EFFECTS OF SEEDLING YEAR MANAGEMENT TREATMENTS ON STANDS AND YIELDS OF MEDIUM RED CLOVER, *TRIFOLIUM PRATENSE* L.

by
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SECTION I. INTRODUCTION

Red clover is an important plant in the agriculture of Maryland. Seeded with timothy, it occupies a major portion of that part of the state's cropland acreage which is used for the production of hay. Being a legume, it is important as a source of nitrogen in the soil, in addition to improving the quality of the hay crop; and being a biennial, it fits exceptionally well into the commonly used three-year rotation of corn, small grain, and hay.

It is obvious, therefore, that failures of red clover stands such as occurred in 1947 and 1948, can cause serious losses to the farmers of the state. This study was undertaken because of these losses, and deals principally with the management practices used in handling the stands through the seedling year, especially after combining of the small grain crop.

SECTION II. LITERATURE REVIEW

Red clover was introduced into the United States over 200 years ago, but until recently, very little scientific work has been done on management practices for seedling stands. Pieters (6), in a U.S.D.A. Farmers Bulletin on red clover culture published in 1921, devotes only the following short paragraph to the subject:

> When seeded with a grain nurse crop no special treatment is given the clover the first season. It develops in the stubble after the grain has been cut and occasionally may afford some pasturage in the fall. If the late summer be especially favorable sufficient growth may be made for a cutting of hay, and in some cases a crop of seed has been secured. The stand, however, is likely to be injured by the cutting, and it is usually best to clip back the growth to check the development of the plants. When seeded in the fall in corn or with rape one or two crops may be expected the next season. It is usually not advisable to pasture spring seedlings the same season with sheep or hogs, as they are likely to injure the young plants. Pasturing with cattle is less injurious.

In a further discussion of red clover as pasture, he advises against close early pasturing and also against too close grazing in the fall, stating that:

> The plants should rather be allowed to go into the winter with some growth upon the crowns in order to prevent winterkilling and also to enable them to store up material in the roots for an early vigorous growth the following spring.

As reasons for clover sickness or clover failure he cites soil exhaustion, improper methods of seeding, nurse crops, etc., unfit seed, diseases, and improper treatment the first autumn.

Fergus (1), in 1931, makes no mention of seedling year management practices in his analysis of clover failure in Kentucky. He considered winterkilling to be the principal cause of failure and stated that "Attempting to grow unadapted clovers, therefore, is the fundamental cause of clover failure in Kentucky". As other factors, he listed soil deficiencies, parasitic diseases, and leaf-hopper injury.

Hollowell (4), in 1934, gave essentially the same reasons for red clover failures, namely, unfavorable soil conditions, unadapted or poor seed, poor methods of seeding, diseases and insects, and improper management. He suggested that the clover should make four to six inches of growth before cold weather but that blooming in the fall of the seedling year should be prevented by grazing or high clipping.

Thatcher, Willard, and Lewis (8), in 1937, recommended low clipping during the first summer to control weeds. They stated that since the weeds recover from stem buds, while the red clover recovers from crown buds, the lower the growth is clipped, the better the weeds will be controlled without injury to the clover. They also suggested removing the clippings if they are heavy enough to injure the stand.

In discussing the effect of clipping on root reserves and root systems they stated that red clover was definitely injured by clipping after September 1, but the yield the following year was usually somewhat greater following late August clipping than when it was not cut at all. Their experiments also showed that allowing red clover to produce seed the first year did not necessarily cause failure of the stand. They considered that failures which do follow this practice are due to the late cutting which is generally involved, and which does not allow sufficient time for the plants to replenish the root reserves necessary for winter survival.

They also found that red clover could be clipped twice during the first summer without injury, although they did not recommend the practice.

Willard and Lewis (12), in 1947, reported on several years of observations and experiments on management of first year stands in Ohio where the combine had been used to harvest the small grain. In 1938, questionnaires sent to county agents in western Ohio revealed that there was considerable difference of opinion as to whether combined straw left on the field was beneficial or detrimental to the clover stands.

Of informal experiments conducted during 1939, 1940, and 1941, they wrote: "Cutting the stubble immediately after combining and removing straw, stubble, hay, and weeds never resulted in damage, contrary to the alleged 'protective' effect of the straw on legume seedlings". Their observations indicated that higher yields of better quality hay were obtained from plots which had been clipped and the material removed. They also observed that clover in the clipped plots withstood a severe dry spell better than that in areas from which the straw was not removed.

Nine different management treatments were studied from 1943 to 1945 and the results substantiated the previous observations. In addition, it was found that in late August of the first year, a second cutting could be made, which yielded about 1500 pounds of clean clover hay per acre, and did not injure the stand.

On the basis of these observations and experiments, Willard, Thatcher, Dodd, and Jones (13) recommend that after combining, the stubble should be clipped low and all material removed. If excessive growth develops during the summer, it should be removed for hay by September 1. Following binder harvest, they recommended clipping once about the middle of August. They also suggest pasturing the new seedlings in early summer if the stock are removed as soon as the growth is grazed down.

Greathouse and Stuart (2) noted the importance of building up and maintaining a high level of reserves, especially sugar, in red clover roots. They observed that many clover plants died in early spring, after growth had started, and from analyses made in early spring, they concluded that the plants died of starvation, or were so weakened that disease entered, before photosynthetic area and activity became great enough to produce the materials necessary for proper life activities. They also found that carbohydrate reserves were utilized earlier in the spring than nitrogen reserves.

In Finland, Virtanen and Nurmia (9) studied the effect of time of cutting on the carbohydrate content of roots of second year red clover plants. Red clover in uncut plots was overshadowed by tall grass. Under those conditions the carbohydrate root reserves were depleted and remained low throughout the season.

A first cutting was made on all other plots in late June before flowering, and of these plots, one fourth were not cut again, one fourth were cut a second time in late August at full bloom, one fourth in early September at late flowering, and the remaining fourth in early October when the plants were mature and had formed seed. They found that the later the second cut was made, the more rapid the replacement of reserves, and the higher the final percentage in late October. The minimum of root reserves was reached in about two weeks after cutting, regardless of the cutting date, but replacement of reserves took six weeks in the summer as against two weeks in the fall.

They also found that cutting did not significantly affect the content of sucrose and other soluble sugars, the values for which were very low during June, July, and August, but rose towards autumn.

SECTION III. OBJECTIVES OF STUDY

The broad objectives of this study were to determine when and why losses occurred in red clover stands, and what could be done in the way of management practices to minimize these losses. More specifically, it was desired to determine (1) the importance of removing combined straw, (2) the importance of clipping, (3) the best time or times for clipping, and (4) the importance of removing mowed growth. It was also desired to determine (5) the effect of management practices on disease and insect incidence, and (6) the effect of management practices on the storage of root reserves, as indicated by chemical analysis for total available carbohydrates and for nitrogen.

Stand counts, or plants per square foot, were used to follow the seasonal effects of the various treatments, while the final criterion was yield of clover hay at harvest time.

SECTION IV. MATERIALS AND METHODS

<u>Locations</u>. The work was carried on for two years at four locations each year with the help of cooperating farmers who had planted strains of red clover locally adapted to their respective areas. In all but one case, timothy was seeded in the fields with the red clover.

In 1948, two of the trials were located in Talbot county near Easton, one, designated as "C" on Mattapex silt loam, and the other, "M_1" principally on Othello silt loam with a small portion on Fallsington fine sandy loam. A third trial, "L", was located in Montgomery county near Ashton on Chester loam, and the fourth, "E", was in Washington county near Clear Spring on Murrill gravelly silt loam.

In 1949, two trials were again located in Talbot county near Easton, one, "D", on Mattapex silt loam, and the other, "M_2", on Matapeake very fine sandy loam. A third, "O", was located in Montgomery county near the village of Unity on Manor loam, and the fourth, "H", was in Frederick county between Jefferson and Middletown on Chester loam.

<u>Treatments</u>. In all cases, the clover was seeded in wheat which was combined during the first half of July. Sixteen different management treatments were used as follows:

1. No clipping; combined straw left on field.

2. No clipping; combined straw removed within one week after combining.

3. Combined straw removed within one week after combining; mowed between September 1 and 15; growth removed.

4. Combined straw removed within one week after combining; mowed

between September 1 and 15; growth left.

5. Mowed within one week after combining; nothing removed.

6. Mowed within one week after combining; everything removed.

7. Mowed within one week after combining; everything removed; mowed again first half of September; growth removed.

8. Mowed within one week after combining; everything removed; mowed again first half of September; growth left.

9. Mowed six weeks after combining; nothing removed.

10. Mowed six weeks after combining; everything removed.

11. Mowed at about first frost; nothing removed.

12. Mowed at about first frost; everything removed.

13. Mowed within one week after combining, at six weeks, and at about first frost; everything removed at each treatment.

14. Combined straw removed within one week after combining, mowed when red clover seedlings are about half bloom stage; everything removed.

15. Combined straw left on field; mowed when red clover seedlings are about half bloom stage; everything removed.

16. Combined straw removed within one week after combining; mowed when red clover seedlings are about half bloom stage, and again between September 1 and 15; everything removed at both treatments.

This schedule of treatments was followed as closely as possible, although there was some unavoidable deviation. In 1948, the first treatments were made during the second week of July and the half bloom treatments were made during the first ten days of August. The six-week treatments were made during the first two weeks of September at the same time as the first half of September treatments. The last treatments were made during the first two weeks of November.

In 1949, the first treatments at two locations were delayed until late in July and the six week treatments were made from the twelfth to the twenty-fifth of August. The half bloom treatments were made at the end of August and the September treatments were made from the twenty-first to the twenty-fourth of September. The last treatments were made during the second week of November.

Differences in growing conditions at the various locations were taken into consideration when planning the order in which locations should be treated.

Plot Layout. During the first year that the trials were run, five replicates were used and the sixteen treatments were arranged in five randomized complete blocks, and for these blocks a 1 x 16 arrangement was used. The trial area was laid out in a single large block with no spaces between single plots or between rows.

In the second year there were four replicates, and a 4 x 4 arrangement was used for the randomized complete blocks. The trial area was laid out in four rows of plots with three foot alleys maintained between the rows in order to prevent tramping or otherwise disturbing adjacent plots when treatments were made.

The size of individual plots was 10 x 20 feet. All plot corners which lay along the perimeter of the over-all trial area were marked with bricks, which were set flush with the ground surface so as not to interfere with machinery. Plot areas were fenced where necessary to prevent grazing by livestock. All field data were recorded directly onto mimeographed copies of the plot designs.

Procedures and Equipment. All mowing was done with small power mowers of the front mounted reciprocating cutter bar type. These machines cut a three foot swath and were adjusted so that they cut at a height of approximately two inches.

At the time of harvest of the first set of plots in 1949, eighteen inches were cut off each end of each of the plots and a three foot strip or an area of 51 square feet was harvested from the center of each plot.

In the harvest of the second set of plots in 1950, the three foot alleys, which had been included in the plot layout, were mowed, and a three foot strip or an area of 60 square feet was harvested from the center of each plot.

In the seedling year treatments during 1948, field weights were taken for the full 200 square feet from all the plots where the treatment called for cutting and removal of growth. In the seedling year treatments in 1949, field weights were in general taken for a three foot strip, or 60 square feet, except where growth was light, in which case the weights were taken for the full 200 square feet.

Immediately after cutting, material to be removed was raked and then weighed to the nearest one tenth pound. Growth cut and left was not weighed.

Moisture samples were taken of all material in the hay crop harvest and all material cut and removed in seedling year treatments. In 1948 and 1949, composite samples of each treatment at each location were brought to the University farm in rubberized bags. The material was put through a small forage chopper and 200 to 300 gram samples were dried in a forced draft oven for 36 hours at 60° to 70° C.

