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The Occurrence of Mesocarpic Stone Cells in the Fruit of Cultivated Highbush Blueberry

R.E. Gough
Department of Plant and Soil Science, University of Rhode Island, Kingston, RI 02881

Additional index words. brachysclerids, blueberry, anatomy, *Vaccinium corymbosum*

Abstract. **Stone cells of highbush blueberry (*Vaccinium corymbosum* L.) were distributed primarily toward the periphery of the fruit; they apparently differentiated from ground parenchyma shortly after anthesis. Secondary cell wall material continued to be accreted through harvest, with lamellations about 1μm in width. The lignified walls were heavily pitted, with pits contiguous with those of adjacent stone cells. The number of stone cells may be correlated positively to the length of the growth season for each cultivar.**

Stone cells, also termed sclereids, occur in the fruit of several species (6). These often are associated with the endocarpic region, but may be found throughout the pericarp. Because of their obvious economic importance, stone cells in the flesh of the pear fruit (*Pyrus communis* L.) have been studied extensively (2, 4, 5). Yarbrough and Morrow (1) reported that both endocarpic and mesocarpic sclereids occur in the fruit of several species of *Vaccinium*, including cultivars of *V. australe* Small. Some species had more or larger stone cells than other species and hence were "grittier" and less palatable. The objective of this study was to examine the ontogeny, composition, and distribution of mesocarpic stone cells in highbush blueberry.

Materials and Methods

Mature 'Earliblue', 'Collins', and 'Coville' plants were maintained on a Narragansett Silt Loam soil with a sawdust mulch at Kingston, R.I. The cultivars were early-, mid-, and late-ripening, respectively. Fruits were harvested at 6 color stages of maturity and ripeness during the 1977 growing season. The stages are as follows: 1) immature green (IG), with the largest berries a dark green color over 100% of their surface, about 8 days after full bloom; 2) mature green (MG), with the berries a light greenish-white color with the calyx just beginning to turn pink, about 28 days after full bloom; 3) green-pink (GP), with the surface coloration of these berries about 75% green and 25% pink; 4) pink-green (PG), with the fruit about 75% pink and 25% green in surface coloration; 5) blue-pink (BP), with the fruit surface about 75% blue and 25% pink; and 6) blue (B), with the berries blue on about 90% of their surface and about 10% pink coloration around the scar.

Ripe fruit were prepared for macroscopic investigation according to Crist and Batjer (2). Longitudinal and transverse freehand sections about 1.5 mm thick were dehydrated through an acetone/water (50%, 75%, 90%) series into acetone (100%) and finally into cedarwood oil. Cleared sections were suspended in glass cells and mounted in Canada balsam. Sections were photographed using Ektachrome film and bottom illumination.

Samples of fruit were fixed in Randolph's Modified Navashin Solution for microscopic investigation, dehydrated with an ethanol/t-butanol series and embedded in Paraplast Plus. Tissue was sectioned at 12 μm and stained in either Heidenhain's iron alum hematoxylin or in safranin O and fast green FCF, and then mounted in Canada balsam. Tissue was stained in 1% aqueous crystal violet, 0.001% aqueous methyl red; or treated with phloroglucinol/HCl, IKI/H^2SO_4, or chloroiodide of zinc according

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to Johansen (3) and Berlyn and Miksche (1) for determination of approximate stone cell composition.

Sections were examined with a Wild M20 Phase Contrast microscope fitted with an eyepiece micrometer. Photos were taken with an AO Microstar microscope equipped with a 545 Polaroid attachment. All counts and measurements were made from at least 20 samples. The mean number of stone cells found in basal regions of blue fruit was calculated by determining the number in the area of a specific microscopic field of view (1540 μm^2).

The full-bloom stage was reached when the first corollas fell. These dates in 1977 for 'Earliblue', 'Collins', and 'Coville' were May 9, May 9, and May 17, respectively.

Results and Discussion

The blueberry fruit in longitudinal cross-section is shown in Fig. 1. The darker epidermal and hypodermal layers (E, H) are delineated from the remainder of the cortex by an outer ring of vascular bundles (V). The mesocarp tissue (M) is relatively homogenous in appearance and has a 2nd and 3rd ring of bundles. The carpels have 10 locules (L) and 5 large, highly lignified placentae (P), to which are attached many seeds (S). The locules, surrounded by a stony endocarp layer, extend into the mesocarp (LP). Stone cells are distributed unevenly throughout the mesocarp, with their highest frequency of distribution in an area ranging from about 1.4 mm beneath the epidermis to the berry surface (Fig. 2). Stone cells are found rarely in the intercarpellary areas and sepallary tissue. They can be found infrequently in the interior to the vascular bundles and nectariferous tissue, and the largest numbers are found exterior to the outer bundles (Fig. 3). All cultivars examined had similar areas of stone cell distribution, though the mean number per 1540 μm^2 varied among cultivars, with 'Earliblue' having the fewest (1.50 ± 1.28) and 'Coville' the most (19.50 ± 12.00). 'Collins' was intermediate with 4.31 ± 3.11.

