CHAPTER I

INTRODUCTION

The problem of frost resistance in plants is an old and important one not only from the point of view of basic knowledge to the biologist interested in living systems that survive in freezing temperatures, but also for the agriculturalist to whom it remains a practical problem. Therefore, its significance reaches beyond the doors of a research laboratory and the paths of an experimental field to many world societies.

The bulk of the work done on frost hardiness of plants has been thoroughly treated by Levitt (1956), and has led to some general conclusions concerning the nature of this environmental resistance. First, there are numerous reports of an increase in the osmotic value of the cell sap upon increased cold hardiness. This increased osmotic value is reported to be due to a conversion of starch to sugar with the result that the cell is frozen at a lower temperature, less water is withdrawn from the cell, and the cell is less subject to contraction and the subsequent mechanical injury. The significance of this process in overall frost hardiness is somewhat questioned since it

does not occur in all frost resistant plants. Second, observations have revealed changes in the nature of the protoplasm during frost hardening, such as changes in viscosity, increased hydration or greater amounts of bound water, etc. As suggested by Levitt et al (1961) protein hydration depends on the number of polar groups of the molecule, and a hypothesis was developed that suggested this might involve sulfhydryl (-SH) groups. It was also suggested that since a number of enzymes must be involved in the frost hardening process, an increase in -SH groups might be necessary, as a number of enzymes are activated by these groups. As the investigation continued and data were accumulated, new hypotheses developed. The disulfide (-SS-) changes that accompany the changes in -SH groups must also be determined in order to establish whether the latter changes are due to oxidation, reduction or synthesis. It was also important to measure separately the -SH groups of proteins from those of amino acids (cysteine) and peptides (GSH).

One of the basic problems involved in an investigation of sulfhydryls is the method used for measuring these readily reacting, easily oxidized groups. A number of

methods have been developed all of which have their advantages and disadvantages. During the course of the investigation as data were gathered on the sulfhydryl-disulfide relations in frost resistant plants, several side experiments were conducted with the intention of contributing additional knowledge to our methods of measuring these groups. While some of the data gained in the early part of the investigation have been published (Levitt et al, 1961, and Schmutz et al, 1961), much data were not included and additional experiments were performed to verify and expand on these preliminary results. The methodology of the problem is treated here in detail for the first time. In view of the unquestioned relationship between -SH and frost hardiness established as a result of these investigations, it is essential that this fundamental work on the methodology should be available for future investigations of the problem.