

Opportunities for Agronomic Manipulation of Ascorbic Acid Yield in Fruit Crops: A Case Study with *Ribes nigrum* L.

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ABSTRACT

This work focuses on developing an understanding of how agronomic manipulation might enhance fruit L-ascorbic acid (AsA) yields of blackcurrant and how this information might be best used, in field-grown crops. A series of pot and field experiments were carried out to determine the effects of manipulating plant nitrogen, soil moisture and plant leaf canopy light interception, in interaction with the use of reflective mulches. Results show that using 'excessive' nitrogen (ammonium nitrate) enhanced leaf canopy area, but at the expense of fruit AsA concentration which declined, in 'Hedda' and 'Baldwin' grown in pots. In the field, nitrogen application to 'Baldwin' and 'Ben Lomond' showed no stimulation of bush growth or fruit yield with increasing concentration, while fruit AsA concentration and total AsA yield declined. To evaluate the impacts of soil moisture, different rates of trickle irrigation were applied to field-grown 'Baldwin' and 'Ben Lomond'. Plants not receiving irrigation had reduced shoot growth compared with those receiving irrigation, but drought stress did not influence fruit yield per bush. Optimal irrigation did have a cumulative positive effect on fruit yield. There was no impact of drought stress on berry AsA concentration, but a large positive enhancement on total AsA yield per bush. Enhancement of bush solar radiation interception was achieved using light reflective plastic soil mulches. Mulches increased bush canopy vegetative growth and were accompanied by small increases in fruit yield. There was no influence on fruit AsA concentration, or total bush yield of AsA initially, but in the second growing season total bush AsA yield increased. A combination of irrigation and light reflective mulch, in field-grown 'Baldwin' and 'Ben Lomond', enhanced fruit yields and the total AsA yield per bush after two consecutive years of treatment. For 'Baldwin', irrigation was the factor mainly responsible for this enhancement, while for 'Ben Lomond' it was the light reflective mulch that was most important. The implications of how field agronomy might influence the processes which determine AsA biosynthesis are discussed.

Keywords: agronomy, antioxidants, ascorbic acid, blackcurrant, irrigation, mulching, nitrogen, *Ribes nigrum*, solar radiation interception, vitamin C

Abbreviations: AsA, ascorbic acid; ROS, reactive oxygen species

INTRODUCTION

Ribes nigrum

Blackcurrants (*Ribes nigrum* L.) grow as bushes of approximately 1-1.5 m in height with fruit being borne on short shoots (strigs) that originate on wood produced the previous year. Flowers are initiated in late summer of the year prior to fruiting, once summer shoot growth has terminated; winter dormancy follows where no further obvious morphological bud development takes place (Brennan 1996). Fruit yield appears to be under strong genetic control with differences between cultivars being maintained across different years. Environmental and cultural practices, particularly in the year that the fruit-bearing wood develops, can have a considerable influence on shoot growth and fruit bud formation, and subsequently fruit yields and quality (Webb 1978; Toldam-Andersen and Hansen 1997). Commercial blackcurrant production is limited to regions in temperate zone climates that are able to satisfy the required level of chilling, i.e. Northern and Eastern Europe, in particular, the former USSR, Poland, Germany, and the UK (Brennan 1996; Atkinson *et al.* 2004). In 2006/07 the UK planted blackcurrant area had increased from 1956 ha in 1997, to 2596 ha producing 15,600 metric tonnes of fruit (Defra 2008). Prior to the 1950s fruit yields were around 2.5 tonnes ha⁻¹, however due to improvements in plant health (certified stock), site selection, irrigation, herbicides and pest and disease control, yields had increased to 5-6 tonnes

ha⁻¹ by the 1970s (MAFF 1977). Despite the majority of the commercial UK blackcurrant crop being used there is a lack of consistency in fruit quality between years.

Health benefits of AsA

Blackcurrant fruit contain a variety of health promoting phytochemicals (e.g. cyanidin, delphinidin and gallic acid) and are a particularly rich dietary source of ascorbic acid (AsA or vitamin C), having approximately four times the concentration found in some citrus fruits (Miller and Rice-Evans 1997; Kalt 2001; Davey *et al.* 2004). However, there is wide variation in AsA concentration between blackcurrant cultivars. Juice values for AsA concentration in 10 cultivars ranged from 67 mg 100 g⁻¹ for 'Hedda' to 204 mg 100 g⁻¹ for 'Blackdown' (Heiberg *et al.* 1992). A more recent study by Viola *et al.* (2000) extended the range to 263 mg 100 g⁻¹ with an unnamed selection (8932, SCRI). AsA is a vital dietary component for humans (fruit and vegetables provide 86% of AsA in UK diet) and deficiency can lead to diseases such as scurvy (Davey *et al.* 2000). It is now recognised that there are considerable benefits to consuming plant-derived antioxidants, such as AsA, at higher doses than the recommended daily allowance (RDA) (Block 1991; Diplock *et al.* 1998; Prior and Cao 2000; Kalt 2001; Prior *et al.* 2007). The multi-functional abilities of AsA beyond its antioxidant and enzymic cofactor status now suggest that it may also act as an anti-cancer forming agent (Olsson *et al.* 2004; Chen *et al.* 2008). AsA has also been

implicated as a protectant against coronary heart disease and some degenerative aspects of ageing, but supporting evidence is not always forthcoming (Liu 2003; Heart Protection Study 2002). It remains unclear whether sufficient AsA intake can be achieved by dietary consumption, rather than by clinical administration (Borst 2008; Frei and Lawson 2008), particularly as pressure increases to elevate the current RDA of AsA two- or three-fold (Carr and Frei 1999; Levine *et al.* 1999).

AsA biosynthesis

AsA has been identified with a number of roles in plants which includes the protection against reactive oxygen species (ROS) by acting as an antioxidant in photo-protection, as an enzyme co-factor, and as a factor controlling plant growth regulation (Smirnoff and Pallanca 1996; Davey *et al.* 2000; Conklin 2001; Debolt *et al.* 2007). The plant biosynthetic pathway for AsA was only discovered comparatively recently and differs from that in animals (Wheeler *et al.* 1998; Loewus 1999), subsequently there have been several suggested alternative pathways, which relate to specific organs, tissues or developmental stages (Agius *et al.* 2003; Wolucka and van Montagu 2003; Lorence *et al.* 2004; Ishikawa *et al.* 2006). Understanding of the biochemistry of AsA synthesis and recycling has advanced in recent years, but much of the work has been undertaken in photosynthetic tissues using model plants such as *Arabidopsis thaliana* and very little attention has been devoted to the mechanisms governing the accumulation of AsA concentration in sink tissues such as fruits. Conklin (2001) suggests that elevated AsA concentration could be achieved by altering turnover or synthesis and there is evidence for both (see also Chen *et al.* 2003). On the other hand, the activity of the enzyme l-galactono-1,4-lactone dehydrogenase (GalLDH) is linked to AsA regulation, where the decline in AsA concentration corresponds to leaf tissue age and senescence (Borraccino *et al.* 1994; Bartoli *et al.* 2000). GalLDH activity is also known to be positively correlated with light and AsA concentration (Smirnoff 2000). More recently the control of AsA biosynthesis has been unravelled further (Smirnoff *et al.* 2001; Wolucka and van Montagu 2003; Mieda *et al.* 2004; Hancock and Viola 2005; Debolt *et al.* 2007; Linster and Clarke 2008) with its metabolic integration being mediated *via* the last enzyme step GalLDH in AsA biosynthesis (Millar *et al.* 2003). Recent studies with blackcurrant showed that AsA biosynthesis occurred *in-situ* in the fruit using imported photoassimilates (Hancock *et al.* 2007). Some earlier studies have suggested that AsA accumulated in source leaf phloem tissues prior to transportation to sink tissue (Franceschi and Tarlyn 2002). There is still much to be understood before we are able to manipulate AsA biosynthesis to enhance its concentration in fruit (Linster and Clarke 2008). Our interests lie in developing approaches that can be used to enhance dietary intake through relatively short-term agronomic solutions to provide fruit with high AsA concentration and provide the industry with the means to optimise yields of AsA per bush (i.e. fruit AsA concentration × the bush yield).

