

Stress-induced metabolic differences between populations and subspecies of *Artemisia tridentata* (sagebrush) from a single hillside

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Abstract

Big sagebrush or *Artemisia tridentata* Nutt. ssp. *vaseyana* grows at slightly higher, cooler, and drier sites than does *A. tridentata* ssp. *tridentata*. The two sagebrush subspecies and natural hybrids between them are found along an elevational gradient in Salt Creek Canyon, near Nephi, UT, USA, where the parent populations are separated by 85 m in elevation and 1.1 km along the transect. In 1993, three gardens were established with seedlings from five populations from different elevations planted in each garden. Physiological measurements of carbon isotope ratios, chlorophyll fluorescence, and respiratory heat and CO₂ production show adaptation to the site of origin. When transplanted to foreign sites, stress was noted. Sagebrush has persistent leaves that are metabolically active all year. Seasonal changes in temperature promote metabolic responses in sagebrush that differ with population and garden.

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1. Introduction

Artemisia tridentata Nutt. (or big sagebrush) is one of the most widespread and economically important shrubs in western North America. It serves as a primary source of food for sage grouse, antelope and deer, and generally occurs in deep soil with some water availability. Big sagebrush is found from the

Dakotas and New Mexico to California, Oregon and Washington and from British Columbia to Baja California [1]. Big sagebrush and its subspecies have been prominent since the late Tertiary or early Quaternary [2]. The present distribution of subspecies and contact zones between them was probably established at the end of the last glaciation [3]. Basin big sagebrush (ssp. *tridentata*) grows at lower elevations than mountain big sagebrush (ssp. *vaseyana*) Differences in volatile compounds between the subspecies have also been noted [4]. Natural hybrids between the subspecies often occur when parent populations are

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in close proximity. Hybrids may also be produced by controlled pollination [5].

Success of parental subspecies and hybrids between them in the face of environmental stresses is often assessed by growth measurements and changes in morphology. A more sensitive assessment could be made using physiological parameters. Stem water potential and gas-exchange respiration rate have been measured in parental and hybrid big sagebrush grown in common gardens [6]. Significant differences were found among habitats and source populations. Carbon isotopic fractionation associated with degree of stomatal closure in some cases was correlated with productivity [7]. Chlorophyll fluorescence was a sensitive indicator of plant stress [8,9].

Aerobic respiration has two aspects: catabolism and anabolism. In catabolism, organic substrates are oxidized to produce CO₂, heat, and ATP. Anabolism uses the ATP and organic substances for plant growth, reproduction, defense, etc. Calorimetry measures the rate of heat (R_q) produced from both catabolism and anabolism. The rate of CO₂ production (R_{CO_2}) indicates the rate of catabolism and is often measured with an infrared carbon dioxide analyzer, but can also be determined calorimetrically. The two measurements (R_q and R_{CO_2}) allow calculation of plant growth rates as a function of temperature [10,11]. When metabolic heat loss (R_q) exceeds energy made available through catabolism of carbohydrate (R_{CO_2}), the plant is considered to be stressed [12].

Predicted growth rate of structural biomass (rate of anabolism (R_{SG})) is related to the two measured variables as in Eq. (1):

$$R_{SG}\Delta H_B = 455R_{CO_2} - R_q \quad (1)$$

where ΔH_B is the enthalpy change for formation of biomass from photosynthate and Thornton's constant ($-455 \pm 15 \text{ kJ mol}^{-1}$ of O₂) is incorporated to calculate the rate of energy generated by catabolism. Thus, growth rate in terms of energy is proportional to the difference between the measured values of R_{CO_2} and R_q . The temperature dependencies of R_{CO_2} and R_q are different [13]. The difference between $455R_{CO_2}$ and R_q therefore changes with temperature and this difference can be used to predict growth rate changes with temperature [11].

Specific growth rate (R_{SG}) may also be expressed as a function of substrate carbon conversion efficiency

(ϵ) and respiration rate (R_{CO_2}):

$$R_{SG} = R_{CO_2} \left[\frac{\epsilon}{1 - \epsilon} \right] \quad (2)$$

Thus, growth rate is directly proportional to the product of respiration rate and efficiency. Combining Eqs. (1) and (2) to eliminate R_{SG} gives Eq. (3):

$$\left[\frac{\epsilon}{1 - \epsilon} \right] \Delta H_B = -\frac{R_q}{R_{CO_2}} + \left(\frac{1 - \gamma_P}{4} \right) 455 \quad (3)$$

which relates the ratio of R_q/R_{CO_2} to ϵ . Values of R_q/R_{CO_2} measured as a function of temperature can thus provide information on substrate carbon conversion efficiency (ϵ) and the oxidation state of the substrate carbon, γ_P [10].

