ECOLOGICAL STUDIES ON THE PRODUCTIVITY AND FRUIT QUALITY OF MANGOSTEEN (*Garcinia mangostana* L.)

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Ecological Studies on the Productivity and Fruit Quality of Mangosteen (*Garcinia mangostana* L.)

A Thesis

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Declaration

This thesis describes the research and results of original work done by the candidate, Eko Setiawan, for his Ph.D. degree at the Graduate School of Natural Science and Technology, Okayama University. Any assistance and collaboration received from others has been appropriately acknowledged. The research and results herein described are valid and reliable, but no responsibility for reproducibility can be assumed. This thesis has not been previously submitted to any other University or academic institution, neither in whole or in part, for the award of a degree or other professional or academic titles.

Certified by the supervisor,

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Contents

General Introduction

Mangosteen (*Garcinia mangostana* L.) is a tropical, evergreen fruit tree believed to originate in the Sunda Islands and the Malay Peninsula (Osman and Milan, 2006; Yaacob and Subhadrabandhu, 1995). This plant is named the "*Queen of Tropical Fruits"* because of its characteristic taste and flavor (Diczbalis and Westerhuis, 2005; Popenoe, 1915). Mangosteen is consumed as fresh fruit and is considered highly nutritional because it contains large amounts of antioxidants (Salakpetch, 2005).

Growers have found that young mangosteen plants require artificial shade to stimulate growth (Salakpetch, 2005). Hence, mangosteen orchards, which are generally found in villages, tend to contain mixed stands with several other fruit species, such as durian (*Durio zibethinus*), rambutan (*Nephelium lappaceum*), and longkong (*Lansium domesticum*). By intercropping trees in this manner, growers are guaranteed an income when mangosteen trees are still juveniles. After mangosteen trees start to flower, growers usually decide whether to grow mangosteen as the main crop or continue with mixed crops. Mangosteen trees start to flower as early as 4 to 5 years after being planted as seedlings, if provided with adequate amounts of fertilizer and water; however, the fruit size of young trees is usually small and yields are quite low.

Mangosteen trees are pyramidal in shape with symmetrical branches, and grow to a height of between 6 to 25 m (Nakasone and Paull, 1998; Yaacob and Subhadrabandhu, 1995). Canopy structure influences the transmission of sunlight to the bottom and inner positions of the canopy (Setiawan *et al.*, 2006; 2012). The leaves are relatively thick, leathery, and simple in structure and are positioned opposite one another. The flowers are 4 to 5 cm in diameter, fleshy, and may have separate male or both male and female (hermaphrodite) parts on the same tree. Flowers are terminal, solitary, or paired, with 4 sepals and 4 petals, green on the outside and yellow-red on the inside, and with numerous stamens (Nakasone and Paull, 1998).

Growth and Juvenile phases

Mangosteen trees are usually propagated from seeds, which produce true-to-type seedlings. The slow growth of mangosteen is attributed to: (a) poor growth of its root system (no root hairs, poor branching, and easily broken and disturbed by adverse environments, resulting in very limited contact surfaces between the roots and soil medium), (b) poor nutrient and water uptake, (c) low photosynthetic rate, (d) low cell division rate in the apical meristem, and (e) long dormancy of the shoot phase (Poerwanto *et al.*, 2008; Ramlan *et al.*, 1992; Wieble *et al.*, 1993; Yaacob and Subhadrabandhu, 1995). The seedlings should be grown in polybags containing fertilizer and manure and maintained in 50% shade in a nursery for 2 years. After this initial period, the seedlings should be strong and large enough for transplantation to the field (Salakpetch, 2005). In Thailand, general practice also includes the use of a complete fertilizer (N-P_2O_5-K_2O: 16-16-16) and regular irrigation with about 70% of daily pan evaporation water, which is reported to accelerate growth of mangosteen seedlings under nursery conditions. In addition, foliar fertilizer sprays may be used. Animal manure (preferably chicken) is also recommended to promote the growth of young mangosteen plants. Under these conditions, plants produce secondary branches, with leaf number and area also increasing, before transplanting.

The development of technology to improve productivity and fruit quality of mangosteen is important to increase export and meet domestic market demands for better quality fruit. The vegetative method of propagation through top-grafting may shorten the time to initial flowering to 4 to 5 years, although the growth of top-grafted mangosteen is slower than that of non-grafted seedlings (Poerwanto, 2002; Rukayah and Zabedah, 1992). In comparison, 2–3 years is required to grow the rootstock. Grafting has been attempted in Thailand, Australia, and Malaysia to reduce the juvenile period; however, a reliable technique is yet to be established. In Thailand, research has shown that if tertiary limbs are selected as scions, the grafted plants tend to grow horizontally after planting in the field (Salakpetch, 2005).

Productivity and Fruit Quality

The total mangosteen production by Thailand, Malaysia, the Philippines, and Indonesia, majority of the mangosteen producing countries in Southeast Asia, in 1998 has been reported 150,000 ton (Osman and Milan, 2006). Most fruit in these markets are obtained from orchards planted in backyards or from mixed fruit orchards, with very few fruits being produced via commercial cultivation. Consequently, detailed data about mangosteen production in these 4 countries is not readily available (Osman and Milan, 2006). Mangosteen production has remained relatively static, although there is some recent evidence of increased production. The fruit quality of mangosteen varies across production centers because mangosteen plantations are managed traditionally, with production systems depending on natural conditions (Dorly *et al.*, 2008). Osman and Milan (2006) reported that the quantity of mangosteen production is generally lower than other fruits, such as banana, pineapple, citrus, mango, rambutan, langsat, and durian, in all countries where it is produced. Propagation with seeds is relatively easy; however, the juvenile phase of the plant may last 10 to 15 years. As a result, these plants often have low productivity because of poor flowering and fruit set, as well as pigmentation (termed gamboge, a mustard yellow pigment) problems (Poerwanto *et al.*, 2008; Rukayah and Zabedah, 1992). Yaacob and Subhadrabandhu (1995) reported that the fruit load for the first crop of 6- to 8-year-old trees is about 10 kg per tree, depending on canopy size, with fruit load increasing with age. Phonrong and Sdoodee (2005) reported that mangosteen trees produce more than 1500 fruits/tree (around 119.89 kg/tree), although the fruit size is relatively small.

In 1999, only about 19,174 tons of Indonesian mangosteen was produced; however, this dramatically increased to 25,821 tons in 2001 and 79,073 tons in 2003 (Osman and Milan, 2006). Indonesian mangosteen is mostly exported to Europe, the Middle East, Taiwan, Hong Kong, and Singapore. However, increased levels of export have not been supported by increased fruit production and quality. Low productivity is mostly attributable to the poor flowering and fruit set of this plant (Poerwanto *et al.*, 2008; Setiawan *et al*., 2006; Setiawan and Poerwanto, 2008). Only 5–10% of the harvested fruit passes the quality standards for export (Osman and Milan, 2006; Setiawan *et al.,* 2006). One of the biggest issues of harvested fruit is the high percentage of visible scars on the skin surface, which are caused by thrips (tiny slender insects with wings that belong to the order Thysanoptera). These insects cause silvering of the skin and pale yellow to brown discoloration (Affandi and Emilda, 2010). Salakpetch (2005) reported that mangosteen plants should be kept well protected from major pests, such as chilli thrips (*Scirtothrips dorsalis* Hood), mangosteen thrips (*Scirtothrips oligochaetus* Karny), leaf miners (*Phyllocnistis* sp. and *Acrocercops* sp.), and leaf eating caterpillars (*Stictoptera columba* [Walker], *Stictoptera cucullioides* [Guene], and *Stictoptera signifera* [Walker]).

Climatic Requirements

In terms of agro-climatic requirements, mangosteens preferentially grow on deep, fertile, well-drained, and slightly acidic soils with high organic content. The plants do not grow well in soils with a high pH. Mangosteen requires high humidity and an annual rainfall of 1200 mm or more, without prolonged dry periods. Mangosteen appears to require an uninterrupted water supply of 15 to 30 days, with a short dry season that initiates flowering (Nakasone and Paull, 1998). Some productive mangosteen plants are found growing near the banks of streams, lakes, ponds, or canals, where their roots are almost constantly wet. The optimum temperature for growth ranges from 25 to 35 °C (Osman and Milan, 2006). Most mangosteen species grow in the warm and humid tropics of South and Southeast Asia. Specific climatic requirements limit the distribution of mangosteen to the equatorial band between 10 °N and 10 °S latitude (Verheij, 1992). However, mangosteen may be cultivated at higher elevations, although growth rates are much slower (Nakasone and Paull, 1998).

Study Objectives

The objectives of this study were to: (1) investigate the effects of sector (position in the canopy) and tree age on the productivity and fruit quality of mangosteen; (2) clarify the meteorological conditions of mangosteen orchards; (3) monitor seasonal changes in carbon and nitrogen contents, the C-N ratio, SPAD value, and chlorophyll content of leaves, with special reference to flower bud differentiation; (4) determine the quantity and components of sugars, organic acids, and amino acids of fruit (aril) in relation to sector and tree age; and (5) evaluate the partitioning of ^{13}C-photosynthates in branches of different sectors in relation to fruit quality.

Chapter 1

Productivity and Fruit Quality of Mangosteen Grown in Commercial Orchard

1.1 Introduction

The quality of fruit produced by mangosteen trees (*Garcinia mangostana* L.) varies markedly among individual trees according to season and growing conditions (Malip and Masri, 2006; Nakasone and Paull, 1998). The commercial quality of mangosteen fruits is evaluated according to the following criteria: size and weight, scarring of skin surface due to thrip infestation or other damage, and sugar and acid content of the fruit (aril). Some researchers report that the highest quality mangosteen fruit is born on branches close to the trunk, or on internal branches that are not exposed to direct sunlight (Nakasone and Paull, 1998; Yaacob and Subhadrabandhu, 1995). We have previously reported that the quality of mangosteen fruits varies substantially among bearing positions in the tree canopy (Setiawan *et al.*, 2006).

In Indonesia, the productivity and fruit quality of mangosteen are relatively low (Setiawan and Poerwanto, 2008; Setiawan *et al.*, 2006). Low productivity is mainly caused by poor flower bud differentiation and fruit set. It is well known that carbohydrate levels in mangosteen tree are closely related to the induction of flower buds (Weibel *et al.*, 1993). Blooming is not uniform, and the average flowering period is approximately 40 days. In 6- to 8-year-old trees, the first crop produces approximately 10 kg of fruit, depending on canopy size, and this increases with tree age (Yaacob and Subhadrabandhu, 1995). Phonrong and Sdoodee (2005) reported that mangosteen trees produced more than 1500 fruits/tree (around 120 kg/tree), although fruits were small size.

The quality of mangosteen fruit is usually evaluated using Codex Stan 204-1997, which requires that fruit size to be more than 62 mm, minimum weight of 76 g, have no scars, undamaged calyx, and is fresh green in color to be considered first grade (Diczbalis, 2009; FAO, 2005). In Indonesia, only 5 to 10 % of harvested fruit passes the quality standards for export (Osman and Milan, 2006; Setiawan *et al*., 2006), with one of the biggest issues being the high percentage of visible scars on the skin surface caused by thrips (*Scirtothrips dorsalis* Hood and *Selenothrips rubrocintus* Giard), which cause a silvering of the skin, and pale yellow to brown discoloration (Affandi and Emilda, 2010). Khalid *et al.* (2012) also reported that the fruit quality in citrus may be evaluated by external features, such as rind colour, size, and rind texture, and physical and biochemical characters of its internal features, like seedless, juice and vitamin C content, total soluble solid (TSS), titratable acidity (TA), and TSS/TA ratio. Chaisrichonlathan and Noomhorm (2011) reported that the internal physical disorders of smooth-surface in mangosteen fruits were higher than coarse-surface ones.

Fruiting position in canopy can also affect fruit quality, with Moon *et al.* (2011) reporting that fruit diameter, fruit weight, pulp weight, and pulp thickness were higher in citrus fruits harvested from the top position of a tree than in the middle or bottom positions. This research was done to understanding the effect of fruiting position and tree age on mangosteen tree in relationship with productivity and fruit quality. In this chapter, first, I investigated the productivity and fruit quality in two locations of commercial orchard and then compared their productivity and fruit quality of mangosteen among sectors (position in the canopy) and location. Second, I investigated the effect of sector and tree age on the productivity and fruit quality of mangosteen.

1.2 Effect of Location on Productivity and Fruit Quality in Mangosteen

1.2.1 Materials and Methods

Research site

The study was conducted at a commercial orchard in Bogor district (site 1 = 6° 61´ 69 S, 106° 64´ 42 E) and Purwakarta district (site 2 = 6° 68´ S, 107° 56´ E), West Java province, Indonesia (Fig. 1). Seven 25-years-old (young tree) mangosteen trees of similar size were selected for study in each site from July 2003 to February 2004 (*on-year*). The research was set in a Randomized Complete Block Design (RCBD) in seven replications with one tree per replicate.

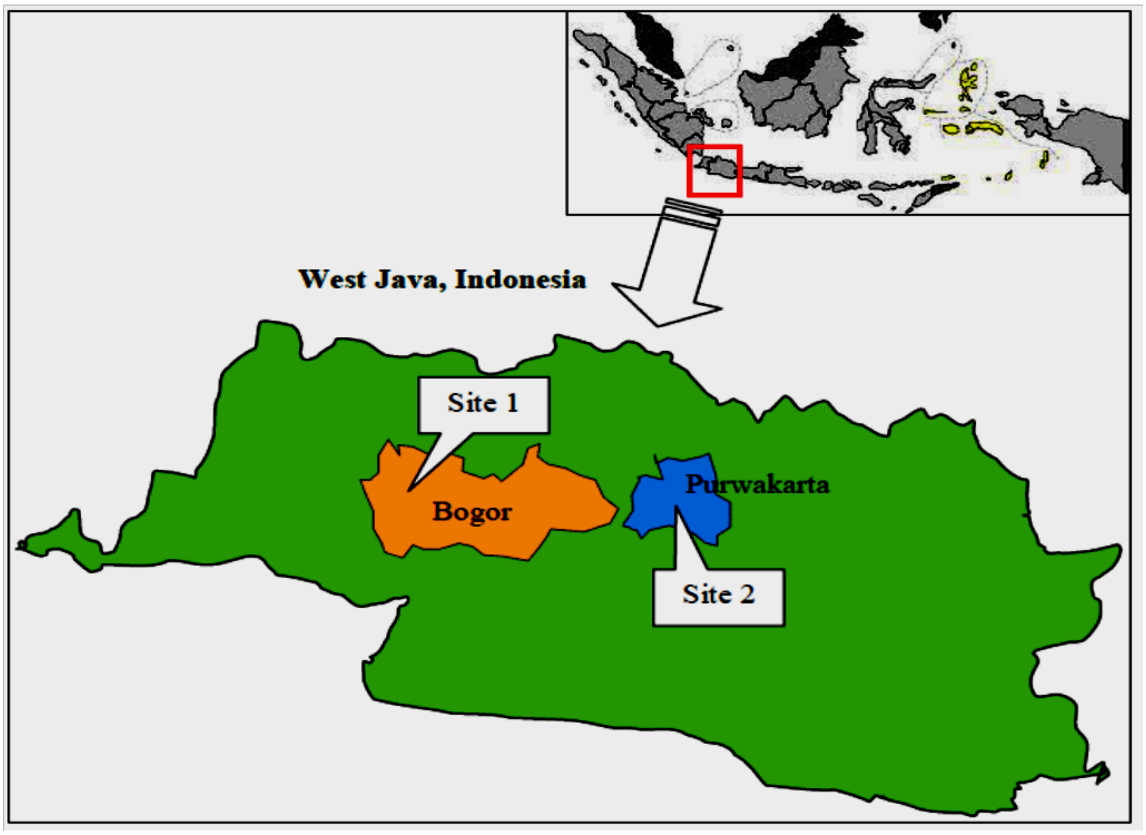

Fig. 1. The research sites at Bogor and Purwakarta district, West Java province, Indonesia.

Plant materials

The canopy of each tree was divided into 9 sectors, based on tree height (bottom, middle, and top) and width (inner, center, and outer), as follows: Sector 1 = inner bottom, Sector 2 = center bottom, Sector 3 = outer bottom, Sector 4 = inner middle, Sector 5 = center middle, Sector 6 = outer middle, Sector 7 = inner top, Sector 8 = center top, and Sector 9 = outer top (Fig. 2).

