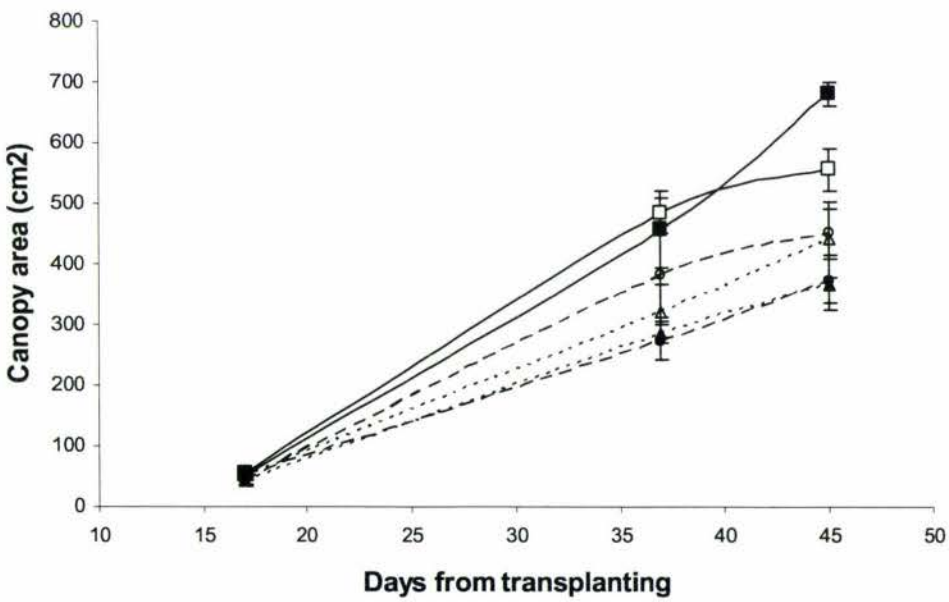


Fig. 4.4.5 Canopy cover per lettuce head, means of 4 plants per treatment at 17, 37 and 45 days from transplanting (DAT). Treatments were ○ = Ctrl, ● = C+sp, △ = DC, ▲ - = DC+sp, □ = Org, ■ = Bd. Treatment codes are shown in Table 3.3. Bars show standard error of means.

Plants in management treatments given compost had significantly greater canopy cover at 37 and 45 DAT. At 45 DAT lettuces in the Biodynamic treatment covered the greatest area and those in Control + sprays and DAPCAN + sprays treatments the least

area. Effect of and interactions between the 2 variable management factors, fertiliser amendment and silica spray can be seen in canopy cover measurements. The greater leaf area in the compost treatments indicates that the compost promoted horizontal growth whereas the silica spray on treatments not receiving compost appeared to reduce horizontal growth. This finding is consistent with observation that the spray treatments had a slightly more upright form and with earlier research (Koepf, 1993) that found that the silica spray promotes more vertical growth, shown by long, less divided roots and small short leaves, rather than horizontal growth. It is also consistent with the tendency for the plants to be smaller in the spray treatments. However, it is interesting to see that the Organic and DAPCAN treatments had slowed down in horizontal growth relative to other treatments, by the last measurement whereas the Biodynamic treatment lettuces kept growing. This is consistent with anecdotal evidence from pastoral farmers who find that application of the biodynamic preparations extends the grass-growing season.

Table 4.4.2 Lettuce canopy cover (cm^2, mean of 4 plants per treatment) at 37 and 45 DAT and lettuce longest root length (cm, mean of 8 plants per treatment) at 28 DAT and mean of 12 plants per treatment at 47 DAT. Treatment codes as in Table 3.3.

Treatment	Canopy cover (cm^2)		Root length (cm)	
	37 DAT	45 DAT	28 DAT	47 DAT
Ctrl	381.1 ab	451.8 c	17.8 ab	26.1 a
C + sp	273.4 b	371.8 cd	15.4 b	27.2 a
DC	321.7 b	441.1 cd	16.8 ab	25.7 a
DC + sp	284.2 b	366.5 d	16.8 ab	24.9 a
Org	486.0 a	556.2 b	15.3 b	25.8 a
Bd	456.2 a	680.3 a	19.1 a	28.7 a
Fertiliser				
None	327.3 b	411.8 b	16.6 a	26.6 a
DAPCAN	303.0 b	403.8 b	16.8 a	25.3 a
Compost	471.1 a	618.3 a	17.2 a	27.3 a
Spray*				
No sprays	351.4 a	446.4 a	17.3 a	25.9 a
sprays	278.8 a	369.2 b	16.1 a	26.0 a

Letters indicate differences between means by t-test at 5% level. Means with the same letter are not significantly different.
* Means of control and DAPCAN treatments only

Root length

Significant treatment differences in longest root lengths of harvested plants were recorded for the 28 DAT harvest but not for the 15 DAT and 47 DAT harvests (Table 4.4.2, Figure 4.4.6).

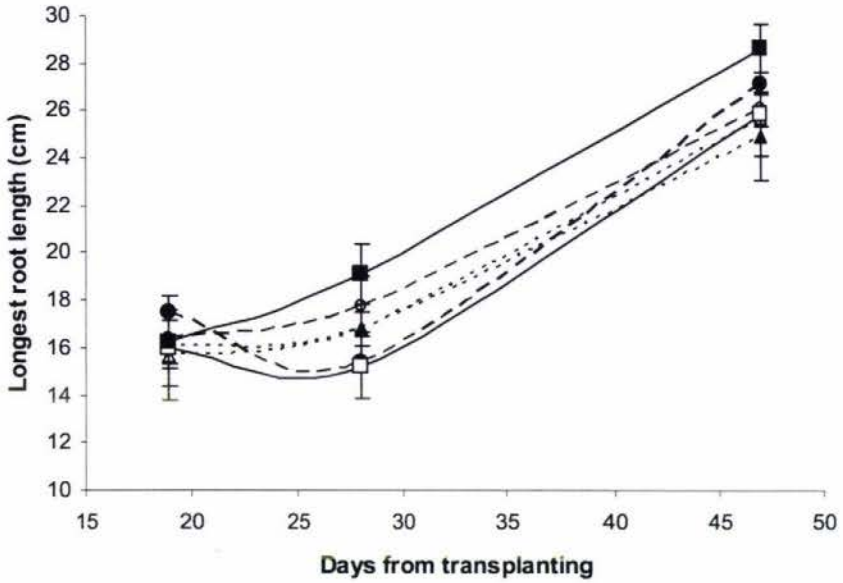


Fig. 4.4.6 Effect of management treatment on longest root length measured at each harvest time, 19, 28 days from transplanting (DAT). (mean of 8 plants per treatment) and 47 DAT (mean of 12 plants per treatment). Treatments were ○ = Ctrl, ● = C+ sp, △ = DC, ▲ = DC+ sp, □ = Org, ■ = Bd. Treatment codes are shown in Table 3.3. Bars show standard error of means.

At 28 DAT the Biodynamic treatment plants had significantly longer roots than plants from the Organic and Control + sprays treatments. At 47 DAT there were no significant differences. As a wider group, application of silica sprays did not significantly increase the length of the longest roots compared to the same treatment not sprayed (Table 4.4.2), which is inconsistent with observations by Koepf, (1993) (Table 2.3.3) that application of the silica spray may increase root length. Such results, however, could be expected to be dependent upon the underlying soil physical and chemical condition. That no effects were observed in this trial may indicate that the soil alone presented few limiting factors to root growth.

Root profile measurements

In general, the treatments that had shorter roots were found to have greater number of roots, as measured by root profiles (Figure 4.3.7).

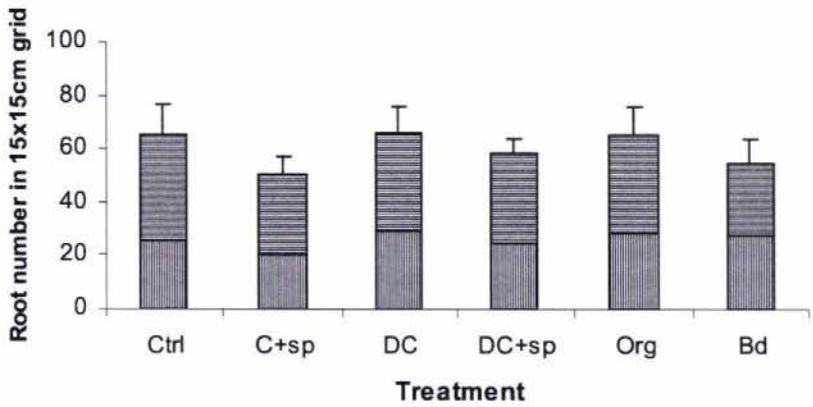


Fig. 4.4.7. Root numbers counted in a 15 cm² grid placed 2 cm from the outer edge of plant leaves of one plant per plot at 43 days from transplanting. ▥ = 7.5 – 15 cm below ground, ▤ = 0 – 7.5 cm below ground. Bars indicate standard error of means.

There was considerable within treatment variability in root numbers. In general it appeared that plants in treatments that received the silica spray had fewer numbers of roots, indicating that the spray may have inhibited secondary root growth.

4.4.2 Dry matter content of plants

Analysis of the % content of dry matter in roots and heads showed significant treatment differences at 28 DAT, but less difference at 47 DAT (Table 4.4.3, Figure 4.4.8).

Table 4.4.3. Dry Matter (DM) % of heads and roots at 28 and 47 days from transplanting (DAT) Means of 8 plants (28 DAT) and 12 plants (47 DAT) for each treatment. Treatment codes in Table 3.3.

Treatment	Head DM%		Root DM%	
	28 DAT	47 DAT	28 DAT	47 DAT
Ctrl	6.23 b	6.02 ab	6.63 ab +	9.93 a
C + sp	7.36 a	5.90 b	7.53 ab	10.80 a
DAPCAN	6.43 b	5.32 c	6.94 ab	11.81 a
DC + sp	7.21 a	6.38 a	8.39 a	11.89 a
Organic	6.19 bc	5.60 bc	6.61 ab	10.35 a
Biodynamic	5.50 c	5.97 ab	6.31 b	10.61 a
Fertiliser				
None	6.80 a	5.96 a	7.08 a	10.36 b +
DAPCAN	6.82 a	5.85 a	7.66 a	11.85 a
Compost	5.84 b	5.79 a	6.49 a	10.48 ab
Spray*				
No sprays	7.35 a	5.67 b	6.78 a	10.87 a
sprays	6.40 b	6.14 a	7.96 a	11.35 a

* Means of control and DAPCAN treatments only

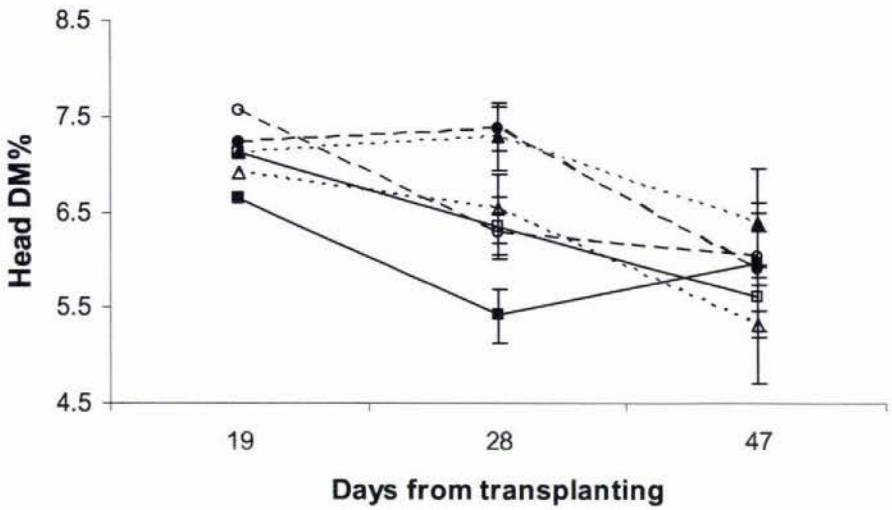


Fig. 4.4.8 Head dry matter (DM) % of plants from each treatment. Mean of 4 plants at 19 days from transplanting (DAT), mean of 6 plants at 28 DAT, mean of 12 plants at 47 DAT. Treatments were ○ = Ctrl, ● = C+ sp, △ = DC, ▲ = DC+ sp, □ = Org, ■ = Bd. Treatment codes are shown in Table 3.3. Bars show standard error of means.

At 28 DAT from planting the dry matter % of both heads and roots was significantly higher for the Control + sprays and DAPCAN + sprays treated plants than the biodynamic plants, reflecting their "condensed" appearance. By 47 DAT there was less difference between treatments, although plants of the DAPCAN + sprays treatment contained significantly higher shoot DM% compared to Control + sprays plants. At 47 DAT the DAPCAN treatment plants also contained significantly higher root DM% compared to control treatment plants but there was no significant effect of spray treatment. The lower DM% of the Control + sprays shoots indicated they recovered better from the water stressed condition recorded at 28 DAT than had the DAPCAN + sprays plants. High root DM, and high root: shoot ratio (Table 4.4.1), indicate less efficiency in leaf production of DAPCAN treatment plants compared to plants in other treatments. It is noted that whereas spray treatment was associated with significant DM % increase in the DAPCAN treatment at 47 DAT, spray did not result in such a large DM% increase in Control plants.

The lack of differences in % DM content of heads at 47 DAT is reflected in the fact that regression analysis shows a linear relationship between head fresh weight and dry weight at 47 DAT (Figure 4.4.9).

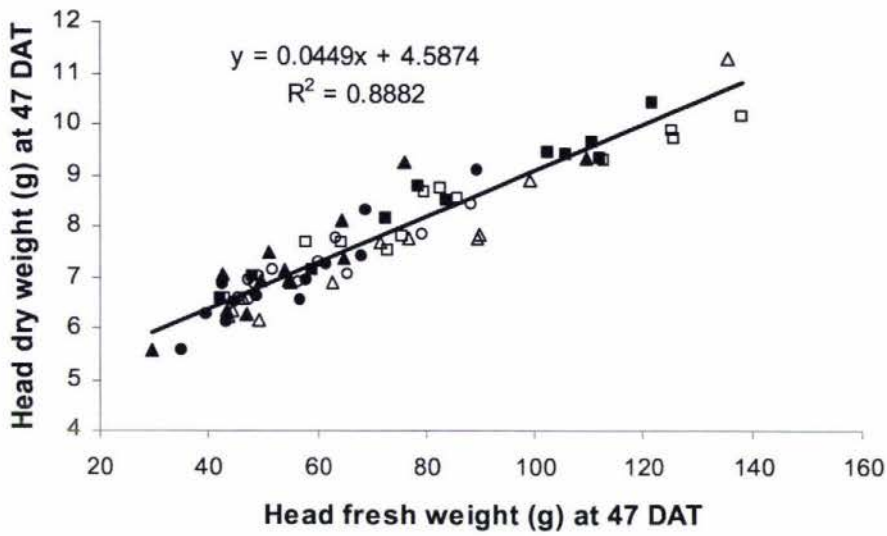


Fig. 4.4.9 Relationship of lettuce head dry weight (g) with head wet weight (g) at 47 DAT Treatments were ○ = Ctrl, ● = C+ sp, △ = DC, ▲ = DC+ sp, □ = Org, ■ = Bd. Treatment codes are shown in Table 3.3. Trend-line, equation and R^2 value are for all treatments combined.

Analysis of dry matter content is helpful for comparing nutrient concentrations between treatments. Organically grown plants have often been found to contain higher DM% (eg Mäder et al., 1992). However, from a food consumption point of view, nutrient content on a fresh weight basis is more relevant, particularly for a leafy vegetable such as lettuce, and is therefore preferable for comparison of nutrient value between treatments (Heaton, 2002).

4.4.3. Can pre-planting mustard yield be used as a co-variate to control variation in lettuce yield caused by plot and block differences?

Mustard harvest weights for each plot were also included as a covariate in the analysis of variance of lettuce yields to see if they accounted for some of the variability. The mustard yield, however, accounted for a negligible plot to plot variation in lettuce yield. Regression analysis of mustard yield with weight of lettuce plants harvested showed no significant relationship. There was slightly more relationship with lettuce weights at 28 DAT (Figure 4.4.10) than with weights at 47 DAT.

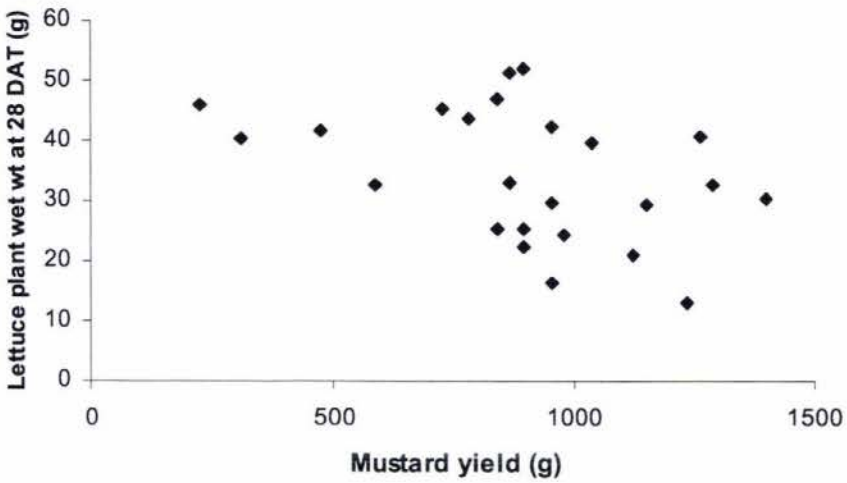


Fig. 4.4.10 Relationship between lettuce whole plant fresh weight (mean of 2 plants per plot) at 28 days from transplanting (DAT) and mustard yield from an area 69x76 cm in the centre of each plot 21 days before lettuces were transplanted into those plots.

There was, if anything, a slight inverse relationship between mustard yield and lettuce weights at 28 DAT from the same plots. This indicates that although the mustard yield was not a good predictor of lettuce yield, it may have removed some of the available nitrogen, resulting in less nitrogen availability in plots that had highest mustard yield. The lack of relationship also indicates that different management treatments could have had more effect on lettuce growth than the quantity of available nitrogen in the soil at the beginning of the trial.

4.5. Elemental analysis of leaves

Element, protein and vitamin content are determinants of lettuce product quality. The concentration of major and minor elements in lettuce leaf dry matter was analysed in material harvested at 28 and 47 DAT. The results are discussed in regard to how soil climatic conditions and treatments of compost, soluble fertiliser and biodynamic field sprays could have affected uptake and leaf concentrations of each element. Implications for product quality are also discussed.

4.5.1 Nitrogen content of lettuce leaves

At 28 days from transplanting the nitrogen (N) percentage of leaf dry matter was significantly higher for compost treatments and significantly lower in the Control+sprays treatment (Figure 4.5.1 and Table 4.5.1). At 47 DAT there were no significant differences in leaf % N between treatments, but the Control+sprays treatment showed a possible trend to the highest concentration.

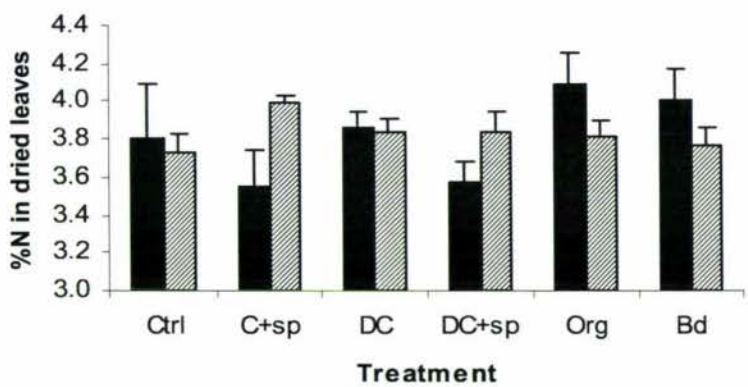


Fig. 4.5.1 Percentage N in leaf dry matter for lettuces harvested at 28 (■) and 47 (▨) days after transplanting (DAT). Bars represent standard error of means. Ctrl = Control, C+sp = Control+ sprays, DC = DAPCAN, DC+sp = DAPCAN + sprays, Org = Organic, Bd = Biodynamic

At 28 and 47 DAT the %N in dried lettuce leaves ranged between 3.58 and 4.09%, values considered to reflect adequate N nutrition (HortResearch, 1995, see Table 4.5.2). A decline in % N with increasing plant weight generally occurs (Greenwood, 1983).

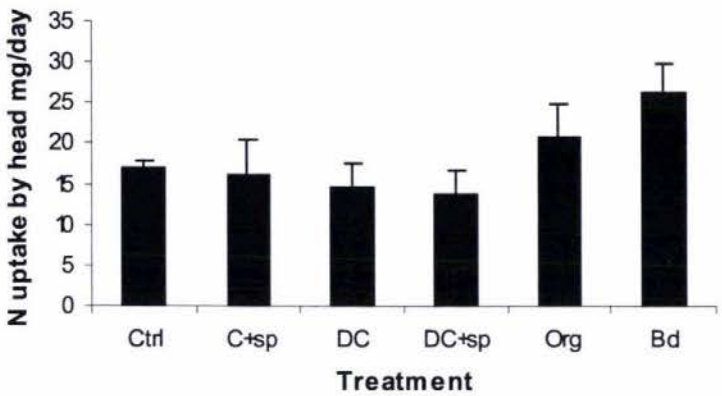


Fig. 4.5.2 Nitrogen uptake per lettuce head (mg N per day per plant head) for the growth period between 28 – 47 DAT. (Treatment codes as for Fig 4.5.1). Bars represent standard error of means.

Lettuces treated biodynamically (Bd), followed by the organic lettuces (Org), had the fastest N uptake rates between 28 and 47 days (Figure 4.5.2). Uptake rate by lettuces in the biodynamic treatment was significantly higher than that of lettuces in the DAPCAN treatments (Table 4.5.1). It is not known whether rate of uptake had slowed by the second harvest date, as leaf N analysis was for 2 harvests only. Measurement of plant N and calculation of N uptake does not take account of leaf nitrate (NO_3-N), which is not included in Kjeldahl digestion. The NO_3-N contribution to total leaf N was small (4 - 5% of total leaf N, Table 4.5.3), based on leaf dry matter and extractable NO_3–N (measured in Section 4.5.3). The leaf NO_3-N contents at 47 DAT ranged from 0.14 – 0.2% (Table 4.53). (The small difference in leaf % N uptake (Figure 4.5.1) between control and fertilized plots indicates that nitrogen content of the soil was not a limiting factor for lettuce growth). If soil N had been more limiting, treatment effects on plant %N uptake may have been more evident.

Table 4.5.1 Nitrogen and protein content of lettuce leaves at 28 and 47 DAT (Treatment codes in Table 3.3)

Treatment	%N (dry wt)		N uptake (mg/plant/day)	Protein content (mg/100g fresh wt)	
	28 DAT	47 DAT	28-47 DAT	28 DAT	47 DAT
Control	3.81 abc	3.75 a	16.91 ab	1.47 ab	1.40 ab
C + sprays	3.55 c +	3.99 a	16.12 ab	1.62 a +	1.47 a
DAPCAN	3.85 abc	3.84 a	14.53 b	1.54 ab	1.27 c
DC + sp	3.58 bc	3.84 a	13.77 b	1.61 a+	1.48 a
Organic	4.09 a +	3.81 a	20.69 ab	1.57 ab	1.34 bc
Biodynamic	4.01 ab	3.77 a	26.11 a	1.43 b +	1.38 abc
Fertiliser					
None	3.68 b	3.87 a	16.51 ab	1.55 a	1.44 a
DAPCAN	3.71 b	3.84 a	14.15 b	1.57 a	1.38 a
Compost	4.05 a +	3.79 a	23.40 a	1.50 a	1.36 a
Spray*					
No sprays	3.83 a	3.79 a	15.72 a	1.51 a	1.34 b
Sprays	3.561 a	3.91 a	14.94 a	1.62 a	1.47 a

Different letters indicate significant difference by t-test at 5% level. + indicates significant difference by Tukey test at 5% level.
* Means of control and DAPCAN treatments only

4.5.2 Crude protein content of leaves

Crude protein in 100g fresh leaves was estimated by multiplying % N in dried leaves by 6.25 and by % dry matter.

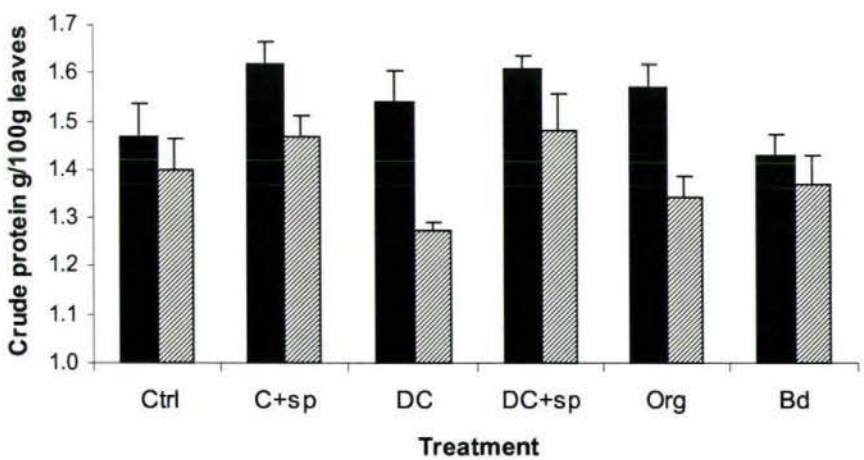


Fig. 4.5.3 Estimated crude protein content of fresh lettuce leaves (mg/100 g head) at 28 (■) and 47 DAT (▨) (Treatment codes in Fig 4.5.1). Bars represent standard error of means

Estimated crude protein content (Figure 4.5.3 and Table 4.5.1) shows larger differences between treatments at both harvest times and changes between harvests than the

corresponding differences in % N content. This difference reflects the difference in lettuce weights and dry matter content at those times. At the final harvest, 47 DAT, treatments receiving field sprays contained significantly more protein than those not receiving sprays. It is not known how much of this was true protein as total N analysis does not differentiate between protein and other nitrogenous compounds such as free amino acid content. However, this result indicates that there was greater activity of nitrogen assimilation enzymes such as nitrate reductase, in sprayed plants.

All treatments had higher crude protein content than the average for field lettuces in New Zealand Food Composition Tables (Athar, 2001), but lower than average for hydroponic lettuce types (1.1 g/100 g and 1.9 g/100 g fresh leaves respectively, see Table 2.4.1).

Relationships between N uptake, root size and canopy area

The soil treatment effect on N uptake (Figure 4.5.2) could result either from differences in the amount of plant available N supplied by soil amendments or from the amendment stimulating soil organic matter decomposition or root growth. Relationships between plant properties and N uptake were investigated. Strong relationships existed between the total amount of N per lettuce head and plant root wet weight (Figure 4.5.4).

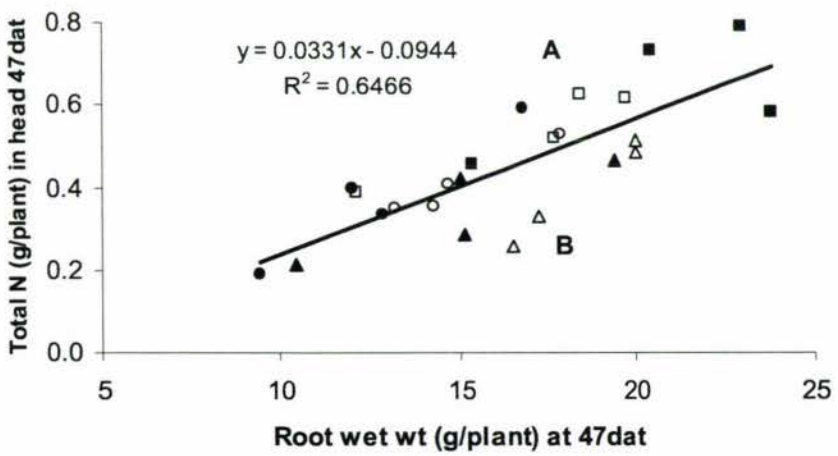


Fig. 4.5.4 Relationship between estimated total N content of lettuce head and root wet weight at 47 DAT. Regression line, equation and R^2 for all treatments. Symbols show treatment levels. Control (○) Control + sprays (●) DAPCAN(△) DAPCAN + sprays (▲) Organic (□) and Biodynamic (■) treatments.

Higher root masses and N uptake were associated with the compost treatments (Figure 4.5.4). Most of the A outliers (above the line) were compost treatment plants, and there was a consistent treatment effect of DAPCAN on B outliers (below the line).

There was no significant relationship between N uptake and root length (data not shown). A relationship was found between lettuce canopy cover and N content of lettuce heads (Figure 4.5.5), indicating that plants that took up more nitrogen grew bigger.

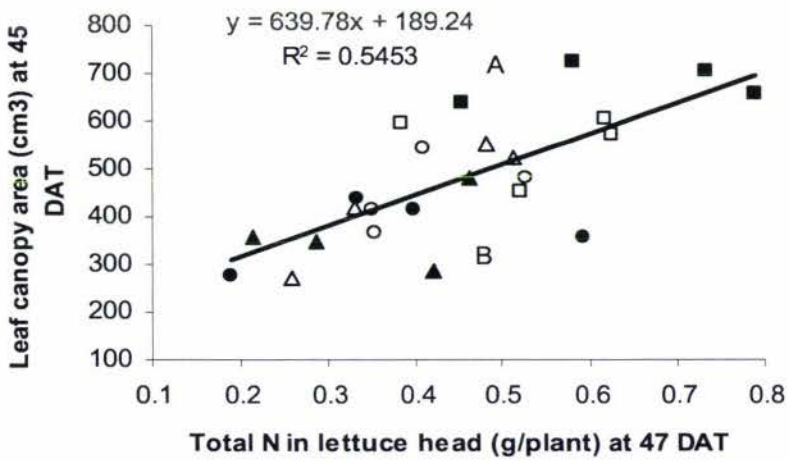


Fig. 4.5.5 Relationship between estimated total N content of lettuce head at 47 DAT and leaf canopy area at 45 DAT as estimated from photos taken from above. Regression line, equation and R^2 for all treatments. Control (○) Control + sprays (●) DAPCAN(△) DAPCAN + sprays (▲) Organic (□) and Biodynamic (■) treatments.

In this relationship, the 'A' outliers (above the regression line) were consistently compost treatments, whereas the 'B' outliers below the line were 2 replicates of the spray treatment (with control and with DAPCAN)(Figure 4.5.5). The compost treated plants (Organic and Biodynamic) in general were larger plants, and had higher leaf N concentrations (Figures 4.5.4, 4.5.5). It is not known whether more nitrogen was made available through decomposition mediated by soil organisms in the compost treatments, or improved water retention by compost, made N more available. The compost could have introduced a greater diversity of organisms (e.g. more protozoa and nematodes), which could have increased nitrogen release from micro-organisms (Ingham, 1998). Soil in biodynamic and organic systems has been found to have higher biodiversity, which has been related to greater efficiency of nutrient utilisation (Mäder et al., 2002).

Possibly, growth stimulants from humic materials and/or soil organisms stimulated plant and/or microbial growth, enabling more N uptake. Humic substances appear to increase the ATPase enzyme activity and the intake of nitrate ions through plant cell membranes by plasma membrane proton pumps (Pinton et al., 1999). Humic substances also increase microbial activity, releasing more N for plant uptake (Figliola et al., 1994).

Relationships between nitrogen uptake and soil respiration and AM fungi colonisation

The potential for soil treatments to stimulate microbial decomposition activity (soil respiration) and thereby increase N mineralisation was investigated by comparing plant N uptake between 28 and 47 days and soil respiration activity at 28 days.

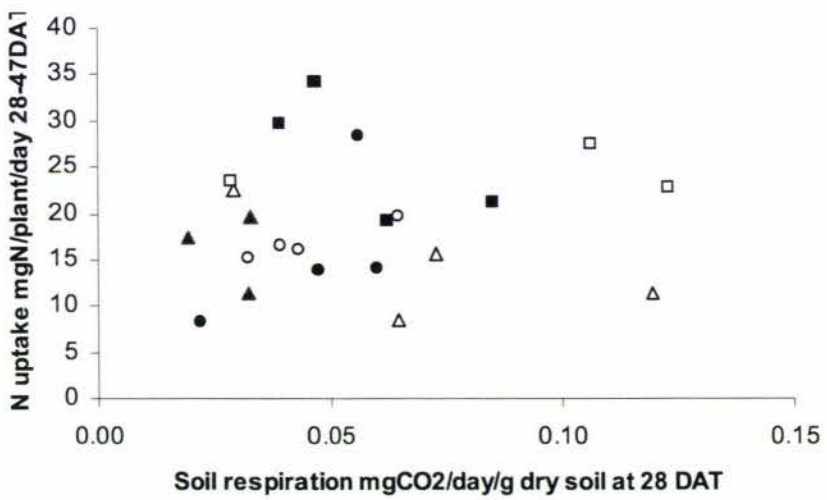


Fig. 4.5.6 Relationship between nitrogen uptake/plant from 28 to 47 DAT and soil respiration at 28 DAT for Control (○) Control + sprays (●) DAPCAN(△) DAPCAN + sprays (▲) Organic (□) and Biodynamic (■) treatments.

There was no relationship between nitrogen uptake and soil respiration Figure 4.5.6. However, the graph is included because it shows the wide range of respiration rates within soil treatment types. Relationships between soil respiration rate and supply of plant available N in composted soil are likely to be complex. High rates of respiration may reflect a phase of rapid microbial growth and N immobilisation rather than mineralisation and release of plant available N (this is discussed further in Section

4.7.1). In addition, soil respiration rates measured at one point in time ex-situ may not fairly reflect decomposition activity over a period of plant growth.

..

Apparent different non-significant trends of control treatments compared to DAPCAN and compost amendment treatments in Figure 4.5.6 may relate to different C:N ratios and/or different microbial populations in different treatments. Such differences could have resulted in different rates of nitrogen release and net mineralisation. However, the large variability within treatments and limitations of the one soil respiration test prevent any conclusions being drawn.

There was no relationship between nitrogen uptake and AM fungi colonisation (data not shown). Azcon et al (1996) found that inoculation of a sterilized loam soil low in organic matter with *Glomus fasciculatum* increased uptake of nitrate, nitrate reductase activity and protein content of lettuces compared with uncolonised plants. However, AM fungi colonisation in plant roots and their contribution to N uptake is increased by lower soil N and P status (Alloush et al., 2000). As soil N and P content were higher in my trial, AM fungi are unlikely to have had a large role in N uptake.

A further possible factor in the higher N uptake of lettuces in composted plots is that more N as ammonium and/or amino acid was available from the compost. A combination of ammonium and nitrate-N results in highest growth rates (Mengel and Kirkby, 1982).

4.5.3 Leaf nitrate content

Nitrate concentration in leaf sap was tested using Merck strips and by acetic acid extraction of dried leaf material to see if differences in N assimilation could be detected. Merck strip tests showed considerable variation in nitrate levels (Table 4.5.2, Appendix I). Note that for some measurement times only 2 or 3 replicates of each treatment were tested and within treatment variation was quite high.

Results of Merck strip tests at 21, 39 and 45 DAT were from 3-4 treatment replications and were therefore analysed for covariance by a SAS mixed procedure which is used for repeated measurements on the same plants. The data for 39 DAT showed some

significant differences between treatments, but there were none at the 45 DAT testing time (Table 4.5.3)

Table 4.5.3 Mean nitrate levels in leaf sap 39 DAT at 7pm (Merck strip) and in dried leaf material at 28 and 47 DAT and nitrate N: total N ratios (Treatment codes in Table 3.3).

Treatment	NO_3 mg/l in cell sap (Merck strip)		%NO_3-N in dry lvs (acetic acid extracted)		NO_3-N :tot N*	
	39 DAT	45 DAT	28 DAT	47 DAT	28 DAT	47 DAT
Ctrl	633.3 ab	825.0 a	0.203 a	0.143 a	0.053 a	0.038 a
C + sp	433.3 c	850.0 a	0.145 b	0.194 a	0.040 bc	0.049 a
DC	700 a	1050.0 a	0.179 ab	0.199 a	0.046abc	0.052 a
DC + sp	600 ab	975.0 a	0.132 b	0.184 a	0.037 c	0.048 a
Org	500 bc	900.0 a	0.208 a	0.177 a	0.051 ab	0.047 a
Bd	533.3 bc	875.0 a	0.230 a	0.136 a	0.058 a	0.036 a
Fertiliser						
Control	533.3 b	837.5 a	0.174 b	0.168 a	0.047 ab	0.043 a
DAPCAN	650 a	1012.5 a	0.155 b	0.192 a	0.042 b	0.050 a
compost	516.7 b	887.5 a	0.219 a+	0.157 a	0.054 a	0.041 a
Spray**						
No sprays	666.7 a	937.5 a	0.191 a	0.171 a	0.050 a	0.045 a
Sprays	516.7 a	912.5 a	0.139 b	0.189 a	0.039 a	0.048 a

* Calculated from acetic acid extraction results
** Means of control and DAPCAN treatments only
Different letters indicate significant difference by t-test at 5% level.
+ indicates significant difference by Tukey test at 5% level.

