FACTORS AFFECTING CAROTENE CONTENT OF ALFALFA

by

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TABLE OF CONTENTS

INTRODUCTION

Alfalfa hay has long been considered as of exceptional value in animal nutrition. Its value is partly due to its importance as a source of vitamin A in the form of its precursor, carotene, in addition to its protein, carbohydrate, fat, and mineral content. The importance of vitamin A upon the health, reproduction, lactation, growth, and fattening of animals as well as its value in the human diet has been pointed out by Black et al. (1939), Meigs (1939), and Booher et al. (1939). The prominence of alfalfa as a hay crop in Kansas is emphasized by the fact that 795,000 acres were devoted to hay production in 1945.[1] The increase in the artificial drying or dehydration of alfalfa, and the increasing utilization of alfalfa meal as a source of carotene, or provitamin A, is well recognized.

Considerable experimentation has been done on the preservation of carotene content of alfalfa including methods of field curing, dehydration and storage. It appears that a study of factors affecting the carotene content of the alfalfa plant would be of great economic and practical importance both in obtaining its highest feeding value and in increasing its value as a source of carotene.

The objective of this study was to determine the inherent nature of alfalfa in respect to carotene content, the influence

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[1] Supplied in letter by Mr. H. L. Collins, State Agricultural Statistician for Kansas.

of seasonal and environmental factors, effect of fertilizers, relationship of carotene to protein content, and to determine what methods or practices could be used to increase the vitamin A potency of alfalfa.

REVIEW OF LITERATURE

Relatively little work has been done concerning the inherent nature of alfalfa in respect to carotene content. Ham and Tysdal (1946) reported that alfalfa strains and hybrids differed inherently in their carotene content, as some of the strains ranked consistently high while others were consistently low. It appears that an increase in carotene content may be obtained by selecting strains with a higher percentage composition of carotene. Mitchell (1946), although not directly concerned with a study of the carotene content of alfalfa, presented data which indicated that four varieties (Buffalo, Ranger, Grimm, and Turkestan) did not differ consistently in carotene content. Hamner and Maynard (1942) reported that many investigators are in agreement that carotene content of tomatoes varies markedly with variety.

Johnson (1936) has shown that leafhopper-yellowed alfalfa contained much less carotene than did green alfalfa of the same variety grown from an adjacent area upon which leafhopper injury had been eliminated. These results were confirmed by Ham and Tysdal (1946). They stated that, in general, plants with less visible leafhopper injury had a higher carotene content. It appears evident that selection of strains resistant to leaf-

hopper yellowing would be of importance in increasing carotene content of alfalfa in areas subjected to leafhopper infestations.

Hauge (1934) found the vitamin A potency of the leaves about ten to fourteen times that of the stems. Ham and Tysdal (1946) reported that on the average the leaves of a number of strains of alfalfa contained 77.1 percent of the total carotene contained in the plant, varying from 59.0 to 94.6 percent. They reported a range in proportion of leaves to stems from 36.8 percent leaves to 70.3 percent leaves in plants of different strains. Thus the percentage of leaves is an important factor determining the carotene content of alfalfa.

Johnson (1938) reported that after five months' storage in a warm place, non-yellowed, high-vitamin alfalfa carried its superiority through storage better than leafhopper-yellowed, low-vitamin alfalfa. It appears that anything that can be done to increase the initial carotene content of alfalfa will be of value even after storage of hay under conditions favorable for rapid destruction of carotene.

In respect to stage of growth, it was demonstrated by Hauge (1934) that vitamin A value of young alfalfa (10 to 12 inches high) was much greater than that of alfalfa in the bloom stage. Douglas et al. (1933) presented evidence which indicates that alfalfa cut at the early bloom stage contains more carotene than that cut at other stages of growth. Snyder and Moore (1940) reported that carotene content of herbages including alfalfa is much greater during the earlier stages of growth than after they reach the usual harvesting stage of maturity. Ham and Tysdal

(1946) obtained results showing that new growth contained from two to five times as much carotene as did older alfalfa plants of the same strain. In general the newer, more succulent growth is high in carotene content and low in amount of dry matter. They also reported that the leaves found in new growth were exceedingly high in carotene as compared to those found in older growth, while the carotene content of the stems of young material was little higher than in the older stems. Meenen's results (1945) indicate that carotene is continually being built up in the leaf, at least until the blooming period, as he found that older leaves from an alfalfa plant contained more carotene than new leaves. He stated that probably the reason for the reduction in carotene content as the plants mature is due to loss of leaves.

The literature dealing with environmental factors and fertilizer treatments affecting carotene content of plants is limited and confusing. Very little experimental work has been done on the effect of these factors on carotene content of alfalfa. Therefore, it was decided to present a review of the literature concerning the influence of fertilizer treatments and environmental conditions on the carotene content of other crops as well as alfalfa with the view that it may bear some relation to the effect of these various factors on the carotene content of alfalfa.

Smith and Wang (1941) reported that the carotene content was greatest in grassland herbage when the plants were young and tended to decline at flowering, followed by further decreases on ripening. Dutcher (1932), studying the vitamin content of leafy

plants and vegetables, found that young, rapidly growing tissue contained a higher vitamin A value than old mature plants. The carotene content of plants tends to reach a maximum at an early stage of growth, either before or at the beginning of flowering, followed by a marked decline (Virtanen, 1936). Hamner and Maynard (1942) reported that many investigators are in agreement that the vitamin A potency of tomatoes increases during ripening. The oldest leaves in turnip greens appeared to be lower in carotene than younger leaves as indicated by Bernstein et al. (1945). They found no correlation between amount of growth and carotene content.

Atkeson et al. (1937) studied a number of pasture plants including alfalfa for their carotene contents at various stages of growth, and at regular intervals during pasture season. All showed a relatively high carotene content early in the summer. The carotene content tended to decrease markedly during the midsummer, followed by an increase in carotene content during the fall rains. Moon (1939) found the carotene content of grass was low during the spring, but he accounted for this by the fact that growth was slow to start due to an unusual drought, and the samples were contaminated with dead grass from the previous season. Carotene increased in June and showed marked increases in autumn.

In 1939 Powers reported the addition of borax to soils presumably deficient in boron increased the vitamin A content 30 percent as well as increasing the yield of alfalfa. These results were confirmed by Maynard and Beeson (1943). They reported

that Beeson in unpublished work showed a significant increase in carotene content of alfalfa due to the application of boron to a soil deficient in that element. Mitchell (1946) studying the enzymic nature of the carotene-destroying system of alfalfa presented data representing one determination from two series of alfalfa fertility plots which indicated only a slight difference in carotene content due to the application of fertilizers.

Honeywell and Dutcher (1930) observed the greater the chlorotic condition, the less vitamin A is found. Spinach grown on soils that were limited in manganese supply contained less vitamin A than normal spinach. Whittemore (1934) reported no appreciable effect upon the vitamin A value of spinach by the application of nitrogen, phosphorus, potassium, and manganese on soils deficient in these elements, even though yields were very low without the addition of fertilizer. Ijdo (1936) showed that a higher level of nitrogen resulted in a greater carotene content of spinach. An increase of potassium in the soil caused a decrease in carotene content at low nitrogen levels, but very little at high nitrogen levels. The vitamin A value of various vegetables was found by Scheunert and Wagner (1939) to be affected little by fertilization, except under conditions of extreme depletion of mineral supply. They found that the average carotene content deviated only a small amount under the most varied conditions of origin and season.

Hamner (1945) stated that the evidence with respect to the relation of vitamin content of plants and any one of the major elements is not conclusive. He reports that little work has been

done dealing with the influence of minor elements on the vitamin content of plants. Hamner found no appreciable effect of many different fertilizer treatments on the carotene content of turnip greens even though these treatments caused marked influence on growth and development of plants. He stated that any mineral nutrient deficiency which will cause chlorosis in plants is likely to cause a decrease of carotene content of the leaves. The treatments which give the highest crop yield per acre are very likely to give the highest vitamin yield per acre.

Smith and Wang (1941) found that the effects of manuring on grassland herbage were not spectacular except that ammonium sulphate increased the carotene content of rye grass. They reported the effects of lime and slag on carotene content were much less marked, and there were no obvious effects due to environment. Moon (1939) reports that nitrogen increased the carotene content of pasture grass 28 percent. Carbonate of lime or superphosphate did not affect the carotene content significantly. As a result of increased dry matter production, the yield of carotene per unit area was increased by use of fertilizers. Maynard and Beeson (1943) pointed out that increase in carotene content due to the application of nitrogen as reported by Moon was probably due to the stimulation of growth of herbage. Since carotene content is highest in young actively growing tissue, it would appear that any factor that stimulated growth might indirectly increase carotene content.

Ellis and Hamner (1943) indicated that increasing supplies of nitrates resulted in increasing carotene content of tomatoes,

but the differences were slight. No measurable effect on carotene was obtained on tomatoes grown in nutrient solutions varying widely in the supply of calcium, magnesium and sulphates, even though great variations in yield and growth of the plants occurred. Little effect on carotene was observed when plants were grown in different localities and in different soils. In an experiment designed to study the influence of various micronutrients on carotene content of tomato fruits, Lyon et al. (1943) found no effect of these elements on the carotene content of the fruits. They also reported that environmental factors did not influence the provitamin A value significantly.

