HARDINESS STUDIES WITH CLONAL SELECTIONS OF FRENCH CRAB APPLE SEEDLINGS

by

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INTRODUCTION

Man is ever pushing back the barriers impinging on the successful extension of the range of crop plants. And no such extension is more vigorously pushed nor has been the subject of research for any longer period of time than that with perennial fruit crops. It is this nature of relative permanence which has primarily made that statement a fact.

Probably coincident with, for a good many years, and antecedent to the selection and development of hardy intermediate stocks and rootstocks, by a few years, has been work on varieties of fruits combining satisfactory use characteristics with outstanding growth qualities and hardiness and disease resistance. But the difficulty of obtaining all such qualities in at least a comparative degree has led to the search for and use of intermediate stocks and rootstocks possessing various desirable attributes, especially since the most frequent and most serious type of winter injury is on the trunk and about the crotches of the scaffold branches.

A method was developed at Kansas State College by Filinger and Cardwell (1941) for the direct measurement of electrical resistance in firm stems and twigs, and the application of drop in resistance values to a determination of killing in these plant materials. Raspberry canes were the experimental plant parts used and killing was accomplished both by boiling some samples and by plunging others into liquid air. The reduction was slightly greater after boiling in each instance.

The experiments reported in this thesis are an extension of work done by Doctors Filinger and Cardwell in the following two respects:

1. A determination of whether intermediate temperature treatments below freezing could be translated into intermediate percentage reduction in resistance and, therefore, whether partial injury could be demonstrated.

2. Utilization of differential reductions in resistance at a particular temperature treatment to evaluate hardiness of the clones of the K series French Crab as under stocks and intermediate stocks.

REVIEW OF LITERATURE

Oskamp (1918) noted injury on peaches and apples in Indiana following the hard winter of 1917-1918. He charges its occurrence to the late maturity of these parts of the tree relative to the remainder above ground.

Hibernal and Virginia Crab as intermediate stocks largely circumvent this difficulty, though as reported by Maney (1938) and Maney and Plagge (1935), there are a number of varieties with which one or the other will not unite or unite only poorly, and, furthermore, there are variations in size at maturity of varieties grafted on the latter. Too, performance varies locally, as, for instance, in the susceptibility of Virginia Crab to fire blight and of Hibernal to sun-scald as reported by

Leslie in Manitoba and quoted by Gourley and Howlett (1941, p. 486).

However, as Maney (1943) points out, Virginia Crab and Hibernal are superior stocks relative to the objectives of the Iowa Agricultural Experiment Station: (1) Vigorousness, (2) Hardiness, (3) High production, (4) Resistance to various tree disorders, and (5) Production of standard sized trees. These, with slight variations, are well-adapted for the care of any breeding or selection programs. But, though the universal objectives, meeting them in one locality does not insure that they will be met in like degree elsewhere, as has been shown above.

French Crab has been a satisfactory understock as concerns the vigor of the scions growing upon it, withal that it is lacking in a number of the other qualities mentioned previously. Considering the diversity of its parentage, varieties grafted to it show surprising uniformity in growth. Yerkes, Sudds, and Clarke (1938) found them only slightly greater in variability than similar varieties on clonal stocks. This is, as Gourley and Howlett (1941, p. 481) suggest, very likely due to the unintentional selection which goes on in every step of nursery practice from the time of selecting the best of a mixture of seeds for planting.

Considering the undoubted heterozygosity of French Crab seedlings, the difficulty in evaluating individual hardiness factors in a plant and the lack of knowledge of how many fruit characters are inherited, makes the selection of seedlings of this sort for hardiness, as well as for other attributes, fraught with potentialities.

Selections of French Crab seedlings were made and are growing in the Kansas State Orchard row and are being studied for compatibility, structural strength, precocity of bearing, blight resistance, and hardiness. These seedlings have been designated as the K-series. This is necessarily a long process in all stock studies on the points of compatibility, precocity of bearing, and hardiness. But, the testing for hardiness is especially haphazard under natural conditions and years are required for a fair test. This is because slight amounts of injury in the less rigorous winters may go unnoticed, or be easily mistaken for some other disorder. Furthermore, injury under a given set of conditions may not be indicative of what will occur under others, so that to assign a hardiness rating which will be satisfactory over a wide range of conditions requires years of observation under natural conditions.

Artificial freezing has offered a means of speeding up the process of testing, provided it gives results comparable to those observed in the field. Edgerton (1950) has found that such experiments simulating a given set of conditions in the winter outdoors provide an entirely reliable guide to the cold weather performance of the buds of fruit plants. Observation of the same killing of parenchyma cells and the closing of vessels by wound gum in artificial freezing studies as under natural conditions was noted by Waring and Hilborn (1937).

Artificial freezing methods have been employed for some years in hardiness studies - even before the advent of electrical refrigeration. But all such experiments have depended upon visual detection of damage to tissues or observation of subsequent growth to assess the results of the experiment.

Tests of the electrical conductivity of plant material were begun in 1897 by Stewart and by the coworkers Bugarsky and Tangl, according to Miller (1938, p. 99). Later (about 1912 to 1918) Osterhout used the method extensively to study the resistance of Laminaria to the passage of an electrical current after treatment with salt solutions of differing concentrations. And, although he did not work with temperature effects upon resistance, he postulated a theory explaining his data and suggested that temperature would produce like results (Osterhout, 1921).

Controversy has existed over the validity of conductivity or resistance as a measure of changes in permeability and a question whether, if they are valid measures, this can be used as a test of hardiness.

The work of Dexter, Tottingham, and Graber (1930) appears to mark the beginning of expanded use and more general acceptance of electrical methods in hardiness studies, with conductivity of exosmosed solutions being the measure of injury and, later, direct measurements on the tissue.

Swingle (1932) adapted the method of the above workers to the study of rootstocks, but did not accept it as applicable to all studies of hardiness. He pointed out that the studies in

1930 were with hard materials, yet he checked his results by the "freeze and wait" visual method, as he called it, in his care to insure accuracy.

More lately Stuart (1939) has used Swingle's method in a study of the hardiness of more than 50 varieties of apple roots. His results checked quite well with ratings of relative hardiness as gained by observations over a great period of years.

MATERIALS AND METHODS

Experiment with Detached One-year-old Twigs

November 20, 1949, samples of one-year-old wood, representing 20 clones of the K series, were collected from the experimental block at the Kansas State College horticultural farm. Each clonal sample of 15 twigs represented five specimens, of varying diameter, from the west side of each of three trees, except K12 and K38, of which only one tree of each variety was available for sampling.

In the former of these two exceptions, five samples were taken from the southwest side of the tree, five from the northwest, and five from the northeast. One from each side was used for a treatment. One treatment only was used on the five samples taken at random from the single tree representing the other clone.

After binding and labeling, these bundles of 7-inch long twigs were placed immediately in moist peat moss and the moss covered with damp burlap. In this environment they were placed in cold storage at a temperature of approximately 40 degrees F. and checked for condition weekly until used.

Prior to being cut and placed in cold storage, the lowest outdoor temperature to which these twigs had been subjected was 16° F.

Subsequent to the collecting of the K series twigs, it was decided to include several of the Malling stocks in the tests. These were collected December 2, 1949 in the same manner as the earlier samples. However, these trees were all much smaller, so some changes in the system for taking samples were necessary clone by clone. These are described in the notes of Table 1. Even where three trees were available for taking twig specimens, it was not possible to localize the collection to one side of the tree. And in some cases, terminal growth was not sufficient to permit taking 7-inch-long samples. All such deviations are also described in the footnotes mentioned above.

A final group of material consisted of Yellow Siberian and Manchu Crab twigs received from the South Dakota Experiment Station for propagation at the horticultural farm. Twigs remaining after sufficient had been set aside for propagation were utilized in this project. Handling subsequent to receipt of the material was identical with that of the other twigs.

