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Thermochimica Acta 394 (2002) 211–217

thermochimica acta

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Metabolic response to temperature for six populations of winterfat (*Eurotia lanata*)

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Received 10 September 2001; received in revised form 7 March 2002; accepted 9 March 2002

Abstract

Eurotia lanata (Pursh) Moq. [*Krascheninnikovia lanata* (Pursh) Guldenstaedt.] (winterfat) is a boreal cold-desert subshrub, seldom more than 2 ft tall, that thrives in dry climates at cool temperatures. Diaspore collections from Saskatchewan, Wyoming, Colorado, and New Mexico were cleaned and placed on moistened filter paper in petri dishes. Seeds maintained at 0, 5, 10, 15, and 20 °C for seven days germinated at all temperatures with no evidence of acclimation. At radicle emergence (ca. 3 mm), seeds were placed in calorimeter ampoules. Heat rate (R_q) was measured at a given temperature, then a vial containing NaOH solution was added to measure the rate of CO_2 evolution (R_{CO_2}) for the same tissue at the same temperature. This procedure was repeated for each of the populations at temperatures ranging from 0 to 25 °C. Metabolic efficiency and predicted specific growth rates were calculated from these measurements. Optimum temperature for germination, metabolism, and early seedling growth ranged from 5 to 25 °C. Seedlings differed in response to temperature reflecting the climate at the site of origin. Published by Elsevier Science B.V.

Keywords: Germination; Metabolism; Respiration rate; Temperature; Winterfat

1. Introduction

Winterfat is a small cold-desert subshrub that thrives in dry climates at cool temperatures. Stems, leaves, and dispersal units (diaspores) are covered with a dense mix of short and long white hairs that aid in water retention [1]. Foliage is 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 and the other from the cold deserts of Asia [2].

Populations within a species (accessions) are adapted to the particular microclimate of their origin and may, or may not, grow as well when moved to a 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 respiratory metabolism in winterfat diaspores

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0040-6031/02/$ – see front matter. Published by Elsevier Science B.V.
PII: S0040-6031(02)00253-8

collected from six locations. When metabolic heat loss exceeds energy made available through catabolism of carbohydrate, the plant is considered to be stressed [3].

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 (Pi) to ATP, the rest is lost as heat.

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

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

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

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 (R_q) from both catabolism and anabolism. The rate of CO_2 production (R_{CO_2}) 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 Eq. (3):

$$R_{SG}\Delta H_B = 455R_{CO_2} - R_q \quad (3)$$

where ΔH_B is the enthalpy change for the formation of biomass from photosynthate and Thornton's constant ($-455 \pm 15\,\text{kJ mol}^{-1}$ 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_{CO_2} and R_q. The temperature dependencies of R_{CO_2} and R_q are different [4]. Thus, the difference changes with temperature [5].

Predicted specific growth rate may be expressed as a function of the substrate carbon conversion efficiency (ε) and respiration rate (R_{CO_2}) as in Eq. (4):

$$R_{SG} = R_{CO_2}\left[\frac{\varepsilon}{(1-\varepsilon)}\right] \quad (4)$$

Combining Eqs. (3) and (4) to eliminate R_{SG} gives Eq. (5):

$$\left[\frac{\varepsilon}{(1-\varepsilon)}\right]\Delta H_B = -\frac{R_q}{R_{CO_2}} + \left(1 - \frac{\gamma_P}{4}\right)455 \quad (5)$$

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, i.e. γ_P [4].

2. Materials and methods

Diaspores from *Eurotia lanata* (Pursh) Moq. [*Krascheninnikovia lanata* (Pursh) Guldenstaedt.] (winterfat) were hand-collected from Pine Bluffs, WY; Sterling, CO; and Matador, Sask., Canada [6]. The second site is located in Shortgrass prairie while the first and third are located in Mixed prairie (Table 1). Additional diaspores were collected from three locations in the Sevilleta National Wildlife Refuge about 100 km south of Albuquerque, NM, USA (Table 1). The Northside and Southside sites are separated about 25 km along a north–south gradient in Transition

Table 1
Sources and habitats for winterfat (*Eurotia lanata*) diaspores and a summary of metabolic data in response to temperature (low and high stress) of germinated seeds[a]

Site	Location	Elevation (m)	Community	Temperature response (°C)		
				Low	Optimal	High
Pine Bluffs	41°10′N, 104°09′W	1554	Mixed prairie	0	5–15	18
Sterling	40°37′N, 103°13′W	1181	Shortgrass	0	5–15	16
Matador	50°42′N, 107°43′W	685	Mixed prairie	0–5	10–15	20
Northside	34°25′N, 106°39′W	1600	Chihuahuan prairie 2	5–25	25+	
Westside	34°20′N, 106°55′W	1539	Great Basin prairie 7	10–25	25+	
Southside	34°12′N, 106°48′W	1533	Chihuahuan prairie 8	10–25	25+	

[a] Calorimetric measurements were made every 5 °C from 0 to 25 °C.

