# Assimilate partitioning in *Vitis vinifera* during the reproductive period

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## Objectives of Proposed Research

1. Investigate the relationship between abscisic acid (ABA) and auxins and the regulation of carbon partitioning.

2. Investigate the origin of ABA present in the fruit during ripening and attempt to modify the partitioning pattern through manipulation of endogenous ABA levels using mefluidide (ABA biosynthesis promoter) or fluridone (ABA biosynthesis inhibitor).

3. Investigate whether auxin interacts with ABA to inhibit carbohydrate partitioning to the fruit.

## Introduction

Agricultural yields can be increased by changing the pattern of carbohydrate partitioning to favor the harvested portion of the plant over unused plant parts. In viticulture therefore, yields are determined by the amount of carbohydrate partitioned to the fruit rather than to other organs. Breeding and clonal selection have been used to improve yield and fruit quality and also allow for the selection of vines adapted to the characteristics of specific sites. An appreciation of environmental effects on photosynthesis has led to the use of canopy management practices that increase photosynthetic efficiency and thereby increase the assimilates transported to the fruit. A basis for further improvement of grapevine yield may come from an understanding of the physiological regulation of carbohydrate partitioning within the vine.

It is not until veraison that the fruit becomes a strong assimilate sink[1] (Candolfi-Vasconcelos and Koblet, 1991; Candolfi-Vasconcelos *et al.*, 1994b). By learning how to manipulate the grapevine so that translocation favors movement of assimilates into the fruit earlier, viticulture production could be increased. But, how grapevine allocate assimilates to competing sinks is not understood nor are the factors that cause increased partitioning to the fruit at veraison. There is evidence that growth regulators[2] are involved in the regulation of fruit sink strength. Several studies point to abscisic acid (ABA) as an important influence on the changes that occur in the grapevine at veraison (Coombe, 1976; Coombe and Hale, 1973; Düring *et al.*, 1978; Kataoka *et al.*, 1982; Palejwala *et al.*, 1985). ABA accumulation in the fruit parallels sugar accumulation (Coombe, 1976) and advances the onset of ripening (Coombe and Hale, 1973; Düring and

[1] Carbohydrates or assimilates are translocated from the sites of production or storage (sources) to the sites where they are needed (sinks) such as actively growing tissues. Sink strength reflects the ability of a tissue to attract assimilates from the leaves or storage organs (sources).

[2] Growth regulators or phytohormones are organic chemicals that are synthesized by plants and regulate growth and development.

Alleweldt, 1984; Düring *et al.*, 1978). Applications of ABA to leaves and fruit have been reported to increases sink strength (Dewdney and McWha, 1979; Düring and Alleweldt, 1984; Düring *et al.*, 1978; Setter et al., 1981). Auxin[3] (IAA) may inhibit ABA accumulation and ripening (Davies et al., 1997). IAA is present in the grape berry in high concentrations early in fruit development but its concentration decreases rapidly just before veraison when ABA increases (Cawthon and Morris, 1982).

The data presented here is from an experiment in a larger study we are conducting to establish the relationship between ABA and regulation of assimilate partitioning and how IAA affects the activity of ABA. We will also investigate the origin of the ABA present in the fruit during ripening and attempt to modify the carbohydrate partitioning pattern through manipulation of ABA and IAA levels. The work is being conducted at Oregon State University's Lewis Brown Farm in Corvallis, Oregon. The data being presented was obtained while conducting preliminary experiments during the summer of 2001.

## Materials and Methods

In June of 2001 we constructed a pot-in-pot (PIP) system at Lewis Brown Farm. PIP systems are used in nursery production of trees and shrubs. The PIP system enables us to grow grapevines in pots but in conditions very close to those that field grown plants experience. The vines are exposed to normal sunlight and wind and the roots are insulated by the ground. PIP systems employ a socket pot set permanently in the ground with approximately 3 inches of lip above ground. Plants are grown in insert pots that are placed into the socket pot. Drainage tile and 15 cm of gravel beneath the socket pots allow for good drainage from the pots. The system built at Lewis Brown Farm has pots 37 cm in diameter, 43 cm in depth and 46 L in volume. This large volume pots was selected to allow unrestricted root growth. 400 socket pots are set in five rows of 80 pots with 30 cm between pots. Irrigation was provided by drip lines with micro sprinklers in each pot.