The freezer used in this experiment was a war-surplus box bearing the brand name of "Kold-Hold." Freezing compartment volume was approximately 8 cubic feet. Radiation effects were reduced by enameling the walls white and providing a surface of corrugated cardboard to support the twigs 4 inches above the bottom of the freezing compartment. A Bristol Thermograph provided a record of temperature fluctuations and an accurate Centigrade thermometer placed on the cardboard platform beside the thermocouple served as a check on the thermograph. No circulation of air was effected in the box, but twigs were spread in a layer one-half inch high or less on the supporting surface to minimize temperature stratification influence.

Resistance, the measure of the condition of the twigs, was determined with a Boyoucous Model C Moisture Bridge manufactured by the Wood and Metal Products Company. This model employs a 1000 cycle alternating current and uses a set of earphones to detect the null point.

Contact on the twigs was made by a device designed in the Kansas State Physics Department. It consisted of two pairs of electrician's pliers having parallel-acting jaws and spaced at near 6 inches by a fiber spacing bar. Extensions were fastened to the plier jaws to give greater capacity and it was through these extensions that portions of steel needles were inserted. This device is pictured attached to the stem of one of the K series in Plate I. The Boyoucous apparatus is pictured in its relationship to the contacts in the same plate. As used in these experiments, whenever it was necessary to rest the contact device on a surface, the same fiber clip board was used, since preliminary work showed variation in resistance with the material on which the contrivance was placed. Of several tested,

EXPLANATION OF PLATE I

A. Potted tree with contacts inserted for measuring resistance.

B. Boyoucous Model C Moisture Bridge with headphones for detecting null point.

PLATE I

A B

conductance was least through the clip board.

Additional apparatus and materials used consisted of a large bell jar beneath which twigs were placed between resistance readings and several polyethylene refrigerator bags in which all twigs were kept constantly after removal from cold storage. A small inside and outside caliper graduated in 32nds was used for diameter measurements on the twigs.

Just prior to the freezing of a particular group of twigs at one of the treatment temperatures selected, a composite sample of one twig picked at random from the group from each of the three trees sampled was made up in the cold storage room and each twig labeled as to clone number and specimen number. This latter corresponded to the tree from which the twig was taken.

When the freezer had been adjusted for the temperature of treatment, the twigs to be frozen at that particular temperature were removed from keeping storage in polyethylene bags and the resistance immediately determined. In making the resistance readings, each twig was removed from one bag and placed promptly in another following the location of the null point. Largely, this was a matter of two minutes.

The duration of the freezing period in every instance was 24 hours, except with the treatment with liquid air, in which groups of twigs were kept until boiling ceased. Twigs were retained in the polyethylene bags while frozen in the former instances, to minimize moisture loss, but were spread out as previously described.

At the termination of the 24-hour period, the material was placed, still in the moisture-proof bags, beneath a bell jar in a saturated atmosphere. Two hours later the first post-freezing readings were taken, but thawing was not always complete at this time. This was especially true of the samples frozen in liquid air and those subjected to -35° C. In these instances readings were taken at one-minute intervals on the first twig until the results became stable. By that time, it was found, all other twigs had completely thawed and determinations were taken on the entire group. In neither case did the thawing time reach three hours.

Since, in preliminary probing, mold growth was found to occur on plant material kept under experimental post-treatment conditions, bags and twigs were liberally dusted with wettable dusting sulfur from one to two times following the taking of the first resistance data. Tips of twigs were also dipped in the dust. Fungal growth was not completely inhibited, however. Hence, data subsequent to the first determinations are presented, but may not be valid.

Following the first post-freezing readings, two additional checks of resistance were made -- the first of these being approximately 19 hours after the initial determination following removal from the freezer and the second about 48 hours later. At some nonuniform time between the first and last readings of resistance of treated twigs, each was calipered at the point at which it was pierced by the contacts and the diameter recorded in 32nds of an inch.

Five treatments were utilized in this experiment: -18° C., -25° C., -30° C., -35° C., and freezing in liquid air. The last was selected as a measure of maximum damage with least change in the chemical state of the colloidal systems of the cells. Minus 35° C. was selected as the lowest temperature achievable with the freezing apparatus on hand and low enough that, theoretically, an upward gradation of "percentage resistance drops" might be obtained. The remaining treatment temperatures were arbitrarily selected as gradations upward to the approximate centigrade equivalent of 0° F.

Experiment with One-year-old Twigs of Potted Two-year-old Trees

Fourteen clones were represented in the 67 two-year-old trees dug from the horticultural farm nursery row on September 28, 1949 and heeled-in in the greenhouse before any freeze or frost had been experienced. Only in clones K5, K3, and K20 were there but four trees. These clones were not own-rooted, but had been grafted on French Crab seedling roots for facility in propagation.

The trees, when dug, showed great variability in root system type and these differences seemed to be, in the main, intraclonal. This clonal characteristic was translated into differential clonal survival by the time the trees were subjected to the freezing treatment. In clones K5, K17, and K20, mortality was sufficiently high that they were not included in the experiment.

Damp burlap was on hand and the roots were immediately wrapped in this material after digging.

Six weeks after heeling-in, when 10-inch clay pots were available, the trees were transplanted into a sandy silt loam similar to that in which they had been growing in the nursery.

From the time of placing in the pots, difficulty was experienced in maintaining the moisture content, so, during the period December 4 to December 10, 1949, all containers were plunged in the greenhouse soil with an east to west alignment by clones. This arrangement is shown in Plate II.

Moisture control in the pots was considered a necessity in order that the moisture content of all plant material might be at approximately the same level at the time of treatment. Preliminary work with Cornus racemosa had shown that a 0.35 inch rainfall occurring within five and one-half hours of measuring resistance was detectable in a 40,000 to 120,000 ohm drop in resistance. This observation was recorded in February when there had been no precipitation for two weeks prior to the test and then only 0.2 inch as snow.

Control was effected by the method of Boyoucous and Mick (1940), utilizing the same resistance bridge as was used for measurement of resistance through twigs. Standard plaster of paris blocks containing electrodes, which were manufactured by the maker of the bridge, were embedded in the soil of each pot five inches below the rim and midway between the axis of the

EXPLANATION OF PLATE II

Pots containing trees of 14 unhardened clones plunged in greenhouse soil with alignment from side to side of plate.

PLATE II

plant and the side of the container. This arrangement with the connection to the moisture bridge is illustrated in Plate III.

Just prior to a run with a representative of each of the clones used, the moisture level in the pots was stabilized at as close to 500 ohms as possible. This is the median of the range which Boyoucous and Mick (1940) state represents the limits of resistance for all soils at field capacity. Accurate control was difficult, since there appeared to be inter-pot movements where heavy additions of water were made and also no tendency for resistance to be lowered at all if the addition of water was not equal to a given minimum at a given resistance reading above 500 ohms. Stated otherwise, it appeared that a given amount of water did not uniformly moisten the pot to its 10-inch depth but brought a given upper stratum to a particular moisture level before a lower stratum was moistened at all.

The final pre-freezing moisture estimation was made the morning of a treatment, at which time a section of a one-year-old twig, as near 6/32nds by 7/32nds inch in diameter as possible at a distance of six inches apart, was selected for the resistance measurement area. A white tag delimited this section. In the event that no such section was available, the portion of greatest diameter on a one-year-old twig was chosen. Resistance was read immediately on these six-inch segments.

