

## **Developing Sumptuous Apricots<sup>©</sup>**

Jill Stanley, Ross Marshall and Arlene Nixon  
The New Zealand Institute for Plant & Food Research Limited, 990 Earnsclough Rd, RD  
1, Alexandra 9391, New Zealand  
Email: jill.stanley@plantandfoodresearch.co.nz

Mike Malone and Shayna Ward  
The New Zealand Institute for Plant & Food Research Limited, Private Bag 1401,  
Havelock North 4157, New Zealand

Emily Buck  
The New Zealand Institute for Plant & Food Research Limited, Private Bag 11600,  
Palmerston North 4474, New Zealand

Jinquan Feng, Shane Olsson, Allan Woolf and Roger Harker  
The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169,  
Auckland 1142, New Zealand

### **INTRODUCTION**

New Zealand currently exports 1000-1500 metric tonnes of apricots annually worth \$NZ 6-7 M. The largest market is Australia, followed by Europe and U.S.A. where much lower fruit volumes are exported. Export returns in the last 3 years have remained relatively stable, despite the high value of the New Zealand dollar and world economic downturn. There is potential to achieve a high return for export apricots if the New Zealand industry can supply fruit in the international market during March when there are few competing apricots present. This opportunity can be realised through the production of late season apricot cultivars with long storage capability and retention of excellent flavour. Commercial apricot cultivars are generally only stored for a maximum of 3 weeks in cold storage because of rapid softening and development of storage disorders. New apricot cultivars selected for their capacity to maintain quality for up to 6 weeks of cold storage would open up the possibility of sending apricots by sea freight to more distant markets, such as Asia and Europe, thereby reducing costs and the carbon footprint.

Storage life and quality in apricots can be influenced by a range of factors including genetic, developmental, and postharvest conditions. A 5-year research programme to investigate these factors and to develop “Sumptuous Summerfruit,” including late season apricots, began in 2008. The apricot aspects of the programme encompass three main objectives: breeding new improved cultivars, developing molecular tools for faster breeding for sweetness and long storage, and increasing our knowledge on postharvest fruit quality and control. These targets will ensure the development of new elite breeding lines and cultivars with enhanced storage capability and expand our involvement in more distant export markets. This paper will outline some of the methodologies used and discuss progress to date on these three aspects.

### **BREEDING NEW LATE SEASON APRICOT CULTIVARS**

#### **Origin and History**

The Plant & Food Research (PFR) apricot breeding programme at Clyde, Central Otago began in 1976, essentially to find a replacement for the ‘Moorpark’ cultivar and to develop cultivars for export over a wide maturity period. Several apricot cultivars have been released from the apricot breeding programme to date. The early work resulted in the release of the Clutha series. This range of cultivars was developed from a cross between ‘Sundrop’ and ‘Moorpark’ and is well adapted to the hot, dry summers and cold winters of the Otago Region. ‘Cluthagold’ was released in 1988 and became a very successful commercial cultivar. It matures in late January in Central Otago. It has proved

to be an outstanding apricot with good cropping, handling and shipping qualities, attractive appearance, and good eating quality. Other Clutha series that are still commercially grown include 'Cluthastar' (1992) and 'Cluthasun' (1993).

A second generation derived mostly from 'Cluthagold' as a parent produced a further four apricots; 'Benmore' (1999), 'Dunstan' (1999), 'Alex' (1998) and 'Vulcan' (1998). Of this series, 'Vulcan' was the most popular with growers. 'Vulcan' initially was perceived as a prolific cropping cultivar, yielding large, attractive, good eating fruit, with a highly attractive sheen and maturing mid-season. With a high chilling requirement, the cultivar was well adapted to Central Otago but was only marginal in the lower-chill northern region of Hawke's Bay. However, cold springs have shown that the cultivar is bud tender, resulting in pistil death. Thus its cropping potential has not been fully achieved and 'Vulcan' has gradually been removed from production. A highly coloured, mid-season selection derived from this second generation was released and named 'Cluthafire' in 2001.

