A SEARCH FOR FACTORS DETERMINING WINTER HARDINESS IN ALFALFA

A SEARCH FOR FACTORS DETERMINING WINTER HARDINESS IN ALFALFA

By

Clive R. Megee

A THESIS

PRESENTED TO THE FACULTY

OF

MICHIGAN STATE COLLEGE

OF

AGRICULTURE AND APPLIED SICNECE

IN

PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

East Lansing

1 9 3 5

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to Dr. C. E. Millar, Dr. G. J. Bouyoucos and Dr. C. H. Spurway of the Soils Department, Professor H. C. Rather of the Farm Crops Department, and Dr. R. P. Hibbard of the Botany Department for their helpful suggestions during the course of the investigation of this problem and in the preparation of this paper.

A SEARCH FOR FACTORS DETERMINING WINTER HARDINESS IN ALFALFA

INTRODUCTION

In the northern part of the United States winter killing of alfalfa is often prevalent and the seeding of winter hardy strains is of paramount importance in maintaining satisfactory stands. Field tests to determine the relative winter hardiness of the various strains of alfalfa have contributed much toward enabling Michigan to become the second highest state in the acreage of alfalfa in the United States. The hardy strains of alfalfa, such as the Hardigan and Grimm are usually able to withstand low temperatures without winter killing while the non-hardy strains, such as the Arizona Common and Hairy Peruvian often are killed the first winter. There are many strains occupying an intermediate position of winter hardiness between these extremes.

During the past 15 years the writer (14) has conducted many field tests to determine the relative winter hardiness of the various strains of alfalfa of the United States and many foreign countries. In order for a field test to reach a high degree of dependability it is necessary that it extend over a period of from 4 to 8 years and this involves a serious delay as well as considerable expense in conducting the test. While such tests have been of much value in determining the relative winter hardiness of the various strains of alfalfa, they have in no way explained the phenomenon of winter hardiness.

Increase in colloid content and osmotic pressure of root sap, and decrease in moisture content of alfalfa tissue have been suggested as an explanation for winter hardiness. Should it be possible to locate some one or more factors definitely correlated with winter hardiness that could readily be determined in the laboratory, this problem of the agronomist would be greatly simplified.

The object of this paper is to present data on the relationship between winter hardiness of alfalfa and various properties of alfalfa root tissue as measured by electrical conductivity, moisture equivalent, swelling, heat of wetting of oven dry tissue, respiration, freezing point depression, moisture content and the rate of loss of moisture. Should any of the above factors prove to be directly correlated with winter hardiness, the fact would be an important step in determining and possibly explaining the phenomenon of winter hardiness.

It is generally recognized that damage to plants may result from winter injury due to several causes, such as low temperature, heaving of soil and plants by the building up of layers of ice in the soil, suffocation under an ice crust, and the desiccation of plant tissues due to dry winds. In this paper only the first form, winter injury due to low temperature, is considered. In the experiments here reported, the other three forms of winter injury were almost, if not entirely, absent.

REVIEW OF LITERATURE

No attempt will be made to review the voluminous literature on the subject of winter hardiness. Harvey (12) gives a very complete bibliography on the low temperature relations of plants. Chandler (5), Dexter (6), Graber (10), Gortner (9), Harvey (11), Maximov (13), Newton (15) and Rosa (16) not only give their views but also have reviewed the work of other investigators. Dexter (6) upon mentioning the work of other investigators states; "It may be briefly stated that most, if not all, of the theories of winter hardiness are built around the idea of the water relation of the plants and that structural, osmotic, or colloidal protection from water withdrawal or ice formation form the basis of explanation for individual or varietal differences in hardiness".

MATERIAL USED

Three strains of alfalfa of known winter hardiness; the Hardigan, Utah Common and Arizona Common, were selected for these experiments from among a large number of strains representing most of the seed producing sections of the world. Field tests of these strains for winter hardiness were previously conducted by the writer over a period of eight years. Plots seeded in 1921 were continued until 1929. Other plots seeded in 1922, 1923 and 1924 were continued for periods ranging from three to five years. Table 1 shows the comparative winter hardiness of these three strains under Michigan conditions.

Table 1. Comparative winter hardiness as indicated by yield of Hardigan, Utah Common and Arizona Common alfalfas at East Lansing, Michigan.

	Yields of hay expressed in per cent of Hardigan yields									Yield per acre
	1922 %	1923 %	1924 %	1925 %	1926 %	1927 %	1928 %	1929 %	Ave. %	Ave. Tons
Hardigan	100	100	100	100	100	100	100	100	100	4.8
Utah Common	93.7	82.9	90.7	65.9	50.2	47.0	64.6	57.6	69.1	3.3
Arizona Common	63.3	10.8	19.6	11.7	21.9	0.0	0.0	0.0	15.9	0.8

The Hardigan is a variegated strain of alfalfa developed at the Michigan Agricultural Experiment Station and is very winter hardy under Michigan conditions. The Utah Common is a strain of only medium winter hardiness and has been grown for many years in the alfalfa seed producing section of the Uinta

Basin of Utah. The Arizona Common is from the Yuma Valley of southwestern Arizona and is one of the least winter hardy strains of alfalfa.