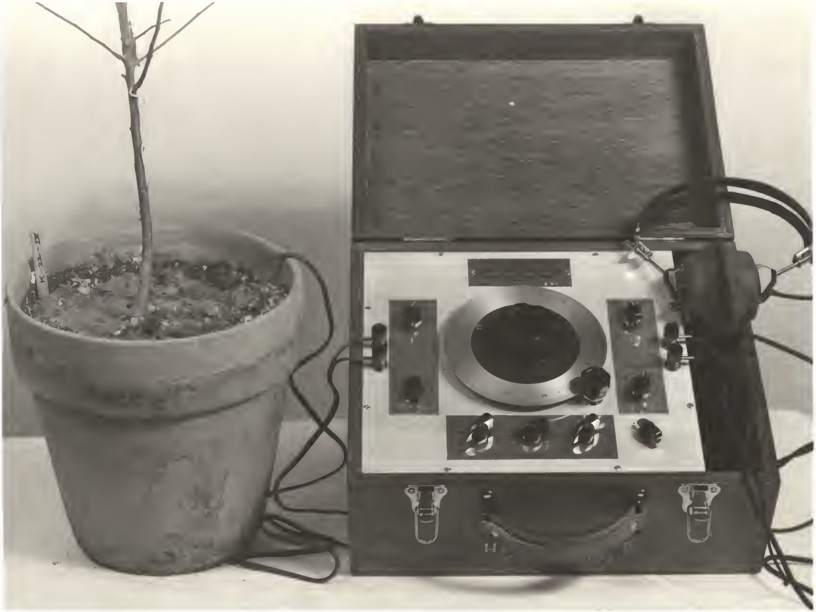
Following removal to the building housing the freezer (a walk-in blast cooler operating at about -20° C., in this case), each pot was surrounded by a minimum of three inches of sawdust.

EXPLANATION OF PLATE III

A. Potted two-year-old tree of K series showing wire lead to plaster of paris block buried in soil.

B. Boyoucous Moden C Moisture Bridge.

PLATE III



A B

All was contained in a standard bushel basket and a split burlap bag covered the sawdust to prevent blowing in the moving air within the cooler. This amount of insulation was calculated to be sufficient to prevent the soil in the pots from freezing for six hours at a temperature in the box an adequate margin, since the freezing time was three hours. A centigrade thermometer inserted through the sawdust to the soil line and checked at half-hourly intervals (as was a like thermometer suspended from a twig of a tree in the center of the group) did not fall below $+10^{o}$ C. in any case.

Returned to the greenhouse, resistance was checked two hours after removal from the freezing unit and daily thereafter for the first week. Subsequently a weekly determination was made until resistance figures were well above normal resistances for the group.

With the first run only, plaster of paris blocks were removed from the pots immediately after treatment and water added as the soil appeared dry until it was realized that, even in the injured state of the young trees, resistance readings were fluctuating with moisture changes in the soil. The blocks were re-embedded. Moisture determinations were taken daily thereafter and moisture content corrected to keep resistance between the buried electrodes at about 500 ohms on this and the remaining runs, until the experiment was discontinued.

Six weeks following the experimental treatment of each run, all plants in the group had succumbed. Before discarding the plants, a record was made of the linear stem distance above the level to which sawdust had been packed at which growth had occurred. Note was also made of the distance from the tip of a twig to the uppermost contact point.

PRESENTATION OF DATA

Experiment with Detached One-year-old Twigs

Basic data pertinent to this phase are presented in Table 1. Therein should be noted the increasing percentage drop in resistance with decrease in the temperature of treatment. This is true with but two exceptions -- K19 at treatment temperatures of -25° C. and -30° C. and K12 at the same freezing temperatures. The former is rather insignificant, but the latter is more marked and difficult to explain, especially since all twigs in this particular clone came from the same tree. However, there was a consistency in that, in each treatment, one twig came from each of three different sides of the tree.

The standard deviation here, though above the mean standard deviation for the treatment, is not the highest in the group by six clones. Thus, the upsetting of the order cannot be attributed to extreme variability in response.

The average percentage drop in resistance at the treatment temperature -30° C. is more nearly comparable to other averages at the temperature than is the drop in resistance of K12 at -25° C. to the responses of the other clones at that temperature. This would strongly suggest that the disorder were in

Table 1. Average clonal or varietal percentage drop in resistance at various treatment temperatures.

Clone or variety	Treatment temperatures -18° C.	-25° C.	-30° C.	-35° C.	Liquid air
K1 1 yr. wood		53.4		61.7	85.4[2]
K1 2 yr. wood	11.1	44.3	57.0	69.1	88.4
K2	14.2	37.0	68.4	76.7	86.2
K3	14.7	48.8	55.0	58.5	82.0
K4	18.3	51.8	62.8	76.1	88.0
K5	35.1	44.4	60.5	73.1	90.2
K12[3]	18.5	63.0	56.1	69.4	81.0
K16	24.9	40.7	59.8	71.6	79.8
K17	18.0	47.6	63.2	71.7	87.2
K18	25.7	58.9	63.5	77.1	86.5
K19	28.2	52.5	50.7	63.1	79.8
K20	35.1	58.7	69.2	73.8	84.2
K21	9.8	57.6	66.9	74.5	79.7
K22	13.9	42.3	59.2	72.0	81.6
K23	27.7	36.8	70.1	80.8	83.7
K29	12.2	58.0	65.4	78.3	83.6
K33	15.9	57.9	66.5	73.6	83.5
K38		48.2[4]			
K43	17.7	53.6	62.2	73.7	79.1
K48	0.7	40.4	56.7	63.6	77.1
K56	9.4	41.0	59.0	73.7	82.4
M IV[5]	7.6[2]	36.6			73.8

Table 1. (concl.).

Clone or variety	Treatment temperatures -18° C.	-25° C.	-30° C.	-35° C.	Liquid air
M IX	8.0	46.2	59.0[6]	65.1	77.1
M XIII	8.7	30.1	66.7	76.2	83.5
Yellow Siberian[7]		28.4			68.6
Manchu Crab[7]		29.9		38.9	63.5[8]

[1] Three determinations, each from a different tree of the same clone or variety, averaged, unless otherwise indicated.

[2] Average of resistance drop of four twigs.

[3] All samples from same tree but one each from NE side, NW side, and SW side for each treatment.

[4] Average of determinations on five twigs, all from the same tree.

[5] One sample from E side and two from W in liquid air treatment; two from W and two from E in -18° C. treatment, and two from E and one from W side of same tree in -25° C. treatment.

[6] One twig shorter than others. Contact points 4 5/8 inches apart.

[7] Material from South Dakota.

[8] Contact lengths all less than six inches. One percentage used in calculating average obtained on second resistance reading after freezing because of tendency of first post-freezing reading to "drift" upward.

this latter bracket.

A study of the values calculated for the individual twigs in this treatment shows those from the NW and SW sides to be disproportionately high (Table 2) and, in fact, the twigs from the NW side of this specimen of K12 consistently show a percentage fall in resistance greater than the treatment mean with

Table 2. Individual percentage resistance drop and standard deviation by clone and treatment.

