Translocation and Partitioning Patterns of ^{14}C Photoassimilate from Light- and Shade-adapted Shoots in Greenhouse-grown ‘Chardonnay’ Grapevines (*Vitis vinifera* L.)

Justine E. Vanden Heuvel,¹ Evangelos D. Leonardos, John T.A. Proctor, K. Helen Fisher, and J. Alan Sullivan²

Department of Plant Agriculture, University of Guelph, Guelph, ON Canada N1G 2W1

ADDITIONAL INDEX WORDS. sucrose, starch, carbohydrate, roots

ABSTRACT. Potted ‘Chardonnay’ grapevines (*Vitis vinifera* L.) with either two or three shoots were grown in a greenhouse for one month and then transferred to a phytotron room, where either one or two shoots were shaded. Twenty-four days after transfer, leaves at the fifth node of either the light-adapted or shade-adapted shoot were exposed to a 2-hour pulse of $^{14}\text{CO}_2$. Both light environment and number of shade shoots on the vine had a significant effect on photosynthate partitioning within the plant following a 22-hour chase. Leaves fed with $^{14}\text{CO}_2$ on a light-adapted shoot translocated 26.1% and 12.7% more radioactivity to the roots and trunk, respectively, than leaves from shade-adapted shoots. Photosynthates were exported from light-adapted leaves to shade-adapted shoots (1.3% of total ^{14}C in plant). The number of shaded shoots and the light environment of the fed leaf had a large effect on partitioning of photosynthates among ethanol-insoluble, water-soluble, and chloroform-soluble fractions within the leaf. Recovered ^{14}C in the water-soluble fraction of the fed leaf appeared to be affected more by number of shoots than by light environment of the fed leaf. The data suggest that there is a sink effect on initial carbon partitioning patterns in grapevine leaves. Sink strength may have a greater role than light environment. A large proportion of interior leaves versus exterior leaves may be costly with respect to the carbohydrate budget of a vine.

Grapevine (*Vitis vinifera*) trellising systems differ in the percentage of leaves located in the interior of the canopy vs. the exposed canopy surface area (Katerji et al., 1994; Schultz, 1995; Smart et al., 1990). Leaves in the interior of dense grapevine canopies can be severely shaded. The interior of a grapevine canopy has been characterized as a dark environment from berry set onward, with <1% of ambient photosynthetic photon flux (*PPF*) (Smart, 1985), and a red to far-red ratio (*R:FR*) of 0.1 or lower (Dokoozlian and Kliewer, 1995).

Schultz et al. (1996) determined that reducing *PPF* to 12 mol·m⁻²·d⁻¹ depresses maximum net photosynthesis (*P_n*) and stomatal conductance values to ≈60% to 65% of that of light-adapted grape leaves. The reduction in *P_n* was caused primarily by a decrease in carboxylation efficiency, while the CO_2 compensation point remained unaffected (Schultz et al., 1996). Shade-adapted leaves operated at a higher internal CO_2 partial pressure, indicating a lower stomatal conductance/ carboxylation efficiency ratio than light-adapted leaves. This reduction in carboxylation efficiency indicated a loss of Rubisco activity, which was coupled to a reduction in ribulose 1,5-biphosphate regeneration capacity, as indicated by the lower CO_2 saturated *P_n* rates in the field (von Caemmerer and Farquhar, 1981). Iacono et al. (1995) reported only a reduction in stomatal conductance but not *P_n* or carboxylation efficiency when shading half of a field grown vine with 50% shade cloth at veraison.

Stitt and Quick (1989) suggested that a hierarchy of regulatory mechanisms exists which allows partitioning to be changed without necessarily leading to a reduction in the rate of photosyn-

thesis. The amount of stored assimilate that is available for export or use in the leaf is determined by the regulation of carbon allocation between starch synthesis and synthetic processes in the chloroplast and cytosol (Geiger and Servaites, 1991). Carbon that exits the chloroplast can be used for synthesis of sucrose, for respiration, or for synthesis of compounds that remain in the leaf.

The role of severely shaded leaves with respect to carbohydrate budgets of dense grapevine canopies is not well known. Estimations of the contribution of interior grapevine leaves to vine carbon balance range between 22% (Williams, 1996) and 30% (Smart, 1974) of total CO_2 assimilation. Cartechini and Palliotti (1995) concluded that shaded *Vitis vinifera* L. grapevines can adapt to modified light intensity [30% photosynthetically active radiation (*PAR*)] and produce a given amount of photoassimilates, guaranteeing a certain productivity, even when grown under shaded conditions. Shade-grown vines had lower total leaf area per vine, specific leaf weight, alcohol-soluble carbohydrates and starch content in the leaves, as well as lower soluble solids in the berries compared to light-adapted vines. These differences indicated that light- and shade-adapted plants utilize photoassimilates differently at both structural and storage levels.

