Chapter 2

Literature Review

1. Origin and distribution of lychee

Lychee is doubtless proof that man has used it for along time. Most authors tell us that the species have been cultivated for some 3000 years, but it has been said to date back forty centuries. Although it is impossible to determine a date with any accuracy, lychee is reported to have been cultivated at least as far back as 1766 BC. The lychee is adapted to the warm sub-tropic, cropping best in regions with brief cool dry frost-free winters and long hot summers with high rainfall and humidity (Menzel and Simpson, 1987). Lychee cultivation has been reported since 1500 BC by the people of Malayan descent and has been growing for thousands of years in southern Guangdong province of China. From China, it reached Mayanmar (Burma) by the end of 17th century and was introduced to India about 100 years later. Lychee reached Madagascar and Mauritius around 1870 and was introduced to Hawaii in 1873 by a Chinese trader. It arrived in Florida, from India, between 1870 and 1880 and was introduced to California in 1897. Lychee was brought to Australia by Chinese immigrants in 1954 and arrived in Israel sometime between 1930- 1940. Presently, lychee is grown in central and south America, parts of Africa and throughout Asia. Thailand are now major lychee producing countries in the world (Ghosh, 2001).

2. Climate of lychee growing areas

Lychees are grown commercially from 17° and 32° latitude, and are usually found at low elevation in the subtropics and from 300-600 m mean sea level (MSL) in tropical locations, with cool or cold winters and warm to hot summers (Menzel, 1983). Rainfall is generally highest in summer and least winter or spring. The only exceptions are near Cairns in northern Australia (lat. 17^0 S) where they are grown along the coastal flats and in some areas of South Africa (lat. 25^0 S) where they are found at 600-800 m MSL. In both these locations, winters are dry and the Tai So

and/or Maritius cultivars are grown. Tropical lowland types have also been developed for the central plains of Thailand north of Bangkok and in a few selected areas of Indonesia. The range in climates in the main production areas is much narrow for lychee compared with other tropical fruits such as citrus and banana that are grown across several climatic zones (Menzel, 2001). Moderate day/night temperatures (20°-15° / 15°-10°C) increased vegetative growth and reduce flowering in lychee (Menzel and Simpson, 1988). Lychees need a dry period to encourage vegetative dormancy and especially a period of cool weather to initiate flowering. Minimum temperature of about 10° -15° C and rainfall less than 60 - 80 mm. in winter, are required. However, these requirements vary depending on varieties, for example the lychees in the central region of Thailand require short period of dryness and cool for flowering whereas the northern lychees need longer period. Mild conditions are required during flowering (February - March) to ensure pollination. Temperatures below 5° C can affect flowering, this is one of the reason why lychee is not commonly grown in the hill areas in Chiang Mai and Chiang Rai where the temperature in winter is too low. Hot dry winds during blooming can devastate fruit set. Low humidity can upset flowering while heavy rain or misty conditions during the flowering period will reduce the fruit setting as pollen and bee activity are reduced. Warm humid conditions are optimal for fruit growth and vegetative growth (March - October). In some month when a long dry period occur, irrigation become quite essential, since uniform soil moisture is most important for high fruit production during critical periods at flowering, fruit set and the latter part of fruit development. Low humidity, hot dry winds and fluctuating soil moisture can devastate yields or cause fruit splitting (Subhadrabandhu, 1990).

Lychees respond quickly to changes in the environment and cultivars are not readily adaptable to a wide range of localities. For this reason, cultivars must be selected specifically for each district. In general terms, for regular cropping lychee requires cool, dry winter periods to encourage vegetative dormancy prior to flowering, mild dry weather during flowering and warm, wet period for successful fruiting (Subhadrabandhu, 1990).

3. Lychee in Thailand

In Thailand, the lychee cultivars can be divided according to the adaptability into two groups.

Lowland or Tropical cultivars

The cultivars in this group do not require a cool period for flowering. These cultivars are sometimes classified as lowland lychee or tropical lychee since they are commercially grown in the central region of Thailand. The growing areas of these lychee are in Umpawa and Bangkontee districts in Samut Songkhram province near Bangkok. Some leading cultivars in this group are Chau Ra Kum, Dang Pha Yom, Haew, Khiew Waan and Kom (Subhadrabandhu and Yapwattanaphun, 2001a). Kom is an important cultivar of lowland group (Anupunt and Sukvibul, 2005).

Subtropical cultivars

The cultivars in this group require a longer cool period for flowering. These cultivars are commercially grown in the northern part of Thailand where the climate is classified as subtropical. Large orchards are found in Chiang Mai, Chiang Rai and Lamphun provinces, with some areas in Petchaboon, Nan and Prae provinces. These lychee cultivars may have been introduced to Thailand much later than the first group. The characters of some leading cultivar are as Kwang Choia, Kim Cheng, Chakrapad, O-Hia and Hong Huay (Subhadrabandhu and Yapwattanaphun, 2001a). Hong Huay is an important cultivar of upland group (Anupunt and Sukvibul, 2005). Lychees are less adaptable than other tree crops. Selecting a suitable site for required variety is essential for the profitable yields. It is essential that lychees must grown in sites where conditions and management of the crop will compliment with the trees natural fruiting cycle. The natural growth cycles of lychee in Thailand is shown in figure 1.
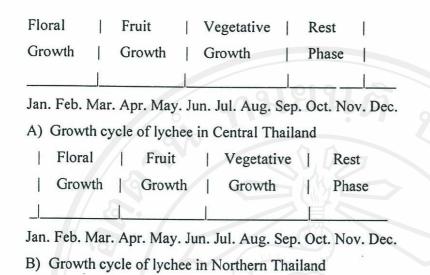
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Floral    |  Fruit    |  Vegetative  |  Rest    |
Growth    |  Growth   |  Growth      |  Phase   |
_____|_____|_____|____|_____
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Jan. Feb. Mar. Apr. May. Jun. Jul. Aug. Sep. Oct. Nov. Dec.

