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NOVA
– the National Olive Variety Assessment Project

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Foreword

The current interest in Australia in the Mediterranean diet has led to an increased demand for olive products, which has seen imports rise above $AUS100 million per year since the mid 1990's and has provided the stimulus for the recent investment and expansion of the local olive industry (Sweeney and Davies, 1998). However, olives and olive oil are international commodities with 444 500 tonnes of oil and 326 500 tonnes of table olives exported in 1999/2000, predominantly from the major production areas around the Mediterranean Sea (International Olive Oil Council, 2001a).

For the Australian industry to be sustainable, it must be competitive on the international market. This can only be achieved by adopting high quality techniques in management and production technology and ensuring that the local industry uses the best varieties suitable for Australian conditions to achieve optimal yields and quality.

Unfortunately, the selection of suitable varieties is a far from straightforward matter for the Australian olive industry. There is uncertainty over the true identity of olive varieties in Australia and there is limited reliable performance data for any olive variety under the wide range of Australian conditions and the industry relies on overseas information.

The National Olive Variety Assessment Project (NOVA) has been established to resolve the confusion in variety identity and to assist olive producers in making informed varietal choices from the comparative physiological information on the performance of olive varieties in Australia.

This project was funded from RIRDC Core Funds which are provided by the Federal Government.

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Simon Hearn
Managing Director
Rural Industries Research and Development Corporation

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Executive Summary

There is enormous potential for olive oil and table olive production in Australia as an import replacement and value-added export industry. Australia currently imports over $AUS 100 million per year and as much of Australia is climatically suitable for growing olives, this has stimulated investment and establishment of olive groves across the country. It is imperative that best management practises for intensive cultivation and latest technology is employed for the Australian industry to compete with imports and achieve success in overseas markets.

One of the major challenges in the establishment of the olive industry has been the selection of the best varieties suitable for Australian conditions to achieve optimal yields and quality. There has been limited reliable information and performance data for any olive variety under the wide range of Australian conditions and the industry has relied mainly on Northern hemisphere research and information. Another major issue that has confronted the Australian olive industry is that of ensuring the correct varietal identity of a particular tree, as there is a great deal of confusion in olive variety identification. Performance characteristics of a specific genotype are the basis on which a selection is made for a particular usage or physical situation. Correct identification is critical since mistakes may not become apparent for some years.

The National Olive Variety Assessment (NOVA) project, has been established to help resolve the confusion in olive variety identity as well as to evaluate the performance of all known commercial olive varieties in Australia and how some of them perform in different climatic regions of Australia. There are two major components to the NOVA project:

A. The National Olive Collection established at the University of Adelaide's Roseworthy Campus.

B. The evaluation of olive varieties in commercial situations on grower properties across Australia.

The National Olive Collection is a replicated experimental trial planted with most of the known olive varieties currently available in Australia. One hundred accessions were sourced from nurseries and old government collections across Australia. Eighty-seven of these accessions were provisionally regarded as different olive varieties. Planting was in two stages in late 1998 and 1999.

An important part of evaluating this collection has been the ability to DNA fingerprint all 600 trees, using randomly amplified polymorphic DNA (RAPD), to ensure their true varietal naming. The DNA fingerprints of the 100 accessions were compared to those of a number of named varieties obtained from international and Australian collections.

It was found that a number of varieties planted at Roseworthy matched with the correct international standards including: Arbequina, Barnea, Coratina, Frantoio, Hojiblanca, Kalamata, Koroneiki, Leccino, Manzanillo de Sevilla, Nevadillo Blanco, Pendolino, Picual, Sevillano, Souri and one of the Verdales. This is a reassuring result for the Australian industry as these are all popular variety choices.

However there is also clearly confusion with other varieties. Of the 100 NOVA accessions tested (which were supposedly 87 different varieties), only 53 different genotypes were detected. While it was not surprising to find some synonyms, it was remarkable that 14 differently named varieties were of the same genotype as the Italian Frantoio. This is particularly significant as many growers believing they have different varieties to enhance cross-pollination may infact only have a single variety with subsequent deleterious impacts on pollination efficacy and fruit set.
The plethora of variety names is also confusing for variety selection and labelling of varietal oils and table fruit. As well, the product end-use will depend on the type of olive produced. The variety names Belle de Espagne and Big Spanish are likely to be associated with table fruit, whereas the

accessions grown in the NOVA trial were genetically similar to Frantoio and Arbequina respectively, which are both oiling varieties with small fruit.

Not only were there many misnamed varieties in the NOVA collection, in 11% of the samples, the 6 replicate trees were not identical and the anomalous trees are being removed from the collection. This result highlights the difficulties in initially recognising specific varieties and subsequently ensuring that lines are reliably maintained.

The physiological data being evaluated includes tree vegetative growth since planting, and fruit physical and chemical characteristics from 2002. Many of the same fruit attributes were measured for the commercial scale evaluation and the results are summarised with the National Collection data.

Height and butt diameter growth has been measured on all the trees in the National Collection. Barnea was clearly the tallest variety but there was no one variety with a significantly larger diameter.

Not all varieties in the National Collection had produced sufficient fruit for analyses by 2002 and these varieties may not be suitable for early fruit production in this environment.

The fresh weight of fruit per trees that had yielded in the National Collection was measured and is an important value for the table olive varieties. For these varieties, only Gordal Sevillana had a significantly lower yield than the others of the table olive varieties that had yielded.

The oil yield per tree is most important for the oil producing varieties. The best performing varieties in the National Collection in terms of oil yield at this early stage of the trial are: Areccuzo, Picual, Barnea, Arbequina, Oblitza and Group VII.

In 2001, olive growers from around Australia submitted fruit samples from their olive varieties for analyses. For both the National Collection and the Commercial Scale results, there was no significant effect of fruit maturity on the oil percentage in the dry flesh within the range of fruit maturities at the $p < 0.05$ level. There were however large differences in oil content observed across the different varieties examined. If Picual is used as an indicator of a recognised high oil yielding variety, then a number of varieties with higher yields than Picual, showed promise as high oil yielders to the extent that oil % in dry flesh is a predictor of oil yield. These varieties are: Arbequina, Areccuzo, Barnea, Columella, Coratina, Correggiola, Frantoio, Group VII, I77, Kalamata, Koroneiki, Leccino, Manaiki, Mediterranean, Nevadillo Blanco, Paragon and WA Mission.

High water content of fruit can make commercial oil extraction difficult due to oil/water emulsions being formed during malaxation. This study shows the relative differences between varieties in water content of the fruit. Generally the varieties with higher fruit water contents are considered table olive varieties such as UC13A6, SA Verdale and Manzanilla de Sevilla. It is possible that irrigation management can be used to control fruit water content before harvest, particularly those varieties that naturally have high water content, if they are to be processed for oil. If the water regime does affect the water content of the fruit, those varieties that naturally have high water content may not be suitable for oil production in climates with high rainfall preceding and/or during the harvest period.

The range of fatty acid composition for most of the varieties fall within the accepted limits for fatty acid composition of Virgin Olive Oil. A number of varieties did however record levels of linolenic acid higher than the 1% limit set for virgin olive oil. This may have been an anomaly due to a cooler than usual ripening season but producers need to be aware that this could be an issue if they wish to export their oil.

The results indicate that less mature fruit may produce healthier oil in respect to stearic acid content. Although there was an apparent effect of maturity on linoleic and palmitoleic (increasing with maturity) and oleic (decreasing with maturity), they were not statistically significant.

The data does indicate a trend towards higher levels of oleic acid in fruit from more southerly latitudes. Oleic acid is considered favourable in olive oil due to enhanced oxidative stability and superior nutritional quality.

At this stage, the fruit analyses from the commercial properties have revealed much about the performance of the varieties. However more data is required on total yields, health and vigour to gain a fuller picture on variety suitability for different regions of Australia.

The National Collection of olive varieties at Roseworthy is unique and the DNA typing of this collection has made enormous inroads into the positive identification of olive varieties in Australia. This database should be utilised by the industry. However, the trees have yet to reach maturity and data collection and evaluation needs to continue for a number of years to gain a full picture of the variety production potential.

The physiological data the NOVA collection is providing for each accession will be important to compare with the DNA fingerprinting results in the future. Varieties with similar RAPD fingerprints but differing in agronomic qualities could be studied to find genetic markers for those traits.

1. Introduction

There is enormous potential for olive oil and table olive production in Australia as an import replacement and value - added export industry. Imports of olives and olive oil products are currently valued at more than $110 million per annum. From 1989-2001, the average annual growth rate of olive oil imports to Australia has been 7.5% (International Olive Oil Council, 2001a). There is the opportunity in Australia to replace at least some of these imports with a locally manufactured product, as much of Australia is climatically suitable for growing olives. The opportunity also exists for exports into Japan, SE Asia, United Kingdom and North America where consumption of olive products is also rapidly increasing.

There have been several attempts to establish an olive industry in Australia over the last 150 years and they have failed due to Australia's inability to compete with cheap olive product imports. The industry has been characterised by a predominance of small growers and processors using traditional management techniques. There is a great deal of optimism that this current attempt will succeed due to a reduction in subsidised production in the EEC, the advent of mechanised harvesting and a significant increase in demand for olive products from non-traditional olive consuming countries. Economic analyses carried out by PIRSA for RIRDC (Hobman, 1995) have indicated that a successful Australian industry will require high yields under intensive cultivation and latest technology to replace olive oil and table olive imports and compete in the international market place. Large, intensive developments are now being planned and established in Australia.