In 1950, as each plot was cut and weighed a 1½ to 2 pound sample was put into a cloth bag and weighed in the field to the nearest gram. These samples were placed in a corn dryer at the farm for several days for preliminary drying, and were then placed in the forced draft oven for four to five hours at 60° to 70° C.

In 1949, the amount of clover in the hay harvest was determined by estimating the percentage of clover in the plots before they were mowed, and then applying this percentage to the total plot yields.

In 1950, the percentage of clover in the material harvested was estimated by examination of the samples used for moisture determinations, and the percentages applied to the total plot yields as in the previous season.

Stand counts were made on both sets of trials, using a metal hoop about 15 inches in diameter, which was placed on the ground and all the red clover plants within the circle counted. Three such counts were made within each plot and the plot and treatment totals converted to plants per square foot.

When the first count was made in the fall of 1948, the ring was thrown three times at random within each plot. Before the next count was made, it was decided to mark the locations where the ring was placed so that each subsequent count would be made on the same area. Two inch squares of red plastic film pinned to the ground with nails proved to be very satisfactory markers, inasmuch as they were easy to see, were not disturbed by mowing or raking, and were very resistant to the weather, remaining in good condition after fifteen months in the field. Exact replacement of the counting ring was assured by aligning the heads of the nails with two distinct marks which had been filed into the ring at diametrically opposite points.

During the counting operation, and at other times when it was convenient to visit the plots, close inspections were made for the presence of insects and diseases. Advice and assistance in this phase of the work was obtained from the Entomology Department and from the Plant Pathology Division of the Botany Department of the University of Maryland, and from U.S.D.A. personnel.

Samples of diseased plants were brought into the laboratory and the fungi cultured and isolated. Field inspection for Fusarium involved sectioning the roots and crown of plants dug at random from the plots. In the spring of 1950, the plots at one location were sprayed with chlordane to control spittle bug because an unusually heavy infestation threatened to eliminate the clover from the plots.

It was noted in the fall and early winter that field mice tended to concentrate in the plots where heavy cover was present and to stay out of the plots where cover was light. The damage from field mice feeding on the crowns of the clover plants was not uniform for all treatments, and it was felt that migration into the plot area from the surrounding clipped fields had occurred. Therefore, the mice were controlled with a phosphorus poison dusted onto apple slices which were placed in the runways.

To determine root storage of total available carbohydrates and nitrogen, samples were dug from selected plots in December of each year. These plots were those receiving treatments 1, 3, 6, 7, 10, 13, 15, and 16.

As soon as possible after the roots were dug, they were washed thoroughly and autoclaved under five pounds pressure for fifteen minutes to kill the plants and prevent loss of carbohydrate by respiration or enzymatic action. Top growth was then removed, the crowns and roots dried, and the number of roots and the dry weight for each sample was noted. The dried roots were ground in a Wiley mill with a 40-mesh screen and were stored in airtight bottles until analyzed.

Total available carbohydrates were determined according to the method described by Weinman (10, 11). Commercial takadiastase, purchased in the local drugstore, gave good results with low blank values after being dialyzed in a cellophane bag in running tap water for four days.

Duplicate analyses were made of each sample and two aliquots were taken from each duplicate to be reduced by Fehling's solution. When more than one percent variation was obtained among the values the analysis was repeated.

Nitrogen was determined by the micro-kjeldahl method, using a Kemmerer-Hallet (5) steam distillation flask. Complete directions for the analysis were furnished by the Horticulture Department of the University of Maryland, and included modifications involving collecting the ammonia in boric acid and using brom cresol green-methyl red combination indicator in the titration. Duplicate analyses were made which were required to be within five hundredths of one percent.

SECTION V. RESULTS

Stand Counts. The average number of red clover plants per square foot for each treatment for the three counts made during 1948-1949 are shown in Table 1, and for the eight counts made during 1949-1950 in Table 2.

Table 1. Red clover stand counts made in 1948-49.

Treatment No.	Average Date of Count		
	Oct. 3	Apr. 15	Jul. 9
	Plants per square foot 1/		
1	7.0	4.5	3.0
2	6.8	4.6	2.6
3	9.0	8.6	3.9
4	8.4	6.7	3.5
5	8.1	6.3	3.3
6	8.7	7.6	3.5
7	10.7	9.8	4.9
8	10.3	9.0	4.4
9	7.9	7.1	3.4
10	9.0	8.7	3.7
11	7.4	4.6	2.5
12	6.2	5.7	3.2
13	12.1	11.8	5.7
14	10.9	9.1	4.0
15	11.0	8.8	4.0
16	13.5	11.1	5.2

1/ Averages of 20 plots per treatment (5 replicates ar 4 locations).

Table 2. Red clover stand counts made in 1949-50.

Treatment No.	Jul. 21	Aug. 16	Sep. 13	Oct. 8	Nov. 27	Feb. 12	Apr. 10	Jun. 25
	Plants per square foot 1/							
1	21.1	13.1	10.4	7.7	5.1	4.6	3.8	2.0
2	19.4	15.6	10.4	6.9	4.7	4.3	3.9	2.2
3	19.3	15.3	10.4	8.1	7.0	6.5	5.7	2.8
4	19.8	15.1	10.4	7.4	6.0	5.5	4.6	2.5
5	20.4	11.2	11.0	7.4	5.8	5.2	4.4	2.2
6	18.8	16.8	12.8	9.4	6.8	7.1	6.0	3.3
7	19.1	16.9	13.4	10.8	9.0	8.9	7.4	3.5
8	20.4	16.7	12.8	9.8	7.4	6.8	5.6	2.8
9	20.4	12.6	10.4	9.2	7.4	7.2	5.7	3.1
10	20.4	13.4	14.2	11.9	9.1	9.4	8.0	3.4
11	22.6	13.0	10.6	7.6	5.6	4.8	3.6	1.9
12	21.4	12.8	9.2	6.6	5.2	4.6	3.7	1.9
13	20.9	17.8	16.0	13.0	11.6	10.8	8.8	4.5
14	20.3	16.7	13.0	10.5	8.5	9.1	7.5	3.6
15	19.7	13.4	13.0	11.0	8.6	9.0	7.4	3.8
16	21.4	15.4	12.4	10.4	9.0	8.6	7.7	3.3

1/ Averages of 16 plots per treatment (4 replicates at 4 locations).

Table 3 shows the treatments listed in descending order according to the number of plants per square foot for the counts made in 1948-1949, and for the counts made in 1949-1950 from October through June. Valid comparisons among all treatments cannot be made prior to October because the effects of September treatments would not be apparent for at least a month. The fact that treatments 11 and 12 were not made until November can be disregarded in October comparisons inasmuch as these treatments when made showed no significant effect on subsequent stand counts.

This table shows that even though the relative position of most of the treatments varied from month to month, certain treatments, namely 7, 10, 13, 14, 15, and 16, were always within the upper half while another group, namely 1, 2, 4, 5, 11, and 12, were always within the lower half. The remaining treatments, 3, 6, 8, and 9, were about average between the other two groups.

Table 3. Management treatments by number listed in descending order according to number of red clover plants per square foot on dates indicated.

Rank	Average Date of Count							
	1948-1949			1949-1950				
	Oct. 3	Apr. 15	Jul. 9	Oct. 8	Nov. 27	Feb. 12	Apr. 10	Jun. 25
	Treatment Number							
1	16	13	13	13	13	13	13	13
2	13	16	16	10	10	10	10	15
3	15	7	7	15	7	14	16	14
4	14	14	8	7	16	15	14	7
5	7	8	14	14	15	7	15	10
6	8	15	15	16	14	16	7	16
7	10	10	3	8	9	9	6	6
8	3	3	10	6	8	6	9	9
9	6	6	6	9	3	8	3	8
10	4	9	4	3	6	3	8	3
11	5	4	9	1	4	4	4	4
12	9	5	5	11	5	5	5	5
13	11	12	12	4	11	11	2	2
14	1	11	1	5	12	1	1	1
15	2	2	2	2	1	12	12	11
16	12	1	11	12	2	2	11	12

Analyses of variance of the October and April stand counts for the two years are shown in Tables 4 and 5. High significance was obtained in all cases except between years for the October count.

The high significance for blocks and for locations indicates that factors other than the management treatments used had considerable effect on stand survival. The high significance shown for the treatment by location and treatment by year interactions, indicates that these other factors, such as soil type, fertility, moisture, or weed competition, which varied between blocks and especially between locations, had some influence on the relative effects of the treatments used. However, when the calculated F values for the interactions are compared to the calculated F values for the treatments themselves, it is obvious that these interactions, even though highly significant statistically, are of relatively small practical importance, especially in the April counts.

The fact that the differences between years were not significant in October, but were highly significant in April, reflects the influence of the winter weather on stand survival. The winter of 1948-1949 was relatively mild with an early spring and little loss of stand, while that of 1949-1950 was characterized by an extended warm period in February, followed by severe cold and dry weather, which caused considerable reduction in stand at the Montgomery and Frederick county locations.

Table 4. Analysis of variance. Red clover plants per square foot for sixteen treatments in October 1948 and 1949.

Source	S.S.	D.F.	M.S.	F. (calc.)
Total	985,239	560		
Blocks	49,506	28	1,768	4.33 **
Treatments	193,761	15	12,917	31.66 **
Locations	439,407	6	73,235	179.50 **
Years	469	1	469	1.14
Tr. x Loc.	115,266	90	1,281	3.14 **
Tr. x Yr.	21,469	15	1,431	3.51 **
Error	165,361	405	408	

Table 5. Analysis of Variance. Red clover plants per square foot for sixteen treatments in April 1949 and 1950.

Source	S.S.	D.F.	M.S.	F. (calc.)
Total	638,994	560		
Blocks	55,206	28	1,972	6.99 **
Treatments	214,363	15	14,291	50.68 **
Locations	126,580	6	21,097	74.81 **
Years	51,309	1	51,309	181.95 **
Tr. x Loc.	64,870	90	721	2.56 **
Tr. x Yr.	12,279	15	819	2.90 **
Error	114,387	405	282	

Table 6 shows the two year treatment averages of plants per square foot for October and for April, arranged in descending order. The least significant difference (L.S.D.) between treatments for October is 0.93 at the five percent level, and 1.22 at the one percent level, and for April is 0.78, and 1.02 respectively.

From the standpoint of hay yield, April is the more important of the two months. Spot checks and partial counts made during May indicate that there is very little loss of stand once growth is well started in the spring.

Comparison of the L.S.D. with the differences between treatments in April shows that 13 with the highest stand count is significantly better than 16 which has the second highest count. Treatment 16 is, in turn, significantly better than 7 which ranks third. Treatments 7, 10, 14, 15, rank from third to sixth respectively and there are no significant differences among them. All of this group are significantly better than treatment 8, which ranks seventh. Treatment 15 is significant over 3, which ranks eighth, and is highly significant over 6, which ranks ninth. It should be noted that with the exception of 8, which has the growth left at the second clipping, all of these nine treatments best in relation to stand count, call for one or more clippings with removal of the mowed growth.

Table 6. Two year average stands of red clover in October and April.

Rank	October		April	
	Treatment	Plants per sq. ft.	Treatment	Plants per sq. ft.
1	13	12.55	13	10.30
2	16	11.95	16	9.40
3	15	11.00	7	8.60
4	7	10.75	10	8.35
5	14	10.70	14	8.30
6	10	10.45	15	8.10
7	8	10.05	8	7.30
8	6	9.05	3	7.15
9	3	8.55	6	6.80
10	9	8.55	9	6.40
11	4	7.90	4	5.65
12	5	7.75	5	5.35
13	11	7.50	12	4.70
14	1	7.35	2	4.25
15	2	6.85	1	4.15
16	12	6.40	11	4.10

L.S.D at 5% level — .93
at 1% level — 1.22

L.S.D. at 5% level — .78
at 1% level — 1.02

<u>Yields</u>. Table 7 shows the average treatment yields at the first cutting for each year, and for the two years combined, arranged in descending order. The least significant difference between two year averages is 166

at the 5% level and 218 at the 1% level. Comparison of these least significant differences shows no significant differences among the first six treatments, 16, 7, 13, 10, 8, and 3, in that order. Only the first two, 16 and 7, are significant over 14 which is in eighth position. It should be noted that this group of treatments is identical with the eight highest treatments in the April stand count, even though they are not in the same order.

