INVESTIGATIONS OF THE HARDINESS OF PLANTS BY MEASUREMENT OF ELECTRICAL CONDUCTIVITY[1]

S. T. DEXTER, W. E. TOTTINGHAM, AND L. F. GRABER

In a preliminary report, DEXTER, TOTTINGHAM and GRABER (**3**) have described a method of estimating the hardiness of plants as applied to three varieties of alfalfa, in which exosmosis of electrolytes from suitably frozen tissue into distilled water is determined electrometrically, and the magnitude of the electrical conductivity associated with the known hardiness of the samples. They found a progressive hardening of Grimm alfalfa, a hardy variety, as the winter season approached, and little or no hardening in a very tender strain, Hairy Peruvian. Utah Common alfalfa was intermediate in response.

The principle of increased permeability and electrical conductivity of injured tissue is a familiar one in plant physiology, while the method of electrical conductance as a measure of changing electrolyte concentration has been more or less standard for many years. (See OSTERHOUT (**16**); DIXON and ATKINS (**4**); HOAGLAND and DAVIS (**9**); HIBBARD and MILLER (**6**); HOTTES and HUELSEN (**10**); PANTANELLI (**17**); MERRILL (**13**); STILES and JØRGENSON (**21**).) In the papers listed above the principle has been applied to injuries resulting from freezing, from the action of various chemicals, from pathological causes, and from mechanical rupture.

In view of the fact that the chemical and physical theories of winter-hardiness of plants have been amply discussed in recent publications, and since the bibliography has been so fully covered there, the consideration of this phase of the problem will be dismissed by reference to several papers which furnish suggestive discussions and inclusive citations of the literature. MAXIMOW (**12**) has reviewed the various physical and chemical studies of the hardiness problem in which compositional or osmotic and colloidal properties of plants are shown to be associated with hardiness. He cites, as well, numerous recent investigations in which growth recovery from controlled freezing treatment is used to estimate the degree of hardiness. NEWTON (**15**), WEIMER (**22**), STEINMETZ (**20**), ROSA (**18**) and CHANDLER (**2**) present excellent discussions, full bibliographies and additional data in regard to several chemical and physical methods of investigating the hardiness of plants. It may be stated briefly that most, if not all, of the theories of winter-hardiness are built around the idea of the water-relations of the plant, and that structural, osmotic or colloidal pro-

[1] Contribution from the departments of Agronomy and Agricultural Chemistry, University of Wisconsin, Madison, Wis. Published with the approval of Director.

tection from water-withdrawal, or ice-formation form the basis of explanation for individual or varietal differences in hardiness.

The positive recognition of hardiness in plants, and the practical estimation of the degree of injury from freezing are matters which do not seem to have been adequately considered, nor have the various studies furnished convenient means for their accomplishment. MAXIMOW (**12**) in concluding his review of the various methods previously put forth states "we may observe that, notwithstanding a series of successful attainments, the study of the internal factors conditioning hardiness has not yet established any reliable and easily determinable symptoms for use in breeding work. For this reason, refrigerating machines—are being more and more used for the acceleration of the breeding of frost resistant varieties." Thus, the recent work of STEINMETZ (**20**), WEIMER (**22**), MARTIN (**11**), STEINBAUER (**19**), BRIERLEY and HILDRETH (**1**), HILL and SALMON (**8**), HARVEY (**5**) and others shows that varieties of alfalfa, wheat, red clover, apples and other plants can be distinguished from one another on a hardiness basis when suitably frozen after a period of hardening. In these experiments, the injury to the tissue exposed to cold temperatures is estimated by the degree of growth recovery or by microscopic observation.

The work of DEXTER, TOTTINGHAM and GRABER (**3**) has indicated that injury from controlled freezing treatments may be conveniently determined by measurement of the degree of exosmosis of electrolytes and that alfalfa varieties so examined fall into the proper order of hardiness. It is true that such measurements have not yet been adequately related to the rate or degree of recovery from freezing injury, but specific determination of injury in itself is an important step in a critical study of hardiness and its related problems.

The rapidity with which the estimation of injury can be accomplished and the convenience of the precise numerical values obtained by electrical conductance, makes the application of the method to plants other than alfalfa a matter of some interest. Moreover, as suggested above, a modification of the method might well be devised in which conductivity measurements of the tissues themselves would be made, subsequent to freezing injury, since increased conductivity might be anticipated in the injured tissues. To these ends, a series of experiments has been performed, investigating some important aspects of the hardiness problem as related to several crops under various environments.