Barnes (1936) found no influence of nitrogen, phosphorus, potassium, manganese, or magnesium upon the carotene content of carrots. He also concluded that soil moisture, air temperature, and length of day had no effect on vitamin A. Regardless of the nature of fertilization, the carotene content of carrots was fairly uniform (Scheunert and Wagner, 1939). Ott (1937) found that the carotene of carrots increased to an optimum with potassium fertilizers, but further addition of fertilizers caused a decrease. Swanson et al. (1940) found the vitamin A content of sweet potatoes was not significantly affected by the type of fertilizer used in the soil.

Pfutzer and Pfaff (1935) reported that full fertilization on a considerable variety of plants gave an increase in carotene content and plant yield, thus resulting in a very high carotene yield per unit area. Guthrie (1929) demonstrated that an insufficient supply of nitrate nitrogen decreased the amount of

carotene present in the soybean plant.

A significant correlation between carotene content of pasture grasses and percentage of crude protein present was reported by Thomas and Moon (1938). Later work by Moon (1939) indicated that carotene was most closely associated with protein content under fertilizer treatment that had no effect on these two constituents. Under fertilizer treatments that produced increases in carotene or protein, the correlation between the two was not so close. It appeared to Moon that the relationship between carotene and protein is not a direct one but merely a secondary effect. Wynd and Noggle (1945) found that in the case of immature oat leaves the percentage yield of carotene seemed to be affected by the same soil properties which influence the percentage yield of protein.

Wynd and Noggle (1945) showed that higher pH values increased the concentration of carotene and yield of dry matter of immature oat leaves. This was apparently due to increasing amounts of nitrogen present in soils with the higher pH values. Virtanen et al. (1933) found that plants grown at an optimum pH have a higher carotene content than those grown under a more acid condition, and the greatest amount of carotene is found where the nitrogen supply is sufficient to secure maximum growth. It is postulated as a general principle that maximum carotene content and maximum growth go hand in hand so that carotene must be regarded as an essential growth factor in the plant. Similar results were reported by Virtanen again in 1936. Moon (1939) reported that carbonate of lime applied in sufficient amount to

raise the pH of the soil from 5.63 to 7.31 did not increase the carotene content of pasture grass.

In a review of the literature, Maynard and Beeson (1943) indicated that the vitamin content of plants depend upon a number of factors such as varietal differences, climatic and seasonal variations, and, to a small extent, fertilizer practices. They stated that:

> A critical study of the mass of data dealing with the effect of soil type and nutrient supply suggests that both have a much less influence than variety and climate. It is also clear, however, that only a few of the many possible relationships have been studied.

It appears that the influence of fertilizers and soil factors on vitamin content of plants is not conclusive.

Hamner and Maynard (1942) found that environmental factors, such as exposure to light, produced variations in the carotene content of tomatoes. Smith (1936) found that tomatoes grown in the greenhouse did not contain as high a quantity of carotenoid pigments as did the tomatoes grown in the field. Full exposure to light favored the maximum carotenoid content. Pfutzer and Pfaff (1935) reported that the carotene content and the plant yield for some plants were increased by additional neon illumination in the greenhouse. It was observed by Ellis and Hamner (1943) that tomatoes grown in the greenhouse during either summer or winter were always lower in carotene than when grown out-of-doors. They also noted little effect on carotene when plants were grown in different localities.

On the other hand, Bernstein et al. (1945) indicated that turnip greens grown in the greenhouse during the wintertime had

much higher carotene values than any of those produced during the summer. Differences in carotene content at different dates of harvesting and variations occurring due to location indicate that environmental factors are of great importance in determining vitamin values. Guthrie (1929) found that increasing the duration of illumination results in a decrease of total carotenoids. Reducing the light intensity to 12 percent of normal sunlight results in an increase in carotene. He also reported that a reduction in nitrate supply results in an increase in percentage of carotene under the light conditions prevailing in the greenhouse during the winter. The opposite is true in the spring, with higher light intensities.

From a review of the literature it appears that, in general, the carotene content of plants has not been found to be consistently influenced by different fertilizer treatments. However, any nutrient deficiency that will cause visible chlorosis of the plant will decrease carotene. Climatic and environmental conditions such as exposure to light apparently affect vitamin A content of some plants. The application of boron seems to be beneficial in increasing the carotene content of alfalfa on soils deficient in that element. Young alfalfa seems to be higher in percentage composition of carotene than older alfalfa, and the carotene content fluctuates with the season, generally being highest in the spring and in the fall. It appears that carotene content is influenced by the inherent nature of alfalfa. An increase in carotene content seems possible. Ham and Tysdal (1946) pointed out:

It appears the breeder may increase the carotene content of alfalfa by selecting for (a) resistance to leafhopper yellowing, (b) a high percentage of leaves, (c) retention of leaves and green color which involves selection for resistance to all leaf diseases, and (d) strains which have a higher percentage composition of carotene.

MATERIALS AND METHODS

Equipment and facilities for experimentation was furnished by the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture; the Departments of Agronomy and Chemistry, Kansas Agricultural Experiment Station; and the Kansas Industrial Development Commission. Carotene determinations were made under the supervision of the Department of Chemistry and in their laboratories.

The plants sampled in this investigation were grown either in the greenhouse or on a sandy loam soil on the Soil Conservation Service Nursery, with the exception that samples were obtained from Cherokee type soil from the alfalfa fertility plots on the Southeast Kansas Experiment Field located at Columbus, Kansas.

The sampling of plant material involved the collection of samples as quickly as possible and bringing them directly to the laboratory where the samples were hot-water blanched for ten minutes. This was done to inactivate the enzyme system which causes rapid destruction of carotene in alfalfa (Mitchell and Hauge, 1946).

The plant material was dehydrated in a circulating hot-air oven at a temperature of 70° F. for periods of time ranging from three to five hours for different series. The material was ground in a Wiley mill, thoroughly mixed, divided, percentage of moisture determined, and subsequent carotene analysis carried out on each of the two duplicate samples. Unless otherwise stated, all samples were prepared in this manner. Whenever it was necessary to store the samples before analyzing, they were preserved in air-tight bottles at a temperature of -25° C.

Carotene determinations were made by the method of Silker, Schrenk, and King (1944) which consists of extracting the dehydrated sample with Skellysolve B and acetone; the extracts were concentrated and an adsorption column consisting of a mixture of magnesia and Hyflo Super-Cel was employed to adsorb the pigments. The carotene was separated from the other pigments by washing the column with a 4-percent solution of acetone in Skellysolve B. The various carotene fractions were not separated. Color density measurements were made on a Beckman quartz spectrophotometer. The total carotene was reported as milligrams per 100 grams of dry matter.

Protein determinations were made on some of the samples. These values were computed from the total nitrogen content as determined by the Kjeldahl method (Assoc. of Off. Agr. Chemists, 1940).

Inheritance Studies

During the summer of 1944, in order to determine the inherent variability of alfalfa plants, the leaves from 48 clonal lines (asexually propagated cuttings from a single plant) of three plants each were stripped and analyzed for carotene content. The leaves were stripped by grasping the stem of the plant near the base and drawing the hand upward, removing leaves and tips of stems. These plants were space planted in a field plot at random. At a later date the plants were sampled for carotene by carefully picking off the leaves, thus eliminating any portion of the stem or petiole.

On the basis of carotene determinations, 13 parental plants, part of which appeared to be high and others which appeared to be low in carotene content, were selected from the 48 lines. All of these lines were highly self-sterile, with desirable agronomic characteristics selected from a strain of Kansas Common.

Further tests to confirm the results of the original carotene analysis were carried out by sampling whole plant material from the 13 parental plants on three occasions during the summer of 1945.

The polycross progeny of the 13 maternal plants were analyzed for carotene content. These plants were the progeny from seed produced on the highly self-sterile clonal lines that were subject to outcrossing with other lines grown in the same nursery, thus only the maternal parent was known. Two determinations were made by sampling the progeny consisting of two groups of 50 plants of

each line produced in the greenhouse. Other determinations were made by gathering stems at random and compositing the material from plants growing in two-row plots which were replicated four times in the polycross progeny yield test.

Greenhouse Fertility and Soil Moisture Studies

A controlled experiment was conducted in the greenhouse under two levels of soil moisture referred to in this study as "high" and "low". Each moisture level was divided into limed and unlimed treatments which were further subdivided into no fertilizer treatment, nitrogen, phosphorus, and nitrogen plus phosphorus treatments. This made a total of 16 combinations of moisture and fertility levels with six pots in each treatment.

The soil used in this test was prepared by mixing five parts of Geary clay loam obtained from an eroded hillside on the Agronomy Farm with one part of washed silica sand. The soil had a pH of 5.02 as determined by a potentiometer using the glass electrode method (Assoc. of Off. Agr. Chemists, 1940). A qualitative test indicated only a small amount of available phosphorus present, and previous experiments indicated that crops responded to phosphorus applications. Finely chopped wheat straw was mixed with the soil at the rate of three and one-half tons to the acre to lower the available nitrogen, as the previous crop was a green manure crop of soybeans.

The wilting coefficient of the prepared soil was determined to be 10.98 percent by calculation from the moisture equivalent

as determined in the usual manner. The field water-holding capacity was assumed to be 32 percent. This was determined by saturating 12-inch columns of soil one inch in diameter with water and allowing the columns to drain on sand in a perpendicular position for 24 hours before determining the percentage of moisture.

