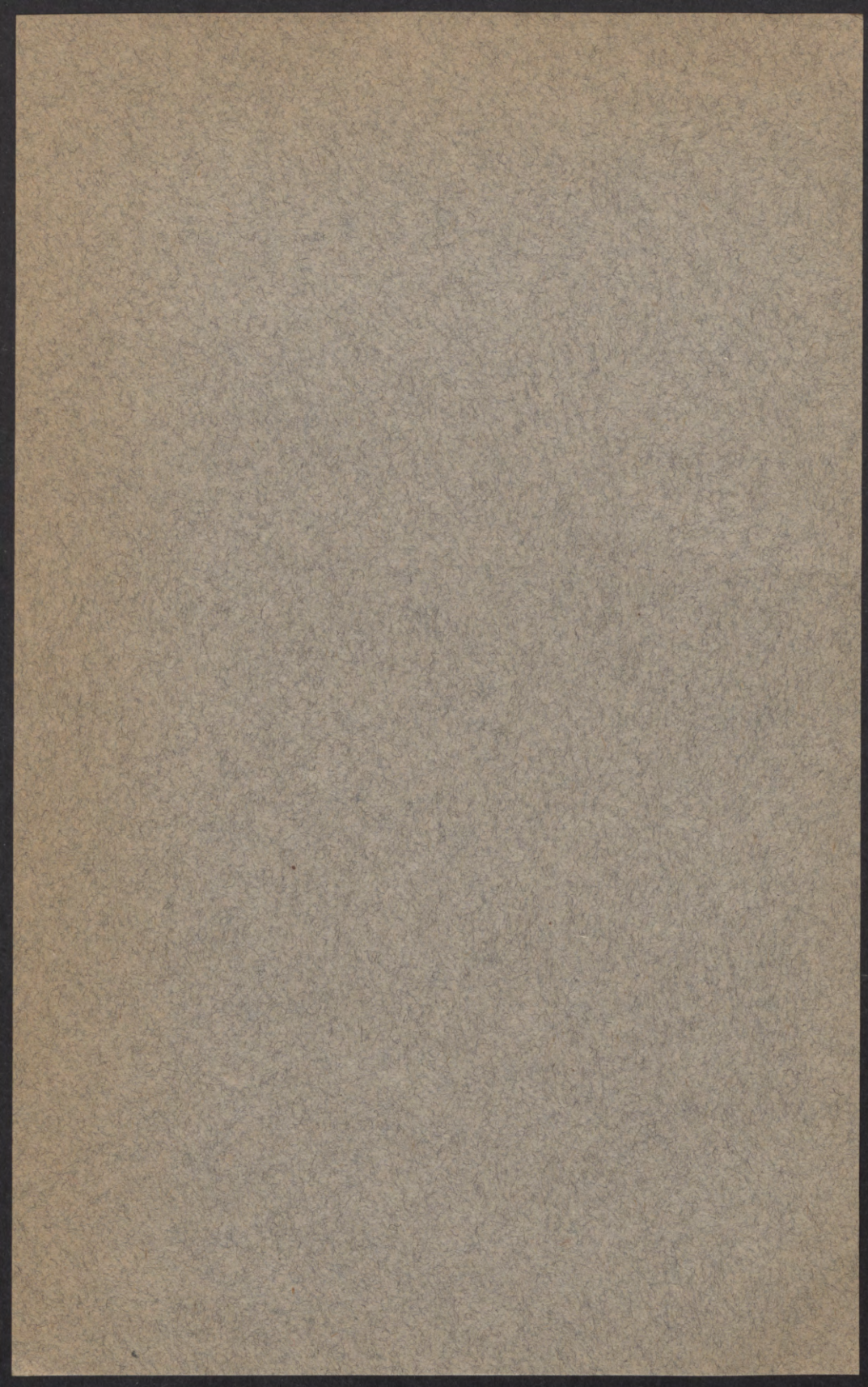*University of Minnesota*
Agricultural Experiment Station

Determination of Hardiness in Apple Varieties and the Relation of Some Factors to Cold Resistance

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DETERMINATION OF HARDINESS IN APPLE VARIETIES AND THE RELATION OF SOME FACTORS TO COLD RESISTANCE[1]

By Aubrey Claire Hildreth

INTRODUCTION

With the introduction of apple culture into the central northwest states and the neighboring Canadian provinces, horticulturists were faced with the serious problem of winter hardiness. In the attempt to find varieties which would endure the severe winters of this region, practically all of the standard varieties and thousands of seedlings were planted out, most of them with discouraging results. The problem has been complicated by the fact that our winters are not all equally severe. As Macoun (41), and Dorsey and Bushnell (18) have shown, test winters which seriously injure all but the hardiest varieties have usually not occurred oftener than every seven to ten years. Sometimes nearly twenty years have elapsed between them. Thus many varieties flourished for a time only to be eliminated by a test winter. This trial-and-error method, while effective in separating the hardy from the tender forms, is slow and expensive, and the need for a reliable and more rapid method of testing is patent to every pomologist.

Unfortunately, most of the varieties which have stood the test of time fall short of the standard for desirable commercial apples. Fruit breeders are making progress in overcoming this fault. Hardiness, however, is the first essential of a variety in this climate, and in order to determine the hardiness of his productions the fruit breeder must resort to long and expensive field tests. At the University of Minnesota Fruit Breeding Farm, where apple breeding is conducted on an extensive scale, this field hardiness test is becoming an increasingly difficult problem. The space, care, and record keeping necessary for the thousands of trees which will ultimately be eliminated by a test winter constitute no small item of expense. Some simple test for hardiness by which even a part of the tender individuals could be weeded out in the nursery row would greatly facilitate the work of developing hardy commercial varieties of apples.

This paper deals with a laboratory method of determining hardiness in apple varieties, together with a study of some factors which previous workers have suggested as being associated with cold resistance in plants.

ACKNOWLEDGMENTS

PREVIOUS WORK

The relation of plants to low temperatures has been given serious study for nearly two hundred years, and the literature on the subject is voluminous. Several reviews have recently appeared—Blackman (9), Chandler (12), Harvey (29), Rosa (58), Newton (51), and others— and only the more salient points in the progress of our knowledge in this field will be repeated here.

THEORIES ON THE MECHANISM OF KILLING BY COLD

Almost two centuries ago Duhamel and Buffon (20) advanced the theory that death from cold was due to rupture of the plant cells by ice formation inside them. Göppert (24) disproved this theory and attributed death to some other action of cold on cells, as no visible rupture occurred. Sachs (59) further refuted the idea of cell rupture by showing that ice normally forms in intercellular spaces and not inside the cells. From experiments and practical considerations, Sachs concluded that rapid thawing was the cause of death. Müller-Thurgau (46), however, could find no deleterious effects from rapid thawing, nor could Molisch (45), Winkler (75), or Chandler (12), with few exceptions.

Müller-Thurgau (46) proved that death was not caused primarily by cold, but resulted only on ice formation. Investigations by Molisch (45), Voigtländer (69) and Wright and Taylor (76) support this view. Müller-Thurgau (46) believed cold injury to be the result of desiccation by crystalization of the water of the cell. Matruchot and Molliard (42) and Greely (27) emphasized the similarity in effect of freezing, desiccation, and plasmolysis.

Gorke (26) believed that incident to water withdrawal for ice formation, proteins were precipitated out owing to increased salt concentration. He also noted increased acidity on freezing. Harvey (29) found increased hydrogen-ion concentration to result from freezing and believed this an additional factor in protein precipitation.

Schaffnit (63), Lidforss (38), Schander and Schaffnit (64) and Newton (50) agreed that protein precipitation is an important factor in freezing to death, tho Chandler (12) was inclined to doubt its occurrence. Maximov (43) concluded that in water withdrawal the plasma membrane suffers injury and the osmotic properties of the membrane are consequently disturbed.

Lepeschkin (36) regards cold injury as resulting from a denaturing of the protoplasm following water withdrawal to form ice. In addition to desiccation he regards the mechanical effect of protoplasmic contraction as well as pressure from ice masses as important in causing irreversible coagulation of the protoplasm.

Schander and Schaffnit (64) picture the steps in the process of freezing to death essentially as follows:

1. Ice formation in intercellular spaces.
2. Streaming of water from cells to crystalization centers.
3. Frost plasmolysis and concentration of cell sap.
4. Coagulation of all membranes and disturbance of osmotic functions.
5. Permeation of concentrated solutions of electrolytes and acids leading to coagulation of protoplasmic proteins, resulting in—
6. Death of the cell.

THEORIES ON THE MECHANISM OF COLD RESISTANCE IN PLANTS

The ability of certain plants to endure considerable cold, early attracted attention; and Greek philosophers reasoned that plants, like animals, develop heat for protection. This idea was still prevalent at the beginning of the nineteenth century, when it was overthrown by experiments of Nau (49), Shübler (65) and Göppert (24).