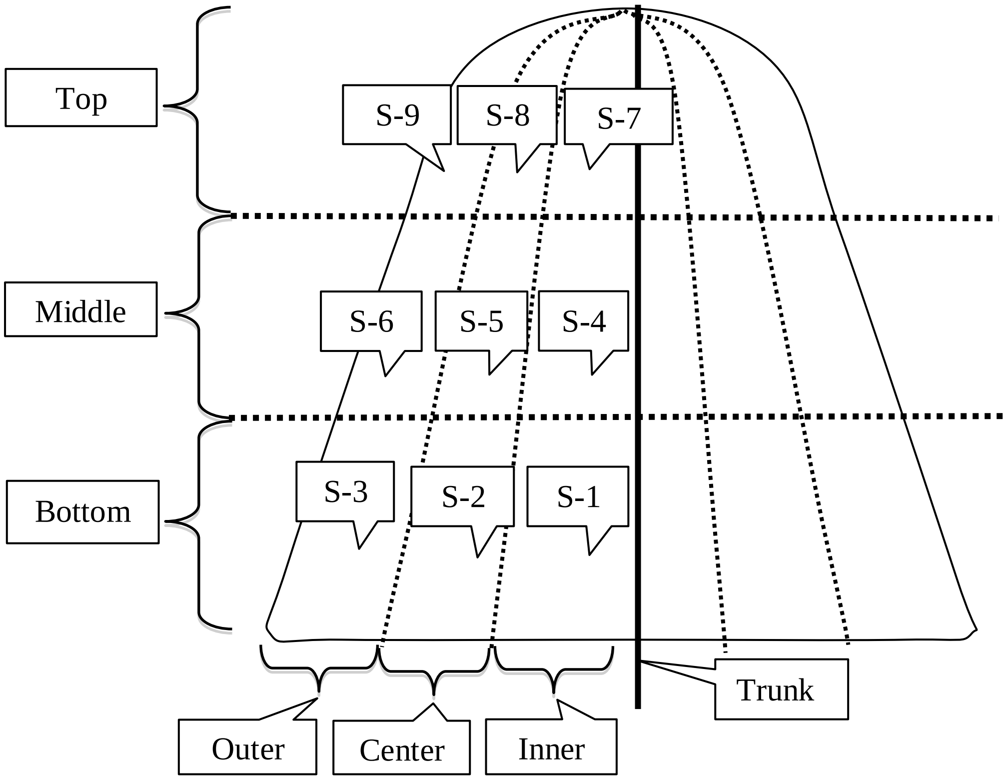

Fig. 2. Illustration of the sectors (positions in the canopy, S-1 to S-9) defined for study of mangosteen trees.

Productivity and fruit quality

The phenological development of mangosteen trees was monitored in 2003. The dates of new vegetative shoot, flower bud differentiation, and fruit harvested were recorded. The number of new vegetative shoots and flowers in each sector was counted from February to September, 2003. Whereas, the number of fruits produced in each sector was counted from October to November, 2003. Furthermore, the flower and fruit drop were counted, then the initial and final fruit set was calculated. The measurement of fruit development was done every week only in Bogor orchard (site 1). All fruit were harvested from each sector from December 2003 to January 2004 at the light greenish yellow with 51 to 100 % scattered pink spot (stage 2), which is the stage that fruit is usually harvested (Palapol *et al.*, 2009). The percentage of the entire fruit surface covered with scars was calculated. Width (transversal) and length (longitudinal) diameters and skin thickness was measured by calipers (Mitutoyo, Japan). Each fruit was separated into pericarp, aril, and seed, and then weight was recorded with an analytical balance. The edible portion of the fruit (aril) was stated as a percentage and could be estimated by (aril/fruit) weight × 100 %. The number of fleshy segments, the number of seeds, and the percentages of yellow latex in aril were counted. Total soluble solid (TSS; °Brix) and titratable acidity (TA; %) were analyzed at Center for Tropical Fruit Studies, Bogor Agricultural University (IPB). TSS was measured using a refractometer (PAL-1, Atago, Tokyo Tech). TA was measured using the following method: distilled water was added to 10 g of crushed fruit in the measuring flask until the volume reached 100 ml, then it was filtered. A few drops (approx. 2-3 ml) of the phenolphthalein indicators were added into 25 ml of filtrate, then titrated with 0.1 N NaOH until a stable pink colour was formed. The productivity and fruit quality of mangosteen was compared among sectors (position in the canopy) and location.

Codex standard for mangosteen (Codex Stan 204-1997; FAO, 2005)

This standard applies to commercial standardization of mangosteens (*Garcinia mangostana* L.), that is supplied fresh to the consumers, after preparation and packaging.

In all classes, subject to the special provisions for each class, and the tolerances allowed; the mangosteens must be:

- Whole, complete with the calyx and pedicel intact,
- Sound, produce affected by rotting or deterioration such as to make it unfit to consumption is excluded,
- Clean, practically free of any visible foreign matter,
- Practically free of pests affecting the general appearance of the product,
- Practically free of damage caused by pests,
- Free of abnormal external moisture, excluding condensation following removal from cold storage,
- Free of any foreign smell and/or taste,
- Fresh in appearance, have a shape, colour and taste characteristic of the species,
- Free of latex,
- Free of pronounced blemishes,
- Allowing the fruit to be cut open normally.

1) Classification

   Mangosteens are classified into two classes defined below:

   a) Extra Class, mangosteens in this class must be of superior quality. They must have the characteristic of the variety and/or commercial type. They must be free of defects.

   b) Class I, mangosteens in this class must be of good quality. They must be characteristic of the variety and/or commercial type. The following slight defects, however, may be allowed, provided these do not affect the general appearance of the produce, the quality, the keeping quality and presentation in the package: slight defects in shape and slight defects on the peel and calyx such as bruising, scratches or other mechanical damage. The total affected area should not exceed 10 %.

2) Provisions concerning sizing

Size is determined by the weight or the diameter of the equatorial section through the fruit, in accordance with the following Table 1.

Table 1. Size code, weight and diameter of mangosteen fruit (FAO, 2005)

Size code	Fruit weight (g)	Fruit diameter (mm)
A	30 - 50	38 - 45
B	51 - 75	46 - 52
C	76 - 100	53 - 58
D	101 - 125	59 - 62
E	> 125	> 62

Statistical analysis

A one-way analysis of variance (ANOVA) was performed to test whether there were significant differences in productivity and/or fruit quality among sectors (positions in the canopy) and location. Means were compared using Duncan's Multiple Range Test. All statistical tests were carried out using SPSS version 16.0 (IBM SPSS Inc., Chicago) and differences with p-values less than 0.05 were considered significant.

1.2.2 Results

a) Bogor district

Productivity

Table 2 showed the number of new vegetative shoots, flower, and fruit production differed among sector in Bogor district. The strong vegetative shoot was observed in Sector 6, following in descending order by top position of canopy such as

Sectors 7 to 9 than in bottom one such as Sectors 1 to 3. In this research, the large number of flower and fruit set was found in Sector 4, following in descending order by Sectors 1, 2, and 5. Very few flowers and/or fruits were born from Sectors 3, 6, and 1, and no flower and fruit was produce in Sectors 7 and 8. Although the flowers and fruits drop was highest from Sectors 4, 1, 2, and 5, but the number and fruit weight per sector was higher in Sector 4, following in descending order by Sectors 1, 5, and 2. Outer position of canopy such as Sectors 3, 6, and 9 was low in the number of fruit and fruit weight per sector. The percentage of fruit set both in initial and final fruit set was highest in Sectors 6 and 9, and the lowest in Sector 2. Productivity (number of fruit per m^3 of canopy) is 1.48 fruit/m^3 (canopy size = 5.4 × 5.2 m in tall and width, respectively).

Table 2. The number of new vegetative shoots, flower, fruit set, flower and fruit dropped, fruit harvested, percentage of initial and final fruit set, and fruit weight per sector in 25-years-old mangosteen trees as affected by different sectors at Bogor district in 2003 (*on-year*)

Sector	Number of						% of fruit set		Fruit weight per sector (g)
	Shoots	Flower	Flower dropped	Fruit set	Fruit dropped	Fruit harvested	Initial	Final	
1	2.0 d [z]	22.0 b	4.1 a	17.9 b	9.0 a	8.9 b	83.0 b	53.0 c	602.6 b
2	1.7 d	13.0 c	4.6 a	8.4 c	5.7 a	2.7 cd	64.1 c	29.3 d	209.7 c
3	2.7 d	2.0 d	0.0 b	2.0 d	1.0 b	1.0 d	100.0 a	50.0 c	86.0 c
4	6.7 d	33.3 a	5.3 a	28.0 a	8.4 a	19.6 a	84.2 b	69.4 b	1471.4 a
5	14.7 c	10.0 c	1.5 b	8.5 c	2.2 b	6.3 bc	88.1 b	78.4 b	547.8 b
6	37.7 a	1.5 d	0.0 b	1.5 d	0.0 b	1.5 d	100.0 a	100.0 a	112.7 c
7 [y]	29.0 b								
8 [y]	26.0 b								
9	27.1 b	1.0 d	0.0 b	1.0 d	0.0 b	1.0 d	100.0 a	100.0 a	90.2 c

[z] Value with different letters in each column indicate significant difference by *Duncan's Multiple Range Test, p<0.01.*

[y] No flower and fruit.

Fruit quality

The widest size of fruits harvested from Bogor district was found significantly at Sectors 9, 3, and 5, whereas the longest size of fruits harvested was found at Sector 3 (Table 3). The small of width and length size was obtained from Sectors 2 and 1. The pericarp thickness was varied greatly among the sectors, range from 6.3 to 7.5 mm in fruit harvested from Sectors 6 and 3, respectively. Fresh weight of fruit harvested from Sector 9 was the highest, whereas Sector 1 was the lowest. Consequently, fresh weight of aril was greater in Sector 9 than Sector 1. There was no significant different among sectors in both fresh weight of pericarp and seed. The percentage of edible portion of the fruit (aril) was high in Sector 9 and low in Sectors 1 and 4. The number of fleshy segment was high in Sector 3 and low in Sector 9. There was no significant different among sectors in the number of seed. The range was from 0.8 to 1.3 in Sectors 2 and 5, respectively.

Table 3. Fruit size, pericarp thickness, fresh weight, percentage of edible portion, and number of seed and fleshy segments in 25-years-old mangosteen trees as affected by different sectors at Bogor district in 2003 (*on-year*)

Sector	Fruit size (mm)		Pericarp thickness (mm)	Fresh weight (g)				% of edible portion	Number of fleshy segments	Num ber of seed
	Width	Length		Fruit	Pericarp	Aril	Seed			
1	52.5 c [z]	46.0 b	6.8 c	68.0 c	39.4	24.8 d	1.0	36.4 c	6.3 bc	1.0
2	52.0 c	46.2 b	7.1 b	67.9 c	39.5	24.7 d	0.9	36.6 c	5.9 d	0.8
3	57.0 ab	50.0 a	7.5 a	86.0 ab	45.5	36.8 b	1.4	42.8 b	7.0 a	1.0
4	54.0 bc	47.0 ab	7.0 bc	75.6 bc	44.1	27.5 cd	1.2	36.4 c	6.1 cd	1.2
5	55.0 abc	47.4 ab	7.2 b	83.7 ab	47.4	31.7 c	1.5	37.9 c	6.1 cd	1.3
6	54.3 bc	48.5 ab	6.3 d	82.4 ab	47.8	30.1 cd	1.2	37.2 c	6.5 b	1.0
7 [y]										
8 [y]										
9	57.5 a	47.0 ab	7.0 bc	90.2 a	42.2	44.1 a	1.1	48.8 a	5.0 e	1.0

[z] Value with different letters in each column indicate significant difference by *Duncan's Multiple Range Test, $p<0.01$.*
[y] No fruit.

Fruit harvested from Sectors 9 and 6 in Bogor district had the higher percentage of scars than those harvested from Sectors 3 and 2 as shown in Table 4. In this research, TSS content was higher in Sector 2 and lower in Sector 3 about 16.3 and 14.0 °Brix, respectively. Whereas TA content was high in Sector 9 and low in Sector 3 about 1.15 and 0.97 %, respectively. The high percentage of fruit 'E' size used Codex Standard was found in Sector 5 followed by Sectors 4 and 1. Whereas, fruit ‘D’ size was high in Sector 6, followed in descending order by Sectors 5, 4, 1, and 2. The percentage of ‘Extra’ class was highest in Sector 3, followed in descending order by Sectors 2, 5, 1, and 4. Otherwise, the percentage of ‘I’ class was highest in Sectors 6 and 9, followed in order by Sectors 4, 1, 5, and 2.

Table 4. Percentage of scar in skin surface, TSS and TA content, and percentage of fruit classing and sizing used Codex Standard in 25-years-old mangosteen trees harvested in purple dark stage as affected by different sectors at Bogor district in 2003 (*on-year*)

Sector	% of skin with scars [z]	TSS (°Brix)	TA (%)	% of fruit size used Codex Standard					% of fruit class	
				A [w]	B	C	D	E	Extra [v]	I [u]
1	15.0 bc [y]	15.7 b	1.02 bc	9.7	45.2	35.5	8.0	1.6	46.8	53.2
2	6.3 c	16.3 a	1.01 bc	10.4	52.7	31.6	5.3	-	52.8	47.2
3	0.0 c	14.0 c	0.97 c	-	-	100.0	-	-	100.0	0.0
4	18.4 bc	15.3 b	1.13 a	8.7	31.4	43.1	11.7	5.1	39.5	60.5
5	18.2 bc	15.3 b	1.02 bc	2.6	23.7	44.7	21.1	7.9	47.3	52.7
6	32.5 ab	15.5 b	1.08 ab	-	66.7	-	33.3	-	0.0	100.0
7 [x]										
8 [x]										
9	50.0 a	15.4 b	1.15 a	-	-	100.0	-	-	0.0	100.0

[z] Mangosteen fruits affected by thrips typically show silvering of the skin, and pale yellow to brown discoloration that may cover the entire fruit surface.
[y] Value with different letters in each column indicate significant difference by *Duncan’s Multiple Range Test, p<0.01*.
[x] No fruit.
[w] Size code (A = 30−50 g and 38−45 mm, B = 51−75 g and 46−52 mm, C = 76−100 g and 53−58 mm, D = 101−125 g and 59−62 mm, and E = > 125 g and > 62 mm base on fruit weigh and diameter, respectively).
[v] Superior quality, fruit free from defects.
[u] Good quality, the total fruit slight defects in shape, bruising on the peel and calyx, scratches or other mechanical damage not exceed 10 %.

Fruit growth

Fruit diameter increased throughout the season in pattern fitted well by sigmoid model (Fig. 3). Fruit mass growth during the first four weeks after flowering was slow. Fast growth was occurred from 5 to 9 weeks after flowering, and then become slower after 10 weeks after flowering.

