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CAROTENE AND PROTEIN CONTENTS OF ALFALFA AS INFLUENCED BY VARIETY AND CERTAIN ENVIRONMENTAL FACTORS

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SAMPLING METHODS AND DRYING CONDITIONS OF ALFALFA are here critically discussed in relation to carotene and protein contents. The preferred sample for variety comparisons is a definite number of culms per plot, with the effect of sample variation minimized by use of replicates.

Hot-water blanching results in loss of dry weight and an apparent increase in carotene content. Steam blanching is therefore preferred. Effective blanching is also possible by placing small samples in a large oven at 130° C.

Drying at 130° C resulted in about 5 per cent more carotene destruction than was obtained by drying in vacuo at 65° C. No significant differences were found in air drying at 65°, 100°, and 130° C; but this probably would not be true with other drying conditions or use of functionally different equipment.

Many varieties were compared. Six standard varieties were studied during six cuttings. No variety was consistently higher than others in either carotene or protein content. Other comparisons between more varieties and strains, but involving fewer cuttings, confirm this similarity. Any differences found could be attributed to differences in leafiness.

Physiological factors are important. As alfalfa matures, carotene content is maximum in the pre-bud stage, decreases rapidly from then until about the 1/10 bloom stage, and less rapidly thereafter. Total dry weight and total carotene per culm increase at a fairly uniform rate until after seed pods are formed and then decrease. The per cent oven dry weight increases and the protein content decreases with increasing maturity. The carotene content of alfalfa cut at the 1/10 bloom stage increased 23 per cent from June to October. Some variation occurs during the day and from day to day.

These data indicate the necessity of sampling at the same stage of maturity for variety comparisons. Seasonal trends are very important for comparisons of carotene contents but not for protein contents.

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CAROTENE AND PROTEIN CONTENTS OF ALFALFA AS INFLUENCED BY VARIETY AND CERTAIN ENVIRONMENTAL FACTORS[1]

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ALFALFA has long been of prime importance as a hay and forage crop because of its high protein and carotene contents. Protein is generally stable during storage, but may be lost in leaf shatter through poor management in handling the crop.

Carotene, on the other hand, is very unstable and losses may be severe during harvest operations and storage. In spite of customary large losses of carotene, alfalfa products are of great importance in the feeding of livestock and poultry because of their content of *beta*-carotene (provitamin A). This is largely due to the initial high content of this nutrient rather than to superior methods of preventing loss.

Ham and Tysdal (1946) stated that certain crosses may be consistently different in carotene content from others. Thompson (1949) has recently discussed the desirability of obtaining alfalfa varieties with higher carotene content and indicated that differences exist among common varieties. This study was undertaken to fill a need for further and more comprehensive survey work on contents of both carotene and protein.

While extensive comparisons were being made of the carotene contents of different varieties, hybrids, and selections from the alfalfa-breeding project at this station, several factors influencing the results of carotene determinations were evaluated. A rapid and satisfactory method of sampling and comparing the carotene contents of alfalfa varieties was developed. Consideration of these factors may be applicable to studies of other constituents in alfalfa and related crops.

MATERIALS AND METHODS

The alfalfa was grown on the University Experiment Station Farm at Davis, California, in a field of uniform Yolo fine sandy loam. The alfalfa was planted in close-drilled rows (6 inches apart), in plots $3\frac{1}{2} \times 16$ feet in

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size (6×6 Latin square design), and irrigated once or twice a month. Plots were at no time infested with insects or subject to any conditions of disease. Samples were taken during 1949 and 1950. Sampling was done from 8 to 11 a.m., P.S.T.

A random sample, consisting of 25 culms (each culm taken from a different plant) cut at 1 to 1½ inches from the ground, was blanched with steam at 5 p.s.i. for 1 to 3 minutes. The samples were cut into 4- to 6-inch lengths and spread in wire trays, $2 \times 9 \times 18$ inches, made of ⅛-inch hardware cloth. They were dried in a 4 cubic foot Aminco Forced-Draft Electric Constant Temperature Oven previously heated and set for 130° C with minimum recirculation of air. Twelve to 14 samples were usually dried at one time. The samples were watched closely and removed as soon as the large stems were brittle. Drying seldom required more than one hour. The dried samples were ground in an intermediate Wiley mill to pass a 40-mesh screen. After mixing, they were stored below 12° C. Using the above methods it was possible in one hour to sample and blanch 20 to 30 samples, dry and grind 12 samples, or analyze an average of 7 or 8 samples.

Samples were analyzed for carotene by the Zscheile-Whitmore method (1947) for dried alfalfa meal. Carotene concentrations were determined using a Klett photometer with a blue filter. Carotene contents in parts per million were calculated from the dry sample weights without regard to variations in moisture content. Data are presented as averages (of six samples unless otherwise noted), $\pm$ the standard errors of the means. Protein analyses were made by the standard Kjeldahl-Gunning-Arnold method for organic and ammoniacal nitrogen as adopted by the Association of Official Agricultural Chemists (1945, p. 27). Moisture contents were determined by the Electric Air-Oven method adopted by the Association of Official Agricultural Chemists (1945, p. 405).

ANALYTICAL FACTORS

Sampling Methods

In determining the sampling method to be used, consideration must be given to the type of material under investigation, the purpose for which the analysis is made, the time involved, and the facilities available for handling the samples. Since the leaves contain 80 to 90 per cent of the carotene in alfalfa, Zscheile and Whitmore (1947) used picked leaves. Mitchell and King (1948) recommended using whole plants, pointing out the great variability of carotene distribution in the plant, a fact also recognized by Thompson (1949). To get greater uniformity among samples in controlled drying experiments, Griffith and Thompson (1949) used whole alfalfa cut to a uniform length from the tip. All of these methods have advantages and disadvantages. Thus, the picked-leaf method of Zscheile and Whitmore and the method of Griffith and Thompson result in greater uniformity among individual samples and are useful in controlled experiments where such uniformity is desirable. On the other hand, they do not provide good samples for comparing varieties, since neither method considers possible variation in leaf-to-stem ratios for the entire plant. The latter factor varies with variety and with the physiological state and the stage of development of a given variety. This latter cause of variation has not heretofore been fully investigated, although its importance was recognized by Ham and Tysdal (1946).

Since the culm age from a given alfalfa plant varies widely and, as will be shown later, carotene content varies with maturity, this factor must be considered in sampling. An attempt to select culms of uniform age (or size) would be time-consuming, and in addition would not consider possible differences in culm age variation among varieties. Soil variability, the heterogeneity of plants from even the more uniform varieties, and the necessity of keeping the sample size within reasonable limits were also considered in choosing the sampling method.

TABLE 1

EFFECT OF BLANCHING METHODS ON OVEN DRY WEIGHT AND CAROTENE CONTENT

(California Common, third cutting)

Treatment	Oven dry weight, grams	Carotene content (oven dry wt. basis)	
		p.p.m.	Total mg
1. Unblanched (dried directly)	10.22±.12	401±12	4.10±.15
2. Blanched in autoclave (5 lbs. 2 min.)	10.16±.13	407± 4	4.14±.04
3. Blanched in boiling water (5 min.) and squeezed before drying	8.23±.04	493± 5	4.06±.04

A definite number of culms taken at random from all sections of a given plot or row considers most of the factors enumerated, but, as shown by standard errors in the tables, may result in considerable variation among replicates. However, the consistently small standard errors of the averages of at least six replicates from a given plot or from separate small plots in the same general area (table 18) indicate that the sampling method is satisfactory. Six replicates are considered adequate, and only slight reduction of the standard error is accomplished by use of 12 samples (table 2). Twenty-five-culm samples at the $\frac{1}{10}$ bloom stage weighed 25–35 grams when dry and represented the maximum size of sample that could be handled regularly.

