A STUDY OF CAMBIUM SPLITTING AND OF STARCH RESERVE IN ALFALFA

by

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INTRODUCTION

Winter killing has long been recognized as one of the most common causes of the loss of alfalfa stands throughout the northern alfalfa growing section of the United States.

For a number of years, observations at the Upper Peninsula Experiment Station at Chatham indicate that alfalfa stands are short lived with resultant thin stands being common after the first hay year. Alfalfa at the Chatham Station, however, and in other parts of the Upper Peninsula, is generally well protected from freezing temperatures during the late fall, winter and early spring, by a blanket of snow a foot or more in depth. This is in contrast to Lower Michigan, where alfalfa is generally subjected to intermittent severe freezing because of a lack of snow cover. Periodical shallow freezing of the ground occurs in the Upper Peninsula only in early fall prior to the establishment of a snow cover, and in the spring for a short period after the snow has disappeared. The alfalfa starts to grow immediately after the snow disappears in the spring, about May 1, resulting in occasional slight freezing of the new top growth.

In order to determine the reason for the rapid thinning-out of the alfalfa, a study was undertaken to obtain quantitative data on cambium injury and the relation of cutting treatments on starch reserve levels. Plots were laid out and work begun in the spring of 1939 at the Upper Peninsula Branch Experiment Station at Chatham, Michigan.

LITERATURE REVIEW

The following literature review is intended to include only those citations particularly pertinent to the present study.

Rather and Harrison (8) emphasized the "critical periods" during which time alfalfa should not be cut or closely grazed. The fall period during which the cutting or close grazing of alfalfa was likely to prove most injurious because of winter freezing varied with geographic locations. Ray splitting due to winter freezing was found in roots devoid of starch. Bell (1) found that Hardigan roots subjected to -7°C to -8°C for a period of 3 hours, resulted in formation of rifts which extended radially along the parenchyma cells of the rays. But when Hairy Peruvian roots were subjected to the same treatment, cambium splitting resulted, with very little ray splitting. An increase in starch content in both varieties during the course of the experiment was accompanied by decrease in cold resistance, while decrease in intensity of starch reserve was accompanied by increase in cold resistance. Jones (5) subjected 5 year old Kansas Common alfalfa plants to temperatures about -14°C and found that the rays of the xylem and phloem were badly split and that the cambium cells were frequently collapsed. It was apparent that many of the openings in the rays were closed during the growing season either by mechanical pressure, enlargement of neighboring cells, or perhaps by a combination of both. Grimm and Hardigan, representatives of the most hardy varieties, showed less evidence of healing than the less hardy varieties.

Tysdal (10) measured the hardiness of alfalfa varieties by their enzymatic responses and reported that the non-hardy and hardy varieties did not differ widely--that there was no great seasonal difference in the original activity of the diastatic enzyme. However, a higher concentration of sugar could be produced from a limited amount of starch by enzymes taken from plants in the fall, a higher concentration could be produced from the hardy varieties than from the less hardy varieties. The hardier the variety, the higher was the protected activity, (the diastatic activity after the extract has been subjected to a temperature of 70^{o} for 10 minuts) in the fall. After mid-winter, however, the difference diminished, and toward spring no one variety showed a distinct superiority.

The relationship of organic root reserve to the permanency of alfalfa stands has been studied rather extensively. Grandfield (2) reports that when growth started in the spring, and after each cutting, there was a rapid decline of total carbohydrates and of nitrogen until a minimum was reached, after which there was a rapid increase. Janssen (4) reports that the organic root reserves, including the sugars, starch, hemicellulose, and nitrogen compounds, vary with the type of treatments given the aerial parts of the plants; and, conversely, the amount of top growth produced by perennial plants is partially dependent upon the organic reserves in the roots. Hildebrand and Harrison (3) state that alfalfa and smooth bromegrass both became weakened when cut at low levels continuously and at frequent intervals. Megee (7), Steinmetz (9), and others have pointed out that the presence of abundant organic reserves does not insure against winter injury, since the plants must also have an heredity ability to successfully withstand cold.

Mark (6) reported that starch began disappearing in early fall and constituted a very small portion of the root reserve, if any, after October; and that digestion was most rapid in the hardy varieties.

MATERIALS AND METHODS

(A) Alfalfa Treatments.

Six plots for subsequent cutting treatments were laid out on 2-year old stands of Hardigan alfalfa in 1939 and 1940, which had been previously cut on September 15. The cutting treatments for the 1939 plots were as follows: Plot A - 1st cutting July 21; Plot B - 1st cutting June 26; Plot C, D, E, and F - 1st cuttings June 2, and 2nd cuttings August 17, 31, September 14 and 28, respectively. The cutting treatments for the 1940 plots were as follows: Plot 1 - no cutting; Plot 2 - 1st cutting July 2; Plots 3, 4, 5, and 6 - 1st cuttings July 2, 2nd cuttings August 17, 31, September 14 and 28, respectively.

