DWARFISM IN LOW CHILL HIGHBUSH BLUEBERRY (*Vaccinium corymbosum*)

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
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This thesis is dedicated to Karen, David Manuel, Gabriel Roberto, Ana Faustina and María Teresa.

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Plant dwarfism was studied in the highbush blueberry (*Vaccinium corymbosum* complex hybrid) breeding program at the University of Florida between the fall of 2002 and spring of 2005. Morphological studies included comparisons among dwarf and normal populations for plant height, internode length, leaf area and number of sprouts or branching. Inheritance studies were conducted by crossing dwarf x normal, dwarf x dwarf and normal x normal plants, and by making field observations on more than 10,000 seedlings from normal x normal crosses grown in high-density seedling nurseries in the breeding program.

All of the studied morphological traits were significantly different between normal and dwarf populations. Plant height, internode length and leaf area of normal plants were 2.5, 1.7 and 2.7 times that of dwarf plants respectively. Dwarf plants were also characterized by a high number of branches when

compared to normal. Based on a logistic regression analysis, plants that had more than 5 branches and were less than 16 cm tall at 10 months had a 99.4 % probability of being dwarf. Conversely, the probability of a dwarf plant was 0.9% for plants with fewer than three branches and a height exceeding 16 cm.

The fertility of the studied dwarf plants was normal. The genotype of dwarf plants appears to be simplex (Aaaa), with the nulliplex form being lethal. Most crosses between normal plants that segregated dwarfs segregated in an 11:1 and 27:8 normal to dwarf ratio, supporting the hypothesis that triplex and duplex plants have normal growth habit. Triplex to duplex cross produces dwarf to normal ratios of 11:1. Duplex to duplex cross segregates in a 27:8 normal to dwarf ratio. Segregation ratios in a few dwarf to dwarf and dwarf to normal crosses did not fit the proposed model.

CHAPTER 1
INTRODUCTION

Mature plant height is influenced by both environment and genotype. Environmental influences can significantly reduce plant height and produce dwarfed plants, as in the case of the bonsai, which, due to constant pruning and small containers, yields dwarfed plants (Garvey, 1985). Environmentally induced dwarfism is not passed to its progeny, and it can be tedious work to keep the plant dwarf. A dwarf genotype achieves its dwarf phenotype without unusual environmental influence and may pass this trait to its progeny.

Dwarf genotypes are of interest to most breeding programs, because the shorter stature plants can have advantages in common horticultural practices. Dwarf genotypes can reduce the cost of controlling tree size (a significant operational cost for normal height plants), and can maximize the use of available space. Dwarf plants can also be of horticultural and ornamental use when height is a limitation in protected agriculture or in indoor landscapes.

Dwarf plants have been important in agriculture. The spectacular increase in grain yield during the “Green Revolution,” particularly in wheat and rice, can be attributed largely to the dwarf traits introduced into the cultivars (Hedden, 2003). Horticultural crops that benefit from short sized cultivars or rootstocks include apples (Atkinson and Else, 2001, Johnson et al., 2001), cherries (Edin et al., 1996, Franken-Bembenek, 1996), bananas and raspberries (Keep, 1969). Advantages offered by dwarf plants include easier harvest, less need for

trimming and pruning to control height, and suitability for high-density planting designs.

Dwarf trees may also be useful for studying the genetic control of physiological factors affecting shoot growth (Sommer et al., 1999). To date, there has been much published research that used dwarf mutations to study plant regulators. Curtis et al. (2000) discovered a feedback control of GA 20-oxidase gene expression in *Solanum dulcamara* by over-expressing the pumpkin gene CmGA20ox1 that induced semi-dwarf plants in *S. dulcamara*. Molecular and genetic studies of dwarf mutants of arabidopsis, tomato and pea have helped explain the role of brassinosteroid biosynthesis and regulation (Li and Chory, 1998).

Dwarf growth habit has been previously observed and studied in highbush blueberry (*Vaccinium corymbosum* complex hybrids). In 1984, Draper et al. studied dwarf selections and concluded that their data appeared not to fit tetraploid genetic ratios for a single locus; furthermore plant height in crosses involving dwarfs appeared to follow a continuous distribution. In the University of Florida blueberry breeding program, dwarf plants have been observed segregating from southern highbush crosses in which both parents had normal stature. These dwarf seedlings are characterized by reduced height and by compact and multiple-sprouted canopy, similar to the dwarf *Vaccinium ashei* (FL 78-66) described by Garvey and Lyrene (1987).

The objective of this research was to study the inheritance and morphology of different southern highbush dwarf phenotypes observed in the University of Florida blueberry breeding program.

CHAPTER 2
LITERATURE REVIEW

Morphology of Dwarf Plants

Dwarf plants are characterized by reduced height when compared to normal plants. Dwarfness results from either smaller and/or fewer cells (Gale and Youssefian, 1985) and thus, shorter and/or fewer internodes. Bindloss (1942) reported fewer cell divisions in the stem of a dwarf *Lycopersicon esculentum* L. compared to a normal plant. Pelton (1964), as cited by Garvey (1985), reported precocious secondary cell wall thickening in the dwarf columbine (*Aguilegea vulgares* L. cultivar 'Compacta'). This was believed to result in smaller cells and dwarf plants.

Other morphological traits that have been described in dwarf plants include smaller canopy (Fideghelli et al., 2003); higher number of sprouts (Wareing and Phillis, 1978; Draper et al., 1984; Garvey and Lyrene, 1987), smaller leaves (Draper et al., 1984), smaller reproductive organs, smaller fruits, and smaller root weight and depth (Gale and Youssefian, 1985).

In blueberries, dwarf plants have been observed and studied. Draper and colleagues in 1984 reported dwarf selections of *V. corymbosum* with shorter internodes and smaller leaves. They also mentioned a bushy appearance to each shoot caused by high sprouting. Garvey and Lyrene (1987) observed and studied dwarf selections of *V. ashei*. The dwarf plants were described as short-saturated and compact-growing.

Genetics and Physiology of Plant Dwarfism

Reports on the inheritance of dwarfing genes are numerous and diverse. There are good examples of simple inheritance like the recessive *dw* gene in peach (Hansche et al., 1986), the monogenic recessive dwarfing genes in raspberry: *fr, n* and *dw* (Knight and Scott, 1964; Jennings, 1967) and the single dominant gene for compact habit in 'Wijcik' apple (Lapins and Watkins, 1973). Keep (1969) mentioned the digenic 'sturdy dwarf' and 'crumpled dwarf' in raspberry.

There are also complex inheritances that can not be explained by a known inheritance ratio. Examples include the *Vaccinium* dwarfs studied by Draper and colleagues (1984) and by Garvey and Lyrene (1987). These dwarf phenotypes might be the product of several interacting genes.

Aneuploidy can also affect the stature of plants and cause dwarfs. With aneuploids, fertility is significantly reduced because the abnormal chromosome number affects meiosis, producing some non-viable gametes.

Three major groups of hormones are most often reported in association with dwarf phenotypes. The most commonly mentioned group is gibberellins (GA), followed by auxins and brassinosteroids. A fourth group, cytokinins has been implicated in some dwarfs.

Gibberellins are usually associated with shoot and cell elongation, internode length and other plant developmental processes like fruit enlargement (Berhow, 2000). Gibberellin was named after the fungus *Gibberella fujikuroi*, which causes elongation in infected rice seedlings. Foliar application of GA (either GA_3 or GA_4)

in apple (*Malus domestica* Borkh) decreased shoot number but increased total shoot length and total bud number (Kurshid et al., 1997).

Mutations affecting GA synthesis, deactivation and reception are usually identified via a shoot elongation screen (Ross et al. 1997). Short plant (dwarf) mutants can be categorized as responsive or non-responsive depending on their response to exogenous gibberellins.

Ladizinsky (1997) described a dwarf phenotype in *Lens* characterized by short internodes, short leaf axis and smaller convex leaflets. This dwarf, when sprayed with GA, responds positively by elongation of the internode and leaf-axis. Dwarfs that respond in this way to GA applications are referred to as GA responsive or GA sensitive dwarfs. Goldman and Watson (1997) described a monogenic dwarf mutant in red beet (*Beta vulgaris* L. subsp. Vulgaris) that is also sensitive to GA.

Mackenzie-House et al. (1998) gives a good example of a non responsive dwarf mutant. Application of GA to *Pisum sativum* L. plants that carry the *lrs* mutation reduces internode length, GA synthesis and cell elongation.