Sample Preparation

Blanching. It is well recognized that blanching is necessary to inactivate carotene-destroying enzymes. The method of blanching, however, markedly influences the results, as shown in table 1. For this experiment the culm tips of whole alfalfa were aligned and cut to 10 inches. After mixing, samples of 50 grams (green weight) were weighed, and four replicates were subjected to each of the indicated conditions. All samples were dried at 130° C for 45 minutes. It is evident that carotene retention was comparable in the three treatments and that the apparent carotene content was virtually the same in treatments 1 and 2. Hot-water blanching and squeezing, however, resulted in about 20 per cent loss of dry matter and a corresponding increase in apparent carotene content. These data agree in general with results reported by Bailey and Dutton (1945) on similar changes during the blanch-

TABLE 2

CAROTENE, PROTEIN, AND MOISTURE CONTENTS AND OVEN DRY WEIGHTS OF ALFALFA DRIED UNDER DIFFERENT CONDITIONS

(California Common, fifth cutting)

Treatment	Drying temperature, deg. C	Oven type	Drying time, hours	Number of samples	Moisture content, per cent	Oven dry weight, grams	Carotene content*		Protein* content, per cent	Date
							p.p.m.	Total mg		
1	100	Air	2.3	12	....	34.4±1.2	265±3	9.09±.25	16.6±0.1	9–12
2	130	Air	1.0	12	2.5–3.5	34.7±1.0	273±5	9.40±.15	17.7±0.3	9–12
3	65	Air	5.5	12	6.5–7.5	36.0±1.0	263±4	9.47±.28	16.3±0.2	9–13
4	130	Air	1.0	12	4.5–6.0	35.8±1.1	269±6	9.64±.37	16.6±0.2	9–13
5	65	Vacuum	30.0	6	3.5–4.0	34.4±1.0	291±7	10.00±.14	17.3±0.4	9–14
6	130	Air	1.0	6	7.0–8.0	33.4±2.4	272±10	8.97±.49	16.8±0.3	9–14
7	130	Air	2.0	6	7.0–7.5	32.1±1.5	243±3	7.80±.35	16.7±0.1	9–14
8	130	Air†	0.8	6	....	32.3±0.7	254±5	8.18±.27	16.1±0.2	9–14

* Moisture contents not considered.
† Air entrance opening decreased 1/3; exit opening unchanged.

ing of carrots. Unless carotene is reported in terms of green weight, as in the Zscheile-Whitmore method for green leaves (1947), or the loss of dry matter is accurately determined in those cases in which carotene is determined on dried material that has been hot-water-blanched, this method will give erroneous results.

Placing unblanched samples in the oven previously heated to 130° C resulted in effective blanching of the tissue. However, steam blanching is recommended as a rapid and effective means of handling numerous samples without carotene loss before drying.

For field blanching, the use of a pressure cooker (Griffith and Thompson, 1949) and a portable gasoline burner provides a very satisfactory method. In using a pressure cooker, the culms may be bent and the sample tied in a bundle to increase the number of samples handled. It is essential to drive out all air with steam before closing the vent to insure high enough temperatures for effective blanching. At the conclusion of blanching the steam may be released rapidly. If necessary, the sample may be kept several hours in the shade before drying.

An alternative method was to autoclave samples in the laboratory soon after picking.

Drying. In the choice of an expedient drying method, one must sometimes balance known carotene losses in rapid drying against the longer time required for a lower loss. When a limited number of samples is to be dried, the time factor is of little importance, and conditions may be adjusted for minimum loss. When larger numbers of samples must be handled with limited time and drying facilities, larger losses from drying at higher temperatures may be tolerated if the loss is uniform among samples. There is little doubt that drying at low temperatures under high vacuum results in the least amount of carotene destruction of any drying method with which we are familiar. However, this method is not practical when a large number of samples is to be dried. Drying at 130° C was a common practice followed in the University of Chicago Botany Laboratory at Riverside, California (unpublished data), where it was found that losses were fairly uniform and were 10 per cent or less with the ovens used.

Table 2 presents the results of an experiment which tested various drying conditions. The samples were taken from 12 plots of California Common on three successive days. In sampling, two 25-culm samples were taken as rapidly as possible from a given plot, and the plots were sampled in the same order each day. All samples were blanched in steam at 5 p.s.i. for 2 minutes in an autoclave within ½ to 1 hour after sampling was started. Then the samples were separated so that no two from the same plot were subjected to the same drying treatments. The temperature of the plant material slowly increased as drying progressed. After drying, the samples were removed from the oven and weighed immediately. Some groups of samples were ground at once after removal from the oven, while others could not be ground until 1 to 2 days after drying. The ground samples were stored in tightly stoppered bottles at –12° C. Moisture was determined on 2-gram portions of the samples in November by drying in open vessels placed in a convection oven for 2 hours at 100° C and then cooled in a desiccator.

Results of the tests are complicated by the moisture differences among the treatments and, as will be shown later, strict comparisons can be made only on samples dried in a single day. Since the moisture contents given in table 2 were determined on samples that had been stored for some time, they are merely indicative of the moisture content when the samples were placed in the bottles after grinding. They do not indicate the moisture contents as the samples came from the original drying oven.

The carotene contents of the vacuum-dried samples averaged 302 p.p.m. and the samples at 130° C for one hour (treatment 6) 295 p.p.m. on a moisture-free basis. Using these figures the average total carotene for the two sets of samples would be 10.4 and 9.85 mg, respectively. Assuming no loss from vacuum drying, the loss from drying at 130° C in the oven was 2.3 per cent on the basis of p.p.m. carotene and 5.4 per cent on the basis of total carotene. Probably slight loss does occur even in vacuum drying. Changes due to isomerization of carotene during sampling and analysis were not considered of practical importance. Five per cent is a conservative estimate of loss, which is considerably less than differences of usual practical importance. Drying for an additional hour at 130° C resulted in at least 11 per cent destruction of the carotene present after one hour of drying (treatments 6 and 7).

In treatment 8, table 2, the entrance opening for air was changed to reduce the volume of entering air, resulting in a loss of carotene. It is thus very important to determine the characteristics of a given oven for such work. With the oven as employed, with maximum air openings and prompt removal of samples after drying, there were no significant differences in carotene content of samples dried at 65°, 100°, and 130° C.

Protein-content differences are not considered significant and, in general, follow the moisture differences.

Mitchell and King (1948) found increasing carotene loss with increasing temperature, results not substantiated in this experiment. Differences in drying conditions in the oven could account for this discrepancy, since sample weights were very different (30-fold), and oven loads relative to drying capacities probably differed also.

