Chapter 2

Theory and Literature reviews

2.1 Mango

Botany and ecological habitat of mango

Mango, the genus *Mangifera*, belongs to the order Sapindales in the family Anacardiaceae which is a family of mainly tropical species with 73 genera, with a few representatives in temperate regions. The other distant relatives of *Mangifera* are cashew (*Anacardium occidentale*), gandaria (*Bouea gandaria*), pistachio (*Pistacia vera*), marula (*Sclerocarya birrea*), ambarella (*Spondias cytherea*), yellow mombin (*Spondias mombin*) (Campbell, 1992). Apart from edible fruit, Anacardiaceous species also yields other valuable products such as wood, gums and resins, wax and varnishes and tanning materials. It is also a family well known for the dermal irritation produced by some of its members, including some *Mangifera spp*. whose resinous sap may induce allergic reaction.

Mango trees, grown from seeds are known as "seedlings", have a long straight bole. Tree is sympodially branched. The shape of the canopy depends on the space available for its development. Tree is medium to large (10-40 meters in height) and evergreen with symmetrical. Its rounded canopy ranges from low and dense to upright and open. Bark is usually dark grey-brown to black. It is rather smooth and superficially cracked or inconspicuously fissured, peeling off in irregular and rather thick pieces. The tree forms a long unbranched tap root (up to 6-8 meters and more) plus a dense mass of superficial feeder roots. The leaves are simple, estipulate, alternately arranged, 15-45 centimeters in length. The inflorescence is pseudo-terminal, originating from a bud, together with the new leafy sprout; there are cultivars with lateral inflorescence. Hermaphrodite and male flowers are produced in the same panicle, usually with a larger number of the latter. The size of both male

and hermaphrodite flowers varies from 6 to 8 mm in diameter. The pollen grains are of variable shapes. The fruit is more or less compressed, fleshy drupe. It varies considerably in size, shape, colour, presence of fiber, flavour, taste and several other characteristics. The most characteristic feature of the mango fruit is the formation of a small conical projection developing laterally at the proximal end of the fruit, known as the beak.

Mango can be found in a wide range of climate, and is particularly well adapted to topical and subtropical climates. However, temperature and rainfall are essential. For optimum growth and productivity, the optimum temperature needed is 25 ˚C. The temperature which is less than 10 ˚C or higher 42 ˚C, is not conducive for good growth. Mango can be grown upto 1200 meters above sea level. However, the growth suffers above 600 meters (Dinesh, 1997). In areas of heavy rainfall, the rate of regulative growth is high but occurs at the expense of fruiting, and crop yield is low. In some moist localities, mango does not set fruit, whereas in comparatively drier localities it yields a commercial crop. For profitable cropping, the dry season should be well ahead of flowering. Soil moisture content of 50 percent is recommended for flowering of mangoes in Thailand. Mango trees are grown on a wide range of soil. However, they prefer a deep rich fertile soil because of the long tap root. The soil should be well drained and without a hard pan. In many places deep sandy loam and clay loam soil with pH ranging from 5.5 to 7.0 are found suitable for the mango (Bamroongrugsa and Yaacob, 1989).

Off-season mango

The commercial mango in Thailand can be classified into two types depending on maturing stages: ‘raw-eating’ and ‘ripe-eating’. The first type named ‘starchy mango’ or ‘crispy mango’ is increasingly reputable because of its fresh taste. This type includes the following varieties: Khiew Sawoey, Rad, Fahlun and others. The other type of mango is the ‘ripe-eating’, that is, the fruit is consumed when ripe. The type includes Nam Dok Mai, Chok Anan, Okrong, Pimsen, Mahachanok and many others. Among these, Khiew Sawoey has the largest growing areas compared to the

others. It was estimated by Thailand Department of Agricultural Extension in 2000-2002 that the total mango growing areas for the whole in country was 2,184,518 rais and 1,718,217 rais had already produced fruits with the total fruit yield of 1,670,106,924 kilogram (Department of Agricultural Extension, 2004).

The application of paclobutrazol in off-season mango

Mango has been defined as an economic crop in which the produce is mainly used for domestic consumption, while only a few varieties are exported. In Thailand, the peak harvest period of mango is in the mid-summer months (April-May) with short harvesting season. Flowering occurs just after the cool period (dry period, 10-15 ˚C) from December to January. A large volume of the fruits together with short storage life causes excessive supply of the fruits in the market, leading to low price. For this reason, fruit growers in Thailand have had a great interest in producing fruits at a time out of harvesting season. Nowadays mangoes off-season fruit production is made possible by applying triazole paclobutrazol (PBZ) and also many other methods. However, it can be said that at the PBZ application is the most popular method for growers in Thailand to produce off-season mango (Nartvaranant *et al*., 2000). When PBZ is applied as soil drench, it is more effective than foliar spray (Tongumpai *et al.*, 1996). However, the off-season fruit production techniques are complex, and the yields vary from year to year. Several factors were responsible for this yield variation including individual varieties of fruit trees, climatic conditions, mango cultivars, orchard management, and location and most importantly the experience of mango growers (Subhadrabandhu and Tongumpai, 1989; Nartvaranant *et al*., 2000).