The next release from the programme was 'Clutha Summer' tested as D14/1. 'Clutha Summer' was developed from open pollinated seedlings of 'Goldstrike'. It produces very large apricots that ripen in the early season (early January) in Central Otago. Some fruits have weighed up to 130 g. Fruit shape is round-ovate, flesh is orange and skin colour is bright orange with about 70% deep red blush. The flesh is firm and juicy with a sweet apricot flavour and semi-cling stone. 'Clutha Summer' is a high chill cultivar with potential to replace 'Sundrop' in Central Otago, but to date it has not performed well in Hawke's Bay because of its high chill requirement. New Zealand Plant Variety Rights was applied for 'Clutha Summer' in 2010.

The various releases from the breeding programme have helped meet the demand for mid-season apricots for export, largely to Australia. The current aims of the breeding programme now include breeding for late maturing apricots for export to a wider array of markets. Emphasis has been put on generating seedling populations with high-coloured, good-eating fruit that mature from February through to mid-late March.

### **Breeding Methodologies**

The breeding programme consists of hybridisation, seed extraction and seedling cultivation, progeny evaluation and selection, and grower trial followed by cultivar release.

In spring, usually at the start of September, apricot hybridisation is conducted under cover. To develop late maturing progenies, we have used a local (Roxburgh) 'Moorpark' seedling known as Cold Creek sport. This seedling, which matures in March, has been crossed with high-quality selections to allow further development of the desired traits of late maturity, high fruit colour, and high flavour. The crosses are harvested at maturity, and the stones extracted and sent to Hawke's Bay where the stones are cracked and the seed recovered. The seed is then stratified for 2 to 3 months at 0°C and planted in the greenhouse when the radicles emerge. The resulting seedlings are shipped back to Clyde in October-November for planting. Seedling progeny take about 3 to 4 years to fruit when they are then evaluated in the field and laboratory, and best selections go on to the second stage of trialling. The selections are propagated in a nursery on 'Golden Queen' rootstock and planted both on site and at various grower properties. The plants are then evaluated for up to 60 traits to determine if they will be further trialled, kept for breeding purposes, or culled.

### **Progress**

We currently have over 12,000 seedlings on the research station at Clyde. There are also over 200 advanced selections on trial at the station and over 20 selections in grower trials. Two late selections currently in grower trial in Roxburgh will both have additional trees planted over the next 2 years to enable evaluation on a larger scale.

## MOLECULAR TOOLS FOR APRICOT

Molecular tools can help increase the breeding efficiency within breeding programmes. Two key tools for this are deoxynucleic acid (DNA) markers and genetic linkage maps.

### Background and Principles

Deoxynucleic acid markers are a means by which variations (alleles) at particular points (loci) in the DNA are visualised. The same technology is used in DNA fingerprinting to identify individuals. These markers can be developed from genes or non-genic parts of DNA and once developed, they can then be screened over the parents and the progeny of a controlled cross and used to construct a genetic linkage map. This is achieved by using the frequency within the progeny alleles from different marker loci present together; the higher the frequency, the closer the markers are positioned on the map. In this way, traits can also be added to the map.

Once a genetic linkage map is constructed and the trait information added, regions involved in traits of interest can be identified and information from the map can be used in the breeding programme in two key ways. One is by positioning the trait on the map so that the number of genetic loci underlying the trait (also known as quantitative trait loci, or QTL) can be identified, which can be used to develop new breeding strategies for the trait. The other use is more direct and is the application of the DNA markers themselves to the breeding effort. If DNA markers tightly linked to beneficial traits are identified, they can be used as a selection tool by the breeders to identify potential parents as well as elite seedling material before the trait may even be expressed visibly. For example, if a marker tightly linked to fruit sugar has been identified, samples of seedling tissue can be taken soon after germination. These can then be screened with the marker and those individuals with the preferred allele linked to the beneficial trait can be selected for further trials, and those without that allele can be removed. This is known as marker-assisted selection (MAS) and reduces both the time spent waiting for the trait to reveal itself and the number of seedlings needed to be maintained within the breeding programme. When DNA markers linked to multiple traits are identified several markers can be selected at once, reducing the numbers of seedlings further, thus increasing the breeding efficiency in the development of new cultivars.

