National Strawberry Varietal Improvement Program - Subtropical Regions

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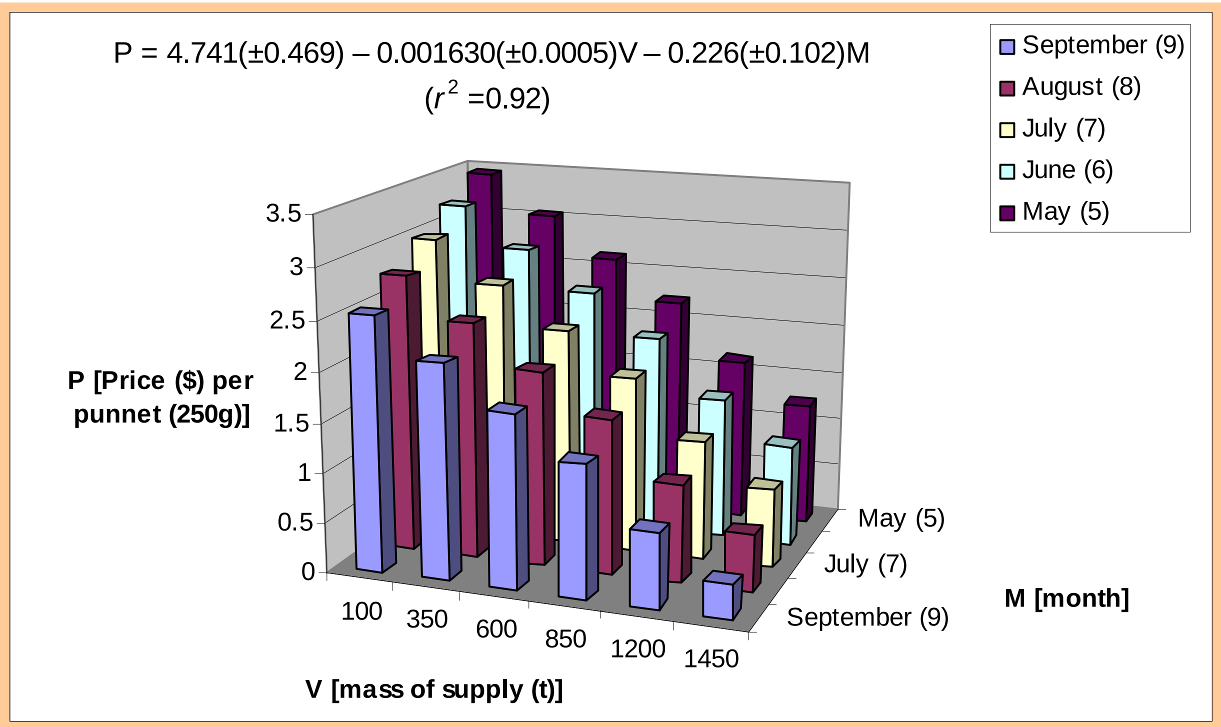

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Purpose of report

This report documents research in subtropical Australia focusing on Queensland to improve the profitability and sustainability of the Australian strawberry industry by producing cultivars with improved production and quality characteristics to reduce costs and to increase demand.

Mark Herrington was project leader.
Mark Herrington, Louella Woolcock and Sam Price conducted experiments on breeding and selection.
Lien Ko coordinated and conducted the experiments on DNA applications.
Michelle Paynter conducted the experiments on Fusarium wilt.

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Industry summary

Strawberries are a delightful dessert fruit that in Australia are produced in the subtropics from late autumn through early spring and in temperate areas during the remainder of the year. The subtropical industry is valued at $160m and focused in Queensland. Previous breeding programs and associated introductions have been pivotal in building an efficient runner supply with increased choices available to fruit growers, because all runner growers to strive to produce high quality planting material. The breeding program was designed to produce cultivars which would comprise ~ 15% of subtropical production with improved production characteristics to reduce costs and high consumer satisfaction to increase demand. The program was guided by the national industry's Strawberry Breeding Steering Committee.

The two promising cultivars released in this project were 'Suncoast Delight' and 'Aussiegem'. They have predicted 'whole of industry' gross margin increases of 10 and 31 % respectively – (*when the latter is planted at higher density [54000plants per ha]).* These complement six earlier releases, including 'DPI Rubygem', which together currently occupy 14.6% of the low chill runners planted in Queensland and contribute ~ $40 m per year to industry i.e. about $280 m over the last 7 years. A breeding line with very high anthocyanin levels was identified and may offer future potential to target health benefits and 'high-health lifestyle' markets.

A number of environmental and genetic factors influence the productivity of strawberry. Productivity can be described as: $Y = fn\ (n, r, x, w, t, a)$. Where Y = yield of fruit per ha (in kg); n = number of crowns per unit area; r = number of trusses per crown; x = number of berries per truss per time; w = mean berry weight (in kg); t = duration of cropping; a = area.

'Festival' is resistant to Fusarium wilt. Inheritance studies indicate that selection for resistance is achievable. Fusarium resistance screening is now routinely implemented in the selection process.

Routine DNA cultivar verification is effective and is now incorporated into the breeding program.

We raised the level quantitative genetic breeding techniques in the program, and better integrated this with economic outcomes through a modified gross margin spreadsheet. This showed a relationship between fruit production and price. National data would be helpful to develop a more 'manufacturing 'approach to production.

The industry has early access to overseas cultivars e.g. 'Fortuna' because of linkages, collaborations and systems developed through the DAFF Qld breeding program. High quality cultivars are available in a range of supply streams so growers can better match runner supply with individual farm needs.

Technical summary

Australia produces approximately 1% of the world's delicious strawberries, with an annual crop (2007/08) of around 58,000 tonnes and a farm-gate value of $A310 million (Strawberries Australia Strategic Plan 2009-2013). The majority of Australia's strawberry production is consumed domestically as fresh fruit.

The breeding programs and associated introductions have been pivotal in building an efficient runner supply with increased choices available to fruit growers because all runner growers strive to produce high quality planting material. To ensure the Australian industry has access to improved, locally-adapted varieties into the future, DAFF, HAL and SAI invested in breeding for targeted (subtropical) environments as part of the national breeding project. The subtropical program aimed to commercialise 2-4 low chill (subtropical) cultivars, improve breeding efficiencies and strengthen international linkages by selection, data analysis and publication. The project reviewed day neutrality and associated traits and productivity, assessed the inclusion of appropriate DNA based technology, evaluated selection for Fusarium wilt, developed a spreadsheet to determine the effects of traits on gross margins and implemented quantitative genetics approaches for a range of traits including resistance to rain damage. For selection trials strawberry clones were planted as 'plugs' 1 to 4 plots of 2 to 6 plants in double rows on raised polythene covered beds at appropriate times: 7 Feb-30 March at Maroochy Research Facility (MRF), and at Bundaberg Research Facility (BRF). Cultural conditions were as per recommendations of Agrilink (1997). Fruit were generally harvested once weekly from April/May through August.

A number of environmental and genetic factors influence the productivity of strawberry. Productivity, in the context of yield components, can be described as: $Y = fn\ (n, r, x, w, t, a)$. Where Y = yield of fruit per ha (in kg); n = number of crowns per unit area; r = number of trusses per crown; x = number of berries per truss; w = mean berry weight (in kg); t = duration of cropping; a = area. Primary components of yield include berry number and berry size. Factors such as planting densities and day neutrality influence productivity through these components.

The genetics of day neutrality is often confounded by the environments where the segregating populations are tested and the evaluation methods used. The genetic control of day neutrality is likely quantitative and complex, and so should be evaluated in the environments of relevance. Chilling requirement and temperature tolerance may be important characteristics to investigate. Any DNA marker techniques for day neutrality would likely require QTL analyses, but with only marginal gains to be made this does not appear promising at present.

A series of 14 strawberry leaf samples from plants grown at MRF could not be correctly matched for day neutrality by markers developed for southern genotypes by AgGenomics probably because these markers appear to be population specific and other target traits may be more suited to marker assisted selection. Initial issues with DNA extraction from MRF material were overcome but similar issues in extraction and restriction enzyme action were also apparent in some of 32 cultivars under the DArT system e.g. 'Earlisweet' completely failed to be digested. Further work is warranted if the system is to be implemented in more detail.

Losses caused by Fusarium wilt are expected to increase and are already high in Western Australia (WA). We tested genetic similarity between isolates from WA and Queensland using Vegetative Compatibility Group (VCG) based on nitrate non-utilising mutants and found a high level of variation. Isolates from WA belonged to one VCG, while most other isolates belonged to single-member VCG's. This may complicate selection for resistance should a differential response among 'resistant' genotypes occur. Inoculum concentration of 10^5 spores/ml was adequate to induce symptoms. 'Kabarla' is highly susceptible and 'Albion' moderately susceptible. 'Sugarbaby' and 'Camarosa' showed slight susceptibility to only one isolate. 'Festival' was resistant to all isolates tested. Narrow sense heritability calculated from the partial diallel crosses was moderate (0.39) and so the population should be responsive to phenotypic recurrent selection. Some predicted breeding values for progeny (278) were better than any of the parents. Fusarium resistance screening is now routinely implemented.