Manipulation of AsA concentration in fruit

The work described here is part of our strategy to optimise fruit AsA production through the development and application of environmental and agronomic knowledge (Harris 1975). A number of external factors are known to influence cultivar variation in AsA production and fruit concentration including geographical location, climate and various nutritional factors (Nilsson 1969; Chrapkowska and Rogalinski 1975; Given 1985; Hancock and Atkinson 2007). To us this suggests that there may be opportunities to increase AsA fruit concentrations and bush yields if we understand the mechanisms by which these factors have their influence and how they may interact. Linster and Clarke (2008) highlighted in their recent review the importance of AsA in con-

trolling plant growth and development, and its protective role against environmental stresses such as UV, high temperatures, ozone, high radiation and flooding (see review Blokhina *et al.* 2003). Our strategy is therefore based on the notion that AsA concentration is likely under some form of environmental regulation and this might be used practically to enhance its concentration within fruit.

There is already some suggestion, with certain crops, that the notion of environmental factors influencing AsA production can occur (Grace and Logan 1996; Logan *et al.* 1996; Lee and Kader 2000). Radiation has been shown to influence AsA concentration in apple tissues; exocarp AsA concentration of the parts of the apple that received the greatest radiation dose had approximately twice as much AsA than samples from areas receiving lower light levels (Davey *et al.* 2004). Increasing the amount of available radiation through the use of reflective mulches has been shown to increase fruit AsA concentration in some cultivars of strawberry and cantaloupe melons (Atkinson *et al.* 2006; Fonseca 2008). Radiation intensity affects AsA concentration in leaves (Smirnoff and Pallanca 1996) which, through AsA phloem transport may subsequently influence storage organs AsA concentration as is the case with potato tubers (Tedone *et al.* 2004). Temperature effects on fruit AsA concentration have not been widely studied but elevated temperatures in controlled environment chambers reduced AsA concentration in blackcurrant (Redalen 1993) and kiwifruit (Richardson *et al.* 2004).

There are contradictory reports as to how mineral nutrition, particularly nitrogen, influences AsA concentration. For example, enhanced plant nitrogen was positively correlated with AsA concentration, and other antioxidants, in strawberry fruit (Wang and Lin 2003). However, the opposite was true with citrus fruits (e.g. Valencia oranges), tomatoes, strawberries, onion, cabbage, lettuce and spinach (Embelton *et al.* 1980; Nagy 1980; Pankov 1983; Sørensen 1984; Haynes and Goh 1987; Montagu and Goh 1990; Sørensen *et al.* 1994; Takebe *et al.* 1995; Toor *et al.* 2006). In various potato cultivars, tuber AsA concentration appears to be negatively correlated with nitrogen fertilizer application (Augustin 1975). A higher ratio of AsA to dehydroascorbate (DHA) was found in nitrogen-limited spinach and soybean plants, compared to nitrogen-sufficient controls (Robinson 1997). One simple explanation for this deleterious nitrogen response on AsA concentration at least in fruit has been attributed to an increase in plant foliage, with increasing shading and reduction of light interception by shaded fruits (Lee and Kader 2000). This implies that nitrogen fertilization has to be optimised to achieve maximal AsA concentration (Rupp and Tränkle 2000).

Mild drought stress generally appears to increase the AsA concentration of crops (Sørensen *et al.* 1995), but the situation with fruit crops is less clear. There is a suggestion that under drought stress, AsA concentration decreases in drought-sensitive plants or cultivars, but remains at higher concentrations in drought-tolerant ones, such as tomato (Shalata *et al.* 2001) and wheat (Sairam and Srivastava 2001). The AsA concentration in leeks (Sørensen *et al.* 1995) and broccoli (Toivonen *et al.* 1994) appears to be inversely correlated with rainfall or irrigation and generally increases with moderate water deficit. Intelligent use of a regulated plant water supply is now being used as an effective means of influencing vegetative growth; it can also have other benefits to agriculture/horticulture (Stoll and Jones 2001). The mechanisms by which vegetative growth is restricted can be activated by the drying of one part of the plant's root system (Davies *et al.* 2000). The ensuing effects are reduced stomatal conductance, a restriction of leaf area, and an increase in the plant's water use efficiency. Collateral effects observed in many crops are enhancement in nutritional quality of the harvestable product (fruit and seeds), with respect to increases in acids, soluble solids and sugars.

MATERIALS AND METHODS

Pot experiments

Two-year-old bushes of the blackcurrant 'Baldwin' were obtained from Hargreaves Plants Ltd, Spalding, Lincs. The bushes were potted in the winter of 2002 into 4-litre pots containing nutrient-free compost (Bulrush Peat Co.) The potted bushes were placed outside on a free draining gravel bed. Also, potted bushes of blackcurrant 'Hedda' were obtained *via* SCRI from Norway, in May 2002. In order to receive adequate chilling (requirement for more chilling than 'Baldwin'), the potted 'Hedda' bushes were placed in a +2°C dark, humid cold store in January 2003 for 4 weeks. The bushes were then re-potted into nutrient-free compost as for 'Baldwin', and placed outside on the same gravel bed.

Field experiments

The experimental site was at East Malling Research, East Malling Kent, UK (51°17'N, 00° 27'E). The soil was predominantly Malling Series, with approximately 100-200 cm sandy loam topsoil (Jarvis *et al.* 1984). The field experiments were carried out on blackcurrant bushes that were planted in a plot of seven rows, each row containing 180 bushes. The rows were 3 m apart and the bushes were 0.5 m apart within the rows. Bushes in the outer two rows were guard plants and not used in the experiments. Each row was arranged in two groups of 90 plants, one group of the cultivar 'Baldwin' and the other of cultivar 'Ben Lomond'. The two-year-old 'Baldwin' bushes were planted into the field site in December 2002 and 'Ben Lomond' bushes in November 2003. All field experiments were carried out in this planting using different bushes to carry out different experiments.

Fruit harvesting and shoot growth measurements

For all experiments, the total yield was recorded. Fruit were collected into three size classes by passing a 10-20% random subsample through sieves of >11.2, 8-11.2 and 5.6-8 mm. For each size class, the total weight of berries was determined and a subsample was taken, frozen in liquid nitrogen and stored in a freezer at -80°C, until required for subsequent AsA analysis.

Shoot growth measurements were carried out during the winters of 2004, 2005 and 2006. The number of new shoots and extension growth of new shoots produced during the previous growing season was measured.

AsA analysis

Fruit total AsA concentration (oxidised + reduced AsA) was measured by HPLC-UV. Approximately 20 g frozen weight of berries were homogenised in a blender with 10 volumes of 25 mM ammonium acetate buffer pH 4.0. The homogenate was centrifuged at 12,000 rpm in a cooled (4°C) ultracentrifuge (MSE Europa 24M) for 15 min, and the volume of the resulting supernatant was measured. 300 µl of 5% trifluoroacetic acid (TFA) (Fluka UK) and 30 µl of 500 mM of the reducing agent tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (Fluka UK) were added to a 3 ml aliquot of supernatant and left at 4°C for 30 min. 500 µl of the resulting solution was pipetted and placed into a Whatman Mini-UniPrep HPLC vial incorporating a 0.45 µm polypropylene filter. HPLC conditions were as follows: Varian Chromsep Polaris C18-A 3 µ 150 × 2 mm column, fitted with a Chromsep 10 × 2.0 mm Polaris C18-A 3 µ guard cartridge; column temperature 30°C; mobile phase 0.5% TFA in aqueous solution run isocratically with a flow rate 0.2 ml min⁻¹. The chromatographic system consisted of a Waters Alliance 2690 HPLC system connected to a Waters 996 photodiode array detector. Detection of AsA was at 245 nm, and quantitative analysis was by means of an external standard calibration curve, generated by injecting varying concentrations of an L-ascorbic acid (Aldrich UK) standard solution. This external calibration methodology follows that of Walker *et al.* (2006).

Experimental design and statistical analysis

All experiments were designed with consultation and approval of a

qualified biometrician. Treatment differences were determined using analysis of variance (Genstat software Ver. 9.1, Rothamsted Experimental Station, UK).