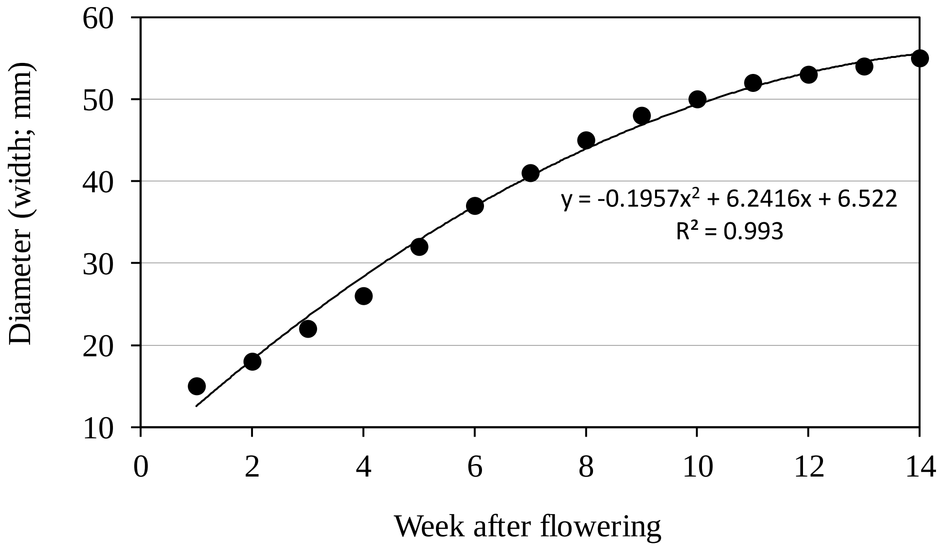

Fig. 3. Seasonal changes in diameter of mangosteen fruit.

b) Purwakarta district

Productivity

The number of new vegetative shoots, flower, and fruit production differed among sectors in Purwakarta district was shown in Table 5. In this research, the strong vegetative shoot was observed in Sector 6, following in descending order by Sectors 7, 5, 8 and 9. Bottom canopy such as Sectors 1 to 3 was low in the number of vegetative shoots. The number of flower and fruit set was highest in Sectors 5, 4, and 2, whereas in Sector 9 was no produce of flower and fruit. The flowers drop was highest in Sector 2, and lowest in Sector 6. Fruits drop was high in Sector 5, followed in descending order by Sectors 7, 4, and 2. The number and fruit weight per sector was high in Sectors 4, 5, and 2 compared to other sectors. The percentage of initial fruit set was highest in Sector 6 and the lowest in Sector 1 about 96.0 % and 81.1 %, respectively, whereas the final fruit set was high in Sector 7 about 77.4 %, and low in Sector 3 about 44.6 %. Productivity (number of fruit per m^3 of canopy) is 3.4 fruit/m^3 (canopy size = 4.6 × 4.1 m in tall and width, respectively).

Table 5. The number of new vegetative shoots, flower, fruit set, flower and fruit dropped, fruit harvested, percentage of initial and final fruit set, and fruit weight per sector in 25-years-old mangosteen trees as affected by different sectors at Purwakarta district in 2003 (*on-year*)

Sector	Number of						% of fruit set		Fruit weight per sector (g)
	Shoots	Flower	Flower dropped	Fruit set	Fruit dropped	Fruit harvested	Initial	Final	
1	2.0 e[z]	12.9 bc	2.4 ab	10.5 b	4.1 ab	6.3 b	81.1 c	56.3 ab	549.4 b
2	0.9 e	38.0 ab	7.0 a	31.0 a	13.1 ab	17.9 a	84.3 bc	63.5 ab	1237.8 a
3	0.6 e	7.0 c	1.0 ab	6.0 b	2.9 b	3.1 b	84.4 bc	44.6 b	245.3 b
4	10.1 d	42.6 a	4.9 ab	37.7 a	15.0 ab	22.7 a	89.7 ab	64.1 ab	1783.8 a
5	19.6 b	43.6 a	6.9 ab	37.7 a	21.4 a	19.4 a	91.5 ab	69.7 ab	1439.9 a
6	45.9 a	6.2 c	0.2 b	6.0 b	2.2 b	3.8 b	96.0 a	53.8 ab	480.8 b
7	21.9 b	9.5 c	2.6 ab	8.2 b	16.6 ab	5.0 b	92.3 ab	77.4 a	371.4 b
8	15.1 c	14.0 bc	2.5 ab	11.5 b	5.0 ab	6.5 b	89.6 ab	73.4 ab	335.0 b
9[y]	13.6 cd								

[z] Value with different letters in each column indicate significant difference by *Duncan's Multiple Range Test, p<0.01.*
[y] No flower and fruit.

2. Fruit quality

Table 6 showed that fruit harvested from Purwakarta district was no significant difference between sectors in TSS. The percentage of TA was high in Sector 4 and low in Sector 5 about 1.09 and 0.86 %, respectively. The high percentage of fruit 'E' sizes used Codex Standard in Purwakarta district was found in Sector 6 followed in descending order by Sectors 1, 4, 7, 5 and 2. Whereas the fruit 'D' size was high in Sector 1, followed in descending order by Sectors 4, 7, and 5. The percentage of 'Extra' class was highest in Sector 3, followed in descending order by Sectors 6, 2, 5, 1, 7, and 4. On the other hand, the percentage of 'I' class was highest in Sector 8, followed in order by Sectors 4, 7, 1, and 5.

Table 6. TSS and TA content, and percentage of fruit classing and sizing used Codex Standard in 25-years-old mangosteen trees harvested in purple dark stage as affected by different sectors at Purwakarta district in 2003 (*on-year*)

Sector	TSS (°Brix)	TA (%)	% of fruit size used Codex Standard					% of fruit class	
			A [x]	B	C	D	E	Extra [w]	I [v]
1	17.1	0.95 ab [z]	13.7	38.6	20.4	20.5	6.8	29.4	70.6
2	17.2	0.96 ab	22.4	40.0	25.6	8.8	3.2	34.4	65.6
3	17.2	0.94 ab	13.6	50.1	27.3	9.0	-	49.9	50.1
4	17.2	1.09 a	9.3	38.4	25.8	20.2	6.3	22.7	77.3
5	17.5	0.86 b	19.9	27.1	34.6	14.0	4.4	29.4	70.6
6	17.8	0.91 ab	8.6	21.7	48.0	8.7	13.0	39.0	61.0
7	17.9	0.94 ab	15.0	45.0	20.0	15.0	5.0	25.0	75.0
8	17.8	0.94 ab	38.5	61.5	-	-	-	7.7	92.3
9 [y]									

[z] Value with different letters in each column indicate significant difference by *Duncan's Multiple Range Test, p<0.01*.
[y] No fruit.
[x] Size code (A = 30–50 g and 38–45 mm, B = 51–75 g and 46–52 mm, C = 76–100 g and 53–58 mm, D = 101–125 g and 59–62 mm, and E = > 125 g and > 62 mm base on fruit weigh and diameter, respectively).
[w] Superior quality, fruit free from defects.
[v] Good quality, the total fruit slight defects in shape, bruising on the peel and calyx, scratches or other mechanical damage not exceed 10 %.

Mangosteen phenology

Mangosteen tree produced new vegetative shoots in February, and then fell into dormancy from March 18th to April 19th. Flowering occurred in August after the drought condition (Table 7). Fruit was growth around 13 weeks and harvested during December 2003 to January 2004.

Table 7. The phenological development of mangosteen trees in 2003

Phenology	Start	Finish	Duration (days)
Shoot growth	Feb 14th	Feb 28th	15
Dormancy	Mar 18th	Apr 19th	32
Dry condition	July 1st	July 31st	31
Flowering	July 30th	Aug 15th	16
Fruit harvest	Dec 7th	Jan 20th	44

1.3 Effect of Sector (Position in the Canopy) and Tree Age on Productivity and Fruit Quality in Mangosteen

1.3.1 Materials and Methods

Plant materials

Mangosteen trees of 3 different ages (young, 20-years-old; middle, 35-years-old; and old, 50-years-old; n = 5 per age) growing in a commercial orchard in Bogor district, Indonesia, were studied in May 2010 (*off-year*). The canopy of each tree was divided into 9 sectors, based on tree height (bottom, middle, and top) and width (inner, center, and outer) (refer to Fig. 2).

Productivity and fruit quality

The number of fruits produced in each sector was counted in August and September, 2010. One fruit was harvested from each sector in mid-October at the mature green stage (i.e., yellowish white with to light green skin color), which is the stage that is usually harvested (Palapol *et al.*, 2009). The percentage of the entire fruit surface that was covered with scars was calculated, and fruit size and weight were recorded. Each fruit was separated into pericarp, aril, and seed, and the number of fleshy segments was counted. After the fresh aril had been weighed, it was dried in an oven at 70 °C for 4 days, following which its dry weight was measured, then ground into a powder using a mill.

Statistical analysis

A two-way analysis of variance (ANOVA) was performed to test whether there were significant differences in productivity and/or fruit quality among sectors (positions in the canopy) and trees at different ages. Means were compared using Duncan's

Multiple Range Test. All statistical tests were carried out using SPSS version 16.0 (IBM SPSS Inc., Chicago) and differences with *p*-values less than 0.05 were considered significant.

1.3.2 Results

1. Productivity

The number of fruits produced was the highest in Sector 1, followed, in order, by Sectors 5, 4, and 2; very few fruits were produced in Sectors 7, 6, 3, and 8, and no fruits were produced in Sector 9 (Table 8). Fruits harvested from Sectors 5 and 4 were significantly longer than those harvested from Sector 3, and were also wider and had greater fresh weight of the whole fruit and the aril than fruits from other sectors, although these differences were not significant. There was no significant difference among sectors in the percentage of edible portion of the fruit (aril) or the number of fleshy segments. Fruits harvested from the outer position of canopy such as Sectors 3, 6, and 9, had a higher percentage of scars than those fruit from the inner one such as Sectors 1, 4, and 7, but this difference was not significant. The different size and fruit surface was harvested from different sector and tree age as shown in Fig. 4.

The number of fruits per tree significantly increased with tree age, from an average of 11.6 fruits in young trees to 39.0 fruits in middle-aged trees and 152.4 fruits in old trees. Fruit size and fresh weight of the whole fruit and aril were also significantly larger in middle-aged and old trees than in young trees. There was no significant difference among trees of different ages in the percentage of edible portion of the fruit (aril), the number of fleshy segments, or the percentage of scars.

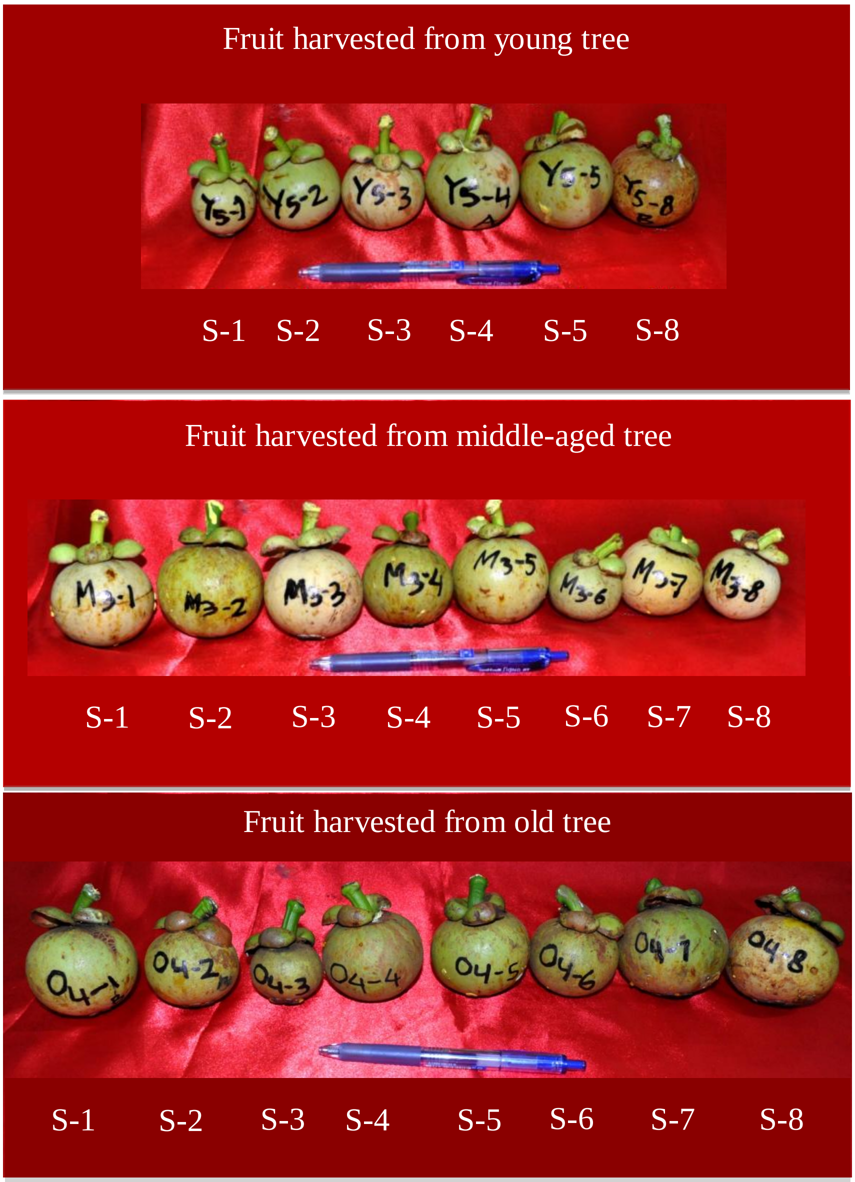

Fig. 4. Size of fruit harvested from different sectors of young, middle-aged, and old trees.

There was a strong linear relationship between fruit size and fresh weight of the whole fruit, with high coefficients of determination (R^2 = 0.940 for width, and R^2 = 0.912 for length) (Fig. 5). There was also a positive correlation between both fresh and dry weight of the aril and fresh weight of the whole fruit, although the coefficients of determination were not as high (Fig. 5).

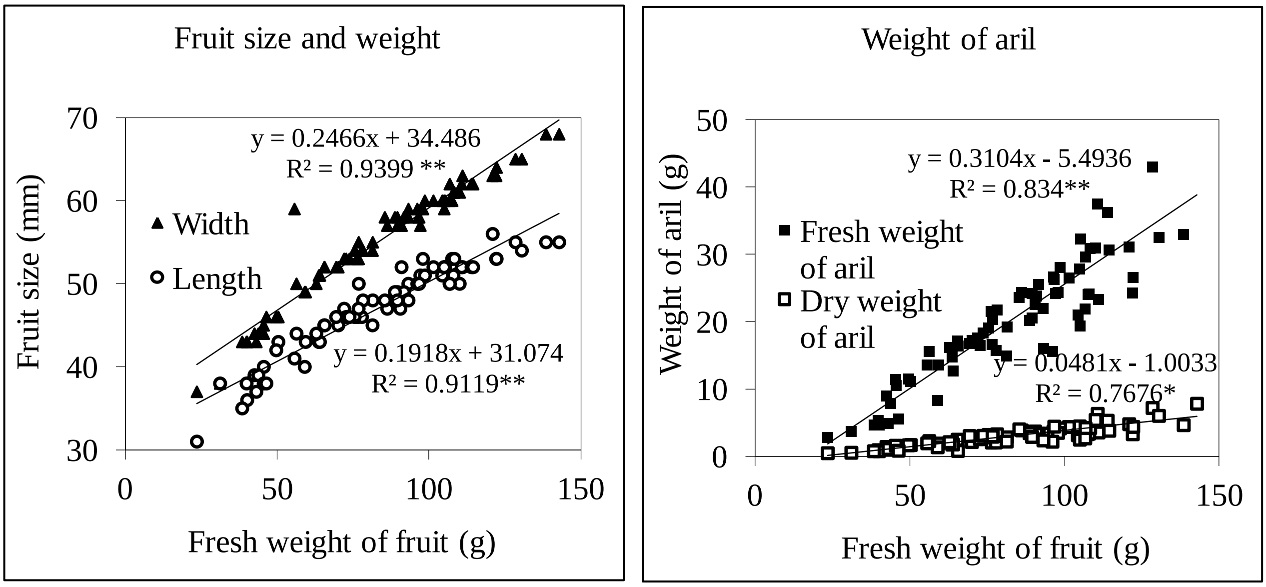

Fig. 5. Correlation between fresh weight of the fruit and fruit size (left) and weight of the aril (right).

Note: *,** indicate significant difference by *Duncan's Multiple Range Test*, at $p<0.05$ and $p<0.01$, respectively.

2. Fruit quality

Fruits harvested from Sectors 5 and 4 were significantly longer than those obtained from Sector 3, and were also wider and had greater fresh weight of the whole fruit and the aril than fruits from other sectors, although these differences were not significant (Table 8).

There was no significant difference among sectors in the percentage of edible portion of the fruit (aril) or the number of fleshy segments. Fruits harvested from the outer position of canopy, such as Sectors 3, 6, and 9, had a higher percentage of scars than those harvested from the inner one such as Sectors 1, 4, and 7, but this difference was not significant.

Fruit size and fresh weight of the whole fruit and aril were also significantly larger in middle-aged and old trees than in young trees. There was no significant difference among trees at different ages in the percentage of edible portion of the fruit (aril), the number of fleshy segments, or the percentage of scars. Productivity (number of fruit per m^3) is 0.24 fruit/m^3 in young tree canopy; 0.58 fruit/m^3 in middle-aged; and 1.51 fruit/m^3 in old trees (canopy size = 7.1 × 6.8 m in young; 7.3 × 9.2 m in middle-aged; and 9.1 × 6.8 m in old trees in tall and width, respectively).

