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## CARBON AND NITROGEN ALLOCATION TO MALE AND FEMALE REPRODUCTION IN ROCKY MOUNTAIN DOUGLAS-FIR (*PSEUDOTSUGA MENZIESII* VAR. *GLAUCA*, PINACEAE)<sup>1</sup>

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We measured carbon (respiration, photosynthesis, and production) and nitrogen allocation to male and female cones of Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) to quantify gender-specific: (1) resource allocation to reproduction, and (2) contribution to carbon costs of reproduction via photosynthesis. We also measured foliar photosynthesis and nitrogen concentration ([N]) near and far from female cones to examine the relationship between reproduction and foliar physiology. Over one growing season, male cones required only 8% of all carbon allocated to reproduction, with females consuming the remaining 92%. Female cones, however, had maximum instantaneous refixation rates of 54%, which, integrated over the season, offset 6% of their total carbon requirements, while male cones were completely dependent on vegetative tissues for carbon. Male cones received 22% of all nitrogen allocated to reproduction and female cones received the remaining 78%. Foliage near female cones had elevated photosynthesis during the early stages of cone development and consistently lower [N] than foliage far from cones. Although female cones may photosynthesize, the annual sum of carbon fixed by reproductive structures is minor in comparison to the total carbon allocated to production and respiration.

**Key words:** carbon; gender; nitrogen; photosynthesis; Pinaceae; *Pseudotsuga menziesii*; resource allocation; respiration.

The carbon costs of reproduction of many herbaceous, woody, and crop plants is partially offset by photosynthesis of the reproductive tissues (Bazzaz, Carlson, and Harper, 1979; Linder and Troeng, 1981; Werk and Ehleringer, 1983; Jurik, 1985; Williams, Koch, and Mooney, 1985; Koppel, Troeng, and Linder, 1987; Reekie and Bazzaz, 1987a, b; Dick, Smith, and Jarvis, 1990; Whiley, Schaffer, and Lara, 1992; Galen, Dawson, and Stanton, 1993; Ogawa et al., 1995; Ogawa and Takano, 1997). This photosynthetic capacity is important for understanding carbon allocation among male, female, and vegetative tissues.

Reproductive allocation theory assumes that the availability of plant resources, such as carbon or nitrogen, is fixed and that a trade-off exists between allocating resources to male vs. female tissues (reviewed in Charnov, 1982; Goldman and Willson, 1986; Charlesworth and Charlesworth, 1987; Charlesworth and Morgan, 1991). Both theoretical and quantitative research have concluded that female reproduction is generally more carbon-demanding than male (Goldman and Willson, 1986; Ashman, 1992). This conclusion is based on the greater biomass of female reproductive tissues, including the seeds and fruit, relative to that of males. However, resource allocation is an active process and biomass reflects a state at one point within that process. When resource allocation to reproduction is considered a process, it becomes

informative to include measurements of both carbon loss by respiration and carbon gain by photosynthesis of the reproductive tissues. In many native and crop species, female organs photosynthesize, thus contributing to their own carbon needs, while male structures are usually non-photosynthetic, so their carbon demands are met entirely by vegetative tissues (Galen, Dawson, and Stanton, 1993; Eckhart and Chapin, 1997).

In addition to supplying carbon to a plant's resource pool, reproductive organs also act as carbon and nutrient sinks. The strong carbon sink of developing fruits increases photosynthetic rates of neighboring foliage in herbaceous and crop species, thus increasing total available carbon (de Jong, 1986; Reekie and Bazzaz, 1987a; Bazzaz and Ackerly, 1992). Such an increase in carbon availability opposes the assumption that resource availability is fixed. However, reproductive tissues are also sinks for nitrogen from neighboring foliage (Chapin, 1989; Ashman, 1994). Nitrogen concentration ([N]) is usually positively correlated with photosynthetic rates (Field and Mooney, 1986). Therefore, any depletion of foliar nitrogen due to reproductive nitrogen demand may reduce foliar photosynthesis, confounding the increased photosynthesis associated with a reproductive carbon sink. Therefore, the sink activity of plant reproductive tissues has several counteracting effects on resource allocation within the entire plant.

The integration of photosynthesis and respiration in whole-tree carbon budgets is becoming increasingly common (reviewed in Ryan et al., 1994, and Sprugel et al., 1995). However, most carbon budgets assume carbon allocation to reproduction is negligible, despite evidence of high respiratory rates in reproductive tissues. For exam-

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ple, in a review of the genus *Pinus*, Ryan et al. (1994) found that female cones commonly had high respiration rates similar to those of fine roots and foliage. Linder and Troeng (1981) quantified respiration of female cones of *Pinus contorta* and estimated that the carbon allocated to a single year of female cone production and respiration may equal as much as 10–15% of a tree's annual stem wood production and respiration. However, female cone photosynthesis may offset 16–31% of these high respiratory costs over the entire growth period of the cones (Linder and Troeng, 1981; Koppel, Troeng, and Linder, 1987; Dick, Smith, and Jarvis, 1990). Few data are available on the costs of male reproduction in coniferous trees, yet they may be a significant carbon investment for the tree due to their large numbers. Although cone respiration may be a large carbon sink, neither it nor cone photosynthesis has been well studied and consequently it is difficult to draw conclusions regarding the magnitude of carbon allocation to reproduction.

We examined the seasonal course of carbon allocation to respiration and growth, nitrogen allocation to growth, and photosynthesis in male and female cones of Rocky Mountain Douglas-fir [*Pseudotsuga menziesii* var. *glauca* (Mirb.) Franco]. We used these measurements to: (1) quantify the resource allocation of carbon and nitrogen of each gender, (2) determine the contribution of each gender to the carbon costs via photosynthesis, and (3) examine the relationship between foliar photosynthetic rates and reproductive activity.

