Whole-Plant Gas Exchange and Reductive Biosynthesis in White Lupin[1]

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Simultaneous measurements of CO_2 (CER) and O_2 (OER) exchange in roots and shoots of vegetative white lupin (*Lupinus albus*) were used to calculate the flow of reducing power to the synthesis of biomass that was more reduced per unit of carbon than carbohydrate. On a whole-plant basis, the diverted reductant utilization rate (DRUR which is: $4 \times [CER + OER]$) of shoot tissue was consistently higher than that of roots, and values obtained in the light were greater than those in the dark. An analysis of the biomass being synthesized over a 24-h period provided an estimate of whole-plant DRUR (3.5 mmol e^- $plant^{-1}$ d^{-1}), which was similar to that measured by gas exchange (3.2 mmol e^- $plant^{-1}$ d^{-1}). Given that nitrate reduction to ammonia makes up about 74% of whole-plant DRUR, root nitrate reduction in white lupin was estimated to account for less than 43% of whole-plant nitrate reduction. The approach developed here should offer a powerful tool for the noninvasive study of metabolic regulation in intact plants or plant organs.

Plants use CO_2, H_2O, mineral nutrients, and light energy to produce carbohydrate and other organic compounds during plant growth. Although the composition of biomass varies depending on species and environmental conditions, it is almost always more reduced than Glc per mole of carbon due to the presence of highly reduced molecules such as fatty acids, lignin, and reduced minerals such as nitrogen and sulphur. This is reflected in the fact that the heat of combustion of biomass (15–30 kJ g^{-1} dry weight; Griffin, 1994; Gary et al., 1995; Spencer et al., 1997; Eamus et al., 1999) is greater than that of Glc (15 kJ g^{-1} dry weight). In addition, the elemental composition of biomass ($CH_{1.3\text{-}1.8}N_{0.01\text{-}0.06}O_{0.5\text{-}0.6}$; McDermitt and Loomis, 1981; Williams et al., 1987; Walton and Fowke, 1995; Walton et al., 1999) has less oxygen and a higher relative carbon content than does Glc or other carbohydrates (CH_2O).

In theory, the synthesis of biomass that is more reduced per unit of carbon than Glc should be reflected in a greater rate of gas production (CO_2 in non-photosynthetic tissues, O_2 in photosynthetic tissues) than gas uptake (O_2 in non-photosynthetic tissues, CO_2 in photosynthetic tissues), integrated over the growth of a plant. This is because the production of gases in plants is associated with the production of reducing power, whereas the uptake of gases are generally indicative of photosynthetic CO_2 fixation or oxidative phosphorylation.

Therefore, quantification of the CO_2 (CER) and O_2 (OER) exchange rates of plant tissues (where production is positive and uptake is negative) can be used to obtain a noninvasive measure of the biosynthetic processes occurring within those tissues. A gas exchange ratio (GER) defined as:

$$GER = -(CER)/(OER) \quad (1A)$$

for tissues having net CO_2 production (i.e. equivalent to respiratory quotient), or defined as:

$$GER = -(OER)/(CER) \quad (1B)$$

for tissues having net CO_2 uptake (i.e. equivalent to photosynthetic quotient), would yield values greater than 1.0 when there is reductive biosynthesis, but less than 1.0 when the tissues that are synthesizing a more oxidized biomass.

Previous studies have reported large variations in the GER of plant tissues during growth. In barley, leaves reducing nitrate had GER values of 1.16 and 1.51 in the light and dark, respectively, compared with values for mutant leaves lacking nitrate reductase of 1.02 and 0.96 in the light and dark, respectively (Bloom et al., 1989). Cramer and Lewis (1993) reported low values for GER in roots of wheat and maize, although values measured in nitrate grown plants (1.0–1.1) were significantly higher than that those in NH_4^+ grown plants (0. 5–0.6). Even larger nitrate-induced increases in GER have been reported in algae (Weger and Turpin, 1989), a finding consistent with the fact that algae have a much lower carbon:nitrogen ratio than herbaceous plants.

Fock et al. (1972) measured CER and OER in photosynthesizing leaves of 18 species and found average GER values of 0.79 for leaves at 400 $\mu L\ L^{-1}$ CO_2 in air. These low GER values for photosynthesizing leaves were challenged by Kaplan and Bjorkman

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(1980), who reported values of 1.04 to 1.14 in the C_3 species *Encelia californica* and the C_4 species *Atriplex rosea*. However, Tolbert et al. (1995) reported that GER values in tobacco leaves in the light decreased from about 1.0 at 21% (v/v) O_2 and 350 μL L^{-1} CO_2 to a value of 0 at 27% (v/v) O_2 and 350 μL L^{-1} CO_2. Moreover, higher pO_2 and lower pCO_2 were reported to result in the net uptake of CO_2 and O_2 (Tolbert et al., 1995).

These large variations in the GER may reflect the large biochemical diversity that exists in plant tissues or the inherent difficulties there are in measuring OER in an atmosphere that contains a high background O_2 concentration (about 20.9% [v/v] or 209,000 μL L^{-1}). For example, even if plant metabolism generates a 100 μL L^{-1} O_2 changes in the composition of the air around the plant, that still represents an atmospheric pO_2 change of only 0.05%.

This study uses a new differential oxygen analyzer capable of measuring less than 5 μL L^{-1} O_2 differentials against a background of air (Willms et al., 1997, 1999). The instrument was incorporated into a whole-plant gas exchange system having separate shoot and root chambers so that continuous CER and OER measurements on a single plant could provide insights into the temporal and spatial variations that occur in reductive biosynthesis during the vegetative growth of a plant.

Nitrate-grown white lupins (*Lupinus albus* cv Manitoba) were chosen as the study organism since previous work (Atkins et al., 1979; Pate et al., 1979) has shown that nitrate reduction was predominantly in the roots. Roots have lower gas exchange rates than shoots, thereby making it easier to obtain precise measurements of OER, CER, and the differences between them. Plants were chosen at various times during vegetative growth (18–33 d after sowing) to test two hypotheses: first, that the reductive biosynthesis in nitrate-grown white lupins should be higher in the root than in the shoot, and second, that spatial (root versus shoot) and temporal (light versus dark period) variations in reductive biosynthesis should integrate to give net reductive biosynthesis (GER > 1.0) in the whole plant over a 24-h day.

