

Volume 6 (Relatives of Temperate Fruits) of the book series, "Wild Crop Relatives: Genetic, Genomic and Breeding Resources ed C. Kole 2011 p179-197

9 *Rubus*

J. Graham* and M. Woodhead

Scottish Crop Research Institute, Dundee, DD2 5DA, UK

*Corresponding author: **Julie.Graham@scri.ac.uk**

Abstract

The Rosaceae family consists of around 3, 000 species of which 500 belong to the genus *Rubus*. Ploidy levels range from diploid to dodecaploid with a genomic number of 7, and members can be difficult to classify into distinct species due to hybridization and apomixes. Species are distributed widely across Asia, Europe, North and South America with the center of diversity now considered to be in China, where there are 250-700 species of *Rubus* depending on the taxonomists. *Rubus* species are an important horticultural source of income and labor being produced for the fresh and processing markets for their health benefits. Blackberries and raspberries have a relatively short history of less than a century as cultivated crops that have been enhanced through plant breeding and they are only a few generations removed from their wild progenitor species. *Rubus* sp. are typically found as early colonizers of disturbed sites such as pastures, along forest edges, in forest clearings and along roadsides. Blackberries are typically much more tolerant of drought, flooding and high temperatures, while red raspberries are more tolerant of cold winters. Additionally, they exhibit vigorous vegetative reproduction by either tip layering or root suckering, permitting *Rubus* genotypes to cover large areas. The attractiveness of the fruits to frugivores, especially birds, means that seed dispersal can be widespread with the result that *Rubus* genotypes can very easily be spread to new sites and are very effective, high-speed invaders. Nevertheless, wild *Rubus* species represent a valuable genetic resource and protecting this biodiversity for future *Rubus* breeding programs is essential as breeders respond to changes in environmental and growing conditions.

Keywords : *Rubus*, raspberry, blackberry,

9.1 Basic Botany of the Species

The genus *Rubus* is composed of a highly heterozygous series of some 500 species, with a range of ploidy levels from diploid to duodecaploid (Jennings 1988; Meng and Finn 2002). Hundreds of species are divided botanically under 15 subgenera, many of which have been used in breeding (Jennings et al. 1991; Knight 1993; Finn 2001; Finn et al. 2002a b). Members of the genus can be difficult to classify into distinct species for a number of reasons, including hybridization between species and apomixes (Robertson

1974; Dickinson et al. 2007; Evans et al. 2007). Cytological data are being generated and used in an attempt to gain insight into the relationships of the genus (Wang et al. 2008). The subgenus *Rubus* is divided into 12 sections with most of the cultivated blackberries being derived from the *Allegheniensis*, *Arguti*, *Flagellares*, *Rubus*, *Ursini*, and/or *Verotrivialis* (Gustafsson 1943; Finn 2008). Red and black raspberries along with many of the wild harvested species from around the world are in the *Idaeobatus* subgenus.

Commercially, the most important raspberries are the European red raspberry, *R. idaeus* L. subsp. *idaeus*, the North American red raspberry *R. idaeus* subsp. *strigosus* Michx and the black raspberry (*R. occidentalis* L.). *Rubus* subgen. *Idaeobatus* is distributed principally in Asia but also in east and South Africa, Europe and North America. In contrast, subgen. *Eubatus* is mainly distributed in South America, Europe and North America (Jennings 1988). The members of subgenus *Idaeobatus* sp. are distinguished by the ability of their mature fruits to separate from the receptacle. The subgenus is particularly well represented in the Northern Hemisphere. The place of origin of raspberry has been postulated to be the Ide Mountains of Turkey (Jennings 1988). The center of diversity is considered to be in China, where there are 250-700 species of *Rubus* depending on the taxonomists (Thompson 1997). Jennings (1988) and Roach (1985) have given extensive accounts of early domestication. Records were found in the 4th century writings of Palladius, a Roman agriculturist, and seeds have been discovered at Roman forts in Britain; hence, the Romans probably spread cultivation throughout Europe.

Rubus species are prostrate to erect, generally thorny shrubs producing renewal shoots from the ground (called canes). They are perennials only because each bush consists of biennial canes, which overlap in age. Leaves are compound with 3-5 leaflets, the middle one being the largest; margins serrate to irregularly toothed. Small (0.5-1.5 cm), white to pink flowers are initiated in the second year of planting. The gynoecium consists of 60-80 ovaries, each of which develops into a drupelet. There are 60-90 stamens. The flowers of *Rubus* are structurally rather similar to those of strawberries, with five sepals, five petals, a very short hypanthium, many stamens, and an apocarpous gynoecium of many carpels on a cone-like receptacle. They produce an aggregate fruit, composed of individual drupelets, held together by almost invisible hairs. In *Rubus* each carpel will develop into a small drupelet, with the mesocarp becoming fleshy and the endocarp becoming hard and forming a tiny pit that encloses a single seed. Each drupelet usually has a single seed, though a few have two. Canes grow one year and fruit the next, but there are also primocane varieties, which fruit in the first year. The biennial growth cycle of stems begins when a bud from below soil level develops and elongation of the internodes carries the growing point, protected by leaf scales, to the soil surface. At the surface, leaves expand to form a tight rosette around the growing point. Elongation of the shoot starts in spring and continues until autumn, by which time the shoot will have attained a height of 2 to 3 m. In red raspberries (*R. idaeus* L.), shortening days and falling temperatures in late summer cause shoot elongation to cease and dormancy to set in. This is a gradual process extending over several weeks and once a stage of complete dormancy is reached it is not readily reversible. Black raspberries (*R. occidentalis* L.) or purple raspberries (hybrids between red and black raspberries) and most blackberries differ from red raspberries both in time when dormancy begins and

intensity of dormancy attained. In these fruits, growth continues well into autumn. The initiation of flower buds usually starts at the same time as the canes begin to acquire dormancy. In the spring of the second year, vegetative primocanes become fruiting canes. The fruit is composed of a large number of one-seeded drupelets set together on a small conical core (Jennings 1988). Commercial blackberries are classified into three categories based on cane type: trailing, semi-erect, and erect (Strik 1992). Trailing types and semi-erect habit blackberries are crown-forming and the primocanes trail on the ground surface until lifted and staked. Erect blackberries grow upright, but less vigorously than the semi-erect types, and instead of being crown forming, they sucker beneath the soil line.

The genomic number of *Rubus* is seven and species representing all ploidies from diploid to duodecaploid are found in nature. The range in size is from 1- 4 μm (Jennings 1988). The diploid genome has been estimated to be 275 Mbp. Self-incompatibility systems occur in some *Rosaceous* species and it is common among many of the diploid *Rubus* species (Keep 1968). In contrast, all polyploid species are self-compatible as are the domesticated forms of the diploid raspberries.

Rubus species are an important horticultural source of income and labor. In most countries fruit from *Rubus* species is produced for the fresh market. Fruit for processing is usually used in the food and beverage industries where it is used to produce wine, beer and soft drinks, preserves and desserts. Fruits may also be frozen or canned. Blackberry production is rapidly increasing (Strik 1992; Clark 2005; Clark et al. 2007; Strik et al. 2007) with an estimated 140,292 MT commercially harvested from 20,035 ha in 2005. Europe leads the world in acreage (7,692 ha), while North America has the highest production (59,123 MT). Serbia (69%) dominates European production however, a number of countries have significant production. In North America, the US, particularly Oregon State, is the major producer. However, Mexican production has been rapidly increasing. California and Arkansas are the only other states in the US with over 1,000 MT production. Central American production (1620ha) is predominantly from Costa Rica and Guatemala where in addition to harvest from managed stands, a great deal is harvested from feral stands. South American production (1597 ha) is predominantly from Ecuador and Chile. Asian production has been rapidly increasing with over 1,550 ha of new plantings, predominantly in China. The production in Oceania is mainly in New Zealand although the area planted is small with only about 259 ha. African production is only reported in South Africa but has been initiated in Morocco, Algeria and Kenya and possibly others. The bulk of the fruit is grown for processing applications in the Pacific Northwest US, Serbia, and China whereas fresh market sales are the focus of the industry elsewhere.

Raspberry is an important high-value horticultural industry in many parts of the world providing employment directly in agriculture, and indirectly in food processing and confectionary. Production is estimated at 482,763 MT (2005) (<http://FAOSTAT.FAO.ORG>). Europe is estimated to produce around half of all production (*Rubus idaeus* L.). Most raspberry production is concentrated in the northern and central European countries, although there is an increasing interest in growing cane fruits in southern Europe, e.g. in Greece, Italy, Portugal and Spain. The major production areas of red raspberries in North America are the Pacific Northwest (Oregon, Washington and British Columbia), California, the eastern US (New York, Michigan, Pennsylvania and

Ohio) and a rapidly expanding industry in Mexico. While adapted to many of the same areas as the other cultivated *Rubus*, black raspberry (*R. occidentalis*) cultivation is concentrated in Oregon in the western US, in Ohio, Pennsylvania and New York in the eastern US and in Korea.

9.2 Conservation Initiatives

A number of studies have been carried out to characterize the levels of genetic variation in wild species and to examine the turnover of wild populations. In the Tayside region of Scotland, a study of wild raspberry populations has been carried out over a ten year period. Initially Graham et al. (1997) examined the spatial genetic diversity in wild accessions of red raspberry from four sites. Most of the variability detected using random amplified polymorphic DNA (RAPD) markers was observed between the collection sites. Within sites, increasing diversity coincided with greater spatial separation. None of the wild populations were closely related to any of the commonly grown commercial cultivars and were all spiny. A larger study examined a wider range of wild *R. idaeus* from 12 sites across a greater area and compared the accessions to the cultivar 'Glen Moy'. Again, greater genetic similarity was found within each population collected, which indicates a hindrance to gene movement across geographic locations. This barrier to gene flow was partly explained by a separation of flowering period, with altitude proving to be important here (Graham et al. 2003). Marshall et al. (2001) examined some of the wild accessions studied by Graham et al. (1997) to determine whether these populations were adaptively differentiated from each other. Plants were brought into a common environment and 20 traits assessed. A consistent north-south trend was identified confirming substantive differentiation between populations. Similar studies using phenotypic characteristics have been carried out on 12 wild raspberry populations in Russia (Ryabova 2007) where wild populations were examined for characteristics, which may be useful in cultivated raspberries. Ten years after these initial Scottish studies had been carried out, these wild red raspberry plants at 12 sites were re-examined for changes in population size and to address an earlier finding, which demonstrated significant population differentiation over a small scale (Graham et al. 2009a). Reductions in plant numbers was observed at almost all sites. Given that each population had unique alleles, which could be identified even in a small number of plants, this loss of plants also equates to a loss of alleles. The studies of Marshall et al. (2001), reinforces the value of this wild germplasm having demonstrated plants from these locations (spanning an altitudinal cline from 5 to 600 m) exhibit significant variation in flowering and fruiting period in a common environment. In the light of current climate change implications these populations represent a huge genetic resource that could be utilized within commercial red raspberry breeding programs to develop new cultivars, better adapted to a changing natural environment.

