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Raspberry Breeding

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ABSTRACT

Raspberries have been gathered since the beginnings of history and have been carried to temperate regions worldwide from their Eurasian place of origin. In the last two hundred years deliberate attempts to produce improved types were considerably accelerated as an understanding of reproductive biology was gained and crosses and formal breeding programs were initiated. Initial breeding utilised selections from Europe and North America, resulting in great improvements. These have been focused and expanded with the development of primocane fruiting types and through the use of other *Rubus* and subgenus Idaeobatus accessions for breeding. The advents of specialised tools and techniques to assess plant material, to examine their genetic structure and inheritance and even to modify the plant genome have also made a major contribution to the present range of new cultivars. This review gives an overview of raspberry genetic resources, environmental and pest and disease challenges encountered in raspberry cultivation, specialised techniques used in the development of new genotypes and propagation of the elite new cultivars. The best is yet to come!

Keywords: breeding methodology, resistance, *Rubus idaeus*
Abbreviations: IQF, individually quick frozen; **MAS**, marker-assisted selection; **MH**, machine harvest; **RBDV**, *Raspberry bushy dwarf virus*

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ORIGIN AND HISTORY OF THE CROP

Red raspberries (*Rubus idaeus*) are native to temperate regions of Europe, Asia, and North America (Jennings 1988). Those occurring throughout North America and into Eastern Asia are usually designated *Rubus idaeus* subsp. *strigosus* and those occurring in Europe, from near the polar circle to the mountains of the Caucasus in Asia Minor, designated *R. idaeus* subsp. *vulgatus* (Jennings 1988). Records of raspberry in human history goes back to ancient Greeks were cultivated raspberries are first mentioned by Pliny the Elder who wrote about the people of Troy at the base of Mount Ida that gathered 'Ida' fruits. Later the name 'ida' was used by Linnaeus for the species name *idaeus*. The genus name *Rubus* was derived from the word *rubra,* meaning red in Latin. Gardening books from the early 1800's list less than 25 cultivars growing in English and North American Gardens. Most cultivars dating from this period are hybrids of the European and North American species *R. idaeus* and *R strigosus* (Daubeny 1983). It appears that domestication of raspberries started about five hundred years ago. The crop did not become commercially important in North America until after 1865, when an industry was founded on the 'Cuthbert' cultivar, a chance seedling found in what is now New York City that is likely a cross between a European cultivar Hudson River Antwerp and *R. strigosus* (Darrow 1937).

Early commercial cultivars of red raspberries were derived from the European wild red raspberry *R. idaeus*, the American wild red raspberry *R. strigosus* and the North American wild black raspberry *R. occidentalis* (Darrow 1937). Self-pollination and inbreeding in raspberries results in dwarfs, weakness, luck of hardiness, low productivity and partial or complete sterility. Early in the 1900's, nurseryman G. Pyne was successful in obtaining new cultivars by selecting self-sown seedlings. His most successful cultivar was 'Pyne's Royal'. J.J. Kettle introduced 'Lloyd George', a chance seedling found in the woods of Kent in 1919. 'Pyne's Royal' and 'Lloyd George' were used extensively by the East Malling research program. The use of controlled crosses in North America started at an earlier date than in Europe (Jennings 1988). 'Latham' and 'Chief' red raspberries were released from the Minnesota program in 1918 and 1930 from crosses between 'King' and 'Louden' and self-pollinated 'Latham' respectively. They both became important to the developing industry and were used

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by breeders in developing new cultivars. An important development in the development of new red raspberry cultivars was the identification of primocane fruiting types that bear fruit on first-year canes.

There are now about 45 raspberry breeding programs in 22 countries, but only a few are particularly active (Finn and Hancock 2008; Kempler *et al*. 2011). The objective of most programs is to develop high yielding cultivars with some improved resistance to pest and diseases and improved fruit quality for hand harvest (supplying the fresh market), or machine harvest (for processing and sales).

In the United Kingdom, the East Malling program was responsible for the release of the 'Malling' cultivars, including 'Malling Promise', 'Malling Jewel', and a range of late fruiting types including 'Malling Joy' and 'Malling Leo'. The most significant contribution of the East Malling program to modern raspberry production has been the outstanding primocane fruiting cultivar 'Autumn Bliss' which made commercial production of primocane fruiting raspberries possible in the UK. The Scottish program at SCRI released the 'Glen' cultivars, including 'Glen Moy' and 'Glen Prosen', which are spineless and offer excellent fruit quality and appearance. 'Glen Ample', released in 1994, has become the standard for fresh market production in the UK, along with 'Tulameen' (Daubeny and Kempler 2003; Finn and Hancock 2008).

The breeding programs in the Pacific Northwest of North America, at Washington State University (WSU; Puyallup, Wash.), Agriculture and Agri-Foods Canada (AAFC; Agassiz, BC) and the U.S. Dept. of Agriculture-Agricultural Research Service in Oregon (USDA-ARS; Corvallis) have benefited from many years of collaboration with one another and with the U.K. programs. The USDA-ARS floricane releases, 'Willamette' and 'Canby', and the primocane cultivars 'Summit' and 'Amity' are still commercially important. 'Meeker', developed by WSU and released in the 1960s, is still the processing industry standard in the PNW and many other growing regions (Moore and Daubeny 1993; Malowicki *et al.* 2008a). The WSU program has recently released two cultivars with excellent root rot tolerance: 'Cascade Delight', which displays excellent fruit quality and is suited for the fresh market, and 'Cascade Bounty' which is suited for mechanical harvesting and the processing market.

The AAFC program has been one of the most prolific and important programs in the world. The 1977 releases 'Chilcotin', 'Skeena' and 'Nootka' had excellent fruit quality and high yields for fresh market. The breeders there also took advantage of germplasm exchanges with the U.K. and were successful at developing outstanding selections from crosses between British Columbia selections and some of the 'Glen' series, particularly 'Glen Prosen' (Finn 2006). This resulted in the release of 'Chilliwack' in the mid-1980s and 'Tulameen' in 1989. 'Tulameen' set new standards for fresh market quality especially with outstanding flavour and has become the fresh market standard in Europe. The AAFC program remains active with the recent releases of 'Cowichan', a RBDV resistant cultivar, and the high quality machine harvestable 'Chemainus' and 'Saanich' which are now being widely planted (Kempler *et al.* 2005a, 2005b, 2006, 2007).

'Heritage', the historically most important primocane fruiting cultivar in North America, was released in 1969 from New York Agricultural Experiment Station (Geneva), and it became the standard in growing regions where cold winter temperatures caused damage to canes of floricane fruiting raspberries (Daubeny 1996). Further, primocane fruiting cultivars from this program include 'Ruby' ('Watson') and 'Crimson Giant'. 'Ruby' has not achieved commercial success but has been surpassed by 'Crimson Giant' which is being propagated for large plantings, especially in Morocco after it's commercial release in 2011 (Weber pers. Com.). Breeding in Eastern North America has also resulted in the high yielding and high quality primocane fruiting cultivars 'Caroline', 'Anne', and 'Josephine', derived from elite cultivars from North America and from the UK. They were released from the cooperative program centered at the University of Maryland, in cooperation with Virginia Tech University, Rutgers University, and the University of Wisconsin – River Falls. Primocane fruiting production has become the standard in California where companies such as Driscoll's Strawberry Associates have developed cultivars and a dual crop, primocane/floricane, production system where the plants are in the ground for only 18 months (Finn and Knight 2002).

In Russia, the use of Eastern Bloc floricane fruiting genetics and primocane fruiting genetics from East Malling and the Eastern Bloc has resulted in outstanding new primocane fruiting cultivars with extremely large fruit, very high yields per cane and strong, upright growth. Elite cultivars include 'Bryanskaya Divo', 'Penguin', 'Atlant' and 'Gerakl'. Production season of these cultivars is very early, allowing the majority of fruit to be produced before the onset of winter frosts, and enabling a long period between harvests for fresh market because of improved fruit quality (Hall pers. obs.).

BOTANICAL DESCRIPTION AND GENETIC RESOURCES

Typical description of raspberry

Raspberries are a deciduous herbaceous plant producing biennial canes from perennial roots. New canes grow up from the crown or from root suckers each spring, usually growing up to two metres tall in a managed plantation. In ideal conditions, cane growth can be 5 m or more in length, which is excessive for normal production methods. After the longest day of summer or after reaching a certain stage of maturity, flower initials differentiate in axial buds on the canes. These usually remain dormant until after leaf drop, acquisition of sufficient winter chill to break dormancy, and the onset of favourable conditions in the springtime.

In spring, axial buds on canes begin to grow and produce fruiting laterals, usually with 10-20 flowers containing numerous ovules, each with its own stigma, style and ovary attached to a central receptacle. The receptacle and its attached female organs are surrounded by a whorl of stamens with anthers adjacent to the stigmas, and these are usually surrounded by five petals and calyces. After pollination, individual ovules grow into drupelets which bind together to form an aggregate fruit. Drupelets usually ripen simultaneously and can be harvested as a single fruit, with an abscission zone at the base of each drupelet allowing the fruit to be picked off the receptacle. In cultivation, fruit number per lateral has been increased to 60 or more in some clones. Increased fruit number per lateral is an important component of yield that must be addressed in a breeding programme developing new raspberry types.

In the wild, red raspberry accessions are usually biennial bearing, producing a crop only on second year floricanes that have grown as primocanes the previous summer. However, a low percentage of wild plants also show atypical fruiting behaviour, with flowers and fruit being produced on the top of new canes later in late summer and autumn. This trait traditionally was known as "autumn fruiting" in England, "fall bearing" in North America, "remontant" in France and Spain and "Herbsternte" in Germany, although the terms "everbearing" and "tip fruiting" also have been used (Keep 1988). The term "remontant" is also used in Russia. Expression of this trait was most advanced in the wild selections 'Lloyd George' (*R. idaeus*) from the UK and 'Ranere' (*R. strigosus*) from the eastern USA. These cultivars form fruit buds on canes in mid to late summer, beginning at the tips and flowering and fruiting downward toward the base (Keep 1988).

Extensive breeding in the USA, in England and in Europe produced a range of advanced selections, 'Heritage', 'Zeva Herbsternte', 'Lyulin', 'Summit' and 'Sweetbriar', derived mainly from 'Lloyd George' and 'Ranere'. Further

advances in breeding were obtained at East Malling using *R. arcticus* as a source of earliness for primocane fruiting, resulting in the release of 'Autumn Bliss'. With the advent of new cultivars like 'Autumn Bliss', and even earlier selections beginning to fruit in early summer, the terms "autumn fruiting", "fall bearing" and "Herbsternte" became redundant. Thus it was suggested by Whitey Lawrence in Oregon to rename the trait "primocane fruiting" and this term has become the common English designation for fruiting on first year raspberry canes. 'Autumn Bliss' has been used extensively in breeding around the world since its release in 1984 and many of the cultivars subsequently produced have incorporated 'Autumn Bliss' genetics in their background. The common approach with this has been to cross 'Autumn Bliss' or similar selections from East Malling with high quality floricane fruiting types, especially with early fruiting types. This has led to a range of new cultivars in Australia, Italy, New Zealand, Switzerland, USA, UK, Eastern Europe and the Russian Federation.

Growth habit in red raspberries varies from low arching or nearly prostrate to very upright. The degree of uprightness depends on the environment; some plants that are quite upright in a cooler environment become almost prostrate in a warm environment. For example, the cultivar 'Malling Leo' is reasonably upright in the UK but the plant was almost prostrate in when grown in New Zealand. The cultivar 'Chilliwack' is very upright when grown in the Pacific Northwest, New Zealand and in Victoria, Australia but when long cane plants are moved to Northern New South Wales new growth is significantly less upright.

Internode length is usually greater in more vigorous clones, in cultivated raspberries and in better growing conditions, especially in the absence of competition from weeds. However, in some breeding programmes, selection for short internode length can result in increased productivity, especially where growing conditions are excellent. At each node on a primocane, raspberries have a pinnately compound leaf with 3-5 leaflets, sometimes with fused lateral leaflets. In some clones, leaf blades may be extended from the leaf petiole by petiolules 1 cm or more, but in other clones the petiolule length is minimal. Leaf blades also vary considerably in size and closeness from overlapping to free and the leaf blade varies from smooth to deeply furrowed. The margin of the leaf blade also may be smooth to deeply toothed.