Moisture Content

This factor was not considered in the major part of the work herein reported but merited consideration in greater detail. From moisture data of table 2 it is seen that differences within single treatments are small and cannot explain differences in standard error, nor can they account for major differences in carotene content. As a general practice, samples should be ground immediately after removal from the oven, and the moisture content of samples should be determined at once, or ground samples should be maintained under constant conditions until a relatively constant moisture content has been reached.

Leaf-to-Stem Ratio

In this study twelve 15-culm samples of alfalfa at 1/10 bloom were divided into leaf-plus-petiole and stem fractions. The leaf-plus-petiole fraction constituted 58 per cent of the total dry weight and contained 92 per cent of the total carotene. This carotene value agreed well with the values of 90 per cent reported by Zscheile and Whitmore (1947) and 93 per cent given by Ham and Tysdal (1946).

EFFECT OF VARIETY

Varietal factors such as leafiness, differential response to seasonal influences, dormancy period, resistance to disease and insect attack, and adapta-

TABLE 3

SEASONAL STUDY OF SIX ALFALFA VARIETIES, THIRD CUTTING

Variety and sample series*	Plot						Average
	1	2	3	4	5	6	
			Carotene content, p.p.m.				
Hairy Peruvian							
A	272	260	232	242	278	256	257±7
B	278	283	240	276	260	291	271±7
California Common							
A	254	266	250	262	256	268	259±3
B	254	278	256	276	278	270	269±4
Buffalo							
A	272	240	266	228	266	272	257±8
B	266	278	287	256	276	293	276±6
Argentina	253	240	254	253	259	261	253±3
Indian	259	250	245	250	278	287	262±7
African	266	271	258	250	257	276	263±4
			Protein content, per cent				
Hairy Peruvian							
A	18.6	18.9	17.9	18.0	19.0	17.9	18.3±0.2
B	18.3	18.8	17.1	17.0	18.0	16.7	17.6±0.3
California Common							
A	17.6	18.6	18.2	18.7	18.2	18.4	18.3±0.1
B	18.1	18.8	18.2	19.0	18.4	17.7	18.4±0.2
Buffalo							
A	19.8	18.7	18.6	19.0	18.6	18.2	18.8±0.2
B	19.2	18.2	17.5	17.4	18.9	18.0	18.2±0.3
Argentina	17.8	17.0	18.0	17.8	17.7	18.3	17.8±0.2
Indian	19.6	19.9	19.7	19.5	18.0	18.4	19.2±0.3
African	20.1	19.4	18.6	18.4	18.9	19.0	19.1±0.2

* Series A sampled June 23; series B sampled June 24; Argentina sampled July 1; Indian and African sampled July 15, 1949.

bility to soil and climatic conditions may also affect carotene content and should be considered in evaluating variety comparisons. Differential soil fertility and moisture relations may also be important.

Many samples in this study were taken at the $\frac{1}{10}$ bloom stage of varieties grown under similar conditions throughout a season. Unless noted otherwise, the time of sampling was carefully chosen to obtain comparable stages of development for each variety or strain. Studies described below have demonstrated this to be a very important and essential precaution.

These studies started with the third cutting, in June, 1949. Six standard

TABLE 4

SEASONAL STUDY OF SIX ALFALFA VARIETIES, FOURTH CUTTING

Variety	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Average
				Carotene content, p.p.m.			
Hairy Peruvian	275	286	288	315	293	307	294±6
California Common	282	294	276	309	294	323	296±7
Buffalo	295	276	325	303	304	321	304±7
Argentina	290	301	285	295	289	306	294±3
Indian	276	288	264	283	283	279	279±3
African	293	270	276	265	268	286	276±4
				Protein content, per cent			
Hairy Peruvian	21.1	21.1	19.6	21.0	21.7	20.4	20.8±0.3
California Common	20.2	21.3	19.6	18.7	20.3	20.8	20.1±0.4
Buffalo	21.0	21.2	21.3	21.0	21.2	21.8	21.2±0.1
Argentina	18.5	19.8	19.4	20.0	20.7	20.6	19.8±0.3
Indian	21.8	21.4	21.2	21.3	20.9	21.1	21.3±0.1
African	21.2	20.4	20.5	20.2	20.0	20.9	20.5±0.2

Hairy Peruvian, California Common, and Buffalo were sampled July 22; Argentina was sampled August 2; Indian and African were sampled August 5, 1949.

TABLE 5

SEASONAL STUDY OF SIX ALFALFA VARIETIES, FIFTH CUTTING

Variety	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Average
				Carotene content, p.p.m.			
Hairy Peruvian	297	309	285	277	292	277	290±5
California Common	295	320	258	301	303	257	289±11
Buffalo	299	315	297	278	315	306	302±6
Argentina	299	297	294	272	299	324	298±7
Indian	293	299	287	276	293	284	289±3
African	305	310	284	307	301	307	302±4
				Protein content, per cent			
Hairy Peruvian	18.2	19.9	18.3	18.6	19.6	18.8	18.9±0.3
California Common	18.9	18.6	17.6	17.5	20.0	18.9	18.6±0.4
Buffalo	19.5	19.5	18.5	18.2	20.0	20.1	19.3±0.3
Argentina	20.2	19.5	20.2	18.7	21.4	19.2	19.9±0.4
Indian	19.4	20.8	18.0	18.7	18.0	19.3	19.0±0.4
African	19.5	19.8	18.0	19.6	18.3	20.4	19.3±0.4

Hairy Peruvian, California Common, and Buffalo were sampled August 25; Argentina, Indian, and African were sampled September 12, 1949.

TABLE 6

SEASONAL STUDY OF SIX ALFALFA VARIETIES, SIXTH CUTTING

Variety	Plot						Average
	1	2	3	4	5	6	
	Carotene content, p.p.m.						
Hairy Peruvian	274	326	332	291	322	319	312±9
California Common	319	329	285	325	337	305	317±8
Buffalo	332	310	313	313	323	301	315±4
Argentina	274	281	293	278	295	298	287±4
Indian	327	330	332	329	337	318	329±3
African	351	338	347	353	327	362	346±5
	Protein content, per cent						
Hairy Peruvian	17.6	20.0	19.2	18.0	19.6	18.6	18.8±0.5
California Common	19.4	19.0	17.2	18.0	18.6	17.8	18.3±0.3
Buffalo	19.6	19.4	18.7	18.0	17.7	18.2	18.6±0.3
Argentina	19.2	18.9	20.2	19.8	21.3	19.9	19.9±0.3
Indian	22.3	22.0	22.4	20.6	19.8	20.9	21.3±0.4
African	22.2	21.3	20.6	20.8	20.5	21.4	21.1±0.3

Hairy Peruvian, California Common and Buffalo sampled October 5; Indian and African sampled October 14; Argentina sampled October 28, 1949.

TABLE 7

SEASONAL STUDY OF SIX ALFALFA VARIETIES, SEVENTH CUTTING

Variety	Plot						Average
	1	2	3	4	5	6	
	Carotene content, p.p.m.						
Hairy Peruvian	261	250	252	271	317	274	271±10
California Common	321	335	262	286	327	278	302±12
Buffalo	327	307	328	271	313	325	312±9
Argentina	337	315	307	282	268	308	303±9
Indian	292	307	305	282	310	298	299±4
African	325	307	300	272	286	298	298±7
	Protein content, per cent						
Hairy Peruvian	22.6	23.7	23.0	23.5	23.5	21.9	23.0±0.3
California Common	23.0	22.5	22.6	24.2	23.1	22.3	22.9±0.3
Buffalo	24.3	23.9	24.9	23.1	24.8	24.0	24.2±0.3
Argentina	25.1	29.3	27.4	29.2	29.0	30.6	28.4±0.8
Indian	24.0	25.4	24.7	24.1	23.8	24.7	24.4±0.2
African	23.3	24.2	23.3	23.2	22.7	23.4	23.3±0.2

Sampled December 8, 1949.

