AN ANALYSIS OF DIFFERENTIAL YIELDS IN ALFALFA (MEDICAGO SATIVA L.) WITH SPECIAL REFERENCE TO FACTORS AFFECTING NET PRODUCTION AND PHOTOSYNTHETIC ACTIVITY

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This is to certify that the

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AN ANALYSIS OF DIFFERENTIAL YIELDS IN ALFALFA
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ABSTRACT

AN ANALYSIS OF DIFFERENTIAL YIELDS IN ALFALFA (MEDICAGO SATIVA L.) WITH SPECIAL REFERENCE TO FACTORS AFFECTING NET PRODUCTION AND PHOTOSYNTHETIC ACTIVITY

by Frederick W. Fuess

Field and controlled-environment studies were conducted to determine the causes of differential production of alfalfa (*Medicago sativa* L.) under four cutting systems to determine specifically why alfalfa cut three times yields more than when cut twice per season. Net production, leaf area, leaf distribution and light interception were measured weekly and leaf area indexes and net assimilation rates were calculated. Net photosynthetic rates of excised alfalfa leaves from both the controlled-environment chamber and the field were measured in a Warburg respirometer.

Alfalfa cut three times per season yielded 0.77 ton more for an average of two years than when cut twice primarily due to a measured net leaf loss of 0.53 ton more in the two- than in the three-cutting treatment, accounting for two-thirds of the difference in total yield. The remaining one-third of the difference appears to have been due to increased rates of net photosynthesis in the leaves of the three-cutting treatment which were younger on the average than the leaves in the two-cutting treatment.

Frederick W. Fuess

Leaf area indexes (LAI) reached an average ceiling value of 5.16 during the first cutting. LAI did not serve as an adequate predictor of seasonal production but showed a positive relationship to yield for individual cuttings in the frequently-cut alfalfa.

Net photosynthetic activity in alfalfa decreased with age. Leaves more than three weeks old were less than one-seventh as active photosynthetically as five-day old leaves.

The Warburg respirometer appears precise enough to support the use of this method in determining comparative rates of photosynthetic activity in alfalfa leaves.

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A special note of gratitude is expressed to my wife, Marilyn, without whose moral, mental and financial support this study would not have been possible and to Frederick IV and Cynthia, our children, for their cooperation and devotion during the past three years.

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INTRODUCTION

The importance of alfalfa as a hay crop in Michigan and in the northeastern half of the United States has been well established. In Michigan, approximately 15% of the 10 million acres of cropland are utilized for the production of either alfalfa hay or an alfalfa-grass hay. In the United States, alfalfa hay occupies 8% of the total cropland. The amount of alfalfa hay produced per acre in Michigan varies from well over five tons per acre on the fertile, well-drained soils to less than one ton in the marginal agricultural areas.

Alfalfa management practices have undergone several changes in the past sixty years. During the early 1900's, it was believed that frequent cutting stimulated growth and increased yields. After the results of long-term studies reviewed by Graber et al. (10) showed in the 1920's that frequent cutting resulted in lowered yields in subsequent years, agronomists in the Great Lakes states recommended that alfalfa be cut twice during the season at the full bloom stage. With the advent of practical leafhopper control, more wilt-resistant and winter-hardy varieties, increased emphasis on quality, and shorter rotations, three harvests before September 1 have been recommended in the northern part of the Great Lakes area. Since the late 1950's, a few studies in the North Central states have indicated that a three-cutting system produces more hay than either a two- or four-cutting system for as long as three and possibly four years. Few attempts, however, have been made to determine the factors which are responsible for the yield differences except as the cutting treatment affects the yield in subsequent years.

Consequently the purpose of the studies reported herein has been to obtain fundamental information concerning the leaf production, light interception, net production, and photosynthetic efficiency of alfalfa (*Medicago sativa* L.) cut one, two, three, or four times per season in an attempt to answer the specific question: Why does a three-cutting system yield more hay than a two-cutting system over a period of three to four years?

REVIEW OF LITERATURE

Yields and Carbohydrate Reserves

During the first two decades of this century, alfalfa management systems were based primarily on empirical observations rather than scientific investigations. These early recommendations have been reviewed by Graber _et al_. (10). Since the late 1920's, many studies have been conducted to measure the effects of time and frequency of cutting on the yield and longevity of alfalfa stands with special emphasis on the effects of cutting treatments on the carbohydrate reserves and winter survival. An excellent review of these investigations from the early 1930's until 1950 has been presented by Willard (27).

Studies in the last 5 years have indicated that some of the recently-released varieties which combine wilt resistance and winter hardiness will withstand more frequent cutting than the less hardy, wilt-susceptible varieties in common use in the 1930's. Folkins _et al_. (9), working in Ontario, Canada, reported no significant decrease in yield in the third year after cutting a Vernal alfalfa-timothy stand three times before August 16. Over the three-year period, the three-cutting system produced more dry matter than either a two- or a four-cutting system.

A three-year study in Michigan (20) showed that Vernal alfalfa when top-dressed annually with 83 lbs. K yielded one ton more hay per acre per year when cut three times instead of twice a year with the last cutting being on September 1; the yield increase was only 0.4 ton when not top-dressed.

A study in Wisconsin (17) using two alfalfa varieties, two fertility levels, two levels of insect control, and five cutting treatments over a three-year period has shown that three harvests before September 1 produced 22% more dry matter and 49% more protein than a two-cutting system. The only treatment which favored the two-cutting system was the low fertility system with no insect control and differences were only evident in the third harvest year.

The carbohydrate reserves of roots of Vernal alfalfa not cut or subjected to a two- or three-cutting system were measured by Smith (16). A noticeable drop in the carbohydrate content of the roots was evident after each defoliation. By mid-November, however, the percentage of total available carbohydrates in the roots was approximately the same in all cutting treatments.

Leaf Area, Distribution and Light Interception

Watson (23) has suggested that the "measure of leaf area which is relevant to the comparison of agricultural yields, that is, of weights of different crops produced per acre of land, is the leaf area per unit of land, which it is proposed to be called the Leaf Area Index (LAI)". It has recently been pointed out by Watson (26) that total dry matter production varies with size, activity, and duration of the photosynthetic system. His data indicate that the size of the photosynthetic system is the greatest variable as indicated by the fact that varieties of potatoes and sugar beets with the highest mean leaf area produced the highest total yield.

Many investigators have reported leaf area indexes for various crops but no reports of leaf area measurements in alfalfa were found. Davison and Donald (5) measured the leaf area of subterranean clover planted at densities of from 1 to 50 plants per square link (63 square inches). Their results show that all swards tended toward a common leaf area index of 8.7 at the end of the growing season. Maximum dry matter production of the subterranean clover was reached when the leaf area index was between 4 and 5. In studies with white clover, Brougham (4) found that the LAI never exceeded 5.5. Donald and Black (7) reviewed the leaf area indexes of other crops.

An analysis of the apparent importance of leaves at different locations on the stem has shown that the total LAI may be an overestimation of the total photosynthetic area. Of the maximum LAI of 5.5 in a white clover sward, Brougham (4) found that only 60 percent of the leaves were fully-expanded, active leaves; the remaining 40 percent of the leaves were either young and folded or dead and dying. Senescense of lower leaves became evident approximately 35 days after defoliation when the leaf area index reached 4.

Studies with corn (13) show that the production of dry matter per unit of leaf area by the top, middle, and bottom leaves was of the ratio of 4 to 2.2 to 1. The study suggests that the low production of the lower leaves was due to a reduction in the light intensity from shading by the upper leaves. Similar results have been reported by Donald (8) who has stated that under a dense leaf canopy, the lower leaves may become parasitic.

The relationship between light interception and LAI as reported by Brougham (3) has shown that the growth rate of a legume-grass pasture increases until complete light interception at ground level is approached and thereafter growth remains at almost a constant rate. The leaf area index of 4 in clover and 7 in timothy, at which 95% light interception occurred, has been defined as the "critical LAI". This value appears to be similar to the "optimum LAI" as defined by Donald (8). Leaf areas tend to increase beyond this "optimum" level until the rate of death of old leaves equals the rate of appearance of new leaves which Donald has called the "ceiling LAI". He notes that plants continue to increase in size after the ceiling LAI is reached as the stems continue to elongate. Maximum yield is not reached until the net photosynthesis of the whole plant reaches zero.

Net Assimilation Rate

Studies of net assimilation rates of many crops have been presented by many investigators and the techniques used to measure the assimilation rates have varied. Early studies which attempted to correlate yield with morphological measurements were difficult to interpret because of variations in weather conditions during the growing season. A complete review of the literature concerning the early attempts to measure net assimilation rates is presented by Watson (25). Talling (19) reviewed the more recent studies with special emphasis on photosynthetic studies in the field. Only a few of the more pertinent investigations are presented herein.

In one of the earliest studies of assimilation, Thomas and Hill (21) measured the changes in CO_2 concentration above an alfalfa plot enclosed in a small glass chamber. Their data show that the assimilation rate of an alfalfa crop 48 inches tall was only twice as great as that of plants 6 to 8 inches tall despite the fact that the taller alfalfa had 3.6 times the leaf weight.

A mathematical approach to assimilation rates, originally suggested by Briggs et al. (2), was refined and expanded by Watson (25). He defined net assimilation rate (NAR) as the change in dry weight of the plants per unit area of leaf per day. In reality, the NAR as defined by Watson is a measure of photosynthetic efficiency rather than of growth.

One of the major errors cited in the calculation of NAR has been the incomplete recovery of the root system. However, differences in the NAR of wheat calculated from the total dry weight including the recovered roots and calculated from the dry weight of the shoots alone were small (24).

Studies of the assimilation rates of individual leaves have been conducted to determine the optimum conditions for maximum photosynthesis under the artificial conditions present in such a test. The temperature and light intensity at which isolated leaves have the greatest rate of apparent photosynthesis appears to vary with the species tested. Light intensities between 1,200 and 2,000 foot candles and temperatures between 20 and 30° C. have been recommended (1) (6).

The age of the leaf appears to have a greater effect on the photosynthetic rate than the chlorophyll content of the leaf. Moss (14) has reported that the yellowish-green corn leaves are as effective photosynthetically as dark green leaves of the same age. Pea leaves have been reported to reach their maximum photosynthetic rate when they are still folded and have not turned dark green (15).

PART I

EFFECTS OF CUTTING FREQUENCY ON YIELD, NET PRODUCTION, LEAF AREA, LEAF DISTRIBUTION, LIGHT INTERCEPTION AND NET ASSIMILATION RATE

Material and Methods

The experiments were conducted on a tile-drained Conover silt loam of high fertility. The sites used in 1961 and 1962 were located within 500 feet of each other and were part of the experimental fields of Michigan State University.

1961

A series of Vernal alfalfa plots in their third harvest year was used in 1961. These plots were seeded in 7-inch rows in April, 1959, with a grain drill equipped with band seeding tubes and were sprayed to control weeds. Two harvests were taken from the plots in both 1959 and 1960. The area was top-dressed with 13# of P and 72# of K per acre in April, 1961.

The following four cutting treatments were imposed with a self-propelled mower with a 3-foot cutting bar set to cut the forage to a 2-inch height:

Number of cuttings per season	Dates of cutting
One	August 30
Two	June 21, August 30
Three	May 31, July 12, August 30
Four	May 17, June 21, August 2,[a] August 30

[a]Cut July 26, 1962

A split-plot design with five replications was used in 1961. The four cutting treatments which occurred at random in each replication occupied the whole plots which were 6 by 68 feet in size. Each whole plot was divided into seventeen 4- by 6-foot sub-plots.

The sub-plots were not randomized but were harvested in consecutive order to facilitate the sampling and cutting of the experimental area. Each sub-plot was harvested only once during the season. Only those sub-plots which had not been previously sampled were cut with the mower.

At weekly intervals from May 10 to August 30, 1961, an area five rows wide (35 inches) by 36 inches was cut to ground level by hand. This sample was removed from the sub-plot leaving a border of one and one-half feet on each side and one foot on the harvested end of the plot. A sub-sample of from 12 to 20 stems was selected at random from the harvested material of two of the five replications. It was found that the leaf percentage and leaf area of the plots exhibited the greatest variability during early periods of regrowth. Therefore, the larger sub-samples were taken immediately after the cutting treatments were imposed.

The stems of the sub-sample were cut into 6-inch segments immediately after collection and placed in plastic bags in an insulated box. Stems were cut individually and the point of attachment of the petiole determined the 6-inch segment into which the leaf was placed. The plastic bags were stored in a cooler at 40° F for not more than three days. The remainder of the forage from the 35- by 36-inch sub-plots was placed in a paper bag and dried to a constant weight at 140° F. Yields are reported on an acre basis as tons of hay containing 12% moisture.

Leaf area and leaf distribution were determined by removing the leaflets from the petiolules and petioles with a pair of tweezers. The petiolules, petioles, and stems were grouped together and will hereafter be referred to as stems. An "Ozalid" print was made of the leaflets from one replication (Figure 1). The leaves and stems from each 6-inch segment were dried, weighed, and the dry weight ratio of leaves to stems determined.

The "Ozalid" prints of the leaflets were cut out, dried, and weighed. The weight of a known area of Ozalid paper was determined and the area:weight ratios of the leaves in each 6-inch segment was calculated. The leaf area index and leaf distribution of each sub-plot was determined for each of the five replications from the leaf:stem ratio and the area:weight ratio.