## MATERIALS AND METHODS

**Study species and site**—*Pseudotsuga menziesii* var. *glauca* is the Rocky Mountain variety of Douglas-fir and is common in low- to mid-elevation forests of the interior western United States and Canada. It is a monoecious tree that produces cones on a 2-yr cycle by initiating reproductive buds in the early summer of one growing season and completing bud emergence, pollination, fertilization, seed development, and dispersal in the following (Owens, 1991). This study was conducted during the 1997 growing season at two sites within 300 m of each other in a forest near Deary, Idaho (latitude 46°45' N, longitude 116°33' W, elevation 884 m). On nine dates between 3 May and 14 September, samples were collected from at least six of 13 different trees of approximately equal age, height, and light exposure in order to capture the entire course of cone development. Intervals between sampling dates were shorter during early cone development in order to capture the high rates of change of photosynthesis and respiration associated with the rapid growth rates during this period. Capturing this variability would then improve our annual estimates of carbon fluxes from cones. Sampling intervals increased as growth, photosynthesis, and respiration rates stabilized. Trees were ~80 yr-old, 30 m tall, and had fully exposed, south-facing canopies with crowns extending the full height of the trees. We collected 2–6 samples per tree for each type of needle and cone.

**Field measurements**—Gas exchange measurements were made with the LI-6200 Portable Photosynthesis System (LI-COR Instruments, Lincoln, Nebraska, USA). Measurements were made between 0900 and 1400 local time to insure full sunlight exposure on the south-facing sites. Samples were cut from the trees and measurements begun within 1 min. Cones and needles were randomly selected from throughout the canopy between 1.5 and 11.5 m above the ground.

Male cones were sampled on the first three sampling dates (3 May, 11 May, and 18 May), after which they senesced. *Pseudotsuga menziesii* male cones develop on a 1-yr-old shoot in clusters of 3–15 cones between needles. In order to measure gas exchange of the male cones

while minimizing handling of the fragile cones and resultant pollen loss, needles were removed from the twig around the cones and the entire shoot was placed in the cuvette for respiration measurement ( $R_{\text{tot}}$ ). Then, on several samples, the cones were removed from the twig and twig respiration was measured ( $R_{\text{twig}}$ ). Linear regression between measured total cone and twig respiration predicted twig respiration for those samples for which only  $R_{\text{tot}}$  was measured ( $N = 30$ ,  $r^2 = 0.83$ ). Cone respiration ( $R_{\text{cone}}$ ) is the difference between  $R_{\text{tot}}$  and  $R_{\text{twig}}$ . An independent sample  $t$  test between  $R_{\text{tot}}$  measurements made with needles removed immediately prior to placement in the cuvette and  $R_{\text{tot}}$  measurements with needles removed several hours prior showed no significant wound respiration on the twigs resulted from needle removal ( $df = 28$ ,  $t = -0.41$ ,  $P = 0.680$ ).

Net respiration of female cones was measured in full sunlight on each of the nine sampling dates. The cones were then shaded for 1 min, and gross respiration ( $R_{\text{cone}}$ ) was measured. Gross respiration was also measured on some cones after 30 min of shading, and a paired  $t$  test showed no significant effect of these shade times on  $R_{\text{cone}}$  rates ( $df = 3$ ,  $t = 0.125$ ,  $P = 0.908$ ). Photosynthesis was calculated as the difference between net respiration and  $R_{\text{cone}}$ . The temperature response ( $Q_{10}$ ), or change in respiration with a 10°C change in temperature, was estimated on male and female cones during one diel period on 18 May. Temperature and respiration of male and female cones were measured every 3–4 h over a temperature range of 14°–31°C.

Net photosynthesis of needles was measured in full sunlight. Needles were sampled from 1-yr-old shoots that both did and did not terminate with a female cone. On those shoots with cones, the cone was left attached to the twig, but was not placed in the cuvette with the needles.

Daily maximum and minimum temperatures were measured with a thermometer located ~400 m from the field site. For the dates on which no temperature data were recorded, a regression using nearby weather station temperatures was used to predict field site temperatures.

**Laboratory measurements**—After field measurements were made, samples were kept frozen until they could be oven dried at 60°C for 24 h and weighed. Rates were then calculated per kilogram dry mass. Carbon and nitrogen concentrations were determined using a LECO 600 CHN analyzer (LECO, Inc., St. Joseph, Michigan, USA). Due to the low mass of individual samples, particularly at early dates, samples (i.e., male cones, female cones, needles) were pooled for each date and ground using a mortar and pestle. Three subsamples of a pooled sample per date were analyzed.

**Calculations**—Relative growth rates (RGR) were calculated for male and female cones for each sample date using the following equation:

$$\text{RGR} = (\ln W_2 - \ln W_1) / (t_2 - t_1) \quad (1)$$

where  $W_2$  was the dry mass in kilograms at date 2 and  $W_1$  was the dry mass at the previous date. Total allocation of carbon to production for an average male and female cone was estimated as the final average dry mass multiplied by the average carbon concentration.

We normalized all respiration rates to a common temperature of 15°C using the gender-specific  $Q_{10}$  for statistical comparisons and seasonal scaling. The relationship between respiration and temperature was expressed as (Lavigne, 1987):

$$R = R_{15} Q_{10}^{(T-15)/10} \quad (2)$$

where  $R$  is measured respiration rate per kilogram tissue per second,  $R_{15}$  is respiration at 15°C,  $Q_{10}$  is the ratio of respiration rate at some temperature and the rate at 10°C higher than that temperature, and  $T$  is temperature (°C). Equation 2 was linearized through logarithmic transformation:

$$\ln(R) = \beta_0 + \beta_1 [(T - 15)/10] \quad (3)$$

where  $\beta_0$  is  $\ln(R_{15})$  and  $\beta_1$  is  $\ln(Q_{10})$ . Values of  $\beta_0$  and  $\beta_1$  were estimated by linear regression.

