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Pollen Flow and the Effect on Fruit Size in an 'Imperial' Mandarin Orchard

H.M. Wallace
Faculty of Science, University of the Sunshine Coast, Maroochydore D.C., 4558 Australia

B.J. King
Department of Plant Science, University of Adelaide, Waite Campus, Glen Osmond, SA 5064 Australia

L.S. Lee
Centre for Plant Conservation Genetics, Southern Cross University, P.O. Box 157, Lismore, 2480, Australia

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Abstract. **Pollen source is known to affect the fruit size and quality of 'Imperial' mandarin, but no study has determined the appropriate orchard design to maximize the beneficial effects of pollen source. We determined the parentage of seeds of 'Imperial' mandarin using the isozyme shikimate dehydrogenase to characterize pollen flow and the effect on fruit size in an orchard setting. Two blocks were examined: 1) a block near an 'Ellendale' pollinizer block; and 2) an isolated pure block planting. Fruit size and seed number were maximum at one and three rows from the pollinizer ($P \leq 0.05$). Isozyme results were consistent with all seeds being the result of fertilization by the 'Ellendale' pollinizer. In the pure block planting, fruits in rows 5–11 inside the block were very small with no seeds. This indicates poor pollen flow resulting in a reduction in fruit quality for the pure block. These results emphasize the importance of pollinizers in orchard design, and bees in orchard management. They suggest that each row should be planted no more than three rows from the pollinizer to maximize the benefits of the pollen parent in self-incompatible cultivars such as 'Imperial'.**

Adequate pollination is an important consideration in the management of commercial mandarin orchards. In particular, the genotype of the pollen may have a strong influence on the numbers of fruit set and may produce xenia effects, i.e., effects on seed and fruit characters. In mandarins, the genotype of the pollen is known to influence size and seediness of fruits in cultivars such as 'Ellendale' (Vithanage, 1991) and 'Page' (Hearn et al., 1968, 1969). Pollen source is particularly important for self-incompatible cultivars such as 'Imperial'. In self-incompatible cultivars, the consequence of poor pollination or self pollination in some circumstances may be low fruit set, reduced fruit size, and reduced sugar content (Wallace and Lee, 1999).

Since pollen source may have effects on crop size and quality, it is important to determine the most appropriate planting ratio of pollinizer trees to commercial cultivars in order to maximize the benefits of cross pollination. Pollen flow and dispersal distance have been assessed in many crops by examining pollen parents of seeds using isozymes. Many authors report decreasing pollen flow with increasing distance from the pollinizer, e.g. Stern et al. (1993) and Degani et al. (1995) for 'Mauritius' and 'Floridian' lychees. In some crops, pollen flow may be very restricted (Jackson and Clarke, 1991) whereas in other crops, pollen may disperse relatively long distances (Granger, 1997). All of these studies examined single-seeded fruit. The observed pollen flow could theoretically be the result of dispersal of, and pollination by, a single pollen grain. In citrus, there is a known correlation between seed number and fruit size in some cultivars (Krezdorn, 1967; Vithanage, 1991), therefore multiple pollen grains on each stigma are required to produce large fruit. There are few studies on pollen dispersal in other multiseeded fruit. In apples, increasing distance to pollinizers is related to decreasing seed number and higher incidence of misshapen fruit (Brookfield et al., 1996). Currently there are no data on pollen flow in citrus orchards and the impact of pollinizer proximity on fruit size of citrus.

The purpose of this study was to investigate pollen flow in 'Imperial' mandarin, a monoembryonic cultivar of citrus known to be self-incompatible (Wallace and Lee, 1999). This would enable determination of the most appropriate orchard design to maximize the benefits of pollinizer rows on fruit production.

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Materials and Methods

Study site. The experiment was carried out in a commercial orchard in the Central Burnett region (25°S), Queensland, Australia. Fruits were sampled from two blocks of 'Imperial' mandarin: 1) a block next to a pollinizer ("pollinated" block); and 2) a "pure" block planting. The "pollinated" block was planted 10 m from a block of 'Ellendale' mandarin and consisted of 17 rows of 'Imperial', each row containing ≈40 trees.

Trees were spaced at 5 × 3 m. Trees were ≈8 years old with an open canopy. The nearest cultivar besides 'Ellendale' was ≈1 km away. The "pure" block was isolated from the nearest pollinizer ('Ellendale') by ≈500 m and barriers including a river and natural vegetation. There were 95 trees in each row and 20 rows in the block. Trees were ≈25 years old and the canopy between trees had closed to form a hedgerow. Honeybees were introduced to the orchard 3 weeks prior to flowering, and placed in groups of 4–6 hives within 500 m of both pure and mixed blocks.