Fifteen roots were dug at random from the respective plots at two week intervals in 1939, 1940 and 1941 on various dates, as shown in Tables 1 and 2. The roots were washed thoroughly, and a sample, approximately 3/8" in length, was taken from each to be pickled in alcohol-formalin solution for further study.

The pickled root samples were dehydrated and embedded in paraffin. Sections 15 microns in thickness were cut from each of 10 roots, fixed on a glass slide and stained for starch with Grams iodine solution.

(B) Determination of Starch Reserves.

With the aid of a photronic cell, projecting microscope and a galvanometer, as described by Bell (1), starch readings were obtained of each root. The slides on which the root slices were fixed, were inserted between a standard light and a photronic cell, causing a registration of amperes on the galvanometer in direct proportion to the amount of light passing through the roots. Those roots containing large amounts of stored starch intercepted a greater portion of the light beam than did the roots containing lesser amounts, and this in turn resulted in lower galvanometer readings. The galvanometer was adjusted so that a "depression" reading was obtained; and allowance was made for the root structure and the glass slide, the final reading being that caused by starch only. Plates 1, 2, 3 and 4, are photomicrographs showing the starch content of alfalfa roots and the "depression" or starch reading recorded in microamperes.

Two readings were made of roots 4.5 mm or less in diameter, one through the outer portion in areas 2 mm in diameter, and the other through the center of the root. Four readings were made of roots larger than 4.5 mm in diameter, two each of the outer and inner portion, using correction factors on the outer readings to compensate for the difference in total area of the two portions of the root.

(C) Determination of Cambium Injury.

A microscopic study was made of all of the roots to determine the presence and intensity of cambium injury or splitting. The cambium injury varied in intensity and was classified into three groups as follows: (1) slight "partial" - less than half of the total cambium area injured, (2) extensive "partial" - more than half injured, (3) "total" - all of the cambium area injured.

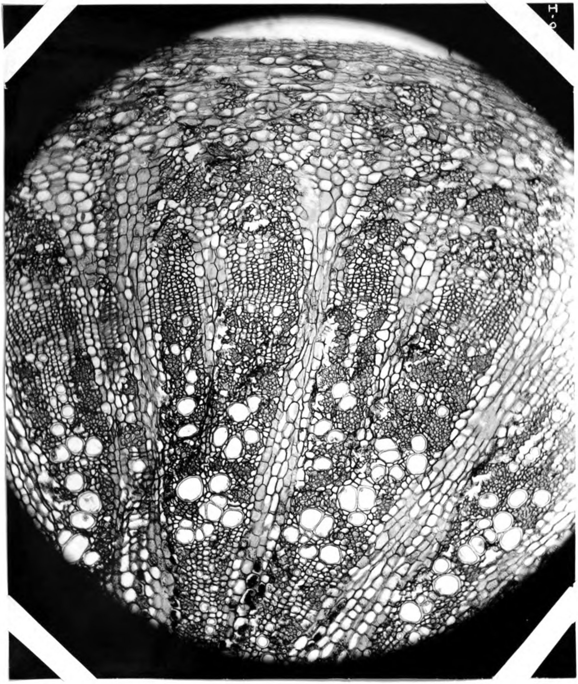

Plate 1. Photomicrograph of a portion of an alfalfa root having a starch reading of approximately a8.0 microamperes.

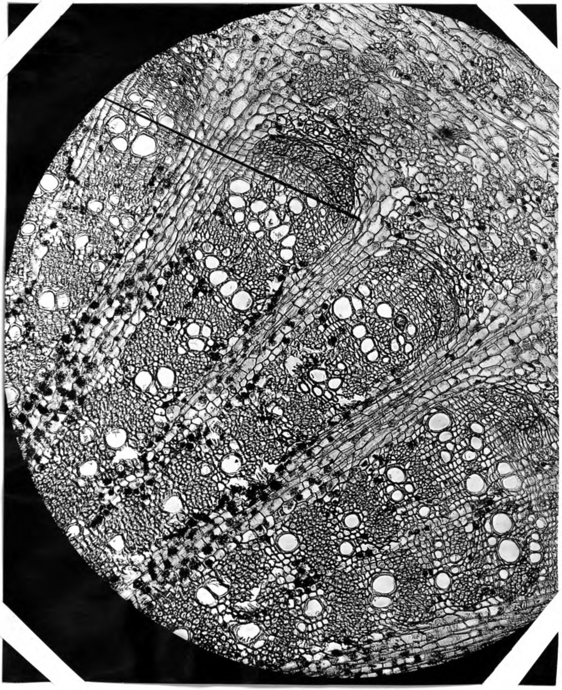

Plate 1. Photomicrograph of a portion of an alfalfa root, practically devoid of starch, having a starch reading of approximately 1.20 microamperes.