TABLE 8

SEASONAL STUDY OF SIX ALFALFA VARIETIES, FIRST CUTTING

Variety	Carotene content, p.p.m.						
	Plot						Average
	1	2	3	4	5	6	
Hairy Peruvian	204	192	198	198	191	163	191±6
California Common	202	190	195	191	211	216	201±4
Buffalo	157	217	185	181	187	150	179±10
Argentina	180	204	226	213	203	209	206±6
Indian	197	222	191	163	150	190	185±10
African	196	197	171	203	211	166	191±7

Sampled April 24, 1950.

TABLE 9

SEASONAL SUMMARY OF SIX ALFALFA VARIETIES, SIX CUTTINGS

Variety	Cutting number						Over-all seasonal average
	1	3	4	5	6	7	
	Carotene content,* p.p.m.						
Hairy Peruvian	191	257	294	290	312	271	269
California Common	201	259	296	289	317	302	277
Buffalo	179	257	304	302	315	312	278
Argentina	206	253	294	298	287	303	274
Indian	185	262	279	289	329	299	274
African	191	263	276	302	346	298	279
Cutting average	192	258	290	295	318	297	275
	Protein content, per cent						
Hairy Peruvian	....	18.3	20.8	18.9	18.8	23.0	20.0
California Common	....	18.3	20.1	18.6	18.3	22.9	19.6
Buffalo	....	18.8	21.2	19.3	18.6	24.2	20.4
Argentina	....	17.8	19.8	19.9	19.9	28.4	21.1
Indian	....	19.2	21.3	19.0	21.3	24.4	21.0
African	....	19.1	20.5	19.3	21.1	23.3	20.7
Cutting average	....	18.6	20.6	19.2	19.7	24.4	20.5

* Each figure is the average of samples from 6 plots (tables 3–8)

varieties were sampled throughout a one-year period, omitting the second cutting, which was not sampled.

Tables 3 to 8 are arranged in parallel fashion to permit easy comparison of plots and varieties for both carotene and protein contents. The tables present in detail analyses for six varieties for each plot in the replicate series. In table 3 the B series of samples were taken one day later than the A series. In almost all cases the B value is higher than the corresponding A value of the previous day. The differences in the A and B averages are equal

TABLE 10

STUDY OF SIXTEEN ALFALFA VARIETIES AND STRAINS, THIRD CUTTING

Variety or strain	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Average
Carotene content, p.p.m.							
Buffalo	299	274	277	252	272	270	274±6
African	314	262	258	240	273	244	265±10
Atlantic	319	287	244	273	304	302	288±10
California Common B	278	262	264	234	276	292	268±8
Indian	283	269	267	246	267	234	261±7
California Common	289	272	270	264	280	293	278±5
Atlantic (California grown)	302	267	267	278	297	298	285±7
97	289	301	276	278	301	291	289±4
H2	286	250	252	265	290	310	276±10
H3	269	264	284	240	261	282	267±6
H4	278	282	243	260	260	297	270±8
48	284	272	260	244	271	266	266±5
105	280	266	257	252	266	289	268±6
106	284	279	295	256	266	256	273±7
84	295	276	281	252	283	267	276±6
89	288	274	282	287	283	297	285±3
Over-all average	...	...	...	...	...	...	274
Protein content, per cent							
Buffalo	23.7	21.4	21.5	20.4	20.0	20.7	21.3±0.5
African	25.0	20.0	20.0	20.0	19.0	19.2	20.5±0.9
Atlantic	23.6	23.6	19.7	21.7	21.2	21.3	21.8±0.6
California Common B	21.3	20.5	20.6	20.6	19.6	20.6	20.5±0.2
Indian	21.2	21.6	20.0	20.3	17.7	19.3	20.0±0.6
California Common	23.2	22.6	21.4	20.1	19.1	20.6	21.2±0.6
Atlantic (California grown)	22.4	22.8	21.7	22.0	22.0	21.4	22.0±0.2
97	21.9	21.5	20.2	20.4	20.7	20.0	20.8±0.3
H2	23.0	22.8	20.6	19.5	20.0	20.5	21.1±0.5
H3	21.5	21.4	20.9	21.3	20.0	20.9	21.0±0.3
H4	22.1	22.2	20.8	21.5	20.5	20.4	21.2±0.3
48	21.2	21.0	20.5	19.6	18.8	20.3	20.2±0.4
105	20.4	21.8	20.5	18.9	18.6	19.0	19.9±0.6
106	20.9	21.8	21.0	20.3	17.0	19.5	20.1±0.7
84	21.8	21.5	22.4	20.9	19.0	20.4	21.0±0.5
89	20.7	20.2	20.0	20.2	19.1	20.4	20.1±0.2
Over-all average	....	....	....	....	....	....	20.8

Sampled June 6 to 30, 1949.

to or greater than the standard errors of the means. Table 9 presents a summary of tables 3 to 8, with over-all seasonal averages for each variety, which represent 36 individual plot samples. Averages of six varieties are presented for each cutting. In carotene content no variety is consistently higher than the others throughout the six cuttings, and the over-all averages are almost identical. The seasonal influence discussed later is evident throughout this series. Seasonal trends are not evident in the protein content, but the over-

TABLE 11
STUDY OF SIXTEEN ALFALFA VARIETIES AND STRAINS, FOURTH CUTTING

Variety or strain	Plot						Average
	1	2	3	4	5	6	
Carotene content, p.p.m.							
Buffalo	303	294	289	313	293	314	301±4
African	295	288	269	299	283	292	288±4
Atlantic	317	294	260	310	313	302	299±8
California Common B	274	274	314	280	279	314	289±8
Indian	254	282	305	275	297	285	283±7
California Common	238	302	298	285	266	278	278±10
Atlantic (California grown)	331	300	271	298	348	298	308±11
97	296	308	316	277	313	284	299±7
H2	277	266	272	302	307	290	286±7
H3	272	284	287	314	272	276	284±6
H4	284	295	287	285	293	299	291±2
48	291	294	301	301	303	288	296±3
105	272	286	275	252	275	297	276±6
106	273	293	284	293	264	292	283±5
84	273	253	321	278	269	302	283±10
89	293	310	312	329	297	309	308±5
Over-all average	...	...	...	...	...	...	291
Protein content, per cent							
Buffalo	21.5	21.6	20.6	20.1	17.6	20.1	20.2±0.6
African	19.3	19.6	18.9	18.4	18.6	18.1	18.8±0.2
Atlantic	21.7	21.3	18.8	19.3	21.5	22.1	20.8±0.5
California Common B	20.1	20.2	21.6	19.9	19.6	20.0	20.2±0.1
Indian	19.9	20.3	21.2	19.4	19.0	18.9	19.8±0.3
California Common	20.2	20.8	20.2	19.5	19.5	19.4	19.9±0.2
Atlantic (California grown)	21.9	20.7	20.4	20.5	21.4	20.2	20.8±0.3
97	19.4	20.2	20.1	20.2	19.2	19.8	19.8±0.2
H2	20.0	19.7	19.9	20.2	20.2	19.7	19.9±0.1
H3	20.1	19.6	20.1	20.3	18.4	19.3	19.6±0.3
H4	21.0	20.3	20.9	20.8	19.8	20.2	20.5±0.2
48	19.2	18.4	18.5	17.6	18.9	18.8	18.6±0.2
105	19.1	19.2	18.6	17.9	18.5	19.0	18.7±0.2
106	19.3	19.2	19.4	19.0	17.1	18.4	18.7±0.4
84	17.9	16.7	21.5	18.7	19.3	18.5	18.8±0.6
89	19.4	19.4	18.7	19.4	18.7	18.8	19.1±0.1
Over-all average	....	....	....	....	....	....	19.6

Sampled July 28 and 29, 1949.

all averages are similar for the six varieties. Protein contents do not vary in a fashion parallel to carotene contents.