Laterals vary considerably in length from short and rudimentary, up to a metre long or more, although long lateral types are usually rejected as potential cultivars. Typically laterals at the cane tip are short and longer closer to the ground. When plants have grown taller than the support structure, some management systems require cutting off canes at the top wire and allowing longer laterals grow to the top of the structure. Other systems bend the cane tips over and tie them further along the top wire, allowing the shorter laterals to grow and produce fruit. Within the lateral usually the tip or king fruit are the largest, followed by the tip fruit on each sub lateral. In most cultivars, the third and greater levels of fruit are substantially smaller than primary or secondary fruits. However, some new cultivars have similar sized fruit from each part of the fruiting lateral.

Fruits of most cultivars have 60-80 drupelets, but some recent cultivars have twice as many or more. Drupelet weight is usually around 0.04 g, but may vary from 0.02 to 0.07 g. Continuous selection for large drupelet size in Scotland gave rise to a whole programme full of selections with large drupelets. Fruit shape in wild raspberries is typically round in *R. strigosus* and round to conical ovate in *R. idaeus*. Amongst breeding populations unusual shaped fruits include some clones with cylindrical shaped fruit and others with very chunky fruit. Fruit weights in the wild typically are from less than 1 g to 3 g and in rare populations significantly heavier than that. In breeding populations fruits have been reported of weights up to 23 g from Russia, in advanced populations containing the mutant gene L for large fruit size (Kichina 2005). Non gene L populations also have yielded fruit of up to 20 g also in Russian breeding populations (Kazakov *et al.* 2007).

In spite of the tremendous range of improvements in raspberry, the number of ancestral mother clones involved in the development of modern raspberries is very limited (Dale *et al.* 1993). There remains a very large resource of raspberry germplasm that exists in the wild, both in the red raspberry, *R. idaeus* and *R. strigosus* and in other wild Idaeobatus species around the world. Both red raspberries and the wild Idaeobatus species contain considerable diversity (Graham *et al.* 1997; Marshall *et al.* 2001; Patamsytė *et al.* 2004; Ercisli *et al.* 2008; Graham *et al.* 2009). However, almost everywhere where there are wild stands of red raspberry and other Idaeobatus there is an ongoing erosion of diversity and loss of breeding material of future potential (Graham *et al.* 2009).

Raspberry germplasm collections have been instigated and maintained in formal collections at Corvallis, Oregon at the United States Department of Agriculture National Clonal Germplasm Repository, at the Canadian Clonal Genebank collection in Harrow Ontario, at the Nordic Genebank, at the Dresden-Pilnitz Genebank, Germany, and the Scottish Crop Research Institute, and there is a State Register of Cultivars in the Russian Federation.

Incorporation of new genetics in breeding

Many of the raspberry breeding programmes worldwide have very limited or no use of wild accessions of *Rubus idaeus*, *R. strigosus* or other Idaeobatus species in their new cultivar development programs, preferring to use crosses within the limited genepool from which most raspberry cultivars are derived (Dale *et al.* 1993). However, in most of the larger programs, there has been ongoing use of new genetics for incorporation in raspberry breeding.

In the early years of the New York State breeding program at Geneva, a range of *R. strigosus* clones, especially 'Ranere', were used for breeding, resulting in significant new genetic combinations especially utilised in the development of primocane fruiting cultivars. Also the breeding programme at the Virginia Polytechnic Institute used 'Ranere' for breeding. This resulted in the cultivar 'Cherokee', which has continued to be used for breeding, giving root rot resistance and primocane fruiting character to its progenies. This has been followed by significant use of new accessions of this species in Oregon and recently in British Columbia where emphasis has been directed towards resistance to root rot and to the North American aphid vector of the Mosaic Virus complex *Amphorophora agathonica* (Daubeny and Stary 1982; Kempler and Daubeny 2008). In addition, the WSU breeding program at Puyallup, the old Maryland program and the new North Carolina breeding program at Raleigh have made extensive crosses between red raspberries and other Idaeobatus species and they have assembled a range of interesting new interspecific hybrids. Much of the diversity within these hybrids has yet to be explored (Finn *et al.* 2002).

In the UK, there has been significant use of wild *R. idaeus* accessions both at East Malling and Dundee and these programs have also used other Idaeobatus species and more distant relatives widely. At East Malling, many of the species have been crossed with red raspberry and then a concerted program of back crossing while selecting for economic traits has resulted in some outstanding new selections and cultivars, including 'Autumn Bliss', derived from *R. arcticus* giving a breakthrough in primocane fruiting. The East Malling program incorporated a significant improvement in fruit firmness from *R. occidentalis* and this has been widely incorporated in breeding programs around the world. Other species used at East Malling have included *R. cockburnianus* (high fruit numbers per lateral), *R. crataegifolius* (upright growth, stout, branched canes, bright red, easily harvested fruit and pest and disease resistance), *R. odoratus* (early primocane fruiting, root rot resistance), *R. spectabalis* (earliness, early primocane fruiting, root rot

resistance), and *R. lasiostylus* (large, easily harvested fruit, fruit rot resistance, root rot resistance) (Knight 1993; Knight and Fernández Fernández 2008). In Scotland, the old cultivar 'Burnetholm' (entirely *R. idaeus*) was used extensively as a source of genetic spinelessness and fruit firmness in East Malling selections derived from *R. occidentalis* was widely adopted. Presently, almost all SCRI crosses produced in the program are derived from this material. In the 1980s, *R. pileatus* was used for crossing at SCRI for fruit rot resistance and cane disease resistance. Many selections from that period incorporated this species in their background (Jennings 1982, 1983; Jennings and Williamson 1982).

In New Zealand, the breeding program incorporated a lot of material from SCRI during the 1980s, especially genetics derived from *R. pileatus* and containing genes for spinelessness. Inbreeding in this material resulted in selection for very large fruit size, some clones with fruit of over 11 g in weight. In addition, the species *R. niveus* (low chill adaptation and extreme earliness), *R. occidentalis* (bud moth resistance) and *R. parvifolius* (low chill adaptation, light and bright fruit colour) have been extensively used for crossing in this program (Stephens *et al.* 2002; Hall *et al.* 2005).

As in East Malling and Scotland, utilisation of new *Rubus* genetics in raspberry breeding has mostly been targeted at incorporating traits observed in the wild accessions, rather than simply mining the material for new genetic combinations which could give economic advantage to selections and cultivars derived from them. The latter approach has been used very successfully in blueberries by Paul Lyrene in Florida, and could be of particular value in breeding new raspberries for the future.

ECONOMIC IMPORTANCE

The economic importance of raspberries internationally has changed significantly within the last twenty years, particularly since the commercial release of 'Tulameen', the first modern high quality raspberry suitable for fresh market, in 1989 (Daubeny and Anderson 1991). In the 1990s, 'Tulameen' was followed by 'Glen Ample' and 'Isabel', which went on to become the most important cultivars for almost ten years (Daubeny 1995; Wilhelm and Fear 1999; Daubeny 2000). Since that time 'Isabel' has been replaced by other cultivars and 'Maravilla' has taken its place for production in some locations.

These cultivars have become the mainstay of fresh market production in Europe and North America and they have made it possible to reliably send fruit through marketing channels to supermarkets and fruiterers giving at least a 2-3 day shelf life in the market without spoilage. The result of having these cultivars in the marketplace has been that retailers have refused to accept fruit of many other cultivars, consumers have realised that they can buy fruit that are not half rotten when they get them home and they also have had a pleasurable eating experience. Fruits are firm, do not leak juice and are visually attractive.

Production of fruits for fresh market also has been transformed with much of the production now coming from under protection, where glass, or polythene tunnels keep the fruit dry even in taxing weather conditions. Fruit produced from tunnels or glasshouses is higher quality and does not suffer from post-harvest rots in the same way as fruit produced outdoors, even in good climatic conditions. A consequence of the improved quality delivered to marketers and consumers has been the demand for year-round availability. This, in turn, led to the development of out of season production, firstly in the Southern Hemisphere and then into warm temperate regions and the sub-tropics. In North America production has increased in California and it has extended into Southern California and further south into Mexico and other Central American countries. In Europe, out of season production has developed across Southern Europe, especially in Spain and Portugal and into North Africa.

Table 1 World production of raspberries 2005 (Kempler *et al.* 2009).

Country	Area harvested (Ha)	Production (tonnes)	Yield (t/Ha)	
Russian Federation	34,000	176,000	5.1	*
Serbia and Montenegro	16,500	90,000	5.1	
United States of America	6,840	82,826	12.1	
Poland	17,200	65,000	3.8	*
Chile	10,500	64,000	6.1	
Ukraine	5,000	27,000	5.4	*
Germany	5,900	20,000	3.4	F
China	2,200	15,300	7.0	
Canada	2,958	15,000	5.1	F
United Kingdom	1,430	12,200	8.5	
Spain	1,400	7,000	5.0	F
Hungary	1,200	6,724	5.6	
Azerbaijan	1,400	6,300	4.5	*
France	1,303	5,742	4.4	
Korea	1,000	4,700	4.7	
Mexico	380	4,253	11.2	
Romania	200	4,200	21.0	F
Bulgaria	1,200	3,000	2.5	F
Norway	282	1,719	6.1	
Kyrgyzstan	600	1,700	2.8	*
Bosnia and Herzegovina	427	1,700	4.0	F
Moldova	300	1,500	5.0	*
Italy	178	1,421	8.0	
Switzerland	158	1,285	8.1	
Croatia	278	800	2.9	F
Finland	418	608	1.5	
Australia	230	600	2.6	F
Netherlands	50	500	10.0	F
New Zealand	300	390	1.3	F
Estonia	400	300	0.8	F
Belgium	30	275	9.2	
Slovakia	80	200	2.5	F
Sweden	130	190	1.5	F
Ireland	44	100	2.3	F
Zimbabwe	50	80	1.6	F
Denmark	30	65	2.2	F
Morocco	16	50	3.1	F
Czech Republic	25	28	1.1	F
Total	114,639	616,091		

* = Unofficial figure | F = FAO estimate

Production in these lower chill locations has required the development of new techniques, such as "long cane" production, where canes are grown under high chill conditions, dug and planted in a warmer location to bring forth very early production. A modification of this technique has also been developed where the "long canes" have been produced under the warm temperate or sub-tropical conditions, water stressed to cause leaf drop, then dug and chilled to satisfy chilling requirements. This technique has predominantly been used in Europe and in Australia.

In North America, high quality cultivars of primocane fruiting, and dual cropping raspberries have been used in more southern locations to provide out of season production, although further south only the primocane crop is grown. Primocane production is also gaining favour in European lower chill areas as newer high quality cultivars are becoming available.

On both sides of the Atlantic, commercial fresh market production has dramatically increased, so that in some areas production for fresh market now outstrips that for processing. Fresh market sales are continuing to increase, in spite of the world-wide recession and this is likely to be a growth area for the future due to increased consumer demand and the health promoting properties of this fruit.

In Russia, a range of new cultivars of both primocane and floricane fruiting types has also become available within the last 20 years. This has resulted in the increase of production there for both fresh and processing markets. Production there has also increased significantly with the avail-

ability of improved cultivars and much of the production in Russia is from small scale or *dacha* (a small plot of land in the country, often with a summer cottage) plots.

In both Europe and North America, raspberries are popular in farmer's markets and production is increasing for sales through these outlets. Production in Serbia and Poland has increased significantly, mostly in small, 0.5-1.5 ha plantings. World-wide production is estimated in **Table 1**.

BREEDING OBJECTIVES

The objectives of all the breeding programs are to develop high yielding cultivars with improved fruit quality that are suited for the fresh market or for processing. In addition most programs are breeding for improved pest and disease resistance including resistance to *Phytophthora rubi* (Wilcox & Duncan) Man in't Veld (*Pfr*), cane diseases (*Botrytis cinerea* Pres.:Fr., *Didymella applanata* [Niessl] Sacc. and *Elsinoe veneta* [Burkolder] Jenk) and *Raspberry bushy dwarf virus* (RBDV). Disease resistance is increasing in importance as a breeding objective as consumers require high-quality raspberry fruit and at the same time public and environmental pressure is less tolerant of the use of chemicals for pest and disease control. Progress towards resistance to major diseases has been made through greater understanding of the inheritance of these traits, and the use of novel and traditional germplasm sources.