Sagebrush in Salt Creek Canyon has been intensively studied for ecological, morphological, and biochemical differences among the two subspecies and the hybrid swarm between them [3]. Physiological studies on early morning and midday stem water potential using the Scholander pressure bomb technique and on CO₂ evolution as measured by infrared gas analysis showed significant differences between populations and between sites [6]. Research reported here used different techniques to extend and expand earlier physiological work.

2. Materials and methods

Mountain sagebrush (*A. tridentata* ssp. *vaseyana* (Rydb.) Beetle) grows at slightly higher, cooler, and drier sites than does valley sagebrush (*A. tridentata* ssp. *tridentata* Nutt.). Natural hybrids between the two subspecies are often found in locations such as Salt Creek Canyon, located 10 km east of Nephi, UT, USA, where the parent populations are separated 85 m in elevation and 1.1 km in distance on the east-facing slope of the canyon. Previous studies [3,5,6] have shown that much of the hillside between the parent populations is occupied by hybrids. Common gardens were established at the valley (1775 m elevation), middle of the hybrid zone (1790 m) and mountain (1860 m) locations. Hourly air temperature data were measured at five locations—each of the gardens plus 1835 and 1885 m—with HOBO loggers from the Onset Computer (Bourne, MA). Temperatures were

recorded from 1 April to 30 June 2001 along the elevational transect in Salt Creek Canyon, near Nephi, UT.

Seed was collected from the parent locations and from three areas in the hybrid population zone. Seed was germinated and seedlings grown in containers in the greenhouse. In the spring of 1993, 60 seedlings were randomly planted in each of the three fenced common gardens, 12 from each of the five populations (mountain, high elevation hybrid, mid-elevation hybrid, low-elevation hybrid, and valley). Details of the study site and garden design have been described earlier [3,5,6].

Calorimetry was used to determine small differences in high and low stress temperatures of sagebrush from different locations grown in common gardens. About 100 mg fresh weight of stem tips and leaves were placed in each of three ampoules of a microcalorimeter (Hart model 7707, Pleasant Grove, UT, or Calorimetry Sciences Model 4100, American Fork, UT). Each plant sample was run in triplicate with three plants from each garden-population. After 15–20 min thermal equilibration, metabolic heat rate (R_q) was measured for an additional 15–20 min. The ampoules were removed from the calorimeter and a small vial filled with 40 μ l of 0.4 M NaOH placed in the ampoule with the tissue. Again a 15–20 min thermal equilibration was necessary, followed by measurement of the sum of heat from metabolism plus heat of carbonate formation ($-108.5 \text{ kJ mol}^{-1}$). The difference in heat produced with and without NaOH gave the rate of CO_2 evolution (R_{CO_2}) by the plant tissue. After the NaOH was removed the heat rate (R_q) was measured again as before. These values were measured [13] at eight temperatures: 20, 15, 10, 5 or 25, 30, 35, and 40 °C.

Quantum yield of photosystem II was measured by giving a pulse of 680 nm light to dark-adapted shoot tips and photosystem II fluorescence at 695 nm recorded 10 s later (Morgan Scientific CF-1000 Chlorophyll Fluorescence System, Wilmington, MA). The quantum yield of photosystem II was expressed as the ratio of variable to maximal fluorescence (F_v/F_m). A decrease in the quantum yield (lower F_v/F_m ratio) indicates greater environmental stress. For instance F_v/F_m ratios of 0.45–0.65 indicate significant stress while values of 0.75–0.85 are in the normal range. Each measurement was made three times on each of three shoot tips for each plant.

Sagebrush shoot tips collected at the same time as chlorophyll fluorescence was measured, were oven-dried (70 °C), placed in quartz tubes with manganese dioxide (as a source of oxygen), combusted at 800 °C, and the $\delta^{13}\text{C}$ value of the CO_2 measured on an isotope ratio mass spectrometer. The $\delta^{13}\text{C}$ values were expressed in per mil (‰) relative to Pee Dee belemnite (PDB).