A) Growth cycle of lychee in Central Thailand

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|  Floral   |  Fruit   |  Vegetative  |  Rest
|  Growth   |  Growth  |  Growth      |  Phase
_|_____|_____|_____|_____
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Jan. Feb. Mar. Apr. May. Jun. Jul. Aug. Sep. Oct. Nov. Dec.

B) Growth cycle of lychee in Northern Thailand

Figure 1 Growth cycle of lychee in Central and Northern Thailand
(Subhadrabandhu, 1990)

Lychee production zones in Thailand are located in the central lowlands, and uplands of both the eastern and western regions. The most areas are in the north. Chiang Mai (8,322 ha) and Chiang Rai (5,763 ha) are the two major provinces that contribute more than 60 percent of the overall acreage (22,937 ha). Lychee growers are smallholders with planting ranging from a few trees to orchards of a few areas. Upland growers may possess up to several thousands trees, however, such growers are few in number. With the production areas ranging from the tropical lowlands to the sub-tropical climate of the north and northeast regions, the fruiting season could lengthen up to a full 3 month period. Earliest harvesting could be from mid-March in the central region through last fruiting in mid-June in the northern region (Table 1). There were several areas where selected cultivars could fill up any gap of supply throughout this 3 month period. Thus, better confidence in supply could strengthen marketing planning strategy (Sethpakdee, 2002).

Table 1 The harvesting time of lychee in Thailand separated by the production zone.

Production zone	Month			
	March	April	May	June
Chanthaburi	——			
Samut Songkhram	—			
Kanchanaburi		—		
Nakhon Ratchasima		—		
Phayao			—	
Chiang Mai and Chiang Rai			——	

4. Botany of lychee cv. Kom, Hong Huay and Chakrapad

Lychee cv. Kom

Compound leaves are small to large, 26 cm wide and 21cm long, with 3-4, usually 4 pairs of leaflets. Petioles are green in both adaxial and abaxial, with a thickness of 0.17 cm. Leaflets are oblong-shaped, green, small to large, about 2.5 cm wide, 13 cm long and 0.06 cm thick, with undulate margin, acuminate apex, cuneate base and smooth surface. Flowering habit is very profuse. Inflorescences are small to large, 6 cm wide and 12.5 cm long. Florets are profuse and in cream color. Fruit are spheroid in shape and small in size, about 2.8 cm in width, 2.9 cm in length and 12 g in weight. The skin is dark red and weighs 1.9 g per fruit. The aril is dull white and 8 g, with 14% TSS. Seeds are ovoid in shape, 1.3 cm in width, 2 cm in length and 2.3 g in weight (Subhadrabandhu and Yapwattanaphun, 2001a).

Lychee cv. Hong Huay

Compound leaves are medium to large, about 26 cm wide and 25 cm long, with 3-4, usually 4 pairs of leaflets. Petioles are dark green in adaxial and green in abaxial, with a thickness of 0.24 cm. Leaflets are dark green, oval-shaped and small to large in size, i.e. 4 cm wide, 14 cm long and 0.09 cm thick, with undulate margin, cuspidate apex, acute base and smooth surface. Flowering habit is profuse.

Inflorescences are small to large, around 9 cm wide and 17 cm long. Florets are profuse and in cream color. Fruit are ellipsoid in shape, medium in size, 3 cm in width, 3.2 cm in length and 17 g in weight. The red skin weighs 3 g per fruit. The aril is dull white and about 10 g with 14.8% TSS. Seeds are ellipsoid in shape, 1.41 cm in width, 2.4 cm in length and 3.5 g in weight. Among these subtropical lychees cv. Hong Huay is the most popular one and is commonly grown in northern Thailand. The next largest cultivars in terms of acreage in the country are 0-Hia, Chakrapad and Kim Cheng (Subhadrabandhu and Yapwattanaphun, 2001a).

Lychee cv. Chakrapad

Compound leaves are small to large, around 22 cm wide and 18 cm long, with 3-4, usually 4 pairs of leaflets. Petioles are dark green in both adaxial and abaxial, with thickness of 0.16 cm. Leaflets are dark green in color, lanceolate in shape and small to large in size, i.e. 3.5 cm wide, 11 cm long and 0.05 cm thick, with entire margin, acute apex, cuneate base and smooth surface. Flowering habit is sparse. Inflorescences are small to large, around 8 cm wide and 16 cm long. Florets are profuse and in cream color. Fruit are heart in shape and large in size, i.e. 4.3 cm wide and 4.4 cm long. Single fruit weight is about 32 g. The red skin weighs about 3.5 g per fruit. The aril is dull white and weighs about 25 g, with a TSS content of 15 %. Seeds are ovoid in shape, about 1.8 cm in width, 2.6 cm in length and 1 g in weight (Subhadrabandhu and Yapwattanaphun, 2001a).

5. Factors of flowering

The deals with the factors governing shoot initiation, floral induction, and flower development in lychee and longan, the centre of origin for both crops is southern China, and the limits of commercial production are generally between 12° and 35° latitude. Commercial plantings in China occur primarily in the southern provinces, ranging from 18° to 25° N latitude, in Guangdong (about 68% of total production), Guangxi, Fujian, Hainan, Yunnan and in Taiwan (Ghosh, 2001).

The main lychee-growing area in Vietnam is near Hanoi, whereas longan is more important in the south, near the Mekong Delta. Most of the Thai production is center around Chiang Mai (19° N latitude), but commercial orchards

growing tropical cultivars can be found as far south as Samut Songkhram (13^0N latitude) (Subhadrabandhu, 1990; Subhadrabandhu and Yapwattanaphun, 2001a).

