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PHYSICAL AND CHEMICAL CHANGES ASSOCIATED WITH THE DEVELOPMENT OF THE LOWBUSH BLUEBERRY FRUIT *VACCINIUM ANGUSTIFOLIUM* AIT.

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SUMMARY

Growth curves, changes in soluble solids, titratable acidity, and pH during the ontogeny of lowbush blueberry fruit were studied for a total of 16 clones. The growth curve was characterized by three distinct phases which regression equations showed to be a cubic parabola. Soluble solids increased slowly early in fruit development, but accumulated rapidly at onset of ripening. Citric acid increased initially, and then decreased abruptly at the beginning of ripening. The rate of increase in fruit pH accelerated as the growing season progressed.

Developmental stages with delimited physiochemical characteristics were identified and provided an index of physiological maturity.

INTRODUCTION

Suitable indices for determining the maturity of blueberry fruits have been sought by many investigators. Bailey (3) indicated that for the highbush blueberry, the elapsed time from full bloom to maturity varied considerably between clones. The blooming period extended over several weeks, making it impossible to obtain uniformly ripening fruits. Similar findings have been reported for the lowbush blueberry (4). Workers studying the highbush blueberry (12) and lowbush blueberry (5) used color changes as an indicator to differentiate stages of physiological maturity.

Young (13) was the first investigator to ascertain that highbush blueberry fruits from several cultivars exhibit a growth curve of a sigmoid type with three distinct stages. Hindle *et al.* (7) reported similar findings.

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Fruit acids, pH, and soluble solids are closely related to the palatability and keeping quality of blueberry fruits. Citric acid is the predominant organic acid in blueberry fruit, although small amounts of malic acid and traces of oxalic acid may also be found (10). Woodruff and Dewey (12) reported a positive linear correlation between the actual sugar and soluble solids of fresh highbush blueberry fruit.

The objective of this investigation was to determine the growth characteristics, changes in the soluble solids, pH, and titratable acidity for the purpose of defining and describing stages in the growth of the blueberry fruit.

MATERIALS AND METHODS

In 1966 and 1967, several clones in typical commercial fields in Maine were selected to provide experimental material for this investigation, since specific cultivars have not been developed for the lowbush blueberry. Clones were selected in early spring, while dormant, on the basis of vigor, size, abundance of stems and flower buds, and freedom from insect, disease or winter injury.

No precise method exists for ascertaining the physiological age of developing blueberry fruit, especially before the color changes. In these studies, the date of pollination was used to provide a common reference point in the ontogeny of the fruits.

Flowers of lowbush blueberry clones are largely self-sterile (1) and cross-pollination by insects is essential for achieving appreciable fruit set (9). Prior to the onset of anthesis, the clones were covered with cheesecloth to prevent insect visitation. When a given clone approached "full bloom" (80-90% of the blossoms open), the cheesecloth cover was removed for two days allowing insects to visit and pollinate the blossoms. Each clone was covered again until after total petal fall, to prevent additional cross-pollination. Six honeybee hives were placed adjacent to the experimental plots to ensure adequate pollination.

Size and color were used to separate fruits into specifically defined stages of development. Maturity classes were established as described below:

(1) *Immature Green:* Fruits were entirely green in color and small in size. Detached fruits from this stage, if held under suitable conditions, failed to develop anthocyanin pigments.

(2) *Mature Green:* Fruits were green in color and showed visual signs of chlorophyll breakdown by the appearance of a pale cream color in the region of the calyx end. Usually these fruits were larger than those of the Immature Green stage.

If detached and held under suitable conditions, they proceeded to ripen and develop pink, red or even blue coloration.

(3) *Green-Pink:* Fruits were largely pale green with traces of pink coloration evident around the calyx end. If detached, they continued to ripen.

(4) *Pink-Red:* Fruits were largely pink in color with very little, if any, green present. Red coloration was evident at the calyx end.

(5) *Red-Blue:* Fruits were reddish in color with dark red or blue appearing at the calyx end. No green or pink color was present.

(6) *Blue:* Fruits were entirely blue in color.

Growth Studies. Ten randomly selected stems from each clone were tagged. To identify individual fruits, cotton threads of various colors were tied to the pedicel of 10 fruits randomly selected on each tagged stem. The diameter of these fruits was measured along the transverse axis with a vernier caliper, accurate to 0.1 mm. The blueberry fruit is not a perfect sphere and cross diameter measurements do not accurately represent the true volume. Young (13) and others, however, found this method to provide an adequate indicator for the changes in the size (volume) of blueberry fruit. Growth measurements were initiated two weeks after pollination and were continued three times per week for the duration of the experiment.

