A STUDY OF COLD RESISTANCE AND STARCH RESERVES IN ALFALFA

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A STUDY OF COLD RESISTANCE AND STARCH RESERVES IN ALFALFA

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INTRODUCTION

Under Michigan conditions some varieties of alfalfa, such as Hardigan and Grimm, exhibit a high degree of winter hardiness, while Hairy Peruvian and others are decidedly non-hardy and consequently have no place in the agriculture of the state.

Many workers have attempted to establish the relationship between physiological and morphological characteristics of alfalfa and its capacity to resist the injurious effects of freezing temperatures. The present experiment was designed to further such studies by obtaining additional information regarding the relation of starch reserves to cold resistance in alfalfa roots. In view of the plant material on hand and the findings of other workers to date, it appeared that the problem might be attacked by studying this relationship as it existed within individual plants. For this purpose, the plants of F_2 progenies resulting from original crosses between Hardigan and Grimm (female parents) and Hairy Peruvian (male parent) were used, rather than individual plants of a variety relatively pure, genetically, for winter hardiness.

LITERATURE

Literature dealing with the subjects of organic reserves in alfalfa and the cold resistance of plants is voluminous. No attempt is made here to review it in detail. For comprehensive reviews of the subject of organic reserves in alfalfa the papers of Grandfield (5), Graber et al. (4), Mark (9), and Willard et al. (20) should be consulted. Papers dealing with the low temperature relations of plants have been thoroughly reviewed by Harvey (6), Rosa (15), Newton (12), Maximov (10), and others.

Other pertinent literature will be referred to as the several phases of the experimental results are discussed.

MATERIALS AND METHODS

In September, 1937, several plants of Hardigan and Grimm alfalfa were dug from field plots, brought into the greenhouse, and transplanted into clay pots. Of these, only two plants of each variety were later used as female parents in the crosses made. Two Hairy Peruvian plants which had been growing in the greenhouse were used as the male parents. Throughout the course of the experiment all plants grown in the greenhouse were in clay pots or flats containing coarse sand, and were given nutrient solution at weekly, or more frequent, intervals.

The crosses between the parent plants were made during the first three months of 1938. For the purpose of maintaining identity, they were given numbers as follows: Grimm 4 X Hairy Peruvian 1, Cross 4; Grimm 1 X Hairy Peruvian 2, Cross 5; Hardigan 4 X Hairy Peruvian 2, Cross 6; and Hardigan 1 X Hairy Peruvian 1, Cross 7. Individual F_1 plants of the first-named cross were then designated as 4-1, 4-2, 4-3, etc. The same system of identifying F_1 plants was used throughout.

The F_1 seedlings were grown in flats in the greenhouse and were later transplanted into clay pots. During the last two months of 1938 they were self-pollinated by manually "tripping" the flowers with a needle. F_2 seed was harvested from them during January and February, 1939.

On May 9, 1939, all F_1 plants, about 230 in number, were transplanted into the field. These plants were spaced at 30-inch intervals in rows 30 inches apart. At the same time, 22 plants resulting from self-fertilizing Hairy Peruvian 1 were transplanted. Seed from these inbreds and from all F_1 plants was harvested by cutting the plants down to the crown in early September, 1939. After harvesting the plants, growth was resumed and considerable root storage took place previous to killing frosts.

F_2 seedlings were grown in the greenhouse until July 3, 1939, when, at the age of six weeks, they were transplanted into the field. They were spaced at 8- to 10-inch intervals in rows 30 inches apart, and were not harvested for either hay or seed after the above date.

The field soil varied from sandy loam to loam, and had been uniformly cropped during the previous five years.

A. Technique of Cross-pollination

The technique employed in controlled cross-pollination was similar to that used by many workers, and involved the use of suction in emasculation of the flowers. The suction equipment consisted of a glass tube having an inside bore of approximately 1 millimeter, and gum rubber tubing which connected the capillary tube with an ordinary laboratory filter pump. The glass tube had been drawn out at one end until the inside bore was only slightly greater than the size of an alfalfa anther.

Only flowers in which the anthers had dehisced were chosen for crossing operations. Racemes of the female parent were thinned to from three to six flowers. Using small scissors, the standard of each flower was then clipped off as short as possible. By exerting a slight, steady pressure against the base of the keel, the staminal column was made to emerge slowly so that little pollen was thrown from the anthers. Emasculation was accomplished by holding the smaller end of the glass tube in contact with the exposed anthers. With short upward strokes of the tube the ten anthers were quickly removed by the suction and drawn within the tubing. Following emasculation, a fine stream of water from a syringe was directed upon the face of the stigma to remove any pollen which might have adhered. Final examination of the stigma was made with a small hand lens to determine completeness of pollen removal. A single flower from the male parent was then picked from its raceme and held at its base with forceps while the standard was gently removed with the fingers. Using a needle, the flower was "tripped" as gently as possible. The anthers were then brushed directly against the stigma of the emasculated flower until it was apparent that the entire stigmatic surface was well covered with pollen. The cross-pollinated flowers of the female parent plant were tagged for identification and allowed to remain unbagged. Emasculated flowers were left occasionally as a check upon the thoroughness of the technique.

