Predicting Plant Growth Rates From Dark Respiration Rates: an Experimental Approach

Bruce N. Smith
C. Mel Lytle
Lee D. Hansen

Abstract—A desirable quality in native plants used for revegetation is the ability for rapid growth and establishment, whether transplanted as seedlings or directly seeded. Photosynthetic rates are variable but not correlated with growth rate. Dark respiration rates measured by microcalorimetry, infrared gas analysis (CO_2), or oxygen electrodes are strongly correlated with growth rate for desert shrubs and crop plants. Respiration of germinating seeds effectively predicts performance not only of seedlings but also mature plants. This allows for rapid screening of available accessions to predict growth performance. This method is faster and less expensive than determining $^{13}C/^{12}C$ ratios of plant tissue and works for C_4 plants as well as C_3 plants.

Revegetation in arid environments requires plants that can grow rapidly under harsh conditions. Metabolic measures made at an early stage that indicate future rapid growth would thus be of great value. Photosynthetic differences exist but do not seem to be correlated with growth rate (Frank and others 1986). Water use efficiency, as measured by $^{13}C/^{12}C$ ratios, has been widely used to predict growth rate (Condon and others 1987; Farquhar and others 1982), however this test is expensive and works only for C_3 plants under water stress. Dark respiration has been said to be positively correlated with plant growth (Hay and Walker 1989), but others hold a contrary view (Amthor 1989). Thornley (1970) proposed a model for plant growth based on respiration: $P - R_m = R_x +$ storage, where P is the gross photosynthetic rate, R_m is the respiration rate needed to maintain life processes, R_x is the rate of carbon metabolized for growth, defense, stress reduction, etc., and storage is the rate of production of starch, lipid, or biomass which can be converted to energy. In practice, several of these parameters, especially R_m, are difficult to measure. This research is an attempt to experimentally define parameters that may prove useful in predicting plant growth.

Bruce N. Smith and C. Mel Lytle are Professor and Graduate Student, respectively, in the Department of Botany and Range Science; Lee D. Hansen is a Professor in the Department of Chemistry, Brigham Young University, Provo, UT 84602.

Materials and Methods

Shrub seeds were obtained from Durant McArthur at the Intermountain Research Station's Shrub Science Laboratory and from Howard Stutz of Brigham Young University. Other plant materials were obtained from the Botany greenhouse and from commercial sources. Specific respiration and photosynthetic rates were measured using a Lycor-2600 infrared gas analyzer, a Hansatech oxygen electrode, and a Hart Scientific model 7707 differential scanning calorimeter. Isotope ratios were determined on an isotope ratio mass spectrometer.

Results and Discussion

Bushbean (*Phaseolus vulgaris* L.) cv. Tendergreen seed was soaked for one hour in aerated, distilled water, then planted in a light soil mix and placed in a growth chamber with 15 hours in light at 23°C and 9 hours in dark (21°C). Gas exchange was measured on whole seedlings, or leaves, with an infrared gas analyzer at 24°C. The dry seed had a very low rate of dark respiration. As seen in Table 1, respiration increased rapidly and continuously with seedling growth and development. Green tissue appeared after 7 days with some fixation of CO_2. However CO_2 uptake in the light did not exceed CO_2 evolution in the dark until the plants were nearly two weeks old. The photosynthesis to respiration ratio is an indication of energy available from photosynthesis.

Another way to follow development with time is to use leaves of different ages from the same plant. The smallest

Table 1—Photosynthetic rate (P_{CO2}) and dark respiration rate (R_{CO2}) in leaves from Bushbean cv. Tendergreen germinated and grown in a growth chamber

| | μg CO_2/g fr wt/min | | |
age (days)	P_{CO2}	R_{CO2}	P_{CO2}/R_{CO2}
dry seeds		+0.04	
0 (1 hr soak)		+1.1	
1		+4.6	
2		+7.8	
7	+8.7	+16.5	
11	+7.8	+20.3	
13	-24.2	+23.5	1.0
14	-45.8	+27.5	1.3
15	-83.5	+34.9	2.4

and youngest leaves are at the stem tip and leaves become progressively larger and older down the stem. Leaves may be retained for a very long time. Leaves from a six-month old hybrid of *Atriplex canescens* x *A. obovata* grown in the greenhouse were removed in order of growth (and size) and analyzed for metabolic activity with an infrared gas analyzer at 24°C. Table 2 shows that the youngest leaves had the highest rates of dark respiration, decreasing with leaf number to a low and steady rate. By contrast the photosynthetic rate was much lower in younger leaves and higher in older leaves. There was a fair correlation between leaf number and leaf area, the older leaves becoming much larger (Table 2). Ratios of photosynthesis to respiration increased with leaf age to very high values.