Polynomial regression analysis was employed to statistically treat the growth data, and a growth curve was characterized for the lowbush blueberry fruit.

Determination of Soluble Solids, pH and Titratable Acidity. The level of soluble solids in developing blueberry fruits was determined in the field using a hand refractometer as described by Woodruff and Dewey (12).

Fruits used in the pH and titratable acidity determinations were harvested, separated into the various developmental stages, placed in plastic bags, sealed in 1 pint plastic containers and held at –25° C for later chemical analysis. Samples for pH and titratable acidity measurements were taken on the same dates that determintions of soluble solids were made. Titratable acidity was determined by the official A.O.A.C. method (2). The soluble solids, pH, and titratable acidity data were statistically treated utilizing a split plot design.

RESULTS AND DISCUSSION

Fruit Growth. Cubic regression equations for data averaged for all measured berries from three of seven clones are presented in Fig. 1. Growth curves of all tested clones were similar.

The cubic, or quartic parabolic growth curves of three representative blueberry fruits that attained different sizes on a given stem are shown in Fig. 2. Although the fruits attained different ultimate sizes, their growth curves were similar and characterized by three specific phases. The first was associated with a rapid increase in diameter (Phase I). A second period was characterized by a slower increase in berry size (Phase II). This was followed by another period of accelerated growth (Phase III). The growth curves of large and medium sized berries exhibited a fourth phase (Phase IV) during which size either did not change or decreased. Phase I for all fruits appeared to be of similar duration. The duration of Phase II varied greatly and ranged between 8 days for a large berry, 15 days for a medium size berry, and 25 days for a small berry (Fig. 2). Phase III for all fruits appeared to be of a similar duration. The large berry attained its maximum size and initial color changes earlier than did the medium and small berries. The R^2 values for cubic regression equation ranged between 0.9100 and 0.9881.

Maturation rate appeared to be related to the duration of stages II and III. The final, rapid increase in size occurred largely after the blue color began to appear. Small berries tended to have a flatter growth curve than larger berries. Time of development and ultimate size of berries were inversely related. Mature blueberry fruits remained on the plant for 10-12 days after they had fully ripened.

Fruit Composition. Changes in soluble solids, pH and titratable acidity of lowbush blueberry fruits as influenced by stage of development are presented in Table 1.

The concentration of soluble solids tripled as the fruits progressed from the Immature Green to the Blue stage. Differences observed among the means of the six physiological stages were statistically significant in both test years.

The pH of blueberry fruits was found to increase throughout the developmental period. Significant differences in pH level were observed only in the later stages of fruit development.

Titratable acidity, expressed as percent citric acid, showed a steady decline after the Mature Green stage. The levels for a given stage were remarkably constant between years. At full maturity (Blue stage), the concentration of citric acid was only one-fourth that of the Mature Green stage.