Table 8. Number, size, and weight of mangosteen fruits as affected by different sectors and tree ages at Bogor district in 2010 (*off-year*)

Sector / tree age	Number of fruits per sector / tree	Fruit size (mm)		Fresh weight (g)		% of edible portion	Number of fleshy segments	% of skin with scars [z]
		Width	Length	Fruit	Aril			
Sector								
1	23.2 a [y]	54.4	47.5 ab	81.8	20.6	25.2	5.6	10.0
2	16.0 ab	54.0	46.3 ab	79.4	19.0	23.9	5.9	20.4
3	2.2 b	51.2	43.9 b	68.2	15.2	22.3	6.1	22.0
4	19.6 ab	56.8	48.9 a	89.8	22.5	25.0	5.8	19.1
5	21.3 a	57.0	49.2 a	92.9	23.1	24.8	5.9	18.0
6	2.8 b	56.3	47.0 ab	90.5	21.4	23.6	6.0	30.0
7	4.4 b	55.6	45.5 ab	80.3	18.8	23.4	5.9	21.3
8	1.7 b	55.8	47.2 ab	87.2	23.1	26.5	5.8	30.8
9 [x]								
Tree age								
Young	11.6 b	51.1 b	43.9 b	64.9 b	15.7 b	24.2	5.9	18.8
Middle	39.0 ab	57.0 a	48.8 a	91.3 a	21.9 a	23.9	5.8	18.0
Old	152.4 a	55.4 a	47.2 a	86.0 a	21.4 a	24.9	5.8	21.5

[z] Mangosteen fruits affected by thrips typically show silvering of the skin, and pale yellow to brown discoloration that may cover the entire fruit surface.
[y] Value with different letters in each column indicated significant difference by *Duncan's Multiple Range Test, $p<0.05$.*
[x] No fruit.

1.4 Discussion

Productivity

The first research showed the productivity and fruit quality of mangosteen was significantly different among sectors and locations. Regardless of location, the number of vegetative shoots, flower and fruit was significantly different among sectors. In both Bogor and Purwakarta orchard, the number of vegetative shoots was high in Sector 6, followed in descending order by top position of canopy such as Sectors 7 to 9 than in bottom one such as Sectors 1 to 3. During flowering, it is suggested that the number of shoots reduced by 35-50 % (Sdoodee and Phonrong, 2006). The strong flush vegetative growth seen on the outer position of canopy, particularly in young trees, suggests that fruit set and vigorous shoot growths are competing for nutrients. Salakpetch (2005) reported that the branches at the top position of canopy should be removed to improve the light penetration and interception within the canopy and to encourage development of lateral branches inside the canopy.

In this research, the number of flower and fruit set in Bogor orchard was highest in Sector 4, although in Purwakarta orchard, not only Sector 4 but also in Sectors 5 and 3. This indicated many flowers and/or fruits are borne in middle and bottom position of canopy, and from inner and central position of canopy. Regardless of the sector, the number of vegetative shoots, flower, fruit, and percentage of fruit set in Purwakarta orchard was higher than Bogor orchard. In first research, no flower or fruit was produced in Sectors 7 and 8 in Bogor orchard, whereas in Purwakarta orchard only in Sector 9. Reasons for the different results between two locations of research may caused by meteorological conditions. Boonklong *et al.* (2006) previously reported that mangosteen productions in the eastern region of Thailand increased when drought period occurred before flowering which increased the number of flowers because as the number of rainy days decreased. Sdoodee and Phorong (2006) had reported that high crop load was correlated with tree dry matter production, and an increase of crop load decreased vegetative and root growth. In Thailand, optimizing crop load or number of fruits of the tree, is important for enhancing fruit quality, because >70 g fruit is an acceptable size for export leading to maximize returns.

Mangosteen is usually characterized by a lack of profuse flowering and irregular fruiting during the early stage of tree maturity. In second research, the number of fruits produced was found to vary greatly among sectors, being higher in Sectors 1, 5, 4, and 2, and lower in Sectors 3, 6, 7, and 8. The number of fruits produced also significantly increased with age of tree. Sdoodee *et al.* (2008) reported that mature mangosteen trees produced good yields of large fruits at a moderate crop load of approximately 1001–1500 fruits per tree, and Nakasone and Paull (1998) found that old trees usually produced 3000 fruits per tree, and that production decreased with age. Therefore, our results may suggest that 50 years is not considered \`old\` for mangosteen trees.

Besides, it was remarkable that the fruits harvested in 2010 were of very poor quality. The results presented here suggest that mangosteen production is associated with the drought period. In this research, the drought period in 2010 was such short that the small number of flower occurred because not enough to break of dormancy bud. Similarly, Boonklong *et al*. (2006) reported that mangosteen productions could be higher in a year that has a longer drought period. Mangosteen production in the southern and eastern regions of Thailand increased with drought period and concurrently increased the number of flower.

Fruit quality

Our result showed mangosteen fruit quality varied largely among sectors, tree age, and locations. However fruit over 70 g is an acceptable size for export. There was a strong correlation between fruit size and weight. The same way, high flesh weight fruit was followed by high fresh weight in pericarp and aril. The seed was only small portion in mangosteen fruits, with fresh weight of seed less than 1.5 g. The percentage of edible portions in Bogor orchard was range from 36.4 to 48.8 %. The percentage of scars in

Bogor orchard was significantly different among sectors, the highest in Sector 9, followed by Sector 6. Regardless of sector, TSS content in fruit harvested from Purwakarta was higher than Bogor orchard, whereas TA content was high in fruit harvested from Bogor orchard. TSS content in fruit harvested from Purwakarta ranged from 17.1 to 17.8 °Brix, whereas TSS content in fruit from Bogor ranged from 14.0 to 16.3 °Brix. TA content in fruit harvested from Purwakarta ranged from 0.86 to 1.09 %, whereas from Bogor ranged from 0.97 to 1.15 %.

Reference to fruit quality using Codex Standard, the fruit code ‘E’ size was determined to be >125 g in weight and >62 mm in size. Regardless of the sector, the percentage of fruit ‘E’ size was less than 8 % and 13 % in fruit harvested from Bogor and Purwakarta orchard, respectively. The fruit ‘E’ size in Purwakarta orchard was found in Sectors 6, 1, 4, 7, 5 and 2, whereas in Bogor orchard only in Sectors 5, 4, and 1. However, an acceptable size for export was over than 70 g, this means fruit with class ‘A’ and ‘B’ are not acceptable for export. Regardless of sector, around 26.3 to 66.7 % and from 30.3 to 63.7 % of class ‘A’ and ‘B’ fruit harvested from Bogor and Purwakarta orchard, respectively, was rejected. Fruit from Purwakarta with ‘Extra’ class was less than 50 % in spite of the sector, whereas from Bogor, fruit with ‘Extra’ class was found only in Sectors 1 to 5. Similarly, previous reports by Setiawan and Poerwanto (2008) and Setiawan *et al.* (2006), suggested that fruits harvested from bottom and middle position of canopy, and from inner and central position were of good quality. Few of fruit of ‘Extra’ class was rejected due to problems caused by calyx and pedicel not intact, scars in surface of skin, etc.

The presence of scars, a skin disorder caused by thrips, strikingly reduces the market value of mangosteen fruits (Affandi and Emilda, 2010). In the second research, the percentage cover of scars on the skin surface was higher in fruits harvested from the outer position of canopy such as Sectors 3, 6, and 9. Similarly, Yaacob and Subhadrabandhu (1995) reported that good quality fruits are borne on branches close to the trunk. We have previously found that the light intensity was the highest in Sector 9, followed by Sectors 8, 6, and 3 on both cloudy and sunny days (Setiawan *et al.*, 2012). Therefore, this may suggest that scar development is promoted by an increase in light intensity. Similarly, Affandi and Emilda (2010) reported that the scars developed from broken tissue of the fruit shell, probably as a result of the high-heat intensity from sunlight, which directly hits the shell surface during fruit growth. Further investigation is needed to determine the factors that cause scarring in mangosteen fruits and the underlying mechanism of scar development.

1.5 Summary

Mangosteen productivity was investigated among sectors (position in the canopy) and location of orchard in first research, and compared among sector and tree age in second research. The first research, comparing our results for the sector in both locations of orchard show the number of flower and fruit was highly produced in Sectors 1, 2, 4, and 5, whereas in top position of canopy such as Sectors 7 to 9 had few flower and fruit. On the other hand, new vegetative shoot was produced mainly at Sectors 6 to 9. Fruits harvested from the Sectors 1, 4, 7, 5 and 2 were of good quality. Good quality fruit with ‘E’ size was less than 15 %, and fruit rejected was around 26.3 to 66.7 % and from 30.3 to 63.7 % fruit harvested from Bogor and Purwakarta orchard, respectively.

The second research, the number of fruits in younger trees was few, whereas in older trees produced a larger number of fruits. Younger trees were dominated by branches with flushes of new vegetative shoots, whereas in older trees branches had mature shoots mostly. The number of fruits was largest in Sector 1, followed in order by Sectors 5, 4, and 2, whereas in Sectors 8, 3, 6, and 7 it was very few, and there was no fruit in Sector 9. The position branches on Sectors 1, 2, 4, and 5 was close to the trunk. Sector 9 was produced vegetative shoots, also in Sectors 6 and 8. The results demonstrated clearly the production of mangosteen fruits was increases with tree age. The results also suggest that this fruit production vary largely among sectors. The largely number of fruits was produced on branches close to the trunk.

Chapter 2

Meteorological Conditions of Mangosteen Orchard in West Java, Indonesia

2.1 Introduction

Mangosteen is adapted to a well distributed of 1200 mm annual rainfall (Osman and Milan, 2006; Yaacob and Tindall, 1995). Boonklong *et al.* (2006) reported that the excessive rainfall causes major problems in flowering, pests, diseases and fruit quality. Trees tend to bear in alternate years, and bearing varies from tree to tree. Nakasone and Paull (1998) previously reported that mangosteen trees tended to flower after vegetative growth flushes and especially after dry condition. Also they reported that mangosteen growth is slow below 20 °C and the trees are die at 3 to 5 °C; the upper temperature limit is 38 to 40 °C with leaves and fruit being susceptible to sunburn. The critical agro-meteorological variables associated with agricultural production are rainfall, air temperature and drought period (Boonklong *et al.*, 2006). In general, tropical-fruit production is normally limited by the available soil moisture and many fruit trees, such as mango and litchi, require a dry period to stop vegetative growth and induce flowering (Nakasone and Paull, 1998; Sdoodee, 2007). The duration of the dry period for mangosteen is approximately 15 to 30 days (Nakasone and Paull, 1998; Salakpetch, 2000).

The production seasons for mangosteen among locations show some distinct different effect of several climatic regimes (Chapter 1). Nakasone and Paull (1998) reported that Malaysia, Indonesia, Philippines, Thailand, and Vietnam generally have similar production seasons with fruits present from May to January. In the South-East Asia region, in September and October, mangosteen may not be available for the market because this is the end of the seasons of Thailand, Malaysia, and Indonesia. The Philippine crop occurs at a time when Thailand, Malaysia, and Indonesia production is low since its production season is later. Thus, during this period, there is a window of opportunity for the Philippine mangosteen fruits to become available in August to November (Osman and Milan, 2006).

Inconsistency in the flowering and fruiting in mangosteen is a common problem; its production always remains unstable from the immature to the mature tree stage, when the production can vary from tree to tree and from one season to another (Malip and Masri, 2006). Assimilate accumulation and partitioning among sinks in mangosteen trees depends on seasonal growth patterns of vegetative and reproductive sinks (Weibel *et al.,* 1993).

Several environmental factors vary with the depth in a leaf canopy, but the most important with respect to photosynthesis is light, in both quantity and quality (Araujo *et al.*, 2008). Feng-li *et al.* (2008) reported that light interception of tree canopy is crucial for the external and internal fruit quality, such as fruit size, color, TSS, and TA content. However, previously we reported that the distribution of light intensity in mangosteen trees was different among position canopy. This research was done to understand the effect of meteorological conditions in orchard to productivity and fruit quality in mangosteen. In this chapter, I describe the meteorological conditions such as light intensity, rainfall, temperature, and relative humidity for commercial mangosteen orchards as affected by sectors and tree ages.

2.2 Materials and Methods

Plant materials

Mangosteen trees of 3 different ages (young, 20 years old; middle, 35 years old; and old, 50 years old; $n = 5$ per age) growing in a commercial orchard in Bogor, Indonesia, were studied in May 2010. The canopy of each tree was divided into 9 sectors, based on tree height (bottom, middle, and top) and width (inner, center, and outer) (refer to Fig. 2 in Chapter 1).

Meteorological conditions of mangosteen orchard

Light intensity in mangosteen orchards was measured by light meter (LI-250A, LI-COR) on sunny and cloudy days in each sector in August of 2010. The monthly rainfall data from 2000 to 2010 was collected from Bogor Meteorological Station. Data on monthly temperature (maximum and minimum) and relative humidity (RH; %, observed in morning = 7.00 a.m., noon = 1.00 p.m., and evening = 6.00 p.m.) also was collected.

Statistical analysis

A one-way analysis of variance (ANOVA) was performed to test whether there were significant differences in meteorological conditions among sectors (positions in the canopy) and trees of different ages. Means were compared using Duncan's Multiple Range Test. All statistical tests were carried out using SPSS version 16.0 (IBM SPSS Inc., Chicago) and differences with p-values less than 0.05 were considered significant.

2.3 Results

a) Light intensity

Light intensity in mangosteen orchards increased rapidly with respect to time of day and reached a maximum at 10:30 a.m., then gradually decreased (Fig. 6). Light intensity was significantly different among sectors, but no significant difference was observed among tree ages (Fig. 7). As shown in Fig. 7, the highest light intensity on cloudy days was observed in Sector 9, resulting in 300 μmol•m^{-2}•s^{-1} for all tree ages, followed by Sectors 8, 6, and 3, whereas in Sector 1, it was the lowest ranging from 200 to 300 μmol•m^{-2}•s^{-1} (Fig. 7).

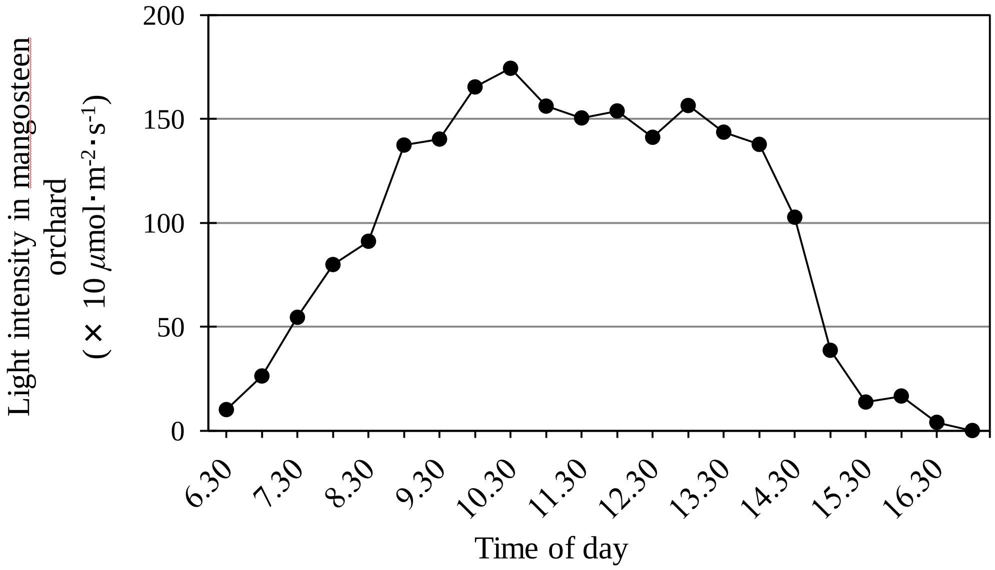

Fig. 6. Diurnal changes of light intensity during a sunny day in the mangosteen orchard.

