

## SHORT COMMUNICATIONS

### FREEZING PROCESSES IN WHEAT STEMS\*

By W. V. SINGLE† and C. R. OLIEN‡

The study of the formation of ice crystals in wheat stems presents a number of technical difficulties.

Single (1964) showed that freezing boundaries do not spread uniformly throughout supercooled stems, but may be immobilized at the nodes, leaving regions of unfrozen tissue at temperatures as low as  $-5^{\circ}\text{C}$ . This situation is not adaptable to direct observation by light microscopy, nor, on account of its extreme instability, by methods which disturb the cells in any way.

Techniques developed by Olien (1961, 1964) for the study of ice formation in barley crowns were found to be applicable. These rely on measurement of the ability of tissues to transmit low voltage electrical current, which for practical purposes travels only as a result of electrophoresis in the interprotoplasmic fluid, providing a very sensitive indication of any change in physical state of this medium.

#### *Methods*

Wheat plants of the variety Lerma Rojo were grown under controlled conditions with a 14-hr photoperiod of approximately 2000 f.c., and day and night temperatures of 21 and  $10^{\circ}\text{C}$  respectively. Prior to treatment they were conditioned at  $5^{\circ}\text{C}$  for approximately 1 week. Two methods of testing were employed.

*Method 1.*—Stem sections 5 cm long were excised, and the centre 2 cm wrapped first in plastic film, then in several layers of aluminium foil. They were mounted in pairs across a copper strip 2 cm wide, having grooves of appropriate depth to allow a snug fit of the wrapped sections, in contact with a thermoelectric heat sink.

The ends of the sections were embedded in moist carbon paste in electrical contact with a millivolt recorder and a square wave power supply giving current of the order of 5 V at 5 c/sec.

For testing, the mounted specimens were placed in a refrigerator at  $1^{\circ}\text{C}$ , and the continuous measurement of electrical resistance along each was commenced. The temperature of the wrapped middle sections was then lowered to a selected level (between usually  $-2^{\circ}\text{C}$  and  $-5^{\circ}\text{C}$ ) by means of the thermoelectric block. At this point, crystallization of water was initiated by insertion into the chilled tissue of a fine hollow probe filled with ice. Nine pairs of stems were tested in this way, covering periods of 30 min to 3 hr with minimum temperatures varying from  $-3^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$ .

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† New South Wales Department of Agriculture, Agricultural Research Station, Tamworth, N.S.W.

‡ Crops Research Division, United States Department of Agriculture, located at the Department of Crop Science, Michigan State University, East Lansing, Michigan.

*Method 2.*—Whole plants were frozen in a cold chamber in which the relative humidity was maintained at 90–95%. Electrical contact was made through small platinum electrodes of area approximately 2 mm<sup>2</sup>, coated with moist carbon paste, and pressed gently against the stems, which had previously been brushed with carbon to remove the cuticle and thereby establish good contact. The paste was allowed to dry before measurement was commenced. Temperatures were recorded with fine wire thermocouples inserted above and below the stem sections under study. Freezing of the tissue was initiated by touching the stem several centimetres away from the electrodes with a copper rod previously chilled in solid carbon dioxide. In all, five stems were tested in this manner.

To ensure that the tissues were not disturbed by the electrical treatment, direct current of increasing voltage was passed through representative stem sections. A linear relationship between voltage and current density was established with voltages ranging up to several times greater than those used in actual freezing trials, indicating that cell damage by electrical effects was most unlikely. Attempts to trace the path of a direct current through the tissues by following the movement of charged dye, although complicated by mass water flow in the vascular system, gave reasonable indications that in wheat stems ions are not channelled into discrete paths. Electrical phenomena recorded may therefore be assumed to be representative of all types of tissue between relevant contacts.

