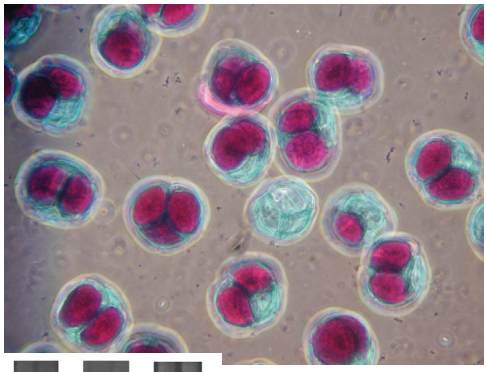
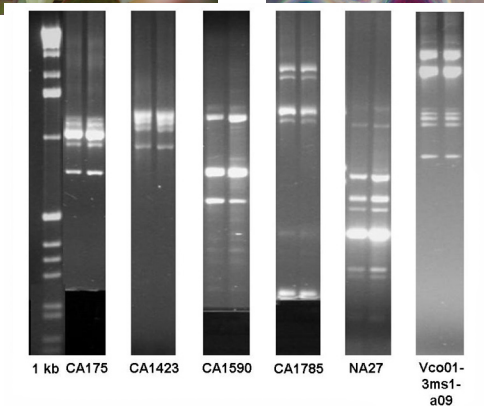
ISSN 1070–1524





Recent Advances in the Biology and Genetics of Lowbush Blueberry

Daniel J. Bell

Lisa J. Rowland

John Smagula

Frank Drummond

Technical Bulletin 203 October 2009

MAINE AGRICULTURAL AND FOREST EXPERIMENT STATION
THE UNIVERSITY OF MAINE

THE UNIVERSITY OF MAINE

MAINE AGRICULTURAL AND FOREST EXPERIMENT STATION
5782 WINSLOW HALL
ORONO ME 04469-5782

5-5-38900

A member of the University of Maine System

Recent Advances in the Biology and Genetics of Lowbush Blueberry

Daniel J. Bell
Research Scientist
University of Maine/United States Department of Agriculture, Agricultural Research Service

Lisa J. Rowland
Research Geneticist
United States Department of Agriculture, Agricultural Research Service

John Smagula
Professor of Horticulture
Department of Plant, Soil & Environmental Sciences
University of Maine

Frank Drummond
Professor of Insect Ecology/Entomology
School of Biological Sciences, University of Maine

Maine Agricultural & Forest Experiment Station
5782 Winslow Hall
University of Maine
Orono, ME 04469-5782

ACKNOWLEDGMENTS

This research was supported by various sources over the course of Daniel J. Bell's doctoral studies from January 2005 to June 2009. Included among these were teaching assistantships from the School of Biology and Ecology at the University of Maine and research assistantships and summer support from various sources in Professor Frank Drummond's lab, such as funding from the Wild Blueberry Commission and the Maine Agricultural and Forest Experiment Station. Daniel Bell also received 1½ years of financial support from Lisa J. Rowland of the U.S.D.A. in Beltsville, MD. In addition, we want to thank David Yarborough who throughout Daniel Bell's Ph.D. study was a most valuable resource and a good friend. His contributions to this technical bulletin are significant.

Contents

ECONOMIC IMPORTANCE TO MAINE AND NUTRITIONAL VALUE

People have harvested wild blueberries since antiquity. Within the past century, however, increased efforts in "tending" fields have increased their harvest potential. Today, Maine is the single largest producer of lowbush blueberries (*Vaccinium angustifolium* Ait. Section *Cyanococcus* A. Gray, Ericaceae) in the world (Yarborough 2009). The state produces 99 percent of the lowbush blueberries in the United States and 40 percent of the world's supply. Overall blueberry production in the U.S. is comprised of two-thirds cultivated highbush blueberry (*Vaccinium corymbosum* L.) with the remaining one-third being lowbush (Smagula and Yarborough 2004; Yarborough 2009). A small percentage is comprised of rabbiteye blueberry (*Vaccinium virgatum* Ait.). In Maine in 2008, from a harvested area of approximately 24,000 ha, the annual yield of lowbush blueberry was more than 40.8 million kg. The economic value to Maine, and particularly to the rural communities of Washington and Hancock counties in "Downeast" Maine, was approximately $75 million dollars (Yarborough 2009). Also, not measurable in direct economic terms, the wild, lowbush blueberry fields are a highly visible symbol of Maine's agricultural heritage and are part of the culture of Downeast Maine.

In addition to blueberries' economic value, recent nutritional research has identified numerous health benefits from a diet that includes blueberries (Xianli 2004). Wild blueberries naturally contain substances called anthocyanins, which are derived from the color pigments in blueberries and act as antioxidants. Blueberries have recently been shown to have one of the highest antioxidant activities compared to the other fruits tested including cranberries, apples, and red and green grapes (Prior et al. 1998; Wolfe and Liu 2007). Antioxidants are associated with health benefits such as retarding age-related diseases like Alzheimer's and enhancing memory (Duffy 2008). Other health benefits linked to antioxidants include reducing eye strain, preventing macular degeneration, and exhibiting anti-cancer activity (Cho et al. 2004; Kalt et al. 2007). Rimando (2004) reports reduction in the risk of heart disease. A key component of the marketing of blueberries emphasizes increasing public awareness of these benefits.

MANAGEMENT/CULTURAL PRACTICES

As a managed agricultural ecosystem, lowbush blueberry is unique. The seeds from which the vast expanses of Maine's "blueberry barrens" arose were never sown directly by human beings. Rather, after land clearing by natural (fire, glaciers) or man-made (fire, tree removal) events, the blueberries, which had colonized landscapes earlier via a process called ecological succession (a transformation from their native origins in the natural forest understory), are released and flourish. Contemporary cultivation methods, which primarily focus on optimizing conditions for growth by limiting competition and deterring disease, has produced an "artificially prolonged seral stage" (Hall et al. 1979) in which the blueberry barrens have remained for many decades.

Lowbush blueberry fields visually appear as a mosaic of individuals (genotypes), which are referred to as "clones." Clones are genetically distinct individuals that grow vegetatively by a slowly expanding underground network of stems called "rhizomes." Whitton (1964) has stated that the term is technically inaccurate since clones are unique and only represent a single individual; nonetheless, it is the adopted term. Properly, according to the scientific clonal literature, a unique genetic individual is a "genet" while the numerous genetically identical branches arising from the genet are called "ramets" (Eriksson 1989; Harper 1978). Assuming that every clone in Maine is unique, Yarborough (2009) has estimated from average coverage calculations that there are perhaps some 6.5 million individual clones in Maine in managed fields alone. Based on estimates of rhizome growth rates, clones have life spans that exceed 200 years (Eaton and Hall 1961; Vander Kloet 1976; Drummond et al. 2008). Interclonal variation is highly pronounced in many biological traits, e.g., age, height, color and hue, phenology, and yield. Although these expanses of lowbush blueberry are a managed and integral component of Maine's agricultural economy, natural processes over perhaps millennia (Borns 2004; Drummond et al. 2008) produced the distribution and genetic variation evident in the millions of diverse lowbush blueberry clones. Undeniably, at least in this regard, the system is correctly referred to as "wild" (limited selection pressure by humans).

Being wild, blueberries are used as food (including the leaves for some animals) by black bear (*Ursus americanus*), white-tailed deer (*Odocoileus virginianus*), and numerous other small mammals and birds, particularly migratory robins (*Turdus migratorius*) (Eaton 1957; Galleta 1975; Hall et al. 1979; Martin et al. 1951). Prior to the 17th century, Native Americans burned areas of forest to en-

courage blueberry growth (Russell 1976). Later, European settlers began to clear forests for blueberry production, and by 1860, more than 200,000 acres were harvested and blueberry, shipped by train, was a major export to other states (Russell 1976). By the early 20th century, growers began more actively managing blueberry land and canning the fruit for long-term preservation (Wood 2004).

Cultivation of lowbush blueberry has always been focused on the management of wild stands. In early years, pruning by burning occurred infrequently, but by the 1930s, most blueberry fields were burned every third year in Maine (Shoemaker 1948). Standard modern cultivation practice has demonstrated that yields are maximized when the crop is grown on a two-year cropping cycle, with alternating vegetative and fruiting (yield) years (DeGomez 1988). Prior to the 1980s, following the yield year, fields were either burned with straw or oil burners. Mowing has replaced burning, however, as a more cost-efficient and environmentally friendly alternative (Yarborough and Hoepler 2001). Presently a majority of the managed blueberry land in Maine is mowed (Yarborough pers. comm.).

Production practices have been designed to optimize conditions for growth while minimizing damage due to disease and insect pests and competition from weeds. The intensity and integration of these practices have increased, especially in the latter half of the 20th century. One high-profile example of this has been the increased use of fertilizers (Yarborough and Smagula 1993), pesticides (Drummond 2000; Yarborough and Bhowmik 1988), and rented honey bees (Drummond 2002; Karmo 1957, 1958; Wood 1961). Increasingly, cultivation practices are being designed to be environmentally friendly and highly integrated, such as integrated crop management practices (Yarborough et al. 2001).