Tables 10 and 11 present data for two successive cuttings of five common varieties and 10 strains. With one exception (California Common) carotene contents for the fourth cutting exceeded those for the third, while the reverse relation obtained with the protein content for all varieties. Four of the same varieties reported for the same cuttings in tables 3 and 4 (different plots)

TABLE 12

CAROTENE AND PROTEIN CONTENTS OF VARIETIES AND PROGENY ROWS FROM SELECTIONS

(Third cutting)

Variety or selection	Harvest date (1949)	Carotene content, p.p.m.	Protein content, per cent
Indian-70-1 (California grown)	June 28	190	18.2
Cossack	July 1	258	18.0
African	July 1	241	17.2
Indian	June 28	226	19.0
Buffalo	July 1	220	17.5
Arizona Chilean	July 1	208	16.0
Argentina	July 1	252	19.0
Iran (Nematode resistant)	June 28	246	18.4
Oregon Creeping	July 1	274	19.8
Dakota Common	June 28	242	19.7
Utah Common	July 1	260	17.8
Kansas Common	June 28	230	18.6
*Kansas Common (yellow)	June 28	165	20.6
Hardigan	June 28	244	22.3
South African	July 1	257	18.8
Orestan	June 28	247	20.6
Nebraska Common	June 28	242	20.4
Meeker Baltic	June 28	256	22.6
Hardestan	July 1	239	19.3
Arabian	June 28	210	17.9
Chilean-40-13	June 28	234	20.4
Chilean-40-12	July 1	229	17.7
Broadleaf-40-1	July 1	198	17.0
Turkestan-40-2	July 1	226	18.2
Ecuador-40-3	July 1	231	17.7
Afghanistan-40-4	July 1	227	17.2
Indian-40-5 (2)†	June 28	203	17.2
Indian-40-5 (1)†	June 28	208	17.6
Atlantic	July 1	226	17.7
Ranger	July 1	243	18.5
Ladak	July 1	257	20.1
Grimm	July 1	250	19.2
Hairy Peruvian	July 1	212	17.5
California Common-43116	July 1	201	17.2
Argentina-70-4	July 1	244	19.1
Argentina-70-5	July 1	262	19.7
Argentina-70-6	July 1	262	19.2
Argentina-70-7	July 1	253	20.2
Average		235	18.7

* A yellow plant from Kansas Common, and not included in average.
† Different selections of Indian-40-5.
Numbers following variety names are Uniform Nursery numbers.

did not show this reversal effect with protein contents; the fourth cutting values all exceeded the third cutting figures. Again no great differences are evident among these varieties and strains. These data indicate that average differences of around 1 per cent in protein content and 20 p.p.m. carotene are of little significance in comparisons between varieties or cuttings. They are probably due to physiological conditions. Greater variations are often found among individual plots of a single variety. The selected strain B, of California

TABLE 13

CAROTENE AND PROTEIN CONTENTS OF VARIETIES AND PROGENY ROWS FROM SELECTIONS AND CROSSES BETWEEN SPECIFIC SELECTIONS

Fourth cutting

Variety, strain, or cross	Carotene content, p.p.m.	Protein content, per cent
California Common	246	17.8
California Common × Nebraska-17	215	17.6
African × Nebraska-17	238	19.6
Ranger	243	19.2
Indian × Nebraska-17	236	18.0
African × Nebraska-52	269	20.0
*African	244	20.2
African × Nebraska-54	214	19.1
California Common × Nebraska-54	249	20.2
*Indian × Nebraska-35	283	23.5
California Common × Nebraska-7	243	19.5
Indian	211	18.3
African × Nebraska-33	227	18.1
African × Nebraska-53	246	21.7
Indian × Nebraska-45	232	18.3
California Common × Nebraska-33	257	19.3
Nebraska 52 × California Common-10-39-1	268	21.2
California Common-10-39-1 × Indian	250	21.2
California Common-10-39-1 × African	247	19.4
California Common-10-39-1	250	19.3
Indian	259	18.5
California Common-0-26-9 × Indian	221	17.9
California Common-0-26-9 × African	260	19.3
Nebraska 35 × California Common-0-26-9	234	18.0
California Common-0-26-9	241	18.3
African	274	19.2
*Indian × California Common-10-81-2	297	23.3
Nebraska-7 × California Common-10-81-2	274	20.4
California Common-10-81-2	269	18.6
African × California Common-10-81-2	250	20.5
Nebraska-54 × California Common-10-68-1	251	18.4
Average	245	19.5

* Sampled July 28 at 1/10 bloom.
Others sampled August 5, 1949, were of different maturity and past 1/10 bloom. The first-named parent of crosses was female. Numbers refer to specific plant selections; absence of a number indicates bulk seed of the variety named.

Common, did not differ from California Common, nor did California-grown Atlantic differ from Atlantic from seeds produced elsewhere.

Table 12 includes data from 23 varieties and 15 selections for the third cutting. This large group of varieties and selections, each represented by only a single sample and analysis, presents a considerable range of values, from 190 to 274 p.p.m. carotene and from 16.0 to 22.6 per cent protein. The yellow selection of Kansas Common is excessively low in carotene, as in chlorophyll content, and simply shows that strains very low in carotene can be found. In general, protein values are parallel to carotene values, but the very limited number of samples would invalidate any generalization on this subject from these data, as discussed in the previous paragraph. Several

TABLE 14

CAROTENE AND PROTEIN CONTENTS OF PARENT AND HYBRID
Third cutting

Strain	Plot 1	2	3	4	5	6	Average
Carotene content, p.p.m.							
California Common (Wilt Resistant, bed 7)*	246	245	243	242	241	232	241±2
Turkestan	252	262	203	239	238	226	237±8
Protein content, per cent							
California Common (Wilt Resistant, bed 7)*	19.3	19.2	20.3	20.1	19.5	18.8	19.5±0.2
Turkestan	19.0	19.0	18.9	20.2	19.8	19.1	19.5±0.2

Sampled June 29, 1949.
* Progeny from cross California Common × Turkestan. Turkestan is the source of wilt resistance and winter hardiness.