R. idaeus wild accessions from a Lithuanian germplasm collection were examined for genetic diversity using RAPD loci (Patamsytė et al. 2004). Soil acidity rather than geographic distance was significantly correlated to observed polymorphisms indicating an environmental effect on diversity within populations. DNA probes from two variable number tandem repeat (VNTR) loci were utilized to examine diversity in wild populations of *R. moluccanus* L. in the Philippines (Busemeyer et al. 1997). The results

were similar to that of Graham et al. (1997, 2003), finding greater similarity present within populations at each location than between locations. Additionally, apomictic reproduction was ruled out in these populations because no identical VNTR patterns were identified.

Research on natural populations of arctic raspberry has shown genetic diversity at levels near 50% for among and within population estimates (e.g., Lindqvist-Kreuze et al. 2003). Genetic diversity has been examined in natural populations of black raspberry (*R. coreanus*) in Korea using inter simple sequence repeat (ISSR) markers (Hong et al. 2003) and overall genetic relationships among populations were associated with geographic location. Black raspberry (*R. leucodermis*) populations have also been evaluated for traits of importance for use in red and black raspberry breeding (Finn et al. 2003). A study on 63 natural populations of *Rubus strigosus* across North America (Marking 2006) using chloroplast sequence and I-SSR found the majority of the variation to be within populations (79.5%).

Cloudberry (*Rubus chamaemorus*) is a highly valued berry in Scandinavia and northern Russia and has potential for domestication (Korpelainen et al. 1999). It reproduces primarily through clonal growth (Makinen and Oikarunen 1974) and although sexual reproduction occurs rarely, this is obviously important for colonizing new habitats. *R. chamaemorus* exhibits large morphological differences but genetic studies on Finnish populations indicate that the levels of genetic diversity within populations was quite low, comprising 2 to 4 clonal genotypes per population (Korpelainen et al. 1999). This has implications for domestication and breeding programs because the clear morphological variability observed may be largely influenced by environmental conditions. Therefore, plants for breeding programs should be selected from populations located some significant distance apart (Korpelainen et al. 1999).

A number of formal and informal gene bank collections exist around the world. These include a *Rubus* collection of over 140 species and 302 named cultivars and selections with seed or clones available on request, housed in the USDA-ARS National Clonal Germplasm Repository (Hummer and Finn 1999). Another *Rubus* collection of over 140 accessions is maintained in a field collection and in protected culture at the Canadian Clonal Genebank (Luffman 1993). A genebank inventory is published annually. In the UK field collections of over 150 accessions exist at SCRI in Scotland and at East Malling Research in England (A. Dolan, pers. comm.). Collections resulting from botanical surveys in Columbia consist of ten *Rubus* species recorded in open and/or disturbed habitats (Rivera et al. 1997). Plant material and seeds from exploration trips in Sakhalin territory are stored in gene banks including an orange *Rubus chamaemorus*, and a dark purple cloudberry (*R. pseudo-chamaemorus*) (Sabitov et al. 2007). Genotypes from seven Chinese provinces have been established in Jiangsu province and evaluated for a range of characteristics (Yin et al. 1996).

In Europe, efforts are being made to conserve the biodiversity of berries (Bartha-Pichler 2006) with an interest in the conservation of genetic resources. The 'GENBERRY' project, partly funded by the European Community, has been designed to ensure that agricultural biodiversity of small berries is preserved, characterized and used to improve varieties adapted to local European regions. Strawberry (*Fragaria x ananassa*) and raspberry (*Rubus idaeus*) represent the two main cultivated small berries.

The project is focusing on the construction of core collections, the development of a passport data list, the selection and definition of appropriate primary and secondary descriptors, characterization of genotypes using molecular markers, identification of health nutritional compounds and diseases evaluation for a large subset of the collections and the establishment of the European small berries database sustained by a continuous long term network (Denoys-Rothan et al. 2008). Romanian (Rusu et al. 2006a) and Bulgarian red raspberries (Badjakov et al. 2006) have been studied to determine their similarity with European and American germplasm using simple sequence repeat (SSR) markers. Badjakov et al. (2006) analyzed 28 raspberry genotypes from the Bulgarian germplasm collection including 18 Bulgarian cultivars and breeding lines, eight accessions from outside Bulgaria and two wild species accessions, *R. occidentalis* and *R. adiene* using RAPD markers. They created a genetic similarity tree with two clusters, which corresponded to two pedigree groups among the Bulgarian genotypes. They also analyzed the 28 accessions with four SSR loci, demonstrating high levels of diversity within the collection (Badjakov et al. 2006). Weber (2003) analyzed genetic diversity in cultivars of black raspberry (*R. occidentalis*) and red raspberry using RAPD markers and found that black raspberry genotypes showed on average 81% genetic similarity. This compared well to the 70% similarity measured among red raspberry cultivars in Europe (Graham et al. 1994). Of the 16 genotypes investigated, five cultivars accounted for 58% of the observed variability in black raspberry, and none of the black raspberry cultivars were more than two generations from at least one wild ancestor.

9.3 Role in Elucidation of Origin and Evolution of Allied Crop Plants

Blackberries and raspberries have a relatively short history of less than a century as cultivated crops that have been enhanced through plant breeding and they are only a few generations removed from their wild progenitor species. The improvements that have allowed these plants to be commercial cultivated crops are well documented: including increased yield, improved harvest efficiency, abiotic and biotic stress tolerance, increased fruit quality for fresh and processed markets, altered plant architecture, etc. Roach (1985) and Jennings (1988) gave accounts of the early domestication of red raspberry (*Rubus idaeus* L). During the 19th Century, the North American red raspberry (*R. idaeus* subsp. *strigosus* Michx) was introduced into Europe and subsequently crossed with the European sub-species (*R. idaeus* subsp. *vulgatus* Arrhen.). Five parent cultivars dominate the ancestry of red raspberry; 'Lloyd George' and 'Pynes Royal' entirely derived from *R. idaeus* var. *vulgatus* and 'Preussen', 'Cuthbert' and 'Newburgh' derived from both sub-species. Controlled crossing began slightly earlier in the US than the UK with the introduction of 'Latham' in 1914 (McNicol and Graham 1992). There are many excellent reviews on blackberry and raspberry breeding, genetics and germplasm including (Darrow 1937; Daubeny 1996; Waldo 1968; Oydvin 1970; Sherman and Sharpe 1971; Jennings 1988; Hall 1990; Jennings et al. 1991; Moore 1984; Clark and Finn 2008).

The development and application of molecular markers has allowed improvements in taxonomical classification to be made as well as providing tools for the development of genetic linkage mapping, fingerprinting and assessments of diversity to be undertaken in raspberry. Marker development has been reviewed by Antonius-Klemola (1999), Hokanson (2001) and Skirvin et al. (2005). As well as the deployment of anonymous DNA markers such as RAPDs (Graham et al. 1994; 1997; Weber, 2003) and amplified fragment length polymorphisms (AFLPs) (Graham et al. 2006), SSR, EST-SSR and single nucleotide polymorphism (SNP) markers have been developed (Graham et al. 2002, 2004, 2006; Stafne et al. 2005, Lewers et al. 2005; Lopes et al. 2006, Woodhead et al. 2008; McCallum et al. 2010) and can be used to characterize *Rubus* accessions.

Alice and Campbell (1999) produced a *Rubus* phylogeny of 57 species including multiple raspberry species based on ribosomal internal transcribed spacer region (ITSR) sequence variation. The *Rubus* subgenus *Idaeobatus* of the Pacific region was studied in comparison with species from other subgenera to evaluate biogeographic and phylogenetic affinities of *R. macraei*, using chromosome analysis and chloroplast gene *ndhF* sequence (Morden et al. 2003). Their results showed that *R. macraei* is most similar to blackberry species of the subgenus *Rubus*. Moreover they discovered that *R. macraei* and *R. hawaiiensis* are derived from separate colonizations from North America and that similarities between them are due to convergent evolution in the Hawaiian environment.

Trople and Moore (1999) calculated genetic similarities among 43 *Rubus* species and raspberry genotypes based on marker profiles from six RAPD primers. The similarity indices were relatively low between the species (0.15 to 0.52) with much higher indices for multiple accessions within species (0.62 to 0.82) (Trople and Moore

1999). In another study, 40 species of *Rubus* were analyzed, including many raspberry types, using RAPD markers and showed that molecular classification of species agreed with the traditional classification of *Rubus* in most cases, except for three species in the subgenus *Malachobatus* that clustered with the raspberry types in subgenus *Idaeobatus* (Pamfil et al. 2000). However, their RAPD based taxonomy could not explain differential success of interspecific hybridization within each subgenus.

9.4 Role in Development of Cytogenetic Stocks and Their Utility

A wealth of useful germplasm exists within and between the different *Rubus* species but the complex ploidy series that exists is an obstacle to its efficient introduction into commercially important species. In order to facilitate the exploitation of existing genetic diversity several strategies have been examined. The ploidy level of diploid *Rubus* species has been increased using colchicine on germinating seeds to generate tetraploids (Jennings and McNicol 1989; Jamieson and McLean 2008). The resulting tetraploid black and purple raspberries were reported have larger fruit and set fruit more uniformly than diploids in cold conditions at flowering (Jennings and McNicol 1989) and increased ploidy influenced flower size, flower fertility drupelet numbers, seed size and leaf morphology (Jamieson and McLean 2008). Colchicine has also been used on tissue cultured shoot tips to generate non-chimeral autotetraploids in *R. allegheniensis* and *R. rusticanus* (Gupton 1989). Allopolyploids were made between colchicine doubled raspberry autotetraploids and different blackberry polyploids to make hybrid berries (Knight and Rosati 1994). This material has been analyzed to confirm the nature of the hybrids using genomic and fluorescent in situ hybridization (Lim et al. 1998). A similar approach has been used with primocane fruiting, diploid *R. idaeus* cultivars and the resulting regenerant autotetraploids used as female parents with tetraploid and octaploid blackberries or with hexaploid hybrid berries to produce allotetraploid hybrids (Lim and Knight 2000). Although it is technically feasible to incorporate genetic diversity between different *Rubus* species, few of the hybrids were considered to have commercial potential – most produced poor, astringent fruit which would be commercially unacceptable (Lim and Knight 2000).

Blackberry breeding would also benefit from the efficient introduction of wild intra- and interspecific germplasm into breeding programs and this would be assisted if the ploidy level of blackberries could be reduced to the diploid level (Naess et al. 1998). Ploidy reduction can happen spontaneously in plants, but it occurs rarely and methods have been developed to increase the incidence of this process, including interspecific hybridization, interploidy crosses, improved selection techniques, chemical and physical treatments of pollen and seed parent and in vitro culture of male and female gametophytes (reviewed by Kimber and Riley 1963; Magoon and Khanna 1963; Lacadena 1974; Chu 1982; Yang and Zhou 1982). Diploid progeny of blackberries have been observed (Yarnell and Blackhurst 1947; Crane and Thomas 1949; Einset and Pratt 1954; Jennings et al. 1967) and more recently strategies to produce dihaploids from tetraploid blackberry cultivars using selection of twin seed, interspecific hybridization and pollen irradiation techniques have been reported (Naess et al. 1998). Of these, pollen irradiation was the most successful technique (Naess et al. 1998) and paves the way for incorporating wild *Rubus* germplasm into commercial cultivar breeding.