Borner et al. (1999) studied two dwarfing genes in barley (*Hordeum vulgare*), the recessive *gai* and *gal* dwarfing genes. Both were on chromosome 2H and both reduced plant height, but the *gal* phenotype was sensitive to exogenous GA, whereas the *gai* phenotype was insensitive.

Auxins are also directly involved in regulation of stem elongation (Little et al., 2003), and they might be present at lower levels in dwarf plants than in normal tall plants (Yang et al., 1993). An abrupt growth response was observed

by Yang et al. (1996) in dwarf mutants (GA_1-deficient *le*) of light-grown pea (*Pisum sativum* L.) after applying IAA. The lag time was only 20 minutes, and the plants reached a growth rate up to ten times higher than the control. The elongation was in the older elongating internodes. Gibberellins also caused an elongation response (mainly in less than 25% of expanded internodes). The authors concluded that auxins and gibberellins control separate processes that together contribute to stem elongation. A deficiency in either leads to a dwarf phenotype.

Brassinosteroids have also been observed to cause dwarf plants, mainly due to their role throughout plant growth and development. Yin et al. (2002) and Schaller (2003) reported that plants with defective brassinosteroid biosynthesis and perception have cell elongation defects and severe dwarfism.

Since prolific sprouting is characteristic of many dwarf types and is the result of the growth of many axillary buds (Wareing and Phillis, 1978), and since the interaction among auxins and cytokinins has been reported to control apical dominance (Sachs and Thimann, 1967), cytokinins should also be considered when investigating plant dwarfism mechanisms, because cytokinins can disrupt apical dominance and cause the multiple sprouting or bushy branches reported with the dwarf phenotype by Draper et al. (1984).

Highbush Blueberry Domestication

The domestication of highbush blueberries (complex hybrids of *Vaccinium corymbosum* L.) started early in the 20th century with the work of Frederick Coville, a botanist who made the first selections for breeding purposes in the first US blueberry breeding program (Coville, 1937).

From the beginning, highbush blueberries were hybridized with other species in *Vaccinium* section Cyanococcus (Moore, 1966). Some of the other species used to improve the highbush blueberry included lowbush blueberry (*V. angustifolium* Ait.) and the myrtle blueberry of Florida (*V. myrsinites* Lam.). Early in the breeding of blueberries it was noticed that some species did not hybridize, and it was determined by the cytological work of Longley in 1927 (cited by Coville, 1937) that the primary cause was the difference in ploidy – diploids when crossed with tetraploid species did not hybridize, or if they did, they produced a few low vigor plants (Coville, 1937).

Draper and Hancock (2003) mentioned the work of Darrow and Sharpe, who selected a *V. darrowi* (Camp) plant that they found near Tampa, Florida. They named it Florida 4B, and this plant has been used extensively in the southern highbush blueberry breeding programs to reduce the high chill requirement of the northern highbush (Sharpe, 1954; Sharpe and Darrow, 1959; Sharpe and Sherman, 1971; Lyrene and Sherman, 1984). It is important to mention that this diploid plant hybridizes with the tetraploid *V. corymbosum* because it produces unreduced gametes (Lyrene, Vorsa and Ballington, 2003).

Other interspecific crosses involving tetraploid cultivated blueberries with non tetraploid species included hexaploid *V. ashei* (Darrow, 1949; Lyrene and Sherman, 1984) and diploid *V. elliottii* (Lyrene and Sherman, 1983; Lyrene and Sherman, 1985).

The rabbiteye blueberry (*V. ashei*) was grown commercially in Florida starting around 1893, and by the late 1920's approximately two- to three-

thousand acres were in cultivation (James, 1924; Clayton, 1925; Mowry and Camp, 1928; Lyrene and Sherman, 1979). By 1930, the industry had declined rapidly, mainly because the quality of the Florida blueberries was low compared to New Jersey and Michigan blueberries. The northern blueberries were produced on clonally-propagated cultivars based on *V. corymbosum* developed by the U.S. Department of Agriculture (USDA) (Lyrene and Sherman, 1977). To support the blueberry industry in the southeast, breeding efforts with *V. ashei* were started in 1940 in Tifton, Georgia (Brightwell, 1971) and with highbush blueberries in Florida in 1948 (Sharpe and Sherman, 1971).

The Florida blueberry breeding program has focused mainly on the improvement of the tetraploid *V. corymbosum*, rather than on the hexaploid *V. ashei*. Lyrene and Sherman (1977) mentioned various reasons for this, including the fact that Georgia already had an active rabbiteye breeding program, and none of the *Vaccinium* species in Florida can be easily crossed with *V. ashei* without producing pentaploids. Further, early ripening (the most significant advantage for Florida) and low chilling were not readily available in *V. ashei* germplasm.

Low-chill highbush blueberries from Florida, based on *V. corymbosum* and *V. darrowi* hybrids, has resulted in an early-season blueberry industry in Florida and Georgia. To date, the annual shipment of fresh-market blueberries from Florida is about 4 million pounds and is increasing yearly. The estimated wholesale value (farm gate value) is about $20 million per year. Almost all early-

season varieties grown in Florida came from the University of Florida blueberry breeding program (Dr. Paul Lyrene, personal communication).

CHAPTER 3
MATERIALS AND METHODS

It was noticed in the Florida tetraploid highbush breeding program that some crosses between two plants of normal stature segregated dwarfs. Two of these dwarf plants, 00-266 and 00-08, were selected by Dr. Paul Lyrene before the study reported here was carried out, because of their desirable traits for breeding (early leafing and high fruit quality). Other dwarf selections were made during the study period from the high density seedling nurseries of 2002 and 2003. The high density nursery (Stage One) consisted of about 120 different crosses of normal stature plants, about 90 seedlings per cross, producing a highly diverse blueberry population.

Morphological Studies

Two analyses were carried out for morphological traits with the objective of contrasting normal and dwarf types. The first was a multiple comparison analysis for particular traits (e.g. internode length). The second was a logistic regression analysis that modeled the response (the probability of being a dwarf) for given parameters (i.e. height and sprouting).

Multiple Comparison Analysis

To classify and distinguish dwarfs from normal plants, internode length, leaf area, and plant height were measured from different clones representing the dwarf and normal types.

The dwarf plants studied for internode length and leaf area measurements were selected from the high density nursery (Stage One) of 2003, except for 00-266 and 00-08 which had been selected and propagated previously by Dr. Paul Lyrene. Dwarf plants were selected subjectively by visual inspection, but the dwarf plants appeared to be qualitatively different from their normal full-siblings, and there were few or no plants whose classification was not obvious. The normal plants selected for contrast with the dwarfs also had a diverse background and were used previously in the breeding program (some of them are known cultivars, e.g. ‘Emerald’ and ‘Jewel’)

All plants used in this study were at least one year old. They were grown in black 3-liter pots filled with peat, outside in full sun, watered as needed, and fertilized with Tracite 20-20-20 with minor elements (Helena Corp.) about once a month during the growing season. The measurements were taken at the end of August 2003.

The plants for the height study were selected from the high density seedling nursery of 2002. From these seedlings, approximately 36 plants were selected as dwarfs in the spring of 2003 and were transplanted to fallow ground at one end of the nursery to keep them from being shaded by taller plants.

The height of these 36 dwarf plants as well as of 36 randomly selected tall plants from the Stage Two nursery of 2003 was determined. The Stage Two nursery of 2003 was the group of selected plants (based on their desired breeding attributes) from the high density nursery of 2002 after the unselected plants were removed.

A one side t-test for two populations (dwarf vs. normal) was conducted to determine if there were statistical differences between the two populations for the traits studied. Analyses of variance and multiple comparisons were also conducted. Tukey's *W* procedure with alpha=0.05 was performed to determine if there were statistical differences between the means of each clone for the studied traits.

Internode length

The average lengths of three-internode stem segments were determined for 12 clones: six dwarf (00-08, 00-266, 03-105, 03-112, 03-115 and 03-118) and six normal ('Emerald', 'Jewel', 00-204, 00-206, 00-59, 98-325). The three internodes measured started at the fourth node counting from the tip of a randomly selected stem and ending at the seventh node from the tip. The fourth internode was selected as the starting point to avoid measuring internodes that were still elongating. Internode length was measured for ten stem segments of each clone.