Sampling methods. Every 5th, 10th, and 15th tree in every second row was sampled from the pollinated block. For the pure block, every 15th, 30th, and 45th tree was sampled. Sampling commenced at row one for both blocks. From each tree, five fruits were sampled randomly. Fruits were taken to the laboratory, where they were weighed and the seeds extracted using a kitchen juicer. Seed number was counted for each fruit, and seeds were retained for isozyme analysis.

Isozymes. Seeds of unknown parentage and seeds of known parentage were sampled for isozyme analysis. All seeds of unknown parentage from one fruit of each tree for each of the trees sampled in the pollinated block were sampled and processed for isozymes. Seeds of known crosses with 'Imperial' as the female parent ('Imperial' x 'Ellendale', and 'Imperial' x 'Imperial') produced using the methods described by Wallace and Lee (1999) were included on all gels for comparison. In addition, leaf samples from 'Imperial', 'Ellendale' and 'Murcott' were included in all gels.

Isozymes were extracted using a modification of the method of King et al. (1995). The embryos were removed from the seed coat and ground in a mortar and pestle in 0.6 mL of extraction buffer chilled to 5 °C. The resulting solution was transferred to Eppendorf tubes and centrifuged at 12000 rpm for 2 min. Samples were analyzed using polyacrylamide gel electrophoresis (PAGE) using the optimal conditions described by King et al. (1995). Samples were run using a PAGE system consisting of a gel buffer of 0.5 M Tris HCl (pH 7.5) in 5% acrylamide. The electrode buffer consisted of 0.07 M $NaBO_3$ (pH 7.5). Gels were run for 10 h at an initial current of 5 mA and a constant voltage of 60 V. Samples were then stained for shikimate dehydrogenase (SkDH) (Richardson et al., 1986).

Statistical analysis. Fruit weight data for both blocks and seed number for the "pollinated" block were square-root-transformed to adjust for skewness, and analyzed using a two way analysis of variance (sources of variation were row number and tree). Where significant differences were detected, means were compared with a Tukey's HSD test. Seed number data for the pure block were not normally distributed and were analyzed using a Kruskal-Wallis test. Isozymes were analyzed using a Chi-square test.

Results

Fruit size and seed number. In the "pollinated" block, fruit size and seed number decreased significantly with increasing distance from the 'Ellendale' pollinizer row ($P \leq 0.001$). Fruit sampled from Row 1, adjacent to the pollinizer were significantly larger than fruit from rows 5–15 (Tukey's HSD, $P \leq 0.05$, Fig. 1).

A similar pattern was observed for seed number (Fig. 1b). The row adjacent to the pollinizer had significantly more seeds than all other rows (Tukey's HSD, $P \leq 0.05$). The regression between fruit weight and seed number was significant ($P \leq 0.001$, $r^2 = 0.542$, intercept = –24.44, slope = 3.213).

The pure block (isolated from pollinizers) also showed a decrease in fruit weight with increasing distance from the edge of the block ($P \leq 0.001$). Only rows 1 and 3 produced commercially sized fruits (>80g), and fruits from rows 5–11 were significantly smaller (Fig. 2a). Seed numbers were much lower in the pure block than in the pollinated block (Figs. 1b and 2b) and no effect of distance from the edge of the block was found for seed numbers (Kruskal-Wallis, $P = 0.338$). The regression between fruit weight and seed number was not significant for the pure block ($r^2 = 0$, $P = 0.88$).

Isozymes. Bands for SkDH using PAGE were described by King et al. (1995) as MM for 'Imperial', and FS for 'Ellendale'. In the controlled crosses between 'Ellendale' and 'Imperial', S was clearly distinguishable from M, but there was poor separation of the F and M bands. As a consequence, 50% of controlled crosses were clearly distinguishable as progeny of 'Imperial' x 'Ellendale' (MS), and 50% of controlled crosses (MF) were not distinguishable from 'Imperial' selfs (MM).

Exactly 50% of the genotypes taken from across the rows in the pollinated block were MS, indicating pollination by 'Ellendale'. The other 50% of seeds were not distinguishable as either 'Imperial' selfs (MM) or 'Ellendale' parentage (MF). If all seeds were the result of pollination by 'Ellendale', 50% would show the MS band given Mendelian segregation of the isozyme genotypes. The proportions of observed MS were not significantly different from 50% (expected ratios) and were not significantly different across rows (Table 1, $\chi^2 = 0.412$). This indicates that all seeds are likely to be the result of pollination by 'Ellendale'. Furthermore, 'Imperial' is self-incompatible and self-pollination is rare (Wallace and Lee 1999) and it is therefore likely that the MM genotype was very rare. Due to very low seed numbers, seeds