The high soil moisture pots were maintained near the field water-holding capacity (25%), and the low level was maintained at 13 percent, which was slightly above the wilting coefficient. The limed pots received finely divided $CaCO_3$ applied at the rate of two and one-half tons per acre. One hundred pounds per acre of available nitrogen was added as ammonium nitrate to pots receiving nitrogen. The phosphorus treatment consisted of adding the equivalent of 100 pounds per acre of available phosphoric acid added in the form of 43-percent treble superphosphate. The nitrogen plus phosphorus treatments received the equivalent of 100 pounds of available nitrogen and 100 pounds of available phosphoric acid per acre.

The equipment used in this experiment was designed by Grandfield (1941). It consisted of seven-inch glazed pots equipped with a perforated copper coil to insure uniform distribution of water throughout the soil mass. The watering was done as illustrated in Plate I. To maintain the soil at the desired moisture levels, they were brought to their proper weight each day by using a scale which weighed to an accuracy of 10 grams. The pots were moved daily to a new location on the bench in such a manner that at the end of every eight days each pot had made a

EXPLANATION OF PLATE I

The scales, watering device, and technique of watering the potted alfalfa plants to maintain the soil at the desired moisture levels are shown in Plate I.

PLATE I

complete cycle and was at its original position, thus reducing to a minimum the effects of light and temperature variations in the greenhouse due to location.

To eliminate the inherent variability of different alfalfa plants, cuttings from a single plant were used in this experiment. The plants were allowed to develop to the full-bloom stage, from which 96 plants of nearly the same size and stage of development were selected for the experiment. While the plants were becoming established, the soil moisture was maintained at 20 percent. After the plants had become established, all the top growth was removed. The various fertilizer treatments and lime were applied by placing the material in small holes probed at intervals and at different depths in the soil around the plant. Half of the pots in each treatment were reduced to the 15-percent moisture level, and half were raised to the 25-percent level to obtain the desired treatments previously mentioned. After the first samples were taken, these moisture levels were changed to 12 and 30 percent, respectively, in order to obtain greater differences.

After the plants had developed to the full-bloom stage they were sampled by clipping off the top growth and compositing the material from three pots in each treatment, thus giving duplicate samples in each treatment. In addition to carotene determination, the yield of oven-dry hay and the percentage of leaves were determined by separating the leaves from the stems after drying.

The experiment was continued and at the second cutting the plants were harvested in the same manner.

Upon reaching the bloom stage the third time the plants were sampled by carefully picking off the leaves and compositing the material from all six pots in each treatment. In this case the leaves and stems were analyzed separately, from which the carotene content of the whole plant was calculated. Observations on stage of maturity and height of each plant were recorded at each sampling date. The pH from a representative soil sample of each replication was determined at the end of the experiment by the glass electrode method.

Field Fertility Studies

Carotene determinations were made on alfalfa obtained from a series of fertility plots at the Southeast Kansas Experiment Field located at Columbus, Kansas. Approximately one-pound samples were secured by collecting a number of random samples within each plot. The plants were immediately frozen with cracked dry ice and placed in an insulated container to prevent loss of carotene until they reached the laboratory. Soil samples for pH determinations were taken from each plot in a similar fashion.

Field Soil Moisture Studies

Relatively dry areas of soil were observed occurring in spots in the polycross yield nursery due to a faulty overhead

sprinkling system following a week to ten days after the plot was irrigated during a dry period in July, 1946, when little rainfall was received. Samples for carotene analysis were obtained from plants of the same strain growing adjacent to each other, part of which received sufficient moisture for normal growth while the others were growing under drought conditions. Seven strains were sampled.

It was observed that there was a slight difference in growth between the outside and inside rows of Kansas Common used as a border plot consisting of two rod rows spaced eight inches apart. The more luxuriant growth in the outside row was assumed to be due, at least partially, to a more abundant moisture supply. In this case, samples were obtained by picking off the leaves from each row. The stage of maturity and height of the plants in all instances were recorded.

EXPERIMENTAL RESULTS

The results herein reported are concerned with studies on the inherent nature of alfalfa in respect to carotene content, seasonal influence, the effect of various fertilizer treatments, the influence of soil moisture, and the relationship of carotene content to percentage composition of protein in alfalfa.

Inheritance Studies

Since no direct data were available on the variations in carotene content of different varieties and strains of alfalfa

when study on this problem began, it was considered desirable to determine if the carotene content of alfalfa is inherited and to obtain as much information as possible concerning the mode of inheritance. This would be of value in breeding for an increased carotene content.

The results of two carotene determinations of 48 clonal lines are presented in Table 1. The analysis of variance was calculated on these data by Paterson's (1939) method, and the results are shown in Table 2. A significant difference at the 5-percent level between the carotene content of the clonal lines was obtained. A difference between means (Table 1) greater than 8.20 is significant at the 5-percent level, while a difference of 10.77 is required for significance at the 1-percent level. The means of the two determinations ranged from 48.40 milligrams of carotene per hundred grams of dry matter to 67.95. The highest ranking mean differed statistically only from those lower than twenty-fourth in rank. The difference in carotene content between dates is largely due to the difference in sampling technique.

Hauge (1934) and Ham and Tysdal (1946) have reported that most of the carotene is found in the leaves of the alfalfa plant. As these determinations represent analysis of the leaves only, it indicates there was actually a difference in the concentration of carotene between the plants and the differences obtained were not due merely to variations in the percentage of leaves of the plants.

Table 1. The carotene content of leaves from forty-eight clonal lines of alfalfa on samplings made in 1944 from field plots.

Clonal line number	Milligrams of carotene per 100 grams of dry matter			
	Sampled on July 24, 1944 leaves stripped[1]	Sampled on Sept. 9, 1944 leaves picked[2]	Average	Rank
K-38-43	40.1	95.8	67.95	1
K-38-50	40.1	90.5	65.30	2
K-38-55	40.3	89.7	65.00	3
K-38-24	44.8	84.8	64.80	4
K-38-18	44.5	84.2	64.35	5
K-38-39	42.4	84.7	63.55	6
K-38-58	42.1	84.3	63.20	7
K-38-14	40.3	85.3	62.80	8
K-38-26	41.8	83.5	62.65	9
K-38-28	45.1	80.1	62.60	10
K-38-64	38.4	86.4	62.40	11
K-38-59	39.5	85.2	62.35	12
K-38-22	40.9	83.6	62.25	13
K-38-9	41.3	81.9	61.60	14
K-38-23	42.0	81.2	61.60	15
K-38-47	44.0	78.8	61.40	16
K-38-3	40.0	82.1	61.05	17
K-38-10	41.2	80.6	60.90	18
K-38-60	40.9	79.9	60.40	19
K-38-8	30.0	90.0	60.00	20
K-38-17	43.1	76.9	60.00	21
K-38-38	41.9	77.9	59.90	22
K-38-35	37.0	82.7	59.85	23
K-38-20	40.8	78.9	59.85	24
K-38-2	32.0	86.4	59.20	25
K-38-42	40.3	78.1	59.20	26
K-38-80	42.2	73.2	58.70	27
K-38-25	35.6	81.4	58.50	28
K-38-72	35.5	80.9	58.20	29
K-38-56	34.1	81.8	57.95	30

Table 1 (concl.).

Clonal line number	Milligrams of carotene per 100 grams of dry matter			
	Sampled on July 24, 1944 leaves stripped[1]	Sampled on Sept. 9, 1944 leaves picked[2]	Average	Rank
K-38-36	34.8	80.6	57.70	31
K-38-68	39.8	75.2	57.50	32
K-38-21	37.2	77.4	57.30	33
K-38-65	37.4	76.0	56.70	34
K-38-57	28.9	84.4	56.65	35
K-38-4	30.0	82.9	56.45	36
K-38-40	36.7	75.4	56.05	37
K-38-31	37.0	74.6	55.80	38
K-38-45	30.4	80.4	55.40	39
K-38-34	39.7	71.0	55.35	40
K-38-5	30.4	79.7	55.00	41
K-38-1	27.6	81.2	54.40	42
K-38-19	35.7	72.2	53.95	43
K-38-44	35.5	72.3	53.90	44
K-38-63	37.1	69.6	53.35	45
K-38-30	32.8	73.6	53.20	46
K-38-54	30.5	75.6	53.05	47
K-38-7	22.2	74.6	48.40	48

Least significant difference between averages at the 5-percent level = 8.20, and at the 1-percent level = 10.77.

[1]The leaves and tips of the stems were removed by grasping the stem of the plant near the base and drawing the hand upward.

[2]The leaves were picked from the petiole by hand.

Table 2. Analysis of variance of carotene content of leaves from forty-eight clonal lines.

Factor	Degrees of freedom	Sum of squares	Variance	Calculated F	Table readings of F (P = 0.05)	Table readings of F (P = 0.01)
Total	95	46,455.17				
Between clonal lines	47	1,437.80	30.591	1.7496*	1.62	1.98
Between sampling dates	1	44,195.58	44,195.580	2,527.7728**	4.05	7.21
Error	47	821.79	17.484			

* Significant.
** Highly significant.

