Horticulture Innovation Australia

Final Report

Assisting the development of the avocado oil industry in Australia and New Zealand

Dr Allan Woolf
HortResearch Ltd

Project Number: AV03007

AV03007

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Woolf A, Requejo-Jackman C, Olsson S, McGhie T, Wohlers M, Hofman P, Stubbings B, Wong M, Wang Y.

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Woolf A, Requejo-Jackman C, Olsson S, Wohlers M.
The Horticulture and Food Research Institute of New Zealand Ltd
Mt Albert Research Centre
Private Bag 92169
Auckland Mail Centre, Auckland 1142
NEW ZEALAND
Tel: +64-9-815 4200
Fax: +64-9-815 4201

McGhie T
The Horticulture and Food Research Institute of New Zealand Ltd
Palmerston North Research Centre
Private Bag 11030
Manawatu Mail Centre, Palmerston North 4442
NEW ZEALAND
Tel: +64-6-356 8080
Fax: +64-6-354 6731

Hofman P, Stubbings B
Horticulture and Forestry Sciences
Maroochy Research Station
PO Box 5083, Sunshine Coast Mail Centre
Nambour, Queensland 4560
AUSTRALIA

Wong M, Wang Y
Institute of Food, Nutrition and Human Health
Massey University
Private Bag 102-904, Auckland
NEW ZEALAND

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Research Scientist

Date: 31 July 2007

Group Leader, Quality Systems

Date: 31 July 2007

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Assisting the Development of the Avocado Oil Industry in Australia and New Zealand

Allan Woolf, et al.

Research Provider: The Horticulture and Food Research Institute of New Zealand Ltd

Horticulture Australia Limited Project Number AVO3007

Project Leader: Dr. Woolf A.
The Horticulture and Food Research Institute of New Zealand Ltd
HortResearch Mt Albert
Private Bag 92 169
Auckland Mail Centre
Auckland 1142
NEW ZEALAND
Tel: +64-9-815 4200
Fax: +64-9-815 4201

Key Personnel: Requejo-Jackman C, Olsson S, Wohlers, M.
The Horticulture and Food Research Institute of New Zealand Ltd
HortResearch Mt Albert
Private Bag 92 169
Auckland Mail Centre
Auckland 1142
NEW ZEALAND
Tel: +64-9-815 4200
Fax: +64-9-815 4201

McGhie T.
The Horticulture and Food Research Institute of New Zealand Ltd
Palmerston North Research Centre
Private Bag 11030
Manawatu Mail Centre
Palmerston North 4442
NEW ZEALAND
Tel: +64-6-356 8080
Fax: +64-6-354 6731

Hofman P, Stubbings B
Horticulture and Forestry Sciences
Maroochy Research Station
PO Box 5083, Sunshine Coast Mail Centre
Nambour, Queensland 4560
AUSTRALIA

Wong M, Wang Y
Institute of Food, Nutrition and Human Health
Massey University
Private Bag 102-904
Auckland
NEW ZEALAND

The purpose of this report: Final Report on Avocado Oil Study in Australasia.

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MEDIA SUMMARY

The flesh of avocado fruit contains approximately 8-24% oil for fruit harvested in the commercial harvest season. Some procedures for extracting the oil produce high oil yields, but the oil loses its characteristic flavour, colour and health benefits. “Extra virgin” or “cold-pressed” oils are extracted using more “gentle” procedures so that much of the colour, flavour, and specific “health-promoting” compounds of the fresh fruit are retained. These include “good” (mono-unsaturated fatty acids) oils, Vitamin E, plant pigments (antioxidants providing disease protection), plant sterols, fibre and Vitamin B6.

Commercial production of extra virgin avocado oil commenced in New Zealand in 2001, and in Brisbane, Australia in 2004. Several factors can affect the efficiency of oil extraction, and also oil quality, and these factors need to be well understood to develop a viable avocado oil industry. This project investigated the oil yield from the main avocado cultivars from some of the major production regions in Australia and New Zealand through the harvest season and from a range of growers over two seasons. Oil quality (content of some health-promoting compounds) was also studied in some samples.

In Australia and New Zealand, both dry matter and oil content increased as the fruit matured. In New Zealand, these tended to reach a maximum in about January, but this varied in Australia because of the larger differences in growing climate. Maximum oil yield (laboratory-based extraction) varied from about 10% in early season fruit, to about 29% in late season fruit with some regional differences. Commercial extraction using cold-pressed techniques resulted in lower yield comparatively, but the results suggest commercial yields of 25% could be possible for harvested fruit later in the season. There was little difference in maximum oil yield from fruit of the same maturity from Australia or New Zealand, suggesting similar commercial potential in both countries. However, New Zealand growers generally harvest for fruit with higher potential yield, so that commercial viability in New Zealand may be higher. This factor may change if the Australian industry changes its minimum fruit maturity level.

One of the key challenges for an avocado processing industry in Australia and New Zealand is consistency of fruit supply, particularly of fruit with adequate oil yield and fruit quality. This will be the major factor determining long-term commercial viability.

TECHNICAL SUMMARY

The flesh of avocado fruit contains approximately 8-30% oil for fruit harvested in the commercial harvest season. Avocado oil has been commercially extracted for many years using a range of extraction processes. Extraction using organic solvents or high temperatures (about 70°C) produces the highest oil yield, but the quality is low because of loss of its characteristic flavour, colour and health benefits. As a result, oils extracted in this way are used primarily for industrial purposes in the pharmaceutical and cosmetic industries.

"Extra virgin" or "cold-pressed" oil are extracted using more "gentle" procedures and the oil is extracted by tissue grinding, then slow stirring and oil separation at less than 50°C. This results in high-value culinary oil with distinctive colour, flavour, and high concentrations of a range of "health-promoting" compounds. These include mono-unsaturated fatty acids (the "good" lipids), tocopherols (Vitamin E), plant pigments (antioxidants providing disease protection), sterols, fibre and folate (Vitamin B6). However, the oil yield by cold pressing is significantly lower than by solvent extraction and improvements to yield and quality would provide commercial benefits.

Commercial production of extra virgin avocado oil commenced in New Zealand in 2001, and in Australia in 2004. Business plans were based on preliminary data of oil yield from the main commercial cultivar in both countries ('Hass'). Several factors are known to affect oil content, particularly fruit maturity, but maybe also growing region, and grower-to-grower differences within a production region. In addition, different cultivars might have differing oil yields and oil quality.

This project investigated the oil yield from the main avocado cultivars grown in the major production regions in Australia and in New Zealand. Fruit were taken from several growers within each region and several times during the commercial harvesting season. The percentage dry matter and the oil yield (based on laboratory extraction procedures) were determined. The project concentrated on the major avocado cultivars ('Hass' and 'Shepard' in Australia, and 'Hass' in New Zealand), with some work done on less common cultivars.

In Australia and New Zealand, both dry matter and oil content increased in more mature fruit. In New Zealand, these tended to reach a maximum in about January. In most cases, the sampling period in Australia was shorter, so this response was not observed as frequently. The maximum oil yield varied from about 10% in early season fruit, to about 29% in late season fruit. This equates to an average 13% yield from commercial cold-pressed procedures. Commercial yield of cold-pressed avocado oil appeared to be the same in fruit of the same maturity (dry matter) in both Australia and New Zealand. However, the average yield in New Zealand tended to be higher than in Australia, mainly because more of the fruit were harvested at a higher dry matter level. This factor may change if the Australian industry changes its minimum fruit maturity level.

Although there are may be some differences in the chemical makeup of 'Hass' oils from New Zealand and Australia, these differences are not large enough to be significant from a commercial perspective, where oils can be blended prior to bottling.

This work has provided important foundational data on the key cultivar in Australasia - 'Hass' - in terms of maturity (dry matter), maximum oil yield (i.e. laboratory-based solvent extraction), concentrations of "healthful" phytochemicals, and cold-pressed oil yields. Although 'Hass' is likely to remain the primary cultivar for oil processing, the study of other cultivars has indicated their usefulness or otherwise for production of novel oils, or blending with 'Hass' oil.

The major challenges for an avocado processing industry in Australia and New Zealand is consistency of fruit supply, particularly of fruit with adequate oil yield and fruit quality. This will be the major factor determining long-term commercial viability.

INTRODUCTION

AVOCADO OIL

Avocado oil has been commercially extracted for many years using a range of extraction processes. While solvent extraction produces the highest oil yield, the resulting oil quality is low and the oil is mainly used in pharmaceutical and cosmetic products. Other techniques use high temperatures (≅ 70°C), which results in poor quality oil requiring significant refining after extraction, and an oil with few, or none of the health promoting compounds found in extra virgin oils. "Extra virgin" or "cold-pressed" oils are extracted at low temperatures. The resulting oil is then re-centrifuged ("polished") to remove trace amounts of water. This results in a high-value culinary oil with distinctive colour, flavour, and high concentrations of a range of "health-promoting" compounds. Generally, the yield of oil by cold pressing is significantly lower than by solvent extraction.

Recognition of the health benefits of avocado and its oil has increased interest in its culinary uses. This has been further stimulated by the commercial development of "cold-pressed extra virgin" avocado oil and demand is now rapidly increasing.

Development of a viable commercial avocado oil industry provides an alternative use for externally low quality, small, or reject fruit. This has both direct and indirect benefits to avocado growers: firstly, a financial return for "reject fruit", and more significantly, the indirect benefit of a positive impact on local market prices due to reduced supply (estimated to be worth $4M p.a. to the New Zealand growers in 2002) improves grower profitability. In addition, there are "co-marketing" benefits in terms of greater awareness in consumers of the nutrition and health benefits of both avocado fruit and its extracted oil.

COLD-PRESSED OIL EXTRACTION AND OIL YIELD

Currently, cold-pressed avocado oil extraction is carried out using mechanical extraction methods where the temperature of the flesh and oil is kept below 50°C. The process is carried out by removing the seed or stone and much of the skin tissue, grinding the flesh to a paste to break the cells, and then slow mixing of the paste in a malaxer to help to release the oil. The oil is then separated from the solid and water phases in a horizontal decanting centrifuge ("expeller"). Further removal of all water from the oil is achieved in high speed centrifuges, a process sometimes referred to as polishing (Wong et al. 2007).

The yield of oil in this system will depend on the maturity at harvest and on fruit ripeness. Maximum oil yield obtained from ripe avocados varies between 10 to 25% of the original fresh weight of the intact fruit. Oil content is generally higher in more mature fruit, but this relationship may vary with different cultivars. It is unclear at this stage whether grower and location can also affect oil yield (at the same stage of fruit maturity), and whether increasing maturity has the same effect across different growing regions. Clearly, the final oil yield has a significant impact on the viability of commercial oil extraction. Thus, baseline information on the maximum oil available for extraction is required to determine the maximum potential oil yield from fruit of various cultivars, regions, and maturities. In addition the impact of these factors on the chemical composition of the oil needs to be considered.

OIL COMPOSITION

One of the key marketing advantages for extra virgin avocado oil is its health-related composition. Avocados are high in mono-unsaturated fatty acids (the "good" lipids). However, avocados also contain significant concentrations of other beneficial compounds, including tocopherols (Vitamin E), plant pigments (antioxidants providing disease protection), sterols, fibre and folate (Vitamin B6).

Mono-unsaturation of the lipids

A diet high in mono-unsaturated fatty acids (MUFAs) is recommended for a healthy lifestyle. The Mediterranean-style diet recommends abundant plant foods and olive oil as the principal sources of dietary lipids. This diet has favourable effects on lipoprotein concentrations, endothelium vasodilation, insulin resistance, metabolic syndrome, antioxidant capacity, myocardial and cardiovascular mortality (Serra-Majem et al. 2006). The lipid content of avocados is 15–20% saturated fats, 60–70% mono-unsaturates and about 10% poly-unsaturates. Avocados and avocado oil have very similar lipid profiles to olive oil and hence can be included as a healthy addition to the Mediterranean diet.

Vitamin E

Avocados contain high concentrations of the antioxidant α-tocopherol (Vitamin E). 'Hass' flesh can contain as much as 2.89 mg/100 g (Lu et al. 2005). Requejo-Jackman et al. (2005) reported concentrations ranging from 5.5 to 9.0 mg/100 g in cold-pressed oil of several cultivars grown in New Zealand.

Pigments

Avocados also contain high concentrations of carotenoids and chlorophylls, generally responsible for the yellow and green colour in plants. Carotenoids have been studied for their antioxidant properties and protection against diseases. Alpha- and β-carotene have pro-Vitamin A activity, which has been associated with reduced risk of cancer and other chronic diseases. Avocados contain high concentrations of lutein, which is known to reduce age-related macular degeneration (AMD; Richer et al. 2004). Lu et al. (2005) reported 2.93 µg/g fresh weight (FW) in the pulp of 'Hass' avocados. Ashton et al. (2006) showed that carotenoid concentrations in avocado skin and flesh decrease with ripening after harvest.

There is also a strong correlation between ingestion of foods with high chlorophyll concentration and a decreased risk of certain types of cancer (Minguez-Mosquera et al. 2002). Chlorophyll concentrations are highest in avocado skin (approximately 186 µg/ g FW), and decrease from the dark green flesh to the yellow flesh (38 to 2.2 µg/ g FW respectively; Ashton et al. 2006).

Plant sterols

Avocados also contain high concentrations of plant sterols, especially β-sitosterol (Piironen et al. 2003). Plant sterols decrease cholesterol absorption if 1.5 to 2.0 g of sterols are consumed per day. Avocado oil from several cultivars contain 2.25 to 4.3 mg/ g FW of sterols, with the highest concentration found in 'Hass' (Requejo-Jackman et al. 2005).

Folate and fibre

Avocados contain high folate concentrations (0.04 mg/100 g), which is recommended for women of child bearing age to reduce the risk of neural birth defects (South African Avocado Growers Association; www.avocado.co.za).
The dietary fibre contents are also high at 6.72 g/100 g (Li et al. 2002).

PROJECT BACKGROUND

Olivado New Zealand Limited (Olivado NZ) started in New Zealand in 2001 six years ago with the aim of producing very high quality cold-pressed avocado oil primarily for culinary use. This product is one of a number of avocado products produced by Olivado NZ (see www.olivado.co.nz). To date Olivado NZ has achieved sales of more than NZ$3.5M and is currently exporting to 11 countries. After only eight months in production the company received a marketing award and since then has been awarded three other gold medal food awards. Olivado NZ is also the only company world-wide to have successfully stabilised cold-pressed avocado oil, increasing its shelf life from six to more than 24 months. Olivado NZ expanded their processing business into Australia in 2004 by building a plant in Cleveland, Brisbane. They sought initially to concentrate on 'Hass' using their New Zealand experience as a guide to commercial best practice. More detailed information on Australian 'Hass' oil yields and the effect of maturity and production location were required to fine-tune practices for Australian conditions.

There was strong pressure from Australian growers for processing of the cultivars 'Shepard', 'Wurtz', 'Sharwil' and 'Reed' as well. Response was difficult in the absence of information on yields and oil quality. These lower production volume cultivars might have potential for blending if they had high concentrations of specific "health" compounds.

This project aimed to answer some of the above questions, to assist in the commercial success of an avocado oil component to the Australian avocado industry. The company, directors and research partners in this project (HortResearch, Massey University and the Department of Primary Industries and Fisheries, Queensland (DPI&F)) formed a team with a wide breadth of skills, including leading expertise in avocado oil extraction, processing and storage, avocado postharvest handling systems, and marketing and promotion.

This project was funded by HortResearch New Zealand (with funds from the New Zealand Foundation for Research Science and Technology - Opportunities for New Zealand's Emerging Horticultural Crop Industries - C06X0203), and the Queensland Department of Primary Industries and Fisheries (DPI&F).

OVERVIEW OF AUSTRALIA AND NEW ZEALAND AVOCADO INDUSTRIES

Australian avocado production is slightly larger than that of New Zealand, but there are significant differences in relation to the range of cultivars grown and export focus.

Australia

Currently, Australian avocado production generally matches domestic demand, so there is little incentive for export. Only about 1% of Australian production is exported (Table 1). Australia also produces a range of cultivars, estimated to consist of 70% 'Hass', 15% 'Shepard', 6% 'Fuerte', 6% 'Wurtz', and 3% "other" cultivars ('Fuerte', 'Pinkerton', 'Edranol', 'Bacon', 'Gwen', 'Hazzard', 'Zutano', 'Nabal' and 'Rincon').
The Bundaberg area is the largest avocado production area in Australia, followed by North Queensland (on the Atherton Tablelands behind Cairns), southeast Queensland (including the

Sunshine Coast and inland such as the Blackall Ranges, Mt Tambourine), Western Australia (primarily Perth and the Pemberton areas), northern and central New South Wales (including Alstonville, Coffs Harbour), and the Tristate (bordering on Victoria, New South Wales and South Australia (Figure 1)). These production areas provide a range of harvesting times, so that 'Hass' can be harvested from early April (Atherton Tablelands) to January/February (Western Australia).

Table 1. Total production and the volumes exported and processed, for the major avocado production countries in the 2004/2005 marketing year [a] (Wong et al. 2007).

Country	*Production (metric tonnes)*	*Exported (metric tonnes)*	*Processed (metric tonnes)*	*% of crop 'Hass' cultivar*
Australia	32,000	320	1,200	75
New Zealand	22,000	15,000	1,850	98
Chile	177,000	136,412	300	93
Israel	77,000	45,000	1,000	32
Mexico	934,282	180,165	25,000	95
South Africa	85,000	38,000	13,600	36
Spain	55,000	48,000	n.r.	75
United States	162,721	1,431	n.r.	95

n.r. – none reported.

[a] Marketing year varies for each country: Australia, New Zealand and Spain, July/June; Chile and South Africa, January/December; Mexico, August/July; United States, November/October.

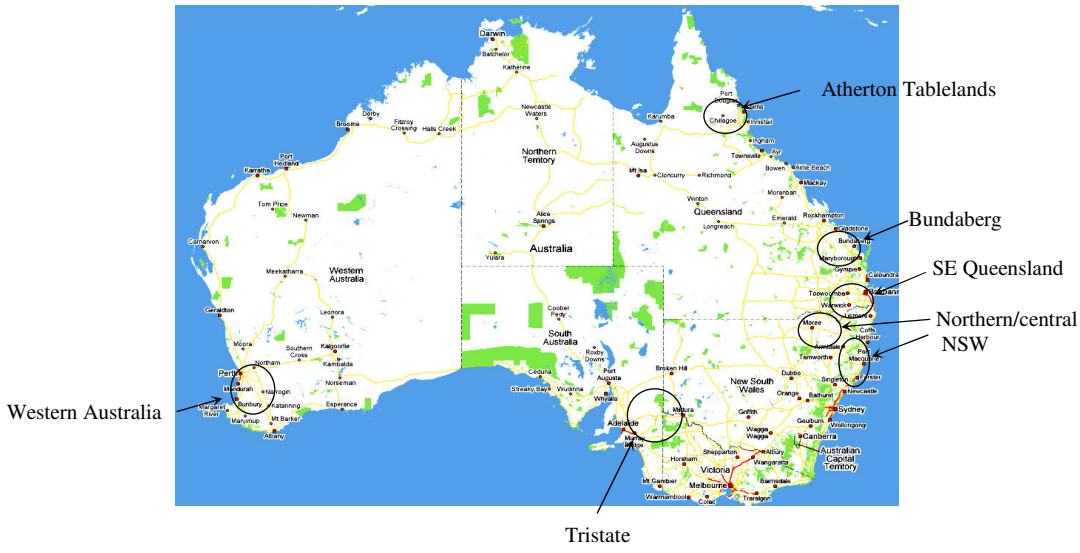


Figure 1. Major avocado production areas in Australia.

New Zealand

The New Zealand industry is largely based on 'Hass', and has a much stronger export focus than Australia (Table 1). New Zealand avocado production is estimated to be more than 95% 'Hass', and only 'Hass' fruit are exported. In 2005, production was 22,000 tonnes and export accounted for more than 75% of production. Other cultivars grown include (in approximate order of volume); 'Reed', 'Fuerte', 'Zutano', 'Bacon' and 'Hayes'. The key growing regions are the Far North (mostly around Kaitaia), Whangarei, Auckland, Bay of Plenty (Katikati to Te Puke), Opotiki, and Gisborne (Figure 2).

Figure 3 shows the detail for each of the six growing regions. Some predominantly local market orchards exist in coastal frost-free areas in Taranaki and north of Wellington.

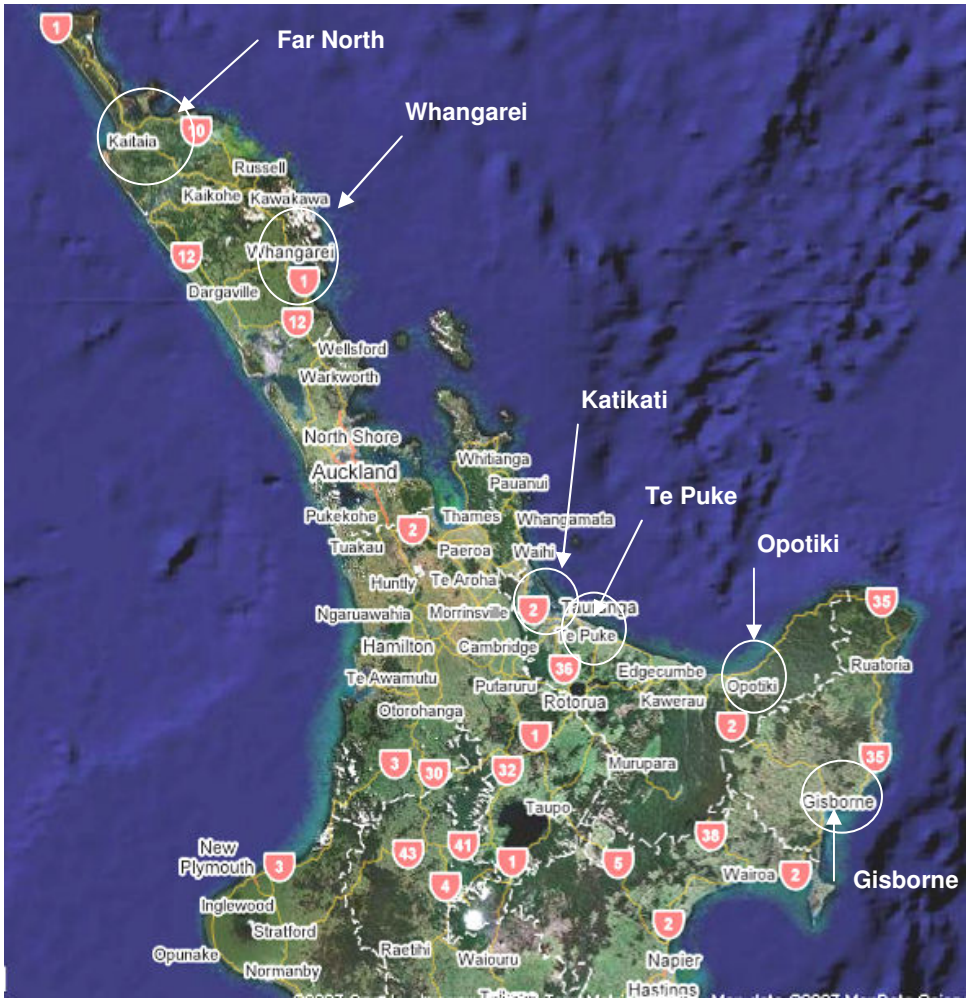
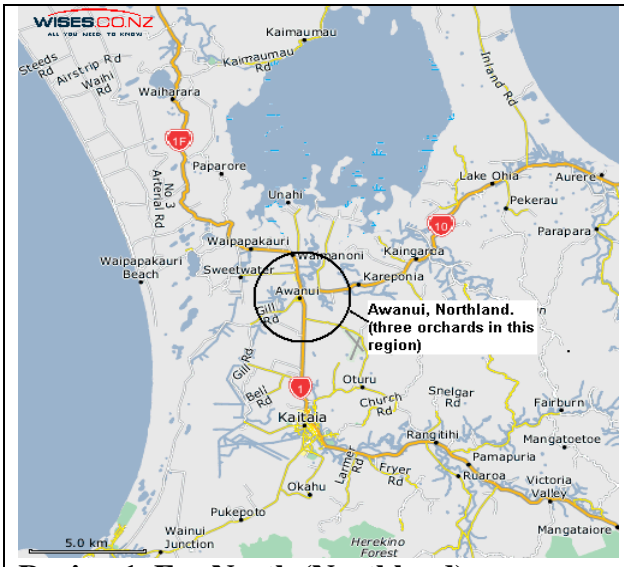
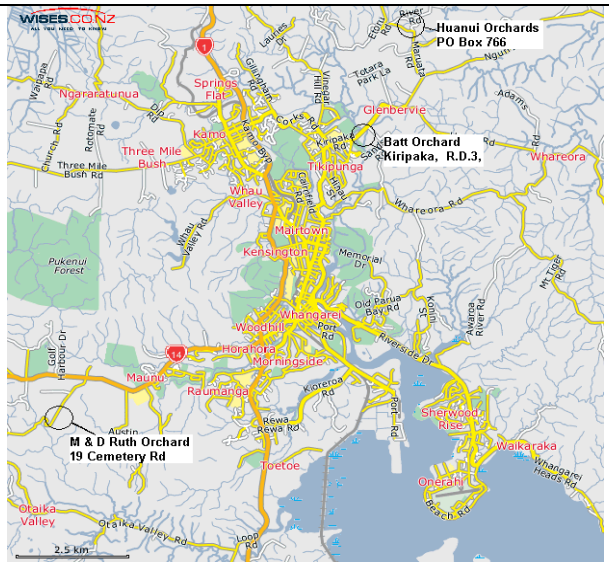


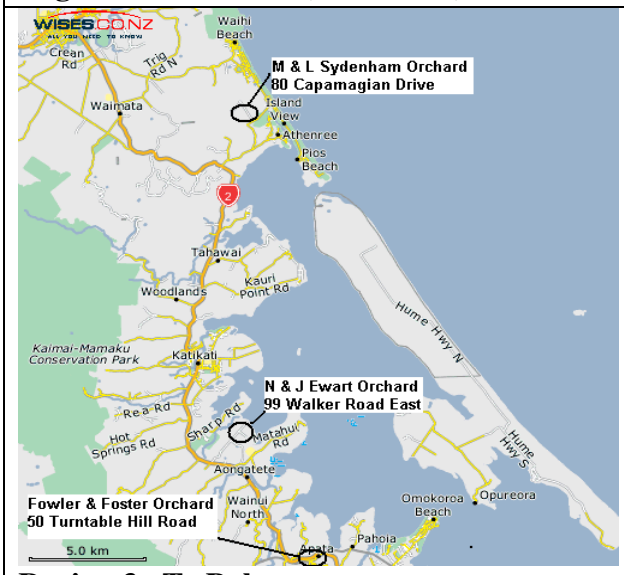
Figure 2. Major avocado production areas in New Zealand.