Table 7. Dry weight yields of red clover for first cutting at all locations. Treatment averages for each year and for two years.

Rank	1948-49		1949-50		2 year average	
	Treatment	Lbs. per acre	Treatment	Lbs. per acre	Treatment	Lbs. per acre
1	7	1680	16	930	16	1270
2	16	1620	14	880	7	1270
3	10	1610	13	860	13	1210
4	13	1560	7	850	10	1190
5	3	1520	8	810	8	1140
6	8	1470	15	800	3	1130
7	15	1360	10	780	15	1080
8	9	1270	3	740	14	1060
9	12	1260	9	620	9	940
10	4	1240	4	610	4	930
11	14	1230	6	560	12	890
12	6	1020	5	450	6	790
13	5	990	12	410	5	720
14	11	890	2	360	11	610
15	1	860	1	360	1	610
16	2	700	11	330	2	530

L.S.D for two year average, at 5% level — 166
at 1% level — 218

Table 8 shows the two year average yields at the second cutting. These averages are for five locations only, two being harvested twice in the first season and three in the second season. As nearly as can be determined from the limited amount of observation which was possible, the stand losses which occurred between the first and the second harvest at the other three locations were due to a combination of factors, principally Fusarium, dry weather, low fertility, and insects. Again it should be noted that the eight highest treatments are identical with, though not in the same order as, the eight treatments which are highest in the April stand count and highest in first cutting yields.

Table 8. Two year averages for five locations of dry weight yields of red clover for second cutting.1/

Rank	Treatment	Pounds per Acre
1	13	800
2	16	780
3	14	760
4	7	750
5	15	680
6	10	670
7	8	660
8	3	650
9	9	580
10	4	530
11	6	480
12	5	380
13	12	370
14	11	360
15	2	330
16	1	320

1/ Two locations harvested in 1949; three locations harvest in 1950.

The analysis of variance for the first cutting yields for the two years is shown in Table 9. Significance at the 5% level was shown for block differences and for treatment by year interactions, while high significance was shown for all other sources of variation. As with the plant counts, the high calculated F value for the treatments compared to the treatment interactions indicates that the effects of season or location on treatments are of relatively little practical importance. The treatments tend to fall into high, medium, and low groups with the interactions changing the order within groups, but only occasionally causing a treatment to change to a different group.

Table 9. Analysis of variance. Red clover yields at first cutting of sixteen treatments for two years.

Source	S.S.	D.F.	M.S.	F. (calc.)
Total	674,817	558		
Blocks	12,951	28	463	1.68 *
Treatments	73,303	15	4,887	17.71 **
Locations	302,783	6	50,464	182.84 **
Years	115,261	1	115,261	417.61 **
Tr. x Loc.	52,237	90	580	2.10 **
Tr. x Yr.	7,144	15	476	1.72 *
Error	111,039	403	276	

Location yields, which show an exceptionally high significance, are given in the following brief table which also includes second cutting location yields.

Table 10. Dry weight yields of red clover. Average of all treatments at each location used in two years.

	Location and Year of harvest							
	1949				1950			
	C	L	M_1	E	D	M_2	O	H
1st cutting	1920	1420	1090	625	1500	695	325	80
2nd cutting	510	256	—	—	1170	550	358	—

Factors which, from observations, are believed to be at least partly responsible for these considerable differences will be mentioned briefly here.

At the "C" location, which produced the highest average yield for the first cutting, there was no timothy planted with the clover, and therefore the only competition suffered by the clover was from weeds which volunteered in the poorer treatments. The lack of competition from an associated grass is considered to be an important factor in the production, at this location, of a clover yield five hundred pounds, or 35% greater than that at the "L" location which had the next highest 1949 yield, and 420 pounds, or 28% higher than that at the "D" location which had the best 1950 yield. At the latter two locations there were moderate amounts of timothy and ryegrass respectively, while other factors such as fertility levels, moisture, and stand counts were essentially the same.

Moderate stands of timothy were also present at the "M_1" and "M_2" locations, but average yields were considerably lower than at the two previously mentioned locations. This is considered to be due to a relatively lower fertility level, and the fact that the lowest stand counts in their respective years were at the "M_1" and "M_2" locations.

At the "E" location, stand counts were relatively high and early spring growth was normal, but by mid-May the plants on more than half of the plot area appeared stunted and unhealthy, and by June none of the plants were as large as they normally should have been. The cause or causes of this abnormality were not readily apparent and sufficient observation to determine them was not possible. It was suspected that drought and low fertility might have been at least partly responsible, as even the competing weeds and timothy did not appear to be growing with their normal vigor.

The low first cutting yields at the "O" and "H" locations are primarily due to spittle bug injury. In late April, the plants in these stands were making good growth, and there were sufficiently good stand counts to make a good yield, at least on the better treatments, when the severe spittle bug infestation developed. The plants were stunted and had the appearance of being "sucked dry", some even showing a reddish coloration of the leaves similar in appearance to potassium deficiency. The "H" location was treated with a chlordane spray which is believed to have been effective against the insects, but which was too late to save the clover. The clover at both of these locations was also subject to competition from fairly heavy timothy stands.

The second cutting yields in 1949 show the effect of dry weather during July, while those for 1950 show that growing conditions were more normal during that year.

The high significance between years shown in Table 9 is largely a reflection of the spittle bug injury at the "O" and "H" locations and the poor stand at the "M_2" location. The 1949 average yield per acre for all treatments for the first cutting was 1260 pounds and for 1950 was 650 pounds.

Seedling year average yields were also calculated for all treatments where growth was removed. These are shown in Table 11.

Table 11. Two year averages of seedling year dry weight yields from red clover experimental plots.

Treatment No.	Material harvested Straw	Straw & Stubble	Straw Stubble Clover & Weed	Stubble Clover & Weed	Clover
	Pounds per Acre				
2 1/	1400	——	——	——	——
3	1400	——	——	2350	——
4	1400	——	——	——	——
6	——	1950	——	——	——
7	——	1950	——	——	1480
8	——	1950	——	——	——
10	——	——	2710	——	——
12	——	——	2150	——	——
13	——	1950	——	——	1620
14	1400	——	——	2110	——
15	——	——	2820	——	——
16	1400	——	——	2110	420

1/ Treatments 1, 5, 9, and 11 have nothing removed.

Treatments 2, 3, 4, 14, and 16 give straw for bedding, the yield of which depends on the small grain crop and how the combine is set. By clipping the stubble before gathering the straw, as in treatments 6, 7, 8, and 13, the yield of bedding material may be increased 30 to 35%, in addition to controlling weeds and making it possible to harvest clean clover hay later in the summer. This is done in treatment 7, which gave an average of 1480 pounds of clover hay per acre in early September. In treatment 13, 1620 pounds of clover was produced which was harvested at three subsequent clippings in 1948 and two in 1949. This treatment simulates rotational grazing.

Material harvested as in treatment 10 or 15 during August, will make poor hay at best, due to the straw and stubble which has been left on the field, and to weeds, if they are present. Material from treatment 12 will be poor even as bedding due to the fact that by November most of the straw is partially decomposed and the dead weed and clover growth is woody and not very absorbent.

The quality of the material harvested from plots which have had the straw removed, as in treatments 3, 14, and 16, depends to some extent on how much stubble was left on the field, but principally on how much weed growth is present. In treatment 16, a second clipping in September following the one in August, gave an average of 420 pounds of clover hay per acre.

The treatments which are the best in relation to yield of forage or bedding during the seedling year, 3, 7, 10, 13, 14, 15, and 16, are the same group which maintain the higher stand counts and produce the most clover in the hay crop.

Root Reserves. The results of the chemical analyses for total available carbohydrates and for total nitrogen are shown as treatment averages at locations in Tables 12 and 13 respectively. The overall averages and the location averages are also shown in these tables. Only three locations are reported for the 1949-1950 season since the stands in the plots at the "M_2" location were too poor to furnish a full set of replicated samples.

The analyses of variance for carbohydrate and nitrogen percentages are shown in Tables 14 and 15 respectively. High significance was obtained in all but two cases, blocks and treatment by year interactions for nitrogen showing significance only at the 5% level. As in the case of stand counts and of yields, the calculated F value for treatments is so much greater than that for the interactions, that the latter do not assume any great practical significance.

For carbohydrates, treatment differences are approximately equal to location differences, while for nitrogen, treatment differences are considerably less than those between locations. This indicates that carbohydrate reserves can be controlled to a considerable extent by the management practices used in this study, but that nitrogen reserves are more dependent on other factors. This same conclusion can also be drawn from a comparison of treatment and location averages for percentage of carbohydrates and nitrogen (tables 12 and 13). For carbohydrates, the treatment averages range from 24.3% to 31.6%, a difference of 7.3%, while the location averages range from 26.0% to 32.6%, a difference of 6.6%. For nitrogen, the treatment averages range from 2.59% to 2.79%, a difference of only 0.20%, while the location averages range from 2.42% to 3.07%, a difference of 0.65%, or over three times as great as the treatment range.

Another interesting comparison is between the "C" and "D" locations in Table 12. At the "C" location, where tall growing weeds were prevalent, the treatment range is 15.7% from treatment 10 which is high, to treatment 1 which is low. At the "D" location there were very few weeds and the treatment range is only 2.8% with treatment 1 being high and 13 being low. This indicates that weeds which will overshadow the plants must be controlled in order for carbohydrate reserves to be stored. These two locations were considered to be the best in regard to fertility and moisture and they are also the highest two in nitrogen root reserves (table 13). These facts lend further support to the conclusion that carbohydrate reserves are increased by clipping practices which control weeds and allow more sunshine to reach the plants, while nitrogen reserves are more dependent on other growing conditions.

Table 12. Carbohydrate percentages in red clover roots in late December. Averages for treatments given by locations.

Treatment No.	1948-49 C	1948-49 M_1	1948-49 E	1948-49 L	1949-50 O	1949-50 H	1949-50 D	Treatment Averages
			Percent of carbohydrate					
1	14.7	29.3	29.3	22.0	22.0 1/	22.2	30.9	24.3
3	29.9	33.8	33.4	30.9	31.6	29.9	29.8	31.4
6	24.7	32.3	28.1	26.1	27.2	23.2	30.8	27.5
7	27.1	33.2	34.9	32.6	31.9	30.7	30.7	31.6
10	30.4	34.9	34.3	30.9 1/	29.3	29.7	30.2	31.5
13	27.4	30.6	33.7	30.1	28.1	27.6	28.1	29.5
15	25.3	33.2	31.7	30.7	30.3	31.0	30.7	30.4
16	28.5	33.9	33.3	30.4	31.6	31.4	30.7	31.4
Location Averages	26.0	32.6	32.3	29.2	29.0	28.2	30.2	

1/ Treatments 1 at "O", and 10 at "L" calculated by missing plot formula.

Table 13. Nitrogen percentages in red clover roots in late December. Averages for treatments given by locations.

Treatment No.	1948-49 C	1948-49 M_1	1948-49 E	1948-49 L	1949-50 O	1949-50 H	1949-50 D	Treatment Averages
			Percent of nitrogen					
1	2.73	2.69	2.56	2.80	2.72 1/	2.60	3.06	2.73-
3	2.70	2.65	2.42	2.64	2.61	2.38	3.11	2.63
6	2.63	2.80	2.43	2.85	2.61	2.62	3.00	2.70
7	2.77	2.54	2.31	2.68	2.58	2.48	2.98	2.62
10	2.75	2.68	2.37	2.74 1/	2.78	2.73	3.20	2.73+
13	2.74	2.56	2.32	2.44	2.61	2.52	3.00	2.59
15	2.86	2.80	2.57	2.80	2.74	2.70	3.10	2.79
16	2.76	2.75	2.38	2.63	2.67	2.49	3.13	2.68
Location Averages	2.74	2.68	2.42	2.70	2.66	2.56	3.07	

1/ Treatments 1 at "O", and 10 at "L" calculated by missing plot formula.

