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Blackberry Breeding and Genetics

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ABSTRACT

Blackberry (*Rubus* L. subgenus *Rubus* Watson) improvement has made substantial progress with over 400 cultivars named originating from wild selections to many releases from breeding efforts. Public breeding has been ongoing for over 100 years. The result of these improvements is commercial production for processing and fresh markets in a number of countries. There has been excellent progress made in improving several very important traits. Fruit quality has been and continues to be a high priority in breeding, with selection for improved processing quality along with enhanced postharvest handling potential for fresh market expansion. Fruit size has been advanced and a range of berry sizes from small to very large exist among released cultivars. A number of plant characters have been addressed, with thornlessness becoming more common in recent introductions. Likewise, enhanced yield potential, improved disease resistance, and improved cane management characteristics have been achieved. More recently, primocane fruiting has been incorporated into blackberry, expanding production options. Breeding has been done using classical breeding methodology, crossing complementary parents and selecting improved progeny. Research using molecular methods has been limited in blackberry, and has not contributed substantially to cultivar improvement. Although breeding efforts in blackberry are more limited than the major berry crops, a continuous supply of new cultivars should result from ongoing programs. These, along with previous cultivar developments, will contribute to an ever-increasing number of cultivar options resulting in an increased of production of this increasingly popular berry crop.

Keywords: breeding, cultivar development, fruit quality, molecular genetics, selection, small fruits

Abbreviations: AA, antioxidant activity; **ACY**, total anthocyanins; **BCE**, **CE**, before current epoch, current epoch**; BYVaV**, *Blackberry yellow vein associated virus*; **BYVD**, blackberry yellow vein disease; **BVY**, *Blackberry virus Y*; **cDNA**, complementary DNA; **EMBRAPA**, Empresa Brasileira de Pesquisa Agropecuária; **INSV**, *Impatiens necrotic spot virus*; **IQF**, individually quick frozen; **ORAC**, oxygen radical absorbance capacity; **PCR**, polymerase chain reaction; **RAPD**, random amplified polymorphic DNA; **RBDV**, *Raspberry bushy dwarf virus*; **SSR**, simple sequence repeat; **TDZ**, thidiazuron; **ToRSV**, *Tomato ringspot virus*; **TPH**, total phenolics; **TRSV**, *Tobacco ringspot virus*; **USDA-ARS**, United States Department of Agriculture – Agricultural Research Service

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ORIGIN AND HISTORY OF THE CROP

Blackberry species (overall considered *Rubus* L. subgenus *Rubus* Watson by most authorities), which are grouped with plants designated as caneberries or brambles, have been harvested from the wild as a food source by humans wherever they have been in proximity to one another. Efforts to develop cultivars can be traced into the late 1800s, however the incredible advances in improved cultivars that have moved blackberries from a wild harvested crop to a large scale commercial crop have taken place within the past 70-80 years. Excellent comprehensive reviews have recently been published by Clark *et al.* (2007), and Finn (2008), with other valuable reviews by Darrow (1937), Ourecky (1975), Jennings (1988), Hall (1990), and Daubeny (1996).

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Invited Review

Hummer and Janick (2007) presented an extensive review of the development of *Rubus* in archeological, artistic, and written historical records. The Newberry Crater near Bend, Oregon (USA) has food remnants of *Rubus* that date to 8,000 BCE. Native American culture has a long oral tradition of using raspberries, blackberries, and their relatives for their medicinal characteristics as well as food items (Moerman 1998). In Europe, the writings of Eschylus and Hippocrates, dating to around 500 BCE, discussed caneberries for their medical value (Hummer and Janick 2007). The Hebrew Bible referenced thorny plants that are presumed to have been *Rubus* species. Later, herbals such as Dioscorides' De Materia Medica, written about 65 CE, presented the benefits of the use of blackberries in medical treatments. Hummer and Janick (2007) identified a series of important artworks that depicted blackberries including ones in the *Juliana Anicia Codex*, (approx 512 CE), paintings by Jan Bourdichon (1503-1508) that illustrate the *Horae ad isum Romanum* (1503-1508), a drawing of a *Rubus* plant by Leonardo da Vinci (1510-1512), and a wood cut by Leonhart Fuchs (1544) from the herbal *De Historia Stiripum* as examples.

Blackberries were being mentioned in gardening books by the 1660s, however, since they were so common where people lived there seemed to be little interest in bringing them into cultivation or in developing cultivars (Jennings 1988). When documented selection efforts finally were begun in the 1800s, it was the unusual albino or pink genotypes from the wild that seemed to predominate (Hedrick 1925). 'Dorchester' was the first cultivar named in 1841 and 'New Rochelle' (syn. 'Lawton'), released in 1854, was the first to be widely planted (Hedrick 1925). 'Aughinbaugh', 'Eldorado', 'Lucretia', and 'Snyder' were also important cultivars in the mid to late 1800s in the USA (Hedrick 1925; Ourecky 1975; Moore 1984; Jennings 1988; Clark *et al.* 2007). Although blackberries are a minor crop among fruits, there have been hundreds of cultivars named ranging from wild selections to those developed from multiple cycles of selection (see Clark *et al.* 2007 for a listing of most blackberry cultivars developed as of the date of that publication). Blackberry cultivation began using wild selections and chance discoveries and these genotypes have provided the basis for genetic improvement in breeding since the early 1900s. One of the early achievements was the mixing of blackberry species in the eastern USA and blackberry and raspberry species in the western USA to develop a tremendously diverse germplasm pool. The early cultivars were selections from the wild and not from documented breeding programs like that of Judge James H. Logan in Santa Cruz, California (USA) (Logan 1955). Logan released 'Loganberry', the result of a blackberry × raspberry cross, in 1890 and it is still grown commercially today. While Logan was very much running a breeding program, apparently the population from which 'Loganberry' was selected was a surprise to him as he said (Oregon Horticulture Society, undated but likely in the early 1920s): "It has been repeatedly stated in public prints that I entertained the idea when I planted those seeds of a cross between the raspberry and blackberry. I am sorry to disturb one of the supposed truths of history, but candor compels me to say that such is not the case. I did not even deem such a cross possible, and did not know what I had done until May, 1883, when the plant first fruited." His 'Black Logan' was also widely grown and has been a valuable parent in breeding programs. The great horticultural personality of the time, Luther Burbank, developed or found 'Phenomenal'/'Burbank's Logan' that was nearly indistinguishable from 'Loganberry' (Darrow 1925; Clark *et al.* 2007). A man from Louisiana (USA), Byrnes M. Young, who could not grow 'Loganberry' nor 'Phenomenal', made a cross between the latter and the regionally adapted 'Austin Mayes' to produce 'Youngberry' in 1905 (Christy 2004; Clark *et al.* 2007). While 'Youngberry' was not widely grown, it found a niche market in some parts of the world and is often found mixed in New Zealand 'Boysenberry' plantings (H. Hall pers. obs.), and it is prominent in the pedigree of the widely grown 'Olallie' and 'Marion'. 'Boysenberry' is another early cultivar with an uncertain origin that is still widely grown. Wood *et al.* (1999) did a thorough examination of the 'Boysenberry' history as part of an introduction to a paper discussing disease problems. 'Boysenberry' was discovered by Rudolph Boysen on John Lubben's farm in northern California. Boysen moved to southern California where the genotype grabbed the attention of United States Department of Agriculture – Agricultural Research Service (USDA-ARS) plant breeder George Darrow from Beltsville, Maryland (USA) who convinced a local fruit grower, Walter Knott, to trial the selection. Knott went on to develop a thriving business that started as a farm with a dining room serving 'Boysenberry' pie and became the Knott's Berry Farm empire. While Wood *et al.*'s (1999) explanation of the historical origins of 'Boysenberry' is well researched, there is still no certainty of its genetic origins. 'Boysenberry is often cited as being from a raspberry × blackberry hybridization; however, its similarity to 'Youngberry' has led some to hypothesize it is a cross of a "Loganberry-like" genotype with an eastern trailing blackberry such as 'Lucretia' or 'Austin Mayes' (Nybom and Hall 1991; Hall *et al.* 2002). Similar hybrid berries were also developed in Europe during the same time period including 'Laxtonberry', 'Veitchberry', 'Mahdi', and 'Kings Acre' (Darrow 1937). The widespread use of 'Boysen' and 'Logan' derivatives has brought an extensive amount of red raspberry (*Rubus idaeus* L. *Idaeobatus*) background into modern blackberry germplasm, and the recent release 'Newberry' demonstrates how these and other red raspberry sources are still potentially rich sources of new germplasm (Finn *et al.* 2010a).

As breeding programs were being initiated, selections from the wild such as 'Evergreen' (*R. laciniatus* Willd.) and later its thornless chimera 'Thornless Evergreen' became important commercial cultivars (Waldo 1977). The genetically thornless 'Everthornless' was later developed from somaclonal plants of 'Thornless Evergreen' (McPheeters and Skirvin 2000).

The Texas Agricultural Experiment Station (College Station, Texas, USA) is credited with having the first public breeding program having begun in 1908 (Darrow 1937). The original emphasis was in developing genotypes that were adapted to hot climates with low chilling requirement. 'Nessberry' was developed using *R. trivialis* L. germplasm and has been valuable as a parent of the low-chill 'Brazos'. Soon after this, the John Innes Horticultural Institute in England, the New York State Agricultural Experiment Station (USA), and the USDA-ARS in Georgia (USA) began programs. The John Innes program developed 'Merton Thornless', which is still the source of thornlessness in all modern tetraploid cultivars. The New York program developed several erect cultivars in the 1950s including 'Bailey', 'Hedrick', and 'Darrow', the last of which is still occasionally grown due to its winter hardiness. The USDA-ARS program in Georgia served as the basis for the USDA-ARS programs in Maryland and Oregon (USA).

While there have been many programs worldwide since these first breeding programs, few are still active (Finn and Clark 2008; Finn and Knight 2002). The major current breeding efforts worldwide are with the University of Arkansas (USA), the USDA-ARS in Oregon, and the private program run by Driscoll's Strawberry Associates (Watsonville, California).

The USDA-ARS Beltsville program was responsible for incorporating thornlessness from 'Merton Thornless' into the first outstanding thornless cultivars released in the late 1960s and early 1970s including: 'Black Satin', 'Smoothstem', 'Thornfree', and 'Dirksen Thornless' (Scott and Ink 1966). The USDA-ARS had a significant effort at their station in Carbondale, Illinois in the 1960s until it was closed in the early 1970s. 'Hull Thornless' and the very important 'Chester Thornless' came from these programs and the last release was 'Triple Crown' in the 1990s (Galletta *et al.* 1998b). This group of breeding material and cultivars is called "semi-erect" and the plants are characterized as being thornless, with vigorous, erect canes that grow 4-6 m long from a crown and arch to the ground. Their fruit is similar in quality to the erect blackberries and plants are very productive.

The New Zealand Institute for Plant and Food Research Ltd. (formerly HortResearch) was one of the most valued and aggressive programs in the 1980s and 1990s. Since 2005, their blackberry effort has dropped to a low level. Begun in 1980, their blackberry and "hybridberry" program had several objectives, the most important being the development of new "Boysenberry-like" cultivars (Hall *et al.* 2002). They blended germplasm from the USDA-ARS (Oregon) and the Scottish Crop Research Institute (Dundee, Scotland, UK) as well as other available cultivars and developed the 'Lincoln Logan' source of spinelessness (S_{fL}) (Hall *et al.* 1986a, 1986b, 1986c). Their most important releases have been 'Ranui', 'Waimate', 'Karaka Black', and 'Marahau' (Hall and Stephens 1999; Clark and Finn 2002; Hall *et al.* 2003).