Many reports indicated that gibberellins enhanced vegetative growth and inhibited flowering of mango (Tomer, 1984.; Chen, 1987, Davenport *et al*., 2000). PBZ specifically inhibits a gibberellin synthesis, prevents the cell elongation and decreases the concentration of gibberellin (Nakajima *et al*., 2001). Tongumpai *et al*. (1991) found that soil application of PBZ in ‘Khiew Sawoey’ resulted in a significant increase in flowering and slightly earlier flowering whereas a combination of soil application of PBZ (6g/tree) and foliar spray of potassium nitrate 8 weeks after PBZ

treatment induced very early flowering. For 'Tommy Atkins' a single soil application of PBZ resulted in earlier flowering due to an earlier inflorescence initiation in apical buds of mature spring shoots, which had not flushed during the fall (Perez-Barraz *et al*., 2000). Moreover, PBZ was found to be more effective as less number of growth flushes was observed. As a result of decrease in flush number, the production of malformed panicles (too compact) was also minimized (Tahir *et al*., 2002). Tongumpai (1997) reported that single and multiple foliar application of PBZ at 1,000 and 2,000 ppm were applied to mango tree cv. Nam Dok Mai in June to induce off-season flowering. In this study, the treated trees initiated flowering 29-41days earlier than the controls. The amount of flowering shoot of all PBZ treated trees was 2 times greater than that of the control. The canopy sizes of the treated trees were significantly reduced by 19.33 percent in height and 15.81 percent in spread in one year. This gave the optimum canopy size for high density planting while the canopy of the control trees became overlapping. The PBZ effect on growth lasted. For only one year after which normal growth resumed. The treated trees flowered more profusely flowering (91.5%) and considerably earlier 49 days than the controls (Charnvichit *et al*., 1991). In addition, Tongumpai *et al*. (1989) also found that 'Cultar' (PBZ) applied as a collar drench at the rate of 1.0 g *a.i.* per meter canopy diameter induced flowering 3 to 5 months after treatment in easy-to-flower cultivars of mango. This made possible off-season production and substantially increased growers' income. Mango cv. Khiew Sawoey was less responsive to 'Cultar' but off-season flowering was enhanced by follow-up sprays of potassium nitrate (KNO_3).

Junthasri *et al*. (2000) and Nartvaranant *et al*. (2000) oriented that the technique in producing off-season mango has been adopted in Thailand since 1986. Paclobutrazol, a plant growth retardant, was used in combination with thiourea for producing as well as breaking of flower buds. The studies on application methods showed that soil drenching of PBZ is more effective for the induction of flowering in mango as compared to foliar spray. The rate of paclobutrazol application depended on the size of tree canopy as well as on mango cultivars. For most cultivars, the rate of PBZ applied is generally determined by multiplying the diameter of tree canopy (expressed in meter) with 1.0-1.5 g of active ingredients of PBZ. At 120 days after the

application of PBZ, 0.5% thiourea is usually sprayed to some cultivars for breaking of buds. Using this method, inflorescences are visible within 2.5 to 4.0 months after the paclobutrazol application depending on cultivar. Flower induction in mango is not major problem for Thai growers, as they can control the flowering in both 'on-season' and 'off-season' periods. However, the mango production problems that need further research are few or none fruit set and pre-harvest fruit drop in both 'on-season' and 'off-season' production. Thus, Tongumpai (1999) and Nartvaranant *et al*. (2000) summarized the procedure to control flowering of mango in Thailand based on the research findings as adopted in the following diagrammatic scheme in Figure 2.1.

Off-season mango production in others countries

In the Southwest region of Bahia state, Brazil. The most cultivated long time ago of mango is ‘Tommy Atkins’. The mango tree production presents a biennial bearing, in function of natural bearing alternance, low flowering and fruit set, less yield, etc. They are trying to produce off–season mango, many methods have been used, such as girding, potassium or nitrates spraying. At presently, found that the mango trees which received paclobutrazol in concentration 0.5 and 1.0 g *a.i.* per meter of canopy in diameter with potassium or calcium nitrate spraying, after 90 to 120 days after paclobutrazol application produced flowering and yield earlier (Jose and Reboucas, 2000). In India, paclobutrazol as soil drench in ‘Alphonso’ mango produced significantly effective minimum outbreak of vegetative flushes and gave 3-4 weeks early, profuse flowering (>80%), convenient and cost effective (Burondkar and Gunjate, 1993). Also in Mexico, soil application of paclobutrazol in ‘Tommy Atkins’ resulted in earlier flowering due to and earlier inflorescence initiation in apical buds of mature spring shoots, which had not flushed during the fall. Further work is necessary to manipulate the vegetative flushes and to better understand floral determination in mango (Perez-Barraza *et al*., 2000). Sergent *et al*. (1996) also found that high paclobutrazol doses (15 g *a.i*.) with high potassium nitrate doses (36 g/L) were effective in maintaining high yield and reduced the biennial bearing of ‘Haden’ mango in Aragua state, Venezuela. However, Paclobutrazol is not to be injected: (l) into trees that do not appear healthy, (2) into fruit or nut trees that will be harvested

within one year after application, and (3) into sugar maple or any other trees that are or could be tapped for sugar (Cornell University, 1985).