There was a significant difference in sap NO_3 concentration ($p = 0.0131$) between DAPCAN, compost and control treatments and between the DAPCAN and Control + sprays treatments for sap tests at 39 DAT, and there were no significant differences between treatments at 45 DAT. The generally high nitrate levels on 9.12.02 (47 DAT) could have resulted from a flush of N mineralisation in the soil due to significant rainfall after a dry period. Lower nitrate levels with increased lettuce maturity are generally expected.

Nitrate levels vary with intensity of solar radiation during the day (Maynard et al., 1976). Tests made in 2 sequences, at the same time of day on different days and at different times of day appeared to show some differences between treatments, but as most of the means plotted are not from full data sets and there were few significant differences between means, the graph in figure 4.5.7 only indicates possible differences.

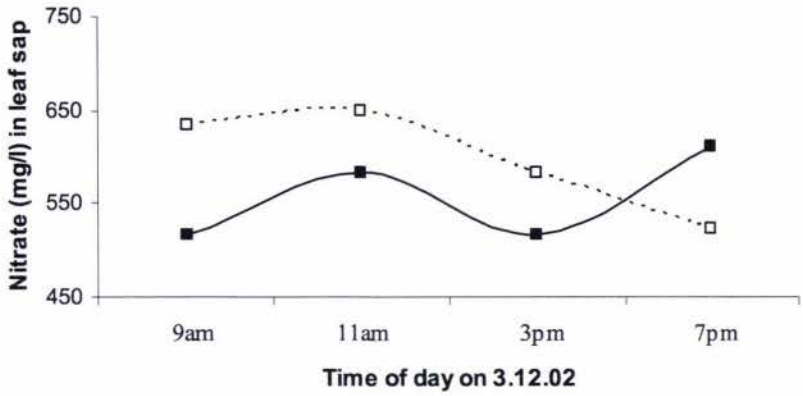


Fig 4.5.7 Variation in diurnal fluctuation of mean leaf sap nitrate contents by spray (■) and non-spray (□) treatments

There were also greater differences between treatments in leaf NO3-N concentration measured by acetic acid extraction at 28 DAT than at 47 DAT (Table 4.5.3). At 28 DAT leaves in the compost treatments contained significantly more nitrate than those in other treatments, which could mainly relate to a higher N uptake. Nitrate concentration in leaves at 28 DAT was likely mainly related to rate of uptake of nitrate, with generally highest concentrations in plants in the compost treatments, which were bigger, so growing faster. Also, plants with lower nitrate content could have been taking up more of their nitrogen as ammonium. There could have been differences in form of nitrogen taken up between compost and DAPCAN treatments although it was probably all mostly nitrate. However, for plants taking up nitrogen released by microbes in plots with high microbe activity such as in the Organic treatment, there could have been more ammonium or amino acid N relative to NO_3 uptake.

The lettuces from the Biodynamic treatment showed a large change between the 2 harvest dates, from having high nitrate content at 28 DAT, to having much lower mean nitrate content at 47 DAT. This could be because those lettuces had reached maturity and were taking up less nitrate at 47 DAT. At 47 DAT the nitrate content of the Biodynamic lettuces was 46% lower than that of lettuces from the DAPCAN treatment, but as there was so much variability, this was not a statistically (t-test at the 5% level) significant difference.

Conversion of Merck-strip NO_3 (mg NO_3/L) results to NO_3-N (NO_3 as mg N/L) by multiplying by 14/62 and expression of dried leaf NO_3-N concentrations as ppm showed similar levels of concentration for each test method, but there was not good correlation between nitrate levels measured by acetic acid extraction and by Merck strip for the same plots (Figure 4.5.8).

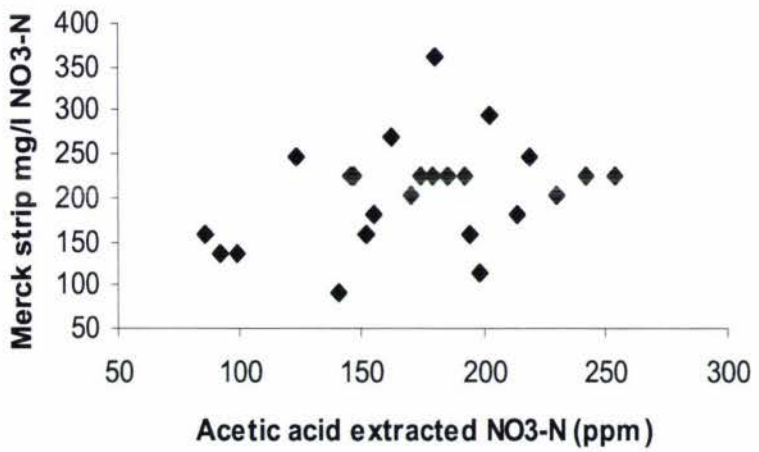


Fig 4.5.8 Relationship between nitrate-N measured by Merck strip in leaf cell sap at 45 DAT and acetic acid extracted nitrate- N at 47 DAT.

The lack of relationship between results of the 2 nitrate testing method for leaves of similar age but harvested 2 days apart (Fig 4.5.8) reflects the large variability in leaf nitrate content between plants within treatments and also indicates that one or both testing methods was probably unreliable. The large within treatment variability can be seen by looking at the pooled variance and treatment F-value, which were 232.6 and 1.2 respectively for the Merck-strip-NO_3, 45 DAT results and 0.044 and 1.32 respectively for the dried leaf NO_3-N 47 DAT results shown in Table 4.5.3. There was no relationship between nitrate content of leaves and plant weight at 47 DAT (data not shown).

Ratio of nitrate N: Total N

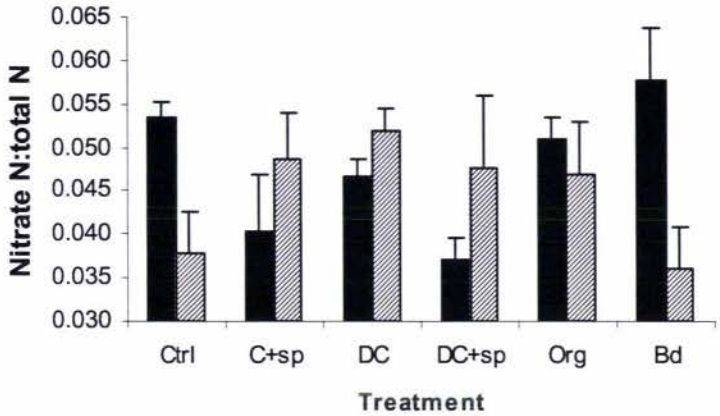


Fig 4.5.9 Ratio of nitrate-N % to total N % in dried leaf material at 28 DAT( ) and 47 DAT( ) Treatment codes as in Table 3.3. Bars represent standard error of means.

The ratio of nitrate % to total N% in dried leaf material (Table 4.5.3) provides an indication of the relative difference between rates of nitrate uptake and rates of assimilation of nitrate into amino acids, protein and other nitrogenous compounds. Fig 4.5.9 indicates that this ratio decreased between 28 and 47 days from transplanting in the Control and Biodynamic treatments, but increased in the Control + sprays and DAPCAN with and without sprays treatments. Statistical analysis revealed that this ratio was not significantly different for the sprayed treatments than the unsprayed treatments at 28 DAT. Within treatments, sprays had some significant effects. For example, the Control + sprays (C+sp) and DAPCAN + sprays (DC+sp) treatments had the lowest ratios. Those lettuces had taken up less nitrogen than in other treatments at 28 DAT. It is possible that the difference mainly related to an increased ability of plants to assimilate nitrogen at lower uptake rates. The differences could also relate to differences in plant metabolism induced by spraying the silica spray. Research discussed by Matt et al. (2002) and Lillo and Appenroth (2001) indicates that changes in light concentrations and wavelengths can affect plant signaling and phosphorylation/dephosphorylation activity, which in turn affect nitrate reductase activity. This could have resulted in differences in quantity and form of amino acid and protein production.

There were no significant differences between treatments in NO_3: total N at 47 DAT (Table 4.5.3). There is therefore no evidence of lower nitrate concentration and higher

crude protein: nitrate ratio in leaves of Biodynamic and other sprayed treatments compared to DAPCAN treatment at 47 DAT. As the soil was initially high in nitrates (see Table 4.1.5, Section 4.1), lettuces of all treatments may have taken up nitrate preferentially to ammonium and most plants contained high levels of nitrate.

Biodynamic literature suggests that spraying the silica spray will increase the "light" or "terrestrial silica" effect in relation to the "dark" calcium effect, which may result in changes in plant metabolism under different nutrient supply conditions. However, there was no general statistically significant difference (at 95% confidence interval) between sprayed and unsprayed treatment NO_3: Total N ratios at 47 DAT, even though the Biodynamic and Control treatments had considerably wider ratios than the ratio for the DAPCAN treatment. The high variability between nitrate and total N concentrations in individual plants within treatments meant that significant differences between treatment ratio means would have to exceed 0.017 (47 % of the lowest treatment mean) being found.

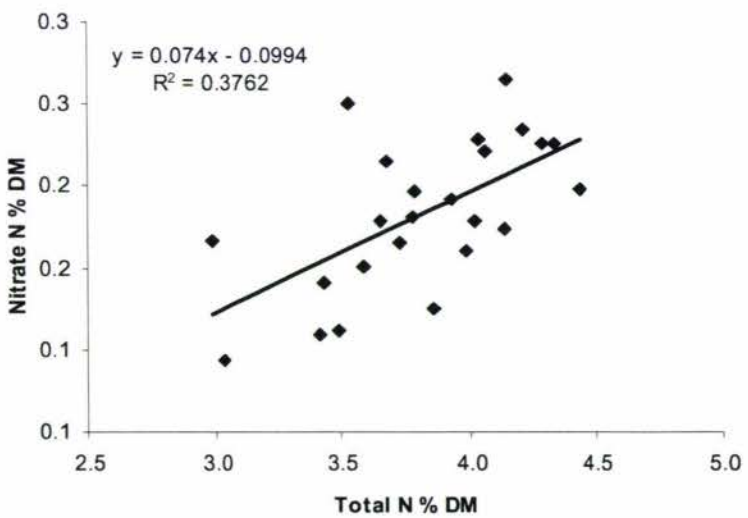


Fig 4.5.10 Relationship between Total N % and Nitrate N % in dried leaf material at 47 DAT

Regression analysis to look for a relationship between nitrate N in leaf sap and total N in dried leaf material (Figure 4.5.10) showed that nitrate as a percentage of plant dry weight was weakly ($R^2 = 0.38$) related to the percentage N content of the plant. The nitrate content variability resulted from either the rate of nitrate supply to the plant or nitrate reductase activity within the plant.

Implications for food quality

The crude protein content of leaves from all trial treatments was high compared to the average for New Zealand field-grown lettuces and compared to that type grown in USA (see table 2.4.1). It is advantageous for human nutrition that lettuces have low levels of nitrate in leaves and higher crude protein: nitrate ratio. Control and biodynamically grown lettuce had the lowest NO_3: Total-N ratios at harvest (47 DAT), which may be a significant nutritional improvement over the other treatments. Due to the large experimental error in this field trial this difference could not be proved to be significant at the 95% confidence interval. A grower however may be prepared to accept a lower confidence interval when assessing the ability of biodynamic treatments to produce lettuces of lower NO_3: Total-N ratios.

4.5.4 Phosphorus content of leaves

At 28 DAT there was a marked range in leaf % phosphorus (P) concentration, ranging from almost adequate (0.37 – 0.38%) in Organic and Biodynamic treatment lettuces to marginally deficient (<0.3%) in the Control treatment lettuces (Figure 4.5.11 and Table 4.5.4).

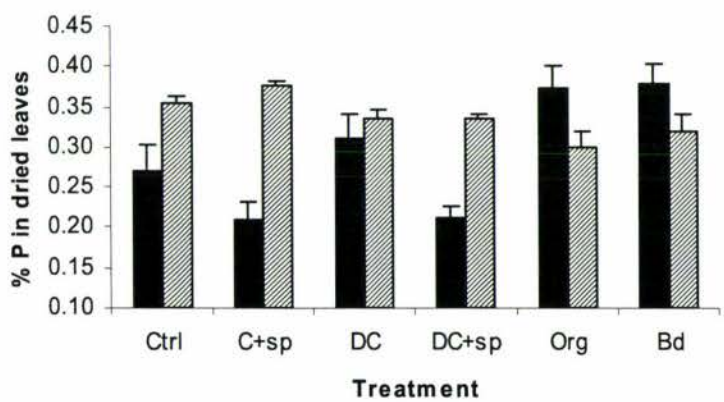


Fig 4.5.11 % P in leaf dry matter for lettuces harvested at 28 (■) and 47 (▨) days after transplanting (DAT). Bars represent standard error of means. Treatment codes are shown in Table 3.3

Table 4.5.4 Phosphorus content of dried lettuce leaves at 28 and 47 DAT. And P uptake 28-47 DAT. Treatment codes as in Table 3.3.

Treatment	%P (dry wt)		P uptake (mg/plant/day)
	28 DAT	47 DAT	28-47 DAT
Ctrl	0.27 bc	0.36 ab	1.75 ab
C + sp	0.21 c	0.38 a	1.64 ab
DC	0.31 ab	0.33 abc	1.28 b
DC + sp	0.21 c	0.34 abc	1.27 b
Org	0.37 a	0.30 c	1.42 ab
Bd	0.38 a	0.32 bc	2.27 a
Fertiliser			
None	0.24 b	0.37 a	1.70 a
DAPCAN	0.26 b	0.34 ab	1.28 a
Compost	0.38 a	0.31 b	1.84 a
Spray*			
No sprays	0.29 a	0.35 a	1.51 a
sprays	0.21 b	0.36 a	1.46 a

* Means of control and DAPCAN treatments only
Different letters indicate significant difference by t-test at 5% level. + indicates significant difference by Tukey test at 5% level.

Plants grown on composted plots had highest % P content at 28 DAT (Table 4.5.4). This could be because of the apparently higher water retention in composted soil relative to uncomposted soil which had a soil water deficit (Figure 4.4.2, Section 4.4). This could have enabled more diffusion of P to roots, and the greater root mass (Figures 4.3.2, 4.3.3) enabled more uptake of soil and compost P, as found by Scaife and Barnes (1977).

All % P concentrations at 28 DAT were low in comparison to the 0.4 -0.6% P levels recommended for new wrapper leaves at half size (HORT Research 1995) It seems possible that the ability of the trial plants to access P was one of the main limitations on growth during the period 0 – 28 DAT. A good supply of P is particularly important for lettuce yield (Ladebusch and Melzer, 1999). P deficiency was particularly evident in the Control+sprays and DAPCAN + sprays treatments at 28 DAT (0.21%P). Low temperatures and low water availability are likely to have affected P supply. The silica spray appears to have led to reduced water uptake, which would have also reduced P uptake.

At 47 DAT the organic treatment plants had significantly lower % P in leaves than those in the control plots with no compost or fertiliser. Calculation of P content per 100g fresh leaves reveals that the %P levels at 47 DAT were at the lower end of the usual range, but not far from the average for similar lettuce types (Table 4.5.1, USDA (1998) and Souci et al. (1994).

The increase in P concentration at 47 DAT compared to 28 DAT for control and DAPCAN treatments can be contrasted with the usual decline in P concentration with increasing lettuce age found when most P is supplied in fertiliser at the beginning of the growth period (Greenwood et al., 1980).

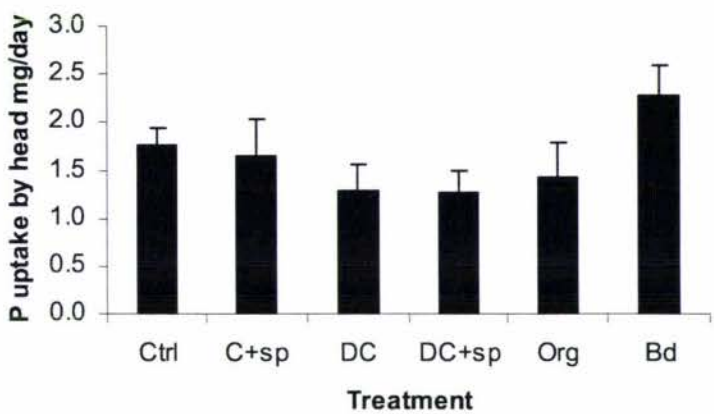


Fig 4.5.12 Phosphorus uptake by lettuce head (mg N per day per plant head) for the growth period between 28 – 47 days after transplanting (DAT). Bars represent standard error of means (Treatment codes as in Table 3.3)

The results shown in Table 4.5.4 and Figure 4.5.12. indicate that P uptake between 28 and 47 DAT was significantly higher for lettuces in the Biodynamic treatment than lettuces in both DAPCAN treatments. P uptake between 28 and 47 DAT was not related to P supply in DAPCAN and compost amendments because % P in control heads at 47 DAT was significantly higher than that in DAPCAN and compost treatment heads. The cause of higher P uptake in the Biodynamic treatment, however, requires further investigation as the effect is not a simple effect of spray application because differences in P uptake compared for sprays vs non-sprays (grouped treatments) (Table 4.5.4) were not significant. It is possible that some interaction between composts and sprays produces the higher P uptake in the Biodynamic treatment. It is likely that P mineralisation by soil organisms enabled more P uptake in the Biodynamic treatment,

whereas added inorganic P may have been made non-plant available by sorption to soil surfaces, or added P (DAPCAN) inhibited the quantity of net P mineralisation.

Relationships between P uptake and size of roots

There was a significant relationship between P uptake and root weight at 47 DAT (Figure 4.5.13), which explained 59% of P uptake.

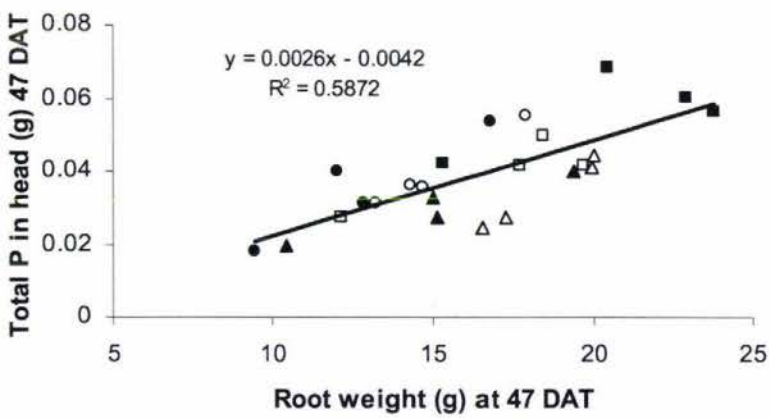


Fig 4.5.13 Relationship between estimated total P content of lettuce head and root wet weight at 47 DAT for all plots. (See figure 4.5.6 for symbol key). Regression line, equation and R^2 for all treatments

Larger root mass would have had a larger surface area enabling more uptake of soluble P which is relatively immobile in soil and can move only by diffusion in soil water to the root (Barber, 1984). It should be noted that 3 of the 4 Biodynamic treatment plots had the highest root mass, which may explain the greater P uptake (28-47 DAT, Table 4.5.5) by the plants in those plots. The higher rate of P uptake in the Biodynamic treatment between 28 and 47 DAT was associated with a high N uptake (Table 4.5.1) and the longest root length (Table 4.4.2).

In addition, increased partitioning of photosynthate to roots has been documented as a response to low P uptake (P deficiency) in some plants (Marschner, 2002). This could have occurred in the Control + sprays and DAPCAN +sprays treatments, which had larger root: shoot ratios (Table 4.3.1) than plants from other treatments.

Relationships between P uptake and soil respiration and AM fungi colonisation

No significant relationship could be found between P uptake and soil respiration

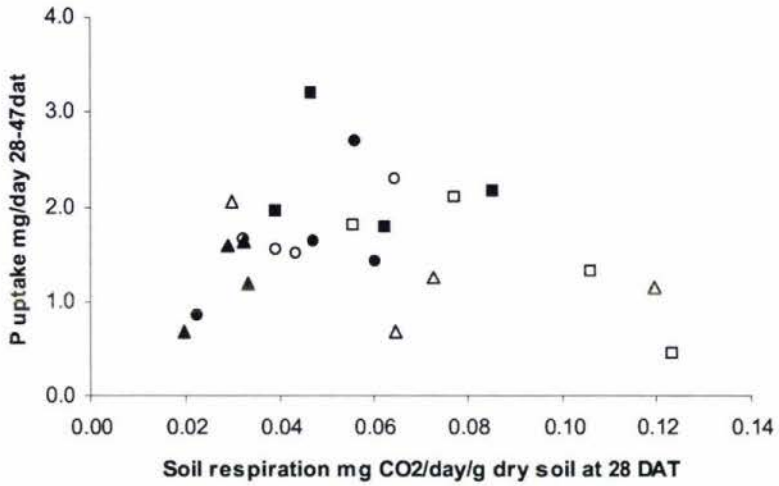


Fig 4.5.14 Relationship between phosphorus uptake from 28 to 47 DAT and soil respiration at 28 DAT (See figure 4.5.6 for symbol key).

Highest soil organism respiration activity (Figure 4.5.14, respiration assay) was not associated with highest P uptake in lettuces. No general conclusions can be drawn from one ex situ soil respiration test at one point in time. More information is required on the C: P ratio of the soil organic matter being mineralised under field respiration conditions.

It is also expected that compost addition to soil may enhance P availability. Oberson (1993) found that mobility of phosphate ions measured by ^{32}P isotopic exchange was higher in manured soil than minerally fertilised plots, thought to be due to different, more labile bonds with organic matter. (The manure effect is normally through organic moieties competing with P for sorption sites and causing more P to be desorbed) However, matured compost made principally from vegetable matter is quite different from manure. Mäder et al. (2000) found phosphorus flux through the microbial biomass was faster in organic soils, and more phosphorus was bound in the microbial biomass and nutrients are less dissolved in the soil solution in organic systems. Oberson (1993) found that soil acid phosphatase and ATP activity was greater on farmyard manure than minerally fertilised plots, where P supply was the same. This is consistent with greater P release into forms available to plants and soil microorganisms. The same processes

could be expected to be enhanced in the Biodynamic treatment and may partly explain the greater P uptake by lettuces.

No relationship of P uptake with AM fungi colonisation could be found, indicating that AM fungi may not have made a major contribution to plant P uptake.

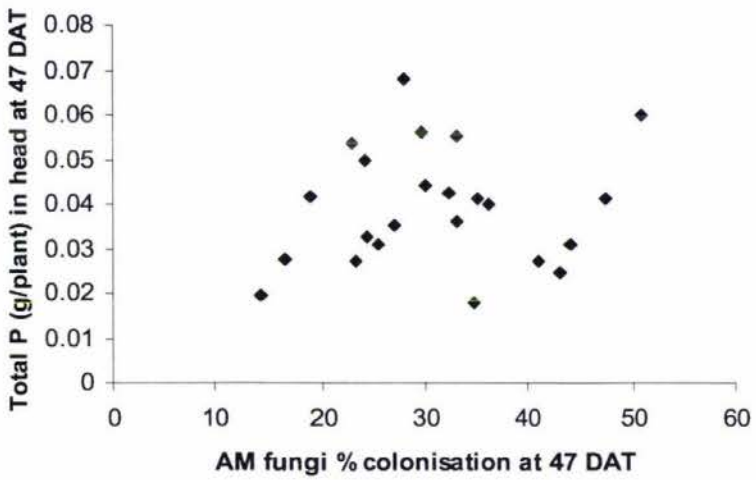


Fig 4.5.15 Relationship between total P in leaf DM and AM fungi % colonisation at 47 DAT

As P uptake did not relate positively or negatively to AM colonisation, it is likely that P concentration in soil solution and its supply to lettuce plants was not limiting between 28 and 47 DAT, which was the period when most P was taken up by rapidly growing plants.

Implications for nutrition

Phosphorus is necessary for metabolizing protein and activating enzymes in both plants and humans. Phosphorus was considered by (Pfeiffer 1981) to be important for assimilation of light energy. Recent research has shown that the phosphorylation/dephosphorylation reaction is particularly important for the functioning of enzymes such as nitrate reductase (Lillo and Appenroth, 2001) and for protein phosphorylation in general, regulating many cellular processes including DNA synthesis, transcription and mitosis and response to extracellular signals (Hardie, 1996). P nutrition of the plant is therefore important not so much in supplying P in the plant product but in affecting quantity and quality of protein in the product.

4.5.5 Calcium, Magnesium and Potassium content of leaves

There were significant differences between treatment means in lettuce leaf concentrations of potassium (K), calcium (Ca) and magnesium (Mg) at both 28 and 47 days from transplanting (Table 4.5.5, Figure 4.5.15).

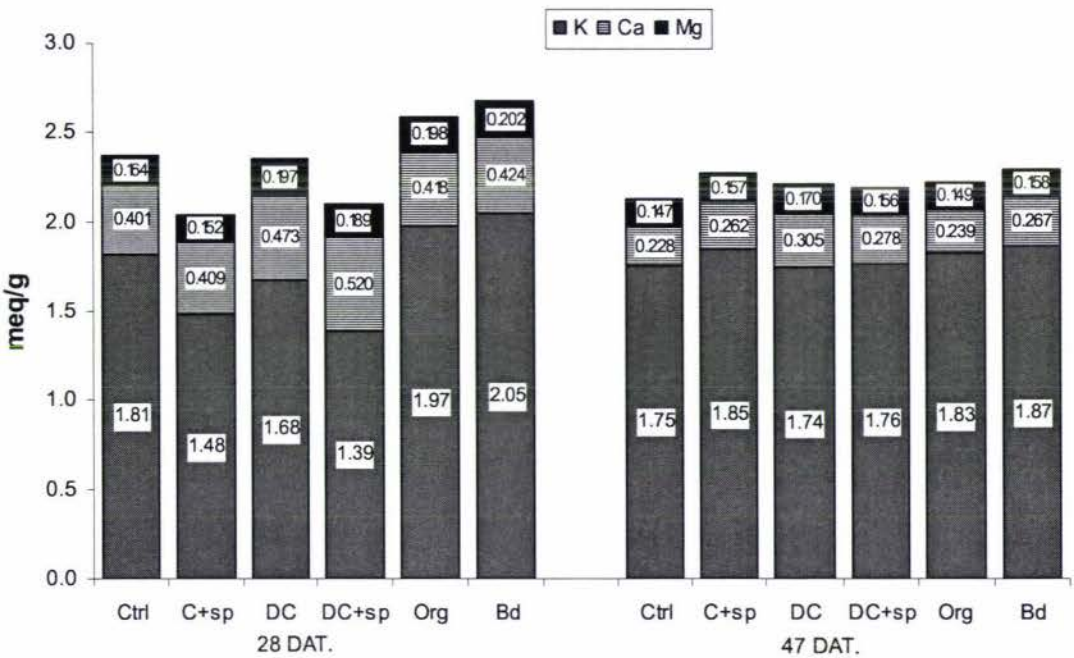


Fig. 4.5.15. Major cation content of lettuce leaves in milli-equivalents per gram leaf dry matter at 28 and 47 days from transplanting. Treatment codes in Table 3.3

Table 4.5.5 Potassium, magnesium and calcium content of leaves (meq/g dried leaves) at 28 and 47 DAT. Treatment codes in Table 3.3.

	Potassium		Magnesium		Calcium	
Treatment	**28 DAT**	**47 DAT**	**28 DAT**	**47 DAT**	**28 DAT**	**47 DAT**
			meq/g dried leaf material			
Ctrl	1.811 ab	1.749 ab	0.164 bc	0.147 b +	0.401 c +	0.228 b +
C+ sp	1.477 c	1.847 ab	0.152 c	0.157 ab	0.409 c +	0.262 ab
DC	1.677 bc	1.739 b+	0.197 a	0.169 a +	0.473 b	0.305 a +
DC + sp	1.393 c	1.760 ab	0.189 ab	0.156 ab	0.532 a	0.278 ab
Org	1.972 a	1.828 ab	0.198 a	0.149 b +	0.418 c	0.239 b
Bd	2.049 a	1.868 a+	0.202 a	0.158 ab	0.424 bc	0.267 ab
Fertiliser						
None	1.644 b	1.798 ab	0.158 b	0.152 a	0.405 b	0.245 b
DAPCAN	1.535 b	1.750 b+	0.193 a	0.163 a	0.502 a	0.292 a +
Compost	2.010 a	1.848 a+	0.200 a	0.154 a	0.421 b	0.253 ab
Spray*						
No sprays	1.744 a	1.774 a	0.179 a	0.158 a	0.441 a	0.267 a
sprays	1.435 b	1.804 a	0.166 a	0.156 a	0.458 a	0.270 a

* Means of control and DAPCAN treatments only
Different letters indicate significant difference by t-test at 5% level. + indicates significant difference by Tukey test at 5% level.

At 28 DAT lettuce leaves from the organic treatments contained significantly higher milli-equivalents (meq) of K per g dry matter than Control + spray and DAPCAN lettuces (Table 4.5.5). Possibly the compost provided increased water holding capacity and cation exchange capacity. However, the DAPCAN plants contained significantly more calcium than the other treatments. Mg concentration was lowest in the control treatments. K concentration was significantly lower in sprayed treatment plants than in non-sprayed treatments.

At 47 DAT the biodynamic treatment lettuces contained significantly higher meq/g of K than DAPCAN treatment lettuces. This was partly because there were greater total concentrations of K, Ca and Mg but also because there was a greater concentration of K and less of Ca and Mg in the biodynamic treatment lettuces. Higher uptake of K and N and lower availability of Ca in organic treatments would have depressed uptake of Ca as monovalent K ion is taken up preferentially to divalent Ca ion. DAPCAN treatment lettuce leaves contained significantly more meq/g of Ca than control and compost treatment lettuce leaves at both 28 and 47 DAT, possibly because they were supplied with more in the calcium ammonium nitrate. There was also significantly higher Mg concentration at 47 DAT in DAPCAN plants compared to organic and control plants. Note that the biodynamic sprays appear to have had the effect of reducing treatment differences in calcium and magnesium concentrations. This effect of reducing differences by the biodynamic preparations was discussed by Koenig (1988).

Potassium, magnesium and calcium percentage of dried leaves is also shown (Table 4.5.6) as leaf analysis results are generally expressed in those units.

Table 4.5.6 Percentage potassium, magnesium and calcium in dried leaves at 28 and 47 DAT. Treatment codes in Table 3.3.

	Potassium		Magnesium		Calcium	
Treatment	**28 DAT**	**47 DAT**	**28 DAT**	**47 DAT**	**28 DAT**	**47 DAT**
			% in dry leaf matter			
Ctrl	7.06	6.82	0.20	0.18	0.78	0.46
C+ sp	5.76	7.20	0.18	0.19	0.79	0.52
DC	6.54	6.78	0.24	0.20	0.98	0.53
DC + sp	5.43	6.87	0.23	0.19	1.04	0.54
Org	7.76	7.13	0.24	0.18	0.84	0.48
Bd	7.90	7.29	0.24	0.19	0.85	0.53

The cation concentrations shown in Table 4.5.6 can be compared with recommended concentrations by HORT Research (1995) for wrapper leaves of, of 6-8% K, 1.4-2% Ca and 0.5 – 0.7% Mg (Table 2.4.2). A lettuce with half-size head would correspond more to the 47 DAT than the 28 DAT data. By that standard, all the trial K levels are within the normal range, but Mg and Ca levels are low. However, comparison of trial lettuce cation levels in 100g fresh leaves with average levels for field-grown lettuces in the NZ Food Composition Tables (Athar, 2001) (Table 2.4.1) indicates that the trial lettuces contained much higher K, about average Mg and almost average Ca. The apparent discrepancy could be because field-grown lettuces are generally harvested when hearts have fully developed, whereas the trial lettuces were harvested more as a leaf lettuce type, when hearts were half-developed.

High concentration of K in leaves relates to fairly high soil levels (Table 4.1.1), which were unusual for a previously organic pasture with no history of K fertiliser application, but could be because the area was used intermittently as a night-camp by dairy cows.

At 28 DAT the sprayed treatment plants contained significantly less K but the concentrations of other cations were not significantly different from those of other treatments. The lower K concentration may indicate more balance between K and Ca uptake. According to Koepf (1993), the horn-manure spray (preparation 500) helps to improve plant calcium nutrition. However, there may not have been a spray effect in this trial because of the strong correlation between K and N concentrations and higher K concentrations at 47 DAT.

The calcium content of vegetables is important in the human diet, particularly for vegetarians, who are at greater risk of osteoporosis. Although bioavailability of different calcium sources and human variability in calcium absorption complicate the issue, maintaining dietary calcium intake is generally recommended (Fairweather-Tait, 2002). Magnesium is also important, for example for protein synthesis (Stipanuk, 2002).

The content of most other cations, particularly sodium, hydrogen and aluminium, was not measured. Cation ratios were calculated because not only absolute levels but also relative levels of cations are being found to affect animal and human health.

Calcium: phosphorus and cation ratios

Links between improved animal health, growth and reproductive capacity and calcium: phosphorus ratios of stock feed have been found (Olechnowicz, 2001; Nedeva R, 2002; Cockell et al., 2002). Low calcium: magnesium ratio in water and milk has been related to lower human cardiovascular disease risk (Seelig et al, 2001, Moss and Freed, 2003). High potassium: calcium +magnesium ratio in pasture has been linked to increased incidence of cattle tetany (grass staggers) (Jefferson et al, 2001, Cherney et al, 2002).

Significant treatment differences were found for Ca: P and K: Ca +Mg ratios, but not for Ca: Mg ratios (Table 4.5.7)

Table 4.5.7 Ratios of calcium: phosphorus (calculated from %/g dry matter at 47 DAT), calcium: magnesium and potassium to calcium + magnesium (calculated from meq/g DM at 47 DAT).

Treatment	Ca: P (%/g DM basis)	Ca: Mg (meq/g DM basis)	K: Ca+Mg (meq/g DM basis)
Ctrl	1.29 b	1.56 a	4.67 a
C+ sp	1.39 ab	1.67 a	4.44 ab
DC	1.85 a	1.78 a	3.77 c
DC + sp	1.66 ab	1.80 a	4.06 bc
Org	1.69 ab	1.60 a	4.73 a
Bd	1.61 ab	1.69 a	4.41 ab
Fertiliser			
None	1.34 b	1.62 a	4.55 a
DAPCAN	1.76 a	1.79 a	3.92 b
Compost	1.65 ab	1.65 a	4.57 a
Spray*			
No sprays	1.57 a	1.67 a	4.22 a
sprays	1.53 a	1.72 a	4.31 a

* Means of control and DAPCAN treatments only

The Ca: P ratio was significantly larger for the DAPCAN compared to the control treatment lettuce leaves. Nedeva (2002) found that increasing the Ca: P ratio from 1.2:1 to 2.5:1 negatively affected growth rate and feed conversion of pigs, however Cockell et al. (2002) found that reducing the Ca: P ratio from 1.7 to 1 increased kidney calcification in rats and Olechnowicz et al. (2001) found that a low blood serum Ca: P ratio increased the calving-conception interval in cows. These research findings indicate that the ratio of calcium to phosphorus in food may have health effects. The biodynamic

preparations may affect the balance between cations and anions in soil, plants and animals (see Section 2.3.3) but no significant effects were found in this trial. However, the trial results show that this ratio in lettuce leaves can vary according to soil management.

No significant treatment difference in the calcium: magnesium ratio was found, although DAPCAN treatment lettuces appeared to have slightly higher ratios (Table 4.5.7). People living in an area of Finland where the Mg content of water is low and food has a high Ca: Mg ratio, have the highest cardiovascular disease mortality in the world (Seelig et al, 2001) Moss and Freed (2003) discussed links between high Ca: Mg ratio in milk and coronary heart disease.

The K: Ca +Mg ratio was significantly lower for the DAPCAN treatment lettuces than for the control and organic treatment lettuces (Table 4.5.7). Plant and animal magnesium deficiency induced by high potassium uptake was a concern of Albrecht (1975) who linked it to grass tetany (staggers) in cattle. Cherney et al (2002) showed how high dairy manure application increased the K: Ca +Mg ratio up to 3.26 in orchard grass, whereas unmanured grass had a ratio of 0.34-1.74. A ratio of 2.2 was considered to be the critical ratio for tetany risk. The ratios found in the trial lettuces are all higher than those levels, but it is likely that the ratio would be higher in lettuces than in grass.

These results show that the calcium supply from the compost and control treatments appeared to be insufficient in relation to K supply for optimum concentration in the leaf, whereas application of calcium ammonium nitrate to the soil appears to have increased calcium concentration in leaves which could have nutritional benefits.

Relationships between cation and nitrogen uptake

There is a close correlation between N and K uptake (Figure 4.5.16) because K is always present as soluble K^+ ions allowing rapid uptake of NO_3^- ions for charge-balancing (Marschner, 2002).