At PFR we have initiated the development of these molecular tools for apricot by utilising markers and information from peach, a close relative of apricot. It has previously been shown that markers and map information can frequently be transferred between close relatives including among species within the *Prunus* genus (Lambert et al., 2004). In the case of apricot, this enables us to tap into the huge amount of information available in peach [see Arús et al., (2012) for a comprehensive review] including thousands of markers, many maps including those that reveal QTL in the peach genome which are involved with fruit sweetness, and, more recently, the whole genome sequence of peach. In addition, we have developed new markers based within genes known to be involved in the traits of interest. This is known as a candidate gene (CG) approach, and the variation in the trait observed across different cultivars is likely to be caused by variation in the genes involved in those traits. Therefore markers based on those genes are likely to underlie the major QTLs identified and therefore be good candidates for use in marker-assisted selection.

The following progress updates includes the use of peach molecular resources in the development of CG based markers and summarises the next steps we are taking to develop molecular tools for application in the apricot breeding programme.

### Progress

A key advance for *Prunus* mapping research was the development of interspecific linkage maps. One map constructed from a cross between almond [cultivar ‘Mission’ (syn. ‘Texas’)] and peach (‘Earlygold’), also referred to as the TxE map (Joobeur et al., 1998), became a core resource for molecular research in *Prunus*.

We used the TxE map in the development of several CG based markers. A total of 15

new CG markers were developed from genes important in the sugar pathway, such as sucrose synthase and invertase enzymes, and those involved in ripening and softening, such as enzymes in the ethylene pathway. These markers were placed onto the TxE map (Fig. 1), and their relation to QTL already known for sugar related attributes (Fig. 1) was inferred from shared markers between the TxE map and other maps (Etienne et al., 2002; Quilot et al., 2004). Of the 15 CG markers developed so far, five are found within QTL for aspects of sweetness (indicated with starburst in Fig. 1). All of these markers were then tested in the parents of several potential mapping populations being developed for apricot, and eight were found to be transferable to apricot (as indicated by the dot by CG markers 1, 2, 4, 7, 10, 11, 12, and 13, Fig. 1).

