CONFIDENTIAL

HORTLINK

Annual Report

Project title*:* Developing a raspberry toolkit for marker-assisted breeding for premium sensory characters in UK fruit. HL0170/SF 76

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PROJECT STRUCTURE

1. Collaborators: Scottish Crop Research Institute
   Strathclyde University
   Horticultural Development Council
   Mylnefield Research Services Ltd
   Kentish Garden
   P. Thomson
   ReDeva
   Marks and Spencer

2. Investigator(s) being funded (Name and start date):
   Susan McCallum July 2006
   Dzeti Zait April 2006
3. Project Manager: Dr Jonathan Snape, MRS
   Scientific supervisors Dr Alistair Paterson and Dr Julie Graham

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Grower Summary

Headline

- Through marker assisted breeding the quality traits of taste colour, fruit weight and brix are being examined to develop markers for breeding improved varieties in future.

Background and expected deliverables

Poor quality and flavour, short shelf-life and limited availability of raspberries lead to consumer disappointment. When raspberries are set at high prices, such factors can discourage repeat purchases. Through breeding, the raspberry industry is fundamentally interested in improving genetic traits such as sweetness, flavour intensity, berry conformation (drupelet structure and cohesion) colour and firmness.

Non-controversial state-of-the-art molecular breeding technologies of proven success in other crops (e.g. tomato and peach) can now be applied to raspberry to ensure new varieties have specific sensory characters and quality parameters.

In raspberry, this is now possible through the SCRI genetic map of raspberry (Graham *et al*., 2004, 2006), recently refined (with contribution from HortLink 0169 funding for root rot resistance) to 7 linkage groups (representing the 7 chromosomes) with many EST markers (Tierney *et al* 2007 in prep). This allows the study of inheritance of important traits and will allow knowledge and technology transfer from other major *Rosaceous* crops (notably the peach and apple) that have made good progress.

Summary of the project and main conclusions

Objective 1. Sensory and compositional analysis of sugars and acids

Replicate clones of the Glen Moy x Latham population were established at three sites: one experimental field site, one commercial field site and a site grown under protected cultivation.

An appropriate sampling strategy was determined in order to select fruit from each of the widely differing progeny at an equal stage of ripeness. Based on the sampling from 2006, a pick order was established for the 2007 sampling across 3 sites.

Fruit was collected in 2006 from the field site and assessed by sensory assessors for sweetness, sourness and flavour intensity. Fruit from 2007 was collected across the three sites (Invergowrie – open and tunnel; Blairgowrie –

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tunnel) and assessed as in 2006.

Fruit from the field site was assessed in 2006 in the lab for colour. This was done both visually and using a Minolta reflective colour meter. Percentage Brix and berry weight were measured. Correlation between visual colour score and a parameter from the colour meter was established. These measurements were repeated in 2007 across all three sites.

Fruit was frozen and transported to Strathclyde University for the quantification of sugars and acids. This has been carried out for 2006 fruit using chromatography with advanced HPLC column matrices. Statistical design was used to ensure that replicate analyses were not significantly different and thereafter sugars and acids were quantified in progeny replicates. Data was sent to SCRI and BIOSS for analyses.

- Data analysis demonstrated a significant correlation between the sensory parameters of sweetness and sourness with the compositional analyses of sugars and acids.

- Flavour intensity is also highly correlated with sweetness and sourness but not with individual sugars and acids.

- Flavour intensity but no other sensory or compositional trait was significantly correlated with brix.

- Weight and colour show only weak correlations with other traits.

Variation between year to year and site to site has been found for some traits. However individual traits are highly correlated across seasons and sites.

Objective 3. Molecular data enhancing deliverables

Raspberry genes likely to be responsible in part for the main traits of interest will be studied. Initially a number of genes with alleles that may have an effect on sweetness, sourness and flavour intensity have been identified. These include genes for transporters into fruit, sugar metabolism and organic acid synthesis. Such genes have not been sequenced in raspberry and so a strategy of examining genes from closely related species and then using oligonucleotide primers designed on the basis of these sequences to pull out the raspberry equivalent was adopted. To date 18 raspberry sequences have been identified.

Once sequence information was available for raspberry genes of interest, Glen Moy and Latham were examined for differences in DNA sequence and any difference (polymorphism) detected was used to map the genes onto linkage groups**.**

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- Mapping of the phenotypic traits by sensory and biochemical trait scores will be effected and QTL map locations compared with candidate gene loci.

Financial benefits

This work will lead to the enhancement of breeding programmes in future and lead to the production of high quality UK fruit.

Action points for growers

There are no action points to offer to growers from this work to date.

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PART 1 - TECHNICAL

A. PROJECT TIMETABLE

1. Project start date: 1 July 2006

2. Project end date: 30 June 2009

3. Management Meeting dates: 30 January 2007, SCRI Dundee
   26 September 2007

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B. OBJECTIVES / MILESTONES

Task	Objective	Proposed date	Duration	Date Completed
	1. Sensory and compositional analysis of sugars and acids			
1.1	Establish clones of the mapping population for study under 3 conditions		12 months	Complete
1.1.1	Propagation and hardening underway for field planting in MRS protected cultivation site and P. Thomson's farm site.		12 months	Complete
1.1.2	Planting of the two additional populations above.			Complete
1.2	Devise an appropriate sampling strategy and collect fruit with breeders scoring of traits	June 2006	1 month	Complete
1.2.1	Establish field sampling strategy for 2006 and collect fruit from 1 site.	June-Aug 2006	3 months	Complete
1.2.2	Establish order of pick and collect selected samples across three sites	June-Aug 2007	3 months	Complete
1.2.3	Carry out 'breeders' assessment of colour, brix, weight, firmness for 2006 season.	July-Aug 2006	2 months	Complete

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1.2.4	Carry out 'breeders' assessment of colour, brix, weight, firmness for 2007 season.	July-Aug 2007	2 months	Complete
1.3	Quantification of key berry metabolites and sensory characteristics for taste.			
1.3.1	Set up sensory panels and score the sensory characteristics of sweetness, sourness and flavour intensity 2006.	June-Aug 2006	3 months	Complete
1.3.2	Set up sensory panels and score the sensory characteristics of sweetness, sourness and flavour intensity 2007.	June-Aug 2007	3 months	Complete
1.3.3	Quantification of key berry metabolites 2006	July 06-April 07	9 months	Complete
1.3.4	Quantification of key berry metabolites 2007	July 07-April 08	9 months	Underway
	2. Correlating sensory data with consumer perceptions and breeders views	July 07- Sept 08	15 months	
	3. Molecular data enhancing deliverables			
3.1	Identify and map candidate genes for quality traits	July06-Jan 08	18 months	Underway
3.1.1	Identify raspberry gene sequences for candidate genes related to fruit quality attributes	July06-Jan08	18 months	Underway

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3.1.2	Examine raspberry gene sequences for polymorphism between Latham and Glen Moy and map based on the polymorphism identified	July06-June08	24 months	Underway
3.2	Examine synteny between the *Prunus* reference and the *Rubus* map, anchoring where possible to allow direct access to QTLs, ESTs and candidate genes mapped in *Prunus*.	July07-Jan09	18 months	Underway
	4. Mapping of QTLs			
4.1	Collate and organise data in suitable form for mapping and liaise with BiOSS to determine QTL locations for key traits.	Sept06-Mar09	30 months	Underway
	5. Assess allele diversity in wide gene pool			
5.1	Identify markers linked to key traits.	March 2009	4 months	
5.2	Select a pool of germplasm to examine allele diversity of identified markers	March 2009	4 months	

2 Comment on any differences in the above dates:

None

3 Give the objectives for the next six months:

1.3 Complete quantification of key berry metabolites for 2007 fruit.
3.1 Continue to identify and analyse candidate gene polymorphism in Latham and Glen Moy
3.1 Map candidate genes where polymorphism can be identified
3.1.1 Develop EST library (library of genes being expressed at a particular

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stage) in fruit at various
ripening stages from Latham and Glen Moy

1.2 Analyse 2006 and 2007 data with BioSS and map data as appropriate.

4. Describe the overall objectives which have changed and give the reasons for the changes:

Overall objectives remain unchanged.

Additionally we decided to measure colour using a meter as samples were available in the lab for other analysis.

C. PROJECT RATIONALE

1. What new factors have arisen which might affect the original rationale for the project?

None

2. Detail any **Problems or Opportunities** which might affect the future progress of the project?

A second PhD student at Strathclyde is assessing genes involved in aroma which will add to our knowledge of flavour, Mrs Angzzas Mohd Kassim (Malaysian Government)(December 2005 – November 2008). To date, she has quantified anthocyanins and selected aroma volatiles in 2006 fruit from progeny. Two French MSc students (studying Flavour and Fragrance Chemistry at University of Le Havre) have been involved in flavour volatiles and anthocyanin quantification and an Indian (University of Strathcyde) in non-volatiles analyses.

A collaboration has been established with HortResearch in New Zealand with a group working on apple and blueberry. They have a number of candidate genes from apple which are thought to be important in fruit colour. Discussions are underway on how best to proceed with the collaboration and a post-doc from HortResearch recently visited the group to move this forward.

A post-doc (Dr. Mary Woodhead) has been appointed at SCRI to work on raspberry BAC screening and this technology will be available to this project as required. Large insert genomic libraries (BACS) are invaluable tools for physical mapping, positional cloning and as a scaffold for whole genome sequencing. *Rubus idaeus* is an ideal candidate for BAC library construction, since it is diploid (2n = 2x = 14) and has a very small genome (275 Mbp). Indeed, the genome size of raspberry is only twice that of the model plant *Arabidopsis*, making it highly amenable to complete

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physical map construction, and thereby providing a platform for map-based gene cloning and comparative mapping with other members of the Rosaceae. The availability of a detailed genetic linkage map, together with a deep coverage bacterial artificial chromosome library, will be of great value in the identification of the genetic factors that underpin a wide range of commercial characteristics such as appearance, genetic resistance, texture and sensory (taste and aroma) attributes of fruit. Once QTLs have been identified and markers closely associated with traits selected, BAC screening can provide information on the actual gene content of identified regions.

Data mining from the EST library developed in HL0169 has identified potential candidate genes that may impact on quality and these will be tested for polymorphism between Glen Moy and Latham for mapping.

D. TECHNOLOGY TRANSFER OUTPUTS

1. Papers published in refereed journals during the period:

   None

2. Other reports produced during the period:

   HortLink leaflet produced on the project

3. Transfers of technology (movements of people or artefacts, including software) -

   A presentation of the project was given at Fruit for the Future 2007.

   Presentations have been given by both Susan and Dzeti at Strathclyde University as a requirement for their thesis.

   Visit from A post-doc from HortResearch New Zealand to discuss transfer of apple sequences of potential value in raspberry.

E. PROGRESS TOWARDS EXPLOITATION

1. Extent of progress towards exploitation (new products, processes or materials):

   Data has shown variation between the fruit samples from different lines of the Glen Moy x Latham mapping population for the key factors of sweetness, acidity sugars, acids and also colour, firmness and brix.

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The links between attributes have been briefly explored for 2006 data and underway for 2007 data. Data from 2006 has shown no significant variability between clones and replicates of the same lines for any of the parameters measured. This allowed analysis of more progeny to be carried out across 3 sites in 2007 enabling the effect of different environments to be assessed, as well as seasonal influences.

Data from 2006 has demonstrated that significant correlations exist between quality parameters as assessed by sensory panels' and the concentrations of sugars and acids in the fruit.

Only a weak correlation could be identified in 2006 between brix and the parameter of flavour intensity, no other correlations with brix could be identified.

For 2007 the correlation between brix and flavour intensity became highly significant at all 3 sites, and a weak correlation between brix and sweetness at the open field site was detected.

Between 2006 and 2007 for the field site, only brix showed a significant difference.

Significant variation was detected in fruit quality traits between the field and protected sites in 2007, however individual traits were significantly correlated from site to site.

Sugars and acid analysis is underway for 2007 to allow further comparisons to be made.

Assessment of colour by both visual estimate and colour meter analysis are highly correlated.

Identification of raspberry sequences and polymorphism between parents has allowed mapping to proceed for candidate genes identified at the project start.

2. Patents (identify geographical coverage):

None

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Project Summary

Purchase and consumption of raspberries forms the end of a supply chain. Currently however factors such as poor flavour, limited shelf-life, short availability and quality compromise lead to consumer disappointment and with high price, discourage repeat purchases. Growers expend capital and effort in establishing specialised raspberry cultivation and need to achieve profitability by sale of fruit perceived of premium quality and readily available for harvest from robust plants.

Traits of fundamental interest across the season to supply chain stakeholders are considered to be: sweetness and flavour intensity, berry conformation (drupelet structure and cohesion), colouration and firmness.

What are required are the application of non-controversial state-of-the-art molecular breeding technologies of proven success in other crops (e.g. tomato and peach) that can ensure new varieties have specific sensory characters and quality parameters.

In the raspberry this is now possible through the SCRI genetic map (Graham *et al*., 2004, 2006), recently refined (with contribution from HortLink 0169 funding for root rot resistance) to 7 linkage groups (representing the 7 chromosomes) with many EST markers (Tierney *et al* 2007 in prep). This has made possible studies of inheritance of important traits and will allow knowledge and technology transfer from other major *Rosaceous* crops notably the peach and apple that have made good progress.

Objectives of the project

Quantitatively inherited characteristics account for the majority of variability, including in fruit character, selected during raspberry breeding. Traits linked to consumer perceptions of high quality will be defined and quantified in progeny of the Glen Moy x Latham cross by integrating compositional and sensory analyses, supported by consumer studies, with molecular marker profiles and modelling.

Quality trait identification will centre on sensory panel assessments of appearance (berry structure and pigmentation) and flavour (concentrating on sweetness, flavour intensity and acidity) supported by instrumental compositional analyses – quantifications of fruit sugars, acids and pigments. Consumer studies involving a multiple retailer will establish relationships between quality traits and consumer judgements of premium character. Quantitative and qualitative data will be complemented by detailed market data on the UK industry in terms of consumer demands, purchasing and market share provided by a major marketing company.

