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Highbush Blueberry: Cultivation, Protection, Breeding and Biotechnology

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ABSTRACT

Highbush blueberry is one of the most commercially significant berry crops. It is mainly cultivated in the United States and Canada, but also in Europe, Australia, Chile and New Zealand. Production of this crop is likely to increase in response to increased consumer demand for healthy foods, including the antioxidant-rich blueberry. This review describes several issues and developments in sustainable blueberry farming, including agronomical and cultural techniques (mulching, irrigation, the beneficial effects of mycorrhizae and fertilization), disease management (biology and control of common and emerging diseases), pest management, pollinators (effects on fruit set and production), conventional breeding and molecular techniques for examining and engineering blueberry germplasm. This paper describes past problems and current challenges associated with the commercial production of highbush blueberry, as well as new approaches and techniques for improving crop quality and future perspectives for innovative research.

Keywords: diseases, germplasm, pests, production, *Vaccinium*

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INTRODUCTION

Blueberry is currently cultivated on a relatively small scale and, therefore, is considered a minor crop. The high concentrations of antioxidants and other beneficial compounds in blueberries suggest that the demand for this crop among health-conscious consumers may increase in the near future. If demand does increase as predicted, the amount of area under cultivation will most likely increase, as well. Of all the cultivated species and hybrids in the genus *Vaccinium*, highbush blueberry is notable for its ability to provide satisfactory productivity levels with minimal inputs. To date, few research efforts have been dedicated to highbush blueberry in Europe, even though some highly innovative approaches have been explored and tested with success in North America.

European cultivation of highbush blueberry began after 1920, in the Netherlands. In the following years, cultivation

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Invited Review

Fig. 1 (**A**) Highbush blueberry flowers. (**B**) Bumble bee visiting blueberry flowers. (**C**) Highbush blueberry cultivation in spring. (**D**) Blueberry cluster before ripening. (**E**) Ripe berries. (**F**) Highbush blueberry cultivation in autumn. (**G**) *Armillaria* root rot on blueberry crown: mycelium and rhizomorphs. (**H**) Berries infected by *Colletotrichum* spp.

spread to Poland and Germany, where the first European blueberry breeding products, crosses of North American genotypes, were introduced (Pliszka 1997). However, highbush blueberry cultivation did not expand into southwestern Europe until the 1980s (Strik and Yarborough 2005). Today, European highbush blueberry production is mainly concentrated in Germany, Poland, France, the Netherlands, Lithuania, Romania, Italy and Spain.

It is difficult to estimate worldwide production and consumption of highbush blueberry: some papers describe specific productions in North America (Strik 2006), South America (Bañados 2006), Europe (Pliszka 1997) or other minor countries. Worldwide updated reports on blueberry production and cultivation are available on-line on the FAO database (FAOSTAT 2006, URL: http://faostat.fao.org), but they provide information regarding all blueberry species together (named generally "blueberries") or the sum of cranberries and blueberries.

In 2004, the world production of "blueberries" reached 240,786 tons with a harvested area of 51,756 ha: the most important countries were Canada (26,058 ha) and USA (17,980 ha) while in the European Union (25 member states) the harvested area was approximately 5,800 ha (FAOSTAT 2004).

Strik (2006) reported that in 2003 the highbush and rabbiteye blueberry planted area in the USA was about 22,390 ha with an increase of 13% from 1992 to 2003. In Canada, in the same period, the planted area to highbush blueberries increased 102%, reaching almost 4,400 ha (Strik 2006).

Chile and Argentina are the most important blueberry producers in South America. In 2004, blueberry surface area

(both highbush and rabbiteye) was estimated in 2,500 ha in Chile and about 1,200 ha in Argentina (Bañados 2006). Most of their production is for the export as fresh product to the USA and Europe (Bañados 2006).

Few data are available on blueberry production and cultivation for each European country, therefore information must be gathered from different sources such as grower associations and extension services. To our knowledge, the estimated blueberry surface area is currently about 1,800 ha in Germany and 1,900 ha in Poland with a production of 8,000 and 4,000 tons respectively. In France, in the Netherlands and Spain blueberry cultivated area is more than 200 ha while in Italy the total area is about 200 ha.

Blueberries are also cultivated in Oceania (New Zealand and Australia) and Asia (Japan and China).

AGRONOMY AND CULTIVATION

Blueberry requires acidic, well-drained soils, with optimum acidity ranging from pH 4.5 to 4.8 (Eck *et al*. 1990). Blueberry has a shallow root system (located mainly at depths smaller than 60 cm) and, especially on clay soils, should be mulched with a deep layer (at least 10 cm) of organic mulch, such as bark, sawdust or leaves. Mulching increases the amount of organic matter in the soil, keeps moisture in the soil, protects roots from heat and helps to control weeds.

Where available and economically feasible, traditional organic mulches, such as pine bark can be used (Magee and Spiers 1995). Where pine bark is unavailable or expensive, lower cost organic and inorganic materials can be used, and several alternatives have been explored. White-over-black plastic mulch was found to be as good as pine bark in terms of plant growth and fruit yield (Magee and Spiers 1995), and a combination of coal ash, composted sewage and leaf compost also gave good results (Black and Zimmerman 2002). Tire chips could be a potential mulch and substrate component (they reduce high soil temperatures and weed growth and are not phytotoxic to blueberry plants), but more information is needed before recommending their use in commercial fields (Krewer *et al*. 1997). Derivates from industrial processes, such as fresh pine telephone pole peelings (25% bark, 75% elongated fibers of cambial wood) and pine fence post peelings (75% bark, 25% fibers), may be excellent, low cost substitutes for milled pine bark (Krewer *et al*. 2002).

Organic mulch can be a source of root rot pathogen inoculum, or promote the growth of these pathogens. For example, *Armillaria* spp. has been found on coniferous bark used as mulch in highbush blueberry plantings (Prodorutti *et al*. 2005).

The blueberry root system is not only shallow, but also has a limited water uptake capacity (Holzapfel *et al*. 2004). Therefore, the amount of water applied and irrigation scheduling and distribution will significantly impact production. Economic and technical parameters, such as the type of soil, plant spacing, availability and quality of water, available labor resources and the costs of all of these inputs must be considered for the selection of the most appropriate irrigation system for a particular blueberry field (Holzapfel *et al*. 2004). Microjet and drip irrigation systems are currently the most commonly used in blueberry plantings, while sprinkler irrigation is used mainly for frost protection and cooling (Caruso and Ramsdell 1995; Holzapfel *et al*. 2004). The use of overhead sprinkler systems is not recommended in regions with severe fungal disease problems, because high levels of relative humidity in the foliage promote infections by several pathogens. Drip irrigation is recommended in soils with high water-holding capacities; emitters should be placed at least 40 cm away from the crown in order to avoid overwatering the shallow blueberry roots (Holzapfel *et al*. 2004).

Ericoid mycorrhizal fungi form symbiotic associations with the roots of blueberry and other ericaceous species. *Hymenoscyphus ericae* (Read) [Korf & Kernan] and *Oidiodendron griseum* Robak are the most common species found in association with *Ericales* (Goulart *et al*. 1993). Highbush blueberry has a root system devoid of root hairs, but the role of root hairs is often taken over by mycorrhizae (Eck *et al*. 1990). The extensive hyphae of these fungi increase the volume of soil that can be accessed by the plant and improve nutrient uptake. Moreover, ericoid mycorrhizal fungi protect roots against infection by pathogens, such as *Phytophthora* spp., increase the plants' tolerance for stressful environmental conditions and influence plant growth and yield (Goulart *et al*. 1993; Koron and Gogala 2005). Intensive use of chemicals and intensive mineral fertilization and soil compaction prevent the development and the establishment of populations of mycorrhizal fungi in blueberry plantings (Koron and Gogala 2005).

