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ORIGINAL PAPER

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# Gene flow in cherry orchards

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**Abstract** A survey of Australian cherry orchards identified the cherry cultivars Sunburst, Summit, Merchant, Sam, Sylvia, Tieton, Kordia, Regina, Empress, Nordwunder and Ulster as having low fruit-set associated with poor pollination. Unique orchard sites across Australia where low fruit-set was not a problem for these cultivars were located, and pollen gene-flow-analysis conducted using 6-PGD, GOT, G6PD, GPI, IDH, FDP and SKDH isozyme markers. Pollenisers for the above-mentioned cultivars were determined and Stella was a polleniser for eight of them. Stella's predominance was linked to it reaching full bloom before the other cultivars; anther dehiscence occurs sometime after flowers open, as such newly opened flowers; the most-fertile stage was mainly exposed to Stella pollen. Sunburst a self-compatible cultivar showed no evidence of self-fertilisation. The majority of pollenisers were found to be within 20 m of the tree under examination. Isozyme profiles for 22 cultivars not previously analysed are also presented.

## Introduction

Prior to 1990, the majority of cherry trees planted in commercial orchards in Australia were self-incompatible. In a situation such as this, cross-compatible cultivars must be planted in close proximity to ensure good fruit-set and satisfactory fruit yields. With the introduction of self-compatible cultivars, such as Stella (Lapins 1971), Lapins and Sunburst (Lane and Schmidt 1984), it was thought that the need for polleniser trees could be eliminated. The importance of pollenisers for both self-incompatible and self-compatible cultivars was emphasised by work in South Australia (Granger 1997), that showed satisfactory cropping of the self-compatible cultivar Stella was largely a result of outcrossing rather than selfing.

During the period from 1989 through 2000 the number of cherry trees planted in Australian orchards increased from 600,000 to 1.7 million (Granger 2002). The majority of cultivars planted during this time were imported from overseas breeding programs. More recently, Australian cherry growers reported poor yields, over several seasons, from the imported cultivars, and suspected poor pollination was the problem.

The aim of this study was to determine pollen gene flow in cherry orchards and, therein, effective pollenisers for imported cherry cultivars.

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## Materials and methods

### Industry survey

Cherry industry associations across Australia were contacted, and grower members surveyed to determine cherry cultivars with low yield problems thought to be a result of poor pollination. In addition any orchards where the problem-cultivars from the grower-survey were performing well, were identified, and it was in these orchards that the study was based to construct gene-flow patterns and deduce pollen parents (Jackson and Clarke 1991), and, in effect determine pollenisers of the problem cultivars.

### Growth stage

The stage of the growth of trees targeted for sampling at each site was recorded in October.

### Leaf sampling and isozyme extraction

Leaves from the tips of extending shoots were collected from one tree of each cultivar on cool days (maximum temperature not exceeding 20°C). Leaves were placed in labelled plastic bags and immediately stored on ice in an insulated container. Leaves were then transported to the laboratory and extracted; when this was not possible, leaves were held at 2°C for up to 7 days prior to extraction. Four hundred mg of leaf tissue, 150 mg of polyvinyl polypyrrolodine and 2 ml of tris-citrate extraction buffer pH 8.0,

were ground in a mortar and pestle at room temperature. Samples were then centrifuged for 15 min (3,000 G) and the supernatant used for electrophoresis (Granger et al. 1993).

### Embryo sampling and isozyme extraction

Two- to three-hundred mature fruit were harvested from each target tree at each site. Fruit were taken from the outside of the canopy at a height of 1.5 m. The fruit was stored at 4°C for 2–3 weeks and then the flesh removed. Adhering flesh was cleaned from the seed coat by scrubbing seeds under running water. Seeds were air-dried, dusted with copper oxychloride, sealed in plastic bags and stored at 4°C. Isozyme analysis began as soon as possible after harvest. Seeds were cracked, the seed-coat removed and extractions made as described above for leaves. Except that 100 mg of embryo tissue was used, 0.5 ml of extraction buffer and 37.5 mg of polyvinyl polypyrrolodine was added.