We raised the level quantitative genetic breeding techniques in the program, and better integrated this with economic outcomes. This involved creating a database in Katmandoo from historical clonal data 2004-9, including extensive pedigree information. The unbalanced data was analysed under ASREML for variances, heritabilities and breeding values. Narrow-sense heritability of resistance to rain damage calculated for seedlings was low (0.21 ± 0.15) and not significantly different from zero; however, broad-sense heritability estimates were moderate in both seedlings (0.49 ± 0.16) and clones (0.45 ± 0.08) from the first population and similar in clones (0.56 ± 0.21) from the second population. Immersion of fruit in deionised water produced symptoms consistent with rain damage in the field ($r^2 = 0.90$) and could be used in selection for resistance to rain damage.

We developed methods to assess the economic impact of changes to the production system, and identify plant traits that would influence outcomes from the system.
From May through September *P* (price; $\$\cdot\text{punnet}^{-1}$), *V* (monthly mass; tonne of fruit on the market) and *M* (calendar month; i.e. May = 5) were found to be related ($r^2 = 0.92$) using data from 2006 to 2010 for the Brisbane central market. Increasing yield increased the gross margin to a maximum. A redistribution of 10% of total yield from September production to May production increased the gross margin by 23%. Increasing fruit size increased gross margin: a 75% increase in fruit size (to ≈ 30 g) produced a 22% increase in the gross margin. The modified gross margin analysis developed in this study allowed simultaneous estimation of the gross margin for the producer and gross value of the industry.

We used the results of multiple years data (2004-2009) generated in the normal course of the evaluation of early clonal selection. Narrow-sense heritabilities for traits ranged from 0.11 to 0.78 so the population should respond well to selection. Additive genetic correlations were abundant but the only two greater than 0.7 i.e. between total and early yield and between resistance to bruising and abrasion were positive, while the highest negative correlation (r=-0.65) was between acidity and flavour. A breeding line with very high anthocyanin levels was identified and the potential offered to target health benefits and 'high-health lifestyle' markets should be investigated.

'Suncoast Delight' and 'Aussiegem' were released with predicted 'whole of industry gross margin' increases of 10 and 31 % respectively – (*when the latter is planted at higher density [54000plants per ha]).* These complement six earlier releases that together contribute $41 million per year to industry value (i.e. $280 m over last 7 years) and comprise 14.6% of the 'low chill' plantings in Queensland.

Contents

1.0.0. General Introduction

An economic evaluation of the Australian strawberry breeding program was commissioned by Horticulture Australia Limited (HAL) in early 2008. The report was a comprehensive review of the previous program's success with some key issues stemming from it, namely;

- · Key issues for strawberry breeding include competition amongst runner suppliers and whether foreign breeding programs can appropriately support the Australian industry. Only 10% of all strawberry varieties planted in Australia is derived from Australian breeding programs.
- · If adoption of improved Australian varieties could be increased to 15%, cost-benefit analysis indicates the breeding program would generate positive net economic benefits.
- · A number of risks including access to genetic material, potential productivity and quality superiority of Australian varieties and uptake amongst runner suppliers hinder economic benefits being realised.

As a consequence of the findings of this report, a National Strawberry Varietal Improvement Proposal was developed by Strawberries Australia Incorporated (SAI) in consultation with the Strawberry Industry Advisory Committee, DAFF and HAL. The objectives of the plan were to:

> "Develop a strawberry varietal improvement program focused on;
> (i) maximizing access to superior varietal strawberry germplasm from overseas sources under commercial arrangements favourable to the Australian Industry and
> (ii) maximizing uptake by Australian strawberry growers of Australian-bred varieties."

The review sought to resolve the various issues identified for a strong return on investment in this vital area of R&D for the strawberry industry. All alternatives were investigated as part of the proposal preparation phase and run through a number of key decision making processes including identifying risks and barriers to success, future review and evaluation, key determinants for a successful varietal improvement program and strategies for achieving success. The resulting proposal was deemed the most appropriate model to take the industry into the future with the highest likelihood of succeeding and ultimately returning in excess of the 15% adoption targets as outlined by the economic evaluation of the Australian strawberry breeding program for generating positive net economic benefits.

Strawberry Industry Strategic Plan 2009-2013 indicates Australia had 2007/2008 and annual crop of around 58,000 tonnes with a farm-gate value of $310 million. The strawberry industry summary (from project proposal) developed in the HAL commissioned review included: that Australia produces approximately 1% of the world's strawberries. The majority of Australia's strawberry production is consumed domestically as fresh fruit.

The breeding programs have had a very significant impact on the industry and have contributed in no small way to the outstanding industry growth achieved over the past decade. For example, the Queensland industry has grown from $8 million in 1991 to over $120 million in 2006. Similarly, strawberry production in Victoria has increased from 8,000 tonnes in 1987 to 15,000 tonnes in 2006 with a current farm gate value of $65 million. At the same time the industry in that state has become more efficient as this production is derived from less area (300 ha in 2006, compared to 400 ha in 1987) and by less growers (110 in 2006 compared with 400 in 1987).

The breeding programs have been pivotal in building a viable runner supply program in Queensland which increases choices available to fruit growers. Currently Queensland runner growers produce more than 20 million runners in a national market of ~72 million plants with about 32 - 36 million in Queensland alone (Strawberry Industry Strategic Plan 2009-2013). This has encouraged all runner growers to strive to produce high quality planting material.

Development of new strawberry varieties with improved flavour and quality is a key issue for industry viability. To ensure the Australian industry has access to improved, locally-adapted varieties into the future, it was proposed that DAFF continues to invest in breeding for targeted environments (subtropical) as its contribution to the national breeding project.

Consequently a program was initiated within the context of improved breeding efficiencies and strengthening international linkages to:

- Commercialise 2-4 (short day low chill) varieties with high consumer appeal and outstanding agronomic characteristics, which capture at least 15% of the total Queensland/NSW market by 2012 and 20% of the total Queensland/NSW market by 2015.

- Commercialise one variety with high consumer appeal and outstanding agronomic characteristics for the northern WA (Perth) growing districts.

Additionally the program was designed to provide intellectual input for the efficient conduct of strawberry breeding programs, firstly by a continual assessment process and secondly by further fostering international collaboration by scientific publication on relevant topics related to project needs.

The continual assessment process within the breeding and selection techniques was designed to include:- A review on yield, day neutrality and associated traits and productivity: Assessment and inclusion of appropriate DNA based technology (including appraisal of marker technology): Evaluation and selection for specific disease tolerances: Implementation of quantitative genetics approaches including; prepare data base and populate with historical data sets for analysis; integrate into parental selection, seed production, and progeny selection: Preparation of advanced clones for commercialisation (virus indexing etc).

The proposed publication schedule was integrated into the other outputs and included:

- ‘The incidence, costs and genetic parameters associated with rain damage on strawberries grown in south-east Queensland.’

- 'The influence of plant and fruit traits on the costs associated with production of strawberries grown in south-east Queensland.'
- 'The genetic variances and correlations and heritabilities associated with economically important plant and fruit traits of strawberries grown in south-east Queensland.'
- 'The definition and validation of a multi-trait selection procedure for strawberries grown in south-east Queensland'.

The features of the research are reported here.

2.0.0. Experimental program

2.1.0. General materials and methods - field culture

For selection trials strawberries were planted at appropriate times at Maroochy Research Facility (MRF), and at Bundaberg Research Facility (BRF). Cultivars and breeding lines were planted 28 February to 23 March at MRF. Seedlings were planted between 7 February and 24 March at MRF, and between 23 February and 30 March at BRF. MRF is located at Nambour 27^{o}S, 153^{o}E near sea level (33m), 15km from the coast. The site slopes north and soil is a grey loam. BRF is located at Bundaberg 25^{o}S, 152^{o}E near sea level (23m), 15km from the coast. The soil is a Euchrozem, (red loam) and the area has never been fumigated and is essentially flat (less than 1% and facing NE). The MRF trial breeding selection area had not been fumigated for at least 4 years prior to planting. Several disease organisms are known to commonly occur in the MRF field because *Fusarium* spp., *Colletotrichum gloeosporioides* and *Macrophomina phaseolina* have previously and regularly been isolated from wilting plants in the MRF selection fields.

Runners for clonal trials were produced from potted plants. Individual mother plants were dug from the field early in the spring (October) and established in 10 L pots in a screen house. Runners produced on these plants over summer were positioned into steam sterilised 'Vitality Premium Hi-Draw 90/10' Power-Blend® mix (sourced from Growing Media Queensland Pty Ltd) in 'plugs' in Bowman® 60 cell trays , established and grown attached to mother plants until approx two weeks prior to field planting. Seed were germinated in peat/vermiculite mix, then at about 6 weeks after incubation seedlings were transplanted to 'plugs' as with clones.