During the last several decades, yields have increased severalfold in Maine (Yarborough 2004) due to practices such as the optimal use of fertilizers through leaf tissue analysis for N and P and soil analysis for pH. An organic "pad," the result of decades of leaf litter accumulation, is characteristic of an unplowed wild blueberry soil. Most rhizomes and roots are found in the upper 3 in. of soil, where the organic matter content can be 10% to 14%. This is also where most of the nutrients are located. In Maine, soil samples are taken to determine pH, but not nutrient content, since positive correlations between yield and soil nutrient levels have not been found. Soil pH ranges from 3.9 to 6.2, and sulfur is recommended to lower the pH to 4.0. A reduction in soil pH makes the nutrient environment more favorable for blueberries and less so for weeds, thereby reducing weed pressure and the need for herbicides. In a

recent study in blueberry plots, using sulfur to lower soil pH to about 4.0 had no negative effect on yield, compared with unadjusted plots at pH 4.6.

Fertilizer recommendations are based on composite leaf tissue samples taken at 90% to 100% tip dieback, the cessation of the spring growth flush from the rhizome. This usually occurs during the first week of July in Maine. At tip dieback, the changes in nutrient concentrations are minimal. Maine and Canadian standards of satisfactory levels of nutrients in leaf tissue at tip dieback have been established. Although nutrient deficiency symptoms are rarely observed, yield has been increased when leaf nitrogen (N) and phosphorus (P) concentrations have been raised to above the N standard (1.6%) and P standard (0.125%). Potassium, with a standard of 0.4%, does not appear to be deficient in Maine soils. Fertilizing with other nutrients such as iron, boron, zinc, and copper have not improved growth or yield. The University of Maine Analytical Laboratory currently gives growers recommendations for fertilizer based on N and P concentrations of leaf samples. Monoammonium phosphate or diammonium phosphate are the most commonly applied fertilizers to correct N and P deficiency in lowbush blueberry.

Monitoring techniques have been developed for insect pests and action thresholds have been established (Drummond 2000). These pest management tactics allow growers to determine when, what kind, and the dosage and frequency of pesticide application that is necessary to deter damages from weeds, diseases, and insect pests such as blueberry spanworm and thrips (Yarborough and Drummond 2009). Even the expensive investment in irrigation has been shown to be cost effective (Seymour et al. 2004). There has also been a decrease of hand-harvesting and an increase of mechanical harvesters, with approximately 80% of the fields in Maine now machine harvested (Canfield 2007; Yarborough 2001).

HABITAT

Lowbush blueberry is a low-growing prostrate shrub endemic to North America. Under the Raunkiaer life-form classification system, it is a chamaephyte (stem height ranging between 20 and 50 cm). Its Canadian range extends from Newfoundland to Lake Winnipeg in Manitoba. Its latitudinal range extends from 57° N, in Quebec to 38° N in Virginia (Hall et al. 1979).

From studies of evolutionary history, researchers surmise that lowbush blueberry has been associated with a variety of severe environments including acidic, poorly drained sandy soils and

extreme temperature fluctuations (Vander Kloet 1978). In undisturbed settings, these habitats include exposed headlands, raised bogs, outcroppings, mountain summits, and the herb-dwarf shrub stratum of open to shaded pine-spruce understory (Hall et al. 1979). The species is regarded as shade intolerant and does not flower at less than 50% full sunlight (Hall et al. 1979). Thus, its tenacious clonal habit, in which it can persist for very long periods of time without sexual reproduction, conveys a fitness component suitable for survival under such circumstances (Klimes et al. 1997). Also, symbiotic mycorrhizal (fungal) associations have been documented in blueberry, which may aid in mineral absorption since the species does not possess root hairs (Addoms and Mounce 1931). This symbiotic association presumably augments nutrient uptake of P (MacArthur 1955) and N, which can come from protein compounds in the soil (Litten and Smagula 2002)

These life history traits, coupled with its prolific fecundity during the infrequent sexual recruitment periods and its ability to disperse seeds long distances through the consumption of berries by mammals and birds, explain in part the success of lowbush blueberry in the harsh habitats of the northeastern coastal regions of North America (Eaton 1957; Hill and Vander Kloet 2005). In recent times, due to its enhanced ability as a primary colonizer, its most visible natural occurrence is in disturbance communities resulting from geological events, clearcut forests, natural and man-made fires, and the abandonment of agricultural lands. According to Whitton (1964), lowbush blueberry was primarily harvested on the extensive coastal plain that is still called the blueberry barrens located near Cherryfield and Harrington, Maine. This treeless, glacier-scarred landscape, referred to geologically as the Pineo Ridge (Borns 2004), still represents a significant portion of the harvested areas in Maine.

CLASSICAL TAXONOMY

Although noted and catalogued by Lamarck in 1783 under the name of *Vaccinium pennsylvanicum* and further documented by Gray (1848) under the same name, it was not until Camp's comprehensive monograph (Camp 1945) that the taxonomic status of the lowbush blueberry was officially codified under the rules of modern botanical nomenclature. Due to the high frequency of polyploidy, both autopolyploidy (a whole genome duplication event in which the chromosome number of the species has effectively doubled), and allopolyploidy (a doubling of the chromosome number, generally

thought to create a new species, by the merging through sexual hybridization of two different but related species), the genus Vaccinium and the section *Cyanococcus* (taxonomic grouping below species) have long been considered difficult ones for classification and have been subject to frequent revisions (Bruederle and Vorsa 1994; Galleta and Ballington 1996; Lyrene et al. 2003; Ortiz et al. 1992; Vander Kloet 1972, 1988; Watson and Dallwitz 1992). The following taxonomic description of *V. angustifolium* is after Camp (1945) as presented in Whitton (1964):

Plants in dense and sometimes extensive colonies, 5–20 cm high, (twigs of the current season, green). Leaves deciduous, green; the lower surface shining, non-glandular, glabrous (or rarely with some pubescence on the veins); usually narrowly elliptic, 0.4–1 cm wide, 1–3 cm long; the margin sharply serrate. Corolla cylindraceous, 3–5 mm long, usually white. Fruit bright blue, 5–7 mm in diameter, of excellent flavor.

Camp (1945) recognized a sub-family within the Ericaceae (Heath family), the Vacciniaceae. This distinction was based primarily upon fruit structure (fleshy) and a wholly or partially inferior ovary. Within this sub-family he recognized two genera, *Vaccinium* and *Gaylussacia*, the only two genera that occur in the temperate latitudes. Today, as per the most recent taxonomic treatments and revisions of the group, the "true, cluster-fruited" berries are within the section *Cyanococcus* (Bruederle and Vorsa 1994; Camp 1945; Galleta and Ballington 1996; Vander Kloet 1988) and include all the cultivated blueberry species. The only wild representatives of the section Cyanococcus are in North America (Hancock and Draper 1989). On a worldwide basis, the genus *Vaccinium* contains approximately 400 species. One or more representatives of this genus can be found on all continents except Antarctica and Australia (Ballington 2001; Lyrene et al. 2003).

Vander Kloet (1978) states that since the publications of both Fernald's (1950) and Gleason's (1952) respective floras of the northeastern United States, the taxonomic treatment of lowbush blueberry has been the "centre of considerable controversy." Camp (1945) noted high levels of intraspecific variation within section *Cyanococcus,* which Bruederle and Vorsa (1994) described as consisting of a large number of morphological variations both in sexual and vegetative features, characteristics of the flower, fruit, leaf, twig, and general habit. In his doctoral dissertation, Vander Kloet (1972) used 44 eco-morphological traits in a clustering model (Rubel's) to reduce Camp's 24 basic populations into 10 distinct species. As even more evidence of the group's complexity, the taxonomy of

Vaccinium has undergone numerous revisions during the last few decades (Bruederle and Vorsa 1994; Galleta and Ballington 1996; Lyrene et al. 2003; Ortiz et al. 1992; Vander Kloet 1972, 1988; Watson and Dallwitz 1992) and is still considered unresolved (Boches et al. 2006). With regard to *V. angustifolium*, although individual lowbush clones appear "different" from each other, it is impossible to estimate levels of genetic similarity among clones in a field or across great distances by visual observation alone. Thus, other methods based on genetic markers have been developed and shown to be useful (Bell 2009; Bell et al. 2008, 2009).

GENETIC COMPOSITION: PLOIDY AND HYBRIDIZATION

The complex and polymorphic nature of the genus *Vaccinium* may in part be due to the interaction of a variety of genetic, physiological, and historical characteristics. Camp (1942) summarized these as follows: (1) the lack of significant reproductive barriers between homoploids, (2) functional self-sterility, (3) a high incidence of polyploids, (4) general shade and alkaline intolerance, and (5) given the antiquity of the genus, the accumulated impact of geological events and changes in distribution and gene flow patterns over many millennia.