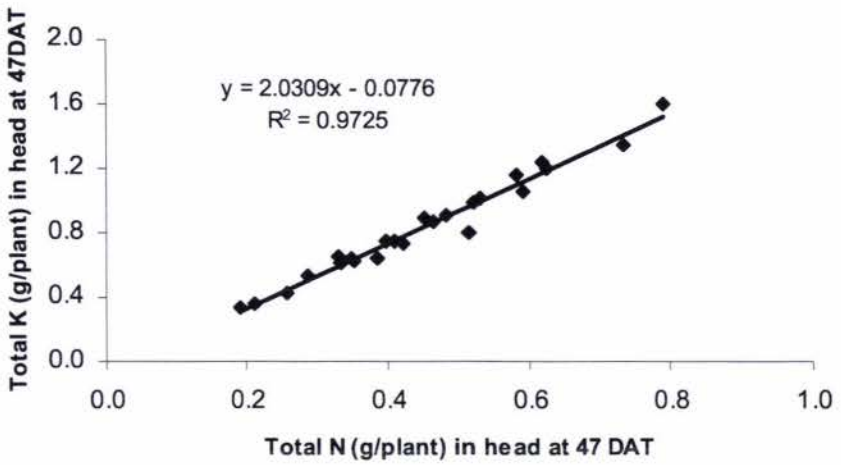


Fig. 4.5.16 Relationship between total potassium and nitrogen concentrations in leaves at 47 DAT

High K uptake by the lettuces, particularly in the compost treatments, (Table 4.5.6) would have contributed to larger plants as it promotes synthesis of Ribulose bisphosphate carboxylase and increased CO_2 assimilation, increased photosynthetic O_2 production and increased transport of photosynthates (Marschner, 2002). Marschner cites research by Wyn Jones and Pollard (1983) showing the importance of maintaining sufficient K concentration in plant cells for protein synthesis. Magnesium concentration in leaves, which is important for photosynthesis, was also found to be related to nitrogen concentration (Figure 4.5.17).

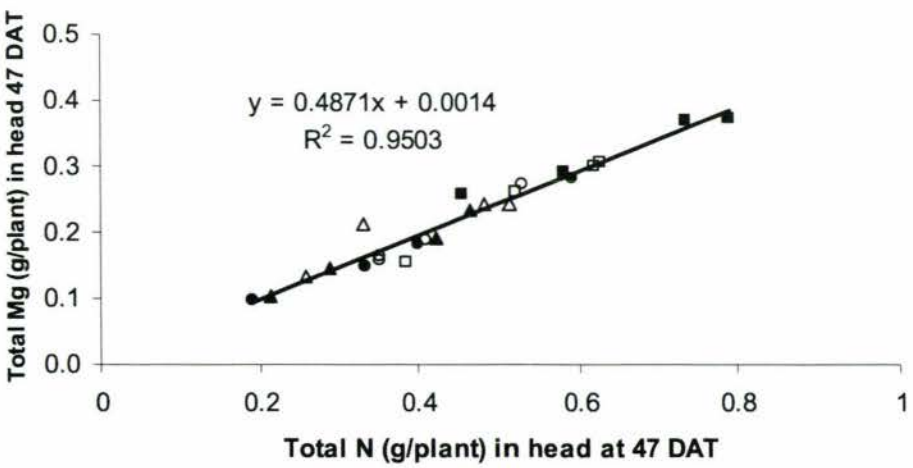


Fig. 4.5.17 Correlation between total magnesium and nitrogen concentrations in leaves at 47 DAT for control (○) control + sprays (●) DAPCAN(△) DAPCAN + sprays (▲) Organic(□) and Biodynamic (■) treatments

Relationship between cation uptake and AM fungi colonisation

No relationships could be found between K and Ca uptake and AM fungi colonisation of plant roots (data not shown), although it appeared there was a slight trend towards a positive relationship between Ca uptake and AM fungi colonisation. This could have related to the increased supply of Ca from the DAPCAN fertiliser. Ingham (2002) has found that fungi take up Ca from the soil, reducing leaching of soluble Ca.

4.5.6 Trace element content of lettuce leaves

Significantly higher concentrations of iron (Fe), zinc (Zn) and copper (Cu) were found in dried lettuce leaf samples from DAPCAN treatments than in those from compost treatments (Table 4.5.8, Figures 4.5.18 – 4.5.20). Only samples from the four treatments shown were analysed for trace element concentrations due to expense. Selenium concentration was also tested but was below the minimum detectable level of 0.005 g/mL for all samples tested.

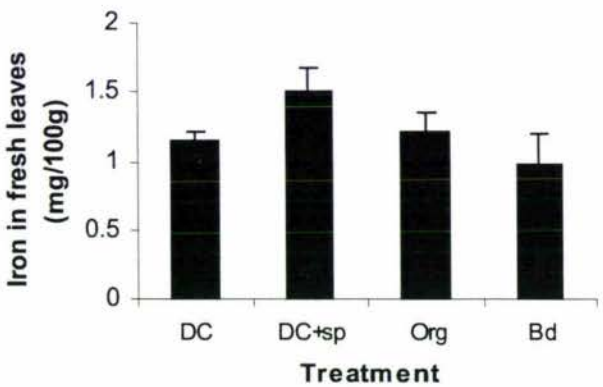


Fig. 4.5.18 Iron content in 100g of fresh leaves based on concentration in leaf dry matter of 4 plants per treatment at 47 DAT. Treatment codes as in Table 3.3. Bars represent standard error of means.

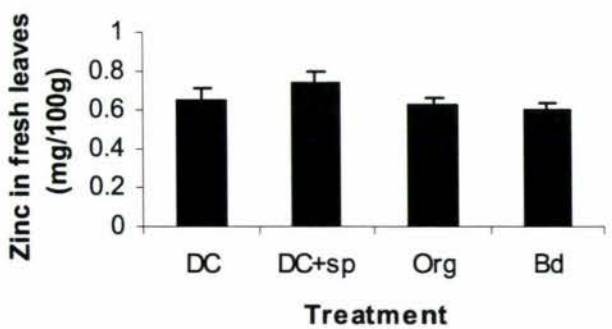


Fig. 4.5.19 Zinc content in 100 g of fresh leaves based on concentration in leaf dry matter of 4 plants per treatment at 47 DAT. Treatment codes as in Table 3.3. Bars represent standard error of means.

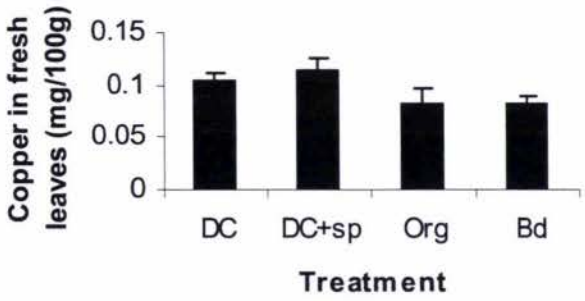


Fig. 4.5.20 Copper content in 100 g of fresh leaves based on concentration in leaf dry matter of 4 plants per treatment at 47 DAT. Treatment codes as in Table 3.3. Bars represent standard error of means.

Table 4.5.8. Iron, zinc and copper concentrations in dried leaves at 47 DAT. Treatment codes in Table 3.3.

	Iron	Zinc	Copper	Iron	Zinc	Copper
	mg/g dried leaves			*mg/100 g fresh leaves*		
DC	0.213 ab	0.121 a	0.019 a	1.14 b	0.65 ab	0.103 ab
DC + sp	0.244 a	0.121 a	0.018 ab	1.51 a	0.74 a	0.113 a
Org	0.212 ab	0.112 ab	0.015 bc	1.21 ab	0.63 ab	0.083 ab
Bd	0.163 b	0.100 b	0.014 c	0.97 b	0.60 b	0.082 b
DAPCAN	0.228 a	0.121 a	0.019 a	1.32 a	0.69 a	0.108 a
Compost	0.187 b	0.106 b	0.014 b	1.09 b	0.61 a	0.083 b

On a dry-weight basis, highest concentrations of iron, and zinc were found in leaves from the DAPCAN + sprays treatment and highest concentration of copper in the DAPCAN treatment. On a fresh-weight basis, highest concentration of iron, zinc and copper were found in the DAPCAN + sprays treatment lettuces. The lowest concentrations of iron, copper and zinc on a dry-weight and fresh weight basis were found in the biodynamic treatment lettuces.

Iron uptake by dicotyledonous plants such as lettuce is mainly as Fe^{2+} (Marschner, 2002). Iron uptake is enhanced by low soil pH and plant exudation of organic acids, which is increased if plants are stressed and short of P (Jones et al 1966). A possible reason for the higher iron and Cu concentration in the DAPCAN plants (Tables 4.5.8, 4.2.4) was that the DAPCAN application will have raised the soil solution ionic strength and lowered soil pH (Wang et al. 1995), mobilizing Fe^{2+} and increasing the concentration of Cu^{2+} in soil solution (Liao et al., 2000). Ongoing nitrification of the added ammonium-N will have also lowered pH. The lowered pH and exuded organic

acids from lettuce roots will have enhanced the reduction of Fe^{3+} to Fe^{2+} (Marschner, 2002) and accelerated Fe uptake. Application of calcium ammonium nitrate has been found to increase uptake of Zn and Cu in grass and clover (Whitehead, 2000).

DAPCAN plus silica spray produced the highest Fe, Zn and Cu concentrations, but as a single treatment its result was not significant. Increased exudation of organic acids, however, could also have been an effect of application of the biodynamic field sprays. Remer (1995) claimed that concentrations of malic, citric and oxalic acids were high in carrots to which a homoeopathic D7 silica spray had been repeatedly applied. Those levels were not quantified, but measurements of iron content of the carrots by Bathophenanthroline test showed 17 – 33% (not statistically analysed) higher iron concentration in the treated compared to untreated carrots. However, trial results are inconclusive in this regard as iron content of the Biodynamic treatment (organic + sprays) lettuces appeared to be lower than in other treatments. In addition, when treatments were grouped, there was no significant difference in micronutrient concentrations in sprayed compared to unsprayed plants. This may suggest a possible reduction-of-differences effect of the biodynamic treatment as the biodynamic preparations act principally as regulators of nutrient uptake, rather than always enhancing uptake (Koenig, 1988), so may have reduced uptake of iron. There was no evidence of an antagonism between zinc and iron and copper which sometimes occurs (Pendias, 2002)

As copper is held tightly to cation exchange sites in humus rich soils (Mengel and Kirkby, 1982) less could have been available for uptake in the compost treatment soils. However, amino acids assist copper transportation within the plant (Liao et al. 2000).

Soil micronutrient concentrations were not tested before the trial, but an earlier soil test showed slightly deficient levels of zinc and copper. As the trial area was previously intermittently used by dairy cows as a night camp, it is possible that the cows had been given zinc and copper supplements, which led to high levels being excreted on to the pasture and consequent high concentrations in the lettuces.

Implications for nutrition

The concentrations of iron in the trial lettuces were about the same as the average 1mg/100g fresh leaves in the NZ Food Composition tables (Table 4.5.1), but the DAPCAN +sprays treatment lettuces contained a higher concentration (1.5mg/100g). High iron concentration in a food product is generally not a problem as the body regulates its iron levels through how much is excreted via the gastrointestinal tract and iron toxicity (hemochromatosis) is generally due to a genetic problem (Groff et al, 1995). In addition, most of the iron in vegetables is non-heme iron which is less readily available.

The concentrations of, zinc and copper were high compared to average for lettuce leaves in NZ Food Composition Tables (Table 2.4.1). Zinc concentrations were about 3 times the average 0.2 g/100 g fresh leaves for NZ lettuces, and copper concentrations of 0.08 – 0.11 mg/100 g fresh leaves were higher in comparison to the average range of 0.3 – 0.8 mg/100 g fresh leaves (Souci et al, 1994, Table 2.4.1). Zinc toxicity impairs immune function, increases coronary heart disease and reduces Fe and Cu availability, however, zinc toxicity is not likely from plant foods as its bioavailability is low, and deficiency is more likely (Welch, 1993). Copper is similarly more likely to be deficient.

Relationships between trace element concentration and other nutrients and AM fungi colonisation

A possible relationship between copper and nitrogen concentrations in lettuce leaves was looked for because copper uptake and transport within the plant is in association with amino acids. (Liao et al. 2000) A fairly close relationship $R^2 = 0.7$) was found (Figure 4.5.21).

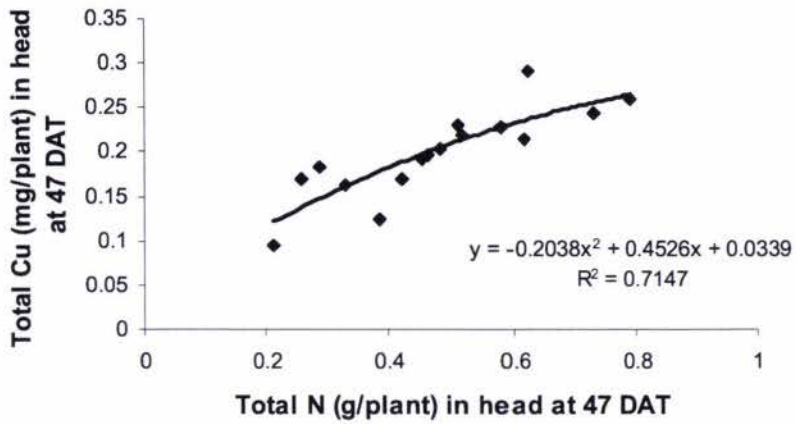


Fig.4.5.21 Relationship between total estimated copper and nitrogen uptake by a lettuce head at 47 DAT.

A relationship between zinc and phosphorus concentrations in lettuce leaves was looked for because a negative relationship is often found either because high phosphorus uptake can reduce zinc uptake, or low zinc increases root membrane permeability, enabling higher phosphorus uptake (Marschner, 2002).

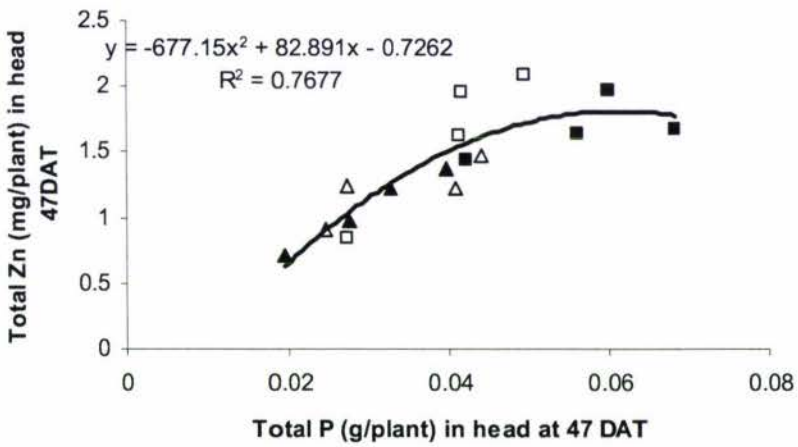


Fig 4.5.22 Relationship between total estimated zinc and phosphorus uptake by a lettuce head at 47 DAT for DAPCAN(△) DAPCAN + sprays (▲) Organic (□) and Biodynamic (■) treatments.

A curved relationship was found between Zn and P concentrations (Figure 4.5.22)

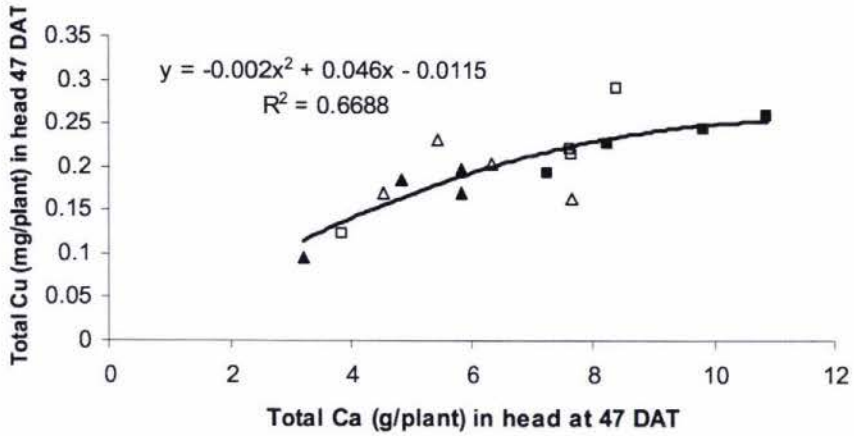


Fig.4.5.23 Relationship between total estimated copper and calcium content of lettuce heads at 47 DAT for DAPCAN(△) DAPCAN + sprays (▲) Organic (□) and Biodynamic (■) treatments.

There was a relationship between copper and calcium concentrations in leaves (Figure 4.5.23). This may be because both Ca and Cu uptake are significantly related to N uptake or that higher free soil solution Ca in this trial was associated with lower pH that also mobilised Cu.

No relationships between micronutrient concentrations and AM fungi colonisation were found (data not shown).

4.6 Amino acids, antioxidants and sugar content of leaves

4.6.1 Amino acids

Analysis of amino acid content of dried leaf samples did not result in any significant differences between treatments for any amino acid because the cost of the test precluded analysis of more than 2 samples for each of 3 treatments. However, some small differences between treatment were evident, and were fairly consistent for all amino acids measured (Table 4.6.1, Figure 4.6.1). Trial lettuce amino acid contents were generally higher than the average contents for the Romaine type lettuce, which is the lettuce type with highest amino acid content, listed in the USDA nutrient database.

Highest levels of all amino acids measured were found in the control with sprays treatment, and least of almost all in the DAPCAN treatment. The higher levels in the control treatment can be compared to the results of Brunsgaard et al. (1994) who found that the total protein content of crisphead lettuce increased from 16.00% with a fertilisation rate of 50 kg N/ha to 20.13% at 150 kg N/ha, but levels of essential amino acids fell with increasing fertilisation rate. No effect from the biodynamic field sprays or other management treatments on amino acid contents can be determined as insufficient treatments and numbers were analysed.

There was little difference between treatments in proline content which has found to be related to water stress and to colonisation by some species of AM fungi (Ruiz Lozano et al. (1995).

Protein quality score

The quality of protein can be assessed by its content of the essential amino acids that cannot be synthesized in the human body (Stipanuk, 2000). The protein quality score is calculated by dividing the quantity of each essential amino acid (in mg/g protein) by the quantity in a reference protein. The amino acid with the lowest score is the limiting amino acid. A recent recommendation for protein quality scoring, the MIT (Massachusetts Institute of Technology) score (Young and Borgonha, 2000) uses the estimated actual requirements of an adult human, rather than a food source. Quantities of most essential amino acids in the trial lettuces were found to be more than adequate compared with recommended levels (Table 4.6.2).

Table 4.6.1 Amino acid content of fresh lettuce leaves (mg/100mg). Mean of 2 samples / treatment compared to average quantities for 2 varieties in the USDA nutrient database.

Amino Acid	DC[1]	C+sp[1]	C+sp/DC[1]	Bd[1]	Bd/DC[1]	Romaine[2]	Butterhead[2]
				mg/100g fresh leaves			
Aspartic acid	0.167	0.220	1.32	0.185	1.11	0.177	0.141
Threonine	0.076	0.098	1.29	0.080	1.05	0.074	0.059
Serine	0.065	0.076	1.17	0.065	1.00	0.049	1.039
Glutamic acid	0.219	0.274	1.25	0.239	1.09	0.227	0.180
Proline	0.074	0.095	1.28	0.075	1.01	0.060	0.048
Glycine	0.082	0.104	1.27	0.083	1.01	0.071	0.057
Alanine	0.093	0.121	1.30	0.095	1.02	0.070	0.055
Valine	0.088	0.115	1.31	0.093	1.06	0.087	0.069
Methionine	0.026	0.033	1.27	0.026	1.06	0.020	0.016
Isoleucine	0.073	0.095	1.30	0.076	1.04	0.105	0.083
Leucine	0.131	0.169	1.29	0.136	1.04	0.098	0.078
Tyrosine	0.057	0.072	1.26	0.056	0.98	0.040	0.032
Phenylalanine	0.083	0.106	1.28	0.087	1.05	0.068	0.054
Histidine	0.044	0.056	1.27	0.046	1.05	0.028	0.022
Lysine	0.097	0.126	1.30	0.102	1.05	0.105	0.084
Arginine	0.094	0.125	1.33	0.099	1.05	0.088	0.070
Lysine:Arginine	1.026	1.011		1.033		1.193	1.200

[1] Trial results – treatment codes in Table 3.3

[2] USDA Nutrient Database for Standard Reference, Release 12 (March 1998)

Table 4.6.2 Calculation of protein quality scores from amino acid mg/g crude protein for trial lettuce essential amino acids treatment means, recommended values and an average lettuce. Treatment codes in Table 3.3.

Amino acid	Treatment DC	C+ sp	Bd	Recommended+	Average lettuce ++
			mg/g protein		
Threonine	60.95	67.92	59.53	25	45
Valine	69.85	79.76	69.14	35	54
Methionine	20.83	22.86	19.04	25*	25*
Isoleucine	58.38	65.60	56.45	35	65
Leucine	104.62	117.27	100.96	65	61
Tyrosine	45.62	49.69	41.22		
Phenylalanine	66.56	73.84	64.11		67**
Histidine	35.15	39.14	34.14		17
Lysine	77.36	87.39	75.67	50	65

+ MIT recommended reference pattern for adults (Young and Borgonha, 2000)
++ Average lettuce amino acid contents (NutritionData, 2002)
* Methionine + cysteine
** Tyrosine + phenylalanine

Tryptophan and cysteine were not measured for the trial lettuces as they are not extracted by the method used. Tryptophan was found to be the limiting amino acid in average lettuce scoring recorded by NutritionData (2002), with only 63% of the recommended 10mg/g protein. All other essential amino acids were stated to be almost or more than adequate quantities. However, that analysis used the Chemical Score (FAO, 1985), which is based on requirements by infants, which have higher protein requirements than adults, and uses egg as a reference protein, which is different from vegetable protein. The Chemical score also does not allow for digestibility. Young and Borghona (2000) discussed the importance of including a measure of digestibility in the scoring. However, digestibility is measured for rats, which is not the same as for humans, and rats require more sulphur containing amino acids. Young and Borghona recommended using scores developed by MIT as shown in Table 4.6.2.

The Biodynamic lettuces contained lowest quantities of essential amino acids per g crude protein, and the control with sprays highest (Table 4.6.2). This is also consistent with the finding of Brunsgaard et al. (1994) who found that higher levels of N fertilisation resulted in a significant drop in essential amino acids.

Lysine: arginine ratio

Another measure of protein quality is the lysine: arginine ratio. Lysine is an essential amino acid required for functions such as tissue repair and calcium absorption, but too much lysine in relation to arginine can increase degradation of arginine by the kidneys (Austic, 1998). A low lysine: arginine ratio in the diet has beneficial health effects such as reducing atherosclerosis (Rajamohan and Kurup, 1990)

There was no significant difference between treatments for this ratio (Table 4.6.1). However, the Control + sprays treatment lettuces appeared to contain slightly higher levels of arginine and a lower lysine: arginine ratio than lettuces from the other treatments. All the trial lettuces had lower lysine: arginine ratio than the average calculated from average amino acid levels in the USDA Nutrient Database (1998).

4.6.2. Antioxidant content of leaves

Analysis of antioxidant content of the leaves at final harvest by the Ferric Reducing Ability of Plasma (FRAP) assay (which uses antioxidants as reductants of Fe^{3+} to Fe^{2+} in a redox-linked colorimetric reaction, (Bente et al, 2002) showed some significant treatment differences when analysed for variance by SAS (Table 4.6.3).

Table 4.6.3 Mean Ferric Reducing Ability of Plasma (FRAP) of lettuce leaves at 47 DAT. Treatment codes in Table 3.3.

Treatment	Mean umol FRAP /g dried leaves	Mean umol FRAP 100/g fresh leaves
Ctr	71.05 a	1005.58 ab
C + sp	72.78 a	1054.38 a
DC	61.50 ab	726.00 dc
DC + sp	66.70 ab	875.19 bc
Org	58.63 b	631.16 d
Bd	56.58 b	669.47 d
Fertiliser		
None	71.91 a	1029.98 a
DAPCAN	64.10 ab	800.59 b
Compost	57.60 b	650.31 c
Sprays*		
No spray	66.28 a	865.8 a
Spray	69.74 a	964.8 a

* Mean of control and DAPCAN treatments only

Lettuces that did not receive either compost or soluble fertilizer contained highest mean umol FRAP/g leaf dry weight. There was a larger difference between treatments, in the same order, when compared on a fresh weight basis (Figure 4.6.1, Table 4.6.3).

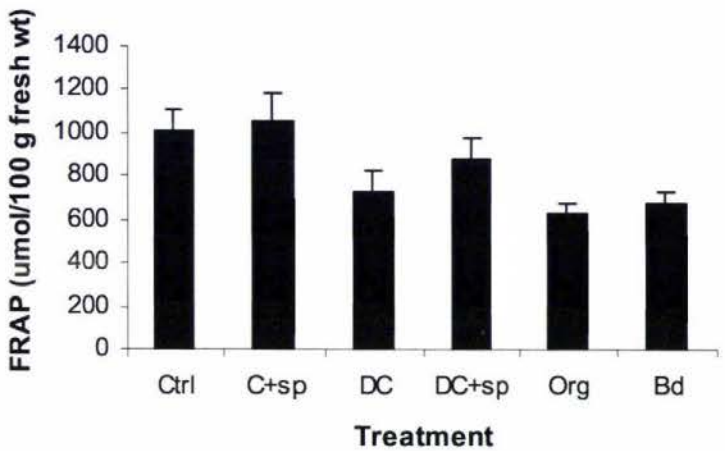


Fig. 4.6.1 Mean Ferric Reducing Ability of Plasma (FRAP) in lettuce leaves at 47 DAT in each treatment. Treatment codes in Table 3.3. Bars indicate the standard error of means.

Lettuces from the control treatments had significantly higher FRAP/100g fresh leaves. Sprayed plots had higher FRAP content, but this difference was not significant at the 95% confidence level. Largest within treatment variations were in the Control and DAPCAN with and without field-sprays treatments. The FRAP levels can be compared with those found by Bente et al. (2002) who recorded 70 and 600 FRAP µmol/100g for crisphead and Lollo Rosso lettuces respectively. Higher levels would be expected for darker and redder coloured leaves. The level for Lollo Rosso lettuces corresponds quite well to levels found in the Organic and Biodynamic treatment lettuces, but the other trial treatment lettuces contained higher levels.

Antioxidants measured are likely to include α-tocopherol, vitamin C, ß-carotene, other carotenoids, polyphenolic acids, sulphides, flavonoids, and lignans (Bente et al, 2002). The lettuces which had highest antioxidant content were from treatments which were most stressed and developed a red colour during the first 28 days of the experiment (Tables 4.2.3, 4.2.4). It is therefore likely that the flavonoids which could have been produced in those lettuces as a reaction to stress, contributed to the high levels found in plants from those treatments. Bergman et al. (1999) discussed the formation of antioxidants such as phenylpropanoids in plants as a reaction to stress and found that stressors could include mycorrhiza and rhizobacteria. Organically-grown food has been

found to contribute more of a flavonoid antioxidant (quercetin) to the diet in one trial (Ginder-Pederson, 2003).

The levels of antioxidants in lettuces are generally low compared to some other foods e.g. leaf lettuce contains 265 ORAC (oxygen radical absorbance capacity) units/100g compared to 890 in broccoli and 2400 in blueberries (The ORAC measure was found to be closely related to the FRAP measure (Bente et al., 2002)). However, the levels found in the trial lettuces indicate that lettuce can make a contribution to dietary antioxidants.

There was an inverse relationship between antioxidant content and total lettuce head dry weight (Figure 4.6.2).

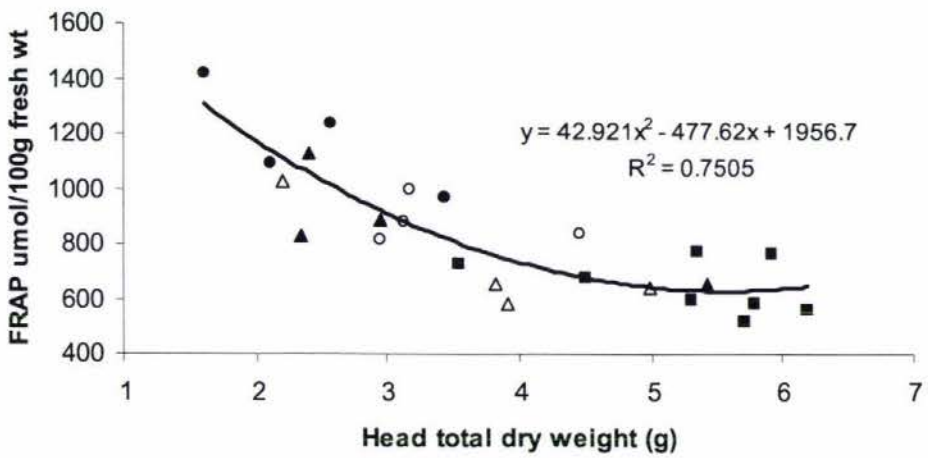


Fig. 4.6.2 Relationship between lettuce head dry weight and FRAP/100mg leaves. Symbols show treatment levels. Control (○) control + sprays (●) DAPCAN(△) DAPCAN + sprays (▲) Organic(□) and Biodynamic (■) treatments. Regression line is for all treatments.

There was no significant relationship between FRAP concentration of leaves and total N uptake (Figure 4.6.3), because of considerable variability between plants. However a trend of reduced FRAP concentration with increasing N content could be seen. One reason for the poor correlation could be that the dried leaf contents of 3 plants were amalgamated for analysis of N content so there could be less correlation than if N content had been analysed for the same plant as was analysed for FRAP concentration.

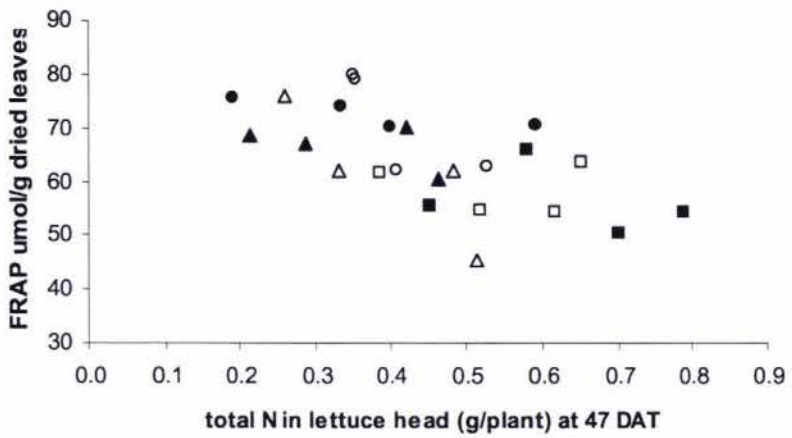


Fig. 4.6.3 Relationship between FRAP concentration and total N in lettuce leaves at 47 DAT. Symbols show treatment levels. control (○) control + sprays (●) DAPCAN(△) DAPCAN + sprays (▲) Organic (□) and Biodynamic (■) treatments.

4.6.3 Leaf Brix readings

Refractometer Brix readings of soluble solids were made for the same plant sap samples used for nitrate determination. The Brix level is an estimate of the percentage by weight of soluble solids, mostly sugars, in cell sap. As for the nitrate results (Table 4.5.2), means at most times are from small data sets and therefore indicative only (Table 4.6.4) (Appendix I).

Analysis of Brix levels for lettuces recorded at 7pm, 41 DAT, 9 hours after spraying with the silica spray showed a significant difference between the Brix level for Control + spray and DAPCAN plants (Table 4.6.5).

Table 4.6.5 Lettuce leaf sap Brix levels at 7 pm 41 DAT.

Treatment	Mean Brix level	Fertiliser	Mean Brix level
Ctr	4.07 ab	None	4.87 a
C + sp	5.67 a	DAPCAN	4.00 a
DC	3.47 b	Compost	4.40 a
DC + sp	4.53 ab	**Sprays***	
Org	4.30 ab	No spray	3.77 a
Bd	4.50 ab	Spray	5.10 a

* Mean of control and DAPCAN treatments only
There was no significant difference at 5% level between data with the same letter

Although it appeared that all the sprayed treatments had higher Brix levels, the differences were not significant because of the high variability within treatments. There

would have had to be at least 1.1 Brix unit difference between means for a significant difference at 0.05% level by t-test. However, SAS mixed procedure analysis showed a significant difference between control treatments sprayed and those not sprayed ($p = 0.0344$). All the levels are quite low. Harrill (1998) wrote that levels of 4 or less are poor and that high quality lettuces can have Brix levels of about 12.

Differences had largely disappeared by the last test at 45 DAT, not long before final harvest. Figure 4.6.4 provides an indication of the mainly non-significant differences between treatments recorded over time. Diurnal changes in Brix levels are usual, as sugars generally accumulate with sunlight hours during the day.

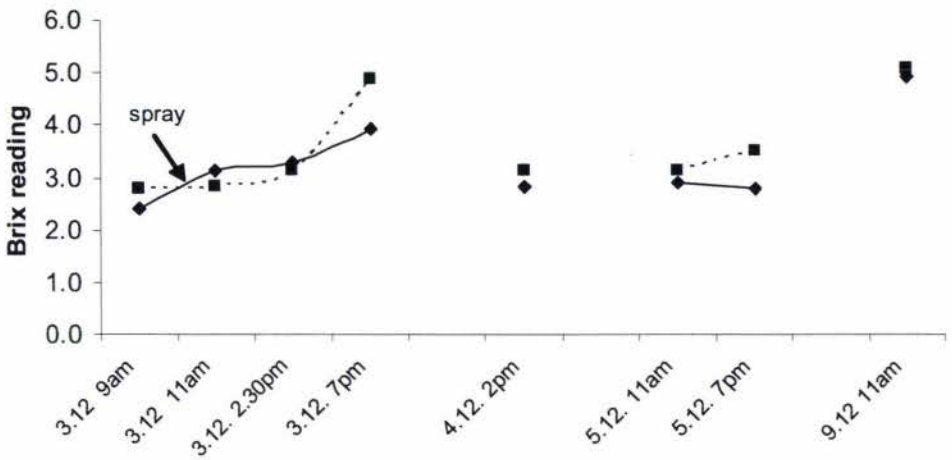


Fig. 4.6.4 Diurnal variation of Brix level and possible effects of the silica spray. ♦ = no sprays, ■ = sprays

4.7 Soil microbial activity and arbuscular mycorrhizal fungi inhabitation of roots

4.7.1 Soil microbial activity

Soil respiration tests undertaken at 28 days from planting (DAT) indicated significantly highest carbon dioxide emission in soil from the Organic plots and lowest in the DAPCAN + sprays plots (Figure 4.7.1, Table 4.7.1).

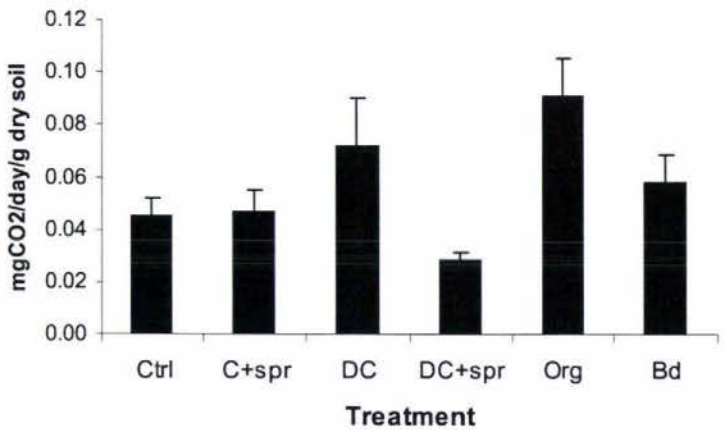


Fig. 4.7.1 Carbon dioxide respiration in soil samples taken from each plot 28 days after transplanting. Treatment codes in Table 3.3. Bars represent standard error of means.

Table 4.7.1 Soil respiration at 28 DAT. (Treatment codes in Table 3.3)

Treatment	Soil respiration mg CO_2/day/g	Fertiliser	Soil respiration mg CO_2/day/g
Ctrl	0.045 bc	None	0.046 b+
C + sp	0.047 bc	DAPCAN	0.050 ab
DC	0.072 ab	Compost	0.075 a+
DC + sp	0.029 c +	**Spray***	
Org	0.091 a+	No sprays	0.058 a
Bb	0.059 abc	Sprays	0.038 b

* Mean of control and DAPCAN treatments only
Means with the same letter are not significantly different.
+ indicates 95% confidence by t-test, but not significant by Tukey test

Soil from compost treated plots and from plots not receiving biodynamic field-sprays emitted significantly higher CO_2 than soil from control plots and plots that were sprayed with field-sprays. DAPCAN plots also had high microbial activity. As this test was conducted once during the trial on soil taken into the laboratory it can only serve as a snapshot to actual field conditions during the trial. The standard error bars in Figure

4.7.1 indicate the large variability within treatments, particularly in the DAPCAN and Organic treatments.

