ROOT ANATOMY AND GROWTH HABIT OF SOME ALFALFA VARIETIES IN RELATION TO WILT RESISTANCE AND WINTER HARDINESS

Thesis for the Degree of M. S.
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Jagdish Seth

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This is to certify that the

thesis entitled

Root Anatomy and Growth Habit of Some Alfalfa Varieties in Relation to Wilt Resistance and Winter Hardiness.

presented by

Jagdish Seth

has been accepted towards fulfillment of the requirements for

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Major professor

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Jagdish Seth

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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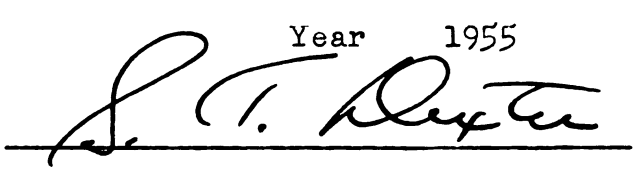
Approved 

THESIS

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THESIS ABSTRACT

Five bacterial wilt resistant and five wilt susceptible varieties of alfalfa, ranging from very hardy to non winter hardy in each group, were planted in the field and in the greenhouse with continuous long and long-to-short days. Roots were dug from the field in October, December and February and from the greenhouse cultures for microscopic study on triple-stained slides.

The slides were examined and scored or measured for variations in cell and tissue structure and size, and for starch content by staining with iodine.

In no way could the anatomy of the wilt resistant varieties be differentiated from that of the susceptible varieties. Short days caused a notable decreased and recumbent top growth in the winter hardy varieties. The annual growth rings of the field-grown roots of hardy varieties showed large xylem vessels formed during long days and small during shorter days. The same contrast in vessel size was seen in the greenhouse-grown roots of hardy varieties. The contrast in xylem size due to change in day length was less prominent in non winter hardy varieties.

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Dedicated

to

My father - whose encouragement and

inspiration led me to come to the

United States for graduate study.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks and appreciation to Dr. S. T. Dexter, Dr. C. M. Harrison and Professor H. M. Brown of the Farm Crops Department, for their guidance and assistance during the course of this study.

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INTRODUCTION

Michigan has one of the largest alfalfa acreages in the U.S.A. Mostly the winter hardy varieties of alfalfa are grown. In selecting an alfalfa variety, wilt resistance and winter hardiness are of primary importance. In Michigan, the crop of alfalfa is often injured by severe winters and therefore the choice of an alfalfa variety with a high degree of winter hardiness is important. Wilt resistance becomes very important when a field is to be left for three or more years.

Alfalfa varieties respond differently in growth behavior to day-length at various seasons of the year. The different growth characteristics of several varieties of alfalfa observed under field conditions suggest that there might be differences in the root structure which could be seen in cross-section. Similarly, one would expect chemical or anatomical differences associated with wilt resistance.

As a result a study was started in October, 1953, to 1) compare the anatomy of field grown roots of wilt resistant and wilt susceptible alfalfa varieties that varied from highly to slightly winter hardy; and 2) compare the growth

behavior and root anatomy of such alfalfa varieties when grown in the greenhouse under short or long day conditions.

REVIEW OF LITERATURE

Much work regarding wilt resistance and winter hardiness in alfalfa has been reported. Peltier et al (11) showed differences in the root anatomy of wilt resistant and wilt susceptible alfalfa varieties. Wilt resistant varieties had smaller xylem vessels than those susceptible to wilt. Rochlin (15) indicated that the infection of club root of cabbage was less in those plants, the roots of which had a compact xylem than in those where the xylem strands were divided by wide medullary rays.

Angell (2) showed that in onions phenolic compounds such as protocatechuic acid and catechol are toxic to fungi and consequently develop disease resistance in the plant. Hatfield et al (7) found that there was a correlation between strong pungency and disease resistance in onion. Brown (4) indicated that susceptibility of a potato tuber when attacked by a certain fungus depended largely on the chemical action of the tuber which allowed the fungus to develop. Greathouse et al (6) observed that the presence of berberine in roots of M. trifoliolata was a possible factor in the resistance to root rot.

Oakley et al (10) showed that different varieties of alfalfa reacted very differently to length of day. In

general southern varieties grew tall in short days, while northern varieties stayed short. Tysdal (16) observed that the hardier varieties of alfalfa responded markedly to short day length under which they hardened off much more than under a normal day length. Peltier et al (12) reported that under normal conditions, hardy varieties became dormant earlier and hardened more rapidly than non-hardy varieties. Jones (8) found that phloem and phloem rays of Kansas Common alfalfa were affected by winter injury and were then split. The cambium cells were also disrupted.

