BOUND WATER AND HIGH TEMPERATURE TOLERANCE STUDIES OF SEVERAL VARIETIES OF ALFALFA

by

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TABLE OF CONTENTS

	Page
INTRODUCTION	2
REVIEW OF LITERATURE	3
Bound Water	3
Drought Resistance	9
MATERIALS AND METHODS	16
EXPERIMENTAL RESULTS	26
Bound Water	26
High Temperature Studies	40
DISCUSSION	62
SUMMARY	67
ACKNOWLEDGMENT	69
LITERATURE CITED	69

INTRODUCTION

Drought is a major limiting factor in crop production in Kansas and has been especially important during the last several years. Extreme deficit of soil moisture combined with severe atmospheric drought has centered attention upon the seed of developing crop plants that are capable of producing good yields under conditions of both soil drought and atmospheric drought.

Until recently the identification of varieties and individual plants possessing drought resistance has been only by observation in the field. This is a long-time process as drought conditions are not existent every year in the locality in which it is desired to test the particular variety or individual.

Alfalfa, long one of the important crops of Kansas, has suffered from drought along with other crops. Alfalfa is used for hay, forage, and seed production, and has an important use in rotations on many Kansas farms. Vavilov (41) has stated that it is probably impossible to breed crop plants for resistance to soil drought but that it should be possible to develop varieties of crop plants that are resistant to atmospheric drought. It follows that any test or criterion that would give an indication as to the drought resistance would greatly aid the developing of desirable plants with minimal effort.

With the above thoughts in mind, it is the purpose of this thesis to report the results of some studies on alfalfa which have been made in an effort to determine an indication of the drought resistance of some of the varieties of alfalfa which are commonly grown in Kansas, and to secure a means of measuring drought resistance that could be used in the development of superior varieties.

REVIEW OF LITERATURE

Bound Water

According to Jones and Gortner (17) the first work of a quantitative nature on the amount of freezable water in plant material was done by Muller-Thurgau in Germany about 1880. He froze tissue of apple and potato in an ice and salt bath and then determined the amount of water that froze by placing the material in a water calorimeter and measured the amount of heat necessary to melt the ice that had been formed in the sample.

Since that time there has been a great deal of work done on the amount of freezable water in materials of a colloidal nature, both organic and inorganic. The work of

Foote and Saxton (7, 8, 9), in determining the effect of freezing upon certain inorganic hydrogels, has been an outstanding piece of work. They found that all of the water in hydrogels did not freeze at the freezing point of water, but as the temperature was lowered there was more water frozen out of the material. However, they could never get all of the water frozen out of the material they were testing. They classed the water into three classes, free, capillary, and combined water. The free water was that which could be frozen at a temperature of from 0° C. to -6° C.; capillary water as that water which could be frozen out of the material as the temperature was lowered more; and combined water was the difference between the total amount of the other two types and the amount which was added to the sample. Their work was done with the dilatometer and was the first extensive work reported in which this means of expressing bound water was used.

Following the work of Foote and Saxton, bound water studies have been conducted on a wide variety of materials. Bouyoucos (4) has demonstrated that there is water in soils that will not freeze even at a temperature of -78° C. He classifies the types of soil moisture into three groups: free water, that which freezes at a little below 0° C.; capillary-adsorbed, that which will freeze as the temperature is lowered to -78° C.; and combined water, all that is not accounted for by the other two groups. Robinson, (32) working on the resistance of insects to cold, has stated that the colloids present in the insect tissues and body liquids withdraw and bind free water under conditions of falling temperatures. Some of the properties of water are changed in such a way as to protect the insects during the winter.

The work on plant material has shown a remarkable resemblance to that done on other colloidal material. As the result of this, workers investigating bound water, have been in general agreement that the water binding properties of plant substances are largely due to the colloidal material in the cells of the plant. Gortner (11) states that proteins possess the power of becoming strongly hydrated and the large amount of water present in living organisms is probably mainly held through this affinity of the proteins for water. He further states that in the plant kingdom, however, the structural elements and intercellular constituents are largely of a carbohydrate nature, polysaccharides in most cases. These polysaccharides are, in many cases, highly hydrophilic and adsorb relatively large amounts of water. Certain of the phosphatides, such as lecithin, are in themselves extremely hydrophilic, exist in the colloidal state and react as typical hydrophilic colloids. Newton and Gortner (29) found that colloidal substances in plant cells compare favorably in imbibitional capacity with various other substances well known for their hydrophilic properties. In fact, of the materials tested, both organic and inorganic, none equalled the plant colloids in water binding properties. These authors, in studying the amount of hydrophilic colloids present in expressed plant fluids, found that bound water corresponds so regularly with the content of the hydrophilic colloids as to indicate a close relationship. They concluded that bound water was a more stable characteristic than osmotic pressure. Newton and Martin (30) feel, as a result of their work on the cactus, that colloids play a more important part in adaptation to drought than do the structural characteristics of the plant. Bound water of expressed juice has been used by these workers to successfully list wheats and grasses studied with respect to their drought resistance. In this regard Gortner (11) says that expressed juice will probably give a minimum amount of bound water. Lott (20) felt that he was able to secure a more accurate picture of the forces which exert or express themselves in bound water by the use of the plant tissue rather than expressed juice. Novikov (31), working in Russia, found that the non-freezing water changes during the day, increasing from morning to noon and diminishing toward evening. He feels that the change depends upon the accumulation of the soluble products of photosynthesis.

There is a decided lack of uniformity in the definition of bound water. The definition is largely dependent upon the conditions under which the investigator conducted his experiment and the methods used in determining the bound water content of the material tested. Briggs (3) says that in general the idea of bound water carries with it a picture of a portion of the water in a system in which it is associated with the colloidal phase with such strength that it no longer exhibits those properties characteristic of water; i.e., it is no longer available to act as a solvent nor can it be separated from the colloidal phase by freezing or subjection of the system to pressure as in an ultrafilter. This water can, however, be easily removed from the sample by heating to 90° C. in a constant temperature oven or to ordinary temperatures in a vacuum oven. Jones and Gortner (17) emphasize the fact that bound water is an indeterminate term and that bound water values, as experimentally determined, may be expected to vary from system to system, the variation may be due to many factors not the least of which is the method selected for the measurements. Gortner (11), in an address to the Farraday Society, stresses the fact that there is no sharp line of demarcation between free and bound water, but that we must postulate an insensible gradation between molecules of water having the normal activity of pure water and molecules where this activity has been so reduced that such molecules have become to all intents and purposes a part of the solid on which they are adsorbed.

There is ample proof, however, that in plants and animals there is water which is not existent in the free state. The variety of methods used in determining the amount of bound water makes it impossible to make a direct comparison of percentages of bound water but relative amounts, determined in various experiments by several investigators using different methods, can be compared in a general way. Sayer (34) found that in the materials he tested there was a close agreement between three of the most common methods, the dilatometric, cryoscopic, and calorimetric. He states that the dilatometric method has the advantage in that it can be used on either solid material or the juices of plants and other liquids as well.

Drought Resistance

Drought studies at the present time indicate that there is no one morphological or physiological characteristic of plants which will determine their resistance to either soil or atmospheric drought. Studies are being made in an effort to break the complex problem down into its component parts to find the exact cause of resistance. Some of the major works in drought resistance are reviewed.

It has long been known that plants may suffer from severe conditions of atmospheric drought even when there is an abundance of moisture in the soil. This observation has led investigators to develop methods of reproducing the atmospheric type of drought in the laboratory. Aamodt (1), working in Canada, has been able to produce conditions of atmospheric drought similar to conditions in the dry areas of Alberta, by means of a "chinook" machine. The injury produced artificially by means of blowing air heated at 110^{o} F. at a rate of six miles per hour over wheat plants enclosed in a glass tunnel was similar to that caused by natural drought. The results show that he was able to differentiate among varieties with respect to resistance to atmospheric drought. The differential injury corresponds with observations made in the field. Hunter, Laude, and

Brunson (16) have used a heat chamber which was automatically controlled for tests of inbred lines of corn at high temperatures. They tested 14-day old seedlings at a temperature of 140° F. for a period of 6.5 hours. The lines which were the most heat tolerant in the artificial test, in general, were also possessed with the greatest ability to withstand drought conditions in the field. Shirley (36) described a machine for securing controlled conditions of temperature and relative humidity in which he studied the effect of high temperature and low relative humidity upon one, two, and three-year old seedlings of Picea canadensis. He was able to maintain the relative humidity at 15 per cent by passing the air over calcium chloride to dehydrate it. The temperature was automatically controlled at about 40° C. He made notes only on the length of the time the seedlings would live in that condition and was able to discern considerable differences among the seedlings depending upon the treatment prior to testing. Grandfield and Zink (13) developed a chamber for controlling temperature and relative humidity in which the plants are placed under glass and exposed to the natural light during the test. The temperature control is automatic and the relative humidity is controlled by sulphuric acid. The machine is being used by Grandfield to to study seed set of alfalfa at various temperatures from 60° to 120° F., and at various relative humidities ranging from 10 to 100 per cent.

Studies with artificially produced high temperatures have advantage in that the plants are actually under conditions which are controlled as desired. The plants cannot evade the conditions because of small size, they cannot escape by early maturity, nor are they able to go into dormancy previous to being exposed to the severe conditions of the test. The plants can be tested at a time convenient to the investigator. The work of Hunter, Laude, and Brunson (16) indicates that plants can be tested in the seedling stage which makes it possible to test a large number of individuals in a short time. The investigator need not wait for the conditions to occur naturally, which might take several years, but can produce the conditions at will.

Various studies have been made of xerophytic plants in an effort to determine the particular type of structure which would explain their persistence in arid places. There seems to be no uniform characteristic which accounts for this property of drought resistance. Maximov (23) has shown that xerophytic plants have a high transpiration rate which is contrary to the earlier general belief. He feels that the principal basis for drought resistance in plants is their capacity to endure a great loss of water. He found that xerophytic plants were capable of losing one half of their water content without injury whereas mesophytic plants showed a reduction in dry weight, depletion of food reserves, and shedding of leaves with much less water loss.

Experimental studies of the water requirement of various plants have been conducted in the hope of securing a relation between drought resistance and the amount of water necessary to produce a unit of plant growth. Work done by Shantz and Piemeisel (35) shows that there is no close association between the water requirement of plants and their drought resistance. They found that some drought resistant grasses had a high water requirement while certain drought susceptible species, rice and buckwheat for example, had a relatively low water requirement. Alfalfa was shown to have a higher water requirement than corn, sorghum, or millet. This was especially true of the later cuttings. It was found that the water requirement increased for each cutting. They also concluded that the difference between varieties of alfalfa tested was so small as to make it impossible to distinguish between them by means of their water requirements. The work of Dillman (5) corroborates the work done by Shantz and Piemeisel. Kiesselbach (18) reports that the water requirement was approximately the same for two varieties of corn, one which had been grown in New York under humid conditions for many years, and the other under relatively dry conditions in western Nebraska. He feels that there is no such thing as a definite water requirement which is constant for any kind of crop. The water requirement is much larger in an infertile soil than in a fertile soil. Kiesselbach concludes that drought resistance qualities of certain crops must lie elsewhere than in a low water requirement.

