18 *Vaccinium* Species (Small-Fruited Berries): In Vitro Culture and the Production of Food Colorants and Phytochemicals

M.A.L. SMITH[1]

1 Introduction

1.1 Distribution and Importance of the Plant

The genus *Vaccinium*, from the heath family (Ericaceae), includes a wide range of popular berry species of economic importance, including the American cranberry (*V. macrocarpon* Ait.), the wild lowbush blueberry (*V. angustifolium* Ait.), cultivated highbush and rabbiteye blueberries (*V. corymbosum* L. and *V. ashei* Reade), bilberry (*V. myrtillus* L.) and lingonberry (*V. vitis-idaea* L.). While these crops are well known throughout the world, in many cases, their individual distributions are quite narrow (Lyrene and Perry 1988; Scorza and Welker 1988; Kalt and Dufour 1997). Wild lowbush blueberry, for example, is localized in the extreme northeastern United States and maritime provinces of Canada (Fig. 1A,B); bilberry is grown only in a few European countries with an isolated pocket of distribution in the Rocky Mountain region of the USA, and cranberry production, which until recently was confined to the eastern and western coasts of the USA, has recently expanded into higher elevations in South America. The harvested berries are marketed fresh, frozen, and in some cases, sweetened and dried (personal communication, D. Nolte, Decas Cranberry Co.). They are also popular components in bakery items, dried cereals, jams, juices, and numerous related edible products.

Less well-known species of *Vaccinium*, including wild plantings, are limitedly distributed in pockets throughout the world. *V. pahalae* Skottsb., common name ohelo, is typical of this latter category. Ohelo is a creeping shrub which is only known to grow on the main island of Hawaii, in bog-type areas, weathered lava flows, and on mountain slopes. It is expected to hybridize freely with other wild *Vaccinium* species which colonize this precarious environment. The edible berries range from dark red to pale yellow, and are eaten raw or used in baking. This particular plant is the focus of a rich island folklore, and was considered to be sacred to the volcano goddess Pélé. In fact, a legend states that ohelo berries were customarily thrown into the center of volcanic

[1]University of Illinois, Department of Natural Resources and Environmental Sciences, Urbana, Illinois 61801, USA

Biotechnology in Agriculture and Forestry, Vol. 51
Medicinal and Aromatic Plants XII (ed. by T. Nagata and Y. Ebizuka)