RESULTS

Time Course of CO_2 and O_2 Gas Exchange in Shoots and Roots

The gas exchange system described here provided continuous measurements of CO_2 and O_2 exchange in the shoot and root of individual plants. Six replicate measurements, each made over a 2- to 4-d period, were carried out with white lupin plants that ranged in age from 18 to 33 d old and plant dry weight ranged from 1.2 to 4.5 g $plant^{-1}$. Detailed results are presented for a single 24- to 25-d-old plant, whereas mean values (±SE) for all six replicate plants are presented only for summary data.

The 24- to 25-d-old gas exchange plant was harvested on d 26, and its biomass was provided in Table I, along with the mean dry weights of six replicate plants from the same population harvested at d 23 and another six plants harvested at d 25. These plants were used to calculate the relative growth rate for roots, stems + petioles and leaves of 0.071, 0.069, and 0.079 Δg dry weight g^{-1} dry weight d^{-1}, respectively (Table I).

In the shoot of a typical 24- to 25-d-old plant, CER values were about −0.88 mmol CO_2 $plant^{-1}$ h^{-1} in the light, but in the dark, CER was in the opposite direction (0.15 mmol CO_2 $plant^{-1}$ h^{-1}), and only 17% of the magnitude during the light (Fig. 1A). The OER values were of a similar magnitude (Fig. 1B), but were in the opposite direction of the CER values (Fig. 1A). Assuming that all of the daytime CO_2 exchange was associated with leaves, the average leaf area-specific photosynthesis rate was 10 μmol CO_2 m^{-2} s^{-1}. These measurements were carried out when the temperature and relative humidity in the shoot chamber was 28°C and 86%, respectively, in the light, and 25°C and 75% in the dark. Root temperature was maintained at 24°C ± 0.6°C in the light and dark (data not shown).

Since roots only respire, CER values were always positive (production) and OER values were always negative (uptake; Fig. 1B). The magnitude of the CER and OER values ranged from ±50 to 80 μmol $plant^{-1}$ h^{-1}, and values obtained at dark were only 4% lower

Table I. *The dry wts and RGR of a population of white lupin plants (n = 6) from which was selected the experimental plant used for the gas analysis measurements reported in Figures 1, 2, and 4*

The experimental plant was harvested on d 26 and was found to have a total wt of 2.90 g dry wt including 1.18 g dry wt leaves, 0.89 g dry wt stems + petioles, and 0.83 g dry wt roots.

Items	Plant Dry Wt		RGR[a]
	23 d	25 d	Δg dry wt g^{-1} dry wt d^{-1}
Leaves	0.96 ± 0.05	1.12 ± 0.04	0.079
Stems and petioles	0.82 ± 0.06	0.94 ± 0.06	0.069
Roots	0.77 ± 0.05	0.89 ± 0.06	0.071
Whole plant	2.55 ± 0.10	2.95 ± 0.09	0.074

[a] The exponential RGR was calculated according to Radford (1967) using the equation of RGR = (ln DW_2 − ln dry wt)/(d_2 − d_1), where d is days and the subscripts "1" and "2" refer to values for d 23 and 25, respectively.

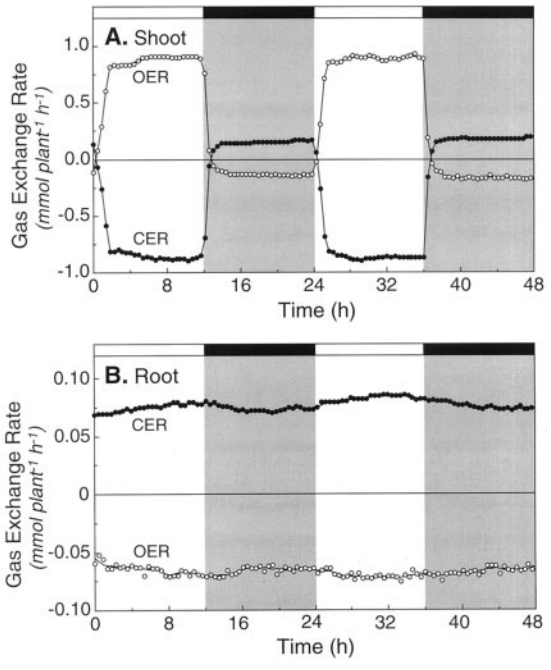

Figure 1. Continuous measurements of the CER (●) and OER (○) in the shoot (A) and root (B) of a 24- to 25-d-old white lupin plant. The black bar at the top of A and B, and the shading within A and B, denotes the 12-h night periods. The photoperiod, temperature, and humidity were 12 h/12 h, 28°C/25°C and 86%/75%, day/night, respectively, in the shoot chamber. The temperature was maintained at 24°C ± 0.6°C in root chamber.

than those obtained during the photoperiod. Expressed per gram of dry weight root, CER values were about 98 μmol g^{-1} dry weight root h^{-1}, a value similar to that reported previously for roots of white lupin (Pate et al., 1979). Since the shoot:root ratio of the plants was 2.3:1 (Table I), the average gram of shoot biomass had a specific gas-exchange rate six times that of root tissue during the light, but only 1.4 times that of roots at dark.

Daily Budget for CER and OER

Measured values for CER and OER in a single plant (Fig. 1) were integrated over 24 h to calculate the daily CO_2 and O_2 interchanges within the whole plant (Fig. 2). About 60% of the net CO_2 assimilation was incorporated into plant biomass production, whereas 40% was released again in plant respiration (Fig. 2A). Of this carbon loss, about one-half was attributed to shoot respiration in the dark, whereas root respiration in the light and dark accounted for the remainder (Fig. 2A).

