# L-Ascorbic Acid Accumulation in Blackcurrant Fruit (*Ribes nigrum* L.)

**Paul G. Walker[1], Simon D.A. Pont[1], Roberto Viola[1], Robert D. Hancock[1]**

[1] Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA. United Kingdom.
[2] IASMA, Via E. Mach 1, I-38010 San Michele all'Adige, Trento, Italy.
Rob.Hancock@scri.ac.uk

Scottish Crop Research Institute

## Introduction

In recent years significant advances have been made regarding the metabolism of L-ascorbic acid (AsA) in plants. Evidence has been presented for the existence of several biosynthetic pathways and more details regarding AsA recycling and degradation have been elucidated[1]. In addition, details regarding the biochemical and genetic control of biosynthetic pathway flux are beginning to emerge.

However, many of the studies undertaken to date have used photosynthetic tissues and with the exception of apples[2], much less is known regarding the accumulation of AsA in heterotrophic tissues such as tubers and fruits. In the present study the biochemical, physiological and environmental factors affecting AsA accumulation in blackcurrant fruit were examined. Based on current data, a model for AsA accumulation is presented.

## Materials and Methods

### Plant Material and Growth Conditions

All blackcurrant genotypes were grown outdoors at the Scottish Crop Research Institute, Dundee, Scotland (56°27'N, 3°04'W) and subjected to standard industry fertilisation and pest control regimes. The genotypes used in the biochemical studies; Hedda, Baldwin and 8982-6 contained an average of 0.71 ± 0.21, 1.96 ± 0.09 and 2.58 ± 0.25 mg AsA $gFW^{-1}$ in ripe fruit. Fruit stages were defined as green 4-8 mm (1), green 8-12 mm (2), green-red (3), red-green (4), red (5) and black (6).

### Extraction and Quantification of AsA and Sugars

Blackcurrant fruit were ground to a powder in liquid $N_2$ and extracted in 5% metaphosphoric acid containing 5 mM TCEP[3]. AsA was quantified by HPLC using a cation interaction column[4].
Sugars were extracted from powdered blackcurrant fruit in 20 volumes of 70% ethanol at 80°C for 1 h. After the removal of ethanol under reduced pressure, sugars were quantified by strong anion exchange HPLC with pulsed amperometric detection[5].

### Measurement of AsA Biosynthesis and Turnover

AsA biosynthesis was measured in detached, bisected flowers or fruit using [U-$^{14}$C]mannose as a substrate as described[6] with the exception that the buffer solution consisted of 50 mM MES pH 6.5, 300 mM mannitol. AsA turnover was measured after incubation of bisected flowers or fruit with [1-$^{14}$C]AsA for 2 h followed by a chase of 24 h. In both cases [$^{14}$C]AsA was extracted in 5% perchloric acid and quantified by radio-HPLC[6].

## Results

### L-Ascorbic Acid is Accumulated Early in Fruit Development

Flowers and fruit of 3 blackcurrant genotypes were harvested throughout development and fruit fresh weight and AsA concentration recorded. The data presented in Fig. 1 shows that all cultivars, regardless of ripe fruit AsA concentration, showed the same pattern of accumulation which occurred only during fruit expansion and had ceased by the time fruit reached stage 3. Although 8982-6 had a higher AsA concentration in ripe fruit, Baldwin fruit contained more AsA per berry due to their larger fruit size.

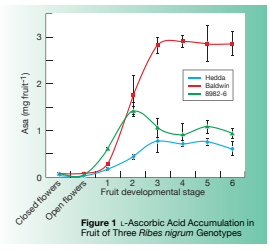



**Figure 1** L-Ascorbic Acid Accumulation in Fruit of Three *Ribes nigrum* Genotypes

### L-Ascorbic Acid Accumulation is Associated with Biosynthetic Capacity

In order to determine if fruit biosynthesis was responsible for AsA accumulation, the incorporation of [U-$^{14}$C]mannose (an intermediate of the *de novo* pathway) into [$^{14}$C]AsA was quantified throughout fruit development. Biosynthetic capacity was highest in flowers and remained high during fruit expansion (stages 1-2) but rapidly declined as fruit ripened further (stages 3-6; Fig. 2). In addition to the developmental correlation between biosynthetic capacity and AsA accumulation, there was also a correlation between biosynthetic capacity in expanding fruit (stages 1-2) and ripe fruit AsA content between genotypes. These data were interpreted to suggest that the major source of fruit AsA is *in situ* synthesis from imported sugars.