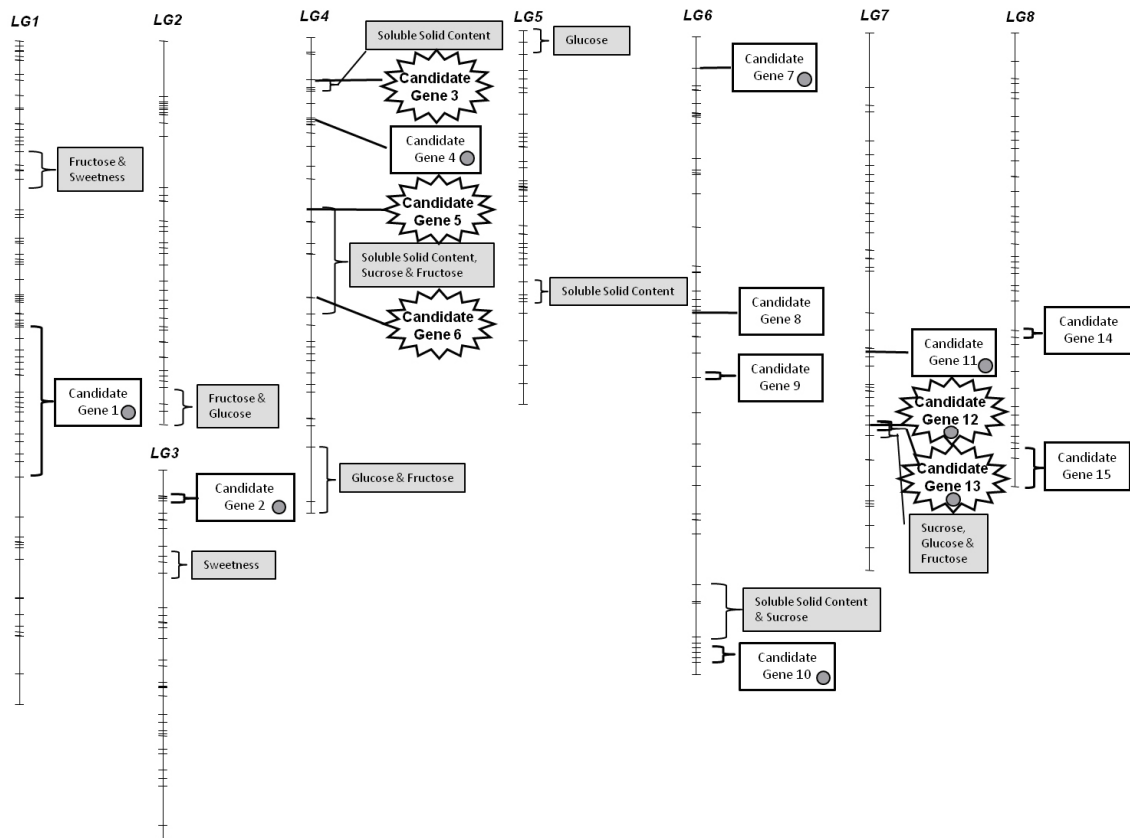


Fig. 1. An outline of the TxE *Prunus* reference map (Joobeur et al., 1998) with, in grey, putative QTL for various aspects of sweetness in peach (Etienne et al., 2002; Quilot et al., 2004). 15 candidate genes markers are positioned onto the genetic map. Those in starbursts are five candidate genes markers co-locating with the QTL in peach. Those with including a dot (candidate genes 1, 2, 4, 7, 10, 11, 12, and 13) indicate candidate gene markers which are transferable to the apricot samples screened.

### Future Work

Several potential apricot populations suitable for map construction are currently under development, and initial fruit sweetness and potential storability have been assessed. The next steps are to continue with the marker screening on these populations, including published markers available for apricot as well as peach and almond. Later in 2012, PFR will be screening 9000 peach SNPs (single nucleotide polymorphism-based DNA markers) using an Illumina<sup>®</sup> Infinium chip over a subset of these apricot populations to assess their transferability and build an apricot map. This chip was developed using the

recently sequenced peach genome and it is expected that a good percentage of the SNPs will be transferable to apricot.

This molecular research will provide insight into the genetics behind sweetness and storability in apricots. Any markers tightly linked to these traits will be assessed for their transferability across different genetic backgrounds and then used within the apricot breeding programme in the development of improved sumptuous apricots for both local and international markets.

### **APRICOT POSTHARVEST AND SENSORY RESEARCH**

This element of the programme aims to develop an understanding of the effects of preharvest, harvest, and postharvest factors on apricot fruit quality in order to assist growers and marketers to improve fruit quality of existing cultivars delivered to consumers, assist with the development of molecular markers for long storage and evaluate storage potential of new selections.

#### **Consumer Preferences**

A previous consumer survey found that 40% of supermarket consumers were dissatisfied with apricots they had purchased, with the major reasons being fruit having no flavour, being too hard, or being mealy and soft (Bruhn et al., 1991). Stonefruit which has been coolstored too long or in inappropriate temperatures would give the consumer a bad experience due to the development of storage disorders, such as mealiness, gel formation, loss of juiciness, excessive softening, or rubbery flesh (Lurie and Crisosto, 2005; Stanley et al., 2010). Our consumer study focussed on consumer preferences when apricots had been stored for less than 2 weeks and did not have any negative chilling injury symptoms. Sensory evaluation by the consumer panels indicated that there was a significant preference for apricots with a higher soluble solids concentration (SSC) ( $P \leq 0.05$ ). This result is consistent with those for other fruit crops (Burdon et al., 2004; Crisosto and Crisosto, 2005; Palmer et al., 2010). Unlike some other fruit such as apples, apricots do not contain starch that can be converted to sugar after harvest (Kurz et al., 2010). In apricot, therefore, the SSC at commercial harvest is very important. This confirms that there is a need to select new cultivars with higher SSC at harvest.