Regardless of tree age, the highest light intensity on sunny days was found in Sector 9, and it was 800.7, 750.3, and 770.4 μmol•m^{-2}•s^{-1} for young, middle-aged, and old trees, respectively (Fig. 7). In contrast, the lowest was found in Sector 1, and it was 50.3, 40.2, and 40.9 μmol•m^{-2}•s^{-1} for young, middle-aged, and old trees, respectively. Light intensity in outer position of canopy such as in Sectors 3, 6, and 9 was higher than in inner one such as Sectors 1, 4, and 7. Light intensity was also higher in top position of canopy than in bottom one.

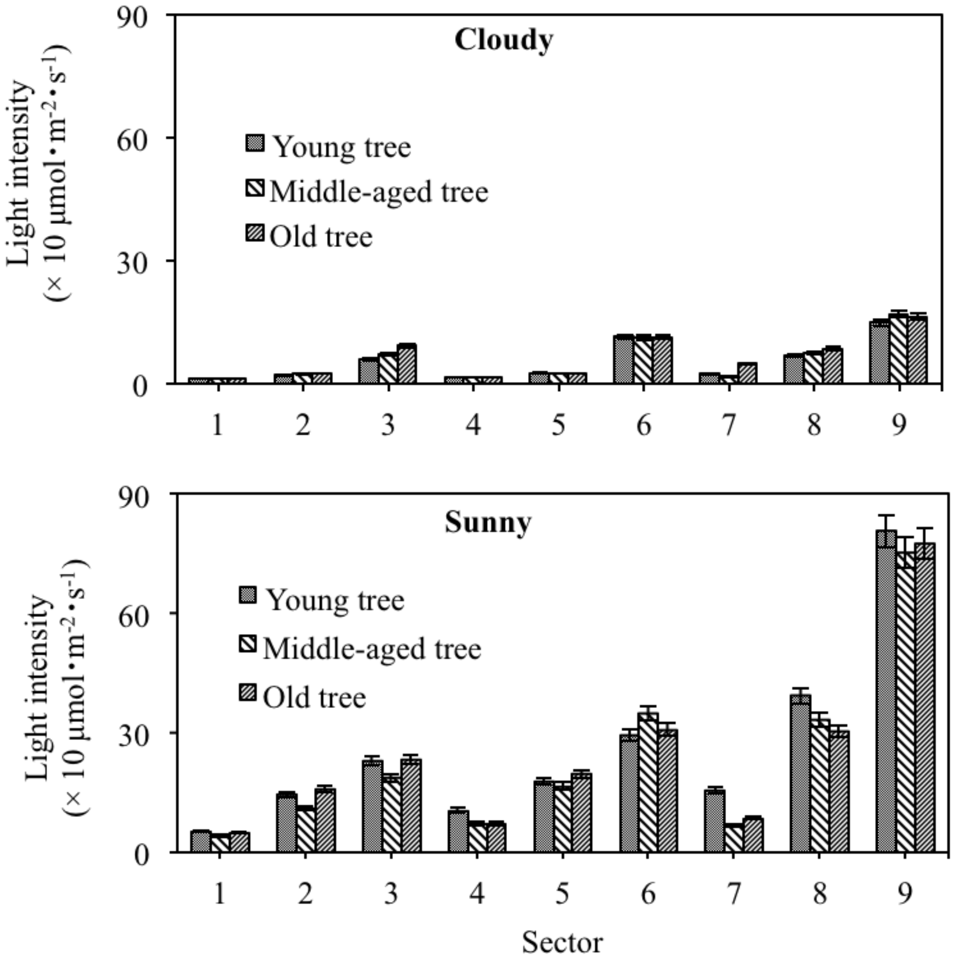

Fig. 7. Light intensity of cloudy and sunny days in mangosteen orchards as affected by different sectors and tree ages.
Light intensity was measured in August 2010. Vertical bars indicate ± SE (n=5).

b) Rainfall

Indonesia has a tropical monsoon climate. The climate shows a distinct seasonal pattern of rainfall. Bogor area has the high rainfall average above 300 mm per month. The pattern of rainfall in Bogor area is almost same every year with the heavy rainfall period was occurred between October and December, then rainfall gradually decrease at the end of December until June (Fig. 8). Drying period occurred from July to August (case 2003 and 2009), followed by the flowering period from September to October. In

2010, however, the phenological pattern of mangosteen trees (Table 9) was changed markedly owing to irregular rainfall (Fig. 8). Climate in 2010 was anomalous, and a drought occurred in April inducing flowering of mangosteen in May. The pattern of monthly rainfall and dry season in 2010 was change and made effect on mangosteen flowering. There was an increasing trend in yearly rainfall during 2006 to 2010 (Fig. 8). The annual rainfall was 2808, 3240, 4014, and 4944 mm during from 2007 to 2010, respectively.

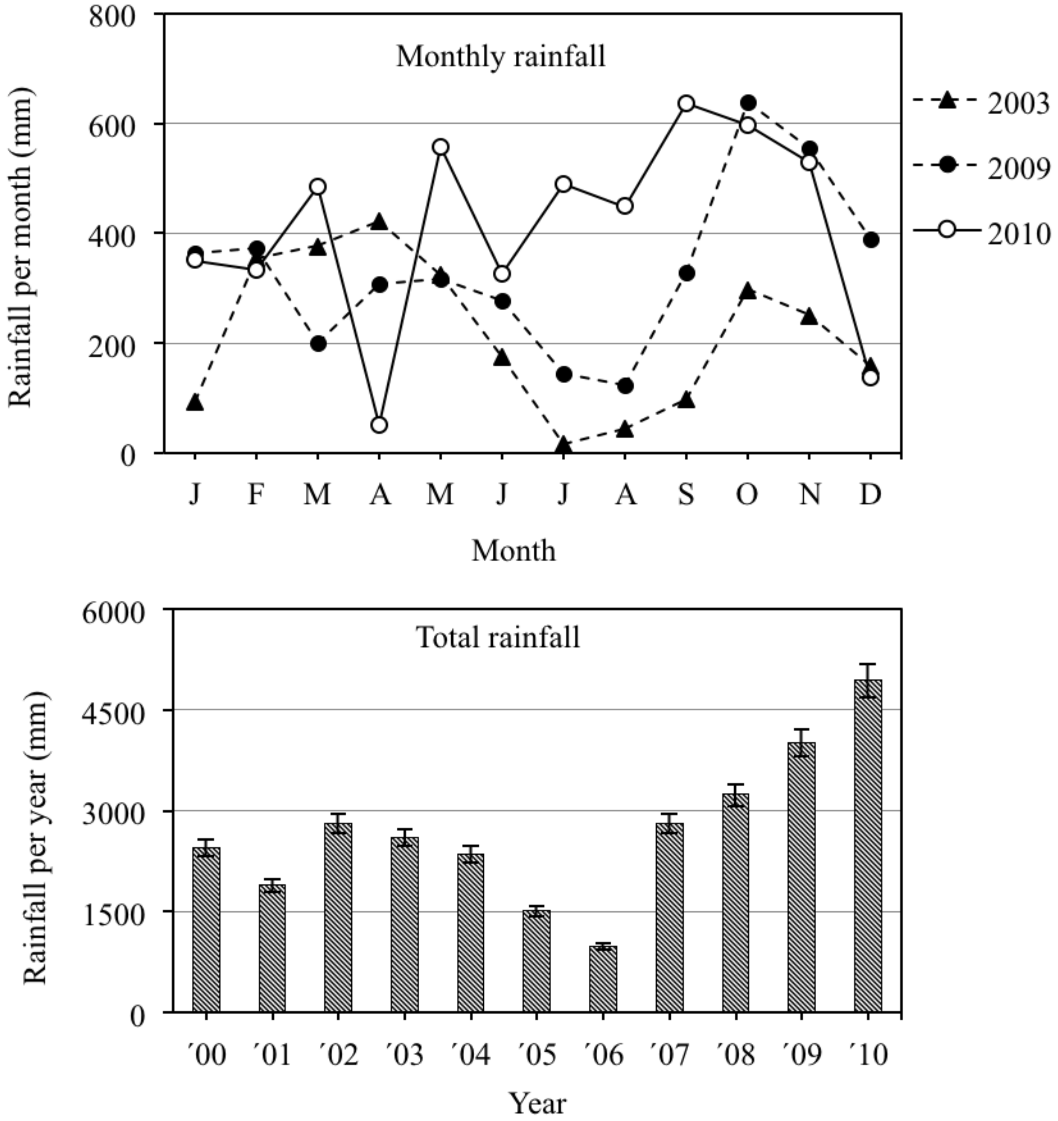

Fig. 8. Seasonal changes of rainfall per month in 2003, 2009, and 2010, and total rainfall per year in mangosteen orchards.

c) Temperature and relative humidity

Average daily maximum and minimum temperatures were ranged from 29.6 to 32.8 °C and from 21.2 to 22.7 °C, respectively (Fig. 9). Maximum temperature was high in June to September. Bogor district is a tropical temperature type remains relatively constant throughout the year and seasonal variations are dominated by precipitation.

Relative humidity (RH) in Bogor was highest in morning then gradually decreased at noon and increased at evening. The average of RH at mangosteen orchards in Bogor was ± 90 %, ± 65 %, and ± 80 % in morning, noon, and evening, respectively (Fig. 9). Both in morning and evening the average of RH was almost stable every month, except in January was highest and during August to September was lowest. Whereas RH in noon was highest in January then decreased from February to December. Regardless of time, RH in August and September was lowest compared another month.

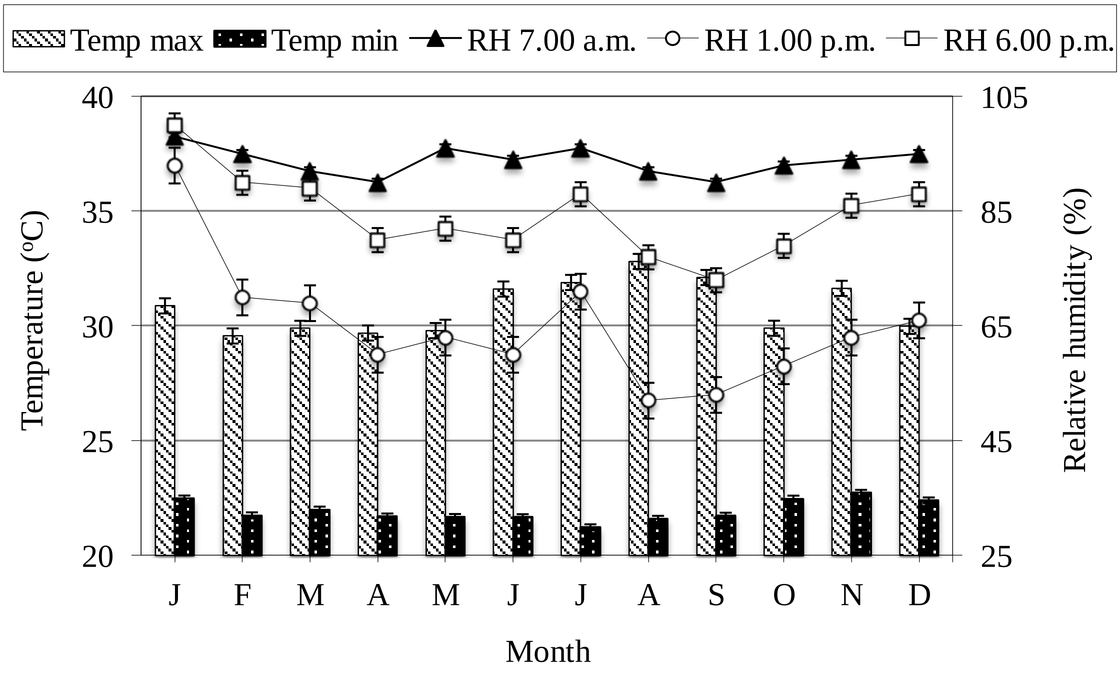

Fig. 9. The seasonal changes of maximum and minimum air temperature and relative humidity.
RH (%) was recorded at 7.00 a.m., 1.00 p.m., and 6.00 p.m. in the mangosteen orchard.

2.4 Discussion

In this research, light intensity in outer position of canopy such as in Sectors 3, 6, and 9 was higher than in inner one such as Sectors 1, 4, and 7. However, the high light penetration was correlated with new vegetative shoots. Table 2 and 5 in Chapter 1 showed that in Sectors 6 to 9 produced more new vegetative shoots compared other sectors. Issarakraisila and Settapakdee (2008) reported that an increase of light intensity from 25 % of full sunlight to higher light intensity levels increased the thickness of lamina and stomata frequency. Maximum growth was found when tree exposed to light intensity condition of 40 %. In Indonesia, shade is offered by other trees in the traditional mixed orchards and home gardens. The results presented here show light intensity was also higher in top position of canopy than in bottom one. We have previously reported that dense canopy structure in top position protect light availability in bottom and inner positions of canopy (Setiawan and Poerwanto, 2008; Setiawan *et al.*, 2012). Ramlan *et al.* (1992) reported that increasing light intensity results in increased photosynthesis until 700 μmol•m^{-2}•s^{-1} when light saturation is achieved. Higher dry weights were attained when plants were grown under 50 % shade (Goenaga and Rivera, 2005; Nakasone and Paull, 1998). Sdoodee and Phonrong (2006) reported that an excessive crop load of mangosteen caused adverse effect on fruit size, and this led to a marked decrease in yield of large-size fruit. The bagging of mangosteen fruits has been potential to improve fruit quality and added benefits on visual appearance (Ratanamarno *et al.*, 2005).

Throughout growth and development of fruit, water stress greatly affects the final yield, and mangosteen trees need a drying period to induce flowering. Salakpetch and Poonnachit (2006) previously reported that the irrigation strategies after attaining a water stress condition could stimulate flowering of mangosteen in Thailand. In 2003 and 2009, mangosteen bloom in West Java started in September after imposing dry conditions from July to August, and fruits are harvested from December to January. In 2010, however, the phenological pattern of mangosteen trees changed markedly owing to irregular rainfall. Climate in 2010 was anomalous, and a drought occurred in April following with flowering of mangosteen in May (Fig. 8). Apiratikorn *et al.* (2012) reported that the phenology in many fruit trees, including mangosteen, has been changed due to climatic variability. The phenological pattern changed because of irregular rainfall distribution in the mangosteen trees also was studied in Thailand during the period 2008 to 2010, and affected yield and fruit quality (Boonklong *et al.*, 2006; Sdoodee and Udom, 2002). Similarly, Sdoodee and Chiarawipa (2005) reported that the drought period in southern Thailand usually occurred from February to March, and a short dry period occurred during July to August. Our results coincide with other studies reporting that mangosteen trees need a dry period to induce flowering (Boonklong *et al.*, 2006; Nakasone and Paull, 1998; Sdoodee and Chiarawipa, 2005). A marked change in rainfall distribution affected the phenological change in flowering, productivity and fruit quality (Apiratikorn *et al.*, 2012). The results of the current study also indicated that fluctuations in rainfall and climatic variability cause uncertain flowering of mangosteen. This evidence indicated that Bogor had been under the influence of erratic rainfall and drought pattern. Fig. 8 shows that there has been an increase in rainfall trends in Bogor during 2006 to 2010 year. Fluctuation of annual rainfall was prominent and could cause shortened drought and intense flooding.

In this research, average daily temperatures ranged from 29.6 to 32.8 °C for maximum and from 21.2 to 22.7 °C for minimum temperature. The temperature was high during June to September, because summer season has drought condition and low rainy. Temperature remains relatively constant throughout the year. Osman and Milan (2006) reported that mangosteen thrived in the temperature range of 25 to 32 °C but growth become affected when the temperature falls below 25 °C. Similarly, Weibel *et al.* (1993) previously reported that the photosynthetic rate in mangosteen is steady in temperature range over a 27 to 35 °C and shade of between 20 to 50 %.

In this research, the average of relative humidity (RH) was low at noon then increased at evening. This may suggest high evaporation at noon because of high light intensity and temperature. Mangosteen orchard usually had rainfall at evening to night, it suggests increase in RH at evenings. Both in the morning and evening the average RH was almost stable every month, except in January which was highest and during August to September was lowest. Condition during January was characterized by a lot of rainy, in contrast during August which had drought condition and high temperature.