From the 48 clonal lines, 13 plants - part of which appeared to be high and others which appeared to be low in carotene carotene content - were selected for further study. The results of two seasons' analyses, representing six determinations on the 13 parental plants are presented in Table 3. A significant difference between plants was obtained by the analysis of variance of these data. An F value of 2.48 was obtained, while an F value of 2.50 was required for significance at the 1-percent level. Thus the probability of obtaining these differences purely by chance is about one in one hundred times. The average carotene content ranged from 27.38 to 39.30 milligrams of carotene per 100 grams of dry matter. The four clonal lines ranking highest in carotene differed statistically from the three lines ranking lowest in carotene content. The differences between dates of sampling may be attributed to seasonal influence and variations in sampling technique. While the different dates of sampling do not constitute replication, it should provide a good check on the consistency of the behavior of the plants in respect to carotene content. It appears from these results that plants differ genetically in carotene content. This seems likely with the well known heterozygosity of alfalfa.

It appeared the best method of testing the performance of the parental plants was to determine the carotene content of their polycross progeny. It was recognized that only the female parent of the progeny would be known, as the parental plants were highly self-sterile, and the majority of the progeny would

Table 3. The carotene content of the thirteen parental clones.

Clone number	Milligrams carotene per 100 grams dry matter						
	Sampled on 8/24/44[1]	Sampled on 9/19/44[2]	Sampled on 9/27/44[3]	Sampled on 6/11/45[3]	Sampled on 7/13/45[3]	Sampled on 9/18/45[4]	Average
K-38-3	40.0	82.1	33.8	23.7	21.1	35.1	39.30
K-38-43	40.1	95.8	28.2	20.3	14.2	25.8	37.40
K-38-24	44.8	84.8	24.0	20.5	19.0	26.7	36.36
K-38-64	38.4	86.4	27.7	22.2	20.2	24.6	36.58
K-38-58	42.4	84.3	32.5	21.2	16.3	21.1	36.30
K-38-28	45.1	80.1	18.5	23.3	17.9	28.2	35.51
K-38-55	40.3	89.7	21.2	17.5	18.0	21.7	34.73
K-38-50	40.1	90.5	16.3	19.4	15.4	25.3	34.50
K-38-25	35.6	81.4	28.9	21.2	10.9	26.8	34.13
K-38-5	30.4	49.7	30.6	17.2	14.3	25.2	32.90
K-38-7	22.2	79.1	17.4	18.3	16.1	31.4	30.75
K-38-19	35.7	72.2	14.6	16.1	15.8	28.6	30.50
K-38-30	32.8	73.6	17.0	13.3	7.1	20.5	27.38

L S D between averages = 5.75 at the 5-percent level and 7.55 at the 1-percent level.

[1]Leaves stripped.

[2]Leaves picked by hand.

[3]Samples consisted of whole plants which were dehydrated but not blanched.

[4]Samples consisted of whole plants which were dehydrated (not blanched), ground and stored at 3° C. for one month.

be outcrossed plants. However, this would give the desired results of evaluating the ability of the maternal plants to transmit their character for carotene content to their hybrid progeny.

The results of this experiment representing five dates of sampling are presented in Table 4. The difference between strains was statistically significant at the 5-percent level and approaches significance at the 1-percent level, as shown by the analysis of variance of these data. A difference between the means in Table 4 greater than 3.47 is significant at the 5-percent level, while 4.57 is required for the least significant difference at the 1-percent level. These data indicate that alfalfa strains vary significantly in carotene content.

The relationship of the carotene content of the polycross progeny to the carotene content of their maternal parents is shown in Plate II. The graph was constructed from data representing the averages of all carotene determinations of the parental and polycross progeny plants. As the carotene determinations on the maternal clones and their polycross progeny were made at different dates and at different locations, their actual carotene content is not comparable but it shows their relative relationship. A correlation coefficient of 0.537 was obtained between the maternal parents and their progeny which was significant at the 5-percent level. A regression coefficient of 0.9237 of the polycross progeny on the maternal parents was significant at the 5-percent level when the "t" test was applied. It appears the progeny tends to regress towards the mean carotene content.

Table 4. The carotene content of the polycross progeny from the thirteen parental clones.

Strain number	Milligrams carotene per 100 grams dry matter					
	Sampled on 2/26/45[1]	Sampled on 2/28/45[2]	Sampled on 6/5/45[3]	Sampled on 8/20/45[4]	Sampled on 10/1/45[4]	Average
K-49-3	52.4	61.3	45.8	16.4	27.6	40.70
K-49-55	53.8	55.3	38.3	15.5	28.3	39.04
K-49-7	50.5	56.6	39.1	19.9	25.4	38.30
K-49-64	48.3	57.6	38.6	18.2	28.2	38.18
K-49-50	49.5	55.6	37.0	12.6	29.8	36.90
K-49-24	46.4	56.2	40.9	19.6	20.7	36.76
K-49-58	49.0	58.0	34.9	20.2	21.5	36.72
K-49-43	47.3	56.0	35.0	16.5	26.0	36.16
K-49-25	46.9	56.5	39.5	10.8	26.7	36.08
K-49-5	48.2	55.9	37.1	15.3	23.1	35.92
K-49-28	45.1	56.3	35.7	11.5	28.5	35.42
K-49-30	37.6	56.4	37.9	15.3	28.3	35.10
K-49-19	41.4	51.7	36.7	11.8	23.3	32.98

L S D between averages = 3.474 at the 5-percent level and 4.566 at the 1-percent level.

[1]Samples taken from 50 seedlings in greenhouse. Analysis run on fresh sample.

[2]Same as 1, except hot-water blanched 10 minutes.

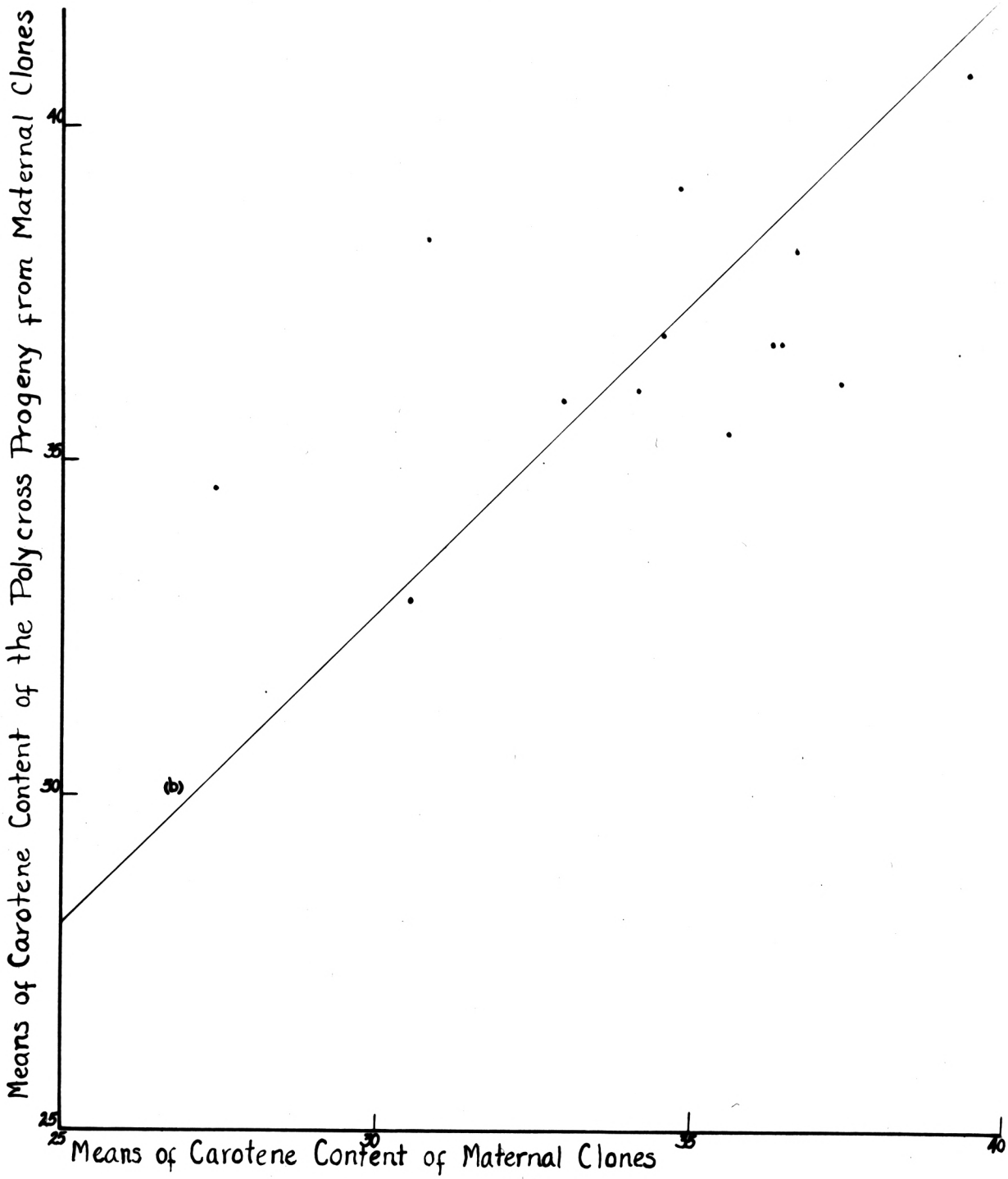
[3]Samples taken from 50 other seedlings in greenhouse and plants dehydrated but not blanched.

[4]Samples taken from polycross yield test in the field. Not blanched.

EXPLANATION OF PLATE II

Plate II is a scatter diagram of the relation of the carotene content of the polycross progeny to the carotene content of their maternal parents. The straight line is the regression line of the polycross progeny on their maternal parents.