It has been observed that alfalfa plants sometimes live through the winter entirely devoid of leaves. In view of this observation the material used in the laboratory tests consisted of the root and a portion of the crown, the top being removed just above the lower crown buds. Although containing a portion of the crown, this material is referred to as the root.

The roots used were of the first season's growth and were grown on a Hillsdale sandy loam soil. Roots were used from both fertilized and unfertilized plots. The amounts of superphosphate and muriate of potash necessary to apply to the soil in order to have a good supply of available phosphorus and potassium present as determined by the Spurway Method (17) were found to be equivalent to the nutrients in 2400 pounds per acre application of an 0-8-24 fertilizer. These materials were applied to a series of plots on May 29 and worked in as the soil was prepared for seeding which was done June 8. On September 4 a like application was made as a top dressing on another series of plots. On the same date an application of 500 pounds per acre of nitrate of soda as a top dressing was made on a third series of plots. The fertilized plots were 25 x 300 feet and the varieties of alfalfa were seeded in quadruplicate plots 25 x 330 feet running across the fertilized and unfertilized plots. This arrangement provided roots of both hardy and non-hardy alfalfas grown on fertilized and unfertilized soil.

EXPERIMENTAL RESULTS

THE RELATION BETWEEN WINTER HARDINESS AND THE LIBERATION OF ELECTROLYTES BY ALFALFA ROOT TISSUE EXPOSED TO LOW TEMPERATURE.

It is a matter of frequent observation that as the living protoplasm of a cell is injured it becomes more permeable and the electrolytes pass out of the cell. As the injury becomes more intense the permeability increases, and additional electrolytes are liberated. To determine the amount of electrolytes liberated when alfalfa roots were submitted to low temperature, electrical conductivity determinations were made on water extract of roots of hardy and non-hardy alfalfas collected from fertilized and unfertilized plots. The method suggested by Dexter (6) was used.

Extreme care must be exercised in preparing alfalfa field material for electrical conductivity tests. Hardigan, though one of the most uniform strains of alfalfa, is not a pure line selection owing to its method of pollination. Consequently, some variations will be found in the winter hardiness of different individual plants. Also an entirely uniform soil cannot be secured for field work. To eliminate these variations in so far as possible, plants were selected from various parts of the plot and after thorough washing and removal of the external moisture, the roots were cut into three-fourths inch pieces and the pieces thoroughly mixed so that the ten gram sample used would represent the plot as a whole.

The samples were then placed in Pyrex glass tubes and the tubes held in alcohol slush in a cold chamber. The alcohol slush was found to be very efficient in maintaining the material at a constant temperature, variations seldom exceeding one degree. Samples taken in October and November were frozen at -9° C for four hours, but as the season advanced it was found necessary to freeze later samplings for a longer period, or at a lower temperature. At the end of the freezing period 75 c.c. of distilled water were added and the tubes placed in a water bath maintained at 2° C. Exosmosis was allowed to proceed for twenty hours, at the end of which time conductivity readings were made by means of a Wheatstone bridge. The Wheatstone bridge readings are expressed in specific conductivity ($x\ 10^{-6}$) in reciprocal ohms (mhos) per gram of dry matter. Before making the reading each tube was inverted three times to secure an equal distribution of the electrolytes through the solution. Student's method was applied to data from triplicate samples and in most instances differences greater than 8 per cent were found to be significant.

Electrical conductivity tests were made on samples of roots taken October 16, 1933, from the Hardigan and Arizona Common varieties from plots receiving no fertilizer treatment and from plots receiving each of the fertilizer treatments. No varietal or plot differences were found. Hardening had not developed to a point where a distinction could be made between hardy and non-hardy alfalfas.

On November 23, 1933, the test was repeated. The roots were frozen four hours at -9° C and 20 hours were allowed for exosmosis.

Table 2. Effect of freezing on the liberation of electrolytes from roots of hardy and non-hardy alfalfas grown on fertilized and unfertilized soil. Test made November 23, 1933.