The development of commercial mycorrhizal fungi for the nursery industry is an innovative aspect of blueberry cultivation. Inoculation with ericoid mycorrhizal fungi has been shown to improve the productivity and quality of nursery plants, and also reduce the need for supplemental fertilizer (Scagel 2005). The choice of method of plant inoculation is critical for the successful application of mycorrhizae. Micropropagated blueberry plantlets can be inoculated by placing the rooting shoots in agar medium containing mycorrhizal fungi and a superimposed layer of sterilized peat (Eccher and Noè 2002). Koron and Gogala (2005) successfully inoculated one-year-old plants with mycorrhizal fungi grown in a mixture of vermiculite and peat (at a 1:3 ratio) that had been soaked with a liquid nutrient medium. Scagel (2005) reported the use of a method in which potted blueberry plantlets were inoculated with an aqueous suspension of different ericoid fungi. Wide-scale use of mycorrhizae-amended plants may reduce the use of fertilizers and chemical products, but the high costs and technical hitches associated with inoculated plant production and the small market for these inoculated plants, have prevented the extensive use of this technique (Koron and Gogala 2005).

Most blueberry plantings require nitrogen applications each year, while other nutrients are generally applied only as needed. Ammonium sulfate is the preferred nitrogen source for blueberry, especially if the soil pH is relatively high (above 5.0), because it tends to decrease soil pH levels. Nitrogen is usually split in multiple soil applications during the spring, using granular formulation on the surface of the mulch, in order to increase nitrogen efficiency: this is particularly important on sandy soils (Eck *et al*. 1990). The application method strongly influences the effects of nitrogen on yield and plant growth. Fertigation through drip irrigation has been shown to provide superior results, as compared to surface applications of nitrogen, probably because of the easy availability of nitrogen placed in the root zone (Finn and Warmund 1997).

Foliar fertilization can effectively supply mineral nutrients during periods of maximum demand by the crop and low availability in the soil (Widders and Hancock 1994). Nitrogen sprays can benefit nitrogen-deficient blueberry plants, but bushes receiving appropriate soil applications of nitrogen did not show any significant yield response to sprays (Widders and Hancock 1994).

POLLINATION AND POLLINATORS

A large amount of data is available on pollination of the numerous *Vaccinium* species and their hybrids, but as flower structure and size varies within the genus, generalizations must be applied cautiously.

Highbush blueberry flowers are 6-10 mm long, with calyxes made up of five elements. The white petals unite to form a tubular or bell-shaped corolla that hangs with its open end downward before pollination (McGregor 1976; **Fig. 1A**). The nectar is secreted at the base of the style, so insects have to push their tongues between the filaments of the anthers in order to reach it (Free 1970). Flowers are self-fertile, with the degree of self-fertility varying among cultivars, but highbush blueberry greatly benefits from cross-pollination. Cross-pollinated plants set more fruit, and these

fruits are bigger than those of self-pollinated flowers (Free 1970; McGregor 1976). Insect pollination is, therefore, essential (McGregor 1976).

In some specific situations, honeybees and other pollinators can be highly detrimental to the crop. They may transport conidia of mummy berry to the floral stigmas (Dedej *et al*. 2004) or transmit pollen-borne viruses (BlShV and BBLMV) from infected to healthy plants (Childress and Ramsdell 1987; Bristow and Martin 1999).

Honeybees, bumblebees and solitary bees

Bumblebees, with their long tongues, can easily reach the base of blueberry flowers, as compared to honeybees that cannot reach the nectar of varieties with long flowers (**Fig. 1B**). Bumblebees are the most frequent and active pollinators in several blueberry cultivation areas (MacKenzie and Eickwort 1996). In other regions, honeybees result the most numerous and efficient pollinators (Goodman and Clayton-Greene 1988; Dedej *et al*. 2004). In some areas, such as Maryland (USA), native solitary bees, such as *Andrena* spp. and *Colletes* spp., are among the insects that frequently visit blueberry flowers (Batra 1997).

Differences among the prevalent pollinators in different areas may be related to one or more of the following factors: local environmental conditions, the presence or absence of domestic honeybees, number and species of wild pollinators, composition of the competitive flora and differences in the flowers of different blueberry cultivars (quantity, timing and composition of nectar and corolla length).

Honeybees have been used to ensure adequate pollination in situations where native bee activity is insufficient, but, since honeybees are not particularly attracted to blueberry, they may prefer visiting other competing flowers in the area surrounding the field (Batra 1997). For this reason, other more manageable bees (bumblebees and solitary bees) were evaluated for use as alternative commercial pollinators of highbush blueberry. *Bombus impatiens* Cresson was found to be an efficient pollinator, improving fruit production and quality, especially on blueberry grown in plastic tunnels where bees are usually less suitable (Sampson and Spiers 2002). Solitary bees [i.e. *Osmia ribifloris* Cockerell, *O. cornifrons* Radoszkowski, *Megachile rotundata* Fabr., *Anthophora pilipes* Smith and *Habropoda laboriosa* (Fabr.)] are also promising commercial pollinators of blueberry, because they are easy to manage, forage even in unsuitable weather and prefer *Vaccinium* spp. flowers to those of other species (Batra 1997; MacKenzie *et al*. 1997; Sampson and Cane 2000).

To maximize cross-pollination, two or more cultivars with similar bloom periods are planted in alternating pairs of rows or, ideally, mixed throughout the field (Pritts 1997). Pesticide applications in and around the planting, type of groundcover and nutrient and water management can affect pollination in blueberry crops (Pritts 1997). The availability of suitable nesting sites, abundant food sources and clean water, indirectly influences pollination by supporting large wild bee populations (Pritts 1997).

A BRIEF REVIEW OF RECENT HIGHBUSH BLUEBERRY DISEASE RESEARCH

As a comprehensive treatise of blueberry diseases is beyond the scope of this paper, interested readers are referred to the review by Caruso and Ramsdell (1995). In this discussion of important blueberry pathogens, we highlight aspects of the most innovative approaches for the diagnosis and control of diseases, and for understanding the biology and epidemiology of the different pathogens. In contrast to other horticultural crops (i.e. apple, pears, peaches, etc.), there are few widespread highbush blueberry diseases. Most diseases of this crop are linked to specific environments, or occur only occasionally. Several hypotheses can be suggested to explain the fortunate lack of widespread blueberry diseases. Blueberry is of recent interest for agriculture, and, in many regions, can still be considered a minor crop. Therefore, monoculture is limited to relatively small areas. Blueberry plantings are often scattered and somewhat isolated. The low intensity of blueberry monoculture may limit the spread of airborne and soilborne pathogens. Most of the commercial plantings outside the US are very young (up to 15- to 20-years-old) and pathogen populations have not had much time to establish themselves. In addition, it seems that most highbush blueberry pathogens are specific to species of the genus *Vaccinium*. The lack of wild relatives of blueberry in several areas of recent cultivation provides these crops with a degree of isolation from some of the more damaging diseases. The currently cultivated varieties are not the output of intense breeding programs and, therefore, are relatively robust and not highly susceptible to diseases. In contrast to the situation in many other horticultural crops, disease control in blueberry is relatively simple. However, if diseases like phomopsis twig blight and canker, mummy berry and anthracnose, are introduced into the growing area, they can be very destructive.