9.5 Role in Classical and Molecular Genetic Studies

Domestication has resulted in a reduction of both morphological and genetic diversity in red raspberry (Haskell 1960; Jennings 1988) with modern cultivars being genetically similar (Dale et al. 1993; Graham and McNicol 1995). Similar work on the genetic relatedness of black raspberries using RAPD markers was carried out and raised similar concerns with the need for greater incorporation of more diverse germplasm into black raspberry breeding (Weber 2003). Relatedness in blackberries has also been examined using pedigree analysis with similar findings recommending the diversification of the gene pool (Stafne and Clark 2004). This restricted genetic diversity is of serious concern for the future of *Rubus* breeding, especially when seeking durable host resistance to intractable pests and diseases for which the repeated use of pesticides in some regions is ineffective, unsustainable or unacceptable for certain selected markets, such as 'organic production'. The gene base can and is being increased by the introduction of unselected raspberry clones and species material (Knight et al. 1989). However, the time required to produce finished cultivars from this material can be considerable, particularly if several generations of backcrossing are required to remove undesirable traits.

Breeding in *Rubus* is carried out by hybridizations between cultivars and/or species with desirable characteristics for multiple generations. Each cycle of crossing involves a cycle of glasshouse screening and field observation. Prior to the advent of molecular markers, inheritance and genetic mapping studies were limited to simple morphological traits (Ourecky 1975; Jennings 1988). These studies generally utilized phenotypes that are deleterious in the recessive form so that they are undesirable to maintain in breeding programs. The advent of biotechnology has resulted in a fundamental shift in the development of genetic linkage maps and their use in variety development. Classical breeding, which selects parents and their desirable offspring based on an observable phenotype, is being integrated with techniques that can identify and manage genetic variability at the molecular level (protein or DNA). The ability to detect genome-wide variability has led to the characterization of genetic variation within, not only coding regions (i.e. genes and their morphological manifestations), but also in non-coding regions as well, which make up large portions of plant genomes. These developments have enabled the construction of genetic linkage maps of red raspberry containing numerous genetic markers that are phenotypically neutral, which have been used to identify genomic regions associated with phenotype. Corresponding mapping in blackberry and other *Rubus* species has lagged due to their complex genetic make up and/or low economic importance.

Early work on linkage analysis of morphological traits by Crane and Lawrence (1931) and Lewis (1939) documented aberrant segregation ratios among populations segregating for fruit color (*T*) and pale green leaves (*g* or *ch_l*) in red raspberry (Crane and Lawrence 1931; Lewis 1939). Further work showed genetic linkage among five genes (waxy bloom *b*, apricot or yellow fruit *t*, pale green leaf *g*, red hypocotyl *x* and pollen tube inhibitor *w*), producing the first genetic linkage group for *Rubus* (Lewis 1939, 1940). Sepaloid *sx₃* was later added to the linkage group between *b* and *t* (Keep 1964). Crane and Lawrence (1931) and Lewis (1939, 1940) also postulated on a linkage

between a semi-lethal allele with the unlinked *h* gene. Jennings (1967) added further evidence to this linkage, proposing the symbols *w_t* for the locus linked to the fruit color *t* locus and *wh* linked to the hairy locus (*h*) in place of *w* that Lewis (1939) used (Jennings 1967).

Subsequent work in red raspberry has further elucidated the inheritance of hairiness and fruit color as well as numerous other traits. Associations between the *H* allele for cane hairiness and resistance to spur blight, cane *Botrytis* and cane blight have been recognized (Jennings 1988; Knight and Keep 1958). This same gene also has been associated with susceptibility to cane spot, powdery mildew and western yellow rust (Jennings and McGregor 1988; Jennings and Brydon 1989). Similarly, the recessive gene *s* for spine-free canes and the dominant *B* for waxy bloom on canes can reduce spur blight incidence (Jennings 1982, 1988). No other linkage groups based solely on morphological traits have been proposed. Daubeney (1996) lists 72 individual loci or alleles that have been identified, many of which are part of an allelic series for aphid resistance (Daubeney 1996). Corresponding work in blackberry and other *Rubus* species has been largely absent, probably due to the complex genetics of blackberry and the relatively unimportant economic impact of other species.

The first genetic map of raspberry using markers was developed by Graham et al. (2004) utilizing SSR and AFLPs for a population of ‘Latham’ × ‘Glen Moy’. SSR markers were developed from both genomic and cDNA libraries from the cultivar ‘Glen Moy’. QTL analysis for variability in spine density identified two associated regions on linkage group 2. Graham et al. (2006) later added 20 SSR markers to the ‘Latham’ × ‘Glen Moy’ map along with analyzing data on the *H* gene for cane hairiness and resistance to multiple fungal pathogens. The *H* gene was mapped to linkage group 2 and associated closely with resistance to cane *Botrytis* and spur blight. Unlike previous reports no association between resistance to cane spot or yellow rust and gene *H* was identified.

Raspberry root rot caused by *Phytophthora fragariae* var. *rubi* is probably the most destructive disease in raspberry plantations (Wilcox et al. 1993; Wilcox and Latorre 2002). Resistance to *Phytophthora* root rot (PRR) is found in a number of wild *Rubus* species including *R. coreanus*, *R. crataegifolius* (Jennings 1988) and *Rubus idaeus strigosus*, the native North American red raspberry but less so in those derived from *Rubus idaeus vulgatus*, the European red raspberry (Pattison and Weber 2005). Generating crosses between *R. strigosus* and *R. idaeus* can facilitate the identification of the genes underpinning this resistance. Using a ‘Latham’ (*R. idaeus strigosus*) × ‘Glen Moy’ (*R. idaeus vulgatus*) mapping population two regions, one on each of two linkage groups have been identified and further research aimed at confirming these in a second population through glasshouse and field trials has been completed (J. Graham, K. Smith and I. Tierney, unpublished data). BAC clones have been mapped into the resistance regions and are being sequenced to identify the genes in the region (J. Graham and K. Smith, unpublished data). Using a RAPD-based linkage map from a cross between ‘Latham’ and ‘Titan’, Pattison and Weber (2005) also identified markers clustered on two linkage groups, which were associated with disease score QTL for PRR using bulk segregant analysis. Recently, this work has been extended to include AFLP, RAPD and resistance gene analog polymorphism (RGAP) markers in other ‘Latham’ and ‘Titan’ populations (Pattison et al. 2007). Considerable progress towards identifying markers

and ultimately the gene(s) responsible for *R. strigosus*-based resistance to this disease is being made and this can be incorporated into raspberry breeding programs, allowing the rapid identification and selection of durable resistant genotypes.

Aphids, particularly *Amphorophora idaei* (Borner) and *Aphis idaei* van der Goot), are one of the most damaging arthropod pests in raspberry (Gordon et al. 1997), due to direct feeding damage to susceptible cultivars and because they act as vectors for virus transmission (McMenemy et al. 2009). Over the past 40 years, breeding for host plant resistance to raspberry aphids, has reduced the need for pesticides and controlled the spread of aphid borne viruses (Birch et al. 2005). However, insect pests are constantly adapting and overcoming plant resistance genes. Several types of aphid resistance genes, minor/multi-gene and single major genes, e.g. A1 and A10 with different mechanisms have been used against *A. idaei* in sequence by raspberry breeders, but in the UK each type of major gene resistance has been broken. To date, minor gene-based aphid resistance remains durable in raspberry but it provides only partial resistance (Birch et al. 2005). Efforts to identify new sources of aphid resistance from wild species and other cultivars is underway, as is the development of molecular markers to speed up the selection of promising genotypes (Birch et al. 2005). This will be facilitated by efforts to map aphid resistance genes by anchoring marker data from appropriate segregating populations to the published raspberry genetic maps (Sargent et al. 2007). Determining the map location of a number of aphid resistance genes from various sources will allow the discrimination of different genes and gene pyramiding in new raspberry cultivars.

Attempts to develop markers for viral resistance genes have been carried out for raspberry leaf spot and raspberry vein chlorosis utilizing the 'Latham' × 'Glen Moy' cross of Graham et al. (2004). Field screening was carried out to measure symptom production of leaf spot and vein chlorosis in two different environments. These traits were analyzed for significant linkages to mapped markers and resistance loci were found on linkage groups 2 and 8 (Rusu et al. 2006b).

Mapping health related compounds is a major goal in raspberry research. The emergence of metabolomics makes possible the simultaneous analysis of multiple metabolites at specific time points. In *Rubus* a metabolomic approach has been used to identify bioactive compounds in a segregating mapping population planted under two different environments (Stewart et al. 2007). As a greater understanding of the relative importance and bioavailability of the different antioxidant compounds is achieved, it may become possible to develop and identify those raspberry genotypes with enhanced health-promoting properties from breeding programs (Beekwilder et al. 2005). Progress in mapping anthocyanins has been made by Kassim et al. (2009). Quantitative trait loci (QTL) for eight antioxidants mapped to the same chromosome region on linkage group 1 of the map of Graham et al. (2006), across both years and from fruits grown in the field and under protected cultivation. QTL for seven antioxidants also mapped to a region on LG 4 across years and for both field and protected sites. Candidate genes including a basic helix-loop-helix (bHLH) (Espley et al. 2007), a no apical meristem (NAM/CUC2_-like protein (Ooka et al. 2003) and a basic leucine zipper (bZIP) transcription factor (Holm et al. 2002; Mallappa et al. 2006) underlying the mapped anthocyanins were identified (Kassim et al. 2009). A similar approach is underway for other *Rubus*

antioxidants allowing the QTL and the underlying genes to be determined, with the ultimate aim of identifying favourable *Rubus* alleles to be used in breeding programs.

9.6 Role in Crop Improvement through Traditional and Advanced Tools

Rubus breeding is hampered by several genetic problems including polyploidy, apomixes, pollen incompatibility and poor seedling germination. The highly heterozygous nature of the germplasm requires evaluation of large seedling populations. Breeding is based on a generation by generation improvement in breeding stock through selection and intermating of individuals showing promise of producing superior progeny. This average improvement in the progeny of breeding stock resulting from intermating selected parents is called response to selection (see review Hansche 1983). Several excellent reviews of blackberry and raspberry breeding have been written in the past few years including Finn and Knight (2002a,b), Clark et al. (2007), Finn (2008), Finn and Hancock (2008).