Miller (25) reported that the nature of the root system may play an important part in the prevention of incipient wilting. Sorghum was found to have twice the transpiration rate per unit of leaf area as corn but the leaf area of sorghum was only 25 per cent as great as that of corn. Upon investigating the nature of the root system Miller found that while corn and sorghum had the same number of roots of the first order, sorghum had twice as many roots of the second order. He believes that the greater number of finer roots of the sorghum, together with the smaller leaf surface, are instrumental in keeping the water supply of the leaf sufficient to retard incipient wilting. Garver (10), in a study of the root development of various varieties of alfalfa, states that the Hairy Peruvian variety has a small distinct tap root, comparatively few branch roots, and few fibrous roots, which are distributed rather uniformly over the root system. The northern grown common alfalfas show a distinct tendency to produce branch roots and fibrous roots. Ladak, which is a cross between *Medicago sativa* and *Medicago falcata*, has an abundant development of fibrous roots. In this respect it exceeds all other alfalfa root systems studied. Tumanov (38) noted that sickle alfalfa (probably *M. falcata*), lost fewer leaves than other varieties under drought conditions. This may be due to the superior root system. The southern common alfalfas are more nearly like the Hairy Peruvian variety in root development. Aamodt and Johnston (2) found that among the varieties of wheat tested by them, those with the finer and more branched root systems were better able to withstand periods of atmospheric drought in their chinook machine and that these same varieties were more resistant to drought according to field observations.

The stomatal action of alfalfa is a distinct type. As described by Miller (27) the stomata, under favorable conditions of moisture, close partially for a short time during the middle of the day. This period of midday closing increases to complete closure as the conditions become less favorable. The stomata may even remain closed during the entire day and open during the entire night. This action would be conducive to the conservation of water by the plant under adverse conditions.

There has been some investigation into the chemical composition and changes in plants in an effort to find an explanation of drought resistance. Lvof and Fichtenholz (21), working on tobacco, have found that there is a mass hydrolysis of starch in the more resistant varieties during wilting, saccharose being the main product formed upon hydrolysis. They conclude that during wilting there is a stimulus in the plant which causes the hydrolysis of starch producing an effective saccharose. Drought resistant plants have this faculty in a greater degree than those not resistant. The ability to produce an active saccharose is developed in the early phases of wilting and produces a hardening of the plant. Vassiliev and Vassiliev (40) and Vassiliev (39) report their experiments with wheat during wilting indicate that as the water supply decreases and the water deficit in the tissues increases, the process of hydrolysis of the complex combinations of carbohydrates into simpler ones becomes predominant. When the supply of water increases the reverse process of synthesis takes place intensively and the amount of soluble carbohydrates decreases.

Vassiliev feels that the role of sugars in plants undergoing the influences of drought is analogous to their role in the plants exposed to frost. In both cases the sugars are substances protecting the plant organism against adverse conditions. Newton (28) has made the same observation in his study of the cold resistance of plants. He further states that the precipitation of the colloids under low temperatures appears to be similar to their coagulation by heat, and that the precipitation is delayed when sucrose is present in the cells of the plant. It has also been observed that enzymes are capable of tolerating a much higher temperature when there is ample sucrose present in the cells. Loomis (19) reports that corn plants fail to form starch under drought conditions, indicating that the substances which normally are used for starch synthesis are used by the plant to exist under drought conditions. He found that there was an increase in sucrose content, particularly in the stalks, but the total carbohydrate content did not increase during the drought.

MATERIALS AND METHODS

The material used in the bound water determinations reported herein consisted of the first six inches of alfalfa root immediately below the crown. Three varieties of alfalfa, Kaw, Kansas Common, and Ladak, growing in plots at the Agronomy Farm were selected for the tests. These plots were planted in the fall of 1936.

Five varieties of alfalfa were tested in the high temperature chamber. They were Kaw, Kansas Common, Ladak, Hairy Peruvian, and Oklahoma Common. Seed of these varieties was planted in four-inch porous clay pots in the greenhouse. A mixture of six parts black silt loam, one part sand, and one part compost was used to fill the pots. The seed was not scarified. Enough was planted in each pot to secure at least five uniform plants per pot. Shortly after germination the pots were uniformly thinned to five plants each. The pots were watered enough to keep the soil at the optimum water content.

Kansas Common alfalfa, according to Waters (42), probably originated in Persia and was brought to Kansas by way of California. It is well adapted to Kansas, very leafy, and yields as well as the better strains of other varieties. It was first introduced into Kansas about 1870 and the present strain is probably a descendant of these first introductions. Ladak is a cross between Medicago sativa and Medicago falcata. The variety is very cold resistant and gives a good yield, especially for the first cutting. Kaw, an introduction from France, resembles the Turkestans as to type and growth habits. Oklahoma Common is a regional type of common alfalfa adapted in Oklahoma where the winters are less severe than in Kansas. Consequently it is less winter hardy than Kansas Common. Hairy Peruvian, introduced from Peru in 1903, is grown in the southern part of the United States where the winters are very mild and the alfalfa may grow all the year long. It will seldom survive the winter in Kansas.

The dilatometric method of determining bound water was selected for this experiment as it was best suited to the materials and equipment at hand. This method has been used by Novikov (31), Bouyoucos (4), Foote and Saxton (7, 8, 9), Rosa (33), Lott (20), and McCool and Millar (22). Sayer (34), in a comparison of the dilatometric, cryoscopic and calorimetric methods, found these methods to be in close agreement on some materials tested by him.

The freezing apparatus shown in Plate I consisted of a small battery jar six inches deep and four inches in diameter, being imbedded in sphagnum moss in a two-gallon earthen jar. Over the surface of the moss was run melted parafin to keep the moss from spilling and from becoming soaked with water. Two such freezing jars were made as well as a smaller one which was used to cool the ligroin before it was poured over the sample. This type of freezing jar was satisfactory as it was possible to maintain the desired temperature over a period of eight hours.

The freezing bath consisted of a mixture of ice, salt and water. It was possible to maintain a constant temperature over long periods by the addition of more salt, ice or water. A thermometer was placed in the bath and frequent readings taken to insure the maintenance of a constant temperature in the bath.

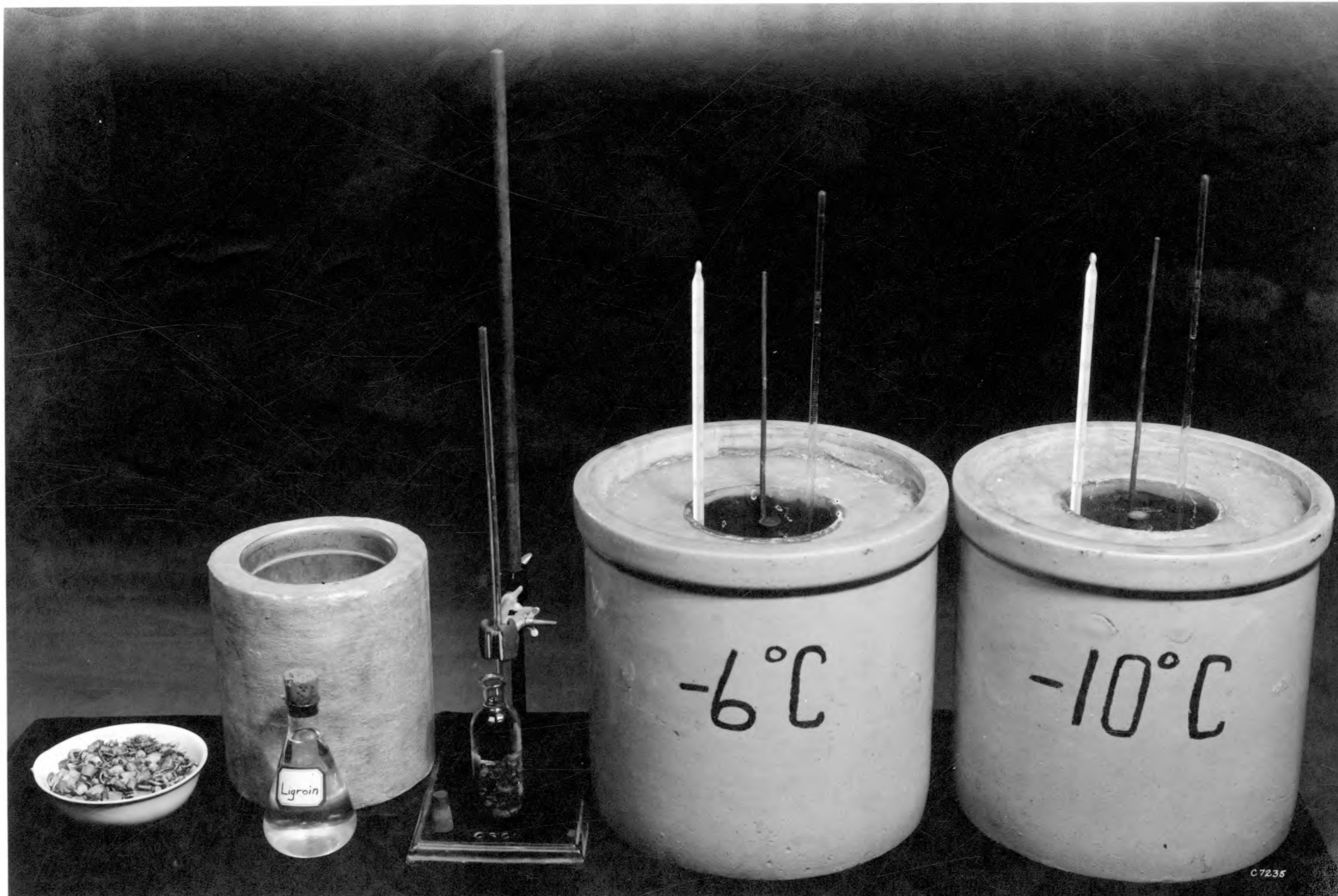
The dilatometers used had a capacity of approximately 50 ml. and were of the small-mouthed type. The side arm, the portion of the dilatometer held by the burette clamp as shown in Plate I, was sufficiently long to insure the meniscus of the ligroin being above the surface of the bath when contraction had ceased. The side arm had a capacity of 1 ml. and was graduated to 0.001 ml. Ligroin (petroleum ether) was used in the dilatometers as it is not miscible with water and also it rapidly assumes the temperature of the freezing bath.