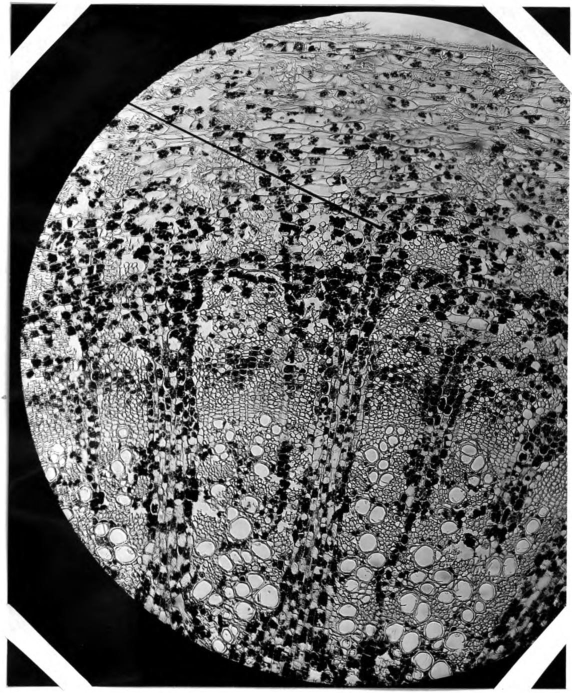

Plate 3. Photomicrograph of a portion of an alfalfa root having a starch reading of approximately 16.0 microamperes.

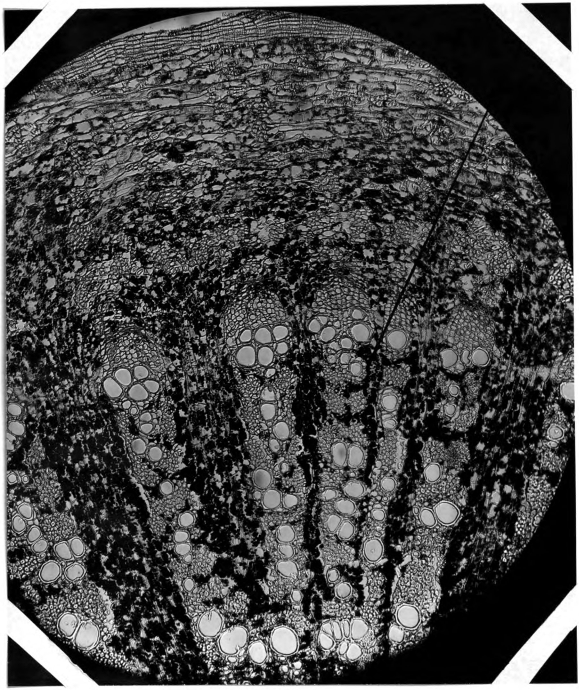

Plate 4. Photomicrograph of a portion of an alfalfa root having a starch reading of approximately 32.0 microamperes.

Experimental Results

(A) Effects of First Cutting on Starch Reserve.

An average of the starch readings from the ten roots collected every two weeks is graphically shown in figures 1 and 2, and tables 1 and 2. The intensity of starch reserve of the alfalfa roots in 1939 and 1940 was comparatively low when growth was resumed in the spring, about May 1. During May there was a gradual decrease of starch reserve in the roots, the starch probably being used for initiation of new top growth. The roots collected June 16 showed a replenishment of starch which continued accumulating until the first cuttings were made. It was observed in the field that the alfalfa grew very rapidly after June 1, and as indicated by the data, more food material was manufactured than was needed for top growth, resulting in storage of starch in the roots.

Two dates of first cutting of alfalfa were studied in 1939. A first cutting was made on one plot June 26 and on the other July 21. The roots of the alfalfa collected on July 1, the nearest root collection date to the time of top removal on June 26, were partially filled with starch. There was a sudden drop in starch content when growth resumed after the cutting, reaching a minimum about 20 days later, after which there was a rapid replenishment. Roots collected on July 16 from the plot cut later on July 21, had approximately twice the amount of starch as those collected July 1 from plants cut on June 26. The alfalfa was in full bloom on July 16 and probably had reached its maximum storage of starch. The decline of starch reserve after the cuttings was found to be considerably greater in the alfalfa cut on July 21 when compared to that cut on June 26, but both reached maximum storage of starch at the same time, September 16. There was a gradual decrease of starch after September 16, and the

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Fig. 1. INTENSITY OF STARCH RESERVE OF HARDIGAN ALFALFA IN 1939.

Fig. 2. INTENSITY OF STARCH RESERVE OF HARDIGAN ALFALFA IN 1940.