Rather et al (13) found that due to winter freezing of alfalfa ray, splitting occurred in roots which were devoid of starch. Rather et al (14) observed that alfalfa roots were lowest in starch reserves during the period of most active growth in the spring. They also found that the stand of a winter hardy variety of alfalfa was associated with reserve foods, especially starch, in the roots of alfalfa plants, as they reached the dormant season in the fall. Weimer (17) indicated that the roots of non-hardened alfalfa with large amounts of carbohydrate reserves froze less easily than roots that had been deprived of light for some time.

Bell (3) observed tnat an increase in starch content of both hardy and non-hardy alfalfa resulted in a decrease in cold resistance, while a decrease in starch content of both hardy and non-hardy alfalfa resulted in an increase in cold resistance.

MATERIALS AND METHODS

The basic design of this experiment was to provide alfalfa varieties that were similarly paired as to winter hardiness and contrasted as to wilt resistance. Thus Ladak is similar in growth behavior and winter hardiness to Rhizoma but they contrast widely in wilt resistance. Similar is the relation with Vernal and Naragansett, Ranger and Atlantic, Buffalo and Talent, and Caliverde and California Common. These varieties provide both resistance and susceptibility to bacterial wilt over a wide range of winter hardiness.

Ladak, Ranger, Buffalo, Rhizoma, Naragansett, Atlantic, Talent and California Common alfalfa seed was sown on August 14, 1952. Ten roots of each variety were dug from the field at three different dates: October 15, December 17, 1953, and February 25, 1954. These roots were dug at ten different places in each plot. Those roots which showed yellowness, were discarded to avoid diseased roots. There was a considerable range in diameter among the roots of each variety. The ten roots were washed and a section, an inch long, was selected about two inches below the crown. The ten sections of roots of each variety were pickled* in a solution of formalin alcohol for about a week. The roots were embedded

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* Killed and fixed in a solution of 60 c.c. of 95 percent ethyl alcohol, 40 c.c. of water and 4 c.c. of formalin and then dehydrated in alcohol of varying concentrations.

in paraffin and cut into cross sections of 10 microns thickness. Ten sections, one from each of ten roots of a given variety, were mounted on a glass slide. Ten such slides were made from each lot of ten roots from each of the eight varieties and from the three dates of collection. A representative from each lot of 10 was then stained with a triple stain consisting of safranin, crystal violet and orange G.for later examination under the microscope.

Seed of ten varieties of alfalfa, viz., Ladak, Vernal, Ranger, Buffalo, Caliverde, Rhizoma, Hardigan, Atlantic, Talent and California Common was planted in the greenhouse in clay pots on November 12, 1953. Two pots of each variety were planted and grown under a long day until the plants were about four inches high. They were then thinned to about thirty plants in each pot. One culture of each variety was given a photoperiod of fourteen and the other eight hours. The long day lot received normal day together with six hours of fluorescent light. The short day lot was given only normal daylight. The exact duration of light was controlled by covering the plants with a black cloth. The positions of the cultures were changed at intervals to equalize the effect of the light source. Ten representative plants from each variety were harvested carefully on February 23, 1954. Observations were taken regarding heights, weights, and growing habits of each under different photoperiods.

The dry weight of the roots and tops of each variety was taken after drying the roots and tops to constant weight in a drying oven. Ten fresh plants were photographed (Figure 1) to show the characteristic growth habit of each variety under different light treatments. These roots were sampled, pickled, sectioned and mounted on slides with ten sections per slide as with the field grown roots. The remaining plants in the cultures were taken outside of the greenhouse on February 23 and left in freezing temperatures until April 5, 1954. Six weeks after returning them to the greenhouse, ten representative plants of each previously frozen variety and of different light treatments, were harvested. Slides were made of the roots of these previously frozen alfalfas.

The following lots of slides were available for study under the microscope:

One section each of ten individual roots of each of eight varieties, viz., Ladak, Ranger, Buffalo, Rhizoma, Naragansett, Atlantic, Talent and California Common from each of the following dates:

1) Fall -- dug from field October 15, 1953.

2) Winter -- dug from field December 17, 1953.

3) Spring -- dug from field February 25, 1954.

Also one section each of ten individual roots of each of ten varieties, viz., Ladak, Vernal, Ranger, Buffalo,

Caliverde, Rhizoma, Hardigan, Atlantic, Talent and California Common from each of the following treatments:

1) Long day in greenhouse.

2) Long day frozen.

3) Short day in greenhouse.

4) Short day frozen.

On each slide there were the cross-sections of ten roots of one variety harvested on a single date. Observations under the microscope were made of angularity of xylem vessels, size of xylem vessels, width of xylem ray, thickness of bark, thickness of wood, abundance of fibers in the vascular bundles, presence of xylem vessels in the center, contrast in the annual rings formed, width of cambium and starch content.

