FACTORS AFFECTING, AND METHODS FOR MEASURING LOW TEMPERATURE TOLERANCE OF ALFALFA, *MEDICAGO SATIVA* L.

by

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INTRODUCTION

Alfalfa production has increased remarkably in the past 25 years. This has been due partly to increased acreage, in turn, influenced greatly by a dependable supply of high quality seed of improved varieties. Of considerable importance, has been the introduction of new varieties which combine resistance to bacterial wilt (Corynebacterium insidiosum) and winter injury. Most of the newer varieties have sufficient wilt resistance to withstand natural conditions found in North America. The resistance to low temperature injury is not so complete. The newer varieties have varying degrees of winter hardiness which limit their utility in some areas.

It is generally recognized that winter killing is one of the most common causes of the loss of stands of alfalfa throughout the northern alfalfa-growing sections of North America. Much has been written on the value of planting only hardy varieties, as well as on the relationship of cutting frequency, fall management and soil fertility to the longevity of the stand. Relatively little work has been done in the evaluation of the newer varieties for winter hardiness as influenced by these cultural practices.

The purpose of this study was to measure the influence of variety, fertility, clipping treatments, fall management, stand density and soil temperature on the winter hardiness of alfalfa. The effects were measured over a three year period involving several self-contained experiments. In measuring the effect of these factors on low temperature hardiness, two criteria were used. The major criterion was the ability of treated plants to withstand artificially controlled freezing temperatures. Yield also was used as a measure of the effects of the cultural treatments, and their influence on survival.

REVIEW OF PERTINENT LITERATURE

Low temperature hardiness is a subject which has concerned many people for many years. As a result, the literature pertaining to this subject is voluminous. This review will, where possible, deal only with low temperature hardiness as it pertains to cultivated forage plants and more specifically to the legumes, especially alfalfa.

In order to facilitate the presentation of this material, the subject matter has been divided into four major areas; mechanisms of low temperature hardiness, cultural practices affecting low temperature hardiness, morphological factors associated with low temperature hardiness, and methods of measuring low temperature hardiness.

Mechanisms of Low Temperature Hardiness

The literature contains many theories on low temperature hardiness. They are concerned with the relationship between hardiness and structural characteristics (13) (58) (78) (86), water content of the plants (4) (57), cell sap concentration (30) (31) (53) (83), and such protoplasmic factors as viscosity (15) (44), hydrophillic properties (15) (85), membrane permeability (15) (43), and nitrogen content (7) (51).

In certain early work, Hunter (36) expressed the opinion that some winter hardy plants escaped injury because of the heat evolved in their life processes. Aristotle expressed this concept by saying that life cannot exist without its "natural heat". Hunter's later work (37) showed the internal temperature of tree trunks to be nearly always warmer than the surrounding air. He also observed that the sap obtained from tree trunks froze at a higher temperature than the trunk itself. From such evidence, he concluded that all frozen trees are dead trees. Others (11, p. 275) (87, pp. 244-248) expressed similar views and this "Caloric theory" became the prevalent one accepted and expounded in the early part of the nineteenth century.

The prevalence of this theory did not prevent the publication of experimental results in disagreement with it. According to Levitt (44), Du Petit-Thouars and Dubanel actually observed ice crystals inside of plants that proved to be alive on thawing. They also reported the freezing of apples for two months without apparent injury. They raised the question, is there any limit to the low temperature that plants can attain without injury?

Stuckey and Curtis (76) have shown that in the case of higher plants, protoplasm can survive long exposures to the lowest temperatures attainable (liquid hydrogen - 250°C). However, this is true only if the protoplasm is in a dry state. The seeds and other plant parts that survive the lowest temperatures are readily killed if allowed to take up water.

Chandler and Hildreth (10) immersed fully hydrated cells of higher plants in sugar solutions and gained complete protection from low temperature injury. They emphasized that protection occurs only if the solutions are of such concentration to plasmolyze the cells.

Wiegand (86) working with the buds from eight species of trees has shown that plant tissue can survive a much lower temperature if undercooled than if permitted to freeze. Under natural conditions, he demonstrated that tissues may be undercooled to -26.5^{o} C before freezing occurs.

Chambers and Hale (9) pointed out that the lipid plasma membrane inside the cell favors undercooling of the cell sap. The water outside the cell is not so protected and therefore freezes after a slight undercooling. They suggested that the lipid layer also keeps the ice crystals which are formed in the intercellular spaces from "seeding" the cell sap. Lusena and Cook (48) have shown that once the ice forms in the intercellular spaces the vapor pressure drops below that inside the cell, and water diffuses from the cell through the plasma membrane into the regions of ice formation.

Whether or not ice forms inside the cell depends on the rate of exosmosis of the water to the extracellular ice loci. This concept was advanced by Scarth and Levitt (64). Since the permeability of hardy cells to water is high, and the cells frequently are small, exosmosis will be more rapid from hardy than from nonhardy cells. Furthermore, because of the higher osmotic pressure of the hardy cells, less water has to leave them at any one temperature. Thus, hardiness may involve the prevention of the fatal intracellular freezing. Direct observation by Siminovitch and Scarth (66) has confirmed this, for unhardened cells do freeze intracellularly more readily than hardened cells.

Gortner (24) and Stiles (75) postulated that if injury is due to a mechanical disruption of the protoplasmic structure, it should be prevented by reducing the crystal size. Since the structure of protoplasm is submicroscopic, the crystals would also have to be of this size or smaller. Luyet (49) has shown that when freezing is rapid enough to produce ice crystals too small to be seen under the microscope, no injury occurs. This requires extremely rapid freezing and cannot be duplicated in nature. It seems obvious that the prevention of injury by maintaining ice crystals of submicroscopic dimensions is impossible at temperatures to which plants are normally exposed. This agrees with the fact that all observed cases of ice formation in the protoplasm have been fatal.

The slower water loss by hardy plants has led many investigators to postulate an increased water binding power in frost-hardy plants. This has been called bound water and also colloidally bound water. Dexter (16), Grandfield (27), Greathouse (29), and Carroll and Welton (8), working with alfalfa, red clover and Kentucky bluegrass have shown a very distinct correlation between frost hardiness and the bound water content of these plants. On the other hand, Steinmetz (74), Martin (52), and Van Doren (82) working with alfalfa and wheat found no correlation between frost hardiness and bound water content. Megee (53) reported no difference in the rates of water loss between hardy and nonhardy alfalfa varieties. Perhaps the reason for these conflicting results stems from the difficulty in measuring colloidally held water with any degree of accuracy. Meyer (54) indicated that the main difficulty was due to the deviations of sucrose from the laws of ideal solutions which are sufficient to result in large and spurious estimates of colloidally bound water.

According to Levitt (43) most of the results showing a correlation between bound water and hardiness have been due to osmotically bound water. Osmotically active substances are those which are molecularly dissolved. A vast number of direct determinations of solute concentration have in general shown a higher concentration in hardy than in nonhardy plants. Newton (56), Martin (52), Tysdal and Salmon (81), Granhall (28) and Greathouse and Stuart (31) working with small grains and red clover found a positive correlation between solute concentration and frost hardiness. Negative results reported by Steinmetz (74), Weimer (84) and Megee (53) with alfalfa, and Salmon and Fleming (62), working with small grains were obtained primarily when comparing varieties. In most instances, both the hardy and the nonhardy varieties showed the same increase in solute concentration with hardening. In many cases the very workers who succeeded in demonstrating the most striking correlations when some varieties, where compared, failed completely in the case of others. It seems from these results that the increase in cell sap concentration is unable by itself to account for all the increase in frost hardiness that normally accompanies it.

Evidence has accumulated from exact analyses which leaves little doubt that sugar accumulation is the cause of the increased cell sap concentration upon hardening. Newton (56) has shown that total electrolytes remain constant in wheat plants, while sugars which account for only 4% to 8% of the osmotic effect in unhardened plants are responsible for 10% to 40% in the hardened plants. According to Dixon and Atkins (19) and Lewis and Tuttle (46), the relationship is very similar in evergreens.

Graber, et al. (25) reported that injury to alfalfa plants was increased by low concentrations of carbohydrates. Bula and Smith (7) have shown that total carbohydrates in roots and crowns of alfalfa, red clover and sweetclover reach a peak in mid-October. At this time, the carbohydrates are chiefly in the form of starch. By December, the available carbohydrates were largely total sugars and the highest level of cold resistance occurred at this time. The highest level of total sugars was found in sweetclover, and a lower level of total sugars was found in red clover than alfalfa.

The occurrence of a starch minimum and a sugar maximum accompanying the increase in low temperature hardiness of alfalfa has been investigated by Ireland (38) who also noted a reconversion of the sugar to starch in the early spring. Greathouse and Stuart (31) working with red clover report the same general reaction. Mark (51) although unable to place the alfalfa varieties in the proper hardiness groups upon the basis of their chemical composition, was able to demonstrate the partial disappearance of starch and the increase in total sugars with increases in low temperature hardiness. That both temperature and day length are involved in this carbohydrate conversion was advanced by Tysdal (78). Working with several alfalfa varieties, he found the hardier varieties to respond markedly to a short day length in the conversion of starch to sugar. This response, however, was confined to certain temperature ranges. Practically no response to a 7-hour day at 20° C or at 0° C was observed, but at temperatures of 10° C to 12° C, the response was very marked. The intensity of light was also found to be an important factor but only when it fell below a minimum necessary for maintaining a healthy plant.

The most widely held concept is that the conversion of starch to sugar is caused by a temperature drop, Bula and Smith (7) and Arny (1). However, Rosa (61), pointed out that the sugar formation directly due to the temperature drop was relatively slight, otherwise all plants, hardy as well as nonhardy, would show the same sugar accumulation at low temperatures, and it would happen equally at all times of the year. Siminovitch, et al. (67) proposed that the conversion of starch to sugar is due to the activation of a specific enzyme as a result of the shortening photoperiod and temperature drop. Arreguin and Bonner (2) were successful in demonstrating this enzyme activity in potato tubers. Ewart, et al. (22) suggested that a naturally occurring sulfhydryl complex was responsible for the seasonal changes in the carbohydrates. Tysdal (79) concluded that hardy alfalfa varieties have a greater protected diastatic power than nonhardy varieties. Protected diastatic power, as determined by Tysdal is a measure of the resistance of the enzymes to precipitation. His results suggest a correlation between enzyme stability and protoplasm stability.

Cultural Practices

The agronomist is interested in the effects of management on winter hardiness. Crops of great potential value may be ineffectively used when knowledge of helpful practices is lacking or not applied. Proper management may completely alter the winter survival and agronomic value of many crops.

Cultural factors which have been shown to influence the winter hardiness of alfalfa include; time of harvest, fall management, soil moisture level, surface cover, and fertility level. In most instances, winter injury means the cumulative effect of low temperature, ice smothering, cleavage due to heaving, and diseases favored by low temperatures.

Many workers including Kiesselbach and Anderson (39), Nelson (55), Dawson, et al. (12), Salmon, et al. (63) and Garver (23) have all noted a relationship between cutting alfalfa at immature stages and stand loss, especially during the winter. Tysdal and Kiesselbach (80) were able to demonstrate varietal differences in winter survival when harvested at immature stages. The late fall removal of an additional cutting, overgrazing in the fall, and cutting at the bud stage, and allowing little time for fall growth have all been noted as reducing winter survival of alfalfa. Rather and Harrison (60) suggested that alfalfa should be permitted to develop sufficient top growth in the fall to enable it to accumulate ample quantities of starch in the roots as reserve food to carry the plants through the winter and to initiate vigorous growth in the spring. If a fall cutting of hay or fall pasturage is needed, least injury will result if the alfalfa is allowed to accumulate root reserves. Also, the cutting or grazing should be late enough in the fall so cold weather will prevent subsequent growth and the resultant depletion of root reserves. Rather and Dorrance (59), working with grazing trials on alfalfa noted that alfalfa pastured after the first of September in Michigan had less dry matter per 100 roots and had fewer and much less vigorous crown buds than roots from plots which were not grazed in the fall. The heaving of plants was very evident in the fall-pastured alfalfa, while in the plots which were not fall pastured, there was no evidence of heaving. At Ames, Iowa, Mark (51) showed that cutting Grimn alfalfa in late August and again in early October prevented the normal fall accumulation of reserve carbohydrates, and resulted in the complete winter killing of the plants during the winter of 1934-35. The roots of the plants which were not cut after August 29 were 75 percent larger than those given a late cutting on October 8. Under Kansas conditions, Grandfield (26) noted that maximum concentration of carbohydrates in alfalfa roots occurred in late October. He also found that for maximum fall carbohydrate accumulation, there must be 8 to 10 inches of top growth. Removal of the top growth when growth ceased in the fall, resulted in a lower carbohydrate content than when the top growth was left intact. Permitting the aftermath to remain during the winter also resulted in a more vigorous growth and an increased yield of the first cutting the following spring.

To illustrate the importance of a snow cover in protecting perennial plants, Steinmetz (74) reported an air temperature of -21.5° C, the snow temperature at the surface of the soil was -6.2° C, while the soil temperature 2 inches below the surface was -1.2° C. The removal of the snow cover in late winter with alternate thawing and freezing which reduces the resistance to low temperatures, causes great damage to overwintering plants. Kinbacher and Jensen (40) suggested that U. S. Weather Bureau records have limited usefulness in determining the cause of differential spring survival. They found the extreme diurnal fluctuation near the ground, when there was no snow cover, subjected the plant to rigorous environmental conditions. Since such differences exist, they recommend the use of temperature records at plant level for interpretation of winter hardiness data.