The main determinant of increased microbial activity appears to have been nitrogen and possibly also calcium and/or phosphorus additions, rather than the form of delivery. Scheller (1996) discussed the "priming effect" of nitrogen on microbial growth. Haynes and Swift (1988) demonstrated the effect of lime additions, and interaction with phosphate additions on microbial activity. The calcium ammonium nitrate applied in the DAPCAN treatment added extra calcium, nitrogen and phosphorus. Other factors that could have contributed to the highest microbial activity recorded for the organic plots, are improved water holding capacity from compost addition, and growth stimulating effects of humic compounds in the compost. Vaughan and Malcolm (1985) discussed the effects of humic substances on cell respiration, ATP production and enzyme activity, which affect growth and activity of soil organisms and plants.

Application of the sprays appears to have reduced the amount of microbial activity in the DAPCAN and Biodynamic treatments. This effect of the sprays could be an indirect effect, from reduced plant growth and photosynthesis resulting in less root exudates available for microbial uptake. Water shortage stress, which was more marked in the plots receiving field-sprays but no compost, could have contributed to the lower microbial activity recorded for those plots, through plant synthesis of ethylene, which inhibits microbial growth (Lynch and Brown, 1997). Carpenter-Boggs (2000) found significantly higher soil respiration in compost treated compared to minerally fertilised soils. Compost containing biodynamic preparations and treatment with biodynamic field-sprays resulted in slightly but not significantly higher respiration values. Mäder et al. (2000) found that long-term biodynamically managed soil had a significantly higher microbial diversity than other management systems, and an associated lower metabolic quotient, indicating that the soil communities were able to use organic substances more for growth than for maintenance.

High microbial activity is not necessarily beneficial to crop growth, as the soil biota may be immobilising nutrients that are then unavailable for plant uptake, although Gunapala and Scow (1998) found that this seemed to only apply to soils fertilised with soluble fertilisers and that under an organic system, higher microbial activity was

associated with greater plant nutrient uptake. Lynch (1983) pointed out that the interrelationships in the rhizosphere can be very complex. A better measure of microbial activity could have been the metabolic quotient (qCO2 = soil basal respiration/soil microbial biomass), which indicates energy efficiency of the microbial community, as discussed by Sparling (1997).

Measurements of microbial activity also give no indication of what species of microbes are present. The population types and diversity in the different treatments were not necessarily the same. Mäder et al (2002) found significantly more microbial diversity (measured by Shannon index) under biodynamic compared to conventional management, and under controlled conditions the smaller but diverse biodynamic community decomposed more ^{14}C-labeled plant material than the ones of the conventional soils.

Relationships between soil microbial activity and plant nutrient uptake and weight

There was no simple relationship between soil respiration and plant phosphorus uptake (Figure 4.7.2).

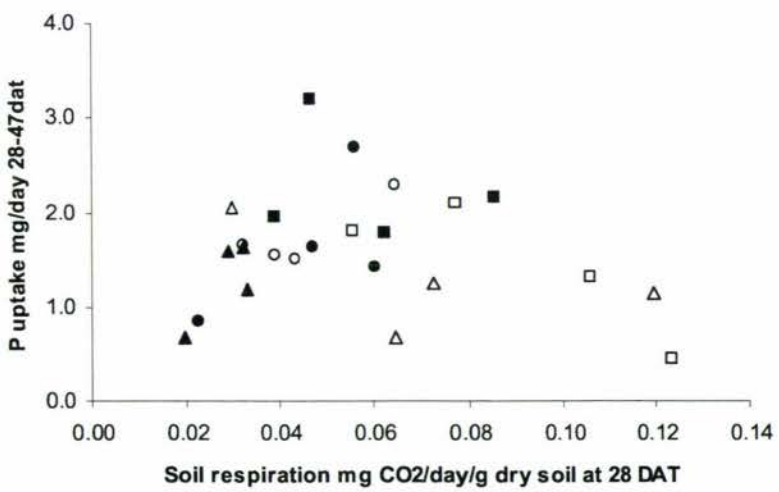


Fig. 4.7.2, Relationship between soil respiration at 4 weeks and estimated phosphorus uptake by plant head, 28- 47 days after transplanting . Symbols show treatment levels. Control ○) control + sprays (●) DAPCAN(△) DAPCAN + sprays (▲) Organic □) and Biodynamic ■) treatments.

No relationship was found between soil respiration and nitrogen or calcium uptake or plant wet and dry weight at 28 DAT (data not shown).

4.7.2 Arbuscular mycorrhizal (AM) fungi colonisation of lettuce roots

There was a significant difference between mycorrhizal colonisation of lettuce roots from the DAPCAN (37.25%) and DAPCAN + spray (22.75%) treatments (Figure 4.7.3, Table 4.7.2).

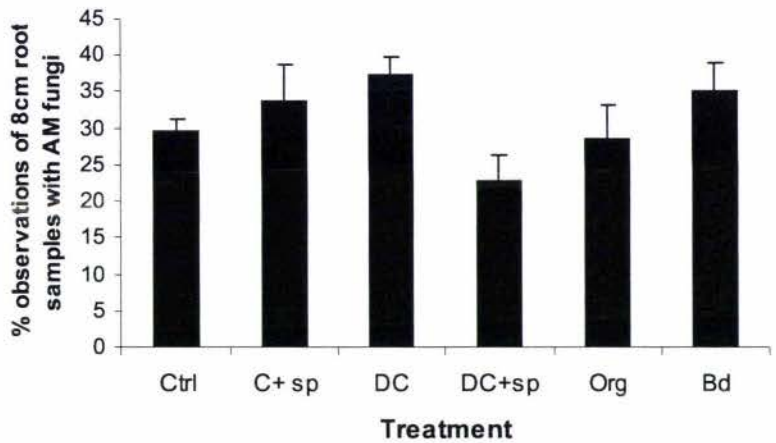
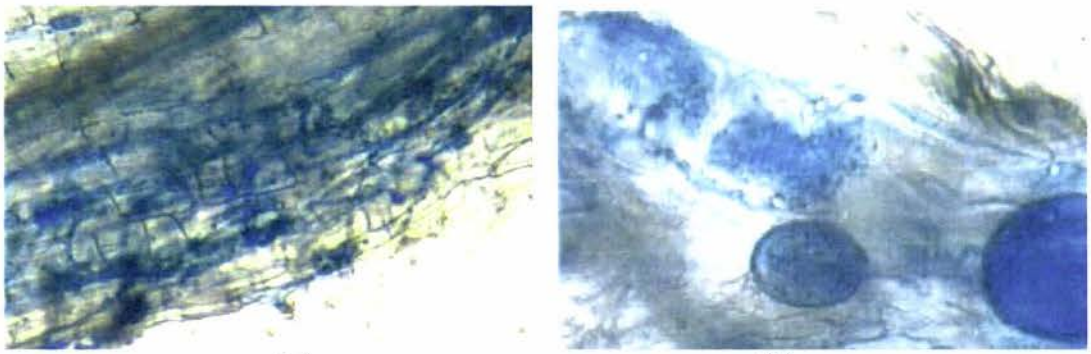


Fig. 4.7.3 Percentage of root observations in which AM hyphae with or without arbuscles or vesicles were present as measured with a microscope cross-hair at *200 magnification. Bars represent standard error of mean observations for each treatment.

The numbers of times a microscope cross-hair intersect passed through root only, hyphae, hyphae and arbuscles or hyphae and vesicle was recorded for at least 60 observations made approximately every 10 mm over approximately 8 cm of stained root for each of 2 slides for each sample, following the method recommended by Brundrett et al. (1996). All observations are likely to be under-recorded. This is because not all roots and hyphae took up the stain properly and also some of the colonisation was by a species that was likely to be the fine endophyte mycorrhiza, *Glomus tenuis*, which is harder to identify and distinguish from non-mycorrhizal fungi than other species with larger hyphae and arbuscles. Percentage of observations rather than % of root colonised was recorded because the variability in staining success and root width made for some variation in root area observed. On most slides observations were made on about 8cm of roots. Some observations are shown in Plates 4.7.1 – 4.7.3.



(a) (b)

Plate 4.7.1 Arbuscles and vesicles in lettuce root (stained with Trypan-blue) from Control treatment (a) at x 100 (b) at x 200 magnification

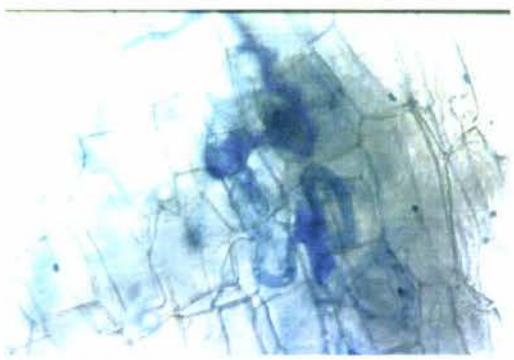


Plate 4.7.2 AM hyphae in lettuce root from Control treatment at x 200 magnification

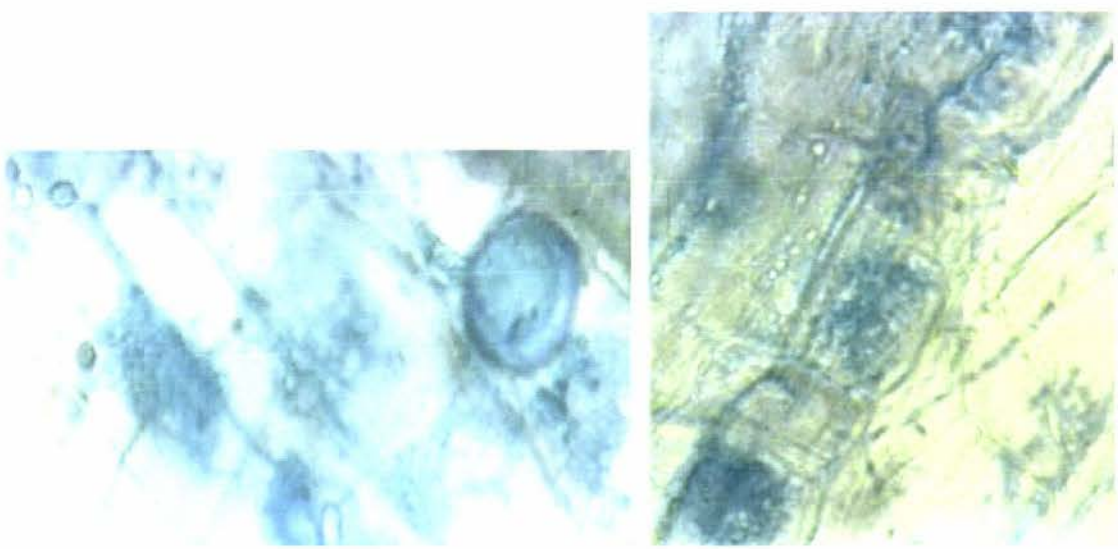


Plate 4.7.3 Hyphae, arbuscles and vesicle in lettuce roots (stained with Trypan-blue) at x 200 from (a) organic (b) DAPCAN treatment

There appeared to be some relationship between the amount of root branching observed and AM fungi colonisation, but the most densely branched roots found for lettuces growing in the organic plots did not show the highest level of colonisation.

Table 4.7.2 Mycorrhizal colonisation of lettuce roots

Treatment	% root observations with AM fungi	Fertiliser	% root observations with AM fungi
Ctrl	29.8 ab	None	32.5 a
C + sp	35.3 ab	DAPCAN	30.0 a
DC	37.3 a +	Compost	31.8 a
DC + sp	22.8 b +	**Spray***	
Org	28.3 ab	No sprays	33.5 a
Bb	35.3 ab	Sprays	29.0 a

* Mean of control and DAPCAN treatments only
Means with the same letter are not significantly different.
+ indicates 95% confidence by t-test, but not significant by Tukey test

Average colonisation rates were less than the 41-68% lettuce root colonisation by 2 AM fungi species, *G. mosseae* and *G. fasciculatum* in pots with varying levels of nitrogen fertilization measured by Azcon et al. (2001), but were more similar to the 30% colonisation of non-inoculated field-grown lettuce roots found by Berg et al (2001) and the 24-43% colonisation rate of field grass-clover roots recorded by Mäder et al. (2000).

It is interesting to note that the between treatment differences is a similar result to the comparative levels found for microbial respiration, and adds support to the hypothesis that in the DAPCAN+ spray plants there was less carbon transported down the plants which could be used by AM fungi and soil biota. Percentages for other treatments were intermediate between these levels, with no significant difference between fertilisation or spray treatments. Roots from Biodynamic and Control + sprays plots contained more AM fungi than roots from the Organic and Control plots, but these differences were not significant. Overall, there was little difference between colonisation of roots from composted and non-composted, and sprayed and non-sprayed plots.

The apparent higher colonisation rate for the DAPCAN plots can be compared with the findings of Mäder et al. (1999) who found highest colonisation in unfertilised plots, and least colonisation in soluble fertiliser plots in the long-term DOC trial comparing biological and soluble fertiliser systems. The difference between those results and my trial results could be because different species of AM fungi were involved. Azcon et al. (2001) found similar high colonisation rates by *Glomus fasciculatum* for N fertilisation rates of 84 – 252mg N/kg, whereas colonisation rates by *G. mossae* declined from 56.6% at 84 mgN/kg to 41.2% and 43.1% at 168 and 252 mgN/kg respectively.

The trial soil may not have been ideal for AM fungi. Mäder et al. (1999) found in an inoculation experiment that it was principally soil properties and not number of infectious AM propagules that determined root colonisation. It is likely that using a freshly cultivated paddock previously used for dairy cow grazing contributed to a fairly high initial level of microbial activity and good supply of mycorrhizal hyphae and spores. Smith and Read (1997) discussed how pasture provides a good supply of infective propagules and spores, which are reduced by tillage and fallowing. Lack of soil cover between plants during the first weeks of the trial could have reduced AM fungi viability.

About one third of the AM fungi colonisation recorded was by fine endophyte, possibly *Glomus tenuis,* which is generally found in acid soils (Rillig, 2002). This species was more often recorded on roots of plants from field sprayed plots. This observation leads to questions as to whether application of the biodynamic sprays did result in more acidification in the rhizosphere, and how this may have affected soil organisms and plant nutrient uptake. Research has shown that effects of AM fungi on nutrient uptake by plants vary considerably according to species of fungi (Azcon et al., 2001), so it is possible that the sprays favoured different fungi species which affected plant nutrition. It appears that at least some of the AM fungi species may have had a more "parasitic" effect on the lettuce plants, reducing net availability of nutrients, at least in the more stressed plant control and DAPCAN + sprays treatments during the first 28 DAT. This was the finding by Graves (2002) for maize seedlings grown in winter when light quantity and quality was reduced.

The lack of much significant difference between colonisation measured for the different treatments and the high variability of most other plant growth and nutrient composition parameters measured, mean that relationships with plant nutrient uptake and leaf nutrient composition cannot be established. In addition, in any interpretation of results it should be remembered that roots of only one plant per plot were sampled at one point in time, whereas the presence of active hyphae and arbuscles can be quite transient as an arbuscle only lasts a few weeks (Brundrett et al., 1996). The results and possible factors affecting them are further discussed with these limitations in mind.

Miller and Jackson (1998) found that the factors most increasing mycorrhizal colonisation of field-grown lettuces were larger C: P and N: P ratios, and high P fertilization was highly negatively correlated with colonisation. In my trial I attempted to make N and P additions the same for composted and soluble fertiliser plots, although the organically composted plots had larger C: P because the added compost C: P ratio was higher (40:1) than the mean soil and biodynamic compost C:P (24.7 and 26.7) respectively. However, the organic composted plots had lower mycorrhizal colonisation. Ryan et al. (2000) found that colonisation by arbuscular mycorrhizal fungi of white clover (*Trifolium repens*), perennial ryegrass (*Lolium spp.*) and *Paspalum* was lower in conventionally managed pastures than in biodynamic pastures but phosphorus levels and host plant species were the main determinants of colonisation.

Interactions between AM fungi, composts and water availability

There was no evidence of any interaction between increased water availability in composted plots and mycorrhizal colonisation in the trial results. Other research has shown that AM fungi increase water acquisition by plants, partly because of improved nutrition enabling enhanced growth (Clark and Zeto, 2002). Possibly AM fungi made a substantial contribution to water uptake by the trial lettuce plants because at the end of the trial, when some plants were removed for analysis, other plants growing beyond the area of soil disturbed by removing plants wilted. Tartafdar and Rao (2002) quoted several research results showing increased aggregation of soil particles by AM fungi. For example, Bethlenfalvey and Barea measured 13.0 and 2.6 water stable aggregates over 1mm diameter in pots of pea plants, with and without AM fungi respectively. AM fungi have also been found to improve soil aggregation through production of the glycoprotein, glomalin (Rillig et al., 2002). However, as the greatest colonisation by AM fungi was found in the DAPCAN treatment in which there was possibly more reduction of plant growth through less water availability than in the composted plots; AM fungi appear to have had less effect on soil aggregation than compost.

The possibly reduced colonisation by AM fungi found in composted treatments compared to the DAPCAN treatment suggests that the compost may have reduced AM colonisation, possibly through provision of more available P and N or through altering the balance of the soil biota community to one less favourable to AM fungi. This possibility is supported by the finding of Sainz et al. (1998) who found that addition of

vermicomposted urban waste increased Olsen-P and other mineral elements in soil and shoot P, Ca, Mg, Cu, Mn and Zn concentrations, but caused a significant reduction in root length colonised by AM fungi. However, Mäder et al. (2002) reported that root length colonised by mycorrhizae in the long-term DOC trial organic treatment, which used composted farmyard manure, was 40% greater than in conventional systems. Composts might also contribute to AM fungi propagules. Brechelt (1990) found that chicken litter/leaf and dairy cow manure/leaf composts enhanced spore numbers of *Glomus* spp. compared with those found in plots treated with raw dairy cow manure and soluble fertiliser.

Relationships between AM fungi colonisation and plant nutrient uptake and weight

No significant relationship between AM fungi colonisation of roots and leaf nutrient composition could be found. The scatter of treatment levels is shown in Figure 4.7.4.

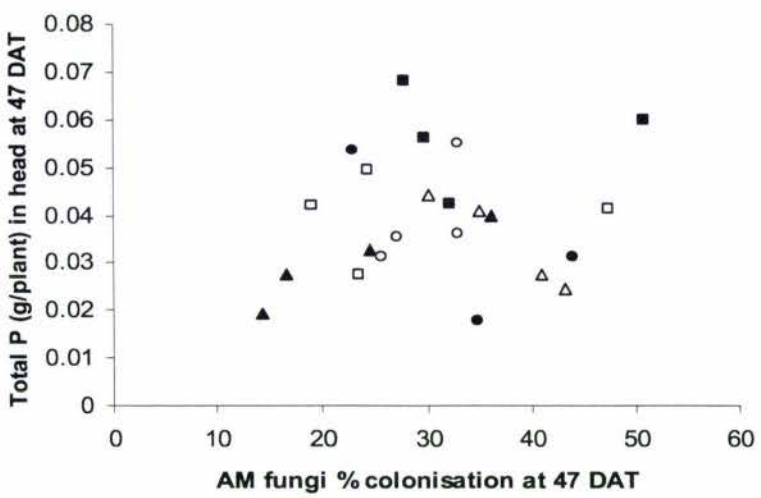


Fig. 4.7.4 Relationship between estimated lettuce leaf uptake of P and AM fungi colonisation of roots of lettuce plants. Symbols show treatment levels. Control (○) control + sprays (●) DAPCAN(△) DAPCAN + sprays (▲) Organic (□) and Biodynamic (■) treatments.

Many researchers have found relationships, particularly in controlled, pot conditions, e.g. Clark and Zeto (2002) reviewed the effects of AMF on enhancing or reducing acquisition of nutrients in plants. The nutrients enhanced most in host plants grown in many soils of high and low soil pH were found to be P, N, Zn, and Cu, while K, Ca, and Mg are enhanced when plants are grown in acidic soils. AM fungi have been found to alter the amount and form of N uptake by plants, increasing ammonium uptake which they prefer over nitrate (Cooper, 2002). As greatest AM fungi colonisation was found in plants in DAPCAN treatments, which also had higher leaf concentrations of Ca and

Mg, it is possible that higher availability of Ca from the DAPCAN treatment helped to increase AM fungi colonisation. Vestberg et al. (2002) found AM spore density correlated positively with high amounts of extractable Ca and P in some plots of a field cereal rotation trial, and Liu et al. (2002) found the correlation between abundance of extraradicular hyphae and concentrations of Ca and Mg in field maize shoots was significant in soils where available Ca or Mg was relatively low. However it is harder to discern relationships when so many factors and interrelationships are involved.

No relationship was found between AM fungi colonisation of lettuce roots and calcium uptake (Figure 4.7.5).

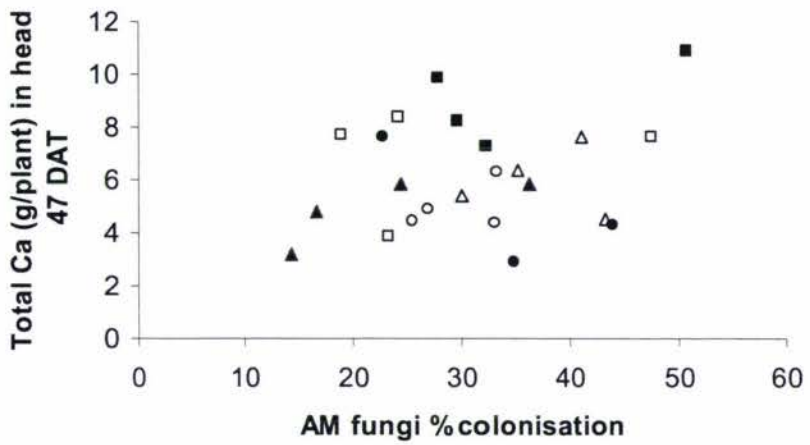


Fig. 4.7.5 Relationship between estimated lettuce leaf uptake of Ca and AM fungi colonisation of roots in plants. Symbols show treatment levels. Control (○) control + sprays (●) DAPCAN(△) DAPCAN + sprays (▲) Organic (□) and Biodynamic (■) treatments.

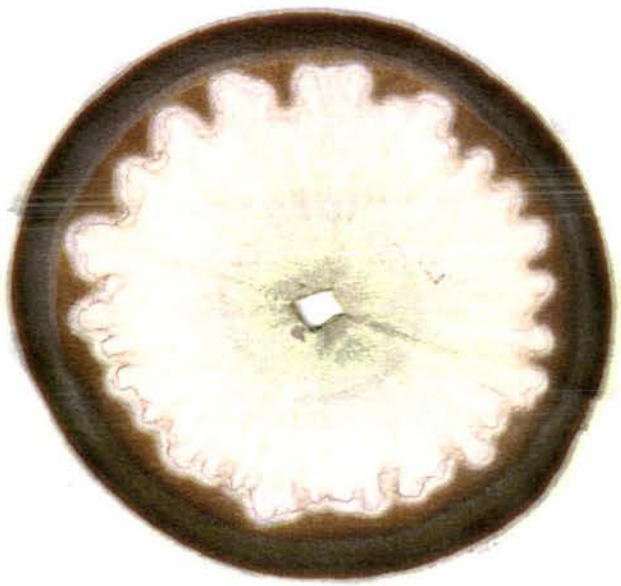
No relationship was found between percentage fungi colonisation and final wet or dry weight of the plants from which the root samples were taken.

No firm conclusions can be drawn from the soil respiration and AM fungi colonisation results presented here as to the effects of different management soil treatments and sprays on rhizosphere soil biota and root symbiosis and possible links with plant nutrient uptake and nutrient composition. From the results of other research reviewed, there is evidence that such relationships can be found, but colonisation by AM fungi may be fairly unimportant for lettuce nutrition as suggested by Berg et al. (2001) and Ryan and Graham (2002). Absence of increased soil respiration or AM fungi colonisation in control plots indicates that the fairly high rates of N and P applied in the compost and DAPCAN treatments did not inhibit soil biota activity.

4.8 Capillary dynamolysis, taste and self decomposition

4.8.1 Capillary Dynamolysis

Capillary dynamolysis of lettuce sap at 35 and 47 DAT resulted in chromatograms shown in Plates 4.8.1 - 4.8.4.



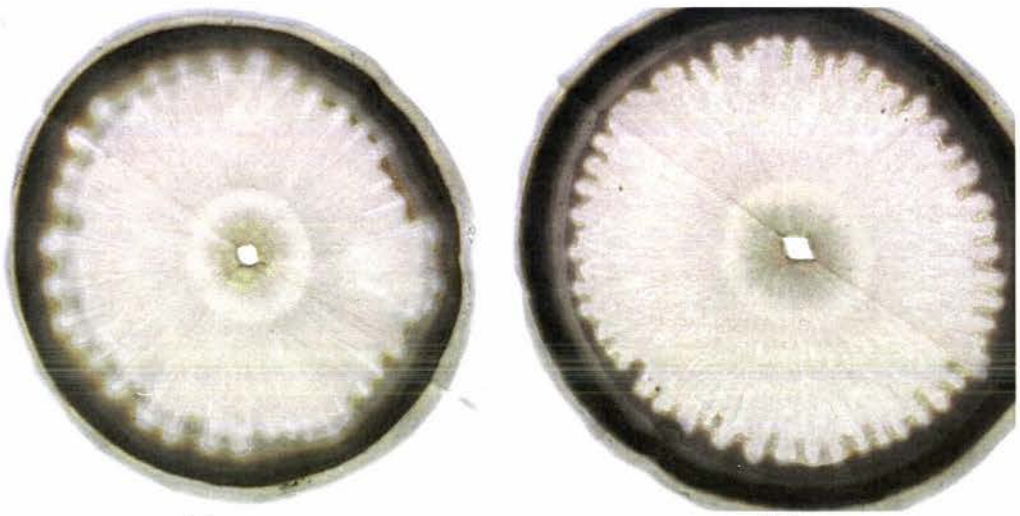
(a)

(b)

Plate 4.8.1 Chromatograms made from lettuce sap at 35 days from transplanting (DAT)
(a) lettuce from the Control + sprays treatment, (b) lettuces from the Biodynamic treatment.

The chromatograms in Plate 4.8.1 can be compared with those in Plate 4.8.2 which show less lively movement and a more mineralized appearance grayer colour. The pink

colour should not be compared as the chromatograms in Plate 4.8.2 had faded before being scanned.



(a) (b)

Plate 4.8.2 Chromatograms made from lettuce sap at 35 DAT (a) lettuce from DAPCAN treatment, (b) lettuce from organic treatment.

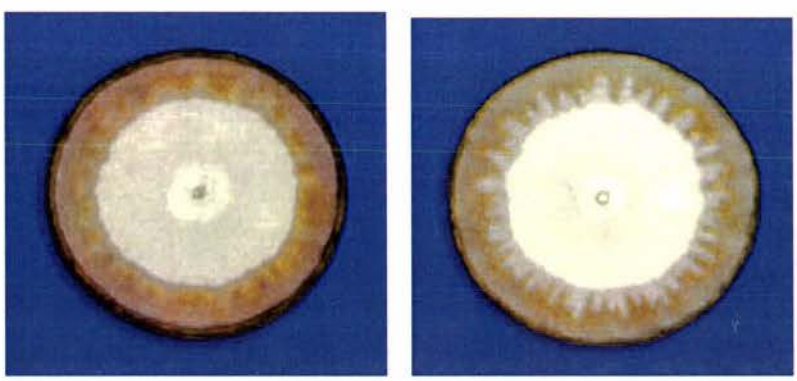
(a)

(b) (c)

(d) (e)

Plate 4.8.3 Chromatograms of lettuces at 47 DAT.- (a) Control (b) DAPCAN, (c) DAPCAN + sprays, (d) organic, (e) biodynamic.

None of the chromatograms in Plate 4.8.3 were very well formed because of difficulty in elution at the sap dilutions used and dry conditions. The chromatograms from lettuces that had been sprayed with the biodynamic fieldsprays (c and e, Plate 4.8.3) appeared to be slightly more ordered. The DAPCAN treatment lettuce chromatograms had grey circles (also in the biodynamic treatment in Figure 4.8.1), which could be an indication of more mineralisation. All the trial plots were relatively "organic" compared to soil which has been continuously cropped using soluble fertilizers for many years (shown in Plate 4.8.4. (a).



(a) (b)

Plate 4.8.4. Chromatograms made from young maize leaves by Pfeiffer (Galaxy Nutrients, 2001) Chromatogram (a) is from maize grown with mineral fertilizers and chromatogram (b) from organically grown maize.

Tingstad (2001) made rising capillary dynamolysis pictures from carrot juice. She found that carrots which were grown with compost or mineral fertilisers and organic and biodynamic treatments could be correctly differentiated by this method, both by blind panelist trial and by digital image analysis. Haegel et al. (2000) compared chemical analysis measurements such as potassium and magnesium content with parameters such as "ripeness" differentiation" and "vitality" evaluated by chromatography in carrots and found significant relationships.

4.8.2 Lettuce sensory evaluation

Evaluation of lettuce appearance, aroma, texture and flavour by a panel of 10 people yielded rather variable results.

Appearance, aroma and flavour

Conversion of the 5-point category scales for general appearance, aroma and flavour to scores and analysis of these scores showed little differences between lettuces from different treatments (Table 4.8.1, Figure 4.8.5). The scales ranged from very good (scored 5) to very poor (scored 1). The data were not statistically analysed because there were not large differences between treatment means except for aroma, and number of panelists was quite low.

Table 4.8.1 Mean scores for lettuce leaf appearance, aroma, and flavour of lettuce samples assessed by 10 panelists using 5-point category scales. Treatment codes as in Table 3.3.

Sample	Treatment	Appearance	Aroma	Flavour
f	Ctrl	4.3	2	2.0
b	C+ sp	3.5	1.5	2.2
d	DC	3.7	1.5	2.0
a	DC+ sp	4.1	3.1	2.3
e	Org	4.2	0.8	2.0
c	Bd	4	0.7	2.0

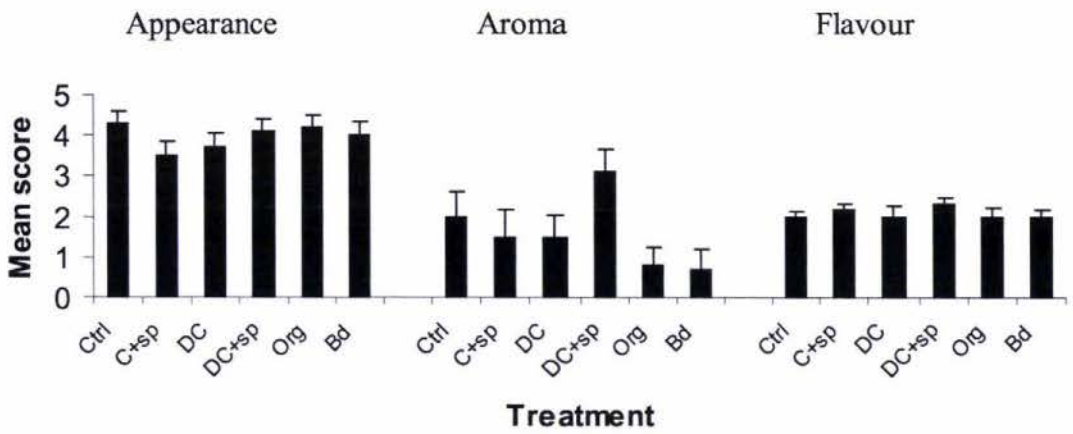


Fig. 4.8.5 Mean scores for lettuce appearance, aroma and flavour for sample leaves from each treatment assessed by 10 panelists using descriptive category scales. Treatment codes as in Table 3.3. Bars represent standard error of means.

The samples generally scored well for appearance. Many samples were considered to have no aroma: an interesting finding was that the DAPCAN +spray lettuce leaves appeared to have most aroma. There was little difference in ranking for flavour, but more differences when panelists were asked to score for sweetness and bitterness.

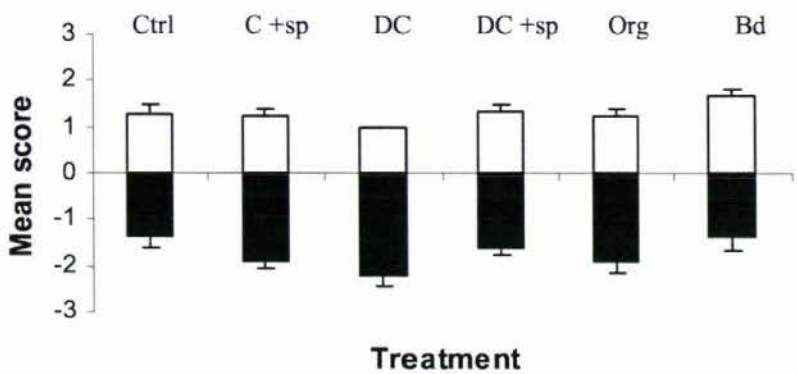


Fig. 4.8.6 Mean scores for lettuce sweetness (⬚) and bitterness (■) for sample leaves from each treatment assessed by 10 panelists using descriptive category scales. Treatment codes as in Table 3.3. Bars represent standard error of means.

Most panelists considered many of the samples to be bitter. Harvesting early in the morning on a dull day would have contributed to this bitterness; however, commercially grown lettuces are often harvested early in the morning. Analysis of variance showed no statistically significant differences between treatment means (Table 4.8.2, Figure 4.8.6).

Table 4.8.2 Mean scores for lettuce leaf bitterness and sweetness assessed by 10 panelists using 3-point intensity scales. Treatment codes as in Table 3.3.

Treatment	Bitterness	Sweetness
Ctrl	1.4 a	1.3 a
C+ sp	1.9 a	1.2 a
DC	2.2 a	1.0 a
DC + sp	1.7 a	1.3 a
Org	1.9 a	1.2 a
Bd	1.5 a	1.7 a
Fertiliser		
None	1.67 a	1.3 a
DAPCAN	1.94 a	1.0 a
Compost	1.72 a	1.4 a
Spray*		
No sprays	1.83 a	1.1 a
sprays	1.78 a	1.4 a

* Mean of Control and DAPCAN treatments only

The intensity scales for sweetness and bitterness had only 3 categories – not bitter, bitter and very bitter. Better results might have been obtained using intensity scales with more categories. Meilgaard et al. (1999) discuss how panelists tend to use only the middle section of a scale rather than the extreme points and recommend evaluating how many steps a panelist can meaningfully employ and then adopting a scale twice that length. A further factor was the insufficient time for training and establishing a common reference point for bitterness and equal differences between points on the scale. Statistical analysis revealed a significant p-value (0.0435) for difference between panelists.

Sweetness and bitterness were scored separately. In general samples that scored high for sweetness scored low for bitterness and vice versa. The biodynamic lettuce leaves scored highest for sweetness and equal lowest for bitterness with control lettuce leaves. Although from their comments these were the attributes most noticed by panelists, there did not, however, seem to be much relationship between sweetness and bitterness score and overall ranking of samples.

Ranking

Mean scores for ranking by panelists are shown in Figure 4.8.7.

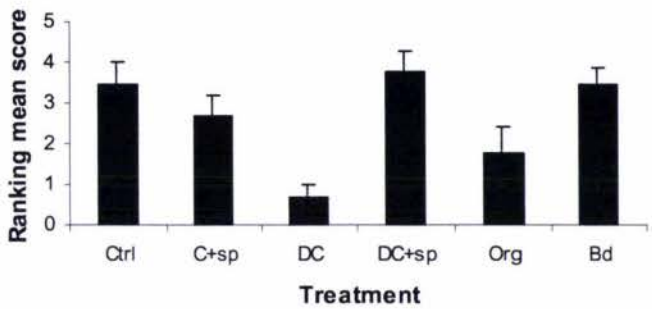


Fig. 4.8.7 Mean scores for ranking of lettuce sample leaves sensory quality for from each treatment assessed by 9 panelists. Treatment codes as in Table 3.3. Bars represent standard error of means.

Using Friedman's statistical method for nonparametric analysis (Meilgaard et al., 1999)

$T = ([12/(n)(k)(k+1)](\sum R^2))-3(n)(k+1)$

Where k = number of samples, n= number of subjects, R1, R2...=rank sum for each sample.

The result for T was much higher than the chi-square value for 95% confidence level, so there was no statistical difference in ranking scores between samples. Many more panelists would have been needed to obtain any statistical differences between ranking scores.

DAPCAN +sprays treatment lettuce samples were ranked highest (Figure 4.8.7), which was an interesting result as they were not the sweetest or least bitter sample (Figure 4.3.6). However, the panelists considered they had the greatest aroma and flavour, so this must have been important for that group of panelists. It is possible that this group of panelists was not representative of the general population because most grew vegetables in home gardens and would therefore be more familiar with the fresh taste of lettuces rather than the generally blander flavour of lettuces purchased from a shop. DAPCAN lettuces were ranked lowest which was consistent with scoring for sweetness and bitterness. Although no statistical difference between taste values for samples could be shown, it is interesting to see the trend to perceived improved flavour in lettuces from treatments given biodynamic field-sprays, other than the Control +sprays treatment. This is consistent with the findings of Lammerts van Bueren et al. (1988) who found taste differences between lettuces grown with and without the biodynamic preparations.

Results of sensory comparisons between organically and conventionally grown vegetables have generally not given consistent or meaningful results (Bourn and Prescott, 2002).