Each root section was either measured or given an individual score in regard to each of the characteristics mentioned above. For measuring the diameter of xylem vessels, an ocular micrometer was used while for measuring the thickness of cambium, bark or wood, the stage micrometer was used. Characters such as angularity of xylem vessels, abundance of fibers, starch content, etc. were given scores.

EXPERIMENTAL RESULTS

Lengths of Alfalfa Tops and Roots

The average top and root lengths of ten alfalfa plants are given in Table II.

Under short days, Ladak the most hardy variety of alfalfa, had the least top height, while the least hardy variety California Common, had the greatest height. Light seemed to have very little effect on root lengths of the different alfalfa varieties. There was however, a definite influence of light on the top growth. The top heights under different light treatments were analysed and an analysis of variance is given in Table I.

Green Weights of Alfalfa Tops and Roots

The weights of tops and roots of each variety of alfalfa consisting of ten plants were taken on the same day they were harvested and the figures are given in Table II.

The non winter hardy alfalfa did not show much response to long days. The hardy varieties except Vernal and Ranger added much top growth under long days. Under short days, Vernal, Buffalo, and California Common had more top growth than the others. Two or three tall Vernal plants in the

TABLE I

ANALYSIS OF VARIANCE FOR TOP HEIGHTS UNDER SHORT AND LONG DAYS

Sources of Variance	DF	SS	MS	F
Total	19	230.13	12.11	
Varieties	9	18.66	2.07	1.90
Winter hardy: non winterhardy	1	9.11	9.11	8.35*
Within winter hardy	4	6.15	1.53	1.40
Within non winter hardy	4	3.40	0.85	0.78
Length of day	1	201.61	201.61	184.96**
V x L (error line)	9	9.88	1.09	

*Significant at 5% level
**Significant at 1% level

TABLE II

AVERAGE LENGTHS AND WEIGHTS OF PAIRED ALFALFA VARIETIES (ten plants of each variety) GROWN IN THE GREENHOUSE UNDER DIFFERENT LIGHT TREATMENTS

Varieties Decreasing in order as to winter hardiness	Length in Inches							
	Tops				Roots			
	Short Day		Long Day		Short Day		Long Day	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	6.00	7.00	13.50	14.00	6.00	8.00	7.50	8.00
Vernal -- Hardigan W.R. W.S.	8.50	7.00	15.00	14.00	8.50	7.00	7.00	7.00
Ranger -- Atlantic W.R. W.S.	7.00	7.50	12.00	16.50	7.00	6.50	6.00	5.50
Buffalo -- Talent W.R. W.S.	9.00	7.50	14.00	14.50	6.50	6.50	7.00	6.50
Caliverde--California Common W.R. W.S.	9.50	10.00	13.50	15.50	6.50	7.00	5.50	5.50

*Wilt Resistant - W.R.
**Wilt Susceptible - W.S.

TABLE II (cont.)

Varieties Decreasing in order as to winter hardiness	Green Weight in Grams							
	Tops				Roots			
	Short Day		Long Day		Short Day		Long Day	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	12.5	16.00	40.50	34.50	3.70	5.00	8.10	7.00
Vernal -- Hardigan W.R. W.S.	29.50	12.00	30.50	20.00	6.70	3.30	4.10	4.50
Ranger -- Atlantic W.R. W.S.	19.00	14.50	23.50	21.50	6.00	3.60	4.60	2.80
Buffalo -- Talent W.R. W.S.	32.00	19.00	30.00	23.00	6.40	4.80	5.50	4.50
Caliverde-California Common W.R. W.S.	21.00	32.50	20.50	30.00	4.10	5.90	3.60	4.00

TABLE II (Cont.)

Varieties Decreasing in order as to winter hardiness	Dry Weight in Grams							
	Tops				Roots			
	Short Day		Long Day		Short Day		Long Day	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	2.00	3.00	9.00	6.50	1.00	2.00	2.50	2.00
Vernal -- Hardigan W.R. W.S.	5.00	3.00	6.00	2.50	2.00	1.00	1.50	2.00
Ranger -- Atlantic W.R. W.S.	4.50	3.50	4.50	4.00	2.00	1.00	1.50	1.50
Buffalo - Talent W.R. W.S.	4.50	3.50	6.00	3.00	2.50	1.50	2.00	1.50
Caliverde-California Common W.R. W.S.	4.00	6.00	2.50	5.00	2.00	2.00	1.50	1.50

culture were mainly responsible for including this variety with California Common and Buffalo since most of the plants were much shorter. Under short days, the root weight of Vernal, Ranger, Buffalo and California Common exceeded that of the rest of the varieties. Under long days, Ladak, Rhizoma and Buffalo showed more root growth when compared with the other varieties.

