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DIFFERENTIATING APPLE SPORTS BY POLLEN ULTRASTRUCTURE

A thesis presented in partial fulfilment of the requirements
for the degree of
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Alastair John Currie

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ABSTRACT

Cultivars are plants that form distinct, uniform and stable phenotypes. New cultivars can be protected by Plant Variety Rights (PVR) which allow the owner exclusive rights to the propagation and sale of the plant material. Current PVR identification methods for apple cultivars require detailed records of tree, flower and fruit characteristics to differentiate the new cultivars from known cultivars. This method is slow, expensive and unable to cope with the increasing numbers of sports. Biochemical identification methods such as isozymes, restriction fragment length polymerisation (RFLP), random amplified polymorphism DNAs (RAPD), and minisatellite probes, can quickly and objectively differentiate cultivars, but cannot differentiate apple sports. Previous research suggested that pollen ultrastructure could be an alternative method for plant identification. This thesis is concerned with the development of a technique to differentiate apple sports using pollen exine patterns.

Scanning electron microscopy was used to capture images of the apple pollen grain and the exine surface. A digital image analysis algorithm was developed to extract quantitative data from the pollen grain dimensions and pore characteristics, and a Fast Fourier transform extracted quantitative data from the ridge patterns. Statistical methods were applied to the data to differentiate the sports.

Pollen harvested from apple flowers in the spring were wider than pollen harvested from flowers forced out of season under artificial conditions. Significant differences between trees were found for pollen grain length:width ratio, percent pore coverage, pore area and pore length but further research is required. However, apple cultivars types 'Red Delicious' and 'Gala' were successfully differentiated by pore and pollen grain variables, and 'Aversang' and 'Ultrared' sports of 'Red Delicious', and 'Splenola' and 'Galalea' sports of 'Gala' were successfully differentiated by exine ridge patterns and pollen grain measurements.

Differentiation of apple sports by pollen requires further development but may be one of the only quick, objective identification methods that can differentiate sports. Sport

differentiation would greatly aid PVR establishment and enforcement.

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1. INTRODUCTION

Cultivars are cultivated varieties of a crop and are the product of change to the genome, for example sexual reproduction or mutation. The cultivars that are the products of mutations are called sports and can either occur naturally or be induced with a mutagenic agent (for example radiation). In Brooks and Olmo's Fruit and Nut register there are approximately 2500 cultivars listed for the period between 1962 and 1991. Over 12% of these cultivars are sports. Sports form an even more significant proportion of apple cultivars; nearly 31% of the commercial cultivars in the Brooks and Olmo Register 1962-1991 are sports (Brooks and Olmo, 1962, 1963, 1964, 1965, 1966, 1967, 1968, 1969, 1970, 1971, 1972, 1973, 1974, 1975, 1978, 1982, 1983, 1984, 1991).

If it can be demonstrated that a cultivar is new, distinct, homogenous, and genetically stable then the developer may apply for a plant patent. In New Zealand new varieties have only been covered by plant patent laws since 1975 (Whitmore, 1992). The current laws dealing with plant patents are in the Plant Variety Rights (PVR) Act of 1987 which is based on the International Union for the Protection of New Varieties of Plants (UPOV) convention. The PVR office has to establish that each applicant for PVR status meets the criteria of distinctness, homogeneity and genetic stability. Applicant plants are planted in the field beside other known, similar cultivars and are observed over several seasons to remove the influence of environment. This can be quite a large undertaking. The Brooks and Olmo Register recorded 57 sports of Delicious between 1962 and 1991 (Brooks and Olmo, 1962, 1963, 1964, 1965, 1966, 1967, 1968, 1969, 1970, 1971, 1972, 1973, 1974, 1975, 1978, 1982, 1983, 1984, 1991). Once the PVR is established the developer usually licences nurseries to propagate the plant material and charges a royalty on each tree sold. The produce of the new cultivar can also be protected by marketing the fruit under a trade mark which can be licensed out as well (Selby, 1995). Over the years there has been a move away from government backed research for the public good and a move towards research for commercial returns which has meant an increase in interest in developing and patenting cultivars for financial returns.

In New Zealand, apples are an important crop. The annual return from fresh apple

exports was about $346.3 million in 1992/3, which represented more than 38% of the total export fruit sales (Fruit Research Council, 1994). Many cultivars of apple are used for export and each cultivar has minimum thresholds for colour, size and other quality factors. Mutations in the genome can enhance the quality of the fruit, and increase the value of the crop. The New Zealand Apple and Pear Marketing Board (NZAPMB) encourages the discovery of naturally occurring, new, improved sports. Recently there has been an increase in the numbers of sports as applicants for PVR status (Whitmore, 1992) and this may either be a reflection of the NZAPMB encouragement, the opportunity for financial gains from PVR, or the apple crop may be prone to mutation.

The numbers of cultivars submitted with little morphological or agronomic difference from established cultivars is a major concern for the PVR office (Whitmore, 1992). Plants can be identified or differentiated by differences in agronomy, morphology, or biochemistry. Field observation of morphology has been adequate for most apple cultivars but requires the use of a large area to grow the standards as well as the applicant plant, and an expert to record the large amounts of data (PVR evaluation form, appendix B). Although most of the measurements are objective, some like colour require judgement. Unfortunately biochemical markers do not, so far, offer appropriate solutions to differentiate sports. DNA techniques like polymerase chain reaction (PCR), restriction fragment length polymerisation (RFLP), and minisatellite probes do not show sufficient polymorphism to differentiate apple sports. Isozyme techniques can differentiate some sports, but not all, and isozymes are sensitive to the environment. Other simple biochemical tests can be applied in specific cases, for example phenol test for wheat, but cannot be applied to all crops. There is a need for a plant identification method for PVR that will easily and objectively differentiate sports.

The aim of this project was to develop a system that would differentiate apple sports using pollen grain ultrastructure. Scanning electron microscopy was used to capture the image and a new digital image analysis algorithm was developed to extract data from the image. Statistical methods were then applied to the data to differentiate the sports. This method could further be used to assist with the establishment of PVR status as well

as identifying cultivars and sports. Although the technique was developed on apples, it could also be applied to other crops.