One of the major issues for growers to consider is choice of olive variety. There is very little scientific performance data for any olive varieties grown under the wide range of Australian conditions and the industry relies on overseas information. Another major problem is that of ensuring the correct varietal identity of a particular tree. Performance characteristics of a specific genotype are the basis on which a selection is made for a particular usage or physical situation. Correct identification is critical since mistakes may not become apparent for some years.

Uncertainty over the identity of varieties in Australia has already resulted in large planting's of mis-identified trees. This has resulted in inadequate cross-pollination, fruit tonnage and oil yields because of lower fruit set and the use of generally inferior varieties. Subsequently there has been a reduction in projected financial returns.

Confusion in olive variety identification in Australia exists for the following reasons:

1) It is not possible in the great majority of cases to distinguish between olive varieties on the basis of the phenotypic characteristics of vegetative growth or fruit. This is due to a natural homogeneity of general appearance and the broad range of minor variability attributable to local climatic and edaphic conditions.

2) Much of the planting material being used in Australia at present is sourced from old "colonial" groves or Government collections where records are incomplete, unreliable or no longer exist, leading to confusion about the identity of individual specimens (Burr, 1998). As well, the names of some varieties that occur in early records are no longer known in the industry. These trees may have been 're-discovered' as unknown varieties and subsequently renamed as something else.

3) There is no guarantee that the names under which these varieties were introduced into the country, officially and otherwise, were correct in the first instance as synonyms may historically have been used for genetically identical plants. This comment can equally apply to later importations. Until the mid 1960's olives were usually imported into Australia as ornamental plants and no details of variety or provenance was required by the Australian Quarantine and Inspection Service (AQIS) (Anthony Wicks, pers. comm).

4) In addition to the confusion caused by material being mis-named at source is the problem of mistaken identification or labelling occurring within propagation facilities in Australia. This might be due to lack of appreciation of the implications in earlier times or breakdown in control systems in contemporary facilities.

Regardless of the planting decisions now being made, the information derived from this project will be valuable to improve management of current plantings, for the development and management of future plantings and for the correction of planting errors by topworking.

This project will enable olive producers to make informed varietal choices from the comparative physiological information on the performance of most of the known olive varieties in Australia, grown under intensive, irrigated conditions.

2. Objectives

The National Olive Variety Assessment (NOVA) project, has been established to help resolve the confusion in olive variety identity as well as to evaluate the performance of all known commercial olive varieties in Australia and how some of them perform in different climatic regions of Australia. There are two major components to the NOVA project:

A. The National Olive Collection established at the University of Adelaide's Roseworthy Campus.

B. The evaluation of olive varieties in commercial situations on grower properties across Australia.

3. Methodology

National Collection

Experimental materials and culture

The National Collection was established to scientifically evaluate most of the known olive varieties in Australia, at the University of Adelaide Roseworthy campus (-34^{0}52'S, 138^{0}69'E), a dryland cropping research farm, 50 km north of Adelaide, South Australia. Roseworthy has a Mediterranean-type climate with an average annual rainfall of 440mm with 330mm (75%) occurring between the months of April to October and an average annual Class A Pan evaporation of 1957 mm (Adams et al, 2000). The site was formerly used for dryland wheat production. This study was conducted between July 1999 and November 2002.

The average combined depth of topsoil and upper subsoil is 40cm. Topsoils are subangular blocky pedality but upper subsoils are primarily prismatic pedality. Textures range from sandy loam to sandy clay loam and light medium clay. The upper subsoils with clay texture and prismatic structure had reduced permeability in their present state. These layers were ameliorated with gypsum incorporated by deep ripping to a depth of 0.8 metres at a rate of 5 tonnes per hectare. This treatment was applied to all tree rows.

A carbonate layer that contains high concentrations of fine soil carbonate in light sandy clay loams and clay loams, occurs at an average depth of 40cm. This carbonate layer has only moderate permeability and excessive irrigation may result in water logging problems in the clay subsoils, particularly in spring when the soil profile is already wet from winter and crop water use rates are relatively low. An irrigation schedule based on an objective soil water monitoring program was installed to minimise the risk of water logging. The average values of the EC of the saturated soil paste extract (ECe) and pH (1:5 soil/0.01M $CaCl_2$ extract) were 0.5dS/m and 7.6 respectively in the upper subsoil and 0.8dS/m and 8.6 respectively in the carbonate layer.

Soil nutrient analysis showed the primary concern to be low levels of nitrogen, sulphur and copper. Single super phosphate (9% P) with 1% Cu was broadcast across the site at a rate of 300 kg/ha and then incorporated along the rows pre plant.

The National Collection is a resolvable incomplete block design, in order to limit the observable cultural effects due to soil variability, consisting of 3 replicates by 2 trees per replicate of 100 accessions sourced from nurseries and old government collections across Australia (Table 1). Eighty-seven of these accessions were provisionally regarded as different olive varieties. Thirteen of the accessions had the same name as others in the trial but were of different provenances or planted at a different time. Not all accessions were ready to plant at once so the trial was planted in two stages in late 1998 and 1999. Six varieties (Frantoio, Barnea, Picual, Hojiblanca, Arbequina and Manzanillo) were repeat planted to enable comparison of all accessions between the two stages of planting. Tree spacing is 6 metres within rows by 7 metres between rows. A barrier row of olive trees was planted around the 3 replicates.

The trees were mostly struck from cuttings although some that were difficult to strike were grafted onto Frantoio or feral olive rootstock. They were approximately 12 months from striking or grafting when planted although there were large differences in initial height and diameter that is addressed in the discussion of the results. Ammonium nitrate (34% N) was sprinkled around each tree at 15g/m^2 after planting. For the first irrigation season from mid February until mid April 1999, 2.5g of urea (46%N) was applied to each tree every 2 weeks. For the 2000 irrigation season (November-April), 2.5g of ammonium nitrate per tree was applied every week through the fertigation system. For the 2001 irrigation season, the equivalent of 2.5g N/tree was applied each week using a proprietary fertigation mix.

Annual leaf tissue tests in January monitored tree nutrient levels and extra nutrients other than nitrogen will be applied in future on an "as needs" basis.

Weeds were suppressed along the tree rows using contact and residual herbicides. A covercrop of ryecorn between tree rows was sown each winter and slashed in November to control weeds mid-row and increase soil organic matter. Leaf chewing curculio beetles (*Otiorhynchus cribricollis*) were controlled with spot sprays of alphacypermethrin. Individual trees affected with black scale (*Saissetia oleae*) were sprayed with petroleum based summer oil when the crawlers hatched.

Pruning

In order to develop a canopy reflecting as much as possible the natural growth habit of the variety but still enable the trees to be mechanically harvested in the future, the single trunk, free canopy system was employed (Gucci and Cantini, 2000)

Irrigation

Irrigation was applied by in-line drippers with a 3.6 L/h flow rate. Lines were placed 0.5 m either side of the tree row to give two drip lines per tree row. Drippers were spaced at 0.75 m intervals along the drip line. When new driplines were buried in September 2001 due to line damage from hares, the dripper flow rate was changed to 2.9 L/h and spacings to 0.6m. Lines were still placed 0.5 m either side of the tree row however they were buried to a depth of 0.1 m. Root intrusion of the buried inline drippers was prevented by dissolving minute quantities of trifluralin herbicide (3 ppb at the drippers) into the water at each irrigation. Flow to each replicate was monitored with an in-line meter. Irrigation water was mains water of potable quality.

The irrigation schedule was based on soil moisture monitoring using tensiometers in the first year and EnviroSCAN® probes in subsequent years. Irrigation was applied before crop water stress occurred as the aim was to keep the trees in a well watered condition. However, due to the moderately impermeable subsoil, care was taken not to over water the trees. The trees received approximately 53 mm in 1998/99, 65 mm in 1999/2000 (a very wet summer), 148 mm in 2000/2001 and 200 mm in 2001/2002. Exact irrigation quantities are not possible to report in the first three years due to ongoing chewing damage of irrigation lines by hares.

DNA Fingerprinting

All 600 trees in the trial had their leaf DNA analysed using the randomly amplified polymorphic DNA (RAPD) technique (Guerin et al, 2002). The DNA fingerprints of the NOVA accessions were compared with DNA from standards that were considered most likely to match. Where possible, the DNA fingerprints of the NOVA trees were compared with DNA fingerprints from international standards sourced from the following collections, with the codes used in this paper shown in parentheses: The Olive World Collection, Centro de Investigacion y Desarollo Agrario, Cordoba, Spain (Spain); The Volcani Centre, Bet-Dagan, Israel (Israel); CORIPROL, Pescia, Italy (Italy1); Consiglio Nationale delle Ricerche, Instituto di Ricerca Sulla Olivicultura, Perugia Italy (Italy2); Foundation Plant Material Service, University of California, Davis, California USA (USA and Mexico); Subtropical Plants and Olive Trees Institute of Chania Agrokipio, Chania, Greece (Greece); Jouve-Racamond Nursery, Avignon, France (France).