TABLE 1. Average ( $\pm 1$  SE) dry mass, carbon concentration, carbon content, nitrogen concentration, and nitrogen content for male cones of *P. menziesii* on each sampling date. The standard errors for carbon and nitrogen concentration are for bulked samples, not for individual cones.

Date (day of year)	N	Dry mass (g/cone)	[C] (mol C/kg dry mass)	Carbon content ( $10^{-3}$ mol C/cone)	[N] (mol N/kg dry mass)	Nitrogen content ( $10^{-3}$ mol N/cone)
3 May (123)	23	0.022 ( $\pm 0.002$ )	42.07 ( $\pm 0.06$ )	0.94 ( $\pm 0.07$ )	1.52 ( $\pm 0.03$ )	0.034 ( $\pm 0.002$ )
11 May (131)	17	0.014 ( $\pm 0.001$ )	40.73 ( $\pm 0.04$ )	0.57 ( $\pm 0.05$ )	0.84 ( $\pm 0.01$ )	0.012 ( $\pm 0.001$ )
18 May (137)	19	0.011 ( $\pm 0.001$ )	41.79 ( $\pm 0.02$ )	0.48 ( $\pm 0.03$ )	0.74 ( $\pm 0.05$ )	0.009 ( $\pm 0.001$ )

Annual respiration from male and female cones was estimated using gender-specific  $Q_{10}$ 's and site temperature data with the following equation:

$$R_t = R_{10} Q_{10}^{(T-15)/10} I_o (\beta_2 A_d) 86400 \tag{4}$$

where  $R_t$  is total respiration (in micromoles per kilogram per day),  $R_{10}$  is the respiration rate at 10°C,  $T$  is the mean daily temperature,  $I_o$  is a function to correct for diurnal variation in temperature (Ågren and Axelsson, 1980),  $\beta_2$  equals  $(\ln Q_{10})/10$ ,  $A_d$  is the daily temperature amplitude  $(T_{max} - T_{min})/2$ , and 86400 converts seconds to days. Linear interpolation was used to estimate  $R_{10}$  between measurement dates. Annual allocation of carbon to respiration was estimated as the sum of respiration for all days during which cones were respiring.

Seasonal photosynthesis for female cones was calculated by estimating daily photosynthetic rates via linear interpolation between measurement dates. We then assumed that cone photosynthesis was active for 2 h less than total daylength each day based on the light response of the cones (S. McDowell, unpublished data). This estimate of photosynthesis is an upper limit because it assumes no light limitation. However, light limitation for the cones was minimal because of few cloudy days during the measurement period at this site, the well-lit south-facing canopies, and the minimal within-canopy shading of cones due to cone position on branch tips. The annual percentage of cone carbon needs supplied by cone photosynthesis was calculated as the sum of photosynthesis for the season, divided by total cone respiration and production.

We determined the relationship between respiration and [N] for male and female cones via linear regression of the mean respiration rates and [N] from each date. Likewise, mean photosynthetic rates for female cones from each date were regressed against tissue [N] to determine the relationship between female photosynthesis and nitrogen.

All statistical analyses were done with the SYSTAT 5.03 statistical package (Wilkinson, 1992) with  $\alpha = 0.05$  level of significance.

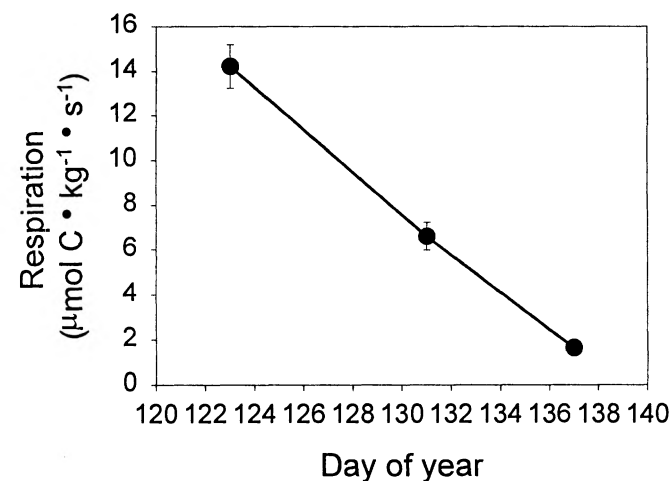


Fig. 1. Respiration rates of male cones of *P. menziesii*. Error bars represent  $\pm 1$  SE.

RESULTS

**Male cones**—Growth of male cones was rapid, but their lifetimes were short. When they emerged from the bud on 1 May, the cones were purple. They achieved their greatest measured dry mass within 2 d of emergence (Table 1). RGR was not calculated because growth was finished before the second sampling date on 11 May. The cones lost 36% of their dry mass, 67% of their nitrogen content, and 39% of their carbon content by 11 May (Table 1). Male cones senesced and began to fall within 3 wk of budbreak.