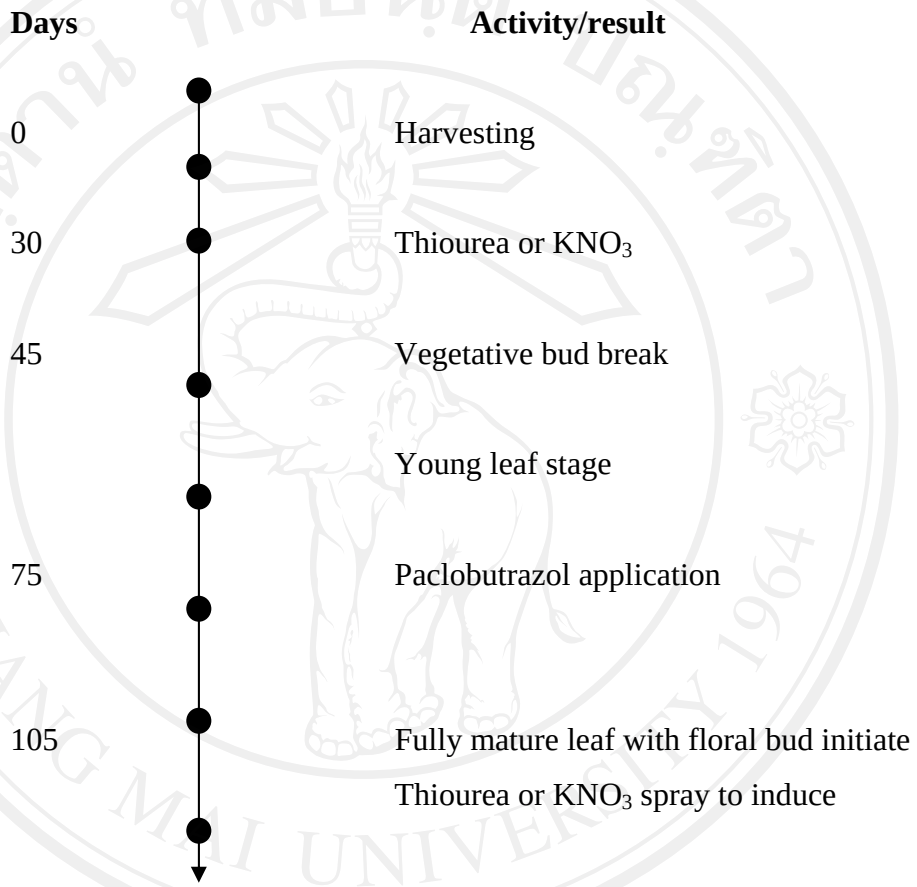

Figure 2.1 The schematic for control flowering of mango using paclobutrazol and thiourea. (Tongumpai, 1999; Nartvaranant *et al*., 2000)

2.2 Paclobutrazol (PBZ)

Chemical properties

Paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1H-1, 2, 4-triazol-1-yl) pentan-3-ol] is used as a plant growth regulator and fungicide. The molecular weight is 293.8 g/mol and melting point is approximately 164-168˚C. It has been commonly used in agriculture. The chemical structure is displayed in Figure 2.2. The acceptable daily intake (ADI) for paclobutrazol established with CODEX is 0.1 mg/kg body weight. The maximum residue limits (MRL) is the highest concentration of a chemical residue that is legally permitted or accepted in a food. For MRL of paclobutrazol in fruits vary from 0.05 to 1.0 mg/kg, which would result in 0.05 mg/kg per apple (FAO, 2002).On the other hand, New Zealand Food and Safety Authority's regulation (2005) indicates MRL of paclobutrazol in avocados and stone fruit at 0.01 mg/kg. Very little is known about the dissipation characteristics of this compound in the environment. Due to soil adsorption, paclobutrazol and its major decomposition products have low mobility in soil as paclobutrazol is strongly bound to organic matter (Singh, 2002; Dy, 2003). The lack of polar functional groups in this molecule explains why it is absorbed into the hydrophobic sites on organic matter. Thus, causes of non-biological loss of paclobutrazol in soil are few since it is non-volatile and thermally stable (Jackson *et al*., 1996; Gevao *et al*., 2000). The chemical also does not photo-degrade after exposed to simulated sunlight for 10 days. Paclobutrazol degrades aerobically in soil with half-life of about 1-7 months depending upon soil type (Cornell University, 1985). The half-life of this substance in water is 24.4 days (Castro, *et al*., 2004). The addition of this substance disintegrates in soil about 200 days, depending on soil type (USEPA, 2000). Its solubility is 35 mg/l at 25C and the compound has high potential to leach into surface and ground water.

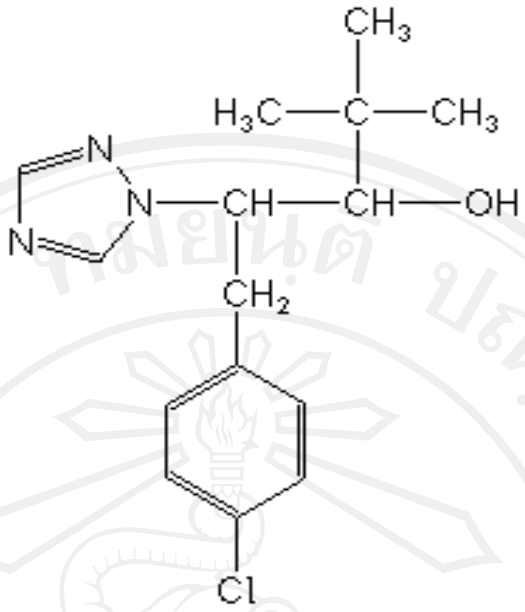

Figure 2.2 Chemical structure of paclobutrazol

Mode of action, classifiable and reactionary of paclobutrazol

Today plant growth regulators are used to improve long term reliability (reduce tree growth and extend pruning cycles), enhance customer relations (extend pruning cycles thereby reducing property intrusions), increase health of the trees (combine health benefits with reduced pruning for sensitive customers), and reduce line clearance costs (reduce trim and chip time; and reduce biomass). Plant growth regulators can be classified into three distinctly different groups (Rossi, 1998):

Class A plant growth regulators interfere with the production of gibberellins late in their biosynthetic pathway.

Class B plant growth regulators interfere with the production of gibberellins early in their biosynthetic pathway.

Class C plant growth regulators are mitotic inhibitors, disrupting cell division.

Rademacher (2000) demonstrates that the 4 different types of inhibitors of gibberellic acid are known:

A) Onium compounds, such as chlormequat chloride, mepiquat chloride, chlorphonium, and AMO-1618, which block the cyclases copalyl-diphosphate synthase and ent-kaurene synthase involved in the early steps of GA metabolism.

B) Compounds with N-containing heterocycle, e.g. ancymidol, flurprimidol, tetcyclacis, **paclobutrazol**, uniconazole-P, and inabenfide. These retardants block cytochrome-P450-dependent monooxygenases, thereby inhibiting oxidation of ent-kaurene into ent-kaurenoic acid.