Table 14. Analysis of variance. Carbohydrate percentages in red clover roots for eight treatments for two years.

Source	S.S.	D.F.	M.S.	F. (calc.)
Total	469,289	243		
Blocks	27,450	25	1,098	2.42 **
Treatments	152,044	7	21,721	47.95 **
Locations	122,465	5	24,493	54.07 **
Years	4,832	1	4,832	10.67 **
Tr. x Loc.	78,494	35	2,243	4.95 **
Tr. x Yr.	10,159	7	1,451	3.20 **
Error	73,845	163	453	

Table 15. Analysis of variance. Nitrogen percentages in red clover roots for eight treatments for two years.

Source	S.S.	D.F.	M.S.	F. (calc.)
Total	132,738	243		
Blocks	5,299	25	212	1.71 *
Treatments	10,297	7	1,471	11.86 **
Locations	73,048	5	14,610	117.82 **
Years	10,037	1	10,037	80.94 **
Tr. x Loc.	11,472	35	328	2.65 **
Tr. x Yr.	2,396	7	342	2.76 *
Error	20,189	163	124	

Table 16 shows the treatment averages of percent of carbohydrate and nitrogen root reserves arranged in descending order. For carbohydrates, treatments 7, 10, 3, and 16 are high with no significant difference between them. Treatments 7 and 10 are just significant over 15, while 3 and 16 are highly significant over 13. Treatment 13 is highly significant over 6 which in turn is highly significant over 1. For nitrogen, 15 is significantly the highest, with 10, 1, 6, and 16 appearing in that order with no significant difference between them. Treatments 3, 7, and 13 are the lowest and there is no significant difference within this group.

Table 16. Average percent of carbohydrates and nitrogen in red clover roots.

Rank	Carbohydrate		Nitrogen	
	Treatment #	Average %	Treatment #	Average %
1	7	31.6	15	2.79
2	10	31.5	10	2.73
3	3	31.4	1	2.73
4	16	31.4	6	2.70
5	15	30.4	16	2.68
6	13	29.5	3	2.63
7	6	27.5	7	2.62
8	1	24.3	13	2.59

L.S.D. at 5% level — 1.04
at 1% level — 1.37

L.S.D. at 5% level — .055
at 1% level — .072

The roots and crowns on which the foregoing analyses were made were weighed prior to grinding, and the average air dry weight per root calculated for each sample. The analysis of variance for the root weights for the two years is shown in Table 17. Highly significant calculated F values were obtained for all but the year differences.

Table 17. Analysis of variance. Average red clover root weight for eight management treatments for two years.

Source	S.S.	D.F.	M.S.	F. (calc.)	
Total	40,207,792	243			
Blocks	3,781,512	25	151,260	1.92	**
Treatments	2,421,425	7	345,918	4.38	**
Locations	11,329,517	5	2,265,903	28.71	**
Years	12,148	1	12,148	— —	
Tr. x Loc.	7,704,928	35	220,141	2.79	**
Tr. x Yr.	2,094,888	7	299,270	3.79	**
Error	12,863,374	163	78,916		

The treatment averages arranged in descending order are shown in Table 18. There is no significant difference between the best six treatments, 16, 10, 7, 3, 15, and 6, however each was significant over 1 and 13.

Table 18. Average air dry weight of red clover roots dug in December for chemical analysis.

Rank	Treatment	Average root weight 1/
1	16	.885
2	10	.885
3	7	.875
4	3	.870
5	15	.860
6	6	.780
7	1	.655
8	13	.635

L.S.D. at 5% level — 0.135
at 1% level — 0.180

1/ Dry weight in grams, average of 32 plots per treatment with 10 to 15 roots per plot.

<u>Correlations</u>. Comparisons of the tables which have been presented indicate that certain correlations exist. The most obvious of these is that between yield and plants per square foot. The treatment averages for all sixteen treatments for clover yields and plants per square foot in April at the various locations were compared, giving a total of 123 comparisons with a positive correlation coefficient of .701, which is highly significant.

Comparisons of plot yields to plot stand counts in April for the eight treatments which were selected for chemical analysis were divided into two groups, one in which the stand counts were less than ten plants per square foot, and another in which the stand counts were ten or more. The correlation coefficients for these groups had to be calculated separately by locations, due to location yield differences which were caused by factors other than stand density.

Table 19. Correlation of red clover hay yields with April stand counts of less than ten and of ten or more plants per square foot at five locations.

Location	Less than 10 pl. per sq. ft.		10 or more pl. per sq. ft.	
	n	r	n	r
C	15	+.605 **	25	-.0084
M	33	+.407 *	7	-.169
E	24	+.531 **	16	+.0035
L	25	+.398 *	7	-.592
O	25	+.565 **	7	-.501

Table 19, above, shows the numbers of comparisons and the correlation coefficients for the two groups at the five locations which had plots with over ten plants per square foot. Either significance or high significance was obtained at all locations when the plant counts were under ten plants per square foot, while no significance was shown at any location when the counts were over ten. These correlations indicate that stands of over ten plants per square foot do not significantly increase yield, and that if eight to ten good plants per square foot are present during the spring growing season, the yield will be controlled by other factors, among which are fertility, moisture, insects, and weed or grass competition.

Comparisons of first cutting yields to second cutting yields gave correlation coefficients ranging from +.688 to +.937, and comparisons of each year's average April stand counts to each year's average late June stand counts gave a correlation coefficient of +.965. These were highly significant in all cases.

It is possible to compare yields with total available carbohydrates, with nitrogen, and with average root weight, but due to the time interval between December, when the root samples for chemical analysis were dug, and June, when harvest was made, and to the numerous other factors which exert their influence during this time, it is believed that the indirect relationships involved are not sufficient to make the comparisons valid.

The carbohydrate and nitrogen percentages for individual plots were compared, giving a total of 261 comparisons with a negative correlation coefficient of -.185 which is just significant at the 1% level. In the carbohydrate percentage to average root weight comparisons, a positive correlation coefficient of +.458 was obtained for the 261 comparisons. Nitrogen percentage compared to average root weight gave a positive coefficient of +.154 which is significant at the 2% level.

These last two correlation coefficients indicate, as might be expected, that the larger plants contain a greater percentage of root reserves, and therefore stand a better chance of surviving the winter and are better able to initiate vigorous growth in the spring. The negative correlation shown by the carbohydrate and nitrogen percentages is believed to support the statement made earlier that carbohydrate content is strongly influenced by the management practices used, but that nitrogen content is primarily influenced by other factors. Assuming that this is true, the amount of nitrogen in the roots would be fairly constant from one treatment to another, while the amount of carbohydrate would vary, and of course, as the amount of carbohydrate increased, the percent of nitrogen would decrease. The negative correlation obtained supports this point. The fact that both carbohydrate and nitrogen are positively correlated with root weight, while being negatively correlated with each other, may be explained by the fact that the amount of carbohydrate is approximately ten times that of nitrogen.

Diseases. The most prevalent disease observed in this study was a crown and root rot caused by Fusarium species. During the first winter a number of cultures were made from plants showing a brown discoloration of the stele of the tap root or the center of the crown, and Fusarium was isolated in every case. The color of the mycelium in culture ranged from white to pink and lavender, indicating that several different strains were present.

In March of each year random samples of plants from all locations were dug and inspected for the presence of Fusarium. They were classified into four groups according to the degree of infection, and the percentages of infection were calculated as shown in the following table:

Table 20. Red clover roots and crowns infected by Fusarium at all locations in March of 1949 and 1950.

Year	Degree of Infection				Total %
	None	Light	Medium	Heavy	Infected
1949	19%	42%	24%	15%	81%
1950	27%	35%	20%	18%	73%

Many of the infected plants were large and growing vigorously even though the tap root or crown, or both, showed serious damage. It was found by spot checks which were made in May of 1949, and represented all treatments, that the number of plants had been reduced less than 7% since the April count in spite of the fact that approximately 80% of the stand was infected with Fusarium.

These observations indicate that this disease does not seriously impair the spring growth of clover provided the plants come through the winter with sufficient reserves to get growth well started. Plants with sufficient photosynthetic area and adequate feeding roots can, if given good growing conditions, produce a good crop of clover even if the tap root and part of the crown have been destroyed. However, when the hay crop is harvested, the photosynthetic area is removed and the plants must depend on root reserves to initiate second growth.

A comparison of the April stand counts to the June, or after harvest counts, shows an average loss for all locations of 51%, most of which occurred after harvest. Inasmuch as the recovery of the plants depends primarily on how severely the tap roots and crowns have been damaged, and since Fusarium was present in over 75% of all plants inspected during the spring months, it seems reasonable to believe that this disease is primarily responsible for the loss which occurs within a short time after harvest.

Fusarium is considered to be a secondary infection, entering the roots only after they have been injured by some other cause. In Pennsylvania, Henderson (3) considered the clover root borer to be largely responsible for the entry of Fusarium into clover roots in that area. However, this insect was not found at the locations where this study was made. Observations made when sectioning roots to determine the extent of infection, indicate that in many cases entry to the crown might have been made through decayed stubs of crown buds or cut stems, and to the roots, through breaks in the epidermis. The causes of these breaks were undetermined, although some appeared to be caused by trampling or mechanical injuries, and others could have been caused by various soil insects, such as wireworms or the so-called free living nematodes.

Another disease which shows a brown discoloration in the root and crown is caused by a virus infection. However, it is easily distinguished from Fusarium by the fact that the cambium layer is discolored rather than the stele, and in addition the leaves present an abnormal and stunted appearance. Only a few isolated plants were found which were infected with virus, and it was considered to be of minor importance.

At the "O" location in 1949, the disease known only as "Black Patch" was especially severe. It was identified and the causal organism cultured by Dr. LeFebvre, Forage Pathologist of the Forage Crop Division, U.S.D.A., but as no spores have so far been found or produced, it has not been identified as to genus and species. The disease attacks the leaves causing the entire leaflet to dry and turn brown, and it is named from the very dark, practically black, color of the mycelium.

At the location where it was severe, early clipping and removal of growth proved to be a striking preventive measure. Plots which were mowed in July showed almost no infection, even though they were adjacent to unmowed plots which were 90% to 95% infected. It was also controlled by the August and September mowings which were made after the disease had become established quite uniformly throughout the plots which had not been clipped earlier. In general, the later the cutting was made the lower the plant counts obtained, but those plants which recovered grew normally with no further sign of the disease. The plants which survived in the uncut plots were so few and so small that it was impossible to secure an adequate sample from the #1 treatment for chemical analysis without digging much of the plot area.

Other diseases which occurred but were not serious, were Powdery Mildew, Erysiphe polygoni D.D., and a fall leaf spot from which Stemphylium sarcinaeforme (Cav.) Wiltshire was isolated. Neither southern anthracnose, Colletotrichum trifolii Bain and Essary, nor sclerotinia, Sclerotinia trifoliorum Erika, was observed at any of the locations in this study.

