

## Effect of Age (Position on Stolon) on the Carotene Content of Leaf Blades and Leaf Stalks of Ladino Clover

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(Figs. 1-2; Tables 1-4; Plate 1)

### INTRODUCTION

Recently Ladino clover has become more widely grown and used in Japan with the major purpose of providing an economical and highly nutritious feed for livestock. Ladino clover under good management and wise use as pasture, silage or hay may reduce significantly the cost of supplying protein often bought as concentrates. Furthermore, from the standpoint of carotene content Ladino clover assumes very important role. It is well known that carotene is a substance which changes into Vitamin A in the body. As Vitamin A essentially serves as a stimulus for the formation of new cells, this nutrient is necessary for the maintenance of good health of live stock.

However, commercial concentrates normally do not supply this essential vitamin and are often very deficient in carotene. Therefore, for animals which do not graze nor are allowed access to Ladino clover, special arrangement should be made to include Vitamin A in their diet. When fresh Ladino clover is available, carotene is ingested in amounts vastly exceeding the requirements for the formation of Vitamin A. In this respect Ladino clover can be stressed, being by far the best source of carotene.

The most important part of Ladino clover associated with carotene content is the leaf. Therefore, the proportion of leaf blades to leaf stalks serves as a good index of carotene content. Leaf blade of Ladino clover contains most of carotene and the total carotene content is determined largely by the percentage of leaves to stems.

With regard to the relation between carotene content and green color it is said that the deeper the green color, the higher the carotene content. Chlorophylls are always accompanied by yellow pigments, carotene and xanthophyll. This means that carotene with other carotenoids and green pigments, chlorophylls, are related to photosynthesis.

It has recently been shown that in the absence of carotene and other carotenoid pigments the photosynthetic apparatus is rapidly destroyed and it is suggested, therefore, that the essential function of carotenoids including carotene in the grana of plant cells is to prevent or minimize photooxidative damage by chlorophyll.

The objectives of this experiment were first to ascertain the effect of age on the proportion of leaf blades to leaf stalks, second, to determine the effect of the percentage of leaf blade to leaf stalk on the carotene content, and third to demonstrate whether a direct relationship exists between green pigments, chlorophylls and carotene content of

Ladino clover.

## MATERIALS AND METHODS

These experiments were conducted under field conditions during the growing season of 1963, 1964 and 1965 at the Forage Research Field of Kamo, Miyuki and Okinogami of the Department of Fisheries and Animal Husbandry, Hiroshima University.

Ladino clover under favorable soil and climatic conditions often attains a height of 35–40 cm or more. In these experiments the oldest leaves near the base of stolons were at least 30 days older than the youngest ones that grew directly from the nodes of the same stolons and its stalks height was 40 cm. The stalks of the youngest leaves just developing on the top at the nodes of stolons were 7.5 cm in height. Every sample was collected by removing them in the morning on different days. Approximately 10–15 fresh leaf blades with stalks were collected to study the effect of position on the proportion of leaf blades to leaf stalks at the stolons and were used for the chemical analysis. At the same time a study was attempted to determine the effects on the carotene content of flowers at various flowering stages and also of stolons.

Carotene analysis was performed according to the method of the Association of Vitamin Chemists, Inc. U.S.A., while chlorophyll analysis was carried out according to the established procedures of the official methods of analysis of A.O.A.C. (Association of Official Agricultural Chemists)

## RESULTS

Table 1 shows that the proportion of leaf blades to leaf stalks varies with advancing age or the position at the node of stolon. Young small new leaves at the height of 7.5 cm have a higher percentage of leaf blades by weight in comparison with its leaf stalks than the large fully developed old leaves.

Although there were a few exceptions, the proportion of leaf blades generally decreased with advance in age. When top young small leaves were harvested at the height of 7.5 cm, leaf blades ranged from 34.60 to 40.06% by weight and leaf stalks from 65.40 to 59.94%. When it grows to the height of 20 cm with advance in age (perhaps older than 15 days) the leaf blades make up 31.33–33.71% and the stalks may range from 68.67 to 66.29%; when it reaches 30 cm in height, the proportion of leaf blades falls to 27.48–29.93%. In this experiment the fully developed old large leaves near the base of the stolons were 40 cm high (about 30 days older). The percentage of leaf blades at this age decreased to 25.52–26.25%. Thus, with advancing age leaves on the stolon of Ladino clover decrease in the proportion with leaf blades and increase in leaf stalks.