### Electrophoresis

Sheets of cellulose acetate 30 cm×30 cm×200 $\mu$m (Chemetron) were cut into 15 cm×30 cm gels. Gels were supported in perspex boxes divided into two compartments and furnished with platinum wire for electrodes. Electrophoresis was conducted at 2°C at 200 V for 1.5 h. Extracts to be examined for 6-PGD, GOT and G6PD were run in 0.02 M phosphate buffer pH 7.0, and GPI, IDH, FDP and SKDH in 0.05 M pH 7.8 tris-maleate buffer (Granger et al. 1993).

### Staining

Staining protocols for 6-PGD, GOT, G6PD, GPI and IDH were according to Richardson et al. (1986). FDP and SKDH activities were revealed using a protocol adapted from starch-gel electrophoresis described by Soltis et al. (1983). Stained gels were incubated in an oven at 37°C and photocopies made as the isozymes developed. Scoring the resultant banding patterns was carried out by using the nomenclature developed for cherry isozyme genotypes (Granger et al. 1993; Granger 1996).

## Results

Cherry cultivars in Australia with pollination difficulties were identified in descending order of importance as: Sunburst, Summit, Merchant, Sam, Sylvia, Tieton, Kordia, Regina, Empress, Nordwunder and Ulster.

Pollination of the cultivar Sam had been investigated in a previous study (Granger 1997) and was not re-examined.

The growth stage of trees representing target and potential polleniser-cultivars at each location is shown in Table 1. Site 1 was a cherry orchard located at Legana in the State of Tasmania, site 2 an orchard at Crystal Creek, Victoria, and sites 3 and 4 orchards at Yark, also in Victoria, owned by Messrs. Rouget and Sibley respectively. In general the growth stage of trees at Yark were advanced by approximately 1–3 days compared to Crystal Creek, and 3–7 days as compared to Legana.

Many of the cultivars at the trial sites had undergone isozyme analysis in previous studies (Granger et al. 1993); however, they were re-analysed in this study to verify that they were true to type. Table 2 lists varieties not previously reported and their corresponding isozyme genotypes.

**Table 1** Growth stages of target trees and potential pollinisers at sites in Tasmania and Victoria, Australia, October 2000. Blank entries indicate that a cultivar was absent at that site

| Variety | Legana | Crystal Creek | Yark |
|---|---|---|---|
| 13N-7-39 | | Petal fall | |
| Bing | Late petal fall | | |
| Bing (old strain) | Shuck fall | | |
| Blackboy | Shuck fall | | |
| Durone di Vignola | | | Late petal fall-shuck fall |
| Early Burlat | | | 15–20 mm fruit |
| Empress | | | 15 mm fruit |
| F12-1 root-stock | Full bloom | | |
| Florence | Full bloom | | |
| Georgia | | Petal fall | |
| Hedelfingen | Petal fall | | |
| Kordia | | Petal fall | |
| Kristen | Post shuck fall | | |
| Lambert | Full bloom | Full bloom | |
| Lapins | Shuck fall | Late shuck fall | |
| Late Noble | | Full bloom | |
| Lewis | | Initial fruit-set | 15 mm fruit |
| Merchant | Full bloom | Petal fall | 15 mm fruit |
| Mermat | Full bloom | | |
| Merpet | Full bloom | | |
| Napolean | Late petal fall | | |
| Nordwunder | | Petal fall | Petal fall |
| PC7616-4 | | Late petal fall | |
| Rainier | Full bloom | | |
| Regina | | Petal fall | Late petal fall |
| Salmo | | Petal fall/shuck fall | |
| Simone | | Post shuck fall | |
| Sommerset | | Shuck fall | |
| Stella | Late petal fall | | |
| Summit | Full bloom-petal fall | Petal fall | Late petal fall |
| Sunburst | Full bloom | | Shuck fall |
| Sylvia | | Full bloom | Petal fall |
| Tieton | | Petal fall/shuck fall | |
| Ulster | | Petal fall/shuck fall | Shuck fall |
| V69062 | | | Petal fall |
| Van | Petal fall | Shuck fall | |
| Vega | Petal fall | | 10 mm fruit |
| Victor | Petal fall-shuck fall | | |
| Vista | | | Shuck fall |