Where international standards were not available, Australian standards were sourced from named trees in olive variety collections planted at government research stations in the early 1900's. These collections are at Wagga Wagga, NSW (Wagga Wagga), Blackwood, SA (Blackwood), and Roseworthy, SA (Roseworthy). Many of the NOVA trees were also sourced from these collections so in some instances, where no other standards were available, the Australian standard was from the same source as the NOVA tree. In some cases, standards for comparison were only available from

commercial nurseries (Nursery), a private SA property (Keith) or in a few instances, no comparators were available at all.

Fruit Yield

From 2000, when fruit on individually bearing trees was as close to the Maturation Index (MI) of 3 (Hermoso *et al.*, 1997) as possible, the fruit was hand harvested, weighed and sent to the laboratory for analysis.

Fruit Analyses

Ten olives (or with small samples as close to 10 as possible) with a MI of approximately 3, where the skin is reddish and the flesh buff-coloured, were selected from each sample, weighed and cut with a scalpel to remove the flesh. The stone was then scrubbed clean and weighed. The flesh to pit ratio was determined by expressing the weight of the flesh (whole olive weight minus the stone weight) divided by the weight of the stone.

Approximately 5 g of the flesh was weighed, dried to constant weight at 80^{o}C (usually 24 hrs), and extracted with n-hexane (BDH, Australia) in a Soxhlet extractor for 10 hr. Hexane was removed on a rotary evaporator to constant weight and the flask re-weighed to estimate the oil yield.

Fatty acid profiles of the oils were determined by gas chromatographic analysis of the fatty acid methyl esters (FAME) (International Union of Pure and Applied Chemistry, 1991). 100 μL of oil were derivatised by heating with 1 mL of freshly made sodium methoxide (0.5 M) in anhydrous methanol in a capped tube for 60 min at 60^{o}C. After cooling to room temperature, 2 mL of hexane and 5 mL of deionised water were added, mixed by vortexing and centrifuged at 3800 rpm for 10 min.

The hexane supernatant (1 mL) was transferred to a GC autosampler vial, and the fatty acid methyl esters measured on a Shimadzu GC-14A gas chromatograph fitted with a SGE BP20 capillary column, (50m x 0.32 mm ID) operating isothermally at 220^{o}C with a run time of 15 min. Nitrogen was the carrier gas and injector and flame ionisation detector temperatures of 300^{o}C were used. Peaks were identified by comparison with authentic standards (Mix C, Altech USA) and composition quantified on an Area % basis.

Tree Growth

The butt diameter at 0.45 m from the ground and the height of every tree was measured at planting and then in April and September/October each year.

Statistical Analysis

The definition of variety was that obtained from DNA analyses as described in Guerin et al, 2002 (Tables 1 and 2).

Overall, there were only 27 of the 53 different DNA typed varieties with sufficient fruit for analyses, i.e. fruit from three or more trees per variety. Some of the variables had a large range in mean between the varieties (e.g. the fresh weight of fruit ranged from nothing to several kilograms), with a corresponding large range in variance. A logarithmic transformation was therefore used to give approximate homogeneity of variances. The replicate effects were removed using a covariate technique.

Although most of the fruit was close to MI of 3, there was a range of maturities of the fruit when processed. This affected in particular the fresh weight of the fruit. MI was included as a covariate and data was adjusted to a mean MI of 3.2. As described previously, there were separate planting dates so planting date was also included as a covariate.

For the height and diameter analyses, the estimates of the means and the standard errors of the difference between pairs of varieties were obtained by using Restricted Maximum Likelihood from within Genstat. In that analysis, the varietal effects were considered fixed and the replicated effects were random. The initial size (height or diameter) of the trees was included as a covariate to correct for a nursery effect.

Unfortunately there had to be a range of planting dates due to availability of stock, incorrect identification or tree losses. This was included as a simple covariate based on tree age.
The trial was designed as a resolvable incomplete block design with two tree plots. This enabled the trial to be analysed as a randomised complete block or as an incomplete block – the latter method should remove more of the field variation. This did not occur so the simpler randomised block analysis was used.

Commercial Scale Evaluation

Plant Material and Sampling

In 2001, olive growers from different regions in Australia (Fig. 1) submitted fruit samples from their olive varieties for analysis of fatty acid profiles and fruit characteristics (Sweeney et al, 2002)

Each participating grower collected a random sample of 100 olives from five trees of a single variety (20 olives per tree), evenly spaced along the diagonal in the area of the orchard containing that variety. More than 90% of the samples came from trees aged between 3 and 8 years old. Samples were collected when the olives were as close to a MI of 3 as possible although samples with large variations in MI were received.

To minimise spoilage of fruit, clean, dry samples were delivered as quickly as possible (maximum 3 days in the post, usually less) to the laboratory and stored at 4^0C until processed.

Fig. 1 – Map of Australia showing sample sites

Fruit Analyses

The fruit was analysed as for the National Collection.

Statistical analyses

Residual (restricted) maximum likelihood (REML) in GENSTAT,® (Version 5, Release 4.1, Lawes Agricultural Trust) was used to adjust the varieties for site means and maturity (linear adjustment) where the site effects were considered random and the variety effects were considered as fixed.

Although the majority of olives selected for analysis were at a MI of approximately 3, some samples had only very green or very ripe olives and ranged between a MI of 1-7. This effect was removed by using MI as a covariate so that all the results were adjusted to the mean MI of 3.4.

4. Detailed Results

National Collection

DNA Analyses

Table 1 shows all the accessions in the NOVA collection and from where they were sourced, and the standards used for comparison and from where the standards were sourced (Guerin et al, 2002). Where there was no match with the standard or there was still uncertainty about the correct identity of the NOVA accession, the DNA fingerprint from the NOVA accession was then compared with other fingerprints in the database. The final column in Table 1 shows that in many cases the NOVA accessions DNA fingerprint matched another standard not previously compared or a group of differently named accessions in the NOVA collection had identical DNA fingerprints.

Table 2 shows groups of differently named NOVA accessions with identical fingerprints (Guerin et al, 2002). In some instances the DNA fingerprints matched a known international standard and the group is named after this standard. In other instances the DNA fingerprints matched no known international standard and these groups were numbered I-VII.

Table 1. Details of varieties in the NOVA collection, the NOVA variety source, planting date, rootstock where used, the standards that were used for the RAPD analyses, the source of the standard, whether a positive match was identified and whether another match of the NOVA variety was found. (n/a – not available)

NOVA Accession	NOVA Source	Planting Date	Rootstock	DNA Standard	DNA Standard Source	Match	Other Match
Amelon	Wagga Wagga	14/9/1999		Amelon	Wagga Wagga	no	
Arbequina 1	Nursery	3/12/1998		Arbequina	Spain	yes	
Arbequina 2	Nursery	14/9/1999		Arbequina	Spain	yes	
Areccuzo	Roseworthy	14/9/1999		n/a			
Ascolano	Blackwood	14/9/1999		Ascolano	USA	yes	
Atro Rubens	Wagga Wagga	14/9/1999		Atro Rubens	Wagga Wagga	yes	
Atroviolacea Brun Ribier	Blackwood	21/12/1999		Atroviolacea Brun Ribier	Blackwood	yes	
Attica	Wagga Wagga	14/9/1999		Attica	Wagga Wagga	yes	Californian Mission
Azapa	Nursery	3/12/1998		n/a			
Barnea 1	Nursery	3/12/1998		Barnea	Israel	yes	
Barnea 2	Nursery	14/9/1999		Barnea	Israel	yes	
Barouni	Nursery	3/12/1998		Barouni	Nursery	yes	
Belle de Espagne	Wagga Wagga	14/9/1999		Sevillano	Israel	no	Frantoio
Benito	Nursery	3/12/1998		n/a			
Big Spanish	Wagga Wagga	14/9/1999		Sevillano	Israel/Spain	no	Arbequina
Black Italian 1	Nursery	3/12/1998		Black Italian	Blackwood	no	Verdale (USA)
Black Italian 2	Blackwood	21/12/1999		Black Italian	Blackwood	yes	
Blanquette	Wagga Wagga	14/9/1999		Blanquetta	Spain	no	Group IV
Blanquette - Early	Blackwood	21/12/1999	Frantoio	Blanquetta	Spain	no	
Blanquette - Late	Blackwood	14/9/1999		Blanquetta	Spain	no	Group II
Boothby's Lucca	Roseworthy	14/9/1999		Lucca	Blackwood	yes	Frantoio
Borregiola	Blackwood	21/12/1999	Frantoio	Frantoio	Italy2/Spain/Greece	no	Group V
Bouquettier	Blackwood	14/9/1999		Bouquettier	Roseworthy	yes	Group II
Bouteillon	Blackwood	21/12/1999		Bouteillon	Wagga Wagga	yes	Frantoio
Buchine	Blackwood	21/12/1999		Buchine	Blackwood	yes	
Californian Mission 1	Blackwood	21/12/1999		Mission	USA/Mexico	yes	
Californian Mission 2	Nursery	3/12/1998		Mission	USA/Mexico	no	Verdale (USA)
Columella	Wagga Wagga	14/9/1999		n/a			
Coratina	Nursery	3/12/1998		Coratina	Spain	yes	
Corregiola 1	Yanco	14/9/1999		Frantoio	Italy2/Spain/Greece	yes	
Corregiola 2	Nursery	21/12/1999		Frantoio	Italy2/Spain/Greece	yes	

