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An improved method for the determination of winter hardiness in fruit trees

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Summary

The electrical conductivity method has long been an accepted means of determining winter hardiness in plants. However, it has essential disadvantages as the results are affected by changes in temperature and contamination of shoots or electrodes. Tre present paper therefore offers a modification which instead of involving measurement of the electro-conductivity (EC) of at water extract of thawed shoots uses direct estimation of the underlying principle i.e. the concentration of potassium ions (K+).

The degree of frost injury may be assessed by determining the percentage of K+ ions released by frost treatment (calculated from the total concentration of K+),, or by comparing the levels of K+ concentration in solutions from frozen and intact shoots, respectively.

Introduction

Various methods are used to determine frost injury and winter hardiness in the laboratory, e.g. grafting of frozen shoots /10/, observation of colour changes /8, 11/, etc. A method by which changes in electro-conductivity either directly in frozen tissues, or in tissue extracts, serve to indicate the degree of winter hardiness has found wide application. For more than 50 years/2/ it has helped plant breeders to select earlier maturing and consequently more cold resistant varieties /9, 15, 16/, and has also been useful in studies on the influence of fertilization and cultivation on frost hardiness /3, 6, 7, 14/.

A method of measuring winter hardiness in terms of electroconductivity in extracts from thawed tissues presupposes, that the quantity of electrolytes diffusing out is proportional to the degree of frost injury /15/. The present report discusses some disadvantages of the electro-conductivity method and suggests the use of the potassium ion concentration instead, in order to improve the accuracy of studies on winter hardiness.

Material and method

The effects of autumn application of Alar on the winter hardiness of apple trees were studied during the winter 1970-71. 13-year-old trees of the cultivars "Milton", "Close", "Red James Grieve" and "Rogers McIntosh" were used for the present experiment. Six shoots from each tree were collected, placed together with a thermograph in a cardboard box having double lining and put in a freezer. The temperature and the duration of artificial freezing were selected according to out side temperatures and the natural winter-hardiness of the trees involved. Temperatures ranging between −17° to −32° C were applied for periods of 6 to 12 hours. Great care was taken to ensure that freezing and thawing took place slowly and gradually. After a subsequent period of about 10 hours at temperature above freezing, the shoots were cut into sections of about 5 mm each and placed in beakers containing an amount of distilled water exactly 10 times the weight of the shoot. The beakers were left at room temperature for 24 hours. After shaking, the electrical conductivity of the solution was

measured by means of an ohm-meter (*Helweg Mikkelsen & Co.*, Copenhagen), by transfering part of the solution to a cell with an inner diameter of 5 mm and a distance between the electrodes of 15 cm. After each measurement the cell was rinsed out three times with distilled water.

As soon as all samples had been measured, the concentration of potassium was determined by means of a flame photometer (*Evans Electroselenium Ltd.*, Halstead, Essex, England). The samples were then boiled on a water bath for 7 minutes and left at room temperature for another 24 hours. Water lost by evaporation was then replaced to make up the initial weight, and the EC and the concentration of K^+ ions were measured as above.

On 15th October 1971, 20 shoots were taken from each of the cultivars "Close", "Milton" and "Red James Grieve". One half of the shoots were frozen for a period of 15 hours at −18°C, after which they were cut and soaked in distilled water as were remaining control intact shoots. The EC and the concentrations of K^+, Na^+, Ca^{++} and Mg^{++} ions were than measured. A duplicate experiment was carried out on 15th November, 1971.

On 1st December 1971 groups of 10 shoots from trees of "Rogers McIntosh" were treated at −18°C for periods of 0, 1, one and half, two and half, four and half and twenty two and half hours and the values for EC and the ion concentrations were determined.

In the autumn of 1971 the electro-conductivity was measured on a *Philips* apparatus type *GM 4249*. The K^+ and Na^+ concentrations were measured on the Evans Electroselenium photometer, and the concentration of Ca^{++} and Mg^{++} on a *Beckman Atomic Absorption* apparatus.

Results

The idea of using the concentration of potassium ions as a measure of frost injury developed because a linear correlation was observed between the electro-resistance (ER) and the K^+ concentration in solutions derived from thawed shoots. Mathematical treatment of data yielded high coeficients, i.e., r from −.889 to −.912 (Fig. 1.)