PLATE II


Means of Carotene Content of the Polycross Progeny from Maternal Clones
40
35
30
25
(b)
25
30
35
40
Means of Carotene Content of Maternal Clones

However, in general, it shows the maternal plants have the ability to transmit their character for carotene content to their progeny.

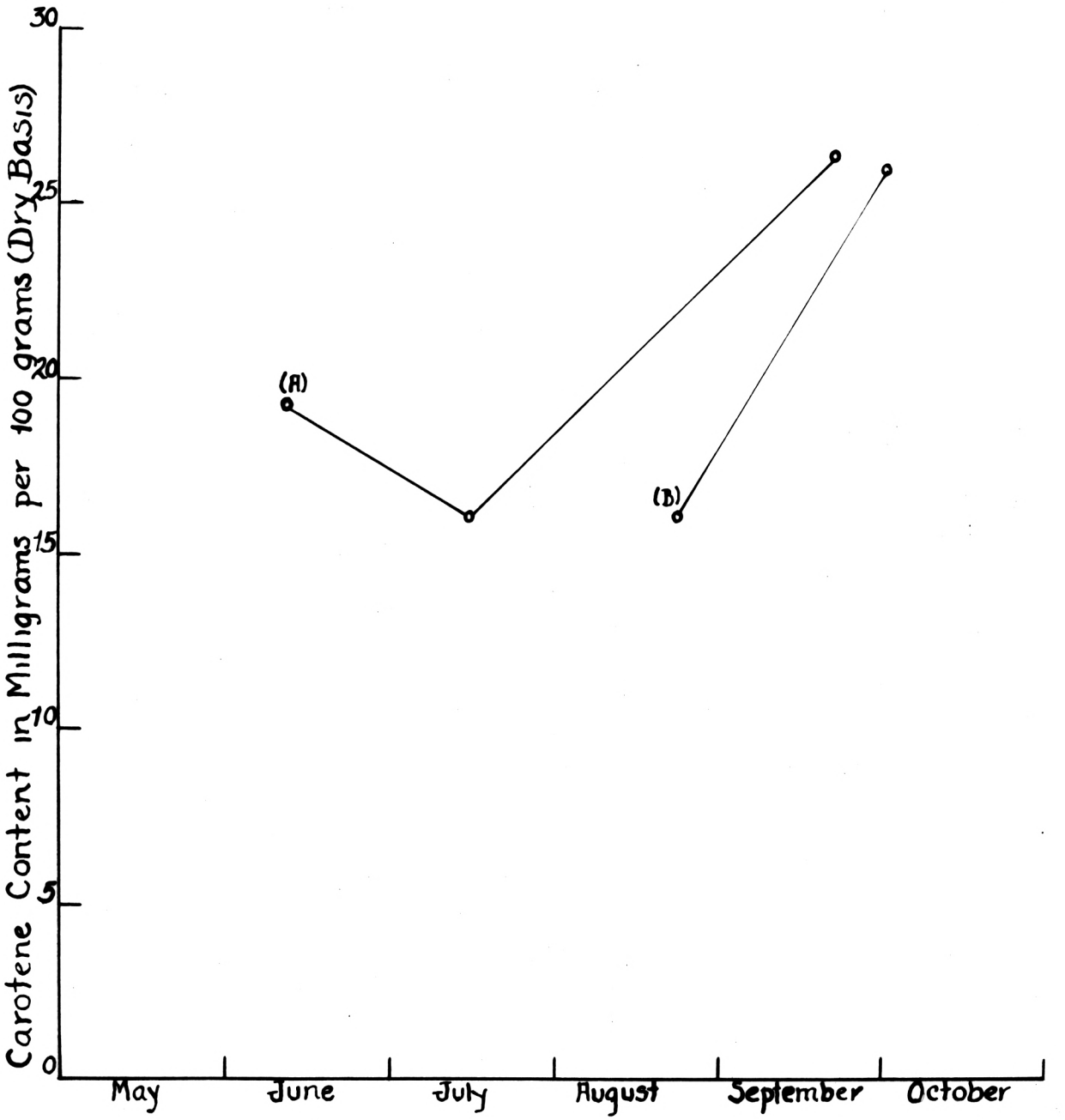
Seasonal Influence

The seasonal effect on carotene content of alfalfa is shown graphically in Plate III. The values in line (A) for each of the sampling dates represent the average carotene content of the 13 parental clones. The plants were in the early bloom stage at each date of sampling, and the same sampling technique was used for each determination. Thus it is unlikely the differences in carotene content between the dates is due to a difference in age of the plants or to a difference in sampling technique. The carotene content for each time the plants were sampled represents new growth since the previous cutting, as the plants were sampled by completely removing the top growth. The values in line (B) were obtained in a similar manner and represent the average carotene content of the 13 polycross progeny strains. Each individual strain and clone sampled showed the same general seasonal trend as diagrammed in Plate III. The carotene content of alfalfa appears to be high in the spring, followed by a marked decline during the midsummer (July and August). Samples taken in the fall showed a very striking increase in carotene content. Seasonal factors apparently have a marked influence on carotene content.

EXPLANATION OF PLATE III

The seasonal influence on the carotene content of alfalfa is shown in Plate III.

PLATE III


Carotene Content in Milligrams per 100 grams (Dry Basis)
30
25
20
15
10
5
0
(A)
(B)
May
June
July
August
September
October

Greenhouse Fertility and Soil Moisture Studies

It was decided to study the effect of some of the major plant elements on the carotene content of alfalfa, as little information was available on the influence of fertilizers on alfalfa, and the results with respect to other crops were not conclusive. The effect of various fertilizer treatments and soil moisture levels under greenhouse conditions on the carotene content of alfalfa is presented in Table 5, and the results of the analysis of variance of these data in Table 6.

In Table 5 the first and second cuttings represent the average of duplicate plant samples from each treatment. No significant differences could be associated between duplicate samples of plant material from an analysis of variance of the data from the first two cuttings. The third cutting represented duplicate laboratory determinations on each treatment. Therefore, it was decided to use the average of duplicate plant samples in the first two cuttings and the average of the laboratory duplicates in the third cutting in computing the analysis of variance of these data, as there was little difference between duplicates.

There was no significant response in carotene content due to the application of nitrogen, phosphorus, or nitrogen and phosphorus fertilizers. There was a marked and highly significant difference of soil moisture levels on carotene content. Under conditions of low soil moisture the plants were materially and consistently higher in carotene content than under conditions

Table 5. Effect of soil moisture and fertility treatments on the carotene content of alfalfa plants grown in pots under greenhouse conditions.

Treatment	Cuttings	Milligrams of carotene per 100 grams dry matter				
		Low soil moisture		High soil moisture		Average of fertilizer treatments
		Unlimed	Limed	Unlimed	Limed	
Nitrogen treatments:						
Nitrogen	1st[1]	16.8	24.0	20.8	25.3	
	2nd[2]	32.9	42.3	25.2	26.3	
	3rd[3]	33.5	34.1	17.1	17.8	26.34
Nitrogen and Phosphorus	1st	22.1	25.9	19.7	20.9	
	2nd	35.7	34.2	24.4	28.6	
	3rd	29.0	31.8	15.9	20.1	25.69
Average:	Unlimed 24.42					
	Limed 27.60	28.33	32.05	20.52	23.17	
No nitrogen treatments:						
No fertilizer	1st	22.6	25.7	20.9	19.6	
	2nd	33.8	31.3	27.5	31.8	
	3rd	32.1	28.8	19.3	15.8	25.76
Phosphorus	1st	23.2	22.8	21.6	24.4	
	2nd	37.1	36.2	29.8	26.5	
	3rd	31.5	30.8	15.4	15.8	26.25
Average:	Unlimed 26.23					
	Limed 25.79	30.05	29.26	22.42	22.32	
Average of all treatments		29.19	30.66	21.47	22.74	
Average of all:	Low moisture 29.92			High moisture 22.10		
Average of all:	Unlimed 25.32					
	Limed 26.70					

L S D between means of all the soil moisture or lime treatments at the 5-percent level = 0.94 and at the 1-percent level = 2.42.

L S D between means of lime treatments = 1.98 at the 1-percent level under "Nitrogen Treatments".

No significant difference between lime treatments under "No Nitrogen Treatments".

[1]Whole plants sampled and analyzed for carotene on March 27, 1946.

[2]Whole plants sampled on May 7, 1946, and stored for three weeks at 25° C. before analyzing.

[3]Carotene determinations were made on leaves and stems separately on June 15, 1946, from which the carotene content of the whole plant was calculated.

Table 6. Analysis of variance of carotene content of alfalfa plants produced under soil moisture and fertility treatments.