Investigations

CONDITION OF THE ROOTS IN THE FIELD IN WINTER

In order to investigate the condition of alfalfa roots in the field in winter, samples of roots were dug from the deeply frozen ground on January

10, 1930. These roots were two years old, from plats which had received but two cuttings in the summer of 1929. Twenty-gram samples of roots were prepared in the usual manner (see Dexter, *et al.* (**3**) for details) and without further freezing treatment beyond that received in the field, were exposed to exosmosis into 50 cc. of water at 25° C. for ten hours. Table I gives the results of this trial.

TABLE I

SPECIFIC CONDUCTIVITY ($\times 10^6$, 25° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACT FROM ALFALFA ROOTS FROZEN ONLY IN THE FIELD. AN INTERVAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS AT 25° C.

VARIETY	SPECIFIC CONDUCTIVITY
Grimm	360
Utah Common	634
Hairy Peruvian	1690

These data give definite evidence of severe field injury in the case of Hairy Peruvian, considerable injury in the case of Utah Common and but very little injury in the case of Grimm alfalfa. Conductivity of checks, in the fall, on unfrozen tissue approximated 200. Field counts in the following spring indicated almost complete loss of plants in the Hairy Peruvian plats, about 50 per cent. loss in the Utah Common plats, and under 10 per cent. loss in the Grimm plats.

A similar experiment on the same date with three varieties of winter wheat indicated precisely the same condition. The magnitudes of the electrical conductivity values and hardiness were correlated with larger exosmosis from the tenderer strains. From a ten-gram sample of crowns, exosmosis for seven hours into 40 cc. of water gave specific electrical conductivities ($x\ 10^6$) of 423 for Wisconsin Pedigree no. 2 wheat, 478 for Michigan Amber wheat and 558 for Dawson wheat. These values are quite in accord with the order of hardiness of these varieties as determined by field trials at the Wisconsin Experiment Station.

EXPERIMENTS ON GRAINS GROWN IN THE GREENHOUSE

To continue the work throughout the winter, several varieties of small grains were planted in beds and in wooden flats in the greenhouse on Jan. 27, 1930. The temperature of the greenhouse varied considerably and rarely reached a temperature as low as 60° F. during the first few weeks. Preliminary studies indicated very marked hardening effects in these greenhouse plants, with much lower exosmosis and electrical conductivity from frozen wheat crowns which had been previously exposed for a few hours to temperatures approximating 35° F.

Consequently a series of experiments was run to test several varieties in respect to hardening in a more extensive way. Samples were prepared, on each date, by removing the roots and a portion of the leaves from the individual plants leaving a clean, etiolated crown about an inch long. From such material, without washing, 2.5 gm. samples were secured, placed in Pyrex tubes and frozen in an air chamber at $-9°$ C. for 1.5 hr. After thawing at room temperature, 20 cc. of distilled water were added to each tube and exosmosis permitted to proceed at 25° C. for 10 hr. before conductivity measurements were made. Samples were first taken on March 7 from the greenhouse maintained at approximately 60° F. and subjected to the freezing-exosmosis treatment described. The house was then cooled as much as the prevailing weather would permit. It rarely reached a temperature below 50° F. although on two successive days the house was cooled to below 40° F. for a few hours. On these two cooler days, flats containing lumps of soil in which the wheat was growing were placed outside for an additional hardening treatment at a temperature of about 32° F. Samples were prepared from the greenhouse material and from the outside plats on March 15. Table II presents the data for the three hardening treatments. The first treatment represents, roughly, wheat grown for six weeks in a 60° F. house; the second, wheat with an additional week in a 45° F. house; and the third treatment, wheat with a further additional hardening of 12 or 15 hr. at a temperature approximating 32° F.

TABLE II

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 25° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF CROWNS OF SEVERAL VARIETIES OF WHEAT AND RYE FROZEN AT $-9°$ C. FOR 1.5 HOURS. AN INTERVAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS AT 25° C.

