

Short Communication

Effects of Low Temperature on Respiration and Uptake of Rubidium Ions by Excised Barley and Corn Roots¹

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ABSTRACT

The effect of temperature upon ion uptake and respiration was investigated with excised roots of corn (*Zea mays*) and barley (*Hordeum vulgare*). A strong inhibition ($Q_{10} = 5$ to 8) of ion uptake was observed at temperatures below 10 C. At higher temperatures more normal temperature dependencies ($Q_{10} = 1.3$ to 2) were obtained. When the data were plotted according to the Arrhenius relationship, two different activation energies were indicated above and below 10 C. Other studies have related such changes with temperature in activation energy of processes to changes in membrane properties induced by temperature. These results suggest that such phase transitions may affect ion uptake processes. If so, then differences among species in their capacity to maintain normal root function at low soil temperature and to resist low temperature stress may be related to differences in the physical properties of cellular membranes.

It is well known that low soil temperatures may affect plant growth and that there are great differences among species in their tolerance of low soil temperature (2, 6-8, 13). Presumably these effects are due to an inhibition of root function by low temperature. Comparative studies have shown that roots of plants native to habitats with cool soil temperatures tend to have a higher capacity for ion uptake and respiration at cool temperatures than plants native to warmer soil habitats (2). These differences in metabolic capacity of the roots must play a role in adaptation of plants to differing thermal regimes. Little is known, however, of the basic effects of temperature on root function. In this paper we report studies of the effect of temperature upon short term assays of ion uptake and respiration of excised roots of barley and corn. Ion uptake processes are shown to be strongly inhibited at low temperature. Analysis of the apparent Arrhenius activation energy of ion uptake over a wide temperature range suggests that this inhibition may be the result of an effect of low temperature on the physical properties of a cellular membrane involved in ion uptake. A preliminary account of this work has been published (1).

MATERIALS AND METHODS

Barley seeds (*Hordeum vulgare* cv. Mariout Ferry Morse) and corn seeds (*Zea mays* cv. WF9 × Bear 38) were rinsed three times with fresh 0.5 mM CaSO₄ solution, and germinated on acrylic-framed polyethylene-coated Fiberglas mesh screens floating on 3 liters of aerated 0.5 mM CaSO₄ solution in polyethylene boxes. These boxes were placed in the dark for 5 days at 28 ± 0.5 C. The

seedlings were rinsed daily with deionized H₂O and transferred to clean boxes with fresh CaSO₄ solution. At the end of the growth period the seedlings were rinsed with CaSO₄ solution and the roots extending below the screen were excised for the experiments described below.

O₂ consumption at normal O₂ concentration was measured on samples of excised roots (100 mg) in an O₂ electrode (Rank Bros., Bottisham, Cambridge, England) after 15 to 20 min preconditioning at the measurement temperature. The assay is nondestructive hence a single sample could be used for the temperature range from 5 to 40 C. Similar results were obtained with upward or downward sequences of temperature change.

Rubidium uptake was measured as ⁸⁶Rb accumulation from a solution containing 0.5 mM CaSO₄ and 0.05 mM [⁸⁶Rb]RbNO₃ (approximately 0.07 μCi/μmol). This concentration of Rb was assumed to be rate-saturating for the low concentration isotherm mechanism (4). Separate samples of excised roots with a fresh wt of 200 to 300 mg in a "tea bag" (5) were conditioned for 20 min in a 0.5 mM CaSO₄ solution at the measurement temperature. The "tea bags" were then transferred into 300 ml of labeled solution at the appropriate temperature for either 20 min below 15 C or 10 min above 15 C.

After the uptake period, any ⁸⁶Rb present in the readily exchangeable "free spaces" of the roots was removed by desorption in 0.05 mM RbNO₃ and 0.5 mM CaSO₄ at 0 C for 1 min and 30 min at 25 C. The ⁸⁶Rb retained by the roots was determined by immersing the roots in 10 ml of liquid scintillation fluid (Toluene, Triton X100, Omnifluor, water, 1000:500:6.66:166) and counting the vials after they had stood overnight. Control experiments indicated 80% efficiency of counting by this procedure.