NOVA Accession	NOVA Source	Planting Date	Rootstock	DNA Standard	DNA Standard Source	Match	Other Match
Cucco	Wagga Wagga	14/9/1999		Cucco	Wagga Wagga	yes	Gordal Sevillana
Del Morocco	Roseworthy	14/9/1999		Del Morocco	Roseworthy	yes	Group VII
Dr Fiasci	Wagga Wagga	14/9/1999		Dr Fiasci	Wagga Wagga	yes	
Emu Flat	Keith	22/12/1998		Emu Flat	Keith	yes	Frantoio
Frantago	Nursery	14/9/1999		Frantoio	Italy2/Spain/Greece	no	Group VI
Frantoio 1	Nursery	3/12/1998		Frantoio	Italy2/Spain/Greece	yes	
Frantoio 2	Nursery	14/9/1999		Frantoio	Italy2/Spain/Greece	yes	
Frantoja	Blackwood	14/9/1999		Frantoio	Italy2/Spain/Greece	yes	
FS17	Nursery	14/9/1999		n/a			
Gaeta	Blackwood	14/9/1999	Frantoio	Gaeta	Blackwood	yes	Group V
Gros Reddeneau	Blackwood	14/9/1999		Gros Reddeneau	Blackwood	yes	Verdale Aglandau
Hardy's Mammoth	Blackwood	14/9/1999		Hardy's Mammoth	Blackwood	no	Verdale Aglandau
Hojiblanca 1	Nursery	3/12/1998		Hoji Blanca	Spain	yes	
Hojiblanca 2	Nursery	14/9/1999		Hoji Blanca	Spain	yes	
I77	Nursery	3/12/1998		n/a			
Institute	Blackwood	14/9/1999		Institute	Blackwood	yes	
Jumbo Kalamata	Nursery	3/12/1998	Frantoio	n/a			
Kalamata	Nursery	28/1/1999	Feral	Kalamata	Italy2/Israel	yes	
Katsourela	Nursery	28/1/1999	Feral	Katsourela	Nursery	no	
Koroneiki/Maniataki/Badska	Nursery	22/12/1998	Feral	Koroneiki	Greece/Spain	yes	
Large Fruited	Wagga Wagga	14/9/1999		Large Fruited	Wagga Wagga	no	Group III
Large Fruiting	Blackwood	14/9/1999		Large Fruiting	Blackwood	yes	Group III
Large Pickling	Roseworthy	14/9/1999		Large Pickling	Roseworthy	yes	
Leccino	Nursery	3/12/1998		Leccino	Italy2/Israel	yes	
Leccure	Roseworthy	14/9/1999		Lucque	France	no	Frantoio
Longue d'Ascoli	Blackwood	14/9/1999	Frantoio	Longue d'Ascoli	Blackwood	yes	Group V
Lucca	Blackwood	14/9/1999		Lucque	France	no	Frantoio
Manaiki	Nursery	3/12/1998	Feral	Manaiki	Nursery	yes	
Manzanillo 1	Nursery	3/12/1998		Manzanilla de Sevilla	Spain	yes	
Manzanillo 2	Nursery	3/12/1998		Manzanilla de Sevilla	Spain	yes	
Manzanillo 3	Nursery	21/12/1999		Manzanilla de Sevilla	Spain	yes	
Marchiosa	Roseworthy	14/9/1999		Marchiosa	Roseworthy	yes	Verdale Aglandau
Marcocarpa	Wagga Wagga	14/9/1999		Marcocarpa	Wagga Wagga	yes	Group I
Mediterranean	Nursery	28/1/1999	Feral	Frantoio	Italy2/Spain/Greece	yes	
Morihioso	Blackwood	14/9/1999	Frantoio	Morihioso	Blackwood	yes	Group V

NOVA Accession	NOVA Source	Planting Date	Rootstock	DNA Standard	DNA Standard Source	Match	Other Match
Nab Tamri	Nursery	3/12/1998		n/a			Gordal Sevillana
Nevadillo Blanco	Wagga Wagga	21/12/1999		Nevadillo Blanco	USA	yes	
O de Grasse	Wagga Wagga	14/9/1999		O de Grasse	Wagga Wagga	yes	Group VII
Oblitza	Wagga Wagga	14/9/1999		Oblitza	Wagga Wagga	yes	
Oblonga	Wagga Wagga	14/9/1999		Oblonga	Wagga Wagga	yes	Group VI
Oje Blanco Doncel	Wagga Wagga	14/9/1999		Hoji Blanca	Spain	yes	
Palermo	Blackwood	21/12/1999		Palermo	Roseworthy	no	Group III
Palsano	Roseworthy	14/9/1999		Palsano	Roseworthy	yes	Frantoio
Paragon	Nursery	3/12/1998		Frantoio	Italy2/Spain/Greece	yes	
Pendolino	Nursery	3/12/1998		Pendolino	Italy2/Spain/Israel	yes	
Pendulina	Wagga Wagga	14/9/1999	Frantoio	Pendolino	Italy2/Spain/Israel	no	Group I
Picholine	Blackwood	21/12/1999	Frantoio	Pecholene	Italy1	no	Group V
Picual 1	Nursery	3/12/1998		Picual	Spain	yes	
Picual 2	Nursery	3/12/1998		Picual	Spain	yes	
Picual 3	Nursery	14/9/1999		Picual	Spain	yes	
Pigale	Roseworthy	14/9/1999		Pigale	Wagga Wagga	no	
Polymorpha	Wagga Wagga	14/9/1999		Polymorpha	Wagga Wagga	yes	Group I
Praecox	Wagga Wagga	14/9/1999		Praecox	Wagga Wagga	yes	
Pueblana	Blackwood	14/9/1999		Pueblana	Blackwood	yes	Frantoio
Queen of Spain	Nursery	3/12/1998	Frantoio	Sevillano	Israel	no	
Regalise de Languedoc	Blackwood	14/9/1999		Regalise de Languedoc	Blackwood	yes	
Rouget	Blackwood	14/9/1999		Rouget	Blackwood	yes	
Rubra Baillon d'Aise	Blackwood	21/12/1999		Rubra Baillon d'Aise	Blackwood	yes	Group IV
Salome	Blackwood	14/9/1999		Salome	Blackwood	yes	Verdale Aglandau
Sevillano	Nursery	3/12/1998		Sevillano	Israel	yes	Gordal Sevillana
Souri	Nursery	22/12/1998		Souri	Israel	yes	
Tarascoa	Roseworthy	14/9/1999		Tarascoa	Wagga Wagga	no	Verdale Aglandau
UC13A6	Nursery	3/12/1998		UC13A6	Nursery	yes	
Verdale 1	Wagga Wagga	14/9/1999		Verdale	USA	no	Group II
Verdale 2	Nursery	3/12/1998		Verdale	USA	yes	
Verdale 3	Blackwood	14/9/1999		Verdale	USA	no	
Volos	Nursery	21/12/1999		n/a			
Wallace	Nursery	3/12/1998		n/a			Koroneiki
WA Mission	Nursery	3/12/1998		Mission	USA/Mexico	no	Frantoio

Table 2. Lists of variety names from the Roseworthy trial with identical DNA fingerprints. Groups I-VII contain accessions that had identical fingerprints but did not match any international standard.

Frantoio (Italy2)	**Verdale Aglandau (France)**	**Gordal Sevillana (Spain)**	**Verdale (USA)**	**Hojiblanca (Spain)**
Belle de Espagne Boothby's Lucca Bouteillon Correggiola Emu Flat Frantoio Frantoja Leccure Lucca Mediterranean Palsano Paragon Pueblana WA Mission	Gros Reddeneau Hardy's Mammoth Marchiosa Salome Tarascoa	Cucco Nab Tamri Sevillano	Black Italian 1 Californian Mission 2 Verdale 2	Hoji Blanca Oje Blanco Doncel
Koroneiki (Greece)	**Arbequina (Spain)**	**Mission (USA)**		
Koroneiki Wallace	Arbequina Big Spanish	Attica Californian Mission 1		

Group I	**Group II**	**Group III**	**Group IV**	**Group V**
Marcocarpa Pendulina Polymorpha	Blanquette Late Bouquettier Verdale 1	Large Fruited Large Fruiting Palermo	Blanquette Rubra Baillon D'Aise	Borregiola Gaeta Longue de Ascoli Morihioso Picholine
Group VI	**Group VII**			
Frantago Oblonga	Del Morocco O'de Grasse			

Fruit Analyses

Table 3 shows fresh weight per tree, oil yield per tree, whole fruit weight, percentage oil in dried flesh, percentage moisture in the whole fruit, and flesh to pit ratio for all varieties in the survey where three or more trees had sufficient fruit. There were significant ($P<0.001$) differences between varieties, so a protected least significant difference can be used. The data in Table 3 are backtransformed means, so the corresponding least significant difference becomes a least significant difference ratio. For example in fresh weight per tree, the ratio of yield between Areccuzo and Arbequina is less than 6.07 so there is no significant difference. However, the ratio between Arbequina and Barouni exceeds 6.07 so those two varieties differ at the $P < 0.05$ level.

Unfortunately, Koroneiki had its fruit removed by starlings before it was due to be harvested however observations indicated it would have produced similar yields as Arbequina.

Table 4 shows the means of the fatty acid concentrations of the same varieties. There was no significant effect of the replicate blocks, maturity of the crop or tree age. No corrections were therefore applied to the mean.