Dry Weights of Alfalfa Tops and Roots

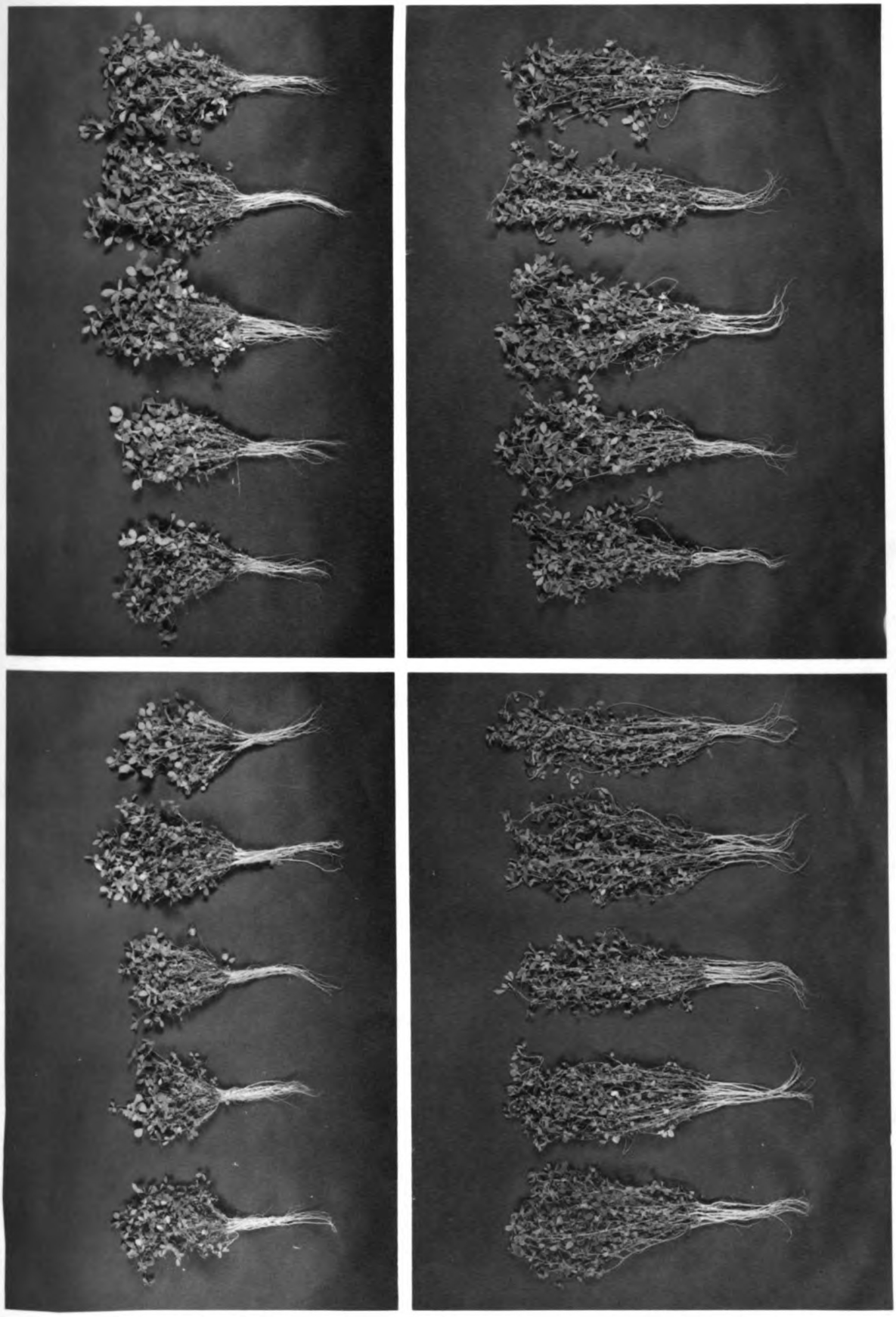
Under short days, Ladak had the lowest dry weight of tops, while California Common had the highest. Under long days, Ladak had the highest weight of tops while Hardigan and Caliverde had the lowest. There was very little difference in dry weights of roots among all the varieties under both short and long days.

Photographs

Ten plants of each variety and treatment were photographed and are shown in Figure 1. Under short days non hardy varieties, e. g. Talent, Buffalo, California Common and Caliverde showed more foliage growth than the hardy varieties, e. g. Rhizoma, Ladak, Hardigan and Vernal. Under long days there was very little difference in the top growth of the hardy and non hardy varieties of alfalfa. All the

Figure 1. Alfalfa plants given short (above) and long (below) photoperiods. Varieties from left to right are:

1. Rhizoma
2. Ladak
3. Hardigan
4. Vernal
5. Atlantic
6. Ranger
7. Talent
8. Buffalo
9. California Common
10. Caliverde



alfalfa varieties showed more growth under long days than under short days.