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Genetic inheritance of quality traits will be mapped on the genome through the collection of high quality data from three environments over 2-3 seasons. By analysing a population segregating for the defined traits of interest Quantitative Trait Loci (QTL) positions can be determined on linkage groups. Once QTL locations have been determined marker assisted breeding strategies can be established.

Candidate gene approaches should locate associations between genes involved in relevant metabolic pathways and QTLs. Knowledge of metabolic pathways involved in uptake and regulation of synthesis of sugars and organic acids as well as pigments will be utilised to identify primary candidate genes. These will include transporters and transcription factors important in specific biochemical pathways. This is an important area of this research, as to improve fruit quality we must first identify and characterise control processes, and determine where the key polymorphisms exist. Candidate gene mapping is a good way of supporting roles of identified QTLs in traits of interest. Ultimately we need to identify key alleles for the desired traits and these are likely to be favourable alleles for the gene or transcription factors that control gene expression.

Early selection with molecular markers (MAS) will allow accurate screening of seedlings years before field evaluation can be completed.

Background and expected deliverables

In traditional breeding programmes, selections are broad-based and largely simultaneous for multiple fruit quality traits - firmness (shelf life potential), overall acceptability, freedom from storage effects (adverse storage flavour changes) and appearance (berry dimensions, drupelet cohesion and colouration): traits cannot be deconvoluted from the whole. Currently release of a new variety takes anywhere from 8-15 years and varieties with desirable features can also retain deleterious traits from parents.

A strategy of identification of physical genetic markers linked to specific character traits inherited through simple Mendelian and quantitative trait loci, correlated with molecular markers will yield targeted breeding over a shorter time-frame. Identification of markers linked to quality traits represents a quantum leap in crop breeding terms facilitating a reductionist approach. Breeding for specific premium sensory qualities, retained in fruit cropping throughout the summer assisted by protected cultivation, will allow UK growers to meet retailer demand, stimulate consumer interest, and compete effectively with imports.

Progress to date

Objective 1. Sensory and compositional analysis of sugars and acids

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Replicate clones of the Glen Moy x Latham population were established at three sites: one experimental field site, one commercial field site and a site grown under protected cultivation.

An appropriate sampling strategy was determined in order to select fruit from each of the widely differing progeny at an equal stage of ripeness. Based on the sampling from 2006, a pick order was established for the 2007 sampling across 3 sites.

Fruit was collected in 2006 from the field site and assessed by sensory assessors for sweetness, sourness and flavour intensity. Fruit from 2007 was collected across the three sites (Invergowrie – open and tunnel; Blairgowrie – tunnel) and assessed as in 2006.

Fruit from the field site was assessed in 2006 in the lab for colour both visually and using a Minolta reflective colour meter, %Brix and 10 berry weight. Correlation between visual colour score and a parameter from the colour meter was established. These measurements were repeated in 2007 across all three sites.

Fruit was frozen and transported to Strathclyde University for quantification of sugars and acids. This has been carried out for 2006 fruit using chromatography with advanced HPLC column matrices. Statistical design was used to ensure that replicate analyses were not significantly different and thereafter sugars and acids were quantified in progeny replicates. Data was sent to SCRI and BIOSS for analyses.

Objective 3. Molecular data enhancing deliverables

Raspberry genes likely to be responsible in part for the main traits of interest will be studied. Initially a number of genes with alleles that may have an effect on sweetness, sourness and flavour intensity have been identified. These include genes for transporters into fruit, sugar metabolism and organic acid synthesis. Such genes have not been sequenced in raspberry and so a strategy of examining genes from closely related species and then using oligonucleotide primers designed on the basis of these sequences to pull out the raspberry equivalent was adopted.

Once sequence information was available for raspberry genes of interest, Glen Moy and Latham were examined for differences in DNA sequence and any difference (polymorphism) detected was used to map the genes onto linkage groups**.**

Mapping of the phenotypic traits by sensory and biochemical trait scores will be effected and QTL map locations compared with candidate gene loci.

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F. Project Report

Introduction

In traditional breeding programmes, selections are broad-based and largely simultaneous for multiple quality traits - overall acceptability, freedom from storage effects (and adverse storage flavour changes), firmness (related to shelf-life) and appearance (berry dimensions, drupelet cohesion and colouration). Strategies are not available so that individual traits can be de-convoluted from the whole. At this time, release of a new variety takes anywhere from 8-15 years. Another important factor is that varieties with desirable features can retain deleterious traits from parents. A strategy of identification of molecular genetic markers linked to specific character traits inherited through simple and quantitative trait loci, could yield targeted breeding over a shorter time-frame. Identification of markers linked to quality traits represents a quantum leap in crop breeding terms through facilitating a reductionist approach. Breeding for specific premium sensory characters, retained in fruit cropping across the summer assisted by protected cultivation, would allow UK growers to meet retailer demand, stimulate consumer interest, and compete effectively with imports.

Aim of project

To utilise a key genetic cross between two very different raspberries to locate genes/markers on chromosomes central to sensory quality and retailer requirements thus allowing the development of clearly targeted marker-assisted breeding in this valuable soft fruit crop.

Progress achieved to date on objectives

Objective 1. Sensory and compositional analysis of sugars and acids

Task 1.1 Establish clones of the mapping population for study under three

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conditions (commercial, protected and research).

Materials and Methods

In order to evaluate the effect of environmental conditions as well as the genetics on the key traits of interest, three sites were established for study. Mother plants from the original Glen Moy x Latham cross were maintained for propagation in a gauze house. The mother cane was cut from the pot and re-planted, and the root material remaining in the pot was chilled for 6 weeks. After this time the root from each mother plant was put in trays with compost and placed in a warm glasshouse. Plants growing from the root material served as a source for the establishment of replicated field trials. Once plants were established and grown they were placed outside under rain shelters to acclimatise. Plants were then maintained for two seasons as long canes before use as planting stock.

Results

Three sites were established as follows: Field site at SCRI (M24), Protected site at SCRI (poly), 'Commercial' protected site at Blairgowrie (P Th) (with thanks to P. Thomson). The data collected across the three sites in years 2 and 3 will allow us to partition variability into genetic and environmental.

Task 1.2.1 Establish field sampling strategy for 2006 and collect fruit from one site.

Materials and Methods

The whole emphasis of the project is to develop markers linked to the key quality attributes of sweetness and sourness followed by some other visual characters like colour and size. To map these traits requires an accurate assessment of the key characteristics both by sensory means and biochemical measures and

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examination of how these parameters correlate. The progeny arising from the Latham x Glen Moy cross segregate widely for a number of key characteristics, including time to ripe fruit and fruit colour that will impact on the ability to visually select and then collect fruit at the same stage of ripeness across all progeny. For this study it was crucial to determine as closely as possible when material from each of the progeny was ripe, as this would affect any data collected from the material and ultimately the accuracy of mapping. For mapping purposes a second factor is the variation in the characteristics of interest from year to year and also from site to site.

In order to determine this, it was important initially to know what the variability was from clone to clone and rep to rep within a year at a single site. In year one, the aim was therefore to develop an appropriate sampling strategy for the trial with two objectives

1. Devise a sampling strategy to allow the collection of ripe fruit from the progeny, taking into account the variability in ripening of the progeny from the Glen Moy x Latham cross.
2. Determine variability within and between clones and replicates at one site.

For point 1, it was decided that the best way forward to allow selection of fruit at the ripe stage, was to follow the process of ripening of each plant individually based on a visual assessment whereby the latest stage present on each bush was allocated a grade as follows:

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Stages of ripening

1= buds
2= bud break/open flowers
3= fruit set
4= green fruit
5= green/red fruit
6= red fruit
7= over ripe fruit

Fruit was therefore assessed on a daily basis and as soon as the majority of fruit on each plant reached stage 6, it was picked into bags, and each bag was labelled with the clone number and the rep number. Fruit was always picked from one side of the plant to avoid any effect of sun/shade. After picking, the fruit in bags were placed into ice boxes for transport to the cold room and subsequent sensory and fruit analyses, or to the freezer with subsequent transport to Strathclyde University for chromatographic analyses.

For point 2, it was decided to sample clones within a repetition and clones at a second repetition of as many lines as the season allowed, to look at variation within and between clones and replicates at one site.

Results

Ripening profiles were collected for the population at the SCRI field site. This assessment started in the middle of May and involved scoring plants two or three times a week initially and then on a daily basis as the season progressed. This gave a standardisation point for fruit ripening by following the progress of each plant individually. Fruit was then picked from progeny when the majority of

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berries on the plant reached stage 6. This data will be analysed to determine if an order of collection can be developed for next year across the 3 sites, as complete assessment of ripening for all selected samples across three sites would be difficult to implement.

In order to identify raspberry samples varying for the phenotypic traits of interest in this project a large number of samples were collected for analysis from the outdoor field trial at SCRI in 2006 season : i) for analysis of fresh chilled fruit; ii) for freezing and transport to Strathclyde for chromatographic analyses.

Task 1.2.2 Establish order of pick and collect selected samples across three sites (2007).

Materials and Methods

Fruit sampling in 2007 was from three different sites comprising of an outdoor site at SCRI, protected cultivation site at SCRI and a commercial protected trial in Blairgowrie.

Due to variability in key traits across the mapping population, in 2006 each line was assessed across the season for stage of ripening. This was not feasible across three sites in 2007 and therefore a pick order was established from the 2006 data to use as a guide for picking across three sites in 2007.

However due to the possibility of environmental variability affecting the pick order

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all three sites were visited at least 2 times per week across the season to avoid errors in pick order.

To obtain a subset of around 150-190 samples for analysis in 2007, samples from 2006 were analysed to identify a narrower range of lines for fruit collection based on outliers.

Results

Pick list was drawn up by sorting 2006 data based on date fruit was collected in 2006.

Key samples identified for analysis.

Task 1.2.3 Carry out 'breeders' assessment of colour, brix, weight and firmness for 2006 season.

Materials and Methods

Four clones of each sample were analysed for colour composition, soluble solid content (% Brix is used as an order of sweetness) and 10 berry weight. Fruit was also picked into punnetts and a visual assessment made of appearance (Table 10).

Colour

Colour was assessed visually after picking into a punnett on a scale of 1-5 with 1 being pale red and 5 purple/red (table 10) and also instrumentally using a Minolta Chroma meter CR-100/CR-110. This allowed each sample to be measured in

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terms of chromaticity under two separate formats Yxy and L*a*b*, by analysing the reflectance ratio between the emitted light from the measuring head and the light reflected from the samples (Minolta operating manual). The Yxy measurements assess the Brightness of samples (illuminance and reflection) represented by the Y, the saturation of colour (colour intensity/ chroma) as denoted by the x and the wavelength of individual colours (red, yellow, green and blue) represented by the y. Whereas the L*a*b* looks more specifically at individual colour composition with L* measuring brightness to darkness, a* green to red spectrum (-a = greenness) and b* blue to yellow spectrum (-b = blueness).

Due to the variation found between the samples in terms of their consistencies, careful preparation of the fruit was necessary to prevent inaccurate measurements being recorded. This was achieved by carrying out extensive testing prior to the commencement of sample analysis and involved direct comparisons between blenders and sample weights used.

Two different blenders were used, (a hand held domestic blender and a table top commercial Waring® blender (Model 38BL41) along with 25g, 30g and 35g of fresh raspberries. The Waring® blender produced the most consistent smooth results for all varieties tested whereas the hand held blender failed to produce a smooth result for drier fruit with lumps collecting within the blenders bulbous guard.

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Testing also found that while 25g of fresh fruit produced sufficient sample for colour analysis, 35g created a more consistent puree for all samples with the elimination of the majority of lumps. Other tests involved further sieving of pureed fruit samples as well as the addition of 50mls of deionised water to samples prior to blending. Subsequent analysis however found sieving required far more initial fresh fruit (approximately 50 to 60g) which for some progeny would not leave enough for sensory/biochemical analysis. The addition of water to samples found some became more dilute than others affecting the accuracy of the subsequent colour measurement. Based on initial results, it was decided that all samples would undergo preparation and analysis as follows:

Thirty five grams of fresh fruit is weighed and placed into the Waring® blender and blended on full power for 10 seconds. The puree is then mixed for a few seconds and further blended for another 10 seconds. Fifteen grams of puree is then transferred into a sterile Petri dish which is gently shaken to provide an even surface for analysis. Ten grams of this puree was also analysed to allow for subsequent statistical analysis to be carried out measuring any reflectance differences obtained from a reduced sample thickness. The Chroma meter was then set out as per the operating manual and blanked using the accompanying tile (101974) with a reflectance of Y: 87.4, x: 0.308 and y: 0.315 and results were recorded for Yxy and, L*a*b* values and colour deviation.

Comparisons of chromaticity across a range of parameters, from fresh and frozen fruit, were carried out on a range of named cultivars to determine how

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freezing would affect the measurements. This would allow us to determine if fruit could be frozen when large volumes of samples require analysis simultaneously.

Results

Initial results obtained for colour meter analysis was examined in collaboration with Biomathematics and Statistical Scotland (BioSS) in respect of any significant differences obtained for the different weight measurements analysed or between clones and reps. This involved transferring results into GENSTAT software where a direct comparison can be made between the two treatment variants.

A summary of statistics for each individual measurement (Yxy, L*a*b*) for both 15g and 10g was carried out and resulted in no significant difference being recorded for the separate weights or between the different reps. Although significant differences exist between individual progeny (table 1.2.3.1 in appendix shows the minimum and maximum values of the samples) the measurements for the different clones and reps remained consistent. Graphical representations of some of the colour parameters are shown in figures 1.2.3.1-1.2.3.6 in the appendix.

The effect of freezing named cultivars on a range of colour parameters is given in tables 1.2.3.2 and 1.2.3.3 in the appendix.