Three year old Pinot noir vines (clone FPMS 2A grafted on 101-14 rootstock) vines with two shoots were potted in insert pots using sandy loam soil and placed in the PIP system. Vines were irrigated regularly (4L approximately twice weekly) to maintain optimal soil moisture conditions. Treatments and sample dates were randomly assigned to the vines. On August 2, one of the two shoots on each vine was pruned to four nodes and the cluster and all leaves except the apical leaf were removed.

### *Carbohydrate partitioning*

A $^{13}CO_2$[4] labeling method modified from Candolfi-Vasconcelos et al. (1994a) is being used to determine the pattern of carbohydrate partitioning. The leaf remaining on the pruned shoot was

[3] Auxin is a phytohormone involved in the regulation of cell enlargement, maintenance of apical dominance and the initiation of root formation in cuttings. IAA, indoleacetic acid, is a naturally occurring auxin

[4] There are two stable isotopes of carbon (proton numbers 12 and 13) and four radioactive ones (10, 11,14,15). Carbon 12 is the most abundant in nature. Tracing studies have traditionally used the radioactive carbon 14. We prefer to use the non-radioactive carbon 13 for our labeling experiments.

enclosed in a plastic bag and supplied with $^{13}CO_2$ enriched air through an inlet. $^{13}CO_2$ was supplied for 3 hours.

Starting at lag phase and continuing through maturity, six control and six $^{13}C$ labeled vines were destructively harvested 24 hours after labeling. Labeling and vine harvest were done every 2 weeks (3,17, and 30 August, 17 and 28 September). Clusters were cut from the vines and immediately immersed in liquid nitrogen. Frozen clusters were packed in dry ice and stored in a -35°C freezer for later growth regulator analysis. The grapevines were separated into roots, shoot, shoot tip, mature leaves, lateral shoots, lateral leaves, labeling shoot, and labeling leaf. Fresh weights of all vine parts were taken. The vine parts were dried and dry weights were measured. The vine parts were all ground to a fine powder and are being analyzed by the OSU isotope analysis lab using mass spectrometry to determine the molar abundance ratio of vine part samples ($^{13}C/^{12}C$).

Calculations adapted from Candolfi-Vasconcelos *et al.* (1994a) will be used to determine the relative sink strength (RSS, Relative sink strength of each organ as it relates to the sum of the sink strength of all plant organs) of each vine part. RSS will be estimated based on the amount newly fixed carbon relative to the previously fixed carbon in the labeled vines.

Additional 12 cluster samples were taken at weekly intervals. Six clusters were frozen as described previously and will be used for growth regulator analysis. The remaining six clusters were crushed to determine °Brix, pH, and TA.

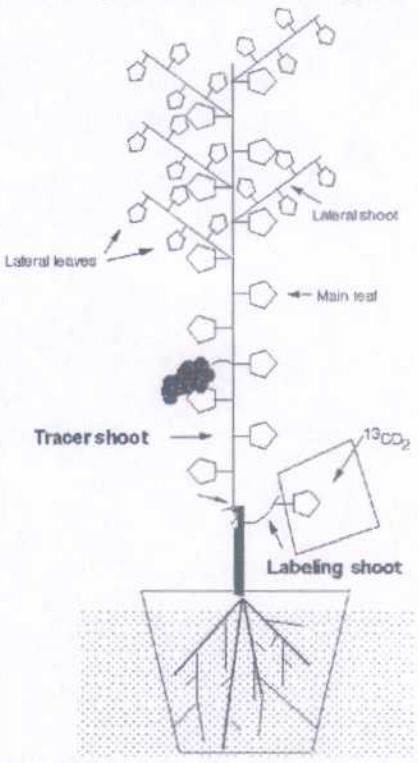


Figure 1: Diagram of the $^{13}CO_2$ labeling technique. Adapted from Candolfi-Vasconcelos *et al.* (1994).