Variety	Unfrozen tissue from second hardening treatment (March 15)	Frozen after hardening treatment		
		First (60° F.) (March 7)	Second (45° F.) (March 15)	Third (32° F.) (March 15)
Wheat				
Wisconsin Pedigree no. 2	141	1764	920	726
Ashkoff	164	1123	987	747
Gladden	154	1362	765	774
Honor	86	1630	765	695
Marquis	305	2355		
Progress	324	2415		
Rye				
Wisconsin Pedigree no. 2	168	882		
Spring rye	303	2155		

That hardening occurred in these winter wheats as the temperature was lowered is clearly shown. However, no definite correlation of freezing injury and electrical conductivity with varietal hardiness is evident at any stage of this hardening treatment. It is very apparent from the figures given that the hardening process was by no means complete. From data obtained at a later date from field plats (see table V) this hardening treatment would appear to be insufficient to develop the characteristic varietal differences in hardiness which occur in the field under normal fall and winter environment. It is interesting to observe that, although the hardy types, Wisconsin Pedigree no. 2 wheat and Ashkoff wheat, had assumed a very recumbent type of growth, they were in an unhardened condition on March 7 and were very sensitive to freezing. The very high values for conductivity which are found with the unfrozen tissues of spring grains are of interest and are due perhaps to the very succulent type of tissue produced under greenhouse conditions.

Chloride tests on the exudates from the wheat crowns gave values varying in a manner comparable to the conductivity values, and could be used to distinguish, at least roughly, the hardened from the unhardened tissues.

Further studies of the hardiness of alfalfa in the autumn of 1930

On June 27, 1930, several additional varieties of alfalfa were seeded in rows and in field plats for study the following season. In the continuation of the work with alfalfa, several modifications of the procedure seemed necessary and desirable. The June seedlings were of such a size that no attempt was made to have equal numbers of plants in the twenty-gram samples from the various varieties. From the row cultures it was impossible to obtain strictly comparable samples. Poor stands in some of the rows and excessive crowding in others gave roots and plants of widely divergent sizes and development. To the contrary, samples from the field plats were comparable, and gave satisfactory checks on duplicates. In the plats used in 1929 (now three years old) winter-killing and insect-injury of the roots made sampling somewhat difficult. No attempt was made to use eight roots in samples weighing twenty grams, as in 1929 (See Dexter *et al.* (**3**), but the samples consisted, in 1930, of twenty gm. of representative root tissue without reference to the number of roots.

Additional low temperature facilities in 1930 permitted the use of a freezing chamber (7 cu. ft.) stirred with an electric fan, and, on occasion, of a large liquid bath. Either of these could be held at the desired temperature by thermostatic controls. The tissues were permitted to thaw by immersion of the tubes in an ice-water bath. After an hour or two, water at 0° C. was added to the tubes and exosmosis was permitted to continue for a period of twenty hours at this temperature, when electrical conductivities were determined. Exosmosis is somewhat less complete in this method than in the method used in 1929 (10 hours at 25° C.) but there is obviously less opportunity for complicating fermentations. In addition, the values given, being specific conductivities at 0° C. rather than at 25° C., must be multiplied by a constant (about 1.82) if they are to be compared with the previous figures. However, among themselves they are entirely comparable.

A series of row cultures of ten hardy strains introduced by H. L. WESTOVER, of the U. S. Department of Agriculture, was examined throughout the season. These varieties might be classed as Turkestan since they were selected in various parts of that area. All of these varieties were of a more or less dormant type. Every variety showed the same hardening effect that was reported in 1929, although some reached much lower values than others. From original conductance of approximately 750 for all varieties on October 1, final values of from 286 to 466 were reached on December 5. The varieties were rather uniform in hardiness on December 5, except for two or three varieties from the Khiva district which were hardier than the rest, as anticipated by WESTOVER. Since the varieties are unnamed, no value can be gained in this paper by enumerating the many conductivities taken on the various dates throughout the fall.

Another series of hardy varieties, in field plats, were compared as shown in table III. Several tender varieties are included for comparison. The data in this table indicate wide differences in hardiness between the very

TABLE III

SPECIFIC CONDUCTIVITIES ($x\ 10^6$, 0° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF ALFALFA ROOTS FROZEN 4 HOURS IN A LIQUID BATH AT −10° C. THE ROOTS WERE DUG ON DEC. 10, 1930, FROM SLIGHTLY FROZEN GROUND. AN INTERVAL OF 20 HOURS WAS ALLOWED FOR EXOSMOSIS AT 0° C.