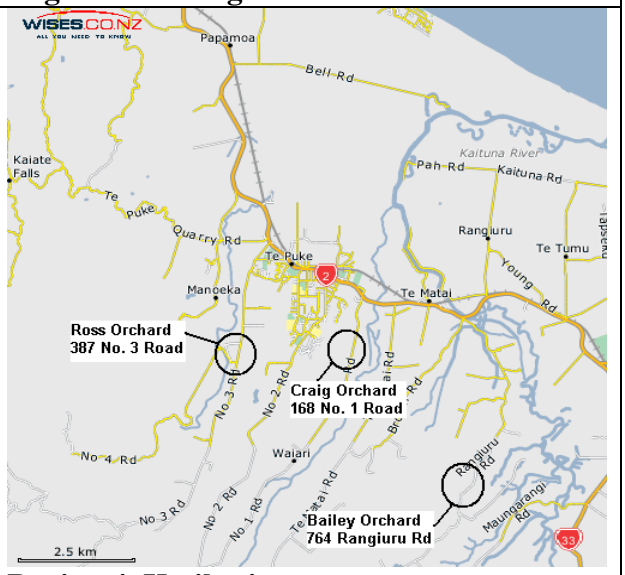
Region 1. Far North (Northland)



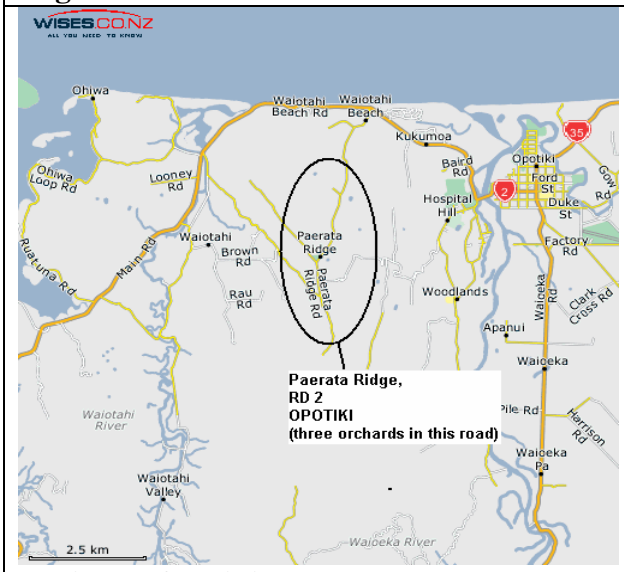
Region 2. Whangarei



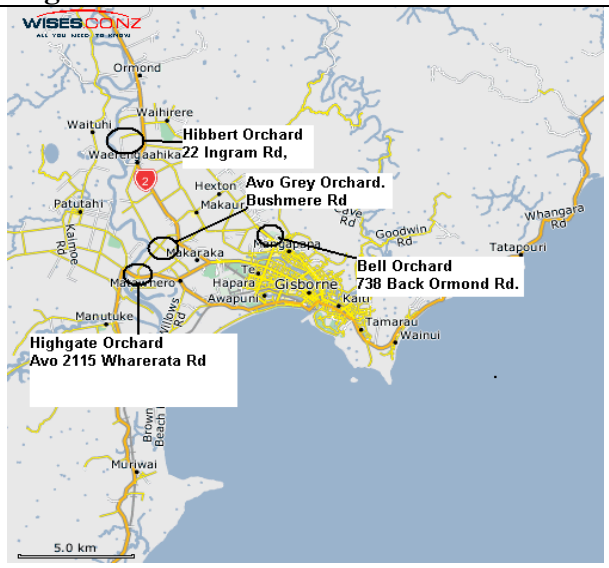
Region 3. Te Puke



Region 4. Katikati



Region 5. Opotiki



Region 6. Gisborne

Figure 3. Detailed maps of orchard locations within each of the six growing regions examined in the New Zealand research project.

HAL PROJECT OVERVIEW / RATIONALE

'Hass' is by far the most significant avocado cultivar in Australia and New Zealand, and indeed in the avocado trade in the world markets. Consequently, most emphasis was placed on determining the influence of maturity and regional factors on oil yield from this cultivar. Knowledge of the effect of all major growing areas on avocado oil yield and quality would have been beneficial, but project resources were insufficient. Therefore, the project focused on the major production areas of the Atherton Tablelands, Bundaberg and coastal and inland southeast Queensland. It was rationalised that the inland areas of southeast Queensland (such as Bellthorpe, Maleny) would be typical of Northern and Central New South Wales. Studies on fruit from Western Australia and the other smaller production areas would have been valuable, but their relatively smaller volumes, lack of on-the-ground collaborators for fruit sampling, distance from the DPI&F laboratories, and distance to the oil processing plant made this region a lower priority.

'Shepard' is a significant early cultivar in Australia, so information on its potential for oil production was also required. 'Shepard' samples were only obtained from the warmer growing areas of the Atherton Tablelands and Bundaberg, since the cultivar does not produce well in cooler areas. There was some concern that the early season 'Shepard' fruit (February from North Queensland and March from Bundaberg) would have low oil yield because of their low dry matter, and may not be suitable for commercial extraction. This needed verification. Growers expressed willingness to "late hang" blemished and under-sized fruit for later harvest when oil content and perhaps quality was likely to be higher. This project attempted to provide preliminary answers to these questions.

Some minor work on the lesser cultivars ('Sharwil', 'Wurtz' and 'Reed') in Australia was also conducted. This was restricted to samples from only one region (two at the most) because of resource limitations.

Oil composition is a significant commercial parameter, since understanding the concentrations of health-promoting compounds assists with accessing retail chains, and gives significant market advantage. However, because of budgetary constraints, only limited examination of the concentrations of beneficial health compounds was carried out. Information on 'Hass' oil composition had been gained from previous New Zealand work, but little work has been done on the other cultivars. Composition studies would also indicate whether the lesser cultivars may contain high concentrations of specific 'health promoting' components, which could be valuable in blending to provide niche products and/or be used as dietary supplements.

The samples were processed to determine maximum potential oil yield using a laboratory-based solvent extraction system. Commercial "cold-pressed" oil yields are reported to help to relate laboratory results to commercial potential. This component of the project was considered as in-kind contribution from Olivado NZ.

It is important to note that although the work in each country was linked, the HAL project only funded the Australia portion of the work, and full chemical and data analysis is continuing in the New Zealand work (HortResearch/Foundation for Research, Science and Technology (FRST)-funded).

MATERIALS AND METHODS

EXPERIMENTAL OVERVIEW

Avocados of several cultivars were sampled from commercial orchards in Australia and New Zealand. Flesh tissue samples were taken soon after harvest and the maturity measured by dry matter analysis. Oil was extracted from the tissue using a solvent-based laboratory technique, and in some cases, a range of compounds were measured in the oil. Cold-pressed oil yield was carried out in commercial factory conditions by Olivado NZ.

Australia

Avocado samples were obtained from commercial orchards in the five main production areas in Queensland (Figure 1). Figure 4 shows a more detailed map. These were:

- Region 1: Atherton Tablelands; Mareeba, Walkamin just south of Mareeba and relatively warmer, Tolga and Kairi further south of Mareeba and generally cooler than Mareeba and Walkamin
- Region 2: Bundaberg area; close to Bundaberg, South Kolan (west of Bundaberg) and Childers (south of Bundaberg)
- Region 3: Coastal Southeast Queensland (SEQ); Beerwah, Woombye and Glasshouse Mountains on the Sunshine Coast (just south of Nambour)
- Region 4: Blackall; Inland southeast Queensland; Montville and Bellthorpe (inland from Nambour, higher altitude)
- Region 5: Crows Nest (inland southeast Queensland, higher altitude, near Toowoomba).

For the major cultivars, fruit were obtained from 3-4 orchards within each production area in the 2004 and 2005 seasons. For most locations, samples were taken at the start, in the middle and at the end (early, mid and late season) of the "typical" commercial harvest for each orchard (Table 2). In some instances, this resulted in a relatively short sampling period because of the short time between the start and end of the commercial harvest.

In most instances, the early season sample was taken one week before the start of commercial harvest on that orchard, so that the dry matter of that sample reflected that of the first commercial harvest. For the 2004 season in the Atherton Tablelands, sampling commenced well after the start of the commercial harvest because of late project approval, and continued well past the end of the commercial harvest.

For the minor cultivars, usually only 1-2 production areas were sampled and from three growers where possible.

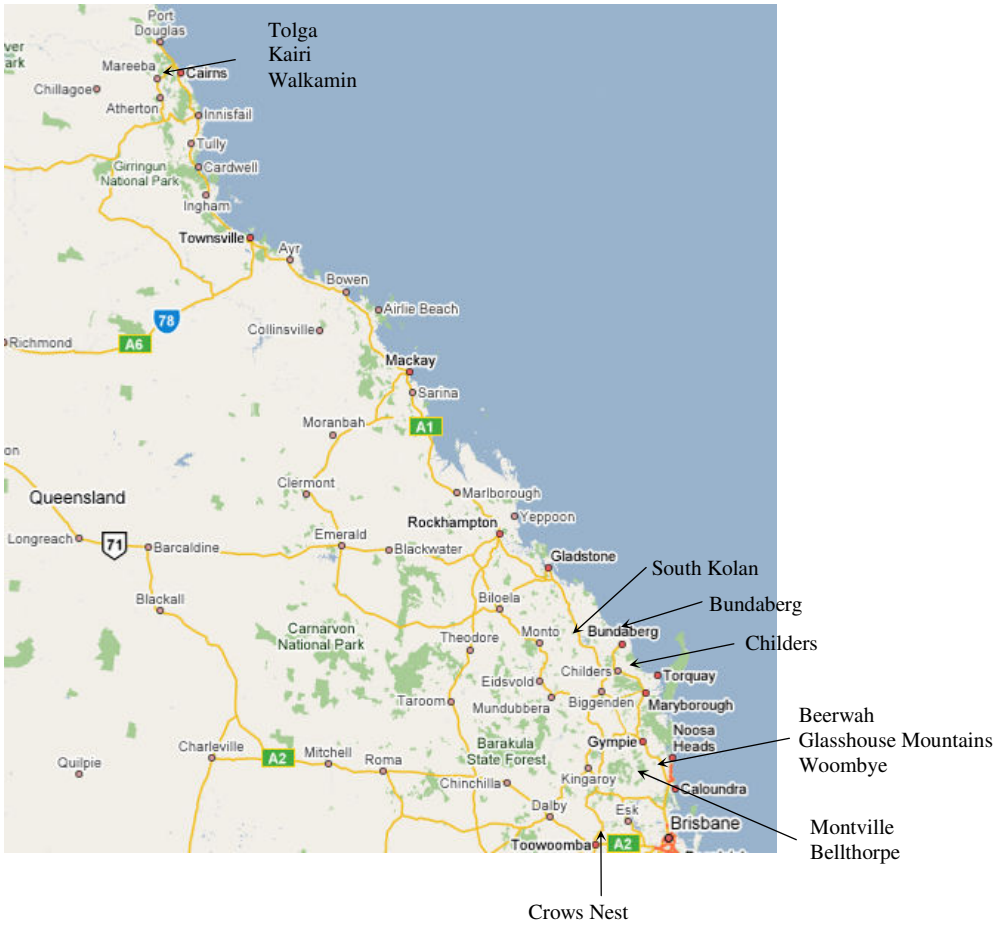


Figure 4. Location of the production areas and locations sampled in Queensland for avocado oil analysis.

Table 2. 'Hass' and 'Shepard' avocado samples collected during 2004 and 2005 from the five main production regions in Queensland. Samples were usually collected at the start, middle and end (Early, Mid and Late; E, M, L) of the typical commercial harvest for each orchard. The flesh was analysed for % dry matter (DM) and % oil content. In limited cases, measurements of the fatty acids, β-sitosterol, α-tocopherol and pigments were carried out.

Region	Maturity E/M/L	Month	DM
'Hass' 2004			
Atherton T.	-		
	M	May-June	√
	L	May-July	√
Bundaberg	E	April	√
	M	May-June	√
	L	June-July	√
Coastal SEQ	E	April-May	√
	M	May-July	√
	L	June-July	√
Blackall	E	June-July	√
	M	Jul.-Aug.	√
	L	Aug.-Oct.	√
Crows Nest	E	July	√
	M	July-Aug.	√
	L	October	√
'Hass' 2005			
Atherton T.	E	April	√
	M	May	√
	L	May	√
Bundaberg	E	April	√
	M	May	√
	L	May-June	√
Coastal SEQ	E	April-May	√
	M	May-July	√
	L	June-Aug.	√
Blackall	E	July-Sept.	√
	M	July-Aug.	√
	L	Sept.-Oct.	√
Crows Nest	E	July	√
	M	August	√
	L	Oct.-Nov.	√
'Shepard' 2004			
Atherton T.	M	March	√
	L	April	√
Bundaberg	E	March	√
	M	March	√
	L	Mar.-Apr.	√
'Shepard' 2005			
Atherton T.	E	February	√
	M	March	√
	L	April	√
Bundaberg	E	March	√
	M	March	√
	L	Mar.-Apr.	√

ACTIVITIES IN NEW ZEALAND – FRUIT COLLECTED

Avocado samples were obtained from commercial orchards in six production areas in the North Island of New Zealand (Figure 2). Detailed maps for each of the growing areas are shown in Figure 3. These included:

- The Far North: Awanui just north of Kaitaia
- Whangarei: Glenbervie area (north-east of Whangarei)
- Katikati: South Waihi, North Aongatete
- Te Puke: No 1 and No 3 Roads (south-west of Te Puke)
- Opotiki: Paerata Ridge (north-west of Opotiki)
- Gisborne: Makaraka and Waerengaahiki (north-west of Gisborne).

For 'Hass' samples, fruit were obtained from three orchards within each production area in the 2003/04, and 2004/05 seasons. Samples were taken over as wide a range of maturities (harvest dates) as possible, and generally commenced before reaching the standard commercial start point (24% DM).

As part of this project we collected fruit from a wide range of cultivars including: 'Fuerte', 'Reed', 'Zutano', 'Pinkerton', 'Hayes', 'Santana', 'Esther', 'Dailey', 'Fujikawa', 'Bacon', 'Pioneer', 'Houston' and 'Gwen'. For these "minor" cultivars, usually only 1-2 production areas were sampled and grower numbers were limited by availability; indeed, for some of these cultivars (e.g. 'Dailey' 11) the number of trees is limited. Only some of the data for the minor cultivars will be presented here, as chemical analysis is continuing.

DETAILED METHODOLOGY

Fruit collection

Twenty medium sized avocado fruit were sampled at least three times during the commercial season (early, mid and late) from tagged trees within one typical block. In some instances late in the season, fruit samples were taken from different trees within the orchard, as the original block had already been strip-picked.

The fruit samples were transported to the research laboratory in plastic bags and inside polystyrene containers to reduce water loss. In Australia, samples from North Queensland were air-freighted to Maroochy Research Station (MRS, Nambour) within 24 hours of harvest. Samples from the Bundaberg area and southeast Queensland were transported to the laboratory in an air-conditioned car within six hours of harvest. For all samples, the fruit were processed within 24 hours of harvest. In New Zealand, samples were sent to the HortResearch laboratory in Mt Albert, Auckland and were processed within 24 hours. For Australia, all samples were collected by project staff, while in New Zealand, samples were collected by the growers. See Appendices 1 and 2 for detailed sampling procedures.

Dry matter determination

The total weight of the 20 fruit was determined then two core samples (yielding four plugs of flesh) taken from each fruit using the Hofshi coring machine (Arpaia et al. 2001). The skin and seed coat was removed from each plug. One plug from each fruit was placed in a pre-weighed dish, then weighed, dried in a domestic food drier at 65°C for 48 hours or until constant weight, then re-weighed and the percentage dry matter determined.

Oil content

The other three plugs of flesh from each fruit were placed in three separate pre-weighed dishes labelled as replicates A, B and C. After the 20 fruit had been sampled, the skin and seed coat was removed, the tissue plugs cut in half, weighed, then immediately frozen in liquid nitrogen (N_2), heat-sealed in foil bags containing an oxygen absorber sachet (Ageless®, FX-20E, Mitsubishi Gas Chemical Company Inc., Japan) and stored in a freezer at –25°C. Several times during the season, the samples were packed in dry ice and sent to HortResearch (New Zealand) following strict quarantine measures. At the HortResearch laboratories (Mt Albert, Auckland) the imported tissue was freeze dried (20°C) and stored in oxygen-free N_2 (99.99% purity) flushed laminated foil bags with an oxygen absorber sachet (Ageless®, FX-20E, Mitsubishi Gas Chemical Company Inc., Japan) at –25°C until oil extraction. The sample was ground to a fine powder immediately before solvent extraction of the oil.

Accelerated Solvent Extraction (ASE). The standard method of hexane extraction has typically been the Sohxlet, which involves boiling samples in hexane for many hours. This tends to degrade oil components. We have refined a new methodology using the Accelerated Solvent Extraction Unit (ASE® 300 Dionex Corporation, Sunnyvale California; Figure 5). A weighed ground sample of approximately 20 g was placed in a 100 ml stainless steel closed cell fitted with a cellulose filter. Extractions were performed under minimal light (0.001 $\mu mol\ s^{-1}\ m^{-2}$) using 100% hexane (liquid chromatography LiChrosolv®). Extraction conditions involved a 5 min sample heating time to 60°C followed by 100 min total extraction time at 10,000 kPa. The run was split into five cycles of 20 min with a N_2 gas purge cycle of 90 s. Here and elsewhere, all N_2 used was oxygen free (99.99% purity). The oil dissolved in the solvent was collected in dark glass bottles, which were N_2-flushed during and after extraction by the ASE® 300.

Oil Drying/Storage. After oil extraction, the hexane was removed over 2 hours at 30°C using a Rapid Vap unit (RapidVap N_2 Evaporation Systems, Labconco® Corp. Kansas City, MO) under flowing N_2. The oil yield was expressed as percent oil per dry weight of avocado tissue. The oil samples were poured into dark glass bottles, flushed with oxygen-free N_2, and stored at -80°C until analysis.

Figure 5. **The Dionex Accelerated Solvent Extraction (ASE® 300) used to determine oil content in freeze-dried avocado tissue. The stainless steel cells that contain the ground avocado tissue are shown (12 per run), with the collection bottles underneath. One collection bottle is shown on the right showing the hexane/oil mix (the green pigmentation is clearly visible).**

Oil composition

In a limited number of cases, analysis was conducted for fatty acids (i.e. the concentrations of monounsaturates, polyunsaturates, and saturated fatty acids), pigments (chlorophylls and carotenoids), tocopherols (e.g. α-tocopherol or Vitamin E) and plant sterols (e.g. β-sitosterol).

Fatty acid analysis. The fatty acids were saponified and methylated using the method described by Hartman & Lago (1973). A Hartman reagent was prepared comprising ammonium chloride dissolved in methanol plus concentrated sulphuric acid. The fatty acids were saponified with 0.5M methanolic-potassium hydroxide and methylated with the Hartman reagent. Hexane was added to extract the fatty acid methyl esters and the supernatant layer was injected into the gas chromatograph (GC), with a DB-wax capillary column heated to 250°C. The individual fatty acid composition of the oils was determined as a percent of the total peak area.

Carotenoid and Chlorophyll Compositional Analysis: This was carried out using the HPLC procedure outlined in Ashton et al. 2006.

Vitamin E and β-sitosterol.

The α-tocopherol and β-sitosterol contents in the extracted oils were analysed simultaneously using the HPLC method developed by Indyk (1990). Both compounds were eluted on the same chromatogram. The oil was saponified in an ethanol (containing pyrogallol) and potassium hydroxide solution. The unsaponifiable fraction containing the tocopherols and sterols was extracted with a hexane and di-isopropyl ether mixture. After centrifugation, the unsaponifiable fraction was evaporated to dryness under nitrogen. The residue was dissolved in ethanol then injected into the HPLC system. The Shimadzu HPLC system consisted of a 5 μm C18 Luna column (150 mm × 4.6 mm); detection was by UV-Vis detector at 200 nm. HPLC grade methanol was used as eluent after filtration and degassing, at a flow rate of 0.7 ml/min. External standards were used and peak areas were used to calculate sample concentrations for both α-tocopherol and β-sitosterol.

Statistical Analysis

The statistical results for the Australian data were generated by using the 'GLM' procedure while the New Zealand data were analysed by using the 'MIXED' procedure, both using the SAS/STAT software, Version 9.1 of the SAS® System for Windows XP (SAS Institute Inc., 1990). The percentage of dry matter and oil were regressed on the factors year, time of season, region, and grower within region, as well as the higher order interactions. The significance of the factors and their interactions were assessed using an F test on the type III sum of squares to account for the unbalanced nature of the data, with all non significant interactions dropped from the model in a sequential manner. Least squares means were computed for the year, time in season, and region. The pairwise comparisons of these means were adjusted for multiple testing using the Tukey-Kramer method for unbalanced data.

RESULTS AND DISCUSSION

AUSTRALIA: 'HASS' DRY MATTER AND OIL CONTENT

Percentage Dry Matter

There were significant differences (P<0.001) in dry matter between the regions (average of the two seasons and three times in the season; Table 3). The dry matter average for Atherton Tablelands, Bundaberg and Coastal south east Queensland (SEQ) were similar; however, the average dry matter for Crows Nest was significantly higher than that for Atherton Tablelands, Bundaberg and Coastal SEQ, and the dry matter from Blackall was the highest (Figure 6). This generally reflects the preference for Atherton Tablelands, Bundaberg and Coastal SEQ to access the early season market, and Blackall and Crows Nest, the later market.

As expected, there were significant differences in dry matter between early, mid and late season (P<0.0001), and between growers within a region (P<0.0038).

Table 3. Statistical analysis of differences between the mean dry matter and oil content for 'Hass' avocados harvested from the five main production regions in Queensland. The results were averaged across the 2004 and 2005 seasons.

Region	% Mean+	*P* value for across regions				
		Region 1	Region 2	Region 3	Region 4	Region 5
Dry matter						
1. Atherton Tablelands	24.5	*	0.07	0.99	<0.0001	0.0003
2. Bundaberg	22.5	0.07	*	0.01	<0.0001	<0.0001
3. Coast SE Queensland	24.8	0.99	0.01	*	<0.0001	<0.0001
4. Blackall	34.7	<0.0001	<0.0001	<0.0001	*	<0.0001
5. Crows Nest	27.9	0.0003	<0.0001	<0.0001	<0.0001	*
Oil content						
1. Atherton Tablelands	13.3	*	0.07	0.99	<0.0001	0.0003
2. Bundaberg	11.3	0.07	*	0.01	<0.0001	<0.0001
3. Coast SE Queensland	13.5	0.99	0.01	*	<0.0001	<0.0001
4. Blackall	22.6	<0.0001	<0.0001	<0.0001	*	<0.0001
5. Crows Nest	16.6	0.0003	<0.0001	<0.0001	<0.0001	*

+ Statistically adjusted mean for multiple testing using the Tukey-Kramer method for unbalanced data. Means within a column followed by different letters are significantly different (α =0.05)

In all instances, dry matter increased during the early harvests (Figure 6). In most cases, dry matter continued to increase with later harvests, but this was not a universal trend.

Overall, there was a trend for higher dry matter earlier in the season for more northern growing regions, although there was also significant variation within each region between growers. Although the relatively limited number of harvests limits our confidence, it appears that the maximum dry matter level achieved was generally around 30%, with the exception of the Blackall region where amounts as high as 40% were observed.

For the Atherton Tablelands, the commercial harvest season is relatively short because of the desire to access the early market. In 2004, the fruit from the warmer site (Walkamin) had higher dry matter than those from the cooler site. Grower differences were not so obvious in 2005, because of similar climatic conditions between the sites (no samples from Walkamin).

In the Bundaberg region, fruit from the more coastal site (Bundaberg) had a higher dry matter at the same harvest date than those from the more inland sites (South Kolan and Childers). Dry matter differences were less obvious in coastal southeast Queensland because of relatively minor microclimatic differences. For Blackall, there was no consistent location effect on dry matter between the two years, while in the Crows Nest region, Crows Nest 2 had consistently lower dry matter than Crows Nest 1.

Table 4 indicates the actual and predicted dates at which each region would reach 21% dry matter (the current commercial minimum maturity level in Australia), based on the average results across the sampled orchards within each region, and using regression tools (Figure 7). In some instances, these are indicative only and based on extrapolation of relatively limited data (especially for Blackall and Crows Nest). The results were consistent between the two years. There was only 12-15 days' difference within each year between the regions in reaching 21% dry matter. This is supported by the results of HAL project AV06025, where the dry matter of fruit from the cooler areas of the Atherton Tablelands, and Bundaberg, coastal southeast Queensland, Blackall and Crows Nest ranged from only 19.5 to 22.5% dry matter when harvested between 9 and 18 April 2007 (Table 5). The small dry matter difference between the regions was somewhat surprising, given there was an almost four-month gap between the start of harvest at the Atherton Tablelands and at Crows Nest (Table 4). The main reasons for the later start of harvest in the cooler regions are to access the later market, and because of low fruit weight in April/May (observations from project AV06025) in spite of the fruit having reached approximately 21% dry matter.

