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Chlorophyll Content of Persistent-Green and Normal Snap Bean Pods (*Phaseolus vulgaris* L.)[1]

H. F. Mayland and Leslie L. Dean[2]
Agricultural Research Service, U. S. Department of Agriculture, and University of Idaho

Abstract. Chlorophyll concns decreased rapidly as pods matured from the 1-day-old fruit stage. Total chlorophyll a + b per pod was influenced by pod length as well as pigment concn. Apparent chlorophyll synthesis occurred in field-grown green snap bean fruits up to 11-mm diam pods (sieve size 5 or 6), but a net decrease occurred with further maturation. Dry-mature persistent-green pods contained 300 ppm chlorophyll on a dry matter basis, whereas normal-green pods had none. Lack of consistent agreement between visual and chemical methods to rank cultivars for pod color suggested differences in chlorophyll distribution in external versus internal tissue.

Color intensity in green snap bean pods decreases with increasing pod size and physiological maturity. Loss of green color may parallel increases in fiber (texture) and seed content, and decreases in other quality factors. Decreased color intensity creates a serious problem for commercial processors since color influences the esthetic quality (4). Utilization of the persistent-green character (PC) in snap beans (for processing) has been suggested (3) as a means of increasing color uniformity among pods of various physiological maturities.

Intensity of the green color decreases more rapidly with physiological aging in normal than in PC pods. It was assumed that chlorophyll concns were correlated with pod color, hence the chlorophyll loss from developing pods of normal cultivars would be much more rapid than in PC Pods. It was anticipated that small immature pods of PC and normal lines might not differ greatly in chlorophyll concn. Visual evaluation of large pods, however, suggested that PC lines had greater chlorophyll concns than normal cultivars. This becomes apparent at seed maturity when PC pods remain green while normal-green pods become tan or light brown. The large-sieve and mature PC pods remain green longer possibly because chlorophyll does not degenerate as rapidly as in normal pods, a fact that has already been established for excised leaves from these plants (2). Persisent green pods may be able to synthesize chlorophyll for a longer period of time than do normal-green cultivar pods. Another possibility is that the enzyme which destroys chlorophyll may be inactivated in the PC pods while some chlorophyll yet remains. Bouwkamp and Honma (2) postulated that leaves from PC plants contained higher endogenous levels of some kinetin-like substance than did leaves from normal cultivars. This could explain the greater tendency of PC lines to abscise older leaves following plant stress, but it is not clear how this might affect rates of chlorophyll formation or degradation in pods.

The objectives of this study were to identify the differences in chlorophyll yield components between several PC breeding lines, and normal-green snap bean cultivars, and secondly, to determine if ranking by chlorophyll concn correlated with visual color grading of pods.

Materials and Methods

Pods were harvested from 4 experimental PC lines and 2 normal-green snap bean cultivars grown during 1969 under irrigation at the Twin Falls, Idaho Branch Experiment Station. The experimental lines were XIda 121-15-3 (since named 'Custer'), XIda 69-3, XIda 266-5, and XIda 68-5381, while the normal-green cultivars were 'Tendercrop' and 'White Seeded (WS) Tendercrop'.

The pods were harvested August 11, 52 days after planting, immediately hand-graded to sieve sizes 3 and under, 4, 5, 6, and 7 and over, and randomly divided into 8 subsamples each containing 10 to 14 pods (6 to 8 g total dry wt). Sieve sizes 1, 2, 3, 4, 5, 6, and 7 and over correspond to <5.8, 5.8 to<7.3, 7.3 to <8.3, 8.3 to <0.5, 9.5 to <10.7, 10.7 to <11.7 and ≥ 11.7 mm diam, respectively. Pods were weighed and then freeze-dried to less than 0.5% water content and reweighed. The freeze-dried material was ground to pass a 40-mesh sieve and stored in plastic vials at −20C until analyzed.

In a second experiment, XIda 69-3 and 'WS Tendercrop' were grown under winter greenhouse conditions. Harvested samples consisted of the following pod sizes: 2- (1-day-old), 3-, and 3-5-mm diam, sieve sizes 1 to 5; nearly mature (30% dry matter); and mature (85% dry matter). Samples were treated similarly to those collected from the field.

In another experiment, pods from 10 green snap bean lines grown under field conditions were harvested at sieve size 6,

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[2]Research Soil Scientist, Snake River Conservation Research Center, Kimberly, Idaho and Research Professor of Plant Pathology, Bean Research Laboratory, University of Idaho, Twin Falls, respectively.

[3]Hildebolt, W. M. 1969. The influence of preservation methods of the color and other quality attributes of green beans (*Phaseolus vulgaris* L.). Unpublished Ph.D. Thesis, Ohio State University.

blanched, and visually ranked. Total chlorophyll was then determined.