Chihuahuan and Plains Grassland dominated by both black grama [*Bouteloua eriopoda* (Torr.) Torr.] and blue grama [*B. gracilis* (H.B.K.) Lag. ex Steudel]. The Westside site is about 20 km west of the other sites in Great Basin Grasslands characterized by galleta [*Hilaria jamesii* (Torr.) Benth.] and Indian ricegrass [*Oryzopsis hymenoides* (R&S) Ricker] [7].

The seeds were first removed from the utricle and enclosing bracts to decrease fungal growth during germination [1]. The threshed seeds were soaked in Tween solution (10%) for 10 min, and then in dilute sodium hypochlorite (1%) for 45 min. Treated seeds from the northern populations were placed on moistened filter paper in petri dishes kept in beakers partially submerged in coolant baths for 7 days maintained at 0, 5, 10, 15, and 20 °C to study the effects of temperature on germination and metabolism.

After 7 days, radicles emerged to about 3 mm. Seedlings (about 100 mg fresh weight) were placed in each of three ampoules of an isothermal microcalorimeter (Hart Scientific Model 7707 or Calorimetry Sciences Corporation Model 4100). After thermal equilibration for 15–20 min at a given temperature, the metabolic heat rate (R_q) was measured for another 15–20 min. The ampoules were removed from the calorimeter and a small vial filled with 40 μl of 0.4 M NaOH was placed in the ampoule with the tissue. Again a 15–20 min thermal equilibration was necessary, followed by a measurement of the sum of the heat from metabolism and CO_2 reaction with the NaOH for 15–20 min (R_{CO_2}). The reaction of CO_2 with the NaOH solution to form carbonate produces $-108.5\,kJ\,mol^{-1}$. After the NaOH was removed the heat rate (R_q) was measured again as before [4,5]. The tissue was then run at another temperature. A total of seven calorimeters were used. There were six replicates for each measurement. The uncertainty for measuring R_q was ±5% and for R_{CO_2} ± 20%. Measurements were made on each sample at five or six temperatures: 25, 20, 15, 10, 5, and 0 °C. It was not possible using our methods to measure R_{CO_2} at temperatures below freezing as the dilute NaOH froze.

3. Results

Germination at various temperatures had no effect on time of germination or metabolism. Seeds germinated as rapidly at 0 °C as they did at 20 °C with essentially 100% germination at all temperatures, in agreement with previous work [8]. Seeds germinated at different temperatures did not differ in metabolic response to temperature. For example, seeds germinated at 5 °C had the same metabolic response as seeds germinated at 20 °C [9].

Growth in terms of energy can occur only when the rate of energy generation from oxidation of carbohydrate ($455R_{CO_2}$) exceeds the rate of heat loss (R_q). Metabolic data for winterfat populations from Pinebluffs, WY (Fig. 1A), Sterling, CO (Fig. 1B) and Matador, Sask., Canada (Fig. 1C) differ from each other (Table 1). Metabolic heat rates and respiration rates are compared for the three populations of winterfat from the Sevilleta National Wildlife Reserve, New Mexico in Fig. 2. The Northside (Fig. 2A), Westside (Fig. 2B), and Southside populations (Fig. 2C) also were metabolically different from one another (Table 1).

Differences between northern (Colorado, Wyoming, and Saskatchewan) and southern (New Mexico) populations are illustrated (Fig. 3) by comparing metabolic efficiency as indicated by the R_q/R_{CO_2} vs. temperature of the Pinebluffs and the Northside populations. Note that a smaller ratio of R_q/R_{CO_2} indicates greater efficiency. Values greater than 455 μW per mg dry weight represent either physical damage or a shift to another substrate (e.g. lipid).