In terms of O_2 exchange, only 34% of the net O_2 released in photosynthesis was offset by respiratory O_2 consumption (Fig. 2B). Again, shoot respiration in the dark accounted for about one-half the O_2 consumption, whereas root respiration in the light and dark consumed the remainder. As a result, the net daily production of O_2 was about 13% higher than the net carbon gain. This was due to 2.6% more O_2 production than CO_2 fixation in photosynthesis, and 12% less O_2 uptake than CO_2 evolution in respiration (Fig. 2).

Gas Exchange Ratios and Diverted Reductant Utilization Rate (DRUR) in Shoots and Roots during the Day and Night

Integrated over the 12-h light or dark periods, and in all six replicate plants, the production of CO_2 or O_2 was of a greater magnitude than the corresponding uptake of O_2 or CO_2, respectively. Therefore, the GER values were consistently greater than 1.0 (Fig. 3A). During the light period, root GER was significantly greater than that in shoots, but since the total shoot gas exchange dwarfed that of roots (Fig. 1), whole-plant GER during the light was more a reflection of the shoot than the root (Fig. 3A).

In the dark, GER values for roots and shoots were not significantly different, ranging from 1.12 ± 0.01 to 1.17 ± 0.03 ($n = 6$), although the average GER for the whole plant in the dark (1.15 ± 0.02) was significantly greater than the whole-plant GER during the light (1.07 ± 0.01). Values for the 24- to 25-d-old experimental plant described in Figures 1 and 2 (○, Fig. 3A) were similar to the mean values for the six replicate gas exchange plants.

To provide information on the relative contribution of shoots and roots to "reductive biosynthesis" during the light and dark, calculations were made of the DRUR (Eq. 2) for each 2-h interval over the 2-d study period. Plant-to-plant variation in DRUR was large, ranging from 2.0 to 5.5 mmol e^- $plant^{-1}$ d^{-1}. To see through this variability and identify diurnal and organ-specific trends in DRUR, values were plotted as a percentage of whole-plant DRUR for the 24-h period (Fig. 3B).

Roots, every 2-h period of the day, consumed about 3% (light) or 2% (dark) of the whole-plant DRUR for

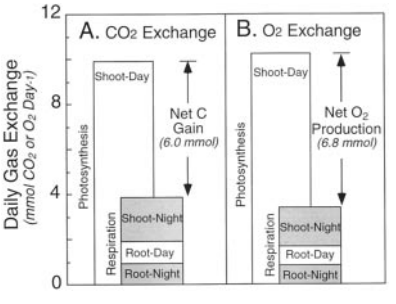

Figure 2. Net CO_2 (A) and O_2 (B) exchanges in the 24- to 25-d-old white lupin plant described in Figure 1. Shoot-day values are shown as photosynthetic CO_2 uptake (A) or O_2 production (B). Respiratory CO_2 loss (A) or O_2 uptake (B) for root-night, root-day, and shoot-night are presented in stacked bar graphs so that the difference between the sum of respiration and the photosynthetic values will illustrate the net carbon gain or the net O_2 production of the plant over the 24-h period. Values are means of three diurnal measurements obtained sequentially using a single plant.

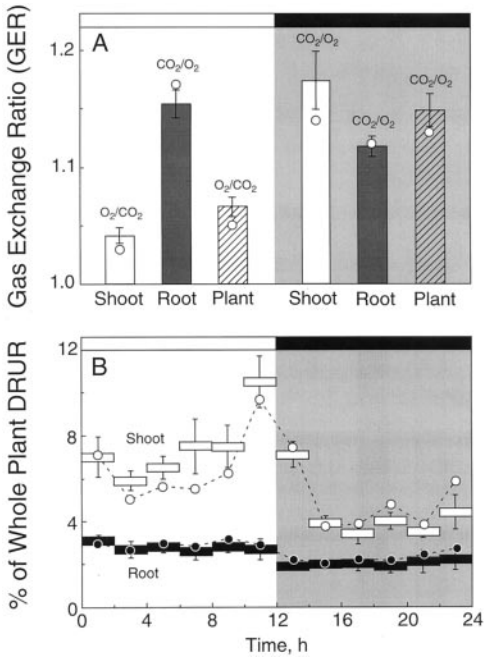

Figure 3. The imbalance between CO_2 and O_2 exchange in shoots and roots of white lupin plants quantified as either gas exchange (GER = [CO_2 production]/[O_2 uptake] or [O_2 production]/[CO_2 uptake]) (A) or as a percentage of whole-plant DRUR (4 × [CER + OER]) (B). In A, bar graphs denote GER values (±SE, $n = 6$) for 18- to 33-d-old shoots (□), roots (■), or whole plants (▨) during the light (white background) and dark (shaded background) periods. The circles represent the same values that were obtained for the 24- to 25-d-old experimental plant for which data is provided in Figures 1 and 2. In B, average DRUR values were calculated for each 2-h period of the day, and to minimize the effects of plant-to-plant variability, values were expressed (± SE, $n = 6$) as a percent of the total DRUR measured for each plant (Average whole-plant DRUR = 3.5 ± 0.6 mmol e^- $plant^{-1}$ d^{-1}). ○ and ● represented the same values for shoot and root, respectively, that were obtained for the 24- to 25-d-old experimental plants for which data was provided in Figures 1 and 2.

a 24-h period. In contrast, shoots contributed 6% to 10.5% of daily whole-plant DRUR for each 2-h period in the light, and about 4% for each 2-h period in the dark (Fig. 3B). Therefore, the shoot DRUR in the light period was about 1.8 times higher than that in the dark period, and the DRUR values of shoots were about 2.5 and 1.6 times higher than that of the root in the light and dark period, respectively (Fig. 3B). A similar pattern was obtained for the 24- to 25-d-old experimental plants described in Figures 1 and 2.

Relative Contribution of Shoot and Root to Reductive Biosynthesis

The results of Figure 3B for the 24- to 25-d-old experimental plant were integrated over a 24-h period and were used to calculate the relative contribution of shoots and roots in the light and dark to the daily whole-plant DRUR (Fig. 4A). The whole-plant DRUR was calculated as 3.2 μmol e^- $plant^{-1}$ d^{-1}. A larger proportion (68%) of DRUR was found in the shoot compared with the root (32%) and within each organ, DRUR values were greater in the light than in the dark (Fig. 4A).