Plants were grown in double rows on raised black (MRF) or white (BRF) polythene covered beds with 1 to 4 plots of 2 to 6 plants each set in randomised complete block designs. Each plot comprised two rows per bed with 35-50 cm between plants within a row and 26-35 cm between rows within a bed. Bed centres were 1.25-1.5 m apart and bed tops were approximately 70 cm wide. Inter-bed walkways were mulched with pine-flake, sugarcane mulch or civic compost at MRF but not mulched at BRF.

Basic fertiliser rates and crop maintenance schedules were based on commercial soil analysis - dolomite was broadcast and incorporated before the beds were formed, the remainder was applied during bed formation. Plants were subsequently fertilised primarily through the trickle irrigation system with occasional foliar applications. Irrigation was as required by Enviroscan. Bare rooted runners from MRF or Stanthorpe were initially established using over-head or mini-sprinklers. Pest and disease control was based on recommendations given by commercial integrated pest management consultants following fortnightly assessments. Predatory mites were used for two-spotted mite control.

Fruit were generally harvested once weekly from April/May through August and variably into early September, weighed and counted. The standard cultivars used for comparison were usually Festival and Rubygem.
Data were analysed by analysis of variance using GenStat 11th Edition(Lawes Agricultural Trust, Rothamsted, UK) and or ASReml (Release 2.0. VSN International, Hemel Hempstead, HP1 1ES, UK, Hampstead).

2.2.0 Breeding and selection techniques - Reflective assessments

The continual assessment process within the breeding and selection techniques included development and reflection on the following aspects:
2.2.1. A review on yield, day neutrality and associated traits
2.2.2. Assessment and inclusion of appropriate DNA based technology (including appraisal of marker technology),
2.2.3. Evaluation and selection for specific disease tolerances,
2.2.4. Implementation of quantitative genetics approaches: prepare data base and populate with historical data sets for analysis; review productivity data; integrate into parental selection, seed production, and progeny selection.
2.2.5. Cultivar development and release: Preparation of advanced clones for commercialisation (virus indexing etc),

2.2.1. Review on yield, day neutrality and associated characteristics *(given as part of milestone 106 [April 2010)])*

A number of environmental and genetic factors influence the productivity of strawberry. Productivity, in the context of yield components, can be described as: Yield = *fn* (n, r, x, w, t, a). Where Y = yield of fruit per ha (in kg); n = number of crowns per unit area; r = number of trusses per crown; x = number of berries per truss; w = mean berry weight (in kg); t = duration of cropping; a = area. Primary components of yield include berry number and berry size. Factors such as planting densities and day neutrality influence productivity through these components. Day length interacting with temperature has traditionally been considered a major determinate of environmental adaptation. The introduction of the day neutral characteristic has changed the capability of production timing but its genetics is poorly understood. This brief review considers day neutrality (DN), and to a lesser extent, some associated characteristics impacting on strawberry production.

Chilling

Cultivars differ substantially in their chilling requirement, which is the cumulative hours below a threshold temperature, to satisfy their 'rest periods' (Bigey 2002; Hancock *et al.* 1996; Lieten 2006; Yanagi and Oda 1993). Some effects of inadequate chilling, such as lack of vegetative growth, poor pollen quality and misshapen fruit, can be alleviated by night-break lighting of 8-9 illumination episodes of 15 minutes each (Bigey 2002; Lieten 2006). While no studies of the inheritance of chilling requirements have been published, genetic control is apparent because cultivars that originate in and are adapted to warm areas such as southern USA, the Mediterranean region, and Africa have the shortest rest periods (lowest chilling requirement) between annual crops and can grow and ripen fruit during the short days of winter (Hancock *et al.* 1996). Such cultivars include 'Missionary', 'Florida Ninety', 'Tioga', 'Selva', 'Pajaro' and more recently 'Sweet Charlie' and 'Camarosa' (Bigey 2002). *F. chiloensis* was an important contributor to the low chill requirement in Californian cultivars with short rest periods (Hancock *et al.* 1996), while 'Missionary' contributed to the trait in Florida and Queensland cultivars, e.g. 'Florida Ninety' resulted from a back-cross to 'Missionary' (Darrow 1966), and in Queensland 'Missionary' was a parent of 'Redlands Crimson'.

Temperature tolerance

Species and cultivars also differ in their tolerance of high temperature (Hancock *et al.* 1996). Selected clones of *F. virginiana* were found to maintain photosynthetic capability at high ($30^o/25^oC$ day/night) temperatures while other *F virginiana, F. ananassa* and *F. chiloensis* did not (Serce *et al.* 2002). No study of the inheritance of high temperature tolerance has been found but genetic control is apparent because cultivars such as 'Missionary', 'Blackmore' and 'Pocahontas' possess the trait and which both 'Blackmore' and 'Pocahontas' have 'Missionary' in their ancestry (Darrow 1966). These cultivars originated in and are adapted to warm areas of southern USA. In a study they were found to be more heat tolerant than many other *F x ananassa* cultivars, with the 'Missionary', 'Blackmore' having tolerance levels similar to *F. virginiana* accessions (Darrow 1966). Abdelrahman (1985) found 'Raritan' was less sensitive to $40^o/30^oC$ day/night than two other cultivars in the trial.

Day length response (Day Neutrality)

Strawberry genotypes are often classified according to their flowering response to day-length, as short-day (SD), day-neutral (everbearing; DN), and long day plants (Hancock 1999). In addition flowering is significantly influenced by temperature and by the interaction between temperature and photoperiod in *F. ananassa* (Battey *et al.* 1998) and also in *F. virginiana* and *F. vesca* (Sonsteby and Heide 2008a; b). The genotype x environment interaction influences the earliness of the start and the lateness of the finish of harvest. Therefore yield and its distribution, together with temporal price structures may have a profound effect on the economics of production. Shaw and Larson (2005) evaluated seedling populations for yield distribution for 3 years in California to determine genetic parameters. They found narrow sense heritability ranged from 0 to 0.24 for early season yield, while for the proportion of an individual clone's total yield expressed early heritability ranged from 0 to 0.53.

Most reports of early yield are made in the context of temperate fruit production and there are no reports in the literature found so far on the genetic control of flowering in a subtropical environment, although some researchers class southern California as warm (Bringhurst and Voth 1975) and perhaps subtropical (Hancock 1999). However the influence of both temperature and day-length could be expected to create a quantitative genetic response for early yield and early flowering. Powers (1945) found the time of flowering in F_1 strawberries was nearly as early as the early parent and earliness was partially dominant. This is consistent with work of (Scott *et al.* 1972). Barritt *et al.* (1982) found that the genetic inheritance parameter 'general combining ability' (gca) was more important than 'specific combining ability' (sca) for early flowering in populations produced by crossing day-neutral and short-day plants. Crosses that produced the highest number of day-neutral progeny also produced the highest proportion of early flowering plants.

Day-neutrality is conferred by a single recessive gene in the ever-bearing cultivars 'Baron Solemacher' and 'White Bush' of the diploid *F. vesca* (Brown and Wareing 1965). Furthermore, molecular markers have been found (Cekic *et al.* 2001) that are closely linked (SCAR flanking 1.7 and 3.0 cM) to the seasonal flowering locus in *F. vesca* (Albani *et al.* 2004). However according to Weebadde *et al.* (2008) the marker of Cekic *et al.* (2001) did not segregate in *F.* x *ananassa* while the testing in *F.* x *ananassa* of the SCAR markers of Albani *et al.* (2004) is yet to be finalised. In Australia AgGenomics Pty Ltd, a subsidiary of Genetic Technologies Limited, established a collaborative program with HAL to develop genetic markers to identify strawberry plants with the day-night neutral characteristic (Genetic Technologies Limited Annual Report 2005, p.11).

The genetics of day-neutral flowering in the octoploid *Fragaria* x *ananassa* are still being elucidated (Luby *et al.* 2008) despite the fact that the day-length response, especially day-neutrality (ever-bearing) trait, has been the subject of many experiments and reviews with varying conclusions regarding its genetic control. For example Hancock (1999) cites 9 studies from 1919 to 1991 in which the mechanism of control ranges from single recessive (Darrow 1937) to single dominant (Ahmadi *et al.* 1990). There were two complementary dominant genes at three loci and four recessives reported (Powers 1954) as well as other options. Hancock (1999) offered several possible explanations for the differences in results among the studies, including: a) there may be multiple sources of day-neutrality now operating in octoploid breeding populations; b) the genetic background of individual breeding populations may be variable enough to influence the expression of day-neutrality; and c) many ever-bearing clones of strawberry may be misclassified as short-day plants, because many day-neutral individuals do not express the ever-bearing trait until the second year (Barritt *et al.* 1982). Hancock (1999) considered it most appropriate to view the inheritance of day-neutrality (ever-bearing ability) as a quantitative trait controlled by a few major genes and many minor ones. This was consistent with the fact that at the time of writing in 1999, no homozygous ever-bearing genotypes of *F.* x *ananassa* had been reported (Hancock 1999).