Lychee growing in South Africa is limited to the north-east of the country ($25°$ S latitude). Production in Madagascar ($13-25°$ S latitude) is primarily by small landholders on the coastal plains along the eastern shore with some production at higher elevations. The small islands of Mauritius and Reunion ($20-22°$ S latitude) in the Indian Ocean have irregular but substantial production of lychees (Ghosh, 2001). Lychee and longan plantings in Australia span the eastern coast from Cairns ($17°$ S latitude) to northern New South Wales ($32°$ S latitude) (Menzel, 2001). Those areas producing less than 1000 t of lychee and longan per year include Indonesia ($3-10°$ S latitude), Spain ($39-41°$ N latitude) (Menzel, 2001), southern Florida ($25-27°$ N latitude) (Campbell and Campbell, 2001), northern Israel ($32-34°$ N latitude) (Goren *et al.,* 2001).

Annual temperatures vary markedly across this range of latitudes. Polar air normally reaches the higher latitudes during winter, but does not always have the power to reach the more tropical lower latitudes where it might reduce temperatures, except at higher elevations. It is well established that the lower the latitude in which lychees or longans are grown, the less dependable is flowering (Menzel, 2001). This is because cool temperatures are necessary for floral induction (Groff, 1943; Young and Harkness, 1961; Menzel and Simpson, 1994). In contrast, the upper limit of production is governed by the occurrence of frosts around the time of anthesis. Best yields occur when temperatures periodically approach freezing, followed by warm days and nights that allow flower development and pollination (Menzel, 1983). Various reviews have considered the impact of weather and tree physiology on flowering (Subhadrabandhu and Yapwattanaphun, 2001a). Using this background information, a model for vegetative and reproductive growth of lychee and longan that is similar to those of mango and citrus is proposed (Menzel and Waite, 2005).

5.1 Phenology and shoot development

In lychee and longan, periods of stem elongation are separated by periods of stasis, when the terminal bud is at rest (quiescent), or a terminal

inflorescence has formed. The interval between successive episodes of stem elongation depends on pruning, available water and nutrients, and the weather. For the part of stem developing during the current episode of stem elongation as a 'shoot' (whether vegetative or floral), and define this in recognition of the loose way in which the term is used in the literature (Menzel and Waite, 2005).

The pattern of shoot and flush development is fundamental to flowering and fruit production in lychee and longan. Batten and McConchie (1995) showed that flowering of lychee could be induced after a new shoot had been initiated, provided that the new bud was no more than a few millimetres long. This research, along with work on other tropical trees is consistent with the idea that an inductive signal acts on actively growing buds. Flushes tend to occur on groups of stems that are connected by a common scaffold within the canopy (Zheng et al., 2001). Spatial variation in the timing of flush development over a canopy can lead to variations in flowering.

5.2 Types of shoots

Under normal circumstances, one of three shoots initiates growth from apical or axillary buds in resting stems (Robbertse et al., 1995). Vegetative shoots produce only leaves at each node, generative shoots give rise to inflorescences, and mixed shoots bear both leaves and inflorescences at each node. A fourth type of shoot referred to as a "leafy inflorescence", is distinct from mixed shoots. It commences producing only leaves, but makes a transition midway to form lateral inflorescences in the distal portion of the shoot. These are defined as vegetative to floral or V/F transition shoots. Stems and shoots have an apical meristem that is never fully committed to form only leaves or flowers. Surrounding the resting meristem in both lychee (Naphrom et al., 2001) and longan are spiralling whorl of nodes, each composed of a primordial compound leaves and a lateral meristem. The primordial leaves of the outermost whorl form protective bracts that surround the meristem. The bracts are variable in size, similar to miniature leaves, but with a brownish cast. At initiation, the pre-existing primordial leaves and/or lateral meristems within each node begin to develop, depending on the type of shoot, i.e. vegetative, generative or mixed. This early development of pre-existing lateral meristems constitutes the first phase of

shoot differentiation. The apical meristem then forms more nodes, providing further leaf primordia and lateral meristems, before returning to rest in the case of a vegetative shoot, or differentiating in the case of an inflorescence (Menzel and Waite, 2005).

5.2.1 Vegetative shoots

In warm weather, the pre-exist tiny primordial leaves develop to form true compound leaves. If favourable conditions persist throughout the initiation and differentiation of new nodes, the new primordial leaves develop into compound leaves at the distal part of the shoot. The lateral meristems at the base of each expanding leaf are repressed, and form a spiral of a few lateral nodes, each forming a primordial leaf and lateral or axillary meristem. The primordial leaves of the outer-most spiral of nodes form the minute bracts that protect the new lateral buds.

Vegetative shoot development takes 6 weeks or longer, depending on the weather. During this period, the apex produces about eight leaves before returning to rest. Expanding leaves are initially light colour internodes alternating with long internodes. Lychee intercalations are less compressed than those of citrus or mango, but are easily recognized (Menzel and Waite, 2005).

5.2.2 Reproductive shoots

Reproductive shoots typically form in the apical and/or lateral buds when shoots emerge during cool weather. In contrast to vegetative shoots in which true compound leaves arise from the primordial leaf tissues, the leaf primordia remain relatively quiescent, and the lateral meristems at each node are instead evoked. The lateral meristems organize themselves to form short-lived apical meristems, which give rise to primordial leaves, and secondary lateral meristems, which may develop into tertiary or higher levels of branching, with leaf development repressed at each level. If inductive conditions continue during the formation of new nodes at the terminal apical meristem, then the same pattern of lateral meristem development prevails, thus extending the length of the thyrsoid or panicle. Elongation of the articulated thyrsoidal structures at each node terminates in dichasial floral structures (Menzel and Waite, 2005).

5.2.3 Mixed and transition shoots

Initiation of mixed shoots occurs in mild weather, at temperatures lower than those required for purely vegetative shoots, and higher than those for purely floral shoots. In this case, both leaf primordia and lateral meristems develop, resulting in shoots with a leaf and an inflorescence at each node.