TABLE 15

CAROTENE AND PROTEIN CONTENTS OF IMPROVED STRAINS AND HYBRIDS
Fourth cutting

Variety or strain	Plot 1	2	3	4	5	6	Average
Carotene content, p.p.m.							
California Common B*	268	251	276	263	272	250	263±4
California Common–49†	287	270	273	249	270	256	267±5
California Common (Bed 33)‡	287	266	257	248	289	262	268±7
Caliverde§	274	283	248	278	278	249	268±6
Arizona Common–21–5	274	266	249	230	257	264	257±6
Nebraska–54 × African¶	278	294	284	282	284	275	283±3
Over-all average	...	...	...	...	...	...	268
Protein content, per cent							
California Common B	19.9	19.3	....	18.4	19.3	19.4	19.3±0.2
California Common–49	20.0	19.7	19.5	18.6	18.1	18.6	19.1±0.3
California Common (Bed 33)	20.5	20.1	19.7	19.0	19.5	18.2	19.5±0.3
Caliverde	20.1	20.0	17.8	19.4	19.4	18.9	19.3±0.3
Arizona Common–21–5	19.8	18.8	19.4	18.2	18.7	20.0	19.2±0.3
Nebraska–54 × African	20.5	20.6	19.5	20.2	20.6	19.3	20.1±0.2
Over-all average	....	....	....	....	....	....	19.4

Sampled July 19, 1949.
* B-Selection of F. N. Briggs.
† Dwarf resistant.
‡ Nematode tolerant.
§ Caliverde is resistant to wilt, mildew, and leaf spot.
¶ Hybrid.

TABLE 16
CAROTENE AND PROTEIN CONTENTS OF IMPROVED STRAINS AND HYBRIDS
Fifth cutting

Variety or strain	Plot						Average
	1	2	3	4	5	6	
Carotene content, p.p.m.							
California Common B	282	299	319	317	311	317	307±6
California Common-49	312	321	310	298	297	293	305±4
California Common (Bed 33)	290	319	316	320	303	293	307±5
Caliverde	318	310	293	307	327	307	310±5
Arizona Common-21-5	290	295	310	317	324	312	308±5
Nebraska-54 × African	329	334	319	330	315	319	324±3
Over-all average	...	...	...	...	...	...	310
Protein content, per cent							
California Common B	19.8	19.7	19.9	18.6	19.2	20.7	19.7±0.3
California Common-49	21.2	20.2	18.9	18.9	19.5	19.4	19.7±0.3
California Common (Bed 33)	20.9	21.0	20.2	20.4	19.8	18.7	20.2±0.3
Caliverde	20.6	20.0	20.1	19.0	19.3	18.7	19.6±0.3
Arizona Common-21-5	22.0	20.6	20.0	19.4	19.7	19.8	20.2±0.4
Nebraska-54 × African	20.6	20.8	20.9	20.4	20.3	19.7	20.4±0.2
Over-all average	....	....	....	....	....	....	20.0

Sampled August 25, 1949.

generalizations might be drawn concerning carotene contents of certain groups of strains. The Indian selections are below average, those from Africa and Argentina are higher than the average, while two Chilean strains are about average. Utah Common is the highest of the "Common" group, which ranges from 201 to 260 p.p.m. Hairy Peruvian, Buffalo, Indian, and African are considerably lower than in the plots of table 3 for the same cutting.

Table 13 permits comparison of four varieties, three strains, and 22 crosses between selected strains and other selections or varieties for the fourth cutting. Differing maturity stages at time of sampling complicate interpretation of these data. The over-all average carotene value is almost identical with that of the group reported in table 12 for the third cutting or other selections, but much lower than values of table 11 for the fourth cutting. The range of 211 to 297 p.p.m. carotene is of size comparable to that of table 12. Protein values range from 17.6 to 23.5 per cent. The crosses Indian × California Common-10-81-2 and Indian × Nebraska-35 have very high values for both carotene and protein. These were sampled at $\frac{1}{10}$ bloom and are therefore comparable to values of table 12, in which many varieties and strains had comparable carotene contents but none had as high values for protein content.

Table 14 compares samples from a derived wilt-resistant strain of California Common and Turkestan, one of its parents. It is clear that parent and

TABLE 17

CAROTENE AND PROTEIN CONTENTS OF IMPROVED STRAINS AND HYBRIDS

Sixth cutting

Variety or strain	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Average
Carotene content, p.p.m.							
California Common B	321	337	329	315	332	324	326±3
California Common-49	336	311	329	324	307	339	324±5
California Common (Bed 33)	336	326	327	350	297	327	327±7
Caliverde	335	318	324	318	308	285	315±6
Arizona Common-21-5	336	330	329	318	323	328	327±3
Nebraska-54 × African	338	338	346	324	356	350	342±5
Over-all average	...	...	...	...	...	...	327
Protein content, per cent							
California Common B	19.5	19.5	19.2	18.5	18.7	19.3	19.1±0.2
California Common-49	19.3	18.7	18.8	19.4	18.9	20.1	19.2±0.2
California Common (Bed 33)	20.0	19.2	20.0	19.6	18.1	19.8	19.4±0.3
Caliverde	20.6	19.4	19.5	19.4	18.8	18.8	19.4±0.3
Arizona Common-21-5	19.6	19.3	19.3	19.1	19.1	19.1	19.2±0.2
Nebraska-54 × African	20.8	20.3	19.7	20.8	20.4	20.7	20.4±0.2
Over-all average	....	....	....	....	....	....	19.4

Sampled October 5, 1949.

hybrid are similar in both carotene and protein contents and not greatly different from the other parent (California Common) in the same cutting (table 3).

Tables 15 to 17 compare three successive cuttings of five important selections and one cross. The selections from a Common (Chilean) source all had remarkably similar values for carotene and protein contents. The cross Nebraska-54 × African contained appreciably higher contents in all cases except for protein in the fifth cutting (table 16). This consistent trend over three cuttings suggests a possible superiority, which may be due to increased leafiness of this cross. The seasonal upward trend of carotene values is evident in this series of observations.

PHYSIOLOGICAL FACTORS

Maturity

The importance of maturity as a factor to consider in carotene analyses is illustrated by the data of table 18. For this experiment, California Common plots were sampled twice a week during most of the period from July 15 (13 days after cutting) to September 16 (ripe-seed stage). Twenty-five-culm samples were taken from each of six plots, except for the initial samples that consisted of 50 culms each because of their small size. On and after August 8,

TABLE 18
VARIATION OF OVEN DRY WEIGHTS, CAROTENE, AND PROTEIN CONTENTS OF 25 CULMS WITH MATURITY
California Common, fifth cutting; fourth cutting date July 2