Clone	Tree sample	-18° C.	Std. dev.	-25° C.	Std. dev.	-30° C.	Std. dev.	-35° C.	Std. dev.	Liquid air	Std. dev.
		Percentage drop in resistance (Detached twigs)									
K1	#1			44.6)				60.7)		82.0)	
1 yr.	#2			63.6)	9.8			60.0)	2.3	87.5)	3.2
wood	#3			52.0)				64.4)		87.8)	
K1	1	00.0)		40.0)		65.7)		62.1)		87.4)	
2 yr.	2	10.1)	11.7	33.1)	13.9	50.5)	7.8	73.8)	6.6	92.8)	3.9
wood	3	23.4)		60.0)		54.9)		61.5)		85.0)	
	1	8.8)		27.2)		75.2)		78.7)		88.6)	
K2	2	19.1)	5.1	48.1)	10.5	66.8)	6.1	76.0)	1.6	85.1)	2.0
	3	14.8)		35.7)		63.2)		75.6)		85.0)	
	1	9.9)		39.4)		60.0)		65.7)		79.1)	
K3	2	24.0)	8.0	56.6)	8.7	49.1)	5.5	54.2)	6.2	81.8)	3.0
	3	10.3)		50.5)		56.1)		55.8)		85.1)	
	1	10.4)		56.5)		68.9)		78.3)		90.4)	
K4	2	29.0)	9.6	50.4)	4.1	63.9)	6.6	79.5)	4.8	86.2)	2.1
	3	15.5)		48.6)		55.8)		70.5)		87.6)	
	1	30.2)		53.7)		62.8)		69.2)		90.3)	
K5	2	32.3)	6.7	36.0)	8.8	54.7)	5.1	75.0)	3.4	88.7)	1.4
	3	42.8)		43.6)		64.2)		75.3)		91.6)	
	1	12.7)		49.1)		56.7)		75.8)		81.0)	
K12	2	27.0)	7.5	68.7)	12.1	63.0)	7.1	70.5)	6.9	82.1)	1.0
	3	15.9)		71.3)		48.8)		62.0)		80.0)	
	1	32.7)		32.8)		57.8)		69.8)		82.4)	
K16	2	7.6)	15.0	45.6)	6.9	58.1)	3.3	73.9)	2.0	76.7)	2.8
	3	34.5)		43.7)		63.7)		71.3)		80.3)	

Table 2. (cont.).

Clone	Tree sample	Percentage drop in resistance (Detached twigs) -18° C.	Std. dev.	-25° C.	Std. dev.	-30° C.	Std. dev.	-35° C.	Std. dev.	Liquid air	Std. dev.
K17	1	26.5)		34.3)		50.1)		58.8)		83.1)	
	2	11.6)	7.6	33.3)	23.8	71.8)	11.5	80.0)	11.3	88.0)	3.7
	3	16.0)		75.2)		67.7)		76.4)		90.5)	
K18	1	43.2)		50.0)		60.0)		79.1)		76.5)	
	2	18.3)	15.1	77.9)	16.4	64.3)	3.2	79.0)	3.3	86.5)	0.9
	3	15.7)		48.8)		66.3)		73.3)		85.7)	
K19	1	10.6)		52.0)		56.7)		61.0)		79.7)	
	2	42.1)	16.0	46.6)	6.2	45.1)	5.8	71.3)	7.3	79.6)	0.3
	3	32.1)		59.1)		50.5)		57.1)		80.3)	
K20	1	51.3)		61.8)		67.6)		71.5)		85.3)	
	2	25.1)	14.1	65.7)	8.9	70.5)	1.4	75.1)	2.0	82.8)	1.2
	3	29.0)		48.6)		69.7)		75.0)		84.5)	
K21	1	1.7)		56.6)		67.2)		75.3)		79.7)	
	2	16.0)	7.3	64.9)	6.7	69.0)	2.2	74.7)	0.8	80.9)	1.1
	3	11.9)		51.9)		64.6)		73.6)		78.7)	
K22	1	36.4)		46.4)		64.6)		73.8)		80.6)	
	2	3.0)	19.4	38.8)	3.8	56.4)	4.6	70.7)	1.5	80.2)	2.0
	3	2.5)		41.8)		56.6)		71.6)		84.0)	
K23	1	26.3)		38.9)		71.3)		80.6)		81.6)	
	2	27.1)	1.7	37.3)	2.3	72.0)	2.5	82.8)	1.8	86.2)	2.3
	3	29.7)		34.3)		67.2)		79.1)		83.4)	
K29	1	22.2)		39.4)		67.8)		78.5)		83.9)	
	2	13.0)	10.3	74.8)	17.7	65.8)	2.5	78.1)	0.2	83.7)	0.3
	3	1.5)		60.0)		62.8)		78.4)		83.2)	

Table 2. (concl.).

Clone	Tree sample	Percentage drop in resistance (Detached twigs) -18° C.	Std. dev.	-25° C.	Std. dev.	-30° C.	Std. dev.	-35° C.	Std. dev.	Liquid air	Std. dev.
K33	1	16.9)		74.5)		82.2)		85.0)		89.2)	
	2	15.1)	0.9	49.4)	14.3	56.2)	13.7	66.7)	9.9	79.6)	5.0
	3	15.9)		50.0)		61.2)		69.3)		81.9)	
K43	1	16.0)		57.6)		64.5)		76.0)		80.0)	
	2	18.6)	1.4	61.5)	10.3	62.1)	2.2	74.6)	2.8	78.6)	0.7
	3	18.5)		41.9)		60.0)		70.5)		78.8)	
K48	1	2.2)		45.7)		54.5)		66.5)		73.7)	
	2	0.0)	1.2	40.9)	5.4	64.0)	6.4	61.4)	2.6	83.7)	5.6
	3	0.0)		34.8)		51.6)		63.0)		74.0)	
K56	1	12.0)		30.1)		63.1)		72.3)		83.0)	
	2	10.0)	2.8	34.0)	15.6	55.7)	3.7	72.6)	2.2	82.7)	0.6
	3	6.4)		59.0)		58.2)		76.4)		81.7)	
M IX	26	3.8)		39.0)		55.5)		75.5)		77.8)	
	28	16.9)	7.6	39.6)	11.9	61.0)	3.0	59.4)	8.9	73.9)	2.9
	29	3.5)		60.0)		60.6)		60.6)		79.6)	
M XIII	NW	11.6)		36.1)		63.9)		75.3)		78.1)	
	SW	12.5)	5.8	27.8)	4.6	78.7)	3.5	75.6)	0.8	89.5)	5.7
	E	2.0)		28.4)		65.5)		76.8)		82.9)	
M IV	W #1	10.6)								75.9)	
	W #2	7.8)*	10.1	29.5)						73.3)	1.8
	E #1	16.8)		40.7)	6.2					72.3)	
	E #2			39.8)							
Manchu Crab	#1			26.5)				31.2)		66.5)	
	#2			28.3)	4.4			40.0)	7.2	61.8)	2.6
	#3			35.0)				45.6)		62.2)	
Yellow Siberian	#1			28.4)						68.6)	
	#2			30.2)	1.7					67.2)	1.5
	#3			26.7)						70.2)	

* Percentage resistance increase.

which it is calculated. This may have no meaning in this case but suggests an interesting problem as to whether resistance determinations on various sides of a tree might not show greater variation than determinations between twigs from the same side of various trees of the same clone or variety.

Even a cursory look at Table 1 reveals the disproportion in the resistance drop between -18° C. and -25° C. relative to those between other treatments, even though there are two degrees more of temperature difference. A calculation (from the 21 clones which were subjected to all treatments) of the average resistance drop per degree between the above-mentioned temperatures is 4.3 percent and between each two consecutive increasingly lower temperature treatments, 2.7 percent, 2.0 percent, and 0.07 percent, respectively. This same trend is delineated in the graphs, Figs. 1 to 6, inclusive.

Thus, it is evident that the curve of change in resistance follows a decreasing gradient. And, if the supposition, yet to be argued, that the degree of fall in resistance directly measures the amount of injury is valid, then the increment amount of injury decreases with decreasing temperature.

The bulk of the argument for correlating resistance change to injury in this case will be left to the section "Discussion."

If an increasing permanency of resistance drop can be demonstrated and this correlated with the degree of fall in resistance, then there is a strong case for directly associating degree of injury with resistance drop.