Pest and disease resistance

Root rot is the most significant disease internationally, and the primary reason for replanting in many raspberry production regions in the world. The disease symptoms usually occur in wet, saturated heavy soils and include reduced vigour, severe root rot, leaf chlorosis, plant wilting and collapse of the root system (Wilcox 1989). Different species of *Phytophthora* have been associated with the symptoms but *Pfr* is the most virulent (Wilcox 1989). The disease can be controlled with improved drainage and fungicide but the most effective is the use of resistant cultivars. Sources of resistance have been identified in 'Latham', 'Asker', 'Boyne', 'Newburgh', 'Durham', 'Chief', 'Chilliwack', 'Cherokee', 'Pathfinder', 'Sumner', 'Sunrise' and 'Cascade Bounty' and clones of *Rubus* species material including *R. crataegifolius* Bunge, *R. coreanus* Miq., *R. glaucus*, *R. lasiostylus* Focke, *R. odoratus* L., *R. phoenicolasius*, *R. pileatus* Focke, *R. spectabilis* Pursh., *R. strigosus*, and *R. sumatranus* Miq. as well as the blackberry *R. ursinus* Cham. et Schlecht, which can be successfully hybridized to red raspberry (Barritt *et al.* 1981; Seemüller *et al.* 1986; Bristow *et al.* 1988; Kennedy and Duncan 1993; Finn and Hancock 2008; Knight and Fernández Fernández 2008b; Hall *et al.* 2009; Kempler *et al.* 2011). The PARC breeding programme has identified *Phytophthora* resistance in seedling populations of *R. strigosus* from six sites, one each in BC, New York, Minnesota, North Carolina, Ontario, and Quebec (Lévesque and Daubeny 1999).

Breeding for root rot resistance successfully resulted in recent years the release of resistant cultivars like 'Encore' using 'Cherokee', 'Cascade Delight', and 'Cascade Bounty' using 'Latham' and the recently released 'Ukee' using 'Chilliwack' and a clone of *R. strigosus* as sources of resistance (Sanford *et al.* 2001; Moore 2004, 2006; Kempler pers. obs.). The method of disease screening at the PARC program involves a semi-hydroponic system in which plants are grown in vermiculite in cone-shaped containers suspended in pans of a standard greenhouse nutrient solution (see **Fig. 1**). The plants used are from tissue culture to ensure uniform, disease-free material. They are inoculated with a suspension of macerated hyphae of the pathogen, and water is added to the pans to flood the plants for two weeks. After an additional two weeks, the plants are visually rated for root rot symptoms using a scale from 1 to 8. The WSU program has a long history of screen for *Pfr* in the greenhouse and under natural high disease pressure in the field (Hoashi-Erhardt *et al.* 2008). The New York program is screening in the field and also with a hydroponic system in the greenhouse (Pattison *et al.* 2004).

Fig. 1 Greenhouse screening for *Phytophthora* induced root rot in a semi-hydroponic system. Plants are grown in vermiculite in containers suspended in pans of nutrient solution. (Left to right, cultivars: 'Malahat', 'Cowichan', 'Ukee' and 'Latham'; in each of three treatments: SCRH isolate ATCC 90442, Local isolate from an infected field in the Fraser Valley, British Columbia and non-inoculated control.

Resistance to *Pfr* is heritable and is conducted through major genes. A dominant two-gene model is suggested, with additive and non-additive components. This is supported by genetic mapping developed from generational means analysis and it is confirmed that moderate progress for the development of new resistant cultivars could be achieved through recurrent mass selection (Nestby and Heiberg 1995; Pattison *et al.* 2007). The spread of raspberry production into warmer climates and high tunnels has resulted in an increase in incidence of Verticillium wilt (*Verticillium albo-atrum* Reinke & Berthier and *V. dahliae* Kleb.). Research is needed to identify sources of resistance.

Raspberry bushy dwarf virus (RBDV) is a pollen transmitted virus that spreads rapidly and has been reported in red raspberry, black raspberry and Boysenberry (Converse 1988). The virus is found in all growing regions and a more virulent resistance-breaking strain RB-RBDV is found in Russia, Serbia, and the UK (Jones and McGavin 1998). Kempler *et al*. (2011) listed close to 100 cultivars known to be resistant to RBDV, conferred by the *Bu* gene, but only 'Haida' and 'Schonemann' are resistant to RB-RBDV. The objective of breeding for resistance is very important in the priorities of the PNW, SBL and the UK breeding programs. Molecular markers to identify RBDV resistant genotypes have been identified by Weber and a protocol for screening seedling and resistant genotypes (Kempler pers. comm.). A genetically modified (GMO) RBDV resistant 'Meeker' cultivar was developed by Martin (Martin *et al.* 2004; Malowicki *et al.* 2008b), but because of public and industry concerns with GMO's, it was never released to the public.

Aphids and aphid-borne viruses Breeding for resistance to the aphid vectors is a major objective of several programs including East Malling, SCRI, AAFC-PARC and the USDA-ARS program in Corvallis in their black raspberry breeding (Dossett and Finn 2008; Kempler *et al*. 2011). The approach of breeding for vector resistance has been used by the East Malling program for more than 50 years (Knight and Keep 1958). *Amphorophora idaei* Börner and *A. agathonica* Hottes, found in Europe and North America respectively, are vectors of several viruses: *Raspberry mosaic virus complex* (RMD), *Raspberry leafspot* (RLSV), *Raspberry leaf mottle* (RLMV), *Rubus yellow net* (RYNV), *Black raspberry necrosis* (BRNV), *Raspberry vein chlorosis* (RVCV), and *Raspberry leaf curl* (RLCV) (Keep 1989). Breeding for vector resistance, together with planting clean stock is effective in preventing virus spread where these virus diseases are important. Currently five biotypes of *A. idaei* and several genes for resistance have been identified. Gene A_1 derives from *R. idaeus* confers resistance to bio-

type 1 and 3 is expressed as a single dominant allele. Gene A_{10} derives from *R. occidentalis* which confers resistance to biotypes 1 to 4 (Jones *et al.* 2001; Birch *et al.* 2002). New biotypes of the North American aphid (*A. agathonica*) have also appeared but the new biotypes do not colonize resistant plants (Daubeny and Stary 1982). All of AAFC-PRAC releases are immune to aphids and 'Algonquin' has been identified as homozygous for gene A_{g1}. Its resistance was inherited from 'Haida' and 'Canby' (Daubeny and Sjulin 1984; Daubeny *et al.* 1991).

Grey mold (*Botrytis cinerea*) is a serious disease that causes yield losses and reduced shelf life in outdoor production of raspberries. When raspberries are cultivated in tunnels or green houses, infection is markedly reduced. For outdoor production the disease is the main reason for significant use of fungicide spray programs (Mason and Dennis 1978). Higher incidence occurs on cultivars that are leafy, have drooping laterals and fruit are tightly clustered; low incidence of fruit rot is associated with open plant habit, upright laterals and widely spaced fruit. Sources of resistance come from *R. pileatus*, *R. occidentalis*, *R. crataegifolius* and *R. coreanus* (Stephens *et al.* 2002; Finn and Hancock 2008; Hall *et al.* 2009).

Cane and leaf diseases Spur blight (*Didymella applanata*) is a serious disease that infects leaves and canes resulting in reduced vigour and yield. Jennings (1983; 1988) suggested that gene *H*, which causes hairy canes, combined with spine free and dense waxy bloom traits, gives rise to resistant canes. Resistance to cane botrytis (*B. cinerea*) is correlated with spur blight resistance, and cane pubescence. Anthracnose, also known as cane spot [*Elsinoe veneta* (Burkh.) Jenkins] resistance is also reported by Jennings (Jennings 1983, 1988; Jennings and McGregor 1988) to be associated with gene *H* that controls pubescent canes. However, molecular mapping confirms the association of gene *H* with resistance to spur blight and cane botrytis but not with anthracnose or yellow rust (Graham *et al.* 2006). Cane blight (*Leptosphaeria coniothyrium* [Fuckel] Sacc.) enters the primocanes through wounds and potentially can cause significant damage in fields that are mechanically harvested where the spring-loaded catcher plates rub against the new primocanes. Sources of resistance are found in *R. coreanus, R. mesogaeus* Focke, *R. pileatus* and *R. odoratus* (Finn and Hancock 2008). Resistance is also associated with the spinelessness gene *s* from the old cultivar 'Burnetholm' and it is found in 'Helkal', 'Julia', 'Pocahontas' and 'Tomo' (Hall *et al.* 2009).

Yellow rust (*Phragmidium rubi-idaei* (D.C) Karst. Syn. *P. imitans* Arth.) occurs in wet growing seasons when all succulent plant parts are infected and vigour is reduced. Resistance in 'Latham', 'Chief' and 'Boyne' is conferred by gene *Yr* that prevents sporulation. A second source of resistance is found in cultivar 'Meeker' where a polygenic incomplete resistance causes a delay in the appearance of pustules and reduction in their size and number (Jennings 1988). Use of 'Marcy' in breeding populations has also given resistance to material developed in New Zealand and this has been transferred through several generations of breeding. In contrast the use of SCRI cultivars in New Zealand has resulted in progenies with significant rust susceptibility.

Raspberry leaf spot (*Sphaerulina rubi* Demi. & Wilc.) occurs at the southern limits of the raspberry growing regions in the United States and Europe. Under warm, humid conditions, cultivars can be killed. Most cultivated raspberries are susceptible to the disease with the exception of the red raspberries 'Ranere', 'Dixie', 'Pyne's Royal', 'Bath Perfection', 'Citria', 'Fertodi Rubina' and 'Iskra' and the purple/black raspberries 'Potomac' and 'Evens' (Hall *et al.* 2009). Further resistance has been identified in the Asiatic species *R. biflorus* Buch.-Ham. ex Sm., *R. microphyllus* L. f., *R. inopertus* (Focke) Focke, *R. innominatus* S. Moore, *R. mesogaeus*, *R. crataegifolius* (syn. *R. morifolius* Siebold ex Franch & Sav, *R.* wrightii Gray), *R. niveus*, *R. parvifolius*, *R. phoenicolasius*, *R. rosifolius* Sm., and *R. thibetanus* Franch. (syn. *R. veitchii* Rolfe), (Keep 1989). However, few modern cultivars can withstand pressure from this disease under warm humid conditions and little effort has been put into breeding for resistance.

Powdery mildew [*Podosphaera macularis* (Wallr.) U. Braun & S. Takam. syn. *Sphaerotheca macularis* (Fr.) Jaczewski and *S. humili* (DC.) Burr.] is a widespread disease that reduces fruit quality of infected fruit. Screening for the disease can be achieved very easily during the early stages in the greenhouse propagation process when susceptible individuals segregate. Breeding for resistance to this disease becomes important when breeding for fresh market cultivars grown in tunnels where conditions are favourable for the disease and plants and fruit are both affected. Sources of resistance include most black and purple raspberries (with the exception of 'Black Hawk', 'Dundee' and 'Munger' black raspberries and 'Cardinal' purple raspberry that are susceptible), as well as several *Rubus* sp. (Keep 1989; Finn and Hancock 2008; Hall *et al.* 2009).

Crown gall (*Agrobacterium tumefaciens* [Smith & Townsend] Conn) disease is most important during the propagation stage in the nursery where it can spread with the propagation stock. The disease causes crown gall on a wide range of dicotyledonous (broad-leaved) plants, especially members of the rose family among them raspberry. The bacterium transfers part of its DNA to the plant which integrates into the plant's genome, causing the production of tumours. The fact that *A. tumefaciens* attacks so many different families of plants indicates that the parasitic activity of this bacteria is not highly specialized. However, results of observation show that most varieties are congenial hosts. Some are semi-congenial and a few can be identified as resistant to infection (Süle 1978). Vrain and Copeman (1987) suggest that an interaction between increased inoculum level of root lesion nematodes (*Pratylenchus penetrans*) and incidence of crown gall on root system. In trials it was found that cortical endoparasitic nematodes have a predisposing effect on wound pathogens. They also suggest that the basis of resistance in 'Willamette' is physiological and not related to the availability of wounds. Griffin *et al.* (1968) could not distinguish between physiological resistance and disease escape when wound sites were absent, where in the cultivar 'September', resistance to nematodes may be the reason for it being resistant to crown gall also. The cultivar 'Canby' was reported susceptible to crown gall in the presence of *Meloidogyne hapla* and was considered susceptible in the absence of nematodes by Zurowski *et al.* (1985).