C) Structural mimics of 2-oxoglutaric acid, which is the co-substrate of dioxygenases that catalyze late steps of GA formation. Acylcyclohexanediones, e.g. **prohexadione-Ca** and trinexapac-ethyl and daminozide, block particularly 3ss-hydroxylation, thereby inhibiting the formation of highly active Gas from inactive precursors, and

D) 16, 17-Dihydro-GA5 and related structures act most likely by mimicking the GA precursor substrate of the same dioxygenases. Enzymes, similar to the ones involved in GA biosynthesis, are also of importance in the formation of abscisic acid, ethylene, sterols, flavonoids, and other plant constituents. Changes in the levels of these compounds found after treatment with growth retardants can mostly be explained by side activities on such enzymes.

Triazole plant growth regulators such as diclobutrazol, uniconazol, hexaconazol, propiconazole, flutriafol and **paclobutrazol** are class B plant growth regulators that act as anti-gibberellin compounds much earlier in the biosynthetic pathway by inhibiting the cytochrome P-450-mediated monoxygease reactions involved in the isoprenoid pathway. Triazols also induce a variety of other responses in plants including reduced or altered sterol biosynthesis (Khalil and Rahman, 1995), increased chlorophyll concentration (Khalil and Rahman, 1995), increased photosynthetic rate (Archbold and Houtz, 1988), delayed senescence and increased stress tolerance (Basiouny and Sass, 1993; Zhu *et al*., 2004). It has also been reported that ABA levels were increased in plants grown under triazole regulation (Tafazoli and Beyl, 1993). It is suggested that the lowered gibberellic acid and increased ABA levels increases stress tolerance during chilling or freezing (Lurie *et al*., 1995). Paclobutrazol is one of the triazol that the best enhances stress tolerance (Wang and

Steffens, 1985) and helps to increase the non-structural carbohydrates (Phavaphutanon, *et al*., 2000).

The cytochrome P-450 dependent oxidation of ent-kaurene to ent-kaurenoic acid is specifically inhibited in the gibberellin pathway by paclobutrazol as the compound blocks three separate steps in the terpenoid pathway for the production of gibberellins (Figure 2.3). One of the main roles of GA in trees is the stimulation of cell elongation. When gibberellin production is inhibited, cell division still occurs, but the new cells do not elongate. The result is shoots with the same numbers of leaves and internodes compressed into a shorter length. For many years this was considered to be the sole response of trees to treatment with paclobutrazol. However, research has demonstrated that blocking a portion of the terpenoid pathway causes shunting of the accumulated intermediary compounds above the blockage. The consequence is an increase in the production of the hormone abscisic acid and the chlorophyll component phytyl, both beneficial to tree growth and health. The unique structure of paclobutrazol that allows it to bind an iron atom in the enzymes essential for the production of gibberellins also has the capacity to bind enzymes necessary for the steroid production in fungi as well as those that promote destruction of abscisic acid (Figure 2.3). The result is that paclobutrazol-treated trees have greater tolerance to environmental stresses and resistance to fungal disease infections. Morphological modifications of leaves induced by treatment with paclobutrazol such as smaller stomatal pores, thicker leaves, and increased number and size of surface appendages on leaves may provide physical barriers to some fungal, bacterial and insect infection (Chaney, 2004; 2005).

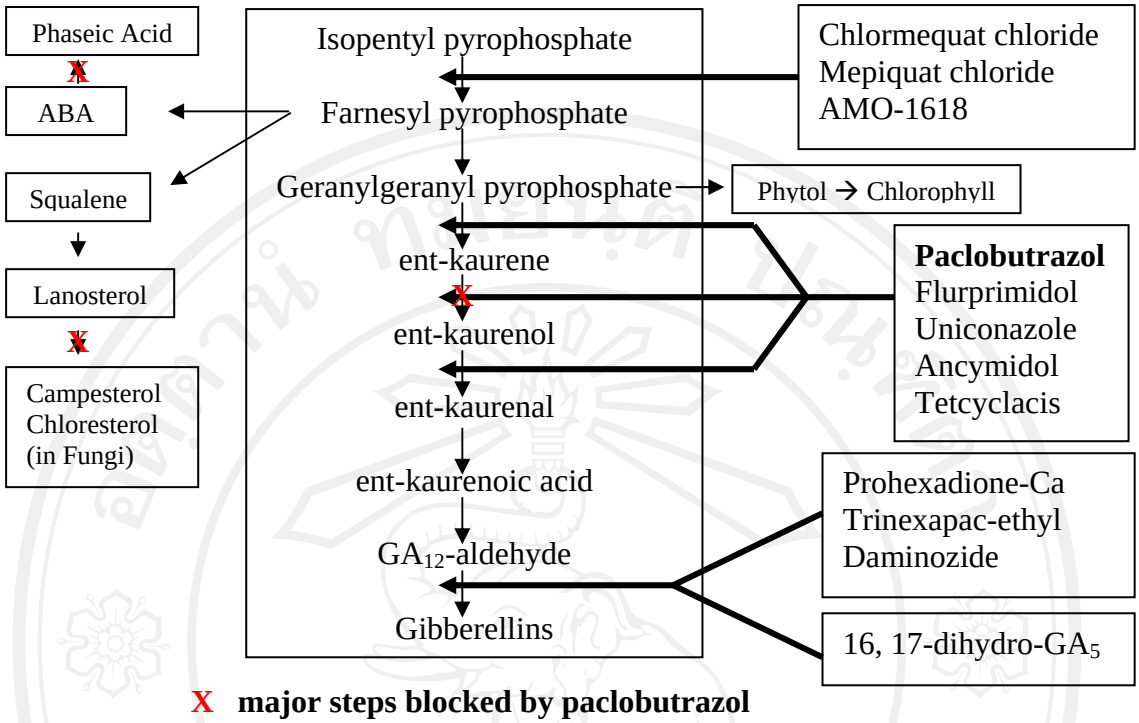

Figure 2.3 Biosynthetic steps of gibberellins and site of inhibition by various growth retardants (Rademacher, 2000; Chaney, 2004; Chaney and Bai, 2004; Swain *et al*., 2005).