Fertilizer Treatment	Portion Root Used	Hardigan	Arizona Common	Difference Per Cent
		mhos	mhos	
None	Upper 5 inches	37.0	53.7	45.1
	Lower&Fibrous	61.5	70.5	14.6
0-8-24	Upper 5 inches	50.5	64.5	27.7
Sept.4	Lower&Fibrous	75.1	77.5	3.1

The results, presented in Table 2, show that the Hardigan roots were much more resistant to low temperature than were those of the Arizona Common. Resistance to damage from low temperature developed most rapidly in the upper portion of the tap root while it was delayed at lower depths and in the small lateral and hair roots. Heavy applications of an 0-8-24 fertilizer on September 4 caused resistance to damage by cold to develop slowly. No difference was found in the average air-dried weight of hardy and non-hardy alfalfa roots, consequently the weight of the root is not a factor in winter hardiness.

In contrast to the results presented above, no differences were found in the winter hardiness of the alfalfa roots from the plots receiving no fertilizer, the roots from plots receiving fertilizer on May 29, and the roots from plots receiving nitrate of soda on September 4.

The electrical conductivity tests were repeated on samples taken December 16, and December 18. The relationship of Hardigan to Arizona Common and the effects of different soil treatments had not changed. Somewhat more resistance to liberation of solubles through freezing had developed, but just how much could not be determined because the roots dug December 16 were frozen for fifteen hours at -9° C and those dug December 18 were frozen for six hours at -15° C.

Another lot of Hardigan roots was dug January 15, the object of this test being to determine the relative winter hardiness of the roots from plots receiving no fertilizer treatment with those from the plots receiving 2400 pounds of 0-8-24 on September 4, and also to determine the relative amounts of electrolytes liberated when the roots were not cut, cut into 3/4 inch lengths, into 3/8 inch lengths, and when ground. The roots were frozen 15 hours at -9° C and

20 hours were allowed for exosmosis at 2° C. After the determinations were made the samples were boiled for 15 minutes, 20 hours allowed for exosmosis at 2° C, and the electrical conductivity redetermined.

Table 3. The effect of freezing upon the liberation of electrolytes from roots of hardy alfalfa when grown on fertilized and unfertilized soil and when tap and fibrous roots were cut at different lengths and when ground. Test made January 15, 1934.

Part of Roots Used	Size of Pieces	No soil treatment		Fertilized Sept.4		% Difference Frozen	% Difference Boiled
		Frozen	Frozen & Boiled	Frozen	Frozen & Boiled		
		mhos	mhos	mhos	mhos		
Tap	Uncut	25.2	121.3	30.5	125.0	21.0	3.0
Tap	3/4 in.	28.7	137.2	34.7	139.5	20.9	1.6
Tap	3/8 in.	34.3	143.6	41.2	144.5	20.1	0.6
Tap	Ground	52.8	151.5	60.5	153.2	14.5	1.7
Fibrous	3/4 in.	49.0	151.6	54.7	155.2	11.1	2.3
Fibrous	Ground	63.4	156.2	68.6	158.0	8.2	1.1

The results in Tables 2 and 3 show that the roots from plots receiving an 0-8-24 fertilizer on September 4 hardened less rapidly during November than the roots from the other plots, but during late December and January they hardened more rapidly. The injurious effect of low temperature upon alfalfa roots receiving fertilizer during September would be determined largely upon whether the soil temperature was low during the fall or whether it was low during the winter. During 1933-1934 the soil temperature was not low until after January 15 and all plots of Hardigan came through with very little, if any injury. None of the Arizona plots had been injured to any appreciable extent by January 15, but winterkilled 99% by spring. The test ran January 15 showed that all plots of alfalfa had made a considerable increase in winter hardiness since November 23. This increase was greater than is shown by comparing the figures in Tables 2 and 3, since the roots dug November 25 were frozen four hours at -9° C, while those dug January 15 were frozen 15 hours at -9° C and the latter liberated less electrolytes.

Data in Table 3 also show that grinding tap roots and grinding and cutting fibrous roots failed to bring out differences in winter hardiness by the electrical conductivity method to the extent that was brought out with uncut roots and with tap roots cut in lengths of 3/4 inch and 3/8 inch. The results secured after boiling show that the greater liberation of electrolytes from the roots from plots receiving fertilizer on September 4 was due to less hardening and not to an excess of salt stored in the roots. The results secured when the tests were repeated during the season 1934-35 were the same, except that the amount of electrolytes released after boiling was somewhat more variable, likely due to the extreme drought of the summer of 1934. This fact did not change the relationship of one variety to another or of one plot treatment to another in comparison with the results of 1933-1934. The electrical conductivity test was found to be valuable in determining the relative winter hardiness of different alfalfas.

PARTIAL CHEMICAL ANALYSIS OF HARDY AND NON-HARDY ALFALFA ROOTS

A chemical analysis* of the roots was made to determine whether there was a relationship between winter hardiness and the chemical composition of the roots. The results of the chemical analysis are shown in Table 4.

Table 4. A comparison of partial chemical analysis of roots of hardy and non-hardy alfalfas.

