

Waxes and Other Surface Characteristics of Fruit and Leaves of Native *Vaccinium ellioti* Chapm.¹

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Abstract. Fruit color of selections of native *V. ellioti* Chapm. varied from black to blue. Wax on fruit was occasionally found to be globular in form but most often waxes were found to range from flat plates to upright platelets and from horizontal rods to long upright rods. Combinations of wax forms occurred in varying densities on the fruit of native selections. Upright rods and platelets accounted for the blue fruit color. Mature black fruit usually had a limited amount of platelet wax and no rodlet structures. β -Diketones were absent or nearly absent on these mature black fruit. Black fruit had some of the higher weight loss measurements. The paraffin content of the fruit wax was relatively high at one location for some black selections and appeared to reduce weight losses of these selections to levels similar to the bluer selections. Leaves had only globular wax forms, less wax than fruit, and considerable variation in the density of acerate trichomes. Leaf hair density and distribution on leaves was independent of variation in wax forms on fruit.

Surface waxes of fruit, including blueberries, are important because they affect fruit color (3, 12) and retard fruit desiccation and deterioration (1, 3, 13). Were it not for upright surface wax forms (wax bloom), most dark colored fruit would appear black (3, 12). This is important for fruit with characteristic bloom colors such as blue blueberries and some plum cultivars. Since there is much genetic variability for wax formation (1, 4, 7, 9, 10, 14, 15) and this may influence keeping quality and consumer preference, fruit breeders should not ignore wax genetics in their breeding programs.

Seven species of native blueberries are abundant and widespread in Florida, and all are being considered for use in breeding blueberries adapted to the southeastern United States. Fruit color, blue to black, varies among and within 6 of the species. In Florida all *Vaccinium arboreum* Marsh fruit appear to be black. *V. myrsinites* Lam. and *V. fuscatum* Ait. usually produce black fruit, though fruit from some plants are slightly glaucous. *V. ashei* Reade, *V. ellioti* Chapm., and *V. stamineum* L. each encompass great plant-to-plant variation in fruit color. *V. darrowi* Camp is probably the most consistently blue-fruited of the 7 species, though fruit are nearly or entirely black on some bushes.

V. ellioti, the species chosen for this initial study, is native from southeastern Virginia south to Florida and west to Louisiana and Arkansas (5). Observed characteristics of this species in the wild potentially valuable in breeding include tolerance for clay and dry sandy soils, early ripening fruit, disease resistance, longevity, and a low chilling requirement. Throughout their native range in Florida, plants of *V. ellioti* are highly variable in fruit color with fruit color ranging from black to blue. In this study, we examined the waxes on fruits of native *V. ellioti* plants that varied from the darkest to the lightest-colored fruit forms of the species and compared weight loss to fruit color and wax types. Our objectives were to see how fruit color was related to types and quantities of surface waxes and if black fruits dehydrated more rapidly than blue ones. Lower leaf surfaces also were examined for surface structure and wax forms. The

goal of this work was to determine if some leaf and fruit characteristics such as hairs and wax varied sufficiently within the wild population to justify their consideration as important genetic characteristics that would segregate in future breeding work.

Materials and Methods

Leaves and fruit from native *V. ellioti* plants were collected on May 8, 1978, at 3 sites (Rock Church, New Hope, Graham South) along the Santa Fe River, about 24 km north of Gainesville, Florida. The sites were 8 to 16 km apart. At each site, 4 to 5 plants were selected for sampling on the basis of differences in fruit color and the presence of 50 or more mature fruit on the bush. Ten mature fruit per bush were collected for weight loss measurement. Surface areas were calculated from length-width measurements and formulas for surface areas of appropriately shaped spheroids. Picking scars were covered with Duco cement to prevent water loss through the scar area. Weight loss was measured at room temperature and humidity (about 23°C and 60% relative humidity) over a 48 hr period and calculated as $\mu\text{g}/\text{mm}^2$ per 48 hr.

