Microcalorimetric Studies on Metabolic and Germination Response to Temperature for Three Populations of Winterfat (*Eurotia lanata*)

Tonya Thygerson
D. Terrance Booth
Jennifer M. Harris
Lee D. Hansen
Bruce N. Smith

Abstract–*Eurotia lanata* (Pursh) Moq. (winterfat) is a boreal cold-desert subshrub, seldom more than 2 feet tall, that thrives in dry climates at cool temperatures. Diaspore collections from Matador, Saskatchewan, Canada; Pine Bluffs, Wyoming; and Sterling, Colorado, were cleaned and placed on moistened filter paper in petri dishes maintained at 0, 5, 10, 15, and 20 °C to study germination. Seeds germinated at all temperatures but seedlings were not acclimated to cold by germination temperature. At radicle emergence (ca. 3 mm), seeds were placed in calorimeter ampules. Heat-rate (q) was measured at a given temperature, then a vial containing NaOH solution was added to measure the rate of CO_2 evolution (R_{CO2}) for the same tissue at the same temperature. This procedure was repeated for each of the populations at temperatures ranging from –10 to +20 °C. Metabolic efficiency and predicted specific growth rates were calculated from these measurements. Optimum temperature for germination, metabolism, and early seedling growth was about 10 °C. Stress was noted near 20 and –5 °C. Acclimation during germination had no effect. Differences between the three populations correlated with altitude rather than latitude.

Winterfat is a small cold-desert subshrub that thrives in dry climates at cool temperatures. Stems, leaves, and dispersal units called diaspores are covered with a dense mix of short and long white hairs that aid in water retention (Booth and Haferkamp 1995). Foliage and fruit are retained throughout the winter. Winterfat is excellent forage for both wildlife and domestic cattle and is a good source of protein and vitamin A. In North America, winterfat is found from Canada to Mexico, and from Manitoba to British Columbia and the Dakotas and Nebraska west to the Great Basin. The genus consists of only two species, one from North America, the other from the cold deserts of Asia (Mozingo 1987).

Populations within a species (accessions) are adapted to the particular microclimate of their origin and usually do not grow well when moved to a slightly different location. The purpose of this work is to examine how plants adapt their respiratory metabolism to match the temperature of their native climate. In this study, calorimetry was used to determine the temperature response and high and low stress temperatures of winterfat diaspores collected from three locations. When metabolic heat loss exceeds energy made available through catabolism of carbohydrate, the plant is considered to be stressed (Smith and others 2000).

Aerobic respiration has two aspects: catabolism and anabolism. In catabolism, organic substrates are oxidized to produce CO_2. Part of the energy produced by oxidation is used to convert ADP and inorganic phosphate to ATP, the rest is lost as heat.

$$\text{substrate} + O_2 + \text{ADP} + P_i \rightarrow CO_2 + H_2O + \text{ATP} + \text{heat} \quad (1)$$

ATP produced in catabolism is transient, but is used for cellular work, including anabolism as shown below:

$$\text{substrate} + \text{ATP} \rightarrow \text{growth} + \text{ADP} + P_i + \text{heat} \quad (2)$$

In anabolism, heat and new plant tissue are produced and ATP is hydrolyzed back to ADP and phosphate. A calorimeter measures the rate of heat loss (q) from both catabolism and anabolism. The rate of CO_2 production (R_{CO2}) measures the rate of catabolism. With carbohydrate as the specific substrate, predicted growth rate of structural biomass or rate of anabolism (R_{SG}) is related to the two measured variables as in equation 3.

$$R_{SG}\Delta H_B = 455R_{CO2} - q \quad (3)$$

where ΔH_B is the enthalpy change for the formation of biomass from photosynthate and Thornton's constant (–455 ± 15 kJ mol^{-1} of O_2) 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_{CO2} and q. The temperature dependencies of R_{CO2} and q are different (Hansen and others 1994). The difference between $455R_{CO2}$ and q therefore changes with temperature and this difference can be used to predict growth rate changes with temperature (Criddle and others 1997).

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Tonya Thygerson and Jennifer M. Harris are Undergraduate Students; D. Terrance Booth is a Senior Scientist, USDA-ARS, High Plains Grassland Research Station, Cheyenne, WY 82009. Lee D. Hansen is a Professor, Department of Chemistry and Biochemistry; Bruce N. Smith is a Professor, Department of Botany and Range Science, Brigham Young University, Provo, UT 84602.