For the Atherton Tablelands and Bundaberg regions, the dry matter of the early season sample was sometimes below the recommended minimum 21% dry matter (Table 4, Figure 6). The early season sample was generally taken just before the start of commercial harvest for each grower.

Table 4. Australian 'Hass' avocados harvested from several regions in 2004 and 2005: The projected date at which fruit reached 21% dry matter (DM; averaged across the 3-5 growers per region, and extrapolated where required), the date of the first sample (approximately equivalent to the start of commercial harvest on the sampled orchards), and the dry matter at the first sampling (close to the start of commercial harvest). SEQ = South East Queensland.

	Region				
	Atherton	Bundaberg	Coastal SEQ	Blackall	Crows Nest
Projected date at 21% DM					
2004	10 Apr.*	20 Apr.	5 Apr.*	25 Apr.*	25 Apr.*
2005	20 Apr.	30 Apr.	20 Apr.	-	2 June*
Date of the first sample (early harvest)					
2004		4 May	22 Apr.	22 Jun.	20 Jul.
2005	6 Apr.	13 Apr.	15 Apr.	6 Jul.	27 Jul.
% Dry matter of the first sample					
2004		20.5- 21	23- 25	25.5- 36	24- 27.5
2005	19-20.5	20- 20.5	19.5- 22	25- 33	24.5- 27

*Predicted by extrapolation of the regression line in Figure 7

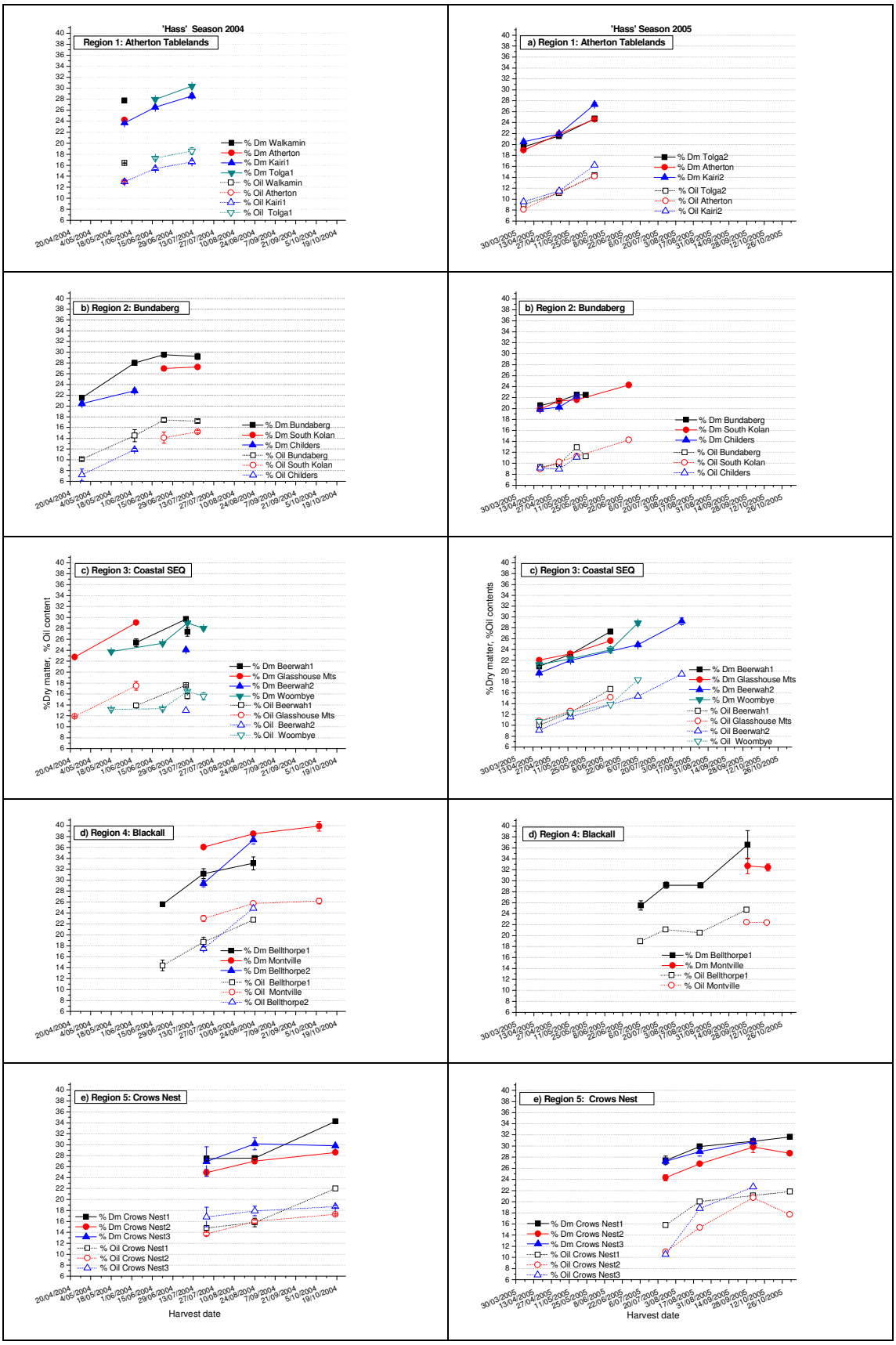
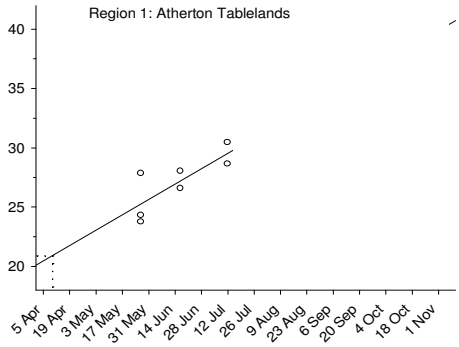
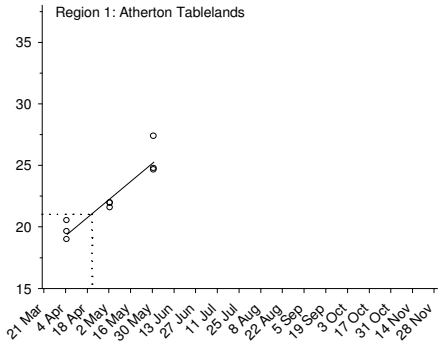
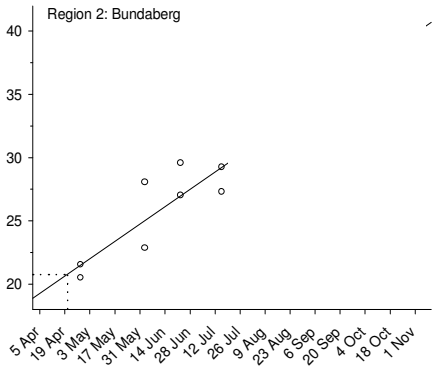
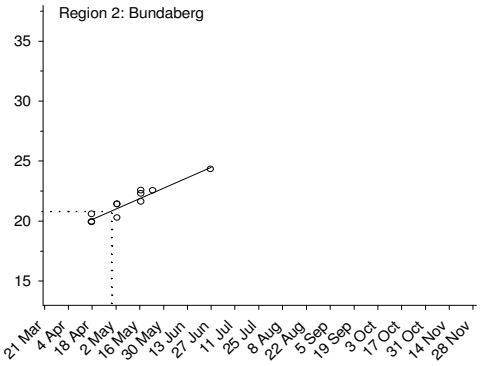
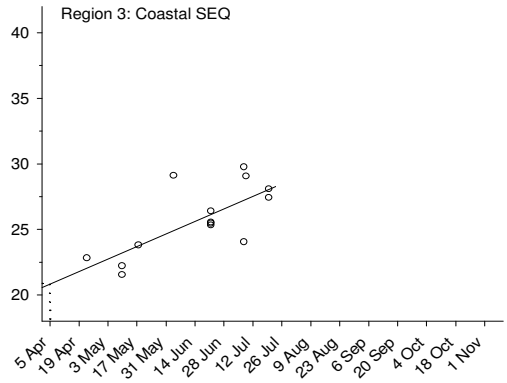
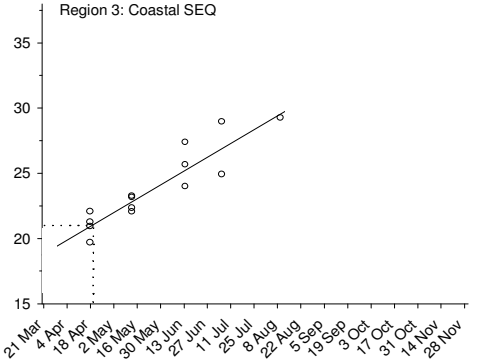
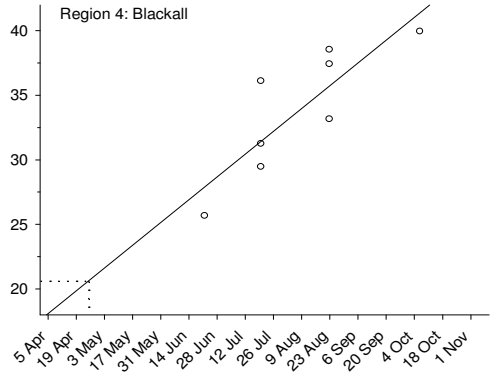
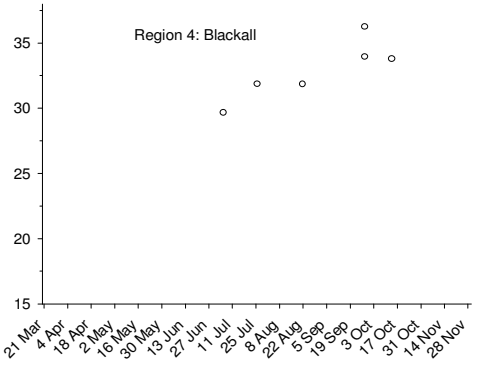


Figure 6. Percentage dry matter and oil content (percentage fresh weight basis) of Australian 'Hass' avocados harvested from five main producing regions in 2004 and 2005. Coastal SEQ = coastal south east Queensland. Values are the means of three replicates of 20 fruit. Vertical bars = standard errors of mean.

‘Hass’ Season 2004	‘Hass’ Season 2005
	
	
	
	

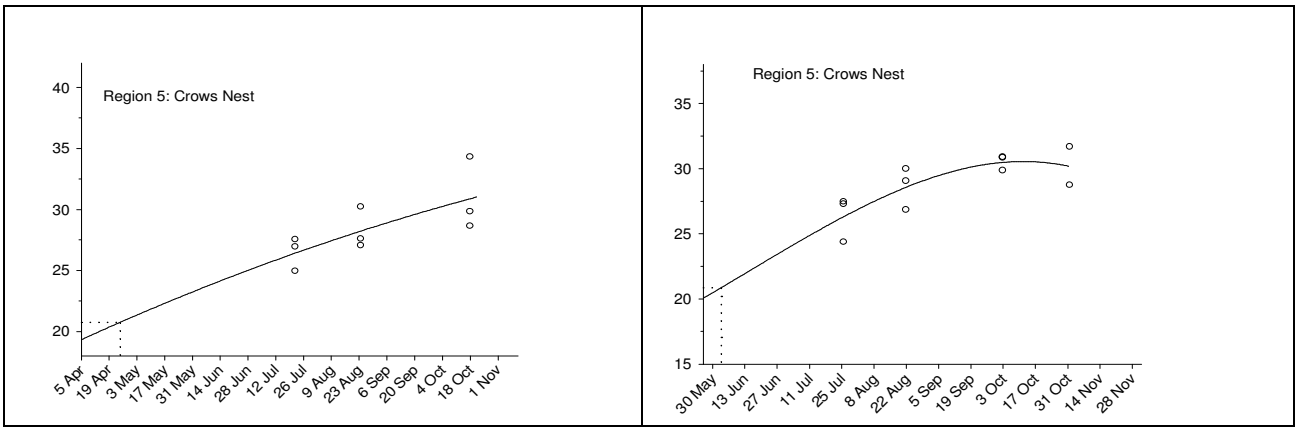


Figure 7. Prediction of when 'Hass' avocado fruit reached the 21% dry matter standard in the main avocado production regions in Queensland during the 2004 and 2005 production seasons. Each data point represents the mean of 3 replicates from each grower for each time in the season. The dotted line indicates the estimated date for 21% dry matter. Coastal SEQ = coastal south east Queensland.

Table 5. The dry matter (DM) of 'Hass' avocados sampled from several regions in Australia during April 2007 for the HAL project 'Australian consumers' perceptions and preferences for 'Hass' Avocado project (number AV06025).

Region	Harvest date	Average DM
Atherton Tablelands	18 April	21.4
Atherton Tablelands	18 April	21.9
Atherton Tablelands	18 April	24.4
Atherton Tablelands	18 April	24.9
Bundaberg	16 April	22.2
Bundaberg	16 April	22.5
Coastal SE Queensland	9 April	20.3
Coastal SE Queensland	13 April	19.5
Blackall	9 April	19.6
Blackall	9 April	19.7
Crows Nest	9 April	21
Western Australia	19 April	38.5

Percentage Oil Content

Oil content followed very similar patterns to dry matter, including little change or a reduction in late season in some instances. There were significant differences ($P<0.001$) in dry matter between the regions (average of the two seasons and three times in the season). The dry matter for Atherton Tablelands, Bundaberg and Coastal SEQ were similar. However, the dry matter for Crows Nest was significantly higher than Atherton Tablelands, Bundaberg and Coastal SEQ, and the dry matter from Blackall was the highest (Figure 6). This generally reflects the preference for Atherton Tablelands, Bundaberg and Coastal SEQ to access the early season market, and Blackall and Crows Nest, the later market.

As expected, there were significant differences in dry matter between early, mid and late season ($P<0.0001$), and between growers within a region ($P<0.0038$). The differences between regions were similar, as with dry matter.

Oil content was 11.4% less than dry matter (average over all regions, seasons and time in season). The range between regions across years was 10.8-12.3%.

Australia: Relation between dry matter and oil content

There were strong, significant relationships between dry matter and oil content, irrespective of growing region or season (Figure 8, Table 6). Table 6 indicates small differences in the regression relationships between region and season. There was little consistent difference in the estimated oil content at 24% dry matter, suggesting that similar maximum oil yields can be obtained from these regions.

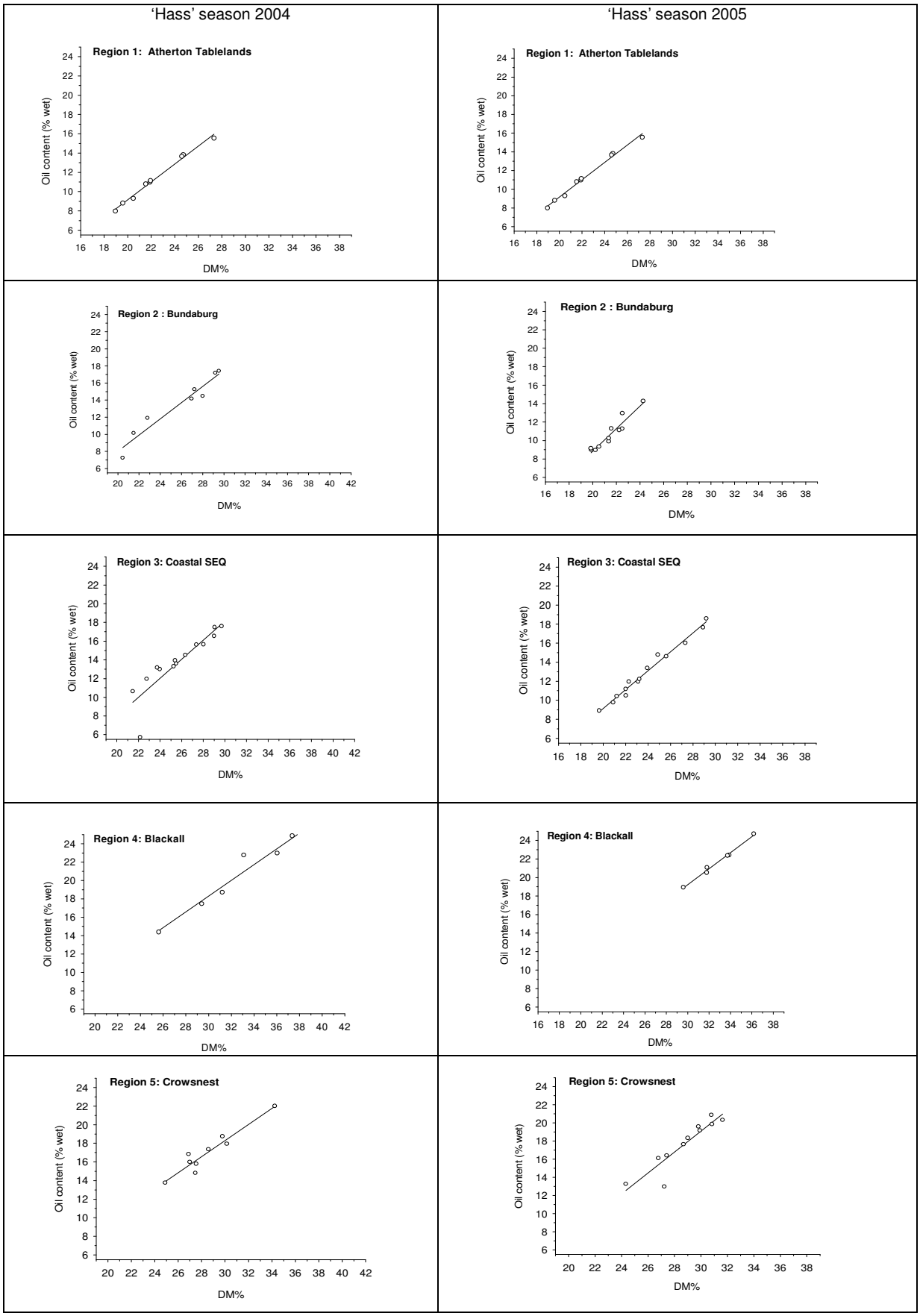


Figure 8. Relation between the % dry matter (DM) and the oil content (percentage fresh weight basis) in 'Hass' avocado fruit flesh obtained from the main production regions in Queensland (Australia) in 2004 and 2005. The regression equations are presented in Table 6.

Table 6. The regression characteristics between percentage dry matter (DM) and oil content of 'Hass' avocado fruit flesh obtained from the main production regions in Queensland (Australia) in 2004 and 2005. The linear regression equation is based on: oil content (%fresh weight) = a*(% dry matter) + c. The linear regression graphs are presented in Figure 8. Coastal SEQ = coastal south east Queensland.

Growing region	a	c	r^2	Oil content at 24% DM
2004				
Atherton T.	0.87	-7.78	0.97	13.1
Bundaberg	0.95	-10.95	0.94	11.8
Coastal SEQ	1.02	-12.44	0.79	12.0
Blackall	0.86	-7.36	0.97	13.2
Crows Nest	0.86	-7.64	0.91	13.1
2005				
Atherton	0.93	-9.41	0.99	12.9
Bundaberg	1.22	-15.57	0.91	13.7
Coastal SEQ	0.99	-10.69	0.98	13.1
Blackall	0.86	-6.69	0.99	14.0
Crows Nest	1.15	-15.47	0.84	12.2

NEW ZEALAND: 'HASS' DRY MATTER AND OIL CONTENT

Percentage Dry Matter

Regional differences: As found in Australia there were significant differences ($P<0.001$) in dry matter accumulation between the growing regions (average of the two seasons and multiple harvests in the season; Table 7, Figures 9 and 10). Statistical analyses showed no differences in the (adjusted) mean dry matter percentage between Opotiki and Te Puke regions (approximately 33.6%), or, interestingly, between Whangarei and Gisborne regions (approximately 34.5%). However, mean dry matter for Whangarei was significantly higher than for the Far North ($P<0.0001$), and was also the highest mean dry matter overall i.e., among all regions over the two productions seasons studied (Table 7). In contrast, the lowest mean dry matter was recorded for the Katikati region at 30.9% for the period of study.

Timing of harvest differences: As would be expected, the effect of harvest time by year (i.e. harvests during the season) on dry matter content was highly significant ($P<0.0001$). An important difference to note between the work carried out in Australia from that in New Zealand is that fruit were harvested at 5 – 6 times in the season (compared with 3 – 4 times in Australia), and over a more protracted harvest time. This meant that as fruit matured we were able to record a progressive increase in the rate of dry matter accumulation, which tended to slow down or in some cases reach a plateau towards the end of the production season. Thus, as the season progressed, the data collected showed higher significant differences in dry matter percentage among the earlier harvests (harvest #1 through to harvest #4) and smaller differences among the later harvests #4, #5 and #6 (Table 8).

Grower differences: Within a region, grower effect on dry matter percentage was also significant ($P<0.0001$) for the period of study. However, the differences among growers within a region significantly changed from Year 1 to Year 2 ($P<0.0001$). These results suggest that orchard management practices and/or microclimate or soil conditions play a significant role in fruit maturation. Grower differences ranged from very small, such as in Whangarei, to large as in the Te Puke region, and these were not necessarily a reflection of geographic distance from each other.

Table 7. Statistical analysis of differences between the mean dry matter and oil contents for 'Hass' avocados harvested from the six main production regions in New Zealand. Mean values are the average of the two production seasons, 2003/2004 and 2004/2005 and of multiple harvests in the season.

Region	Mean % Dry matter	Mean+ % Dry matter	*p* value for across regions					
			Far North	Whangarei	Te Puke	Katikati	Opotiki	Gisborne
Dry matter								
1. Far North	32.3	32.5b	*	<0.0001	0.0572	0.0229	0.3972	0.0085
2. Whangarei	34.7	34.7c	<0.0001	*	0.3811	<0.0001	0.3428	0.9769
3. Te Puke	32.5	33.8bc	0.0572	0.3811	*	<0.0001	0.9986	0.9346
4. Katikati	31.3	30.9a	0.0229	<0.0001	<0.0001	*	0.0004	<0.0001
5. Opotiki	34.7	33.5bc	0.3972	0.3428	0.9986	0.0004	*	0.8427
6. Gisborne	31.1	34.3c	0.0085	0.9769	0.9346	<0.0001	0.8427	*

+ Statistically adjusted mean for multiple testing using the Tukey-Kramer method for unbalanced data. Means within a column followed by different letters are significantly different (α=0.05)

Table 8. Statistical analysis of the effect of harvest time on dry matter content for 'Hass' avocados harvested from the six main production regions in New Zealand. Mean values are the average of the two production seasons, 2003/2004 and 2004/2005 and of multiple harvests in the season.

Harvest number and time in the production season	Mean % Dry matter	Mean+ % Dry matter	September /October	November	December	January/ February	March	April
1.September/October	26.4	26.3a	*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
2. November	30.2	30.1b	<0.0001	*	<0.0001	<0.0001	<0.0001	<0.0001
3. December	33.0	33.2c	<0.0001	<0.0001	*	<0.0001	<0.0001	<0.0001
4. January/February	35.2	35.6d	<0.0001	<0.0001	<0.0001	*	0.0597	<0.0001
5. March	35.9	36.6de	<0.0001	<0.0001	<0.0001	0.0597	*	0.4489
6. April	36.9	37.9e	<0.0001	<0.0001	<0.0001	<0.0001	0.4489	*

+ Statistically adjusted mean for multiple testing using the Tukey-Kramer method for unbalanced data. Means within a column followed by different letters are significantly different (α=0.05)

Seasonal differences. As we have observed in our previous work (Requejo-Tapia et al. 1999 and White et al. 2000; Figures 11 and 12), there is a trend for dry matter to reach a plateau after January. This occurred more clearly in some regions than others, with clear plateaus in the Far North and Whangarei, some indications for the Katikati and Gisborne regions, and for Opotiki in the 2004/05 season. This is supported by previous data where the Far North orchard (Clark) showed a plateau in both the 1998/99 and 1999/2000 seasons Requejo-Tapia et al. 1999 and White et al. 2000, while this was less clear in the Te Puke orchard (Bailey).

In the present study statistical analyses showed that mean dry matter averaged across all regions and harvests significantly increased by approximately 1% from Year 1 to Year 2 (P = 0.0038). Gisborne exhibited the highest yearly (mean) dry matter increase (6%) while Whangarei remained unchanged and the Te Puke region decreased by 1.6% (from one season to the next) (P<0.0001) (Table 9).

Table 9. Seasonal changes in dry matter content as a percentage for 'Hass' avocados harvested from the six main production regions in New Zealand. Mean values are for all harvests in the season.

Region/season	% Dry matter +	
	2003/2004	2004/2005
1. Far North	31.55a	32.72b
2. Whangarei	34.88b	34.83c
3. Te Puke	34.94b	33.34b
4. Katikati	30.87a	30.91a
5. Opotiki	33.6b	33.98bc
6. Gisborne	31.14a	37.30d

+ Statistically adjusted mean for multiple testing using the Tukey-Kramer method for unbalanced data. Means within a column followed by different letters are significantly different (α=0.05)

Seasonal mean dry matter shows that the Far North and Gisborne regions appear to be more advanced in the second season (2004-05) than in the first season (2003-04) for the same harvest time. Significant differences year to year are known to occur (Figure 13) and this is the primary reason why maturity monitoring is carried out in New Zealand, something that is clearly best practice. It is also interesting to note that there are suggestions that grower-to-grower variability differs between seasons, as can be seen for the Far North region where greater differences are observed in the first season, and remarkably few differences in the second season (Figure 9).