B. Mechanical Injury in Root Tissues

To compare the nature of mechanical injury resulting from freezing under field conditions, as reported by several investigators, with that resulting from artificial freezing, the following procedure was adopted. Sections of unfrozen roots from six cold resistant Hardigan plants and from four non-resistant Hairy Peruvian plants, all of which had been dug from the field on December 8, were imbedded in paraffin in the usual manner. Other sections of the same roots were artificially frozen at a temperature of $-7^{o}C.$ to $-8^{o}C.$ for $3\frac{1}{4}$ hours and then were likewise imbedded. All roots were sectioned transversely at a thickness of 15 microns, mounted on slides, and stained with safranine O, crystal violet and orange G.

C. Determination of Cold Resistance

Dexter, Tottingham, and Graber (2) have described a method of determining cold resistance of alfalfa roots. They point out that tissue injury as a result of freezing involves disorganization of cells and that, upon disorganization, the cells lose their ability to control diffusion of soluble contents. The outward diffusion of electrolytes can be determined by electrical conductivity measurements and from these the degree of injury estimated.

The method of determining cold resistance in this experiment was based upon the work of the above writers and differed only in details from the procedure they describe. Each sample consisted of 1 gram of root tissue from a single plant, and comprised three adjacent sections of root from either side of a middle section located 1¼ inches beneath the crown. The middle section was placed in a formalin alcohol fixative, and preserved for starch determination. The test tubes, each containing the six sections of root tissue, were placed in a water bath at +2° C. for 11 to 15 hours so that all sections might attain a uniform temperature before freezing. The roots were frozen at -7°C. to -8°C. in an alcohol slush, for a period of 3¼ hours. Upon removal they were allowed to stand at room temperature for a period of 1¼ hours. Five cc. of distilled water were then added to each test tube, and exosmosis was allowed to proceed at +2°C. for a period ranging from 25 to 27½ hours before resistance measurements were made with a conductivity cell and a Wheatstone bridge.

On five of the dates of sampling, test tubes containing sections of Hardigan roots were handled in the manner described above, except that they were not subjected to freezing. This was done to provide a check upon the effects of freezing.

D. Determination of Starch Reserves

Chemical analysis is the usual method of determing quantitatively the presence of starch in plant tissue. In this experiment, however, the method of determination was based upon the fact that starch grains assume a dark blue color when treated with an iodine solution.

The middle section from each root, previously fixed in formalin alcohol, was imbedded in paraffin in the usual manner and cut transversely at a thickness of 15 microns. The sections were then fixed to glass slides, stained in Gram's iodine solution for a period of ten minutes, mounted in Canada balsam and covered with a Number 2 cover glass.

Plate I shows the equipment, and its arrangement, used in the determination of intensity of starch reserves. By means of a projecting microscope the image of an individual root was focused directly upon a photronic cell. This in turn was connected with a sensitive galvanometer, the readings from which indicated, relatively, the amount and intensity of light falling upon the cell. It was found necessary to use an automatic voltage stabilizer because of sharp fluctuations of voltage in the current delivered to the microscope lamp.

Those roots containing large amounts of stored starch intercepted a greater portion of the light beam than did the roots containing lesser amounts, and this in turn resulted in lower galvanometer readings. Preliminary trials indicated that the "depression" values obtained in this manner correlated closely with the comparative amounts of starch as observed with the naked eye. Because the iodine stain faded, particularly in the presence of light, starch determinations were made within six hours after staining.

It was found after numerous trials that reliable readings could be obtained only when the intensity of the uninterrupted light beam was constant and when interruption by a glass filter resulted in a constant "depression" as indicated on the galvanometer. In order to eliminate variations in readings

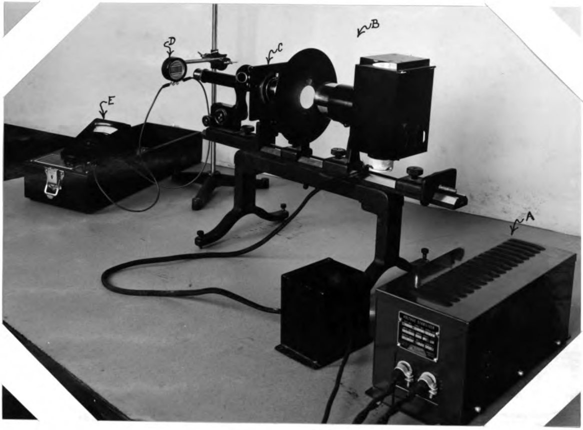

Plate I. Arrangement of equipment used in determination of intensity of starch reserves in alfalfa roots. A, voltage stabilizer; B, projecting microscope; C, microscope stage; D, photronic cell; E, galvanometer.