The highest respiration rates we measured on male cones were within 2 d of emergence from the bud (Fig. 1). Average  $R_{cone}$  on 3 May (day of year 123) was  $14 \mu\text{mol C}\cdot\text{kg}^{-1}\cdot\text{sec}^{-1}$ , or  $0.31 \times 10^{-3} \mu\text{mol C}\cdot\text{cone}^{-1}\cdot\text{sec}^{-1}$  (Fig. 1). Within 1 wk,  $R_{cone}$  decreased by >50%, and by 18 May (day of year 137), it had fallen to  $1.7 \mu\text{mol C}\cdot\text{kg}^{-1}\cdot\text{sec}^{-1}$ , or  $0.019 \times 10^{-3} \mu\text{mol C}\cdot\text{cone}^{-1}\cdot\text{sec}^{-1}$  (Fig. 1). At this date, no temperature response was found ( $N = 30$ ,  $r^2 = 0.04$ ). Therefore, a  $Q_{10}$  of 2, the prevailing temperature response value for most plant tissues, was used to normalize rates to 15°C (Amthor, 1989). Respiration rates, averaged for ~20 cone samples per date, were well correlated with mean tissue [N], although this relationship was not significant ( $N = 3$ ,  $r^2 = 0.921$ ,  $P = 0.180$ ). Over the 18-d growing season of male cones, the respiratory cost of an average male cone equaled  $0.19 \times 10^{-3} \text{ mol C}\cdot\text{cone}^{-1}\cdot\text{yr}^{-1}$ . The production cost of an average male cone, including a mean [C] of 41.53 mol C/kg (Table 1), was  $0.91 \times 10^{-3} \text{ mol C}\cdot\text{cone}^{-1}\cdot\text{yr}^{-1}$ . Thus, the total carbon cost of an average male cone of *P. menziesii* at 15°C was  $1.1 \times 10^{-3} \text{ mol C}\cdot\text{cone}^{-1}\cdot\text{yr}^{-1}$ . Photosynthesis was not apparent on any sampling date.

**Female cones**—Female cones emerged from the buds on approximately the same date as the male cones, but completed their life cycle over 4 mo. They emerged upright and, like the males, purple. Within 1 wk the cone bracts began turning green, and after another week the cones turned downward and the scales also started turning green. Most female cones were entirely green by 31 May (day of year 151), 4 wk after budbreak. By 14 September (day of year 257), the cones had turned completely brown and were beginning to fall from the trees.

The dry mass of the female cones increased rapidly for the first 2 mo after emergence (Table 2). The dry mass continued to increase until the final sampling date when the cones lost 0.5 g, or 11% of their maximum dry mass, from the previous sampling date. The maximum RGR of  $0.10 \text{ g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$  was reached on 20 June, after which it continually declined (Table 2). Carbon and nitrogen contents of the cones increased throughout the growing sea-

TABLE 2. Mean dry mass, relative growth rate, carbon concentration, carbon content, nitrogen concentration, and nitrogen content ( $\pm 1$  SE) of female cones of *P. menziesii* on each sampling date. The missing SEs for some nitrogen data are due to a reagent failure during carbon, hydrogen, and nitrogen (CHN) elemental analysis. The standard errors for carbon and nitrogen concentration are for bulked samples, not for individual cone samples.

Date (day of year)	<i>N</i>	Dry mass (g/cone)	RGR ( $g \cdot g^{-1} \cdot d^{-1}$ )	[C] (mol C/kg dry mass)	Carbon content ( $10^{-3}$ mol C/cone)	[N] (mol N/kg dry mass)	Nitrogen content ( $10^{-3}$ mol N/cone)
3 May (123)	21	0.086 ( $\pm 0.008$ )	—	40.10 ( $\pm 0.03$ )	3.5 ( $\pm 0.3$ )	2.08 ( $\pm 0.02$ )	0.18 ( $\pm 0.02$ )
11 May (131)	17	0.149 ( $\pm 0.014$ )	0.072	39.49 ( $\pm 0.13$ )	5.9 ( $\pm 0.5$ )	2.04 (—)	0.31 (—)
18 May (137)	21	0.224 ( $\pm 0.018$ )	0.058	39.65 ( $\pm 0.03$ )	8.9 ( $\pm 0.7$ )	1.67 ( $\pm 0.03$ )	0.38 ( $\pm 0.03$ )
31 May (151)	22	0.863 ( $\pm 0.109$ )	0.104	40.89 ( $\pm 0.03$ )	35.3 ( $\pm 4.5$ )	1.21 ( $\pm 0.04$ )	1.04 ( $\pm 0.13$ )
20 Jun (171)	19	2.333 ( $\pm 0.160$ )	0.050	38.65 ( $\pm 0.15$ )	90.2 ( $\pm 6.2$ )	0.66 (—)	1.54 (—)
6 Jul (187)	17	3.309 ( $\pm 0.196$ )	0.022	38.73 ( $\pm 0.03$ )	128.2 ( $\pm 7.6$ )	0.64 ( $\pm 0.02$ )	2.12 ( $\pm 0.13$ )
22 Jul (203)	16	4.167 ( $\pm 0.353$ )	0.012	39.14 ( $\pm 0.07$ )	163.1 ( $\pm 13.8$ )	0.59 ( $\pm 0.02$ )	2.46 ( $\pm 0.21$ )
22 Aug (235)	12	4.473 ( $\pm 0.272$ )	0.003	40.59 ( $\pm 0.06$ )	181.5 ( $\pm 11.1$ )	0.52 ( $\pm 0.01$ )	2.33 ( $\pm 0.14$ )
14 Sep (257)	12	3.999 ( $\pm 0.347$ )	-0.005	40.06 ( $\pm 0.04$ )	160.2 ( $\pm 13.9$ )	0.33 ( $\pm 0.02$ )	1.32 ( $\pm 0.11$ )

son until a decrease of both on the final sampling date (Table 2).

