OBJECTIVE MEASUREMENT OF HARDINESS IN AZALEA

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ABSTRACT

OBJECTIVE MEASUREMENT OF HARDINESS IN AZALEA

by Robert L. Gonderman

Azaleas are among our best landscape materials, but are restricted to limited ecological sites. In breeding Azaleas to adapt them for diverse new habitats, the desired characters must be found among the parents and recombined in their progeny. These must then be tested to determine their qualities so that selection of superior clones may be made.

Hardiness is one trait of major importance to be added to species with otherwise desirable landscape characteristics for satisfactory performance in colder areas. Hardiness, an internal, physiologically developed, sequential process is not an easily discernable overt character. Therefore, some means to determine its presence and degree of intensity is needed. This work is an investigation into a method to devise a usable technique for plant breeders.

To furnish a base, plants of known hardiness established in campus plantings were measured every two weeks through the major part of the year, so that quantitative standards might be established. Azaleas chosen as base selections were specimens of calendulaceum, nudiflora, poukhanense and cultivars of 'Maxwelli alba', 'James Gable,' 'Alaska,'

'Polar Bear', 'Gloskey Pink', and 'Corsage'.

Measurements were made by employing a weak flow of direct electricity through a twig portion one centimeter long. Measurements were recorded in kilo-ohms of resistance, using a small battery-powered, portable multi-meter.

Contacts of three types were employed. The anvil consisted of two metal blades spaced one centimeter apart, and firmly supported by a cork base. Twigs were cut to length, and placed in contact with the electrodes. The second type consisted of two electrolytic rubber pads under tension of a spring clip. Here the rubber pads served as contacting electrodes.

The third device consisted of two needle electrodes one centimeter apart firmly secured through a hand grip. This was merely plugged into the twig. Measurements could be made as rapidly as they could be recorded.

Differential readings after freezing are largely due to exosmosis which follows frost injury to cells. The base plants were tested for degree of resistance to electrical conduction every two weeks throughout fall, winter, and spring. This revealed differences in their hardiness patterns over this period. These measurements constituted a base of quantitative values against which measurements of other plants were compared, to predetermine their degree of hardiness.

The technique was then applied to hybrid plants of

unknown parentage. Recovery and survival observations taken in spring corresponded closely to the predictions of hardiness derived from fall measurements.

This method for measurements of plant hardiness is relatively rapid, inexpensive, convenient, quantitative, and repeatable.

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By

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INTRODUCTION

Man has long been intrigued by the problems of lack of hardiness in plants. Since our primordial ancestors moved from their tropical epicenter and began to exchange their nomadic ways for the more stable pastoral existence, freezing and death of desirable plants has plagued them. Present archeological findings (Anderson, 1945; Vavilov, 1926; Suneson, 1956) reveal that man practiced plant selection in prehistoric times to obtain crops with desirable adaptations. As civilization moved northward, hardier plants became increasingly important for survival during adverse seasons. Selections over long periods of time have changed several genetically plastic weeds into desirable plants adapted to cold climates (Dobzhansky, 1955 and Harlan, 1956).

Although all plants respond in various ways to environmental stimuli, hardy plants differ from tender plants in their physiological adaptation to toleration of freezing (Duhamel and Buffon, 1737; Sachs, 1860; Levitt, 1951; Gorke, 1924). Breeding programs are in the process of increasing hardiness of various food, fiber, feed, drug and landscape plants today. The introduction of hardiness into woody ornamentals has lagged, with many of them being discovered by chance, rather than being the result of art and science of the geneticist.

Versatility of plants for the landscape has created a horticultural commodity that demands increasingly more attention than it formerly did. The psychological effects of the various facets of the art of landscaping are well-known as yet only to a comparatively few people. This is changing. Our technology gives man more leisure time to devote to interests outside of his primary field. The endeavor to assure survival through food, clothing and shelter is not the totality of man's needs. In our society, recreation is important. Public parks with an organized program save money for the community in terms of alternative costs for crime prevention and juvenile delinquency.

Hardiness in plants is a complex trait, consisting of a balance between external conditions and internal physiological processes that result in a cumulative response (Luyet and Gehenio, 1940). Comprehensive accounts of frost history such as those of Scarth (1944), Levitt (1940) and Vasilyev (1956) show that hardiness is the result of a number of factors such as rapidity of ice formation, culture, light, nutrition, temperature and genetic factors influencing physiological processes.

Considering the overall complexity of the conditions and processes which induce hardiness, it is necessary to follow the organismal approach.

The chief methods for determining hardiness in the past have been:

1. Degree of injury as determined visually (Sachs, 1860).
2. Leaching or loss of pigments or electrolytes (Dexter, 1930).
3. Changes in electrical resistance of exosmosed electrolytes (Dexter, Trottingham and Graber, 1932; and Fillinger and Cardwell, 1941).
4. Percent of regeneration after treatment (Greenham and Daday, 1957).

Generalizations about the amount of natural hardiness to be expected from a plant can only be valid within the limitations imposed by the vagaries of the environment. Largely, the range of wild and cultivated plants are determined by their resistance to frost damage toward the extremes in the distance from the equator, and the degree and duration of rest period toward the areas approaching the equator (Scarth, 1944)

Many of these phases still defy scientific investigation--theories change; new facts are discovered; and new postulations promulgated.

OBJECTIVE

Because of a need for increased hardiness in plants, attempts have been made toward discovering a method for determining hardiness. In recent years, several methods have been developed, but present methods inculcate various working deficiencies which cause them to be of relatively small use to the average plant breeder. Time, laboratory equipment, training and expense discourage use of many methods.

Investigations were initiated in an attempt to discover a rapid, inexpensive, convenient, reproducible method of determining hardiness, and one which could be stated in quantitative terms.

REVIEW OF LITERATURE

Hardening Process

Resistance to frost depends largely on the character of the organized protoplasm, as revealed by the study of living cells in the intact organism (Stuart, 1940; Dexter, 1933; Maximov, 1929 and Kessler, 1935).

Early reports of cell size, ploidy, plasmolysis, rapid thawing, conductance of electricity by electrophoresis have not proven to be reliable guides. Several other correlations have been investigated with varying degrees of success. In such attempts, utilization of the organism as an organized biological entity, embodying the syndrome of interrelated processes is essential. The parts, as cytoplasm, cells, vascular system, hormonal system, enzyme system, transpiration, and activities of the roots are important as factors. To explain the accumulated effect of hardiness, the entire plant must be projected as a unit against the background of its micro-climate, and related to its physiological responses en toto.

Metabolism coupled with its physiological reactions is affected by the environment impressed on the genetic constitution of the plant (Dexter 1933a). The standard hardening conditions induce physiological changes which resist frost injury (Maximov, 1929). Since energy from food reserves are

used in this process, it follows that healthy, vigorous plants are more apt to become successfully hardened, to maintain life over winter, and to burgeon into new growth in spring (Dexter, 1933 and Wilhelm, 1935).

The key factors appear to be protoplasmic or at least cellular, but include integrally the associated factors which reinforce cytoplasmic responses (Luyet and Gehenio, 1940). Among these is the enzyme system as suggested by Downs and Butler (1960). The role of colloids in the protoplasm may be more important than has been generally reported.

Dexter and his associates have investigated hardiness rather extensively, and have concluded that a major precondition for frost resistance is an increase in dry weight and a decrease in free water, associated with increased protoplasmic permeability to salts.

The course of the process is related to the retention of efficient leaf area into the cold inducing period. Cultural factors, as low nitrogen level, restricted soil moisture, adequate summer growth, early fall cover crops, and mulch after soil temperatures have decreased to forty-five degrees or after freezing, do modify the microclimate, with attendant reactions by the plant toward stimulation of processes leading to the hardened condition (Daday and Greenham, 1960; and Tysdal, 1933).

Several morphological regions of hardiness have been delineated, such as bud, bark, cambium, root and shoot. In

this paper, the entire organism will be the unit under study.

Due to the reactions of several physiological processes, the overwintering plant becomes dormant at the same time that hardiness develops as is inferred from the reports of Chandler (1941a), Harvey (1918) and Rosa (1921). Studied further, one is led to the conclusion that this condition is a prerequisite leading to the resting condition, which is resistant to environmental fluctuation.

Environmental conditions are complexes of triggering mechanisms for the stepwise sequence of hardening. It appears clear that no one factor is all important, but that the organized physiological response is due to the combined forces involved during the onset of the standard hardening conditions found at the approach of normal freezing temperatures.

Environmental Effects:--Plant reactions leading to winter hardening are induced by the interplay of external environmental forces and physiological conditions (Platt, 1937). The physiological conditions are in turn governed by the genetic constitution and its influence exerted through control of the enzymatic system, according to Harris (1934), Woolley and Wilsie (1961) and Samish (1954).

A great diversity of environmental stimuli, as edaphic, biotic, nutritional, and insolational, effect the hardening process. Seymour (1944) observed that the steady frosts of midwinter do less damage than late spring or early fall frosts, which find plants unprepared.

Most hardy woody plants respond to hardening conditions of the environment in several ways: new growth hardens; loses its excess moisture; winter buds form with protective coatings; and leaves drop. One change induces subsidiary changes, and all combine into the reaction chain of the organism. Tumanov (1931) reported that decreased photoperiodic duration as well as decreased temperatures were necessary for the hardening process to begin. Long and Melcher in 1943 found that leaves exposed to shortening days developed a growth inhibitor, Seymour (1944) believes that much alleged tenderness of Azaleas is due to the deleterious effects of late summer drought, rather than primarily winter cold. The colder, shorter days of fall reduce the availability of nitrogen and soil moisture. The cooler nights decrease transportation of carbohydrates out of the cell, but bright sunny days produce a good supply, so that an accumulation and conversions result in the cell. Tysdal in 1933 reported that short days increased frost resistance and that this was more important than intensity of insolation.

Harvey (1930) suggested that hardening responses to temperature began at five degrees Centigrade. He also found that more rapid changes followed a twelve hour alternation between ten and zero degrees centigrade.

Thus hardy plants are induced to begin the hardening process by effects of the environment.

The Role of Metabolism in the Hardening Process:--Metabolic activities which promote and conserve accumulation of soluble carbohydrates serve as one of the initial processes leading to changes which induce hardiness. This in turn increases osmotic pressure and hydrophily (Scarth, 1944 and Levitt, 1951).

During the advent of fall weather, sugars are stored in the cells at the same time that they are decreasingly consumed from the same cells by respiration and translocation. The balance gradually changes to favor sugar accumulation. Chandler (1941a) and Chandler and Chandler (1943) found that sugar content increased as starch content decreased. Harvey (1930) and Tysdal (1933) found the best temperature to be a zero to ten degree Centigrade fluctuation. This gives the best level for metabolic activities on sugar accumulation and its conversion to other plastic substances. (Tumanov, 1931 and Hedlund, 1917). Sisakjoh and Rubin (1939) and Tysdal (1933) concurred. They found the greatest activity of invertase to be at zero Centigrade.

Thus metabolism is necessary not only to supply plastic substances but also to furnish energy for conversion of soluble proteins, and oils which also play a role in cytoplasmic resistance to freezing temperatures.

Cell Permeability in the Hardening Process:--Permeability of the cell to water is important in that water from the vacuole passes through the cytoplasm, along with water from the

cytoplasm itself, to form ice crystals intercellularly. This appears to be a protective mechanism against lethal intracellular crystallization. Western investigators have long agreed that permeability increased with hardiness (Scarth, 1944 and Levitt, 1955), and now belief appears to be generally accepted (Vasilyev, 1956). Scarth (1944) reported that the hardier the cell, the greater the permeability. Dexter's experiments have led him to the conclusion that an increase in dry substance and an increase in protoplasmal permeability to salts is essential to the process of hardening.

Non-Solvent Space:--The fraction of the cell not in solution extends to half the volume. In seeds and spores, which are ordinarily very hardy, this increases to a very large fraction of the volume, and little space for freezable water remains. Sulukadze (1945) reports that seventy to eighty percent of the water contained in the cell at freezing temperatures becomes frozen.

Lidforss (1896) followed by John (1931) and Harvey (1933) state that cold temperature intensifies hydrophilic processes. Several insoluble compounds become colloidal in nature to constitute non-solvent space, leaving very little water to freeze. Thus, this is a contributing factor in hardiness.