Percentage Oil (fresh tissue basis)

Regional differences: As oil is the main component of the dry matter in the tissue, it was not surprising to find similar responses to those of the dry matter. Thus, we found that for the two years and all harvests collected, oil accumulation could be affected by the region from which the fruit originated ($P<0.0001$). Statistical analyses showed no differences in the adjusted mean oil percentage between Gisborne, Opotiki and Te Puke regions, all at approximately 22.6%, whereas oil percentage in Gisborne significantly differed from that in Katikati. Mean oil content for Whangarei was significantly higher than for the Far North ($P<0.0001$), and was also the highest in all regions. The lowest mean oil content overall was recorded for the Katikati region, at 19.5% for the period of study (Table 10).

Table 10. Statistical analysis (*P* values) of differences between the mean oil contents for 'Hass' avocados harvested from the six main production regions in New Zealand. Mean values are the average of the two production seasons, 2003/2004 and 2004/2005 and of multiple harvests in the season.

Region	Mean % Oil (fresh basis)	Mean+ % Oil (fresh basis)	*p* value across regions					
			Far North	Whangarei	Te Puke	Katikati	Opotiki	Gisborne
Dry matter								
1. Far North	21.3	21.0ad	*	<0.0001	0.0572	0.0229	0.3972	0.0085
2. Whangarei	23.5	23.67b	<0.0001	*	0.3811	<0.0001	0.3428	0.9769
3. Te Puke	21.3	22.43a	0.0572	0.3811	*	<0.0001	0.9986	0.9346
4. Katikati	19.7	19.48c	0.0229	<0.0001	<0.0001	*	0.0004	<0.0001
5. Opotiki	21.01	22.64a	0.3972	0.3428	0.9986	0.0004	*	0.8427
6. Gisborne	19.9	22.92e	0.0085	0.9769	0.9346	<0.0001	0.8427	*

+ Statistically adjusted mean for multiple testing using the Tukey-Kramer method for unbalanced data. Means within a column followed by different letters are significantly different (α=0.05)

Timing of harvest differences: The time of harvest had an important effect on oil content (P<0.0001). Similarly to dry matter, results showed that oil in the tissue accumulated at higher rates during the first half of the production period (September – December) than during the second half (January- April) with a tendency to reach a plateau by February. Thus, as the season progressed, the data collected showed higher significant differences in oil percentage among the earlier harvests (harvest #1 through to harvest #4) and smaller differences among the later harvests #4, #5 and #6 (Table 11).

Table 11. Statistical analysis (P values) of the effect of harvest time on oil contents for 'Hass' avocados harvested from the six main production regions in New Zealand. Mean values are the average of the two production seasons, 2003/2004 and 2004/2005 and of multiple harvests in the season.

Harvest number and time in the production season	Mean % Oil (fresh basis)	Mean+ % Oil (fresh basis)	September /October	November	December	January/ February	March	April
1. September/October	15.5	15.47a	*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
2. November	19.5	19.16b	<0.0001	*	<0.0001	<0.0001	<0.0001	<0.0001
3. December	21.9	22.01c	<0.0001	<0.0001	*	<0.0001	<0.0001	<0.0001
4. January/February	24.2	24.61d	<0.0001	<0.0001	<0.0001	*	<0.0001	<0.0001
5. March	24.6	25.01de	<0.0001	<0.0001	<0.0001	0.9646	*	<0.0001
6. April	25.2	25.95df	<0.0001	<0.0001	<0.0001	0.1340	0.5583	*

+ Statistically adjusted mean for multiple testing using the Tukey-Kramer method for unbalanced data. Means within a column followed by different letters are significantly different (α=0.05)

Grower differences: Within a region, grower effect on oil content was also significant (P<0.0001) for the duration of this study. Grower differences ranged from very small i.e. in Whangarei and the Far North, to large, such in Te Puke and Gisborne areas. Microclimates and individual cultivation practices have shown to affect fruit dry matter content and these were reflected in the accumulation of the oil (Figures 9 and 10).

Seasonal differences. The data collected revealed a small change in the mean oil content from the 2003/04 to the 2004/05 seasons (P = 0.0474) across all regions and harvests. However, this overall increase might have been driven by only the Gisborne area, which showed the largest increase (approximately 5%) from year one to year two of this study, while the other regions means increased by a maximum of 1.5% (Table 12).

Table 12. Seasonal changes in oil contents as a percentage for 'Hass' avocados harvested from the six main production regions in New Zealand. Mean values are for all harvests in the season.

Region/season	% Oil (fresh weight basis) +	
	2003/2004	2004/2005
1. Far North	20.74a	21.34b
2. Whangarei	23.77b	23.56d
3. Te Puke	23.48b	22.09bc
4. Katikati	20.15a	18.66a
5. Opotiki	22.42b	23.32cd
6. Gisborne	20.59a	25.59c

+ Statistically adjusted mean for multiple testing using the Tukey-Kramer method for unbalanced data. Means within a column followed by different letters are significantly different (α=0.05)

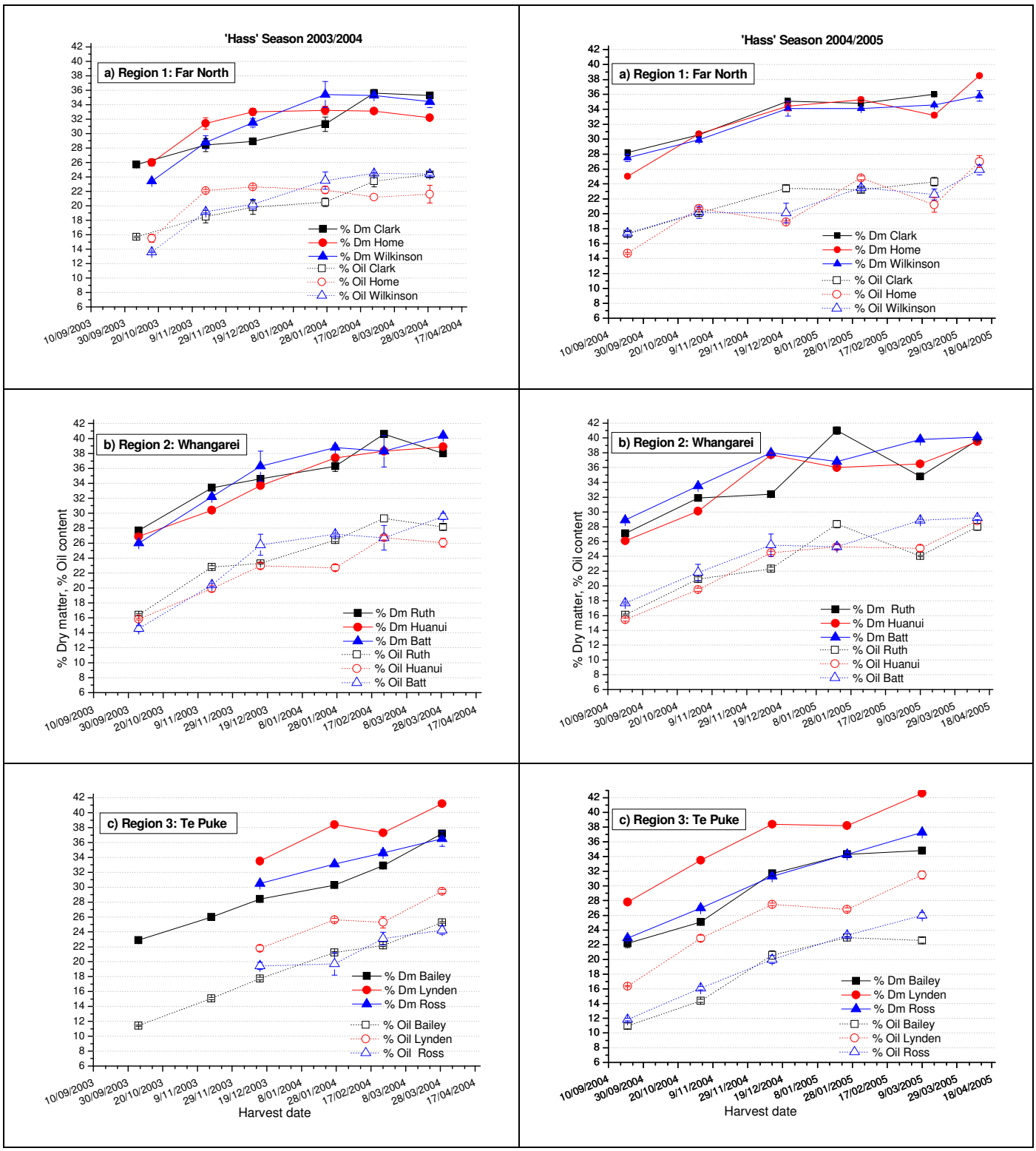


Figure 9. Percentage dry matter of 'Hass' avocados harvested from the Far North, Whangarei and Te Puke regions in New Zealand during the 2003/2004 and 2004/2005 production seasons. Each point is the mean of triplicate samples from 20 fruit. Vertical bars = standard errors of mean (SEM).

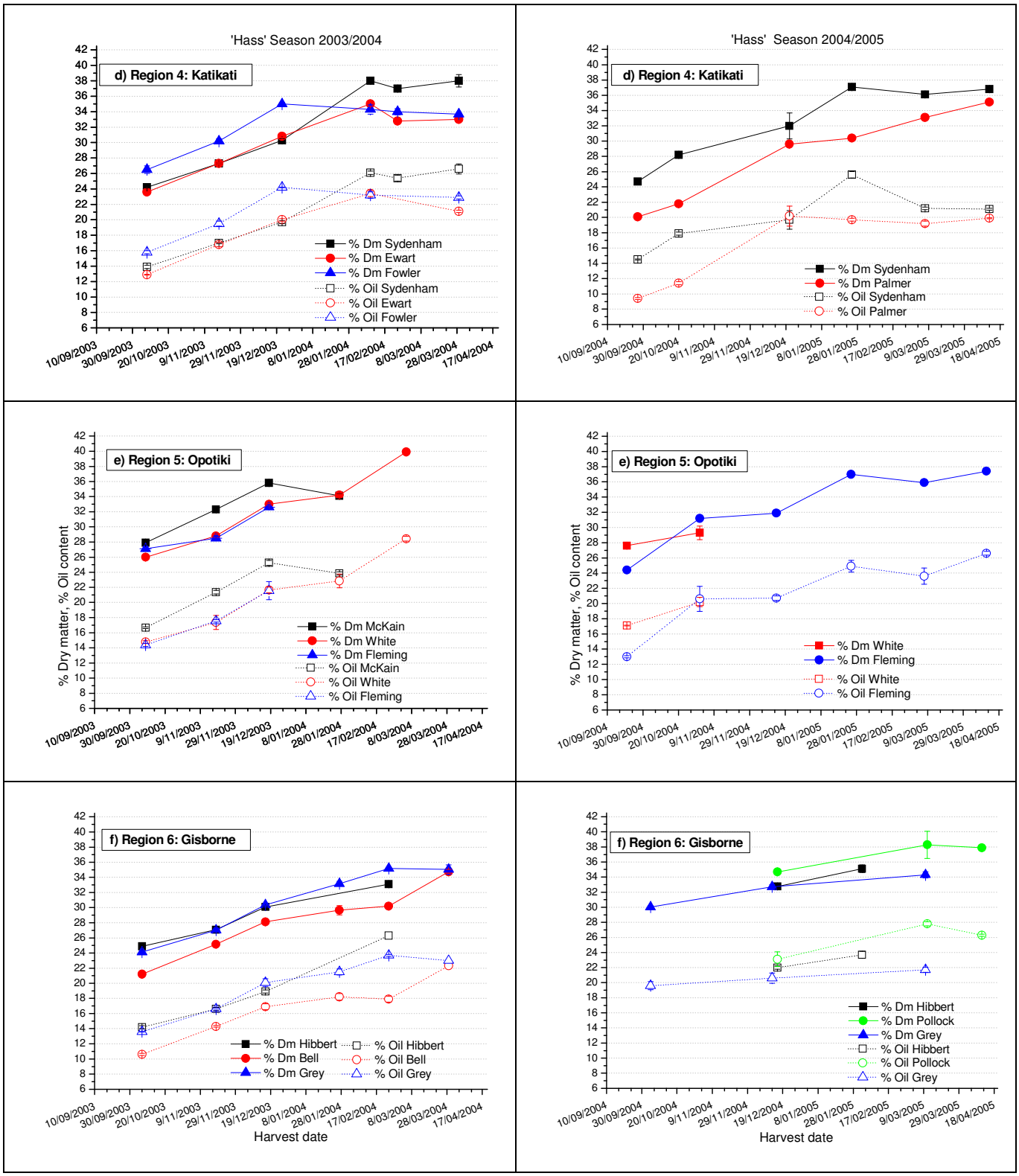


Figure 10. Percentage dry matter of 'Hass' avocados harvested from the Katikati, Opotiki and Gisborne regions in New Zealand during the 2003/2004 and 2004/2005 production seasons. Each point is the mean of triplicate samples from 20 fruit. Vertical bars = standard errors of mean (SEM).

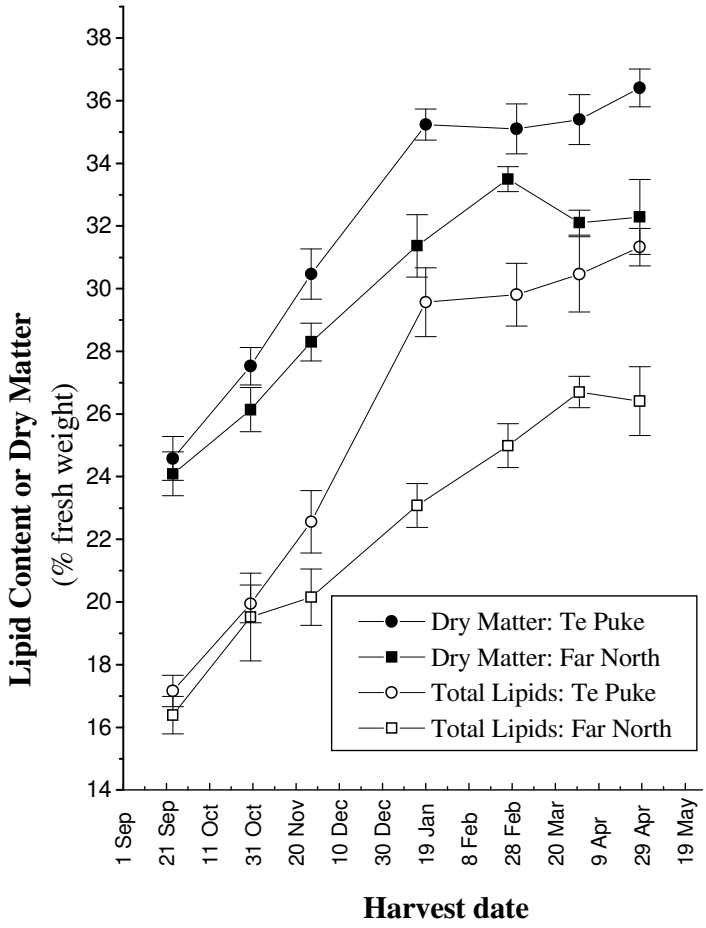


Figure 11. Mean oil content and dry matter of 'Hass' avocado fruit harvested from one orchard in Te Puke and one in the Far North from September to April 1998-1999. Each point is the average of four replicates of five fruit. Vertical bars = standard errors of the mean (SEM, extracted from Requejo-Tapia et al. 1999).

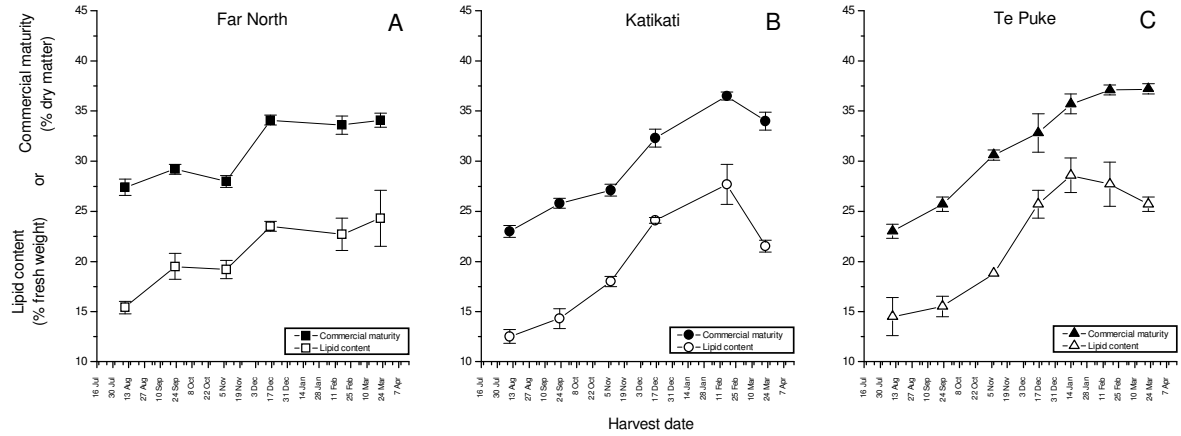


Figure 12. Mean oil content and dry matter of 'Hass' avocado fruit harvest from one orchard in the (A) Far North, (B) Katikati and (C) Te Puke from August 1999 to March 2000. Each point is the average of four replicates of five fruit. Vertical bars = standard errors of the mean (SEM, extracted from White et al. 2000).

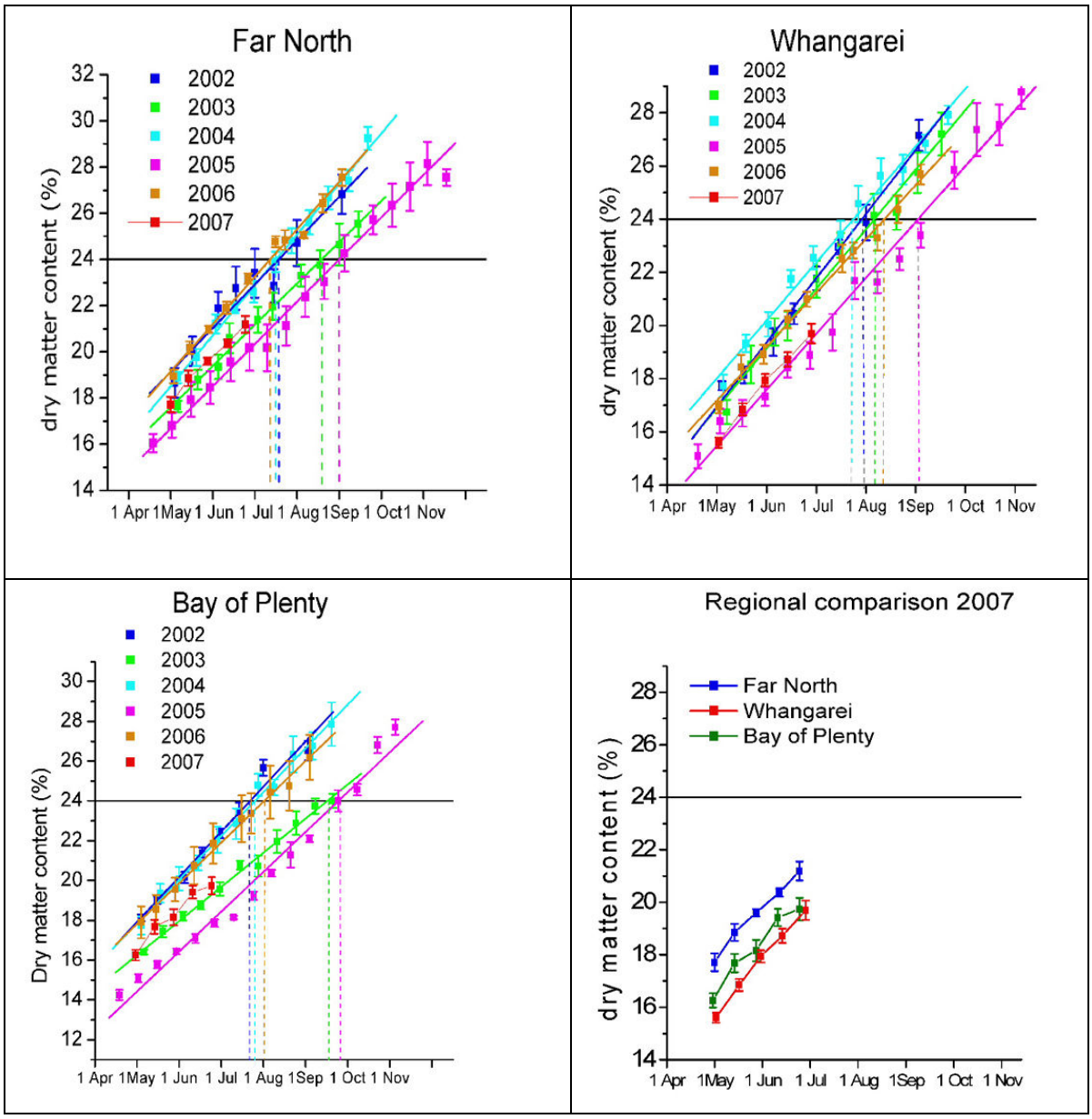


Figure 13. Dry matter accumulation for the years 2002-2007 for three New Zealand growing regions (Far North, Whangarei and the Bay of Plenty (Katikati/Te Puke), and a regional comparison for the 2007 season. Data and graphs from the New Zealand Avocado Industry Council (AIC) website (http://nzavocado.co.nz/monitoring-results.html).

New Zealand: Relation between dry matter and oil content

As observed in Australia, and in previous New Zealand work (Requejo-Tapia et al. 1999 and White et al. 2000) there were strong, significant relationships (r^2 from 0.8 to 0.95) between dry matter and oil content, irrespective of growing region or season (Figure 14). A linear regression fitted to these graphs showed a positive correlation between percentage dry matter and oil described by Y= -5.9713 + 0.85344X for the 2003/2004 season, while the equation Y= -5.90868 + 0.83497X described the relationship for the 2004/2005 season. The linear regressions for each growing regions for each season are shown in Figures 15 and 16.

We have observed that overall, the difference between oil and dry matter ranged from approximately 10 to 12%, depending in regions and season. This "rule of thumb" appeared to hold true in most regions.

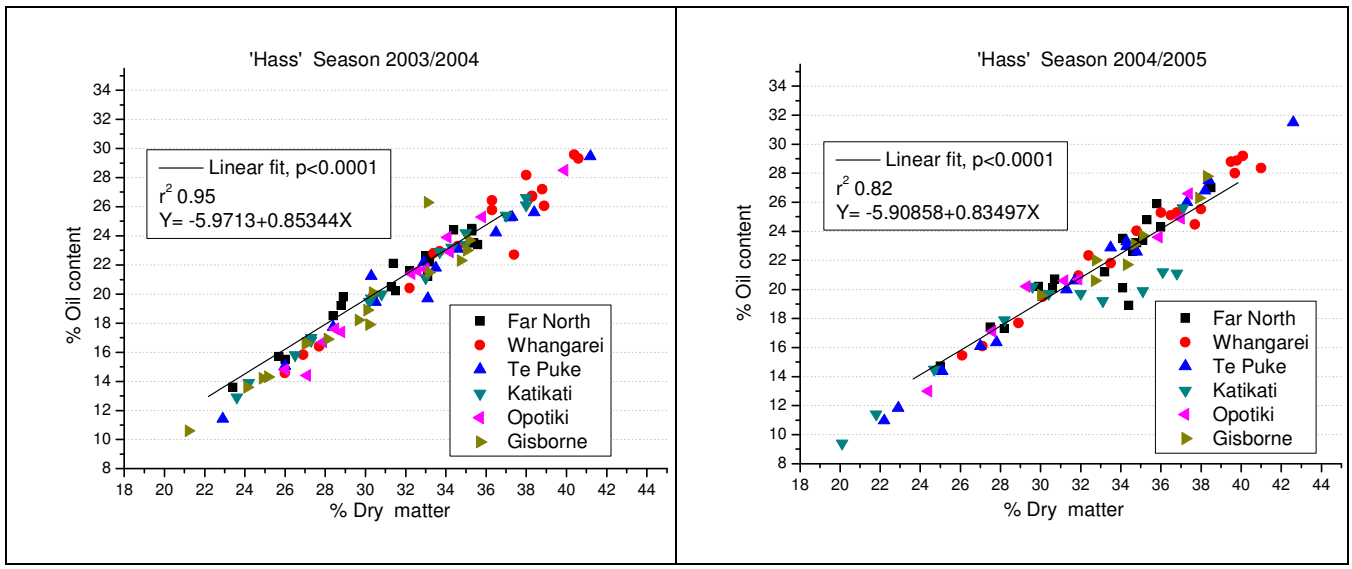


Figure 14. Correlation of dry matter with oil content for the 2003/04 season (left) and the 2004/05 season (right) for all harvests and orchards for each of the five main New Zealand 'Hass' avocado growing regions. Each point is the mean of triplicate samples from 20 fruit.