The USDA-ARS program in Oregon was started in 1928 and was responsible for developing an entirely new blackberry industry based on the trailing blackberry. The original primary germplasm pool included wild selections from the Pacific Northwest USA of trailing, dioecious *R. ursinus* Cham et. Schlt. as well as 'Loganberry', 'Youngberry', 'Himalaya', 'Santiam', and 'Mammoth'. The plants are crown-forming, have very long canes that trail along the ground if not trained to a trellis, tend to have excellent fruit quality, but have poorer winter hardiness than the other types. The first cultivars from this program included 'Pacific', 'Cascade', 'Chehalem', and 'Olallie, released from 1942-1950, and these were instrumental in establishing an industry (Waldo and Wiegand 1942; Waldo 1948, 1950). These were followed by the release of 'Marion' in 1956 (Waldo 1957), which is still an industry standard in the region, and 'Kotata' (Lawrence 1984). During the 1970s-1980s, improving the thornless germplasm pool was a program goal that resulted in the release of the first trailing, thornless cultivar 'Waldo', which carries thornlessness from 'Austin Thornless' (Lawrence 1989). While the release of 'Waldo' was important, the germplasm pool from which it came led to the thornless 'Black Diamond', 'Black Pearl', and 'Nightfall' that have been widely planted (Finn *et al.* 2005a, 2005d, 2005e). In a moderate climate, trailing blackberries tend to be earlier ripening than the semi-erect or erect blackberries and in these climates the earliest ripening trailing genotypes (i.e. 'Metolius' and 'Obsidian') are the earliest ripening of all blackberries (Finn *et al.* 2005b, 2005c). This program also extensively re-sampled the wild *R. ursinus* germplasm and from a selections attained from these more recent samples crossing has been conducted. One cross of the newer wild selections × the thornless 'Waldo' resulted in 'Wild Treasure' which is a thornless, very small-fruited, machine-harvestable cultivar with excellent processed fruit quality (Finn 2001; Finn *et al.* 2010b).

The University of Arkansas is primarily responsible for the development of the erect blackberries from eastern North American species. They are characterized by plants that produce stiff, upright canes that are 1-4 m tall and the plants sucker to produce a hedgerow. Erect cultivars such as 'Eldorado' can be traced back to the 1800s, however, the focused effort to develop this erect blackberry type as a viable commercial crop began in Arkansas in 1964. The erect blackberries are tetraploid and share a similar genetic background with the semi-erect types and generally have comparable fruit characteristics. However, since much more effort has gone into the erect cultivars in the past 20 years than the semi-erect cultivars, the erects now have superior fruit quality especially as related to shipping in the fresh market. In the 1980s, 'Navaho', developed with the 'Merton Thornless' source of thornlessness, was the first thornless, erect cultivar. With each succeeding decade the cultivars from this program have had better fruit quality and more recently, thornless cultivars have become standard. A new type of blackberry has recently been developed at Arkansas with the primocane-fruiting trait. Plants with this trait flower and fruit very late in the season on current-season canes (Clark 2008). Released in 2005, 'Prime-Jan'® and 'Prime-Jim'® were the first cultivars of this type, followed by 'Prime-Ark® 45' in 2009 with improved fruit quality (Clark *et al.* 2005; J.R. Clark pers. obs.). Primocane fruiting was critical to the worldwide expansion of the red raspberry industry and should have a similar impact on blackberry production.

Driscoll Strawberry Associates, Inc. (Watsonville, California) has been breeding red raspberries since the 1930s and their blackberry program was started in 1991. The blackberry program is one of the larger efforts in the world. While their cultivars are not available outside of the company, they have played a critical role in the expansion of the fresh raspberry and blackberry industry, the acreage devoted to their cultivars is large, and their breeding program is among the most important in the world.

Public programs though smaller are productive and active elsewhere as demonstrated by the recent development of 'Tupy' (also known as 'Tupi' in the trade) from EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) Brazil (Clark and Finn 2002), 'Loch Maree', 'Loch Ness', and 'Loch Tay' from the Scottish Crop Research Institute (Jennings 1989; Clark and Finn 2006; Finn and Clark 2008), and 'Čačanska Bestrna' from the Serbian Research Institute (Belgrade) (Clark and Finn 1999; Stanisavljevic 1999).

The major accomplishments in blackberry followed a similar pattern in each major program and type. A germplasm pool was assembled that lead to cultivars that were commercially viable and that later had outstanding traits. Then sources of thornlessness were incorporated into this germplasm as they were discovered. In the trailing blackberries, the first major accomplishment was the development of commercially viable cultivars from their high ploidy germplasm pool. In turn, breeders developed cultivars with exceptional processing and fruit quality characteristics and that were machine harvestable. After the discovery of 'Austin Thornless', it was incorporated in breeding programs to develop high quality thornless cultivars suited for processing. The USDA-ARS in Illinois and Maryland developed the semi-erect cultivars with thornlessness that had been previously isolated in 'Merton Thornless'. Thornless cultivars were later developed that had better cold hardiness, very high productivity, and excellent postharvest handling characteristics. By merging germplasm from several different sources, the University of Arkansas program developed erect-caned cultivars with improved fruit size and quality adapted to the mid and upper southern regions of the USA. While initially a regional novelty, blackberries have become a commercially viable industry worldwide in a very short amount of time. As the thornlessness of 'Merton Thornless' was merged into the germplasm, thornless cultivars with exceptional post harvest handling capacity were developed. The other critical accomplishment that was tied with the incorporation of this thornlessness was that this germplasm had resistance to rosette/double blossom (caused by *Cercosporella rubi* [G. Wint.] Plakidas), one of the most limiting factors in blackberry production in the southern USA. Separately, The New Zealand Institute for Plant and Food Research Ltd. Developed the 'Lincoln Logan' source of thornlessness that was a major accomplishment as it further facilitated breeding thornless types and incorporated more raspberry germplasm into blackberry germplasm.

Recently, there has been a dramatic expansion of production in central Mexico where low-chill requiring blackberries thrive at higher elevation with a significant modification of traditional production practices. The original work in Texas and later at EMBRAPA in Brazil to develop low-chill germplasm was a major accomplishment. 'Brazos', developed in Texas, was the most important cultivar in Mexico for several years and its replacement, 'Tupy', from Brazil has significantly better quality with low-chill require-

Fig. 1 Blackberry breeding. Well formed, attractive, ideal blackberry fruits ('Black Diamond', (**A**); 'Natchez', (**B**)). Pictures taken by authors. Reversion of blackberry where fruit picked fully black turned red after harvest occurring usually after refrigeration or freezing (**C**); Prime-Ark® 45 fruit with very little reversion after refrigerated storage for 7 days (**D**). Pictures taken by authors. Evaluation of adaption of plant (**E**) and fruit (**F**) to mechanical harvesting. Pictures taken by authors. Cold injury at the base of a bud of 'Thornless Evergreen' (**G**). Picture courtesy B.C. Strik, Oregon State University. (**H, I**) Ultraviolet damage/sunburn on blackberries; (**H**) classic damage to large portions of berries; (**I**) white is damage to individual drupelets. Pictures taken by authors. (**J**) Primocane fruiting of blackberry as seen by terminal flowers on a primocane with ripe fruit harvested from floricanes of the same plant; terminal fruits on a primocane (**K**); (**L**) row of primocane-fruiting blackberry plants. Pictures taken by authors. Red berry mite damage symptoms on semi-erect blackberry (**M**). Pictures taken by authors. Pollinating an emasculated blackberry flower (**N**). Picture courtesy Steve Ausmus, USDA-ARS.

ment. Taking low chilling a step further, the recent development by the University of Arkansas of the primocane-fruiting types that fruit on current season's growth, apparently without the need for chilling, has the potential to expand the industry to new heights as occurred with red raspberry decades ago.

Fruit quality in general has been a driving force in recent breeding efforts. Blackberries that are reliably sweet, do not have crunchy seeds, and are firm enough to ship when ripe are essential for the fresh market. Dual purpose berries that can either be shipped fresh or processed will be important in the future.

BOTANICAL DESCRIPTION AND GENETIC RESOURCES

Blackberries, red raspberries, and black raspberries (*R. occidentalis* L.; *Idaeobatus*) are the primary commercialized species within *Rubus* (*Rosaceae*). Blackberries are classified in the *Rubus* subgenus *Rubus* (formerly *Eubatus*) and typically do not have a species epitaph because the cultivated blackberries are nearly all derived from at least two or more species. A number of other *Rubus* species are regionally important as commercial crops including *R. glaucus*, Benth. in Andean South America, *R. armeniacus* Focke (= *R. procerus* P. J. Muell.) in its native Europe or as a naturalized species in South America; *R. phoenicolasius* Max., *R. coreanus* Miq., and *R. parvifolius* L. in Asia; and *R. chamaemorus* L. and *R. arcticus* L. native to the far reaches of the northern hemisphere (Finn 1999, 2008; Finn and Hancock 2008).

The progenitor species for the cultivated blackberries are all perennial plants with biennial canes. Vegetative canes called primocanes are produced the first year and after a dormant period they are called floricanes. The floricanes flower, fruit, and senesce while new vegetative primocanes are produced. Blackberries are generally large (up to 5 m long canes) and vigorous plants with several species such as *R. armeniacus* considered noxious weeds around the world. The cultivated blackberries have a range of growth habits from prostrate (trailing) to very upright (erect) (Clark *et al.* 2007).

Flowers and fruit are born in a panicle-like or racemose-cymb, with primary fruit ripening prior to secondary, quaternary, or tertiary (Hummer and Janick 2007). The flower receptacle has multiple ovaries, styles, and stigmas and is surrounded by white or pink petals; double flowers are not uncommon. The flowers and pollen are well adapted for insect, particularly bee pollination. After fertilization, an aggregate fruit is produced that consists of the central torus (receptacle) surrounded by a number of fleshy drupelets that each contains a seed (pyrene) (**Fig. 1A, 1B**). The trait that distinguishes raspberries from blackberries is where the abscission zone forms at fruit maturity. The abscission zone forms at the base of the blackberry receptacle and the torus picks with the fruit whereas the abscission zone forms between the raspberry drupelets and the torus so that the torus remains on the plant when the fruit is picked.

The number of species already mentioned that make up the heritage of the cultivated blackberries point to a very diverse background and potential for further introgression of new sources of variability. Many species in the *Allegheniensis*, *Arguti*, *Caesii*, *Canadenses*, *Flagellares*, *Rubus*, *Ursini*, *Verotriviales*, *Idaeobatus*, and *Lampobatus* have been utilized in breeding (Jennings *et al.* 1992; Finn *et al.* 1999b, 2002a, 2002b; Clark *et al.* 2007; Finn 2008). Thornlessness, traits related to plant architecture, phenology, fruit quality, and abiotic and biotic stress resistance were some of the traits identified that have been introgressed into cultivated germplasm. While the species used in developing the cultivated types are largely American or European in origin, Asia, specifically China, has a wealth of diversity that should be useful in breeding for environmental and disease tolerances (Jennings *et al.* 1992). Although crosses between diploids and tetraploids or with other subgenera often lead to sterility, crosses among other ploidy levels within the *Rubus* subgenera are commonly fertile and crosses with members of the *Idaeobatus* are often successful and have led to several important cultivars (Finn 2001; Finn *et al.* 2002a, 2002b; Clark *et al.* 2007).