Predicted growth rate vs. temperature for Pinebluffs is compared with that for Northside in Fig. 4. $R_{SG}\Delta H_B$ values lower than zero indicate no growth or dormancy.

4. Discussion

Table 1 summarizes data for the six populations studied. Metabolic data presented here indicate that these closely related populations are differently adapted to temperature at their respective sites. Similar calorimetric measurements of metabolism have shown differences between cultivars of corn (*Zea mays* L.) [10], soybean [*Glycine max* (L.) Merr.] [11], and populations of cheatgrass (*Bromus tectorum* L.) [12].

We plan to expand this study to include winterfat populations from more diverse environments. We

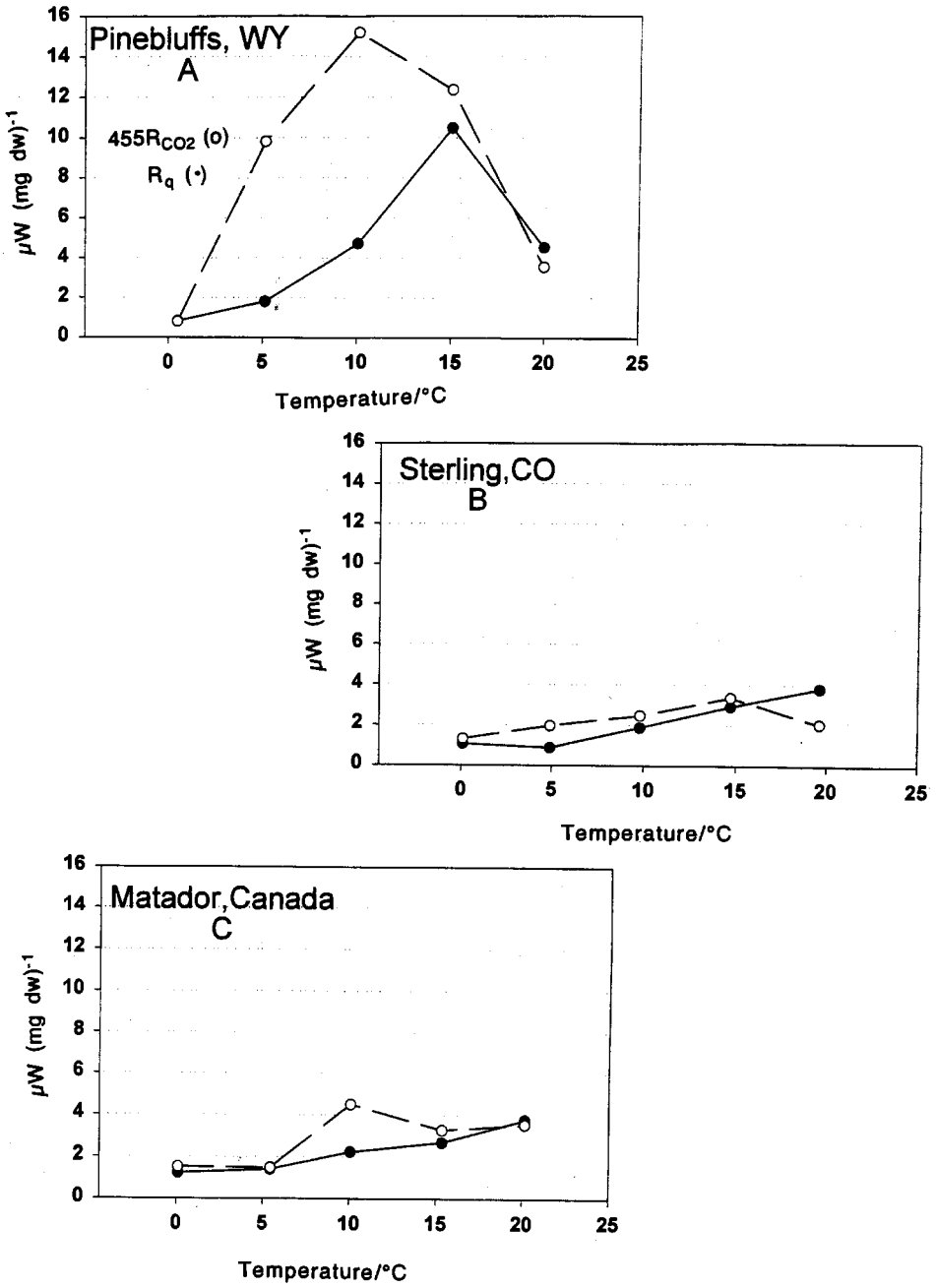

Fig. 1. Metabolic heat rate (R_q) (●) and respiration rate ($455R_{CO_2}$) (○) as microwatts (μW) per mg dry weight were measured at different temperatures for winterfat seedlings from Pinebluffs, WY, USA (A); Sterling, CO, USA (B); and Matador, Sask., Canada (C).