In a more detailed study of the problem during the autumn of 1971, large and statistically significant differences were found between the EC and the concentrations of K^+, Na^+, Ca^{++} and Mg^{++} ions in water extracts from frozen and intact shoots. In Fig. 2 the results of two experiments carried out on 15th

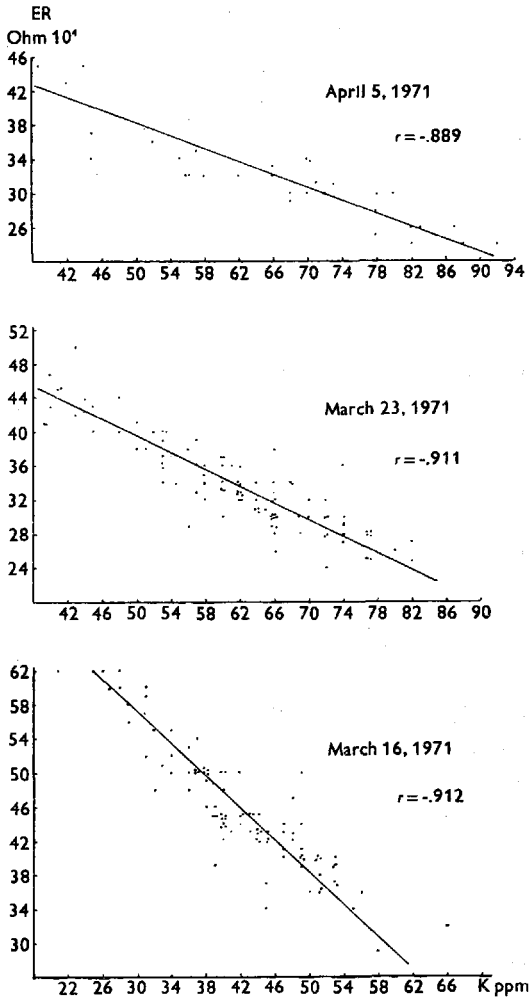

Fig. 1. Correlation between electroresistance (ER) and K^+ concentration of solution.

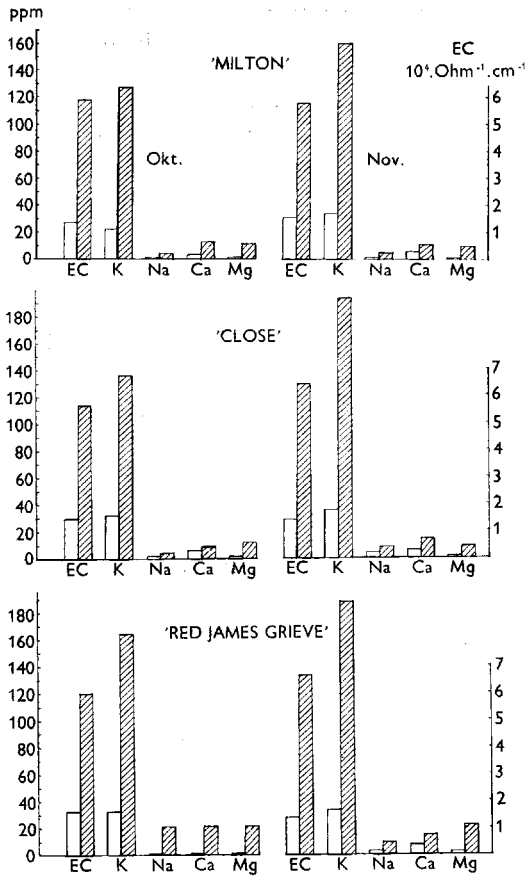

Fig. 2. Electro-conductivity (EC) and ion concentration in solutions, prepared from intact (open) and frozen (stippled) shoots.

October and 15th November 1971, with three different apple cultivars are shown.

The figure clearly shows the sharp increase in the values of EC and the concentration of K+, Na+, Ca++ and Mg++ ions resulting from the release and diffusion into the solution of the ions because of the artificial frost damage to the shoots.

In terms of average values from all treatments, the EC increased from 1.44 x 10^{-4} to 6.05 x 10^{-4} ohm cm $^{-1}$; the concentration of K+ from 31.1 to 161.5 ppm; Na+ from 1.8 to 6.6 ppm; Ca++ from 5.0 to 12.2 ppm; and Mg++ from 0.6 to 10.9 ppm.

On December 1st 1971 groups of shoots were exposed for different lengths of time to artificial frost, resulting in different degrees of frost injury. The Fig. 3 indicates the levels of the EC and the concentration of ions as average from 10 shoots. The values of the EC and the K+ concentration are seen to increase with increasing duration of exposure.