Table 1. Starch Readings of Alfalfa Roots Collected in 1939. (In Microamperes)

Date Collected	Plot A 1st Cutting 7/21	Plot B 1st Cutting 6/26	Plot C 1st Cutting 6/26 2nd Cutting 8/17	Plot D 1st Cutting 6/26 2nd Cutting 8/31	Plot E 1st Cutting 6/26 2nd Cutting 9/14	Plot F 1st Cutting 6/26 2nd Cutting 9/26
May 1	5.92					
May 16	3.56					
June 1	.88					
June 16	2.36					
July 1	16.52	13.00				
July 16	28.92	11.13				
August 1	15.64	22.80				
August 16	14.88	26.12				
September 1	30.88	35.52	24.20			
September 16	32.56	35.36	22.04	25.00		
October 1	24.24	29.24	18.76	16.56	27.60	
October 16	29.36	28.04	18.68	22.12	24.40	32.68
November 1	25.44	28.56	18.52	17.11	16.24	22.16
November 16	16.76	15.12	16.00	11.88	11.24	15.48

Table 2. Starch Readings of Alfalfa Roots Collected in 1940 - 1941 (In Mircroamperes)

Date Collected	Plot 1 No Cutting	Plot 2 1st Cutting 7/2	Plot 3 1st Cutting 7/2 2nd Cutting 8/17	Plot 4 1st Cutting 7/2 2nd Cutting 8/31	Plot 5 1st Cutting 7/2 2nd Cutting 9/14	Plot 6 1st Cutting 7/2 2nd Cutting 9/28
April 1, 1940	4.24					
April 16, 1940	3.36					
May 1, 1940	2.96					
May 16, 1940	2.40					
June 1, 1940	.72					
June 16, 1940	4.60					
July 1, 1940	14.76					
July 16, 1940	25.48	10.00				
August 1, 1940	30.00	8.88				
August 16, 1940	32.56	26.68				
September 1, 1940	25.70	28.52	22.76			
September 16, 1940	26.00	29.96	14.48	18.64		
October 1, 1940	23.28	28.84	14.96	18.76	23.52	
October 16, 1940	22.40	18.40	14.48	16.88	20.08	19.48
November 1, 1940	19.80	21.08	9.56	14.20	18.60	19.04
November 16, 1940	14.08	18.68	4.92	13.44	14.44	13.84
April 16, 1941	4.28	4.24	2.96	3.20	5.60	3.72
May 1, 1941	1.44	2.00	3.52	2.60	2.60	2.12
May 16, 1941	1.56	.60	1.84	1.80	.40	1.24
June 1, 1941	9.03	15.04	8.04	7.00	13.68	4.96
June 16, 1941	16.36	25.96	19.36	26.00	27.72	17.32

roots collected November 16 from both plots had practically the same amount of starch reserve.

The 1940 results on first cutting of alfalfa are quite comparable to those of 1939. The alfalfa which received no cutting reached maximum storage of starch August 16, a month earlier than the alfalfa cut at ½ bloom on July 2. The alfalfa roots collected on July 1 contained half as much starch as those collected on August 16 from alfalfa not cut. There was a gradual decline of starch in the fall.

(B) Effects of Second Cutting on Starch Reserve.

The effects of second cuttings on August 17, 31, and September 14 and 28, were studied in 1939, 1940 and 1941. Having the starch readings of the individual roots, it was possible to determine the effect of second cuttings on the starch reserve. This was done by comparing the starch reserve of roots from plants which had been cut twice in relation to those cut once. These comparisons were made on the dates the second cuttings were removed and further comparisons were made on roots from these plots collected November 16. The difference in starch reserve may be considered as due to the second cutting.

In 1939, as shown in table 3, the alfalfa roots from plots receiving a first cutting June 26, and second cuttings August 17 and September 28, respectively, contained slightly more starch on November 16 than those which received only a first cutting June 26. The alfalfa roots from plots receiving second cuttings August 31 and September 14, respectively, were slightly lower in starch on November 16 than those receiving a first cutting June 26, resulting in a decrease in starch reading due to the second cuttings of 3.24 and 3.98, respectively.

A comparison on a percentage basis of the starch reserve of roots collected November 16 from plots receiving second cuttings with those from the plot cut only on June 26, and used as a check plot, was as follows: August 17 - 105.8%; August 31 - 78.5%; September 14 - 74.4%; and September 28 - 102.3% of the check.

The 1940 results on the effects of second cuttings were not comparable to those of 1939, as shown in table 4. It was found that roots collected from plants cut July 2 and again August 17, were practically devoid of starch on November 16; while those from the plot cut July 2 only were comparatively high in starch. When the starch content in the roots from the check plot, which was cut only on July 2, was considered as 100% on November 16, those of the August 17, 28 and September 14 and 28, rated 27, 72, 77 and 74%, respectively. The loss of starch from 100% to 27% as a result of cutting a second time on August 17, was much greater than the other losses due to cuttings on the later dates.

Alfalfa roots collected in the spring of 1941 from the 1940 plots, were lower in starch reserve than of those collected November 16 the previous fall, as shown in table 2. On April 16, 1941, the roots from the August 17, 31 and September 14 and 28 2nd cuttings contained 69.6, 75.6, 131.3, and 87.5% respectively, as much starch as those from the July 2 cutting.