Stone cell development was similar in all 3 cultivars studied. Therefore, their development in 'Coville' only will be discussed. Certain cells in the area of the future stone cells had prominent nuclei and nucleoli at the immature-green stage. These cells were

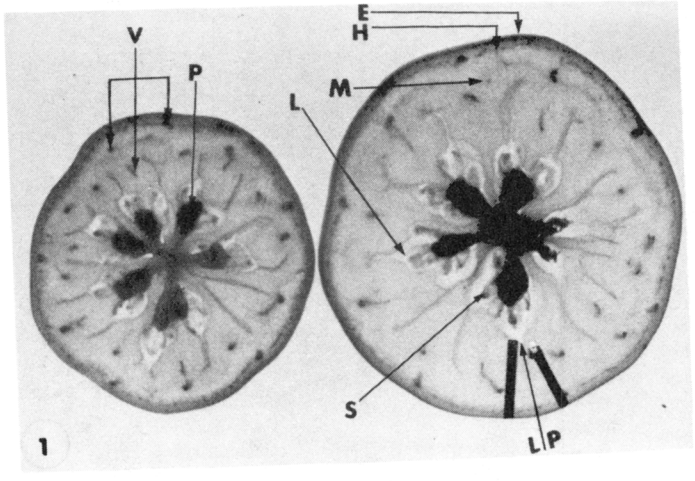

Fig. 1. Transverse section through a blue fruit of 'Collins' blueberry showing the darker epidermal (E) and hypodermal (H) layers, the lighter mesocarp area (M), 3 rings of vascular bundles (V), 5 carpels with 10 locules (L), 5 woody placentae (P), and many seeds (S). Note: the extension of the locules into the mesocarp usually terminate in a rather well-delineated point (LP) ×9.

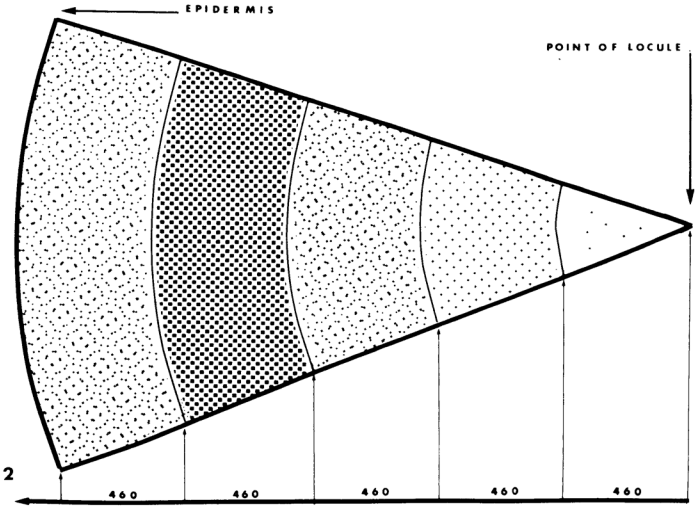

Fig. 2. Diagrammatic representation of the fruit sector in Fig. 1. The degree of shading represents the frequency of stone cell distribution. The numbers along the baseline represent the distance from the locule point in μm. Note that high distribution frequencies occur within the outer 1400 μm of berry tissue, with highest frequency occurring 460 to 920 μm below the berry surface.

primarily uninucleate but on occasion binucleate. Most of them also exhibited granular, parietal cytoplasm and a relatively large, central vacuole. Cell division and enlargement still were occurring in the relatively homogenous cortical parenchyma.

Cell division in the mature-green stage was restricted almost entirely to the hypodermal area of the fruit. The stone cells were prominent and mostly vacuolate, and some intercellular spaces were visible between these and contiguous parenchyma cells. The centripetal accretion of secondary wall material had initiated, thickening the wall to about 6 μm as compared to 1.5 μm for the surrounding parenchyma cells (Fig. 4). The mean stone cell size (80 μm) approximated that of the surrounding parenchyma. Pitting in the stone-cell wall had become clearly visible.