Some Characteristics of Alfalfa Varieties Observed in the Greenhouse

Short days. The leaves of Rhizoma, Ladak, Hardigan and Vernal were smaller than those of other varieties. The varieties of Rhizoma, Ladak, Vernal and Atlantic were recumbent while the rest of the varieties were erect. Rhizoma, Hardigan, Buffalo, California Common and Caliverde had uniform shoots while others did not. Vernal had the most branching at the base and a general lack of uniformity. Talent and Caliverde had very little branching at the base. Rhizoma had the greatest amount of root branching. Ladak, Hardigan, Atlantic and California Common had hairy roots while the others had very little hairiness.

Long day. All varieties of alfalfa had uniformity in the size of shoots except Atlantic, which had shoots of differing sizes. Vernal, Atlantic, California Common were recumbent while the other varieties were erect. The roots of Atlantic and California Common were very hairy. Hardigan, Atlantic, Ranger and Caliverde had very thin roots while Buffalo had thick ones.

Anatomical Studies

An average of scores and measurements of cross-sections of thirty roots per variety (ten roots of each variety dug in October, December, and February) and of forty roots from each variety from the greenhouse, are shown in Tables III and IV.

Field roots. Varieties could not be distinguished from one another as regards angularity of xylem vessels, size of xylem vessels, width of xylem parenchyma ray, width of xylem ray, thickness of bark, thickness of wood, abundance of fibers, presence of xylem vessels in the center and width of cambium.

The contrast between the size of xylem vessels in the growth rings was distinct in winter hardy varieties but not in the winter tender ones.

As regards the starch content of the cells, it was found that there was a trend toward early conversion of starch to sugar in winter hardy varieties. The figures are given in Table V.

Greenhouse roots. Varieties could not be distinguished one from another as regards angularity of xylem vessels, size of xylem vessels, width of xylem parenchyma ray, width of xylem ray, thickness of bark, thickness of wood, abundance of fibers, presence of xylem vessels in the center and width of cambium.

TABLE III

AVERAGE SCORE OR SIZE OF CELL STRUCTURES OF TEN ROOTS OF EACH ALFALFA VARIETY DUG AT THREE DATES FROM THE FIELD (average of three dates)

Varieties Decreasing in order as to winter hardiness	Angularity of Xylem Vessels [1]		Size of Xylem Vessels in m.m.		Width of Parenchyma Ray in m.m.		Width of Xylem Rays in m.m.	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	4.52	4.00	.042	.038	.106	.110	.19	.21
Vernal -- Naragansett W.R. W.S.	--	4.91	--	.059	--	.130	--	.22
Ranger -- Atlantic W.R. W.S.	4.93	3.93	.045	.054	.116	.140	.20	.20
Buffalo -- Talent W.R. W.S.	5.31	4.80	.037	.035	.140	.130	.21	.22
Caliverde-California Common W.R. W.S.	--	4.70	--	.040	--	.130	--	.26

[1] 1=least, 10=most
* Wilt Resistant - W.R.
**Wilt Susceptible - W.S.

TABLE III (Cont.)

Varieties Decreasing in order as to winter hardiness	Size of Bark in m.m.		Size of Wood in m.m.		Abundance of Fibers[1]		Xylem Vessels in the center[1]		Contrast in Annual Rings[1]	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	.71	.72	1.28	1.34	4.32	4.95	7.59	6.58	5.1	5.20
Vernal -- Naragansett W.R. W.S.	--	.76	--	1.52	--	5.00	--	7.83	--	4.48
Ranger -- Atlantic W.R. W.S.	.76	.79	1.33	1.54	4.10	4.38	5.66	7.60	3.7	4.23
Buffalo -- Talent W.R. W.S.	.78	..83	1.63	1.62	4.82	4.74	5.31	5.85	4.1	3.39
Caliverde-California Common W.R. W.S.	--	.73	--	1.54	--	4.31	--	8.56	--	2.68

TABLE IV

AVERAGE SCORE OR SIZE OF CELL STRUCTURES OF TEN ROOTS OF EACH ALFALFA VARIETY PLANTED IN GREENHOUSE UNDER DIFFERENT LIGHT TREATMENTS AS WELL AS FROM PLANTS FROZEN OUTSIDE
(Average of short day, long day, short day frozen, long day frozen)