Intra-cultivar variation was evident; Bing OB260 had a single allele difference compared to Bing, while St. Margaret grown at Crystal Creek had different genotypes at the 6-PGD, FDP and IDH loci, compared to St. Margaret sampled previously (Granger et al. 1993)

Ron's Seedling grown at Legana was different to Ron's Seedling grown at Crystal Creek at the 6-PGD, GOT and GPI loci. A comparison between Simone and Lapins (thought to be the same cultivar by Australian cherry growers) showed a difference at two isozyme loci (Table 2).

**Table 2** Cherry cultivar isozyme genotypes (genotypes in bold denote differences between two cultivars at a particular isozyme locus)

| Variety | Isozyme genotype | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6-PGD | G6PD | GOT | GPI | FDP | SKDH | IDH |
| 13N-7-39 | aa | aa | bc | aa | ab or bb | ab | ab |
| Bing[a] | aa | ab | bc | ab | **aa** | aa | aa |
| Bing OB260[c] | aa | ab | bc | ab | **ab** | aa | aa |
| Durone di Vignola | aa | ab | ab | aa | bb | aa | bc |
| Early Burlat | aa | ab | ab | aa | ab | aa | ab |
| Empress | aa | ab | ac | aa | aa | aa | ab |
| Florence | aa | ab | bc | aa | – | aa | aa |
| Georgia | ab | ab | ac | aa | bb | ab | aa |
| Kristen | bb | ab | bc | aa | bb | ab | aa |
| Late Noble | ab | ab | – | aa | aa | aa | aa |
| Lewis | ab | ab | bc | aa | aa | aa | aa |
| St. Margaret[b] | **aa** | aa | – | aa | **aa** | aa | **ac** |
| St. Margaret[c] | **ab** | aa | ac | aa | **bb** | aa | **bc** |
| Mermat | aa | ab | bc | aa | ab | ab | aa |
| Olympus | ab | ab | bc | aa | ab | aa | aa |
| PC7616-4 | ab | ab | bc | ab | ab | ab | aa |
| Regina | aa | ab | bc | aa | bb | aa | aa |
| Ron's Seedling[b] | **ab** | ab | **bc** | **ab** | ab | ab | aa |
| Ron's Seedling[a] | **aa** | ab | **ac** | **aa** | ab | ab | aa |
| Simone | ab | ab | ac | ab | **bb** | **ab** | aa |
| Lapins[c] | ab | ab | ac | ab | **ab** | **aa** | aa |
| Sommerset | ab | aa | bc | aa | aa | ab | aa |
| Sylvia | ab | ab | bc | aa | ab | aa | aa |
| Tieton | aa | ab | ac | aa | bb | ab | aa |
| V69062 | aa | bb | bc | aa | aa | ab | aa |

[a] Legana
[b] Crystal Creek
[c] Previously reported

**Table 3** Cherry cultivars and corresponding pollinisers

| Cultivar | Pollinisers (% of pollen genes contributed to embryos) |
|---|---|
| Empress | 64% Noir de Guben, 36% Ron's Seedling |
| Georgia | 80% Lambert, 20% Stella |
| Kordia | 32% Margaret, 68% unidentified |
| Merchant 1a[a] | 50% Van, 50% Sunburst |
| Merchant 1b | 77% Van, 17% Sunburst, 6% Stella |
| Merchant 3 | 25% Sunburst, % n.d. Vega, Empress, Burlat |
| Merchant 4 | 34% Sunburst, % n.d. Burlat, Empress, 13N-7-39 |
| Nordwunder | 100% Stella |
| Regina 2[b] | 65% PC7616-4, 32% Georgia, 3% Stella |
| Regina 4 | 82% Sylvia, 18% V69062 |
| Summit 1[c] | 70% Van, 30% Stella |
| Summit 2 | 58% Merchant, 34% Stella, 8% Van |
| Sunburst 1[d] | 100% Stella |
| Sunburst 4 | 50% Vista, 50% Merchant |
| Sylvia 2[e] | 61% Van, 39% Stella |
| Sylvia 4 | 50% Sunburst, 50% Durone di Vignola |
| Tieton | 97% Stella, 3% PC7616-4 |
| Ulster | 61% Summit, 31% Vista, 8% unidentified |