Müller-Thurgau (46) investigated the undercooling of plant tissues and considered this ability of some importance in cold resistance. He also demonstrated that undercooling occurred in outdoor plants on a still cold night. D'Arsonval (15) thought that undercooling occurred to a considerable degree in small cells. Voigtländer (69) showed, however, that undercooling to any extent was not general under natural conditions; and Rein (56) showed that there was no relation between cell size and resistance to low temperatures.

Contemporary theories of cold resistance generally accept as a basis Müller-Thurgau's principle that killing is a result of water withdrawal for ice formation. However, they center around two different steps in the process of freezing to death as outlined by Schander and Schaffnit (64). As Newton (51) has pointed out, there are two steps

in this scheme where protective forces come into play, one in preventing the water outgo from the cell, the other in preventing disorganization of the protoplasm.

Around these two points have developed two schools of thought on hardiness, the one attributing cold resistance to the cell's ability to limit the amount of water frozen out; the other being not so much concerned with preventing excess water loss from the cell contents as with the immunity of the protoplasm to the effects of water loss.

Adherents of the former school agree that the imbibitional forces of the cell are the chief means of preventing water loss on freezing. Müller-Thurgau (46) was perhaps the first to suggest the importance of the water-retaining power of the protoplasm in cold resistance. More recently Hooker (31), Rosa (58), Strausbaugh (68), Newton (51), Dorsey and Strausbaugh (19) and Dunn and Bakke (21) point out the importance of the imbibitional forces of cell colloids in cold endurance.

Means by which plant cells escape injury following water loss on freezing have been considered by many investigators. Sugar increase in plants on exposure to low temperatures has been observed by Müller-Thurgau (47), Lidforss (37), Fischer (23), Apelt (2), Appleman (3), Chandler (12), Harvey (29), Rosa (58), Newton (50, 51) and others. Lidforss (38) was perhaps first to offer a plausible explanation of the significance of this transformation. He showed experimentally that sugars have the ability to protect proteins from precipitation on freezing, and concluded that in this manner the increase of sugars in plants toward winter serves as an adaptation to low temperatures. Schaffnit (63) Schander and Schaffnit (64), and Newton (51) have confirmed the results of these experiments in regard to the protective action of sugars. Blackman (9) pointed out, however, that too wide generalizations can not be made, as the sugar beet and sugar cane, altho high in sugar, develop very little cold resistance; and that sugarless bacteria, on the contrary, are very resistant. Zacharowa (77) has recently studied the freezing of young roots in detail and finds the meristematic region of the tip much more resistant to cold than the cortex or the central cylinder. The latter regions showed appreciable quantities of reducing sugars, while the hardy meristematic region was practically devoid of carbohydrates but was rich in protein materials.

Schaffnit (63) noted that proteins of hardened plants were less readily precipitated than those of unhardened plants. He concluded, therefore, that the proteins themselves change by cleavage of the larger molecules to a simpler and less labile form. Harvey (29) found indication of cleavage of proteins in hardened cabbages, the hardened plants having more than twice as much amino nitrogen as the un-

hardened. He mentions, however, that it is "not necessary nor probable that cleavage as far as to the amino acids should occur to prevent precipitation on freezing." Newton (51) failed to find any consistent differences in amino-acid content of hardy and tender wheat varieties, altho the amino nitrogen increased in all varieties with the approach of winter.

As to means of protection against increased acidity on freezing, Harvey (29) found that the more easily injured mid-rib and petiolar tissues of the cabbage were less highly buffered than the rest of the leaf. Some recent studies of Zacharowa (77) on root tips are of interest in this connection. She found that the meristematic region in seedling root tips was extremely resistant to cold while the remainder of the root was killed at a temperature slightly below freezing. Micro-chemical tests showed the resistant portion to be decidedly alkaline, while the other tissues were acid in reaction.

MEANS OF DIFFERENTIATING HARDY AND TENDER PLANTS

The differentiation of hardy from tender plants has received attention from many investigators and several points of difference have been reported.

Molisch (45) and Pfeffer (53) considered high osmotic pressure of importance in cold resistance. Ohlweiler (52) found direct correlation between osmotic pressure and hardiness in a number of plants. Chandler (12) found that in a given plant an increase in osmotic pressure was accompanied by an increase in cold resistance. His results were substantiated by Rosa (58), working with vegetable plants. Harris and Popenoe (28) believed that high osmotic pressure may be associated with cold resistance in the avocado.

Apelt (2) and Rein (56), however, showed that in general there is no correlation between high osmotic pressure and cold resistance in plants. Salmon and Fleming (61) found no correlation between sap density and hardiness in various cereal plants, nor could Newton (51) in studies on wheats of different degrees of hardiness.

Small size of cells was advanced by Molisch (45) as contributing to cold resistance, a view further supported by D'Arsonval (15), who estimated that in very small cells water is held under considerable capillary force and that its freezing point is in consequence materially lowered. Wiegand (74) observed that trees with small cells required lower temperature for ice formation. Rosa (58) found small cells characteristic of vegetable plants in a hardened condition. Rein (56), however, by an extensive survey of plant species, proved conclusively that size of cells bears no fundamental relation to cold resistance.

High hydrophyllic colloid content was considered by Hooker (31) and Rosa (58) as characteristic of cold resistant plants. Strausbaugh (68) and Newton (51) concurred in this view. Rosa (58) and Hooker (31) attributed the hydrophyllic properties to pentosans. Newton (51), however, found no correlation between pentosan content and hardiness in wheats; and De Long (16) was not able to substantiate Hooker's results on the relation between pentosan content and hardiness in apples. Newton (51), working on the hydrophyllic colloid theory of cold resistance, devised an ingenious method of testing wheats for hardiness. The material, after freezing, was subjected to a given pressure in a hydraulic press and the amount of juice squeezed out was measured. Imbibitional powers and cold resistance were inversely proportional to the quantity of juice obtained. On the other hand, Steinmetz (67) using the same apparatus, was unable to measure hardiness in alfalfa roots by this means. More recently, Dunn and Bakke (21) have found a high degree of correlation between hardiness in apple varieties and the hydrophyllic colloids of the cortex as measured by the ability to absorb certain dyes.

Low moisture content was found associated with hardiness by Johnson (34), Strausbaugh (68), Shutt (66), and others. Beach and Allen (8) found a similar relation in general, altho they reported that following prolonged cold the shoots of hardy varieties of apple have the higher moisture content. Their data on this point, however, show wide fluctuations and several inconsistencies. The same authors studied many morphological characters of apples and found little to differentiate them as hardy or tender. Firmness of wood seemed fairly reliable, but there were notable exceptions.

Bakke, Radspinner, and Maney (5) worked out a hardiness index factor involving the moisture content and the freezing point depression by which they were able to classify apple varieties according to hardiness.

Dorsey (17) and co-workers (18, 19) used the extent of browning, killing back, and bud injury following test winters as means of determining hardy and tender tree fruits. As this method is based on the plant's actual ability to endure winters, it is necessarily reliable, altho its practicality depends upon the frequency of test winters.

Beach and Allen (8) experimented with artificial freezing as a means of separating hardy and tender apple varieties. Altho unable to control temperatures closely, their results indicate that hardiness might be determined by this method.

In view of the fact that the mechanism of freezing to death or of cold resistance is little understood, it seemed to the writer that for practical testing some simple method was needed by which the whole

complex of factors concerned in cold resistance could be measured at once. For this purpose artificial freezing seemed most promising.

The idea of subjecting plants to low temperatures to determine their cold resistance is not new. On the contrary, the method has been employed by practically every investigator of the low temperature relations of plants. However, with the exception of the work of Beach and Allen (8), this means has not been employed, so far as the writer is aware, to test the hardiness of horticultural varieties with a view to determining their climatic adaptation. Various means of securing low temperatures have been used in experiments on plants. Early workers, as Göppert (24), simply exposed their material outside when the atmospheric temperature reached the desired point. Later, Sachs (59), Müller-Thurgau (46), Molisch (45), Winkler (75), and others used freezing mixtures of salts and snow or ice. Schaffnit (63) employed CO_2 expansion as a means of cooling. Temperatures of liquid air and other gases of low liquefaction point have been used frequently on seeds and spores. Harvey (29) and Potter (55) employed mechanical refrigeration in testing for hardiness.

ARTIFICAL REFRIGERATION TEST FOR HARDINESS
METHODS AND MATERIALS

In preliminary experiments with artificial refrigeration, conducted in 1923-24, freezing was accomplished by blowing CO_2 through an expansion coil placed inside an ordinary thermal jug. The coils were surrounded with methyl alcohol and material to be frozen was placed in wide-mouthed bottles, sealed, and immersed in the alcohol surrounding the coil. It was impossible to maintain the temperature for any length of time within several degrees of the desired point, altho in spite of this fluctuation satisfactory differences in hardiness of varieties were detectable.

A mechanical refrigerating plant was later installed, and all freezing determinations reported in this paper were made by this method. The apparatus (Fig. 1) consists essentially of an ammonia compressor and an insulated chamber cooled by the direct expansion of ammonia into coils lining the walls, and is capable of producing temperatures below —40° C. It is equipped with automatic temperature controls and also a device for varying the temperature at any desired rate. For the most part, control of ±0.6° C. of the desired temperature was possible; however, for temperatures around —40° C., fluctuations of ±1° C. usually occurred. Material to be frozen was placed loosely on wooden racks in the freezing chamber and carefully insulated from the coils. The air in the chamber was constantly stirred by an electric fan. Temperature control was verified by thermographic records.