#### **Preharvest Factors**

A number of preharvest factors likely to affect fruit quality after postharvest storage are being studied, including crop load, position in canopy, and the type of wood the fruit is borne upon. In general lower crop loads established by thinning fruitlets at pit-hardening resulted in increased SSC for 'Cluthagold' fruit (Stanley, 2011). At higher crop loads [6-8 fruit per cm<sup>2</sup> trunk cross-sectional area (TCA)], fruit were mealier after 2, 4, or 6 weeks of coolstorage than lower crop loads (2-4 fruit per TCA) ( $P \leq 0.05$ ). These results differed from peaches, where fruit from lower crop loads had a higher incidence of mealiness after storage (Lurie and Crisosto, 2005). We also found that fruit from the first harvest had significantly higher SSC than the two subsequent harvests (SSC of 12.4, 11.0, and 11.3 for harvests 1, 2, and 3 respectively, SE=0.22,  $P \leq 0.01$ ), possibly because harvest 1 fruit were harvested from the higher-light regions of the tree.

Whilst our results suggested growers should be thinning apricot trees to lower crop loads, a reasonable crop load is required to ensure adequate yield and profit to growers. Crop loads of approximately 2 fruit per cm<sup>2</sup> TCA resulted in estimated yields of 5 to 10 tonnes per hectare, whereas yields of around 8 fruit per cm<sup>2</sup> resulted in yields of 20 to 25 tonnes per hectare (commercial crop loads were approximately 4 to 6 fruit per cm<sup>2</sup> TCA). There is potential for genetic improvement in SSC and reduction in storage disorders (such as mealiness) at higher crop loads. Postharvest fruit quality at different crop loads needs to be assessed on advanced selections.

#### **Harvest Factors**

The maturity at which apricots are harvested affected the quality of the fruit and the fruit

behaviour in storage. Fruit harvested at advanced maturity will bruise easily during transportation to the markets and is more likely to arrive soft and overripe. However, if growers harvest fruit when they are too firm, other problems can develop. ‘Cluthagold’ and ‘Larclyd’ (marketed as ‘Genevieve’) apricots harvested when very firm (Maturity 1), were lower in SSC and often did not soften to an acceptable “eating” firmness after storage (1 to 2.5 kgf) (Table 1). ‘Cluthagold’ fruit harvested in the standard maturity harvest range (Maturity 2; around 4 to 6 kgf) had higher SSC and softened normally after removal from the coolstore. ‘Larclyd’ fruit harvested at a standard maturity range did not soften fully after storage and became slightly rubbery, possibly because of heavy rain prior to harvest. In general, more gel formation and mealiness developed after coolstorage in Maturity 2 fruit (Table 1). The proportion of free juice, measured using the technique developed by Lill and Van der Mespel (1988), dropped slightly as fruit was held in coolstore and ripened after coolstore for Maturity 1 fruit of both cultivars. The proportion of free juice of fruit ripened after 3 weeks of coolstorage was approximately 50% and 10% that of apricots at harvest for Maturity 2 ‘Larclyd’ and Cluthagold’ fruit respectively (Table 1). The protocol for assessing fruit harvested at different maturities could be used for evaluating how much variation there was in postharvest fruit quality from different maturities for each new selection.

Table 1. Characteristics of ‘Cluthagold’ and ‘Larclyd’ apricots at harvest, after 3 weeks at 0°C and after subsequent shelf-life of 4 and 6 days at 20°C, for two different maturities (based on colour at harvest). Data include flesh firmness (FF), soluble solids concentration, proportion free juice, mealiness score [from 0 (not mealy) to 3 (very mealy)], gel formation score [from 0 (no gel) to 3 (>50% flesh with gel)] and rubbery score [from 0 (not rubbery) to 3 (very rubbery and penetrometer will not pierce flesh)].

	‘Cluthagold’		‘Larclyd’	
	Maturity 1	Maturity 2	Maturity 1	Maturity 2
<b>At harvest</b>				
FF (kgf)	8.0	5.9	6.7	4.3
SSC (%)	9.5	12.3	11.1	12.3
Free juice (%)	29.4	25.8	20.5	21.5
<b>After 3 weeks at 0°C</b>				
FF (kgf)	7.9	5.2	6.7	5.6
Mealiness score	0.00	0.00	0.00	0.00
Gel breakdown score	0.00	0.00	0.00	0.00
Free juice (%)	30.2	20.8	17.4	14.9
Rubbery score	0.1	1.3	0.0	0.3
<b>After 3 weeks at 0°C and 4 days at 20°C</b>				
FF (kgf)	5.6	1.4	4.7	3.2
Mealiness score	0.00	0.38	0.04	0.42
Gel formation score	1.09	1.66	2.31	2.06
Free juice (%)	22.1	1.2	13.7	9.3
Rubbery score	0.5	0.5	0.5	0.8
<b>After 3 weeks at 0°C and 6 days at 20°C</b>				
FF (kgf)	5.4	1.1	4.2	3.8
Mealiness score	0.00	0.42	0.00	0.04
Gel formation score	0.34	2.28	0.66	1.3
Free juice (%)	17.9	0.7	15.0	11.5
Rubbery score	0.8	0.5	1.2	1.4