Random samples of 50 roots of each variety were dug from the plots at eight o'clock the morning of the test. Immediately after digging they were wrapped in a wet cloth and brought to the laboratory as quickly as possible. Here they were washed and trimmed to a uniform length of six inches after the tops and crowns had been removed. As soon as the roots were washed they were dried with a paper towel and cut in small pieces, not over 10 mm. in size. This size allowed their being easily and quickly placed in the dilatometer and also hastened the freezing process. After the roots were cut up they were placed in the blast of a fan to evaporate from the surface the water that might have remained after drying with the paper towel. When the excess water on the surface was evaporated four samples of ten grams each were weighed from this material. Care was taken to secure uniform random samples. Two of the samples were placed in a constant temperature oven for a moisture determination and the other two samples were used for the bound water determination. The oven was kept at 90° C. and the samples were allowed to remain at this temperature until they had attained a constant weight. In all weighing a chainomatic balance sensitive to 0.001 gm. was used.

The procedure in the freezing test was as follows: the ice, salt, and water were mixed in the jars in the proper portion to bring the temperature to -6° C. in the first bath and to a -10° C. in the second bath. No salt was placed in

Plate I. Equipment used in the dilatometric method of determining bound water.

Plate I.



the smaller freezing jar in which the ligroin was cooled. Next about 75 ml. of ligroin was put in a small Erlenmeyer flask and this was placed in the small freezing jar and cooled to 0° C. A 10-gram sample of material was then placed in the dilatometer, the cooled ligroin poured over it, and the dilatometer stoppered with a rubber stopper and immersed in the -6° C. bath as quickly as possible. Speed was essential in this operation to prevent the ligroin from becoming warmed more than was necessary. It was also important in this operation to get all of the air out of the dilatometer. This was easily accomplished by pushing a small copper wire down the side-arm and working the bubbles to the surface. After placing the dilatometer in the -6° C. bath the level of the meniscus of the ligroin in the side-arm was closely watched and the point at which it stopped falling was recorded. This point was quickly reached. As the ice formed in the sample the ligroin was pushed up the side-arm of the dilatometer. Frequent readings were taken during the expansion period until the ligroin in the side-arm had reached equilibrium. This high point was recorded. The dilatometer was then placed in the -10° C. bath where it was allowed to remain until it had reached equilibrium. As soon as equilibrium was reached, the dilatometer was brought back to the -6° C. bath and allowed to reach equilibrium again. This second reading was used as a check on the first reading and also to be sure that all of the water that was freezable at -6° C. was frozen. In all cases sufficient time was allowed to elapse after the ligroin had stopped motion in the side-arm to be sure that an equilibrium had been established. All samples were run in duplicate and as nearly at the same time as was possible.

The method of calculating the amount of bound water is the same as used by Bouyoucos (4) and Rosa (33). The use of the dilatometer is based upon the fact that one gram of water increases approximately one-tenth in volume upon freezing. Therefore, the amount of the expansion upon freezing, which is shown by the distance that the ligroin had raised in the side-arm, multiplied by 10 gives the number of ccs. of ice that was formed in the sample. The per cent of bound water can, therefore, be determined by dividing the number of ccs. of ice formed by the total moisture content of the sample, and then subtracting this from 100 to get the percentage of bound water, or unfreezable water at -6° C.

The method used in the study of the response of several varieties of alfalfa to high temperatures consisted of placing the alfalfa in a high temperature chamber which is located in the Agronomy greenhouse at Kansas State College. This high temperature chamber is six feet square, nine feet high, and is well insulated. High temperatures are secured in the chamber by drawing air into the chamber over electric heaters which are enclosed in a small compartment outside the heat chamber. The temperature is automatically controlled by means of thermo-couples which are wired to automatic relays. The range of temperature is from ordinary greenhouse temperature to 160° F. Relative humidity is controlled independently of the temperature by means of a spray of water in the stream of air that is drawn into the chamber. It was possible to secure good control of both temperature and relative humidity. A small fan inside the chamber kept the air in constant circulation, thus overcoming stratification of the air and difference in the temperature at various locations inside the chamber. The plants were subjected to a five-hour treatment in all cases. The temperature was maintained at 130° F. ±1 and the relative humidity maintained at 30 per cent ±1 per cent. The plants were given all the water they could use during their exposure to the high temperatures. This was accomplished by standing the pots in shallow pans. The plants that were tested in the morning were covered the night before to insure their receiving no light until they were placed in the chamber at 7 o'clock A.M. The plants that were tested in the afternoon were allowed to stand on the benches in the greenhouse until noon and were then moved directly into the high temperature chamber. The morning tests were started at 7 o'clock A.M. and ran until 12 N. The afternoon tests were started at 12 N. and continued until 5 o'clock P.M. Two pots of each variety, each pot having five plants, were used in every test. After the test, the plants were placed upon the benches in the greenhouse and the soil moisture kept at optimum. Notes were taken on the injury one week after the test. One week later, another reading was made in order to check upon the first record and also to secure a reading upon the recovery of the plants. Injury was recorded in per cent of plants killed or the per cent of the above ground portion that was killed.

EXPERIMENTAL RESULTS

Bound Water

The studies relative to bound water were conducted on the roots of Ladak, Kaw and Kansas Common alfalfa which had been planted on September 17, 1936, and from which the samples were taken during the period July 12 to December 31, 1937, at more or less regular intervals. As the summer progressed there was a deficiency of soil moisture and severe atmospheric conditions were experienced. It was hoped that there could be some light thrown upon the drought resistance of the three varieties of alfalfa as a result of the study.

Gortner (11) says that the technic of Newton and Gortner in using expressed juice for bound water studies probably does not yield a true measure of the relationship between bound water and free water as present in the cells and tissues of the plant. This same view is held also by Lott (20) who states that possibly (when the juice is studied) the specific substances which it is desired to study may be left in the residual tissue or at best only a part of it may be extracted. In view of this the root tissue instead of expressed juice was used in the measurement of bound water. Although the roots were cut up it is believed that more nearly natural conditions existed than if expressed juice were used.

Bound water in this experiment is considered to be that portion of the total moisture which does not freeze at a temperature of -6° C. The values for bound water are relatively high in this experiment because the freezing was done at a comparatively high temperature, -6^{o} C. The drought condition during the summer would also tend to make the bound water content high during the period in comparison with the winter period.

The results of the study of bound water in alfalfa roots shows that the difference between the three varieties at any one date is small and can probably be accounted for by experimental error. The varieties, on the average, differed not more than about 7 per cent in bound water content at any one date. This is shown in Table I and figure 1. The varieties also varied as to their relative position in bound water content on the various dates. This would seem to indicate that they are very similar as to their water-binding properties. The close agreement of the varieties as to the amount of total moisture in the root and the variation as to rank among the varieties also were noted. This information is included in Table I and figure 1. In view of these data the discussion of the experiments is based on the average results of the three varieties for each date.

The data presented in figure 2 seem to show four rather distinct periods of bound and free water relationships existent in the roots of alfalfa during the period July 12 to December 31, 1937.

Table I. Percentage of total moisture and bound water in roots of three varieties of alfalfa. July 12 to December 31, 1937.

Variety	7-12	7-19	7-26	8-2	8-23	9-9	9-27	10-4	10-11	10-18	10-25	11-20	12-31
	Total Moisture*												
Ladak	63.7	68.9	67.7	66.5	65.0	61.5	56.5	55.4	60.4	60.9	60.3	63.4	65.5
Kaw	58.5	66.1	70.2	64.8	63.7	61.9	59.5	55.9	61.3	61.4	60.6	64.6	66.9
Kansas Common	56.4	64.9	70.7	65.1	62.0	59.5	58.0	58.2	63.6	62.3	63.4	65.2	65.4
Average	59.5	66.7	69.5	65.5	63.6	60.9	58.0	56.5	61.8	61.5	61.4	64.4	65.9
Amount bound	45.3	56.8	60.5	54.9	54.3	53.0	54.6	52.7	58.0	59.1	56.6	59.2	63.1
	Bound Water - Percentage of Total Water												
Ladak	66.6	89.1	84.5	81.2	83.1	88.6	94.7	97.3	93.4	97.1	90.9	87.8	97.0
Kaw	69.2	85.6	85.8	83.4	82.3	89.5	92.9	92.8	94.3	93.5	95.1	94.9	97.0
Kansas Common	92.5	80.7	90.8	86.9	90.7	83.2	94.8	89.7	96.9	97.6	90.4	94.6	93.1
Average	76.1	85.1	87.0	83.8	85.3	87.1	94.1	93.3	93.9	96.1	92.1	92.4	95.7

* Percentages of water figured in the weight of wet sample.

Figure 1. Per cent of total moisture and bound water in the roots of three varieties of alfalfa. July 12 to December 31, 1937.

Key:

——— = Kansas Common

—·—· = Ladak

----- = Kaw

Figure 1.

