

# Production technologies for low-chill temperate fruits

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# Foreword

PRODUCTION of temperate fruits in tropical and sub-tropical regions of the world is rapidly increasing.

There is a growing demand in Asia for temperate stone fruit, which can fetch much higher prices than the abundant, local tropical fruit. Regions in the northern hills of Thailand, Vietnam and Lao PDR are climatically suitable for growing such fruit and, because of the high value of the product, this enterprise can provide options to diversify farming systems and also help stem the movement of rural people from those regions to the cities.

In Thailand, it especially offers an economically viable alternative to the growing of opium poppies. As well, sedentary fruit tree culture in these hilly regions can be a more sustainable use of land than the current practices of shifting agriculture and arable cropping.

Vietnam already grows many temperate fruit crops and the average revenue from these can be two or three times higher than that of rice. With better-adapted and higher-quality varieties, many more areas could be suitable for such fruit production. Laos also has suitable areas and there has been some attempt to grow varieties brought in from neighbouring countries but production can certainly be enhanced by selecting and promoting better varieties.

Stone fruit is also popular in Australia with low-chill varieties grown in sub-tropical areas becoming significant as better varieties have been developed; the value of the industry is expected to increase. Production in Australia and Asian countries is complementary as fruit comes into season at different times.

Along with long-term support from many other organisations, ACIAR has funded research and development in low-chill temperate fruits since 1997 in Thailand, Laos and Vietnam with the aim of helping to develop a sustainable temperate fruit industry for low-chill varieties. Considerable progress has been made with on-farm commercial production systems developing and fruit appearing in major markets, especially in Thailand.

This research and development is presented and discussed in these reports from the Second International Workshop on Production Technologies for Low-chill Temperate Fruits. The workshop coincided with a review of the ACIAR project which resulted in a decision to extend the work on developing the industry in these countries. The excellent organisation and contributions of Thai collaborators and all presenters made the workshop a valuable and enjoyable experience and are gratefully acknowledged.

ACIAR is very happy to publish these reports from the workshop for the benefit of researchers, extension officers and farmers with an interest in this area.



*Peter Core*  
*Director*  
*Australian Centre for International Agricultural Research*

*April 2005*

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# Opening Address

TEMPERATE fruit production in Thailand was the result of an idea of genius from HM King Bhumibol Adulyadej to cope with the complicated problem of opium cultivation, the practice of slash and burn agriculture and the poor livelihood of people living in the highlands of northern Thailand. He initiated the Royal Project in 1969 in order to conduct research relating to highland agriculture which was a very new area for Thailand at that time.

Since then, continuing research has contributed to commercial success of several temperate fruit crops — namely Japanese apricot, peach, Japanese plum, Asian pear and persimmon. A few promising crops such as kiwifruit and nectarine may become commercialised in the near future.

Production of temperate fruit crops in the highlands of sub-tropical Asian countries has an advantage over production in the temperate zone of the Northern Hemisphere because fruits may be harvested a few months in advance. For example, early harvesting of peach in Thailand could begin in late March. However, different cultivars and cultural practices suitable for low-chill areas need to be carried out.

The 2nd International Workshop on Production Technologies for Low-chill Temperate Fruits was held to demonstrate this fact. The organisers hoped that Thailand's success could be replicated by others who are facing similar problems.

Another aim of this workshop was to honor the late Professor Suranant Subhadrabandhu who was among the pioneers of temperate fruit research in Thailand. Not only was Prof. Suranant our great mentor, but he also motivated us all to be dedicated researchers. Without him, the success of temperate fruit research in Thailand would not have come this far.

*Dr. Unaroj Boonprakob  
Chairman of organizing committee  
The 2nd International Workshop on  
Production Technologies for Low-chill Temperate Fruits*

# PART I

## Papers of Oral Presentations



# Temperate fruit research in a changing world

Santhad Rojanasoonthon<sup>1</sup>

## Background

NORTHERN Thailand is one of the most important regions of the country from a socio-economic, agro-ecological and political perspective. The five provinces of Mae Hong Son, Chiang Mai, Lamphoon, Chiang Rai and Payao account for about one-quarter of the country's forest area and most of the hilltribes live in these provinces. The hilltribe population has been expanding rapidly and with it the practice of shifting cultivation.

In the well-known 'Golden Triangle' region more than half the world's heroin and other drugs are being produced from opium.

Recognising the problem, His Majesty, King Bhumibol Adulyadej created the Royal Project Foundation with the primary goal of crop replacement. H.S.H. Prince Bhisatej Rajani has led the project from its inception.

The Foundation's objectives are to help the hilltribes: for humanitarian reasons, to reduce the destruction of natural resources, to stop opium cultivation, to conserve soil and make proper use of the land and to produce cash crops for the benefit of the Thai economy. Its three major activities are research, development and technology transfer, and marketing.

## Research

The research of the Royal Project Foundation addresses the issues of quality and market supply. The Foundation calls upon researchers from various organisations, including universities and government agencies, to assist in its projects. The Royal Project Foundation pioneers research into new crops and management practices.

## Development and technology transfer

The second stage involves pilot testing of all research results, establishing the proper channels for production, training, consultation and technology transfer. The Royal Project Foundation encompasses 37 centres with 306 villages, covering 2000 sq km and 102 379

inhabitants. It extends over the provinces of Chiang Mai, Chiang Rai, Lamphoon, Mae Hong Son and Pra Yao.

## Marketing

The Royal Project Foundation has trialed and grown 12 varieties of fruit trees, 20 species of flowers and 60 new vegetable and herb crops. Other products include pot plants and canned food. There is an emphasis on quality and ensuring product freshness and food safety.

Research is undertaken on post-harvest, packaging and transport. Products from the Royal Project are marketed under the registered brand name 'Doi Kham' meaning Golden Mountain.

## Why grow temperate fruit trees in Thailand?

### Soil and water conservation

Temperate fruit trees are perennial and so can help conserve soil and water and counter erosion effects where forests have been cleared.

Fruit trees also replace shifting cultivation by hill tribes as they can earn income from fruit every year so do not need to move around.



An emphasis on quality: 'Tropic Beauty' peaches are packed on foam trays at Royal Ang Khan Research Station.

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## Climate

Although Thailand is located in the tropics, the climate in the high mountainous area of the north could be regarded as temperate or, more accurately, semi-temperate. Tropical and sub-tropical fruit cannot be produced due to the low winter temperatures.

## Market demand

Although many kinds of tropical fruits are produced in Thailand, temperate fruits are welcomed in the local market. Thus, cultivating temperate fruit in the highlands can lead to import replacement on local markets.

## Research plan for temperate fruit (2002–2006)

A working group has been established to develop a breeding program for fruit trees and to guide the direction of research for each species.

The priorities for research work are based on marketing value and future marketing challenges. The first group is fruits that can be promoted for commercial growing. These include strawberry, persimmon, peach and nectarine, Asian pear, Japanese apricot, plum, avocado and passionfruit; the second group is fruit trees that show good performance for commercial growing: grape, kiwifruit, macadamia nut, papaya, dragon fruit, star fruit and mango; and the third group is species under adaptability testing which show good performance for future commercial production — raspberry, bayberry, Chinese chestnut, atemoya, litchi, cape gooseberry, blueberry, loquat, pomegranate and guava for processing. The fourth group is those which show potential for study — pecan, walnut, almond, cherry and Chinese jujube.

## Effect of free trade with PR China

Since the opening of the market with the Peoples Republic of China, relatively low-priced, good quality

fruit has been entering the market from China. Table 1 shows the variation in price between different varieties and markets.

The challenge for Thailand's temperate fruit industry is to produce and market fruit in the one-month window before fruit from China arrives. This means more selective market distribution to produce fruit of a desirable quantity and quality which is fresher than the imported produce.

Planning ahead to meet this challenge will require cultivars that are adaptable to high altitude areas. Research on breeding and selection of high-quality fruit with a commercially viable yield is essential.

Physiological studies are necessary to understand the mechanisms for production. How trees adapt to the Thai climate must be understood before good tree management can be programmed.

## Research direction in 2005

Overall, value chain production should be concerned with factors such as production for competition with local and imported products; production which does not deteriorate natural resources and the environment. The priorities for the workplan are (1) marketing; (2) rehabilitation and conservation; (3) human relation enhancement; (4) performance indicators; (5) evaluation and assessment — a three-step procedure: reduce-refrain-quit; retain-correct-increase; start-test-perform.

### *His Majesty's views of world environmental problems*

*“Environmental problems will cause the world to change. The problem will be something that everyone will experience, but not everyone will know. It is thus our duty as citizens of the world to have knowledge and a clear understanding in order for us to find the ways to solve the problem correctly.”*

**Table 1.** Variation in retail price of apples at different outlets in Thailand.

Place	Apple selling price
High class market, supermarket	cv. Delicious, 20 Baht each cv. Fuji, 100 Baht each
Fruit strollers along footpaths	cv. Delicious, 5 Baht each cv. Fuji, 10–20 Baht each

# Trends and progress of low-chill stone fruit breeding

David H. Byrne<sup>1</sup>

## Abstract

Breeding towards the development of commercially acceptable low-chill stone fruit began almost 100 years ago in California. Since then, mainly through work done in the USA (California, Florida, Texas) and Brazil (Pelotas, Sao Paulo), and later in Mexico and South Africa, a solid base of low-chill peach and plum germplasm has been developed. With the emergence of commercially useful varieties and a global produce market wanting a year round supply of stone fruit, additional programs were initiated in Asia and Australia in the 1990s. At present, most of the commercial low-chill varieties available are yellow-fleshed peaches and plums, both with high acidity in their flesh. Much work needs to be done, not only to improve upon the current varieties but also to expand the harvest season and types of stone fruit available for growers in these mild winter areas. It is essential that the low-chill varieties mimic the range of fruit types (flesh colours, shapes, acidity), quality (high soluble solids, post harvest qualities), and adaptabilities (disease and pest resistance, tolerance to heat, drought, salt, calcareous soils) that are found in their high-chill cousins. This will involve much introgression work to extract these traits from the high-chill germplasm but, given a solid base of low-chill germplasm, this work should progress relatively rapidly. Thus, even though it took almost 100 years to develop the solid base of commercially useful germplasm, given the current efforts being expended and the demands of the global produce market, I expect that the varietal offerings for the low-chill zones of the world will increase manifold over the next 25 years.

## Chilling zones

ALTHOUGH there are many models to predict the accumulation of chilling (Erez, 2000), we have used a model developed in the southeastern USA which relates the mean monthly temperature of the coldest month(s) (<http://aggie-horticulture.tamu.edu/stonefruit/chillacc.htm>) to the plant's chilling requirement (Fig. 1). With this tool we can estimate the chilling accumulated at any site. Much of the Southeast Asia region is within the tropics and thus receives no chilling temperatures. Beginning with about 20° latitude, there is a positive accumulation of chilling which increases with the latitude. Generally more than 1000 chilling units are accumulated above 26° latitude whereas in Australia low- to medium-chill areas are found at latitudes of up to 35° south (Table 1). The chilling accumulated is affected by the ocean currents and local geography, especially the altitude in tropical and sub-tropical zones. With cooler temperatures at the higher

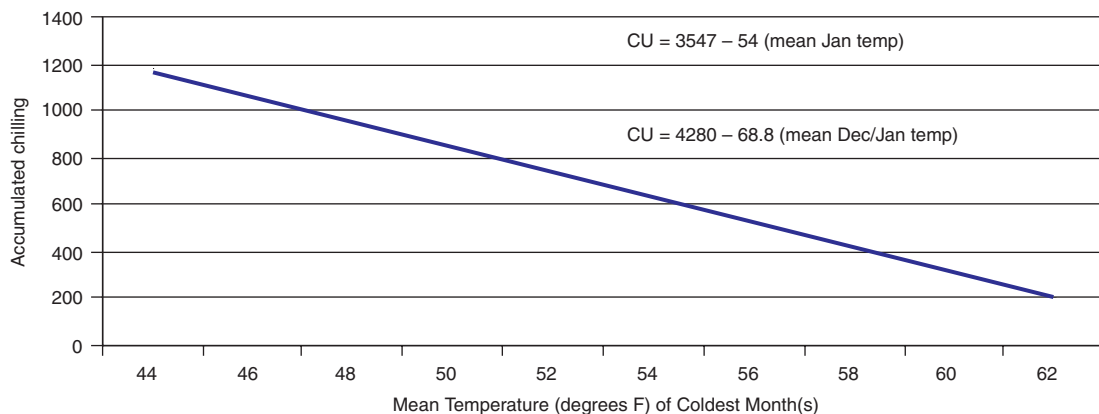
altitudes, it was estimated in Taiwan that with each increase in altitude of 100 m there is an increase in chilling accumulation of 27 chilling units (Ou and Chen, 2000). When using the expected temperature decrease with altitude of 1°F or 0.56°C per 100 m, the estimated chilling increase was 54–61 units per 100 m altitude, a figure higher than reported in the Taiwanese work.

**Table 1.** Estimated mean chilling accumulation in South-east Asia and Australia as a function of latitude.

Latitude	Chilling range	
	Southeast Asia	Australia
< 20	None	
20–23.9	0–630	0–700
24–27.9	100–1500	50–500
28–31.9	> 1000	300–1100
32–35.9	> 1000	450–1300
36–39.9	> 1000	> 850

Chilling accumulation estimated by using the following equation  
CU = 3547 – 53 (mean coldest month temperature in degrees F).

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**Figure 1.** Mean temperature of coldest month(s) and accumulated chilling.

Although the mean chilling received is useful, it is the year-to-year fluctuations that are critical to examine when choosing varieties that would be well adapted to the region (Table 2). For example, varieties that require 850 CU are poorly adapted in College Station even though the mean chilling is 860 CU because they would fruit poorly 30% of the years due to insufficient chilling (Fig. 2). Thus, the most appropriate chilling range (and most consistent in production) for this site would be varieties requiring between 450 and 600 CU. If cold weather during bloom was not a problem, lower chill varieties could also be used. This data gives an idea of what varieties to test but ultimately, it is in situ testing of varieties that gives the best measure of the required chilling range.

Mild winter regions of the world are classified into three major adaptation zones: the transitional, medium, and low-chill zones by chill requirement of commercial cultivars. The transitional-chill zone includes regions in which most commercial cultivars have a chill requirement of 650–950 chilling units (CU). This zone experiences years in which the higher chill varieties produce poorly due to mild winters. This is true of the southern San Joaquin

valley in California on the West Coast and central Georgia and the coastal plain of South Carolina on the East Coast of the USA, some regions of southern Australia, and the southern and higher altitude regions of South Africa. The medium-chill zone includes areas where cultivars with a chill requirement of about 400–650 CU can be grown consistently. This would include areas such as southern Georgia, northern Florida, southern Louisiana, south central Texas, southern Spain, the highlands in Mexico and other sub-tropical areas, and lower altitude areas of South Africa. The peach varieties that mark the higher limits of the medium chill zone would be ‘June Gold’, ‘June-prince’, ‘Springcrest’, and ‘TexRoyal’ and the lower limit would be ‘Flordaking’, ‘TexKing’, and ‘Flordacrest’. The low-chill zone is characterised by frequently receiving less than 400 CU. Varieties grown in these areas include ‘Flordaprince’, ‘TropicPrince’, ‘EarliGrande’, ‘TropicBeauty’, and ‘TropicSnow’.

### Historical context — worldwide

The active breeding for lower-chill peach and nectarine varieties was initiated in the transitional chill zone of California (USDA at Palo Alto, Armstrong, Chaffey Junior College, and University of California at Riverside) at the beginning of last century. These programs released ‘Babcock’ which was used along with other varieties by the Armstrong program to develop a range of nectarines and peaches. The Armstrong peach (‘Flamingo’, ‘June Gold’, ‘Robin’, and ‘Springtime’) and the nectarine (‘Armking’, ‘Panamint’, and ‘Palomar’) varieties contributed substantially to the programs in the lower-chill zones as a source of germplasm.

**Table 2.** Estimated chilling accumulated at various sites in the USA.

Location	Latitude	Mean CU	Range CU
Fresno, CA	36.8	1080	790–1270
College Station, TX	30.6	860	520–1280
Victoria, TX	28.9	600	295–960
McAllen, TX	26.2	235	0–565

Chilling accumulation estimated by using the following equation  $CU = 3547 - 53$  (mean coldest month temperature in degrees F).

From the late 1930s to the 1960s, peach breeding programs began in the USA (Florida, California, Georgia, Louisiana, and Texas), Brazil (Pelotas and Campinas), India (Saharanpur, Uttar Pradesh) and South Africa. In addition, a few medium-chill varieties were released by breeding programs in the transitional chill zone in California (Zaiger's Genetics and USDA, Fresno) and in Georgia (USDA). In particular, the USDA program in Georgia was instrumental in getting the Florida effort going by cooperating with them on some of the initial crosses. The four most active programs in the mild winter regions (Florida, Pelotas, Campinas, and South Africa) have worked independently except for an occasional exchange of germplasm.

As the Armstrong program ended in the 1980s, new programs were initiated in California (Sun-World, Bakersfield), south Georgia (Attapulcus, cooperative program with the University of Georgia, University of Florida and USDA, Byron, Georgia), Texas (low-chill program in south Texas), and Mexico (Chapingo and Queretaro). The US programs have been building on the germplasm developed by Florida, Georgia (USDA, Byron, GA), and California (various programs). More recently, new efforts have been initiated in Sicily, Italy (Tiziano Caruso), Taiwan (Taiwan Agricultural Research Institute, Taichung), China (Lirong Wang, Zhengzhou Fruit Research Institute and Jiang Quan, Institute of Pomology and Forestry, Beijing), Thailand (Unaroj Boonprakob, Kasetsart University, Bangkok), Japan (I. Kataoka, Kagawa University) and Australia (Bruce Topp, Department of Primary Industries and Fisheries, Nambour, Queensland). The programs in China and Japan are directed to develop lower-chill cultivars suitable for protected culture production.

This recent increased interest in the development of medium- and low-chill stone fruit is fuelled by better transportation and a demand for a year-long supply of high-quality peaches and nectarines, combined with the well-adapted commercial types developed over the last 50 years by various programs in the USA (spearheaded by the Florida program) and South America (Brazil) (Byrne and Bacon, 1999; Byrne et al., 2000).

Until recently, the vast majority of low-chill varieties have been developed and released by public programs in the USA, Mexico, and Brazil. In contrast, only 33% of the peach and nectarine varieties released from 1990 until 1996 in the world were developed and released by publicly funded programs (Fideghelli et al., 1998). This is changing in two ways.

1. Privately funded breeding efforts are increasing.
2. Beginning in the 1990s due to decreased public funding and increased commercial interest in stone fruit production in medium and low-chill zones, public programs have begun patenting their releases and are developing commercial partnerships to finance their breeding research. Although these arrangements are working, it has led to less germplasm exchange among the public breeding programs.

Another aspect of this trend is the decrease in basic research in germplasm development, genetics and breeding technology that is primarily done by the public programs (Frey, 1996, 1998). Thus the stone fruit industry needs to get more involved to encourage consistent and increased governmental funding for this research which will ensure the long-range success of breeding programs.

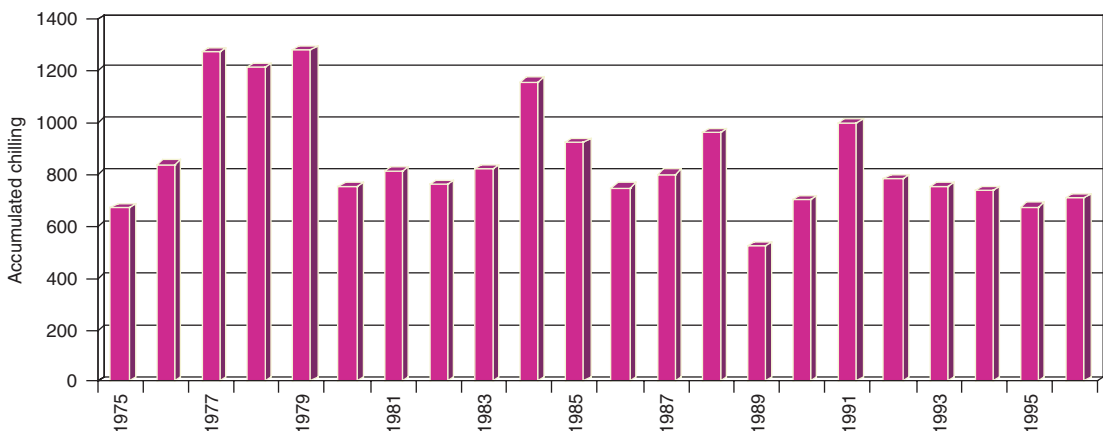


Figure 2. Accumulated chilling at College Station from 1976 to 1996.

## Present germplasm and variety situation

The species, *Prunus persica* (L.) Batsch, includes two economically important crops: the peach and nectarine. The species originated in China and spread through Persia to Europe and the Americas. For centuries, this species was propagated by seed and consequently, as it spread, the species was selected for adaptation. At present there are populations of *Prunus persica* adapted to a wide range of climates ranging from the tropics to the cold higher latitudes throughout the world. Although most of the genetic improvement work has been done in the high-chill zones, Brazil (Pelotas, Sao Paulo) and the USA (Florida, Texas, California) have been the leaders in the development of stone fruit varieties adapted to mild winter areas (Barbosa et al., 1995; Bruckner, 1987; Byrne et al., 2000; Rasiera et al., 1992; Sherman et al., 1992).

Diversity studies of stone fruit (peach/nectarine, almond, plum, apricot and cherry) have indicated that among these crops peach germplasm is the least variable (Byrne, 1990; Byrne, 1989). Inbreeding analyses done on various populations of peaches (Eastern cultivars, Florida low-chill cultivars, and California processing germplasm) (Scorza et al., 1985, 1988; Gradziel et al., 1993; Byrne and Bacon, 1999) have shown that peaches share much common parentage within the various germplasm pools. For example, the peach cultivars from the Eastern USA have six commonly used parents and show very high levels of inbreeding. The available nectarine cultivars trace back to four cultivars. Further studies using isozyme polymorphisms, morphological/phenological traits, RAPDs (randomly amplified polymorphic DNA) and, most recently, SSRs (microsatellites or single sequence repeats) have been done in the USA and Europe. Unfortunately,

these studies have focused on improved germplasm and included few accessions from low-chill or Chinese germplasm. Nevertheless, these studies indicate that the US commercial peach and nectarine germplasm is relatively uniform (narrow) and that higher levels of diversity may exist among Chinese germplasm, low-chill germplasm and criollo non-melting peaches in Spain (Aranzana et al., 2003a; Arulsekar et al., 1986; Byrne, 1990; Ibanez et al., 1993; Messeguer et al., 1987; Mowrey et al., 1990; Perez et al., 1993; Warburton and Bliss, 1996; Werner, 1992).

In a recent germplasm collection trip to China, the centre of diversity for peach, I was surprised to realise that, although there were a few local low-chill varieties, there was no commercial peach production industry in the mild winter region of southern China. An analysis of the founding clones of the germplasm adapted to mild winter areas developed in the Americas has shown that their ancestors comprised the following groups of cultivars.

- a. Clones integral to the development of the high-chill peach germplasm such as 'J. H. Hale', 'Elberta', 'Fay Elberta', 'July Elberta', 'Boston', 'Mayflower', 'St. John' (Scorza et al., 1985; Scorza et al., 1988).
- b. Three low-quality, soft, small-fruited, white-fleshed peaches from south China: 'Peento', 'Okinawa', and 'Hawaiian'.
- c. Local selections and varieties from Mexico ('Carlos', 'Mexican Cling', S100, 871B, Guanaquato, I7-4, Celaya Criollo, and Lucero).
- d. Local varieties from Brazil ('Delicioso', 'Precoce Rosado', 'Admiravel', '15 de Novembro', 'Rei da Conserva', 'Perola de Itaque', and 'Taichi').

The relationships among the high-chill USA developed peach varieties, the low-chill Chinese peaches and the local Latin American selections are not well understood. Nevertheless, this analysis, as well as isozyme and RAPD analyses, indicate that the low- and medium-chill germplasm is more diverse in origin than the high-chill germplasm developed in the USA (Byrne and Bacon, 1999; Byrne, 2002).

Presently, although there are hundreds of peach and nectarine varieties used in the USA and throughout the world, only about 10% are medium- to low-chill varieties and most of these are yellow-fleshed peaches. The majority have been released from the USA (Florida, Louisiana, Texas, and California) and Central and South America (Mexico, Brazil) (Della Strada et al., 1996; Fideghelli et al., 1998; Okie, 1998). Thus, commercially there are few varieties to choose from for these mild winter regions, especially if something beyond a yellow peach is needed. But it should be noted that there is an excellent base of well-adapted commercially acceptable germplasm in



Stone fruit breeders need to expand on the available germplasm to improve the varieties available.

both melting and non-melting flesh available to work with as well as a vast amount of naturalised peach populations that have been propagated for many centuries throughout the medium- and low-chill regions of the world in South America, southern Europe, northern Africa, southern Asia and Australia. Consequently, what needs to be done is to expand upon this germplasm to improve the varieties available.

### Important goals

*Expand adaptation range.* The 'mild winter region' is as diverse or more so than the traditional high-chill regions. It contains areas that are ideal for stone fruit production with hot, dry, sunny summers that favour tree growth and fruit development without excessive disease pressure. An example is the 'Mediterranean' type subtropical climates typically situated along the western sides of continents as seen in the borderlands of the Mediterranean Sea, central and coastal southern California, central Chile, the southern tip of South Africa, and parts of southernmost Australia. The mild winter region, however, also contains areas of marginal to extreme environmental conditions for stone fruit production. These include the sub-tropical-humid zones, typically located on the eastern sides of continents, which have hot, humid, rainy summers that favour disease development. Such areas are plagued with less consistent winter dormancy conditions, caused by conflicting air masses of tropical and polar origin and perennial spring freeze danger during bloom.

Thus, beyond developing a complete set of varieties for the various levels of chilling, efforts need to be made to develop varieties that fruit consistently and develop good quality (especially fruit shape, colour and firmness) under a range of chilling conditions, have the ability to set fruit under high temperatures during bloom, grow without excessive blind node development in hot summer climates, and have good resistance to the various disease (bacterial leaf spot, rust, powdery mildew, peach leaf curl), and pest (aphids, mites, thrips) problems.

For frost-free zones, work needs to be done to develop varieties that can be cropped twice a year or, alternatively, varieties in which the bloom can be easily manipulated to time harvest for specific periods. In areas where protected culture is economical, lower-chill and early ripening varieties well adapted to this unique environment need to be developed.

*Extend ripening season.* In the programs in North America, the emphasis has been to develop early ripening varieties to extend the harvest season forward by 30 or more days over the traditional high-chill growing regions to capture the lucrative early fruit market. By contrast, in the Latin American programs, although early varieties were developed, many

mid season and late ripening varieties were also released to support their local produce/processing industry (Byrne et al., 2000). This work has resulted in increased peach production in many areas, most noticeably South America (Brazil, Bolivia, Uruguay, and Ecuador), and northern Africa (Algeria, Egypt, Morocco and Tunisia) (FAOSTAT, <http://apps.fao.org/>). Although some of this fruit is destined to be marketed internationally, much is still sold in the region. Consequently, for the global market objective, the emphasis is the development of varieties ripening in April and May in the northern hemisphere and October and November in the southern hemisphere. But, there is still a need to develop mid- and late-season varieties to supply fresh fruit for the local market as generally 90–95% of the production is consumed locally. A good example of this would be the work being done by Dr. Tiziano Caruso in Sicily where he is improving the medium-chill, late ripening (September to November) varieties traditionally grown in Sicily. There they have a strong local market based on the influx of tourists from more northern climates during this fall season.

*Increase the fruit types available.* Over the last 50 years, the supermarket produce section has changed dramatically with a greatly increased number of items sold. Not only are there more varieties and types of any given fruit but also there is more competition from exotic fruits and another class of convenience food — the minimally processed fresh product. Fifty years ago, the yellow peach was king. Now nectarines compose about one-third of the 'peach' market (USDA, 2001). In addition, there are white and yellow flesh types, low-acid and high-acid types. Recently we have begun to see flat (pantao) peaches in the supermarkets and soon we will see red- and orange-fleshed peaches and nectarines. At the present time, among the medium- and low-chill varieties available, are mainly yellow-fleshed peaches. Although these varieties have proven that it is possible to produce marketable fruit in low- and medium-chill zones, much work needs to be done to expand the fruit types available to more colours (orange, yellow, white, red), flavours (low, medium, and high acid), and types (peach, nectarines, and pantaos).

*Improve fruit quality.* Compared to other crops, such as pome fruit and grapes, the consistency of the quality of stone fruit is poor. This does not refer to external qualities as these are efficiently standardised by packing, but rather to internal quality (firmness, soluble solids, acidity). Although, traditionally, internal quality assessments rely on destructive tests, we are beginning to see the commercial use of non-destructive systems to measure quality using acoustical and near infrared systems. This gives us the

ability to select individual fruit for minimum fruit quality and put higher quality standards on the varieties that are developed. This is essential to increase peach consumption. Unfortunately, peach quality is a complex trait, which is dependent on cultural conditions (Crisosto et al., 1997; DeJong et al. 2002) as well as the genetics of the variety (Byrne, 2002). Nevertheless, it is clearly essential to have sufficient firmness for ease of handling and sufficiently high soluble solids for good consumer acceptability.

Whereas the US based programs have traditionally emphasised the development of melting flesh types for the fresh market, the two Mexican and the Brazilian (Pelotas) programs have worked with non-melting flesh germplasm. More recently, this flesh type has been used to develop early ripening varieties with better firmness and flavour for the fresh market. This approach has been promoted in the low chill zone by Wayne Sherman of the University of Florida and has resulted in several recent releases ('UFGold', 'UFQueen', 'UF2000', 'Gulfprince', and 'UFO'). Additional ripening traits, such as the stony hard (low-ethylene) trait (Goffreda, 1999), the slow ripening trait from 'Fantasia' (Brecht et al., 1984), and the flesh types of the varieties 'Yumyeong' and 'Grezzano' need to be incorporated into the low-chill germplasm (Byrne, 2002).

Peaches are expected to be sweet and recent surveys indicate that soluble solids less than 10% for acid varieties and 11% for low acid peaches/nectarines are generally unacceptable to consumers (Crisosto et al., 2003). Unfortunately, many common low and medium-chill, early ripening varieties typically have soluble solid levels of 8–12° Brix. This needs improvement and excellent progress is being made in the high-chill germplasm. Unfortunately, there are breeding obstacles to the development of a peach/nectarine that is large, early ripening and has high soluble solids since there is a negative correlation between total soluble solids and fruit development period and fruit size (Souza et al., 1998, 2000; Byrne, 2002). Nevertheless, recent work in my program and that of the USDA program in California has shown it is possible to combine high soluble solids with good fruit size and a fruit development period of less than 100 days.

As the public becomes more aware of the health benefits of fruits and is being told to eat a colourful diet there is a potential to create a new market for varieties specifically developed for their health benefits. Recent work has shown that carotenoids (orange/yellow pigments), anthocyanins (red pigments), and general phenolics (colourless) found in peaches have antioxidant properties that protect against various pathological conditions such as inflammation, cancer, atherosclerosis, and other circulatory problems (Prior

and Cao, 2000; Wargovich, 2000; Cevallos-Casals et al., 2002; Gil et al., 2002; Tomas-Barberan et al., 2001). Such 'health enhanced' varieties would provide a new product that could be sold fresh or processed (total crop or as an outlet for the cull fruit) into extracts that are natural sources of antioxidants, antimicrobials, and colourants (Byrne, 2002).

Another important trend is the increase in convenience foods. In the case of produce, there has been a rapid increase in the number of pre-cut, minimally processed items in the produce section of the grocery store. Although much of the development has to do with post-harvest treatment and packaging strategies to prolong the self life of these products, the selection of the appropriate varieties for such uses will be important as this industry develops and expands into the stone fruit arena.

### **Improved postharvest durability**

As the produce market becomes more global and a year-round supply of produce is required, fruit varieties need to have the appropriate post-harvest characteristics that allow a shipping/marketing period of several months without losing quality or experiencing internal breakdown. This implies that we need to be able to control the ripening process. A peach that could be harvested mature ripe, held in storage, and then induced to ripen once put into the retail market would be ideal. In peach there are genes such as stony hard (Goffreda, 1992) and slow ripening (Brecht and Kader, 1984) that control ethylene and the rate of maturation as are found in tomato, a well studied fruit ripening system. A promising research approach would be to use the existing information on other crops to understand and identify genes in peach that control ripening.

The most common post-harvest problem is chilling injury or internal breakdown, which includes flesh browning and mealiness (wooliness). Although much work has been devoted to controlling this problem by manipulating the storage conditions (mainly temperature) only recently has good data been developed to compare varietal differences (Crisosto et al., 1999). This is the first step in developing rapid evaluation techniques to evaluate seedling trees for their resistance to chilling injury during storage. The physiological and genetic basis of this and other post-harvest traits needs to be further studied.

### **Potential of low- and medium-chill stone fruit development**

Peach breeding programs of the world have churned out 60–70 new peach/nectarine varieties per year for the last 20 years trying to keep up with the changes in the produce market, production constraints/practices,



Dr David Byrne and Dr Unaroj Boonprakob inspect a display of peach varieties at Chiangmai Royal Agricultural Research Center, Khunwang.

and new production areas. The work has been productive and we are seeing significant movement towards fruit diversification, improved adaptability to disease and pest pressures, and improved quality and post-harvest traits, especially in the high-chill germplasm.

After many decades of a low level of effort, starting with low-quality varieties, the productivity and quality of the medium- and low-chill peach/nectarine varieties is at a level where their potential is readily visible. As the produce market becomes more global and the demand for year-round supply of fruit increases, the opportunity to supply fruit during the late-early seasons (October to November in the south and April and May in the north) of each hemisphere increases as well. To capitalise on this opportunity, the current crop of medium- and low-chill cultivars (mainly yellow-fleshed peaches) needs to be expanded to include a complete range of fruit types with improved adaptability, quality, and postharvest durability. Given the recent increase of breeding activity for these zones, new varieties are being released more frequently and will continue to be until there is a complete series of varieties available that have the same quality as their high-chill cousins.

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# Producing super-sweet peaches and nectarines under sub-tropical climates

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## *Abstract*

New systems are being developed to produce super-sweet peaches and nectarines under sub-tropical conditions of Australia. Most low-chill stone fruit, because of their short fruit development period, exhibit low sugar concentration (9–10° Brix). Slightly higher concentrations (11–13° Brix) can be produced in northern Thailand under drier growing conditions. A minimum acceptable consumer standard for sugar concentration in stone fruit would be 11° Brix, depending on the sugar:acid balance.

Stone fruit studies on fruit fly exclusion netting as an environmentally friendly means of preventing fruit fly damage and other insect pest damage provided additional benefits. It changed the microclimate inside the enclosure, resulting in fruit with Brix concentrations as high as 15°. Other management techniques also increase fruit sugar concentrations by an additional 10–20%. Applying foliar growth retardants of pro-hexiodione-Ca (Regalis) or paclobutrazol during early fruit development restricted shoot extension growth; and applying ethylene inhibitors such as AVG 1 week before first harvest delayed fruit maturity and extended the period for carbohydrate accumulation and conversion to sugar.

New studies being initiated to produce super-sweet fruit include: micro-climatic modification techniques (exclusion netting), reflective mulches, new training and trellising systems to increase light interception and the best management practices (described above). These systems and practices will be imposed on new, high sugar-accumulating varieties bred at the Maroochy Research Station. These varieties alone, without special practices, can exhibit on average 17° Brix. However, to be able to guarantee that 100% of the fruit reach a minimum standard of 15° Brix, a totally new management system will need to be developed. Other technologies to assess sugar concentration of the fruit after harvest, using near infra-red spectroscopy (NIR) are also being evaluated and will eventually be used in grading and sorting of the fruit so as to deliver to consumers a consistently high-quality product.

## **Introduction**

New systems are being developed to produce super-sweet peaches and nectarines under sub-tropical conditions of Australia. Most low-chill stonefruit, because of their short fruit development period, exhibit low sugar concentration (9–10° Brix). Slightly higher concentrations (11–13° Brix) can be produced in northern Thailand under drier growing conditions. Based on Californian studies, a minimum acceptable consumer standard for sugar concentration in peach would be 11° Brix for yellow-fleshed, high acid

(≤ 0.7%) varieties and 12° Brix for white-fleshed, low acid (≤ 0.4%) varieties, depending on the sugar: acid balance (Crisosto et al., 2003). They found that consumer acceptance for two Californian varieties of peach was related to ripe soluble solids concentration (RSSC) but not to ripe titratable acidity (RTA).

Peach quality is complex and depends on cultural conditions (Crisosto et al., 1997; DeJong et al., 2002) as well as the genetics of the variety (Byrne, 2002). New technologies that are being evaluated on new improved sugar accumulating varieties include use of exclusion netting; growth retardants; ethylene inhibitors and UV light inhibitors.

This paper reports on the preliminary findings from a series of studies on these new technologies. The potential synergistic effects of these technologies, when applied in combination, are also being studied.

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## New varieties

Low-chill peach and nectarine breeding commenced at the DPI&F Maroochy Research Station in 1998 with the aim of producing varieties with high fruit quality adapted to sub-tropical growing environments. Several selections are currently on test that produce fruit, under standard commercial growing conditions, with sugar levels in the range of 13 to 18% total soluble solids. In this breeding program eating quality is being improved by a combination of:

- Incorporation of different flesh texture characteristics, such as non-melting flesh, which will allow fruit to be harvested closer to the physiological tree-ripe stage and yet have enough firmness to be handled through the supply chain. The end result will be sweeter fruit with higher flavour.
- Hybridising standard low-chill peach and nectarine germplasm with medium and high-chill germplasm containing higher fruit sugar levels. Medium-chill genotypes with sweet fruit have been selected and are currently being used as parents in breeding for lower-chill peaches and nectarines.
- Selecting for longer fruit development periods (FDP) to allow higher accumulation of sugars in fruit. In southern Queensland and northern NSW there are many sites where spring frosts are not a problem. At these locations 100 chill unit varieties with 120 day FDP will bloom in early July and still reach market before the critical December period (when an influx of high-chill fruit occurs).

## Exclusion netting

Exclusion netting, with mesh size less than 2 mm, has been trialed in a number of countries including Israel, Thailand and Taiwan to exclude major insect pests such as fruit fly (Lloyd et al., 2003). Studies in Queensland showed that exclusion netting totally excluded fruit fly from netted stone fruit orchards under high external fruit fly pressure (Lloyd et al., 2003). Temperatures under exclusion netting were monitored continuously on an hourly basis from October 2001 to June 2002 at the Maroochy Research Station, Nambour, Queensland. Results showed that exclusion netting raised maximum air temperature by about 5°C compared with ambient, but the minimum temperatures were not affected (Fig. 1). Spectral composition of the light under the netting was changed slightly.

Exclusion netting increased the sugar concentrations of nectarine cvs. Sunwright and White Satin by 30%, compared with bird and bat netting (Table 1) presumably due to high heat units accumulated under the netting. Exclusion netting also significantly improved fruit colour and fruit size. In other studies, exclusion netting has been shown to increase sugar concentrations of some varieties by as much as 40%.

Overseas research in Israel (Shahak et al., 2002) has shown that the colour of the netting, particularly red netting, can significantly change the spectral transmittance properties of light below the netting, leading to significant improvements in fruit quality. Consequently, the effects of netting colour on tree physiology need to be fully investigated.

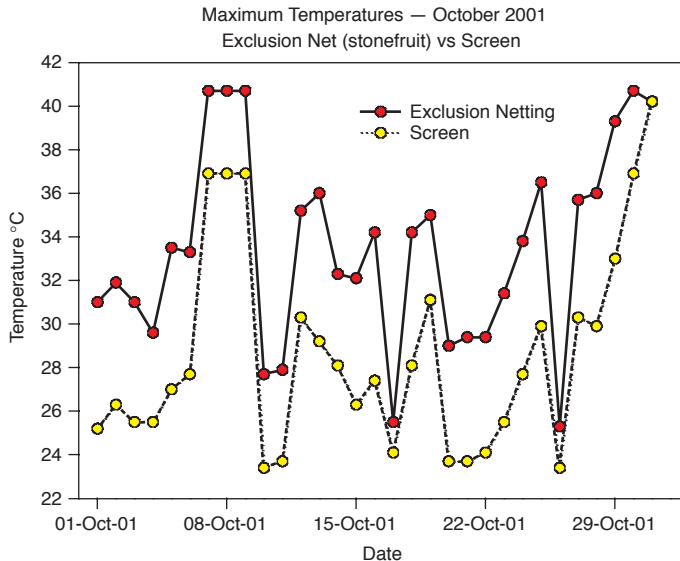


Figure 1. Daily maximum temperatures for October 2001.

**Table 1.** Effects of different types of netting, training systems and mulching on fruit quality of nectarine cvs White Satin and Sunwright.

Type of netting	Variety	Training system	Type of mulch	Average fruit weight (g)	Brix (°)	Firmness (kg)	Colour (%)	Colour Intensity (1-5)
Exclusion net	Sunwright	Vase	Straw	98.0	13.2	10.2	64	4.4
	White Satin	Vase	Straw	95.0	13.2	5.9	59	4.1
Bird and bat net	Sunwright	Vase	Straw	79.9	10.3	7.8	46	4.2
	White Satin	Vase	Straw	76.3	10.1	7.4	43	3.7

### Reflective mulching

Recent studies in Australia and New Zealand have shown that various types of reflective mulch can significantly improve fruit quality by increasing the amount of reflective light intercepted by the tree canopy. Various types of mulch are currently under trial eg 'Extendaday®' and 'Tyvek®'. Sugar concentrations of the fruit may be increased by 2° Brix and fruit size by 15%.

### Growth control

Excessive vegetative growth and poor light penetration into the peach tree canopy during fruit development may reduce fruit size and quality of early-season stonefruit. Excessive vegetative growth can be controlled using paclobutrazol applied as a soil application (George and Nissen, 1987; George and Hieke, 1996). Fruit size increases of up to 30% have been achieved where shoot extension growth at stone-hardening and harvest is reduced to 10 cm and 50 cm, respectively (George et al., 1994). However, soil applications of paclobutrazol are slow-acting, particularly on heavier textured soils and continual use of the product may result in the build up of soil residues (George et al., 1993; 1994).

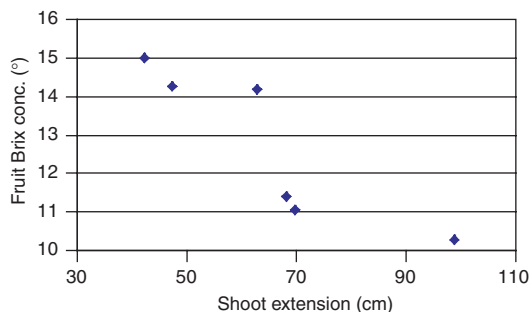
More rapid response may be achieved through foliar application of growth retardants. Foliar applications of paclobutrazol have been shown to be partially successful in controlling early-season growth of some low-chill stone fruit cultivars (George and Nissen, 1987) but in other studies there has been little or no effect on growth, yield or fruit quality. A new growth retardant, prohexadion-Ca (BASF Regalis), is being evaluated in Israel and Queensland on a range of temperate fruits (Erez, 2003; George, unpublished data) and has been shown to be effective as a foliar spray in controlling vegetative growth of peach in Israel. It has been reported to have a stronger growth retarding effect on peach plants than paclobutrazol (Erez, pers. comm). Prohexadione-Ca is primarily transported acropetally via the xylem. Its mode of action is to inhibit the late steps of GA biosynthesis.

Compared with paclobutrazol, the active ingredient decomposes very rapidly in the soil and the biological half-life is about 10–14 days. This characteristic would be highly advantageous to commercial growers, as it would give a wider range of choices to control vegetative growth. In preliminary trials conducted at the Maroochy Research Station at Nambour, Queensland, Regalis has been shown to improve sugar concentration by about 10% (Table 2).

**Table 2.** Effects of prohexadion-Ca on fruit quality of two nectarine cvs at Nambour, Queensland.

Prohexadion-Ca rate	Fruit colour (1-10)	Firmness (kg)	Brix (°)
cv. White Satin			
Control	4.8	7.4	10.3
Regalis 2g/L	5.3	8.0	11.0
Regalis 4g/L	5.7	7.5	11.4
cv. Sunwright			
Control	5.7	7.6	14.2
Regalis 2g/L	6.0	7.9	15.0
Regalis 4g/L	5.9	9.2	15.1

Brix concentrations (Figure 2) and average fruit weight were negatively correlated with shoot growth ( $r=-0.90$ ,  $-0.80$ , respectively,  $P<0.05$ ).



**Figure 2.** Brix concentrations vs. shoot extension. Pooled data for the two varieties.

**Table 3.** Effects of AVG on fruit quality of two stonefruit cvs at Nambour, Queensland.

ReTain conc.	Timing	Firmness (kg)	Brix (°)
Cv Sunwright			
Control		7.4	9.9
0.83g/L* ReTain	27 September (15 days prior to first harvest)	7.7	10.3
0.83g/L ReTain	7 October (5 days prior to first harvest)	9.6	11.3
0.83g/L ReTain	27 September and 7 October	11.1	10.9
1.66g/L Retain	27 September	7.7	10.8
Cv. Flordaprince			
Control		6.8	11.6
0.83g/L* ReTain	27 September (15 days prior to first harvest)	9.5	11.5
0.83g/L ReTain	7 October (5 days prior to first harvest)	10.2	11.5
0.83g/L ReTain	27 September and 7 October	10.3	11.6
1.66g/L Retain	27 September	7.6	11.3

### Ethylene inhibitors

Ethylene is a highly potent plant hormone that is involved in plant processes such as fruit maturation. An approach to manipulate ripening could be achieved by applying substances, which inhibit ethylene production such as aminoethoxyvinylglycine (AVG). ReTain (aminoethoxyvinylglycine: AVG), a newly registered commercial product, is an ethylene biosynthesis inhibitor that delays fruit maturation if applied before harvest and increases fruit set if applied after bloom in pears and apples. Studies on peaches (Vizzotto et al., 2002; Bregoli et al., 2002; Rath and Prentice, 2004) and apples (Brackmann and Waclawovsky, 2001) have shown that, if AVG is applied prior to harvest, fruit size, firmness, sugar concentrations and storage life may all be significantly increased. Our preliminary studies showed that ReTain increased sugar concentrations of low-chill nectarine by between 0 to 15%. The lack of response in some varieties appears to be due to a significant increase in fruit size due to ReTain.

### Complete management system

Preliminary evaluation of the above technologies has shown that all of them alone can significantly improve the sweetness of stone fruit. It is anticipated that a positive synergistic response would be achieved when these technologies are applied in combination to the new, higher sugar accumulating varieties under exclusion netting. The ultimate aim of this research would be to guarantee internal eating quality to the consumer by using non-invasive NIR grading equipment.

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# Temperate fruit development in Vietnam

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and Doan Nhan Ai<sup>1</sup>

## Abstract

Vietnam is evaluating a range of new fruit crops, including low-chill temperate fruits such as: peach, plum, nectarine, apricot, pear and persimmon and sub-tropical fruits such as avocado. These crops have been selected as they appear to have the best potential to improve the living standard of ethnic minorities as well as being environmentally sustainable. Due to the growing demand for temperate fruits in Vietnam and the high adaptability of these fruits in upland and mountainous areas, the Vietnamese government plans to establish 150 000 hectares of temperate fruits by 2010.

Although some gains in temperate fruit production have been made recently, including newly introduced varieties, the lack of elite varieties and information about temperate fruit management are still major problems that need to be addressed.

## Introduction

VIETNAM is located in the Indochina Peninsula of Southeast Asia. The country stretches along almost 15° of latitude with a 3000 km long coastline.