Growth habit

The ideal floricane raspberry should have erect canes, be spineless, produce adequate cane numbers and have medium height. Fruiting laterals should be moderately long, upright and strongly attached, capable carrying the fruit weight and have the fruit well spaced and not bunched (Daubeny 1999). The ideal primocane fruiting cultivar according to Daubeny (1999) should produce abundant canes that branch to produce high numbers of fruiting nodes. Development of canes that are strong and short can be selected to eliminate the need for trellising and supports. All these characters are heritable and genetic variability exists among the present germplasm (Daubeny 1996; Finn *et al.* 2002). Spines for red raspberry are not a significant problem with most existing cultivars in commercial production. However use of other species such as *R. arcticus* in breeding has resulted in cultivars like 'Autumn Bliss' with objectionable spininess. Other species like *R. coreanus*, *R. niveus* and *R. occidentalis* give even spinier types that are unsuitable for commercial production. In the UK, cultivar development has widely used gene *s* from 'Burnetholm' for spineless and its inheritance has been studied and identified (Lewis 1939; Jennings 1988; Jennings and Brydon 1990; Daubeny 1996). Almost all modern cultivars developed in the UK and New Zealand have spinelessness from this source. In British

Columbia a dominant source of spinelessness has been used giving few spines in most of the cultivars developed. Inheritance of this character has not been studied closely.

Yield

In raspberries, yield and yield components are complex additively inherited traits with significant genetic interactions (Dale 1989; Dale *et al.* 1989; Daubeny 1996). In floricane types, the yield depends on cane number, fruiting laterals per cane, fruit number per lateral and fruit weight. Cultivars with compact growth habit and short internodes produce high number of laterals, although any excess in any of the yield component can have a negative effect (Jennings 1980). Gene L_1 which enhances fruit size, has proven to be very unstable and most breeding programs have actively worked to eliminate it (Jennings 1988). However, in Russia Kichina (2005) successfully used gene L_1 germplasm to develop very large fruited cultivars and these are successfully propagated by selecting only the canes with large stipules at the leaf base. Yield in primocane types depends on cane number and amount of branching. Because of the short fruiting season of the primocane raspberries, earliness is also an important yield component, enabling a greater percentage of production to be achieved before the onset of winter (Keep 1988; Kazakov and Evdokimenko 2007).

Fruit weight and/or fruit size is a significant yield component and this can be improved with breeding by 1) increasing drupelet size, 2) increasing drupelet number and 3) increasing the thickness of the fruit in the middle. Lower fruit weight is associated with a large internal cavity, girdling of the fruit (with reduced fruit thickness midway between the calyx and fruit tip), with fewer drupelets and smaller drupelet size. Increasing fruit size is a significant step in increasing yield within raspberry germplasm. With increased number of drupelets and no increase in drupelet size berries become less coherent and may be unable to hold together effectively. On the other hand if increase in drupelet size is the major factor for increased fruit size then berries may be unsuitable for IQF freezing in liquid Nitrogen with drupelets exploding when dropped into liquid N. In addition pyrene (seed) size is also associated with drupelet size and large drupelets need to be accompanied by excellent adherence of the pulp to the seed or the fruit will be objection-ably seedy. Even with good pulp adherence to the seed, pumping of pulp or other processes plant may remove the pulp envelope from the seed and the processed products will be seedy. For effective use of increased fruit size/ weight as a useful component of yield fruits need to have both increased drupelet numbers and size, especially around the equatorial region of the fruit and if used for processing then IQF fruit producers may need to avoid the use of liquid Nitrogen for freezing. For future processing types processors will need to learn how to handle bigger fruits as this component of yield is the key to increasing productivity. For fresh market types, large fruit size is the key for reducing costs of hand harvest and overall production.

Quality

Fruit quality aspects, including size, shape, shelf life, color, firmness, skin strength, seed size, flavour and fruit nutritional content are key part in the development of new raspberry cultivars. The importance of the different traits is determined by the intended use of the fruit. Processed fruit are harvested and frozen within a short time. They do not need to be large (but will be larger in future) or very firm but should have good skin strength, dark color and intense flavour, be high in soluble solids and titratable acidity and have a low pH. In contrast fresh market quality is measured in terms of large size, a great fresh flavor, low acidity, excellent skin strength and internal firmness, non-darkening light red color, glossy appearance, small seeds, and high nutritional content.

End use

Raspberries have many uses. Fresh raspberry fruit have greatly increased in popularity during the last twenty years and supermarket and fresh produce markets are supplied year round with fresh fruit from production areas around the world. Nevertheless many raspberries are grown for processing and stored as individual quick frozen (IQF) and block or concentrate frozen pulp until used. Pulp is often screened to remove solid parts for juice production. Drying by heat or freeze drying is used to make raspberry fruit leather. Because of its convenience, IQF is becoming more popular as the preferred source for fruit. There is a need for cultivars that will IQF well, producing product that can be used in bakeries, dairy products and ice cream and dessert makers. Raspberry seeds may be separated from the pulp which is then mixed with sugar and cooked to produced seed free jam or jelly. There is increasing interest in producing products for health concerned consumers that are looking for products containing raspberry without added sugar. Processed raspberries are also used as an additive to flavour medication to make it more palatable. In addition, many studies suggest that consumption of a phytochemical rich diet that includes fresh or processed raspberries contributes towards reducing the risk of many chronic diseases including certain type of cancers and cardiovascular diseases (Seeram 2006; Rao and Snyder 2010).

Environmental adaptation

The optimum environments for growing raspberry are characterized as mild maritime climates with moderate summer temperatures, ample sunshine, without high light intensity that can result in sun damage, adequate rainfall that is spread throughout the year, with dry period during the fruiting season, low winter temperatures to supply the needed chilling requirements but not so low as to result winter damage to canes and buds (Daubeny 1996). The increase in worldwide production means that breeding programs are selecting for adaptation to marginal environmental conditions that are considered less than optimum.

The subjects of adaptation to low temperature, winter hardiness, and low chilling requirements have been extensively covered by some, mostly recent reviews (Daubeny 1996; Finn and Hancock 2008; Hall *et al*. 2009; Kempler *et al.* 2011). The inheritance of winter hardiness is genetically complex and can be characterized by several key factors that include late bud break, early fall hardening and long deep dormancy that will not break with temperature fluctuation (Finn and Hancock 2008; Hall *et al*. 2009). Good winter hardiness is present in wild populations of northern *Rubus* species like *R. strigosus*, *R. idaeus*, *R. arcticus*, *R. sachalinensis*, *R. deliciosus*, *R. coreanus* and *R. crataegifolius* (Hall *et al*. 2009).

Extension of production regions

With the limited availability of new good land for raspberry production in many raspberry growing regions and with the demand for year round production for marketing of fresh fruit there is a strong drive in raspberry breeding for development of cultivars adapted for growing in soils less suitable for raspberry production and in regions where climatic conditions cannot sustain long term growing of existing cultivars due to lack of chill, heat, and pressure from diseases. In the Pacific Northwest of the USA and Canada lack of suitable land has resulted in use of heavier and wetter soils that favor the development of *Phytophthora* root rots and root rot resistance is critical for growing in these conditions.

In the South Eastern part of the USA the greatest limiting factor for raspberry production is susceptibility to leaf spot. Susceptible cultivars are defoliated and unable to survive under the high temperatures and high humidity conditions in this region. Leaf spot resistance exists in a few cultivars including ‘Dormanred’ and ‘Mandarin’ but this needs

to be combined with fruit quality and high yield for the development of modern cultivars suitable for competing in the fresh market alongside recent cultivars grown in California and in cooler regions.

In Southern California, Mexico, Australia, New Zealand, Spain and other countries around the Mediterranean the key limiting factor for raspberry production is lack of sufficient chill or sufficient winter to allow growth year after year. This has been compensated for by the use of long cane production, use of primocane fruiting types and through the use of short cycle production by companies such as Driscoll Strawberry Associates. For the future of production in these regions and in other locations such as Kenya, Tanzania, Peru and at higher elevations in Southern Asia new cultivars will be developed that are more suitable for these conditions.

In Australia, the most significant other factor limiting raspberry production is high temperatures, giving rise to significant sunburn and sun scald of fruit under hot conditions. Raspberry germplasm contains significant variability in response to high temperature conditions and breeding by Graham McGregor in Victoria was beginning to see results in ability to handle elevated temperature conditions by the time the program was terminated on his death. This work needs extending for the future of raspberry production in Australia and other high temperature locations.

Production type

The history of raspberry production has largely revolved around floricane fruiting and the use of these types for the production of high quality fruit for the fresh market. Recent developments in breeding have seen a great improvement in fruit quality and production of primocane fruiting types and it appears that in future production of at least fresh market raspberries will largely be reliant on primocane fruiting and/or dual-cropping cultivars. Fruit quality and yields of primocane fruiting types have greatly improved and it is clear that new primocane fruiting cultivars will be able to compete effectively with floricane fruiting cultivars and will displace them, especially for production in warm climate locations for out of season production.

Large-scale commercial production of red raspberries was initially developed in the 19th century in regions close to large cities or locations where process fruit were produced for shipment to distant markets preserved by. While SO_2 preservation is no longer practiced and harvest may be by machine, large scale commercial plantings continue to be grown. Nevertheless smaller scale production has not ceased around the world with considerable production in third world countries coming from small plantings of less than 1.5 ha. In the west there also is an increasing amount of raspberry production in small plantings for sale at farmer's markets. Requirements for raspberries suitable for small-scale production are different to large-scale commercial production and there is a new developing place for breeding proprietary cultivars for the small scale production and home garden markets (Kobelt pers. comm.).

Long cane production also has different requirements than for other raspberry cropping and there is also a place for the development of specific cultivars suitable for this practice.

Low chill adaptation and winter hardiness

High chill cultivars will not grow under low chill conditions, and adaptation is necessary for long-term production of raspberries in regions with minimal winter cold. The effect of lack of chill in raspberries is to reduce or even eliminate bud break, to limit flower initiation and frequently results in cessation of growth and plant death after 2-3 years. No floricane fruiting cultivars have been developed which are capable of growing year after year without regular annual periods of winter chill. However, in the wild, especially in Asia there are Idaeobatus species that grow and fruit every year without receiving chill. These species could be used to develop raspberry cultivars that do not require chill for production.

Selection for the primocane fruiting trait in raspberry breeding has to some extent resulted in inadvertent selection for low chill adaptation. However, few primocane fruiting raspberry cultivars are capable of continuous cycling of primocane production in a low chill environment. The cultivar 'Summit' is a possible exception to this as it has been grown in Mexico for at least 12 cycles of production without running out due to lack of chill (Lopez-Medina pers. comm.)

Table 2 Other domesticated *Rubus* species and their growing regions.

Species	Growing region
R. chamaemorus L.	Scandinavia
R. arcticus L.	Cylactis
R. phoenicolasius Miq.	Japan
R. parvifolius L	China
R. niveus Thunb.	China
R. glaucus Benth	Central and South America
R. coreanus Miq.	China
R. crataegifolius Bunge	China
R. occidentalis L.	North America

CLASSICAL BREEDING

The most widely grown domesticated *Rubus* species are the red raspberries *R. idaeus* var. *vulgatus* Arrhen and *R. idaeus* var. *strigosus* Michx. *R. idaeus* is native to much of Europe and Asia, it is not as hardy as its North American relative and the fruit is dull and conical. The 'Lloyd George' cultivar is derived from this species. *R. strigosus* is native in the mountains from Georgia to Pennsylvania, north to Canada and from the Maritimes Provinces across Canada into the Pacific Northwest (Kempler *et al.* 2002). It is extremely variable and much hardier then the European species and the berries are bright red.

The other most important species from the subgenus *Idaeobatus* that have been domesticated are listed in **Table 2**. *R. occidentalis*, the North American black raspberry, ranges further south and not as far north as *R. strigosus.* It is much sweeter, seedier than and not as hardy as the wild red raspberry. Hybrids between black and red raspberry are called purple raspberries. The purple raspberry is more vigorous and if fertile is more productive than both black or red raspberry and the fruit less seedy (Darrow 1937).

Additional domesticated species are the Andes black raspberry, *R. glaucus*, grown in northern South America, *R. niveus*, a tropical species from India to Thailand and the Philippines, and the wineberry, *R. phoenicolasius* from Japan.

Breeding methodology

Raspberries have biennial canes that require a dormant period prior to flowering. Flower bud initiation starts in late summer to early fall when day length becomes shorter and temperatures are lower than 13°C (Dale and Daubeny 1987). Exceptions to the biennial trait are the primocane fruiting types that initiate flowers under long day conditions in the spring and flower and fruit late in the summer and the fall. While raspberry is a protandrous species, a significant level of self-pollination occurs (Daubeny 1971).