2.5 Summary

Light intensity as affected by sector (position in the canopy), tree age and meteorological condition such as rainfall, relative humidity and temperature was investigated. Light intensity in mangosteen orchards increased rapidly with respect to time of day and reached a maximum at 10:30 a.m., then gradually decreased. Light intensity was significantly different among sectors, but no significant difference was observed among tree ages. The highest light intensity on cloudy days was observed in Sector 9, followed by Sectors 8, 6, and 3, whereas in Sector 1, it was the lowest. Regardless of tree age, the highest light intensity on sunny days was found in Sector 9, and the lowest was found in Sector 1. Light intensity in outer position of canopy such as in Sectors 3, 6 and 9 was higher than in inner one such as Sectors 1, 4, and 7. Light intensity was also higher in top position of canopy than in bottom one. In 2003 and 2009, mangosteen trees in West Java regularly faced a drought period from July to August, followed by a flowering period from September to October. In 2010, however, the phenological pattern of mangosteen trees changed markedly owing to irregular rainfall. Climate in 2010 was anomalous, and a drought occurred in April inducing flowering of mangosteen in May. Daily temperatures ranged from 29.6 to 32.8 °C for maximum and from 21.2 to 22.7 °C for minimum temperature. Maximum temperature was high in June to September. Rainfall induced significant climate change, compared to light intensity, temperature, and relative humidity in relationships to the phenological pattern of mangosteen tree in 2010 and effect to fruit production.

Chapter 3

Seasonal Changes in Carbon and Nitrogen Contents and C-N Ratio of Mangosteen Leaves

3.1 Introduction

Specific information on the effects of fertilizers on mangosteen is lacking, and many existing recommendations are based on empirical trials and traditional practices. Leaf analysis has been a guide in diagnosing nutritional problems and a basis for fertilizer recommendation for mangosteen trees. Inconsistency in the flowering and fruiting in mangosteen is a common problem; its production always remains unstable from the immature to the mature tree stage, when the production can vary from tree to tree and from one season to another (Chapter 1; Malip and Masri, 2006). Excess fertilizer application is expensive and leads to N losses by leaching with negative impacts upon the environment. However, to provide an optimal final yield and the desired fruit quality, trees must be sufficiently be fertilized (Boussadia, *et al.*, 2010). These responses of photosynthesis may be related to changes in leaf N concentration, and it has been shown that N partitioning within leaves changes with growth irradiance in such a way that it almost maximizes photosynthesis (Araujo *et al.*, 2008).

Carbon and N metabolism are the two basic of metabolic processes in crop. They directly affect the formation and transformation of the photosynthetic product, mineral nutrition absorption, and protein synthesis. Hong-Biao *et al.* (2008) reported that the ratio of carbon and N affect source-to-sink ratio and yield in cotton boll. N is the most important element for higher plants, and plant productivity is to a large extent determined by N nutrition. N shortage results in a marked decrease in plant photosynthesis in many crops. This is to be expected, because more than half of the total N in leaf is allocated to the photosynthetic apparatus (Makino and Osmond, 1991). Cronje *et al.* (2011) reported that the leaves and fruit of citrus in the inside position of the dense canopy have higher levels of N, P, and K compared to the outside position.

The leaf chlorophyll concentration is often well correlated with leaf N status and photosynthetic rates (Evans, 1983). Jaroonchon *et al.* (2010) reported that a portable chlorophyll meter (SPAD) has been considered satisfactory for predicting leaf chlorophyll and N levels in pummelo. SPAD value are instantaneous and do not involve destructive sampling. These advantages provide a rapid, quantitative and inexpensive field estimation of N content in leave for proper N management. However, canopy microclimate not only impacts on differences in fruit internal quality, but also on differences in accumulation of mineral nutrients in leaves and fruit (Cronje *et al.*, 2011).

The relationship between chlorophyll meter readings and the N content of mangosteen leaves has not been reported. Very little information is known regarding the carbon (C) and nitrogen (N) contents in the leaves of the mangosteen trees. The productivity and fruit quality of mangosteen varied greatly among sectors and tree age (Chapter 1). This research was done to understand relationship between C-N ratio of mangosteen leaves with fruit quality and productivity. Accordingly, in this chapter, I describe the seasonal changes in carbon and nitrogen contents, C-N ratio, SPAD value, and chlorophyll content of leaves as affect by different sector (position in the canopy) and tree age. Moreover, three types of branches with different growth aspects namely branches with fruit, dormant bud, and vegetative shoots were investigated to compare the carbon and nitrogen contents, and C-N ratio among its types of branches.

3.2 Effect of Sector and Tree Age on Seasonal Changes in Carbon and Nitrogen Contents, C-N Ratio, SPAD Value, and Chlorophyll Content of Mangosteen Leaves

3.2.1 Materials and Methods

Plant materials

Mangosteen trees of 3 different ages (young, 20 years old; middle, 35 years old; and old, 50 years old; n = 5 per age) growing in a commercial orchard in Bogor, Indonesia, were studied in May 2010. The canopy of each tree was divided into 9 sectors, based on tree height (bottom, middle, and top) and width (inner, center, and outer) (refer to Fig. 2 in Chapter 1).

C-N ratio of leaves

Six leaves were collected from each sector at monthly intervals from May to September 2010. Leaf sample was taken from the mature leaf pair at the number two from shoot tip positions. Leaf samples were washed, dried in an oven, and ground into powder with a mill. Carbon and nitrogen contents of leaves were determined by CN analyzer (MT700MC, Yanaco, Tokyo).

Chlorophyll content of leaves

Six leaves of similar age and position were selected in each sector in August, and their SPAD value was determined by chlorophyll-meter (green meter) (SPAD–502, Konica Minolta). The leaves were collected, then chlorophyll was extracted by 85 % acetone and the content was determined by spectrophotometer (UV–VIS 1800, Shimadzu).

Statistical analysis

A two-way analysis of variance (ANOVA) was performed to test whether there were significant differences in C-N ratio among sectors (positions in the canopy) and trees of different ages. Means were compared using Duncan's Multiple Range Test. All statistical tests were carried out using SPSS version 16.0 (IBM SPSS Inc., Chicago) and differences with *p*-values less than 0.05 were considered significant.

3.2.2 Results

a) Carbon

Carbon content of leaves varied largely among sectors and tree ages. However, in young tree, high-carbon content was observed in top position of canopy compared to bottom and middle positions, also it was high in outer position than inner one in spite of the month (Fig. 10). In middle-age tree, carbon content in middle position of canopy was high during May to June, whereas from July to September was high in Sectors 7 and 3. Furthermore, in middle-aged tree, high-carbon content was observed from outer position of canopy than inner one especially in the bottom and middle height. On the other hand, in the old tree, the high-carbon content was found in bottom position of canopy than top one, and also in outer position of canopy than inner one (Fig. 10). The pattern of seasonal change carbon content in the middle-aged and old tree was similarly. Regardless of sector and tree age, the lowest carbon content was observed in May for young and old tree, but it was in June for middle-aged trees. Carbon content was increased in June for young and old tree, in July for middle-aged tree then gradually decreased until August, for all tree age.

b) Nitrogen

Regardless of the sector, the pattern of seasonal change of nitrogen content in the young and middle-aged tree was similarly in all month (Fig. 11). Both in young and middle-aged tree, nitrogen content was low in May followed in order by increased in June then gradually decreased in July, increased in August, and stable in September in spite of the sector. On the other hand, nitrogen content in the old tree was lowest in May, followed in order by increased from June to July after that gradually decreased in August and September. Regardless of the month and trees age measured, nitrogen content of leaves was higher in bottom position of canopy such as Sectors 1 to 3 than in middle and top ones such as Sectors 5 to 9. The value of nitrogen content was around from 1.3 to 1.6 % in young and middle-aged, and 1.2 to 1.6 % old trees.

c) C-N ratio

Regardless of tree age, the C-N ratio of leaves was higher in top position of canopy compared to bottom one. There was no significant difference in C-N ratio of leaves among tree ages; it was around from 32.6 to 41.4 % for young, from 32.2 to 39.6 % for middle-aged, and from 32.2 to 42.0 % for old trees. Regardless of the sector, the pattern of seasonal changes of C-N ratio in the young and middle-aged tree was similarly in all month (Fig. 12). In spite of the sector, both in young and middle-aged tree, C-N ratio was decreased from May to June then slightly increased in July followed in order by gradually decreased until September. On the other hand, C-N ratio in the old tree was highest in May followed in order by gradually decreased until July, then increased slightly in August.

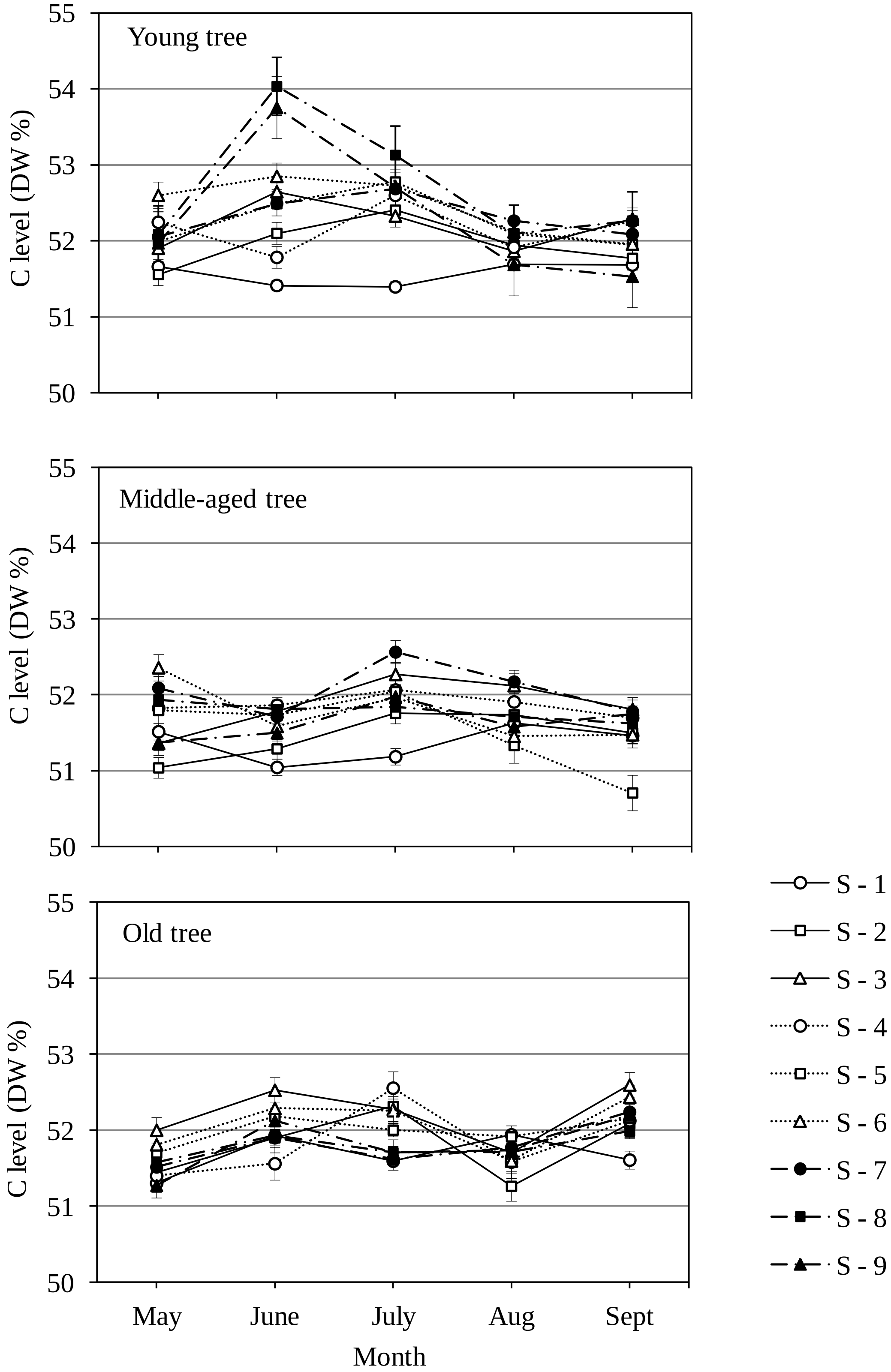

Fig. 10. Seasonal changes of carbon contents of mangosteen leaves in different sectors in young, middle-aged and old trees.
Vertical bars indicate ± SE (n=5).

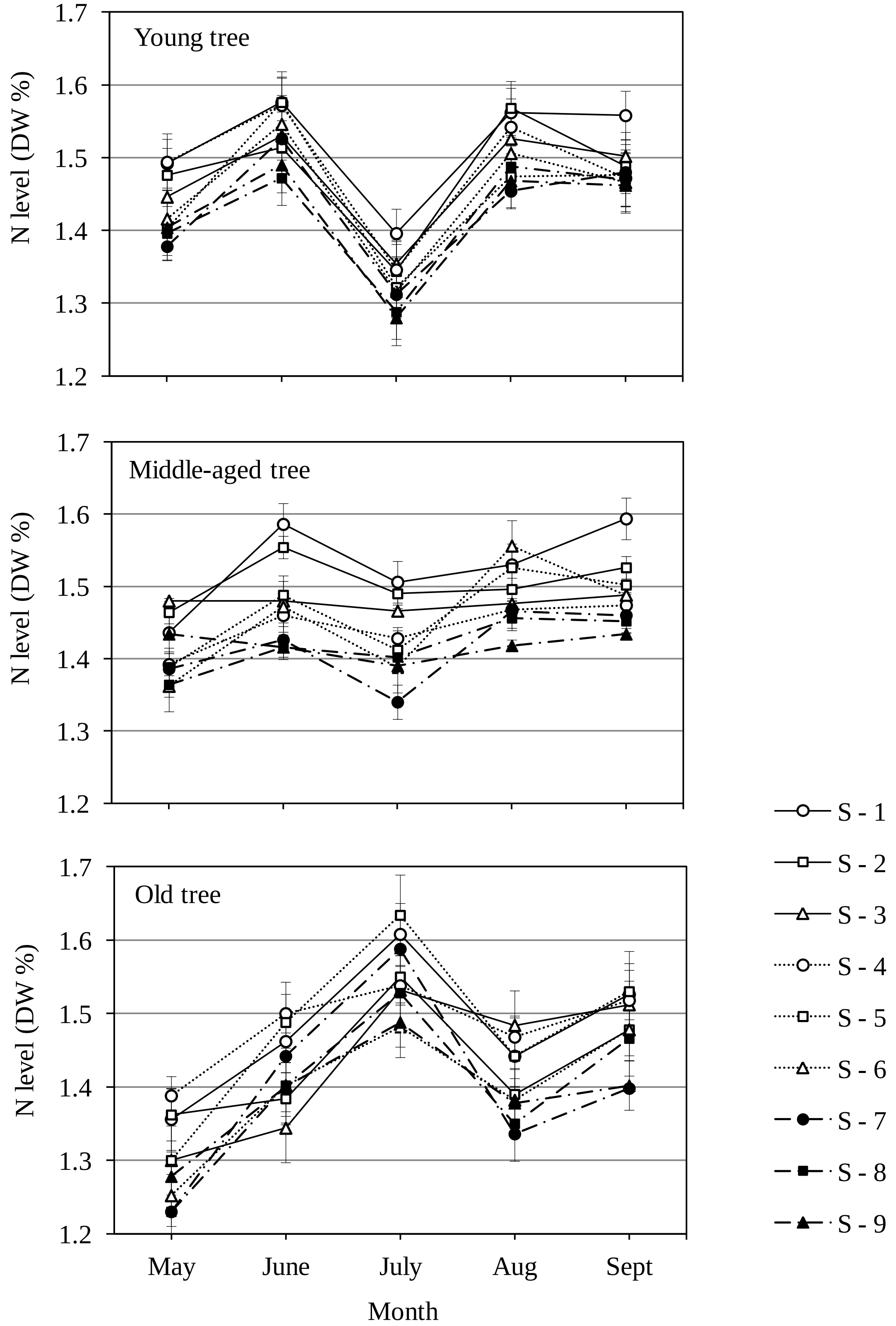

Fig. 11. Seasonal changes of nitrogen contents of mangosteen leaves in different sectors of young, middle-aged and old trees.
Vertical bars indicate ± SE (n=5).