Leaves, through sink–source interactions, are involved in the regulation of carbon allocation within the whole plant (Dickson and Isebrands, 1991). The partitioning response of plants, in particular the ratio of dry-matter partitioning to roots vs. stems and leaves, is a revealing stress indicator (Mooney and Winner, 1991). Vanden Heuvel (2002) determined a partitioning response in shaded (10% *PAR*) greenhouse-grown ‘Chardonnay’ grapevines that indicated utilization of carbohydrates in shade leaves and vines is affected highly by light environment, as demonstrated by a root dry weight reduction of 84% when shading level of whole plants was increased from 0 (no shade) to 99%. Leaf area ratio (i.e., leaf area/whole plant dry weight) in shade shoots was

Received for publication 17 May 2002 Accepted for publication 26 Aug. 2002. This research is a portion of a thesis by Justine E. Vanden Heuvel. The authors thank Dennis Murr for his assistance with this manuscript.

¹Current address: University of Massachusetts Cranberry Station, East Wareham, MA 02538; e-mail justinev@umext.umass.edu.

²To whom reprint requests should be addressed; e-mail asullivan@uoguelph.ca.

Fig. 1. Diagrammatic representation of the four treatments. Arrows denote leaf fed $^{14}\text{CO}_2$. 1L = one light-adapted shoot + one shade-adapted shoot with one light-adapted leaf fed $^{14}\text{CO}_2$; 1S = one light-adapted shoot + one shade-adapted shoot with one shade-adapted leaf fed $^{14}\text{CO}_2$; 2L = one light-adapted shoot + two shade-adapted shoots with one light-adapted leaf fed $^{14}\text{CO}_2$; and 2S = one light-adapted shoot + two shade-adapted shoots with one shade-adapted leaf fed $^{14}\text{CO}_2$. PAR at the treated leaves was 350 to 450 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for the light-adapted shoots, and 65 to 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for the shade-adapted shoots.

greater than in light-adapted shoots, similar to the results of Schultz and Matthews (1993).

Understanding the liabilities vs. contributions of light-adapted and shade-adapted leaves during a season is important for modelling whole-plant carbon assimilation (Schultz et al., 1996), and may also play an important role in determination of optimal management practices. It is hypothesized that shade leaves, while photosynthesizing, partition little photoassimilate to permanent structures of the vine. The objectives of this study were to: 1) determine photoassimilate import and export patterns from light- and shade-adapted shoots; and 2) determine the partitioning of photoassimilate in light- and shade-adapted leaves.

Materials and Methods

PLANT MATERIAL. Dormant vines of *Vitis vinifera* L. 'Chardonnay' clone 96 on Couderc 3309 (C3309) rootstock were potted into 12-L nursery pots with PRO-MIX Plug Mix (Premier Horticulture, Red Hill, Pa.) on 7 June 2001 in Guelph, Ont., Canada. The potted vines were placed in a greenhouse with no supplemental irradiance provided. Vines were maintained with either two or three shoots, and further shoot extension was prevented by pinching when plants attained ten leaves. All developing axillary shoots were removed to reduce within-plant shading (Edson et al., 1993). Vines were watered and fertilized as required (20N–20P–20K at 2 g·L⁻¹). The temperature regime in the greenhouse was 25 ± 2/15 ± 2 °C (day/night).

TREATMENTS. On 6 July 2001, 12 plants each with two shoots and 12 plants each with three shoots were moved onto three benches in a phytotron room. Plants were placed with either one or two shoots under a 75% shade cloth hung over a portion of the bench. The four treatments (Fig. 1) consisted of: one light-adapted shoot + one shade-adapted shoot with one light-adapted leaf fed with $^{14}\text{CO}_2$ (1L); one light-adapted shoot + one shade-adapted shoot with one shade-adapted leaf fed with $^{14}\text{CO}_2$ (1S); one light-adapted shoot + two shade-adapted shoots with one light-adapted leaf fed with $^{14}\text{CO}_2$ (2L); and one light-adapted shoot + two shade-adapted shoots with one shade-adapted leaf fed with $^{14}\text{CO}_2$ (2S). Six treatment replications were placed in a

randomized complete block design (two blocks per bench). Plants were subjected to an 18-h photoperiod with a day/night temperature regime of 25/15 °C. Temperature underneath the shade did not differ by more than ±0.2 °C compared to unshaded portions of the bench. Levels of PAR were measured with a quantum sensor (LI-COR, Lincoln, Nebr.) at the top leaf of the plants three days before $^{14}\text{CO}_2$ exposure. Light levels were between 550 to 650 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the light and between 130 to 160 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under the shade. At the height of the $^{14}\text{CO}_2$ -treated leaves, PAR levels on light-adapted shoots ranged from 350 to 450 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and

from 65 to 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ on shade-adapted shoots.