Manipulation of nitrogen supply

Pot experiment (2003)

An initial pot experiment was carried out to determine the effect nitrogen application rates had on fruit AsA concentration in plants of 'Baldwin' and 'Hedda'. Potted bushes were arranged in a split-plot design: 2 cultivars × 5 nitrogen treatments × 6 replicates, with 2 main plots (cultivars) within each replicate block and 5 sub-plots (nitrogen concentrations) within each main plot.

Irrigation was by means of an automatic fertigation system and five concentrations of nitrogen were applied: 19 mg l⁻¹ (N1), 131 mg l⁻¹ (N2), 243 mg l⁻¹ (N3), 467 mg l⁻¹ (N4) and 915 mg l⁻¹ (N5). All other macro- and micro-nutrients were kept constant and were fertigated at the following rates: P at 40 mg l⁻¹, K at 156 mg l⁻¹, Ca at 82 mg l⁻¹, Na at 34 mg l⁻¹, B at 0.3 mg l⁻¹, Cu at 0.1 mg l⁻¹, S at 113 mg l⁻¹, Fe at 3 mg l⁻¹, Zn at 0.7 mg l⁻¹, Mo at 0.5 mg l⁻¹ and Cl at 4 mg l⁻¹. From mid-April, two 15 min fertigation events were applied daily through 2 l h⁻¹ drippers. The pHs of the fertigation solutions, and the pH of the compost, were monitored regularly throughout the experiment.

Fruit were harvested from the bushes in July and subsequently graded and frozen as described above. The blackcurrant bushes were then harvested destructively to determine total leaf area, leaf fresh and dry weight, and dry weights of old shoots (previous season's growth), new shoots (current season's growth), and the amount of root dry matter.

Field experiments

The experiments consisted of five treatments based on a standard application rate of 76 kg ha⁻¹ as calcium ammonium nitrate, which were as follows: N1 - No fertiliser; N2 - half standard application rate (38 kg N ha⁻¹); N3 - standard application rate (76 kg N ha⁻¹); N4 - double standard application rate (152 kg N ha⁻¹); N5 - quadruple standard application rate (304 kg N ha⁻¹). A standard rate of nitrogen application (76 kg N ha⁻¹) was made, in both years, in mid-April and used throughout, a further application was applied to N4 and N5 treatments in mid May and subsequent applications to treatment N5 in mid-June and July. Experiments were carried out with 'Baldwin' and 'Ben Lomond' bushes. Each experiment had five nitrogen treatments applied in a four replicate randomised block design across 90 bushes. Each treatment was represented once in each of the four blocks. Each replicate plot contained two bushes, and plots were separated by two guard bushes. The fruit were harvested in late July and a sub-sample was frozen in liquid nitrogen and stored at -80°C prior to AsA analysis.

Manipulation of water supply

The experiment comprised of three treatments: Control - no applied irrigation; partial drying (PD) - alternate irrigation to one side of plant; and full - irrigation applied to both sides of plant. The treatments were applied in a seven-replicate complete randomised block design with each experimental plot containing three bushes. Each plot was separated from the next plot by a single guard bush. Experimental treatments were applied to the 'Baldwin' bushes in 2004 and 2005; and only in 2005 for the 'Ben Lomond' plants. Black plastic mulch was laid along the full row (i.e. including 'Baldwin' and 'Ben Lomond') in mid-April 2004. The plastic extended for 1 m from either side of the bush and edges were buried in the soil.

Year 2004

Irrigation was applied *via* boot lace drippers, positioned 10 cm from the base of each bush. From mid-May, water was applied three times a day for 10 min per event with 2 l h⁻¹ emitters. For the fully irrigated plants, two drippers, one on each side of the plant, were used. For the partial drying treatment, irrigation was initially on the West side of the bush, and was switched to the East side in

late May 2004. This approach was used to allow part of the root system to dry out and mimic soil drying. All irrigation treatments ceased in mid-September. Fruit were harvested in mid-July and a sub-sample for AsA analysis was frozen in liquid nitrogen and stored at -80°C.

Year 2005

The experimental set up was similar to that described above, with irrigation being applied three times daily for 5 min per event for 'Baldwin' and 2 min per event for the smaller 'Ben Lomond', from mid-April. For the partial drying treatment, irrigation was initially on the West side of the bush and then switched to the East side at the beginning of June. 'Baldwin' was harvested in late-July, while 'Ben Lomond' was harvested in early-August; fruit was graded as described above. A sub-sample for AsA analysis was frozen in liquid nitrogen and stored at -80°C.

Manipulation of light interception

The effect of two different reflective mulches on influencing incident radiation in the crop canopy and their subsequent effect on crop growth and AsA production was determined. The treatments used were as follows: 'None' - bare soil; 'Extenday' - reflective material (supplied by FAST Ltd, Faversham Kent); 'Solarmat' - solarisation matting (supplied by Capatex Agro-textiles, Nottingham). The 'Extenday' and 'Solarmat' were laid in 1.25 m strips on either side of each bush, abutting the edges of each side sheet under the bush, i.e. mat around the entire bush. The ground was cultivated to produce a concave trench at approximately 50 cm either side of each bush and the reflective mulch was applied directly to this contoured soil base to reflect radiation back into the canopy. The treatments were applied in seven replicate complete randomized blocks. Each experimental plot contained three bushes and was separated from the next plot by a single guard bush. The reflective mulches were laid in mid-April 2004 for 'Baldwin' bushes and left *in-situ* for two years. For 'Ben Lomond' the reflective mulches were laid in late-March 2005 and used for one growing season. 'Baldwin' fruit were harvested mid-July in both years while 'Ben Lomond' was harvested early August. Fruit was graded as described above and sub-samples frozen in liquid nitrogen and stored at -80°C for AsA analysis. Arrays of photosynthetically active radiation (PAR) sensors were used to log data (Delta-T Devices, Cambridge, UK) over three months (May to July) in 2004 and meaned.

Multi-application treatment – irrigation and reflective mulch

The most promising agronomic factors as identified above were included into a multi-combination treatment (2 × 2 factorial) experiment to determine their combined impact on fruit AsA yields. To achieve this, light reflective mulches were used in combination

with supplementary irrigation and their impact on fruit yield, AsA concentration and AsA yield per bush measured. The treatments were as follows: No mulch and no irrigation (control); No mulch with irrigation; mulch without irrigation; mulch plus irrigation. The 'Extenday' reflective mulch used was laid out in the same way as described earlier. Water was applied via automatic trickle irrigation using boot lace drippers and 2 l h⁻¹ emitters positioned 10 cm from the base of each bush, on both sides. These treatments were applied to bushes of 'Baldwin' and 'Ben Lomond' in four replicate complete randomized blocks. Each experimental plot contained three bushes. Each plot was separated from the next plot by a single guard bush.

Year 2006

Water was applied three times a day for 5 min per event from early May 2006 until the beginning of June when the time of each individual irrigation event was increased to 15 min. The fruit were harvested from all bushes in late July for 'Baldwin' and 'Ben Lomond' and were graded using the techniques outlined above. A sub-sample for AsA analysis was frozen in liquid nitrogen and stored at -80°C.

Year 2007

The experimental treatments used in 2006 were re-applied in 2007. The reflective mulches laid in 2006 remained *in-situ*. Water was applied three times a day for 10 min per event from mid April. The fruit were harvested and graded from all bushes of both cultivars in late July and sub-sample for AsA analysis was frozen in liquid nitrogen and stored at -80°C.

RESULTS

Manipulation of nitrogen supply

Pot experiment (2003)

'Hedda' bushes produced a larger crop yield than 'Baldwin' for each treatment level (Table 1), although this was not statistically significant. There was no statistically significant overall N-treatment effect on crop yield. Nitrogen-treatment had a significant effect on leaf, new shoot and root dry weight and leaf nitrogen concentration, but there were no significant differences between the values in the two cultivars. Leaf dry weight was lowest for N1 plants followed by the N2 plants and was greatest for those receiving N3 and N4. The N5 treatment resulted in a decrease in leaf dry weight compared to the N3 and N4 plants. This was confirmed with the total leaf area data which showed a similar pattern. New shoot dry weight was lowest for those plants in the N1 treatment, with those receiving treatments N2, N3 and N4 having similar shoot dry weights, and those

Table 1 The effect per blackcurrant bush of N application on dry weights of leaf, new shoot growth, old shoots, roots and on total, leaf area and fruit fresh weight, of 'Hedda' (H) and 'Baldwin' (B) after fruit harvest in July 2003.