Toxicity of paclobutrazol

Paclobutrazol is of slight to moderate acute toxicity to mice, rats, guinea pigs and rabbits. The toxicological evaluation of the liver effects for mouse is at 15 mg/kg body weight/day, rat at 10 mg/kg body weight/day, and dog at 75 mg/kg body weight/day (www.inchem.org, 2004). A few studies of toxicology of paclobutrazol deal with its potential toxic effects. Castro *et al*. (2004) reported that paclobutrazol at 10 x ADI was administered to rats in order to evaluate maturational and behavioural aspects of offspring development. This dose however did not promote evidence of maternal toxicity and the weight of gravid uterus, fetus, and ovary on day 16 and 20 of pregnancy were not affected. Also, the substance did not affect body weight gain of the dams and offspring. However, the pups' survival at weaning was impaired by paclobutrazol. Beyond that, study of the functional state of rat pups' nervous system

at different stages of postnatal development revealed some differences in treated ones. Damage was observed in the expression of acoustic startle reflex and altered locomotion and/or rearing in an open-field apparatus in treated pups depending on their age. There were no observed alterations in swimming behaviour. In other words, the pirimicarb and paclobutrazol had comparable rates of dermal penetration and lag times of around 18 hours. Methiocarb had a considerably shorter lag time. Dermal penetration continued for extended periods after exposure had ended. With lag time sometimes considerably longer than a normal working day, biological monitoring at the end of exposure may seriously underestimate the acute exposure. There may be implications for regulatory guidelines, which often require only 24 hour observation periods (Nielsen and Nielsen, 2000). In addition, Nielse and Andersen (2001) evaluated that the effect of nonylphenolethoxylate on dermal penetration of three extensively used pesticides, methiocarb, paclobutrazol, and pirimicarb, and the protection against dermal penetration offered by protective gloves made of latex or nitrile. There was a general tendency, though not statistically significant for all pesticides, for nonylphenolethoxylate to decrease the percutaneous penetration of the three pesticides. The nitrile generally offered better protection against percutaneous penetration of pesticides than did latex, but the degree of protection decreased over time and depended on the pesticide used.

Translocation of paclobutrazol in plant

Paclobutrazol, a triazole compound and plant growth regulator, is applied as a foliar spray (apply to leaves, a good coverage spray that multiple application was effective in promoting uniform off-season flowering in mango), soil drench (apply to roots, more popular and convenient), trunk injections (directly to the vascular system of the stems, using pressure). The translocation of paclobutrazol from root uptake has been assumed to occur primarily though xylem. However, a few direct experimental data and clear explanations have been provided to support this assumption. As to the letter, Sterrett (1985) found that 27 days after pressure injection of [^{14}C] paclobutrazol into the trunk of young apple trees, 23% of the ^{14}C-activity was in the shoots; 11% was in the xylem and phloem tissue the graft and apex; 58% remained in the xylem and phloem tissue near the injection site; and, 8% was in the roots. Similarly, the gas chromatography-mass spectrometry confirmed that paclobutrazol was taken up through roots and transported primarily in the xylem through the stems and accumulated in leaves.

In a case of apple seedling, no detectable basipetal movement of paclobutrazol was found (Wang *et al*., 1986). Quinlan and Richardson (1986) also found that paclobutrazol translocation pattern of M.26 apple moved though the xylem. It exerted its effected on shoot growth by inhibiting gibberellin biosynthesis in the shoot tip and the expanding leaves. This contributes to an understanding of the requirements for efficient orchard application of foliar sprays of paclobutrazol. In addition, radioactive detection by X-ray film radiograms shows that an acropetalic translocation of paclobutrazol or paclobutrazol labeled residues mainly to the terminal site of growth and the minor basipetal translocation was also observed. Zilkah and David (1993) found that the labeled paclobutrazol was applied to pot soil of avocado seedlings and was observed that it was concentrated mainly at the sprouting points of the terminal inflorescences and in its new vegetative growth. It can be concluded that paclobutrazol can be taken up by almost each part of the avocado canopy and roots. The uptake through the inflorescences was the most effective. Paclobutrazol translocates mainly to vegetative sinks, where it inserts the growth retardation. Also

with experiment of Hamid and Williams (1997) resulted that the translocation pattern of paclobutrazol of Sturt's Desert Pea was readily translocated acropetally within a shoot (via xylem) but not basipetally (via phloem).

Previously, a number of reports indicate only the translocation of paclobutrazol via transpiration stream. In contrast, some studies suggest that some translocation may also occur via the phloem. According to Witchard (1997a), paclobutrazol was detected in both xylem and phloem sap of *Pistachia* with highest dosage (7.5×10^4 mg of paclobutrazol/cm^3) 6 months after trees were injected with the compound. As paclobutrazol is believed to be exclusively xylem mobile in plants, its detection in the phloem exudates is an unexpected discovery. Likewise, from castor plant (*Ricinus communis* L.), paclobutrazol was detected in xylem and phloem sap collected above the point of introduction (taken 20 cm above/below) but not in xylem sap below this point (Witchard, 1997b). The concentration was always lower in the phloem (Witchard, 1997b). It shows that paclobutrazol is not exclusively xylem mobile as previously believed. This might be due to the lateral translocation from xylem to phloem.

Residues of paclobutrazol in soil and fruits

Paclobutrazol was absorbed through the roots and transported primarily through stem before accumulation in the leaves (Wang *et al*., 1986). The amount of paclobutrazol residue abandoned in the soil and fruits would appear to depend on the methods of application, dozes and the crop. Singh and Bhattacherjee (2005) reported that the paclobutrazol residues in the soil collected during the third year form the root zone of Chausa mango trees was in the rang of 0.4898-1.0005 µg/g (applied at 2 and 8 g *a.i.*/tree) by gas-liquid chromatography with electron capture detector (ECD). In addition, Sharma and Awasthi (2005) found a little persistence of paclobutrazol residues in the soil over 8 months after application. Subhadrabandhu *et al*. (1999) also studied the residues of paclobutrazol and reported that the remaining was found upto 11 months in soil. Similarly, Mcarthur and Eaton (1989) found that paclobutrazol was still detected in the soil 50 weeks after application at cherry orchard. Soil residues of paclobutrazol have been estimated by indirect bioassay method (Jacyna and Dodds, 1995).