Photosynthesis was not noticeable until 1 wk after budbreak when the bracts began turning green. The maximum photosynthetic rate per unit dry mass of  $9.59 \mu\text{mol C} \cdot \text{kg}^{-1} \cdot \text{sec}^{-1}$  was reached on 11 May (day of year 131),  $\sim 10$  d after budbreak (Fig. 2). By the end of August, the cones were photosynthesizing at  $< 5\%$  of their initial rate. Maximum photosynthetic rate per cone was achieved on 31 May (day of year 151), and remained relatively constant through the end of July (Fig. 3). No photosynthesis occurred on the final sampling date.

Female respiration rates followed a declining trend similar to that of the photosynthetic rates. Maximum  $R_{\text{cone}}$  of  $20.0 \mu\text{mol C} \cdot \text{kg}^{-1} \cdot \text{sec}^{-1}$  was measured on 11 May (day of year 131), the same date as the maximum photosynthetic rate (Fig. 2). Respiration rates per unit dry mass decreased dramatically between 31 May (day of year 151) and 20 June (day of year 171), and more gradually thereafter. During this same period, respiration rates per cone and total cone carbon demand (production + respiration) increased (Figs. 3, 4). On the final sampling date, respiration of the brown cones was barely apparent.

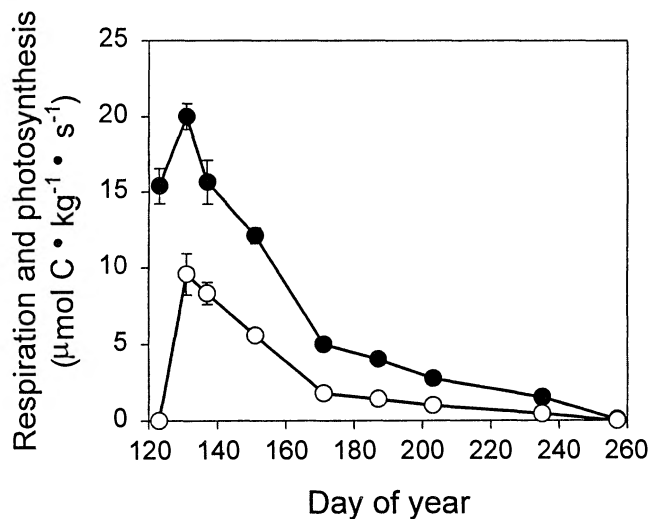
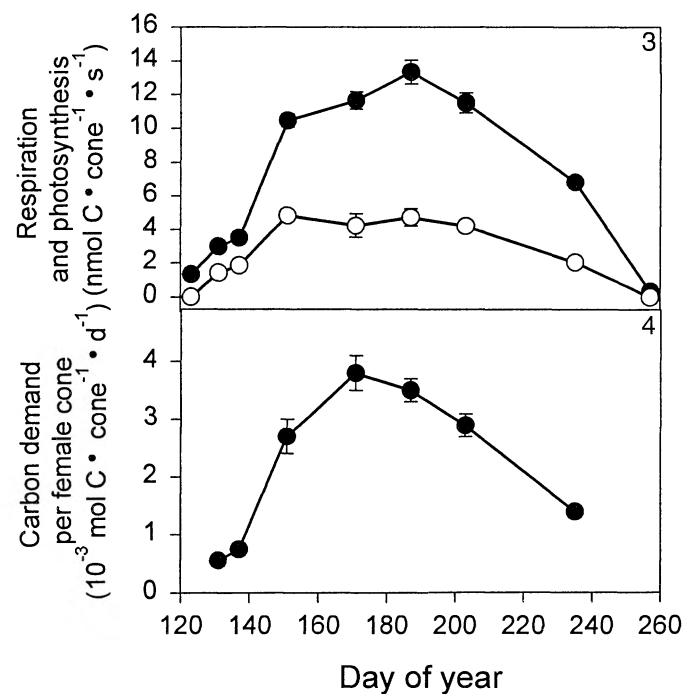


Fig. 2. Respiration (filled circles) and photosynthetic rates (open circles) of female cones of *P. menziesii* per unit dry mass. Error bars represent  $\pm 1$  SE.

Respiration rates of female cones did not statistically differ between canopy positions ( $F_{2,141} = 0.145$ ,  $P = 0.865$ ). The  $Q_{10}$  was 1.84 for the female cones ( $N = 17$ ,  $r^2 = 0.350$ ).

The percentage of daytime respiratory carbon costs met by female cone photosynthesis declined during the growth period (Fig. 5). Photosynthesis as a percentage of respiration ranged from a maximum on 18 May (mean  $\pm 1$  SE =  $54 \pm 2.3\%$ ) to a low on 22 August (mean  $\pm 1$  SE =  $30 \pm 3.9\%$ ) (Fig. 5). Over the entire 136-d growing season, an average female cone respired  $0.15 \text{ mol C} \cdot \text{cone}^{-1} \cdot \text{yr}^{-1}$  and photosynthesized  $0.02 \text{ mol C} \cdot \text{cone}^{-1} \cdot \text{yr}^{-1}$ , thus photosynthesis reduced annual respiratory costs by 13%. The production of an average female cone was  $0.18 \text{ mol C} \cdot \text{cone}^{-1} \cdot \text{yr}^{-1}$ . Therefore, the



Figs. 3–4. Carbon demand per female cone of *P. menziesii*. 3. Respiration (filled circles) and photosynthetic rates (open circles) per female cone. Error bars represent  $\pm 1$  SE. 4. Total carbon demand per female cone (production plus respiration per cone per day). Error bars represent  $\pm 1$  SE.