Treatment and statistics	Dry weight (g)										Leaf area (m ²)		Leaf nitrogen (%)		Fruit fresh weight (g)	
	Leaf		New shoot		Old shoot		Roots		Total		H	B	H	B	H	B
	H	B	H	B	H	B	H	B	H	B	H	B	H	B	H	B
N1	30	25	18	6	95	56	35	44	178	132	0.46	0.60	1.65	1.87	126	123
N2	59	57	33	30	100	63	26	36	217	186	0.90	0.74	3.13	3.07	202	148
N3	68	73	36	38	86	71	38	37	228	219	1.05	1.09	3.81	3.46	228	172
N4	74	68	35	32	81	69	28	37	218	206	1.17	0.93	3.95	3.66	191	159
N5	68	59	21	23	91	50	29	22	209	155	0.92	0.89	4.30	3.80	216	137
SED																
Cultivar (df=5)	4.1		3.6		3.0		3.9		11.3		0.148		0.070		30.6	
Nitrogen (df=37)	5.3		3.9		6.1		3.7		14.5		0.111		0.118		35.0	
Nitrogen.cultivar (df=37) - (for comparisons within cultivar)	7.5		5.5		8.6		5.2		20.5		0.157		0.166		49.5	
P-value																
Cultivar	ns		ns		<0.001		ns		0.043		ns		0.04		ns	
Nitrogen	<0.001		<0.001		ns		0.004		<0.001		<0.001		<0.001		ns	
Nitrogen.cultivar	ns		ns		0.051		0.098		ns		ns		ns		ns	

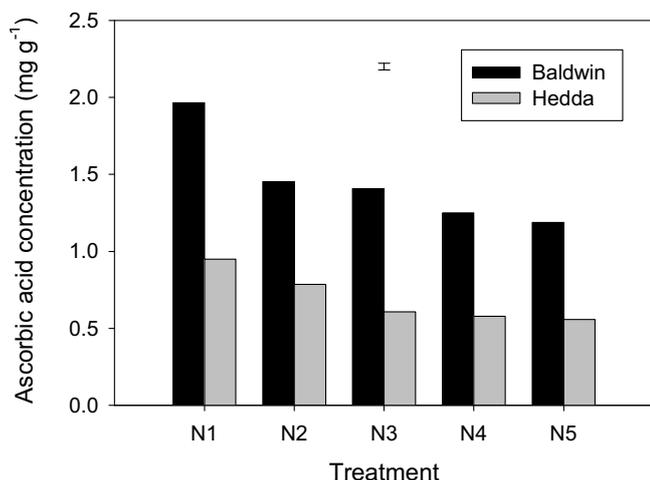


Fig. 1 The effects of different levels of applied nitrogen on AsA concentration of blackcurrant fruits >11.2 mm diameter for 'Hedda' and 'Baldwin' in a pot experiment. The vertical bar is the standard error of difference between means (SED), for comparison of treatments within cultivar, 30 degrees of freedom (d.f.).

receiving N5 having a reduced new shoot growth when compared with N2-N4. For 'Baldwin' root dry weight was lowest in the N5 plants and highest in those receiving N1 treatment. Total dry weight was lowest in N1 plants, increasing for N2 and increasing further and approximately similar, for N3 and N4, and then decreasing with N5. There was a significant cultivar effect on total dry weight. 'Hedda' had a higher dry weight than 'Baldwin'; due to the former having a greater old shoot mass.

The level of nitrogen application had a significant effect ($P < 0.001$) on AsA concentration, with AsA concentration declining as nitrogen application increased from N1-N5 (Fig. 1). Results for ripe berries of the larger size category (diameter >11.2 mm) are shown in Fig. 1. There was a significant cultivar effect ($P < 0.001$), with berries from 'Hedda' containing approximately half the AsA concentration found in 'Baldwin'.

Field experiment

Total fruit yield was not statistically different between N treatments for 'Baldwin' (Table 2). Also, fruit yields within each of the three berry size categories did not differ between nitrogen treatments. There was a (non-significant)

Table 4 The effect per blackcurrant bush of N application on growth of 'Baldwin' and 'Ben Lomond' bushes during 2005.

Treatment and statistics	'Baldwin'		'Ben Lomond'	
	№ of new shoots	Total shoot growth (cm)	№ of new shoots	Total shoot growth (cm)
N1	15	340	32	1211
N2	20	492	31	1246
N3	13	321	33	1291
N4	16	379	33	1225
N5	23	459	30	1268
SED (df=12)	2.2	99.4	2.8	116.7
P-Value	0.004	ns	ns	ns

difference in the AsA concentration of berries in the >11.2 mm size category with N5 treatment. When calculating the yield of AsA per bush (AsA concentration × fruit yield) there was a slight trend for AsA yield to decrease with increased nitrogen application, with the N1 bushes having 50% more AsA than N5 bushes.

For 'Ben Lomond', total fruit yields, and the yields in each size category, did not differ between nitrogen treatments (Table 3). The majority of berries were in the large size category. Fruit AsA concentration of berries >11.2 mm showed some statistically significant differences with a tendency for concentration to decline with increasing nitrogen concentration, but the pattern was unclear. Total bush AsA was lowest (not significant) for the N5 bushes and levels were similar in the N1-N4 bushes. Total shoot growth for 'Baldwin' and 'Ben Lomond' bushes did not differ between the nitrogen treatments in 2005, but the number of new shoots showed statistically significant differences for 'Baldwin' (Table 4).

Manipulation of water supply

Year 2004

The amount of irrigation applied to bushes of 'Baldwin' had no effect on total harvest yield per bush, and there was very little difference in the yield of berries within each size class (Table 5). However, bushes that received the partial drying treatment and full irrigation had a higher proportion of berries in the largest size class (>11.2 mm diameter), with those not irrigated having a higher proportion of fruit in the 8-<11.2 mm size category. AsA concentration was greatest in the berries within size class 8-<11.2 mm diameter and similar for the larger (>11.2 mm) and smaller (5.6-<8 mm)

Table 2 The effect per blackcurrant bush of N application on fruit yield in each of three size classes (mm) and on total fruit yield, AsA concentration (mg g⁻¹ fresh weight) and total AsA yield for 'Baldwin' in 2005.

Treatment and statistics	Fruit yield (g)			Total	AsA concentration (mg g ⁻¹)		Total AsA (g)
	>11.2	8-<11.2	5.6-<8		>11.2	8-<11.2	
N1	329	1050	156	1532	2.25	2.96	4.17
N2	303	865	108	1276	2.22	2.47	3.07
N3	152	1064	220	1436	2.07	2.59	3.65
N4	156	1115	232	1503	2.12	2.52	3.73
N5	282	793	158	1233	1.87	2.32	2.77
SED (df=12)	102	127	51	171.1	0.120	0.307	0.381
P-Value	ns	ns	ns	ns	0.055	ns	0.01

Table 3 The effect per blackcurrant bush of N application on fruit yield in each of two size classes (mm) and on total fruit yield, AsA concentration (mg g⁻¹ fresh weight) and total AsA yield for 'Ben Lomond' bushes in 2005.

Treatment and statistics	Fruit yield (g)		Total	AsA concentration (mg g ⁻¹)		Total AsA (g)
	>11.2	8-<11.2		>11.2	8-<11.2	
N1	225	35	260	1.80	1.92	0.47
N2	238	45	283	1.76	2.06	0.49
N3	290	52	343	1.51	2.07	0.50
N4	225	50	275	1.75	2.13	0.48
N5	196	29	225	1.64	2.06	0.37
SED (df=12)	41.7	14.3	49.6	0.077	0.1177	0.084
P-Value	ns	ns	ns	0.005	ns	ns

Table 5 The effect per blackcurrant bush of different methods of irrigation on fruit yield in each of three size classes (mm) and on total fruit yield, AsA concentration (mg g⁻¹ fresh weight) and total AsA yield for 'Baldwin' in 2004 and 2005.