Texture

Words used by panelists to describe the lettuce texture were quite variable, so that no clear differences between treatments could be seen. "Crunchy" was used to describe the DAPCAN + spray lettuces slightly more often than for other treatments, and "firm" most often to describe the Control + spray lettuces. These two treatment types were least often described as soft. "Tough" was used to describe the DAPCAN and Organic treatment lettuces more than for lettuces from other treatments.

4.8.3 Lettuce self decomposition test

No significant differences between treatments were found for mean weight loss from loss of water by lettuce leaves kept in unsealed plastic bags in a drawer for 17 days from harvest Table 4.8.3, Figure 4.8.8).

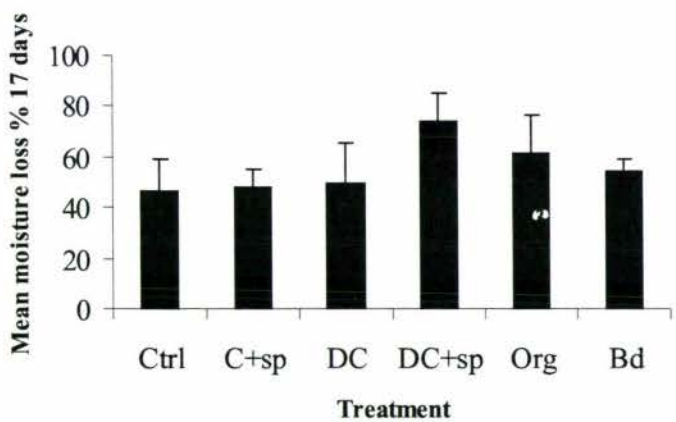


Fig. 4.8.8 Mean % moisture loss over 17 days of 3 samples per treatment of 3 lettuce leaves kept in an unsealed plastic bag in a drawer for 17 days after harvesting. Treatment codes as in Table 3.3. Bars represent standard error of means.

The control treatment lettuces had slightly lower water loss and DAPCAN + sprays treatment lettuces highest water loss which was not consistent with leaf dry matter content shown in Table 4.3.3. Moisture loss may have related to permeability of cell walls, which was likely to be greater for plants that took up more nitrogen.

Table 4.8.3 Mean weight loss (g/day) and deterioration score of lettuce leaves from each treatment over 17 days from harvest. Treatment scores in Table 3.3.

Treatment	Weight loss (g/day)	Decomposition score
Ctrl	0.020 a	3.00 a
C+ sp	0.025 a	2.00 a
DC	0.029 a	2.33 a
DC + sp	0.042 a	2.00 a
Org	0.036 a	2.67 a
Bd	0.032 a	3.67 a
Fertiliser		
None	0.023 a	2.50 a
DAPCAN	0.036 a	2.17 a
Compost	0.034 a	3.17 a
Spray*		
No sprays	0.025 a	2.67 a
sprays	0.033 a	2.56 a

* Means of control and DAPCAN treatments only

There was no significant difference between treatment means for decomposition of lettuce leaves (Table 4.8.3, Figure 4.8.9), and decomposition did not relate very well to water loss. Maturity may have been a factor in decomposition as generally leaves from more mature trial lettuces decomposed more. The lettuces from the compost treatments were not found to store better than lettuces from other treatments. This finding can be contrasted with that of Samaras cited in Koepf (1993). Samaras (1978) found less spoilage, shrinking and bacterial and enzyme activity of carrots and potatoes grown in organic compared to mineral systems, and less in those grown with field sprays. Potatoes from organic treatments in the long-term Swedish K-experiment were found to have less fungal deterioration and evaporation loss after 6-7 months storage than those from treatments with soluble fertilisers (Granstedt and Kjellenberg, 1996).

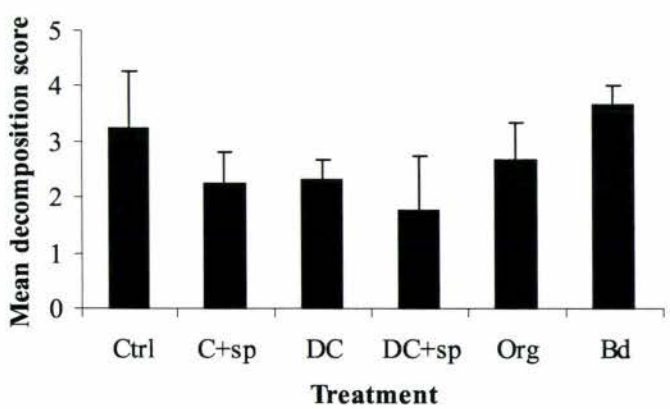


Fig. 4.8.9 Mean decomposition score of 3 samples per treatment of 3 lettuce leaves kept in an unsealed plastic bag in a drawer for 17 days after harvesting. Treatment codes as in Table 3.3. Bars represent standard error of means.

One factor that may have affected results was that lettuce leaves all had to be washed to remove soil before weighing at harvest time. Although they were all carefully dried with paper towels, some may have been damper than others, or they could have been damaged by drying (however, this would have been a random effect so should not have affected differences in treatment means).

4.9 Preliminary Conclusions from Field Trial

4.9.1 Preliminary conclusions

This section draws some preliminary conclusions from the field trial in order that supplementary trials can be conducted. A full discussion of the field trial results is given in Section 6.

For the field trial described in Sections 3 and 4, lettuces (cv. Canasta) grown in Te Puke series sandy loam, amended with organic composts, gave higher yields at all harvest times than lettuces grown in soil that was unfertilised (control) or treated with soluble fertilisers containing estimated equivalent N and P as the composts.

Compared to plants grown on soil treated with soluble fertilisers and control plots, plants grown on organically amended soil had lower DM% and lower concentration of antioxidants and most elements measured other than N, P and K. Lower nutrient concentrations probably resulted from faster growth due to the high available N and K status from the soil and the large quantity (8.35kg/m^2) of compost added to provide lettuces with their target N content at harvest.

Compared to lettuce plants grown in soil amended organic treatments, plants fertilised with soluble fertilisers (DAPCAN) contained higher concentrations of Ca, Mg, Fe, Zn and Cu, had higher Ca: P and lower K: Ca+ Mg ratios. The higher Ca uptake was likely related to provision of extra calcium in the fertiliser. Although not statistically significant at the 95% confidence level, these lettuces also showed a trend to higher nitrate concentrations, greater nitrate-N: total N ratios and lower concentrations of essential amino acids.

At final harvest, on average lettuce plants from plots receiving biodynamic field-sprays had higher DM%, longer, less branched roots and higher concentrations of P, amino acids, antioxidants and protein in leaves. However, the effects of the sprays on each amendment treatment (control, DAPCAN and compost) were not consistent at a single harvest or over time.

At 28 DAT the silica spray applied to the DAPCAN + sprays and Control + sprays treatments had opposite effects to effects on the Biodynamic treatment. This meant that a comparison of the 3 sprays treatments with the 3 no-sprays treatments showed few consistent effects. For example, mean lettuce head DM% at 28 DAT for the Organic treatment was 6.19% whereas Biodynamic treatment (sprayed) was 5.5%. For the DAPCAN and Control treatments however, the sprays increased DM%, increasing it to (6.43 to 7.21%) and (6.23 to 7.36%) for the corresponding spray treatments. When combined as groups there was no significant difference between means for sprays and no sprays. The pooled sample variance was 0.479, so it is likely that the high DM% in the DAPCAN + sprays and Control + sprays treatments was an effect of the sprays, and not just an apparent effect caused by within treatment variability. Similar but less extreme effects of the sprays were found for N, P and K concentrations in leaves and for shoot: root weight ratios, which were all greater in Biodynamic and less in DAPCAN + sprays and Control + sprays treatment plants. Large errors between plants also meant that few differences from spray application on a single treatment could be detected.

Inconclusive effects of treatments were due partly to the large natural plant to plant (within replication) variation which meant that on average treatment effects had to be greater than 20% (root length), 8 % (leaf DM), 16-57% (P concentration) 47% (light wavelength absorption), 43% (sap nitrate concentration), 13 - 55% (Brix), 127% (soil respiration), of the treatments means to be significant. Much of this error is associated with spatial soil variability affecting seedling vigour. To conclusively show small (15 – 20%) differences between different management treatments care has to be taken to minimise variability between plants and soil and large numbers of replications are needed. High fertility of the trial soil would also have reduced the size of treatment effects.

Thus while some interesting differences were observed when silica spray was applied to lettuces it was not possible to statistically validate the effects of the sprays either in the short term or longer term.

4.9.2 Supplementary Glasshouse trial

It was decided to conduct a glasshouse trial to look for evidence of possible changes in plant metabolism from spraying the biodynamic silica spray. This would enable:

- Reduction in treatment differences in more controlled conditions, which should enable short term effects such as small differences in leaf nitrate and sugar concentrations between treatments to be statistically significant.
- Reduction in numbers of influencing factors, particularly soil moisture status, by keeping all factors constant between treatments except for spraying with the silica spray. (In the field trial, some of the sprayed plots also had added soluble fertiliser and some also had added compost and biodynamic compost preparations and preparation 500).
- Investigation into whether application of the silica spray might change factors that affect plant metabolism, such as net photosynthesis and light absorption.

5 GLASSHOUSE TRIAL

Introduction

This trial was conducted in order to investigate the effects of biodynamic silica spray application on lettuces grown under more uniform temperature and moisture conditions.

5.1 Method

5.1.1 Trial design and lettuce cultivation

Trial design

There were three treatments in this trial:

- No spray (N), (sprayed with water at the first 2 silica spray times, but not sprayed at the final spray time,
- Sprayed three times with horn-silica spray (S), and
- Water-sprayed plants (W), which were sprayed with equivalent amounts of water at each of the silica spray times.

Fifteen pots were prepared for planting with lettuces for each treatment, to enable 5 replicates of each treatment at each of 3 harvests.

Collection of soil and preparation of pots

On 14.4.03 soil was taken from the top 15cm of two of the control treatment plots used in the trial reported in sections 3 and 4 and air-dried. On 17.4.03 the air-dry soil and some of the biodynamic compost used as described in section 3 (but 6 months older) were separately passed through 5mm sieves. Compost (350 g, containing 43.5 % moisture) and soil (1042 g) of were weighed out and mixed together. This quantity of compost provided the same quantity of nitrogen as was applied to each plant in the first trial. The mixture was placed in a plastic pot, 125 x 125cm x 110 cm deep. Distilled water (400 mL) was added to each pot, and the pots covered with a sheet of newspaper. The temperature of the greenhouse was kept at about 22°C.

Lettuce transplanting

On 23.4.03, lettuces (cv. Cos Little Gem) were transplanted into the 46 previously prepared pots (Table 5.1.1) and further distilled water was added until the soil in each pot reached 80% of pot capacity, predetermined as described below under water supply.

The lettuce plants had been grown from seed from one plant that had been previously raised organically. The day chosen for transplanting was when the moon was in a descending phase, in the constellation of Capricorn, to optimize rooting and establishment of the plants (Thun, 2002). As the lettuces were initially stressed through having their roots washed at transplanting time, about 5 mL of a vermicompost and water spray was applied to each one on 5.5.03.

Application of biodynamic preparation 500

A 1g portion of preparation 500 (horn-manure) was stirred for 1 hour in about 5lt of distilled water in a bucket (Procter, 1996). 1 g of horn-clay was also included. This is a preparation that was alluded to by Steiner but has not generally been applied by biodynamic farmers until recently. Its stated purpose is to assist the movement of the calcium and silica forces enhanced by preparations 500 and 501 to be taken into the plant. About 3 mL of the mixture was applied to the surface of each pot by flicking drops by hand after transplanting.

Water supply

The soil and compost were initially damp and 400mL water was added to each pot, which brought them almost to field capacity. Field capacity was determined by weighing 2 pots containing the same weights of soil and compost mixed, watering from the top until water started to come out of the bottom, leaving them for 48 hours then reweighing. The weight of water required to bring the pot to 80% of pot capacity was calculated. Whenever average weight of 10 weighed pots dropped below 60% of field capacity all pots were adjusted back to 80% pot capacity. As some plants grew faster than others and took up more water, pot weights became more variable, so from 6 weeks from planting, every pot was numbered and weighed separately, and watered to keep them at similar soil moisture content.

Application of biodynamic horn-silica preparation

On 8.5.02 the plants were ranked in order of size and alternate pots allocated to spray and non-spray treatments. The sprayed plants were taken out of the greenhouse for spraying to ensure no spray reached non-sprayed plants. BdMax 501 (7.5 mL) spray concentrate was diluted in 500 mL distilled water and sprayed above the selected lettuce plants. After 20 min they were returned to the greenhouse. The same quantity of

distilled water only was sprayed above the other plants. The BdMax spray contains a potentised homoeopathic dilution of the horn-silica spray and was used instead of preparing a spray by stirring horn-silica preparation in water for one hour. This was to increase speed of application and because the effect of the spray appears to be the same as the stirred spray except but more concentrated on the immediate application area.

Lighting

The tables on which the plant pots stood were rotated every 2-3 days to counteract possible inequality in lighting. On 28.5.02 plants were given an extra 4 hours lighting per day by 2 lights installed above the plants so that all plants were lit as equally as possible, to compensate for decreasing day-length and increase growth. On 5.6.02 artificial lighting time was extended to 8 hours per day.

Pest management

On 3.6.03 conditions were humid and the plants became infected with aphids. To avoid contamination of leaf surfaces which could have affected trial results the plants were not sprayed, but aphids were removed with a small brush as far as possible and a sticky trap was hung above them. The plants that were receiving the silica spray were sprayed on 4.6.03 as that spray can be used for pest management of sucking insects when conditions are too humid. All plants were sprayed with silica or water as described in the previous paragraph. The soil moisture content was allowed to reduce.

5.1.2 Measurements

Table 5.1.1 Calendar of Trial Measurement Dates

Date	17.4	23.4	8.5	4.6	17.6	18.6	19.6	20.6
Days from transplanting			15	42	55	56		
Prepare pots	✔							
Transplant		✔						
Spray 500		✔						
Spray silica			✔	✔		✔		
Leaf Measurements*						✔ ✔ ✔		
Merck nitrate test+						✔ ✔	✔	
Capillary dynamolysis					✔		✔	
Harvest							✔	
Sap KCl extraction								✔
Freeze drying								✔

* measurements included leaf spectral reflectance, photosynthesis, stomatal resistance

\+ remove 3 young mature leaves per plant, test sap for leaf sap analysis of nitrate, soluble solids.

Capillary dynamolysis

On 17.6.03 sap was extracted from 2 or 3 young mature leaves from each of 2 sprayed and 2 unsprayed plants by chopping the leaves, mashing them by pestle and mortar, placing them in a piece of thin plastic then squeezing the sap through pinholes. The sap was then used in circular capillary dynamolysis tests. The method used was adapted from the method described by Knorr (1982) similar to that described for compost testing in section 3.1.2. The sap was diluted with sodium hydroxide solution and immediately placed in a plastic lid in a Petri dish and eluted into the filter paper. The dishes were placed in a dessicator containing warm water to provide a humid atmosphere. Several dilutions of sap were tried, to make the NaOH about 1% of the total solution as recommended by Pfeiffer (1988). In all cases there appeared to be too much sodium hydroxide and the circular chromatograms obtained were less clear than those previously made from sap with no sodium hydroxide added. The same solutions were eluted into strips of Whatmans No 1 filter paper placed vertically in plastic lid using the rising capillary dynamolysis method described by (Kolisko and Kolisko, 1978).

Nitrates

On 18.6.03 at 9am, 2-3 young mature leaves were removed from each of 4 sprayed and 4 unsprayed plants. The sap was extracted by the method described above. 1mL of the sap was diluted with 200mL deionised water and tested for nitrate concentration with a Merck nitrate strip. The rest of the undiluted sap was refrigerated.

Leaf spectral reflectance

The soil surface of 4 sprayed and 8 unsprayed plants was covered with aluminium foil to prevent water loss from the soil surface (Plate 5.1.1 a), then those pots were weighed. At 11 pm the plants were all measured for leaf spectral reflectance. This was done using an 8-channel reflectometer held by a clamp so that a plant could be placed directly underneath the aperture. A piece of white card was held between the plant and reflectometer in such a way that it could be slid over or away from the plant (Plate 5.1.1 b). Reflectance for each of 8 wavelengths was recorded for the white card then readings repeated immediately for a plant. This procedure was repeated for the next plant.

(a) (b)

Plate 5.1.1, Lettuce plants and equipment prepared for reflectometer reading

Plant water use

The pots used for the leaf spectral reflectance measurements were also used to measure water use by repeated weighing of the whole pot during the trial. Results of the pot water loss were expressed as water use per g plant fresh weight.

Net photosynthesis and stomatal resistance

Four other plants from each treatment were measured for photosynthesis and stomatal resistance using a Lister – 6200 portable photosynthesis system. Leaf surface temperature readings were also taken but later found to not be reading properly.

Programme of measurements

Table 5.1.2 Programme of measurements, Glasshouse Trial

	18.6.03	19.6.03
Final spray applied	12.00	
Plant weight	11am, 12.30,2,3,4.30pm	12.00, 2pm
nitrate	9am, 3pm	3pm
Spectral reflectance	11.30am (Time 1) 12.30pm (Time 2) 2.30pm (Time 3) 4pm (time 4)	
Photosynthesis, stomatal resistance	11.30am, 12.30, 2.30pm	
Brix		3pm

At 12.00 midday, 8 previously sprayed plants were sprayed with silica spray, 8 others were sprayed with distilled water, and 8 were not sprayed. This was not the usual time

to spray – it is generally recommended to apply the silica spray at dawn. Pettersson (1979) reported a higher level of food-quality index for plants sprayed with horn-silica spray at dawn compared to plants sprayed later in the day. However, a later time was chosen when sunlight intensity would be relatively stable. In fact there was considerable variation in light intensity and relative wavelength intensity as there was varying cloud cover. In the afternoon cloud cover increased, resulting in lower but less variable light intensity.

Ten minutes after spraying the plants were reweighed and 30 minutes later tested again for leaf spectral reflectance, photosynthesis and leaf surface temperature. Care was taken to try to place the pots under the reflectometer in the same position as for previous readings, and to use the same leaves for photosynthesis readings. These measurements were repeated again at the times shown in Table 5.1.2. At 3pm leaves were harvested from plants not used for other measurements and tested for nitrate as described above.

On 19.6.03 plants were reweighed at 12 and 2pm to compare moisture loss. After 2pm these plants that had been used for spectral reflectance measurements were harvested and the heads weighed. Every other leaf from each of 2 sprayed and 2 non-sprayed plants was removed, washed, dried, weighed, placed in a plastic bag and frozen prior to freeze drying for amino acid analysis. Samples of leaves were also taken from 4 plants per treatment for sap extraction and testing for nitrates. The sap was also tested for soluble solids by a hand-held refractometer and some of the sap was used for capillary dynamolysis tests.

Laboratory analysis for nitrates in lettuce leaf sap

Leaf sap saved and refrigerated from expression at the 3 measurement times was diluted 1:200 in 2M KCl extractant solution (Westerman, 1990), filtered and analysed for nitrates by Technicon Autoanalyser.

Amino acid analysis

Standard amino acid profiles of freeze-dried leaf material were analysed by a similar method to that reported by Fierabracci et al. (1991) by the Institute of Food, Nutrition and Human Health Laboratory, Massey University.

5.2 Glasshouse Trial Results and Discussion

5.2.1 Observations on Plant growth

The lettuce seedlings took approximately 10 days to recover from transplant shock, possibly because prior to transplanting, soil was washed from their roots. Many of the original leaves died back, but new growth started within a few days (Plates 5.2.1, 5.2.2). Growth was slow, but increased at 35 DAT when day-length was increased with artificial lighting.

Plate 5.2.1 Lettuce plants after transplanting

Plate 5.2.2 Lettuce plants 35 DAT

The plants sprayed with the silica spray grew more slowly than those not sprayed. At 6 weeks from transplanting conditions were very humid and the plants became infested with aphids. A further silica spray appeared to slightly increase infestation of sprayed plants relative to unsprayed plants, possibly due to increased stress.

From 6 weeks from transplanting a few plants developed tip-burn, indicating calcium deficiency. There was no apparent difference in incidence between sprayed and unsprayed plants.

5.2.2 Plant water loss by lettuces at 56 DAT, immediately after spray application.

In the 4 hours after spray application, there were no significant differences between treatments in the weights of water that the lettuce plants lost by transpiration. (Figure 5.2.1, Table 5.2.1).

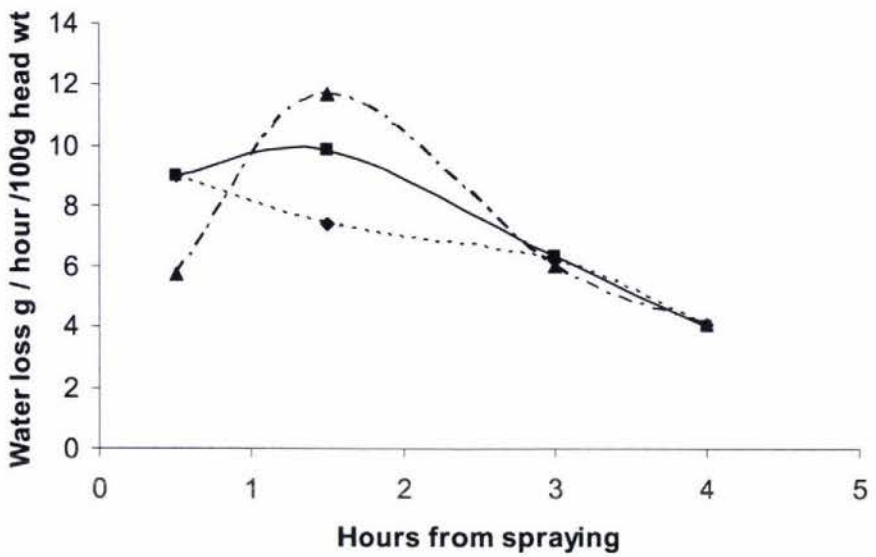


Fig. 5.2.1 Weight loss (g) per hour per 100 g fresh lettuce head, as an indicator of water loss by transpiration). N (●) no spray, S (■) silica spray, W (▲) water spray.

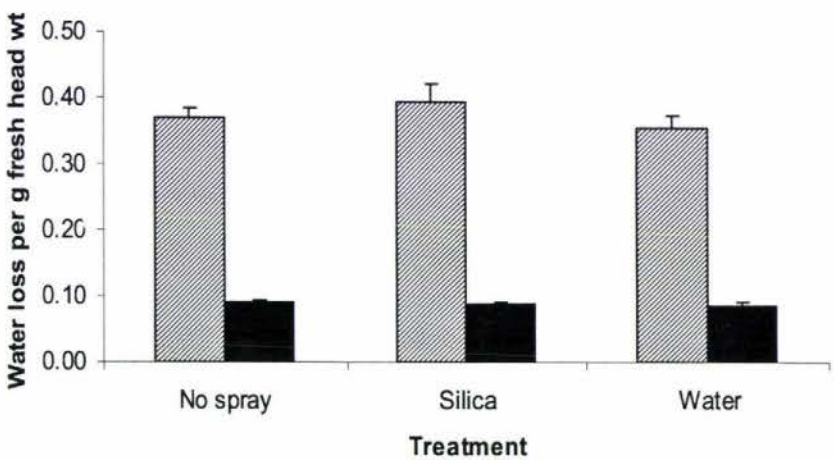


Fig. 5.2.2 Estimated water losses from lettuce plants during 5 hours (from one hour before to 4 hours after spraying) on day 1 (▨) and during 2 hours (from midday to 2 pm) on day 2, (■) Bars represent standard error.

Table 5.2.1 Estimated mean water losses by lettuce plants in each treatment from one hour before spraying

	Estimated Water loss (g/100 g plant fresh wt)		
Time	**First 5 hours, day 1**	**Last 2 hours, day 2**	**Total period 29 hours**
No spray	37 a	9 a	89 a
Water	35 a	10 a	88 a
Silica	39 a	9 a	93 a

When the weight loss was normalized to a standard plant fresh weight of 100g, it was apparent that spray treatments had no significant effect on the amount of water lost by transpiration either in the short term (5 hours after spray application) or in the longer-term (29 hours after spray application, Table 5.2.1, Figure 5.2.5).

5.2.3 Leaf spectral reflectance and light absorption

Estimates of light absorption by lettuce leaves were determined by measuring the difference in spectral reflectance of sunlight from a white card and a growing lettuce plant. This estimate makes no allowance for transmittance of light through the leaves. As the sunlight was varying with changing cloud cover, each reading of leaf reflectance is expressed as the percentage of reflectance from the white card taken immediately before the plant reading. There was not very much difference in light absorption recorded for lettuces from the different treatments either before or after spray application, particularly for the mid-spectrum light wavelengths (Figures 5.2.3, 5.2.4).

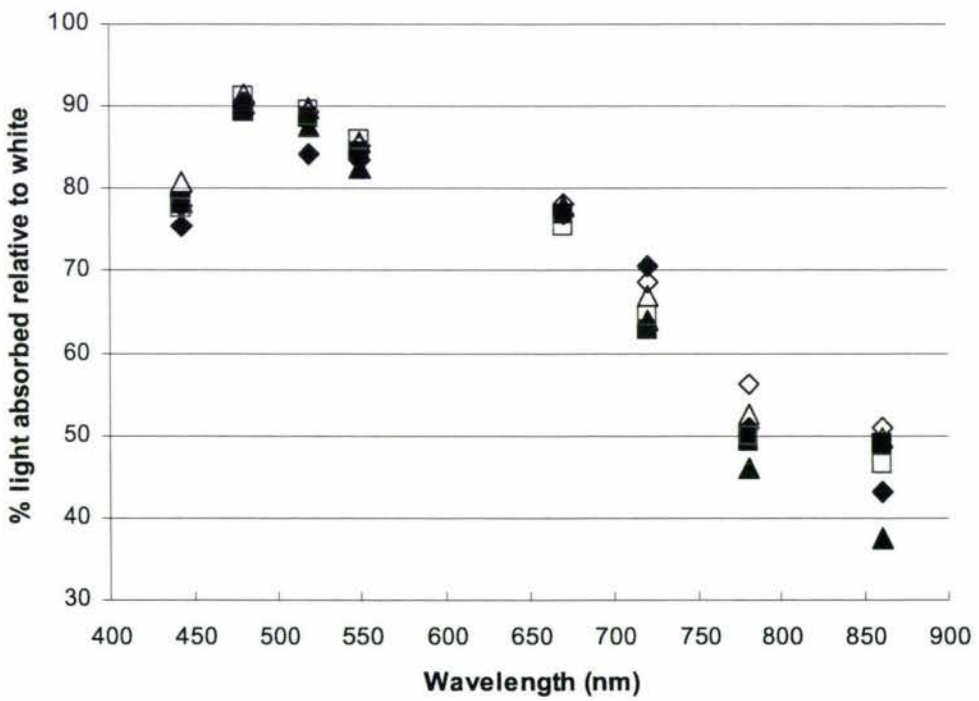


Fig. 5.2.3 Percentage of light absorbed (100*(reflectance white – reflectance plant) /reflectance white) by lettuce plant leaf surface relative to absorbance by a white card at a range of wavelengths, as measured by a reflectometer above the plant before (closed symbols) and one hour after (open symbols) spraying (mean of 4 reps). ♦ = no spray, ■ = silica spray, ▲ = water spray. Treatment and time codes are explained in Method sections 5.1.1, 5.1.2.

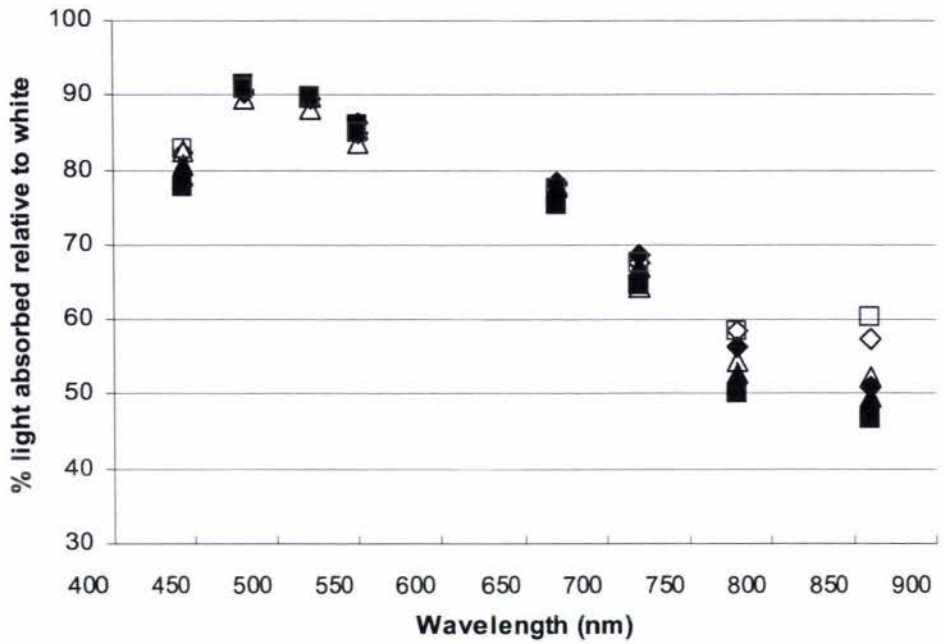


Fig. 5.2.4 Percentage of light absorbed (100*(reflectance white – reflectance plant /reflectance white) by lettuce plant leaf surface relative to absorbance by a white card at a range of wavelengths by plants 30 minutes (closed symbols) and 150 minutes (open symbols) after spraying. (mean of 4 reps) . ♦ = no spray, ■ = silica spray, ▲ = water spray. Treatment and time codes are explained in Method sections 5.1.1, 5.1.2.

There were very few significant differences in estimated mean light absorption for any of the wavelengths measured at different times after spray application (Tables 5.2.2, 5.2.3). The natural variation in percent absorption by plants of the same treatment was high. For example the pooled co-efficient of variance for 780 nm (far-red) light at the second reading time was 14.7 % and there needed to be at least 23.56 difference between means (47 % of the lowest mean) for a significant difference between treatment means.

Table 5.2.2 Percentage absorption relative to white card of 443 nm (blue) and 670 nm (red) wavelengths by sprayed and unsprayed lettuces before and after spraying (Means of 4 replications per treatment)

	Blue light – 443 nm				Red light –670 nm			
Minutes from spraying	**-30**	**30**	**150**	**240**	**-30**	**30**	**150**	**240**
No spray	75.34a	77.88a	82.31a	82.34ab	76.87a	78.04a	78.33a	74.56a
Water	78.31a	80.69a	82.67a	82.02 b	77.86a	77.94a	77.67a	73.87a
Silica	78.14a	77.74a	82.89a	83.28 a	76.81a	75.40a	77.54a	73.72a

Treatments and measurement times as explained in Sections 5.1.2 and Table 5.1.2.

Blue light

No significant differences in blue light absorption treatment means were recorded except between water and silica sprayed plants 150 minutes after spraying (Table 5.2.2, Figure 5.2.5).

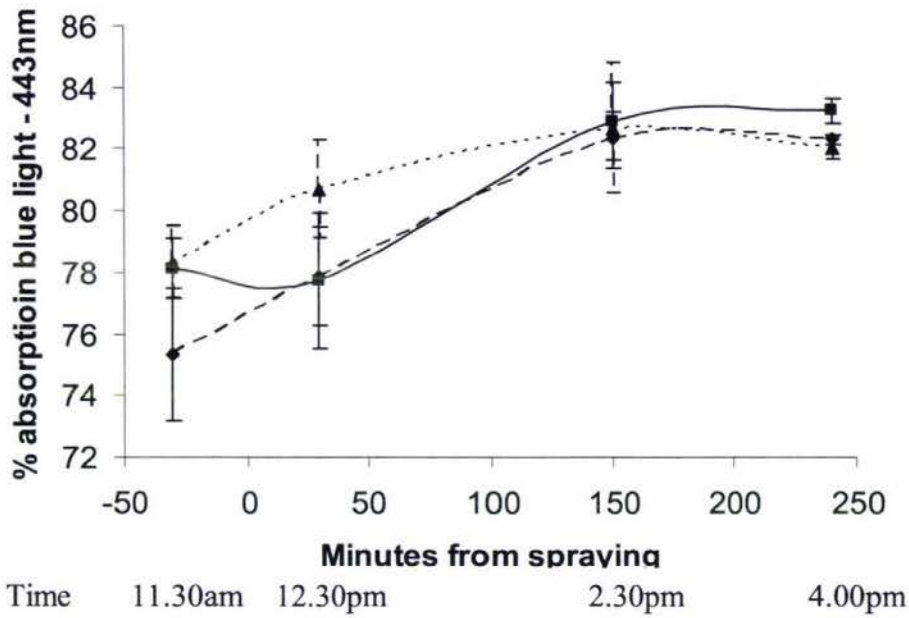


Fig. 5.2.5 Percentage absorption relative to white card of 443 nm wavelength (blue) by lettuces from each treatment (mean of 4 reps) at each measurement time before and after spraying. N (●) no spray, S (■) silica spray, W (▲) water spray. Treatment and time codes are explained in Method sections 5.1.1, 5.1.2. Bars represent standard error.

A slight reduction in blue light absorption by the silica sprayed plants was recorded 30 minutes after spraying, whereas absorption of lettuces in the other treatments increased. This was not a significant difference from the other treatments because the no-spray treatment plants' absorption was lower than the others before spraying. Absorption of blue light by all plants increased until 2.30 pm indicating that the increased absorption related to the time of day. By 4 hours after spraying percentage absorption of blue light relative to white light of plants in the silica sprayed treatment (S) was found to be significantly higher by t-test compared to the water-sprayed (W) plants. This indicates that the difference was due to the silica rather than the water in the spray.

Changes in leaf light absorption could have resulted from changes in transpiration rate. Agarie et al. (1998) recorded reduced rates of transpiration of rice plants given 100 ppm silica in the nutrient solution, mainly attributable to reduction in the rate of transpiration through stomata pores. Increases in leaf water conductance from blue light pulses were greater in plants not given silica, indicating that silica may influence stomatal opening

in response to environmental stimuli by regulating water potential in the epidermal cells of leaves (Agarie et al., 1999). Briggs and Liscum (1997) discuss how blue light has been found to activate signals which induce proton extrusion in leaf guard cells, and this is the mechanism which induces stomatal opening.

It appears that decreased blue light absorption by lettuces immediately after spraying with silica may have increased stomatal opening and increased transpiration, but this effect had reversed by 150 minutes after spraying, when blue light absorption had decreased. This could have led to more stomata closure relative to the other treatments.

Briggs and Liscum (1997) discuss how blue light has been found to initiate inhibition of plant growth, which could explain the reduction in growth of lettuce plants sprayed several times with silica. It is possible that the spray changed absorption in ultraviolet wavelengths also, which were not measured. Solar UV-A and UV-B radiation have been shown to have inhibitory effects on lettuce growth (Krizek et al., 1998).

Red light

No significant differences between treatments in red light absorption were recorded (Table 5.2.2, Figure 5.2.6). An apparent drop in percentage absorption of red light after spraying, similar to that of blue light was recorded in silica sprayed plants, but by the last measurement there was little difference between treatments.

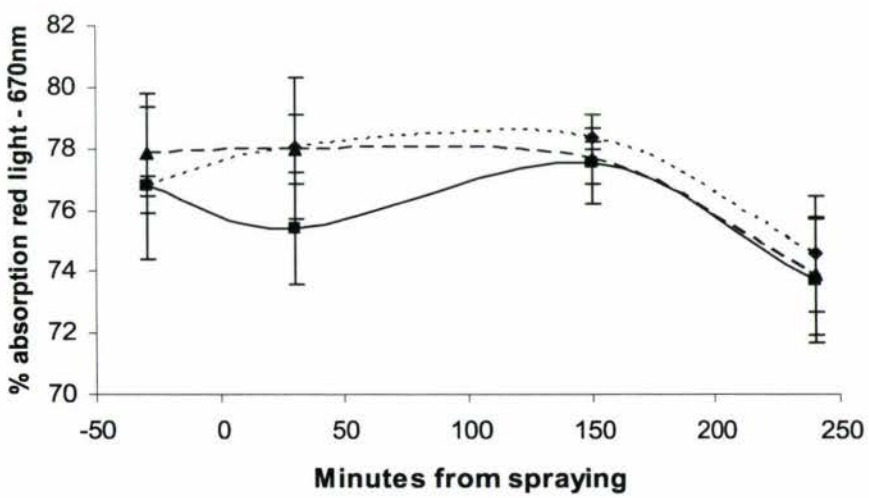


Fig. 5.2.6 Percentage absorption relative to white card of 670nm wavelength (red) by lettuces from each treatment (mean of 4 reps) at each measurement time before and after spraying. N (●) no spray, S (■) silica spray, W (▲) water spray. Treatment and time codes are explained in Method sections 5.1.1, 5.1.2. Bars represent standard error.