Plumeria is a small tropical understory tree with tufts of leaves at the ends of branches. New leaves are formed at the apex as older leaves senesce and fall off, retaining the tufted appearance. The leaves are large, dark green and leathery. Table 3 shows both similarities with and differences from the data in Table 2. Younger leaves have higher rates of respiration than older leaves, and photosynthetic rate is lower in the younger leaves and increases with leaf number up to about 12. In contrast to *Atriplex*, in *Plumeria* the very oldest leaves are smaller and have decreasing rates of photosynthesis (Table 3). Differences between *Atriplex* and *Plumeria* can be understood and explained on the basis of growth habit. *Atriplex* is a sun plant with higher rates of photosynthesis and higher photosynthesis to respiration ratios in the oldest leaves while *Plumeria* is a shade plant. As the older *Plumeria* leaves begin to senesce, nutrients are reabsorbed and distributed to growing tissue. As the old leaf becomes chlorotic, it falls off the stem.

Seedlings of the three subspecies of big sagebrush (*Artemisia tridentata*) and two geographic races of *Kochia prostrata*) were grown in the greenhouse for four months under well-watered conditions. Shoot length was measured, carbon isotopes determined in leaf tissue and dark metabolic heat rates determined using a differential scanning microcalorimeter in the isothermal mode at 25°C. Results are shown in Table 4. Within species the metabolic heat rates were clearly a much better indicator

under these conditions of plant growth rates than were $^{13}C/^{12}C$ ratios.

Pea plants (*Pisum sativum* L. cv. Azad) were grown in natural light for 15 to 20 days. Disks (7 mm diameter) were cut under water from the first (young) and fourth (old) pair of leaves counting from the top. Disks were incubated in Petri dishes containing a medium of 0.02 mM $CaCl_2$, 2 mM bicarbonate in 10 mM HEPES-KOH at pH 7.8 under weak light (75-100 μE m^{-2} s^{-1}) for 12 hours followed by 12 hours dark with continuous shaking. The area of leaf disks immediately after cutting and after 24 hours in the Petri dishes was measured on enlarged xerox copies of the disks. Oxygen uptake or production was monitored at 25°C using a Clark-type oxygen electrode. Light intensity of 1,000 μE m^{-2} s^{-1} was used for photosynthesis. Disks were incubated with metabolic inhibitors for one hour. Gas exchange measurements were made with inhibitor present.

Disks from younger leaves of pea plants expanded five times as rapidly as disks from older leaves. As seen in Table 5, rates of photosynthesis and dark respiration were more than twice as great in young leaves as in old leaves. Plant species either possess or can develop an alternative oxidase system (the physiologic function is still not understood). Sodium azide (NaN_3) inhibits cytochrome activity

Table 2—Photosynthetic rate (P_{CO2}) and respiration rate (R_{CO2}) as a function of leaf number (age) and leaf area from tip to base of a six–month old seedling of an *Atriplex* hybrid (*obovata x canescens*). Photosynthesis (P_{CO2}) means CO_2 uptake (−), while dark respiration (R_{CO2}) means CO_2 production (+). Photosynthesis to respiration ratios are an indication of energy available from photosynthesis for growth.

| leaf # | leaf area cm^2 | μg CO$_2$/g fr wt/min | | |
		P_{CO2}	R_{CO2}	P_{CO2}/R_{CO2}
3,4	0.75 cm^2	−96.9	+42.1	2.3
5,6	1.52	−100.8	+25.2	4.0
7,8	1.35	−92.1	+20.3	4.5
9,10	3.08	−128.0	+9.1	14.1
22,23	5.61	−134.9	+8.1	16.7

Table 3—Photosynthetic rate (P_{CO2}) and respiration rate (R_{CO2}) as a function of leaf number and leaf area starting from the stem tip of *Plumeria*. Photosynthesis means CO_2 uptake (−), while respiration means CO_2 production (+). Photosynthesis to respiration ratios (P_{CO2}/R_{CO2}) are an indication of energy from photosynthesis available for growth.