Leaf area

The leaf area (LA) of dwarf and normal types was estimated by measuring leaf length (L) and leaf width (W), then calculating the area using the formula for a rhombus (LA= W * L / 2). Five leaves were measured for each of 18 clones, nine dwarf (00-08, 00-266, 03-105, 03-112, 03-114, 03-115, 03-116, 03-117, 03-118) and nine normal type ('Emerald', 'Jewel', 95-174, 97-118, 98-325, 00-59, 00-116, 00-206, 00-204). The measured leaves were mature and picked at random.

Height of the plant or length of the longest shoot

The height of the plants was measured from the soil level to the tip of the longest shoot.

Logistic Regression Analysis

Plants used in this study originated from seed sown in pots of peat in June 2003. In August, the seeds were germinated in a controlled temperature chamber at about 10°C with continuous illumination. After germination was at about 50%, the seedlings were moved to a greenhouse. They were transplanted at 2cm by 2cm spacing to trays of peat in September 2003. They were grown in a greenhouse until May 2004, being watered daily by hand and fertilized every 3 weeks with Tracite 20-20-20. At the end of May, when the plants were about 10 months old, they were visually classified into dwarf and normal phenotypes. Measurements were taken for each class as described below.

Categorical data analysis was conducted using SAS (the SAS System V.9), to model the log of the probability that the plant was dwarf given the predictor parameters: length of the longest shoot (measured as described previously) and branching (the number of sprouts or branches on the longest shoot, divided by the length of that shoot measured in cm), by a multiple logistic regression analysis. Both predictors were treated as categorical variables. Scores were assigned to each predictor category, and backward elimination of predictors was conducted to select the most appropriate model as described by Agresti (1996). (For detailed information on logistic regressions see Appendix).

The benefit of this analysis is that it allows the study of various parameters (i.e. length of the longest shoot and branching) as well as their interaction, as

predictors for a categorical response (i.e. dwarf vs. normal blueberries). It was noticed that dwarf blueberries had high sprouting and low stature. Both parameters were predictors of dwarf blueberry plants. These parameters were also preferred over others, because they were the easiest to measure.

Inheritance Studies

Field observations from crosses of the University of Florida blueberry breeding program were made in the fall of 2002, 2003 and 2004 to study the inheritance of dwarfness. Controlled crosses between selected dwarf plants and normal plants were carried out in a greenhouse in the fall of 2002 and were evaluated during the summer and fall of 2004. The evaluation consisted of classifying the progeny plants as either normal or dwarf by their physical appearance (the normal being taller and with normal branching, while the dwarf smaller and with high branching) to obtain inheritance ratios.

Normal to dwarf ratios from field observations of the breeding program and from the controlled crosses were analyzed statistically by a chi-square test to see how well they fit various hypothesized ratios.

Field Observations

The field observations were made in Stage One high density nurseries planted in 2002, 2003 and 2004. All of the clones used for the crosses were of normal stature phenotype. Stage One is the first field stage in the blueberry breeding program before any selection is done. For each nursery, the seeds were planted in December. The seedlings were transplanted to trays of peat and grown in a greenhouse until May. Then, they were transplanted to a fumigated field nursery (Stage One) at a spacing of 15 cm between plants and 45 cm

between rows (about 15 plants per m^2). The evaluations were carried out in October 2002, November 2003 and November 2004, after the plants had been growing in the field nursery for 10 to 11 months.

Controlled Crosses

Dwarf plants were selected from the 2002 Stage One high density nursery based on their vigor and attractive architecture, except for 00-266 and 00-08, which had been selected previously. The plants were prepared for winter crossing by keeping them in the greenhouse and not allowing them to enter dormancy. Controlled pollination as described by Galletta (1975) was started in January and continued until the end of March. Various cross combinations were tried: dwarf to dwarf (00-266 x 00-08, 00-266 x 03-105, 00-08 x 03-105 and 03-112 x 00-08), dwarf to normal (00-266 x 01-21, 00-266 x 'Emerald', 00-08 x 'Emerald') normal to dwarf (01-21 x 03-112, 'Jewel' x 00-266), normal to normal (03-120 x 'Southern Belle', 03-54 x 'Santa Fe', 03-73 x 'Jewel', 'Emerald' x 'Sapphire') and self (00-266 and 00-08).

The mature fruits were harvested, and the seeds were extracted following the method used in the blueberry breeding program. The berries were processed in a food blender with water for a few seconds, after which most of the seeds were obtained by washing away the flesh and skin of the berries. The seeds were then dried at room temperature and stored in a refrigerator at 7°C until they were sown in pots with peat moss and germinated in a chamber with a temperature of about 10°C in the summer of 2003. The seedlings started to germinate in early August, and were then transplanted to trays, 48 seedlings per tray, in September. A total of 96 seedlings, two trays filled to capacity, were

grown for each controlled cross in the greenhouse. Each cross was evaluated for dwarf to normal ratios in February, when the plants were about 6 months old and still growing in greenhouse trays.

Only the dwarf plants were transplanted to the 2004 Stage One high density nursery. A follow up evaluation was performed in October 2004 to check for possible short normal plants that could have been erroneously classified.

CHAPTER 4
RESULTS AND DISCUSSION

Morphological Studies

Multiple Comparison Studies

The studied dwarf plants were short, with a compact look similar to the descriptions of dwarf blueberries given by Draper et al. (1983), and Garvey and Lyrene (1987) (Figure 1). Among the dwarf plants observed in the field, leaf size, plant height and branching were variable, just as these characteristics are variable among seedling blueberries that are not dwarfs.

Figure 1. The author with dwarf and normal highbush blueberry, May 2005.

The internodes of the dwarfs were significantly shorter than those of normal plants (P = 0.0001, see Table 1 and 2). The average dwarf internode was somewhat over half the normal length. Nevertheless, dwarfs 00-08 and 00-266 were not significantly different from normal 00-206, and only dwarf 00-266 was not significantly different from normal cultivar 'Jewel' (Figure 2).

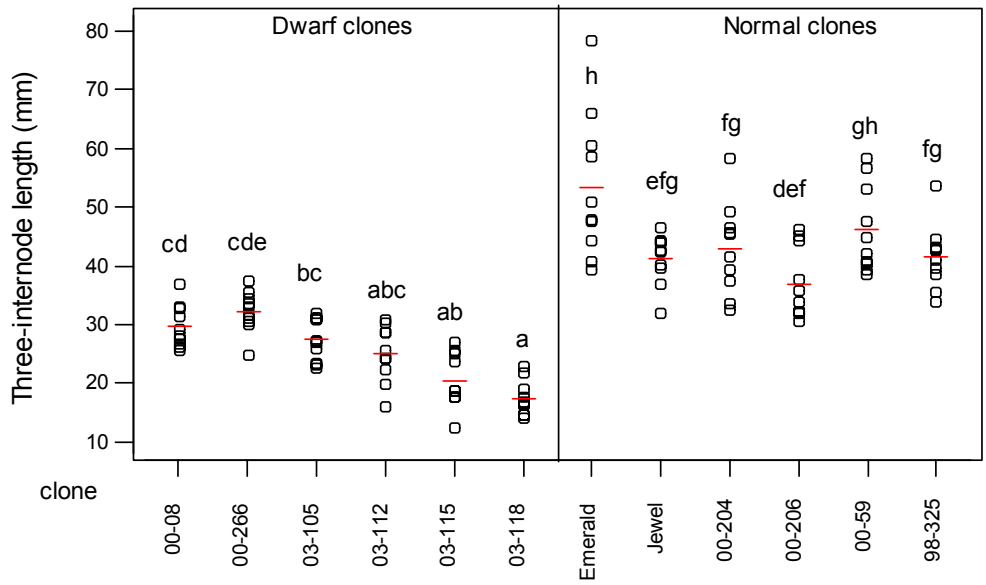

Figure 2. Dotplot of length of three internodes (mm) by clone. The group means are indicated by horizontal red lines and each circle represents an observation. The first 6 plants were considered dwarf and the last six normal. The letters on top of each clone represent the statistical grouping according to Tukey's *W* procedure.

Leaf area was also smaller in the dwarfs compared to normal plants (Table 3 and 4). Nevertheless, leaf areas of dwarf genotypes: 03-114, 03-105, 00-08 and 00-266 were not statistically different from the normal genotypes 98-325, 97-118, 95-174, 00-59, and 00-204 (Figure 3). Also dwarfs 03-105, 00-08 and 00-266 were not statistically different from the normal cultivar 'Jewel'.