Hancock *et al.* (2002) and other authors noted that the environments where the segregating populations were tested and the evaluation methods used can significantly affect the expression (detection) of day-neutrality (Hancock *et al.* 2002; Serce and Hancock 2005). Hot days appear to inhibit flower bud formation in most day-neutral types (Durner *et al.* 1984). It is important to note, however, that the original source of Californian day-neutrality *F. virginiana* ssp. *glauca* was not day-neutral *per se* but continued flowering through the long days once triggered by short days. Bringhurst and Voth (1976) suggested that the mechanisms which normally delay flowering in *F. virginiana* are confounded in those backgrounds of *F.* x *ananassa* which lack a rest requirement and have a low chill requirement, thus resulting in the expression of day-neutrality. Sugimoto *et al.* (2005) used a Japanese breeding population derived from the Watsatch source of day-neutrality, from which the California DN is derived. They found a RAPD-marker linked to and flanking (11.8 and15.8 cM) the dominant gene regulating day-neutrality. This is consistent with the single dominant gene theory proposed for this source (Bringhurst and Voth 1976).

In a project to reconstitute *F.* x *ananassa* on a wider germplasm base, Luby *et al.* (2008) found gca and sca effects differed with location. They were significant at Minnesota where gca and sca variances (with % of total variance) were 2.6 (13.5%) and 1.8 (9%) for spring bloom, and 18 (7%) and 37 (14%) for percentage of progeny expressing day-neutrality. By contrast in Ontario, one third of the parental genotypes had no individual progeny expressing day-neutrality. The relatively high sca:gca ratio (2:1) for the percentage of progeny expressing day-neutrality at Minnesota is consistent with other studies suggesting day-neutral flowering in octoploid strawberries is under polygenic control (Hancock *et al.* 2002; Serce and Hancock 2005) and that some short-day *F.* x *ananassa* carry some of the genes required for day-length insensitivity (Luby *et al.* 2008). Luby *et al.* (2008) found results consistent with Barritt *et al.* (1982) that populations of plants that flowered early in spring tended to have a significantly higher proportion of day-neutral plants.

Weebadde *et al.* (2008) used linkage mapping and a QTL approach on seedlings of ‘Tribute’(DN) x ‘Honeoye’(SD), to determine the number of loci regulating day-neutrality. This cross was found in an earlier study by Serce and Hancock (2005) to segregate 1:1 DN:SD. Weebadde *et al.* (2008) generated a map using AFLP then phenotyped replicate plants at five different locations in the USA (California, Minnesota, Michigan, Maryland and Oregon). They found significant genotype x environment interaction and speculated that different loci regulate day-neutrality in various areas due to climatic variation noting that when temperatures are below 15°C all genotypes then seem to behave in a photoperiod insensitive manner (Darrow 1966; Hancock 1999), while when temperatures are above 26°C flowering is inhibited regardless of the photoperiod (Durner *et al.* 1984). Weebadde *et al.* (2008) concluded that day-neutrality was likely inherited polygenically as eight QTL were identified that were either shared among locations or were location specific and none of the QTL explained more than 36% of the phenotypic variation. Weebadde *et al.* (2008) suggest that 'what is described as day-neutral flowering pattern in strawberries is better defined as remontancy where multiple genes influence repeat flowering in addition to those regulating photoperiod insensitivity.' These reports indicate that the time when flowering commences and ends is under complex quantitative genetic control and so affects the duration of production and consequently yield.

The node of flowering and the inflorescence structure described in the flowering diagram in the report on *Fragaria vesca* by (Brown and Wareing 1965) show marked similarities to these attributes in tomato, where the 'sp' gene of tomato confers determinate flowering. The 'sp' gene of tomato (Pnueli *et al.* 1998) is an ortholog to the CENTRORADIALIS and TERMINALFLOWER1 genes which maintain an indeterminate state of inflorescence meristems in *Antirrhinum* and *Arabidopsis*, respectively. According to K. Folta (pers com) from University of Florida the diploid strawberry appears to have elements of a sequence consistent with TERMINALFLOWER1 of Arabidopsis, but further work would be required to explore this possibility. Since this/these genes are thought of as the "integrators" and not the DN sensor-signal conduit *per se*, one would expect them to be just pawns of the upstream processes, but one could imagine that day neutrality could come from a loss or gain of function in these genes that would divorce them from the upstream controls (K. Folta, pers com). This area could be investigated further.

Conclusion

The genetics of day neutrality is often confounded by the environments where the segregating populations are tested and the evaluation methods used. Because a number of processes are involved in the expression of day neutrality, the genetic control of day neutrality is likely quantitative and complex, and so should be evaluated in the environments of relevance. Any DNA marker techniques for day neutrality would likely require QTL analyses, but with only marginal gains to be made this does not appear promising at present. By contrast, much initial progress may be made by exploring the occurrence of flowering orthologs in the diploid *F. vesca* with a 'desktop analysis', and then looking for their presence in the *F. x ananassa*. Chilling requirement and temperature tolerance may also be important characteristics to investigate or account for when increasing the adaptability of strawberry and ensuring a consistency of expression of day neutrality.

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2.2.2. Assessment and inclusion of appropriate DNA based technology (including appraisal of marker technology)

This aspect was approached from three directions:
2.2.2.1. Assessment of capacity to identify day neutrality in sample via the provider AgGenomics.
2.2.2.2. Collaborative genotyping of sample population through DArT project.
2.2.2.3. Implementation of cultivar/breeding line identification through commercial laboratory.
Additionally an overall discussion is given in:
2.2.2.4. General discussion

2.2.2.1. Assessment of capacity to identify day neutrality in sample via AgGenomics.

Materials and methods
A series of strawberry leaf samples (14 genotypes in 2009 following the 23 sent in late 2007) collected under standard protocols from plants grown in field and glasshouse at Maroochy Research Facility were sent to AgGenomics for DNA extraction, DNA-based cultivar identification and classification of day neutral response based on protocols they had developed. The 'blind' cultivar samples (BC) comprised six cultivars sent to AgGenomics for clonal sample-matching. The 'blind' day length samples (BN) representing 'day neutral' types included a range of day-neutral classifications and origins e.g. 'Tribute' from north East USA, 'Selva' from California, 'Earliblush' from Queensland program and 'Lowanna' from Victorian program as well as short day cultivars including 'Earlisweet' and 'Festival'.

Results and discussion
Although initially there had been major issues reported with DNA extraction from plant material grown in Queensland, in the final tests all pairs of the 'blind' cultivar 'BC' samples were correctly matched. This indicates that local technology and infrastructure are capable of identifying and matching to reference material. However, the available day neutral markers developed by AgGenomics for southern Australian cultivars appear to be population specific because the markers did not successfully validate day neutrality in the 'BN' test lines sent for analysis. Additionally AgGenomics deregistered itself in June 2012 and so no further work can be expected to come from that local technology. Nevertheless technology in this marker area is developing rapidly and may be available from other providers. Additionally other target traits (e.g. disease resistances or fruit traits such as firmness) may be more readily suited than day neutrality to marker assisted selection.

2.2.2.2. Collaborative genotyping of sample population through DArT project

Materials and methods
We collaborated in a consortium coordinated through DArT (Diversity Array Technologies in Canberra, www.diversityarrays.com) with other lead strawberry researchers in France (Dr. Beatrice Denoyes-Rothan: INRA, UREF, Villenave d'Ornon) and Spain (Dr Iraida Amaya: IFAPA-Centro de Churriana, Málaga) with the aim to expand the knowledge of the genetics and potential markers in strawberry populations. This involved sampling leaves of 32 genotypes under standard procedures including freeze dried tissue, which were despatched to

DArT who extracted DNA and performed microarray analysis and on occasions sent a sub-sample of DNA to France for assessment by their techniques (microsatellite technology).

Results and Discussion

Again, there were initial major issues reported with DNA extraction from plant material grown in Queensland. Other researchers (e.g. pers. com. Dr. Beatrice Denoyes-Rothan) have also noted that some genotypes presented difficulties with extraction and digestion. Subsequent modifications to extraction protocol improved recovery with 521 of 776 markers having P values greater than 90 but discordant values of 10 or more occurred with seven of the cultivars and one cultivar, 'Earlisweet', completely failed to be digested by the restriction enzymes. Principal Component Analysis of the SSR pattern reported from Spain using 7 SSR markers from different linkage groups positioned the Queensland material with cultivars from USA and modern Europe (post 1970) rather than with 'old European' cultivars. DArT found the Queensland material difficult to work with e.g. indicating that '..out of 70 organisms for which we have developed our technology…. some RE inhibitor in strawberry leaves spoiled this effort…'. This suggests further work is warranted if the system is to be implemented in more detail.

With the new understanding of the limitation that 'some genotypes are difficult or impossible to digest' one can adjust expectations of the level of outcomes and further extractions, digestions and DArT analyses may still be warranted.

Our initial analysis using the suite of markers and genotypes available in the population of the May 2010 DArT data did not identify any potential marker for day-neutrality. Further interrogation of the data with the deletion of 'Earliblush', 'Rubygem' and 'Sweet Charlie' from the data set (because of apparent anomalies found from Spanish data) may be warranted. Moreover through other sections of this project we now have additional phenotypic data, including data on quantitative traits suitable for QTL (quantitative trait loci) determination, which could be included in more detailed analyses but is beyond the scope of the present project.