Serious loss due to winterkilling, which is not strictly a disease in the pathological sense, but a physiological result of reserve depletion, occurred during the second winter at the "H" location. As mentioned before, the first winter was very mild and there was little loss of stand, in fact, some plots especially those under treatment 12 were observed to make growth and actually increase in size during the winter months. During the second winter, an extended warm period during the last half of February caused premature initiation of growth with consequent depletion of reserves. This warm period was terminated about the first of March by a cold spell which was most severe at the "H" location, due to its being located in the Middletown valley, which is considerably further inland and at a higher elevation than the other locations used that year. About the middle of March when the Fusarium samples were collected, practically all plants in the owner's field and many in the plot area presented a withered appearance, and a number of these were brought to the University of Maryland Plant Pathology Division of the Botany Department. No disease was found other than the Fusarium which was present in a few of these plants, but since it was also present in approximately 75% of surviving plants, it could not be designated as the critical factor in plant survival at this location. When the preceding weather conditions were taken into consideration, it was concluded that the plants had died because root reserves had been depleted below the level necessary to initiate growth a second time after the early March freeze. This conclusion is substantiated by the work of Smith (7) in Wisconsin, who found that total available carbohydrates in red clover roots decreased from 31.9% on November 15, to 13.6% on April 1, merely in keeping the plants alive, as no new growth was apparent on April 1. Since Smith reports a 60% loss of red clover stand during the winter months, it is presumed that the April percentage is the average for only the strongest 40% of the plants which were present in November, and, could the 60% which died have been represented in the April sample, the percentages of reserves would have been lower than 13.6%.

It is obvious from these results that unless plants enter the winter period with a high carbohydrate root reserve, they cannot be expected to survive severe winters, especially if forced to initiate spring growth more than once. The carbohydrate percentage at the "H" location averaged 28.2%, which is second to the lowest of the seven locations sampled and analyzed.

Insects. The insect which caused the most damage in these trials was the spittle bug, Philaenus spumarius L., which was responsible for stunting the plants and reducing the clover yields to very low figures at the "O" and "H" locations. As mentioned previously, a chlordane spray was applied at the "H" location, and from the observations made on the number of adults at harvest time, it was considered to have been reasonably effective and, had it been applied earlier at the beginning of the infestation, it might have saved the clover yield.

In the early spring of 1949 there was a fairly heavy infestation of the clover leaf weevil larvae, Hypera punctata (F.), at the "C" location.

Both the larvae and the adults feed on young clover leaves, but in this case little damage was done due to natural control of the insect by a parasitic fungus Empusa sphaeroperma Fres., which infected and killed practically the entire infestation.

In early October of 1949, it was observed that many of the small new leaves on plants at the "O" location in Montgomery county were being killed. Examination showed that the damage was caused by a small lepidopterous larva which was identified by Dr. Poos of the Bureau of Entomology and Plant Quarantine of the U.S.D.A. as the clover head caterpillar, Laspeyresia interstinctana Clemens, which is known to attack the clover crown growth if heads are not present. This insect bores into and down the petiole of a young leaf, emerges near the base of the petiole within the protection of the stipules, and feeds on the young growth at the tip of the stem. A number of plants which were known to be infested were marked and were examined again in November, at which time no trace of the insects could be found. From observations of plants in various plots under different treatments, and of plants in other fields in the county, it was concluded that damage was slight provided the plants were growing vigorously, but that it could be severe if the plants were growing slowly or not at all due to drought, low fertility, or excessive weed competition. When growth is rapid, the insect damages one or two leaves by eating the newly produced tissues, but when the plant is growing very slowly, the growing point itself may be eaten out, which stops further growth of the stem.

The clover head caterpillar was found again in the spring after the first harvest, and it was considered to be a contributing factor to the almost complete loss of stand on even the best treatments at the "H" location where the soil moisture was quite low and growth was poor and slow during June. Two other similar insects found at this location on July 1 were identified through Dr. Poos as a species of Curculionidae, *Hypera nigrirostris* (F.), and a species of Tortricidae, possibly *Argyrotaenia velutinana* (Wlk.)

The larva of a small fly which was identified as a species of *Oscinella* of the Chloropidae family, was found in the fall, but only on small dead plants which had been overshadowed by dense weed growth. Since it was never found on live plants it was not considered to be of importance in relation to the clover stand.

Field mice tended to concentrate in the plots which had the taller and denser growth or cover in the late fall, notably treatments 1, 2, 5, 6, 9, 14, and 15, and to remain almost completely out of the plots where the growth was short and the cover light, such as treatments 3, 7, 13, and 16. The crowns of clover plants growing along the edges of mouse runways were often damaged severely by mice. Since this damage was not evenly distributed among all treatments, and since it was thought quite possible that mice had migrated from the surrounding clipped fields to the unclipped plots, control measures were taken against the mice. Apple slices treated with phosphorus poison and placed directly in the runways eliminated practically all damage from this source.

SECTION VI. DISCUSSION

The following discussion will be limited as much as possible to the objectives of the study as listed on page 6.

<u>When and Why Losses Occur</u>. Stand count results show that there is considerable reduction in the number of red clover plants during July, August, and September of the seedling year. It is believed that the primary cause of these first summer losses is plant competition, or crowding out of the weaker plants by the stronger and more vigorously growing ones. This belief is supported by the observation that the individual plants were usually smaller on the plots which had the higher stand counts. These plots were in general those which were mowed more than once during the summer, the clippings keeping the plants smaller and thereby reducing crowding or competition between plants.

The next most important factor in summer losses is considered to be shading. In less than four weeks between the July and August stand counts, plots which were mowed and had all growth removed lost 19.3% of their stand, those that had only the straw removed lost 21%, those on which the straw was left lost 36.7%, and those which were mowed and had the cut growth plus the straw left on, lost 43.5%. It can be seen from these figures that the denser the shade on the seedling plants, the greater the stand losses. The question naturally arises at this point as to whether or not conditions on the unclipped plots were more favorable to disease, and whether or not this might be the reason for the greater stand loss. However, close observation throughout the summer failed to turn up any evidence of disease other than the "Black Patch" infection mentioned previously. However, this disease can be ruled out inasmuch as it was equally severe on plots where the straw was left on and where it was removed, and was not severe on the plots which were clipped and the cut growth left on. Also, in the Ohio experiments (12) Willard could find no disease responsible for the greater loss of stand when the straw was left on the field.

A clue pointing to shading as the responsible factor is found in the work of Virtanen and Nurmia (9), who found that second year red clover plants which were overshadowed by grass and tall weeds used up their root reserves during the summer and did not replace them. This seems to indicate that the clover plant requires a certain amount of direct sunlight in order for the chlorophyll to produce sufficient carbohydrates to supply the plant needs. In a seedling plant these needs are three-fold, to build the tissues of new root and top growth, to build a root reserve, and to furnish energy necessary for the roots to take up both water and nutrients from the soil. If second year plants overshadowed by grass and tall weeds cannot produce enough carbohydrate for maintenance, it seems reasonable that seedling plants cannot be expected to produce enough under the same conditions to increase in size and plant weight.

Willard (12) noted that plants on clipped plots withstood drought better than those on unclipped plots, an observation which was also made in this study. One reason for this may be the fact that clipping reduces the transpirational area, and another is no doubt the fact that clipping reduces the competition for moisture by controlling weeds. Another possible reason might well be that clipping allows direct sunlight to reach the plants and this could cause increased photosynthetic activity, which may cause the plant to make more efficient use of the limited amount of moisture available under drought conditions.

Competition for nutrients is also considered to be a factor in the first season losses, especially if the plants have to compete with weeds which are more efficient feeders, and which, if the fertility level is low, may reduce the supply of available nutrients to below the level at which the less efficient clover plants can survive.

Under some conditions, diseases may also be an important factor in summer stand losses, however this occurred at only one location in this study and the disease was controlled by the better management practices.

To summarize the causes of first summer losses, it may be said that a certain amount of loss appears to be inevitable, even under the best of management practices, and that this loss is due primarily to competition for growing space, or the crowding out of the weaker plants. Reasons for additional loss under less favorable treatments include shading by straw or clipped growth left on the field, diseases under some conditions, and competition from weeds for light, moisture, and nutrients. The latter conclusion is substantiated by the fact that at locations where there were very few or no weeds, reasonably good stand counts were maintained on unclipped plots.

Fall losses, during October and November are considerably less than summer losses, and are considered to be due to essentially the same factors. However, competition between clover plants is less because fall growth is slower, and weed competition is less because many of the annual weeds die out during early fall. Insects, such as clover head caterpillar, may cause some loss in early October under unfavorable growing conditions, and if weather conditions are favorable for diseases such as mildew or the fall leaf spots, these could kill weaker plants during the fall, as well as weaken the stronger ones so that they would be more apt to die during the winter months.

Whether or not excessive losses occur during the winter months will depend largely on the strength of the plants or the amount of root reserves present, and on the severity of the winter weather. There are three common types of winter injury, actual freezing of the root or crown tissues by extremely cold temperatures, depletion of root reserves before spring growth starts as reported by Smith (7), and heaving, caused by alternate freezing and thawing of the soil which tends to lift the crown above the surface of the soil exposing it and part of the tap root to desiccation by sun and wind. Heaving may also cause breaking of many of the smaller roots.

The first type of injury, actual freezing, is not expected to be common in Maryland, except in the western counties with high elevations. Reserve depletion and heaving, however, were both noted in this study, and may be expected to be found anywhere in the state when weather and other conditions are favorable for their occurrence.

Reserve depletion losses, which have been discussed previously (p. 37) are believed to be responsible for most of the loss which occurred between February and April stand counts. Heaving was observed during both winters on plots where the ground was bare around small plants, but injury from this source was not extensive and was limited principally to treatment 12 in which growth was clipped and removed in early November.

The sharp drop in stand count immediately after hay harvest is considered to be due to a combination of factors, the principal one being Fusarium, which was present in 75% to 80% of the plants at all locations in the spring of both years. This disease which has been discussed previously, (p. 35), destroys the tissues in which the root reserves are stored, thereby preventing the initiation of second growth and normal recovery after harvest.

No other disease was observed to be present during the after-harvest period, but insects, principally the clover head caterpillar, are believed to have killed some plants which might otherwise have recovered. The spittle bug infestation in May at two of the locations no doubt weakened the plants to a point where recovery was considerably below normal. In addition to the direct injury caused by these insects sucking the juices from the plants, an indirect detrimental effect was produced by stunting the plants which were then overshadowed by timothy. This probably prevented a normal build-up of reserves, and in turn tended to prevent normal recovery.

Low fertility and low soil moisture are also considered to be contributing factors in the after harvest stand losses in fields where these conditions exist.

Until control measures for Fusarium are developed, or resistant strains of clover produced, it seems apparent that after harvest stand losses similar to those in this study will continue to be experienced. However, it is believed that these losses can be minimized by maintaining a good fertility level, by keeping insects in check as much as possible, and by planting a shorter, earlier maturing strain of timothy, which will shade the clover less, and will also remove the temptation to mow later than is best for the clover in order to get greater tonnage from the timothy. It is felt that there will be an increase in the quality of hay produced, which will offset any decrease in quantity from planting a shorter and earlier timothy.

Effects of Management Practices on Stands and Yields. The results of this study show that the management practices during the first summer have considerable influence on stand survival and on yields the following spring. The phases of management considered were the importance of removing combined straw, the importance of clipping, the best time or times for clipping, and the importance of removal of mowed growth.

When the plots were laid out and the first treatments made, straw was removed from the plots which were to receive treatments 2, 3, 4, 14, and 16, and was left on the plots which were to receive treatments 1, 9, 10, 11, 12, and 15. Within less than a month, and before any additional treatments had been made, the first set of plots with the straw removed averaged 21% loss of stand, while the set with the straw left on had lost nearly 37%. However, by the end of the season there was no practical difference between 1 and 2, neither of which had received further treatment, and furthermore, 10 and 15, which had the straw left on until they were clipped, were equally as good as 14 and 16, which had the straw removed after combining and were clipped later in the summer.

This indicates that in this study, merely removing the straw was of little practical importance, although if the straw cover had averaged a ton or more per acre instead of approximately three fourths of a ton, it might have assumed greater importance. The presence of tall growing weeds at most of the locations tended to eliminate the effects of straw removal, due to the fact that these shaded the clover plants even more than the straw cover, in addition to competing for moisture and nutrients.