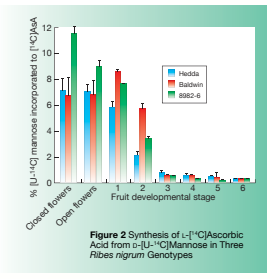



**Figure 2** Synthesis of L-[$^{14}$C]Ascorbic Acid from D-[U-$^{14}$C]Mannose in Three *Ribes nigrum* Genotypes

### Cessation of L-Ascorbic Acid Accumulation is Associated with Increased Turnover

Fruit AsA turnover rates were low (~1% total pool $h^{-1}$) during flowering and fruit expansion (stages 1-2), increasing to ~3.5% total pool $h^{-1}$ in ripening and ripe fruit (stages 3-6; table 1). It was concluded that cessation of AsA accumulation occurred through a combination of decreased biosynthesis and increased turnover.

AsA Turnover (% Total pool $h^{-1}$)

| Developmental Stage | Hedda | Baldwin | 8982-6 |
|---|---|---|---|
| Flowers | 1.09 ± 0.11 | 0.82 ± 0.06 | 0.97 ± 0.24 |
| Fruit 1-2 | 0.45 ± 0.21 | 1.41 ± 0.15 | 1.14 ± 0.21 |
| Fruit 3-5 | 3.74 ± 0.33 | 3.22 ± 0.53 | 3.56 ± 0.55 |
| Fruit 6 | 3.90 ± 0.25 | 3.63 ± 0.17 | 3.78 ± 0.46 |

**Table 1** L-[$^{14}$C]Ascorbic Acid Turnover in Developing Fruit of Three *Ribes nigrum* Genotypes

### Blackcurrant Fruit Show a Biphasic Pattern of Sugar Accumulation

The ultimate substrate for AsA synthesis in fruit must be sugars imported from other parts of the plant. In order to determine whether substrate limitation might influence AsA biosynthesis sugar accumulation was quantified during fruit development. In all genotypes examined, sugar accumulation followed a biphasic pattern (Fig. 3) with an initial accumulation during fruit expansion (stages 1-3) followed by a brief plateau (stages 3-4) and then a second phase of accumulation (stages 4-6). It was postulated that the first phase of sugar accumulation resulted from starch breakdown and mobilisation while the second phase resulted from translocation of sugars from source leaves.

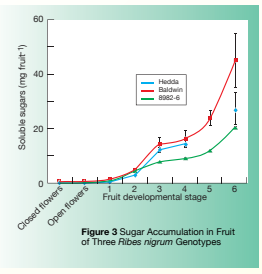



**Figure 3** Sugar Accumulation in Fruit of Three *Ribes nigrum* Genotypes

### AsA Accumulation in Blackcurrant Fruit is Positively Correlated with Solar Radiation

In order to determine environmental effects on blackcurrant AsA accumulation, historical records of fruit AsA concentration from known cultivars grown in SCRI breeding plots were correlated with different meteorological parameters (rainfall, temperature, sunshine hours, total solar radiation). The most significant correlation observed was that between post-harvest solar radiation (Aug-Oct) and the fruit AsA concentration in the following year ($R^2$ = 0.47, Fig. 4). This data is consistent with the hypothesis that greater post-harvest radiation allows increased accumulation of starch reserves providing a greater substrate pool for fruit AsA biosynthesis in the following year.

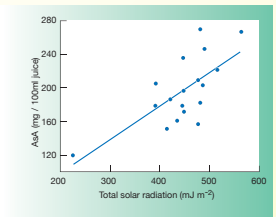
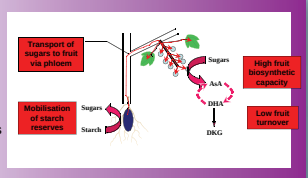



**Figure 4** Correlation between Post-Harvest Solar Radiation and Fruit L-Ascorbic Acid Concentration in the Following Year (cv. Ben Tirran)

## A Model for L-Ascorbic Acid Accumulation in Blackcurrant Fruit

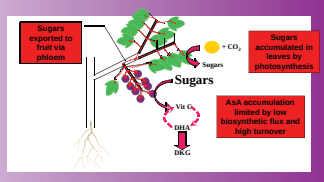
### Spring

Fruit and leaves still in development. Mobilisation of starch reserves provide substrates for developing tissues and AsA biosynthesis



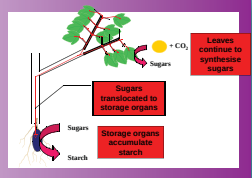

### Summer

Starch reserves depleted. Leaves fully developed and exporting photosynthetically generated sugars. AsA accumulation in fruit limited by low biosynthetic flux and high turnover.




### Autumn

Fruit have been harvested. Photosynthesis continues resulting in starch accumulation. Current work is investigating the link between starch accumulation and fruit AsA concentration in the following season.

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