Protoplasmal Viscosity:--Scarth in 1944 reported on the difficulty of measuring the viscosity of the protoplasm, as well

as the fact that it varies with the physiological condition of the plant (Wilhelm, 1935).

Kittsley and Noble (1955) and Kessler (1935) also report variation with differences in specific gravity, solute concentration, osmotic pressure, and pH.

Rosa (1921) observed that hardiness was determined mainly by hydrophilic colloids. His work was amplified by Newton (1924) who concluded that hardiness was concomitant with the water holding capacity of the cell colloids. Harvey (1933), Martin (1934), Scarth and Levitt (1937) and Kessler and Ruhland (1938) found increased viscosity of the protoplasm upon hardening.

Siminovitch and Levitt (1941) believed that the protoplasmal permeability and consistency change with increasing hardiness, and that increasing hydrophily precedes them.

Increasing viscosity decreases vital activity, which induces relative dormancy under which cells are more resistant to unfavorable external conditions.

The Role of Osmotic Pressure in the Hardening Process:-- Scarth (1944) reports that increasing osmotic pressure is due largely to conversion of starch to soluble sugars, with the greatest change being in hardiest cells. Ackerman (1927) felt the osmotic pressure was a function of the concentration of hydrophilic sugars. The work of Dexter over a period of years showed that hardening is related to the amount of plastic

substances present. Metabolic activities, which promote the increase of sugars, promote hardiness (Rosa, 1921). This increasing concentration of plastic substances attracts and holds water through an increase in the osmotic pressure (Newton, 1924a)

Kessler (1935) found that osmotic pressure in the cells rises with increasing frost resistance in fall.

Increase of osmotic pressure without other related factors in the hardening process appears to have little affect on hardiness (Scarth, 1944 and Levitt, 1951).

Role of Physico-chemical Factors in the Hardening Process:-- Biological physico-chemical changes accompany the changes of organized cellular resistances leading to hardiness. Cell sap characters are related to frost resistance as moisture content, sap concentration and sugar content, which forms an inter-related group (Levitt, 1951). However, correlations with survival in nature indicate that more fundamental factors must be operative.

As early as 1896, Lidforss found that fats and oils in the cell resisted freezing. These lipids fluctuated, appearing to be independent of temperature. Scarth in 1944 reported that lipids regulate cell wall permeability.

Siminovitch (1949) found twice as many soluble proteins at pH 5 than at pH 7 in hardy trees. He also noticed that increasing hydrophily increases protein and lipid content.

Proteins undergo changes during the hardening process, splitting into simpler, more soluble, less coagulable forms. Only the water soluble proteins increase . Rich storage of reserves occur when growth stops, but the manufacture of carbohydrate nutrients continues in the processes of natural resistance to cold according to Hedlund (1917). Various investigators have reported on soil solution uptake, as a more or less passive process combined with semipermeability of root tissue membranes (Epstein, 1955; Hylmo, 1953; Mitchell et al., 1960 and Hoagland and Broyer, 1936).

Harris (1934) was convinced that properties of tissue fluids were due to soil solution properties as well as genetically controlled synthesis. Later, Hurd-Karrer in 1939, reported that liming the soil had no effect on the pH of plant juices, and that little difference was found in plants under good growing conditions. Location effects related to soil nutrient concentrations were also reported.

Wilhelm (1935) found increased potassium, when combined with deficiencies of nitrogen and phosphorus, contributed to prolonged duration of sugar concentration in protoplasm.

Gladwin (1917) was unable to influence the degree of winter-killing of grapes by nitrogen, potassium or phosphorus fertilization, but Yasuda (1927) did so by using high potassium combined with low temperatures. Dickey and Poole in 1961 found a direct relationship between soil nutrients

and their concentration in leaf tissues. Inverse relationships existed between concentration of the nitrogen-calcium-magnesium group and the potassium-phosphorus group. In 1956, Levitt reported that while potassium and phosphorus increased hardiness, an excess produced adverse affects on physiological processes. This was corroborated to a degree by the work of Arland in 1932.

Goodall and Gregory (1947) reported on the complications entailed in determining soil solution concentrations by foliar testing, but other factors remain to be discovered as yet.

Vasilyev, writing in 1961, concluded that pH is a concomitant factor, and changes to a relatively small degree with season or hardiness. Hewitt (1952) found low tolerance to salt accumulation, and that high osmostic pressure depressed growth. (Haywood and Long, 1943 and Gauch and Wadleigh, 1945).

The information obtained from the chemical approach is of interest as it applies to the problem.

Role of Bound Water in the Hardening Process:--Chandler (1941b) postulated that all molecules of bound water in a micelle are strictly orientated and polarized. Newton (1924b) reports that tender and hardy plants have approximately the same amount of bound water in summer. Scarth (1944) reports a consistent increase in waterbinding hydrophilic colloids as a feature of hardening.

The fall weather with bright sun and cool nights increase the concentration of sugars which in turn binds water to its molecular framework. This bound water is less subject to freezing. Maximov (1929) and Chandler (1941a and 1941b) found that the soluble carbohydrates, especially the pentosans, were instrumental in binding water against the pressures of crystallization. John (1931) and Simin vitch and Levitt (1941) agree with this conclusion. According to Frey-Wyssling (1950) and Phillis and Mason (1951), water bound more closely to the micelles by proximity attraction is less likely to freeze than the more distal, loosely-bound molecules. This factor is of importance as a matter of degree within its major role in protection.

Molecular Level:--Since physiological processes can often be readily approached and comprehended at the molecular level, some considerations are pertinent here.

Decrease in temperature decreases the kinetic energy of the molecule, and activities are reduced accordingly. The less soluble a substance, the slower is its diffusion, transportation rate, and reaction capacity (Newton, 1924b). Solutions and colloids thus become more viscid as the Brownian movement slackens its activity, as colloids gel, and all associated activities decrease their activity (Chandler, 1941a and Northen, 1942). The higher energy water molecules are lost to intercellular space, and only the more sluggish remain

to bombard and combine with protein molecules, which leads to a greater measure of dessication. Increased propinquity causes greater attraction and cohesion between chemical bonds in the cytoplasm, which shrinks, thickens and becomes more gelatinous (Chandler, 1941b and Derjaguin, 1960). The ions of salts and acids remaining are more concentrated and bind polar substances more firmly (John, 1931). This in turn raises the osmotic pressure, which resists greater dessication until an equilibrium point is attained. Lipids appear and coat the surface of the plasmolyzed cytoplasm, further encysting it against adverse effects (Samish, 1954).

Since all cells must respire sufficiently to provide energy for life processes, there appears to be a threshold beyond which minimal vital processes of the cell cannot operate. For hardy plants this may be the balance between degree of hardiness necessary to resist freezing conditions, yet maintain life.

All the factors involved in induction of hardiness must be considered in a research program concerned with the physiology of hardiness.

Environment, metabolism, permeability, viscosity, physico-chemical reactions, bound water, and molecular behavior are all factors affecting the process. Since the hardening effect is achieved through their combined activities, an understanding is essential for planning of experimental work and interpretation of results. Results of work by previous

investigators in relation to the overall problem of hardiness can be used as a basis of consideration when attempting a new attack on the problem. Without the knowledge obtained through the survey of literature, this investigation into better methods for detection of hardiness would not have been possible. Through knowledge obtained in this manner, the basic natural reading was established as a part of the measurement of hardiness.

Freezing Process

The conditions which the hardening process impresses upon the normally hardy cell are present in nature at the onset of usual freezing temperature. It is upon this syndrome of cellular conditions that freezing influences are exerted, the shrunken protoplasm, gelled colloids, lipidinous coating, high soluble carbohydrate content, concentration of soluble proteins, and reduced metabolism (Samish, 1954).

All cells must respire foods to gain energy for metabolism to sustain life processes. Measurable respiration has been demonstrated at minus twenty degrees Centigrade. This has been generally believed to be commensurate with the minimal state of activity during the hardened and resting condition (Newton and Anderson, 1931 and Dexter, 1933).

During the usual course of natural freezing, ice crystals form first in the intercellular spaces. This

crystallization provides a concentration gradient which draws water from the cells. The lower the temperature, the greater is the proportion of water which freezes, according to Siminovitch and Levitt (1941).

Chandler and Chandler (1943) agree with Maximov (1929) that plants which do not store cellular carbohydrates are much more susceptible to frost injury. Both concur that the amount of water that does freeze under high soluble sugar content, does so only after considerable undercooling. In a Molar solution of sucrose, approximately half of the water freezes at minus 4.336° C., but at two Molar strength, only traces of ice occur.

Akerman (1927) computed changes in sugar solution concentration when water was frozen out of it. From in initial 1/4 N, the concentration doubles at minus 0.98° C., quadruples at minus 1.86° C., increases twelvefold at minus 7.44 and sixteenfold at minus 14.88° C. He believed the process theoretically would be accelerated by metabolizing living cells. There is an increase due to hydrolytic processes, which cause dissolution of previously insoluble compounds, and an increase in plastic, water-binding, substances. Various other physiological processes affect the freezing process inter-relatedly. These processes fall into the organismal concept here also.

In freezing, water from the vacuole is drawn through the cytoplasm and to the ice crystals of the intercellular

space. Increasing cell permeability protects against intracellular freezing, as the outflow of water serves to concentrate cell solutions till their freezing points drop to the actual temperature as observed by Scarth (1944).

Freezing Injury:--Frost injury to plants endemic to the temperate zone depends on ice formation. The usual locus of ice is outside of the cell in intercellular spaces. The size of crystals decreases and their dispersion increases with increased rate of freezing. Upon slow freezing, large extracellular ice masses can mechanically disrupt transport and collapse dehydrated cells with high osmotic pressure.

Intracellular freezing is usually fatal to the cell. Death is probably due to crystals which ramify through the cytoplasm, disorganize essential structure, and possibly cause precipitation of proteins, as was postulated by Scarth in 1944. Lozino-Lozinskiy (1948) micromanipulated cytoplasm, showing that it was somewhat resistant to mechanical damage.

Frost injury produces several visible changes. The cells may be completely separated from each other. Protoplasts undergo plasmolysis, coagulation and contraction. The dead protoplasm may show fluidity. The cell loses its semipermeable properties and solutes are subject to equilibrium with the external media (Luyet and Gehenio, 1940). Frequently a frothy structure may be seen (Scarth, 1944).

Injury to Cellular Components:--In testing, it appears that

cambium tissues and ray parenchyma are especially susceptible. Allen and Asai (1943), upon microscopical examination found the pericycle and phloem rays to be injured, in addition to cambium. Injured cells soon become discolored.

Protoplasmal Damage:--Inability of roots in winter to absorb sufficient soil moisture often results in dessication, plasmolysis and impairment of vital functions leading to death. This type of inability to resist winter freezing damage may be attributed to drought or frozen soil moisture. Death of terminal parts are also hastened by sun and winds which aid in dessication of cell contents. When half the water freezes out of the cell, the resulting dessication, plasmolysis, and ionic concentrations may cause severe damage or death.

Damage may occur in spring due to late frosts after dormancy has been broken by the burgeoning process as a consequence of early spring warm periods. Burgeoning may induce colloid hydration in the cytoplasm (Kessler, 1935 and Kessler and Ruhland, 1938).

Vasilyev (1956) reports the general acceptance now in Russia of the underlying idea of chemical destruction of protoplasm through concentration by hydrolysis, dessication and plasmolysis. Vasilyev reports that the commonest form of winter-killing is cell dehydration caused by freezing of the water necessary for the protoplasmal activity necessary to maintain intracellular life processes.

The factors involved in the freezing process are complex, and must be studied intensively to understand the physiological processes entailed.

Freezing injury of cellular components and death of the cell are matters affecting the experimental readings. Limitations could be placed upon readings with death of cells by realizing the affects upon extracellular moisture.

It is freezing damage to the plasma membrane which allows exosmosis of intracellular solutions that is measured in terms of lowered resistance to conductivity of electricity.

The Cell Wall

Several investigators have studied the cell wall intensively, and ascribed to it no major factor in the physiology of the cell nor contribution to hardiness. Chambers and Chambers (1961) report the primary cell wall to be very thin network of cellulose fibrils embedded in a matrix of amorphous pectic material.