ECONOMICAL IMPORTANCE, USES, AND AREAS OF PRODUCTION

Blackberry production has undergone expansion in the past 15 to 20 years and has become a substantially more important crop during this period (Strik *et al.* 2007). In the USA and some countries of Europe, it has become the "fourth" berry in the fresh marketplace, after strawberries (*Fragaria* × *ananassa* Duchesne.), blueberries (*Vaccinium* species) and red raspberries. In a survey conducted in 1990, North American production was 4,385 ha with about 75% of that in the Pacific Northwest (Clark 1992; Strik 1992) and about 90% of the Pacific Northwest production was for processing. The remainder was scattered across various areas of the continent, and was mostly sold by pick-your-own, on-farm, or other locals sales. A substantial shift developed in the mid to late 1990s, when off-season shipments of fruit into North American markets from Chile, Guatemala, and Mexico began to increase. By the early to mid 2000s, Central Mexico became the largest region of blackberry production in the world, with this production predominantly for the fresh market and for export to retail markets in the United States and Europe. This production, which extends from late October until June, has provided blackberries in large quantities sufficient enough to maintain a stable presence on retail market shelves. With the addition of domestic production in these countries, blackberries have become available, or nearly so, twelve months of the year. Also, domestic fresh market production has increased substantially in the United States, with increased production for fresh market especially in California and the South. As reported by de Carvalho *et al.* (2009), blackberry shipments in U.S. terminal markets increased 530% from 1999 to 2008, and statistics collected did not include all shipped fruit in the U.S so the increase was likely greater than reported.

Further, Europe was the 2nd most productive region after North America (Strik *et al.* 2007). In their survey, Serbia accounted for 69% of European production. Asia was in 3rd place with about half the production of North America. China accounted for all of the known Asian production. The Serbian and Chinese production was predominantly for processing. Likewise, increases in production for fresh market in the UK and other European countries have contributed to the expanded domestic supply. Strik *et al.* (2007) estimated there to be 20,035 ha of blackberries planted and commercially cultivated worldwide with an additional 8,000 ha of fruit harvested from the wild for a total estimate of 140,292 Mg (Strik *et al.* 2007). Production area has increased since the time of this survey also, as their report predicted. There are several reasons that blackberries have increased in production and these include: 1) new cultivars with improved shipping characteristics, 2) improved handling facilities along with enhanced marketing and promotion, 3) the interest by consumers in "new" crops plus interest in crops with high antioxidant levels, and 4) the recognition that blackberries were more profitable to grow due to longer-lived plantings than some of their *Rubu*s relatives such as red raspberry.

Blackberries are important in fresh and processed markets. Fresh fruit is sold mainly in clam shells or other similar packaging. The units sold vary substantially from 125 g up to over 600 g packages, depending on market requirements. The primary processed products are individually quick frozen (IQF), bulk frozen whole fruit, or products including puree, juice, and canned or dried berries. From these basic wholesale products a range of products are made for both the institutional food service industry and retail markets.

Blackberries are quite widely adapted in many temperate regions of the world. A key climate factor is winter temperatures, as they are not adapted to areas with extreme winter cold. The best performance is on well-drained, fertile soils with adequate moisture. Blackberries are fairly well adapted to regions with high summer temperatures. They are not routinely grown in arid climates, however. Compared to red raspberries, blackberries in general are more heat tolerant, less winter cold tolerant, and more tolerant of heavy soils. Although the cultivated types have been limited to temperate regions (due to dormancy requirements), the introduction of primocane-fruiting in blackberries could provide for expanded production in no- or low-chill environments. Likewise, primocane-fruiting could allow production in more harsh winter environments where overwintering of canes is not required. However, blackberry fruit are susceptible to sunburn, particularly in regions with intense sunlight and low humidity.

BREEDING OBJECTIVES

Although blackberries have not had the attention of many major fruit crops, currently there is much excitement in improving blackberries for expanded production and markets worldwide. Overall, breeding goals in various programs share in some areas of focus while differing in others due to type of blackberry, use and market, and genetic variability available (Clark and Finn 2008). A primary focus is always quality, and most agree that to further expand blackberry consumption, particularly for fresh market, increased quality is imperative. Advances in quality from the early wild selections and first improved cultivars have been substantial in the last 20-30 years. This progress made has elevated blackberry from being a fruit often harvested wild to one that is now routinely found on retail market shelves throughout the world.

As with any crop, limitations in genetic variability and breeding methodology are the major limits to improvement. Adequate variability exists for improvement of most major traits such as fruit characteristics including quality and size and plant characters including thornlessness, cane architecture, disease and insect resistance, adaptation, and yield. Several traits that continue to be limiting are heat tolerance in primocane-fruiting types, fruit resistance to sun and rain damage, seediness, floricane winter hardiness, and virus and other some other disease resistances.

Fruit quality

Fruit quality has moved to the top characteristic for improvement of most blackberry breeders. This is due to increased consumer pressure for an enhanced eating experience, and that many other traits such as large fruit size and thornless canes have already been improved and incorporated in more recent cultivar advances.

Clark (2005a) proposed that enhanced quality, particularly sweetness, is the key to expansion of fresh-market blackberries. Flavor is also critical for processed blackberries. Fruit flavor can be divided into other components such as acidity, aromatic content, and astringency in addition to sweetness. Other common quality components include fruit firmness, shape, strength of skin, texture, seediness, color, and nutraceutical content along with other characters such as ease of fruit removal at harvest.

The range of flavors in blackberries in the world is substantial. Distinct flavors of the *Ursini* section are widely desired and bring a premium price. Within this group, the aromatic flavors provided by 'Marion' are an excellent example which also has a pleasant balance of sweetness and acidity. High acidity is important for anthocyanin stability in processed products and when balanced with high soluble solids the berries have a full, intense flavor. Further, trailing types differ in flavor as previously reported (Kurnianta 2005; Yorgey and Finn 2005). Cultivars developed from eastern USA-derived germplasm are very different from the

Ursini and related types, and are desired by many consumers who are familiar with the flavors of wild eastern species. Further, aromatic flavor components of a given genotype can have substantial environmental influences (Wang *et al.* 2005).

In general, a soluble solids content of at least 10% provides for a "sweet" eating experience for fresh blackberries. Some of the more common erect-caned cultivars such as 'Navaho' and 'Ouachita' provide 10-12% soluble solids contents. Soluble solids content can be further increased with crossing of high sweetness parents, and enhancements up to 15% soluble solids or more are possible. Fortunately, high soluble solids can be paired with excellent postharvest performance as evidenced by 'Ouachita' and even more so by 'Navaho'. Among the trailing genotypes, cultivars such as 'Boysen' had higher soluble solids levels (11-13%) compared to 'Chester Thornless' (8%) and it has been reported that 'Boysen' can have over 15% soluble solids (Fan-Chiang 1999; Siriwoharn *et al.* 2004). Another approach to perceived high soluble solids is by decreased acidity level in berries. However, the problem with this approach can be a resulting "flat" flavor when acidity falls too low to give a full and balanced flavor profile. Very important also is astringency in blackberries. Although not always one of the initial flavor components sensed during eating, astringency can impact the eating experience negatively and also result in a bitter aftertaste. Low astringency can be selected for in seedling populations even when parents of the progeny express astringent flavor (J.R. Clark pers. obs.). A very exciting horizon in blackberry breeding is the combining of excellent aromatic flavors of the *Ursini* with the eastern USA-derived germplasm. However this combination has not been achieved in commercial cultivars.

The impact of fruit ripeness or maturity is very dramatic for blackberries. The sweetest berries are those that are very mature to the stage of dull black (after loss off fruit glossiness). Maturity of fruit of blackberries greatly affects fruit quality, particularly the sugar and acid levels. Dull-black fruit were found to be the sweetest compared to mottled or shiny black fruit but were also softer (Perkins-Veazie *et al.* 2000). Volatiles were much higher in dull-black compared to shiny-black fruit (Perkins-Veazie *et al.* 2000; Siriwoharn *et al.* 2004). However, fruits that were at the shiny black stage were found to be superior for postharvest handling (Perkins-Veazie *et al.* 1997). Therefore, the challenge is to combine traits of high soluble solids and flavor components in a shiny black berry with excellent firmness for commercial shipping and handling. Breeding emphasis in the past 15 to 20 years has shifted substantially to postharvest handling potential. This has led to the development of a commercial shipping industry for blackberries. A major cooperative effort was undertaken between the University of Arkansas and the USDA-ARS, Lane, Oklahoma (USA) in the early 1990s to focus on postharvest evaluation of blackberry genotypes (Perkins-Veazie and Clark 2005). Their initial effort was on evaluation of cultivars in various temperatures and times of storage, and 'Navaho' had exceptional performance in storage compared to several thorny, commercial cultivars. 'Navaho' remained firm while retaining fully black drupelet color (Perkins-Veazie *et al.* 1996, 1999).Cultivars released from the University of Arkansas program after 'Navaho' were also found to be very good in handling (Perkins-Veazie and Clark 2005; Clark and Moore 2008). Further, 'Navaho' was also found to be storable for up to 21 days (Perkins-Veazie *et al.* 2000).

The primary characters assessed in postharvest evaluations for fresh fruits include appearance, firmness, flavor, and lack of fungal or other pathogen development. Limitations such as presence of decay, leakage of juice, mushy fruit, and substantial red drupelet color greatly reduce consumer appeal, while shiny, fully black berries are very attractive on market shelves (Perkins-Veazie and Clark 2005). Attractiveness usually includes a focus on retention of black color as well as fruit glossiness. The development of red drupelet color, termed "reversion" by some, is a major concern in fresh market blackberries (**Fig. 1C, 1D**). Fortunately, retention of black color can be selected for but postharvest evaluation must be done to verify that this trait is present; it cannot be determined by field evaluation. Fruit firmness is usually evaluated subjectively after storage. It is important to note that postharvest firmness retention cannot be judged in the field. However, fruit firmness is greatly impacted by environment, particularly rainfall near harvest. Perkins-Veazie and Clark (2005) found that fruits exposed to rainfall within 4 days of harvest could have greatly reduced postharvest storage potential. They also found that several years of evaluation were needed to fully determine a genotype's postharvest potential, primarily due to environmental effects. In breeding for fresh-market shipping potential, a uniform protocol for evaluation must be in place to reliably evaluate selections. One major cultural practice that is increasing in use is the production of blackberries under high tunnels resulting in an environment that prevents rainfall on the berries. For more reliable postharvest performance, high tunnels offer a substantial advantage in areas with rainfall risk during the harvest season. However, if seedling and selection evaluation is conducted under tunnels, then postharvest potential may not be fully expressed under tunnel conditions that provide a reduced selection pressure for environmental influences such as wind and rainfall compared to the selection pressure under open-field production.