Parameters measured by the colour meter were examined for correlations with

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the visual score made on the fruit on a 1-5 scale. Analysis of the data showed a highly significant correlation ($p<0.001$) between the visual score and the Hue (Y measurement). The lighter the visual score, the higher the Y measurement. Likewise the darker the fruit was the lower the Y measurement.

Brix testing

Materials and Methods

Brix testing is a measure of the total soluble solids (TSS) in a given weight of plant extract. The Brix scale is based on a sucrose solution and how the solution's concentration relates to the refractive index. Due to the presence of other substances however, (salts, minerals, proteins) the Brix % represents the total concentration of all soluble solids within each sample analysed. (Pocket Pal-1 instruction manual). The relationship between brix and the measurements of the actual sugars and acids will allow us to more accurately understand how brix relates to taste.

Two berries from each sample were randomly selected and placed into a cut piece of muslin before two or three drops of juice were squeezed onto the refractometer (PAL-1) prism. The refractive index of each sample was then measured and recorded as a % of total soluble solids. As each sample was subsequently blended for colour analysis a few drops of pureed sample were also analysed on the Brix meter in order to allow a direct comparison to be carried out between the TSS content of both raspberry juice and puree.

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Results

Statistical analysis of Brix results (table 1.2.3.4 and fig. 1.2.3.7 appendix) recorded significant ($p<0.001$) differences between individual progeny but no significant differences between clones, reps or involving the two different treatment techniques of fruit juice and fruit puree.

Sample weight

Materials and Methods

Ten berries from each sample were randomly selected and weighed into a sterile Petri dish in order to give a quick accurate measurement of individual fruit size. Some samples were noted for their poor druplet cohesion, and this will be scored in all samples over all environments as from next season allowing mapping of this trait also.

Results

Results of the 10 berry weights are presented graphically in fig. 1.2.3.8 (and given in table 1.2.3.5 in appendix) where significant variation in 10 berry weights exists between the progeny, but measures on the same progeny across clones and reps are consistent with no significant differences.

From the results collected in 2006 all parameters (berry weight, brix, colour and firmness) showed significant differences between progeny but not within clones

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and reps of individual progeny. This will allow individual progeny to be sampled across 3 sites in 2007.

Task 1.2.4 Carry out 'breeders' assessment of colour, brix, weight and firmness for 2007

Materials and Methods

Colour, brix, weight and firmness were assessed using protocols developed in 2006 (task 1.2.3). Instead of collecting data from replicated lines within a site, data were collected on individual lines across three sites. Data for 2007 was then examined alongside the 2006 data.

Results

Colour

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Table 1.2.4.1 shows analyses of the colour data for 2007 and comparison with 2006 data. Figure 1.2.4.1 shows the comparison of visual and Y readings for 2006 and 2007 data and table 1.2.4.2 summarises the Y parameter across seasons and sites. The Y score is significantly higher for 2006 than 2007 but across sites in 2007 no significant differences exist. The commercial samples (protected) of 2007 (PT07) had a greater representation of darker colours, and the chromaticity measurement (PT07Y) had a greater concentration of lower Y readings. There is a highly significant inverse correlation between visual score and Y parameter.

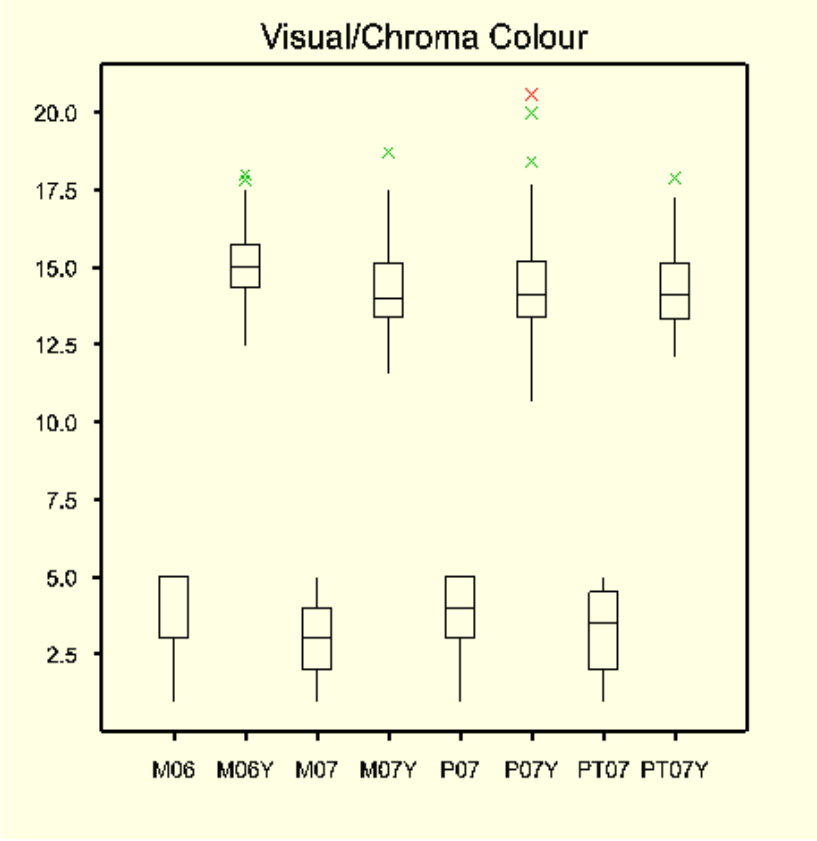

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Figure 1.2.4.1 Visual colour assessment and Y Chromaticity measurements. (M06) M24 2006 (M06Y) M24 2006 Y, (M07) M24 2007, (M07Y) M24 2007 Y, (P07) Polytunnel 2007, (P07Y) Polytunnel 2007 Y, (PT07) Commercial site 2007 (PT07Y) Commercial site 2007 Y

Brix

A summary of brix results can be seen in table 1.2.4.3 for the two seasons and the three environmental locations. The differences between seasons (Brix 06 and M24) and sites (M24, PTh and poly) is apparent. The field site M24 recorded a significant decrease in % brix from 2006 to the 2007 season. In 2007 the protective sites were not adversely affected by the weather and have a significantly larger mean % brix value compared to the M24 site.

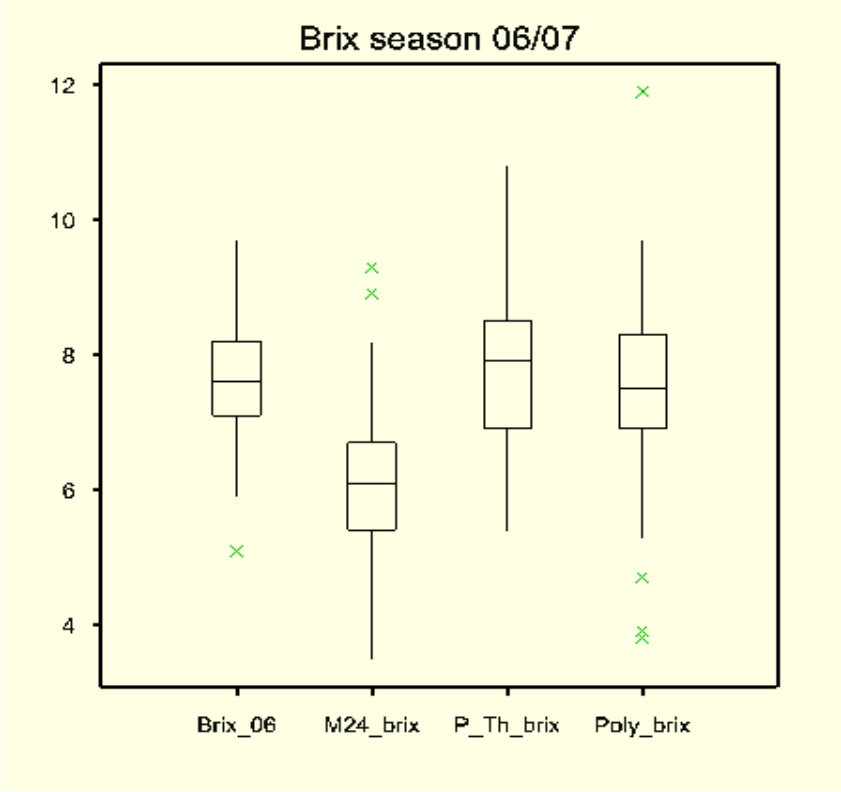

Figure 1.2.4.2 % Brix for the mapping population for season 2006 M24 (Brix_06)

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and season 2007 M24, Commercial site (P_Th) and SCRI Polytunnel (Poly).

Sample weight

Comparative analysis was carried out for the weight results (Table 1.2.4.4) for season 2007, with significant differences in overall weight being noted for almost all progeny grown under protective cultivation. Many individual samples showed increases of up to three and four times those found for the open field. Results between seasons for the field site M24 showed a slight but non significant drop in 2007.

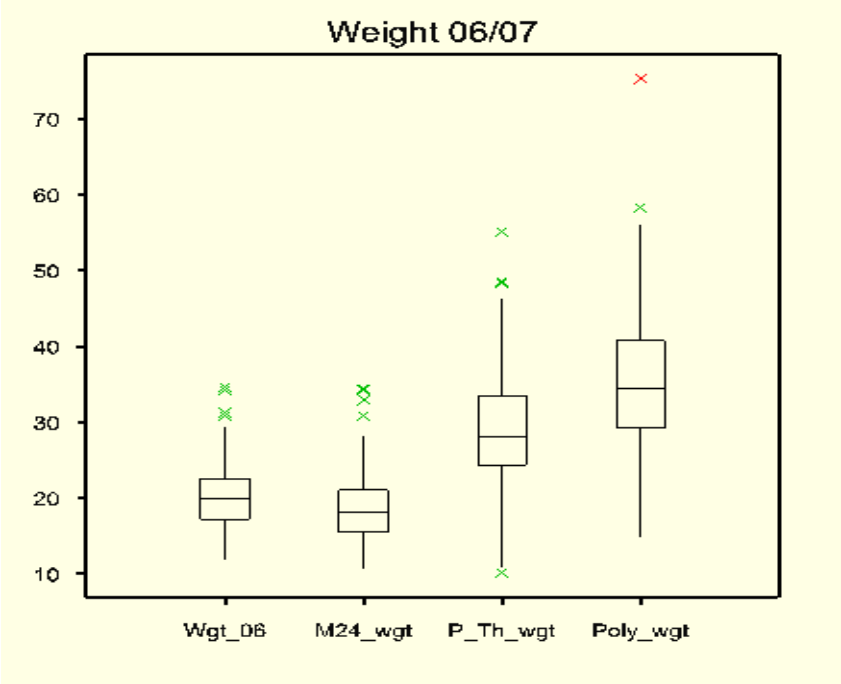

Figure 1.2.4.3. Box plot figures showing the differences found between progeny at the three different sites. (M24 season 06, M24 season 07, commercial 07 and

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polytunnel 07.

Task 1.3.1 Set up sensory panels and score sensory characteristics of sweetness, sourness and flavour intensity 2006.

Materials and Methods

Sensory analysis was done using progenies belonging to the mapping population (*Glen Moy* x *Latham*). For each progeny there are 2 plants each, in replicate 1 and replicate 2.

Panelists were chosen from healthy volunteers aged 18 – 60 years at SCRI.
Before the start of the experiment, potential assessors were selected based on their ability to rank sweetness and sourness in raspberry fruit purees supplemented with either food-grade artificial sweetener (0.31% and 0.73% per 150g puree) (Splenda, Washington, USA) or citric acid (1.35% and 2.67% per 150g puree) (E 330 Citric Acid, Young's Home Brew, West Midlands, UK). For each attribute (sweetness or sourness) 3 samples were given, with assigned randomly generated numeric codes. Assessors were asked to rank them numerically from 1(*least*) - 3(*most*). A sample of the ranking analysis sheet is given below.

Name**:* ***Age: **Date:**

Sensory Evaluation of raspberries

(1) Rank these samples based on sweetness:

731	**571**	**351**
___	___	___

(2) Rank these samples based on sourness:

537	**931**	**793**
___	___	___

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Figure 1.3.1.1 Ranking form for sweetness and sourness to select assessors

The panellists chosen obtained a result of more than 50% right answers.

The experimental presentation of samples was established with DesignExpress software in order for the collected data to be statistically significant. A total of 15 samples were analyzed daily everyday, in 2 sessions, with 6 samples in each session. The panelists numbered between twelve and fifteen, depending on availability. Fruit samples were pureed using hand-held blenders and placed into clear plastic cups labeled with random numbers. Approximately 10 g of fruit puree was given to each assessor. Then sweetness, sourness and flavour intensity were scored on 7-point scales on prepared forms. Assessors were asked to rinse their mouths between samples to minimise carry over effects. Named varieties were included in the sensory studies for comparison.