Leaves from several twigs, 30 to 50 mature fruit, and 10 to 15 immature fruit were sampled for scanning electron microscopy (SEM) and wax analysis. For SEM, sections (3.5 × 5 mm) were cut from 10 leaves and 5 to 10 mature and immature fruit surfaces. These sections were fastened to plastic Petri dishes using a smear of citrus oil (1). After drying in a desiccator, 2 × 3 mm sections were mounted on aluminum stubs using silver conductive glue or "Avery" self-adhesive paper tacks. The prepared stubs were coated with 100 Å of gold-palladium (60:40) on a "Technics" sputter coater and observed on a JEOL JSM-35 Scanning Electron Microscope operated at 10 or 15 kV and 80 to 100 μA current. The remaining fruit in each sample were extracted for surface wax by totally immersing and agitating in 100 ml chloroform (55 to 58°C) for 1 min. The chloroform-wax solution was filtered, reduced to dryness with a rotary evaporator, and oven dried overnight at 35°C in a preweighed flask. The dried waxes were made up to 1% solutions in chloroform and stored in a freezer for subsequent chromatographic analyses. Additional leaf and fruit wax samples were prepared from a black and blue-fruited selection at the New Hope site. Leaves (800 to 900) and all available fruit (68 to 230) were extracted from the black and blue-fruited plants in 500 ml of solvent.

Thin layer chromatography (TLC) was used to fractionate the epicuticular waxes into their constituent classes. Replicate 5 μl origin spots of 1% wax solution on 250 μm silica gel G plates were developed using benzene-acetic acid (99:1) as the

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TLC solvent. Separated spots were detected by spraying with 0.05% aqueous Rhodamine-6G and observing under both long and short wavelengths of UV light. These spots were identified using Rf values and chromatography with standards (6). Ultra-violet spectral absorption was used to confirm the presence of β -diketones (6).

Results and Discussion

Observation by SEM of lower leaf surfaces from all the selected plants revealed acerate leaf hairs (11). Varying densities over the main veins occurred. None to frequent occurrence was observed over the stomated blade surface of the various selections (Fig. 1). The epicuticular wax on the abaxial leaf surfaces were relatively formless with occasional globular forms (Fig. 1). The relative densities of leaf hairs and globular wax are presented in Table 1. The lack of any rodlet wax, particularly in the stomatal antechambers, might result in lower stomatal resistances to water loss during the dry spring period. *Vaccinium ashei* has rodlet wax occlusions in the stomatal pores that

appear to influence stomatal resistance (2, 6). On the other hand, native plants of *V. ellioti* with high densities of trichomes on the stomated leaf surface would have higher boundary layer resistances which would lower their transpiration rates.

With a few exceptions, fruit from the selected plants had prominent wax forms when viewed by SEM (Fig. 2). These were usually mixtures and included globules, flat plates, upright platelets, horizontal rodlets, and vertical rodlets. The wax forms on each selected plant are summarized in Table 1. No selection was considered to consistently have globular wax forms. The variation in wax form within the single species *V. ellioti* in these native stands appeared to be analogous to and perhaps greater than those reported among the cultivated *Avena* species (4). This suggests that considerable genetic variability exists in wax formation since different wax forms are related to different wax chemical groups (8, 15).

Fruit color, the basis for selecting the plants, was related to wax form (Table 1). Mature black fruit had no rodlet wax. Medium black fruit usually had less rodlet wax than blue fruit.

Table 1. Summary of leaf and fruit surface characteristics as viewed by SEM, relative presence of paraffin and β -diketone wax classes on mature fruit, and weight loss of mature fruit of wild selections of *V. ellioti*.