(C) Cambium Splitting

In addition to the studies of the comparative amounts of starch in the alfalfa roots subjected to various cutting treatments, extensive study was made of tangential cambium splitting occurring in many of the roots. Cambium splitting was found to range from "partial" to "total"

Table 3. Effects of Second Cutting on Starch Reserve - 1939

Plot	Date of Cutting 1st	Date of Cutting 2nd	Date Collected	Starch Reading	Decrease In Starch	Decrease Due To 2nd Cutting
C	6/26	8/17	8/16 11/16	26.12 16.00	10.12	.88*
B	6/26	None	8/16 11/16	26.12 15.12	11.00	
D	6/26	8/31	9/1 11/16	35.52 11.98	23.64	3.24
B	6/26	None	9/1 11/16	35.52 15.12	20.40	
E	6/26	9/14	9/16 11/16	35.36 11.24	24.12	3.98
B	6/26	None	9/16 11/16	35.36 15.12	20.14	
F	6/26	9/28	10/1 11/16	29.24 15.48	13.76	.36*
B	6/26	None	10/1 11/16	29.24 15.12	14.12	

* - Increase.

Table 4. Effects of Second Cutting of Starch Reserve - 1940.

Plot	Date of Cutting		Date Collected	Starch Reading	Decrease in Starch	Decrease Due to 2nd Cutting
	1st	2nd				
3	7/2	8/17	8/16	26.68	21.76	
			11/16	4.92		13.76
2	7/2	None	8/16	26.68		
			11/16	18.68	8.00	
4	7/2	8/31	9/1	28.52		
			11/16	13.44	15.08	5.44
2	7/2	None	9/1	28.52		
			11/16	18.68	9.69	
5	7/2	9/14	9/16	29.96		
			11/16	14.44	15.52	4.24
2	7/2	None	9/16	29.96		
			11/16	18.68	11.28	
6	7/2	9/28	10/1	28.84		
			11/16	13.84	14.00	3.84
2	7/2	None	10/1	28.84		
			11/16	18.68	10.16	

splitting, varying in proportion with the various collections of roots, as shown in tables 5, 6, and 7. Plats 5, 6, and 7, are photomicrographs showing an uninjured root, "partial" and "total" splitting, respectively.

Cambium splitting was not found in any of the roots collected May 1, 1939. "Partial" splitting, varying from slight to extensive, was found in roots collected May 16, and two of the roots collected June 1 showed "total" splitting. The peak of splitting was June 16 when two of the roots showed slight and eight extensive "partial" splitting of the cambium. Roots collected July 16 from plants that did not receive a first cutting, were free of cambium splitting.

Following the June 26 cutting, there was an increase in "partial" splitting, reaching a peak July 16, after which there was a decrease, with no splitting in roots collected September 1. The roots collected a week previous to the July 21 cutting did not show cambium splitting, but those collected after the cutting showed splitting until October 16.

Cambium splitting was not found in any of the roots collected from plots receiving second cutting treatments.

Similar results were obtained in 1940, with "partial" splitting occurring in some of the roots collected May 16. Most severe splitting was found June 16, after which there was less severe splitting, with no splitting in those collected July 16. An increase in splitting occurred after the first cutting of July 2. As in 1939, cambium splitting was not found in any of the roots collected from plots receiving second cutting treatments.

Cambium splitting was found in the spring of 1941 in all of the 1940 plots. The intensity and total number of roots showing cambium splitting

Table 5. Alfalfa Roots Injured With Cambium Splitting - 1939

Date Collected	Date Cut	Starch Reading	Cambium Splitting in Roots				Per Cent of Roots Splitting
				Partial			
			None	Slight	Extensive	Total	
May 1		5.92	10				
May 16		3.56	1	4	5	)	
June 1		.88	1		7	2)	90%
June 16		2.36		2	8	)	
July 1		16.52	2	3	5	)	
July 16		28.92	10			)	
July 1	June 26	13.00	1	8	1	)	
July 16	June 26	11.13	1	5	4	)	
August 1	June 26	22.80	1	7	2	)	75%
August 16	June 26	26.12	7	3		)	
September 1	June 26	35.36	10			)	
August 1	July 21	15.64		5	4	1)	
August 16	July 21	14.88		1	8	1)	
September 1	July 21	30.88	3	5	2	)	60%
September 16	July 21	32.56	8	2		)	
October 1	July 21	24.24	9	1		)	
October 16	July 21	29.36	10			)	

Table 6. Alfalfa Roots Injured With Cambium Splitting - 1940.

Date Collected	Date Cut	Starch Reading	Cambium Splitting in Roots				Per Cent of Roots Splitting
				Partial			
			None	Slight	Extensive	Total	
May 1		2.96	10				
May 16		2.40	4	3	3		
June 1		.72	1	2	7		
June 16		4.60	1		8	1	78%
July 1		14.76	2	6	2		
July 16		25.48	3	5	2		
August 1		30.00	10				
July 16	July 2	10.00		4	6		
August 1	July 2	8.88	3	6	1		76%
August 16	July 2	26.68	4	6			
September 1	July 2	28.52	10				

Table 7. Alfalfa Roots Injured With Cambium Splitting - 1941.