The incorporation of novel resistance/tolerance to pests and diseases is regarded as essential for the development of cultivars suitable for culture under integrated pest management (IPM) systems. Sources of resistance in diverse *Rubus* sp. to many pests and diseases have been identified and exploited in conventional cross-breeding (Keep et al. 1977; Jones et al. 1984; Jennings 1988; Knight 1991; Williamson and Jennings 1992). However, germplasm bearing single resistance genes, when planted over extensive areas, can eventually be overcome by the rapid evolution of new biotypes of pests, so that new types of host resistance are required to sustain plant protection (Birch et al. 2002; Jones et al. 2002). Pest and diseases of raspberry in Europe have been extensively reviewed in Gordon et al. (2006).

Pattison et al. (2007) combined generational means analysis with molecular markers and QTL analysis to map resistance to Phytophthora root rot in a BC₁ population of NY00-34 ('Titan' × 'Latham') × 'Titan'. Separate genetic linkage maps of NY00-34 and 'Titan' were developed using RAPD, AFLP and RGAP and analyzed for QTL associated with various parameters of root rot resistance assayed in a hydroponic system (Pattison et al. 2004).

Agrobacterium-mediated genetic transformation techniques for *Rubus* have been described in the literature (Graham et al. 1995b; Mathews et al. 1995; Kokko and Karenlampi 1998) and transgenic raspberry plants have been generated that are resistant to raspberry bushy dwarf virus (Malowicki et al. 2008) and that contain a modified auxin synthesizing gene to enhance fruit productivity (Mezzeti et al. 2004). Should potentially favourable alleles conferring desirable characters be identified in wild *Rubus* species it may be technically feasible for these to be incorporated into existing commercial cultivars. Whether genetic modification is a commercially acceptable way to producing improved *Rubus* cultivars remains to be seen.

9.7 Genomics Resources Developed

The advances in genomics technologies have led to a massive increase in the numbers of DNA sequences held in public databases and the numbers of *Rubus* sequences are

very likely to increase rapidly as efforts are under way to sequence EST libraries generated from different tissues and developmental stages. At SCRI cDNA libraries have been generated from leaves (approximately 6,500 clones), canes (approximately 8,000 clones) and roots (approximately 7,300 clones) and new generation sequencing has been used to identify gene transcripts in ripening fruit (J. Graham, K Smith, M Woodhead and S McCallum, unpublished data). Besides providing sequence information on genes expressed in these tissues, these resources are being used to identify gene based markers for use in the genetic mapping programs. A project to characterize bud dormancy phase transition in woody perennial plants at a molecular level generated a total of 5,300 ESTs from endodormant (true dormancy) and paradormant (apical dominance) raspberry meristematic bud tissue (Mazzitelli et al. 2007). PCR-products from these cloned cDNA fragments have been spotted onto glass slides and have been used in microarray experiments to identify genes that show differential expression. At present, approximately 380 clones exhibit up- or down-regulation during the endodormancy – paradormancy transition. Some of these ESTs, including one encoding a MADS-box gene, a MYB gene and several containing SSRs have been identified and mapped in the ‘Latham’ × ‘Glen Moy’ mapping population and these underlie ripening QTL (Graham et al. 2009b).

Genes up-regulated during fruit ripening have been identified using classical plus/minus screening of cDNA libraries (Jones et al. 1998) and RNA fingerprinting techniques (Jones et al. 2000). Amongst the genes identified were cell wall hydrolases involved in fruit softening and ACC oxidase (Jones et al. 2000) involved in the ethylene biosynthetic pathway.

The phenylpropanoid pathway is important in raspberry, as end products contribute to the color and aroma of the fruit and are involved in other processes such as lignin production. Aroma and color in raspberry fruit are partly derived from the polyketide derivatives benzalacetone and dihydrochalcone, which are formed during fruit ripening as a result of the action of several enzymes, polyketide synthases (PKS), benzalacetone synthase and chalcone synthase (CHS) during fruit development. A number of PKS genes have been characterized from raspberry (Zheng et al. 2001; Kumar and Ellis 2003a). Kumar and Ellis (2003a) reported the PKS gene family in *Rubus* consists of at least 11 members and expression analysis of three cDNAs showed they exhibited tissue-specific and developmental patterns of expression, with two cDNAs up-regulated during fruit ripening. More recently, the cloning of a raspberry benzalacetone synthase (PKS4) has been reported (Zheng and Hradzina 2008).

Genes encoding 4-coumarate:CoA ligase, an enzyme that activates cinnamic acid and its derivatives to thioesters, which then serve as intermediates for the production of phenylpropanoid-derived compounds that influence fruit quality have also been studied. Kumar and Ellis (2003b) have characterized the 4-coumarate: CoA ligase (4CL) genes in raspberry found there are three genes, which are differentially expressed in various organs and during fruit development and ripening. Based on the expression patterns and substrate utilization profiles of the recombinant proteins, they suggest that 4CL1 is involved in the biosynthesis of phenolics in leaves, 4CL2 in cane lignification and 4CL3 in the flavonoid and/or flavor pathway in fruit. These genes are also being targeted in the *Rubus* mapping program (Woodhead, Graham and Smith, pers. comm.).

The first publicly available red raspberry BAC library from the European red raspberry, 'Glen Moy' has been constructed comprising over 15,000 clones with an average insert size of approximately 130 kb (6-7 genome equivalents). Hybridization screening of the BAC library with chloroplast (*rbcL*) and mitochondrial (*nad1*) coded genes revealed that contamination of the genomic library with chloroplast and mitochondrial clones was very low (> 1%) (Hein et al. 2005). Initial screening of the BAC library employed probes for chalcone synthase, phenylalanine ammonia lyase and a MADS-box gene involved in bud dormancy (I Hein and S Williamson, pers. comm.). More recently, the library has been probed with genes involved in fruit quality genes (M Woodhead and S McCallum pers. comm.) and a peach ever-growing gene (A. G. Abbott, pers. comm.) and with markers underlying QTL for *Phytophthora* root rot resistance.

9.8 Scope for Domestication and Commercialization

Berries are extremely high in antioxidants, exhibiting up to four times more antioxidant capacity than non-berry fruits, 10 times more than vegetables and 40 times more than cereals (Halvorsen et al. 2002). They contain high levels of the antioxidant vitamins A, C and E and very high levels of non-essential but strongly antioxidant phenolic compounds. Phenolics can account for 90% or more of the overall antioxidant capacity found in berry fruit (Deighton et al. 2000), the most readily visible of which are the anthocyanin pigments. These pigments impart the deep, vibrant colors of berries and can be found at concentrations of up to 500 mg 100 g FW⁻¹. Berries represent a significant dietary source of anthocyanins, as only 24 out of 100 common foods contain anthocyanins and non-berry anthocyanin containing foods typically contain less than 100 mg 100 g FW⁻¹ (Wu et al. 2006).

The shift in focus from vitamin C and micronutrients towards the polyphenolics causes something of a challenge for any breeding effort, since the polyphenolics are chemically diverse and the content of individual health-promoting compounds varies in raspberry fruit due to both developmental and genetic factors (Beekwilder et al. 2005). However, with the emergence of metabolomics the simultaneous analysis of multiple metabolites at specific time points is now feasible. In *Rubus* a metabolomic approach has been used to identify bioactive compounds in a segregating mapping population planted under two different environments (Stewart et al. 2007). As a greater understanding of the relative importance and bioavailability of the different antioxidant compounds is achieved, it may become possible to develop and identify those raspberry genotypes with enhanced health-promoting properties from breeding programs (Beekwilder et al. 2005) and to mine for favourable alleles from wild *Rubus* accessions for inclusion into breeding programs.

The traditional medicinal uses of different *Rubus* species are well known and have recently been reviewed (Patel et al. 2004). *Rubus idaeus* leaves and roots are anti-inflammatory, astringent, decongestant, ophthalmic, oxytocic and stimulant (Triska 1975; Grieve 1971; Foster and Duke 1990; Chevallier 1996). A tea made from them is used to treat diarrhea and as a tonic for pregnant women (only in the last three months of pregnancy) to strengthen the uterus in preparation for childbirth (Foster and Duke 1990). Externally the leaves and roots can be used to treat tonsillitis, mouth inflammations and as a poultice to treat sores, conjunctivitis, minor wounds and burns (Bown 1995;

Moerman 1998). The Kiowa and Apache made a tea from the roots of *Rubus occidentalis* species to treat stomach ache and blackberry root tea was part of the treatment for hemorrhaging and hemophilia (<http://www.biosurvey.ou.edu/shrub/rubocc.htm>). *Rubus fruticosus* roots infused in water with pennyroyal (*Mentha pulegium*) were used in the treatment of bronchitis and asthma and the leaves used as an astringent against bacterial infections (Beith 1995). Raspberry leaf tea is probably the most widely known herbal product associated with *Rubus* but the value of natural components within *Rubus* are still being realized.

Fruits from *Rubus* are antiscorbutic (i.e. prevent scurvy) and diuretic (Chiej 1984) and recent evidence suggests that fruit polyphenol components may affect activities of digestive tract enzymes, and may provide a means for controlling diseases like type 2 diabetes (McDougall and Stewart 2005). The antioxidant compounds present in black raspberry berries are being evaluated for the topical treatment of human pre-malignant oral lesions (Mallery et al. 2007, 2008) and freeze dried black raspberries and raspberry extracts are being assessed for the prevention of esophageal and colon cancer (Stoner et al. 2007). Raspberry fruits contain complex phenolic compounds, e.g. ellagitannins, which are strong antimicrobial agents against, for example, *Salmonella* and *Staphylococcus* (Puupponen-Pimia et al. 2005) and may have applications in both medicine and the food industry in the future.

It has been reported that topical application of raspberry ketone (4-(4-hydroxyphenyl)butan-2-one), the compound that gives raspberry fruit their characteristic aroma, can promote hair re-growth in some humans with alopecia and increase skin elasticity (Harada et al. 2008) and in mice prevents and improves obesity (Morimoto et al. 2005).

Patel et al. (2004) recently reviewed the volatile components occurring in a number of *Rubus* species. Not only do different *Rubus* species produce different types of volatile, the relative proportions of the volatiles produced can change during plant growth in raspberry and those emitted during flowering act as attractants to pollinating insects (Robertson et al. 1995). The total amount and type of volatiles produced from ripe blackberry fruit varies significantly between cultivar (Qian and Wang 2005) and in raspberry, although ethyl acetate at 12-18% was found to be the major detectable volatile product of ripe raspberry fruit (Robertson et al. 1995), it is not the major aroma compound. This is attributed to raspberry ketone, a compound widely used in perfumery, in cosmetics, and as a food additive to impart a fruity odor. This natural compound (also known as 4-(4-hydroxyphenyl)butan-2-one) is a derivative of the phenolic pathway and although it is the primary aroma compound of red raspberries (Hradzina 2006) it is found in low quantities in plants, between 1 to 17 $\mu\text{g}/100\text{g}$ FW (Borejsza-Wysocki et al. 1992). Demand for raspberry ketone is growing considerably and although it can be produced by organic synthesis, work is underway to better understand how this compound is produced in planta. A raspberry gene encoding a benzalacetone synthase (or polyketide synthase 4, PKS4) has recently been reported (Zheng and Hradzina 2007) and paves the way for producing this flavor compound using alternative strategies such as microbial fermentation. Due to the sensory importance of this compound in fresh and processed raspberry products (Larsen et al. 1991), it would be desirable to screen raspberry germplasm for genotypes containing naturally high

levels of raspberry ketone to improve the sensory characteristics of fruit through breeding.