VARIETY	AGE OF PLANTS	SPECIFIC CONDUCTIVITY
Canadian Variegated	Seedling 5 mos.	377
Grimm	Seedling "	384
Ladak	Seedling "	322
Hardistan	Seedling "	281
Turkestan	Seedling "	364
Cossack	Seedling "	384
Hairy Peruvian (row)	Seedling "	593
Hairy Peruvian (plat)	Three years	513
Argentine (row)	Seedling 5 mos.	546

dormant types, as Hardistan or Ladak, and the vegetative types such as Hairy Peruvian and Argentine. Duplicate samples from the field plats gave good check values for conductivity whereas the row cultures are sometimes uncertain in this respect.

Application of the test to red clover varieties

On December 9, red clover roots were dug from field plats which had been planted with barley as a nurse crop the preceding spring. The samples were prepared in the usual manner, using twenty-gram samples from the thoroughly washed and surface-dried roots. These samples were placed in Pyrex tubes, frozen four hours in a liquid bath at $-10°$ C. and thawed at 0° C. in ice-water. Exosmosis into 50 cc. of water at 0° C. was permitted to continue for 20 hours. Table IV indicates that a difference in hardiness existed between the Wisconsin strain grown in this state for fifty years, a variety from Missouri, and one imported from Italy. These results show that this test will differentiate the hardiness of roots of these three varieties of red clover.

TABLE IV

Specific conductivities ($\times 10^6$, 0° C.), expressed in reciprocal ohms, of water extracts of clover roots frozen 4 hours in a liquid bath at $-10°$ C. The roots were dug on Dec. 9, 1930, from slightly frozen ground. An interval of 20 hours was allowed for exosmosis at 0° C.

Variety	Specific conductivity
Wisconsin	730
Missouri	900
Italy	1171

Application of the test to winter-wheat sown in field plats at the usual rate per acre

Samples were prepared in the manner previously described, from plants dug from field plats of wheat on three dates in the fall. The samples consisted of 100 gm. of crowns. These were frozen on open screens in front of an electric fan in the freezing chamber. They were thawed in beakers immersed in ice-water. After thawing, the samples were placed in flasks, to which 750 cc. of water at 0° C. were added. Exosmosis was allowed to proceed for 16 hours at 0° C. Table V presents the results of these trials. In this table it should be noted that the freezing treatment was made more severe on December 6 than on the previous dates, since pronounced hardening had by this time rendered the plants of the hardier varieties unsusceptible to the milder freezing treatment. The table indicates the same tendency that has been described before. Following the hardening process

TABLE V

Specific conductivities ($\times 10^6$, 0° C.), expressed in reciprocal ohms, of water extracts of winter wheat crowns frozen as indicated. An interval of 16 hours was allowed for exosmosis at 0° C.

Variety	Freezing treatment on date specified		
	Oct. 25	Nov. 7	Dec. 6
	– 10° C. 2 hours	– 10° C. 2 hours	– 20° C. 2 hours
Minhardi	725	243	338
Wisconsin Pedigree no. 2	707	309	540
Trumbull	767	527	684

which occurred with cold weather, lower values of electrical conductance are found in exudates from the frozen tissues of varieties known to be hardy than from similar frozen tissues of tenderer strains, where greater injury from freezing occurred. In this particular case, Minhardi, a very frost-resistant wheat, is shown to be hardier than Wisconsin Pedigree no. 2 (a Turkey selection) which in turn is hardier than Trumbull.

Experiments with other small grains

Several varieties of rye, oats and barley, planted in rows gave results of a very similar nature. Table VI gives an excerpt from abundant data on several dates during the fall. The table shows the considerable degree of difference of the various varieties of rye to withstand severe freezing after all varieties had been exposed to snow and cold weather in the field. The winter and spring varieties of grain were clearly differentiated by a suitable freezing treatment at –5° C., while at –10° C. both were so severely injured that the difference is much less marked. On Oct. 15, very hardy varieties of winter wheat or rye were nearly as much injured at –5° C. as at –10° C. On the contrary, the southern winter varieties had hardened in the warm fall weather to such an extent that they were not damaged to any great degree at –5° C. but were very severely hurt by the more severe freezing treatment. There seems, therefore, to be a decided tendency for the southern winter varieties to harden in milder weather than the very hardy northern strains. An unusually warm period in November brought out the further fact that, with the exception of Minhardi winter wheat, all the ten varieties examined, showed rather definite breaking of the hardened condition with the elevated temperature, and greater injury from freezing following this period.