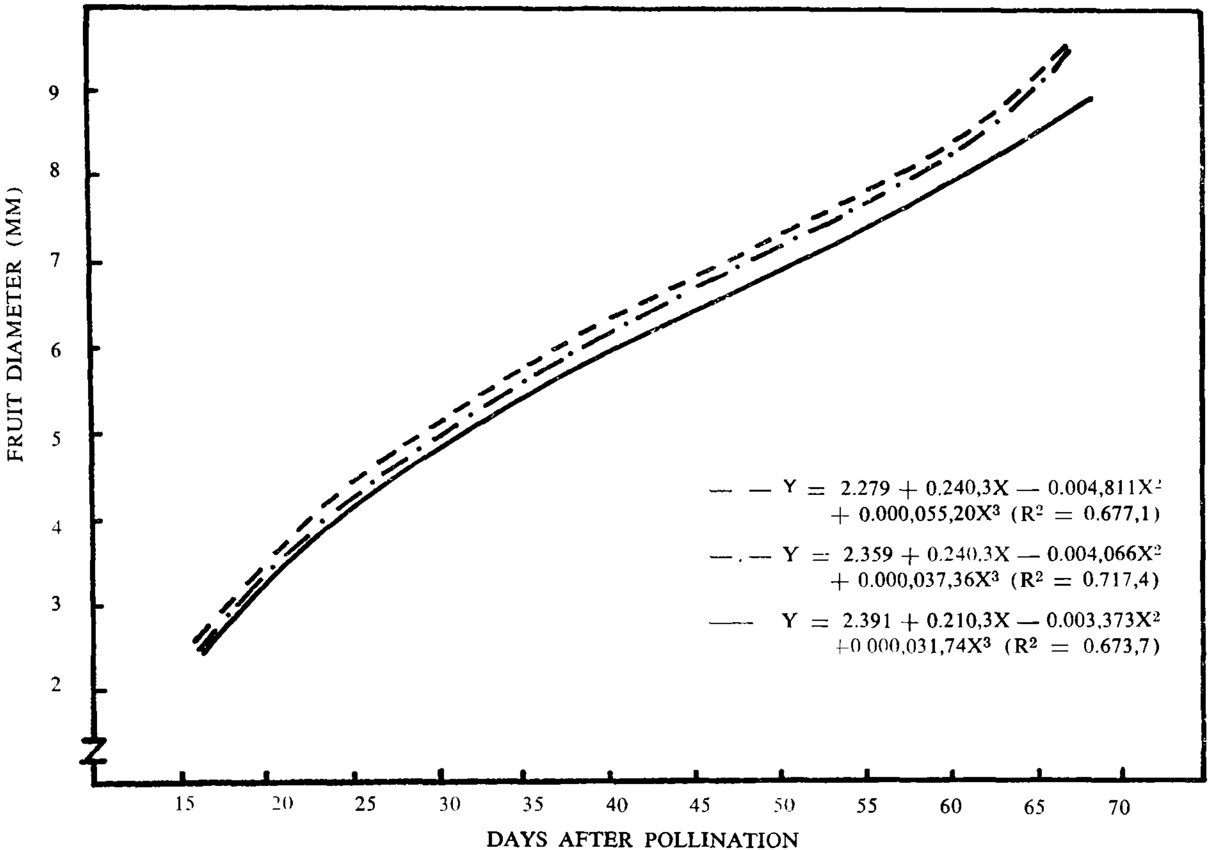

FIGURE 1. Growth curves of lowbush blueberry fruits from 3 clones with each point representing averaged measurements of a minimum of 84 berries.