9.9 Some Dark Sides and Their Addressing

Rubus sp. are typically found as early colonizers of disturbed sites such as pastures, along forest edges, in forest clearings and along roadsides. Blackberries are typically much more tolerant of drought, flooding and high temperatures while red raspberries are more tolerant of cold winters. Most species are polyploid, facultatively apomictic and pseudogamous (i.e. pollination is required to trigger endosperm development in the seed (Gustafsson 1943)). Additionally, they exhibit vigorous vegetative reproduction by either tip layering or root suckering, permitting *Rubus* genotypes to cover large areas (Werlemark and Nybom 2003). The attractiveness of the fruits to frugivores, especially birds, means that seed dispersal can be widespread with the result that *Rubus* genotypes can very easily be spread to new sites. The overall effect - *Rubus* sp. are very effective, high-speed invaders (Greimler et al. 2002; Baret et al. 2004).

Like many plant species that have since become invasive weeds, *Rubus* spp. typically moved around the world by humans who introduced them as food crops or as a result of trading activities (Ellison and Barreto 2004). Certain *Rubus* species have become very problematic in some regions of the world (Daehler 1998) where they produce very dense, impenetrable thickets which make it impossible for native flora to germinate and establish and they can also form hybrids with native species. As well as contributing to the obvious loss in biodiversity, these weeds pose a serious financial problem to agriculture and efforts to find effective solutions to control them continue.

There are many alien *Rubus* species and here we present several examples and the problems they pose and in some cases, strategies employed to control them.

Blackberry (*Rubus fruticosus* L. aggregate) is an important weed in both agricultural and natural ecosystems in Australia, covering some 8.8 million hectares. It is regarded as one of the worst weeds in Australia because of its invasiveness, potential for spread and economic and environmental impacts (<http://www.weeds.gov.au/publications/guidelines/wons/pubs/r-fruticosus.pdf>).

At least 15 different but closely related species of blackberry are naturalized in Australia (Evans et al. 2005). Accurate taxonomic keys are important to determine which taxa are contributing to the problem and DNA fingerprinting is important as a tool in this area (Evans et al. 1998, 2007). This is particularly relevant for applying biological control measures because blackberry leaf rust (*Phragmidium violaceum*) is ineffective against some European blackberry species and all American blackberry species (Scott et al. 2008). An additional biological control agent, the redberry mite, *Acalitus essigi* (Hassan), is under evaluation to aid control of blackberry (Scott et al. 2008) but other measures such as herbicides, mechanical removal and burning are still required to control this weed.

In Chile, two weedy species of blackberry, *R. constrictus* Lef. & M. (native to central Europe) and *R. ulmifolius* Schott (native to the Mediterranean), both introduced in the second half of the 19th century, have become naturalized and were estimated to cover 5 million hectares by 1973 (Ellison and Barreto 2004). As in Australia, the use of

Phragmidium violaceum has proved effective in controlling these species, particularly *R. constrictus*, the more problematic of the two weeds, without affecting the commercial species *R. idaeus* L., and *R. loganobaccus* (loganberry). The rust hastens normal defoliation and infected stems do not lignify properly which increases susceptibility to infection by other pathogens and to frost damage (Oehrens and Gonzalez 1977). Such weakened plants are less competitive and are displaced by native species (Oehrens 1977).

In Hawaii there are two endemic *Rubus* species: *R. hawaiiensis* A. Gray, a major component of the forest ecosystem above 200 m elevation and *R. macraei* A. Gray, which is less widely distributed. Both have North American ancestry (Howarth et al. 1997; Morden et al. 2003) and are partly sympatric with seven alien *Rubus* species that are naturalized in the Hawaiian islands (Randell et al. 2004) including *Rubus rosifolius*, a native to Australia. Apart from the threat of these *Rubus* species to invade and dominate existing forest, hybrids between *R. rosifolius* and *R. hawaiiensis* have been found (Randell et al. 2004). Studies have shown the hybrids to be sterile so although this may preclude genetic assimilation of *R. hawaiiensis* by *R. rosifolius* and the acquisition of favorable, adaptive alleles by *R. rosifolius* from *R. hawaiiensis*, nevertheless the production of inviable seed by *R. hawaiiensis* represents a loss of reproductive effort and may have a negative impact on the species, and the hybrids may well have an advantage and pose a competitive threat (Randell et al. 2004).

Rubus armenicus is now a serious invasive weed in the USA and Australia. Native to Armenia in southwest Asia, it was introduced to Europe in 1835, and Australasia and North America in 1885. It was valued for its large, sweet fruit, similar to that of common blackberries (*Rubus fruticosus*) and attractive for domestic and commercial fruit production (cultivars 'Himalayan Giant' and 'Theodore Reimers' are particularly commonly planted (Ceska 1999)).

R. alceifolius Poir., a bramble, is native to southeastern Asia and Malaysia and has been introduced to the Indian islands of Madagascar, Mayotte, La Reunion and Mauritius where it is a serious weed, and to Queensland, Australia (Amsellem et al. 2001). Reductions in the level of genetic diversity of the populations in areas of introduction were found and within the Indian islands, each population examined was characterized by a single clone which was closely related to individuals from Madagascar (Amsellem et al. 2000). Amsellem et al. (2001a, 2001b) suggest that there is a switch in the reproductive biology in this species. In its native range *R. alceifolius* produces seed sexually, the plants in Madagascan populations are hybrids between *R. alceifolius* and native populations of *R. roridus* and produce seed mostly apomictically whilst plants from Reunion Island (where *R. alceifolius* was introduced in 1850) produce seed exclusively apomictically. Considerable variation in fertility and vegetative growth in this species on Reunion Island has been described; fruit set is decreased in plants at increasing elevations but this may be compensated for by greater vegetative growth (Baret et al. 2004).

Gene flow between distantly related *Rubus* species has been demonstrated by the presence of naturally occurring hybrids between *R. caesius* (a facultatively agamous tetraploid blackberry) and diploid *R. idaeus* in Europe (Alice et al. 2001), and the presence of hybrids between *R. alceifolius* and *R. roridus* in Madagascar (Amsellem 2001). Luby and McNicol (1994) surveyed wild and feral *Rubus* populations in Scotland

for evidence of the escape of two genes, L_1 (for fruit size and plant morphology) and s (for spinelessness) introduced into raspberry cultivars by traditional breeding in the previous 20-30 years. The L_1 gene was not found and very low frequencies of the s gene (0.004) were found in wild *R. ideaus* populations within the locale of the commercial planting area but not in populations remote from commercial production. Thus, should transgenic *Rubus* crops be deployed, there is potential for escape into sympatric wild populations, although this was considered to probably be infrequent (Luby and McNicol 1994). More recently, Graham et al. (2009a) demonstrated that limited gene flow into a wild *R. ideaus* population occurred but that pollen movement was hindered between populations at different altitudes, probably because of differences in flowering time. This gene flow into one population was identified by the gain of one new allele into progeny at the site; however work also showed that three alleles were lost from parents to progeny highlighting the flux in genetic diversity in natural populations.

9.10 Recommendations for Future Actions

With the narrowing genetic base of our cultivated fruits, coupled with the increasing demands from consumers, new breeding methods are required to meet demands. Concern over the environmental impact and sustainability of agricultural and horticultural practices is leading to a greater emphasis on pest and disease resistance, as well as the ability of plants to withstand local environmental stresses. The changes in environmental, cultural and agronomic practices within the industry will impact strongly on the nature of the germplasm required for the future. Greater conservation of genetic resources and utilization of diverse, locally adapted germplasm will be required for the future viability of *Rubus* production. Nevertheless, the development of molecular and genetic tools to link genotype to phenotype in *Rubus* mapping populations segregating for key characteristics and the identification of favourable alleles from diverse germplasm may allow for more rapid and targeted deployment of genes controlling these important traits, whether by marker assisted breeding (MAB) or through other means such as genetic modification (GM).

The changing climate is already a major consideration for soft fruit growing due to the succession of mild winters leading to poor bud break in some fruit species and this may be addressed by employing locally adapted germplasm.

References

- Alice LA, Campbell CS (1999) Phylogeny of *Rubus* (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. *Am J Bot* 86:81-97
- Alice LA, Eriksson T, Eriksen B, Campbell CS (2001) Hybridization and gene flow between distantly related species of *Rubus* (Rosaceae): evidence from nuclear

ribosomal DNA internal transcribed spacer region sequences. *Syst Bot* 26: 769-778

- Amsellam L, Noyer JL, Le Bourgeois T, Hossaert-McKey M (2000) Comparison of genetic diversity of the invasive weed *Rubus alceifolius* Poir (Rosaceae) in its native range and in areas of introduction, using AFLP markers. *Mol Ecol* 9: 433-455
- Amsellem L, Chevallier MH, Hossaert-McKey M (2001a) Ploidy level of the invasive weed *Rubus alceifolius* Poir (Rosaceae), in its native range and in areas of introduction *Plant Syst Evol* 228:171-179
- Amsellam L, Noyer JL, Hossaert-McKey M (2001b) Evidence for a switch in the reproductive biology of *Rubus alceifolius* (Rosaceae) towards apomixes, between its native range and its area of introduction. *Am J Bot* 88: 2243-2251
- Antonius-Klemola K (1999) Molecular markers in *Rubus* (Rosaceae) research and breeding. *J Hort Sci Biotechnol* 74: 149-160
- Badjakov I, Todorovska E, Kondakova V, Boicheva R, Atanassov A (2006) Assessment the genetic diversity of Bulgarian raspberry germplasm collected by microsatellite and RAPD markers. *J Fruit Ornament Plant Res* 14:61-76
- Baret S, Maurice S, Le Bourgeois T, Strasberg D (2004) Altitudinal variation in fertility and vegetative growth in the invasive plant *Rubus alceifolius* Poiret (Rosaceae), on Reunion island. *Plant Ecol* 172: 265-273
- Bartha-Pichler B (2006) Endangered biodiversity of berries in Switzerland – a National Berry Collection in Riehen (Basel-Stadt, Switzerland) and strategies for conservation of ProSpecierara facing the increasing gene erosion. *Mitteilungen der Naturforschenden Gesellschaften beider Basel* 9: 33-45
- Beekwilder J, Jonker H, Meesters P, Hall RD, van der Meer IM, de Vos CHR (2005) Antioxidants in raspberry: On-line analysis links antioxidant activity to a diversity of individual metabolites. *J Agri Food Chem* 53: 3313-3320
- Beith M (1995) *Healing threads—traditional medicines of the Highlands and Islands*. Polygon, Edinburgh, UK
- Birch ANE, Jones AT, Fenton B, Malloch G, Geoghegan I, Gordon SC, Hillier J, Begg G (2002) Resistance-breaking raspberry aphid biotypes: Constraints to sustainable control through plant breeding. *Acta Hort* 585, 315-317
- Birch ANE, Gordon SC, Brennan R, Jones AT (2005) Breeding for resistance to the large raspberry aphid: an update on durability of current genes and future prospects. *Bull OILB/SROP* 28: 21