TABLE VI

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 0° C.), EXPRESSED IN RECIPROCAL OHMS, OF WATER EXTRACTS OF SMALL GRAIN CROWNS, FROZEN AS INDICATED. AN INTERVAL OF 16 HOURS WAS ALLOWED FOR EXOSMOSIS FROM A 2-GRAM SAMPLE INTO 15 CC. OF DISTILLED WATER AT 0° C.

VARIETY	DATE	FREEZING TREATMENT	SPECIFIC CONDUCTIVITY
Rye			
Wisconsin Pedigree no. 2 winter rye	Nov. 29	2 hours –20° C.	626
Abruzzi winter rye	"	"	769
Italian winter rye	"	"	898
Spring rye	"	"	1024
Oats			
Red rust-proof winter oats	Oct. 15	2 hours –5° C.	333
Wisconsin Pedigree no. 7 spring oats	"	"	665
Red rust-proof winter oats	Oct. 15	2 hours –10° C.	907
Wisconsin Pedigree no. 7 spring oats	"	"	925
Barley			
Union winter barley	Oct. 15	2 hours –5° C.	312
Wisconsin Pedigree no. 38 spring barley	"	"	475
Union winter barley	Oct. 15	2 hours –10° C.	898
Wisconsin Pedigree no. 38 spring barley	"	"	887

ROW VERSUS NATURAL FIELD SPACING

With the small grains planted in thick rows, as with the alfalfa, less satisfactory results were obtained, poorer checks of duplicate samples were secured, and in general a less reliable indication of winter-hardiness could be gained than when the plants were growing in dense, but uniform, populations under natural field conditions. Probably the crowding, uneven covering by soil, snow and surface materials, and the competition for mineral matter contribute to these discrepancies. In the case of alfalfa, the variation in the growth of individual plants in the row was extreme. It does not appear, however, that excessive growth of the seedling plants is necessarily conducive to hardiness and moreover we have clear preliminary evidence that deficiencies, either in regard to mineral nutrition or organic foods, may be restrictive in the full development of the hardened condition. Further investigations of these relations are in progress.

Determination of injury of alfalfa roots by measurement of electrical conductivity of the tissue itself after freezing

In that a test of injury would be useful which involved very small portions of tissue and which would not necessitate sacrifice or even severe disturbance of the mother plant, the following method was devised. One-centimeter sections were cut from various parts of three-year-old roots of Grimm, Utah Common and Hairy Peruvian alfalfa. These sections were quickly weighed, and fastened with fine wire so that they could be suspended in tubes for freezing. The tissues were frozen by suspending the tubes for four hours in a liquid bath at the desired temperature. Thawing was allowed to proceed for several hours in the water-bath at 0° C. After warming to room temperature the root sections were placed between two electrodes actuated by a spring clamp and the electrical resistance of the tissue itself measured. The reciprocal of this resistance gives the conductivity of the section, which is divided by the weight of the section to reduce all values to approximately equal cross-sections and, therefore, comparable values. Preliminary experiments on November 5 had shown that the electrical resistance of the root section decreases very considerably as a result of freezing, and may be, in general, from one-half to one-tenth of its former value. The Grimm sections showed decidedly less change in resistance and conductivity than those from Utah Common alfalfa, while Peruvian sections were still more markedly affected. It had also appeared that pieces of small roots and especially pieces taken at some distance below the crown were more tender than large pieces, especially when taken near the crown. It therefore seemed desirable to investigate the following points in a more extensive experiment.

1. The variation in hardiness of individual plants within a variety as compared with other varieties.
2. Variation in hardiness due to position of the section on the root.
3. Variation due to the size of the root.
4. Comparison of the conductivity of the tissues themselves with values for hardiness obtained by the exosmosis method.

On November 10, eight average, uniform roots and four large roots were selected from each of the three varieties. From each of the 36 roots a two-centimeter section at the crown was removed and retained to be added to the residue of the root for exosmosis. From the first four average roots (1 to 4) of each variety, two one-centimeter sections were cut, adjacent to each other at the upper end of the remaining root. From the other four average roots (5 to 8) the upper one-centimeter section was retained, then six centimeters of root removed below it and another one-centimeter section secured. The same procedure was followed using two of the larger roots (9 and 10) for adjacent sections, and two (11 and 12) for separated sections. These sections were weighed, suspended by fine wire in moistened tubes, frozen and thawed as previously described. The results are presented in table VII. From this table it is very evident that decided differences existed in the hardiness of individual plants of any given variety on November 10. It is not assumed that these values are a measure of the ultimate hardiness of the individual plants, since they would be expected to vary somewhat in rate of hardening as winter weather approached. It