Breeding for resistance or tolerance to fungal and bacterial pathogens can have clear advantages, but resistant varieties have not yet been developed for some diseases. Little information is available on biocontrol of highbush blueberry diseases. *Bacillus subtilis* QRD137 was successfully applied against mummy berry (Scherm *et al*. 2004) and *Gliocladium virens* increased leaf area and number, as well shoot and root dry weights (de Silva *et al*. 2000).

Diagnosis of fungal pathogens is mainly based on morphological identification, while viruses are generally detected with serological methods (ELISA). Molecular based diagnosis has not been developed yet for most of highbush blueberry pathogens.

Blossom and fruit diseases

Botrytis cinerea Pers.:Fr. (Botrytis blossom blight) and *Monilinia vaccinii-corymbosi* (Reade) Honey (Mummy berry disease) are the two most important pathogens that attack highbush blueberry fruits.

Outbreaks of botrytis blossom blight occur occasionally, but they can be very destructive. Symptoms of this disease are sometimes confused with those of frost injury, since *B. cinerea* can invade injured tissue following a spring frost. The fungus also attacks uninjured blossoms, tender, green twigs, and leaves in early spring, causing infected flowers and twigs to quickly turn brown or black and die. Often the fungus advances from infected flower clusters into the stem, girdling it and killing all of the flowers above the infection point. It can also occasionally cause preharvest fruit rot and, more frequently, postharvest decay during cold storage.

M. vaccinii-corymbosi is a major problem for North American blueberry production. The fungus overwinters in fruit mummies (pseudosclerotia) that drop to the soil at harvest. In the spring, the only source of *M. vaccinii-corymbosi* primary inoculum is the apothecia, which are produced on the pseudosclerotia. The released ascospores cause leaf and shoot blights. From these infections, conidia responsible for the infection of open flowers (secondary infection) are produced. Later, a subsequent infection of the developing fruit occurs and, as infected fruit matures, the fungal mycelia forms melanized entostroma, which leads to the formation of the pseudosclerotium (Cox and Scherm 2001a).

There are major differences between the two pathogens *B. cinerea* and *M. vaccinii-corymbosi*. *B. cinerea* inoculum is almost always present in the field, as conidia are ubiquitously produced on several substrates, while *M. vaccinii-corymbosi* primary inoculum is only produced by apothecia on infected fruits from the previous year. In the case of *B. cinerea*, it is crucial to protect the susceptible flowers when weather conditions are favorable for infection, because once inoculum is present in a field, it is very difficult to

control the disease. Since rain increases the rate of infection, it may be advisable to cover the crop with plastic tunnels or some other material. On the other hand, *M. vaccinii-corymbosi* infections can be prevented by elimination of the mummified berries, which are the source of primary inoculum. Based on the assumption that reducing *M. vaccinii-corymbosi* overwintering inoculum (apothecia) can result in a reduction of the risk of primary infections in the spring and evidence that most ascospores are deposited close to the apothecia in which they were produced (Cox and Scherm 2001b), different approaches for disease control can be tested. Treatment of pseudosclerotia of *M. vaccinii-corymbosi* with desiccants and herbicides negatively affected multiple aspects of apothecia germination, suggesting that these chemicals may have positive, antifungal side effects in the field (Cox and Scherm 2001a). The combined use of soil cultivation implements that result in deep burial of pseudosclerotia with those that reach the pseudosclerotia located near the plants may also reduce the risk of disease (Ngugi *et al*. 2002).

It is difficult to protect flowers against *B. cinerea* and *M. vaccinii-corymbosi* with sprayed pesticides. During the flowering period, new flowers open almost every day and the target area for fungicide applications is tiny and unexposed. Most systemic pesticides do not accumulate to sufficient concentrations in the susceptible part of the flowers (Ngugi and Scherm 2006). Two innovative approaches have been applied to these problems. In the first approach, bees or other pollinators were used as carriers of the control agent, allowing for the effective use of fungicidal materials that persist for only a short time period, such as biocontrol agents (Dedej *et al.* 2004). Pollinators can constantly visit new flowers, providing them with protection as soon as they open. Electrostatic treatments, which incorporate electrostatic force to increase the mass transfer of the viable bacterial biocontrol agent *B. subtilis* onto stigmatic surfaces of blueberry flowers, have also been studied. The population density of biocontrol agent electrostatically deposited using charged sprays on the stigma exceeded by 4.5-fold that deposited by conventional hydraulic spraying (Law and Scherm 2005).

Blueberry anthracnose (caused by *Colletotrichum acutatum* Simmonds) can affect the postharvest fruit quality of highbush blueberries. Plants are susceptible to infection not only during the blooming period, but at all stages of fruit development (bloom to ripe berry). Once the pathogen has become established in a new growing area, it can be highly destructive. Infections are visible as masses of orange spores on the surfaces of affected fruit (**Fig. 1H**).

Several cultivars of blueberry show relatively low susceptibility to foliar and/or fruit infections. Even though leaf infections do not cause significant economic losses and little correlation has been observed between foliar and fruit responses to anthracnose infection, breeding new cultivars with resistance to foliar infections may assist in the reduction of inoculum levels in the field. This is particularly important because *C. acutatum* overwinters primarily in vegetative tissue. An estimation of narrow-sense heritability suggested the additive inheritance of blueberry resistance to anthracnose (Polashock *et al*. 2005; Ehlenfeldt *et al*. 2006).

Other fruits pathogens, such as *Alternaria tenuissima* (Kunze:Fr.), *Phyllosticta vaccinii* Earle and *P. elongata* Weidemann, are occasionally reported in blueberry, but are not significant sources of field losses.

Leaf and stem diseases

This group includes fungi that cause leaf spots, twig blights and cankers.

Leaf pathogens primarily reduce photosynthesis in the infected tissues but, when they induce premature defoliation (i.e. *Septoria albopunctata* Cooke, *Gloeosporium minus* Shear and *Dothichiza caroliniana* Demaree & M.S. Wilcoxis), they affect subsequent flower bud formation. Older leaves are more likely to die prematurely, as compared with younger leaves, and, by staying on the plant for a longer period of time, also to accumulate higher levels of disease, which increases the risk of defoliation. The risks posed by these pathogens must not be underestimated and maintaining disease-free foliage is important for maximizing the quality and quantity of yields in the following growing season (Ojiambo and Scherm 2005).

The causal agent of powdery mildew, *Microsphaera vaccinii* (Schwein.) Cooke & Peck, is present at economically insignificant levels, in most blueberry orchards in the US, but has not yet been reported in other countries. As powdery mildew infections are generally promoted by dry and warm conditions, the use of plastic tunnels to prevent *B. cinerea* infections could possibly increase the future risks posed by this currently unimportant disease.

Upright dieback, caused by *Phomopsis vaccinii* Shear (teleomorph *Diaporthe vaccinii* Shear in Shear, N. Stevens, & H. Bain), is only present in North America and Chile. Due to the risk of its spread into Europe, it has been added to the A1 list of quarantine diseases of the European and Mediterranean Plant Protection Organisation (www.eppo.org). For quarantine pathogens, prompt and precise identification of infected plant material is crucial. However, diagnosis of *P. vaccinii* using traditional phytopathological methods is quite difficult because there is a long latency period between infection and the appearance of symptoms, which are often undistinguishable from damage caused by other factors. The isolation of *P. vaccinii* is also tricky because it is easily overrun by other fungi and, once it has been isolated, seldom produces the pycnidia necessary for morphological identification. The use of serological and DNA-based techniques can improve diagnosis success rates. Immunoassays, including direct tissue blot immunoassays and plate-trapped antigen enzyme-linked immunosorbent assays, have been successfully used to detect the pathogen in plant tissues (Gabler *et al*. 2004).