2.2.2.3. Implementation of cultivar/breeding line identification through commercial laboratory

Materials and methods

The prime need for cultivar release and commercial production of advanced genotypes is to ensure trueness to type. The third approach therefore was to link into systems currently using DNA technology, in the basic form, for cultivar identification. Therefore we linked into developments in project BS07018 (Variety Quality Management in Strawberry, using DNA genotyping as a Tool) which had identified an appropriate laboratory – i.e. established protocols based on microsatellite technology, reliable results, technical expertise, cost effective and approachable, and which had a sufficiently large and expanding data base to allow discrimination among cultivars now and into the future.

Results and Discussion

We used the laboratory identified in project BS07018 which was: California Seed & Plant Lab., Inc. (Contact Details: : Sukhi Pannu, Director, Testing Services, California Seed & Plant Lab., Inc., 7877 Pleasant Grove Rd, Elverta, CA 95626. Lab: 916-655-1581 - Mobile: 916-204-8329 - Fax: 916-655-1582 . Email : spannu@calspl.com . www.calspl.com). Freeze dried samples of advanced breeding lines are now routinely prepared and sent to the lab to produce a reference data set that is then available for comparison through commercial production systems. This has led to the timely identification of at least one situation where the 'wrong' cultivar appears to have been supplied from an overseas program. The early recognition likely saved industry from the potential consequences of the mix-up.

2.2.2.4. General discussion on DNA aspects

Routine cultivar identification is effective and is now incorporated into the breeding program. That some genotypes are difficult or impossible to digest must be accounted for in future DNA related work. Further extractions, digestions and DArT or other systems analyses are still warranted. As well, a first step may be to link the additional phenotypic data, including data on quantitative traits suitable for QTL as developed in this project with the DArT or SSR set available. The progress in this project puts us in a good position to be aware of developments and apply them in a cost effective manner as they are recognised as useful.

2.2.3. Evaluation and selection for specific disease tolerances

2.2.3.1. Fusarium resistance in strawberry

Among the diseases expected to constrain economic strawberry production in Australia, especially in 'warmer' areas, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *fragaria* (*Fof*) is considered likely to be considered the major disease (D Hutton pers. com). Losses are expected to increase as effective fumigation is reduced. Increases in disease outbreaks of the pathogen especially in Western Australia have already occurred. The search for alternative disease management strategies places emphasises on the development of resistant cultivars. The breeding program therefore aims to incorporate resistance into the program. This requires knowledge of the disease, its variation, sources of genetic resistance and knowledge of genetic parameters that will aid efficient deployment of resistance into commercial genotypes.

Currently we do not know the diversity of *Fof* in Australia. Information gained though the study of Vegetative Compatibility Groups (VCG) is useful for determining genetic relatedness of *Fusarium* isolates and to give a better understanding of population origin and distribution. VCGs have been used to study populations of many *Fusarium* species and can decipher if it is sexually and DNA compatible, and give a good understanding of the pathogenic and genetic variation within and among formae speciales of *F. oxysporum*.

Aspects of this work are reported here and will be discussed in more detail in papers currently in the 'draft' stage and anticipated to be published within the next 12 months.

The program determined:

- The diversity of *Fusarium oxysporum* in Australia using VCG techniques

- The pathogenicity of eight *F. oxysporum* strains and the effect of inoculation methods and inoculation concentration with *Fof* isolates on strawberry (to determine pathogenicity, variability, and suitability for screenings)

- The variation of susceptibility to Fusarium wilt in strawberry germplasm, selections with high resistance to *Fof* and associated genetic parameters including heritability and breeding values for resistance.

2.2.3.2. The diversity of *Fusarium oxysporum* in Australia using VCG techniques

Materials and methods

To test genetic similarity between isolates, nitrate utilising mutants (*nit* mutants) of *F. oxysporum* were generated and paired to verify VCGs. This is determined by the isolate's ability to form a prototrophic heterokaryon when grown on a minimal medium with $NaNO_3$ as the sole nitrogen source. Heterokaryon formation represents two isolates belonging to the same VCG and able to exchange genetic material. If isolates differ at a locus of alleles governing vegetative incompatibility they are vegetatively incompatible and unable to form a stable heterokaryon. Isolates belonging to the same VCG are considered to be clones or closely related strains descended from a common ancestor. VCGs are also informative in differentiating between pathogenic from non-pathogenic strains, although a VCG can be associated with non-pathogenic isolates.

Results and discussion

Most of the isolates paired belonged to single-member VCGs, with the exception of isolates from Western Australia which all belonged to the one VCG, and several isolates from the Sunshine Coast region (Queensland) that shared the same VCG (but different from the WA VCG). Our results indicate a high level of variation associated with *Fusarium* species isolates in Australia. This may complicate selection for resistance if the isolates show a differential response among 'resistant' plants.

2.2.3.3. The pathogenicity of eight *Fusarium oxysporum* strains and the effect of inoculation methods and inoculation concentration with *Fof* isolates on strawberry (to determine pathogenicity, variability, and suitability for screenings)

Materials and methods

In order to determine a reliable *Fof* screening protocol glasshouse trials were undertaken at MRF in June 2010. These trials aimed firstly to provide data on cultivar performance with regard to resistance/susceptibility using current popular strawberry cultivars and secondly to determine best inoculation treatments.

The first trial tested strawberry cultivars to verify germplasm responses to two highly pathogenic isolates (N18553 & N18549). The chosen cultivars included: 'Kabarla', 'Festival', 'Sugarbaby', 'Camarosa', and 'Albion'.

To determined spore quantity requirements for adequate inoculation and occurrence of disease symptoms, a second glasshouse trial was undertaken using the conventional dip method as a carrier of *Fof*. Four treatments of spore concentration were tested on the highly susceptible 'Kabarla' cultivar.

Results and discussion

Results from this genotype trial showed 'Kabarla' to be highly susceptible to both isolates and 'Albion' to be moderately susceptible. 'Sugarbaby' and 'Camarosa' showed moderate to slight susceptibility to one isolate only. 'Festival' was highly resistant to both. The highly susceptible cultivar ‘Kabarla’ provides us with a reliable positive control plant for use in screening for *Fof* and for further use in research. As an alternative the cultivar ‘Albion’ can be used. ‘Festival’ provides a source of resistance.

The inoculation trial confirmed that concentrations from 1.0×10^5 to 1.0×10^6 spores/ml were adequate for disease response. Using the lower concentration of 1.0×10^5 spores/ml lessens our harvest requirements of *Fof* spores and the associated resources needed for their multiplication and storage.

This research will help facilitate current and ongoing breeding selections for Fusarium resistant cultivars

2.2.3.4. The variation of susceptibility to Fusarium wilt in strawberry germplasm, selections with high resistance to *Fof* and associated genetic parameters including heritability and breeding values for resistance.

<u>Materials and methods</u>
A partial diallel cross involving seven parents; two highly resistant to *Fof* (‘Festival’ and ‘Sugarbaby’), two susceptible (‘Maroochy Jewel’ and ‘Kabarla’) and the remaining three intermediate; was performed for use in glasshouse resistance screenings to identify the best performing lines and determine their suitability as parents. The resulting progeny were evaluated for their susceptibility to *Fof*. Two highly virulent isolates of *Fof* (N18553 and N18549) were combined as inoculum. Data from disease severity ratings was analysed using a General Linear mixed model (GLMM) incorporating a pedigree to produce Best Linear unbiased predictors (BLUP) for Breeding values.

<u>Results and discussion</u>
The continuous variation in disease response, from highly susceptible, moderate, to resistant implies a quantitative effect. Estimate of the narrow sense heritability (additive genetic effects) was 0.39 and consequently the population should be quite responsive to phenotypic recurrent selection. The individual predicted breeding values for each of the progeny (278) and 7 parents show several lines from the families to have good predicted resistance, with many of the progeny predicted to have a better breeding value than any of the parents. This is promising for the development of resistant cultivars.

2.2.3.5. General discussion on Fusarium resistance in strawberry

Adequate genetic resistance appears available and moderately selectable. Achieving higher levels of resistance is practical although no association with other traits has yet been determined, nor has the potential differential response among isolates for resistance been completely defined and this should continue to be monitored. Routine population screening has now been implemented in the seedling and clonal evaluation process and over time this will generate more information on association among traits and raise the overall level of resistance in the population.

2.2.4. Implement quantitative genetics approaches: prepare data base and populate with historical data sets for analysis; review productivity data; integrate into parental selection, seed production, and progeny selection.

A key process for continual assessment and improvement was to raise the level of quantitative genetic breeding techniques in the program and to better integrate this with economic outcomes.
To allow this we needed: suitable data; suitable analyses methods; and a defined context in which to evaluate the process.

2.2.4.1. Suitable data

Materials and methods
To provide suitable data we created a data base in Katmandoo by reformatting relevant historical data, including extensive pedigree information. The focus here was to maximise available genetic diversity and relationship structures, but still have sufficient data on a range of traits to allow adequate analyses. To meet these criteria we chose data from the first clonal trials (i.e. the first vegetative generation produced from selected seedlings) in the years 2004 through 2009, with the facility to continually add each following year's data as it becomes available.