between slides, cover glasses and balsam layers of different-thicknesses, the following precaution was taken. Several readings were obtained from each slide when the light beam was made to pass between adjacent root sections and was interrupted only by the slide, cover glass and the balsam layer. The average of the "depression" values so obtained was then deducted from the "depression" value for each root section upon that slide. These differences, then, represented the comparative amounts of the light beam intercepted by the root sections. Only one reading was obtained from each root, the light beam passing through as near the center of the root as possible. This procedure was decided upon only after it had been noted that intensity of starch reserves in the central area correlated closely with the intensity throughout the entire root. There were exceptions, however, particularly noticeable in one progeny in which starch was lacking from the central portion of many of the root sections. An attempt was made to correct for this by allowing the beam of light to pass through an area slightly off-center.

EXPERIMENTAL RESULTS AND DISCUSSION

A. Mechanical Injury to Roots as a Result of Freezing

Numerous workers have observed that root tissue of alfalfa is subject to injury by freezing, particularly when organic reserves have been depleted (14) (4) (20). In Michigan, this latter condition may be expected most commonly during late winter and early spring. Death of plants following root injury appears to be largely dependent upon the extent and severity of cell disorganization.

Jones (8) describes the nature of injury in roots of alfalfa plants that had been severely frozen under field conditions. He also describes repair of parenchymatous tissue by meristematic activity of adjacent parenchyma cells. Injury was always found where cells had been separated by rifts along the middle lamella. However, from his observations he inferred that injury was primarily a direct effect of freezing, and not primarily, or necessarily, a consequence of mechanical injury. Weimer (19) found that splitting of tissue was so commonly associated with death of the cells that the two must be considered together. He further observed in histological studies of 16 varieties that splitting of rays was more or less severe in all alfalfa plants during the winter, but that, if the injury was not too severe, practically all of the cracks were closed during the following growing season.

Plates II, III, IV and V show the condition of roots of Hardigan and Hairy Peruvian varieties before being frozen and after having been artificially frozen at -7°C. to -8°C. for a period of 3¼ hours.

It will be noted that there was no apparent injury in the unfrozen roots of either variety. This was expected as the soil had been frozen to a depth of only one-half inch prior to the date of sampling.

In the case of the frozen Hardigan roots, considerable injury was apparent, chiefly in the form of rifts which extended radially along the parenchyma cells of the rays. Parenchymatous tissue in the center of the root (not shown) was also

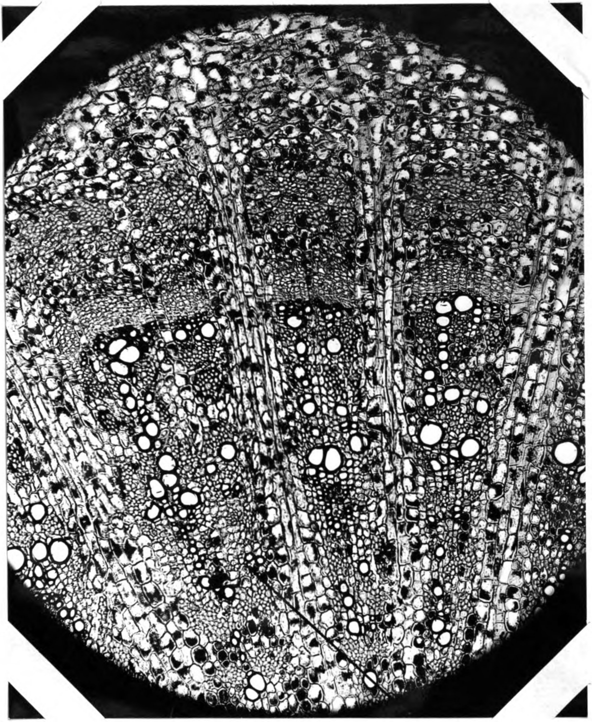

Plate II. Photomicrograph of typical unfrozen Hardigan alfalfa root as dug from the field on December 8, 1939.