Moreover, the persistence of paclobutrazol in soil further complicates agricultural environment by affecting non-target succeeding crop (Bhattacherjee and Singh, 2002). Paclobutrazol residue in soil may result in contamination of nearby water bodies, which in turn may also be a hazard to human and animal health, and may influence the soil microbial activity (Sharma and Awasthi, 2005). Soil microbial count of a mango orchard soil where paclobutrazol was frequently applied was shown to be reduced by up to 58 % and it may be recommended for use in short rotation cropping systems (Jackson *et al*., 1996; Silva *et al*., 2003).

The maximum residue limits (MRLs) of paclobutrazol accepted by FAO in apple and stone fruits are 0.5 mg/kg (previously, 0.2 mg/kg) and 0.05 mg/kg (FAO, 2002), respectively. Paclobutrazol was found to persist 2-5 years in apple and 1-3 years in peach (Singh and Ram, 2000), cranberry (Mcarthur and Eaton, 1989), and 1-2 years in apricot (Jacyna *et al*., 1989). Bicchi *et al*., (2001) found 0.006 mg/kg of triazol pesticide residues detected in apple and pear pulps. Also Sharma and Awasthi,

2005 reported that the paclobutrazol residues in whole fruit or pulp of mango fruits were found to be below detectable limits (limit of detection 0.001 µg/g). Therefore, Subhadrabandhu *et al*. (1999) reported that when applied paclobutrazol at 8 grams per tree on 'Nam Dok Mai' mango; no chemical residues were detected in the mature fruit. However, very little amount of paclobutrazol is required to induce flowering and fruiting in fruit crops (Browning *et al*., 1992).

Effect of paclobutrazol on fruit qualities

The experiment of Singh and Dhillon (1992) reported that the foliar and soil drench treatments of Cultar at 0, 10, 20, 40, and 60 g/tree in cv. Dusehri did not significantly affect fruit weight and pulp stone ratio. Foliar application of Cultar increased the TSS of the fruit, whereas soil application of Cultar was less effective than foliar application increased the acid content of the fruit compared with the controls. Only the soil application of Cultar (20 g/tree) significantly increased the sugar acid ratio, compared with the controls and all other treatments, except foliar spray application (60 g/tree), which was at par. The increase in sugar acid ratio may be attributed to the decreased acid content of the fruit and increased TSS of the fruit. It was also reported that the fruit yield was significantly highest (27.2 kg/tree) with the soil application of Cultar (20 g/tree) as compared to all other treatments.

Janthasri *et al*. (2001) found that when three-year-old wax apple trees cv. Phetch Toon Klao were treated with paclobutrazol by drenching at the rate of 0, 1, 2, and 4 g *a.i.*/tree or by spraying at 0, 500, 1000 and 2000 mg/L, 40 and 90 days after pruning, the quality of harvested fruits such as total soluble solid, titratable acidity, vitamin C, total non-structural carbohydrates and the ratio of sugar acid did not differ significantly among the treatments.

Khader (1989) illustrated that when paclobutrazol (PP333) was applied as foliar spray at 250, 500, 1000, 2000 or 3000 mg/L in 'Dashehari' mango (*Mangifera indica* L.) tree, the concentrations of 2000 and 3000 mg/L treatments were significantly higher (9.1-9.3) in TSS values but total acidity values were lower than

the control at the beginning of the harvest. A similar trend was found with the sugar acid ratio with significantly higher values in fruits when treated with 2000 or 3000 mg/L PP333. However, all treatments had a uniform level of ascorbic acid at harvest were observed.

Voon *et al*. (1991) found that the work conducted with Cultar by ICI agrochemicals and various co-operators in Thailand, Malaysia and Indonesia. Fruit numbers were increased significantly. Nonetheless, Fruit size was usually not affected but in some cases increased. They also explained that fruit size is related more to fruit load on the tree than to the direct effect of Cultar. To sustain yield increases, it is important that nutrients, irrigation and management are provided. It is also important not to over crop and this can be achieved by applying optimal Cultar rates for each cultivar per tree size.

Yeshitela *et al*. (2004) reported that when ten-year-old 'Tommy Atkins' mango trees in the Rift Valley of Ethiopia was treated with PBZ by soil drench and spray at 0, 2.75, 5.50, 8.25 g *a.i.* per tree, the main effect of method and rate PBZ application significantly affected the total fruit numbers at harvest. The results showed that higher numbers of fruit were obtained from soil drenching than spray applications. Applications of 8.25 g *a.i.* per tree PBZ increased the weight of harvested fruit by 152.87% when compared with the control. Average weight of fruit was not significantly affected by PBZ application. In addition, Soil drenched trees had a significant higher TSS (14.77) value in their fruits than foliar sprayed trees (14.26). The other fruit quality parameters observed in this study (TA, sugar acid ratio, reducing and total sugar) was significantly affected only by PBZ rates.

Winston (1992) found that PBZ was applied after harvest as a foliar spray, a band along the drip line, or a collar drench in trials over 3 years on 3-, 4-, and 5-year-old trees of mango cv. Kensington Pride. In particular, collar drenches of 4 and 8 mL *a.i.*/tree, applied for 2 consecutive years, reduced summer growth. Flowering and cropping were significantly increased by paclobutrazol in a year of inadequate winter stress, while a trend towards increased yield was noted under more normal conditions.

Yield increases were due to fruit numbers rather than size. Rate of PBZ >4 mL *a.i.*/tree caused unacceptable compaction of flower panicles.