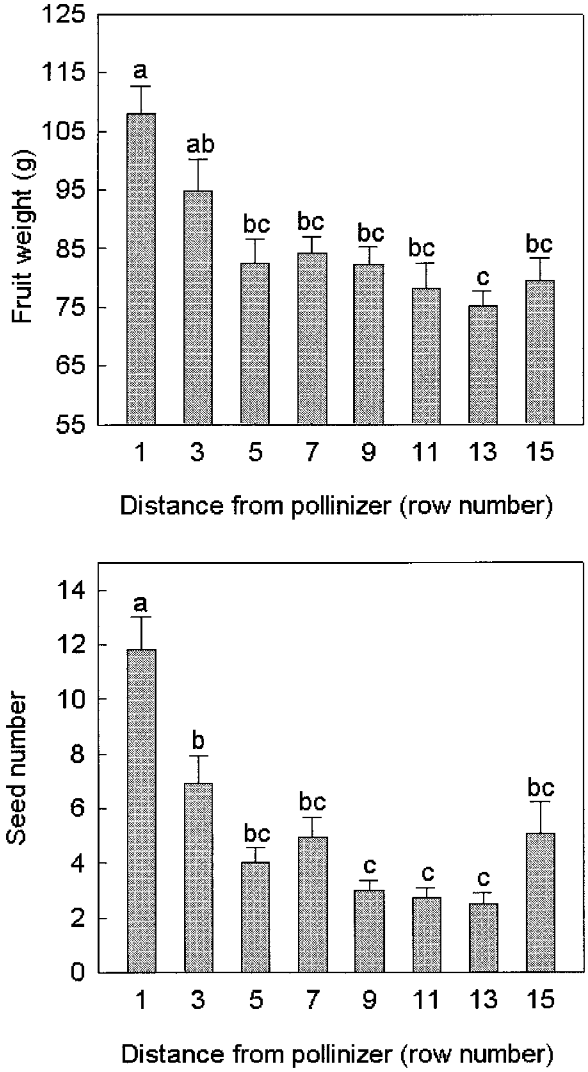

Fig. 1. The effect of distance from the pollinizer row on (**a**) fruit weight, and (**b**) seed number of 'Imperial'. Means and SE bars are presented. Rows with different letters are significantly different (Tukey's HSD, $P \leq 0.05$).

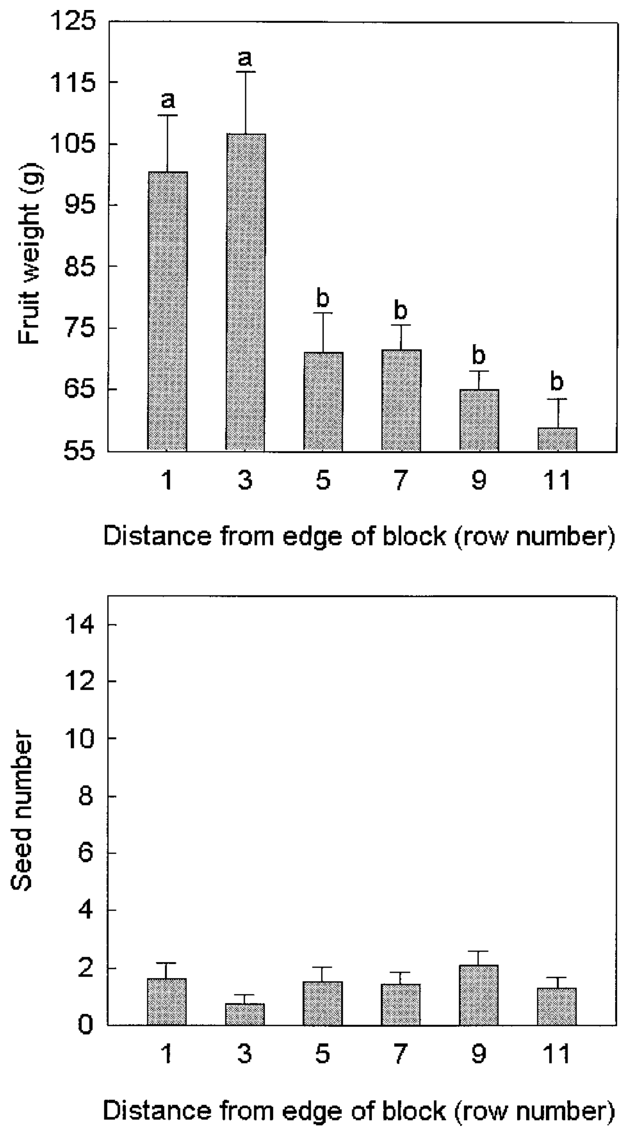

Fig. 2. Mean and standard error of (**a**) fruit weight and (**b**) seed number in a pure block of 'Imperial' with increasing distance from the edge of the block. Means and SE bars are presented. Rows with different letters are significantly different (Tukey's HSD, $P \leq 0.05$).