Sampling location	Mature fruit color	Leaf surface ^z			Young fruit ^z				Mature fruit ^z				Wax classes ^y		Weight loss ^x µg/mm ² per 48 hr	
		Main vein	Blade	Wax pebbles	Plates and Platelets		Rodlets		Plates Platelets		Rodlets		Relative presence			
					Flat	Upright	Flat	Upright	Flat	Upright	Flat	Upright	Paraffins	β -diketones		
Rock Church	Black	+++	+	-	-	++	-	-	-	++	-	-	++	-	3.7 b	
	Med. Black	+++	+	++	-	+++	+	(long)	-	+++	-	++	++	+	2.8 b	
								+++ (short)								
	Med. Blue	++	-	+	-	+	+++ (fused rodlets)	++ (short)	+++	++	-	++ (short)	+	++	3.4 ab	
	Blue	++	+	+	-	+++ (fused rodlets)	-	+++ (short)	-	+++	-	+++ (short)	++	++	3.1 ab	
New Hope	Black	++	-	+	+	-	-	-	-	++	-	-	+	-	4.2 b	
	Med. Black	++	++	-	+	-	++	-	+++	+++	-	-	+	trace	2.9 a	
	Med. Black	++	+	++	-	+++	-	+++ (short)	+++	++	-	-	+	+	2.3 a	
	Med. Blue	+++	++	++	-	+++	+++	+++ (short)	+++	+++	+++	-	++	++	3.8 b	
	Blue	+++	++	++	++	+	+++	-	++	+++	-	+++ (short)	++	++	2.5 a	
Graham South	Black 1	ND	ND	ND	++	+	-	++ (short)	-	+++ (small)	-	-	++	-	3.6 b	
	Black 2	+++	++	+	-	++	-	-	+++	++ (small)	-	-	+++	-	3.3 ab	
	Med. Black	++	+	++	+++ (fused rodlets)	+	-	+++	+++	+	-	+++	+	+	3.8 b	
	Med. Blue	+++	+	++	++	++	++	++ (short)	+	+++ (fused rodlets)	++	-	+++ (short)	+	+	3.1 a
	Blue	+++	++	+	+++	++	+++	++	+++	+++	+++	+++	++	+++	3.5 ab	

^zUltrastructure by SEM
- Absent
+ Some present
++ Easily observed
+++ Profuse

^yWax TLC
- Absent
+ Trace to light spot
++ Moderate spot
+++ Dark, large spot

^xWeight loss statistical comparison are for each location, significant at 5% level if followed by different letter.