Transition shoots commonly appear in lychee when shoots emerge during rapidly changing temperatures. These initiate one type of shoot, either vegetative or generative, which finish as the other. Batten and McConchie (1995) described such shoots as "leafy inflorescences", produced when plants were transferred from warm to cool conditions soon after bud break. Vegetative to flowering (V/F) transition shoots occur in south Florida when cold weather arrives soon after the initiation of vegetative shoots. Bud determination in mango is also progressive and sensitive to fluctuations in vegetative/floral signals. Transferring mango plants from warm to cool conditions soon after the initiation of vegetative shoots resulted in a high proportion of V/F shoots, while transferring plants initiating floral shoots from cool to warm conditions resulted in a high proportion of F/V shoots.

5.3 Role of growth substances in shoot initiation

Initiation is the onset of shoot development in resting buds, regardless of the type of shoot. It commences with cell division and elongation of dormant cells in the leaf primordia (vegetative shoots), lateral meristems (generative shoots) or both (mixed shoots), followed by cell division in the apical meristem, with subsequent new lateral leaf primordia and meristems in the distal portion of the shoot. Davenport (1990) proposed that shoot initiation is cyclic, occurring two or more times per year, but may also be stimulated by pruning, defoliation and irrigation. Shoot initiation in citrus and mango is regulated by the ratio of a shoot initiation promoter and a shoot initiation inhibitor. It was further proposed that cyclic changes in these components also regulate shoot initiation in lychee and longan. A high promoter:inhibitor ratio favours shoot initiation, and a low ratio favours continued rest.

Evidence elicited from citrus and mango indicates that the promoter is a cytokinin (CK) produced in growing root tips and transported in xylem to the buds. This concept is supported by the observation of higher concentrations of endogenous dihydrozeatin (DHZ) in xylem sap of vigorous rootstocks with frequent flushes on Alphonso mango, compared with low levels in non-vigorous, dwarfing rootstocks producing fewer flushes. The inhibitor may be an auxin that is produced in, and transported at relatively high concentrations from, expanding leaves, tapering off as they age. It is proposed that auxin synthesized in young leaves inhibits growth of resting buds, and is transported to roots that are connected to stems that have recently flushed. As those stems age, the growing roots synthesize CKs that are transported in xylem, and accumulate in these same stem buds. This is despite the fact that most of the water in the xylem flows out of the leaves (Menzel and Waite, 2005).

Although a distinct alternation in root and shoot growth has been demonstrated in large avocado trees such alternation was not apparent in young lychees (Marler and Willis, 1996). These relationships may explain why flushes occur frequently in young trees, due to the close proximity of stem, and roots. Consistent with this hypothesis is the observation that pruning or defoliation stimulates shoot initiation. Such treatments not only remove the source of auxin production, but also increase CK concentrations in xylem sap which would quickly shift the CK:auxin ratios to favour bud break (Menzel and Waite, 2005).

5.3.1 Auxins

Auxins are primarily synthesized in young leaves and seeds and trans-ported to root tips. Unlike CKs, they typically inhibit shoot initiation. Indole-3-acetic acid (IAA) is the natural auxin in angiosperms (Davies, 1995). Shigeura (1948) was the first to report that foliar application of the sodium salt of naphthalene acetic acid (NAA) in November and December (late autumn and early winter) inhibited initiation of vegetative lychee shoots in autumn, in Hawaii. This delay in flushing promoted flowering in winter, whereas stems that flushed during autumn did not flower. The most effective application depended on the concentration used. For example, four bi-weekly sprays were necessary with 50 mg/l, commencing in late September. Higher concentrations, e.g. 150-400 mg/l, required fewer applications, commencing in

October or November. Applications of 100-150 mg/l of the sodium salt of NAA to lychees in Florida in early autumn discouraged vegetative flushes and increased flowering and yields over controls in most years. No increases in yield were observed when the weather discouraged vegetative flushing before flowering. Responses in other experiments were inconsistent when treatments were applied during vegetative flushes in late summer or early autumn (Nakata, 1955), or when high rainfall and high leaf nitrogen concentrations promoted frequent leaf growth. Endogenous concentrations of auxin in terminal shoots decreased as they aged. Chen (1990) observed higher concentrations of IAA in young lychee leaves than in mature leaves. Liang *et al.* (1987) also noted initiation of generative shoots only when concentrations of IAA were low. Auxin is also the primary hormone regulating root initiation and development and the high concentrations that suppress shoots may promote root growth. New roots, in turn, are sources of CKs that promote shoot initiation. Such an interaction between stems and roots might account for the observed rise in CK concentrations as auxin concentrations tall (Menzel and Waite, 2005).

5.3.2 Gibberellins (GAs)

More than 100 GAs have been described, but only a few have a specific physiological role. GA_1 is considered to be the primary GA involved in shoot elongation (Rademacher, 2000), while GA_3 inhibits flowering in citrus and mango (Davenport, 2003). For mango, the effect is through the inhibition of shoot initiation (Kachru *et al.*, 1971). Few reports are available describing the effects of exogenous GAs on shoot initiation or flowering of lychee or longan. The application of GA_3 at 200 mg/l to lychees in mid-October and late November did not affect flower initiation in January compared with unsprayed controls. The gibberellin GA_3 at 50-1000 mg/l did not affect growth or fruit set when applied five times to Brewster lychee trees, from before flowering to fruit set (Young *et al.*, 1960). Chen (1990) found that the dominant GAs in lychee were 1,4, 17 and 20 and in longan were 1, 8, 9 and 32. In general, the concentrations of GAs in the two species are lowest during rest (Naphrom *et al.*, 2001). Chen (1994) found that the highest concentrations of free GA_1-like and GAs-like substances in Ken Fur longan were present during leaf flushing. In contrast, the concentrations of GA_9-like and GA_{32}-like substances increased substantially at the

start of bud dormancy and flower initiation, respectively. Chen proposed that GA_{32}-like substances were involved in floral induction in this species. Other experiments showed that injection of GA_4 or GA_7 into the centre of the shoot apex induced flowering.