Date Collected	Date Cut in 1940	Starch Reading	Cambium Splitting in Roots				Per Cent Of Roots Splitting
			None	Partial		Total	
				Slight	Extensive		
April 16	None	4.28	9	1		)	
May 1	None	1.44		4	6	)	
May 16	None	1.56			7	3)	82%
June 1	None	9.03			9	1)	
June 16	None	16.36		4	6	)	
April 16	July 2	4.24	8	2		)	
May 1	July2	2.00			8	2)	
May 16	July 2	.60			3	7)	84%
June 1	July 2	15.04		5	4	1)	
June 16	July 2	25.96		6	4	)	
	July 2 &						
April 16	August 17	2.96	5	3	2	)	
May 1	" "	3.52	1	4	5	)	
May 16	" "	1.84		2	4	4)	80%
June 1	" "	8.04		3	7	)	
June 16	" "	19.36	4	6		)	
	July 2 &						
April 16	August 31	3.20	10			)	
May 1	" "	2.60		2	7	1)	
May 16	" "	1.80			7	3)	78%
June 1	" "	7.00			9	1)	
June 16	" "	26.00	1	4	5	)	
	July 2 &						
April 16	September 14	5.60	8	2		)	
May 1	" "	2.60	2	5	3	)	
May 16	" "	.40		1	4	5)	74%
June 1	" "	13.68			8	2)	
June 16	" "	27.72	3	6	1	)	
	July 2 &						
April 16	September 28	3.72	7	3		)	
May 1	" "	2.12			8	2)	
May 16	" "	1.24			6	4)	80%
June 1	" "	4.96			7	3)	
June 16	" "	17.32	3	5	2	)	

varied slightly between the plots of roots collected April 16 to June 16, inclusive. But in general, there was no significant difference between the various plots, which had received different cutting treatments the previous year.

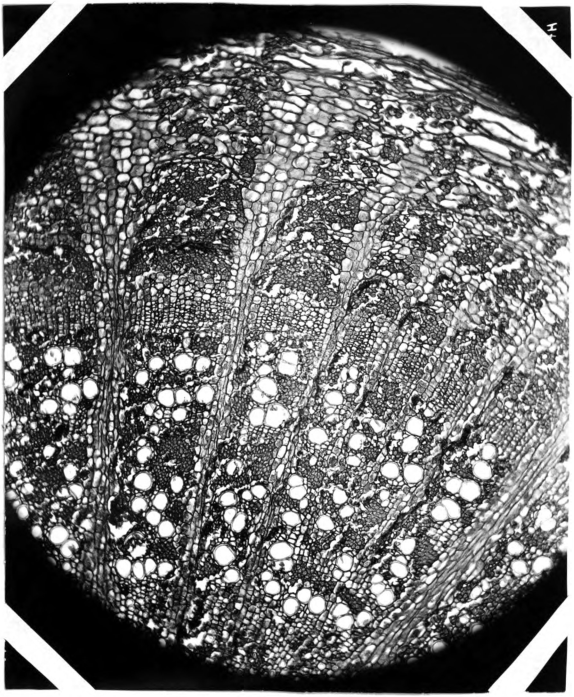

Plate 5. Photomicrograph of a portion of an alfalfa root with structure in good condition.

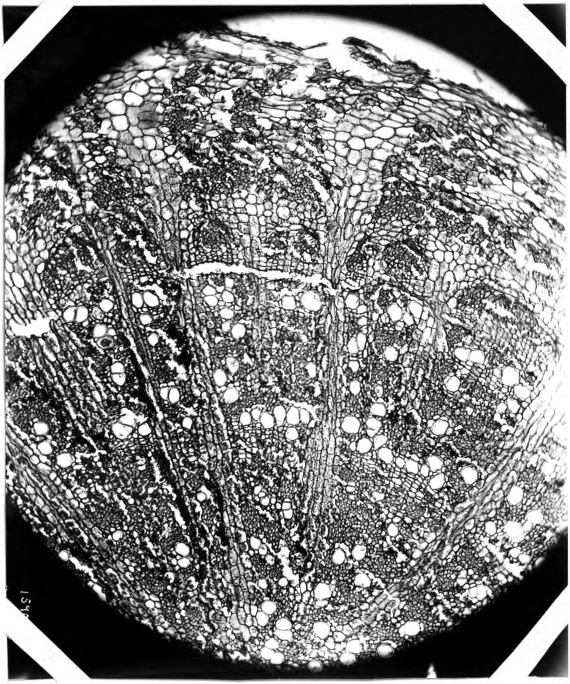

Plate 6. Photomicrograph of a portion of an alfalfa root showing "partial" splitting of the cambium.