| leaf # | leaf area cm^2 | μg CO$_2$/g fr wt/min | | |
		P_{CO2}	R_{CO2}	P_{CO2}/R_{CO2}
3	182 cm^2	−70.7	+63.0	1.1
6	410	−96.9	+34.0	2.9
9	506	−82.6	+49.6	1.7
12	654	−101.2	+40.4	2.5
15	570	−54.4	+33.5	1.6
18	330	−38.6	+25.3	1.5

Table 4—Shoot growth, carbon isotopic ratios, and metabolic heat rates from leaves of races or subspecies of *Artemisia tridentata* and *Kochia prostrata* .

subspecies	shoot length (cm)	$\partial^{13}C$ vs PDB (o/oo)	metabolic heat rate (μw/mg)
Artemisia tridentata			
wyomingensis	4.66	−34.91	8.46
tridentata	7.24	−35.01	10.56
vaseyana	7.57	−34.96	10.74
F$_2$ hybrid tridentata x vaseyana	7.65	−34.21	12.69
Kochia prostrata			
U 10	8.15	−23.88	9.36
U11	9.01	−18.28	14.27

Table 5—Photosynthetic rates (P_{O_2}) and respiration rates (R_{O_2}) in young and old pea leaves inhibited by 1 mM NaN_3 and 10 mM salicylhydroxamic acid (SHAM)

	young leaves		old leaves	
	rate	inhibition %	rate	inhibition %
P_{O_2} (μmol O_2 produced m^{-2} s^{-1})				
control	5.53	0	2.17	0
+ NaN_3	0.56	90	0.80	63
+ NaN_3 + SHAM	-0.40	107	0.08	96
R_{O_2} (μmol O_2 consumed m^{-2} s^{-1})				
control	1.86	0	0.83	0
+ NaN_3	1.33	28	0.51	39
+ NaN_3 + SHAM	0.21	89	0.24	71

Table 6—Respiraton rates (R_{CO_2}) in younger and older phyllodes from a single leaf of *Acacia auriculariformis* after exposure to metabolic inhibitors. For dark-adaptation, an entire leaf was removed from the tree and kept with the petiole in water in the dark for 24 hours.

	young		old	
	R_{CO_2}	inhibition	R_{CO_2}	inhibition
	(μmol CO_2/m^2/s)	(%)	(μmol CO_2/m^2/s)	(%)
light-adapted				
control	8.10	0	3.26	0
+ NaN_3	6.83	16	2.90	11
+ NaN_3 + SHAM	4.24	48	0.52	84
+ NaN_3 + SHAM				
+ NaF	3.10	62	0.52	84
dark-adapted				
control	4.13	0	3.84	0
+ NaN_3	2.53	39	3.31	14
+ NaN_3 + SHAM	7.44	0	5.30	0

while salicylhydroxamic acid (SHAM) inhibits the alternative pathway of electron transport in mitochondria. Photosynthesis proved to be very sensitive to both inhibitors, even more so than dark respiration. The difference in respiration between young and old leaves was largely due to the alternative path. Maintenance respiration has been estimated as the steady-state flux of CO_2 after 48 hours in the dark at a constant temperature (McCree 1974). Such treatment in older leaves caused a complete elimination of the cytochrome path that returned with recovery in the light. Starvation (48 hours in the dark) in younger leaves mostly decreased the alternative path. Our results indicate this method of measuring maintenance respiration is severely flawed.

Similar results were obtained by comparing younger and older phyllodes from a single, large compound leaf from six-year old trees of *Acacia auriculariformis* A. Cunn. ex Benth. In this case gas exchange was measured with an infrared gas analyzer. Juvenile phyllodes from near the leaf tip had twice the rate of dark respiration as did mature phyllodes from near the leaf base (Table 6). Keeping the phyllodes in the dark for 24 hours completely eliminated the alternative path from both juvenile and mature phyllodes. The difference in respiration between juvenile and mature phyllodes was due to cytochrome respiration, glycolysis and basal respiration. Carbon dioxide evolution in the presence of all three inhibitors (NaN_3, SHAM, and NaF), termed basal respiration, is probably due to direct oxygen uptake by phenol oxidases, ascorbic acid oxidases, etc.

In conclusion, dark respiration can predict plant growth but is complex enough that one must know a great deal about the biology of the particular plant. Thornley's model (1970) for respiration and growth is too simplistic and needs drastic revision. We intend to pursue this idea in several other systems in the coming months.

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