Some researchers have investigated the role of GAs on shoot initiation and flowering through application of plant growth retardants such as paclobutrazol, often combined with other chemicals that inhibit specific enzymes involved in GA biosynthesis. Applied as foliar sprays or root drenches, these retardants affect the phenology, morphology and hormonal fluxes of lychee and longan (Chaitrakulsub *et al.*, 1989b; Menzel and Simpson, 1990). The effects on flowering are highly variable, with no change recorded in some experiments, and enhancement in others. These findings are somewhat at odds with the situation in citrus and mango, where growth retardants promote flowering (Menzel and Waite, 2005).

5.3.3 Cytokinins (CKs)

Cytokinins are substituted adenine compounds that induce cell division in the presence of auxin in tobacco pith or similar tissue culture systems (Horgan, 1984). Their effects on plants, however, extend far beyond this limited definition. An overview of CK biochemistry and action can be found in McGaw and Burch (1995).

Synthetic CKs, N6-benzyladenine (BA), its riboside (BAR), and kinetin, mimic the action of the native hormones in some plants. Roots are the major source of CKs in higher plants , although other tissues with active cell division also produce or metabolize CKs. The current view is that the majority of CKs are synthesized in the root tips near the cap during active root growth, and distributed to stems through transpiration. However, the root cap is hydraulically isolated from the remainder of the root, raising the question of how these substances access the transpiration stream (Menzel and Waite, 2005).

Shoot initiation in lychee and longan is intricately associated with CKs. Chen and Ku (1988) reported that foliar application of kinetin at 200 mg/l to 5-month-old Haak Yip (Heiye) lychee stems in the autumn, about 2 weeks after ethephon (200 mg/l) treatment, stimulated bud break 1 month earlier than in control stems. This treatment resulted in 60% of the stems flowering, and in combination with ethephon

raised the flowering rate to 80% compared with no flowering in the controls. It was not revealed whether the absence of flowering in the controls was due to a lack of bud break or to the initiation of leaves. Chen (1991), however, found that 100 µg kinetin in a 5 µl drop of 1 M acetic acid in autumn, about 2 weeks after ethephon (200 mg/l) treatment, stimulated bud break 1 month earlier than in control stems. This treatment resulted in 60% of the stems flowering, and in combination with ethephon raised the flowering rate to 80% compared with no flowering in the controls. It was not revealed whether the absence of flowering in the controls was due to a lack of bud break or to the initiation of leaves. Exogenous application of CKs, presumably during cool weather, resulted in greater bud break and more branched inflorescences than in controls. The reported similar results for mango when a synthetic CK, thidiazuron, was applied during cool weather. The same product applied during warm weather resulted in many lateral vegetative shoots, supporting the concept that CKs are involved in shoot initiation, but not in floral induction (Menzel and Waite, 2005).

Further evidence of the role of CKs comes from measurements by bioassay, of CK activity, which increased in lychee buds and xylem sap (Chen, 1990; Naphrom et al., 2001) before floral initiation. In longan, glucosylated conjugates of zeatin (Z) and its riboside (ZR) accumulated in resting buds, but they were hydrolysed to free CKs during shoot initiation. The apices of initiated shoots had concentrations of free-CKs several-fold higher than those of resting buds. The evidence for the role of CKs in floral induction from measurements of endogenous hormone levels is scant. Buds of lychee and longan (Huang and Huang, 1996) had a higher concentration of CKs in "on" flowering years than "off" flowering years, and generative longan shoots had higher CK concentrations than vegetative shoots (Chen et al., 1997). In general, high CK activity promotes shoot initiation, but not flowering. Cytokinin and ABA concentrations promoted flower bud morphogenesis while GA inhibited it (Qiu et al., 2001).

5.3.4 Ethylene

Ethylene is a phytohormone involved in abscission, ripening and senescence (Abeles et al., 1992). It was thought to have a role in the floral induction of mango on the basis of the effectiveness of smudge smoke (ethylene is one of its

constituents) and ethephon (an ethylene precursor) on flowering. However, Davenport and Nunez-Elisea (1991) found either no response or a vegetative response with ethephon, and suggested that any effect of ethylene on flowering was due to a change in the timing of shoot initiation, not a direct effect on induction lychee, there are several reports of ethephon enhancing the number of flowering shoots (Sittichaikasem, 1974; Subhadrabandhu, 1986; Subhadrabandhu and Koo-Duang, 1987), and one where vegetative shoots were enhanced (Subhadrabandhu and Koo-Duang, 1987). As argued above, the effect of ethylene or lychee may be on shoot initiation, not floral induction. The argument is strengthened by research on longan, which showed that ethephon increased CK concentrations and bud break (Qiu *et al.*, 2001)

Ethephon has been tested for its ability to defoliate young vegetative flushes occurring in late autumn, when it is too warm for flowering. Subsequent shoots do not appear before spring, when temperatures are too warm for flowering (Olesen *et al.*, 1999). Defoliation of the autumn shoots stimulates the initiation of lateral shoots a few weeks after treatment. If cool weather coincides with this, the shoots will flower.

5.3.5 Paclobutrazol

Paclobutrazol is taken up passively through roots, stem tissue and foliage. Movement within the plant is acropetal, moving in the xylem to leaves and buds. There is no phloem mobility. Paclobutrazol is believed to move relatively slowly to active sites in the sub-apical meristems from reservoirs of compound in soil or stem tissue. The practical consequences are that:

- activity from sprays usually occurs more rapidly than that from soil applications.
- activity may persist into the year after treatment if the reservoir of available paclobutrazol is not totally depleted.