1962

The experimental area used in 1962 was band seeded with Vernal alfalfa on summer-fallowed ground on August 7, 1961, at a rate of

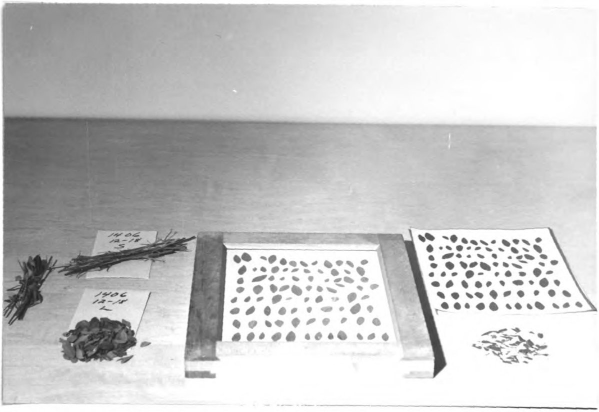


Fig. 1.--Ozalid print method used to determine leaf area in 1961 showing (left to right) the green leaves and stems, the printing frame, the developed print and the cut-out leaf prints (lower right).

11-1/2 pounds of seed per acre. A total of 140 pounds of phosphorus and 260 pounds of potassium per acre was applied in three applications before and at the time of seeding. The field was clipped with a tractor-drawn rotary mower on April 21, 1962, to remove the large weeds.

A split-plot design with four replications was used in 1962. The four cutting treatments comprised the whole plots and occurred at random in each replication. The whole plots, 12 by 20 feet in size, were each divided into 18 sub-plots, 12 by 6 feet in size. The larger size of both the whole plots and the sub-plots in 1962 afforded a border of 4-1/2 feet on each side and 3 feet on the harvested end of the 35- by 36-inch sampling area. The borders helped to prevent shading of the newly-cut whole plots by the uncut plots.

The sampling procedure in 1962 remained the same as in 1961. However, the number of stems selected as a sub-sample was decreased to 10 in those plots with growth of over 24 inches. The leaf area: weight ratio was determined for two replications rather than one as in 1961.

In 1962, the leaf area:weight ratio was determined by weighing leaf disks of known area. Forty-eight leaflets were selected at random from the leaves present in each 6-inch segment. The leaflets were stacked on a rubber stopper in groups of six and a disk 0.23 square centimeters in area was obtained with a sharpened, hollow steel rod from as near the center of the leaflets as possible (Figure 2) since a comparison of the "Ozalid" print and disk methods indicated that the disk method gave results which were consistently 2% below the print method if the disks were removed from the center of the leaflets. However, if the disks did not include the mid-vein of the leaf, the

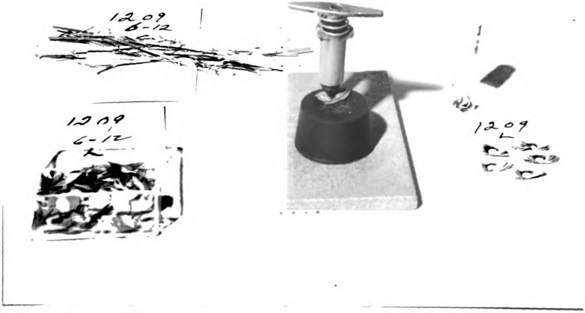


Fig. 2.--Leaf punch used to determine leaf area in 1962 showing (left to right) the green leaves and stems, the punch with leaflets in place, the leaf disks (upper right) and the sampled leaflets.

area tended to be overestimated by as much as 20% as compared with the print method.

Weekly samples were taken from May 3 to August 30, 1962. The cutting treatments imposed on the whole plots followed the same schedule as in 1961 with the exception that the date of the third harvest of the 4-cutting treatments was advanced to July 26, 1962. The plots were sprayed weekly with one pound of DDT per acre to control leafhoppers.

Light interception was measured using a Weston Model 756 illumination meter fitted with a quartz window. The photosensitive cell of the light meter was fitted into a small aluminum U-bar (Figure 3). The end of the U-bar was fitted with a small hardwood point to facilitate passage through the forage. A small, round bubble-type level was attached to the aluminum bar to insure horizontal placement of the photosensitive cell in the forage. An aluminum frame was constructed to hold the bar and photosensitive cell at 6-inch increments from the surface of the ground (Figure 4). The aluminum bar with the photosensitive cell in place was pushed between the rows of forage a distance of 2, 2-1/2, and 3 feet and light readings were taken at each distance. Readings were made between 11 a.m. and 1 p.m. on clear days.

The net assimilation rate (NAR) for each weekly interval was calculated using the following formula:

$$NAR = \frac{W_2 - W_1}{(t_2 - t_1)\ \dfrac{L_1 + L_2}{2}}$$

where W_1 and W_2 equal the dry weight of the forage and L_1 and L_2 the total leaf area at times t_1 and t_2 respectively. The NAR is expressed as grams of dry forage per square meter of leaf area per day (g/m^2/day).

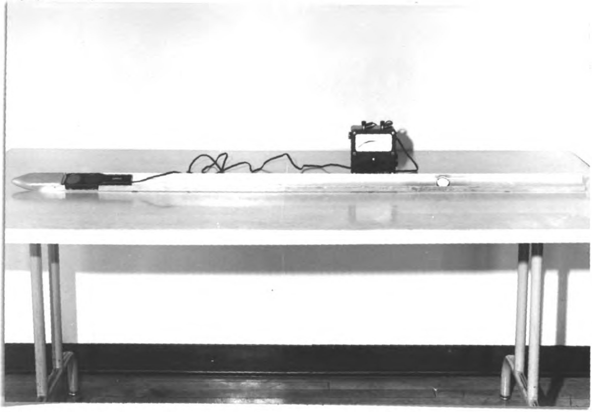


Fig. 3.--Aluminum U-bar probe used to measure light intensities in alfalfa showing the leveling bubble on the U-bar and the Weston 765 illumination meter.

Fig. 4.--Aluminum frame used to support the U-bar in a horizontal position at six-inch increments from the ground level.

Statistical significance was determined using Duncan's new multiple-range test (18). Where applicable the least significant ranges at the 5% level are reported for p = 2 (LSD) and p = the maximum numbers of entries (R).

Weather Conditions

The monthly precipitation in inches and deviation from normal recorded at the United States Weather Bureau station located approximately one mile from the experimental areas were as follows:

Month	1961		1962		Normal[a]
	Total	Deviation	Total	Deviation	
April	3.55	.73	1.23	-1.59	2.82
May	1.04	-2.72	1.82	-1.94	3.76
June	2.17	-1.21	3.42	.04	3.38
July	2.71	.01	2.41	- .29	2.70
August	3.97	1.13	1.85	- .99	2.84
Total	13.44	-2.06	10.73	-4.77	15.50

[a]47-year average.

Rainfall from the beginning of the growing season to the time of the last harvest date in 1961 and 1962 was 2.06 and 4.77 inches below normal, respectively. In 1961, below normal rainfall in May and June was partially compensated for by above normal rates in July and August. The 1962 season was very dry with below normal rainfall in every month except in June when precipitation was only 0.04 inches above normal. Near drought conditions were experienced in the latter part of July since more than one-half of the total monthly rainfall fell on July 3.

Experimental Results

Yield

The average yields from each harvest and the total yields of alfalfa managed under the four cutting treatments are shown in Table 1. Alfalfa cut three times was significantly higher in yield for the two years than any of the other cutting treatments. The yield of 5.37 tons of hay under the 3-cutting treatment was 0.77 tons greater than under the 2-cutting treatment and 0.63 tons greater than under the 4-cutting treatment. The 3-cutting system yielded much more--2.47 tons--than the plots cut only once on August 30.

There was no difference in yield between 1961 and 1962 in the 2- or the 3-cutting treatments. The 1-cutting treatment, however, produced 0.57 ton more hay in 1961 than in 1962. The difference in yield may have been due to the very dry season in 1962. Visual observations indicated that regrowth from crown buds was noticeably decreased and the limited amount of regrowth produced was not vigorous enough to penetrate the dense cover of mature forage in 1962.

The difference of 1.28 tons between the 1961 and 1962 yields in the 4-cutting treatment appears to have been caused by several factors. A severe infestation of leafhoppers in July and August of 1961 reduced the number of leaves and stunted the growth, especially during the last period of regrowth. The very warm spring of 1962 was responsible for at least a part of the increased yield in that year as approximately 40% of the total difference in yield of the 4-cutting treatment can be

TABLE 1.--Yield in tons of hay per acre and percent produced by each cutting of alfalfa harvested one, two, three, and four times per season in different locations

Number of cuttings per season	Date of cutting	1961		1962		Average	
		Tons	Percent	Tons	Percent	Tons	Percent
one	August 30	3.18 a[1]	100	2.61 a	100	2.90 a	100
two	June 21	3.57	77	3.15	69	3.36	73
	August 30	1.04	23	1.44	31	1.24	27
	Total	4.61 b		4.59 b		4.60 b	
three	May 31	2.40	44	2.62	49	2.51	47
	July 12	2.00	37	1.89	35	1.94	36
	August 30	1.00	19	.83	16	.92	17
	Total	5.40 c		5.34 c		5.37 c	
four	May 17	1.32	32	1.84	34	1.58	33
	June 21	1.25	30	1.56	29	1.40	30
	August 2[2]	1.02	25	1.20	23	1.11	24
	August 30	.51	13	.78	14	.65	13
	Total	4.10 b		5.38 c		4.74 b	

[1]Yields in same column with same letter are not different at 5% level.

[2]Harvested July 26, 1962.

accounted for in the first harvest on May 17.

The percentages of the total yield harvested at each cutting date were similar in 1962 and 1961. In all cases, the first harvest was responsible for the largest part of the total yield--73, 47, and 33% in the 2-, 3-, and 4-cutting treatments, respectively. Under the 4-cutting system, 87% of the total yield was in the first three cuttings and only 13% was produced in the last cutting.

Net Production

The net production curves (based on harvestable material) shown in Figures 5 and 6 were constructed from weekly production data collected from the 35- by 36-inch sub-plots. Since the data representing the period prior to the first defoliation of each cutting treatment were combined and averaged, all curves representing net production from the beginning of the season until the time of the first harvest are identical.

Except for minor differences in net production attributable to leafhopper damage in 1961 or dry weather in July and August in 1962, the general pattern was similar in the two years on the two different areas.

Accumulated net production per acre increased steadily under the 1-cutting treatments from the beginning of the growing season. Maximum yields of 3.92 tons on July 5, 1961 (Figure 5) and 3.80 tons on July 12, 1962 (Figure 6) were obtained. After these maximum levels were reached the tons of harvestable material produced per acre decreased. The more severe decline in harvestable material under the 1-cutting system during August of 1962 than in August of 1961 appears

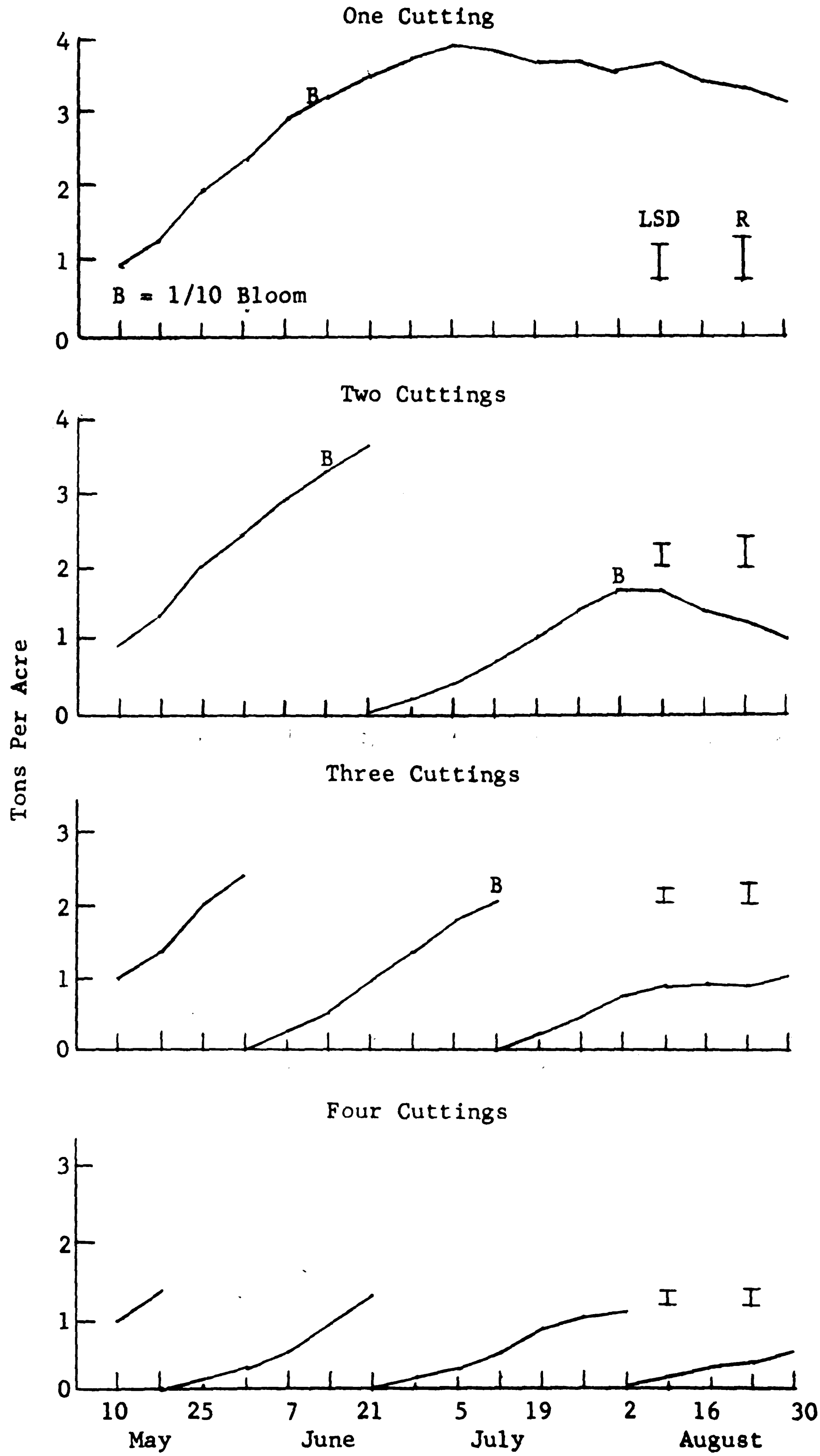


Fig. 5.--Tons of hay produced per acre by alfalfa cut one, two, three or four times per season. Weekly determinations, 1961. LSD and R at 5% level.