Processing quality evaluation has some additional variables for consideration. The ease of fruit separation from the plant in mechanical harvesting is of high importance for processed blackberries. Fruit separation is a secondary objective in breeding for the fresh market also. Fruit firmness is not as important for processing fruits compared to fresh market fruits; however, berries for processing must have firmness that is adequate enough for harvesting and sorting processes with very little damage resulting in attractive frozen fruit appearance. Drupelet skin breakage is largely unacceptable in fresh-market cultivars while some breakage is acceptable for processing berries. Berries for processed markets must possess intense flavor and color along with high soluble solids and titratable acidity levels, low pH, and a low perception of "seediness" (Finn *et al.* 1997; Hall *et al.* 2002). These traits must be retained when the berries are frozen and later used. In breeding for processing, selections are routinely screened for harvestability by machine harvesting with the berries subsequently sorted and frozen as individually quick frozen (IQF) fruit (**Fig. 1E, 1F**). To complete the evaluation process, fruit samples are often evaluated for fruit chemistry including pH, titratable acidity, soluble solids, and possibly anthocyanin content. As selections advance in the breeding program, processed samples are prepared as IQF, pureed, and occasionally juice for evaluation by panels for appearance, flavor, color, and overall quality (Hall *et al.* 2002; Finn *et al.* 2005a, 2005b, 2005c; Yorgey and Finn 2005).

Blackberries can have substantial seed content, and this trait is unacceptable to some consumers. More specifically, the feel of seeds in the mouth is very important. Consumers can perceive some trailing genotypes as "seedless" or of having low levels of seediness (Finn *et al.* 1997), a perception apparently due to seed shape and endocarp thickness (Takeda 1993). Erect blackberry seeds were generally ellipsoidal and smaller than those of eastern semi-erect blackberries that were "clam shaped" (Takeda 1993). Takeda further reported that trailing blackberries such as 'Marion' had seeds that were flat with a soft, thin endocarp. In an inheritance investigation, seed size was found to be quantitatively inherited with partial dominance for small size (Moore *et al.* 1975); therefore progress in crossing and selecting for small seeds should be successful. Progenies derived from crosses between eastern erect and western trailing blackberries show a range of seediness (C. Finn pers. obs.). Fortunately, large fruit can be attained with moderate to small seed size in breeding, with 'Siskiyou' an excellent example (J.R. Clark pers. obs.; Strik *et al.* 1996;

Finn *et al.* 1999a, 1999b).

Blackberry shape can vary substantially, and in general there is no overall consensus on what is the most desired shape. Shape uniformity is imperative, however. Fruits that have irregular drupelet size, do not have even shape, or are double fruits are not acceptable. Most individuals agree that a berry with uniform barrel, round or conical shape and uniform drupelets is most desirable (Clark *et al.* 2007). For the fresh market, shape can impact berry placement in the punnet or clamshell. Long berries can provide for very attractive placement in the vessel resulting in excellent market appeal (**Fig. 1A, 1B**).

Much interest has developed in the last 10 years on the nutraceutical and/or antioxidant content of blackberries (Wang and Lin 2000; Clark *et al.* 2002; Moyer *et al.* 2002; Perkins-Veazie and Kalt 2002; Wada and Ou 2002; Bushman *et al.* 2004; Cho *et al.* 2004; Siriwoharn *et al.* 2004; Cho *et al.* 2005; Connor *et al.* 2005a, 2005b). Substantial variation was found among cultivars with two- to four-fold differences in oxygen radical absorbance capacity (ORAC) depending on year in the University of Arkansas breeding program (Clark *et al.* 2002). Perkins-Veazie and Kalt (2002) reported no ORAC differences between shiny- and dull-black fruit or for fruit stored for 7 days although values differed among genotypes. Wang and Lin (2000) found differences in ORAC between green, red, and ripe fruit of three semi-erect cultivars as well as significant differences among the cultivars. Conner *et al.* (2005a, 2005b) evaluated genotype and environmental effects for anthocyanins, phenolics, and antioxidant activity from cultivars grown in New Zealand and Oregon for two years. Antioxidant activity (AA) as determined by FRAP, total phenolics (TPH), total anthocyanins (ACY) as well as individual anthocyanins were measured. AA and TPH were not significantly different among cultivars and locations but the variation between years within location and the genotype × environment interactions were significant. The genotype × environment interaction was also significant for total and individual ACY. Correlations between ACY and AA were much lower ($r = 0.63$) than they were for TPH and AA ($r = 0.97$). Results indicated that genetic variation for anthocyanins, total phenolics, and antioxidant levels exist and that breeding for enhanced levels would likely be possible.

Fruit size

Fruit size has long been a primary objective in all breeding efforts and is also important as a yield component of blackberries (Darrow 1937; Sistrunk and Moore 1973; Ourecky 1975; Caldwell and Moore 1982; Jennings 1988; Daubeny 1996). Early in blackberry breeding, large fruits were a major focus in parent selection, either from the wild or from early cultivars. Fruit size inheritance was examined and was found to be quantitatively inherited in erect-caned blackberries (Caldwell and Moore 1982). In trailing genotypes, variability was documented for not only size but also drupelet set (Strik *et al.* 1996). As breeding continued into the late 1980s to early 1990s, cultivars were released that had fruit weight of 10-15 g (Hall 1990; Finn *et al.* 1998). Large fruit size is exciting to achieve in breeding, but excessive fruit size (possibly over 15 g) is usually not desired for processed or fresh market use. Large berries can be very difficult to place in clamshells or punnets due to the impact of the lid or cover on the berries, plus difficulties of large berries allowing the stated package weight to be achieved. In general, the ideal berry weight for fresh market uses is 8-10 g. Blackberries that are too large cannot be used in frozen berry mixes as they dwarf other berries in the mixes such as blueberries and raspberries.

Plant productivity

Blackberry plant productivity or yield varies substantially among types, cultivars, cultural management systems, and locations of production. Due to the complex nature of the genetics of yield, breeding for increased productivity can be a substantial challenge. An evaluation of yield components has been done for a limited group of cultivars, with the most attention given to the trailing cultivar 'Marion' along with limited investigations on erect genotypes (Bell *et al.* 1995a, 1995b; Cortell and Strik 1997a, 1997b; Takeda and Peterson 1999; Himelrick *et al.* 2000; Takeda 2002; Takeda *et al.* 2002, 2003). However, these reports do not address a genetic variability approach to yield, but rather address physiological or cultural management aspects. Fruit weight (or size) can be a key component of yield. In a report by Eyduran *et al.* (2008), they found that berry weight variation was accounted for by the variables cane height, number, diameter, and yield for eight cultivars. These cane variables can also affect the overall "yield" of a plant. As with most crops, blackberry breeders try to achieve high yields of high quality fruits in breeding. However, if fruit quality suffers (including aspects such as soluble solids content, berry size, flavor components, etc.) then high yield is of little true value to growers. It has been observed that excessive yields can lead to reduced vigor and primocane renewal of plants on erect types, possibly reflecting limits on how high yields can be increased (J.R. Clark pers. obs.). As with most quantitative traits, breeding for increased productivity is approached by hybridizing high yield parents and selecting for outstanding offspring, with subsequent research in cultural management of the resulting genotype contributing to the optimum product for growers (Clark *et al.* 2007).

Plant adaptation

In general, blackberries are considered to be adapted to a wide range of climates and soils. In fact, their invasive nature has led to them being considered a noxious weed in some areas of the world, especially Australia, Chile, and western North America. Blackberries are not usually found in arid environments, however. The two major environmentally limiting factors to blackberry production are lack of chilling for the dormancy requirement of floricanes and low winter temperature damage to floricanes and floricane buds (**Fig. 1G**). Breeding for broader adaptation has expanded in recent years as interest in production of blackberries has increased. The most extensive expansion has been in areas with low or no chilling provided for a "dormant" season (Clark *et al.* 2007).

Moore (1984) indicated that lack of winter hardiness of canes was the major limit to expanded production in more northern areas of North America. Similar limitations exist in other regions of the world. Cultivars including 'Illini Hardy' and 'Chester Thornless' (Moore 1997; Galletta *et al.* 1998a) provided for cultivars with increased cane hardiness. The approach to hardiness breeding has been to hybridize winter hardy parents and select hardy offspring. However, since field selection is the primary or only approach to seedling screening, variations in winter low temperatures (particularly with warmer winters as experienced in some regions of the world in the last 10-15 years) can lead to reduced advances in selecting for hardiness. Following selection, multiple years and locations for testing for winter hardiness are needed to verify that true progress is made for this difficult to manage trait.

Traditionally, cultivated blackberry production has been confined mostly to temperate climates. Therefore, chilling requirement information for blackberries has been limited, although some research in this area has been done in recent years. Carter *et al.* (2006) reported that Arkansas-developed cultivars ranged from near 300 hours to 900 hours of chilling (hours below 7°C). Additionally, Warmund and Krumme (2005) estimated chill for a group of USA-developed cultivars and recommended an appropriate model for chilling requirement measurement for blackberry. Since the early to mid 1990s, production in subtropical to tropical climates has expanded, including areas that provide for no chilling hour accumulation. The greatest example is in Central Mexico, primarily in the Mexican states of Michoacán and Jalisco. This production has been achieved in an area with no dormant period. In this region, the plants grow vegetatively in the traditional rainy season of June through August, and then a series of cultural manipulations are applied including defoliation with chemicals, pruning, and application of growth regulators. These treatments have been refined over the years for dependable fruit production from late October to early November until May or June (J. Lopez-Medina pers. comm.). The resulting fruit is shipped primarily to the USA and EU, and provides for a stable supply of blackberries on retail market shelves for this period. The cultivars used in this management system were first 'Brazos' and later 'Tupy'. These were both developed in lower-chill locations (College Station, Texas and Pelotas, Rio Grande do Sul, Brazil, respectively), not in an area with the climate such as central Mexico. Breeding in tropical climates would likely further enhance selection for adaptation and allow for production without as much cultural manipulation.

Fortunately, the inclusion of primocane fruiting in blackberry potentially allows an approach to impact winter injury and chilling requirement limitations. Primocane fruiting should allow avoidance of injury to canes as the canes do not require overwintering and exposure to damaging temperatures. However, evaluations of the first primocane-fruiting cultivars Prime-Jim® and Prime-Jan® in northern USA locations St. Paul, Minnesota and Geneva, New York showed two limitations: 1) flowering and fruiting was not completed prior to freezing temperatures, and 2) crown and root kill was experienced in some years (particularly when no snow cover was in place) (J. Luby and C. Weber pers. comm.). Breeding for earlier flowering and fruit maturation is ongoing at the University of Arkansas with evaluations conducted in colder and shorter-season locations to address these limitations. With primocane fruiting plants, chilling requirement is eliminated for fruit production. This trait appears to have substantial potential value for use in no- or low-chill locations, and should reduce production costs compared to the current intense management system required for reliable fruit production (see later section on primocane fruiting).

Another component of plant adaptation in blackberry is in floricane bloom and harvest period. There is substantial variation among genotypes in both traits. The expansion of blackberry production for retail sales has put increased emphasis in breeding to expand the fruit maturity time for early and later periods. Within floricane-fruiting genotypes, transgressive segregants usually result from crossing within early or late parents. Fortunately, blackberries are not as early blooming as high-risk crops such as peaches, and bloom on blackberries is not as concentrated, so breeding for late bloom is not as high a priority trait.

Issues of sun damage occur at times in blackberry production, with the impact being most substantial on fruit. Currently, many believe that sunburn damage is greatest in lower-humidity climates with high light intensities. Examples of these environments include the Willamette Valley of Oregon, Central Valley of California, and some regions of Australia (J.R. Clark pers. obs.). Sunburn can cause drupelets to develop a white appearance, with either individual or groups of drupelets affected (**Fig. 1H, 1I**). Heritability of heat reactions has not been investigated, however, but it is clear that some segregants in breeding populations are markedly more susceptible than others (H. Hall pers. comm.).