3. Results

Photosystem II fluorescence detected stress in only two cases—mountain plants in the basin garden and basin plants in the hybrid garden (Table 1). Carbon isotopic values for sagebrush collected in 1995 on different dates, did indicate greater stress at the highest temperature [12]. However for samples collected on 6 November 1999, the only noticeable trend in carbon isotopes was for greater stress for plants in the mountain garden (Table 1) as indicated by more negative $\delta^{13}\text{C}$ values.

Fig. 1 illustrates the calorimetric data obtained from sagebrush tissue of a particular origin, collected at a given time and place. Only at temperatures where the catabolic rate (R_{CO_2}) exceeds the rate of heat loss (R_q), can growth occur (Fig. 1A). Temperatures that exceed these limits are stressful. The efficiency of conversion

Table 1
Sagebrush photosystem II fluorescence (F_v/F_m) and carbon isotope ratios

Garden-source	F_v/F_m	$\delta^{13}\text{C}$ vs. PDB (‰)
Basin-basin	0.805	-27.44
Basin-low elevation hybrid	0.772	-25.66
Basin-hybrid	0.768	-27.59
Basin-high elevation hybrid	0.781	-29.67
Basin-mountain	0.543	-26.30
Hybrid-basin	0.680	-30.47
Hybrid-low elevation hybrid	0.793	NA ^a
Hybrid-hybrid	0.729	-26.85
Hybrid-high elevation hybrid	0.820	NA
Hybrid-mountain	0.773	-31.35
Mountain-basin	0.777	-29.07
Mountain-low elevation hybrid	0.784	-29.40
Mountain-hybrid	0.724	-29.19
Mountain-high elevation hybrid	0.779	-28.27
Mountain-mountain	0.788	-32.93

^a Not available.