Roots dug October 16, 1934

Variety	Treatment	H_2O %	Protein %	Ash %	Fat %	Crude Fiber %	N.F.E. %	C H O %
Hardigan	None	7.39	15.60	2.11	0.66	16.68	57.56	74.24
Arizona C.	None	7.26	14.99	2.61	0.77	18.04	56.33	74.37
Hardigan	0-8-24	7.15	14.57	3.32	0.63	14.34	59.99	74.35
Arizona C.	May 29	7.22	14.63	2.04	0.48	17.02	58.69	75.71
Hardigan	0-8-24	7.22	15.16	2.63	0.69	14.60	59.70	74.30
Arizona C.	Sept.4	7.04	14.29	2.80	0.39	17.33	58.15	75.48
Average			14.87	2.58	0.60	16.33	58.40	74.74

Roots dug December 20, 1934

Variety	Treatment	H_2O %	Protein %	Ash %	Fat %	Crude Fiber %	N.F.E. %	C H O %
Hardigan	None	5.27	19.08	2.23	1.76	23.39	48.27	71.66
Utah C.	None	6.56	17.71	2.25	1.59	21.19	50.70	71.89
Arizona C.	None	5.92	16.34	2.17	1.47	25.68	48.42	74.10
Hardigan	0-8-24	5.63	17.81	2.87	1.32	18.30	54.57	72.37
Utah C.	May 29	5.98	17.50	2.53	0.97	19.02	53.65	72.67
Arizona C.		6.03	15.71	2.63	1.32	21.25	53.41	74.66
Hardigan	0-8-24	5.46	17.98	3.16	1.99	19.74	51.67	71.41
Arizona C.	Sept.4	5.76	16.41	2.85	1.28	23.84	49.86	73.70
Average			17.31	2.59	1.46	21.55	51.32	72.80

*The writer appreciates the assistance of the Chemistry Section of the Michigan Agricultural Experiment Station in determining the chemical analysis.

The chemical analysis of the alfalfa roots afforded no positive indication concerning factors causing winter hardiness. There are, however, some consistent differences in the protein and crude fiber content of the Hardigan and Arizona Common roots. On December 20, the date when a certain degree of winter hardiness had developed, the protein content of the Hardigan was higher than that of the Arizona Common and the increase in protein content at this date over that of October 16 was greater in the case of the Hardigan than for the Arizona Common. The crude fiber content of the Hardigan alfalfa was lower in all cases than that of the Arizona Common at both dates of sampling and the crude fiber content of Arizona Common increased markedly over that of the Hardigan between the two dates. The sum of the protein and nitrogen free extract content of Hardigan was slightly but consistently greater than that of the Arizona Common.

THE MOISTURE CONTENT OF THE ROOTS OF HARDY AND NON-HARDY ALFALFAS

The moisture content of many winter hardy plants decreases as the fall and winter seasons advance. In order to determine the relationship between winter hardiness and moisture content, the amount of moisture in the roots of Hardigan and Arizona Common strains of alfalfa was determined at intervals during the fall and winter months. An electrically controlled oven maintained at 90° C was used for this purpose. The results are presented in Table 5.

Table 5. The moisture content of the roots of hardy and non-hardy alfalfas from October to February.

Date Roots Dug 1934-35	Per Cent Moisture	
	Hardigan	Arizona Common
Oct. 24	66.7	67.0
Nov. 19	60.0	61.3
Dec. 22	62.9	63.0
Jan. 1	62.4	62.3
Jan. 21	64.0	64.0
Feb. 6	68.4	68.5
Feb. 20	68.2	66.7
Growing	76.4	76.8
Average	66.1	66.2

The results show that no varietal differences in the moisture content of the roots existed neither during the fall nor winter months nor when the roots were in a growing condition. Hardened roots of winter hardy strains of alfalfa were low in moisture, as were the roots of the non-hardy alfalfas. Consequently, there is no significant correlation between winter hardiness and moisture content.

THE RATE OF LOSS OF MOISTURE FROM THE ROOTS OF HARDY AND NON-HARDY ALFALFAS

Protection against water withdrawal has been advanced as of importance in preventing winter injury. In case this is true the Hardigan should release its moisture more slowly at low temperatures than should the less winter hardy Arizona Common. The relative loss of moisture of the roots of both the Hardigan and Arizona Common was determined. Hardened living roots were dug February 20, and slowly reduced in moisture content in desiccators held at 2° C and at -9° C. The samples were weighed daily and the daily loss of moisture calculated. The results are shown in Table 6.

Table 6. The rate of loss of moisture from roots of hardy and non-hardy alfalfas held at 2° C and -9° C.