In these studies on hardiness in apple trees, terminal growths of the last season have been used entirely. Several considerations led to the selection of this particular portion. Partial or total killing of this part is perhaps the most common form of injury to apple trees in cold climates. Macoun (40) has shown that killing back of this growth is an indication of "inherent tenderness of the variety, or of immaturity of wood." Furthermore, there is not the complicating factor of injury in previous winters that might be encountered in older parts of the tree. From the practical standpoint, terminal growths are easiest to obtain and their removal causes little damage to the tree.
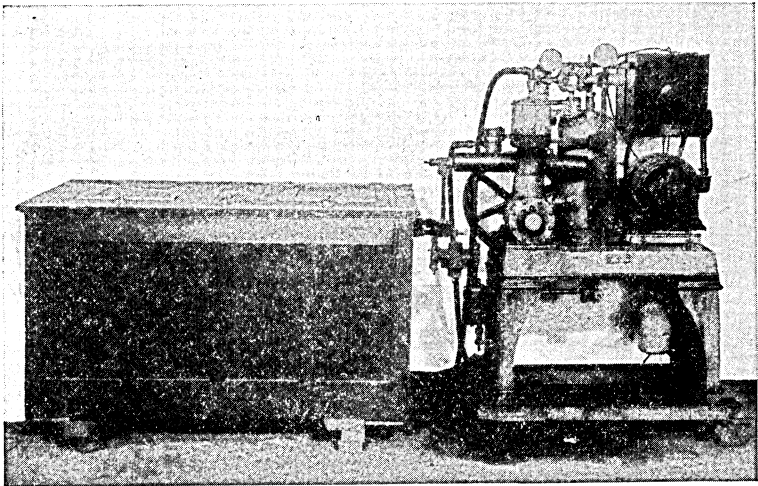
Fig. 1. Artificial Refrigeration Plant Used in Freezing Determinations

Only shoots normal in growth and more than five inches long were used, and care was taken to exclude anything in the nature of water sprouts. After collection in the field, the shoots were wrapped in waterproof paper and carried to the laboratory, where they were kept outside until used. In the growing season the leaves were stripped off at the time of collection and the shoots were packed in damp sphagnum to prevent wilting.

FREEZING DETERMINATION

From ten to fifty shoots of each variety were used in a freezing determination, and when varieties were compared at any temperature, all varieties were in the freezing chamber at the same time and were therefore subjected to the same treatment. The chamber was always cooled to the temperature of the outside air in which the twigs were kept before the samples were put in. In the usual determinations

the rate of temperature fall was 5° C. per hour. When the desired degree of cold was reached, the temperature remained constant for three hours and then rose to room temperature at the rate of 5° C. an hour. Cold injury to woody stems is not at once evident and the material after freezing must be kept for some time under suitable conditions before the extent of the injury can be determined. Various treatments were tried on the frozen twigs, such as packing in wet sawdust or sand, keeping in a cool and a warm room, placing in vessels of water, and keeping under a belljar. The method used was largely a matter of convenience, little difference being noted so long as drying out was prevented and sufficient time allowed for changes following low-temperature injury to develop. The method finally adopted was to place the samples, with cut ends down, in a deep vessel like a garbage can, add a few inches of water, place on top a loosely fitting cover, and keep at room temperature. A layer of green sphagnum on the bottom of the vessel was found helpful in preventing slimy masses of organisms that tended to collect at the base of the shoots. The partially saturated atmosphere in the can prevents excessive drying, uninjured buds open, and in a week observations may be made on amount of killing. Extent of injury was judged by growth of buds, killing back of tips, and amount and intensity of browning in pith, wood, cortex, and buds.

COMPARISON WITH FIELD EXPERIENCE

The proof of the efficacy of any artificial test for hardiness must be the conformity of its results to field experience. In Table I is shown the type of data secured from freezing determinations on shoots of different apple varieties. This material was collected on February 8, when trees were in a thoroly hardened condition. The trees from which the shoots were taken were growing at the University of Minnesota Fruit Breeding Farm, were of bearing age, and were comparable in vigor.

It will be seen that there were considerable differences in the extent of injury to the different varieties, some being killed outright while others showed practically no injury. Arranging the varieties in order of their apparent hardiness, they rank as shown in the left-hand column of Table II. In the right-hand column of this table, the varieties are grouped according to the general opinion regarding their hardiness in Minnesota. The arrangement within the groups is of no significance. This list is an adaptation from the recommendations in the Minnesota State Horticultural Society Circular for 1925. These recommendations represent the accumulated experiences of practical fruit growers and professional horticulturists and are, therefore, the most reliable information at present available as to the hardiness of these varieties in this region.

TABLE I

RESULTS OF FREEZING APPLE TWIGS COLLECTED FEBRUARY 8, 1925, AT −40° C. FOR THREE HOURS

Variety	Extent of injury*						
	Terminal bud	Lateral bud	Bark	Wood	Pith	Killing back	Remarks
Paragon	Dead	Dead	Some life	Dead	Dead	All	No growth
McIntosh	Injured	Medium	No injury	Slight	Dark	None	
Jonathan	Dead	Badly injured	Slight	Dark	do	4 in.	Practically dead
Hibernal	Trace	Slight	None	Trace	Slight	None	Buds growing
Wealthy	Medium	do	do	do	do	do	do
University	Nearly dead	Injured	do	Medium	Medium	do	do
Charlamoff	Slight	Slight	do	None	Slight	do	Good condition
Duchess (Oldenburg)	Medium	Trace	do	Trace	Trace	do	do
Patten	Slight	Medium	do	do	Dark	do	do
Wolf River	Dead	Badly injured	do	Slight	Medium	2 in.	No growth
Delicious	do	Nearly dead	do	do	Dark	do	do
Northwestern	Nearly dead	Badly injured	do	Medium	Medium	None	
Fameuse	Badly injured	Medium	do	do	Dark	do	
Windsor Chief	Injured	Injured	do	Slight	do	......	Poor sample
King David	Dead	Badly injured	Some life	Dark	do	All	Probably dead
Black Ben	do	Nearly dead	do	do	do	do	do
Lansingburg	All dead	All dead	do	Dead	Dead	Dead	No growth

* The terms "dark," "medium," "slight," and "trace" refer to intensity of browning.

TABLE II

COMPARISON OF ARTIFICIAL TEST WITH FIELD EXPERIENCE

Artificial test Arranged according to hardiness	Horticultural Society rating Varieties grouped according to hardiness
Charlamoff	Duchess
Hibernal	Hibernal
Duchess (Oldenburg)	Patten
Patten	
Wealthy	Charlamoff
Windsor Chief	Wealthy
McIntosh	
Fameuse	Windsor Chief
University	
Northwestern	University
Wolf River	McIntosh
Jonathan	Jonathan
Delicious	Fameuse
King David	King David
	Delicious
Black Ben*	Northwestern
Paragon*	
Lansingburg*	Wolf River

* Not recommended by the Horticultural Society.

Some exceptions may be taken to this grouping. Charlamoff, for example, is not classed with the hardiest standard apples. Recent experiences in the trial orchards in the northern part of the state indicate that Charlamoff is fully equal in hardiness to Duchess or Hibernal, and it is almost certain that at the next revision of this list Charlamoff will be classed with the hardiest varieties. As another instance, Canadian horticulturists seem inclined to place McIntosh somewhat higher in the scale of hardiness (16), and no doubt their observations justify this arrangement. However, in the writer's opinion, this does not hold true for Minnesota. Under usual Minnesota orchard conditions, McIntosh has a tendency to make a second growth in the fall, a condition noted in certain plants in Missouri by Bradford (7). Consequently, undue winter injury may follow as a result of immaturity. This point merely illustrates the extreme complexity of the hardiness problem and indicates that the relative hardiness of varieties may vary in different regions.

From Table II it is evident that there is a close correlation between the results of this artificial freezing test and field experience. It is true that certain varieties seem somewhat out of place in the artificial test, particularly Charlamoff, which has already been discussed, and Wolf River. From the standpoint of the practical application of such a test, however, these deviations are of little significance. The important thing to note in the artificial test is that not one of the generally

recognized hardy varieties was ranked with the tender ones, and no tender ones were classed as hardy. In the three years in which these experiments have been conducted, hundreds of samples from different sources have been tested with essentially the same results. Fluctuations in the position of certain varieties occurred from time to time, but the grouping was always the same. Invariably the hardier varieties stand well toward the top and the tender ones at the bottom.