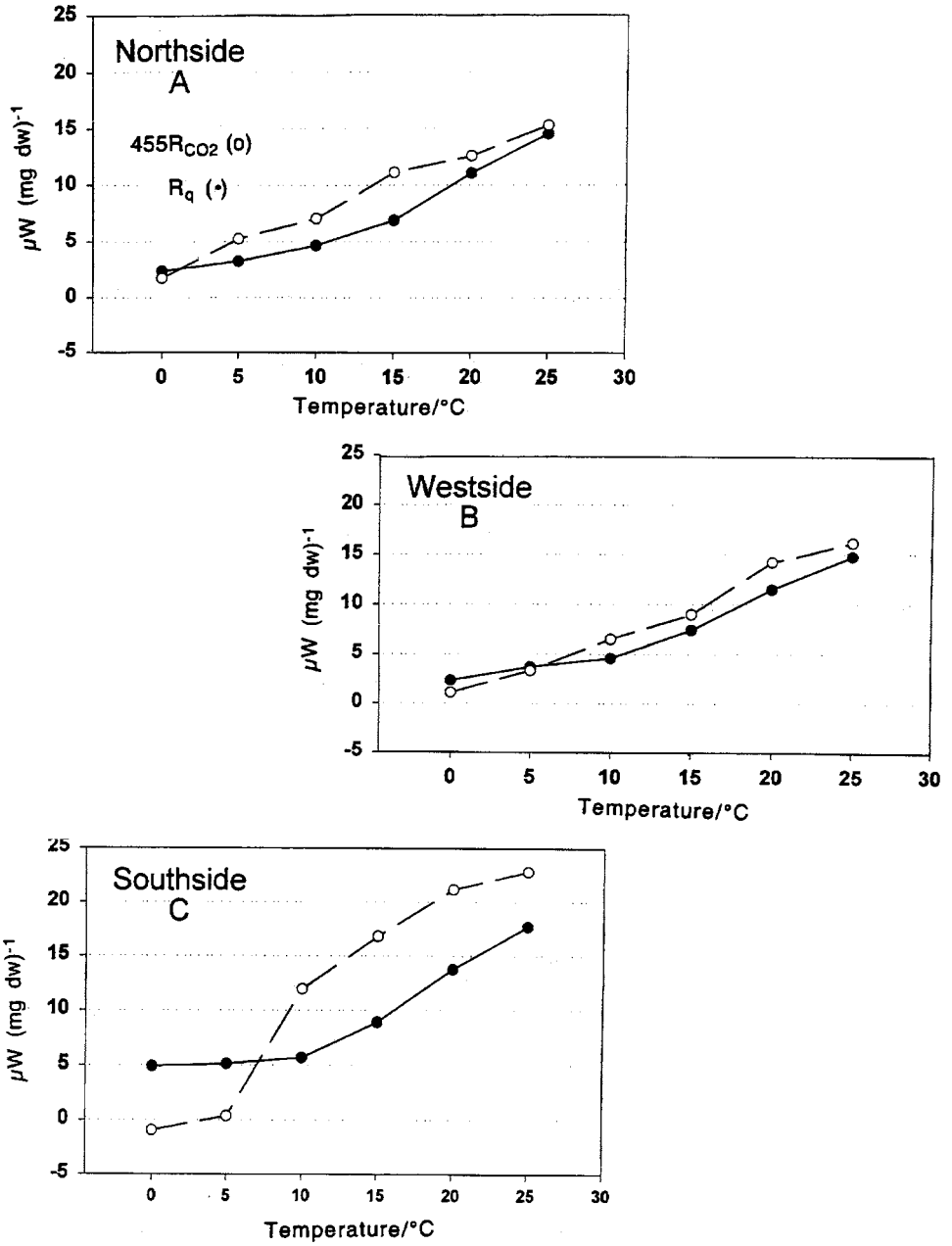

Fig. 2. Seeds from three different locations within the Sevilleta National Wildlife Refuge in New Mexico were germinated and measured metabolically as in Fig. 1. The populations were designated as Northside (A), Westside (B), and Southside (C) and the metabolic data presented as in Fig. 1. Note the difference in scales between Figs. 1 and 2.