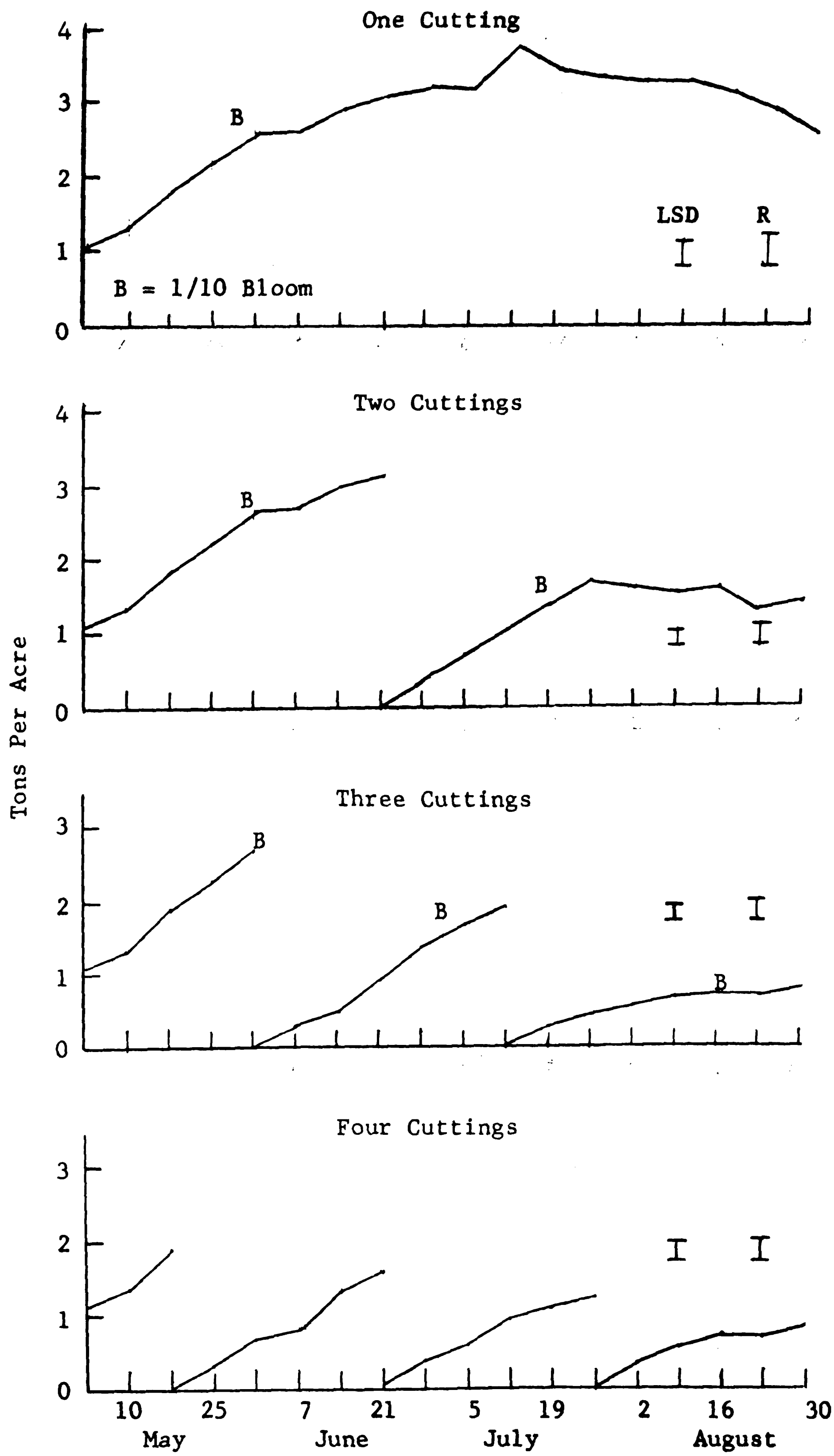


Fig. 6.--Tons of hay produced per acre by alfalfa cut one, two, three and four times per season. Weekly determinations, 1962. LSD and R at 5% level.

to have been due to the drouth conditions which existed. Since the first cutting in the 2-, 3-, and 4-cutting systems was made before maximum production was obtained, decreases in weekly yield were not evident during the period prior to the first defoliation.

In the 2-cutting treatment, production rates during the first six weeks of regrowth in 1961 and the first five weeks in 1962 closely paralleled the production curves representing the early part of the season. The amount of harvestable material produced under the 2-cutting system decreased in late August of both years. A severe leafhopper infestation in 1961 appears to have been partially responsible for the decrease in the production. In 1962, weekly applications of an insecticide were successful in controlling the leafhoppers yet there was an actual decrease in harvestable material near the end of the season because of drouth.

As the number of cuttings increased from two to four, the production rate in each successive regrowth period decreased. In contrast to the 1- and 2-cutting treatments, however, there was a gradual increase in production and at no time was there a decrease in production between one week and the next in the 3- and 4-cutting treatments. The dry weather in 1962 did not seriously affect the growth in the 3- or 4-cutting treatments.

Leaf Area Index

The ratio of leaf area (one surface only) to land area is expressed as the leaf area index (LAI). Weekly determinations of the LAI of alfalfa grown under four different cutting treatments are shown for 1961 in Figure 7 and for 1962 in Figure 8.

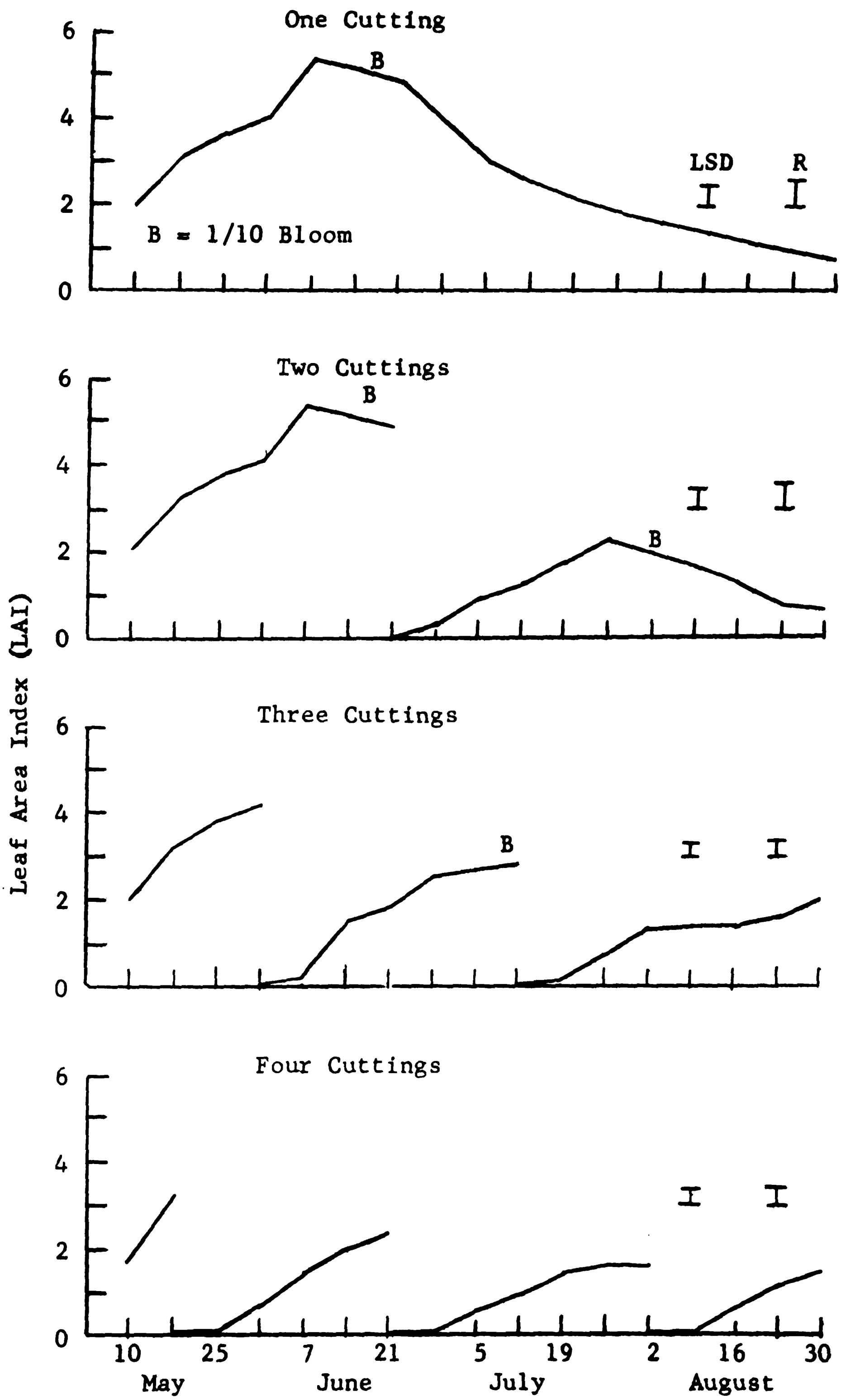


Fig. 7.--Leaf area indexes (LAI) of alfalfa cut one, two, three and four times per season. Weekly determinations, 1961. LSD and R at 5% level.

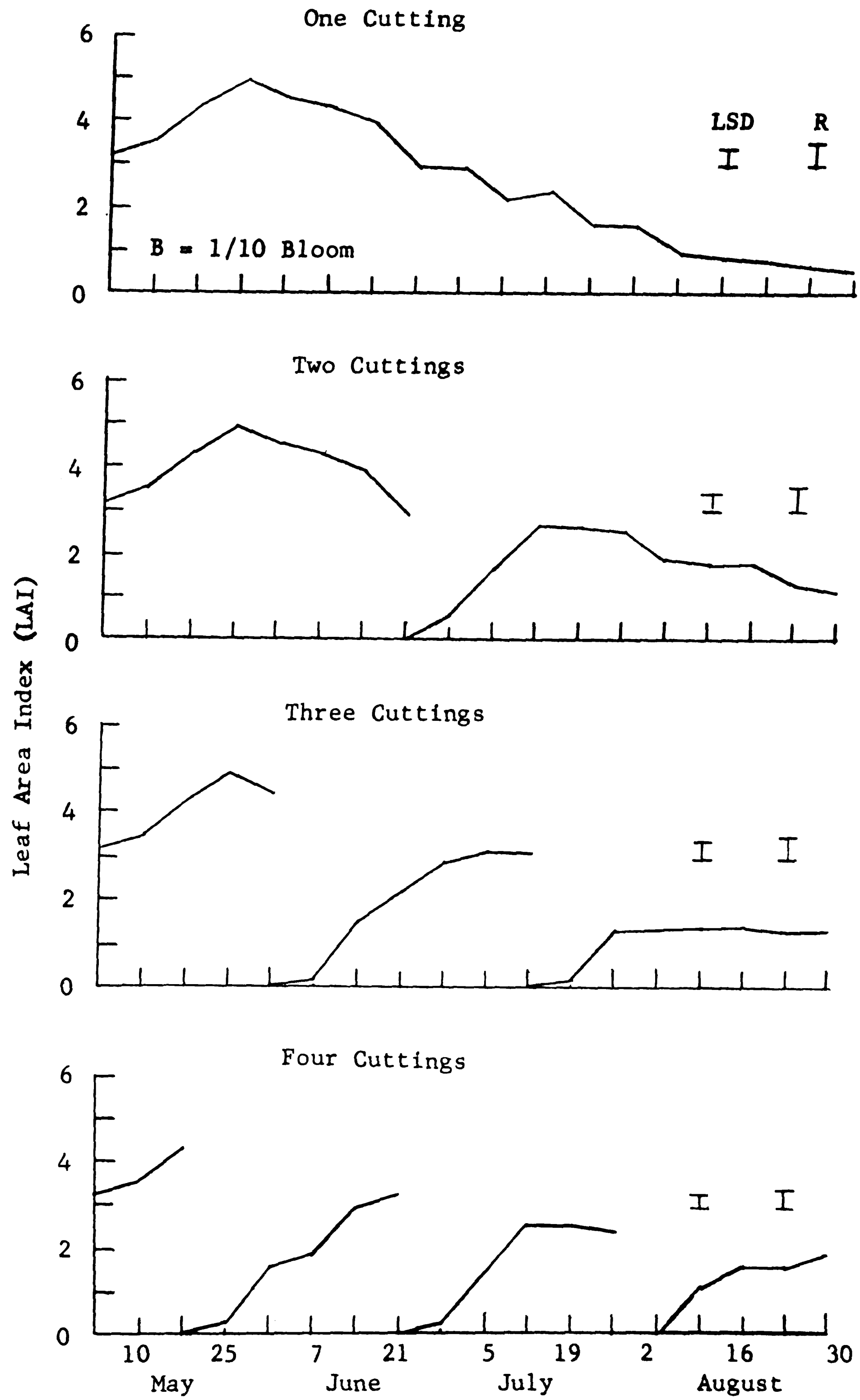


Fig. 8.--Leaf area indexes (LAI) of alfalfa cut one, two, three and four times per season. Weekly determinations, 1962. LSD and R at 5% level.