A serious objection may be made against any artificial refrigeration method of testing hardiness on the ground that it does not represent actual outdoor conditions. The question may well be raised as to whether winter injury is caused by low temperature or by some accompanying environmental factors. The close agreement of the freezing tests and field experience, and the similar nature of the injury indicate that the degree (and perhaps duration) of cold is the chief factor concerned in winter killing in this region. It is true that the winters in which greatest damage to fruit trees has occurred are not the ones having the lowest temperatures as recorded by the weather bureau. Aside from atmospheric conditions, however, there are many physiological factors within the plant which are of utmost importance in relation to cold injury. Late growth, over-bearing, disease and insect ravages all serve as modifying influences, so that the cold resistance of a tree at the coming of winter may vary from year to year. It is significant, however, that through the vicissitudes of recurring test winters certain varieties have persisted in cold regions, while others succumbed. This indicates that altho the absolute cold resistance may fluctuate, the relative order of hardiness of varieties in a given region is, in general, maintained, a matter of extreme importance in any artificial test for hardiness.

In the application of the test here proposed it can not be assumed that because a variety could withstand $-40°$ C. in the cold chamber, it would necessarily be adapted to a region where this temperature is not exceeded. Just how far one can go in this direction is yet to be determined. The test, however, is meant to be comparative rather than absolute. In actual practice the untried variety would first be grown under similar conditions with standard varieties of known hardiness. The terminals of all would be frozen at the same time and the injury compared. In this way the hardiness could be expressed as "hardy as Wealthy," "hardier than Hibernal," "tender as Winesap," etc.

In addition to determining the cold resistance of varieties during the winter when they are in a fully hardened condition, the hardiness can be determined at any stage of activity throughout the year. When applied in the fall, the artificial freezing test furnishes a reliable guide

to the rate of hardening off, or in other words, it becomes a test for early maturity of wood. The importance of this factor in determining the adaptation of a variety to northern climates has been thoroly emphasized by Waldron (70), Macoun (39), Beach and Allen (8), and others.

RELATION OF FACTORS OTHER THAN LOW TEMPERATURE TO COLD INJURY

EFFECT OF LENGTH OF EXPOSURE TO LOW TEMPERATURES

As previously stated, the standard time of freezing in these experiments was three hours. This period was adopted because a study of the weather records for this region showed that the lowest temperatures have been of short duration, usually not more than two hours. The effect of length of exposure to low temperatures is of both practical and theoretical interest. Potter (55) logically points out that if death be due to the removal of water from the protoplasm on freezing, additional injury would not be expected so long as the temperature remained constant. However, if we adhere to the other school of thought on freezing to death and think of cold injury as due to some change in the aggregation of protoplasmic colloids, then the time factor assumes more importance, for the influence of time on changes in colloidal systems is well known.

Many writers have laid emphasis on the prolonged cold in test winters. Potter (55) has proved experimentally that length of exposure exerts a marked influence on injury in apple roots, killing being proportional to duration of freezing.

Table III shows some results of freezing apple shoots at —30° C. for three hours and for twelve hours. In every instance the longer exposure increased the injury.

TABLE III

EFFECTS OF DIFFERENT LENGTHS OF EXPOSURE TO LOW TEMPERATURES—COLLECTED JANUARY 2, 1926. FROZEN AT —30° C.

Variety	Extent of injury					
	Terminal bud	Lateral bud	Bark	Wood	Pith	Killing back
Frozen 3 hours						
Winesap	Dead	Partly dead	None	Medium	Dark	2 inches
Wealthy	Uninjured	Uninjured	do	Trace	Slight	None
Duchess (Oldenburg)	do	do	do	None	None	do
Hibernal	do	do	do	do	do	do
Frozen 12 hours						
Winesap	Dead	Dead	Dead	Dead	Dead	Entirely
Wealthy	do	Slight	None	Medium	Dark	1 inch
Duchess (Oldenburg)	do	Trace	do	do	Medium	½ inch
Hibernal	Nearly dead	do	do	Slight	do	None

EFFECT OF RATE OF FREEZING AND THAWING

Müller-Thurgau (46) showed that rapid freezing tends to produce small and irregular ice crystals while slow freezing gives larger and regular ice masses. In very rapid freezing he found ice crystals to form inside the cells, altho normally they were extracellular. Regarding the relative injury from rapid and slow freezing, Pfeffer (53) believed that there is no difference. Winkler's experiments (75), however, proved that a rapid temperature fall is much more injurious than a gradual lowering. His results have been substantiated by Chandler (12), and more recently by Carrick (11) and Potter (55).

On the effects of rapid and slow thawing, there is a divergence of opinion. Sachs (59) believed rate of thawing to be the determining factor in killing by low temperatures. Göppert (25) and Molisch (45) proved that injury results at the time of freezing. Müller-Thurgau (46) was unable to detect any deleterious effect from rapid thawing, altho later (48) he found that fruits of apple and pear were exceptions. Molisch (45) supported Müller-Thurgau's general conclusion that rate of thawing is without influence, but noted an exception in the case of leaves of *Agave americana* L. Chandler (12) found, in addition, that lettuce leaves were injured more by rapid thawing. Wiegand (74) found no relation between rate of thawing and extent of injury. Winkler (75), in experiments with deciduous and evergreen twigs, could detect no difference in injury by rapid or slow thawing, nor could Carrick (11), working with roots of several plant species. Akerman (1) found that plants subjected to temperatures slightly above the lethal point are generally more injured by rapid than by slow thawing. His method of rapid thawing was by immersion in luke-warm water. He found no differences in injury from rapid and slow thawing in air.

Some limited tests were made on apple twigs to determine the effect of rate of freezing and thawing. All terminal growths of a thrifty Wealthy apple tree were collected on December 30. They were sorted into five comparable lots of twenty shoots each for freezing. Lots 1, 2, 3, and 4 were cooled at the usual rate of 5° C. an hour. When the cold chamber reached —35° C., lot 5 was put in as quickly as possible, transferring it from a temperature of —10° C.; and freezing at —35° C. was continued for three hours. At the end of this time lot 1 was quickly immersed in rapidly flowing tap water at a temperature of 15° C. Lot 2 was plunged into a mercury bath at 30° C. for three minutes with constant stirring. In view of the high thermal conductivity of mercury this method gives perhaps the maximum rate of thawing it is possible to secure without increasing the temperature beyond the critical point. The other lots were thawed

slowly. The treatments and resultant injury are given in Table IV. The figures in the last column represent the estimated percentage of injury, total possible injury being taken as 100 per cent.

TABLE IV

EFFECT OF RATE OF FREEZING AND THAWING ON WEALTHY APPLE SHOOTS FROZEN 3 HOURS AT —35° C.

Lot No.	Rate of cooling	Rate of thawing	Per cent injury
1	5° C. an hour	Immersed in running water at +15° C.	64.4
2	do	Plunged in mercury at +30° C.	65.5
3	do	Average 2½° C. an hour	8.8
4	do	Cold chamber at 5° C. an hour	6.6
5	Suddenly from—10° to —35° C.	Cold chamber at 5° C. an hour	65.6

The rapid freezing and rapid thawing gave approximately equal amounts of injury and both are markedly more severe than when temperature changes were gradual. These results are entirely in accord with those of Akerman (1). These extremes are of only theoretical interest, as such conditions are probably never encountered in nature. The nature of the injury in both rapid freezing and rapid thawing was noticeably different from the usual type of cold injury to dormant twigs. The buds and cortical region suffered most, while wood and pith showed less injury. Normally the reverse is true, as can be seen from Tables I and III. The greater injury to buds and cortex was probably due to their being more exposed and consequently subject to more sudden temperature changes.

EFFECT OF WINTER DESICCATION ON COLD INJURY

In addition to low temperatures, the factor most commonly thought of in connection with winter injury is desiccation. Horticultural literature abounds in references to this phenomenon and the following quotations from horticultural writers show the sinister regard with which winter drying is held. Warder (71) says, "Exposure to long continued cold, with severe winds, seems to dry up the juices of the plants, in some instances, and thus effect their destruction. This, in the far north, is believed to be a frequent cause of the evil" (winter killing). Waugh (72), in speaking of winter killing of young growth, says, "It has been shown that this damage occurs largely through the drying out of the young shoots, due to the fact that, while some evaporation goes on in winter, the trees cannot take up water from the frozen soil to make good this evaporation. The young shoots become so thoroughly dried out during cold weather that the tissues are killed."

On the whole question of winter injury through desiccation, there is much circumstantial evidence but there are few data. The popularly

accepted view of the cause of such injury was expressed by Müller-Thurgau (46) as early as 1886. By experiments he proved that twigs of apple and other trees exposed outside on two cold nights lost weight. He believed that soil and stem in frozen condition were poor conductors of moisture and unable to replace water lost by evaporation from ice crystals in the frozen branches. In consequence of the water loss, the tissue died. Sachs (60) had earlier indicated the retardation of conduction with low soil temperatures by showing that in cold soil the transpiration rate of plants was materially reduced.