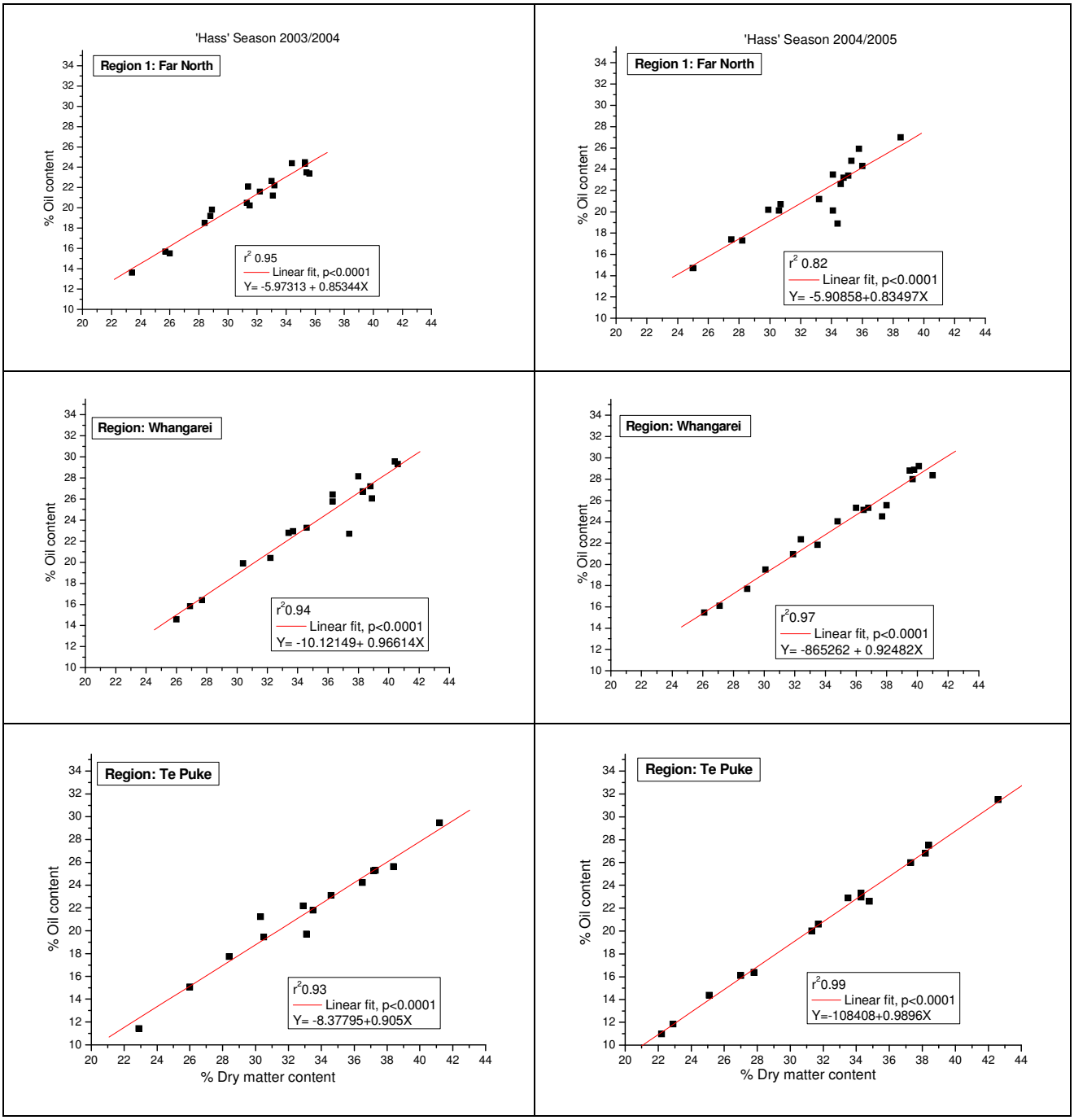


Figure 15. Correlation of dry matter with oil content for the 2003/04 session (left) and the 2004/05 season (right) from the Far North, Whangarei and Te Puke 'Hass' avocado growing regions in New Zealand, for three orchards/region. Each point is the mean of triplicate samples from 20 fruit.

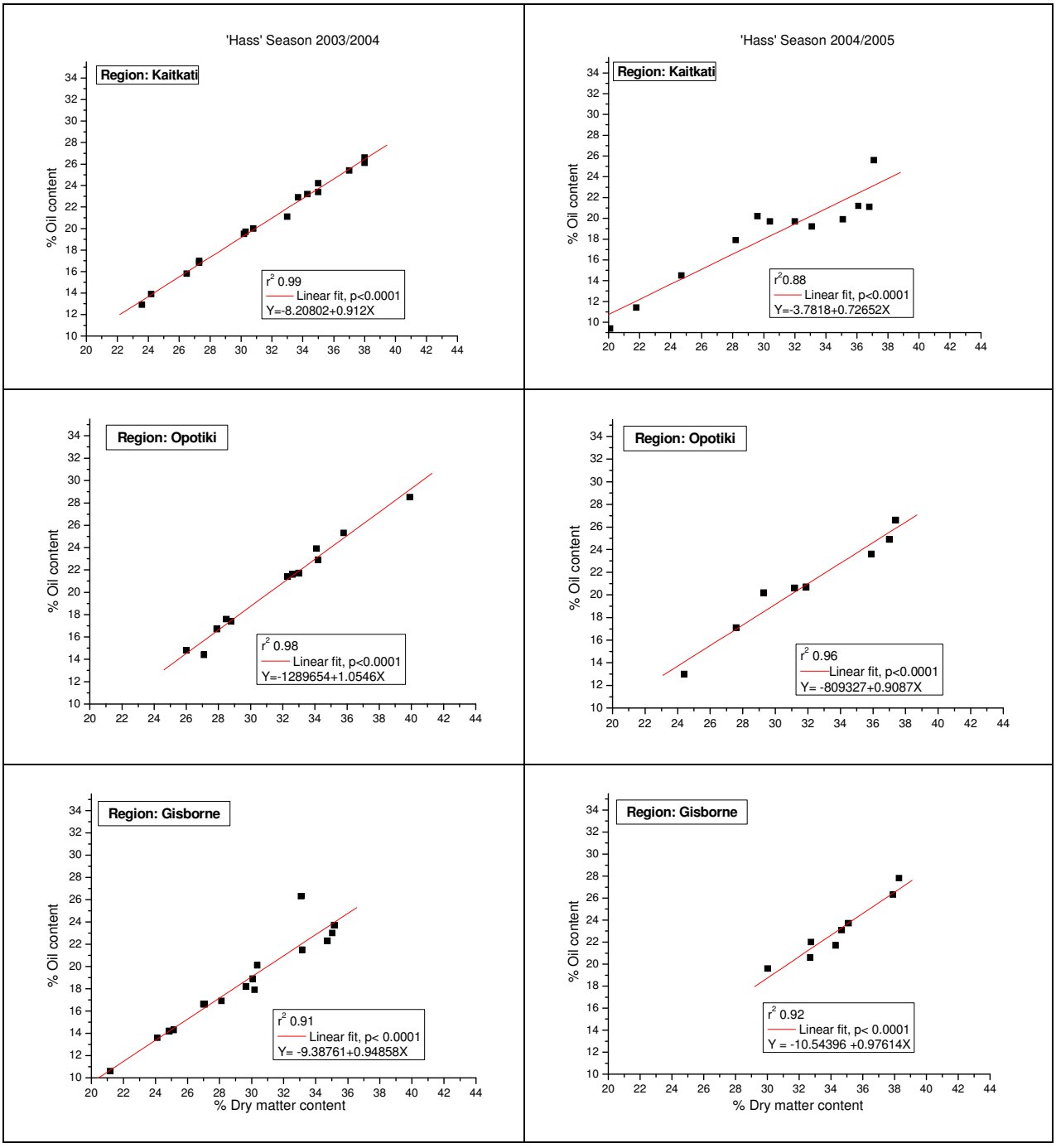


Figure 16. Correlation of dry matter with oil content for the 2003/04 season (left) and the 2004/05 season (right) from the Katikati, Opotiki and Gisborne 'Hass' avocado growing regions in New Zealand, for three orchards/region. Each point is the mean of triplicate samples from 20 fruit.

OTHER CULTIVARS: DRY MATTER AND OIL CONTENT

Australia: 'Shepard'

'Shepard' is an early cultivar, growing only in the Atherton and Bundaberg regions. The commercial harvesting season is relatively short because of the preference to access the early market.

When averaged over the two seasons, there were significant differences in dry matter between regions (P=0.006). Fruit from the Atherton Tablelands had higher dry matter on average than Bundaberg, but this may be because sampling started relatively late in 2004 because of late project approval. There was no significant difference between growers within a region.

As expected, there were significant differences between time in the season, with early harvest fruit having significantly lower dry matter than those from later harvests (P<0.0001). The percentage dry matter generally increased with later harvests, but there was notable difference in the rate of increase between years (Figure 17). The date when 21% dry matter was reached was similar between the two years for the Bundaberg region (Figure 18). The estimated date for the Atherton Tablelands was different between years, but the estimate for 2004 was based only on two harvests (Table 12).

Often, the dry matter of the early harvest sample in both regions was below the 21% commercial minimum maturity standard (Table 12, Figure 18). This probably reflects the desire to access the early market with this cultivar.

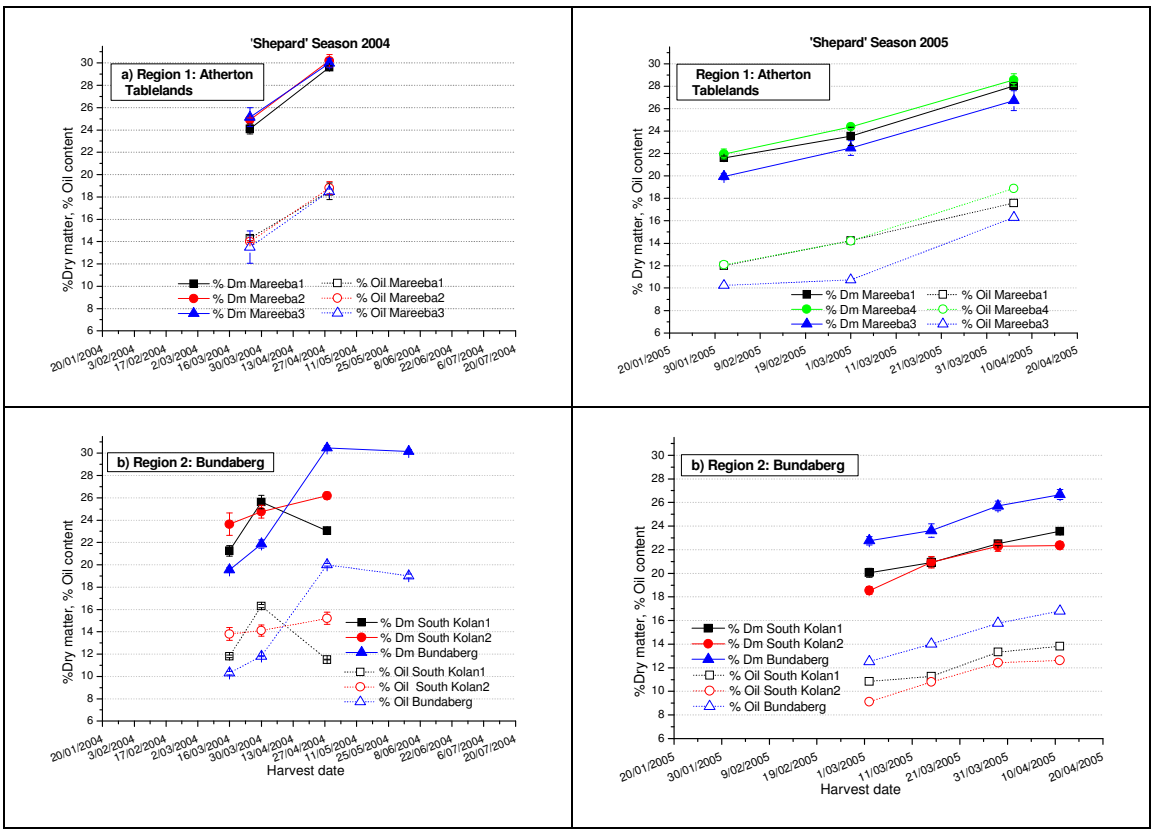


Figure 17. Percentage dry matter and oil content of Australian 'Shepard' avocados harvested from five main producing regions in 2004 and 2005. (Coastal SEQ = coastal south east Queensland). Values are the means of three replicates of 20 fruit. Vertical bars = standard errors of the mean.

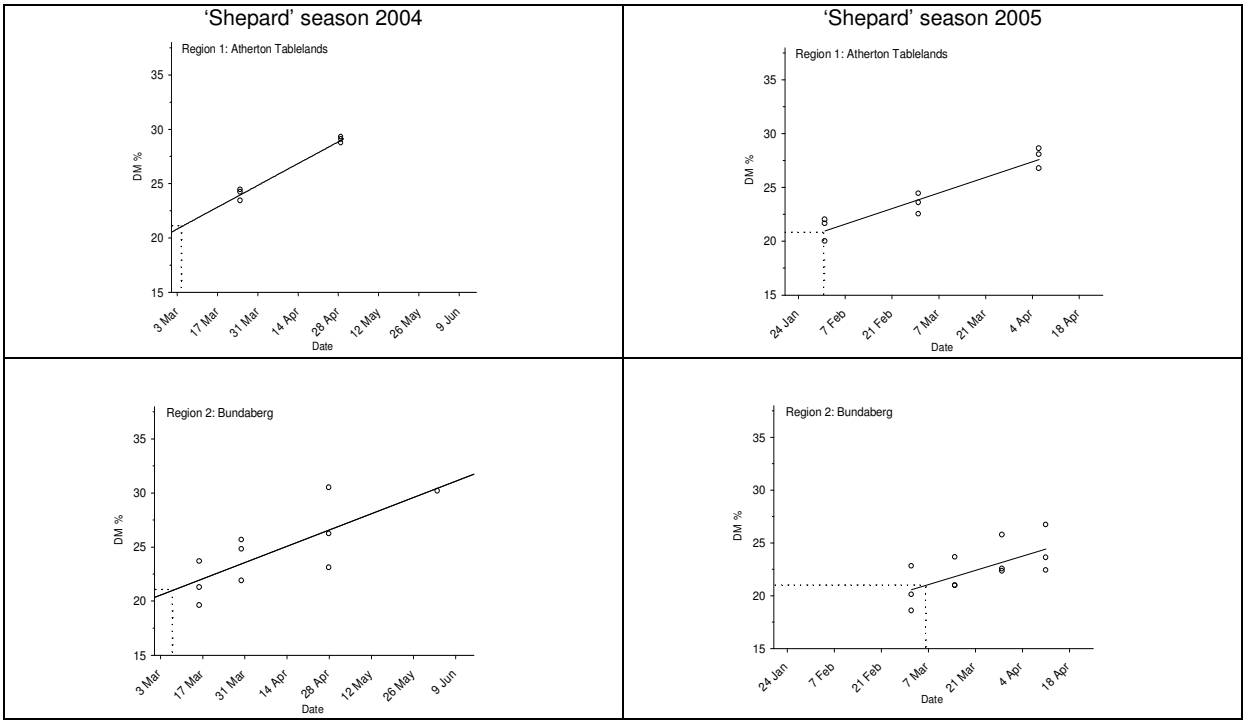


Figure 18. Prediction of when 'Shepard' avocado fruit reached the 21% dry matter (DM) standard in the main avocado production regions in Queensland during the 2004 and 2005 production seasons. Each data point represents the mean of 3 replicates from each grower for each time in the season. The dotted line indicates the estimated date for 21% dry matter.

Table 12. Australian 'Shepard' avocados harvested from several regions in 2004 and 2005: The projected date at which fruit reached 21% dry matter (DM; averaged across the 3-5 growers per region), the date of the first sample (approximately equivalent to the start of commercial harvest on the sampled orchards), and the dry matter at the first sampling (close to the start of commercial harvest).

	Region	
	Atherton T.	**Bundaberg**
Season	**Date at 21% DM**	
2004	4 Mar.[1]	7 Mar.
2005	1 Feb.	6 Mar.
Date of first sample (early harvest)		
2004		16 Mar.
2005	2 Feb.	2 Mar.
% Dry matter of the first sample		
2004		19.5-23.8%
2005	20-22%	18.8-23.3%

[1]Projected from only two harvest dates

When averaged over years and time of season, fruit from the Mareeba region had higher oil content than those from the Bundaberg region (P=0.01), again probably because of the late samplings in 2004 from the Mareeba region. As expected, early fruit had lower oil content than later in the season, but there were no significant differences between growers within each region.

Again, oil content changed in a similar pattern to dry matter (Figure 17). Thus, there were strong, significant relationships between dry matter and oil content, irrespective of growing regions or seasons, (Figure 19, Table 13). Table 13 indicates only small differences in the relationships between region and season. There was little difference in the estimated oil content at 24% dry matter, suggesting that similar maximum oil yields can be obtained from these regions.

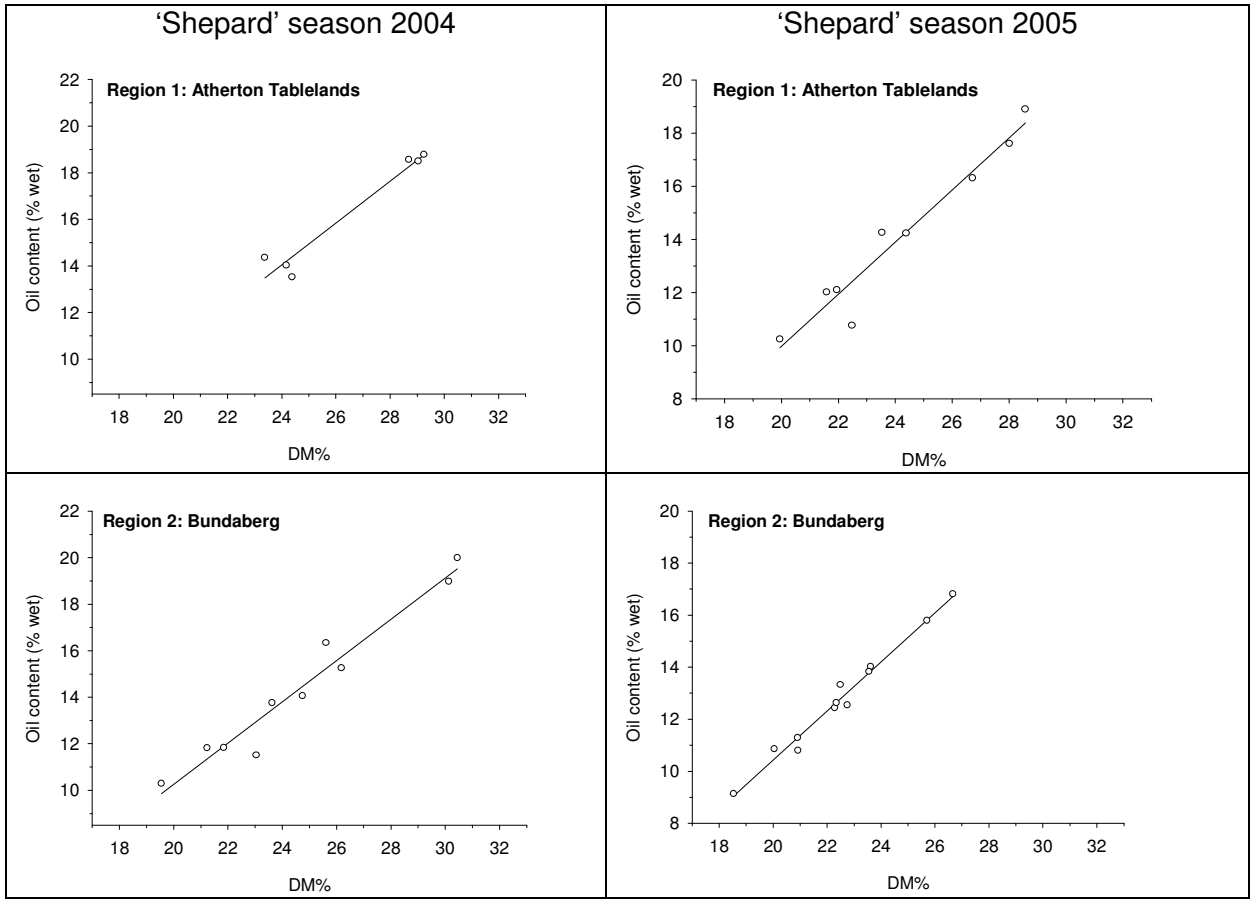


Figure 19. Relation between the percentage dry matter and the oil content (percentage fresh weight basis) in 'Shepard' avocado fruit flesh obtained from the main production regions in Queensland (Australia) in 2004 and 2005. Each data point is the average of the three replicate samples for each grower and time of season. The regression equations are presented in Table 13.

Table 13. The regression characteristics between percentage dry matter (DM) and oil content of 'Shepard' avocado fruit flesh obtained from the main production regions in Australia in 2004 and 2005. The linear regression equation is based on: oil content (%fresh weight) = a*(% dry matter) + c. The linear regression graphs are presented in Figure 19.

Growing region	a	c	r^2	Oil content at 24% DM
2004				
Atherton T.	0.90	-7.51	0.95	14.0
Bundaberg	0.89	-7.48	0.95	13.8
2005				
Atherton T.	0.98	-9.65	0.94	13.9
Bundaberg	0.94	-8.44	0.98	14.2

The difference between dry matter and oil content averaged 10.1% units over all regions and years, with very little difference between regions and years (9.7-10.2% units). The range between growers was 9.0-11.1% units.

Australia: Other minor cultivars

For the other minor cultivars, only occasional samples were taken. The results are presented in Figures 20 to 23, and Table 14. Generally dry matter and oil content increased with later harvests. As with 'Hass' and 'Shepard', there were very strong relationships between dry matter and oil content. The difference between average % dry matter and oil content was between 9.5 and 10.2% for all minor cultivars tested. There was also a strong linear relationship between these parameters (Figure 24).

For most of the minor cultivars, estimated oil content at 24% dry matter was similar to 'Hass' and 'Shepard' (Table 15).

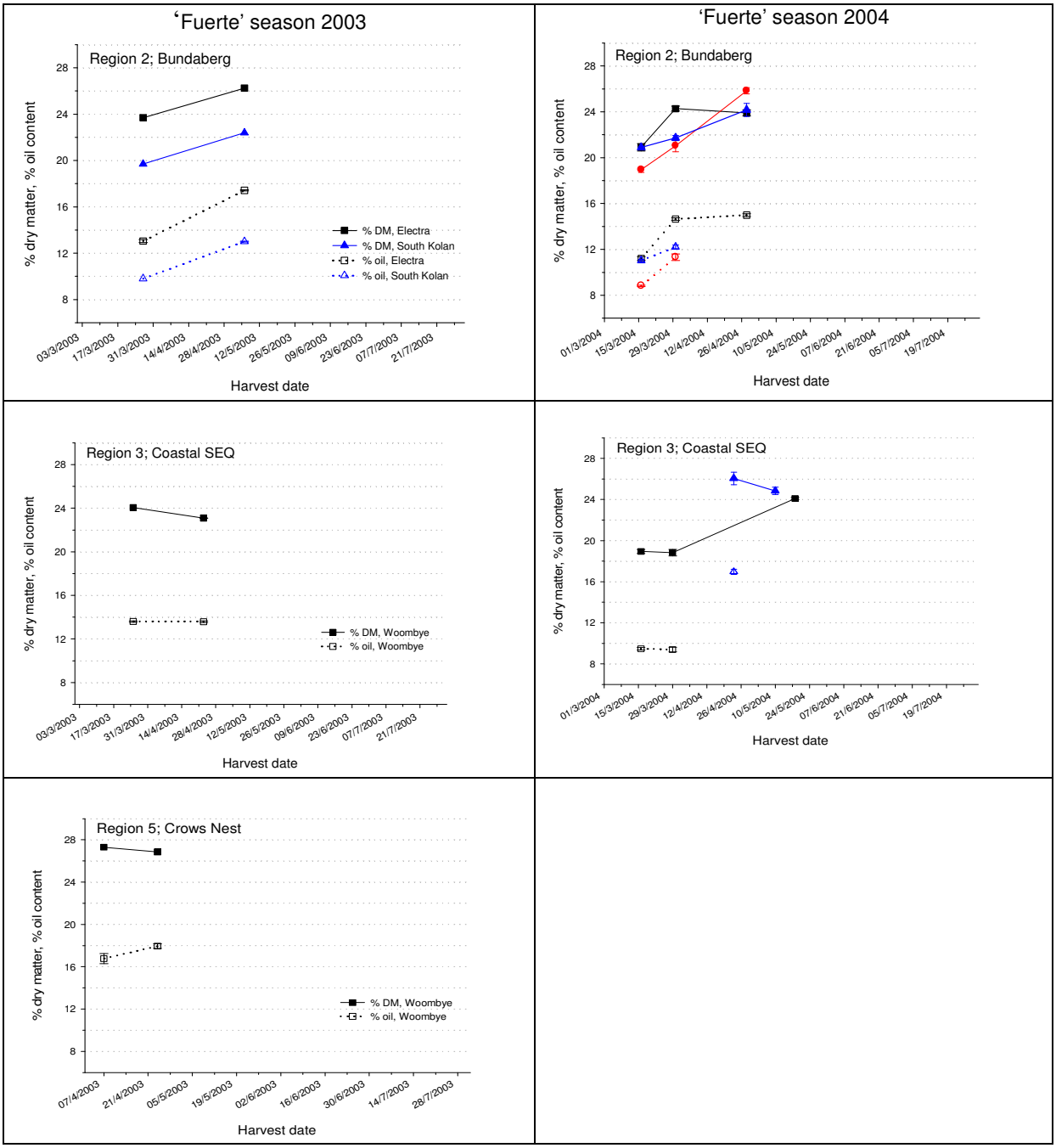


Figure 20. Percentage dry matter and oil content of 'Fuerte' avocados harvested from the Bundaberg, coastal region of Queensland (South East Queensland) and Crows Nest in Australia during the 2003 and 2004 production seasons. Each point is either one sample, or the mean of triplicate samples from 20 fruit. Vertical bars = standard errors of mean (SEM).