Year	Treatment and statistics	Fruit yield (g)			Total (g)	AsA concentration (mg g ⁻¹)			Total AsA (g)
		>11.2	8-<11.2	5.6-<8		>11.2	8-<11.2	5.6-<8	
2004	None	441	637	26	1105	2.19	2.53	2.14	2.6
	PD	540	596	19	1155	2.14	2.55	1.93	2.7
	Full	572	620	22	1215	2.21	2.54	2.09	2.9
	SED (df=12)	65.8	51.0	2.37	103.6	0.048	0.054	0.069	0.27
	P-Value	ns	ns	0.047	ns	ns	ns	0.026	ns
2005	None	410	1273	138	1821	2.06	2.35	2.42	4.1
	PD	484	1685	220	2390	2.13	2.42	2.26	5.6
	Full	497	1779	260	2536	2.17	2.38	2.33	5.9
	SED (df=12)	74.7	113.5	30.2	154.5	0.060	0.050	0.099	0.37
	P-Value	ns	<0.001	<0.001	<0.001	ns	ns	ns	<0.001

PD = partial drying

Table 6 The effect of different methods of irrigation on new shoot and total shoot growth of 'Baldwin' bushes during 2004 and 2005.

Treatment and statistics	2004		2005	
	Nº of new shoots	Total shoot growth (cm)	Nº of new shoots	Total shoot growth (cm)
None	23	892	45	1828
Partial drying	24	1200	48	1911
Full	25	1257	46	1935
SED (df=12)	1.5	64.2	2.7	131.8
P-Value	ns	<0.001	ns	ns

berries. The different methods of irrigation did not influence AsA concentration for the berries 5.6-<8 mm diameter, but partial drying reduced the concentration compared to full irrigation or no irrigation for the smallest berries (5-8 mm diameter). The method of irrigation had no effect on AsA yield per bush. Full or partial irrigation had a significant effect on the growth of the bushes; total shoot length was increased by both irrigation treatments (Table 6). Total shoot lengths per bush were 41 and 35% greater for those receiving full and partial irrigation respectively, compared to those not receiving irrigation.

Year 2005

The total fruit yield of 'Baldwin' bushes grown under full or partial drying was significantly increased when compared to those bushes not receiving any irrigation; particularly so for the berries in the 8-<11.2 and 5.8-<8 mm size categories (Table 5). This was probably a consequence of increased shoot growth in the irrigated bushes during the previous year. Irrigation had no impact on 'Baldwin' fruit AsA concentration for any of the berry size categories, but the yield of AsA per bush increased by approximately 25% with irrigation, full or partial drying. This difference in AsA yield can be attributed to an increase in berry yield as AsA concentration did not differ between the treatments.

Total shoot growth of 'Baldwin' bushes was greater in 2005 than in 2004, but there was no difference between irrigation treatments (Table 6). The total fruit yield of 'Ben Lomond' bushes did not differ between the irrigated and non-irrigated treatments, and neither did the yield in each of

Table 8 The effect per blackcurrant bush of different methods of irrigation on new shoot and total shoot growth of 'Ben Lomond' during 2005.

Treatment and statistics	Nº of new shoots	Total shoot growth (cm)
None	20	530
Partial drying	26	609
Full	22	550
SED (df=12)	2.7	67.8
P-Value	ns	ns.

the two size categories (Table 7). Fruit AsA concentration and total AsA yield per bush did not differ between the treatments. Irrigation did not affect the amount of shoot growth produced by the 'Ben Lomond' bushes (Table 8).

Manipulation of light interception

Year 2004

Measurements using light sensors showed that 'Extenday' reflected 37% of the PAR hitting the ground covered by the mulch, while the 'Solarmat' reflected 19% and bare earth reflected 9% (Fig 2). There were no differences in air temperatures recorded within the bushes, at two different heights, which could be attributed to mulching. There were small differences in soil temperatures, with the mulch reducing temperatures compared to bare soil (Fig. 2).

Total bush shoot length was increased by 'Extenday' and 'Solarmat' by 30 and 16%, respectively, when compared to the bare soil treatment. Total harvest yield in 'Baldwin' was 4% greater for bushes planted into 'Extenday' and 11% greater for those in 'Solarmat' than for those in bare soil (Table 9). However, these effects were not statistically significant. The reflective mulches increased the growth of the bushes, most likely due to the increased radiation intercepted by the leaf canopy (Table 10). This was due to an increase in the average shoot length; there was no increase in the number of new shoots.

Year 2005

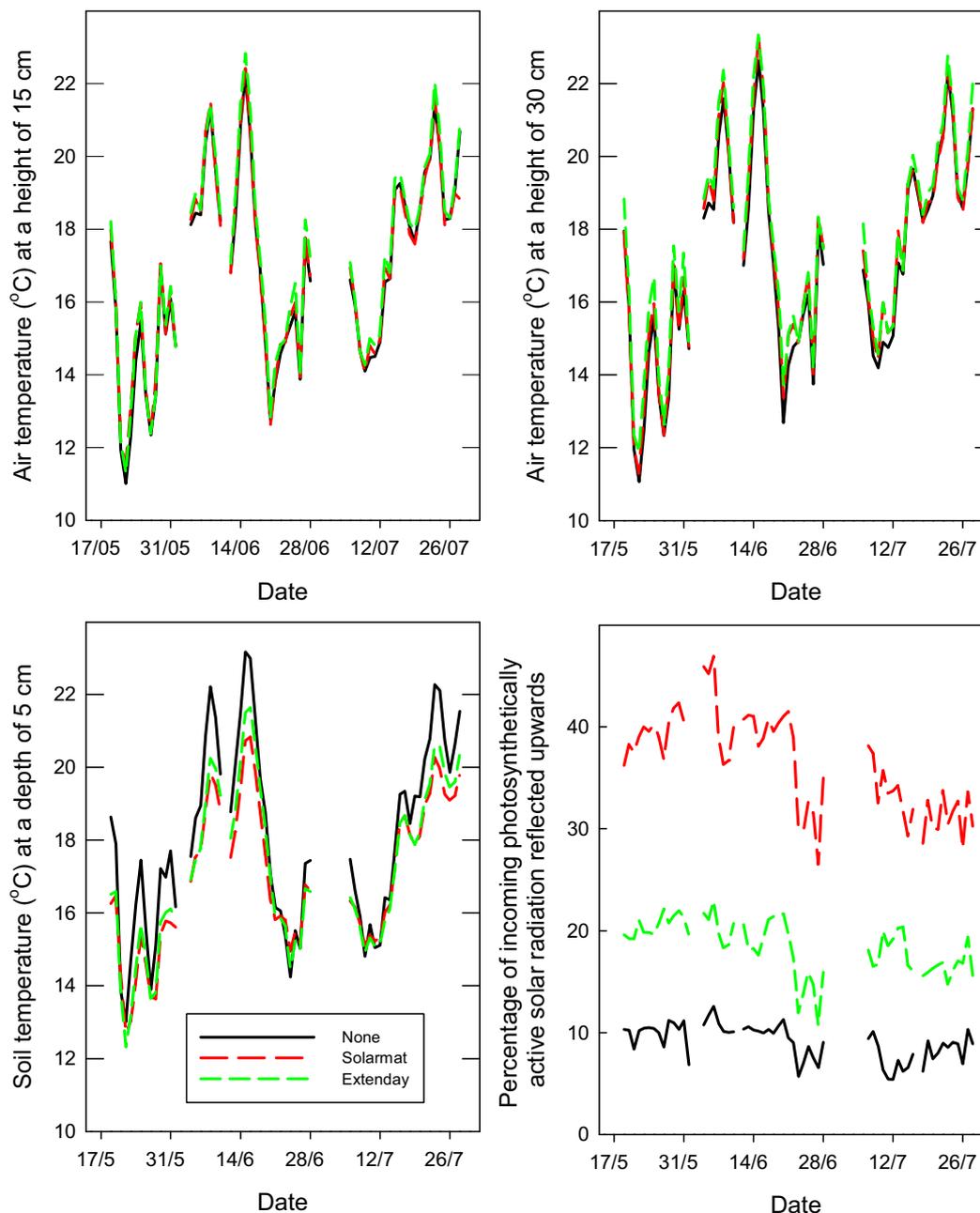
Both reflective mulches increased total yields by approximately 15% (Table 9) but fruit AsA concentration was not

Table 7 The effect per blackcurrant bush of different methods of irrigation on fruit yield in each of two size classes (mm) and on total fruit yield, AsA concentration (mg g⁻¹ fresh weight) and total AsA yield for 'Ben Lomond' in 2005.