At the end of the elongation phase, internal apposition of fibrils in parallel orientation were found. These are layered in a series of three rotations. Alignment shifts from one rotation to the next in a regular pattern, but all converge at the poles. This forms the secondary rigid cell wall. The calcium pectate matrix, as a colloidal gel, becomes rigid as it is affected by different chemicals at this stage (Proline and Preston, 1961)

The stretching of the primary wall is due to the properties of the long chain macromolecules, formed of helical, longitudinal repeating units, according to Swanson (1960). The cell wall has come to occupy less importance in physiology as its main contribution is mechanical.

Plasma Membrane

The general features of the molecular structure of the plasma membrane and the fundamental pattern of organisation have been revealed by Robertson (1962), Swanson (1960), Chambers and Chambers (1961), Walker (1958), Bedford, Meyer, and Preston (1958) and others.

Studies of ionic exchange between the enveloping solution of the free space and the interior of the cell indicate that molecules of various kinds enter or exit against the concentration gradient through the expenditure of energy in active transport (Epstein, 1955 and Harris, 1956).

The plasma membrane forms the outer boundary of the cytoplasm. This membrane is common to all cells of biological organisms, but plants, in addition, have a cell wall for support. The plasma membrane may be elastic and pliable, or rigid and unyielding, depending upon the function of the cell (Brachet, 1961 and Robertson, 1962).

Models generally indicate the presence of a double layer of lipid molecules sandwiched in right-angled orientation to, and between, two layers of long-chain protein molecules.

The cell membrane has been reported to be strongly acidic and well buffered (Holter, 1961; Harris, 1954 and Doty, 1957). Considerable work has been done on this subject, and composites of form and function are gradually forming.

The structure of the plasma membrane is characterized by an internal double layer of lipids with the non-polar end groups of the molecule adjacent to each other. The polar end groups meet with the polar groups of the amphoteric protein molecules at the outer faces. The proteins on the surfaces are orientated in a reticular network of chains which allows elasticity, mechanical strength, filtration, and enzyme localization, in the opinion of Holter (1961). The latter may be important in conversion of insoluble to soluble derivatives to pass the cell membrane (Levitt, 1951 and Levitt and Siminovitch, 1940).

The thickness of the membrane has been reported to be from seventy-five to 100 angstrom units. Diameter of the pores also have been estimated variously, with Soloman (1960) computing a diameter of seven Angstroms in erythrocytes. (Robertson, 1962)

Temporary imbalances of the equilibrium may tend to cause variability in the degree of permeation. Thus the membrane is semi-permeable, permitting entry of some ions but excluding others (Rothstein, 1955a). Through such selective permeability, the ionic content of the cell is maintained at a relatively constant level. Dynamic equilibrium achieves a

balance under which metabolism can produce carbohydrates for energy, growth, and processes leading to hardiness.

Since the semi-permeable plasma membrane largely controls the amount and kind of ions which accumulate in the film of moisture on the cell wall, the effects upon conductance under an electro-motive force would vary with conditions prevailing at the time of testing.

The post freezing readings would be affected if the plasma membrane was damaged and its selective permeability destroyed, as this would allow equalization of intra and extra cellular solutions.

Free Space

The apparent free space has been defined as that fraction of tissue into and out of which ions can move freely by diffusion without any permeable membrane between this volume and the external medium.

While there have been controversies over the subject and its potential anatomical ramifications, they will not be covered in the scope of this paper.

The freely diffusable ions in the solution which adheres as a thin film over the outer layer of the cell wall and which is essentially the end point of the circulatory transport system of the organism has been useful in explaining biological phenomena of physiological nature (Jones, 1961).

This surface film within the organism <u>en situ</u> under

ordinary growing conditions varies little. The ion accumulation is narrowly variable under normal growing conditions. Under the stress of dessication, the film thins and resistance rises. The accumulation of excess salts may also have a degree of influence upon the conductance of electrons, but this would normally be a constant from plants in the same uniform area.

The transport of ions from root to shoot has been generally considered passive in nature (Epstein, 1955; Hylmo, 1953 and Mitchell, 1960).

It is generally accepted that ion absorption and accumulation are independent (Kramer, 1957). The ions move passively over the moist film interface of the cell wall. This extra-cellular solution is considered to be part of the continuous liquid system in plant tissues (Olien, 1961). It is this space which allows conduction of electrons by ions. Levitt (1956) postulated surface film of 0.02 millimeter from tests of solutions absorbed on experimental tissues.

Robertson (1959) and Cole and Curtis (1938) reported that cells were separated by a space of 110 to 150 Angstrom units which contains a material of low electron density. This space varies with metabolic changes.

Since the cellular membrane resists passage of direct currents of electricity, the electrical resistances used in this study do not include variables of cytoplasm or cellular inclusions. Thus the flow of electrons

from the electrical forces applied are limited to the free space (Kramer, 1957).

Colloids

The majority of biological colloids are lyophilic (Samish, 1954; Willhelm, 1935 and Newton, Brown and Anderson, 1931). The viscosity of colloids derives from amphoteric molecules with hydrophilic forces which align at interfaces and produce surface activity (Livingston, 1938 and Fisher, 1924). Gelation results from numerous strong linkages between protein molecules and their attracted spheres of water (Frey-Wissling, 1948; Derjaguin, 1960 and Nedelsky, 1945), within a reticulated meshwork of intertwining fibrils (Robertson, 1962; Francis and Morse, 1956 and Swanson, 1960).

Several workers have investigated gelation and viscosity. Newton (1924b) found no difference between colloids of tender and hardy plants during the growing season. He noted an increase in viscosity of colloids associated with lowered temperatures, as did La Verne,(1949), John (1931), Buhlert (1906) and Mudra (1932). The rapid increase in viscosity of hydrophilic sols with increasing concentration of solute is ascribed to the hydration sphere of the micelles in a review by Meyer and Anderson (1952).

Scarth (1944) states that a high colloidal content of cytoplasm is necessary for resistance by hardened cells, as does Dexter (1935) and Levitt (1935).

Daniels and Alberty (1955) reported that colloids under dehydration shrink to a certain extent, then tenaciously maintain the remaining liquids over a long period of time. Lebedincev (1930) found the water-binding power of colloids to be directly related to both frost and drought resistance. Nizenjkov (1939), however, found the inverse relationship with drought resistance.

Gels appear to have an increasing effect on diffusion, conductivity, and velocity of reactions as rigidity increases (Langley, 1960; Samish, 1954 and Siminovitch and Levitt, 1941). Increases in viscosity lead to gelation. Since protein sols are amphoteric, the activity of their gel-sol relationships depends on the pH of the media (Anderson, 1939; Chandler, 1941a and Mudra, 1932). Generally in hydrophilic colloids, stability is determined by the electrical charge on the molecule and the hydration sphere (Anderson, 1939; Chandler, 1941b and Derjaguin, 1960).

Too great a degree of stability leads to an undesirable rigidity. In the investigations of Siminovitch and Levitt (1941) it was found that protoplasm of hardy cells does not become rigid as does that of tender plants at freezing temperatures.

The most generally agreed characteristic change accompanying hardiness was a sharp increase in water-holding capacity of cellular colloids (Newton, 1924; Martin, 1934; Chandler, 1941a and Meyer, 1932).

It might be postulated from reports in the body of the literature that more viscid colloids permit a slow and steady rate of respiration which maintains life in protoplasm during adverse periods.

It is apparent that colloids play a basic role in the resistance of protoplasm to frost injury, to rest period, and to the gradual return to the burgeoning of growth in spring.

They also play a part in dessication of extracellular solutes upon hardening, as well as gradual release during the depths of winter's cold. These affect readings of conductance of electricity as used in measurements of hardiness.

Permeability

Continued cell life depends upon a dynamic equilibrium of water, salts and organic matter in the cytoplasm. Control is largely maintained by a membrane only one ten-millionth of a millimeter thick. Ions and molecules of various kinds pass in and out under a controlled system. Pressure from the vacuole pushes the cytoplasm outward for intimate contact with the cell wall, which facilitates exchange through the membrane by the aqueous phase containing food, wastes, and metabolic products. More rapid exchange occurs in small cells due to the higher surface to volume ratio.

The plasma membrane is capable of controlling the

processes of energy conversion and expenditure in accordance with the complex pattern of cellular nutrient and energy exchange (Holter, 1961).

Molecules in the solution in the plant normally dissociate into ions which carry an electrical charge. This forms a force in permeability equal to the differential electrical potential.

Passive transport is derived from cellular environment. If energy from the cell is employed in passing an ion across the membrane, this is active transport. Ions can be attracted, held or released against the concentration gradient.

Soluble substances can dissolve in the lipid layer of the membrane for penetration. Others enter through the pores, so that the size of the ion may be a regulatory factor. In addition, the pores may contain ionic charges. Holter (1961) reported that the factors altering permeability were mainly ionic. In his opinion, the movement of solutes through the cell membrane is affected by molecular size, partition coefficient, concentration gradient, electrical charge, and active transport. Other workers generally agree with this view. Harris (1954) adds the diameter of the hydrated ion and osmotic pressure. Tumanov (1931) inferred that food exhaustion and accumulation of metabolic wastes could lead to an acid condition which could become toxic and thus affect permeability of the cell. Katchalsky and Miller (1951) reported that an increase in acidity of the medium increased

ionization or electrical charges.

Permeation is proportional to the percent of undissociated molecules, and this is influenced by pH changes to and from the isoelectric point. Easier entry of monovalent ions as compared to divalent ions has lead Giese (1952) to postulate larger hydration spheres due to the attraction and orientation of water molecules.

Rothstein (1955b) postulated an enzyme type carrier with localized areas in the cell wall. Hope and Robertson (1953) postulated that increasing frost injury to cell membranes might lead to leaching and dessication resulting in death to the cell. Permeability could also be affected by the increasing concentration of acids and salts during plasmolysis of cold hardiness conditions. Volume computed as 4/3 Pi r cubed, leads to possible increases in concentration by eight to sixty-four times. This magnitude could dissolve the pectocellulose matrix in the cell walls or the lipids of the membrane and allow rapid passage of solutions such as is seen after freezing.

Siminovitch and Levitt (1941) stated that permeability and consistency change with alteration in frost resistance, and that hydrophily appears to control these changes. Newton's experiments (1922) showed that hardened plants released very little juice under pressure. Later, Newton (1924) found that frozen hardened plants also retained their juice equally as well, while tender frozen plants released their

juices freely. Using this information, Dexter, Trottingham, and Graber (1930) expressed the belief that this could be used as a means of measuring the injury to the semipermeable cell membrane. Dead plants, whether tender or hardy, released approximately the same quantity of juice. These factors are basic foundations for modern investigations of plant hardiness.

Hardening Conditions

Adequate conditions for hardiness of some plants can be induced by gradually lowering temperatures and decreasing daylight hours (Angelo, 1938; Harvey, 1930; Scarth, 1944 and Stuart, 1940).

Duration of treatment and degree of intensity of application are the function of the growing stage of the plant and its innate capacity to respond to hardening environments.

Adequate hardening conditions appear to be temperatures fluctuating ten to twenty degrees diurnally, just above the freezing point, combined with light duration reduction to approximately eight hours.

Standard Hardening Conditions:--The term standard hardening conditions will be used as denoted above. Valid measurements of hardiness can only be made under the influence of these environmental effects.

Breeding

In selecting species and varieties of plants to grow in the north or south, factors of winter hardiness or rest period must be given attention. Breeding methods can recombine desirable features into a composite plant to meet the demands of increasing urbanization and the need for better landscape plants.

Azaleas at present are enjoyed for their pyschological impressions in landscaping almost exclusively in limited areas of natural adaptation.

Azalea species are known that are indigenous to wet or cold or neutral soils or exposure to sun, and have various degrees of environmental adaptation. Thus gene pools exist for these characteristics. In the control of a competent breeder, recombinations of gene patterns can genetically adapt azaleas for greater service to man.

Phenotypic effects of gene frequencies for size, color, foliage appearance and form are easily selected. Physiological hardiness and rest periods are not so easily detected. Breeders may have to carry thousands of seedlings till a "test winter" to determine hardiness before introduction or use in breeding. Weather records indicate that winter conditions are variable.

The search for methods to determine the hardiness of plants has been the subject of several investigations in recent years. If possible, the desirable plants should be

selected and tender plants discarded before large amounts of time, labor, and land use has been expended. This would also speed the cycle for a more successful breeding program.

Programs have been recently developed, but have not proven as applicable as might be desired due to the need for laboratory equipment, training, time and expense involved. A rapid, inexpensive, non-complicated system is needed to adequately measure the hardiness of segregating generations of progeny, new introductions, and seedlings of superior merit phenotypically. Such has been the object of the basic research of this paper.