$^{14}\text{CO}_2$ EXPOSURE. Plants were exposed to $^{14}\text{CO}_2$ on 30 July 2001. Two hours after the beginning of the photoperiod the leaf at the fifth node of each shoot was sealed in a poly bag. The bag was sealed around the petiole by using a band of plasticene and a thin wire to create an airtight seal. $^{14}\text{CO}_2$ (0.875 MBq/leaf) generated from $\text{NaH}^{14}\text{CO}_3$ was injected with a syringe into each bag. The injection site in the bag was then sealed using an airtight tape. Leaves were left in the bags to assimilate the $^{14}\text{CO}_2$. After 2 hours (Motomura, 1990), any remaining $^{14}\text{CO}_2$ was flushed from the sealed bag through a potassium hydroxide trap. The poly bags were then removed from the leaves.

PARTITIONING OF ^{14}C -INTERMEDIATES IN THE FED LEAF. A leaf disk (area = 0.95 cm²) was taken with a cork borer from each fed leaf at the end of the 2-h pulse as well as at the end of the chase period (i.e., 24 h from beginning of pulse) from either side of the midvein. Partitioning of the recently fixed ^{14}C in the source leaf was determined after rapid triple-extraction of the intact leaf disks in 5 mL boiling ethanol (80%). The extract was vacuum-dried, rehydrated and partitioned against 2 chloroform : 1 water (by volume). The radioactivity in each fraction (EtOH-insoluble, H₂O-soluble, CHCl₃-soluble) was determined by liquid scintillation counting of aliquots (model LS-6800; Beckman Instruments Inc., San Ramon, Calif.). The EtOH-insoluble fraction is composed primarily of starch, the H₂O-soluble fraction is composed of sugars, amino acids, and organic acids, while the CHCl₃-soluble fraction is composed mostly of pigments and lipids.

DETERMINATION OF TRANSLOCATION PATTERNS. Following a 22-h chase period, plants were then divided into: fed leaves, shoots containing fed leaves (including non-fed leaves), non-fed shoots and leaves, shade non-fed shoot (2S treatment only), roots, and trunk. Area of fed leaves was determined using a leaf area meter (LI-3100; LI-COR, Lincoln, Nebr.). All samples were then placed in an 80 °C oven for 48 h, with the exception of the trunks which were dried for 96 h. Samples were ground in a Wiley mill to a 40 mesh size. Subsamples of 25 mg were taken from all organs with the exception of the fed leaf, where 10 mg subsamples were removed.

Two subsamples of each of the ground plant organs were combusted with a biological oxidizer (BIO-OX; RJ Harvey Instrument Co., Hillsdale, N.J.) and the radioactivity generated was determined by liquid scintillation counting with ^{14}C scintillation cocktail (RJ Harvey Instrument Co., Hillsdale, N.J.). Radioactivity per mg dry weight was calculated. Allocation or distribution measurements were expressed as total radioactivity recovered in each plant part and as a percentage of the total ^{14}C recovered in the whole plant.

STATISTICAL ANALYSIS. The Statistical Analysis System v.8

Table 1. Leaf dry weight, labelled shoot dry weight, non-labelled shoot dry weight, and specific leaf weight of potted vines of 'Chardonnay' as affected by shoot number and light environment.

Treatment ^z	Labelled shoot dry wt (g)	Nonlabelled shoot dry wt (g)	Leaf dry wt (g)	Specific leaf wt (g·m ⁻²)
1L	9.5 ± 0.8 a ^y	6.0 ± 0.8 b	0.44 ± 0.06 a	46.3 ± 2.8 ab
1S	5.9 ± 0.8 b	9.9 ± 0.8 a	0.32 ± 0.06 b	37.7 ± 3.1 bc
2L	7.9 ± 0.8 ab	9.0 ± 0.8 a	0.44 ± 0.06 a	51.6 ± 2.8 a
2S	5.9 ± 0.8 b	8.3 ± 0.8 a	0.26 ± 0.06 b	31.8 ± 2.8 c
P	0.0136	0.0125	0.0145	0.0008

^zTreatments: 1L = one light-adapted shoot + one shade-adapted shoot with one light-adapted leaf fed ¹⁴CO₂; 1S = one light-adapted shoot + one shade-adapted shoot with one shade-adapted leaf fed ¹⁴CO₂; 2L = one light-adapted shoot + two shade-adapted shoots with one light-adapted leaf fed ¹⁴CO₂; and 2S = one light-adapted shoot + two shade-adapted shoots with one shade-adapted leaf fed ¹⁴CO₂.

^yMeans within columns are separated by *t* tests, *P* ≤ 0.05.

(SAS Inc., Cary, N.C.) for personal computers was used to analyze the data. Analysis of Variance using General Linear Model Procedure in SAS (Proc GLM, SAS) was performed separately for each response variable (e.g., total ¹⁴C, % ¹⁴C) and the significance of each model factor was evaluated. Model assumptions were tested through residual analysis and were determined to have been met. Contrast statements were used to define significant differences among the treatments, and estimates of contrast differences were generated (Bowley, 1999). The Type I error rate (α) was set at 0.05 for all statistical tests.