Sequence analysis of the ITS rDNA of *Phomopsis* isolates has been used successfully in a number of studies to identify unknown isolates from both diseased and asymptomatic hosts and can be useful for the identification of isolates that no longer produce pycnidia. Analyses including all available alignment positions can be used to separate isolates of *P. vaccinii* from those of other taxa, including closely related strains, but alignment of the ITS regions across the whole genus *Phomopsis* is problematic. This is due to the large number of insertion and deletion events, which makes the number of ambiguously aligned positions quite large. Removing ambiguously aligned positions from the analysis may obscure the relationships between closely related taxa, due to the large number of significant positions that would be discarded. After analysis of the first grouping of taxa using all available alignment, closely related taxa should be analyzed separately for the accurate determination of relationships (Farr *et al*. 2002).

Botryosphaeria corticis (Demaree & M.S. Wilcox) Arx & E. Mueller, *Fusicoccum putrefaciens* Shear (teleomorph *Godronia cassandrae* Peck *f. sp. vaccinii*), *Pseudomonas syringae* van Hall and *B. dothidea* (Moug.:Fr.) Ces. & de Not. cause cankers and stem blights. To date, none of the tested blueberry cultivars have been found to be completely resistant, but there are great differences in their respective susceptibilities (Storming and Stensvand 2001; Smith 2004). The use of tolerant cultivars is an important tool for managing these diseases. The inoculum of these pathogens is present on infected twigs or branches, and any practice that reduces the amount of infected material in the field is beneficial. Winter pruning and the removal of infected twigs and branches during the growing season can be critical for control of Phomopsis twig blight and Fusicoccum canker.

The bacterium *Agrobacterium tumefaciens* can cause pea-sized to large, round galls on low branches and at the base of canes, but since blueberries are grown on acidic soils and the crown gall bacterium does not grow well in

acidic environments, *A. tumefaciens* infections of blueberry are uncommon.

Root diseases

Root rot diseases caused by *Armillaria mellea* (Vahl:Fr.) P. Kumm., *A. ostoyae* (Romagnesi) Herink and *Phytophthora cinnamomi* Rands affect blueberry crops when these fungi are present in the soil and environmental conditions are favorable for disease development. Symptoms are first seen on the above-ground portions of the plant (chlorosis and reddening of the leaves, small leaves, defoliation, branch dieback, death of entire canes, stunting, and death of the entire bush), but are always subsequent to root damage. In the case of *P. cinnamomi*, the very fine, absorbing roots turn brown and black and larger diameter roots may also be discolored. *Armillaria* spp. mycelia and rhizomorphs are mainly found under the bark of old roots (**Fig. 1G**).

Avoiding the establishment of pathogen populations in the soil and careful site selection are key factors for the prevention of these diseases. Particular care must be taken if a new crop is planted on ground previously covered by forest, where additional pathogen species, other than the very common *A. mellea*, may be present (Prodorutti *et al*. 2006). When these diseases are already present in the field, growing non-host crops for few years before blueberry can dramatically reduce the amounts of long-lasting inoculum present in the environment. Since *Armillaria* species are not specific pathogens of *V. corymbosum*, they infect more than 200 plant species, the presence of infected roots (of various plant species) is an important and likely source of inoculum. *P. cinnamomi* also attacks a number of additional plants from the family *Ericaceae*.

P. cinnamomi is common in the United States, but it is also present in New Zealand (Cheah and Hunt 1988) and has recently been reported in Europe (Tamietti 2003). *Armillaria* species are widespread around the world.

Phytophthora root rot is usually associated with poorly-drained soils and the highest disease incidences are correlated with frequent waterlogging (de Silva *et al*. 1999). Mulching, that could theoretically increase the availability of *Armillaria spp*. inoculum, does not significantly affect the risk posed by phytophthora root rot (de Silva *et al*. 1999). Most cultivars are susceptible to this disease, although some cultivars may tolerate some degree of infection better than others.

Resistance to *P. cinnamomi* has been shown to be partially recessive and quantitatively inherited (Clark *et al*. 1986). To the best of our knowledge, there have been no reports of resistance or tolerance to *Armillaria* spp.

Diseases caused by viruses or phytoplasma

Plants infected with viruses or phytoplasma cannot be cured with pesticides, therefore planting healthy, virus-indexed plants obtained from a trustworthy nursery is essential. Resistance to viruses or phytoplasma is desirable, but sometimes only tolerant cultivars are available. These cultivars do not show symptoms and produce normal fruits, but they are still capable of hosting the pathogen and constitute a reservoir of inoculum that, in the presence of an active vector, can be spread to nearby susceptible varieties, producing heavy damages. When virus- or phytoplasma-infected plants and their vectors are present in the cropping area, efforts should be focused on reducing the risk of disease transmission by direct control of vectors and prompt elimination of both symptomatic plants and alternative hosts of the pathogen. However, the quick removal of infected plants can be difficult due to the long incubation of some blueberry viruses (i.e. blueberry shoestring virus and blueberry scorch virus).

The most advanced techniques of serology and molecular biology have been applied to the detection and characterization of blueberry viruses and phytoplasma. Commercial antibodies are available for most blueberry viruses.

Blueberry scorch virus (BlScV), also known as sheep pen hill disease, is the causal agent of a blighting disease of highbush blueberry. Symptoms caused by BlScV range from complete blighting of flowers and young leaves and twig dieback in the most sensitive cultivars to no visible damage in the most tolerant cultivars. The complete genome sequence of an isolate from New Jersey has been published (Cavileer *et al*. 1994). Thorough molecular characterization and phylogenetic analyses have demonstrated a considerable divergence of strains from western Canada from strains from the eastern United States. A reverse transcription polymerase chain reaction (RT-PCR) assay, enabling the detection of RNA viruses, has been developed for the detection of one strain of BlScV (Halpern and Hillman 1996), opening the door to the wide use of PCR-based methods in routine diagnoses of blueberry viruses.

DNA sequencing of blueberry red ringspot virus (BRRV) demonstrated that this virus is more similar to members of the genus provisionally named "Soybean chlorotic mottle-like viruses", rather than members of the genus Caulimovirus, in which it had been placed previously (Glasheen *et al*. 2002).

Blueberry shock ilarvirus (BlShV) causes symptoms similar to those caused by BlScv, but, after a few years of symptoms, the infected plants usually recover (Bristow *et al*. 2002). These non-symptomatic plants may act as reservoirs of inoculum. Like other ilarviruses, BlShV is present in pollen, therefore wind and pollinators can play signifycant roles in the spread of the disease (Bristow and Martin 1999).

Blueberry shoestring sobemovirus (BBSSV) is the most widespread virus disease of highbush blueberry. It is transmitted in a persistent and circular manner by the aphid *Illinoia pepperi* (MacGillivray). The long latent period makes roguing of infected plant unfeasible for commercial production, and very few cultivars shows resistance. Therefore, whenever possible, efforts are focused on controlling the aphids.

Tobacco ringspot virus (TRSV), tomato ringspot virus (TmRSV), peach rosette mosaic virus (PRMV), and blueberry leaf mottle virus (BBLMV) all occasionally cause damage to highbush blueberry (Caruso and Ramsdell 1995).