### *Results and Discussion*

Figure 1 shows typical relationships between current and temperature in a freezing wheat stem. Current is shown in arbitrary units, after correction for the effect of temperature on the viscosity of water. The positions of the electrodes are shown in the accompanying sketch. As three earth contacts were used, the current measured at each location was susceptible to some influence from the remainder of the section. This was checked during the course of the experiment by disconnecting earth leads in pairs. Generally it was noted that approximately 80% of the current measured at each point passed to the opposite earth contact, so that the values shown in Figure 1 tend to diminish the contrast in behaviour between the different parts of the stem. The manipulation of the positions of the earth contacts also served to show that resistance at the contacts themselves did not play a major part in determining current densities. This was further supported by general similarity in behaviour of tissues treated according to the two methods described. In method 1, very large moist contacts were used and these were kept above the freezing point of water to obviate any possibility of sensible resistance at these points.

From the data of Olien (1964) it is to be expected that, as freezing proceeds, electrical resistance of the tissue will increase due to the immobilization of ions external to the protoplasts, as long as living cell membranes remain intact. This relationship should be reversible in a quantitative way. This was never completely realized in wheat stems in this series of experiments, although there were indications from preliminary trials that leaves behave in this fashion as long as temperatures remained above about  $-3^{\circ}\text{C}$ . The patterns for contacts I and III in Figure 1 came as close to this situation as any recorded for stems. There was a distinct fall in current flow with freezing and for a time thereafter as the temperature was reduced; however,

it is clear that this was not completely reversible, as when the temperature rose again a disproportionate increase occurred.

Subsequent histological examination of the tissue showed collapse of cells in both regions, with open space where ice crystals had presumably formed. These were particularly evident beneath the epidermis at the node, and beside the vascular bundles in the lower internode section which was in the process of rapid elongation at the time of freezing. It seems from Figure 1 that the tissues at contact III suffered some injury almost from the time of initial ice formation and that the resultant slow release of electrolytes tended to balance the expected fall in conductivity of the original free water phase.

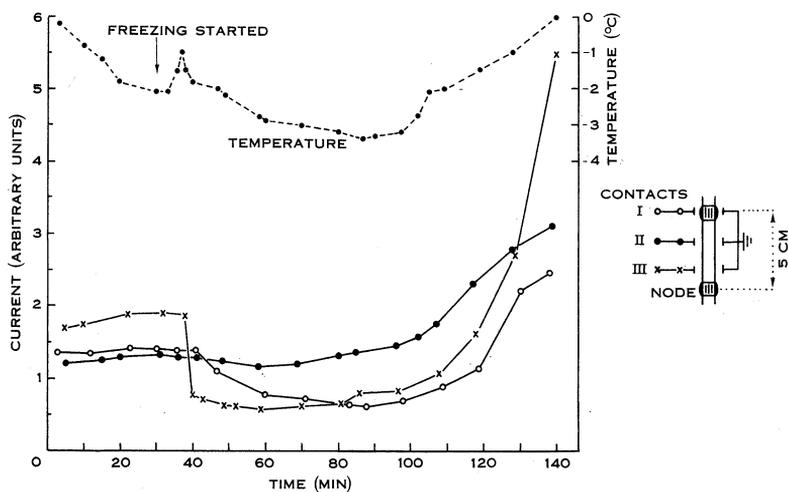


Fig. 1.—Current flow across three sections of an elongating wheat stem.

Contact II produced a pattern which was typical of mature internodes. The flow of current changed little on freezing and commenced to increase while the temperature was still falling, indicating a complete lack of tolerance to ice formation. Later examinations revealed very few living protoplasts although cell walls remained intact, and superficially there was little damage.

The apparent general susceptibility to freezing injury of stem tissue is difficult to reconcile with the ability of nodes to prevent the spread of an ice front at temperatures recorded ( $-5^{\circ}\text{C}$  and below). It is possible, however, that the tendency for ice to form at preferred sites causes redistribution of moisture before damaging temperature levels are realized, and the relatively dry areas so formed provide the ultimate barriers to the freezing boundary. Obviously further information concerning the role of moisture in such a system is required, and experiments to explore this have been planned.

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