Crosses can be made in the field, greenhouse or growth chamber using individual plants that are free of *Tobacco streak virus* (TSV) and all strains of RBDV. Flower buds just beginning to show petals are emasculated using pointed forceps to cut a complete circle into the base of the sepals that when pulled away, removes the sepals, petals and anthers, leaving the gynoecium with the styles and stigmas unharmed. Paper, glassine or other semitransparent bags that are weather proof, and do not allow heat build-ups, are placed on the laterals covering all the flowers. Additional

mature flower buds can be emasculated in 2-3 days, but any small buds are removed. Flowering laterals on the male parent are also bagged to provide flowers as a pollen source that is not contaminated with unknown pollen. Two to three days after emasculation, open flowers from bagged laterals on the pollen parent are harvested and placed in Petri plates to dry. Later these can be used directly as a "brush" to transfer pollen to the stigmatic surfaces or the dishes with the dried flowers can be shaken and the pollen that collects on the plate surface transferred to the female flowers with a camel hair brush or a glass rod. The process is repeated at 2-3 day intervals until it appears that the flowers are not receptive anymore, when the stigma and style start to brown. While weather-dependent, the flowers can be receptive for 7 to 10 days. Laterals are re-bagged after each pollination and 70% alcohol is used to clean hands and tools and prevent pollen contamination. Variations of this process are reported by Ourecky (1975), Jennings (1988), Daubeny (1996), Finn and Hancock (2008) and Hall *et al.* (2009).

Pollen can be extracted by removing anthers and drying them under incandescent light. The dry anthers are then crushed with a glass rod to release the pollen. The dry pollen can be stored for four or more weeks in a desiccator with calcium chloride at 5°C. For out of season pollination, dormant plants are bought into a warm environment (15°C with 16-h day length) and the same process as for field pollination is followed.

Bags are kept on the laterals covering the fruit until it ripens. Harvested ripe fruit can be placed in the refrigerator until the whole cross has been harvested or sufficient fruit have been harvested for seed extraction. Fruit is covered with water and about 10 drops of pectinase are added to the slurry. The fruit may be simply mashed with a fork or, if very carefully done, the fruit can be pureed with a few quick pulses with a low speed blender with reversed or protected blades to prevent damaging the seeds. The slurry is kept at room temperature for 12-24 h, more water is added, and the viable seeds settle to the bottom of the container and the pulp and hollow seeds can be decanted off. Seeds are air dried before being stored in seed envelopes. Seed may be stored for a few months at room temperature before sowing as this seems to keep the seed from going into a deeper dormancy. Seed that needs to be stored longer may be refrigerated at a temperature between 1 to 5°C or at -18°C until sowing. Refrigerated seed stored in a desiccator will remain viable for many years (Ourecky 1975; Daubeny 1996).

Seed germination

Rubus seeds require scarification treatment that involves cutting the seed coat using abrasion, thermal stress or chemicals to physically remove much of the endocarp making it permeable and encouraging germination. Seeds also require stratification to simulate winter conditions so that germination may occur. The procedure described by Daubeny (1996) and Ourecky (1975) has been used by the AAFC-PARC breeding program and other programs with satisfactory results. Dry seeds are placed in glass test tubes or polycarbonate vials and kept in a crushed ice bath for 15-20 min treatment with enough concentrated H_2SO_4 to cover the seeds. Recent research examining endocarp thickness and evaluation of different duration of acid treatment has shown that 15 minutes of acid scarification is appropriate for most seed lots but there are some that require less that 15 min or seed will be destroyed and others that require up to 30 minutes to elicit maximum germination (Hall pers. obs.). After acid treatment, tube contents are poured into filter mesh and washed for 5 min under running tap water to remove any acid residue. The seeds are then immersed for 1 week in a 1% solution of calcium hypochlorite followed by a wash in running water for 5 min. Seed may be stored in moist sand or directly on moistened peat in the germination flat at 5°C for 6 weeks to satisfy stratification requirements. Stratification may not be necessary if the seed has been cool-stored for 6 weeks or more before scarification. In this case sowing of scarified seed immediately after treatment may be very successful (S.N. Jennings pers. obs.).

Seed is sown on light soilless potting medium and covered with a small amount of sand and placed in a 25°C, 16-h day length and high humidity environment. Seedlings are more often killed by excessive than too little watering. Intermittent mist that keeps the seeds damp but not soaked is ideal. Six weeks of stratification are not needed if seeds are treated immediately after harvest with sulphuric acid and calcium hypochlorite (Dale and Jarvis 1983). When only small amounts of seeds are available it is possible to nick through the seed coats and expose the embryo or to use *in vitro* germination procedures (Ke *et al.* 1985; Nesme 1985; Finn and Hancock 2008).

Germination begins within 3-4 weeks (Dale and Jarvis 1983). When the first true leaves appear, seedlings are ready to be transplanted into larger pots. At this time, selection for spineless canes expressed by gene *s* can be made where spineless segregates are devoid of stalked glands at the edge of the cotyledon leaves while on the spiny plants glandular hairs are present (Hall *et al.* 2009). Seedlings may be screened at this early stage for resistance to the large raspberry aphid, the vector of the raspberry mosaic virus complex. The aphid vectors are *Amphorophora idaei* in Europe and *A. agathonica* in North America. *Amphorophora idaei* has several biotypes that are differentiated by their abilities to overcome plant resistance genes. Bioassays of aphid field populations showed a strong shift towards A_1 resistance-breaking biotypes since the 1960's (Jones *et al*. 2001; Birch *et al.* 2002). *Amphorophora agathonica* that attacks plants previously identified as being resistant have been found, however these 'biotypes' have not reproduced well on resistant plants and, so far, have not been shown to be a threat in the field (Daubeny, Pepin and Levesque 1992; Kempler pers. obs.). Screening for reaction to *A. agathonica* in the AAFC-PARC program is done prior to field planting on seedlings with at least three leaves. Aphids are reared according to Forbes *et al*. (1985) and three aphids are placed on each plant every 3-4 days for three weeks. Plants that are not colonized by aphids are classed as resistant. Additional observations are made in the field to identify escapes from the common biotype or susceptibility to a resistance-breaking biotype. Young seedlings have also been screened for reactions to root rot caused by *Phytophthora rubi* in the AAFC-PARC program. The seedlings are grown in individual pots of soil and at the five true leaf stage the roots are inoculated with a mycelial suspension of the pathogen. Above-ground symptoms of root rot usually appear within 10 weeks and susceptible genotypes are dead within 15 weeks while resistant seedlings grow vigorously (Daubeny 1996). Pattison *et al.* (2004) developed an effective hydroponic procedure to conduct screening for resistance. Young seedlings may also be pre-field screened for resistance to other diseases and pests according to the relative importance of the problem and the practicality of the screening. DNA screening of the plant population can also be used to identify individuals with desirable traits before moving plants into the field.

Assessment of seedlings

Seedlings are then planted in the field, typically at 75 to 150 cm spacing within the row. The AAFC-PARC program plants at 90 cm within the row as this allows a reasonable distinction between individual plants. However at this close spacing propagation stock must be harvested carefully to ensure genotype integrity. The between-row spacing depends on local farming practices. The 240 cm between row spacing used by the AAFC-PARC program allows for a tractor to pass between rows. For most crosses, a progeny size of 100 seedlings will give a good representation of the potential of the specific combination. Larger progeny size may be valuable when the parents involved are especially genetically diverse or when primocane fruiting segregates

are sought from crosses between floricane fruiting and primocane types, as in the seedling populations that produced 'Erika' and 'Sugana', each from around 6,000 plants of the cross 'Tulameen' x 'Autumn Bliss'. Each year, the AAFC-PARC program plants 2,000 to 5,000 seedlings after prescreening for susceptibility to the aphid vector *A. agathonica*, which eliminates about 30% of the seedlings. The AAFC program makes 25 to 60 crosses annually with an average of 60 to 80 seedlings per progeny. Seedlings are planted early in the spring and immediately irrigated. Some programs plant in the fall to reduce problems with weed control. Dormant primocanes of young plants that will be evaluated for their floricane crop may be cut back in order to save on labor in pruning and training. If grown well and under ideal conditions, some programs may successfully make selections of superior primocane-fruiting genotypes towards the end of the growing season. However, since developing cultivars that are resistant to RBDV is a main objective of the AAFC-PARC program, the canes are left to flower and to add another year of exposure to RBDV infection.

Usually selection takes place in the second or occasionally the third year after planting. During the fruiting season, fields are walked every 2-5 days and plants are selected according to the objectives of the program. In the AAFC-PARC program notes are collected only on the selected plants and selection is done according to the desired plant habit, fruit characteristics (especially flavor) and suitability for the fresh or processing market. A selection rate of 0.5 to 1.5% in the seedling field is common in most breeding programs but occasionally it may be up to 10 percent (Hall *et al*. 2009). Leaf tissue from each selection is tested for the presence of RBDV. Selections that test positive are discarded mainly because selections that became infected in the field after short exposure (2-3 seasons) are very susceptible but also because it is time consuming to use heat therapy to produce a virus-free clone. This would not be appropriate if the parents had not been tested prior to using them to produce the cross.

Propagation

Soon after a selection is made, stem nodes are collected from the primocanes to establish the genotype *in vitro* plants. Nodes that are collected late (September-October in British Columbia) have already initiated flowers and will produce no vegetative buds (Sønsteby and Heide 2008; Kempler pers. obs.). In the AAFC-PARC program, selections are also transplanted into a "repository field" where they can be used as parents for crossing and where they are also tested for RBDV every year. Enough plants are propagated in tissue culture over the winter for early spring planting in first year trials.

Assessment of selections

If the selection has the potential to be suited for mechanical harvesting, 10 plants are planted in an unreplicated plot at 75-90 cm between plant spacing where it will be harvested in the 2nd and 3rd year after planting with a commercial harvester. A gap between plots allows excellent separation of the harvested fruit between the selections and collection of the fruit into separate trays. Machine harvest evaluation early in the evaluation of several genotypes was critical in allowing for the relatively rapid release of the AAFC-PARC cultivars 'Chemainus', 'Saanich', 'Nanoose' and 'Ukee'. The selections are assessed weekly and rated numerically for yield, overripe fruit, unripe and green fruit, fruit color and firmness, fruit integrity and suitability for mechanical harvest (plant growth habit). Their possible suitability for IQF processing is also inferred from fruit qualities. Clones that show promise are propagated and planted in large scale growers' trials.

In the AAFC program three-plant-plots that are replicated three times are planted with promising selections along with standard commercial cultivars. Two years after planting, when the plants are well established, they are evaluated for horticultural parameters like total yield, fruiting season, fruit size, firmness, soluble solids concentration (Brix), flavor, and pre- and postharvest fruit rot that is mostly caused by *Botrytis cinerea*. If sufficient labor is not available for harvest, yield estimates may be made using yield component estimates (Daubeny *et al.* 1996). Fruit samples are collected and frozen immediately after harvest. They are used to determine titratable acidity, pH, soluble solids and anthocyanin concentration during the winter months. In recent years, there has been an increased interest in the health benefits of the fruit and so the anthocyanins, ellagic acid content and level of antioxidant activity (e.g. ORAC, TEAC, or FRAP) and other traits may be measured.

Plant growth habit is also evaluated throughout the growing season to assess whether the clones have a desirable growth habit. Ideally, the plants have upright spine-free or nearly spine-free canes that carry strongly attached, short to medium length, upright laterals with fruit that is well spaced and not bunched. Plants should have a sufficient number of new replacement primocanes that are strong, straight and long enough to reach the trellis wires. During the late winter months and before bud break, selections are examined for their reaction to various cane diseases including spur blight, cane Botrytis and anthracnose and during the summer months the foliage is inspected for cane blight, powdery mildew, yellow rust and other diseases that are present in the area. The plots are rated on a numerical scale and compared against standard cultivars. This information is used to help choose the parents for crosses during the process of introducing improved resistance into the germplasm.