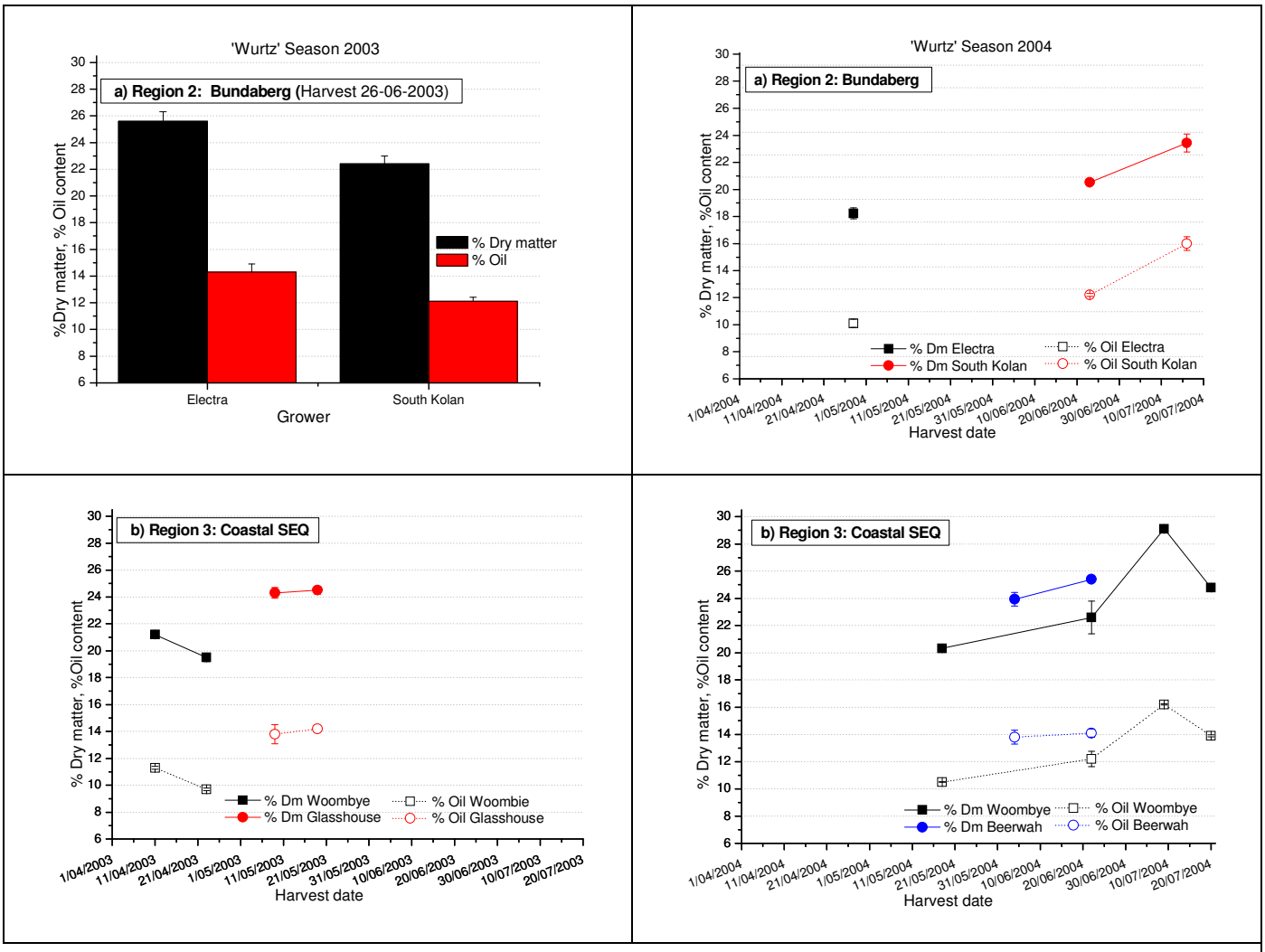


Figure 21. Percentage dry matter and oil content of 'Wurtz' avocados harvested from the Bundaberg and the coastal region of Queensland (South East Queensland) in Australia during the 2003 and 2004 production season. Each point is the mean of triplicate samples from 20 fruit. Vertical bars = standard errors of mean (SEM).

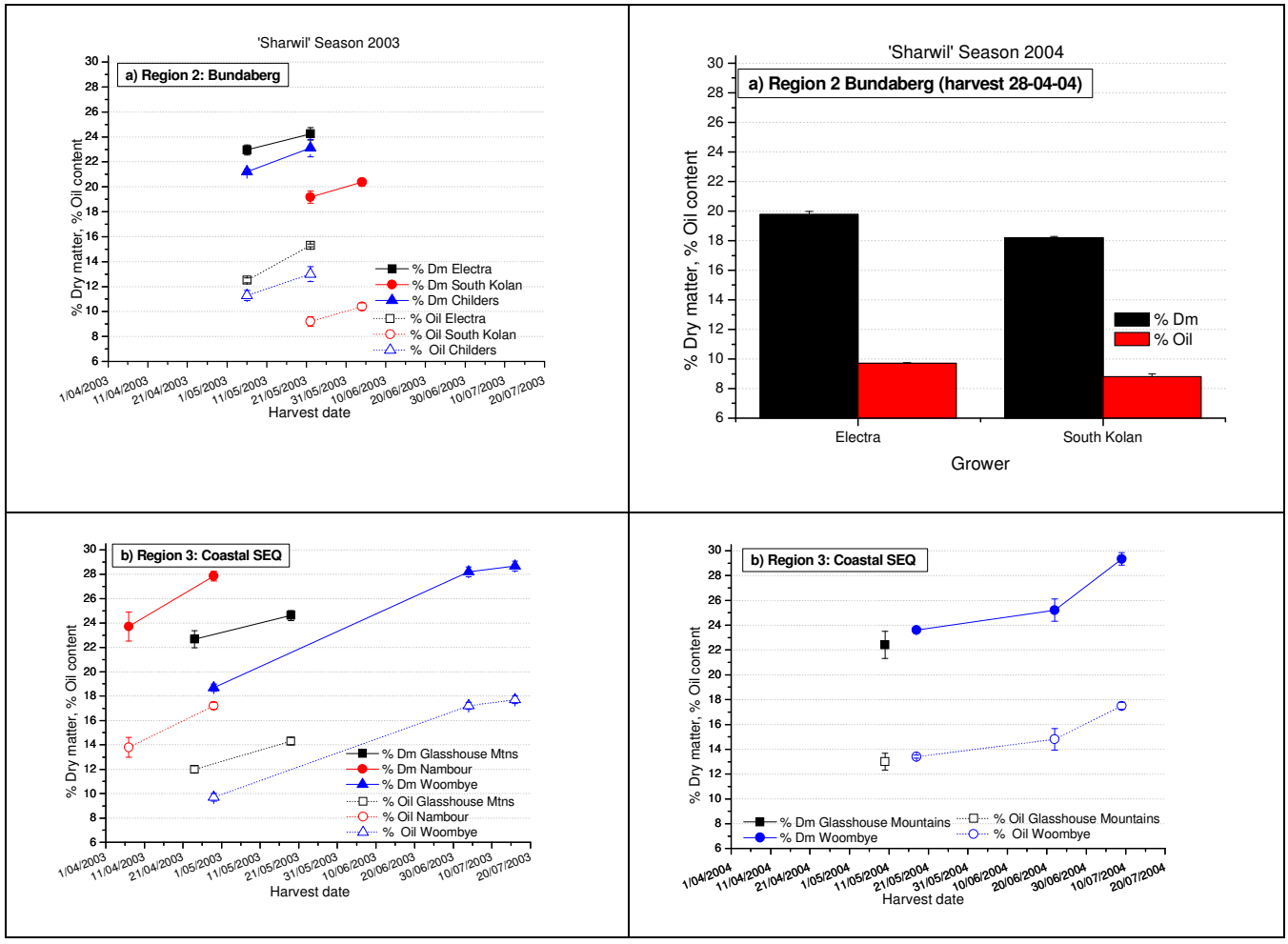


Figure 22. Percentage dry matter and oil content of 'Sharwil' avocados harvested from the Bundaberg and the coastal region of Queensland (South East Queensland) in Australia during the 2003 and 2004 production season. Each point is the mean of triplicate samples from 20 fruit. Vertical bars = standard errors of mean (SEM).

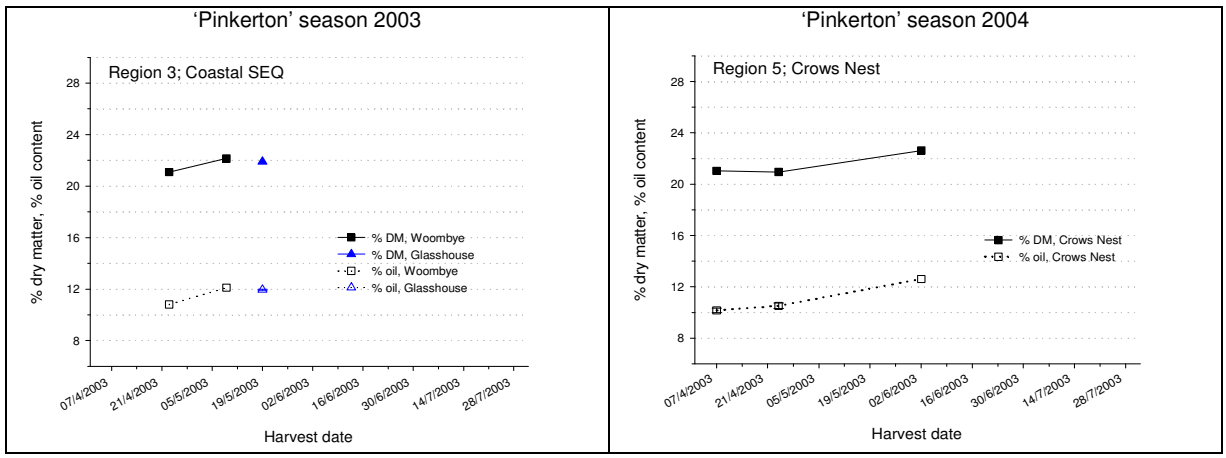


Figure 23. Percentage dry matter and oil content of 'Pinkerton' avocados harvested from the coastal region of Queensland (South East Queensland) and Crows Nest in Australia during the 2003 production season. Each point is either one sample, or the mean of triplicate samples from 20 fruit. Vertical bars = standard errors of mean (SEM).

Table 14. Percentage dry matter (DM) and oil content of several minor avocado cultivars harvested from the Woombye district of coastal south east Queensland in Australia during the 2003 and 2004 production season. Data are either one sample, or the mean of triplicate samples from 20 fruit. E, M, L = Early, Mid, Late.

Variety	Harvest date	Time in season	%DM	% Oil
2003				
'Rincon'	3/04/2003	L	24.6	13.5
'H77'	25/03/2003	E	21.9	12.4
	8/04/2003	M	22.4	12.8
	23/04/2003	L	22.1	14.5
'Hazzard'	16/07/2003	L	23.1	8.6
'Edranol'	16/07/2003	L	24.5	16.3
'Reed'	20/08/2003	L	23.7	13.3
2004				
'Reed'	9/07/2004	E	17.5	7.1

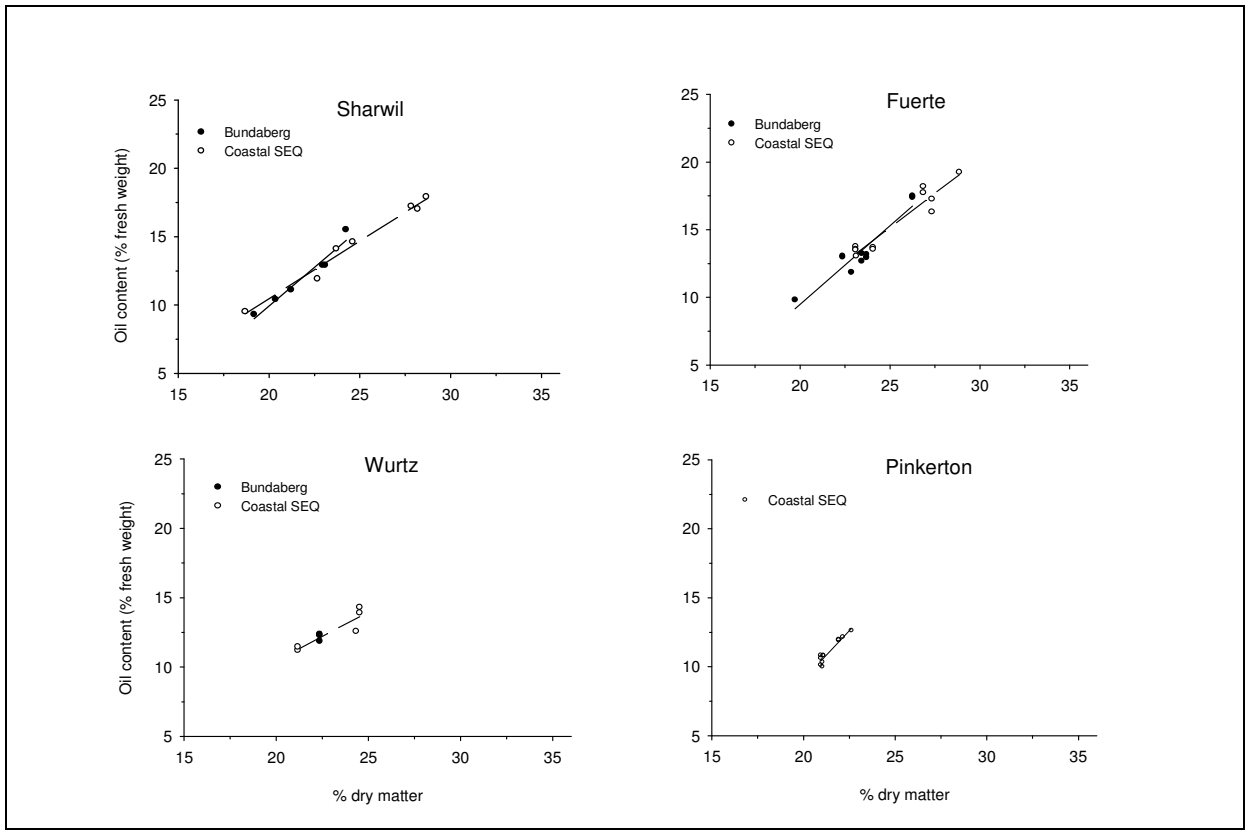


Figure 24. Relation between the percentage dry matter and the oil content (percentage fresh weight basis) in the flesh of several avocado cultivars grown in the Bundaberg and coastal south east Queensland regions in 2003. Each data point is the average of the three replicate samples for several growers and times of season.

Table 15. The estimated oil content at 24% dry matter (DM) for several cultivars grown in the Bundaberg and Coastal South East Queensland regions. The estimates were based on either the regression lines (Figure 24) or from single samples where insufficient data were available for accurate regression.

Variety	% DM	Oil (%)
From regression equation		
'Sharwil'	24.0	13.8
'Fuerte'	24.0	14.1
'Wurtz'	24.0	13.2
'Pinkerton'	24.0	14.8
From single samples		
'Reed'	23.7	13.3
'Hazzard'	23.1	8.6
'Edranol'	24.5	16.3
'Rincon'	24.6	13.3

DRY MATTER AND OIL CONTENT RELATIONSHIP IN AUSTRALIA AND NEW ZEALAND.

Comparing Australia and New Zealand results

The maturity of fruit from New Zealand, and therefore also the oil content, were significantly higher than that of Australia, primarily because New Zealand fruit is harvested at a higher dry matter level (minimum industry level of 24%). New Zealand harvests fruit later because of the cooler temperatures, and Australia aims to harvest its fruit early for the local market. Moreover, New Zealand exports to Australia after Australian growers (other than Western Australia) have completed their harvest. This also meant that fruit sampling carried out in New Zealand could be done over a longer period. This explains the observed plateau of dry matter in New Zealand after about January, while in Australia this was much less likely to be observed.

Figure 25 shows dry matter and oil content for all data measured in the two countries for 'Hass' from 2003 to 2005. The correlation is very strong ($P < 0.0001$) with an r^2 of 0.96 described by the formula $Y = -8.75937 + 0.92839X$. It is clear from the spread of data that regardless of dry matter content, (range 20 to 40%), dry matter is highly correlated to oil content. The close and positive relationship between dry matter and oil holds true over two different growing seasons in two very different countries, across an extremely wide range of growing environments (growing regions).

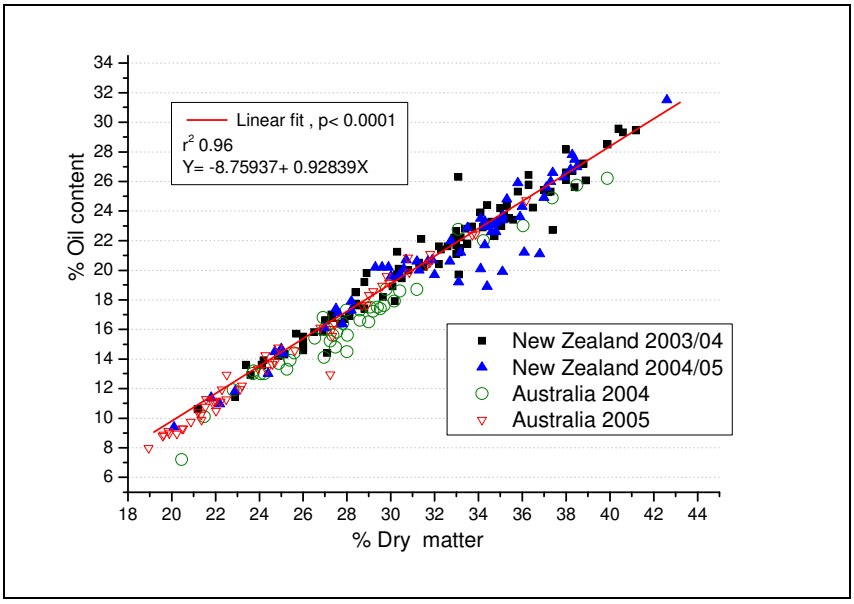


Figure 25. Relation between the percentage dry matter and the oil content (percentage fresh weigh basis) in the flesh of 'Hass' avocado grown in both New Zealand and Australia from 2003 to 2005. Each data point is the average of the three replicate samples for several growers and times of season.

HEALTHFUL COMPOUNDS IN EXTRACTED OIL

Australia

As noted previously, funding allowed only limited analysis of the makeup of the oil and thus the results are preliminary in nature, since there is little replication and no examination of multiple orchards.

Preliminary results of commercially extracted cold-pressed oil from 'Shepard' showed that it is mostly composed of unsaturated fatty acids (68%), and that the saturated fatty acids were at approximately 28%. Cold-pressed oil from Australian 'Hass' differs from that in New Zealand in that ≅ 78% of the oil is composed of unsaturated fatty acids and approximately 22% of the oil is saturated fatty acids, whereas in the oil extracted from New Zealand 'Hass', approximately 89% is unsaturated fatty acids and approximately 12% is saturated fatty acids. These differences could be due to climatic and cultural conditions.

The following results are from oil extracted using laboratory-based solvent extraction. The fatty acid profiles show that 'Sharwil' grown in Coastal Southeast Queensland (Region 3) had higher concentrations of monounsaturated fatty acids (MUFAs) than those grown in Bundaberg (Region 2; mean of 64.6% v. 60.7%, respectively) and this concentration seemed to increase in more mature fruit (Figure 26). In fruit from Bundaberg and Coastal Southeast Queensland, 'Wurtz' had a higher content of polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs) than 'Hass'. The concentration of MUFAs in 'Wurtz' was similar in both regions. For the early season samples from the Coastal southeast Queensland region, 'Reed' fruit sampled had lower concentrations of MUFAs and higher concentrations of PUFAs and SFAs than fruit of 'Sharwil' and 'Wurtz'. Even though 'Wurtz' fruit had higher amounts of PUFAs, they also contained higher amounts of SFAs. Growing region influenced only the fatty acid profile of 'Sharwil', where Coastal Southeast Queensland produced fruit

with higher proportions of MUFAs. Comparison of early, mid and late harvests showed a slight increase in MUFAs and a corresponding decrease in PUFAs and SFAs from early to mid to late season in 'Wurtz' from Bundaberg and 'Sharwil' from Coastal Southeast Queensland (Figure 26).

For 'Sharwil' the α-tocopherol concentration increased from early to late season fruit. For 'Sharwil' grown in Bundaberg, the α-tocopherol concentration had increased to 0.24 mg g^{-1} by mid season and in Coastal southeast Queensland, the α-tocopherol concentration had not increased to 0.24 mg g^{-1} until the late season harvest. For 'Wurtz' avocados, the concentration of α-tocopherol decreased from early to late season. Early in the season, 'Wurtz' had high concentrations of α-tocopherol (range 0.28 to 0.31 mg g^{-1}), in both Bundaberg and Coastal Southeast Queensland, but declined to 0.16 to 0.20 mg g^{-1} by late season. 'Reed' avocados had an α-tocopherol concentration for early season fruit mid way between those of 'Sharwil' and 'Wurtz'. The greatest influence on α-tocopherol concentrations in the fruit was harvest time and the best time to harvest for optimum concentrations of α-tocopherol depended on the cultivar (Figure 27).

The concentrations of β-sitosterol did not vary as significantly as those of α-tocopherol (Figure 28 v. Figure 27). Growing region showed a slight influence on β-sitosterol concentration, with higher concentrations of β-sitosterol in 'Sharwil' and 'Wurtz' from Bundaberg than from Coastal southeast Queensland. 'Sharwil' had higher concentrations of β-sitosterol than 'Wurtz', but 'Reed' from early harvest had the highest concentration at 4 mg g^{-1}, compared with early season 'Sharwil' from Bundaberg (3.8 mg g^{-1}). In 'Wurtz' and 'Sharwil', late harvested fruit appeared to have slightly lower β-sitosterol concentrations than the fruit from the early and mid harvests (Figure 28).

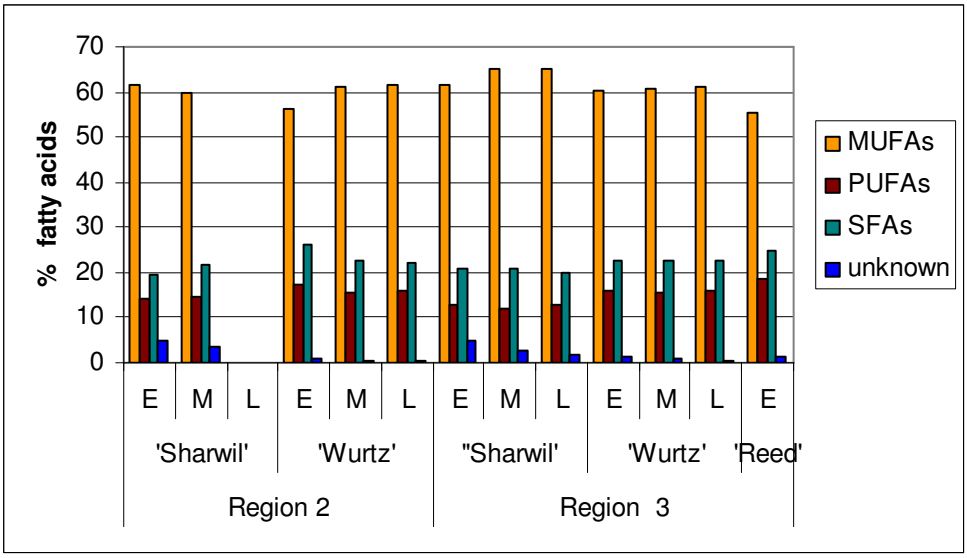


Figure 26. Fatty acid composition of avocado fruit harvested from Region 2 (Bundaberg) and Region 3 (Coastal Southeast Queensland) during the 2004 season. E-early season, M-mid season, L-late season. MUFAs-monounsaturated fatty acids, PUFAs-polyunsaturated fatty acids, SFAs-saturated fatty acids.

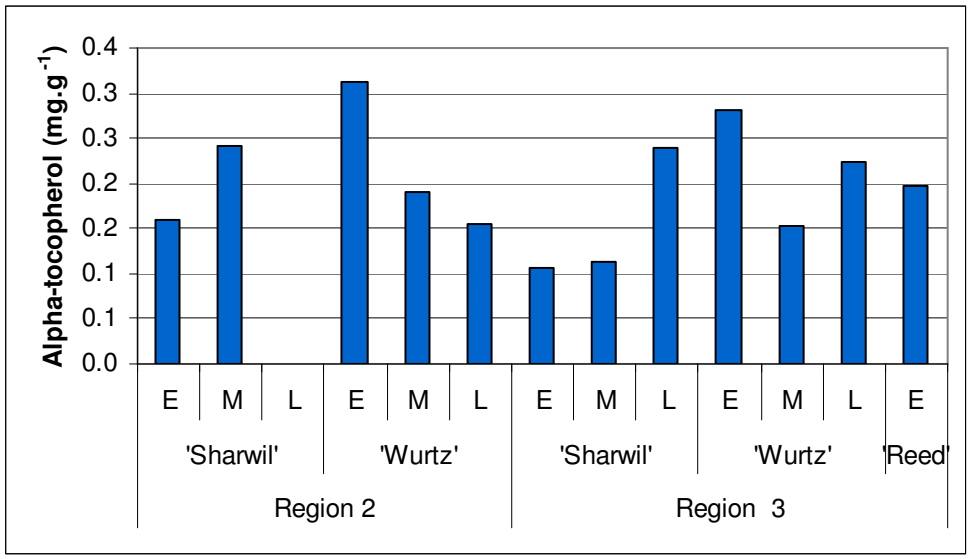


Figure 27. Alpha-tocopherol concentration (mg g^{-1}) in avocado fruit harvested from Region 2 (Bundaberg) and Region 3 (Coastal southeast Queensland) in 2004. E-early season, M-mid season, L-late season.