For the experimental sensory analysis, approximately 15g of fruits were weighed out for each sample. Fruit was pureed using a hand blender and a 2g (approx.) aliquot of fruit puree was placed in a 15 ml matte disposable plastic cup, assigned randomly generated numeric codes and given to each assessor. A total of 6 samples were given for each session. One plastic spoon was provided for each assessor per session and assessors were asked to wipe the spoon between samples with the provided paper towel. One cup of drinking water was provided for mouth rinsing between samples. These precautions were taken to ensure minimal sensory fatigue and carry over effects. The taste attributes were scored on a 7-point scale. Below is a sample of the analysis form for scoring of the taste components:

Sensory analysis of raspberries

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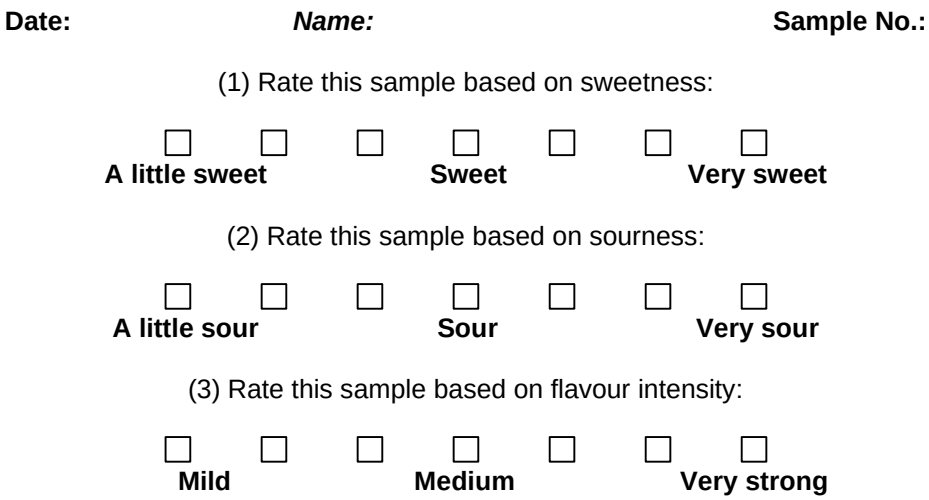

Figure 1.3.1.2: Scoring form for sweetness, sourness, and flavour intensity of samples

For each sample, the mean of the individual flavour attributes were calculated. Using Minitab (version 14.1), One-way analysis of variance (ANOVA) was done to investigate variability between replicates of each sample.

Results

Sensory scores were carried out on over 300 samples from the Glen Moy x Latham population as well as a range of named varieties. The ranking of named varieties based on the parameters sweetness, sourness and flavour intensity are presented in table 1.3.1.1 in appendix alongside other quality parameters. Good variability and spread of sensory scores for progeny are shown in table 1.3.1.2 (appendix) along with the parental means.

Regression analysis was done on all 3 flavour attributes and it was revealed that sweetness and sourness have a positive correlation with flavour intensity, whereas sweetness is negatively affected by sourness. All 3 attributes

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contributed less than 20% variability towards one another. The regression equations for all three relationships are as follows:

Sweetness = 0.65789 + 0.53726 (**Flavour intensity**)
Sourness = 1.61503 + 0.42449 (**Flavour intensity**)
Sweetness = 3.85708 – 0.38642 (**Sourness**)

From the results it can be deduced that sweetness and sourness are inversely related. When sweetness scores mid-range to high (4-6), the scores for sourness is low, and vice versa. Examining the data exhibiting relationship of sweetness:sourness ratio with other parameters, we can see that only sweetness and sourness are giving variation and there is very little variation caused by sample number. This is indicative that between repetitions, there is little variability, with the exception of a few outliers.

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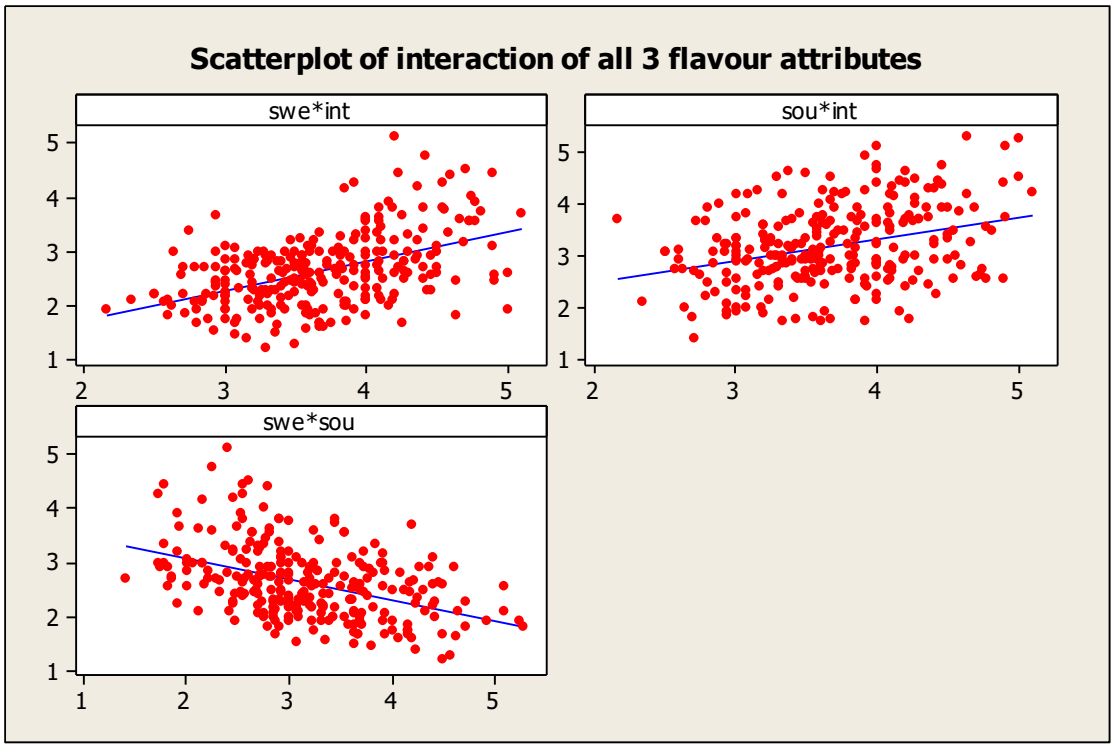

Figure 1.3.1.3 Scatterplot to show regression relationship of interaction between sweetness and intensity (*upper left corner*), sourness and intensity (*upper right corner*) and sweetness and sourness (*lower left corner*) of sensory data in 2006.

Task 1.3.2. Set up sensory panels and score sensory characteristics of sweetness, sourness and flavour intensity 2007.

Materials and Methods

The samples used for 2007 were harvested over 3 sites; polytunnel (poly), open field (M24) and commercial site (P Th). It was determined through results of the previous year that only one replicate was needed for the data to still be statistically significant. All samples from the polytunnel and open field site were chosen, whereas samples exhibiting extreme values for the 3 attributes were chosen from the commercial site.

The experiment was carried out in a similar fashion to 2006 but at purpose-built

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facilities at the University of Strathclyde with the advantage of using FIZZ sensory software (Biosystemes, France) for experimental design, to record and analyse experimental data. It was also possible to evaluate experimental fruit under red lighting to eliminate bias from appearance. Therefore, the assessors used software to record scores, rather than marking these on prepared forms.
Assessors were again chosen through ranking of fruit samples supplemented with either Splenda® or citric acid. Pureed samples of the progenies were scored once again, for sweetness, sourness and flavour intensity with a 7-point scale.
A total of 24 samples were scored each day, with 2 sessions of 12 samples each.

Results

The data obtained was important in comparing with the previous year data to examine for both variation and consistency. In conjunction with that, the data was also analysed for variation between sites. This is to investigate the influence of environment in flavour development.

Data was analysed in Genstat and significant variation was found between the field and the protected sites for all sensory attributes ($p<0.001$) (Table 1.3.1.3). Berries grown under protection scored significantly higher for sweetness and flavour intensity and lower for sourness compared to field grown berries. Interestingly, no significant difference was detected in the sensory attributes of sweetness, sourness and flavour intensity from the field site from 2006 to 2007 (table 1.3.2.1).

Regression analysis was carried out to investigate interaction of all 3 flavour attributes and this has shown a consistency over the 2 years. The scatterplot below visually exhibits this.

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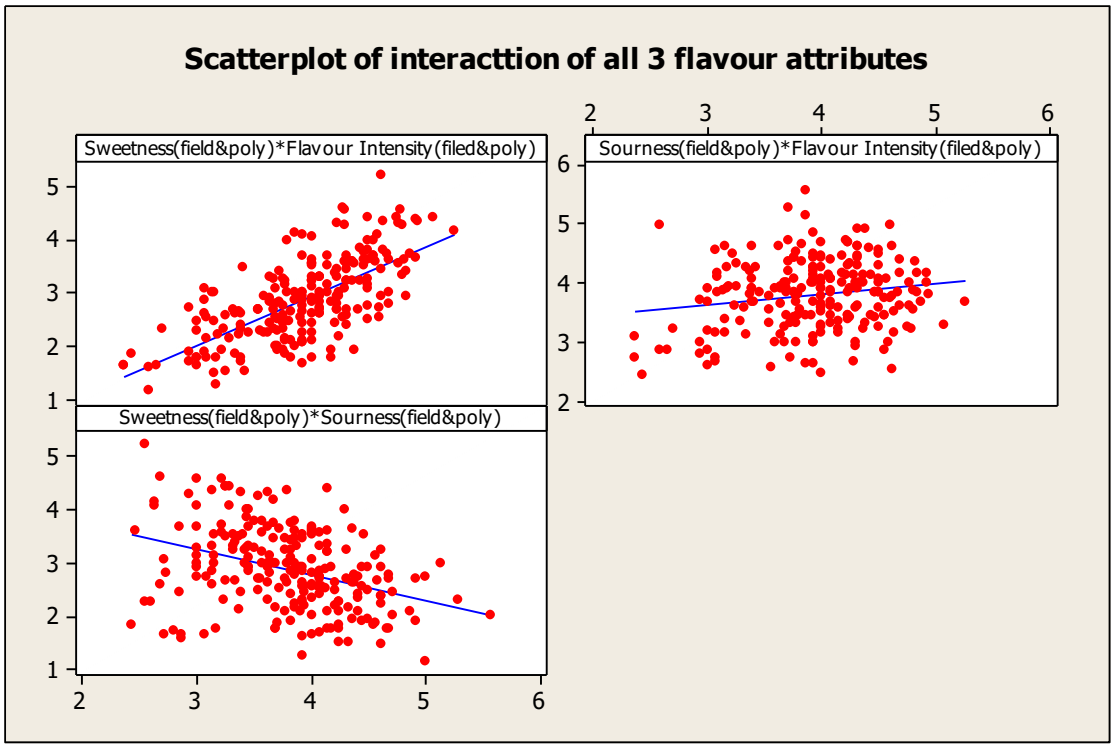

Figure 1.3.2.1 Scatterplot to show regression relationship of interaction between sweetness and flavour intensity (*upper left corner*), sourness and flavour intensity (*upper right corner*) and sweetness and sourness (*lower left corner*) of sensory data 2007.

Task 1.3.3 Quantification of key berry metabolites 2006

Materials and methods

Fruit were collected and transported to Strathclyde University and the biochemical analysis of the key metabolites is underway.

The analysis of sugars and organic acid content of the raspberries was done through High Precision Liquid Chromatography (HPLC). For sugars analysis, *sucrose, fructose* and *glucose* were the main sugars investigated and for organic acids, *citric* and *malic* acid.

A standard curve was plotted by running standard solutions at specific decreasing concentrations and regression analysis was performed to reveal

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correlation between individual sugar concentration and area under the curve of chromatograms.

For sugars, concentrations of 0.05M, 0.04M, 0.032M, 0.0256M, 0.02M, 0.016M, 0.013M, 0.01M, 0.008M, 0.007M, 0.005M, 0.005M, 0.004M, 0.003M, 0.0027M, 0.0022M, 0.002M, 0.0004M were used. Triplicate injections were done for each concentration and an ANOVA analysis was done on the raw data using Minitab (version 14.1). The linear relationships in the sugars are:

Sucrose concentration (M) = (Area under the curve) + 12091 / 5587950
Fructose concentration (M) = (Area under the curve) + 2417 / 446178
Glucose concentration (M) = (Area under the curve) + 4847 / 1185395

With these linear equations, total sugars content in each progeny can be calculated from the chromatogram.

The same procedure was carried out for the organic acids analysis. The linear equations for the organic acids present are:

Malic acid (M) = (Area under the curve) – 1177947 / 61554817

Citric acid (M) = (Area under the curve) + 219130 – $e^{1.97}$ / 0.8

The organic acid content in samples was calculated the same way as the sugars.

The berries were harvested starting 7th July 2006 to 7th August 2006, put into polyethylene bags, labelled with the unique progeny code and the replicate number, stored at –20^{o}C until analysis. For the sugars and organic acids analysis, the extraction process was modified from Sturm *et al* 2003. Approximately 0.3g of defrosted fruit was weighed out into 1.5ml centrifuge tubes. The fruit was pureed using sterile toothpicks. Distilled sterile water was added and made to mark (1.5ml). After vortexing the mixture for 20 seconds, it is

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then centrifuged at 13000rpm for 30 minutes. 800μl supernatant was collected and put into a filter unit placed in a 2ml centrifuge tube (Alltech Micro-spin® Centrifuge Filter Tubes, 0.45μM, regenerated cellulose). This was centrifuged at 13000rpm for 10 minutes. 20μl of the extract was used for each analysis.

The HPLC parameters for sugars analysis were as follows; mobile phase is degasses deionised distilled water (conductivity 18Ω), flow rate of 0.6mL/min, column temperature set at 85ºC. An ion exclusion column was used to separate the sugars (Varian MetaCarb Pb Plus, 7.8mm x 30mm). For detection, a low temperature evaporative light scattering detector was used (Sedex Model 55, Sedere, France) set at 91ºC. This was connected to an integrator (Varian 4950) for data collection in the form of chromatograms.
The retention times for the sugars were:

Sucrose – 10 minutes
Glucose – 13 minutes
Fructose- 19 minutes

For the organic acids analysis, the HPLC parameters were as follows; mobile phase is degassed 4mM sulphuric acid, at a flow rate of 0.4mL/min, with the column operating at a temperature of 65ºC. An ion exclusion column was also used to separate the acids (Varian MetaCarb H Plus Column, 7.8mmx30mm). A UV-Visible variable wavelength detector set at 215nm (Varian 3090 UV-Vis Detector) was used. This unit was connected to data management software ChromPerfect LSi (Justice Scientific, New Jersey, US) to process chromatograms.