Longley (1927) was the first to document 24 bivalents at synapsis, specifically during diakinesis, which is the phase of meiosis (sexual cell division) where recombination of genetic material from homologous chromosomes occurs. Longley (1927) thus established that his two voucher specimens, of what is now known as *V. angustifolium*, from Massachusetts and New Hampshire, were tetraploid. Newcomer (1941) later substantiated this work. Neither of these researchers, however, provided any cytological evidence that V. angustifolium was an autotetraploid. According to Whitton (1964) and others, the absence of multivalents does not exclude the possibility of autotetraploidy. Curiously, Camp (1942) thought that V. angustifolium was a diploid, and he believed that its rarer, wild derivative *Vaccinium lamarkii* was a tetraploid. Darrow et al. (1942, 1944) put forth evidence in support of this conclusion. However, according to Whitton (1964) and Vander Kloet (1978), both Camp and Darrow based their conclusions on erroneously identified voucher specimens that were probably the diploid *Vaccinium boreale* Hall & Aald. Today, therefore, the binomial, *V. lamarkii* Camp is no longer valid and the correct binomial is *V. angustifolium*. In the first large-scale cytological study of chromosome counts in wild

clones (n = 275) from eastern Canada, Hall and Aalders (1961) established that *V. angustifolium* was indeed a tetraploid (2n = 12x = 48). More recent evidence supports this now unchallenged conclusion (Bent and Vander Kloet 1976; Hersey and Vander Kloet 1976; Whitton 1964).

The question of whether *V. angustifolium* is an allo- or autotetraploid, however, is more difficult and remains more one of probability than certainty. Whitton (1964) concludes his doctoral dissertation "favoring" allopolyploidy for a variety of reasons. Using the evidence available at the time, Whitton (1964) based his conclusion on a possible series of events: (1) the initial production of a fertile hybrid between *V. boreale* and *V. myrtilloides,* (2) significant introgression towards *V. boreale,* and (3) a genome duplication event that resulted in chromosome doubling in the hybrids. From an evolutionary perspective, Whitton (1964) goes on to speculate that such an allopolyploid hybrid complex would possess an array of plants that possibly could have been capable of exploiting the rapidly changing habitat landscape characteristic of the post-glaciation colonization periods of the last 13,000 years.

Later, Vander Kloet (1977, 1978) proposed two theories that supported this conclusion. These were that either it is an allotetraploid of *V. boreale* x *Vaccinium pallidum* Ait. or *V. boreale* x *V. myrtilloides* Michx. According to Hokanson and Hancock (1993), Vander Kloet's two theories seem to have been based primarily on the superficial resemblance of artificial hybrids between the two possible progenitor species and *V. angustifolium*. However, in this same paper, Hokanson and Hancock (1993) reanalyzed the data of Hall and Aalders (1963) who studied the inheritance and segregation ratios of the "white-fruit" character. Hall and Aalders (1963) concluded, using a two-factor model in which both loci had to be in the homozygous recessive condition for the white-fruit character to be manifested, that the inheritance was disomic. Hokanson and Hancock (1993) tested an alternative hypothesis using a single-locus model in which four recessive copies at one locus were required for the white-fruit trait. They used the "Yates" chi-square correction, which purportedly strengthens the power of the test when sample sizes are small (Strickberger 1985). Their results indicated that the segregation ratios were closer to tetrasomic than disomic inheritance. They cited also additional circumstantial evidence of the generally complete inter-fertility between *V. angustifolium* and the known autotetraploid *V. corymbosum* (Krebs and Hancock 1989). They state that, if true, this would be the only example of a successful hybridization between an autotetraploid and an allotetraploid (Steb-

bins 1950). In summary, as the title of the Hokanson and Hancock (1993) article reads, it is concluded that *V. angustifolium* "may be an autotetraploid."

Currently, the issue remains unresolved, but it is not merely an academic question. In an autotetraploid, tetrasomic inheritance would allow the genetic variability in the original progenitor to segregate, but it would take much longer to reach fixation in the homozygous state than with disomic (diploid-like) inheritance (Bingham 1980). Put another way, Haldane (1930) has shown that autotetraploids approach homozygosity at one-third the rate of diploids and thus may be more buffered against inbreeding depression (and possibly "near-parental" inbreeding depression) than allotetraploids with disomic inheritance. This may be an important dynamic in the breeding system in lowbush blueberry. As stated before, clones can be very old and quite large. Observations of bee foraging point indirectly to the conclusion that much self-pollen deposition must be occurring (an area of study currently underway via molecular approaches). Therefore, tetrasomic inheritance patterns would be expected to "buffer" the deleterious effects of such selfing, which is considered the most severe form of inbreeding depression. This is a highly evocative and expanding area of research in the genetics of yield in lowbush blueberry.

BIOCHEMICAL MARKERS: ISOZYMES/ALLOZYMES

Biochemical molecular markers (isozymes/allozymes) were first used in immunological studies of the 1960s (Altukhov and Salmenkova 2002). The advent of protein electrophoresis allowed the separation of proteins by size and charge on gels and allowed insight into the hidden, hereditary variation within organismal genomes. One of the earliest studies using biochemical markers in blueberry genetics was Vorsa et al. (1988), in which isozyme variation and inheritance in blueberry were described. Since then, isozymes/allozymes have been used in several genetic studies including the documentation of tetrasomic inheritance in highbush blueberry (Krebs and Hancock 1989).

Although still useful and even complementary to newly emergent technologies, DNA markers offer a much greater resolution of genetic variability than is generally obtainable from isozymes (Altukhov and Salmenkova 2002). The invention of the polymerase chain reaction (Mullis et al. 1986) has made possible the amplification of billions of copies of short segments of DNA in short periods

of time that can be used for further analyses such as fingerprinting, determining genetic relationships and population genetics.

GENOMIC DNA MARKERS IN LOWBUSH BLUEBERRY: RAPD MARKERS

Burgher et al. (2002) were the first to use RAPD (randomly amplified polymorphic DNA) markers in lowbush blueberry. They showed that RAPD technology (Williams et al. 1990), which is based on arbitrary PCR primers (and therefore presumably are distributed across the entire genome, both coding and non-coding regions), could be used to identify and examine relationships between specific clones of lowbush blueberry from a selection of 26 genotypes of varied origins from Maine and Nova Scotia. RAPD primers are generally 10 bases in length and the fragments that are amplified from them are scored as dominant markers (present/absent). Burgher et al. (2002) showed that the Maine accessions grouped together as expected using both principal coordinate and clustering analysis, but that the Nova Scotia accessions did not. Since they could discriminate individual clones (intraspecific), Burgher et al. (2002) concluded that RAPDs could be used to study closely related genotypes within fields as would be needed in pollination genetic studies.

RAPD MARKERS: SGS STUDIES IN OTHER *VACCINIUM* SPECIES

Since 2000, several researchers have used RAPD markers to study the patch structure, or the genetic homogeneity of patches, of other wild, rhizomatous *Vaccinium* species, including bog whortleberry and deerberry (Albert et al. 2004, 2005; Garkava-Gustavsson et al. 2005; Kreher et al. 2000; Persson and Gustavsson 2001). These studies have attempted to describe genetic relationships as a function of physical distance and to characterize the patterns of "patches" in terms of clonal theory, phalanx or guerilla (Lovett-Doust 1981), and the type and frequency of seedling recruitment and dispersal. The observations in these studies show that although there is a broader tendency for phalanx organization of patches, visually apparent patches themselves often consist of numerous genets, i.e., the G/N ratio (unique genotypes/total number of samples) was often much less than unity. Also, the majority of genetic diversity was found within populations, but significant between-field (populations) structuring was demonstrated by AMOVA (Analysis of Molecular

Variance). Furthermore, between-field differentiation tended to increase with increasing distance between fields. Importantly, Albert et al. (2005) found that clones within fields demonstrated random structure, i.e., spatial distance and genetic similarity were not correlated. None of these studies, however, has used molecular markers to experimentally address questions involving the genetics of yield and reproduction in *V. angustifolium*.

Recently, another kind of molecular marker termed ISSR (inter-simple sequence repeat) was used to discriminate between 43 *V. angustifolium* clones in Nova Scotia (Debnath 2009). ISSR markers possess a similar benefit to RAPDs in that no previous knowledge of the genome is necessary for their use. Principal coordinate analysis revealed two distinct clusters among the 43 clones, which were collected from 10 communities located in four Canadian provinces. The authors cite that the method provides a cost-effective way to develop a germplasm collection for future use in evaluating existing genetic resources in breeding programs.

EST-PCR (EXPRESSED SEQUENCE TAG-POLYMERASE CHAIN REACTION) MARKERS

A decade ago, RAPD technology was criticized due to concerns about reproducibility between labs (Powell et al. 1996). Partly to address this concern, but also to develop a marker from actual gene sequences, Rowland et al. (2003a, 2003b) developed expressed sequence tag-PCR (EST-PCR) markers from highbush blueberry. These were developed from mRNAs expressed in cold-acclimated and nonacclimated buds. Currently, there are approximately 5,000 of these cDNA sequences or ESTs available in GenBank. Rowland et al. (2003a, 2003b) showed that primers designed near the ends of these ESTs amplified polymorphic fragments useful for cultivar identification and genetic relationship studies in highbush and rabbiteye blueberry and were potentially useful in Vaccinium species as distantly related as cranberry.