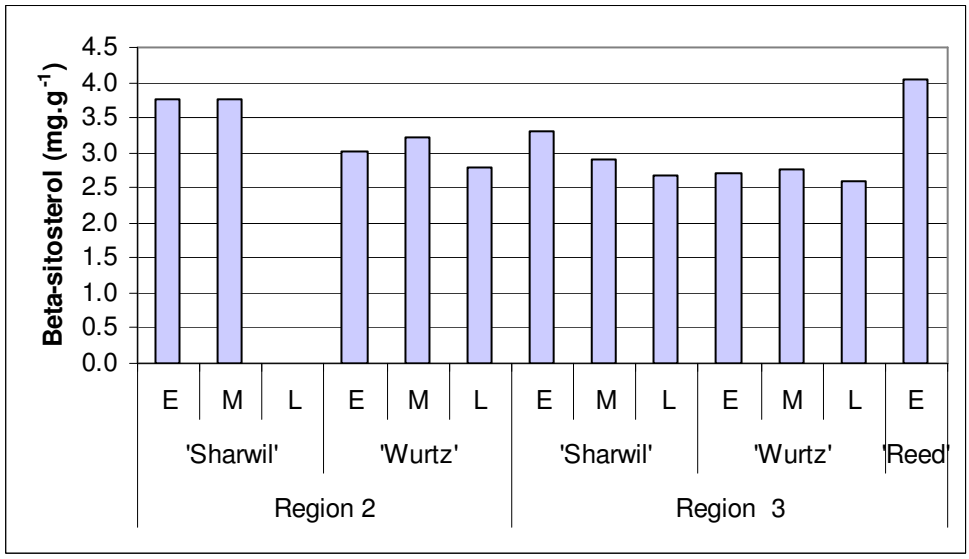


Figure 28. Beta-sitosterol concentration (mg g^{-1}) in fruit harvested from Region 2 (Bundaberg) and Region 3 (Coastal southeast Queensland) in 2004. E-early season, M-mid season, L-late season.

New Zealand: 'Hass'

Fatty Acids

Avocado oil is similar in its fatty acid makeup to olive oil (Table 16). On average it contains high amounts (≅ 75%) of monounsaturated fatty acids (oleic acid - C18:1 and palmitoleic acid - C16:1), and about 13% polyunsaturated (linoleic acid - C18:2 and linolenic acid - Cl8:3) and saturated fatty acids (palmitic acid - C16:0 and stearic acid - C18:0).

Pigments

The concentrations of plant pigments in avocado oil are high, as might be expected from its strong green colour. Avocado oil has a concentration of 1.6 µg/g of lutein, a pigment believed

to be of significance in eye-health. This pigment is believed to protect the cells of the macula from light-induced damage.

Alpha-tocopherol
Vitamin E (α-tocopherol) is a powerful antioxidant and the concentrations measured in New Zealand cold-pressed avocado oil were approximately 0.070 – 0.019 mg/g oil. These are very favourable and comparable to those in olive oil. Vitamin E is important not only for human health, but it also helps to stabilize oil and reduce its degradation in the bottle.

Beta-sitosterol
Plant sterols are also important important healthful compounds in avocado oil. The main plant sterol present in avocado oil is β-sitosterol, but we have measured low concentrations of other sterols such as D-5-avenasterol, campesterol and stigmasterol. Beta-sitosterol concentrations in New Zealand avocado oil were approximately 2.2-4.5 mg/g oil, which are significantly higher than that in olive oil.

Table 16. Composition of avocado oil extracted from New Zealand-grown 'Hass' avocados.

	Approximate average value
Typical Values at extraction	
% free fatty acids (as oleic acid)	0.28
Peroxide value (mEq/kg)	0.57
Fatty Acids (% of total)	
Oleic acid (monounsaturated)	71.46
Palmitoleic acid (monounsaturated)	4.09
Linoleic acid (polyunsaturated)	11.61
Linolenic acid (polyunsaturated)	0.53
Palmitic acid (saturated)	12.31
Stearic acid (saturated)	0.26
Pigments	
Chlorophylls (μg/g)	
Total chlorophylls	13.3
Chlorophyll a	4.9
Chlorophyll b	5.1
Pheophytin a	1.1
Pheophytin b	2.2
Carotenoids (μg/g)	
Total Carotenoids	1.9
Lutein	1.6
Neoxanthin	0.2
Violaxanthin	< 0.5
Antheraxanthin	< 0.5
Tocopherols (mg/g)	
Alpha-Tocopherol	0.11
Beta tocopherol	< 0.01
Gamma tocopherol	< 0.01
Delta tocopherol	< 0.01
Sterols (mg/g)	
Beta-sitosterol	3.28
D-5-Avenasterol	~ 0.3
Campesterol	~ 0.2
Stigmasterol	< 0.1

New Zealand: Other minor cultivars

Preliminary data are presented below, showing the lutein and chlorophyll concentration in laboratory cold-pressed extracted avocado oil from 5 cultivars, and one cultivar from two locations ('Hayes'; Figure 29). However, work is continuing in the extraction of oil from 13 cultivars other than 'Hass'. 'Pinkerton' showed a significantly higher concentration of these plant pigments, and concentrations in 'Santana' were very low. 'Hass', the predominant cultivar processed currently worldwide, had intermediate concentrations of these chemicals. The concentrations of α-tocopherol varied less than those of β-sitosterol (Figure 30), and significantly, 'Hass' appeared to have one of the highest concentrations, although 'Fujikawa' was similar to that of 'Hass'.

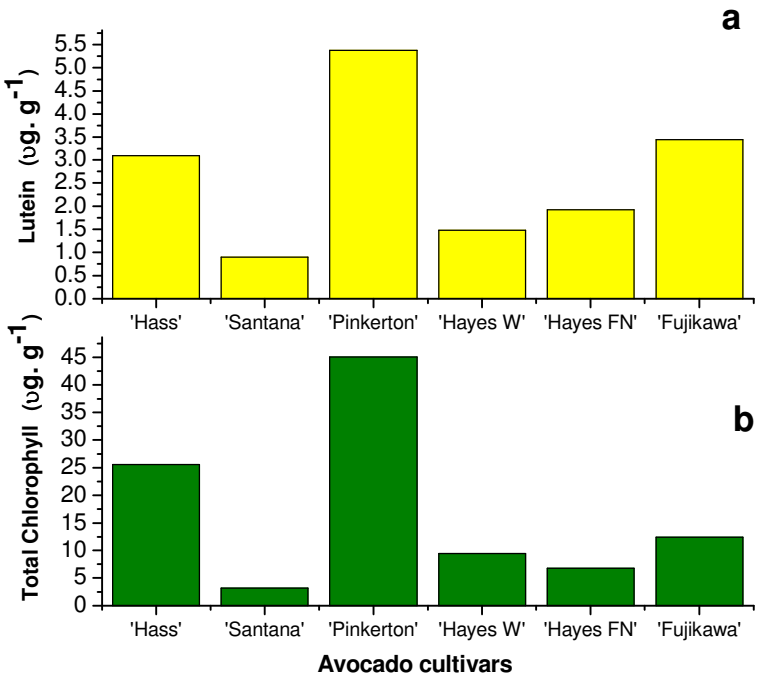


Figure 29. Concentration of lutein (a) and total chlorophylls (b) in laboratory cold-pressed oil from five avocado cultivars grown in New Zealand. Hayes W = 'Hayes' grown in Whangarei; Hayes FN = 'Hayes' grown in the Far North.

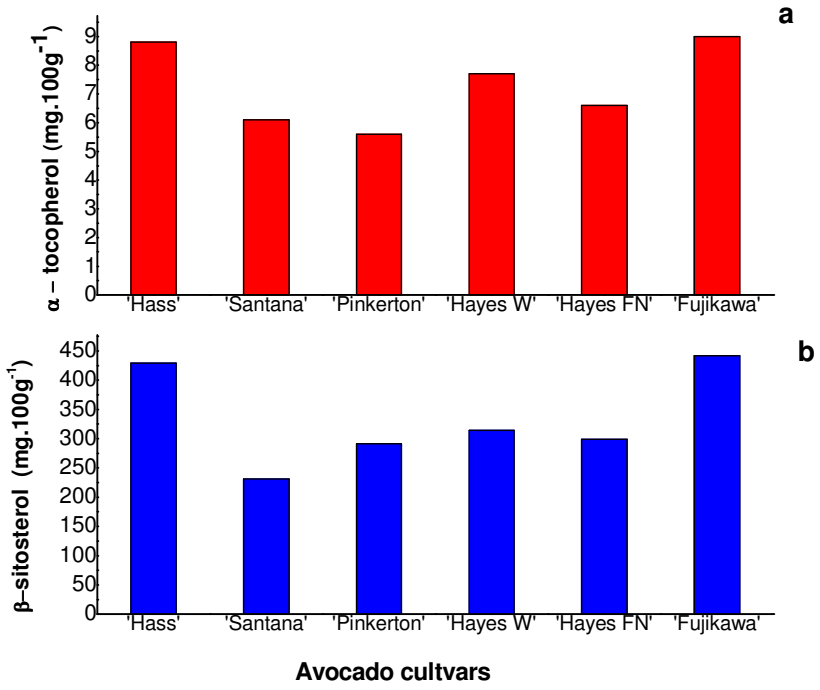


Figure 30. Concentration of Vitamin E (α-tocopherol (a) and β-sitosterol (b) in cold-pressed avocado oil from five cultivars grown in New Zealand. Hayes W = 'Hayes' grown in Whangarei; Hayes FN = 'Hayes' grown in the Far North.

COLD-PRESSED OIL YIELDS

Australia

Olivado NZ's oil extraction operations in Australia commenced in July 2004 in a purpose-built factory in Cleveland, Brisbane. The processing plant was modelled on the New Zealand unit (Alfa Laval), with some modifications. Oil yield results presented in this section are on a weight/weight basis (w/w), with the weight of oil yielded divided by the entire fresh fruit weight. This differs from the presentation in the laboratory-based oil extraction where data are presented as proportion of oil from the flesh of the fruit. Flesh makes up about 68% of the fruit weight (Wong et al. 2007).

2004 season

At the start of extraction (July 2004), 'Hass' avocados in the region contained approximately 25% dry matter. Based on results above, this would mean there was ≅ 15% oil available on a % oil in the flesh basis (which converts to roughly 10.2% available oil on a whole fruit basis). Commercial cold press extraction yields averaged 8.7% (w/w) in July to 13.1% (w/w) in September. Processing fruit availability gradually increased from approximately 40,500 kg to 126,000 kg in September.

2005 season

Olivado NZ started processing 'Shepard' fruit in March through until the end of April, whereas processing of 'Hass' fruit started in May and continued until October 2005. In the case of 'Shepard', Olivado NZ cold processed 370,358 kg of fruit with a seasonal average oil yield 7.7% (w/w). In the case of 'Hass', 277,760 kg of fruit were processed during the season, where the cold-pressed yields averaged 10.7% (w/w).

2006 season

Limited commercial extraction of cold-pressed oils from three minor cultivars found the yields to be 5.5% for 'Wurtz', 8% for 'Sharwil', and 9% for 'Reed'. These were significantly lower than the yield from 'Hass' (season average of 11% in Australia and ≅ 15% in New Zealand).

New Zealand

2006
Annual commercial cold-press extraction yields for 'Hass' averaged 11% in Australia, while in New Zealand extraction yields were about 13.5%.

GENERAL DISCUSSION

This work has sought to examine the dry matter and oil concentrations in as wide a range of cultivars as possible, and to do this at multiple times in the season. This has highlighted some of the genetic variability that exists in the dwindling number of cultivars present in Australasia. Further work on other cultivars than 'Hass' may be warranted in the future should the use of pollinizers become more widespread.

The work in New Zealand predominantly also looked at a wide range of the healthful compounds found in avocado: the fatty acid makeup (high in monounsaturated fatty acids), β-sitosterol, α-tocopherol (Vitamin E), and plant pigments, particularly lutein. This area of work has also been novel in that it has been able to show what pre- and postharvest factors are important in determining variability in healthful phytochemical concentrations (data not shown).

CONTINUITY OF OIL SUPPLY

Perhaps the key commercial factor facing oil extractors is achieving a reliable production of avocado oil that can provide oil to the markets over a calendar year. This is a combination of two factors, sourcing of adequate numbers of good quality avocados, and maximising oil yield.

Several key factors will influence the commercial viability of an avocado oil industry. These are, among others:

Supply/demand on local or export markets. A key factor that affects fruit availability is the complex interaction of supply/demand economics. This has been compounded in a number of years by problems in both Australia and New Zealand with fruit supply. Climatic effects have led to large swings in yields. Along with the resulting demand for local and/or export fresh fruit, this meant that the tonnages available for processing have varied widely. For example, in New Zealand there were 187 tonnes in 2005 and 1,282 tonnes in 2006, but only 190 tonnes in 2007. In addition, fluctuations in supply within a given season can also affect fruit availability for processing. For example, in 2005 in Australia, the supply of fruit to the domestic market was consistent, and at a level that maintained high consumer demand and fresh fruit prices. The prices that could be offered for processed fruit could not match those offered by the fresh fruit market, even for lower quality fruit that in other years might be reject grade.

This places processors in a difficult position to provide oil for the developing markets, particularly when supermarket chains require consistent supply. While price is one tool that the processor can use, there is an economic ceiling above which processing is simply uneconomic.

External quality/packout and quality standards. The standards set by the industry and/or packhouse will affect the proportion of fruit rejected for fresh fruit consumption. Clearly the quality of fruit produced by a given orchard/harvest time will also affect rejection rate and therefore the proportion of fruit destined for oil processing.

Oil content

Harvest timing from a maturity perspective. As avocados accumulate oil as they mature (as measured by dry matter content), the timing of harvest is critical for the amount of oil

available. We found average oil concentrations ranged from 8 to 26% (fresh weight) throughout the commercial harvest season.

Commercial harvest indices. These are significant since most avocados used for oil processing are fruit rejected from the fresh fruit market because of defects. Since avocados accumulate oil as they mature, if the commercial maturity standards are lower, then the oil content in reject fruit will be lower. On this basis, New Zealand's higher minimum maturity standard (24%) will mean average oil yields over the season will be higher than in Australia, where the standard is 21%.

Late hanging. The ability (or willingness) to leave reject fruit (small or externally unacceptable) on the tree after commercially acceptable fruit are harvested is another factor. For example, if small fruit are left on the tree at the end of the commercial harvest season, they can increase in size (and oil content) and be harvested solely for oil processing, if this fits with orchard management practices. This is a practice that the oil companies would welcome as routine practice.

Orchard maturity differences. Although we are not clear why, or what management practices influence maturity, there is no doubt that orchard differences occur, and that these can occur over relatively small distances. Tree age can also influence fruit maturity where fruit on trees less than 5 years old tend to mature earlier. However, in this work, we endeavoured to obtain fruit from trees of at least 8-10 years of age (see Appendix 1).

Growing region effects on maturity. As is well known (AIC data - http://nzavocado.co.nz/monitoring-results.html; Figure 13) and confirmed here, the growing region has a significant impact on fruit maturity in terms of both timing and maximum dry matter attained. In Australia, the commercial harvest period is strongly dictated by the desire to access the early or late market windows. Therefore, the Atherton Tablelands and Bundaberg regions have a lower seasonal average oil content because of few late-hanging fruit. This could have a significant effect on the viability of an avocado oil industry in Australia, compared with in New Zealand. It would be interesting to evaluate effects of later harvests on oil content in these regions.

Country. A wider interpretation of growing region is, of course, the country in which it is grown. Clearly there are large differences between Australia and New Zealand, and for example, California, where temperatures and rainfall differences can be extreme. These can affect tree growth, tree health, irrigation practices and fruit quality (particularly the expression of ripe rot which is higher in wetter growing environments). All of the above will affect maturity, and thus oil yield.

Season. This work has shown significant differences in the dry matter and oil accumulation patterns between seasons, being in some cases more significant than the grower or regional differences, both in terms of timing of maturity changes.

Cultivar. Clearly there are differences between cultivars and these interact with all the above factors.

Cold-pressed oil yield

Although the maximum amount of oil available for extraction (as determined by laboratory-based chemical extraction techniques) is a key factor, the most important issue for the oil processor is the yield of cold-pressed oil. Cold-pressed oil yield varies with fruit maturity, but may also be influenced by grower, region and season differences. The experience of Olivado

NZ has shown that early season fruit have poorer yields than late season fruit, even when dry matter is taken into consideration. This was not addressed in this work, but is an area that requires further study.

Oil quality

The concentration of beneficial phytochemicals in avocado oil may vary with cultivar, maturity, and production location. Although of secondary importance, these amounts may influence the harvest timing and indeed desirability of a fruit for processing. This is somewhat the case with 'Shepard' where the concentration of saturated fatty acids is significantly higher than for 'Hass', and for some fruit lines, the oil would have more than 20% saturated fatty acids and thus would not be eligible for the New Zealand Heart Foundation Tick (which requires a saturated fatty acids content of less than 20%).

Other aspects of fruit quality

The effects of orchard management practices such as fungicide sprays, and postharvest management factors that affect fruit quality are important, particularly in relation to ripeness, variability, and the levels of physiological and pathological discords. Fruit are partly ripened before processing. Therefore, any factors that negatively affect ripe flesh quality can reduce oil quality. For instance, the distance from orchard or packhouse and the processing plant can have significant impact in terms of economics (cost of transport), timeliness, flexibility, and the impact of transport on fruit quality. While these effects would likely be less for processed fruit than for the fresh market, they can still be significant. They include inadequate in-field and postharvest disease control, damaged fruit in the reject bin, poor cool chain management and delays between harvest and processing (either from long transport distances, or imbalance between fruit supply and processing capacity requiring storage). These factors are clearly more problematic in Australia than in New Zealand, where travel times may be in the order of days, rather than hours.

This project examined only those issues relating to oil content, and to some extent oil quality. Some other significant issues, such as fruit supply, can only be addressed through commercial negotiation. However, certain commercial practices (e.g. chain management and fruit storage) can assist in addressing some fruit supply issues, and these may require further research and development.

VIABILITY OF GROWING FOR OIL ONLY

One question that is often asked is whether avocados might be grown solely for oil processing. Clearly when oil supply is uncertain, this is one avenue that processors might consider. Overall, it appears unlikely that this will be economic in Australasia for the following reasons. Firstly, avocados require high quality land, which is therefore of higher value. Secondly, the savings that might be made in growing only for oil are not that large (such as fewer sprays for pests and diseases). Generally, to maintain adequate tree health, particularly avoiding root rot, requires a high level of husbandry (phosphine application by trunk injection, and good plant nutrition). Finally, it is unlikely that mechanical harvesting could be developed. It may be possible to develop processing-only production systems, but these would probably have to be based on using a very productive cultivar to minimise vegetative growth, very effective canopy management systems to minimise pesticide application and harvesting costs, pruning/training systems for mechanical harvesting, *Phytophthora*-tolerant rootstocks, and phosphonate foliar sprays for *Phytophthora* control.

Alternatively, growing for oil might be successful in other countries, such as the Pacific Islands or elsewhere where currently fruit have no, or limited markets and labour and/or land costs are relatively low.

A HIGH HEALTH AVOCADO OIL?

As noted above, avocado oil has a range of health-promoting phytochemicals that are important from a marketing perspective. A logical progression of these health benefits is to maximise, or potentially concentrate the health compounds such that the oil becomes more health focussed. This oil could then be either promoted as a "super high health" product line for culinary use, or encapsulated (or similar) and sold as a health supplement. Although our analysis of phytochemical components continues in the wide range of cultivars (mainly in the New Zealand work), we can see already that there are significant differences in concentrations of phytonutrients between cultivars, sometimes as much as 5-fold. These differences do suggest that cultivars might be sourced, or indeed grown specifically for their "high health' phytochemical-containing properties.

Olivado NZ have already released an oil in 2005 specifically aimed at the health market (Figure 31). "Omega Plus Oil" is a blend of avocado, olive and flaxseed cold-pressed oils combined to achieve the optimal combination of Omega 3, 6 and 9. This ratio (1:3:25) has been shown to be optimal for human heart health.

Figure 31. Olivado NZ "Omega Plus Oil", which combines cold-pressed avocado, olive and flaxseed oils to deliver oils with the optimal combination of Omega 3, 6 and 9.

A point that should be noted is that measurement of dry matter in this work was carried out using the "Hofshi coring machine" rather than the current standard industry techniques. This was carried out primarily to achieve a higher fruit replication, standardisation between New Zealand and Australia researchers, and fast throughput of many samples. Data from California (Arpaia et al. 2001; Figure 32) show a very high correlation (r^2 of 0.994) of the corer

technique with the standard "opposing vertical eighths" technique. The New Zealand industry has not moved to this technique because it is believed that in the early season, the plugger may slightly underestimate maturity. We do not believe these differences will be significant, and minimum dry matter amounts can be adjusted to take account of these effects. A discussion of the relative merits of these techniques is outlined in an *Orchardist* article (Woolf et al. 2003; Appendix 3).

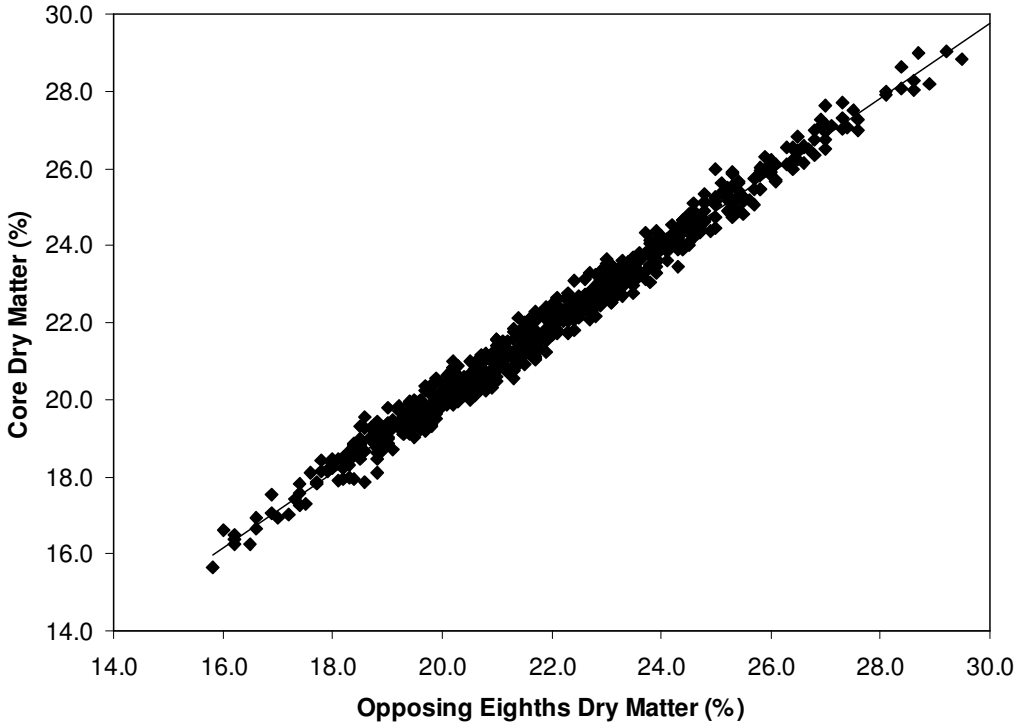


Figure 32. Correlation of standard Californian dry matter measure (Opposing eighths) for avocado fruit with the Corer technique. n=722, y = -0.080845 + 0.997240x. r^2 = 0.9941 (Arpaia et al. 2001).

CONCLUSION

This work has provided important foundational data on the key cultivar in Australasia - 'Hass' - in terms of maturity (dry matter), maximum oil yield (i.e. laboratory-based solvent extraction), concentrations of "healthful" phytochemicals, and cold-pressed oil yields. The key outcome from an oil processing perspective is that fruit should be harvested with high dry matter levels to ensure good oil yields, and the timing differs between growers and between growing regions, although region is a more significant factor. The data provided here provide a solid template for planning the processing season, since although there are differences between years, these are not as large as the regional effects. Although 'Hass' is likely to remain the primary cultivar for oil processing, the study of other cultivars has indicated their usefulness or otherwise for production of novel, "high health" oils, or for blending with 'Hass' oil.

TECHNOLOGY TRANSFER

Between the work carried out in New Zealand and in Australia, there has been a wide range of technology transfer activities other than the many meetings, emails and phone conversations that were held between HortResearch, QDPI and Olivado NZ. HortResearch has also played a significant role in the New Zealand Oils & Fats Specialist Group (Dr. Laurence Eyres), and this provides many opportunities for interaction and discussions, and presentations at joint conferences. Outputs include popular articles, reports, book chapters, scientific conference presentations, international refereed publications, and a Masters' thesis (awarded First Class Honours).

Following is a bibliography summarising the range of technology transfer activities.

Popular article

1. Hofman P, Requejo-Jackman J, Stubbings B, Roberto M, Woolf A 2006. Avocado oil processing in Australia- Benefits and Prospects. Talking Avocados. 16(4): 14-17.
2. Requejo-Jackman C, Wong, M Wang Y, McGhie T, Petley M, Woolf AB 2005. Preliminary results on oil quality and composition from avocado cultivars. The Orchardist. 78(10): 54-58.
3. Requejo C, Wong M, McGhie T, Eyres L, Boyd L, Woolf A 2003. Cold pressed avocado oil – a healthy development The Orchardist. 76(9): 56-58.

Reports

1. Woolf A 2005. CONFIDENTIAL Report. Assisting the development of the avocado oil industry in Australia and New Zealand. Report to Horticulture Australia Ltd, Project AV03007. HortResearch Client Report No. 15150. HortResearch Contract No. 18942.
2. Requejo-Jackman C, Hofman P, Stubbings B, Wong M, Wang Y, Olsson S, Woolf A 2006. Assisting the development of the avocado oil industry in Australia and New Zealand. Report to Horticulture Australia Ltd, Project AV03007. HortResearch Client Report No. 15152. HortResearch Contract No. 18942.
3. Requejo-Jackman C, Wong M, Wang Y, Olsson S, McGhie T, Hofman P, Stubbings B, Woolf A 2006. CONFIDENTIAL. Assisting the Development of the Avocado Oil Industry in Australia and New Zealand. Confidential Report to Horticulture Australia Ltd, Project AVO3007. HortResearch Client Report No. 15153. HortResearch Contract No. 18942.
4. Woolf A 2005. CONFIDENTIAL. Assisting the development of the avocado oil industry in Australia and New Zealand. Report to Horticulture Australia Ltd, Project AV03007. HortResearch Client Report No. 15150. HortResearch Contract No. 18942.
5. Requejo-Jackman C, Hofman P, Stubbings B, Wong M, Wang Y, Olsson S, Woolf A 2005. CONFIDENTIAL. Assisting the development of the avocado oil industry in Australia and New Zealand. Report to Horticulture Australia Ltd, Project AV03007. HortResearch Client Report No. 15150. HortResearch Contract No. 18942.