Varieties Decreasing in order as to winter hardiness	Angularity of Xylem Vessels[1]		Size of Xylem Vessels in m.m.		Width of Parenchyma Ray in m.m.		Width of Xylem Rays in m.m.	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	4.65	4.06	.030	.031	.052	.057	.082	.095
Vernal -- Hardigan W.R. W.S.	3.57	3.90	.032	.030	.055	.057	.090	.085
Ranger -- Atlantic W.R. W.S.	4.16	3.55	.033	.035	.065	.057	.080	.082
Buffalo -- Talent W.R. W.S.	3.67	3.84	.035	.033	.070	.075	.087	.085
Caliverde - California Common W.R. W.S.	4.28	4.91	.031	.036	.062	.067	.090	.095

[1] 1=least, 10=most
* Wilt Resistant - W.R.
**Wilt Susceptible - W.S.

TABLE IV (Cont.)

Varieties Decreasing in order as to winter hardiness	Size of Bark in m.m.		Size of Wood in m.m.		Abundance of Fibers[1]		Xylem Vessels in the Center[1]		Contrast in L. and Sh. day Xylem Vessels[1]	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	.40	.38	.44	.40	3.01	2.69	8.25	9.18	4.25	4.02
Vernal -- Hardigan W.R. W.S.	.37	.34	.41	.36	2.81	2.28	8.96	9.72	5.35	3.00
Ranger -- Atlantic W.R. W.S.	.43	.32	.41	.38	2.58	2.88	9.25	9.47	4.55	2.90
Buffalo -- Talent W.R. W.S.	.49	.44	.47	.45	2.96	2.87	7.87	8.22	1.29	3.10
Caliverde-California Common W.R. W.S.	.38	.41	.38	.49	3.04	3.04	9.17	9.06	0.71	0.0

TABLE V

AVERAGE OF SCORE ON STARCH CONTENT AND WIDTH OF CAMBIUM OF TEN ROOTS OF EACH ALFALFA VARIETY GROWN IN GREENHOUSE UNDER DIFFERENT LIGHT TREATMENTS

Varieties Decreasing in Order as to winter hardiness	Starch[1]							
	Long Day		Short Day		Long Day Frozen		Short Day Frozen	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	1.60	2.30	2.45	1.25	1.90	2.90	2.60	2.90
Vernal -- Hardigan W.R. W.S.	3.11	1.77	3.44	0.88	0.70	2.60	2.20	2.00
Ranger -- Atlantic W.R. W.S.	2.30	1.70	0.60	2.22	2.37	1.50	1.90	2.33
Buffalo -- Talent W.R. W.S.	2.70	2.00	2.22	2.30	3.30	2.70	3.20	2.90
Caliverde-California Common W.R. W.S.	1.20	1.66	0.11	4.12	1.50	1.00	3.10	1.60

[1] 1=least, 10=most

* Wilt Resistant - W.R.
**Wilt Susceptible - W.S.

TABLE V (Cont.)

Varieties Decreasing in order as to winter hardiness	Cambium Width in m.m.							
	Long Day		Short Day		Long Day Frozen		Short Day Frozen	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	.10	.15	.12	.14	.20	.16	.11	.15
Vernal -- Hardigan W.R. W.S.	.11	.10	.16	.12	.13	.15	.13	.12
Ranger -- Atlantic W.R. W.S.	.10	.12	.14	.11	.12	.14	.15	.15
Buffalo -- Talent W.R. W.S.	.10	.10	.14	.14	.12	.13	.17	.15
Caliverde-California Common W.R. W.S.	.10	.11	.13	.16	.16	.16	.16	.17

The contrast between the size of xylem vessels produced in long and short days was sharp in winter hardy alfalfa varieties but not in the winter tender ones.

Slides were stained with Iodine-Potassium iodide solution for starch studies. Those root sections which stained darker than others were given higher scores. It was found that there was a trend towards early conversion of starch into sugar in winter hardy varieties. The figures are given in Table VI.

A sample of actual data obtained by observing various slides under the microscope, is given in Table VII. The data of only two alfalfa varieties, viz., Ladak and California Common has been included in that table. These data will enable the reader to see the range of variation of measurements obtained with 10 roots of two varieties as regards the eleven characteristics.

TABLE VI

AN AVERAGE OF SCORE ON STARCH AND WIDTH OF CAMBIUM OF TEN ROOTS OF EACH ALFALFA VARIETY DUG AT THREE DATES FROM THE FIELD

Varieties Decreasing in order as to winter hardiness	Starch[1] Fall October 15, 1953		Winter December 17, 1953		Spring February 25, 1954	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	1.40	5.30	1.70	1.20	0.77	2.00
Vernal -- Naragansett W.R. W.S.	--	2.60	--	1.90	--	2.12
Ranger -- Atlantic W.R. W.S.	1.30	1.00	0.80	1.10	1.70	1.77
Buffalo -- Talent W.R. W.S.	4.30	1.90	2.00	2.10	1.50	1.77
Caliverde -California Common W.R. W.S.	--	2.60	--	2.00	--	2.00

[1] 1=least, 10=most
* Wilt Resistant - W.R.
**Wilt Susceptible - W.S.