The extraction procedure for chlorophyll (used in this paper as a + b) was essentially that given by AOAC (9), method 6.097. Chlorophyll was extracted from 0.35 g freeze-dried tissue by 5-minute homogenization in a high-speed blender along with 0.1 g $CaCO_3$ and 45 ml 85% acetone (15% H_2O). Sample flasks were cooled in an external ice bath during the homogenization procedure. Homogenates were filtered through F sintered-glass filter funnels under approximately 600 mm Hg vacuum. The filtrate was made to 50 ml vol with 85% acetone and 15 minutes after homogenization the transmittance at 660 mm was determined.

Results from the acetone procedure were calibrated against results of ether extracts from freeze-dried 'Sanilac' bean leaves [AOAC (9), procedure 6.100]. Data were statistically analyzed by the Duncan's Multiple Range Test (8).

Results and Discussion

Chlorophyll concn decreased as pod size increased in both PC and normal cultivars (Table 1). The two PC lines, XIda 69-3 and XIda 121-15-3, were generally higher in chlorophyll concn than the normal cultivars or the 2 other experimental PC lines. Visual color evaluation of XIda 121-15-3 supports the finding that this PC line is superior in color intensity to the normal-green snap bean cultivars used in this study. The 2 remaining PC lines had generally lower concns of chlorophyll than did normal lines, especially in the larger pod sizes. The ranking of bean cultivars based on chlorophyll concns in dry material was not the same as that based on visual selections for raw dark green colors.

Table 1. Chlorophyll concentration in various sized bean pods and cultivars computed on dry matter basis.

Line or Cultivar	Pod sieve size ≤3	4	5	6	≥7
			mg/g[z]		
XIda 69-3	0.93a	0.79a	0.67a	0.59a	0.39a
XIda 121-15-3	0.87b	0.65b	0.52c	0.39b	0.32b
Tendercrop	0.79e	0.65b	0.53b	0.36d	0.27d
WS Tendercrop	0.85c	0.58e	0.49d	0.38c	0.29c
XIda 266-5	0.82d	0.64c	0.48e	0.35e	0.27d
XIda 68-5381	0.84c	0.59d	0.39f	0.29f	0.23e
Mean	0.85	0.65	0.51	0.39	0.29

[z]Columnar data followed by the same letter are not significantly different at 1% probability.

Chlorophyll concn decreased rapidly with increasing pod size as illustrated for one bean line from each type (Fig. 1). Supplementary information on small and mature pods was obtained from the greenhouse material. Chlorophyll concn was greatest in the 2-mm diam, 1-day-old pods. The curves illustrate the greater chlorphyll concns in XIda 69-3 compared to the normal cultivar even for the small pods.

Chlorophyll concn data were obtained for field grown XIda 69-3, 'Tendercrop' and 'WS Tendercrop'.pods collected in 1966, blanched, and frozen until analyzed in 1969. Chlorophyll concn (data not shown) were about 0.1 mg/g dry matter less than for field samples collected in 1969, but ranking was the same. Such concn differences between years might be due to environmental responses of the growing beans or to the blanching and storage treatment.

The decrease in chlorophyll concn (Table 1) with increased pod size could result from a dilution of chlorophyll by additional pod dry matter production with little or no increase in chlorophyll. Hall (5) suggests, however, a very rapid increase in total pod chlorophyll followed by a rapid decrease with additional maturation. Thus, changes in chlorophyll content may be more than a simple dilution effect by dry matter