No. days	Held at 2° C.				Held at -9° C.			
	Total loss %		Loss per day %		Total loss %		Loss per day %	
	Hardigan	Arizona C.	Hardigan	Arizona C.	Hardigan	Arizona C.	Hardigan	Arizona C.
1	23.0	21.0	23.0	21.0	14.0	18.2	14.0	18.2
2	33.8	32.4	10.8	11.4	25.6	27.0	11.6	8.8
3	48.2	45.8	14.4	13.4	35.6	38.8	10.0	11.8
4	59.0	57.4	10.8	11.6	43.8	48.0	8.2	9.2
5	62.4	62.6	3.4	5.2	47.6	52.4	3.8	4.4
6	64.0	65.4	1.6	2.8	48.6	53.4	1.0	1.0
7	64.8	66.2	0.8	0.8	60.0	62.4	11.4	9.0
8	65.0	66.4	0.2	0.2	63.0	63.8	3.0	1.4
9	65.4	66.8	0.4	0.4	65.0	64.6	2.0	0.8
10	65.6	66.8	0.2	0.0	66.8	65.2	1.8	0.6
11	66.0	67.4	0.4	0.6	67.4	66.0	0.6	0.8
12	68.2	69.2	2.2	1.8	68.6	66.0	1.2	0.0

On the eighth day samples of roots of both Hardigan and Arizona Common were removed from the desiccator held at 2° C. The roots were transplanted into flower pots filled with sandy soil and held at favorable conditions for growth. Growth was resumed, although 65 per cent of the original weight of the roots had been lost due to the loss of moisture. The results show that both hardy and non-hardy alfalfa roots can withstand a heavy loss of moisture without being severely injured. The results also show that there is no consistent difference between hardy and non-hardy alfalfas in either the rate of loss of moisture or total moisture lost, and that the loss of moisture was not correlated with winter hardiness.

THE RELATIVE RATE OF RESPIRATION OF ROOTS OF HARDY AND NON-HARDY ALFALFAS

Respiration is the process of energy release in the living cell. The end products of aerobic respiration are CO_2 and water and the intensity of respiration may be determined by measuring the amount of CO_2 liberated over a given period of time. Plants become dormant as the fall and winter seasons advance and the process of energy release slows down.

In order to determine the relationship between winter hardiness and the rate of energy release, the relative rate of respiration of hardy and non-hardy alfalfas was determined. The roots were placed in 500 c.c. Erlemeyer flasks fitted with rubber stoppers and in-take and out-let tubes. The tubes were kept stoppered except when connected to the train for carbon dioxide determinations. The train was set up as follows: air pump, potassium hydroxide solution, ascarite, phosphoric anhydride, concentrated sulphuric acid, sample, distilled water, potassium hydroxide. Duplicate twenty gram samples were used. The apparatus was checked repeatedly for leaks. Each sample was connected into the train for five minutes, a previous test having shown that the air was completely changed in two minutes. The results are shown in Table 7.

Table 7. The relative rate of respiration of hardy and non-hardy alfalfa roots held at different temperatures.

Condition of Roots	Temperature Centigrade	Average Daily Liberation of CO_2	
		Hardigan Grams	Arizona Common Grams
Not hardened	21°	0.1151	0.1185
Not hardened	10°	0.0163	0.0157
Not hardened	2°	0.0139	0.0129
Hardened	15°	0.0400	0.0384
Hardened	2°	0.0145	0.0140

The results show no difference in the rate of respirtion between roots of hardy and non-hardy alfalfas.

THE RELATION BETWEEN WINTER HARDINESS AND THE PHYSICO-CHEMICAL BEHAVIOR OF THE COLLOIDAL MATERIAL IN ALFALFA ROOTS

The great importance of colloidal materials in the functioning of living matter immediately raises the question of the relationship between winter hardiness and the physico-chemical nature of the colloidal material in hardy and non-hardy alfalfas. Moisture equivalent (3), swelling (4), and heat of wetting (2) afford good indices of physico-chemical differences of colloidal materials. The above mentioned indices were used to study the relationship between winter hardiness and the nature of the colloidal materials present in hardy and non-hardy alfalfas.

Roots of the hardy and non-hardy alfalfas from plots receiving the various fertilizer treatments were dug at dates ranging from October 21, to December 17. These roots were thoroughly washed, dried at air temperature, and ground to a very fine meal. Each sample was thoroughly mixed to facilitate sampling.

THE MOISTURE EQUIVALENT OF COLLOIDAL MATERIAL OF THE ROOTS OF HARDY AND NON-HARDY ALFALFAS

The moisture equivalent was determined as follows: A Büchner funnel fitted with a filter paper was filled with ground roots and the funnel tapped gently twenty times to secure a uniform settling of the material. The funnel was then placed in a beaker of water for four hours and the excess water removed by means of suction filtration for fifteen minutes. The material was then removed from the filter, weighed, and placed in an electrically controlled oven held at ninety degrees Centigrade for sixteen hours. The material was then weighed and the amount of water adsorbed was calculated. The results are presented in Table 8.