Table 3 - Fresh weight per tree, oil yield per tree, whole fruit weight, percentage oil in dried flesh, percentage moisture in the whole fruit, and flesh to pit ratio for all varieties in the survey where three or more trees had sufficient fruit. LSR is the minimum ratio required for two varieties to differ.

Variety	**Fresh Weight of fruit per tree (gms)**	**Oil yield per tree (gms)**	**Average fruit weight (gms)**	**% oil in dry flesh**	**% water in whole fruit**	**Flesh:pit ratio**
Arbequina	2247.0	351.1	2.2	62.4	64.2	8.5
Areccuzo	5089.4	557.8	2.4	54.9	69.1	8.8
Azapa	2568.1	197.0	7.2	29.8	66.3	11.8
Barnea	3174.2	462.9	4.4	63.7	68.3	10.8
Barouni	195.6	21.4	10.2	40.1	63.9	10.7
Columella	919.5	118.7	4.8	52.6	65.5	9.4
Coratina	925.8	131.6	4.7	50.2	58.1	7.8
Frantoio	308.6	48.6	3.7	54.9	59.2	7.9
Gordal Sevillana	191.2	21.0	10.9	43.7	68.1	13.9
Group III	448.2	37.1	3.7	38.9	69.9	10.9
Group VI	1203.8	96.2	2.8	40.3	66.7	6.8
Group VII	2044.5	263.9	3.4	51.4	64.5	9.1
Hojiblanca	985.2	81.3	4.6	35.9	68.0	10.4
I77	940.9	134.7	6.0	53.5	62.0	8.5
Jumbo Kalamata	270.0	32.6	12.2	41.5	64.2	14.4
Kalamata	163.1	26.6	5.1	52.0	57.7	9.3
Katsourela	411.6	49.7	4.4	44.3	63.1	9.9
Large Pickling	1454.0	183.4	3.0	48.6	62.7	8.3
Manaiki	97.1	16.2	4.7	54.7	61.4	12.4
Manzanillo	915.2	83.8	6.3	37.6	68.6	13.9
Oblitza	3323.8	277.6	6.2	42.3	71.8	11.9
Pendolino	322.4	27.7	2.4	36.8	62.7	6.4
Picual	4504.6	468.3	4.4	49.4	69.2	9.3
Rouget	2195.8	176.4	2.8	46.4	74.0	12.2
UC13A6	770.1	36.3	10.8	18.8	72.3	12.7
Verdale (SA)	1133.8	69.6	7.3	35.6	73.0	9.1
Verdale Aglandau	408.8	49.9	4.3	46.1	64.6	10.4
LSR	6.07	6.85	1.26	1.30	1.10	1.18

Table 4 - Mean percentage composition of six fatty acids from olive trees at the NOVA site. The table includes the maximum Standard Error of the Difference between varieties, the least significant difference and the percent of the variation explained by the varieties. The accepted limits for fatty acid composition of Virgin Olive Oil (International Olive Oil Council 2001b) are shown in the first row.

	% Composition					
Variety	**Palmitic**	**Palmitoleic**	**Stearic**	**Oleic**	**Linoleic**	**Linolenic**
Accepted Limits	7.5-20	0.3-3.5	0.5-5.0	55.0-83.0	3.5-21.0	≤ 1.0
Arbequina	14.63	2.49	1.56	66.38	13.20	0.68
Areccuzo	12.35	0.67	1.50	67.88	15.37	1.40
Azapa	16.20	2.55	2.22	62.60	14.68	0.92
Barnea	10.43	1.05	1.97	73.38	12.02	0.60
Barouni	12.13	1.93	1.60	73.47	9.23	0.70
Columella	14.70	2.80	1.18	59.30	20.25	0.90
Coratina	10.54	0.28	1.80	77.80	7.90	0.78
Frantoio	12.25	1.40	1.85	70.90	12.10	0.78
Gordal Sevillana	12.99	1.12	1.86	66.11	14.99	1.32
Group III	14.57	1.97	2.23	68.77	10.73	1.03
Group VI	11.03	1.05	1.33	78.30	6.03	1.28
Group VII	13.45	1.26	2.34	70.15	10.36	1.03
Hojiblanca	11.17	0.30	2.87	74.67	9.00	0.95
I77	10.12	0.24	1.44	77.82	8.50	0.90
Jumbo Kalamata	13.54	1.56	2.94	66.14	14.34	0.82
Kalamata	8.90	0.90	1.58	76.47	10.60	0.53
Katsourela	17.40	2.23	2.00	54.83	21.93	1.07
Large Pickling	16.98	2.78	1.97	66.93	9.87	1.02
Manaiki	11.40	0.47	2.40	64.23	20.20	0.50
Manzanillo	13.24	1.87	2.92	72.97	7.39	0.73
Oblitza	12.05	0.47	2.58	71.18	11.22	1.18
Pendolino	13.95	1.72	1.33	74.22	7.40	0.90
Picual	11.99	1.45	2.30	79.31	3.59	0.87
Rouget	15.26	1.78	2.10	61.22	17.70	1.30
UC13A6	15.37	2.73	1.23	67.07	12.73	0.53
Verdale (SA)	13.78	1.48	2.07	65.07	14.80	1.51
Verdale Aglandau	14.93	2.43	1.98	65.28	13.00	1.08
SED (max)	0.91	0.30	0.21	2.35	1.35	0.20
LSD (95%)	1.79	0.59	0.41	4.66	2.67	0.39
F Ratio	18.34	26.08	22.34	26.66	44.09	9.38
Variation explained	75%	85%	80%	85%	87%	65%

Tree Growth

The results of the analyses are summarised in Table 5. The varieties were defined by DNA testing on each tree. The height and diameter measures are corrected for planting date and height or diameter at the time of planting. Replicate effects were also removed.

Significance can be gauged using the standard errors of the differences (SEDs) that are estimates for maximum, average or minimum replication (as shown at the bottom of the tables). Any difference that exceeds two SEDs differs significantly at the $P < 0.05$ level.

Table 5 Mean Height and Diameter of 53 varieties of olives.

Variety	Height (cm)	Diameter (mm)	Number of Trees
Amelon	266.4	62.67	6
Arbequina	264.5	55.61	18
Areccuzo	220.1	57.46	6
Ascolano	296.0	60.44	6
Atro Rubens	215.5	39.46	6
Atroviolacea Brun Ribier	295.3	45.16	6
Azapa	250.8	59.07	6
Barnea	396.1	86.39	12
Barouni	273.8	72.93	6
Benito	279.0	75.34	6
Black Italian (Blackwood)	300.7	52.61	6
Blanquette - Early [1]	169.1	41.00	5
Buchine	250.5	56.11	6
Columella	301.0	62.63	6
Coratina	225.1	58.64	6
Dr Fiasci	299.9	68.46	6
Frantoio [2]	274.5	68.29	96
FS17	265.9	56.48	6
Gordal Sevillana	269.2	60.24	18
Group I [2]	288.3	68.81	18
Group II	274.0	64.22	18
Group III	266.6	63.03	17
Group IV	310.7	61.70	12
Group V [1]	318.5	57.93	29
Group VI	309.9	64.54	6
Group VII	254.4	68.34	12
Hojiblanca	308.7	64.63	17
I77	269.2	53.55	6
Institute	256.4	49.31	6
Jumbo Kalamata [1]	259.4	61.97	6
Kalamata [1]	237.7	56.08	6
Katsourela [1]	203.0	42.98	6
Koroneiki [1]	274.4	65.67	12
Large Pickling	245.7	69.29	6
Leccino	319.0	79.24	4
Manaiki [1]	310.8	72.36	6
Manzanillo	295.2	66.75	17
Mission (Californian)	326.8	64.51	12
Nevadillo Blanco	268.7	65.36	6
Oblitza	321.4	60.79	6
Pendolino	307.4	75.41	6

Picual	294.4	63.00	15
Pigale	301.2	84.79	6
Praecox	243.6	56.75	6
Queen of Spain [1]	258.6	68.83	4
Regalise de Languedoc	250.0	37.04	6
Rouget	272.9	72.5	6
Souri	219.8	50.28	3
UC13A6	315.9	87.18	6
Verdale (Blackwood)	252.7	74.35	6
Verdale (SA)	239.3	51.24	18
Verdale Aglandau	266.5	58.11	30
Volos	324.7	59.44	3
SED Average	16.4	5.68	
SED Maximum	25.2	8.78	
SED Minimum	6.6	2.26	

[1]Grafted
[2]Some trees from this group grafted

The analyses were consistent across blocks. An estimate of this can be made by calculating the sum of squares explained by the varieties expressed as a percentage of the total between plot sum of squares (less that explained by planting date and initial size). The amounts explained respectively for height and diameter were 66% and 67%.

Commercial Scale Evaluation

Fruit Analyses

Table 6 shows; the number of samples per variety, average MI, whole fruit weight, percentage water in the whole fruit (raw and adjusted data), percentage oil in dried flesh (raw and adjusted data) and flesh to pit ratio for all varieties in the survey, where four or more samples were submitted. Table 7 shows the adjusted means of the fatty acid composition for the same varieties and Table 8 shows the effect of maturity on adjusted fatty acid percentage.