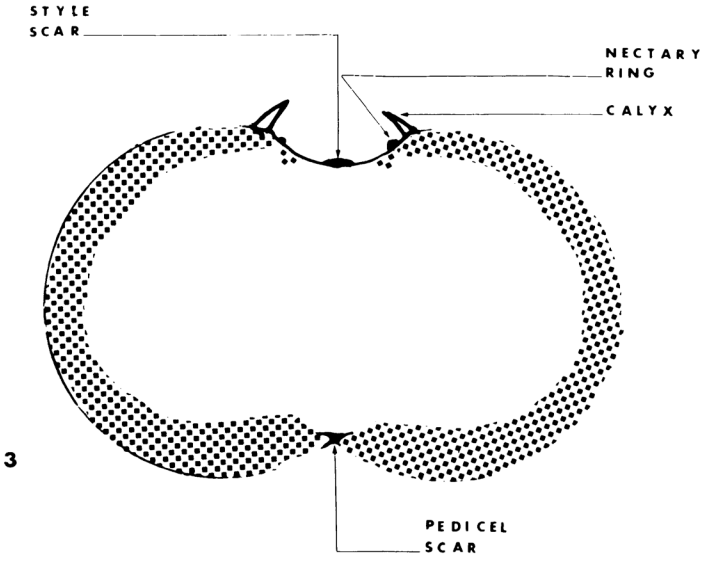

Fig. 3. Diagrammatic representation of a longitudinal cross-section of a blueberry fruit. Shaded area represents stone cell distribution. Note that few stone cells are present within the nectary ring and none in the inner portions of the fruit. The heaviest distribution appears to be in the basal lobes surrounding the pedicel scar.

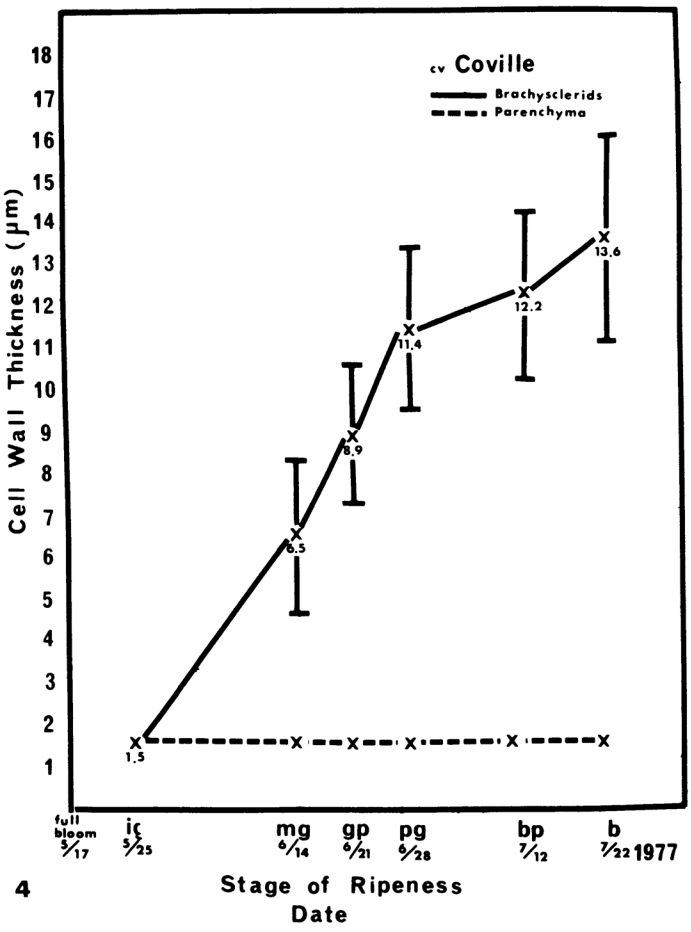

Fig. 4. Development of the mesocarpic stone cell (brachysclereids) in blueberry fruit expressed as increases in cell wall thickness over time. Each plot represents the mean and SD of 20 samples.

In the later stages (GP, PG, BP, B), stone cells remained essentially the same as in the MG stage, except for continuous secondary wall deposition (Fig. 4). This deposition occurred at its most rapid rate between the MG and PG stages and decreased somewhat during the final stages of ripening. It did appear, however, that development of the stone cell was continuous through harvest.

In many cases, the mature stone cell appeared completely vacuolate, though in some cases the lumen contained a brownish, granular substance which appeared to be tanniniferous in nature (Fig. 5).