Carter and McCain (1993) found that reflectance sensitivity increased substantially in the 400-760 nm range with peak sensitivity near 695 nm as chloroplast relative water content decreased. However, all the soil for all plants in this trial contained similar moisture content, so differences in light absorption immediately after spraying could not have been due to differences in plant moisture content. However, further experiments in which light transmittance was measured in addition to leaf reflectance and absorption (Carter and Knapp, 2001) showed increased transmittance of light by leaves of stressed plants, which was shown to relate to reduced chlorophyll concentrations.

Red light absorption is likely to affect photosynthesis rate as the chlorophyll *a* molecules have absorption maxima at 670 and 700 nm (Hader and Tevini (1987). No significant treatment differences in far-red light (780 nm) absorption were found (data not shown).

Table 5.2.3 Red: far-red ratio of light absorption and percentage absorbance relative to white card of 860 nm (infrared) wavelength by lettuces before and after spraying. Treatment codes and time are explained in Section 5.1.1, Table 5.1.2.

	Red: Far-red ratio				Infra-red – 860 nm			
Minutes from spraying	**-30**	**30**	**150**	**240**	**-30**	**30**	**150**	**240**
No spray	1.09 a	1.15 a	1.16 a	1.30 a	43.00 a	50.98 a	57.35ab	39.87a
Water	1.23 a	1.22 a	1.21 a	1.36 a	37.60 a	49.68 a	52.38 b	30.84a
Silica	1.26 a	1.21 a	1.15 a	1.26 a	53.13 a	46.64 a	60.34 a	35.48a

Treatments and measurement times as explained in section 5.2.2
Different letters indicate significant differences between treatments by SAS t-test at 0.95%.

Red: far-red ratio

The red: far-red ratio (R: FR) appears to trigger light/shade responses in plants (Smith, 1982). No significant treatment differences in red: far-red ratio were found for any of the measurement times (Table 5.2.3, Figure 5.2.7).

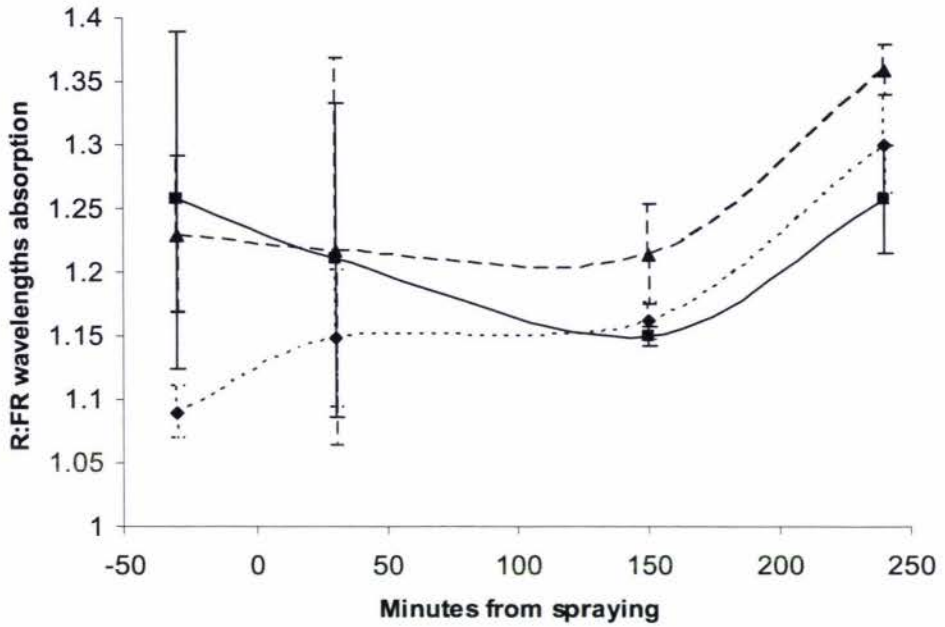


Fig. 5.2.7 Ratio of percentage absorption relative to white card of 670 nm (red) and 720nm (far-red) wavelengths by lettuces from each treatment (mean of 4 reps) at each measurement time before and after spraying. N (●) no spray, S (■) silica spray, W (▲) water spray. Treatment and time codes are explained in Method sections 5.1.1, 5.1.2. Bars represent standard error.

The consistent but non-significant reduction in red light absorption and decrease in the red: far-red ratio from spraying with the silica spray could result in a "shade" effect on plant growth, which is the opposite effect to that described by Koepf (1993).

Sleeman and Dudley (2001) found that *Brassica rapa* plants that grew under high R : FR light showed higher rates of photosynthesis and stomatal conductance and higher water-use efficiency relative to plants under comparatively lower R : FR. Garcia-Martinez and Gil (2002) discussed how red and far-red light effects on lettuce growth appears to be via phytochrome which inhibits stem elongation, either by a decrease in the content of active gibberellins or a reduction of gibberellin sensitivity of the stem. Fritz et al (1997) considered that the silica spray had a gibberellin-like effect on dwarf bean growth.

Near Infrared light

Percentage absorption of near infra-red (860 nm) wavelength light showed some interesting apparent changes in the silica sprayed lettuces, although only the difference between mean absorption by silica- and water-sprayed lettuces at the third measurement time was statistically significant (Table 5.2.3, Figure 5.2.8).

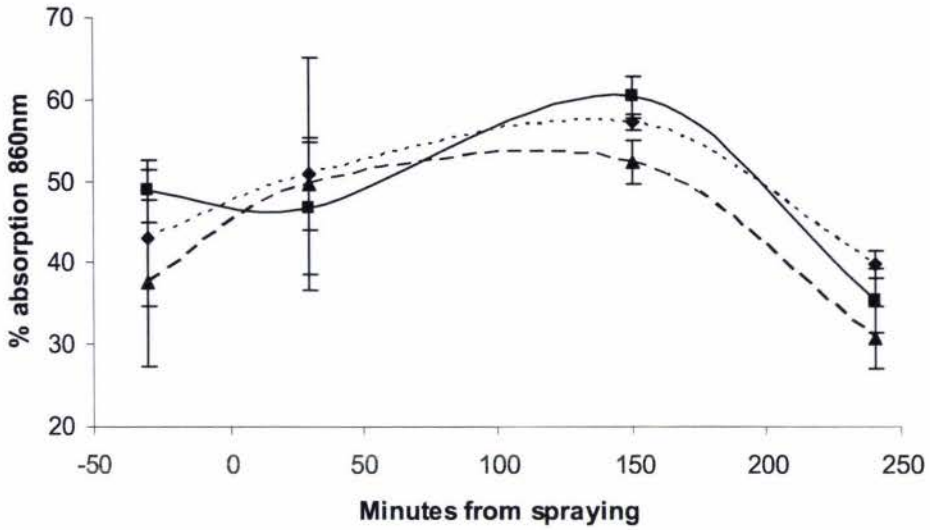


Fig. 5.2.8 Percentage absorption relative to white card of 860nm wavelength (infrared) by lettuces from each treatment (mean of 4 reps) at each measurement time before and after spraying. N (●) no spray, S (■) silica spray, W (▲) water spray. Treatment and time codes are explained in Method sections 5.1.1, 5.1.2. Bars represent standard error.

Before the last spray, absorption of the near infra-red wavelength by silica treatment lettuces was higher than that of the other treatment lettuces which indicates there could have been some lasting effect from previous silica sprays, however the difference was not significant. There was then a drop in absorbance straight after spraying, but by 2½ hours after spraying the silica sprayed lettuces were absorbing significantly more light at this wavelength than the water sprayed lettuces. However by 4 hours after spraying the silica sprayed plants were absorbing less than the non-sprayed plants. This indicates that there may not have been any real, lasting differences between treatments.

Changes in absorption of infrared wavelengths could have resulted from changes in water transpiration and water stress induced by the silica spray. However, Carter (1993) has found that there is generally only consistent response in leaf reflectance of infrared wavelengths when there is severe leaf dehydration, which was not the case in this trial.

5.2.4 Photosynthesis and Stomatal resistance

Photosynthesis

Analysis of measurements of net photosynthesis of lettuce leaves before and after spraying with water or silica spray showed no significant differences between treatment.

means (Table 5.2.4 and Figure 5.2.9). After spraying, however, between and within treatment variance decreased.

Table 5.2.4 Mean treatment readings for photosynthesis and stomatal resistance in leaves of 4 lettuce plants per treatment before and after spraying. Treatment codes and time are explained in Section 5.1.1, Table 5.1.2.

Treatment	Photosynthesis			Stomatal resistance		
Minutes from spraying	-30	30	150	-30	30	150
N	8.633 a	8.656 a	2.625 a	0.277 a	0.385 a	0.375 a
W	10.025 a	8.433 a	2.651 a	0.270 a	0.402 a	0.360 a
S	7.412 a	7.925 a	2.585 a	0.364 a	0.389 a	0.350 a

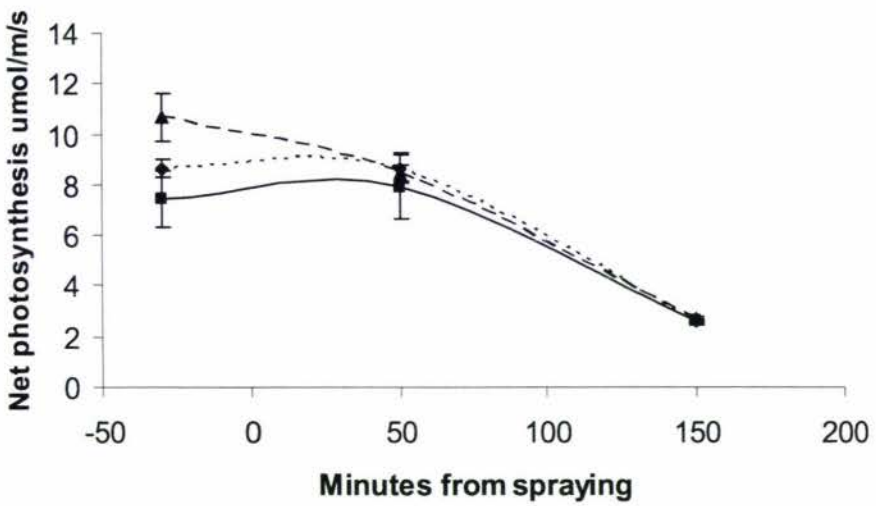


Fig. 5.2.9 Net carbon dioxide assimilation by plant leaves in each treatment before and after spraying (4 plants per treatment). N (♦) no spray, S (■) silica spray, W (▲) water spray. Treatment codes and measurement times as explained in section 5.2.2. Bars represent standard error of means.

The reduction in net photosynthesis for all treatments shown in the last reading was due to increased cloud cover. The slight drop in red light absorption in silica sprayed plants measured by leaf reflectance shortly after spraying (Figure 5.2.6) did not appear to affect net photosynthesis, and the continued lack of difference between treatments in red light absorption is consistent with lack of differences in net photosynthesis.

This result is consistent with results reported by Koenig (1988) who found very little difference in net CO_2 assimilation in plants to which various combinations of biodynamic sprays were applied. Measurements of photosynthesis of grape leaves

sprayed with the same silica spray used in this trial failed to find significant differences from control plant rates. (McArtney et al., 2003).

Stomatal resistance

No significant differences between treatment means were found for stomatal resistance measurements (Table 5.2.4 and Figure 5.2.10).

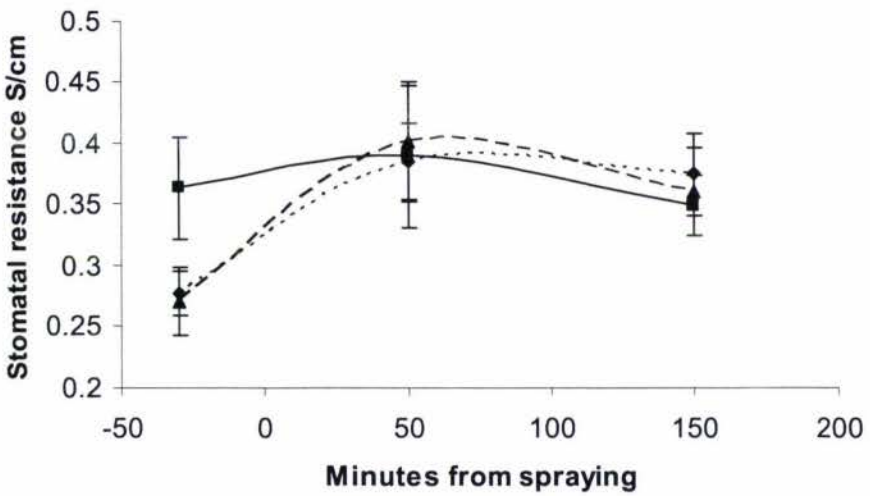


Fig. 5.2.10 Stomatal resistance in plant leaves of sprayed and unsprayed plants (4 plants per treatment) before and after spraying). N (♦) no spray, S (■) silica spray, W (▲) water spray. Treatment codes and measurement times as explained in Sections 5.1.1, 5.1.2. Bars represent standard error of means.

Stomatal resistance in non-sprayed and water sprayed plants increased compared to that of silica sprayed plants shortly after spraying. This is consistent with the slightly greater water loss measured for silica sprayed plants reported in Table 5.2.1.

The small decreased variation in stomatal resistance in the silica sprayed plants is interesting to compare with the results obtained by Koenig (1991). He measured stomatal oscillations in dwarf bean plants over a number of days, comparing plants to which different combinations of sprays were applied. He found that plants sprayed only with preparation 500 and those sprayed with both preparation 500 and 501 in a daily rhythm showed reduced diurnal stomatal oscillation, whereas plants sprayed with preparation 501 only showed increased stomatal oscillation. In my trial the plants sprayed three times with preparation 501 (silica spray, Table 5.1.1) and once with preparation 500, showed less stomatal oscillation immediately after spraying than those that had only received the preparation 500 (water spray and no spray treatments).

5.2.5 Nitrate content of leaves

No significant differences in mean leaf sap nitrate (NO_3) –N concentrations between treatments were found (Table 5.2.5 and Figure 5.2.11). NO_3-N content of leaf sap was estimated to be higher and inter-plant variation was greater using Merck strips than the results from KCl extraction and Autoanalyser. This is to be expected as the Merck measurement only provides a rough guide.

Table 5.2.5 Mean NO_3–N mg/L in lettuce leaf sap from leaves of 4 lettuce plants Per treatment as measured by Merck strip and by KCl extraction and autoanalyser at each measurement time before and after spraying. Treatment codes and time are explained in Sections 5.1.1, Table 5.1.2.

Treatment	Merck strip test			KCl extraction		
	mg/L NO_3–N					
Hours from spraying	**-3**	**3**	**27**	**-3**	**3**	**27**
No spray	1375 a	2300 a	1200 a	1003 a	1120 a	901 a
Spray	1250 a	1850 a	1300 a	1031 a	1210 a	988.5 a

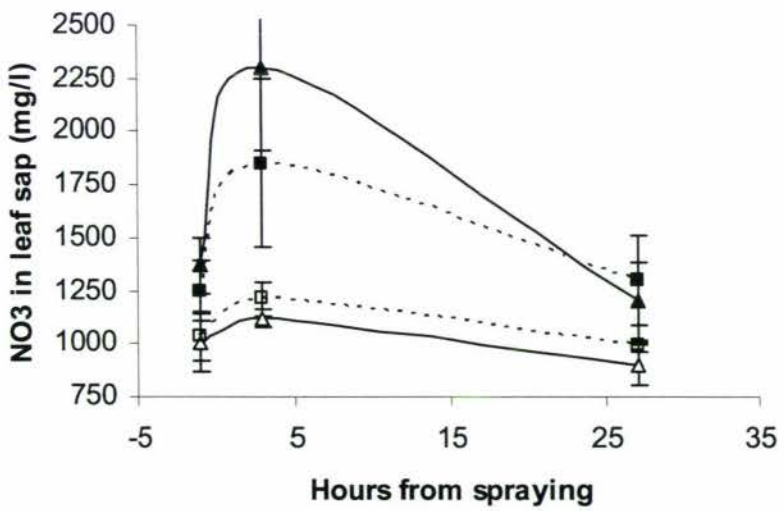


Fig. 5.2.11 Mean NO_3–N (mg/L) in lettuce leaf sap of 4 plants per treatment at each measurement time as measured by Merck strip (closed symbols) and by KCl extraction and autoanalyser (open symbols). ▲ = no spray, ■ = silica spray. Treatment codes and measurement times as explained in Sections 5.1.1, 5.1.2. Bars represent standard error of means.

A rise in NO_3 concentration between 9 am and 3 pm on the day of spraying was found for all treatments, higher for the no-spray treatment lettuces in the Merck measurements, but higher for the silica sprayed lettuces when measured by autoanalyser. It is surprising to find any rise, as nitrate levels are generally highest in the morning and decrease during the day as light activates nitrate reductase enzymes,

however, the cloudy conditions and drop in light intensity are likely to have been the reason (Maynard et al., 1976). The rise in NO_3 levels is an indication that NO_3 was being taken up into the leaves at a faster rate than it could be assimilated.

The lack of difference in nitrate concentrations in between sprayed and non-silica sprayed lettuces may suggest that lower nitrate levels reported for plants grown by biodynamic management do not result from the silica spray alone. The lower nitrate concentrations found in biodynamically grown vegetables by Klett (1968) and Mader (1992) are likely to have been the result of the combined effect of all the biodynamic preparations and organic management. In the greenhouse trial the silica spray did not assist plants to assimilate the high nitrogen provided by the compost and soil as expected. This may be because nitrogen assimilation in the lettuces was unlikely to have been optimum for either treatment as evidenced by the poor, stressed growth of the lettuces. This was possibly related to low calcium uptake and a possible soil boron deficiency, indicated by tip-burn in some plants, as found by Crisp (1976). Calcium and boron appear to interact in cell walls, leading to impaired transport of calcium in boron-deficient plants (Marschner, 2002).

5.2.6 Soluble solids content of leaves

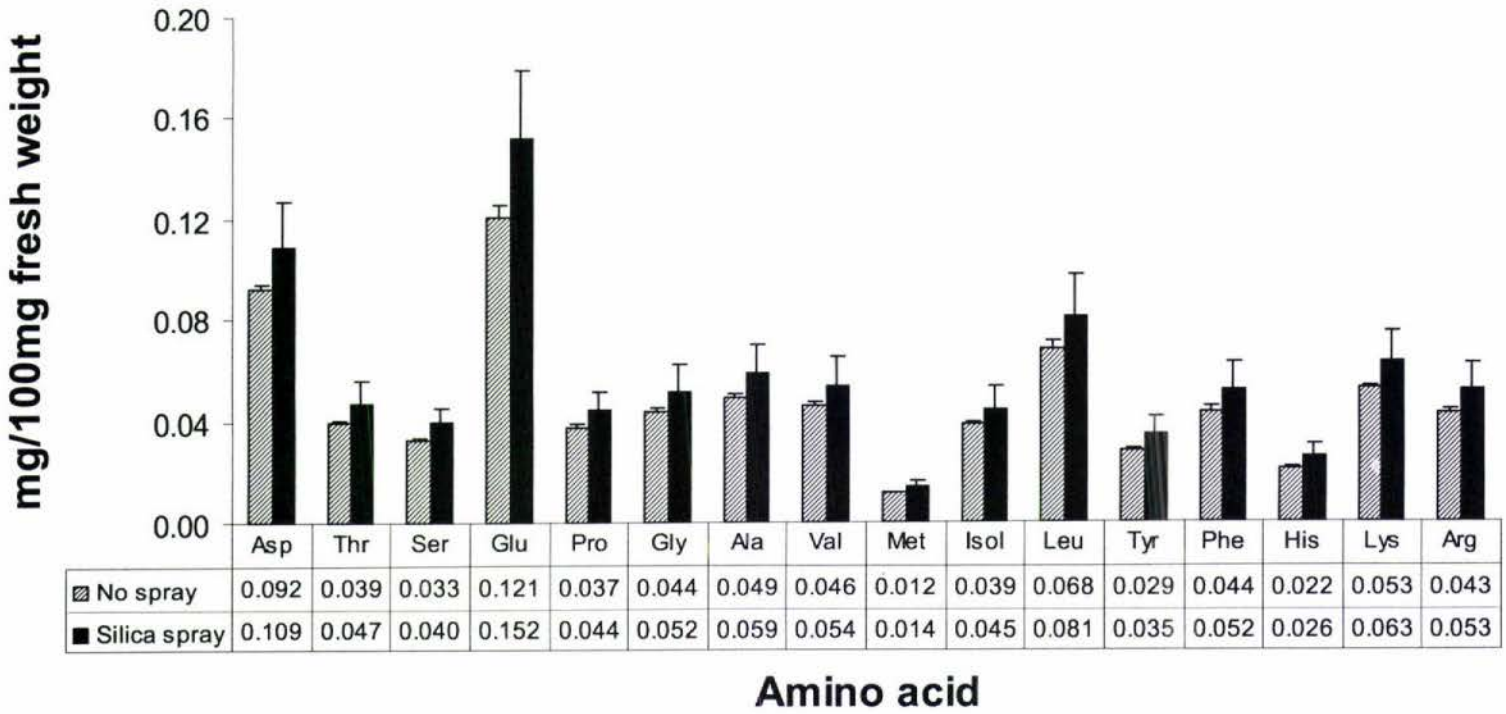
No significant differences between treatment means were found for Brix readings of soluble solids content of lettuce leaf sap (Table 5.2.6). A slightly higher level for the silica sprayed lettuces was recorded but this was not a significant difference because of the considerable variation between plants within treatments.

Table 5.2.6 Mean refractometer reading for lettuce leaf sap from each treatment 27 hours after spraying in °Brix (equivalent to % sucrose content)

Treatment	°Brix
No spray	3.350 a
Spray	3.775 a

Letters indicate that no differences were significant by t-test at 0.05% level

All readings for soluble solids were low. Harrill (1998) recommended that the Brix level should be 6 in an average quality lettuce and 10 in one of excellent quality. A slightly lower reading would be expected for plants not fully mature.



	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Isol	Leu	Tyr	Phe	His	Lys	Arg
No spray	0.092	0.039	0.033	0.121	0.037	0.044	0.049	0.046	0.012	0.039	0.068	0.029	0.044	0.022	0.053	0.043
Silica spray	0.109	0.047	0.040	0.152	0.044	0.052	0.059	0.054	0.014	0.045	0.081	0.035	0.052	0.026	0.063	0.053

Fig. 5.2.12 Amino acid content of lettuce leaves grown with (■) and without (▨) silica field spray treatment. Mean of 2 samples per treatment. Bars represent standard error of means.

5.2.7 Amino acids

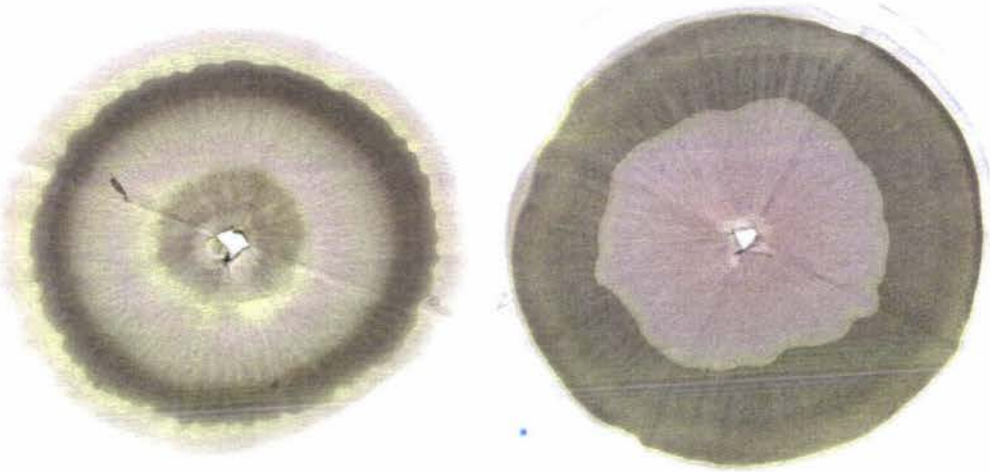
None of the differences between treatment means of amino acid concentrations shown in Figure 5.2.12 were significantly different because only 2 replicates of the treatments were analysed. One of the silica sprayed lettuce plants contained considerably higher levels of all amino acids measured than the other plants analysed. Remer (1995) measured, by descending chromatography, up to twice the concentration of some free amino acids in cabbages treated up to 12 times with the silica preparation 501 compared to control plants (no statistics provided). Remer also repeatedly measured amino acids, by the same analysis method, in carrots treated weekly with a D7 spray potentised from the silica preparation. Levels of aspartic acid, glutamic acid and alanine were consistently higher in the silica sprayed carrots. However, the trial lettuce amino acid analysis was of all amino acids, both free and in protein, and measurement method was more accurate than that used by Remer.

Content of essential amino acids is of more interest than that of the acids measured by Remer. In the glasshouse trial, lysine: arginine ratio, a measure of protein quality (Section 4.6.1), was slightly less for sprayed (1.203) than for non-sprayed (1.252) plants. Low lysine: arginine ratio in the diet has beneficial health effects such as reducing atherosclerosis (Rajamohan and Kurup, 1990). Essential amino acid levels measured by Zaimenko (1998),discussed in section 2.3.4, were up to 6 times higher for arginine and 2.3 times higher for histidine in *Anthurium* plants treated with a boiler-waste silica compound compared to control plants. Lysine: arginine ratios in *Aglaonema* plants treated with the same compound were 0.62 compared to 0.69 in control plants: both readings were very low compared to those for the trial lettuce plants. Effects of the biodynamic silica spray on amino acids appear to be similar to those from other silicon compounds.

5.2.8 Capillary dynamolysis

Chromatograms prepared from lettuce cell sap before and after spraying with the last silica spray gave disappointing results (Plate 5.2.3). This was mainly because sodium hydroxide solution was used to extract sap components as recommended by Pfeiffer

(1988), but the concentrations of sodium hydroxide used appeared to be too high, masking some of the effects.



(a) (b)

Plate 5.2.3 Lettuce sap chromatograms of greenhouse lettuce 57 days from transplanting made before the last silica spray (a) sprayed with silica spray, (b) not sprayed

The chromatograms in Plate 5.2.3 show considerable differences partly due to a lower sap concentration in (a). The different centre of (a) could either be due to the difference in concentration or to the stress during growth of the silica sprayed lettuces. No conclusions can therefore be drawn from these chromatograms.

5.2.9 General conclusions: Glasshouse trial

The results indicate that the silica spray appeared to have little short-term effect on net carbon dioxide assimilation, and net photosynthesis, which is consistent with other research results (Atkinson, 2003). It remains possible that the spray may slightly affect light properties and light absorption by plant leaves at some wavelengths, such as blue light and near-infra-red. This may trigger signals that lead to changes in stomatal conductance, water transpiration and carbon and nitrogen metabolism in the plant. The lack of effects of the spray on plant water use, leaf nitrate, or soluble solids content, in this trial indicate that such changes did not occur, or were too small to be measurable. The lack of change in leaf nitrate could be because the high nitrogen supply from the soil and compost overrode any such effect. Other factors could be that that forced growth in the glasshouse causes lettuce to develop calcium and boron deficiency, affecting transport of solutes in the plant and deficient plant uptake of calcium, which is involved in the plant signaling response (Ward and Shroeder, 1997).

6 FINAL DISCUSSION AND RECOMMENDATIONS

6.1 Discussion

Lettuce growth and nutrient concentration in the field trial

In drawing conclusions from plant growth experiments the conclusions should be kept in context with the soil type, soil management and weather patterns under which the plants were grown. Under the growing conditions of the field trial conducted, some notable changes in lettuce yield and composition resulted from using different soil amendments.

The smaller DM% and lower concentration of antioxidants and most elements measured other than N, P and K found in plants grown in composted treatments, compared to those on soil treated with soluble fertilisers and control plots, probably result from faster growth due to high availability of N and K from the soil and the large quantity (8.35 kg/m^2) of compost added to provide lettuces with their target N content at harvest. In hindsight less compost could have been added because all lettuces had N concentrations higher than values reported for commercial crops, so net N mineralisation was probably higher than the 30% N release estimated before the trial. Research has shown that plant nutrition from net mineralisation by soil organisms (Koopmans and Bokhurst, 2000) and AM fungi colonisation (Azcon et al., 1996) is improved at lower rates of nitrogen fertilisation.

Compared to lettuce plants grown in compost amended treatments, plants fertilised with soluble fertilisers (DAPCAN) contained higher concentrations of Ca, Mg, Fe, Zn and Cu, had higher Ca: P and lower K: Ca+ Mg ratios (measures that have been related to animal health by Cockell et al. (2002) and Jefferson et al. (2001)). The higher Ca uptake was likely related to provision of extra calcium in the fertiliser). Although not statistically significant at the 95% confidence level, these lettuces also showed a trend to higher nitrate concentrations, greater nitrate-N: total N ratios and lower concentrations of essential amino acids.

The higher DM%, longer, less branched roots and higher concentrations of P, amino acids, antioxidants and protein in leaves of plants sprayed with biodynamic field-sprays

are sometimes associated with applying phytohormones such as auxins, gibberellins or abscisic acid to plants (Wein, 1997). The mechanisms resulting in the significant treatment mean differences in P, protein, amino acids, and antioxidant concentrations and in nitrate: total N, and cation ratios require further investigation in other growing environments. The mechanisms causing these differences in nutrient assimilation could possibly relate to factors influencing the soil supply of specific nutrients and their interactions with climatic, light and warmth factors. Some answers may be found in rhizosphere processes, microbial activity and AM fungi colonisation.

The biodynamic silica spray has been found to promote effects of "light" and "warmth" in "vertical" growth, i.e. deep, less divided roots and small, thick, short leaves, whereas water, humus and N ("shade" factors) promote shallow branching roots and large elongated leaves (Koepf, 1993) (See Table 2.3.3) These effects were observed in the field trial lettuces (Section 4.2), the former effect in DAPCAN + sprays and Control + sprays treatments and the latter in the Organic treatment. The Biodynamic treatment lettuces, although also receiving the sprays and growing deep roots, exhibited some "shade" effects, such as low DM%, likely brought about by high N supply. The apparently different effects of the silica spray on the DAPCAN + sprays and Control + sprays treatments on the one hand and the biodynamic treatment on the other are discussed in Section 4.9.1.

No statistically significant treatment differences were found in sensory evaluation. However trends in scores were highest for plants that had been sprayed with the biodynamic sprays, particularly for the DAPCAN + sprays treatment lettuces which had high DM%, flavour, aroma and crunchiness. Leaves from these plants also lost least water and deteriorated least when stored. Chromatograms indicated possible differences between lettuce sap from different treatments, but laboratory technique would need to be improved before any conclusions could be drawn.

Inconclusive effects of treatments were due partly to the large natural plant to plant (within replication) variation which meant that on average treatment effects had to be greater than 20% (root length), 8 % (leaf DM), 16-57% (P concentration) 47% (light wavelength absorption), 43% (sap nitrate concentration), 13 - 55% (Brix), 127% (soil respiration), of the treatments means to be significant. Much of this error is associated

with spatial soil variability affecting seedling vigour. This shows that in order to conclusively show differences between different management treatments, care has to be taken to minimise variability between plants and soil and large numbers of replications are needed. The high fertility of the trial soil would also have reduced size of treatment effects.

A higher soil respiration rate than in the Control treatment was associated with treatment with organic compost (Table 4.7.1). Possibly more hydrolysing and P solubilising enzymes such as phosphatase were produced in compost treatments, initially increasing P availability, because mean P concentrations in lettuces from compost treatments were higher at 28 DAT. It is possible that the compost preparations included in the biodynamic compost helped to increase P and K activity and uptake in plants. No research results showing effects of the compost preparations on leaf mineral concentrations were found, but as they have been shown to increase yields (Pfeiffer, 1983), increased mineral uptake is possible. Lower soil respiration rates (Table 4.7.1), indicating reduced soil organism activity, appeared to be associated with application of the silica spray. However, no conclusions can be drawn from one ex situ respiration test. Mader et al. (2002) found highest microbial diversity and higher efficiency of nutrient uptake in a biodynamic treatment. Further investigation would be needed to find whether the biodynamic sprays induce changes in plant metabolism and composition of root exudates which favour different soil microbe populations, such as reducing proliferation of bacteria populations which immobilize nutrients but increasing activity of other soil organisms which may produce more enzymes.

Despite repeating the silica spray applications on glasshouse grown lettuces (section 5) with lower replicate variability, few significant results were recorded. However, it appeared that lettuces sprayed with the silica spray had decreased absorption of most light wavelengths immediately after spraying, followed by increased water transpiration and stomatal conductance, increased absorption of blue and near infra-red light wavelengths and higher sugar concentrations after 2 hours. These small fluctuations could have had an effect on cell signalling, hormone and enzyme activity. Research has shown rapid responses by plants to changes in light wavelengths. For example Briggs and Liscum, (1997) discussed experiments by Kaldenhoff et al, 1993 which showed that when seedlings grown under red light were transferred to blue light, the *AthH2* mRNA

level increased rapidly, and this effect was also induced by application of abscissic acid. (AthH2 appears to encode a protein that acts as a water channel).

Nutrition aspects

In general, nutrient contents of lettuces were within or above average ranges (Table 2.4.1), but those ranges are quite wide, and there are considerable differences between varieties. The nutritional measurements and tests do not take bioavailability of nutrients for consumers into account. Consumers have expectations on what a lettuce should be like, which was demonstrated in the taste test. The panelists who tasted the trial lettuces preferred lettuces with higher DM, stronger aroma and taste and crunchy texture, without the bitter taste caused by high nitrate content. However, lettuce leaves are usually fairly watery, without a high DM content as in other crops such as carrots. Soil mineral imbalances will produce effects on nutrient composition that may not be discernible by sight or taste. For example in this trial the reduced concentrations of calcium, iron and zinc in the organically amended treatments.

Nitrates and nitrogen metabolism

At 28 DAT nitrate concentrations in leaves were significantly higher in the organic treatments than in the control and DAPCAN treatments. This effect had disappeared at 48 DAT although the nitrate concentration of cell sap in all treatments remained high. These high cell sap nitrate concentrations are indicative of the adequate supply of nitrogen in the soil and the relative immaturity of lettuce growth sustained by non-nutrient limited vegetative growth. As the soil was initially high in nitrates, lettuces probably took up nitrate preferentially to ammonium and plants contained high levels of nitrate at 28 DAT.

Nitrate reductase activity in plants depends on light quantity and quality, being higher with more blue light and higher red: far-red as discussed in Section 2.4. The leaf spectral reflectance results from the glasshouse trial indicated possible effects on these light properties from spraying the horn-silica preparation. Treatment differences were not found in nitrate concentration in the glasshouse grown lettuces. This is a very interesting area and the effect of silica spray application on nitrate reduction, and whether the traditionally stirred preparation 501 and the potentised silica spray have the same effect, should be investigated further.

Linking the biodynamic approach to plant biochemistry

Some aspects of the growth and nutritional qualities of lettuce plants were shown to be affected by application of composts, soluble fertilisers and biodynamic spray preparations. It seems possible that the "calcium" and "silica" processes, said to be regulated by preparations 500 and 501, are expressed in plant signalling mechanisms and phosphorylation/dephosphorylation, which affect production and activity of enzymes and thus nutrient assimilation and integration of the growth and differentiation factors in the product as discussed by Bloksma et al. (2001). It is possible that changes in hormonal balance and interrelationships with soil organisms and AM fungi also affect these processes. Changes in amino acid and antioxidant concentrations are some measurable effects of such processes.

Silica, light and plant cell signalling

In the greenhouse trial, applying the silica preparation increased plant stress, as shown by increased aphid attack and decreased growth of sprayed plants. Water use measurements before and after the last spray application indicated higher water loss from sprayed plants, lower light absorption particularly of blue and near infrared wavelengths straight after application, but slightly higher stomatal conductance and absorption of blue and near infrared wavelengths a few hours later. More detailed analysis of photosynthetic, gas and water exchange processes are required to determine if these observations are consistent with all silica spray applications.

If these effects are consistent, then in the field trial (Section 4) spray treatment may have initially induced an increase in stomatal opening, causing more water loss, which may have been exacerbated by the initial dry soil conditions. After a few hours water stress could have led to abscisic acid production and closure of stomata to reduce transpiration loss via calcium ion signalling. This would reduce carbon dioxide intake and photosynthesis. The resulting decreased photosynthetic efficiency may have been the cause of the set back in plant growth. The red colouring observed and greater antioxidant concentrations measured in sprayed plants may be related to higher anthocyanin production. This could have resulted from stress and/or possible increased UV radiation absorbance (as found by Tosserams et al., 2001) which could have been a direct effect of the silica spray. Changes in plant metabolism from applying the silica

spray were not conclusively shown in this trial, but further investigation of possible effects such as changes in cell pH and organic acid concentration and exudation by roots should be investigated.

Some of the measured effects of the biodynamic silica spray, such as increased essential amino acid concentrations, appear to be the same as effects of applying silica compounds to soil or nutrient solution (Zaimenko, 1998). As the silica spray contains very low concentrations of silica, this effect may be mediated by changes in plant hormone balance and/or cell signalling mechanisms. This hypothesis is backed up by small variations in blue and near infra-red light wavelengths estimated leaf absorption measured after spraying the silica spray.