The influence of soil moisture on hardiness of alfalfa has been investigated by Tysdal (78). Reducing the soil moisture as low as the wilting point did not markedly increase the hardiness of any of the varieties. The rapidity and degree of freezing of the dry soil as compared to the moist soil, far overshadowed any hardening effect of the low moisture. Plants in the high moisture soil invariably gave the highest percentage survival when frozen for the same length of time as the plants in the low-moisture soil. If the plants were frozen until the temperature in the high-moisture soil was the same as in the low-moisture soil, the survival was slightly in favor of the plants in the low-moisture soil. Plants kept severely wilted for 10 to 14 days and then frozen were much more resistant to cold temperatures than those plants which were watered normally. When soil moistures were equalized immediately before the freezing tests there was little difference in hardiness after two weeks of exposure to different soil moisture levels.

Information on the influence of mineral nutrition on hardiness of alfalfa is very limited. Magistad and Truog (50) have shown nitrogen to reduce the hardiness of corn tissues. Ellet and Wolfe (21), Worzella and Cutler (88) and Livingston and Swinbank (47) all working with winter wheat, have attributed the reduction in hardiness with applications of complete fertilizer or manure to the nitrogen fraction. Potassium and phosphorus applications, in contrast to nitrogen, have generally been found to increase hardiness. Koperzinskii (41) found both potassium and phosphorus to increase the hardiness of red clover. Magistad and Truog (50) found the same relationship with corn. Skinner and Reed (68) have shown increases in hardiness in various vegetable crops with calcium applications. Dexter (14) reported the effects of nitrogen on winter wheat and showed that plants high in nitrogen may completely fail to harden if not illuminated.

Kucinski, et al. (42) noted that alfalfa grown on plots which did not receive potash was more subject to winterkilling, and was often replaced by thick stands of grass and weeds the following year. Seay, et al. (65) found that topdressing alfalfa in the fall with muriate of potash increased the number of living plants present the following year. Attoe and Truog (3) found that winter survival of alfalfa was promoted by potash fertilization. Competition between grass species and alfalfa for potash may eliminate alfalfa from the mixture according to Blaser and Brady (5). This result was most striking when nitrogen fertilizer was applied.

Morphological Factors

In a search for factors associated with low temperature hardiness, several morphological characteristics have been suggested as criteria for selection. Wiegand (86) demonstrated that small cell size is associated with hardiness and suggested that it protects the plant by favoring undercooling in the tissues. The smaller cell size as reflected in smaller plants may help explain why Smith (69) found common White clover to survive 4 weeks of encasement in solid ice while Ladino clover was killed within 2 weeks. Early work by Blinn (6) with alfalfa in Colorado showed the association between hardiness, deeper set crowns and smaller leaf area to be significant. Garver (23) found, that between alfalfa varieties and species of widely differing degrees of cold tolerance, there were striking differences in root type. The root systems of the hardy types had much more of a tendency to branch than did the nonhardy types. Intermediate forms were not sufficiently distinct to be distinguished from one another. Kiesselbach and Anderson (39) reported striking differences in root branching characteristics between southern grown Common and yellow-flowered alfalfas. Smith (70) found Ladak, Grimm and Cossack varieties to be most heavily branched, while New Mexico Common and Buffalo were predominantly tap rooted. Ranger was intermediate with respect to the number of branches arising from the primary root. Prostrate growth has been associated with hardiness. Smith (71) investigated this relationship with alfalfa and found less hardy types produce a greater number of tall plants and a fewer short plants following early fall cutting. Within the same variety he was able to detect differences in winter survival between erect and prostrate types. Heinrichs (34) worked with creeping-rooted alfalfas and found a significant negative correlation between winterkilling and creeping-rootedness. It appeared that killing of the main crown actually stimulated development of new shoots from creeping rootstocks.

Methods of Measuring Low Temperature Hardiness

The most common method of testing for low temperature hardiness in plants is by field survival. However, this type of evaluation measures winter injury which includes much more than just cold injury. Winter hardiness results should be examined with care in observing extenuating circumstances. In some cases where heaving is involved, it may be spring rather than winter survival, or it may be disease resistance which is actually measured. Another complicating feature of using field survival as a measure of differential injury is the scarcity of "test winters" in which conditions are precisely right for differentiating hardy types.

Probably the most common field measure of the comparative winter hardiness of a variety is the "percentage stand counts" or estimates thereof. In other cases "percentage ground cover" or "vigor" in the spring may be used when few or none of the plants actually die. According to Sprague and Graber (73) yield of dry matter per given area may prove effective as a method of judging injury.

A method for determining survival up to any given time is outlined by Worzella and Cutler (88). The injury to the plants may be determined by bringing samples from the field into the greenhouse at intervals during the winter and observing the ability of the plants to initiate new growth. Holmes and Robertson (35) used a portable, bottomless field growth chamber with which they revived plants at given intervals in the field. They were able to detect differences of 5%-10% in winterkilling over a two week period by this technic.

Steinmetz (74) measured the development of hardiness in alfalfa by measuring the depression of the freezing point of naturally hardened root tissue. He used a thermo-electric method suggested by Harvey (33). He found it to be a very rapid method of making determinations, but in general, was unable to differentiate between hardy and nonhardy varieties. He also concluded that no correlation exists between the degree of freezing point depression and resistance to killing by freezing. Greenham and Daday (32) used a modification of this method by impaling root tissue of red clover and alfalfa on electrical needle tips and determining the resistance index.

During the past 25 years the use of refrigeration equipment has become established as an essential tool in cold hardiness studies. With the use of refrigeration, the concept of cold hardiness as a definite developmental process was clearly established. Several arrangements for artificial lighting, temperature and humidity controls are described in the literature, Steinmetz (74), Peltier (57), and Dexter, et al. (17).

Several methods have been used to grow and harden the plants to be tested in the freezing chambers. Steinmetz (74) dug alfalfa roots from field plots at intervals during the year and subjected them to controlled freezing, both in the soil and in the open air. Dexter, et al. (17) describe a method of freezing prepared alfalfa roots in test tubes. The electrical conductivity of the solution resulting from exosmosis of electrolytes from the thawed roots into distilled water was measured. Peltier and Tysdal (58) grew alfalfa seedlings in flats in the greenhouse and subjected them to various hardening treatments of two weeks duration at several ages, usually about one month. They obtained results which correlated with field trials when they included several check varieties in each flat and discarded the edge rows.

When hardening plants in preparation for freezing treatments, research workers have recognized that actual hardiness attained under local conditions is more significant than maximum hardiness attained under artificial conditions. As a result, Steinmetz (74), Bula and Smith (7), and Dexter, et al. (18) preferred to let the alfalfa plants grow and harden in the field before bringing them in for freezing treatments.

Three difficulties arise in the use of the refrigerator to determine winter hardiness. Only by long experience can one select a freezing temperature that will differentiate the hardy and nonhardy variety. Also the length of time of exposure to the freezing temperature is important and difficult to determine. And if temperature and time of exposure are correctly determined, what is the best way to determine the extent of the injury? Timmons and Salmon (77) working with field grown alfalfa plants placed major emphasis on the degree of injury and recorded the percent dead, the percent dead and badly injured, and the percent injury. Peltier and Tysdal (58) measured the percentage survival in terms of a control alfalfa variety. They found the most satisfactory temperatures for differentiating alfalfa varieties was between -10° C and

-20° C, but a short exposure to a relatively low temperature was found better in determining differences than a long exposure to a higher temperature. They used a temperature of -18° C for periods ranging from 1 to 4 hours.

Dexter, et al. (18) working with alfalfa roots of varying ages found the measurement of the electrical conductivity of the exosmosed solution to be a satisfactory means of measuring cold injury. They used a temperature of -10° C and an exposure time of 4 hours, with an interval of 20 hours for exosmosis at 0° C. Bula and Smith (7) used the same technic except the thawing temperature was 3° C. In all cases, the alfalfa plants used were tested in the fall of the seeding year. Smith[1] suggested that when two year old plants are being tested, the exposure time should be extended but the freezing temperature should not exceed -11° C as he found all alfalfa plants, irregardless of type or age, were killed at temperatures below -11° C.

Smith, Dale, Agronomy Department, University of Wisconsin, Madison, Wisc. Electrical conductance method for measuring low-temperature hardiness of alfalfa. Private communication. 1956.

METHODS AND MATERIALS

The primary objectives of this study were to evaluate the influence of various management practices on the low temperature hardiness of several alfalfa varieties and to compare methods of determining hardiness. To realize these objectives, four field experiments were utilized.

In the fall of 1956, root samples were taken from a 3 year old alfalfa varietal test plot and subjected to low temperatures. Subsequent recovery and electrical conductivity measurements were made. During the fall months of 1957 and 1958, root samples were taken from a spaced-row alfalfa experiment and tested in the same general manner as in 1956. These plots were seeded in the spring of 1957. In the fall of 1957, two alfalfa tests were laid out, one, to determine the influence of mulch cover and spacial influence on survival, and the other to measure the influence of seeding date and mulch cover on survival. Plant counts and forage yield were the only measures of survival utilized in these two experiments.

Experiment With Three-Year Old Roots

In 1953, a randomized complete block experiment (split-plot design) was laid out in field 1000-C on the Agronomy Farm, Ames. It included four fertilizer treatments, eight alfalfa varieties and two systems of cutting management.

Fertilizer treatments were assigned to the whole plots and included a check (P_0K_0), yearly applications of phosphorus at 53 pounds per acre (P_1K_0), yearly applications of potassium at 100 pounds per acre (P_0K_1), and both phosphorus and potassium at the above rates (P_1K_1).

Eight varieties of alfalfa were included as sub-plots on each fertilizer treatment. The varieties used were Atlantic, A-224, Buffalo, Grimm, Ladak, Narragansett, Ranger and Vernal.

In 1954, the cutting treatments were imposed as sub-sub-plots on the eight varieties. Each variety plot was divided in half; one half was cut for hay, the other clipped to simulate grazing. These cutting treatments were used throughout the 1954 and 1955 growing seasons. In 1956, the entire experiment was harvested at the hay stage.

In the fall of 1956, the roots of four varieties including A-224 (regional Clone), Buffalo, Ladak and Vernal were sampled according to the schedule in Table 1, and processed according to the indicated method.

At the time of each root harvest, each sub-sub-plot (cutting treatments) was sampled. With four replicates, four fertility treatments, four varieties, and two cutting treatments there were three or six root samples taken from each of 128 plots at each sampling date.

When the root samples were to be frozen and subjected to electrical conductivity analysis, three uniform roots were

Table 1. Schedule of root harvests of four alfalfa varieties. 1956

Date of root harvest	Method of determining hardiness
August 3	C[a]
October 2	R[b]
October 18	C + R
November 1	R
November 15	C + R

[a]Hardiness determined by electrical conductivity of exosmosed solution from artificially frozen roots as described on page 34.

[b]Hardiness determined by recovery after artificial freezing as described on page 33.

dug, and the four-inch portion immediately below the crown of each root was used as the sample. If the roots were to be checked for regrowth after freezing, three uniform roots were dug, and the crown, plus the four-inch portion immediately below was used.

At the time of the second root harvest, all eight varieties were checked for type-of-crown characteristics. The purpose of this was to measure the degree of erectness exhibited by these adapted varieties after three years of differential clipping management. This was done by measuring the angle between the vertical and the lowest crown shoot. A wide angle would indicate a more prostrate type of crown.

Spaced-Row Experiment

On September 1, 1956, a split-plot factorial experiment was laid out on the east end of the south ten acres of the Agronomy Farm, Ames. This field had been in oats in 1956 and corn in 1955 when it was heavily fertilized with nitrogen, phosphorus and potash. Four-hundred pounds of 4-16-8 were applied in each of these two years. This study included such factors as between-row-spacing, varieties of alfalfa, and fall clipping treatments. The three fall clipping treatments and the two varieties (Vernal and Buffalo), were assigned to the whole plots (36 feet x 15 feet). The sub-plots (9 feet x 15 feet) consisted of four row spacing treatments (6", 12", 24", and 42").

The alfalfa was seeded with a Planet Jr. hand seeder, at a rate of twelve pounds per acre in six-inch rows or about one-gram in each fifteen-foot row of the sub-plots. In the 6, 12, 24, and 42 inch spaced-row sub-plots, the number of rows per plot were 17, 9, 5, and 3, respectively. This left a six-inch border on all the sub-plots, except the 42-inch row plots which had a one-foot border. The treatments were replicated four times.

The fall germination and stand were excellent and went into the winter with about a six to eight inch top growth.

Table 2. Minimum air and soil temperatures in $^{\circ}$ F at the Agronomy Farm, Ames. 1954-1958

Year	Date of minimum soil temper- ature	Soil depth (inches)	Snow cover	Soil temper- ature	Air temper- ature	Date of minimum air tem- perature
1954	Jan. 21	1	Trace	7		
	Jan. 21	2 1/4	Trace	6		
	Jan. 21	4	Trace	8	-15	Jan. 21
	Jan. 21	8	Trace	11		
1955	Dec. 20	1	2"	14		
	Dec. 9	2 1/4	Trace	14	-19	Jan. 27
	Dec. 20	4	2"	14		
	Dec. 20	8	2"	16		
1956	Jan. 23	1	3"	14		
	Jan. 23	2 1/4	3"	17		
	Jan. 23	4	3"	15	-23	Jan. 21
	Jan. 23	8	3"	18		
1957	Jan. 23	1	Trace	7		
	Jan. 23	2 1/4	Trace	10	-30	Jan. 14
	Jan. 23	4	Trace	13		
	Jan. 23	8	Trace	19		
1958	Feb. 9	1	4"	14		
	Feb. 9	2 1/4	4"	12		
	Feb. 9	4	4"	15	-2	Feb. 17
	Feb. 11	8	4"	16		

During the winter of 1956-1957 air temperatures reached a record low of -30° F on January 14. The recorded soil temperature on January 15 under a six-inch snow cover was 22° F throughout the surface eight inches of soil. On January 23, the air temperature was $+1^{\circ}$ F, but as the snow cover had disappeared, the soil temperatures ranged from 7° F at the

Table 3. Average index[a] of winter survival (1956-1957) of two alfalfa varieties in four row spacing treatments

Variety	Row spacing 6"	12"	24"	42"
Buffalo	3.83	1.60	0.57	1.0
Vernal	3.44	1.88	0.50	1.0

[a]Recovery index from 0 (all dead) to 5 (complete survival).

one-inch level to 19° at the eight inch level. The minimum air temperatures and the minimum soil temperatures at various depths for the past five years are shown in Table 2.