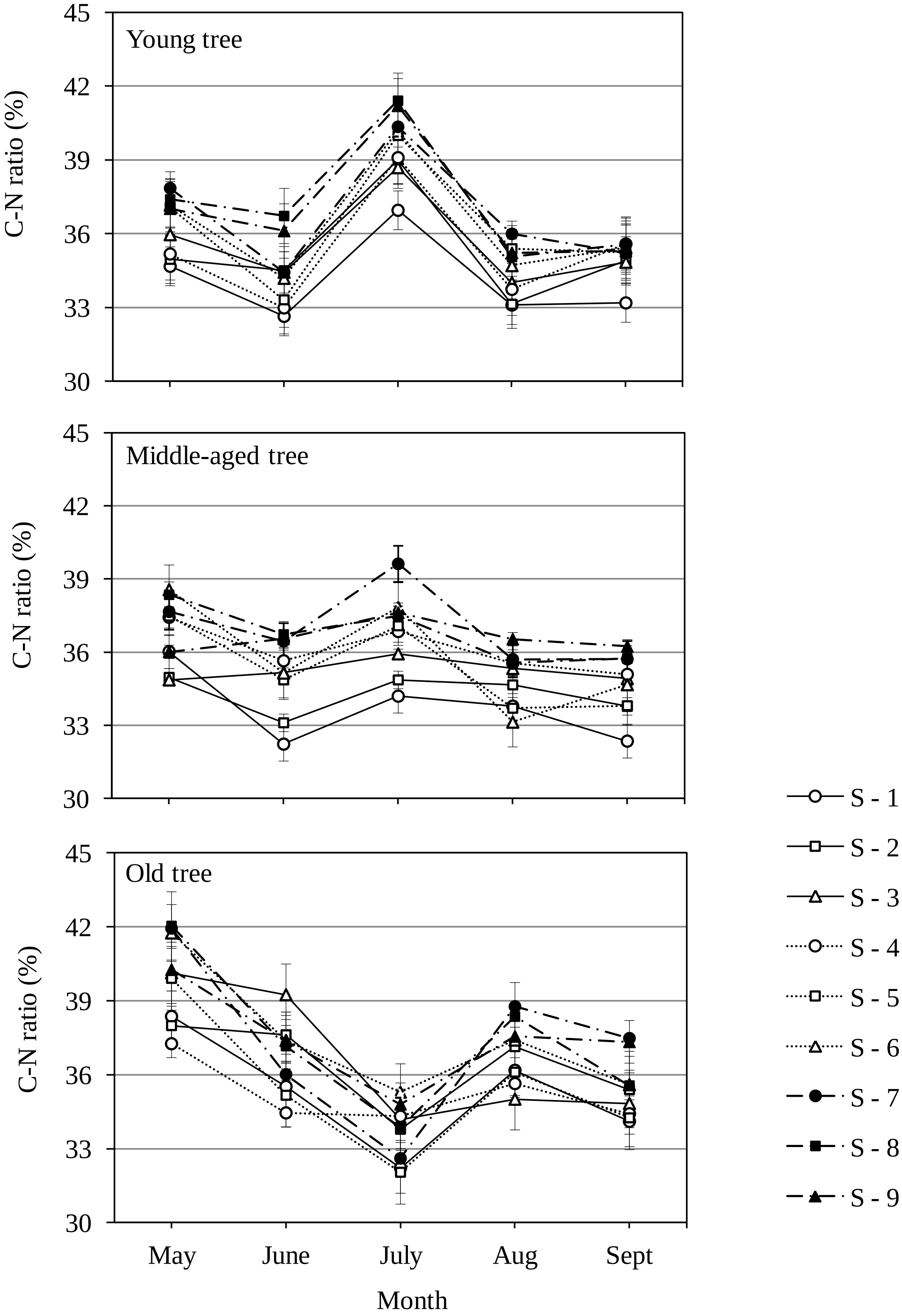

Fig. 12. Seasonal changes of C-N ratio of mangosteen leaves in different sectors in young, middle-aged and old tree.
Vertical bars indicate ± SE (n=5).

SPAD value and chlorophyll content of leaves

The content of chlorophyll a was significantly different among sectors and tree ages. Fig. 13 showed chlorophyll a of leaves was higher in top position of canopy such as Sectors 7 to 9 than in bottom one such as Sectors 1 to 3. On the other hand, content of chlorophyll b was not significantly different among sectors. Both chlorophyll a and b content of leaves was higher in young and middle-aged trees compared to old ones.

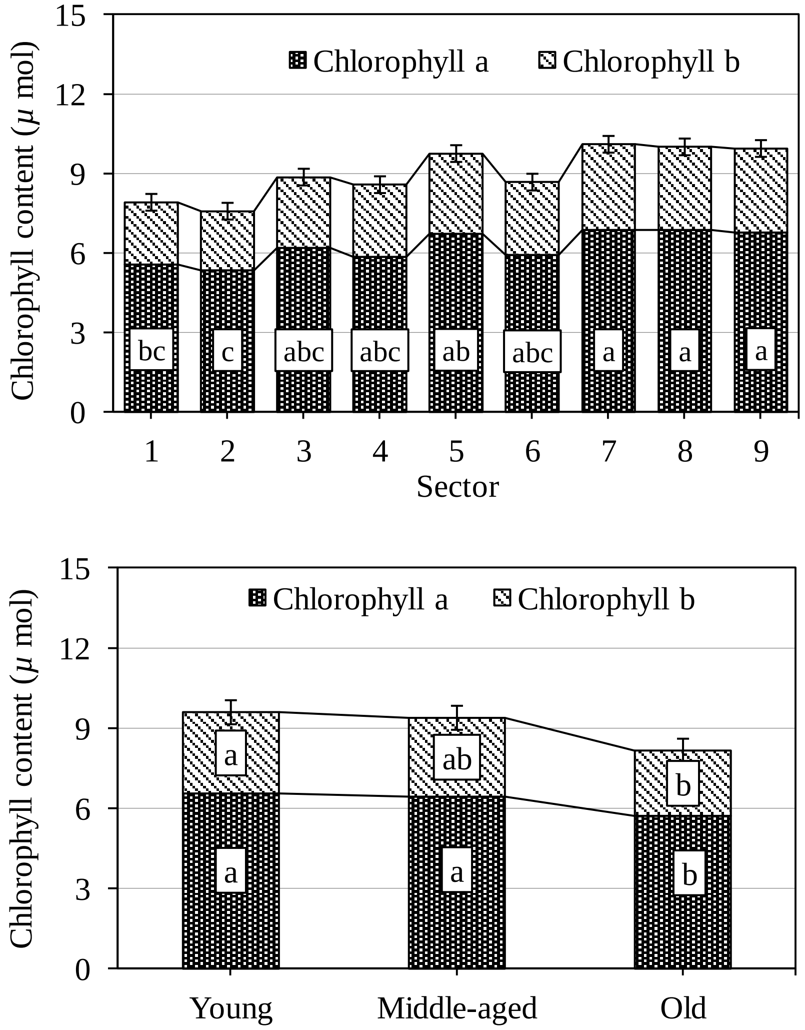

Fig. 13. Content of chlorophyll a and b in mangosteen leaves as affected by different sectors and tree ages.
Different letters indicate significant difference by *Duncan's Multiple Range Test, p<0.05.*

For all sectors, the SPAD (green meter) value of leaves was ranged from 67.5 to 79.1 (Fig. 14). SPAD value in both young and middle-aged trees was higher in inner position of canopy such as Sectors 1, 4, and 7 than in outer one such as Sectors 3, 6, and 9, while in old trees, it was higher in outer position of canopy than in inner one. In spite of tree age, SPAD value of leaves in middle and top positions of canopy such as Sectors 5 to 9 was high compared to bottom one such as in Sectors 1 to 3.

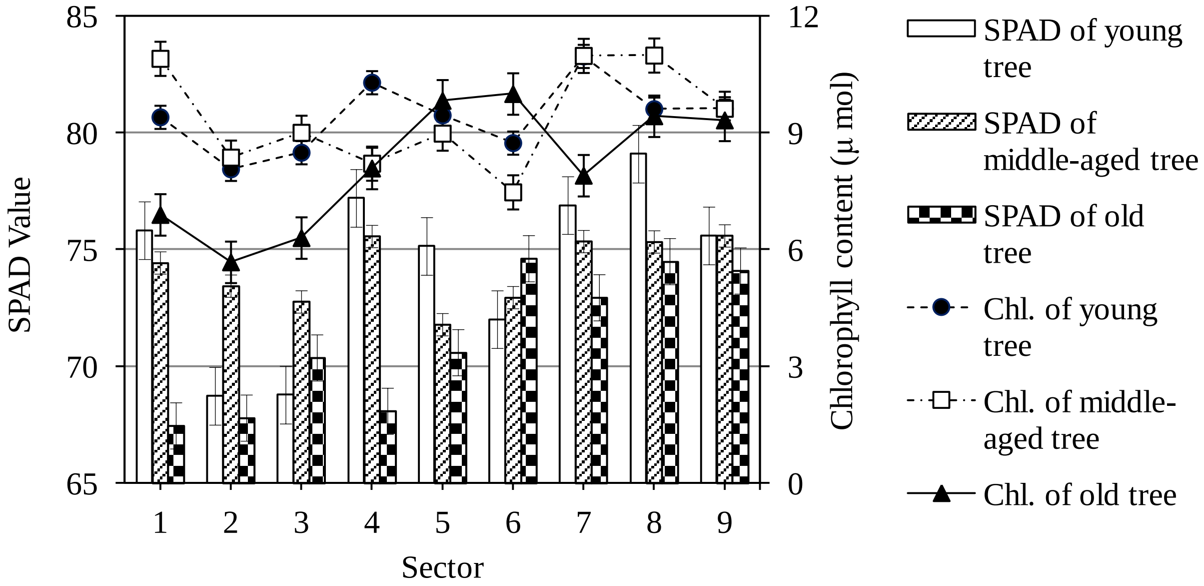

Fig. 14. SPAD value and chlorophyll content of mangosteen leaves as affected by different sectors and tree ages.
Vertical bars indicate ± SE (n=5).

Fig. 15. Change of leaf colours collected from young (tip, left) to old (based, right) on branch.

The SPAD value was significantly different among sectors and tree ages. The new leaves are light green colour and soon acquires the characteristic dark-green colour, and relatively thick and coriaceous (Fig. 15). In mangosteen, the SPAD value of leaf was high ranged from 60 to 85. Regardless of tree age, nitrogen content of leaf in top position of canopy such as Sectors 7 to 9 was low compared to bottom and middle ones, whereas SPAD value of leaf in top position of canopy was higher than bottom and middle ones (Fig. 16).

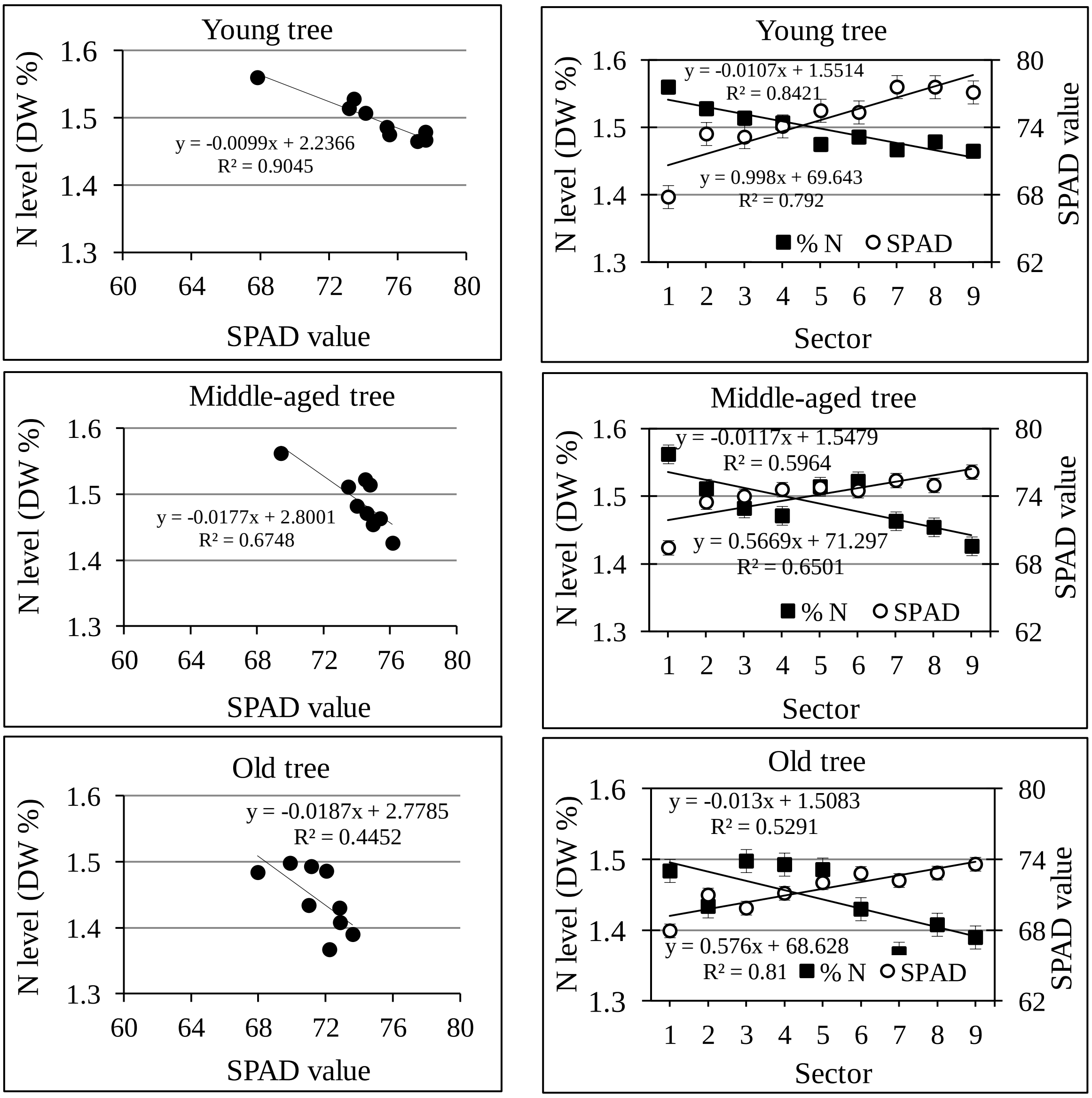

Fig. 16. Relationship between N level and SPAD value in mangosteen leaves, N level, and SPAD value per sector in young tree, middle-aged tree, and old tree.

3.3 Effect of Branch Types on Carbon and Nitrogen Contents, and C-N Ratio of Mangosteen Leaves

3.3.1 Materials and Methods

Plant materials

Mangosteen trees of 3 different ages (young, 20 years old; middle, 35 years old; and old, 50 years old; $n = 5$ per age) growing in a commercial orchard in Bogor, Indonesia, were studied in May 2010. The canopy of each tree was divided into 9 sectors, based on tree height (bottom, middle, and top) and width (inner, center, and outer) (refer to Fig. 2 in Chapter 1).

C-N ratio of leaves

Leaf sample was taken from the mature leaf pair at the number of two from shoot tip position. Six leaves were collected from three types of branches showing different growth aspects, A: branches with fruit, dormant bud, and vegetative shoots, B: branches with fruit and dormant bud, C: branches with dormant bud and vegetative shoots in August 2010 (Fig. 17). Leaf samples were washed, dried in an oven, and ground into powder with a mill. Carbon and nitrogen contents of leaves were determined by CN analyzer in the same way mentioned above.

Statistical analysis

A two-way analysis of variance (ANOVA) was performed to test whether there were significant differences in C-N ratio among sectors (positions in the canopy) and trees of different ages. Means were compared using Duncan's Multiple Range Test. All statistical tests were carried out using SPSS version 16.0 (IBM SPSS Inc., Chicago) and differences with p-values less than 0.05 were considered significant.