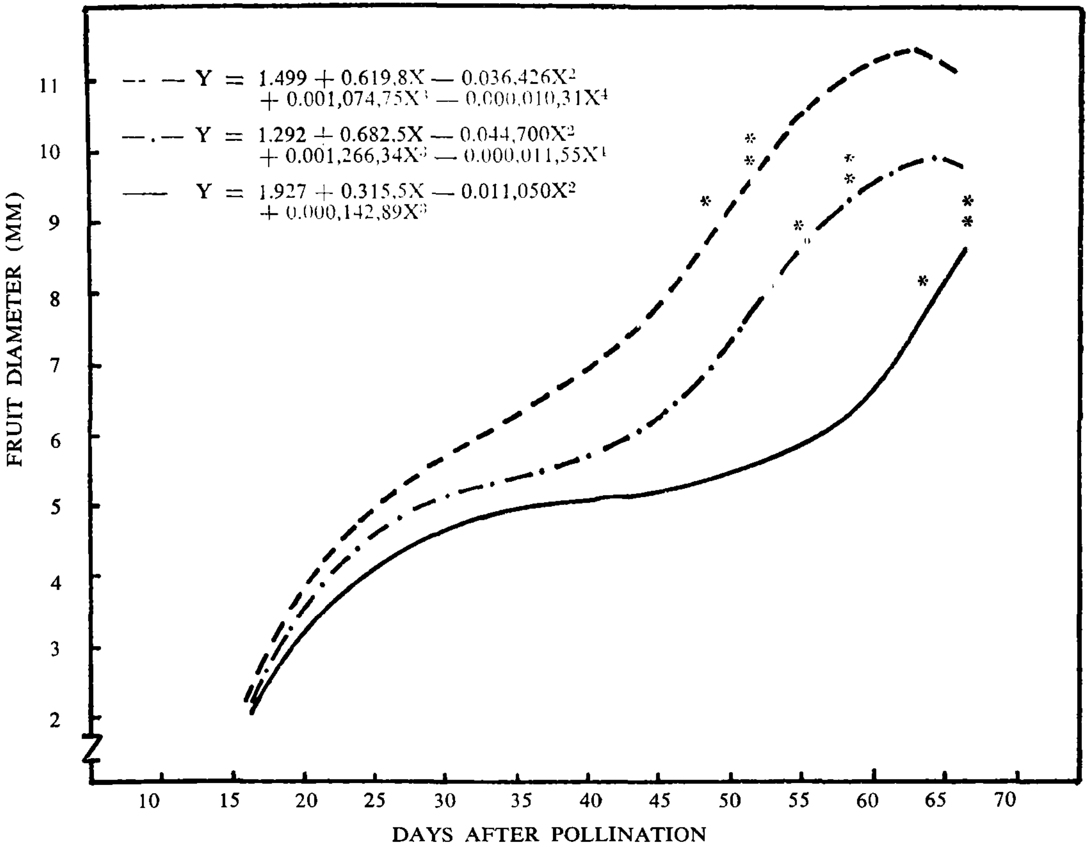

Figure 2. Parabolic growth curves for 3 lowbush blueberry fruits that attained various sizes. Color change of berries is indicated by: * Red Blue; and **Blue.

Table 1.

Soluble solids, pH, and titratable acidity of lowbush blueberry fruit as influenced by stage of development (means of 3 clones).

Stage of Fruit	Soluble Solids (%)		pH		Titratable Acidity[1]	
Development	1966	1967	1966	1967	1966	1967
Immature Green	4.40 f[2]	3.08 f	2.863 e	2.984 d	1.20 b	1.27 a
Mature Green	6.22 e	4.26 e	2.865 e	3.006 d	1.44 a	1.38 a
Green Pink	7.69 d	5.63 d	2.937 d	3.017 d	1.20 b	1.20 a
Pink Red	9.61 c	6.97 c	3.026 c	3.069 c	1.10 c	0.93 b
Red Blue	11.58 b	8.80 b	3.267 b	3.139 b	0.77 d	0.70 c
Blue	13.90 a	10.93 a	3.716 a	3.630 a	0.37 e	0.34 d

[1] % citric acid.
[2] Means in a column followed by the same letter(s) do not significantly differ at the 5% level of probability.

Physiological changes in the developing blueberry fruits showed no consistent chronological control. Fruits from clones pollinated on the same date grew and developed at highly variable rates, as indicated by size, color and chemical composition.

Color and size facilitated the segregation of fruits into several physiological stages during the ontogeny of the fruits. It should be noted, however, that with blueberry fruits, size and color may vary greatly from clone to clone. Thus, intensity of color and size limitations should be defined and imposed according to the characteristics of a specific clone.

Proposed Terminology for the Development of The Blueberry Fruit. From these findings, and those reported earlier by Ismail and Kender (8) utilizing the same blueberry clones, it is evident that following fertilization certain well-defined physical, chemical, and physiological changes occur in the blueberry fruit (Fig. 3). Changes in growth rate, percent soluble solids, fruit pH, fruit acid, and respiration rate appear to follow definite patterns. Distinct periods can be identified in the development of the blueberry fruit, namely: pre-maturation, maturation, ripening and senescence. Based on these findings, and in accordance with terminology proposed for fruit development by Gortner *et al.* (6), the following definitions are proposed for various stages of the development of the blueberry fruit.

Development. This time interval includes the first two periods (Pre-maturation and Maturation), and extends from fertilization until the fruit reaches maximum size and attains optimum chemical properties.

Pre-maturation. The period of fruit development extending from fertilization to the end of Phase II of the growth curve. During this time the respiration rate decreases rapidly, with no appreciable changes in fruit pH, acid or percent soluble solids.

Maturation. The period of fruit development immediately following Pre-maturation. It begins with the onset of Phase III of the growth curve. The blueberry fruit attains maximum edible quality and full size with rapid changes in the chemical and physiological properties of the fruit taking place. At the onset of Maturation, a rapid increase in percent soluble solids is evident. The decline in respiration rate continues and fruit pH and acid increase. Successful completion of Maturation necessitates that the fruit remain attached to the plant at least during the first half of this period.

Ripening. The onset of Ripening is characterized by marked physiochemical changes. The decrease in respiration rate ceases and a rapid upsurge occurs, followed by a second decrease in respiration rate. Thus, the climacteric rise occurs during ripening. The fruit acid ceases to increase and sharp decrease ensues. Fruit pH increases at an accelerated rate. The percent soluble solids in the fruit doubles during this period and chlorophyll disappears rapidly while synthesis and accumulation of anthocyanins occurs. Fruit reaches the maximum in size and percentage of soluble solids during this period. Ripening takes place in a Mature blueberry fruit whether attached or detached from the plant. After ripening is completed, the fruit is fully developed.