Blueberry stunt it is caused by a phytoplasma. The only known carrier of this phytoplasma is the sharp-nosed leafhopper (*Scaphytopius* spp.; Maeso Tozzi *et al*. 1993), though other vectors probably exist. Affected plants are dwarfed with shortened internodes and excessively branched. They are not vigorous and produce small, hard, fruits lacking flavor. Diseased plants produce small, downward cupped leaves with mottled areas (yellow along the margins and between the lateral veins) that turn prematurely red in late summer.

Outlook on current and future studies of blueberry pathology

Research on highbush blueberry diseases has mainly focused on problems present in North America, where the species originated and has been extensively cropped for several decades. In other regions, such as Europe, South America and Japan, where it has only recently been introduced, there have been few reports of pathogens or severe disease outbreaks and, consequently, research has been limited.

In conclusion, it seems that disease management in highbush blueberry crops in new cultivation areas should require only limited use of pesticides. A high level of care must be taken to avoid the introduction or establishment of new diseases, applying strict phytosanitary measures to plant material coming from infected areas and using disease-free planting stocks. Before starting cultivation in a new region, it may be worthwhile to survey the area for alternative hosts and potential vectors of *V. corymbosum* diseases. Considering disease resistance or tolerance as an im-

portant aspect in breeding programs and the development of commercial biocontrol agents can help in areas where diseases are already established.

RELEVANCE AND DISTRIBUTION OF HIGHBUSH BLUEBERRY PESTS

Similar to research on blueberry diseases, most research on insect pests of highbush blueberry has been carried out in North America and there are few publications dealing with insect pests in Europe and other production regions. Few indigenous insects attack highbush blueberry in new cultivation areas. The absence of the most economically significant insect pests, which attack blueberry in its native country, in these areas or insufficient study may explain this lack of information.

Some native North American *Vaccinium* pests migrated from patches of wild blueberries to commercial fields (Eck *et al*. 1990), while other pests were introduced into North America from other continents, such as the Japanese beetle (*Popillia japonica* Newman), which is native to Asia. It was introduced into North America in 1911, where it became a more serious pest than in its own area of origin (www.eppo.org).

The key insect pests of highbush blueberry in North America, other then the Japanese beetle, are the blueberry maggot (*Rhagoletis mendax* Curran) and the cranberry fruitworm (*Acrobasis vaccinii* Riley) (O'Neal *et al*. 2005a). Chemical insecticides are also applied against blueberry gall midge (*Dasineura oxycoccana* Johnson), plum curculio (*Conotrachelus nenuphar* Herbst.) and blueberry bud mite (*Acalitus vaccinii* Keifer). Infestations of Putnam scale (*Aspidiotus ancylus* Putnam), blossom weevil (*Anthonomus* spp.) and the sharp-nosed leafhopper (*Scaphytopius magdalensis* Prov.) may occasionally require treatments (Eck *et al*. 1990).

The blueberry maggot and the cranberry fruitworm have not been reported in Europe, while *P. japonica* was identified in the Azores (Portugal). The blueberry maggot and Japanese beetle are classified as quarantine pests by the European and Mediterranean Plant Protection Organization (www.eppo.org).

The blueberry gall midge has recently become more important in the southern United States. This midge, native to North America, was recently introduced into Europe (Bosio *et al*. 1998).

What is eating my fruit? Insects which damage berries

Adult Japanese beetles, blueberry maggot larvae and cranberry fruitworms feed on ripening berries, as well as harvested fruits, making them unmarketable. In the US, there are stringent quality standards for fresh and processed blueberries, including zero tolerance for contamination by these insects (O'Neal *et al*. 2005b).

The Japanese beetle (Coleoptera: Scarabaeidae) is an univoltine insect. Larvae feed on roots of turf and overwinter in the soil. They pupate in June and adults emerge and start feeding on fruits about two weeks later, with population levels peaking in July and August (Szendrei *et al*. 2005).

The blueberry maggot (Diptera: Tephritidae) is also univoltine. It overwinters as pupa in the soil and adults emerge over a prolonged period, from late June to early August. About 10 days after emergence, female flies start to lay eggs under the fruit skin. Maggots feed for about three weeks inside ripening and harvested fruits.

In blueberry fields, damage from uncontrolled feeding by cranberry fruitworm, *A. vaccinii* (Lepidoptera: Pyralidae), may exceed 50% (Sarzynski and Liburd 2004). As in the case of the blueberry maggot, berries infested with this insect may be harvested and packaged without the detection of the larvae, which are later found by consumers. Larvae of *A. vaccinii* also feed on wild blueberries and cranberries, from which they can move to nearby comercial fields, causing serious problems. One cranberry fruitworm larva will feed on five to eight berries to complete its development and produce silk webbing which will make the entire cluster unmarketable (Sarzynski and Liburd 2004).

To meet quality standards, several applications of broad-spectrum insecticides (organophosphate and carbamate) are applied before harvest (O'Neal *et al*. 2005b). Public concerns about health and environmental risks associated with pesticides, increasing costs and the recent withdrawal of several inexpensive insecticides from the market (Cappaert and Smitley 2002) have boosted interest in integrated pest management, including the use of cultural practices and biological control (Szendrei *et al*. 2005).

Among the examined cultural practices, tillage was found to have a strong negative effect on Japanese beetle populations, because mechanical manipulation of their habitat affects the survival and development of larvae, which feed on grass roots, and adults, which prefer to lay eggs in grassy areas. Tilling the row middles of commercial blueberry fields has been shown to lead to a reduction (up to 72%) in the concentrations of *P. japonica* larvae, as compared with fields with untilled row middles (Szendrei *et al*. 2005). These results were the same regardless of whether the ground was tilled in spring or autumn. Cover crops can also be a valid alternative to tillage for management of *P. japonica*. Acid-tolerant cover crops that are not attractive to beetles can also provide additional benefits by mitigating the soil compaction caused by field machinery, and reducing erosion and pesticide runoff. Different cover crop species will have different effects on insect populations. For example, buckwheat (*Fagopyrum esculentum* Moench) followed by Alsike clover (*Trifolium hybridum* L.) and perennial ryegrass (*Lolium perenne* L.), grown for three years in row middles, consistently and significantly reduced adult beetle populations, even though to a lesser extent compare to bare ground plots where they were generally absent (Szendrei and Isaacs 2006).

Classical biological control could represent a powerful method for reducing the spread and the growth of populations of imported highbush blueberry pests. For example, Japanese beetle is considered an insignificant pest in its place of origin (Asia) where several natural enemies are present (Cappaert and Smitley 2002). Importation of these enemies may help to control the beetle in countries where it has no native enemies, like the US. Recently, in the US, mushy *P. japonica* grubs and grubs that were turning yellow or red were found. These larvae, probably infected with nematodes, viruses or bacteria, may represent the beginning of natural biocontrol.

Restrictions on the availability of broad-spectrum insecticides have spurred the development of new, low toxicity compounds, some of which are also certified as organic insecticides (Barry *et al*. 2005). Several of these compounds (spinosad, pyrethrum and azadirachtin) have been shown to control blueberry maggot, making them excellent candidates for incorporation into IPM and organic management programs (Barry *et al*. 2005).

In addition to more environmentally-friendly pesticides, several other innovative methods for the control of blueberry maggot have been developed recently. Biodegradable, baited spheres (9 cm diameter) treated with 2% imidacloprid successfully controlled *R. mendax* in highbush blueberry field trials. Results showed that the deployment of imidacloprid-treated spheres provided pest control similar to that provided by conventional organophosphate sprays, but without contaminating fruits with the insecticides (Stelinski and Liburd 2001). The "attract-and-kill" approach, using plastic and biodegradable traps coated with the insecticides fipronil and imidacloprid, was also found to be effective (Barry *et al*. 2004).