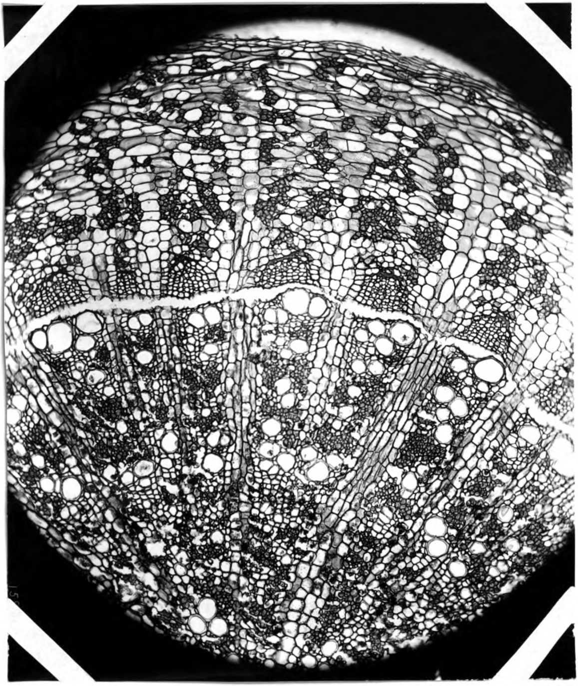

Plate 7. Photomicrograph of a portion of an alfalfa root showing "total" splitting of the cambium. This type of injury probably would result in subsequent death of the plant.

DISCUSSION OF RESULTS

In this study of root reserve, it was found that growth initiation in the spring, and after each cutting, resulted in a rapid decline of starch until a minimum was reached, after which there was a rapid increase. The initiation of growth in alfalfa in the spring, or that following any cutting, comes from the food material stored in the roots. In the process of photosynthesis the green portion of the plant manufactures carbohydrate, which is necessary in building the frame work of the plant, both root and top. As the plant approaches maturity, these materials are manufactured faster than they are utilized in top growth, the surplus being stored in the roots largely in the form of starch.

Cutting the alfalfa at 1/10, 1/2 or full bloom, did not prevent the plants from storing a large amount of starch for the winter. The alfalfa cut at 1/10 bloom decreased less in starch reserve after the cutting and started to restore starch sooner than alfalfa cut at later stages of growth. This can be explained by the fact that this alfalfa had a more rapid renewal of top-growth than that cut later. However, on November 16 they all had practically the same amount of starch reserve.

A gradual decrease of starch occurred during the fall, which is believed by some authorities to be due to a conversion of starch into sugar (sucrose) as colder weather approaches. This conversion must be kept in mind when studying the effects of a second cutting on the starch reserve. Thus, there can be two principle reasons for a decrease in starch in plants receiving a second cutting: (1) Conversion of starch into sugar, representing a physiological process directly associated with increased cold resistance, and (2) initiation of top growth. The latter one is governed by cultural practices and can be influenced by varying the date of the second cutting.

The difference in behavior of the second cutting treatments in 1939 and 1940 might be explanable when considering the minimum temperature records for September and October of those two years, as shown in figure 3. In 1939 the first killing frost was September 24, when the temperature dropped to 28°F. Cold weather following this frost materially retarded alfalfa growth. In 1940, the first killing frost was October 16, when the temperature dropped to 24°F. The number of days between a second cutting and first killing frost, probably explains why the August 17 cutting in 1940 was most critical from the standpoint of starch reserve. There was a lapse of 60 days between the second cutting and first killing frost, presumably allowing sufficient time for the alfalfa to make considerable top growth, using much of the starch that was reserved in the roots, but not having sufficient time to restore food in the form of starch. With the other second cuttings of both years, there was less growth after the cuttings, and the reserve starch was used only moderately.

The average date of first killing frost at the Chatham Station for the past fifteen years has been September 20. From the standpoint of first killing frost occurring September 24 in 1939 and October 16 in 1940, the 1939 results are probably more normal than those of 1940.

Yields from the second cuttings were not determined, but from observations it can be stated that it is impracticable to make second cuttings prior to September 15 for maximum forage yield. In previous years, the second cuttings were made about September 15, which according to our findings would not be detrimental to the alfalfa from the standpoint of starch reserve, causing rapid dying out of the alfalfa.

Freezing injury in the form of ray splitting was not found in any of the roots collected during the three years. The alfalfa was not subjected