^wND indicates no data

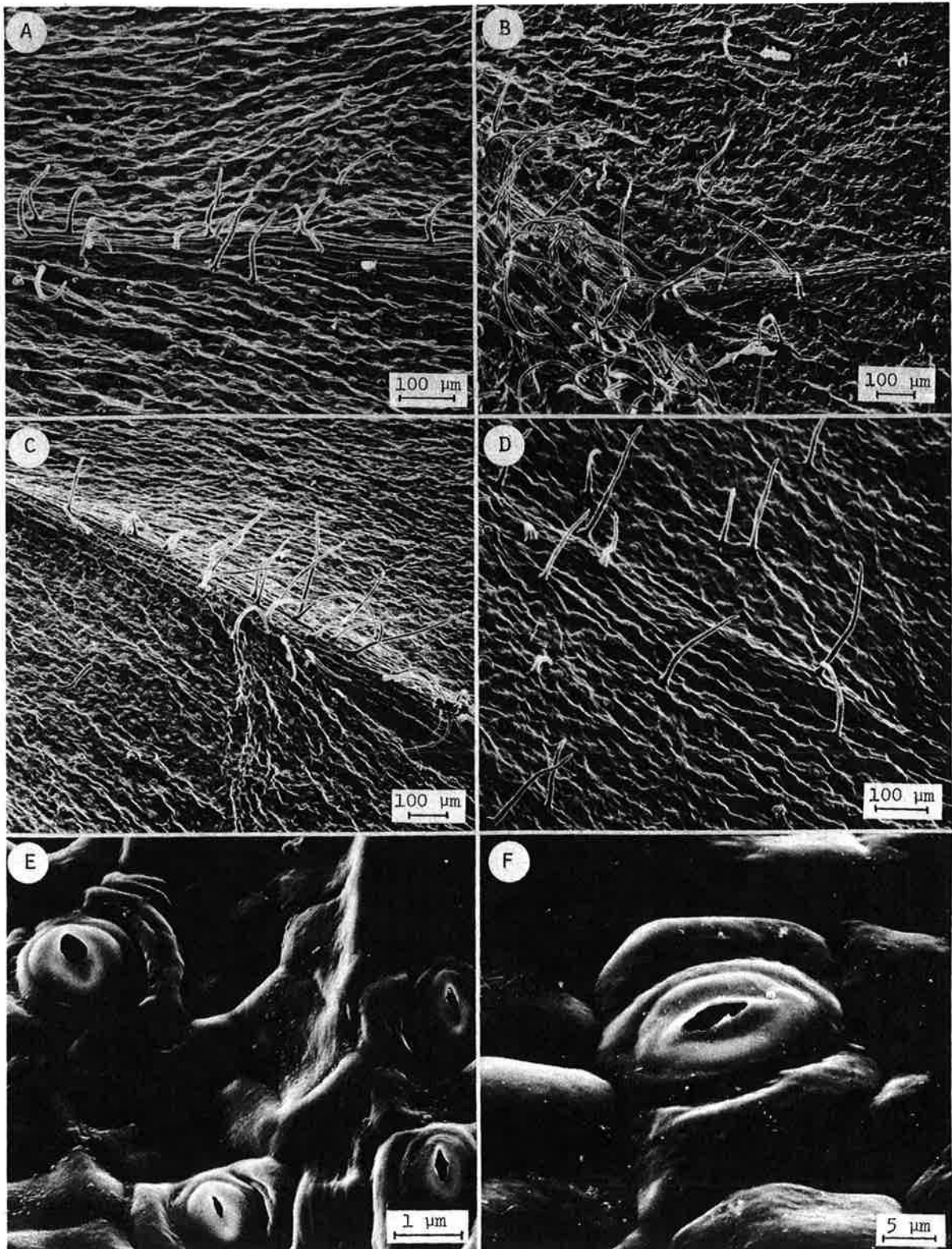


Fig. 1. Characteristics observed on lower (abaxial) leaf surfaces of selected wild plants of *V. ellioti* from 3 sites along the Santa Fe River, Florida. Variations in leaf hair densities were observed over the main veins, low (A) to high (B) densities, and over the stomatal surface, none (A, C), few (B), and relatively many (D). Except for a few pebbles, little wax structure was observed over these abaxial surfaces (E, F).