The change may be seen to take place very rapid intially and to level off subsequently, as the quantity of diffused ions may be at initial stage depends on the number of frozen cells. The shape of the curve for the concentration of K+ ions suggests that all cells gradually have frozen during the first two and half hours. Throughout the subsequent two hours period the cells remained in an identical condition and consequently there is no difference between the levels of samples "12^{30}" and "14^{30}". The later increase in the values for the K+ concentration and the EC seems to be due to dehydration of the cells and accompanying irreversible physical-chemical processes.

It is also evident from Fig. 3 that the levels of Ca++, Na+ and Mg++ in solution prepared from intact shoots ("10 a.m.") and from shoots frozen for periods up to twenty two and half hours show very little differences. Consequently changes in the concentrations of these ions cannot serve as a reliable measure of frost injury.

Discussion

The electro-conductivity measured represents the amount of electrolytes diffused into the solution as a result of the freezing of tissues affecting the permeability of the cell membranes /16/, and consequently it offers a guide to the degree of frost damage. However, the shoots are not free from impurities such as surface dust and remains of protective sprays, which may diffuse into the solution and affect the readings. Repeated rinsing with distilled water as recomended by *Way* /14/ is no guarantee of the removel of all foreign matter and more

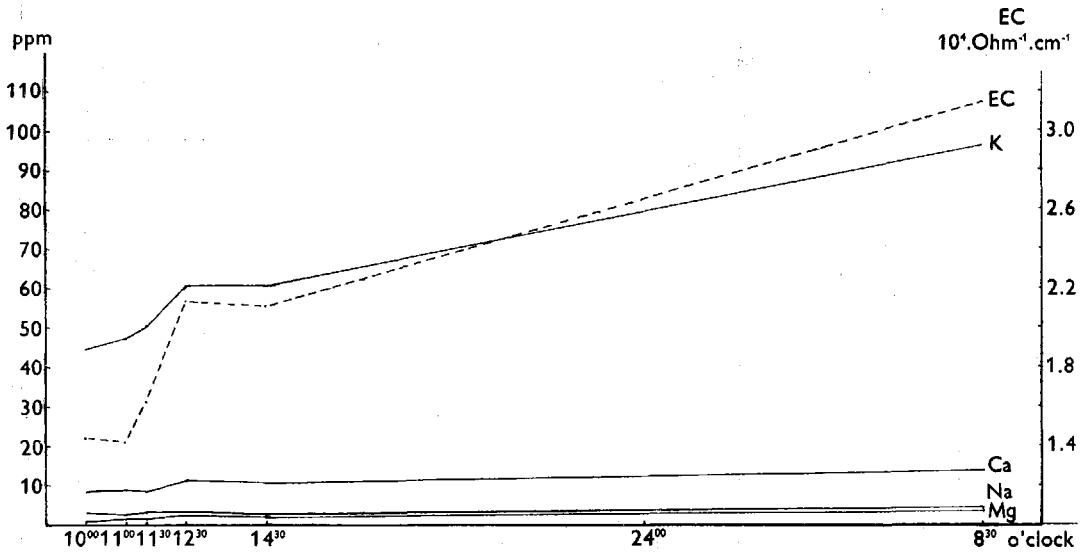

Fig. 3. Influence of exposure time of artifical frost (—18 °C) on the electro-conductivity and ions concentration.

extensive rinsing introduces the risk of accidentally washing some of the ions concerned out of the shoots.

It is also necessery to consider the inevitable electrolytic dissociation of the solution and the accumulation of ions on the electrodes changing their conductivity. Rinsing of the electrodes with water after each measurement does not suffice to remove accumulated ions.

The EC of solutions is well known to depend to a great extend on the temperature /1/. The so-called "room temperature" within any one laboratory also changes, while the temperature inertia in individual containers depends on their thickness and amount of the solution present. In practice the temperature changes may be slight, but they will still affect the ohmmeter readings. The flame photometer readings are not temperature dependent and the concentration of K^+ is constant at any temperature.

Wilner /15/ also established high correlation coefficient between the EC and the K^+ concentration, i.e., from .805 to .932. If this correlation has been observed by other workers it may be assumed that little importance has been attached to the K^+ concentration as a measure of frost damage largely because of the difficulty and slowness of conventional methods of classical analytical chemistry. With the equipment available to-day the determination becomes nearly as fast and easy as that of electrical conductivity.

Hence it now becomes far more rational to measure not the ensuing change in electrical conductivity, but its root cause, i.e., the initial change in the concentration of ions, and more particularly that of K^+, which offers much higher accuracy and gives a more reliable overall impression of the condition of the cells.