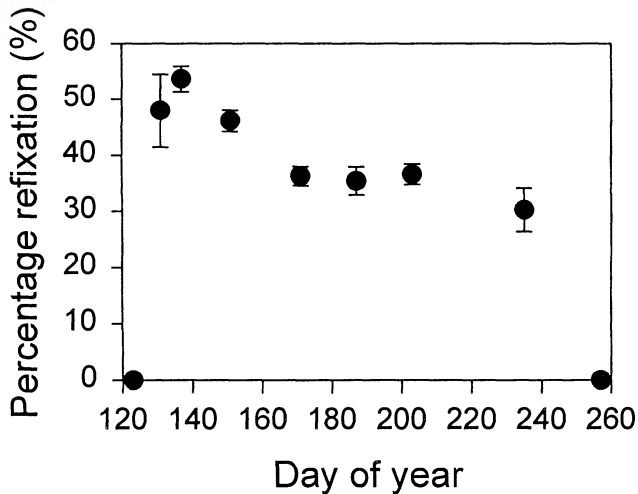


Fig. 5. The ratio of instantaneous photosynthesis and respiration of female cones of *P. menziesii*. Error bars represent  $\pm 1$  SE.

total carbon cost of a female cone was  $0.33 \text{ mol C}\cdot\text{cone}^{-1}\cdot\text{yr}^{-1}$ , with respiration consuming 46% of the total carbon allocation and dry matter production the remaining 54%. The total cost was reduced to  $0.31 \text{ mol C}\cdot\text{cone}^{-1}\cdot\text{yr}^{-1}$  when cone photosynthesis was included. In other words, 6% of the carbon cost of female reproduction of *P. menziesii* was covered by cone photosynthesis. Changes in nitrogen concentration of female cones through the year were strongly correlated with both respiration rates ( $N = 9, r^2 = 0.945, P < 0.0001$ ) and photosynthetic rates ( $N = 9, r^2 = 0.988, P < 0.0001$ ) (Fig. 6).

**Vegetative activity associated with reproduction**—The correlation between reproductive activity and needle photosynthetic rates was noticeable only in the 1st mo of measurements (Fig. 7). On 18 May (day of year 137), the photosynthetic rates of needles near cones was  $19.2 \mu\text{mol C}\cdot\text{kg}^{-1}\cdot\text{sec}^{-1}$ , while those of needles without any

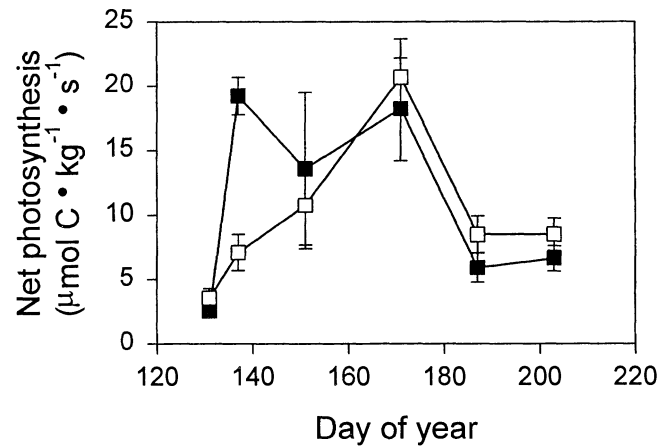


Fig. 7. Mean net foliar photosynthetic rates of current needles of *P. menziesii* on shoots terminating with female cones (filled squares) and needles on shoots without cones (open squares). Error bars represent  $\pm 1$  SE.

cones was  $7.1 \mu\text{mol C}\cdot\text{kg}^{-1}\cdot\text{sec}^{-1}$  ( $df = 14, t = -10.72, P < 0.0001$ ). Needle photosynthetic rates near cones declined to rates similar to those of needles without cones within 2 wk and remained statistically similar for the remainder of the season ( $df = 38, t = -0.322, P = 0.749$ ) (Fig. 7). Nitrogen concentration of needles near cones was significantly lower than needles without nearby cones for all sampling dates except the first (Fig. 8). Photosynthesis per unit nitrogen was higher for needles near cones than for needles without nearby cones for all dates, but this difference was significant only on 18 May ( $df = 14, t = 6.788, P < 0.0001$ ). Averaged over the whole year, foliar photosynthesis per unit nitrogen was slightly higher for needles with cones than without ( $15.3$  and  $12.1 \mu\text{mol C}\cdot\text{mol N}^{-1}\cdot\text{sec}^{-1}$ , respectively), although the difference was not statistically significant ( $df = 12, t = 0.671, P = 0.259$ ).

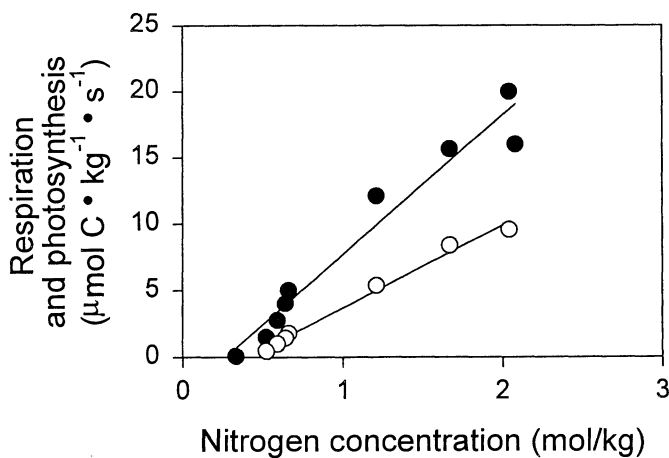


Fig. 6. The correlation between mean nitrogen concentration with both respiration (filled circles;  $r^2 = 0.944, P < 0.0001$ ) and photosynthetic rates (open circles;  $r^2 = 0.988, P < 0.0001$ ) of female cones of *P. menziesii*.