Table 1—Sources and habitats for winterfat seeds (after Bai and others 1999).

Site	Location	Community	Seed weight g/100 seeds
Pine Bluffs, Wyoming, U.S.A.	41°10'N, 104°09'W elevation 1554 m	mixed prairie	0.18
Sterling, Colorado, U.S.A.	40°37'N, 103°13'W elevation 1181 m	shortgrass prairie	0.23
Matador, Saskatchewan, Canada	50°42'N, 107°43'W elevation 685 m	mixed prairie	0.25

Predicted specific growth rate may also be expressed as a function of the substrate carbon conversion efficiency (ε) and respiration rate (R_{CO2}).

$$R_{SG} = R_{CO2}\,[\varepsilon/(1-\varepsilon)] \quad (4)$$

Combining equations 3 and 4 to eliminate R_{SG} gives equation 5

$$(\varepsilon/1-\varepsilon)\Delta H_B = -q/R_{CO2} - (1-\gamma_P/4)455 \quad (5)$$

which relates the ratio of q/R_{CO2} to ε. Values of q/R_{CO2} 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 (Hansen and others 1994).

Winterfat diaspores collected from three locations were compared at several temperatures using calorimetry.

Materials and Methods

Diaspores from *Eurotia lanata* (Pursh) Moq. (winterfat) were collected from Matador, Saskatchewan, Canada; Pine Bluffs, Wyoming; and Sterling, Colorado (table 1; Bai and others 1999). The diaspore was first removed from the seed to decrease fungal growth during germination (Booth and Haferkamp 1995). The threshed seeds were soaked in a tween solution (10 percent) for 10 minutes, then in dilute sodium hypochlorite (1 percent) for 45 minutes. Then the seeds were placed on moistened filter paper in petri dishes kept in beakers partially submerged in coolant baths maintained at 0, 5, 10, 15, and 20 °C to study acclimation effects on germination and metabolism.

At the time of radicle emergence (to about 3 mm), seeds (about 100 mg fresh weight) were placed in each of three ampules of a microcalorimeter (Hart Scientific model 7707 or Calorimetry Sciences Corporation MCDSC model 4100). After 15 to 20 minutes thermal equilibration at the desired temperature, the metabolic heat rate (q) was measured for another 15 to 20 minutes. The ampules were removed from the calorimeter and a small vial filled with 40 µl of 0.4 M NaOH placed in the calorimeter ampule with the tissue. Again a 15 to 20 minute thermal equilibration was necessary, followed by measurement of the sum of the heat from metabolism and CO_2 reaction with the NaOH for 15 to 20 minutes. After the NaOH is removed the heat rate (q) is measured again as before (Hansen and others 1994; Criddle and others 1997). The reaction of CO_2 with the NaOH solution to form carbonate produces –108.5 kJ mol^{-1}. Dividing the difference in the measurements with and without NaOH solution present gives the rate of CO_2 evolution (R_{CO2}) by the plant tissue. The tissue was then run at another temperature. Measurements were made on each sample at 7 temperatures: 20, 15, 10, 5, 0, –5, and –10 °C.

Results and Discussion

Acclimating germinating seeds at various temperatures had no effect on germination or metabolism. Seeds germinated as rapidly at 0 °C as they did at 20 °C with essentially 100 percent germination at all temperatures in agreement with previous work (Bai and others 1998a). Also seeds germinated at a given temperature, say 5 °C, showed no different metabolic response at 5 °C than did seeds germinated at 15 °C (Bai and others 1998b). Desiccation and cold hardiness are often linked in winterfat (Hou and others 1999).

Since growth in terms of energy can occur only when catabolic energy generation rate ($455R_{CO2}$) exceeds total heat loss (q), metabolic data (fig. 1) for the winterfat population from Pinebluffs, Wyoming, indicated cold stress near 0 °C and heat stress at about 18 °C. Please note that a smaller ratio of q/R_{CO2} indicates greater efficiency (fig. 2) with values greater than 455 µW mg^{-1} dry wt. representing