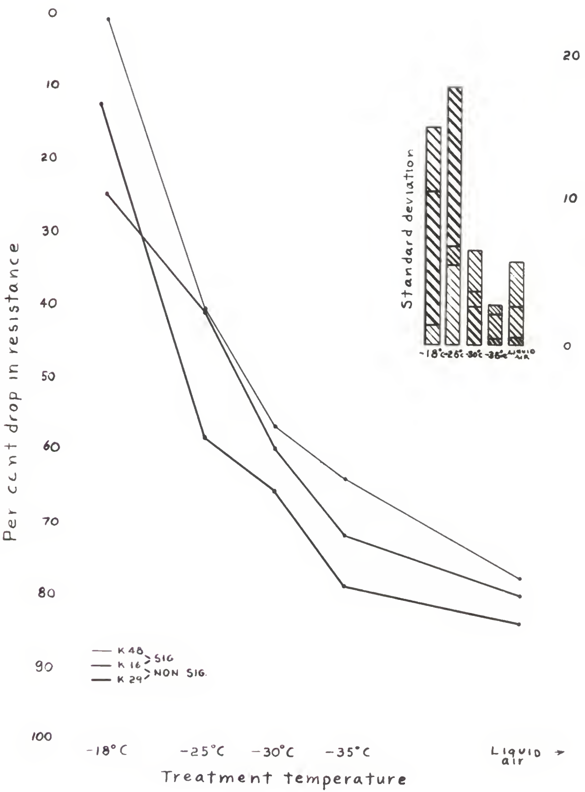


Fig. 1. Percentage resistance decreases of three K series clones as temperature is decreased.

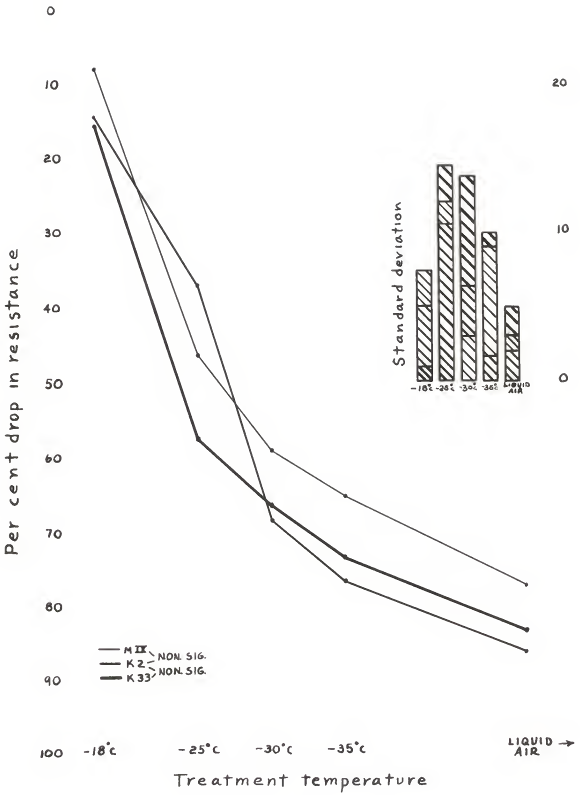


Fig. 2. Percentage resistance decreases of three K series clones as temperature is decreased.

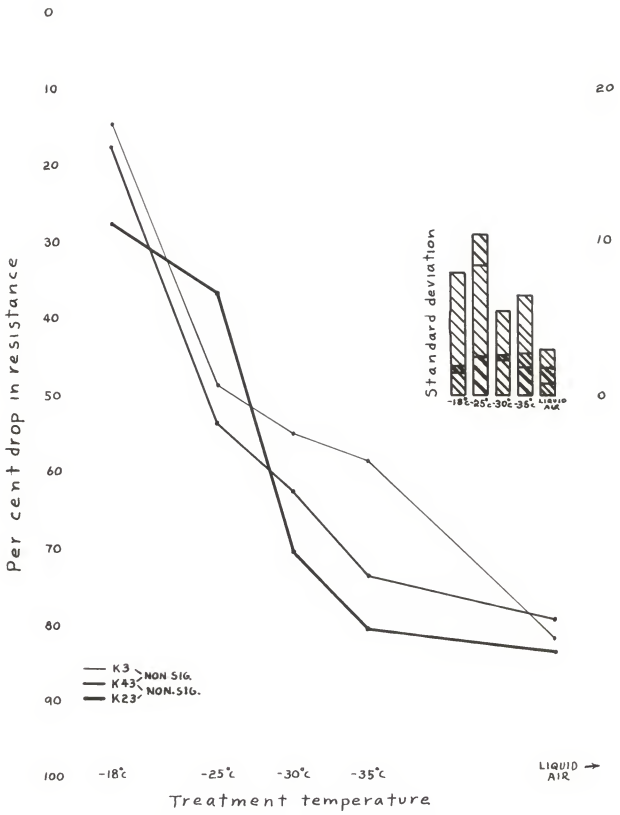


Fig. 3. Percentage resistance decreases of three K series clones as temperature is decreased.

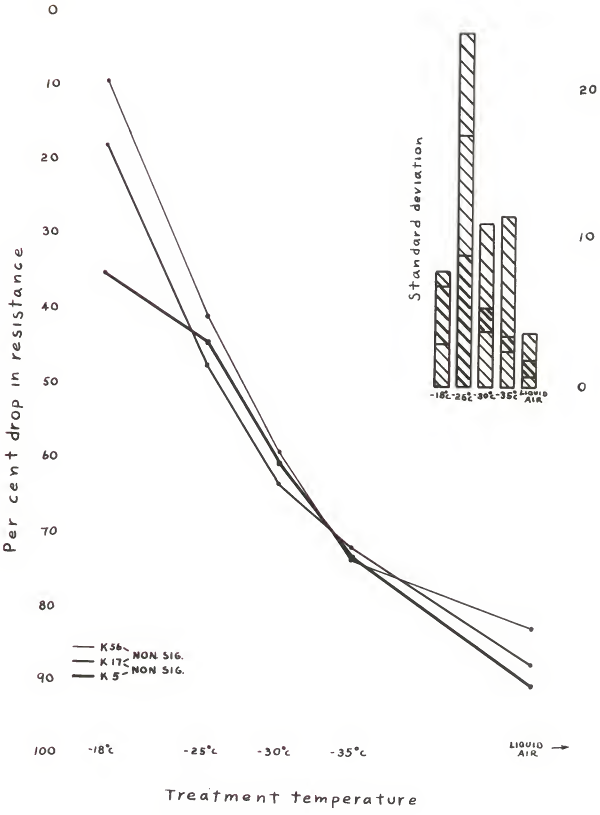


Fig. 4. Percentage resistance decreases of three K series clones as temperature is decreased.

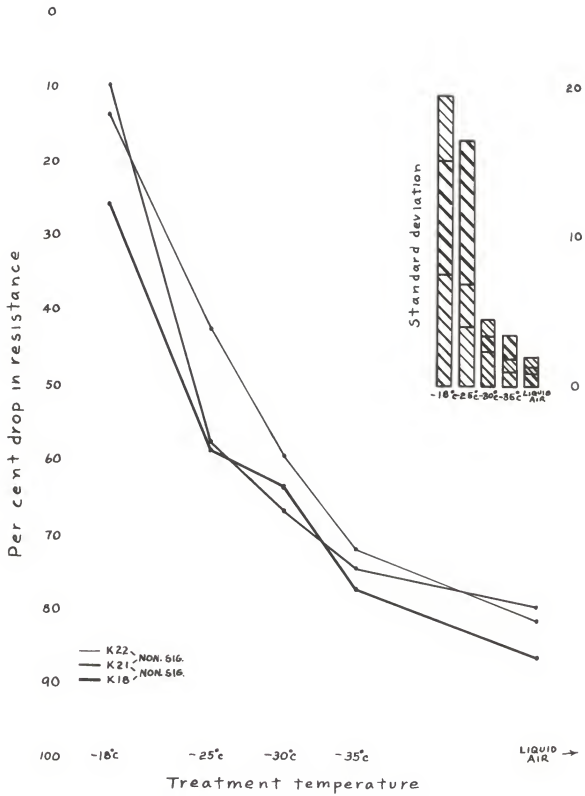


Fig. 5. Percentage resistance decreases of three K series clones as temperature is decreased.