Investigations by Waldron (70) and Buffum (10) show greater injury when trees enter winter with dry soil. Undoubtedly root injury is here chiefly concerned, as many indications show that dry soils favor root killing. Craig (14) found root killing more prevalent in light, dry soils. Emerson (22) experimentally proved greater root injury in dry soils, which results were later substantiated by Potter (54). Emerson also showed that dry soils freeze deeper than wet ones. Potter (55), in artificial freezing experiments, proved that soil with low moisture content responds more quickly to air temperature fluctuations than damp soil. He also found that at the same temperature roots in moist soil are injured more than in dry soil, if there is any difference, thus sustaining an opinion expressed earlier by Chandler (12). Carrick (11), in freezing experiments with roots, found no consistent differences in injury at a given temperature with either moist or dry soils. Potter (55) concludes that if roots under natural conditions are injured less in moist soils, it is due to a difference in temperature. Experiments of Howard (32) under outdoor conditions are in accord with Potter's (55) findings on rate of temperature change in dry and wet soils. He failed, however, to find any differences in minimum temperatures and concludes that soil moisture exerts some protective action against cold injury. His results are open to a possible criticism, as temperature measurements were made inside a box with a perforated bottom, plunged in the ground. This might conceivably interfere with conduction of heat from the lower levels. More experiments are needed on this subject, but at present the evidence seems to favor the view expressed by Chandler (12) and Potter (55)—that temperature, rather than moisture, is the important factor in root injury.

In regard to desiccation of above-ground portions, Bailey (4) found apple twigs to lose appreciable moisture in winter in New York State. Sandsten (62), at the Wisconsin station, cut off 8-year-old apple trees and weighed them at intervals during winter to determine the water loss. Table V is adapted from his data.

TABLE V
DECREASE IN WEIGHT OF APPLE TREES IN WINTER (AFTER SANDSTEN)

	1902-03 Weight of tree				1903-04 Weight of tree	
	No. 1	No. 2	No. 3	No. 4	No. 1	No. 2
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
December 5	...	...	...	...	26.2	24.2
December 19	36.6	24.6	35.7	30.4	26.3	24.8
January 9	35.5	23.2	34.3	30.0	26.4	24.4
January 30	35.6	24.0	35.0	30.0	26.0	23.9
February 20	34.5	23.0	33.8	28.7	25.9	24.2
March 19	...	...	...	...	25.9	24.0
March 20	33.2	22.6	33.0	27.6	...	...
March 26	...	...	...	...	25.6	23.6
March 27	32.6	22.0	32.4	27.4	...	...
April 3	31.4	21.4	31.6	26.5	...	...
Loss	5.2	3.2	4.1	3.9	0.6	0.6
Per cent of weight lost	14.2	13.0	11.4	12.8	2.2	2.4

The results show marked differences in the two winters. In 1902-03, loss was near 15 per cent total weight, while in 1903-04 it was little more than 2 per cent. The latter year was described as humid. It is interesting to note that in 1902-03, when the greater loss in weight occurred, loss during two months of the presumably colder part of the year was less in every case than in approximately six weeks of warmer spring weather. No statement is made as to whether these trees showed any signs of winter injury.

Experiments of Ivanoff (33) at Petrograd, with evergreen trees severed from the roots, show that the roots exert only a small influence on the water content of leaves in winter. With deciduous twigs cut off and exposed to temperatures below freezing, he found southern species tended to lose moisture more rapidly than northern ones. He concludes, therefore, that the ability to resist winter drying is the chief factor in determining the northern range of plant species.

Some preliminary experiments on winter drying conducted in 1924-25 seem worth reporting. The object of these studies was to determine the amount of water loss from apple twigs during winter in Minnesota, its relation to temperature, and its effect in promoting winter injury. Terminal shoots were collected in winter and cut surfaces were sealed with grafting wax. They were held separately in an upright position by ordinary spring clothespins nailed on a board. This support was fastened in a fully exposed location about eight feet above the ground. The twigs were weighed outside at intervals. The data are shown in Table VI and are represented graphically in Figure 2. Both samples lost weight for the duration of the experiment, the only break occurring after a rain on February 9, when the McIntosh sample,

which had lost more, presumably by reason of longer exposure, gained weight. No comparison of varieties was intended or is possible from these data, as the two samples were put out at different times. The data for the two varieties are included merely to show that water loss is not peculiar to one variety.

TABLE VI

LOSS OF WEIGHT OF TWIGS CUT OFF AND EXPOSED OUTSIDE DURING WINTER

Date	McIntosh 25 terminals		Wealthy 14 terminals		Average loss per day
	Weight	Total loss	Weight	Total loss	
	gm.	gm.	gm.	gm.	gm.
December 17, 1924	201.65	....	....	....	....
December 20, 1924	201.10	0.5	....	....	....
December 23, 1924	200.6	1.0	....	....	....
December 27, 1924	198.9	2.7	....	....	....
January 1, 1925	....	....	194.6	....	....
January 7, 1925	194.5	7.1	192.8	1.8	0.25
January 12, 1925	192.7	8.9	192.0	2.6	0.16
January 16, 1925	191.8	9.8	191.7	2.9	0.07
January 24, 1925	185.9	15.7	188.1	6.5	0.45
January 31, 1925	182.5	19.1	186.0	8.6	0.3
February 9, 1925	183.0	18.6	183.7	10.9	0.25
February 17, 1925	176.5	25.1	179.9	14.7	0.47
March 3, 1925	166.0	35.6	172.8	21.8	0.5
March 27, 1925	....	....	155.3	39.3	0.72
April 8, 1925	....	....	137.5	57.1	2.25
Per cent of original weight lost	....	17.4	....	29.3	....
Final per cent of moisture	34.5	....	....	....	....

The slope of the curves shows that the rate of water loss was gradual during periods of low temperature, but increased as spring opened, despite the fact that the moisture content of the twigs was being constantly diminished. These results are opposite to what would be expected if the winter desiccation rate were equal to the spring rate. In such case we should expect the logarithmic curve typical of the dehydration of colloids, which would show a high initial rate with a rapid flattening out of the curve as the last increments of moisture were more firmly held. This low evaporation rate in winter is in agreement with findings of Kosaroff (35), who showed that coniferous trees transpire from 300 to 400 times more in summer than in winter. The average daily loss for the periods between weighings is shown at the top of Figure 2. The broken line represents the average daily minimum temperatures for the same periods. Except for the rainy period, about February 9, the evaporation curve of the twigs agrees fairly closely with the temperature curve.

On March 3, part of the McIntosh sample taken for moisture determination showed a moisture content of 34.5 per cent. A similar determination made on shoots which had been left intact on the same

tree from which the original sample was taken showed a moisture content of 45.5 per cent. It is obvious that during this time considerable conduction of moisture had occurred to the twigs left on the tree.[1] Kosaroff (35) has also found that water is conducted to leafless twigs in winter; in evergreen trees, however, he found that the rate of water movement in the twigs is slowed down more, proportionately, than the rate of evaporation from the leaves.
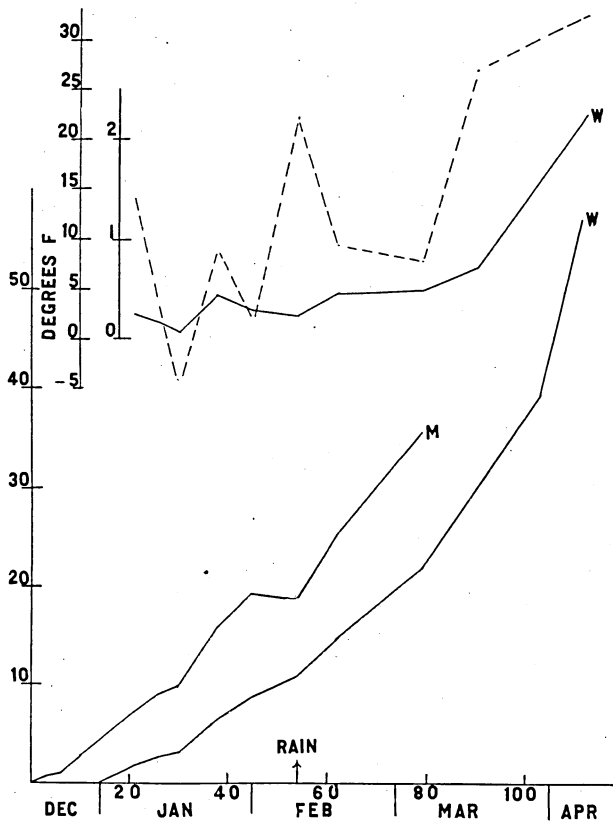
Fig. 2. Effect of Winter Desiccation on Apple Terminals
The lower curves show the total loss of weight of two varieties exposed at different times. Number of days is shown as abscissa and loss in grams as ordinates.
Above, the solid line represents the average daily rate of moisture loss of the Wealthy. The broken line shows the average daily minimum temperature for the same periods.

The remainder of the McIntosh sample used in these studies was placed in water in the greenhouse and all buds opened apparently normally. The Wealthy sample set up on January 1 lost 29.3 per cent of its original weight by April 8, altho nearly two-thirds of this

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[1] The temperature of the air and possibly of trunk and branches was frequently above freezing during this period, altho the soil was frozen throughout.

loss occurred during the last month of exposure. Growth tests were not made on this material, but the entire sample was sectioned for study. It showed none of the appearances characteristic of winter injury.