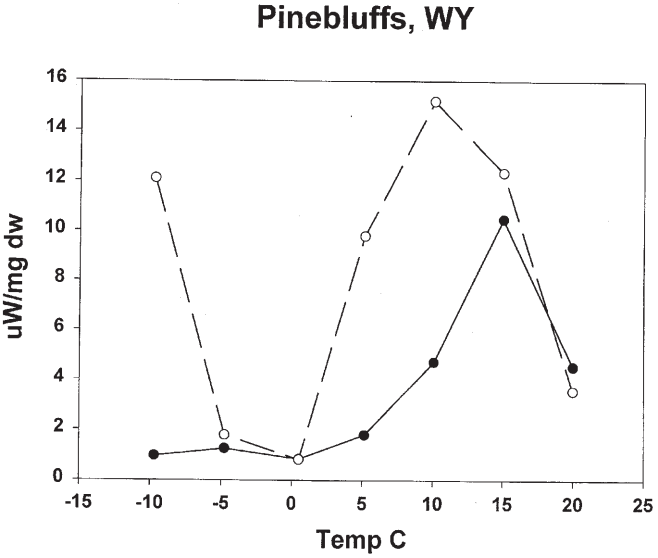

Figure 1—Winterfat seedlings from Pinebluffs, Wyoming, U.S.A.; metabolic heat rate (q), (●), and respiration rate ($455R_{CO2}$), (o), as µW per mg dry wt. versus temperature measured at 5 °C intervals.

a shift to another substrate (for example, lipid) or physical damage. Negative points in figure 3 occur because the substrate was assumed to be carbohydrate. Relative specific growth rates (R_{SG}) less than zero occur then this assumption is invalid.

Winterfat seedlings from Sterling, Colorado, (fig. 4) were cold-stressed at about –8 °C and heat stressed at about 16 °C with good efficiency (fig. 2) and growth (fig. 3) predicted between those temperatures.

Seedlings from Matador, Saskatchewan, Canada, (fig. 5) were cold-stressed at about –6 °C and heat stressed at about 19 °C with maximum efficiency (fig. 2) and growth (fig. 3) between 8 and 12 °C.

Seed weights decreased (Bai and others 1999) with increasing elevation (table 1). The highest elevation site probably has the shortest growing season. However, once daytime temperatures are above freezing, rapid growth can occur. Note that the Pinebluffs' population (figs. 1, 2, and 5)

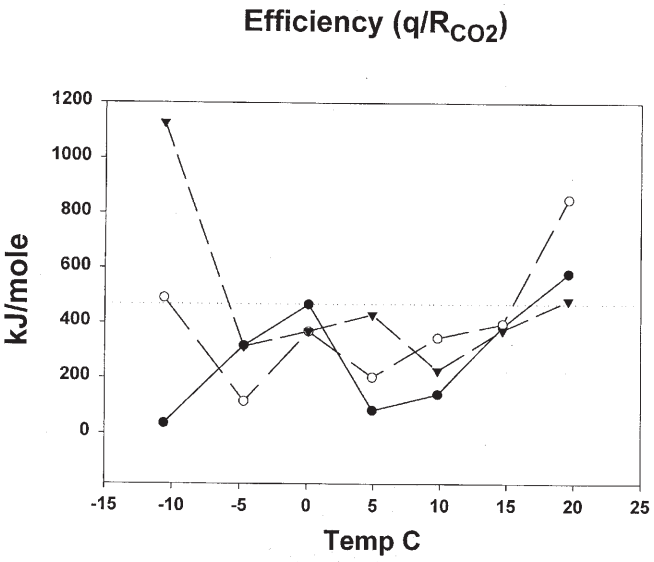

Figure 2—Comparison of the metabolic efficiency (q/R_{CO2} in kJ/mole) of the three populations versus temperature. Pinebluffs (●), Sterling (o), and Matador (▼). Note: smaller numbers mean greater efficiency.