Treatment and statistics	Fruit yield (g)			Total	AsA concentration (mg g ⁻¹)		Total AsA (g)
	>11.2	8-<11.2			>11.2	8-<11.2	
None	502	70		572	1.70	1.85	0.98
Partial drying	532	64		597	1.60	1.78	0.97
Full	504	56		560	1.57	1.78	0.89
SED (df=12)	64.9	11.0		71.8	0.058	0.107	0.114
P-Value	ns	ns		ns	ns	ns	ns

Table 9 The effect per blackcurrant bush of reflective mulch on fruit yield in each of three size classes (mm) and on total fruit yield, AsA concentration (mg g^{-1} fresh weight) and total AsA yield for 'Baldwin' in 2004 and 2005.

Year	Treatment and statistics	Fruit yield (g)			Total	AsA concentration (mg g^{-1})			Total AsA (g)
		>11.2	8-<11.2	5.6-<8		>11.2	8-<11.2	5.6-<8	
2004	No mulch	363	569	25	957	2.32	2.77	2.22	2.5
	'Extenday'	401	568	24	993	2.40	2.80	2.89	2.6
	'Solarmat'	362	665	32	1059	2.43	2.89	2.17	2.9
	SED (df=12)	37.4	71.6	4.8	88.5	0.047	0.054	0.064	0.230
	P-Value	ns	ns	ns	ns	ns	ns	ns	ns
2005	No mulch	245	1088	193	1526	2.51	2.57		3.91
	'Extenday'	256	1190	342	1787	2.33	2.74		4.83
	'Solarmat'	235	1248	245	1729	2.39	2.70		4.53
	SED (df=12)	45.9	98.2	51.0	115.3	0.093	0.082		0.288
	P-Value	ns	ns	0.019	0.072	ns	ns		0.01

**Fig. 2** The effects of different light reflective plastic mulches on mean air temperature in blackcurrant bushes at heights of 15 and 30 cm from ground level, and mean soil temperatures recorded at a depth of 5 cm under the mulches. The amount of photosynthetically active radiation being reflected by the mulches is also shown relative to incident radiation.

affected by the reflective mulches. However, the total yield of AsA per bush (AsA concentration \times fruit yield) was significantly increased through the use of reflective mulches, with 'Extenday' increasing ascorbic acid yield by 23% and

'Solarmat' by 15%. Shoot growth at the end of the second growing season was about half of the previous years growth, and unlike the previous year there was no significant increase in shoot growth from use of reflective mulch

Table 10 The effect per blackcurrant bush of reflective mulch on new shoot and total shoot growth of 'Baldwin' during 2004 and 2005.

Treatment and statistics	2004		2005	
	№ of new shoots	Total shoot growth (cm)	№ of new shoots	Total shoot growth (cm)
No mulch	25	780	15	323
'Extenday'	28	1015	19	413
'Solarmat'	26	908	18	408
SED (df=12)	2.0	72.7	2.1	46.4
P-Value	ns	0.024	ns	ns

Table 12 The effect per blackcurrant bush of reflective mulch on new shoot and total shoot growth of 'Ben Lomond' during 2005.

Treatment and statistics	№ of new shoots	Total shoot growth (cm)
No mulch	27	1294
'Extenday'	22	1632
'Solarmat'	20	1403
SED (df=12)	2.3	94.2
P-Value	0.022	0.011

Table 11 The effect per blackcurrant bush of reflective mulch on fruit yield in each of two size classes (mm) and on total fruit yield, AsA concentration (mg g⁻¹ fresh weight) and total AsA yield for 'Ben Lomond' in 2005.

Treatment and statistics	Fruit yield (g)			Total	AsA concentration (mg g ⁻¹)		Total AsA (g)
	>11.2	8-<11.2			>11.2	8-<11.2	
No mulch	321	76	398	1.84	1.92	0.61	
'Extenday'	341	69	410	2.02	1.90	0.64	
'Solarmat'	375	94	478	1.93	1.93	0.67	
SED (df=12)	46.5	18.3	56.3	0.078	0.089	0.094	
P-Value	ns	ns	ns	ns	Ns	ns	

(Table 10).

The use of reflective mulch did not have any effect on the total fruit yield of 'Ben Lomond' bushes, or the yield in the two size classes (Table 11). Fruit AsA concentration and total AsA per bush (AsA concentration × fruit yield) was also unaffected by use of reflective mulch. Shoot growth was increased by the use of 'Extenday' reflective mulch; bushes in this treatment had 26% more growth than those in the no mulch treatment (Table 12). The bare soil treatment produced more shoots than the reflective mulch treatment; however the average length per shoot was significantly reduced (not tabulated).

Multi-application treatment – irrigation and reflective mulch

Year 2006

In the first year irrigation and reflective mulching were applied together, total fruit yield of 'Baldwin' was unaffected (Table 13). There was no significant difference in yield of berries of size >11.2 or 8-<11.2 mm, although non-irrigated

bushes had significantly higher berry yield in the smallest size categories. Irrigated bushes had a higher proportion of berries in the largest size category compared to those not irrigated. Mulched bushes also had a higher proportion of berries in the largest berry size category. Mulching and irrigation had no effect on fruit AsA concentration or total fruit AsA yield per bush. Shoot growth was increased with the use of irrigation (Table 15). Mulch increased shoot growth of the bushes, but this effect was not significant. Most shoot growth was found in those bushes receiving irrigation and mulch, but the number of new shoots was unaffected by irrigation or mulch.

Total fruit yield in 'Ben Lomond' increased with irrigation, irrespective of mulch (Table 14). The benefits of irrigation were apparent with increased fruit yield in the larger size category for 'Ben Lomond'. Fruit AsA concentration of berries >11.2 mm was not affected by irrigation and mulch; however for berries of size 8-<11.2 mm there was a reduction in AsA concentration for those grown with mulch. Irrigation of 'Ben Lomond' showed a positive trend of increasing total AsA yield per bush, however this was not statistically significant. Total shoot growth and the number

Table 13 The effect per blackcurrant bush of a combination of reflective mulch and irrigation on fruit yield in each of two size classes (mm) total fruit yield, AsA concentration (mg g⁻¹ fresh weight) and total AsA yield for 'Baldwin' in 2006 and 2007. Treatment key: -M-I - no mulch and no irrigation (control), -M+I - no mulch with irrigation, +M-I - mulch without irrigation, +M+I - mulch plus irrigation.