TABLE VII

VARIATIONS IN ELECTRICAL CONDUCTIVITY OF SECTIONS OF ALFALFA ROOT TISSUES FROZEN 4 HOURS AT –10° C., ON NOVEMBER 10, 1930. THE RECIPROCAL OF THE RESISTANCE OF A ONE-CENTIMETER SECTION IS DIVIDED BY THE WEIGHT OF THE SECTION TO GIVE THE CONDUCTIVITY VALUES PRESENTED

Root	Position of section	Conductivity ($\times 10^6$) divided by weight of section		
		Grimm	Utah Common	Hairy Peruvian
1	Crown	498	891	1675
	Adjacent	745	972	1755
2	Crown	365	1410	2038
	Adjacent	407	1435	2000
3	Crown	521	618	1960
	Adjacent	617	576	2000
4	Crown	1165	892	4380
	Adjacent	1365	1100	4080
5	Crown	607	1040	1815
	6 cm. below	925	1408	1935
6	Crown	342	1302	1200
	6 cm. below	967	1400	1305
7	Crown	607	1565	1192
	6 cm. below	1209	2050	1485
8	Crown	685	1350	....
	6 cm. below	1513	1423	....
9	Crown	192	545	1105
	Adjacent	246	631	1215
10	Crown	697	776	1465
	Adjacent	823	761	1580
11	Crown	358	792	1025
	6 cm. below	598	1338	1510
12	Crown	623	1020	1242
	6 cm. below	1053	1357	1360

is apparent that at this time of year the position of the tissue on the root is of enormous importance in the case of Grimm, of some importance in the case of Utah, and of relatively little importance in the case of Hairy Peruvian roots. The crown tissue of Grimm roots is very much less affected by freezing than the tissue more deeply buried in the soil, while crown tissue of Hairy Peruvian is but slightly different from that at other points on the root. It would appear that the crown tissue of Grimm roots has become hardened by exposure to cold, while that of the tender variety is almost unchanged. The variation due to the size of root is relatively slight, although these data are insufficient to draw definite conclusions. Further data, by this method, later in the season, appear to indicate that the small Grimm roots from field plats are more severely injured by a given freezing treatment. In this test, as in the field, large roots of Utah Common and Hairy Peruvian seem to be distinctly more winter-resistant than smaller roots. Perhaps the small roots are more completely frozen in each case and it may be that the small size of these roots is but a reflection of unfavorable growing conditions which would affect hardiness adversely. It should be remarked that the winter of 1929–30 had killed most of the Hairy Peruvian in the field and that this group of roots no doubt represents the more hardy residue from natural selection. Most of the surviving roots of Hairy Peruvian were very large. The same statements are in a measure true of Utah Common, since over half of the plants in the field were killed during that winter, while the loss of Grimm plants was less than ten per cent. These facts no doubt explain the very great variation in the Grimm roots and the relatively more uniform condition of those of the tenderer varieties. Table VIII summarizes the results presented in table VII as to the range of hardiness of the crowns within each of the three varieties.

TABLE VIII

A SUMMARY OF TABLE VII. RANGE AND AVERAGE OF CONDUCTIVITIES OF ALFALFA CROWN TISSUES AFTER FREEZING 4 HOURS AT –10° C., NOVEMBER 10, 1930

VARIETY	RANGE OF CONDUCTIVITIES OF 12 FROZEN CROWN SECTIONS	AVERAGE OF 12 CROWNS	AVERAGE OF 4 LARGE CROWNS
Grimm	192 to 1165	555	468
Utah Common	545 to 1565	1017	783
Hairy Peruvian	1025 to 4380	1736	1209

Three grams of the residue of each root were frozen and exosmosis allowed to proceed for 20 hours into 15 cc. of water in the usual way. In every case, outstandingly low values of conductivity of the exudate corresponded to low values of conductivity of the tissue itself. Since residues of the roots were used, it was quite impossible to secure samples for exosmosis identical to those used for the measurement of tissue conductivity. In some cases, the sample for exosmosis had a higher position on the root than the one-centimeter sections of which the resistance was measured; in other cases the opposite was true. However, the two methods checked in a satisfactory manner, and outstandingly hardy or tender roots were in all cases indicated by both methods, in spite of the necessary discrepancies in sampling.