- Borejsza-Wysocki W, Goers SK, McArdle RN, Hrazdina G (1992) p-Hydroxyphenylbutanone levels in raspberries determined by chromatographic and organoleptic methods. *J Agri Food Chem* 40, 1176-1177
- Bown D (1995) *Encyclopaedia of Herbs and their Uses*. Dorling Kindersley, London, UK
- Busemeyer DT, Pelikan S, Kennedy RS, Rogstad SH (1997) Genetic diversity of Philippine *Rubus moluccanus* L. (Rosaceae) populations examined with VNTR DNA probes. *J Trop Ecol* 13:867-884
- Ceska A (1999) *Rubus armeniacus* - a correct name for Himalayan Blackberries. *Botanical Electronic News* 230: <http://www.ou.edu/cas/botany-micro/ben/ben230.html>
- Chiej R. (1984) *Encyclopaedia of Medicinal Plants*. MacDonald, Edinburgh, UK
- Chu CC (1982) Haploids in plant improvement. In: IK Vasil, Snowcroft WR, Frey KJ (eds) *Plant Improvement and Somatic Cell Genetics*. Academic Press, New York, NY, USA, pp 129-158
- Clark JR (2005) Changing times for eastern United States blackberries. *HortTechnology* 15:491-494
- Clark JR, Stafne ET, Hall HK, Finn CE (2007) Blackberry breeding and genetics. *Plant Breed Rev* 29:19-144
- Clark JR, Finn C (2008) Trends in blackberry breeding. *Acta Hort* 777:41-48
- Chevallier A (1996). *The Encyclopedia of Medicinal Plants*. Dorling Kindersley, London, UK
- Crane MB, Lawrence WJC (1931) Inheritance of sex, colour and hairiness in the raspberry, *Rubus idaeus* L. *J Genet* 24: 243-255
- Crane MB and Thomas PT (1949) Reproductive versatility in *Rubus*. III. Raspberry-blackberry hybrids. *Heredity* 3: 99-107
- Daehler CC (1998) The taxonomic distribution of invasive angiosperm plants: ecological insights and comparison to agricultural weeds. *Biol Conserv* 84: 167-180
- Dale A, Moore PP, McNicol RJ, Sjulín TM, Burmistrov LA (1993) Genetic diversity of red raspberry varieties throughout the world. *J Am Soc Hort Sci* 118: 119-129

- Darrow GM (1937) Blackberry and Raspberry Improvement. . USDA Yearbook of Agriculture
- Daubeny HA (1996) .Brambles. In *Fruit Breeding* Volume 11. Vines and Small. Fruits. Edited by J. Janick and J.N. Moore. Hoboken, NJ: John Wiley Inc, USA
- Deighton N, Brennan R, Finn C, Davies HV (2000) Antioxidant properties of domesticated and wild *Rubus* species. *J Sci Food Agri* 80: 1307-1313
- Denoyes-Rothan B, Sasnauskas A, Rugienius R, Chartier P, Petit A, Gordon S, Graham J, Dolan A, Hofer M, Faedi W, Maltoni ML, Baruzzi G, Mezetti B, Snachez Sevilla JF, Zurawicz E, Korbin M, Coman M, Mladin P (2008) Genetic resources of European small berries according to Genberry project. *Sodininkuste IR Darzininkyste* 27(2) 371-377
- Dickinson TA, Lo E, Talent N (2007) Polyploidy, reproductive biology, and Rosaceae: understanding evolution and making classifications. *Plant Systematics Evol* 266: 59-78
- Einset J and Pratt C (1954) Hybrids between blackberries and red raspberries. *Proc Am Soc Hort Sci* 63: 257-261
- Ellison CA, Barreto RW (2004) Prospects for the management of invasive alien weeds using co-evolved fungal pathogens: a Latin American perspective. *Biol Invas* 6: 23-45
- Espley RV, Hellens RP, Putterill J, Stevenson DE, Kutty-Amma S, Allan AC (2007) Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. *Plant J* 49: 414-427
- Evans KJ, Symon DE, Roush RT (1998) Taxonomy and genotypes of the *Rubus fruticosus* L. aggregate in Australia. *Plant Protect Quart* 13: 152-156.
- Evans KJ, Jones MK, Roush, RT (2005) Susceptibility of invasive taxa of European blackberry to rust disease caused by the uredinial stage of *Phragmidium violaceum* under field conditions in Australia. *Plant Path* 54: 275-28
- Evans KJ, Symon DE, Whalen MA, Hosking JR, Barker RM, Oliver JA (2007). Systematics of the *Rubus fruticosus* aggregate (Rosaceae) and other exotic *Rubus* taxa in Australia. *Aust Syst Bot* 20: 187-251
- Finn CE (2001) Trailing blackberries: From clear-cuts to your table. *HortScience* 36:236-238
- Finn CE, Knight VH (2002a) What's going on in the world of *Rubus* breeding? *Acta Hort* 585:31-38

- Finn CE, Swartz HJ, Moore PP, Ballington JR, Kempler C (2002b) Use of 58 *Rubus* species in five North American breeding programs-breeders notes. *Acta Hort* 585:113-119
- Finn C, Wennstrom K, Link J, Ridout J (2003) Evaluation of *Rubus leucodermis* populations from the Pacific Northwest. *HortScience* 38: 1169-1172
- Finn CE (2008) Blackberries. In: *Temperate Fruit Crop Breeding: Germplasm to Genomics*. Ed Hancock, JF. Springer, Netherlands pp 83-114
- Finn CE, Hancock JF (2008) Raspberries. In: *Temperate Fruit Crop Breeding: Germplasm to Genomics*. Ed Hancock, JF. Springer, Netherlands pp359-392.
- Foster S, Duke JA. *A Field Guide to Medicinal Plants. Eastern and Central N. America*. Houghton Mifflin Co. 1990
- Gordon SC, Woodford JAT, Birch ANE (1997) Arthropod pests of *Rubus* in Europe: pest status, current and future control strategies. *J Hort Sci* 72: 831-862
- Gordon SC, Williamson B, Graham J (2006) Current and Future Control Strategies for Major Arthropod Pests and Fungal Diseases of Red Raspberry (*Rubus idaeus*) in Europe. In: *CROPS: Growth, Quality and Biotechnology*, WFL Publisher, Finland, pp 925-950
- Graham J, McNicol RJ, Greig K, Van de Ven WTG (1994) Identification of red raspberry cultivars and an assessment of their relatedness using fingerprints produced by random primers. *J Hort Sci* 69: 123-130
- Graham J, McNicol RJ (1995) An examination of the ability of RAPD markers to determine the relationships within and between *Rubus* species. *Theor Appl Genet* 90:1128-1132
- Graham J, McNicol RJ, Kumar A (1995b) *Agrobacterium*-mediated transformation of soft fruit *Rubus*, *Ribes*, and *Fragaria*. *Meth Mol Biol* 44:129-33
- Graham J, Squire GR, Marshall B, Harrison RE (1997) Spatially dependent genetic diversity within and between colonies of wild raspberry *Rubus idaeus* detected using RAPD markers. *Mol Ecol* 81: 533-542
- Graham J, Smith K, Woodhead M, Russell J (2002) Development and use of SSR markers in *Rubus* species. *Mol Ecol Notes* 2: 250-252

- Graham J, Marshall B, Squire GR (2003) Genetic differentiation over a spatial environmental gradient in wild *Rubus idaeus* populations. *New Phytol* 157: 667-675
- Graham J, Smith K, MacKenzie K, Jorgenson L, Hackett CA, Powell W (2004) The construction of a genetic linkage map of red raspberry (*Rubus idaeus* subsp. *idaeus*) based on AFLPs, genomic-SSR and EST-SSR markers. *Theor Appl Genet* 109:740-749
- Graham J, Smith K, Tierney I, MacKenzie K, Hackett CA (2006) Mapping gene *H* controlling cane pubescence in raspberry and its association with resistance to cane botrytis and spur blight, rust and cane spot. *Theor Appl Genet* 112: 818-831
- Graham J, Woodhead M, Smith K, Russell J, Marshall B, Ramsay G, Squire G (2009a) A decade of change; new insight into wild raspberry populations using SSR markers. *J Am Soc Hort Sci* 134:109-119
- Graham J, Hackett C, Smith K, Woodhead M, Hein I, MacKenzie K, McCallum S (2009b) Mapping QTL for developmental traits in raspberry from bud break to ripe fruit. *Theor Appl Genet* 118: 1143-1155
- Greimler J, Stuessy TF, Swenson U, Baeza CM, Matthei O (2002) Plant invasions on an oceanic archipelago. *Biol Invas* 4: 73-85
- Grieve M (1971) *A Modern Herbal*. (Accessed at <http://www.botanical.com/botanical/mgmh/mgmh.html>)
- Gu Y, Wang CY, Zhao CM, Sang JZ, Li WL (1996) Evaluation of *Rubus* genetic resources. *J Plant Res Environ* 5: 6-13
- Gupton CL (1989) Production of non-chimeral colchiploids in *Rubus* species by tissue culture. *Euphytica* 44: 133-135
- Gustafsson A (1943) The genesis of the European blackberry flora. *Lunds Univ Arsskrift* 39: 1-200
- Hall HK (1990) Blackberry breeding. *Plant Breed Rev* 8:249-312
- Halvorsen BL, Holte K, Myhrstad MW, Barikmo I, Hvattum E, Remberg SF, Wold AB, Haffner K, Baugerod H, Andersen LF, Moskaug JO, Jacobs DR, Blomhoff R (2002) A systematic screening of total antioxidants in dietary plants. *J Nutr* 132: 461-471
- Hansche, P.E. (1983) Response to selection. *In: Methods in Fruit Breeding* eds. Moore and Janick pp. 154-171.