Prophylactic insecticide applications for the control of cranberry fruitworm can be optimized if based on the numbers of adult moths captured in pheromone-baited traps,

paying attention to the fact that appropriate placement of the pheromone traps (height and location within a planting) are crucial for accurate monitoring of male moth populations (Sarzynski and Liburd 2004).

Bud pests and disease vectors in highbush blueberry in North America

Pests that develop specifically inside buds cause significant damage to North American highbush blueberry crops. The most relevant are the blueberry gall midge, *Dasineura oxycoccana* (Diptera: Cecidomyiidae) and the blueberry bud mite, *Acalitus vaccinii* (Acari: Eriophyidae). Blueberry gall midge larvae feed exclusively on *Vaccinium* spp. bud tissue, inducing necrosis and bud abortion. The blueberry bud mite lives and feeds inside the fruit buds of both highbush and lowbush blueberry, causing poor plant growth and fruit set, particularly in the tops of plants (Isaacs and Gajek 2003).

Biological control of these bud pests, based on encouraging native natural enemies by limiting the use of broad-spectrum insecticides, can be quite successful in commercial fields. The main natural enemies of the gall midge, in the US, are eulophid wasps (parasitoids) and the predatory larvae of the hoverfly [*Toxomerus geminatus* (Say) Mets] (Sampson *et al*. 2002). A fungal parasite, *Hirsutella thompsonii* Fisher, and several species of predatory mites (tydeid and phytoseiid) have been found in association with the blueberry bud mite (Isaacs and Gajek 2003).

Chemical control of blueberry gall midge can be based on pre-bloom applications of malathion (up to 94% larva mortality). But, biopesticides, like spinosad, are also effective and help in the preservation of natural enemies (Sampson *et al*. 2002). Chemical control of *A. vaccinii* is based on postharvest applications of products such as endosulfan, as an improved management program that retains the activity of biological control agents has not yet been developed (Isaacs and Gajek 2003).

Insects are not only responsible for direct crop damage, but can also act as disease vectors. Several species of aphids (Hemiptera: Aphididae) colonize blueberry bushes, but the most serious aphid pests are those capable of transmitting viruses. Blueberry aphid [*Illinoia pepperi* (MacGillivray)] is the vector of blueberry shoestring virus. It is also a vector of blueberry scorch virus, which is also transmitted by *Ericaphis* spp. aphids (Terhune *et al*. 1991; Raworth 2004). In Canada, several species of aphids that act as vectors for blueberry scorch virus have been identified. Among these, the most important are *Ericaphis fimbriata* (Richards), *Aphis fabae* Scopoli, *Brachycaudus helichrysi* (Kaltenbach), *Hyalopterus pruni* (Geoffroy), *Hyperomyzus lactucae* (L.), *Myzus persicae* (Sulzer), *Rhopalosiphoninus staphyleae* (Koch), and *Rhopalosiphum padi* (L.) (Raworth 2004; Raworth *et al*. 2006). The "sharp-nosed leafhopper", *Scaphytopius* spp. (Homoptera: Cicadellidae), is the only known vector of blueberry stunt disease (Maeso Tozzi *et al*. 1993). Direct control of these insect vectors is an indirect way of protecting plants from these viruses and phytoplasma.

Insect pests in new areas of highbush blueberry cultivation

In Europe, as in other regions where highbush blueberry was introduced only recently, there are generally few infestations of insect pests that seriously affect the crop. For example, in Andalusia (southwestern Spain), several insect pests of highbush blueberry have been reported, including aphids, leaf rollers, an identified gall midge and hairy cetoniid beetles, but control measures have usually not been required (Barrau *et al*. 2003). The potential pests include native species that have adapted to highbush blueberry or non-indigenous ones imported from North America. In Italy, where highbush blueberry cultivation has increased significantly over recent years, the major pests are species of otiorhynchid weevils (*Otiorhynchus* spp., Coleoptera: Curculionidae) that damage roots, noctuid moths [e.g. *Operophtera brumata* (L.), *Conistra vaccinii* (L.) and *Eupsilia transversa* (Hufnagel)] which feed on foliage and flower clusters and some species of scale insects and aphids (Grassi and Forno 2004). All of these pests were introduced into blueberry fields from nearby crops and plants. In the 1990s, a new gall midge (*Jaapiella vacciniorum* Kieffer), known to develop in galls in the shoot tips of bilberry (*Vaccinium myrtillus* L.) in the Alps, was detected in highbush blueberry orchards in northeastern Italy (Grassi and Forno 2004).

Following the recent report of blueberry scorch virus in Europe (northern Italy) (Ciuffo *et al*. 2005), it will be necessary to evaluate the abilities of native species of aphids to transmit this disease.

Advanced biological techniques are currently applied in commercial fields against weevil larvae. Nematodes (*Heterorhabditis* spp.), which are applied to the field through the irrigation system, infect and kill otiorhynchid weevils in the soil. At the present time, this is the only effective method available, as chemical control has proven unsuccessful, against *Otiorhynchus* weevils in highbush blueberry.

AVAILABILITY OF A WIDE VARIETY OF *VACCINIUM* GERMPLASM

Blueberries are common worldwide and the genus *Vaccinium* includes about 400 species, on all continents except Australia and Antarctica, which exhibit a high degree of morphological diversity (Luby *et al*. 1991). The sections of the genus *Vaccinium* that have made the most significant contributions to today's commercial cultivars are *Cyanococcus*, *Vitis-idaea*, *Myrtillus*, *Vaccinium* and *Oxycoccus*. Blueberry germplasm resources have been divided into three main groups (Lyrene and Ballington 1986; Ballington 2001). These are i) cultivated species of *Vaccinium* section *Cyanococcus*, including 10-26 species, according to different taxonomic classifications; ii) non-cultivated species of *Vaccinium* section *Cyanococcus* and iii) species from other sections. Like other members of the *Ericaceae* family, they are mostly long-living, woody shrubs or vines.

The cultivated and semi-cultivated blueberries in the first group, derived from *Cyanococcus*, are related to a few main species: *V. corymbosum* L., *V. angustifolium* Ait., *V. virgatum* Ait., *V. elliottii* Chapm. and *V. darrowii* Camp. *V. corymbosum* is tetraploid (2n=4x=48) and has a very narrow genetic base. *V. angustifolium* is tetraploid, native to the northeast of the US and Canada and commercially significant. It is grown on rocky and dry uplands and typically harvested from managed, perennial fields (Burgher-MacLellan and MacKenzie 2004). *V. virgatum* is a hexaploid species that is commercially known as rabbiteye. It can be grown in different habitats and is better adapted to open fields than the highbush varieties, due to its high tolerance of drought, high temperatures and a wide range of soil pH levels, as compared with other *Vaccinium* species. *V. elliottii* is diploid, very suitable to low chill areas, with a crown-forming, usually upright, growth habit and habitat preferences similar to those of *V. virgatum*. There are two races of *V. darrowii*, both of which are evergreen (Lyrene 1986) and, unlike other species, have no chilling requirements.

The second group includes several non-cultivated species of the section *Cyanococcus*, such as *V. amoenum* Ait., *V. constablaei* Gray, *V. tenellum* Ait., *V. hirsutum* Buckley and others, that have been particularly important in interspecific hybrids. The third group includes the *Vaccinium* sections *Oxycoccus*, *Vitis-idaea*, *Myrtillus*, *Pyxothamnus*, *Bathodendron*, *Polycodium* and *Hemimyrtillus* (Ballington 2001). Cross-breeding of species from the section *Oxycoccus* with *Cyanococcus* has produced interesting results (Lyrene and Ballington 1986) that might be valuable for gene resources.