Table 1. Length of three-internodes (mean ± 2 SE) of dwarf and normal plants (average of 10 measurements).

Pedigree	Type[y]	Three-internode length (mm) (Mean ± 2 SE[z])
00-08	D	29.76 ± 2.32
00-266	D	32.24 ± 2.18
03-105	D	27.52 ± 2.32
03-112	D	25.12 ± 3.01
03-115	D	20.45 ± 2.93
03-118	D	17.47 ± 1.90
Emerald	N	53.41 ± 7.80
Jewel	N	41.30 ± 2.68
98-325	N	41.51 ± 3.45
00-59	N	46.18 ± 4.65
00-206	N	36.99 ± 3.82
00-204	N	42.98 ± 4.91

[y] D=Dwarf, N= Normal
[z] SE= SD/($n^{1/2}$)

Table 2. Results of the t-test for the length of three internodes of dwarf plants vs. normal plants

Type[z]	N	Mean	Std.Dev.	SE Mean
D	6	25.43	5.62	2.3
N	6	43.73	5.60	2.3

[z] D=Dwarf, N= Normal

95% CI for μ dwarf type – μ normal type: (-25.6, -11.0)
Ho: μ dwarf type = μ normal type; Ha: μ dwarf type < μ normal type
t = -5.65 P-value = 0.0002 DF = 9

Table 3. Individual leaf area (mean ± 2 SE). Average of 5 measurements of dwarf and normal plants.

Clone	Type[y]	Leaf area (mm^2) (Mean ± 2 SE[z])
00-08	D	507.1 ± 150.2
00-266	D	565.0 ± 114.0
03-105	D	443.9 ± 161.4
03-112	D	211.9 ± 54.6
03-114	D	382.2 ± 95.8
03-115	D	211.6 ± 62.6
03-116	D	199.8 ± 72.6
03-117	D	145.8 ± 22.8
03-118	D	136.8 ± 43.2
Emerald	N	1242.2 ± 149.0
Jewel	N	780.3 ± 170.0
95-174	N	672.3 ± 133.0
97-118	N	620.0 ± 202.0
98-325	N	683.8 ± 93.0
00-59	N	761.2 ± 172.4
00-116	N	1079.0 ± 226.0
00-206	N	953.0 ± 330.0
00-204	N	668.6 ± 46.0

[y]D=Dwarf, N= Normal
[z] SE= $SD/(n^{1/2})$

Table 4. Results of the t-test for the leaf area of dwarf plants vs. normal plants

Type	N[y]	Mean[z]	Std.Dev.	SE Mean
Dwarf	9	312	164	55
Normal	9	829	215	72

[y] N= number of observations
[z] Square mm

95% CI for μ dwarf type – μ normal type: (-711,-324)
Ho: μ dwarf type = μ normal type; Ha: μ dwarf type < μ normal type
T = -5.74 P-value = 0.0000 DF = 14

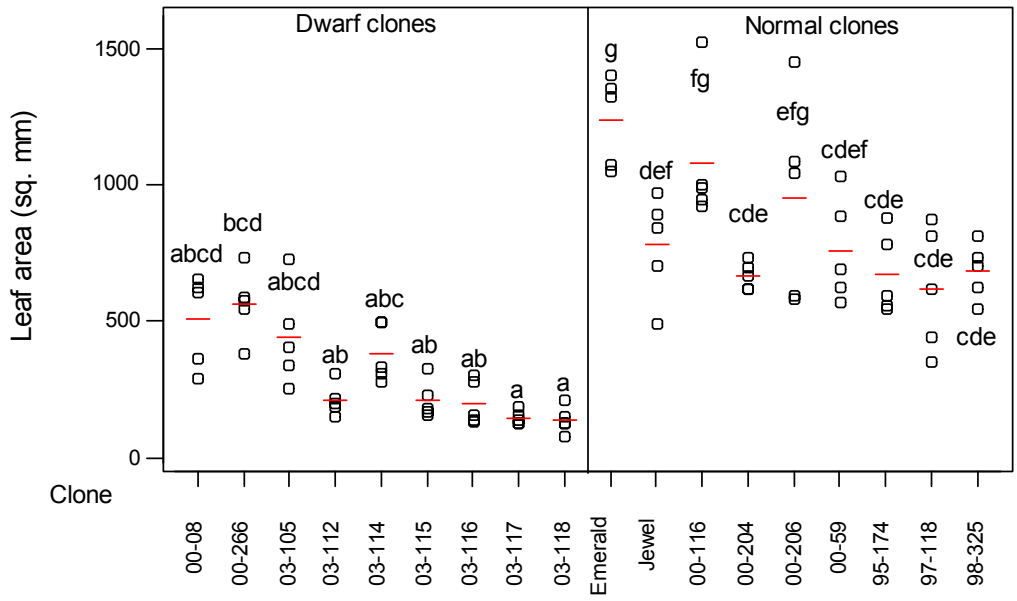

Figure 3. Dotplot of leaf area (mm^2) by clone. Group means are indicated by horizontal red lines and each circle represents an observation. The letters on top of each clone represent the statistical grouping according to Tukey's *W* procedure.

Plant height was significantly different among dwarf and normal plants. Three year old normal plants were 2.5 times taller than three year old dwarf plants, both from the 2003 Stage 2 nursery (Figure 4). The analysis of variance reported an F value of 309.1 with one degree of freedom for plant type (P value= 0.000).

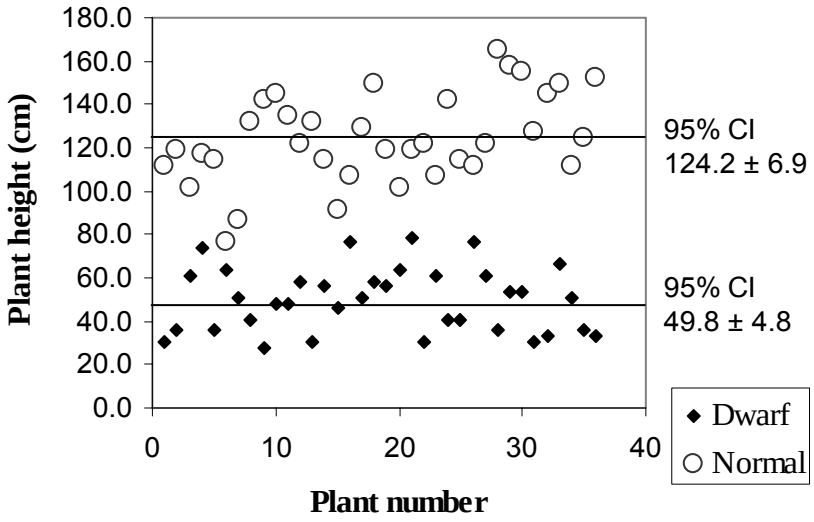

Figure 4. Scatter plot of three year old dwarf and normal plant heights (cm) from the 2003 Stage 2 nursery. Plant numbers are the sequence in which the plants were measured.

Logistic Regression Analysis

The total number of plants observed (373) had a large range for both variables (44.0 cm for shoot length and 18.9 cm for number of branches), which was due mainly to the differences between dwarf and normal types. Dwarf plants were shorter, with a mean of 11.8 ± 0.5 cm and a 95% confidence interval (CI) of 4.9 to 18.7 cm, whereas the mean length of normal plants was 22.1 ± 0.7 cm with a 95% CI of 11.1 to 33.0 cm. Dwarf plants also had more sprouts, with a median of six branches compared to a median of three branches for normal plants.

In order to determine the number of categories to use for each predictor and to assign their ranges, a scatter plot of shoot length versus branch number was made (Figure 5). Two categories were assigned for shoot length – plants with shoots less than or equal to sixteen centimeters and plants with shoots longer than sixteen centimeters, and three categories for branch number – plants

that had three branches or fewer, those that had between four and five branches and those that had more than five branches. The categories were chosen in such a way that each category would include both dwarf and normal plants so that the chi square approximation would be valid (Table 5).