The climate is tropical with monsoonal winds and a cold winter in the north. The agricultural ecology is, therefore, quite diverse. After the problem of food security was significantly solved, the Government of Vietnam paid a great deal of attention to the development of fruit crops, including temperate fruit crops in upland and mountainous regions in the north and central highlands where the living standard of ethnic minority people is still low and environmental conditions are not well protected. The advantages of temperate fruit development in the mountainous area in the north and some areas in the central highlands of Vietnam can be summarised generally as follows.

There is a growing demand for temperate fruit in Vietnam, particularly in big cities and places where the population is concentrated. High mountainous areas in the north and the central highlands with cold winters and receiving sufficient chilling are suitable for growing low-chill, temperate fruits. Varieties requiring medium-chill units can be grown in some locations. Ethnic minorities traditionally grow temperate fruits at an altitude of 800–2000 m above sea level. Some

selection of low-chill temperate fruit cultivars already exists, such as Tam Hoa, Hau, Ta Van plums; Mau Son, Vang peach; Ngan Son, Nau pear etc.

## Present situation of low-chill temperate fruit production in Vietnam

Of the country's seven agro-economic regions (Table 1) mountainous areas in the north and central highlands can be partly exploited for low-chill temperate fruit cultivation. It is estimated that about 150 000 ha in the



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Dr Le Duc Khanh assessing the nectarine cultivar 'Sunwright' at Moc Chau, Vietnam.

northern upland and about 20 000 ha in Lam Dong province (central highlands) are suitable for temperate production and these fruits are currently under-exploited in Vietnam. The mid-land and mountainous regions in the north of the country have a diversified agricultural ecology in which certain areas located at high altitude, such as Moc Chau (Son La province); Sapa, Bac Ha, Muong khuong (Lao Cai province); Dong Van, Pho Bang (Ha Giang province); Trung Khanh (Cao Bang province); Mu Cang Chai (Yen Bai province); and Cao Loc (Lang Son province) favour the growth of low-chill temperate fruit.

### Temperate fruit production regions

As mentioned above, temperate fruit particularly low-chill fruit, can be grown in mountainous areas in north and central Vietnam. The following regions are considered to be of high potential.

*Moc Chau (200 km northwest of Hanoi).* The elevation at Moc Chau ranges from 200 to 1800 m, with an average of 1000–1200 m. Frosts are rarely observed in the region. Humidity remains high throughout the year at 85% and rainfall averages 1500 mm. At present, about 3700 ha of fruit trees are cultivated, of which an estimated 1380–2240 ha are plum that produces 13 500–18 880 tons of fruit per annum, much of which is consumed locally but some of which is also sent to Hanoi.

*Bac Ha (northern highland region, 300 km north-northwest of Hanoi).* Bac Ha is an elevated highland district (1400 m asl) located in a valley and well suited to temperate fruit production. In this area, plums are the major crop planted with a total area of 2500–2700 ha, producing 16 000 tonnes in the off-year and 44 000 tonnes in the on-year. As well as the main original Chinese cultivar (Tam Hoa), some new varieties of peach, grape and pear have been recently introduced.

*Sapa (northern highland region, 360 km northwest of Hanoi).* Sapa is surrounded by steep mountainous



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Immature local peach for sale in the Moc Chau fruit markets, Vietnam.

terrain which makes the area well suited to temperate fruit production. Some local cultivars of peach, plum and persimmon have been traditionally grown in this area but productivity and quality are still the main problems that must be addressed.

*Ha Giang province (northern highland region, more than 400 km north-northwest of Hanoi).* Ha Giang is an elevated highland province (600–2000 m asl) located in a valley. Various types of temperate fruit, such as pear, plum and peach, have been traditionally grown in most districts of the province. Of 9093 ha of fruit crops cultivated, more than 300 ha of peach and plum and 142 ha of pear are grown in the whole province, producing more than 1700 tonnes annually.

*Other locations.* Apart from the above mentioned areas, low-chill temperate fruit are also planted in other provinces of the northern highland region, such as Lang Son, Cao Bang, Quang Ninh and Bac Can, and the central highlands (Lam Dong in particular). Table 2 shows the area of the main temperate fruits cultivated in these provinces.

**Table 1.** General information about the development of temperate fruit in northern Vietnam.

No	Regions	Area (Million ha)	% of total	Altitude (m)	Temp (min) – Temp (max) (°C)	Rainfall/year (mm)
1	North mountain & midland	9.8	30.0	100–3140	12–32	1600–2500
2	Red river delta	1.0	3.8	12–14	16–32	1700–1800
3	North central coast	5.2	17.5	100–2710	25–32	2450–2890
4	South central coast	4.6	13.9	10–105	22–32	1000–1300
5	Central highland	5.5	16.6	2200–2600	23–32	2100–2000
6	Northeast – South	2.3	7.0	100–1000	26–32	1960–2000
7	Mekong river delta	4.0	12.1	7–10	26–31	1950–2010



**Table 2.** Area of temperate fruit grown in the northern provinces.

No	Province	Fruit crop			
		plum	apricot	peach	pear
1	Lao Cai	2942	383	114	35
2	Son La	1699	1056	15	—
3	Thai Nguyen	—	130	—	—
4	Bac Can	607	2890	—	—
5	Cao Bang	605	355	14	172
6	Lang Son	625	365	20	80
7	Quang Ninh	65	65	—	—
8	Ha Giang	—	—	—	—
9	Yen Bai	—	—	—	—
	Total	6513	5224	163	287

**Table 3.** Mean monthly temperature of Vietnamese temperate fruit growing regions.

Region	Month												
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Av.
Hanoi	16.4	17.0	20.2	23.7	27.3	28.9	28.9	28.2	27.2	24.6	21.4	18.2	23.5
Moc Chau	11.8	13.1	16.8	20.2	22.5	23.1	23.1	22.4	21.2	18.9	15.7	12.8	16.5
Bac Ha	10.8	12.2	16.0	19.7	22.5	23.7	23.7	23.1	20.8	19.2	15.6	12.1	18.4
Sapa	8.5	9.9	13.9	17.0	18.3	19.8	19.8	19.5	18.1	15.6	12.4	9.5	15.2

**Table 4.** Number of days with frosts.

Region	Month												
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Av.
Hanoi	0.03	0.03	0	0	0	0	0	0	0	0	0	0	0.2
Moc Chau	2.00	0.20	0	0	0	0	0	0	0	0	0.60	2.3	5.1
Bac Ha	1.60	0.10	0	0	0	0	0	0	0	0.10	0.40	1.3	3.5
Sapa	2.00	0.20	0.04	0	0	0	0	0	0	0.03	0.80	2.3	5.4

**Table 5.** Estimated chilling received at selected sites in Vietnam.

Sites	Latitude (° North)	Elevation (m)	Mean temperature of the coldest month (°C)	Chilling unit (C.U.)
Ha Noi	21.01	8	16.4	125
Da Lat	11.60	800	16.4	125
Moc Chau	20.80	1,000	11.8	402
Bac Ha	22.70	1,400	10.8	485
Sapa	23.00	1,580	8.5	731

**Table 6.** Average temperature (°C), rainfall (mm), and humidity (%) by district in Lao Cai.

Districts	Factors	Month												Av. Temp & humidity Total annual rainfall
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	
Muong Khuong	Av. temperature	11.6	13.0	16.7	21.0	23.4	24.1	24.5	23.9	22.8	20.3	16.5	13.5	19.3
	Rainfall	32.2	43.5	52.4	102.7	202.9	282.6	390.7	368.9	191.6	131.0	82.8	32.2	1913.5
	Humidity	90	90	88	87	85	87	88	88	87	86	88	88	88
Bac Ha	Av. temperature	10.8	12.2	16.2	19.7	22.5	23.5	23.7	23.1	21.8	19.2	15.6	12.1	18.4
	Rainfall	18.1	30.4	42.7	120.6	165.4	259.9	328.8	362.6	237.5	124.7	64.2	19.1	1774.0
	Humidity	89	89	87	85	84	86	87	88	87	87	88	88	87
Lao Cai city	Av. temperature	16.0	16.8	20.6	24.0	26.8	27.6	27.7	27.3	26.3	23.8	20.2	17.3	22.9
	Rainfall	20.7	35.5	59.9	119.7	209.0	236.3	301.3	330.5	241.2	131.2	54.6	24.5	1764.4
	Humidity	86	85	84	84	83	86	86	87	86	86	87	86	86
Sapa	Av. temperature	8.5	9.9	13.9	17.0	18.3	19.6	19.6	19.5	18.1	15.6	12.4	9.5	15.2
	Rainfall	55.8	79.2	105.5	197.2	353.2	392.9	453.0	478.1	332.7	208.7	121.6	55.1	2833.0
	Humidity	88	85	82	83	84	87	88	89	90	89	90	87	87
Than Uyen	Av. temperature	14.0	15.5	19.2	22.4	24.5	25.0	25.1	24.9	24.1	21.8	18.1	14.6	20.8
	Rainfall	33.7	39.7	56.5	166.0	238.7	391.2	409.4	406.8	176.0	78.6	49.9	20.8	2066.9
	Humidity	82	80	78	79	81	85	86	86	82	80	81	81	82

**Table 7.** Local selections of temperate fruit.

Region	Species/Variety	Scientific name	Characteristics
Moc Chau	Purple plum	<i>Prunus salicina</i>	Purple skin, yellow flesh, small seed, 30–50g/fruit, slightly sweet
	Peach	<i>Prunus persica</i>	Var. Meo, white flesh, green skin with yellow blush, sour, 10–15 fruit/kg
	Pear	<i>Pyrus spp.</i>	Var. H'mong, rose skin and flesh, 5–7 fruit/kg, susceptible to fruit fly
Bac Ha	Plum	<i>Prunus salicina</i>	Var. Ta Van, yellow skin, slightly sweet
	Peach	<i>Prunus persica</i>	Var. Meo (mentioned above)
	Persimmon	<i>Diospiros kaki</i>	Various astringent and non-astringent cultivars
	Pear	Chinese cultivars	Yellow and brown skins (up to cultivars)
Sapa	Apple	<i>Malus douneriex</i>	Local cultivars named Son Tra (small-sized fruit)
	Persimmon	<i>Diospiros kaki</i>	Astringent and non-astringent cultivars
	Plum	<i>Prunus salicina</i>	Hau (late cultivars), Ta Hoang Ly, Ta Van, Violet plum
	Peach	<i>Prunus persica</i>	Van Nam (Chinese originated cultivar), Meo, Mau Son

### **Climatic conditions of regions where temperate fruit growing is planned**

The temperature and frost incidence data for the regions have been collected and are presented in Tables 3 and 4. This information is used to calculate the chilling units (CU) for each region (Table 5).

Rainfall related to atmospheric humidity and radiation are other important factors that affect the yield and quality of temperate fruits (Table 6). Generally, high humidity at some sites in the rainy season can harm trees, whereas radiation should not be a major problem.

### **Temperate fruit species grown in Vietnam**

A wide range of local species of temperate fruits are grown throughout Vietnam. Recently, some low-chill temperate fruit cultivars have also been introduced in some areas, of which, promising ones are also evaluated and observed (Table 7).

### **Problems to be solved**

In order to promote the development of temperate fruit in Vietnam, the industry needs to focus on the following issues, which are considered as the main constraints.

1. High humidity favours the development of insects and diseases. Pests would be best controlled using IPM strategies.
2. Knowledge about the best varieties of crops to grow, effective cultivation practices, harvest and post-harvest technology and marketing is limited and needs to be significantly improved.
3. Establish nurseries (and upgrade existing ones) with appropriate management and production technologies to provide fruit growers with healthy planting materials
4. Search out and disseminate information about the adaptability and management of low-chill temperate fruits.

# Innovative fruit production systems for peach and nectarine in Australia and Southeast Asia

R.J. Nissen<sup>1</sup>, A.P. George<sup>1</sup>, A. Lloyd<sup>2</sup> and G. Waite<sup>1</sup>

## *Abstract*

New training and trellising systems are being developed for temperate fruits in Australia. The most productive and efficient trellising system with improved fruit quality appears to be the open Tatura system. Poor fruit size is a major problem of early-season cultivars. Crop-loading studies have established indices, such as fruit number per canopy surface and butt cross-sectional area, to provide a simple guide for optimum fruit thinning levels. Best management practices have been developed for the use of growth retardants, for optimising leaf nitrogen concentrations and for controlling rates of timing of irrigation. Regulated deficit irrigation (RDI) improves fruit sugar concentrations by restricting water application during stage II of fruit growth. RDI can also be used after harvest to restrict vegetative growth and enhance floral bud differentiation. New pest and disease control measures are being developed using a new generation of fruit fly bait products. These 'soft' insecticides, such as Spinosad (Dow AgroSciences), are used in significantly lower concentrations and have lower mammalian toxicity, than the organophosphates currently registered for use in baits in Australia. In addition to bait sprays, fruit fly exclusion netting has proven to be highly effective in eliminating fruit fly and many other insect pests from the orchard. This type of netting has been shown to increase sugar concentrations of peach and nectarine fruit by as much as 30%. Economic analyses have shown that the break-even point can be reduced from 10 to six years using these new production systems.

## **Introduction**

FRUIT quality is directly related to consumer satisfaction and purchasing patterns. Poor fruit size and quality are major problems in early-season, low-chill stone fruit cultivars. This has resulted in uneconomical and unsustainable farming practices due to poor tree training, excessive crop loading, and incorrect management of pests, diseases, irrigation and nutrition.

In many fruit crops, approaches to improve light interception include planting design, pruning and tree training. These are critical determinants of tree productivity with internal tree shading severely reducing yield and fruit quality (Jackson, 1980; Palmer et al., 1992). Studies have shown that peach, nectarine and plum trees need good light penetration into the tree

canopy with a minimum of 20% full sunlight transmitted to the fruiting sites. Arriving at the correct crop load level is critical with cropping capacity varying with tree age, variety and environment.

Management practices also directly impact on fruit size and quality and the interactions between vegetative growth, adequate nutrition, irrigation and pest and disease control practices are major determinants of fruit size and quality. One of the most serious pests of fruits and vegetables is the fruit fly (Tephritidae), which causes substantial losses in terms of both quantity and quality. Fruit flies are recognised worldwide as the major pest of horticultural production at both the commercial and subsistence levels, from the cold temperate regions of the globe to the heart of the tropics. Countries such as Thailand, Laos and Vietnam experience pre-harvest fruit and vegetable losses as high as 70–100%. As a result, fruit flies are seen as a major contributor to the ongoing problems of hunger, poor food nutrition and poverty, especially in rural communities. Thus, introduction of simple, practical, in-field solutions to the fruit fly problem will have a direct and positive influence on household food security for the rural citizens.

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Consumers are now requesting that their fruit and vegetables are grown in environmentally friendly systems. This impacts directly on the cost of production and growers not using best management practices will become unsustainable and, therefore, uneconomical. New production systems need to be fully tested and an economic evaluation carried out to determine their viability.

### Orchard design and training systems

New planting systems and training systems have been developed to improve orchard production and efficiency. A major consideration in the design of these systems is the level of light interception into the tree canopy as this has a major impact on yield potential. Tree spacing and canopy characteristics (height, width, shape and leaf density) are elements which we can manipulate to capture a higher proportion of incoming light (Corelli and Sansavini, 1989). Economic fruit yields and quality are a function of the efficiency of light utilisation and distribution in the canopy (Jackson, 1980; 1985). Once a mature tree has filled the allotted space, excessive vegetative growth reduces light penetration into the canopy, affecting floral bud development, fruit set and fruit quality.

Close planting of trees, greater than 1000 trees per hectare under traditional orchard designs and training systems, results in poor light distribution and penetration into the canopy. These orchards become uneconomical within a short period of time. New orchard designs and training systems have been developed, which allow the close planting of trees that can double the yield per hectare.

Compared with central leader or free standing, vase trained trees, the open Tatura system produces greater cumulative yield during the first 3 years after planting (Van den Ende et al., 2001). The traditional vase trained trees, planted at low densities of less than 400 trees per hectare, can yield from 15 to 25 tonne per hectare. In contrast, the open Tatura system can produce yields as high as 40–50 tonne per hectare. The open Tatura training system provides the highest levels of light intercepted and transmitted through the canopy (Figs 1 and 2).

### Crop loading and cultural practices

There are many indices or indicators of yield efficiency. These include:

- Yield per butt circumference
- Yield per butt cross-sectional area
- Yield per canopy surface area
- Yield per canopy volume, full and part cone

Yield efficiency based on a tree circumference or butt cross-sectional area are the most commonly used

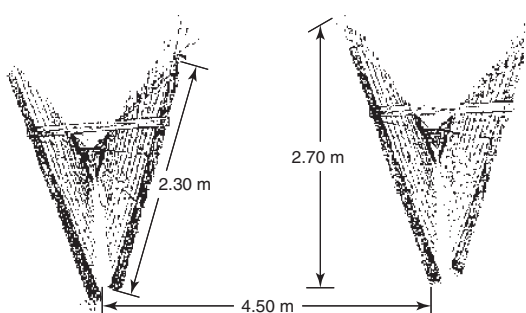
indicators for trees under the age of 5 years, but as a tree ages, other measures such as canopy surface area and canopy volume may be more appropriate. This is due to the effects of cultural practices, such as the use of growth retardants (eg paclobutrazol), which increase butt circumference, average fruit weight and consequently tree yield, but reduce vegetative growth, consequently reducing the yield efficiency expressed on a butt circumference or cross-sectional basis (Nissen et al., 2002; Nardi 2001).

Our studies have shown that average fruit weight decreases rapidly with increasing crop loads but the rate of decline is reduced by applying additional



R.J. Nissen

**Figure 1.** Stone fruit trees trained onto the open Tatura trellis system.



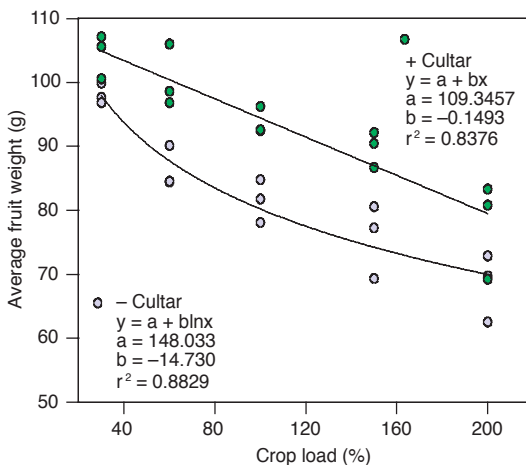
**Figure 2.** Dimensions and spacings for the open Tatura trellis system (Bas van den Ende 2001).

nitrogen and paclobutrazol because paclobutrazol alters the competition between vegetative and fruit growth in favour of the fruit. The pattern of decline in fruit weight is typical of almost all varieties, whether high- or low-chill. Growers of low-chill varieties leave about two fruit per centimetre of butt circumference (Fig. 3). However, based on the findings of our studies, we would recommend leaving either 20 fruit per sq metre of canopy surface area or 50 fruit per cubic metre of canopy volume

Cultural practices such as the use of paclobutrazol advance maturity by about 10 days and increase fruit size grades by between 1 and 2 grades thus significantly increasing monetary returns compared to untreated trees. Fruit sugar concentrations (total soluble solid) for low-chill stone fruit are low due to the short fruit development period. Studies by Nissen et. al. (2002) show that sugar concentrations of the fruit decrease by about 0.1° Brix for each increase of 10 fruit per tree. Paclobutrazol, when combined with additional nitrogen, improved sugar concentrations by about 1° Brix (Fig. 4). This synergistic response contradicts many other studies that have shown that nitrogen alone reduces sugar concentration.

Fruit firmness increases with increasing crop load, presumably due to a similar number of thicker-walled cells in smaller-sized fruit compared with large fruit. Paclobutrazol significantly increases fruit firmness due to a reduction in vegetative growth, enabling the fruit to compete more strongly for nutrients such as calcium and boron.

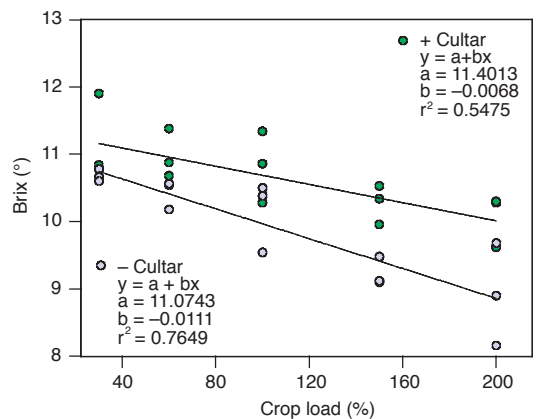
Our studies show that gross returns per tree increased up to normal crop loading levels (250 fruit per mature tree, 6 years of age, planted 3 × 4 m



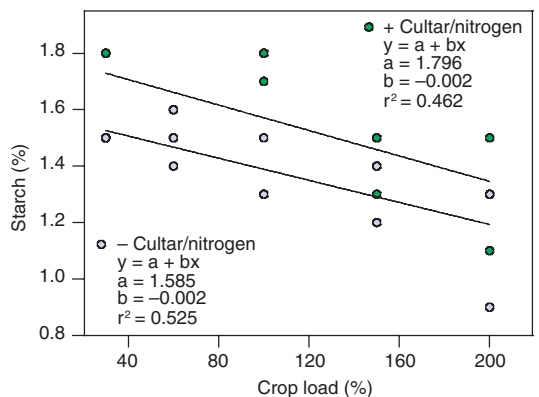
**Figure 3.** Effects of crop load and paclobutrazol on average fruit weight of cv. Flordaprince, Maroochy Research Station 1998.

spacing × 2 m high). By increasing fruit number per tree up to 150% and 200% above the commercially accepted practice, returns increased slightly but fruit quality was severely affected. If we were to extend loading levels to 300% and 400% above normal crop loading levels, returns would decrease due to production of smaller, lower-quality fruit and increasing growing, harvesting and packaging costs.

However, it may not be possible to sustain such high crop load level in the long term. At high crop load levels, the higher percentage of smaller, poorer-quality fruit may deplete trees of their carbohydrate and nutrient reserves leading to biennial bearing. At very heavy crop load levels this may even lead to tree death. At 200% crop load level the percentage starch decreased by 30% in the shoots (Fig. 5).



**Figure 4.** Effects of crop load and paclobutrazol on Brix concentration (°) of cv. Flordaprince, Maroochy Research Station 1998.



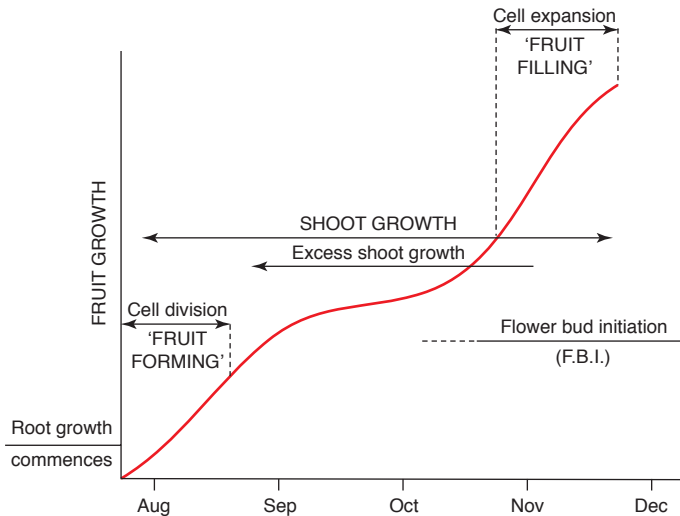
**Figure 5.** Effects of crop load and management treatments on shoot starch concentrations.

## Irrigation

The water use over a season relates closely to the development stage of the tree — both canopy coverage and the fruit growth stage. The development pattern is vital to understanding the sensitivity to water and other stresses. The water use, and therefore irrigation requirements, for stone fruit are greatly influenced by the fruit growth curve (Fig. 6). Large-scale irrigation systems are not required to produce high-value horticulture crops. Direct pumping and small reservoirs, weirs, bores and wells, etc., are more appropriate. Table 1 provides a broad annual guide to water requirements for bearing trees (Year 2 and onwards) on a palmette system in coastal Australia.

For management reasons, irrigation requirement may be different from total tree water use. For

example, excessive vegetative vigour can be controlled by restricting irrigation at the critical period during fruit development and after harvest. This irrigation management is termed ‘regulated deficit irrigation’ (RDI). In much of Southeast Asia, water deficits will occur in at least 4 months each year and irrigation will be essential for good commercial production. For early-maturing, low-chill stone fruit cultivars, due to their shorter fruit development period and a truncated stage two of fruit growth, RDI may be less effective. Also, in eastern Australia, the summer rainfall pattern coincides with the fruit development period of low-chill cultivars and this would make RDI difficult to implement. For countries such as Thailand, because the ‘dry season’ coincides with the flowering and fruit development period, regulated deficit irrigation may be feasible.



**Figure 6.** Idealised growth pattern for a later maturing, low-chill peach or nectarine (adapted from Menzies 1992).

**Table 1.** Water requirements for bearing trees in Australia (ignoring rainfall).

Month	Stage of growth	mm per week	Litres per tree per week
August (winter)	Dormancy	15	150
September (spring)	flowering/early fruit set	20	300
October (spring)	Harvesting	25–30	350
November (early summer)	Harvesting	30	250
December (mid summer)	floral initiation	25	250
January (late summer)	Completion of vegetative flush	20	200
February (early autumn)	Hardening off flush	25	200
March (mid-autumn)	Hardening off flush	20	200
April to July (late autumn)	Leaf senescence/early dormancy	no irrigation necessary	no irrigation necessary

Initial studies were conducted to determine if RDI could be used with low-chill cultivars with much shorter fruit development periods (80–120 days). Our results indicate that mild to moderate water stress (up to –50 kPa) may be beneficial if applied just prior to stone-hardening, after the completion of the cell division period and up to 3 weeks prior to harvest, particularly in warm–subtropical regions where fruit growth in the final stage is excessive. However the short fruit development period of early-season, low-chill cultivars may limit the period to which the RDI can be applied to about 30 days, and for later-season chill cultivars to about 45–50 days.

Further studies are needed to evaluate higher soil moisture stress levels (–50–200 kPa), similar to those used with high-chill cultivars in Victoria, and a longer duration of stress from flowering to the end of stage two of fruit growth. Preliminary evaluation on light clay soils in Thailand indicates that soil moisture stress levels up to –200 kPa reduced yield and fruit firmness by about 20% but fruit sugar concentrations were increased by a similar 2–3° Brix (Nopkoonwong et al., 2002). The maximum stress level where there appeared to be little or no effect on fruit growth was achieved at about –80kPa.

For low-chill varieties, RDI may have a greater application to control excessive growth after harvest. Further studies are needed to elucidate these effects. Table 2 presents the critical application times and minimum watering rates for hill tribe villages in northern Thailand using principles of deficit irrigation.

## New pest control measures

### Species of fruit fly

The major species of fruit fly present in Australia are: Queensland fruit fly, Mediterranean fruit fly, and Papaya fruit fly. The major species of fruit fly in Thailand is *Bactrocera dorsalis*. In Thailand, the usual control measure is to bag the fruit before it starts

to colour. The fruit fly species of greatest economic importance in the north of Vietnam are *Bactrocera dorsalis*, *B. correcta*, *B. pyrifoliae*, *B. cucurbitae*, *B. tau* and *B. latifrons*. In the south of Vietnam the most important species are: *Bactrocera dorsalis*, *B. correcta*, *B. cucurbitae*, *B. tau* and *B. carambolae*.

In Australia, cover sprays of persistent insecticides, such as fenthion, are applied to control fruit fly. This causes environmental problems through leaving chemical residues on the fruit and in the environment. This problem would be exacerbated in Vietnam because of the extensive network of canals and watercourses throughout the farming districts and the use of these watercourses for producing other food supplies, bathing, washing clothes, etc.

There are several ways to monitor fruit flies and the best strategy uses a combination of these techniques. The methods are:

- Fruit fly traps (male pheromone used as an attractant, these need checking regularly)
- Visual monitoring
  - checks made of fruit and foliage for adult flies
  - fruit sampling, monitoring of early-set fruit for stings and eggs under the surface.

### Cover sprays

Two broad-spectrum insecticides are normally used to control fruit fly. These are fenthion and dimethoate. Both these chemicals have been widely used for fruit fly control for many years but in recent years there has been a strong move to develop alternative treatments. This has been driven by consumer and environmental concerns over pesticide usage and residues, problems associated with long withholding periods which interfere with harvesting, and the fact that broad spectrum insecticides are detrimental to beneficial insects and are therefore not compatible with Integrated Pest Management (IPM) programs. Another concern is uncertainty about the long-term availability of the currently used chemicals.

**Table 2.** Water requirements for bearing trees (3 years and older) in northern Thailand with no rainfall using deficit irrigation. Trees are watered at a minimum of four critical times; all trees are heavily mulched to conserve moisture.

Season	Months	Plant growth stages	Minutes of watering by hand-held hose	Number of 10-litre buckets	Litres of water per tree
Winter	November–December	Dormancy		No irrigation necessary	
Dry	December–January	Flowering	7–8	15	150
Dry	February–March	2–3 weeks after flowering	7–8	15	150
Hot	March–April	4 weeks before harvest	10–12	20	200
Hot	May–June	two weeks before harvest	7–8	15	150
Wet	June–October	Vegetative Growth		No irrigation necessary	



Various natural products such as neem oil and natural pyrethrum have been investigated as cover sprays or repellents for fruit fly control but none has proven to be particularly effective. New generation bait products based on 'soft' insecticides are being developed. These are used in much lower concentrations and have much lower mammalian toxicity than the organophosphate insecticides registered for use in baits in Australia. The new generation, microbially produced insecticide, Spinosad (Dow Agro-Sciences), which has obtained organic certification in the US, appears to offer the best prospect as a new cover spray treatment for fruit flies. However, further research is needed on Spinosad to determine its effective application rates.

### Protein baiting

As an alternative to cover sprays, fruit fly may be controlled by applying bait sprays. The advantage of bait sprays is that they are only applied to a small part of the tree, such as the trunk or foliage. Consequently, insecticide residues are not left on the fruit. Bait sprays consist of a combination of an insecticide, eg chlorpyrifos, plus an attractant for fruit fly, eg yeast autolysate. About 50 ml of the mixture is applied to the lower leaves of each tree every 7 days and reapplied after rain. If fruit fly infestation is high, bait spraying may need to be switched to cover sprays (spray the whole tree) of fenthion or trichlorfon. Trees are sprayed every 7 days until harvest. A suggested application schedule for Thailand is presented in Figure 7.

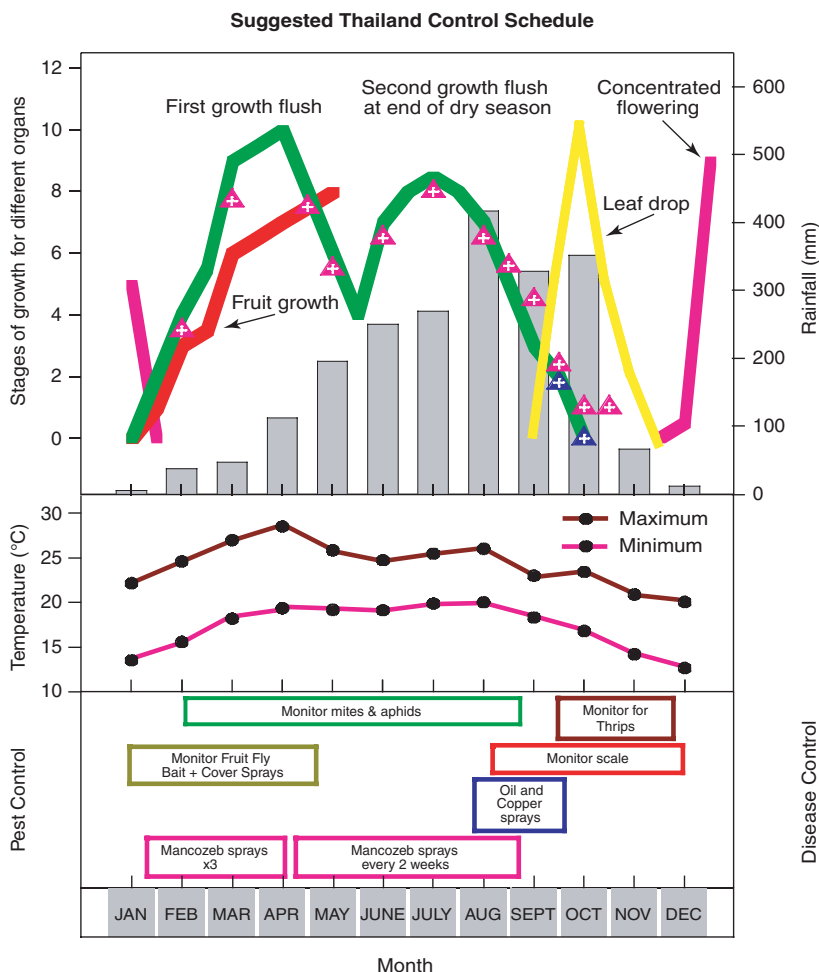


Figure 7. Suggested application schedule to control fruit fly in stone fruit orchards in Thailand.

Two new commercial baits include attractant protein sources mixed with insecticides Spinosad (Dow AgroSciences) or fipronil (BASF) as insect toxicants. DPIF researchers are developing these new baits to provide the same level of control for Queensland fruit fly as currently used standard bait. The new baits are applied at lower rates (5–7 L/ha) than standard baits, which are applied at 15–30 L/ha, depending on the crop type. Both bait formulations include thickening agents which prolong the effective life of the bait on foliage but current recommendations are that the baits should still be applied on a weekly basis, the same as the standard bait.

### Physical barriers

Physical barriers that exclude the adult insect and thereby prevent oviposition into fruit provide a non-chemical method of control. These are highly suitable for both conventional and organic production on a number of different scales. On the smallest scale, applicable to organic home gardeners, various types of bags can be used to completely enclose individual fruit to protect them from fruit fly and other insect pest damage.

On a larger scale, small mesh net fabric can be used to fully enclose individual trees when fruit are susceptible to attack. A 2 mm mesh net made from long lasting, translucent fibre that minimises the shading factor has been used to exclude fruit flies and a variety of other insect pests such as macadamia nut borer, fruit spotting bug, fruit piercing moth, and yellow peach moth. Provided the net is correctly erected and maintained, this technology has the potential to significantly reduce pesticide usage in conventional production, and to provide a practical and appropriate method for organic pest control in a range of crops.

Exclusion netting involves a high initial capital cost and it will not be appropriate for all crops, but in some crops where conventional hail/bird/bat netting is already being extensively used (eg stone fruit, pome-fruit, kiwifruit, persimmon), it will provide

new options for both conventional and organic producers at relatively little extra cost. Recent trials over 2 seasons at Maroochy Research Station, Nambour, Queensland compared fruit fly infestation in peaches under exclusion netting (with no additional fruit fly treatment) to that in an adjacent block under conventional hail net (Figs 8 and 9).

Fruit fly trap catches in the area during the trials ranged from 50–350 flies per trap per week. Infestation levels under exclusion netting were zero in both seasons. The infestation level in the chemically treated block was zero in the first year (Fig. 10) and 0.25% in the second year. As well as the economic viability, the researchers also investigated the effects of this small mesh size on environmental conditions and crop parameters under the net. Preliminary results indicate that fruit quality and yields can be significantly increased under the exclusion netting. Fruit maturity is advanced by about 7–10 days due the higher heat units accumulated under the netting.

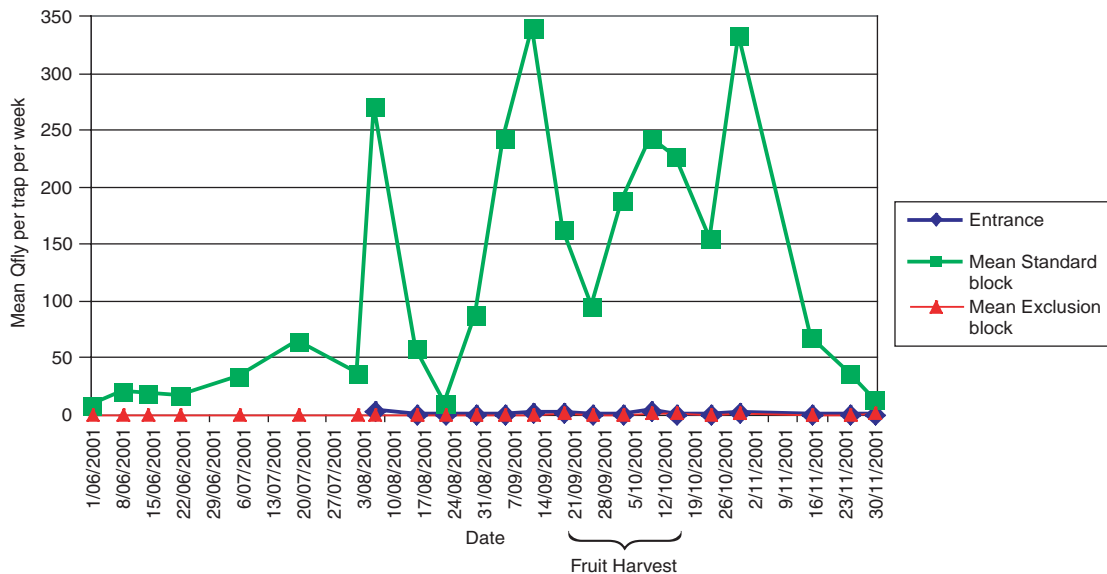


**Figure 8.** Close up of the 2 mm translucent monofilament fibre exclusion netting.



**Figure 9.** View of Maroochy Research Station stone fruit trial blocks with total exclusion net (white) and bird and bat net (black).

R.J. Nissen



**Figure 10.** Fruit fly trap catches in stone fruit in 2001.

### Particle film technologies

A new kaolinite product Surround™ has been developed in the USA for protecting fruit crops from heat stress, sunburn and frost. This product can also be used as a biopesticide. Due to its ability to reduce heat stress on the tree, Surround™ can improve photosynthesis, fruit size and fruit set. It can also improve fruit colour due to a greater proportion of transmitted and diffuse light within the canopy. In the USA, it is reported to control leafrollers and leafhoppers and to suppress mites, codling moth, plum curculio, apple sucker, stinkbugs, apple maggot and thrips. Surround® is sprayed on as a liquid, which evaporates, leaving a protective powdery film on the surfaces of leaves, stems, and fruit. Conventional spray equipment can be used and full coverage is important. The film works to deter insects in several ways. Tiny particles of the clay attach to the insects when they contact the tree, agitating and repelling them. Even if particles do not attach to their bodies, the insects find the coated plant or fruit unsuitable for feeding and egg-laying. In addition, the highly reflective white coating makes the tree less recognisable as a host (Dufour, 2001; McBride, 2000).

Trials with Surround® in Israel on nectarines have shown that female Mediterranean fruit flies avoided landing on treated fruits and no infestations occurred (Mazor and Erez, 2004). Similarly, Saour and Makee (2004) found kaolin film effectively controlled olive fruit fly (*Bactrocera oleae*) in olives. Results showed successful season-long suppression

of *B. oleae* compared to insecticide sprays of dimethoate that failed to protect olives for the same period after the last spray application (Saour and Makee, 2004). Due to the completely dry fruit development period in Thailand, Surround® may act as a highly effective protectant of temperate fruits. Surround® can be washed off by heavy rain and has to be reapplied, so its usefulness in high rainfall regions is limited, but in areas of low rainfall this technology would be highly applicable and beneficial.

### Economic evaluation

New innovative production systems for stone fruit tested at Maroochy Research Station were evaluated for their economic viability using economic analysis programs. Market prices are highly volatile. Variation between seasons and within seasons significantly affects profitability so price data was collated from years 1996 to 2000 and averaged, then used in the analysis. Industry standard training systems were evaluated for a non-netted and netted orchards (Table 3).

**Table 3.** Comparison of tree number per hectare under different training systems.

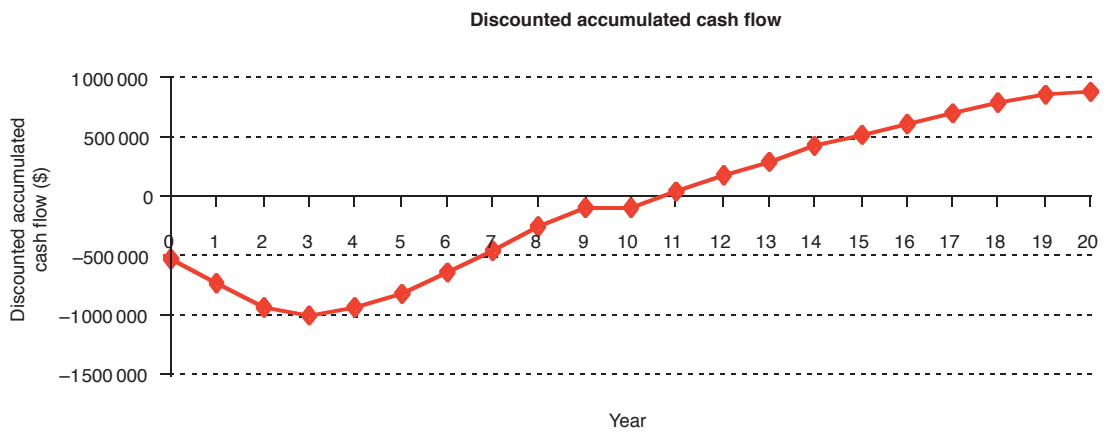
Training system	Tree spacing (m)	Tree number per hectare
Tight vase	3	833
Palmette	3	1111
Open Tatura	1	2222

Development, fixed, variable and capital costs were adjusted for each training system and a discounted accumulated cash flow generated.

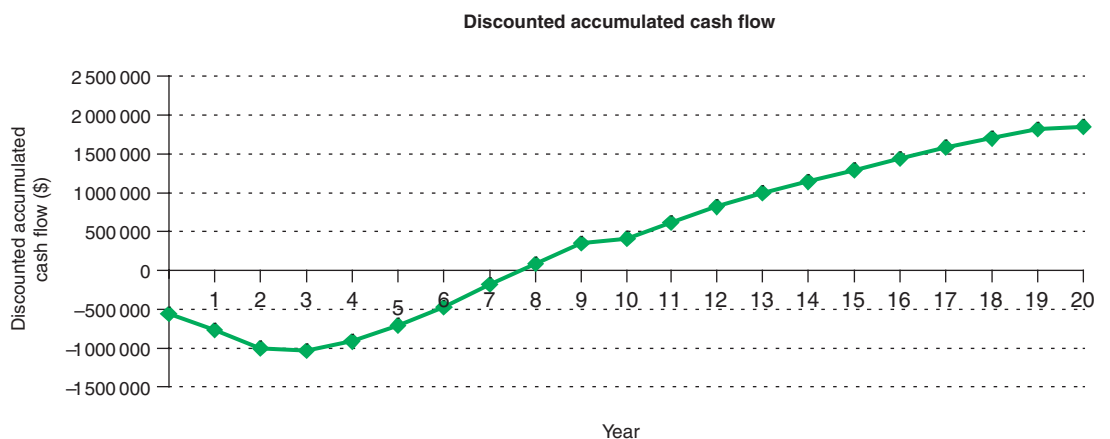
Our findings show that non-netted orchards will be non-viable. The greatest impact on the break-even point is the number of trees planted per hectare. This is due to increases in productivity (yield). Comparing total exclusion-netted orchards under an open Tatura system to a tight vase system, the break-even point is advanced by about three years. Comparing a total exclusion netted orchard under an open Tatura system to a palmette system, the break-even point is advanced by four years (Figs 11 and 12).

An increase in market access has not been accounted for in this economic analysis. A significant increase in the number of potential markets, due to fruit fly freedom status, is a major benefit of utilising total exclusion netting. Also, significant benefits due to decreased use of pesticides, providing consumers with a clean green product may realise increased returns.

In conclusion, this analysis of total exclusion netting has shown that the extra costs associated with enclosing a stone fruit orchard under such a netting structure did not reduce its viability. To recover the higher cost of a total exclusion netting system, growers must use high-density, high-yielding plantings such as open Tatura and best management practices.



**Figure 11.** Discounted accumulated cash flow of palmette system under total exclusion netting.



**Figure 12.** Discounted accumulated cash flow under total exclusion netting for open Tatura stone fruit orchard.

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# Breeding of low-chill peach cultivars under plastic to achieve early-season production

Maneethon Sutasinee, Naoko Kozai, Kenji Beppu and Ikuo Kataoka<sup>1</sup>

## Abstract

Growing peaches under plastic increases their marketability in Japan. For high-chill domestic cultivars, growers start heating after the accumulation of 900 hours of chilling. To commence forcing earlier, a low-chill trait is essential. In this paper we report our observations on the growth of low-chill peach under plastic and preliminary trials on the breeding of low-chill cultivars. Under forced conditions, rooted cuttings of low-chill cultivars 'Fukushu', 'Premier', 'Okinawa' and 'Tsukuba No.1', performed well even at 450 chilling hours (CH). We compared the initial growth of potted plants of 'Premier' and 'Hakuhou' grafted on low-chill 'Newbelle' (150 CU) and high-chill 'O'Henry' (800 CU) seedling rootstock. Even at 450 CH, bud break of 'Premier' started 10 days after heating commenced. The rate of leaf bud break on 'Newbelle' rootstock was higher than that on 'O'Henry' rootstock. In 'Hakuhou', bud break was delayed and sporadic. 'Hakuhou' grafted on 'Newbelle' peach showed a slightly earlier and higher percentage of bud break compared with 'O'Henry' rootstock and the percentage of flowering on 'Newbelle' rootstock was also slightly higher. The seedlings from crosses between high-chill 'Hakuhou' and low-chill 'Flordaprince', 'Flordaglo', 'TropicSnow', 'EarliGrande' and 'Red Angkhang' had a relatively lower chilling requirement and flowered 1–4 weeks earlier than 'Hakuhou' in the field. The fruit of some seedlings had relatively high total soluble solid (TSS), above 12%, but weight was less than 100 g.

## Introduction

In 2001, the total area of peach trees planted in Japan was 10 600 ha, producing 175 000 t of fruit. Peaches are grown in all parts of the country, except Hokkaido. The leading prefecture is Yamanashi, followed by Fukushima, Nagano and Yamagata. In the west of the country, Wakayama is the leading producer, followed by Okayama and Kagawa (Fig. 1).

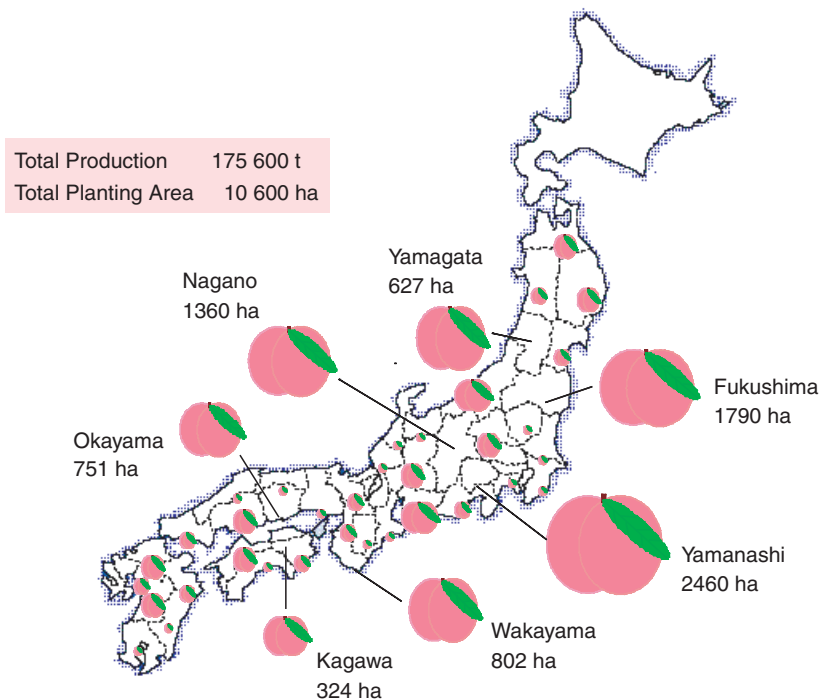
Major cultivars are listed in Table 1. The number of days required for ripening ranges from 70 to 130 days. Early cultivars such as 'Chiyohime' and 'Takei Hakuhou' ripen 70–80 days after flowering and are harvested in late June. The late-harvest cultivar 'Hakuto' appears in the market in mid- to late-August. Generally, fruit are as large as 200–250 g with relatively high TSS (12–14%). New cultivars are mostly derived from 'Hakuhou' and 'Hakuto', as these have excellent fruit quality with high TSS. In recent years, 'Akatsuki' as a mid-season cultivar and 'Kawanakajima Hakuto' as a late-season cultivar are becoming popular and the area

planted to these cultivars is increasing rapidly. Most Japanese peach cultivars have skin blush and white flesh. In some cultivars, such as 'Hakuto' or 'Shimizu Hakuto', fruit are continuously bagged during ripening and kept whitish yellow until harvest. Most of the cultivars have melting flesh and a short shelf life.