However, shoot biomass in the study plants (2.07 g dry weight) was larger than root biomass (0.83 g dry weight), resulting in a shoot:root ratio (2.5) that was only slightly larger than the ratio of DRUR in shoots and roots (2.1). Since the shoots and roots had similar relative growth rates (RGR, Table I), if the DRUR values were normalized for biomass accumulation by plant organs, shoot and root values would be similar.

Deposition of DRUR Products in Plant Organs

Values of DRUR coefficient (β_{DRUR}, units of μmole e^- mg^{-1} element) were calculated from a knowledge of the net electron demand for nitrogen and sulfur reduction (items 1 and 2, Table II), or from the net reductant flow (per unit of carbon) associated with the synthesis of various organic matter constituents (items 4–10, Table II). Per milligram of nitrogen, sulfur, or organic matter product, the β_{DRUR} was highest for nitrogen and sulfur reduction (571 and 250 μmol e^- mg^{-1}, respectively), but also high for the synthesis of lipids and lignin from carbohydrate (102 and 28 μmol e^- mg^{-1}, respectively). Organic acid, being more oxidized per carbon than carbohydrate, had a negative β_{DRUR} (−36 μmol e^- mg^{-1}; Table II).

By combining the β_{DRUR} values with measured or estimated values for biomass composition, an estimate was obtained for the RD (units of mmol e^- Δg^{-1} dry weight) that would be associated with the synthesis of each biomass component (Table II). Nitrate reduction dominated the overall reductant demand associated with each tissue, accounting for 82% of leaves, 63% of stems + petioles, and 65% of roots.

When these values for reductant demand were applied to the white lupin plant that was harvested at 26 d following 2 d of gas analysis measurements (24–25 d), an estimate was obtained for the DRUR that should have been associated with 1 d biosynthesis of new leaves, stems + petioles, and roots (Fig. 4B). The whole-plant DRUR of 3.5 mmol e^- $plant^{-1}$ d^{-1} was predicted to be slightly greater in a 26-d-old plant than that which was measured in the same plant at 24 to 25 d old (3.2 mmol e^- $plant^{-1}$ d^{-1}, Fig. 4A). The biosynthesis of new leaves was predicted to account for 56% of whole-plant DRUR (Fig. 4B), whereas values for stems + petioles and roots were 19% and 25%, respectively.

DISCUSSION

Whole-Plant CO_2 and O_2 Exchange

Previous studies have reported simultaneous measurements of CO_2 and O_2 exchange in plant tissues or organs (Myers, 1949; Fock et al., 1971, 1972; Kaplan and Bjorkman, 1980; Bloom et al., 1989; Weger and

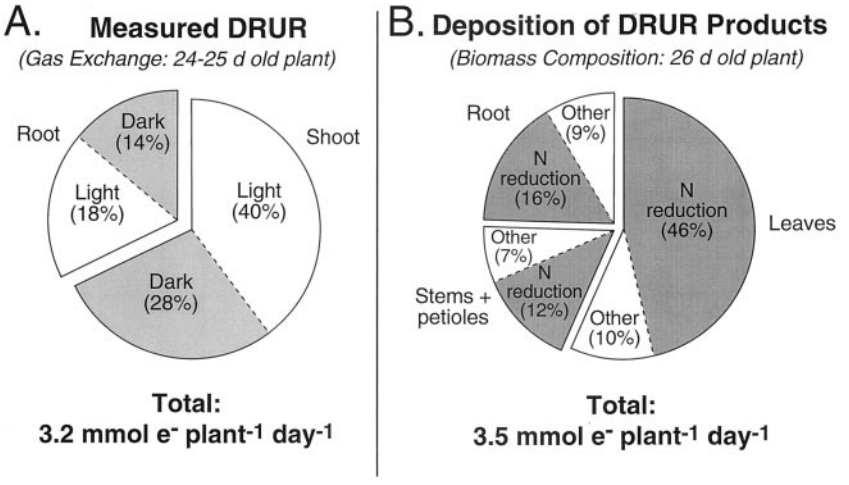

Figure 4. The contribution of shoot and root tissues to the daily DRUR measured by gas exchange (A) or calculated from the growth of biomass (B) in white lupin plants. The measured values in A were obtained from the average data for the 24- to 25-d-old plant as reported in Figure 3B. The values for B were obtained from the results and calculations of Tables I and II, as described in the text.

Turpin, 1989; Cramer and Lewis, 1993; Tolbert et al., 1995; Scheurwater et al., 1998; Van Der Westhuizen and Cramer, 1998; Willms et al., 1999). However, to our knowledge this is the first report of long-term, continuous, simultaneous measurements of CO_2 and O_2 exchange within a whole plant.

This study was made possible through the use of a differential oxygen analyzer (Willms et al., 1997), which was able to measure less than 2 μL L^{-1} O_2 differentials between a reference and analytical gas stream having the composition of air (20.95% [v/v] or 209,500 μL L^{-1} O_2). Given that the gas exchange system used here (Fig. 5) allowed the plants to generate differentials of 180 to 250 μL L^{-1}, and contained a single calibration system for the O_2 and CO_2 analyzers (Willms et al., 1999), sufficient precision was available to measure small differences between CO_2 and O_2 exchange.

In roots and shoots, and during light and dark, the production of CO_2 or O_2 was consistently higher than the corresponding uptake of O_2 or CO_2 in the same plant organs. This is the gas exchange signature of a plant tissue that is synthesizing biomass, which is more reduced per unit of carbon than carbohydrate. For example, in photosynthetic tissues in which O_2 production is greater than CO_2 fixation, the difference between the two exchanges represents the flow of reducing power that is diverted to alternative pathways such as nitrate reduction (Bloom et al., 1989; Weger and Turpin, 1989) or oil synthesis (Willms et al., 1999). In a similar manner, in respiring tissues where CO_2 production is greater than O_2 uptake, the difference between the two exchanges represents the flow of reducing power into processes such as nitrate reduction (Bloom et al., 1989; Weger and Turpin, 1989), oil synthesis (Willms et al., 1999), or other biochemical pathways that results in a final biomass that is more reduced per unit of carbon than the initial biomass.