Year	Treatment and statistics	Fruit yield (g)			Total	AsA concentration (mg g ⁻¹)		Total AsA (g)
		>11.2	8-<11.2	5.6-<8		>11.2	8-<11.2	
2006	-M-I	656	670	68	1393	1.48	1.72	2.23
	-M+I	770	493	36	1298	1.52	1.72	2.07
	+M-I	795	526	38	1359	1.48	1.74	2.17
	+M+I	991	469	34	1493	1.37	1.67	2.21
	SED (df=9)							
	Mulch or irrigation	133.3	84.5	7.6	199.4	0.048	0.050	0.321
	Mulch.irrigation	188.5	119.2	24.1	282.0	0.067	0.070	0.454
2007	Mulch	ns	ns	0.069	ns	ns	ns	ns
	Irrigation	ns	ns	0.04	ns	ns	ns	ns
	Mulch.irrigation	ns	ns	ns	ns	ns	ns	ns
	-M-I	646	714	80	1439	3.23	3.63	4.9
	-M+I	668	783	106	1556	3.23	3.56	5.3
	+M-I	932	956	141	2029	3.19	3.03	6.3
	+M+I	980	1184	185	2348	3.16	3.46	7.8
2007	SED (df=9)							
	Mulch or irrigation	140.9	100.7	14.2	241.5	0.072	0.252	0.76
	Mulch.irrigation	450.8	142.4	45.4	341.5	0.102	0.357	1.09
2007	Mulch	ns	ns	0.036	ns	ns	ns	ns
	Irrigation	0.063	0.011	<0.001	0.019	ns	ns	0.033
	Mulch.irrigation	ns	ns	ns	ns	ns	ns	ns

Table 14 The effect per blackcurrant bush of a combination of reflective mulch and irrigation on fruit yield in each of two size classes (mm) total fruit yield AsA concentration (mg g⁻¹ fresh weight) and total AsA yield for 'Ben Lomond' in 2006 and 2007. Treatment key: -M-I - no mulch and no irrigation (control), -M+I - no mulch with irrigation, +M-I - mulch without irrigation, +M+I - mulch plus irrigation.

Year	Treatment and statistics	Fruit yield (g)			Total	AsA concentration (mg g ⁻¹)		Total AsA (g)
		>11.2	8-<11.2	5.6-<8		>11.2	8-<11.2	
2006	-M-I	1077	521	20	1619	1.48	1.62	2.47
	-M+I	1220	438	16	1674	1.49	1.54	2.53
	+M-I	1384	385	11	1780	1.49	1.59	2.70
	+M+I	1577	389	15	1980	1.33	1.46	2.71
	SED (df=9)							
	Mulch or irrigation	84.9	28.1	2.23	106.7	0.046	0.031	0.203
	Mulch.irrigation	120.1	39.7	3.15	150.9	0.064	0.044	0.287
	P-value							
	Mulch	0.08	ns	ns	ns	ns	0.007	ns
	Irrigation	0.004	0.009	0.036	0.057	ns	ns	ns
Mulch.irrigation	ns	ns	ns	ns	ns	ns	ns	
2007	-M-I	1475	199	2	1676	3.08	3.56	5.25
	-M+I	1600	256	4	1860	3.15	3.48	5.89
	+M-I	1383	230	5	1618	3.12	3.29	5.09
	+M+I	1667	312	5	1984	3.17	3.44	6.36
	SED (df=9)							
	Mulch or irrigation	118.6	27.3	0.9	126.6	0.097	0.136	0.417
	Mulch.irrigation	167.7	38.6	1.3	179.0	0.138	0.193	0.589
	P-value							
	Mulch	ns	0.031	ns	0.058	ns	ns	0.047
	Irrigation	ns	ns	0.043	ns	ns	ns	ns
Mulch.irrigation	ns	ns	ns	ns	ns	ns	ns	

Table 15 The effect per blackcurrant bush of a combination of reflective mulch and irrigation on new shoot and total shoot growth for 'Baldwin' and 'Ben Lomond' in 2006. Treatment key: -M-I - no mulch and no irrigation (control), -M+I - no mulch with irrigation, +M-I - mulch without irrigation, +M+I - mulch plus irrigation.

Treatment and statistics	'Baldwin'		'Ben Lomond'	
	N ^o of new shoots	Total shoot growth (cm)	N ^o of new shoots	Total shoot growth (cm)
-M-I	37	73	62	117
-M+I	40	93	70	138
+M-I	42	103	57	107
+M+I	49	128	65	143
SED (df=9)				
Mulch or irrigation	5.1	13.4	2.4	7.0
Mulch.irrigation	7.2	18.9	3.5	9.9
P-value				
Mulch	ns	ns	0.008	0.03
Irrigation	ns	0.040	0.049	ns
Mulch.irrigation	ns	ns	ns	ns

of new shoots were increased by the mulch treatments (Table 15), but irrigation did not increase shoot growth.

Year 2007

Total fruit yield and fruit yield in each of the three fruit size classes for 'Baldwin' bushes was positively influenced by irrigation (Table 13). Whilst mulch did increase total fruit yield for irrigated and non-irrigated bushes the effect was not statistically significant. However, those bushes receiving irrigation and mulch had the greatest fruit yield and harvested 63% more fruit than those receiving the control treatment. The proportion of fruit in each size category did not differ between the treatments. Neither mulch nor irrigation had any effect on fruit AsA concentration, but total AsA yield per bush was significantly increased by irrigation. Mulch increased yield of AsA but the effect was not statistically different. The bushes receiving mulch and irrigation together yielded the greatest quantity of AsA, an increase of 60% when compared to the control bushes.

Total fruit yield of 'Ben Lomond' was increased by use of reflective mulch when in combination with irrigation

(Table 14). Irrigation did not significantly increase total yield, but yields were 10% higher in bushes that received mulch and irrigation, compared to bushes that received neither treatment. Fruit AsA concentration was not affected by irrigation or mulching. The total yield of AsA per bush was increased by the use of mulch. The greatest yield of AsA occurred in bushes that received mulch and irrigation, being 21% greater than in those not receiving either treatment.

DISCUSSION

The success with which we have been able to manipulate AsA has been achieved predominantly in two ways. Firstly, to simply use measures of fruit AsA to determine if actual fruit AsA concentration increases in response to treatment. Knowledge, when this work began, of fruit AsA biosynthesis was limited for perennial fruit crops. However, the established fundamental biological regulatory role of AsA, and redox systems in general, in plants led us to hypothesise that elevating fruit AsA concentration would not be simple. Our second approach was to determine if treatments also impact on the yield of fruit per plant (bush). This is important with respect to the economics of commercial fruit production. For any practical science-based development to be taken up commercially requires that the users are confident in being able to apply the technology and that it is financially beneficial. Other concerns such as the environmental impacts, food production standards and legislation, along with consumer requirements, should also be considered. To determine if our treatments increased yield of AsA at the bush level (or yield per unit land area) we have combined measures of fruit AsA concentration (mg g⁻¹ fruit) with those of fruit yield (g bush⁻¹) to obtain AsA yield per bush (mg AsA bush⁻¹).

Manipulating plant nitrogen supply

Initial pot experiments with 'Hedda' and 'Baldwin' showed a very clear response to increases in applied nitrogen. Destructive leaf tissue analysis confirmed that nitrogen uptake changed with nitrogen application up to an optimum. Field-based studies supported the notion that at a supply rate greater than 152 kg N ha⁻¹, there was little further agronomic value (particularly yield gain) of adding further nit-

rogen. Importantly, fruit size also increased with plant nitrogen status. But this appeared to be at the expense of fruit AsA concentration. This conclusion was consistent with respect to cultivars with known differences in AsA production, i.e. 'Hedda' and 'Baldwin', low and high ($\times 2$) AsA concentrations, respectively (Viola *et al.* 2000). Both cultivars responded similarly to increasing nitrogen concentration. A decline in AsA concentration suggests a possible antagonistic interaction between plant nitrogen status and AsA biosynthesis/catabolism. The interlinking of carbon and nitrogen metabolism beyond the requirements for growth and development is well known (Van Dam *et al.* 1996; Fritz *et al.* 2006).

Another explanation might be that nitrogen supply acts through the control of growth which dilutes cellular contents. Enhanced nitrogen application is documented indirectly encouraging cell expansion, increases in leaf area and canopy development. Vacuolar sequestration of nitrate appears to be the most likely mechanism driving leaf expansion (Millard 1988). With monocots leaf elongation rate induced by increased nitrogen supply extends the cell division phase yielding increases in leaf mesophyll cell number accompanied by a 'compensatory' increase in epidermal cell length (MacAdam *et al.* 1989). With dicots the effect is dependent on the timing of nitrogen enhancement in relation to leaf development stage. Once the cellular complement of a leaf is achieved (pre-determined) nitrogen status only influences leaf cell size and not cell number (Roggatz *et al.* 1999). The decline in fruit AsA concentration and the link with an increase in mean fruit size could be simply explained by this growth dilution response.