In a recent work, Bell et al. (2008) demonstrated that these same EST-PCR markers designed from highbush blueberry also proved useful for estimating genetic relationships between clones within cultivated fields of lowbush blueberry in Maine. One known pedigree of four genetic individuals, derived from breeding experiments in the 1970s, was appropriately identified as a family. Moreover, Bell et al. (2009) showed that these markers could also be used to estimate spatial genetic structure (SGS) at three scales: (1) within a clone (clonal fidelity), (2) between clones within fields, and (3)

between fields of clones. An important finding in this study was the scale at which significant SGS appeared in fields of wild, lowbush blueberry. At the within-field level, clones were not organized in space; however, at the between-fields level (distances between fields ranging from 12.5 to 65 km) significant between-population differentiation was found via analysis of molecular variation (AMOVA). Simply, although clones within fields exhibited random spatial structure, on the whole individuals were more genetically similar to each other (related) than were clones from different fields. Particularly, the random structure of clones within fields may have important implications for possible management practices such as bee placement.

SSR MOLECULAR MARKERS

Generally, simple sequence repeat (SSR) molecular markers are considered to be one of the most polymorphic and reproducible DNA marker available. These snippets of sequence repeats are caused by mistakes in DNA replication (replication slippage) and consist of short tandem repeat sequences (1-6 bp), which are often flanked by unique, conserved regions of DNA (Tautz and Rentz 1984; Weber and May 1989). Microsatellites (SSRs) are ubiquitous across the genomes of most organisms (Li et al. 2002). They can sometimes be treated as codominant and have become the preferred marker for forensics and DNA fingerprinting in animals and plants (Wunsch and Hormaza 2002). A drawback for many researchers, however, is that, as opposed to RAPD markers, SSRs have to be developed de novo for unrelated species and this is expensive and time consuming. Fortunately, this work has been started and is progressing in blueberry.

Boches et al. (2005) described 30 microsatellite primer pairs that were developed from the EST libraries of Rowland et al. (2003a, 2003b). Since then, Boches et al. (2006) have shown that these SSRs can be used in the assessment of genetic diversity in both wild and cultivated highbush blueberry. In tetraploids, like highbush and lowbush blueberry, exact allelic copy number cannot be discriminated with current technologies. However, even with this current limitation, their microsatellite analysis was useful for measuring heterozygosity in 69 blueberry cultivars and wild accessions of highbush blueberry showing an average of 17.7 alleles per locus (Boches et al. 2006). This is higher than that of maize (12.2 alleles), which underscores how genetically polymorphic blueberry is. The availability of these types of markers, like EST-PCRs and

SSRs, in blueberry allows questions of clonal fidelity, spatial genetic structure, and genetics of yield to now be addressed in lowbush blueberry (Bell et al. 2009).

LIFE HISTORY: GROWTH, REPRODUCTION, AND SURVIVAL

Understanding the biological success and distribution of lowbush blueberry requires an appreciation of its bimodal life history, as both a clonal and sexual organism. Competition, according to Milne (1961), is universally accepted as an integral component of organismal interaction and is generally viewed to be synonymous with Darwin's (1859) "struggle for existence." Lowbush blueberry has been referred to as a "poor inter-specific competitor" in terms of open-field succession (which it curiously and prolifically occupies in Maine via human intervention), but is regarded as a highly successful primary colonizer in those same fields and in its more natural occurrence in the harsh, isolated habitats described earlier.

Its life history, as viewed along a long time line, is dualistically capable of long periods of vegetative persistence in extremely harsh habitats and when conditions permit, periods when it produces and disseminates large numbers of seeds contained in berries. The risks and perhaps limited periodicity involved in sexual reproduction is likely to be a part of the reason for an increase in clonal life form presence in alpine regions (Körner 2003). When sexual recruitment does take place, berries and therefore seeds of lowbush blueberry are dispersed significant distances away from their maternal parents via migratory birds and other frugivores. Furthermore, they have been recently found to persist much longer in seed banks (> 15 years) than previously thought (Hill and Vander Kloet 2005). In short, lowbush blueberry possesses both a potent sexual and asexual combination of life-history strategy for coping with the problems of regeneration and dissemination in a harsh and challenging environment.

Grime (1977) extends the r-K selection theory of MacArthur and Wilson (1967) through a model that constructs primary strategies for survival in vascular plants. Grime classifies two axes, (1) the intensity of disturbance and (2) the intensity of stress. From a two-way classification of these axes, three viable dual classifications derive, listed here in order of their occurrence along an r-K continuum: (1) ruderal (high disturbance, low stress); (2) competitive (low disturbance, low stress); and (3) stress-tolerant (low disturbance, high stress). These ideas are discussed here to underscore

that lowbush blueberry has been somewhat "domesticated." In its more native occurrences, as per Grime's categories, the species might lie within the ruderal category (initial colonization both in severe natural conditions and in cleared, abandoned pasture lands) and as a stress-tolerant organism in the severe habitats such as rocky outcroppings. In the latter case, it is capable of taking hold (via clonal ability) in ecologically stressed environments. However, in the managed field context, upon which this review is centered, it is artificially forced to remain in a seral stage of succession (Hall et al. 1979) by the eradication of competitors and disease, via selective herbicides and pesticides. Thus, though its clonal nature may better serve in natural settings for its distribution and habitat, it is the sexual component that agricultural management practices optimize. Under the optimized conditions of light, water, nutrients, and pollinators, its sexual prowess and extreme fecundity in berry production renders this crop valuable to Maine's agriculture.

ASEXUAL—CLONAL GROWTH

The estimated 6.5 million clones or unique genotypes under cultivation in Maine each started by the establishment of a single seed. The concept of a clone is a tenuous one and has often been described as a poorly defined concept in plant biology. In fact, as per the definition of Cook (1983), a clone should be reserved for a vegetatively propagating plant, which by inherent design separates its "zygotic individual" (genet) from its "physiological individual" (ramet). In this strict sense, lowbush blueberry may not fit. Hay and Kelly (2008) have stressed the need for biologists to include the "trait" of disintegration and fragmentation as an integral component of the definition of clones. In one of his examples, Cook (1983) cites *Viola blanda,* which spreads by underground rhizomes in such a way that eventually the originating center dies and thereby cleaves into two distinct clonally identical daughter individuals. This is not the case with lowbush blueberry, since it does not possess such a design (clones are unique and have no duplicates) and thus, should perhaps not be termed clones (Whitton 1964). Lowbush blueberry does grow extensively underground via a network of rhizomes that take hold and root variously at slowly extending positions away from the center, sending up ramets vertically from those points. In addition, perhaps largely due to this mode of vegetative persistence, lowbush blueberry clones are exceptionally long lived.

Clonality may be a bet-hedging strategy (Cook 1979, 1983) to maximize persistence when sexual reproduction is not possible.

However, Cook (1979), among others, presented still further explanations as to its (clonality) fitness value. Being underground stems, rhizomes of lowbush blueberry possess growth rings. Interestingly, exhuming rhizomes and counting these rings has only resulted in age estimations of approximately 21 years (Hall et al. 1979) when it was known that the entire clone was much older. It seems as if the older portions of rhizomes senesce and die, while development by meristematic multiplication of ramets continues. Cook (1979), along with Eriksson (1989, 1993) argue that this is a form of "risk spreading" (lessening risk of genet death). The clonal individual seems to possess "immortality" in its ability to continually produce vegetatively by the proliferation of ramets while older components senesce and die. Indeed, Hamilton et al. (1987) argue that studying clonal biology means studying the "population demographics of ramets," not zygotic individuals. Although theoretically this is intriguing, perhaps the most important characteristic of the prodigious belowground growth in rhizomes is the storage and buffering capacity this affords the whole plant. Lowbush blueberry is resilient to destruction, for example, by fire, either wild or set by people. Fire destroys the aboveground portions of the clone, while the belowground rhizome is left to send up shoots later at a more conducive time. Lowbush blueberry is a resilient organism.

SEXUAL REPRODUCTION

Lowbush blueberry produces terminally situated racemes of hermaphroditic (possesses both male and female reproductive organs in a single flower) flowers. Generally considered 5-merous, it possesses a distinctly bell-shaped (urceolate) corolla that tends to hang downwards. This is thought to facilitate buzz-pollination via "sonication" (vibration of flight muscles) by the bumble bee (Bombus spp.). It has 10 stamens in two whorls of five that are highly specialized as poricidal anthers. These are thought to act as dispensers of pollen and regulate its delivery (Harder 1990; Harder and Barclay 1994). The style generally protrudes from the corolla opening and is receptive only on its surface. Meiosis is completed first in the anthers, then about a week later in the ovules (Bell and Burchill 1955). Then, when flowers open between mid-May and mid-June (in Maine), both male and female gametes are mature. Pollen (2n) is shed in tetrads from the anthers. It has been estimated that pollen takes about three to four days to reach the ovules (Bell 1957). The average number of ovules per flower as cited by Bell (1957) is 64.2. Bell (1957) stresses in his classic anatomical growth and

development paper of the lowbush blueberry seed, that the species produces an inordinate number of aborted seeds, even in outcrossed, supplemental hand pollinations. His data show that the average number of perfect seeds (germinable) was 13.3, and imperfect seeds (aborted, not germinable) 49.9, and concludes that this species is a hybrid (see previous discussion in Genetic Composition: Ploidy and Hybridization section).