Senescence. Senescence is the period that immediately follows fruit development and where growth of the fruit ceases, pH continues to increase, while the percent soluble solids, fruit acid, and respiration rate decrease.

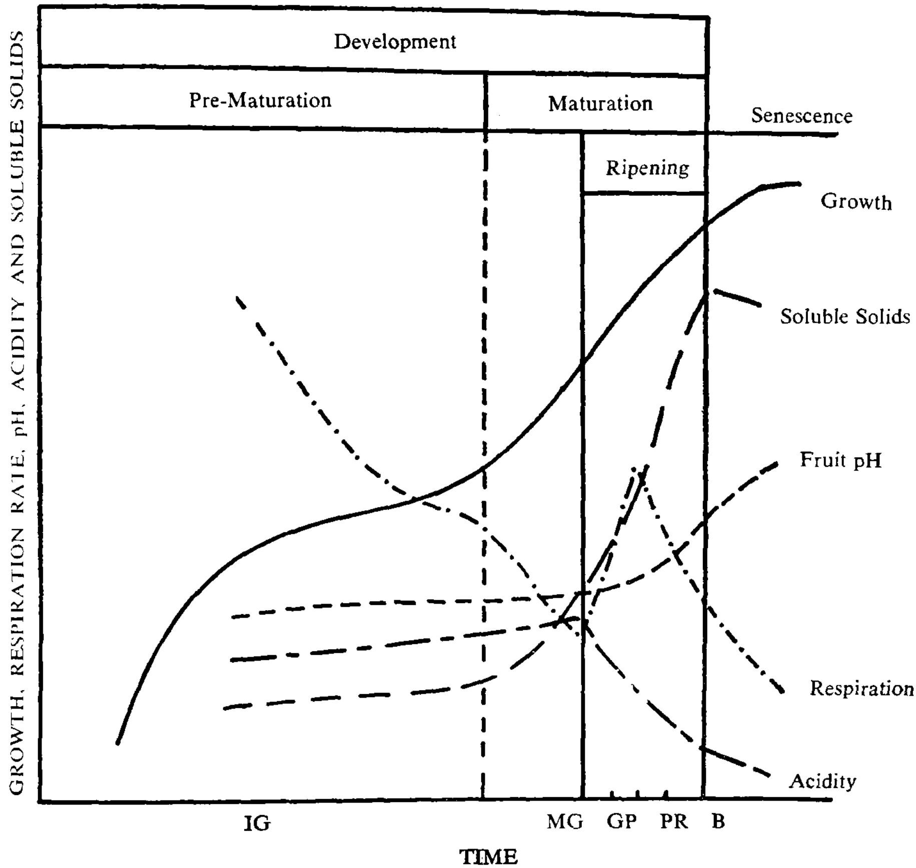

FIGURE 3. Growth, chemical, and respiratory patterns associated with the development of the blueberry fruit, IG, Immature Green; MG, Mature Green; GP, Green Pink; PR, Pink Red; RB, Red Blue; and B, Blue.

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APPENDIX

Polynomial regression equations for averaged growth curves of lowbush blueberry fruits from seven clones, 1967.

Covariance analysis between growth curves for fruits on one stem in Clone 4-67, 1967.

Covariance analysis between growth curves for fruits on ten stems in Clone 6-67, 1967.

Table 1-A

Polynomial regression equations for averaged growth curves of lowbush blueberry fruits from seven clones, 1967.

Clone	No. of Berries	No. of Observations	Cubic Regression Equation	R^2
1-67	92	2116	$Y = 2.279 + 0.240{,}3\ X - 0.004{,}811\ X^2 + 0.000{,}055{,}20\ X^3$	0.677,1
2-67	84	1932	$Y = 2.361 + 0.208{,}4\ X - 0.002{,}166\ X^2 + 0.000{,}013{,}12\ X^3$	0.801,7
3-67	85	1955	$Y = 2.359 + 0.240{,}3\ X - 0.004{,}066\ X^2 + 0.000{,}037{,}36\ X^3$	0.717,4
4-67	87	2001	$Y = 2.391 + 0.210{,}3\ X - 0.003{,}373\ X^2 + 0.000{,}031{,}75\ X^3$	0.673,7
5-67	88	2024	$Y = 2.563 + 0.213{,}4\ X - 0.002{,}653\ X^2 + 0.000{,}024{,}82\ X^3$	0.525,3
6-67	91	2093	$Y = 2.554 + 0.175{,}2\ X - 0.001{,}751\ X^2 + 0.000{,}015{,}26\ X^3$	0.808,2
7-67	83	1909	$Y = 2.567 + 0.225{,}2\ X - 0.003{,}625\ X^2 + 0.000{,}036{,}81\ X^3$	0.720,4