[a] Merchant 1a: Legana, top block; Merchant 1b: Legana, bottom block, Merchant 3: Yark, Rouget, Merchant 4: Yark, Sibley
[b] Regina 2: Crystal Creek, Regina 4: Yark, Sibley
[c] Summit 1: Legana, Summit 2: Crystal Creek
[d] Sunburst 1: Legana, Sunburst 4: Yark, Sibley
[e] Sylvia 2: Crystal Creek, Sylvia 4: Yark, Sibley

Pollen parents or pollenisers were deduced from the isozyme patterns observed in embryos as described by Jackson and Clarke (1991). In most cases more than one cultivar provided pollen to the cultivar under examination. The frequency of pollen isozyme alleles in embryos was used to assign the proportion of embryos produced by each pollen donor. Where the pollen genes could not be uniquely determined, allele frequency was not calculated. Table 3 lists cultivars identified in the survey and their corresponding pollenisers. The proportion of pollen genes donated by each polleniser is expressed as a percentage.

## Discussion

The objective of the project was to determine pollinisers for difficult to pollinate cultivars using deduced pollen gene flow in Australian cherry orchards, and this has been achieved.

As expected, the growth-stage of cultivars varied between sites. Cultivars at Legana were one growth-stage (3–7 days) behind the same cultivars at Crystal Creek, and cultivars at Crystal Creek were a growth stage behind those at Yark. Crystal Creek and Yark are nearby one another in northern Victoria at a latitude of 37°S, while Legana, near Launceston in Tasmania, is at approximately 41°S. Later development of cultivars at Legana is associated with the site being further south. The difference between Crystal Creek and Yark is related to microclimate effects. There were exceptions; Lambert, for example, was at full bloom at Legana and Crystal Creek at the same time, and Nordwunder was at petal fall at Crystal Creek and Yark at the same time. The differences in growth stages are related to the time of bud break which is dependent on sufficient winter chill, followed by the accumulation of heat units,

specific to each cultivar (Thompson 1996). Chill and heat units vary from site to site.

The results suggest that, in the majority of cases, pollinisers flower just before the variety of interest. For example at Legana in this study, Sunburst was planted on Tatura trellis 1 m between trees and 5 m between rows, Lambert was planted at every tenth tree along the row as a polleniser, because it was known to flower at the same time as Sunburst. As expected Lambert and Sunburst reached full bloom at the same time, yet Lambert did not contribute pollen genes to any of the Sunburst embryos. Instead, Stella trees, 4 rows or 20 m away and 3–7 days ahead in flowering, provided all of the pollination to Sunburst. The only case wherein later flowering cultivars were effective-pollinisers was with Empress. It reached full bloom at Yark on September 20, while its polliniser Noir de Guben reached full bloom 8 days later on September 28. While pollination by later-blooming cultivars may seem impossible, it should be noted that full bloom indicates that the majority of flowers, on a particular tree, are fully open, and a proportion remain unopen while others are finished. In this example the later-blooming flowers of Noir de Guben acted as pollenisers for Empress. The results lead to the question of why earlier flowering-cultivars predominate as pollinisers? This may be related to the effective flowering period. McGregor (1976) states that when the cherry flower opens, the stigma is receptive, but the anthers are closed. Furthermore, pollination on the first day after anthesis is much more effective than pollination on the second day. Vezvaei and Jackson (1995) showed that in almond, flowers are more fertile when newly opened than at other stages of development. Full bloom for any cultivar varies from year to year (Granger 1997), and flower development varies within cultivars and trees. The timing of flower opening for cultivars plays an important role in determining effective pollinisers.