SELF-FERTILITY

In general, the floral morphology is technically described as "herkogamic," i.e., it displays a physical separation of male and female reproductive organs within the same flower. This is thought to inhibit the deposition of self-pollen on the same flower (autogamy). From my own dissections (Bell 2009), this seems effective as self-pollen clings to the side of the flaring trumpet-shaped style and falls out and away from the receptive surface of the stigma. Furthermore, caged or bagged flowers set no fruit (Bell 2009). Lowbush blueberry has been thought to be self-infertile as a result of gametophytic self-incompatibility (GSI) since it possesses two of the hallmark attributes of GSI, bi-nucleate pollen and wet stigmas (de Nettancourt 2001; Igic and Kohn 2001; Vander Kloet and Lyrene 1987). No evidence of stylar arrest of pollen tubes via RNase, however, has been documented, which means that lack of self-fertility must be caused by other post-zygotic factors. Furthermore, in highbush and lowbush blueberry pollen chase experiments, in which self-pollen is "chased" by outcrossed pollen three days later, increased numbers of aborted seeds result because outcrossed pollen (applied after self pollen) is precluded from fertilizing already fertilized ovules by self-pollen. This reveals that self-pollen reaches and fertilizes ovules, and thus experimentally eliminates the possibility that a functional pre-zygotic SI system is operative (Hokanson and Hancock 2000; Krebs and Hancock 1988, 1990, 1991). Therefore, self-infertility in both highbush and lowbush blueberry is thought to be due to early-acting inbreeding depression.

POLLINATORS

The lowbush blueberry crop is pollinated primarily by bees (Delaplane and Mayer 2000). Other pollinator taxa, however, have been observed visiting blueberry flowers (Drummond unpublished data; Stubbs et al. 2007) suggesting that the diversity of pollinators might also include Apocrita wasps (especially hornets [Vespidae]),

ants (Formicidae), flower flies (Syrphidae), and humming birds (Trochilidae). Without pollinators, near zero fruit set results. The most important native pollinators include *Andrena* spp., *Megachile rotundata* F., *Halictus* spp., and *Osmia* spp. (Drummond and Stubbs 1997b; Javorek et al. 2002). Since the 1950s, use of the domesticated honey bee, *Apis mellifera* L. (Karmo 1958), has increased dramatically. However, recent concerns about the disappearance of bees in what is called colony collapse disorder (Cox-Foster et al. 2007), along with a host of other disease concerns, has spurred renewed interest in the introduction and/or management of other bees.

The following exotic bee species have been found to have potential for commercial management in lowbush blueberry in Maine: *Anthophora pallipes* (Stubbs and Drummond 2000), *Osmia ribifloris* (Stubbs et al. 1994), and the alfalfa leafcutting bee, *Megachile rotundata* (Stubbs and Drummond 1997a, 1997b). Management tactics, including screening of potentially hazardous insecticides and stocking-density guidelines, have been developed for the alfalfa leafcutting bee in lowbush blueberry (Stubbs and Drummond 1997b, 1998, 1999). Only a few acres are currently being pollinated with this bee in Maine, but it is being used to pollinate several thousands of acres of lowbush blueberry in the Canadian Maritimes and Quebec. Species native to Maine that have been investigated are the Maine blueberry bee, *Osmia attriventris* (Drummond and Stubbs 1997a; Stubbs et al. 2000), and *Bombus impatiens* (Stubbs and Drummond 2001b; Stubbs et al. 2001). Efforts have also been focused on surveys of the native bee fauna associated with lowbush blueberry and its conservation (Drummond and Stubbs 2003; Stubbs et al. 1996, 1997; Stubbs and Drummond 2001a). In addition, Drummond (2002) maintains an active education program in pollination tactics for lowbush blueberry using the honey bee.

Commercial bumble bees, *B. impatiens,* are now used on about 2,000 acres of lowbush blueberry in Maine each year (Frank Drummond pers. commun.). The recommended stocking densities are cited as three to four colonies (200-worker-strength colonies) per acre by Stubbs et al.(2001). A more recent study in Quebec also suggests that bumble bees can be an effective commercial pollinator of lowbush blueberry (Desjardins and Olivereira 2006).

BREEDING

According to Hall (1983), a breeding program on lowbush blueberry was begun in 1961 through the initial collection of superior clones, principally based on berry size. These clones were collected

from the wild in areas of eastern Canada and Maine. In a series of short papers that commenced in the mid-1970s, the results of these early breeding efforts were described and included the release of several named cultivars and selections. They were Augusta (Aalders et al. 1975), Blomidon (Hall and Aalders 1982), Brunswick (Aalders et al. 1977), and Chignecto (Hall et al. 1977) among others. Breeding of lowbush blueberry is complicated by its ploidy, heterozygosity, the complexity of quantitatively inherited traits such as yield, and perhaps most severely by the difficulty and three- to six-year timeframe for establishing fruit-bearing progeny.

Hall (1983) noted that this breeding program resulted in a statistically significant improvement in both size and yield. This conclusion was based on experimental evidence presented in his paper showing that yield diminished progressively from crosses involving only select clones, select clones sired by open pollinated clones, and average clones sired by unknown open donors. Although this was not analyzed as a diallel, which is a full or partial factorial crossing design generally analyzed by statistical methods of Griffing (1956), he demonstrated that there was promising evidence that the phenotypic selections could result in a genetic advance in breeding, i.e., that there was a heritable genetic component involved in yield. This early observation that clones could convey through phenotypic selection quantitatively complex traits, such as yield, to offspring has been recently corroborated and expanded by Bell (2009).

In this work, Bell (2009) experimentally demonstrated via field one-source hand crosses, that self-fertility was a significant predictor of outcross fertility. Also, from a random selection of five clones that were crossed in all possible combinations in an experimental design referred to as a complete diallel (Griffing's Model 2, Method 3), it was shown that both general (GCA) and specific (SCA) combining abilities were significant, which translated to a moderately high narrow-sense heritability for three yield traits measured. Basically, if parents were high yielders, they tended to predictably convey this trait to their progeny. Also, highly self-fertile clones tended to be good yielders. This work revealed possibilities for a renewed era in breeding in which clones would be screened for self-fertility, either conventionally by hand crosses, or possibly via a molecular marker approach. These clones could then be used in further breeding experiments such as diallels to identify both (1) individual high-producing clones (significantly positive GCA) and (2) at a second hybrid level, specific combinations of clones that showed significantly positive SCA, or what is known as hybrid vigor. The identified clones could then be bred to produce seed families after

the methods of Jamieson (2008a, 2008b), which could be used to fill in bare areas of fields.

Hall (1983) adds that yield was not based solely on the size and weight of berries. Other whole-plant characteristics such as floral density per stem, stem density, and exogenous factors such as pollinator presence all contribute to the whole-plant horticultural yield. Thus, it has been clearly shown that genetic improvement via clonal selection and breeding is possible.

PROPAGATION METHODS

The time period to fruit-bearing maturity, via any of the known propagation methods, is currently a significant limiting factor in the implementation of breeding advances in lowbush blueberry. The three best-known methods of propagation have been reviewed by Litten and Smagula (2000). They are via seedlings, softwood cuttings, and micropropagation, and each has its own set of advantages and disadvantages. Jamieson and Nickerson's (2003) findings, showing that seedling propagation results in wider rows of plants over time than cuttings, represents one of the best recent attempts at developing an efficient method of propagation. Such horticultural technology is a mandatory prerequisite to the establishment of genetically superior clones developed through breeding. Also, Jamieson (2008a, 2008b) has shown that hybrid seed produced from crosses between select clones is an effective approach for producing plants of superior yield and fruit quality.

SUMMARY AND GOALS FOR FUTURE DEVELOPMENT

The lowbush blueberry industry in Maine is not immune from the possible effects of global climate change and increased competition. In a recent quote in the Boston Globe, Dr. David Yarborough from the University of Maine states: "That's the fear, Quebec could outstrip Maine in the future" (Daley 2007). Quebec is opening up more acreage to lowbush blueberry production every year due to the decreasing frequency of "killer spring frosts" that had always limited expansion in the past. Thus, research aimed at further elucidating the complex reproductive genetics, particularly leveraging newly developed molecular markers, should be started in order to understand the specific biological causes of the greatly varying yield between clones in this system. Such knowledge may spur the development of new management practices such as filling in bare areas of fields with superior clones derived from seed families

(Jamieson 2008a, 2008b). Recent publications by Bell et al. (2008, 2009) are evidence that this work has begun. To remain a leader in wild, lowbush blueberry production, Maine needs to invest in new molecular approaches to better understand the genetic basis of yield in this species.