Leaf area, in the 1-cutting treatment, increased from the beginning of the season until June 7, 1961, when a ceiling LAI of 5.44 was reached. In 1962, unseasonably warm weather in early May hastened leaf development and the ceiling LAI of 4.89 was reached on May 25. After reaching the ceiling level in both years, the LAI of the 1-cutting treatment decreased gradually until the first harvest was removed on August 30. At this time, the LAI was similar for the two years--0.80 in 1961 and 0.62 in 1962. Abscission of leaves and a decreased rate of leaf formation as the plants approached maturity appear to have been responsible for these declines. The loss of dry weight which accompanied the decrease in LAI resulted in a decrease in net production during the latter portion of the season (Figures 5 and 6). Since the rates at which LAI decreased in 1961 and 1962 were similar, at least a part of the more pronounced decrease in harvestable material in 1962 than in 1961 was probably due to the dry weather conditions.

In the 2-cutting treatment, the LAI reached a ceiling level 2 and 4 weeks before the first cutting date in 1961 and 1962. The leaf area declined steadily from these ceiling levels until the first harvest on June 21. Since the ceiling LAI was reached two weeks earlier in 1962 than in 1961, a more noticeable decrease in leaf area occurred before the June 21 cutting date in 1962 than in 1961. After cutting, leaf area in the 2-cutting treatment increased in both years and reached fairly similar ceiling levels of 2.27 on July 26, 1961, and 2.60 on July 12, 1962. An adequate insect control program in 1962 tended to stabilize the LAI during July. Leaf area did decrease, however, during August of 1962 indicating that only a part of the decrease in August of 1961 was due to leafhopper damage. By August 30 when the 2-cutting treatment was harvested for the second time, the LAI had dropped to 0.68 in 1961 and 1.10 in 1962.

Except during the first harvest period of the 3-cutting system in 1962 when a ceiling LAI was reached on May 25, leaf area increased after each cutting and a significant decline in leaf area was not obtained under either the 3- or 4-cutting treatments in either year. The decreased rate at which LAI changed during the latter portion of each regrowth period indicated, however, that the ceiling LAI was being approached. As in the case of production, leaf area did not increase as rapidly and the maximum leaf areas indexes during each harvest decreased with each successive defoliation. In the 3-cutting treatment, the LAI curves were very similar in 1961 and 1962 except for the last two weeks in August when favorable precipitation in 1961 caused a greater increase in the LAI than in 1962.

Seasonal average leaf area indexes for the two years decreased from 2.60 to 2.12 to 1.62 as the number of defoliations per year increased from two to three to four (Table 2). The reduction of the LAI to zero after each defoliation and the relatively slow recovery during the first two weeks of regrowth were responsible for these low averages. The average leaf areas of the 1- and 2-cutting treatments were not significantly different despite the fact that the LAI of the 2-cutting system was reduced to zero after the first defoliation.

There appears to be a positive relationship between the average LAI for a single cutting period and the amount of forage produced during the same period. When two years are compared, the year with the cutting period having the highest LAI likewise had the highest yield.

TABLE 2.--Average leaf area indexes of alfalfa cut one, two, three, or four times per season

Number of cuttings per season	Date of cutting	1961	1962	Average
one	August 30	2.84 a[1]	2.34 b	2.59 a
two	June 21	4.07	3.92	4.00
	August 30	1.32	1.79	1.55
	Average	2.45 b	2.74 a	2.60 a
three	May 31	3.25	4.07	3.66
	July 12	1.91	2.14	2.03
	August 30	1.24	1.17	1.20
	Average	1.95 c	2.30 b	2.12 b
four	May 17	2.56	3.66	3.11
	June 21	1.29	1.97	1.63
	August 2[2]	1.01	1.81	1.41
	August 30	.82	1.23	1.02
	Average	1.23 d	2.00 c	1.62 c

[1]Areas in the same column with same letter are not different at 5% level.

[2]Harvested July 26, 1962.

Leaf Distribution

Leaf distribution as expressed by the percentage of total leaf area and the LAI in each 6-inch segment of the alfalfa stems is shown in Tables 3 and 4 for the two years. Only total leaf area was measured on the first two sampling dates in 1961 and after August 2 in the 1-cutting treatment in both years. In both years abscission of lower leaves started about three weeks before maximum LAI was reached. During these early periods, however, the rate of formation of new leaves exceeded the rate of leaf loss which resulted in increased total leaf areas from one week to the next. It is recognized that the changes in leaf distribution were in part due to stem elongation. Visual observations, however, indicated that the greatest cause of the decreasing leaf area in the lower portion of the stems was the abscission of lower leaves.

In both years, the percent of the leaf area and the LAI in each 6-inch segment of the lower portion of the stems dropped during May until on May 31 only 19 and 9% of the total leaf area was located in the lower 12 inches of the stems in 1961 and 1962, respectively. From May 31 until June 21 when the 2-cutting treatments were harvested for the first time, there was a continual loss of leaves in this area until only 2% in 1961 and 1% in 1962 of the total leaf area was in the lower 12 inches of the forage. Leaf area indexes in this lower area were correspondingly low and near zero. Decreases in the LAI of the 6-inch segments were less pronounced after June 21 due to the more uniform abscission of leaves from the entire stem.

A LAI of from 1.00 to over 2.00 was present in at least one of

TABLE 3.--Leaf area indexes and percentages of total leaf area located in 6-inch segments of alfalfa stems 1961

Number of cuttings per season	Six-inch segments	May										June							
		3		10		17		24		31		7		14		21		28	
		LAI	%	LAI	%	LAI	%	LAI	%	LAI	%	LAI	%	LAI	%	LAI	%	LAI	%
one	0-6	a		b		b		0.30	8	0.17	4	0.11	2	0.00	0	0.00	0	0.04	1
	6-12							0.94	25	0.62	15	0.49	9	0.05	1	0.10	2	0.00	0
	12-18							1.88	52	1.53	37	1.25	23	0.62	12	0.49	10	0.24	6
	18-24							0.64	17	1.81	44	2.07	38	1.91	37	1.37	28	0.96	24
	24-30											1.25	23	1.60	31	1.51	31	1.20	30
	30-36											0.27	5	0.77	15	0.98	20	0.72	18
	36-42													0.21	4	0.34	7	0.44	11
	> 42															0.10	2	0.40	10
	Total			1.96		3.16		3.76		4.13		5.44		5.16		4.89		4.00	
two	0-6							0.30	8	0.17	4	0.11	2	0.00	0	0.00	0	0.31	100
	6-12							0.94	25	0.62	15	0.49	9	0.05	1	0.10	2		
	12-18							1.88	50	1.53	37	1.25	23	0.62	12	0.49	10		
	18-24							0.64	17	1.81	44	2.07	38	1.91	37	1.37	28		
	24-30											1.25	23	1.60	31	1.51	31		
	30-36											0.27	5	0.77	15	0.98	20		
	36-42													0.21	4	0.34	7		
	> 42															0.10	2		
	Total			1.96		3.16		3.76		4.13		5.44		5.16		4.89		0.31	
three	0-6							0.30	8	0.17	4	0.15	100	0.88	60	0.24	13	0.10	4
	6-12							0.94	25	0.62	15			0.58	40	1.02	55	0.85	34
	12-18							1.88	50	1.53	37					0.59	32	1.55	62
	18-24							0.64	17	1.81	44								
	Total			1.96		3.16		3.76		4.13		0.15		1.46		1.85		2.50	
four	0-6							0.10	100	0.62	100	1.24	86	0.26	13	0.09	4	0.09	100
	6-12											0.20	14	1.09	54	0.71	31		
	12-18													0.67	33	1.23	54		
	18-24															0.25	11		
	Total			1.96		3.16		0.10		0.60		1.44		2.02		2.28		0.09	

TABLE 3--Continued

Number of cuttings per season	Six-inch segments	July 5 LAI	July 5 %	July 12 LAI	July 12 %	July 19 LAI	July 19 %	July 26 LAI	July 26 %	August 2 LAI	August 2 %	August 9 LAI	August 9 %	August 16 LAI	August 16 %	August 23 LAI	August 23 %	August 30 LAI	August 30 %
one	0-6	0.00	0	0.03	1	0.02	1	0.00	0	0.07	4	b		b		b		b	
	6-12	0.03	1	0.00	0	0.00	0	0.00	0	0.02	1								
	12-18	0.31	10	0.10	4	0.11	5	0.02	1	0.03	2								
	18-24	1.02	33	0.69	27	0.65	30	0.27	14	0.18	11								
	24-30	0.99	32	0.89	35	0.65	30	0.52	27	0.48	30								
	30-36	0.37	12	0.51	20	0.37	17	0.33	17	0.11	7								
	36-42	0.28	9	0.26	10	0.22	10	0.19	10	0.27	17								
	> 42	0.09	3	0.08	3	0.15	7	0.60	31	0.45	28								
	Total	3.09		2.56		2.17		1.93		1.61		1.40		1.20		1.00		0.80	
two	0-6	0.95	100	0.49	38	0.31	17	0.23	10	0.10	5	0.05	3	0.08	6	0.01	1	0.01	1
	6-12			0.76	59	0.99	55	1.25	55	1.02	51	0.91	52	0.67	51	0.41	49	0.25	37
	12-18			0.04	3	0.51	28	0.79	35	0.82	41	0.70	40	0.53	40	0.36	43	0.32	47
	18-24									0.06	3	0.09	5	0.04	3	0.05	6	0.09	14
	24-30															0.01	1	0.01	1
	30-36																		
	36-42																		
	> 42																		
	Total	0.95		1.29		1.81		2.27		2.00		1.75		1.32		0.84		0.68	
three	0-6	0.14	5	0.03	1	0.09	100	0.90	100	0.40	32	0.34	24	0.38	27	0.20	12	0.25	13
	6-12	0.70	26	0.42	15					0.85	67	0.87	62	1.01	71	1.10	68	1.18	60
	12-18	1.54	57	1.66	59					0.01	1	0.20	14	0.03	2	0.32	20	0.53	27
	18-24	0.33	12	0.70	25														
	Total	2.71		2.81		0.09		0.90		1.26		1.41		1.42		1.62		1.96	
four	0-6	0.53	100	0.50	54	0.35	26	0.41	25	0.27	17	0.09	100	0.68	100	0.63	58	0.30	21
	6-12			0.42	46	1.00	74	1.17	72	1.24	79					0.45	42	0.96	67
	12-18							0.05	3	0.06	4							0.17	12
	18-24																		
	Total	0.53		0.92		1.35		1.63		1.57		0.09		0.68		1.08		1.43	

[a]Not sampled in 1961.

[b]Only total leaf area determined.