During the winter of 1972 the new method was used with increased accuracy. Fifty shoots from each treatment were frozen, cut into sections and mixed thoroughly. For each treatment ten samples were prepared by soaking 4 g of the mixture in 40 ml of water. The accuracy was increased because of the inclusion of a larger number of shoots in the study, and the cV was reduced to 5 per cent, whereas for single shoots samples the cV value was above 12 per cent. This tends to reduce the time involved.

The laboratory method designed to assess the winter hardiness of fruit trees is rapid and does not depend on the outside temperature,

but there are certain factors affecting the success and accuracy of the experiments:

– Winter hardiness in plants is well known to be a quality which disappears almost completely during the summer months /5, 11, 13/. Shortening of the photoperiod induces the first phase, and the first cold nights the second one, during which the plants are able to survive at very low temperatures. When the weather becomes warmer, the hardiness is reduced. Hence it is important to take into consideration the natural hardiness of the trees during the period under investigation, and to choose suitable temperatures. It would be wrong, for example, to test during October – November at –30° C, and in mid-winter, when the trees show their highest frost resistance, at –10° C.

– The artificial frost temperature must be fairly low to injure the material with least frost resistance; but at the same time it must be sufficiently »moderate« not to destroy the material with greatest frost resistance /13/.

– In order to avoid selecting one temperature at random, it is advisable to include a larger number of shoots which are then frozen in batches at different temperatures at 5° intervals, e.g., –15°, –20°, –25° and –30° C.

– The temperature of freezing should be 5–10° lower than the minimum temperature recorded in the locality /11/.

– In accordance with Fig. 3 the exposure time must be more than two and half hours, while some authors recommend from 6 to 12 hours /11/.

– The processes of freezing and thawing should take place slowly and gradually, at a recomended speed of approximately 1-2° per hour /11, 16/.

In an attempt to increase the accuracy of the determination of the degree of frost injury, some workers /4, 16/ have, after the electroconductivity measurements of the solution, placed the samples in a boiling water-bath for a few minutes; subsequent water lost by evaporation was replaced and the samples were left for another 24 hours before being measured again. The boiling is intended to kill cells still left intact after freezing, in order to release their ions and so to determine the total potential electro-conductance. However other authors /12, 14/ claim that boiling is unnecessery and consider a comparison between the EC values for solution prepared from frozen and intact shoots, respectively, sufficient.

Similary, the K^+ concentration may be used as a measure of the degree of frost injury in one of two ways as follows.

1. By determining the amount of K^+ released by frost treatment (before boiling) as percentage of the total K^+ content (as determined after boiling).

2. By using the K^+ concentration in a solution prepared from intact control shoots as a basic value = 100, calculating from that the precentage increase in K^+ resulting from frost injury.

Table 1. Effect of duration of artifical freezing, calculated in two ways (1st December 1971, test)

Exposure time in hours	Results, calculated from the total amount of potassium[1])	Results, calculated as K^+ concentration from control shoots = 100
0	17.6 a[2])	100.0 a
1	18.5 a	104.5 b
1½	20.2 a	114.2 c
2½	24.2 b	136.9 d
4½	24.3 b	137.8 d
22½	38.6 c	218.0 e

[1]) The values for K^+ released from frozen shoots (Fig. 3) as a function of time are set in proportion to the total amount of K^+ (about 250 ppm) released at boiling of same shoots.

[2]) Vlaues not followed by the same letter differ significantly at level 95 per cent.

Table 1. represents the data resulting from different periods of exposure and calculated as under 1. or 2. above. The second column shows the shoots to have been significantly damaged just after two and a half hours frost treatment; there is no apparent difference in frost injury between shoots treated for two and a half and four and a half hours. However twenty two and a half hours treatment caused

most significant frost injury as compared by shorter exposure.

The data in column 3 show significant injuries to shoots exposed to one hour of treatment only, and also confirm the absence of any difference in degree of frost injury between shoots treated for two and a half and four and a half hours, respectively.

Although both methods of calculation show a similar trend, we believe the first one to be the more correct one, since it uses absolute values for all samples (the second one employs relative values, putting the average of all intact control shoots at 100). Hence it is advisable to use the total concentration of K^+ for calculations of the degres of frost injury, although it does involve rather more work than the second method.

Conclusion

Present data confirm that the concentration of potassium ions in extracts from frozen and subsequently thawed shoots may serve as a valid measure for comparative studies of winter hardiness in trees, such as may be required for:

a) breeding experiments, e.g., attempts at developing new cultivars with increased winter hardiness;

b) studying the effects of cultivation or growth retardants on the frost resistance of fruit trees;

c) testing the winter hardiness of newly introduced varieties.

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