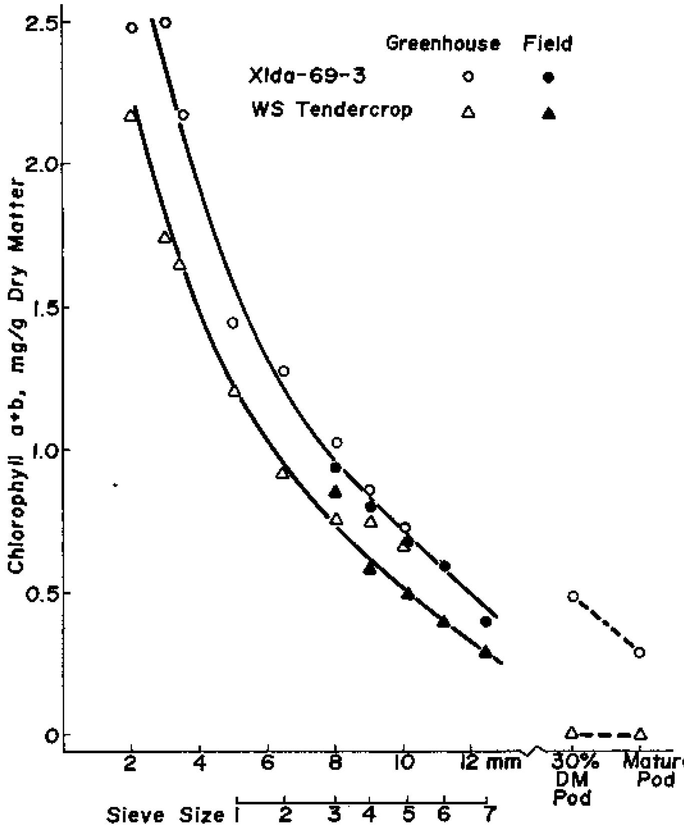

Fig. 1. Chlorophyll concn in 5 sieve sizes of a field grown PC and normal-green cultivar, supplemented with data from 8 small and 2 mature pod groups grown in the greenhouse.

accumulation.

Chlorophyll data were therefore expressed as yield per pod (Table 2). Since the pods are sized as a function of their diam, the changes in rank in Table 2 from those in Table 1 result from differences in pod length, density, and physiological maturity. Total pod chlorophyll increases with increasing pod size (Fig. 2), but for field samples reaches a maximum in sieve sizes 5 or 6, whereas pod mass continues to increase (Table 3). The pod size at which maximum chlorophyll accumulates does not appear to be associated with type since it occurs in sieve size 5 for 1 normal and several PC lines, but it occurs at sieve size 6 for several others. Plant growth under greenhouse conditions is generally quite different from growth in the field. Although this may account for the differences shown in Fig. 2, the plotted data illustrate the concept of chlorophyll accumulation in developing pods.

Ranking these snap bean lines in order of chlorophyll concn on a fresh-wt basis did not separate the PC lines from the normal cultivars. Breeder selection criteria for PC lines included a green, mature pod (compare chlorophyll concns of mature

Table 2. Chlorophyll yield in various sized bean pods and cultivars.

Line or cultivar	Pod sieve size ≤3	4	5	6	≥7
			μg/pod[z]		
XIda 69-3	169b	243b	276bc	318ab	313ab
XIda 121-15-3	223a	289a	333a	343a	335a
Tendercrop	141bc	221bc	264c	303bc	301b
WS Tendercrop	114c	214bc	296b	292c	295bc
XIda 266-5	175b	221bc	266c	279cd	272cd
XIda 68-5381	126c	208c	250c	249d	259d
Mean	158	233	281	297	296

[z]Columnar data followed by the same letter are not significantly different at 1% probability.

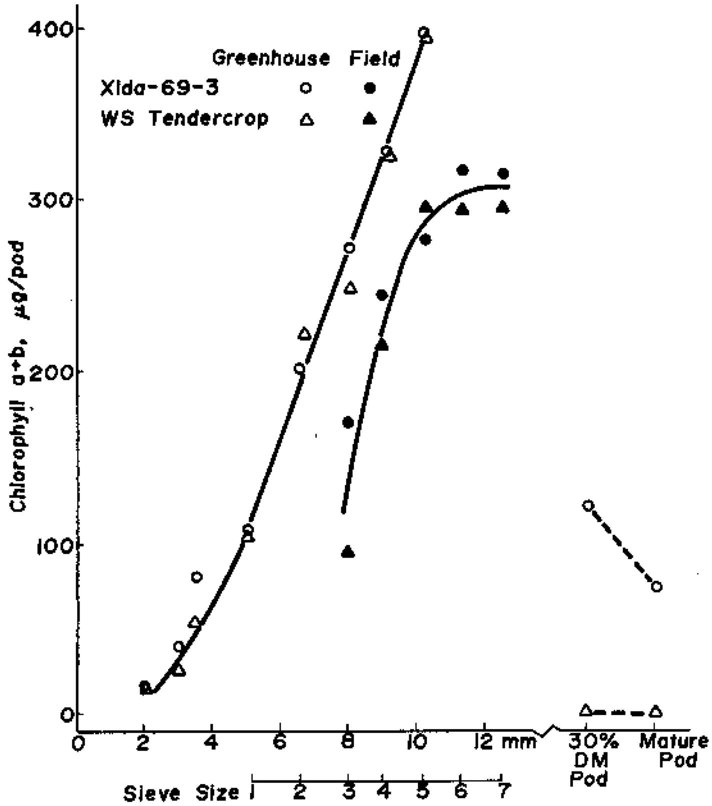

Fig. 2. Chlorophyll yields per pod from 5 sieve sizes of a field grown PC and normal-green cultivar, supplemented with data from 8 small and 2 mature pod groups grown in the greenhouse.