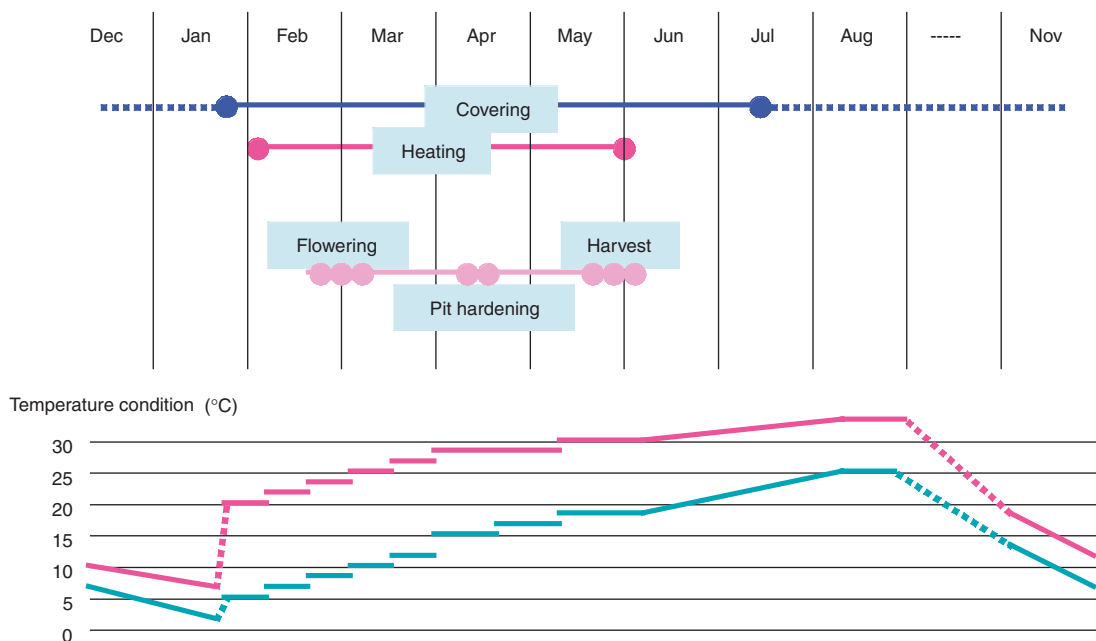
Under natural conditions, starting with early cultivars, the harvest period lasts only 2 months until late August or early September. With many fruit coming onto the market, the price falls rapidly. Consequently, the advantages of growing under plastic are extending the harvest period and obtaining a high price on the early market.

Figure 2 (Kagawa Prefecture, 1998) shows the steps in growing 'Hikawa Hakuhou', an early-ripening cultivar, under plastic. Trees are covered with plastic at the end of January and 1 week later heating starts. Initially, the air temperature is kept at 20°C maximum and 5°C minimum, then it is raised one degree every 10 days up to 28°C, until pit hardening. By early March, the trees are in full bloom. Fruit are harvested from late May to early June. After harvest, heating is stopped, the cover is removed in mid-July and trees are returned to natural conditions.

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**Figure 1.** Peach production in Japan (Agricultural statistics 2001).



**Figure 2.** Conditions of forcing culture of 'Hikawa Hakuhou' peach in Kagawa.

**Table 1.** Major peach cultivars in Japan.

Days for ripening	Cultivar	Fruit weight (g)	TSS (%)	Planting area (ha)
71–80	Chiyohime	180	10–11	72
	Takei Hakuhou	220	10	252
81–90	Hikawa Hakuhou	220	11–12	790
91–100	Kanoiwa Hakuhou	260	11–13	190
	Yahata Hakuhou	200	11–12	300
101–110	Hakuhou	230	12–13	1871
	Akatsuki	230	12–14	1376
	Asama Hakuto	250	12–13	443
	Shimizu Hakuto	250	12–13	438
	Ohkubo	250	11–12	460
	Chikuma	250	12–13	107
111–120	Nagasawa Hakuhou	280	12–13	344
	Kawanakajima Hakuto	300	12–14	1122
121–130	Hakuto	300	12–13	124

Forcing early ripening in this way extends the season of high-chill cultivars one month before that of early-season cultivars under natural conditions. However, it is difficult to achieve more advanced harvest and obtain higher marketability for these cultivars as growers cannot start heating until the chilling requirement is satisfied. A way to extend the season could be to cultivate low-chill peaches using this method. In this report, we review the growth of low-chill peaches under plastic and preliminary trials on breeding low-chill cultivars for this type of cultivation.

## Materials and methods

### Experiment 1. Growth habit of low-chill peach grown under plastic

Two-year-old own rooted plants of low-chill cultivars 'Fukushu' (ex Taiwan), 'Premier', Okinawa, Tukuba No.1. and high-chill cultivar 'Hakuto' were used for experiments. Plants were transferred from the field to a glasshouse kept above 20°C after receiving 450 and 900 chilling hours (CH). Dates and percentage of floral and vegetative bud break were recorded.

### Experiment 2. Effect of rootstocks with different chilling requirements

Two-year-old grafted plants of high-chill cultivar 'Hakuhou' (900 CU) and low-chill cultivar 'Premier' (150 CU) on either high-chill 'O'Henry' (900 CU) or low-chill 'Newbelle' (150 CU) rootstocks were used. They were forced at 20°C after receiving 350 and 700 hours of chilling, or left under natural conditions. Dates and percentage of floral and vegetative bud break were recorded.

### Experiment 3. Character of seedlings from crosses between high-chill 'Hakuhou' and several low-chill cultivars

'Hakahou' as pollen parent was hybridised to several low-chill cultivars: 'Flordaprince', 'Flordaglo', 'Earli-Grande', 'TropicSnow' and 'Red Angkhang'. Two-year-old seedlings of these crosses were used. Under field conditions, flowering time and fruit characters were observed. The percentage bud break of shoot cuttings collected after accumulation of 350 CH and 700 CH, was recorded. Cuttings were held at 25°C.

## Results and discussion

### Experiment 1

Even after 450 hours of chilling, flower and leaf bud break started immediately after the start of forcing in all low-chill cultivars. In 'Okinawa' and 'Fukushu' leaf bud break slightly preceded flower bud break. In contrast, there was little or no bud break with high-chill Japanese peach 'Hakuto' with this amount of chilling (Fig. 3). Flowering started 18 days after forcing and reached full bloom 25 days after chilling. Under natural conditions in the field with 1400 hours of chilling, low-chill cultivars break almost 1 month earlier than high-chill varieties. Low-chill cultivars flowered 3 weeks earlier than high-chill cultivars (data not shown).

Low-chill cultivars used in this study showed a high percentage of bursting even at 450 CH and they started flowering within 4 weeks after heating began. By using these low-chill cultivars it should be feasible to start heating much earlier. It usually takes 1 month to reach full bloom after heating starts when Japanese high-chill cultivars are forced after



receiving 900 chilling hours. In this experiment, low-chill cultivars reached full bloom within 2 weeks after heating. This implies a higher sensitivity to temperature for flowering, and a possibility to reduce production costs by lowering the forcing temperature.

### Experiment 2

After 450 hours of chilling, the low-chill scion 'Premier' broke immediately after forcing, reaching

almost 100% bud break. Flower bud break was not affected by the rootstock, whereas leaf bud break was enhanced with low-chill rootstock. With the high-chill scion 'Hakuhou', bud break occurred 40 days after forcing, at a rate considerably lower than that of the low-chill scion. In the high-chill scion, both flower and leaf bud break were enhanced by the low-chill rootstock (Fig. 4). In the low-chill scion, flowering occurred 20–25 days after forcing and was

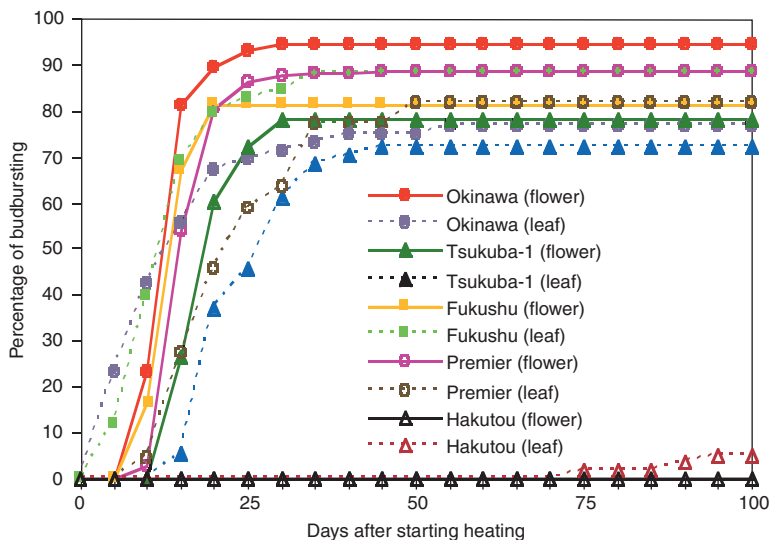


Figure 3. Bud burst of low- and high-chill peach cultivars at 450 CH.

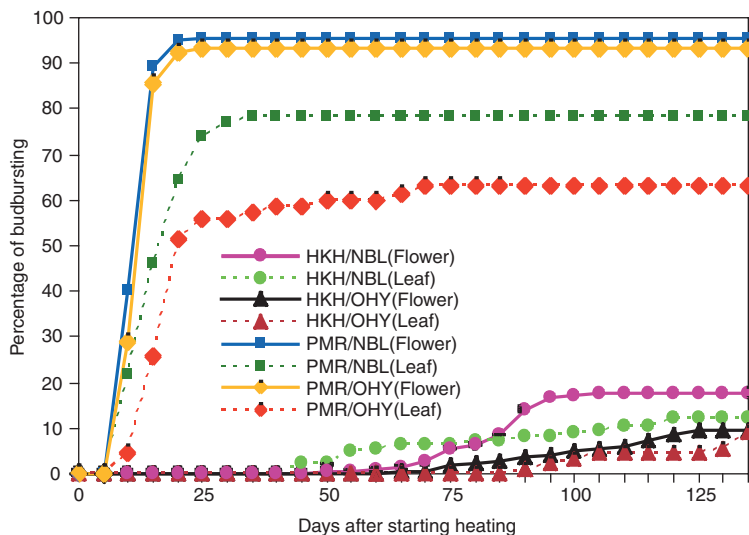


Figure 4. Bud burst of low-chill 'Premier' and high-chill 'Hakuhou' grafted on the low-chill 'Newbelle' and high-chill 'O'Henry' seedling rootstocks.

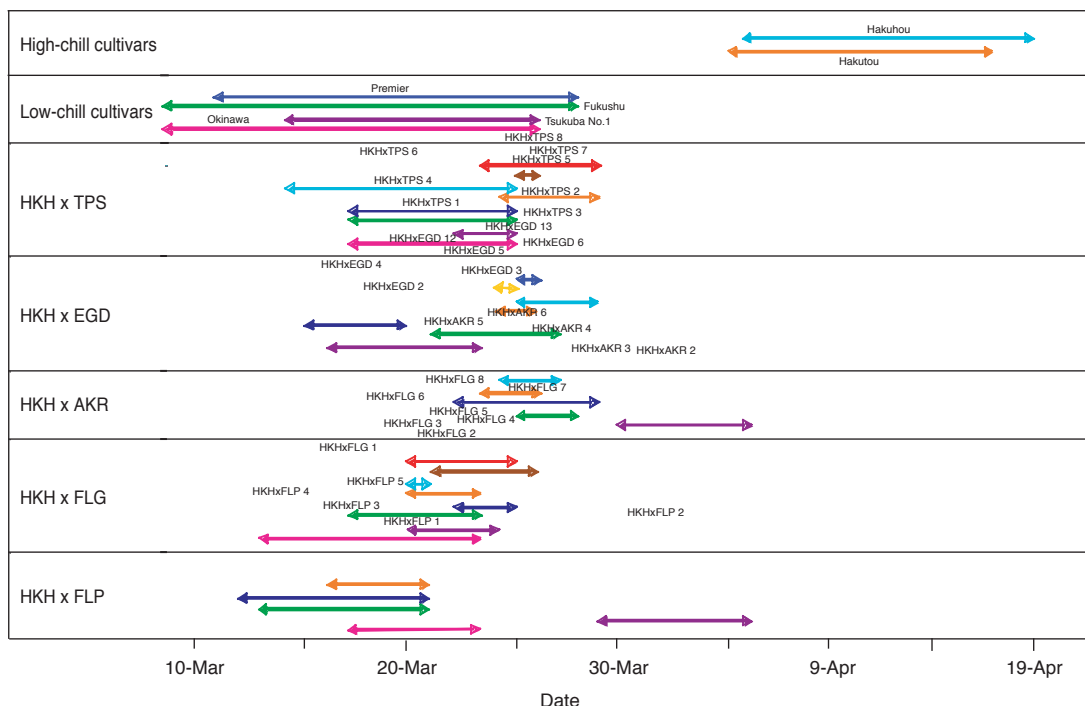
not affected by rootstock. With the high-chill scion, flowering on the low-chill rootstock preceded that on the high-chill rootstock. Under natural conditions, the low-chill scion ‘Premier’ broke much earlier than the high-chill scion ‘Hakuhou’. In both cultivars, flower bud break preceded leaf bud break. The rate of bud break was as high as 75% in both scion and rootstock combinations. Under natural conditions, the low-chill scion flowered about 1 month earlier than the high-chill scion. The chilling of rootstock did not affect the timing of flowering (data not shown).

George and Erez (2000) suggested that rootstock may influence time of bud break in three ways: (1) by having a vigour control effect which indirectly affects bud dormancy; (2) by having a direct effect through earlier root development and movement of growth regulators; (3) by having a chilling requirement that needs to be satisfied in the same way as the chilling requirement of the scion cultivar. They pointed out that in Australia low-chill rootstocks, such as ‘Nemasun’ and ‘Okinawa’, must be used in regions receiving <400 chill units to avoid delayed and sporadic bud break problems. In this experiment, bud break of the high-chill scion was slightly enhanced by the low-chill rootstock under low-chill conditions. However, the rootstock effect was not

observed under other conditions. This suggests that low-chill rootstock helps promote bud break when chilling conditions are insufficient for the scion but has little effect on bud break when chilling requirements are fulfilled. Young and Werner (1984) reported that low temperature treatment on rootstock promoted bud break of apple but not peach. Kataoka and Yamamoto (1995) observed that root growth of ‘Yahata Hakuhou’ trees grafted on high-chill seedling rootstock occurred even under low-chill conditions at 350 CH. The promoting effect of low-chill rootstock may be attributable to the sensitivity of rootstock to root temperature rather than the chilling requirement of rootstock.

### Experiment 3

After 350 hours of chilling, low-chill cultivars broke 6–12 days after heating began, whereas high-chill ‘Hakuhou’ and ‘Hakuto’ were not affected. In contrast, most of the seedlings grown from crosses between ‘Hakuhou’ and low-chill cultivars showed high bud break immediately after forcing. Most of these seedling crosses flowered 2–3 weeks earlier than high-chill cultivars such as ‘Hakuto’ and ‘Hakuhou’ and were close to the flowering time of low-chill cultivars such as ‘Premier’ (Fig. 5).



**Figure 5.** Flowering period of cross seedlings of high-chill ‘Hakuhou’ with low-chill cultivars.

**Table 2.** Fruit character of seedlings from crosses of high-chill ‘Hakuhou’ with low-chill cultivars under natural conditions.

Cross seedling	Weight (g)	Fruit size (cm)			TSS (%)	Harvest date
		Length	Suture diam.	Cheek diam.		
HKHXFLG1	64.2	4.7	5	5	8.2	12 June
HKHXFLG4	60.3	4.8	5	4.5	12.5	12 June
HKHXFLG5	55.2	4.9	4.7	4.3	13.2	12–14 June
HKHXFLG7	75.1	5	5.2	4.9	13.8	2 July
HKHXFLG8	57.7	4.6	4.9	4.7	15.9	25 July
HKHXFLP1	55.8	4.5	4.8	4.8	8.9	12–14 June
HKHXFLP3	74.2	5	5.3	5.3	10.2	12–14 June
HKHXFLP4	159.8	6.3	6.9	6.9	11	25 July
HKHXFLP5	88.3	5.2	5.5	5.7	10	3 July
HKHXEGD2	89.4	5.2	5.5	5.4	12	7 July
HKHXEGD3	96	5.1	5.8	5.8	11.9	2 July
HKHTPS3	62.2	4.5	4.8	4.8	13.4	2 July
HKHXTPS4	71.1	5	5.1	4.8	12.8	2 July
HKHXTPS5	78.7	5.1	5.4	5.2	13.4	25 July
HKHXTPS6	84.5	4.9	5.7	5.5	13.3	25 July
HKHXTPS8	172.2	6.7	7	6.9	12.8	25 July
HKHXAKR6	84.8	5.4	5.2	5.3	14.5	25 July

Progenies of ‘Flordaglo’ and ‘Flordaprince’ produced fruits with red skin and white flesh. Most of the seedlings of a cross with ‘TropicSnow’ produced fruit with slightly blushed or white skin. Some of the fruits had relatively high TSS above 12%, but the fruit was quite small (Table 2).

The low-chill trait seems useful for advancing maturity under protected culture as well, extending the growing area of peach under tropical and subtropical conditions (Sherman and Rodriguez, 1987; Byrne and Bacon, 1989; Byrne et al., 2000) In this preliminary trial we were able to demonstrate the possibility of attaining a lower-chilling trait with high TSS and melting white flesh by crossing high-chill Japanese cultivars with low-chill cultivars. Now, we are evaluating the performance of seedlings from a cross between a low-chill cultivar and a high-chill Japanese cultivar to obtain low-chill, high-quality peach suitable for growing under plastic.

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# Breeding low-chill stone fruit in Thailand

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## *Abstract*

Historically, opium under wild cultivation in highland areas of northern Thailand, was a threat to national security. In addition, shifting cultivation in hilly topography speeded up environmental degradation. Temperate fruits were introduced into the highland regions of northern Thailand for three reasons: to replace opium crops; because they suited the climate and to conserve and maintain the ecology. Stone fruits, particularly peach, have shown great domestic and export potential due to their non-competitive marketing opportunity. However, only a few obsolete varieties were being cultivated, resulting in limited production areas. So, a collaborative stone fruit breeding program with Texas A&M University was initiated in 1997 to develop new varieties suitable for commercial production in the highland areas of northern Thailand. Several new varieties and advanced selections have been evaluated in the Thai climate and some have been selected for future commercial planting. Thousands of hybrids were planted annually with several objectives, such as adaptability to subtropical highland climate; extending harvesting seasons from February to May; improving fruit qualities, particularly sugar content; and disease resistance, particularly leaf spot and rust. Progress is reported in this paper.

## **Introduction**

AGRICULTURAL research activities of the Royal Project Foundation have focused on temperate fruit crops (Subhadrabandhu and Punsri, 1987). The logical reasons for this were: (1) temperate fruit trees could be cultivated economically as a substitute crop to opium poppy; (2) the highland climate is to some extent conducive to the production of temperate fruit crops; (3) fruit tree production is agriculturally friendly to the environment of the highlands; and (4) cultivation of fruit trees could replace the slash and burn agriculture commonly used by highlanders. The proposed temperate fruits were pome fruit, such as apples, pears and quince, and stone fruit, such as peaches, nectarines, almonds, Japanese apricots, plums and cherries.

In 1965 imported peach budwood was grafted onto native peach trees in the highlands. The objective was to replace inferior fruit qualities of native peach with superior ones of the imported cultivars.

In the following years, several types and cultivars of stone fruit were tested and evaluated in the highlands of Thailand. These included 87 peach cultivars, 12 nectarine cultivars, 13 plum cultivars, 14 almond cultivars, 14 apricot cultivars, three cultivars of Japanese apricots and five cultivars of sweet cherry. After several years of multi-location evaluation, the results indicated that most highland areas in northern Thailand, with an altitude over 1000 m had a climate suitable for some stone fruits such as peaches, Japanese plums and Japanese apricots.

Continuing research activities have contributed to successful commercial production of peaches, Japanese plums and Japanese apricots. Annual production of these fruit crops was approximately 130, 160 and 800 metric ton, respectively. Nevertheless, a steady increase in production over previous years seems to have leveled off in recent times. The limitation for greater production is due to several factors, depending on the type of crop. However, the main limiting factor for stone fruit production is the lack of cultivars and the inferior quality of the existing cultivars.

In peach, there are only three commercial cultivars: 'EarliGrande', 'Flordabelle' and 'Flordaprince'. These cultivars are so obsolete that they have not been used commercially anywhere for a long time. Fruit qualities, particularly the low firmness, have

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contributed to high yield loss during harvesting, packing and transportation. The harvest season for peach is very short — from early to late April. In plums, ‘Gulfruby’ and a couple of Taiwanese selections are the leading cultivars. These cultivars produce very small fruit with a strong sour flavor if not fully ripened. Harvesting at full ripening is impossible due to flesh softening. In Japanese apricots, there are only a couple of Taiwanese selections but fruit size is too small by processors’ standard.

These problems could not be solved through research into cultural practices alone because genetic composition of fruit trees plays a major role in determining fruit quality and adaptation. A breeding program, aimed at developing new cultivars with suitable adaptation and superior fruit quality, needs to be conducted to solve these problems. With genetically improved cultivars and a greater range of cultivars to choose from, expansion of production could be resumed. Then the benefits from growing temperate fruit tree crops could spread to more people living in the highlands.

The stone fruit improvement program was initiated in 1997 by the Royal Project Foundation and Kasetsart University in collaboration with Texas A&M Stonefruit Breeding Program. There were several key objectives: (1) adaptability to abiotic stress such as mild winter climate, acidic soil and drought, and to biotic stress such as rust and shot hole diseases; (2) improving fruit qualities such as flavor, aroma and attractiveness and (3) diversity of products such as extending harvesting period, novelty traits, and processing type.

## Materials and methods

### Testing low-chill cultivars and selections

Available cultivars and selections were introduced for local testing. These clones were mainly from three sources: Texas A&M Stonefruit Breeding program (TAMU), University of Florida Stone Fruit Breeding Program (UF), and the Brazilian program (BZ). Thirty-one clones ranging in chilling requirement (CR) between 150–450 chilling units (CU) were from TAMU; 13 clones ranging between 150–450 CU were from UF; three clones were jointly released cultivars (‘TropicBeauty’, ‘TropicSnow’ and ‘Tropicsweet’) from these two programs; and 17 clones ranging between 150–600 CU were from BZ.

These clones were either budded or grafted on native peach as a seedling rootstock. Two to four trees of each clone were then planted in experimental plots at two locations: Royal Angkhang Agricultural Station, Chiang Mai (N19° 54.51', E99° 2.58' altitude 1400 m) and Chiang Mai Royal Agricultural

Research Center at Khunwang, Chiang Mai (N18°, E98° altitude 1100 m). In winter, Angkhang had an average minimum temperature of 5.4°C and maximum temperature of 21.1°C, while Khunwang had an average minimum temperature of 11.9°C and maximum temperature of 17.9°C. Temperatures during fruit growth at Angkhang were between 7.1°C and 26.2°C and at Khunwang were between 18.3°C and 22.6°C. Average annual precipitation at Angkhang was 2000 mm and at Khunwang it was 1700 mm. Based on climatic data, clones with CR greater than 450 CU were not planted at Khunwang. Trees were planted at 4 × 4 m spacing and trained to an open centre system. Completely randomised design with single-tree replication was carried out.

### Hybridisation to create new genotypes

Each year during November to February, several controlled pollinations were done at both locations to create new hybrids. Low-chill clones, that showed promising adaptability from the testing program, were selected as female parents. Some tested clones, with marginal adaptability but possessing interesting traits such as non-melting flesh or subacid flavor, might be used as pollen parents. Other sources of pollen parents were provided by collaborating partners. These were Texas A&M Stonefruit Breeding Program, several Japanese institutes, and Beijing Institute of Forestry and Pomology.

## Results and discussion

### Testing low-chill cultivars and selections

*Performance of clones from TAMU.* For adaptability evaluation, most TAMU clones had a CR between 150–250 CU except for a couple of selections which had a CR of about 450 CU. Very low-chill clones (less than 200 CU) flowered intensely at both testing locations; however, flowering and leafing were more uniform and concentrated for the trees at Angkhang. Tree growth, in terms of trunk diameter and canopy size, was moderate to vigorous. Most clones could be adequately cultivated at a spacing of 4 × 4 m or 4 × 5 m for the open centre training system. None of the tested clones from TAMU showed any resistance to either rust or shothole. Tree yields ranged from moderate to high with about 20–40 kg per tree. The yield per tree is equivalent to about 12.5–25 metric ton per hectare. Harvesting began in late March and lasted to early May. Fruit size ranged from small (80 g) to medium (150 g). As expected, earlier harvested clones produced lower yield and smaller fruit than the later harvested clones. Other prominent fruit qualities were yellow flesh, firmness, good shape (round to flat-round), high colour, less fuzz and high

acidity. Currently, four advanced selections are in the process of joint release with Texas A&M Stonefruit Breeding program.

**Performance of clones from UF.** For adaptability evaluation, most UF clones had a CR of between 150–450 CU. The lowest CR was found with ‘Flordaglo’. Flower and leaf buds of UF clones were well formed. Several nectarine cultivars (‘Sunblaze’, ‘Suncoast’, ‘Sungem’, ‘Sunraycer’ and ‘SunWright’), regardless of their CR, showed high flower bud density. Clones with CR less than 200 CU produced more uniform and intense blooming and leafing. Tree growth was similar in degree with TAMU clones. Yields were low to moderate with about 10–25 kg per tree (6.2–15.6 metric ton per ha). Harvesting season was similar to TAMU clones. The distinctive traits of UF clones were related to fruit quality. UF clones tended to produce soft to very soft fruit, prominent suture and apex, high fuzz and less color. Poor qualities such as low firmness and difficult-to-pack shape would contribute to less commercial yield. None of the UF clones was recommended for planting in the highlands of northern Thailand. However, some were used in the early phase of the breeding program.

**Performance of cultivars jointly released from TAMU and UF.** These cultivars were originally from UF germplasm and underwent evaluation and selection under south Texas conditions where the climate was mild in winter and very hot in summer. The climatic condition resulted in selected clones that were adapted to very low-chill regions and maintained acceptable fruit qualities, particularly fruit shape. Performance of these cultivars in the highlands of northern Thailand was comparable to TAMU clones. The best performer was ‘TropicBeauty’ (Fig. 1) which could be used to replace the obsolete cultivars presently cultivated.

Credit line to come



**Figure 1.** ‘TropicBeauty’ peach; yellow flesh, 70% blush, medium size, round, semi-free, firm, ripens in mid April.

**Performance of clones from BZ.** For adaptability evaluation, most clones had higher CR (ranging between 300–500 CR) than either TAMU or UF clones. The exceptions were ‘Premier’ and ‘Diamante’ which had about 150 CU. Flower and leaf buds were well formed except ‘Pilcha’. Tree growth was moderate to vigorous, therefore, suggested spacing for commercial production was 4 × 6 m. A few clones, particularly ‘Jade’, showed moderate tolerance to shot hole and rust. Yield was moderate to high with about 20–40 kg of fruit per tree. The harvest season was later than for cultivars from TAMU and UF, beginning from late May and lasting to early June. Fruit size was medium (140–160 g) with round to oblong shape. Prominent suture and apex were common for most BZ clones. Because some BZ clones included cultivars for processing, we observed that many exhibited the non-melting flesh trait. The best performer was ‘Jade’ (Fig. 2), which is being tested at other locations.

### Hybridisation to create new genotypes

Several low-chill clones that were well adapted to the tropical highlands of Thailand were chosen for crosses. Traits that should be found among selected parents were good shape (round to oval with no prominent suture and apex) and good firmness (greater than 20 N). These clones were mainly selections from TAMU. Some marginally adapted clones possessing interesting traits such as non-melting flesh, sub-acid flavor and late harvesting season were used in crosses with well adapted clones.

Pollens of Japanese cultivars were introduced for crosses with low-chill clones. Intended traits for incorporation from Japanese sources were large fruit size (200–300 g), low acid and high soluble solid (12–14°Brix). Because all Japanese cultivars had

Credit line to come



**Figure 2.** ‘Jade’ peach; yellow flesh, large size, round, cling, non-melting, ripens in early May.

very high CR (greater than 1000 CU), hybrid seedlings resulting from these crosses must go through a couple of cycles of selfing and backcross to low-chill parents in order to recover the low CR trait.

Pollens of Chinese cultivars were also introduced for crosses with low-chill clones. Traits of interest were honey peach, melting firm flesh, large size (greater than 300 g) and saucer shape. With the exception of Peen Tao (saucer shape), which had a low to medium CR, all Chinese cultivars had comparable levels of CR to the Japanese varieties. Therefore, hybrids of Chinese cultivars would follow similar breeding strategies to those of Japanese cultivars.

About 30 hybrids have been selected for further evaluation. In order to speed up the selection process, budwood of selected progeny was propagated for evaluation at Angkhang and Khunwang. It was planned that evaluation would be done over 3 years at both locations in order to screen for any advanced selections.

## Conclusion

Testing several low-chill peaches from different sources revealed that TAMU clones were better adapted to the highlands of northern Thailand. Useful traits were found in other sources and these were incorporated through the breeding process. 'TropicBeauty' was introduced as a replacement for obsolete cultivars. Four advanced selections were in the process of joint release with Texas A&M Stonefruit Breeding program. Once available, these clones would extend the harvest season for as long as 2 months from mid March to mid May.

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# Gene expression of water channels on 'Kosui' Japanese pears treated by hydrogen cyanamide

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## Abstract

Dormancy has been defined as acclimatisation to cold climate as well as hardening. The water content of plant parts, ie buds, decreases slightly after induction of dormancy, from autumn into winter and thereafter increases conspicuously towards spring. Endo-dormancy is broken by chilling temperatures in January in most regions of Japan. As previously reported, cyanamide (range of 0.5–1%) has been shown to be effective in breaking dormancy of some deciduous fruit trees. Interestingly, the efficacy of cyanamide varies with the position of buds on the dormant shoot. Water movement is implicated in the dormancy-breaking process, as postulated by Faust et al. (1997). We measured changes in membrane permeability and dehydration in the tissue of dormant buds of 'Kosui' Japanese pears and compared the gene expression of water channels of cyanamide-treated shoots with controls. The RNA, extracted from the dormant buds at different times, was used for semi-quantitative RT-PCR using PIP1 ( plasma membrane intrinsic protein) specific primers. The expression of the water channel gene was stronger in control shoots and the buds located lower down the shoot, irrespective of the cyanamide treatment. It is suggested that a decrease in the gene expression of the water channel could therefore be related to bud break. The effects of lack of chilling on bud dormancy will also be discussed in terms of the expression of this gene during the induction of dormancy.

## Introduction

BECAUSE they lack the ability to move freely, plants must adapt themselves to different environments. So far, the genes responsible for response to some environmental stresses such as drought, cold and salinity have been found but further studies on function of these genes need to be conducted. As previously reported (Gemma, 2002), hydrogen cyanamide is supposed to be a potential agent to break dormancy in deciduous fruit trees grown in regions with a mild winter climate. However, this chemical is not absolutely effective at breaking profound dormancy, ie endo-dormancy. A study on developing quantitative analysis of stress-induced genes will be available to elucidate gene function on regulatory mechanisms against stresses. These findings will be applied to develop methods to control bud dormancy. Recently, cDNA microarray analysis

has been used to analyse the response of *Arabidopsis* to stresses such as cold (Seki et al., 2002). They reported that water channel protein as a functional category was up-regulated by cold stress, being encoded by the RD28 gene. Therefore, this gene was supposed to be one of the cold stress-inducible genes.

This study was conducted on Japanese pear treated with hydrogen cyanamide and cold privation to evaluate water channel expression of dormant buds at different states of dormancy. The aim is to develop techniques to control or manipulate bud dormancy.

## Materials and methods

### Changes of water channel gene expression during endo-dormancy and effects of temperature

'Kosui' Japanese pears were grown in the experimental orchard at the University of Tsukuba. Dormant shoots about 1 m long were taken from 'Kosui' trees on September 30 2003. The base of each shoot was submerged in water as described previously (Gemma, 2002), and exposed to different tempera-

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tures for one and half months. The following treatments were applied: (1) ambient temperature as the control; (2) 25°C as cold privation; and (3) 4°C as chilling treatment. Table 1 shows the different chilling requirement for each treated shoot on different dates when dormant buds at different positions were sampled for the further gene expression survey irrespective of temperature treatment. As well, temperature-treated shoots were transferred to warm conditions (>10°C) and time of bud break was recorded.

**Table 1.** Chilling hours of each shoot exposed to different temperatures.

	September 30	October 14	October 30	November 14
Cont.	0	15 hrs	62 hrs	130 hrs
25°C	0	—	—	—
4°C	0	360 hrs	744 hrs	1104 hrs

RNA was isolated from the buds by CTAB based extraction method. The 0.5–1 g of buds was homogenised in liquid nitrogen with a mortar and pestle. The resulting powder was suspended in 5 ml of CTAB extraction buffer and 0.275 ml of 2-mercaptoethanol was added to the suspension which was incubated at 65°C for 10 min. The suspension was then purified twice with an equal volume of chloroform: isoamyl alcohol (24:1) solution. A quarter volume of 10 M LiCl was added to the supernatant and incubated at –20°C for 2 hours. After the centrifugation, the precipitate was dissolved in TE buffer and precipitated with ethanol. The precipitate was suspended with TE buffer and extracted with TE saturated phenol (pH 9.0), TE saturated phenol: chloroform: isoamyl alcohol, and chloroform: isoamyl alcohol. A quarter volume of 10 m LiCl was added to the supernatant to precipitate RNA. The RNA was suspended with an appropriate volume of TE buffer.

Water channel (PIP1) gene transcripts were detected by RT-PCR using an mRNA selective PCR kit (Takara, Kyoto, Japan) and southern blot hybridisation. A forward primer (PyPIP2: 5'-TGTTGCCCATGGTTA CCAAGGG-3') and a reverse primer (PyPIP2: 5'-AGATGATGGC AGCGCCAAGACTCC-3') were designed as gene specific primers for amplifying the PIP1 gene in Japanese pears. Total RNA (0.4 ug) was used for the RT-PCR. Products of the RT-PCR were separated by electrophoresis on 2% agarose gel and then transferred to Hybond-N<sup>+</sup> (Amersham) and cross-linked under UV light. A probe of JP-PIP1, was labeled with the DIG high-prime DNA labeling/detection kit II (Roshe Diagnostics). The membrane was hybridised at 42°C overnight, washed once with 2 × SSC containing 0.1% SDS for 15 min at 25°C

and once with a solution of 0.1 × SSC, 0.1% SDS for 30 min at 65°C. The substrate for alkaline-phosphate reaction was CSPD<sup>TM</sup> (Roshe Diagnostics). As a control RT-PCR reaction, forward 5'-GCCGAC CCAGTTCTCCTCAC-3' and reverse 5'-TCCTGT TCATAG TCAAGAGC-3' primers for the actin gene were designed, followed by RT-PCR and southern blot hybridisation.

### Effects of hydrogen cyanamide treatment on eco-dormancy, bud break and expression pattern of water channel gene

On January 15 2003, 1% cyanamide was painted on all the dormant buds on shoots of 1-year-old 'Kosui' grown in containers. Forcing in a greenhouse (>10°C) commenced 1 day later. The rate of bud break was recorded for buds located in different positions. Buds were sampled 5 and 22 days after each treatment from the upper part comprising terminal to fifth bud positions, the middle part from eighth to 14th bud positions and the lower part from 17th to 24th bud positions for gene expression analysis as well as changes of water.

### Results and discussion

As shown in Table 1, shoots subjected to ambient temperature under nature received insufficient chilling up to November 14 whereas those exposed to 4°C received sufficient chilling hours to break dormancy of 'Kosui' flower buds (700–800 h by October 30) and leaf buds (1000–1300 h by November 14). The expression of *JP-PIP1* in buds changed with time and varied between the different positions; upper parts with floral and leaf mixed bud, middle parts with leaf buds and lower parts with leaf buds (Fig. 1). Figure 2 shows the level of expression by relative amounts for the different parts. The control was consistent, in particular, for both the middle and lower parts. This corresponded with their dormant state, ie bud break was still not observed 2 months later (Table 2). In contrast, chilled shoots showed a drastic change in expression of *JP-PIP1* (Fig. 2). The decrease was especially remarkable on November 14. In fact, upper buds and lower buds burst 14 days and 20 days respectively after transfer into warm conditions (Table 2).

**Table 2.** Days to bud break after transfer to the greenhouse.

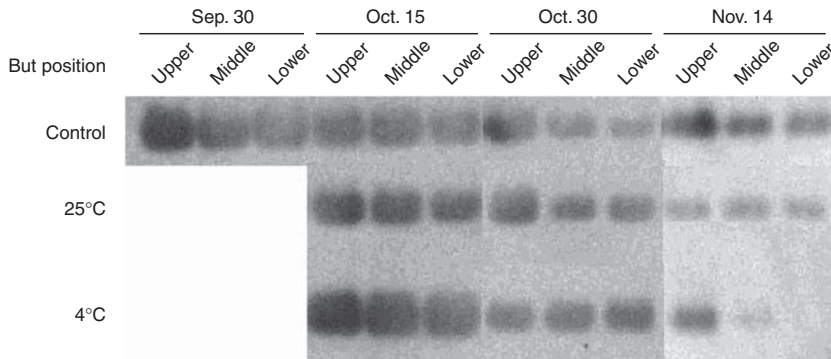
	Upper part	Middle part	Lower part
Cont.	30 days	—	—
25°C	—	—	—
4°C	14 days	—	20 days

Transfer was done on November 14, not determined even after 2 months.

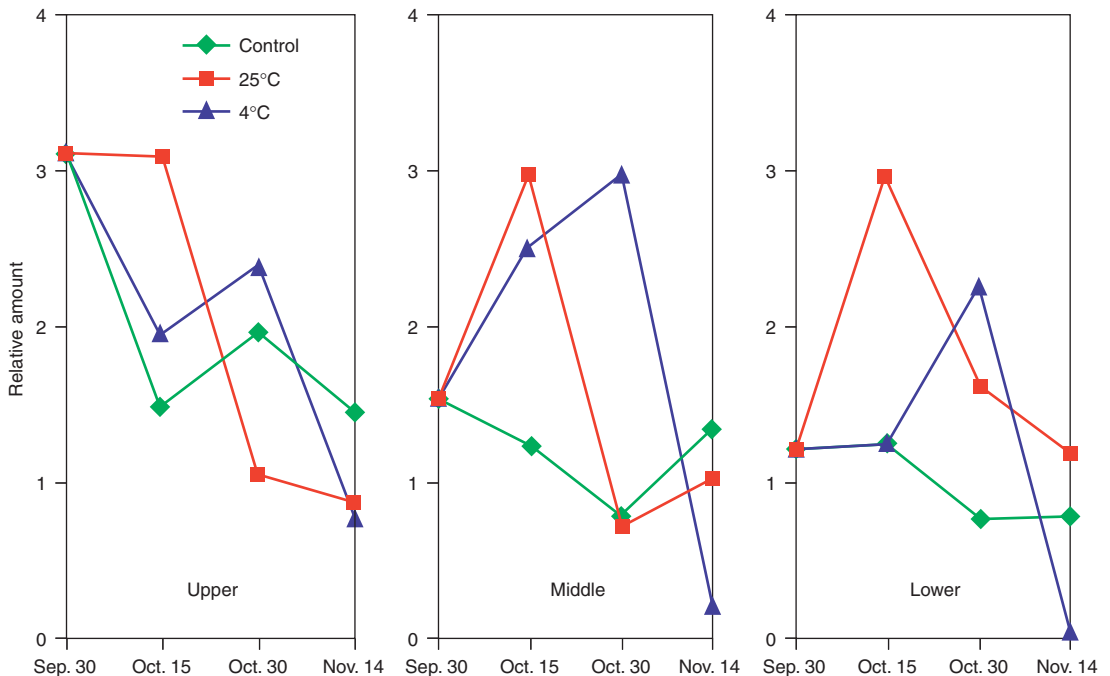
With other species, low temperature (cold stress) up-regulated the water channel gene in *Arabidopsis* (Seki et al., 2002) and plasma membrane aquaporins (*JrPIP2,1* and *JrPIP2,2*) were increased in walnut trees throughout autumn–winter (Sakr et al., 2003). Our finding is a contradictory result, but there were surges of expression of *JP-PIP1* in advance of bud break. Cold privation treated shoots showed a similar surge and decrease in expression of the water

channel gene even though there was a small time lag compared to the 4°C treatment. The reason for this is unclear.

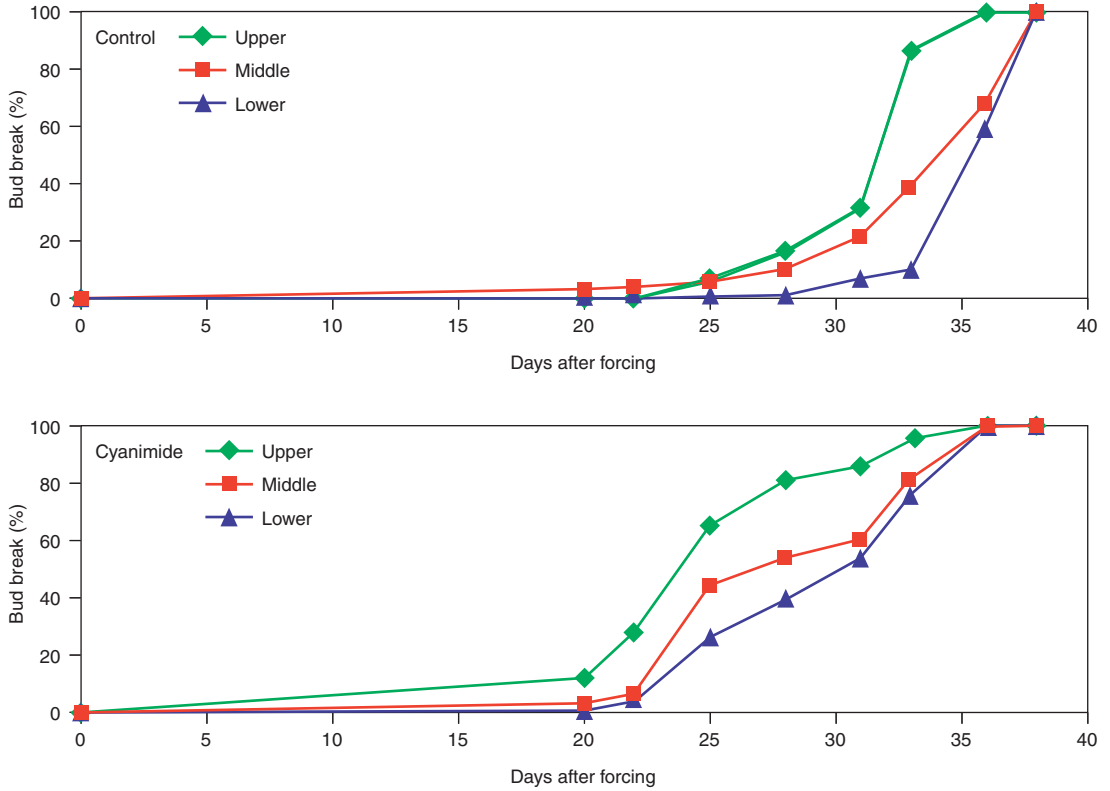
In the experiment using 1-year-old trees grown in containers, earlier bud break was observed for cyanamide-treated trees compared with controls. Bud break on the upper shoots was first followed by the middle and lower parts (Fig. 3). The expression level of *JP-PIP1* in cyanamide-treated buds was lower than



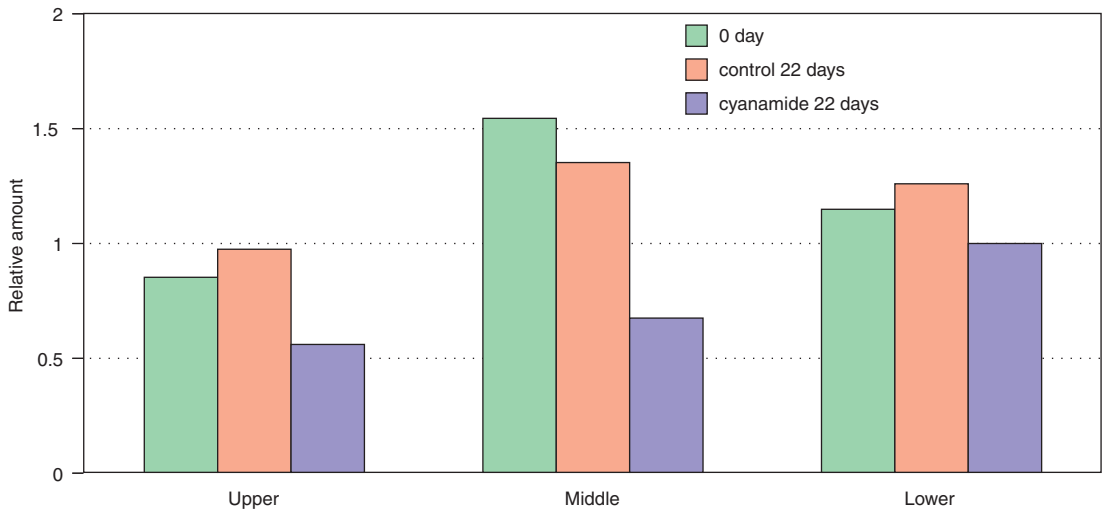
**Figure 1.** Expression level of *JP-PIP1* in ‘Kosui’ buds under various temperature conditions.