GER, DRUR, and Reductive Biosynthesis

In the present study with white lupin, the calculated values for gas exchange (GER = [CO_2 production]/[O_2 uptake] or [O_2 production]/[CO_2 uptake]) were consistently greater than 1.0, but less than 1.2 (Fig. 3A). This range of values was similar to those obtained in many previous studies (Kaplan and Bjorkman, 1980; Bloom et al., 1989), but differed from other reports for leaves (GER = 0.79; Fock et al., 1971, 1972), shoot tissue (GER = −2 to 1; Tolbert et al., 1995), and roots (GER = 0.5–1.1; Cramer and Lewis, 1993; Van Der Westhuizen and Cramer, 1998).

One problem in the interpretation of GER values is that they are very sensitive to variations in specific gas exchange rates of the tissues. For example, in the present study, the GER in the shoot in the light (1.04 ± 0.01) was lower than that of roots (1.15 ± 0.02), but it contributed a larger proportion (40%) of whole-plant DRUR than did the root (18%; Fig. 4A). This illustrates the value of the DRUR term over the GER term as a quantitative measure of reductant flow to biosynthetic processes. In essence, the difference between CER and OER provides more valuable information than the ratio of the two gas exchanges (Willms et al., 1999).

When DRUR was calculated for each 2-h period, the values were consistently positive, but higher in shoots than in roots, and greater in the light than in the dark (Fig. 3B). This pattern was observed in the 24- to 25-d-old study plant for which detailed data was provided (Figs. 1 and 2; Table I), and in the five other white lupin plants (ages ranging from 18–33 d) that were treated in a similar fashion (Fig. 3B).

NO_3^- assimilation in leaves may be higher in the light than in the dark due to an enhanced nutrient uptake from the roots and to the potential for the light reactions of photosynthesis to provide directly, the reductant demand for nitrate reductase (Bloom et al., 1989).

Table II. *Calculation of the theoretical reductant demand (RD, units of mmol e^{-2} Δg^{-1} dry wt of each plant part) associated with the biosynthesis of various mineral and organic constituents of leaf, stem + petiole, or root tissues*

RD was calculated as the product of relative composition of leaves, stems + petioles (S + P), or roots and the DRUR coefficients (β_{DRUR}, units of μmol e^- imbalance between CER and OER per milligram of compound produced). β_{DRUR} values were calculated from known biochemical pathways as described in the text, where the products of metabolism are more (positive value) or less (negative value) reduced per unit carbon than the substrates from which they are derived.

Item No.	Biosynthesis of Organic Compounds	Biomass Composition[a]			β_{DRUR}[b]	RD[b]		
		Leaves	S + P	Roots		Leaves	S + P	Roots
		mg Δg^{-1} dry wt			*μmole e^- mg^{-1} element or compound*	*mmole e^- Δg^{-1} dry wt*		
	Mineral Elements							
1	NO_3^- (N^{5+}) → NH_3 (N^{3-})	33.0	12.8	18.0	571	18.9	7.30	10.3
2	SO_4^{2-} (S^{6+}) → SH^- (S^{2-})	2.2	3.5	8.7	250	0.550	0.875	2.18
3	Other minerals	25.0	25.0	25.0	0	0	0	0
	Organic Matter							
4	$(CH_2O)n$ → Protein	179.0	84.1	84.1	5.2	0.928	0.436	0.436
5	$(CH_2O)n$ → Free amino acids	20.6	9.4	9.4	−5.1	−0.106	−0.048	−0.048
6	$(CH_2O)n$ → Nucleic acids	6.2	2.9	2.9	−23.0	−0.142	−0.067	−0.067
7	$(CH_2O)n$ → Lipids	25.0	25.0	25.0	102	2.55	2.55	2.55
8	$(CH_2O)n$ → Lignins	80.0	80.0	80.0	28.4	2.27	2.27	2.27
9	$(CH_2O)n$ → Organic acids	50.0	50.0	50.0	−36.1	−1.81	−1.81	−1.81
10	$H_2O + CO_2 \rightarrow (CH_2O)$	579.0	707.3	696.9	0	0	0	0
11	Total	1,000	1,000	1,000	–	23.1	11.5	15.8

[a] Values for nitrogen and sulphur composition were measured elemental contents in plant white lupin tissues. The nitrogen content was corrected for changes in the NO_3^- pool size in tissues. For other components, the organic composition of plant biomass was according to Penning De Vries et al. (1974). [b] Calculated from known biochemical pathways assuming Suc, NH_3, and reduced sulphur were the starting material for synthesizing other organic compounds. The amino acid composition of protein was assumed to be the same as Rubisco (Ramshaw, 1982), and the free amino acid composition was as described by Chu et al. (1974). The theoretical DRUR production was obtained as the product of β_{DRUR} and the corresponding biomass composition.

The greater increase in shoot DRUR in the last 2 h of the light period may also be attributed to NO_3^- assimilation, especially if carbohydrates pools are filled as has been described previously for algal systems (Huppe and Turpin, 1994). In contrast, the higher shoot DRUR during the first 2 h of the dark period may be similar to previous studies (Scaife and Schloemer, 1994; Delhon et al., 1995) in which nitrate reduction did not decline until about 2 h in the dark. However, other reductive biosynthesis in plant such as fatty acids biosynthesis might also be involved in the dynamic changes of DRUR. For example, Willms et al. (1999) reported higher DRUR values in soybean fruit in the light than in the dark, and suggested that this may be due to light stimulated oil synthesis in developing fruits.

Comparison of Measured DRUR and the Deposition of DRUR Products

In the 24- to 25-d-old white lupin plant, the whole-plant DRUR over a 24-h period was measured at 3.2 mmol $plant^{-1}$ d^{-1}, with 68% of this being attributed to the shoot tissue during the light and dark (Fig. 4A). Although the measured DRUR provided information on the site of reductive biosynthesis, the ultimate deposition of the reduced products within plant tissues may be very different, since the xylem and phloem may redistribute highly oxidized (e.g. organic acids) or highly reduced (e.g. reduced nitrogen, lipids, etc.) compounds around the plant after they have been synthesized.