Perhaps, as a result of these low temperatures, the field survival of the plants was unsatisfactory. In the spring, the differential survival of the plants was recorded and Table 3 shows the survival rating associated with the treatments. It will be noted that the plants in the six-inch-row plots exhibited a high degree of survival.

As a result of this differential survival the experiment was plowed-up and reseeded on April 15, 1957, in exactly the same manner as described above. During the first summer, the plots became overrun with grassy weeds which required extensive hand weeding during July. The plots were sprayed with D.D.T. and toxophene on August 2. By late summer the plants

Table 4. Schedule of fall clipping treatments, and root sampling of two varieties of alfalfa under four spacing treatments during the fall months of 1957 and 1958

Date of root harvests 1957	Date of root harvests 1958	Plots sampled in 1957 root harvests[a]	Date of fall clipping 1957	Date of fall clipping 1958
Sept. 13	Sept. 16	Only plots clipped Sept. 13	Sept. 13	Sept. 16
Oct. 1	Oct. 11	Only plots clipped Sept. 13 and Oct. 1	Oct. 1	Oct. 11
Oct. 28	Oct. 21	All plots	Oct. 30	Nov. 1
	Nov. 4			
	Nov. 20			

[a]In 1957, only the indicated plots were sampled at each root harvest. In 1958, all plots were sampled at each root harvest.

were well established and ready for the fall clipping treatments and root sampling. Table 4 indicates the schedule followed in applying the clipping treatments and sampling the roots for the two years, 1957 and 1958.

The electrical conductivity and recovery methods were used to determine hardiness at each root harvest in the 1957 and 1958 seasons. However, in 1957, the freezing equipment broke down, and other equipment with less precise temperature controls was used. The freezing temperatures were difficult

Figure 1. General view of alfalfa plots in the spaced-row experiments

Figure 2. Alfalfa root sampling operation, showing different fall clipping treatments

to maintain and it was impossible to get differential recovery data. This was also the case for the first sampling date in 1958. In 1958, forage yields were determined. All the plots were harvested three times, on June 19, July 25, and September 2. The plots were sprayed with D.D.T. and Toxaphene on August 5. Figure 1 shows the general layout of the plots. Figure 2 shows the fall clipping treatments and the root harvesting operation.

Two-Dimensional Spacing Experiment

Because of the differential survival of the alfalfa plants during the winter of 1956-1957 another study was initiated in 1957 at the Agronomy Farm, Ames.

In this experiment the spacial arrangement of individual plants as it affects survival was studied in conjunction with different types of surface cover and root association. On August 19, Ranger alfalfa was seeded by hand into micro-depressions at various spacings. Table 5 shows the spacing arrangements and seeding rates for the three treatments.

The spacing treatments were assigned to the whole plots (9 feet x 3 feet) with the surface cover treatments applied in strips to the sub-plots (3 feet x 3 feet). The surface cover treatments consisted of chopped oat straw applied October 9, at the rate of two tons per acre, oats broadcast at the time of seeding at the rate of three bushels per acre,

Table 5. Plant spacing treatments of Ranger alfalfa. 1957

Dimensions of area allotted to each seed (inches)	Square inches per seed	Pounds of seed per acre
1 3/4 x 2	3 1/2	8.15
2 x 2 1/2	5	5.70
4 x 4	16	1.78

and a check treatment which was left without any applied surface cover. At the time the straw mulch was applied, the oats on the broadcast plots were cut to a height of about eight inches.

An auxiliary planting was made adjacent to the experiment from which plants were transplanted into missing plant areas on September 14.

The treatments were replicated four times, but heavy rains from August 2 to September 3 washed out two of the replicates. As it was too late to reseed the entire experiment and be certain of having the plants established before cold temperatures set in, the two remaining replicates were used throughout the experiment.

Permanent quadrats were established in each sub-plot and stand counts made in October of 1957 and April of 1958. Yield data were taken during the 1958 growing season when three harvests were made.

Time of Fall-Seeding Experiment

In the area adjacent to the two-dimensional spacing experiment described above, another experiment was conducted to test the effect of fall seeding date on alfalfa survival under the surface cover and root association treatments previously described.

Ranger alfalfa was seeded with a Tysdal broadcast seeder at the rate of twelve pounds per acre. The dates of seeding were August 17 (early seeding), September 2 (middle seeding), and September 16 (late seeding). The date-of-seeding treatments were assigned to the whole plots (15 feet x 3 feet) and the surface cover treatments applied in strips to the subplots (5 feet x 3 feet). Oats were broadcast on the plots as they were seeded at the rate of three bushels per acre. The chopped oat straw used as a mulch cover was applied on October 9, at which time the oats were clipped to a height of about eight inches. The experiment was replicated eight times. Yield data were taken during the 1958 cropping season when the plots were harvested three times.

Methods Used in Determining Low-Temperature Hardiness

As previously discussed and indicated in Table 1, two methods were used in determining the hardiness of alfalfa roots to low temperatures.

One method was the recovery or regrowth technic, which determined the hardiness of the crowns and roots by observing the amount of regrowth after freezing at a prescribed temperature. When this method was to be employed, three uniform and representative roots were dug from each test plot. Care was taken to ensure that diseased or damaged roots were not used. The crowns were trimmed to approximately 1 1/2 inches above the cotyledonary node and four inches of the tap root were left below the crown. The lateral roots and root hairs were trimmed off. The root samples were then washed and brushed in cold water and placed in the freezer, one layer deep, between moist burlap. The temperature of the freezer was maintained at -8° C. The roots remained in the freezer for twenty-four hours. Thermograph readings indicated that it took six hours for the temperature to equilibrate at -8° C, after each batch of samples was placed in the freezer. The temperature of the freezing chamber was kept uniform with a circulating air fan.

At the end of the twenty-four hour freezing period, the root samples were placed in a thawing chamber at 2° C for another twenty-four hour period. They were then stored at 8° F for eighteen hours. Following this thawing treatment, the roots were transplanted into a pit sand bench in the greenhouse. The plants were watered and sprayed to control mold and insects as needed. After twenty-one days the amount of regrowth that had occurred was rated according to an index ranging from one to five in 1956, and from one to nine in 1958. The lower numbers in each index indicated poor recovery.

The other method used to determine hardiness was the electrical conductivity technic. In this method three uniform and representative alfalfa roots were dug from each test plot. Care was taken to ensure that diseased or damaged roots were not used. The crowns were discarded and the four inch portions immediately below the crowns retained for freezing.

Great care was exercised in washing and drying the roots preparatory to freezing. All root hairs and lateral roots were removed and the roots gently brushed in cold water to remove all external impurities. They were then dried on absorbent towels and weighed. In 1956, the entire four inch root was weighed and the weight recorded to give three subsamples from each plot. In 1957 and 1958 the three roots from each plot were composited and a single ten-gram sample taken for freezing. Following weighing the samples were rinsed twice in distilled water and dried of superficial moisture with clean gauze cloth and placed in lightly stoppered eight inch test tubes. The samples were frozen in the same manner as described above.

After freezing, fifty milliliters of distilled water was added to each test tube. The test tubes were then tightly stoppered and allowed to exosmose at a constant temperature of $+2^{o}$ C for twenty-four hours. At the end of this time, the liquid in each test tube was decanted into a cylinder and the specific conductance determined in mhos x 10^{-5}. The instrument used to measure the conductivity was a Solu-Bridge Soil Tester Model RD 15 with a temperature correction dial and an immersion dip-cell. One reading was made on each sample. In 1956, the average of the three readings from each plot was taken as the average reading. In 1957 and 1958, the single reading on the composited sample was used.

Weather Summary and Data

The weather during the course of this study ranged from normal to very extreme. The growing season of 1956 was characterized by above normal temperatures and far below normal precipitation. The precipitation from January 1 to September 30, was 16.66 inches which was 9.15 inches below the long-term average for this period.

The cropping season of 1957 was normal in most respects. Temperatures were slightly above normal and precipitation was well distributed and about 2 inches above normal for the period from April 1, to September 30.

In 1958, the air temperatures were decidedly below the normal. The average mean monthly temperatures for June, July, August, and September were 4.5, 5.4, 0.3, and 0.2 degrees Fahrenheit below the normals for these months. The 1958 precipitation from January through September was 24.30 inches which was 2.34 inches below normal. The distribution was such that over 23 inches of this amount was received between April 1 and September 30.

Tables 45, 46, and 47, in the appendix show the daily mean 4 inch soil temperatures for 1956, 1957 and 1958 from September 1 to the end of the root sampling period for each year. Also indicated is a daily "cold-unit" determination which is based on deviations of daily mean temperatures below a base temperature of 60° F.

EXPERIMENTAL RESULTS

Results of the various measurements which were made in the several experiments are reported in this section. The treatment means are discussed and, where appropriate, the significant treatment differences are tested at the 5% level, as indicated by Duncan's multiple range test (20).

Three Year Old Roots

A summary of the analysis of variance of the electrical conductance of the three year old roots is presented in Table 6. Electrical conductance was measured on the exosmosed solution obtained from alfalfa root-sections immediately below the crown, which were frozen at -8^{o} C for 24 hours and thawed in distilled water at 2^{o} C for 24 hours.

Error terms have been pooled where the partitioned variances were homogeneous. This analysis indicates that the fertilizers (P, K, and PK) did not have a significant effect on electrical conductance. The four alfalfa varieties differed significantly. The data, shown in Table 7, indicate that Vernal and A-224 and Ladak had the highest degree of resistance to freezing. At the 5% level of significance these three varieties differed significantly from Buffalo.

Cutting treatments had a significant effect on

Table 6. Summary analysis of variance for electrical conductance of alfalfa roots in mhos x 10^{-5} per gram at 25^{o} C, 1956

Source of variation	d.f.	Mean squares[a]
B (Blocks)	3	5.753
F (Fertilizers)	3	1.277
Error (a)	9	1.580
V (Varieties)	3	7.047**
V x F	9	3.046
Error (b)[b]	36	1.554
C (Cutting treatments)	1	19.620**
C x V	3	4.953
C x F	3	4.473
Error (c)[b]	57	1.958
D (Sampling dates)	2	102.855**
D x V	6	5.043**
D x C	2	0.010
Residual[c]	246	0.876
Total	383	

[a]Double asterisk (**) indicates F-test significant at the 1% level.

[b]Pooled sums of squares do not differ significantly as indicated by Bartlett's test, (72).

[c]Coefficient of variation 17.00%.

conductance as shown in Table 8. Under frequent clipping, with the top growth removed when the plants reached a height of 8 inches, the electrical conductance indicates that the plants were unable to harden as rapidly or as completely as plants which were cut for hay.

Table 7. Mean electrical conductance of roots from four alfalfa varieties in mhos x 10^{-5} per gram at 25^{o} C, average of fertilizers, cutting systems and sampling dates. 1956[a]

Vernal	A-224	Ladak	Buffalo
5.29	5.34	5.50	5.89

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

Table 8. Mean electrical conductance in mhos x 10^{-5} per gram at 25^{o} C of alfalfa roots subjected to normal and intense clipping treatments - average of varieties, fertilizer treatments, and sampling dates. 1956[a]

Clipping treatment	Conductance
Hay	5.28
Frequent	5.73

[a]Treatment means differ significantly at the 5% level, Duncan's test (20).

The electrical conductance of the three root harvests differed significantly at the 5% level as shown in Table 9.

The alfalfa varieties differed in their response to the dates of sampling. Table 10 indicates that Buffalo was the slowest variety to attain hardiness as measured by electrical conductivity. The October sampling shows the greatest

Table 9. Mean electrical conductance of alfalfa roots at three sampling dates in mhos x 10^{-5} per gram at 25° C - average of varieties, fertilizer and cutting treatments. 1956[a]

August 31	October 18	November 15
6.48	5.33	4.71

[a]Treatment means differ significantly at the 5% level, Duncan's test (20).

Table 10. Mean electrical conductance in mhos x 10^{-5} per gram at 25° C of roots from four varieties of alfalfa at three sampling dates - average of fertilizer and cutting treatments. 1956

	August 31	October 8	November 15
A-224	6.31	5.28	4.42
Buffalo	6.35	6.11	5.21
Ladak	7.00	4.93	4.57
Vernal	6.23	5.00	4.64

differences in varietal conductance. On November 15, the electrical conductance of Buffalo had not reached the same level as Vernal, A-224 or Ladak.

A summary of the analysis of variance for the recovery index of three-year-old alfalfa crowns is presented in Table

Table 11. Summary analysis of variance for crown recovery index of alfalfa. 1956

Source of variation	d.f.	Mean squares[a]
B (Blocks)	3	2.72
F (Fertilizer)	3	1.59
Error (a)	9	1.09
V (Varieties)	3	13.45**
V x F	9	0.57
Error (b)[b]	36	1.15
C (Cutting treatments)	1	1.10
C x V	3	1.11
C x F	3	0.66
Error (c)[b]	57	1.40
D (Sampling dates)	3	607.64**
D x V	9	1.12
D x C	3	0.37
Residual[c]	369	0.64
Total	511	

[a]The double asterisk (**) indicates F-test significant at the 1% level.