Active compound reaching sub-apical meristems inhibits gibberellin production inhibiting the oxidation of kaurene to kaurenoic acid, a cytochrome P 450 catalyse reaction taking place on microsomes. This, in turn, reduces the rate of cell division without causing any cytotoxicity. The direct morphological consequence is a

reduction in vegetative growth. The reduction in vegetative growth, by altering relative sink strengths within this has the indirect consequence of allowing a greater partition of assimilates to reproductive growth, to flower bud formation, fruit formation and fruit growth. Paclobutrazol is also believed to have effects on plant sterol synthesis and on acid synthesis, producing less saturated forms. These two actions may affect membrane fluidity and may possibly explain observed increased frost tolerance in paclobutrazol treated plant. The agronomic benefits observed following application of paclobutrazol to a wide range of fruit species include: reduce vegetative growth, increase fruit bud formation, increase flowering, greater physiological tolerance of flowers to frost, increase fruit set, improved fruit quality (size, colour, storage properties) and suppression of apple scab and meldew when use in a repeated foliar spray program (ICI, 1984). The maximum level of flowering in paclobutrazol treated trees occurred when the control trees bloomed moderately (40-60% of terminal branches) (Menzel and Simpson, 1990). Paclobutrazol applied as soil drench at 2, 4, 8 g ai / tree were compared to the single spray at the concentration of 125, 250, 500 ppm and the untreated control in the 12 years old lychee cv. Hong Huay. Soil drench treatment did not affect vegetative growth but spray increased the diameter of internode greater than soil drench and untreated control. The treatment did not affect flowering, while soil drench reduced panicle length to be shorter than spraying and control (Chaitrakulsub *et al.*, 1992). Single spray of paclobutrazol follow by ethephon at the concentration of 1,000 : 500 ppm reduced the percentage of leaf flushing about 10 percents compared to the untreated control, but did not affect the percentage of flowering (Chaitrakulsub *et al.*, 1989). A foliar application of paclobutrazol on Maididen Bramley's Seedling apple trees on M.26 and MM.106 rootstocks restricted the growth and improved the flowering (El Hodairi and Canham, 1990). A foliar application of paclobutrazol restricted the growth and stimulated the flowering of Bramley's Seedling and Golden Delicious apple trees (El Hodairi and Canham, 1990). Sprays of paclobutrazol at 500 mg/l on 12 weeks old Delicious apple seedlings significantly reduced vegetative growth and increased root : shoot ratios. Root dry weight, relative growth rate, net assimilation rate and leaf area ratio were not affected (El Hodairi and Canham, 1990).

5.3.6 Potassium chlorate

The properties of potassium chlorate are transparent, colorless crystals or white powder, cooling and saline taste. Soluble in boiling water, Sp. Gr. 2337; mp 368^0 C; bp, decompose at 400° C and giving off oxygen (Hawley, 1981) Potassium chlorate 7.3 g dissolves in 100 ml of water at 20° C (Dean, 1985). Derivation of potassium chlorate is preferably by interaction of solution of potassium chlorate and sodium chlorate or calcium chlorate. Potassium chlorate is moderate toxic, form explosive mixtures with combustible materials such as sulfer, sugar, etc. and strong oxidizing agent. To use this chemical should be careful (Hawley, 1981). Potassium chlorate ($KClO_3$) dissociates into potassium ion (K^+) and chlorate ion (ClO_3^-) when dissolves in water. Plant exposed to chlorate display various system: root growth is severely inhibited and yellow leaves, wilt, and die (LaBrie et al.,1991). Potassium chlorate is a chemical unrelated to any plant growth regulator. It stimulates out-of-season flowering in longan, but not in lychee, and it sometimes damages the leaves (Subhadrabandhu and Yapwattanaphun, 2000, 2001b). The effect appears to be similar to that of potassium nitrate in mango. See Chompoo, E-Daw and Haew are the most responsive cultivars in Thailand (Manochai, 2000). E-Daw flowered 3 weeks after a November (autumn) soil drench at 4-8 g/m^2 (Manochai et al., 1999), while See Chompoo flowered at doses of 1-4 g/m^2 (Khaosumain et al., 1999). For flowering to occur, it was necessary to have mature leaves on resting stems (Manochai et al., 1999). Foliar sprays (Sritontip et al., 1999) and trunk injection (Viriyalongkorn et al., 1999, cited in Subhadrabandhu and Yapwattanaphun, 2001b) were also effective. Protocols for use of the chemical were summarized by Khaosumain et al. (1999) and Subhadrabandhu and Yapwattanaphun (2001b). Application of the chemical to out-of-season, easy-to-flower, Tawai has resulted in the production of longans nearly all year round in Thailand (Subhadrabandhu and Yapwattanaphun, 2001 b). The results of experiments in Taiwan, using gunpowder and potassium chlorate, were somewhat different. Trees flowered after a single application nearly any time of the year, as in Thailand, but rates of 13-26 g/m^2 were required. Potassium chlorate, applied at rates shown to be effective for particular growing areas, has the potential to increase longan production in the tropics. The soil drench and foliar spray of the $KClO_3$ treatments had the highest percentage of flower emergence. The $KClO_3$ and thiourea treatments