The deposition of the biosynthetic products that were more (or less) reduced per unit of carbon than carbohydrate was estimated from the growth rate and composition of biomass in the study plant (Fig. 4B). Although this theoretical, whole-plant DRUR was slightly larger (3.5 mmol $plant^{-1}$ d^{-1}) than that measured by gas exchange (3.2 mmol $plant^{-1}$ d^{-1}), the former was based on a plant that was 1 to 2 d older than the plant used for gas exchange, and there were many assumptions about the precise tissue composition. As a consequence, the whole-plant values for measured DRUR from gas exchange were considered to be in good agreement with those calculated for deposition of DRUR products from tissue composition.

The deposition of DRUR products in the shoot accounted for 75% of whole-plant DRUR, and 58% of whole-plant DRUR products were associated with nitrogen reduction (nitrate assimilation). In contrast, shoot gas exchange accounted for only 68% of whole plant DRUR, suggesting that some of the nitrogen and other reduced products deposited in shoot tissues were, in fact, reduced in the root and translocated to the shoot, presumably in the xylem (Atkins et al., 1979).

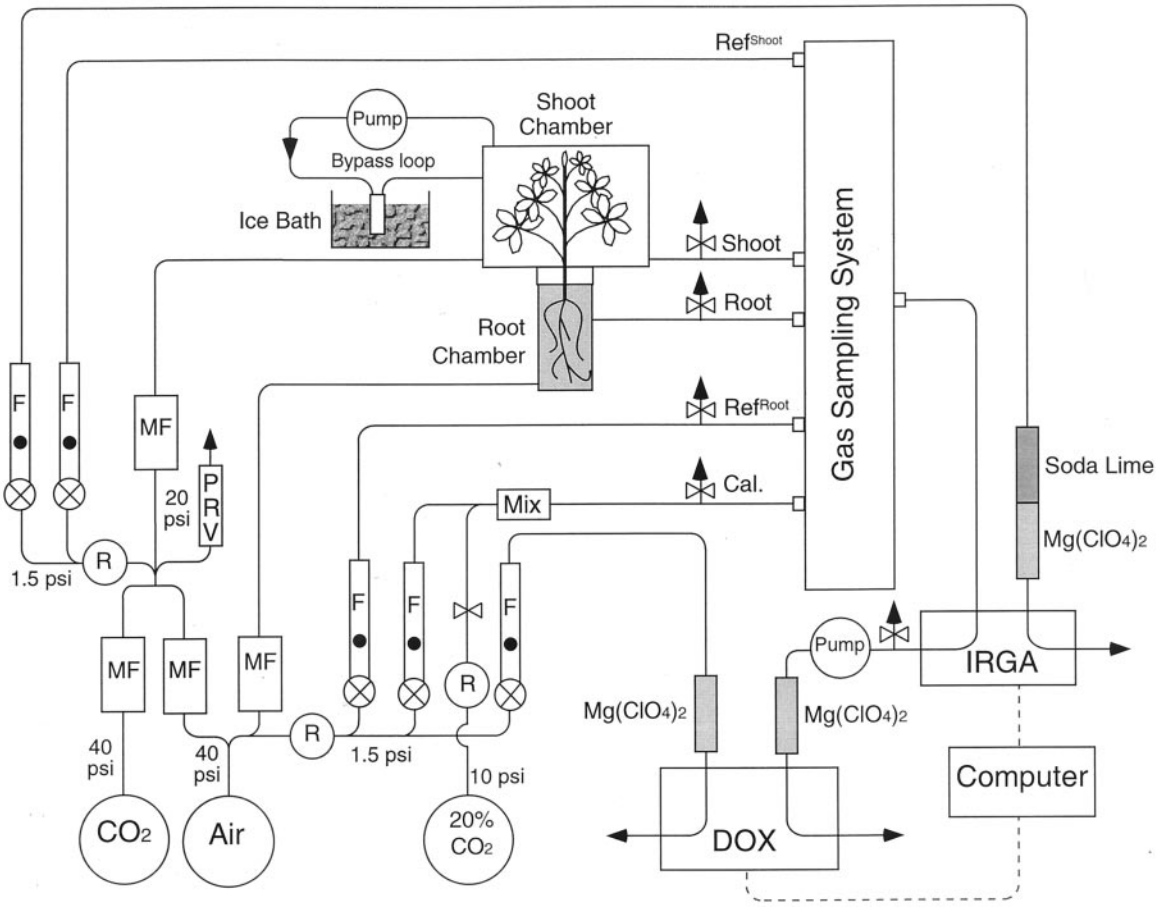

Figure 5. A schematic diagram of a system capable of making continuous, simultaneous measurements of CO_2 and O_2 exchange in intact roots and shoots. See text for description. Cal, Calibration gas stream; DOX, differential O_2 analyzer; IRGA, infrared CO_2/H_2O analyzer; F, flow meter; MF = mass flow controller; PRV, pressure release valve (set at 20 psi); R, downstream pressure regulator; Ref^{Root}, reference supply gas to root; Ref^{Shoot}, reference supply gas to shoot.

The Site of Nitrate Reduction in White Lupin

A previous study (Pate et al., 1979) estimated that over 90% of the nitrogen assimilation in white lupin roots was associated with root nitrate reduction; the shoot having only a minor role in this process. In making this conclusion, they coupled compositional analyses of xylem and phloem sap in white lupin with an empirical model of whole-plant carbon and nitrogen transport.

The present study offers a very different conclusion from that of Pate et al. (1979), highlighting a much more important role for the shoot in nitrate reduction in white lupin plants. Root DRUR measured by gas exchange accounted for only 32% of whole-plant DRUR (Fig. 4A), and on a whole-plant basis, nitrate reduction to ammonia was estimated to account for 74% of whole-plant DRUR (Fig. 4B). Given these constraints, and assuming that the entire DRUR of roots or shoots could be associated with nitrate reduction, root nitrate reduction could account for 8% to 43% of whole-plant nitrate reduction, whereas shoot nitrate reduction could account for 57% to 92% of whole-plant nitrate reduction (calculations not shown).