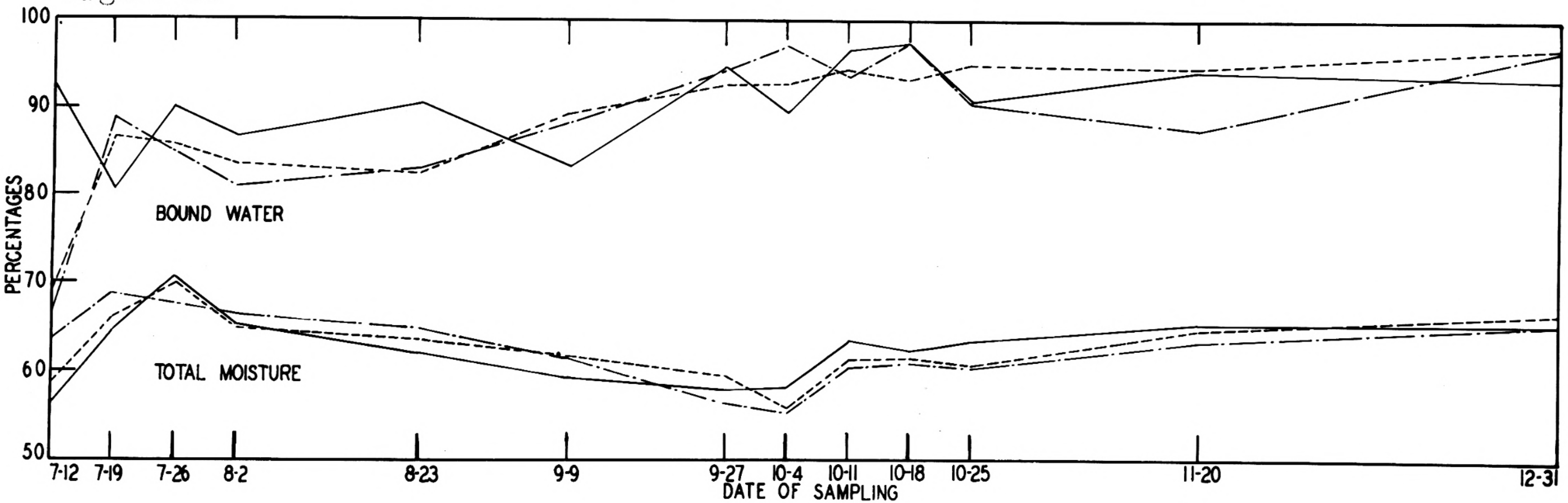
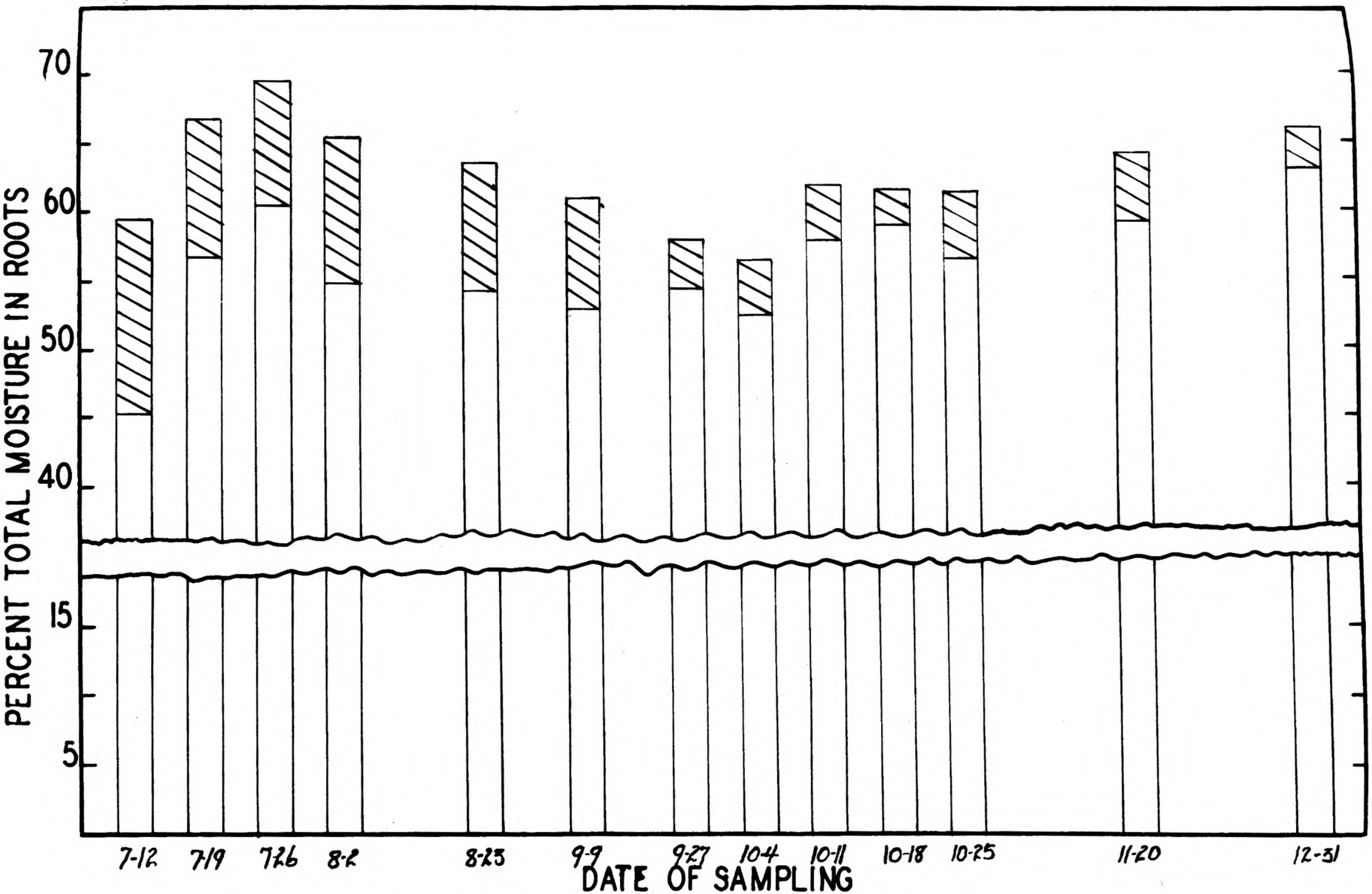

100
90
80
70
60
50
PERCENTAGES
BOUND WATER
TOTAL MOISTURE
7-12 7-19 7-26 8-2 8-23 9-9 9-27 10-4 10-11 10-18 10-25 11-20 12-31
DATE OF SAMPLING

Figure 2. Showing the relative amounts of the total moisture, in the roots of alfalfa, in free and bound states. Column represents the percentage moisture in roots, crosshatch represents the portion in a free state, and not crosshatched represents the portion in a bound condition.

Figure 2.


PERCENT TOTAL MOISTURE IN ROOTS
70
60
50
40
15
5
7-12
7-19
7-26
8-2
8-23
9-9
9-27
10-4
10-11
10-18
10-25
11-20
12-31
DATE OF SAMPLING

The first period noted is for the samples taken from July 12 to July 26 inclusive. There was 59.5 per cent water in the roots on July 12, of which 76.1 per cent was in a bound condition. By July 19 the total water in the roots had increased to 66.7 per cent. This was probably due to .75 inch of rain which fell on July 15 and 16. The proportion of the water which was bound, however, increased to 85.1 per cent. The plots had been cut June 7 and had made some growth up to about July 1. However, there was little rainfall from June 9 until July 12 and as a result there was not an abundance of top growth during this time. Following the rain of 2.74 inches on July 12, there was moisture in the soil available to the plant for growth. The plants did make considerable growth following this rain. As a result of the increasing top growth, which would require considerable quantities of water for development and transpiration, the free water in the roots would be low. This is probably why there was an increase in the amount of bound water in the roots as noted on July 19 even though the total moisture did increase in amount. On July 26, the amount of water in the roots showed an increase of 2.8 per cent over July 19. This was probably due to the moisture in the soil as a result of the rain on July 12 and to two rains on July 24 and 25 amounting to .31 inch. The amount of bound water was only slightly higher than on July 19, being 87.0 per cent of the water in the roots. This was only 1.9 per cent higher than on July 19. It will be noted that during the period July 12 to July 26 the bound water content increased slightly. The total moisture content of the roots showed a steady increase during this period due to an increase in soil moisture as a result of 4.0 inches of rainfall received during the period.

The next period from July 27 to October 4 inclusive, was one of decreasing moisture content in the roots. During the week of July 27 to August 2 there was no measurable rainfall. This was reflected in the total water in the roots which decreased 4.0 per cent from the amount on July 26. The bound water content was 3.2 per cent lower in amount or 83.8 per cent. The next sample was taken on August 23. During this period there were 2.05 inches of rainfall which came in eight rains, only three of which were more than .14 inch. The amount of total moisture in the roots decreased 1.9 per cent during this period. There was 85.3 per cent of the water in a bound condition which was an increase of only 1.5 per cent over August 2. The plots were cut on August 16. By September 9 the moisture in the roots had decreased to 60.9 per cent. There was only .42 inch of rainfall during the period, August 23 to September 9, which would probably account for the decrease noted in total water in the roots. The bound water was 87.1 per cent of the total water or an increase of 1.8 per cent over August 23. The next sample was taken about two weeks later on September 27. The amount of total moisture in the roots had decreased to 58.0 per cent and the amount that was in a bound condition was 94.1 per cent. The decrease in the amount of water in the roots was probably due to the decrease in the amount of soil moisture during this period as only .39 inch of rain was received. Because of this decrease in the amount of total water in the roots and soil, the roots would be under stress to supply the needs of the plant for transpiration and growth. On October 4 the amount of total water had decreased to 56.5 per cent and of this amount 93.3 per cent was in a bound condition. The period was one of decreasing amount of water in the roots, the decrease probably being due to different causes. Probably the drought conditions which prevailed during this period accounted for the reduction of moisture in the roots of samples taken from July 19 to September 9 inclusive. The further reduction in water content of the samples taken on September 27 to October 4 probably was the result of other factors which would cause an increased demand for water by the plant. The rapid development of the crown buds during this period would necessitate large amounts of water for their growth. The crown buds which are the stems of the following year, were very succulent during this period and contained a high per cent of moisture as indicated by the amount of expressed juice extracted under 100 atmospheres pressure (12). The buds were also increasing in dry weight during this period and thus were using water to manufacture the materials required to make up their tissue. The roots were storing large quantities of organic reserves during this period and it would take considerable water to accomplish this. This increase in carbohydrates in the roots would increase the material of a hydrophilic nature present there. According to Gortner (11) the polysaccharoses are in many cases hydrophilic and combine with relatively large amounts of water. As has been shown by Foote and Saxton (7, 8, 9) working with lampblack and inorganic gels of several materials, Bouyoucos (4) with soils, and Robinson (32) with insects, materials of a hydrophilic nature are capable of adsorbing water so tightly that it will not be frozen even at a temperature several degrees lower than the freezing point of water. Such adsorbed water is said to be in a bound condition. Therefore, carbohydrates being hydrophilic, their increase during this period would indicate that there should be an increase of bound water in the roots during this period for it is there that these reserves are stored. The data presented show that this actually occurred.

The next period includes the samples taken from October 4 to October 25. About the first of October the alfalfa went into a semi-dormant condition, ceasing growth but remaining green and physiologically active. The total water in the roots, as determined on October 11, was 61.8 per cent. This increase over October 4 was probably due in part to .31 inch of rainfall during the period October 4 to 11 and in part to the cessation of growth as the alfalfa became semi-dormant. The proportion of the total water that was in a bound condition amounted to 93.9 per cent. This was only .6 per cent higher than on October 4. The total water on October 18 was 61.5 per cent of which 96.1 per cent was in a bound condition. There were 1.69 inches of rainfall falling on four days during this period but it did not penetrate deeply enough into the soil to cause a change in the moisture content of the roots. On October 25 the total moisture content was 61.4 per cent of which 92.1 per cent was bound.

The final period of water relationships is for the samples taken on November 20 and December 31. After October 25 the total water in the roots increased slightly. It was

64.0 per cent on November 20 and 65.9 per cent on December 31. There is very little metabolic activity in the plant during these cold months. The increase in moisture content of the roots was probably due to the replacement of some of the capillary water, as described by Bouyoucos (4), which had been used during September and October. The plants apparently could absorb soil moisture even during this colder season in excess of the amount necessary for the normal functions of the plants. The additional water in the roots was probably adsorbed to the colloidal or hydrophilic material and thus, as would be expected, did not cause an increase in the amount of free water. On November 20 the amount of bound water was 92.4 per cent of the total water and on December 31 there was 95.7 per cent in a bound condition.