**Results and Discussion**

*Fresh weight*

Fresh weights from whole vine harvests are presented in Table 1. There was no significant effect of $^{13}C$ labeling on the distribution of vine fresh weight, nor was there any interaction between $^{13}C$ labeling treatment and date of vine harvest. The date of vine harvest was significant in determining the proportion of fresh weight allocated to all the vine parts except roots, lateral shoots and lateral leaves. There are two flushes of roots growth. The first one occurs during bloom and the second after fruit harvest. During fruit ripening, there is no noteworthy root growth. Although most the proportion of fresh weight allocated to many of the vine parts was

Table 1: Fresh weight of Pinot noir vine organs harvested 3, 17, and 30 August and 17 and 28 September as the proportion of total vine fresh weight.

| Date | Treatment | Total fresh weight (g) | Percentage of total fresh weight | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Shoot | Shoot Tip | Trunk | Roots | Cluster | Mature Leaves | Lateral Shoots | Lateral Leaves | Labeled Shoot | Labeled Leaf |
| 03-Aug-01 | $^{13}$C labeled | 453.47 | 8.46 | 0.35 | 19.32 | 42.94 | 14.20 | 9.42 | 0.50 | 2.73 | 1.55 | 0.51 |
| | Control | 441.83 | 8.34 | 0.31 | 19.67 | 40.95 | 15.93 | 9.32 | 0.58 | 3.05 | 1.36 | 0.48 |
| | Combined | 447.65 | 8.40 | 0.35 | 19.54 | 41.82 | 15.14 | 9.37 | 0.54 | 2.90 | 1.45 | 0.50 |
| 17-Aug-01 | $^{13}$C labeled | 439.67 | 8.46 | 0.56 | 19.93 | 40.44 | 15.25 | 8.80 | 0.81 | 3.77 | 1.50 | 0.46 |
| | Control | 441.28 | 9.27 | 0.34 | 18.83 | 43.22 | 12.57 | 9.39 | 0.78 | 3.42 | 1.65 | 0.52 |
| | Combined | 440.47 | 8.66 | 0.45 | 19.55 | 42.07 | 13.93 | 8.98 | 0.77 | 3.47 | 1.62 | 0.50 |
| 30-Aug-01 | $^{13}$C labeled | 433.36 | 7.19 | 0.25 | 18.24 | 45.55 | 14.82 | 8.82 | 0.53 | 2.77 | 1.34 | 0.49 |
| | Control | 469.69 | 7.60 | 0.51 | 18.43 | 44.80 | 14.98 | 7.95 | 0.69 | 3.04 | 1.54 | 0.46 |
| | Combined | 451.55 | 7.34 | 0.37 | 18.39 | 45.11 | 15.05 | 8.33 | 0.61 | 2.86 | 1.46 | 0.48 |
| 17-Sep-01 | $^{13}$C labeled | 541.55 | 6.15 | 0.09 | 16.76 | 43.35 | 19.45 | 8.20 | 0.77 | 3.73 | 1.12 | 0.37 |
| | Control | 571.49 | 6.50 | 0.11 | 16.29 | 42.43 | 21.34 | 7.58 | 0.68 | 3.43 | 1.28 | 0.37 |
| | Combined | 556.19 | 6.32 | 0.10 | 16.65 | 43.03 | 20.20 | 7.90 | 0.70 | 3.55 | 1.20 | 0.34 |
| 28-Sep-01 | $^{13}$C labeled | 580.46 | 6.15 | 0.18 | 15.52 | 43.57 | 20.07 | 7.42 | 1.02 | 4.67 | 1.05 | 0.36 |
| | Control | 587.29 | 6.35 | 0.11 | 15.23 | 41.08 | 24.60 | 7.06 | 0.65 | 3.32 | 1.24 | 0.35 |
| | Combined | 583.65 | 6.20 | 0.13 | 15.42 | 42.33 | 22.43 | 7.23 | 0.81 | 3.95 | 1.15 | 0.35 |
| Significant F for Treatment | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Significant F for Date | | 0.001 | 0.01 | 0.01 | 0.001 | ns | 0.001 | 0.001 | ns | ns | 0.05 | 0.01 |
| Significant F for Interaction | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Linear regression (date) | $R^2$ | 0.53 | 0.24 | 0.18 | 0.38 | 0.47 | 0.02 | 0.28 | 0.04 | 0.06 | 0.13 | 0.02 |
| | slope | 2.75 | -0.05 | -0.01 | -0.08 | 1.22 | -0.04 | -0.04 | 0.00 | 0.02 | -0.01 | 0.00 |

Table 2: Dry weight of Pinot noir vine organs harvested 3, 17, and 30 August and 17 and 28 September as the proportion of total vine dry weight.