Stella was a polliniser for 8 of the 11 cultivars sampled and can be considered a good general purpose polliniser, except for cultivars that do not overlap the flowering time of Stella, such as the very early flowering cultivar Empress, or the very late Ulster. Sunburst is described as self-compatible (Tobutt et al. 2001) yet none of the embryos sampled resulted from selfing; instead Vista, Merchant and Stella provided all pollen genes in the embryos of Sunburst. Granger (1997) found that the self-compatible cultivar Stella outcrossed in 70% of successful pollinations and selfed in 30%, and concluded that it was necessary to include polliniser cultivars in plantings of self-compatibles; this finding concurs with the results for Sunburst which add further support to this conclusion.

Brandt et al. (1999) found Stella to be the main polliniser for Summit; in this study, Stella contributed about 30% of pollen genes to Summit embryos, while Van and Merchant made larger contributions.

Jackson and Clarke (1991) found that gene flow in almond orchards was concentrated between neighbouring parts of tree canopies and no gene flow occurred beyond 76 m. In comparison, Granger (1997) found that 50% of gene flow in a cherry orchard occurred over 50 m. In this study the main polliniser was usually within approximately 20 m.

Intra-cultivar variation has been reported previously (Granger et al. 1993). This study shows again that different genotypes have been labelled with the same name. For example, Bing OB260 is different to Bing, the former being ab and the latter aa for FDP. Bing OB260 is a selection of Bing made through a clonal selection program focused on Bing in orchards of the Pacific Northwest of U.S.A. (E.L. Proebsting, personal communication). Similarly, cultivars called St. Margaret and Ron's Seedling at two separate locations were found to be genetically different, varying at three isozyme loci. Simone and Lapins are often confused as the same cultivar. Simone differs from Lapins at two isozyme loci; there is little difference between the fruit characteristics of the two cultivars, and it may be that Simone is a mutation of Lapins.

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## References

Brandt B, Witherspoon J, Granger AR, Collins GG (1999) Identification of pollen donors for the sweet cherry cultivars "Stella" and "Summit" by isozyme analysis. Aust J Exp Agric 39:473–477

Granger AR (1996) Inheritance and linkage of isozymes in sweet cherry (*Prunus avium* L.) Theor Appl Genet 93:426–430

Granger AR (1997) Pollen gene flow in South Australian cherry (*Prunus avium* L.) orchards. Aust J Exp Agric 37:583–589

Granger AR (2002) Breeding cherries in Australia to meet demand from overseas and domestic markets. In: Proc 12th Australasian Plant Breed Conf Perth, WA, Australia, Sept 2002, pp 598–560

Granger AR, Clarke GR, Jackson JF (1993) Sweet cherry cultivar identification by leaf isozyme polymorphism. Theor Appl Genet 86:458–464

Jackson JF, Clarke GR (1991) Gene flow in an almond orchard. Theor App Genet 82:169–173

Lane WD, Schmidt H (1984) Lapins and Sunburst sweet cherry. Can J Plant Sci 64:211–214

Lapins KO (1971) Stella, a self-fruitful sweet cherry. Can J Plant Sci 51:252–253

McGregor SE (1976) Insect pollination of cultivated crop plants. USDA Agriculture Handbook No. 496, pp 138–142

Richardson BJ, Baverstock PR, Adams M. (1986) Allozyme electrophoresis. A handbook for animal systematics and population studies. Academic Press, Sydney, pp 99–144

Soltis DE, HauflerCH, Darrow DC, Gastony GJ (1983) Starch-gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and staining schedules. Am Fern J 73:9–27

Tobutt KR, Sonneveld T, Boskovic R (2001) Cherry (in)compatibility genotypes—harmonisation of recent results from U.K., Canada, Germany, Japan and U.S.A. Eucarpia Fruit Breed Sect Newslett 5:41–46

Thompson M. (1996) Flowering, pollination and fruit-set. In: Cherries: crop physiology, production and uses. In: Webster D, Looney NE (eds) CAB International, Wallingford, pp 223–241

Vezvaei A, Jackson JF (1995) Effect of pollen parent and stages of flower development on almond and nut production. Aust J Exp Agric 35:109–113