The results of the bound water studies on alfalfa are in accord with the work done on red clover at the New Jersey Station by Greathouse and Stuart (15). Their season is a little longer than it is in Kansas and the increase in bound water takes place a little later in the fall. However, the general trends shown by their work agree with the results of this experiment.

High Temperature Studies

The first part of the high temperature study referred to as Series I included work with five varieties of alfalfa; viz., Kaw, Kansas Common, Ladak, Hairy Peruvian and Oklahoma Common. Plants of these were tested at weekly intervals beginning one week after planting and continuing for fourteen weeks. Duplicate plantings were made on three successive days and the plants tested on the same day of the week as they were planted. This made it possible to secure a greater number of replications.

The plants were exposed to a temperature of 130° F. with a relative humidity of about 30 per cent, for a period of five hours. The tests were conducted in the morning and the afternoon. In each test there were two pots of each variety, each pot with five plants. This was considered the unit of the experiment.

Injury was measured in per cent of the plants killed and the per cent of the above ground parts that were killed. Notes were taken one week from the time the plants were tested as the injury seemed to show up best at that time. One week after this first reading another was taken to measure recovery and also as a check on the first reading. There was some difficulty with "damping off" of the plants during the first two or three weeks of growth. To insure against this injury being recorded as heat injury, the heat injury readings on the younger plants were made the day following the treatment and the plants checked each day for a period of two weeks.

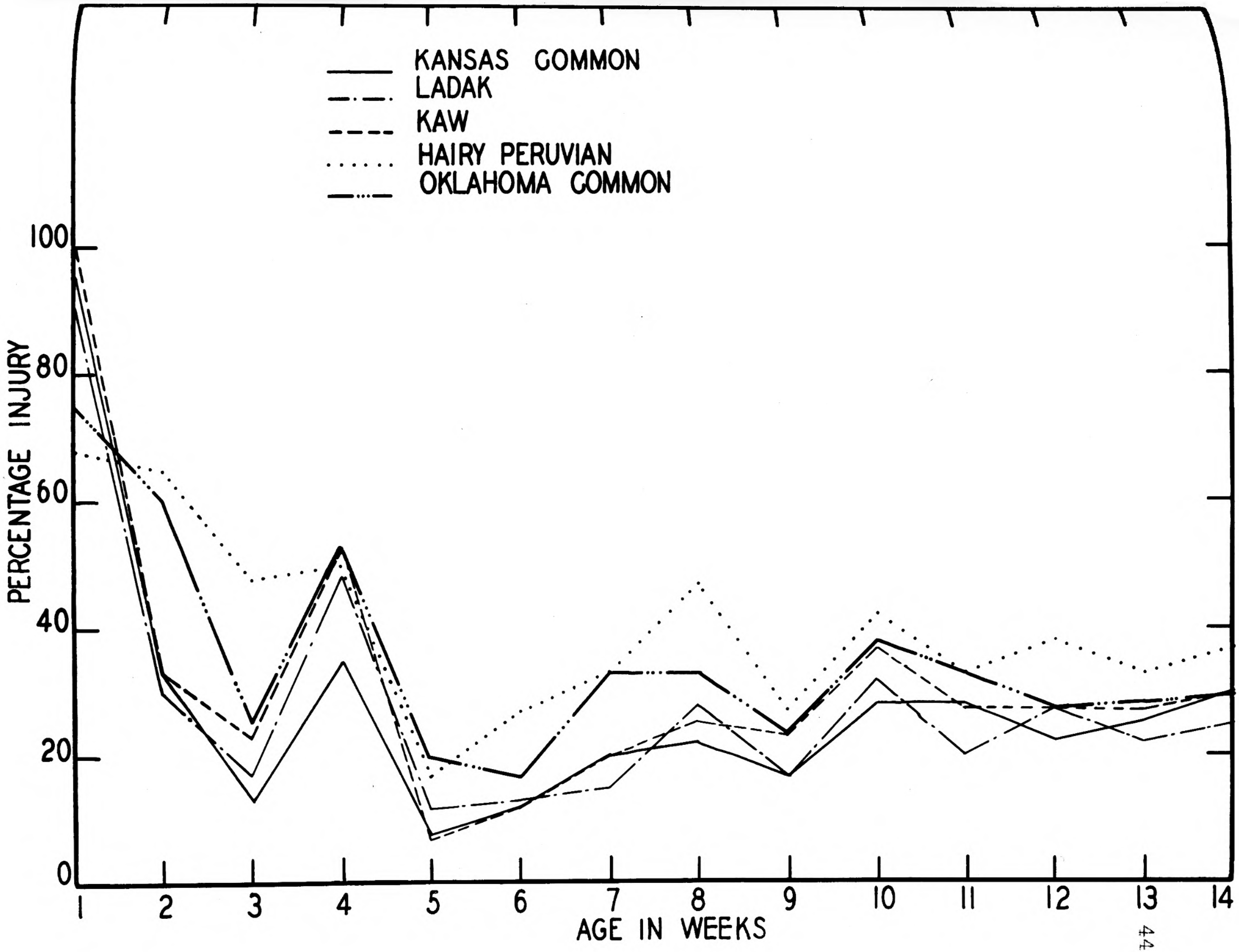
Considering first the tests made in the morning the results of which are presented in Table II and figure 3, we find there is evidence that the southern types, namely, Oklahoma Common and Hairy Peruvian, are more resistant to heat in the very young seedling stage than are Kaw, Ladak or Kansas Common. The injuries to Oklahoma Common and Hairy Peruvian were 75 and 68 per cent respectively, while Ladak, Kaw and Kansas Common were injured 90, 100, and 95 per cent in the order named, at the first test when the plants were one week of age. The injury at the one week age usually resulted in killing the plants although a portion of the injured plants survived. The northern types grew slowly and usually were not so well developed by the end of the first week as were the southern types. They ordinarily had only one leaf while the southern varieties had three or more. In Oklahoma Common and Hairy Peruvian the first leaf in some instances was killed whereas only portions of the other leaves were killed. Such plants often recovered and lowered the per cent of injury for these two varieties below that of the others in which many of the plants had produced only one leaf. The tests made when the plants were two weeks old showed that there was considerable gain in resistance by Ladak, Kaw and Kansas Common. The injury recorded was 30, 33, and 33 per cent respectively. Hairy Peruvian and Oklahoma Common were injured about the same at this age as when the plants were one week old. Their injury was 65 and 60 per cent respectively.

Table II. Average injury to five varieties of alfalfa at 14 ages after five hours heat treatment at 130° F. and 30 per cent relative humidity. Morning treatment.

Age in weeks	Varieties				
	Kansas Common	Ladak	Kaw	Oklahoma Common	Hairy Peruvian
1	95	90*	100	75	68
2	33	30	33	60	65
3	13	17	23	25	48
4	35	48	53	50	50
5	8	12	7	20	17
6	12	13	12	17	27
7	20	15	20	33	33
8	22	28	25	33	47
9	17	17	23	23	27
10	28	32	37	38	42
11	28	20	27	33	33
12	22	27	27	27	38
13	25	22	27	28	33
14	30	25	30	28	37

* Each figure an average of three tests.

Figure 3. Injury to five varieties of alfalfa at 14 ages after five hours heat temperature at 130° F. and 30 per cent relative humidity. Morning treatment.


KANSAS COMMON
LADAK
KAW
HAIRY PERUVIAN
OKLAHOMA COMMON
PERCENTAGE INJURY
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
AGE IN WEEKS

When the plants were more than two weeks old, Oklahoma Common and Hairy Peruvian were injured consistently more by the high temperatures than Ladak, Kaw and Kansas Common. In fact, Hairy Peruvian was injured more than Kaw at every age except four weeks and more than Ladak and Kansas Common at every age. Oklahoma Common was injured as much or more than the latter varieties at all stages except the four-week age when the injury was less than in Kaw and the 14-week age when it was 2 per cent less than Kansas Common and Kaw.

In comparing Oklahoma Common and Hairy Peruvian, the data show that Hairy Peruvian was injured more than Oklahoma Common at nine of the fourteen ages. They were injured the same at three ages and Hairy Peruvian was injured less at only two ages. This would indicate that Oklahoma Common was more heat tolerant, on the average, than was Hairy Peruvian.

Kansas Common and Ladak reacted similarly and were injured less than Oklahoma Common and Hairy Peruvian on all dates except the first, and Kansas Common was injured 2 per cent more than Oklahoma Common on the last age tested. Ladak was injured less than Kansas Common at six ages, Kansas Common less than Ladak at seven ages and they were alike at one age. There was no period when either variety was consistently lower than the other. The average difference between the amount of injury was slight, being only about 4 per cent. The results would indicate that these two varieties were much alike as to heat tolerance, neither being superior to the other.

Kaw was intermediate among the varieties in its reaction to high temperature. It was injured more than any other variety at the first test but from that age on it was injured less than Hairy Peruvian and Oklahoma Common at all but two ages and the injury was the same at one age. In comparing Kaw with Kansas Common, it is found to be injured less than Kansas Common at only two ages and was equal to it at two ages. Kaw was injured less than Ladak at three ages and was equal at one age. The results, therefore, would indicate that Kaw was more tolerant to heat, on the average, than Hairy Peruvian and Oklahoma Common, and a little less tolerant than Kansas Common and Ladak.

Four of the varieties, Kaw, Ladak, Kansas Common and Oklahoma Common, were becoming more alike in their response to high temperature toward the close of the experiment. This was noticed especially for the varieties after they were 11 weeks of age. The plants reached this age the last part of September and during the month of October. At that time all of the varieties except Hairy Peruvian were becoming semi-dormant due to the shortening of the day.

In order to determine the significance of the data, they were subjected to analysis of variance as described by Snedecor (37), the results of which are shown in Table III.

Table III. Analysis of variance of heat injury to five varieties of alfalfa at 14 ages. Morning treatment.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F*	1 per cent point
Between varieties	4	4644.50	1161.13	29.49	3.44
Between ages	13	62645.22	4818.86	122.40	2.30
Between tests	2	321.95	165.98	4.37	4.75
Interaction (var-age)	52	30166.67	580.13	14.74	1.66
Remainder-Experimental error	138	5240.62	37.97		
Total	209	103018.96			

* Ratio obtained by dividing the respective mean square by the mean square of the experimental error.