[b]Pooled sums of squares do not differ significantly as indicated by Bartlett's test (72).

[c]Coefficient of variation 46.76%.

11. The crown recovery index was determined by rating the amount of regrowth which occurred in the 21 day period following artificial freezing at -8° C for 24 hours, and transplanting in pit sand in the greenhouse. Fertilizer treatments did not significantly affect the crown recovery index, but the four varieties differed considerably in the amount

Table 12. Mean crown recovery index of four alfalfa varieties - average of sampling dates, fertilizer and cutting treatments. 1956[a]

Buffalo	A-224	Vernal	Ladak
1.38	1.51	1.86	2.09

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

Table 13. Mean crown recovery index of alfalfa crowns at four sampling dates - average of varieties, fertilizer and cutting treatments. 1956[a]

October 2	October 18	November 1	November 15
0.65	0.47	0.87	4.96

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

of crown recovery after freezing. Table 12 shows that Ladak and Vernal were distinctly more vigorous in crown regrowth than Buffalo or A-224. Sampling date had a great influence on the recovery of the alfalfa crowns. Table 13 indicates the degree of crown recovery with advancing sampling date.

The angle between the vertical and the lowest crown

Table 14. Mean stem angle of eight alfalfa varieties - average of fertilizer and clipping treatments. 1956[a]

Atlantic	Buffalo	Ladak	Grimm	Vernal	Ranger	Narragansett	A-224
27.3	29.6	32.5	32.5	35.4	37.5	37.7	44.2

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

branch was measured on 96 alfalfa plants in each of 8 varieties, in the fall of 1956. These plants were over 3 years old and had been subjected to different clipping management treatments for the two years previous to 1956. Table 14 shows the varietal means for the measured angle. The greater the angle the more prostrate was the plant.

Spaced-Row Experiment

In the spring of 1957, two alfalfa varieties, Buffalo and Vernal were planted in a factorial experiment with 4 row spacings (6, 12, 24 and 42 inches), and three fall clipping treatments. The plants from this alfalfa experiment were sampled three times in the fall of the seeding year and the roots subjected to electrical conductivity measurements.

Table 15. Analysis of variance for electrical conductance of alfalfa roots in mhos x 10^{-5} per 10 grams at 25° C. First root sampling September 13, 1957

Source of variation	d.f.	Mean squares
R (Replicates)	3	75.875
V (Varieties)	1	364.500
Error (d)	3	271.750
S (Spacings)	3	210.208
S x V	3	42.417
S x R	9	158.958
S x V x R	9	55.556

Table 15 gives the analysis of variance for the first root harvest on September 13.

As the fall clipping treatments were not applied until the date of the first sampling, no variance could be computed for this treatment until the following sampling date. None of the treatments demonstrated any significant differences in conductivity at this early date.

Table 16 shows the analysis of variance for the second root harvest. At this time it was possible to compare the early clipped plots with the non-clipped plots.

Row spacing treatments at the second harvest differed significantly. The conductance indicated an increase in hardiness with decreasing row width, as shown in Table 17.

The clipping of the plants on September 13 had a

Table 16. Analysis of variance for electrical conductance of alfalfa roots in mhos x 10^{-5} per 10 grams at 25^{o} C. Second root sampling October 1, 1957

Source of variation	d.f.		Mean squares[a]
R (Replicates)	3		332.438
T (Treatments)	3		
V (Varieties)		1	33.063
C (Clipping dates)		1	14,580.563*
C x V		1	175.562
Error (a)	9		
V x R		3	288.687
C x R		3	454.604
C x V x R		3	91.854
S (Row spacings)	3		348.354*
S x T	9		
S x V		3	17.687
S x C		3	54.937
S x V x C		3	112.104
Error (b)[b,c]	36		91.271

[a]Single asterisk (*) indicates F-test significant at the 5% level.

[b]Pooled sums of squares do not differ significantly as indicated by Bartlett's test (72).

[c]Coefficient of variation 25.1%.

significant effect on the conductance. This effect was even more striking at the third sampling date when the clipping treatment was the only factor to demonstrate significance. These differences are shown in Table 18.

The analysis of variance over all harvest dates shown in Table 19, indicates that the time of sampling had a

Table 17. Mean electrical conductance in mhos x 10^{-5} per 10 grams at 25° C of alfalfa roots from plots of different row widths. Second root sampling October 1, 1957 - average of varieties and fall clipping treatments[a]

Row width			
6 inches	12 inches	24 inches	42 inches
105.50	105.94	112.94	114.50

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

Table 18. Mean electrical conductance in mhos x 10^{-5} per 10 grams at 25° C of alfalfa roots subjected to different fall clipping treatments. Third root sampling October 28, 1957 - average of varieties and row spacings[a]

Clipping treatments		
September 13	October 1	Not clipped
77.19	69.59	63.09

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

Table 19. Summary analysis of variance for electrical conductance in mhos x 10^{-5} per 10 grams at 25° C over all sampling dates. 1957

Source of variation	d.f.	Mean squares[a]
R (Replications)	3	1294.597
D (Sampling dates)	2	37842.302**
D x R	6	606.025

[a]Double asterisk (**) indicates F-test significant at the 5% level.

Table 20. Mean electrical conductance in mhos x 10^{-5} per gram at 25° C of alfalfa roots at three sampling dates - average of varieties, row spacings and fall clipping treatments, 1957[a]

Sampling date		
September 13	October 1	October 28
109.56	109.72	69.96

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

significant effect on electrical conductance.

A survey of the sampling date means show that the first two sampling dates differed very little from each other as indicated in Table 20.

Table 21. Comparison of significant treatments determined by electrical conductance and crown recovery after freezing at -8° C for 24 hours at five alfalfa root sampling dates, 1958[a]

Source of variation	d.f.	Sampling date: Sept. 16 E.C.[b]	Sept. 16 C.R[c]	Oct. 11 E.C.	Oct. 11 C.R	Oct. 21 E.C.	Oct. 21 C.R	Nov. 4 E.C.	Nov. 4 C.R	Nov. 20 E.C.	Nov. 20 C.R
R (Replicates)	3										
V (Varieties)	1					*	*		*		*
C (Clipping dates)	2			*	*	*	**			**	**
V x C	2					**					
Error (a)[d]	15										

[a]Double (**) and single (*) asterisk indicates F-test significant at the 1% and 5% levels respectively.

[b]Electrical conductance of the exosmosed solution from alfalfa root-sections, frozen at -8° C for 24 hours and thawed in distilled water at -2° C for 24 hours.

[c]Crown recovery rating of the amount of regrowth which occurred in the 21 days following freezing at -8° C for 24 hours, and transplanting in pit sand in the greenhouse.

[d]Pooled error term and degrees of freedom used in testing main plot treatments for conductance at 4th sampling date and for regrowth at 3rd, 4th, and 5th sampling dates.

Table 21. (Continued)

Source of variation	d.f.	Sampling date									
		Sept. 16		Oct. 11		Oct. 21		Nov. 4		Nov. 20	
		E.C.	C.R	E.C.	C.R	E.C.	C.R	E.C.	C.R	E.C.	C.R
V x R	3										
C x R	6										
C x V x R	6										
S (Row spacings)	3									**	
S x V	3				**						
S x C	6			*	**		*	**			*
S x C x V	6	*			**						
Error (b)[e]	54										
S x R	9										
S x V x R	9										
S x C x R	18										
S x C x V x R	18										

[e]Pooled error term used for recovery at 3rd, 4th, and 5th sampling dates.

Table 22. Mean electrical conductance in mhos x 10^{-5} per 10 grams at 25° C and mean crown recovery index of alfalfa roots for three fall clipping treatments - average of sampling dates, varieties and row spacings. 1958[a]

	Clipping date		
	September 16	October 11	November 1
Crown recovery	8.35	8.70	8.66
Electrical conductance	90.34	72.92	74.08

[a]Underscored means do not differ significantly at the 5% level as determined by Duncan's test (20).

In the fall of 1958, electrical conductance and crown recovery after artificial freezing at -8° C for 24 hours, was measured at five sampling dates. Table 21 shows a comparison of the results of the analysis of variance for each date. While it is difficult to evaluate these results, there are some general observations which may be made. In measuring the influence of varieties Vernal and Buffalo and fall management treatments on the hardiness of alfalfa, the electrical conductance and crown recovery methods appear equally effective. The two methods of determining hardiness were especially consistent in determining the influence of fall management treatments. Table 22 indicates that the early clipping treatment had a more harmful influence on hardiness than the later clipping treatments. This was also the case in 1957.

Table 22. Mean electrical conductance in mhos x 10^{-5} per 10 grams at 25° C and mean crown recovery index of alfalfa roots for three fall clipping treatments - average of sampling dates, varieties and row spacings. 1958[a]

	Clipping date		
	September 16	October 11	November 1
Crown recovery	8.35	8.70	8.66
Electrical conductance	90.34	72.92	74.08

[a]Underscored means do not differ significantly at the 5% level as determined by Duncan's test (20).

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Table 23. Mean electrical conductance in mhos x 10^{-5} per 10 grams at 25° C and mean crown recovery index of roots for two alfalfa varieties at five sampling dates - average of row spacings and fall clipping treatments[a]

Sampling date	Conductance			Crown recovery		
	Vernal		Buffalo	Vernal		Buffalo
September 16	99.9		99.9	3.00		3.00
October 11	90.9		97.2	6.96		4.83
October 21	82.5	*	92.1	6.42	*	4.96
November 4	53.3		62.7	13.42	*	10.62
November 20	47.9		44.8	17.88	*	14.63

[a]Single asterisk indicates F-test significant at the 5% level.

The difference between the varieties Vernal and Buffalo also was significant. This was particularly evident when the recovery index method was employed. Varietal differences were not detected until late October which is an important factor in winter hardiness. Table 23 shows the varietal means for the various sampling dates.

Row spacing treatments were shown to influence hardiness only at the last sampling date, and then only when the electrical conductance method was used. This is of considerable interest because of the differential survival observed in the spring of 1957, (Table 3) when the wide differences in

Table 24. Mean electrical conductance in mhos x 10^{-5} per 10 grams at 25° C of alfalfa roots grown under four row spacings and averaged over varieties and clipping treatments. Sampled November 20, 1958[a]

6 inches	12 inches	24 inches	42 inches
40.46	47.92	47.13	49.83

[a]Underscored means indicate no significant differences at the 5% level, Duncan's test (20).

survival were attributed to row spacing. Perhaps this can be explained on the basis of maturity, as the plants in 1957 were only seedlings whereas in the fall of 1958, they were two years old. Table 24 indicates the effect of row spacing at the time of the November 20th sampling.

It is noted that roots grown under the six-inch spacing showed a greater degree of hardiness than roots grown under wider spacings, the difference being significant at the 5% level.

The interaction between row spacing and clipping dates was consistently appraised as having a significant influence on hardiness. Table 25 shows the treatment means for the electrical conductance and crown recovery methods of determining hardiness at the various row spacings and fall clipping treatments.

There is greater consistency in the electrical

Table 25. Mean electrical conductance in mhos x 10^{-5} per 10 grams at 25° C and mean crown recovery index of alfalfa roots at four row spacings and three fall clipping treatments. Average of sampling dates and varieties. 1958

Interaction Row spacing	x Clipping date	Electrical conductance	Crown recovery
6 inch	September 16	78.4	8.65
6 inch	October 11	66.7	8.80
6 inch	November 1	72.3	8.45
12 inch	September 16	78.7	8.35
12 inch	October 11	73.2	8.30
12 inch	November 1	73.5	8.85
24 inch	September 16	88.8	7.95
24 inch	October 11	74.8	8.50
24 inch	November 1	76.0	9.10
42 inch	September 16	90.5	8.45
42 inch	October 11	76.0	9.20
42 inch	November 1	75.1	8.25

conductance means than in the crown recovery means. A survey of the conductance means shows that the early fall clipping was the most harmful over all row spacings. The 42-inch row spacing was most effective in increasing electrical conductivity over the three clipping treatments. The six-inch row spacing decreased the conductivity over the three clipping treatments. The twelve-inch spacing resulted in lower conductivity readings than the 24-inch spacing over the three clipping treatments.

Table 26. Summary analysis of variance for electrical conductance of alfalfa roots in mhos x 10^{-5} per 10 grams at 25° C, over all sampling dates. 1958

Source of variation	d.f.	Mean squares[a]
R (Replicates)	3	37.044
S (Sampling dates)	4	52863.783**
S x R[b]	12	357.263

[a]Double asterisk (**) indicates F-test significant at 1% level.

[b]Coefficient of variability 24.54%.

Sampling dates differed significantly in their effect on electrical conductance and crown recovery index. Table 26 shows the significance of sampling date on conductance as determined by analysis of variance.

A survey of the electrical conductance means for each sampling date in Table 27 shows a consistent reduction in conductivity until the fourth harvest when a marked drop was noted.

The effect of sampling date on the crown recovery index is shown in Table 28.

The sampling date means in Table 29, show a consistent increase in crown recovery with advancing sampling date.

Correlation coefficients were determined for electrical conductance and crown recovery index at each of the sampling

Table 27. Mean electrical conductance in mhos x 10^{-5} per 10 grams at 25° C of alfalfa roots at five sampling dates - average of varieties, fall clipping treatments and row spacing. 1958[a]

September 16	October 11	October 21	November 4	November 20
99.46	94.03	87.33	58.00	46.33

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

Table 28. Summary analysis of variance for crown recovery index of alfalfa, over all sampling dates, 1958

Source of variation	d.f.	Mean squares[a]
R (Replicates)	3	31.593
S (Sampling dates)	4	1841.71**
S x R[b]	12	173.98

[a]Double asterisk (**) indicates F-test significant at the 1% level.

[b]Coefficient of variability.

dates at which these two measurements were made in 1956 and 1958. These coefficients are shown in Table 30.