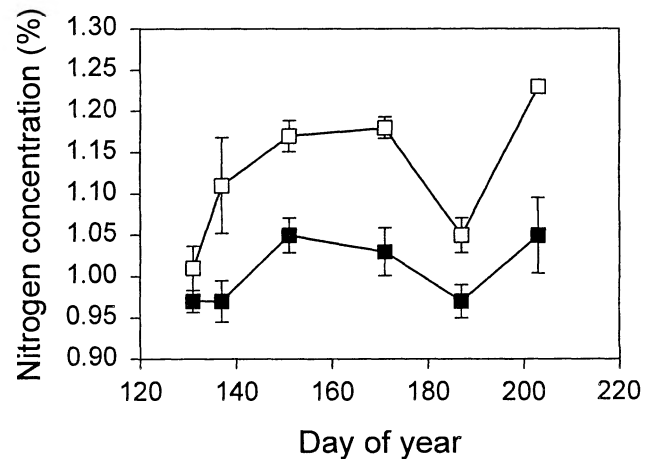


Fig. 8. Mean foliar nitrogen concentration of needles of *P. menziesii* on shoots terminating with female cones (filled squares) and needles on shoots without cones (open squares). Error bars represent  $\pm 1$  SE. The standard errors are for pooled samples, not for individual needle samples.

## DISCUSSION

**Resource allocation to cones**—Male cones of *P. menziesii* are completely dependent on vegetative tissues for their carbon and nutrient needs. The life cycle of the male cones of *P. menziesii*, including emergence from the bud, elongation, and pollen dispersal, was finished in <3 wk. The rapid loss of mass and nitrogen and decrease of  $R_{\text{cone}}$  of *P. menziesii* cones by 11 May, ~10 d after budbreak, was associated with pollen dispersal (S. McDowell, personal observation), confirming that the critical period of development was prior to that date. Although our sampling captured broad patterns of male cone development, it is possible that we missed details of development between 3 and 11 May. Future work with male cone development could benefit from more frequent sampling.  $R_{\text{cone}}$  of male *P. menziesii* cones ( $14 \mu\text{mol C}\cdot\text{kg}^{-1}\cdot\text{sec}^{-1}$ ) was higher than that of expanding *Pinus contorta* male cone buds, which ranged from 6.3 to  $9.9 \mu\text{mol C}\cdot\text{kg}^{-1}\cdot\text{sec}^{-1}$  (Dick, Smith, and Jarvis, 1990). These differences may be in part due to the timing of measurements; ours were made on fully expanded cones while those on *Pinus contorta* were made while the cones were still in bud (Dick, Smith, and Jarvis, 1990). While frequent early measurements are important to capture the carbon fluxes from male cones, it is similarly important to make measurements on mature cones. Although male cones did not photosynthesize, their total carbon and nitrogen requirements are relatively minor.

Whereas the life cycle of female *P. menziesii* cones was longer than that of the male cones, the female cones contributed to their own carbon demands. Photosynthesis, RGR, and  $R_{\text{cone}}$  per unit dry mass were at their greatest during the first few weeks of cone development. These results are similar to those from cones of *Pinus sylvestris*, *Picea abies*, and *Pinus contorta* for which photosynthesis, respiration, and growth peaked in early spring at pollination and then declined throughout the growth period (Linder and Troeng, 1981; Koppel, Troeng, and Linder, 1987; Dick, Smith, and Jarvis, 1990). Similar declining trends have been observed in the photosynthetic rates on fruits of flowering plants (Jurik, 1985; Blanke and Lenz, 1989; Galen, Dawson, and Stanton, 1993). Dehydration and loss of stomatal function on the photosynthesizing fruit or flower surface appear to be the common cause for these reduced photosynthetic rates. Further research on the water balance of female cones of *P. menziesii* may provide more understanding of their declining photosynthetic rates.

**Cone carbon balance**—The percentage of daytime respired carbon met by female cone photosynthesis decreased over the growth period, from 54% on 18 May to 30% on 22 August. Over the entire growing season, cone photosynthesis reduced the respiration costs of reproduction by only 13%, substantially less than that reported for other conifer species. For *Pinus sylvestris*, refixation reduced the seasonal respiratory losses of cones by 31% (Linder and Troeng, 1981). For female cones of *Pinus contorta* it was estimated that daily respiration was reduced 25% by photosynthesis over the entire year (Dick, Smith, and Jarvis, 1990). Annual respiratory costs of *Picea abies* cones were reduced 16–17% by cone photo-

synthesis (Koppel, Troeng, and Linder, 1987). Although the percentage of annual cone respiratory costs met by cone photosynthesis appears to be variable across species, there is a pattern related to the cone development period. The female cones of both *Pinus* species, which develop on a 3-yr cycle (according to Owens, 1991), have higher net refixation, while the cones of *Pseudotsuga* and *Picea*, both of which develop on a 2-yr cycle (Owens, 1991), have lower net refixation rates. Further quantification of the relationship between cone carbon balance and development period may simplify our understanding of carbon allocation to reproduction in conifers.