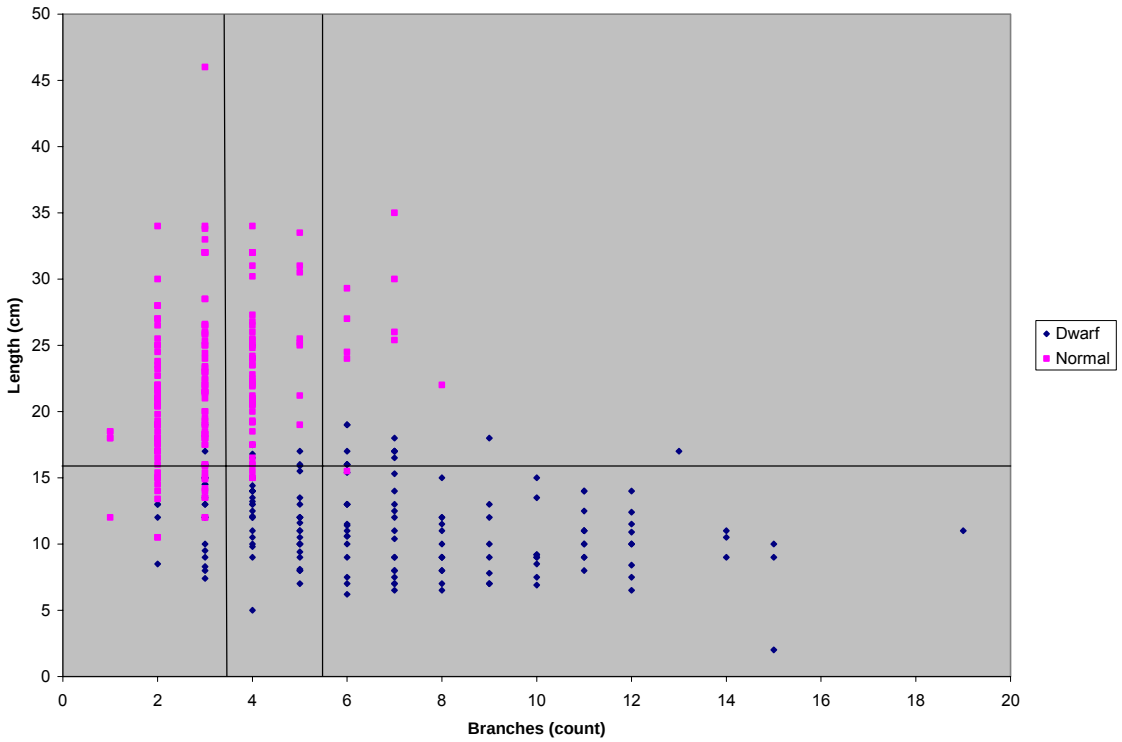

Figure 5. Scatter plot of height versus number of branches for dwarf and normal plants 10 month old.

Using these categories, a logistic regression analysis was carried out to model the odds of a plant being a dwarf (probability of dwarf / probability of normal plants) and to test the main effects of the two predictors, shoot length and branch number, as well as to test for interaction effects. For information on logistic regression analysis see Appendix.

Table 5. Frequency of plants in different categories of length and branch number for each plant type.

Branches (total count of shoots)	Length of tallest shoot (cm)			
	Dwarf plants		Normal plants	
	≤ 16	> 16	≤ 16	> 16
< 4	27	2	22	106
4 – 5	45	6	6	51
> 5	88	10	1	9

First, using the univariate procedure in SAS, the categories were given scores. For branches, the scores were: 3, 4, and 8, which were the medians for each group. The scores for the length groups were the means for each group, 11.6 and 22.9. Multiple logistic regression analysis was performed using all variables, as well as all interactions. A second model was tested using only the length measurements and number of branches as predictors, ignoring potential interactions. The models are given below (see Table 6 and 8). The first model had a good fit with G^2=199.89 and *df*=367. The second model also had a good fit with G^2=199.93 and *df*=369. The likelihood ratio statistics for the interacting terms showed there were no significant interactions between length and branches. The differences in deviances between the two models were small, 0.04 with two degrees of freedom ($P>X^2_2 = 0.980$). Thus, these differences were not significant and it was concluded that dropping the interaction terms would have no effect on the ability of the model to predict the log of the odds of a plant being a dwarf.

Both predictors, height and branch number, were significant. The likelihood-ratio test gave probability values <0.0001 for both predictors. Thus Model 2 was the better model for indicating whether a plant was dwarf or normal.

Table 6. Model 1 and Model 2 equations with logit, odds of dwarf and probability of dwarf, for Model 2 only.

MODEL1: Logit P(Y=dwarf) = 0.1054 – 4.0757 [b≤3] – 2.2454 [4≤b≤5] + 4.3720 [l≤16] – 0.1969 [b<=3]* [l≤16] – 0.2170[4≤b≤5]*[l≤16]

MODEL 2: Logit P(Y=dwarf) = 0.137 – 4.127 [b≤3] – 2.299 [4≤b≤5] + 4.198 [l≤16]

Logit (Y=dwarf) Odds of dwarf Probabiliy of dwarf	Branches (b) ≤ 3	4≤b≤5	Branches (b) > 5
Length (l) ≤ 16	0.208 1.23 0.552	2.036 7.66 0.885	4.335 76.32 0.987
Length (l) > 16	-3.990 0.02 0.018	-2.162 0.103 0.115	0.137 1.15 0.534

Using the simpler model (Model 2), it was found that the odds of getting a dwarf plant, if the number of branches was three or fewer, was 1.6% of the odds of getting a dwarf plant when the number of branches was greater than five, with a 95% confidence of 0.5% to 4.9%. Furthermore, the odds of a dwarf plant with four or five branches was 10% the odds of a dwarf plant with more than five branches (95% CI 3.5% to 28.5%). The odds of getting a dwarf with three or fewer branches was 16% the odds of having a dwarf with four or five branches. Finally, a plant was 66.5 times (95% CI 28.2 to 157.1) more likely to be a dwarf if its height was less than 16 cm than if its height was more than 16 cm.

Table 6 shows that the probability that a plant is dwarf increased as branch count increased for a given length category, whereas the estimated probability of

dwarf types decreased when plant height was taller than sixteen centimeters for a given branch category.

During the analysis of this model it was realized that the odds of a dwarf with fewer than three branches was 1.6% the odds of a dwarf with more than five branches. This number seemed rather small. The odds of getting a dwarf with four and five branches was only 10% the odds of getting dwarfs with more than five branches. It was hypothesized that a simpler model would fit, using only two categories for branches – those with more than five branches and those with five or fewer branches. This model was labeled Model 3. However, Model 3 fit the data poorly. The deviance of this model was 20.13 with only three degrees of freedom and various large residuals, which illustrates the poor fit of this model to the data.

Going back to the second model, the residuals were studied. There were three observations with large residuals, which may have had an effect on the original model. As seen in Table 7 and Table 8, after removing these outliers, the model was again fitted – one with interaction terms (Model 4) and one without interaction terms (Model 5). The observations with the large residuals were two relatively tall plants that had very few branches, which had been classified as dwarfs. From the previous analysis of these data, observations like that seemed very unlikely. Thus, these strong outliers may have shifted the model. The third outlier was an observation of a plant that had classified as normal but was short and had numerous branches. After fitting the data to the remaining observations it was again found through the likelihood-ratio test that the interacting terms were

insignificant and could be dropped from the model (G^2 of model without interacting terms = 173.30; G^2 of model with interacting terms =169.01, thus the difference = 4.29 with 2 degrees of freedom and a P>X^2_2= 0.117). The deviance of Model 5, the model that deleted the three outliers and did not include of interactions, was equal to 173.30 with 366 degrees of freedom, and the model was declared to be a good fit for the data.

Using Model 5, the odds of getting a dwarf with three or fewer branches was 0.74% the odds of getting a dwarf with more than five branches (95% CI: 0.2%, 2.8%). The probability of getting a dwarf with four or five branches was 6.8% the odds of getting a dwarf with more than five branches (95% CI: 6.0%, 7.6%). The odds of getting a dwarf with three or fewer branches were 10.9% the odds of getting a dwarf with four or five branches, similar to the results from Model 2.

Table 7. Model 4 and Model 5 equations with logit, odds of dwarf and probability of dwarf, for Model 5 only.