| Date | Treatment | Total Weight (g) | Percentage of total dry weight | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Shoot | Shoot Tip | Trunk | Roots | Mature Leaves | Cluster* | Lateral Shoots | Lateral Leaves | Labeled Shoot | Labeled Leaf |
| 03-Aug-01 | $^{13}$C labeled | 138.50 | 9.84 | 0.27 | 30.96 | 36.82 | 8.81 | 8.77 | 0.39 | 2.16 | 1.97 | 2.46 |
| | Control | 131.70 | 9.67 | 0.24 | 31.63 | 34.88 | 9.87 | 9.00 | 0.48 | 2.57 | 1.76 | 2.27 |
| | Combined | 135.10 | 9.75 | 0.26 | 31.29 | 35.87 | 9.34 | 8.88 | 0.43 | 2.36 | 1.87 | 2.37 |
| 17-Aug-01 | $^{13}$C labeled | 132.30 | 10.23 | 0.51 | 33.06 | 31.54 | 9.35 | 10.07 | 0.61 | 4.22 | 2.07 | 0.62 |
| | Control | 141.86 | 11.92 | 0.27 | 28.57 | 37.16 | 7.27 | 9.99 | 0.66 | 3.50 | 2.20 | 0.60 |
| | Combined | 137.08 | 11.10 | 0.17 | 30.74 | 34.45 | 8.31 | 10.03 | 0.64 | 3.85 | 2.14 | 0.61 |
| 30-Aug-01 | $^{13}$C labeled | 142.11 | 10.12 | 0.36 | 30.00 | 38.45 | 8.78 | 9.49 | 0.38 | 2.93 | 1.74 | 0.55 |
| | Control | 156.08 | 9.43 | 0.27 | 30.54 | 39.22 | 9.87 | 8.28 | 0.48 | 2.85 | 2.02 | 0.51 |
| | Combined | 149.09 | 9.76 | 0.06 | 30.28 | 38.85 | 9.33 | 8.85 | 0.43 | 2.89 | 1.89 | 0.53 |
| 17-Sep-01 | $^{13}$C labeled | 176.78 | 8.76 | 0.10 | 29.52 | 37.31 | 17.48 | 8.57 | 0.55 | 3.43 | 1.61 | 0.30 |
| | Control | 180.61 | 10.02 | 0.08 | 29.27 | 38.56 | 21.80 | 8.42 | 0.53 | 3.38 | 1.88 | 0.33 |
| | Combined | 178.69 | 9.40 | 0.15 | 29.40 | 37.94 | 19.64 | 8.49 | 0.54 | 3.40 | 1.74 | 0.32 |
| 28-Sep-01 | $^{13}$C labeled | 204.55 | 8.46 | 0.09 | 23.93 | 44.01 | 28.33 | 7.17 | 0.67 | 4.33 | 1.38 | 0.27 |
| | Control | 205.72 | 8.87 | 0.12 | 23.97 | 41.55 | 26.10 | 7.13 | 0.52 | 3.20 | 1.68 | 0.29 |
| | Combined | 205.13 | 8.67 | 0.12 | 23.95 | 42.78 | 22.89 | 7.15 | 0.60 | 3.77 | 1.53 | 0.28 |
| Significant F for Treatment | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Significant F for Date | | 0.001 | ns | 0.001 | 0.010 | 0.001 | 0.001 | 0.001 | ns | 0.050 | ns | 0.001 |
| Significant F for Interaction | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Linear regression (date) | $R^2$ | 0.53 | 0.03 | 0.14 | 0.13 | 0.30 | 0.42 | 0.15 | 0.02 | 0.10 | 0.03 | 0.62 |
| | slope | 1.03 | -0.02 | 0.00 | -0.10 | 0.17 | 0.24 | -0.03 | 0.00 | 0.02 | 0.01 | -0.03 |

*Cluster dry weight was calculated as dry weight = 0.0329(fresh weight$^{1.3402}$).