Results and discussion
Presently the data set includes up to 2010. Although there are limitations as some selection has already occurred, but because the data covers more than six years and a diversity of populations, we believe it is thus acceptably robust. The advantage of this approach is that it captures the required information by using data collected in the normal course of the evaluation process. The alternative is to separately produce specific crosses and then collect data from many plants that would otherwise have little relevance. For example, in the latter process based on separate populations where the sole purpose is to produce genetic parameters this is efficient; however if one also wants to apply selection it is expensive most (>95%) data will be discarded because selection intensities are often only 1 to 3%. By contrast using the population to provide selections and then derive genetic parameters we believe balances inputs and outcomes.

2.2.4.2. Suitable analyses methods

Materials and methods
To analyse the unbalanced data sets for variances, heritabilities and breeding values we employed ASREML modules and included extensive pedigree information including substantial pedigree information from the University of Florida (UF). We regularly exchange strawberry seed material with UF under a formal agreement.

Results and discussion
The results of these analyses are summarised earlier under the publication section and are in the process of being published.

2.2.4.3. Defined context in which to evaluate the breeding process

Materials and methods
To define the context in which to operate and deploy the results of the genetic analyses we constructed a spreadsheet to account for differences in levels of expression of traits on the economic outcome. This involved describing the industry practices, breaking them into components and apportioning costs to the components so that the effect of changes in traits

could be quantified. The analysis also involved estimating the relationship among price and volume of fruit on the market based on data from the Brisbane central market.

Additionally to help discriminate superiority among potential selections the spreadsheet was populated with data on genotypes. Basic data included:
Total yield per plant, proportion of yield in each month (May through September), average fruit size, resistance to rain damage, bush size, fruit display (i.e. position of fruit relative to foliage) resistance to abrasion, resistance to bruising, fruit shape (regularity of appearance), truss type and ease of detaching fruit (picking). In the spreadsheet fruit quality traits are assumed to be at levels that are adequate for the market because the spread sheet does not yet account for differences among quality traits such as flavour, sweetness, acidity and colour. However because for efficiency reasons we do not normally take yield data throughout September so this month requires an assumption. In the absence of other estimates we use the average of some earlier estimates and assume 0.19 as the proportion of yield in September.

Results and discussion
The published results of the industry practice on cost analyses are summarised earlier in this report under the publication section. This study identified that total yield, the proportion of yield that was early (early yield) and fruit size as major influences on economic outcome (i.e. the effect on gross margin). The effect of different proportions of cultivars can also be investigated.

Cultivar releases give improved gross margins (Table 1) but care is required because very high increases in yield can reduce the gross margin. Distribution of yield is often more important that total yield.

The quantitative genetics approach using breeding values has been applied in the selection of parents for resistance to rain damage (see publication in earlier section) and it is anticipated that the approach will be applied more fully to other traits in any future crossing cycles. It is expected that this approach, including applying the economic analysis component, will make cultivar development and deployment more efficient.

Table 1: Gross margin for cultivars ('Aussiegem' and 'Suncoast Delight') released from the project, estimated relative to the gross margin of a planting with 50% Festival and 50% Fortuna.

Variety	Percentage. gross margin increase obtained for first 5% of growers using cultivar [c]	Percentage. gross margin increase obtained at optimum adoption by industry [d]
Rubygem	na	Current cultivar
Festival	na	Current cultivar (Associated introduction UF)
Fortuna	na	Current cultivar (Associated introduction UF)
Suncoast Delight	105	10
Aussiegem	-17[a],21[b]	11[a],31[b]
Advanced selection 9	57	42
Advanced selection 10	70	48

Assumptions included: projected yield for September; Fruit quality of all cultivars was sufficient to maintain the demand/supply curve *(Herrington M, Wegener M, Hardner C, Woolcock L, Dieters M (2012) Influence of plant traits on production costs and profitability of strawberry in southeast Queensland. Agricultural Systems 106:23-32),*

[a] is at standard spacing , [b] is higher density (i.e. 54000 plants per ha) to increase yield ha by 20%

As the proportion of new cultivar (N) increases so the proportion of Festival (E) and Fortuna (T) decrease in equal amounts (i.e. 1=N-(E+T) where E=T.

[c] First adopters to use cultivars derive higher benefits; [d] as use becomes more widespread later adopters derive less benefit.

2.2.5. Cultivar development and release

2.2.5.1. Materials and methods

Promising indexed advanced lines that had shown promise have been made available under non-exclusive licences to runner growers within approved runner schemes.

2.2.5.2. Results

One hundred and eighty four (184) parents were used over the study period to produce 396 cross-pollination combinations (Table 2). Approximately 35100 seedlings from the crosses were progressively selected for 3 seasons at about 5% per season (Table 2). Progress was reported to and guided by the National Strawberry Breeding Steering Committee, virus tested foundation stock and intellectual property management including Plant Breeders Rights was prepared for commercialisation when it became appropriate.

Additionally a breeding line 'BL 2006-221' was identified as having exceptionally high anthocyanin levels.

Under PBR protection three cultivars were initially commercialised including 'Suncoast Delight', 'Aussiegem' and 'Sunblushgem' (this last is no longer offered). 'Suncoast Delight' and 'Aussiegem' although currently only included in a small percentage of plantings (~1%) are creating interest. These, together with the previous releases including 'DPI Rubygem' now comprise ~ 14.6% (i.e. 4.8 million of the 32.7 million) of low chill runners planted in Queensland each year, and about 22% of runners produced in Queensland. Approximately seven million ‘DPI Rubygem’ runners were also planted in Turkey in the 2011/2012 season. Other advanced selections also currently show considerable promise based on estimated gross margin (Table 1).

2.2.5.3. Discussion

The recent commercial availability of ‘Fortuna (introduced), the new ‘Suncoast Delight’, ‘Aussiegem’ together with ‘Festival’ and ‘Rubyygem’ mean the ‘bar’ for future cultivar releases is significantly higher. All these cultivars have featured in recent crossings and when integrated with our genetic backgrounds and breeding strategies their inclusion promises to continue to raise the quality of fruit available to consumers and increase profitability to industry.

Additionally the high anthocyanin breeding line ‘BL 2006-221’ may allow cultivars which target health benefits and ‘high-health lifestyle’ markets to be developed.

That an active genetic improvement program is essential to continue to adapt the crop genetics to the continually changing environment is demonstrated by the estimated improvement in gross margins from recent releases and early stage breeding lines. This endeavour is especially relevant at this time of accelerating climate change, and rapidly rising energy-related and labour-related input costs.

Table 2. Crossing and selection schedule (historic 1990-2012).

Year of cross (nominal)	Year of first selection	Number of parents	Number of crosses (families)	Number of seedlings evaluated	Number of initial selections	Number of prospective clones [a]	Number of provisional test lines (farm 1)	Number of advanced test lines (farm 2)	Number of commercial cultivars	Number of project cultivars commercially marketed [b]
Total	1990- to 2005-	329	723	85608	3034	472	56	35	13	6
Annual Average	1990- to 2005-	25	56	6585	303	43	6	3	1	1.5
2005	2006 (2006-)	38	47	13450	492	97	8	7	2	2
2006	2007 (2007-)	33	133	24350	500	57	11	2	na	na
2007	2008 (2008-)	71	106	6770	176	45	6	2	na	na
2008	2009 (2009-)	36	125	4500	230	37	5	1	na	na
2009	2010 (2010-)	48	122	13000	158	49	3	na	na	na
2010	2011 (2011-)	55	85	14000	246	83	na	na	na	na
2011	2012 (2012-)	45	64	3600	145	na	na	na	na	na

[a] indexing commences at this stage.
[b] In 2012

3.1.0. Fostering international collaboration by scientific publication

Fostering international collaboration by scientific publication on relevant topics related to project needs.

The proposed publication schedule included topics:
3.1.1. 'The incidence, costs and genetic parameters associated with rain damage on strawberries grown in south-east Queensland.'
3.1.2. 'The influence of plant and fruit traits on the costs associated with production of strawberries grown in south-east Queensland.'
3.1.3. 'The genetic variances and correlations and heritabilities associated with economically important plant and fruit traits of strawberries grown in south-east Queensland.'
3.1.4. 'The definition and validation of a multi-trait selection procedure for strawberries grown in south-east Queensland'.

3.1.1. Rain damage: The following publications are associated with: 'The incidence, costs and genetic parameters associated with **rain damage** on strawberries grown in south-east Queensland.'

3.1.1.1. Herrington ME, Woolcock L, Wegener M, Dieters M, Moisander J (2009) Cultivar differences in tolerance to damage by rainfall. Acta Horticulturae 842:483-486

Abstract:
Differences in the level of damage to fruit following six rainfall events, that ranged from 7 to 330 mm, between May and September were determined for cultivars 'Festival' and 'Rubygem' and 43 breeding lines. 'Festival' with an average score of 7.5 was more tolerant than 'Rubygem' (6.0) to rain damage. Relative scores for tolerance to rain-damage were consistent across the rainfall events with no cultivar*rain-event interaction. Evaluation at only one rainfall event may be sufficient to determine a cultivar's relative resistance to rain-damage, providing key cultivars with 'known' levels are included as calibration points.