The fact that the unclipped plots are among the lowest in final stand count, and are the lowest in yields, indicates that clipping is an important factor in the management of red clover. As mentioned previously (p.43) observations at the various locations indicate an inverse relationship between presence of weeds and red clover on unclipped plots. This leads to the conclusion that the importance of clipping, to a great extent, is proportional to the amount of weed growth. Clipping also helps to maintain higher stand counts, especially if repeated, by checking the larger, more dominant plants, and allowing the smaller plants to develop normally.

These conclusions lead to a consideration of the best time or times for clipping the clover stands. Single clippings were made on treatments 5 and 6 in July after combining, on 9 and 10 about the third week in August, on 14 and 15 about the first of September, on 3 and 4 in the third or fourth weeks of September, and on 11 and 12 in early November about the time of the first hard frosts.

Comparisons of stand counts and of yields for these treatments show that mid-August to early September is the best time for clipping seedling stands. The July mowing did not give as good weed control as later clipping, and the clover growth became quite tall and overmature in late September, in which condition it was more subject to leaf spot diseases. Also, it bloomed and set some seed in most cases, which tends to reduce root reserves.

The late September clipping gave only average results, due principally to the fact that stand counts were reduced considerably by weed competition, and at one location by disease, before the clipping was made. The clipping in November gave very poor results.

For timing a single clipping, a good rule of thumb to follow may be to mow when the buds form on the ragweed in the field. This will be approximately mid-August and a single clipping at this time should give good results in central Maryland. On the eastern shore, a single clipping may be made two to three weeks later, if weeds are not prevalent.

Two or more clippings were made on treatments 7, 8, 13, and 16, and the results from these treatments indicate that the first cutting may be made any time in July or early August, and the second about the middle of September, although it may be made a week or more later on the eastern shore of Maryland. Because of the longer growing season on the eastern shore, two or more clippings are especially recommended in order to better control weeds and prevent overmature first year growth.

Treatments 7, 13, and 16 were consistently within the highest four out of the sixteen treatments for spring stand counts and yields for both years. They were also the only treatments to give clean clover hay the first season. Treatment 8 was consistently the highest in stand count and yields among the five treatments in which the mowed growth was left on the field.

Treatments 7 and 8 were cut in July at the same time as 5 and 6, and again in September at the same time as 3 and 4. Since single clipping at either of these dates gave only average results, it is apparent that the difference is due to the double clipping. Treatment 16 was cut in August and again in September and was a very good treatment, although there was much less clover at the second clipping than there was in 7, due to a shorter interval between clippings.

Treatment 13 calls for three cuttings to be made in July, August, and November, but in the first year a cutting was also made in September, making four cuttings that year. This treatment gave the highest spring stand counts in each year, was respectively fourth and third in first harvest yields for the two years, and also gave the highest two year average for second harvest yields.

These results indicate that summer clipping of seedling stands is beneficial rather than detrimental, and that either a hay crop or considerable grazing may be had during the first summer, without injuring the stands or reducing the spring yields. The late clipping of treatment 13 indicates that excess growth may be harvested, or moderate grazing practiced, in the very late fall or early winter, before the top leaves are frozen, but after the weather has become cold enough so that the plants will remain dormant and not use up reserves in initiating new growth. Sufficient growth must be left on the plants so that the surface of the ground is well shaded in order to prevent the possibility of heaving during the winter.

Treatments 4, 5, 8, 9, and 11 were paired respectively with 3, 6, 7, 10, and 12, the only difference within the pairs being that in the former treatments the mowed growth was left on the field, and in the latter it was removed. In treatment 8, which received two clippings, only the second was left on the field. In each of these pairs, the treatment from which the growth was removed gave the higher stand counts and the higher yields, the difference within each pair in most cases being either significant or highly significant. These comparisons indicate that for best results, mowed growth should be removed from the field.

Other Effects of Management Practices. The management practices used were also found to have a definite effect on certain diseases, as well as on the storage of root reserves. Since these factors have already been discussed under the section on Results, they will be mentioned at this point only in relation to management practices.

The incidence of Fusarium, which was the most serious disease present in these trials, was not affected to any apparent degree by the practices used in this study. This is to be expected since it is caused by a soil borne organism and is primarily a root and crown disease, being found in the stem in only one instance.

The disease on which management practices had the most effect was that known as "Black Patch". As mentioned previously, the early clipping treatments prevented its occurrence and the later midsummer clippings effectively checked it. This appeared to be true whether or not the mowed growth was removed from the plots. The fact that the infected growth left on the plots did not reinfect new growth indicates that the mycelium died with the mowed material without producing spores, and therefore it may be assumed that the disease requires a very specific set of conditions for sporulation, and need not become serious if it is recognized in the early stages of its development, and if the stands are clipped before it affects the crowns.

The fall leaf spot diseases were more apt to occur on the treatments which allowed the growth to become tall and mature, and were relatively rare on the later clipped and shorter growth. If leaf spots are present when the clover is mowed, the material should be removed, as these diseases readily produce spores which will remain dormant on dead material until conditions are favorable for their dissemination.

Powdery Mildew did not seem to be affected by the practices used, and it is not expected that sclerotinia would be controlled to any great extent by summer clipping, except that fall conditions might be less favorable for the disease on clipped plots, due to greater air movement and lower humidity near the ground surface under plants which had been kept short by clipping.

It is believed that southern anthracnose, had it been present, would have been affected by the management practices used, inasmuch as a common recommendation for its control is pasturing or the clipping and removal of infected growth to reduce inoculum.

Winterkilling by freezing might be prevented to some extent by treatments such as 10, 14, or 15, which produce a fairly heavy ground cover and thereby tend to insulate the ground surface and the crowns of the plants. Injury by heaving might also be prevented by treatments which produce enough cover to shade the ground, thereby preventing the sun from thawing the ground surface around the plants on mild winter days. The management practices used should also affect winterkilling by reserve depletion, since it has been shown in this study that they do influence the percentage of carbohydrate reserves stored in the roots. Treatments such as 3, 7, 10, and 16, which call for a clipping between mid-August and mid-September, or late September in milder sections, can be expected to produce good carbohydrate root reserves. The fact that 13, a very good treatment in regard to stand count and yields, is slightly lower than the other good treatments in carbohydrate root reserves may be due to the November or after-frost clipping, or to the small size of the individual plants. It is believed to be principally due to the latter reason, since 13 had the lowest average root weight, and there is a highly significant correlation between root weight and carbohydrate percentage.

Treatments such as 6, which are mowed too early in the summer to obtain good weed control, cannot be expected to produce as high a percentage of root reserves due to partial shading by weeds. These early clipped plants also mature in the late fall and may bloom and set seed, which may prevent some root reserve storage. These plants may also initiate new growth in the late fall which also tends to lower root reserves, especially if the photosynthetic area of the mature growth was reduced by leaf spot diseases.

Where weeds are not controlled at all, as in treatment 1, root reserves are at their lowest. It is interesting to note that at one location where there were practically no weeds in the plots, there was very little variation between treatments in carbohydrate reserve, and treatment 1, which was lowest at nearly every other location, was the highest at this location, though not significantly so. Other factors being equal, it appears that red clover root reserve storage is proportional to the area of the top growth exposed to direct sunlight. This seems to indicate that winter survival, in so far as it is dependent on carbohydrate root reserve, is correlated with weed control, provided relatively large plants are produced and the stand is not clipped later than approximately a month before killing frosts.

The clipping treatments used in this study did not appear to have any definite effect on nitrogen reserves. It is believed that the nitrogen percentage of the roots is primarily dependent on other factors such as fertility, moisture, soil aeration, and activity of nitrogen fixing bacteria.

Due to the general mobility of insects, the clipping practices used had very little or no effect on their incidence. Field mice, however, were definitely encouraged and protected by the treatments producing the heaviest ground cover.

SECTION VII. SUMMARY

This study of red clover seedling year management practices was conducted for two years at four locations each year in various sections of Maryland. Sixteen treatments were used to study the effect of frequency and time of clipping, and removal of combined straw and clipped growth on red clover stand maintenance and hay yield. The principal findings of this study are:

1. Major stand losses occurred in July, August, and September of the seedling year, and immediately after harvest of the hay crop in the second year.

2. Better stand maintenance and hay yields were obtained from areas which received two or more clippings during the seedling year, and from which the clipped growth was removed.

3. Combined straw averaged less than three fourths of a ton per acre and its removal was of little practical importance, especially on weed infested fields.

4. The best time for a single clipping was found to be from mid-August to early September. If two clippings are made, the first may be any time from immediately after combining until mid-August, and the second from mid-August to mid-September.

5. The practice of grazing was simulated by several clippings. Results show that mowing and removing growth in July, August, September, and early November, maintained the highest stand count, gave good hay yields for the first spring cutting, and highest yields for the second cutting, even though the plants were somewhat smaller and root reserve percentages slightly lower.

6. Two year averages for spring stand counts ranged from a high of 10.3 plants per square foot to a low of 4.1 plants per square foot, while the two year averages for dry weight yields of clover at first cutting ranged from a high of 1270 pounds per acre to a low of 530 pounds per acre.

7. There was a high correlation between spring stand counts and first cutting hay yields for plant counts of up to ten plants per square foot. No significant correlation was found for counts of ten or more plants per square foot. It is believed that stands of eight to ten strong plants per square foot in the spring growing season will give maximum yields.

8. Plant competition is believed to be the primary factor responsible for loss of plants during the seedling year. On plots receiving the better treatments, this competition was among red clover plants with gradual elimination of the weaker plants. On the poorer treatments, taller growing weeds such as ragweed provided the major competition.

9. Occurrence of Fusarium root and crown rot is considered to be the primary factor responsible for after harvest stand losses in the hay year. Other factors contributing to loss of stand after harvest were insects, drought, low fertility, and pre-harvest shading by timothy.

10. Fusarium root and crown rot was the most serious disease present and was not affected by clipping treatments. The fall leaf spot diseases were much less prevalent in clipped plots and at one location a foliage disease known as "Black Patch" was controlled effectively by clipping.

11. Insects causing appreciable damage were the spittle bug in May of the hay year, and the clover head caterpillar in October of the seedling year and in the following June. These insects were not affected by management practices.

12. Carbohydrate root reserves in samples collected in the latter half of December were affected by management treatments. These were, in

general, highest on plots which were clipped around the first of September, and on which weeds were controlled and over mature growth prevented. Nitrogen root reserves appeared to be affected more by other factors or growing conditions. Although there was a negative correlation between carbohydrate and nitrogen percentages, they both tended to be higher in the larger and heavier roots.