Table 8. The moisture equivalent of the colloidal material in roots of hardy and non-hardy alfalfas.

Date Roots Dug 1934	Fertilizer Treatment		Moisture Equivalent		
	Kind	Date Applied '34	Hardigan	Utah Common	Arizona Common
			per cent	per cent	per cent
Oct. 21	0-8-24	May 29	182	----	172
Nov. 6	None	----	200	----	199
Nov. 6	0-8-24	May 29	187	----	180
Nov. 6	0-8-24	Sept. 4	201	----	194
Nov. 6	Nitrate of Soda	Sept. 4	207	----	198
Nov. 24	None	----	219	----	222
Nov. 24	0-8-24	May 29	221	----	215
Dec. 17	None	----	263	261	265
Dec. 17	0-8-24	May 29	266	262	252
Dec. 17	0-8-24	Sept. 4	256	264	269
Dec. 17	Nitrate of Soda	Sept. 4	260	265	259

The results show a marked change from late October into December in the nature of the colloidal material present in the roots of both hardy and non-hardy alfalfas. Winter hardiness is not correlated with this change in the nature of the colloidal material, since the change takes place to the same extent in both hardy and non-hardy alfalfas.

THE SWELLING OF COLLOIDAL MATERIAL IN THE ROOTS OF HARDY AND NON-HARDY ALFALFAS

A 5 gram sample of material was weighed out and placed in a 50 c.c. graduated cylinder which was then filled with distilled water to the 50 c.c. mark. The material was stirred to secure uniform wetting, shaken, and enough water added to again fill to the 50 c.c. mark. Care was exercised in securing cylinders of the same diameter and calibration. The cylinders were then placed in a chamber maintained at 10° C for 16 hours, at the end of which time readings were made.

Table 9. The swelling of the colloidal material of the roots of hardy and non-hardy alfalfas during the fall and early winter months.

Date Roots Dug 1934	Fertilizer Treatment		Swelling		
	Kind	Date Applied '34	Hardigan	Utah Common	Arizona Common
			c.c.	c.c.	c.c.
Oct. 21	0-8-24	Sept. 4	24.0	----	24.5
Nov. 6	None	----	27.0	----	26.5
Nov. 6	0-8-24	May 29	26.5	----	27.0
Nov. 6	0-8-24	Sept. 4	27.0	----	27.0
Nov. 6	Nitrate of Soda	Sept. 4	27.5	----	26.0
Nov. 24	None	----	33.5	----	33.0
Nov. 24	0-8-24	May 29	33.0	----	31.0
Dec. 17	None	----	34.0	34.0	34.0
Dec. 17	0-8-24	May 29	33.5	34.0	33.0
Dec. 17	0-8-24	Sept. 4	35.0	36.0	35.0
Dec. 17	Nitrate of Soda	Sept. 4	34.0	33.0	35.0
Average Roots Dug Dec. 17			34.1	34.2	34.2

The results show a change from October 21 to December 17 in the physico-chemical nature of the colloidal material present in the roots. This change is shown by the increase in the swelling of the colloidal material. The seasonal change from October 21 to December 17 was the same for the non-hardy as for the hardy alfalfas. Fertilizer treatments did not influence the change. These results support those secured by the moisture equivalent index, in that winter hardiness is not a direct result of certain physico-chemical changes in the colloidal material of the alfalfa roots.

THE HEAT OF WETTING OF COLLOIDAL MATERIAL IN THE ROOTS OF HARDY AND NON-HARDY ALFALFAS

A 20 gram sample of air-dried material was placed in a wide glass tube and allowed to dry in an electrically heated oven at a temperature of 90° C for 16 hours. The tube was then removed from the oven, closed tightly with a rubber stopper, and placed on the desk close to the calorimeter, a liter of distilled water, and another sample of material, so that all samples and equipment would reach the same temperature. After a uniform temperature was established 100 c.c. of water was placed in the calorimeter and the heat of wetting of the plant material ascertained (2). The tube was handled with a heavy cloth so that heat would not be imparted to the material. Great care was taken to have the calorimeter, the water, and the material at the same temperature which was that of the room.

Table 10. The heat of wetting of colloidal material in the roots of hardy and non-hardy alfalfas.

Date Roots Dug '34	Fertilizer Treatment		Heat of Wetting	
	Kind	Date Applied '34	Hardigan	Arizona Common
			Calories	Calories
Nov. 1	None	---	100.0	101.8
Nov. 1	0-8-24	May 29	103.0	100.0
Dec. 17	None	---	131.0	131.2
Dec. 17	0-8-24	May 29	129.3	128.1

A change in the physico-chemical nature of the colloidal material from November 1 to December 17 was shown by the increase in the heat of wetting. The change was similar for both hardy and non-hardy alfalfas. These results support those secured by both moisture equivalent and swelling determinations. Neither moisture equivalent, swelling, nor heat of wetting were found to be an index of winter hardiness in alfalfa.