TABLE VI (Cont.)

Varieties Decreasing in order as to winter hardiness	Cambium Width in m.m. Fall October 15, 1953		Winter December 17, 1953		Spring February 25, 1954	
	W.R.*	W.S.**	W.R.*	W.S.**	W.R.*	W.S.**
Ladak -- Rhizoma W.R. W.S.	.10	.11	.12	.14	.13	.10
Vernal -- Naragansett W.R. W.S.	--	.12	--	.18	--	.14
Ranger -- Atlantic W.R. W.S.	.12	.17	.14	.16	.11	.11
Buffalo -- Talent W.R. W.S.	.13	.13	.12	.19	.17	.20
Caliverde-California Common W.R. W.S.	--	.14	--	.14	--	.15

TABLE VII

REPRESENTATIVE DATA OF TEN SECTIONS, ONE FROM EACH OF TEN ROOTS OF EACH OF THE TWO VARIETIES GROWN IN GREENHOUSE UNDER SHORT DAY PERIOD TOGETHER WITH RANGE OF TEN OBSERVATIONS OF EACH CHARACTERISTIC

No.	Root Characteristics	Ladak		California Common	
		Range	Average of Ten Sections	Range	Average of Ten Sections
1	Angularity of Xylem Vessels[1]	3-5	4.00	3-6	4.75
2	Size of Xylem Vessels in m.m.	1-4	2.54	4-6	5.00
3	Width of Xylem Parenchyma Ray in m.m.	.035 - .070	0.046	.070-.105	0.074
4	Width of Xylem Ray in m.m.	.035 - .175	0.066	.070-.140	.089
5	Size of Bark in m.m.	0.3-0.6	0.44	.4-.6	.49
6	Size of Wood in m.m.	0.1-0.7	0.47	.4-.7	.53
7	Abundance of Fibers[1]	1-4	2.55	2-3	2.37
8	Presence of Xylem in the Center[1]	Present in 9 sections, absent in 1 section	9.00	Present in 6 sections, absent in 2 sections	7.5
9	Contrast between long and short day xylem vessels[1]	4-7	5.1	0-0	0
10	Starch Content[1]	0-5	2.45	4-5	4.12
11	Cambium Width in m.m.	.1-.2	.120	.1-.2	.162

[1] 1= least, 10=most

DISCUSSION

An examination of the data on height of alfalfa plants in the greenhouse experiment indicated that winter hardy alfalfas, e.g. Ladak showed more response to length of day than winter tender ones, e.g. California Common. Ladak grew little under short days while California Common grew tall.

It was observed that under short days the winter hardy varieties were recumbent while the non winter hardy alfalfas were erect. This is in agreement with the conclusion reached by Oakley and Westover (10).

In the hardy varieties of alfalfa, big xylem vessels were formed during spring and early summer while in late summer and early fall the vessels formed were smaller. In non hardy alfalfas, the xylem vessels formed during summer were more nearly the same size as those formed during spring.

The starch content of the winter hardy alfalfas declined sharply from fall to winter, while that of winter tender ones remained almost the same. This indicates that the hardy variety of alfalfa converted starch into sugar more rapidly in winter than in fall, while the winter tender ones were slow in starch conversion in both seasons which is in accord with Bell (3). It was observed that the alfalfa roots dug in winter sectioned easier than those dug in fall. This

may have been caused by the difference in starch content of the roots from the two seasons.

Several hundred individual roots in lots of 10 for each variety, were observed under the microscope for various anatomical characteristics but in no case could a wilt resistant variety be positively distinguished from a wilt susceptible variety. The variation in root structures in cross section between individual roots of a given variety was fully as great as the differences between varieties.

SUMMARY

Ten alfalfa varieties were grown from seed in the greenhouse under two different day lengths. Varieties were paired for similarity in winter hardiness and growth characteristics but contrasted as to wilt resistance in the following manner:

Winter Hardiness or fall dormancy	Wilt Resistant	Wilt Susceptible
1. greatest	Ladak	Rhizoma
2.	Vernal	Hardigan
3.	Ranger	Atlantic
4.	Buffalo	Talent
5. least	Caliverde	California Common

Observations were taken of all the varieties regarding height and weight of roots and tops and growth characteristics. Slides were made from ten roots of each variety grown in the greenhouse in long and short day cultures. Additional slides were made after the cultures had been subjected to freezing temperatures outside the greenhouse for six weeks.