The retention times for the analysed organic acids were as follows:

Citric acid- 14 minutes
Malic acid- 18 minutes

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The samples were similar to the population used for sensory analysis. Replicate injections were made on half of the numbers from each replicate to ensure minimal carry over error from extraction process. The analysis was done on both replicates (replicate 1 and replicate 2) of each progeny to investigate variability between replicates, if any, and to see if correlation exists between biochemical data and soluble solids content (Brix%) data collated during the 2006 harvest period.

Results

To date, sugars and acid analysis has been completed on 143 progeny and no significant variation has been detected from different replicates. There is an even spread of sugars content in the berries throughout the population (table 1.3.2.2), indicating distinct phenotypic differences in the segregating population due to the heterozygous nature of the parents.

From the analysis done to date, the major sugars found in the samples are fructose and glucose, with very few or no samples having sucrose. For the organic acids content, the major fractions were malic and citric acid. There are significant correlations between the concentrations of fructose and glucose and between malic and citric acid within a berry. Highly significant correlations also exist between the individual sugars and the individual acids.

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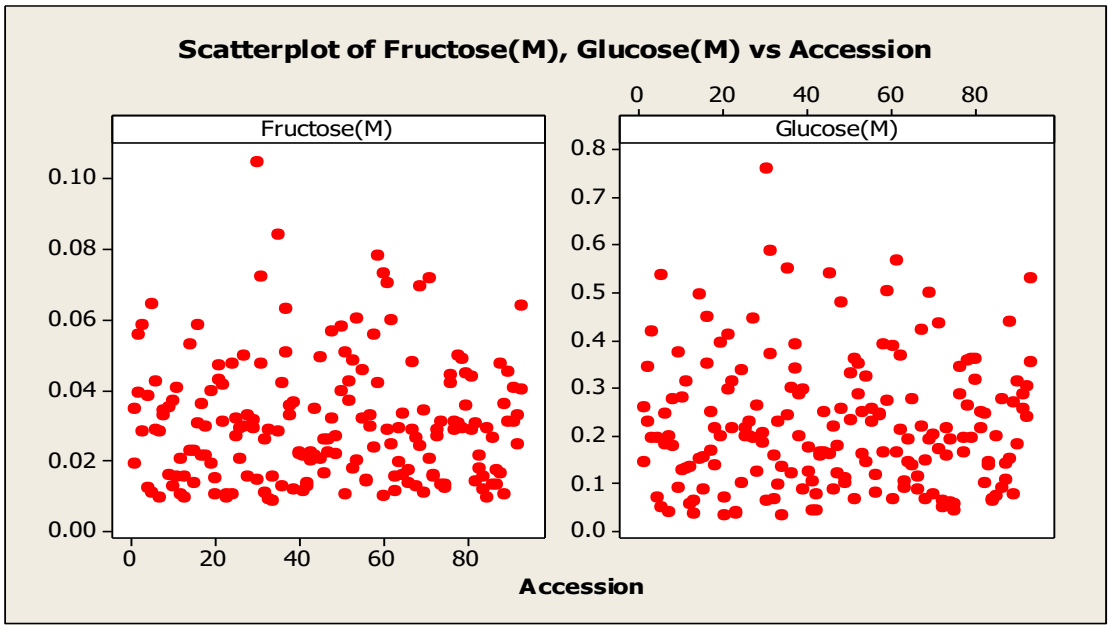

Figure 1.3.3.1: Scatterplot showing distribution of fructose and glucose in samples.

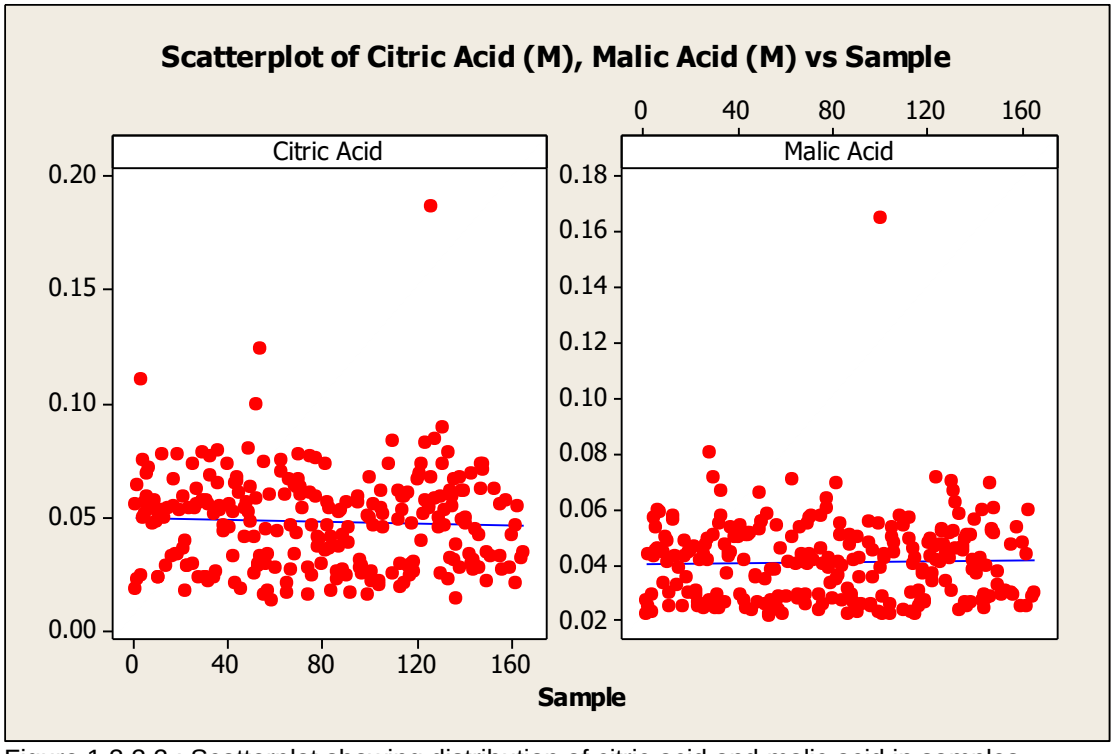

Figure 1.3.3.2 : Scatterplot showing distribution of citric acid and malic acid in samples.

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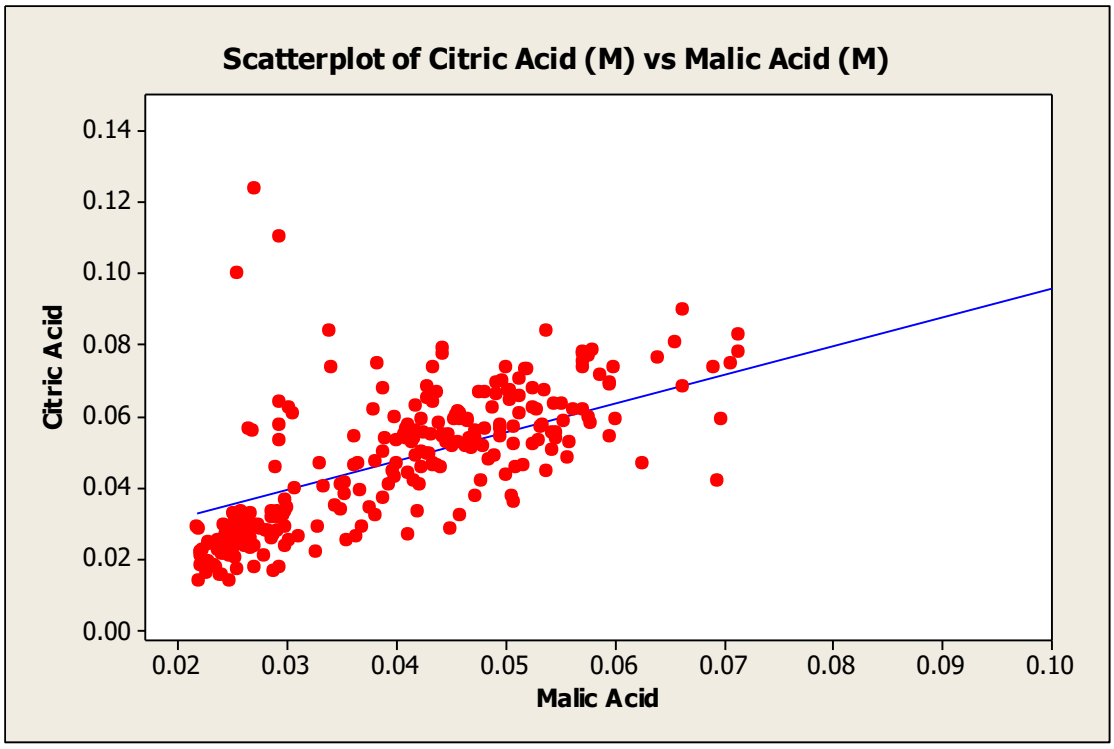

Figure 1.3.3.3: Scatterplot showing regression relationship between citric acid and malic acid in samples.

Discussion of quality data 2006-2007.

Data analysis in 2006 showed no significant variation between reps and clones for all the traits scored (sensory traits: sweetness, sourness, flavour intensity, breeder traits: colour, weight, brix and visual scores and biochemical traits: sugars and acids). Traits did show significant variability between different progeny from the Glen Moy x Latham cross.

For 2006 data, no correlation exists between the brix measure and sensory scores for sweetness or sourness. Nor can any correlation be identified for brix with measures of individual and total sugars and individual and total acids or total sugars and acids. Some correlation exists between brix and flavour intensity but

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significance is low. No correlation was identified between brix and weight or weight and any other trait scored. Brix and colour showed a weak correlation.

Measures of sweetness from sensory panels are significantly correlated ($p<0.001$) with individual measures of glucose and fructose as well as total sugars, total sugars and acids and flavour intensity. There is also an inverse correlation between the sensory perceptions of sweetness and sourness. Sourness correlates significantly with the individual measures of acids and total acids.

Flavour intensity correlates significantly with sensory perceptions of sweetness and sourness but not with any individual or total measures of sugars and acids.

Data from 2007 are still under analysis, with the sugar and acid data currently being collected. Brix measures at all 3 sites significantly correlate with flavour intensity. Brix shows a weak correlation with sweetness under field conditions that was not found for brix and sweetness at the sites under protection. A weak correlation also exists between brix and colour.

For 2007 data again sweetness, sourness and flavour intensity are all significantly correlated. No significant variation was detected in any of the sensory scores (sweetness, sourness, flavour intensity) from year to year, however sweetness, sourness and flavour intensity do show significant variation from open field to protected cultivation. In-fact from site to site all trait scores

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except for colour were significantly different for fruit grown in the open field site. Although the trait scores were significantly different from the open field to the protected sites in 2007, sweetness, sourness, flavour intensity and weight are significantly correlated. A weak negative correlation was detected between weight and sweetness and brix in 2007.

In summary, only the Brix and colour measures varied significantly from year to year, but from site to site sweetness, sourness, flavour intensity, brix and weight but not colour varied significantly (tables 1.3.2.1 and 1.3.2.3). The sugars and acids have still to be measured in 2007 fruit.

Task 1.3.4 Quantification of key berry metabolites 2007

Fruit has been collected across the three sites and frozen for transport to Strathclyde University.

Objective 3. Molecular data enhancing deliverables

Task 3.1.1 Identify raspberry gene sequences for candidate genes related to quality traits.

Materials and Methods

Knowledge of the physiological pathways involved in the uptake and regulation of sugars and organic acids as well as colour was be utilised in order to identify the candidate genes, including transporters and transcription factors important in

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biochemical pathways for the quality traits of interest. An initial set of candidate genes from peach and other *Rosaceae* was identified (table 3.1.1.1). These provide sequence information from similar species that can be used as a probe to pull out the equivalent raspberry gene sequences. These sequences can then be cloned, sequenced and any polymorphisms which exist between the parents detected. Initially the most obvious candidate genes likely to impact on fruit quality have been identified and included transporters of sucrose, water and other small molecules as well as genes with a role in sugar or acid biosynthesis. Primers from related gene sequences were designed using Primer 3 software and used in raspberry on the Glen Moy and Latham parents to see if a product could be obtained. For those that generated a product, the DNA was cloned into pGEM T easy, followed by transformation of plasmid DNA into competent DH5α cells. These were then inoculated onto individual LB plates with ampicillin/IPTG and X Gal for selection of recombinants. Plates were incubated to allow sufficient development of blue/ white colonies, before transferring a selection of white colonies from each plate into individual wells of a 96 deep well plate containing LB and ampicillin. A multi-screen plasmid mini-preparation was carried out before samples were prepared for sequence analysis. Sequencing was carried out using big dye and 1 in 16th reactions.

For those sequences which did not show enough homology to generate a raspberry sequence, new primers for the candidate genes were designed by importing the known peach accession number or gene name of interest into the NCBI database (National Centre for Biotechnology Information). The six most

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similarly aligned sequences were then imported into another database ClustalW, a sequence analysis tool available through European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI). This allowed the alignment of divergent sequences to be directly compared to find the most conserved regions. Selected sequence areas can then be analysed further using primer3 software for optimal primer design.

Fruit has been collected from the Glen Moy and Latham parent at various stages of ripening to allow gene expression patterns to be examined.

Results

Of the primer sequences utilised 18 out of the 20 sequences have now resulted in a clear raspberry PCR product being obtained. Several PCR conditions were tried pertaining to annealing temperature and extension timing but consistent results could not be obtained for the remaining genes. New primers were designed based on alignment of further sequences and of these the status of the candidate gene studies is 3.1.1.2.

Task 3.1.2 Examine raspberry gene sequences for polymorphism between Latham and Glen Moy and map based on the polymorphism identified.

Materials and methods

Single nucleotide sequence polymorphisms (SNP's) between the raspberry Glen

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Moy and Latham parents, and within the two alleles of each parent have been identified for 6 of the 18 genes amplified to date and assays designed to allow mapping to proceed. So far mapping has been attempted for 4 of these sequences 3 of which have been placed on the genetic linkage map.

Discussion

The project is well underway to deliver the objectives.