MODEL 4: Logit P(Y=dwarf) = 0.1054 – 28.4709 [b≤3] – 2.2454 [4≤b≤5] + 28.2597 [l≤16]+0.3106[b≤3]* [l≤16] - 24.1048[4≤b≤5]*[l≤16]

MODEL 5: Logit P (Y=dwarf) = 0.266 – 4.911 [b≤3] – 2.69 [4≤b≤5] + 4. 81 [l≤16]

Logit (Y=dwarf) Odds of dwarf Probability of dwarf	Branches (b) ≤ 3	4≤b≤5	Branches (b) > 5
Length (l) ≤ 16	0.125 1.133 0.531	2.346 10.444 0.913	5.036 153.853 0.994
Length (l) > 16	-4.685 0.009 0.009	-2.464 0.085 0.078	0.226 1.254 0.556

The odds of getting a dwarf with shoot length equal to or less than sixteen centimeters was 122.73 times the odds of getting a dwarf with shoot length

greater than sixteen centimeters (95% CI: 42.68, 353.2). For both predictors, removing the outliers gave stronger evidence that the higher the number of branches and the shorter the plants, the higher the probability of a dwarf phenotype, supporting the field observation that dwarf plants are short and with a higher than normal number of sprouts.

This model has an outcome similar to that of Model 2, but Model 5 showed slightly stronger evidence for what has been observed in the field. Both models had high deviances, showing both are good fits for the data. However, Model 5 has a slightly stronger deviance, as such; the p-value for the intercept of the model is smaller than in Model 2. Thus, Model 5 is a slightly better fit for this data. More importantly, there are no significant residuals when Model 5 is applied to the data, omitting the three outliers. Therefore the best fit for the data is Model 5.

Table 8. Results of fitting five logistic regression models to the dwarf data

Model	Deviance (G^2)	DF	P> X^2 Fit of model	Models compared	Difference	P>X^2
1	199.89	367	1			
2	199.93	369	1	(2) - (1)	0.04 (*df*=2)	0.980
3	20.13	3	0.0002*			
4	169.01	364	1			
5	173.30	366	1	(5) - (4)	4.29 (*df*=2)	0.117

* Not a good fit for the model

It has been shown through categorical analysis that length of the longest shoot and number of branches, when used together, are good predictors of dwarfs in blueberry plants. More specifically, analysis of the data has shown that a plant with more than five branches whose longest shoot is less than sixteen centimeters long has a 99.4 % probability of being dwarf. Conversely, the

probability of having a dwarf plant with fewer than three branches and measuring more than sixteen centimeters is 0.9%.

These results are supported by previous observations suggesting that low or lack of apical dominance, which causes high branching, is associated with dwarfness in *Rubus* sturdy dwarf (Keep, 1969) and in highbush blueberry, *Vaccinium corymbosum* (Draper et al., 1984).

Inheritance Studies

In the fall of 2002, 2003 and 2004, the high density plots of the blueberry breeding program at the University of Florida's Plant Science Research and Education Unit, in Citra, Florida, were studied for dwarf plants.

Each of these three plots consisted of seedlings from about 150 crosses, with 90 seedlings per cross. The parents for each plant consisted of about 200 different southern highbush cultivars and advanced selections and the parents differed for each plot. The parents were all highly heterozygous, and the seedling populations were segregating for many characteristics.

Twenty-five crosses that were segregating dwarfs were identified, and segregation ratios were determined for each cross. Each progeny population was examined for fit to a 3:1 and 11:1 normal:dwarf segregation ratio. The rationale for testing these particular ratios is given below. The dwarf-segregating populations studied could be classified into two groups. The first seven crosses in Table 9 fit an inheritance ratio of 3:1 fairly well. The last 18 fit a ratio of 11:1.

Table 9. Segregation ratios and chi-square tests for normal to dwarf ratios (3:1 and 11:1) for dwarf-segregating normal stature phenotype crosses from the 2002, 2003 and 2004 high density plots.

Pedigree	Nursery Year	Normal (N)	Dwarf (D)	N/D	3:1[y]		11:1[z]	
					X^2_1	P value	X^2_1	P value
01-21 x 96-32	2002	47	20	2.4	0.84	0.359	40.61	0.000
01-20 x Nui	2002	70	18	3.9	0.97	0.325	16.93	0.000
00-43 x S.Belle	2002	67	17	3.9	1.02	0.314	15.58	0.000
Windsor x 98-336	2002	68	14	4.9	2.75	0.097	8.20	0.004
02-38 x 98-325	2003	73	23	3.2	0.06	0.814	30.68	0.000
98-405 x 95-115	2003	74	22	3.4	0.22	0.637	26.73	0.000
NC 2925 x 03-124	2004	69	20	3.5	0.30	0.582	23.29	0.000
97-130 x 93-204	2002	87	8	10.9	13.93	0.000	0.00	0.975
97-61 x 99-220	2002	73	7	10.4	11.27	0.001	0.02	0.893
98-18 x 97-390	2002	80	8	10.0	11.88	0.001	0.07	0.797
01-64 x 97-142	2002	64	5	12.8	11.60	0.001	0.11	0.744
02-20 x 00-61	2003	87	9	9.7	12.50	0.000	0.14	0.712
01-129 x 97-41	2003	84	12	7.0	8.00	0.005	2.18	0.140
02-69 x 00-14	2003	92	4	23.0	22.22	0.000	2.18	0.140
03-47 x S.Belle	2004	74	7	10.6	11.56	0.001	0.01	0.920
03-61 x 90-4	2004	80	8	10.0	11.88	0.001	0.07	0.797
03-01 x S.Belle	2004	69	7	9.9	10.11	0.001	0.08	0.782
03-12 x Emerald	2004	76	8	9.5	10.73	0.001	0.16	0.693
98-406 x Jewel	2004	75	9	8.3	9.14	0.002	0.62	0.430
03-50 x 03-126	2004	85	5	17.0	18.15	0.000	0.91	0.340
02-86 x Sapphire	2004	79	11	7.2	7.84	0.005	1.78	0.182
Sapphire x 95-209-B	2004	69	10	6.9	6.42	0.011	1.93	0.164
Jewel x 02-22	2004	60	9	6.7	5.26	0.022	2.00	0.157
S.Belle x 00-206	2004	85	3	28.3	21.88	0.000	2.79	0.095
03-103 x Santa Fe	2004	83	13	6.4	6.72	0.010	3.41	0.065

[y] AAaa x AAaa and the reciprocal cross
[z] AAaa x AAAa and the reciprocal cross

The dwarf phenotype seems to be a recessive trait with monogenic inheritance. Since crosses between two normal plants segregated dwarf plants, the dwarf genotype cannot include the triplex form (AAAa) because this would imply that one of the parents was a dwarf which was not the case. This limits the possibilities of dwarfs segregating from normal plants to the duplex (AAaa) and simplex (Aaaa) forms, because the nulliplex (aaaa) form cannot occur in the

progeny of a triplex parent that shows chromosome segregation preferentially over random chromatid and maximum equational segregations.

If the duplex genotype is dwarf, then the only combination of normal-statured parents that would segregate dwarfs would be that of crossing two triplex (AAAa). This would produce the duplex genotype of a dwarf with a 0.25 frequency, giving a 3:1 normal to dwarf ratio (Table 10). The fact that 18 crosses did not follow a 3:1 frequency implies that the duplex genotype has a normal instead of dwarf phenotype.

Since duplex plants have normal stature, the nulliplex form is possible for a dwarf segregating from a cross between two normal plants. The genotypic combinations of normal phenotypes that could produce dwarfs are as follows: AAAa x AAaa, AAaa x AAaa and the reciprocal crosses.

Dwarfs produced by crossing a triplex with a duplex will be simplex and would be expected in a ratio of 11:1 normal to dwarf phenotype. A duplex times another duplex can produce dwarfs in a 3:1 ratio, the possible genotypes for dwarf being the simplex and nulliplex at frequencies of 0.22 (8/36) and 0.03 (1/36) respectively (Table 10).

To further test this hypothesis, two dwarf plants (00-266 and 00-08) were self-pollinated, intercrossed with two other dwarf selections (03-105 and 03-112) and backcrossed to normal types ‘Emerald’, ‘Jewel’ and 01-21 (Table 11 and Table 12). See Table 14 for a cross table of all the controlled crosses evaluated in this study.

The two dwarfs that were self-pollinated (00-266 and 00-08) segregated some normal type plants, indicating that they are not nulliplex and are probably simplex (Aaaa) for the normal allele. The 1:3 normal to dwarf segregation did not fit very well when the two clones were self pollinated, so the possibility that the nulliplex is lethal was tested. For this case a 1:2 segregation ratio was expected, and both 00-266 and 00-08 had low chi-square values for this ratio, 0.09 and 0.01 respectively, with very high probabilities (Table 11).