had the highest percentage of leaf flushing (Sritontip *et al.*, 2005). The longan trees treated with $KClO_3$ and NaOCl had flower emergence better than untreated trees. In addition, the trees treated with $KClO_3$ and NaOCl had flower emergence earlier than those treated with $Ca(ClO)_2$. However, The $KClO_3$, NaOCl and $Ca(ClO)_2$ had decreased leaf flushing (Sritontip *et al.*, 2005). Potassium chlorate as soil drench is the most effective treatment to induce flowering in longan (Manochai *et al.*, 1999). Sodium chorate is less popular and can damage longan trees. High concentrations of potassium chlorate may cause leaf fall, restricted growth, panicle malformation and shoot deformity (Subhadrabandhu and Yapwattanaphun, 2001b). The $KClO_3$ treated trees were flowering before the untreated trees (Wangsin and Pankasemsuk, 2005). Already 4 g/m^2 of $KClO_3$ induced more than 80 % of buds to flower and 8 g/m^2 had already a saturating effect, while without $KClO_3$ application there was no flowering in out of season experiments (Manochai *et al.*, 2005). Concerns about the health risks associated with the use of potassium chlorate have been prompted by the knowledge that it is an ingredient of some herbicides, matches, cleaning agents, fireworks and explosives. Potassium chlorate caused an explosion in a longan drying plant in 1999, which claimed many lives in the San Pa Tong district of Chiang Mai. Recent research in Thailand has recorded anaemia, thrombocytopaenia, high serum creatinine, haematuria, pyuria and methaemoglobinaemia, all symptoms of blood, kidney, and urinary tract damage, in a significant proportion of longan growers (Wewatanadate *et al.*, 2001). Most growers did not use any personal protection, such as gloves or respirators, when applying the material. The same care should be used in application of potassium chlorate as with any other hazardous agricultural product (Menzel and Waite, 2005). There are no chlorate and chlorite residues in $KClO_3$-treated longan fruits in all treatments while Cl^- was found with no significant difference in concentration as compared with control (i.e. with no $KClO_3$ treatment) and the application of $KClO_3$ did not affect K^+ ion and Cl^- ion contents in longan fruits (Kanaree and Pankasemsuk, 2005).

5.4 Effects of environment on shoot induction

A simple model of shoot induction has been developed for lychee, citrus and mango based on the balance between putative flowering and vegetative

promoters, neither of which is known, and the time of shoot initiation. Some of the characteristics of the flowering promoter are well understood. Mature leaves are thought to be a major source of the promoter in mango, which is often effective with only a fraction of a leaf per stem. This promoter is graft-transmissible and can be transported over long distances, but is short-lived, effectively being lost within days of leaves being removed. Citrus is somewhat different in that the promoter is thought to be synthesized in the stem tip. Little is known of the flowering promoter in lychee other than that mature leaves are required (Menzel and Waite, 2005).

5.4.1 Temperature

It has long been recognized that, in common with a range of other tropical tree species, cool temperatures promote flowering in lychee and longan. Nakata and Watanabe (1966) determined that cool nights of 14°C induced flowering of lychee under short (6 h) and long (18 h) photoperiods. Thus, lychee was deemed a non-photoperiodic flowering plant. These experiments also demonstrated that cool temperatures stimulate shoot initiation. Cool nights of 10°C or less stimulate shoot initiation and floral induction, if temperatures remain low through early shoot development. Under such conditions, generative shoots appear within 1 month. Menzel and Simpson (1988) conducted experiments on several cultivars of lychee grown in pots and exposed to 15°/10°, 20°/15°, 25°/20° or 30°/25° C until shoot initiation occurred (within 8 weeks in all regimes). There were differences between cultivars but overall, plants exposed to 30°/25° or 25°/20° C initiated vegetative shoots; those exposed to 20°/15° C, a combination of generative, mixed and vegetative shoots; and those exposed to 15°/10° C, generative and mixed shoots, with a higher ratio of generative to mixed shoots than at 20°/15° C. Menzel and Simpson (1995) observed that maximum/minimum temperatures of 25°/20° C or above stimulated vegetative growth in Kwai May Pink and Casino lychees. Floral induction occurred when plants were exposed to temperatures of 15°/5° C for 10 weeks. Although there were some differences between the two cultivars, intermediate temperatures resulted in mixed shoots. Stored starch reserves did not correlate with vegetative or floral development. The longer the period that Wai Chee plants were kept at 15°C (4-10 weeks) before they were transferred to 30°/25°C, the greater was the flowering. A minimum of 4

weeks' exposure was required for floral induction. The greatest proportion of mixed shoots occurred when plants were transferred after 6 or 8 weeks, whereas 10 weeks of cool temperatures gave the maximum proportion of generative shoots. Increasing periods from 1 h to 24 h per day of $20°C$ interrupting a constant $15°$ C, reduced flowering (Menzel and Simpson, 1995). Batten and McConchie (1995) took a different approach by examining the responses of buds at different stages of development. Buds that had not elongated when the plants were transferred from warm to cool conditions, or which were actively growing and no more than a few millimetres long, generally flowered. Actively growing buds that were slightly longer, up to several millimetres in length, generally produced transition inflorescences or no flowers, while buds that were longer still, rarely flowered. The strict conclusion was that temperatures experienced by the whole plant during early shoot development affected bud determination, but the results are also consistent with the hypothesis that buds need to be actively growing to flower.

In order to make field estimates of the critical temperature for flowering, Olesen et al. (2002) assumed that early shoot development was the time of bud determination in lychee. Floral bud determination occurred at mean daily temperatures below $17-19°$ C, a result similar to that reported by Menzel and Simpson (1995) for plants grown in controlled environments. In general lychee var. Mauritius and Brewster stems must be about 15 weeks old to initiate flowering in response to cool night winter temperatures (Zheng et al., 2001). The $KClO_3$ treated and untreated trees were not significantly different of fruit quality, fruit size, seed size, exocarp color, fruit pole strength, fruit volume, total soluble solids, and fresh and dry weight (Kanaree and Pankasemsuk, 2005).