TABLE 4.--Leaf area indexes and percentages of total leaf area located in 6-inch segments of alfalfa stems 1962

Number of cuttings per season	Six-inch segments	May										June							
		3		10		17		24		31		7		14		21		28	
		LAI	%	LAI	%	LAI	%	LAI	%	LAI	%	LAI	%	LAI	%	LAI	%	LAI	%
one	0-6	1.63	51	0.83	24	0.52	12	0.20	4	0.05	1	0.04	1	0.00	0	0.03	1	0.12	4
	6-12	1.56	49	2.48	72	0.99	23	0.68	14	0.36	8	0.30	7	0.15	4	0.00	0	0.03	1
	12-18			0.14	4	1.69	39	1.47	30	1.27	28	1.11	26	0.77	20	0.26	9	0.30	10
	18-24					1.12	26	1.71	35	1.58	35	1.49	35	1.43	37	0.96	33	1.07	35
	24-30							0.83	17	0.95	21	0.90	21	1.08	28	0.87	30	0.88	29
	30-36									0.32	7	0.34	8	0.35	9	0.41	14	0.37	12
	36-42											0.09	2	0.08	2	0.23	8	0.21	7
	> 42															0.15	5	0.06	2
	Total	3.19		3.45		4.32		4.89		4.53		4.27		3.86		2.91		3.04	
two	0-6	1.63	51	0.83	24	0.52	12	0.20	4	0.05	1	0.04	1	0.00	0	0.03	1	0.56	100
	6-12	1.56	49	2.48	72	0.99	23	0.68	14	0.36	8	0.30	7	0.15	4	0.00	0		
	12-18			0.14	4	1.69	39	1.47	30	1.27	28	1.11	26	0.77	20	0.26	9		
	18-24					1.12	26	1.71	35	1.58	35	1.49	35	1.43	37	0.96	33		
	24-30							0.83	17	0.95	21	0.90	21	1.08	28	0.87	30		
	30-36									0.32	7	0.34	8	0.35	9	0.41	14		
	36-42											0.09	2	0.08	2	0.23	8		
	> 42															0.15	5		
	Total	3.19		3.45		4.32		4.89		4.53		4.27		3.86		2.91		0.56	
three	0-6	1.63	51	0.83	24	0.52	12	0.20	4	0.05	1	0.15	100	1.56	100	0.55	25	0.32	11
	6-12	1.56	49	2.48	72	0.99	23	0.68	14	0.36	8					1.59	73	1.26	44
	12-18			0.14	4	1.69	39	1.47	30	1.27	28					0.04	2	1.17	41
	18-24					1.12	26	1.71	35	1.58	35							0.11	4
	24-30							0.83	17	0.95	21								
	30-36									0.32	7								
	Total	3.19		3.45		4.32		4.89		4.53		0.15		1.56		2.18		2.86	
four	0-6	1.63	51	0.83	24	0.52	12	0.27	100	1.63	100	0.93	49	0.66	23	0.51	16	0.26	100
	6-12	1.56	49	2.48	72	0.99	23					0.97	51	1.40	49	1.30	41		
	12-18			0.14	4	1.69	39							0.80	28	0.95	30		
	18-24					1.12	26									0.41	13		
	Total	3.19		3.45		4.32		0.27		1.63		1.90		2.87		3.17		0.26	

TABLE 4--Continued

Number of cuttings per season	Six-inch segments	July 5 LAI	July 5 %	July 12 LAI	July 12 %	July 19 LAI	July 19 %	July 26 LAI	July 26 %	August 2 LAI	August 2 %	August 9 LAI	August 9 %	August 16 LAI	August 16 %	August 23 LAI	August 23 %	August 30 LAI	August 30 %
one	0-6	0.05	2	0.00	0	0.00	0	0.05	3	0.00	0	a		a		a		a	
	6-12	0.07	3	0.05	2	0.02	1	0.06	4	0.08	8								
	12-18	0.23	10	0.12	5	0.34	22	0.23	14	0.23	24								
	18-24	0.58	26	0.21	9	0.40	26	0.14	9	0.19	20								
	24-30	0.67	30	0.60	25	0.30	19	0.23	14	0.16	17								
	30-36	0.29	13	0.71	30	0.20	13	0.23	14	0.12	12								
	36-42	0.23	10	0.43	18	0.11	7	0.18	11	0.11	11								
	> 42	0.15	6	0.26	11	0.14	12	0.50	31	0.08	8								
	Total	2.27		2.38		1.51		1.62		0.97		0.87		0.79		0.70		0.62	
two	0-6	0.93	56	0.62	24	0.26	10	0.13	5	0.02	1	0.00	0	0.00	0	0.00	0	0.00	0
	6-12	0.76	44	1.38	53	1.04	40	0.73	29	0.30	16	0.20	11	0.23	13	0.14	10	0.09	8
	12-18			0.60	23	1.17	45	1.36	54	0.82	43	1.04	58	1.13	63	0.92	68	0.69	63
	18-24					0.13	5	0.30	12	0.76	40	0.56	31	0.43	24	0.30	22	0.32	29
	24-30																		
	30-36																		
	36-42																		
	> 42																		
	Total	1.69		2.60		2.60		2.52		1.90		1.80		1.79		1.36		1.10	
three	0-6	0.27	8	0.13	4	0.13	100	1.28	100	0.77	58	0.69	52	0.56	39	0.59	45	0.27	20
	6-12	1.06	35	1.39	45					0.56	42	0.64	48	0.88	61	0.72	55	1.08	80
	12-18	1.18	39	1.41	46														
	18-24	0.51	17	0.15	5														
	24-30																		
	30-36																		
	Total	3.02		3.08		0.13		1.28		1.33		1.33		1.44		1.31		1.35	
four	0-6	1.42	96	0.61	25	0.45	18	0.12	5	0.13	100	1.07	100	1.44	89	1.32	84	1.22	68
	6-12	0.06	4	1.71	70	1.56	63	1.24	52					0.18	11	0.25	16	0.57	32
	12-18			0.12	5	0.47	19	1.03	43										
	18-24																		
	Total	1.48		2.44		2.48		2.39		0.13		1.07		1.62		1.57		1.79	

[a]Total leaf area only determined.

the 6-inch segments of the 1-cutting treatments from the beginning of the growing season until July 5. In general, these larger leaf area indexes were located between 18 and 30 inches from the base of the stems and represented over 30% of the total leaf area.

Rapid decreases in the percent leaf area and the LAI in the lower 6 inches of the stem were evident in the 2-cutting system from June 21 to August 30. By August 2 only 5% of the total leaf area in 1961 and 1% in 1962 was located in the lower 6 inches of the forage. The higher total leaf area indexes in 1962 resulted in a complete absence of leaves in the lower 6 inches of forage which was heavily shaded.

The relatively short regrowth periods in the 3- and 4-cutting treatments and the relatively low leaf area indexes accounted for a higher percent of the leaves in the lower portion of the stems. In these two treatments, especially in the 4-cutting system, cuttings were made before most of the lower leaves became senescent. Furthermore, higher light intensities reaching the lower leaves as shown in Figure 9 favored increased leaf retention as cutting frequency increased.

Light Interception

The average percent light interception at 0, 6, and 12 inches from ground level is shown in Figure 9 for the 1962 year. Since light readings were taken only on clear days, data obtained during the period three days prior to and three days after the weekly sampling dates were averaged and recorded as the average percent light interception for sampling date. Light readings 6 and 12 inches above ground level are not presented for the 1-cutting treatment after June 21 since lodging

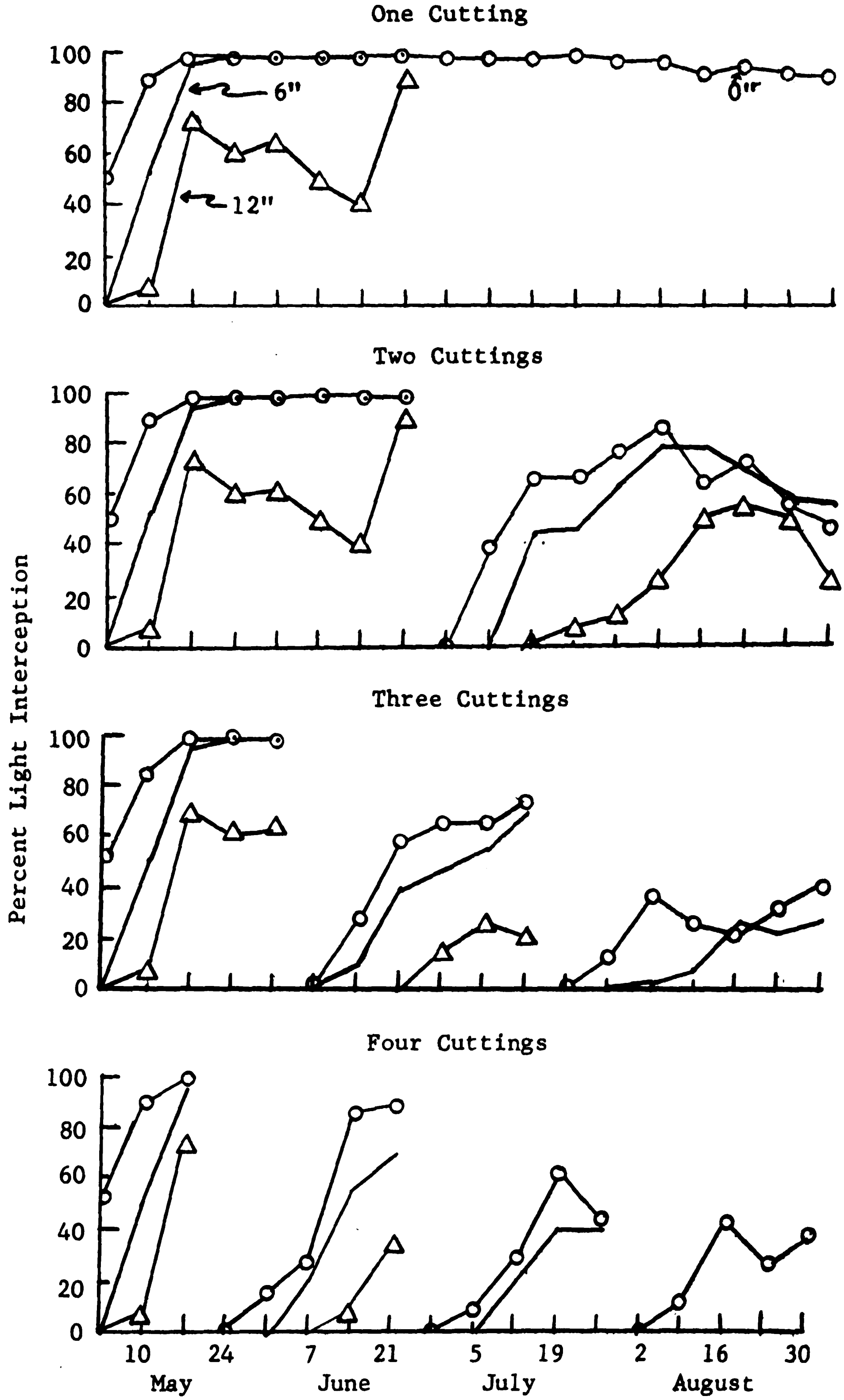


Fig. 9.--Percentage light interception 0, 6 and 12 inches from ground level in alfalfa cut one, two, three and four times per season. Weekly determinations, 1962.

and dense growth made passage of the photosensitive cell of the light meter through the forage impossible without disturbing the natural arrangement of the foliage.

The percentage of light interception at ground level in the 1-cutting treatment was 95% on May 17 and remained high during the remainder of the growing season. There were practically no leaves (12% of total) in the 0-6 inch segment on May 17 and only 4 and 1% of the total leaves were in this lower area on May 24 and 31. Under the 2-, 3-, and 4-cutting systems, light interception at ground level was negligible during the first week of regrowth but increased rapidly during the second and third week of regrowth. Increases in the percent of light interception after the first five weeks of regrowth were generally slight as evidenced by the tendency of the percent light interception to remain relatively stable during the latter portion of each regrowth period in the 2- and 3-cutting treatments. Except for the last regrowth period of the 3- and 4-cutting treatments, light interception at ground level exceeded 50% during some part of each of the regrowth period. Light interception never exceeded 90% after the first cutting was removed in the 2-, 3- and 4-cutting treatments. Decreases in the percent light interception at ground level in the 2-cutting treatments after August 2 were due to decreasing leaf areas which allowed more light to penetrate.

Light interception 6 inches above ground level closely followed the pattern of the ground level readings during the early part of the season. In the 2- and 3-cutting treatments, only 3% of the ambient light reached the 6-inch level from May 24 to the time the first cutting treatment was imposed on May 31 and June 21, respectively. Apparent decreases in the light interception with advancing age appear to have been due primarily to decreasing leaf areas and secondarily to high leaf densities in the area where light readings were made. This latter situation resulted in the shading of the photosensitive cell which caused an overestimation of the percent light interception in some cases.

During the early part of the season, light interception at the 12-inch level fluctuated greatly. At least a part of this fluctuation was due to lodging during the middle of May. As the alfalfa regained its normal upright habit of growth, light interception increased. After the removal of the first hay crop, light interception at the 12-inch level never exceeded 60% in the 2-cutting treatments.

Net Assimilation Rates

Net assimilation rates are presented as a relative measure of the leaf efficiency and are expressed as the grams of dry forage produced per square meter of leaf area per day (Table 5). Data are presented only for those periods when yield and leaf area measurements were available for two consecutive weeks. Since the leaf area remaining after each defoliation was not measured (because of inaccuracies in measuring small amounts of leaves) until the foliage had been allowed to grow for one week, net assimilation rates are not presented for the week following defoliation.

Net assimilation rates remained relatively high during the month of May in the uncut plots but by mid-June there was a noticeable decrease in the NAR. This decrease continued until in early July when the NAR of the 1-cutting treatment dropped below zero. The loss of lower leaves and small branches during this period accounted for a

TABLE 5.--Net assimilation rates in grams of dry forage per square meter of leaf area per day of alfalfa under four cutting treatments

Weekly Interval	1961				1962			
	Number of cuttings				Number of cuttings			
	One	Two	Three	Four	One	Two	Three	Four
May 3-10	a	a	a	a	2.24	2.24	2.24	2.24
May 10-17	4.02	4.02	4.02	4.02[b]	3.60	3.60	3.60	3.60
May 17-24	5.62	5.62	5.62	--	2.44	2.44	2.44	--
May 24-31	2.93	2.93	2.93	11.86	2.30	2.30	2.30	9.24
May 31-June 7	3.16	3.16	--	6.40	0.01	0.01	--	2.11
June 7-14	1.62	1.62	8.31	5.52	2.35	2.35	7.59	6.12
June 14-21	1.36	1.36	6.50	4.99	1.62	1.62	6.32	2.57
June 21-28	1.38	--	5.01	--	0.83	--	4.65	--
June 28-July 5	1.52	8.38	5.20	10.10	- .13	7.87	2.57	7.47
July 5-12	- .79	6.83	2.11	7.21	7.33	4.21	2.40	4.57
July 12-19	-1.56	7.04	--	7.63	-4.99	3.68	--	2.31
July 19-26	0.64	4.94	9.62	3.49	-1.47	3.08	7.10	1.13
July 26-Aug. 2	-2.92	2.97	7.85	0.69	-1.70	-0.26	2.47	--
Aug. 2-9	3.10	.24	2.52	--	+0.44	-1.33	1.67	10.99
Aug. 9-16	-5.68	-5.28	2.22	8.19	-5.30	1.19	1.88	3.01
Aug. 16-23	-3.29	-5.83	-0.70	2.54	-9.91	-4.35	-0.08	-0.68
Aug. 23-30	-4.96	-6.33	0.77	3.53	-12.23	1.43	2.69	1.99
Average	0.38	1.98	3.87	4.76	-0.74	1.77	2.93	3.86

[a]Data not obtained until second weekly interval.