Table 10. Tetrasomic inheritance frequencies following chromosomal segregation. In italics, normal to dwarf segregation ratios when dwarf is either a simplex or nulliplex. In parenthesis, normal to dwarf segregation ratios when the nulliplex is lethal.

AAAA x aaaa		**AAAa x aaaa**		**AAaa x aaaa**		**Aaaa x aaaa**	
AAaa	1	AAaa	1/2	AAaa	1/6	Aaaa	1/2
		Aaaa	1/2	Aaaa	4/6	aaaa	1/2
				aaaa	1/6		
All normal		*1:1*		*1:5 (1:4)*		*All dwarfs*	
AAAA x Aaaa		**AAAa x Aaaa**		**AAaa x Aaaa**		**Aaaa x Aaaa**	
AAAa	1/2	AAAa	1/4	AAAa	1/12	AAaa	1/4
AAaa	1/2	AAaa	1/2	AAaa	5/12	Aaaa	2/4
		Aaaa	1/4	Aaaa	5/12	aaaa	1/4
				aaaa	1/12		
All normal		*3:1*		*1:1 (6:5)*		*1:3 (1:2)*	
AAAA x AAaa		**AAAa x AAaa**		**AAaa x AAaa**			
AAAA	1/6	AAAA	1/12	AAAA	1/36		
AAAa	4/6	AAAa	5/12	AAAa	8/36		
AAaa	1/6	AAaa	5/12	AAaa	18/36		
		Aaaa	1/12	Aaaa	8/36		
				aaaa	1/36		
All normal		*11:1*		*3:1 (27:8)*			
AAAA x AAAa		**AAAa x AAAa**					
AAAA	1/2	AAAA	1/4				
AAAa	1/2	AAAa	2/4				
		AAaa	1/4				
All normal		*All normal*					

The other two dwarfs studied (03-105 and 03-112) also appeared to be simplex because they segregated normal types when crossed with the putative simplex 00-266 and 00-08. Nevertheless, neither of these dwarf to dwarf crosses followed the expected ratios 1:3 or 1:2 (Table 11). 03-105, when crossed with 00-266 and 00-08, produced 29% and 40% of the expected number of dwarf plants expected for a 1:3 segregation ratio. 03-112 also segregated more normal plants than expected. For each of these three cases, the chi-square statistics clearly indicate that they fit neither a 1:3 ratio nor a 1:2.

Table 11. Segregation ratios and chi-square test for normal to dwarf ratios in dwarf x dwarf controlled crosses.

Pedigree		Normal (N)	Dwarf (D)	N/D	1:3[y]		1:2[z]	
					X^2_1	P. value	X^2_1	P. value
00-266	00-266	30	56	0.54	4.48	0.034	0.09	0.760
00-08	00-08	32	63	0.51	3.82	0.051	0.01	0.942
00-08	03-105	43	51	0.84	73.8	0.000	38.00	0.000
00-266	03-105	50	43	1.16	38.0	0.000	15.70	0.000
03-112	00-08	42	48	0.88	22.5	0.000	7.20	0.007
00-266	00-08	40	54	0.74	18.0	0.000	4.69	0.030

[y] Aaaa x Aaaa
[z] Aaaa x Aaaa when the nulliplex is lethal

The cross between putative simplex plants 00-266 and 00-08 also segregated more than the expected number of normal plants for a 1:3 ratio (three times the expected count for normal phenotypes). The ratio of normal to dwarf did not fit a 1:2 ratio, with $X^2_1 = 4.69$ and a probability of 0.030, so the genotypes of these selections is undetermined.

The crosses between dwarf and normal plants gave ratios that could be more easily explained than the crosses described above. As seen in Table 12, for the crosses of dwarf x normal, all but one cross, 01-21 x 03-112, gave seedlings that fit a 3:1 ratio, which was the expected for a simplex times a triplex

(01-21 was a putative duplex based on the high-density plot ratios, see Table 9). 01-21 x 03-112, was expected to have more dwarfs (i.e. 50% of the total population) than the 39% observed. The chi-square test indicated that the segregation ratio in this cross was a poor fit to a 1:1 segregation (P= 0.025). Possibly, as was speculated before, the nulliplex form is lethal. In this case the expected segregation would be 6:5. The chi-square test for a 6:5 segregation supports this speculation (X^2_1= 1.85) with P=0.174. The contradiction is that 00-266 x 01-21 (a putative simplex times a putative duplex) fit a 3:1 ratio and not the expected 6:5 if indeed the nulliplex is lethal or a 1:1 otherwise.

Table 12. Segregation ratios and chi-square test for normal to dwarf ratios in dwarf x normal controlled crosses.

Pedigree		Normal (N)	Dwarf (D)	N/D	3:1[x]		1:1[y]	
					X^2_1	P. value	X^2_1	P. value
00-266 (D)	01-21 (N)	63	24	2.63	0.31	0.577	17.50	0.000
01-21 (N)	03-112 (D)	59	37	1.59	9.39	0.002	5.04	0.025
Jewel (N)	00-266 (D)	75	19	3.95	1.15	0.284	33.40	0.000
00-266 (D)	Emerald (N)	72	19	3.79	0.82	0.364	30.90	0.000
00-08 (D)	Emerald (N)	78	18	4.33	2.00	0.157	37.50	0.000

[x] Aaaa x AAAa
[y] Aaaa x AAaa

Table 13. Segregation ratios and chi-square test for normal to dwarf ratios in normal x normal controlled crosses.

Pedigree		Normal (N)	Dwarf (D)	N/D	3:1[x]		11:1[y]	
					X^2_1	P. value	X^2_1	P. value
03-120	S. Belle	90	6	15.00	18.00	0.000	0.55	0.460
03-54	Santa Fe	90	5	18.00	19.70	0.000	1.17	0.279
03-73	Jewel	86	10	8.60	10.90	0.001	0.55	0.460
Emerald	Sapphire	83	12	6.92	7.75	0.005	2.30	0.130

[x] AAaa x AAaa
[y] AAAa x AAaa

In the normal x normal crosses (see Table 13), some dwarfs were observed. Since the genotype of ‘Jewel’ and ‘Emerald’ is triplex, the genotype of

the plants crossed with them can be determined. In the case of 03-73 crossed with the triplex 'Jewel', the progeny fit an 11:1 ratio, indicating that 03-73 is duplex. 'Sapphire' is also duplex because it also fits an 11:1 ratio when crossed with Emerald. The cross 03-120 x 'Southern Belle' also followed an 11:1 ratio, 'Southern Belle' being the duplex and 03-120 the triplex.

Table 14. Cross table of all the controlled crosses per genotype evaluated in this study.

Female	Male			
	Aaaa	AAaa	AAAa	AA_ _
Aaaa	00-266 x 00-266 00-08 x 00-08 00-08 x 03-105 00-266 x 03-105 03-112 x 00-08 00-266 x 00-08	00-266 x 01-21	00-266 x Emerald 00-08 x Emerald	
AAaa	01-21 x 03-112		03-73 x Jewel	
AAAa	Jewel x 00-266	Emerald x Sapphire 03-120 x S.Belle		
AA_ _				03-54 x Santa Fe

The results found have a few contradictions, in that some of the crosses among the putative simplex dwarfs did not follow the expected ratio (Table 11), and the dwarf x normal cross 00-266 x 01-21 did not follow the expected 6:5 ratio (Table 12). For the cross 01-21 with the dwarf 03-112, the expected 6:5 ratio was obtained.

It appears that the nulliplex is indeed lethal, but this could not be definitively proved because of the aforementioned contradictions. It also appears likely that the duplex form has a normal phenotype; the dwarf phenotypes observed are simplex.

Draper et al. (1984) and Garvey and Lyrene (1987) mentioned in their work that the inheritance of dwarfism was complex, and they attributed its complexity to multiple genes. The analysis of the data presented in this research suggests a simpler inheritance. For most of the cases it supports monogenic inheritance with tetrasomic segregation. The few contradictions are probably due to heterozygosity at other loci, and to the fact that the blueberry germplasm studied was the product of several interspecific crosses that involved lowbush blueberries, *V. darrowi* (the most likely source for the dwarf genes). The possibility of aneuploidy as a cause for dwarfness has been mentioned in the literature, but in the case of the controlled crosses involving the dwarfs: 00-08, 00-266, 03-112 and 03-105, the fertility was normal, suggesting euploidy.