3.1.1.2. Herrington ME, Hardner C, Wegener M, Woolcock LL (2013) Rain-damage on three strawberry cultivars grown in subtropical Queensland. International Journal of Fruit Science 13:52-59

Abstract:
In Queensland, Australia, strawberry (*Fragaria×ananassa* Duchesne) is grown in open fields and rainfall events can damage fruit. Following a rain event, damage was evaluated on three commercial cultivars. 'Rubygem' (80%) had more fruit damaged than 'Strawberry Festival' (55%) and 'Camarosa' (61%). "Etch," where the surface of the fruit is eroded and, consequently, the seeds are raised relative to the damaged surface, was the most frequently occurring (>80%) damage type and was distributed on the body and tip of the fruit, while some fruit (<16%) showed cracking. Fully mature fruit was damaged (>80%) more than partially mature fruit, which differed between 'Strawberry Festival' (16%) and 'Rubygem' (68%). Cultivars that are resistant to rain damage would reduce losses and lower risk for the growers.

3.1.1.3. Herrington ME, Hardner C, Wegener M, Woolcock LL, Dieters MJ (2011) Rain damage to strawberries grown in southeast Queensland: evaluation and genetic control. HortScience 46:1-6

Abstract:
In Queensland, Australia, strawberries (*Fragaria x·ananassa* Duchesne) are grown in open fields and rainfall events can damage fruit. Cultivars that are resistant to rain damage may reduce losses and lower risk for the growers. However, little is known about the genetic control of resistance and in a subtropical climate, unpredictable rainfall events hamper evaluation. Rain damage was evaluated on seedling and clonal trials of one breeding population comprising 645 seedling genotypes and 94 clones and on a second clonal population comprising 46 clones from an earlier crossing to make preliminary estimates of heritability. The incidence of field damage from rainfall and damage after laboratory soaking was evaluated to determine if this soaking method could be used to evaluate resistance to rain damage.
Narrow-sense heritability of resistance to rain damage calculated for seedlings was low (0.21 ± 0.15) and not significantly different from zero; however, broad-sense heritability estimates were moderate in both seedlings (0.49 ± 0.16) and clones (0.45 ± 0.08) from the first population and similar in clones (0.56 ± 0.21) from the second population.
Immersion of fruit in deionised water produced symptoms consistent with rain damage in the field. Lengthening the duration of soaking of 'Festival' fruit in deionised water exponentially increased the proportion of damage to fruit ranging in ripeness from immature to ripe during the first 6-h period of soaking. When eight genotypes were evaluated, the proportion of sound fruit after soaking in deionised water in the laboratory for up to 5 h was linearly related (r^2 = 0.90) to the proportion of sound fruit in the field after 89mmof rain. The proportion of sound fruit of the breeding genotype '2008-208' and 'Festival' under soaking (0.67, 0.60) and field (0.52, 0.43) evaluations, respectively, is about the same and these genotypes may be useful sources of resistance to rain damage.

3.1.2. Traits on costs of production: The following publication is associated with: 'The influence of plant and fruit traits on the **costs associated with production** of strawberries grown in south-east Queensland.'

3.1.2.1. Herrington M, Wegener M, Hardner C, Woolcock L, Dieters M (2012) Influence of plant traits on production costs and profitability of strawberry in southeast Queensland. Agricultural Systems 106:23-32

Abstract:
In Queensland the subtropical strawberry (*Fragaria ×ananassa*) breeding program aims to combine traits into novel genotypes that increase production efficiency. The contribution of individual plant traits to cost and income under subtropical Queensland conditions was investigated, with the overall goal of improving the profitability of the industry through release of new strawberry cultivars. The study involved specifying the production and marketing system using three cultivars of strawberry that are currently widely grown in southeast Queensland under the annual planting production system, developing methods to assess the economic impact of changes to the system, and identifying plant traits that would influence outcomes from the system.

From May through September P (price; \$·punnet^{-1}), V (monthly mass; tonne of fruit on the market) and M (calendar month; i.e. May = 5) were found to be related ($r^2 = 0.92$) by the function (±SE) $P = 4.741(\pm0.469) - 0.001630(\pm0.0005)V - 0.226(\pm0.102)M$ using data from 2006 to 2010 for the Brisbane central market. Sensitive factors in both income and cost elements in the gross margin were subject to sensitivity analysis.

'Harvesting' and 'Handling/Packing' 'Groups' of 'Activities' were the major contributors to variable costs (each >20%) in the gross margin analysis. Within the 'Harvesting Group', the 'Picking Activity' contributed most (>80%) with the trait 'display of fruit' having the greatest (33%) influence on the cost of the 'Picking Activity'. Within the 'Handling/Packing Group', the 'Packing Activity' contributed 50% of costs with the traits 'fruit shape', 'fruit size variation' and 'resistance to bruising' having the greatest (12 to 62%) influence on the cost of the 'Packing Activity'. Non-plant items (e.g. carton purchases) made up the other 50% of the costs within the 'Handling/Packing Group'. When any of the individual traits in the 'Harvesting' and 'Handling/Packing' groups were changed by one unit (on the 1 to 9 scale) the gross margin changed by up to 1%. Increasing yield increased the gross margin to a maximum (15% above present) at 1320 g·plant^{-1} (94% above present). A 10% redistribution of total yield from September to May increased the gross margin by 23%. Increasing fruit size increased gross margin: a 75% increase in fruit size (to ≈ 30 g) produced a 22% increase in the gross margin.

The modified gross margin analysis developed in this study allowed simultaneous estimation of the gross margin for the producer and gross value of the industry. These parameters are sometimes moving in opposite directions.

3.1.3. Genetic parameters of traits. The following publications are associated with: 'The genetic variances and correlations and heritabilities associated with economically important plant and fruit traits of strawberries grown in south-east Queensland.'

3.1.3.1. Chandler CK, Folta KM, Dale A, Whitaker V, Herrington M (2012) Chapter 9. Strawberry. In: Badenes ML, Byrne DH (eds) *Fruit Breeding*, Handbook of Plant Breeding, 8. Springer, New York, pp 305-325

Abstract:
The cultivated strawberry, *Fragaria* × *ananassa* Duch., is a versatile crop in terms of its adaptability to various locations and cultural systems. Breeding efforts started in the early 1800s and continue today in numerous public and private programs. Among these programs and in germplasm repositories, there is still considerable variation available in traits of economic interest. Currently, the biggest opportunity in strawberry breeding is the development of day-neutral cultivars for cool summer climates outside of California while the biggest challenge facing strawberry breeders may be the development of cultivars that can produce fruit with consistent size, appearance, and flavour over an extended period of time. To accomplish this challenge, breeders need to stay focused on these traits as their primary screens. Despite the complexity of the octoploid strawberry genome, new genomics knowledge and biotechnologies make increasing contributions to strawberry breeding.

3.1.3.2. Herrington et al. Genetic parameters for plant and fruit traits in subtropical strawberry (*Fragaria xananassa*). In late draft stage anticipate submission by late January 2013

Abstract:
The results of multiple years data 2004-2009) generated in the normal course of the evaluation and selection of early clonal trials were adjusted for spatial and temporal effects using ASREML then used to estimate variance components and calculate genetic parameters for 17 fruit and plant traits. In most traits the phenotypic (V_P) and total genetic variance (V_{GT}) were high with V_{GT} usually at least 50% of V_P. Additionally additive genetic variance (V_{GA}) was usually more than 80% of V_{GT}. The exceptions were sweetness, fruit shape code and glossiness which had low genetic components. Heritabilities (narrow-sense) reflected these trends ranging from 0.11 to 0.78. Additive genetic correlations were abundant but only two were greater than 0.7 and they were positive. These were between total yield and early yield (r=0.75) and between resistance to bruising and resistance to abrasion (r=0.82). The highest negative correlation, between acidity and flavour, was lower (r=-0.65).

3.1.3.3. Fredericks CH, Fanning KJ, Gidley MJ, Netzel G, Zabaras D, Herrington M, Netzel M (2012) High-anthocyanin strawberries through cultivar selection. Journal of The Science of Food and Agriculture. DOI 10.1002/jsfa.5806

Abstract:
Diets high in fruit and vegetables are known to have significant health benefits. This is in part due to the presence of phytochemicals, which possess potential protective health benefits. This study focuses on the ability of strawberries to be bred for higher anthocyanin content. This is a major contributor to the characteristic colour and nutritional value of ripe strawberries, together with phenolic acids, ascorbic acid and total antioxidant capacity. Anthocyanins in five commercial strawberry cultivars and three breeding lines were assessed. This led to the discovery of one breeding line (BL 2006-221) as an exceptional source of anthocyanins (1 g kg^{-1} fresh weight), with approximately double the levels of current commercial cultivars. Temperature was shown to influence anthocyanin extraction, with 40 ◦C being the best extraction temperature using the accelerated solvent extraction (ASE) method. Hue angle and anthocyanin concentration showed a good correlation (r^2 = 0.69). The new breeding line BL 2006-221 has the potential to be used in the development of phytochemically rich strawberry cultivars. Using hue angle as a screening tool for total anthocyanin concentration and extraction of anthocyanins from strawberries by ASE at 40^o C would support such cultivar development.