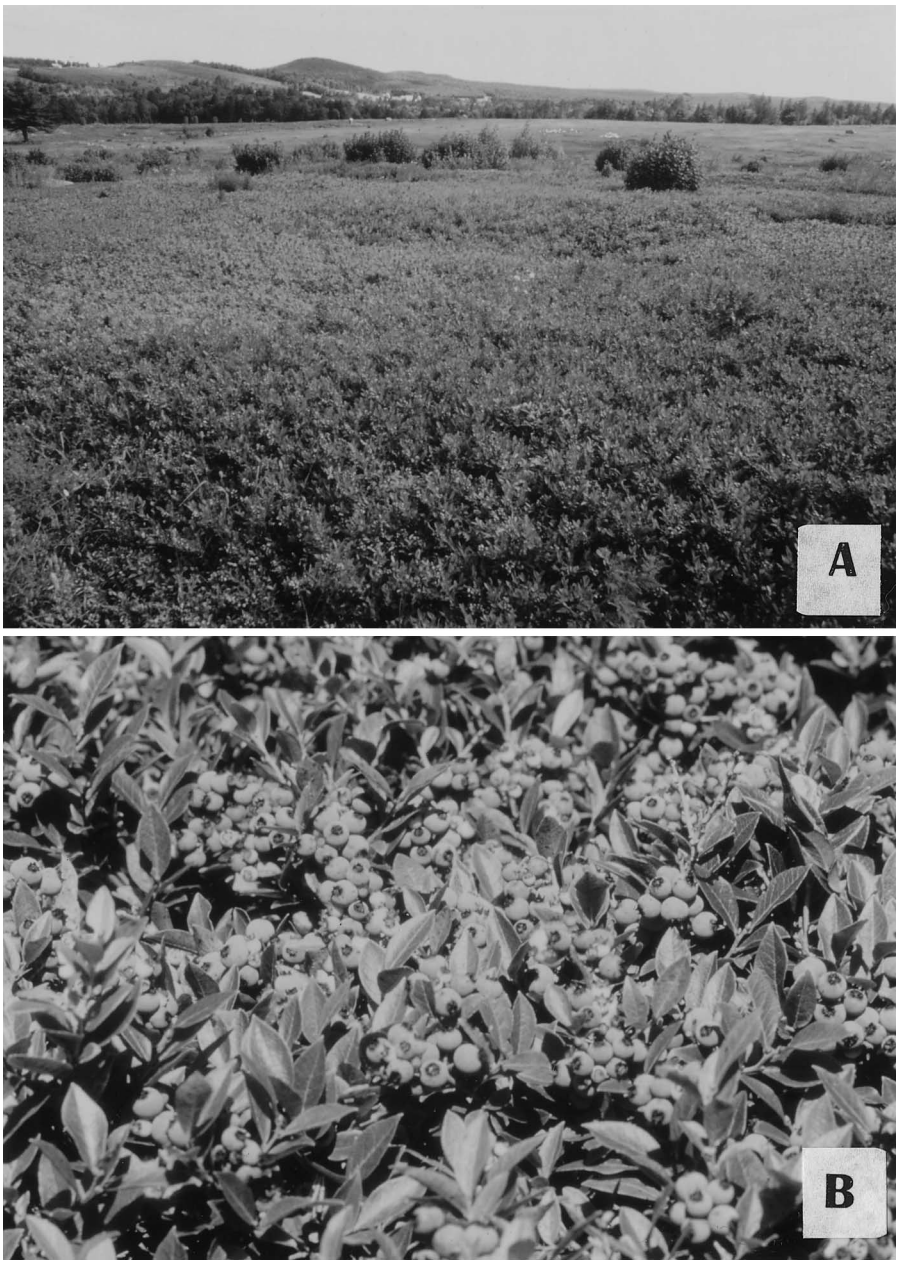


Fig. 1. Wild blueberry (*Vaccinium angustifolium*), one of the prominent members of the genus with known bioactive properties. **A** The low-growing crop spreads over acreages in the maritime provinces of Canada, and the extreme northeastern USA. **B** Prolific berries are available for harvest within a narrow (2–3 week) window of time in the late summer

activity to allay the goddess's wrath; only after this offering was it considered safe to eat the berries (Degener 1984).

1.2 Production of Bioactive Compounds in *Vaccinium* Fruits

Members of the genus *Vaccinium* are primarily grown as a source of edible fruit, but also as landscape ornamentals, and are used to colonize wild areas. In most cases, plants from this genus prefer acid soils and require some inactive dormant cold period in order to successfully produce flowers and fruits. Bog-type environments favor production of some of these fruits (e.g., cranberry and ohelo), and in some cases, production is largely limited to established wild stands, since propagation and new plantings are not easily initiated (wild blueberry). The berries are extracted and marketed as a valuable source of natural anthocyanin pigments for the food-processing industry, as replacements for potentially harmful synthetic pigments and dyes (P. Hereld, the Hereld Organization, pers. comm.). In cranberry, for example, the waste product of the juicing process (the pomace) can be further extracted as a source of the natural anthocyanin pigments (Francis 1989). The flavonoid content (pigments and other polyphenolic compounds) from different species and cultivars has been extensively investigated in an effort to identify donors for valuable extracts (Fuleki and Francis 1967; Wang et al. 1978). More recently, consumer interest in these species has risen due to increasing evidence for the health protective properties of the berries.

While anthocyanins have most frequently been cited as the bioactive constituents of *Vaccinium* extracts, considerable evidence has also indicated that the proanthocyanidin fractions possess considerable anticancer and antiradical activity. Mixtures of flavonoids derived from *Vaccinium* berries may have additive or synergistic biological benefits that cannot be realized from one isolated compound; hence, some of the products derived from the berries are rich in mixtures of proanthocyanidins and anthocyanins (Table 1).

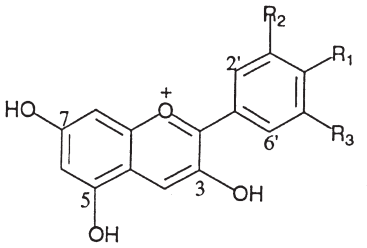
Health benefits associated with consumption of some *Vaccinium* berries have been recognized for centuries, but it is just within the past decade that potent antioxidant, anticancer, cardioprotective, and other bioactive properties have been scientifically demonstrated and widely appreciated. The anthocyanins, the proanthocyanidins, or combinations of these phytochemicals have been reported as the active ingredients from foods and supplements containing *Vaccinium* spp. (Fig. 2). The anthocyanin composition of some of the most important *Vaccinium* members is given in Table 2. The exact proanthocyanidin composition has not been elucidated for these species. Both leaves and fruits of the bilberry have been known as ingredients of traditional European medicines since the 16th century. Bilberry extracts are rich in up to 15 free anthocyanins (3-*O*-arabinosides, 3-*O*-glucosides, and 3-*O*-galactosides of cyanidin, delphinidin, peonidin, petunidin, and malvidin) as well as associated

Table 1. Summary of in vivo/in vitro production and harvest of secondary metabolites in the genus *Vaccinium*