Table 2 presents (position on the stolon) the effect of age of leaves on the carotene content of Ladino clover. And also analysis for carotene content in leaf blades and stalks at various ages is given in Table 2. In this experiment young small leaves just developing

Table 1. Effect of Age (Position on Stolon) on the Proportion of Leaf Blades and Stalks of Ladino Clover

Stage of Growth (Age)	1963 (Kamo)		1964 (Miyuki)		1965 (Okinogami)	
	Blades	Stalks	Blades	Stalks	Blades	Stalks
7.5 cm high (about 3 days old)	37.80	62.20	34.60	65.40	40.06	59.94
20.0 cm high (about 13 days old)	33.71	66.29	31.33	68.67	33.01	66.99
30.0 cm high (about 20 days old)	28.08	71.92	27.48	72.52	29.93	70.07
40.0 cm high (about 30 days old)	26.06	73.94	26.25	73.75	25.52	74.48

Table 2. Carotene Content in Leaf Blades and Leaf Stalks at Various Stages of Ladino Clover

Stage of Growth (Age)	1963 (Kamo)			1964 (Miyuki)			1965 (Okinogami)		
	Blades	Stalks	Entire leaves	Blades	Stalks	Entire leaves	Blades	Stalks	Entire leaves
7.5 cm high (about 3 days old)	1.447	0.156	0.648	1.104	0.150	0.937	1.881	0.240	0.837
20.0 cm high (about 13 days old)	2.263	0.319	0.975	3.718	0.388	1.370	3.997	0.334	1.482
30.0 cm high (about 20 days old)	4.721	0.478	1.521	7.416	0.710	2.811	5.641	0.616	2.120
40.0 cm high (about 30 days old)	5.578	0.578	1.883	9.536	0.750	3.050	6.288	0.633	2.320

on the top of the stolons contained 1.474 mg% carotene on the average and its stalks contained 0.184 mg %. The total carotene of entire leaves (blade and stalk) was 0.638 mg % (3-year average). As the age advanced and it reached the height of 20 cm, the amount of carotene in its blades and stalks was greater than the younger small top leaves taken from the same stolons. At this age entire leaves had an average of 1.297 mg % carotene, leaf blades contained only 3.329 mg %, and stalks had 0.347 mg %. As the leaves further aged, they gradually reached near the base of stolons. Though the proportion of leaf blades was decreased in this stage carotene content of the entire leaves, blades and stalks was increased.

When leaves were harvested at the height of 30 cm, blades contained about 5.690 mg% and the stalks 0.564 mg%. The entire leaves contained 2.084 mg% of carotene on the 3-year average. Fully developed large leaves near the base of the same stolons was 40 cm high and the carotene content in the entire leaves was 4.148 mg% on the average. Their blades only contained 8.046 mg% and the stalks 0.661 mg%.

These results demonstrate that young entire leaves (including blades and stalks) contained less carotene than older leaves taken from the base of same stolons. Although the carotene content was lower at younger age, it was especially small in amount from the top young leaves to the height of 20 cm. In these younger stages leaf blades on the stolons are usually not completely open and the leaf color is pale green or blue.

The results of these experiments also suggested that the leaf blade contained about 90% of the carotene of the entire leaf.

Table 3. (A) Carotene Content of Flowers

Stage of Flowering	Carotene Content		
	Flower heads	Flower stalks	Total
	mg%	mg%	mg%
Flower, in bud stage	1.147	0.237	0.406
Flower, in early bloom stage	0.976	0.361	0.584
Flower, in full bloom stage	1.114	0.574	0.778
Flower, in late bloom stage	0.531	0.760	0.665

Table 3. (B) Carotene Content of Stolons

Length of Stolon	Carotene Content
cm	mg%
Stolon, 25	0.069
Stolon, 35	0.042
Stolon, 43	0.127
Stolon, 46	0.195