Table 2-A

Covariance analysis between growth curves for fruits on one stem in Clone 4-67, 1967

Berry[z] Number	Adjusted Mean	Cubic Regression Equation	R^2
1	6.995	$Y = 2.827 + 0.133,0\ X + 0.001,669\ X^2 - 0.000,023,97\ X^3$	0.985,3
2	4.939	$Y = 1.963 + 0.314,7\ X - 0.010,983\ X^2 + 0.000,132,63\ X^3$	0.981,3
3	3.652	$Y = 2.037 + 0.176,6\ X - 0.005,151\ X^2 + 0.000,047,56\ X^3$	0.910,0
4	7.013	$Y = 2.779 + 0.144,4\ X + 0.000,890\ X^2 - 0.000,010,77\ X^3$	0.988,1
5	5.409	$Y = 2.433 + 0.198,6\ X - 0.005,925\ X^2 + 0.000,087,97\ X^3$	0.966,2
6	5.800	$Y = 2.524 + 0.195,9\ X - 0.004,047\ X^2 + 0.000,050,86\ X^3$	0.953,4
7	5.513	$Y = 2.469 + 0.236,5\ X - 0.007,133\ X^2 + 0.000,093,25\ X^3$	0.982,2
8	4.439	$Y = 2.186 + 0.234,8\ X - 0.008,397\ X^2 + 0.000,104,90\ X^3$	0.928,8
9	6.904	$Y = 2.805 + 0.097,4\ X + 0.002,952\ X^2 - 0.000,032,88\ X^3$	0.980,7
10	4.922	$Y = 2.349 + 0.196,9\ X - 0.006,391\ X^2 + 0.000,089,99\ X^3$	0.981,9
All Berries	5.559	$Y = 2.436 + 0.193,2\ X - 0.004,267\ X^2 + 0.000,054,14\ X^3$	0.805,9

[z] Number of observations per berry = 23.

Source	Degrees of Freedom	Mean Square	Adjusted F Value
Berries	9	30.031,2	44.73**
Error	217	0.671,3	

** Significant at 0.01 level of probability.

Table 3-A

Covariance analysis between growth curves for fruits on ten stems in Clone 6-67, 1967.

Stem Number	Number of Observations	Adjusted Mean	Cubic Regression Equation	R^2
1	230	6.000	$Y = 2.500 + 0.200,4\ X - 0.003,832\ X^2 + 0.000,048,25\ X^3$	0.852,6
2	161	5.330	$Y = 2.387 + 0.185,8\ X - 0.004,455\ X^2 + 0.000,059,27\ X^3$	0.851,1
3	230	6.712	$Y = 2.627 + 0.172,2\ X + 0.000,155\ X^2 - 0.000,016,04\ X^3$	0.922,5
4	230	5.735	$Y = 2.585 + 0.137,4\ X - 0.000,935\ X^2 + 0.000,010,80\ X^3$	0.743,8
5	207	6.050	$Y = 2.488 + 0.173,2\ X - 0.001,360\ X^2 + 0.000,007,20\ X^3$	0.889,6
6	230	6.467	$Y = 2.608 + 0.186,2\ X - 0.001,215\ X^2 + 0.000,002,36\ X^3$	0.770,7
7	230	5.907	$Y = 2.451 + 0.212,8\ X - 0.004,053\ X^2 + 0.000,043,90\ X^3$	0.660,7
8	184	6.368	$Y = 2.681 + 0.166,9\ X - 0.001,037\ X^2 + 0.000,006,95\ X^3$	0.854,9
9	184	6.448	$Y = 2.679 + 0.139,8\ X + 0.000,939\ X^2 - 0.000,021,28\ X^3$	0.860,9
10	207	5.896	$Y = 2.526 + 0.172,3\ X - 0.001,853\ X^2 + 0.000,015,04\ X^3$	0.805,1
All Stems	2093	6.105	$Y = 2.554 + 0.175,2\ X - 0.001,751\ X^2 + 0.000,015,26\ X^3$	0.808,2

Source	Degrees of Freedom	Mean Square	Adjusted F Value
Stems	9	33.208,3	39.34**
Error	2080	0.844,0	

** Significant at 0.01 level of probability.