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Abstract

The main purpose of this study is to investigate the relationship between the dark respiration rate of two herbaceous perennials, *Campanula rotundifolia* L. and *Bistorta bistortoides* (Pursh.) Small, and growing season temperature. The metabolic basis of respiratory temperature response is also of interest. *B. bistortoides* is found in Colorado above 9000' and does not occur on Open Space holdings. *C. rotundifolia* can be found in natural populations from approximately 5700' to 12000'. It is a common plant in the Open Space and Mountain Parks. *C. rotundifolia* and *B. bistortoides* were collected from three sites within each of their elevational ranges. Collection of above ground tissues began as soon as the plants broke dormancy and continued every 14 days until the plants senesced. Between 4 and 7 collections were made at each site. Each individual was divided into leaf, stem, and flower/seed portions. The fresh weight of each tissue was recorded. One half of the individuals were then dried and reweighed. Respiration rate was measured for each tissue at 15 and 25 °C. The molecular composition and tissue cost of each individual will be measured in the next 6 months. Preliminary results show that both respiration rate and biomass accumulation vary along each elevational gradient and that the two species exhibit different respiratory responses to elevation. These results are currently being written into a manuscript which will be submitted to the Journal of Ecology in January.

Objectives and Hypothesis

Dark respiration is a biochemical pathway which provides all aerobic cells with energy in the form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) and carbon skeletons which can be used to build new tissues. Despite this vital metabolic function the process of respiration also results in the loss of carbon in the form of CO_2 from the plant. Lambers (1985) estimates that this loss is between 5 and 30 % of the carbon taken up by the plant during photosynthesis.

The effect of growth temperature on the dark respiration rate of plants is little understood. Because the effect of growth temperature on respiratory characteristics is not understood the effect of changing temperature due to global climate change on respiratory characteristics is also not understood. The ability to predict the change in plant respiration rates which result from global temperature changes is essential to our understanding of the global carbon cycle.

Past studies have shown that plants can increase, decrease, or maintain a constant respiration rate as temperature increases (Atkin and Day 1990, Scholander and Kanwisher 1959, Klikoff 1968, Wager 1941). Approximately 50% of the plant species studied have exhibited an increase in respiration rate at colder growth temperatures. There are two common short-comings to most of these studies. Few studies of been conducted on natural populations of plants; most plants are grown in controlled conditions in laboratories. Secondly, few studies have been designed to make comparisons of the same species in a variety of environments. More commonly congenerics are compared between environments.

The purpose of this study is to investigate the relationship between temperature or, more specifically, altitude and respiration rates of two plants across an elevational gradient. Temperature, growing season length, and atmospheric pressure all decrease with altitude and each of these variables would be expected to result in an increase in respiration rate with altitude. The main hypothesis tested in this study is that respiration rate will increase with altitude or with a decrease in growing season temperature. The metabolic basis of this change will also be studied. Do plants from cooler environments have higher protein concentrations and do they have higher respiratory enzyme activities? Do plants from colder environments have higher tissue costs which in turn drive up respiratory rates?

Hypotheses

1. Respiration rate will increase with altitude or with a decrease in growing season temperature.
2. Plants from colder environments will have higher protein concentrations, respiratory enzyme activities, and tissue costs.

Methods

Species Description

Two herbaceous perennials, *Bistorta bistortoides* (Pursh) Small (Polygonaceae) and *Campanula rotundifolia* L. (Campanulaceae), were studied along elevation gradients in the Rocky Mountains of Colorado. *B. bistortoides* is a common plant of arctic and alpine tundra and is also found in both subalpine and montane meadows in Colorado. Individuals of *B. bistortoides* have a large storage rhizome. In the lower parts of its altitudinal range, *B. bistortoides* is most often found in soils which are saturated with water for most of the growing season (per. observe). *C. rotundifolia* exists naturally in a much wider range of habitats. It can be found from the foothills of the Rockies (5900') up to the rocky fellfields of the tundra (12000'). *C.* rotundifolia is most often found in well-drained, rocky soils. Its metabolism is unusual in that plants produce latex and accumulate fructans instead of starch.

Site Description

The *Bistorta bistortoides* sites range in elevation from 9100' to 11200'. The lowest site was located adjacent to a highway in an old cow pasture. The site is located in *Pinus contorta* forest. For approximately 6 weeks of the growing season this site was inundated with water. *B. bistortoides* were also collected from a subalpine site at 10500'. This site was also very wet for most of the growing season. Plants were collected from an open meadow in *Picea engelmanii* and *Abies lasiocarpa* forest. The highest *B. bistortoides* site was located on Niwot Ridge, a LTER site, at 11200'. This tundra site was at the transition habitat between dry and wet meadow. This was the driest of the three Bistort sites.