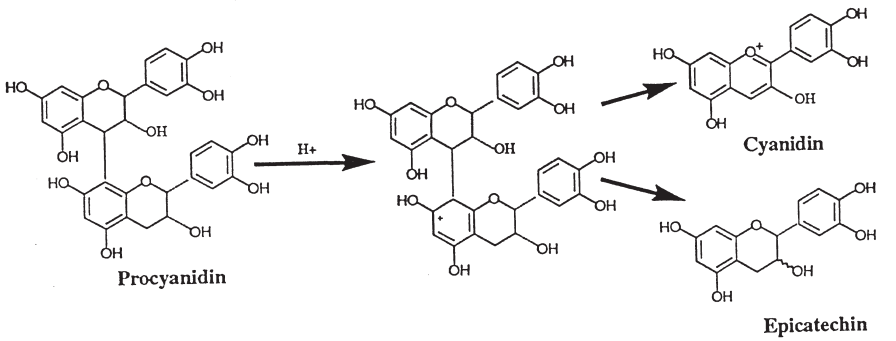
Fruits	Secondary metabolite harvested as pharmaceutical or food supplement	In vivo/ in vitro	Sources or references
Lowbush blueberry (*V. angustifolium*)	Anthocyanins	In vivo	Francis et al. (1966)
Rabbiteye blueberry (*V. ashei*)	Anthocyanins, proanthocyanidins, and carotenoids	In vitro	Nawa et al. (1993)
Bilberry (*V. myrtillus*)	Anthocyanin and proanthocyanidin extract	in vivo	Bettini et al. (1991); Indena Corp. ESA Labs
	Anthocyanin and proanthocyanidin extract; carotenoids	In vitro	Madhavi et al. (1998)
American cranberry (*V. macrocarpon*)	Anthocyanin-rich powdered extract	In vivo	Fuleki and Francis (1967); Decas Cranberry Co.; Ocean Spray Cranberries; Cape Cod Biolab Corp.
	Anthocyanins	in vitro	Madhavi et al. (1995)

Indena Corp. is based in Italy. Decas Cranberry Co, ESA Labs, Ocean Spray Cranberries, Inc., and Cape Cod Biolab Corp. are based in the USA.

proanthocyanidins, and are currently marketed through pharmacies in Europe. The antioxidant capacity of the rich flavonoid complement in bilberry extracts effectively inhibits the oxidative modifications of low density lipoproteins (LDL). Because of this demonstrated capacity, bilberry extracts are consumed in part to inhibit the formation of atherosclerotic plaques in the arterial wall (Laplaud et al. 1997). Research has cited the ability of bilberry anthocyanins to relax coronary artery segments (antagonize contractile responses) as a potential inhibitor of heart disease (Bettini et al. 1991), and to enhance microvascular blood flow (Colantuoni et al. 1991). These same extracts have been lauded for antiulcer and therapeutic activity (Cristoni and Magistretti 1987), and for clinical applications in ophthalmology, including the ability to increase night vision acuity (Morazzoni and Bombardelli 1996). In all these applications, the anthocyanin pigments are designated as the active principles responsible for bioactivity (Kalt and Dufour, 1997). Related fruits from other members of the genus are recognized for other miscellaneous health benefits: blueberries and cranberries as sources of antiadhesins (effective in treatment of urinary tract infections); blueberries as a source of cough suppressants and



The structure of anthocyanidin.



Hydrolysis of proanthocyanidins in acid. Proanthocyanidins are encountered in the form of procyanidins.

Fig. 2. Anthocyanins and proanthocyanidins are both recognized as biologically active phytochemicals in the genus *Vaccinium* and are responsible for antioxidant, cardioprotective, and anticancer properties associated with consumption of the fruits

as a diarrhea remedy; cranberries as donors of cardio-protective phytochemicals (Ofek et al. 1991; Avorn et al. 1994; Kalt and Dufour 1997; Wilson et al. 1998).

While crude extracts from wild blueberry, cranberry and lingonberry fruits each demonstrated inhibition of the promotion stage of chemically induced carcinogenesis, it was the proanthocyanidin-rich fraction which proved to be most highly anticarcinogenic (Bomser et al. 1996; Smith et al. 2000). Proanthocyanidins are one of the major flavonoid classes found in these fruits, and are associated with, but separate from, the anthocyanin pigments. Further research has recently shown that blueberries, on a fresh weight basis, have the highest antioxidant capacity of all previously tested fruits and vegetables in the typical diet, which is indicative of the ability of these fruits to protect against conditions of neurodegenerative changes with aging, cardiovascular disease, and carcinogenesis (Prior et al. 1998). In these tests, the anthocyanin pigments themselves are cited as the active components responsible for antioxidant capacity.