Distribution:--The northern limits of distribution of Rhododendrons appears to be the cold of Lapland, Kamchatka and Siberia according to Watson (1911). The southern range extends to the humid tropics. Of the reported species of the genus, few are hardy in Zone V and VI which comprise a large portion of the densely populated northern United States (Skinner, 1962; Lee, 1958 and Lewis, 1961). Breeding lines descended from hardy varieties are now being developed.

Background for Breeding:--The three species from the arctic and the three from Siberia should contain adequate germ plasm for cold resistence. Vavilov (1926) postulates parallel development such as has attended introduction in Alfalfa and Rye. Axelrod (1959) purports that evolution of modern plants has often been toward hardiness. Stebbins (1950) and

Darlington (1939) also theorize that the variation and evaluation of plants such as the Azalea could lead to recombinations including hardiness, as most of the genes are in a plastic stage of diversionary evolution. Jain and Allard (1960) reported on the genetic background of polymorphism, homeostasis, and coadaptation which may be encountered in various species. Daday and Greenham (1960) have shown the distribution of hardiness in interbreeding gene pools, and that polygenetic inheritance is indicated. Paris (1960), in tracing the parentage of hybrid azaleas revealed the breeders choice of hardy parents to produce progeny selected for introduction to the trade.

Geneticists' results point to several independent Mendelian factors which effect this stepwise process according to Levitt (1941). It is of interest to notice the overdominance which allows selection of progeny either more tender or more hardy than either parent. Since the basis is for several genes which regulate this quantative physiological process, and these are variably expressed in the phenotype as inter-related to vagaries of climatic and edaphic factors, the problems of determination of most suitable parental material are considerable. (Hagberg, 1952 and 1953; Rollins and Howlett, 1955 and Chambers, 1961).

Application in Breeding:--While overt physical traits may be relatively easily observed, noted, and duly utilized in a pedigree program, the internal innate physiological mechanisms

are a vastly different type to distinguish. If these traits can be recognized and especially if they can be determined to a degree of intensity, the breeder is in a much better position to manipulate his parental selections for better results. Such is the case with plant hardiness. With the results of the experiments described in this paper, the breeder has a readily available tool for determining the hardiness of his stock. Thus, fortified with knowledge of inherent hardiness, the work of the breeder will be simplified and reduced. Hardiness can be incorporated as an integral part of the program to introduce this characteristic into plants with many large flowers, evergreen foliage, desirable form, texture, foliage gloss, fall color and season of bloom. It is confidentally expected that improved Azaleas will be produced by breeders through application of the measurement of hardiness.

MATERIALS AND METHODS

Technique of Measurement

Twigs were selected from healthy plants which appeared to be in normal condition for the season. Twigs to be taken were cut above a node, labeled with masking tape, placed in a polyethylene bag which was humidified with damp paper towels, and sealed for transportation to the laboratory.

Twigs were allowed one-half hour at room temperature to thaw, if necessary. Following this, twigs were removed individually and measured by the Ohmmeter, after which they were placed in a second polyethylene bag with the same humidifying materials and sealed. The lot was then placed in a temperature of minus eighteen degrees Fahrenheit, on a mesh bench with adequate air circulation at all times. Materials were frozen for approximately eighteen hours.

A battery-powered, portable Ohmmeter manufactured by the Supreme Instrument Company, Model 345, at Greenville, Mississippi was used to measure resistances. The low voltage, 1 1/2, was selected to avoid intra-cellular complications and avoid injury to membranes of the living cells.

Contacts of three types were used, anvil, spring-clip and matrix.

The anvil was comprised of two metal blades set in

a basal block. Lead wires from the Ohmmeter were soldered to these conducting surfaces. Twigs were cut to 1 cm. length and placed firmly between these contacts for reading.

The second device consisted of electrolytic rubber cushions fused into a spring-clamp arrangement. The electrolytic rubber was supplied by the Shell Chemical Company. The rubber was reduced to a solution with organic solvents and allowed to harden in cushions in the jaws of the spring-clamp arrangement. Twigs were cut as above, placed between the jaws, tension allowed to close the circuit, and readings made.

The matrix consisted of a non-electrolytic hand grip pierced by two needle electrodes placed 1 cm. apart, and attached to the lead wires of the Ohmmeter. These were merely plunged into the twig. Measurements could be made as rapidly as they could be recorded.

Normally four measurements were taken from each twig, and two to three twigs used per plant during seasons of greater variation. As techniques and equipment improved this was reduced to three measurements on two twigs, even though at times it was no longer necessary.

Expressions of Measurements

Resistance to conductance of electrons through the intercellular solution is the basic factor determining readings on the Ohmmeter.

Two readings were necessary to establish the measurement of a sample twig. One reading (a) was taken from the

natural state twig. At times it was taken from the living plant *en situ*. The second reading (b) was taken after the freezing treatment period.

The measurement was at first expressed as an a/b relationship. Later this was reduced to a ratio by the division process.

To be valid, it was found that the division process entailed a conversion of diameters to a constant unit size. The 2.0 mm modal class was chosen.

Later it was found that expressions of readings by ratio did not reflect the magnitude of readings in some cases, and alternate methods were investigated.

The formula $y = m, a + b$, where m is a base constant reading; a, an after-freezing reading; and b, the reading prior to freezing minus 3a, was suggested.

A second formula,

$$b = \frac{b_1 - b_2}{a_1 - a_2}$$

$c + n$, where c is a class level, and n is a base constant, was also considered and tested.

Both formulas have certain merits and certain undesirable features.

Upon trial and examination, it was found that a more convenient formula based on the above could be employed which served the purpose more closely. This was

$$\frac{a}{n_1} + \frac{b}{n_2}$$

where n_1 is a constant relating to a, and n_2 is a constant relating to b. Since this measurement derived from the two readings on a basis of equality, the resulting figure is of magnitudinal character.

A further step in the process of trial and examination was to use base constants of frost killed twigs. Then it was revealed that any pertinent n number could be employed. Thus, for ease in computation the second step of this formula was selected as

$$\frac{a}{1000} + \frac{b}{100}$$

with n_1 and n_2 falling roughly into classes accompanying hardiness in climatic zone V.

In the final stage of evolution, it was seen that the two readings could be reduced to percentages and added, as the most rapid and convenient method. This was adopted as the percentage summation method of expressing hardiness.

Plant Material

Plant material of a wide scope of hardiness was chosen as base plants to establish a range of basic values of measurements.

Rhododendrons, Series Azalea, used in the program or

possibly used as parents of hybrids fall into climatic zones of hardiness as follows:

Zone III. R. nudiflora, roseum.

Zone IV. R. vaseyi, Ghent Hybrids.

Zone V. R. calendulaceum.

Zone VI. R. poukanese (yedoense poukanense), japonicum.

Zone VII. R. Kaempferi Hybrids, Gable Hybrids, molle.

Zone VIII. R. obtusum, macrantha, Kurume hybrids.

More specifically, the Rare Plant List published annually by the Michigan State University Grounds Department, under the direction and cooperation of Milton Baron, is a guide for this area. Dr. Baron reports the following hardiness evaluations:

'James Gable' - hardy here several years.

'Gloskey Pink' - has done well here. Few years of establishment.

'Polar Bear' - believed hardy.

'Corsage' - probably tender here.

'Maxwelli alba' hardy here for many years.

'Alaska' - tender.

poukanense - hardy over many years, several locations.

calendulaceum - dependably hardy.

nudiflora - very hardy.

Standardization

Rigid standardization of techniques, timing, and growth stages was found to be a necessary factor in establishing a measure of reliability to readings.

Severed plant parts continually change and soon can no longer adequately reflect living conditions.

Techniques:--Apical twigs or stems to be measured were removed from intact plant and immediately placed in a polyethylene bag containing a damp paper towel to maintain humidity. These were carried to the laboratory, and, if frozen, allowed a minimum of thirty minutes to thaw at room temperature. Twigs were removed from this microenvironment one at a time as they were measured, after which they were again placed in the same type of container and frozen at minus 18° F. for approximately 18 hours and then read again.

Timing:--When measuring the degree of hardiness, the plant material must conform to the stages of standard hardening conditions as earlier outlined. Otherwise the interplay of the developmental phases of hardiness are prone to be misleading. After material has been severed, it should be used for measurements within a half hour after removal or thawing.

Growth Stages:--For all purposes, it is best to select comparable material from the same growth stage. Obviously green and brown, water sprouts and spurs, seedlings and

mature plants are not easily equated.

It is also best to select twigs of even size if possible. However, the differences in diameter can be transformed to a given dimension--2.0 mm--by arithmetical computations.

Standardization as to loci of measuring was important during physiological changes of fall and spring. During this period, marked changes in hardiness between the tips and lower portion of a twig were apparent, as the twig changed from one condition to another. During periods of stable growing or hardiness conditions, this factor was negligible.

Exposure to air was also controlled, as trials indicated that a moisture loss of approximately one-half milligram per 100 milligrams occurred each fifteen seconds from twigs with leaves. This loss was considerably reduced in plants without leaves, and further reduced with deciduous, winter hardened tissues.

Plants growing under a moisture stress were quick to increase resistance to unfrozen measurements. Thus adequate moisture control is a prerequisite for measurement, unless base correlated plants are included as a standard.

Temperature controls are also important, for, while hardiness is gained slowly, it is lost rapidly under elevated temperatures in the early stages--often in a matter of a few hours.

Twigs selected must also be in a healthy condition.

Measurement of tender twigs with previously frozen tops revealed that injury had occurred internally from one to three centimeters basipetally, and that this was soon followed by dessication.

Salt in concentrations leading to death of the plant from soil applications, had only a small effect on readings of resistance to conductance of electricity. While large differences in soil fertility and alkalinity can affect readings, the usual variations found in nature and gardens were negligible.

Thus, the more precisely variables are controlled, the more precise are the measurements, and the more reliable the predictions will be.

TEMPORAL HARDINESS

In order to establish a foundation of known values for comparative evaluations through the year, Azalea plants of known hardiness were selected as "base" plants. Selected plants were of a broad range of hardiness, established in the trade, and known to the majority of horticulturists in their respective areas.

Azalea 'Polar Bear' was growing in a flower bed with living ground cover, without overhead shade, but with some protection from a north tree belt.

Variety 'Gloskey Pink' was grown in a raised peat bed in the open. These were approximately three to four year plants with one year of growth in the bed. 'Alaska' was under heavy shade, and mulched heavily. 'Corsage' was in a cold frame with lath shading. The others were dispersed at borders of wooded slopes with leaf mulch over the soil.

The soil acidity, measured at two, three and five inches ranged from pH 6.0 to 6.5. While this is comparatively high, all except nudiflora appeared to be healthy and vigorous. Leaves of nudiflora exhibited chlorosis during drier parts of summer, but otherwise plants were healthy and grew well.

Natural state and post-freezing readings were made

following techniques previously described under "Materials and Methods." Measurements were taken each two weeks during fall, winter and spring. During periods in which standardized hardening conditions were absent, readings were largely suspended, as of little value in this study.

A few aberrant ratios were found, in which it appeared that either the pre- or post-freezing physiological processes were advanced temporally over the other in a particular twig.