The correlations between crown recovery index and cold unit accumulation, and between electrical conductance and cold

Table 29. Mean crown recovery index of alfalfa roots at five sampling dates - average of varieties, fall clipping treatments and row spacings. 1958[a]

September 16	October 11	October 21	November 4	November 20
1.00	1.97	1.90	4.00	5.42

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

Table 30. Correlation coefficients for electrical conductance and crown recovery index[a]

Sampling time	1956[b]	1958[c]
1st sampling		
2nd sampling		-0.107
3rd sampling	-0.136	-0.129
4th sampling		-0.120
5th sampling	-0.160	-0.109
Over all sampling dates	-0.942**	-0.988**

[a]Double asterisk (**) indicates significance at the 1% level.

[b]Degrees of freedom 128.

[c]Degrees of freedom 96.

Table 31. Correlation coefficients for crown recovery index, electrical conductance and cold unit accumulation, 1956-57-58

Year	Cold units - accumulated to each sampling date[a] X Electrical conductance	X Recovery index
1956	-0.816 N.S.	+0.962**
1957	-0.994 **	---
1958	-0.956 **	+0.977**

[a]Double asterisk (**) indicates r values are significant at the 1% level.

unit accumulation were determined for each year. In determining cold units, 60° F was used as a base. Deviations below this base, in the daily mean 4 inch soil temperature, determine the number of cold units for a given day. Table 31 shows that the correlation coefficients are highly significant for both methods of measuring hardiness.

The inability to obtain crown recovery data in 1957 explains the absence of an r value for that measurement. The lack of significance between electrical conductance and cold unit accumulation in 1956 could be due to two factors. There were only three conductivity measurements made so the degrees of freedom were limiting. Also, when 3 year old roots are

Table 32. Summary analysis of variance for total yields of alfalfa forage in tons of dry matter per acre, 1958

Source of variation	d.f.	Mean squares[a]
R (Replicates)	3	0.46
V (Varieties)	1	0.06
C (Clipping dates)	2	2.02**
Error (a)[b]	15	0.083
S (Row spacings)	3	18.97**
S x V	3	0.087
S x C	6	0.077
S x V x C	6	0.067
Error (b)[bc]	54	0.081

[a]Double asterisk (**) indicates F-test significant at the 1% level.

[b]Pooled sums of squares do not differ significantly as indicated by Bartlett's test (72).

[c]Coefficient of variation 9.6%.

subjected to conductivity analysis it is difficult to completely remove all of the impurities, and to make certain that only roots which are completely free from disease and deterioration are processed.

The significant relationships between the two hardiness-measuring methods and accumulated cold units for each of the three years that observations were made, are shown in Figures 3, 4, 5 and 6.

Forage yields were taken during the 1958 growing season. The summary analysis of variance in Table 32 indicates that

Figure 3. The relationship between crown recovery, cold unit accumulation, and the date of sampling three-year-old alfalfa roots

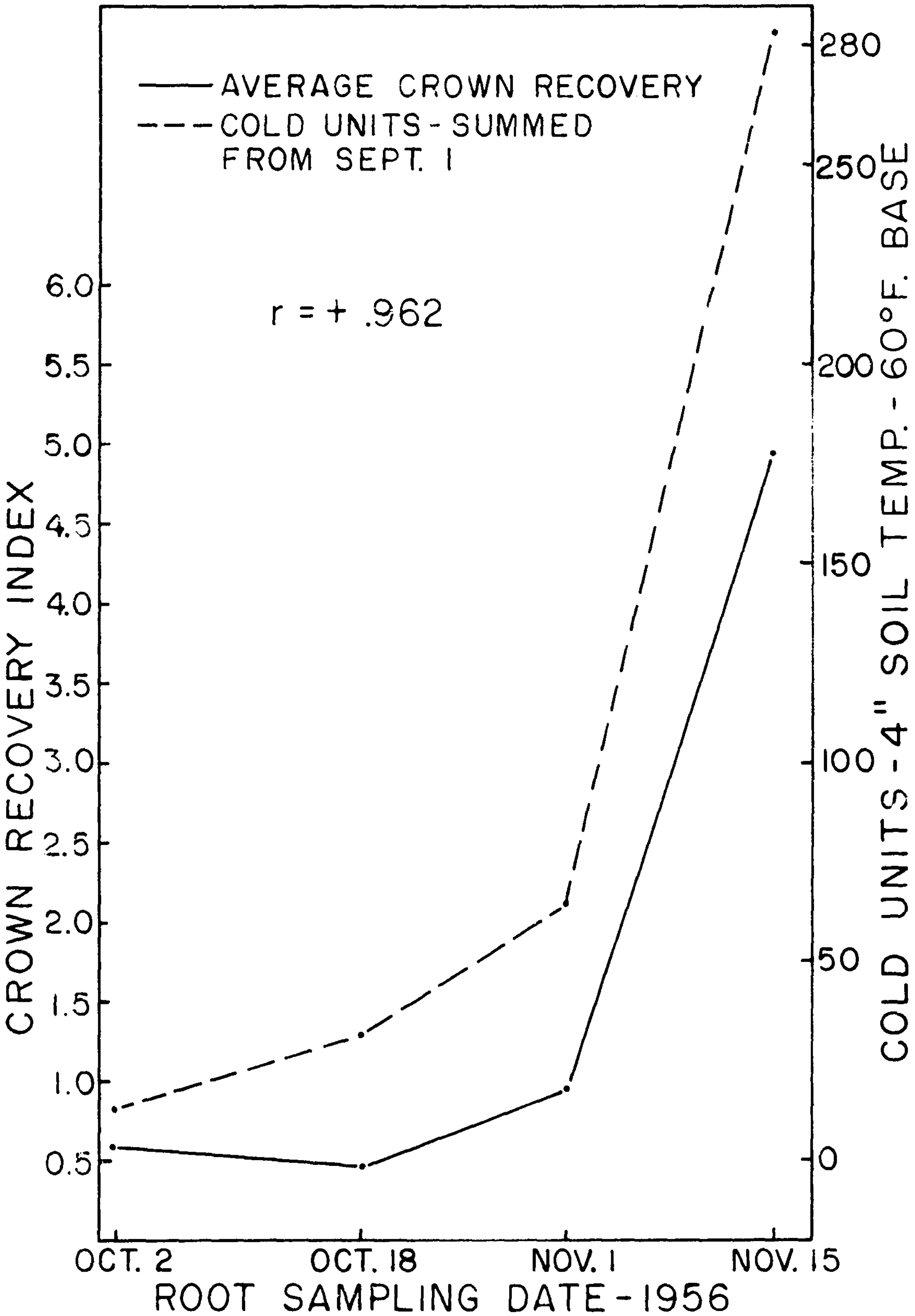

AVERAGE CROWN RECOVERY
COLD UNITS - SUMMED FROM SEPT. 1
r = + .962
CROWN RECOVERY INDEX
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
COLD UNITS - 4" SOIL TEMP. - 60°F. BASE
0
50
100
150
200
250
280
OCT. 2
OCT. 18
NOV. 1
NOV. 15
ROOT SAMPLING DATE - 1956

Figure 4. The relationship between the reciprocal of electrical conductance, cold unit accumulation, and the date of sampling one-year-old alfalfa roots

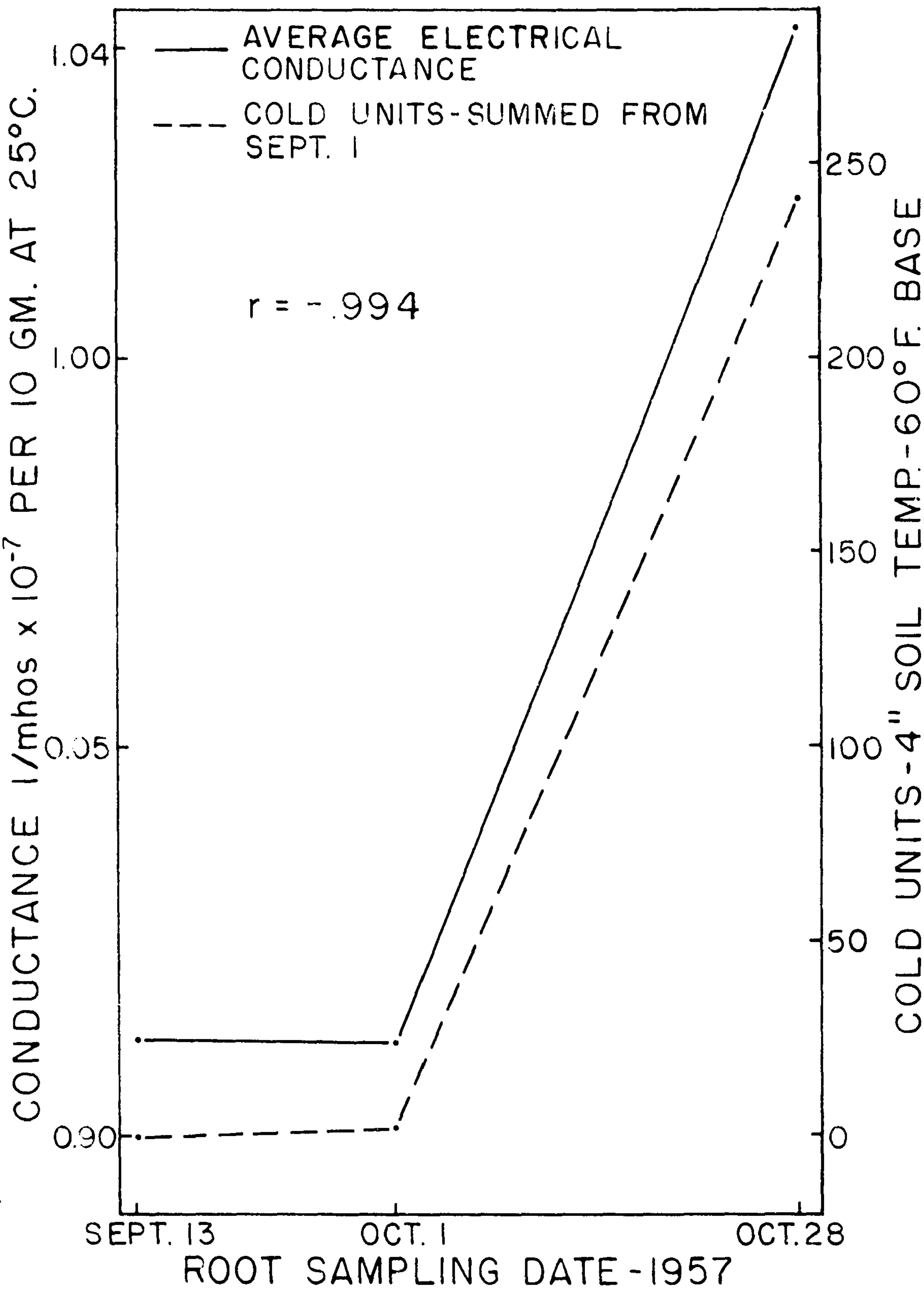
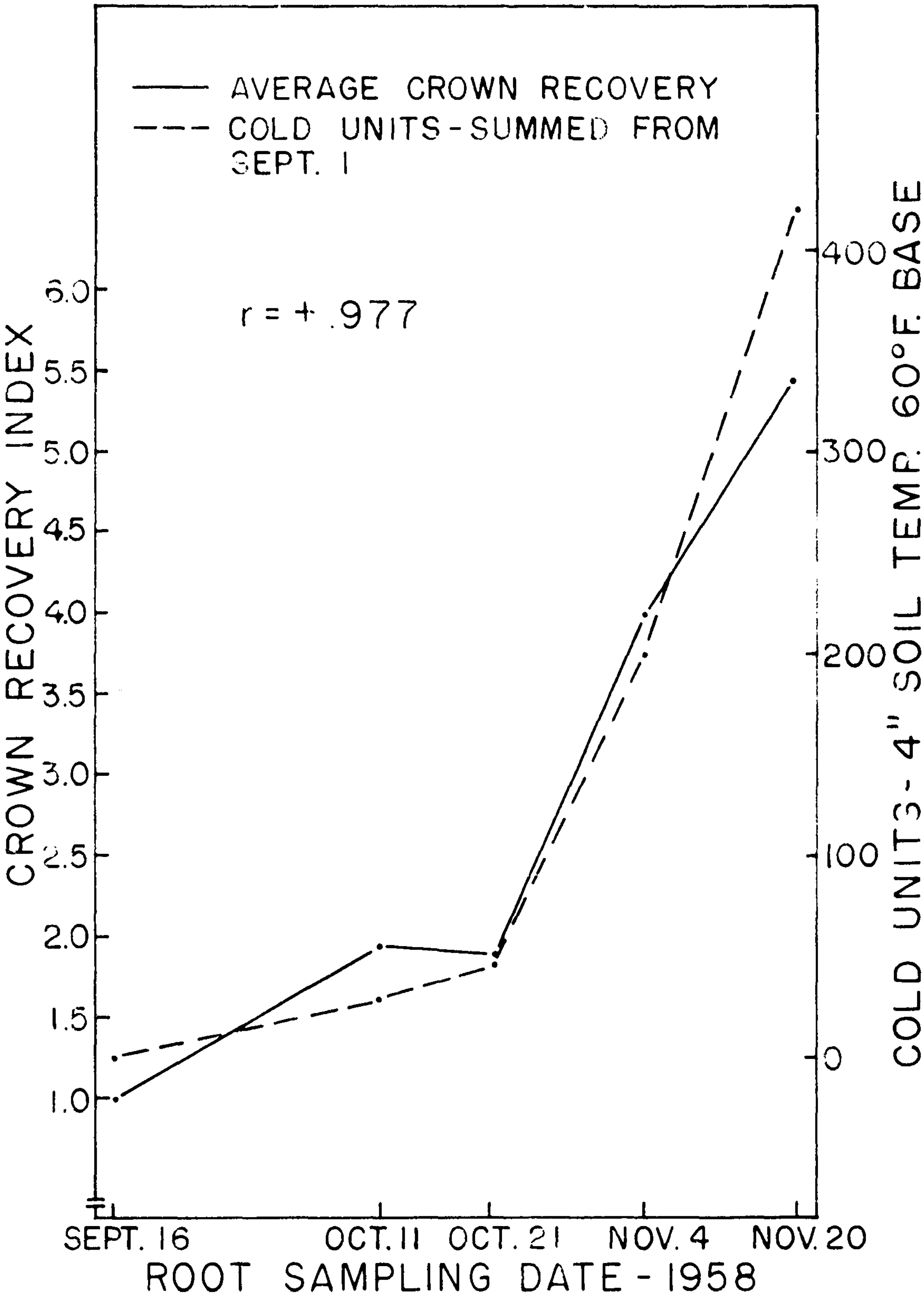
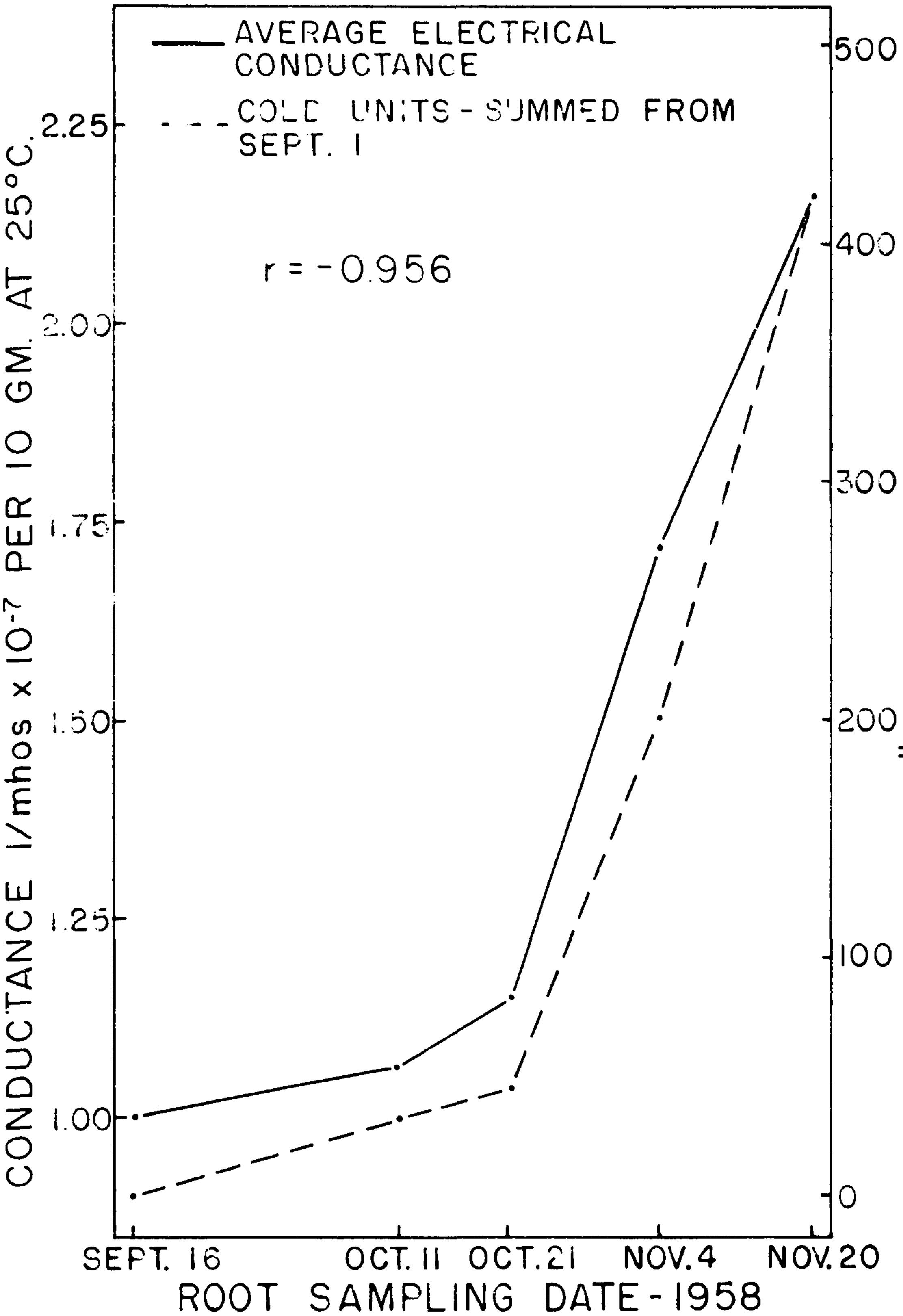