The *Vitis-idaea* section includes lingonberries and cowberries, which have been gathered for many years in Europe,

especially in the northern forests. These are perennial, evergreen, dwarf shrubs, which produce small berries that are still gathered from the wild. Domesticated varieties have been developed; however, they are still inconsistent in terms of production (Gustavsson 2001).

The section *Oxycoccus* is represented in northern and central Europe by *V. oxycoccus* L.; these small cranberry plants grow on ombrotrophic sphagnum bogs and minerotrophic fens in moist forests. The section *Myrtillus*, also found in Europe, includes the species *V. myrtillus* L. that may have received genetic material from globe huckleberry (*V. globulare* Rydb.), dwarf huckleberry (*V. caespitosum* Michx.) and blue huckleberry (*V. membranaceum* Dougl.). Another common plant in Europe belonging to the section *Vaccinium* is bog whortleberry (*V. uliginosum* L.), a long-living species with a mixed breeding system that has been incorporated into the Finnish blueberry cultivar 'Aron' (Lehmushovi 1982).

HIGHBUSH BLUEBERRY BREEDING

Cultivar selection is one of the most important decisions for berry producers. Germplasm collection and conservation, and the incorporation of this germplasm into breeding programs, are strategic assets for successful crop production.

Breeding of improved cultivars of highbush blueberry began after 1900 (Eck *et al*. 1990), when market demand increased and could no longer be satisfied by the quantities of available wild berries. The first highbush blueberry cultivar was named 'Brooks' and was selected by 'Coville' in New Hampshire (USA). This was followed by the introduction of 'Russel', a lowbush blueberry cultivar. Interspecific hybridization between homoploids has been crucial for the development of commercial blueberry cultivars. At the beginning of the 20th century, crosses were made between *V. stamineum* L. and *V. myrtilloides* Michx., *V. melanocarpum* Mohr. and *V. myrtilloides*, and *V. corymbosum* and *V. australe* Small. The genetic diversity of cultivated blueberry has been partially maintained over years of interspecific and intersectional hybridization, but inbreeding is a distinct risk in the current commercial cultivar landscape. Over the years, many efforts have been made to overcome crossing barriers in *Vaccinium*. These efforts have included field studies of native hybrids, ploidy manipulation, the use of mentor pollen (Wenslaff and Lyrene 2000), embryo culture, ovule culture and *in vitro* pollination (Lyrene 1986).

Certain species have been particularly valuable as breeding stock. *V. corymbosum* has been extremely useful source of germplasm for cold hardiness, fruit size and early ripening. *V. darrowii* and *V. elliottii* have been extensively used for the development of cultivars with reduced chilling requirements (i.e. cultivars that can be grown in regions characterized by mild winters), as well as adaptation to less acidic soils and light blue fruit color. *V. elliottii* has also been used as a source of resistance to stem blight.

The development of new blueberry cultivars has focused on the improvement of characteristics desired by both growers and consumers. Blueberry breeding programs, like those of other crops, have aimed for improved yield, improved fruit quality, improved resistance to biotic and abiotic stresses, adaptation to different soils and variations in flowering and fruiting periods.

Breeding for adaptation to non-traditional soils and water stress tolerance

Blueberry production on high pH substrates is difficult. Some genetic variability for soil pH tolerance has been reported and genetic improvement appears to be the only way to increase production on more alkaline soils (Korcak 1986).

Hybrid plants with *V. angustifolium* in their ancestry have been shown to be capable of absorbing and tolerating relatively high levels of magnesium. However, the hybrids with the least *V. corymbosum* germplasm produced the most vigorous growth (Korcak 1986) on upland soil conditions, while *V. ashei* Reade, *V. atrococcum* (Gray) and *V. darrowii* have been identified as potential germplasm sources for adaptability to upland soils (Galletta 1975).

There is a high degree of genetic diversity among *Vaccinium* species also for resistance to water deficit. A screening of interspecific seedlings conducted by Erb *et al*. (1988) showed that the southern species *V. darrowii*, *V. elliottii* and *V. ashei* were more drought resistant than *V. corymbosum*, *V. vacillans* Torr. and *V. myrtilloides*. This study also found that drought resistance appears to be highly heritable when crossing northern and southern species and that clones with half their germplasm from southern were usually drought resistant, in addition to being better adapted to milder winters.

Genotypes with reduced chilling requirements

The expansion of the areas of highbush blueberry production has required the development of commercially-viable cultivars that can thrive in regions where winters are mild (low chilling requirement). The hybrid *V. darrowii* x *V. corymbosum*, called 'US75', played a critical role in the development of southern highbush cultivars with low chilling requirements, like 'Cooper', 'Georgiagem', 'Gulfcoast', 'O'Neal' and 'Sierra', which includes genes from five species (Hancock *et al*. 1995). 'US75' has proved to be particularly able to increase or maintain the water use efficiency (Erb *et al*. 1991). *V. darrowii* and *V. elliottii* have been extensively used as sources for low chilling requirement germplasm and *V. darrowii-tenellum* hybrids were shown to be five times as productive when *V. darrowii* was the seed parent. 'Florida 4B' is a low chilling selection of the diploid *V. darrowii* that has also played a key role in the breeding of highbush blueberries. In addition to being a source of a number of desirable plant and fruit characteristics, it has also been used as a bridge to bring germplasm from other diploid species, which do not have unreduced gametes, into the highbush cultivars (Draper and Hancock 2003).

Cold hardiness and de-acclimation

There is an effort to find the optimal balance between cold hardiness and a low chilling requirement. Lack of cold hardiness and susceptibility to spring frosts significantly limit the current cultivars (Moore 1993), so that developing cold-hardy cultivars is a general priority. Arora *et al*. (1998) suggested that cold hardiness in blueberry may be controlled by a relatively small number of genes that have been previously identified with the dehydrin gene family, which is closely associated with adaptation to low temperature environments. Data suggest that the southern germplasm component in some breeding programs, *V. ashei*, may be the source of genes responsible for faster de-acclimation, whereas both southern species, *V. darrowii* and V. *ashei*, may contribute genes for cold sensitivity (Arora *et al*. 2004). *V. constablaei* Gray may also be useful in breeding programs, as a source of genes for late de-acclimation, which should translate into greater tolerance for spring frost and mid-winter hardiness (Rowland *et al*. 2005). In a paper published in 2007, Dhanaraj *et al*. reported many differences in the types of gene products induced under cold room conditions and those induced in the field, suggesting that cold acclimation under natural conditions could differ from the cold acclimation observed in the many studies that have been performed in artificial environments.

Breeding for disease resistance

For many years, wild *V. corymbosum* has been an important source of resistance to blueberry stem canker. 'Echota' is the first highbush blueberry cultivar with genes for stem canker resistance. The selection 'US41', a colchiploid *V. corymbosum*, has been used as source of resistance to *Phytophthora cinnamomi*. In different studies, individual cultivars have

shown resistance to one of these pathogens, but not necessarily the other; although, overall, the resistances were correlated.

A high level of resistance to mummy berry fruit rot was observed in all accessions of *V. boreale* Hall & Aalders, *V. myrtilloides*, *V. pallidum* Ait. and *V. tenellum*, and most accessions of *V. darrowi* (Stretch *et al*. 2001). Species screenings have identified excellent sources of resistance to mummy berry fruit rot, with *V. boreale* and *V. myrtilloides* showing excellent resistance to both phases of the disease (Ehlenfeldt *et al*. 2002).