RESULTS

Rates of respiration and Rb ion uptake as a function of root temperature for excised barley roots are shown in Figures 1 and 2. Respiration has an optimum near 40 C and below that the rate of respiration falls off exponentially with temperature. As shown in Figure 2 the respiration data fit rather well to a straight line on the Arrhenius plot of 1/T versus the natural logarithm of the rate. This line indicates a constant activation energy of 12.8 kcal mol⁻¹ over the range from 5 to 30 C and is similar to relationships obtained for respiration by intact tissue or for succinate oxidation by isolated mitochondria of species such as potato (11).

The capacity of the excised barley roots for ion uptake also decreases with temperature at temperatures below 35 C. Ion uptake is more dramatically affected by changes in temperature at low temperature than in the range of 20 to 30 C (Fig. 1). The data for ion uptake when graphed as an Arrhenius plot (Fig. 2) fit two different straight lines, with a sharp discontinuity at 10 C. At temperatures from 11 to 30 C an apparent activation energy of 5.3

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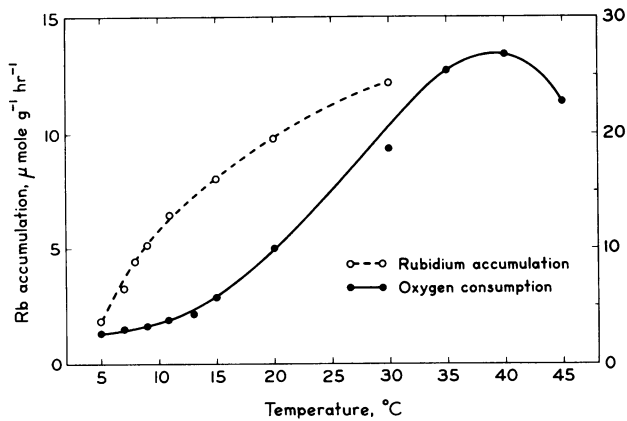


FIG. 1. Rates of respiration and Rb ion accumulation by excised barley roots as a function of temperature.

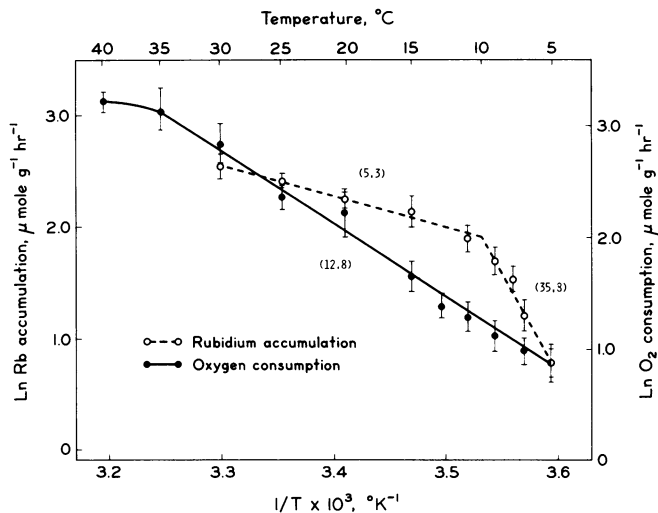


FIG. 2. Rates of respiration and Rb accumulation by excised barley roots as a function of temperature. Data are graphed to the Arrhenius relationship. Numbers in parentheses represent apparent activation energies of the lines connecting the points. Points and bars represent the mean and SE of the mean of several separate determinations.

kcal mol⁻¹ is indicated, while from 5 to 10 C the apparent activation energy is 35.8 kcal mol⁻¹. Ion uptake is thus dramatically inhibited at temperatures below 10 C. Similar strong inhibition of ion uptake was observed in studies with excised corn roots (Fig. 3). The apparent activation energy for ion uptake was 6.4 kcal mol⁻¹ above 10 C and 26.5 kcal mol⁻¹ below 10 C. Figure 3 also shows that the respiratory activity of corn roots was strongly inhibited at low temperature with the data approximating two different apparent activation energies above and below about 10 C.

DISCUSSION

The results presented here indicate a strong and complex effect of temperature upon root function. Ion uptake by both barley and corn roots is affected by temperatures above 10 C ($Q_{10} = 1.4-1.5$), however, it is much more strongly affected by temperatures below 10 C ($Q_{10} = 5-8$). Sharp discontinuities in apparent activation energy similar to those described here have been reported for succinate oxidation by intact isolated mitochondria of several species of higher plants and animals (18). Uptake of sugar by *Escherichia coli* follows a higher activation energy below a critical temperature than above it (9). The ($\text{Na}^+ + \text{K}^+$)-ATPase of animal tissue which is thought to be involved in transport of Na^+ and K^+ across membranes has also been shown to exhibit such sharp changes in apparent activation energy (3).