To assist with the process of identifying resistance and susceptibility, the AAFC-PARC program does not apply any field spray program to control diseases or pests. Although 'Qualicum' was identified to be winter hardy in BC, it showed significant winter injury when tested in other production areas because it is very susceptible to anthracnose. This was not considered important as commercial growers in BC routinely use a spray program to control fruit rot. This fungicide spray program is also very effective in controlling cane diseases including anthracnose, and the release of 'Qualicum', an anthracnose susceptible cultivar, was therefore not a concern in BC (Daubeny and Kempler 1995). Most breeding programs follow a minimal spray program in their selection trials. A typical breeding program might have the basic dormant sprays for cane diseases and a reduced Botrytis fruit rot program. This is important for two primary reasons: 1) sometimes genotypes with some tolerance to biotic stress are overwhelmed with inocula from nearby plots of very susceptible genotypes and 2) for some diseases, despite tremendous efforts on the part of breeders and pathologists; no good resistance has been uncovered. *Botrytis* fruit rot is a very good example of this.

Variation in breeding programs

While there is a scientific basis underlying most raspberry breeding, results delivered from different breeding programs vary from breeder to breeder and region to region. Part of this is due to pest and disease or environmental pressures and due to the germplasm used for breeding. There is an enormous amount of variability hidden in the genomes of raspberry cultivars and selections used for breeding around the world. The usual size of breeding populations of less than 200 seedlings delivers but a small part of the variability available from each cross. In spite of this there is ample diversity available for selection from most breeding programs, except perhaps when selecting to expand the region of environmental adaptation for new cultivars.

Other factors affecting the outcome of breeding programs include bias from a cultural perspective, from local traditions or from the particular artistic flair of the breeder or breeding team. This is most obvious when considering

the shape, color and flavor of the fruit but it is also evident in seasonality, structure of laterals and architecture of the plant.

In Scotland, the breeding by Derek Jennings over a period of 30 years resulted in material with large chunky fruit, light color, spineless canes, early harvest and shorter laterals with fewer larger fruit. After Ronnie McNicol succeeded Derek Jennings in the program in a short time, there was a very high percentage of the seedlings that had a similar size, shape, color and appearance. In contrast the program at East Malling under Elizabeth Keep selected for fruits with a higher number of smaller drupelets, longer, narrower fruit, darker color, a different characteristic flavor, late seasonality and long laterals with high fruit numbers.

Further breeding with materials from these two programs in Russia has resulted in outstanding gene *L1* floricane fruiting spineless selections from a program near Moscow and excellent primocane fruiting selections from a program near Bryansk, each with their own distinguishing characters and both different to the parent programs in the United Kingdom.

In North America, programs in the East have focussed on hardiness, root rot resistance, and primocane fruiting, showing considerable advances in the genetics in three different locations; Nova Scotia, Geneva, New York and in North Carolina, each with a different flair. Similarly in the West programs in California have produced types with a particular individual flair of the breeder or the company running the program. Light color, high yield, dual cropping, high quality fruit with good shelf life and excellent fresh market flavor and presentation, yet discarding some outstanding material because it did not satisfy the desires of the marketing companies commercialising the new cultivars. In Oregon, Washington and British Columbia programs in each of these locations have selected for root rot resistance. In Oregon primocane fruiting was a major theme, RBDV resistance was the focus in Washington, along with introgression of genetics from Asia and in British Columbia the foci have been on aphid resistance, fresh market quality and introgression of genes from *R. strigosus*. Each program has been given the particular flair and focus by the breeder or company and this has resulted in material of a particular character. Plant breeding is as much an art as a science and it is driven by personal preferences as well as local themes and the variation found in the starting point genetics.

KARYOTYPING

Chromosome number, the place of polyploidy

Raspberry chromosomes, like those in other *Rubus* types, are very small (1.5-3 μm) and detailed studies of chromosome structure are difficult or impossible to carry out (Pool *et al.* 1981; Wang *et al.* 2008b). However Bammi (1965) found that preparations of chromosomes of *R. parvifolius* at the pachytene stage of division were easier to spread than other *Rubus* and showed good definition. Chromosomes were found to be highly differentiated and the centromere and its associated chromatic regions were easy to differentiate (Bammi 1965; Jennings 1988). More recent studies have showed that chromosomes in a range of species are easy to differentiate and that it is possible to recognise each of the seven chromosomes within the species studied (Pool *et al.* 1981; Wang *et al.* 2008b).

Red raspberries, like most of the species from the Ideaobatus subsection of the genus Rubus, are diploid, having two sets of the basic 7*x* chromosome genomic constitution, 2*x* =14 (Bammi 1965; Thompson 1997). The subsection also has a few species that are triploid (*R. idaeopsis* Focke), or tetraploid (*R. sachalinensis*, *R. mullerii* F.M. Bailey, *R. nishimuranus* Koidz., and *R. leucocarpus* Arn.), or that have triploid and/or tetraploid variants of the basic 2*x* species (*R. apetalis* Poir., *R. foliosis* D. Don, *R. niveus* Wall, ex G. Don., *R. longipedicellatus* (Gust.) C.H. Stirt., *R. parvifolius* L. and *R. pinnatus* Willd. (Thompson 1997; Wang *et al.* 2008b). *R. longipedicellatus* is also distinguished by giving a pentaploid form. Red raspberry (*R. idaeus* L.) and its hybrids also have some triploid and tetraploid forms and cultivars that are 3*x* (‘All Summer’, ‘Belle de Fontenay’ ‘Erskine Park’, ‘Immer Tragende’ and ‘November Abundance’) and 4x (‘Colossus’ ‘Hailsham’, ‘La France’, ‘Surprise de Autumn’, ‘Perpetuelle de Billiard’) (Fischer *et al.* 1943; Pratt *et al.* 1958; Thompson 1997; Kazakov and Evdokimenko 2007). In addition tetraploid sports of raspberry cultivars are relative common and they have been used significantly in the development of hybrid berries and in distant crosses between raspberry species. Natural tetraploids have been found of many cultivars, including ‘Malling Promise’, ‘Malling Jewel’, ‘Meeker’, and others (Jennings pers. comm.; Hall pers. obs.) and others have been developed artificially; ‘Latham’, ‘Newburg’, ‘Lloyd George’, ‘Carnival’, ‘Malling Promise’, ‘Malling Exploit’, Rubin Bulgarski’, ‘Ottawa’ (Ogoltzova and Kichina 1973; Kichina and Aver'yanova 1981).

Size and structure of chromosomes

Raspberry chromosomes are small, varying from 1.8-3.1 μm in length with chromosomal arm length ratios being from 1.1-7.9 (Jinno 1957; Bammi 1965). In terms of modern examinations the genome compliment of raspberries also is very small, with only 275 Mbp, less than 2x the compliment of Arabidopsis with 145 Mbp. This has made chromosome studies in raspberries difficult in terms of getting a good view of chromosome structure and morphology. However, now that much work is being invested in molecular biological studies in raspberries this small genome compliment has now become an asset making mapping studies and marker assisted selection (MAS) easier and cheaper than with many other fruiting plants.

MOLECULAR MARKERS AND GENOME SEQUENCING

In recent years there have been considerable advances in the area of biotechnology, enabling direct examination of plant DNA and RNA sequences and proteins, correlation of this information with plant traits, and enabling deeper understanding of genetic variation within plants. These techniques have been applied to raspberries making it possible to identify genetic markers from within plant material of a known background and to determine the presence of specific genes or traits in individual seedlings of progenies from that background. Direct detection of variability from within the raspberry using molecular techniques has become a new way of assessing genetic variation within the whole genome and enabled the construction of genetic linkage maps showing regions associated with specific genes or gene aggregations which control complex traits. Associations for polygenic traits are also possible and a detailed understanding of these offers a new future for plant breeding of raspberries (Graham *et al.* 2007; Hall *et al.* 2009; Kempler *et al.* 2011). These techniques offer greatly increased speed and precision in assessing of raspberry germplasm and in the incorporation of specific traits from wild accessions into new cultivars, while leaving behind many deleterious traits.

Value of MAS

RAPD and SSR markers can be used to distinguish between cultivars and they are also useful to show groups of similar origin from amongst larger collections of cultivars. Pedigree relationships can be elucidated and followed and genetic diversity can be shown among cultivars; this examination of selections, cultivars and germplasm can be used to produce an accurate DNA fingerprint for each clone. This does not rely on morphological assessment and it can be determined with accuracy on vegetative plants at any stage of propagation, even within tissue culture and in some cases it can be used to differentiate sports that have arisen from a cultivar.

Markers can also be used to identify segregates from within a population that have inherited an identified trait, making it possible to eliminate the undesired genotypes before resources are invested in planting and assessment in the field. An effective marker is usually closely associated with both the trait in question and also the genetic background from within which it is identified. Often a marker is not effective in identifying segregates for the same trait from within populations that are not closely related. A marker has to be closely associated with the gene or trait within related populations and be subject to minimal crossing over, so that plants falsely identified to have the trait are an insignificant part of the whole.

Efforts put into identification of markers can be very rewarding, especially if traits associated with the markers are important for the commercial success of the new cultivars. Particular value with MAS is achieved when the time to assess a trait is reduced, resources to screen seedlings for the trait in the field are significantly decreased, or when costly and difficult testing methods are required to eliminate undesirable clones. In international programs it is also possible to eliminate segregates that are susceptible to pests and diseases that are not found in the region where the seedlings are grown but resistance to them is desired as an insurance against infestation in the future. For example, *Phytophthora rubi* is not found in New Zealand but is found in the majority of raspberry production regions around the world. In New Zealand it is highly desirable to be able to develop cultivars resistant to this disease in case it arrives in the future.

Identification of markers

Markers for a trait can be identified by examining a carefully phenotyped population in which all individuals have been clearly screened to show that they possess the desired trait or it is clearly absent. Phenotyping can be assisted by cloning each member of a population and planting replicated trials in two or more different environments, especially if that population is to be screened for multiple traits, including resistance to pests and disease, fruit and growth characteristics and ability to withstand environmental extremes. In Scotland, a population of seedlings from the cross 'Glen Moy' x 'Latham' were screened for a range of traits including resistance to *Phytophthora*, cane *Botrytis*, spur blight, cane spot, yellow rust, and also a marker for the presence of gene *H* was determined and linkages between this gene and other traits examined (Graham *et al.* 2006).

Use of MAS in breeding

At SCRI MAS is being used for breeding to identify traits that either are expensive, difficult and/or time consuming to determine. Resistance to *Phytophthora* is difficult to determine accurately and also it is difficult to produce clean material for planting in the field without spreading the pathogen after resistance has been determined. In addition, MAS is being investigated for selecting traits that cannot be determined until mature plants have been grown in the field and testing of these has been completed. These can include fruit quality parameters, yield components, pest and disease resistance, growth habit and plant morphological traits. MAS for resistance to *Phytophthora* is also being incorporated into the Geneva program in New York State and will be utilized for the future of raspberry breeding there.

Cost-benefit analysis

Determination of markers associated with traits in raspberries is an extremely expensive process and in general the cost of using MAS in raspberry breeding cannot be justified in most raspberry breeding programs around the world. However, in locations where it is being used MAS is likely to assist in the selection of new cultivars that will be greatly used for raspberry production in those parts of the world. Use of MAS for selection of pest and disease resistance in seedling populations will shorten the time required to prove genetic resistance in a cultivar and eliminate the requirement for raising the pest or disease to screen the material before plants are evaluated for production and quality traits. If investment is made into identifying markers for important traits then much can be gained by investing further in closer inspection of morphology, growth traits, fruit characters and determination of the invisible traits that can only be seen by chemical, physical and other tests.

Genomics and genome sequencing

As red raspberry is diploid and contains a very small genome it is an excellent subject for map construction and map based gene cloning (Graham and Woodhead 2009). Genetic variation contained in red raspberries from Europe and North America is plentiful and this is extended greatly when the widespread range of Idaeobatus species is included. Adaptation is from the tropics to the Arctic Circle and from sea level to mountain tops throughout Europe, Asia and North America and rather more sparsely into South America, Australia and Africa. Adaptation exists for tolerance of very cold and very hot conditions, high humidity and drought, and resistance is found to pests and diseases, as well as an enormous array of variation in growth, morphology, biochemistry, quality and quantity of production. Close examination of clonal material from this genetic resource will enable the development of knowledge and insight into the complex relationships between genotypes and environmental conditions. In time, this could lead to the ability of researchers to develop markers for polygenetic traits and the identification of gene controlling complex phenotypes (Graham and Woodhead 2009).