Results

LEAF AND VINE GROWTH. Leaf dry weight and specific leaf weight of the fed leaf were reduced in the shade shoots compared to the sun shoots (Table 1). Leaf dry weight was reduced by 48% between the 2L and 2S treatments, while specific leaf weight was reduced by 38% between those treatments. Non-labelled shoots from the 2S and 2L treatments did not differ in weight from the single non-labelled shoot from the 1S treatment, indicating that shaded shoots in the 2S or 2L treatments were smaller than in the 1S or 1L treatments (Table 1). Area of the fed leaf, total vine dry weight, root dry weight, and trunk dry weight were unaffected by treatment (data not shown).

¹⁴CARBON IN EtOH-INSOLUBLE, H₂O-SOLUBLE, AND CHCl₃-SOLUBLE FRACTIONS.

Fig. 2. ¹⁴Carbon (MBq·m⁻²) in EtOH-insoluble, H₂O-soluble, and CHCl₃-soluble fractions recovered from the fed leaf immediately following a 2-h ¹⁴CO₂ pulse (A) and 22 h later (B). Treatments: 1L = one light-adapted shoot + one shade-adapted shoot with one light-adapted leaf fed ¹⁴CO₂; 1S = one light-adapted shoot + one shade-adapted shoot with one shade-adapted leaf fed ¹⁴CO₂; 2L = one light-adapted shoot + two shade-adapted shoots with one light-adapted leaf fed ¹⁴CO₂; and 2S = one light-adapted shoot + two shade-adapted shoots with one shade-adapted leaf fed ¹⁴CO₂. Means within columns are separated by *t* tests, *P* ≤ 0.05.

¹⁴Carbon in the EtOH-insoluble and CHCl₃-soluble fractions differed between treatments immediately following the ¹⁴C pulse, and differed in all fractions 22 h later (Fig. 2). While there was no difference between light and shade-labelled leaves with respect to

Table 2. Mean differences ($\pm$ SE) and level of significance for radioactivity found in EtOH-insoluble, H₂O-soluble, and CHCl₃-soluble fractions in the leaf fed ¹⁴CO₂ immediately following a 2-h pulse and 22 h later (MBq·m⁻²) on a light-(L) or shade-adapted (S) shoot, or a vine with either one or two shade-adapted shoots.

Contrast ^z	Immediately following 2-h pulse				After 22-h chase			
	EtOH-insoluble	H ₂ O-soluble	CHCl ₃ -soluble	Total	EtOH-insoluble	H ₂ O-soluble	CHCl ₃ -soluble	Total
L vs. S	13.9 $\pm$ 0.4	1.2 $\pm$ 6.5	-2.0 $\pm$ 0.5	10.2 $\pm$ 8.5	7.1 $\pm$ 3.1	21.2 $\pm$ 5.9	-1.4 $\pm$ 0.6	26.3 $\pm$ 7.6
<i>P</i>	(0.0023)	(0.8564)	(0.0024)	(0.2630)	(0.0384)	(0.0027)	(0.0278)	(0.0040)
1 vs. 2	9.4 $\pm$ 0.4	-18.2 $\pm$ 6.6	-0.4 $\pm$ 0.5	-7.5 $\pm$ 8.5	3.5 $\pm$ 3.1	7.6 $\pm$ 5.9	1.2 $\pm$ 0.6	11.1 $\pm$ 7.6
<i>P</i>	(0.0251)	(0.0209)	(0.4749)	(0.4035)	(0.2727)	(0.2158)	(0.0749)	(0.1677)

^zL vs. S = light -adapted vs. dark-adapted shoot with leaf labelled with ¹⁴C; 1 vs. 2 = one dark-adapted shoot vs. two dark-adapted shoots. Values are the mean difference between the two effects. A negative value indicates that the mean of the first treatment (e.g., L) is higher than the mean of the second effect (e.g., L > S).

¹⁴C in the H₂O-soluble fraction immediately following the pulse (Fig. 2A), the shade-adapted leaves fed ¹⁴CO₂ contained more ¹⁴C in that fraction than the light-adapted leaves 22 h later (Fig. 2B; Table 2). The EtOH-insoluble fraction from shade-adapted leaves fed ¹⁴CO₂, and in particular the 2S treatment, contained more ¹⁴C than the EtOH-insoluble fraction from fed leaves on light-adapted shoots both immediately following the 2-h pulse, and the 22-h chase (Fig. 2; Table 2). The CHCl₃-soluble fraction from the shade-adapted leaves contained significantly less ¹⁴C than was found in the light-adapted leaves at both sampling periods (Table 2).