A common feature of many of these processes which show such changes in apparent activation energy with temperature is that they are in general located on or in biological membrane systems. Lyons and Raison (11) advanced a hypothesis that this pattern of temperature regulation was the result of a temperature-induced phase transition of the membrane supporting the process. A great deal of evidence has been accumulated to support this hypothesis (16, 17). In addition, membranes may lose some of their semi-permeability at temperatures below the phase transition point and become leaky (14, 15).

There is evidence that membranes of corn undergo such a phase transition at near 10 C (16). Inhibition of the carrier which transports ions across the membrane or leakage of these ions back across the membrane might result at temperatures below this phase transition and could explain the inhibition of Rb accumulation observed. The parallel inhibition of respiration by corn roots also observed at low temperature is probably also related to the occurrence of such a phase transition.

The response of barley to low temperature differs from that of corn in that with corn both ion uptake and respiration have sharp changes in activation energy at 10 C while only ion uptake exhibits this response in barley. If the response of ion uptake by barley to low temperature is caused by a phase change of the lipids of a cellular membrane, then it would appear that ion uptake is more sensitive than is respiration. Alternatively this lack of a parallel effect of temperature on both respiration and ion uptake might be taken as a suggestion that the low temperature inhibition of ion uptake may not be due to a phase transition of the membrane but to some other effect of low temperature—perhaps on the transport enzyme directly. Another possibility which should be considered is that ion uptake may be limited through an effect of temperature on energy available to support ion uptake via an effect of temperature on respiration. The data obtained with corn are consistent with this possibility since respiration and ion uptake are affected similarly above and below 10 C. As noted above, this was not true of barley roots suggesting that the effect of temperature on ion uptake is independent of an effect of temperature on respiration—at least with barley. (Also see Lüttge and Pitman [10] who argue that ion uptake processes by barley roots should consume only a small fraction of the energy available from respiration.)

Further studies of the effects of low temperature on the kinetics of the carrier process, the bidirectional fluxes of ions across the membrane, energy levels of the tissue, and physical properties of the membranes would be required to decide the mechanism(s) of the low temperature inhibition. However, regardless of the mechanism responsible for the effect of low temperature on ion uptake,

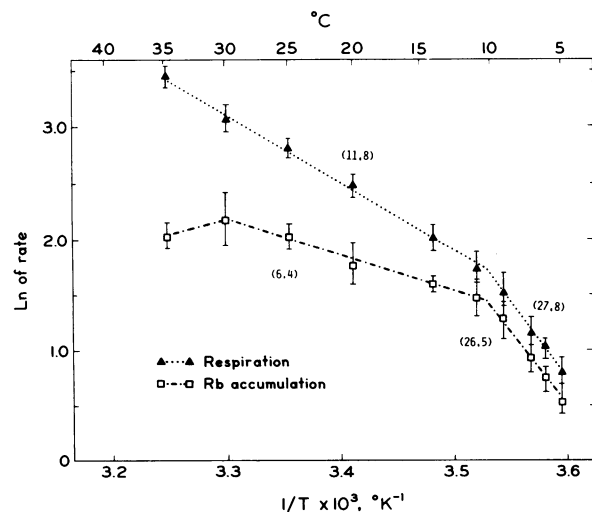


FIG. 3. Rates of respiration and Rb accumulation by excised corn roots as a function of temperature. For details refer to Figure 2.

it seems likely that this would have a significant impact on the nutrition and growth of the intact plant. From the above studies it would appear that root performance would respond normally to root temperature at temperatures above the sharp change in activation energy at 10 C, but performance could deteriorate dramatically at temperatures below that critical temperature. If this is so, the transition temperature might be very important in determining the lower limit for normal physiological performance. In the studies reported here the transition temperature was similar in corn and barley. However, there is evidence of differences among plants in the temperature at which other processes become strongly inhibited by low temperature (16), and for differences among plants in their sensitivity to low root temperature (2, 7, 8).

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