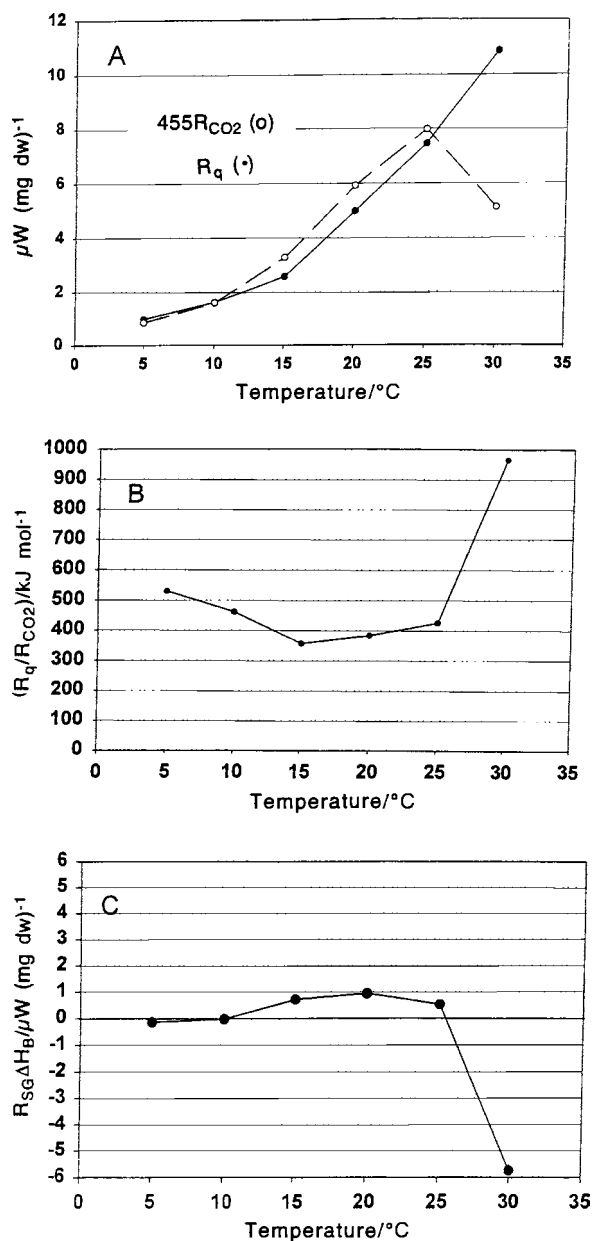


Fig. 1. Calorimetric measurements made on mountain big sagebrush (*A. tridentata* ssp. *vaseyana*) grown in the basin garden (1775 m), in Salt Creek canyon, near Nephi, UT, USA on 23 February 1998. Snow depth was 55 cm and the air temperature was 7 °C. From data such as this, Table 2 was constructed. (A) Heat rate (R_q), ●; respiration rate ($455R_{CO_2}$), ○ (in μW (mg dry weight) $^{-1}$ measured at seven temperatures). (B) Metabolic efficiency (R_q/R_{CO_2} in $kJ\ mol^{-1}$). Note: smaller numbers mean greater efficiency. (C) Relative specific growth rates, $R_{SG}\Delta H_B = 455R_{CO_2} - R_q$ (in μW (mg dry weight) $^{-1}$).

of photosynthate into plant growth as indicated by the ratio of R_q/R_{CO_2} (Eq. (3)) is shown in Fig. 1B. From these measurements, predicted relative specific growth rate (Eq. (1)) was plotted (Fig. 1C). Values from many such plots are summarized in Table 2. Tissue collections and metabolic measurements were made in February–April and July of 1998. At the mountain garden in February, the snow was too deep to correctly identify the individual plants. However, we were able to collect tissue in March at all the gardens. These data suggested that optimal growth occurred at 10–25 °C with low temperature stress from 5 to 10 °C and high temperature stress at 30–40 °C.

Table 2

Calorespirometry data were obtained from tissue collected in different months in 1998 from gardens in Salt Creek Canyon (near Nephi, UT, USA) of basin big sagebrush (*A. tridentata* ssp. *tridentata*), mountain big sagebrush (*A. tridentata* ssp. *vaseyana*), and hybrids between them^a

Garden–source (month)	Temperature response (°C)		
	Low stress	Optimal	High stress
Basin–basin (February)	10	15	30
Basin–basin (April)	<5	15–20	30
Basin–basin (July)	<10	30–40	>40
Basin–hybrid (February)	10	15	20
Basin–hybrid (April)	<5	20–25	30
Basin–hybrid (July)	<5	30	35
Basin–mountain (February)	5	15–25	30
Basin–mountain (April)	5	25–30	35
Basin–mountain (July)	10	20–35	>35
Hybrid–basin (February)	<5	15–25	30
Hybrid–basin (July)	10	15	30
Hybrid–hybrid (February)	5	10, 25	30
Hybrid–hybrid (July)	10	15–25	>35
Hybrid–mountain (February)	<5	10, 25	35
Hybrid–mountain (July)	<5	10, 20	>25
Mountain–basin (April)	<5	5–25	30
Mountain–basin (July)	<10	10, 15	20
Mountain–hybrid (March)	5	10, 15	30
Mountain–hybrid (April)	15	20, 25	30
Mountain–hybrid (July)	<5	10, 30	>40
Mountain–mountain (March)	5	10, 25	30
Mountain–mountain (April)	15	25–35	40
Mountain–mountain (July)	<5	10, 25	35

^a Measurements were made every 5 °C from 5 to 40 °C. Air temperatures when the tissue was collected were 6 °C in February, 20 °C in April, and 31 °C in July. Temperature responses were categorized as low stress, optimal, and high stress for site and source at different times of the year.