TABLE 1

RATIO SEQUENCE FOR BASE PLANTS

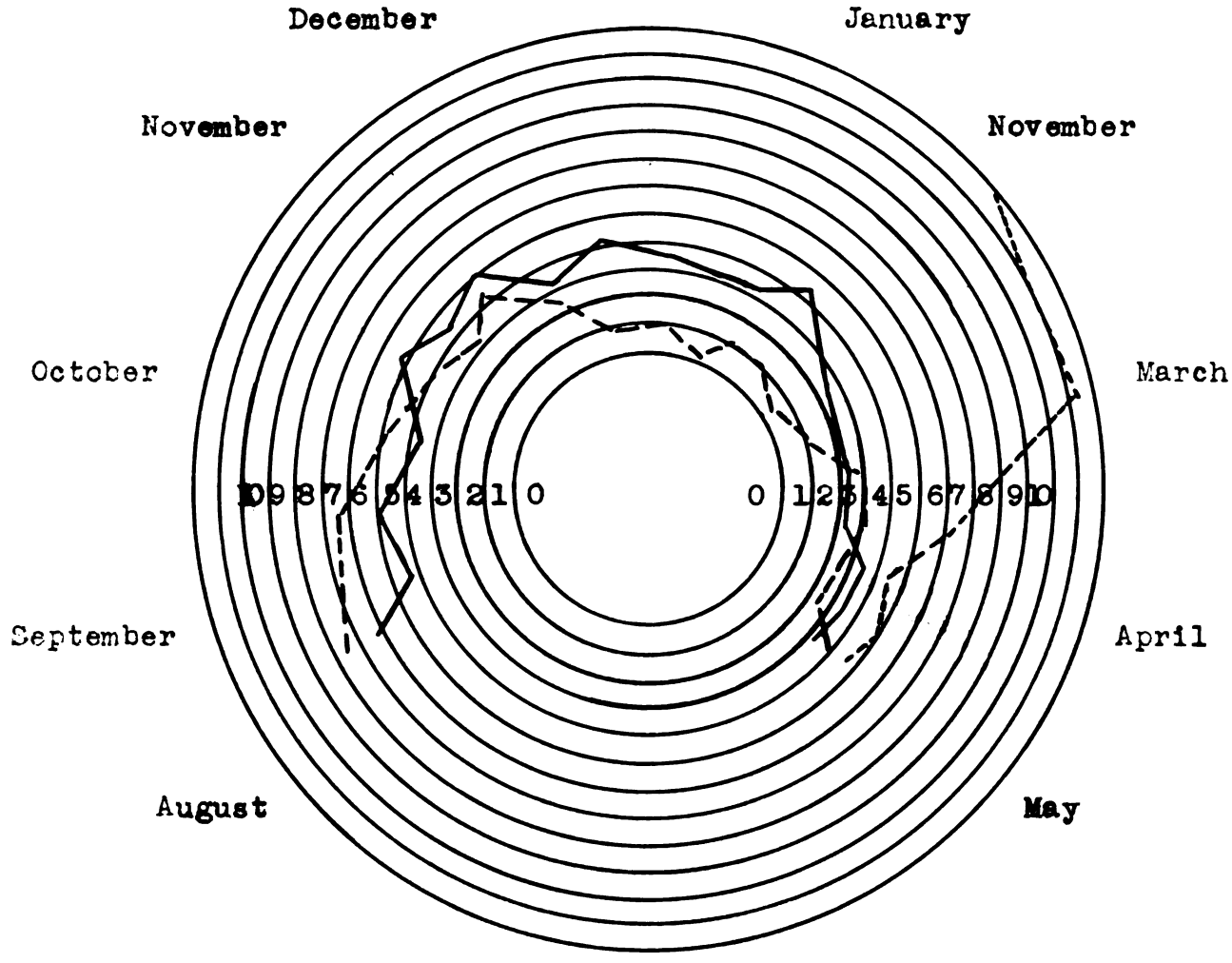
Month	April		May		Aug.	Sept.		Oct.	
Day	13	27	11	26	24	5	20	4	18
Polar Bear	2.2	3.5	3.5	3.6	5.7	4.2	6.4	4.6	4.0
Alaska*	11.7	4.7	5.0	3.8	---	---	---	---	---
Gloskey Pink	3.7	5.0	4.5	6.5	6.6	4.7	6.5	7.3	7.8
mudiflora	---	2.5	2.5	3.6	7.6	7.0	6.6	5.0	4.0
poukanense	---	---	---	3.2	7.6	6.2	8.0	6.5	6.7
calendulaceum	---	---	---	---	5.6	3.2	3.5	3.9	2.9
James Gable	2.2	3.4	3.3	---	6.4	4.2	5.8	3.8	5.1
Maxwelli Alba	2.3	3.4	3.3	3.2	7.6	4.9	5.8	6.3	7.4

Month	Nov.		Dec.			Jan.		Feb.		Mar.	
Day	3	16	1	15	31	12	26	9	23	9	23
Polar Bear	3.4	4.0	3.8	2.5	2.5	1.3	1.3	1.0	---	2.5	2.2
Alaska	---	---	---	---	---	---	---	---	---	---	---
Gloskey Pink	7.1	6.1	6.0	4.3	2.3	2.8	2.8	2.8	--*	---	3.6
mudiflora	3.2	4.2	2.6	1.0	1.0	0.5	1.0	1.0	0.5	1.0	2.7
poukanense	4.1	4.0	2.5	1.5	2.0	1.0	1.7	2.4	2.5	2.3	3.6
calendulaceum	3.4	4.3	1.9	1.5	1.2	1.0	1.2	1.0	1.0	1.0	2.9
James Gable	4.4	5.0	3.3	4.4	3.9	3.1	3.1	2.3	2.4	2.8	3.6
Maxwelli Alba	7.5	5.8	5.6	4.0	3.3	3.8	3.7	2.8	2.7	3.7	6.0

*Twigs injured by freezing were discarded. 'Polar Bear' Azalea on February 23 were under three feet of packed snow. No readings taken. 'Corsage' is not shown, as frost damage had killed it.

GRAPH 1

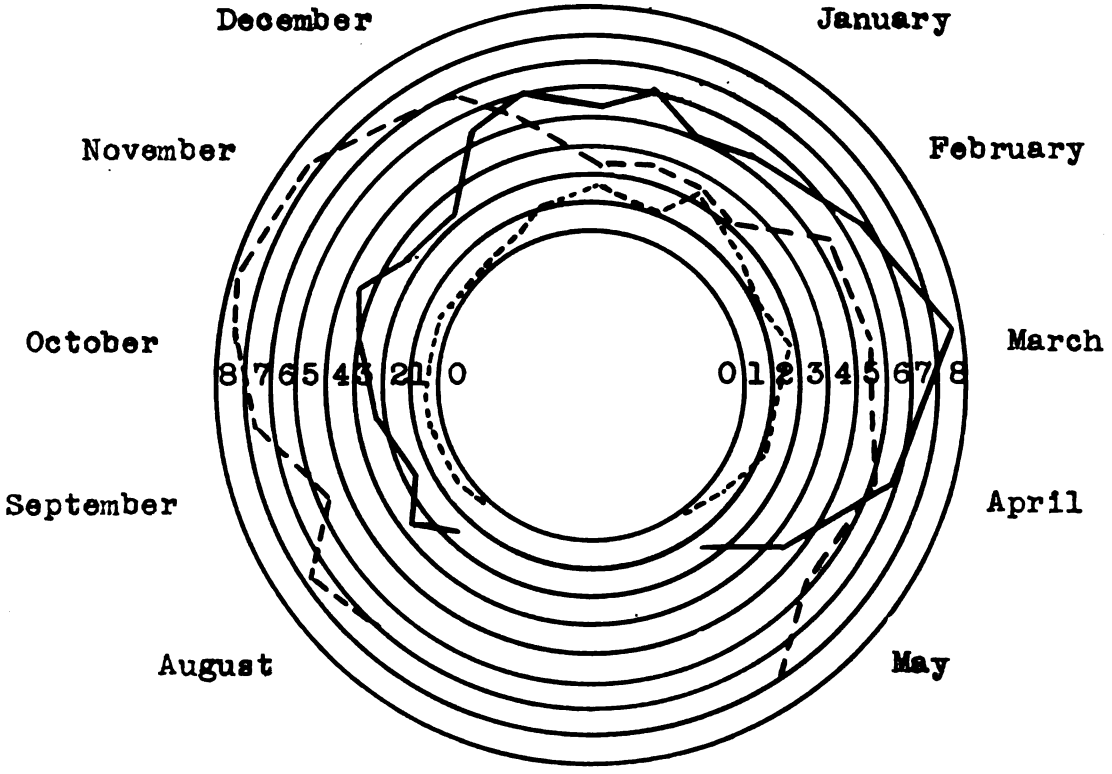
CALENDAR OF RATIOS FOR TENDER, MODERATELY HARDY AND HARDY AZALEAS



R. nudiflora--Long dash line; 'James Gable'--Solid line; 'Alaska'--Short dash line.

GRAPH 2

CALENDAR OF MEASUREMENTS OF RESISTANCE OF 'GLOSKEY PINK' AZALEA



Solid line . . . Natural readings in hundreds of Kilo-ohms.
Long dash line . Ratios derived from readings.
Short dash line Post-freezing readings in hundreds of Kilo-ohms.

EFFECTS OF NUTRIENT LEVELS

Introduction:--Speculation arose as to the possible effects of soil mineral nutrient level on the conductance of electricity through the body of the twigs employed in testing for hardiness. In order to determine the effects of different levels of concentration of the nutrients in the soil solutions, this experiment was designed and executed.

Materials and Methods:--Plant materials were established 'Gloskey Pink' Azaleas growing in three inch clay pots. Plants were selected for uniformity, divided into four plots, labelled, and placed on the greenhouse bench with a 48° F. night temperature. Temperatures and humidity were recorded by hydrothermograph.

Four concentration levels of nutrients were employed: 0.00025 Normal, 0.001 Normal, 0.004 Normal and 0.016 Normal.

Nutrient stock solutions were used as follows:

TABLE 2

NUTRIENT STOCK SOLUTIONS

Nutrient chemical	Conc m Eq	Valence	Mol. Wt.	Grams/liter
KH_2PO_4	1	1	136	136
$(NH_4)_2SO_4$	5	2	132	330
$Ca(NO_3)_2$	3	2	236	354
Mg_2SO_4	1	2	246	123

Nutrient solutions and levels of applications were determined after consultation or review of the work of Hewitt (1952), Hoagland (1935), Ballinger (1961) and Haney (1962).

Micronutrients were prepared after Hoagland's work.

TABLE 3

MICRONUTRIENT STOCK SOLUTIONS

Chemical Nutrient	Amt. in gm/gal.
H_3BO_3	10.6
$MnCl_2$ 4 H_2O	6.8
$ZnSO_4$ 7 H_2O	0.83
$CuSO_4$ 5 H_2O	0.3
H_2MoO_4	0.75
Fe*	10.0

*Fe as Na Fe EDTA (12% metallic iron).

Chemicals were carefully weighed from commercial grades of fertilizer, dissolved in distilled water, and kept in glass containers in darkness. The iron, and the remainder of micronutrients were stored at 40° F. in addition.

These solutions were mixed with distilled water at desired dilution for application.

The first application of nutrient solutions was at the rate of 120 cc per pot to achieve saturation of the soil. Thereafter, 40 cc per pot was uniformly applied, when ever

needed to maintain uniform moisture addition. Solution added was maintained as close to constance as possible, so that variations in reading would by due to variations of concentrations of ions in the aqueous phase of the internal environment.

HARDINESS PREDICTIONS

Laboratory tests of biological behavior have at times proven invalid when compared to actual reactions of organisms in nature. This experiment was designed to determine the validity of the measurements of quantitative hardiness as related to predictions of hardiness.

Hybrid seedlings from winter sowings were planted in raised peat growing beds in the open in June. Widely differing parentages were involved. Parentage being unknown to the author, plants were designated by a grid system and mapped for identification in spring.

Conductance measurements of a number of plants was taken at five different times during the fall and winter, recorded, and later compared to survival and recovery in spring. Those readings made during the course of freezing weather revealed considerable internal undetectable twig injury. Injury was seen upon examination of the cambium. Injured twigs were discarded, as their readings did not reflect valid winter hardiness.

With the advent of burgeoning growth in spring, visual estimation of recovery and survival was made on a six point recovery rating basis as follows:

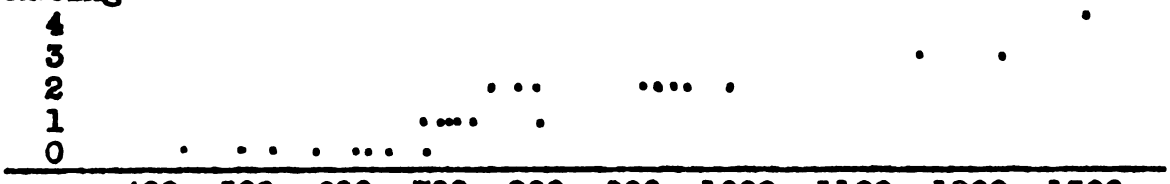
Recovery Rating 0 - Dead.
Recovery Rating 1 - Barely Alive.
Recovery Rating 2 - Frozen back severely.
Recovery Rating 3 - Frozen back to older wood.
Recovery Rating 4 - Terminal twigs dead.
Recovery Rating 5 - Undamaged.