The field trials were established at three environments to allow data collection.

Studies have demonstrated significant variation between progeny for all parameters tested to date. No significant differences were found between clones both within and between replicates at a single site for the parameters tested. The effect of freezing was studied on some parameters and again no significant effect was observed.

Data has now been collected for a range of traits in 2006 and 2007 allowing comparisons to be made across years and sites. Two traits (Brix and colour) are showing significant variation between years. Across sites, all traits, except colour, show significant differences between field and protected sites. All individual traits however are highly correlated from year to year and site to site.

Significant correlation has been identified within and between the sensory parameters and biochemical data. Interestingly Brix scores are highly correlated only with flavour intensity and weakly correlated with colour and weight, but no other correlations were identified.

Candidate gene mapping has been initiated on the genes in which polymorphism

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can be detected. Mapping of the sensory and biochemical data will allow regions of the linkage group(s) to be identified. These regions will be compared with location of candidate genes.

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APPENDIX

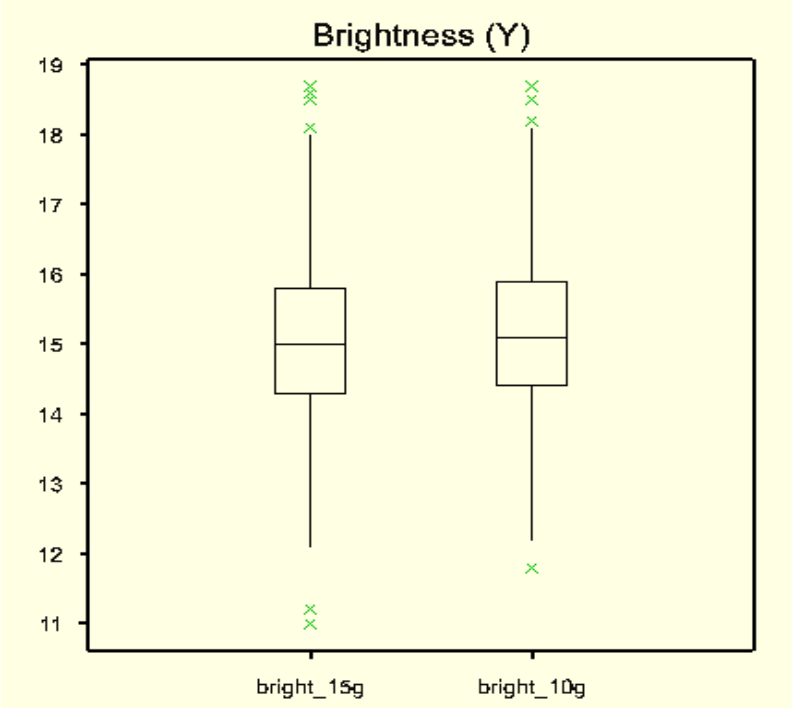

Fig. 1.2.3.1 Graphical representation of mean brightness for 15g and 10g fruit puree prepared as described.

There was no significant difference between the means for the two weights with regard to brightness. Between progeny variations existed for this parameter, with minimum values of 11 and maximum values up to 18.

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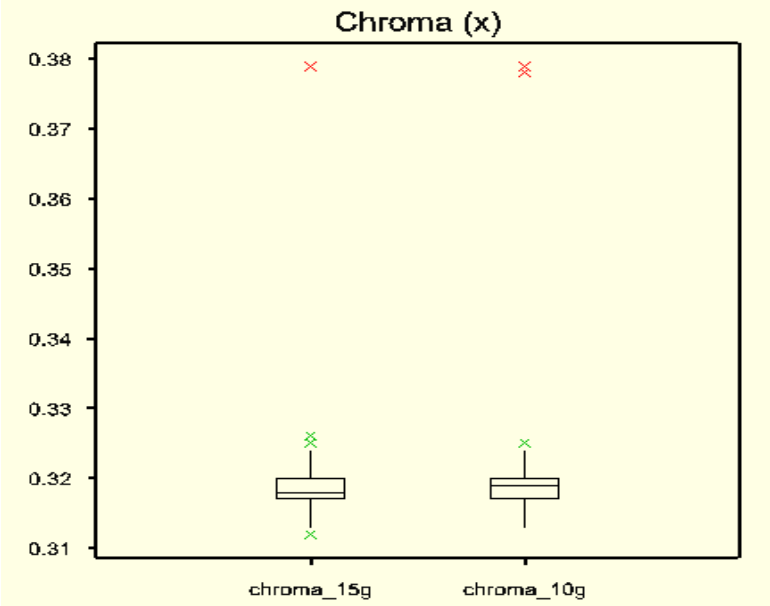

Fig. 1.2.3.2. Graphical representation of mean Chroma (x) for 15g and 10g fruit puree prepared as described.

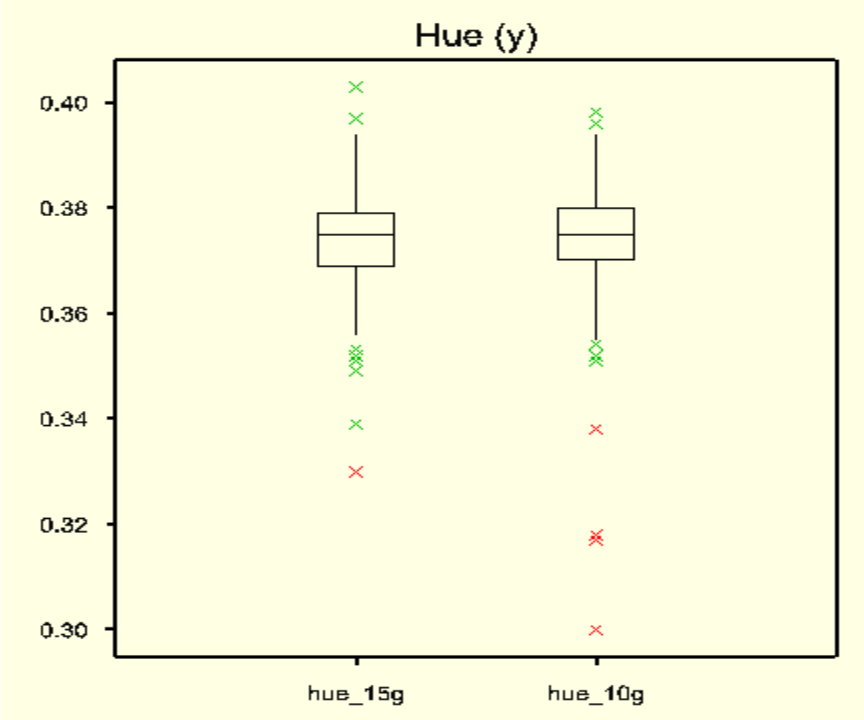

Fig. 1.2.3.3 Graphical representation of mean Hue (y) for 15g and 10g fruit puree prepared as described.

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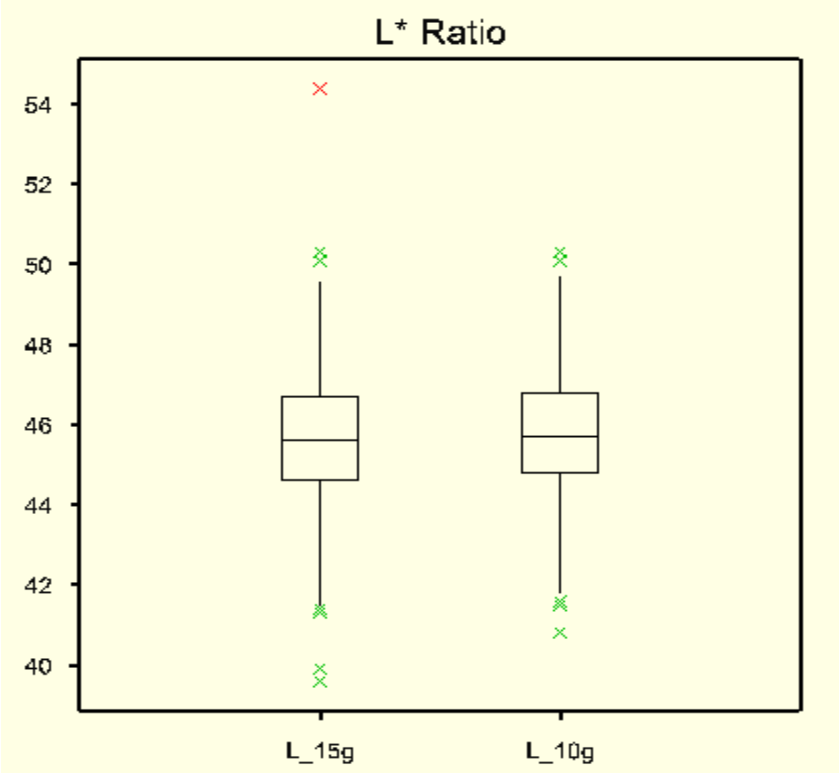

Fig. 1.2.3.4 Graphical representation of mean L* Ratio for 15g and 10g fruit puree prepared as described.

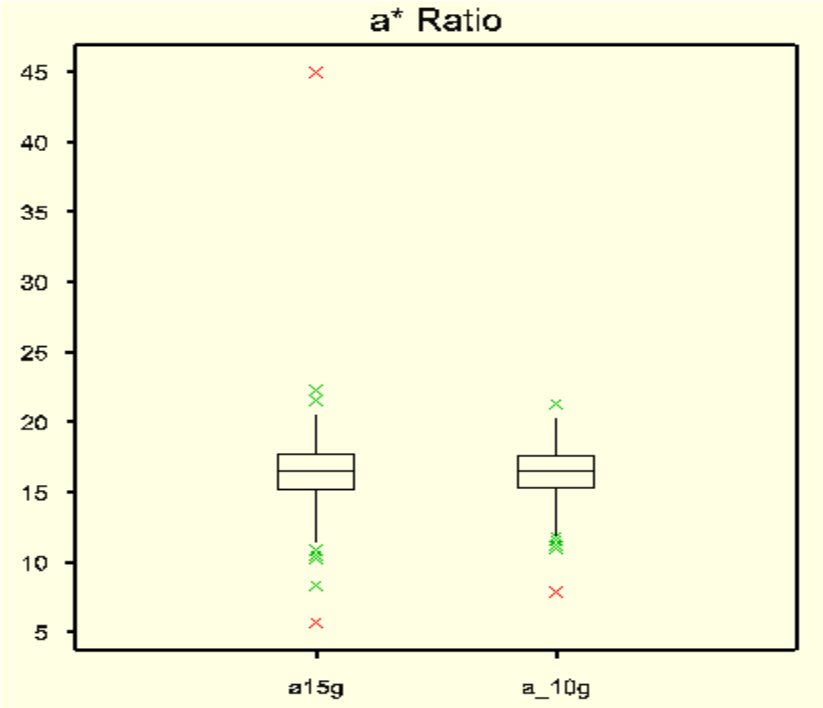

Fig. 1.2.3.5 Graphical representation of mean a* Ratio for 15g and 10g fruit puree prepared as described.

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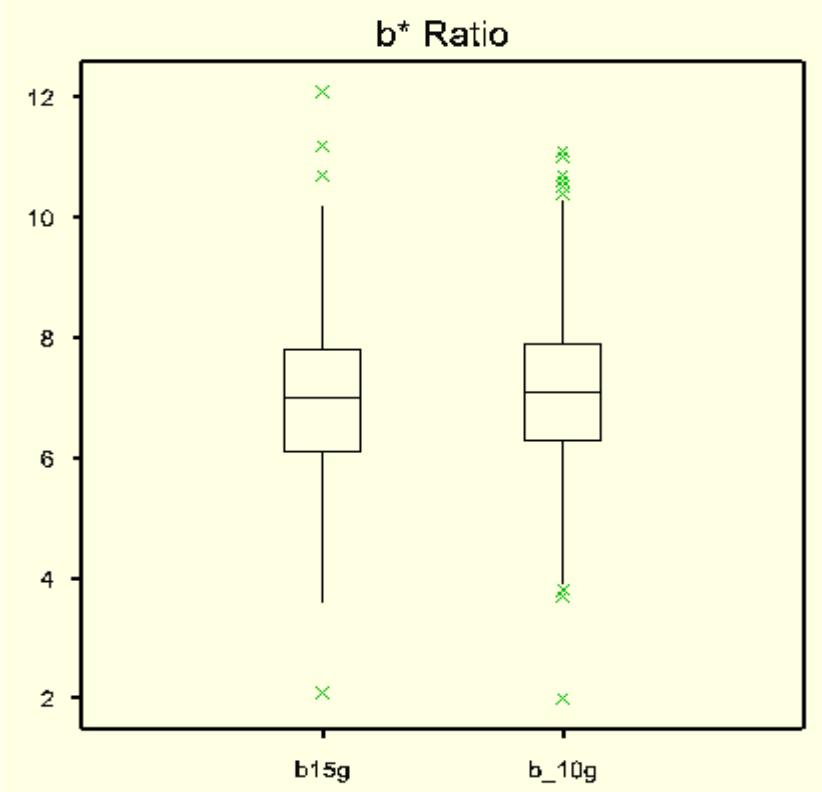

Fig. 1.2.3.6 Graphical representation of mean b* Ratio for 15g and 10g fruit puree prepared as described.