Some additional data on carotene content of the flowers at various flowering stages and the stolons are given in Table 3, A and B. This study shows that the carotene

content in the flowers varies with the stages of flowering and is relatively high at the full bloom stage. However, the carotene content in flower is very small ranging from 0.4 to 0.8 mg%. Stolons of Ladino clover also contain a very small amount of carotene and appear white in color. Thus, the carotene content is very low in the stolons as compared with other parts of Ladino clover, but generally stolons are not taken into account as nutrients of the entire plant and also practically they are not harvested. This is one of the most important reasons why Ladino clover contains relatively large amounts of carotene in comparison with other legumes. Leaves and flowers are the only parts of the plant harvested and Ladino clover does not produce as many flowers as white clover or other legumes.

Ladino clover is one of the most important sources of carotene because leaves are large in size, only a small amount of flowers is produced and stolons are not harvested.

Table 4. Correlations between Carotene and Chlorophylls in Leaves at Various Stages of Ladino Clover

Stage of Growth	Chlorophylls	Carotene
	mg %	mg %
7.5 cm high	8.121	0.837
20.0 cm high	11.917	1.482
30.0 cm high		2.120
40.0 cm high	12.967	2.320

The relation between the carotene content and the green color, chlorophylls, is shown in Table 4. It can be noted that the deeper the green color, the higher the carotene content. The color of leaves was different at different stages of growth. These analytical results show that leaves are not very green when they are young and these contain less carotene. Old fully developed large leaves near the base of the same stolons contain a larger quantity of chlorophylls and also carotene. Thus, the degree of greenness of leaves is a good index of carotene content.

The leaves in the young stage just developed at the top of the stolons contained about 8.121 mg% of chlorophylls and its carotene content was only 0.837 mg%, but older leaves deep green in color contained 12.967 mg% of chlorophylls and 2.320 mg% carotene.

It has been known that beside chlorophyll, all green parts of plants contain carotenoids. Carotenoids are found together with chlorophylls in the chromatophores and are present in either an amorphous or crystalline state.

The chlorophylls and carotenoids of Ladino clover are those pigments which absorb most of the light spectrum except green and yellow. The absorption spectra of chlorophylls and those of carotene of Ladino clover are shown in Fig. 1. HAXO and BLINKS measured the effectiveness of photosynthesis using the same amount of light but different wave lengths and obtained graph in Fig.2 which is very similar to the sum of absorption spectra of both pigments.

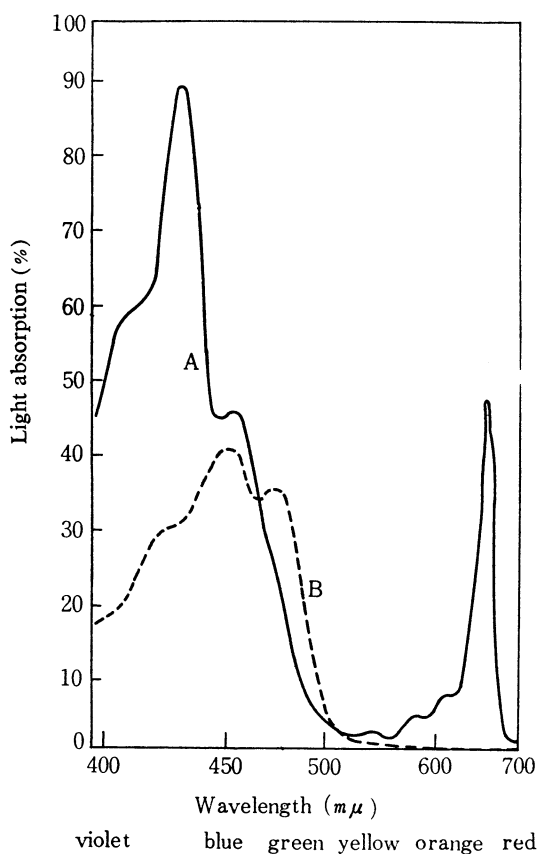


Fig. 1. Absorption spectra of the chlorophylls (A) and of carotene (B) (Ladino clover).