Thornlessness

Blackberries range from being completely thornless to having very dense thorns with varying degrees of size and curvature. In a true botanical definition, blackberry thorns or 'prickles' are actually spines since they are derived from outside the vascular cortex. Thornlessness has been a priority in most breeding efforts and substantial achievements have been in advancing thornlessness in a range of cultivars. Breeders are fortunate to have several sources of thornlessness. A very complete review of the genetics and management of thornlessness in blackberry can be found in a publication by Clark *et al.* (2007).

A very important source of thornlessness is the recessive 4*x* source (designated *s*) derived from *R. ulmifolius* Schott. at the John Innes Institute in the U.K. The use of 'Merton Thornless', which was released from this program, has been very important in advancing thornlessness particularly in erect and semi-erect genotypes. The first improved cultivars developed in the USA using this source of thornlessness were 'Thornfree' and 'Smoothstem' (Scott and Ink 1966), followed by the very successful 'Chester Thornless' (Galletta *et al.* 1998a), all developed by the USDA-ARS. The University of Arkansas used these genotypes and selections in its program that was begun in 1964. The Arkansas program has released the thornless cultivars Navaho, Arapaho, Apache, Ouachita, and Natchez, all using the original 'Merton Thornless' source (Clark and Finn 1999, 2006; Clark and Moore 2008). Several other thornless cultivars using this thornless gene have been released from other programs including the "Loch" series from the Scottish Crop Research Institute, 'Čačanska Bestrna' from Serbia, and proprietary cultivars from Driscoll Strawberry Associates. This source of thornlessness is very stable and provides for entirely thornless plants in all growth stages and plant parts. The major disadvantage in breeding with this source is the recessive nature of the gene, and a second generation of crossing is needed to recover thornless progeny if the initial cross is of thorny × thornless parents. Fortunately, in populations segregating for thornlessness, the thornless progeny can be identified at the cotyledon stage by examination of the margins of the cotyledons for the absence of glandular hairs (one or more hairs indicating a thorny plant) thus facilitating the removal of thorny offspring at a very early seedling age.

'Austin Thornless' is an octoploid and has provided a thornlessness source at the 6x and higher ploidy levels. This dominant source (designated S_f) has been important in breeding trailing types. Plants derived from this source of thornlessness can have thorns on the basal 0.3 m of the cane; these same canes are thornless beyond this point and are commercially thornless since fruit is borne only in the thornless area of the cane. Therefore, the identification of thornless progeny using this source of thornlessness cannot be fully done until seedlings are 20-30 cm tall. Undesirable traits such as sterility, dwarfed plant habit, brittle canes, and tight fruit clusters that were associated with this thornless source in early breeding have largely been overcome in recent years. 'Waldo' was the first cultivar to have this thornless source. Ploidy levels of subsequent releases include 6*x*, 8*x*, and 9*x* and include the cultivars 'Black Diamond', 'Black Pearl', and 'Nightfall' (Finn *et al.* 2005a, 2005d, 2005e).

More recently, Hall *et al.* (1986c) developed a new thornless gene, and this dominant source is designated as gene S_{fl}. A tissue culture technique in which a 'Loganberry'-type clone (L654) was used resulted in a spontaneous embryo from callus tissue. 'Lincoln Logan' was released from this effort and was then used in the New Zealand and the USDA-ARS Oregon breeding programs. Some limitations were found associated with this thornless source initially, but many of these have been overcome in subsequent crossing and the first cultivars with the S_{fl} source are likely to be released in the near future.

Cane architecture

Cane growth habits for blackberry vary from very erect to completely procumbent in nature. Commercially, blackberries are usually grouped in the cane types of erect, semi-erect, and trailing (Strik 1992). Erect-caned blackberries can be fully upright in stature and many of these sucker beneath the soil line resulting in less of a crown-forming plant but rather a more continuous row of canes Most of the more recent erect cultivars were developed with no supporting trellis, and include 'Navaho', 'Arapaho', 'Ouachita', and 'Chickasaw'. However, most commercial production utilizes a trellis with supporting wires to prevent canes from falling into the row middles. Semi-erect blackberries are crown-forming and trellised. With this type, canes grow upward to a height of near 1 m before arching to a horizontal orientation. 'Chester Thornless', 'Loch Ness', and 'Triple Crown' are important semi-erect cultivars. Erect and semi-erect cultivars respond positively to tipping of the canes and are almost always hand harvested. Trailing blackberries are normally crown-forming, and unless supported by a trellis or other structure grow at ground level. Trellising is required for all trailing blackberries. Important cultivars are 'Marion', 'Thornless Evergreen', and 'Black Diamond'. Most trailing types are used for processing and are machine harvested. Cane growth habit is considered a quantitative trait. Crossing within a cane habit usually yields plants with similar form as the parents while crossing erect × trailing usually yields semi-erect progeny. In breeding trailing blackberries, cane flexibility is an important trait as cane management procedures require that the canes be untangled, bundled, and trained to the trellis, all done with minimal cane breakage. However, some genotypes, particularly those whose thornlessness is derived from 'Austin Thornless' such as 'Waldo', are prone to having their canes broken during training. There is a wide range of expression of brittleness among genotypes in populations and it is easy to select thornless genotypes that have flexible canes.

Primocane fruiting

The development of flowers and fruits on primocanes is a new and innovative area of blackberry breeding (Clark 2008) (**Fig. 1J-L**). There is substantial potential to expand production area with the use of primocane-fruiting cultivars in diverse climates with resulting production allowing for fruits to be available for markets more months of the year in more locations in the world.

The wild, North American selection referred to as 'Hillquist" has been the main source of primocane fruiting used thus far in breeding. This diploid source was reported to come from a wild plant found by L.G. Hillquist of Ashland, Virginia USA that was provided to the New York State Agricultural Experiment Station, Geneva, NY in 1949 (Thompson 1995a; USDA 2004). This genotype was observed to have a "rudimentary" level of primocane fruiting. The cross 'Brazos' × 'Hillquist' was made in 1967 at the University of Arkansas and selection Ark. 593 resulted. It was assumed that 'Hillquist' produced an unreduced male gamete to combine with the female gamete of the tetraploid 'Brazos' in this successful cross. Further, Ark. 593 was confirmed as a tetraploid based on numerous subsequent crosses with other tetraploid genotypes. Ark. 593 was not primocane-fruiting. James Ballington of North Carolina State University (USA). selfed Ark. 593 and recovered primocane-fruiting offspring with the breeding selection NC 194 released from this effort (Ballington and Moore1995). Lopez-Medina *et al.* (2000) reported that the primocane trait was recessive. The first primocane-fruiting releases were 'Prime-Jan'® (cultivar APF-8) and 'Prime-Jim'® (cultivar APF-12) in 2004 (Clark *et al.* 2005) by the University of Arkansas. These cultivars were recommended for home garden production primarily, although 'Prime-Jan'® showed some commercial potential. Subsequent evaluation in climates different from Arkansas provided some evidence of commercial potential in California and Oregon (Strik *et al.* 2008). Following 'Prime-Jim'® and 'Prime-Jan'®, Prime-Ark® 45 (cv. 'APF-45') was released in 2009, providing the first primocane-fruiting blackberry with postharvest handling capability for shipping to commercial markets (J.R. Clark pers. comm.; **Fig. 1D**).

In the evaluation of the first primocane-fruiting selections in Arkansas in the late 1990s to early 2000s, it was observed that floricane fruits on these genotypes were larger compared to primocane fruits. Further, it appeared that the high summer heat (over 30°C) in Arkansas during primocane bloom and fruit development was damaging. In testing of 'Prime-Jan'® and 'Prime-Jim'® in Aurora, Oregon, they were observed to have large fruit and substantial yields on primocanes (Clark *et al.* 2005). Primocane fruits in Oregon were also larger than floricane fruits produced in Arkansas. The observed genotype × environment interaction was therefore thought to be due to this different heat exposure. The heat effect on these two primocane-fruiting cultivars was confirmed in work by Stanton *et al.* (2007). They reported that Prime-Jim® and Prime-Jan® flower components were adversely affected by high temperatures, with the greatest impact at 35°C. Further breeding and selection in high temperatures should allow for improvement in heat tolerance. Likewise, selection in more moderate environments than that found in Arkansas should facilitate successful progress in breeding for this plant type.

With primocane-fruiting blackberries, it was very apparent that cultural management procedures of the plants must be developed for maximum production. Pioneering work was done by Bernadine Strik at Oregon State University in cane management, leading to improved methods to impact yields, ripening times, and measure high-tunnel effects (Strik and Thompson 2009).

Disease and insect resistance

Traditionally blackberries were considered to be less impacted by disease and insect organisms than most fruit crops. However, as more and more blackberries are grown, increased disease and insect occurrence is being reported, with fungal diseases being more common than those caused by bacteria or viruses. Most breeding for insect or disease resistance has been done by simply selecting for healthier or non-infested plants in seedling populations rather than using screening or other procedures to identify resistant genotypes.

Several of the more common diseases found in multiple production regions of the world include anthracnose (*Elsinoe veneta* [Burkholder] Jenk.), cane botrytis, and botrytis fruit rot (*Botrytis cinerea* Pers.: Fr.), and cane blight [*Leptosphaeria coniothyrium* (Fuckel) Sacc.] (Ellis *et al.* 1991). The infection of these pathogens can vary greatly depending on berry type, location, inoculum, weather, and planting management.

In the non-western USA states and Eurasia, Botryosphaeria cane canker [*Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not] and *Colletotrichum* spp. can be minor to substantial problems (Clark *et al.* 2007; Finn 2008). Orange rust [*Gymnoconia peckiana* (Howe) Trott.] is seen at times in plantings and usually requires removal of infected plants. If the disease is severe enough, a planting can be devastated by this pathogen. Resistance to orange rust exists in almost all eastern USA cultivars with the exception of 'Navaho' (Clark *et al.* 2007). It can be challenging to fully evaluate for orange rust resistance, however, due to the very infrequent occurrence of the disease (J.R. Clark pers. obs.). For many years in the southern USA, double blossom/rosette was the most limiting disease in blackberry production. Disease impacts ranged from rosettes formed from multiple shoots per node, to sterile flowers, and plant death (Marroquin *et al.* 1990; Ellis *et al.* 1991; Smith and Diehl 1991; Lyman *et al.* 2004). Fortunately, resistance to double blossom/rosette has been identified. All of the thornless Arkansas-developed blackberries are resistant to double blossom in Arkansas and in most other locations in the southern USA. However, under intense disease pressure in Mississippi and Louisiana, resistance is not always maintained (Buckley *et al.* 1995; Gupton and Smith 1997; Gupton 1999; G. Melcher pers. comm.). In general, 'Merton Thornless'-derived cultivars are resistant to this disease.

In selected locations in Chile, Mexico, New Zealand, and the western USA, cane botrytis, cane spot (*Septoria rubi* Westend), purple blotch (*Septocyta ruborum* [Lib.] Petr.), and spur blight [*Didymella applanata* (Niessl) Sacc.]

can be seen in some locations in some years. Fruit rots are not as much of a problem in these areas due to the routinely dry weather during the fruiting season. Also, in these areas plus areas in the UK and EU, downy mildew (*Peronospora sparsa* Berk.) can be a serious problem. It is unknown if full resistance to this pathogen exists, largely due to much of the breeding of blackberries being conducted where the disease is not seen. Raspberry-blackberry hybrids such as 'Boysenberry' and 'Loganberry' can also experience significant downy mildew (Breese *et al.* 1994; Gubler 1991). Powdery mildew [*Podosphaera macularis* (Wallr.) U. Braun & S. Takam] is seen in some areas of the world, particularly in Mexico (Clark *et al.* 2007). With increased plantings in Mexico and other areas that experience downy and powdery mildews, these diseases are likely to become more economically important, and hopefully sources of resistance can be identified and used in breeding.