In an artificial desiccation test, similar Wealthy twigs collected January 1 were waxed on the cut surfaces and placed in a sulphuric acid desiccator at a temperature of 1-3° C. for 24 days. The results were as follows:

	grams
Original weight of sample.........................	138.90
After 24 days in desiccator	110.55
Loss	28.35
	per cent
Weight lost	20.4
Moisture content at beginning......................	44.8
Moisture content after desiccation.................	28.7

Altho these twigs had lost more than a third of their moisture, when set in water in the greenhouse the buds grew normally.

While these very limited experiments do not warrant general conclusions, certain facts are evident:

1. Cold winter weather may have relatively little evaporating effect on twigs.

2. Apple twigs are able to withstand a considerable loss of moisture in winter without injury providing later conditions are favorable for growth. Consequently, only a minimum of water conduction in cold weather would keep them well above the critical point.

3. The fact that shoots left during winter on trees in frozen soil showed considerably more moisture than severed ones, indicates that trees in winter may be able to make up, in part, moisture loss occurring from evaporation.

These remarks should not be construed to mean that "die-back" of tips in winter from deficient moisture, such as Batchelor and Reed (6) describe in the Persian walnut, in California, is at all improbable. Indeed, with a dry soil, a rate of evaporation such as was shown in these experiments in March and April would undoubtedly reduce the moisture content of the twigs below the minimum required for life. However, it seems problematic that this type of injury is in any way related to the killing-back of twigs so common in cold climates and so readily obtained by exposure to artificially produced low temperatures.

RELATION OF CHEMICAL CONSTITUTION TO COLD RESISTANCE

Studies were undertaken to determine the relation between hardiness and chemical constituents in apple twigs. Special attention was given to moisture, sugars, pentosans, and amino nitrogen, because of the significance attached to these materials by previous workers, as mentioned earlier in this paper. Comparison was made between two varieties, a hardy and a tender one, throughout the entire year. By this means it is possible to compare not only the chemical make-up of varieties of different degrees of hardiness, but of the same variety as its hardiness varies from season to season. To establish the hardiness of the varieties at different times in the year, killing tests were made in the cold chamber at each sampling for chemical analysis.

The year 1924-25, in which these experiments were made, proved excellent for hardiness studies, as the weather had many characteristics generally considered conducive to winter injury. The summer of 1924 was unusually cool with much rain, particularly in the latter part of the season. The fall was mild and there was little cold weather until shortly after the middle of December, when the coldest period of the winter was experienced. While not a test winter, there was more than the usual amount of winter injury in Minnesota.

MATERIALS AND METHODS

For these determinations Duchess (Oldenburg) and Jonathan were chosen as typifying respectively hardy and tender varieties. Five Duchess and two Jonathan trees were included. The trees, which were located at the University of Minnesota Fruit Breeding Farm, were under clean cultivation, were in good vigor, and were bearing regularly. Studies were confined to terminal shoots, which were collected and treated as previously described for artificial freezing. At each collection shoots were taken at random from all trees. The material was divided outdoors into lots for chemical analysis and for freezing tests. During freezing weather the chemical sample, wrapped in waterproof paper, was sometimes kept outside over night before preparing for analysis. At other times, however, it was prepared at once on being taken to the laboratory.

To determine the killing points of the varieties, a series of freezings was necessary, at different temperatures of 2- to 5-degree intervals. The freezing tests were carried out as previously described and after suitable time the injury was analyzed as indicated in Table I. Obviously not all possible temperatures were tried out in any test, the aim being rather to establish for each variety two narrow temperature limits, one at which it was still alive and the other at which it was

killed. The killing points recorded in Table VII represent the highest temperature at which there was no life in the sample.

ANALYTICAL PROCEDURE

For chemical analysis the entire twigs were ground in a motor-driven pencil sharpener described in an earlier publication (30), from 25 to 50 terminal growths being required for each composite sample. Mixing was accomplished by thoroly shaking in a large specimen jar fitted with a ground-glass cover. Samples were taken for moisture determination, and 50-gram samples were weighed out for analysis. This material was preserved by dropping it into boiling alcohol to which had been added one gram of calcium carbonate. After boiling for thirty minutes, the jars were sealed and stored until needed. The entire process from beginning of grinding to placing in alcohol required approximately ten minutes.

The preserved samples were further prepared for analysis by extraction with alcohol in Landsiedl extractors for eighteen hours, at which time all gave a negative Molisch test. After making up to volume, the alcoholic extract was treated with chloroform to separate out chlorophyll and lipoid material. Aliquots of the alcoholic phase were taken for sugar and amino-nitrogen determinations, the alcohol being removed in Classen flasks under reduced pressure at a temperature of 40-60° C. Determination of total and reducing sugars and amino nitrogen were made on the alcoholic extract. The residue of the alcoholic extraction was analyzed for pentosans, and for total acid hydrolizable carbohydrates. Organic nitrogen was determined from the residue of the moisture determination. Official methods of analysis of the Association of Official Agricultural Chemists were used throughout except that in sugar determinations, in which the thiosulphate method of determining reduced copper was employed, bromine water was omitted from the process. All determinations were made in duplicate except sugars, which were in quadruplicate.

Complete analytical data, together with killing points of the varieties, are shown in Tables VII and VIII. The last sample, taken July 7, 1925, was from new succulent growth. In all the graphs, data for this collection are plotted first. While not strictly accurate, this seems more logical and furnishes a more complete seasonal picture.

SEASONAL VARIATIONS IN KILLING POINT

Killing points for both varieties throughout the year are shown in Table VII and are represented graphically in Figure 3. The dotted portion of the Duchess curve in Figure 3 indicates that these killing points could not be accurately determined, owing to the difficulty of

maintaining constant temperatures below —41° C. for three hours. It is certain, however, that its killing point lay somewhere below —41° C. from Jan. 3 to March 5.

TABLE VII
Killing Points and Moisture Content of Twigs of Duchess and Jonathan Apple Trees at Different Seasons

| Date	Variety	Killing point	Moisture	Condition of trees and remarks
		deg. C.	per cent	
Oct. 18, 1924	Duchess	—25	48.96	Leaves falling
	Jonathan	—15	49.84	Foliage green, no abscission
Nov. 5, 1924	Duchess	—30	....	Leaves mostly down
	Jonathan	—20	....	Leaves killed by frost
Dec. 4, 1924	Duchess	—35	48.27	No low temperature yet
	Jonathan	—30	46.46	do
Jan. 3, 1925	Duchess	Below —41	47.23	Very cold for 3 weeks
	Jonathan	—41	47.66	do
Feb. 13, 1925	Duchess	Below —41	47.66	Moderate temperature
	Jonathan	—41	45.83	do
March 5, 1925	Duchess	Below —41	....	Warmer, no bud activity
	Jonathan	—40	....	do
March 26, 1925	Duchess	—40	47.09	Buds swelling slightly
	Jonathan	—36	45.79	do
April 10, 1925	Duchess	—30	47.00	Buds opening
	Jonathan	—25	46.15	do
May 10, 1925	Duchess	— 6	51.87	Beginning to bloom
	Jonathan	— 6	52.26	do
July 7, 1925	Duchess	— 3	65.45	In active growth
	Jonathan	— 3	54.30	do
Aug. 22, 1925	Duchess	....	49.29	Terminal bud set
	Jonathan	....	52.96	Terminals still growing
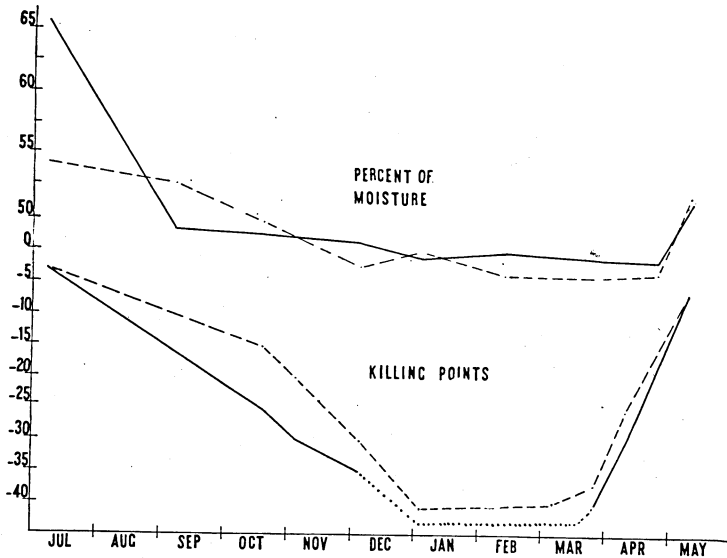
Fig. 3. Seasonal Variations in Killing Point and Variations in Moisture
The lower curves indicate the seasonal variations in killing point. The upper curves show the variation in moisture throughout the year. Solid lines represent Duchess and broken lines, Jonathan.