[b]Indicates cutting date expressed by last date in same line.

--Indicates no data because of minimal growth.

considerable decrease in the dry weight from week to week. These losses combined with lower average leaf areas resulted in high negative net assimilation rates. These negative rates were responsible for the low average NAR of 0.38 in 1961 and -0.78 in 1962 in the 1-cutting treatments.

The NAR of the 2-cutting treatment showed some decline before the first harvest on June 21 but it was high for approximately five weeks after defoliation. During the month of August, net assimilation rates dropped below zero. Despite the negative NAR in the latter part of the season and an assumed rate of zero during the week immediately after cutting, the average NAR under the 2-cutting system was 1.98 in 1961 and 1.77 in 1962.

Net assimilation rates under the 3- and 4-cutting treatments remained high during most of the growing season. There was a noticeable decline in net assimilation rate during the last two weeks of the last cutting of the 3-cutting treatment in both 1961 and 1962. The average NAR of the 3-cutting system differed considerably in the two years (3.87 and 2.93) and the rates were higher than in the 2-cutting treatments in both years. Considerably higher average rates are reported for the 4-cutting treatments (4.76 and 3.86) and again the differences between the average rates in 1961 and 1962 were large.

Two apparent deviations in the 1962 results should be mentioned. The very low NAR in the 1-, 2-, and 3-cutting treatments reported during the weeks of May 31 appear to have been due to very dry conditions during which the increase in weight was slight. On the other hand, the NAR of 7.33 in the 1-cutting treatment reported during the week of July 5 may have been due to increased growth rates caused by very heavy rains in early July.

The very high net assimilation rates during the second week after cutting in all treatments probably were the result of new growth being produced at the expense of stored carbohydrates and also to high photosynthetic rates of young leaves (Part II). Since only the above-ground portion of the plants was harvested, depletion in root weight was not measured. It is assumed that by the end of the third week after defoliation when the alfalfa had reached a height of approximately 12 inches, production of forage based on leaf area was valid.

PART II

PHOTOSYNTHETIC STUDIES OF EXCISED LEAVES IN A WARBURG RESPIROMETER

Materials and Methods

The net photosynthetic rates of leaves both from the field and a controlled-environment chamber were determined. Attempts to compare leaves located near the top and near the bottom of individual alfalfa stems growing in the field proved extremely difficult. Diseases and insects usually damaged the lower leaves before sufficient plant height was attained. A limited number of healthy leaves from field plots was tested on June 12, 1962. Most of the material used, however, was grown in a controlled-environment chamber.

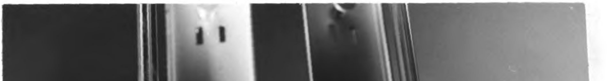
During the winter of 1961-62, approximately 200 clonal alfalfa cuttings were rooted. The parent plant was selected at random from a field of Vernal alfalfa. Three or four rooted cuttings were placed in each of thirty-six 7-inch pots filled with field soil obtained from an old alfalfa field. The pots were divided into six groups of six pots each and each group was cut every six or seven weeks during the summer and fall of 1962. The plants were fertilized regularly with a complete nutrient solution to maintain normal growth.

In December, 1962, five pots of the most productive plants were selected from each group, fertilized immediately after cutting with granular fertilizer at a rate of 22 pounds P and 123 pounds K per acre and placed in the controlled-environment chamber. During the period in the chamber, the plants in each group of five pots were cut at 6-week intervals. Immediately after cutting, the plants were re-fertilized. The pots were watered and rotated daily. Temperature of 50° F. at night and 70° F. during the day were maintained during the growing period. Light was supplied for the 14-hour photoperiod by a bank of both incandescent and fluorescent bulbs which delivered a light intensity of approximately 3,000 foot candles at the surface of the pots. The pots were grouped as closely as possible so that only 10% of the ambient light reached the surface of the pots when maximum growth was attained.

Stems containing leaves to be sampled were cut at ground level and placed in a vial of water in an insulated box for transportation to the laboratory. Each stem was measured and the location of the leaflets to be tested recorded. Leaflets were separated by making a cut as close as possible to the junction of the leaflet and the petiolule or petiole. The age of the leaves was determined by tagging the petiole of the growing leaf as soon as the leaf was completely unfolded.

Net photosynthetic rates were determined in a modified Warburg respirometer. A two-piece flask, approximately 50 milliliters in size, was designed to accommodate large leaflets (Figure 10). Two small plastic bars were attached with small bolts to the top of the flask. The bars were fitted with U-shaped wires which were held in place by small notches at the ends of the bars. A small square of acetate was placed between the wires to support small leaflets and to hold the larger leaflets parallel to the light source. Flasks were calibrated with Brodie solution.

Fig. 10.--Modified Warburg flasks showing the method used to attach the leaflets (upper photograph) and the assembled flask attached to the manometers (lower photograph).



Light was supplied by a series of 300-watt incandescent bulbs located below the plastic bottom of the water bath. A 0- to 75-ohm slide-wire resistor was inserted into the electrical circuit at the base of each light socket. These resistors made it possible to adjust the current to each bulb so that the light intensity at the position of the leaflet was 1,800 foot candles as measured with a Weston Model 765 illumination meter equipped with a quartz window. A variable voltage transformer was inserted in the main circuit to permit reduction of the light intensity of the bulbs on one-half of the apparatus.

To insure an adequate supply of carbon dioxide, a buffer solution was used to provide a carbon dioxide concentration of approximately 15% (calculated) in the leaf environment. The buffer solution consisted of 4 grams of potassium bicarbonate ($KHCO_3$) dissolved in 45 milliliters of a saturated solution of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) and 5 milliliters of distilled water. Three milliliters of the buffer were placed in the bottom of each flask. After coating the ground glass surfaces with lanolin to insure a perfect seal, the bottoms of the flasks were attached to the tops with rubber bands.

After allowing the flasks to equilibrate in the water bath with the lights on for a period of 15 minutes, manometric readings were recorded every 10 minutes for a period of one hour. At the end of each run, the leaflets were printed on "Ozalid" paper, dried and weighed. The net photosynthetic rates were determined on both an area and a dry weight basis using standard methods of calculation (22).

All three leaflets of a trifoliate leaf were used whenever possible, but only the two lateral leaflets were used when the size of the individual leaflets prohibited the use of the entire trifoliate leaf. Sample size varied from 10 to 20 milligrams of dry weight which was equivalent to 3 and 5 square centimeters of leaf area.

Net photosynthetic rates are reported as the net microliters of oxygen evolved per hour per square centimeter of leaf area. Rates were calculated initially on both an area and a weight basis. Some variations in the relative rates were evident when rates were calculated on a weight basis. An increase in the area:weight ratio as the leaves were shaded for longer periods appears to have been responsible for some of these variations. Since leaf area rather than leaf weight had been used as a basis for all other calculations, it appeared that net assimilation rates based on leaf area would be more consistent.

Attempts to measure respiration rates proved futile as changes in manometer readings were slight during the one hour test period. Respiration rates, which are normally considered to be low (12) were, therefore, ignored and net photosynthetic rates (photosynthesis minus respiration), are reported.

Results and Discussion of Net Photosynthetic Studies

Exploratory Investigations

Preliminary investigations were conducted to determine the precision of the Warburg respirometer as a means of determining net photosynthetic rates of alfalfa leaves. Since the two lateral leaflets of the trifoliate leaf of alfalfa are similar in size, shape and age, it was assumed that the net photosynthetic rates of these paired lateral leaflets would be more nearly identical than of a lateral leaflet and the terminal leaflet. A comparison of the net photosynthetic rates of paired lateral leaflets showed that the rates did not differ significantly at a temperature of 20° C. and a light intensity of 1800 foot candles. Measurements of the net photosynthetic rates of paired lateral leaflets at light intensities of 1800, 1350, 900, and 600 foot candles showed a reduction in the average rate of spproximately 20% when the light intensity was reduced to 1350 foot candles. Light intensities of 900 and 600 foot candles caused reduction of 38% and 60%, respectively, as compared to 1800 foot candles. These investigations indicated that the leaves were not light saturated at 1350 foot candles when incandescent lights were used. Lack of data at light intensities higher than 1800 foot candles made it impossible to determine if light saturation occurred at 1800 foot candles. Since, however, all reported data were obtained at 1800 foot candles, the net photosynthetic rates of different leaves are assumed to be comparable.

Initial studies in August 1962 using leaves collected from the field indicated that the net photosynthetic rate of individual leaves of the same age was inversely correlated with the distance of the leaf from the crown. To test this apparent relationship, the net photosynthetic rates of a series of leaves grown in the controlled-environment chamber were measured. Stems, ranging from 3 to 36 inches in length, were selected from material which had been defoliated 2 to 6 weeks previously. A statistical analysis of the net photosynthetic rates of the youngest fully unfolded leaf (as near the same age as it was possible to determine), from each stem showed no correlation between the distance from the crown and the net photosynthetic rate. This was contradictory to the results obtained from the field. The dry conditions in the field in 1962 at the time of sampling may have been responsible for these contradictory results. The short stems obtained from the field in August 1962 had a smaller total leaf area and may not have been subjected to as great a water stress as the leaves on the longer stems with larger leaf areas.

Further analysis of these same data showed that leaf size per se did not influence photosynthetic rates. The net photosynthetic rates of the small, ovate leaflets on the 3-inch stems from the controlled-environment chamber were not significantly different from the larger, more elongated leaflets of the same age from the stems 36 inches long. These studies of leaves grown under the closely-controlled environment, then, showed that in alfalfa (1) net photosynthetic rates of leaves are not a function of distance from the ground level and (2) photosynthetic activity is independent of leaf size.

Principal Investigations

In an attempt to determine the photosynthetic contribution of leaves from different locations on the stems, the net photosynthetic rates of leaves from different locations on a series of 17 stems were determined. Stems ranging from 19 to 40 inches in length were selected from material in the controlled-environment chamber. The stems were selected at random from plants which had been allowed to grow under conditions which shaded the lower leaves for a period of from 37 to 40 days in order to simulate an alfalfa stand under field conditions.

The net photosynthetic rates and the relative position of the leaves on the stems are shown in Figure 11. In all stems, with the exception of numbers 1, 2, 3, and 14, where the rates of the two uppermost leaves were approximately equal, net photosynthetic rates decreased as the distance from the apex of the stem increased. Stem number 13

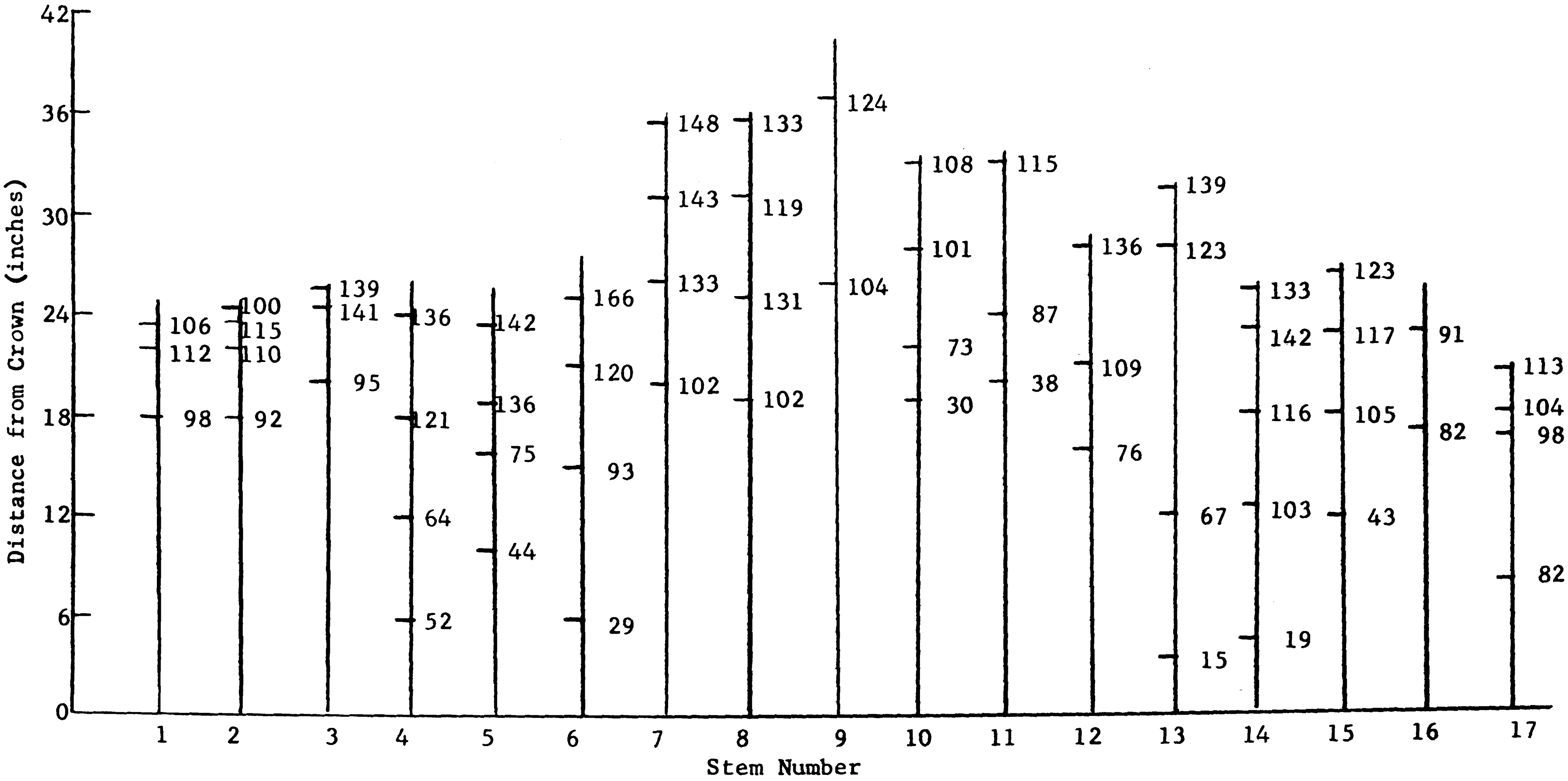


Fig. 11.--Net photosynthetic rates ($\mu l\ O_2/hr/cm^2$) of leaves from different locations on stems grown in the controlled-environment chamber for six weeks. 1962.

showed the greatest range of net photosynthetic rates with the upper leaf (139) nine times as active photosynthetically as the lower leaf (15). Similar results were found in stems number 6 and 14 where the net photosynthetic rates of the upper leaves were six and seven times greater than that of the lower leaves.