There are a number of possible explanations for this discrepancy. First, the white lupin cultivar and growing conditions used in the present study were different from that used previously (Pate et al., 1979), and this might have affected the site of nitrate reduction. Second, the nitrate and amino acid composition of the xylem and phloem saps in the earlier study may have been altered during sampling, and therefore may not have provided a reliable measure of nitrate reduction in plant tissues (Rufty et al., 1982; Andrews, 1986). Third, the DRUR measurements from gas exchange used in the present study may not reflect the site of nitrate reduction in plant tissues. For example, if the shoots were to synthesize highly reduced organic compounds and send them to the root (or if the root were to synthesize highly oxidized compounds and send them to the shoot), the DRUR associated with nitrate reduction would not be able to be distinguished from background rates of reductive (or oxidative) biosynthesis within plant organs.

Allen and Raven (1987) showed there to be a net flux of organic anions from root to shoot to help balance the nitrate anion that is taken up from the medium. Also, organic anions may be synthesized from carbohydrates in the roots and then excreted to and accumulated in the rhizosphere (Loss et al., 1994), thereby lowering the DRUR of the roots. This latter explanation could be tested by measuring DRUR in plant roots and leaves in the presence and absence of added $^{15}NO_3^-$ and ammonia.

Using Gas Exchange to Study Reductive Biosynthesis in Plants

The simultaneous measurements of CO_2 and O_2 exchange, and the use of these data to calculate DRUR offers a potentially valuable tool for the noninvasive study of key metabolic pathways in plants. Integrated over a 24-h period, the measured DRUR of shoots and roots were a good fit to what would be expected from the composition of the plant tissues being synthesized. Further studies are needed to link these DRUR measurements to specific biosynthetic processes, but when this has been done with various tissues and environmental conditions, the method described here should offer a powerful tool for the study of metabolic regulation in intact plants or plant organs.

MATERIALS AND METHODS

Plant Material

Seeds of white lupin (*Lupinus albus* cv Manitoba) were sterilized (0.3% [w/v]sodium hypochlorite for 3 min) and rinsed with water before being germinated in silica sand in 0.72-L pots that could be sealed for gas exchange measurements (Hunt et al., 1989). The plants were watered twice a day with a nutrient solution (Walsh et al., 1987) containing 1 mM KNO_3 (0–19 d) or 5 mM KNO_3 (20+ d) and were maintained in growth chambers (Conviron model PGV 36, Winnipeg, Manitoba) with a 12-h photoperiod and a temperature of 22°C/17°C (day/night). Photosynthetically active radiation was 500 μmol quanta m^{-2} s^{-1} at the plant level and relative humidity was 75%. Gas exchange measurements were conducted over sequential 2- to 4-d periods when the plants were 18 to 33 d old. No nodules were present on the plants used in the study.

The Gas-Exchange System

Plant CO_2 and O_2 gas exchange measurements were conducted using an open gas exchange system with separate shoot and root chambers as shown in Figure 5. Compressed air drawn from outside of the building was provided directly to the root chamber (approximately 150–250 mL min^{-1}), but was mixed with pure CO_2 before being supplied to the shoot chamber (approximately 1–2 L min^{-1}). Sufficient CO_2 was provided to give an effluent gas stream of about 360 ± 20 μL L^{-1}. All flow rates for gas mixing and chamber supply were regulated using thermal mass flow controllers (FMA-100 series, OMEGA Engineering, Stamford, CT). The flow rates through the root and shoot chamber, and the size of the plants used were set to provide CO_2 and O_2 differentials of between 180 and 250 μL L^{-1}. To minimize gas volume and exchange with the environment, all tubing was kept short and made of copper or Bev-A-Line IV (Warehouse Plastic Sales, Toronto).

A shoot chamber (300 mm wide, 300 mm deep, and 180 mm high, 16-L volume) was made with Plexiglas (clear, 4 mm thick) sides and a glass top. It housed the entire shoot of the plant and was tightly sealed onto a Plexiglas (clear, 6 mm thick) plate designed so that the base of the stem could be sealed with Qubitac sealant (Qubit Systems, Canada). Five 12V electric fans located inside the chamber maintained an air flow of about 1.2 m^3 min^{-1} and a copper tube (6.5 mm OD ×1.8 m long) was installed in the chamber so that it could be flushed with cold water to maintain the air temperature at 28°C ± 1°C in the day period and 25°C ± 1°C during the night. Three micro-thermocouples (model N-965, OMEGA Engineering) were used to monitor the air temperature at three different locations within the chamber. To control humidity, a pump was used to draw air (about 2–6 L min^{-1}) from the chamber and pass it through a copper coil to a glass container (69 mL) immersed in an ice bath before returning to the chamber. The flow rate of this bypass pump was adjusted to maintain a relative humidity in the shoot chamber of about 85% during the light period, and 75% during the dark period. The chamber was illuminated from above by an array of 6 × 100 W halogen lamp (General Electric, Canada) to give a photon flux density of 700 μmol quanta m^{-2} s^{-1} (photosynthetically active radiation) at the plant level. The light was passed through a water filter to absorb infrared radiation.

A multi-channel gas sampling system (Layzell et al., 1989) was used to select one of five gas streams for analysis, including the effluent from the shoot and root chambers, reference gas streams similar to those provided to the shoot (Ref^{shoot}) and root (Ref^{root}) chambers, and a calibration gas stream. The calibration gas stream was prepared by mixing (1 L min^{-1}) the outside air (21% [v/v] O_2) with a small volume (0.25–1 mL min^{-1}) of 20.2% (v/v) CO_2 in N_2. This resulted in the calibration gas being enriched in CO_2 and diluted in O_2 by about 50 to 200 μL L^{-1}, but at a precise ratio of 0.96 (Willms et al., 1997, 1999). This gas stream allowed simultaneous calibration of the infrared CO_2 and the differential O_2 analyzers.