Mapping

Several genetic linkage maps have been generated for raspberry, primarily to identify markers for genetic resistance and these are being expanded to include other economic targets. These vary from morphological traits that have previously been associated with resistance, to specific biochemicals that are attracting interest because of their bioactive health properties. In addition plant cells are being mined for genes coding for specific enzymes, proteins and health attributes. These may be utilized for production of industrial and/or biochemical materials, especially if they can be patented and the genes moved into micro-organisms for use as cheap sources of feed-stocks for biological processes that are different in a small way from constructs patented by competitors.

Metabolomics

Metabolomics, "the systematic study of the unique chemical fingerprints that specific cellular processes leave behind" (Wikipedia 2010), has become a useful tool for mining plant variability, establishing the genetic basis to phenotypes, application to functional genomics studies, and examining the genetic interaction with environmental conditions (Fait and Fernie 2009). Use of this tool in raspberries has been limited but it has considerable potential for the future as it can give an instantaneous snapshot of the physiology of the cell. Plant response to environmental variation is quickly visible amongst metabolites and a careful analysis of the products of metabolic processes is a suitable measure for determining the ability of the plant to respond to biotic or abiotic stress. This approach was used to identify bioactive polyphenolic compounds in a segregating mapping population in two different environments (Stewart *et al.* 2007). As the genetic basis and interrelationship of different antioxidant compounds is revealed, it should be possible for breeders to identify raspberry genotypes with enhanced health-promoting properties (Beekwilder *et al.* 2005).

Table 3 Contents of tissue culture media.

	Stage I and II Media			Stage III Media		
	1 (mg/L)	2 (mg/L)	3 (mg/L)	1 (mg/L)	2 (mg/L)	3 (mg/L)
MS salts (Murashige and Skoog 1962)	6600	4330			2165	
Modified MS salts (Murashige and Skoog 1962)				2680		
MT salts (Murashige and Tucker 1969)			2200			880
Additional $MgSO_4$	90					
$CaSO_4$		125			63	
Woody Plant Medium (WPM) vitamins (Lloyd and McCown 1980)	104			104		
zeatin/kinetin			1.0			
indole-3-acetic acid (IAA)					1.0	
indole-3-butyric acid (IBA)	0.1		0.1-0.5	0.1		0.1-0.5
inositol		100			100	
benzylaminopurine (BAP)	0.6	1.0	1.0-4.0			
Thiamine HCl		0.4			0.4	
FeEDDHA	118			78.8		
Sucrose	30,000	30,000	30,000	30,000	30,000	15,000
Plant Agar	6,000	2,000	7,500	6,000	2,000	7,500
pH	5.6	5.6-5.7	5.8	5.6	5.6	5.8

1 (Zawadzka and Orlikowska 2009)

2 PARC Agassiz (Kempler pers. obs.)

3 (Wu *et al.* 2009)

Proteomics

While proteomics was mentioned in a web page on metabolic profiling by Derek Stewart of SCRI in 2003 (Stewart *et al.* 2003), little has been mentioned since then in the literature or in SCRI research publications or posters.

Genetic transformation

Recent advances in biotechnology have allowed scientists to incorporate novel genes into the raspberry genome (Kempler *et al.* 2011). Genetic transformation is the process of altering the genetic code of the plant material creating transgenic plants. This process has been used to confer resistance to diseases, such as the introduction of a RBDV-resistant gene into cultivars such as 'Meeker' (Martin and Mathews 2001; Martin *et al.* 2004). Fruit set remained an issue with the transgenic 'Meeker' plants, but some plants appeared commercially viable, although they have not yet been released for commercial growth (Kempler *et al.* 2011).

Genetic transformation can also be used to increase the nutraceutical value of food crops, such as the incorporation of genes for lysine, iron, zinc and beta carotene into rice (Krishnan *et al.* 2003). Several vitamins and micronutrients contained within raspberry have been identified as having positive health impacts. For example, research suggests anthocyanins and polyphenols (commonly known as antioxidants) such as ellagic acid, which is contained in significant amounts in raspberry, provide anti-aging and anti-cancer benefits (Mullen *et al.* 2002; Funt 2003; Kempler *et al.* 2011). It has been suggested that genetic transformation could be used to increase the concentration of nutraceuticals in raspberry (Stewart *et al.* 2007; Hall *et al.* 2009). However, the biggest challenge to the commercial development and release of transgenic crops is public caution toward consuming genetically transformed food crops (Kempler *et al.* 2011).

IN VITRO CULTURE

Traditional propagation techniques by cutting or suckering can be limited by inability of a genotype to root, transmittance of disease or pest to daughter plants or being too slow and unreliable for the rapid bulk-up of new cultivars (Jennings 1988; Wood and Hall 2001; Wu *et al.* 2009). Micropropagation is a modern laboratory technique that allows genetically identical plants to be multiplied quickly and in large quantities without many of the challenges posed by traditional techniques. It was originally developed because it allowed virus free plants created through heat therapy to be multiplied rapidly (Daubeny 1996). Early *in vitro* propagation of raspberry was conducted by Anderson (1980). Although virus-free varieties have been created through breeding, heat therapy is still used to clean older germplasm, and micropropagation has become an efficient and widely used technique for propagating newly developed selections (Wang *et al.* 2008a).

Raspberry susceptibility to viral diseases makes it necessary to use tissue culture for elite and certified plant production. Despite the technological advances and number of publication on propagation of *Rubus*, there are still difficulties that influence *in vitro* propagation of some *Rubus* cultivars (Donnelly and Daubeny 1986; McPheeters *et al.* 1988; Reed 1990; Bobrowski *et al.* 1996; Gonzalez *et al.* 2000; Pelto and Clark 2000a, 2000b; Tian *et al.* 2005; Zawadzka and Orlikowska 2006).

Micropropagation consists of four stages: Stage I (initiation), Stage II (proliferation or multiplication) and Stage III (rooting) and Stage IV (acclimatization or hardening off) (Kyte and Kleyn 1996).

Culture media

Proliferation (Stage II) media is composed of an agar base supplemented with sucrose or other sugars and micronutrients required for plant growth (see **Table 3**) (Zawadzka and Orlikowska 2009). Nutrients are incorporated into distilled water using a magnetic stir plate. Growth regulators are added to inhibit apical dominance and promote axillary bud development, and pH is adjusted (Daubeny 1996). While **Table 3** provides details of media which will work for many raspberry cultivars all variables need to be tested to find the optimum concentrations for each genotype (Hoepfner and Nestby 1991; Tan pers. comm.). This includes sugars, where some cultivars require glucose and/or fructose, iron source including FeEDDHA, sequestrene and FeEDTA, different phytohormones and concentrations and varying basic salts solutions and concentrations. Recent research points to the beneficial effects of using FeEDDHA to overcome iron chlorosis and increase root number, root length, fresh weight and chlorophyll content of the microshoots (Zawadzka and Orlikowska 2009). Cytokinin levels need to be kept in the range of 0.01-2mg/l to reduce the risk of producing aberrant plants in culture (Tan pers. comm.). With some clones the use of activated carbon in the tissue culture medium is also beneficial, and it results in better growth, plant health and especially rooting of the plantlets in culture.

Once the ingredients are fully dissolved, the media is heated to melt the agar. The media is poured carefully into Petri dishes, test tubes or jars to a depth of about 3 cm and autoclaved at 1.1 kg/cm^2 for 15 minutes (Wu *et al.* 2009). After sterilization, the media is cooled and stored until use.

Rooting (Stage III) media is prepared using a similar methodology, but may contain different growth regulators to promote root growth (Daubeny 1996).

Initiation (Stage I) and sterilization of field and greenhouse materials

Plant materials used for propagation are tested for freedom from common viruses. Bud segments from primocane growth are collected during the spring and summer. Buds collected after differentiation will fail to produce vegetative growth, exhibiting reproductive organs, and are no longer suitable for initiation (Kempler pers. obs.; Hall, pers. obs.). For winter initiation, vegetative buds can be collected from root cuttings that were collected in the fall and produced vegetative growth shortly after (Kempler pers. obs.).

Plant material in the field is likely to be contaminated with many different fungi and bacteria, both topically and endogenous and it may also be infected with viruses. Some laboratories find it very beneficial to heat treat all plants before initiation (Hall, pers. obs.).

Plant materials are initiated by collecting shoot tips of 2-3 cm and stem segments displaying lateral buds of about 3-5 cm in length. Explants are sterilized by immersion in 70% ethyl alcohol for 5 seconds and agitation in a concentration of 1% sodium hypochlorite and two drops of Tween-20® for 25 min (Wu *et al.* 2009) or by rinsing in 10% sodium hypochlorite and agitation twice for ten minutes in 1-5% sodium hypochlorite depending on the sensitivity of the explant (Kempler pers. obs.). To further reduce the level of contamination, especially from endogenous microorganisms, it is worthwhile taking very small meristem explants for initiation (Tan pers. obs.).

Sterilized explants are transferred into test tubes inside a laminar flow hood. Explants are sliced on a slight angle into a single bud segment of approximately 0.8-1.2cm and placed into the media without covering the bud (Kyte and Kleyn 1996). Several explants from the selection can be initiated into the same culture, however this is not recommended because the entire culture can be lost due to a single contamination (Wu *et al.* 2009; Kempler pers. obs.).

Proliferation (Stage II)

Cultures are kept in a growth room. Temperature is maintained at 26 +/- 1°C. 16-h periods of cool-white fluorescent light are followed by 8-h periods of darkness (Wu *et al.* 2009).

Plantlets are closely monitored to ensure that they do not become affected by bacterial or fungal infections. Healthy plantlets are transferred to their own test tubes to continue proliferation, while dead or sick plants are discarded.

After approximately 4-6 weeks in the proliferation stage, the growing bud is cut and transferred into fresh media. If mild contamination by bacteria, mould or fungus occurs, plants are re-sterilized by agitating them in a solution of 5% sodium hypochlorite and then transferring them to fresh media under the laminar flow hood. However, if significant contamination occurs, the test tube is discarded (Kempler, pers. obs.).

Multi-shoot production will start. Cultures are divided into individual shoots and transferred to fresh media every 4-6 weeks. It is possible to use larger jars like mason jars and autoclaveable plastic containers (Hall pers. obs.).

Rooting (Stage III) and acclimatization (Stage IV)

When the desired multiplication has been achieved, plantlets with 3-5 leaves can be transferred in the laminar flow hood to rooting media (**Table 3**) which stimulates the plantlets to elongate and grow roots. Roots may form as soon as 11 days after the plantlets are transferred to rooting media (Wu *et al.* 2009). Plantlets remain in rooting media for 4 weeks, after when they are transferred to the greenhouse. Some clones are slower and begin rooting only after they have been transferred to the soil substrate.

Rooting media is washed from the roots of the plantlets and they are planted in soil and kept in the greenhouse in low light and under mist or covered with plastic domes while they acclimatize.

Germplasm storage

In vitro techniques can also be used in long term storage of germplasm to prevent the loss of genetic diversity. Using media prepared without growth regulators and maintaining the plantlets at 2-4°C, re-culturing times were reduced to 3-4 months, contamination by bacteria and fungi were low, and the plants remained healthy. Cryogenic storage also has been used successfully. No evidence of genetic instability was found (Coman *et al.* 2003; Reed *et al.* 2008).

Protocols for trueness to type

Raspberries are prone to mutate in culture, frequently becoming crumbly fruited and worthless for commercial cultivation. To reduce the possibility for mutation occurring in culture or for propagation of a sport care must be take not to multiply adventitious buds formed around the base of the explant. Cultures should be transferred a limited number of times to prevent mutations from carrying over into subsequent subcultures. Clones should be frequently reinitiated from original breeder stock. Levels of cytokinin need to be kept to relatively low levels (0.01-0.2mg/l) and sufficient initiates need to be started so that propagation targets can be reached without doing more than 10 subcultures. In addition, it is wise to keep track of the propagules from individual or groups of initiates so that if any aberrant types are found then that material can be easily discarded without having to cull or sort large numbers of unaffected material.

FUTURE WORK, PERSPECTIVES

Pest and disease resistance

Pest and disease resistance has been important throughout the history of plant improvement in raspberries, and commercial production of raspberries in most countries requires an ongoing spray program for control of insect pests, nematodes, fungi, bacteria and other biotic assaults on plant growth, plant health and fruit production. However, the use of spray chemicals is more and more difficult with many countries developing lists of permitted chemicals and maximum residue limits (MRLs) allowed for the production of crops entering both animal and human food chains. Cost of the production of new chemicals for pest and disease control is becoming more and more expensive and many effective spray chemicals have been removed from the permitted list, or effectively become unusable due to the minimum waiting period allowed after spray application before harvesting the crop has become too long. Methods of detection have become more and more sophisticated, making it possible to detect lower and lower residue levels and making it difficult to achieve the nil residue requirement in many countries. In addition many countries have a double standard, where marketing of fruit into those countries from outside is much more difficult that local growers as residue level requirements are much more stringent for imported fruit, and local growers may be able to get special allowances to use chemicals that are not allowed to be used on imported fruit.