In Australia, varieties of unknown origin are often given colloquial names (Burr, 1998). Examples in this study include: UC13A6, SA Verdale, Paragon, WA Mission and Mediterranean. As these trees were not DNA tested, they were not grouped into synonyms as per the National Collection. The other varieties in this study are commonly grown in various olive producing regions throughout the world (Fontanazza, 1996).

Levels of oleic acid in different regions

Figures 2 and 3 show observed levels of oleic acid in Manzanilla de Sevilla and Frantoio respectively in different regions of Australia. These two varieties have been highlighted since many fruit samples of these varieties were received in this study. As well, they have consistently proven to be true to type when DNA tested using the RAPD technique (Mekuria *et al.*, 1999). Samples of the varieties labelled Correggiolo and Paragon were included in the Frantoio data as DNA fingerprinting using randomly amplified polymorphic DNA (RAPD) shows that both Paragon (Archer, 1999, Guerin *et al*, 2002) and Correggiolo (Guerin *et al*., 2002, Mekuria *et al.*, 1999) have a high genetic similarity to Frantoio.

Table 6 – Olive varieties with more than 3 samples showing average Maturity Index (MI), fruit weight, percentage water in whole fruit and percentage oil in dried flesh (observed and adjusted) and flesh to pit ratio. The standard error difference (SED) is based on 121 degrees of freedom.

				% water in whole fruit		% oil in dry flesh		
Variety	**Number of samples**	**Average MI**	**Average Fruit Weight (gms)**	**Observed**	**Adjusted**	**Observed**	**Adjusted**	**Flesh:Pit ratio**
Arbequina	10	3.6	2.21	56.0	56.4	48.0	47.7	5.1
Barnea	4	3.5	3.45	61.0	61.3	56.9	56.8	7.3
Coratina	7	3.1	4.46	55.7	55.1	53.4	54.0	6.4
Correggiolo	12	3.3	2.98	48.2	48.6	54.0	54.3	5.1
Frantoio	8	3.8	2.97	46.0	47.7	51.1	50.6	5.1
Kalamata	6	3.9	4.2	52.5	53.4	44.2	43.4	7.4
Koroneiki	4	3.0	1.09	47.8	47.5	50.6	51.4	4.5
Leccino	6	4.3	3.75	48.6	50.7	46.2	44.7	5.8
Manzanilla de Sevilla	41	3.6	5.13	61.8	62.4	44.2	43.9	10.0
Mediterranean	6	3.2	2.84	52.3	51.8	46.2	46.6	4.3
Nevadillo Blanco	5	3.9	3.54	49.1	50.2	50.0	49.2	7.4
Paragon	11	2.8	2.34	44.7	43.4	54.0	55.2	4.5
Pendolino	4	3.2	2.48	59.2	59.3	33.6	34.1	5.8
Picual	12	3.5	4.5	57.5	57.8	44.4	44.3	8.1
SA Verdale	15	3.2	5.7	64.8	64.6	34.1	34.4	6.7
UC13A6	4	3.3	9.13	67.9	67.9	33.7	34.0	10.9
WA Mission	4	3.3	2.26	40.3	40.6	48.8	49.1	4.2
SED Maximum		0.6	0.58	4.2	4.1	7.1	7.0	1.0

Table 7 - Fatty acid % composition of olive varieties in this study with more than 3 samples. The SED is based on 121 degrees of freedom. The accepted limits for fatty acid composition of Virgin Olive Oil (International Olive Oil Council 2001b) are shown in the first row.

			% Composition			
Variety	**Palmitic**	**Palmitoleic**	**Stearic**	**Oleic**	**Linoleic**	**Linolenic**
Accepted Limits	7.5-20	0.3-3.5	0.5-5.0	55.0-83.0	3.5-21.0	≤ 1.0
Arbequina	16.1	2.3	1.7	68.5	9.7	0.5
Barnea	11.7	0.9	1.9	71.5	12.9	0.6
Coratina	11.6	0.7	2.1	75.6	8.8	0.5
Correggiolo	13.5	1.1	2.0	71.3	10.7	0.7
Frantoio	14.3	1.2	2.1	69.1	12.0	0.6
Kalamata	10.6	0.7	1.9	75.8	9.2	0.6
Koroneiki	13.4	1.0	2.5	75.9	5.9	0.7
Leccino	14.2	1.2	2.0	75.0	6.5	0.6
Manzanilla de Sevilla	13.1	1.4	3.0	74.5	6.1	0.6
Mediterranean	14.9	1.5	1.9	70.5	9.7	0.7
Nevadillo Blanco	13.1	0.7	1.5	68.9	14.0	0.8
Paragon	11.7	0.7	2.0	75.7	8.4	0.6
Pendolino	14.2	0.9	1.4	73.6	8.4	0.8
Picual	13.8	1.3	2.5	77.2	3.9	0.6
SA Verdale	16.2	1.3	2.2	66.3	11.5	1.1
UC13A6	14.1	2.1	1.7	72.2	8.6	0.5
WA Mission	15.3	1.6	2.0	66.9	12.9	0.7
SED Maximum	1.2	0.3	0.3	2.9	2.0	0.1

Table 8 – Percentage change of fatty acid composition per unit change in Maturity Index (MI).

	Palmitic	**Palmitoleic**	**Stearic**	**Oleic**	**Linoleic**	**Linolenic**
% change/unit of MI.	-0.164	0.079	0.278	-0.623	0.505	-0.013
SE	0.182	0.044	0.051	0.438	0.296	0.025
t (121 d. of f.)	-0.901	1.795	5.416	-1.422	1.709	-0.502

Fig. 2 – Observed levels of oleic acid in Manzanilla de Sevilla samples

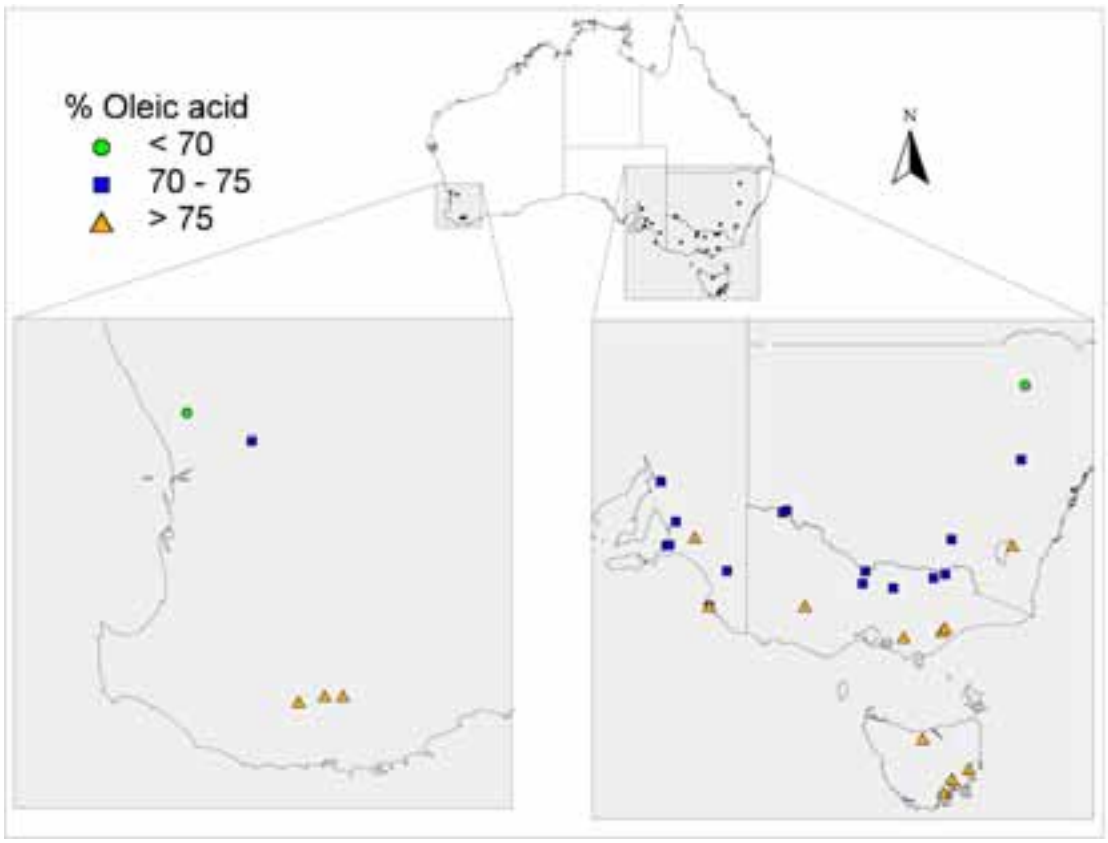
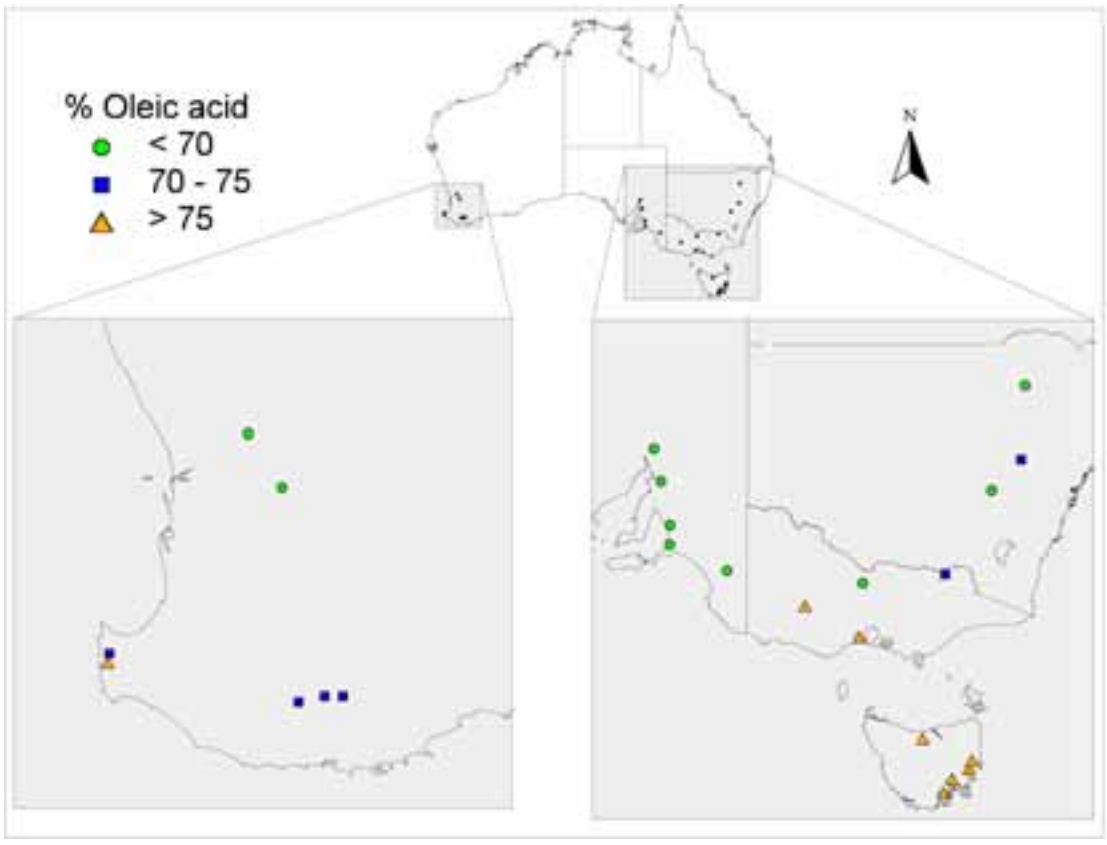