LITERATURE CITED

Aalders, L.E., I.V. Hall, and L.P. Jackson. 1977. Brunswick lowbush blueberry. *Can. J. Plant Sci.* 52:301.

Aalders, L.E., A.A. Ismail, I.V. Hall, and P.R. Hepler. 1975. Augusta lowbush blueberry. *Can. J. Plant. Sci.* 55:1079.

Addoms, R.M., and F.C. Mounce. 1931. Notes on the nutrient requirements and the histology of the cranberry (*Vaccinium macrocarpon* Ait.) with special reference to mycorrhiza. *Plant Physiol.* 6:653–668.

Albert, T., O. Raspe, and A.L. Jacquemart. 2004. Clonal diversity and genetic structure in Vaccinium myrtillus populations from different habitats. *Belg. J. Bot.* 137:155–162.

Albert, T., O. Raspe, and A.L. Jacquemart. 2005. Diversity and spatial structure of clones in *Vaccinium uliginosum* populations. *Can. J. Bot.* 83:211–218.

Altukhov, Y.P., and E.A. Salmenkova. 2002. DNA polymorphism in population genetics. *Russian J. Genetics* 38(9): 989–1008.

Ballington, J.R. 2001. Collection, utilization and preservation of genetic resources in *Vaccinium*. *HortSci.* 36:213–220.

Bell, D.J. 2009. Spatial and genetic factors influencing yield in lowbush blueberry (*Vaccinium angustifolium* Ait.) in Maine. Ph.D. dissertation, School of Biological Sciences, University of Maine, Orono.

Bell, D.J., L.J. Rowland, J.J. Polashock, and F.A. Drummond. 2008. Suitability of EST-PCR markers developed in highbush blueberry for genetic fingerprinting and relationship studies in lowbush blueberry and related species. J. Amer. Soc. Hort. Sci. 133(5): 701-707.

Bell, D.J., L.J. Rowland, D. Zhang, and F.A.Drummond. 2009. Spatial genetic structure of lowbush blueberry, *Vaccinium angustifolium,* in four fields in Maine. *Botany* 87:1-15.

Bell, H.P. 1957. The development of the blueberry seed. *Can. J. Bot.* 35:139–153.

Bell, H.P., and J. Burchill. 1955. Flower development in the lowbush blueberry. *Can. J. Bot.* 33:251–258.

Bent, F.C., and S.P. Vander Kloet. 1976. Reports on Ericaceae. IOPB chromosome number reports L, II. Presented by Askell Love. *Taxon* 25:344–345.

Bingham, E.T. 1980. Maximizing heterozygosity in autopolyploids. *Polyploidy: Biological Relevance,* ed. W.H. Lewis. Plenum Press, New York. pp 471–489.

Boches, P.S., N.V. Bassil, and L.J. Rowland. 2005. Microsatellite markers for Vaccinium from EST and genomic libraries. *Molec. Ecol. Notes* 5:657–660.

Boches, P.S., N.V. Bassil, and L.J. Rowland. 2006. Genetic diversity in the highbush blueberry evaluated with microsatellite markers. *J. Amer. Soc. Hort. Sci.* 131(5): 674–686.

Borns, H.W.J. 2004. The deglaciation of Maine, U.S.A. *Quaternary Glaciations—Extent and Chronology, Part II (North America),* ed. J Ehlers and PL Gibbard. Elsevier, New York. pp 89–109.

Bruederle, L.P., and N. Vorsa. 1994. Genetic differentiation of diploid blueberry, *Vaccinium* sect. *Cyanococcus* (Ericaceae). *Systematic Bot.* 19(3): 337–349.

Burgher, K.L., A.R. Jamieson, and X. Lu. 2002. Genetic relationships among lowbush blueberry genotypes as determined by randomly amplified polymorphic DNA analysis. *J. Amer. Soc. Hort. Sci.* 127(1): 98–103.

Camp, W.H. 1942. A survey of the American species of *Vaccinium,* subgenus *Euvaccinium*. *Brittonia* 4:205–247.

Camp, W.H. 1945. The North American blueberries with notes on other groups of Vacciniaceae. *Brittonia* 25:203–275.

Canfield, C. 2007. Blueberry growers use mechanical harvester. *USA Today* (Aug. 25).

Cho, E., J.M. Seddon, B. Rosner, W.C. Willett, and S.E. Hankinson. 2004 Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. *Arch. Ophthalmol.* 122:883–892

Cook, R.E. 1979. Asexual reproduction: A further consideration. *American Naturalist* 113:769–772.

Cook, R.E. 1983. Clonal plant populations. *American Scientist* 71:244–253.

Cox-Foster, D.L., S. Conlan, E.C. Holmes, G. Palacios, J.D. Evans, N.A. Moran, P. Quan, T. Briese, M. Hornig, and D.M. Geiser. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Scienceexpress 10.1126/science.1146498.

Daley, B. 2007. As frost fades, berry rivalry heats: Temperatures pit Maine, Quebec growers. *The Boston Globe* (October 1).

Darrow, G.M., W.H. Camp, H.E. Fischer, and H. Dermen. 1942. Studies on the cytology of Vaccinium species. *Proc. Amer. Soc. Hort. Sci.* 41:187–188.

Darrow, G.M., W.H. Camp, H.E. Fischer, and H. Dermen, 1944. Chromosome numbers in *Vaccinium* and related groups. *Bull. Torrey Bot. Club* 71:498-506.

Darwin, C. 1859. *The Origin of Species by Beans of Natural Selection or the Preservation of Favored Races in the Struggle for Life*. Murray, London.

Debnath, S.C. 2009. Development of ISSR markers for genetic diversity studies in *Vaccinium angustifolium*. *Nordic J. Botany* 27(2): 141–148.

de Nettancourt, D. 2001. *Incompatibility and Incongruity in Wild and Cultivated Plants,* 2nd ed. Springer-Verlag, New York.
DeGomez, T. 1988. Pruning lowbush blueberry fields. Univ. of Maine Cooop. Ext. Wild Blueberry Fact Sheet No. 229,
Delaplane, K.S., and D.F. Mayer. 2000. *Crop Pollination by Bees.* CABI Publishers, New York.
Desjardins, E.C., and D.D. Olivereira. 2006. Commercial bumble bee *Bombus impatiens* (Hymenoptera: Apidae) as a pollinator in lowbush blueberry (Ericales: Ericaceae) fields. *J. Econ. Entomol.* 99(2): 443–449.
Drummond, F.A. 2000. History of insect pest management for lowbush blueberries in Maine. *Trends in Entomol.* 3:23–32.
Drummond, F.A. 2002. Honeybees and blueberry pollination. Univ. of Maine Coop. Ext. Wild Blueberry Bulletin No. 629.
Drummond, F.A. and C.S. Stubbs. 1997a. Potential for management of the blueberry bee, *Osmia atriventris* cresson. *Acta Hort.* 446:77–83.
Drummond, F.A. and C.S. Stubbs. 1997b. Sampling bee populations in lowbush blueberry in Maine. Proc. 6th Internat. Symp. on Vaccinium Culture. Acta Hort. 446:101-108.
Drummond, F.A., and C.S. Stubbs. 2003. Wild bee conservation for wild blueberry fields. Univ. of Maine Coop. Ext. Wild Blueberry Fact Sheet No. 629.
Drummond, F.A., J.M. Smagula, S. Annis, and D. Yarborough. 2008. Organic wild blueberry production. Maine Ag. & For. Exp. Sta. Bulletin 852.
Duffy, K. 2008. A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainate-induced learning impairment in rats. *Neurobiol. Aging* 29(11): 1680–1689.
Eaton, E.L. 1957. The spread of blueberry seed through manure and by migrating robins. *Proc. Am. Soc. Hort. Sci.* 69:293–295.
Eaton, E.L., and I.V. Hall. 1961. Taxonomy and development. The Blueberry in the Atlantic Provinces. Research Branch, Canada Department of Agriculture Publication 754. pp 7–15.
Eriksson, O. 1989. Seedling dynamics and life histories in clonal plants. *Oikos* 55:231–238.
Eriksson, O. 1993. *Dynamics of Genets in Clonal Plants.* Elsevier Science Publishers, New York.
Fernald, M.L. 1950. *Gray's Manual of Botany,* 8th ed. American Book Co., New York.
Galleta, G.J. 1975. Blueberries and cranberries. *Advances in Fruit Breeding,* ed. J. Janick and J.N. Moore. Purdue Univ. Press, West Lafatette, IN. pp 154–196.
Galleta, G.J., and J.R. Ballington. 1996. Blueberries, cranberries, and lingonberries. *Fruit Breeding, Vol. II: Vine and Small Fruits Crops,* ed. J. Janick and J.N. Moore. John Wiley & Sons, New York. pp 1–107.
Garkava-Gustavsson, L., H.A. Persson, H. Nybom, K. Rumpunen, B.A. Gustavsson, and I.V. Bartish. 2005. RAPD-based analysis of genetic

diversity and selection of lingonberry (*Vaccinium vitis-idaea* L.) material for ex situ conservation. *Genet. Resources & Crop Evol.* 52:723–735.