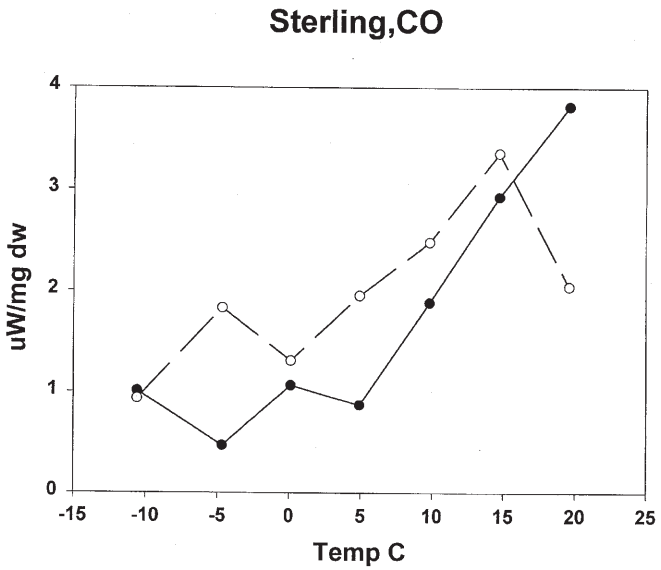

Figure 4—Winterfat seedlings from Sterling, Colorado, U.S.A., as in figure 1.

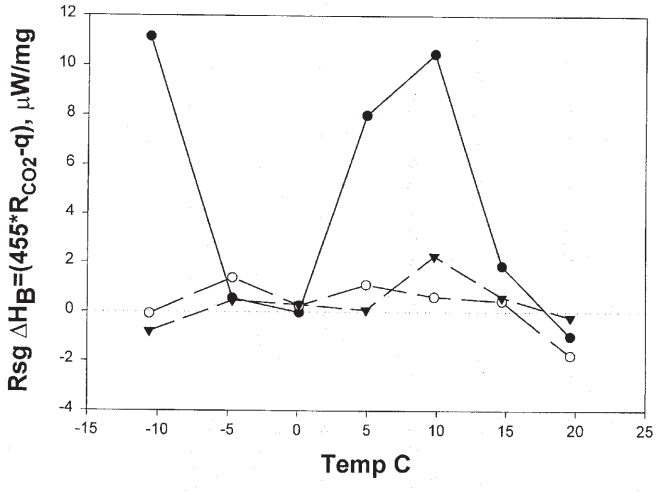

Figure 3—Comparison of the predicted growth rate ($R_{SG}\Delta H_B$ in μW/mg) of the three populations versus temperature. Pinebluffs (●), Sterling (o), and Matador (▼). $R_{SG}\Delta H_B$ values lower than zero indicate temperatures where no growth occurs.

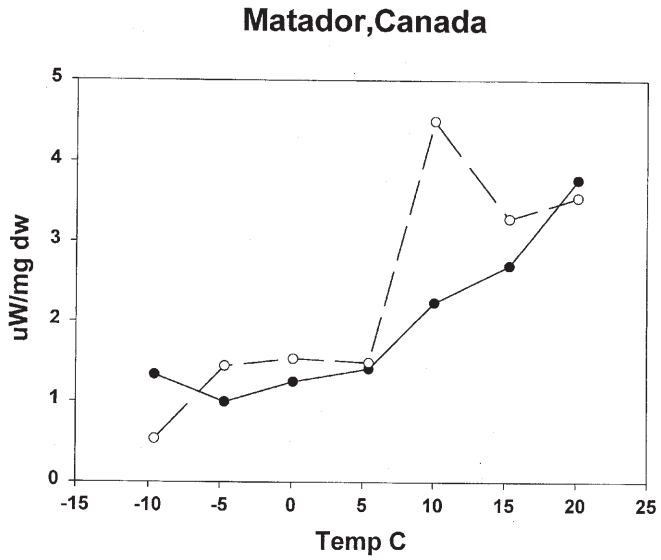

Figure 5—Winterfat seedlings from Matador, Saskatchewan, Canada, as in figure 1.

has a more narrow temperature range for growth, but efficiency of carbon conversion and growth rate exceeds those of both of the other populations. Metabolic data presented here indicate that these three closely related populations are differently adapted to temperature at their respective sites. We plan to expand this study to include winterfat populations across a broader range of environments. This may allow us to determine if the differences noted here among seedling populations also persist for mature plants grown *in situ* or in common gardens.

Conclusions

- Optimum temperature for metabolism and early seedling growth for three populations of winterfat is about 10 °C. Stress is noted below –5 and above +20 °C.
- Metabolic differences among the three populations studied were correlated with altitude rather than latitude, and probably reflect adaptation to different thermal environments.
- Winterfat seeds imbibe water, germinate, and grow at very cool temperatures—even 0 °C. Acclimation had no effect. Thus seeds germinated at 5 °C did no better at that temperature than did seeds germinated at 20 °C.

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