Table 3. Mean fresh wt of individual bean pods and their dry matter content for various sizes and cultivars.

Line or cultivar	Sieve size ≤3	4	5	6	≥7
	g (%)				
XIda 69-3	3.6(5.0)	4.0(7.7)	5.3(7.7)	6.8(8.0)	7.1(11.3)
XIda 121-15-3	3.3(7.9)	5.4(8.3)	6.8(9.4)	8.2(10.7)	8.3(12.7)
Tendercrop	2.4(8.0)	4.3(8.0)	6.0(8.4)	7.6(11.4)	8.8(12.7)
WS Tendercrop	1.7(7.9)	4.5(8.1)	6.1(9.9)	7.2(10.7)	7.9(13.3)
XIda 266-5	2.9(7.5)	4.5(7.5)	6.0(9.0)	6.8(11.7)	7.2(13.7)
XIda 68-5381	2.1(7.1)	4.9(7.1)	7.2(8.9)	8.1(10.7)	9.5(11.8)

pods, Fig. 1, and yields, Fig. 2). It was previously assumed that this PC character was associated with a greater green-color intensity in the large immature pods. Obviously some characteristics accounted for in the visual selection techniques are not accounted for by the chemical analysis of total chlorophyll. Variations in chlorophyll a to chlorophyll b ratio, or in the concn of their respective pheophytins would alter the spectrophotometric absorbances used in this study to calculate total chlorophyll (11). The ratio of chlorophyll a to chlorophyll b apparently changes in maturing grass seeds (10). Bouwkamp and Honma (2), however, found that the ratio remained essentially constant in leaves from both green and tan dry-podded lines of *Phaseolus vulgaris.* We have therefore assumed that the a:b ratio has been constant in this study. The uniformity of chlorophyll extraction from all pods appeared quite satisfactory when checked in the laboratory. However, chlorophyll could be differentially concd in various pod parts. Pods with chlorophyll concd in the exocarp would appear darker than those with the same amount of chlorophyll distributed throughout the exocarp, mesocarp, endocarp, and developing embryos. Thus, some lines might have a heavily pigmented external color, while others have greater internal color. Higher water content would also give the pods a darker appearance.

Measuring fruit quality by selecting aesthetically important pod colors, based upon laboratory analysis of total chlorophyll, would appear possible from the data of Hoffman and Kanapaux (6). Others have also discussed the theoretical (1) and instrumentation aspects (7) of measuring absorption spectra for quantifying color intensity. They have warned, however, that data obtained on dense, highly scattering material be interpreted with caution. The difficulty of obtaining a homogeneous surface causes problems in measuring light absorption characteristics of such fruits as snap bean pods. Inada (7) had difficulty in obtaining homogeneous surfaces if the veins and midribs of bean leaves and their uneven surfaces were included in the material being instrumented.

Hildebolt[3] has commented that:

"... the greenness of any green vegetable is a product of a complex mixture of the different chlorophyll pigments and that many different combinations of value and chroma within the same hue could exist. Depending on the tristimulus values, the green beans may appear of different quality to the human eye, but yet still reflect approximately the same amount of green light. The reflectance colorimeter does not have the ability to interpret quality of light it receives, but only the amount of light. . ."

Optical instruments have been developed to minimize the problem of uneven surfaces and to quantify color quality in a way that correlates with visual grading. Large angle reflectance spectrometers, frequently equipped with 3 wide spectrum photodetectors covering various portions of the visible spectrum, are being used to quantify the tristimulus characteristics of hue, value and chroma. Hildebolt[3] used several modes within each of 2 different reflectance spectrometers to objectively measure color quality of snap beans. These objective methods of determining color quality still did not completely account for characteristics that stimulated visual color grading as illustrated by correlation coefficients of ≤0.7.

Our results illustrate that the chlorophyll concn of immature pods (sieve size 6) does not consistently separate cultivars or breeding lines in the same order as do visual techniques (Table 4). Inadequacies in the chemical procedures are highly unlikely. Therefore, we have concluded that chlorophyll in pods of some snap beans may be concd in the exocarp, whereas in others the chlorophyll may be distributed in the mesocarp, endocarp, or embryo.

Table 4. Visual ranking of 10 cultivars for greenness and corresponding chlorophyll content.

Rank[z]	Line or cultivar	Chlorophyll
		mg/g dry wt.
1	OSU 58	0.51
2	Galagreen	0.40
3	5248	0.37
4	5258	0.32
5	XIda 83-4575	0.34
6	5252	0.33
7	5250	0.33
8	67-6154	0.28
9	Early Gallatin	0.36
10	XIda 83-4589	0.37

[z]Increasing rank value equivalent to decreasing greenness.

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