While there were differences in the EtOH-insoluble and H₂O-soluble fractions between the one vs. two shade shoot treatments immediately following the pulse, they did not differ 22 h later (Table 2). Immediately following the pulse, plants with one shade-adapted shoot contained more ¹⁴C in the EtOH-insoluble fraction than plants with two shade shoots. Plants with two shade shoots, however, had more ¹⁴C in the H₂O-soluble fraction than plants with one shade-adapted shoot (Table 2).

¹⁴C FOUND IN EACH ORGAN. Total ¹⁴C found in each organ, as well as percentage of total plant ¹⁴C found in each organ, differed between treatments (Fig. 3). Light environment of the shoot containing the leaf fed with ¹⁴CO₂ (L vs. S) significantly affected the radioactivity found in the roots, trunk, non-labelled shoot, and the labelled leaf (Fig. 3; Table 3) following the 22-h chase. When a leaf on the light-adapted shoot was fed with ¹⁴CO₂, the percentage of total ¹⁴C

translocated to the permanent structures of the vine (i.e., roots and trunk) increased dramatically (Fig. 3A; Table 3). Translocation of total plant ¹⁴C to the opposite shoot when a leaf on the light-adapted shoot was fed with ¹⁴CO₂ was greater than when a leaf on the shade-adapted shoot was fed with ¹⁴CO₂. The shade leaf, when

Fig. 3. (A) Percentage ¹⁴C recovered in each organ and (B) total ¹⁴C recovered in each organ. Treatments: 1L = one light-adapted shoot + one shade-adapted shoot with one light-adapted leaf fed ¹⁴CO₂; 1S = one light-adapted shoot + one shade-adapted shoot with one shade-adapted leaf fed ¹⁴CO₂; 2L = one light-adapted shoot + two shade-adapted shoots with one light-adapted leaf fed ¹⁴CO₂; and 2S = one light-adapted shoot + two shade-adapted shoots with one shade-adapted leaf fed ¹⁴CO₂. R = root, T = trunk, ODS = other (nonfed) dark shoot (2S treatment only), NLS = nonlabelled shoot, LS = shoot bearing labelled leaf, L = leaf fed ¹⁴CO₂. Means within columns (i.e., organs) are separated by *t* tests, *P* $\leq$ 0.05.

Table 3. Mean differences ($\pm$ SE) and level of significance following the labelling of a leaf on a light- (L) or shade-adapted (S) shoot, or a vine with either one (1) or two (2) shade-adapted shoots with $^{14}\text{CO}_2$ on percentage of total ^{14}C found in each organ 22 h following a 2-h pulse.

Contrast ^a	Root	Trunk	Nonlabelled shoot	Labelled shoot	Labelled leaf
L vs. S	-26.1 ± 3.5	-12.7 ± 1.2	-0.6 ± 0.2	2.1 ± 2.1	37.1 ± 5.2
P	(<0.0001)	(<0.0001)	(0.0137)	(0.3338)	(<0.0001)
1 vs. 2	-2.7 ± 3.5	0.4 ± 1.2	0.4 ± 0.2	-5.2 ± 2.1	5.6 ± 5.2
P	(0.4590)	(0.7826)	(0.0892)	(0.0275)	(0.3040)

^aL vs. S = light -adapted vs. dark-adapted shoot with leaf labelled with ^{14}C ; 1 vs. 2 = one dark-adapted shoot vs. two dark-adapted shoots. Values are the mean difference between the two effects. A negative estimate indicates that the mean of the first treatment (e.g., L) is higher than the mean of the second effect (e.g., L > S).

labelled, exported less total ^{14}C than the light-adapted leaf (Fig. 3; Table 3).

There were no differences between treatments with one or two shade-adapted shoots ($\approx 50\%$ or 66% of total leaf area, respectively) with the exception of the shoot with the light-adapted leaf fed $^{14}\text{CO}_2$, where plants with two shade-adapted shoots contained more of the total ^{14}C than plants with a single shade-adapted shoot (Table 3). While the treatment effect on total ^{14}C found in each organ was significant with the exception of the shoot containing the labelled leaf (Fig. 3), contrasts of total ^{14}C per organ for one vs. two shade shoots were not significant at $\alpha = 0.05$ (data not shown).

Discussion

The results indicate a slow turnover of recently assimilated photosynthate in grapevine leaves. Fed leaves on shade-adapted shoots contained 75.9% and 87.8% (1S and 2S, respectively) of total radioactivity, compared to fed leaves on light-adapted shoots which contained 45.0% and 44.4% of total radioactivity, respectively (Fig. 3). Williams (1996) and references therein have commented on the large proportion of ^{14}C label that remained in the source leaf of grapevines up to 72 h after exposure to $^{14}\text{CO}_2$, whether the plants were potted or grown in the field. Gordon (1986) presented data for the percentage of photosynthate retained in the source leaf for other C_3 species, with retention ranging from a low of 28% in soybean, to 50% in sugar beet, and a high of 65% in pepper. Most C_3 species retain $\approx 30\%$ to 40% of photosynthate in the source leaf.