Factor	Degrees of freedom	Sum of squares	Variance	Calculated F	Table readings of F (P = 0.05)	Table readings of F (P = 0.01)
Total	47	2,078.58				
Between fertilizer treatments	3	3.98	1.326	0.250		
Between limed treatments	1	22.55	22.550	4.256*	4.12	7.42
Between moisture treatments	1	733.98	733.980	138.539**	4.12	7.42
Between cuttings	2	748.59	374.295	70.648	3.28	5.29
Interactions: Fertilizer treatments x lime treatments	3	42.70	14.230	2.685	2.87	4.40
Soil moisture treatments x cuttings	2	341.33	170.665	32.213**	3.28	5.29
Error	35	185.45	5.298			

* Significant.
** Highly significant.

of high soil moisture. A significant difference between limed and unlimed treatments was obtained with a difference of 0.94 between means of all the limed and unlimed treatments being required for significance at the 5-percent level. Apparently environmental factors such as changes in light conditions or temperature are largely responsible for the marked differences between cuttings. An interaction of moisture levels x cutting dates was significant. It is believed that the greater difference between low and high moisture levels in the second and third cuttings was due to an increase in the spread between the two moisture levels for the second and third sampling dates. A significant interaction of fertilizer treatments x lime treatments was not obtained, but it nearly approaches significance at the 5-percent level. This indicates the influence of lime differed with the various fertilizers applied.

The influence of lime and nitrogen on the carotene content is shown in Table 5 under the nitrogen treatments. These data represent all the treatments receiving nitrogen or nitrogen and phosphorus. The analysis of variance of these data demonstrated a significant difference in carotene content associated with a combination of lime and nitrogen treatment. The application of lime with the addition of nitrogen fertilizer increased the carotene content significantly over the unlimed treatments receiving nitrogen.

The effect of lime on the carotene content without the addition of nitrogen fertilizer is shown in the same table under

the no-nitrogen treatments. These data represent the treatment receiving no fertilizer and the one receiving phosphorus. No significant differences could be attributed to the limed and unlimed treatments under these conditions as shown by the computation of the analysis of variance of these data. The application of lime alone or nitrogen alone had no effect on the carotene content, while a combination of the two treatments produced a significant increase in carotene content.

The percentage composition of leaves of the plants determined at each time of sampling is presented in Table 7. The percentage of leaves tends to be closely associated with the average carotene values reported for the various treatments. The low soil moisture plants were significantly higher in percent leaves than the plants produced under high soil moisture. This difference was apparently due to the finer stems produced under the low soil moisture conditions. The percent of leaves was significantly increased with the application of lime. There was no significant difference in percent leaves between fertilizer treatments.

Table 8 represents the carotene determination of the leaves only on the third cutting. It shows the higher carotene content associated with the low soil moisture treatment is not due entirely to a higher percent of leaves under these conditions. A highly significant difference in carotene content between soil moisture treatments was obtained. An examination of these data (Table 8) reveals even a greater difference in carotene content

Table 7. Effect of soil moisture and fertilizer treatments on the percentage of leaves of alfalfa produced in pots in the greenhouse.

Treatment	Cuttings	Percentage of leaves of whole plant (dry basis)				
		Low soil moisture		High soil moisture		Average of fertilizer treatments
		Unlimed	Limed	Unlimed	Limed	
No fertilizer	1st	60.1	60.1	54.8	56.0	
	2nd	58.9	62.0	56.1	58.2	
	3rd	53.2	52.8	47.1	47.9	55.60
Nitrogen	1st	61.1	59.5	50.2	58.6	
	2nd	61.3	61.6	55.0	55.8	
	3rd	51.2	51.6	48.0	49.4	55.28
Phosphorus	1st	60.7	62.2	56.4	56.3	
	2nd	62.6	66.0	54.8	56.1	
	3rd	48.8	48.2	47.0	51.1	55.85
Nitrogen and Phosphorus	1st	59.6	59.1	55.2	51.5	
	2nd	62.6	61.5	53.1	54.7	
	3rd	49.8	51.3	46.5	47.6	54.38
Average		57.49	57.99	52.02	53.60	

Average: Low moisture 57.74 High moisture 52.80

Average: Unlimed 54.75
Limed 55.80

L S D between means of soil moisture or lime treatments = 1.278 at the 1-percent level and 0.972 at the 5-percent level.

Table 8. Effect of soil moisture and fertilizer treatments on carotene content of alfalfa leaves from plants grown in pots in the greenhouse.

Treatment	Milligrams carotene per 100 grams dry matter				
	Low soil moisture		High soil moisture		Average of fertilizer treatments
	Unlimed	Limed	Unlimed	Limed	
No fertilizer	51.2	48.0	30.1	21.0	37.58
Nitrogen	55.5	56.3	25.6	26.0	40.85
Phosphorus	54.5	53.9	24.9	23.2	39.13
Nitrogen and Phosphorus	50.3	53.5	24.8	32.7	40.33
Average	52.88	52.93	26.35	25.73	

Average: Low moisture 52.90 High moisture 26.04

Average: Unlimed 39.61
Limed 39.32

L S D between means of soil moisture treatments = 3.27 at the 1-percent level of significance.

of the leaves between the low and high soil moisture treatments than the differences associated with these treatments produced in the whole plant as shown in Table 5.

The carotene content of the leaves did not differ significantly with the limed and unlimed treatments, with or without the presence of nitrogen. This would indicate the increase in carotene content due to the application of lime and nitrogen, as shown in Table 5, may be due to an increase in percentage of leaves under these conditions. No significant difference in carotene content of the stems could be attributed to any of the various treatments as shown in Table 9. This indicates that any variations in carotene content between the treatments was due largely to differences in the concentration of carotene in the leaves.

Marked differences in growth of plants were obtained as a result of the fertilizer and soil moisture treatments as shown in Table 10. Yields of oven-dry hay were increased due to the application of nitrogen, phosphorus, and nitrogen and phosphorus, over the no-fertilizer treatment. There was essentially no difference in yield between the limed and unlimed treatments with the low soil moisture. It is difficult to explain adequately the lower yield of hay produced with the application of lime under the high soil moisture treatment. The high soil moisture treatment yielded more than twice as much hay per unit area as did the plants growing under low soil moisture. From these results it appears that carotene content is not necessarily

Table 9. Effect of soil moisture and fertilizer treatments on carotene content of alfalfa stems from plants grown in pots in the greenhouse.

Treatment	Milligrams carotene per 100 grams dry matter				
	Low soil moisture		High soil moisture		Average of fertilizer treatments
	Unlimed	Limed	Unlimed	Limed	
No fertilizer	10.4	7.2	9.7	11.0	9.57
Nitrogen	10.4	10.4	9.2	9.7	9.92
Phosphorus	9.6	9.3	7.0	8.0	8.47
Nitrogen and Phosphorus	8.0	9.0	8.1	8.7	8.45
Average	9.60	8.98	8.50	9.35	

Average: Low moisture 9.28 High moisture 8.92

Average: Unlimed 9.05
Limed 9.16

No significant differences between means.

Table 10. Effect of soil moisture and fertilizer treatments on yield of alfalfa hay from alfalfa grown in pots in the greenhouse.

Treatment	Cuttings	Grams of oven-dry hay				
		Low soil moisture		High soil moisture		Average of fertilizer treatments
		Unlimed	Limed	Unlimed	Limed	
No fertilizer	1st	4.04	3.77	10.20	7.75	
	2nd	4.37	4.36	10.03	8.89	
	3rd	5.14	5.13	15.32	13.02	7.68
Nitrogen	1st	4.16	3.82	11.29	10.64	
	2nd	5.69	5.98	11.25	10.49	
	3rd	6.05	6.80	13.53	13.04	8.56
Phosphorus	1st	3.49	4.41	8.98	8.87	
	2nd	4.93	5.82	10.26	9.19	
	3rd	6.35	7.87	17.05	17.41	8.72
Nitrogen and Phosphorus	1st	4.28	3.67	13.09	9.13	
	2nd	6.61	5.54	12.65	10.27	
	3rd	8.18	7.55	18.09	13.41	9.37
Average		5.27	5.40	12.65	11.01	

Average: Low moisture 5.34 High moisture 11.83

Average: Unlimed 8.96
Limed 8.21

L S D between means of soil moisture or lime treatments = 1.117 at the 5-percent level and 1.579 at the 1-percent level.

L S D between means of fertilizer treatments = 1.657 at the 5-percent level and 2.236 at the 1-percent level.

associated with the amount of growth produced.

The average pH values for the various treatments are shown in Table 11. Each of the values represent the average of duplicate determinations from a representative sample of the two replications. The limed treatments had a significantly higher pH value than the unlimed treatments, having an average pH value of 5.64 and 6.62 respectively. This indicates that at least enough lime was applied to change the soil reaction, and that soil acidity may affect carotene content at least in the presence of nitrogen.

In general, the treatments which gave the highest yield of hay per unit area produced the highest total yield of carotene per unit area (Table 12), although not necessarily the highest carotene content. These data show an increase in total yield of carotene per unit area from the addition of nitrogen, phosphorus, or a combination of both over the treatment receiving no fertilizer. The high soil moisture treatment increased markedly the yield of carotene per unit area even though the percentage composition of carotene of the plants under these conditions was significantly lower.

The stage of maturity and height of the plants prior to each sampling were recorded. It is unlikely that differences in carotene content can be attributed to differences in stage of development or the plants under the various treatments, as no difference could be observed. The plants in all the treatments were in the early bloom stage at each time of sampling.

Table 11. Acidity of soil under soil moisture, fertilizer and lime treatments.

Treatment	pH values			
	Low soil moisture		High soil moisture	
	Unlimed	Limed	Unlimed	Limed
No fertilizer	6.05	6.32	5.47	6.40
Nitrogen	5.90	7.10	5.25	6.45
Phosphorus	6.05	6.97	5.35	6.36
Nitrogen and phosphorus	5.90	6.90	5.20	5.92
Average	5.97	6.82	5.32	6.28

Average: Unlimed 5.64 Limed 6.62

Table 12. Effect of soil moisture and fertilizer treatments on total yield of carotene per unit area of alfalfa grown in pots in the greenhouse.