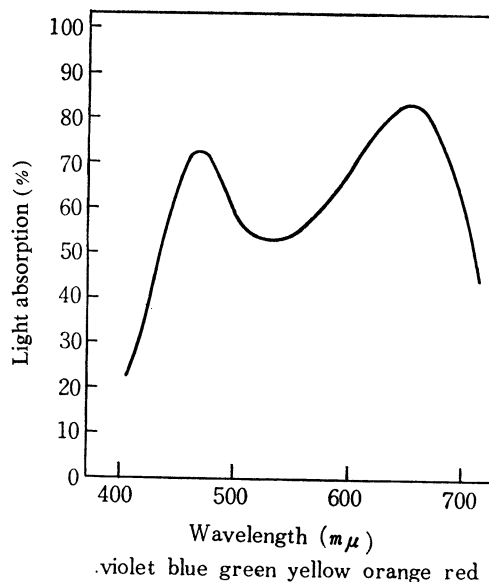


Fig. 2. Effectiveness of photosynthesis using same amounts of light but different wave lengths.

This experiment shows that the total quantity of light absorbed during the process is all engaged in photosynthesis. Furthermore, by comparing Fig. 1 with 2, one can see that at wave lengths of 450–500 ( $m\mu$ ), where the maximum absorption is caused by carotene, photosynthetic reaction was stronger than would have been expected with chlorophyll alone. This indicates that the “yellow companions” also play a definite role. However, since no photosynthesis has ever been discovered in the absence of chlorophyll, one is compelled to assume that the carotenoids, although they absorb light energy, transfer it immediately to the chlorophyll.

#### SUMMARY

The age of leaves of Ladino clover had a significant effect on both the proportion of leaf blades to leaf stalks and also its carotene content. Young small new leaves harvested from the top of stolons of Ladino clover have a higher percentage of leaf blades in comparison with its stalks than large fully developed old leaves of the same stolons. Leaf blades at any growing stage in general contained almost 90 per cent of

the total carotene in the entire leaves. Leaf stalks have only 10 per cent of the total carotene in Ladino clover. Therefore, the most important part of Ladino clover is the leaf blades which is the best source of Vitamin A for live-stock. If leaves are very young just beginning to grow on the top of stolons, the carotene content is very low and the color of such leaves is pale green. These pale green colored leaves were predominant among leaves under the height of 20 cm. At these stages most leaves are not yet completely opened and are not very green.

A significant relationship exists between the carotene content and the green color, chlorophylls of leaves. It is well known that the more green pigments the leaves possess, the higher the carotene content. If leaves contain a large amount of carotene, they usually have deep green color.

Therefore, the best indicator of carotene content is leaf color; if leaves contain a large amount of carotene, they usually have a good green color. Ladino clover is one of the best sources of carotene. This is to be expected because the leaf blades, leaf stalks and relatively small amounts of flowers are the only parts of the entire plant which are harvested. When the stems of any other legumes or grasses are harvested, stolons of Ladino clover should not be included.

In conclusion, the results of this study indicate that Ladino clover should be allowed to grow to the height of 25 cm after each pasturing and also should not be cut or grazed to the height less than 5 to 7 cm.

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## Ladino clover 茎葉中の Carotene 含量に及ぼす Age (Stolon 上の位置) の影響

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Ladino clover の葉身、葉柄中の Carotene 含量は生育期の如何にかかわらず常に葉身中にその大部分 (凡そ90%程度) がみられ、葉柄中には微量 (10%程度) にすぎなかった。花 (頭花、花梗) は花蕾、開花初期、最盛期或は終期等 (Flowering stage) により多少差はあったが葉身に比し Carotene 含量は甚だしく少なく、同様に Stolon も白色を呈し Chlorophyll, Carotene 共に殆んど皆無にひとしい。肉眼的に一見同様と見られる Ladino clover の茎葉比率及び Carotene 含量はその生育時期即ち Stolon 上の位置により相当差がみられ、Stolon 先端上の生育初期の茎葉は基部に近く Age の進んだものに比し葉身きわめて小さく葉柄細く短かい。この時期の葉身の割合は高く全茎葉重量の35—40%を占めているが、その成長につれて葉身は大きく葉柄もより太く伸びるが葉身の割合は反対に減少して40cmに達する頃全茎葉の凡そ25%程度であった。