Gleason, H.A. 1952. *The New Britton and Brown Illustrated Flora of the North-Eastern United States and Adjacent Canada.* New York Botanical Garden, New York.

Gray, A. 1848. *A Manual of Botany of the Northern United States.* American Book Co., New York.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* 9:463–493.

Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111(982): 1169–1194.

Haldane, J.B.S. 1930. Theoretical genetics of autopolyploids. *J. Genet.* 22:359–372.

Hall, I.V. 1983. Genetic improvement of the lowbush blueberry, *Vaccinium angustifolium*. *Can. J. Plant. Sci.* 63:1091–1092.

Hall, I.V., and L.E. Aalders. 1961. Cytotaxonomy of lowbush blueberries in eastern Canada. *Am. J. Bot.* 48(3): 199–201.

Hall, I.V., and L.E. Aalders. 1963. Two-factor inheritance of the white fruit in the common lowbush blueberry, *Vaccinium angustifolium* Ait. Can. *J. Genet. Cytol.* 5(4): 371–373.

Hall, I.V., and L.E. Aalders. 1982. Blomidon lowbush blueberry. *Can. J. Plant. Sci.* 62:519–521.

Hall, I.V., L.E. Aalders, and L.P. Jackson. 1977. Chignecto lowbush blueberry. *Can. J. Plant. Sci.* 57:1217–1218.

Hall, I.V., L.E. Aalders, N.L. Nickerson, and S.P. Vander Kloet. 1979. The biological flora of Canada. 1. *Vaccinium angustifolium* Ait., sweet lowbush blueberry. *Canadian Field-Naturalist* 93(4): 415–430.

Hamilton, N.R.S., B. Schmid, and J.L. Harper. 1987. Life-history concepts and the population biology of clonal organisms. *Proc. Royal Soc. Lond.* 232:35–57.

Hancock, J., and A.D. Draper. 1989. Blueberry culture in North America. *HortSci.* 24:551–556.

Harder, L.D. 1990. Pollen removal by bumble bees and its implications for pollen dispersal. *Ecology* 71(2): 1110–1125.

Harder, L.D., and R.M.R. Barclay. 1994. The functional significance of poricidal anthers and buzz pollination: Controlled pollen removal from Dodecatheon. *Funct. Ecol.* 8:509–517.

Harper, J.L. 1978. The demography of plants with clonal growth. *Structure and Functioning of Plant Populations,* ed. A.H.J. Freysen and J.W. Woldendorp. North-Holland Publishing, Amsterdam. pp 7–48.

Hay, M.J.M., and C.K. Kelly. 2008. Have clonal plant biologists got it wrong? The case for changing the emphasis to disintegration. *Evol. Biol.* 22:461–465.

Hersey, R., and S.P. Vander Kloet. 1976. IOPB chromosome number reports L11. *Taxon* 25:241–346.

Hill, N.M., and S.P. Vander Kloet. 2005. Longevity of experimentally buried seed in *Vaccinium:* Relationship to climate, reproductive factors and natural seed banks. *J. Ecol.* 93:1167–1176.

Hokanson, K., and J. Hancock. 1993. The common lowbush blueberry, *Vaccinium angustifolium* Ait. may be an autopolyploid. *Can. J. Plant. Sci.* 73:889–891.

Hokanson, K., and J. Hancock. 2000. Early-acting inbreeding depression in three species of *Vaccinium* (Ericaceae). *Sex. Plant. Reprod.* 13:145–150.

Igic, B., and J.R. Kohn. 2001. Evolutionary relationships among self-incompatibility RNases. *PNAS* 98(23): 13167–13171.

Jamieson, A.R. 2008a. Developing seed-propagated lowbush blueberry families. *HortSci.* 43(6): 1686–1689.

Jamieson, A.R. 2008b. 'Novablue', a seed-propagated lowbush blueberry family. *HortSci.* 43(6): 1902–1903.

Jamieson, A.R., and N.L. Nickerson. 2003. Field performance of the lowbush blueberry propagated by seed, stem cuttings and micropropagation. *Acta Hort*. 626:423–428.

Javorek, S.K., K.E. Mackenzie, and S.P. Vander Kloet. 2002. Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). *Ann. Entomol. Soc. Am*. 95(3): 345–351.

Kalt W., J.A. Joseph, and B. Shukitt-Hale. 2007. Blueberries and human health: A review of the current research. *J. Am. Pomol. Soc.* 61:151–160

Karmo, E.A. 1957. Pollination of the lowbush blueberry. Nova Scotia Fruit Grower's Association Annual Report 94:93–97.

Karmo, E.A. 1958. Honey bees as an aid in orchard and blueberry pollination in Nova Scotia. *10th Internat. Cong. Ent. Proc.* 4:955–959.

Klimes, L., J. Klimesova, R. Hendriks, and J.M. van Groenendal. 1997. Clonal plant architecture: A comparative analysis of form and function. *The Ecology and Evolution of Clonal Plants,* ed. H. de Kroon and J.M. van Groenendal. Backhuys, Leiden. pp 1–29.

Körner, C. 2003. *Alpine Plant Life,* 2nd ed. Springer, Berlin.

Krebs, S.L., and J.F. Hancock. 1988. The consequences of inbreeding on fertility in *Vaccinium corymbosum* L. *J. Amer. Soc. Hort. Sci.* 113(6): 914–918.

Krebs, S.L., and J. Hancock. 1989. Tetrasomic inheritance of isoenzyme markers in the highbush blueberry, *Vaccinium corymbosum* L. Heredity 63:11–18.

Krebs, S.L., and J. Hancock. 1990. Early-acting inbreeding depression and reproductive success in the highbush blueberry, *Vaccinium corymbosum* L. Theor. *Appl. Genet.* 79:825–832.

Krebs, S.L., and J.F. Hancock. 1991. Embryonic genetic load in the highbush blueberry, Vaccinium corymbosum (Ericaceae). Am. J. Bot. 78(10): 1427–1437.

Kreher, S.A., S.A. Fore, and B.S. Collins. 2000. Genetic variation within and among patches of the clonal species, *Vaccinium stamineum* L. *Molec. Ecol.* 9:1247–1252.

Li, Y., A.B. Korol, T. Fahima, A. Beiles, and E. Nevo. 2002. Microsatellites: Genomic distribution, putative functions, and mutational mechanisms: A review. *Molec. Ecol.* 11:2453–2465.

Litten, W., and J.M. Smagula. 2000. Why not tame the wild blueberry? Maine Ag. & For. Exp. Sta. Misc. Rept. 415.

Litten, W., and J.M. Smagula. 2002. Ericoid mycorrhiza as now understood. *Acta Hort.* 574:25–29.

Longley, A.E. 1927. Chromosomes in *Vaccinium*. *Science* 66:565–568.

Lovett-Doust, L. 1981. Population dynamics and local specialization in a clonal perennial (*Rannunculus repens*). The dynamics of ramets in contrasting habitats. *J. Ecol.* 69:743–755.

Lyrene, P.M., N. Vorsa and J.R. Ballington. 2003. Polyploidy and sexual polyploidization in the genus *Vaccinium*. *Euphytica* 133: 27–36.

MacArthur, M. 1955. Mycorrhiza in the blueberry. Horticulture Division, Central Experimental Farm, Ottawa, Progress Report 1949–53. pp 71–72.

MacArthur, R.H., and E.D. Wilson. 1967. *The Theory of Island Biogeography*. Princeton University Press, Princeton, NJ.

Martin, A.C., H.S. Zim, and A.L. Nelson. 1951. American Wildlife and Plants. Dover Publications, Inc., New York.

Milne, A. 1961. Definition of competition among animals. *Mechanisms in Biological Competition,* ed. F.L. Milnthorpe. Cambridge University Press, London. pp 40–61.

Mullis, K.B., F.A. Faloona, S. Scharf, R.K. Saiki, G. Horn, and H.A. Erlich. 1986. Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. Cold Spring Harbor Symposia on Quantitative Biology.

Newcomer, E.H. 1941. Chromosome numbers of some species and varieties of Vaccinium and related genera. *Proc. Am. Soc. Hort. Sci.* 38:468–470.

Ortiz, R., L.P. Bruederle, T. Laverty, and N. Vorsa. 1992. The origin of polyploids via 2n gametes in *Vaccinium* section *Cyanococcus*. *Euphytica* 61:241–246.

Persson, H.A., and B.A. Gustavsson. 2001. The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf-shape analysis. *Molec. Ecol.* 10:1385–1397.

Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, and S.V. Tingey. 1996. The comparison of RFLP, AFLP, and SSR (microsatalite) markers for germplasm analysis. *Mol. Breeding* 2:225–238.

Prior, R.L., G.H. Cao, A. Martin, E. Sofic, J. McEwen, C. O'Brien, N. Lischner, M. Ehlenfeldt, W. Kalt, G. Krewer, C.M. Mainland. 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J Agric Food Chem* 46:2686–2693

Rimando, A.M., W. Kalt, J.B. Magee, J. Dewey, and J.R. Ballington. 2004. Resveratrol, pterostilbene, and piceatannol in Vaccinium berries. *J. Agric. Food Chem.* 52:4713–4719

Rowland, L.J., A.L. Dhanaraj, J.J. Polashock, and R. Arora. 2003a. Utility of blueberry-derived EST-PCR primers in related Ericaceae species. *HortSci.* 38(7): 1428–1432.