Small, temporary changes in light quality have been shown to activate cell signalling mechanisms such as cystolic calcium and anion channels and phosphorylation/ dephosphorylation, which affect synthesis and activity of enzymes and in turn, rates of nutrient assimilation and plant composition (Briggs and Liscum, 1997, Lillo and Appenroth, 2001). It seems possible that the biodynamic fieldsprays may affect plants in a similar way. It is likely that changes in hormonal balance and interrelationships with soil organisms and AM fungi also affect these processes. The changes measured in amino acid and antioxidant concentrations of the lettuce plants in this study would be seen as measurable effects of such processes. The silica spray does not appear to affect net photosynthesis, but it may increase P uptake and activity, which could provide more energy for plant metabolism. Further investigation would be needed to test these hypotheses.

The results also show that it is over-simplistic to assume that organic management will result in improved product quality (at least as measured by chemical tests). Results vary considerably with soil and climate characteristics and particular practises used, and products may be better in some aspects and not in others.

Large inter-plant variability and large differences between different soil types, climate and site conditions coupled with relatively small differences in nutrient contents explain why it has been so difficult to show any consistent differences between management treatments, let alone between farms with different management systems. Results are

specific to a particular soil and its history, compost composition, water availability, temperature, lettuce variety. Each farm is unique – it develops its own soil communities adapted to its particular conditions and will produce products of different nutrient compositions.

Implications for product quality testing

Field testing of sap nitrates and Brix provided highly variable, inconclusive measurements indicating that these would not be very reliable tests of product quality. However, they do provide some indication, along with tests of sap pH being developed in USA. Tests such as delayed luminescence (Popp et al. 1994), Raman chromatography (Schultz, 2002) and biological assays on human subjects provide more reliable indicators of food quality, but are too expensive for regular food testing.

6.2 Recommendations

Further investigation of the effects of the soil and plant processes influenced by soil amendments and biodynamic preparations should be carried out in controlled, well replicated conditions. This kind of reductionist approach is contrary to the holistic approach favoured in researching organic systems. However, if there is to be more understanding and possible management of nutritional quality of crop products, then effects of contributory factors such as soil mineral status, humus quality, soil organism activity and diversity and application of biodynamic preparations need to be better understood.

Some aspects which particularly need further research are:

- Evaluate humus, micro- and macro-organism and enzyme constituents of different composts and their effects on plant nutrient composition at different rates of application in different soils, with replicated composts.
- Investigate how much and under what conditions, crops take up amino acids, and how this affects their metabolism, nutrient content and life quality.
- Investigate effects of spraying the horn-silica preparation at different times of the day on absorption of different light wavelengths by plants; on cell signalling, phosphorylation, nutrient metabolism in leaves and possible changes of quantity

and constituents of phloem contents and root exudates; and interrelationship with application of the other biodynamic preparations and soil properties.

- Investigate effects of the biodynamic preparations on nutrient assimilation, nitrogen metabolism, production of complex molecules such as essential amino acids and ratios of nutrients.
- Investigate whether effects of the silica spray are the same when it has been stirred by the traditional biodynamic method or applied as a potentised spray.
- Evaluate nutritional quality of organic food products from different soils.
- Explore relationships between soil biota populations and activity, plant metabolism and production of integrated products.

Trials such as were undertaken in this research could be improved by:

- Soil sampling – taking deeper cores than the standard top 15cm, and minimising effects of disturbance and drying on soil organisms to improve applicability of results to actual conditions;
- For time sensitive measurements such as leaf sap nitrate concentrations, a procedure is needed to enable fast measurement;
- For pot trials ways of minimising soil disturbance, particularly in the rhizosphere of transplants, are needed, to reduce adverse effects on soil organisms and mycorrhizae.

REFERENCES

Agarie, S., Uchida, H., Agata, W., Kubota, F., Kaufman, P. B., 1998. Effects of silicon on transpiration and leaf conductance in rice plants (Oryza sativa L.). Plant Production Science 1(2), 89-95.

Agarie, S., Uchida, H., Agata, W., Kaufman, P. B., 1999. Effects of silicon on stomatal blue-light response in rice (Oryza sativa L.). Plant Production Science 2(4), 232-234.

Albanes, D., 1999. B-carotene and lung cancer: a case study. American Journal of Clinical Nutrition, 69.

Albiach, R., Canet, R., Pomares, F., Ingelmo, F., 2000. Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil. Bioresource Technology, 75, 43-48.

Albrecht, W.A., 1975. The Albrecht Papers. Vol. I. Acres, USA.

Alloush, G. A., Zeto, S. K., Clark R. B., 2000. Phosphorus source, organic matter, and arbuscular mycorrhiza effects on growth and mineral acquisition of chickpea grown in acidic soil. Journal of Plant Nutrition 23(9), 1351-1369.

Ames, B. N., 2001. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis 475(1-2), 7-20.

Ames, B.N., Gold, L.S., Willett, W.C., 1995. The causes and prevention of cancer. Proceedings National Academy of Science, U S A. 92, 5258–65.

Aon, M.A., Cortassa, S., 1997. Dynamic biological organization : fundamentals as applied to cellular systems. Chapman & Hall, London.

Apfelthaler, R., 1992. Development of humus research during the past humus et planta symposia. In: Kubat, J. (Ed.). Humus, its structure and role in agriculture and environment, Proceedings of the 10th symposium humus et planta. Elseview, Amsterdam.

Artlip, T.S, Wisniewski, M.E., 2002. Induction of proteins in response to stresses. In: Pessarakli, M.(Ed.). Handbook of Plant and Crop physiology. Marcel Dekker, Inc. New York.

Asghar, H.N., Zahi, Z.A., Archad, M., Khaliq, A., 2002. Relationship between in vitro production of auxins by rhizobacteria and their growth-promoting activities in Brassica juncea L. Biology and Fertility of Soils 35:231-237.

Athar, N., Spriggs, T.W., Taptklis, E., Taylor, G., 2001. The concise New Zealand food composition tables. 5th Edition, Crop and Food Research Ltd., Ministry of Health., Palmerston North, N.Z.

Atkinson, G., 2003. Photosynthesis results. http://bdmax.co.nz

Atkinson, G., 2002. A working manual for gyroscopic astrology. Garuda Trust, Te Puke, New Zealand.

Austic, R E, 1998. Current Research. http://www.ansci.cornell.edu/faculty/austic.html

Azcon, R., Ruiz-Lozano, J.M., Rodriguez, R., 2001. Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (15N) under increasing N supply to the soil. Canadian Journal of Botany 79, 1175-1180.

Azcon, R., M., Gomez, et al., 1996. Physiological and nutritional responses by Lactuca sativa L. to nitrogen sources and mycorrhizal fungi under drought conditions. Biology & Fertility of Soils 22(1/2): 156-161.

Baath, E., 1998. Growth rates of bacterial communities in soils at varying pH: A comparison of the thymidine and leucine incorporation techniques. Microbial Ecology 36(3): 316-327.

Bajpai, R.P., 2000. Possibility of photon emission in the fundamental biological processes involving quantum search of base pairs and amino acids Inauguration Festivities of the International Institute of Biophysics.

Baldock, J.A., Skjemstad, J.O., 1999. Soil organic carbon/soil organic matter. In: Peverill, K.I., Sparrow, L.A., Reuter, D.J. (Eds.)..A Soil analysis and interpretation manual. CSIRO Publishing, Australia.

Barber, S.A., 1984. Soil Nutrient Bioavailability. A Mechanistic Approach.. John Wiley, New York.

Barlow, P., 2001. Getting much more from leaf and soil analysis. Orchardist 74 (4), 36-38.

Barton, L.L, Hemming B.C., (Eds.). 1993. Iron chelation in plants and soil microorganisms..Academic Press Inc.

Bente, L., Halvorsen, K. H., Myhrstad, M. C. W., Barikmo, I., Hvattum, E., Remberg, S. F., Wold, A., Haffner, K., Baugerød, H., Andersen, L.F., Moskaug, Ø., Jacobs, D. R., Blomhoff, R., 2002. A Systematic Screening of Total Antioxidants in Dietary Plants. The American Society for Nutritional Sciences Journal of Nutrition. 132, 461-471.

Benzie, I. F. F., Strain J. J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry 239 (1): 70-76.

Berg, E. S., Eaton, G. K., Ayres, M. P., 2001. Augmentation of AM fungi fails to ameliorate the adverse effects of temporal resource variation on a lettuce crop. Plant & Soil 236(2): 251-262.

Bergmann, H., Lippmann, E., Leinhos, V., Tiroke, S., Machelett, B., 1999. Activation of stress resistance in plants and consequences for product quality Angewandte Botanik 73(5/6), 153-161.

Berthelsen, S., Hurney, A., Kingston, G., Rudd, A., Garside, A. L., Noble, A. D., 2001. Plant cane responses to silicated products in the Mossman, Innisfail and Bundaberg districts. Hogarth, D. M. (Ed.).Proceedings of the 2001 Conference of the Australian Society of Sugar Cane Technologists held at Mackay, Queensland, Australia, 1st-4th May 2001 : 297-303.

Bezdicek, D., Fauci, M., 1997. Nutrient aspects of compost. The Compost Connection, Washington State University Cooperative Extension WSU CSANR.

Bio-Dynamic Farming and Gardening Association, 2002. Using the biodynamic preparations. Resource Directory. Wellington, NZ.

Blakemore, L.C., Searle, P.L., Daly, B.K., 1987. Methods for chemical analysis of soils. (NZ Soil Bureau Scientific Report 80. NZ Soil Bureau, DSIR. NZ.

Bloksma, J., Northolt, M., Huber, M., 2001. Parameters for apple quality and an outline for a new quality concept. Louis Bolk Instituut, Publ.no. FQH 01.

Bloksma J., 2002. Pers.comm.

Blom-Zandstra, M., Lampe, J.E.M., Ammerlan, F.H.M., 1988. C and N utilization of two lettuce genotypes during growth under non-varying light conditions and after changing the light intensity. Physiologia Plantarum, 74, 147-153.

Bolan, N.S., 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants.Plant and Soil 134 (2), 189-207.

Bourn, D., Prescott, J., 2002. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. CRC Critical Reviews in Food Science & Nutrition, 42, (1), 1-34.

Bourn, D.M., 1994. The nutritional value of organically and conventionally grown food – is there a difference? Proceedings of the Nutrition Society of New Zealand, 19, 51 – 57.

Boxall, M.I., 1971. Some effects of soil warming on plant growth. Acta Horticulturae 22. 57-65.

Brandt, K., Molgaard, J.P., 2001. Organic Agriculture: does it enhance or reduce the nutritional value of plant foods? Journal of Science Food and Agriculture, 81, 924-931.

Brechelt, A., 1990. Effect of different organic manures on the efficiency of VA mycorrhiza. Agriculture Ecosystems & Environment 29(1-4), 55-58.

Briggs, W.R, Liscum, E., 1997. Blue light-activated signal transduction in higher plants. In: Aducci P. (Ed.). Signal transduction in plants. Birkhauser Verlag, Basel.

Brinton, W.F., 1997. Dynamic chemical processes underlying Bd horn manure (500) preparation. Journal of Biodynamics Vol 214 Nov-Dec.

Brinton, W.F., 1983. A qualitative method for assessing humus condition. In Knorr D. (Ed.). Sustainable Food Systems. AVI Publishing Company, Westport, Connecticut.

Brown, I.C., 1943. A rapid method of determining exchangeable hydrogen and total exchangeable bases of soils. Soil Science 56. 353-357.

Brown, M. E., Carr, G. R., 1984. Interactions between *Azotobacter chroococcum* and vesicular-arbuscular mycorrhiza and their effects on plant growth. Journal of Applied Bacteriology 56(3), 429-437.

Brundrett, M., Bougher, N., Dell, B., Grave, T., Malajczuk, N., 1996. Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agricultural Research Monograph 32, Canberra.

Brunsgaard, G., Kidmose, U., Sorensen, J. N., Kaack, K., Eggum, B. O., 1994. Influence of growth conditions on the value of crisphead lettuce. 3. Protein quality and energy density as determined in balance experiments with rats. Plant Foods for Human Nutrition 46 (3), 255-265.

Calvet, C., Estaun, V., Camprubi, A., Pinochet, J., 2002. Interactions between arbuscular mycorrhizal fungi and non-symbiotic beneficial microbiota. In: Sharma, A.K., Johri B.N., (Eds.). Arbuscular mycorrhizae interactions in plants rhizosphere and soils. .Science Publishers Inc. Enfield USA.

Camacho-Cristobal, J. J., Maldonado, J. M., Gonzalez-Fontes A., 2002. Effects of phosphate on in vitro nitrate reductase activity from tobacco leaves. Plant Science 163(3), 455-461.

Carpenter-Boggs, L., Reganold, J. P., Kennedy, A. C., 1999. Effects of biodynamic preparations on compost development. Biological Agriculture & Horticulture 17(4), 31.

Carpenter-Boggs, L., Kennedy, A. C., Reganold, J. P., 2000. Organic and biodynamic management: Effects on soil biology. Soil Science Society of America Journal. [print] 64(5), 1651-1659.

Carter, C. 2003 Pers. Comm..

Carter, G. A., Knapp, A. K., 2001. Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. American Journal of Botany 88 (4), 677-684.

Carter, G. A., McCain, D. C., 1993. Relationship of leaf spectral reflectance to chloroplast water content determined using NMR microscopy. Remote Sensing of Environment 46 (3): 305-310.

Carter, G.A., 1993. Responses of leaf spectral reflectance to plant stress. American Journal of Botany 80(3): 239-243.

Chen, J., Ferris, H., 1999. The effects of nematode grazing on nitrogen mineralization during fungal decomposition of organic matter. Soil Biology and Biochemistry 31: 1265-1279.

Chen, Y., Stevenson F.J., 1986. Soil organic matter interactions with trace elements. In The role of organic matter in modern agriculture. In: Chen, Y., Avnimelech, Y. (Eds.)..Marinus Nijhoff Publishers, Dordrecht.

Cherney, J. H., Mikhailova, E. A., Cherney, D. J. R., 2002. Tetany potential of orchardgrass and tall fescue as influenced by fertilization with dairy manure or commercial fertilizer...Journal of Plant Nutrition 25 (7), 1501-1525.

Cisternino, F., Sordo, B., 2003. State of the art and prospects for Raman amplification in long distance optical transmissions. exp.telecomitalialab.com/cms-service/stream/asset?asset_id=2251

Clark, R.B., Zeto, S.K., 2002. Arbuscular mycorrhizae: mineral nutrient and water acquisition In Sharma, A.K., Johri, B.N. (Eds.). Arbuscular mycorrhizae interactions in plants rhizosphere and soils. Science Publishers Inc. Enfield, USA.

Clark, R. B., Zeto, S. K., 2000. Mineral acquisition by arbuscular mycorrhizal plants. Journal of Plant Nutrition 23(7), 867-902.

Clemson University Extension Service, 1996. Sampling guidelines and mineral sufficiency ranges for vegetable crops. hubcap.clemson.edu/~blpprt/bobweb/BOBWEB21.HTM

Clouatre, D., 2003. The top anti-oxidant foods. www.klsdesign.com/anti-ox/allabout-topfoods.html

Cockell, K. A., L'Abbe, M. R., Belonje, B., 2002. The concentrations and ratio of dietary calcium and phosphorus influence development of nephrocalcinosis in female rats. Journal of Nutrition 132 (2), 252-256.

Cooper, K..A., 1984. Physiology of VA Mycorrhizal Associations. In: Powell, C.L., Bagyaraj, D.J. (Eds.). VA Mycorrhiza. CRC Press, Boca Raton.

Cotching, W.E., Allbrook, R.F, Gibb, H.S., 1979. The influence of maize cropping on the soil structure of 2 soils in the Waikato district, NZ. NZ Journal of Agricultural Research 22: 431-438.

Craighead, M.D., Clark, S.A., 1989. Soil testing methods, and modeling the nitrogen requirements for cereals. In: White, R.E., Currie, L.D. (Eds.) Proceedings of the Workshop Nitrogen in NZ Agriculture and Horticulture. Occasional Paper No. 3. Fertiliser and Lime Research Centre, Massey University.

Crisp, P., Collier, G. F., Thomas, T. H., 1976. The effect of boron on tipburn and auxin activity in lettuce. Scientia Horticulturae 5 (3), 215-226

Doran, J.W., Safley, M., 1997. Defining and assessing soil health and sustainable productivity In: Pankhurst, C.E., Doube, B.M. Gupta, V.V.S.R. (Eds.). Biological Indicators of Soil Health. CAB International Wallingford, UK.

Doran, J. W., Sarrantonio, M., Liebig, M. A., 1996. Soil health and sustainability. Advances in Agronomy, Vol 56. San Diego, Academic Press Inc. 56, 1-54.

Drews, M., Schonhof, I., Krumbein, A., 1997. Content of minerals, vitamins, and sugars in iceberg lettuce (Lactuca sativa var. capitata L.) grown in the greenhouse dependent on cultivar and development stage. [German]. Gartenbauwissenschaft 62(2), 65-72.

Dubey, R.S., Pessarakli, M., 2002. Physiological mechanisms of nitrogen absorption and assimilation in plants under stressful conditions. In Pessarakli M. (Ed.). Handbook of Plant and Crop physiology. Marcel Dekker, Inc. New York.

Emery, R.J.N., Atkins, C.A, 2002. Roots and Cytokinins in Waisel, Y., Eshel, A., Kafkafi, U. (Eds.) Plant Roots – the hidden half. Marcel Dekker Inc. New York.

Epstein, E., 1997. The Science of Composting Technomic Publishing Company Inc. Pennsylvania USA.

Fairweather-Tait, S.J., Teucher, B., 2002.Iron and calcium bioavailability of fortified foods and dietary supplements. Nutrition Reviews, 60 (11), 360-367.

FAO/WHO/UNU. 1985. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. WHO Tech. Rep. Ser. No. 724. Geneva, WHO.

FAO/WHO, 1973. Energy and protein requirements. Report of a joint FAO/WHO Ad Hoc Experimental Committee, WHO Tech. Rep. Ser. 522, WHO, Geneva.

Ferris, H., Venette, R. C., Lau, S. S., 1997. Population energetics of bacterial-feeding nematodes: carbon and nitrogen budgets. Soil Biology & Biochemistry 29 (8), 1183-1194.

Fierabracci, V., Masiello, P., Novelli, M., Bergamin, E., 1991. Application of amino acid analysis by high-performance liquid chromatography with phenyl isothiocyanate derivatisation to the rapid determination of free amino acids in biological samples. Journal of chromatography 570:285-291.

Figliolia, A., Benedetti, A., Izza, C., Indiati, R., Rea, E., Alianiello, F., Canali, S., Biondi, F. A., Pierandrei, F., Moretti, R., 1994. Effects of fertilization with humic acids on soil and plant metabolism: a multidisciplinary approach. Note 1: Crop production. In: Senesi, N., Miano, T.M., (Eds.). Humic Substances in the Global Environment and Implications on Human Health. Elsevier Science B.V.

Fliessbach, A. and Mäder, P., 2000. Microbial biomass and size-density fractions differ between soils of organic and conventional agricultural systems. Soil Biology and Biochemistry 32, 757-768.

Follett, R.H., Murphy, L.S., Donahue, R.L., 1981. Fertilizers and Soil Amendments. Prentice-Hall, New Jersey.

Fritz, J., Meyer-Glitza, P., Weidringer, A., Kopke, U., Schuler, C., 1997. Basic studies on the plant treatment preparation horn silica. [German]. Schriftenreihe - Institut fur Organischen Landbau. 4, 231-237.

Fritz, J., 2000. Effects of Horn Silica on lettuce (Lactuca sativa var. crispa) and beans (Phaseolus vulgaris var. nanus). Bonn (2000) 136 S., Landw.F., Diss.v. 28.6.2000.

Frossard, E.M., Bucher, M. F., Ahmad M., Hurrell, R., 2000. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. Journal of the Science of Food & Agriculture, 80(7), 861-879.

Garcia-Martinez, J. L., Gil, J., 2001. Light regulation of gibberellin biosynthesis and mode of action. Journal of Plant Growth Regulation 20(4), 354-368.

Gaspar, M. L., 2002. Cellular and molecular aspects In: Sharma A.K., Johri B.N.(Eds.). Arbuscular mycorrhizae interactions in plants rhizosphere and soils.Science Publishers Inc. Enfield USA.

Gaspar, T .F., Hausman, O., Faivre-Rampant, C., Kevers, Dommes, J., 2002. Auxins in the biology of roots. In: Y. Waisel, A. Eshel & U. Kafkafi (Eds.), Plants Roots: The Hidden Half, Marcel Dekker, New York, 383-403.

Germida, J.J., 1998. Transformations of sulphur. In: Sylvia , D.M., Fuhrmann J.J., Hartel P.G., Zuberer D.A.(Eds.). Principles and Applications of Soil Microbiology. Prentice Hall, New Jersey.

Gerrard, G., 2001. (chromatograms) www.resonant.com.au/chromatography/soils.html

Galaxy Nutrients, 2001. http://www.galaxynutrients.com/01.html

Ghani, A., Sarathchandra, U., Perrott, K. W., Wardle, D. A., Singleton, P., Dexter, M., 1996. Biological and biochemical quality of pastoral soils: spatial and temporal variability Proceedings of the New Zealand Grassland Association 58: 211-218.

Gianquinto, G., Borin, M., 1996. Quality response of crisphead lettuce and kohlrabi to mineral and organic fertilization in different soils. Advances in Horticultural Science, 10 (1), 20-28.

Goh, K. M., Bruce, G. E., Daly, M. J., Frampton, C. M. A., 1999. Sensitive indicators of soil organic matter sustainability in orchard floors of organic, conventional and integrated apple orchards in New Zealand. Biological Agriculture & Horticulture 17(3), 197-205.

Goldstein, W., 1990. Experimental proof for the effects of biodynamic preparations. Michael Fields Agricultural Institute Working Paper, USA. Also In Biodynamics 129, 1-10

Goovaerts, P., Chiang, C. N., 1993. Temporal persistence of spatial patterns for mineralizable nitrogen and selected soil properties. Soil Science Society of America Journal 57 (2), 372-381.

Gottschewski, G.H.M., 1975. Neue Moeglichkeiten zur groesseren Effizienz der toxikologischen Pruefung von Pestiziden, Rueckstanden und Herbiziden. Qualitas Plantarum. 25, 21-42.

Granstedt, A.G., Kjellenberg, L., 1996. Quality investigations with the K-trial, Jaerna and other Scandinavian fertilization experiments..In: Raupp J. (Ed.) Quality of plant products grown with

manure fertilization . Proceedings of the fourth meeting Fertilisation systems in organic farming in Juva, Finland. Institute for Biodynamic Research Vol 9, Darmstadt.

Graves, D.W., 2002. An analysis of field-soil disturbance treatments on arbuscular mycorrhizal fungi. Master of Sciences Thesis, Massey University.

Grayston, S. J., Griffith, G. S., Mawdsley, J. L., Campbell, C. D., Bardgett, R. D., 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biology & Biochemistry 33(4-5), 533-551.

Greenwood, D.J., Barnes, A., Liu, K., Hunt, J., Cleaver, T.J., Loquens, M.H., 1980. Relationships between the critical concentrations of nitrogen, phosphorus and potassium in 17 different vegetable crops and duration of growth.. Journal of Science of Food and Agriculture 31.1343-1353.

Grimme, L.H., 2000. Food quality and nutrition aimed at health: a biological perspective In: Grimme L.H. and Dumontet, S. (Eds.). Food quality, Nutrition and Health 5th Heidelberg Nutrition Forum/Proceedings of the ECBA 1998. Springer-Verlag, Berlin.

Grinder-Pedersen, Rasmussen, S. E., Bugel, S., Jorgensen, L. V., Dragsted, L. O., Gundersen, V., Sandstrom, B. (2003) Effect of Diets Based on Foods from Conventional versus Organic Production on Intake and Excretion of Flavonoids and Markers of Antioxidative Defense in Humans; J. Agric. Food Chem. 51, 5671-5676.

Gunapala, N., Scow, K.M., 1998. Dynamics of soil microbial biomass and activity in conventional and organic farming systems. Soil Biology and Biochemistry 30, 6 805-816.

Gupta, U. C., Gupta, S. C., 2000. Selenium in soils and crops, its deficiencies in livestock and humans: Implications for management. Communications in Soil Science and Plant Analysis, 31(11-14), 1791-1807.

Guyer, P. Q., Hogg, A., White, G., 1984. Grass Tetany. Institute of Agriculture and Natural Resources University of Nebraska, Lincoln, USA. www.ianr.unl.edu/pubs/animaldisease/g32.htm

Hader, D., Tevini, M., 1987. Photobiology. Pergamon Press, Oxford.

Hägel, I., Bauer, D., Haneklaus, S., Schnug, E., 2000. Quality assessment of summer and autumn carrots from a biodynamic breeding project and correlations of physico-chemical parameters and features determined by picture forming methods. In: Alföldi, T., Lockeretz, W., Niggli, U. (Eds.). IFOAM 2000: the World grows organic. Proceedings of the 13th International IFOAM Scientific Conference vdf Hochschulverlag AG an der ETH Zurich.

Haglund, A., L., Johansson, Bergland, L., Dahlstedt, L., 1999. Sensory evaluation of carrots from ecological and conventional growing systems. Food Quality and Preference, 10(1), 23-29.

Hardie, D.G., 1996. The structure and function of the protein kinases: a survey. In: Shewry, P.R., Halford, N.G., Hooley, R., (Eds.). Protein phosphorylation in plants. Clarendon Press, Oxford.

Harrill, R , 1998. Using a refractometer to test the quality of fruit and vegetables. Pineknoll Publishing, Keedysville,

Hart, C.S., Stark, J.M., Davidson, E.A., Firestone, M.K., 1994. Nitrogen mineralisation, immobilization and nitrification. In: Weaver R.W. (Ed. Chair). Methods of Soil Analysis, Part 2. Soil Science Society of America Inc. USA.

Hart, J.W., 1988. Light and plant growth. Unwin Hyman, London.

Hassink, J., Bouwman, L.A., Zwart, K.B., Brussard, l., 1993. Relationships between soil teaxture, physical protection of organic matter, soilbiota and C and N mineralization in grassland soils. Geoderma 57,105-128.

Haynes, R. J., Swift, R. S., 1988. Effects of lime and phosphate additions on changes in enzyme activities, microbial biomass and levels of extractable nitrogen, sulphur and phosphorus in an acid soil. Biology and Fertility of Soils 6 (2), 153-158.

Heaton, S., 2002. Assessing organic food quality: Is it better for you? In: Powell et al. (Eds.), UK Organic Research 2002: Proceedings of the COR Conference, 26-28th March 2002, Aberystwyth, pp. 55-60.

Heller, W. E., 1999. Nitrogen mineralisation of composts in an incubation assay. Agrarforschung 6 (2), 75-77.

Helyar, K.R., Price, G.H., 1999. Making recommendations based on soil tests. In: Peverill K.I., Sparrow L.A., Reuter D.J. (Eds.). Soil Analysis an interpretation manual. CSIRO Publishing.

Hendrix, P.F., Parmalee, R.W., 1986. Detritus food webs in conventional and no-tillage agroecosystems. BioScience 36 (6).

Hoang, L.T., Boehme M., 2001. Influence of humic acid on the growth of water spinach in hydroponic system. Growing media conference, Sweden.

Hodge, A., Stewart, J., Robinson, D., Griffiths, B. S., Fitter, A. H., 2000. Competition between roots and soil micro-organisms for nutrients from nitrogen-rich patches of varying complexity. Journal of Ecology (Oxford) 88 (1), 150-164.

Holland, M. A., 1997. Occam's razor applied to hormonology. Are cytokinins produced by plants? Plant Physiology 115 (3), 865-868.

HortResearch 1995. Fertiliser Recommendations for Horticultural Crops. http://www.hortnet.co.nz/publications/guides/fertmanual/fertinf.htm

Hose, E., Sauter, A, Hartung, W., 2002. Abscisic Acid in roots - Biochemistry and Physiology. In: Waisel Y., Eshel A., Kafkafi U. (Eds.). Plant Roots – the hidden half. Marcel Dekker Inc. New York.

Hunt, A., Ayers, M., 2002. eande.lbl.gov/ECS/aerogels/saoptic.htm Optical Properties of silica aerogels Ernest Orlando Lawrence Berkeley National Laboratory.

ISO 11035: 1994(E). Sensory analysis – identification and selection of descriptors for establishing a sensory profile by a multidimensional approach.

Ingham, E.R., 2002. Workshops given in New Zealand. March, 2002.

Ingham, E.R., 1998. Protozoa and Nematodes. In: Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A., 1998. Principles and Applications of Soil Microbiology. Prentice Hall, New Jersey.

Ingham, R.E., Trofymow, J.A., Ingham, E., Coleman, D.C., 1985. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. Ecological Monographs 55(1).

Jacobs, D.R., Murtaugh, M.A., 2000. It's more than an apple a day: an appropriately processed, plant-centred dietary pattern may be good for your health. American Journal of Clinical Nutrition, 72.

Jalali V. K , Takkar P. N., 1979. Evaluation of parameters for simultaneous determination of micronutrient cations available to plants from soils. Indian Journal of Agricultural Sciences 49 (8), 622-626.

Jefferson, P. G., Mayland, H.F., Asay, K.H., Berdahl, J.D., 2001. Variation in mineral concentration and grass tetany potential among Russian wildrye accessions. Crop Science 41 (2), 543-548.

Jenkinson, D., 1988. In: Russell's soil conditions and plant growth. 11th edition (Wild, A. Ed.) Burnt Mill, Harlow, Essex, England.

Johnstone, P.D., Sinclair A.G., 1991. Replication requirements in field experiments for comparing phosphatic fertilizers. Fertilizer Research 29, 329-333.

Jones, D.L., Darrah, P.R., Kochian, L.V., 1996. Critical evaluation of organic acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. Plant and Soil 180 (1), 57-66.

Jones, D L, Darrah P. R., 1994. Amino-acid influx at the soil-root interface of Zea-mays L. and its implications in the rhizosphere. Plant and Soil 163 (1), 1-12.

Keeney, D.R., Bremner, J.M., 1966. Determination and isotope-ratio analysis of different forms of nitrogen in soils: 4 exchangeable ammonium, nitrate and nitrite by direct-distillation methods. Soil Science Society of America Proc. 30. 53-587.

Killham, K., 1994. Soil Ecology Cambridge University Press.

Klett, M., 1968. Investigation on light and shade as related to mode of production and enhancement of quality by silica preparations. Lebendige Erde, Darmstadt. (In German, reviewed in Koept, H.H., Pettersson, B.D., Schaumann, W., 1976. Bio-dynamic Agriculture, The Anthroposophic Press, Spring Valley, New York.

Knorr, D., Vogtmann, H., 1983. Quantity and quality determination of ecologically grown foods In: Knorr, D. (Ed.). Sustainable Food Systems. The AVI Publishing Company Inc. Westport, Connecticut.

Knorr, D., 1982. Use of a circular chromatographic method for the distinction of collard plants grown under different fertilizing conditions. Biological Agriculture and Horticulture, I, 29-38.

Koehler, B., Folsch, D.W., Strube, J., Lange, K., 2000. The influence of housing systems on the egg quality under particular consideration of the elements fresh grass and lighting conditions. In: Alföldi, T., Lockeretz, W., Niggli, U. (Eds.). IFOAM 2000: the World grows organic. Proceedings of the 13th International IFOAM Scientific Conference vdf Hochschulverlag AG an der ETH Zurich.

Koenig, U.J., 1988. Investigation of phenomena of diurnal rhythms and developmental dynamics with selected crops after application of biodynamic spray preparations. PhD-Thesis, University of Goettingen, Germany.

Koepf, H.H., 1993. Research in biodynamic agriculture: methods and results. Bio-Dynamic Farming and Gardening Association Inc., USA.

Koepf, H.H., Pettersson, B.D., Schaumann, W., 1976. Bio-dynamic Agriculture, The Anthroposophic Press, Spring Valley, New York.

Kolisko, E., Kolisko L., 1978. Agriculture of Tomorrow. 2nd edition (Originally published 1939. Gloucester, John Jennings). Kolisko Archive Publications, Bournmouth, England.

Koopmans, C.J.E., Bokhorst, J., 2001. Nitrogen mineralisation in organic farming systems: validation of the NDICEA model In: Recous, S., Nicolardot, B. (Eds.). 11th Nitrogen workshop. 9-12 September 2001, Reims, p. 403-404. INRA, Reims, France.

Koppenol, M., 2002. Research in biodynamic agriculture. In: A review of New Zealand and international organic land management research. Research and Development Group of the Bio Dynamic Farming and Gardening Association in New Zealand.

Kowalenko, C. G., 2001. Assessment of Leco CNS-2000 analyzer for simultaneously measuring total carbon, nitrogen, and sulphur in soil. Communications in Soil Science and Plant Analysis. 32 (13/14), 2065-2078.

Kowallik, W., 1987. Blue light effects on carbohydrate and protein metabolism. In: Senger, H. Blue Light Responses. CRC Press Inc., Boca Raton, Florida.

Krizek, D. T., Britz, S. J., Mirecki R. M., 1998. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cv. New Red Fire lettuce. Physiologia Plantarum 103(1), 1-7.

Ladebusch, A.A.H., Melzer, O., 1999. Long term trial with increasing amounts of phosphorus, potassium and magnesium applied to vegetable crops. In: Burns, I.G., Bending, G.D., Mulholland, B. Proceedings of the International Workshop on ecological aspects of vegetable fertilisation in integrated crop production in the field. Acta Horticultarae No. 506. Drukkerij Geers, Belgium.

Lairon, D., Spitz N., Termine, E., Ribaud, P., Lafont, H., Hauton, J., 1984. Effect of organic and mineral nitrogen fertilization on yield and nutritive value of butterhead lettuce. Quarterly Plant Foods Human Nutrition, 34, 97 – 108.

Lammerts van Bueren, E., Bisterbosch, L., 1988. Plasticfolie, milieuaspecten, kwaliteit van biiologisch-dynamische spruitpreparaten. Louis Bolk Instituut, The Netherlands.

Landcare Research, 2002. SINDI Soil quality indicators web-based tool. http://sindi.landcareresearch.co.nz/

Lawlor, D.W., 1991. Concepts of Nutrition In Plant growth interactions with nutrition and environment In: Porter J.R., Lawlor D.W. (Eds.). Cambridge University Press.

Lee, D., Nguyen, V., Littlefield, S., 1997. Comparison of methods for determination of nitrogen levels in soil, plant and body tissues, and water. In: Hood, T. M., Benton Jones, J. Jr., (Eds.). Soil and plant analysis in sustainable agriculture and environment. Marcel Dekker, New York.

Leinhos, V., Bergmann, H., 1995. Effect of amino alcohol application, rhizobacteria and mycorrhiza inoculation on the growth, the content of protein and phenolics and the protein pattern of drought stressed lettuce (Lactuca sativa L. cv. "Amerikanischer Brauner"). Angewandte Botanik. 69, 5-6, 153-156.

Levit, G.S. et al. 1999. Geophysiology of cyanobacterial biofilms and the "dissymmetry" principle. Bull. Inst. Océanogr. Monaco Special no. 19, 175-196.

Lewis, D.C., 1999. Sulfur In: Peverill, K.I., Sparrow, A., Reuter, D.J. (Eds.). Soil analysis and interpretation manual. CSIRO Publishing, Australia.

Li, X., George, E., Marschener, H., 1991. Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant and Soil 136, 41-48.

Liao, M.T., Hedley, M.J., Woolley, D.J., Brooks, R.R., Nichols, M.A., 2000. Copper uptake and translocation in chicory (*Cichorium intybus* L. cv Grasslands Puna) and tomato (*Lycopersicon esculentum* Mill. cv Rondy) plants grown in NFT system. II. The role of nicotianamine and histidine in xylem sap copper transport. Plant and Soil 223 (1/2), 243-252.

Lieblein, G., 1993. Quality and yield of carrots: effects of composted manure and mineral fertilizer. Doctor Scientarum Theses 13. Agricultural University of Norway.

Liu, S., Manson, J.E., et al. 2000. Fruit and vegetable intake and risk of cardiovascular disease: the Women's Health Study. American Journal of Clinical Nutrition, 72, 922-8.

Lievegoed, C.B.J., 1951. The working of the planets and the life processes in man and earth. Experimental Circle of Anthroposophical Farmers and Gardeners, Broome Farm, Stourbridge, UK.

Lillo, C., Appenroth, K. J., 2001. Light regulation of nitrate reductase in higher plants: which photoreceptors are involved. Plant Biology 3(5), 455-465.

Liu, A., Hamel, C., Elmi, A., Costa, C., Ma, B., Smith, D. L., 2002. Concentrations of K, Ca and Mg in maize colonized by arbuscular mycorrhizal fungi under field conditions. Canadian Journal of Soil Science. 82 (3), 271-278.

Lorenz, O. A., Maynard, D. N., 1980. Knott's handbook for vegetable growers. John Wiley & Sons, New York, USA.

Lovell, H., 2003. Advanced biodynamic agriculture. A short course prepared for workshops held in the winter of 2003 in Australia and New Zealand. OrGro. Levin.