- Harada N, Okajima K, Narimatsu N, Kurihara H, Nakagata N (2008) Effect of topical application of raspberry ketone on dermal production of insulin-like growth factor-I in mice and on hair growth and skin elasticity in humans. *Growth Horm IGF Res* 18: 335-44
- Hradzina G (2006) Aroma production by tissue cultures. *J Agri Food Chem* 54:1116-23
- Haskell G (1960) The raspberry wild in Britain. *Watsonia* 4: 238-255
- Hein I, Williamson S, Russell J, Powell W (2005) Isolation of high molecular weight DNA suitable for BAC library construction from woody perennial soft-fruit species. *Biotechniques* 38: 69-71
- Hokanson SC (2001) SNiPs, Chips, BACs and YACs: are small fruits part of the party mix? *HortScience* 36: 859-871
- Holm M, Ma LG, Qu LJ, Deng XW (2002) Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in *Arabidopsis*. *Genes Dev* 16: 1247-1259
- Hong YP, Kim MJ And Hong KN (2003) Genetic diversity in natural populations of two geographic isolates of Korean black raspberry. *J Hort Sci & Biotech* 78: 350-354
- Howarth DG, Gardner DE, Morden CW (1997) Phylogeny of *Rubus* subgenus *Ideobatus* (Rosaceae) and its implications toward colonization of the Hawaiian Islands. *Syst Bot* 22: 433-441
- Hummer KE, Finn C (1999) Recent *Rubus* and *Ribes* acquisitions at the USDA ARS National Clonal Germplasm Repository. *Acta Hort* 505: 275-281
- Jamieson AR, McLean NL (2008) Field observations on tetraploid red raspberry genotypes. IX Intl *Rubus Ribes* Symp. *Acta Hort* 777: 183-187
- Jennings DL (1982) Resistance to *Didymella applanata* in red raspberry and some related species. *Ann Appl Biol* 101: 331-337
- Jennings DL (1967) Balanced lethals and polymorphism in *Rubus idaeus*. *Heredity* 22: 465-479.
- Jennings DL, Craig DL, Topham PB (1967) The role of the male parent in the reproduction of *Rubus*. *Heredity* 22: 43-55
- Jennings DL (1988) *Raspberries and Blackberries: Their Breeding, Disease and Growth*. Academic Press, London, UK

- Jennings DL, McGregor GR (1988) Resistance to cane spot (*Elsinoe veneta*) in the red raspberry and its relationship to resistance to yellow rust (*Phragmidium rubi-idaei*). *Euphytica* 37: 173-180
- Jennings DL, Daubeny HA, Moore JN (1991) Blackberries and raspberries (*Rubus*). *Acta Hort* 290: 331-392
- Jennings DL, Brydon E (1989) Further studies on resistance to *Leptosphaeria coniothyrium* in the red raspberry and related species. *Ann Appl Biol* 115, 499-506
- Jennings DL, McNicol RJ (1989) Black raspberries and purple raspberries should be spine free and tetraploid. *Acta Hort* 262, 89-90
- Jones AT, Gordon SC, Jennings DL (1984) A leaf-blotch disorder of Tayberry associated with the leaf and bud mite (*Phyllocoptes gracilis*) and some effects of three aphid-borne viruses. *J Hort Sci* 59: 523-528
- Jones AT, McGavin WJ, Birch ANE (2002) Effectiveness of resistance genes to the largeraspberry aphid, *Amphorophora idaei* Börner, in different raspberry (*Rubus idaeus* L.) genotypes and under different environmental conditions. *Ann Appl Biol* 136: 147-152
- Jones CS, Davies HV, McNicol RJ, Taylor MA (1998) Cloning of three genes up-regulated in ripening raspberry fruit (*Rubus idaeus* cv. Glen Clova). *J Plant Physiol* 153, 643-648
- Jones CS, Davies HV, Taylor MA (2000) Profiling of changes in gene expression during raspberry (*Rubus idaeus*) fruit ripening by application of RNA fingerprinting techniques. *Planta* 211, 708-714
- Kassim A, Poette J, Paterson A, Zait D, McCallum S, Woodhead M, Smith K, Hackett C, Graham J (2009) Environmental and seasonal influences on red raspberry anthocyanin antioxidant contents and identification of QTL. *Mol Nutr Food Res* 53: 625-634
- Keep E (1964) Sepaloidy in the red raspberry, *Rubus idaeus* L. *Can J Genet Cytol* 6:52-60
- Keep E (1968) Incompatibility in *Rubus* with special reference to *R. idaeus* L. *Can J Genet Cytol* 10: 253-262
- Keep E, Knight VH, Parker JH (1977) *Rubus coreanus* as donor of resistance to cane diseases and mildew in red raspberry breeding. *Euphytica* 26: 505-510

- Kimber G, Riley R (1963) Haploid angiosperms. *Bot Rev* 29: 480-531
- Knight RL, Keep E (1958) Developments in soft fruit breeding at East Malling. Rept East Malling Res. Stn. for 1957, 62-67
- Knight VH (1991) Use of salmonberry, *Rubus spectabilis* Pursh., in red raspberry breeding. *J Hort Sci* 66: 575-581
- Knight VH (1993) Review of *Rubus* species used in raspberry breeding at East Malling. *Acta Hort* 352:363-371
- Knight VH, Jennings DL, McNicol RJ (1989) Progress in the UK raspberry programme. *Acta Hort* 262: 93-103
- Knight VH, Rosati P (1994) Prospects of extending the adaptation and production area of *Rubus* in Europe. *Rivista di Frutticoltura e di Ortofloricoltura* 56: 59-63
- Kokko HI, Kärenlampi SO (1998) Transformation of arctic bramble (*Rubus arcticus* L.) by *Agrobacterium tumefaciens*. *Plant Cell Rep* 17: 822-826
- Korpelainen H, Antonius-Klemola K, Werlemark G (1999) Clonal structure of *Rubus chamaemorus* populations: comparison of different molecular methods. *Plant Ecol* 143: 123-128
- Kumar A, Ellis BE (2003a) A family of polyketide synthase genes expressed in ripening *Rubus* fruits. *Phytochemistry* 62: 513-26
- Kumar A, Ellis BE (2003b) 4-Coumarate:CoA ligase gene family in *Rubus idaeus*: cDNA structures, evolution and expression. *Plant Mol Biol* 31: 327-340
- Lacadena JR (1974) Spontaneous and induced parthenogenesis and androgenesis. In: KJ Kasha (ed) *Haploids in Higher Plants*. Univ of Guelph, Guelph, ON, Canada, pp 13-32
- Larsen M, Poll L, Callesen O, Lewis M (1991) Relations between the aroma compounds and the sensory evaluation of 10 raspberry varieties (*Rubus idaeus* L.). *Acta Agric Scand* 41: 447-454
- Lewers KS, Styan SMN, Hokanson SC, Bassil NV (2005) Strawberry GenBank-derived and genomic simple sequence repeat (SSR) markers and their utility with strawberry, blackberry and red and black raspberry. *J Am Soc Hort Sci* 130: 102-115
- Lewis D (1939) Genetical studies in cultivated raspberries. I. Inheritance and linkage. *J Genet* 38:367-379

- Lewis D (1940) Genetical studies in cultivated raspberries. II. Selective fertilization. *Genetics* 25:278-286
- Lim KY, Leitch IJ, Leitch AR (1998) Genomic characterisation and the detection of raspberry chromatin in polyploid *Rubus*. *Theor Appl Genet* 97: 1027-1033
- Lim YK, Knight VH (2000) The successful transfer of primocane fruiting expression from raspberry to *Rubus* hybrid berry. *Euphytica* 116: 257-263
- Lindqvist-Kreuze H, Koponen H, Valkonen JPT (2003) Genetic diversity of arctic bramble (*Rubus arcticus* L. subsp arcticus) as measured by amplified fragment length polymorphism. *Can J Bot* 81: 805-813
- Lopes MS, Maciel G, Mendonca D, Gil FS, Machado ADC (2006) Isolation and characterization of simple sequence repeat loci in *Rubus hochstetterorum* and their use in other species from the Rosaceae family. *Mol Ecol Notes* 6: 750-752
- Luby J, McNicol RJ (1994) Gene flow from cultivated to wild raspberries in Scotland: developing a basis for risk assessment for testing and deployment of transgenic cultivars. *Theor Appl Genet* 90: 1133-1137
- Luffman, M (1993) *Rubus* and *Ribes* germplasm at the Canadian Clonal Genebank. *Acta Hort* 352: 377-379
- Magoon ML, Khanna KR (1963) Haploids. *Caryologia* 16: 191-235
- Makinen Y, Oikarinen H (1974) Cultivation of cloudberry in Fennoscandia. Rep from the Kevo Subarctic Res Sta 11: 90-102
- Mallappa C, Yadav V, Negi P, Chattopphayay S (2006) A basic leucine zipper transcription factor, G-box-binding factor 1, regulates blue light-mediated photomorphogenic growth in *Arabidopsis*. *J Biol Chem* 281: 22190-22199
- Malowicki, SMM; Martin, R; Qian, MC (2008) Comparison of sugar, acids, and volatile composition in raspberry bushy dwarf virus-resistant transgenic raspberries and the wild type 'Meeker' (*Rubus idaeus* L.) *J Agric Food Chem* 56: 6648-6655
- Mallery SR, Stoner GD, Larsen PE, Fields HW, Rodrigo KA, Schwartz SJ, Tian QG, Dai J, Mumper RJ (2007) Formulation and in-vitro and in-vivo evaluation of a mucoadhesive gel containing freeze dried black raspberries: Implications for oral cancer chemoprevention. *Pharm Res* 24: 728-737
- Mallery SR, Zwick JC, Pei P, Tong M, Larsen PE, Shumway BS, Lu B, Fields HW, Mumper RJ, Stoner GD (2008) Topical application of a bioadhesive black raspberry gel modulates gene expression and reduces cyclooxygenase 2 protein in human premalignant oral lesions. *Cancer Res* 68: 4945-4957

- Marking HJ (2006) DNA Marker Analysis of Genetic Diversity in Natural and Cultivated Populations of *Rubus strigosus*, American Red Raspberry. MS Thesis. Pennsylvania State Univ, Univ Park, PA, USA
- Marshall B, Harrison RE, Graham J, McNicol JW, Wright G, Squire GR (2001) Spatial trends of phenotypic diversity between colonies of wild raspberry *Rubus idaeus*. *New Phytol* 151: 671-682
- Mathews H, Wagoner W, Cohen C, Kellog J, Bestwick KR (1995) Efficient genetic transformation of red raspberry, *Rubus idaeus* L. *Plant Cell Rep* 14: 471-476
- Mazzitelli L, Hancock RD, Haupt S, Walker PG, Pont SDA, McNicol J, Cardle L, Morris J, Viola R, Brennan R, Hedley PE, Taylor MA (2007) Co-ordinated gene expression during phases of dormancy release in raspberry (*Rubus idaeus* L.) buds. *J Exp Bot* 58: 1035-1045
- McCallum S, Woodhead M, Hackett CA, Kassim A, Paterson A, Graham J (2010) Genetic and environmental effects influencing fruit colour and QTL analysis in raspberry. *Theor Appl Genet* DOI: 10.1007/s00122-010-1334-5
- McDougall GJ, Stewart D (2005) The inhibitory effects of berry polyphenols on digestive enzymes. *BioFactors* 23: 189-195
- McMenemy LS; Mitchell C, Johnson SN (2009) Biology of the European large raspberry aphid (*Amphorophora idaei*): its role in virus transmission and resistance breakdown in red raspberry. *Agric Forest Entomol* 11: 61-71
- McNicol RJ, Graham J (1992) Temperate small fruit. In: *Biotechnology of Perennial Fruit Crops*(eds Hammerschlag & Litz), CABI Publ, pp 303-321
- Meng R and Finn CE (2002) Determining ploidy level and nuclear DNA content in *Rubus* by flow cytometry. *J Am Soc Hort Sci* 127: 223-227
- Mezzetti B, Landi, L, Pandolfini, T, Spena, A (2004) The *defH9-iaaM* auxin-synthesizing gene increases plant fecundity and fruit production in strawberry and raspberry. *BMC Biotechnology* 4:4
- Moerman D. (1998) *Native American Ethnobotany* Timber Press. Oregon.
- Moore JN (1984) Blackberry breeding. *HortSci* 19:183-185
- Morden CW, Weniger DA, Gardner DE (2003) Phylogeny and biogeography of Pacific *Rubus* subgenus *Idaeobatus* (Rosaceae) species: Investigating the origin of the endemic Hawaiian raspberry *R. macraei*. *Pacific Sci* 57:181-197