**Figure 2.** Expression level of *JP-PIP1* in ‘Kosui’ buds under various temperature conditions.



**Figure 3.** Effect of cyanamide treatment on bud breaking of each bud of 'Kosui' at different positions.



**Figure 4.** Effect of cyanamide treatment on the expression level of *JP-PIP1* in 'Kosui' buds.

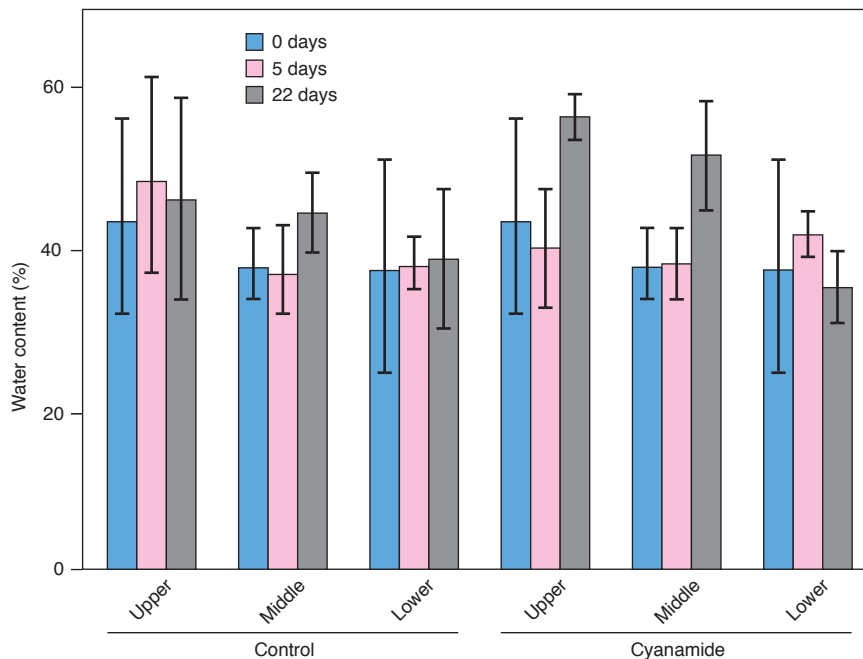
that in control buds (Fig. 4). The number of upper buds in cyanamide-treated trees was less than in the middle and lower parts. The patterns of bud break coincided with changes in water contents 22 days after cyanamide treatment (Fig. 5). Compared with controls, cyanamide-treated buds had higher water content at all positions, with a slope from upper to lower positions. From this evidence, membrane permeability and, in particular, water movement can relate dormancy involving water channel gene expression.

In grape vine treated by hydrogen cyanamide, DGBRPK (grape dormancy-breaking-related protein kinase) might be involved in the perception of cyanamide as a signal of stress, resulting in an increase of the AMP/ATP ratio (Or et al., 2000). There was a differential accumulation of H<sup>+</sup>ATPase mRNA in dormant vegetative buds of peaches during cold-exposure (Gévaudaut et al., 2001) indicating that underlying bud tissues accumulated more sucrose in the bud due to an increase in H<sup>+</sup>/sucrose co-transport. These reports suggest an alternative explanation for dormancy breaking.

In conclusion, *JP-PIP1* increased in the autumn but fell rapidly after low temperature treatment. About 14–20 days after its reduction, bud break occurred. Reduction of the expression level of *JP-PIP1* may be involved in bud breaking of Japanese pears.

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**Figure 5.** Effects of cyanamide treatment on water content of buds.

# Studies on the gene expression of dormant buds of Japanese apricot (*Prunus mume*)

Ryutaro Tao<sup>1</sup>

## Abstract

Bud dormancy in temperate woody perennial plants is a complex process necessary for plant survival in unfavorable environments. In contrast to the well-known characteristics for some external signals involved in bud dormancy, endogenous internal factors controlling endo-dormancy are poorly understood, partially because of the lack of analysis of molecular aspects of dormancy. In this study, Japanese apricot (*Prunus mume*) cultivars with high and low chilling requirements for breaking dormancy were used to investigate changes in gene expression in floral and vegetative buds in response to chilling accumulation and development of cold acclimation. The protein induced at a high level in dormant buds in response to chilling accumulation, and suppressed after dormancy break, was identified. Peptide sequencing revealed that this protein encodes dehydrin [the group of D-11 LEA (late embryogenesis-abundant) protein]. Cultivars with different chilling requirements showed a similar dehydrin expression pattern in response to chilling accumulation, implying that dehydrin is associated with the cold and drought tolerance of dormant buds. Subtractive PCR cloning of cDNAs from floral buds of Japanese apricot revealed that dormancy break is accompanied by the induction of cell-cycle related genes. This suggests that the blocking of cell division in buds and its release with accurate timing are involved in regulating dormancy.

## Introduction

TEMPERATE woody perennial plants exploit bud dormancy as a strategy to survive unfavourable environments, such as cold winters. By mid to late autumn, as night temperatures decline, inhibitory control of bud growth shifts to the bud itself, which is then referred to as endo-dormancy (Lang, 1987). Plants are incapable of emerging from this type of dormancy by removal of terminal buds or defoliation. Resumption of growth and bud break require sufficient exposure to low temperatures, ie satisfaction of the chilling requirement. This requirement is determined genetically (Samish, 1954). One typical way to refer to the chilling requirement for each cultivar is the number of hours between 0 and 7°C necessary for >50% bud break.

Endo-dormancy is the result of physiological changes in response to an internal signal to the bud that prevents untimely growth during seasonal transitions, when environmental conditions often

fluctuate between those permissive or inhibitory to growth. After the chilling requirement is satisfied, buds are capable of resuming growth upon exposure to 2–3 weeks of temperatures near 20°C. Buds with this capability, which lack sufficient exposure to higher temperatures, are termed eco-dormant. In contrast to endo-dormancy, eco-dormancy is imposed by external environmental factors such as cold or drought stress, which induce critical signals that prevent bud growth (Lang, 1987, 1996; Horvath et al., 2003).

Some signals mediating the induction of endo-dormancy have been characterised. The role of plant hormones in endo-dormancy, especially ABA, is well established, and it has been shown that ABA is induced in dormant buds during cold and drought stress. Although a complex set of overlapping hormonal signals is responsive to various environmental and physiological conditions related to dormancy (Horvath et al., 2003), how the signals are involved in dormancy remains to be elucidated. In addition, despite many physiological studies in dormancy, little is known about the molecular aspects of endo-dormancy, ie endogenous processes that induce, maintain, and break the bud dormancy state.

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In this study, Japanese apricot (*Prunus mume*) cultivars were used to investigate changes in gene expression in floral and vegetative buds of a woody perennial in response to chilling accumulation and development of cold acclimation. This species was selected because some early flowering cultivars were available and had the potential to be useful for distinguishing the genes associated with dormancy from those related to cold acclimation. To analyse the gene expression in Japanese apricot buds in response to chilling accumulation, 2D-PAGE analysis was conducted. In addition, subtractive PCR cloning of cDNAs from floral buds of Japanese apricot was performed to identify the genes induced at the transition from bud dormancy to dormancy break.

## Materials and methods

### Experiment 1. Chilling requirement for dormancy break of Japanese apricot cultivars

The Japanese apricot cultivars used in this study were the early-flowering cultivar, 'Niao-ume' (15 years old, seed-grafted), which originated in Taiwan, and a mid-season cultivar, 'Nanko' (14 years old, seed-grafted). Both cultivars were grown at the Horticultural Experiment Center of the Wakayama Research Center of Agriculture, Gobo, Japan. Field chilling was calculated as the number of hours below 7.2°C.

From the beginning of November 2001 until the middle of March 2002 three branches, about 40 cm long, were cut from trees each week. The upper and middle parts of the flower buds on each branch were used to survey the rate of flowering. To survey the rate of vegetative bud break, on the other hand, 10 branches with a length of 10 cm, taken from the middle to upper part of each branch, were used. These branches were placed in water in a growth chamber and maintained at 25°C under cool white fluorescent light with a photoperiod of 16 h. After 1 week in the growth chamber, the buds showing white petals were considered to be flowering, whereas the buds showing green leaves within 3 weeks after cutting were considered to be at bud break.

### Experiment 2. Comparison of protein profiles from vegetative and floral buds of Japanese apricot in response to chilling accumulation

At weekly intervals, vegetative and floral buds were removed from branches, dissected and immediately frozen in liquid nitrogen and stored at -80°C. Acetone powder was prepared from the buds using pre-chilled acetone (-20°C) containing 0.07% 2-mercaptoethanol; and used for protein extraction.

The acetone powder was homogenised with lysis buffer (O'Farrell, 1975) consisting of 8 M

urea, 2% Nonidet P-40, 2% Ampholine pH 3.5–10 (Amersham Biosciences, Uppsala, Sweden), 5% 2-mercaptoethanol and 5% polyvinylpyrrolidone K30. After incubation at 60°C for 10 min, the supernatant was isolated by centrifugation at 15 000 rpm for 10 min. Total protein concentration was determined by the standard Bradford method and the aliquot in a volume corresponding to 200 µg was applied in the two-dimensional polyacrylamide gel electrophoresis (2D-PAGE).

The protein sample was applied to the basic end of the first dimensional gels consisting of 4% acrylamide, 0.2% bis-acrylamide, 8 M urea, 0.2% Nonidet P-40, 2.5% Ampholine pH 3.5–10, and 2.5% Ampholine pH 5–8. The first dimensional gel-electrophoresis was conducted at 200 V for 10 min, at 300 V for 10 min, at 400 V for 150 min, and finally at 800 V for 30 min. Because carrier ampholite reacts with Coomassie Brilliant Blue R-250 (CBB-R250) to make an insoluble precipitation in the gels, the gels were incubated in a solution consisting of 10% TCA and 5% sulfosalicylic acid for 1 hr to remove carrier ampholite (Ampholine). After incubation, the gels were washed gently with double distilled water, equilibrated with the SDS sample buffer (10% glycerol, 6.25 mM Tris-HCl pH 6.8, 2.5% SDS, 5% 2-mercaptoethanol) for 15 min, and subjected to 15% SDS-PAGE for the second dimensional electrophoresis. Then, proteins in the gel were detected by CBB-R250. To determine the internal amino acid sequences, the protein spot of interest was digested with trypsin in the gel as reported by Hellman et al. (1995). The digested peptides were recovered from the gel and separated by the SMART-System (Amersham Biosciences). Several of the purified peptide fractions obtained were dotted on ProSorb (Applied Biosystems, Foster City, CA, USA) and subjected to protein sequencing using a gas-phase protein sequencer (476A, Applied Biosystems). The protein spot pattern of each gel was analysed and the relative intensity of each protein spot was calculated with the PDQUEST (Bio-Rad Laboratories, Hercules, CA, USA) software.

### Experiment 3. Cloning for cDNAs expressed in floral buds in late endo-dormancy stage

Total RNA was isolated from floral buds of 'Nanko' in December 2001 and January 2002 by the Phenol/CTAB method. Buds were homogenised in liquid N<sub>2</sub> with a mortar and pestle to make a fine powder. Phenol/chloroform/isoamylalcohol (25:24:1) was added to the tissue powder in a 50 ml centrifuge tube and mixed with it. After the CTAB buffer (2% CTAB, 0.1 M Tris-HCl pH 9.5, 2 M NaCl, 20 mM EDTA) was added, the homogenate was incubated at 65°C for 15 min with shaking. After subsection

to centrifugation, the supernatant was mixed with 2 M LiCl to precipitate RNA. Then, cDNA was synthesised from the total RNA using a SMART cDNA synthesis kit (BD Biosciences, San Jose, CA, USA) and employed for subtraction cDNA library construction.

The mRNA population in the floral buds in the late endo-dormancy stage was synthesised using cDNA from December floral buds as the ‘driver’ and cDNA from January buds as the ‘tester’ according to the protocol of the PCR-select® cDNA subtraction kit (BD Biosciences). The subtracted cDNAs were cloned into pGEM-T Easy vector (Promega, Madison, WI, USA). The cDNA clones obtained from the library were sequenced using an ABI-Prism DNA sequencer (Amersham Biosciences). The function of the obtained clones was predicted by BLAST search.

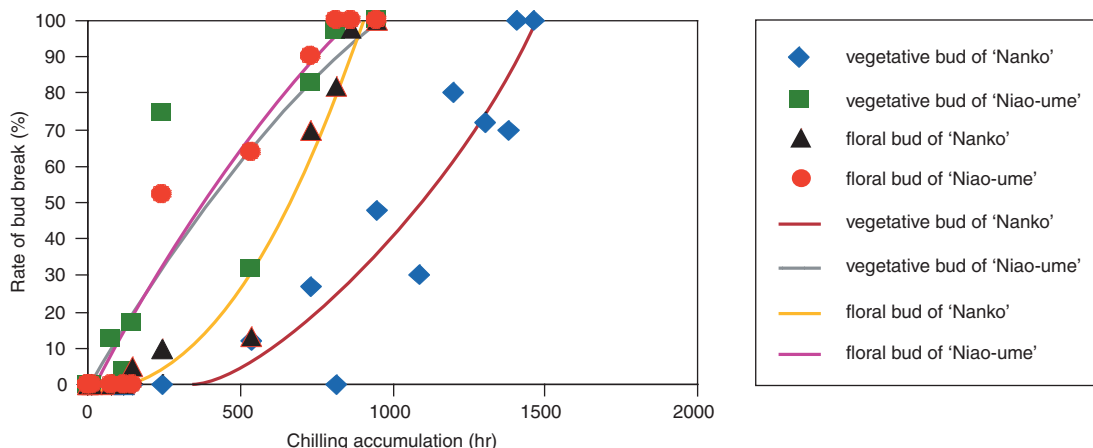
## Results and discussion

### Experiment 1

Under experimental field conditions, chilling accumulation started in the middle of November; hence, we started to collect the branches for this study at the beginning of that month. As shown in Figure 1, the chilling requirements for the dormancy break of the two Japanese apricot cultivars tested differ from each other. Vegetative buds of ‘Nanko’ require about 1100 h of chilling accumulation for dormancy break (50% bud break rate), whereas those of ‘Niao-ume’ require about 300 hours. In addition, the floral buds of ‘Nanko’ require about 600 hours of chilling accumulation for dormancy break and flowering

(50% flowering rate), whereas those of ‘Niao-ume’ require about 300 h (Fig. 1). Many different reports have shown a broad range of chilling requirements for breaking dormancy in fruit tree species (Couvillon and Hendershott, 1974; Egea et al., 2003; Jonkers, 1979; Kester, 1965; Crabbe and Barnola, 1996). For the Japanese apricot, the average chilling accumulation for floral bud dormancy break ranges from 400 h to 800 h. In this study, ‘Niao-ume’ showed a relatively lower chilling requirement for dormancy break compared with the other Japanese apricot cultivars tested so far.

In general, cultivars with a lower chilling requirement are better adapted to subtropical/tropical regions and/or greenhouse production, whereas those with a higher chilling requirement are more suitably cultivated in cold winter regions, which prevents flower damage in the early spring chilling temperature. Average air temperature in Japan has increased in recent years, which increases the need to select for cultivars with a lower chilling requirement. In addition, greenhouse cultivation of fruit tree species is increasing in Japan, which also makes it important to breed cultivars with lower chilling requirements. The ‘Niao-ume’ cultivar used in this study showed a much lower chilling requirement for dormancy break than other commercial Japanese apricot cultivars. This suggested that ‘Niao-ume’ would be a good parent for breeding cultivars suitable for cultivation in sub-tropical zones and/or in greenhouse cultivation. In addition, these cultivars were successfully used for the following experiments to investigate gene expression in dormant buds related to cold accumulation.



**Figure 1.** The bud break rate of vegetative or floral buds of two Japanese apricot cultivars, ‘Nanko’ and ‘Niao-ume’, in response to chilling accumulation.

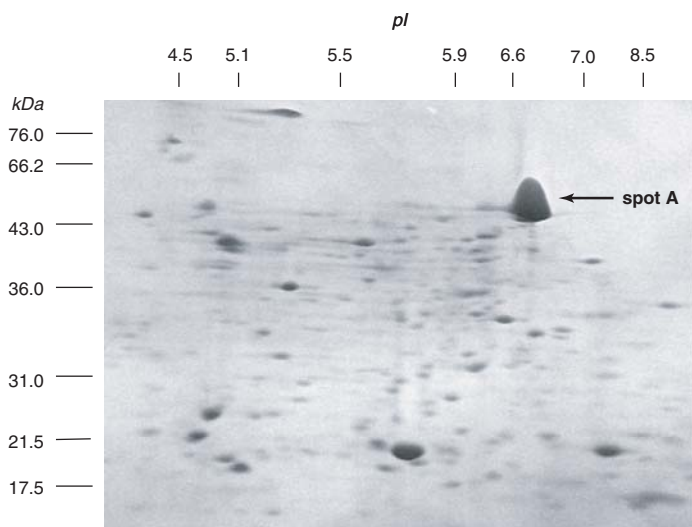
## Experiment 2

To identify changes in gene expression in floral and vegetative buds in response to chilling accumulation, 2D-PAGE profiles of soluble proteins were examined for the low-chill cultivar, 'Niao-ume' and the medium-chill cultivar, 'Nanko'. Samples were taken at several different dormancy levels (Fig. 1). The expression of some protein spots were induced or suppressed in response to chilling accumulation (data not shown). Among these, one spot, spot A, was extensively expressed in the buds during dormancy and then suppressed after the dormancy break (Fig. 2). Spot A had a relative molecular weight of about 65 kDa with a pI of 6.8 (Fig. 2). The internal amino acid sequences of spot A were determined. After the trypsin digestion, three fragments were obtained and sequenced. BLAST search of the three peptide sequences obtained, "LPGGHK", "VGGGG", and "EKLPGGQNVHPK", revealed that these peptide sequences have a similarity with peach dehydrin, indicating that spot A encodes dehydrin.

Dehydrin [the group D-11 LEA (late embryogenesis-abundant) protein] has been found in many plant species (Close, 1996). Dehydrins are induced by environmental stresses such as cold temperature and dehydration and by the plant hormone abscisic acid (Close, 1996). One putative function of dehydrins is alteration of the thermodynamic interactions between macromolecules and water via solute exclusion or direct binding (Close et al., 1993). Thus, they may provide stability to macromolecules, such as nucleic acids and proteins, during desiccation by preventing denaturation or inhibiting ice crystal formation at freezing temperatures, although no directional

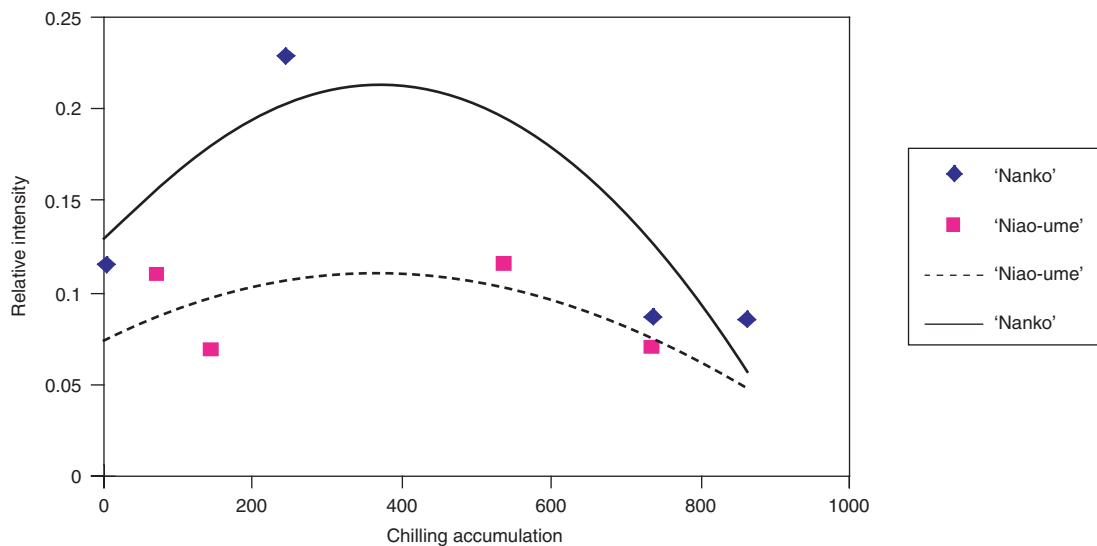
relationship has been shown (Close, 1996). So far, dehydrin has been shown to be induced by chilling accumulation and in response to cold acclimation in floral buds of blueberry (Muthalif and Rowland, 1994). The cDNA encoding peach bark dehydrin was cloned with its expression induced by cold acclimation (Arora et al., 1992; Artrip et al., 1997).

In our experiment, dehydrins were induced in dormant buds and suppressed in response to dormancy break in both of the cultivars tested (Fig. 3). Whereas dormant buds of each cultivar showed different bud break reactions against chilling accumulation, they showed similar dehydrin expression patterns in response to chilling accumulation, suggesting that dehydrin is associated with the cold and drought tolerance of dormant buds rather than dormancy (Fig. 3). Nevertheless, there is a difference in expression of dehydrin in 'Nanko' and 'Niao-ume'. The dormant buds of 'Nanko' accumulate more dehydrin than those of 'Niao-ume' (Fig. 3). Artrip et al. (1997) demonstrated that mRNA encoding dehydrin and dehydrin itself accumulate earlier and to a greater extent, and remain longer in the deciduous peach than the evergreen peach. Since 'Niao-ume' is similar to the evergreen peach in that it has a low chilling requirement for bud break and retains leaves longer, our finding of a lower expression of dehydrin in 'Niao-ume' compared to 'Nanko' is consistent with the findings by Artrip et al. (1997). Evergreen peach tissues are less able to acclimate and do so more slowly than deciduous peach tissues (Arora et al., 1992). Therefore, investigation of the cold hardiness of these Japanese apricot buds would be interesting.



**Figure 2.** 2D-PAGE profile of total proteins from January buds of 'Nanko'.





**Figure 3.** The expression pattern of dehydrin in floral buds of two Japanese apricot cultivars, 'Nanko' and 'Niao-ume', in response to cold accumulation.

### Experiment 3

Bud endodormancy could be regulated by internal signals such as hormones and sugar and external signals such as light and temperature. The genetic mechanism controlling this process is not known. To search for the genetic signals and factors regulating endodormancy break, we used a subtractive PCR hybridisation protocol and isolated the cDNAs induced in the late endo-dormancy stage of floral buds of Japanese apricot.

Among the clones identified, we isolated several cell-cycle-related genes. These include the RING finger protein, myb family transcription factor, and *cdc20* (Table 1). Because bud growth, following the bud dormancy break, is associated with increased cell division, the obtained clones may be related to inducing bud growth. RING finger protein is known to be associated with the degradation of cyclin (CYC) or cyclin-dependent kinase (CDK), which makes mitosis progress properly through the ubiquitin-proteasome pathway (Dewitt and Murray, 2003). The myb family transcription factor is known to be expressed in the G2/M phase transition and is thought to control the transcription level of G2/M phase-specific genes such as B-type CYC (Weston, 1998). *Cdc20* was discovered and identified as a cell cycle switch protein; it often includes WD-40 repeat at the C-terminal. It acts as a member of the E3 complex (ubiquitin ligase, APC/*C<sup>cdc20</sup>*) to degrade CYCA/B, a G2/M phase-specific CYC, through the ubiquitin-proteasome pathway to make cell division progress (Morgan and Roberts, 2002).

**Table 1.** cDNAs isolated by subtractive PCR cloning from December floral buds (late endo-dormancy stage) of Japanese apricot, cultivar 'Nanko'.

Clone homology	BLAST hit score	No. of repeats
Cytochrome P450 monooxygenase	4e-12	2
Unknown function		2
Myb family transcription factor	7e-07	2
<i>Cdc20</i>	3e-75	1
C3HC4-type RING finger protein	2e-04	1
Neutral invertase	9e-52	1
RNA polymerase beta II subunit	4e-41	1

Our results indicate that dormancy break is associated with cell cycle-related gene induction, which suggests that endo-dormancy is maintained by inhibition of the cell-cycle progress. In most cases, cells in buds and shoots appear to be arrested in the G1 phase, before the S phase of the cell cycle (Horvath et al., 2003). Therefore, our experiment supported the idea that some factors inhibiting the transition of G1 to S, such as ICK (an inhibitor of CDK action at the G1/S phase transition) (Wang et al., 1997), might affect continuation of dormancy (Horvath et al., 2003).

Interestingly, we also isolated P450 monooxygenase. It has been suggested that ABA levels are regulated by P450 mono-oxygenase and are associated with axillary bud dormancy in the pea plant (Shimizu-sato and Mori, 2001). This further suggests that P450 mono-oxygenase may act to regulate the ABA level in woody dormant buds. In addition, we

isolated invertase, an enzyme that converts sucrose to hexose. Sugar plays a complex role in dormancy such that it is required for expression of D-type CYC (Riou-Khamlichi et al., 2000; Rolland et al., 2002). G1/S phase-specific CYC interacts with ABA and affects ABA signaling (Xu et al., 1998; Rolland et al., 2002), the involvement of invertase in dormancy might be possible. Further analysis of the expression pattern of these genes might provide clear directions for understanding the induction and breaking of dormancy.

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# Differences in chilling requirement for bud break among tetraploid grape cultivars

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## Abstract

The effect of temperature on bud break of cuttings taken from eight tetraploid grape (*Vitis labruscana*) cultivars during different stages of dormancy (from the beginning of November to the end of January) was studied. The optimum temperature for bud break in cv. Pione was 20–25°C, that in cv. Kyoho was 25–30°C and that in cv. Fujiminori was 25°C. We also evaluated the interaction between date of cutting removal and effects of temperature on subsequent bud break in cv. Pione taken during dormancy. Cuttings taken in July and August broke dormancy best at 25–30°C, whereas those taken at later dates broke dormancy more uniformly, irrespective of temperature.

## Introduction

It is well known that the buds of grapevines and peach trees need to be exposed to low winter temperatures, less than 7.2°C to break dormancy (Faust et al., 1997; Richardson et al., 1974; Samish, 1954). Tetraploid grape (*V. labruscana*) cultivars, including 'Kyoho' and 'Pione', are widely grown throughout Japan (Morinaga, 2001) and commercial production of early-season grapes under protective structures is a common practice (Kubota, 2002). Grapevine buds are usually dormant when forcing starts, because of insufficient exposure to low temperature (Kubota and Miyamuki, 1992; Kubota et al., 1999).

Kubota et al. (2002) previously reported that the response of peach buds to temperature for bud break largely differs among cultivars and rootstocks, although there was no difference among the cultivars growing in Japan. Similar results have been reported in ornamental peaches (Pawasut et al., 2003). However, there is little information about the differences in chilling requirements for bud break among tetraploid grape cultivars.

The purpose of this work was to compare chilling requirements for bud break in tetraploid grape cultivars and to investigate the response of buds to temperature for bud break in grape cv. Pione.

## Materials and methods

### Response of buds to temperature for bud break in tetraploid grape cultivars

From early (late November 1999) to late dormancy (January 2000) canes of eight tetraploid grape (*V. labruscana*) cultivars: 'Kyoho', 'Pione', 'Fujiminori', 'Aki Queen', 'Takao', 'Suiho', 'Takatsuma' and 'Shigyoku' were taken from field-grown vines at the Agricultural Experiment Station, Okayama Prefectural General Agricultural Center. The cuttings (6 cm in length) with single buds were prepared and mounted on plastic foam plates, floated in a water bath, then placed in a growth chamber maintained at either 20, 25, 30 or 35°C. Three replicates of 10 cuttings were made for each treatment (temperature). Buds were regarded as broken when their tips turned green. The number of cuttings which broke dormancy was monitored every day for 60 days after treatment. The accumulated chilling hours (CCH) were calculated as the number of hours below 7.2°C. For November, December and January, the CCHs were 147, 626 and 1152 respectively.

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## Chilling requirement in 'Pione' grapes

The canes of 'Pione' grapevines grown in the research field of Okayama University were collected monthly from late July 2003 to late February 2004. The cuttings with single buds were prepared and mounted on a plastic foam plate, floated in a water bath, then placed in a growth chamber maintained at 20, 25 or 30°C as described above. Each treatment consisted of four replications. CCHs were 0, 0, 0, 0, 6, 291, 719 and 1412 in July, August, September, October, November, December, January and February, respectively.

## Results and discussion

The chilling requirement of grapevine buds is reported to be low compared to other deciduous fruit species (Dokoozlian and Williams, 1995). Horiuchi et al. (1981) reported that bud dormancy of 'Delaware' grape was deep at the beginning of autumn, but the intensity gradually decreased from late autumn through early winter. However, the precise temperature and duration of chilling required for bud break of grapevines, especially for tetraploid cultivars, has not been established. Usually, the effect of temperature on bud break in dormant grapevines was evaluated on the following basis: (1) the fewer number of days to initial bud break after the treatment, indicating promotion of bud break, and (2) the rate of bud break, that is, the uniformity of bud break (Kubota et al., 1999).

Figures 1 and 3 show changes in the rate of bud break after treatment in November and December, respectively. Figures 2 and 4 show the number of days to first and 80% bud break after cuttings in each treatment. For all the cultivars tested, the late treatment resulted in earlier and more uniform bud break. In the November treatment (Figs 1 and 2), uniform bud break occurred in 'Pione', 'Kyoho' and 'Fujimino' at 20 and 25°C, 30 and 25°C and 25°C, respectively, although the cultivars are very closely related genetically. The final percentage of bud break in 'Aki Queen' was higher at 20, 25 and 30°C, whereas in 'Takao' and 'Suiho' it was higher at 20 and 25°C, indicating that the optimum temperature range for bud break in these cultivars is narrow. Aspects of bud break in cuttings of 'Kyoho' and 'Aki Queen' treated in December (Figs. 3 and 4) were different from those treated in November, with the percentage of bud break at 30°C being lower in the former. However, when cuttings of 'Kyoho' and 'Shigyoku' were kept at 20°C, bud break was superior in the December treatment than in the November treatment. In the January treatment (data not shown), superior bud break was observed at 20°C and 25°C for all the cultivars.

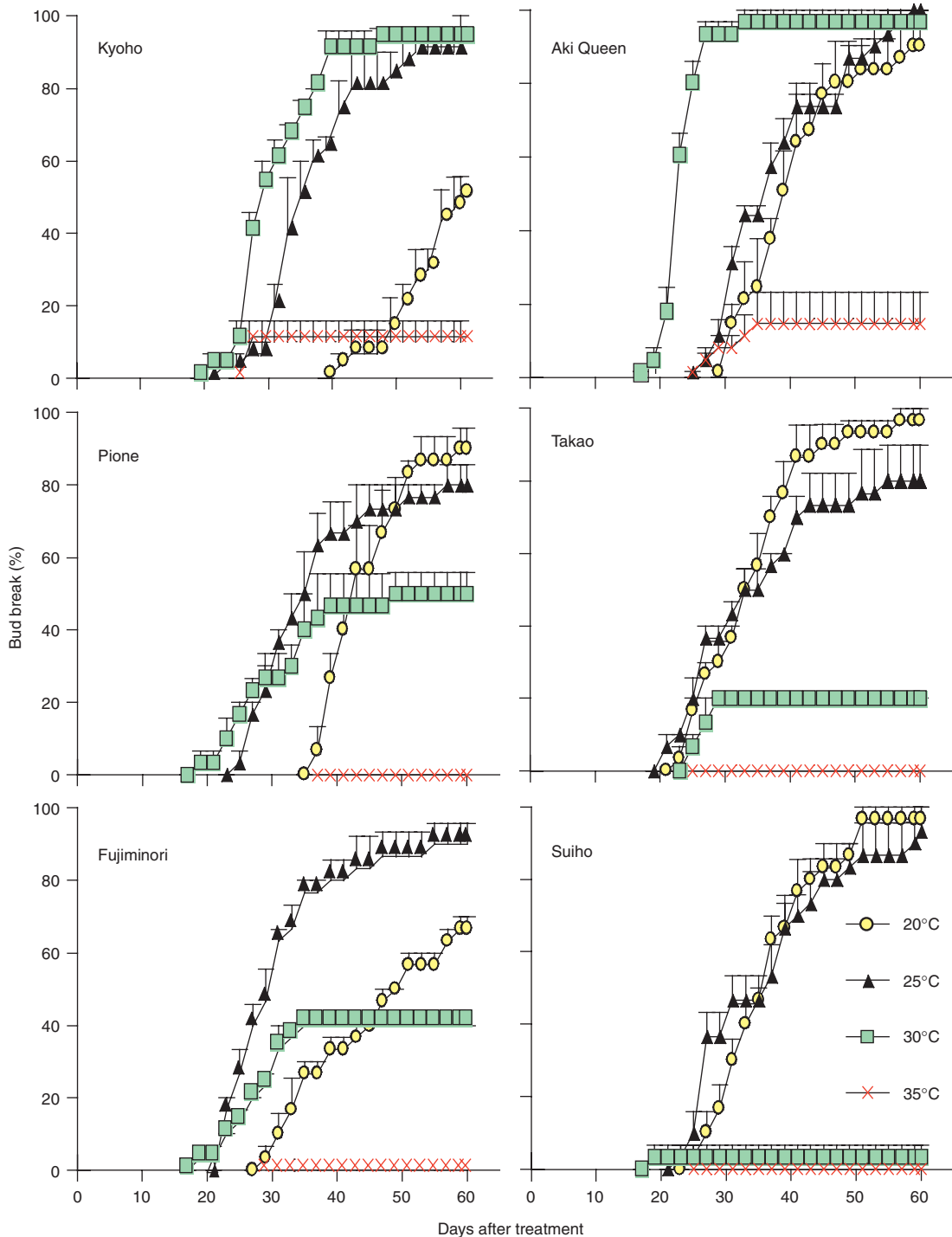
Regardless of the time of treatment, bud break was markedly lower at 35°C for all cultivars. These findings agree with the results of Kubota et al. (2000a). However, the reasons for the different responses by buds of different tetraploid grape cultivars to temperature are not known. Kubota and Miyamuki (1992) and Kubota et al. (2000b) reported that the response of grapevine bud to garlic paste, which is effective at breaking dormancy, significantly varied among the cultivars.

When cuttings of 'Pione' grapes were kept at 25°C or 30°C, early treatment resulted in fewer days required to initiate bud break after the treatment (Fig. 5). However, irrespective of the temperature, late treatment resulted in more uniform bud break. The number of days to first bud break in both October and November treatments was 18, whereas in September and December treatments bud break was initiated in 15 days. Judging from these results, it seems that endo-dormancy of 'Pione' grape occurs from October to November. When temperature was maintained at 25°C or 30°C, late treatment resulted in a higher percent of bud break. At 20°C, the early treatment resulted in a lower final percentage of bud break, but late treatment resulted in a superior bud break, including those cuttings in plots at 25°C and 30°C, although the reason for different responses by different dormant stages to temperatures are not known. In the July and August treatments, final percentage of bud break was lower compared to other treatments, although the initial bud break was accelerated. Late treatment resulted in a small difference among the temperatures in the initiation and uniformity of bud break.

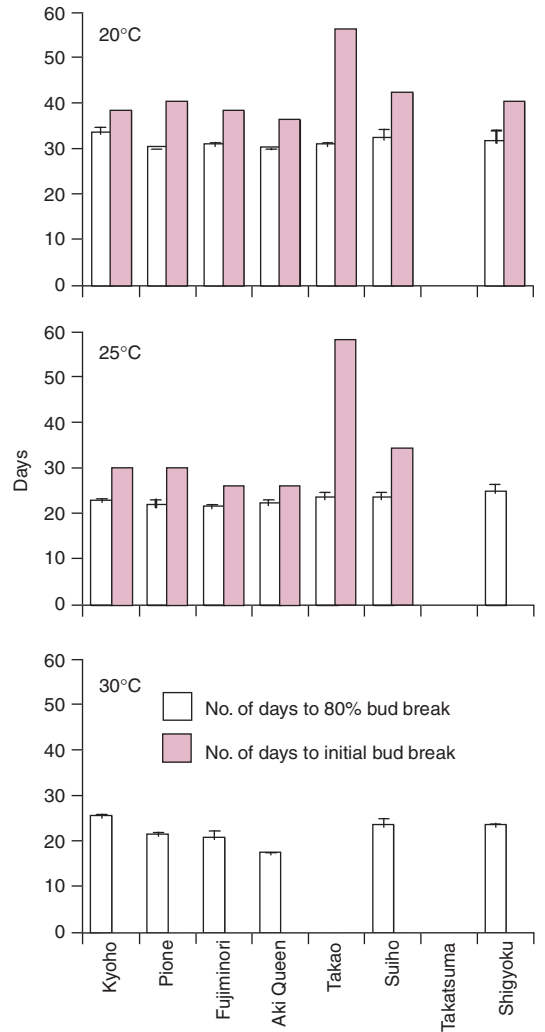
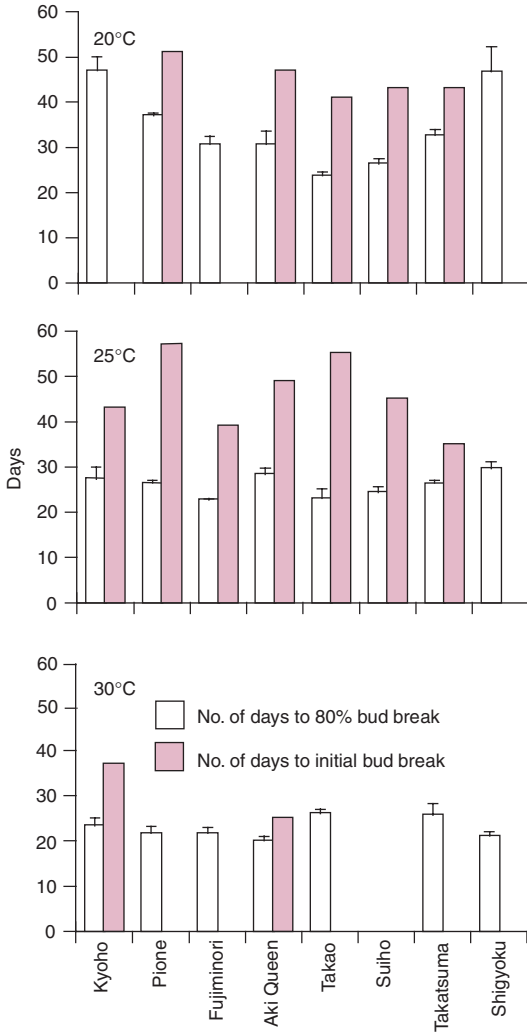
Based on the above observations, we conclude that the responses of grapevine buds to temperature for bud break are different among tetraploid grape cultivars, even though these cultivars are closely related genetically. Further investigations are required to explain the reasons for the different temperature responses of buds of different tetraploid grape cultivars.

## Conclusion

The results indicate that the response of grapevine buds to temperature for bud break differs between tetraploid grape cultivars, even though 'Pione', 'Kyoho' and 'Fujimino' cultivars are genetically closely related. For the November treatment, the optimum temperatures for uniform bud break of cv. Pione were 20–25°C, for Kyoho, 25–30°C and for Fujimino, 25°C. Based on the number of days to initial bud break and the maximum percentage of bud break, it seems that endo-dormancy of Pione grape occurs from October to November.

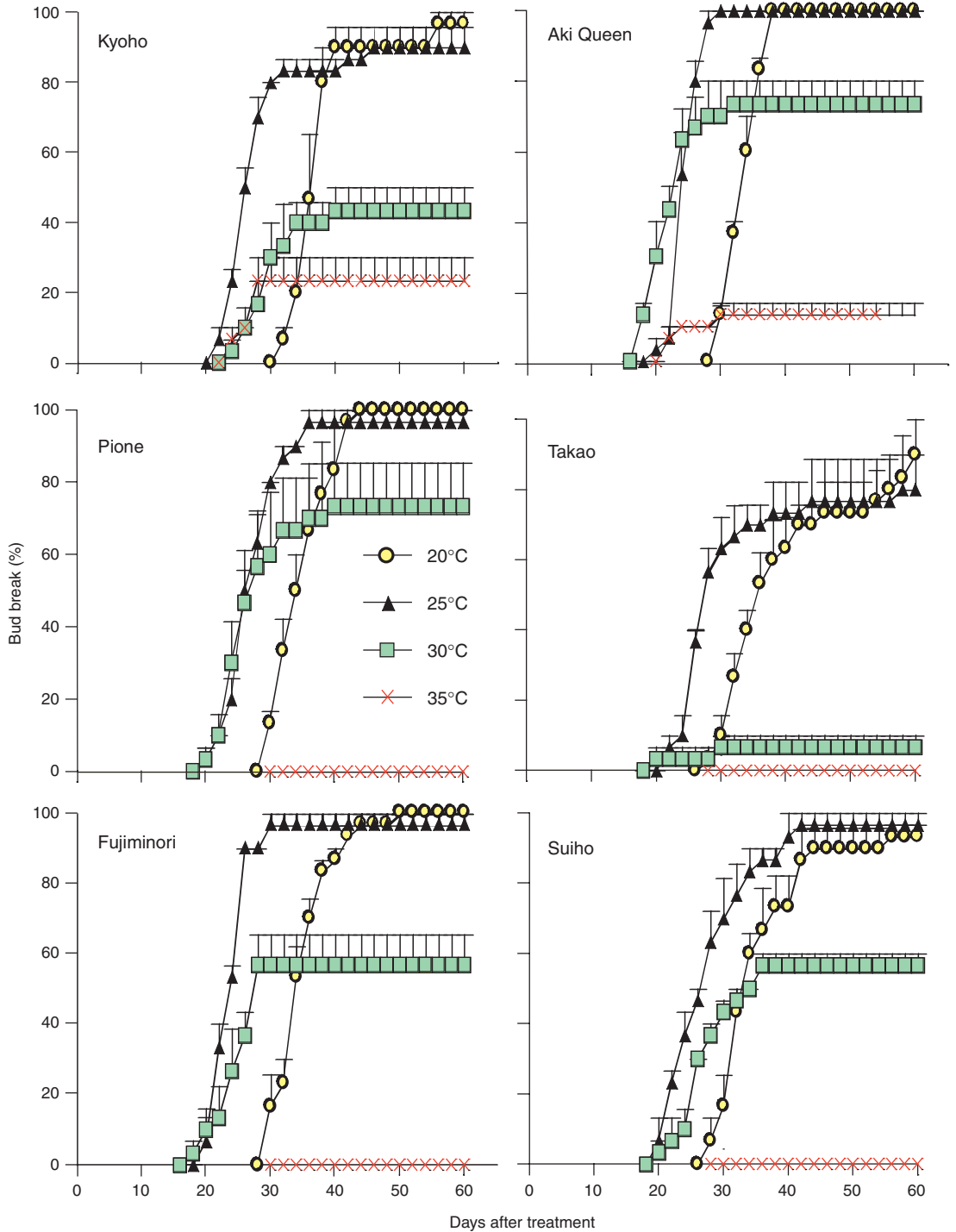


**Figure 1.** Effect of temperature on bud break of single-bud cuttings of six tetraploid grape cultivars (treatment in late November). Vertical bars are the standard error (n=3).

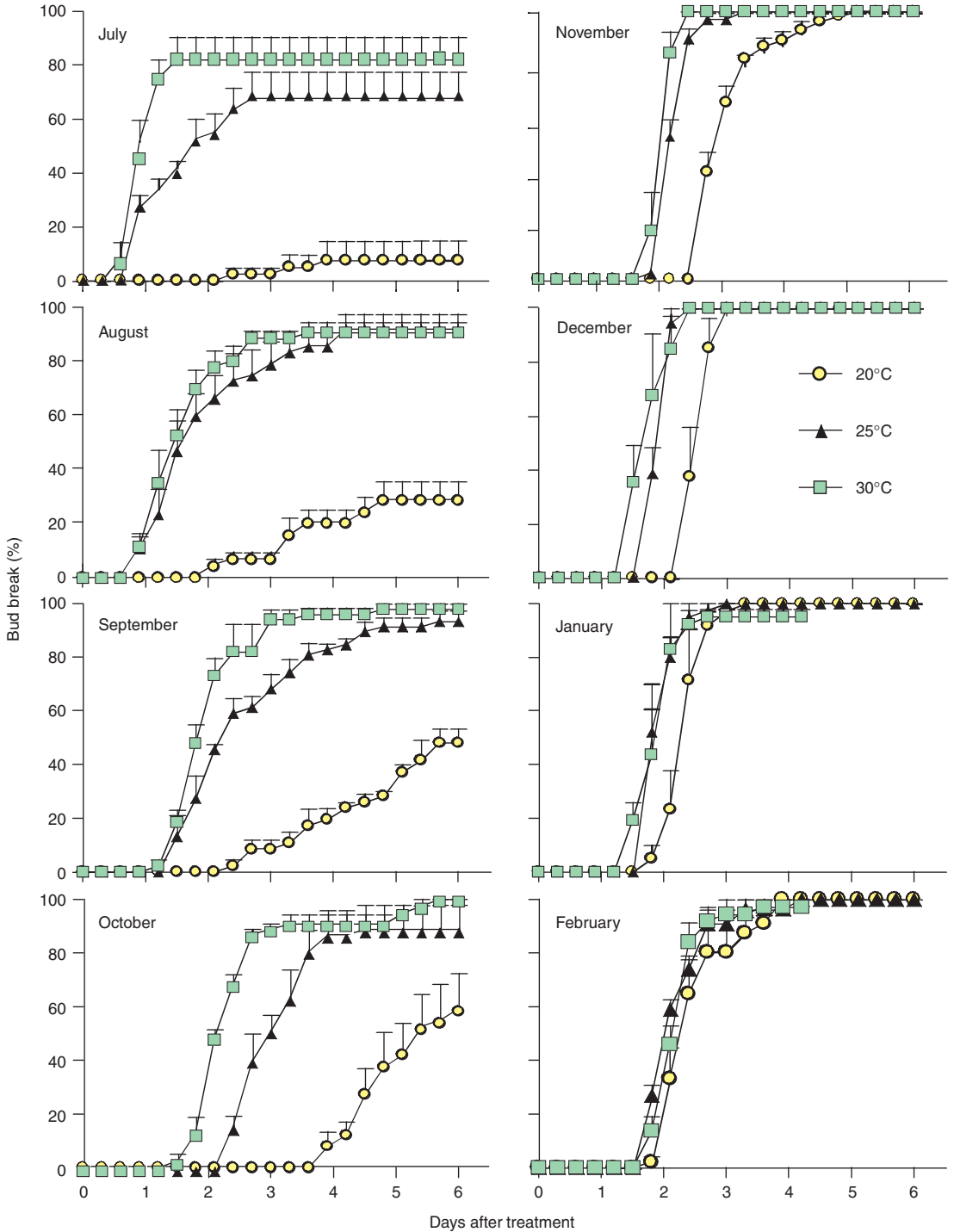


**Figure 2.** Effect of temperature on time of bud break of eight tetraploid grape cultivars (treatment in late November). Vertical bars are the standard error (n=3).

**Figure 4.** Effect of temperature on time of bud break of eight tetraploid grape cultivars (treatment in late December). Vertical bars are the standard error (n=3).



**Figure 3.** Effect of temperature on bud break of single-bud cuttings of six tetraploid grape cultivars (treatment in late December). Vertical bars are the standard error (n=3).



**Figure 5.** Effect of temperature on bud break of single-bud cuttings of 'Pione' grapevines at different dormant stages. Vertical bars are the standard error (n=4).



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# Comparative growth of persimmon seedling rootstocks in Thailand

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## Abstract

Four persimmon seedling rootstocks (*Diospyros kaki* cv. 'Xichu' or 'P2'; *D. lotus*, green fruit and yellow fruit types; and *D. glandulosa*) were compared to investigate the suitability of these species and selections as rootstock for persimmon cultivar 'Fuyu'. *D. lotus* (green fruit type) had significantly higher seed germination than the other rootstocks. One-year-old seedlings differed in tree height and trunk diameter. *D. lotus* (green fruit type) had vigorous growth, but plant dry weight was lower than *D. lotus* (yellow fruit type) and *D. glandulosa* rootstock. *D. glandulosa* had the highest primary root dry weight (PDW) of 7.96 g and *D. lotus* (yellow fruit type) had the highest secondary root dry weight (SDW) of 5.22 g. The SDW/PDW ratio of *D. glandulosa* was higher than the other rootstocks, while the root/shoot (R/S ratio) and nitrogen concentration of the trunk, primary root and secondary root of 'P2' rootstock were higher than for other rootstocks. There were significant differences in grafting success among rootstocks. 'P2' rootstock had the highest grafting take (76%) and produced the greatest dry weight increase 1 year after grafting. Growth of 'Fuyu' scion was the highest on both types of *D. lotus* rootstock. The diameter of the graft-union was different for all rootstocks but the ratio of the diameter of scion and rootstock was about the same for all (1:1). All grafted plants, even on *D. lotus* rootstock, showed no signs of incompatibility and exhibited normal growth in the first year after grafting.