THE SOLUBLE MATERIAL IN ROOTS OF HARDY AND NON-HARDY ALFALFAS AS SHOWN BY THE FREEZING POINT METHOD

The concentration of the material in solution in the plant cell influences substantially the intake, outgo, and translocation of materials in plants.

The freezing point method was used to determine the relation between winter hardiness and the quantity of soluble material in roots of hardy and non-hardy alfalfas. A 5 gram sample of air-dried root material was placed in the freezing tube and 20 c.c. of distilled water was added. The contents of the tube was stirred thoroughly and allowed to stand for twenty minutes. The freezing point was then determined (1), making use of a freezing bath with a temperature of -2.5° C. The contents of the tube was supercooled about one degree before solidification was induced.

Table 11. Freezing point depression of roots of hardy and non-hardy alfalfas during October, November and December.

Date Roots Dug 1934	Fertilizer Treatment		Freezing Point Depression		
	Kind N-P-K	Date Applied '34	Hardigan	Utah Common	Arizona Common
			-°C	-°C	-°C
Oct. 21	0-8-24	Sept. 4	0.56	---	0.56
Nov. 6	None	---	0.61	---	0.60
Nov. 6	0-8-24	May 29	0.60	---	0.57
Nov. 6	0-8-24	Sept. 4	0.62	---	0.61
Nov. 6	Nitrate Soda	Sept. 4	0.66	---	0.63
Nov. 24	None	---	0.64	---	0.63
Nov. 24	0-8-24	May 29	0.68	---	0.67
Dec. 17	None	---	0.52	0.53	0.53
Dec. 17	0-8-24	May 29	0.71	0.70	0.69
Dec. 17	0-8-24	Sept. 4	0.67	0.68	0.66
Dec. 17	Nitrate Soda	Sept. 4	0.68	0.67	0.67

The soluble material in a small number of undried samples was determined by the freezing point method. Immediately upon digging, the roots were washed and ground after removing the external moisture. The determinations were made without addition of water. The freezing point depression of the Hardigan roots averaged $-1.56^{\circ}C$ and of the Arizona Common -1.50° C when the roots were dug December 17. The results showed little difference in the concentration of soluble material in the roots of hardy and non-hardy alfalfas when measured by the freezing point method. This method cannot be used, therefore, to measure the winter hardiness of strains of alfalfa.

DISCUSSION

The relative winter hardiness of the various strains of alfalfa has been quite successfully determined by means of field tests. In order to insure a high degree of accuracy, the field tests must extend over a long period of time. A simple laboratory test for predicting winter hardiness would aid the agronomist in determining the relative winter hardiness of the different strains of alfalfa and also assist in explaining the phenomenon of winter hardiness. In this paper are presented data from comparative studies of roots of hardy and non-hardy strains of alfalfa including determination, by electrical conductivity, of soluble material liberated through submission to low temperatures; chemical composition; moisture equivalent, swelling, and heat of wetting of finely ground root material; amount and rate of loss of moisture; and respiration. These are factors which it was thought might in part determine or explain the phenomenon of winter hardiness.

The electrical conductivity method was found useful in measuring the relative degree of hardening which had taken place in the alfalfa roots at the time the tests were conducted. No differences in hardening between hardy and non-hardy alfalfa were found on October 16, however, marked differences were found on November 23, and at later dates. The varietal differences agreed with the previously conducted field tests. Hardening was found to develop most rapidly in the upper portion of the tap roots, while it was delayed in the lower portion and in the small lateral and hair roots. A heavy application of an 0-8-24 fertilizer on September 4 caused hardening to develop much more slowly during November and December.

Even though the electrical conductivity method was of value in determining the relative winter hardiness of different lots of alfalfa, it did not offer an explanation of the phenomenon of winter hardiness.

Three hypotheses may be suggested to account for the different degrees of winter hardiness found in the different strains of alfalfa. These hypotheses are as follows:

1. Winter hardiness may be caused by a physico-chemical difference in the roots of hardy and non-hardy alfalfas, due to the condition of the material (whether colloidal, in solution, etc.) and composition (whether present as sugar, starch, and protein), and the structure of the tissue present. Factors such as heat of wetting, swelling, moisture equivalent, freezing point depression, chemical analysis, and the amount and rate of loss of moisture afford good indices of physico-chemical differences. These indices showed that there were no physico-chemical differences present in the roots of hardy and non-hardy alfalfas, therefore, affording no support for this hypothesis.