Hardening of isolated root sections

It was thought desirable to investigate the artificial hardening of the lower part of the root, since this point might be of great interest in connection with the applied phases of this work. On November 18, 1930, four roots of Grimm alfalfa were cut into twelve sections each, so that each third section in order of position on the root could be frozen and examined without hardening beyond that received in the field. The remaining sections were similarly divided into two groups to be kept at 0° C. for periods of one and two weeks respectively. Thus, sections 1, 4, 7 and 10 from each root were examined without hardening treatment; sections 2, 5, 8 and 11 were kept at 0° C. for one week; while the remaining sections, 3, 6, 9 and 12, were so hardened for two weeks. The sections to be hardened were cut a few millimeters longer than those used directly, so that they could be freshly cut to the proper length just before freezing, thus avoiding drying and formation of scar tissue on the ends of the sections. The sections were frozen and thawed as in the previous experiment. The conductivity values of the tissue at room temperature divided by the weight of the samples are presented in table IX. The test indicates that very positive hardening effects are obtained by keeping isolated sections at 0° C. for a week at this time of year. The effect varies with the individual root to a marked degree, some roots showing greater ability to harden than others. As a result of two weeks of exposure to a temperature of 0° C. two of the four roots withstood a freezing treatment at –12° C. better than they did one at –10° C. previous to hardening. Although the difference between the upper and lower portions of the root is still evident after two weeks of treatment, it is not as prominent as in roots fresh from the field.

In root 1, section 7 gives a peculiar value entirely out of line with any results obtained in the many samples examined. This unusual result may have been due to super-cooling without crystallization, which is relatively common at higher freezing temperatures (as at –5° C.).

An attempt was made to harden alfalfa roots dug August 27. These roots were in a condition of relatively "low organic reserve." In this case,

TABLE IX

SPECIFIC CONDUCTIVITY ($\times 10^6$) OF ONE CENTIMETER SECTIONS OF FOUR GRIMM ALFALFA ROOTS HARDENED AND FROZEN AS INDICATED. THE RECIPROCAL OF THE RESISTANCE OF A ONE-CENTIMETER SECTION IS DIVIDED BY THE WEIGHT OF THE SECTION TO GIVE THE CONDUCTIVITY VALUES PRESENTED

Section	Treatment		Conductivity			
	Hardened	Frozen	Root 1	Root 2	Root 3	Root 4
1	Field only	4 hrs. –10° C.	667	560	264	362
4	"	"	943	660	313	588
7	"	"	173	693	362	747
10	"	"	1120	767	562	862
	Additional					
2	1 week 0° C.	4 hrs. –10° C.	529	223	163	323
5	"	"	506	339	185	455
8	"	"	508	372	183	840
11	"	"	540	387	260	483
3	2 weeks 0° C.	4 hrs. –12° C.	634	328	285	646
6	"	"	887	385	251	777
9	"	"	814	392	307	1125
12	"	"	877	438	336	1058

ten 20-gm. samples of roots of each of the three varieties (Grimm, Utah Common and Hairy Peruvian) were prepared and placed in Pyrex tubes which were immersed in water at 0° C. for varying lengths of time. With the examination made by the exosmosis method, after freezing at –10° C. for four hours, very little or no hardening appeared to have taken place in the course of a month of such treatment, with any one of the three varieties. Duplicate samples of the three varieties taken from the hardening bath, were examined on five dates throughout the period, and the last of the "hardened" samples were finally compared on September 26 with samples fresh from the field. The "hardened" tissues were found to be very slightly more tender than the field samples. The peculiarity of failure to harden at this time of year should be examined further. It is quite possible that the attainment of complete hardening is dependent upon late fall conditions, involving such factors as short day, dormant condition, organic storage, and the predominance of sugar rather than starch in the roots.

Summary

Investigation has been devoted to a method previously proposed which indicates that the degree of hardiness of plants of certain species can be determined by measuring the extent of exosmosis of electrolytes from suit-

ably frozen tissue. The method has been applied with satisfactory results to a large group of varieties of alfalfa, to three strains of red clover and to over twenty varieties of small grains. Tissues grown under various conditions have been examined by this method and show the marked influence of environment upon the hardening process.