- Morimoto C, Satoh Y, Hara M, Inoue S, Tsujita T, Okuda H (2005) Anti-obese action of raspberry ketone. *Life Sci* 77:194-204.
- Naess SK, Swartz HJ, Bauchan GR (1998) Ploidy reduction in blackberry. *Euphytica* 99: 57-73
- Oehrens EB (1977) Biological control of the blackberry through the introduction of the rust *Phragmidium violaceum* in Chile. *FAO Plant Protect Bull* 25: 26-28
- Oehrens EB, Gonzalez S (1977) Distribution, biological cycle and damage caused by *Phragmidium violaceum* (Schulz) Winter on blackberry (*Rubus constrictus* Lef. & M. and *R. ulmifolius* Schott) in the south-central and southern regions of Chile. *Agron Sur* 5: 73-85
- Oikarinen H (1974) Cultivation of cloudberry in *chamaemorus* populations: comparison of different molecular methods. *Plant Ecol* 143: 123-128
- Ooka H, Satoh K, Doi K, Nagata T, *et al.* (2003) Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. *DNA Res* 10: 239-247.
- Ourecky DK (1975) *Advances in Fruit Breeding*. Purdue Univ Press, West Lafayette, IN, USA
- Oydvín J (1970) Important breeding lines and cultivars in raspberry breeding. *St Forsökag Njos* 1970:1-42
- Patamsytė J, Žvingila D, Labokas J, Baliuckas V, Kleizaitė V, Balčiunienė L, Rančelis V (2004) Assessment of diversity of wild raspberries (*Rubus idaeus* L.) in Lithuania. *J Fruit Ornament Plant Res* 12:195-206
- Pamfil D, Zimmerman RH, Naess K, Swartz HJ (2000) Investigation of *Rubus* breeding anomalies and taxonomy using RAPD analysis. *Small Fruits Rev* 1: 43-56
- Patel AV, Rojas-Vera J, Dacke CG (2004) Therapeutic constituents and actions of *Rubus* species. *Curr Med Chem* 11: 1501-1512
- Pattison JA, Weber CA (2005) Evaluation of red raspberry cultivars for resistance to *Phytophthora* root rot. *J Am Pom Soc* 59: 50-56
- Pattison JA, Wilcox WF, Weber CA (2004) Assessing the resistance of red raspberry (*Rubus idaeus* L.) genotypes to *Phytophthora fragariae* var. *rubi* in hydroponic culture. *HortScience* 39: 1553-1556
- Pattison JA, Suren K, Samuelian SK, Weber CA (2007) Inheritance of *Phytophthora* root rot resistance in red raspberry determined by generation means and molecular linkage analysis. *TheorAppl Genet* 115: 225-236

- Puupponen-Pimiä R, Nohynek L, Alakomi HL, Oksman-Caldentey KM (2005) Bioactive berry compounds-novel tools against human pathogens. *Appl Microbiol Biotechnol* 67: 8-18.
- Qian MC, Wang Y (2005) Seasonal variation of volatile composition and odor activity value of 'Marion' (*Rubus* spp. *hyb*) and 'Thornless Evergreen' (*R. laciniatus* L.) blackberries. *J Food Sci* 70: C13-20
- Randell RA, Howarth DG, Morden CW (2004) Genetic analysis of natural hybrids between endemic and alien *Rubus* (Rosaceae) species in Hawaii. *Conserv Genet* 5: 217-230
- Rivera D, Linares E, Carrizosa MS, Ramirez SC (1997) Botanical survey and germplasm collecting of wild *Rubus* species in the Palmar basin, municipality of Ubaque (Cundinamarca, Colombia): I. Distribucion y ecologia. *Plant Genet Resour Newsl* 111: 40-52
- Roach FA (1985) *Cultivated Fruits of Britain: Their Origin and History*. Blackwell, Oxford, UK
- Robertson KR (1974) The genera Rosaceae in the southeastern United States. *J Arnold Arbor* 55: 352-360
- Robertson GW, Griffiths DW, Woodford JAT, Birch ANE (1995) Changes in the chemical composition of volatiles released by flowers and fruits of the red raspberry (*Rubus idaeus*) cultivar Glen Prosen. *Phytochemistry* 38: 1175-1179.
- Rusu AR, Pamfil D, Graham J, Smith K, Balteanu VA, Groza Gh, Bondrea I, Patrascu B (2006a) Simple sequence repeat (SSR) markers used in *Rubus* species from Romanian flora and north-European and north-American *Rubus* cultivars (*rubus idaeus*). *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. (USAMV-CN)* 63: 252-258
- Rusu AR, Pamfil D, Graham J (2006b) Mapping resistance of red raspberry (*Rubus idaeus* subsp. *idaeus*) to viral diseases - leaf spot (RLSV) and vein chlorosis (RVCV) on the genetic linkage map. *Seria Zootehnie si Biotehнологii* 62: 318
- Ryabova D (2007) Population evaluation in crop wild relatives for in situ conservation: a case study for raspberry *Rubus idaeus* L. in the Leningrad region, Russia. *Genet Resour Crop Evol* 54: 973-980
- Sabitov A, Chebukin P, Hummer KE (2007) Plant exploration for fruit genetic resources in Sakhalin territory. *Acta Hort* 760: 381-388
- Sargent DJ, Fernandez F, Rys A, Knight VH, Simpson DW, Tobutt KR (2007)

- Mapping A1 conferring resistance to the aphid *Amphorophora idaei* and dw (dwarfing habit) in red raspberry using AFLP and microsatellite markers. *BMC Plant Biol* 7(15)
- Scott JK, Yeoh PB, Knihinicki DK (2008) Redberry mite, *Acalitus essigi* (Hassan) (Acari: Eriophyidae), an additional biological control agent for *Rubus* species (blackberry) (Rosaceae) in Australia. *Aust J Entomol* 47: 261-264
- Sherman WB, Sharpe RH (1971) Breeding *Rubus* for warm climates. *HortScience* 6:147-149
- Skirvin RM, Motoike S, Coyner M and Norton MA (2005) *Rubus* spp. Cane Fruit. In: *Biotechnology of Fruit and Nut Crops*. Ed RE Litz , CABI Publ, Wallingford, UK,
- Stafne ET and Clark JR (2004) Genetic relatedness among eastern North American blackberry cultivars based on pedigree analysis. *Euphytica* 139: 95-104
- Stafne ET, Clark JR, Weber CA, Graham J, Lewers K (2005) Simple sequence repeat (SSR) markers for genetic mapping of raspberry and blackberry. *J Am Soc Hort Sci* 130: 722-728
- Stewart D, McDougall GJ, Sungurtas J, Verrall S, Graham J, Martinussen I (2007) Metabolomic approach to identifying bioactive compounds in berries: Advances toward fruit nutritional enhancement. *Mol Nutr Food Res* 51: 645-651
- Stoner GD, Dombkowski AA, Reen RK, Cukovic D, Salagrama S, Wang LS, Lechner JF (2007) Carcinogen-altered genes in rat esophagus positively modulated to normal levels of expression by both black raspberries and phenylethyl isothiocyanate. *Cancer Res* 68: 6460-6467
- Strik BC (1992) Blackberry cultivars and production trends in the Pacific Northwest. *Fruit Var J* 46:207-212
- Strik BC, Clark JR, Finn CE, Banados P (2007) Worldwide blackberry production. *HortTechnology* 17:205-213
- Thompson MM (1997) Survey of chromosome numbers in *Rubus* (Rosaceae: Rosoideae) *Ann. Missouri Bot. Garden* 84: 128-164.
- Triska J (1975) *The Hamlyn Encyclopedia of Plants*. Ed HL Pursey. Pub Hamlyn, London
- Trope DD, Moore PP (1999) Taxonomic relationships in *Rubus* based on RAPD analysis. *Acta Hort* 505:373-378

- Waldo GF (1968) Blackberry breeding involving native Pacific Coast parentage. *Fruit Var J* 22:3-7
- Wang XR, Tang HR, Duan J, Li L (2008) A comparative study on karyotypes of 28 taxa in *Rubus* sect. *Idaeobatus* and sect. *Malachobatus* (Rosaceae) from China. *J Sys Evol* 46: 505-515
- Weber CA (2003) Genetic diversity in black raspberry detected by RAPD markers. *HortScience* 38: 269-272
- Werlemark G and Nybom H (2003) Pollen donor impact on the progenies of pseudogamous blackberries (*Rubus* subgen. *Rubus*). *Euphytica* 133: 71-80
- Wilcox WF, Scott P H, Hamm PB, Kennedy DM, Duncan JM, Brasier CM, Hansen, EM (1993) Identity of a *Phytophthora* species attacking raspberry in Europe and North America. *Myc Res* 97: 817-831
- Wilcox W F and Latorre B A (2002) Identities and geographic distributions of *Phytophthora* spp. causing root rot of red raspberry in Chile. *Plant Dis* 86: 1357-1362
- Williamson B, Jennings DL (1992) Resistance to cane and foliar diseases in red raspberry (*Rubus idaeus*) and related species. *Euphytica* 63: 59-70.
- Woodhead M, Smith K, McCallum S, Williamson S, Cardle L, Mazzitelli L, Graham J (2008) Identification, characterisation and mapping of simple sequence repeat (SSR) markers from raspberry root and bud ESTs. *Mol Breed* 22: 555-563.
- Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE and Prior RL (2006) Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *J Agri Food Chem* 54: 4069-4075.
- Yang HY, Zhou C (1982) In vitro induction of haploid plants from unpollinated ovaries and ovules. *Theor Appl Genet* 63: 97-104.
- Yarnell SH and Blackhurst HT (1947) A polyploid chromosome series from a cross of the Lawton blackberry and the Nessberry. *Proc Am Soc Hort Sci* 49:189-192.
- Zheng D, Schröder G, Schröder J, Hrazdina G (2001) Molecular and biochemical characterization of three aromatic polyketide synthase genes from *Rubus idaeus*. *Plant Mol Biol* 46: 1-15
- Zheng D, Hrazdina G (2008) Molecular and biochemical characterization of benzalacetone synthase and chalcone synthase genes and their proteins from raspberry (*Rubus idaeus* L.). *Arch of Biochem Biophys* 470: 139-145