One objective of the methodology of this experiment was to investigate partitioning patterns of light-adapted and shade-adapted leaves without the confounding effect of different amounts of ^{14}C available for distribution. Total radioactivity found in fed leaves did not differ between treatments immediately following the pulse (Fig. 2; Table 2), indicating that light-adapted and shade-adapted leaves assimilated comparable amounts of $^{14}\text{CO}_2$ during the 2-h feed. Higher photosynthetic rates (Schultz et al., 1996) likely allowed light-adapted leaves to assimilate all $^{14}\text{CO}_2$ in the bag early in the feed, while shade-adapted leaves likely took longer to assimilate a comparable amount. The $^{14}\text{CO}_2$ remaining in the bag was not measured at the end of the pulse. Comparison of $^{14}\text{CO}_2$ delivered in the pulse to total ^{14}C recovered in the leaf immediately following the pulse indicates that, on average, the leaf fed with $^{14}\text{CO}_2$ contained 80% of the fed amount immediately following the pulse. Some photoassimilate was likely exported during the 2-h period.

Different partitioning patterns occurred in fed leaves in the 2S treatment compared to the 1S treatment. Fed leaves in the 2S treatment partitioned a larger percentage of radioactivity into the EtOH-insoluble fraction, compared to the other three treatments

(Fig. 2A), and radioactivity in this fraction decreased during the 22-h chase period in the 2S treatment, while remaining the same in the other three treatments (Fig. 2B). The fed leaf in the 2S treatment also showed different partitioning patterns with respect to the H_2O -soluble fraction. Immediately following the feed, there were no differences among treatments with respect to radioactivity in this fraction, but 22 h following the feed, radioactivity in this fraction had severely decreased in all treatments except the 2S fed leaf. These results are another indication of a slow export rate in grapevine leaves growing in a low light environment. There are several possible explanations for the partitioning patterns observed in the fed leaf of the 2S treatment, one of which is that a portion of the photoassimilate in the EtOH-insoluble fraction found in the leaf following the pulse was converted to the H_2O -soluble fraction during the chase period. The high concentration of H_2O -soluble sugars in the 2S fed leaves following the 22-h chase period (Fig. 2B) may also be an indication of sugar storage in vacuoles in addition to starch storage in chloroplasts.

Mean differences between L vs. S leaves (light- vs. shade-adapted leaf fed with $^{14}\text{CO}_2$) for label in the H_2O -soluble fraction increased greatly in the 24-h chase period. As the difference between L vs. S decreased during that period for the EtOH-insoluble fraction, starch conversion to sucrose may have been greater in the shade-adapted fed leaves than in the light-adapted fed leaves during the night. However, a greater conversion rate of starch to sucrose in the shade-adapted leaves did not result in a large export of photosynthate from the leaf. The conversion of starch in the chloroplast to sucrose in the cytosol in *Phaseolus vulgaris* L. and *Solanum tuberosum* L. is a major energy-requiring process, with export costs accounting for an average of 29% of the dark respiration rate (Bouma et al., 1995). Noguchi et al. (2001) estimated the cost of carbohydrate export in a shade species [*Alocasia odora* (Lodd.)] at about 40% of the ATP production rate, while cost of carbohydrate export in a sun species (*Phaseolus vulgaris* L.) was estimated at 80% of the ATP production rate.

Differences occurred in partitioning among the EtOH-insoluble, H_2O -soluble, and CHCl_3 -soluble fractions between vines with one vs. two shade-adapted shoots, but only immediately following the pulse (Table 2). Leaves fed with $^{14}\text{CO}_2$ on vines with two shade-adapted shoots partitioned less photosynthate into starch, and more into sucrose, likely due to increased demand by the multiple shade shoots on the vine. Interestingly, immediately following the ^{14}C -pulse, recovered radioactivity in the H_2O -soluble fraction of the fed leaf was affected more by number of shoots than by light environment of the fed leaf. Not only do these data suggest that there is a significant sink effect on initial partitioning patterns in grapevine leaves, but also that sink

strength may play a more important role than light environment of the source leaves. However, 22 h following the pulse, mean values for the one vs. two shoot plants were not different.

The amount of ^{14}C in EtOH-insoluble and CHCl_3 -soluble fractions did not change greatly over the 24-h chase period, but radioactivity in the H_2O -soluble fraction of fed leaves in the 1L and 2L treatments decreased drastically during that period (Fig. 2), likely due to export to permanent structures of the vine, as well as use of sucrose for other plant processes. Under conditions such as low light levels where carbon is limited, starch synthesis has priority over sucrose synthesis, resulting in less carbon being exported daily to roots (Mooney and Winner, 1991). Decreases in radioactivity in the H_2O -soluble fraction of fed leaves were less in the 1S treatment, and extremely small in the 2S treatment.