Date	Days after cutting	Oven dry weight		Carotene content		Protein content, per cent	Maturity
		Grams	Per cent	p.p.m.	Total mg		
Initial plots							
July 15	13	2.9±0.27	....	350±9	1.03±.11	34.1±0.3	3-4 inches tall
July 19	17	7.0±0.17	....	358±4	2.51±.05	30.8±0.4	....
July 22	20	10.3±0.5	15.7	353±5	3.63±.15	27.9±0.2	....
July 26	24	16.5±0.5	16.5	344±3	5.67±.21	25.2±0.4	Early bud
July 29	27	19.5±0.9	18.8	309±2	6.00±.24	22.5±0.4	....
Aug. 3	32	25.9±1.7	21.9	298±4	7.70±.41	19.3±0.2	1/10 bloom
Aug. 5	34	30.2±1.0	20.9	271±3	8.22±.35	19.8±0.1	....
Aug. 9	38	32.2±1.7	22.0	272±5	8.74±.45	18.8±0.2	....
Aug. 12	41	46.7±1.8	24.0	255±5	11.9 ±.6	17.6±0.3	1/4 bloom
Aug. 16	45	55.2±1.6	24.5	248±2	13.7 ±.4	16.8±0.1	....
Aug. 19	48	63.4±2.3	24.8	250±3	15.9 ±.6	16.6±0.2	Seed pods present
Aug. 23	52	68.1±3.3	25.8	226±3	15.4 ±.7	16.0±0.2	....
Aug. 26	55	55.8±3.4	26.0	236±5	13.1 ±.6	15.9±0.1	....
Aug. 30	59	71.7±1.1	27.5	215±4	15.4 ±.7	15.6±0.1	....
Sept. 8	68	68.9±4.1	27.9	201±4	13.9 ±.9	15.1±0.3	....
Sept. 16	76	63.0±2.9	30.4	213±7	13.5 ±.9	15.5±0.6	Ripe seed
Adjacent plots							
Aug. 8	37	32.6±1.4	....	267±4	8.69±.4	18.3±0.2	....
Aug. 9	38	35.1±1.6	....	260±4	9.13±.4	17.4±0.2	....
Aug. 23	52	53.5±1.0	25.7	239±4	12.8 ±.4	16.4±0.2	....
Aug. 30	59	60.5±2.4	25.7	226±8	13.6 ±.4	15.4±0.8	....
Sept. 8	68	61.9±2.4	27.2	173±4	10.8 ±.6	15.1±0.2	....
Sept. 16	76	57.5±2.0	28.4	220±4	12.7 ±.3	14.1±0.2	Ripe seed

additional samples were also taken from adjacent (previously unsampled) plots, since it was obvious that after this date excessive thinning of stand in the initial plots was causing misleading results. The dry weights were determined shortly after removal from the oven. Protein and carotene analyses were made on these samples, and results are presented graphically in figures 1 to 4. After August 8, data are presented for both the initial and adjacent plots. Data from the latter are considered the more significant.

The dry weights of 25 culms increased in a linear manner until August 30. The rate of increase averaged about 1.3 grams per day throughout the period of increase. Stand-thinning in initial plots resulted in a marked increase in dry weight after August 9, the average rate of increase between August 9 and 30 being about 1.9 grams per day. During this period the fiber content increased rapidly. The dry weight increased from about 16 to 29 per cent during the course of growth. The rate of increase on a percentage basis, however, was not so constant as the increase in dry weight on an absolute basis and was markedly influenced by irrigations on August 3 and September 3.

Protein contents, plotted in figure 2, showed a steady and consistent decrease, the rate of which became less after 40 days.

The maximum carotene content (fig. 3) was found in the pre-bud stage and decreased rapidly from then until the $1/10$ bloom stage was reached. Thereafter the rate of decrease was less. The marked increase noted on the final determination (76 days) was undoubtedly influenced greatly at this time by a resumption of growth from lateral buds. The rate of decrease in carotene content was probably less than it would have been earlier in the season for a comparable growth cycle since, as will be noted below, the experimental period extended over a period of generally rising carotene content.

Although the carotene content in parts per million decreased markedly, the total carotene per 25 culms (fig. 4) increased throughout most of the experimental period and reached a peak slightly before maximum dry weight was reached. The general practice of harvesting at $1/10$ bloom results in a fairly high carotene content at cutting, but the maximum total carotene yield is not reached at that time. No data are available to determine the stage of cutting that would give maximum carotene yields on a seasonal basis. Other factors would probably be more important than this one in setting the exact stage of cutting.

A near-by field of California Common was sampled on four different dates, as indicated in table 19. On August 2 the alfalfa was at the $1/10$ bloom stage, and the carotene content was 273 ± 5 p.p.m. One week later, samples from the same general area averaged 228 ± 4 p.p.m. This decrease was similar to that observed in table 18 for a corresponding period of time.

The above data indicate the necessity of sampling at the same stage of maturity (according to floral development) in any studies involving variety comparisons of carotene content. Other data supporting these findings will be presented below (tables 19 and 20). Since varieties differ widely in maturity dates, the practice of sampling several varieties a given number of days from a common cutting date can lead to erroneous conclusions with regard to their carotene-producing capabilities.

Time of Day and Sampling on Successive Days

Extensive studies were made of the variation of carotene content during the day. Differences of 4 to 8 per cent were commonly obtained between the carotene contents of samples taken in the morning (8 to 8:30) and afternoon (2 to 2:30), but the differences were not consistent. One day the carotene content of the morning samples might be higher, and the next day the afternoon samples might be higher. Even on the same day, samples taken from alfalfa at different stages of maturity might show higher or lower values for either of the two times of harvest.

Data were obtained which show that differences of 4 to 8 per cent may also be realized if samples are taken at the same time of day on successive days, as illustrated by table 20. The carotene contents of the three varieties were practically the same for a given day but differed significantly at the 1 per cent level between the two days. To establish the cause of such differences would require much work with exact control over environmental conditions. This was not attempted. The results of Thompson (1949) on this subject are

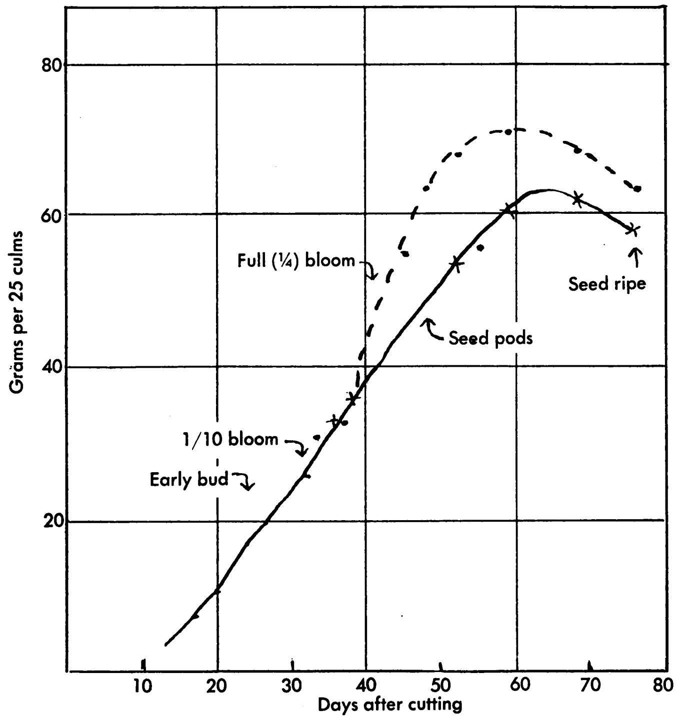

Fig. 1. Sample dry weights. Dotted line represents data for the initial plots in table 18. Solid line beyond point of divergence represents adjacent plots.