TABLE VIII

Chemical Constituents of Twigs of Hardy and Tender Apple Varieties at Various Times of the Year

Date	Variety	Dry matter	Carbohydrates					Nitrogenous material		
			I Reducing sugars as glucose dry wt.	II Total sugars as glucose dry wt.	III Pento-sans dry wt.	IV Acid hydrolyzable alcohol insoluble as glucose	V Sum of II and IV	Total organic N. dry wt.	Amino nitrogen dry wt.	Amino N. as per cent organic nitrogen
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Oct. 18, 1924	Duchess	51.04	1.05	1.84	15.79	30.47	31.95	0.97	0.109	11.23
	Jonathan	50.16	1.03	2.34	14.33	26.78	28.18	0.84	0.089	10.59
Dec. 4, 1924	Duchess	51.73	3.12	6.26	15.10	26.83	33.09	0.97	0.135	13.91
	Jonathan	53.54	2.89	6.00	15.69	25.66	32.66	0.82	0.130	15.85
Jan. 3, 1925	Duchess	52.77	3.25	5.85	19.10	26.77	32.62	0.98	0.142	14.48
	Jonathan	52.34	4.03	6.66	18.92	25.23	31.89	0.80	0.127	15.87
March 26, 1925	Duchess	52.91	1.67	4.23	17.51	26.54	29.77	0.98	0.128	13.06
	Jonathan	54.21	1.60	3.17	19.10	26.41	29.58	0.76	0.146	19.21
April 10, 1925	Duchess	53.00	2.26	3.04	18.82	26.16	29.20	1.10	0.158	14.36
	Jonathan	53.85	2.19	2.76	18.51	24.75	27.51	0.97	0.156	16.08
May 10, 1925	Duchess	48.13	1.46	3.07	20.64	22.39	25.46	0.71	0.195	27.47
	Jonathan	47.74	1.29	2.34	20.70	23.50	25.84	0.82	0.198	24.14
July 7, 1925	Duchess	34.55	1.77	2.61	17.77	24.15	26.76	0.99	0.228	23.03
	Jonathan	45.70	1.35	1.06	15.09	18.48	19.54	0.85	0.149	17.52

Marked variations occurred in killing points of twigs at different seasons. During summer apple shoots in active growth are no more cold resistant than many herbaceous annuals. The hardening-off process begins early in the fall and proceeds gradually until maximum hardiness is attained at about the coldest period of the year. Winkler (75) noted similar tendencies in a number of plant species, and concluded that cold itself produces an adaptation to lower temperatures.

Hardiness, once acquired, is retained until external conditions are suitable for the resumption of growth actively. The rest period of apple trees grown outside in this climate is well over by the middle of January; and if brought into the greenhouse, shoots begin to grow at once and simultaneously lose their hardiness. Consequently, it seems to be lack of conditions favorable to growth and not rest period that determines the length of the hardy period of our northern apples. Hardiness is lost much more quickly in the spring than it is acquired during the fall, and one-year-old twigs in May show little more cold resistance than the succulent summer growth. These results, in general, are in accord with previous investigations on killing points at different times of the year (12, 75). Winkler's (75) killing-point curve plotted for *Populus nigra* agrees in general with that of the apple shown in Figure 3. However, owing perhaps to the shorter winter in Germany, he found the loss of hardiness to begin much earlier than is the case with Minnesota apple trees. A study of these curves shows why a sudden drop to a low temperature in the fall or early winter may be fatal while, later, trees stand much lower temperatures without injury.

Considerable difference in lethal temperatures for the two varieties is evident. In the summer condition there is no difference in the killing points. In the fall, however, the curve for Duchess quickly drops below that of Jonathan, an expression no doubt of earlier maturity. This same relative position of killing points is maintained throughout the winter, Jonathan killing at about $-40°$ C. while Duchess endures lower temperatures. In spring, both varieties lose hardiness at about the same rate, but the Jonathan, having less hardiness to begin with, continues to kill at a higher temperature than Duchess until active growth is resumed in May, when both varieties again have practically the same killing point.

MOISTURE

The moisture determinations made on twigs at various times throughout the year offer little possibility for distinguishing hardy and tender varieties. The moisture content was highest in summer, decreasing toward winter and rising again rapidly in spring with the resumption of growth (Fig. 3). During winter, the moisture content

of the Duchess remained practically constant, while the Jonathan showed a tendency to fluctuate more.   Strausbaugh (68) noted a similar tendency toward variation in moisture of tender plum buds during winter, and Newton (51) finds similar relations in tender wheat varieties. The markedly higher moisture content of Duchess in the July sample is somewhat surprising.   It is probably accounted for by the fact that the Jonathan had made almost twice as much longitudinal growth as the Duchess at this time, and consequently had a much greater amount of hard wood in proportion to the succulent tips than the Duchess. The crossing and recrossing of the moisture curves of the two varieties during winter indicates that the relation between low moisture content and hardiness reported by previous investigators (34, 66, 68) is by no means constant in the apple.

CARBOHYDRATES

The results of carbohydrate analysis are shown graphically in Figure 4.   By adding the values for total sugars (curve II) to those for the acid hydrolyzable, alcohol insoluble carbohydrates (curve IV), the data plotted in curve V were obtained.   These values include the carbohydrates other than the fibrous material and probably represent somewhere near the sum total of carbohydrate reserves in the twigs.

It is evident that carbohydrates, presumably of a reserve nature, are abundant in apple shoots, amounting at certain times of the year to approximately a third of the dry weight.

The curves show the highest amounts present at the sampling of December 4.   Why a maximum should occur at this time is difficult to understand.   It seems improbable that there is translocation of carbohydrates from other parts of the tree to the terminal shoots at this season, for these shoots only a short time before were the seat of great photosynthetic activity.   The most likely explanation is that a higher point than that shown in the curves was reached considerably earlier, probably early in November, when the leaves were killed by frost, altho no analyses were made at this time.

The quantity of carbohydrate reserve falls off gradually throughout the winter and spring, owing, probably, to respiration, altho some may possibly be combined in protoplasmic transformations during the hardening-off process earlier in the winter.

The hardier Duchess had a higher carbohydrate reserve throughout the year, altho during the colder parts of the winter this difference was not great.   The Duchess seems to build up its reserve earlier in the fall while the Jonathan is presumably still using its carbohydrates in late growth.   (Table VII.)

Total sugars show a rapid increase from fall to winter, which is in keeping with the results of previous investigations (23, 63). Toward spring the sugars fall off much like the total reserve carbohydrates. Reducing sugars follow the same general trend as total sugars except that in spring just before the buds open there is a rapid increase, followed by a rapid decline as growth progresses.

The sugar reserves in the two varieties are strikingly similar throughout the year. There is nothing in the data to suggest that the amount of either total or reducing sugars is responsible for the difference in hardiness. In fact, the Jonathan showed slightly higher amounts of both total and reducing sugars in January, altho it would not withstand as low temperatures as the Duchess at this time. The value of sugars in protecting proteins from precipitation on freezing has been thoroly demonstrated in vitro (51, 63). However, there are many plants with very high sugar content (for example, sugar cane and sugar beet) with practically no cold resistance. It might be argued that these plants in process of growth store sugar naturally and do not develop it as a result of exposure to low temperatures. On the other hand, the potato tuber, which normally stores starch, on exposure to low temperatures will show sugar increases greatly in excess of the amount found in apple twigs (3). Apelt (2), however, was able to detect only about half a degree difference in killing point between non-hardened potato tubers and those fully hardened by exposure to low temperatures. Fischer (23) has demonstrated that in woody plants starch is transformed into sugar on exposure to cold, and that with higher temperature starch is again formed. Coville (13) has explained the increase of sugars following low temperatures as due to an increase in the permeability to enzymes, allowing the hydrolytic enzymes to come into contact with the starch. This explanation does not, however, account for the regeneration of starch at higher temperatures noted by Fischer (23).

The increase of sugars on exposure to low temperature, reported by many investigators, leaves little doubt that this phenomenon is general in starch-storing plants, and from the results of experiments in vitro (63, 51) we may conclude that sugars exert a protective action against precipitation of proteins on freezing. However, in view of the present results on the apple and the findings of Apelt (2) and Newton (51), it seems that high sugar content resulting from action of low temperatures may not necessarily result in greater cold resistance in plants. As to increased hardiness without accompanying sugar increase, Harvey (29) was able to develop cold resistance in cabbage plants without appreciable changes in carbohydrates. The lack of carbohydrates in the hardy meristematic portion of roots, as observed by

Zacharowa (77), also indicates that cold resistance may be independent of sugar content.