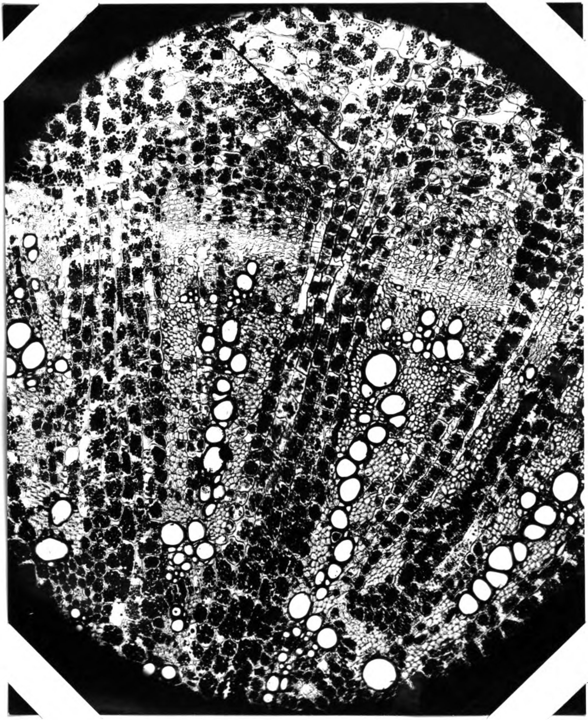

Plate III. Photomicrograph of typical unfrozen Hairy Peruvian alfalfa root as dug from the field on December 8, 1939.

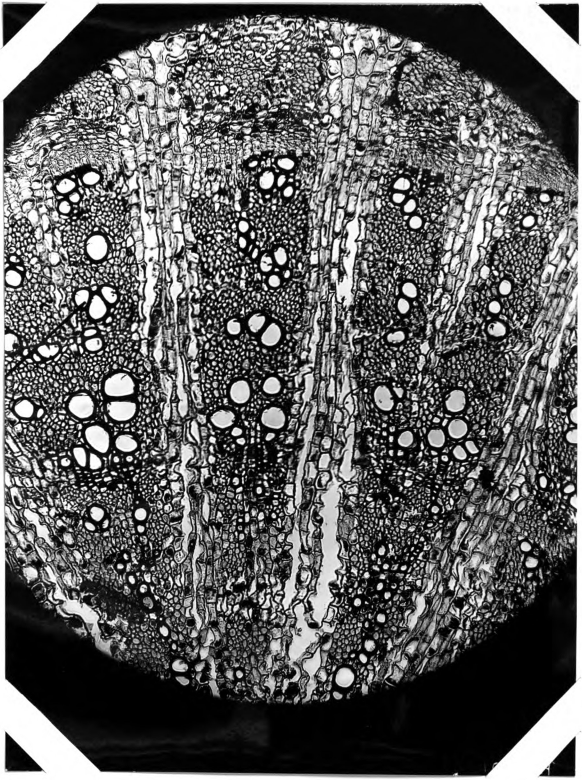

Plate IV. Photomicrograph of typical Hardigan alfalfa root dug on December 8, 1939, after being artificially frozen at -7°C. to -8°C. for $3\frac{1}{4}$ hours.

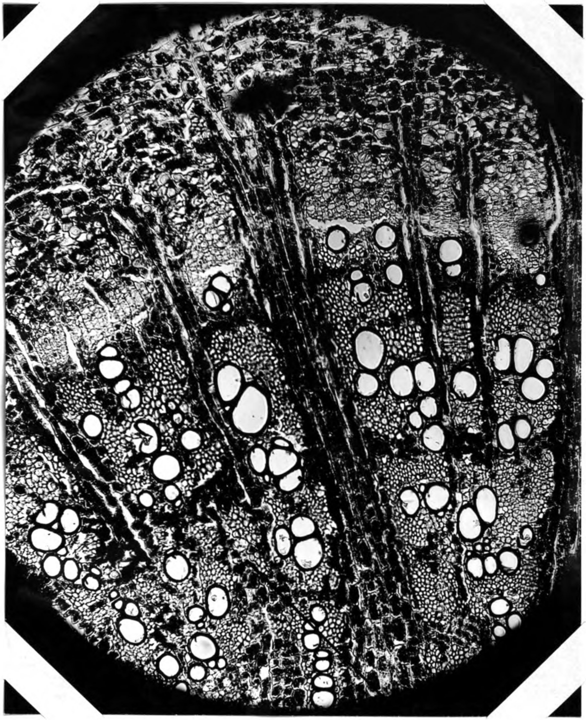

Plate V. Photomicrograph of typical Hairy Peruvian alfalfa root dug on December 8, 1939, after being artificially frozen at -7°C. to -8°C. for 3¼ hours.

disorganized. Although there was almost a complete absence of starch in the roots, the cambium had suffered no injury.

Injury in the roots of the Hairy Peruvian variety was somewhat different in nature, most of it having taken place in the vicinity of the cambium layer. The abundance of starch present may account in part for the less extensive injury in the ray tissue. Whereas the cambium layer of the Hardigan roots showed no effects of freezing, the cambium of the Hairy Peruvian roots was badly split and many of the meristematic cells were collapsed.