Table 1.2.3.1. Summary statistics comparing 15g and 10g weights for Brightness (Y) 2006

Summary statistics	**Y 15g**	Rep 1	Rep 2	**Y 10g**	Rep 1	Rep 2
Number of observations	601	304	297	601	304	297
Number of missing values	15	4	11	15	4	11
Mean	15.04	15.11	14.98	15.14	15.21	15.07
Minimum	11.00	15.00	14.90	11.80	12.40	11.80
Maximum	18.70	18.70	18.60	18.70	18.70	18.50
Standard deviation	1.22	1.24	1.20	1.18	1.22	1.13

Table 1.2.3.2. Summary statistics comparing 15g L*a*b* for Industry standard

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samples 2006

Summary statistics for 15g Colour L*a*b*	Fresh **L***	Fresh **a***	Fresh **b***	Freezer **L***	Freezer **a***	Freezer **b***
Number of observations	9	9	9	9	9	9
Number of missing values	0	0	0	0	0	0
Mean	44.92	16.38	7.311	45.58	18.59	8.667
Minimum	43.50	13.40	5.300	42.40	15.10	6.000
Maximum	46.90	18.50	8.900	48.90	21.50	11.300
Standard deviation	1.05	1.52	1.213	2.41	2.20	1.696

Table 1.2.3.3. Summary statistics comparing 15g Yxy for Industry standard samples 2006

Summary statistics for 15g Colour Yxy	Fresh Y	Fresh x	Fresh y	Freezer Y	Freezer x	Freezer y
Number of observations	9	9	9	9	9	9
Number of missing values	0	0	0	0	0	0
Mean	14.53	0.3759	0.3192	15.06	0.3845	0.3203
Minimum	13.5	0.362	0.317	12.8	0.371	0.317
Maximum	16.00	0.387	0.3220	17.6	0.398	0.3240
Standard deviation	0.77	0.0080	0.0017	1.77	0.009	0.0022

Table 1.2.3.4. Summary statistics comparing % Brix for juice and puree for two separate repetitions from field in 2006

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Summary statistics	**Juice**	Rep1	Rep 2	**Puree**	Rep1	Rep 2
Number of observations	**603**	306	297	**602**	305	297
Number of missing values	**13**	2	11	**14**	3	11
Mean	**7.636**	7.600	7.673	**7.187**	7.243	7.130
Minimum	**3.200**	4.000	3.200	**3.800**	3.800	4.100
Maximum	**12.200**	11.000	12.200	**10.900**	10.900	10.400
Standard deviation	**1.293**	1.253	1.334	**1.149**	1.123	1.173

Table 1.2.3.5. Summary statistics comparing 10 berry weights from field in 2006.

Summary statistics for Weight	Rep 1 Clone 1	Rep 1 Clone 2	Rep 2 Clone 1	Rep 2 Clone 2	Freezer samples
Number of observations	154	151	152	146	34
Number of missing values	0	3	2	8	272
Mean	20.59	20.26	20.45	20.27	19.40
Minimum	9.42	9.03	10.32	8.67	11.72
Maximum	37.00	36.96	36.04	36.80	33.29
Standard deviation	4.95	5.22	4.80	5.48	4.68

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Table 1.2.3.6. Visual Assessment of Quality Parameters from field 2006

Progeny no.	Size	Appearance	Firmness	Colour
	small=1	Bright=b	1=firm	1=pale
	large=4	Dull=d	3=soft	2=pale/mid
				3=mid
				4=mid/dark
				5=dark/purple
GLEN MOY	4	d	3	3
LATHAM	2	b	1	4
1	2	b	1	3
3	3	b	1	3
4	3	b	1	2
7	4		3	3
10	3	b	2	3
11	1	b	1	5
13	4	d	2	5
18	3	b	2	2
19	3	b	1	5
21	3	d	2	5
23	3/4		3	5
24	2/3	b	2	1
34	3	b	3	5
38	3	d	1	3
42	2/3	d	1	3
43	2/3	b	3	3
44	2/3	b	3	5
48	3	d	3	5
53	3	b	3	3
55	3	d	3	3
57	4		3	3
62	1	b	3	3
64	3	b	2	3
68	2	d	3	4
72	3	b	3	5
73	2	b	1	3
82	3	d	3	5
53	3	b	3	3
83	2/3	d	2	5
84	2/3	b	3	1
85	3	d	3	5
88	3/4	b	1	4
89	2	d	1	3
91	4	b	3	5
96	3/4	b	3	4
102	3	d	1	3
105	3	b	1	5
110	2/3	d	3	4
112	3	d	2	3
116	1	b	2	1
118	2/3	b	3	3
127	4	b	2	4

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138	3	b	1	3
140	4	d	3	3
142	3	b	1	2
145	1		3	3
146	2/3	b	3	3
147	4	b	1	2
148	3	d	2	3
149	4	b	2	3
150	3	b	2	3
158	2	b	2	3
160	3	d	3	5
161	2	d	2	2
167	2/3	b	2	3
168	3	b	3	5
169	2	b	1	3
172	3	b	3	4
173	3	b	2	1
178	2	b	1	5
181	2	b	2	5
182	1/2	d	1	3
183	3	b	3	5
184	2/3	d	2	4
193	3	b	3	2
195	3	b	3	3
210	3/4	b	3	3
212	3	b	3	4
216	2/3	b	3	3
222	4	b	2	3
227	3	d	1	4
230	2	b	2	1
237	4	d	2	3
238	2/3	d	3	5
246	2/3		2	1
248	3		2	5
251	1/2	b	2	4
252	3/4	b	1	4
253	2	b	3	1
257	2/3	b	3	3
258	3	d	1	3
267	3/4	d	3	3
270	2/3	d	1	3
276	3	b	3	4
280	3/4	b	3	5

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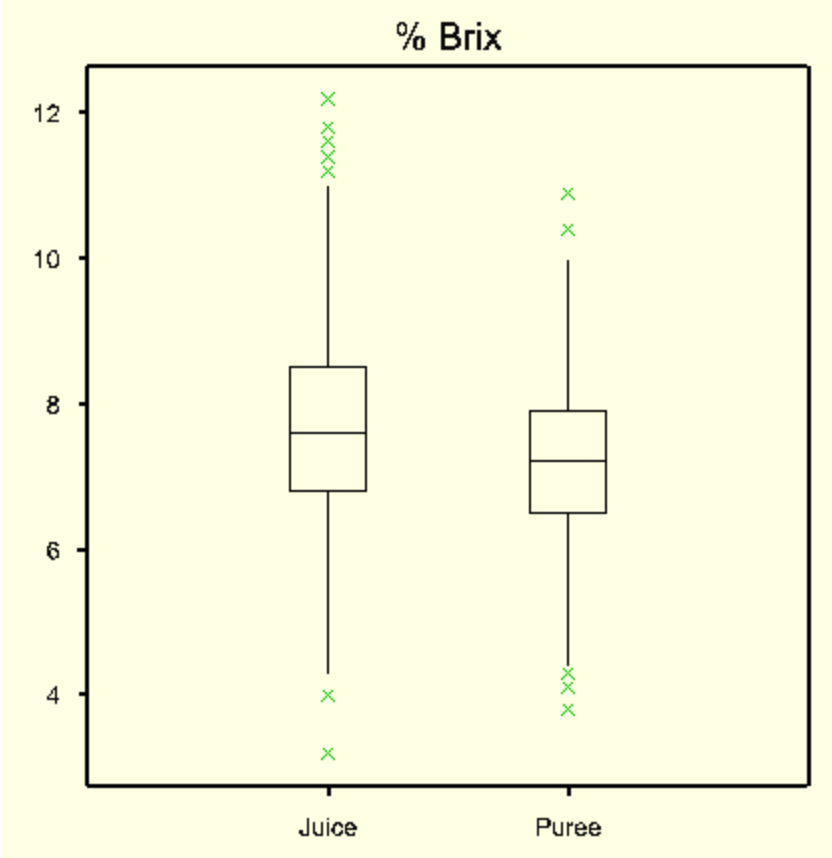

Fig. 1.2.3.7 Graphical representation of %Brix scores from juice or puree.

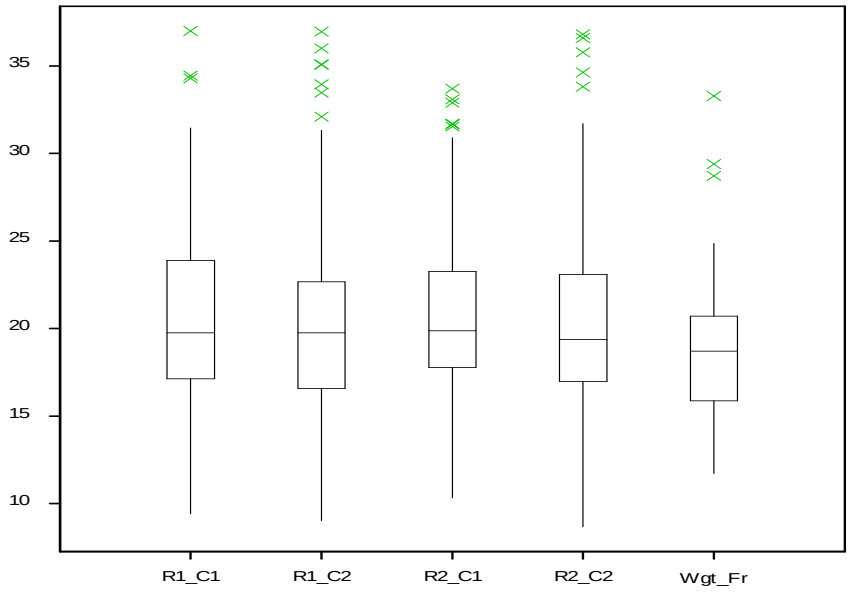

Fig. 1.2.3.8 Box plot showing 10 berry weight comparisons for rep 1 clone 1 and 2 and Rep 2 clone 1 and 2 and frozen fruit.

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Table 1.2.4.1 Measure of visual colour score and Hue (Y parameter) across seasons and sites.

	M24 06	M24 07	P.Th 07	Poly 07	Y M24 06	Y M24 07	Y P.Th 07	Y Poly 07
Latham								
Glen Moy	4	3	3		15.8	14.9	15.7	
R1	3	4		4	14.3	14		12.6
R10	3				15.6			
R102	3	4	5	4	13.4	12.7	13.1	13.4
R105	5	2				13.2		
R11	5	4				15.1		
R110	4	3		4		13.8		14.1
R112	3	2	3	3	15.8	15.2	14.9	15.7
R116	1	2		3	14.3	13.7		14.9
R118	3	3	2	2	16.4	14.4	16.1	16.1
R127	4	2		4	14.5	16.4		15.4
R13	5	3		5	15.1	13.9		13.6
R138	3	1	4	5	14.2	14.3	13.8	14.1
R140	3	2	3	3	14.9	12.6	16.1	15.3
R142	2	3	3	3	16.2	15	15.5	13.6
R145	3		4	5	14.0		13.6	14.1
R146	3	2		4		14.6		13.3
R147	2	2	2	3	16.6	17.5	16.3	15.5
R148	3	3			16.1	14.6		
R149	3	5				12.1		
R150	3		4	3	14.2		12.9	15.5
R158	3	4	2	3	14.8	13.9	16.2	16
R160	5	4	3	5	14.5	15.2	14	13.4
R161	2	1	2	2	16.5	16.6	16.5	15.6
R167	3	2	2	4	15.0	14.7	13.6	13.4
R168	5	2	3	3	15.6	14	13.3	13.9
R169	3	4	3	3	15.2	14	14.1	12.1
R171		5		5	14.0	12.5		14.1
R172	4	2	4	5	16.3	14.9	13.3	14.3
R173	1	2		1	17.8	16.9	16.5	14.9
R178	5	5			14.5	13.1		
R18	2	2	2	3	15.4	16.1	14.6	15.6
R181	5	4	5	5	14.5	13.3	13.5	13.4
R182	3	3				13.9		
R183	5	4	3	4	15.0	14	13.7	10.7
R184	4	4	4	5	14.9	13.7	13.3	13.4
R19	5	5	5	5	13.0	12.9	12.7	12
R193	2	3	2	2	15.9	15.7	15.2	15
R195	3	5		4	14.4	13.1		13.9
R202		1	1	1	16.8	15.7	16.7	20.6

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R21	5	2	2	3	15.8	14.9	15.7	16.1
R210	3	2		4	15.8	15.2		15.8
R212	4	4			14.9	13.5		
R216	3	4		5	14.1	12.4		12
R222	3	3	4	3	14.3	13.6	13	15.4
R227	4	2				14.7		
R228		4				13.9		
R23	5	3		4	16.6	14.3		14.5
R230	1	3				14.7		
R235		3	4	4	12.7	15.2	13.2	14.5
R237	3	1	4	4	16.4	17.1	12.7	13.9
R238	5	3	4	4	15.2	14.6	13.9	14.9
R24	1							
R246	1	2	3	3	15.3	15.8	15.5	14.7
R248	5	4	5	4	14.1	13.5	13.6	14.2
R25								
R251	4	2	3	4	15.4	16.3	14.6	14.9
R252	4	4	2	3	15.1	14.1	13.3	15.2
R253	1	2	1	1	17.3	15.7	17.3	18.4
R257	3	5	5	4	14.5	14.4	13.5	13.5
R258	3	2		3	13.9	14.3		14.2
R267	3	4	2			13.8	15.3	
R270	3		5	5	12.5		12.1	11.6
R276	4			5				10.7
R278		4			15.2	12.8		
R279		3				14.7		
R280	5	5		5	14.6	13.5		13.3
R43	3	5	4	5	13.5	13.8	13.7	14
R44	5	5	4	3	15.1	13.4	12.5	13.7
R48	5	2		3	16.0	16.1		16.8
R53	3	4	5	5	14.3	13.5	12.6	11.3
R55	3	4				13		
R57	3	3		4	16.8	14.5		15.4
R3	3	4		3	15.5	15.2		13.4
R34	5	4	5	4	14.0	12.9	13.2	14
R38	3	2	1	2	16.3	13.2	17.9	15.4
R4	2	3		4	16.1	14.4		14.9
R40		2			15.4	13.8		
R42	3	3	3	3	15.4	12.5	14.2	15.7
R6		2				14.3		
R62	3	3	5	4	13.2	14.2	13	13.1
R64	3	2	4	4	15.4	16	14.6	14.7
R68	4							
R7	3	2	2	3	17.5	16.5	17.1	15.6
R72	5	5	4		14.1	13.4	14.6	
R73	3	2		4	16.5	15.5		12.9
R77		2				16.2		
R82	5	4	4	5	15.1	14.7	14.4	13.7