PENTOSANS

The data on pentosans show some interesting variations with the different seasons (Fig. 4). There is, in general, a decrease in the percentage of pentosans from summer to early winter, followed by a sharp rise to the first of January and a second rise to a maximum in May. There is probably not an actual loss of pentosans from the twig during the late summer and autumn, but the proportion is lowered, owing to the increase of other carbohydrates which occurs at this time. Conversely, in the spring the high content of pentosans may be attributed to a utilization of the more available carbohydrates, resulting in an increased proportion of pentosans, as total sugars and total reserve carbohydrates both decrease during this period. No explanation is offered for the rise in pentosan content in early winter.
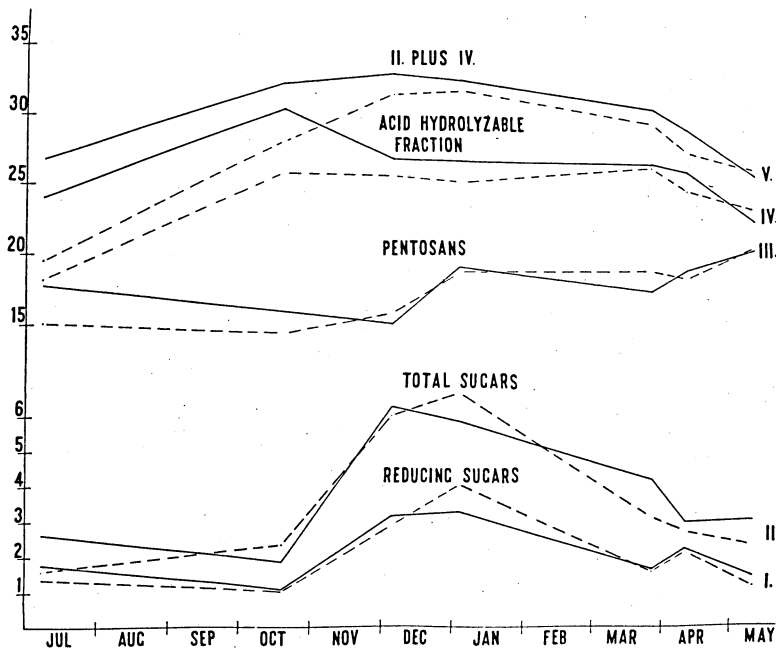

Fig. 4.   Seasonal Fluctuation in Carbohydrates of Apple Twigs
Solid lines represent Duchess and broken lines, Jonathan.
Data for curve V were computed by combining the values for total sugars (Curve II) and acid hydrolyzable, alcohol insoluble carbohydrates (Curve IV).

This increase in pentosans toward winter agrees with the results of Rosa (58), Hooker (31), and Newton (51). The significance attached to pentosans in relation to hardiness by Hooker (31) and Rosa (58) has already been mentioned. De Long (16), however, failed to

substantiate Hooker's (31) results on apple varieties, and McGinty (44) has recently presented data that place Rosa's (58) results in question. Newton (51) was unable to distinguish hardy and tender wheat varieties by their pentosan content.

From the results obtained with apple shoots, there is no evidence of a relation between pentosans and cold resistance. If hardiness in the apple were dependent upon total pentosan content, we should expect apple trees to be hardiest in May and least hardy in the autumn. Between hardy and tender varieties, there is little difference in pentosan content. The Duchess variety showed somewhat higher pentosan content during the growing season, when both were equally tender. However, in the cold period of the year there is no significant difference. This is in complete agreement with determinations of De Long (16) on a number of apple varieties, as shown in the following table adapted from his data. Varieties are listed in descending order of hardiness.

Variety	Pentosans per cent
Duchess	19.09
Crimson Beauty	20.90
McIntosh	20.81
Wealthy	19.37
Fameuse	20.28
Wagener	21.49
Baldwin	19.76
Gravenstein	21.91

It is evident from these results that the hardiness of a variety of apples is not determined by the total quantity of pentosans present in the twigs.

NITROGENOUS MATERIALS

The nitrogen reserve in apple shoots is quite low and the amino-nitrogen content is extremely small. The most notable thing about the organic nitrogen content is its constancy throughout the greater part of the year, as seen in Figure 5. The greatest fluctuation occurs in spring, when there is a sudden rise about the time buds are swelling. The drop in the curves from April to May is probably accounted for in part by the fact that the nitrogen had passed outward from the twigs to the leaves, and to the growing points in the developing shoots. As these parts represented another season's growth than the one studied through the winter, they were removed at the time of collection, and are not included in the analyses.

The percentage of organic nitrogen is appreciably higher in the hardy Duchess variety than in the Jonathan. Whether this is merely a varietal peculiarity or whether it has some relation to the ability to resist low temperatures remains to be determined.

The amino nitrogen is most abundant during periods of vegetative activity.   This is as might be expected, as the amino is the translocation form of organic nitrogen.   There is, however, an increase in amino nitrogen during the hardening-off period in the fall and early winter.   These results are in agreement with Harvey's (29) work on cabbage and with Newton's (51) analyses of wheat plants.
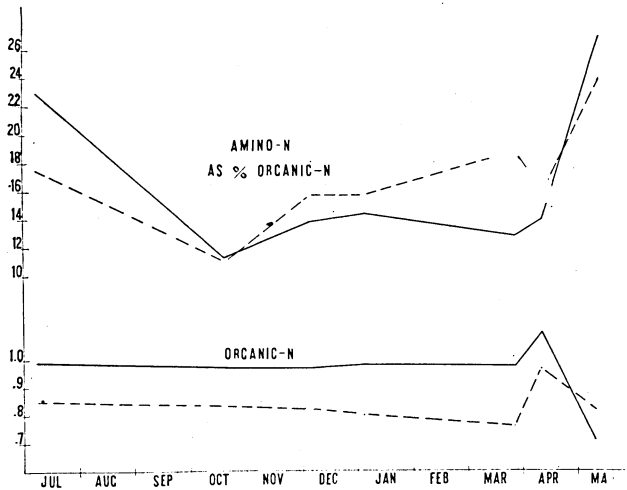

Fig. 5.   Seasonal Variations in Nitrogenous Materials in Apple Twigs
The lower curves show total organic nitrogen and the upper curves show amino nitrogen expressed in per cent of total organic nitrogen.   Solid lines represent Duchess and broken lines, Jonathan.

Schaffnit (63) and Harvey (29) have suggested that as a means of adaptation to lower temperatures the proteins themselves might break down to simpler and less labile forms.   Harvey (29) found evidences of such transformation in the increased amino-nitrogen content of hardened cabbage plants.

If such hydrolytic processes occur in apple twigs, they can best be indicated by expressing the amino nitrogen in terms of percentage of total organic nitrogen.   In Figure 4, these data are shown graphically for the two varieties of apples.   There is a rather sudden increase in the proportion of amino nitrogen in both varieties from October to December.   That this is the result of hydrolysis and not of synthesis, is indicated by the constancy in the amount of organic nitrogen during this period.   The highest points in the curves, however, come at times when the trees are least hardy, consequently a large part of the organic nitrogen in the amino form is no assurance against cold injury.   Comparing the curves of amino nitrogen with the killing curves of the two varieties, it is seen that there is no constant relationship.   The

Jonathan has relatively less amino nitrogen in the fall and more in the winter and early spring, altho its killing points are uniformly higher than those of the Duchess.

CONCLUSIONS

Considering the seasonal variations in killing points together with the differences in hardiness of the two varieties used in these studies, it seems that neither moisture, sugar, pentosans, nor amino nitrogen can be considered as a primary casual factor in cold resistance. From the data on carbohydrates and total organic nitrogen, it appears that the hardy variety is better supplied with these important reserve materials than the tender one. The difference is particularly striking in the case of the nitrogen reserve. Whether this relation holds between hardy and tender varieties in general remains to be determined. If so, the whole problem of ability to resist cold may be placed definitely on a nutritional basis. Certainly there are indications that would support such an assumption. Such conditions as late growth, defoliation, over-bearing, are generally recognized as predisposing factors to winter injury. It is quite evident that these conditions would all tend to influence the amount of reserve materials accumulated.

What a survey of many varieties and species might show as to the relationship between cold resistance and abundant reserves of carbohydrates and nitrogenous materials we can only conjecture. However, the differences here noted offer an interesting hypothesis for further hardiness studies.

SUMMARY

1. Terminal growths of apple varieties of known hardiness have been exposed to artificially produced low temperatures. Classifying these varieties on the basis of the resultant injury, the order agrees closely with that of their hardiness as shown by field experience. The use of this test in determining hardiness of seedlings and varieties of unknown cold resistance seems practical.

2. Degree of cold injury is directly proportional to the duration of exposure to a given low temperature.

3. A rapid fall in temperature above the killing point is more injurious than gradual lowering.

4. When twigs frozen to $-35°$ C. were thawed by immersing in warm mercury or in running tap water, the injury was much greater than when they were thawed slowly.

5. Terminal growths of apple trees cut off and exposed for two and a half months during the coldest part of winter without moisture supply, lost 17.4 per cent of their weight but the buds opened normally when given proper conditions. Another sample lost 29.3 per

cent of its weight but showed no characteristic symptoms of winter injury. The importance of desiccation as a factor in winter killing is questioned.

6. Killing point determinations made on hardy and tender apple varieties showed great variation from season to season. Except during the growing season, the hardy variety was uniformly more resistant to cold.

7. Neither moisture content, sugars, pentosans, nor amino-nitrogen, offered a reliable basis for separating hardy and tender varieties. There was some indication that high reserves of carbohydrates and of organic nitrogen might be correlated with greater hardiness.

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