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SECTION IX. APPENDIX

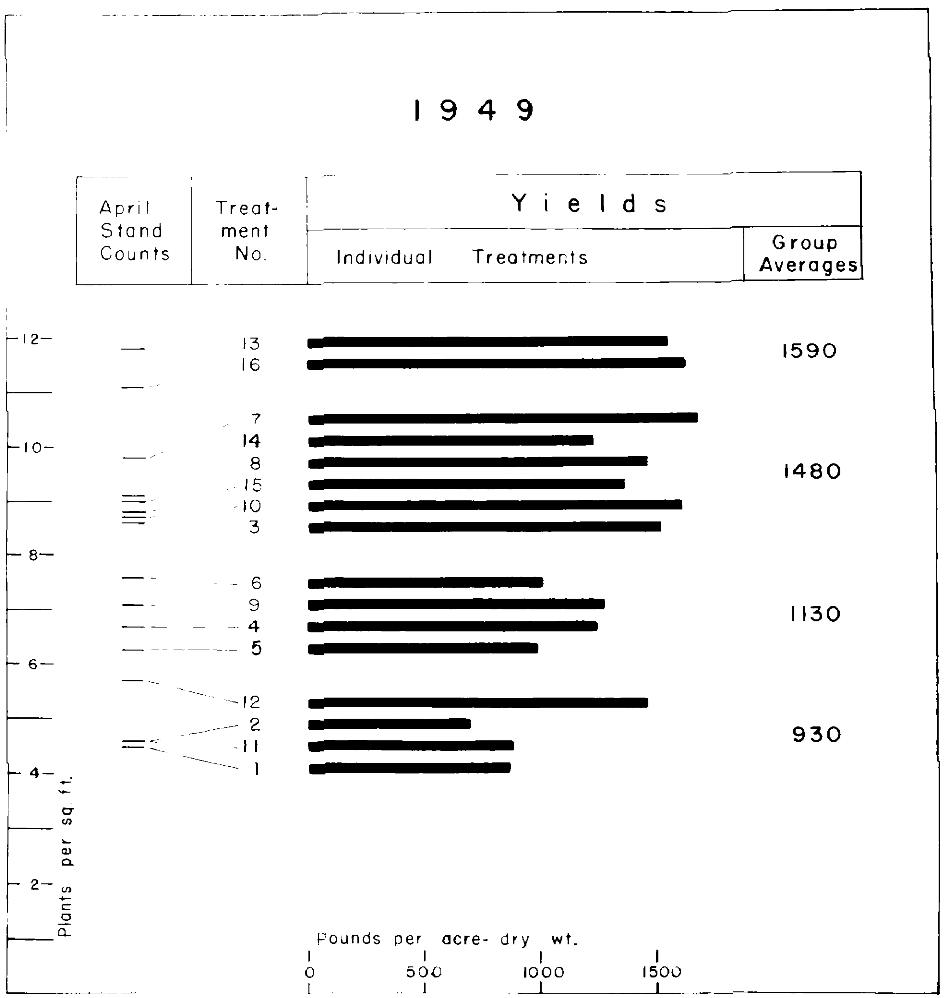

Figure 1. Treatment averages of spring stand counts and first cutting dry weight yields of red clover for four locations in 1949. Treatments are grouped on the basis of stand counts of 4 to 6, 6 to 8, 8 to 10, and 10 to 12 plants per square foot. The highest stand count is for treatment 13 with 11.8 plants per square foot and the lowest is for treatment 1 with 4.5 plants per square foot. The highest yield is for treatment 7 with 1680 pounds per acre and the lowest is for treatment 2 with 700 pounds per acre. The high yield for treatment 12 is believed to be due to exceptionally mild winter weather following cutting and removal of growth in early November.

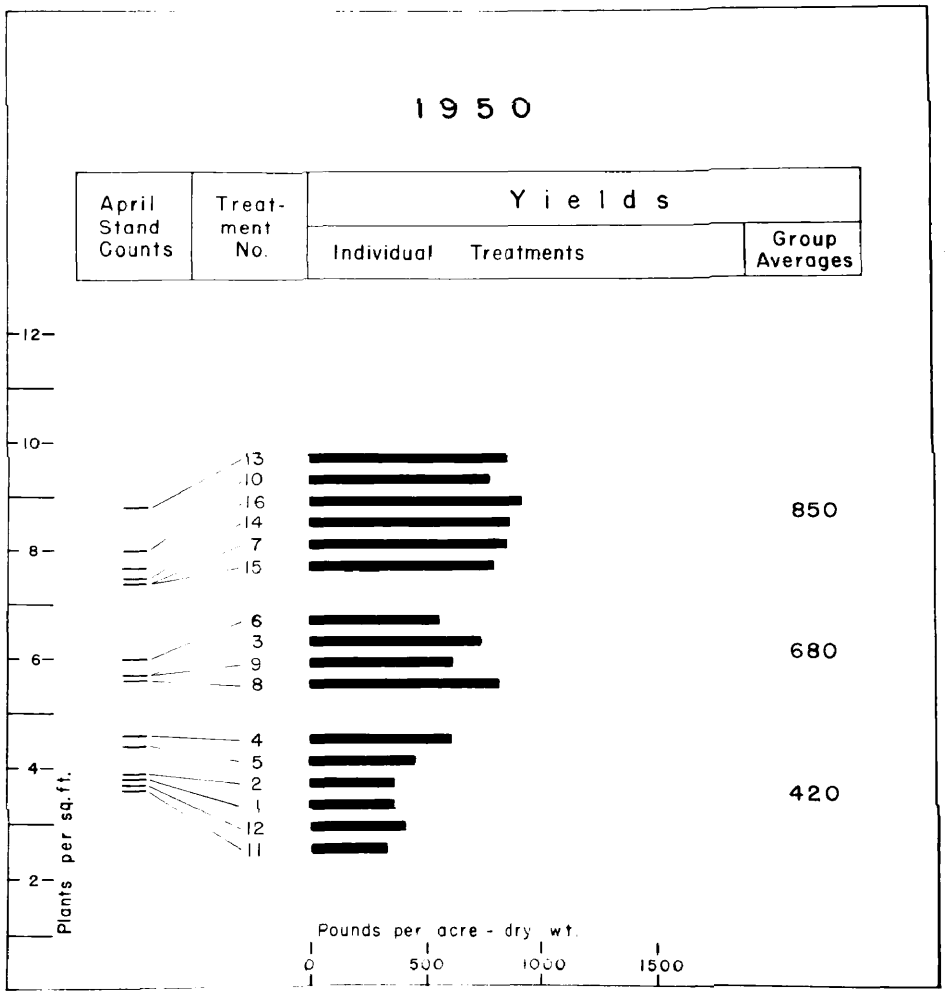

Figure 2. Treatment averages of spring stand counts and first cutting dry weight yields of red clover for four locations in 1950. Treatments are grouped on the basis of stand counts of 3 to 5, 5 to 7, and 7 to 9. The highest stand count is for treatment 13 with 8.8 plants per square foot, and the lowest is for treatment 11 with 3.6 plants per square foot. The highest yield is for treatment 16 with 930 pounds per acre, and the lowest is for treatment 11 with 330 pounds per acre. A severe spittle bug infestation at two locations caused a serious reduction in yields from the 1949 averages.

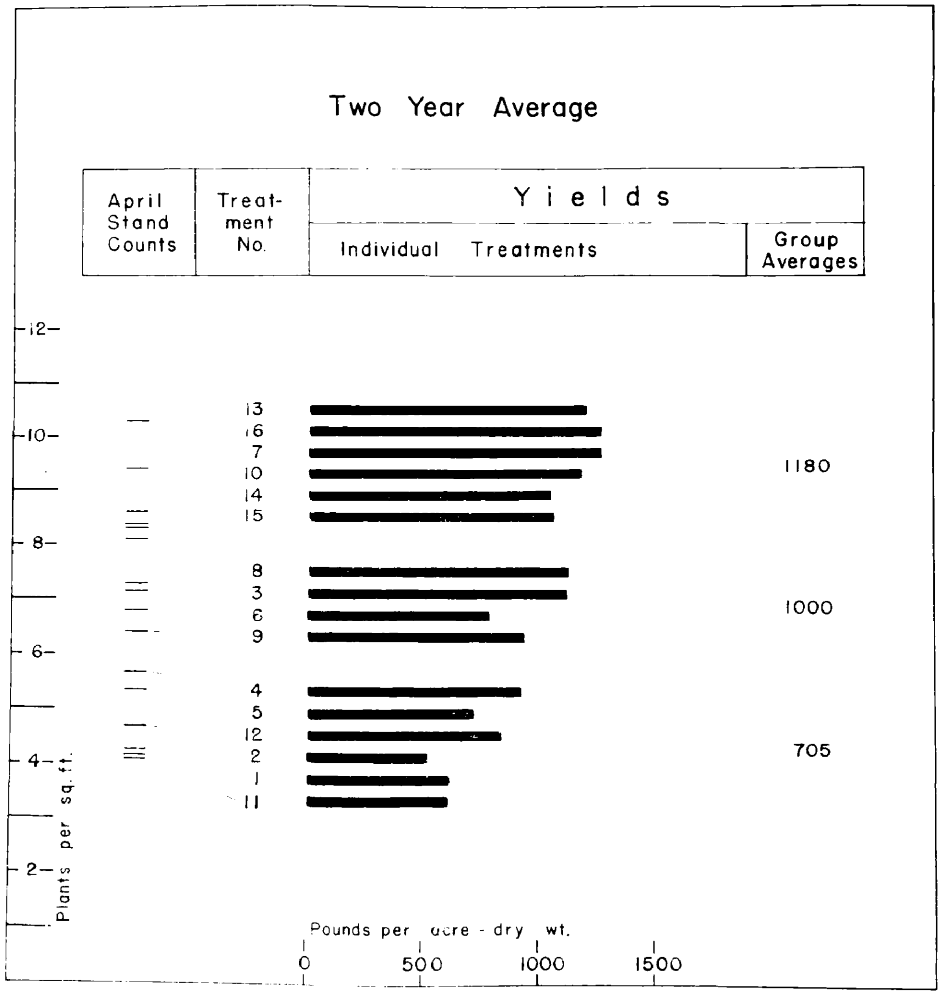

Figure 3. Treatment averages of spring stand counts and first cutting dry weight yields of red clover for eight locations in 1949 and 1950. Treatments are grouped on the basis of stand counts of 4 to 6, 6 to 8, and 8 to 10 (including treatment 13 at 10.3). The highest stand count is for treatment 13 with 10.3 plants per square foot, and the lowest is for treatment 11 with 4.1 plants per square foot. The highest yield is for treatments 16 and 7 with 1270 pounds per acre, and the lowest is for treatment 2 with 530 pounds per acre.

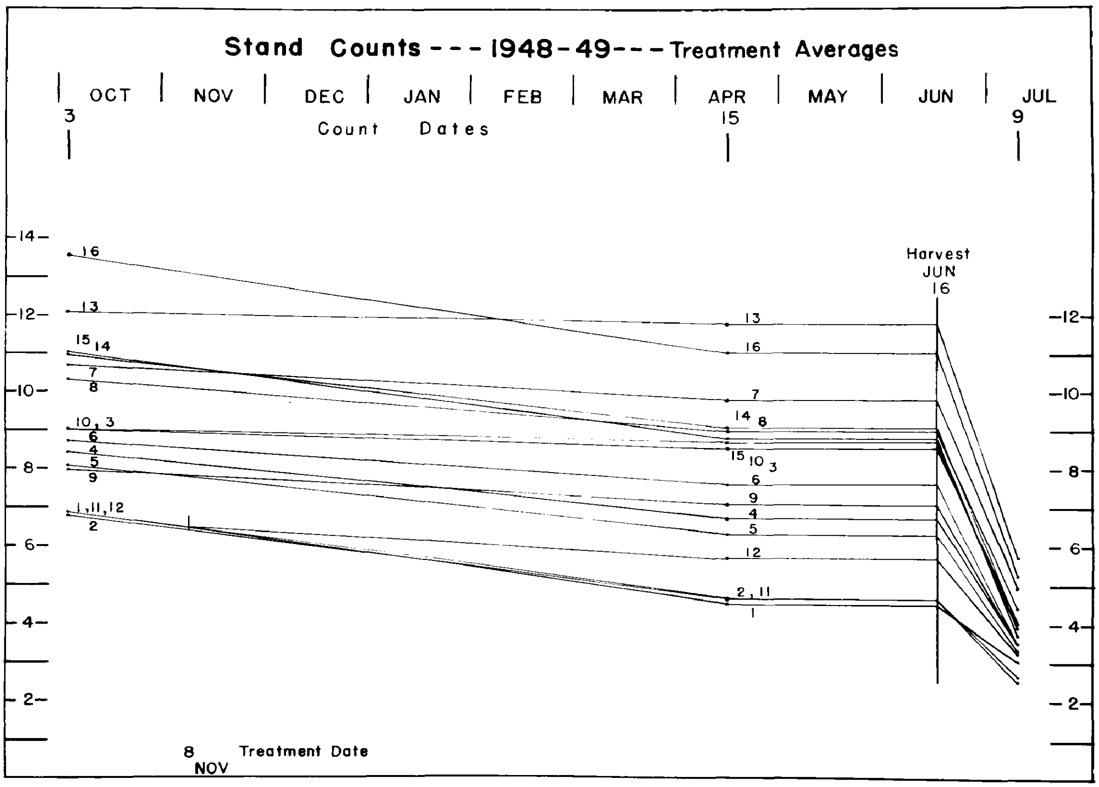

Figure 4. Treatment averages of stand counts made at four locations in the 1948-1949 season. Some loss occurred in all treatments during the fall and winter. Spot check counts indicated almost no loss during the spring growing season, but all treatments suffered a stand loss of approximately 50% within a short time after harvest. Eight out of the nine top treatments are those in which growth was cut and removed one or more times during the summer. The top three are those in which growth was cut and removed two or more times. The two unclipped treatments, 1 and 2, are among the lowest.