Treatment	Cuttings	Milligrams of carotene produced per unit area				
		Low soil moisture		High soil moisture		Average of fertilizer treatments
		Unlimed	Limed	Unlimed	Limed	
None	1st	91.30	96.89	213.18	151.90	
	2nd	147.71	139.60	275.83	282.70	
	3rd	164.99	147.74	295.68	205.72	184.44
Nitrogen	1st	69.89	91.68	234.83	269.19	
	2nd	187.20	252.95	283.50	275.89	
	3rd	202.68	231.88	231.36	232.11	213.60
Phosphorus	1st	80.97	100.55	193.97	216.43	
	2nd	182.90	210.68	305.75	243.54	
	3rd	200.03	242.40	262.57	275.08	209.57
Nitrogen and Phosphorus	1st	94.59	95.05	257.87	190.82	
	2nd	235.98	189.47	308.66	293.72	
	3rd	237.22	240.09	287.63	269.54	225.05
Average		157.95	169.92	262.57	242.22	

Average: Low moisture 163.94 High moisture 252.39

Average: Unlimed 210.26
Limed 206.07

L S D between means of soil moisture treatments = 75.95 at the 1-percent level.

The only marked and consistent difference in height between the treatments was the increased height of plants produced under the high soil moisture treatment over the low soil moisture treatment, as illustrated in Plate IV.

Field Soil Moisture Studies

The carotene content of seven strains of alfalfa showing the influence of soil moisture under field conditions is presented in Table 13. The corresponding heights and stage of development of the plants are recorded in the same table. The data show that plants growing under a limited supply of soil moisture even approaching drought conditions are significantly higher in carotene content than plants produced on soil with a moisture supply sufficient for normal growth. The values reported for Kansas Common represent analysis of the leaves only, thus indicating that leaves from plants produced under conditions of low soil moisture tend to be higher than those grown under high soil moisture. These field observations corroborate with greenhouse studies on the influence of soil moisture on carotene content.

In the field it was observed that a darker green color was associated with the plants growing under a low amount of soil moisture. Under both field and greenhouse conditions the plants grew more vigorously under the high soil moisture levels. In no case did the high soil moisture have any detrimental effects upon the appearance of the plants. The influence of varying amounts of soil moisture in the field on the appearance of the plants is

EXPLANATION OF PLATE IV

Plate IV shows the effect of soil moisture on the growth of potted alfalfa plants. The two pots on the left show the effect of a low amount of soil moisture, and the two pots on the right show the effect of a high amount of soil moisture on plant growth.

PLATE IV


98
7180
3
7181
LBO
9
7552
HAO
17
7633
HBO
IA5361
BROTHERS - LUMBER and COAL - PHONE 391

Table 13. The carotene content of seven strains of alfalfa grown in the field with different amounts of soil moisture at various stages of growth.

Strain No.	Low soil moisture			High soil moisture		
	Height in inches	Stage of maturity % bloom	Carotene content mg/100 gms	Height in inches	Stage of maturity % bloom	Carotene content mg/100 gms
K-49-3	10 to 12	Bud stage	36.3	16 to 18	Bud stage	29.3
K-49-21	10 to 12	10	29.7	16 to 18	10	25.0
K-49-24	8 to 10	Bud stage	32.0	18 to 20	Bud stage	26.8
K-49-28	8 to 10	10	28.7	14 to 16	10	25.3
K-49-58	8 to 10	10	31.9	14 to 16	10	27.1
K-49-63	8 to 10	15	30.2	18 to 20	15	26.5
Kansas[1] Common	11 to 14	10	33.3	16 to 18	10	31.30
Average			31.77			27.33

L S D between means = 2.33 at the 1-percent level.

[1]Represents analysis of leaves only.

shown in Plate V.

Field Fertility Studies

The effects of fertilizer treatments including the application of lime, phosphorus, potash, and manure in various combinations on the carotene content of alfalfa is presented in Table 14. These data represent one determination from an unreplicated series of fertility plots located at Columbus, Kansas. An examination of the data in Table 14 shows the check plots consisting of lime and superphosphate differed little in carotene content. The mean carotene content of the plots was 31.97 with a standard deviation of 2.86. The plot receiving lime alone and all the plots receiving lime, regardless of the nature of the other fertilizers applied, varied within $\pm$ one standard deviation of the mean of all the treatments. The plot receiving no lime was significantly lower than the average of all the fertility treatments receiving lime. The probability of obtaining such a low carotene value for the unlimed plot due purely to chance was about one in one hundred times as determined by its deviation in the normal distribution in terms of the standard deviation as shown by the table of x given by Paterson (1939).

The pH values for the corresponding treatments are reported in Table 14. The no-treatment plot was slightly more acid than the plots receiving lime. The plants responded to the fertilizer treatments as indicated by the marked difference in height of the plants as shown in the same table. The plants were all in the

EXPLANATION OF PLATE V

The effect of variations in soil moisture on the growth of alfalfa plants in the field is shown in Plate V. The smaller plants were grown under a limited amount of soil moisture.

PLATE V

Table 14. The carotene content, percent protein, and height of alfalfa from a series of fertilizer treatments with various soil reactions from field plots at Columbus, Kansas.

Fertilizer treatments	Carotene content mg per 100 gms (dry basis)	Percent protein	Av. height of plants (inches)	Soil reaction pH
Lime	30.8	23.4	8	5.69
Lime and superphosphate	32.0	25.0	14	5.65
Lime, potash, and superphosphate	36.6	23.2	16	5.75
Lime and rock phosphate	31.4	22.8	15	5.89
Lime and superphosphate	32.3	23.5	16	5.85
Lime and manure	32.5	21.6	19	5.58
Lime, manure, and superphosphate	34.2	22.5	18	5.65
Lime and superphosphate	32.2	19.9	16	5.92
Lime, manure, and rock phosphate	32.4	23.1	17	5.62
Lime and superphosphate	32.6	23.4	15	5.78
No treatment	24.7	21.9	8	5.15

bud stage at the time of sampling and no difference in stage of maturity or color of the plants could be observed between treatments.

The alfalfa grown on the fertility plots at Columbus was the first season's growth from fall-seeded alfalfa. Previous experiments in the same field have shown increases in yield of hay due to the application of the various fertilizers. The normal increase from lime at this field is 1.36 tons per acre.

Studies on the Relationship of Carotene Content to Protein Content of Alfalfa

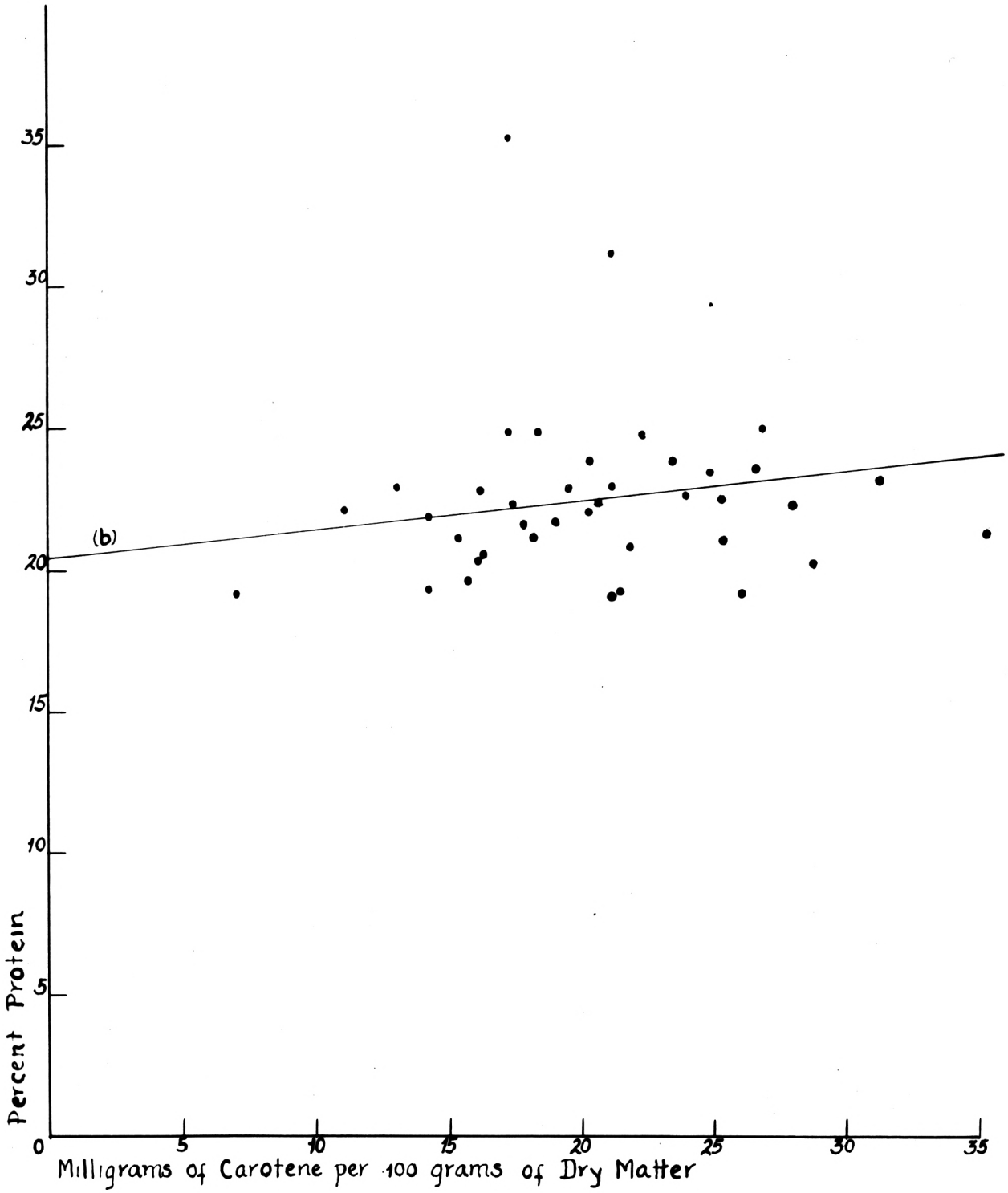
The carotene content and their corresponding protein content of the thirteen parental plants was determined on three dates of sampling. Plate VI shows graphically that there was little relationship between the carotene and protein content of the plants. A correlation of 0.0617 was obtained which was not significant. Although the carotene content and percentage of protein of the plants differed significantly between lines as determined by the analysis of variance of these data, there appeared to be no association between the two.