Survival ratings were then compared to hardiness measurements, and correlations made. Three types of charts were prepared following different methods of comparisons. These were: 1) Recovery ratings plotted against summation percentage measurements; 2) Hardiness measurements compared to measurements of the base plants; and 3) Pre- and Post-freezing readings related to recovery rating.

TABLE 4

OCTOBER 26 HARDINESS PREDICTION SAMPLE

1) Recovery Ratings plotted against Summations



2) Comparative Measurements with Base Plants

Plant Material	Reading	Summation
James Gable	230/52	750
Maxwelli Alba	300/40	700
calendulaceum	375/110	1475
Gloskey Pink	250/35	600
poukanense	350/47	820
Polar Bear	367/90	1267
Rating # 4		1305
Rating # 3		1136
Rating # 2		818
Rating # 1		716
Rating # 0		572

3) Pre- and Post-freezing Readings Related to Ratings

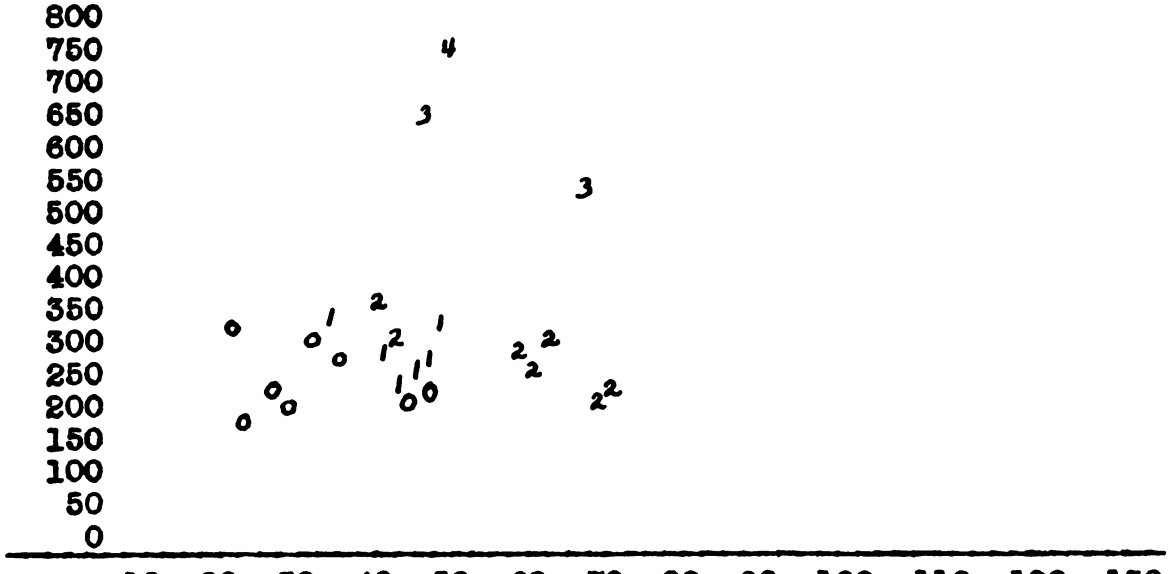
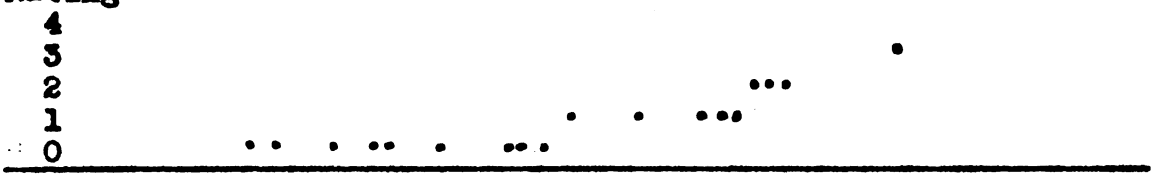


TABLE 5

NOVEMBER 8 HARDINESS PREDICTION SAMPLE

1) Recovery Ratings Plotted Against Summation Measurements



2) Comparative Measurements with Base Plants

Plant Material	Reading	Summation
James Gable	400/80	1200
Maxwelli Alba	350/61	960
calendulaceum	557/130	1807
Gloskey Pink	209/51	719
poukanense	375/95	1325
nudiflora	675/160	2275
Polar Bear	750/150	2250
Rating # 3		1237
Rating # 2		1063
Rating # 1		974
Rating # 0		559

3) Pre- and Post-freezing Readings Related to Ratings

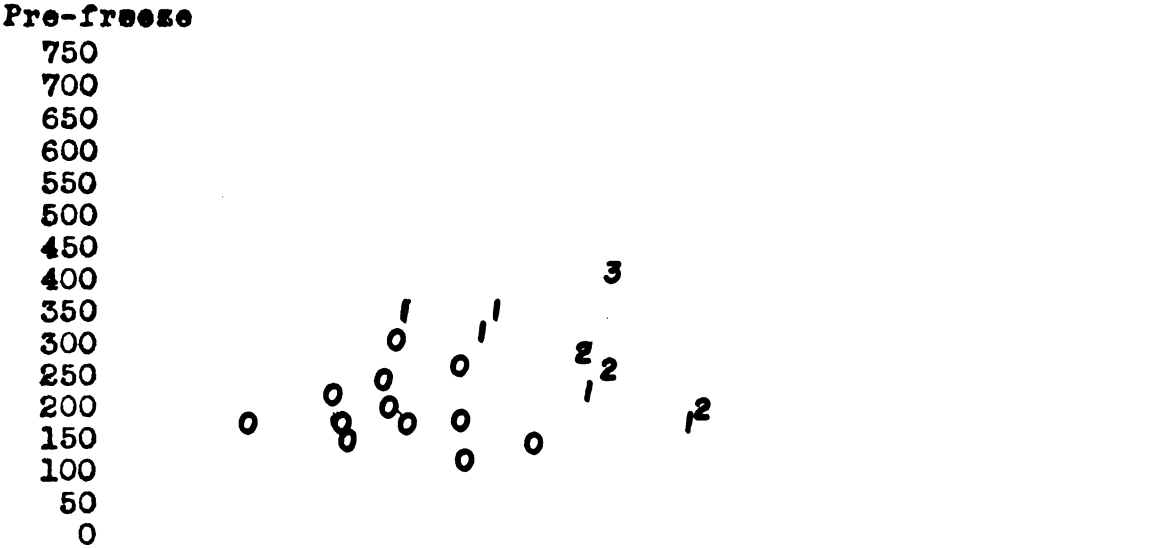


TABLE 6

NOVEMBER 12 HARDINESS PREDICTION SAMPLE

1) Recovery Ratings Plotted Against Summations

2) Comparative Measurements with Base Plants

Plant Material	Reading	Summation
James Gable	400/80	1200
Maxwelli Alba	380/61	940
calendulaceum	557/130	1807
Gloskey Pink	209/51	719
poukanense	375/95	1325
mudiflora	675/160	2275
Polar Bear	750/150	2250
Rating # 3		1500
Rating # 2		1040
Rating # 1		846
Rating # 0		736

3) Pre- and Post-freezing Readings Related to Ratings

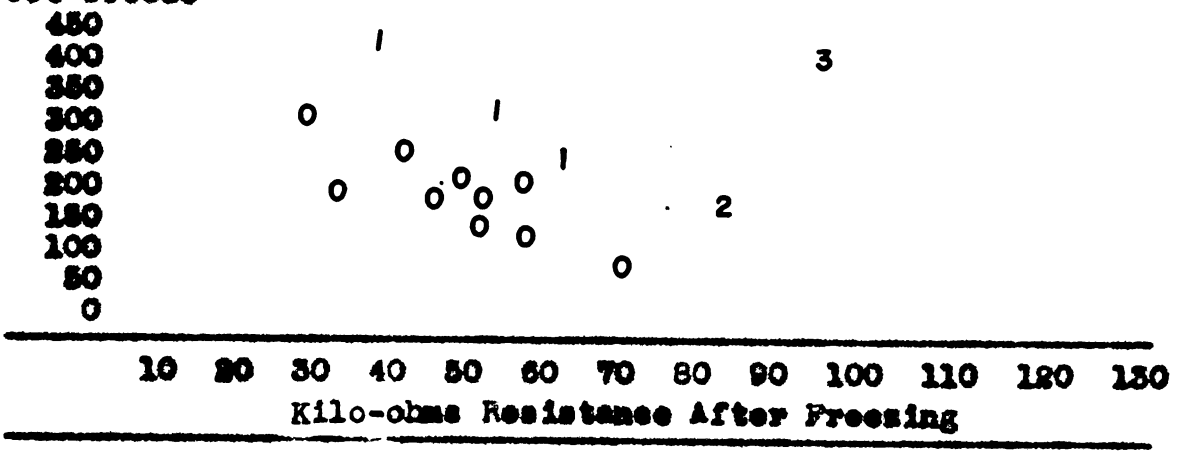
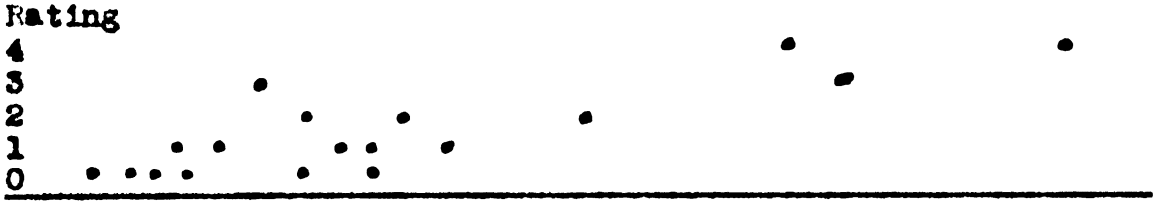


TABLE 7

NOVEMBER 26 HARDINESS PREDICTION SAMPLE

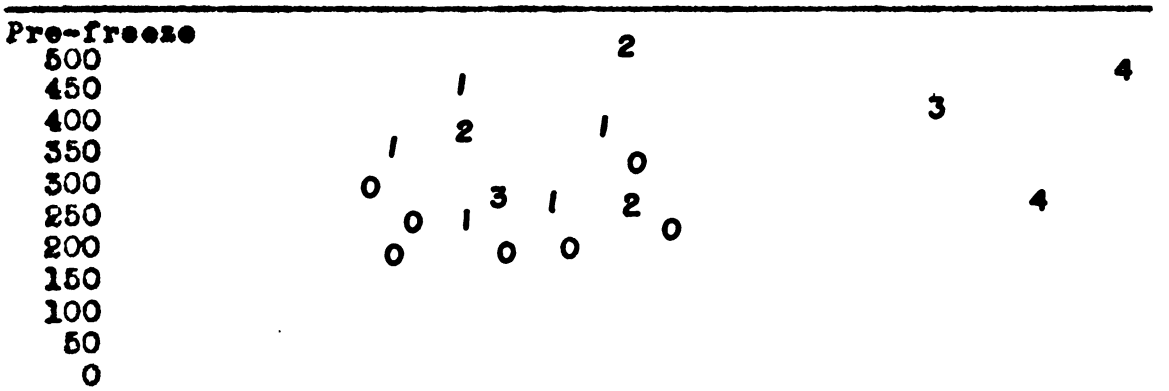
1) Recovery Ratings Plotted Against Summations*



2) Comparative Measurements with Base Plants

Plant Material	Readings	Summations
James Gable	450/135	1800
Maxwelli Alba	508/94	1448
calendulaceum	625/281	3435
Gloskey Pink	433/72	1153
poukanense	433/172	2153
nudiflora	583/223	2813
Polar Bear	875/250	3375
Rating # 4		1650
Rating # 3		1187
Rating # 2		1073
Rating # 1		927
Rating # 0		809

3) Pre- and Post-freezing Readings Related to Ratings



*No diameter corrections available for this date.