Fig. 3 – Observed levels of oleic acid in Frantoio samples (includes Paragon and Correggiolo samples)



5. Discussion of Results

National Collection

DNA Analyses

Standard Matches:
Many of the varieties planted at Roseworthy were sourced from well known nurseries that are selling certified trees or in the process of DNA testing their mother stock. Most of these varieties matched with the correct international standard including: Arbequina, Barnea, Coratina, Frantoio, Hojiblanca, Kalamata, Koroneiki, Leccino, Manzanillo, Nevadillo Blanco, Pendolino, Picual, Sevillano, Souri and one of the Verdales. This is a reassuring result for the Australian industry as these are all popular variety choices.

However there is clearly confusion with other varieties. Californian Mission has been widely mislabelled in Australia, with the accession sourced from a nursery in this study matching with Verdale (USA). However the Californian Mission accession from Blackwood did match with the international standard, as well as an accession named Attica from Wagga Wagga. Similarly, it was believed that the commercially valuable Spanish varieties Hojiblanca and Arbequina had not been introduced to Australia. Consequently they were recently imported and propagated at considerable effort and expense. This study has revealed that they were already in Australia under the synonyms of Oje Blanco Doncel and Big Spanish respectively.

Where no other standards were available, in some cases both the standard and the NOVA samples were originally derived from the same mother tree. In three of these instances (Amelon, Hardy's Mammoth and Large Fruited) the NOVA fingerprint did not even match the standard from the same mother tree. This may have occurred by leaves being taken from different parts of the tree which could have been part rootstock and part scion, or simply a mix up with labelling during collection or in the laboratory.

Synonyms:
The investigation by Guerin et al (2002) into the genetic identity of accessions within the NOVA collection has shown that many of the commercially used varieties known under different names have identical DNA fingerprints ie they are synonyms. Of the 100 NOVA accessions tested (which were supposedly 87 different varieties), only 53 different genotypes were detected. Table 2 lists the 46 NOVA accessions which have found to be synonyms with one of 15 other varieties in the collection.

While it was not surprising to find some synonyms, it was remarkable that 14 differently named varieties were of the same genotype as the Italian Frantoio. The synonyms, Paragon, Frantoja and Correggiola, have previously been reported (Archer, 1999; Mekuria et al., 1999) and the current work confirms these earlier results. The synonyms Emu Flat, Paragon, WA Mission and Mediterranean originated within Australia. This proliferation of new names has arisen through the collection and propagation of trees with good oiling potential but of unknown origin.

Others synonyms of Frantoio found in this study were Belle de Espagne, Bouteillon, Pueblana, Palsano, Lucca, Boothby's Lucca and Leccure. This indicates that Australia may not be the only country with naming confusion as there is documented evidence from the Blackwood collection to show that Pueblana, Bouteillon and Lucca were sourced directly from Italy and Frantoja from France. There is a small chance that all of these varieties were grafted on Frantoio rootstock that eventually overtook the scion material but this is unlikely. The Frantoja accession may simply have been a transcription error.

Oblonga has also been found to be a synonym of Frantoio (Barranco and Trujillo, 2000). However, while the accession named Oblonga from the NOVA trial was genetically identical to another NOVA sample named Frantago, it did not match the Italian Frantoio.

Three accessions of Verdale had different fingerprints with none of them matching the French Verdale Aglandau standard, although five other NOVA accessions did have the same fingerprint as the Verdale Aglandau standard. Verdale 2 matched the standard from USA that has also been shown to match other Australian accessions called 'SA Verdale' (Mekuria et al., 1999). Black Italian 1 and Californian Mission 2 also had matching fingerprints to the USA Verdale. Verdale 1 from Wagga Wagga showed high genetic similarity with Bouquettier and Blanquette late, and Verdale 3 was genetically similar but not identical to Large Pickling.

Tree Growth

Barnea was clearly the tallest variety. However for diameter, no one variety was significantly larger than the rest, although the top three (UC13A6, Barnea and Pigale) were apparently larger than the others, and significantly so compared with all but the other top 10 varieties.

National Collection and Commercial Scale Fruit Analyses

Fresh Weight of Fruit per tree

The fresh weight of fruit value in Table 3 is particularly important for table olive varieties such as Gordal Sevillana, Hojiblanca, Manzanilla de Sevilla, Verdale SA and UC13A6 as growers are generally paid on yield. For these varieties, only Gordal Sevillana has a significantly lower yield than the other table olive varieties.

As indicated in the results, Koroneiki had its fruit removed by starlings but previous observations showed it had a reasonable fresh yield.

Oil Yield per Tree

The oil yield per tree shown in Table 3 is most important for the oil producing varieties. This laboratory derived figure is not directly related to what the oil yield would be from a commercial press however it does give a relative difference between varieties. From these results the best performing varieties in terms of oil yield, in the early stages of this trial are: Areccuzo, Picual, Barnea, Arbequina, Oblitza and Group VII. However, there are many other factors to consider when assessing suitability for oil production such as ease of oil extraction and oil composition.

Fruit Weight

Fruit weight is one of the main variables, along with fruit removal force, considered when assessing the suitability of an olive variety for mechanical shaking at harvest (Civantos, 1996). If all other variables are equal, Tables 3 and 6 show that varieties such as Arbequina, Areccuzo, Koroneiki and Pendolino, which have a low fruit weight, may not be dislodged by mechanical shaking as easily as some of the varieties with heavier fruit such as Jumbo Kalamata, Gordal Sevillana, Barouni and UC13A6. However, a further complication is that many of the larger fruit are table varieties that may not be suitable for mechanical harvest anyway due to susceptibility to bruising.

Oil Content in Dry Flesh

Oil content was represented as a percentage of dry matter rather than fresh weight, as the former variable reaches a maximum value and then stays constant whereas the latter tends to increase with ripening. This occurs because oil synthesis in olives stops after a certain ripening stage whereas the

water content decreases (Di Giovacchino, 1996). In addition, the dry flesh oil percentage is more stable than the whole dry fruit oil percentage (Del Rio and Romero, 1999) and is more useful in characterising olive varieties. However, it cannot be directly equated to the amount of oil that can be extracted from the fruit in a commercial processing plant because of factors such as; ease of oil extraction, polydispersity of flesh to pit ratios and variation in moisture contents across orchards.

For both the National Collection and the Commercial Scale results, there was no significant effect of fruit maturity on the oil percentage in the dry flesh within the range of fruit maturities at the $p < 0.05$ level. There were however large differences in oil content observed across the different varieties examined. If Picual is used as an indicator of a recognised high oil yielding variety then a number of varieties with higher yields than Picual, showed promise as high oil yielders to the extent that oil % in dry flesh is a predictor of oil yield (Tables 3 and 6). These varieties are: Arbequina, Areccuzo, Barnea, Columella, Coratina, Correggiola, Frantoio, Group VII, I77, Kalamata, Koroneiki, Leccino, Manaiki, Mediterranean, Nevadillo Blanco, Paragon and WA Mission. It should be noted however, that the commercial results from Table 6 do not examine total oil yields of the varieties investigated since cropping data is required. For example, a variety with high oil content may not be desirable if the total amount of fruit produced is low.