The results from Figure 11 were summarized by averaging the net photosynthetic rates of the leaves from each 1/4 of the total length of the stem (Table 6). The number of leaves from each section of the stem varied due to errors in technique or abscission of the lower leaves. It is very striking that the average net photosynthetic rates decreased progressively from the top (125) to the bottom (29) of the stems. This difference of the magnitude of 4 to 1 indicates that the youngest leaves had the highest net photosynthetic rates. Although the lowest leaves appeared healthy and turgid, their pale-green color indicated that the chlorophyll content may have been lower than that of the dark-green leaves in the upper part of the stems.

TABLE 6.--Net photosynthetic rates of leaves of alfalfa located in different sections of the stem[1]

Source of stms	Section of stem, % distance from bottom	Number of observations	Average net photosynthetic rates
Controlled-environment chamber	76-100	17	125 a[2]
	51-75	15	99 b
	26-50	7	71 c
	0-25	4	29 d
Field, June 12, 1962	51-100	5	137 a
	0-50	5	113 b

[1]19 to 40 inches long from controlled-environment chamber 21 to 24 inches long from field.

[2]Rates from the same source and in the same column with the same letter are not different at 5% level.

Although the determination of the net photosynthetic rates of upper and lower leaves grown under field conditions was hindered by early abscission of the lower leaves in August, 1962, five stems 21 to 24 inches long were selected from a field of first cutting Vernal alfalfa on June 12, 1963. Because of an abnormally late spring, leaf retention was greater at lower levels of the alfalfa plants than at a similar date in other years. The stems were selected on the basis of the presence of healthy, disease-free leaves in the lower half of the stems. The net photosynthetic rates and the location of the individual leaves in the stems are shown in Figure 12. The younger leaves from the upper portion of the 21- to 24-inch stems had a significantly higher net photosynthetic rate (137) than the older leaves from the lower half of the stem (113) as shown in Table 6. These data indicate that dramatic reductions of the net photosynthetic rate of alfalfa leaves with increasing age, as shown in the case of the alfalfa grown in the controlled-environment chamber, also occur under field conditions.

To test further this apparent relationship between the age of the leaf and the net photosynthetic rate, determinations were made using leaves of precisely-known ages (Table 7) since the leaves were tagged when unfolding. Although there was no significant difference in the average net photosynthetic rates measured 5 and 12 days after unfolding (119 and 121), significant and marked decreases in the net photosynthetic rates occurred as the days since unfolding increased from 12 to 28. The leaves which had been unfolded for 12 days had an average net photosynthetic rate which was more than seven times greater than that of the leaves which had been unfolded 28 days (121 to 17). The sharp decrease in the average net photosynthetic rates between 22

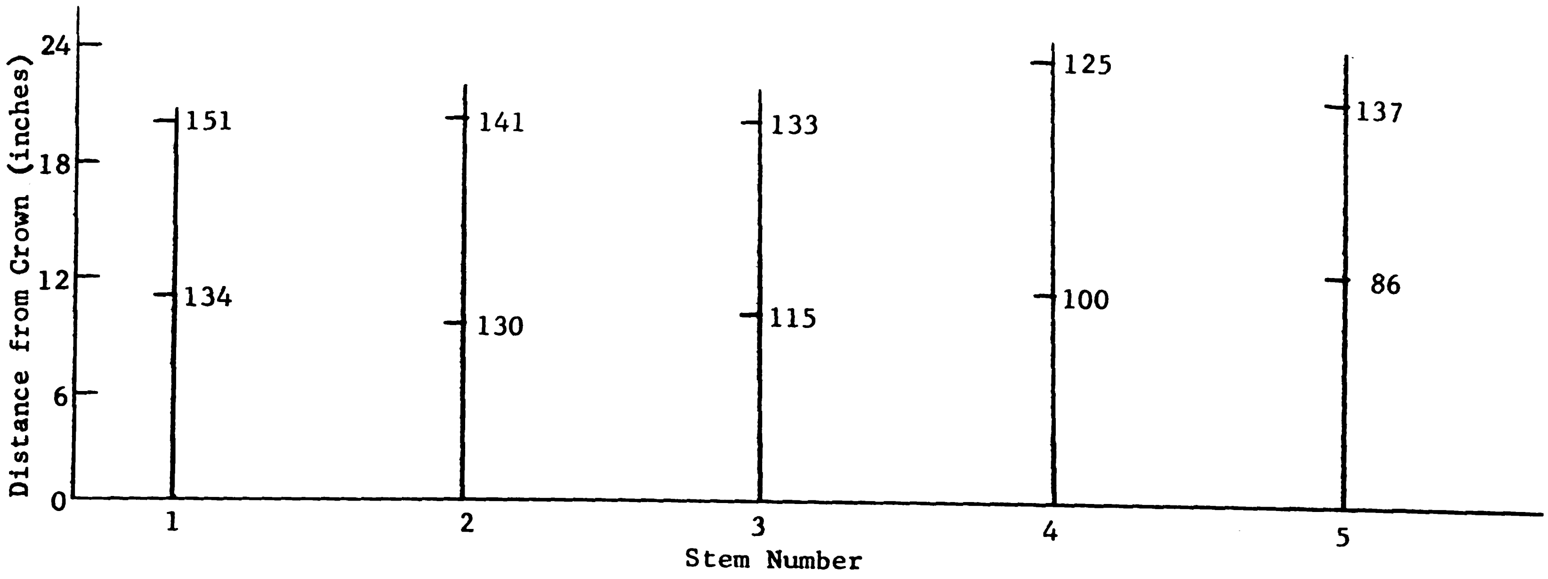


Fig. 12.--Net photosynthetic rates ($\mu l\ O_2/hr/cm^2$) of leaves from different locations on stems from a first cutting stand of alfalfa, June 12, 1961.

and 28 days (80 to 17) appears to have been due to a rapidly accelerated aging of the leaves.

TABLE 7.--Net photosynthetic rates of leaves of different ages of alfalfa grown in a controlled-environment chamber

Days since unfolding	Number of leaves	Net photosynthetic rate
5	4	119 a[1]
12	7	121 a
17	6	112 b
22	6	80 c
28	2	17 d

[1]Rates in same column with the same letter are not different at 5% level.

DISCUSSION OF RESULTS

The objectives of the studies reported herein were to determine the effects of one, two, three and four cuttings on the production and photosynthetic area and efficiency of alfalfa in an attempt to answer specifically why alfalfa cut three times per season (9) (17) (20) yields more than when cut twice for a period of three or four years. The effects of frequent defoliation on root reserves and the longevity of alfalfa stand were not studied. Effects of cutting treatments were intentionally measured for only one season at each of the two locations to prevent the residual effect of previous treatments from influencing yields and other data. Carbohydrate reserves were not measured since it has been well established from previous studies (10) (11) that increased frequency of cutting decreases carbohydrate reserves and causes stand depletion even after the first harvest year. The stand may be vigorous enough for three or four years for near maximum yields under a high fertility program, however, even though it has been partially depleted by frequent cutting.

Since alfalfa yield is a product of photosynthesis, it appeared likely that the average size of the photosynthetic area would be one of the most important factors which determined crop yield. Experimental results presented herein have shown that the average photosynthetic surface (LAI) of alfalfa during the growing season is not, in itself, a suitable index of crop production. The highest 2-year average yields of alfalfa were produced under a 3-cutting system which had an average LAI of 2.12. Significantly lower yields were produced under both the 1- and 2-cutting systems in which the average leaf area indexes were higher--2.59 and 2.60, respectively. There did, however, appear to be a positive relationship between the average leaf area values for shorter periods of time--e.g., the five to seven week period between harvests in the 3- and 4-cutting treatments--and the tons of hay produced. Higher average leaf area indexes during harvest periods were associated with higher yields both within treatments and between years.

Leaf abscission was evident after the forage had reached a height of about 12 inches. The rapid rate of leaf formation during the early part of each regrowth period, however, resulted in an increase in the LAI from one week to the next. Approximately one week before the 1/10 bloom stage was reached maximum leaf area indexes were attained and from that time until the end of the growing period, the decreased rate of leaf formation together with an increased rate of leaf abscission resulted in a decrease in the LAI from one week to the next. In order to get an estimation of leaf loss, determinations were made based on the average weight:area ratio of the leaves in the 6- to 24-inch segments. Loss of leaves resulted in losses of harvestable material equivalent to 409 pounds of hay per acre per unit of leaf area lost. Under the 2-cutting treatment in 1962, a decrease of 1.98 units of leaf area during May and June resulted in a loss equivalent to 810 pounds of hay. The decrease of 1.50 units in the same treatment during August was equivalent to 614 pounds of hay. Thus, under the 2-cutting treatment, 1424 pounds of high quality hay was lost during the growing season due to reductions in LAI alone. Decreases in LAI in the 3-cutting treatments during the 1962 growing season of 0.45 units amounted to a loss of harvestable material equivalent to 184 pounds of hay--1240 pounds less than the 2-cutting treatments. Similar computations for 1961 showed a difference in leaf loss of 875 pounds, averaging 1057 pounds or 0.53 ton more of leaf loss per year under the 2- than under the 3-cutting system. Much of the yield difference favoring the 3- over the 2-cutting treatment is attributable to these decreases in LAI due to leaf loss.

Newly-released insect- and disease-resistant varieties may serve as one method of reducing the rate of loss of older leaves. Natural leaf loss, however, due to senescence presents an entirely different problem. Controlled-environment studies under disease-free conditions in this study indicated that natural abscission of lower leaves occurred five or six weeks after defoliation. Maturation and reduced light intensities appeared to be responsible for at least a part of this natural leaf loss.

Percent light interception at ground level, as in the case of leaf area, was not a good measure of productivity. In the 1-cutting treatment, 95% visible light was intercepted by the forage during all but the first two weeks of the growing season. The yield of the 1-cutting treatment in 1962, however, was less than one-half as large as the 4-cutting treatment in which more than 50% of the ambient light was not intercepted during 2/3 of the growing season. The lack of dry matter production under conditions of high light interception may have been due to the very small photosynthetic area of the plants in the 1-cutting treatments during the latter part of the growing season. Stems which were brown and lignified and infected with black stem disease

(*Ascochyta imperfecta*) had little photosynthetic capacity but were responsible for much of the light interception.

The net production curves, leaf area indexes, percent light interception at ground level and net assimilation rates for the 1962 season are summarized in Figures 13 and 14. Under field conditions, peak light interception occurred early in the spring. Approximately one week after 95% light interception at ground level was attained on May 17, leaf area reached the ceiling level. Production per acre continued to increase for several weeks after the leaf area attained its maximum even though considerable production was lost due to leaf loss. Increases in production after the ceiling LAI was reached appear to have been due to stem elongation, new leaf formation, lignin and cellulose deposition, and seed formation. During the periods of initial regrowth in the 2-, 3- and 4- cutting treatments, leaf area indexes rose sharply but tended to stabilize or even decrease before maximum production levels were reached. After the first defoliation of the 2-, 3- and 4-cutting treatments, light interception never reached 95% and, during most of the periods of regrowth, was below 50%.

Net assimilation rates were generally higher in the 3- than in the 2- cutting treatment which partially explained the higher total yield under the 3-cutting system. Marked decreases in the net assimilation rates during the latter part of each of the cutting periods of the 2-cutting system appear to have been the result of the loss of harvestable material and decreased net photosynthetic rates of the older leaves.