## Introduction

PERSIMMON (*Diospyros kaki* L.) is an important temperate fruit being trialled by the Royal Project Foundation which has been introducing new tree crops to hill-tribe growers as possible substitutes for opium poppy (Subhadrabundhu and Punsri, 1987). Two commercial cultivars are available: 'Fuyu' (non-astringent type) and 'P2' (astringent type).

Generally, grafting of cultivars on seedling rootstock is used for persimmon propagation in Thailand. The date plum (*D. lotus*) was used as rootstock for the astringent cultivar 'P2'. Hodgson (1940) found symptoms of incompatibility when non-astringent varieties were grafted onto *D. lotus* rootstock.

When 'Fuyu' was introduced to northern Thailand, it was grafted on scion variety 'P2' which acted as an interstock between it and the *D. lotus* rootstock. Since there is a big demand for grafted trees of the *Diospyros kaki* cultivar 'Fuyu' and because *D. lotus* is the only species available for rootstock, we evaluated the compatibility between these two species under the climatic conditions of northern Thailand. We also wanted to find a rootstock for 'Fuyu' that is suitable to our climate.

The objective of this research was to compare the growth of some seedling rootstocks as well as their compatibility and effect on 'Fuyu' scion growth in the first year after grafting. These species and selections included *D. lotus* (yellow fruit type) introduced from Taiwan, *D. lotus* (green fruit type) found at the Royal Agricultural Station Angkhang, cultivar 'P2', which is the same species (*Diospyros kaki*) as 'Fuyu' and *D. glandulosa*, an indigenous *Diospyros* spp. of Thailand (Phenglai, 1981). The results will enable better understanding of compatibility and growth of rootstock and provide sufficient information to choose the right rootstock for 'Fuyu' growing in Thailand.

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## Materials and methods

The experiment was conducted at the Royal Agricultural Station Angkhang from December 2002 to January 2004. Seeds of 'P2'; *D. lotus* (green fruit type); *D. lotus* (yellow fruit type) and *D. glandulosa* were tested for their germination (Fig. 1). Each treatment was carried out in four replications using 50 seeds per replication and the percentage of seed germination was recorded. After germination, seedling height and trunk diameter from 20 samples for each rootstock were recorded monthly for 1 year.

After 1 year, just before grafting, total dry weight, primary root dry weight, secondary root dry weight and the ratio between two types of root of 1-year-old seedlings were measured. Total nitrogen concentration (TN) was determined using a Nitrogen Determinator (FP-528 Leco Crop, USA.). Inlay grafting was performed during the dormant season (January 2003) using 100 trees per rootstock. 'Fuyu' scions consisting of one to two buds of the previous season's growth were grafted onto both types of *D. lotus* and 'P2' but not onto *D. glandulosa* due to lack of seedlings. The percentage of grafts which took was recorded in the second month after grafting. The height and dry weight of new shoots, the ratio of scion and rootstock diameter and graft union diameter were recorded. Each rootstock was replicated 10 times using one tree per replication. Data were analysed with a SAS program (SAS Institute Inc., 1997).

## Results and discussion

*Diospyros lotus* (green fruit type) had significantly higher seed germination than other rootstocks. Both the *D. lotus* types had different seed characteristics (Fig. 1) and differences in seed germination may be attributed to their genotypic expression. This indicated that these two types must be from different sources. Among materials tested, *D. kaki* cultivar 'P2' had the lowest germination (Table 1).

After transplantation, seedlings from each type of persimmon showed different growth (Fig. 2).

One-year-old seedlings of *D. lotus* (green fruit type) were the tallest, with the largest stem diameter. *D. lotus* (yellow fruit type) and *D. glandulosa* had the highest dry weights and 'P2' had the lowest growth in all characteristics (Table 2).

**Table 1.** Germination of persimmon seedling rootstocks in Thailand.

Rootstock	Germination (%)
<i>D. kaki</i> 'P2'	34.85 c*
<i>D. lotus</i> ; green fruit	85.07 a
<i>D. lotus</i> ; yellow fruit	59.03 b
<i>D. glandulosa</i>	40.01 c
P > F-test	0.0014

\* Data followed by the same letter are not significantly different at 95% by DMRT.

The root system of the rootstock affects the performance, eg growth of the scion (Hartmann et al., 1997). The root system includes both primary and secondary roots, both of which are involved in water absorption and nutrient uptake (Rom and Carlson, 1987).

For this study, 1-year-old root growth was shown by primary root dry weight, secondary root dry weight and primary and secondary root ratio. The results showed a significant difference between rootstocks. *D. glandulosa* had the highest primary root dry weight (7.22 g) while *D. lotus* (yellow fruit type) had the highest secondary root dry weight (5.22 g) (Table 3). The secondary and primary root ratio of *D. lotus* (yellow fruit type) and *D. lotus* (green fruit type) were higher than the others. The root to shoot ratio of 'P2' was highest because 'P2' had very poor shoot growth compared to its root growth (Table 3).

The total nitrogen concentration in each component of the rootstock is shown in Table 4. Irrespective of rootstock species or type, total nitrogen concentration was higher in the secondary rootstock than in either the primary roots or trunk. Higher nitrogen concentrations in all parts of cultivar 'P2' may be due to



**Figure 1.** Seed characteristics of different rootstocks: A (*D. kaki* 'P2'), B (*D. glandulosa*), C (*D. lotus* green fruit) and D (*D. lotus* yellow fruit).



**Figure 2.** Seedlings from each kind of persimmon showed different growth rates: A (*D. kaki* 'P2'), B (*D. glandulosa*), C (*D. lotus* yellow fruit type) and D (*D. lotus* green fruit type).

its poorer growth. From this study, it appears that nitrogen concentration alone may not be a good indicator of the growth potential of persimmon seedlings. Other biochemical components, such as carbohydrate content, may also be necessary to interpret differences in vegetative growth.

'P2' had the highest level of successful grafts with 'Fuyu' because they are the same species. There was

no difference in the percentage of successful grafts between 'Fuyu' grafted onto *D. lotus* (green and yellow fruit types). Native plants, such as *D. glandulosa*, failed to provide a suitable rootstock for 'Fuyu' due to genetic incompatibility (Lapins, 1959; Hartmann *et al.*, 1997). This study indicated that it is possible to achieve a high percentage of successful grafts of 'Fuyu' with 'P2', and possibly also with *D. lotus* green and yellow fruit types. However, longer-term observations, up to 8 years, will be needed to assess their compatibility (Table 5).

One-year-old 'Fuyu' grafted onto both *D. lotus* rootstock types exhibited better growth than those grafted on other rootstocks. In contrast, the poor growth of cultivar 'Fuyu' on 'P2' rootstock may be due to lack of rootstock vigour. Based on the ratio of scion and rootstock diameter, as well as, graft-union diameter, there were no signs of incompatibility such as overgrowth of the scion on rootstock (Table 6).

Results in this study included some information from *Diospyros* growing in northern Thailand. However, first-year data is still not sufficient to choose a rootstock for 'Fuyu'. Work has started to identify other *D. kaki* cultivars that may have better growth than 'P2' and the results may lead to more progress in propagating sweet persimmon in this area.

**Table 2.** Growth of seedling rootstocks. (data are mean  $\pm$  SE)

Rootstocks	Height	Diameter	Dryweight (g)
	(cm)		
<i>D. kaki</i> 'P2'	18.51 $\pm$ 0.36 d*	0.44 $\pm$ 0.01 d*	6.59 $\pm$ 0.92 c*
<i>D. lotus</i> ; green fruit	43.98 $\pm$ 0.51 a	0.67 $\pm$ 0.01 a	11.45 $\pm$ 0.81 b
<i>D. lotus</i> ; yellow fruit	30.82 $\pm$ 0.57 c	0.55 $\pm$ 0.01 b	16.72 $\pm$ 1.15 a
<i>D. glandulosa</i>	32.92 $\pm$ 0.75 b	0.54 $\pm$ 0.01 c	15.16 $\pm$ 1.67 a
P > F-test	0.0001	0.0001	0.0002

\* Data followed by the same letter are not significantly different at 95% by DMRT.

**Table 3.** Root growth of persimmon seedling rootstocks. (data are mean  $\pm$  SE)

Rootstock	Primary root dry weight (PDW)	Secondary root dry weight (SDW)	SDW/PDW ratio	R/S ratio
	g			
<i>D. kaki</i> 'P2'	3.35 $\pm$ 0.45 bc*	1.79 $\pm$ 0.35 c*	0.51 $\pm$ 0.06 b*	3.49 $\pm$ 0.27 a*
<i>D. lotus</i> ; green fruit	2.71 $\pm$ 0.27 c	3.17 $\pm$ 0.41 b	1.14 $\pm$ 0.09 a	1.07 $\pm$ 0.11 c
<i>D. lotus</i> ; yellow fruit	4.87 $\pm$ 0.46 b	5.22 $\pm$ 0.67 a	1.14 $\pm$ 0.18 a	1.54 $\pm$ 0.12 c
<i>D. glandulosa</i>	7.96 $\pm$ 1.10 a	2.41 $\pm$ 0.28 bc	0.33 $\pm$ 0.04 b	2.22 $\pm$ 0.23 b
P > F-test	0.0001	0.0001	0.0001	0.0001

\* Data followed by the same letter are not significantly different at 95% by DMRT.

**Table 4.** Total nitrogen concentration in various parts of persimmon seedling rootstocks. (data are means  $\pm$  SE)

Rootstock	Nitrogen concentration (%)		
	Trunk	Primary root	Secondary root
<i>D. kaki</i> 'P2'	1.80 $\pm$ 0.05 a*	1.58 $\pm$ 0.10 a*	2.05 $\pm$ 0.10 a*
<i>D. lotus</i> ; green fruit	1.04 $\pm$ 0.05 b	0.88 $\pm$ 0.05 b	1.42 $\pm$ 0.03 b
<i>D. lotus</i> ; yellow fruit	0.94 $\pm$ 0.03 b	0.72 $\pm$ 0.05 b	1.37 $\pm$ 0.06 b
<i>D. glandulosa</i>	0.59 $\pm$ 0.03 c	0.59 $\pm$ 0.03 c	1.31 $\pm$ 0.04 b
P > F-test	0.0001	0.0001	0.0001

\* Data followed by the same letter are not significantly different at 95% by DMRT.

**Table 5.** Success of grafting cultivar 'Fuyu' onto different rootstock

Rootstock	Graft take (%)
<i>D. kaki</i> 'P2'	76.00 a*
<i>D. lotus</i> (green fruit type)	56.67 b
<i>D. lotus</i> (yellow fruit type)	63.33 b
<i>D. glandulosa</i>	7.33 c
P > F-test	0.0001

\* Data followed by the same letter are not significantly different at 95% by DMRT.

### Conclusions

After the first year of the experiment, it can be concluded that:

1. *D. lotus* (green fruit type) had the highest seed germination
2. *D. lotus* (green and yellow fruit types) had the highest growth.
3. 'Fuyu' grafted on 'P2' had the highest percentage of successful grafts, but shoot growth after grafting was poor.
4. There were no symptoms of incompatibility between 'Fuyu' scion and *Diospyros lotus* rootstock. 'Fuyu' grafted on *Diospyros lotus* exhibited better growth than on kaki rootstocks such as 'P2'.

**Table 6.** Growth characteristics of 1-year-old 'Fuyu' scion grafted onto different rootstocks. (data are mean  $\pm$  SE)

Rootstock spp. and type	Height (cm)	Dry weight (g)	Scion/stock diameter ratio	Graft-union diameter (cm)
<i>D. kaki</i> 'P2'	29.2 $\pm$ 1.43 b*	1.28 $\pm$ 0.14 b*	0.87 $\pm$ 0.03	0.85 $\pm$ 0.03 c*
<i>D. lotus</i> (green fruit)	38.9 $\pm$ 1.42 a	2.14 $\pm$ 0.19 a	0.82 $\pm$ 0.03	1.00 $\pm$ 0.04 b
<i>D. lotus</i> (yellow fruit)	40.1 $\pm$ 1.59 a	2.64 $\pm$ 0.25 a	0.79 $\pm$ 0.02	1.13 $\pm$ 0.04 a
P > F-test	0.0001	0.0001	0.1419	0.0001

\* Data followed by the same letter are not significantly different at 95% by DMRT.

### Acknowledgment

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# Influence of different climatic conditions on growth and yield of strawberry plants in Thailand

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## Abstract

Growth and yield of commercial strawberries (*Fragaria* × *ananassa* Duch.) cvs 'Tioga' and 'Tochiotome' were measured under different elevations: Royal Agricultural Research Center (RARC), Royal Phang-da Agricultural Station (RPAS), and Doi Pui Research Station (DPRS) at elevations of 340, 650, and 1300 m, respectively. The results showed that vegetative and reproductive growth were significantly different. At RPAS 'Tioga' had higher fresh and dry weights, and more crowns than the other cultivars, but it had the longest roots at DPRS. At all three sites 'Tochiotome' flowered for fewer days after transplanting than 'Tioga'. At DPRS 'Tioga' had the highest number of inflorescences, while 'Tochiotome' grown at RPAS had the most runners. In this experiment, 'Tioga' at DPRS produced the largest number of fruit and had the highest yield.

## Introduction

IN THAILAND, annual cultural techniques are used for strawberry production. Planting densities range from 50 000–70 000 plants per hectare, with two or three hill-rows on raised beds separated by 0.8–1.0 m and plant spacing of 20–30 cm (Pipattanawong, 1996). Production of runners in the high elevation nursery from May to September results in an acceleration of flower bud initiation. The standard planting time is from early September to late October and harvesting is from early November to late April. Strawberries are grown over an area of about 500 ha with a total yield of 7000–8000 tonnes. About 60–70% of harvested fruits are processed and exported.

Effects of photoperiod and temperature on the vegetative and reproductive growth of strawberry plants are well documented. A number of factors have been shown to cause a decrease in vegetative growth (Piringer and Scott, 1964; Heide, 1977; Durner et al., 1984), runner production (Smeets, 1980), and flower induction (Dennis et al., 1970; Durner and Poling, 1987). Le Mière et al. (1998) found that temperature was positively correlated with the rate of progress to fruiting whereas crown size had no effect. Among the

strawberry cultivars introduced and tested for their adaptability in Thailand, 'Tioga' (Pipattanawong, 1996) and 'Tochiotome' (Akagi, 2001) cultivars are produced for processing and fresh fruit production, respectively. Strawberries are acquiring increasing economic and social importance in different regions of Chiang Mai and Chiang Rai provinces. Therefore, there is a need for a systematic study of the influence of elevations and environmental factors on growth and development in these cultivars. The objective of this experiment was to study the adaptability of 'Tioga' and 'Tochiotome' strawberry cultivars to different climatic conditions.

## Materials and methods

On 15 September 2002, uniformly sized runner plants of two June-bearing strawberries (*Fragaria* × *ananassa* Duch.) were grown in 3.8 L (18 cm diameter) plastic pots containing a mixture of soil and natural compost and left outside in a nursery at Doi Pui Research Station in Chiang Mai province. Observations were made on the cultivars 'Tioga' and 'Tochiotome'. Runners and old leaves of all plants were removed constantly during the experiment.

In late October 2002, plants of each cultivar were divided into three equal groups and moved to the Royal Agricultural Research Center (RARC), Royal Phang-da Agricultural Station (RPAS), and Doi Pui Research Station (DPRS) at elevations of 340 m,

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650 m, and 1300 m, respectively. A 3 × 2 factorial experiment in a completely randomised design with six replications was used in this study with eight plants per experimental unit. All plants were placed on the ground and exposed to the natural climate in the experimental areas. During the growing season, all plants were watered daily and fertilised biweekly with 50 mg per plant of fertiliser (15N-15P-15K). Insecticides and fungicides were applied as required. The number of leaves, number of crowns, petiole length, and mean leaf index (length × breadth of the middle leaflet) to estimate leaf area of the third unfolded leaves (counting from the youngest leaf) (Darrow, 1932) were recorded at two-week intervals. The flowering response of the plants during the experiment was evaluated by counting the total number of flowers and inflorescences per plant. Fruits were harvested at 2–3 day intervals during the growing season, graded, counted, and weighed. At the end of the experiment (30 March 2003), six plants per cultivar in each place were randomly selected, washed, and separated into different plant organs. The whole plants were then dried at 80°C for 48 h and the dry weights of separate organs were determined.

## Results

Figure 1 shows the influence of different elevations on bush size for two strawberry cultivars. In ‘Tochiotome’ plants at RARC, bush size increased after planting until the end of December 2002. Whereas ‘Tochiotome’ grown at RAPS exhibited more growth from the end of December 2002, ‘Tioga’ exhibited little growth at DPRS.

There were no differences in leaf production between the treatments after transplanting to early December 2002 (Fig. 2). In ‘Tioga’ grown at RPAS, the number of leaves increased more rapidly during

late December 2002 to early March 2003 than in the other treatments.

Variation in width and length of leaves of ‘Tioga’ and ‘Tochiotome’ in response to different elevations are shown in Figures 3 and 4. The results indicated that leaf length was more responsive to elevation after transplanting than leaf width.

The influence of different elevations on leaf area in two strawberry cultivars was also studied (Fig. 5). The leaf area of ‘Tioga’ grown at RPAS responded to elevation after the middle of November 2002, whereas that of ‘Tochiotome’ at the same place showed a delayed response from late December 2002. The strawberry plants grown at RARC had smaller leaf areas after December 2002 until the end of the experiment.

The possible influence of elevation on the petiole lengths of strawberry plants was measured under field conditions in this study (Fig. 6). The results showed that ‘Tioga’ and ‘Tochiotome’ grown at RARC had short petiole lengths compared with the other treatments.

The fresh and dry weights of shoots and whole plants of ‘Tioga’ grown at RPAS were heavier than those of the other treatments (Table 1). Whereas ‘Tioga’ grown at RARC had higher root fresh weight than the other treatments, there was no difference in dry weight compared with ‘Tochiotome’ grown at DPRS.

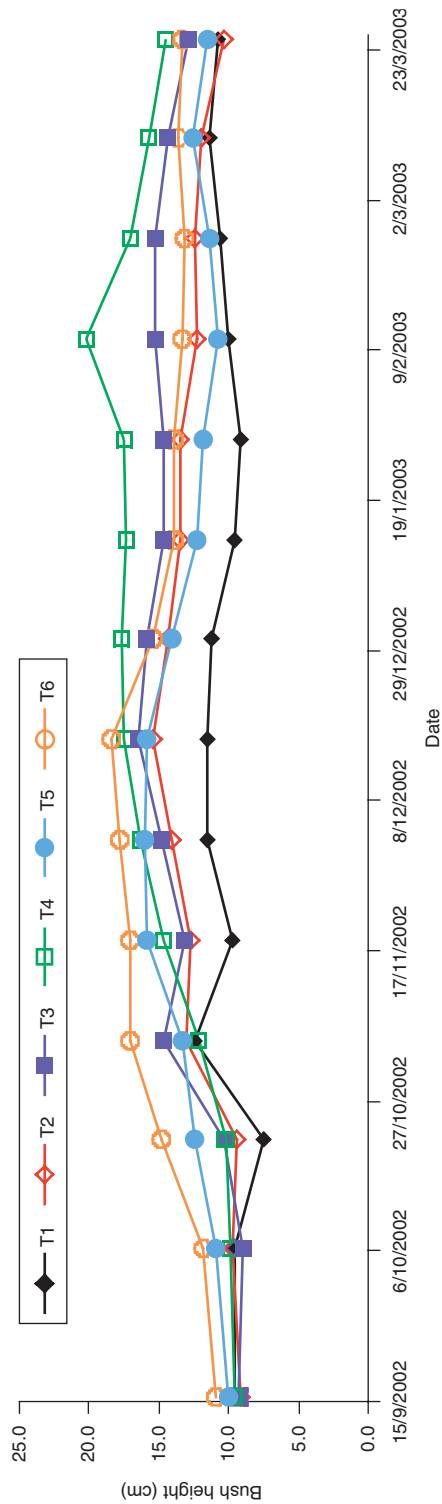
No differences in root lengths were observed between ‘Tioga’ and ‘Tochiotome’ grown at DPRS and ‘Tioga’ at RARC (Table 2). Large differences were found between regions in crown number. For example, the number of crowns in ‘Tioga’ plants at RPAS was more than double that at RARC (3.52 and 7.95 respectively). At RPAS, the ‘Tioga’ plants had significantly larger crown size than the strawberry plants grown at other sites.

**Table 1.** Influence of different elevations on fresh and dry weights of shoot, root and whole plant in two strawberry cultivars.

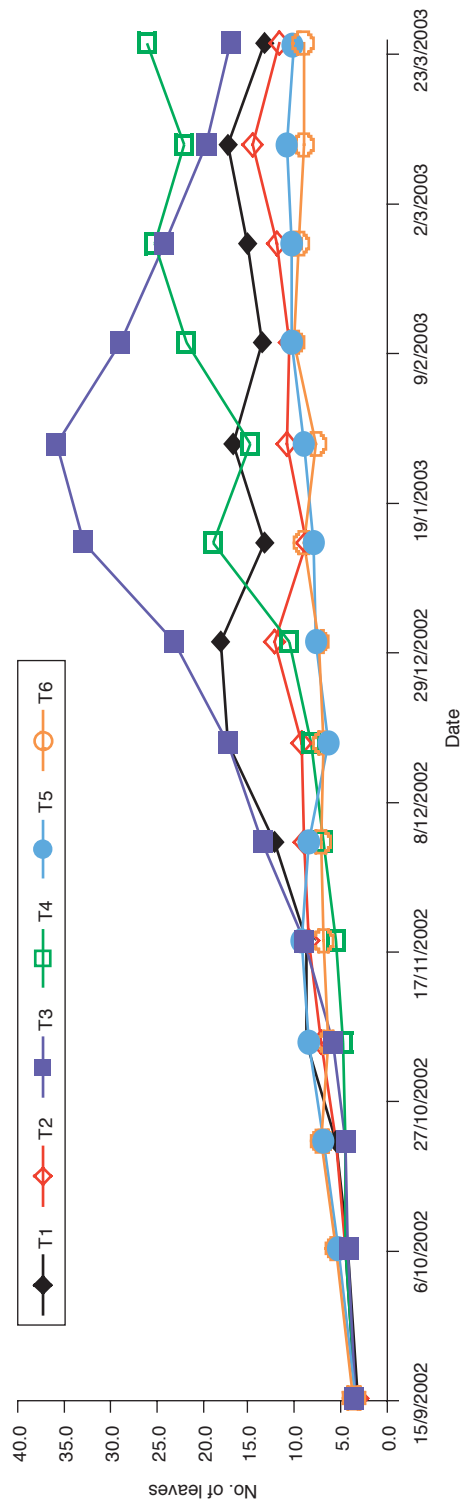
Treatments <sup>a</sup>	Fresh weights (g)			Dry weights (g)		
	Shoot	Root	Whole plant	Shoot	Root	Whole plant
1	46.43 b <sup>b</sup>	9.90 c	56.32 bc	9.92 b	3.58 c	13.52 b
2	44.60 bc	13.45 b	58.05 c	8.87 bc	4.85 ab	13.67 b
3	63.10 a	10.45 c	73.53 a	13.37 a	3.93 bc	17.28 a
4	45.25 b	12.32 bc	57.55 bc	7.82 cd	3.88 bc	11.67 c
5	45.85 b	18.10 a	63.92 b	7.82 cd	5.82 a	13.62 b
6	38.37 c	15.10 b	53.43 c	6.75 d	4.30 bc	11.05 c
F-test	*	*	*	*	*	*
CV (%)	11.89	19.10	11.62	11.62	22.78	11.30

<sup>a</sup> Treatments 1–6 were ‘Tioga’ and ‘Tochiotome’ strawberry cultivars grown at DPRS, RPAS, and RARC, respectively.

<sup>b</sup> Mean separation columns by Duncan’s multiple range test at  $P \leq 0.05$ .

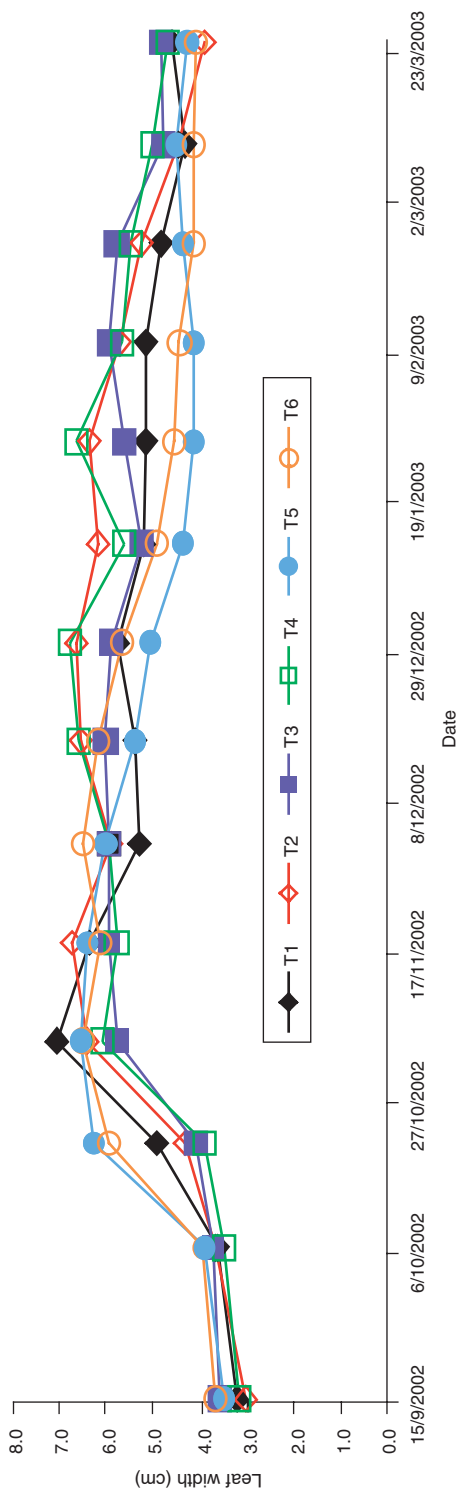


**Figure 1.** Influence of different elevations on bush height (cm) in two strawberry cultivars. Treatments 1–6 were ‘Toga’ and ‘Tochiotome’ strawberry cultivars grown at DPRS, RPAS, and RARC, respectively.

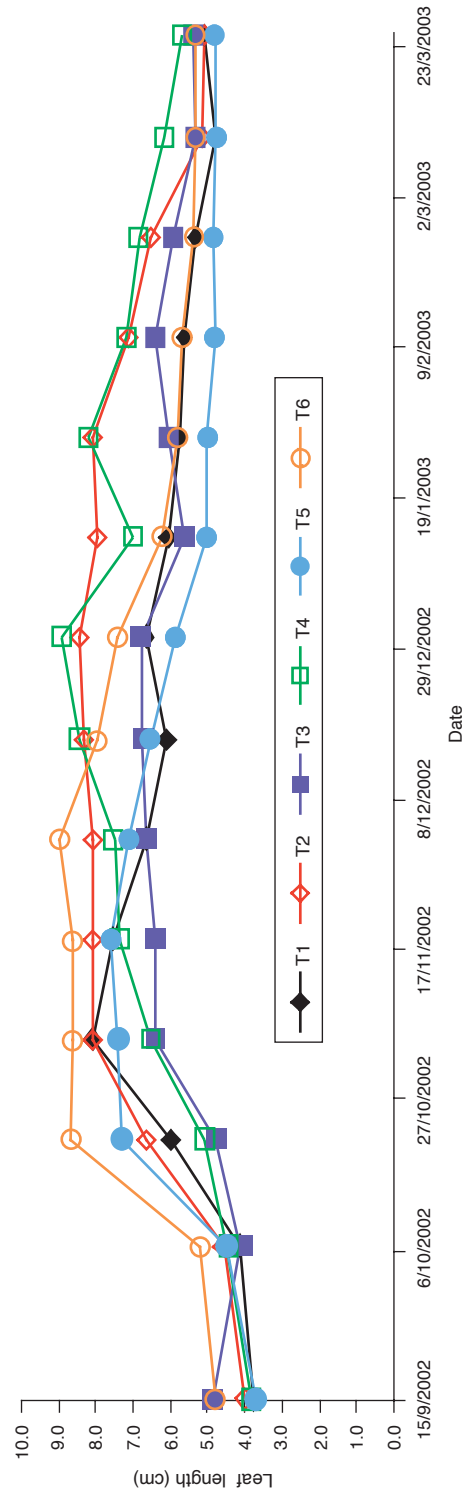


**Figure 2.** Influence of different elevations on number of leaves per plant in two strawberry cultivars. Treatments 1–6 were ‘Toga’ and ‘Tochiotome’ strawberry cultivars grown at DPRS, RPAS, and RARC, respectively.

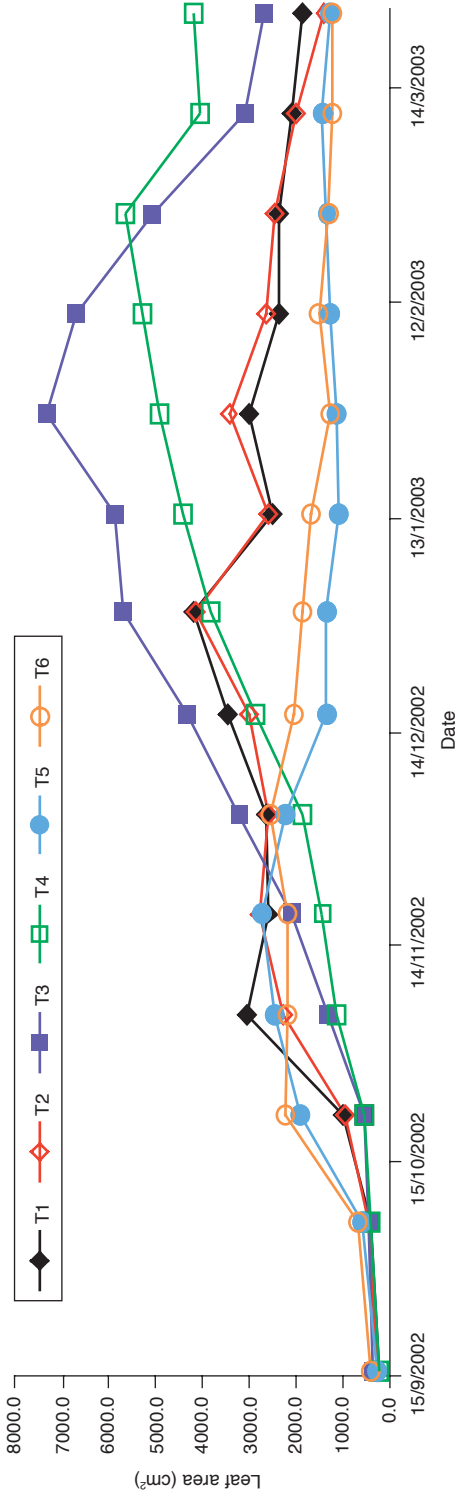




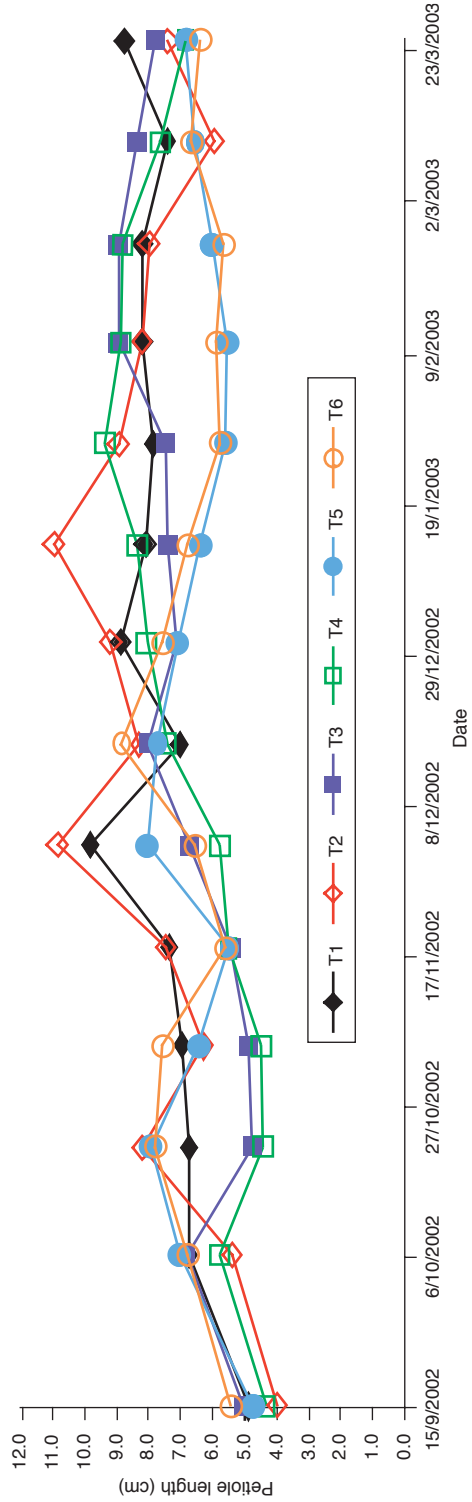
**Figure 3.** Influence of different elevations on leaf width (cm) in two strawberry cultivars. Treatments 1–6 were ‘Tioga’ and ‘Tochtotome’ strawberry cultivars grown at DPRS, RPAS, and RARC, respectively.



**Figure 4.** Influence of different elevations on leaf length (cm) in two strawberry cultivars. Treatments 1–6 were ‘Tioga’ and ‘Tochtotome’ strawberry cultivars grown at DPRS, RPAS, and RARC, respectively.



**Figure 5.** Influence of different elevations on total leaf area (cm<sup>2</sup>) in two strawberry cultivars. Treatments 1–6 were ‘Tioga’ and ‘Tochiotome’ strawberry cultivars grown at DPRS, RPAS, and RARC, respectively.



**Figure 6.** Influence of different elevations on petiole length (cm) in two strawberry cultivars. Treatments 1–6 were ‘Tioga’ and ‘Tochiotome’ strawberry cultivars grown at DPRS, RPAS, and RARC, respectively.

**Table 2.** Influence of different elevations on root volume, root length, number of crowns and crown size per plant in two strawberry cultivars.

Treatments <sup>z</sup>	Root length (cm)	No. of crowns/plant	Crown size (mm)
1	22.15 a <sup>y</sup>	5.60 b	8.05 bc
2	22.10 ab	3.52 c	9.60 a
3	19.72 bc	7.95 a	8.32 b
4	19.57 c	5.05 b	8.32 b
5	21.30 abc	7.63 a	8.48 b
6	19.63 c	5.00 b	7.00 c
F-test	*	*	*
CV (%)	9.90	16.25	11.16

<sup>z</sup> Treatments 1-6 were “Tioga” and “Tochiotome” strawberry cultivars grown at DPRS, RPAS, and RARC, respectively.

<sup>y</sup> Mean separation columns by Duncan’s multiple range test at  $P \leq 0.05$ .

There was no consistent pattern in date of flowering which was highly variable (Table 3). ‘Tochiotome’ grown at DPRS had a significantly lower number of inflorescences while ‘Tioga’ grown at DPRS had the highest number of inflorescences. The number of runners removed from ‘Tochiotome’ grown at DPRS was significantly higher than other treatments.

‘Tioga’ grown at DPRS produced greater yields and had more fruit per plant than the other treatments (Table 4). This cultivar also produced a higher proportion of fruit in the larger fruit size grades.

**Table 3.** Influence of different elevations on number of days from transplanting to the first flower blooming, number of inflorescences and number of runners per plant during reproductive growth in two strawberry cultivars.

Treatments <sup>a</sup>	Number of days	Number of inflorescences	Number of runners
1	67.50 b <sup>b</sup>	10.93 a	3.00 cd
2	40.80 a	7.40 c	9.17 ab
3	69.98 b	8.60 c	4.33 c
4	45.28 a	4.85 d	9.50 a
5	78.90 b	5.73 d	4.67 bc
6	47.23 a	9.22 b	2.50 d
F-test	*	*	*
CV (%)	19.11	14.46	35.38

<sup>a</sup> Treatments 1–6 were ‘Tioga’ and ‘Tochiotome’ strawberry cultivars grown at DPRS, RPAS, and RARC, respectively.

<sup>b</sup> Mean separation columns by Duncan’s multiple range test at  $P \leq 0.05$ .

Temperature data was recorded from September 2002 to March 2003 (Table 5). Mean temperatures for each month differed by about 5°C between DPRS and RARC, with the median temperature at RPAS.

## Discussion

In June-bearing strawberry cultivars, vegetative and reproductive developments are highly sensitive to climatic variables. Both photoperiod and temperature control the perennial cycle of producing runners and flowering (Guttridge, 1985; Le Mière et al., 1996), and this sensitivity can be exploited by growers who produce strawberry runner plants or fruit at certain times of the year (Le Mière et al., 1998). Temperature data was recorded from September 2002 to March 2003 (Table 5). Mean temperatures for each month differed by about 5°C between DPRS and RARC, with the median temperature at RPAS. ‘Tioga’ and ‘Tochiotome’ strawberry plants grown at RPAS have larger bush sizes, more leaves, and larger leaf areas compared with the plants grown at the other two sites. So, temperature affected the vegetative growth of strawberry plants in this experiment. There was a negative relationship between average growing temperature and leaf size in two strawberry cultivars. The plants grown at the highest temperature (RARC) showed smaller leaf size and leaf area, including petiole lengths. For other strawberry cultivars ‘Earliglow’ and ‘Kent’, the optimum day/night temperature for leaf and petiole growth was 25/12°C in Earliglow and Kent cultivars (Wang and Camp, 2000). Le Mière et al. (1998) also found that the canopy development in strawberry cv. Elsanta was more limited at warmer temperatures. Results have indicated the major role of higher temperature decrease on vegetative growth of some strawberry cultivars observed in the field.

Fresh and dry weight data for shoots and whole plants of ‘Tioga’ grown at RPAS indicated that plants had much higher crown production at this elevation. ‘Tochiotome’ at DPRS had the largest crown size but there was little effect of temperature on crown size in the other cultivars. Research has also been carried out on the interaction between the components of growth in the strawberry, often with conflicting results. In one study (Mason, 1987) temperature influenced crown diameter while in another study (Le Mière et al. 1998) it did not. Removal of runners can be used to stimulate and hasten branch crown development in both short-day and day-neutral strawberry types (Hancock, 1999). This shows the importance of studying the influence of culture and environmental factors on cropping in strawberries.

‘Tioga’ grown at DPRS and RPAS produced more runners in this study but had the least runners when grown at RARC. Apparently the lower temperature enables the plants to obtain an adequate runner production. In addition, Durner et al. (1984) have reported that the runners of short-day strawberry plants are produced after flowering at the base of

**Table 4.** Influence of different elevations on total yields, number of fruits per plant, and number of fruit grades by weight in two strawberry cultivars.

Treatments <sup>a</sup>	Total yields (g)	No. of fruits	Number of fruit grades					
			> 25 g	15–25 g	11–15 g	9–11 g	7–9 g	< 7 g
1	1,425.00 a <sup>b</sup>	262.20 a	0.00 b	3.17 b	10.33 a	17.67 a	46.00 a	186.50 a
2	981.20 b	174.80 b	0.17 b	6.50 a	9.50 a	13.17 b	30.17 b	115.80 b
3	761.00 c	130.20 cd	0.00 b	0.33 c	2.17 b	11.33 bc	22.03 b	94.50 b
4	613.20 c	92.83 e	0.00 b	0.67 c	2.00 b	7.00 c	25.67 b	56.50 c
5	649.50 c	166.70 bc	0.17 b	0.17 c	0.83 b	1.17 d	6.83 d	157.50 a
6	614.30 c	122.20 de	1.67 a	1.50 bc	2.17 b	1.50 d	12.17 c	104.00 b
F-test	*	*	*	*	*	*	*	*
CV (%)	16.79	19.73	-	86.40	33.88	35.27	10.56	4.28

<sup>a</sup> Treatments 1–6 were ‘Tioga’ and ‘Tochiotome’ strawberry cultivars grown at DPRS, RPAS, and RARC, respectively.

<sup>b</sup> Mean separation columns by Duncan’s multiple range test at  $P \leq 0.05$ .

**Table 5.** Maximum, minimum and mean temperature (°C) at DPRS, RPAS and RARC (15 Sept 2002 – 31 Mar 2003).

Months	Temp. (°C)								
	Max.			Min.			Mean		
	DPRS	RPAS	RARC	DPRS	RPAS	RARC	DPRS	RPAS	RARC
Sept.	22.0	26.1	27.7	19.1	22.1	21.1	20.8	24.2	25.6
Oct.	22.6	26.4	28.5	18.7	20.8	23.4	20.4	24.1	26.1
Nov.	21.5	25.6	27.0	13.9	17.9	20.5	18.7	22.4	24.2
Dec.	21.2	24.7	25.8	14.7	17.1	19.1	17.9	21.5	23.0
Jan.	17.8	22.6	24.0	14.6	17.5	19.1	16.4	19.4	21.2
Feb.	21.9	23.3	25.0	13.6	18.8	20.3	19.5	20.9	23.0
Mar.	22.8	24.5	29.1	18.6	20.3	22.8	21.2	22.8	26.2

**Table 6.** Maximum, minimum and mean relative humidity (%) at DPRS, RPAS and RARC (15 Sept 2002 – 31 Mar 2003)

Months	%RH								
	Max.			Min.			Means		
	DPRS	RPAS	RARC	DPRS	RPAS	RARC	DPRS	RPAS	RARC
Sept.	93.4	98.1	91.3	76.5	84	80.7	85.9	90.3	84
Oct.	99	91.6	84.3	60.2	65.4	75.3	86.7	76.4	81.5
Nov.	99	93.1	98.4	72.5	72.7	75.3	88	79.5	84.8
Dec.	99	83.3	94.5	61.2	64	77.4	87.1	75.5	85.8
Jan.	99	91.1	98.9	66.4	63	76.5	85.2	72.9	83.9
Feb.	87.4	72.5	85.1	39.6	52.9	60.1	65.5	63.1	68.9
Mar.	96.7	86.2	77.6	38.2	56.3	44.4	69.6	66.7	58.5

new leaves, and are formed most readily during long days (>10 h) when temperatures are in the range of 21–30°C.

The short-day strawberry types actually initiate flower buds either under short-day conditions (<14 h)

or when temperatures are less than 15°C (Guttridge, 1985; Larson, 1994). In this study, all ‘Tochiotome’ plants grown at three elevations flowered within 40–50 days after transplanting, while ‘Tioga’ plants required 68 or more days. The results indicate that

'Tochiotome' is the earlier variety due to an earlier season compared with 'Tioga' in this study. However, the 'Tioga' cultivar is widely planted in Thailand because of its larger fruit size, attractiveness, firmness, productivity, and also good adaptation (Pipattanawong, 2000).

In this study, the strawberry plants received the lowest temperature at DPRS and had already formed flowers before the beginning of the experiment. The duration of cropping was also longer at lower temperatures and so plants grown at lower temperatures would have had longer to produce and transport assimilates to the fruit (Le Mière et al., 1998). Plants grown at DPRS tend to have higher productivity, especially cv. 'Tioga'. Higher temperature did not have a significant negative effect on fruit number. The average size of the fruits produced by the plants in these treatments was smaller. This may be due to the adverse effects of lower night temperature at DPRS and higher day temperature at RPAS and RARC on pollination and early fruit development.

Understanding of the physiology and development of two strawberry cultivars grown at different elevations is of potential value to strawberry growers in Thailand.

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# A trial of rest-breaking chemicals on low-chill peach and nectarine

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## Abstract

Several studies were conducted on rest-breaking chemicals to modulate rest and flowering on low-chill peach cvs. Florida Grande and Florida King and nectarine cv. Sun Wright. Trials were conducted on 5-year-old trees using randomised complete block (RCB) designs, with treatments applied to single tree replicates. Treatments were: control; KNO<sub>3</sub>, 5%; Waiken, 2%; Waiken, 2% + KNO<sub>3</sub>, 5%; Waiken, 4%; Waiken, 4% + KNO<sub>3</sub>, 5%. Results from 2002–2003 showed that the rest-breaking chemical, Waiken, when applied in combination with KNO<sub>3</sub>, can effectively break floral bud dormancy, advance flowering by up to 1–2 weeks and concentrate flowering intensity. These chemicals may indirectly improve fruit size in low-chill peach cv. Florida Grande and nectarine cv. Sun Wright but decrease fruit size in medium-chill cv. Florida King.

## Introduction

IN REGIONS that receive insufficient chilling, dormancy may be broken through the application of selected chemicals such as mineral oil, GA<sub>3</sub>, potassium nitrate (KNO<sub>3</sub>), Thiourea, hydrogen cyanamide (H<sub>2</sub>CN<sub>2</sub>) (commercial names Dormex® and Moregrape®), and fatty acids, eg Waiken. The response of temperate fruit trees to these chemicals which break dormancy is often inconsistent and the efficacy of the chemicals depends on their application rate (Erez, 1983, 1985, 1995). Phytotoxicities can also occur because these chemicals must be applied at concentrations that cause sub-lethal stress to the floral buds (Black, 1952; Erez and Lavee, 1974).

Waiken is a new group of rest-breaking chemicals comprising a mixture of various fatty acid esters. Its mechanism of breaking dormancy is not known but it may expedite changes in endogenous fatty acids, which occur during late dormancy (Wang and Faust, 1998). With very low-chill cultivars of stone fruit,

Waiken alone was effective in advancing and concentrating flowering but this was not the case with medium-chill cultivars.

With very low-chill cultivars, potassium nitrate has been shown to be an effective rest-release chemical (George and Nissen, 1993). Application of this chemical in the early dormancy period can result in advances of flowering, fruit set and fruit maturity of between 10–14 days. Only slight reductions in fruit set as a result of using this chemical have been recorded (George and Nissen, 1988). Potassium nitrate by itself is a rather mild rest-breaking chemical but the efficacy is enhanced when combined with other chemicals (Erez *et al.*, 1971; Erez, 1987; George and Nissen, 1993; Erez, 1995). More recently, the efficacy of potassium nitrate has been improved considerably by combining it with the surfactant Amobreak (North, 1992).

## Materials and methods

The experiment was conducted on peach cvs. Florida Grande, Florida King and nectarine cv. Sun Wright at Chiang Mai Royal Agricultural Research Center, Khun Wang, Thailand, from 2001 to 2003. The trees selected were 4-year-old peach and nectarine trees spaced 3.5 m within row and 4.0 m between rows. In autumn, 36 trees of each cultivar were sprayed with 2.5% KNO<sub>3</sub> eight times. The trees were given

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an interval of 1 week for pre-conditioning before reaching dormancy and then were winter pruned about October. Rest-release chemicals were applied on 7 November 2001 in year 1 and on 20 November 2002 in year 2. The experimental design was a randomised complete block with the following six treatments applied to six single tree replicates: water (control);  $\text{KNO}_3$ , 5%; Waiken, 2%; Waiken, 2% +  $\text{KNO}_3$ , 5%; Waiken, 4%; Waiken, 4% +  $\text{KNO}_3$ , 5%.

The rest-release chemical sprays were applied to individual trees by hand sprayer. Trees were all managed and thinned according to standard practice. Fruit was harvested commercially at the optimum stages of maturity for the local market. The number of fruits removed at each harvest were recorded for fruit diameter, fruit weight and fruit quality. A sample of 5–10 fruit of picking maturity was measured at each harvest. Data were analysed using ANOVA and significant differences between means tested at  $P = 0.05$  level.