The F values as interpreted by Fisher's (6) Tables for the distribution of F, show that there is a highly significant difference between the varieties in their reaction to high temperature. The variation due to the plants being tested at various ages was also highly significant, in fact the variation due to age was the most significant of the variables. The variety-age interaction was also significant. The variation between the tests, which was a measure of the variation resulting from the same ages being tested on successive days, was not significant. This would indicate that there was not enough variation in the repetitions of the high temperature test on successive days to alter the significance of the other variables.

The results of the afternoon treatment of Series I are given in Table IV and figure 4.

Table IV. Series I. Average injury to five varieties of alfalfa at 14 ages after five hours heat treatment at 130° F. and 30 per cent relative humidity. Afternoon treatment.

Age in weeks	Varieties: Kansas Common	Ladak	Kaw	Oklahoma Common	Hairy Peruvian
1	3	15*	35	15	3
2	3	5	7	8	22
3	5	7	18	35	37
4	17	17	23	15	37
5	7	8	8	7	15
6	8	10	12	23	20
7	12	17	22	20	22
8	15	30	27	22	38
9	12	13	20	28	30
10	27	27	32	30	37
11	22	15	28	23	30
12	12	18	28	25	32
13	12	12	28	22	28
14	20	17	32	20	28

* Each figure an average of three tests.

The varieties, on the average, were not injured as severely in the afternoon test as they were in the morning test. Hairy Peruvian was less tolerant to heat than any other variety in this test. It was injured as much or more than any other variety at every age except the first, sixth, and fourteenth ages. In the afternoon treatment, Oklahoma Common was more tolerant to high temperature than Kaw, being injured less than Kaw at 10 ages, the exceptions being the second, third, sixth, and ninth ages. Ladak and Kansas Common were the least injured varieties in this test. Kansas Common was injured as little or less than any other variety at 11 ages, although it was not much superior in tolerance to Ladak except at the first and eighth ages. The results seem to indicate that Kansas Common was a little more heat tolerant than Ladak in this test.

So in the afternoon treatment, the varieties reacted similarly with respect to heat tolerance as in the morning treatment, Kansas Common and Ladak being the most tolerant and Hairy Peruvian the least tolerant. However, Oklahoma Common seemed to be superior to Kaw in the afternoon tests while it was slightly inferior in the morning tests.

Figure 4. Injury to five varieties of alfalfa at 14 ages after five hours heat treatment at 130° F. and 30 per cent relative humidity. Afternoon treatment.

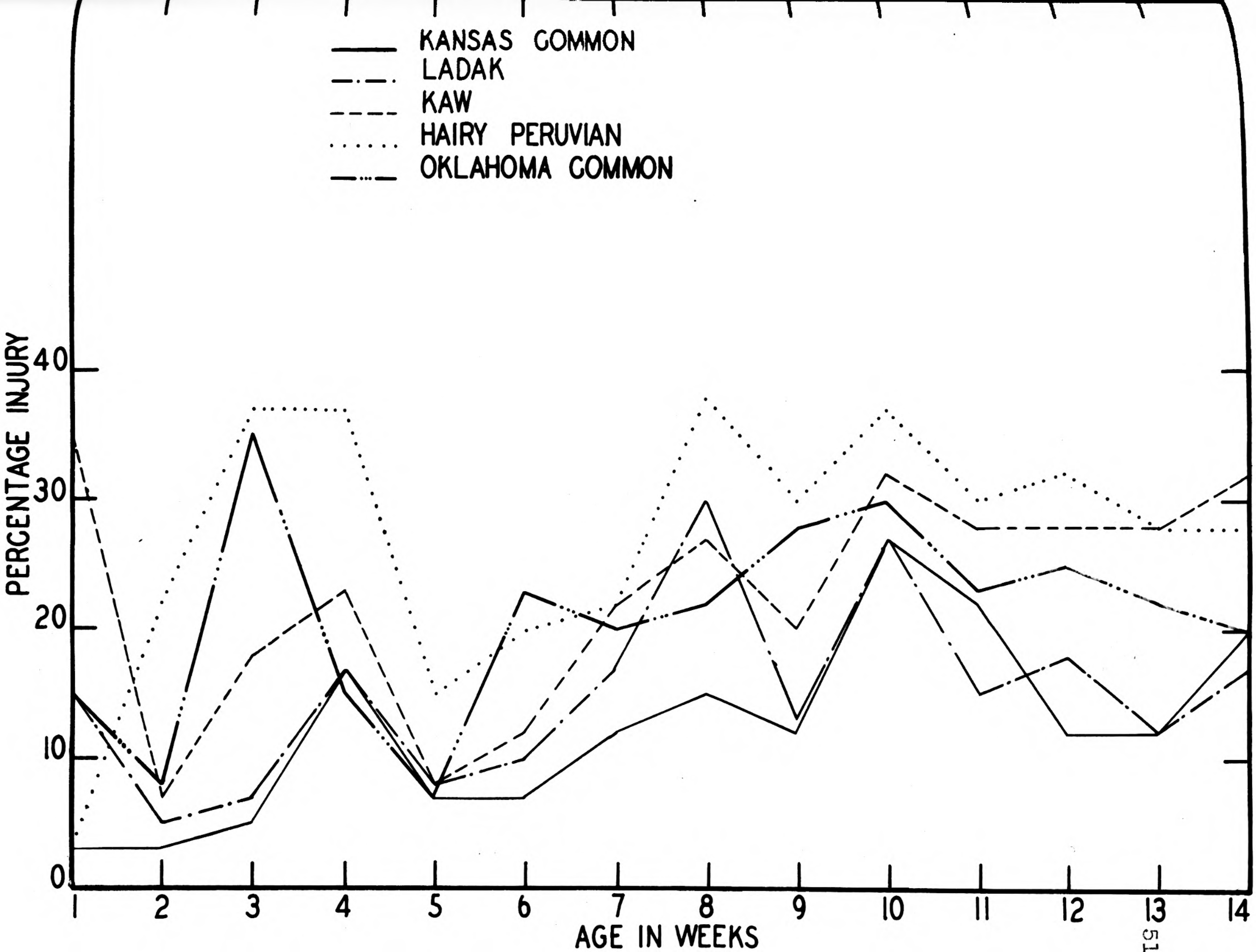

KANSAS COMMON
LADAK
KAW
HAIRY PERUVIAN
OKLAHOMA COMMON
PERCENTAGE INJURY
0
10
20
30
40
1
2
3
4
5
6
7
8
9
10
11
12
13
14
AGE IN WEEKS

Table V. Analysis of variance of heat injury to five varieties of alfalfa for 14 ages. Afternoon treatment.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F*	1 per cent point
Between varieties	4	5725.48	1431.37	43.38	3.44
Between ages	13	6619.53	509.20	15.43	2.30
Between tests	2	434.53	217.27	6.58	4.75
Interaction (var-age)	52	17786.20	342.04	10.37	1.66
Remainder-experimental error	138	4553.79	33.00		
Total	209	35119.53			

* Ratio obtained by dividing the respective mean square by the mean square of the experimental error.

The results of the analysis given in Table V show that the difference between varieties in the test, between the different ages and the interaction between the varieties and the ages are all highly significant. The variation between the tests is also significant although it was not in the morning treatment. A possible explanation of this is that the material was probably less uniform at the beginning of the afternoon than of the morning tests. The plants tested in the morning treatment were covered from 5 o'clock the evening previous to the test and would probably be uniform by the next morning at 8 o'clock when they were placed in the heat chamber. The afternoon treatment followed a period of light from dawn till 12 o'clock noon, and it is possible that the plants responded differently to the environment on the morning of the day on which they were tested. Differences in temperature, humidity, sunlight, and other environmental conditions probably would cause some variation in the heat tolerance of the plants on successive days. However, the F value is only slightly above the 1 per cent point and probably has little importance in relation to the other statistical results since it is much less than the ratios for the other variants.

The second part of the high temperature study was planned so that alfalfa plants of different ages could be tested together whereas in Series I plants of only one age were tested at a time. Ladak and Kansas Common were used in this phase of the experiment and were grown under the same conditions as in Series I. These varieties appeared to be more resistant to heat than the other varieties in preliminary experiments and it was desired to study their resistance more closely. These two varieties were planted at weekly intervals for six plantings. The second week after the first planting there were two ages tested at the same time. Thereafter, one age was added each week until six ages were being tested at one time. This was continued until each age had been tested 14 times. By this means it was possible to observe the comparative heat tolerance of six ages being tested at the same time. There were also six readings recorded for each date of planting except the first and the last where there were only five although the readings were not made on the same day.

The results of this experiment are shown in Table VI and by figure 5.

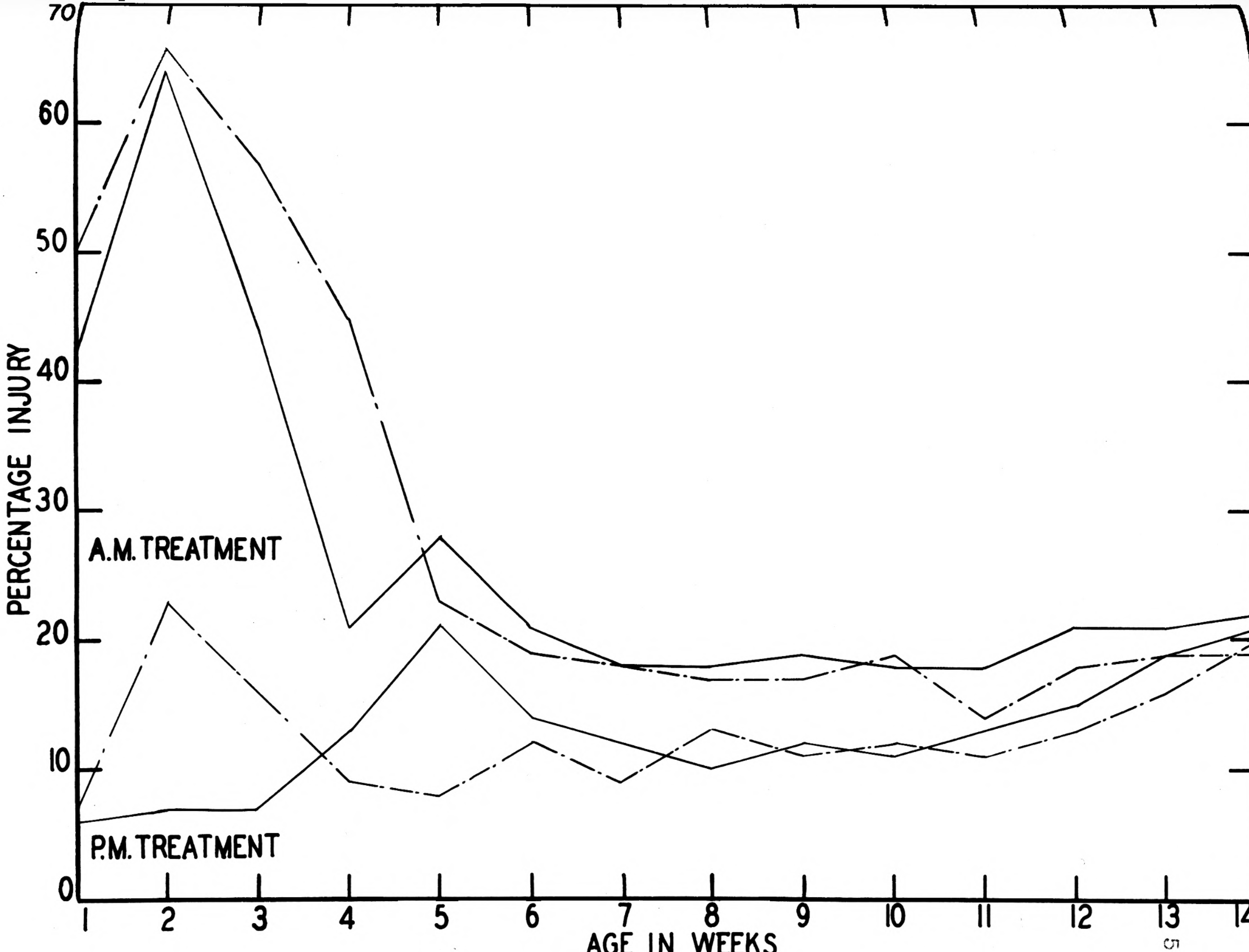
Table VI. Average percentage injury to two varieties of alfalfa by high temperatures when treated in the morning and afternoon.