Mesocarpic stone cells in the blueberry possess a thick, heavily pitted, essentially smooth secondary wall at maturity, composed of several lamellations each about 1 μm in width. The innermost lamellation often is slightly thicker than the rest and appears yellowish in color with a safranin O and fast green FCF stain. Positive staining with methyl red, safranin O, and crystal violet, and a positive test with phloroglucinol-HCl indicate their heavily lignified nature. However, the tests for cellulose employing IKI/H^2SO_4 and chloroiodide of zinc were negative, indicating very little or no cellulose in the stone-cell walls. The wall pits are contiguous with pits in adjacent stone cells or with pit-fields in adjacent parenchyma walls, and are essentially simple in nature. Although some ramiform pitting may occur, however, it is exceedingly difficult to differentiate it from the visual superimpositions of 2 or more simple pits caused by difficulty in resolution.

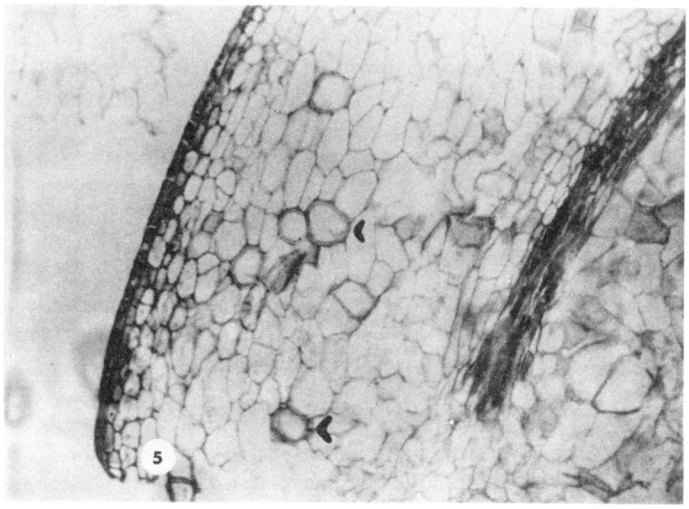

Fig. 5. Mesocarpic stone cells with vacuolate lumen in blue fruit of 'Coville' blueberry (×540).

The stone cells may occur as isolated idioblasts in the berry flesh or in contiguous clusters of 2 or more (Fig. 6). In some cases, cortical parenchyma cells contiguous to a single stone cell form a stellate pattern around that stone cell (Fig. 7). This may be due either to the shrinkage or collapse of the stone cell during maturation as proposed by Crist and Batjer (2) or to the inability of the parenchyma to enlarge to a similar extent in all directions because of attachment of a portion of their wall to the stone cell.

Fruit of the cultivated highbush blueberry contain both endocarpic and mesocarpic stone cells as suggested by Yarbrough and Morrow (7). The mesocarpic stone cells develop in a manner similar to those found in pear fruit (5). In pear, stone cells initially differentiated at random among parenchyma cells soon after anthesis, but never in the epidermis or ovarian tissue. During fruit maturation, the stone cells remained uninucleate while the cytoplasm became increasingly granular. Further, Crist and Batjer (2), also working with pear fruit, reported findings similar

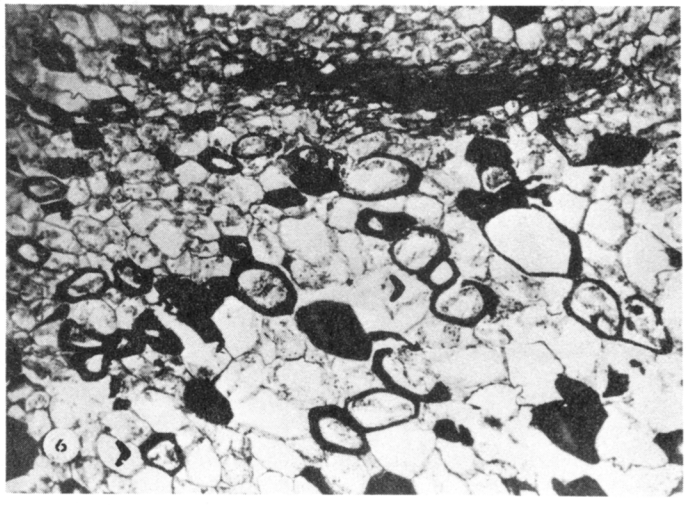

Fig. 6. Mesocarpic stone cells in blue fruit of 'Coville' blueberry may appear as isolated idioblasts or in contiguous clusters of 2 or more (×560).