We estimated the average total carbon cost of reproductive production and respiration of male and female cones for a typical tree at the site. Using binoculars to count cones on representative branches, the numbers of male and female cones per tree were estimated. There were ~50 000 male cones/tree. Total seasonal cone respiration for one male cone equaled  $0.19 \times 10^{-3} \text{ mol C}\cdot\text{cone}^{-1}\cdot\text{yr}^{-1}$ , so the respiratory cost of male cones for one tree was ~9 mol C·tree<sup>-1</sup>·yr<sup>-1</sup>. The dry matter production of male cones equaled ~40 mol C·tree<sup>-1</sup>·yr<sup>-1</sup>. Therefore, the total carbon cost of male reproduction for a single *P. menziesii* tree was ~50 mol C·tree<sup>-1</sup>·yr<sup>-1</sup>. For female cones, there were ~2200 cones/tree. The seasonal respiratory flux of female cones was 0.15 mol C·cone<sup>-1</sup>·yr<sup>-1</sup>, or ~320 mol C·tree<sup>-1</sup>·yr<sup>-1</sup>. With cone photosynthesis included, this rate was reduced to ~280 mol C·tree<sup>-1</sup>·yr<sup>-1</sup>. The production cost of female cones was 390 mol C·tree<sup>-1</sup>·yr<sup>-1</sup>. The total carbon cost of female reproduction for *P. menziesii* using these estimates was 710 mol C·tree<sup>-1</sup>·yr<sup>-1</sup>, or 670 mol C·tree<sup>-1</sup>·yr<sup>-1</sup> with photosynthesis included.

We also estimated the carbon costs of reproduction for a typical closed forest stand of Rocky Mountain *P. menziesii*. Respiration and production of male and female cones were scaled to the stand using an average stem density 0.05 trees/m<sup>2</sup> and assuming cone production was limited to the upper 20% of the tree height. We calculated the annual costs as 6.6 mol C·m<sup>-2</sup>·yr<sup>-1</sup> for female cones with photosynthetic contributions included and 0.5 mol C·m<sup>-2</sup>·yr<sup>-1</sup> for male cone carbon costs. The female cone estimate is greater than total carbon allocation to female cones of *Picea abies* (2.06 mol C·m<sup>-2</sup>·yr<sup>-1</sup>; Koppel, Linder, and Troeng, 1987) and of *Pinus sylvestris* (1.3 mol C·m<sup>-2</sup>·yr<sup>-1</sup>; Linder and Troeng, 1981), but less than aboveground litter estimates of Rocky Mountain *P. menziesii* (13 mol C·m<sup>-2</sup>·yr<sup>-1</sup>; Gower, Vogt, and Grier, 1992; 15 mol C·m<sup>-2</sup>·yr<sup>-1</sup>; N. McDowell et al., unpublished data).

**Effects of reproduction on *P. menziesii* needles**—Although female *P. menziesii* cones cover some of their own reproductive costs, the remaining carbon and all nutrients must be contributed by the vegetative tissues of the trees. Most evidence suggests that the alternative source of cone carbon is photosynthesis of current needles. The biomass of female cones of coastal *P. menziesii* is negatively correlated with current-season stem growth, implying that carbon allocated to reproduction decreases available carbon for vegetative growth (Eis, Garman, and Ebell, 1965; El-Kassaby and Barclay, 1992). In a study of <sup>14</sup>C mobilization in *Pinus resinosa*, Dickmann and

Kozłowski (1970) found that pollinated cones were the priority sink of current needle photosynthate, even while new needles were expanding.

The period of highest cone RGR and respiration per unit dry mass was associated with accelerated photosynthesis in needles adjacent to developing cones, potentially increasing available carbon (Fig. 7). However, carbon demand per cone (production and respiration per cone) continued to increase without an associated increase in vegetative photosynthesis. Throughout the rest of the growing season, needle photosynthesis showed no relationship with developing cones, in spite of declining photosynthetic rates by the cones. In several plant species, the increasing sink strength of a developing fruit is known to elevate photosynthetic rates (de Jong, 1986; Reekie and Bazzaz, 1987a, b). However, in experimental shadings of photosynthetic fruits, vegetative photosynthesis did not increase, but seed number and mass, and thus seed viability, decreased (Hole and Scott, 1981; Galen, Dawson, and Stanton, 1993). For these species, the fruit photosynthetic contributions were critical to seed development, even though the fruit photosynthetic rates declined over the growing season like those of the cones. In a study using labeled carbon applied to cones of coastal *P. menziesii*, 3–4 times as much  $^{14}\text{C}$ -glucose was recovered from seeds as from the cone scales (Ching and Fang, 1963). Thus, the role of cone photosynthesis may be to cover the costs of seed development rather than assist in the carbon costs of other cone tissues such as scales and bracts.

While the carbon demand by female cones of *P. menziesii* appeared to affect needles during the early weeks of cone growth, the demand for nitrogen by cones had a season-long effect. Although cone [N] decreased over the season, the nitrogen content of cones increased as the cones gained mass. Additionally, seed [N] increased throughout the season (S. McDowell, unpublished data). The [N] of needles near cones remained lower than that of needles with no nearby cones for the entire growing season. However, no significant effect of foliar nitrogen depletion on foliar photosynthesis was observed. Perhaps the strong stimulation of foliar photosynthesis via cone proximity counteracted the effect of depleted tissue [N].

**Conclusions**—The results of this study suggest that carbon and nitrogen allocation differ between male and female cones, both in the timing and the amount of allocation. Male cones of *P. menziesii* require <10% of the carbon and about one-quarter of the nitrogen allocated to reproduction in Rocky Mountain *P. menziesii* trees at our site, with female cones consuming the remaining carbon and nitrogen. However, male cones are completely dependent on vegetative tissues for their carbon and nitrogen needs. Female cones, on the other hand, partially offset their carbon costs via photosynthesis. They had maximum refixation rates of 54% of their daytime respiration, but this only represents 6% of their total carbon costs for the full growing season. This research also contributes evidence that female cones on conifers with 2-yr reproductive cycles have lower refixation rates than female cones on conifers with 3-yr cycles (Linder and Troeng, 1981; Koppel, Linder, and Troeng, 1987; Dick, Smith, and Jarvis, 1990).

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