AVERAGE ELECTRICAL CONDUCTANCE
COLD UNITS-SUMMED FROM SEPT. 1
r = -.994
CONDUCTANCE 1/mhos x 10^{-7} PER 10 GM. AT 25°C.
1.04
1.00
0.05
0.90
COLD UNITS-4" SOIL TEMP.-60°F. BASE
250
200
150
100
50
0
SEPT. 13
OCT. 1
OCT. 28
ROOT SAMPLING DATE-1957

Figure 5. The relationship between crown recovery, cold unit accumulation, and the date of sampling two-year-old alfalfa roots

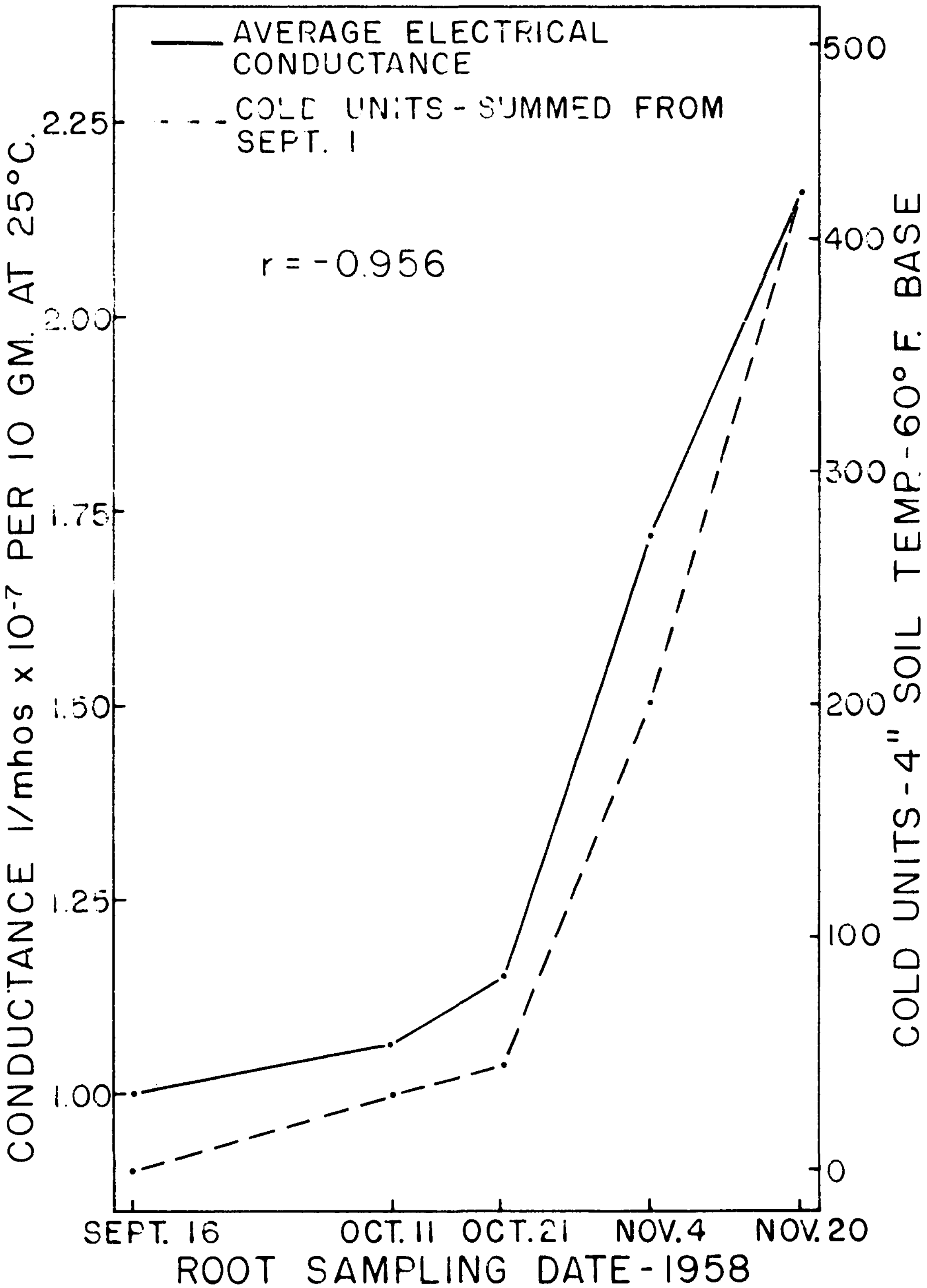


AVERAGE CROWN RECOVERY
COLD UNITS-SUMMED FROM SEPT. 1
r = + .977
CROWN RECOVERY INDEX
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
COLD UNITS- 4" SOIL TEMP. 60°F. BASE
400
300
200
100
0
SEPT. 16
OCT. 11
OCT. 21
NOV. 4
NOV. 20
ROOT SAMPLING DATE - 1958

Figure 6. The relationship between the reciprocal of electrical conductance, cold unit accumulation, and the date of sampling two-year-old alfalfa roots



AVERAGE ELECTRICAL CONDUCTANCE
COLD UNITS - SUMMED FROM SEPT. 1
r = -0.956
CONDUCTANCE 1/mhos x 10-7 PER 10 GM. AT 25°C.
2.25
2.00
1.75
1.50
1.25
1.00
COLD UNITS - 4" SOIL TEMP. - 60° F. BASE
500
400
300
200
100
0
SEPT. 16
OCT. 11
OCT. 21
NOV. 4
NOV. 20
ROOT SAMPLING DATE - 1958



AVERAGE ELECTRICAL CONDUCTANCE
COLD UNITS - SUMMED FROM SEPT. 1
r = -0.956
CONDUCTANCE 1/mhos x 10^{-7} PER 10 GM. AT 25°C.
COLD UNITS - 4" SOIL TEMP. - 60° F. BASE
2.25
2.00
1.75
1.50
1.25
1.00
500
400
300
200
100
0
SEPT. 16
OCT. 11
OCT. 21
NOV. 4
NOV. 20
ROOT SAMPLING DATE - 1958

Table 33. Mean alfalfa forage yields in tons of dry matter per acre for three fall clipping treatments - average of varieties and row spacings. 1958[a]

September 13	October 1	October 28
3.39	3.49	3.87

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

Table 34. Mean alfalfa forage yields in tons of dry matter per acre for four row spacing treatments - average of varieties and fall clipping treatments. 1958[a]

6-inch	12-inch	24-inch	42-inch
4.41	4.15	3.33	2.44

[a]All means differ significantly at the 5% level, Duncan's test (20).

clipping treatments, which were applied the previous fall and row spacings were highly significant.

A survey of the mean forage yields for fall clipping treatments is shown in Table 33.

Forage yields under these fall clipping treatments agree with electrical conductance and crown recovery measurements, both in 1957 and 1958, in that the early clipping

Table 35. Analysis of variance of observed differences in alfalfa plant population between October 14, 1957 and April 10, 1958

Source of variation	d.f.	Mean squares[a]
R (Replicates)	3	28.02*
V (Varieties)	1	17.02
C (Clipping dates)	2	14.39
V x C	2	2.72
Error (a)[b]	15	5.80
S (Row spacings)	3	2.24
S x V	3	4.05
S x C	6	2.06
S x C x V	6	1.20
Error (b)[b]	54	2.26

[a]Single asterisk (*) indicates F-test significant at the 5% level.

[b]Pooled sums of squares do not differ significantly as indicated by Bartlett's test (73).

(mid-September) was the most harmful to the plants.

Table 34 shows the mean forage yields for the four row spacings.

In October of 1957, and again in April of 1958, plant counts were made in marked, three foot segments of one row in each plot. Table 35 shows the analysis of variance of the observed differences between the two plant counts.

The only significant difference was between the replications. None of the treatments were shown to have any significant effect on plant population during the winter of 1957-58.

Table 36. Mean reductions in plants per three-foot of row of alfalfa stands during the period from October 14, 1957 to April 10, 1958

Varieties		Row spacing				Fall clipping date		
Buf-falo	Ver-nal	6"	12"	24"	42"	Sept. 16	Oct. 11	Nov. 1
1.25	0.76	1.61	2.11	1.90	2.44	2.88	1.79	1.38

Even though the analysis was unable to detect significance it is interesting to inspect the mean differences in plant population during this period. Table 36 shows the observed reductions in stand due to the various treatments.

Figure 7 shows the influence of row spacing on alfalfa root development. A wide difference in the number and size of lateral roots at the various rows spacings was noted.

Two-Dimensional Spacing Experiment

In August of 1957, Ranger alfalfa was seeded in a factorial experiment at three rates, so that each plant occupied 3 1/2, 5, and 16 square inches of space. Three cover treatments were applied in the form of a check (no cover), 2 tons of chopped straw per acre, and an oat companion crop. A summary analysis of variance of the total forage yields for 1958 is presented in Table 37.