Because of the tendency of Hairy Peruvian alfalfa to grow until late in the fall, and because the fall months of 1939 were abnormally mild, the meristematic tissue of this variety may have been still active on the date of sampling (December 8). In this condition, the cambium would be especially subject to injury by freezing. In the literature reviewed, no description of repair of injured cambium tissue was found. Further study of alfalfa grown in southern Michigan probably would reveal considerable injury of this type during winter and early spring in non-hardy varieties. This injury in turn would appear to be associated with failure of the plant to recover.

B. Trend of Starch Reserves and Cold Resistance During the Fall of 1939

It has been established by many workers that alfalfa increases in its resistance to cold during the fall months under the influence of shortening day lengths and lowering temperatures. Peltier and Tysdal (13) and others observed that under normal conditions hardy varieties became dormant earlier and hardened more rapidly than non-hardy varieties. This suggests a correlation between dormancy and phsiological processes associated with hardening.

In a study of root reserves and resistance to cold injury Mark (9) found that in each of the varieties of alfalfa analyzed, starch began disappearing in early fall and constituted a very small portion of root reserves after October. However, the varieties showed differences with respect to rate of disappearance of starch during the fall.

Figure 1 shows the intensity of starch reserves and the degree of cold resistance (as indicated by electrical conductivity) in Hardigan and Hairy Peruvian alfalfa roots during the fall of 1939. Although there were fluctuations in both starch reserves and cold resistance from week to week in the two varieties, it will be noted that the trends were as follows:

1. Intensity of starch reserves in Hardigan at the close of the experiment was decidedly less than at the beginning, and less than on any date during the course of the experiment.
2. Intensity of starch reserves in the Hairy Peruvian roots on December 8 was greater than at the beginning of the experiment, and represents the average intensity for the entire period.
3. Contrasting the intensity of starch reserves in the two varieties, Hardigan plants showed a more rapid decrease than did Hairy Peruvian plants.

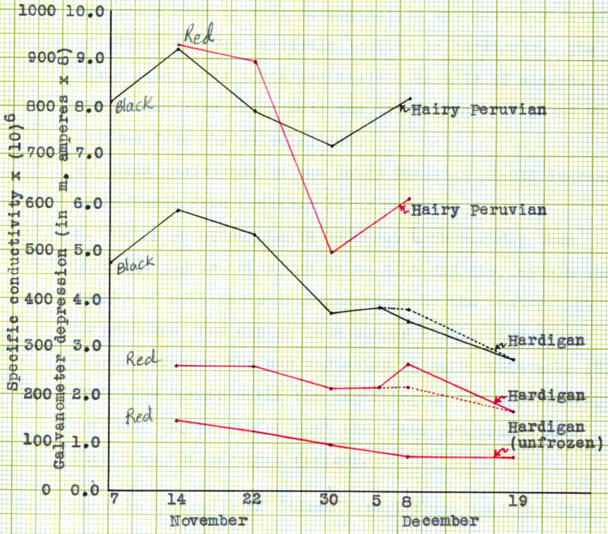

Fig. 1. Electrical conductivity of water extracts and intensity of starch reserves in roots of Hairy Peruvian and Hardigan alfalfas. Conductivity shown in red, starch reserves in black.

4. Cold resistance in the case of Hardigan showed only a slight increase from November 14 to December 19, the date upon which the experiment was terminated. The sharp decrease in cold resistance (increase in electrical conductivity) during the period December 5 to December 8 can be explained by the fact that one of the ten roots used in this determination was entirely devoid of starch and was decidedly lacking in hardiness. The other nine roots contained visible amounts of starch. The dotted line represents the trend when this plant was omitted from the calculations.

5. Cold resistance in the Hairy Peruvian plants increased markedly from November 14 to December 8.

6. Hairy Peruvian plants hardened more rapidly than Hardigan plants, although at no time did they attain the degree of cold resistance possessed by the latter. It is probable that during late summer little difference existed between the two varieties in cold resistance (2). From late summer until the beginning of the experiment, November 14, hardening must then have progressed much faster in the Hardigan variety.

C. Changes in Intensity of Starch Reserves and Cold Resistance

Referring to Figure 1, it is interesting to note that increases in starch content in both varieties during the course of the experiment were accompanied by decreased cold resistance, while decreases in intensity of starch reserves were accompanied by increased cold resistance. Under the treatment received by these plants, they made considerable growth before killing frosts in October rendered them incapable of photosynthetic activity. Ensuing warmer weather failed to stimulate vegetative growth to a visible extent. It is believed, therefore, that these fluctuations in starch content represent conversion of starch to sugar and reconversion to starch rather than marked changes in total carbohydrates. Since the hardening process in many plants has been associated with increased concentration of sugar, it is probable that the sharp decreases of starch during the fall months represent physiological processes directly associated with increased cold resistance.