5.4.2 Water relations

It is a common misconception that drought or a water deficit induces flowering in tropical fruit trees (Menzel and Simpson, 1994). Citrus is one of the few species in which water stress has a direct effect on floral induction. Lychee and mango, however, do not respond in the same way as citrus (Menzel et al., 1989; Chaikiattiyos et al., 1994). A moderate or severe water deficit with dawn leaf water potentials (Ψ_{leaf}) of -2.0 and -3.5 MPa did not induce flowering in lychees grown in a

greenhouse (Chaikiattiyos *et al.,* 1994). Droughted plants only flowered when they were placed in a low temperature regime when re-watered. Several authors have shown that drought can increase flowering and yield in lychee. However, these responses are related to the effects of the water deficit on the timing of shoot initiation rather than on floral induction. Drought is only effective if it coincides with, or is followed by, low temperatures. Shoot initiation is inhibited when Ψ_{leaf} is lower than -2.0 MPa (Menzel *et al.,* 1989). Early experiments in Hawaii demonstrated that a mild drought in Kwai May, for 6 months prior to flowering, prevented vegetative flushing in autumn and promoted inflorescence, compared with irrigated controls that vegetative flushed.

Drought has been used to manipulate autumn vegetative flushing and to improve flowering and yields of lychee in Israel. Irrigation is withheld from mid-September until the commencement of cool, wet weather in December, when the trees initiate inflorescences (Stern and Gazit, 1993). The elevated concentrations of the ribosides of the cytokinins Z and DHZ in "Mauritius" xylem sap during the drought, which were proportional to the level of water deficit in the plants. The use of drought to reduce the risk of autumn flushes requires different strategies in different environments. In some areas, the control of water levels in the root zone is impractical. Although there is no information on the Ψ_{leaf} needed to achieve the desired response (Stern *et al.,* 2003).

5.4.3 Nitrogen

Cobin (1950) observed that "where the nutrition level is high and where abundant moisture and hot temperatures prevail, one vegetative flush will be succeeded by a succession of vegetative flushes without the appearance of a general bloom". The need for vegetative rest during autumn and early winter prompted Cobin to recommend that fertilizer not be applied during this period to promote flowering in early spring in Florida. However, there is no evidence of a direct link between floral induction and leaf or soil nitrogen (Menzel *et al.*, 1989), even though nitrogen is the most important nutrient affecting vegetative flushing (Zheng *et al.*, 2001). Trees received high rates of N displayed an increased frequency and intensity of vegetative flushing and flowering (Davenport *et al.*, 2001).

Management of plant nitrogen concentrations is challenging, making a relationship between flowering and nitrogen difficult to establish. Results of efforts to optimize the rate and time of nitrogen applications for consistent flowering have been inconclusive (Menzel and Simpson, 1988; Menzel *et al.*, 1995; Li *et al.*, 2001). Menzel *et al.* (1992) and Liu and Ma (2001) have concluded that nitrogen concentrations of 1.3-1.5% in leaf dry matter at flowering or fruit set are generally optimal for lychee production in South Africa, Hawaii, India and Australia, with higher concentrations reducing yields in some years. Difficulties in relating fertilizer applications to consistent flowering and yields may be related to the fact that leaf nitrogen concentrations can take months or years to adjust following soil applications, due to storage in soils and trees (Menzel *et al.*, 1995).

5.4.3 Pruning and girdling

Pruning is necessary in order to manage canopy size and shape, especially in high-density plantings. Tip pruning after harvest synchronizes the postharvest vegetative flush throughout the orchard (Goren and Gazit, 1993). Olesen *et al.* (2002) demonstrated the impact of pruning lychees at different times on the subsequent development of vegetative and reproductive flushes. They developed a model for the pruning of trees along the eastern seaboard of Australia, based on the dependency of flush development on temperature and irradiation. Girdling (ringing or cincturing) involves removing a narrow band of bark by cutting through the cambium layer to the wood. The band can be connected to form a closed ring or an overlapping spiral around the trunk, It is generally applied to scaffolding limbs or at the base of tree trunks. Girdling stops shoot initiation while the cut remains open (Menzel and Paxton, 1986) and can increase flowering (Li and Xiao, 2001). Menzel and Paxton (1986) reported that girdling after the postharvest flush had matured delayed the next flush until winter. Girdling disrupts the downward flow of photo-assimilates and auxins from the leaves, which can reduce root growth. The inhibition of root growth may then depress the production of CKs produced in the root tips. Early experiences in Hawaii indicated that the best time to girdle was in early autumn. If vegetative flushes occurred during or after girdling, then the trees generally did not flower (Menzel and Paxton, 1986). The most effective time to girdle in Florida was mid-

September. Complete girdling of scaffolding branches often weakened or killed trees. Girdling only 75% of the circumference was unsuccessful, as was banding branches with butyl rubber (Menzel and Waite, 2005). The girdling and growth retardants suppressed vegetative flush in winter and promoted flowering (Ramburn, 2001). Girdling increased flowering compared with untreated trees, although panicles were shorter (Li and Xiao, 2001). Trunk girdling was better than $KClO_3$ for stimulating flowering (Charoensri et al., 2005).

6. Inflorescence and flowers

The appearance of enlarged leaf primordia and lateral meristems on the elongating main axis are the first indications of floral differentiation in lychee. The emerging inflorescence is initially similar to a vegetative flush, and it is only when the lateral meristems develop into secondary inflorescences but also start producing small leaves in the case of mixed shoots. Each panicle produces tens to hundreds of small flowers, two functional male types (M_1 and M_2), and one functional female (F) type. They identified the inflorescence as a determinate, compound thyrse, because its side branches are cymose (side branches with flat-topped or convex flower clusters). The flowers are borne on partial inflorescences or dichasia. Usually, the first flower to open (number one) is the M_1 the second to open (number two) are two female flowers inserted laterally beneath the number one flower; the third group to open (number three) are four M_2 flowers inserted laterally beneath the number two flowers. The remaining eight (number four) buds are usually insignificant, but in richly branched inflorescences, they may also develop. Many variations on this scheme have been encountered; however, in most of them, the M_1 flowers are absent and the female flowers occupy the first, and sometimes the second, position in the dichasium. Anthesis within the panicle as a whole is from base to tip (acropetal anthesis between dichasia), whereas anthesis within the dichasia occurs from the tip to the base (basipetal anthesis within the dichasia) (Menzel and Waite, 2005).