Variety	Time tested	Ages in weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ladak	A.M.	50*	66	57	45	23	19	18	17	17	19	14	18	19	19
Kan. Com.	A.M.	42	64	44	21	28	21	18	18	19	18	18	21	21	22
Ladak	P.M.	7	23	16	9	8	12	9	13	11	12	11	13	16	20
Kan. Com.	P.M.	6	7	7	13	21	14	12	10	12	11	13	15	19	21

* Each figure for ages two to 13 weeks inclusive is the average of six readings and the others are averages of five readings.

The studies in Series I indicate that differences in repetitions of high temperature tests on different days were

Figure 5. Average percentage injury to two varieties of alfalfa by five hours heat treatment at 130° F. and 30 per cent relative humidity. Morning and afternoon treatments.



70
60
50
40
30
20
10
0
PERCENTAGE INJURY
A.M. TREATMENT
P.M. TREATMENT
1
2
3
4
5
6
7
8
9
10
11
12
13
14
AGE IN WEEKS

of little importance compared with biological differences measured. So, it is believed that the averages of the several readings reported in Table VI, even though each reading was made on a different date, can be considered as replicates without increasing the experimental error appreciably.

Ladak and Kansas Common were very similar in their reaction to high temperatures in this experiment. This tendency was noticed also in Series I. In the morning treatment both varieties were injured severely when one, two, and three weeks of age. Ladak was severely injured at four weeks of age also. Both varieties were injured highest at the second test which was two weeks after planting, Ladak showing 66 per cent and Kansas Common 64 per cent injury. The leaves were very sensitive at this stage and were entirely killed. When all the leaves were killed the plants died. Kansas Common acquired heat tolerance about one week earlier than did Ladak in this test. Kansas Common at three weeks of age was injured 44 per cent while Ladak was injured 57 per cent. One week later Kansas Common injury had dropped to 21 per cent while Ladak was injured 45 per cent. At five weeks of age, Kansas Common suffered 28 per cent injury while Ladak dropped a little lower to 23 per cent. Throughout the remaining ages tested there was very little difference between the two varieties. There was never more than 4 per cent difference, with an average of 2 per cent, in the injury. However, Ladak was injured less than Kansas Common at every age but two, one at which the injury was the same and once when Kansas Common was injured only 1 per cent less than Ladak. After the plants were five weeks old, there were very few in which all the tissue was killed. The injury consisted of the tips of the stems and the outer margins of the leaves being killed. This did not kill the shoot but it did cause cessation of growth.

Considering the afternoon treatment of the same two varieties, we find that there is the same similarity of response. The injury, however, is not nearly as great at the early ages as in the morning treatment. At one week of age Ladak was injured 7 per cent and Kansas Common 6 per cent. The injury increased slightly by the fourth week when the injury to Ladak was 9 per cent and to Kansas Common 11 per cent. By the sixth week the injury to Ladak was 12 per cent and to Kansas Common 14 per cent. The injury remained very uniform from that age until the plants were 11 weeks old. From this time on they became less heat tolerant and at 14 weeks of age Ladak was injured 20 per cent and Kansas Common 21 per cent. At 14 weeks of age the heat tolerance of the two varieties at both morning and afternoon treatments was approximately the same. The tests were started July 28 and the 11-week-old plants were tested late in the fall, from September 27 to November 3, and the 14-week-old plants from October 10 to November 17. During this period the day was shortening and the alfalfa was going into dormancy as was evidenced by the fact that there was little growth. The plants were spreading at the surface of the soil and not growing upward as they normally do during the summer when the days are longer. No artificial light was supplied during the period of the test so it would be expected that the plants would tend toward a dormant condition in the fall. Alfalfa in an adjoining section of the same greenhouse receiving light was making abundant foliage at the time.

In order to determine how nearly Series I and Series II agreed, correlations were calculated for the morning and afternoon treatments of the two varieties, Kansas Common and Ladak. The correlation between the morning treatment of Series I and II for the Ladak was .84±.02, for Kansas Common .81±.03. For the afternoon treatments the correlation between Series I and II was found to be .83±.02 for Ladak and .87±.02 for Kansas Common. According to Fisher's (6) table for significance of "r" with an "n" of 14, the 5 per cent point is .53 and the 1 per cent point is .66. According to this table the values for "r" are highly significant in all cases. This would be an indication that the varieties showed a significantly similar reaction in the two tests.

One of the outstanding differences noted in the high temperature studies was the difference, to the same variety of alfalfa, in injury to the plants tested in the morning as compared with those tested in the afternoon. Table VII shows a comparison of the average percentage injury for all varieties between morning and afternoon treatments.

Table VII. Average percentage injury of each variety in morning compared with afternoon treatments.

Variety	Number of tests	Average percentage injury: Morning treatment	Afternoon treatment	Difference
Kansas Common	28	27.3	12.7	14.6
Ladak	28	28.5	14.0	14.5
Kaw	14	31.6	22.9	8.7
Oklahoma Common	14	35.0	20.9	14.1
Hairy Peruvian	14	40.4	27.1	13.3

Kansas Common, Ladak, Oklahoma Common and Hairy Peruvian showed about the same difference in injury between the morning and afternoon treatments while Kaw showed the least difference. This would indicate that the first mentioned varieties were similar in regard to acquiring tolerance to high temperature during the morning.

The plants tested in the morning were injured more than those tested in the afternoon at every age as is shown in Table VIII.

Table VIII. Average percentage injury of all tests in morning compared with afternoon at each age.

Age in weeks	Number of tests	Average percentage injury		
		Morning treatment	Afternoon treatment	Difference
1	25	69.7	11.6	58.1
2	27	53.5	11.7	41.8
3	27	36.5	16.5	20.0
4	27	40.9	16.3	24.6
5	27	18.5	11.5	7.0
6	27	17.8	13.9	3.9
7	27	20.9	14.4	6.5
8	27	25.0	19.8	5.2
9	27	19.8	16.6	3.2
10	27	28.0	21.9	6.1
11	27	23.0	17.4	5.6
12	27	24.2	17.7	6.5
13	27	23.8	18.0	5.8
14	25	26.2	21.0	5.2

This difference was particularly large for the plants from one to four weeks of age, inclusive. This might be due to the translocation of a high percentage of the photosynthetic products from the leaves of the very young plants, during the night, to the roots for growth and development.

This might leave the plants in a very weakened condition relative to their resistance to high temperature. The plants five to 14 weeks of age, inclusive, did not show as great a difference between morning and afternoon treatments, but the morning treated plants were always injured more than those treated in the afternoon.

DISCUSSION

The proportion of bound water in alfalfa roots seems to be influenced somewhat by the moisture condition in the soil but probably more by the activity and growth of the plant.

The percentage of bound water tended to increase during drought periods in the summer. There was a sharp increase in the percentage of bound water during the fall dormant period when alfalfa was preparing for winter by storage of organic reserves in the roots. This increased the amount of water binding materials present in the roots and, as a result, more water is adsorbed and held in a bound condition. The percentage of bound water remains high during the cold portions of the year.

The results of the high temperature studies indicate that the varieties, in order of decreasing heat tolerance, are divided into three groups: Kansas Common and Ladak; Kaw and Oklahoma Common; and Hairy Peruvian. This is shown in Table VII.

The regional adaptation of the varieties that are grown extensively show them to be located, from north to south, in the following order: Kansas Common, Oklahoma Common, and Hairy Peruvian. Regional adaptation of alfalfa is probably determined by relative winter hardiness more than any other factor. In this respect the varieties are adapted, from north to south, in the following order: Kansas Common, Oklahoma Common, and Hairy Peruvian. This would indicate that the variety most resistant to cold is also the most resistant to high temperature. It might appear inconsistent that a variety of southern adaptation should be the least resistant to high temperature. Newton (28) states that the precipitation of the colloids under conditions of low temperature appears to be similar to their coagulation by heat. It follows that the plant possessing the protoplasm most resistant to cold should be the one most resistant to high temperature. This has been shown in this experiment.

One of the outstanding differences noted in the high temperature studies was the difference in injury, to the same varieties at the same ages, between those receiving high temperature treatment in the morning as compared with those receiving it in the afternoon. The results are shown in Tables VII and VIII.

The stomatal action of alfalfa as described by Miller (27) apparently would not account for the difference in injury between the morning and afternoon tests. The stomata normally open within two to four hours after daylight. This opening is retarded by keeping the plants in the dark, but plants are known to have a daily rythm of opening that is not greatly altered in a short time. Therefore, the stomata probably were partially open when the plants were placed in the heat chamber in the morning. This would make the plants tested in morning and afternoon similar in respect to the condition of the stomata and therefore it is not likely that the difference between the morning and afternoon tests could be due to stomatal action.