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R83	5	3	3		15.1	14.3	13.6	
R84	1	2			16.1	13.2		17.7
R85	5				14.5			
R88	4	3	3	4	14.8	13	14.7	13.7
R89	3	2		2	16.5	13.8		15.3
R91	5	4				13.8		
R96	4		5	5	15.3		13.8	14.4
R2		4				13.4		
R5		5				13.1		
R8		5				13.1		
R9		2	2	3	15.7	14.5	15.9	14.7
R12		3				15.2		
R14			2		13.8		14.2	
R15		3	2	3	16.4	14.8	15.7	14.5
R16		5	5	5	14.8	12.5	13	13.9
R17		3			14.3	16.6		
R20		4	5	4	14.0	14.5	14.6	12.7
R22		5	4	4	14.9	11.6	13.2	14.4
R26		3	5	5	14.8	13.5	13.3	11.8
R27		5	3	5	13.8	12.3	13.7	13.4
R29		3		5	13.5	13.4		13.5
R31		2	3	3	14.9	17.1	14.5	13.9
R32		4				13		
R33		4	3	4	14.3	12.9	12.9	13.6
R39		2	4	3	13.3	15	14.6	14.8
R45		4	5	4	14.2	13.1	13.5	13.3
R49		3	4	5	14.1	12.9	12.4	14
R50		3	2	4	15.2	13.5	13.3	13.2
R51		3		4	15.6	13.9		14.2
R52		4		3	14.3	13		15.4
R56		2	2	3	16.8	16	16.4	16
R58		4	5	3	15.1	13.3	13.3	14.8
R59		5	5	4	12.8	13.6	14.8	11.8
R60		3	5	3	14.6	13.4	13.9	14.3
R61		4	5	5	12.9	12.2	12.9	11.9
R66		5	5	5	14.1	14.2	12.9	12
R74		3	3	5	14.9	16.4	13.6	14.2
R76		3	5	5	15.0	13.8	14.5	14.2
R81		3	5	5	16.1	16.2	14.8	14.4
R93		3		2		13.3		16.4
R94			2		13.0		13.6	
R100		2		3	15.5	15.5		17.1
R103		3		4	14.7	13.5		13.7
R104		4	2	5	14.3	13.9	14.5	13.5
R114		3		4		14.7		12.4
R115		2	2	4	15.5	15.1	15.6	14.4
R122		3		5	15.5	13.1		13
R126		5	4	4	14.8	12.8	14.7	14.8

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R131		3		4	14.9	14.3		13
R132		5	4	5	14.4	13.4	13.2	13.1
R133		4	3	4	14.7	14.6	14.6	15.3
R136		4		5	14.9	13.1		12.3
R141		2	3	3	15.5	13.8	14.8	11.2
R143			4	5	13.8		14.6	12.4
R151		2		5	16.7	16.2		14.6
R159		4		5	14.2	13.8		14.8
R162		3	4	4	14.6	15.3	13.3	14.7
R163		4	4	3		13.2	13.1	13.7
R164		2	2	2	15.0	18.7	15.3	13.9
R165		2	3	2		16.8	13.7	15.9
R174		4	3	5	15.2	13.7	13.9	13.3
R175		2	3	2	16.2	17	16.2	16.7
R176		3		4	15.5	14.4		14.6
R179		4	5	5	15.0	13.4	13	13.7
R185		3		4		14		13.5
R186		2	2	2	17.0	16.5	15.2	17.5
R191		3		2	14.6	13.9		16.1
R196		2	3	4	14.7	13.7	13.4	13.8
R199		2	3		15.4	14.9	15	
R200		3	4	4	14.6	14.8	13.5	13.8
R201		2		1	18.0	16.3		20
R206		4	5	4	14.9	14.7	14.3	13.4
R211		3	5	4	15.7	14.8	15.2	14.4
R213		5	3	5	14.7	14.8	13.1	10.7
R214		4	5	4	14.7	13.3	13.8	14.5
R218		2	5	3	14.4	15.1	15	15.7
R221		3		5	13.7	13.7		13.7
R225		4	4	4	14.6	14.5	14.7	13.8
R226								
R231		5	2	3	14.5	12.5	14.7	14.4
R232		3		3	15.4	15.2		15
R234		2	4	3	16.3	16.9	15.4	16.8
R239		4	2	3	15.7	13.6	15.1	15.7
R240		4		3		12.9		12.1
R241		1	1	1	16.8	16.5	15.5	17.1
R242		5				12.7		
R244		2		5	15.2	16.2		13.6
R249		5		2	15.9	13.3		16.4
R254		3		5		16.2		11.5
R259		4			15.8	13.2		
R260		3		3	14.8	13.4		13.7
R261		3		3	15.7	13.7		11.4
R265		3	4	5	14.6	13.4	13.8	13.9
R268				4	15.4			12.5
R277		2		5	13.9	13.7		12.1
R289		2		3	15.1	16.1		14

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R301		3		3		12.4		12.3
R309		1		2		16.8		15.2
R322				3				13.8
R177		4				14.8		
R271		4				12.6		

Table 1.2.4.2 Comparison of hue (Y) across year and sites.

	Hue Field 2006	Hue Field 2007	Poly 2007	P. Th 2007
Mean	15.04*	14.32	14.22	14.30
Minimum	12.50	11.60	10.70	12.10
Maximum	18.00	18.70	20.60	17.90
SD	1.04	1.31	1.62	1.21

* sig different (p<0.001)

Table 1.2.4.3 Brix statistics for the different seasons 2006 and 2007 across sites

Summary statistics	**M24 06**	**M24 07**	**P Th 07**	**Poly 07**
Number of observations	153	177	105	149
Number of missing values	35	11	83	39
Mean	7.606	6.083*	7.846	7.522
Minimum	5.092	3.500	5.400	3.800
Maximum	9.705	9.300	10.800	11.900
Standard deviation	0.781	1.129	1.166	1.126

* sig different (p<0.001)

Table 1.2.4.4. Ten Berry Weight for M24 season 2006 and the three field sites for 2007

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Summary statistics	M24 06	M24 07	P Th 07	Poly 07
Number of observations	153	177	105	149
Number of missing values	35	11	83	39
Mean	20.34	18.85	29.35	34.74
Minimum	11.87	10.75	10.98	15.00
Maximum	34.60	34.46	55.10	75.31
Standard deviation	4.27	4.60	8.12	9.40

Table 1.3.1.1. Ranking of named varieties from sensory panel 2006.

Ranking of Sweetness	Ranking of Sourness	Ranking of Intensity
Glen Moy	Glen Rosa	Glen Ample
Joan J.	Latham	Tulameen
Malling Leo	Tulameen	Joan J.
Glen Ample	Glen Ample	Malling Leo
Tulameen	Glen Moy	Latham
Latham	Malling Leo	Glen Moy
Glen Rosa	Joan J.	Glen Rosa

Table 1.3.1.2. Variability of mean progeny scores from sensory panels 2006 for sweetness, sourness and flavour intensity compared with mean scores for

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parents

	Glen Moy	Latham	Progeny min-max
Sweetness	5.100	2.600	1.600 - 4.600
Sourness	2.400	3.700	1.700 - 5.200
Intensity	4.200	4.300	2.154 – 5.100

Table 1.3.2.1 Sensory and other analysis of fruit from field site in 2006 and 2007

	2006 mean	2007 mean
Sweetness	2.5	2.5
Sourness	3.90	3.98
Flavour Intensity	3.80	3.70
Brix	7.60*	6.08
Weight	20.34	18.85
Colour (Y)	15.04*	14.32

*Significantly different $p<0.001$

Table 1.3.2.2 Concentrations of sugars and acids from progeny in 2006

	Malic acid	Citric acid	Glucose	Fructose
Minimum	0.02	0.015	0.035	0.01
Mean	0.04	0.05	0.28	0.04
Maximum	0.44	0.18	0.82	0.12
SD	0.02	0.02	0.17	0.02

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Table 1.3.2.3 Mean sensory analysis across 3 sites 2007

	Field 2007	Polytunnel 2007	*Commercial 2007 Subset of samples*
Sweetness	2.5 *	3.24	*3.30*
Sourness	3.98*	3.65	*3.85*
Flavour Intensity	3.70*	4.06	*4.28*
Brix	6.08*	7.52	7.84
Weight	18.85*	34.74	29.35
Colour	14.32	14.22	14.30

*Significantly different p<0.001

Table 1.3.2.4 Correlations between traits

	Sweet	Sour	Flav. int	Glucose	Fructose	Citric	Malic	Brix	Weight	Colour
Sweetness	NA	***	***	***	***	*	*	*	*	-
Sourness	***	NA	***	*	*	***	***		-	-
Flavour intensity	***	***	NA	-	-	-	-	*/***	-	-
Glucose	***	*	-	NA	***	***	***	-	-	-
Fructose	***	*	-	***	NA	***	***	-	-	-
Cirtic acid	*	***	-	***	***	NA	***	-	-	-
Malic acid	*	***	-	***	***	***	NA	-	-	-
Brix	*	-	*/***	-	-	-	-	NA	*	*
Weight	*	-	-	-	-	-	-	*	NA	-
Colour	-	-	-	-	-	-	-	*	-	NA

*** highly significant (p<0.001) * weakly significant (p<0.1)

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Table 3.1.1.1. Candidate genes for initial study in raspberry

Gene name	Code	Function
Transporters		
Tonoplast Intrinsic protein	TIP	Influx of water and small proteins
Sucrose Transporter (plasmalemma	STP1	Sucrose uploading from phloem, Storage of soluble solids
Sucrose Transporter (plasmalemma)	STP2	Sucrose uploading from phloem, Storage of soluble solids
Membrane Intrinsic protein	MIP1	Influx of water and small proteins
Membrane Intrinsic protein	MIP2	Influx of water and small proteins
Membrane Intrinsic protein	MIP3	Influx of water and small proteins
Vacuolar H+ pyrophosphatase	Vp1	Transport of organic acid across Tonoplast
Vacuolar H+ pyrophosphatase	Vp2	Transport of organic acid across Tonoplast
Role in sugar metabolism		
Hexokinase 1	HK1	Glycolysis control
Hexokinase 2	HK2	Glycolysis control
Sucrose synthase	Sus 1	Sucrose and hexose synthesis
Vacuolar acid invertase	Ai1	Hydrolysis of sucrose to glucose and fructose
Role in acid biosynthesis		
Malate dehydrogenase	Mdh1	Malic acid synthesis
Citrate synthase	Cs1	Malic acid synthesis
Isocitrate dehydrogenase	Icdh1	Citric acid catabolism
Isocitrate dehydrogenase	Icdh2	Citric acid catabolism
Galactose dehydrogenase	Vit C	ascorbic acid pathway

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Other genes of interest		
Expansin	EXP	Cell expansion
Sorbitol	Sor	Sugar alcohol obtained from reduction of glucose

Table 3.1.1.2. Candidate gene status in *Rubus*

Candidate Gene	**Primers Used**	**PCR Conditions**	**Sequenced?**	**Cloned?**	**Pyro?**	**Mapped?**
Tonoplast Intrinsic Protein (TIP)	RaspTipR / RaspTipL	57ºC 400bp	Y ? 1 SNP	N	Y	Y LG2
Membrane Intrinsic Protein (MIP2)	Mip2-for / Mip2-rev	57ºC 550bp	Y ? 3 SNP	Y	Y	Y LG2
Galactose Dehydrogenase (GalDH)	AY176585R / AY1765852L	57ºC 500bp	Y ? 2 SNP	Y	Y	Y
Vacuolar H+ Pyrophosphatase (VP1)	Vp1-for / Vp1-rev	57ºC 900bp	Y ? SNP	In Progress		
Membrane Intrinsic Protein (MIP3)	Mip3For / Mip3rev	57ºC 750bp	Y ? SNP	In Progress	Primers ordered for 3 ?SNP's	
Hexokinase (HK1)	Hk1-for / Hk1-rev	57ºC 200bp	Y ? 2 SNP	N	Y All C/A	N
Expansin (Exp)	ExpF / ExpR	57ºC 500bp	Y Short, No obv SNP	In Progress		
Vacuolar acid Invertase (AiL)	AiLF / AiLR	57ºC 200bp	Y ? 1 SNP	In Progress		
Sorbitol (Sor)	Sordhdf / Sordhr	57ºC 900bp	Y Short, No obv SNP	In Progress		
Vacuolar H+ ATPase (AtpvA)	Atpva-for / Atpva-rev	57ºC 900bp	Y Short, No obv SNP	In Progress		
Sucrose Transporter (Plasmalemma) (Stp1)	PrupeStp1F / Stp1R	57ºC 200bp				

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Sucrose Transporter (Plasmalemma) (Stp2)	Stp2-for / Stp2-rev	57ºC 200bp	Y Poor quality sequence			
Hexokinase (Hk2)	Hk2-for / Hk2-rev	57ºC 900bp	Y Poor quality sequence			
Citrate Synthase (Cs1)		57ºC 200bp				
Isocitrate Dehydrogenase (Icdh1)	Redesigned Still no Amplification					
Isocitrate Dehydrogenase (Icdh2)	No Conserved regions to redesign primers					
Sucrose Synthase (Sus 1)		52ºC 800bp				
Vacuolar H+ Pyrophosphatase (VP2)	No Conserved regions to redesign primers					
Membrane Intrinsic Protein (MIP1)	MipRc / MipFc	57ºC 600bp				
AT3G06500 Homologue of neutral invertase / β-fructosidase enzyme	6500F / 6500R	57ºC 800bp	Y ? 1 SNP	In Progress	Primers ordered for 1 ?SNP	
AT3G012240 Acid, CW-bound invertase	12240F / 12240R	57ºC 250bp	Y Poor quality sequence	In Progress		
Malate Dehydrogenase (Mdh1)						

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