Figures 2, 3, 4 and 5 present similar data for the four F_2 progenies studied. The mean air temperature, as reported by the East Lansing station of the U.S. Weather Bureau, for each of the 7 days preceding each date of sampling were averaged together, and these computations are also presented since it is believed they provide an explanation, in part, for the behavior of the plants.

In three of the four progenies an apparent correlation existed between average mean temperature and intensity of starch reserves (r_{xy} = +.84, +.89 and +.78 for progenies 7-19, 6-29 and 5-12 respectively). This relationship is lacking in progeny 4-18 (r_{xy} = +.03), and it has been shown statistically that this is the only instance in which changes in intensity of starch reserves might easily have been the result of inadequate sampling.

Likewise, the correlation between the average mean temperature and electrical conductivity of the water extracts is apparent (r_{xy} = +.70, +.65, +.52 and +.60 for progenies 7-19, 6-29,

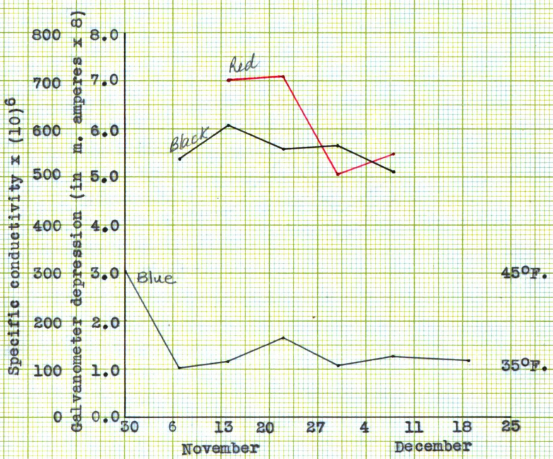

Fig. 2 Electrical conductivity of water extracts and intensity of starch reserves in roots of F_2 progeny 4-18. Conductivity shown in red, starch reserves in black and average mean temperature in blue.

Fig. 3 Electrical conductivity of water extracts and intensity of starch reserves in roots of F_2 progeny 5-12. Conductivity shown in red, starch reserves in black and average mean temperature in blue.

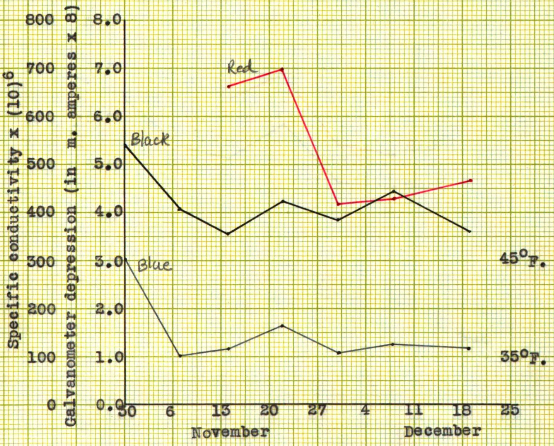

Fig. 4 Electrical conductivity of water extracts and intensity of starch reserves in roots of F_2 progeny 6-29. Conductivity shown in red, starch reserves in black and average mean temperature in blue.

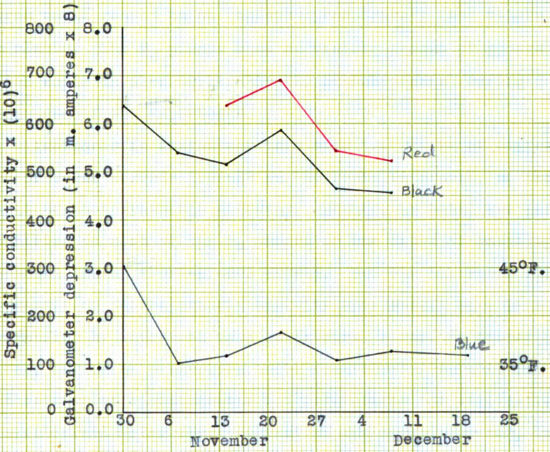

Fig. 5 Electrical conductivity of water extracts and intensity of starch reserves in roots of F_2 progeny 7-19. Conductivity shown in red, starch reserves in black and average mean temperature in blue.

5-12 and 4-18 respectively).

Abrupt decreases in the intensity of starch reserves can best be explained by assuming that the starch was converted to sugar, as it is not probable that the decreasing temperatures would result in an increased respiratory rate within the plants. The F_2 plants made no visible amount of growth (which would involve utilization of carbohydrates) during the course of the experiment.