Meetings and presentations with Olivado NZ Management

In addition to the meetings outlined below, HortResearch and Olivado NZ were in regular contact by email and telephone. The science teams were also in regular contact by email and telephone.

1. May 25 2007. Woolf AB, Lund C, Ashton OBO, Wong M, Requejo-Jackman C, McGhie T 2007. Effect of skin proportion on shelf life and sensory properties of

avocado oil. Presentation to Olivado NZ management - Laurence Eyres and John Ellegard. HortResearch, Mt Albert Research Centre (MARC).

2. October 23 2006. Ashton OBO, Wong M, Requejo-Jackman C, McGhie T, Woolf AB 2006. Effect of skin proportion on "at extraction" quality of avocado oil. Presentation to Olivado NZ management - Laurence Eyres and John Ellegard, HortResearch, MARC.
3. June 18 2006. Woolf AB & Ross G met with John Ellegard to discuss avocado oil opportunities in the USA, HortResearch, MARC.
4. January 26 2006. Woolf AB, & Wong M met with Olivado NZ management. Laurence Eyres and John Ellegard at Novotel, Ellerslie, Auckland.
5. October 26 2005. Woolf AB met with CEOs of both Olivado NZ and The Grove to discuss research and potential collaborations, HortResearch, MARC.
6. October 15 2005. Woolf AB, Lund C, Ashton OBO, Wong M, Requejo-Jackman C met with Olivado NZ management - Laurence Eyres and John Ellegard to overview avocado oil research to date, HortResearch, MARC.
7. 20-22 September 2005. Woolf AB, Hoffman P, Requejo-Jackman C met with Olivado NZ at HortResearch.
8. April 12 2005. MARC, Auckland. Meeting at HortResearch between A Woolf, C Requejo, Olivado NZ Ltd representatives - Laurence Eyres and John Ellegard and Massey University's collaborator Dr M Wong and technician Wang Y to discuss storage trial III results.
9. October 11-13 2004. Brisbane, Australia. Over two days, Allan Woolf met with Chris Nathan (Managing Director of Olivado NZ) and Peter Hofman and Barbara Stubbings (QDPI) to discuss wide range of issues in relation to avocado oil (including the HAL Avocado Oil Project), and avocados.
10. July 6 2004. Woolf AB, Lund C, Ashton OBO, Wong M, Requejo-Jackman C met with Olivado NZ management and parternes for evening meeting to discuss sensory properties of avocado oil. HortResearch, MARC.
11. July 1 2004. Allan Woolf and Cecilia Requejo met with Olivado NZ (John Ellegard and Laurence Eyres) and Marie Wong (Massey University) to discuss FRST avocado oil research and related issues. Greenlane, Auckland.

Masters' thesis

1. Ofelia Batalla Orlinga Ashton 2005. Pigment composition of 'Hass' avocado and the extracted oil. A thesis presented in partial fulfilment of the requirements of the degree of Masters of Technology, Massey University, New Zealand.

Book chapters

1. Woolf A, Requejo-Jackman C, Lund C, McGhie T, Olsson S, Eyres L, Wang Y, Bulley C, Wong M 2007. Avocado oil and Other Niche Culinary Oils in New Zealand. O'Connor, C., Eyres, L. (eds). Handbook of Australasian Lipids. (in press).
2. Wong M, Ashton OBO, Requejo-Jackman C, McGhie T, White A, Eyres L, Sherpa N, Woolf AB 2007. Avocado Oil - The Colour of Quality. Wrolstad, R.E., Culver, C. (Eds). Color Quality of Fresh and Processed Foods. ACS Publishers. (in press).

Refereed publications: Published

1. Ashton OBO, Wong M, McGhie TK, Vather R, Wang Y, Requejo-Jackman C, Ramankutty P, Woolf AB 2006. Pigments in Avocado Tissue and Oil. J. of Agricultural and Food Chemistry 54: 10151-10158.

Refereed publications: In preparation

1. Wong M, Ashton OBO, McGhie T, Lund C, Requejo-Jackman C, Wang C, Roberts C, Woolf AB 2007. Influence of skin level on quality of avocado oil from cold pressed extraction. J. of Agricultural and Food Chemistry.
2. Requejo-Jackman C, Wong M, Wang Y, McGhie T, Ashton OBO, White A, Petley M, Ramankutty P, Eyres L, Woolf AB 2007. Effect of 'Hass' avocado storage period on oil quality and composition. J. of Agricultural and Food Chemistry.
3. Requejo-Jackman C, Olsson S, Wong M, Wang Y, McGhie T, Woolf AB 2007. Effect of fruit maturity, growing region and season on dry matter, oil content and health components of 'Hass' avocado oil.
4. Requejo-Jackman C, Olsson S, Wong M, Wang Y, McGhie T, Woolf AB 2007. Effect of fruit maturity and cultivar on dry matter, oil content and health components of 'Hass' avocado oil.

Scientific presentations

1. Woolf AB, Requejo-Jackman C, Olsson S, McGhie T, Wong M, Wang Y, Ashton OBO, Eyres L 2007. An Overview of avocado oil and factors influencing health component content. NZIFST Conference, Wellington, New Zealand June 18-21.
2. Woolf AB, Requejo-Jackman C, Olsson S, McGhie T, Wong M, Wang Y, Ashton OBO, Eyres L 2006. Healthy functional ingredients in avocado oils and avocados. Functional Foods 2006. Lipids for Lifestyle and Profit. Bruce Mason Centre, Takapuna, Auckland, New Zealand November 16 2006.
3. Wong, M, Requejo-Tapia C, McGhie T, Wang Y, Eyres L, Woolf A 2004. Recent Research On The Health Components In Cold Pressed Avocado Oil. Australasian AOCS Conference Proceedings Adelaide. Fats and Oils – Their Role in Food & Health. Adelaide, Australia 30 November – 1 December.
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5. Hofman P, Stubbings B, Requejo-Jackman J, Woolf A 2004. AV03007: Assisting the development of the avocado oil industry in Australia and New Zealand. Presentation at the AAGF R&D workshop. Brisbane, Australia 25 August 2004.
6. Hofman P, Stubbings B, Requejo-Jackman J, Woolf A 2005. AV03007: Assisting the development of the avocado oil industry in Australia and New Zealand. Presentation at the HAL R&D workshop. Brisbane August 2005.

RECOMMENDATIONS

- Aim to process fruit with as high a level of maturity as possible, which generally equates to as late in the season as possible (although in some regions plateauing was found)
- Encourage growers to late hang as many poor quality or small fruit as possible
- 'Shepard' does not appear to be a viable source of oil, mainly because it is harvested early (low dry matter), and has high concentrations of saturated fatty acids
- At this point it appears unlikely that any of the minor cultivars are of high commercial significance, although 'Pinkerton' may show promise with slightly higher oil yield, and high lutein content
- The oil content and its chemical comparison should be one factor considered in choice of pollinizers, since use of the fruit for oil extraction is an alternative revenue stream
- Healthfulness of 'Hass' avocado oil is high, and this information should be used in marketing of avocado oil, and indeed fresh avocados
- Research ways to maximise cold-pressed oil yield, i.e. to achieve as near as possible to the maximum oil content
- Research ways to maximise the healthfulness of cold-pressed avocado oils, pre- and postharvest, and during processing
- Human trials proving healthfulness of avocado oil would be beneficial, although cost may be beyond the scope of the current avocado oil processing industry.

ACKNOWLEDGEMENTS

We thank Olivado NZ management and staff who have made time and resources available for this and other related avocado oil research. We particularly wish to thank the many growers and packhouse staff in New Zealand and Australia who have taken time and effort to provide fruit for this research. Without their help, work of this extent would not be possible. This work was funded by HAL (Assisting the Development of the Avocado Oil Industry in Australia and New Zealand - AV03007), the New Zealand Foundation for Research Science and Technology (Opportunities for New Zealand's Emerging Horticultural Crop Industries - C06X0203), and the Queensland Department of Primary Industries and Fisheries.

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APPENDICES

APPENDIX 1: LETTER TO GROWERS AND SAMPLING PROTOCOL

Dear Grower,

We are carrying out Government-funded research into avocado oils. The work aims to examine preharvest effects on oil quantity and quality (including the concentration of health components). Our preliminary research undertaken for the Avocado Industry Council showed a correlation between dry matter and oil content in the fruit. There is a need to understand how these variables change in a range of growing regions around the country and how changes in the fruit characteristics over the season influence quality and confer health benefits in processed products, such as oil.

For the present work we are examining how the oil content increases over the picking season. We are examining the effect of cultivar, maturity, orchard and region on oils. For 'Hass' (the main cultivar), this involves obtaining samples from The Far North, Whangarei, Katikati, Te Puke, Opotiki, and Gisborne. We aim to get fruit from three orchards from each of these regions, and to examine maturity effects thoroughly we want to get fruit at 6 times during the season October - April.

We are asking for your assistance in providing us with 'Hass' fruit over this time. At each sampling time we require 25 fruit, which should be couriered to us at Mt. Albert Research Centre, Auckland (see below for details of harvesting and couriering details).

If you could please provide us with an email address or fax (whichever you check most regularly), we will contact you on the week prior to remind you of the upcoming sampling time.

At the completion of each season, we will provide you with a graph of the dry matter patterns of your orchard, and how that compares with the other orchards and regions.

We greatly appreciate your time and effort in assisting with this work.

Allan Woolf and Cecilia Requejo

Harvest protocol and timing for HortResearch avocado oils maturity project

To assist us with processing this number of samples we would appreciate you harvesting on the following dates:

1. Monday 6 or Tuesday 7 October 2003
2. Monday 17 or Tuesday 18 November 2003
3. Monday 15 or Tuesday 16 December 2003
4. Monday 19 or Tuesday 20 January 2004
5. Monday 23 or Tuesday 24 February 2004
6. Monday 5 or Tuesday 6 April 2004.

Some considerations when harvesting our samples are:

1. Select five at least 8-10 year-old trees (healthy, "normal" trees in "average" locations i.e., not beside shelter belts/shaded etc.) This could mean marking the trees with tape (or similar) in that block to ensure fruit are left on tree for the next sampling round. This is particularly important for the later harvests and in this case you might wish to mark clusters of fruit to be left.

2. Second or third grade fruit is OK (i.e. insect damaged, ridging etc.), but please, no fruit which are sunburnt (yellow is acceptable, but not red/black) and/or of generally of unsound nature (rots). However, if possible harvest fruit of moderate size (i.e. approximately 20-24 count for 'Hass'). We realise that this may not always be possible.

3. Fruit should be sampled from all parts of the tree i.e. around the tree, and from a height of between 2 and 8 feet.

4. Fruit should be harvested in the morning and sealed (tied) in a plastic bag when in the tray so as to minimise water loss.

5. Please use bubble wrap or any other material for cushioning the fruit during transport. Plus state FRAGILE on the label.

6. In a piece of paper inside the box (or by e-mail if you prefer) state: harvest date, cultivar name and number of fruit you are sending.

7. Courier overnight to HortResearch at 120 Mt Albert Rd. Mt Albert, Auckland. Attention: Cecilia Requejo.

8. We will reimburse you for fruit and courier costs. We would like to do this at one time in the year, preferably after the end of the last harvest, but before June 30 (end of our financial year). Please post invoice to HortResearch, Private Bag 92169 Auckland. Attention: Allan Woolf.

Please let us know when you have sent the fruit so we can be aware of arrival.

If you have any problems or questions do not hesitate to contact us on:
Allan: 09-8154200 ext. 7050 mob# 0275 744-440 awoolf@hortresearch.co.nz
or
Cecilia: 09-8154200 ext. 7001 mob# 021 731-555 crequejo@hortresearch.co.nz

APPENDIX 2: HAL TISSUE SAMPLING PROTOCOL

NOTE: In the **middle of the season** remember to get one extra tray of fruit for the "**cold press**" sampling.

Samples of fruit from different cultivars and regions and growers will be harvested.

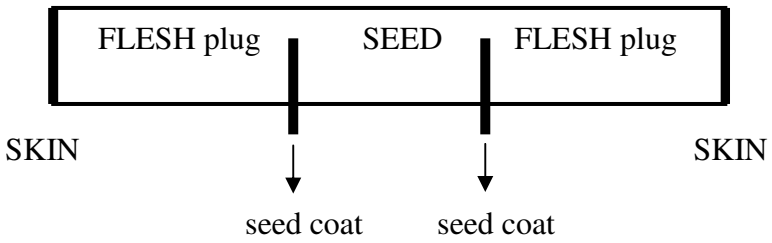
In spreadsheet provided, please record: cultivar, region and grower, harvest date, time in the season (early/mid/late).

Also record:

- Number of trays received from each grower (1 or 2?)
- Number of fruit in tray and the net tray weight (weight of total fruit in tray only with no box).

I. –Tissue Sampling Proceedure for Dry Matter and Oil Quality Determination

Picture of one avocado core: yields 2 flesh plugs



I.1 Tissue Sampling for Industry Dry Matter - Procedure:

1. Take 2 cores per fruit = each fruit yields 4 flesh plugs
2. As you plug, snap the seed out and place 1 plug in one container for a): Industry dry matter, and 3 plugs in another container for b): freeze dried, see below:

   a) The 1 plug for *Industry Dry matter* measurements:
      - With the aid of a scalpel/ blade remove the skin and seed coat (trying to cut off as little flesh as possible)
      - Slice the plug in half longitudinally
      - When all plugs have been sliced, mix/randomise all halves thoroughly
      - Sample three replicates of approximately the same weight in pre-weighed dishes.
      - Measure fresh weight
      - Into oven 65°C for 48 hours (or whatever consistently leads to dry tissue)
      - Important: Discard samples **ONCE** you have entered data and checked that the % dry matter makes sense.

I.2. -Tissue Sampling for Freeze Dried Dry Matter, Oil Yield and Quality Analysis:

b) The 3 plugs for *freeze-dried DM* and further oil yield and oil quality analysis.

- If there are 20 fruit/ tray
- And each plug = approximately 2.5 g.

Then:

- 3 plugs x 20 fruit= 60 plugs x 2.5 g = ***150 g plugged fresh tissue/ tray***
    - Cut off skin and seed coat (as for above)
    - Mix/randomise these plugs
    - Divide into three replicates of approximately same size
    - Sample three replicates of approximately the same weight in pre-weighed dishes
    - Measure fresh weight
    - Freeze samples: If possible, pour at least a small amount of liquid nitrogen over the samples and place them immediately into –20°C or preferably –80°C freezer
    - These samples can be left in freezer until ready to freeze-dry:
    - Freeze dry: Straight from freezer to dryer: …
    - How long to dry? Depends! At HortResearch takes between 10 and 12 hours (shelf temperature was +20°C, but in your case will depend on your machine etc.)
    - Immediately on removal:
        - Measure dry weight (enter into spreadsheet and check dry matter makes sense)
        - Quickly put replicate samples in oxygen-barrier foil bags, one foil bag each replicate, flush with nitrogen gas and seal
    - Labels on bags of tissue should **"FD/ *Sample ID/ Replicate (A, B or C)"***. Enter records in spreadsheet
    - Store samples at ambient (air conditioning would be nice) in cardboard boxes.

3. Shipment of samples will need MAF documentation etc.
4. At HortResearch, the freeze-dried tissue will be ground to a powder, divided into three replicates one replicate taken to extract oil using the Soxhlet method (maximum of 60 g in machine) with hexane and under nitrogen gas to preserve the quality of the components at all times
5. The oil yield will be calculated. This oil will be kept in dark bottles flushed with nitrogen gas
6. The oil will be sent to Massey University (Marie Wong) for:
    - HPLC analysis: Fatty acids, carotenoids, b-sitosterol, x- tocopherol, persin?

II.- Tissue Sampling Proceedure for *Cold-pressed Oil*

We are not interested in yield (i.e. amount of oil from tissue) from this type of extraction; we are interested only in an indication of the colour, taste, other visual characteristics of the oil.

1. In the mid harvest for each cultivar, we require tissue samples for cold press oil extraction (remember to obtain an extra tray of fruit from the mid harvest) for cold press sampling
2. Peter/Barbara will ripen one tray of fruit
3. Ethylene treat (18-20°C, 100 ppm ethylene) for at least 3 days, flush daily, and use hydrated lime for CO_2 scrubbing (new lime each run)
4. At the time when **most** of the fruit are fully ripe (approximately stage 5), record the days to ripen (DTR), and sample all flesh tissue (even if not exactly stage 5 i.e., do not let fruit soften too much)
5. All flesh tissue must be "sound tissue" i.e., free from rots, greying, vascular, browning etc.
6. Quickly divide bulk of tissue in 3 replicates bags (A, B, C) of approximate 600 g each.
7. Flush each bag with nitrogen gas and freeze it as soon as possible (if you can do this with even a little liquid nitrogen that would be nice)
8. Labels on bags of tissue should say **"COLD/ *Sample ID/ Replicate* (a, b or c)"**
9. Store samples at –20°C. The samples will then be delivered to Massey University for cold-press extractions.

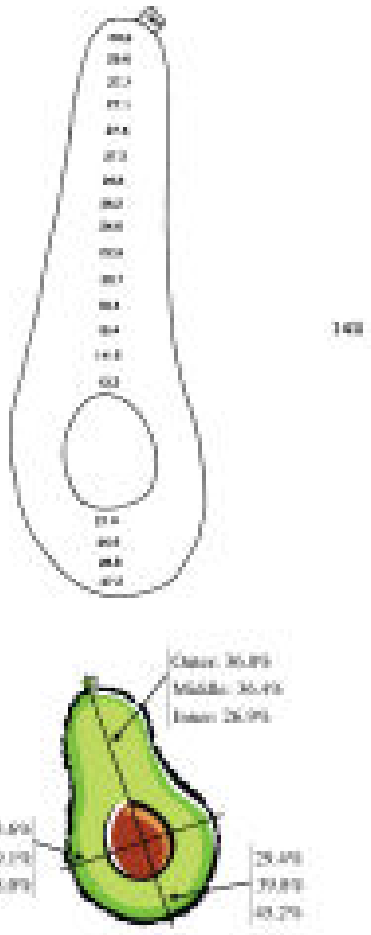
APPENDIX 3. MEASURING AVOCADO MATURITY; ONGOING DEVELOPMENTS

research

Measuring avocado maturity; ongoing developments

Allan Woolf[1], Chris Clark[1], Emma Tavender[1], Vong Phetsomphou[2], Reuben Hofman[2], Mary Lu Arpaia[3], Donella Barkham[4], Marie Wong[5], and Anne White[1] (see below)

Figure 1A & 1B: Diagrams of average distribution of dry matter around avocado fruit (A) 'Pinkerton' (Schroeder, 1966) and (B) New Zealand 'Hass' (Fletcomphou, 2003).



1 The Horticulture and Food Research Institute of New Zealand Limited, Mt Albert Research Centre, Private Bag 92 169, Mt Albert, New Zealand.
2 Institute of Food, Nutrition & Human Health, Massey University, Private Bag 102-904, Auckland, New Zealand.
3 www.avocadosource.com
4 University of California, Dept. of Botany and Plant Sciences, Riverside, CA 92521
5 Avocado Inspection Service, CDFA, Escondido, CA

Dry Matter Variability

longitudinal slices) and plug method have shown extremely high correlation (r2 > 0.95) between the two methods (Arpaia et al., 2003). Figure 3 shows the high level of correlation between the two techniques over a range of growing times and areas in the [illegible]. This work has led to legal changes in the sampling and measurements of dry matter in California (Arpaia, 2003). Preliminary measurements of fruit from three New Zealand orchards at one time in the season also found no significant statistical differences between use of the Hofshi corer, standard New Zealand, and old Californian techniques (Table 1).

Avoguard Cooltrans Ltd has the potential of this system to significantly reduce the labour involved and have registered a modified corer design in New Zealand. The ability to rapidly measure dry matter of individual fruit means that a higher level of accuracy will be achieved by sampling more fruit [illegible] be destroyed. Given the time saving and [illegible] of this technique, it appears a number of other countries will be moving to this system for dry matter measurements.

Dry Matter Variability between Fruit

Due to fruit to fruit variability, how many fruit need to be sampled to achieve a representative sample? Figure 4 shows the distribution of dry matter sampled from one Kaitaia orchard for 80 individual fruit. In October the average dry matter was 27.2%, with dry matter values ranging from 19 to 35%. In January when all fruit have an average dry matter of 34.7%, values ranged from 25

Figure 2. Picture of the Hofshi Coring Device.

Table 1. Mean dry matter values and standard error of the mean (SEM) for 20 fruit from three Far North orchards (September 2003) measured using the three different methods.

	Hofshi System (core)		Current NZ System (slice)		Old Californian system (grated)	
Orchard	Mean	SEM	Mean	SEM	Mean	SEM
1	29.6	0.94	29.2	0.97	29.4	1.03
2	30.6	0.94	30.6	1.06	30.3	1.07
3	31.9	0.73	31.2	1.03	31.7	1.43

[illegible]

Drying Technique

[illegible]

Future Developments

[illegible]

Acknowledgements

[illegible]

References

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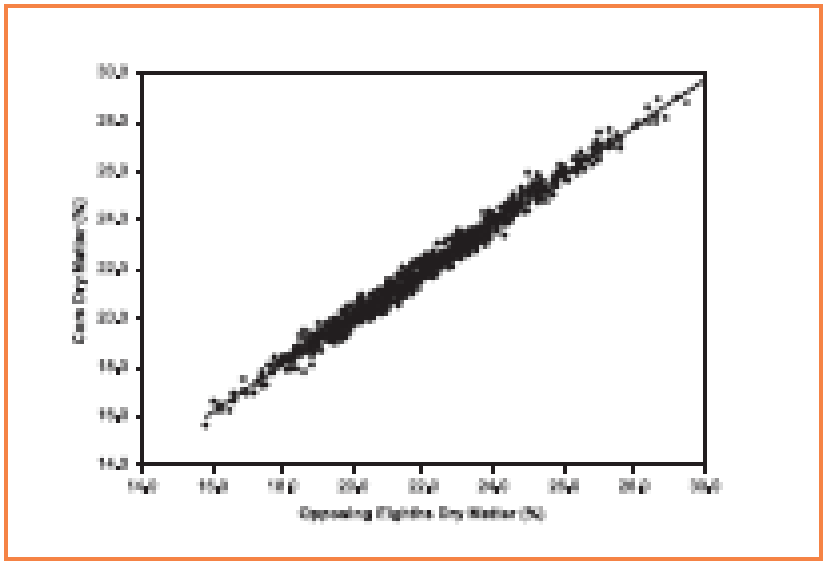

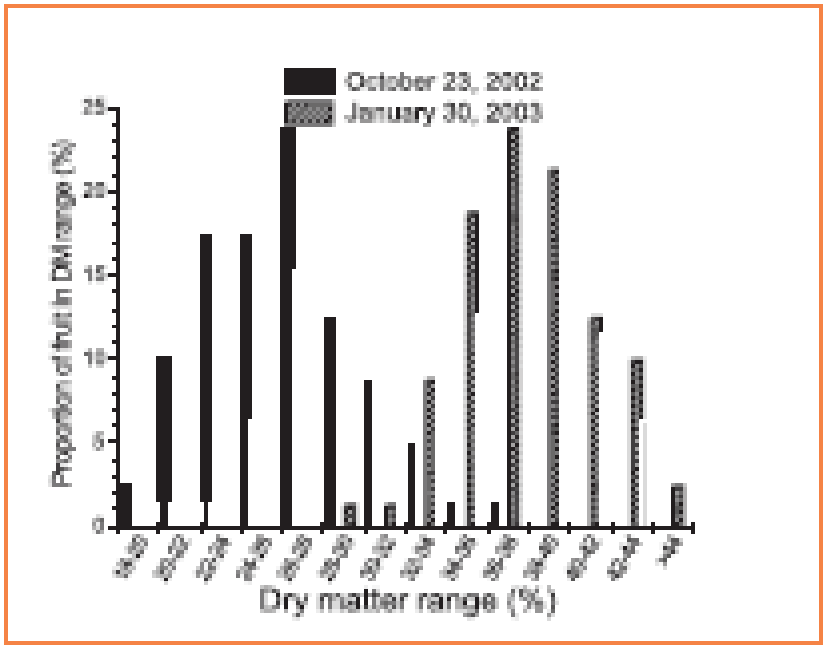
October 23, 2002
January 30, 2003
Proportion of fruit in DM range (%)
Dry matter range (%)

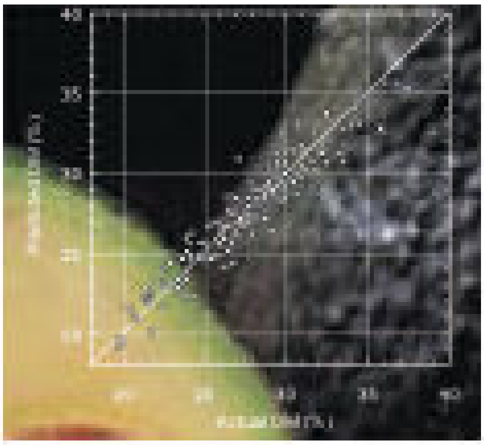

Figure 3 (left). Relationship between core dry matter (%) and opposing eighths dry matter (%) of 'Hass' fruit sampled throughout California from 21/11/01 to 4/01/02 (N=723). Each point represents one fruit. Samples collected as part of the official maturity testing conducted by CDFA Avocado Inspection Service.

Figure 4 (left). Dry matter distribution of 80 individual fruit from one Katikati orchard harvested on two occasions (October 2002 and January 2003).

Figure 5 (right). Actual (measured) dry matter versus predicted dry matter values using NIR model (n = 100). Model has an R^2 of 90%, and an error of measurement of 1.2 % DM.

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