TABLE 8

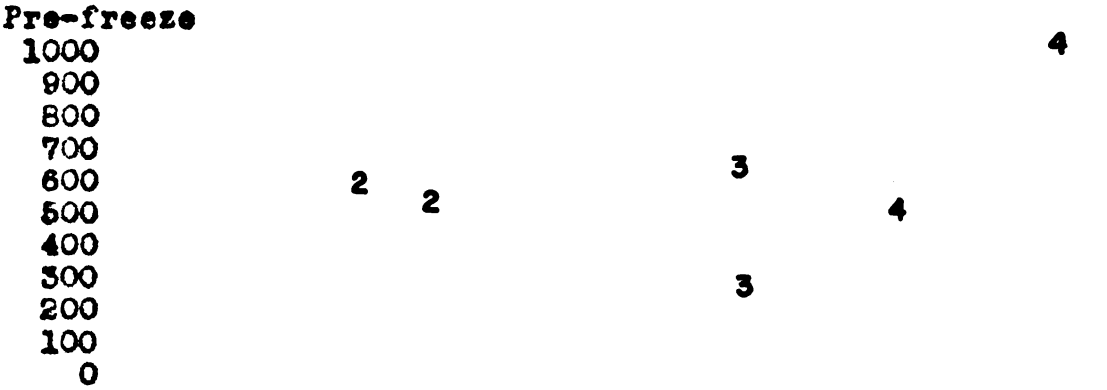
DECEMBER 28 HARDINESS PREDICTION SAMPLE

1) Recovery Ratings Plotted Against Summations*

2) Comparative Measurements with Base Plants

Plant Material	Reading	Summation
James Gable	400/100	1500
Maxwelli Alba	750/150	2250
calendulaceum	800/600	6600
Gloskey Pink	425/183	2250
poukanense	900/450	5400
nudiflora	1000/1000	11000
Polar Bear	1000/400	5000
Rating # 4		2675
Rating # 3		2062
Rating # 2		1755
Rating # 1 (Twigs dead)		----
Rating # 0 (Twigs dead)		----

3) Pre- and Post-freezing Readings Related to Ratings*



*No diameter corrections available.

TABLE 9

TEMPERATURES
FALL 1961, EAST LANSING, MICHIGAN

October		November		December	
1	77-46	1	52-39	1	47-27
2	58-39	2	72-44	2	48-37
3	56-33	3	71-36	3	50-24
4	61-34	4	49-27	4	58-40
5	73-43	5	51-29	5	58-30
6	71-52	6	43-30	6	43-27
7	72-49	7	41-33	7	39-23
8	73-41	8	39-30	8	28-15
9	77-46	9	41-29	9	29-15
10	79-56	10	44-25	10	35-37
11	76-56	11	64-27	11	33-25
12	75-52	12	59-46	12	39-21
13	74-49	13	61-42	13	23-17
14	52-34	14	56-33	14	27-11
15	55-33	15	43-26	15	24- 9
16	58-34	16	61-41	16	31-14
17	74-40	17	47-32	17	34-39
18	72-55	18	38-24	18	34-30
19	71-45	19	33-21	19	34-30
20	50-44	20	40-28	20	32-27
21	57-41	21	38-29	21	29-25
22	60-39	22	37-28	22	29-21
23	61-39	23	45-36	23	28-19
24	60-38	24	45-37	24	28-19
25	57-46	25	54-29	25	30-21
26	49-42	26	54-35	26	34-17
27	52-36	27	53-39	27	34-19
28	53-41	28	33-19	28	20-11
29	60-48	29	40-22	29	18-minus 2
30	62-54	30	43-28	30	28- 5
31	57-47			31	25-15
Soil Temperature Ranges at Three Inch Depth					
	64-51		57-37		43-15

Soil temperature at six inches was within one degree of temperatures prevailing at the three inch depth, measured on Miami Fine Sandy Loam. Azaleas in peat and under mulch over heavier soil, probably had less rapid depth penetration of cold.

RESULTS

Measurement of resistance to conductance of electrons was linear by diameter of twig. Polarization was found to be of no importance under the rapid measurements of the method of testing employed.

Of the types of electrical contacts employed, the matrix with fixed needle electrodes proved to be most satisfactory.

Measurements were sensitive to effects of soil moisture supply. Measurements of different nutrient levels in the soil revealed that differences in supply level of mineral nutrients did affect readings. Plants with highest water to nutrient relationships had the highest resistance. Clones in different locations gave the same measurements if culture and growing conditions were the same.

Measurements of Azaleas of unknown hardiness followed closely the hardiness indicated by survival and recovery in the field over winter. Measurements of a group of unknown plants readily revealed the relative hardiness among them.

Cold hardiness is gained slowly, and gradually becomes increasingly stable and able to resist damage from greater degrees of cold up to a physiological limit.

At the beginning of the hardiness induction process, temperatures in excess of fifty degrees Fahrenheit dissipated

the small amount of hardiness attained.

Measurements of established base plants followed their relative degree of hardiness through the colder parts of fall, winter, and spring. Reliable measurements could be made only after the hardening conditions induced by short days combined with temperatures fluctuating diurnally near the freezing point. During burgeoning and early hardening, readings were of little value for indicating hardiness, as they merely reflected the changes from one condition to the other.

The method of measurements devised and used here was practical for measurement of hardiness under standardized conditions.

Results of Temporal Hardiness

The ratios shown indicate the hardiness of the base selections through the fall, winter and spring. R. nudiflora measured the hardiest, at Ratio 0.5. At negative eighteen degrees Fahrenheit, it did not freeze, but only hardened further in this short period to withstand the effects of the temperature.

Tender 'Alaska' at Ratios of 8.8 and 9.0, died a lingering death, as tops gradually were killed back to areas below the snow, then below the mulch. A few weak shoots occurred in spring, then the entire plant succumbed.

Some twig injury occurred to 'Maxwelli Alba', which had been transplanted, and may not have been completely

established. 'Gloskey Pink', in its exposed situation, incurred considerable twig damage. This would indicate that ratios of 6.0 and 5.6 during standard hardening conditions are near borderline hardiness for open sites or transplanted plants. Reading of 1.0 to 2.5 during standard hardening conditions appear to indicate adequate fall hardiness under the above conditions. Plants measuring between 3.0 and 4.0 during standard hardening conditions appear to be in a range where reliable hardiness would require protection during inclement seasons, and probably should be planted no further north.

It is of interest to find that the hardier plants respond more rapidly to the onset of winter conditions and lose hardiness less rapidly upon the approach of spring growing conditions. Once spring burgeoning occurred, the hardier plants in general responded more rapidly again and achieved a higher magnitude of fluctuation than semi-hardy plants.

The chart of natural and frozen readings and their incident ratios, shows relationships involved. At times these readings can be very informative. In general, if either reading is high, hardiness may be expected, but would not necessarily be shown by the ratio, which involves the division process. This was no problem in this experiment.

The ratios shown here are sufficiently well established that they can be used as a basis for comparative

hardiness estimates for other plants of the same genus as well. Possibly it can be applied to plants of other genera.

Results of Growth at Different Nutrient Levels

TABLE 10

RATIO AND SUMMATIONS OF RESISTANCES OF TWIGS FROM PLANTS GROWN AT DIFFERENT NUTRIENT LEVELS*

Level	March 22		March 26		March 30		April 12	
	Ratio	Sum	Ratio	Sum	Ratio	Sum	Ratio	Sum
1/4 N	3.8	924	3.5	724	2.0	793	3.8	1060
1 N	3.8	924	4.2	877	3.3	995	3.8	1030
4 N	3.8	924	4.1	880	4.1	460	3.1	729
16 N	3.8	924	3.4	530	3.4	312	1.5	523

*March 22nd readings of resistances taken at the beginning of the treatments.

At the end of the experiment, leaves on the 0.016 Normal concentration group appeared to be in a state of moisture stress, curling and assuming an underlying brownish cast. Later most of them died.

The plants under 0.00025 Normal concentration appeared soft, limp and flaccid.

Both 0.001 and 0.004 Normal concentration groups appeared to be normal, growing vigorously.

Variation

Variation of resistance readings were calculated for several classes as follows:

Within a given twig.

Between different twigs of the same plant.

Between plants of the same variety.

Between varieties.

Between seasons.

Determinations of significance were made using the technique of Duncan's Multiple Range Test.

Variation within a given twig:--The least significant range of a typical post-freezing reading of a November twig was 5.29 Kilo-ohms over a trial consisting of three twigs given four readings each. The mean was 51.7 Kilo-ohms.

One of the readings was significantly different at the five percent level.

Variation between twigs:--Four typical twigs were taken from one plant in mid-November, and resistances read five times each. The least significant range was 19.278 Kilo-ohms at the five percent level.

Averages per twig were 98, 116, 106 and 110 Kilo-ohms, with a mean of 107.5. Thus no significant differences were found in averages between twigs at the five percent level.

Variation between plants of the same variety:--Nine large plants of Azalea yedoense poukanense growing in a contiguous area under good cultural conditions were sampled. Three post-freezing readings were made of each plant.

The least significant range was 7.9 Kilo-ohms. No significant differences were found. Averages of readings were 40, 40, 45, 40, 38, 40, 40, 45 and 42 Kilo-ohms, with a mean of 41.1.

Variation between varieties of plants:--Typical twigs were selected from the seven surviving base plants on December 1, 1961. Three twigs of each plant were read four times after freezing. Averages were 72, 94, 135, 172, 223, 250 and 281, with a mean of 175.28, expressed in Kilo-ohms.

With a least significant range of 8.2 Kilo-ohms at the five percent level, all were found to be significantly different.

Variation between seasons:--Typical twigs of R. 'Gloskey Pink' were selected on March 23, May 26, September 5, October 18, and December 28, 1961. These were tested for resistance to electrical conductivity in the natural state. Averages of readings were 143, 168, 350, 425 and 550 Kilo-ohms, with a mean of 327 Kilo-ohms.

With a least significant range of 10.2, all were significantly different.

Comparisons of Measurements by Electrode Type:--

I. Comparison of cushion electrode compared to needle electrodes.

A.

Cushion: 35 30 35 30 Average 32.5 Kilo-ohms

Needle: 35 30 35 30 Average 32.5 Kilo-ohms

B.

Cushion: 30 30 30 27 Average 29.25 Kilo-ohms

Needle: 30 30 30 30 Average 30.0 Kilo-ohms

C.

Cushion: 65 70 60 70 60 60 Average 65.0 Kilo-ohms

Needle: 60 60 60 60 Average 60.0 Kilo-ohms

Averages of the three were Needle 40.83 and Cushion 42.25.

II. Comparison of Anvil to Needle electrode types.

A.

Needle: 30 25 30 25 30 Average 28.0 Kilo-ohms

Anvil: 25 30 22 25 25 Average 25.4 Kilo-ohms

B.

Needle: 60 60 60 60 Average 60 Kilo-ohms

Anvil: 60 60 60 60 Average 60 Kilo-ohms

C.

Needle: 140 120 120 Average 126.6 Kilo-ohms

Anvil: 120 120 120 Average 120.0 Kilo-ohms

Averages of the three readings were Needle 71.63 and Anvil type 68.46 Kilo-ohms.

DISCUSSION OF RESULTS

Standardization of procedures is of utmost importance for validity of readings. Deviation from set standards causes deviations of readings as well.

The equipment employed in this measuring technique can be used in the field for measurements in the natural state, as the portable, battery-operated Ohm-meter fits into a jacket pocket, and the needle electrode matrix is merely plunged into the twig.

The method of measurement devised in this study can be of considerable practical help in the breeder's program for detection of quantitative hardiness in plant materials.

Decreased resistance to flow of electrons after freezing is from exosmosis of cellular fluids caused by loss of selective permeability of cell wall from damage due to freezing. Plants damaged before readings are taken often give aberrant figures. Tender plants should be tested prior to time at which freezing damage occurs. Frozen twigs on the plant, excised parts *in vitro* or dead tissues continue to change in conductivity for some time.

Cultural treatment affects the hardiness of plants and these effects can be measured by the electrical resistance method. For adequate comparisons of hardiness, both test plant and base selection plants must be growing under

approximately the same growing conditions and at approximately the same time.

Most of the Azaleas which were tested hardened in response to winter conditions. Hardening continued to a plateau where no more hardiness was attained. This apparently constituted the physiological capability for resisting freezing damage. Plants of doubtful hardiness may be tested at the degree of temperature for which hardiness determination is desired.

Once the hardiness process was initiated, hardy plants increasingly responded with greater rapidity and intensity to variations of cold temperatures. As temperatures decreased, hardy plants continued to develop hardiness and attain corresponding resistance to freezing damage.

Hardiness of base plants followed a pattern of increasing degree of hardiness with the approach of winter weather. Under controlled conditions, this sequential process of induction of hardiness can be induced in the greenhouse.

Plants of unknown hardiness can be measured without waiting for a "test" winter to eliminate undesirably tender plants. The hardier plants can be selected on a quantitative basis to serve as parent stock for breeders' programs or other desired purposes.