### Postharvest Factors

Coolstore temperatures have a significant effect on apricot fruit quality (Stanley et al., 2010). Apricots stored at 0°C did not soften in storage or softened very slowly, while

those held at 4°C softened rapidly (Fig. 2) and developed much more severe storage disorders (Table 2). Trials evaluating a number of different modified atmosphere bags (low oxygen and high carbon dioxide) showed potential for extending storage time. High carbon dioxide concentrations in some bags resulted in significant off-flavours.

Table 2. Characteristics of ‘Cluthagold’ and ‘Larclyd’ apricots, harvested at Maturity 2, and assessed at harvest and immediately after removal from 0°C or 4°C for 2, 4, or 6 weeks. Data include mealiness score [from 0 (not mealy) to 3 (very mealy)], gel formation score (from 0 (no gel) to 3 (>50% flesh with gel), proportion free juice and rubbery score (from 0 (not rubbery) to 3 (very rubbery and penetrometer will not pierce flesh)).

	‘Cluthagold’		‘Larclyd’	
	0°C	4°C	0°C	4°C
<b>Mealiness score</b>				
0 wks	0.00	0.00	0.00	0.00
2 wks	0.00	0.00	0.00	0.00
4 wks	0.00	0.90	0.00	0.75
6 wks	0.04	1.58	0.00	1.71
<b>Gel formation score</b>				
0 wks	0.00	0.00	0.00	0.00
2 wks	0.00	0.00	0.00	0.00
4 wks	0.00	0.00	0.16	0.41
6 wks	0.47	3.00	0.66	3.00
<b>Free juice (%)</b>				
0 wks	25.8	25.8	21.5	21.5
2 wks	19.8	28.7	17.5	13.7
4 wks	19.6	6.3	12.2	11.2
6 wks	13.0	8.9	13.3	14.7
<b>Rubbery score</b>				
0 wks	0.0	0.0	0.0	0.0
2 wks	0.2	1.2	0.0	0.2
4 wks	1.2	0.4	0.2	0.0
6 wks	2.3	1.0	0.5	0.0

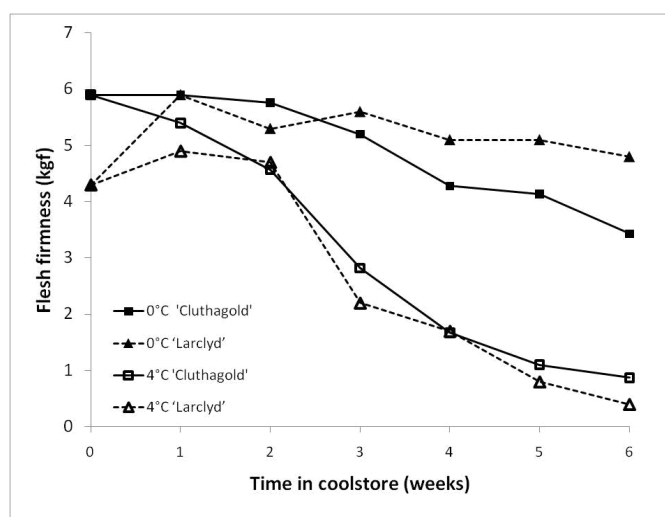


Fig. 2. The effect of coolstore temperature and time in coolstore on flesh firmness of ‘Cluthagold’ and ‘Larclyd’ apricots assessed on the day of removal from coolstore.