## Results

### Times of flowering

#### Year 2001–2002

*cv. Florda Grande.* Treated trees reached 50% open flowering about 1 month after spraying and about 3–6 days before untreated trees (Fig. 1). Treated trees reached full flowering about 2–10 days before the control.

*cv. Sun Wright.* Treated trees flowered (50% open flowering) only a few days before controls (Fig. 2). However, treated trees showed full open flowering 3–12 days before the control treatment.

*cv. Florda King.* Clearly, rest-release chemicals had greater effect on medium-chill cultivars like *cv. Florda King* where they could induce flowering about 2 weeks

earlier than the control treatment (Fig. 3). However, days to full flowering were the same in all treatments.

#### Year 2002–2003

*cv. Florda Grande.* The time to 50% flowering for treated trees was 5–10 days later than for untreated trees. However, treated trees reached full flowering about 2 weeks earlier than the control treatment.

*cv. Sun Wright.* The rest-breaking chemical treatments showed no difference to the control in time to 50% flowering but the treatment seemed to concentrate flowering more than the control, particularly for the application of Waiken, 4% +  $\text{KNO}_3$ , 5%.

*cv. Florda King.* Chemical treatments delayed and moderated flowering by 2–3 weeks but full flowering was completed at the same time as the control treatment.

### Bud break percentage

#### Year 2001–2002

*cv. Florda Grande.* Compared with controls, the Waiken and  $\text{KNO}_3$  combination exhibited a higher bud break percentage for both floral and leaf buds (Table 1).

*cv. Sun Wright.* The application of Waiken, 2% +  $\text{KNO}_3$ , 5% produced the highest floral bud break percentage (Table 2).

*cv. Florda King.* All chemical-treated trees had better floral and leaf bud break percentages than the control. The Waiken sprayed treatments had high percentages of floral and leaf bud break (Table 3).

#### Year 2002–2003

*cv. Florda Grande.* The Waiken 4% treatment exhibited a higher leaf bud break percentage, significantly different from the control treatment (Table 1).



Department of Agriculture regional director Uthai Noppakoonwong and senior researcher Pichit Sripinta assess the results of trials at Chiang Mai Royal Agricultural Research Center, Khun Wang, Thailand

**Table 1.** Bud break, flower bud break and leaf bud break percentages of peach var. Florida Grande in 2002–2003.

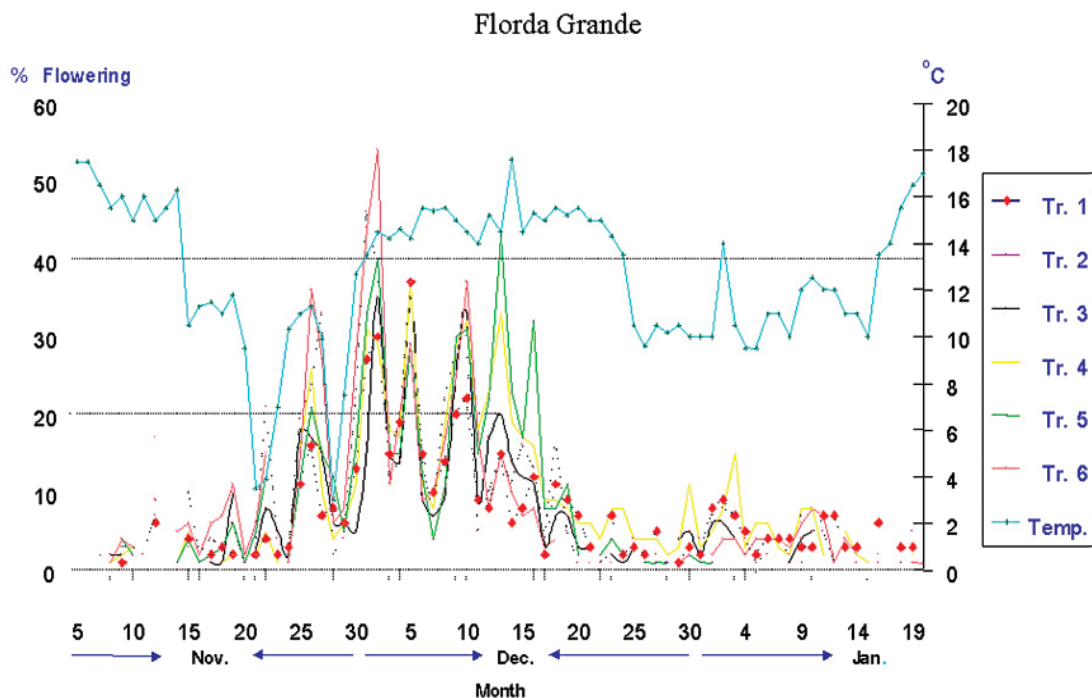
Treatment	% bud break		% flower bud break		% leaf bud break	
	2002	2003	2002	2003	2002	2003
Control	66.1	42.0 ab	69.8 ab	33.6	60.6 b	56.1 ab
KNO <sup>3</sup> 5%	67.6	33.2 b	69.4 ab	29.7	63.9 ab	38.4 b
Waiken 2%	62.4	39.2 ab	62.5 b	29.9	72.1 ab	52.9 ab
Waiken 2% + KNO <sup>3</sup> 5%	76.2	38.4 ab	74.0 ab	26.0	81.2 a	54.8 ab
Waiken 4%	64.7	47.9 a	64.9 b	31.8	76.5 ab	69.6 a
Waiken 4% + KNO <sup>3</sup> 5%	73.7	41.8 ab	80.0 a	30.6	63.2 ab	58.6 a

Means followed by the same letter are not significantly different as determined by LSD at  $P < 0.05$ .

**Table 2.** Bud break, flower bud break and leaf bud break percentages of nectarine var. Sun Wright in 2002–2003.

Treatment	% bud break		% flower bud break		% leaf bud break	
	2002	2003	2002	2003	2002	2003
Control	74.6	46.8	85.5	43.0	40.7	60.3
KNO <sup>3</sup> 5%	81.9	48.6	89.3	43.4	48.6	66.2
Waiken 2%	75.5	46.5	93.7	45.8	45.3	54.8
Waiken 2% + KNO <sup>3</sup> 5%	80.5	45.9	99.0	44.5	40.3	49.4
Waiken 4%	82.8	54.7	95.6	56.4	42.7	50.1
Waiken 4% + KNO <sup>3</sup> 5%	78.1	44.4	94.7	41.3	31.8	52.0

Means followed by the same letter are not significantly different as determined by LSD at  $P < 0.05$ .



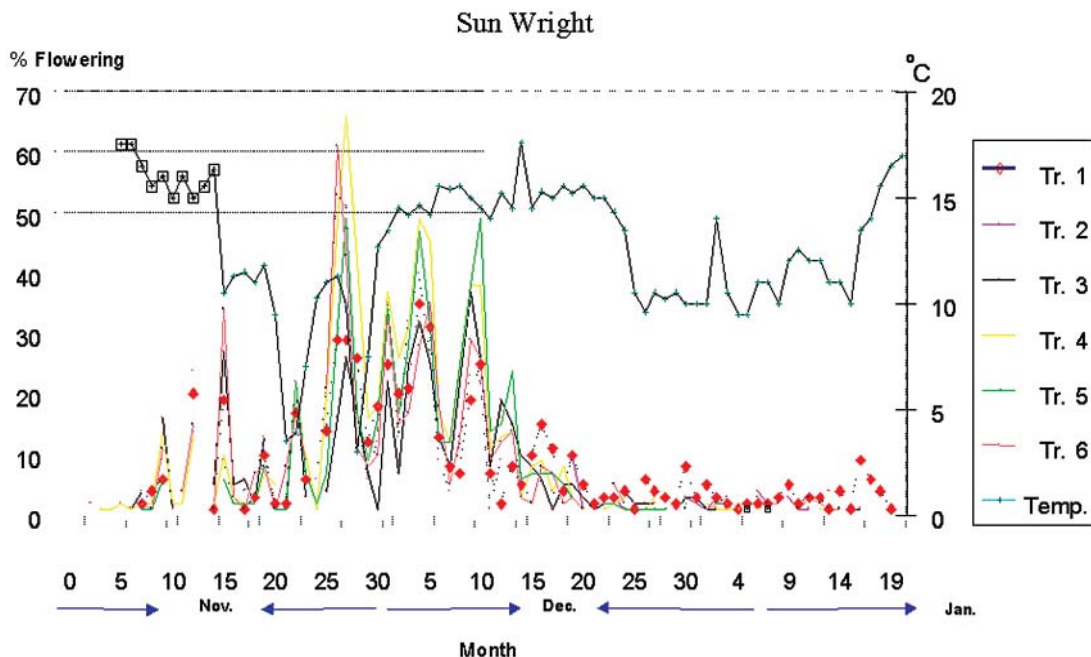
**Figure 1.** Effect of rest-release chemical on flowering in ‘Florida Grande’ peach, 2001–2002.



**Table 3.** Bud break, flower bud break and leaf bud break percentages of peach var. Florida King in 2002–2003.

Treatment	% bud break		% flower bud break		% leaf bud break	
	2002	2003	2002	2003	2002	2003
Control	23.1	24.6	20.4 b	18.9	29.8	12.1
KNO <sup>3</sup> 5%	33.6	34.6	45.1 ab	34.7	27.4	35.2
Waiken 2%	38.1	28.8	55.1 a	26.9	22.4	32.1
Waiken 2% + KNO <sup>3</sup> 5%	33.8	26.2	32.5 ab	28.3	24.1	24.2
Waiken 4%	32.2	34.7	24.6 b	31.0	42.3	39.0
Waiken 4% + KNO <sup>3</sup> 5%	33.2	27.3	39.1 ab	29.5	28.2	27.7

Means followed by the same letter are not significantly different as determined by LSD at P < 0.05.



**Figure 2.** Effect of rest-release chemical on flowering in ‘Sun Wright’ nectarine, 2001–2002.

*cv. Sun Wright.* Waiken 4% had higher bud break and floral bud break percentages than the control treatment. For the leaf bud break chemical, KNO<sub>3</sub>, 5% exhibited the highest percentage bud break (Table 2).

*cv. Florida King.* All chemical treatments, particularly Waiken, 4% and KNO<sub>3</sub>, 5% had high bud break, flower bud break and leaf bud break percentages when compared to the control treatment (Table 3).

### Yield per tree

#### Year 2002

*cv. Florida Grande.* The average yield per tree for all treatments were not significantly different (Table 4).

*cv. Sun Wright.* All chemical treatments had higher yield per tree than the control. The Waiken 4% spray had the greatest yield per tree, significantly different from the control treatment (Table 5).

*cv. Florida King.* Yield per tree of Florida King was very low and there were no differences between treatments (Table 6).

#### Year 2003

*cv. Florida Grande.* All chemical treatments had higher fruit yield per tree than the control (Table 7). The combination of Waiken, 2% and KNO<sub>3</sub>, 5% had the highest yield per tree and was significantly different from the untreated control.

cv. *Sun Wright*. The trees treated with Waiken, 4% had the highest yield per tree but it was not significantly different from the control treatment (Table 8).

cv. *Florida King*. Treatments with Waiken, 4% and Waiken, 4% + KNO<sub>3</sub>, 5% had greater yield per tree than the untreated control (Table 9). The other chemical treatments did not differ from the control.

### Fruit size and fruit quality

The chemical did not directly affect the fruit size and fruit quality of peach and nectarine. It may result in advanced or delayed harvesting times caused by a prolonged or shortened fruit development period.

### Year 2002

cv. *Florida Grande*. There were no differences between treatments in fruit size or fruit quality (Table 4).

cv. *Sun Wright*. The Waiken 2% and 4% sprays, on average, had greater fruit weight than the control (Table 5). There was no difference in fruit firmness, TSS or TA content between treatments.

cv. *Florida King*. The rest-release chemical affected fruit size of cv. *Florida King* peach (Table 6). The average fruit weight of treated trees was significantly smaller than the control (Table 6). The Waiken, 2% and 4% treatments, had higher fruit firmness values than the control. The Waiken, 2% treatment showed a higher TSS content than the control.

**Table 4.** Average yield per tree and fruit quality (fruit size, fruit firmness, TSS and TA) of peach var. *Florida Grande* in 2002.

Treatment	Yield per tree (kg)	Fruit weight (g)	Fruit width (cm)	Fruit length (cm)	Firmness (kg/cm <sup>2</sup> )	TSS (%)	TA (%)
1. Control	13.2	126	6.02	6.35	1.16	10.1	0.58
2. KNO <sub>3</sub> 5%	11.8	126	6.06	6.45	1.15	10.5	0.54
3. Waiken 2%	13.2	124	6.04	6.41	1.22	10.3	0.58
4. Waiken 2% + KNO <sub>3</sub> 5%	14.0	119	5.92	6.31	1.14	10.5	0.61
5. Waiken 4%	12.3	125	6.03	6.36	1.22	10.4	0.62
6. Waiken 4% + KNO <sub>3</sub> 5%	11.3	121	6.17	6.40	1.26	10.3	0.58

Means followed by the same letter are not significantly different as determined by LSD at  $P < 0.05$ .

**Table 5.** Average yield per tree and fruit quality (fruit size, fruit firmness, TSS and TA) of nectarine var. *Sun Wright* in 2002.

Treatment	Yield per tree (kg)	Fruit weight (g)	Fruit width (cm)	Fruit length (cm)	Firmness (kg/cm <sup>2</sup> )	TSS (%)	TA (%)
1. Control	3.03 b	79.8	5.15	5.19	1.57 a	11.7	0.95
2. KNO <sub>3</sub> 5%	4.29 ab	81.5	5.29	5.23	1.40 ab	11.6	0.99
3. Waiken 2%	4.72 ab	89.8	5.45	5.49	1.42 ab	10.8	0.99
4. Waiken 2% + KNO <sub>3</sub> 5%	4.54 ab	84.5	5.39	5.32	1.50 ab	11.2	1.02
5. Waiken 4%	5.65 a	94.4	5.45	5.42	1.48 ab	10.9	1.03
6. Waiken 4% + KNO <sub>3</sub> 5%	3.66 ab	81.1	5.23	5.28	1.13 b	11.4	0.9

Means followed by the same letter are not significantly different as determined by LSD at  $P < 0.05$ .

**Table 6.** Average yield per tree and fruit quality (fruit size, fruit firmness, TSS and TA) of peach var. *Florida King* in 2002.

Treatment	Yield per tree (kg)	Fruit weight (g)	Fruit width (cm)	Fruit length (cm)	Firmness (kg/cm <sup>2</sup> )	TSS (%)	TA (%)
1. Control	0.63	246 a	7.53	8.04	1.10	11.7	0.52
2. KNO <sub>3</sub> 5%	0.52	144 b	6.52	6.91	1.04	11.3	0.64
3. Waiken 2%	0.96	183 ab	7.07	7.31	1.59	13.2	0.51
4. Waiken 2% + KNO <sub>3</sub> 5%	0.49	172 ab	6.73	6.85	1.15	11.6	0.72
5. Waiken 4%	0.24	209 ab	7.28	7.65	1.58	11.2	0.83
6. Waiken 4% + KNO <sub>3</sub> 5%	0.16	145 b	6.23	6.85	1.33	11.2	0.73

Means followed by the same letter are not significantly different as determined by LSD at  $P < 0.05$ .

Year 2003

*Florda Grande*. The rest-release chemical treatments did not have any effect on fruit size of cv. *Florda Grande* peach (Table 7). The chemical appeared to decrease TSS content and increase TA content compared to the control.

cv. *Sun Wright*. Waiken, 4% + KNO<sub>3</sub>, 5% treated trees had an average fruit weight about 10% greater than untreated trees (Table 8). The treatment also increased TSS content significantly compared to the control. The sugar content of Waiken treated trees was up to 15% higher (maximum 14.5 Brix) than the control.

cv. *Florda King*. The results were similar to the previous year (Table 9). The rest-release chemical treatments had smaller fruit size than the control. The TSS content of chemically treated trees was lower than the control but the TA content was no different.

## Discussion

The effect of Waiken on flower bud development was dependent on the application rate and stone fruit variety. The application of Waiken advanced bud burst by about 3–6 days for cv. *Florda Grande* and by 4 weeks for cv. *Florda King*. Waiken also advanced full bloom by 3–10 days and also condensed the flowering period. The higher concentration of Waiken when applied in combination with KNO<sub>3</sub> did

not advance flowering but condensed the flowering period considerably in cv. *Florda Grande* and cv. *Sun Wright*. The chemical application delayed flowering by up to 1–3 weeks at the later application times but showed less effect in closing the flowering periods. At the high rate, Waiken condensed the flowering period and increased the percentage of full bloom peaks.

Based on this trial, the application of Waiken did not advance fruit maturation, possibly because of high temperature during fruit maturation that shortened the harvest period. Most fruits were harvested within 2 weeks. Normally, rest-release treatments that advance flowering also advance fruit maturity. Similarly, concentrated flowering results in a shorter harvest period and, even more, in a shorter period of fruit maturity.

Waiken did not affect fruit size significantly. However, there was a trend to larger fruit with chemical treatments, particularly in cvs *Florda Grand* and *Sun Wright*. This may have been because the chemicals increased floral bud burst and improved early fruit development. Chemicals also increase vegetative bud break and leaf development, which can result in a high rate of photosynthesis and starch accumulation for increasing fruit size. However, for cv. *Florda King*, the fruit size did not improve because this cultivar is very poor when grown in the high humidity conditions of the highlands and leaf bud burst percentage was also low compared to untreated trees.

**Table 7.** Average yield per tree and fruit quality (fruit size, fruit firmness, TSS and TA) of peach var. *Florda Grande* in 2003.

Treatment	Yield per tree (kg)	Fruit weight (g)	Fruit width (cm)	Fruit length (cm)	Firmness (kg/cm <sup>2</sup> )	TSS (%)	TA (%)
1. Control	2.59 b	119	5.89	6.27	2.04	13.2 a	0.62 a
2. KNO <sub>3</sub> 5%	5.25 ab	112	5.79	6.20	1.92	11.4 bc	0.85 bc
3. Waiken 2%	5.69 ab	118	5.95	6.18	1.53	10.6 c	0.70 ab
4. Waiken 2% + KNO <sub>3</sub> 5%	6.37 a	114	5.83	6.19	1.93	12.8 ab	0.83 abc
5. Waiken 4%	3.38 ab	110	5.75	6.15	1.66	11.1 c	1.00 c
6. Waiken 4% + KNO <sub>3</sub> 5%	5.44 ab	110	5.84	5.84	1.43	11.4 bc	0.73 ab

Means followed by the same letter are not significantly different as determined by LSD at  $P < 0.05$ .

**Table 8.** Average yield per tree and fruit quality (fruit size, fruit firmness, TSS and TA) of nectarine var. *Sun Wright* in 2003.

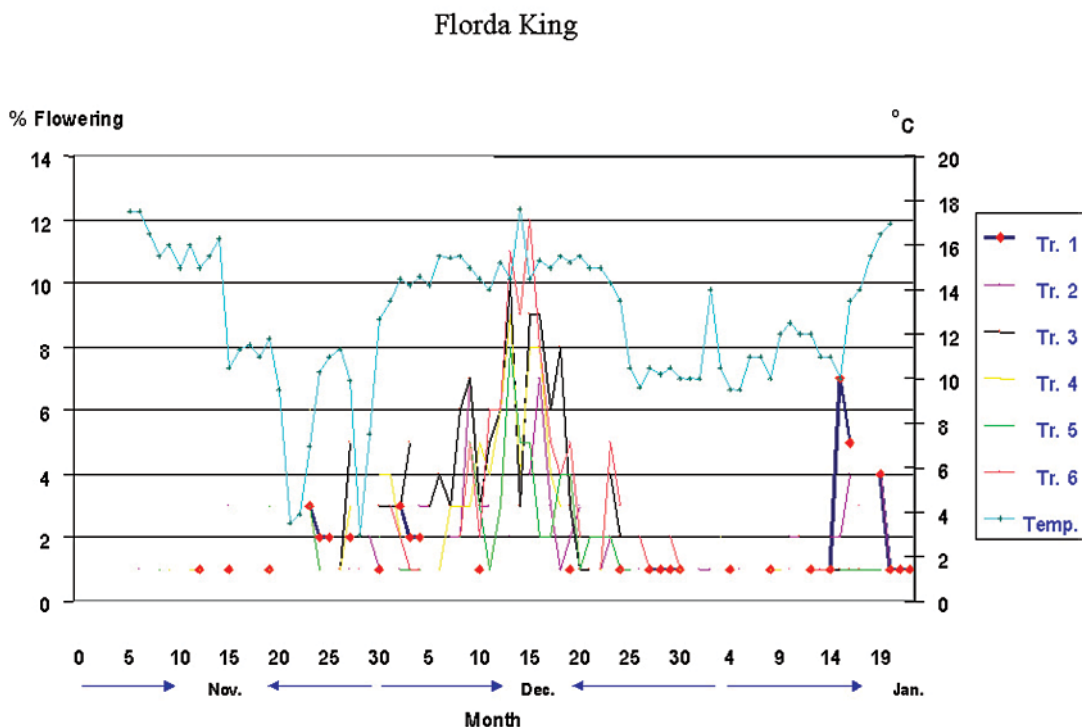
Treatment	Yield per tree (kg)	Fruit weight (g)	Fruit width (cm)	Fruit length (cm)	Firmness (kg/cm <sup>2</sup> )	TSS (%)	TA (%)
1. Control	3.55	71.0	4.89	5.32	1.97	12.5 b	1.52 ab
2. KNO <sub>3</sub> 5%	4.64	75.9	5.00	5.28	1.81	14.2 a	1.41 a
3. Waiken 2%	3.53	70.7	4.92	5.22	2.02	13.5 ab	1.38 a
4. Waiken 2% + KNO <sub>3</sub> 5%	4.19	78.4	5.01	5.27	2.12	13.3 ab	1.68 ab
5. Waiken 4%	5.30	79.7	5.12	5.44	2.03	13.2 ab	1.38 a
6. Waiken 4% + KNO <sub>3</sub> 5%	3.35	79.0	5.08	5.48	2.25	14.5 a	1.85 b

Means followed by the same letter are not significantly different as determined by LSD at  $P < 0.05$ .

**Table 9.** Average yield per tree and fruit quality (fruit size, fruit firmness, TSS and TA) of peach var. Florida King in 2003.

Treatment	Yield per tree (kg)	Fruit weight (g)	Fruit width (cm)	Fruit length (cm)	Firmness (kg/cm <sup>2</sup> )	TSS (%)	TA (%)
1. Control	0.55	260	7.92	8.44 a	1.59 a	10	0.37
2. KNO <sup>3</sup> 5%	0.51	235	7.46	8.34 ab	0.86 ab	9.29	0.24
3. Waiken 2%	0.85	202	7.19	7.68 b	0.47 b	9.53	0.35
4. Waiken 2% + KNO <sup>3</sup> 5%	0.63	191	7.01	8.06 ab	1.43 a	9.77	0.33
5. Waiken 4%	1.37	235	7.32	8.47 a	1.18 ab	9.97	0.36
6. Waiken 4% + KNO <sup>3</sup> 5%	1.46	217	7.42	7.77 ab	1.58 a	9.70	0.38

Means followed by the same letter are not significantly different as determined by LSD at  $P < 0.05$ .



**Figure 3.** Effect of rest-release chemical on flowering in ‘Florida King’ peach, 2001–2002.

The results from these trials indicated that Waiken is a very effective chemical for breaking dormancy in peach and nectarine. Its effect is dependent on rate and time of application. Waiken, 2–4 % KNO<sub>3</sub> and a combination of the two advance and enhance bud break and flowering in Florida Grande, Sun Wright and Florida King. It may delay flowering slightly but still results in a stronger and more concentrated blossom period. Similar results were reported by Erez et al. (1971) who showed that KNO<sub>3</sub> advanced flowering in peach. George and Nissen (1988) trialled KNO<sub>3</sub> with nectarine and found similar results.

The NO<sub>3</sub> ion is efficient in advancing flowering, particularly in low-chill stone fruit cultivars (Erez and Lavee, 1974; George and Nissen, 1988). George et al. (1999) also stated that Waiken induced early flowering in low-chill temperate fruit by 3–14 days and condensed flowering.

Waiken or KNO<sub>3</sub> application alone and in combination stimulated vegetative bud break, floral bud break, and promoted early leaf growth and development in peach and nectarine. The NO<sub>3</sub> ion increases arginine levels that promote flowering (George et al., 1999). The effect on flowering and leaf development

was more pronounced at the higher rates of Waiken. For cvs Florida Grande and Sun Wright, the optimum rate of Waiken seems to be 2–4%. The most effective treatment was Waiken, 2% + KNO<sub>3</sub>, 5%.

All the rest-release chemical and combinations used in this experiment stimulated vegetative bud break and leaf development. Early stimulation of vegetative bud break and leaf growth has been correlated with poor fruit retention in the lower canopy of some high-chill varieties (Erez, 1995). Presumably the increased level of competition between flowers and vigorously growing leaves may reduce flower retention and fruit set.

### Conclusion

The new fatty acid chemical, Waiken, is highly effective in breaking dormancy in low-chill peach and nectarine. Its effect is dependent on the time and rate of application. The optimum rate seems to be 2–4% and it should be applied about 30–45 days before normal bud break. Late applications may delay flowering while spraying too early reduces fruit set and yield. From these trials it could be concluded that:

- Rest-release chemicals advanced flowering and concentrated flowering in cvs Florida Grande, Sun Wright and Florida King by up to 1–2 weeks.
- Waiken alone or in combination with KNO<sub>3</sub> improved bud break for both floral and leaf buds, but the response depends on cultivar and rate of application.
- Waiken and KNO<sub>3</sub> may increase fruit set and result in increased yield per tree in cvs Florida Grande and Sun Wright.
- Waiken, KNO<sub>3</sub> or a combination of the two had no effect on fruit size in cv. Florida Grande but had an indirect effect on fruit size in cv. Sun Wright. For cv. Florida King, fruit size was smaller than in untreated trees.
- Waiken and KNO<sub>3</sub> had no effect on fruit quality of peach and nectarine.

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# Monthly optimal amount of water used by a peach tree

Kumut Sangkhasila<sup>1</sup> and Nattawee Mabangkru<sup>2</sup>

## Abstract

The main objective of this research was to develop a recommendation for monthly optimal water use by peach trees [*Prunus persica* (L.) Batsch]. Several physiological responses were used as indicators to determine what was a sufficient amount of water for a peach tree to use. Responses included diurnal courses of total leaf water potential ( $\psi_{\text{leaf}}$ ), rate of transpiration (TR, measured by sap flow rates), and components of yield and yield quality. These parameters were measured monthly (except the yield components, which were measured only once at harvest). Vapor pressure deficit (VPD) was also measured and was treated as the evaporative demand. Treatments were three levels of irrigation, namely 0.5, 1.0, and 2.0 ETp with three replications. Differences between diurnal courses of  $\psi_{\text{leaf}}$  were not statistically different between irrigation treatments and no TR- $\psi_{\text{leaf}}$  relationships were found in this experiment. Daily rates of TR ranged from 182–497 and 90–290 g of water per cm<sup>2</sup> of trunk X-area per day for 0.5 ETp and 2.0 ETp treatments, respectively. There was no statistical difference between the three irrigation treatments for amount or quality of fruit yield. The results suggested that all three irrigation levels were sufficient for growing peach trees. Daily TR rates, actual peach trunk X-areas and climatic data were needed to formulate the recommendation for the optimum amount of irrigation. Recommended optimum amounts for Royal Angkhang Agricultural Station ranged from 3.65 to 3.88 L per day per tree from August to May, and from 48.58 to 54.20 during June and July.

## Introduction

SUPPLY of sufficient irrigation to fruit trees is obviously essential. Water supports tree growth and so will lead to production of a suitable amount of good quality fruit. Moreover, knowing how much water the trees require will, in turn, indicate the necessary storage capacity of water reservoir to be constructed. This is especially important in highland and mountainous areas where the investment cost of reservoir construction is extremely high. Thus, knowing the water requirement for fruit production will maximize productivity in terms of water use efficiency.

To determine the volume of water required for fruit production, many factors have to be taken into account, including soil and atmospheric conditions

as well as fruit genetics. The atmospheric condition includes the evaporative demand which can be derived with the use of climatic data such as air temperature ( $T_{\text{air}}$ ) and relative humidity (RH). Evaporative demand changes according to seasonal changes in climate. Soil condition includes hydraulic conductivity and water retention. Both indicate the ability of soil to hold water after rain or irrigation. Soil conductivity indicates the ability of soil to transmit water in the root zone. If the rate of transmission is lower than the demand for water, the tree will suffer a water deficit. However, if the demand is very high, trees will respond by partial closure of the stomates, thus decreasing the rate of transpiration (Salisbury and Ross, 1992). Genetic factors include the tree's age and species. Differences in either of these may lead to differences in water requirements.

The aim of this research was to determine the optimal amount of water required for peach production. The responses of peach in terms of leaf water status, transpiration rate, yield, and yield quality were also investigated.

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## Materials and methods

### Experimental units and treatments

Experimental units consisted of nine 3-year-old 'EarliGrande' peach trees with trunk diameters ranging from 12 to 16 cm. At full cover, their leaf areas ranged from 8.8 to 9.5 m<sup>2</sup> per tree. During the course of this experiment, each peach tree was applied with 15-15-15 fertiliser at the rate of 3 kg per plant. The fertiliser was applied in three equal applications in May, October, and December. The ground around the trees was covered with a plastic sheet to prevent the loss of soil water by surface evaporation. Treatments consisted of three replicates and three irrigation levels, namely 0.5, 1.0, and 2.0 ET<sub>p</sub>. These water treatments corresponded to 30, 60, and 120 L of water per week, respectively. Most of the work was carried out at Royal Angkhang Agricultural Station, Chiang Mai, Thailand (N19° 54.51', E99° 2.58').

### Measurements

Diurnal courses of transpiration rate, leaf water potential,  $T_{air}$ , and  $RH$  were measured monthly from June 2002 to June 2003. These variables were automatically logged every 5 min from 0800 h to 1700 h on the dates of measurement.

**Transpiration rate.** Sap flow rate was used as a measure of transpiration rate. If little storage of the water flow pathway is assumed, sap flow rate can be assumed as transpiration rate (Dugas et al., 1994). Dynagauge sensors of a suitable size were attached to the trunks. A heat balance technique was used to compute the flow rate, using the method proposed by Steinberg et al. (1989).

**Total leaf water potential.** A fresh leaf was picked and then wrapped in plastic to protect the transpired water and immediately inserted into the pressure chamber. A small amount of pressure was applied initially and gradually increased. The process continued until a film of water was observed on the leaf panicle. The corresponding pressure was the total leaf water potential.

**Vapour pressure deficit (VPD).**  $T_{air}$  (C) and  $RH$  (%) were used to compute VPD using the following equation:  $VPD = P^{sat}(T_{air}) \cdot RH/100$ , where  $P^{sat}(T_{air})$  is saturation vapour pressure at air temperature.

## Results and discussion

### Effect of irrigation levels on yield and yield quality

Table 1 shows peach yield and yield quality obtained from three irrigation levels. The 1.0 ET<sub>p</sub> treatment gave the highest fruit yield of 14.2 kg per tree, while

the 0.5 ET<sub>p</sub> treatment had the lowest yield. The number of fruits obtained from these three levels of irrigation followed the same pattern. The 1.0 ET<sub>p</sub> treatment also gave the highest fruit density with an average value of 1.52 g per cm<sup>3</sup>. The 2.0 ET<sub>p</sub> gave the highest total soluble contents (Brix) with an average value of 11.3%. Statistical analysis (data not shown) indicated that there was no significant difference among mean values of the number of fruits, fruit mass, fruit density, fruit volume, and total soluble contents obtained from the three levels of irrigation.

### Effect of irrigation levels on diurnal courses of total leaf water potentials

Figure 1 shows diurnal courses of average leaf water potentials which were obtained from peach leaves of the three water treatments. Diurnal courses of VPD are also shown in the same figure. In general, values of VPD are small during the first half of a day with peaks occurring around 1100–1400 h. Responses of leaf water potentials to changes in VPD obtained from the three treatments were the same, ie values of leaf water potential decreased following the increase



R.J. Nissen

Well trained peach tree of the new introduced varieties, Chiang Rai, northern Thailand.

**Table 1.** Yield and quality of peach treated at three different water levels.

	Treatments			
	0.5 ETp	1.0 ETp	2.0 ETp	Average
Fruit quality				
Fruit density (g/cm <sup>3</sup> )	1.10	1.52	1.17	1.26
Fruit volume (cm <sup>3</sup> )	93.67	88.00	82.89	88.19
Total soluble solid (% Brix)	10.6	10.7	11.3	10.9
Number of fruits				
Total	128	143	140	137
Extra grade	0	0	0	0
Grade 1	2	2	0	1
Grade 2	38	83	64	62
Grade 3	75	44	65	61
Poor grade	13	14	12	13
Fruit mass				
Total (kg)	11.5	14.2	12.6	12.7
Extra grade (kg)	0.0	0.0	0.0	0.0
Grade 1 (kg)	0.3	0.2	0.0	0.2
Grade 2 (kg)	4.4	9.1	6.8	6.8
Grade 3 (kg)	6.0	3.6	5.2	4.9
Poor grade (kg)	0.8	1.3	0.6	0.8

in values of VPD. When VPD was >3.5 kPa, the values of leaf water potential increased instead of decreasing. This indicates that at any irrigation level, peach fully responded to the evaporative demand in terms of maintaining its leaf water potential up to 3.5 kPa of VPD. However, if the demand is greater than 3.5 kPa, the plant probably responds by partially closing its stomates. Figure 2 showed the relationship between leaf water potential and VPD obtained from the three irrigation levels. Clearly, leaf water potentials tended to decrease, while values of VPD tended to increase. However, when the value of VPD was greater than 3.5 kPa the leaf water potential increased.

These results indicated that peach did not suffer from the deficit of water, expressed in terms of its water potential, yield and yield quality. Results implied that all irrigation levels used in this experiment provided enough water for the production of fruit.

### Effect of total leaf water potentials on transpiration rates

Figure 3 showed the diurnal courses of sap flow or transpiration rate obtained from 0.5 ETp treatment for several measurement dates. The rates on all measurement dates had the same characteristic. The rate was high from 0800–1100 h and tended to decrease during the second half of the day. Figure 4 shows the relationship between transpiration rate and leaf

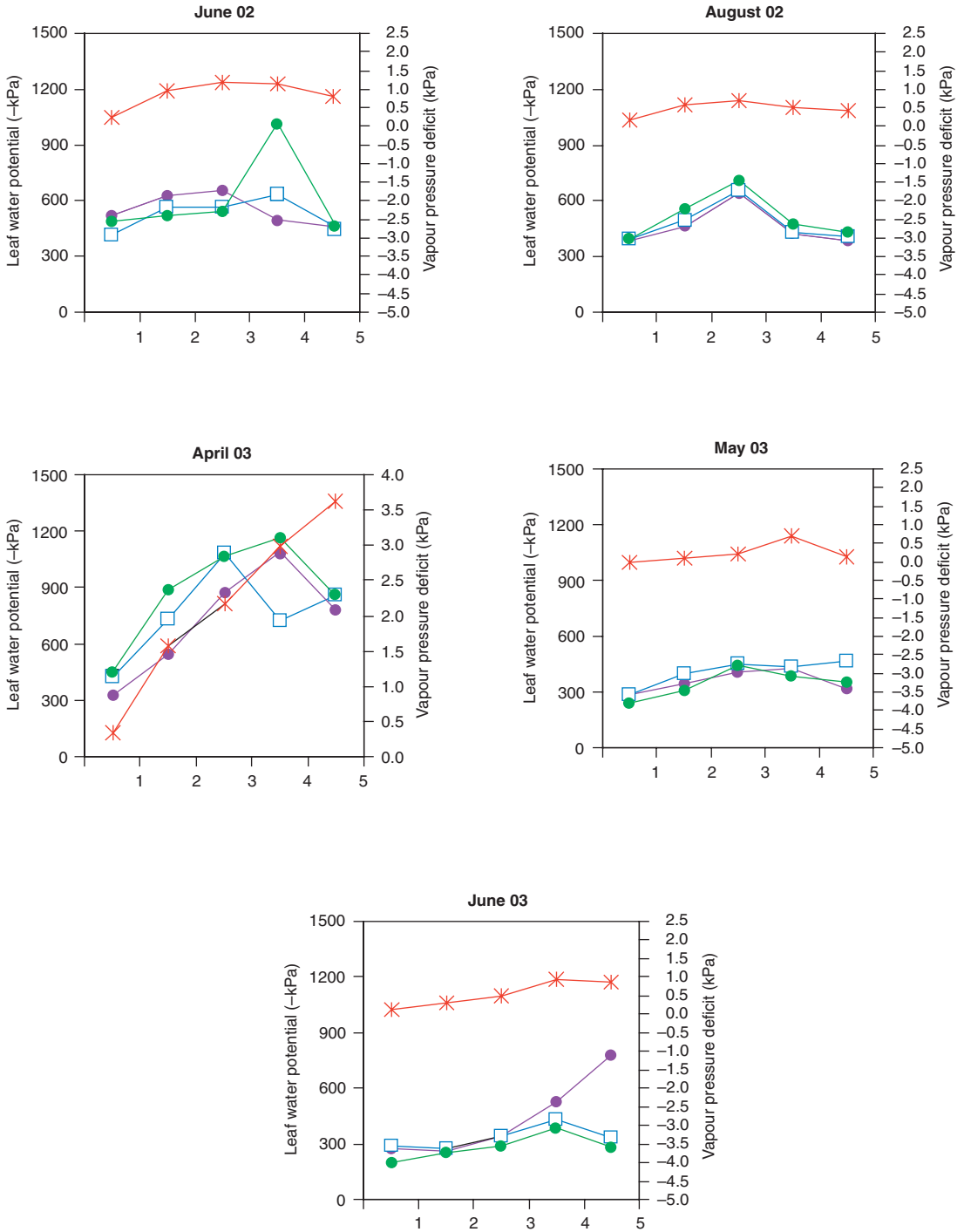
water potential. The high rates of transpiration coincided with leaf water potentials ranging from –500 to –900 kPa. This meant that while leaf water potential was between –900 kPa and –500 kPa, the rate of transpiration was low. At the low values of leaf water potential, the transpiration rate is low because peach can create a low gradient for the flow of water. However, at the high value of water potential the transpiration rate was also low because peach partially closed its stomates.

Figure 5 shows the daily rate of transpiration plotted against the daily maximum value of VPD. The relationship was a parabola with transpiration peak values of 280 g per cm<sup>2</sup> per day corresponding with VPD daily maximum value of 1.5 kPa. The curve explicitly indicated that daily maximum VPD value >1.5 kPa tends to reduce the use of water for peach.

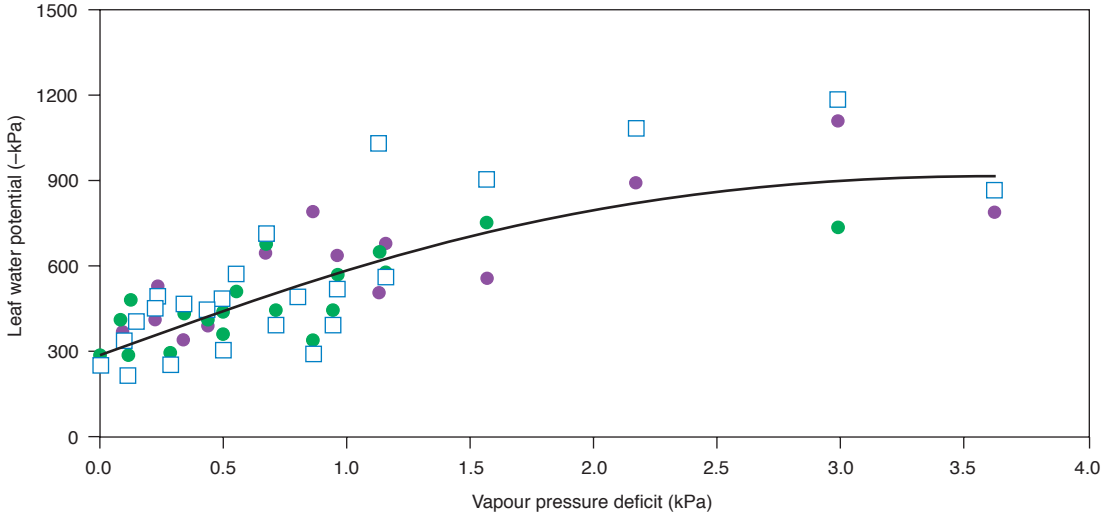
### Application of findings

Figure 5 can be used as a basis for computing the optimal amount of water for peach production. The calculation requires a link between climatic data collected by any climatic station and the daily maximum VPD (x-axis of Figure 5). Figure 6 shows such a link for Royal Angkhang Agricultural Station. Values along the x-axis of Figure 6 were computed by using daily maximum temperature and minimum relative humidity. These data were collected by the Royal Angkhang Agricultural Station.

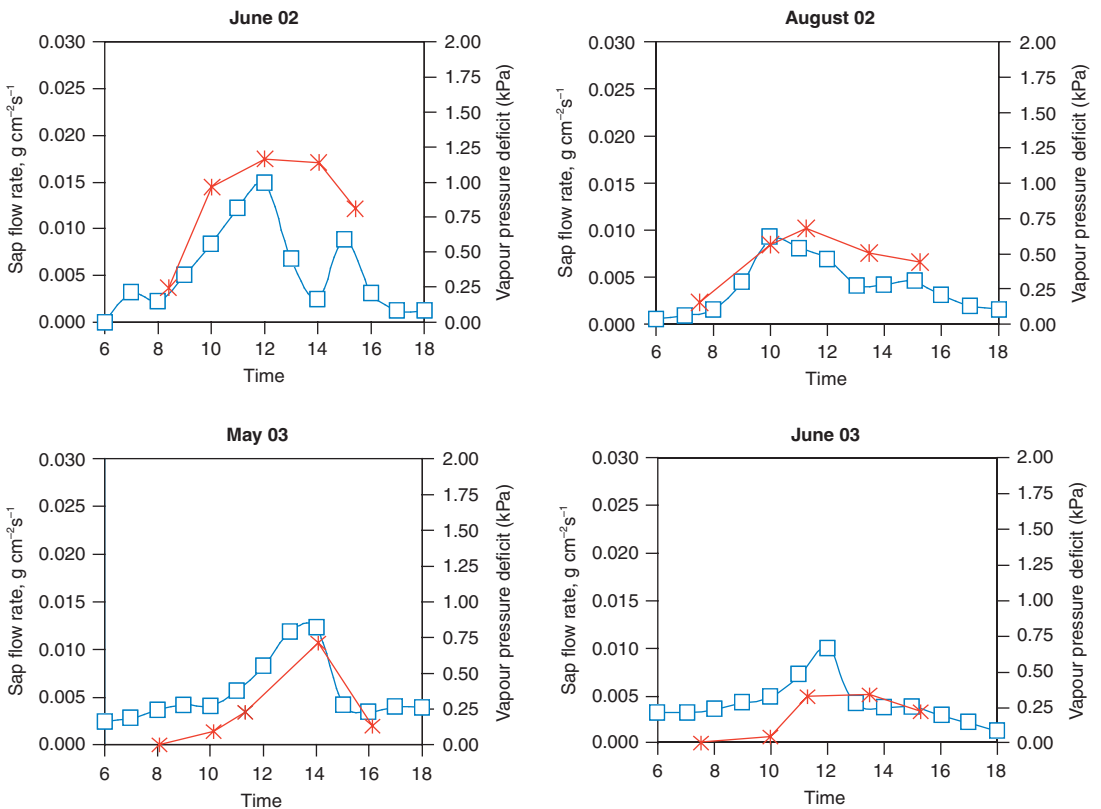




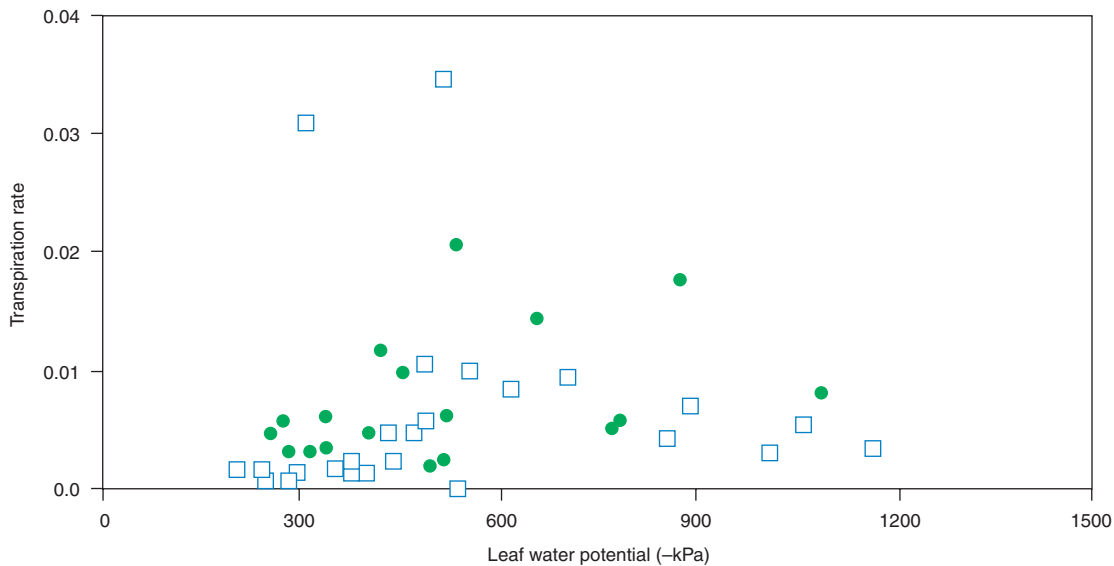
**Figure 1.** Diurnal courses of leaf water potential obtained from three different water treatments (□ 0.5 ETp; ● 1.0 ETp; ● 2.0 ETp; ✕ Vapour pressure deficit). Values of water potential were negative (TIME 1=8:00; 2=10:00; 3=12:00; 4=14:00; 5=16:00).



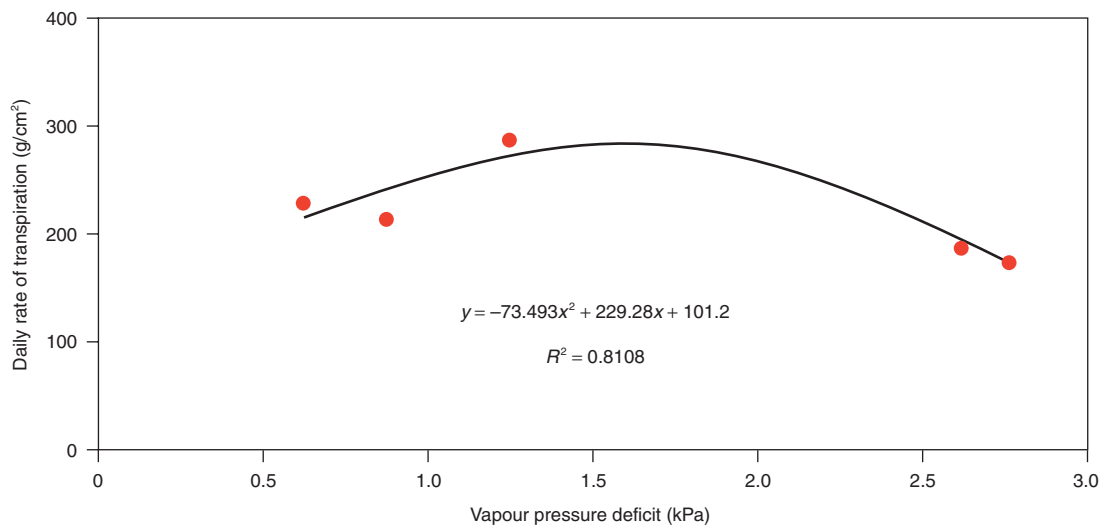
**Figure 2.** The relationship between leaf water potential and vapour pressure deficit. Data were obtained from three different water treatments (□ 0.5 ETp; ● 1.0 ETp; ● 2.0 ETp).