Of the bacterial diseases, crown gall (*Agrobacterium tumefaciens* [E.V. Smith & Townsend] Conn.) is the most commonly seen, and can result in weak plants and substantial yield loss (Ellis *et al.* 1991). Variation in susceptibility to crown gall, fireblight (*Erwinia amylovora* [Burr.] Winslow *et al.*), and Pseudomonas blight (*Pseudomonas syringae* van Hall) have been documented in blackberry (McKeen 1954; Stewart *et al.* 2005).

Interest and occurrence in viruses of blackberry are likely at an all time high as of this writing. For many years, *Raspberry bushy dwarf virus* (RBDV) and several other viruses were major concerns in raspberry production. Conversely, blackberry virus reports were very limited and economic impact small (Converse 1987; Jennings *et al.*1992). RBDV infection has been reported in western and eastern types of blackberry (Wood 1995; Wood and Hall 2001; Strik and Martin 2003). RBDV was commonly found in native western *Rubus* species (e.g. *R. idaeus*, *R. parviflorus*, and *R. spectabilis* Pursch.) (Martin 2002), but was not identified in a survey of *R. ursinus* (a primary progenitor species of the western trailing blackberry) (Finn and Martin 1996). While potentially a serious problem, the erratic nature of transmission and occurrence of RBDV has made it difficult to assess whether breeding for resistance is necessary or possible (Strik and Martin 2003). *Tomato ringspot virus* (ToRSV) and *Tobacco ringspot virus* (TRSV) are commonly identified in most blackberry production regions.

Increased planting of blackberries plus an expanded array of virus identification methods has led to several reports on virus presence, particularly in the southern USA (Chamberlain *et al.* 2003; Guzmán-Baeny 2003; Martin *et al.* 2004; Tzanetakis and Martin 2004; Susaimuthu *et al.* 2006; 2007). Susaimuthu *et al.* (2008) researched viral interactions and their impacts on blackberry plant health, and determined that blackberry yellow vein disease (BYVD) can result from the presence of two viruses, *Blackberry yellow vein associated virus* (BYVaV) and *Blackberry virus Y* (BVY). Further, they found that both viruses were asymptomatic in single infections. They hypothesized that BYVaV is the "synergistic determinant" of BYVD that can cause symptoms at different locations and genotypes during co-infection with other viruses. Tzanetakis *et al.* (2009) investigated viruses present in an infected and symptomatic 'Apache' blackberry plant in South Carolina (USA), and found BYVaV and sequences of *Impatiens necrotic spot virus* (INSV). They concluded that INSV appeared to be one of the major viruses infecting blackberry in the southeastern USA based on this finding plus prior reports of INSV detection in field surveys using ELISA testing. It is now concluded that BYVD is caused by different complexes of viruses, the identity of which changes depending on the geographic area (Tzanetakis *et al.* in press). For example, BYVaV and BVY are widespread in BYVD plants in Arkansas whereas in the Carolinas, where the disease is also widespread, BVY has not been found. Three other viruses, *Blackberry virus X*, TRSV, and *Blackberry chlorotic ringspot* virus along with BYVaV are commonly found in infected plants (Tzanetakis, unpublished data). Breeding for virus resistance may become an important component of blackberry breeding in the future, but at this time, full identification of viruses involved, sources of resistance, and breeding methodologies have not been developed for such an effort.

All production regions have one or more insect problems to contend with. While there are often no standard insecticide programs (Ellis *et al.* 1991), some of the more common insects are: raspberry crown borer (*Pennisetia marginata* [Harris]), red-necked caneborer (*Agrilus ruficollis* [Fabricius]), redberry mite (*Acalitus essigi* Hassan) (damage seen in **Fig. 1M**), strawberry weevil (*Anthonomus signatus* Say), brown and green stink bugs (*Euschistus* spp. and *Acrosternum hilare* Say, respectively), Japanese beetle (*Popillia japonica* Newman), thrips (eastern and western flower thrips, *Frankliniella tritici* Fitch and *F. occidentalis* Pergande, respectively), grass grub (*Costelytra zealandia* White), and foliar nematode (*Aphelenchoides ritzemabosi* [Schwartz] Steiner) (Clark *et al.* 2007). A few production regions have severe pests that require substantial control programs. A good example is in New Zealand where 'Boysenberry', 'Marion', and all other *Rubus* are attacked severely by raspberry bud moth (*Heterocrossa rubophaga* Dugdale) and/or blackberry bud moth (*Eutorna phaulacosma* Meyrick). The green vegetable beetle *Nezara viridula* L. and the leaf roller species including *Epiphyas postivittana* Walker, *Planotortrix exessana* Walker, *P. octo* Dugdale, *Ctenopseustis obliquana* Walker, *C. herana* Felder, and Rogenhofer and *Cnephasia jactatana* Walker can also be severe problems in New Zealand. The New Zealand program identified resistance to bud moth and leaf roller species in black raspberry and has attempted to move this in to blackberry (H. Hall pers. comm.). In the fall of 2008, the spotted wing drosophila (*Drosophila suzukii* Matsumura), a native of Japan, was first reported in California, and it has since become widespread in the western United States in addition to being widespread in China. It was recently identified in Europe. The extent to which this pest will become a problem is not known; however it has the potential to be a major blackberry pest and there is no known host resistance. Formal screening of seedlings and selections in blackberry breeding is not common. Rather, most breeding efforts focus on field evaluations of seedlings and selections for pest resistance and advance those for use in crossing or potential cultivar use. Testing of selections in multiple environments is often done and this can broaden evaluations for pest resistance beyond that of where the breeding is conducted.

CLASSICAL BREEDING

Traditional breeding approaches still predominate in all blackberry breeding programs. The small number of programs devoted to breeding and the complex polyploid nature of the crop suggest that this will probably be the case for the near future. Molecular work in the diploid red and black raspberries may have applications in blackberries and may provide some immediate, but as yet unforeseen, possibilities.

All breeding programs must begin by clearly identifying the goals of the program. If the program is working with a commercial industry, understanding their pressing and long-term needs helps define the goals and gives a priority to the objectives. A germplasm pool that will serve as the basis for the breeding program and that will meet the identified goals must then be assembled. An ideal germplasm pool will have cultivars, and, when possible, advanced breeding materials from strong breeding programs. Gaining access to advanced selections is still possible but is more difficult in the era of intellectual property rights protection. In these early stages, it is also ideal if collaborators can be brought in to the process. For instance, it may be critical to find plant pathologists to work with in order to gain an understanding of a particular disease and to identify sources of resistance.

Complementary hybridization where the parents of each cross are chosen such that the weaknesses of one are matched by the strengths of the other, with the hope that a few of their offspring will have the strengths of both parents and none of the weaknesses as described by Mehlenbacher (1995), is the most common approach to fruit and nut breeding. In blackberries, the parents are highly heterozygous and the first-generation seedling populations usually have substantial segregation. Selections are made in these populations and these are evaluated under increasingly stringent evaluation as the thousands of seedlings become a handful of advanced selections. The approach is essentially phenotypic recurrent selection as the parents from one generation serve as parents for the next generation (Mehlenbacher 1995). While only additive gene effects respond to selection over cycles, in any one generation, all types of genetic variance can be taken advantage of as the desirable genotypes are fixed through asexual propagation.

The approaches to the practical aspects of a breeding program have been outlined in several recent chapters and there have not been any significant changes to these procedures in the past few years (Clark *et al.* 2007; Finn 2008). The process begins with putting together a list of potential parents and then developing a crossing plan addressing program priorities. For a typical cross with the goal of a new cultivar, the parents should be outstanding for most characteristics. Potential parents should be tested for the absence of the pollen-borne viruses such as RBDV.

The procedures for emasculation and pollination are similar to other members of the *Rosaceae*. While most programs conduct their crosses in the field where there are many flowers at the right stage for crossing and where there are usually large amounts of pollen, some breeders prefer doing crosses in the greenhouse under more controlled circumstances resulting in a spread of the springtime work load.

As flowers begin to open, buds that will be the pollen source in the cross are collected at the “popcorn” stage with the buds expanding and showing some petal but before they are open to potential contamination from pollinators. Some breeders prefer to bag flowering laterals that will be the pollen source to allow the flower buds to more completely mature without contamination by pollinators. Harvested buds are cut in half and put under a low-watt incandescent bulb about 20-24 cm away in a protected area to dry overnight. The dried buds are collected and stored in containers (e.g. salve tins, film canisters) in a desiccator placed in a refrigerator or freezer. Pollen at room temperature loses most of its viability in a week and within two weeks when refrigerated.

Emasculation of blackberry flowers is much easier than their red and black raspberry brethren. When the primary flowers have bloomed and the secondary buds are reaching the “popcorn” stage is the ideal time to emasculate flowers on a lateral. This allows one to have several flowers at the right stage that can be emasculated and still fit in a pollination bag. At the “popcorn” stage, the stigmas are not yet mature and the pollen has yet to dehisce. Typically, 3-5 buds on four or more flowering laterals are emasculated for each cross. This should yield a minimum of about 16 fruit with enough seed to produce 100 seedlings or more after taking into account the ways emasculated laterals can be destroyed (e.g. curious birds, storms, tractors, visitors, etc.).

Efficient emasculation is an art and there are many different approaches that are equally effective, but we will describe one. Flower buds are gently rolled over so that you can easily see the base of the flower and then several cuts with a single-edged razor are made around the pedicel that go through the sepal, petal, and stamen whorls simultaneously thereby leaving only the receptacle. Thumbnails, forceps, and scalpels can all be effectively used as well. Since the flowers are immature at this point, a waxed bag is placed over the laterals and secured with paper clips and binders to allow the flowers to fully mature for a few days. Some breeding programs do not bag emasculated flowers as the emasculated flowers are not attractive to pollinators (Finn 1996). However, in climates where rain is common, bagging keeps the flowers dry making it easier to return to the field quickly after rain showers. Two to three days later, the styles will have matured, spread outward, and their color will have changed from bright-green to pale-yellow indicating receptivity. The pollen that will be used in the field should be kept in a cooler with an ice pack throughout the day and the containers filled with pollen should be out of the cooler for as short a time as possible. Pollen is applied with small paint brushes or an index finger (**Fig. 1N**). The brush or other applicator must be sterilized with alcohol between each pollination. A separate brush for each tin/parent speeds up the process and removes the need for the same level of sterilization. Typically the flowers are re-pollinated 2 to 3 days later depending on the weather.

When the fruit is ripe it should be harvested and refrigerated until ready for extraction. While it is possible to extract seed from moldy fruit, it is much easier if done while the fruit are reasonably sound. Fruit are placed in a small container and mashed with 2-4 drops of pectinase and enough water to make a slurry. The slurry is left overnight and then poured through a small strainer and rinsed. The pectinase separates the flesh nicely from the seed and is greatly preferred to blenders with padded blades as the potential for damage to the seeds is eliminated. Seed is spread on paper towels and dried overnight and then placed in labeled envelopes for storage. Seed can be held at room temperature for several weeks without loss in viability. However, for long-term storage, seeds should be kept in a refrigerated desiccator where they can be kept for 10+ years (Clark *et al.* 2007).