There may be other response explanations, where an over-abundant nitrogen supply has been shown to lead to excessive vegetative growth (particularly leaf canopy); this in itself may cause direct competition with fruit for photoassimilates, which indirectly through shading the fruit, causes a decline in its sink strength (Lee and Kader 2000; Ma and Cheng 2003). Using nitrogen at 'excessive' levels promoted bush growth, particularly leaf canopy development. An explanation of this may be that increased shoot and leaf production may cause an increased shading of leaves close to the developing fruit (reduced photosynthesis), as well as shading the fruit itself. This may reduce the carbohydrate available for fruit growth and or the substrates for AsA biosynthesis and leaf chlorophyll. Fruit shading may be a disadvantage if AsA production occurs, or requires photoassimilates, or precursors manufactured in the fruit (as Richardson *et al.* 2004 suggests). There is a wealth of knowledge describing the positive impacts of illumination (light intensity) directly on tomato fruit quality and bioactives (Gautier *et al.* 2009). This may not be a universal picture, particularly relevant to blackcurrant. There is, however, good evidence that a number of fruit secondary metabolites, which act as plant stress protectors (particularly as antioxidants), have enhanced biosynthesis when these organs are illuminated (Loughrin and Kasperbauer 2001; Davey *et al.* 2004; Atkinson *et al.* 2006; Fonseca 2008; Gautier *et al.* 2009).

Nitrogen availability has been shown to modify the behaviour of phenylalanine ammonia lyase (PAL), a key enzyme in the regulatory synthesis of plant phenols. High applications rates of nitrogen reduced flavonoid accumulation in apple fruit, apparently through a down regulation of PAL activity (Strissel *et al.* 2005). While nitrogen deficiency enhanced PAL activity (Kováčik and Bačkor 2007) and increased concentrations of several benzoic and cinnamic acids (Kováčik *et al.* 2007). A link between plant nitrogen status and the production of other secondary metabolites (such as artemisinin), from assimilated carbon, has recently been proposed (Davies *et al.* 2009). Flavonoid biosynthesis, *via* the shikimate pathway, also appears to be negatively responsive to nitrogen enhancement with evidence of gene regulation and molecular links to other stress inducing factors such as high light intensity and carbohydrate shortage (Lillo *et al.* 2008).

In general, the situation with our field-based experiments was not entirely clear, with respect to showing a definitive optimal nitrogen concentration and a clear negative influence on fruit AsA concentration. The most likely reason for this lack of clarity is that Malling soils due to their long history of cultivation and predicted nitrogen mineralization rates (50 to 70 kg ha⁻¹, Greenham 1976) were more than adequate to disguise deficiency through limited field experimental application rates compared to our early pot experiments. However our longer-term field experiment (Year 4, 2005) began to show signs of nitrogen deficiency in the larger bushes of 'Baldwin' which received suboptimal nitrogen fertilisation for at least two years. Here a small but significant negative impact on fruit AsA concentration was apparent.

Manipulation of water supply

Drought-induced responses impact on an extensive range of anatomical, physiological, biochemical and molecular processes in plants (see review of Reddy *et al.* 2004). It appears that when soil dries, roots respond to diminishing availability of water by altering various aspects of shoot behaviour. One of the key responses is stomatal closure which restricts transpiration rate and therefore plant water use and the full expression of tissue stress levels. This response is regulated by chemical messengers that are synthesised in the roots in response to soil drying and can be quantitatively linked to stress intensity (Davies and Zhang 1991). These root-sourced regulators also restrict shoot development and this has led to the suggestion that it may be possible to exploit these chemical signals, during drought stress, to regulate vegetative growth (Dry *et al.* 1996). We hypothesised that these reductions in shoot growth may be beneficial due to the redirection (decline in shoot 'sink' demand) of photoassimilates into reproductive growth (fruit production) at the expense of the shoot. There may also be added benefits with respect to enhancement of fruit bioactives, such as AsA (Dodds 2008). The very nature of the osmotic stress incurred during drought is likely to promote the accumulation of ions and organic solutes in organs such as fruits. These osmolytes protect tissue against reactive oxygen species (ROS), but this is not their primary function. Experiments with grape show that vine water use efficiency increased, whilst shoot vigour was significantly reduced without a yield penalty, there were also significant improvements in fruit quality (enhanced sugar, aroma, flavour and bioactives within fruit). This has been achieved by growing systems which deliver irrigation in a prescribed manner often to only part of the plant's root system (see Cameron *et al.* 2006).

Drought stress was expected to influence fruit AsA concentration as amount of AsA per berry remains relatively constant during blackcurrant fruit development, but the concentration declines as berry size increases during fruit swelling and maturation (Viola *et al.* 2000). Our attempts to regulate bush water supply to plants growing in the field-planting using supplementary irrigation and alternate irrigation impacted on vegetative shoot growth. Bushes not receiving full irrigation had reduced shoot growth. We would assume that field-grown bushes would be much less likely to suffer root restriction effects on shoot growth than pot-grown bushes. As these experiments were continued over more than one growing season it became apparent that, at least with 'Baldwin', the amount of water used to irrigate the field-crop had a positive impact on fruit yield. Ostermann and Hansen (1988) showed that shoot growth was a key component in determining the yield of blackcurrants under drip irrigation, particularly in dry years. Irrigation has often been identified to be a critical component of fruit yield in other perennial crops, at least in part because the impact of drought on yield can be apparent during the actual stress event, as well as in the year after drought, if the formation and production of flower buds has been reduced (Wilson and Jones 1980). To fully describe the implications

of drought and the benefits of irrigation supplementation, requires several seasons of experimentation in the field.

Manipulation of light interception

It is well understood how enhanced interception of solar radiation (PAR), by leaves, enhances plant growth and crop yield by driving the photosynthetic process. This may be particularly relevant as fruit AsA is derived directly from the conversion of D-glucose (Hancock and Viola 2005). It is also apparent that solar radiation, and in particular that which causes photo-damage, such as UV, may influence the accumulation of antioxidants which provide tissue protection against ROS (Saure 1990). This notion is well developed for polyphenol synthesis such as the anthocyanins produced by many fruits. Functional redox coupling between phenolics, AsA and glutathione have been proposed and these link environmental stress responses, secondary metabolites and nitrogen nutrition (Yamasaki and Grace 1998; Blokhina *et al.* 2003). Our aims were to maximise bush solar radiation interception by reflecting solar radiation back into the leaf canopy using reflective polythene mulches on the soil surface below the leaf canopy. We were able to show quantitatively that this approach was effective in enhancing canopy radiation interception. Comparative seasonal measurements of the environment around the bushes showed that the reflective mulch primarily acted through enhanced PAR, not *via* increases in air, or soil temperatures. These reflective mulches increased canopy growth by significant amounts within the first growing season but did not significantly increase fruit yields, with no influence on fruit AsA concentration, or total bush yield of AsA. Longer-term field-based experiments with reflective mulches showed that shoot growth increased, but yield increases were relatively small irrespective of cultivars and there was no elevation of fruit AsA concentration or AsA yield per bush.

Combining irrigation with the use of a reflective soil mulch

Field-based experiments generally did reveal positive treatment effects in the short-term (single season). Our knowledge of perennial fruit research supports the need for experimental evaluation that not only takes into account the year-to-year climatic variation, but also crop development and the seasonal accumulation of plant developmental changes and crop management. Supplementary irrigation, for example, may only be beneficial in years when natural precipitation is low, but irrigation benefits can be cumulative as was shown in the irrigation studies. Our aim from the outset was to carry out field-based experiments to measure the impacts of selected agronomic manipulation over several seasons of growth and cropping, as well as a combination of treatments to evaluate additive effects. This approach may also provide a new strategy for the industry wishing to improve current growing systems. A combination of supplementary irrigation and light reflective soil mulches has been shown, in the field, to significantly enhance fruit yields. The impact on fruit AsA concentration was not as dramatic, but this combination of treatments has increased the total yield of AsA per bush for 'Baldwin'. This is an important achievement given the robust nature with which AsA concentration appears to be regulated biochemically in blackcurrant fruit (Hancock and Atkinson 2007; Hancock *et al.* 2007).

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