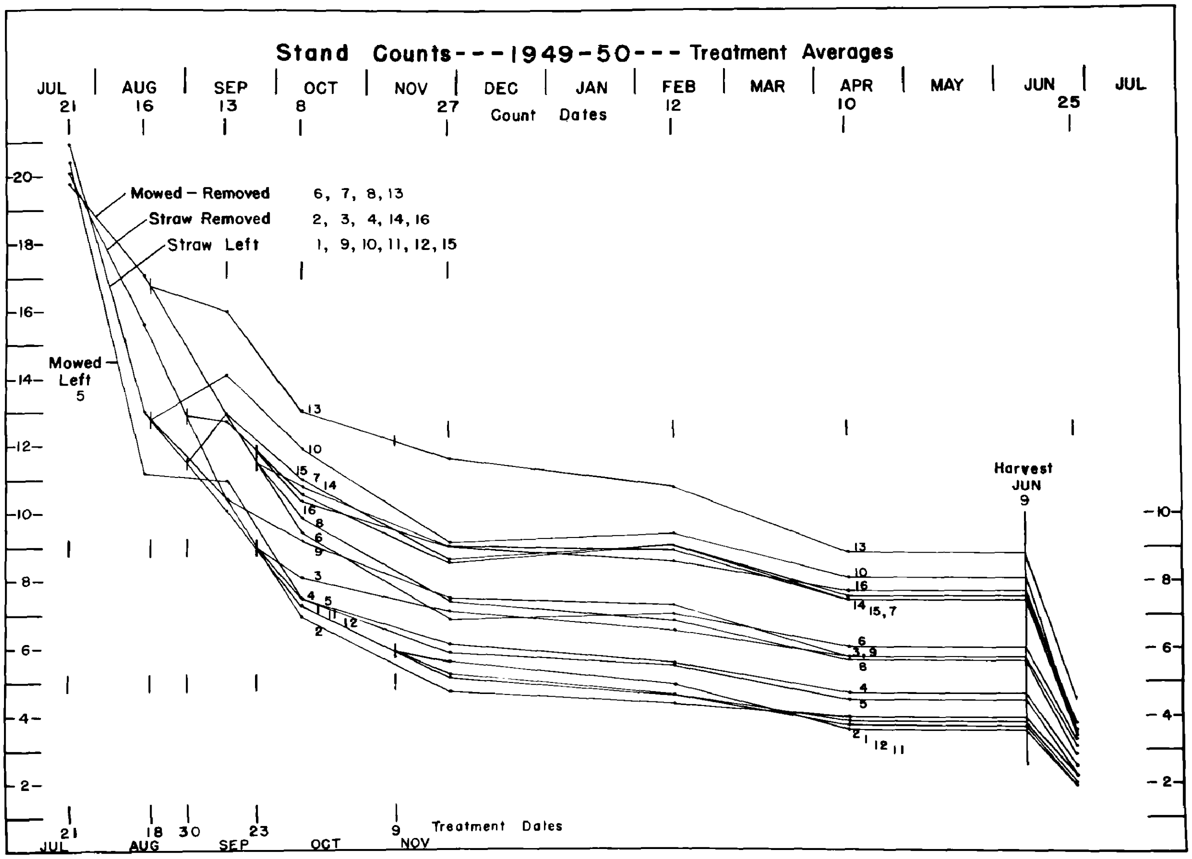

Figure 5. Treatment averages of stand counts made at four locations in the 1949-1950 season. This graph is intended to show that there is loss of stand under all treatments during the summer months, and that management practices can cause considerable variation in the amount of this loss. By November the treatments have segregated into high, medium, and low groups and they remain in these groups throughout the winter and the following growing season.

All treatments in the high group involve clipping and removal of mowed growth one or more times during the summer. The lowest treatments include those which were not clipped until November and those which were left unclipped.

As in the previous year, there was an after harvest stand loss of approximately 50% for all treatments.

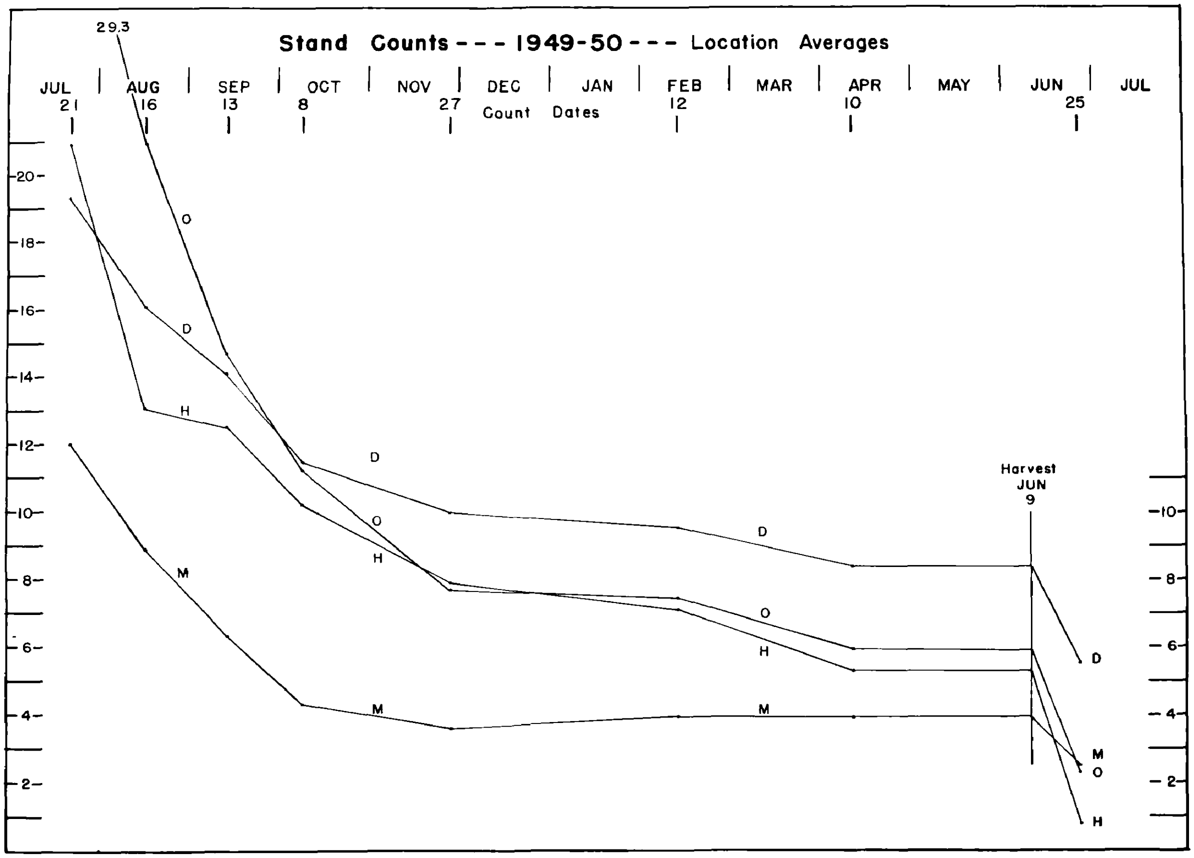

Figure 6. Stand count averages of all treatments for each location used in the 1949-1950 season. At the "D" location, there were few weeds to compete with the clover, and there was also a good fertility level. Weeds were quite prevalent at the "O" and "M" locations and moderately so at the "H" location. Winter loss was greater in Frederick county at "H" than in Montgomery county at "O". It was least on the eastern shore at locations "D" and "M".

The excessive after harvest stand losses at "O" and "H" are believed to be due to a severe spittle bug infestation which stunted and weakened the clover plants prior to harvest. The clover head caterpillar and dry weather also contributed to after harvest loss at the "H" location.

The photographs used for Figures 7 through 18 were all taken on April 28, 1950 at the "O" location.

Fig. 7. Treatment 6. Cut and removed July 16, 1949. Cut too early; low stand count, excessive weed and grass competition.

Fig. 8. Treatment 10. Cut and removed Aug. 16, 1949. Good treatment; shows good stand count.

Treatments 14 and 15, cut and removed Aug. 27, 1949 are similar in appearance.

Fig. 9. Treatment 3. Cut and removed Sept. 22, 1949. Cut late; average stand count.

Fig. 10. Treatment 12. Cut and removed Nov. 8, 1949. Cut too late; stand count severely reduced by weed competition and "Black Patch" disease.

Fig. 11. Treatment 16. Cut and removed twice - Aug. 27, and Sept. 22, 1949. Shows high stand count and good weed control. Treatment 7, cut and removed July 16, and Sept. 22, 1949 is similar in appearance.

Fig. 12. Treatment 13. Cut and removed three times - July 16, Aug. 16, and Nov. 8, 1949. Shows highest stand counts, but slightly smaller plants.

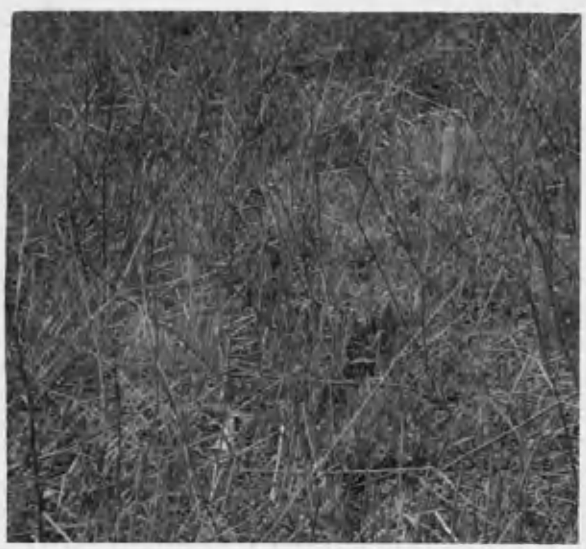

Fig. 13. Treatment 2. Straw removed; no further treatment. Shows almost complete loss of stand due to weed competition and "Black Patch" disease. Weed stalks still standing.

Fig. 14. Shows plastic markers used to mark plant count areas. These have been in the field nine months.

Fig. 15. Comparison of treatments 4 and 3. Both cut Sept. 22, 1949. Growth left on 4 and removed from 3. Shows distinct edge of plots and better stand where growth was removed.

Fig. 16. Comparison of treatments 10 and 2. 10 cut and growth removed on Aug. 16, 1949; 2 not cut. Shows good stand on 10 and only dead weed stalks and some grass on 2.

Fig. 17. Comparison of treatments 6 and 14. 6 cut and growth removed July 16, 1949 - too early for single clipping; 14 cut and growth removed August 27, 1949 - good time for a single clipping.

Fig. 18. Comparison of treatments 12 and 16. 12 cut and growth removed Nov. 8, 1949 - too late for single clipping; 16 cut and growth removed twice - Aug. 27, and Sept. 22, 1949. Note distinct plot outlines of treatment 12.