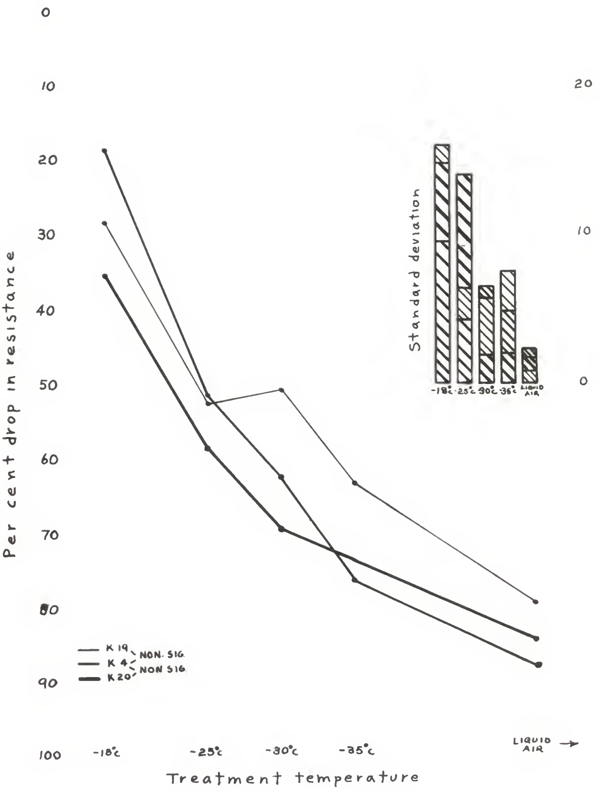


Fig. 6. Percentage resistance decreases of three K series clones as temperature is decreased.

This is fairly well demonstrated in Table 3, which shows resistance readings before treatment and for several days after treatment at various treatment temperatures for six clones. Three of these (marked H) were at the top of the group relative to hardiness (Table 3) and three at the bottom of the group (marked T). The differences between the three hardier clones and the three tenderer are significant. Data for the liquid air treatment are not included, since there was a universal continued resistance decrease over the period of post-freezing readings.

It will be noted in Table 3 that readings following treatment tended to return to normal at the lower treatment temperatures and that where the tendency was the same in two different treatments, the return was more complete at the lower temperature or more of the samples rose in resistance at the lower temperature than at the higher or both. This same tendency is to be noted in other clones on which data are not presented.

That the degree of drop in resistance is also correlated with hardiness is strongly suggested by the values obtained for Yellow Siberian and Manchu Crab, varieties of known hardiness. The data (Table 1), though incomplete, because of a paucity of material, show less resistance change at any of the temperatures at which they were treated than any of the other clones tested.

Table 2 presents interesting relationships between intraclonal standard deviations. This point is also illustrated, and better, for comparative purposes, in the histograms in the upper right hand corners of Figs. 1 to 6, inclusive. Of the 21 clones

Table 3. Comparison of individual resistance readings (in thousands of ohms) of three clones showing low average percentage resistance drops (H) with three showing high average percentage resistance drops (F).

Clone	Tree	Treatment temperature -18° C.				Treatment temperature -25° C.				Treatment temperature -30° C.				Treatment temperature -35° C.			
		Observation															
		Pre-freezing	Post-freezing			Pre-freezing	Post-freezing			Pre-freezing	Post-freezing			Pre-freezing	Post-freezing		
			5-13 -50	5-15 -50	5-17 -50		4-22 -50	4-24 -50	4-26 -50		5-21 -50	5-22 -50	5-24 -50		4-29 -50	5-1 -50	5-3 -50
M IX (H)	26	520	500	500	495	410	250	330	360	315	140	126	142	360	88	98	92
	28	295	245	245	265	285	172	174	184	400	156	112	108	370	150	140	110
	29	285	275	230	235	245	98	82	76	300	118	86	75	290	114	110	88
K20 (T)	1	230	112	88	64	215	82	82	76	340	110	64	60	260	74	60	46
	2	235	176	146	144	280	96	90	82	156	46	25	23	225	56	41	32
	3	265	188	215	305	265	136	158	178	172	52	32	26	240	60	45	37
K48 (H)	1	225	220	205	215	280	152	172	176	220	100	74	72	335	112	104	92
	2	270	270	270	305	210	124	130	132	150	54	44	40	285	110	92	78
	3	315	315	260	310	270	176	240	250	465	225	192	225	390	144	153	146
K18 (T)	1	400	225	270	250	360	180	174	148	345	138	110	108	365	76	58	48
	2	300	245	215	250	290	64	56	48	230	82	56	48	200	42	34	28
	3	285	240	265	280	250	128	150	158	220	74	57	56	330	88	70	60
K23 (T)	1	315	232	285	290	285	174	154	182	230	66	49	45	290	56	53	44
	2	225	164	162	186	265	166	168	210	315	88	64	60	210	36	36	34
	3	420	295	370	420	335	220	156	152	330	108	85	86	335	70	64	54
K3 (H)	1	505	455	500	475	355	215	240	250	325	130	86	70	420	144	116	94
	2	500	380	365	405	295	128	110	94	570	290	240	210	350	160	100	92
	3	530	475	495	505	295	146	144	154	415	182	134	126	510	225	198	174

in Table 2 to be considered here, 18 were amenable to statistical analysis, and their standard deviations are presented graphically as mentioned above.

Standard deviations of 12 of the 21 clones were greatest at the -25° C. treatment and seven of the remaining nine at the -18° C. treatment temperature. There were none of greatest magnitude in the liquid air or -35° C. categories, the last two occurring at -30° C.

Variability within a clone in response to treatment was quite low in liquid air, the average standard deviation being 2.3. However, the resistance decrease was not homologous, the range running from 63.5 percent to 90.2 percent. This suggests the essential similarity in the protoplasm of a clone.

The preponderance of the variation occurring at the second highest treatment temperature probably has an implication in the chemistry of the cell which will be reserved to the next section, where it will be discussed in connection with Osterhout's theories.

It should be pointed out, however, that showing great variation in any treatment apparently has no relationship to the ranking of the clones in Table 4; i.e., the hardier clones did not show their greatest variation at lower temperatures than the tenderer sorts. K48, however, is one of the two which show greatest variation at -30° C.

The basic connotation of the data of Table 3 has been explained previously as well as several suggestive relationships.

Table 4. Summary of results (Detached twigs).

Clone	Clonal mean (All treatments)	Difference from previous value*
K48	47.733	
M IX	51.113	3.380
K3	51.840	0.727
K56	53.146	1.306
K22	53.826	0.680
K19	54.920	1.094
K16	55.393	0.473
K2	56.526	1.133
K43	57.280	0.754
K17	57.553	0.273
K21	57.753	0.200
K4	59.440	1.687
K29	59.540	0.100
K33	59.540	0.000
K23	59.853	0.313
K5	60.693	0.840
K18	62.373	1.680
K20	64.233	1.860

* Difference for significance = 7.055.

It may further be gleaned from this same table that there is some tendency for a sample whose resistance trend following treatment is at variance with the other two to continue that variation to the point where all show continuing drop in resistance following treatment. This, however, is not decided.

The hardier clones in Table 3 show a trend toward a return to normal resistance more often and through lower treatment temperatures than do the tenderer ones. Admittedly, the data for demonstrating this and the assertion of the previous paragraph are meager, but there is a decided suggestion that were there more clones showing significant differences, this could be demonstrated with greater certainty.

That this tendency to rise to normalcy is not strictly a result of desiccation is evident from the fact that there is no such tendency in the liquid air treatment under the conditions of this experiment.

The analysis of variance of this phase of the problem is presented in Table 5. All factors were significant except for trees within the clones, indicating the homogeneity of the trees so far as these experiments were concerned. Undoubtedly the lower treatment temperatures did much more than the higher temperatures to produce this uniformity of response.

Table 5. Analysis of variance; detached twigs.