To prepare the seeds for germination they must be scarified and then put in stratification. For typical, successful crosses, there will be plenty of seed and a standard stratification procedure, described below, can be followed. It takes about 18-22, and up to 28 weeks, to produce a field-ready seedling from seed.

In preparation for scarification with concentrated sulfuric acid, dry seeds are placed in test tubes or small beakers. To ensure even distribution of the acid and to prevent clumping, the number of seeds per vessel should be less than 300. Because most breeding programs have such a wide variety of *Rubus* germplasm, seedlots vary tremendously in seed size and thickness of the pericarp. In general, trailing blackberries require 1-4 hours scarification and semi-erect/erect blackberries require 3-4 hours. Approximately 10 ml acid is poured into each tube, and then stirred using a vortex mixer to coat the seeds. The tube is placed in a rack immersed in an ice bath. The seeds should be stirred periodically and monitored to see if the white embryos become visible at which point they should be removed from the acid. When the scarification is completed, ice water is poured quickly into the tubes and stirred rapidly to dilute the acid and slow the reaction. The seeds are then poured through a strainer and rubbed to remove some of the charred, carbonized surface as they are rinsed under tap water for a few minutes. Seeds are placed in a saturated solution of sodium bicarbonate for 5 minutes and then rinsed again. Finally, they are put in a 1% calcium hypochlorite solution (3 $g \cdot L^{-1}$; based on formulation with 70% active chlorine) with excess calcium hydroxide for 5 to 6 days at 4°C to complete acid neutralization and to remove the carbon layer.

Moist stratification can be done a number of ways but placing the seed on the surface of soilless media in a germination flat in a plastic bag or between layers of paper towels are two common approaches. Seeds placed on media in a germination flat are not covered. The seeds are then stored at 4°C with 16 hours of light for 6-10 weeks. Stratification time varies from cross to cross depending on the parent’s genetic background, so seeds should be checked regularly to see if the flats are still moist and whether germination has begun. Typically, stratification requirements are satisfied in 4-6 weeks for trailing types and 12-15 weeks in erect and semi-erect types.

When stratification is complete, if the seeds are not already in germination flats, they are sowed on the surface of the germination medium and then placed under intermittent mist and bottom heat (24°C) in a greenhouse. Germination usually begins within a week and is largely completed in a month although seedlings may continue to emerge for 3 or more months. When sufficient seedlings have developed true leaves, they are transplanted to a plug tray (50-72 cell) filled with commercial potting soil. Deeper cells are preferred for the best root development. Plugs are grown in the greenhouse at 22-24°C under 16-hour daylength with regular fertilization (balanced fertilizer, 1-2 times/week with 100 ppm N for 2-3 weeks, then 200 ppm N for 3-4 weeks). When roots fill the plugs and outdoor temperatures allow, the flats are moved outdoors under shade cloth for 1 week, then moved to full sun to await field planting after the last frost date.

While most seed lots are germinated using the procedure just described, *in vitro* procedures can be used for small seed lots that are typically from wide crosses (Galletta and Puryear 1983; Galletta *et al.* 1986; Hall 1990; Clark *et al.* 2007; Finn 2008). For trailing blackberry seeds where the embryo from dry seed is physically separated from the seed coat as is described below, the embryo will often germinate with no stratification.

The seed is sterilized prior to stratification with 1 minute in 70% ethanol, followed by 60 minutes on a shaker with a 20-25 ml solution of 10% bleach + 1-2 drops surfactant. The seeds are then placed into sterilized Petri dishes lined with filter paper, sealed with Parafilm® and stratified at 4°C for 6-10 weeks. They should be checked every few weeks and the filter paper remoistened with sterilized water if necessary.

When the stratification is complete the seed is surface sterilized again using 70% ethanol for 1 minute, followed by bleach + surfactant for 1 hour and placed into a tube of sterile water to await dissection. The seed is placed under a dissecting microscope to visually inspect the seeds for viability. Viable seed will be uniformly yellowish or tan, with no blotchiness or variability among seeds, while underdeveloped seed might be dark, grayish, reddish, or black. Grasping the radicle end of the seed, a scalpel is used to sever and remove the half of the seed containing the tips of the cotyledons. Make sure to remove at least half of the seed to facilitate the embryo's separating from the seed coat. Embryos will begin to germinate as quickly as 2 to 4 hours after initial cutting. If the prepared seeds are left in the sterile water for a few hours or overnight, most of the embryos will expand enough to expel themselves from the seed coat thereby separating the embryo from a major source of contamination from bacteria or fungi. The seeds are transferred to germination medium in a 48-well sterile culture plate where the wells are 2/3 filled with autoclaved, ½ strength Murashige and Skoog media with 100 $mg \cdot L^{-1}$ *myo*-inositol, 10 $mg \cdot L^{-1}$ sucrose, and 7 $mg \cdot L^{-1}$ agar.

If the first germination process described (not *in vitro*) is followed the embryos should begin to develop green color and root growth within 10 days. Once germination has started the embryos can be transferred to a test tube for further growth or, if they are allowed to grow in the culture plates until the first true leaves appear, they can be transferred directly to small plug trays with germination often in a peat-based soilless media and later acclimated for outdoor planting.

Seedlings are usually set in the field as soon as possible in the spring. The seedlings are pushed as hard as possible with optimum temperatures and fertilization in order to get the biggest plant in the first year. Ideally, seedlings grow enough the first year to be able to evaluate a crop the second year. While possible with primocane-fruiting types (which normally fruit in the second season on primocanes, not common on first-year seedlings), this is very difficult with floricane types that are more typically evaluated two years after planting. The higher the seedling number produced, the greater the likelihood of rapid improvements. A breeder must balance their land and labor resources with an understanding of the inheritance of the important traits to come up with a suitable number of seedlings. Typically, 100-200 seedlings per cross are established in the field at a plant spacing from 0.25-1.0 m apart within the row and 2-4 m between rows. Populations are often planted in a serpentine pattern. While most selections are made two years after planting, it is not uncommon for programs to evaluate seedlings a second time in the 4th year. However, because of the tremendous expense of holding a field over for a second evaluation, many programs limit selection to just the one year.

The breeder's job is to sift through thousands of seedlings to identify a subset of interesting selections that upon further and more focused evaluation distinguish themselves in a small handful of advanced selections that may in turn yield a cultivar. While the future potential of using molecular markers tied to critical traits to identify the most promising seedlings to move to the field is tantalizing, it is not available yet for blackberry. In the seedling field, evaluations are made quickly with minimal time spent on each plant and very few notes on each selection. Decisions as to whether to keep a genotype are made based on the subjective evaluation of yield, plant health, and fruit quality. Typically 0.5-1.0% of the seedlings are kept as selections. At the time of selection, the breeder typically categorizes the selection into one of three groups: 1) very promising selections that are to be moved as quickly as possible into intensive evaluation, through the propagation of a number of plants that might then be put into replicated trial and maybe even a few plants onto a growers field; there are usually only a few genotypes in this class, 2) a promising selection from which a handful of plants will be propagated for trial in a single, observation plot, where it can be evaluated more carefully to determine if justified in moving into more expensive, replicated trials or grower fields, and 3) selections that are made as part of a germplasm development program where only a few plants are needed and they may not need to be put in a situation where yield and intensive evaluations will be made. Other than in the cases of exotic germplasm or unique characteristics (extreme size, very early/late ripening, etc.), it almost never works out to make a selection only with the intention of using it as a parent; these are invariably passed over in preference of elite clones, with outstanding characteristics and that have been more intensively evaluated.

Once propagated, the selections are next established in trials that are managed similarly to commercial fields. As the season begins, the breeder must evaluate the plots quickly in order to determine whether any genotypes can be discarded before the expensive harvest begins. Typically plots are harvested twice a week. In addition, the plots are evaluated weekly by the breeder to assess traits beyond yield and fruit size that have been defined as important and that likely will include: fruit firmness/skin toughness, color, shape, and flavor, as well as ease of fruit separation, plant vigor, and any particular disease or environmental stress problem. The most promising selections are harvested for yield and often for postharvest fresh-market storage or processing evaluation (see section on Fruit Quality). Commonly about 10% of the advanced selections are identified that combine good yield, good horticultural traits, and excellent fruit quality and these are propagated for further trial. During this time, programs that utilize intellectual property protection consider the collection of botanical data needed for filing for plant patent or plant breeder's rights applications.

While the process is described in a linear fashion, the whole process is a series of overlapping cycles where, in an established program, every step is taking place every year. In a typical year, in the northern hemisphere, seedling germination begins and crosses are planned in early winter. Plants in all plantings are evaluated for winter damage as budbreak commences and soon thereafter, crossing begins with flowering. New plantings are established as soon as the ground is ready and the danger of frost has past. As fruit begins to ripen, evaluations of seedlings and genotypes in trial intensify and continue until harvest is complete. Fruit from successful crosses are harvested as they ripen. Genotypes identified as selections begin to be propagated in late summer. Semi-erect and trailing blackberries are usually propagated from cuttings of mature primocanes while erect blackberries are propagated from root cuttings. Seed from crosses is extracted, scarified, and placed into stratification in later summer/fall in preparation for the cycle to begin again.

No matter how efficient breeders are, the process is slow and the length of time from pollination to naming a cultivar can be as little as nine years if it has outstanding or unique characteristics but it can easily take 13 to 17 years. A number of steps have been sped up to try to get new cultivars out more rapidly. Instead of multiple plantings of a genotype over multiple years and in multiple locations, breeders are more likely to release the new cultivar with much less thorough testing. Since commercial growers have many different environments, management intensities and concerns, the trend has been for more rapidly moving material into commercial settings where it can be evaluated for commercial viability and also to assess whether a genotype might have a specific market niche that the breeder might not be aware of.

KARYOTYPING

Rubus is divided into 15 subgenera and blackberries are classified in the subgenera *Rubus*, which is further divided into 12 sections) The *Allegheniensis*, *Arguti*, *Rubus*, and *Ursini* have been the primary contributors in the pedigrees of cultivated blackberries although red raspberry (*Idaeobatus*) has played a significant role in the development of the western trailing blackberries. Chromosome numbers in *Rubus* range have substantial variation in wild and cultivated genotypes. Reports provide information for this range from 2n = 2x = 14 to 2n = 18x = 126 including odd-ploids and aneuploids (Thompson 1995a, 1995b, 1997; Meng and Finn 1999). The chromosomes are small, 1-3 µm in length, with a nuclear DNA content for the diploid species ranging from 0.56-0.59 pg (Lim *et al.* 1998; Meng and Finn 2002). Historically, manual counting has been the most common method of evaluating chromosomes. More recent work by Meng and Finn (2002) reported the use of flow cytometry and they found that this method was useful to differentiate ploidy level but not the exact chromosome number.

Cultivated blackberries all contain multiple species in their backgrounds (Clark *et al.* 2007) and thus variation in progenitor species chromosome number. The European blackberries were derived from a group of diploid and polyploid species (2n = 28, 42, and 56) and are often referred to as *R. fruticosus* L. agg. (Daubeny 1996). The erect and semi-erect blackberries (4x) and trailing eastern dewberries (2x) were domesticated from diploid and tetraploid species from eastern America. Trailing genotypes originated from polyploid species from western North America, predominantly *R. ursinus* at 2n = 56, 84, with introgression of 4x blackberry and 2x red raspberry through intersectional hybrids such as 'Logan' and 'Tayberry' (2n = 42) and 'Boysenberry' and 'Youngberry' (2n = 49). Resulting trailing cultivars have a wide variation in chromosome numbers from 2n = 42, 49, 56, 63, 72, and 80 and aneuploids such as 'Aurora' (2n = 58) and 'Santiam' (2n = 61) (Thompson 1997; Meng and Finn 2002).