Campanula rotundifolia were collected from three sites which ranged from 5900' to 11200'. The lowest site was in *Pinus ponderosa* forest in the foothill mesas near Boulder, CO. This site is at the lower elevational limit of *Campanula rotundifolia* and in this area *C. rotundifolia* is found only on north-facing slopes in the shade. A mid-elevation site was located at 8500', also in *Pinus ponderosa* forest. Lastly, an alpine site was located near the highest *B. bistortoides* on Niwot Ridge.

Site Quantification

To quantify the environmental characteristics of each site, temperature and soil characteristics were measured (Table 1). Temperature was measured with Onset's Optic Stowaway and Hobo temperature loggers and a Licor data logger. The loggers were in place in each site from before the first collection date until approximately one week after the last collection date. This was not true in the low (5900 ') Campanula site because the data logger failed during the season. A single temperature logger was used to measure the two

Niwot Ridge sites because of the close proximity of the sites. Mean daily temperature, mean low temperature, and mean high temperatures were calculated (Table 1). Soil nitrogen was quantified at each site (Table 1). Soil samples were sieved with a 2 mm^2 screen before nitrogen was measured. The light environment of each site was subjectively quantified in terms of aspect and shadiness (Table 1). The water holding capacity of soil from each site was measured (Table 2). Soil moisture was quantified with Techtronix Cable Tester for two dates at each site. Soil moistures were calculated from soil moisture curves created for each site (Table 2). The soils were sieved through a 5 mm^2 screen prior to the creation of the standard curve.

Tissue Collection

The study was conducted over the entire growing season for each species in each site (Table 3). Plants were collected every two weeks at each site. Collections were made before sunrise. Previous studies have shown that respiration steadily decreases at night until approximately 4 am and then remains constant until the plants become photosynthetically active again at sunrise (Plumb, unpublished data). The above ground portion of 10 to 16 plants was collected. Plants were stored on ice until they could be placed in a refrigerator in the lab. The above ground biomass was divided into three parts: leaves, stems, and reproductive parts (flowers, ripened ovaries, seeds). Each tissue was weighed to obtain a fresh weight. After the respiration rates were measured, one half of the plants collected were dried for 4 hours at 95 °C. The plant tissue was then placed in a 55 °C oven until it was reweighed to obtain the dry weight of each tissue. Portions of the stem and leaf material were trimmed so that they completely filled the area of the oxygen electrode chamber. An attempt was made to include tissue of various age classes. For *C. rotundifolia* whole flowers were used for respiration measurements. The small flowers of *B. bistortoides* were trimmed from the stem for respiration measurements.

Respiration Measurement

The methods of Delieu and Walker (1981) were followed to measure respiration rates. Hansatech Oxygen electrodes were used to measure dark respiration rates at 15 and 25 °C. The temperature of the electrodes was maintained with circulating water baths. The electrode was bathed in a Boric:KCl solution. Respiration rates were measured within 8 hours of plant collection. Plants were stored in a 8 °C refrigerator until respiration rates were measured. The respiration rate of Bistort leaves was found to be stable over this period of time (Plumb, unpub. data). The rate was measured for 6 to 8 minutes after a stable respiration rate was reached. Each sample required approximately 10 minutes to process. The tissue samples used for respiration measurements were dried in the same manner as the whole plant samples.

Respiration rates were measured in umol O_2 consumed per second per kilogram of dry tissue weight (umol O_2/s-kg DW).

Tissue Cost Analysis (Analyses not conducted yet.)

Two methods will be used to assess tissue cost. One will involve the quantification of carbon, nitrogen, oxygen, and hydrogen by elemental analysis (Williams et. al. 1987). Samples of each tissue from an early, mid, and late sampling date from each site will be analyzed. Tissues have been ground to a fine powder for this analysis. The second method will rely on the quantification of concentrations of each molecular constituents (proteins, amino acids, sugars, starch, cellulose, lipids, etc.) in a each tissue. The cost of each constituent pool can then be calculated with the equations of Penning de Vrie et. al. (1974). These measurements will also be conducted on each of the three tissues from early, mid, and late collection dates at each site.

Statistical Analysis

For each collection date the population of plants at each site was placed into a phenologic category. Two-way ANOVAs were run to compare the respiration rate of each species over the entire growing season between sites. Two-way ANOVAs were used to compare respiration at both 15 and 25 °C. ANOVAs were performed only on the phenologic states which occurred in each population across the entire gradient. For instance, the growing season of the *C. rotundifolia* at the Bald Mountain site was considerably longer than at the other two sites. Two collection dates from this site were not used in the analyses because there were not corresponding phenologic states at the Niwot and Shanahan Ridge sites. The sample size for each collection date ranged from 5 to 8 samples for each tissue.

Results

At this time the respiration, biomass, and preliminary germination data have been collected. A manuscript which presents the results of the respiration and biomass study is currently being prepared for submission to the Journal of Ecology in January. Either a reprint or the accepted manuscript can be provided to the Open Space Office. Preliminary analysis of the data suggests that the two species respond very differently to the elevational gradient in terms of respiration. The response of biomass accumulation across the gradient also varies for the two species.