The reduced partitioning of carbohydrate to permanent vine structures by shade-adapted leaves, as in this study, could have a significant effect on fruit yield and composition, and long-term survival of the vine. Shoots with a light-adapted leaf fed with $^{14}\text{CO}_2$ (1L and 2L treatments) translocated 26.1% and 12.7% more radioactivity to the roots and trunk, respectively, than fed leaves on a shade-adapted shoot (1S and 2S treatments) (Table 3). Leaves on light-adapted shoots fed with $^{14}\text{CO}_2$ exported a total of 45.0% and 46.2% (1L and 2L, respectively) of total radioactivity in the plant to the permanent structures of the vine (roots + trunk), while labelled leaves on shade shoots exported 9.8% and 4.0% to the same structures, respectively (Fig. 3). While vines used in this experiment were young, container-grown, and vegetative, the behavior of the shade-adapted leaves indicated that carbon balance of vines may be affected by interior leaves. If this same pattern is seen at the whole-plant level in the vineyard, vines with a higher percentage of interior vs. exterior leaves may allocate fewer resources to root growth, producing smaller root volumes. As roots affect plant growth by processes beyond the functions of supplying water and nutrients, the consequences of reduced energy partitioning to the roots may be great. Reduced root systems may have a significant influence on the development and ripening of the fruit (Coombe, 1992; Ferree et al., 1999), as well as fewer carbohydrates available for winter protection and spring growth (Mullins et al., 1992). Reduced partitioning towards trunk growth may in turn result in lower yield and fruit composition (Koblet et al., 1994).

These data confirm the suggestion (Vanden Heuvel, 2002) that although interior leaves are photosynthesizing at low levels, some sucrose is translocated from exterior leaves to the shade leaves in order to maintain those organs. In the 2L treatment, the shade shoots imported 1.3% of the total radioactivity of the plant (Fig. 3), while the light shoot imported between 0.3–0.5% of total radioactivity. However, in a study of container-grown vines, plants with the six lower leaves shaded produced greater root dry weight than vines with the lower leaves removed (Kaps and Cahoon, 1992). It is unclear how much of a carbon sink light-stressed leaves are with respect to the carbohydrate budget of the vine.

Previous experiments (Vanden Heuvel, 2002) determined that shade-adapted leaves may significantly reduce partitioning of photoassimilate to permanent vine structures such as the roots, and that hypothesis is supported by these findings. Shade-adapted shoots imported photosynthate from fed leaves on light-adapted shoots. The large differences in photosynthate partitioning between the EtOH-insoluble and H_2O -soluble fractions between leaves with either one or two shade-adapted shoots illustrates the large importance of sink strength, particularly compared to light environment of the leaves.

Our experiment was performed on vegetative container-grown vines, therefore extrapolation of the results to the field must be done cautiously. Results from this experiment indicate that a canopy with a high proportion of shaded leaves may yield fewer carbohydrate reserves which could reduce cold hardiness in more northerly temperate regions (Mullins et al., 1992). Yield and fruit composition also may be reduced. Although interior leaves are still photosynthesizing at low light levels, young leaves and growing tips in the vine interior are likely a greater sink than the vegetative shoots used in this experiment. These sinks may result in carbon being imported from the canopy surface. In such a situation, a grape canopy with a high ratio of shaded to exposed leaves would be expected to have fewer carbohydrates available for root, trunk, and fruit growth.