The carotene and protein content of alfalfa which was the result of one determination from the alfalfa fertility plots located at Columbus, Kansas, is shown in Table 14. The correlation coefficient of 0.188 between the carotene and protein content was not significant. It appears that factors affecting the carotene content do not necessarily affect percentage composition of protein in alfalfa.

EXPLANATION OF PLATE VI

Plate VI is a scatter diagram of the relation of the percent protein to the carotene content of thirteen clonal lines of alfalfa representing three dates of sampling. The straight line is the regression line of the percent protein on the carotene content of alfalfa.

PLATE VI


35
30
25
(b)
20
15
10
5
0
Percent Protein
5
10
15
20
25
30
35
Milligrams of Carotene per 100 grams of Dry Matter

DISCUSSION

In regard to the inheritance of carotene content in alfalfa, the data reported in this study indicate that clonal lines and strains of alfalfa differ inherently in carotene content. Some of the clonal lines and polycross strains tended to rank consistently high in carotene content while others were consistently low. The results of carotene determinations on the leaves indicate the difference in carotene content of the clonal lines was actually due to a higher concentration of carotene in the plants, and the differences were not entirely due to the variability in percent leaves between the plants.

It appears that carotene content of alfalfa is inherited, as there was a significant correlation between the carotene content of the polycross progeny and their maternal parents. Although the polycross progeny tended to regress towards the mean carotene content, with few exceptions the maternal parents had the ability to transmit their inheritance factors for carotene content to their polycross progeny.

From these results it appears that one of the most promising methods of increasing the carotene content of alfalfa is by the selection of clonal lines high in carotene content. The ability of those selected plants to transmit their character for high carotene content to their progeny can then be evaluated by determining the carotene content of their polycross progeny.

The conclusion that carotene content of alfalfa is inherited and that an increase in carotene is possible by selection for

higher carotene strains seems to be warranted in view of the evidence reported here and from similar results obtained by Ham and Tysdal (1946).

Atkeson (1937) and Moon (1939) have reported that the carotene values were generally high in the spring and early summer followed by a decline during the midsummer and an increase again in the fall. These results were confirmed by this investigation, thus indicating that seasonal and environmental factors have a marked influence on the carotene content. It appears from these results that higher carotene alfalfa may be obtained from spring and fall cuttings.

Alfalfa plants grown in pots in the greenhouse showed no indication that fertilizer treatments such as the application of nitrogen, phosphorus, or a combination of both on soil deficient in these elements had any influence upon the carotene content of alfalfa; although increases in yield and growth of the plants were obtained due to their application. The addition of lime and nitrogen produced a significant increase in the carotene content of plants over the unlimed pots receiving nitrogen, while an application of lime alone or nitrogen alone had no effect on carotene content. The addition of lime was sufficient to raise the pH of the soil on the average from 5.64 to 6.62.

As most of the carotene is found in the leaves, the increase in carotene content due to the application of lime and nitrogen under greenhouse conditions may be attributed to an increase in the percentage of leaves. An analysis of the leaves

only on the third cutting showed no increase in carotene content due to the application of lime and nitrogen over the unlimed pots receiving nitrogen. However, the corresponding values for the carotene content of the whole plant at the same date of sampling showed slight increases in carotene content due to the application of lime and nitrogen. Thus it appears the increase in carotene content may be accounted for by an increase in percent leaves under these conditions. There was no appreciable difference in carotene content of the stems between any of the treatments.

In field plots the application of phosphorus, potash, and manure in various combinations and rates on limed plots produced no additional increase in carotene content over the plot receiving lime alone, although marked difference in growth was observed. However, all the plots receiving lime were significantly higher in carotene content than the unlimed plot. Under field conditions the application of lime increased the pH of the soil from 5.15 to an average of 5.74.

It appears from greenhouse and field experiments that lime or lime and nitrogen, or perhaps the soil reaction has some influence under certain conditions on the carotene content of alfalfa. Further study should be conducted before definite conclusions can be drawn.

Under greenhouse conditions, plants growing under a low soil moisture supply approaching the wilting coefficient were significantly higher in percentage composition of carotene than

plants produced with the amount of soil moisture near the field water-holding capacity. It may be concluded that conditions were more favorable for growth of plants under the high soil moisture levels as evidenced by the more luxuriant growth. The plants produced under the low soil moisture treatments were smaller and somewhat finer stemmed.

The higher carotene content of the plants produced in the low-moisture pots was apparently not due to a difference in stage of maturity as no consistent differences between the two moisture levels could be detected. The low soil moisture supply produced plants with a slightly higher leaf percentage but the higher carotene content of the plants produced under low soil moisture is not due to a higher leaf percentage as an analysis of the carotene content of the leaves shows a marked increase in carotene content due to a low soil moisture supply.

Field observations gave further evidence which indicates that carotene content is influenced by soil moisture. Alfalfa strains growing under a limited supply of soil moisture even approaching drought conditions were consistently higher in carotene content than the same strains grown in adjacent areas with a sufficient supply of soil moisture for normal growth. Very little difference in stage of development between the two moisture levels could be observed, thus it seems that the differences were not due to differences in stage of maturity of the plants. Determinations on the leaves of Kansas Common under different levels of soil moisture also indicate a higher carotene content

under conditions of low soil moisture. A darker green color was associated with the low soil moisture and high carotene plants under field conditions.

From these data it appears that alfalfa grown in relatively dry areas had a higher concentration of carotene than alfalfa produced in areas having an abundance of rainfall, although further experimental work should be conducted before conclusions can be drawn.

Although marked increases in growth due to soil moisture treatments and to the application of various fertilizers were obtained, there appears to be no consistent relationship between carotene content and amount of growth produced.

The highest total yield of carotene per unit area appears to be associated with treatments which give the highest yield of hay per unit area, although not necessarily the highest carotene content. Thus the application of fertilizers may be beneficial in increasing the total production of carotene if the yield of hay is increased by the fertilizer application. This confirms Hamner's statement (1945), who reports that treatments which give the highest crop yield per acre are the ones most likely to give the highest vitamin yield per acre.

From the results of this investigation, no significant relationship between carotene and protein content was established. Hence the protein content of alfalfa is not necessarily an index to the carotene content.

SUMMARY

In this study experiments were conducted to determine the inherent nature of alfalfa in respect to carotene content, the effect of fertilizers, the influence of seasonal and environmental factors, the relationship of carotene to protein content, and to determine what methods or practices could be used to increase the concentration of carotene in alfalfa.

It was found that clonal lines and strains of alfalfa differed inherently in carotene content. The differences between clonal lines was due to differences in concentration of carotene in leaves and not due necessarily to variations in percent of leaves between the plants. It appears that carotene content is inherited, as a significant regression of the carotene content of the polycross progeny on the carotene content of their maternal parents was obtained. An increase in carotene content by breeding and selection seems possible. The polycross method of selecting lines for combining ability is a satisfactory method of testing the ability of clones to transmit their inheritance factors for carotene content.

The seasonal influence had a marked effect on carotene content. It appears that alfalfa is high in carotene content in the spring followed by a decline during the midsummer and an increase again in the fall.

It appears that fertilizers, in general, have no effect on the carotene content of alfalfa. However, greenhouse experiments indicated that lime and nitrogen may increase the carotene

content, but this was attributed to an increase in percent of leaves under these conditions. Data from a field experiment indicate that lime increases the carotene content. It is possible that under certain conditions lime or nitrogen or both, or the soil reaction, may have an influence on carotene content, but more study is needed before conclusions can be drawn.

Alfalfa growing under a low soil moisture supply, even approaching drought conditions in both the greenhouse and field experiments, were higher in carotene content than alfalfa produced with an adequate amount of soil moisture.

There was no consistent relationship between carotene content and amount of growth produced. In general, treatments which gave the highest total yield of hay per unit area produced the highest total yield of carotene per unit area, but not necessarily the highest carotene content. Hence fertilizers may be beneficial in increasing total yield of carotene if they increase yield of hay.

The protein content of alfalfa is not necessarily an indication of its carotene content as no significant correlation was established between the carotene and protein content of alfalfa.

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