Factors responsible for variability	Degrees of freedom	Sum of squares	Variance	F value	For significance at 5% level	For significance at 1% level
Total	269	156,861.66				
Treatments	4	134,472.89	33,618.22	329.11	2.51	3.62
Between clones	17	4,521.02	265.94	2.60	1.80	2.30
Treatments x clones	68	6,946.22	102.15	1.80	1.69	2.12
Trees in clone	36	2,769.01	76.91	1.36	1.55	1.85
Pooled treatment x trees	144	8,152.52	56.61			

The variation between clones and between treatments was highly significant, the latter being so significant that no comparison is necessary with the Table of F.

Efficacity of some treatments as against others in causing injury in a clone and the relatively lesser effectiveness of the same treatment on other clones while other treatments are perhaps reversed in their action on these given clones or have no differential effect, results in the significance of the treatment by clones interaction. This is reflected in the crossing of interpoint lines in Figs. 1 to 6, inclusive.

In calculating F values, the pooled treatment by trees variance was used in determining that for the factor, "Trees in clones." But since it was not significant, it was used only in ascertaining the F value of the treatment x clone interaction. In accordance with good statistical practice, this last variance was used as the basis of the F calculations.

Though Table 4, a comparison of average resistance drops, shows significant differences between clones, but not between any two consecutive clones, no attempt is made to label any as hardy, moderately hardy, etc. This is avoided for the obvious reason that there are no points of sharp difference between any two adjacent clones, unless it be between K48 and M IX.

But in relating the table of least significant differences for the potted trees (Table 9) to Table 4, some ratings will be attempted.

Experiment with One-year-old Twigs of Two-year-old Potted Trees

The phase of the problem involving fully functioning, unhardened one-year-old twigs of two-year-old potted trees subjected not only to low temperature, but to the desiccating effect of a moderately rapid moving current of air, serves partially as a check on the reliability of a single set of artificial freezing experiments as a guide to hardiness. Unfortunately, however, the clones represented here are not without exception those in the previous portion of the experiment or those suitable for statistical analysis. Eight of the 11 find counterparts in the tests with detached twigs.

The basic data for this test are found in Table 6. The treatment temperature was about midway between the treatment -18° C. and -25° C. in the experiment with hardened twigs, but the results obtained more nearly approximate those obtained at -35° C. in that phase. Again, as previously, some clones showed uniformity in individual readings, while others were quite variable. The variability in the two sets of experiments were not always comparable. For instance, the variability in K3 was less than in any treatment with unattached twigs, while that of K4 and K18 was in the range of the variability of their counterparts at the lower treatment temperatures.

But, this is what should be expected if variability is seen as being in accord with resistance drop rather than with the temperature of treatment. Again, this points to the similarity

Table 6. Percentage drop in resistance of potted two-year-old trees frozen at -21° C. in an air blast.

Clone	Tree No.	Percent resistance drop	Tree No.	Percent resistance drop	Tree No.	Percent resistance drop	Average resistance drop
K1	V	63.3	IV	46.6	III	51.6	53.8
K3	V	61.7	IV	61.8	III	59.0	60.8
K4	V	66.8	III	70.1	I	71.2	69.3
K16	V	70.4	II	68.1	I	77.7	72.0
K18	V	80.0	IV	69.4	I	79.3	76.2
K19	IV	69.0	I	75.5	II	66.3	70.2
K22	V	63.2	I	63.0	IV	77.2	67.8
K43	V	65.7	I	65.5	IV	68.6	66.6
K56	V	70.8	IV	68.8	I	76.3	71.9
K59	V	66.6	IV	65.7	I	72.3	68.2
K60	V	68.8	I	67.0	III	72.6	69.4

of response of the protoplasmic components as injury increases.

Previously, mention has been made of provisions for controlling soil moisture in this test and the response of resistance readings to soil moisture changes.

In the third group of trees frozen, some difficulty was experienced in securing the lowering of the moisture block resistance to the target point of 500 ohms by the necessary treatment deadline. As a result, the trees were placed in the freezing chamber with moisture content of the soil slightly lower than in the other runs. Actually, all but two were within 100 ohms of being at the desired resistance point. This point may be unimportant, but is offered as a possible explanation for the fact that the majority of the resistance declines in the third group were slightly greater than in any other run.

Reference is made again to the statement under "Methods" relative to twig resistance fluctuations with soil moisture level. Data are presented in Table 7. All were taken after treatment.

The intention here is to demonstrate that following freezing treatment, with an increase in soil moisture content, there will be a decrease in twig resistance. It must be recognized that the plaster of paris blocks were buried at half the depth of the pots and that there would, therefore, be no immediate response in these blocks to small additions of water though there might be in the plants, since roots filled the pots. Furthermore, all pots were plunged in soil almost to the rim and plants not immediately being readied for treatment or not already having had a freezing treatment were watered as they appeared dry without exercising care in the addition of exact quantities. Under these conditions there could be and probably were interpot movements of moisture, as it shown by moisture resistance changes without additions of water.

Notwithstanding these circumstances, of the 38 cases presented, 23, or 60 percent, showed the response mentioned. And, if the second day's readings, which, in every case, rose regardless of moisture trend, be excluded, the percentage of occurrence becomes 67 percent.

With reference to the second day readings, where the pot moisture increased from the first to the second day, this decided upward trend was much inhibited. A tabulation of all 24 trees on which it is possible to check this phenomenon shows 21

Table 7. Twig resistance fluctuations with changes in soil moisture content after freezing in seven randomly selected trees.

Clone	Tree	Number of days following treatment 0	1	2	3	4	5	6
K18	I	M620* 68	M540 64	M520 70 (200)	M460 66	M440 60	M460 58	
K18	IV	M560 90	M500 66	M540 108	M540 102	M560 124 (200)	M560 110	M520 98
K22	I	M440 120	M400 88	M400 138	M420 134	M440 150	M440 152	M420 138
K22	IV	M680 98	M600 88	M600 114	M540 118	M520 112	M560 120	
K19	I	M480 66	M430 60	M440 106	M460 100	M480 110	M480 110	M460 102
K56	I	M660 78	M600 70	M580 76	M520 74	M500 66	M540 62	
K4	I	M640 158	M560 140	M540 160 (200)	M480 166	M460 164	M480 158	

* Numbers preceded by M are moisture determinations in ohms; numbers unpreceded by a letter are twig resistances in thousands of ohms; and numbers in parentheses indicate cubic centimeters of water added to pots.

exhibiting a marked increase. Two more showed a slight increase, but were of such small magnitude that they might be attributed to the moisture increases in the pots in those cases.

What this second phenomenon represents is difficult to say. Two possibilities are a trend toward recovery from injury and the beginning of the drying out process in injured twigs. If the first were true, then it must soon be arrested, for some continue to climb well past the original resistance for the twig. In other cases, a fall to the original post-freezing level occurs within a day or two with eventual drying of the twig and a climb to abnormal resistances. This rules out the second as an all-inclusive explanation. Possibly both are represented in the phenomenon.

This leaves the question of why these increases particularly take place on the second day. No answer will be attempted.

There are three factors responsible for the variance in this experiment (Table 8) and of these, only one, variation between clones, is important other than in tabular calculations. Here, as in Table 5, the same high degree of significance is shown. In both experiments clonal differences were responsible for a large amount of the variation existing.

Summarizing results in Table 9, it is seen that the sharpest differences are again at the top of the group. Also, as in the previous summarization, there are no two consecutive clones between which there are significant differences, although K1 is very nearly significantly hardier than K3.

Table 8. Analysis of variance; potted two-year-old trees.