Germination rates have been measured in *Campanula rotundifolia* seeds collected at five sites from 5900 to 11200 feet (Figure 1). Germination rates were measured in a greenhouse at the University of Colorado, Boulder. Further quantification of germination rates is being done in cool and warm growth chambers. Plants from these seeds will be used in further studies which will be conducted at the University of Colorado, Boulder. Germination rate is highest at the mid-elevation site and decreases at sites which are higher and lower elevations. Preliminary data suggest that biomass accumulation is also greatest at the mid-elevation site.

No results are available for tissue cost differences between sites because these analyses have not been conducted yet.

Conclusions

The main focuses of this study, the effect of temperature on respiration rates and the ability to predict plant respiratory responses to global climate change, are probably of little use to the Open Space in terms of management of the Open Spaces. These aspects of the study will be most useful to the Open Space as educational tools which can be used to illustrate the importance, complexity, and connectedness of each piece of the biosphere.

Campanula rotundifolia L. is a relatively abundant plant of the higher elevations of the Open Space. The population from the site near NCAR is at the lower, natural, elevational limit of *C. rotundifolia*. This site probably has some of the minimum environmental requirements for the growth of *C. rotundifolia*. While *C. rotundifolia* is only found on sites with good drainage, this site's soil has very low water holding capacity (Table 2). The soil is also lower in nitrogen concentration than either of the other two Campanula sites studied (Table 2). *C. rotundifolia* at this elevation are also found only in the shade on north-facing slopes which suggests that they are only able to grow at cooler sites. The lower elevational limit may be controlled by any one or a combination of these factors: low nitrogen, high temperatures, or water stress. These observations could be used if restoration of a site included the introduction of *Campanula rotundifolia* L.

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Table 1. Site descriptions. Several environmental variables measured at each site. Temperature was measured with recording data loggers. Soil nitrogen was measured with the Kjeldahl digestion method.

Species	*Site*	*Elevation (m)*	*Temperature (°C): Average Daily, Average Minimum, Average Maximum*	*Soil Nitrogen (% N/g dry soil)*	*Aspect*	*Shadiness*
Campanula rotundifolia	Shanahan Ridge	1800	19.3 ± .18 14.1 ± .42 26.1± .57	.364	north	very shady
	Bald Mountain	2650	14.8 ± .12 9.9 ± .33 21.5 ± .49	.718	south-east	partly shady
	Niwot Ridge	3410	10.0 ± .25 1.4 ± .36 24.2 ± .64	.512	east	sunny
Bistort bistortoides	Peak to Peak Highway	2710	13.8 ± .14 7.7 ± .41 22.9 ± .53	.334	flat	sunny
	Cable Gate Meadow	3200	11.0 ± .41 1.8 ± .48 26.9 ± 1.1	1.111	flat	sunny
	Niwot Ridge	3410	10.0 ± .25 1.4 ± .36 24.2 ± .64	.746	south-east	sunny

Table 2. Soil moisture for two dates at each site. Soil moisture was measured with a Techtronix Cable Tester in meters. Metric measurements were converted to soil moistures with soil moisture calibration curves for each site. Field capacities were measured adding water to the soil until it began to pool at the bottom of a 20 cm soil column.

Species	*Site*	*Field Capacity*	*Date*	*Soil Moisture (% by dry weight)*
Campanula rotundifolia	Shanahan	35 %	7-29-96	26.5 ± 1.93
				not available
	Bald Mountain	75 %	7-30-96	42.6 ± 2.59
			9-14-96	21.6 ± 3.16
	Niwot Ridge,	55 %	7-25-96	16.3 ± 2.74
			9-4-96	20.4 ± 1.87
Bistorta bistortoides	Peak to Peak	not available		not available
				not available
	Cable Gate	not available		not available
				not available
	Niwot Ridge,	not available		not available
				not available

Table 3. Tissue Collection Dates. Plants were collected at two week intervals from the break of dormancy until senescence.

Campanula rotundifolia	**Shanahan Ridge**	**Bald Mountain**	**Niwot Ridge**
	5/20/96	6/10/96	7/12/96
	6/1/96	6/24/96	7/26/96
	6/13/96	7/8/96	8/8/96
	7/1/96	7/22/96	9/5/96
	7/15/96	8/3/96	
	7/29/96	8/16/96	
		8/29/96	
Bistorta bistortoides	**Peak to Peak**	**Cable Gate Meadow**	**Niwot Ridge**
	6/21/96	6/25/96	6/15/96
	7/3/96	7/10/96	7/5/96
	7/17/96	7/24/96	7/19/96
	7/31/96	8/6/96	8/2/96
	8/17/96	8/27/96	8/27/96

Figure 1. Percent germination of *Campanula rotundifolia* seed along an elevational gradient in the Rocky Mountains, CO.