Rowland, L.J., S. Mehra, A. Dhanaraj, E.L. Ogden, J.P. Slovin, and M.K. Ehlenfeldt. 2003b. Development of EST-PCR markers for DNA fingerprinting and genetic relationship studies in blueberry (*Vaccinium,* section *Cyanococcus*). *J. Amer. Soc. Hort. Sci.* 128(5): 682–690.

Russell, H.S. 1976. *A Long, Deep Furrow: Three Centuries of Farming in New England.* University Press of New England, Hanover, NH.

Seymour, R.M., G. Starr, and D.E. Yarborough. 2004. Yield and quality differences of lowbush blueberry (*Vaccinium angustifolium*) in irrigated and rain-fed conditions. *Small Fruits Review* 3(1/2): 45–56.

Shoemaker, J.S. 1948. *Small-Fruit Culture,* 2nd ed. The Blakiston Co., Philadelphia.

Smagula, J., and D. Yarborough. 2004. Factors contributing to the increase in productivity in the wild blueberry industry. *Small Fruits Review* 3:33–43.

Stebbins, D.L. 1950. *Variation and Evolution in Plants.* Columbia University Press, New York.

Strickberger, M.W. 1985. *Genetics,* 3rd ed. Macmillan Publishing Company, New York.

Stubbs, C.S., and F.A. Drummond. 1997a. Blueberry and cranberry (*Vaccinium* spp.) pollination: A comparison of managed and native bee foraging behavior. *Acta Hort.* 437:341–343.

Stubbs, C.S., and F.A. Drummond. 1997b. Pollination of wild lowbush blueberry, *Vaccinium angustifolium* by the alfalfa leafcutting bee, *Megachile rotundata. Acta Hort.* 446:189-196.

Stubbs, C.S., and F.A. Drummond. 1998. Asana, impact on alfalfa leaf-cutting bees and other pollinators. *Arthropod Manag. Tests* 23:52.

Stubbs, C.S., and F.A. Drummond. 1999. Effects of Asana XL on honey bees and alfalfa leafcutting bees pollinators of lowbush blueberry. *Arthropod Manag. Tests* 24:69.

Stubbs, C.S., and F.A. Drummond. 2000. Pollination of lowbush blueberry by *Anthophora pallipes villosula* and *Bombus impatiens* (Hymenoptera: Anthophoridae and Apidae). *J. Kansas Ent. Soc.* 72(3): 330–333.

Stubbs, C.S., and F.A. Drummond. 2001a. B*ees and Crop Pollination—Crisis, Crossroads, Conservation.* Thomas Say Publications, Lanham, MD.

Stubbs, C.S., and F.A. Drummond. 2001b. *Bombus impatiens* (Hymenoptera: Apidae): An alternative to *Apis mellifera* (Hymenoptera: Apidae) for lowbush blueberry pollination. *J. Econ. Entomol.* 94(3): 609–616.

Stubbs, C.S., F.A. Drummond, and S.L. Allard. 1997. Bee conservation and increasing *Osmia* spp. in Maine lowbush blueberry fields. *Northeast Naturalist* 4(3): 133–144.

Stubbs, C.S., F.A. Drummond, and H. Ginsberg. 2007. Effects of invasive plant species on pollinator service and reproduction in native plants at Acadia National Park. Tech. Rept. NPS/NER/NRTR—2007/096

Stubbs, C.S., F.A. Drummond, and E.S. Osgood. 1994. Os*mia ribifloris biedermannii* and *Megachile rotundata* (Hymenoptera: Megachilidae) introduced into the lowbush blueberry agroecosystem in Maine. *J. Kansas Ent. Soc.* 67(2): 173–185.

Stubbs, C.S., F.A. Drummond, and D. Yarborough. 2000. Field conservation management of native leafcutting and mason *Osmia bees.* Univ. of Maine Coop. Ext. Wild Blueberry Fact Sheet No. 301.

Stubbs, C.S., F.A. Drummond, and D. Yarborough. Commercial bumble bee, *Bombus impatiens,* management for lowbush blueberry. Univ. of Maine Coop. Ext. Wild Blueberry Fact Sheet No. 302.

Stubbs, C.S., E.A. Osgood, J.B. Dimond, and F.A. Drummond FA. 1996. Hymenoptera diversity in Maine. *Biological Diversity in Maine,* ed. S.C. Gawler, J.J. Albright, P.D. Vickery, and F.C. Smith. Maine Natural Areas Program, Maine Forest Biodiversity Project, Augusta, ME. pp 81–86.

Tautz, D., and S. Rentz. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Research* 12:4127–4137.

Vander Kloet, S.P. 1972. The North American blueberries revisited: A taxonomic study of *Vaccinium* section Cyannococcus Gray. Ph.D. dissertation, Queen's University at Kingston, Ontario.

Vander Kloet, S.P. 1976. A comparison of the dispersal and seedling establishment of *Vaccinium angustifolium* (the lowbush blueberry) in Leeds County, Ontario and Pictou County, Nova Scotia. *Can. Field-Naturalist* 90:176–180.

Vander Kloet, S.P. 1977. The taxonomic status of *Vaccinium boreale.* Can. J. Bot. 55:281-288.

Vander Kloet, S.P. 1978. Systematics, distribution, and nomenclature of the polymorphic *Vaccinium angustifolium. Rhodora* 80:358–376.

Vander Kloet, S.P. 1983. The taxonomy of *Vaccinium* section *Cyanococcus:* A summation. *Can. J. Bot.* 61:256–266.

Vander Kloet, S.P. 1988. The genus *Vaccinium* in North America. Research Branch Agriculture Canada Publication 1828.

Vander Kloet, S.P., and P.M. Lyrene. 1987. Self-incompatibility in diploid, tetraploid, and hexaploid *Vaccinium corymbosum. Can. J. Bot.* 65(4): 660–665.

Vorsa, N., P.S. Manos, and M.I. van Heemstra. 1988. Isozyme variation and inheritance in blueberry. *Genome* 30:776–781.

Watson, L., and M.J. Dallwitz. 1992. The Families of Flowering Plants: Descriptions, Illustrations, Identification, and Information Retrieval. Version: 14th December 2000.

Weber, J.L., and P.E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Amer. J. Human Genet.* 44:388–396.

Whitton, L. 1964. The cytotaxonomic status of *Vaccinium angustifolium* (Aiton) in commerical blueberry fields of Maine. Ph.D. thesis, Cornell University.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Review* 18:6534–6535.

Wolfe, K.L., and R.H. Liu. 2007. Cellular antioxidant activity (CAA) assay for assessing antixidants, foods, and dietary supplements. *J. Agric. Food. Chem.* 55(22): 896–907.

Wood, G.W. 1961. The influence of honeybee pollination on fruit set of the lowbush blueberry. *Can. J. Plant Sci.* 41:332–335.

Wood, G.W. 1968. Self-fertility in the lowbush blueberry. *Can. J. Plant. Sci.* 48:431–433.

Wood, G.W. 2004. The wild blueberry industry-past. Proc. 9th North American Blueberry Research & Extension Workers Conf., ed. C.F. Forney and L.J. Eaton. Food Products Press, Binghamton, NY. pp 11–18.

Wunsch, A., and J.J. Hormaza. 2002. Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA markers. *Euphytica* 125:59–67.

Xianli, W., G. Beecher, J.M. Holden, D.B. Haytowitz, S.E. Gebhardt, and R.L. Prior. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.* 52(12): 4026–4037.

Yarborough, D. 2001. Progress towards the development of a mechanical harvester for wild blueberries. Wild Blueberry Fact Sheet No. 226, University of Maine Cooperative Extension.

Yarborough, D. 2009. Wild blueberry culture in Maine. Univ. of Maine Coop. Ext. Wild Blueberry Fact Sheet No. 220.

Yarborough, D., and P.C. Bhowmik. 1988. Effect of hexazinone on weed populations and on lowbush blueberries in Maine. *Acta Hort.* 241:344–349.

Yarborough, D., and F.A. Drummond. 2009. 2009 Insect control guide for wild blueberries. Univ. of Maine Coop. Ext. Wild Blueberry Fact Sheet No. 209.

Yarborough, D., and A.L. Hoepler. 2001. Blueberry enterprise budget. Univ. of Maine Coop. Ext. Wild Blueberry Fact Sheet No. 260.

Yarborough, D., M. Hundhammer, and J.A. Collins. 2001. Integrated crop management field scouting guide for lowbush blueberries. Univ. of Maine Coop. Ext. Wild Blueberry Fact Sheet No. 204.

Yarborough, D., and J.M. Smagula. 1993. Lowbush blueberry nutrition series: Fertilizing with nitrogen and phosphorus. Univ. of Maine Coop. Ext. Wild Blueberry Fact Sheet No. 225.