There continues to be a mixture of opinion on if polyploid genotypes are allopolyploids or autopolyploids (Einset 1947; Ourecky 1975; Stafne 2005; Clark *et al.* 2007). In the tetraploids, tetrasomic inheritance appears to predominate although polysomic and disomic inheritance also appears to occur for some traits (Lopez-Medina *et al.* 2000; Stafne 2005). Research on the tetraploid, eastern US-derived germplasm suggests that this material consists of autopolyploids or segmental allopolyploids, as opposed to true allopolyploids (Clark *et al.* 2007).

MOLECULAR TECHNOLOGY

Blackberries are considered a minor crop in the world, and molecular techniques have not been pursued as vigorously in blackberry improvement compared to many economically important crop species. More work has been done with red raspberry at least in part because of its diploidy. The polyploidy found with most blackberry genotypes increases the difficulty of molecular method development. However, some older and some more recent work has been reported in the molecular area and further work could lead to molecular techniques being used in blackberry improvement programs.

Minisatellite DNA probes use in *Rubus* was first reported by Nybom *et al.* (1989) and this initial work found this technique to be useful in identifying and differentiating red raspberry and blackberry cultivars. Further work by Nybom *et al.* (1990) found inter- and intraspecific variation along with some identical fingerprints among raspberry and blackberry genotypes. Nybom and Hall (1991) later confirmed that minisatellite DNA fingerprints were useful for evaluating genetic relatedness and for distinguishing genotype.

RAPD methodology has had some application in blackberries. Coyner *et al.* (2008) used RAPD technology to investigate genetic relatedness of 11 blackberry cultivars that were derived from four different thornless backgrounds. They used 140 random primers and their cluster analysis grouped the cultivars in three distinct clades. They also reported that 98 primers produced 113 cultivar-specific RAPD fragments that could be useful in identifying cultivars. The number of primers capable of distinguishing a single cultivar ranged from one to 24. Zhang *et al.* (2009) investigated genetic relationships using RAPD markers of 17 *Rubus* cultivars including seven blackberries. They found 500 DNA bands amplified by 22 primers and of these 490 were polymorphic.

AFLP and SSR markers were used to investigate *Rubus* species, including the Andean blackberry (*R. glaucus* Benth) diversity in Colombia (Marulanda *et al.* 2007). All species produced specific banding patterns for AFLP and SSR markers. Further, the SSR markers differentiated diploid and tetraploid genotypes of *R. glaucus*. In a study in Turkey by Ipek *et al.* (2009), genetic diversity among a group of blackberry cultivars and their genetic relationship with 'Boysenberry' and raspberry were analyzed using AFLP markers. Results indicated that blackberry cultivars from North America had a narrow genetic background, blackberry genotypes selected from the Bursa province of Turkey shared all AFLP markers with 'Chester Thornless', and that genetic similarity between 'Boysenberry' and other blackberries was low. The results also indicated that AFLP analysis was unable to detect any genetic relationship between 'Boysenberry' and common raspberry cultivars from North America included in their investigation.

The use of SSR markers was reported by Stafne *et al.* (2005) to differentiate progeny and assess genetic similarity within a segregating blackberry population. His results indicated a similarity coefficient averaged over all individuals of 73% for SSR markers. The average similarity coefficients ranged from a high of 80 to 57% for SSR markers. Comparison of the parents ('Prime Jim'® and 'Arapaho') indicated a similarity of 62% for SSR markers. Recently, 10 SSRs were used to effectively genetically fingerprint blackberry accessions in the USDA-ARS, National Clonal Germplasm Repository and these approaches have worked to differentiate genotypes based on leaf tissue as well as on the torus tissue of frozen IQF berries (Bassil *et al.* 2010). SSRs were highly polymorphic and a single SSR primer pair, Rubus 275a, was sufficient to distinguish the tested 16 cultivars. Further studies are needed to optimize DNA sampling from puree in order to reliably detect contamination in concentrate. Alternatively cultivar certification from concentrate might not be possible due to contamination from seed.

Stafne *et al.* (2005) reported that SSR markers found in

Rubus could be useful for mapping. He evaluated SSR primers from Graham *et al.* (2002), Amsellem *et al.* (2001) (derived from *R. alceifolius* Poir.), Lewers *et al.* (2004, 2005) (derived from *Fragaria* × *ananassa*), and *Rosa*. Their results indicated that 29 to 30% of 'Glen Moy' red raspberry-derived SSRs amplified a product in the blackberry cultivars Arapaho and Prime-Jim®, while 25% of the *R. alceifolius* and 19% of the *Fragaria* SSRs amplified a product in the blackberries. No *Rosa*-derived SSRs amplified a product in the blackberries. Lopes *et al.* (2006) identified microsatellite loci in *R. hochstetterorum* Seub., a species native to the Azorean Islands, and 41 SSR markers were identified in a genomic library of this species. These markers achieved cross-species amplification in at least one of the other three tested species of *Rosaceae* including blackberry (*R. fruticosus* aggr.).

An exciting report of progress in molecular techniques for blackberry was made by Lewers *et al.* (2008). They reported the first work in developing an expressed sequence tag library. A cDNA library of 18,342 clones was generated from expanding leaf tissue of 'Merton Thornless', which was a major source of thornlessness used by breeders in the improvement of erect and semi-erect genotypes. Among the most widely expressed of the 3,000 genes annotated were those involved with energy, cell structure, and defense. A total of 667 primer pairs were designed from individual sequences containing SSRs. In additional work in this report, 33 randomly chosen primer pairs were tested with two blackberry cultivars ('Prime Jim'® and 'Arapaho') and 10 of the primer pairs detected an average of 1.9 polymorphic PCR products. They further predict that this library may yield as many as 940 SSR primer pairs that could detect 1,786 polymorphisms and this may be an adequate number to generate a genetic map for association of phenotypic traits to markers. This work is a first substantial step to developing markers to complement the conventional techniques used in current blackberry breeding programs.

In addition, current work is underway through collaboration between the University of Central Arkansas and the National Center for Genome Resources to sequence two full transcriptomes of stem, leaf, and bud tissue from 'Arapaho' and 'Prime-Jim'® using Illumina (next-gen) sequencing. It is expected that these data should represent ~80x coverage of the entire transcriptome of these combined tissues and will provide additional insights to potential markers and genes that may be linked to traits of interest (J.D. Swanson pers. comm.).

We are not aware of marker assisted selection in use in blackberry breeding at this time. The only report of an investigation with this goal in mind was that of Stafne (2005) who investigated RAPD and SSR markers for linkage to floricane/primocane-fruiting and thorny traits. However, he did not find any markers that were adequately linked for use as markers in breeding.

IN VITRO CULTURE AND GENETIC ENGINEERING

In vitro propagation of blackberries is commonly used in commerce. It is an excellent method to introduce new cultivars quickly into the marketplace, and to provide clean planting material for more conventional subsequent propagation. Micropropagation of blackberry usually is done with sequential shoot tip proliferation via axillary budbreak with resulting rooting of shoots on root-induction media. Shoot tip culture is the most efficient method for rapid proliferation and avoids somaclonal variation (Pelto and Clark 2000a). Broome and Zimmerman (1978) evaluated 'Black Satin', 'Dirksen Thornless', 'Smoothstem', and selection SIUS 64-39-2 for micropropagation variables and found variation among genotypes for explant proliferation in different media. Work on a broad number of *Rubus* accessions (256) by Reed (1990) for *in vitro* regeneration and proliferation reported that 69% of blackberry genotypes proliferated successfully on Murashige and Skoog (1962) basal salts.

Tissue culture of meristems or axillary buds can also be done, but these techniques have drawbacks that make shoot tip culture more popular for propagation. Meristem culturing is often essential in conjunction with thermotherapy, to eliminate viruses from the propagation stock (Converse 1987).

Further research has been done to address other *in vitro* improvements and issues such as avoidance of somaclonal variation (Harper 1978; Pyott and Converse 1981; Hu and Wang 1983; McPheeters *et al.* 1988; Skirvin *et al.* 1994), improved efficiency (Slivinski *et al.* 1984; Fernandez and Clark 1991; Pelto and Clark 2000b; Erig *et al.* 2002; Radmann *et al.* 2003), evaluation of various explant material (Harper 1978), or somatic embryogenesis (Cantoni *et al.* 1993).

In vitro culture of seeds and embryos has been used to enhance survival of hybrid seeds (Clark *et al.* 2007). Selonen and Tigerstedt (1989) successfully cultured embryos from a cross of *R. idaeus* × *R. allegheniensis*. Galletta *et al.* (1986) achieved good germination using embryo culture with up to 50% transplantable seedlings from crossing. The blackberry breeding program of the USDA-ARS in Oregon has used *in vitro* methods for germination of seeds from wide crosses or crosses with low seed numbers using a method from Ke *et al.* (1985). This method involves cutting the tops of the seeds off to physically remove some of the barrier to germination.

Limited research has been done in regeneration systems for blackberry for use in genetic engineering (Swartz and Stover 1996; Meng *et al.* 2004). A regeneration efficiency of up to 70% of explants was achieved when leaves were incubated in a thidiazuron (TDZ) pre-treatment medium for 21 days before culture on regeneration medium (Woody Plant Medium with 5 uM benzyl adenine and 0.5 μM indole-3-butyric acid) in darkness for 7 days, and then transferring them to a 16-hour light photoperiod at 23°C for 28 days (Meng *et al.* 2004). With 'Arapaho', it has also been found that the simple treatment of callus on 22 μM also results in a regeneration efficiency of ~70% (J.D. Swanson pers. comm.). Recent work has produced transformed callus and chimeric plantlets using a pre-treatment on TDZ for several weeks in darkness before co-cultivation (J.D. Swanson and N. Gates, pers. comm.). Unfortunately, they are yet to regenerate fully transformed plants. We are not aware of any transgenic blackberries produced to date and there are no active breeding efforts employing transgenic methods in blackberry improvement.

CONCLUSIONS AND FUTURE PERSPECTIVES

The world market for blackberries has greatly expanded in recent years and, with improved cultivars coupled with more aggressive promotion and marketing, this expansion should continue. In the coming years, consumers around the world will require a better and more uniform product and the industry will need to provide that product for the fresh and processed markets. The emphasis on quality, particularly sweetness, flavor, and postharvest handling, will intensify in breeding programs and with consumers. To meet the expanding needs of farmers, marketers, processors and consumers, breeding programs will be required to provide a wider array of cultivar options. To address all world markets and diverse climates for production, there is a need for public and private breeding efforts to expand to meet these needs.

Although the number of public blackberry breeding programs is not as great as in prior years, the genetic and commercial improvement potential is substantial. This potential is based on the expansion in the blackberry production industry, advances in enhancing a number of very important traits, and also the promise of additional genetic and technological opportunities. As fruit quality improves further, blackberries will develop a wider consumer base. Plant improvements including excellent postharvest handling, sweeter fruits, primocane-fruiting growth habit, additional thornless cultivars, wider climatic adaptation, and a range of other traits will play key roles in the expansion of blackberry production. Further, additional emphasis on insect and disease resistance will be required as production expands and climate change becomes more apparent resulting in pest issues become more prevalent.

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