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BRIEF COMMUNICATION

Time Course of Electrolyte Leakage from Various Samples Killed by Frost, Liquid Nitrogen or Boiling

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Abstract. Samples of the same kind (rape leaves, wheat crowns, onion bulbs or spruce needles) killed by a slow freeze-thaw cycle or by immersion in liquid nitrogen showed a similar time course of leakage. Leakage was accelerated in treatments when the sample, immersed in water, was killed by heating for 5, 15 or 30 min in a boiling water bath. The longer the boiling, the sooner the maximum conductivity (L_{km}) was obtained. The L_{km} did not depend on the way of killing but in samples killed by freezing, it was achieved after a longer leakage time. The leakage rate decreased from onion to rape, wheat and spruce. The relative conductivity [%] allowed a better comparison of various treatments and of samples of different kinds of tissues than the specific conductivity [μS cm^{-1}].

The conductivity method has been generally used to assess a degree of injury of plant samples exposed to stress (frost, heat, drought, *etc.*) (LEVITT 1980). In brief, it is based on the proportional relation between the degree of injury and the amount of electrolyte leaked out from the sample into the surrounding deionized water. The results are expressed as specific (absolute) conductivity, relative conductivity or index of injury (FLINT *et al.* 1967, SULLIVAN 1972). In any case it is supposed that the maximum possible value of conductivity is obtained for the sample killed. The objective of this study was to examine whether this value (the maximum conductivity) is identical in the samples killed by the low or by the high temperatures and how this value depends on the leakage time for various samples. It should be stressed that the aim was not to determine the total amount of electrolyte in samples but the maximum (final) attainable amount of electrolyte leaked out from samples killed by various treatments.

Winter wheat crowns, winter rape leaves, onion bulb scales and spruce needles were obtained from experimental plots of our institut.. The needles and the onion

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bulb were gathered in summer, the wheat and the rape at hardening stage in winter. A sample consisted of 5 discs (0.8 cm diameter) of the bulb scales (onion), 12 discs of the leaves (rape), 6 segments (1 cm length) of the crowns and leaf bases (wheat) or 14 segments of the needles (spruce). Test tubes with samples were covered and exposed to one of the following treatments: (1) slow freezing procedure up to −30 °C (12 °C per hour, inoculation by ice crystals at −2 °C) and then thawing for 1 h at +1 °C, (2) quick immersion in liquid nitrogen (−196 °C), (3) to (5) immersion in boiling water (+100 °C) for 5, 15 or 30 min. The tubes in treatments (1) and (2) were filled with 16 ml deionized water after each treatment while in (3) to (5) they were filled beforehand. After equilibration in a water bath at 20 °C the covered tubes were shaken at 150 cycles per min on a shaker at 20 °C. The conductivity of the solution (L_k = conductivity of leachate from a killed sample) was measured at regular intervals over a period of 20 h using a conductivity meter, type OK-102/1 (Radelkis, Hungary). Finally, all the tubes were exposed to boiling water for 15 min and after equilibration at 20 °C, the conductivity of the solution was measured again (L_{km} = maximum [final] conductivity of leachate from the respective sample killed). The results were expressed as a specific conductivity (L_k, L_{km}) in terms μS cm^{-1}, and as a relative conductivity $R_k = L_k/L_{km}$ in per cent. Each value is an average from three tubes (replicates).

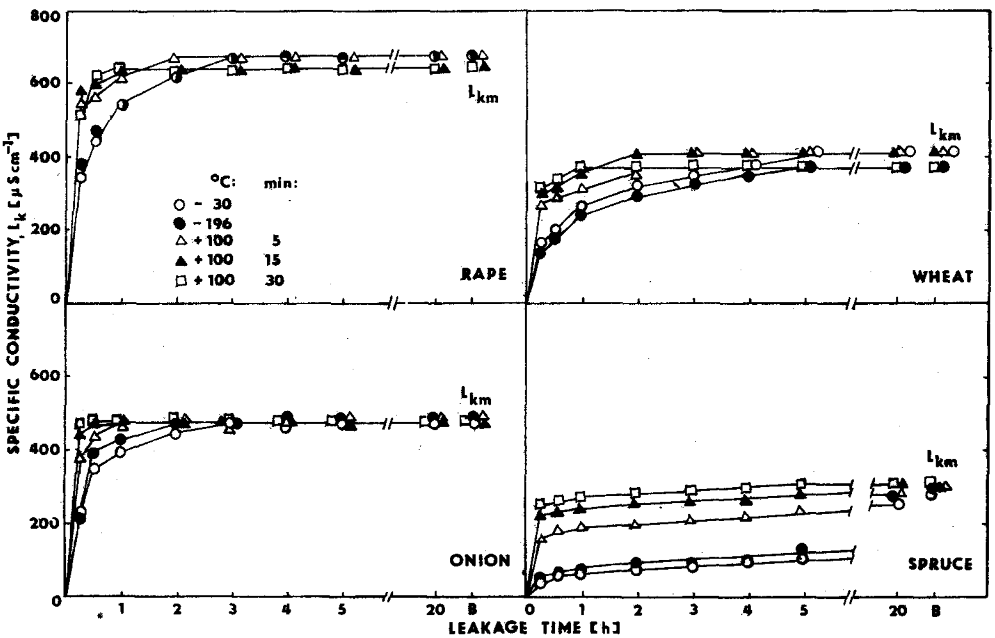

Fig. 1. Time course of specific conductivity from samples of rape leaves, wheat crowns, onion bulb scales or spruce needles killed by freeze-thaw cycle (−30 °C), immersion in liquid nitrogen (−196 °C) or immersion in a boiling bath (+100 °C) for 5, 15 or 30 min. B = the second boiling.