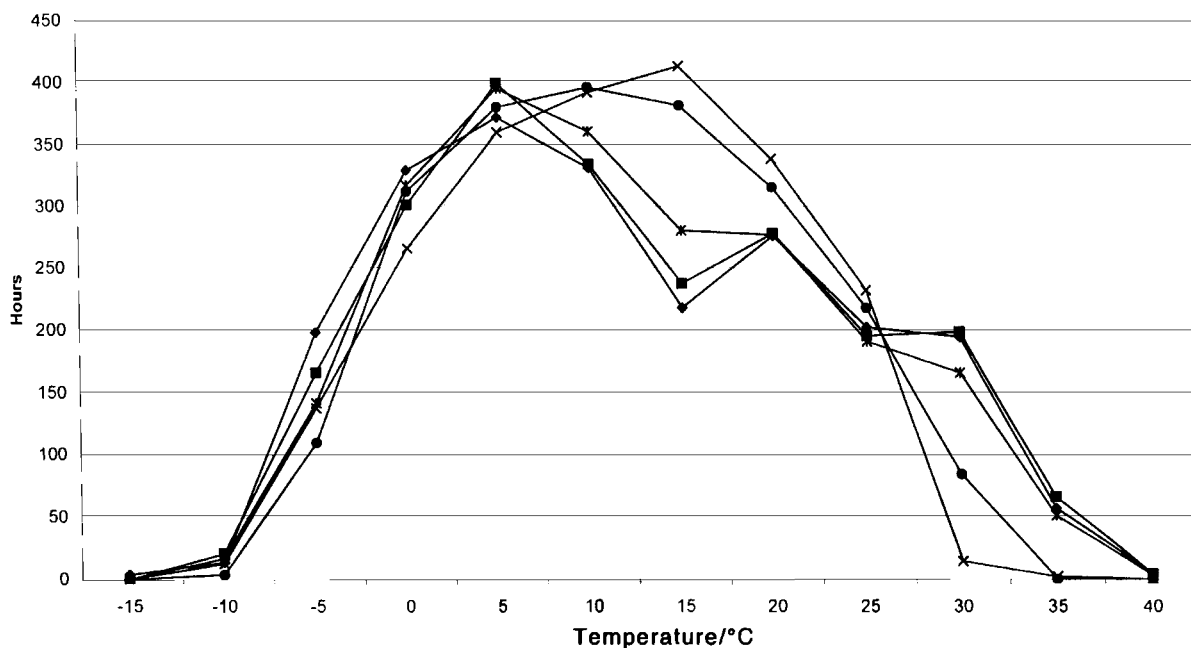


Fig. 2. Air temperatures recorded each hour from 1 April to 30 June 2001 at five stations along an elevational transect in Salt Creek Canyon near Nephi, UT, expressed as the total number of hours at each temperature. The five stations are plotted as meters above sea level: 1885, ×; 1860, *; 1835, ●; 1820, ◆; 1775, ■.

In Fig. 2 the most common temperatures recorded during the spring agree with the temperatures at which (Table 2) sagebrush will grow best.

4. Discussion

Perhaps our most important finding is that sagebrush grows slowly in cold weather and is not stressed by cool temperatures. On the other hand, warmer temperatures produced evidence of stress and reduced growth [14]. In general, the growth curves (e.g. Fig. 1C) look much like the temperature curves in Fig. 2. Note that higher temperatures are experienced as the elevation decreases. This difference probably explains much of the response seen in the transplantation experiment (see Table 2). Sagebrush is well adapted to living in the Great Basin which has cold, wet winters and hot, dry summers. The winters are not extremely cold and it is rare to find the ground frozen, as a blanket of snow usually insulates the surface. Nelson and Tierman [15] found extensive winter injury to big sagebrush in years with low snow cover and consequent

exposure to extreme cold. Sagebrush apparently can become dormant and withstand high summer temperatures, but may have no mechanism for slowing growth during very cold conditions.

Since sagebrush retains leaves all year round, Pearson [16] determined hourly and daily photosynthesis rates on six *A. tridentata* plants grown outside in Rexburg, ID. He found the highest rates of photosynthesis to be in December–February, while the lowest rates of photosynthesis were in July–August when the soil was very dry. Summer drought and high temperatures coincide in the Great Basin and the relative contribution of both stresses must be addressed. In a controlled greenhouse experiment, Booth et al. [17] concluded that for three subspecies of big sagebrush, even under the most severe conditions employed, water was not sufficiently limiting to retard seedling growth. These results were supported by work of Matzner and Richards [18] who found that sagebrush roots could maintain nutrient capacity even under water stress.

More negative carbon isotopic ratios may indicate increased discrimination against the heavy isotope

during diffusion through partially closed stomates [7]. Greater water availability, more extensive root systems, and cooler temperatures allow more open stomates and less isotopic fractionation (Table 1).

As the seasons change from winter to spring to summer, the non-stress temperature spectrum seems to broaden. This may protect tender meristem tissue from sudden changes in temperature. Sagebrush seems to prefer cool to moderately warm temperatures for growth and is well adapted to the cold-desert site in Salt Creek Canyon which has cool, wet winters and hot, dry summers. Individual populations are apparently adapted to local microsites at the relatively fine scales reported in this study.

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