V. angustifolium has proven to be a valuable source of genes for resistance to *Botryosphaeria* spp., while *V. ashei* has been used as a source of resistance to *Scaphytopius magdalensis* Prov., the vector of the blueberry stunt phytoplasma.

Breeding for fruit quality

The blueberry fruit traits which have been targeted by commercial breeding programs are related to what the consumers demand in terms of aroma, texture, postharvest quality and, particularly in the last eight to ten years, nutritional benefits, which we will discuss here only briefly.

Ballington *et al*. (1984) characterized 11 species and found that, although there is a significant amount of variability both among and within species, *V. stamineum* was the best of the examined species in terms of soluble solids, large fruit size and firmness. *V. elliottii* was easily harvested and *V. corymbosum* had a favorable balance between soluble solids and acids. The negative characteristics of wet stem scars and the appearance of blue color before sweetening vary widely among blueberry cultivars (Pritts and Hancock 1992).

Compounds present in blueberry have been shown to protect the nervous system (Sweeney *et al*. 2002; Joseph *et al*. 2003) and blueberry extracts have been found to have some ability to reverse declines in neural and cognitive functioning (Youdim *et al*. 2000). In particular, the anthocyanins found in blueberry have been found to effectively penetrate cell membranes and provide antioxidant protection (Galli *et al*. 2006). However, it has been suggested that the levels of polyphenols in berries are negatively correlated with desirable horticultural traits (Vorsa *et al*. 2002). On the other hand, Kalt (2001) found no correlation between fruit size and anthocyanin content. This same study also found that lowbush cultivars had higher concentrations of anthocyanins and total phenolics, as compared to highbush cultivars. In a screening project conducted in Italy (Giongo *et al*. 2006b) on 38 currently grown cultivars, the highest levels of total polyphenols were found in *V. angustifolium*, while the lowest concentrations were found in *V. corymbosum*. Anthocyanins represented the class of major quantitative relevancy and the second was represented by the hydroxycinnamic acids, primarily *trans*-chlorogenic acid. Genotypes with smaller berries had higher Oxygen Radical Absorbance Capacity (ORAC) values, as well as higher overall levels of phenolics (TPH), anthocyanins (ACY), hydroxycinnamic acids (HCA) and flavonols (FLA) than large-berried genotypes (Howard *et al*. 2003).

BIOTECHNOLOGICAL TOOLS AVAILABLE FOR USE IN BLUEBERRY

The development of DNA-based markers has facilitated species distinction and cultivar identification. Cultivated blueberries contain germplasm from several wild species and are grown around the world, mostly in temperate to tropical climates. Sequencing of the chloroplast *mat*K gene and the nrITS (Kron *et al*. 2002) has allowed researchers to study the phylogenetic relationships between closely related species and clarify our overall understanding of the taxonomy of the *Vaccinieae*. RAPD (random amplified polymorphic DNA sequence) markers have been used to distinguish cultivars and wild accessions (Burgher *et al*. 2002; Giongo *et al*. 2006a) and to discriminate between the three related species, cranberry (*Vaccinium macrocarpon*), lowbush blueberry (*V. angustifolium*) and lingonberry (*V. vitis-idaea*) (Debnath 2005). Already in 1994 a linkage map based on RAPD markers was published that covered 950 cM in 12 linkage groups corresponding to the haploid genome of 12 chromosomes of wild diploid *Vaccinium* species (Rowland and Levi 1994). RAPD markers have the advantage of being extremely easy to detect with only a set of commercial primers, a PCR thermocycler and gel electrophoresis apparatus. However, these markers are dominant, and the results of RAPD analyses are often difficult to reproduce. For these reasons, in identification and mapping studies in other woody perennial species, the use of RAPDs has mostly been replaced by the use of codominant microsatellites (Short Sequence Repeats). These markers are multiallelic and transposable across cultivars or even closely related species. However, they require a relevant input to be developed. For this reason, no frame of a consensus *Vaccinium* map is currently available. Such a framework would greatly facilitate mapping of relevant phenotypic traits.

Recombinant DNA technology has been explored as an alternative to classical breeding for introducing desired traits. Cao *et al*. (2003) have published detailed transformation protocols. Song and Sink (2004) developed an efficient transformation protocol, which they used to transform four relevant highbush blueberry cultivars. According to their published results, southern blotting confirmed that 15% of the produced explants were successfully transformed.

Classical biotechnological tools are used for micropropagation. For over 20 years, highbush blueberry has been propagated *in vitro*, and new selections can be rapidly introduced without any somoclonal aberration (Serres *et al*. 1997; Gajdosova *et al*. 2006).

Published protocols are based on the woody plant medium (Lloyd and McCown 1980), supplemented with various quantities of cytokinins (2iP or/and zeatin) (Chandler and Draper 1986). The reported range goes from 0.018 mM up 2 mg/l zeatin (Gonzalez *et al*. 2000; Zhang *et al*. 2006). Usually sugar levels had to be reduced compared to other woody plants and range from 15 to 60 mM depending on the cultivar. Zhang *et al.* (2006) report substitution of sucrose with sugar for economical reasons. Shoot rooting for commercial purpose is achieved by planting shoots in moss or a similar substrate under reduced light conditions after dipping or soaking them briefly in IBA (or NAA). Rooting and early stages of growth can be enhanced by addition of ericoid endomycorrhizae (Eccher and Noè 2002).

However the various protocols on the techniques and conditions of micropropagation depend not only on the cultivar but also on the specific use (Lopes da Fonseca and Romero Muñoz 2006), therefore a generalization on cannot be stated.

Use of biotechnology in the development of new blueberry cultivars

Blueberry breeding is done on a relatively small scale with relatively few resources. In light of this, few efforts have been directed toward the development of breeding aides, such as molecular markers for relevant traits. Most of the research that has been conducted in this area has been done by the Rowland group at the USDA/ARS fruit laboratory in Beltsville, Maryland (USA). This group has identified and isolated genes associated with cold hardiness in blueberry, including several members of the dehydrin gene family. Their publicly available EST data base can serve as starting point for the development of markers for genes of interest, map construction, fingerprinting, assessments of genetic diversity and marker-assisted breeding.

Highbush blueberry (*V. corymbosum*) is tetraploid, which means that an extremely large population is necessary for mapping any trait. Traits of interest for breeding programs include cold tolerance and low chilling requirements. Enhanced flexibility in both of these traits would allow the

expansion of production areas. The traditional approach of mapping traits based on their segregation patterns in a progeny population was followed by Rowland *et al*. (2003). They combined this approach with the identification and characterization of genes and gene products which respond to cold stress. In this way, they were able to identify a family of genes whose expression is induced by dehydration stress (freezing and drought). These 'dehydrins' are the most abundant proteins in cold-sensitive flower buds during the winter. They are present in all organs and their levels could be correlated to cold hardiness in three genotypes (Panta *et al*. 2001). A particular 14 kDa dehydrin was more abundant in cv. 'Bluecrop' than in the less cold-hardy and drought-tolerant cv. 'Premier' (Dhanaraj *et al*. 2005). Levels of blueberry dehydrins were shown to increase with cold acclimation and decrease with deacclimation and resumption of growth. However, the mapping of a marker derived from a clone of one of the three dehydrin genes, in a diploid *V. caesariense* Mack. population, did not suggest the co-segregation of these genes with the cold hardiness trait (Panta *et al*. 2004).

Marker-assisted breeding and DNA recombinant technology have not yet been applied to the development of new blueberry cultivars. However, the basic tools are available. Micropropagation, on the other hand, is routinely used for the production of high quality plant material.

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