幼時 (7.5cm) は葉身未だ開かず葉柄15cm前後に達する頃半開状となり更らにその生育進み20cmに達する頃完全に開葉する。かかる幼時期 (7.5~15cm) の葉身は淡緑色を呈し葉柄はその色一層淡く基部は殆んど白色にちかい。かかる葉の形成初期は光、温度等の Chlorophyll 生成条件が不十分で未だ Proto-chlorophyll が完全に変化成熟しない状態にある。従って Chlorophyll が未完成の状態で Grana 中に存在する時、常にこれと結合しその普遍的成分である赤橙色色素 Carotene も又少ない。即ち淡緑色を呈する幼葉は Chlorophyll, Carotene 共に少ないことが見られた。反対に一定限度内の日照と温度下では漸次その緑色度を増し Carotene 含量も増加して幼時 (7.5cm) に比し40cmに達したものは平均凡そ3倍程度 Carotene 含量は増加してくる。

以上の実験結果から實際上 Ladino clover の利用に際し余りに若い草地の放牧、刈取等は単に収量減にとどまらず栄養的の損失も多く不得策である。最少限その草丈25cm以上に伸長させ、又刈取放牧は何れも5—7cm以下の低刈或は過放牧等はさける必要がある。

## EXAPLNTATION OF PLATE

- Fig. 1. The general appearance of Ladino clover spreads by mean of creeping stolons as shown in Fig. 1.
- Leaves and leaf stalks may form at the stolon nodes.
- Fig. 2. Leaves and leaf stalks of Ladino clover. Young small new leaf just developing on the top at the stolon, at left, and the older leaf near the base of same stolon, at right. Young leaf is not very green color but as the leaf becomes older (at right) increase greatly its green color as shown in this photograph. Thus, the degree of greenness of leaf is a good index of carotene content.
- Fig. 3. Young leaf blade and leaf stalk on the top of the stolon. As shown in this photograph, young leaves are not yet opened and also are not deeply green colored.





Fig. 1.

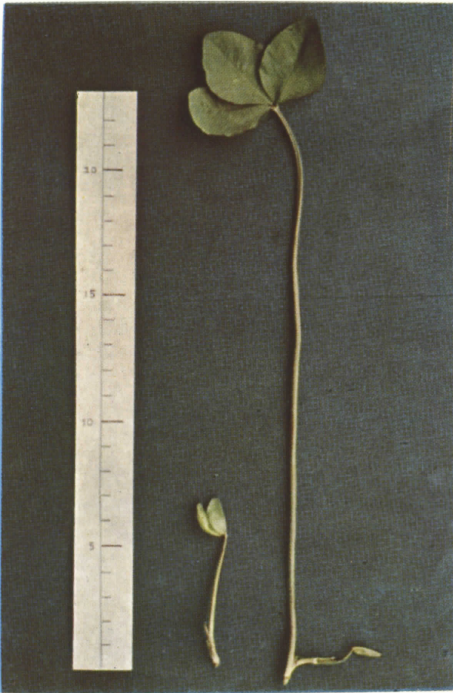


Fig. 2.

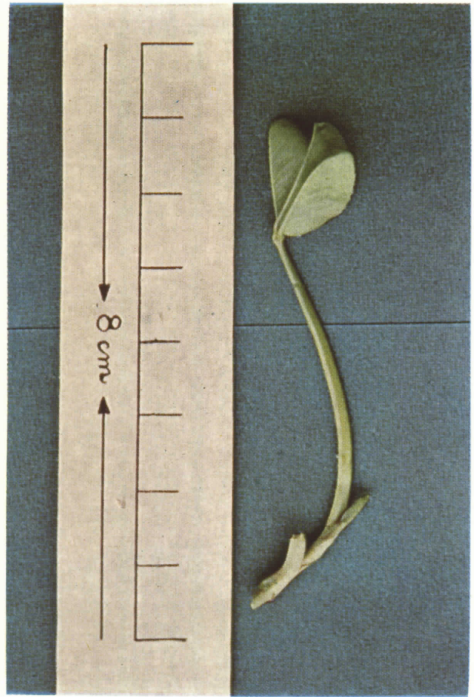


Fig. 3.