The selected gas from the sampling system was passed through an IRGA (LI-6262, LI-COR, Lincoln, NE) before it was subsampled, dried (15-mL column of magnesium perchlorate), and provided to a DOX (S-3A/DOX, AEI Technologies, Pittsburgh). Information on the water vapor content of the effluent gas from the shoot chamber was used to monitor and maintain the humidity in the chamber by manual adjustment of the pump moving gas past the dehumidifier on the bypass loop.

The signals from the IRGA (absolute CO_2 and absolute H_2O) and DOX (differential and absolute O_2, differential

and absolute pressure, and temperature of the differential O_2 block) were collected using Workbench software (Version 4.01, Strawberry Tree Software, Sunnyvale, CA) running on a Macintosh computer (Apple Computer, Cupertino, CA). The same computer and software was used to control the mass flow controllers. The shoot chamber was tested with various combinations of flow rate (400–5,000 mL min^{-1}), CO_2 concentration (0–1,500 μL L^{-1}), and relative humidity (0%, 50%, and 95%). Under steady-state conditions, no significant differences were found in the inlet and effluent gas streams over this range of the measurement conditions (data not shown).

Plant Growth, Nitrogen, and Sulfur Analysis

Plant dry weight and growth rate were measured by harvesting randomly selected plants from the same population at intervals through the study period. In addition, plants randomly selected for gas exchange measurements were harvested at the end of the study. Leaves, stems, petioles, and roots were separated and dried in an oven (70°C) for 5 d before dry weights were recorded. RGR rates were calculated according to Radford (1967) using the equation of RGR = (ln dry $weight_2$ − ln dry $weight_1$)(d_2 − d_1), where the d is plant age in days and subscripts "1" and "2" refer to values obtained at the start and at the end of the study period, respectively. Total nitrogen, sulfur, and nitrate nitrogen were measured in dried plant samples. Nitrogen content was determined by automated combustion method (McGeehan and Naylor, 1988; Sweeney, 1989). Level of nitrate nitrogen was measured by reducing to nitrite at pH 7.5 in a copper-cadmium column (Atkins et al., 1979) and was determined colorimetrically (Wood et al., 1967). Sulfur determination was according to Tabatabi and Bremner (1970) with oxidation temperature at 1,350°C.

Gas-Exchange Calculations

Measurements of CO_2 and O_2 differentials between the inlet (Ref^{shoot} or Ref^{root}) and effluent gas streams of shoots or roots where used to calculate CO_2 (CER) and O_2 (OER) exchange rates (+, production; −, uptake). Since the CO_2 concentration in the gas stream was measured before dehumidification, corrections for the effects of water vapor on CER were carried out according to Long and Hallgren (1985). As described previously (Willms et al., 1999), the OER calculations were corrected for variations in differential pressure between the sample and reference streams, and for any imbalance in CO_2 and O_2 exchange that would have diluted or concentrated the effluent gas stream. For example, if CO_2 production was greater than O_2 uptake, the net gas production (CER and OER) would dilute the O_2 within the effluent stream. This was corrected for using Equation 2 in Willms et al. (1999).

Simultaneous CER and OER measurements of shoots and of roots were used to calculate the DRUR (units of mmol e^- $plant^{-1}$ h^{-1}) for each plant part:

$$DRUR = 4 \times (CER + OER) \quad (2)$$

where 4 is the number of electrons associated CO_2 or O_2 exchange. DRUR is a measure of the energy flow in plant tissues associated with biosynthesis or metabolism to yield a biomass that is more reduced (positive values) or less reduced (negative values) per unit of carbon, than carbohydrate (photosynthesis) or the initial substrate of carbon oxidation (respiration; Willms et al., 1999).

Tissue Deposition of DRUR Products

To determine how the measured DRUR values from gas exchange compared with the deposition of the products of reductive metabolism the following measurements, assumptions, and calculations were carried out:

1. The elemental composition for nitrogen and sulfur were made in leaves, stems + petioles, and roots of the 24- to 25-d-old study plants (items 1 and 2, Table II). Other mineral element (Item 3, Table II) and organic matter constituents (items 4–10, Table II) were assumed as per Penning De Vries et al. (1974). The relative tissue composition was assumed to be stable with time (i.e. mg g^{-1} dry weight = mg Δg^{-1} dry weight).

2. The value of β_{DRUR} (units of μmol e^- mg^{-1} element) was calculated as the number of electrons required to reduce each NO_3^- to NH_3 or each SO_4^{2-} to SH^- (items 1 and 2, Table II). This was based on $8e^-$ per nitrogen or sulfur reduced and atomic weights of 14 and 32 g M^{-1} for nitrogen and sulfur, respectively. Other minerals were assumed to not require reduction before incorporation into tissues (i.e. $\beta_{DRUR} = 0$).

3. The CO_2 and O_2 exchanges (μmol mg^{-1} compound) associated with producing each mg of the various organic constituents of tissues were calculated from the known biochemical pathways for converting carbohydrate into the respective compounds, assuming that the only other products or substrates were NH_3, SH^-, O_2, H_2O, or CO_2. If other compounds were required, pathways were included for how they could be made from carbohydrate. In a similar manner, if other products were made, pathways were included to ensure the full oxidation of these to CO_2 and H_2O. β_{DRUR} values (units of μmol e^- mg^{-1} compound) were calculated as four times the sum of net CO_2 and O_2 exchange, assuming $4e^-$ are associated with each net CO_2 or O_2 exchange. The tissue production of carbohydrate for direct incorporation into biomass (item 10, Table II), or as a substrate for other organic compounds (not shown), had a β_{DRUR} value of 0.

4. The theoretical RD (mmol e^- Δg^{-1} dry weight of each plant part) for the synthesis of leaf, stem + petiole, and root tissues was calculated as the product of the biomass composition and β_{DRUR} (Table II).

5. The theoretical DRUR (mmol e^- $plant^{-1}$ d^{-1}) in the 24- to 25-d-old study plant used for gas exchange measurements was calculated as the product of the theoretical reductant demand (Table II), the RGR, and the tissue biomass at harvest (26 d).

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