Seed of eight of the above varieties was planted in the field in August, 1952. In October and December, 1953, and February, 1954, roots were dug and similar slides made from ten roots of each variety on each occasion.

Microscopic observations were taken of the following factors: angularity of xylem vessels, size of xylem vessels, width of xylem parenchyma ray, width of xylem ray, thickness of bark, thickness of wood, abundance of fibers in vascular bundles, presence of xylem vessels in the center, contrast in the annual rings formed, width of cambium and starch content. Thirty roots of each variety from the field plots and fort from the greenhouse were examined.

CONCLUSIONS

1. The winter hardy varieties showed a wider response to length of day in terms of height and weight of top growth in the greenhouse than did the non hardy ones, but wilt resistant and wilt susceptible varieties could not be distinguished.

2. Little effect of length of day was seen with regard to root length and weight in any group of varieties.

3. The contrast between the size of xylem vessels in the growth rings was distinct in winter hardy varieties, but not in winter tender ones. In no other way could winter hardy be distinguished from winter tender varieties although there was a tendency towards early conversion of starch in winter hardy varieties.

4. Wilt resistant and wilt susceptible varieties could not be distinguished in cross sections of roots by anatomical characteristics of either field or greenhouse grown plants.

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APPENDIX

Description of Alfalfa Varieties Used in the Experiment

Ladak

Ladak was introduced from northern India by the United States Department of Agriculture in 1910. Its flowers are variegated and are largely purple. It has a semi-prostrate growth habit and recovers slowly after cutting. Its yield from the first cutting exceeds that of any other alfalfa variety in northern regions. It is not particularly resistant to wilt but is extremely winter hardy.

Vernal

Vernal was produced at the University of Wisconsin in 1952. It is a synthetic of several winter hardy and highly wilt resistant strains. It is judged to be more wilt resistant, winter hardy and higher in yield than Ranger.

Hardigan

Hardigan was selected from Baltic at Michigan State College. It is a variegated alfalfa and noted for high seed production and desirable forage characteristics. It is fairly resistant to leaf diseases and leaf hopper yellowing. Recovery after cutting is moderate. It is extremely susceptible to bacterial wilt but is quite winter hardy.

Naragansett

Naragansett was developed in Rhode Island through mass selection from several strains of yellow and variegated alfalfas. It is a high yielding variety and has dark green foliage. It is less susceptible to bacterial wilt than Hardigan but is similar to Hardigan with respect to leaf diseases and winter hardiness.

Atlantic

Atlantic was produced at New Jersey from a combination of many different strains. It has been a good forage producer. Its resistance to leaf spot is above average. It has little resistance to bacterial wilt but is fairly winter hardy.

Ranger

Ranger is a synthetic variety developed at the Nebraska Agricultural Experiment Station from wilt resistant selections out of Cossack, Turkistan and Ladak. It shows great variation in habit of growth and flower color. It is wilt resistant but does not produce more forage than other varieties in Iowa (1). It is distinctly variegated in flower color, with occasional yellow flowered plants. It has greater rapidity of recovery after cutting than Ladak or Cossack. It is slightly susceptible to leaf spot diseases, is quite winter hardy and is generally suited to the northern half of the United States.

Buffalo

Buffalo was selected from Kansas Common. It is a purple flowered alfalfa in the common group and is resistant to bacterial wilt. It has only a moderate degree of winter hardiness in Wisconsin (5).

Caliverde

Caliverde is the result of back crossing the characteristics of wilt resistance into the California Common variety and is supposed to be almost identical to California Common except in wilt resistance. It is also resistant to leaf spot and mildews.

Rhizoma

Rhizoma was developed at the University of British Columbia from a cross between a yellow flowered alfalfa and a variegated variety, viz., Grimm alfalfa. It is characterized by having a deep set crown and ability to spread by rhizomes. It is very winter hardy but trials in Wisconsin (5) have shown that it is very susceptible to bacterial wilt. In Iowa (1) it has yielded less than Ranger or Buffalo.

Talent

Talent was produced by the Oregon Agricultural Experiment Station. It was previously introduced in the States from France in 1931. For twenty years it has undergone natural selection in Oregon. It is a high yielding variety,

recovers quickly after cutting and grows late in the autumn. Its flowers vary from light blue to reddish purple. It is susceptible to injury by leaf hoppers and bacterial wilt and is not winter hardy.

California Common

This variety was introduced from Chile into California in 1850. It spread eastward from California and through the years has been modified by natural selection. It is a purple flowered alfalfa. It has some degree of wilt resistance but very little winter hardiness.