Net assimilation rates could not be related to production

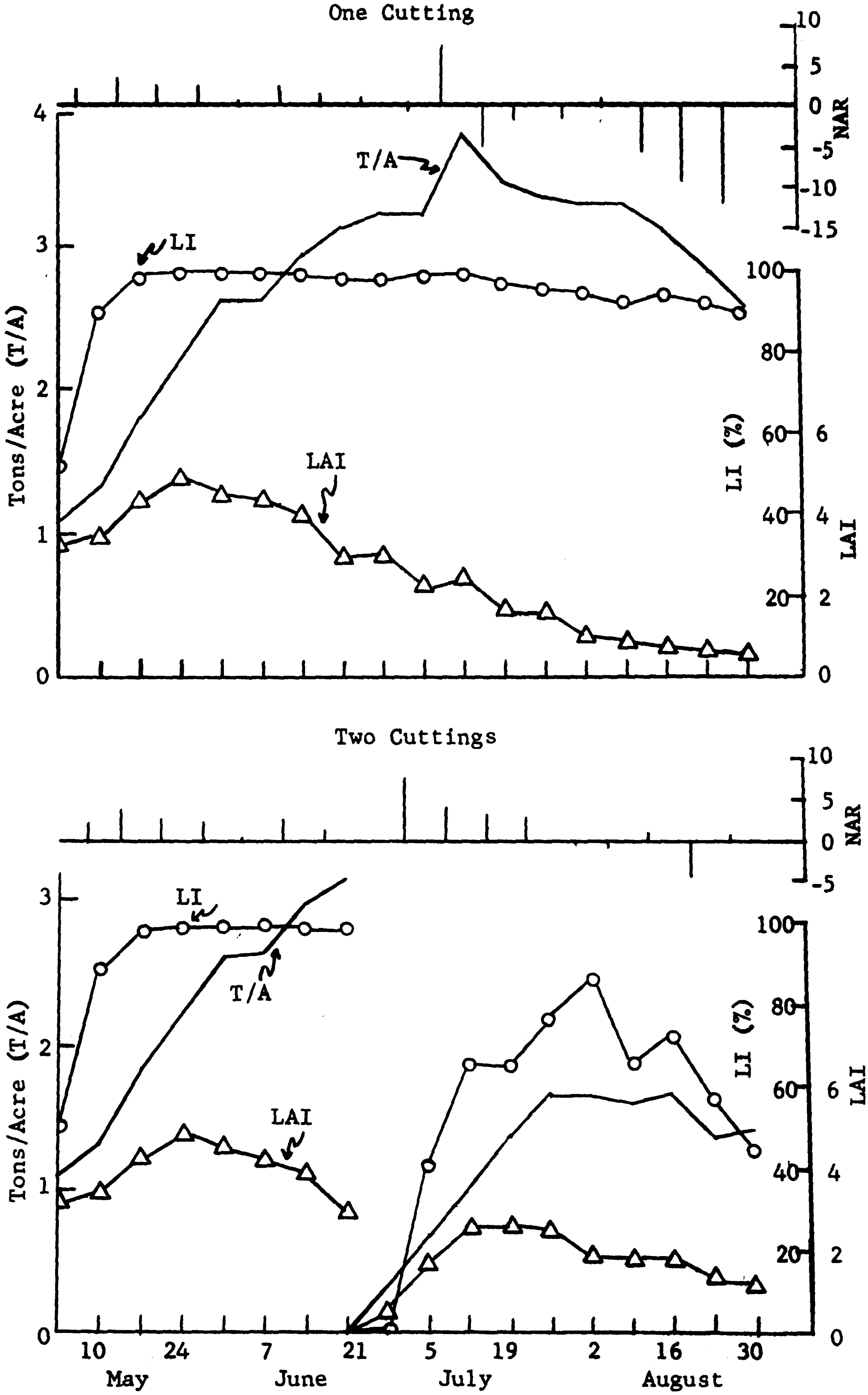


Fig. 13.--Percentage light interception at ground level (LI%), leaf area indexes (LAI), tons of hay per acre (T/A) and net assimilation rates (NAR) of alfalfa cut one or two times per season. Weekly determinations, 1962.

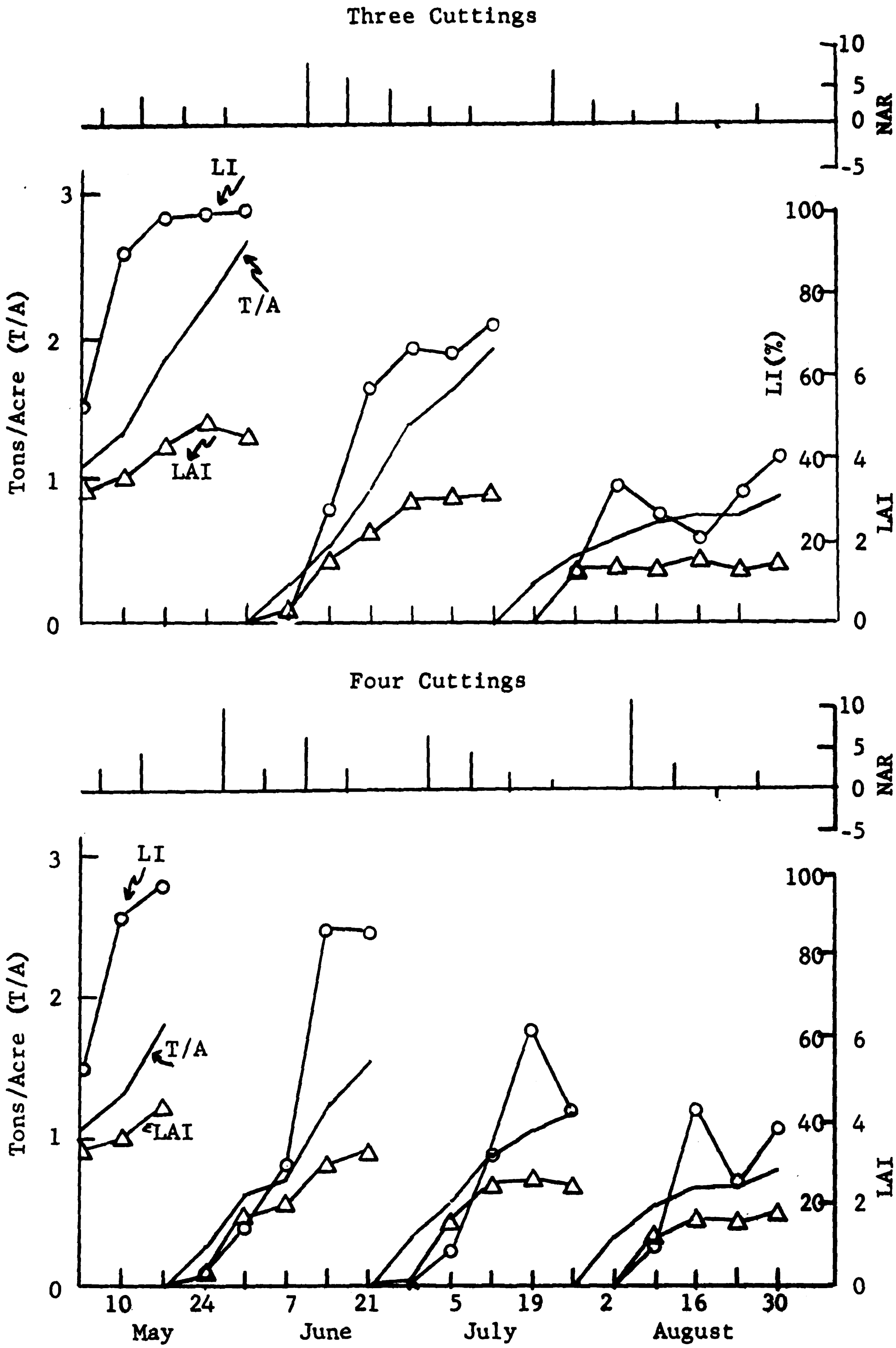


Fig. 14.--Percentage light interception at ground level (LI%), leaf area indexes (LAI), tons of hay per acre (T/A) and net assimilation rates (NAR) of alfalfa cut three or four times per season. Weekly determinations, 1962.

especially in the 1- and 4-cutting treatments. Losses of harvestable material and low average leaf areas during the latter portion of the growing season resulted in a negative average NAR in the 1-cutting treatment. The very much higher net assimilation rates under the 4-cutting treatment were probably due to a depletion of the carbohydrate root reserves rather than higher photosynthetic rates alone, especially during the second week after each defoliation.

Some of the inaccuracies in determining NAR might have been eliminated if root weights had been determined and total leaf production recovered. The practicality, however, of recovering even a major portion of the roots and dead leaves of a perennial crop such as alfalfa seems questionable under field conditions.

The data throw considerable light on the question: "Why does alfalfa yield more under a 3- than a 2-cutting system for a three or four year period?". When alfalfa was defoliated every six or seven weeks under a 3-cutting system LAI seldom reached the ceiling level, leaf abscission occurred at a low rate, and net production was high. Under the 3-cutting system leaf abscission seldom exceeded the rate of formation of new leaves and the amount of harvestable material never decreased from one week to the next. Since net production always continued to increase under the 3-cutting system, higher yields were obtained than in the 2-cutting treatments in which rates were reduced during the latter part of the first harvest period and gradually decreased during the latter part of the second harvest period.

The reduced net production which was obtained during the latter part of each of the two harvest periods in the 2-cutting system was due not only to excessive leaf loss in the lower 12 inches but probably to a decrease in the photosynthetic efficiency of the aging lower leaves. The data in Part II of this study show that the net photosynthetic rate of young leaves of alfalfa grown in a controlled-environment chamber is similar to that of leaves two weeks old but after the third week, the decline in the net photosynthetic activity is marked, being only about one-seventh as great as that of leaves five days old. Limited field data which indicated a decrease in photosynthetic efficiency with increasing age support the data from the controlled-environment chamber. Has the leaf of alfalfa fulfilled its function of food manufacture one month after emergence, then to become a storage organ for a short period of a week or two before abscission? If it is to be harvested after it has completed the major portion of its function as a photosynthetic organ, the harvest schedule would have to be one in which forage is not cut so often as to seriously reduce carbohydrate reserves and reduce longevity of stand but frequently enough, say every six or seven weeks as in the 3-cutting system, to prevent leaf loss and maintain a high level of photosynthetic activity in the leaves rather than infrequently as in the 2-cutting system. This information supports the recommendations to farmers that a 3-cutting system is sound as based on recent data (9) (17) (20) and data in this study which show that under a 3-cutting system it is possible to produce more hay of better quality than under a 2-cutting system.

SUMMARY

Investigations were conducted to determine the causes of differential production of alfalfa under 1-, 2-, 3-, and 4-cutting systems in order to answer specifically why alfalfa cut three times per season yields more than when cut twice for a period of three or four years. Field experiments were conducted at two locations in successive years. Net production, leaf area, leaf distribution and light interception were measured weekly; leaf area indexes and net assimilation rates were calculated. Net photosynthetic rates of excised alfalfa leaves from both a controlled-environment chamber and the field were measured in a Warburg respirometer.

These studies produced the following conclusions:

1. Higher yields of alfalfa were obtained from the 3-cutting than from either the 1- or 2-cutting systems in both years. The two-year average yields were 2.90, 4.60, 5.37 and 4.74 tons per acre when harvested one, two, three or four times, respectively.
2. Leaf losses were evident and light interception at ground level was 50% four weeks before 1/10 bloom in the first cutting. LAI was approximately 3.16 and the plants were 12 to 18 inches tall at this time. Leaf area increased until an average ceiling LAI of 5.16 was obtained one week before 1/10 bloom when the forage was approximately 24 inches tall. The LAI of the 1-cutting treatment decreased to a low of 0.71 on August 30. Under the 2-, 3- and

4-cutting treatments the average LAI decreased with each successive cutting.

3. Decreases in LAI during the growing season were responsible for average losses of 4.46, 2.81, 0.25, and 0.00 units of leaf area under the 1-, 2-, 3- and 4-cutting treatments, respectively.
4. Gradual decreases in harvestable material from mid-July until August 30 resulted in much lower yields in the 1-cutting treatment than in any of the other treatments.
5. Net assimilation rates were higher in the 3- than in the 2-cutting systems. Relative differences in average net assimilation rates among the four cutting treatments indicated that net assimilation rates were not closely related to production in an erect perennial forage crop such as alfalfa where leaf losses and changes in root weights can not be readily measured under field conditions.
6. There were practically no leaves present in areas of the stem where less than 5% of the ambient light penetrated. Interception of 95% of the light by the low amount of leaves and high amount of woody stems during most of the growing season in the 1-cutting treatment indicated high light utilization but production was low under these treatments. As the number of defoliations increased, average percent light interception decreased.
7. Leaves from both the field and the controlled-environment chamber showed a marked reduction in net photosynthetic rates with age. Leaves more than three weeks old were less than one-seventh as active photosynthetically as five-day old leaves from the controlled-environment chamber.
8. Measurements of net photosynthetic rates with the Warburg

respirometer appear to be precise enough to support the use of this method in determining comparative rates of photosynthetic activity in individual leaves of alfalfa.

9. Alfalfa cut three times per season yielded 0.77 ton more for an average of two years than when cut twice primarily due to a measured net leaf loss of 0.53 ton more in the 2- than in the 3-cutting treatment, accounting for two-thirds of the difference in total yield. The remaining one-third of the difference in production appears to have been due to increased rates of net photosynthesis in the leaves of the 3-cutting treatment which were younger on the average than the leaves in the 2-cutting treatment.

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