CHAPTER 5
CONCLUSIONS

Internode length, leaf area and plant height were significantly different between dwarf and normal plants. The average internode length of normal plants was 1.7 times longer than that of dwarf plants. Leaf area was smaller for dwarfs when compared to normal (37.6% the area of a normal plant). When seedlings were three years old, the height of normal plants was 2.5 times taller than the height of dwarf plants.

Dwarf plants were characterized by their high number of branches and low stature. The probability of a dwarf plant from a six month old population is 99.4% for plants with more than 5 branches and height less than 16 cm. Conversely, the probability of a dwarf plant is 0.9% for plants with fewer than three branches and height exceeding 16 cm.

The fertility of the dwarf plants studied in controlled crosses (clones 00-266, 00-08, 03-112 and 03-105) was normal, indicating that aneuploidy was not the reason for the dwarf phenotype.

The genotype of the dwarf plants appears to be simplex (Aaaa), with the nulliplex genotype being lethal. Normal plants that segregated dwarfs when crossed are either triplex (AAAa) or duplex (AAaa). Triplex crossed to duplex and the reciprocal give 11:1 normal to dwarf ratio. Duplex crossed to duplex gives a 27:8 normal to dwarf ratio.

APPENDIX
CATEGORICAL DATA ANALYSIS

Categorical data is a type of data that is measured on a scale that consists of a set of categories (i.e. small, medium or large size of clothing; dwarf or normal height) where only one category applies to each subject.

There could be nominal and ordinal categorical variables. Nominal variables refer to those variables that have unordered scales like race (black, white, hispanic, other) and party affiliation (republican, democrat, independent, other), where the order of listing the categories is irrelevant. Ordinal variables refer to those variables that have order like size of clothing (small, medium, large) and height (short, intermediate and tall), and the statistical analysis should depend on that order. Further, statistical methods designed for ordinal variables cannot be used for nominal variables, whereas statistical methods for nominal variables can be used for ordinal variables, but the information about the order is not used, resulting in the loss of power of the test.

Chi-square Test

In any breeding program, especially after crosses have been made, the understanding of the genetic inheritance of particular characters is important and in some cases necessary to the success of the program.

Inheritance ratios as between dwarf and normal plants can be tested for an inheritance hypothesis by chi-square (X^2) statistics as proposed by Karl Pearson

in 1900. The object of this test is to see if the observed ratios correspond to the expected or hypothesized ones (Watts, 1980).

In a multinomial experiment in which each trial can result in one of *k* outcomes, the expected number of outcomes of type *i* in *n* trials is $n\Pi_i$ where Π_i is the probability that a single trial results in outcome *i* (Ott and Longnecker, 2001).

As proposed by Karl Pearson in 1900, the following test statistic can be used to test the specified probabilities: $X^2 = \Sigma [(n_i - E_i)^2 / E_i]$, where n_i represents the number of trials resulting in outcome *i* and E_i represents the number of trials expected to result in outcome *i* when the hypothesized probabilities represent the actual probabilities assigned to each outcome (Ott and Longnecker, 2001).

If the hypothesized probabilities are correct, the observed cell counts n_i should not deviate greatly from the expected cell count E_i, and the computed value of X^2 should be small. Conversely, when one or more of the hypothesized cell probabilities are incorrect, X^2 should be large.

The distribution of the X^2 value can be approximated by a chi-square distribution provided that the expected cell counts E_i are fairly large. Cochran (1954) indicates that the approximation should be adequate if no E_i is less than 1, and at least 80% of all the E_is are greater than five. Ideally all E_is should be greater than 5.

The chi-square goodness-of-fit test based on *k* specified cell probabilities will have *k*-1 degrees of freedom (*df*). For inheritance studies lexica, *df* equals the number of observed categories minus one, and the formula for calculating the chi-square value is: $X^2 = \Sigma [(\text{observed ratio} - \text{expected})^2 / \text{expected}]$

Logistic Regression Analysis - a summary of Agresti's book on categorical data analysis (1996).

Logistic regression models are a type of General Linear Model (GLM) used to analyze categorical data when the response variable has only two categories (binary response). Thus, its distribution is specified by probabilities of success P(Y=1) and of failure P(Y=0) with binomial distribution.

Because the relation between the probability of success and the observations are usually nonlinear, logistic models are better than linear models. For instance, a fixed change in independent variable X may have less impact when the probability of success is near 0 or 1 than when it is near the middle; a good example would be the probability of doubling the yield when adding *x* amount of fertilizer in different fertility-type soils (rich, medium, poor). The rich soils will have a very low probability, close to zero, because the nutrients available in the soil might be already maximizing the yield potential, thus the fertilizer is not necessary. The poor soils will have high probabilities, close to one, because the fertilizer amendment will significantly improve the nutrients available for sustaining double the yield. The steeper change will occur in soils of medium fertility (the transition between non-likely to respond and always responding) because their response is more variable, less uniform with some observations that will double the yield and some that will not. Overall, if the data are plotted with the fertility-type soil categories (rich, medium, poor) on the x-axis, and the probability of success on the y-axis, the curve will have an "S" shape.

The most important function having this S-shaped curve has the model form: log [P(Y=1)/P(Y=0)] = α + β*x*, where [P(Y=1)/P(Y=0)] is the odds of the response. In this study, the odds of a plant being dwarf.

This function is called the logistic regression function. The random component for the determination of success or failure is binomial. The link function is the logit transformation log [P(Y=1)/P(Y=0)], symbolized by logit P(Y=1). The logit is the natural parameter of the binomial distribution, and because of this it is a canonical link.

To determine if a model is fitting the data set well, goodness-of-fit statistics and residual analysis are useful. When the fitted values are relatively large (exceeding 5) and the number of settings (categories) is fixed, Pearson (X^2) and likelihood-ratio (G^2) goodness-of-fit statistics have approximate chi-squared distributions, and the *df* equals the number of response counts minus the number of model parameters.

The deviance is the likelihood-ratio statistic for comparing model *M* to the saturated model; it is the statistic for testing the hypothesis that all parameters that are in the saturated model but not in model *M* equal zero. For the logit transformation it has the same form as the G^2 likelihood-ratio goodness-of fit statistic for model *M*.

For two models, where M_0 is a special case of M_1, given that the more complex model holds, the likelihood-ratio statistic for testing that the simpler model holds is: $Deviance_0 - Deviance_1$. For larger samples, this is approximately a chi-squared statistic, with *df* equal to the difference between the residual *df*

values for the separate models. This test works well for comparing two models, even when the overall goodness-of-fit test is poor for each model.

In multiple logistic regressions, the significance of a predictor can be tested by the difference in deviance of the model with the predictor and a simpler model without the predictor, this way the effect of predictors and their interactions can be tested to determine if they are significant or if they shouldn't be in the model.

A backward elimination consists in testing different models by the previous method, starting from the most complex model with all the interactions and moving backward to the simplest model possible.

Once the simplest model has been determined, various interpretations can be obtained from it. The odds and probabilities are very useful to study and analyze the data; also confidence intervals (CI) for the odds, and comparisons among the odds of the two categories, i.e. the odds of a dwarf plant vs. the odds of a normal plant, can show trends and enhance the study of the data. For detailed information on how to interpret logistic regressions the book on categorical data from Agresti (1996) is recommended.

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BIOGRAPHICAL SKETCH

David Humberto Baquerizo, born in Guayaquil, Ecuador, in 1978, is the oldest son of Tito and Meche Baquerizo – the parents of six children. From a young age he was delighted by nature. His grandparents “Maruja” and Angel Zambrano, a humble couple from the small town of Manta with a love for agriculture and farm-life, taught him to appreciate simple rural living.

David learned to see nature as a sign of God’s love towards men as he was taught by the Salesians and Jesuits at the schools he attended. This inspired David to venture into studying horticulture.

In 1996 he finished high school at Colegio Javier and moved to Costa Rica to study agriculture at Escuela de Agricultura de la Región Tropical Húmeda (EARTH College) located in the heart of the humid tropics, in Limon province. He graduated with honors in December 1999 after four years of living among howler monkeys and eating “gallo pinto.” He returned to Ecuador with his wife Karen and newborn son David Manuel.

In August 2000, the Baquerizo family emigrated to the US in search of economic stability, because Ecuador was in the middle of a depression. David worked in South Florida in a horticultural related business until August 2002 when he started graduate studies at the University of Florida under the guidance of Dr. Paul Lyrene. Now with four children, the Baquerizo family has become Gator fans and enjoyed living in the beautiful city of Gainesville.