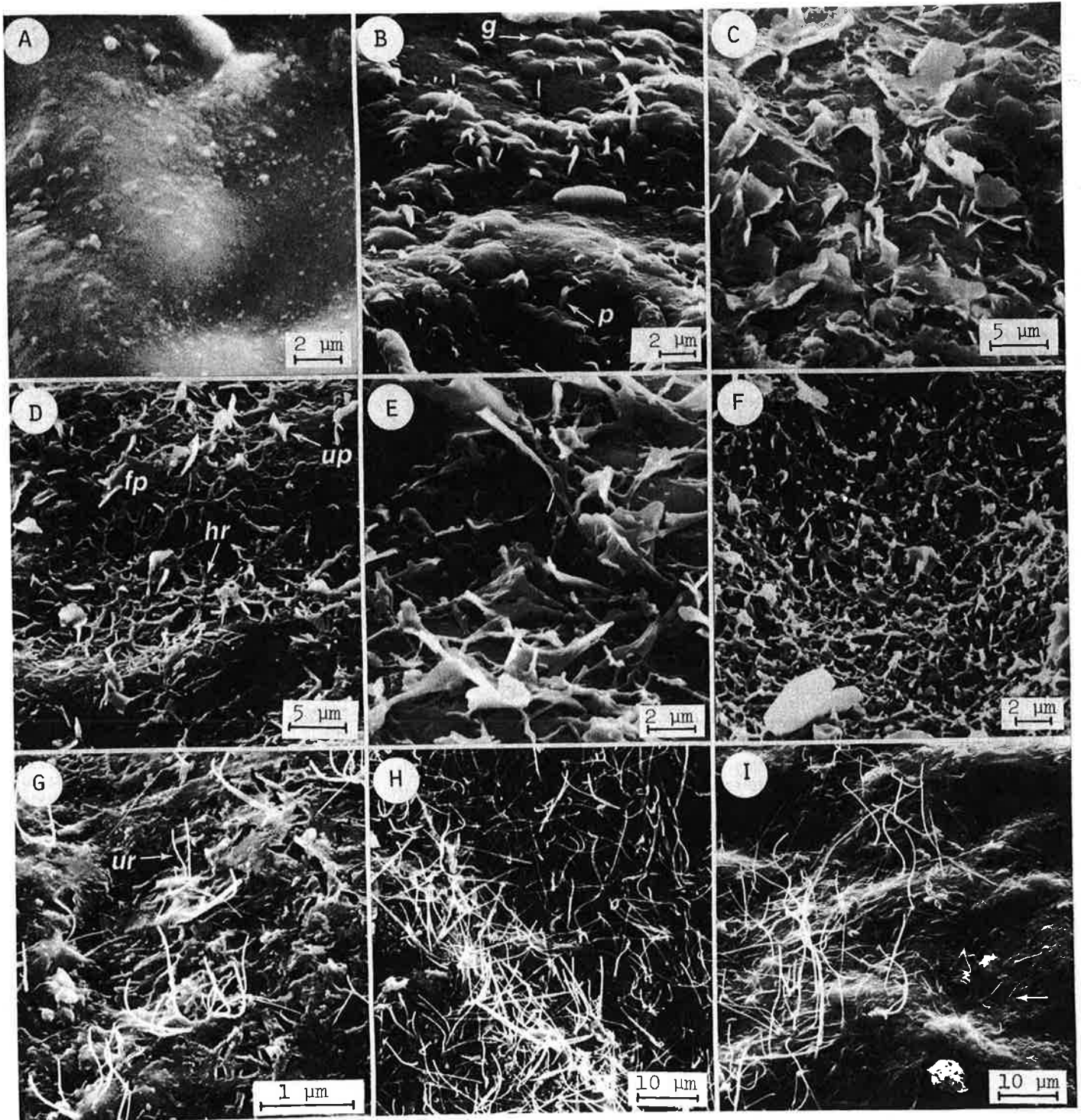


Fig. 2. Surface wax form variation on fruit of *V. elliotii* from selected wild plants at 3 sites along the Santa Fe River, Florida. Surfaces with (A) little wax structure, (B) globular (g) and small platelet mixture (p), and (C, D, E) variations in large upright platelets were observed. Surfaces (D) with horizontal rodlets (hr) with flat (fp) and upright (up) plates (E, F), variations in upright platelets and (G, H, I) long upright rods (ur) occurred. A mature fruit surface is shown in (I) with part of rodlet wax weathered (arrow).

In cases where this was not true, the upright platelet forms were infrequent (Table 1, Graham South). Immature fruit had rodlet wax more frequently than the corresponding mature fruit. These waxes may weather as the fruit matures (6). A weathered area is shown in Fig. 2-1. Rodlet structure and β -diketone wax content were shown to disappear as fruit and leaves matured in *V. ashei* (6). In the present study, platelet wax forms as well as rodlets appeared to contribute to the glaucous effect on those selections having the bluest color.

β -Diketones are the primary wax class responsible for rodlet or tube wax forms (15, 16). These were absent or present in very low amounts on fruit without rodlet structures (Table 1). β -Diketones must be present in relatively high concentration for rodlet formation to occur (16). The TLC plates also revealed variation in the quantities of paraffins on the fruit surfaces of the various selections (Table 1). Black fruit from each site tended to have some of the higher weight loss values (Table 1). The paraffin content of the fruit wax was relatively high for black selections at Graham South and appeared to reduce their weight losses to approx the same levels found for the bluer-fruited selections from that location.