Burondkar and Gunjate (1993) reported that when PBZ was treated at 500, 1000 and 2000 ppm as foliar and 5 g and 10 g per tree by soil drench with 'Alphonso' mango, none of the PBZ treatments impaired and improved any of the fruit quality attributes, the data pertaining to chemical composition of ripe mango fruit .

González and Blaikie (2003) found that the second involved applying PBZ as a soil drench around the trunk of the tree. Commercial fruit yield of PBZ-treated trees was 2-3 times higher than that of control or mango flowering treatment (MFT). The effects of soil drenching with PBZ and foliar spray of Cycocel and Alar on yield and quality of fruits were studied in nine year old mango trees (cv. Alphonso). Fruit yield was enhanced considerably by PBZ at 2.5 or 5.0 g per tree, but 10 g per tree, it reduced yield. Alar at 3000 mg/L was slightly enhanced yield. Higher doses of PBZ (10 g) reduced fruit size and gave an adversely effects to the TSS of fruits while the sugar acid ratio was lower, and also delayed the maturing of fruits on the trees and the ripening of harvested fruits (Kurian and Iyer ,1993).

Wang *et al.* (1986) found that paclobutrazol treatment with apple increased rhamnose, arabinose and galacturonic acid but decreased cellulose. The ratio of xylem to phloem was also reduced by PBZ treatment.

Jacyna and Dodds (1995) found that when twelve-year-old 'Sundrop' apricot (*Prunus armeniaca* L.) trees were treated with a single soil application of PBZ at the rate of 2, 4, and 6 g of active ingredient per tree as soil drench. Although, the effects on fruit quality characteristics were not examined in the first year of the experiment, fruit weight, fruit firmness, and juice refraction were in most instances not affected by PBZ in the second year.

Rizzolo *et al*. (1993) studied the effect of PP333 which was applied two years before, on the quality of Starkspur Golden apples. The experiment was conducted by

sampling fruits from 50 to 180 days after full bloom, and analyzing for soluble solids, starch, titratable acidity, and volatile substances. It was found that the soil treatment influenced starch accumulation, and untreated fruits had the highest amount. However, titratable acidity and soluble solids were not affected. Volatile changed qualitatively and quantitatively during fruit growth and the treated fruits produced lower amounts.

Salazar-Garcia and Vazquez-Valdivia (1996) reported that when 'Tommy Atkins' mango was applied by PBZ at 0, 2.5, 5, 10, 15, 20 and 40 g per tree, all PBZ rates decreased average fruit weight (398 to 301 g). Control trees produced the heaviest fruit (401 g). Fruit diameter was closely related to fruit weight and again the smallest values were for PBZ levels of 15 g per tree and above. TSS of fruit juice were not affected compared with the controls at low rates of PBZ (2.5 and 5 g per tree), but they were significantly higher for trees treated with 10 g PBZ per tree or above. TA for fruits from control and 10 g PBZ per tree gave intermediate values (0.18 and 0.19%). Fruits from trees treated with PBZ at 5 g per trees had the highest acidity (0.29%) and the lowest acidity which corresponded to fruits of treatments with 2.5 and 20 g PBZ per tree (0.15 and 0.14%, respectively).

2.3 Solid Phase Microextraction (SPME)

Solid Phase Microextraction (SPME) is an innovative, solvent free technology that is fast, economical, versatile and commonly used in trace analysis; and can improve the detection limits. SPME is a fiber coated with a liquid (polymer), a solid (sorbent), or a combination of both. The fiber coating removes the compounds from the sample by absorption in the case of liquid coatings or adsorption in the case of solid coatings (Mani, 1999). As shown in Figure 2.4. The primary parameters influencing analyte absorption into the stationary phase are fiber type, extraction time, ionic strength, pH, temperature, sample volume, and agitation. For SPME-GC, analyte desorption is a function of time and temperature. Conversely, solvent type and volume or time is critical for SPME-HPLC modes (Beltran *et al*., 2000; Krutz *et al*., 2003).

SPME technique has been developed to combine sampling and sample preparation in one step (Wardencki *et al*., 2004). In addition, it is a powerful tool in pesticide residue analysis for both qualitative and quantitative determination (Beltran, *et al*., 2000). The extraction may be defined in two modes: Headspace (HS-SPME) and direct immersion (DI-SPME). In HS-SPME, the fiber is exposed in the vapor phase above a gaseous, liquid or solid sample, while for DI-SPME; the fiber is directly immersed in liquid samples. Agitation of the sample is often carried out with a small stirring bar to increase the rate of equilibration. After a suitable extraction time, the fiber is withdrawn into the needle. The needle is then removed from the septum and inserted directly into the injection port of the GC or the desorption chamber of the SPME-HPLC interface. HS-SPME and DI-SPME techniques can be used in combination with any GC, GC-MS, HPLC and LC-MS system (Kataoka *et al*., 2000, Wardencki *et al*., 2004, Meurer *et al*., 2002). The process of fiber SPME is illustrated in Figure 2.5.