Table 1. Isozyme analysis of parentage of 'Imperial' seeds with increasing distance from an 'Ellendale' pollinizer row. The proportions of the MS genotype were not significantly different from expected ratios (based on all seeds having an 'Ellendale' parent) and were not significantly different between rows ($\chi^2 = 0.412$).

Distance from 'Ellendale' (Row number)	n	No. of seeds with known 'Ellendale' parent (MS)	Expected frequency of MS (if all seeds have 'Ellendale' parent)
1	29	13	14.5
3	16	6	8
5	16	11	8
7	9	4	4.5
9	7	3	3.5
11	9	5	4.5
13	6	5	3
15	6	2	3
Total	98	49	49

from the pure block were not analyzed for isozymes.

Discussion

Pollen parent may affect many aspects of 'Imperial' fruit quality including size, seed number and sugar content (Wallace and Lee, 1999). The results of this study demonstrate a significant decrease in quality with increasing distance from the pollinizer row. Quality in this study was determined by fruit size, and the largest fruit was in the row next to the pollinizer. There was a significant reduction in fruit size 3–5 rows distant from the pollinizer and no detectable differences in size among rows 5–15. This suggests that the maximum benefits from pollinizer rows occur in the row nearest to the pollinizer with a rapid decrease with increasing distance from the pollinizer. There was also an associated decrease in seed number 3–5 rows distant from the pollinizer. In our study, fruit size and seed number were correlated in the "pollinated" block. Other studies have found fruit size and seed number to be correlated, with the number of seeds determining quality and size of fruits (Krezdorn, 1967; Vithanage, 1991). Decreasing seed number is the most likely explanation for the decrease in fruit size with increasing distance from the pollinizer row.

There are variable reports on the distance of pollen dispersal in fruit crops. Pollen flow in almonds predominantly took place between neighboring compatible trees (Jackson and Clarke, 1991) and 76 m away there was no pollen flow. In contrast, in cherries, 50% of pollen flow occurred >50 m from the pollen source (Granger, 1997). In avocado, almost all mature fruit up to 36 m distant from the pollinizer (Goldring et al., 1987) or up to 30–50 m from the pollinizer (Degani et al., 1990) resulted from cross pollination. In our study, isozyme analysis suggests that all seeds in the pollinated block originated from 'Ellendale' pollen, indicating pollen dispersal at distances of >50 m. However, the number of successful crosses (as indicated by seed number) per fruit decreased significantly after three rows (≈15 m.) from the 'Ellendale' pollinizer. The most likely explanation is that the number of viable 'Ellendale' pollen grains deposited on each stigma decreased with increasing distance from the 'Ellendale' row. For best pollen flow and fruit quality, pollinizer rows should be planted every six rows to allow all trees to be within three rows of the pollinizer.

'Imperial' is capable of parthenocarpic fruit production (Sykes and Possingham, 1992). In a controlled pollination study at the same orchard Wallace and Lee (1999) found a reduction in size and sugar content in parthenocarpic fruit compared to cross-pollinated fruit. In the pure block, fruits in rows 5–11 showed characteristics of parthenocarpic fruit production; for example they were significantly smaller and usually contained no seeds. This is a result of the isolation of the pure block from compatible pollinizers. However in the edge rows (1 and 3) fruits were a normal size in spite of having few or no seeds. The observed results may have been a consequence of occasional pollination in the edge rows. Alternatively, fruit may have been larger because of a smaller crop load, or light levels in the outer rows may have been higher, allowing more resources for parthenocarpic fruit production. These results indicate the erratic nature of parthenocarpic fruit production in this cultivar. Parthenocarpic fruit production is often erratic for many other cultivars (Krezdorn, 1967; Krezdorn and Robinson, 1958). The importance of seedless fruits for new markets has been emphasized by Sykes and Lewis (1996). Further research is needed into the controlling mechanisms of parthenocarpic fruit production in 'Imperial' to ensure consistent commercial fruit size and quality of seedless 'Imperial' mandarins.

These results emphasize the importance of pollinizers and appropriate orchard design for 'Imperial' production. The commercial implications of this study are that one pollinizer row should be planted at least every six rows of 'Imperial' mandarin so that each row is no more than three rows from the pollinizer. 'Ellendale', 'Murcott' and 'Ellenor' are all appropriate pollinizers for 'Imperial' (Wallace and Lee, 1999). These results also emphasize the need for careful management of pollinators such as the honeybee, *Apis mellifera* L. (Free, 1993) in 'Imperial' mandarin orchards.

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