2. Winter hardiness may be caused by bio-chemical or functional differences brought about by the secretion of substances such as enzymes and hormones, and these differences reflected in the energy release in the cell protoplasm. The rate of respiration was used as an index of energy release and was found to be the same for both hardy and non-hardy alfalfas. The work of Dexter (8) and Tysdal (19) support these results. The acceptance of this hypothesis is not justified.

3. Winter hardiness is an hereditary factor transmitted from generation to generation. This hypothesis has not been

worked out in detail by the geneticists and plant breeders. However, the behavior of succeeding generations of Hardigan, Grimm, Utah Common, Arizona Common, etc., in transmitting different degrees of winter hardiness affords sufficient evidence to warrant the acceptance of this hypothesis.

CONCLUSIONS

A search was made to find factors that could be used in the laboratory to predict the relative winter hardiness of alfalfas.

The results showed that the relative degree of injury of alfalfa roots by low temperature was indicated by electrical conductivity and in this manner the relative winter hardiness of different lots of alfalfas was determined.

No direct relationship was found to exist between winter hardiness and heat of wetting, swelling, moisture equivalent, freezing point, chemical composition, respiration and amount and rate of loss of moisture in roots of hardy and non-hardy alfalfas.

Heredity is the most plausible explanation of the phenomenon of winter hardiness.

For the time being it is necessary to adhere to field tests aided by electrical conductivity tests for determining the relative winter hardiness of different lots of alfalfas.

BIBLIOGRAPHY

1. Bouyoucos, G. J. The Freezing Point Method as a New Means of Measuring the Concentration of the Soil Solution Directly in the Soil. Mich. Agr. Expt. Sta. Res. Bul. 24, 1915.

2. ---------------- Heat of Wetting as a New Means of Estimating the Colloidal Material in Soils. Soil Science 19:153-162, 1925.

3. ---------------- A New, Simple and Rapid Method for Determining the Moisture Equivalent of Soils, and the Role of Soil Colloids on This Moisture Equivalent. Soil Science 27:233-240, 1929.

4. ---------------- Simple and Rapid Methods for Ascertaining the Existing Structural Stability of Soil Aggregates. Jour. Amer. Soc. Agron. 27:222-277, 1935.

5. Chandler, W. H. The Killing of Plant Tissue by Low Temperature. Mo. Agr. Expt. Sta. Res. Bul. 8, 1913.

6. Dexter, S. T., Tottingham, W. E., Graber, L. F. Preliminary Results in Measuring the Hardiness of Plants. Plant Physiology, 5:215-223, 1930.

7. ------------ ---------------- ------------ Investigations of the Hardiness of Plants by Measurement of Electrical Conductivity. Plant Physiology, 7:63-78, 1932.

8. ------------ Respiration Rate and Enzyme Activity as Related to the Hardened Condition of Plants. Plant Physiology, 9:831-837, 1934.

9. Gortner, R. A. Outlines of Biochemistry. John Wiley & Sons, pp. 231-241, 1929.

10. Graber, L. F., Nelson, N. T., Luekel, W. A., and Albert, W. B.
Organic Food Reserves in Relation to the Growth of Alfalfa and Other Perennial Herbaceous Plants.
Wisc. Agr. Expt. Sta. Res. Bul. 80, 1927.

11. Harvey, R. B. Hardening Process in Plants and Developments from Frost Injury.
Jour. Agr. Res. 15:83-112, 1918.

12. ------------- Annotated Bibliography of Low Temperature Relations of Plants.
Burgess Pub. Co., 1935.

13. Maximov, N. A. Internal Factors of Frost and Drought Resistance in Plants.
Protoplasma, 7:259-291, 1929.

14. Megee, C. R. A Comparison of Alfalfa Strains and Seed Sources for Michigan.
Mich. Agr. Expt. Sta. Sp. Bul. 211, 1931.

15. Newton, R. Colloidal Properties of Winter Wheat Plants in Relation to Frost Resistance.
Jour. Agr. Sci. 14:178-191, 1924.

16. Rosa, J. T. Investigations on the Hardening Process in Vegetable Plants.
Mo. Agr. Expt. Sta. Res. Bul. 48, 1921.

17. Spurway, C. H. Soil Testing.
Mich. Agr. Expt. Sta. Res. Bul. 132, 1933.

18. Steinmetz, F. H. Winter Hardiness in Alfalfa Varieties.
Minn. Agr. Expt. Sta. Tech. Bul. 38, 1926.

19. Tysdal, H. M. Determination of Hardiness in Alfalfa by Their Enzymatic Responses.
Jour. Agr. Res. 48:219-240, 1934.

20. Weimer, J. L. Some Factors Involved in the Winter Killing of Alfalfa.
Jour. Agr. Res. 39:263-283, 1929.