Figure 7. The effect of row width on the size and amount of lateral branching of two-year-old alfalfa roots

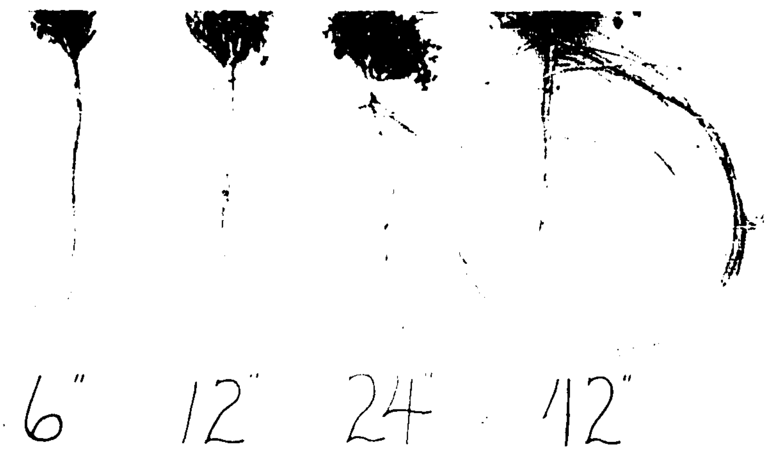

6"
12"
24"
12"

Table 37. Summary analysis of variance for total yields of alfalfa forage in tons of dry matter per acre. 1958

Source of variation	d.f.	Mean squares[a]
R (Replicates)	1	1.73*
S (Spacings)	2	1.03*
Error (a)	2	0.02
C (Cover treatments)	2	5.06*
Error (b)	2	0.18
C x S	4	0.31
Error (c)[b]	4	0.095

[a]Single asterisk (*) indicates F-test significant at the 5% level.

[b]Coefficient of variation 10.7%.

Table 38. Mean forage yields of alfalfa in tons of dry matter per acre for three spacing treatments - average of replicates and cover treatments. 1958[a]

1 plant per 3 1/2 square inches	1 plant per 5 square inches	1 plant per 16 square inches
3.24	2.95	2.43

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

Table 37. Summary analysis of variance for total yields of alfalfa forage in tons of dry matter per acre. 1958

Source of variation	d.f.	Mean squares[a]
R (Replicates)	1	1.73*
S (Spacings)	2	1.03*
Error (a)	2	0.02
C (Cover treatments)	2	5.06*
Error (b)	2	0.18
C x S	4	0.31
Error (c)[b]	4	0.095

[a]Single asterisk (*) indicates F-test significant at the 5% level.

[b]Coefficient of variation 10.7%.

Table 38. Mean forage yields of alfalfa in tons of dry matter per acre for three spacing treatments - average of replicates and cover treatments. 1958[a]

1 plant per 3 1/2 square inches	1 plant per 5 square inches	1 plant per 16 square inches
3.24	2.95	2.43

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

Table 39. Mean forage yields of alfalfa in tons of dry matter per acre for three cover treatments - average of replicates and spacing treatments, 1958[a]

Straw mulch	Check	Oat-companion
3.49	3.31	1.82

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

The spacing of alfalfa plants at different intervals in two dimensions had a significant influence on the yield. Table 38 shows that spacing the plants every 3 1/2 square inches did not significantly increase the yield over the 5 square inch spacing. However, both of these treatments were significantly superior to the 16 square inch treatment.

The cover treatments also demonstrated significant differences. The application of two tons of chopped straw resulted in the highest yield. This was not significantly better than the yield on the check plots which received no cover. Both of these treatments were significantly better than the oat companion-crop treatment, as shown in Table 39.

Plant counts were taken on October 4, 1957 and April 14, 1958 in permanent, square foot quadrats. An analysis of variance of the observed differences in plant population between these two dates is presented in Table 40.

Table 40. Analysis of variance of observed differences in alfalfa plant population between October 4, 1957 and April 14, 1958

Source of variation	d.f.	Mean squares[a]
R (Replicates)	1	8.00
S (Spacings)	2	120.665
Error (a)	2	34.665
C (Cover treatments)	2	129.50*
Error (b)	2	1.50
C x S	4	13.67
Error (c)[b]	4	10.67

[a]Single asterisk (*) indicates F-test significant at the 5% level.

[b]Coefficient of variation 42.6%.

Table 41. Mean reduction in plants per square foot of alfalfa stands under three cover treatments from October 4, 1957 to April 14, 1958[a]

Straw mulch	Oat-companion	Check
9.00	11.50	2.50

[a]Underscored means do not differ significantly at the 5% level, Duncan's test (20).

Table 42. Summary analysis of variance for total yields of alfalfa forage in tons of dry matter per acre, 1958

Source of variation	d.f.	Mean squares[a]
R (Replicates)	7	0.9504
D (Dates of seeding)	2	105.0216**
Error (d)	14	0.5349
C (Cover treatments)	2	0.5758
Error (b)	14	0.2841
D x C	4	3.4911**
Error (c)[b]	28	0.1466

[a]Double asterisk (**) indicates F-test significant at the 1% level.

[b]Coefficient of variation 16.3%.

The cover treatment means as shown in Table 41 indicate that the application of two tons of chopped straw and the oat companion-crop greatly reduced the plant population as compared to the check (non-covered plots).

Date of Fall Planting Experiment

In the fall of 1957 another factorial experiment was set out utilizing Ranger alfalfa, seeded at three different dates, under three cover treatments including a check (no cover), 2 tons of chopped straw per acre, and an oat companion crop.

A summary analysis of variance of the 1958 total forage

Table 43. Mean yields of alfalfa forage at three seeding dates - average of replicates and cover treatments. 1958[a]

August 17	September 2	September 16
4.49	2.23	0.31

[a]All means differ significantly at the 5% level, Duncan's test (20).

yields is presented in Table 42. This analysis shows the time of seeding to be highly significant as also was the date of seeding by cover treatment interaction.

All of the means for the date of seeding treatments differ significantly at the 5% level as shown in Table 43.

The interaction between the cover treatments and dates of seeding was also highly significant. The means for these treatments are arrayed in Table 44.

At the earliest seeding date the oat companion crop reduced forage yields significantly. However, at both of the later seeding dates it resulted in the highest yields of forage. Even though these increases were not highly significant in all cases, they do indicate some benefit derived from the companion crop association when seedings are made later than usually recommended. At each seeding date there were no significant differences in forage yields between the check plots

Table 44. Mean alfalfa forage yields in tons of dry matter per acre under three cover treatments at three dates of fall seeding. 1958[a]

Seeding date	Cover treatment	Forage yields[a]
August 17	Chopped straw	5.01
	Check	5.00
	Oat-companion	3.46
September 2	Oat-companion	2.60
	Check	2.23
	Chopped straw	1.87
September 16	Oat-companion	0.46
	Chopped straw	0.32
	Check	0.17

[a]Sidescored means do not differ significantly at the 5% level, Duncan's test (20).

and those which received a cover of chopped straw.

DISCUSSION

This study was initiated to survey the general effects of cultural practices on the winter hardiness of alfalfa. The results of these various experiments involving different management factors are not conclusive, and any generalizations made from the data should be done with caution. The response pattern of the various alfalfa varieties to electrical conductivity, and crown recovery methods of measuring hardiness had been fairly well established before this study was undertaken (17, 18, 74, 77). However, a comparison of these two methods over a wide range of conditions and over several years had not previously been undertaken. The treatments which were used were not intended to establish a basis for recommendation, but were to serve as comparisons in making deductions regarding the mechanisms of winter-killing.

The study with the three year old plants, which had been subjected to different clipping and fertilizer treatments, indicated the general similarity of the electrical conductance and recovery index methods for measuring hardiness. The recovery index method was more conservative in determining significant treatments. This was most likely due to the fact that when working with old roots it is difficult to insure that only disease-free and non-deteriorating root material is processed. Any contamination of this type would most likely have a greater effect on the electrical conductivity measurements than on the crown recovery index. Conductivity measurements did not rank the four varieties in their usual hardiness pattern as described by other workers (7, 70). The recovery index was more effective in doing this. Ladak and Vernal have been regarded as extremely hardy with Ladak being slightly superior (51). Buffalo, although hardy under average conditions at this latitude, is considered to be lacking in cold hardiness. The two methods were at variance in the placing of Ladak. The conductance data found little difference between Vernal A-224 and Ladak at the 5% level and no significant difference between Ladak and the other varieties at the 1% level. The recovery index method found Ladak to be the most hardy, not differing significantly from Vernal, but distinctly superior to the other varieties at the 5% and 1% levels. As a result of Buffalo being placed at the bottom of the hardiness scale by both of the hardiness measuring methods in 1956, it was decided to compare it with Vernal in a spaced row experiment.

In the fall of the seeding year of the spaced-row study there was no apparent difference in the hardiness of Vernal and Buffalo as determined by electrical conductivity measurements. At the end of the second year significant differences appeared at the third sampling date (October 21), and were evident at each sampling throughout the rest of the season. At each sampling date Vernal was more hardy than Buffalo regardless of the method used in determining hardiness.

The date of root sampling had a highly significant effect on hardiness as measured by the electrical conductance and crown recovery methods. The most striking differences in 1956 were shown by the crown recovery method (Table 13) when a definite increase in recovery occurred after November 1. The conductance data show a more constant advance toward hardiness, with each sampling date differing significantly from the others (Table 9). Although there were no recovery data taken in 1957, the conductance data indicate a sharp increase in hardiness sometime between October 1 and October 28 (Table 20). In 1958 both methods detected a marked increase in hardiness around the first of November (Tables 27 and 29).

When coefficients were determined for the correlation between electrical conductance and the crown recovery index at each sampling in which both measurements were made in 1956 and 1958, it was found that there was no significance. However, when the correlations between these two methods of measuring hardiness were determined over all harvest dates in each year, they were shown to be highly significant (Table 30). The lack of correlation at each sampling date causes concern as to the ability of the two methods used in measuring hardiness to give comparable results. The correlation between the two methods over all the sampling dates involves the ability of the methods to detect the advance in hardiness made by the plants in a given time. Because they were able to do this with comparable accuracy they were significantly correlated but in a negative manner. Further research is needed to determine if the absence of a significant correlation at each sampling date was due to the artificial freezing temperature, length of freezing exposure, or some other factor. This was not done in this study as it would necessitate several freezing chambers with precise temperature controls, and the cost of such equipment precluded further investigation.

It was found that soil temperature was very definitely correlated with the hardiness of alfalfa, and also with the rate of hardening. In searching for a soil temperature parameter which would be simple to evaluate and still quantitatively describe the rate of hardening, it was found that a cold unit accumulation scheme was best. In this scheme a base temperature of 60° F was established. This was selected as a base, because in the history of the Ames weather station there have been very few years when the daily mean soil temperature at the 4 inch depth has dropped below 60° F before September 1. A cold unit is described as a one degree Fahrenheit deviation below 60° F, in the daily mean 4 inch soil temperature. An accumulation of these deviations or cold units up to each sampling date was found to correlate very significantly with electrical conductance and crown recovery measurements over the three year period in which these tests were made. (Table 31).

A survey of the relationship between cold unit accumulation and hardiness of alfalfa as determined by electrical conductance and crown recovery shows a sharp increase in cold unit accumulation sometime between October 1 and November 1. This may depend on the particular year, but regardless of the year, the electrical conductance and crown recovery data show a sharp break at the same time as the cold units (Figures 3, 4, 5, 6). In 1956, this sharp increase in cold unit accumulation occurred sometime after 45 units had accumulated. In 1957 the break came after 25 units and in 1958 after 42 units had accumulated. If it is assumed that the plants are hardy after a certain minimum number of cold units have accumulated we find that this date may come any time within a two week period depending on the year. If a specific date is recommended, after which it is safe to assume that the plants are sufficiently hardy to be clipped or grazed, then according to these data this date would have to be after November 1, in order to apply to all three years. If the 1956 data are disregarded on the premise that they were determined on old root material, then this date would be sometime after October 20, which is about the recommended time for fall aftermath removal of alfalfa for this latitude (51, 26). However, the 1957 data show that the time for fall aftermath removal could be advanced as much as two weeks to October 15, which would be a definite advantage in any livestock management program.

It is difficult to determine exactly how many cold units must accumulate before it can be assumed that the plants have attained maximum hardiness. The data indicate that only in

1956 was sampling continued until near maximum crown recovery was attained. Only in 1958 was there any sign of a leveling off in hardiness as measured by electrical conductance.

Perhaps the best way to make recommendations for fall management practices on the basis of the data would be to allow at least one week and preferably 2 weeks after the sharp increase in cold unit accumulation occurs, before removing the top growth of alfalfa. This would mean that in most years an accumulation of at least 100 cold units should be attained before any fall aftermath removal. This number of cold units was accumulated by October 15 in 1957 and October 25 in 1958, but not until November 3 in 1956, showing once again the variability over years.

The problem of knowing when to stop removing the top growth of alfalfa at the end of the growing season, and when it is safe for the removal of the aftermath has been discussed by many researchers (26, 39, 51, 59, 63, 80). The results of this study indicate that the hardiness of alfalfa as measured by electrical conductance and crown recovery was greatly affected by the timing of the fall clipping treatments. At the time of the last root sampling in 1957 (October 28), electrical conductance measurements indicated a significant difference between those plants which were clipped on September 13 and those which were clipped later (October 1 and October 30). The readings indicated a greater degree of hardiness in those plants which were clipped after September 13. In 1958, the results were very much the same as in 1957, in that there was a significant difference in conductance and crown recovery between plants which were clipped on September 16 and plants which were clipped at a later date (October 11, and November 1). The difference in hardiness was in favor of the two later clipping treatments.

This indicates that mid-September harvesting of alfalfa at this latitude may be injurious to the plants. The cold unit accumulation data also suggest that the harvesting of alfalfa in September would have a harmful effect on the plants.