3.1.4. Multi-trait selection. The following publication is associated with: 'The definition and validation of a **multi-trait selection** procedure for strawberries grown in south-east Queensland'.

This is in the planning draft stage: anticipated submission by April 2013.

4.0.0. General discussion

4.0.1. Outputs

The intended outputs of the project (BS09013) were well met:

Output 1: Commercialisation of 2-6 (short day) low chill varieties with high consumer appeal and outstanding agronomic characteristics, which capture at least 15% of the total Queensland/NSW (subtropical) plant market by 2012 and 20% of the total Queensland/NSW market by 2015 and 22% of the total Queensland/NSW market by 2017.

The release of 'Aussiegem' and 'Suncoast Delight' met the first part of output 1. While for the second part nominated in output 1 is effectively met by the fact that 14.6% of low chill plantings are now occupied by (combined) project produced cultivars.

Output 2: Commercialisation of one variety with high consumer appeal and outstanding agronomic characteristics for the northern WA (Perth area) (Mediterranean/lower subtropical) growing districts

Although no project-derived cultivar was commercialised in WA, 'Festival' and 'Fortuna' (introduced through exchanges facilitated by the project) constituted approx 30% of WA plantings in 2012 and one could argue that this partially met the output 2.

Output 3: The publication outputs were mostly well met although some are still in the drafting and approval process.

Journal papers have been published related to:

- 'The incidence, costs and genetic parameters associated with rain damage on strawberries grown in south-east Queensland.'
- 'The influence of plant and fruit traits on the costs associated with production of strawberries grown in south-east Queensland.'

In draft or advanced draft stage are:

- 'The genetic variances and correlations and heritabilities associated with economically important plant and fruit traits of strawberries grown in south-east Queensland.'
- 'The inheritance of resistance to Fusarium wilt in strawberries grown in Australia'.

Yet to be drafted (anticipated April 2013) is:

- The definition and validation of a multi-trait selection procedure for strawberries grown in south-east Queensland'.

Additional publications were made as described in the publication section of this report.

4.0.2. Outcomes

The intended outcomes of the project (BS09013) will also likely achieve close to prediction:

Judged by predicted gross margin increases (10-31%) for the project released cultivars ‘Aussiegem’ and ‘Suncoast Delight’ the outcome of ‘significantly increasing profitability of strawberries by 2017’ appears likely.

This is especially the case when one notes that the increases in gross margin associated with genotypes which can be further developed and deployed through a proposed ‘follow on’ project are ~ 40%.

5.0.0. Technology transfer

5.1.0. Industry publications:

Herrington M., Woolcock, L., Ko L. and Paynter M. (2012). 'Early cropping improves returns for strawberry growers on Sunshine Coast' In Rowling J (ed) Simply Red, Queensland Strawberries. Queensland Strawberry Industry Promotions Council, Cooroy, Australia Issue 26, June 2012 p.9.

Herrington M., Woolcock, L., Ko L. and Paynter M. (2012). 'Strawberry Breeding – Maroochy-subtropical node of national breeding program' In Rowling J (ed) Simply Red Queensland strawberry industry Newsletter. Queensland Strawberry Industry Promotions Council, Cooroy, Australia Issue 27, September 2012 p.12.

Herrington M.E. (2011). National strawberry varietal improvement program – subtropical regions. Horticulture Australia Limited Strawberry Industry Annual Report, p.2.

Herrington M., Woolcock, L., Price, S., Paynter M. and Ko L. (2011). 'Plant Breeding' 2011 Edition Strawberry R&D Update p 2-4.

Herrington M., Woolcock, L., Paynter M., Price, S. and Ko L. (2010). 'Plant Breeding' 2010 Edition Strawberry R&D Update p 3-6.

Herrington M.E. (2010). National strawberry varietal improvement program – subtropical regions. Horticulture Australia Limited Strawberry Industry Annual Report, p.6.

Herrington M., Woolcock, L., Paynter M. and Ko L. (2009). 'Plant Breeding' 2009 Edition Strawberry R&D Update p 3-6.

Herrington M.E. (2009). Breeding program focuses on profitable high quality strawberries. Horticulture Australia Limited Strawberry Industry Annual Report, p.2.

Herrington M., Woolcock, L., Paynter M. and Ko L. (2009). 'Plant Breeding' 2009 Edition Strawberry R&D Update p 3-6.

Twice yearly Reports to National Strawberry Breeding Steering Committee (April and October, 2009, 10, 11, 12).

5.1.1. Oral presentations to industry included:

QSGA meetings 2 per year
Radio broadcasts

5.1.2. Other Activities included:

Masterclass showcasing Queensland food and wine and strawberries (handouts and info [hotel and restaurant activity at the Brisbane Hilton]) – 300-400 participants (international chefs and food critics etc) (24 to 26 July 2010).

5.2.0. Scientific Publications (refereed):

Herrington ME, Hardner C, Wegener M, Woolcock LL (2013) Rain-damage on three strawberry cultivars grown in subtropical Queensland. International Journal of Fruit Science 13:52-59

Fredericks CH, Fanning KJ, Gidley MJ, Netzel G, Zabaras D, Herrington M, Netzel M (2012) High-anthocyanin strawberries through cultivar selection. Journal of The Science of Food and Agriculture. DOI 10.1002/jsfa.5806

Chandler CK, Folta KM, Dale A, Whitaker V, Herrington M (2012) Chapter 9. Strawberry. In: Badenes ML, Byrne DH (eds) *Fruit Breeding*, Handbook of Plant Breeding, 8. Springer, New York, pp 305-325

Herrington M, Wegener M, Hardner C, Woolcock L, Dieters M (2012) Influence of plant traits on production costs and profitability of strawberry in southeast Queensland. Agricultural Systems 106:23-32

Herrington ME, Hardner C, Wegener M, Woolcock LL, Dieters MJ (2011) Rain damage to strawberries grown in southeast Queensland: evaluation and genetic control. HortScience 46:1-6

Young AJ, Marney TS, Herrington M, Hutton D, Gomez AO, Villiers A, Campbell PR, Geering ADW (2011) Outbreak of angular leaf spot, caused by *Xanthomonas fragariae*, in a Queensland strawberry germplasm collection. *Australasian Plant Pathology* **40**, 286-292

Herrington, M. E. and Price S. (2010). *Fragaria x ananassa* 'Redgem'. Plant Varieties Journal, 23 (3), 99

Herrington, M. E. and Price S. (2010). *Fragaria x ananassa* 'Suncoast Delight'. Plant Varieties Journal, 23 (3), 100

Herrington, M. E. and Price S. (2010). *Fragaria x ananassa* 'Aussiegem'. Plant Varieties Journal, 23 (3), 102

Herrington, M. E. and Price S. (2010). *Fragaria x ananassa* 'Sunblushgem'. Plant Varieties Journal, 23 (3), 99

Herrington ME, Woolcock L, Wegener M, Dieters M, Moisander J (2009) Cultivar differences in tolerance to damage by rainfall. Acta Horticulturae 842:483-486

6.0. Recommendations

- Actively pursue a genetic improvement program comprising subtropical breeding, complementing germplasm introductions and collaborative germplasm exchanges. Such a program based on quantitative genetics principles, including assessment of changes in gross margins, will be critical to meeting the challenges of accelerating climate change, and energy shortages and labour-related input costs.

- Actively seek and understand inheritance and deliberately include and/ or maintain high levels of resistance to ‘wilt’ and ‘crown rot’ diseases that cause plant loss after establishment with initial focus on *Fusarium, Colletotrichum* and *Macrophomina* (and consider *Phytophthora*).

- Focus subtropical breeding on early fruit production, larger fruit size and high consumer quality – within the context of acceptable levels of other traits.

- Further develop diversified cultivars based on high quality and flavour and investigate potential for high anthocyanin (antioxidant) cultivars to target health and ‘high health lifestyle’ market segments.

- Develop a system for industry to have available accurate timely national industry planting and production data including cultivars and plant numbers so as to predict likely production levels.

- Investigate business models that would allow a consistent match of production levels to market requirements, as occurs in manufacturing and finance sectors.

- Test gross margin production spreadsheet against wider industry production data and modify spreadsheet to allow efficient updating for costs, prices and plant trait parameters.

- Extend step-wise a tested gross margin spreadsheet nationally. As a first step include Perth area of Western Australia.

- Extend gross margin analysis spreadsheet to include quality traits.

- Define appropriate overseas markets to target and define cost structure and fruit traits required for competitive entry into these overseas markets.

- Investigate deleting ‘aberrant/unreliable’ data sets to allow re-examining available DNA data and then explore potential when the set is combined with results of trait data developed in this project.

- Consider additional strategies for the promotion of the new cultivars.

- Assess long term applicability of hydroponics, covered structures and available germplasm in relation to potential for future yield potential, robotics and mechanisation.