Increases in starch may have been the result of conversion of sugar already present in the roots to starch, or the result of photosynthetic activity. Either, or both, are possibilities as most of the F_2 plants on the dates of sampling possessed living above-ground growth. Had the increases been the result of photosynthesis activity they should have been accompanied by increased cold resistance, since, as Dexter (1) states, hardening progresses under conditions favorable for synthesizing of carbohydrates but unfavorable for utilization of synthesized materials. In most of the cases, however, increases in starch were accompanied by loss of cold resistance. Therefore, it is reasonable to assume that the increasing temperatures resulted in conversion of sugar to starch and decreased cold resistance.

D. Relation of Intensity of Starch Reserves to Cold Resistance

Numerous experiments have shown that winter injury in alfalfa is closely associated with management practices (4)(5)(14)(20). The explanation for this observed fact is that, to escape winter injury, alfalfa plants must be well supplied with starch at the time they become dormant in the fall. However, as Megee (11), Steinmetz (16), and others have pointed out, the presence of abundant organic reserves does not insure against winter injury, since the plant must also have an hereditary ability to successfully withstand cold. Mark (9) states this postulate somewhat differently when he says that within the Grimm variety a correlation was found to exist between hardiness and root reserves, but that when varietal (genetic) differences were introduced no correlation was found.

It has not been established that, in the hardened state, hardy varieties of alfalfa, as such, are significantly higher in any reserve fraction than non-hardy varieties. On the contrary, several workers have shown that the non-hardy were consistently higher in some of the reserve fractions than the hardy alfalfas. Mark (9) reports that Ladak alfalfa was higher in dextrins, starch, and insoluble nitrogen than Arizona common during the fall, winter, and early spring of 1934 and 1935. He also found that hardy varieties digested starch reserves more rapidly in the fall than did non-hardy varieties. Janssen (7) found that the starch content of Hairy Peruvian alfalfa was higher than that of Grimm during the winter of 1937-38.

In connection with utilization of reserves, Tysdal (18) concluded from his work that hardy varieties have a greater protected diastatic power when in a hardened condition than do non-hardy varieties. This finding, however, does not serve to explain differences in rate of starch digestion between alfalfas differing in inherited hardiness.

It appears from the above and from other work that the level of starch reserves attained by alfalfa before becoming dormant in the fall is governed largely by environment (including cultural practices), whereas differences between varieties in the rate of digestion of starch during the fall and winter is primarily a response to hereditary make-up. During the fall months of most years complete and continued dormancy is not attained. Following relatively short periods of dormancy, weather conditions may be such that the plant resumes photosynthetic activity and even vegetative growth. The effects of alternating dormancy and non-dormancy complicate the relation of starch reserves and winter hardiness to heredity.

As a test for the correctness of the belief that alfalfa plants having the hereditary capacity of comparatively rapid digestion of starch during the fall months also possess the inherited capacity to resist cold injury, simple correlation coefficients were determined between specific conductivity of the water extracts and intensity of starch reserves for each F_2 progeny and for row-grown Hardigan. The results are given in Table 1.

In the row-grown Hardigan, three of the five correlation coefficients were negative. As the season advanced the degree of negative correlation increased, changing from +.43 on November 14 to -.72 on December 8.

In no instance did progeny 4-18 exhibit correlation coefficients which were statistically significant, although all were positive.

Correlations for all four dates of sampling were positive in the case of progeny 5-12. Of these, the plants dug on November 22 and on December 8 exhibited coefficients significant at the 1% point.

Table 1. Coefficients of variability for intensity of starch reserves and electrical conductivity, and correlation coefficients between the two on each date of sampling.

Progeny	Date of Sampling	Number of Plants	Coefficient of variability - conductivity of water extracts	Coefficient of variability - intensity of starch reserves	Coefficient of Correlation
Hardigan	Nov. 14	10	33.6%	94.3%	+.43
Hardigan	Nov. 22	10	31.1%	30.4%	+.14
Hardigan	Nov. 30	10	55.2%	28.1%	-.23
Hardigan	Dec. 5	60	42.0%	54.8%	-.33***
Hardigan	Dec. 8	10	40.4%	62.4%	-.72***
	Weighted Mean		41.2%	54.4%	
4-18	Nov. 14	18	15.7%	9.4%	+.19
4-18	Nov. 22	17	13.6%	15.0%	+.35
4-18	Nov. 30	15	23.9%	9.9%	+.14
4-18	Dec. 8	19	19.8%	11.3%	+.28
	Weighted Mean		19.8%	11.4%	
5-12	Nov. 14	15	14.6%	16.5%	+.28
5-12	Nov. 22	16	11.6%	10.3%	+.70****
5-12	Nov. 30	12	18.4%	31.4%	+.50*
5-12	Dec. 8	13	40.0%	24.6%	+.82****
	Weighted Mean		20.5%	17.7%	
6-22	Dec. 19	29	24.2%	14.4%	+.55****
6-29	Nov. 14	17	28.1%	18.2%	+.53**
6-29	Nov. 22	22	26.0%	15.8%	+.52***
6-29	Nov. 30	20	22.3%	12.9%	+.54***
6-29	Dec. 8	15	26.2%	14.6%	+.80****
6-29	Dec. 19	39	22.5%	15.0%	+.30*
	Weighted Mean		24.5%	15.2%	
7-3	Dec. 12	22	23.3%	14.8%	+.02
7-19	Nov. 14	9	13.4%	16.3%	+.47
7-19	Nov. 22	10	12.8%	8.9%	-.14
7-19	Nov. 30	13	13.5%	6.5%	-.21
7-19	Dec. 8	10	31.9%	8.0%	-.31
	Weighted Mean		17.7%	9.5%	