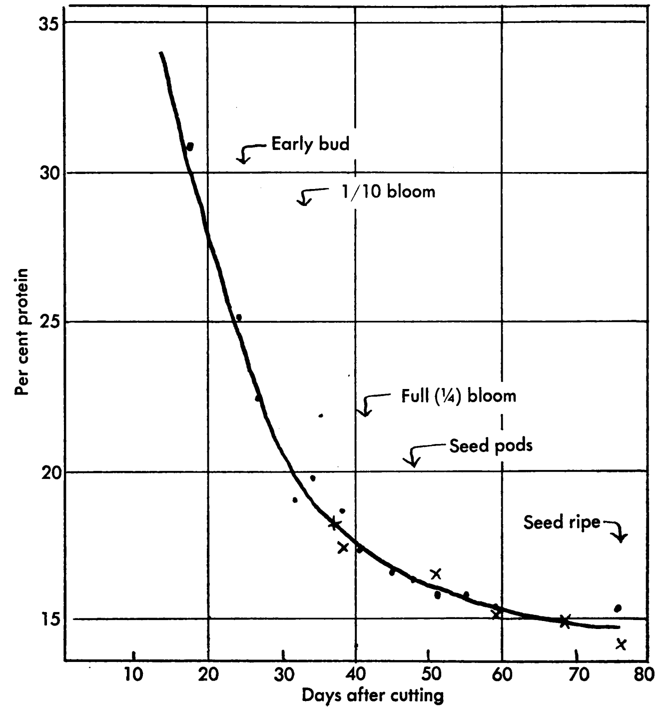

Fig. 2. Protein contents.

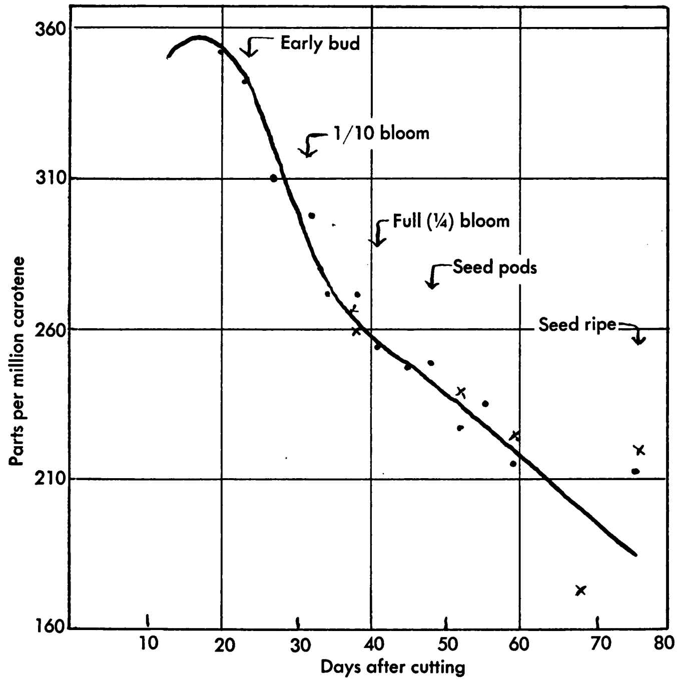

Fig. 3. Carotene contents.

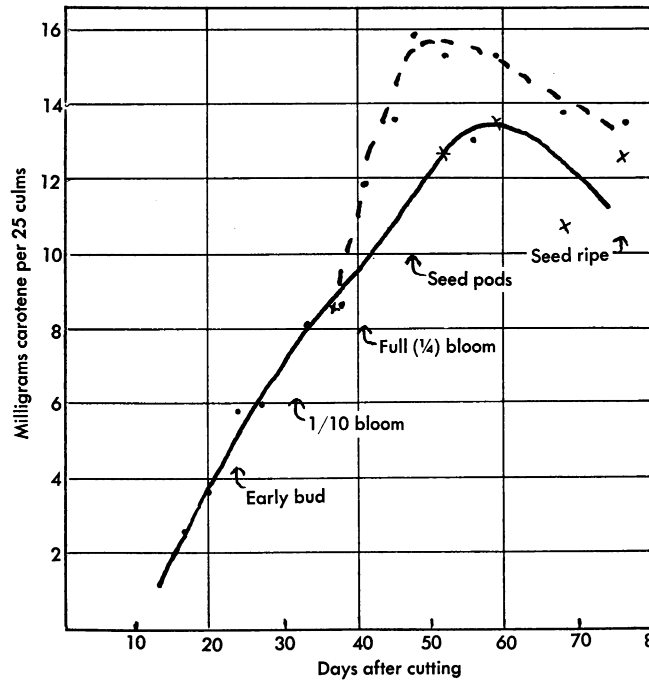

Fig. 4. Total carotene. Dotted line represents data for the initial plots in table 18. Solid line beyond point of divergence represents adjacent plots.

TABLE 19
VARIATION OF CAROTENE AND PROTEIN CONTENTS OF CALIFORNIA COMMON WITH MATURITY AND SEASON

Check*	Sample	July 5, 1949 (Third cutting)		Aug. 2, 1949 (Fourth cutting)		Aug. 9, 1949 (Fourth cutting)		Sept. 26, 1949 (Sixth cutting)	
		Carotene content, p.p.m.	Protein content, per cent	Carotene content, p.p.m.	Protein content, per cent	Carotene content, p.p.m.	Protein content, per cent	Carotene content, p.p.m.	Protein content, per cent
A	1	233	17.1	267	19.2	207	15.8	271	19.3
	2	252	17.2	259	18.8	234	16.5	282	18.7
	3	243	17.6	278	19.3	253	17.4	264	17.4
B	4	242	18.7	235	19.7	215	15.9	279	18.4
	5	250	17.8	270	19.2	223	16.8	290	17.6
	6	236	18.3	278	20.0	231	17.3	262	17.2
C	7	237	18.6	277	19.1	231	16.4	262	16.4
	8	246	17.7	287	19.8	232	17.4	291	17.7
	9	246	17.8	292	19.2	234	16.7	...	....
D	10	267	18.7	256	18.8	226	16.7	274	17.6
	11	233	17.7	291	19.4	215	15.9	317	19.0
	12	245	18.8	283	19.8	236	16.6	...	....
	Average	244±3	18.0±0.2	273±5	19.3±0.1	228±3	16.6±0.2	279±5	17.9±0.3

* These checks were 1/10 acre in size.

TABLE 20
CAROTENE CONTENTS ON SUCCESSIVE DAYS
Third cutting

Variety	Carotene content, p.p.m.	
	June 23	June 24
Hairy Peruvian	257±7	271±7
California Common	259±3	269±4
Buffalo	257±8	276±6

likewise not considered comprehensive enough to demonstrate a clearcut effect of the time of day.

Seasonal Influence

The importance of seasonal influence on carotene content is indicated best in table 9. The periods represent successive cuttings, made at the $\frac{1}{10}$ bloom stage on a date carefully chosen for each of six varieties. At least six samples were taken from each variety, and over-all averages of varieties are presented for each cutting. An upward trend in carotene content is apparent in this period of one year, amounting to 65 per cent, and the value reaches a maximum at the sixth cutting. A similar trend is evident in a shorter period of 12 weeks during which California Common was sampled (table 19). The increase was 14 per cent in this period. Other more limited studies (tables 10, 11, 15, 16, 17) are in agreement with this conclusion. These data show that error may arise from comparisons among different varieties sampled at different seasons of the year.

No similar trends are evident in the protein contents.

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