**Figure 3.** Diurnal courses of sap flow rate or transpiration obtained from the 0.5ETp peach treatment (□ 0.5 ETp; \* Vapour pressure deficit).



**Figure 4.** A plot between leaf water potentials and their corresponding rates of transpiration. Data were obtained from 0.5ETp and 2.0ETp water treatments (□ 0.5 ETp; ● 2.0 ETp).



**Figure 5.** Relationship between the daily rates of sap flow or transpiration and vapour pressure deficit.

Table 2 shows the optimal amount of water recommended for 3-year-old peach at Angkhang Agricultural Station, assuming a trunk diameter of 15.27 cm. Recommendations mostly ranged from 3.7 to 3.9 L/day. However, during summer the rate abruptly increased to 48.6–54.2 L/day.

**Table 2.** Recommended daily amounts of water for a peach tree at Royal Angkhang Agricultural Station.

Month	Recommended Volume (L/plant)		
	Min	Max	Average
January	3.79	3.90	3.86
February	3.80	3.90	3.88
March	2.77	4.88	3.69
April	2.96	3.90	3.65
May	3.44	3.90	3.79
June	42.91	72.26	54.20
July	33.26	72.26	48.58
August	2.78	4.96	3.79
September	2.77	3.90	3.72
October	3.73	3.90	3.86
November	3.81	3.90	3.88
December	2.80	3.90	3.74

Assuming that peach trunk diameter is 15.27 cm.

### Conclusion

In this experiment, the effects of three irrigation levels on leaf water status, yield and yield quality were conducted for peach trees. The three treatments

did not lead to any differences in these parameters. Results suggested that daily rates of transpiration measured from any treated peach trees in this experiment were enough for peach production without trees suffering from water deficit. The relationship between the daily maximum VPD and daily rates of transpiration can be used as a basis for computing the optimal amount of water required for peach production. However, irrigation rates will need to be adjusted for rainfall. To apply this relationship at other locations, a link between the daily maximum VPD and locally collected climatic data is needed. Results of an application on such relationships for Royal Angkhang Agricultural Station were included in this report.

### Acknowledgments

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# Improvement of fruit set and fruit qualities in persimmon ‘Fuyu’ using pollination

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## Abstract

The Royal Project Foundation has supported research into persimmon (*Diospyros kaki* Thunb.) production in the highlands of northern Thailand because this fruit is seen as an alternative crop to opium for hill-tribe farmers. The cultivar Fuyu is replacing Xichu, also known as P2, due to its better fruit qualities. However, ‘Fuyu’ has problems with poor fruit set as well as small fruit size, which are probably caused by low pollination. Since the usual polliniser varieties, eg ‘Prince’, ‘Zenjimar’ and ‘Gailey’, are poorly adapted or not available in Thailand, pollen from P1 (staminate and hermaphrodite flowers), ‘Shogatsu’ and unknown cultivars grown at the Royal Agricultural Station Angkhang were used. Before pollination, germination of the pollen was checked using the hanging drop technique with two concentrations (15 and 30%) of Brewbaker and Kwack media. The results showed that 15% sucrose gave higher pollen germination than 30% sucrose in all treatments. Pollen from ‘Shogatsu’ had the highest germination (17.8%) followed by pollen from P1 staminate and hermaphrodite flowers, which were 14.2% and 11.6% respectively. The unknown cultivars had only 3.4% of pollen germination. Fruit set did not differ between treatments (60–90%) and the control treatment had two seeds per fruit, which means that open pollination also occurred. The only difference between the control and the hand pollination treatment was seed number while other fruit characteristics namely, fruit weight, fruit size, fruit firmness and total soluble solids (TSS) did not differ between treatments.

## Introduction

SINCE 1969 there has been an increase in the production of persimmon in the highlands of northern Thailand. Persimmon has become one of the promising crops for this area. Compared with other temperate fruit trees, persimmon seems to have fewer problems, which made it easy to introduce to farmers. About 498.6 ha (approximately 114 951 trees) have been planted and we have two commercial cultivars available in the market. The first one is an astringent type, cultivar ‘P2’, also called ‘Xichu’, which was introduced from Taiwan. The second cultivar is ‘Fuyu’ which is a non-astringent type introduced from Japan. Even among temperate fruit trees grown in Thai-

land, persimmon may be the easiest one to produce, but we found that some problems still remain. Cv. ‘Fuyu’ is replacing P2 due to its better fruit qualities. But, one serious problem of this cultivar is poor fruit set and small fruit size. Both these factors result in reduced yield, consequently supply cannot match the demand for this cultivar. Fruit drop has many causes, both internal and external (Callan et al., 1978; Pharis and King, 1985; Ryugo, 1988; Whaley, 1993; Kim et al., 1987; Krisanapook et al., 1999). Low pollination can lead to poor or no fertilisation, while high pollination will increase seed formation followed by better fruit set and fruit size. Pollination is necessary for increasing fruit set and fruit qualities of ‘Fuyu’ persimmon (Kim et al., 1997). Normally, cv. ‘Fuyu’ does not produce staminate flowers and so needs to be pollinated by another variety. Because cultivars that are used as pollinisers like ‘Zenjimar’, ‘Prince’, or ‘Gailey’ are not available in Thailand, other cultivars which are grown in Thailand that produce staminate flowers were used in this experiment.

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## Materials and method

### Pollen germination

Pollen was collected from the following cultivars:

'*PI*' or '*Hong Mei*'. This astringent-type persimmon was the early commercial cultivar introduced from Taiwan which has been replaced by other, better-quality cultivars. These cultivars produce three kinds of flower (staminate, pistillate and hermaphrodite). In this study, pollen from both staminate and hermaphrodite flowers was used.

'*Shogatsu*'. One of the old Japanese cultivars.

*Unknown cultivars*. Have been grown in this area for some years.

Pollen was collected 1 day before bloom from 0800 to 1000 h. Anthers were dried in a drying cabinet and pollen inside was put in plastic capsules kept in moisture-trapped vials. Pollen culture was made using the hanging drop technique with two concentrations of sucrose (15% and 30%) of Brewbaker and Kwack media (1963). Prepared slides were incubated for 6 h in a closed moist container. After that, pollen germination was checked under the microscope (40×). Three fields in each slide (six slides in each treatment) were selected at random to check germinated pollen, which was considered as the pollen that produced a germination tube longer than the grain diameter. Numbers of germinated pollen were converted to percentage using the following formula:

$$\text{Percentage of pollen germination} = \frac{\text{Germinated pollen} \times 100}{\text{Whole pollen}}$$

### *Effect of hand pollination on fruit set and fruit qualities*

Ten 16-year-old trees of '*Fuyu*' persimmon grown at the Royal Angkhang Agricultural Station in Chiang Mai province (1400 m asl) were used. Five treatments were applied to two shoots randomly selected on each tree. Each shoot had 5–10 pistillate flowers.

The treatments were as follows: control (no-hand pollination); pollen from '*P1*' persimmon (staminate flower); pollen from *P1* persimmon (hermaphrodite flower); pollen from *Shogatsu* persimmon; and pollen from unknown persimmon.

Hand pollination was carried out on selected '*Fuyu*' pistillate flowers using a paint brush. Before brushing, pollen grains were mixed with talcum powder to increase volume (the ratio of pollen: talcum = 1:20). Hand pollination was repeated the following day. The experiment was conducted in the first week of March 2002.

## Results and discussion

### Pollen germination

Pollen germinated better in 15% sucrose than in 30% sucrose. Thus 15% sucrose should be the concentration used to test pollen germination in persimmon while 30% sucrose may be too high. Although no significant differences were found between treatments, '*Shogatsu*' persimmon had the highest percentage of pollen germination in both sugar concentrations followed by pollen from both types of *P1* flower (Table 1). Pollen germination was lowest for the unknown cultivar. Pollen germination may not be related to flower size since we found that '*Shogatsu*' staminate flowers were smaller than *P1* staminate flowers (Fig. 1). As well, *P1* staminate flowers were smaller than hermaphrodite flowers (Fig. 2) although both types can be used as pollen sources.

### *Effect of hand pollination on fruit set and fruit qualities*

*Fruit set*. One month after pollination, there was still no difference between hand pollination and the control. Fruit drop had still not occurred. Subsequently, the proportion of fruit on the control shoots dropped markedly to 80% and continued to gradually decrease.

**Table 1.** Pollen germination of persimmon '*P1*', '*Shogatsu*' and '*unknown*' cultivars cultured in Brewbaker and Kwack media with two concentrations of sucrose.

Cultivars	Germination (%)		Average*
	15% sucrose	30% sucrose	
<i>P1</i> (staminate)	14.2	1.9	8.04 b
<i>P1</i> (hermaphrodite)	11.6	2.3	6.95 bc
' <i>Shogatsu</i> '	17.8	13.7	15.76 a
Unknown	3.4	0	1.68 c
Average*	11.75 x	4.46y	

\*For each parameter, cultivars or sucrose content followed by the same letter are not significantly different at  $P < 0.05$ .



**Figure 1.** Size comparison of ‘Shogatsu’ staminate flower (left) and P1 staminate flower (right).



**Figure 2.** Size comparison of P1 (A) staminate flower; (B) hermaphrodite flower; (C) pistillate flower.

At harvest time, fruit retention in the control was 61.2%. The pattern of fruit drop for all hand pollinated flowers was the same as the controls. However, the percentage of fruit on the trees at harvest was 75.8–89.7%, higher than in the control (61.2%) but not significantly different. Early fruit drop was due to non fertilisation. Thus, control shoots had less fruit retention than the shoots where flowers were treated with hand pollination. From then on, obviously no fruit drop occurred.

**Table 2.** Fruit set and number of fruit with seed.

Treatment	Fruit set (%)	No. fruit with seeds (%)
Control	61.2	71.1
P1(staminate)	89.7	97.8
P1(hermaphrodite)	75.8	94.3
‘Shogatsu’	88.2	96.1
Unknown	84.7	91.5
F-test	ns	ns
%CV	23.1	8.3

ns = nonsignificant

*Fruit quality.* Thirty fruit in each treatment were randomly selected for determination of fruit quality characteristics. The overall appearance of ‘Fuyu’ fruit — weight, size, flesh firmness and TSS — were not affected by hand pollination (Table 3). However, the number of seed/fruit was significantly different. Hand pollination produced 3.5–4.8 seeds per fruit. However, in the control, we also found some seeds (2.04 seeds/fruit). As well, 91.5–97.8% of fruit in the hand pollination treatments had seed inside while in the control we found that 71.1% of fruit also contained seed (Table 2). This means that open pollination also occurred since ‘P1’ persimmon trees were grown close to ‘Fuyu’ trees. In fact, we considered covering the shoots with nylon nets but found that it was not practical since, even on the same tree, ‘Fuyu’ flowers did not bloom at the same time. Besides, the shoots were quite high off the ground and easy to break. Compare these results to ‘P2’ persimmon (another commercial cultivar that sets parthenocarpic fruit). Xichu had greater fruit drop (about 50%; data not shown). Thus, number of seed should be related to fruit retention in persimmon. In 1996, Keulemans et al. observed a positive correlation between fruit weight and the number of seed. With apple, ‘Fuji’

**Table 3.** Fruit quality of the major cultivars grown in Thailand.

Treatment	weight (g)	width (cm)	length (cm)	firmness (N)	TSS (%)	Seed No.
Control	151.4	6.9	5.5	28.2	12.5	1.9 a
P1(staminate)	149.2	6.9	5.0	27.3	12.8	4.4 b
P1(hermaphrodite)	150.9	6.9	5.5	28.7	12.4	4.6 b
‘Shogatsu’	144.6	6.8	5.6	26.2	12.4	4.3 bc
Unknown	159.0	7.0	5.7	27.8	12.4	3.9 c
F-test	ns	ns	ns	ns	ns	**
%CV	9.4	0.7	7.1	5.8	6	35.5

ns = non significant

\* Values within columns followed by different letters are significantly different at the 99% confidence level (Duncan’s Multiple Range Test).

polliniser can influence fruit weight of apple cv. 'Golden Delicious'. Richardson and Anderson (1996) also stated that hand pollination increased yield, the number of seeds and large symmetrical fruit in a humid climate of cherimoya cv. 'Bay', 'Reretai', 'Spain' and 'White'.

The results from this study confirmed the importance of pollination for fruit setting in 'Fuyu'. However, due to the poor fruit quality of 'Shogatsu' and 'P1', other pollinisers that also have good quality fruit should be introduced to give growers higher incomes. Even seed number in hand pollination treatments was higher than in the control but fruit size was the same. Seed number may not be the only factor that plays a role in increasing fruit size. Other factors, such as fruit thinning, storage carbohydrates and leaf number should also be considered. Thus, further studies are needed to evaluate factors affecting fruit size. Furthermore, other factors, such as pollen storage technique, should also be useful for keeping pollen in good condition and should be tried as well.

### Conclusion

Highest pollen germination was found in 'Shogatsu' flower followed by staminate and hermaphrodite flowers of P1.

The lower sucrose concentration (15%) is more suitable for persimmon pollen germination than the higher (30%) concentration.

Pollination, whether by hand or open, resulted in high fruit retention at harvest time for 'Fuyu' persimmon.

Pollination did not effect fruit qualities of 'Fuyu' except for number of seed.

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## **PART II**

### **Poster Papers**



A young visitor examines the display of stone-fruit products before the poster session at Royal Ang Khan Agricultural Station, April 2004.

# Genetic diversity of local peaches in Thailand based on AFLP markers

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## Abstract

In highland areas of northern Thailand, peach trees have been planted around the hill-tribe villages. Their fruits were mainly used for pickle as they possess small and soft flesh. Recent discovery of rust (*Tranzschelia pruni-spinosae*) resistance in some genotypes has prompted the use of these peaches in the ongoing breeding program to develop resistant cultivars. Due to lack of any genetic information about these local peaches, this study was carried out to estimate their genetic diversity and to determine their similarity to other standard low-chilling peach cultivars. Twenty-three local peach trees found near villages of Royal Project Development Centers (12 locations) were randomly selected for genetic analysis using Amplified Fragment Length Polymorphism (AFLP) markers. Three low-chilling peaches: 'TropicBeauty', 'Premier' and 'Okinawa' were included for the comparison. DNA was extracted from young leaves using a CTAB method. The AFLP analysis showed that 10 primer pairs produced 148 scorable bands in which 121 markers (81.8%) were polymorphic. Genetic diversity among local peaches and genetic similarity of local peaches to standard low-chilling peaches were calculated using Nei and Li coefficients. Cluster analysis was done by UPGMA method. Genetic relationships of 27 peach cultivars showed two main clusters and three sub-clusters within local peach based on AFLP markers. Genetic similarity ranged between 0.54 and 1.00. Local peaches in Thailand were very closely related genetically.

## Introduction

PEACH [*Prunus persica* (L.) Batsch.] is one of the temperate fruits cultivated in Thailand. The local peach was brought by the hill tribe people who migrated to Thailand (Subhadrabandhu, 1987). Because the fruit are very small and soft, they are mainly used for pickling when they are young. Trees are well adapted, exhibiting vigorous growth, which makes them suitable for use as a rootstock. Recently, rust (*Tranzschelia pruni-spinosae*) resistance was observed in some local peach genotypes. Breeders have promoted the use of these peaches in the ongoing breeding program to develop rust-resistant cultivars. Due to a lack of any genetic information, genetic comparison to other peach germplasm would reveal relative and genetic diversity of these local peaches. This information will be useful for future breeding programs.

There are many techniques to evaluate genetic relationships, such as morphological markers, isozyme markers (Torres, 1983) and DNA markers, eg AFLP (Amplified Fragment Length Polymorphism) (Vos et al., 1995), RFLP (Restriction Fragment Length Polymorphism) (Botstein et al., 1980), RAPD (Randomly Amplified Polymorphic DNA) (Welsh and McClelland, 1990; Williams et al., 1990). DNA markers have become a popular choice due to their high selectivity and insensitivity to the environment. Among these the AFLP technique produces a large number of markers and requires no previous information about the markers or genomic sequences. It has been used successfully for estimating genetic relationships in fruit crop species, eg pears (Hayashi and Yamamoto, 2002), apples (Tignon, 2001), mango (Hautea et al., 2001), grapes (Narvaez and Andres, 1998) and peaches (Rajapakse et al., 1995; Dirlwanger et al., 1998; Lu et al., 1998; Aranzana et al., 2001; Arus et al., 2003).

The objectives of this study were to estimate genetic diversity of local peaches and to determine their similarity to other standard low-chilling peach cultivars using AFLP analysis.

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## Materials and methods

### Plant materials

Twenty-seven peach genotypes were used. They were local cultivars (Angkhang and Inthanon groups) and three low-chilling commercial cultivars (Southern China group). One genotype 'ShenzHouMiTao' (Northern China cultivar) was included for comparison (Table 1). These local peach trees, found near villages or Royal Project Development Centers (Fig. 1), were randomly selected for AFLP analysis.

### DNA extraction

The CTAB procedure was modified from Dellaporta et al. (1983) and Agrawal et al. (1992) for use with a 1.5 ml microcentrifuge tube with 50 mg of young fully expanded leaf tissue. The DNA concentration was quantified by visual comparison with SibEnzyme DNA marker 1 Kbp (SibEnzyme, USA) on 0.8% agarose gel (Promega, USA) in 0.5× TBE

buffer. The DNA stock was then diluted to 50 ng/μl working stock with sterile reverse osmosis water.

### AFLP reaction

The purified DNA was digested with *EcoRI* (BioLabs, England) and *Tru9I* (BioLabs, England) restriction enzymes. Digestion was carried out in the volume of 25 μl in 10× NE buffer (50 mM NaCl, 100 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 0.025% Triton X-100), 10× SE bufferW [10 mM Tris-HCl (pH 7.6), 10 mM MgCl<sub>2</sub>, 100 mM NaCl, 1 mM DTT], 1 Unit *EcoRI*, 0.6 Unit *Tru9I* and 50 ng of genomic DNA for 16 h at 37°C. Two different adaptors, one for the *EcoRI* sticky end and another for the *Tru9I* sticky end were ligated to DNA by adding 0.2 μl of a mixture containing 5 pmol *EcoRI* adaptor (BioBasic, Thailand), 25 pmol *MseI* adaptor (BioBasic, Thailand), 10× ligase buffer [50 mM Tris-HCl (pH 7.5 at 25°C), 10 mM MgCl<sub>2</sub>, 5 mM DTT, 1 mM ATP, 25 μg/ml BSA] and 0.2 Unit T4 ligase (SibEnzyme, USA) to the digestion. The ligation was incubated for 12 h at 20°C.

**Table 1.** Peach genotypes used in genetic relationship study.

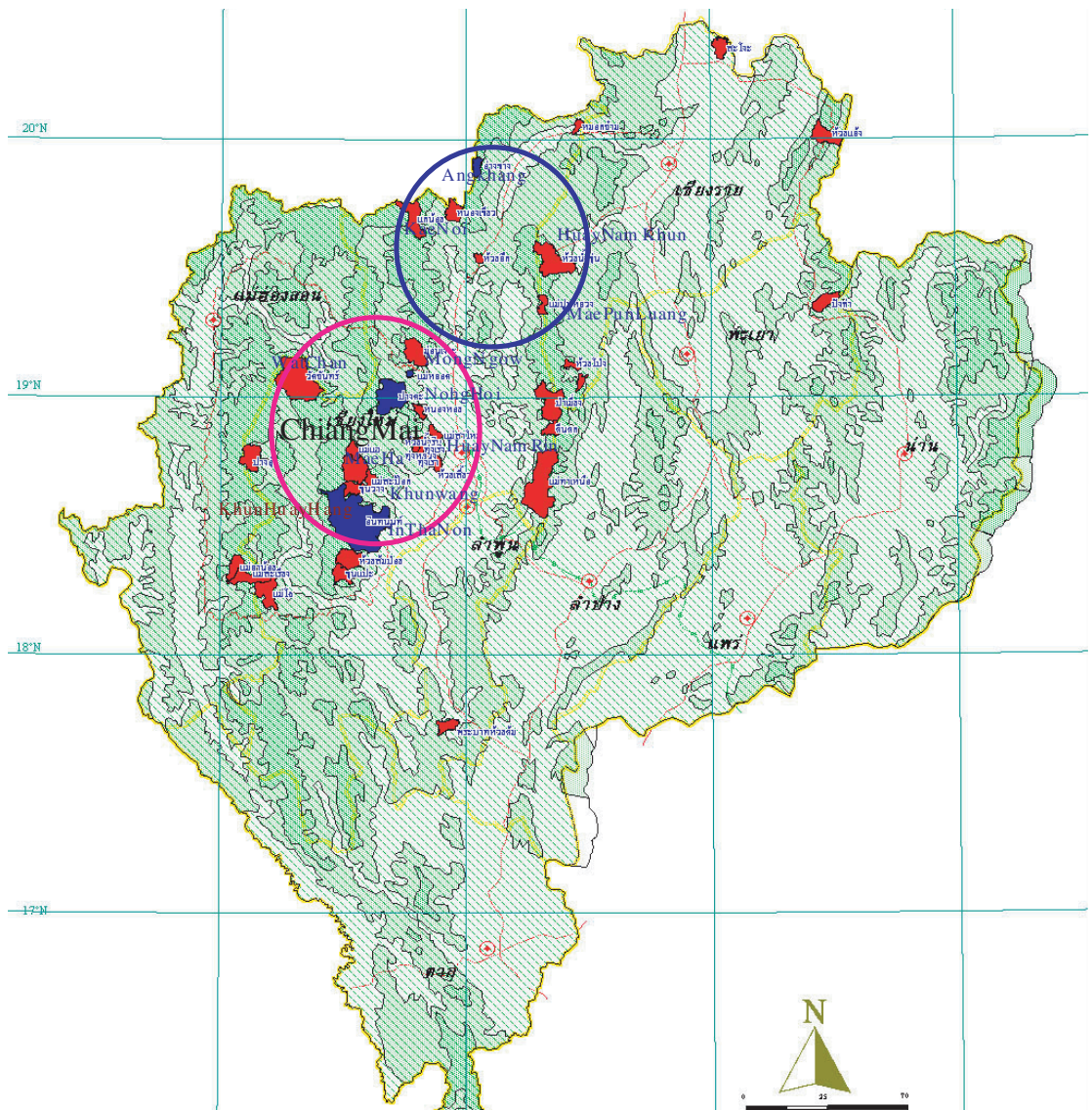
Order	Genotypes	Location*	Note
1	KaeNoi 1	CM, 950–1000 m	large fruit and tip, green ground
2	KaeNoi 2	CM, 950–1000 m	round, small, yellow ground
3	KaeNoi 3	CM, 950–1000 m	round, green-yellow ground
4	MaeHa 2	CM, 1200 m	ovate, large tip, green ground
5	MaeHa 3	CM, 1200 m	
6	MaeHa 1	CM, 1200 m	round, yellow ground, white flesh
7	InThaNon 1	CM, 1300 m	new clone
8	InThaNon 2	CM, 1400 m	old clone
9	KhunHuayHang 1	CM, 1300 m	
10	KhunHuayHang 2	CM, 1300 m	
11	WatChan 1	CM, 1000 m	imported from PangDa Royal Agricultural Station
12	WatChan 2	CM, 900-1200 m	large fruit, large tip, green ground
13	HuayNamKhun 1	CR, 1100 m	small fruit, cling, white flesh, imported from Angkhang Royal Agricultural Station
14	HuayNamKhun 2	CR, 1100 m	small fruit, cling, white flesh, imported from Angkhang Royal Agricultural Station
15	HuayNamRin	CM	
16	MaePunLuang	CM, 1100 m	imported from Banluang Angkhang
17	Local Angkhang	CM, 1400 m	green ground
18	Local Angkhang F10	CM, 1400 m	rust resistance
19	NohgHoi	CM, 1200 m	imported from Doi Pui
20	MongNgow	CM, 1200 m	
21	Local Khunwang	CM, 1300 m	
22	White Angkhang	CM, 1400 m	planted at Angkhang Royal Agricultural Station
23	Red Angkhang	CM, 1400 m	planted at Angkhang Royal Agricultural Station
24	TropicBeauty	CM, 1400 m	medium, yellow flesh round, semi-free, imported from Florida, USA
25	Okinawa	CM, 1200 m	small, white flesh, origin in Florida, USA
26	Premier	CM, 1400 m	white flesh, low acid, Southern China type
27	ShenzHouMiTao	China	fairly firm, sweet, juicy, white flesh, cling, Northern China type

Note \* CM (Chiang Mai); CR (Chiang Rai)

A first preselective Polymerase Chain Reaction (PCR) amplification was performed using *EcoRI* + A and *MseI* + C primer pairs in 2.5 µl 10× Mg<sup>2+</sup> free buffer [10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.1 % Triton X-100], 50 mM MgCl<sub>2</sub>, 2 mM of each dNTP, 6.25 pmol of each primer, and 1 Unit DyNAzyme II DNA Polymerase (Roche, USA). The reaction was carried out in a PTC-0225 Peltier Thermal Cycler (MJ Research Inc., USA) and the samples were subjected to 30 cycles of denaturing at

94°C for 30 s, annealing at 56°C for 60 s, and extension at 72°C for 60 s. The preamplification products were diluted make the starting material for the selective amplification by using 5 µl of preamplified material to 95 µl sterile reverse osmosis water.

For selective amplification *EcoRI* and *MseI* primers with two selective nucleotides were used (Table 2). The PCR reaction was performed in a 20 µl volume of 2 µl 10 × Mg<sup>2+</sup> free buffer, 50 mM MgCl<sub>2</sub>, 2 mM of each dNTP, 5 pmole *EcoRI* primer,



**Figure 1.** Map of 36 Royal Project Development Centers location; ○, local Inthanon group, ○, local Angkhang group.

5 pmole *MseI* primer, 1 Unit DyNAzyme II DNA Polymerase (Roche, USA), and 5 µl of diluted preamplified DNA. The selective amplification was carried out using the following cycling parameters; 1 cycle of 60 s at 94°C, 60 s at 65°C, 90 s at 72°C followed by 10 cycles in which the annealing temperature was lowered by 1°C per cycle, followed by 22 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C.

**Table 2.** Primer pairs and number of AFLP markers used for estimation of genetic relationships.

Primer	AFLP markers		
	Monomorphic	Polymorphic	Total
E-AAC/M-CAC	2	14	16
E-AAC/M-CAT	2	9	11
E-AAC/M-CTC	4	8	12
E-AAC/M-CTT	4	12	16
E-ACC/M-CAT	3	11	14
E-ACC/M-CTA	2	9	11
E-ACC/M-CTC	0	13	13
E-AGC/M-CAC	4	12	16
E-AGC/M-CTT	3	17	20
E-ACG/M-CAG	3	16	19

After completion of a selective PCR, the samples were denatured to single strand by adding formamide buffer containing 99% formamide, 10 mM EDTA, 0.05% bromo-phenol blue, and 0.05% xylene cyanol. The samples were heated for 5 min at 100°C and immediately placed in ice. Six µl of each sample was loaded on a 6% acrylamide/bisacrylamide (19:1), 7.5 M urea and 5 × TBE buffer, 10% APS, Temed gel (14 × 24.5 cm). Electrophoresis was carried out at a constant voltage of 295 V for 210 min in a Dual Slab Gel Unit (DSG-200, C.B.S. Scientific Co., USA). The polyacrylamide gel was visualised by silver staining method (Piyachoknakul, 2002). Gels were dried at room temperature for 2 days.

### AFLP analysis

At least two replications of each sample and primer combinations were successfully done to correctly score the observation. Scoring of data was based on consistent or major AFLP markers. All amplifications were scored as either a present or absent marker. The genetic similarity matrix among genotypes was calculated in SIMQUAL program (Sokal and Sneath, 1963) using Dice (Sneath and Sokal, 1973) similarity coefficients [ $C_{jk} = 2a/(2a+b+c)$ ; where  $a$  is the number of AFLP markers that were present in both  $j$  and  $k$  genotypes,  $b$  is the AFLP marker that was present only in  $j$  genotype and  $c$  is the AFLP marker that was present only in  $k$  genotype].

This similarity coefficient is equivalent to the Nei and Li coefficient (1979). The dendrogram representing genetic relationship based on the similarity coefficient from AFLP markers was constructed by using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) (Sokal and Michener, 1958) in the SAHN program (Sneath and Sokal, 1973). The SIMQUAL and SAHN programs are the packaging of NTSYS-pc 2.00 program (Rohlf, 1997).

## Results and discussion

Ten primer pairs which produced intense and scorable bands were selected. They were tested and PCR amplification yielded approximately 11–20 AFLP markers per primer pair which ranged in size from 100 to 700 bp (Table 2 and Fig.2). Out of 148 reproducible AFLP markers, 121 were polymorphic (81.8%) and 27 monomorphic (18.2%).

### Cluster analysis of 27 peach cultivars

Based on the cluster analysis (UPGMA method) we constructed a dendrogram which showed that the varieties could be divided into two main clusters (Fig. 3).

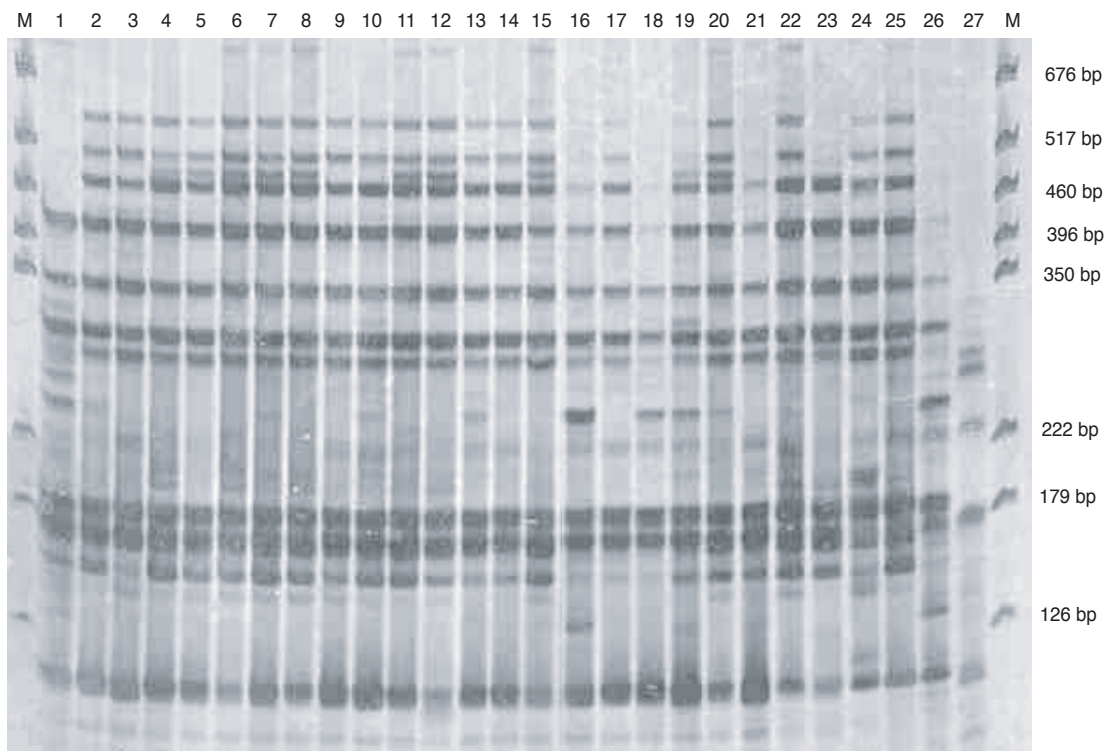
Cluster 1 included 26 peach genotypes with genetic similarity coefficients between 0.79–1.00. This cluster could be divided further into three sub clusters.

Sub-cluster A included clones of KaeNoi group, MaeHa group, InThaNon group, WatChan group, KhunHuayHang group, MongNgow, ‘White Angkhang’, ‘Okinawa’ and ‘TropicBeauty’ with genetic similarity coefficients between 0.88–1.00. Almost all the peach genotypes in this cluster are local peaches, except ‘Okinawa’ which originated in Florida, USA (Okie, 1998) and ‘TropicBeauty’ that was bred in Florida, USA (Rouse and Sherman, 1989). Both cultivars shared a southern China ancestor.

Sub-cluster B included ‘MaePunLuang’, ‘NohgHoi’ and ‘Local Khunwang’ with genetic similarity coefficients between 0.91–0.93.

Sub-cluster C included ‘Local Angkhang F10’ and ‘Premier’ with genetic similarity coefficients of 0.9. Both genotypes were observed as rust (*Tranzschelia pruni-spinosae*) resistant.

Cluster II consisted of one genotype, ‘ShenzHouMiTao’ that originated in northern China (Zailong, 1984). Genetic similarity coefficients between both clusters is 0.54. These results indicated all local peaches in Thailand have a southern China ancestor.



**Figure 2.** Fingerprint of AFLP markers from E-AAC /M-CAC primer pair; m, standard marker; No. 1–27, label of peach genotype in Table 1.

### Genetic relationship between local Angkhang group, local Inthanon group, southern China group and northern China cultivar

Peaches in China could be divided into three groups: a northern group, a southern group and a European or Persian group (Zai-long, 1984). Two groups — the northern and southern groups — were included in this study. Genetic relationship showed a broad genetic diversity of 0.78–0.89 (Table 3) between the local Angkhang group, local Inthanon group, southern China group and the northern China cultivars. Three groups, except the northern China cultivars, being grown successfully in Thailand had genetic similarity between 0.87–0.89. Of these, genetic diversity within the local Inthanon group was lowest (0.9). The result also indicated that the genetic relationships between the local Angkhang and local Inthanon groups is the closest (0.9). Local peaches from the southern China group were more closely related than those from northern China. This indicated that local peaches in Thailand were introduced from southern China. These results agree with the suggestions of Punsri et al. (1995).

**Table 3.** Average of similarity coefficient of peach groups; local Angkhang group (LAK), local Inthanon group (LITN), southern China group (SC) and northern China cultivar (NC).

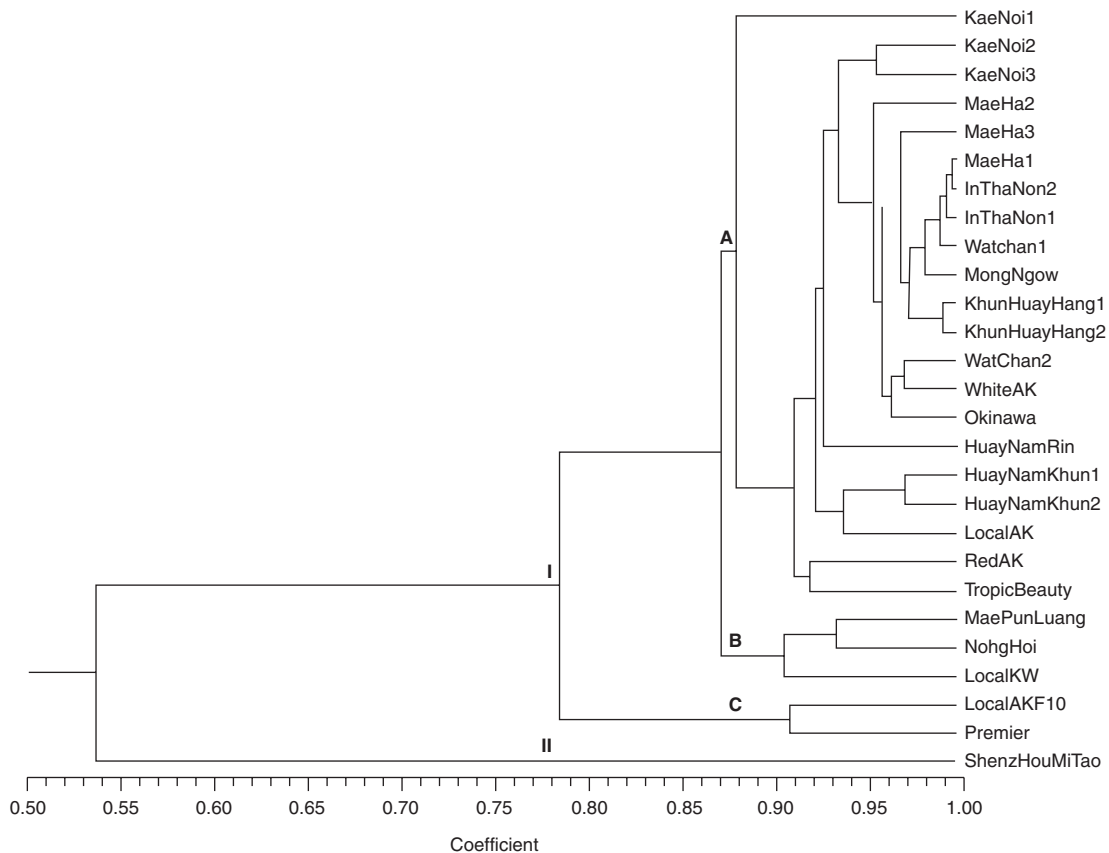
Peach group*	LAK	LITN	SC	NC
LAK	0.87 ± 0.03			
LITN	0.89 ± 0.03	0.90 ± 0.02		
SC	0.87 ± 0.04	0.89 ± 0.03	0.87 ± 0.05	
NC	0.80 ± 0.15	0.86 ± 0.12	0.78 ± 0.16	0.55

\* LAK included 'KaeNoi 1', 'KaeNoi 2', 'KaeNoi 3', 'HuayNamKhun 1', 'HuayNamKhun 2', 'MaePunLuang', 'local Angkhang' and 'local Angkhang F10'

LITN included 'MaeHa 1', 'MaeHa 2', 'MaeHa 3', 'InThaNon 1', 'InThaNon 2', 'KhunHuayHang 1', 'KhunHuayHang 2', 'WatChan 1', 'WatChan 2', 'HuayNamRin', 'NohgHoi', 'MongNgow' and 'Local Khunwang'

SC included 'White Angkhang', 'Red Angkhang', 'TropicBeauty', 'Okinawa' and 'Premier'

NC, 'ShenzHouMiTao'



**Figure 3.** Dendrogram of 27 peach genotypes based on cluster analysis (UPGMA method) on 148 AFLP markers.

### Conclusions

Using 10 primer pairs we were able to score 148 AFLP markers indicating that one primer could reveal 14 markers. Out of these markers, 121 markers (81.8%) were polymorphic.

Genetic relationships of 27 peach cultivars that consisted of local cultivars (Angkhang and Inthanon groups), commercial cultivars (southern China group) and ‘ShenzHouMiTao’ (northern China cultivar) showed two main clusters and three sub-clusters within local peach. Genetic similarity ranged from 0.54 to 1.00 with local peaches in Thailand very closely related genetically.

### Acknowledgment

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# Selection of kiwifruit for the highlands of Thailand

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## *Abstract*

Kiwifruit is one of the temperate-zone fruits that the Royal Project Foundation has aimed to develop into commercial production in the highlands of Thailand due to its high price, a great market demand and low pesticide application. Kiwifruit research has been carried out since 1976. Cultivars such as Bruno, which required high chilling accumulation, were only trialed at the Angkhang Royal Agricultural Station. More recently, research was carried out to evaluate and select for new, low-chilling cultivars of both male and female types. In 2003, following introduction of 19 new cultivars, evaluations for horticultural characteristics were conducted at two centres, the Royal Angkhang Agriculture Station, Fang District, and the Royal Inthanon Agriculture Station (Khun-Huay-Haeng), Jom-Tong district, (1400 and 1200 metres above mean sea level, respectively). These studies revealed that three cultivars (Yellow Joy, Yellow Queen and China no. 4) were productive. The results showed that Yellow Joy, introduced from Japan, had the best production and qualities at the Royal Inthanon Agriculture Station, with an average fruit weight of 120.2 g and an average total soluble solid of 13.8° Brix. It was harvested in October. In addition, 55 out of 591 seedlings from open-pollinated flowers of unknown cultivars produced fruit at the Chiang Mai Royal Agricultural Research Center (Khunwang), Mae Wang district, (1300 m above mean sea level). Some open-pollinated seedlings showed promising characteristics and further tests need to be carried out in other locations. The results suggested that Yellow Joy could be introduced to farmers.

## **Introduction**

THAILAND had introduced many cultivars of kiwifruits including cv. Hayward, Abbott, Monty, Dexture and Gracie from New Zealand. These cultivars were first planted in 1976 at Angkhang Royal Agricultural Station, Fang district in cooperation with Kasetsart University. Researchers evaluated methods to achieve commercial production in the highlands of Thailand. Some cultivars such as cv. Bruno were found to be highly productive at Angkhang Royal Agricultural Station. The vines were easy to plant and required less pesticide application thus making them an ideal species for the local environment. There was also strong market demand for the fruit, which received high prices. However, the highly productive

cv. Bruno could not be grown in other areas besides Royal Angkhang Agricultural Station due to its high chilling requirement. Consequently, the Royal Project Foundation initiated a search for new cultivars with low chilling accumulation and good quality.

## **Materials and methods**

### **Project 1. Study and selection of new cultivars from open-pollinated seedlings**

Test plots were organised and kiwifruit seed introduced from New Zealand. A total of 591 seedlings were planted at Chiang Mai Royal Agricultural Research Center (Khunwang), Mae Wang district in 2000. No chemicals were used to break dormancy as we wanted to select cultivars adapted to low-chilling conditions. After studying the characteristics of each selected cultivar we selected (1) a female cultivar which grew well, had good quality in a low-chill climate and had characteristics that were different from the original cultivar and (2) a male cultivar which grew well and flowered in a low-chill climate where the time of flowering coincided with the time of flowering of the female cultivar.

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## Project 2. Study and selection of new cultivars introduced from another country

The characteristics of the 19 cultivars held at the Royal Project Foundation area at Angkhang Agricultural Station and the Royal Inthanon Agricultural Station were studied. Assessments were similar to those made in Project 1.

### Results

#### Project 1. Study and selection of new cultivars from open-pollinated seedlings

It was possible to classify the seedlings into two species, *A. deliciosa* and *A. chinensis* (139 and 452 plants respectively). We found that 140 plants of *A. chinensis* began flowering in the first year after planting. The progeny could be divided into male cultivar, 99 plants (71%), and female cultivar, 41 plants (29%). When the fruit were ripe in October 2002, they were harvested and assessments were begun on fruit quality (size, shape and colour) and eating quality. Eight female cultivars (8-1-6, 8-1-15, 8-1-11, 1-1-9, 13-1-2, 15-1-13, 16-2-13 and 21-1-14) (Table 1) and six male cultivars (7-1-5, 7-2-2, 8-2-1, 8-1-4, 12-1-9 and 12-2-11) were selected.

In 2003 *A. chinensis* flowered and fruited for the second time and, after studying characteristics of the

fruit, a further three female cultivars (12-2-9, 13-1-8, and 13-2-9) were selected (Table 1).

#### Project 2. Study and selection of new cultivars introduced from other countries

In September–October 2003, *A. chinensis* cv. China No. 4 and Yellow Joy, planted at the Royal Inthanon Agricultural Station and cv. Yellow Queen, planted at the Royal Angkhang Agricultural Station, commenced cropping. Cv. Yellow Joy, which requires low chilling, produced good yield at the Royal Inthanon Agricultural Station, with an average fruit weight of 94.5 g. Cv. Yellow Queen, which is planted at the Royal Inthanon Agricultural Station and cv. Yellow Queen, planted at the Royal Angkhang Agricultural Station, had the biggest fruit size with an average fruit weight of 109.7 g. However, cv. Yellow Queen, which was only planted at the Royal Angkhang Agricultural Station, could not be evaluated for its chilling accumulation. Cv. China No. 4, which was planted at the Royal Inthanon Agricultural Station, produced good yields but fruit were small with an average weight of 64.3 g (Table 2).

In 2003, cvs China No.4, Yellow Joy and Yellow Queen flowered and fruited for the second time. All cultivars had better quality than the previous year with average fruit weights of 120.24, 120.00 and 67.42, and average total soluble solids of 13.76, 12.40 and 15.91°Brix, respectively (Table 2).



Figure 1. Kiwifruit from open-pollinated seedling plants.

**Table 1.** Characteristics of fruit quality of kiwifruit from open-pollinated seedling plants.

Cultivar	Fruit weight (g)	TSS (°Brix)	Fruit width (cm)	Fruit length (cm)	Core width (cm)	Core length (cm)	Fruit pulp colour
8-1-6	48.5	13.8	4.12	4.61	1.35	2.83	yellow
8-1-15	42.5	13.1	3.92	5.12	1.02	2.96	yellow
8-1-11	35.9	14.1	3.82	3.89	0.93	2.05	yellow
10-1-9	62.8	11	4.5	5.57	1.05	3.69	yellow
13-1-2	110	16.2	5.78	6.135	1.68	3.77	yellow
15-1-13	70.4	13.3	4.87	5.23	1.45	2.72	yellow
16-2-13	53.7	14.3	4.34	4.91	1.28	3.03	yellow
21-1-14	63.3	11.8	4.38	5.88	0.91	3.84	green-yellow
12-2-9	85.0	14.1	5.1	5.7	—	—	yellow
13-1-8	59.4	12.9	4.3	5.9	—	—	yellow
13-2-9	66.3	12.1	4.4	6.2	—	—	light-green

**Table 2.** Fruit quality characteristics of kiwifruit introduced from other countries.

Cultivar	Year	Fruit quality characteristic							Location
		Fruit weight (g)	TSS (°Brix)	Fruit width (cm)	Fruit length (cm)	Core width (cm)	Core length (cm)	Fruit pulp	
China	2002	64.30	8.60	5.06	4.91	0.91	4.58	yellow	Royal Inthanon Agricultural Station (Khun-Huay-Haeng)
No. 4	2003	67.42	15.91	4.64	5.55	—	—	yellow	Royal Inthanon Agricultural Station (Khun-Huay-Haeng)
Yellow	2002	94.50	13.50	5.33	5.61	1.70	3.62	yellow	Royal Inthanon Agriculture Station (Khun-Huay-Haeng)
Joy	2003	120.24	13.76	5.73	6.39	—	—	yellow	Royal Angkhang Agriculture Station
Yellow	2002	109.70	13.10	5.51	6.23	1.84	4.75	yellow	Royal Inthanon Agriculture Station (Khun-Huay-Haeng)
Queen	2003	120.00	12.40	5.67	6.61	—	—	yellow	Royal Inthanon Agriculture Station (Khun-Huay-Haeng)
Bruno	2003	92.00	13.65	—	—	—	—	green	Royal Inthanon Agriculture Station (Khun-Huay-Haeng)

**Figure 2.** Kiwifruit cv. Bruno and new cultivars introduced from other countries.

## Conclusion

The results from this trial showed that it is possible to select suitable cultivars within *A. chinensis* because of its lower chilling requirement than *A. deliciosa*. Kiwifruit cv. Yellow Joy could be distributed to the farmer. Our experiments showed that kiwifruit from open-pollinated seedling plants were able to grow satisfactorily in low-chill regions, flower and produce fruit without using chemical dormancy breakers. In future we will continue to select new cultivars from open-pollinated seedlings.

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# Effect of flowering time on development of reproductive organs and fruit set of peaches

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## Abstract

The effects of flowering period on flower characteristics and fruit set were determined by using three 6-year-old 'Flordaglo' trees grown at Angkhang Station. The flowering periods were classified into five terms: term I, before 20 November; term II, from 21 November to 30 November; term III, 1 December to 10 December; term IV, 11 December to 20 December; Term V, 21 December to 31 December. Flowers collected in terms IV and V had smaller pistils and ovaries than those collected in the earlier terms and ovule size was also small. In contrast, the pollen germinability was higher in terms IV and V. Regarding the development of embryo sacs at anthesis, most of the embryo sacs were at megagametophyte or two-nucleate stage in all terms. At 7 days after anthesis, some embryo sacs degenerated in terms I, II, IV, and V. The percentage of fruit set at 30 days after anthesis was lower than 60% in terms IV and V.