Note: 1,2,3, and 4 asterisks indicate significance to 10%, 5%, 2%, and 1% points, respectively (3))

In progeny 6-29 positive correlations between intensity of starch storage and electrical conductivity were significant at the 5% point or more on four of the five sampling dates.

The only F_2 progeny that exhibited negative correlations was 7-19 although none of these was statistically significant. As in the row-grown Hardigan, the magnitude of the negative correlation coefficients increased as the season advanced. The fact that the weighted mean of the several coefficients of variability in progeny 7-19 was less than that of any other progeny may indicate a more nearly homozygous condition with respect to starch storage and winter hardiness. On the basis of the work of Mark (9), and Stewart (17) a negative correlation might be expected.

The extremely high coefficients of variability found in the Hardigan plants are believed to be the result of environmental differences. Roots and top growth varied greatly in size as a result of the thick row-planting and some of the plants appeared to have thrived at the expense of others. Even on November 7, the first date of sampling the Hardigan, the level of starch reserves was highly variable from plant to plant.

Although the evidence presented here is not conclusive, it appears that those plants which have the hereditary characteristic of comparatively rapid digestion of starch during the fall also have the hereditary ability to resist injury from freezing. It also appears that within the Hardigan variety higher levels of starch reserves are associated with increased hardiness. Whether or not this relationship exists within such non-hardy strains as Hairy Peruvian and Arizona Common is problematical.

That extreme hardy alfalfas, even though low in starch reserves, possess greater cold resistance, when in the hardened condition, than do extreme non-hardy alfalfas having an abundance of starch is evidenced by the fact that, of all the roots containing visible starch, only two Hardigan plants failed to show greater cold resistance than the most resistant non-hardy alfalfa plant. A few Hardigan roots containing no visible starch were decidedly lacking in cold resistance and were comparable to the least resistant Hairy Peruvian plants.

SUMMARY

A study of cold resistance and intensity of starch reserves was made during the fall of 1939 with Hardigan and Hairy Peruvian alfalfas and with F_2 progenies resulting from original crosses involving hardy and non-hardy varieties.

Hardigan alfalfa roots, having a low intensity of starch reserves, when artificially frozen exhibited mechanical injury of ray tissue, but no injury in the cambium. Hairy Peruvian roots, having a high intensity of starch reserves, when frozen exhibited not only injury of ray tissue in the vicinity of the cambium, but of cambium tissue as well.

The decrease in starch was more rapid in Hardigan alfalfa than in Hairy Peruvian from November 7 to December 8.

Hairy Peruvian plants hardened more rapidly than Hardigan plants after November 14. However, the Hardigan variety had already attained a much higher degree of cold resistance on November 14, and it maintained this superiority over Hairy Peruvian until December 8, at which time conductivity determinations on the latter were terminated.

In most cases, changes in intensity of starch reserves were accompanied by corresponding changes in electrical conductivity of the water extracts. The several F_2 progenies did not react alike in this respect.

In three of the F_2 progenies intensity of starch storage was positively correlated with field temperatures, while in all four F_2 progenies cold resistance was negatively correlated with field temperatures.

It is believed that fluctuations in intensity of starch reserves were due to conversion of starch to sugar and reconversion to starch rather than to photosynthetic or metabolic activity.

Evidence is presented which indicates that rapid loss of starch reserves during the fall months is associated with inherited cold resistance, but that within the Hardigan variety intensity of starch reserves is correlated positively with cold resistance. This is believed to be an indication that within individual plants, level of starch reserves and level of cold resistance are positively correlated.

Hardigan roots having no visible starch were comparable in cold resistance to the least resistant Hairy Peruvian roots. No Hairy Peruvian roots were found to be devoid of starch.

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