Factors responsible for variability	Degrees of freedom	Sum of squares	Variance	F value	For significance at 5% level	1% level
Total	32	1,627.87				
Between clones	10	1,089.43	108.94	4.45	2.30	3.26
Within clones	22	538.44	24.47			

To compare the rankings in Tables 9 and 4, one of the more obvious points is the position of K18 at the bottom in both tables. Obviously, it must be rated as a tender variety under the experimental conditions. Paradoxically enough, K18 was the last of the clones in the test of potted trees in which the resistance rose above the pre-freezing resistance and could be considered as dried out. The least amount of time required was 21 days and the greatest 29. K18 bears an extremely heavy bark, which probably explains this resistance to desiccation.

Another obvious point is the position of K3 near the top of both tables.

K43 and K22 are out of position relative to each other when both tables are considered, but this is of smaller importance when it is noted that in neither case is the difference between them significant.

On Table 4 the difference between K19 and K18 is just significant, while such is not the case on Table 9. The relationship between K18 and K56 is another such discrepancy.

Table 9. Summary of results (potted two-year-old trees).

Clone	Clonal mean	Difference from previous value*
K1	53.833	
K3	60.833	7.000
K43	66.633	5.800
K22	67.833	1.200
K59	68.200	0.367
K4	69.366	1.166
K60	69.466	0.100
K19	70.266	0.800
K56	71.966	1.700
K16	72.066	0.100
K18	76.233	4.167

* Difference for significance = 8.296

Of seven positional relationships of one clone on Table 9 to the next one above it which also occurs on Table 4, four such occur on both tables. Of six with a two positional difference on the former table, five maintain at least a positional order on the second table. It is evident that a fair correlation exists between the two tables.

DISCUSSION OF RESULTS

One point relative to correlating amount of resistance decrease to amount of injury to a twig has been demonstrated by these experiments and discussed with the presentation of the pertinent data.

Here will be infused references and comments on a much earlier work of a similar nature on Laminaria which seems to bear out the conclusions drawn here. Furthermore, the hypotheses drawn by Osterhout (1921) as to the nature of injury to the cell in the main appear to the writer to be more strongly supported by the results the latter has obtained.

Osterhout stated, "the electrical resistance of [Laminaria] is an excellent index of what may be called its normal condition of vitality."

That has been this author's observation on the material with which he has worked. Tests have shown that the resistance of normal twigs of a given diameter will fall within a certain fairly narrow range on twigs of a given length. And, reducing the length by one-half or two-thirds will reduce the resistance by close to that amount. It has been found also, that at a given resistance measurement length and with shoots of the same diameter, healthy diverse plant materials will give characteristic resistance responses. Several obvious characteristics responsible were viscosity of the sap, succulence of the material, and the presence or absence of pith. Shoots to which reference has been made are, of course, mature ones. No check has been made, but it is thought that twigs or shoots in the earlier stages of growth may show lower resistance than the more mature parts.

Osterhout (1921) asserts that if injury occurs and therefore the resistance falls, the drop in resistance may be considered as giving a measure of the degree of injury.

He said, "if the tissue loses ten per cent of its normal resistance we may say that the injury amounts to ten per cent." This is the conclusion of the writer as a result of the present study.

Additional trends pointed out in the data obtained find support in Osterhout's work. His statement relative to his work treating Laminaria with differing concentrations of sodium chloride is as follows:

> .... if the injury in a solution of sodium chloride amounts to five per cent, the tissue recovers its normal resistance when replaced in sea water. If however the injury amounts to twenty-five per cent the recovery is incomplete: instead of rising to the normal it recovers to only ninety per cent of the normal. The greater the injury the less complete the recovery. When injury amounts to ninety per cent there is no recovery at all.

These exact percentage relationships to recovery cannot be shown in this study because the material used in the first part of the study had been removed from the tree and therefore, was not supplied with water and raw materials with which damaged parts could be reconstructed. The treatment temperature of the potted trees was low enough to cause injury of a degree that little recovery might be expected.

However, Tables 3 and 7 do show trends which undoubtedly have a connection with these statements of Osterhout.

One difference between the response of Laminaria and these apple clones was noted. Failure of the resistance trend to turn upward occurs at a lesser degree of injury on detached twigs than with Laminaria. However, with the potted trees, the 21 cases of sharp upsurge of resistance mentioned, though temporary, was probably an expression of this phenomenon and such occurred with average decreases in resistance of 76 percent. Evidently, reaction of cut twigs was not fully normal.

The preponderance of deviation in resistance decreases occurring at -18° C. and -25° C. is worthy of further study and discussion. A logical suggestion to account for this is a greater fluctuation in the temperature of freezing, but actually, it was less at these two treatment temperatures than at the other two at which the freezer was used. As a freezing unit reaches the limit of its capacity to lower the temperature in a cabinet, the temperature will deviate more around a given desired temperature regardless of range adjustment setting. This is exactly what happened in this case. The deviation on either side of the treatment temperatures in both cases was only one degree.

Another suggestion relating to Osterhout's theories is presented here for consideration. He viewed resistance measurements as an expression of the resistance of the plasma membrane to ionic movements and postulated a series of chemical reactions which constantly rebuilt this membrane in the presence of processes tending to destroy it. Destruction or death was conceived as being a part of the life process. Each of these chemical steps has its own critical temperature of reaction. Inhibition of any of the intermediate steps results in temporary injury, while the destruction of the precursor of all results in permanent injury or death. A hypothetical substance, M, was the material responsible for permeability changes. This is not at variance with present-day theories of genic and plasmagenic control.

If this "chain of reactions with differing critical temperatures" idea be correct, one might envisage a series of points at which resistance might show responses to the maximum inhibition of any one of these processes with the greatest occurring where the production of M were at a minimum and its destructive effect at a maximum. Individual internal conditions such as the reserve of M or, as Osterhout presents it, the thickness of M on the plasma membrane, and any acceleratory or inhibitory internal conditions other than temperature might modify this.

Also, at a critical point any slight temperature differences would be more important than at intermediate points. Here, the inevitable temperature stratification in the freezing chamber may have contributed to the result even though twigs were spread as thinly as possible. This latter plus internal conditions would produce variation; the former would result in a maximum of injury. Both of these characteristics are present in and between the -18° C. and -25° C. treatments.

It has been pointed out that the correlation between Tables 4 and 9 is only fair. Hardiness, of course, is a combination of factors and trees in the orchard may be expected to respond differently, relative to each other, as various climatic, cultural, and pathological factors change. An estimation of the hardiness of a variety is a composite of responses over a number of years. Hence, relative standings will be maintained best when the clones or varieties are farthest apart in their ratings. This is the situation here which applies equally well to the only observation made on the hardiness of these clones under field conditions --

that following the winter of 1947-1948.

It is realized that the treatment temperatures selected were severe, but nothing was known as to whether a gradation of resistance reductions might be obtained. However, Filinger and Cardwell's work had shown what the greatest degree of damage might be. It seemed best, therefore, to work upward at reasonable intervals from the lowest temperature obtainable with the freezer on hand.

SUMMARY

1. Increasingly lower treatment temperatures have produced an ascending gradient of percentage resistance drops.

2. A probable correlation between degree of percentage resistance decrease and injury and hardiness has been demonstrated.

3. Very significant variation has been found between treatments of the phase of the experiment with unattached twigs and between clones in both phases of the experiment. Significant variation has been shown in the interaction between treatments and clones in the first phase.

4. Resistance increases following initial "lows" after the more moderate treatments are probably more than chance variation, especially in the light of corroboration by Osterhout's experiments.

5. Hardier varieties have a tendency toward returning to normal resistance values following treatment at the higher temperatures more often than do tenderer varieties.

6. Fluctuations of resistance due to soil moisture content has been demonstrated in twigs of intact two-year-old apple trees following freezing treatment.

7. A theory has been presented to explain the prevalency of wide variation and the largest temperature increment resistance decrease in the -18° C. and -25° C. treatments.

8. Relative ranking of clones as to hardiness has been made for each experiment in this study and a fair correlation shown between the two tables.

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