Literature Cited

- Bouma, T.J., R. De Visser, P.H. Van Leeuwen, M.J. De Kock, and H. Lambers. 1995. The respiratory energy requirements involved in nocturnal carbohydrate export from starch-storing mature leaves and their contribution to leaf dark respiration. *J. Expt. Bot.* 46:1185–1194.
- Bowley, S.R. 1999. A hitchhiker's guide to statistics in plant biology. Any old subject books, Guelph, Ont., Canada.
- Cartechini, A. and A. Palliotti. 1995. Effect of shading on vine morphology and productivity and leaf gas exchange characteristics in grapevines in the field. *Amer. J. Enol. Viticult.* 46:227–234.
- Coombe, B.G. 1992. Research on development and ripening of the grape berry. *Amer. J. Enol. Viticult.* 43:101–110.
- Dickson, R.E. and J.G. Isebrands. 1991. Leaves as regulators of stress response, p. 3–34. In: J.A. Mooney and W.E. Winner (eds.). *Response of plants to multiples stresses*. Academic Press, New York.
- Dokoozlian, N.K. and W.M. Kliever. 1995. The light environment within grapevine canopies. I. Description and seasonal changes during fruit development. *Amer. J. Enol. Viticult.* 46:209–218.
- Edson, C.E., G.S. Howell, and J.A. Flore. 1993. Influence of crop load on photosynthesis and dry matter partitioning of Seyval grapevines. I. Single leaf and whole vine response pre- and post-harvest. *Amer. J. Enol. Vitic* 44:139–147.
- Ferree, D.C., D.M. Scurlock, and J.C. Schmid. 1999. Root pruning reduces photosynthesis, transpiration, growth, and fruiting of container-grown French-American hybrid grapevines. *HortScience* 34:1064–1067.
- Geiger, D.R. and J.C. Servaites. 1991. Carbon allocation and response to stress, p. 103–127. In: J.A. Mooney and W.E. Winner (eds.). *Response of plants to multiples stresses*. Academic Press, New York.
- Gordon, A.C. 1986. Diurnal patterns of photosynthate allocation and partitioning among sinks, p. 499–517. In: J. Cronshaw, J.W. Lucas, and R.T. Gianquinta (eds.). *Plant biology: Phloem transport*. Alan R. Liss, New York.
- Iacono, F., M. Bertamini, A. Scienza, and B.G. Coombe. 1995. Differential effects of canopy manipulation and shading of *Vitis vinifera* L. cv. Cabernet Sauvignon. Leaf gas exchange, photosynthetic electron transport rate and sugar accumulation in berries. *Vitis* 34:201–206.
- Kaps, M.L. and G.A. Cahoon. 1992. Growth and fruiting of container-grown Seyval blanc grapevines modified by changes in crop level, leaf number and position, and light exposure. *Amer. J. Enol. Viticult.* 43:191–199.
- Katerji, N., F.A. Daudet, A. Carbonneau, and N. Ollat. 1994. Study at the whole plant level of photosynthesis and transpiration of the vine: comparison of traditional and lyre training systems. *Vitis* 33:197–203.
- Koblet, W., M.C. Candolfi-Vasconcelos, W. Zeifel, and G.S. Howell. 1994. Influence of leaf removal, rootstock, and training system on yield and fruit composition of Pinot noir grapevines. *Amer. J. Enol. Viticult.* 45:181–187.
- Mooney, H.A. and W.E. Winner. 1991. Partitioning response of plants to stress, p. 130–141. In: J.A. Mooney and W.E. Winner (eds.). *Response of plants to multiples stresses*. Academic Press, New York.

- Motomura, Y. 1990. Distribution of ^{14}C -assimilates from individual leaves on clusters in grape shoots. *Amer. J. Enol. Viticult.* 41:306–312.
- Mullins, M.G., A. Bouquet, and L.E. Williams. 1992. *Biology of the grapevine*. Cambridge Univ. Press, New York.
- Noguchi, K., G. Chun-Sim, S. Miyazawa, I. Terashima, S. Ueda, and T. Yoshinari. 2001. Costs of protein turnover and carbohydrate export in leaves of sun and shade species. *Austral. J. Plant Physiol.* 28:37–47.
- Schultz, H.R. 1995. Grape canopy structure, light microclimate and photosynthesis. I. A two-dimensional model of the spatial distribution of surface area densities and leaf ages in two canopy systems. *Vitis* 34:211–215.
- Schultz, H.R. and M.A. Matthews. 1993. Xylem development and hydraulic conductance in sun and shade shoots of grapevine (*Vitis vinifera* L.): Evidence that low light uncouples water transport capacity from leaf area. *Planta* 190:393–406.
- Schultz, H.R., W. Kiefer, and W. Gruppe. 1996. Photosynthetic duration, carboxylation efficiency and stomatal limitation of sun and shade leaves of different ages in field-grown grapevine (*Vitis vinifera* L.). *Vitis* 35:169–176.
- Smart, R.E. 1974. Photosynthesis by grapevine canopies. *J. Appl. Ecol.* 11:997–1006.
- Smart, R.E. 1985. Principles of grapevine canopy microclimate manipulation with implications for yield and quality: A review. *Amer. J. Enol. Viticult.* 35:230–239.
- Smart, R.E., J.K. Dick, I.M. Gravett, and B.M. Fisher. 1990. Canopy management to improve grape yield and wine quality—Principles and practices. *S. Afr. J. Enol. Viticult.* 11:3–17.
- Stitt, M. and W.P. Quick. 1989. Photosynthetic carbon partitioning: Its regulation and possibilities for manipulation. *Physiol. Plant.* 77:633–641.
- Vanden Heuvel, J.E. 2002. Photosynthetic capacity and carbon partitioning of light- and shade-adapted leaves in grapevine (*Vitis vinifera* L.). PhD diss., Univ. Guelph, Guelph, Ont., Canada.
- von Caemmerer, S. and G.D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153:376–387.
- Williams, L.E. 1996. Grape, p. 851–881. In: E. Zamski and A. Schaffer (eds.). *Photoassimilate distribution in plants and crops: Source-sink relationships*. Marcel Dekker, New York.