When waxes on most of the leaves and all of the fruit of the black and the blue-fruited plants from the New Hope site were analyzed further, total wax was found to be higher on the leaves and fruit of the blue selection (Table 2). There was more wax per unit surface area on the fruit than on the leaves. Weight loss was lower for the fruit with the higher wax concentration. Total wax content has been shown to be important in the control of cuticular water loss (1, 3, 13), and is usually more important than a single chemical class of waxes.

Separation by TLC of the leaf and fruit waxes from these 2 plants at the New Hope site revealed the absence of β -diketones in the leaf waxes of both plants and from the fruit waxes of the black-fruited plant (Fig. 3). The leaf waxes of both plants contained secondary alcohols which were absent or in trace amounts (Fig. 3B, D) in the fruit waxes. Leaves and fruit of both plants contained primary alcohols, but the fruit waxes contained the most primary alcohols. High concentrations of primary alcohols contribute to upright platelet formation (8).

Wax form, as rodlet and upright platelets, contributed to the glaucous effect (12) that resulted in blue fruit on some native *V. elliotii* plants. These wax forms were related to wax chemistry, and increasing quantities of the waxes led to better control of water loss from the fruit. Better weight loss control was not always associated with the bluer colored fruit. This was partially because the variation in paraffin wax forms do not contribute to wax bloom (12) but do contribute significantly to prevention of water loss (13). For commercial conditions the picking scar characteristics would be a very important horticultural characteristic for water loss and decay control. The picking scars were not a factor in this study since they were coated with plastic for the weight loss studies.

Wax production in the leaves was different from the fruit. This variation between plant parts occurs in other plants (14). Leaf hair distribution or frequency on the leaves did not appear to be associated with any variation in wax form on the leaves

Table 2. Total wax content on leaves and fruit of 2 wild type *V. elliotii* plants from the New Hope area on the Santa Fe River, Florida.

Plant selected	Total wax		Fruit weight loss ^y μg/mm ² per 48 hr
	Leaves ^z μg/cm ²	Fruit ^z μg/cm ²	
Black fruit	44.4	120.0	4.2b
Blue fruit	58.2	171.4	2.5a

^zThese values represent wax from most of the leaves and all of the fruit on these single plant populations.

^ySignificantly different at the 5% level.

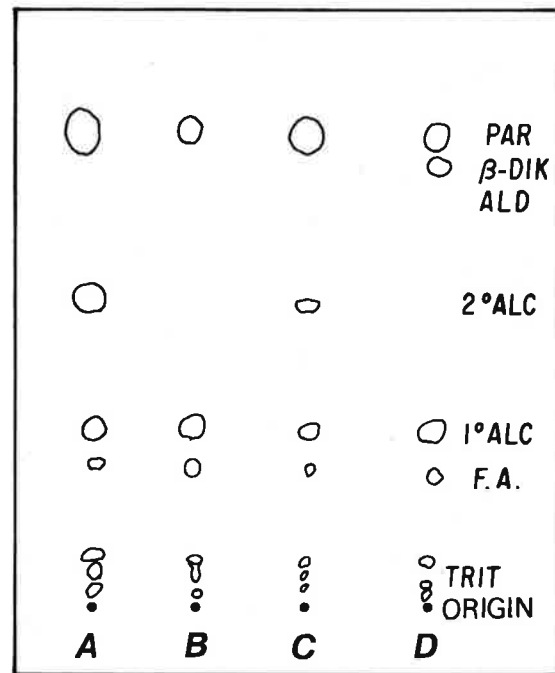


Fig. 3. Reproduction of thin-layer chromatography (TLC) plate showing wax classes present from leaves (A, C) and fruit (B, D) of a black-fruited (A, B) and a blue-fruited (C, D) plant of *V. elliotii*. β -diketones (β -DIK) were absent from the leaves of the black-fruited (A) and the blue-fruited (C) plant and the fruit of the black-fruited selection (B) but were present in the wax of the blue fruit (D). Secondary alcohols (2° ALC) were absent or only a trace in the fruit wax. Paraffin (PAR) contents differed also. Other chemical classes included aldehydes (ALD), primary alcohols (1° ALC), fatty acids (F.A.), and triterpenoids (TRIT).

nor any variation in wax on the fruit (Table 1).