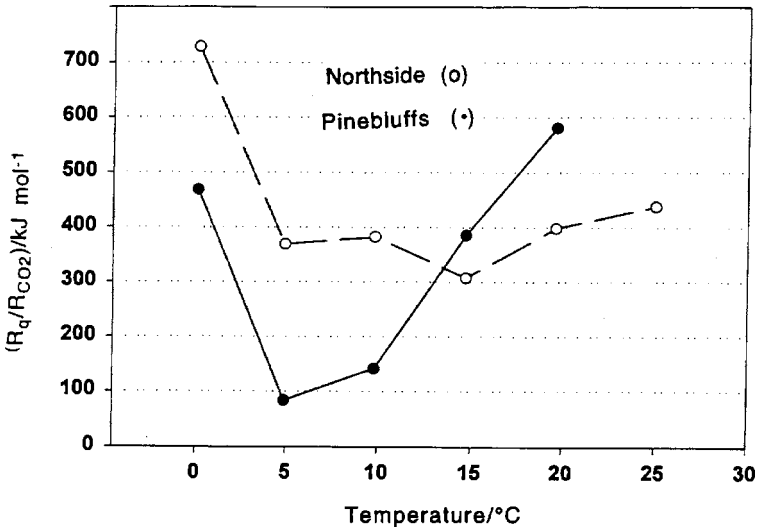

Fig. 3. Two populations, Pinebluffs (●) and Northside (○), compared for metabolic efficiency (R_q/R_{CO_2} in kJ mol^{-1}) at different temperatures. Note: smaller numbers mean greater efficiency.

also must determine if the differences noted among seedling populations persist for mature plants grown in situ or in common gardens.

In conclusion, optimum temperature for metabolism and early seedling growth for the three northern populations of winterfat is about 10 °C with stress noted at 0 °C and above 20 °C (Fig. 1). Seedlings from the New Mexico populations were stressed near 5 °C but indicated good growth at warmer temperatures. Metabolic differences probably reflect adaptation to different thermal environments. Northern populations of winterfat seeds imbibe water, germinate, and

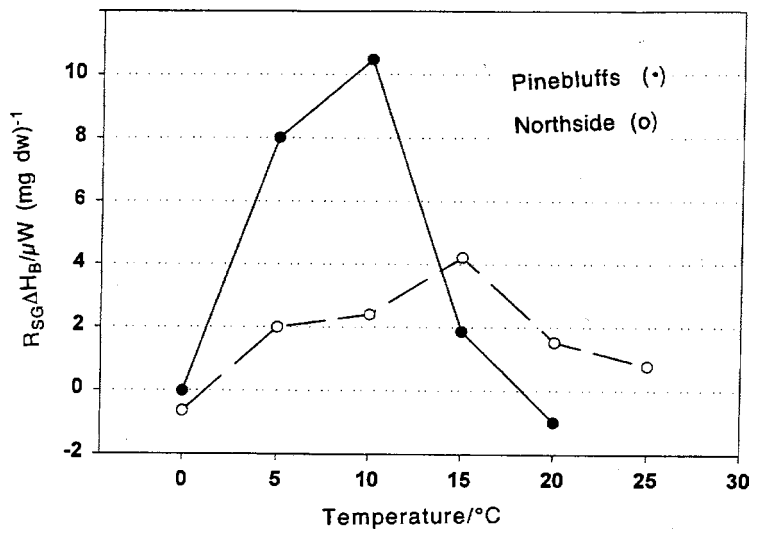

Fig. 4. Pinebluffs (●) and Northside (○) populations compared for predicted growth rate ($R_{SG}\Delta H_B$ in μW per mg dry weight) at different temperatures. $R_{SG}\Delta H_B$ values lower than zero indicate no growth.

grow at very cool temperatures—even 0 °C. A 7-day acclimation seemed to have no effect. Thus seeds germinated at 5 °C did no better at that temperature than seeds germinated at 20 °C.

Acknowledgements

This research was supported in part by funds provided by the Rocky Mountain Research Station, Forest Service, US Department of Agriculture.

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