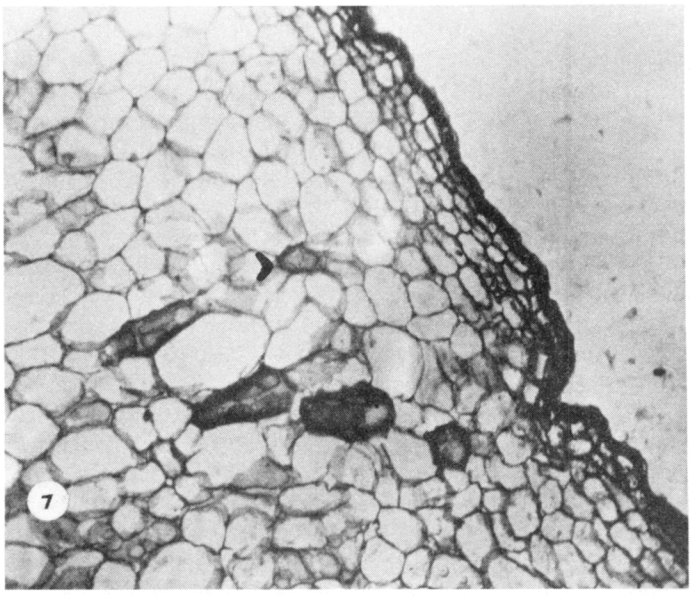

Fig. 7. Stellate pattern of parenchyma surrounding a stone cell in blue fruit of cultivated highbush 'Coville' blueberry (×560).

to those of Sterling (5). They also reported that mesocarpic stone cells in the pear fruit were composed primarily of lignin, and that they accumulated around the carpels and near the fruit periphery. The number of stone cells in the 2 cultivars tested ('Bartlett' and 'Kieffer') was correlated positively with harvest season, i.e., the later the cultivar ripened, the larger the number of stone cells. The above findings are similar to those reported in this study on the cultivated highbush blueberry.

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Postharvest Quality and Storage Life of Grapes as Influenced by Adding Carbon Monoxide to Air or Controlled Atmospheres

E.M. Yahia, K.E. Nelson[1], and A.A. Kader[2]

Department of Pomology, University of California, Davis, CA 95616

Additional index words. *Vitis vinifera*, SO_2 fumigation, decay control, *Botrytis cinerea*, respiration, ethylene production

Abstract. **The effects of 10% carbon monoxide (CO) added to air or controlled atmospheres (2% O_2 with or without 5% CO_2) on quality and storage life of grapes (*Vitis vinifera* L. cv. Thompson Seedless) were compared with those of the conventional SO_2 fumigation treatments for decay control. CO in air reduced respiration and C_2H_4 production rates, and retarded berry browning and softening, but was only partially effective in retarding decay beyond 2 months at 0°C. SO_2 treatments were very effective in controlling the spread of decay, but brown discoloration of the berries increased, especially after 2 months at 0° or 1°. When combined with 2% O_2 with or without 5% CO_2, CO inhibited C_2H_4 production and retarded decay development, but the presence of CO_2 increased brown discoloration of the berries. A combination of 2% O_2 + 10% CO was as effective as SO_2 in controlling decay of grapes held at 0° for up to 4 months and caused less browning and bleaching than SO_2.**

SO_2, although effective in retarding the activity of decay-causing organisms in grapes, including *Botrytis cinerea* Pers., is very corrosive to metals, injurious to most other fresh fruits, and causes injury to grapes if used excessively (4, 5, 10, 11). The optimum SO_2 concentration used in grapes is usually a compromise between that which will provide acceptable control of decay and that which will not cause excessive injury (10).

Controlled-atmosphere (CA) storage has been tested for decay control and extension of the storage life of grapes (13). Uota (16) showed that CA alone did not provide adequate decay control under conditions of relatively high humidity; however, combining CA (5% O_2 and 10% CO_2) with the application of 1000 ppm SO_2 at 7-day intervals gave almost complete decay control. Nassar (8) found that elevated CO_2 concentrations (up to 15%) increased browning of berries and resulted in the accumulation of α-amino butyric and succinic acids and reduction of aspartic and glutamic acids in grapes, but SO_2 fumigation did not change the organic and amino acids examined. Nelson (9) concluded that CA was not very promising for commercial grape storage, since grapes kept at less than 15% CO_2 had higher decay than those subjected to SO_2 fumigation; while 15% CO_2 controlled

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[1]Dept. of Viticulture & Enology.

[2]To whom correspondence should be addressed.