## Ethylene

Apricot is a climacteric fruit and therefore ethylene production is associated with ripening induction (Chahine et al., 1999). Ethylene production increases during ripening of apricot, and significant differences in the ethylene production rate among cultivars has been observed (Bureau et al., 2006). Fruit from apricot cultivars with low ethylene production have longer storage potential than those producing higher ethylene (Gouble et al., 2005). However late season fruit from some low ethylene producing seedlings from PFR's breeding programme have not ripened properly by the end of the season, even when left on the trees. Ethylene production rates of apricots from 79 seedlings from within three families were measured when they reached 2 kgf after being held at 20°C from harvest. Late season seedlings tended to have lower ethylene production rates than earlier maturing seedlings (Fig. 3). These data will assist with development of molecular markers for long storage capability.

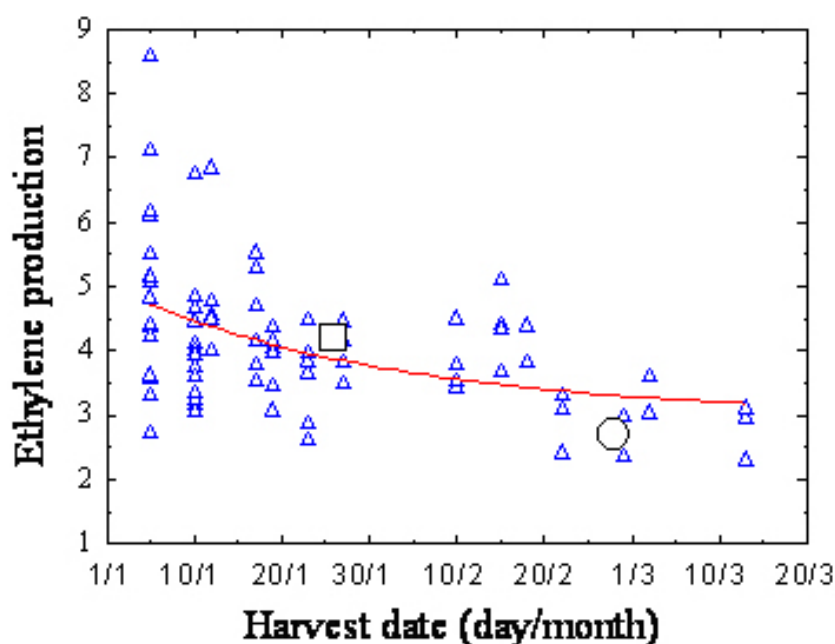


Fig. 3. Relationship between harvest dates and ethylene production rates measured on apricot fruit from 79 seedlings (triangles) when the fruit reached a firmness of 2 kgf after being held at 20°C following harvest at commercial harvest firmness (around 4 to 6 kgf). Data for 'Cluthagold' (square) and 'Larclyd' (circle) are presented for comparison. Ethylene production in the plot is presented in log scale ( $\text{LN}(r\text{C}_2\text{H}_4)$  where  $r\text{C}_2\text{H}_4$  is ethylene production rate expressed as  $\text{nmol C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$ ) for clarity.

## CONCLUSIONS

Crossing with a late season seedling parent has resulted in a large number of seedlings that produced fruit that matured late in the season. Some of these look promising and are already in grower trials.

Progress in developing molecular markers to aid the breeding programme has been made by utilising linkage maps from other *Prunus* species. A total of 15 candidate gene based markers associated with the sugar and ripening pathways have been developed in peach, eight of which are transferable to apricot. A new apricot genetic linkage map will be constructed within the next year using a 9000 SNP chip screen. To identify putative markers linked to fruit sweetness and storability, seedlings within the mapping



populations have been phenotyped for SSC and ethylene production rates, and these data will be positioned onto the map.

A better understanding of pre- and postharvest physiology of apricots has highlighted a number of key traits which may need to be examined when evaluating new selections. These include the capacity for fruit to maintain high SSC at medium to high crop loads, high SSC when fruit are firm, and fruit with low ethylene production rates. Fruit quality needs to be assessed from fruit harvested at different maturities followed by multiple removal times from storage.

We are confident some of the new selections will provide the potential for growers to increase production of high-value, late season apricot cultivars and achieve high returns from exporting high-quality fruit.

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