Jensen[1] has also claimed that the ability to plasmolyse in onion cells is not a reliable index to their viability, because he observed some abnormal cells in which vacuoles were normally stained by neutral red solution and tonoplasts still retained their permeability, having the cytoplasmic layers torn outside the tonoplasts. In a previous report[3] I stated that such abnormal cells have not yet been observed during several years study in the parenchyma cells of the cortex in woody plants, but some parenchyma cells are found in which the ectoplasts still retain their semi-permeability though their tonoplasts are torn and their cytoplasmic layers are mixed with vacuolar content (see plate 1, 2 ref. 2) However, such abnormal cells are easily distinguishable from the normal by their appearance. Thus, in the parenchyma cells in twigs of woody plants, unlike the case of onion cells, it may be possible to determine the relative degree of viability in parenchyma cells, upon the basis of both their vital staining with neutral red solution and the appearance of plasmolysed cells, at least in one and the same series of experiments. However, judging[3] of the intactness of twig as a whole cannot be made only on the basis of plasmolysis test in parenchyma cells just subsequent to, or even after, many days of thawing, because inner cortex, pith ray, and pith periclinal tissue are less resistant to freezing than parenchyma cells of cortex. Accordingly, to demonstrate the intactness of a treated twig as a whole, it was planted in moist sand and its capacity tested to continue normal development at least for three months after planting, as mentioned in my reports[4]. Even in the tetrazolium[5] test used by Parker, it may be said that judging the intactness in plants means only determination of a relative value of viability of certain tissues in twig or leaf without testing the whole of it as is done by the plasmolysis method.

In my previous communication in *Nature* (and ref. 4) it was stated that almost all the easily freezable water in a cell may be drawn from the cell interior by extracellular freezing at about −30° C., and that the cells and tissues in this state are not injured even when immersed directly in liquid nitrogen, provided they can sufficiently withstand such pre-freezing at −30° C. Moreover, it was reported that below this temperature the intensity of cold seems not to exert any important effect upon woody plants, at least as long as the intense cold does not extend over a long period. Against this view, Parker has pointed out that one gains the impression from my article that if a plant can be cooled to −30° C. without damage, it can be cooled down far below this without any damage. As a result of Parker's experiment the winter resistance of several conifers could range all the way from a few degrees below freezing to below −190° C.

In the pre-freezing method, to cool plant material to −30° C. does not mean that the temperature of the material only reaches −30° C., but that its material attains at least the state of equilibrium at −30° C. and the state in which almost all the easily freezable water in a cell is drawn from the cell interior. To assure this condition, in the case of freezing in comparatively large material as a twig, I pre-froze it for 16 hr. (overnight) in all cases as mentioned in previous papers[4,5]. In the cooling method used by Parker, when a certain temperature-level was reached in the flask containing a shoot from branch, the flask was removed from the chilling apparatus, then rewarmed. In such cooling method, even if plant material is cooled to −30° C., it is doubtful whether almost all the freezable water in the cell interior can be drawn out or not within such short period of time.

The grade of frost resistance of a woody plant differs according to the manner in which it is estimated. If the grade of frost-resistance is represented by the lowest temperature at which the twigs are able to survive freezing for an hour, especially when the cooling-rate in a temperature-range below −30° C. is comparatively slow as 4 deg. C./hr., there can be found fairly great differences in the grade of frost injury in the twigs treated at an arbitrary different temperature below −30° C. This is particularly the case of less hardy species. However, when the grade of frost resistance is represented by the minimum temperature at which the twigs are able to survive freezing for a full day, there is found, in many cases, scarcely any difference in the grade of frost resistance between the material treated at −30° C. and that at −70° C. (ref. 7). It may be said, therefore, that the discrepancy between our two points of view is due mainly to the difference in the manner of representation of the grade of frost resistance. However, to clarify this problem, it seems necessary to continue further work in many woody plants.

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Pollen Germination in some Gramineae: *Pennisetum typhoideum*

Pollen of grasses is notoriously difficult to culture, and even in Nature fertilization usually fails if pollen is not transferred directly from the anthers to the stigma[1,2]. Several previous attempts to germinate the pollen grains of the Gramineae have been unsuccessful[1–6]. In this laboratory attempts were made to germinate pollen grains of *Pennisetum typhoideum* (varieties *T*.25 and *T*.55, *I.C*.1472), *Zea mays* (*T*.41 var. Kanpur) and several varieties of *Hordeum vulgare*, *Sorghum vulgare* and *Triticum aestivum* by the hanging-drop technique (for details of technique see Vasil[7]). Best germination was obtained in *Pennisetum typhoideum*, while in the remaining plants pollen tubes longer than 600μ could not be obtained even after the addition of hormones, vitamins, mineral salts and stylar and ovarian extracts.

The pollen grains of *P. typhoideum T*.25 germinate within 10 min. of their inoculation in 10–40 per cent sucrose solutions and the optimum germination (62 per cent) occurs in 30 per cent sucrose where the tubes attain a length of 2,132μ (Fig. 1). With the addition of 0·01 per cent boric acid best growth is obtained in 25 per cent sucrose. In this medium 78 per cent of the grains germinate and the pollen tubes attain a length of 4,320μ (Fig. 1). Pollen grains of the variety *T*.55, *I.C*.1472 require lower concentrations of sugar: in 12·5 per cent sucrose there is 40 per cent germination and the tubes are 316μ long. Here also the percentage of germination is improved on the addition of 0·01 per cent boric acid, and