Ludwig-Muller, J., Kaldorf, M., Sutter, E. G., Epstein, E., 1997. Indole-3-butyric acid (IBA) is enhanced in young maize (Zea mays L.) roots colonized with the arbuscular mycorrhizal fungus Glomus intraradices. Plant Science (Limerick) 125 (2), 153-162.

Lynch, J., Brown, K. M., 1997. Ethylene and plant responses to nutritional stress. Physiologia Plantarum 100 (3), 613-619.

Lynch, J.M., 1983. Soil Biotechnology. Blackwell Scientific Publications, Oxford.

MAF, 1990. Towards Sustainable Agriculture. Organic Farming MAF Policy Position Paper 2 Ministry of Agriculture and Fisheries Wellington, New Zealand.

McCullough, M. L., Feskanich, D., Stampfer, M. J., Giovannucci, E. L., Rimm, E. B., Hu, F. B., Spiegelman, D., Hunter, D. J., Colditz, G. A., Willett, W. C., 2002. Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. American Journal of Clinical Nutrition, 76, (6), 1261-1271.

MacIsaac, S.A., Sawhney V.K., 1990. Protein changes associated with auxin-induced stimulation and kinetin-induced inhibition of lateral root initiation in lettuce (Lactuca sativa) roots. Journal of Experimental Botany 41 (229), 1039-1044.

McKenzie, H.A., Wallace, H.S., 1954. The kjeldahl determination of nitrogen: a critical study of digestion conditions-temperature, catalyst and oxidizing agent. Australian Journal of Chemistry, 7, 55-70.

McLaren, R.G., Cameron, K.C., 1996. Soil Science : sustainable production and environmental protection Oxford University Press, Auckland.

MacRae, R.J., Hill, S.B., Mehuys, G.R., Henning J., 1990. Farm scale agronomic and economic conversion from conventional to sustainable agriculture. Advances in Agronomy, 43,155 – 198.

Mäder, P., Fliesbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil Fertility and Biodiversity in Organic Farming Science v.296, 5573.

Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., Niggli, U., 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. Biology and Fertility of Soils 31 (2), 150-156.

Mäder, P. Pfiffner, L., Niggli, U., Plochberger; V, A., Boltzmann, L., Balzer, U., Balzer, F., Besson, J. M., 1993. Effect of three farming systems (bio-dynamic, bio-organic, conventional) on yield and quality of beetroot (Beta vulgaris L. Var. Escuelenta L.) in a seven year crop rotation. Acta Horticulturae, 339, 11 – 31.

Maloney, P.E., van Bruggen, A.H.C., Hu, S., 1997. Bacterial community structure in relation to the carbon environments in lettuce and tomato rhizospheres and in bulk soil. Microbial Ecology 34: 109-117.

Marschner, H., 2002. Mineral nutrition of higher plants. 2nd edition. Academic Press. Elsevier Science Ltd, UK.

Marschner, H., 1991. Plant-soil relationships: acquisition of mineral nutrients by roots from soils In: .Porter, J.R., Lawlor, D.W. (Eds.). Plant growth interactions with nutrition and environment. Cambridge University Press.

Mathur, S..P., 1991. Composting processes In: Martin, A.M., (Ed.). Bioconversion of Waste Materials to Industrial Products. Elsevier, New York.

Matt, P., Geiger, M., Walch-Liu, P., Engels, C., Krapp, A., Stitt, M., 2001. The immediate cause of the diurnal changes of nitrogen metabolism in leaves of nitrate-replete tobacco: a major imbalance between the rate of nitrate reduction and the rates of nitrate uptake and ammonium metabolism during the first part of the light period. Plant, Cell & Environment 24(2), 177-190.

Matt, P., A. Krapp, Haake, V., Mock, H. P., Stitt, M., 2002. Decreased Rubisco activity leads to dramatic changes of nitrate metabolism, amino acid metabolism and the levels of phenylpropanoids and nicotine in tobacco antisense RBCS transformants. Plant Journal 30(6): 663-677.

Maynard, D. N., Barker, A. V., Minotti, P. L., Peck, N. H., 1976. Nitrate accumulation in vegetables. Advances in Agronomy 28, 71-118.

Mead, R., 1988. The design of experiments. Cambridge University Press, UK

Meilgaard, M., Civille, G.V., Carr, B.T., 1999. Sensory evaluation techniques. CRC Press, Boca Raton, Florida.

Mengel, K., Kirkby, E.A. 1987. Principles of Plant Nutrition. International Potash Institute, Bern.

Meyer, A., 1997. Historical changes in the mineral content of fruits and vegetables: a cause for concern? In: Agricultural Production and Nutrition. Proceedings of an international conference, Boston, Mass.

Miller, R.L., Jackson, L.E., 1998. Survey of vesicular-arbuscular mycorrhizae in lettuce production in relation to management and soil factors. Journal of Agricultural Science 130 (2) 173-182.

Miller, R. M., Miller, S. P., Jastrow, J. D., Rivetta, C. B., 2002. Mycorrhizal mediated feedbacks influence net carbon gain and nutrient uptake in Andropogon gerardii. New Phytologist. 155, 1, 149-162.

Moghimi, A., Lewis, D.G., Oades, J.M. 1978. Release of phosphate from calcium phosphates by rhizosphere products. Soil Biology and Biochemistry, 10, 277-281.

Moir, J.L., Scotter, D.R., Hedley, M.J., 2000. A climate-driven, soil fertility dependent, pasture production model. New Zealand Journal of Agricultural Research, 43(4), 491-500.

Molloy, L., 1998. Soils in the New Zealand landscape : the living mantle. New Zealand Society of Soil Science Lincoln, N.Z.

Moody, P.W., Bolland, M.D.A., 1999. Phosphorus In: Peverill, K.I., Sparrow, L.A., Reuter, D.J. (Eds.). Soil Analysis an interpretation manual. CSIRO Publishing.

Moss, D.N., 1984. Photosynthesis, respiration and photorespiration. In: Tesar, M.B. Physiological basis of crop development. American Society of Agronomy Inc. and the Crop Science Society of America, Inc., Wisconsin, USA.

Moss, M., Freed, D., 2003. The cow and the coronary: epidemiology, biochemistry and immunology. International Journal of Cardiology 87 (2/3), 203-216.

Mullen, M.D., 1998. Transformation of other elements. In: Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A. (Eds.). Principles and Applications of Soil Microbiology. Prentice Hall, New Jersey.

Muller, C., Scheible, W. R., Stitt, M., Krapp, A., 2001. Influence of malate and 2-oxoglutarate on the NIA transcript level and nitrate reductase activity in tobacco leaves. Plant, Cell & Environment 24(2), 191-203.

Murata, T., Goh, K. M., 1997. Effects of cropping systems on soil organic matter in a pair of conventional and biodynamic mixed cropping farms in Canterbury, New Zealand. Biology and Fertility of Soils, 25 (4), 372-381.

Muths, C., 2001. Healing with coherent emissions of light. Nexus:23 Oct-Nov. About the Coherence of Biophotons (1).

Myrold, D., 1987.Relationship between microbial biomass nitrogen and a nitrogen availability index. Soil Science Society of America Journal 51:1047-1049.

Nappi, P., Barberis, R., Consiglio, M., 1992. Chemico-physical and biological parameters for evaluating compost quality. In: Balis et al. (Eds.). International Symposium of Compost Recycling of Wastes. Acta Horticulturae 302, 267-278.

Nardi, S., Pizzeghello, D., Reniero, F., Muscolo, A., 1999. Biological activity of humic substances extracted from soils under different vegetation cover. Communications in Soil Science & Plant Analysis 30(5/6), 621-634.

Navarro, M., Pujola, M., Solivia, M, Garau, M., 1992. Nitrogen mineralization study in compost. In: Balis, C. et al, (Eds.). International Symposium on compost recycling of wastes. Acta Horticulturae 302, 279.

Nedeva, R., 2002. Influence of the different ratio of calcium and phosphorus in the compound feeds on the productivity of growing and finishing pigs. Bulgarian Journal of Agricultural Science 8 (2/3), 261-268.

Nillson, T., 1979. Yield, storage ability, quality and chemical composition of carrot, cabbage and leek at conventional and organic fertilizing. Acta Horticulturae, 93, 209 – 223.

NutritionData, 2002. Food composition analysis and nutrition facts for lettuce nutritiondata.com/facts-001-02s01wr.html

Oberson, A Oehl, F.; Langmeier, M.; Fliessbach, A.; Dubois, D.; Mader, P.; Besson, J. M.; Frossard, E. 2000. Can increased soil microbial activity help to sustain phosphorus availability? In: Alföldi, T., Lockeretz, W., Niggli, U. (Eds.). IFOAM 2000: the World grows organic. Proceedings of the 13th International IFOAM Scientific Conference vdf Hochschulverlag AG an der ETH Zurich.

Olechnowicz, J., Grudzka-Grzelak, E., Antosik, P., Winnicki, S., 2001. Calcium and phosphorus levels in blood serum of cows during the perinatal period. Folia Universitatis Agriculturae Stetinensis, Zootechnica (No.42), 135-140.

Olsen, S.R., Cole, C.V. Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular No. 939.

Pakrou, N., Dillon, P., 2000. Key processes of the nitrogen cycle in an irrigated and a non-irrigated grazed pasture. Plant & Soil. [print] 224 (2), 231-250.

Pamidi, J, Goh, K.M., McLaren, R. G., 2001. Comparison of three different methods of determining soil sulphur mineralization in relation to plant sulphur availability in soils. Biology and Fertility of Soils 34, 131-139.

Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., 1997. Biological Indicators of Soil Health. CAB International Wallingford, UK.

Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., Grace, P.R., (Eds.). 1997. Soil Biota:management in sustainable farming systems. CSIRO, Australia.

Parfitt, R. L., Hume, L. J., Sparling, G. P., 1989. Loss of availability of phosphate in New Zealand soils. Journal of Soil Science, UK. 40 (2), 371-382.

Paul, E.A., Clark, F.E., 1989. Soil microbiology and biochemistry. Academic Press, San Diego.

Paul, E.A., 1984. Dynamics of organic matter in soils. In Biological Processes and Soil Fertility. Tinsley, J., Darbyshire, J.F. (Eds.). Martinus Nijhoff/Dr W Junk The Hague.

Pendias, A.K., Pendias, H., 1992. Trace elements in soils and plants. CRC Press, Boca Raton.

Pettersson, B. 1978. A comparison between conventional and biodynamic farming systems as indicated by yields and quality. Proceedings of the International Research Conference IFOAM, Wirz Verlag, Aarau.

Peverill, K.I., 1999. In: Peverill, K.I., Sparrow, .A., Reuter, D.J. (Eds.). Soil analysis and interpretation manual CSIRO Publishing, Australia.

Pfeiffer, E., 1984. Chromatography applied to quality testing. Bio-dynamic Farming and Gardening Association, Inc., USA.

Pfeiffer, E., 1983. Soil fertility The Lanthorn Press, Great Britain.

Pfeiffer, E., 1958. Sub-nature and super-nature in the physiology of plant and man. Mercury Press, Spring Valley, New York.

Phoenix, V. R., Konhauser, K. O., Adams, D. G., 1999. Photosynthetic controls on the silicification of cyanobacteria. Geochemistry of the earth's surface. Proceedings of the 5th International Symposium, Reykjavik, Iceland, 15-20 August.

Piccolo, A., Nardi, S., Concheri, G., 1992. Structural characteristics of humic substances as related to nitrate uptake and growth regulation in plant systems. Soil Biology & Biochemistry 24 (4), 373-380.

Pinton, R., Cesco, S., Iacolettig, G., Astolfi, S., Varanini, Z., 1999. Modulation of NO3- uptake by water-extractable humic substances: involvement of root plasma membrane H+ ATPase. Plant & Soil 215(2), 155-161.

Pippard, P., 1995. Iron and Anaemia In: Iron Nutritional and physiological significance. The Report of the British Nutrition Foundation Task Force. Chapman Hall.

Popp, F., 1999. Macroscopic Quantum Coherence, Proceedings of an International Conference Boston University, edited by Boston University and MIT, World Scientific.

Popp, F., Gu, Q., Li, K., 1994. Biophoton emission: experimental background and theoretical approaches. Modern Physics Letters B Vol. 8, Nos. 21 and 22. 1269 – 1296. World Scientific Publishing Company.

Pramer, D., Schmidt, E.L., 1964. Experimental Soil Microbiology. Burgess Publishing Company, USA.

Prasad, M., Spiers, T. M., 1984. Evaluation of a rapid method for plant sap nitrate analysis. Communications in Soil Science and Plant Analysis 15 (6), 673-679.

Procter, P., Cole G.L., 1997. Grasp the Nettle. Random House, New Zealand.

Rajamohan, T., Kurup, P.A., 1990. Antiatherogenic effect of a low lysine: arginine ratio of protein involves alteration in the aortic glycosaminoglycans and glycoproteins. Journal of Biosciences. 15(4), 305-11.

Rao, T.P., Yano, K., Yamauchi, A., Tatsumi, J., Iijima, M., 2001. Regulation of phosphorus solubiization by light conditions on shoot legumes. In: Horst W.J.et al. (Eds) Plant nutrition-Food security and sustainability of agro-ecosystems. 174-175. Kluwer Academic Publishers, Netherlands.

Rauma, A. L., Mykkanen, H., 2000. Antioxidant status in vegetarians versus omnivores. Nutrition 16(2), 111-119.

Raupp, J., 1996. Quality investigations with products of the long-term fertilization trial in Darmstadt. In: Raupp, J. (ed.): Quality of plant products grown with manure fertilization. Proc. 4th Meeting Concerted Action Fertilization Systems in Organic Farming (AIR3-CT94-1940), Juva/Finland, 6-9 July, 1996; 13-33

Raupp, J., Konig, U. J., 1996. Biodynamic preparations cause opposite yield effects depending upon yield levels. Biological Agriculture & Horticulture, 13 (2), 175-188.

Raupp, J 2000. The well-proportioned farm organism. Just a pleasing image of a mixed farming system or rather a basic requirement for functioning organic husbandry? In: Alföldi, T., Lockeretz, W., Niggli, U. (Eds.). IFOAM 2000: the World grows organic. Proceedings of the 13th International IFOAM Scientific Conference vdf Hochschulverlag AG an der ETH Zurich.

Reddy, K.R., Feijtel, T.C., Patrick, W.H., 1986. Effect of soil redox conditions on microbial oxidation of organic matter. In: Chen, Y., Avnimelech, Y. (Eds.). The role of organic matter in modern agriculture. Martinus Nijhoff Publishers, Dordrecht.

Redinbaugh, M. G., Huber, S. C., Huber, J. L., Hendrix, K. W., Campbell, W. H., 1996, Nitrate reductase expression in maize leaves (Zea mays) during dark-light transitions. Complex effects of protein phosphatase inhibitors on enzyme activity, protein synthesis and transcript levels. Physiologia Plantarum 98 (1), 67-76.

Reeds, P.J., 1988. Nitrogen metabolism and protein requirements. In: Blaxter, K., Macdonald, I. (Eds.). Comparative Nutrition. John Libbey.

Reganold, J.P., Palmer, A.S., Lockhart, J.C., Macgregor, A.N., 1993. Soil quality and financial performance of biodynamic and conventional farms in New Zealand. Science, 260, 344.

Ren, H., Endo, H., Hayashi, T., 2001. Antioxidative and antimutagenic activities and polyphenol content of pesticide-free and organically cultivated green vegetables using water-soluble chitosan as a soil modifier and leaf surface spray. Journal of the Science of Food and Agriculture, 81, 15, 1426-1432.

Ruiz Lozano, J.M., Azcon, R., Gomez, M., 1995. Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: physiological and nutritional plant responses. Applied Environmental Microbiology. 61,456-460.

Remer, N. 1995. Laws of life in agriculture. Trans. Castellitz K., Davies B. Bio-Dynamic Farming and Gardening Association Incs. Kimberton, USA.

Roper, M.M., Ophel-Keller, K.M. 1997. Soil microflora as bioindicators In Biological Indicators of Soil Health (Eds Pankhurst C., Doube B.M., Gupta V.V.S.R.) CAB International, Wallingford, UK.

Rillig, M. C., Wright, S. F., Eviner, V. T., 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant and Soil. 238 (2), 325-333.

Russell, E.J., 1988. Russell's soil conditions and plant growth. 11th edition (Wild, A. Ed.) Burnt Mill, Harlow, Essex, England.

Ryan, M. H., Small, D.R., Ash, J.E., 2000. Phosphorus controls the level of colonisation by arbuscular mycorrhizal fungi in conventional and biodynamic irrigated dairy pastures. Australian Journal of Experimental Agriculture 40(5), 663-670.

Ryan, M.H., Graham, J.H., 2002. Is there a role for arbuscular mycorrhizal fungi in production agriculture? Plant and Soil, 244 (1/2), 263-271.

Sainz, M. J., Taboada-Castro, M.T., Vilarino, A., 1998. Growth, mineral nutrition and mycorrhizal colonization of red clover and cucumber plants grown in a soil amended with composted urban wastes. Plant and Soil. 205 (1), 85-92.

Saggar, S., Hedley, M.J., White R.E., R.E., Perrott, K.W., Gregg, P.E.H., Cornforth, I. S., Sinclair, A.G., 1999. Development and evaluation of an improved soil test for phosphorus, 3: field comparison of Olsen, Colwell and Resin soil P tests for New Zealand pasture soils. Nutrient Cycling in Agroecosystems 55 (1), 35-50.

Saggar, S., Hedley M.J. White R.E. 1990. A simplified resin membrane technique for extracting phosphorus from soils Fertiliser Research 24 173-180

Samaras, I., 1978. The keeping quality of differently fertilised vegetables with special consideration of physiological and microbiological parameters, (German). Ph.D Thesis, Giesssen.

Sarapatka, B., 2000. Impact of different farming systems and soil properties on soil enzyme activity. In: Alföldi, T., Lockeretz, W., Niggli, U. (Eds.). IFOAM 2000: the World grows organic. Proceedings of the 13th International IFOAM Scientific Conference vdf Hochschulverlag AG an der ETH Zurich.

Saunders, W.M.H., 1965. Phosphate retention by New Zealand soils and its relationship to free sesquioxides, organic matter and other soil properties. New Zealand Journal of Agricultural Research 8:30-57.

Scaife, M.A., Barnes, A., 1977. The relationship between crop yield and petiole nitrate concentration at various growth stages. Plant and Soil 46 (3), 705-712.

Scheffer, F., Kickuth, R., Schlimme, E., 1967. Die Wirkung von Rhizosphaerenprodukten des weissen Senfes auf Eisen-II Phosphat (Vivianit). Z. f. Pflanzenern. U. Bodenk., 116, 53-62.

Scheller, E., 2000. Importance of protein and amino acid metabolism in soil for plant nutrition and soil fertility In: Alföldi, T., Lockeretz, W., Niggli, U. (Eds.). IFOAM 2000: the World grows organic. Proceedings of the 13th International IFOAM Scientific Conference vdf Hochschulverlag AG an der ETH Zurich.

Scheller, E., 1996. Fundamentals of organic agriculture. Proceedings Vol. 1 of the 11th International Scientific Conference of IFOAM in Copenhagen 1996. Editor: Troels V. Oestergaard 47 – 59.

Schiller, H., 1971. Gruedlanduengung und unspezifische Rindersterilitaet. Oberoesterreich und Landw.-Chem. Bundesversuchsanstalt, Linz.

Schmidt, R. E., Zhang, X., Chalmers D. R., 1999. Response of photosynthesis and superoxide dismutase to silica applied to creeping bentgrass grown under two fertility levels. Journal of Plant Nutrition 22(11), 1763-1773.

Schmidt, S., Stewart, G.R., 1999. Glycine metabolism by plant roots and its occurrence in Australian plant communities. Australian Journal of plant physiology 26 (3), 253-264.

Schutter, M. E., Sandeno, J. M., Dick, R. P., 2001. Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems. Biology and Fertility of Soils 34 (6), 397-410.

Searle, P.L., 1967. Determination of carbon, calcium carbonate and sulphur in soil using hifh-frequency induction furnace equipmenet. N.Z.Soil News 5, 168-180.

Seelig, M. S., 2001. Epidemiology of water magnesium: evidence of contributions to health. In: Rayssiguier, Y., Mazur, A., Durlach, J. (Eds.). Advances in magnesium research: nutrition and health.211-218.

Senger, H., 1987. Sun and shade effects of blue light on plants. In: Senger, H. Blue Light Responses. CRC Press Inc., Boca Raton, Florida.

Sharma, A.K., John, B.N., 2002. Physiology of nutrient uptake by arbuscular mycorrhizal fungi. In: Sharma, A.K., Johri, B.N. (Eds.).Arbuscular mycorrhizae interactions in plants rhizosphere and soils. Science Publishers Inc. Enfield, USA.

Shinjini, B., Sunita, T., 2001. Zinc and cognitive development. British Journal of Nutrition, 85 (Suppl.2) S139-S145.

Siomos, A.S., Beis, G., Papadopoulou, P.P., Nasi, P., Kaberidou, I., Barbayiannis, N., 2001. Quality and composition of lettuce (cv. 'Plenty') grown in soil and soilless culture. Acta Horticulturae. No.548, 445-449.

Sidlauskas, G., 2000. The influence of nitrogen, phosphorus and potassium content in spring oilseed rape (Brassica napus) plants at different growth stages on seed, protein and fat yield. Zemdirbyste, Mokslo Darbai (No. 72), 118-135.

Simonne, E., Simonne, A., Wells, L., 2001. Nitrogen source affects crunchiness, but not lettuce yield. Journal of Plant Nutrition 24 (4/5), 743-751.

Singh, R., Adholeya, A., 2002. Plant and fungal responses to colonization. In: Sharma A.K., Johri, B.N. (Eds.). Arbuscular mycorrhizae interactions in plants rhizosphere and soils. Science Publishers Inc. Enfield, USA.

Sleeman, J. D., Dudley, S. A., 2001. Phenotypic plasticity in carbon acquisition of rapid cycling Brassica rapa L. in response to light quality and water availability. International Journal of Plant Sciences, 162(2), 297-307.

Smith, H., 1982. Light Quality, photoreception, and plant strategy. Annual Review of Plant Physiology. 33, 481-518.

Somers, G.F., Beeson, K.C., 1948. The influence of climate and fertilizer practices upon the vitamin and mineral content of vegetables. Advances in Food Research, 1, 291 – 324.

Sorenson, J.N., 1999. Nitrogen effects on vegetable crop production and chemical composition. In: Burns I.G., Bending G.D., Mulholland, B. Proceedings of the International Workshop on ecological aspects of vegetable feretilisation in integrated crop production in the field. Acta Horticultarae No. 506. Drukkerij Geers, Belgium.

Soil Bureau Bulletin 26, 1968. Soils of New Zealand. Department of Scientific and Industrial Research, Wellington, NZ.

Souci, S.W., Fachmann, W., Kraut, H. 1994. Food Composition and Nutrition Tabels. Medpharm Scientific Publishers Stuttgart.

Sparling, G.P., Schipper, L.A., 2002. Soil quality at a national scale in New Zealand. Journal of Environmental Quality 31, 1848-1857.

Sparling, G.P., Rijkse, W., 1998. A pilot survey linking stage 1 state and pressure indicators in Bay of Plenty. Ministry for the Environment. Technical paper No. 28. New Zealand.

Sparling, G.P., 1997. Soil Microbial Biomass, activity and nutrient cycling. In: Pankhurst, C., Doube, B.M., Gupta, V.V.S.R. (Eds.) Biological Indicators of Soil Health. CAB International, Wallingford, UK.

Steiner, R., 1993. Agriculture. A series of lectures given in 1924. Gardner, M. (Ed.). Translated by Creager, C.E., Gardner, M. Bio-Dynamic Farming and Gardening Association Inc., Kimberton, USA.

Stipanuk, M.H., 2000. Biochemical and physiological aspects of human nutrition W.B. Saunders Company Philadelphia.

Stolz, P., Stube, J., Buchmann, M., Hiss, C., 2000. Better dietary protein-quality of beans cultivated biodynamically than by hydro-culture. In: Alföldi, T., Lockeretz, W., Niggli, U. (Eds.). IFOAM 2000: the World grows organic. Proceedings of the 13th International IFOAM Scientific Conference vdf Hochschulverlag AG an der ETH Zurich.

Subramanian, K. S., Charest, C., 1995. Influence of arbuscular mycorrhizae on the metabolism of maize under drought stress. Mycorrhiza 5 (4), 273-278.

Sunlarp, S., 1999. The effect of cultivar, nutrient solution concentration and season on the yield and quality of NFT produced lettuce (Lactuca sativa L.): a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Science at Massey University.

Tanimoto, E., 2002. Gibberellins In: Waisel Y., Eshel A., Kafkafi U., (Eds.). Plant Roots – the hidden half. Marcel Dekker Inc. New York.

Tarafdar, J.C., Rao, A.V., 2002. Possible role of arbuscular mycorrhizal fungi in development of soil structure. In: Sharma, A.K., Johri, B.N. (Eds.). Arbuscular mycorrhizae interactions in plants rhizosphere and soils..Science Publishers Inc., Enfield, USA.

Tate, R.L., 1987. Soil organic matter : biological and ecological effects. Wiley, New York.

Tate, R.L., 2000. Soil microbiology. John Wiley, New York.

Technicon, 1976. Industrial methods for NH_3-N and TKN extract analysis. No. 329-74 W/A. Technicon Industrial Systems. Tarrytown, New York.

Tegethoff, U., 1987. Untersuchungen ueber den Einfluss von im biologisch-dynamischen Landbau eingesetzten Pflegemitteln auf Morphologie und Physiologie einiger Gemuessearten. Ph.D. Thesis, Bonn.

Tei, F., Benincasa, P., Guiducci, M. 1999 Nitrogen fertilization of lettucee, processing tomato and sweet pepper: yield, nitrogen uptake and the risk of nitrate leaching. In Burns I.G., Bending G.D., Mulholland B. Proceedings of the International Workshop on ecological aspects of vegetable fertilisation in integrated crop production in the field. Acta Horticultarae No. 506. Drukkerij Geers, Belgium.

Terry, N., Rao, I.M,. 1991.Nutrients and photosynthesis: iron and phosphorus as case studies. In: Porter J.R, Lawlor D.W. (Eds.). Plant growth interactions with nutrition and environment, Cambridge University Press.

Thun, M., 1979. Working on the Land and the Constellations. Lanthorn Press, East Grinstead, UK.

Thun, M., 2002. Experiences of working with the biodynamic preparations. Journal of the Biodynamic Agricultural Association. U.K.

Tingstad, A., 2001. Kvalitet og metode : med udgangspunkt i stigbilleder til kvalitetsvurdering af fødevarer, Den kgl. Veterinær og Landbohøjskole, Kemisk Innstitut. (English summary of PhD thesis, University of Copenhagen, Denmark).

Tosserams, M., Smet, J., Magendans, E., Rozema, J., 2001. Nutrient availability influences UV-B sensitivity of Plantago lanceolata. Plant Ecology. [print] 154(1-2). June, 159-168.

Triglia, A., La Malfa, G., Musumeci, F., Leonardi, C., Scordino, A., 1998. Delayed Luminescence as an Indicator of Tomato Fruit Quality. Journal of Food Science, Vol. 63, No. 3.

Turner, M.J., 2001. The importance of VA mycorrhizal fungi in copper uptake by natural associations of white clover and ryegrass, and the use of ^{14}C to trace hyphae active in transport. B.Ap.Sc. (Hons) Thesis, Soil Science, Massey University.

Twine, J.R., Williams, C.M., 1971. The determination of phosphorus in Kjeldahl digest of plant material by automated analysis. Communications in Soil Science and Plant Analysis 2, 485-489.

USDA, 1998. Nutrient Database for Standard Reference, Release 12 (March 1998).

Valaja, J., K. Suomi, Alaviuhkola, T., Mela, T., 1997. Effects of variety, soil type and nitrogen fertilizer supply on the nutritive value of barley for growing pigs. Agricultural and Food Science in Finland 6(4), 295-303.

Vancura, V., 1988. Plant metabolites in soil. In: Vancura, V., Kunc, F. (Eds.). Soil Microbial Associations. Elsevier, Amsterdam.

Van Steensel, F., 2002. The soil system: the development and functions of soil. In: A review of New Zealand and international organic land management research. Research and Development Group of the Bio Dynamic Farming and Gardening Association in New Zealand.

Van Steensel, F. 1995. Farm management and Soil Quality. Masters Thesis. Massey University.

Vaughan D., Malcolm R.E. (Eds.) 1985. Soil Organic Matter and Biological Activity. Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht.

Vestberg, M., Kukkonen, S., Saari, K., Uosukainen, M., Palojarvi, A., Tuovinen, T., Vepsalainen, M., Niemi, M., 2002. Cropping system impact on soil quality determinants. Agricultural and Food Science in Finland 11 (4), 311-328.

Villeneuve, F., 1997. Growing soil and vegetable quality. Example: the carrot. SO - Infos (Paris). 136, 41-44.

Waisel, Y., Eshel, A., Kafkafi, U. (Eds.). 2002. Plant Roots – the hidden half. Marcel Dekker Inc. New York.

Waldon, H.,S., Gliessman, Buchanan M., 1998. Agroecosystem responses to organic and conventional management practices. Agricultural Systems, 57(1), 65-75.

Ward, J.M., Schroeder, J.I., 1997. Roles of ion channels in initiation of signal transduction in higher plants In: Aducci, P. (Ed.). Signal Transduction in Plants. Birkhaeuser Verlag, Switzerland.

Ward, R., 1995. Iron overload and toxicity. In: Iron Nutritional and physiological significance. The Report of the British Nutrition Foundation Task Force. Chapman Hall.

Wang, W. J., Chalk, P. M., Chen, D., Smith, C. J., 2001, Nitrogen mineralisation, immobilisation and loss, and their role in determining differences in net nitrogen production during waterlogged and aerobic incubation of soils. Soil Biology & Biochemistry 33 (10): 1305-1315.

Wang, H.L., Hedley, M.J., Bolan, N.S., 1995. Chemical properties of fluidized-bed boiler ash relevant to its use as a liming material and fertiliser. New Zealand Journal of Agricultural Research, 38 (2), 249-256.

Waring, S.A., Bremner, J..M., 1964. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. Nature 4922: 951-952.

Warman, P. R., Havard, K. A., 1998. Yield, vitamin and mineral contents of organically and conventionally grown potatoes and sweet corn. Agriculture Ecosystems & Environment 68(3), 207-216.

Warman, P. R., Havard, K. A., 1997. Yield, vitamin and mineral contents of organically and conventionally grown carrots and cabbage. Agriculture Ecosystems & Environment, 61(2-3), 155-162.

Watanabe, S., Fujiwara, T., Yoneyama, T., Hayashi, H., 2001. Effects of silicon nutrition on metabolism and translocation of nutrients in rice plants. In: Horst W.J.et al (Eds) Plant nutrition-Food security and sustainability of agro-ecosystems. 174-175. Kluwer Academic Publishers, Netherlands.

Watkinson, J. H., Kear, M. J., 1996. Sulfate and mineralisable organic sulfur in pastoral soils of New Zealand. II. A soil test for mineralisable organic sulfur. Australian Journal of Soil Research 34 (3), 405-412.

Weibel, F. P., R. Bickel, Leuthold, S., Alfoldi, T., 2000. Are organically grown apples tastier and healthier? A comparative field study using conventional and alternative methods to measure fruit quality, Acta Horticulturae, 517, 417-426.

Weir, R.G., Cresswell, G.C., 1995. Plant Nutrient Disorders 3. Vegetable crops. Inkata Press.

Welch, R. M., 1997. Agronomic problems related to provitamin A carotenoid-rich plants. European Journal of Clinical Nutrition, 51, S34-S38.

Welch, R.M., 1993. Zinc concentrations and forms in plants for humans and animals. In: Robson, A.D. Zinc in soil and plants. Kluwer Academic Publishers, Australia.

Wellinghausen, N., 2001. Immunobiology of gestational zinc deficiency. British Journal of Nutrition; 85 (Suppl. 2) S81-S86.

Werf, A. van der, Nagel, O. W., 1996. Carbon allocation to shoots and roots in relation to nitrogen supply is mediated by cytokinins and sucrose: opinion. Plant and Soil 185 (1), 21-32.

Westerman, R.L. (Ed.) 1990. Soil Testing and Plant Analysis. Soil Science Society of America, Inc., Madison, Wisconsin USA.

Whitehead, D.C., 2000. Nutrient Elements in Grassland Soil-Plant-Animal Relationships. CABI Publishing, UK.

Wilkinson, S., Davies, W.J., 1997. Xylem sap pH increase: a drought signal received at theapoplastic face of the guard cell which involves the suppression of saturable ABA uptake by the epidermal symplast. Plant Physiology, 113:559-573.

Wijk, C. van, Neuvel, J., Berg, W. van den, 2002. Effects of soil phosphate level and phosphate application rate on the yields of four field vegetables. Editors: Booij, R.; Neeteson, J.Acta Horticulturae(No.571), 225-231.

Woese K., Lange, D., Boess, C., Boegl, K.L., 1997. A comparison of organically and conventionally gown foods – results of a review of the relevant literature. Journal of Science of Food and Agriculture, 74, 281 – 293.

Worthington, V., 2001. Organically grown food – is it better for you? The Journal of Alternative and Complementary Medicine, 7:2, 161 – 173.

Woods End Research Laboratory, 2000. Interpretation of compost tests. Journal of the Woods End Research Laboratory 1998-2000 Vol 1 No.4. http //www.woodsend.org

Wright, D.M., 2001. How is Organic defined? Organics Working Group Report: Facilitating the efficient and sustainable development and growth of the New Zealand organic sector. Ministry of Agriculture and Forestry, NZ.

Yamagata, M., Ae, N. 1999. Direct acquisition of organic nitrogen by crops Jarq-Japan Agricultural Research Quarterly 33 (1), 15-21 JAN 1999.

Yanishevskaya, O. L., Yagodin, B.A., 2000. Effect of Si, Mn and Cr on the productivity and product quality of vegetable crops. [Russian]. Agrokhimiya. 2000. No. 5, 47-51.

Young, V. R., Borgonha, S., 2000.Nitrogen and amino acid requirements: the Massachusetts Institute of Technology amino acid requirement pattern. [Journal Article] Journal of Nutrition, 130, (7S),1841S-1849S.

Zaimenko, N.V., 1998. Effect of organosilicon preparation on activity of redox processes and content of some assimilates in plant leaves. Fiziologiya i Biokhimiya Kul'Turnykh Rastenii, 30, 5.

Zimmer, G.F., 2000. The biological Farmer. Acres, USA, Austin, Texas.

APPENDIX I

Table 4.5 2 Mean nitrate levels in lettuce leaf sap at different times after application of silica spray to some treatments (Treatment codes in Table 3.3)

Time from spraying	- 1 hour	1week	- 1 hour	1hour	5 hours	9 hours	28 hours	48 hours	56 hours	6 days
Date, time	9.11. 11am	15.11 3pm	3.12 9am	3.12 11am	3.12. 3pm	3.12. 7pm	4.12. 2pm	5.12. 11am	5.12. 7pm	9.12 11am
Ctrl	200	450	550	500	500	633	500	600	800	825
C+ sp	333.3	450	600	450	500	433	600	800	700	850
DC	316.7	600	600	550	400	700	750	800	400	1050
DC + sp	300	600	850	700	700	600	450	700	500	975
Org	267	350	400	700	650	500	450	800	400	900
Bd	267	526	450	800	550	533	450	500	600	875

Table 4.6.4 Mean levels of soluble solids (Brix level) in lettuce leaves for each treatment. Treatment codes in Table 3.3

	before spray	41 DAT			42 DAT	43 DAT		45 DAT
	9am	**11am**	**3pm**	**7pm**	**2pm**	**11am**	**7pm**	**11am**
Ctrl	2.85	3.5	3.75	4.07	2.85	2.75	4.00	4.84
C+sp	2.8	2.9	3.5	5.67	3.3	3.45	3.70	5.09
DC+spray	1.4	3.25	2.5	3.47	3.65	3.35	2.20	4.98
DC+sp	3	2.75	3.5	4.53	2.8	2.5	3.20	5.13
Org	3	2.65	3.6	4.3	2	2.65	2.2	4.98
Bd	2.6	2.85	2.4	4.5	3.35	3.5	3.6	5.09

APPENDIX II

Lettuce sensory evaluation questionnaire – Te Puke – 9.12.02

Panelist name ....

Please answer each question for each sample and circle the words that correspond most closely with your evaluation (1 per question).

Sample number .....

Appearance

very good good OK poor very poor

Aroma

Does it have an aroma? yes no

Is the aroma very good good OK poor very poor

Texture

Please circle as many words as fit this sample

hard tough firm crunchy soft flabby

other (please specify) .....

Flavour

strong flavour mild flavour no flavour

Sweetness - very sweet sweet not sweet

Bitterness very bitter bitter not bitter

other (please specify) .....

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Note: The above questions were repeated 5 times, so that each panelist completed all questions for each of 6 samples

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Please rank all the samples in the order of preference (most liked = 1, least liked = 6)

A B C D E F

Thank you very much for your help