According to Miller (26) the insoluble carbohydrates, including starches, pentosans and others, are not formed in large amounts until mid-morning. This would indicate that the alfalfa treated in the morning would have no reserve carbohydrate to draw upon during the test as these insoluble reserves disappear from the leaves almost entirely by midnight. This lack of polysaccharides in the leaf would reduce the amount of material of a colloidal nature and so would reduce the amount of bound water in the leaf according to the views of Gortner (11). Thus, a larger per cent of the water would be capable of being lost by the plant during the morning test. Such would not be the case with the plants put in the chamber at noon. They would have built up a reserve of the polysaccharides in the leaf, increasing the water binding power of the protoplasm and thus there would be less water available for transpiration. Vassiliev and Vassiliev (40) and Vassiliev (39) state that under conditions of drought the hydrolysis of the complex carbohydrates to sugars takes place intensively. They consider that the sugars protect the plant from drought in much the same manner as they protect the plant from cold. The plants tested in the afternoon would have a supply of reserve carbohydrates to draw upon for the hydrolysis and, as a result, would have a larger amount of the sugar available to protect the plant under the adverse conditions of the test.

To accomplish the hydrolysis of the reserve carbohydrates to sugars, as Vassiliev (39) suggests is the case, it would be imperative that the enzymes which act upon these reserves be in a medium and in an environment in which their action is not impaired. The tests were made at 130° F. which is 54.4° C. This would not affect the changing of the sucrose to fructose and glucose as the enzyme, invertase, is not destroyed or inactivated until a temperature of 65° C. is reached, in fact the temperature for the greatest activity for this enzyme is between 55° and 60° C. according to the data presented by Miller (27). This would indicate that the hydrolysis of sucrose should proceed at a maximum rate as far as the temperature to which the plants were subjected might be a factor. The diastases or amylases in the water extract of several crop plants according to Miller (27) in reporting work done by Chrzaszcz were found to be completely destroyed at temperatures above 60° C. but the optimum temperature ranged from 49° to 59° C. According to this work the temperature of the test should not be prohibitive to the hydrolysis of the starch to maltose. However, at that temperature only maltose would be formed from the hydrolysis of starch by invertase. The maltases have an optimum temperature for activity between 38° and 40° C. and are probably completely destroyed at 50° C. (27). In this case the maltose would probably not be reduced to glucose under the conditions of the test and so the starch as a source of hexoses or simple sugars for the protection of the plant would be of no value. However, maltose would be formed in appreciable amounts and this might possibly aid the plant in protecting itself against the severe conditions of atmospheric drought.

Another factor which might account for the greater damage to the plants treated in the morning would be the precipitation of the colloids. This precipitation could be caused by the increased salt concentration or acidity due to the rapid withdrawal of water from the cells. This has been studied extensively in connection with frost resistance by Newton (28) and others. Newton feels that coagulation by dehydration during freezing should be comparable to coagulation by heat and the results should be similar. He found that sugar, sucrose or dextrose, if present in the cells, prevented appreciably the "salting out" or precipitation of the colloids. The fact that plants manufacture sucrose during the day would lead one to deduce that plants tested during the afternoon would be less injured than the ones tested during the morning before any photosynthesis takes place.

SUMMARY

The bound water studies, conducted on the roots of Kaw, Ladak and Kansas Common alfalfa growing in plots during the period from July 12 to December 31, 1937, did not show a distinct varietal difference. Therefore, bound water measurements did not indicate differences in the relative drought resistance of these varieties.

The percentage of bound water in the roots seemed to be influenced somewhat by the moisture conditions in the soil, but probably more by the activity and growth of the plant.

The proportion of total water in a bound condition increased during the dry period in the summer. The per cent of bound water increased markedly during the time the organic food reserves were being stored in the roots. This high percentage of bound water was maintained during the winter.

The high temperature studies indicated that the varieties, in order of decreasing heat tolerance, were: first, Kansas Common and Ladak; second, Kaw and Oklahoma Common; and last, Hairy Peruvian.

The study further indicated that the varieties were tolerant to high temperature in the same order as they are tolerant to cold. Kansas Common and Ladak are grown farther north than Oklahoma Common and Kaw, which, are in turn, grown farther north than Hairy Peruvian.

The varieties tested were injured more severely at every age when treated in the morning than in the afternoon.

The younger plants, one to four weeks of age, inclusive, were injured to a greater degree by high temperature than the more mature plants, from five to 14 weeks of age.

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LITERATURE CITED

(1) Aamodt, O. S.
A machine for testing the resistance of plants to injury by atmospheric drought. Can. Jour. Res. 12:788-95. June, 1935.

(2) ________ and Johnston, W. H.
Studies on drought resistance in spring wheat. Can. Jour. Res. C14:122-52. March, 1936.

(3) Briggs, David R.
Water relationships in colloids. II. "Bound" water in colloids. Jour. Phys. Chem. Part 1. 36: 367-86. Jan. 1932.

(4) Bouyoucos, C. J.
Classification and measurement of the different forms of water in the soil by means of the dilatometer method. Mich. Agr. Exp. Sta. Tech. Bul. 36, 48 p. Sept. 1917.

(5) Dillman, A. C.
The water requirements of certain crop plants and weeds in the Northern Great Plains. Jour. Agr. Res. 42:187-238. Apr. 1931.

(6) Fisher, R. A.
Statistical methods for research workers. 6th ed. Edinburgh. Oliver and Boyd, 339 p. 1936.

(7) Foote, H. W. and Saxton, Blair.
The effect of freezing on certain inorganic hydrogels. Jour. Amer. Chem. Soc. Part 1. 38:588-609. March, 1916.

(8) ________
The freezing of water absorbed in lamp black. Jour. Amer. Chem. Soc. Part 1. 39:627-30. Apr. 1917.

(9) ________
The effect of freezing on certain inorganic hydrosols II. Jour. Amer. Chem. Soc. Part 1. 39:1103-25. June, 1917.

(10) Garver, Samuel.
Alfalfa root studies. U. S. Dept. Agr. Bul. 1087, 28 p. 1922.

(11) Gortner, Ross A.
Outlines of biochemistry. N. Y. John Wiley and Sons, 793 p. 1929.

(12) Grandfield, C. O.
The storage of food reserves in roots and crown buds of alfalfa. Data from unpublished work. 1937.

(13) ________ and Zink, Frank S.
A humidity and temperature control cabinet for growing plants. Jour. Agr. Res. 54:503-8. Apr. 1937.

(14) Greathouse, Glen A.
Unfreezable and freezable equilibrium in plant tissues as influenced by sub-zero temperatures. Plant Phys. 10:781-88. 1935.

(15) ________ and Stuart, Neil W.
A study of the physical and chemical properties of red clover roots in the cold-hardened and unhardened condition. Univ. of Md. Agr. Exp. Sta. Bul. 370, 18 p. 1934.

(16) Hunter, J. W., Laude, H. H., and Brunson, A. M.
A method for studying resistance to drought injury in inbred lines of maize. Amer. Soc. Agron., Jour. 28:694-99. Jan. 1936.

(17) Jones, Ivan D. and Gortner, Ross A.
Free and bound water in elastic and non-elastic gels. Phys. Chem., Jour. Part 1. 36:387-436. Jan. 1932.

(18) Kiesselbach, T.
Transpiration as a factor in crop production. Neb. Agr. Exp. Sta. Res. Bul. 6, 214 p. June, 1916.

(19) Loomis, W. E.
The chemical composition of drought injured corn plants. Amer. Soc. Agron., Jour. 29:697-702. Aug. 1937.

(20) Lott, R. V.
Correlation of chemical composition with hardiness in brambles. Mo. Agr. Exp. Sta. Res. Bul. 95, 22 p. Dec. 1926.

(21) Lvof, S. D. and Fichtenholz, S. S.
Concerning the question of the biochemical foundations of drought resistance. Acta Inst. Bot. Acad. Sci. U.S.S.R. 149-233. 1936. (German summary 215-21 translated to American).

(22) McCool, M. M. and Millar, C. E.
Use of dilatometer in studying soil and plant relationships. Bot. Gaz. 70:317-19. Oct. 1920.

(23) Maximov, N. A.
The plant in relation to water. London. Allen and Unwin, 451 p. 1929. (Authorized English Translation by R. H. Yapp).

(24) ________
Internal factors of frost and drought resistance of plants. Bul. Appl. Bot. Gen. and Plant Breed. 22:40-41. 1929. (Russian with English summary).

(25) Miller, E. C.
Comparative study of the root systems and leaf areas of corn and sorghum. Jour. Agr. Res. 6: 311-33. May, 1916.

(26) ________
Daily variation of the carbohydrates in the leaves of corn and sorghums. Jour. Agr. Res. 27:785-808. March, 1924.

(27) ________
Plant Physiology. New York. McGraw-Hill, 900 p. 1931.

(28) Newton, Robert.
Colloidal properties of winter wheat plants in relation to frost resistance. Jour. Agr. Sci. 14:177-91. Apr. 1924.

(29) ________ and Gortner, Ross A.
A method of estimating the hydrophilic colloids in expressed plant fluids. Bot. Gaz. 74:442-46. Dec. 1922.

(30) ________ and Martin, W. M.
Physio-chemical studies on the nature of drought resistance. Can. Jour. Res. 3:336-427. Oct. and Nov. 1930.

(31) Novikov, V. A.
Jour. Exp. Agron. of the S. E. (U.S.S.R.) II. Part 1:47-66. 1936. (English translation).

(32) Robinson, W. A.
Water binding capacity of colloids a definite factor in winter hardiness of insects. Econ. Ent., Jour. 20:80-88. Feb. 1927.

(33) Rosa, J. T.
Investigations on the hardening process in vegetable plants. Mo. Agr. Exp. Sta. Res. Bul. 48, 97 p. Dec. 1921.

(34) Sayer, J. W.
Methods of determining bound water in plant tissue. Jour. Agr. Res. 44:669-88. May, 1932.

(35) Shantz, H. L. and Piemeisel, P. L.
Water requirements of plants at Akron, Colorado. Jour. Agr. Res. 34:1093-1190. June, 1927.

(36) Shirley, H. L.
A method of studying drought resistance. Sci. 79:14-16. Jan. 1934.

(37) Snedecor, George W.
Statistical methods. Ames, Iowa Collegiate Press. p. 214-17. 1937.

(38) Tumanov, I. I.
Wilting and drought resistance. Bul. Appl. Bot. Gen. and Plant Breed. 22:1-2:144-46. 1929. (Russian with English summary).

(39) Vassiliev, I. M.
The effect of drought on the carbohydrates of wheat plants. Bul. Appl. Bot. Gen. and Plant Breed. 27:5:47-69. 1931. (Russian with English summary).

(40) __________ and Vassiliev, M. G.
Changes in the carbohydrate content of wheat plants during hardening for drought resistance. Plant Phys. 11:115-25. 1936.

(41) Vavilov, I. N.
Studies on the origin of cultivated plants. Bul. Appl. Bot. Gen. and Plant Breed. 16:2:139-248. 1926.

(42) Waters, H. J.
From Persia to Kansas. Kans. St. Bd. Agr., Quarterly Report. 35:171-85. 1916.