Thus from a breeding perspective it is becoming important that control of pests and disease is managed through the development of resistant cultivars. Genetic variability in raspberries and the subgenus Idaeobatus is enormous and it

is possible to find resistance to most of the serious pests and diseases which limit raspberry production around the world. However, even when resistant cultivars have been produced, the resistance may be transitory as pests and diseases may quickly overcome the resistance. Thus for long term solutions to individual pests or diseases multiple resistance genes may need to be pyramided into new cultivars, especially when the pest is very adept at overcoming individual resistance genes. Using traditional breeding techniques it is difficult or impossible to pyramid multiple resistances into a single genotype. Nevertheless, this is possible by using markers associated with different resistances and being able to detect more than one of them in a new cultivar.

Incorporation of new genetics

Key to the development of durable resistance to pests and diseases in new cultivars is the identification and introgression of genes from cultivated raspberries or their wild relatives. This is a time consuming process, often requiring up to 5 generations of breeding improvement from the initial cross to the developed new cultivar. Keys to reducing the numbers of generations to achieve this process are starting with the best parents of cultivated raspberry to cross with the wild type, especially choosing those traits which complement the failings of the wild type. A large population in the F1 generation is also helpful, especially if there is considerable infertility in this material. If reduced fertility is a severe problem but there are still a few drupelets set on flowers of the F1 hybrids, then an open pollinated F2 is often helpful, allowing reassortment of genes in the F2 generation and frequently complete restoration of fertility (Hall pers. obs.). Once fertility has been restored then back crossing it is much easier to achieve success in producing a new commercial cultivar in subsequent generations, but if back crossing is carried out to cultivated types with sub-fertile selections from the F1 or later generations it may take several generations to restore full fertility, if possible at all.

Using MAS and mapping of the genomes of both the cultivated raspberry parent and the wild type utilised for incorporation of new resistance genes it may be quicker and easier to select for genotypes with a genome complement similar to the original cultivated type but containing the desired added resistance genes from the donor accession. Use of mapping and MAS for achieving this has not yet been reported in raspberries.

Move towards primocane fruit production and away from floricane fruiting

Production of raspberries is costly and the longer the plant has to be kept healthy and growing, the more expensive the inputs are maintain plant health, to manage plant growth and to produce the crop. Thus from a simple financial perspective it is desirable to reduce the time from planting to producing an economic return. In addition, the requirements of floricane fruiting raspberries are much greater in terms of pruning and training and the required environmental conditions in the first full year of growth are much more exacting.

Nevertheless, floricane fruit production and floricane fruiting cultivars have been the norm since cultivation of raspberries began. Initially this was because the primocane fruiting trait had not been developed in any cultivar to enable the production of an economic crop. Even until the present date floricane production has remained the usual production method for processed fruit. Cultivars such as 'Meeker' continue to produce yields much greater than the best floricane fruiting cultivars available. Yields obtained by the best growers are in the order of 2-3 kg per plant (3 m row spacing, 0.5 m between plants) or as much as 3.8-5 kg per plant when plants are 0.92 m apart (3 feet). The highest recorded yield of a floricane fruiting cultivar within a trial plot (in California) was over 30 kg per plant on a selection that had all new canes removed (Hall pers. obs.).

Commercial production of primocane fruiting cultivars in an annual typically has yielded between 900 g and 2.7 kg per plant, almost up to the yields of the grower fields in the Pacific Northwest, but an attempt to produce a primocane fruiting cultivar for machine harvest production in Washington State was not successful in the long term. However, the possibility of higher primocane fruiting yields was indicated in production of primocane fruiting, or dual cropping cultivars by Sweetbriars, the forerunner of Driscoll Strawberry Associates. When plants were grown in an 18 month cycle for dual cropping, they yielded as much as 14 kg over two harvests (the first primocane harvest followed by the second floricane harvest with all new canes removed).

In 2007, a manuscript with information about a Russian breeding program developing new primocane fruiting types was seen in China showing some outstanding new primocane fruiting types (Hall pers. obs.). A copy of this book was obtained and translated and in 2009 a visit was made to the breeding program in Russia. Fruiting plants were seen in the field that were easily recognised as the cultivars described in the book seen in 2007. Outstanding primocane fruiting cultivars were observed with fruit weights of 10-12 g and extremely high yields, some of which would have achieved the claimed 6 kg plus production per plant. In addition, some plants produced more than 600 fruits per cane, giving an obvious production unseen in Western breeding programs or commercial production. With the use of this genetics it appears possible to develop primocane fruiting cultivars that could compete successfully with present floricane fruiting types in commercial production for processing.

Growth habit, self-support, and elimination of support structures

In the Russian primocane fruiting breeding program described above, there were no support structures for any of the selections, cultivars or seedlings. These plants produced their high yields without canes falling onto the ground or being damaged through breakage. Whilst there may be little wind which could cause damage in that location and most of the plants were not bolt upright, there were some that were completely upright, healthy, productive, semi-dwarves that could easily be grown in windy conditions in other parts of the world with minimal damage. In Canada, England, New Zealand and Scotland, breeding for upright growth habit has produced healthy plants with sturdy, upright canes. Combination of these genetics with the sturdy caned primocane fruiting genetics from Russia would result in outstanding selections that stand fully upright and eliminate the requirement for the use of trellising and support structures.

Machine harvest for the fresh market

Development of cultivars suitable for machine harvest has been accelerated in recent years with programs in British Columbia and Washington State giving rise to a range of new selections suitable for machine harvest. Many cultivars and selections can be harvested by machine but there are several attributes that are required for a successful cultivar. Key cultivar attributes needed for optimum machine harvest performance include early and uniform detachment, leaving little or no unharvested fruit on the plant. In addition there needs to be little or no damage either to the plant or to remaining fruit after the passage of the machine. With several of the newer selections and cultivars some fruit is removed in suitable condition for fresh market, but this is contaminated by fruit which is overripe and unsuitable for any other use than processing, and even this use would not result in a premium quality product. There is potential for selection of very easily harvested cultivars that have little overripe fruit contaminating the sample and these could be used for fresh marketing.

Adaptation for MH design

Several different manufacturers have developed machines for commercial harvest of raspberries. Most of the successful designs have used the common design feature of straddling the row for harvest and use of beaters to shake the fruit off while the machine passes. Fruit are caught on catching plates or catching pans and then transferred to moving belts past human graders and into containers for processing.

While the general designs of machine have been similar, the design of shaker heads has varied significantly. Initial shaker head design used slappers or beaters which were facing backwards and slapped or swayed the plant as the machine moved forward. This design is sometimes still used for black raspberries but for red raspberry cultivars it has been superseded by rotary shaker heads that turned as the machine moved forward. The first of the rotary head machines was built with large drums and short metal fingers that transferred the shake to the bush but more recent designs have used smaller shaker heads with long fingers made of fibreglass or plastic.

The first successful machine harvest cultivar was 'Willamette' and this was most successfully harvested by a machine with the shake applied backwards and forwards in the rotation axis of the shaker head. 'Willamette' plants are well suited to this shaker action as laterals are short, especially in the top of the plants. When 'Meeker' was introduced as a new cultivar harvest success with this machine design was not as good as with 'Willamette' as fruiting laterals are much longer and could easily be damaged by an along-the-row shake. In addition, that design was very successful in telegraphing the shake along the row in front of the machine so that fruit dislodged before the machine was there to collect the fruit. Both of these issues were resolved by re-designing the shaker heads to apply a vertical shake to the bush.

Two different designs of vertical shake heads have come into common use. The first method where the shake is applied by a rotating counter shaft above the head, and the shake is balanced by counterweights, so that little shake is transferred to the machine. The second design utilised a crank-shaft to generate the shake movement and a leaf spring to soak up the movement at the bottom of the shaker head, again with little shake transferred to the machine itself.

Both the vertical shake and the horizontal shake machines have a significant fault in that they cannot remove all the fruit from the middle of the top of the bush. For longer lateral cultivars, such as 'Meeker', this is particularly significant early in the harvest season when the shaker heads are held well apart to reduce damage to the bush. Up to 20% of the ripe fruit of this cultivar remains on the bush in the beginning of the harvest season.

In recent years, a new machine has been under development which uses fans to buffet the bush and remove the fruit. However, these machines have not yet gained a significant hold on the process raspberry commercial harvest.

New raspberry cultivars suitable for machine harvest should have vigorous, upright cane growth, well attached, medium length laterals that are malleable rather than brittle and strongly attached at a right angle from the cane, medium to large fruit with excellent firmness and skin strength, and ease of harvest that has its onset at a targeted stage of fruit ripeness. Ease of harvest of raspberries is generated by either the development of smooth receptacles with little vascular trace attachment and a small or absent clasp around the receptacle or, like 'Meeker', by having a receptacle that inflates at the desired stage of ripeness. The harvested product must be uniform in ripeness and quality and the fruit remaining on the bush must remain undamaged after the pass of the machine.

Year round production

In recent years, consumption of raspberries has significantly shifted from predominantly processed fruit to much greater consumption of fresh fruit, especially in Australia, Europe and North America. This shift has accompanied the development of high quality fresh market types and the advent of the supermarket as the predominating sales location for raspberries in the developed nations. As supermarkets began to supply a higher and higher percentage of fresh market raspberries, they demanded that their suppliers provide high quality fruit produced in a protected environment throughout the year.

Production through part of the year was achieved by using a range of floricane and primocane cultivars that covered both the summer months and autumn. From warm locations, earlier production of floricane fruiting cultivars allowed spring production as well. However, the winter months were not filled and the supermarkets also were not happy with some of the cultivars in the sequence. They demanded the same or similar high quality floricane fruiting cultivars throughout the year. The first step to supplying fruit through winter months was made through growth and production in traditional Southern Hemisphere production regions and air freight to Northern Hemisphere markets. This was successful but transport costs were high. Recently, two additional approaches have been used. First, in North America, high quality primocane fruiting cultivars were developed in California and these were grown in southern California and Mexico where production could be achieved to fill all the gaps in the marketing calendar. Second, in Europe, the system of long-cane production with the floricane fruiting cultivars 'Tulameen' and 'Glen Lyon' was developed. These cultivars were grown in cooler Northern climes and after sufficient day length shortening and chilling was received to cause flower initiation then plants were dug and transported to Southern Spain or Southern Portugal for production during the winter months under tunnels. A further modification of this system has seen canes of both 'Glen Lyon' and 'Tulameen' grown in the Iberian peninsula and either chilled artificially at that location prior to growing on and fruit production, or shipping the canes further North to produce fruit late in the harvest season in tunnels or under glass.

In recent years, this system of long cane production has been supplemented by tunnel production of high quality primocane fruiting types in Southern Spain, Portugal and Morocco to supply the autumn and winter markets without having to undergo the expenses of long cane production. In time, this method is likely to replace the long cane production method.

Resistance to heat, scald and sunburn

In Australia, accompanying the shift in production from outdoors into protected culture under glass or in tunnels and the shift to producing fruit from warmer sites closer to the equator fruit damage from the sun's radiation or from high temperatures has become an important issue for growers, reducing fruit quality when these climatic conditions were prevalent. Temperature conditions can reach over 40°C in Victoria, a significant production region for this market almost every summer. When temperatures are over 30°C, sunburn of the fruit is common, with white drupelet disorder being the frequent result in fruit that are exposed to direct solar radiation. When higher temperatures occur, fruit inside the bush also is damaged through a scalding action, resulting in the fruit becoming soft and turning a dull, muddy red colour.

The response of different genotypes to this heat stress varies, with some clones being severely damaged while others remain almost untouched. Clearly there is a genetic basis for resistance to heat stress and a breeding program directed at reducing losses through this problem will have a good chance of success.

Other environmental stresses

Resistance to drought, high water tables, cold and other environmental stresses is found in cultivated raspberries or in wild relatives. The use of modern research techniques and biotechnology offers great potential for use by breeders to further expand the range of raspberry production and to reduce losses due to environmental stresses.

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