The influence of the spacial arrangement of alfalfa plants on winter hardiness has not been investigated in any detail. It is of interest not only to the farmer who wishes to use the proper seeding rates, but to the researcher, particularly the plant breeder, who sets out plants in wide-spaced rows and makes selections for various factors based on performance. If plants in wide rows are more susceptible to winter-killing than they would be if placed in narrower rows, then performance would not be a satisfactory criterion for selection.

The results of this study over a three year period indicate that there was a tendency toward decreased hardiness as reflected in higher conductance readings, as the row width was increased from 6 inches to 42 inches (Tables 17 and 24). This difference was even more marked when seedlings from a fall seeding were subjected to the rigorous conditions of the winter of 1956-57 when the minimum soil temperature reached 13° F at 4 inch depth. Under these conditions there was a three to four-fold increase in survival of the 6 inch-row plots over the 42 inch-row plots (Table 3).

Differences in winter survival between wide and narrow row spacings cannot be explained on the basis of the amount of lateral root branching associated with the width of row. Striking differences were noted in root branching (Figure 7). The wide rows exhibited a large amount of lateral branching which decreased as the row width was reduced. Smith (70) and Kiesselbach and Anderson (39) found the opposite to be true when comparing the hardiness and branching characteristics of several alfalfa varieties.

Arranging fall seeded alfalfa plants in a two-dimensional pattern so that each plant occupied an area of 3 1/2, 5 and 16 square inches, resulted in no significant decreases in plant numbers during the 1957-58 winter when the minimum soil temperature at the 4 inch depth reached 14° F. There was no significant difference in forage yields between the 3 1/2 square inch-spaced plots and those spaced at 5 square inches. This means there was very little difference between a seeding rate of 8.15 pounds of alfalfa per acre and 5.70 pounds per acre when forage yield was measured, and little difference between these two seeding rates and 1.78 pounds per acre when winter survival was measured.

The mulching of fall-seeded alfalfa as a protective measure against winter killing resulted in a drastic reduction in plant population. The application of 2 tons of chopped straw per acre, and the association of an oat companion crop, both reduced the winter survival significantly over those plants which received no cover treatment. These treatments also had a significant effect on the subsequent forage yields. The straw mulched plots yielded the most forage although they did not differ significantly from the check plot yields (Table 39).

In the date-of-seeding experiment where the alfalfa seed was broadcast at the rate of 12 pounds per acre, there were no significant differences in forage yield between the two cover treatments and the check. The date of seeding was a highly significant factor in determining forage yields the following year (Table 43). The application of some type of cover became an important factor in increasing forage yields as the fall seeding date was delayed (Table 44). This is of considerable practical importance when fall seeding is delayed beyond the recommended time.

In general, this study was an attempt to survey a wide range of factors as they affect the winter hardiness and performance of alfalfa. In most cases the results should not form the basis for any great changes in the present management of this crop. However, they may indicate the possibilities of realizing greater forage production through the wise interpretation and application of the general underlying principles supported by the data. Any management recommendations based on climatological data should be tested over an extended period of time. In those cases where this applies to this study, caution should be exercised in interpreting the results.

SUMMARY AND CONCLUSIONS

The general objective of this study was to evaluate the response of several alfalfa varieties to various cultural treatments including fertility, cutting management, stand density, and surface cover. The influence of these factors on the low-temperature hardiness of alfalfa was also determined.

Two methods were used in determining low-temperature hardiness. The electrical conductance method measured the conductivity in mhos x 10^{-5} at 25° C of the exosmosed solution from root sections frozen at -8° C for 24 hours and thawed at 2° C for 24 hours. The crown recovery method measured the amount of regrowth which occurred in the 21 days following freezing at -8° C for 24 hours, thawing at 2° C for 24 hours, and transplanting into pit sand in the greenhouse.

In 1956, the three year old roots of four alfalfa varieties, namely Buffalo, A-224, Ladak and Vernal were sampled 5 times from August 31, to November 15, and subjected to conductivity and crown recovery analysis. These varieties had been clipped to simulate grazing and also cut for hay during the two years previous to 1956. They had also received separate and combined treatments of phosphorus and potash in each of these 3 years.

In 1957 the roots of two alfalfa varieties, Buffalo and Vernal were sampled three times, from September 13 to October

28, and subjected to electrical conductance measurements. These varieties were seeded in the spring of 1957, at four row spacings, namely 6, 12, 24, and 42 inches, and fall clippings were made on September 13, October 1, and October 30.

In 1958 forage yields were measured, and in the fall the roots were sampled 5 times, from September 16 to November 20, and subjected to conductivity and crown recovery analysis. Fall clipping treatments were applied on September 16, October 11, and November 1. Stand counts were made in October, 1957 and again in April, 1958.

Cold units were accumulated in each fall of the three years (1956-57-58), and comparisons made between this parameter and hardiness, as measured by the electrical conductance and crown recovery methods. A cold unit is described as a one degree Fahrenheit deviation below a 60° F base in the daily mean soil temperature at a depth of 4 inches.

In the fall of 1957 two experiments were seeded, utilizing Ranger alfalfa. One study involved the spacing of single plants in areas of 3 1/2, 5 and 16 square inches, under three mulch cover treatments of 2 tons of chopped straw per acre, an oat companion crop, and a check treatment which was left uncovered. The other experiment was broadcast seeded on three different dates, August 17, September 2, and September 16. The same mulch cover treatments as above were utilized.

The results of the study are summarized as follows:

1. The influence of fertilization on hardiness was not statistically significant.
2. The two methods used in measuring hardiness found Vernal and Ladak to be the most hardy and Buffalo to be lacking in winter hardiness. A-224 was intermediate in hardiness.
3. Plants which had been clipped frequently demonstrated less hardiness than plants which had been harvested for hay.
4. Measurements on the three year old crowns of eight alfalfa varieties showed A-224 to be the most prostrate, followed by Narragansett, Ranger and Vernal, Grimm and Ladak were next, and Buffalo and Atlantic were the most erect.
5. The date of root sampling had a highly significant effect on the hardiness measurements in each of the three years, 1956-57-58.
6. The time of fall clipping significantly influenced hardiness, with the mid-September clipping having the most harmful effect in both years 1957-58.
7. Row width, while not found to consistently influence hardiness, did demonstrate a general increase in hardiness as row width was decreased. This was most noticeable when the alfalfa was seeded in the fall.

8. The spacing of fall-seeded alfalfa in two-dimensions had a marked effect on forage yield, but no significant influence on winter survival.
9. The covering of fall-seeded alfalfa with a straw mulch significantly reduced the winter survival, but increased the forage yields over the check.
10. The date of fall-seeding alfalfa had a significant effect on forage yields. The application of some type cover became an important factor in increasing subsequent forage yields as the seeding date was delayed.
11. Correlations between electrical conductance and cold unit accumulations, and between crown recovery and cold unit accumulations were highly significant.
12. A close association between cold unit accumulation and the hardiness of alfalfa was postulated.

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APPENDIX

APPENDIX

Table 45. Daily mean 4 inch soil temperatures and cold units from September 1 to November 15, 1956

	September		October		November	
Day of month	Mean 4 inch soil temperature	Cold units	Mean 4 inch soil temperature	Cold units	Mean 4 inch soil temperature	Cold units
1	57.5	2.5	62.0	---	52.5	7.5
2	68.0	---	63.5	---	52.5	7.5
3	71.5	---	62.0	---	53.5	6.5
4	64.5	---	62.0	---	51.0	9.0
5	60.5	---	59.5	0.5	52.5	7.5
6	57.0	3.0	62.0	---	47.5	12.5
7	58.0	2.0	56.5	3.5	40.5	19.5
8	61.5		59.5	0.5	38.0	22.0
9	58.5	1.5	55.0	5.0	37.0	23.0
10	66.0	---	54.0	6.0	38.0	22.0
11	67.0	---	59.0	1.0	42.0	18.0
12	67.5	---	62.5	---	39.5	20.5
13	68.0	---	65.0	---	41.5	18.5
14	63.5	---	61.5	---	46.5	13.5
15	65.0	---	59.0	1.0	42.5	17.5
16	68.0	---	59.5	0.5		
17	61.0	---	60.0	---		
18	63.0	---	60.0	---		
19	63.5	---	59.5	0.5		
20	58.5	1.5	58.0	2.0		
21	63.5	---	56.5	3.5		
22	68.0	---	55.5	4.5		
23	64.0	---	51.5	8.5		
24	64.0	---	52.5	7.5		
25	66.5	---	55.5	4.5		
26	69.0	---	47.0	13.0		
27	68.5	---	44.5	15.5		
28	68.5	---	50.0	10.0		
29	61.5	---	52.0	8.0		
30	57.0	3.0	55.5	4.5		
31			51.5	8.5		

Table 45. Daily mean 4 inch soil temperatures and cold units from September 1 to November 15, 1956

	September		October		November	
Day of month	Mean 4 inch soil temperature	Cold units	Mean 4 inch soil temperature	Cold units	Mean 4 inch soil temperature	Cold units
1	57.5	2.5	62.0	---	52.5	7.5
2	68.0	---	63.5	---	52.5	7.5
3	71.5	---	62.0	---	53.5	6.5
4	64.5	---	62.0	---	51.0	9.0
5	60.5	---	59.5	0.5	52.5	7.5
6	57.0	3.0	62.0	---	47.5	12.5
7	58.0	2.0	56.5	3.5	40.5	19.5
8	61.5		59.5	0.5	38.0	22.0
9	58.5	1.5	55.0	5.0	37.0	23.0
10	66.0	---	54.0	6.0	38.0	22.0
11	67.0	---	59.0	1.0	42.0	18.0
12	67.5	---	62.5	---	39.5	20.5
13	68.0	---	65.0	---	41.5	18.5
14	63.5	---	61.5	---	46.5	13.5
15	65.0	---	59.0	1.0	42.5	17.5
16	68.0	---	59.5	0.5		
17	61.0	---	60.0	---		
18	63.0	---	60.0	---		
19	63.5	---	59.5	0.5		
20	58.5	1.5	58.0	2.0		
21	63.5	---	56.5	3.5		
22	68.0	---	55.5	4.5		
23	64.0	---	51.5	8.5		
24	64.0	---	52.5	7.5		
25	66.5	---	55.5	4.5		
26	69.0	---	47.0	13.0		
27	68.5	---	44.5	15.5		
28	68.5	---	50.0	10.0		
29	61.5	---	52.0	8.0		
30	57.0	3.0	55.5	4.5		
31			51.5	8.5		

Table 46. Daily mean 4 inch soil temperatures and cold units from September 1 to November 1, 1957

	September		October	
Day of month	Mean 4 inch soil temperature	Cold units	Mean 4 inch soil temperature	Cold units
1	77.0	---	62.5	---
2	72.5	---	61.0	---
3	68.0	---	60.5	---
4	69.0	---	59.0	1.0
5	67.0	---	57.5	2.5
6	65.0	---	58.5	1.5
7	64.5	---	58.0	2.0
8	66.0	---	57.5	2.5
9	67.0	---	52.0	8.0
10	64.5	---	48.5	11.5
11	65.0	---	48.5	11.5
12	64.0	---	51.0	9.0
13	61.5	---	53.0	7.0
14	61.0	---	54.5	5.5
15	62.5	---	58.5	1.5
16	62.0	---	56	4.0
17	63.5	---	50	10.0
18	64.0	---	47.5	12.5
19	63.5	---	46.5	13.5
20	59.0	1.0	45.5	14.5
21	60.5	---	48.5	11.5
22	59.0	1.0	52.5	7.5
23	59.0	1.0	54.0	6.0
24	60.5	---	46.0	14.0
25	63.5	---	39.5	20.5
26	61.5	---	38	22.0
27	60.0	---	37.5	22.5
28	58.0	2.0	39.5	20.5
29	57.5	2.5	42.5	17.5
30	61.0	---	43.0	17.0
31			43.5	16.5

Table 47. Daily mean 4 inch soil temperatures and cold units from September 1 to November 20, 1958

	September		October		November	
Day of month	Mean 4 inch soil temperature	Cold units	Mean 4 inch soil temperature	Cold units	Mean 4 inch soil temperature	Cold units
1	67.5	---	53.0	7.0	47.0	13.0
2	73.0	---	55.5	4.5	47.0	13.0
3	75.0	---	59.0	1.0	47.5	12.5
4	72.5	---	62.0	---	48.0	12.0
5	71.0	---	58.0	2.0	49.5	10.5
6	71.0	---	59.0	1.0	43.5	16.5
7	71.0	---	62.5	---	43.5	16.5
8	71.0	---	63.5	---	46.0	14.0
9	72.0	---	62.5	---	44.5	15.5
10	66.0	---	53.5	6.5	43.0	17.0
11	66.0	---	51.5	8.5	45.5	14.5
12	66.0	---	56.0	4.0	43.5	16.5
13	67.0	---	61.0	---	49.0	11.0
14	65.0	---	63.5	---	52.0	8.0
15	68.0	---	65.0	---	51.0	9.0
16	62.0	---	64.0	---	54.5	5.5
17	61.5	---	57.5	2.5	55.0	5.0
18	61.5	---	56.0	4.0	40.5	19.5
19	65.0	---	59.0	1.0	40.0	20.0
20	65.5	---	61.0	---	40.0	20.0
21	66.0	---	59.0	1.0		
22	65.5	---	54.0	6.0		
23	67.5	---	53.5	6.5		
24	72.5	---	52.0	8.0		
25	67.5	---	49.5	10.5		
26	64.5	---	48.5	11.5		
27	62.0	---	50.0	10.0		
28	59.5	0.5	46.5	13.5		
29	61.0	---	46.5	13.5		
30	59.0	1.0	47.0	13.0		
31			47.0	13.0		