The specific conductivity showed greater variability, especially in wheat (Fig. 1). This variability was not reduced if the specific conductivity was expressed on a dry matter basis of the sample (not present here). It was probably caused by nonhomogeneity of the segments. The relative conductivity (Fig. 2) allows a better comparison of various treatments as well as of samples of different kinds of tissues. Time courses of leakage of various treatments had similar trends in all kinds of samples but the leakage rate slowed down from onion to rape, wheat and spruce. It means that the rate of leakage may decrease from samples with softer (scales of onion bulb) and more simple (leaves of rape) tissues to samples with more complex (crowns of wheat) and more rigid (needles of spruce) structures. The size and the shape of segments and discs, of course, may influence the rate, too.

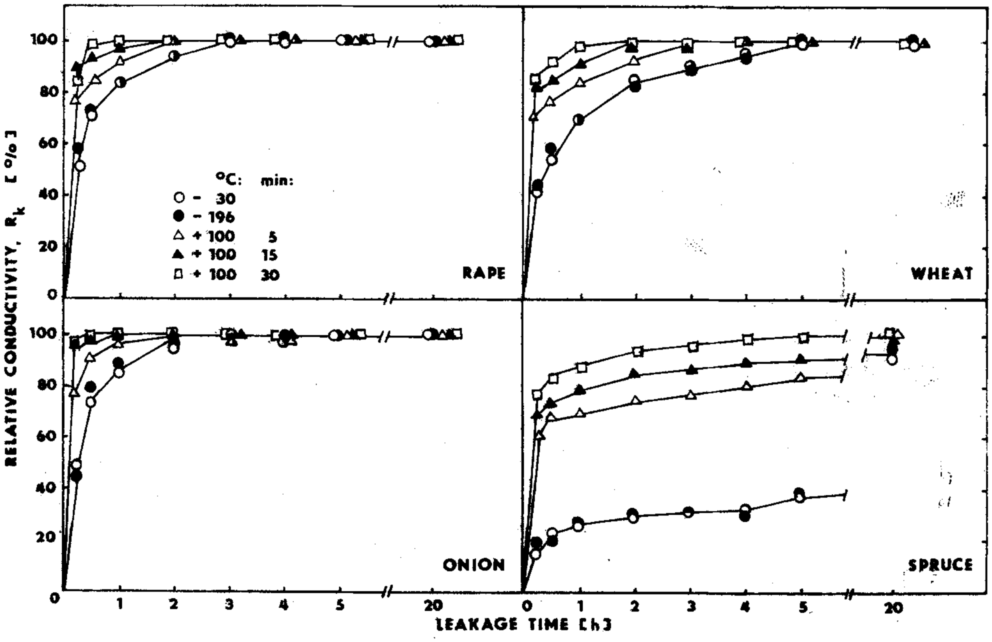

Fig. 2. Time course of relative conductivity from samples of rape leaves, wheat crowns, onion bulb scales or spruce needles killed by freeze-thaw cycle (−30 °C), immersion in liquid nitrogen (−196 °C) or immersion in a boiling bath (+100 °C) for 5, 15 or 30 min.

In all plant samples a longer leakage time for the maximum conductivity (L_{km}) was always found for samples killed by freezing than for those killed by boiling. Nevertheless, the magnitude of an absolute value L_{km} did not depend on the way of killing. The longer the boiling, the shorter leakage time was necessary to obtain L_{km}. When boiling 30 min the L_{km} was obtained immediately (onion), after 30 min (rape), 1 h (wheat) or 4 h (spruce) of shaking the samples (Fig. 1). We conclude that determination of maximum conductivities for calculation of relative conductivities of injuried samples are more rapid and efficient by boiling the samples.

The samples killed by a slow freeze-thaw cycle (−30 °C) and those killed by quick immersion in the liquid nitrogen (−196 °C) showed the same time course of leakage. In these treatments the maximum conductivity (L_{km} *i.e.* R_k = 100 %) was achieved after 2, 3, 5 or more than 20 h of shaking for onion, rape, wheat and spruce samples, respectively (Figs. 1 and 2). ARONSSON and ELIASSON (1970), HALLAM and TIBBITS (1988) have also shown a very long time of leakage (more than 20 h) to attain the maximum conductivity in some kinds of frost-killed samples. The long times of leakage indicate that one does not have to obtain 100 % relative conductivity every time, as is usually *a priori* assumed in frost-killed samples. How this effect can alter estimations of the degree of injury in frost-stressed samples and how it depends on the number, size and shape of segments in a sample is the subject of our next study.

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