Discussion of Temporal Hardiness

Since hardiness is a developmental physiological process, a given set of conditions, which may suffice for one stage cannot be imposed upon an earlier stage of this consecutive process. Measurements taken here indicate that hardiness continues to develop as the intensity of winter cold increased.

A diurnal temperature fluctuation of ten to twenty degrees Fahrenheit should be maintained for more natural physiological reactions. Plants held at forty degrees F. under constant light soon became adjusted, and continued to grow.

Hardy plants under standard hardening conditions responded rapidly to lower temperatures by increasing their ability to resist frost damage. Rhododendron nudiflora after a period of time under these hardening conditions responded to temperatures of minus eighteen degrees F. by hardening further to tolerate affects of the colder temperature during the eighteen hour period of "freezing."

Thus, measurements must be taken at stages of growth induced by standard hardening conditions to be reliable guides. Some plants are slow to attain hardiness, while the majority of them seem to lose it rapidly under the influence of warm temperatures, especially during early steps of the hardening process. The hardening process begins gradually,

and appears to become cumulative, responding more rapidly with time.

While measurements may be taken from the first frosty days to spring burgeoning, it must be taken before tender plants are frozen and injured, as this produces aberrant readings. Tissues already dead from any cause, obviously cannot be injured by experimental exposure to cold. The hardiness of many temperate zone plants may be evaluated under the standards imposed by standard hardening conditions.

Discussion of Effects of Nutrient Levels

At the 0.00025 Normal nutrient concentration level, four days after beginning treatment, the resistance decreased. This could be due to the increased moisture of the plant. Four days later, this natural state resistance was still lower, but the post-freezing resistance rose. This could be attributed to a thicker film on the cell wall before freezing, and to less intra-cellular ions available for release after injury.

Twelve days later, the natural resistance was approximately two and one-half times as high, as less ions became available for free space conduction. Post-freezing readings also rose again, assumtively for the same reason. As ions are metabolized by the plant, fewer are free to cross the membranes damaged by freezing. Thus fewer electrons flow under an electromotive force, and resistance rises.

At the 0.001 Normal level of nutrient supply, the readings remained near the original level, but a gradual resistance to post-freezing reading occurred.

At 0.004 Normal supply, resistance gradually declined, though not sharply overall. This was felt to be due to the increased ions available to carry electrons through the moisture film of the cell wall.

At 0.016 Normal, resistance decreased rather sharply. It is thought that this was due to the increased number of ions available.

Discussion of Hardiness Prediction
October 26, 1961

Predictions of hardiness, judged by measurements, corresponded well with results of the winter test in the open.

Because of the long warm fall, the standard hardening conditions had not been operative sufficiently long to induce adequate hardiness, especially in the protected sites occupied by base specimens. In this pre-hardened condition, adequate comparisons of hardiness related to base selections could not well be made.

Relative hardiness among the progeny was shown by the measurements, as indicated by the charts.

It is thought that the large cultural and site differences between sheltered base plants and exposed test plants may have contributed to this difference in readings.

Overlaps of summation ratings occurred to a small extent. Since overlapping of the same character seemed a constant factor in other samples, the prediction is reduced to a range near the quantitative measurement. Since hardiness is a continuous gradation, and ratings are arbitrarily placed upon them for classification, minor digressions of small degree may be expected.

Chart three reveals interrelations between the two readings involved and their area of expected hardiness.

Discussion of Hardiness Prediction Samples: November 8 and November 12, 1961

The first freezing temperatures occurred the night of November fourth. Test plants in their dry, open site were more affected than protected base plants. This difference was apparent for about two weeks, till temperatures near freezing brought the effects of standard hardening conditions into force.

On November the eighth, inadequate time under standard hardening conditions had occurred for hardiness to be attained by either the base or test plants to a much larger degree than the previous (October 26th) reading.

In Chart 1 relative recovery ratings between plants of the sample correspond very well with measurements.

Table 8 of the November eighth sample (four days after freezing temperatures) shows that recovery rating three

has approximately the same relative hardiness as Azalea 'James Gable.' However, since these plants were not equated for age, establishment, culture or site, they would not be expected to react entirely similarly.

The November twelfth Table 6 reveals the increase in hardiness by test plants in four days of standard hardening conditions.

Older plants of the 'Gloskey Pink' base selection with more extensive root systems grown under like conditions responded to this differential. These did not harden as rapidly, and suffered considerable early winter twig injury.

In Table 6, the rating groupings reveal their relationship to the pre- and post-freezing readings more clearly than in the previous less hardened sample group. Higher readings indicate greater hardiness.

Discussion of Hardiness Prediction Sample: November 26, 1961

By November 26th, almost three weeks of temperatures which fell near freezing at night, 39° to 21° F., and only six warm, 64° to 51° F., days had occurred. This duration of standard hardening conditions brought considerable increasing hardiness to plants and allowed time for hardening processes to affect base selections. It also allowed considerable freezing processes to influence the exposed test varieties.

During this period, no diameter readings were taken

and conversions to uniform diameter figures could not be undertaken. Without diameter corrections, considerably greater variation is shown. The larger variations from expectations thus are less meaningful, as they may be due to diameter differentials. Averages however, still apply since twigs are from same populations.

The figures of Table 7 show that base plants had hardened considerably. Recovery rating #4 was commensurate with hardiness of Azalea 'Maxwelli Alba.' Rating #3 fell into the range of Azalea 'Gloskey Pink.' With their like sites and size differentials, recovery was much as expected from the measurements.

Recovery ratings below three were below any of the base selection group. Those test plants in which measurements fell below recovery rating two, approximated readings of the tender Azalea 'Alaska' for February. Reactions to the winter season were similar, in that both died or were barely alive in spring.

Discussion of Hardiness Prediction Sample: December 28, 1961

By December 28th, hardiness was almost as deeply established as any winter period of testing.

Wind, sun, and snow cover were variables which affected the exposed plants of the test site to a greater extent than the base plants of sheltered sites.

Minimum temperatures had not been severe. Gradual

reductions having occurred since the 24th of October, falling from just above freezing to ten above zero, with five warm days considered to be of no significant consequence during this period.

For this sample, no diameter measurements were available for conversions to uniform size figures. Until this time they had not been needed. Thus more, but less important variation is expected. Averages, as in the previous sample, apply.

Summation figures were about twice as high.

Rating #4 measurements compared with 'Maxwelli Alba' and 'Gloskey Pink' in hardiness.

Rating #3 was somewhat more tender than this.

Rating #2 compared to large evergreen protected Azalea 'James Gable.'

Ratings below #2 had dead twigs and were discarded.

The information in Tables 6 and 7 follow much the same pattern as in preceding hardiness prediction samples

RELATIONSHIP BETWEEN SURVEY OF LITERATURE TO EXPERIMENTS

Hardening Process:--A review of investigations into the hardening process was needed to evaluate the process as a whole. When this was done, the various phases of the process could be seen in perspective. The sequential relationship revealed that application of experimental techniques should be on a temporal basis as hardiness developed.

While reports in the literature revealed that resistances of tender plants were less, it also revealed that this method varied too greatly for reliability. The same excess variability existed with post freezing readings. However, their significance as such remained as a factor to be considered. Thus it was reasoned that the two conditions might be related as proportional to each other. If so, the differential of the resistance to conductance before and after freezing might be an index to the degree of damage sustained.

Several methods of measuring the exosmosed electrolytes have been reported in the literature. Some of these were cumbersome, or time consuming, or required special laboratory equipment or trained personnel. These were sifted with the objective of finding a quick, easy, convenient method. Several methods were tried, as were described under "Materials

and Methods."

Reports of measurements of resistance to conductance in fall, winter and spring gave contradictory results. Readings were taken every two weeks throughout this period in an attempt to determine the changes that took place.

Many plants were reported to form concomitant xerophytic characters with hardiness. It was surmised that the exosmosed solutions may exist for a time as an intercellular film on the cell wall. If so, it might be possible to establish contacts for an electrical force, and to measure the differential resistance. If this proved to be practical, plants of known hardiness could then be measured, and used as a quantitative basis for known hardiness. When this was done, plants of unknown hardiness could be measured the same way, and comparisons made between them on the basis of the quantative resistances.

Freezing Process:--The survey of literature in this area gave a well-rounded picture of another important process affecting plants at low temperatures.

The effects of freezing injury of the cell causes loss of selective permeability of the plasma membrane. Upon thawing, exosmosis of electrolytes from the cell occurs. In the recent past, this solution has been permitted to diffuse from tissues into water, and the added conductivity measured electrically as a means of determining the extent

of damage.

Free Space:--The water free space has been defined as that area in which ions can diffuse freely and become equilibrated with the external solution. The apparent free space is located in the intercellular spaces with the plasmolemma being the barrier to free diffusion. This knowledge concerning the free space was of importance because of its role in electron conductance. Learning that this is part of the continuous water system of the plant brought the conclusion that readings of resistance were related to the film of moisture on the cell wall, especially in the rays, cambium, xylem and phloem.

Biochemical Considerations:--Metabolism and ion exchange through the cell wall and membrane change the relative concentration of ions and the solvent. These changes affect resistance to conductance. Thus, the condition of the plant would be a matter for consideration if constancy were to be achieved.

Affects of varying pH of the soil might also be a factor influencing solution concentrations and consequently readings. Reports in the literature revealed this to be of relatively minor importance, but plans were made to circumvent possible aberrant readings from this source.

Cell Wall:--Information relative to the role of the cell wall led to its classification as a concomitant feature. The cell

wall furnishes mechanical support to protoplasm, and forms a boundary where the plasmolemma and the extracellular solution meet and form an interface. Anionic binding sites on the cell wall are considered important by some workers.

Plasma Membrane:--It was considered essential to learn the role of the plasma membrane. The weak flow of electricity was limited by this membrane, and thus intracellular inclusions were excluded from the apparent need for experimentation.

Since the membrane permits ions of some electrolytes to enter while others are caused to remain in the extracellular solution, the electrical conductance is changed.

The exosmosis of cellular fluids is through this membrane, which is damaged by freezing in non-hardened plants. It is this extracellular fluid which reduces resistance after damage by freezing.

Permeability:--For better understanding of the physiological processes by which cells live, die and proliferate, permeability of the cell walls, the plasmolemma, protoplasm and vacuolar membranes was surveyed in the literature. Permeability of the membranes gives the intracellular balance needed for metabolism and growth of organisms. The solution brought from the roots through the circulatory system bathes the cell walls and supplies raw materials for functioning of the cells.

When the cell dies, selective permeability is lost

as is osmotic pressure, and intracellular fluids are able to diffusion into extracellular spaces. This is the solution measured after freezing and thawing.

Colloids:--Colloids play an important role in dehydration, hysteresis, hardening, burgeoning, and resistance to frost injury. Tender plants do not develop high viscosity of colloids at low temperatures, consequently it was thought that greater mobility of ions would lead to less resistance than that found in hardier plants.

As colloids became more viscous with the dehydration of hardening, it was postulated that a gradation of resistances would be found commensurate with differences in hardiness.

Combining the ideas implied in the above reports, the two readings were postulated to express measurements.

CONCLUSIONS

The method of comparison of resistance to electrical conduction may be used to compare the degree of hardiness of plants with an unknown hardiness to measurements of plants of known hardiness for determination of relative hardiness. With this information, the reaction of a plant in regard to winter cold resistance may be pre-determined on a relative basis.

Plants may be tested for any degree of cold tolerance desired. To be reliable, testing must be done after hardening conditions have induced the physiological reactions resulting in tolerance to a given degree of cold.

Cold hardiness is gained slowly and is easily dissipated by elevated temperatures during early phases of the hardening process. The degree of hardiness increases over a long period until limited by the physiological capability of the plant to resist freezing damage.

Conclusions for Nutrient Level:--The differences in concentration of soil nutrients appears to affect readings of electrical conductance to some extent. Under the concentrations found under average growing conditions in gardens, this effect probably is negligible, but could be important. This indicates that measurements of plants growing in areas of high

or low soil nutrient concentrations should be measured with a "base selection" plant from the same area so that adequate comparisons of hardiness may be made.

Measurements of Azaleas tested in the fall corresponded closely with results of recovery and survival in spring.

The method of measurement devised during this study for determination of hardiness is relatively rapid, inexpensive, convenient, quantitative and repeatable.

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