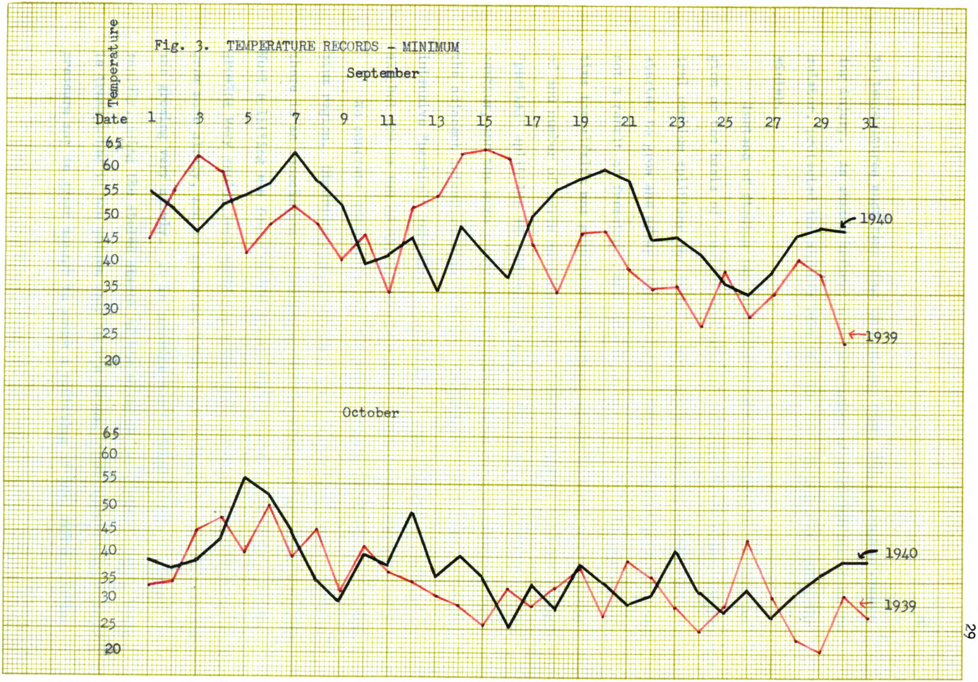

Fig. 3. TEMPERATURE RECORDS - MINIMUM

to temperatures much less than 32°F at any time, thus, escaping freezing injury. As stated before, a thick blanket of snow serving as an insulator, protected the alfalfa from extreme low temperatures during the winter.

Instead of finding ray splitting as would be expected in this region on the basis of previous experimental findings in Michigan, tangentral cambium splitting was found in the spring after the alfalfa had started to grow and after first cuttings, thereby indicating that it was not a result of freezing. Most splitting was found in roots during the time the alfalfa was growing most rapidly, reaching a peak in intensity of and number of roots splitting, when lowest in starch reserve. Slight "partial" splitting was generally found in the fascicular regions of the cambium, and the more intensified splitting involved the fascicular and the adjacent interfascicular regions. A number of those classified as intensive "partial" splitting involved all of the fascicular region and all but one or two of the interfascicular regions of the cambium.

No concrete explanation can be given for this splitting of the cambium region. Observing the pattern and time of splitting, it appears that there is an association of splitting with rapid growth and starch reserve. Most splitting was found in the spring during which time the alfalfa was growing very rapid, less splitting after first cuttings when the alfalfa grew more slowly, and no splitting after second cuttings when the alfalfa was growing very slowly. It is probable that alfalfa which is growing rapidly, using the stored starch of the roots in the form of sugar, which is transported in the phloem than in the xylem. This difference in osmatic pressure may be great enough to cause a splitting of the cambium tissue located between the two regions. The cambium being meristematic tissue, is probably very sensitive to pressure when cell division is most rapid, occurring simultaneously with rapid growth of the plant.

It is most probable that knitting of root tissue takes place in many of the roots not too severely injured, although evidence of knitting could not be found. It is quite evident that the rapidity of the alfalfa dying out at the Station is not in proportion to the amount of cambium splitting found during the seasons studied.

If the dying out of the alfalfa is due to cambium splitting, the 1941 results indicate that there is no critical period at which time second cuttings should not be made. This is substantiated by the fact that practically the same amount of splitting was found in roots the following spring from plants receiving no cutting, only one cutting, and two cuttings, with the latter one at early, medium and late dates in the fall.

It is highly probable that the cambium splitting is detrimental to the alfalfa to a certain extent, but the results obtained in this experiment are not sufficient to correlate it with the gradual dying out of the alfalfa.

SUMMARY

A study of cambium splitting and intensity of starch reserve was made of roots collected in 1939 from a two-year old stand of alfalfa, and in 1940 and 1941 from an adjacent field two and three years old, respectively.

First cuttings of alfalfa at 1/10, 1/2, and full bloom, did not prevent the plants from storing considerable amount of starch for the winter. The maximum starch reserve was practically the same for the different cuttings, but were reached at different dates.

A gradual decrease of starch reserve occurred in the fall, resulting in comparatively low content of starch by November 16.

The August 17 cutting in 1939 was less critical from the standpoint of starch reserve than that of 1940, when the first killing frost was September 24 and October 16, respectively.

Freezing injury was not found in any of the roots collected during the three years. The alfalfa was well protected from low temperatures during the winter months with a thick blanket of snow.

Splitting of the cambium region, varying in intensity and amount, was found in the alfalfa roots in the spring and after first cuttings, immediately following renewal of growth. Most splitting was found in roots practically devoid of starch.

There was no material difference in the amount and intensity of cambium splitting in roots from plants subjected to different dates of cutting.

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