Water in whole fruit

For the 2001 commercial survey, the percentage of water in the flesh was significantly affected ($p \leq 0.01$) by fruit maturity. With each unit of increasing MI, the water content decreased by 1.9%. High water content of fruit can make commercial oil extraction difficult due to oil/water emulsions being formed during malaxation (Di Giovacchino, 1996). This study shows the relative differences between varieties in water content of the fruit. Generally the varieties with higher fruit water contents are considered table olive varieties such as UC13A6, SA Verdale and Manzanilla de Sevilla (Burr, 1998). Table 6 also shows that these three varieties had the highest average weight compared to all other varieties.

Flesh to pit ratio

Flesh to pit ratio is an indicator of suitability of olives for table fruit with a ratio greater than 5:1 being regarded as desirable (Burr, 1998). Tables 3 and 6 shows that many of the varieties have ratios greater than 5:1, indicating their suitability for table olive production. However, there are many other factors such as size, shape, ease of pitting, colour and texture that are also of great importance (Garrido Fernandez *et al.*, 1997) that are not considered in this study.

Fatty acid composition

The ranges of fatty acid composition for most of the varieties listed in Tables 4 and 7 fall within the accepted limits for fatty acid composition of Virgin Olive Oil (International Olive Oil Council, 2001b). However the exception is for linolenic acid where a number of varieties recorded levels higher than the 1% limit. The 2002 ripening season at Roseworthy was unusually cool and may account for the high linolenic acid levels (Mailer, 2003).

Table 8 shows that within the range of fruit maturities received for the commercial survey, stearic acid, which is a minor component of the fatty acid profile, was the only one which was significantly affected by maturity ($p \leq 0.001$), increasing with increasing maturity. Although there was an apparent effect of maturity on linoleic and palmitoleic (increasing with maturity) and oleic (decreasing with maturity), they were not statistically significant.

Levels of oleic acid in different regions

A high level of oleic acid is considered favourable in olive oil due to enhanced oxidative stability (Smouse, 1996) and superior nutritional quality (Kritchevsky, 1996). There are many studies on location effects on oleic acid in Mediterranean countries which have been summarised in Boskou, (1996) and Fiorino, (1996). These studies indicate that oleic acid levels decrease in line with more southerly latitudes in Northern Hemisphere countries. However, there are no similar studies in Australia. This survey indicates that location may affect levels of oleic acid in the fatty acid profile of the oil. A trend is apparent (Figs. 2 and 3) showing that the samples of fruit from Manzanilla de Sevilla and Frantoio that come from more southerly latitudes in Australia exhibit higher oleic acid levels than samples from more northerly latitudes.

6. Implications

National Collection

DNA Analyses

The identification of genetically identical synonyms is of enormous significance to the Australian olive industry. Many of these supposedly different varieties have been popular choices due to their good oiling reputation. It is now possible to ensure that groves, which are planned to contain different varieties, do not inadvertently contain genetically identical material with consequent deleterious impacts on pollination efficacy and fruit set (Wu et al., 2000), and ultimately financial return.

The plethora of variety names is also confusing for variety selection and labelling of varietal oils and table fruit. As well, the product end-use will depend on the type of olive produced. The variety names Belle de Espagne and Big Spanish are likely to be associated with table fruit, whereas the accessions grown in the NOVA trial were genetically similar to Frantoio and Arbequina respectively, which are both oiling varieties with small fruit.

Not only were there many misnamed varieties in the NOVA collection, in 11% of the samples, the 6 replicate trees were not identical and the anomalous trees are being removed from the collection. This result highlights the difficulties in initially recognising specific varieties and subsequently ensuring that lines are reliably maintained.

Care must be taken in interpretation of the results to confine them to the individual trees tested and not to extrapolate to all accessions of the same name, as they may have different genotypes. For example the Palermo fingerprint from Blackwood did not match the Palermo fingerprint from Roseworthy and the Tarascoa from Roseworthy did not match Tarascoa from Wagga Wagga.

Mekuria et al. (1999) and Gemas et al. (2000), have shown that intra-variety variation in olives has been detected using the RAPD technique. However, not all clonal selections can be distinguished by DNA fingerprinting where differences have arisen through somatic mutation and may occur at only one or more sites in the genome (Bowers et al., 1993). Small changes in the genetic structure would be difficult to detect using RAPD, or any other genotyping method, but may affect the agronomic performance of the tree (Guerin et al, 2002).

The NOVA collection is also providing physiological data for each accession that will be important to compare with the DNA fingerprinting results in the future. Varieties with similar RAPD fingerprints but differing in agronomic qualities could be studied to find genetic markers for those traits (Guerin et al, 2002).

Tree Growth

Tree size is an important consideration for straddle harvesters which can generally only harvest trees less than 2.5m tall. However, pruning can reduce the height of the taller varieties although naturally smaller varieties would probably require less pruning management.

Smaller varieties that still produce high fruit yields may be more efficient at converting water and nutrients to fruit than more vegetative varieties and so may be more suited to regions where water supplies are limited. There is insufficient data on these young trees to draw any conclusions however this will be monitored as the trees mature.

National Collection and Commercial Scale Fruit Analyses

Fresh Weight and Oil Yield of Fruit per tree

Although the Roseworthy trees are not mature, these results do give an indication of which varieties may produce higher yields at a young age in this environment, which is important for early economic returns in an olive venture. However, as mentioned in the discussion, there are many other issues to consider in the suitability of olives for either oil or table fruit production. The other varieties in the trial not included in Table 3 did not have sufficient fruit for statistical analysis, which could mean they are not suitable for early fruit production in this environment.

Although Koroneiki was not the only small fruited variety at the site, it was the only variety that suffered significant starling damage. This may be because it was one of the later maturing varieties and other food sources for the starlings had disappeared which could indicate that small fruited, late maturing varieties are more vulnerable to bird attack in this environment. This could also mean that in areas of environmental sensitivity, small fruited, late maturing olives have a higher potential to turn feral from seed dispersal by birds than large fruited, earlier maturing olives.

Fruit Oil and Water Content

If maturity does not affect oil content on a dry weight basis (within the range of fruit maturities received) then growers need to focus on water content of fruit for ease of processing. The optimum water content to maximise oil yield is approximately 55-65% (G. Jones, pers comm)

The question arises as to whether irrigation management can be used to control fruit water content before harvest, particularly those varieties that naturally have high water content, if they are to be processed for oil. If the water regime does affect the water content of the fruit, those varieties that naturally have high water content may not be suitable for oil production in climates with high rainfall preceding and/or during the harvest period.

Fatty acid composition

The linolenic acid levels for many of the varieties was higher than the IOOC limit of 1.0% set for virgin olive oil although it has been observed that linolenic acid levels in SA Verdale decrease with maturity (Jones *et al.*, 2001). Those producers using these varieties for virgin oil production should be aware of this factor. If these oils are tested in export markets and found to exceed the allowable linolenic acid limits, the virgin classification of the olive oil may be in question.

As a saturated fatty acid, stearic acid is less desirable than unsaturated fatty acids in the human diet (Grande Covian, 1996). These results indicate that less mature fruit may produce healthier oil in respect to stearic acid content.

Levels of oleic acid in different regions

The data does indicate a trend toward higher levels of oleic acid in fruit from more southerly latitudes however it may have been caused by another factor and requires further investigation.

7. Recommendations

The DNA typing of the National Collection at Roseworthy has made significant advances into the positive identification of olive varieties in Australia. The database should be utilised by the industry, particularly by propagation facilities, to ensure positive identification of olive varieties in the future.

The collection at Roseworthy is unique in that every tree has been DNA typed as well as being evaluated physiologically. The collection can provide the Australian industry with reliable genetic material and could be the basis of a plant improvement collection for the industry.

However the trees have yet to reach maturity and data collection and evaluation needs to continue for a number of years to gain a full picture of the variety production potentials. Sensory evaluation will give more quality information on the olive oil and this will be assessed on individual varieties from Roseworthy in Stage II of the project.

At this stage the fruit analyses from the commercial properties has revealed much about the performance of the varieties. However more data is required on total yields, health and vigour to gain a fuller picture on variety suitability. This information will be more fully captured in Stage II of the project.

If linolenic acid is inherently high in olives grown under Australian conditions, then action should be taken by the Australian industry to increase the allowable limit under the IOOC classification guidelines.

Irrigation management for controlling fruit water content is an important issue for the Australian industry and warrants further research, as the varieties that naturally have high water content have already been widely planted for oil production, either under irrigation or in these climate zones.

The laboratory method described for extracting oil from the olive samples, while useful, does not duplicate conditions in commercial processing plants. An experimental processing facility is needed to monitor quality of oil produced under realistic commercial processing conditions with the type and quality of fruit being processed. Such a machine should be of the order of 50kg/hr. With this small processing mill it would be possible to forge the links between fruit maturity and quality at harvest and oil quality and yield in an environment which relates to that in commercial mills. In particular several parameters need to be investigated (in addition to fatty acids and oil content). These include: total phenolics and the compounds responsible for bitterness and pungency in olive oil: oleauropin and deacetoxy.

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