An alternate method for the determination of frost resistance is presented, in which the electrical conductivity of the tissue itself is determined after freezing. With three varieties of alfalfa of known hardiness, wide varietal differences are shown, as well as marked differences in hardiness of individual plants within a given variety. The upper root tissue of a hardy variety appears to harden first, and prior to winter temperatures is shown to be decidedly more resistant to freezing injury than the more deeply buried parts. But little difference in hardiness of different parts of the root is found in the case of the most tender strain. The hardening of isolated sections of root tissue taken from the field in November is shown to occur in a refrigerator at 0° C. while hardening treatment during rapid regeneration of new growth in August was not successful.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

LITERATURE CITED

1. BRIERLEY, W. G., and HILDRETH, A. C. Some studies on the hardiness of certain species of *Vaccinium*. Plant Physiol. **3**: 303–308. 1928.
2. CHANDLER, W. H. The killing of plant tissue by low temperature. Missouri Agr. Exp. Sta., Res. Bull. 8. 1913.
3. DEXTER, S. T., TOTTINGHAM, W. E., and GRABER, L. F. Preliminary results in measuring the hardiness of plants. Plant Physiol. **5**: 215–223. 1930.
4. DIXON, H. H., and ATKINS, W. R. G. Osmotic pressure in plants: I. Methods of extracting sap from plant organs. Sci. Proc. Roy. Dub. Soc. **13**: 422–433. 1913.
5. HARVEY, R. B. Length of exposure to low temperature as a factor in the hardening process in tree seedlings. Jour. Forestry **28**: 50–53. 1930.
6. HIBBARD, R. P., and MILLER, E. V. Biochemical studies on seed viability: I. Measurements of conductance and reduction. Plant Physiol. **3**: 335–352. 1928.
7. HILDRETH, A. C. Determination of hardiness in apple varieties and the relation of some factors to cold resistance. Minn. Agr. Exp. Sta., Tech. Bull. 42. 1926.

8. HILL, D. D., and SALMON, S. C. The resistance of certain varieties of winter wheat to artificially produced low temperatures. Jour. Agr. Res. **35**: 933–937. 1927.

9. HOAGLAND, D. R., and DAVIS, A. R. The composition of the cell sap of the plant in relation to the absorption of ions. Jour. Gen. Physiol. **5**: 629–646. 1923.

10. HOTTES, C. F., and HUELSEN, W. A. The determination of quality in sweet corn seed by means of the optical measurement of leached materials. Jour. Agr. Res. **35**: 147–166. 1927.

11. MARTIN, J. H. Comparative studies of winter hardiness in wheat. Jour. Agr. Res. **35**: 493–535. 1927.

12. MAXIMOV, N. A. Internal factors of frost and drought resistance in plants. Protoplasma **7**: 259–291. 1929.

13. MERRILL, M. C. Electrolytic determination of exosmosis from the roots of plants subjected to the action of various agents. Ann. Missouri Bot. Garden **2**: 507–572. 1915.

14. MOORE, J. C., REEVES, R. G., and HIXON, R. M. Electrodialysis as a means of studying biochemical differences in abnormal apple tissue. Plant Physiol. **2**: 313–324. 1927.

15. NEWTON, R. The nature and practical measurement of frost resistance in winter wheat. Univ. of Alberta, Res. Bull. 1. 1924.

16. OSTERHOUT, W. J. V. Injury, recovery, and death, in relation to conductivity and permeability. (Philadelphia and London.) 1922.

17. PANTANELLI, E. Alterazioni del ricambio e della permeabilita cellulare a temperature prossime al congelamento. Atti. Della R. Accad. Lincei Ser. 5, **28**: 205–209. 1919.

18. ROSA, J. T. Investigations on the hardening process in vegetable plants. Missouri Agr. Exp. Sta., Res. Bull. 48. 1921.

19. STEINBAUER, G. Differences in resistance to low temperatures shown by clover varieties. Plant Physiol. **1**: 281–286. 1926.

20. STEINMETZ, F. H. Winter hardiness in alfalfa varieties. Minnesota Agr. Exp. Sta., Tech. Bull. 38. 1926.

21. STILES, W., and JØRGENSON, I. The action of various organic substances on the permeability of the plant cell. Ann. Bot. **31**: 47–76. 1917.

22. WEIMER, J. L. Some factors involved in the winter killing of alfalfa. Jour. Agr. Res. **39**: 263–283. 1929.