## Introduction

IN PEACH growing regions of the subtropical highlands of Thailand, marginal chilling causes sporadic bloom and extension of the flowering period by several weeks (Subhadrabandhu, 1995). This may induce a fluctuation in fruit set. However, there is little information about the influence of flowering time on flower quality and fruit set in this area. In this paper, the effects of flowering time on the development of reproductive organs and fruit set were determined.

## Materials and methods

Three 6-year-old peach 'Flordaglo' (150 CU), which were grafted on local peach grown at Royal Angkhang Station, were used. Vegetative and floral bud-break were recorded weekly from 15 November 2003 until 31 January 2004. The flowering period was classified into five terms: Term I, before 20 November; Term II, 21–30 November; Term III, 1–10 December; Term IV, 11–20 December and Term V, 21–31 December. To determine the effects of flowering time on fruit set, between 100 and 180 flowers were marked in each term. The number of

flowers was counted every 10 days from anthesis until 30 days after. At anthesis, and seven days after anthesis, in each term, five flowers per tree were collected to record their morphology — weight, length of pistil and ovary, and pollen germination on agar medium. The development of primary ovules at, and seven days after anthesis, was also observed anatomically. The size of ovule and nucellus were measured. Primary ovule development was classified into seven stages: before or at embryo sac cell differentiation, megagametophyte, two-nucleate, four-nucleate, eight-nucleate with unfused polar nuclei, eight-nucleate with fused polar nuclei, and degeneration.

Temperature was recorded from 1 November 2003 to 31 January 2004. Chilling accumulation was calculated as Chill Hour below 7.2°C (CH) and Chill Unit (CU). CU was determined using the Utah model (Richardson et al., 1974).

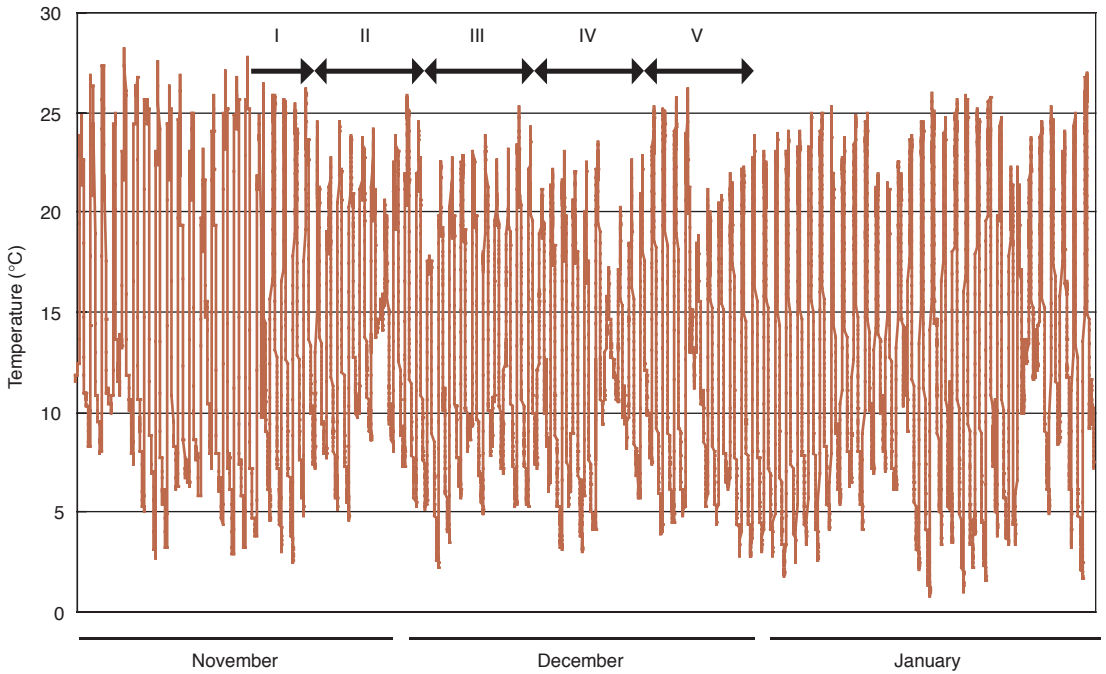
## Results

The average daily maximum temperature was 26.4°C in Term I and 22.0–23.8°C in Terms II–V (Fig. 1). Chilling units started to accumulate from 6 November 2003 (Fig. 2) to reach 150 CU in mid-December.

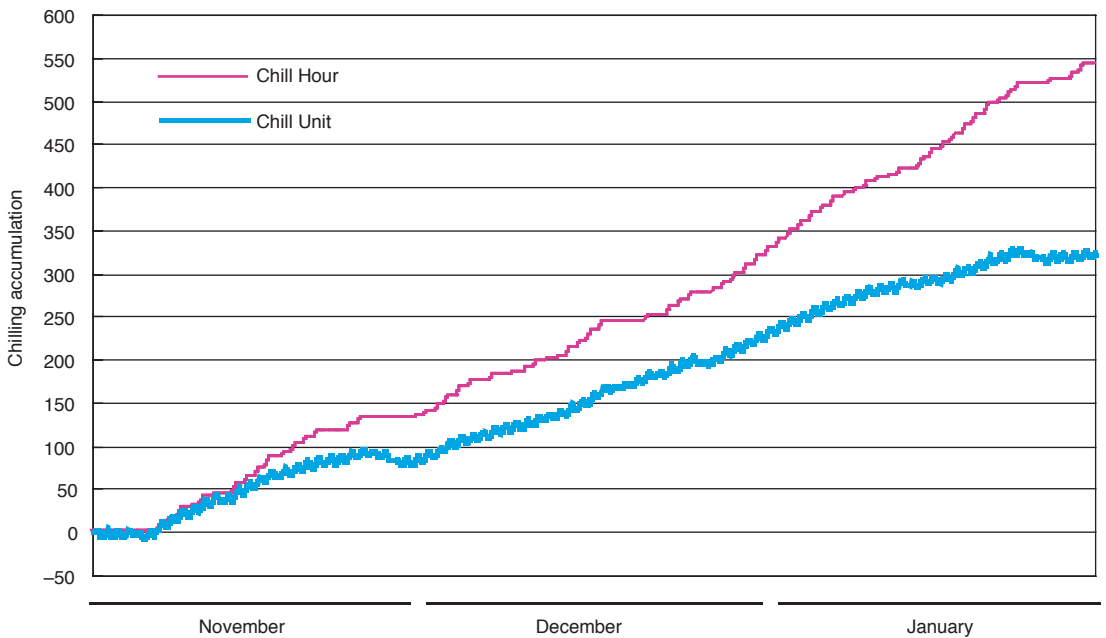
Figure 3 shows the pattern of bud break and flowering. Floral bud break finally reached about 70% by mid-December, while leaf bud break continued until late January. Flowering occurred in mid-November, and lasted until late December.

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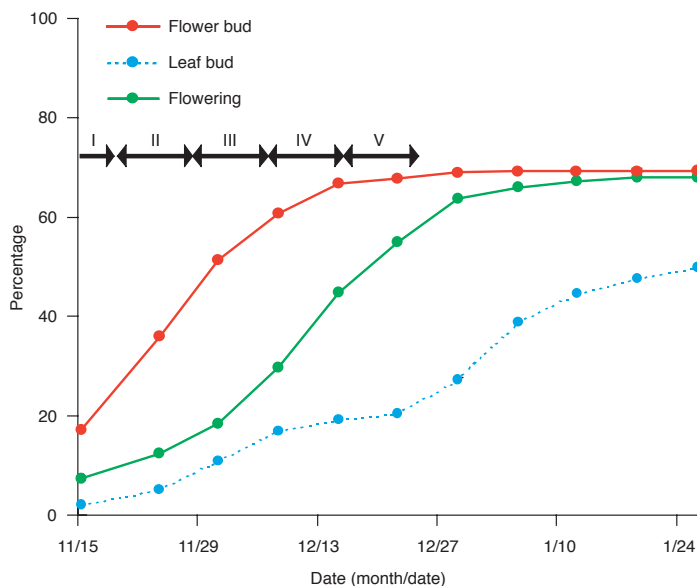
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**Figure 1.** Change of temperature in Angkhang. Arrows denote the flowering period classified into 5 terms: I, before 20 Nov.; II, from 21 Nov. to 30 Nov.; III, 1–10 Dec.; IV, 11–20 Dec., V, 21–31 Dec.



**Figure 2.** Chilling Hour and Chilling Unit accumulation in Angkhang.



**Figure 3.** Budbreak and flowering pattern of ‘Flordaglo’ peach. Arrows denote the flowering period classified into 5 terms: I, before 20 Nov.; II, 21–30 Nov.; III, 1–10 Dec.; IV, 11–20 Dec.; V, 21–31 Dec.

The percentage of fruit set 30 days after anthesis was higher than 70% in terms I–III and 62.6% and 51.8% in terms IV and V, respectively (Fig. 4). Flowers in terms IV and V were quickly abscised within 20 days.

Flowers collected in terms IV and V were smaller than those in earlier terms, while pollen germination was higher in the later terms (Table 1). Ovule size was also small in terms IV and V (Table 2). Regarding the development of embryo sacs at anthesis, most embryo sacs were at megagametophyte or two-nucleate stage in all terms (Table 3). Seven days after anthesis some embryo sacs degenerated at terms I, II, IV and V (11.8–37.5%).

## Discussion

By the later stages of flowering (terms IV and V) small flowers had developed and by 30 days after anthesis a considerable numbers of flowers had abscised (Fig. 4). Poorer fruit set observed in the later flowering term appeared to be related to pistil development but not to pollen germination. Previous research indicates that high temperatures adversely affect the development of reproductive organs for sweet and sour cherry (Beppu et al., 1997; Cerovic and Ruzic, 1992), prune (Thompson and Liu, 1973), almond (Pimienta and Polito, 1983), and peach (Kozai et al., 2002). In peach, poor pistil development in ‘Hakuho’ was reportedly caused by temperatures above 25°C (Kozai et al.,

2002). However, this critical temperature was recorded in term I rather than in the later terms. This suggests that high temperature was not the cause of poor development of pistils in the later stages of flowering.

Regarding chilling accumulation, Dennis (2000) reported that lack of chilling resulted in poor set because flowers were poorly developed. Lam-Yam and Parisot (1990) indicated that insufficient chilling leads to poor flower bud development. However, we found that even though the flower buds that opened in terms IV and V received more chilling than those in the earlier terms (Fig. 2), they developed smaller pistils and set fewer fruit (Fig 4, Table 1). This suggests that lack of chilling accumulation was the cause of reduced fruit set in terms IV and V.

In deciduous trees such as peach, flower development from bud break to early fruiting depends on starch reserves. Rodrigo et al. (2000) found large differences in starch content among flowers at anthesis in apricot. Pre-stored starch in the flower supports initial flower development. Depletion of starch in the ovule reportedly leads to ovule degeneration in peach, apricot, and almond (Arbeloa and Herrero, 1991; Rodrigo and Herrero, 1998; Pimienta and Polito, 1982). Additionally, it has been suggested that pistils provide nutritive support for the growing pollen (Herrero and Hormaza, 1996). The poor fruit set observed in our experiment may be related to nutritional deficiencies. The reduction of starch reserves may be accelerated by earlier floral and vegetative budbreak.

**Table 1.** Morphology of flower organ at and seven days after anthesis, and pollen germination in ‘Flordaglo’.

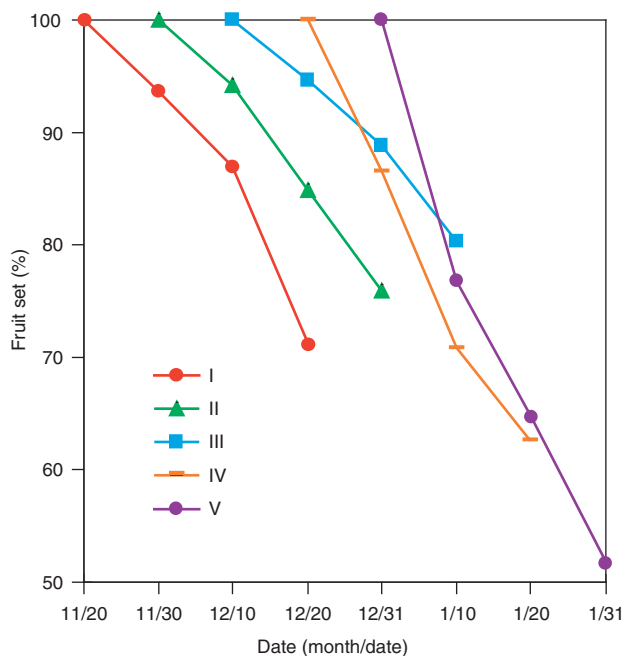
Term	0 day			7 day		Pollen germinability (%)
	Weight (g)	Pistil (cm)	Ovary (cm)	Pistil (cm)	Ovary (cm)	
I	0.142 ± 0.005*	1.74 ± 0.04	0.20 ± 0.01	2.09 ± 0.09	0.37 ± 0.03	22.6 ± 4.4
II	0.153 ± 0.003	1.74 ± 0.01	0.21 ± 0.01	2.09 ± 0.04	0.46 ± 0.04	25.4 ± 8.0
III	0.163 ± 0.015	1.70 ± 0.12	0.20 ± 0.01	2.04 ± 0.06	0.42 ± 0.05	32.7 ± 10.0
IV	0.126 ± 0.018	1.54 ± 0.14	0.19 ± 0.02	1.84 ± 0.12	0.31 ± 0.10	39.0 ± 6.1
V	0.115 ± 0.016	1.53 ± 0.14	0.17 ± 0.02	1.84 ± 0.20	0.33 ± 0.12	40.1 ± 2.9

\* SE

**Table 2.** Ovule and nucellus size at and seven days after anthesis in ‘Flordaglo’.

Term	Ovule (im)		Nucellus (im)		
	Length	Width	Length	Width	
0 day	I	590 ± 23*	278 ± 21	268 ± 65	124 ± 39
	II	596 ± 24	289 ± 8	219 ± 32	110 ± 11
	III	594 ± 20	295 ± 8	253 ± 30	123 ± 13
	IV	504 ± 53	245 ± 5	215 ± 29	100 ± 4
	V	470 ± 81	250 ± 40	218 ± 40	100 ± 17
7 day	I	977 ± 84	382 ± 15	458 ± 56	203 ± 18
	II	1176 ± 91	468 ± 12	682 ± 14	279 ± 14
	III	1040 ± 97	441 ± 11	579 ± 80	269 ± 43
	IV	860 ± 132	351 ± 28	414 ± 61	174 ± 19
	V	790 ± 229	346 ± 69	389 ± 146	180 ± 60

\* SE



**Figure 4.** Percentage of fruit set: I, before 20 Nov.; II, from 21–30 Nov.; III, 1–10 Dec.; IV, 11–20 Dec.; V, 21–31 Dec.



**Table 3.** Effect of flowering term on the development of embryo sac of 'Flordaglo'.

Stage of development	Term				
	I	II	III	IV	V
<i>At antithesis</i>					
Before or at embryo sac cell differentiation	23.1	13.3	14.3	33.3	30.8
Megagametophyte	7.7	20.0	28.6	40.0	38.5
Two-nucleate stage	46.2	40.0	35.7	13.3	23.1
Four-nucleate stage	7.7	13.3	14.3	6.7	7.7
Eight-nucleate stage					
Unfused polar nuclei	0.0	13.3	0.0	6.7	0.0
Fused polar nuclei	0.0	0.0	0.0	0.0	0.0
Degenerated embryo sac or ovule	15.4	0.0	7.1	0.0	0.0
No. of ovules examined	13	15	14	15	13
<i>Seven days after antithesis</i>					
Before or at embryo sac cell differentiation	6.7	0.0	13.3	0.0	23.5
Megagametophyte	0.0	0.0	0.0	0.0	0.0
Two-nucleate stage	0.0	0.0	0.0	0.0	0.0
Four-nucleate stage	6.7	0.0	0.0	5.9	5.9
Eight-nucleate stage					
Unfused polar nuclei	13.3	0.0	20.0	41.2	17.6
Fused polar nuclei	46.7	62.5	66.7	29.4	41.2
Degenerated embryo sac or ovule	26.7	37.5	0.0	23.5	11.8
No. of ovules examined	15	16	15	17	17

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# Time of floral initiation and flowering of Japanese apricots at the Royal Agricultural Station Angkhang

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## Abstract

Timing of floral bud initiation in fruit trees is important for the correct timing of cultural practices such as pruning and chemical and fertiliser application. The objective of this study was to determine the time of floral initiation and flower bud break in two low-chill Japanese apricot cultivars; 'Taiwan' (TW) and 'Khunwang1' (KW1) at the Royal Agricultural Station, Angkhang. One-year-old short spurs of these trees were collected every 2 weeks (June–October 2003). Floral initiation and development were recorded with a stereomicroscope. Flower initiation was characterised by morphological changes of the vegetative bud to a floral meristem. Sixty-five per cent of flowers were initiated by early August in 'TW' and 68% in 'KW1' in late August. Anthesis occurred in late December. On the same spur, floral initiation and development of the basal buds occurred faster than in middle or terminal buds.

## Introduction

JAPANESE apricot (*Prunus mume* Sieb. et Zucc.) is being trialled commercially as a substitute crop for the opium poppy in northern Thailand. The Royal Agricultural Station at Angkhang had many varieties of Japanese apricot in its collection. The tree is fast growing and can withstand long periods of dryness (Subhadrabandhu et al., 1990). Since it is a deciduous tree, it requires a period of low temperature for breaking bud dormancy. The most limiting factor for growing temperate fruit trees in the warm tropics is the inadequate exposure to chilling temperatures in winter. Flower bud formation can be a problem, especially at low altitude where excessive vegetative growth often delays flower bud initiation (Faust, 2000)

In Japan, initiation of flower buds of Japanese apricot begins in October and is completed in mid-December. Flowering occurs from February to March (Tzonev and Yamaguchi, 1999). In highland areas of northern Thailand, Japanese apricot trees flower from late December to mid January. Flowers usually occur in two or three flushes and fruit mature in April. In

general, fruits on the same tree cannot be harvested at the same time due to uneven maturity.

Thus, this study aimed to determine the timing of floral initiation and organogenesis in two low-chill Japanese apricot cultivars; 'Taiwan' (TW) and 'Khunwang1' (KW1) at the Royal Agricultural Station Angkhang. Fruit growers need a better understanding about the onset of the flowering process in order to develop a system of pruning and to select other tree management practices which aim to improve fruit set and control yield.

## Material and methods

Flower bud development was studied on two 13-year-old, low-chill Japanese apricot cultivars — 'TW', a commercial cultivar and 'KW1', a promising new cultivar. They were planted at the Royal Agricultural Station Angkhang, Chiang Mai Province, Thailand. One-year-old spurs were collected every 2 weeks from June to October 2003 and buds fixed in 50% FAA solution (formaldehyde: acetic acid: 70% ethanol = 5: 5: 90). Buds were dissected by removing bud scales under a stereomicroscope. For each collection, these buds were classified as being at one of five stages: (1) flower initiation (2) sepal formation; (3) petal formation; (4) stamen formation and (5) carpel formation. Percentage of floral bud break was recorded every 4 days from October to January in both cultivars.

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## Results and discussion

Japanese apricot bud differentiation was considered to have five stages. Flower initiation occurs at the apex then later becomes broadened to exhibit a dome shape that produces bract primordia on the periphery of the apex. In this experiment there were significant differences in the timing of flower initiation (Table 1). The first evidence of initiation was in early August for 'TW' and in late August for 'KW'. Stage 2 began with the initiation of five sepal primordia and was completed on 9 August. Stage 3, where petal primordia alternate with the sepal primordia, occurred on 23 August. The stamen primordia form acropetal to the petal primordia (stage 4). Stage 5 in both cultivars occurred in early October (Fig. 1). Carpel initiation occurred 2–3 months after flower initiation. Full bloom occurred in late December. There were no significant differences in the timing of stage 5 and flowering. However, not all the buds formed at the same date and therefore buds were not synchronised (Fig. 2). Thus, the flowering period occurred shortly after breaking of dormancy. A shorter post-dormancy stage compared with more temperate regions appears to be due to a more rapid rise in temperature during this period.

It was possible that, on the same spur, floral initiation and development of the basal buds occurred faster than in middle or terminal buds. This may cause a longer flower bud break period from late October until late January. Thus, at the end of these processes, flower bud formation of 'TW' was faster than 'KW'. Similar research by Lam-yam et al. (1990) on peach has shown that the morphological development of flower primordia is more rapid for the

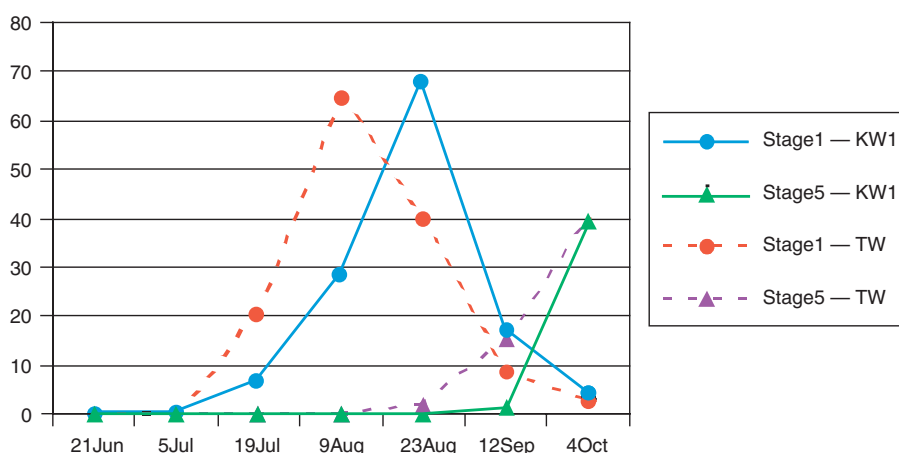
varieties with higher chilling requirement. Furthermore, we noticed that 'KW' exhibited more vigorous vegetative growth. Thus, it had a lower number of flower buds formed than 'TW'.

From these results, we have gained a better understanding of the flower initiation process of Japanese apricot grown in our climate. Our studies have clarified our views about erratic blossoming and staggering, which lead to poor fruit set and fruit quality. Comparing our Japanese apricots to those growing traditionally in cold temperate climate zones, the difference in the behavior of buds leads us to select a training system adjusted for growth habit. Pruning experiments, combined with the trials of chemical growth retardants, should be tried to complete the proper management for this plant in the future.

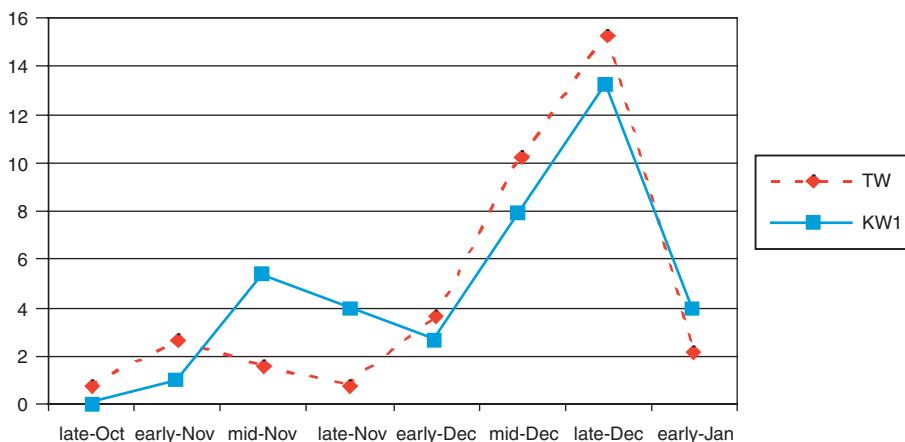
**Table 1.** Per cent flower buds initiated at different times in two cultivars of Japanese apricot

Date	Per cent flower bud <sup>a</sup>	
	TW	KW
21 June	0.00 d	0.00 c
5 July	16.03 d	3.33 c
19 July	30.00 c	11.67 bc
9 August	65.59 a	19.25 bc
23 August	55.42 b	67.96 a
12 Sept.	12.50 d	25.87 b
4 Oct.	2.08 d	2.78 c
Pr > F	0.0139	

<sup>a</sup> Means within the same parameter followed by similar letter are not significantly different at the 5% level by Duncan's Multiple Range Test.



**Figure 1.** Percentage of flower buds at stage 1 and stage 5 of Japanese apricot in TW and KW1 cultivars.



**Figure 2.** Flowering period of Japanese apricot TW and KW1 cultivars.

### Conclusion

Flower initiation in two Japanese apricot cultivars ‘TW’ and ‘KW’ occurred in early and late August, respectively. Floral initiation for cv. ‘TW’ is earlier than cv. ‘KW’. Time of floral bud differentiation of both cultivars overlap. Floral initiation occurs from August until October and flowering from December in both cultivars.

### Acknowledgment

We would like to express our gratitude to the Royal Project Foundation for allowing us to conduct the experiment and for the financial support of the Thailand Research Fund.

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# Time of floral initiation in low-chill peaches and nectarines in the highlands of northern Thailand

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## Abstract

Time of floral bud initiation was observed for four low-chill peaches; TXW1293-2, TX2293-2, 'Flordacrest' and 'TropicBeauty' and two nectarines; 'Sunblaze' and 'Mayglo', budded on two different rootstocks; 'Nemaguard' and 'White Angkhang'. Trees were planted at two sites; the Angkhang Royal Agricultural Station and the Chiang Mai Royal Agricultural Research Center Khunwang. The objective was to evaluate the influence of genotypes, rootstocks and sites on time of flower bud initiation. Current season shoots were collected every 2 weeks from April to August in 2002. Lateral buds were dissected and inspected under a stereo-microscope. Flower initiation was characterised by the morphological change of vegetative bud meristem to flower primordia. Statistical differences were found for genotypes and sites but not for rootstocks. Significant interactions were observed between genotypes and sites with ranking of cultivars changing only slightly between sites, indicating that genotypic expression was similar at both sites. Flower initiation occurred earlier at Khunwang than at Angkhang by an average of 3–4 weeks.

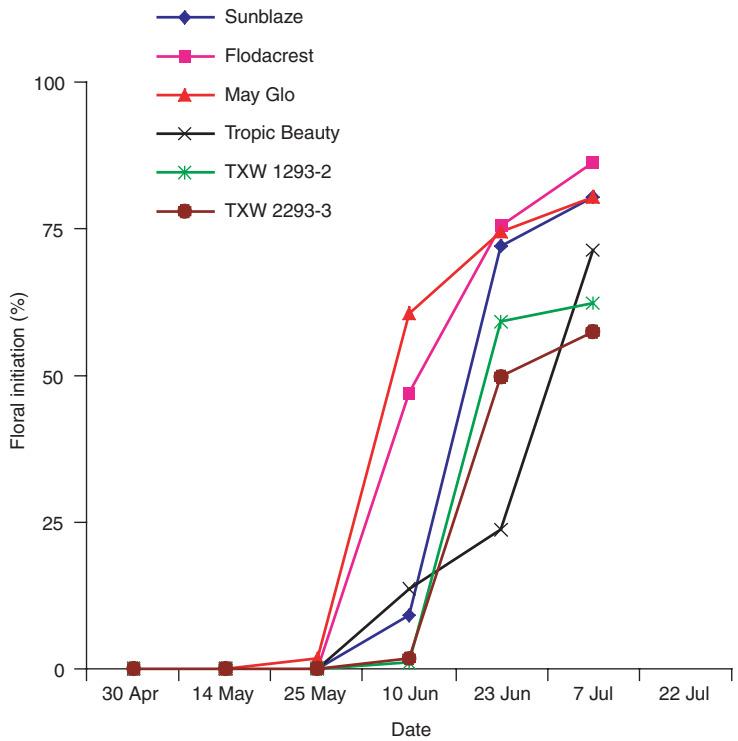
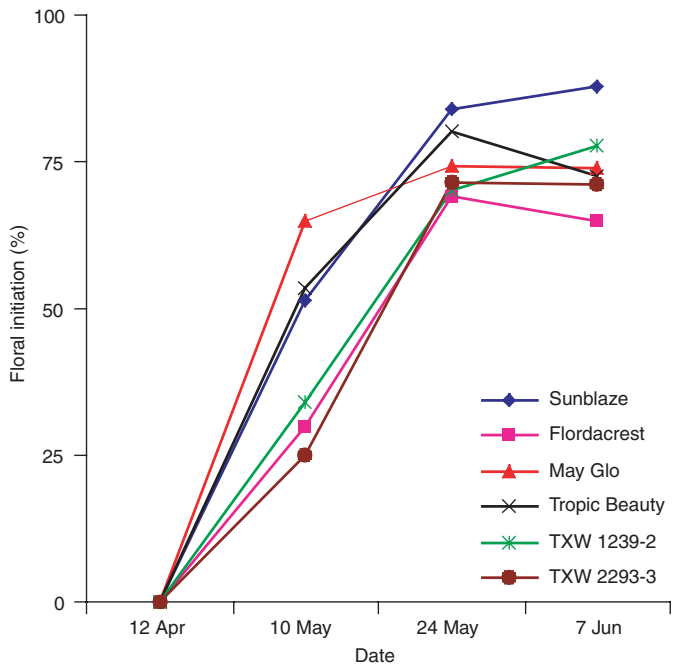
## Introduction

BUDS OF peaches and nectarines are simple and either flower or vegetative buds. Flower buds form on 1-year-old branches (Schaffer, 1994). The fruit of peach and nectarine contain one seed and a hard endocarp, which is why they are called stone fruit (Erez, 2000). Flower buds are initiated from late June to late July in warm climates (Raseira and Moore, 1987) and their formation depends on several factors. The most important factor is carbohydrate reserves. Low carbohydrate reserves adversely affect formation and development of flower buds (Schaffer, 1994). Carbohydrates are formed and stored from harvesting to dormancy. Factors important for increasing carbohydrate reserves are low vegetative growth (Lam-Yam and Parisot, 1990) and low relative humidity (Schaffer, 1994). The flower bud formation period depends on cultivar, rootstock type, environment and cultural practice or training and pruning (Warriner et al., 1985). At present, there is no information about time of flower bud initiation for stone fruit in Thailand. Results of this study can be used to compare new selections and cultivars from the breeding program or effects of cultural practices (Erez, 2002).

## Materials and methods

Four peach cultivars; 'TropicBeauty', TXW1293-2 (very low-chill cultivars; 150 Chilling Units; CU), 'Flordacrest' and TX2293-3 (low-chill cultivars; 400–450 CU) and two nectarine cultivars; 'Sunblaze' (very low-chill cultivar; 250 CU) and 'Mayglo' (low-chill cultivar; 400 CU) were used in this study. They were budded onto 'Nemaguard' and 'White Angkhang' rootstock in August 1998. Trees were planted at the Chiang Mai Royal Agricultural Research Center Khunwang and the Angkhang Royal Agricultural Station. Fifteen 1-year-old branches per tree were collected randomly in the first week of April 2002. After that, two 1-year-old branches were collected randomly from around the periphery of the canopy every 2 weeks until August 2002. Branches were cut and soaked in Copenhagen solution (10 absolute alcohol: 1 glycerol: 8 water) (Pisoksantiwatthana, 2001). Bud scales on the flower buds were left and observed under stereo-microscope. The characteristics of the apical domes were observed. The apical dome of the vegetative bud is round and swollen (Fig. 2) whereas the apical dome of the flower bud is flat (Fig. 3). For 1-year-old shoots, we recorded the date when vegetative buds changed to 50% flower buds. Time of flower bud initiation for cultivars, sites and rootstock were compared. Data were analysed by the general linear model procedure and Duncan's Multiple Range Test in SAS program (SAS Institute, Carry, N.C.).

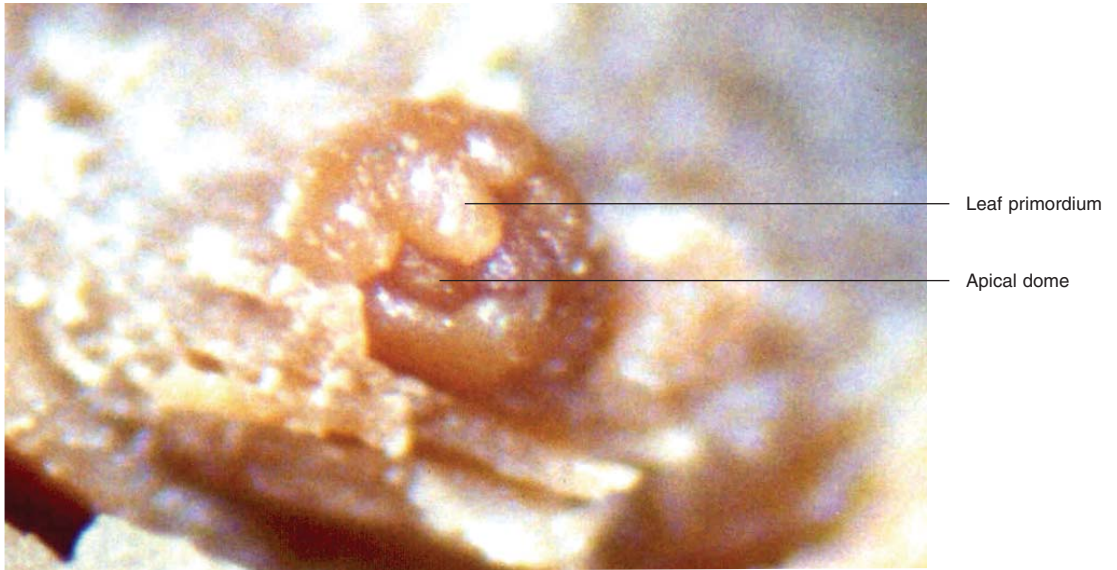
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**Figure 1.** Date of floral initiation of peaches and nectarines at Khunwang (top) and Angkhang (below).



**Figure 2.** Vegetative bud shows a dome-shaped axillary meristem and leaf primordia.



**Figure 3.** Flower bud initiation shows flattening apical dome and rounding of leaf primordia.

**Table 1.** Average date for 50% floral initiation of peach and nectarine in 2002

Factor	Date of floral initiation <sup>1</sup>
<b>Cultivar (A)</b>	
Mayglo	147.1 d
Sunblaze	155.5 c
Flordacrest	157.4 bc
TXW1293-2	161.0 bc
Tropic Beauty	164.3 ab
TXW2293-3	170.6 a
Prob. F-test	
	0.0001
<b>Site (B)</b>	
Angkhang	177.5 a
Khunwang	138.7 b
Prob. F-test	
	0.0001
<b>Rootstock (C)</b>	
White Angkhang	157.81
Nemaguard	159.23
Prob. F-test	
	0.24
A and B interaction	
	0.019

<sup>a</sup> Means followed by different letters are statistically different at the 95% probability level according to Duncan's Multiple Range Test.

## Results and discussion

The time of flower bud initiation (TFI) of 'Mayglo' was significantly earlier (147.1 days) than all other cultivars. The TFIs of 'Sunblaze', 'Flordacrest' and TXW1293-2 were not significantly different, with average times of 155.5, 157.4 and 161 days, respectively. The TFI of cv. TX2293-3 was later (170.6 days) than the other cultivars with the exception of 'Tropic Beauty'. The TFI of 'TropicBeauty' (164.3 days) and TX2293-3 are not significantly different (Table 1). The results showed that chilling requirement did not affect the TFI as these two variables do not appear to be correlated. Fifty per cent TFI of trees at Khunwang (10–24 May) is earlier than that at Angkhang (10 June – 7 July) (Fig. 1). The average TFI at Angkhang and Khunwang is 138.7 and 177.5 days, respectively. The times are significantly different because the vegetative period at

Khunwang is longer than at Angkhang. As well, temperatures during the growing season at Khunwang are higher than that at Angkhang. All these factors affect the TFI (Erez, 2000). The effects of rootstocks, 'Nemaguard' and 'White Angkhang' on TFI were not significantly different. Genotype and site interaction affected the TFI but this only changed the ranking of cultivars slightly between both sites indicating that genotypic expression at one site was correlated with the other site.

## Conclusions

The TFI ranking of all six cultivars from earliest to latest is 'Mayglo', 'Sunblaze', 'Flordacrest', TXW1293-2, 'TropicBeauty' and TX2293-3, respectively. Sites had an effect on the TFI. The TFI at Khunwang and Angkhang is 138.7 and 177.5 days, respectively. Rootstocks had no effect on the TFI. Genotype and site interaction affected the TFI but the ranking between cultivars was only slightly changed.

## Acknowledgment

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# Effect of ReTain® and potassium chloride on peach fruit quality in the subtropical highlands of Thailand

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## Abstract

The objective of the study was to increase fruit quality of peach cv. Tropic Beauty, growing in the subtropical highlands of Thailand. A number of chemical products were applied pre-harvest to improve fruit quality of peach grown at the Chiang Mai Royal Agricultural Research Center during 2003–2004. The chemicals tested were: ReTain®, Aminofit finishing® and potassium chloride. The results showed that ReTain® sprayed either once or twice prior to harvest can delay fruit ripening and concentrate harvesting times. Both ReTain® and Aminofit finishing® increased fruit size and fruit firmness.

## Introduction

Ethylene is a plant hormone that is involved in plant processes such as fruit maturation. An approach to manipulation ripening could be achieved by applying substances that inhibit ethylene production such as aminoethoxyvinylglycine (AVG). Retain® (AVG), a new commercial product, is an ethylene biosynthesis inhibitor that delays fruit maturation if applied before harvest (Williams, 1980). Retain® increases fruit set if applied after bloom in pears and apples. Aminofit finishing® is a product of amino acid and polypeptide biosynthesis, with low molecular weight, that can penetrate through plant cells directly. It can improve the efficiency of chlorophyll synthesis and can improve fruit quality variables such as sugar content. Potassium is the nutrient that generally improves fruit sweetness, increasing TSS content and thus eating quality. This study was conducted to evaluate the effect of different chemicals on yield and improve quality of low-chill peach cv. Tropic Beauty, growing under warm sub-tropical climates of Thailand.

## Materials and methods

Experiments were conducted in 2003–2004 at Chiang Mai Royal Agricultural Research Center, 19°N, altitude 1300 m asl. Trees selected were uniform, 6-year-old cv. 'Tropic Beauty' peach, spaced 3.5 m within the row and 4 m between rows. Trees were trained to a vase system and pruned in late winter. Experimental design was a randomised block, with 5 treatments and 3 replicates using single tree treatment. Treatments were:

1. Water (untreated control).
2. ReTain® at the rate of 16.6 g/20 L water + surfactant organosilicone 10 ml/20 L water, single spray applied 7 days prior to harvest.
3. ReTain® at the rate of 16.6 g/20 L water + surfactant organosilicone 10 ml/20 L water, two sprays applied at 7 and 14 days prior to harvest.
4. Aminofit Finishing® at the rate of 50 ml/20 L water, three sprays at 14, 21 and 30 days prior to harvest.
5. Potassium chloride (0-0-60) at the rate of 200 g/20 L water, three sprays applied at 14, 21 and 30 days prior to harvest.

The commercial formulation of AVG was supplied by Valent BioSciences as ReTain®. Trees were thinned to a set number of fruit based on their size. All other cultural practices were applied as per normal phonology cycle.

Trees were harvested at normal commercial stage of fruit maturity, with a change in ground colour from green

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to yellow-red at intervals of 3–4 days, for yield and fruit quality assessment. The individual weights of 20 fruits, randomly chosen, were recorded for each tree.

At peak harvest, 20 fruits from each datum tree were tested for sugar concentration (Brix) from expressed juice, using a digital refractometer and fruit firmness was tested at two points on the fruit surface with a penetrometer (8 mm tip).

## Results and Discussion

### Harvesting time

In 2003, all treatments were harvested from 21 February to 21 April. In the 2004 season, a single spray of ReTain® was found to delay and shorten the harvesting period (15–24 March). The trees that received two sprays of ReTain® had an even shorter harvesting period (10–17 March). All other treatments, including the control, had a more protracted harvesting period which commenced 2 weeks earlier (26 February to 30 March).

In 2003, all treatments required five picks to complete harvesting. By contrast, in 2004, two sprays of ReTain applied prior to harvest reduced the number of harvest picks from five to two (Table 1).

**Table 1.** Number of harvest picks of peach cv. ‘Tropic Beauty’ in 2004.

Treatment	No. of harvest picks
Control	5 bc
ReTain® (spray at 7 days prior to harvest)	4 ab
ReTain® (spray at 7 and 14 days prior to harvest)	2 a
Aminofit Finishing®	7 c
Potassium chloride (0-0-60)	6 bc

\* Means within the same parameter followed by the similar letter are not significantly different ( $P < 0.05$ ).

**Table 2.** Fruit harvested on each picking date (%) for peach cv. Tropic Beauty in 2003.

Treatments	Total fruit harvested (%)				
	1 (21/2/04)	2 (28/3/04)	3 (1/4/04)	4 (8/4/04)	5 (21/4/04)
Control	12.3	21.5	11.7	40.0	14.5
ReTain® (spray at 7 days prior to harvest)	2.95	6.57	8.18	56.0	26.3
ReTain (spray at 7 and 14 days prior to harvest)	4.20	28.4	14.2	25.5	27.7
Aminofit Finishing®	3.45	18.4	19.6	49.9	8.65
Potassium chloride (0-0-60)	4.70	25.7	11.7	29.8	28.1

\* Means within the same parameter followed by the same letter are not significantly different ( $P < 0.05$ ).

### Harvest distribution

In 2003, all treatments had the same harvest distribution, except treatment 2 where one spray of ReTain delayed the harvest peak, with more than 80% of fruit picked on the last two harvest dates (Table 2).

In 2004, for the Aminofit Finishing® and potassium treatments, most fruit were harvested between 26 February and 10 March (1st – 3rd picking dates). The treatments with two sprays of ReTain®, 7 and 14 days prior to harvest, showed a peak harvest on 10 March, with more than 80% of the total fruit harvested. One spray of ReTain®, 7 days prior to harvest, showed a more even harvest pattern (Table 3).

### Fruit quality

Compared with controls, treatments increased fruit firmness of ‘Tropic Beauty’ peach in both 2003 and 2004 (Table 5). Compared with controls, ReTain® sprays applied at 7 and 14 days prior to harvest, and potassium chloride sprays, improved TSS concentration in 2003 but not in 2004. Compared with controls, treatments increased titratable acid (TA) for both 2003 and 2004.

ReTain® (AVG), applied 7 and 14 days prior to first harvest, increased average fruit weight by about 10% and yield per tree by about 20%. (Table 4). AVG delayed fruit maturity by about 7 days. AVG improved fruit colour intensity of ‘Tropic Beauty’ peach by about 50%. Jerie (2000) found that AVG increased sugar content in temperate fruits but our studies showed only slight increases in one season only. AVG, applied 7 days before harvest, significantly increased fruit firmness by 30% to 50%. In terms of fruit quality, Willams (1980) showed that AVG significantly increased fruit firmness when applied as a pre-harvest spray to apple. The increases in fruit firmness in this study are comparable to the findings of Launder and Jerie (2000) who reported increases in fruit firmness in a wide range of stone fruit varieties by 12% to 60%.

**Table 3.** Fruit harvested on each picking date for peach cv. 'Tropic Beauty' in 2004

Treatments	Total fruit harvested (%)							
	1 (26/2/04)	2 (3/3/04)	3 (10/3/04)	4 (15/3/04)	5 (17/3/04)	6 (20/3/04)	7 (24/3/04)	8 (30/3/04)
Control	38.3	9.33	41.06	0	5.33	0	6.04	0
ReTain® (spray at 7 days prior to harvest)	0	0	0	25.8	30.5	23.5	20.2	0
ReTain® (spray at 7 and 14 days prior to harvest)	0	0	81.0 a	0	19.0	0	0	0
Aminofit Finishing®	19.7	21	42.0 b	3.75	9.33	0	2.11	2.11
Potassium chloride (0-0-60)	26.7	18	40.7 b	9.00	6.33	3.60	0	0

\* Means within the same parameter followed by the similar letter are not significantly different ( $P < 0.05$ ).

**Table 4.** Yield per tree and fruit size of peach cv. 'Tropic Beauty' in 2003–2004

Treatments	Fruit weight per tree (kg)		Fruit size				
	2003	Average fruit wt. (g)	Fruit width (cm)		Fruit length (cm)		
			2003	2004	2003	2004	
Control	3.65	142 ab	113	6.46 ab	5.52	6.10	5.48
ReTain® (spray at 7 days prior to harvest)	4.48	141 ab	110	6.46 ab	5.79	6.31	5.73
ReTain® (spray at 7 and 14 days prior to harvest)	4.66	154 a	118	6.65 a	5.45	6.46	5.26
Aminofit Finishing®	4.74	126 b	110	6.23 b	5.63	6.12	5.58
Potassium chloride (0-0-60)	3.64	134 ab	114	6.32 ab	5.62	6.10	5.17

\* Means within the same parameter followed by the similar letter are not significantly different ( $P < 0.05$ ).

**Table 5.** Fruit quality of peach cv. Tropic Beauty in 2003–2004

Treatments	Fruit firmness (kg/cm <sup>3</sup> )		TSS content Brix		TA content %		TSS/TA Ratio	
	2003	2004	2003	2004	2003	2004	2003	2004
Control	1.54	1.50	9.59	11.3 a	0.60 b	1.50	16.0	7.53
ReTain (spray at 7 days prior to harvest)	2.02	1.85	9.51	9.34 c	0.72 ab	1.85	13.2	5.05
ReTain (spray at 7 and 14 days prior to harvest)	2.38	1.69	10.79	11.0 ac	0.79 a	1.69	13.7	6.51
Aminofit Finishing	2.19	1.84	9.76	9.66 bc	0.74 ab	1.84	13.2	5.25
Potassium chloride (0-0-60)	1.89	1.80	10.31	10.5 abc	0.64 ab	1.80	16.1	5.83

\* Means within the same parameter followed by the similar letter are not significantly different ( $P < 0.05$ ).

Studies on peaches (Vizzotto et al., 2002; Bregoti et al., 2002) and apples (Brackmann and Waclawovsky, 2001) have shown that AVG application prior to harvest significantly increased fruit size, firmness, sugar concentration and storage life. AVG appears to reduce and delay fruit maturity. These responses may be an advantage for low-chill, early maturing stone fruit varieties that, because of their short fruit development

periods, exhibit small fruit size, medium firmness and low sugar concentrations. Consequently, AVG has the potential to be used as a management tool for controlling the timing of harvest and for allowing fruit to ripen more slowly, thus improving fruit size. In addition, AVG may assist in maintaining the post-harvest flesh firmness required to withstand handling during marketing (Lawes and Woolley, 2001).

## Conclusion

One spray of ReTain<sup>®</sup>, at about 7 days prior to first harvest, was highly effective in increasing fruit size, fruit firmness and sugar concentration. Earlier times of application and higher rates than 0.83 g/L did not appear to improve efficacy and may have had some detrimental effects on fruit quality. AVG has the potential to be a key management tool in low-chill stone fruit orchards as an aid to improving fruit size and firmness, which are generally poor for short fruit development cultivars and also to control pre-harvest drop. The following conclusions can be drawn from this study: ReTain<sup>®</sup> delayed and concentrated harvesting in 'Tropic Beauty' peach; ReTain<sup>®</sup> sprayed at 7 and 14 days prior to harvest can increase fruit size; and ReTain, Aminofit and potassium chloride can improve fruit quality e.g. fruit firmness.

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