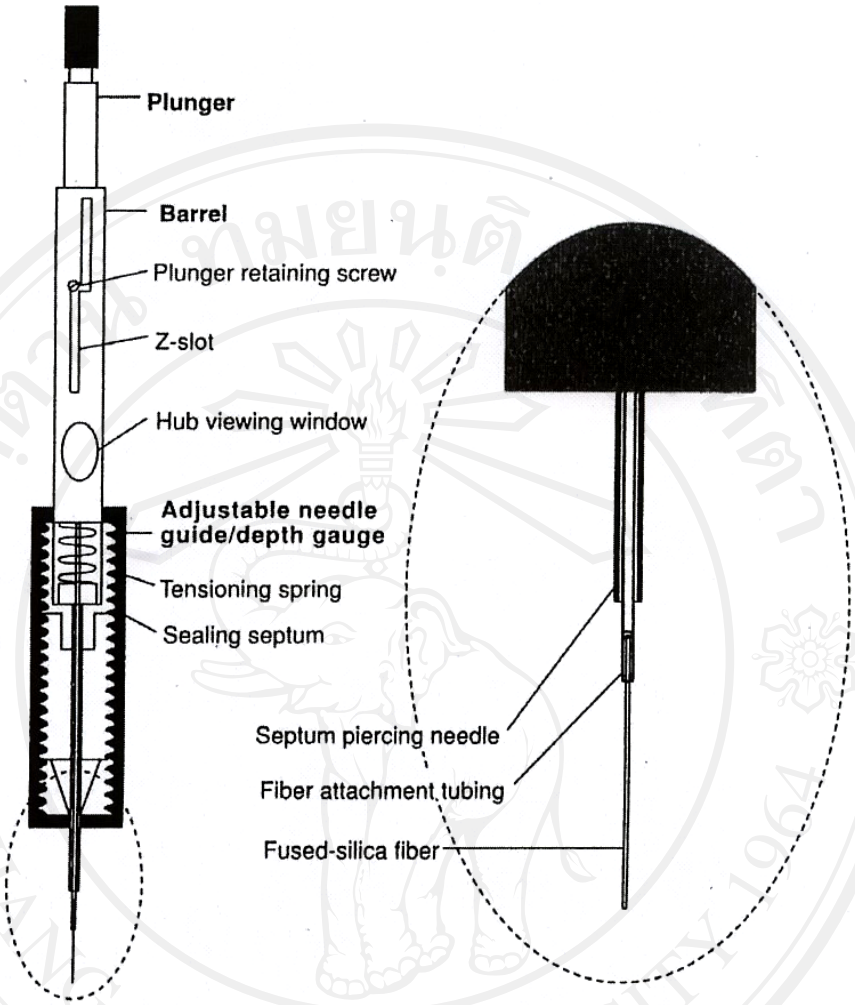

Figure 2.4 Commercial SPME device made by Supleco (Kataota *et al*., 2000).

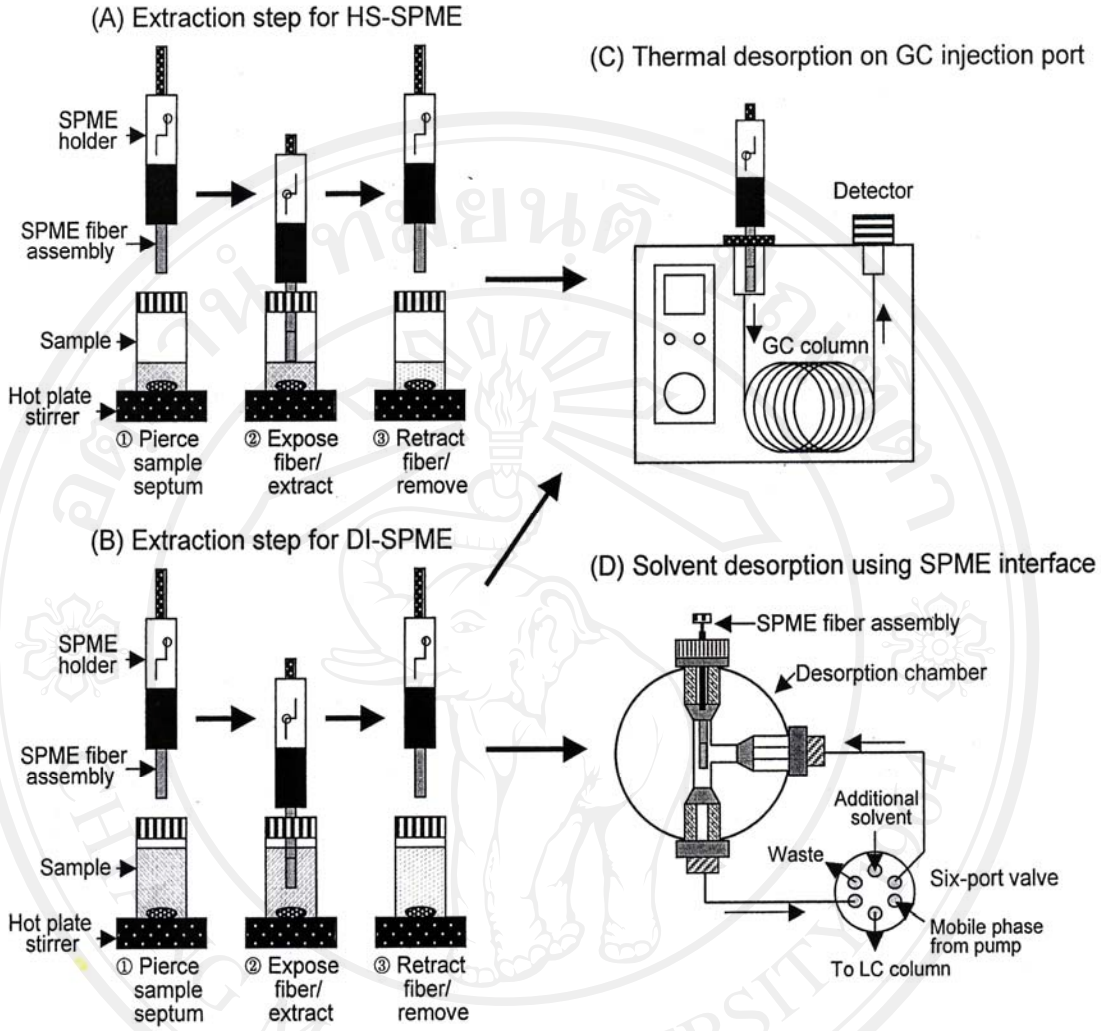

Figure 2.5 Extraction process by headspace and immersion fiber SPME, and desorption systems for GC and HPLC analyses (Kataota *et al*., 2000).