Since wax form affected both fruit color and weight loss, it should be an important consideration in any breeding program. Although some of the differences in color intensity may be due to variations in internal concentration of pigments, wax bloom was primarily responsible for blue to black variation. The presence of several fruit color classes at each site and variability in 2 or more wax classes suggests that multiple gene control, similar to that reported for *Brassica* (10), may be involved in wax metabolism in *V. elliotii*.

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Maturation Temperature Influences on Seed Quality and Resistance to Mechanical Injury of Some Snap Bean Genotypes¹

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Abstract. Of 10 genotypes of bean (*Phaseolus vulgaris* L.) studied, all produced better quality seeds at low maturation temperatures. Resistance to mechanical injury was also maximal in low temperature matured seeds. In general, the colored seeded genotypes unlike the white seeded genotypes, tolerated a wide range of maturation temperatures. However, 'Spartan Arrow', which has colored and large seeds was susceptible to mechanical injury at all maturation temperatures, and the white seeded line 26W showed good tolerance at all temperatures. It appears that it will be possible to breed white-seeded lines showing improved tolerance of high seed maturation temperatures.

Mechanical damage to seed is a serious problem in the bean industry. The source of the problem has been seen as threshing injury and rough postharvest handling (10, 12), low seed moisture content at harvest (1, 13), low Ca and Mg in the cell wall and cotyledons (2), low lignin content and thin seed coat (7) and size and shape of the seed (5). Dickson and Boettger (4) have demonstrated that crossing and selection for resistance to transverse cotyledon cracking and mechanical damage is an effective way to produce lines with resistance to mechanical damage and they developed some breeding lines which are resistant to mechanical damage (3).

Our previous studies have revealed that high maturation temperatures reduce seed quality in the white-seeded 'Apollo' (9). Maturation temperatures above 21°C day/16°C night (12 hr/day) give seed with a low germination percentage, and a high susceptibility to mechanical damage. In the present investigation, the influence of maturation temperature on seed quality and on susceptibility to mechanical damage was tested on 7 mechanical damage resistant breeding lines, and on 3 other non-resistant bean cultivars.

Materials and Methods

White seeded 24W, 25W, 26W and 27W and colored seeded 28C, 30C and 31C F₈ or F₉ bean selections and 'Slimgreen' were received from M. H. Dickson, New York State Agricultural Experiment Station, Geneva. 'Apollo' was obtained from New World Seeds, Sydney and 'Spartan Arrow' from Gordon Smith,

Harrington, N.S.W. The numbers used in previous reports for the breeding lines under investigation are as follows:

No. used in the present report	Referred to in Dickson and Boettger (3, 4) as
24W (white seeded)	539
25W	543
26W	573
27W	571
28C (colored seeded)	534
30C	537
31C	527

The lines 25W, 26W, 27W, 28C and 31C have been reported to be resistant to mechanical damage (3, 4); and 24W and 30C have also been reported to be quite resistant to mechanical damage, transverse cotyledon cracking and seed coat shattering (3). The seeds obtained from different sources were multiplied in a glasshouse at 25°C day/18°C night (12 hr/day).

Plants were grown in 5 liter pots containing a mix of 3 sandy loam soil:1 peat:1 vermiculite:1 sand in a glasshouse at 24°C/19°C. No artificial light was given. The average day lengths in Sydney for the months of September, October, November and December during which the plants were grown, are 11.8, 12.9, 13.9 and 14.4 hr, respectively. We added 763g superphosphate, 572 g sulphate of ammonia, 572 g blood and bone, 477 g sulphate of potash, 667 g hydrated lime, 143 g magnesium carbonate and 49 g Ess-Min-El micronutrient (Lane, Sydney) per m³ of mix. Plants were watered at the roots each second day and fed with Aquasol liquid fertilizer (Hortico, Sydney) 3 weeks after sowing and once during pod development.

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