Table 3: Main, lateral, and labeled leaf number and area of Pinot noir vine organs harvested 3, 17, and 30 August and 17 and 28 September

| Date | Treatment | Mature Leaves # of Leaves | Mature Leaves Leaf Area ($cm^2$) | Lateral Leaves # of Leaves | Lateral Leaves Leaf Area ($cm^2$) | Labeled Leaf Area ($cm^2$) |
|---|---|---|---|---|---|---|
| 03-Aug-01 | $^{13}C$ labeled | 22.17 | 1589.24 | 43.00 | 613.26 | 83.04 |
| | Control | 22.50 | 1565.44 | 44.67 | 694.37 | 76.23 |
| | Combined | 22.33 | 1577.34 | 43.83 | 653.81 | 79.63 |
| 17-Aug-01 | $^{13}C$ labeled | 20.67 | 1381.83 | 40.00 | 775.71 | 75.58 |
| | Control | 21.83 | 1514.28 | 39.67 | 723.75 | 87.40 |
| | Combined | 21.25 | 1448.05 | 39.83 | 749.73 | 81.49 |
| 30-Aug-01 | $^{13}C$ labeled | 23.33 | 1356.32 | 41.33 | 550.76 | 71.38 |
| | Control | 21.83 | 1301.92 | 47.50 | 661.33 | 76.14 |
| | Combined | 22.58 | 1329.12 | 44.42 | 606.04 | 73.76 |
| 17-Sep-01 | $^{13}C$ labeled | 28.67 | 1555.11 | 61.83 | 974.80 | 64.17 |
| | Control | 25.50 | 1485.70 | 57.00 | 922.33 | 68.82 |
| | Combined | 27.08 | 1520.41 | 59.42 | 948.56 | 66.28 |
| 28-Sep-01 | $^{13}C$ labeled | 26.00 | 1433.64 | 73.83 | 1239.00 | 63.08 |
| | Control | 24.17 | 1422.62 | 55.67 | 924.60 | 65.47 |
| | Combined | 25.08 | 1428.13 | 64.75 | 1081.80 | 64.27 |
| Significant F for Treatment | | ns | ns | ns | ns | ns |
| Significant F for Date | | 0.001 | ns | 0.01 | 0.001 | 0.05 |
| Significant F for Interaction | | ns | ns | ns | ns | ns |
| Linear regression (date) | $R^2$ | 0.20 | 0.01 | 0.22 | 0.17 | 0.25 |
| | slope | 0.08 | -1.39 | 0.43 | 7.50 | 0.00 |

Table 4: Cluster weights, soluble solids concentration (°Brix) , pH, and titratable acidity (g/L) of Pinot noir clusters harvested between lag phase and veraison.

| Date | Cluster weight (g) | °Brix | pH | TA (g/L) |
|---|---|---|---|---|
| 03-Aug-01 | 70.37 | 5.8 | 2.73 | 39.81 |
| 10-Aug-01 | 45.04 | 6.2 | 2.42 | 40.43 |
| 17-Aug-01 | 55.48 | * | | |
| 24-Aug-01 | 83.02 | 5.0 | 2.57 | 34.69 |
| 30-Aug-01 | 70.37 | | | |
| 07-Sep-01 | 117.93 | 12.8 | 2.96 | 20.70 |
| 17-Sep-01 | 121.95 | | | |
| 21-Sep-01 | 104.58 | 18.9 | 3.49 | 10.16 |
| 28-Sep-01 | 130.49 | | | |
| Significance | 0.01 | 0.001 | 0.001 | 0.001 |
| Linear regression (date) $R^2$ | 0.40 | 0.63 | 0.34 | 0.31 |
| slope | 1.31 | 0.34 | 0.02 | -0.61 |

*The soluble solids, pH, and TA of clusters harvested from $C^{13}$ labeled vines have not been measured.

significantly different among the sample dates, the changes in fresh weight did not follow a linear pattern.

*Dry weight*

The total dry weight paralleled changes in fresh weight (Table 2). As with fresh weight proportioning, dry weight changes of different plant parts did not follow a linear pattern when regressed against time.

*Leaf number and area*

There were no significant differences among the mean main leaf area over time but lateral leaf number and area did increase (Table 3). Growth of the main shoots slowed or stopped by late August/early September. Therefore apical dominance was broken and lateral shoots and leaves proliferated.

*Fruit Chemistry*

Not surprisingly, fruit maturity parameters were among the measurements most influenced by sample date (Table 4). As the vines shifted from lag phase to veraison, berries and clusters acquired weight, sugars accumulated and acids dropped. Véraison occurred around the second week in September

**Future Work**

Clusters harvested and frozen during the 2001 season will be analyzed using HPLC and fluorometric detection to determine the quantities of IAA and ABA in the fruit.

In 2002, growth regulator levels in the fruit will be manipulated using ABA and IAA promoters and inhibitors. Vines will be labeled with $^{13}C$ as in 2001 and the sink strength of vine organs and growth regulator quantities will be analyzed.

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