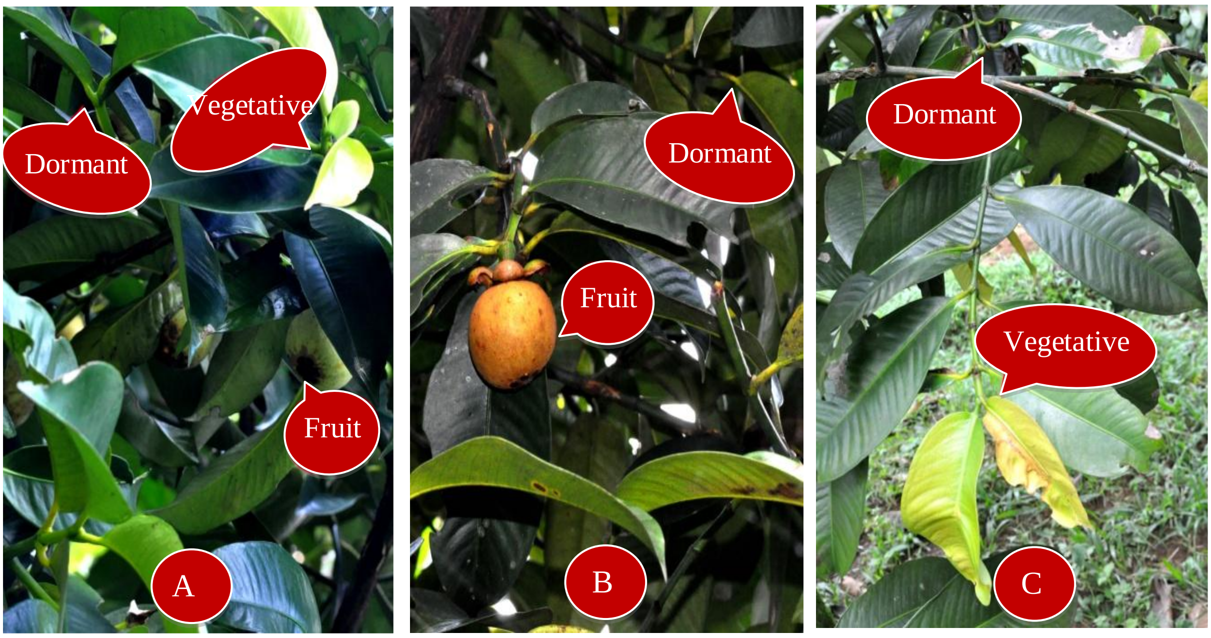

Fig. 17. Three branch types of mangosteen trees with differing growth aspects. A: branches with fruit, dormant bud, and vegetative shoots, B: branches with fruit and dormant bud, C: branches with dormant bud and vegetative shoots.

3.3.2 Results

Fruit production of mangosteen varied markedly from the tree to tree and branch to branch, because trees are composed of 3 different types of branches, A: branches with fruit, dormant bud, and vegetative shoots, B: branches with fruit and dormant bud, and C: branches with dormant bud and vegetative shoots (Fig. 17). Carbon content of leaves was highest in branches with fruit, followed in branches with dormant bud, and branches with vegetative shoots; their values were around 52.4, 52.0, and 51.4 %, respectively (Fig. 18). High nitrogen content of leaves was observed in branches with vegetative shoots, whereas in branches with fruit and dormant bud, it was the lowest. There was significant difference in nitrogen content of leaves in branches of different types; the value was 1.6, 1.5, and 1.7 %, in branches with fruit, branches with dormant bud, and branches with vegetative shoots, respectively. The C-N ratio of leaves was higher in branches with fruit and dormant bud, compared to vegetative shoots. The C-N ratio of mangosteen leaves was 33.3 % for branches with fruit, 33.8 % for dormant bud, and 30.7 % for vegetative shoots.

Carbon content of mangosteen leaves was higher in branches with fruit than in branches with dormant bud (Fig. 18). No significant difference was observed in the nitrogen content of leaves between branches with fruit and branches with dormant bud, although it was higher in the latter. Consequently, the C-N ratio of leaves was 36.3, and 33.7 % in branches with fruit and in branches with dormant bud, respectively.

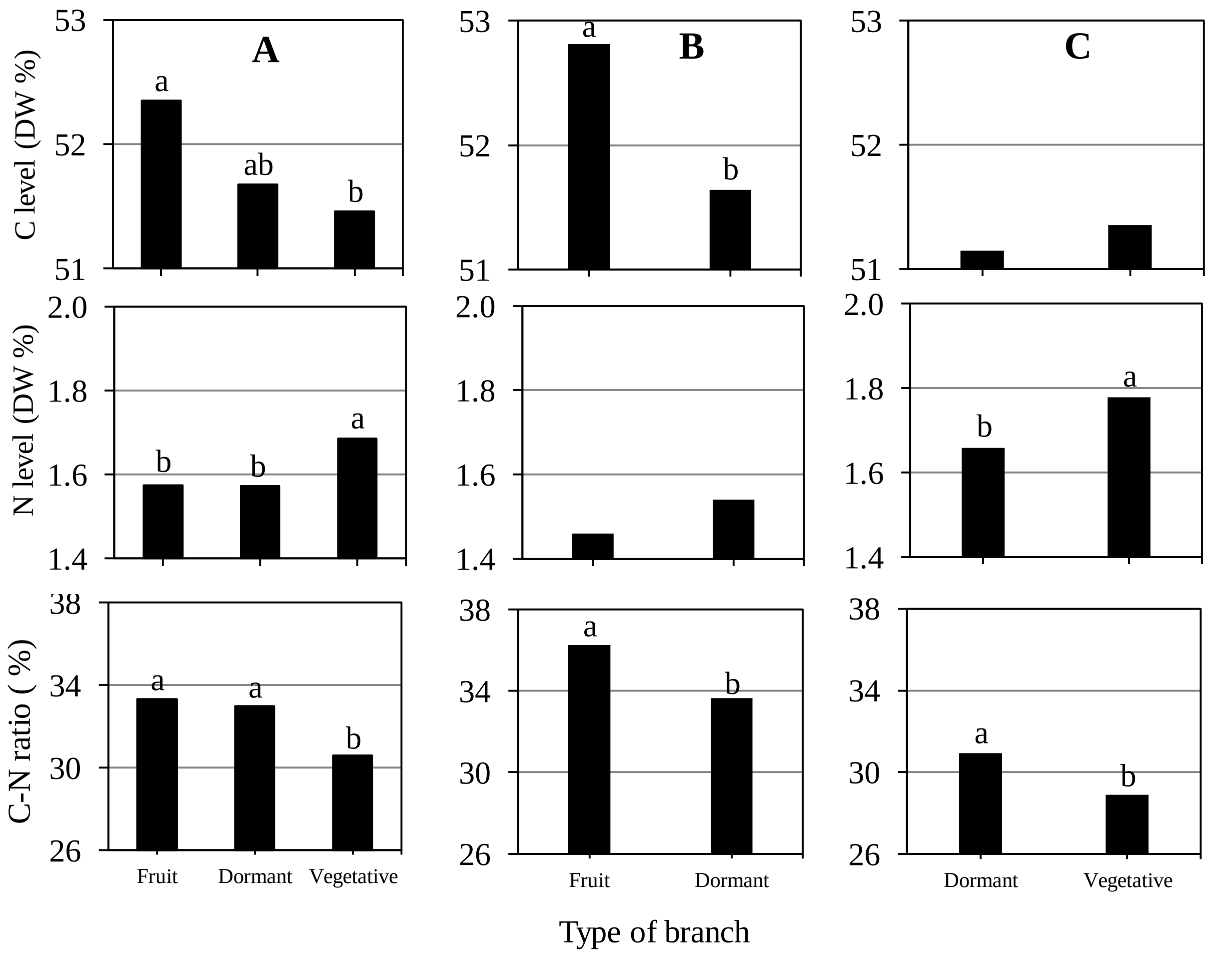

Fig. 18. Carbon, nitrogen and C-N ratio of mangosteen leaves in three types of branch with differing growth aspects. A (left series): branches with fruit, dormant bud, and vegetative shoots, B (central series): branches with fruit and dormant bud, and C (right series): branches with dormant bud and vegetative shoots.
Different letters on column indicate significant difference by *Duncan's Multiple Range Test*, at *p<0.05*.

Fig. 18C shows that carbon contents of mangosteen leaves in branches with dormant bud and vegetative shoots was lower, the value being around 51.1 to 51.4 %. Nitrogen content of leaves in branches with vegetative shoots was higher compared to branches with dormant bud, being 1.8 and 1.7 %, respectively. The C-N ratio of leaves was 30.9 and 28.9 % for branches with dormant bud and vegetative shoots, respectively. Nitrogen content of leaves in branches with dormant bud and vegetative shoots (Fig. 18C) was higher compared to branches with fruit, dormant bud, and vegetative shoots (Fig. 18A) and branches with fruit and dormant bud (Fig. 18B).

3.4 Discussion

Regardless of the sector, both in the young and old tree showed the seasonal trends in the carbon content of the leaves was low in May followed by an increased in June then gradually decreased until August. On the contrary, in the middle-aged tree was low in June followed by an increased in July then gradually decreased until September. This result may suggest different carbon content in leaves caused the phenological response such as to produce new vegetative shoots, bud dormancy, and induction of flowering among different sector and mangosteen tree. The lower carbon content in leaves during May might be due to the transformation and subsequent utilization of the stored starch by flower, while carbon content in leaves gradually decrease during July to August suggest that carbon were used for fruit development. These findings are in general coincided with those reported by Bacha (1975) on pomegranate.

As shown in Fig. 10, the data showed clearly that the nitrogen level (dry weight %) in the mangosteen leaves was low during May, in spite of sector and tree aged. This decrease might be due to the translocation of N to the produced flower fruits and/or new vegetative shoots. For young trees, the nitrogen level was lowest in July (Fig. 10A). This may suggest that nitrogen compound was required for growth of new vegetative shoots. The lower N also occurred during July both in young and middle-aged tree and during August in the old tree might suggest the translocation of N to fruit development. In Malaysia, it has been reported that flushing starts in July and August when nitrogen content decreases to the lowest level of 1.18 % (Malip and Masri, 2006). Nitrogen remained high from August to September, because there were no vegetative shoots in young trees. In May, when flowering began in old trees, the concentrations of nitrogen remained lowest from 1.2 to 1.4 % (Fig. 11C). In old trees, drought conditions in May caused the C-N ratio increase, and this fact may promote flower bud differentiation. This indicated that the dried condition and C-N ratio in the mangosteen leaf show significant effect on the flowering. Hong-Biao *et al.* (2008) reported that the maximum C-N ratio was significantly correlated with dry weight per boll and boll weight in cotton. The monitoring in Malaysia during 2004 had shown that during flushing, nitrogen was low but reversed during flowering, implying that nitrogen was heavily used for vegetative shoot development (Malip and Masri, 2006). The seasonal change in nitrogen content was varied largely among tree ages and month, may suggested the blooming is not uniform. Regardless of month and tree age measured, nitrogen content of leaves was higher in bottom position of canopy such as Sectors 1 to 3 than in middle and top ones such as Sectors 5 to 9. This may suggested in bottom position of canopy was high in photosynthetic rate compared to middle and top ones. Makino and Osmond (1991) reported that the photosynthetic rate increased linearly with nitrogen content in leaves.

In spite of tree age, both in SPAD value and chlorophyll content was high in top position of canopy such as in Sectors 7 to 9 than in bottom and middle positions (Sectors 1 to 6). In contrast, the nitrogen level in middle and top positions of canopy was lower compared to bottom one. In this research, the young leaves with green light colour have low SPAD value and in the older leave with dark-green colour was high SPAD value. However, the nitrogen content in young mangosteen leaves was higher than in older one. This may suggest that the nitrogen content in mangosteen leaves varied contrarily with SPAD value. Meletiou-Christou *et al.* (1994) reported that the nitrogen content was high in young expanding leaves and decreased considerably during the growth period of Mediterranean evergreen sclerophylls. Similarly, Escobar *et al.* (1999) reported that the concentrations of N, P, K, Zn, and B in olive leaves were higher in younger leaves than in older one. Our results contrasts results observed in pea and wheat, in which chlorophyll content increased in parallel with leaf-nitrogen content because nitrogen partitioning into chloroplasts and mitochondria (Makino and Osmond, 1991). This difference in results may be due to the different colour and thickness of leaves. Nakasone and Paull (1998); Yaacob and Subhadrabandhu (1995) previously reported that new leaves are light green then change to dark-green colour, relatively thick and coriaceous, and believe to live for several years.

In this research, SPAD value and total chlorophyll content in the old tree were lowest compared to the young and middle-aged tree. The results presented here suggest that lower SPAD value and chlorophyll content could be due to higher nitrogen level in the old mangosteen tree. Nitrogen level in the old tree during July was high compared other tree ages in all month. This may suggest productivity in the old tree was higher than in the young and middle-aged tree. Boussadia *et al.* (2010) reported that

N–deficient in olive plants had significant lower nitrogen and chlorophyll a contents in leaf, also showed a significant reduction in their photosynthetic capacity.

In this study chlorophyll a/b ratio increased gradually with age; it was 2.20, 2.25, and 2.95 in the young, middle-aged, and old tree, respectively. Issarakraisila and Settapakdee (2008) reported that chlorophyll a/b ratio in seedlings was low as an average of 0.808. Furthermore, Issarakraisila and Settapakdee (2008) reported that the large size of canopy affects the transmission of sunlight into the bottom and inner positions of canopy. Increase in tree age resulted in the increase of height and width of canopy.

3.5 Summary

Tree differing in age and fruiting position in canopy were used to study the C-N ratio in the leaf of mangosteen and its effect on flower induction and fruit growth. In young trees, carbon content of leaves was higher in top position such as in Sectors 7 to 9 than in bottom one such as Sectors 1 to 3, whereas in middle-aged and old trees, the carbon content was varied largely among sectors and month. Regardless of month and tree age measured, nitrogen content of leaves was higher in bottom position of canopy such as Sectors 1 to 3 than in middle and top ones such as Sectors 5 to 9. Consequently, the C-N ratio of leaves was higher in top position of canopy compared to bottom one. In spite of tree age, SPAD value of leaves in middle and top positions of canopy such as Sectors 5 to 9 was high compared to bottom one such as in Sectors 1 to 3. The content of chlorophyll a was significantly different among sectors and tree ages. In this study, the nitrogen content in mangosteen leaves had contrarily with SPAD value. In mangosteen leaves, the high SPAD value has low nitrogen content and in the contrast, low SPAD value means the high nitrogen content. SPAD value in both young and middle-aged trees was higher in inner position of canopy such as Sectors 1, 4, and 7 than in outer one such as Sectors 3, 6, and 9, while in old trees, it was higher in outer position of canopy than inner one. Chlorophyll a of leaves was higher in top position of canopy such as Sectors 7 to 9 than in bottom one such as Sectors 1 to 3. On the other hand, content of chlorophyll b was not significantly different among sectors. Both chlorophyll a and b content of leaves was higher in young and middle-aged trees compared to old one. The results demonstrated the chlorophyll a/b ratio increased gradually with tree age.

Chapter 4

Contents and Components of Sugars, Organic Acids, and Amino Acids of Aril as Affected by Sector and Tree Age

4.1 Introduction

The fruits during ripening intensify their taste and flavor changes in the composition of different compounds are to be expected (Boggio *et al.,* 2000). In the overall taste and flavor of many fruits can be affected by a number of components and the balance between sugars, non-volatile organic acids, and amino acids (Barboni *et al.*, 2010; Boggio *et al.*, 2000). The most important sugars of kiwi fruit are glucose, fructose and sucrose (Barboni *et al.,* 2010). Martin (1980) reported that soluble solids, total acidity and ascorbic acid content in mangosteen fruits decreased during storage while reducing sugars increased. Quantification of acids such as citric, malic, and tartaric, typically present in fruit juices has been carried out routinely to evaluate the quality in many fruits. Arslan and Özcan (2011) and Basson *et al.* (2010) reported even though the organic acids are minor components of fruits, in combination with sugars, they are important attributes of the sensory, flavor, and final fruit quality. Citric, malic, and tartaric acids are commonly found in fruits and berries (Arslan and Özcan, 2011; Barboni *et al.,* 2010). Citric acid is a major organic acid complexion with oxidant metals, and therefore, it has a synergistic reduction effect with ascorbic acid, and on the other hand; malic acid is an important indicator of the freshness of fruits or tubers (Arslan and Özcan, 2011).

As sink organs, fruits are dependent on the translocation of sucrose, amino acids, and organic acids to the developing fruit cells; and the rate of import of these photoassimilates from the leaves is regulated by the metabolic activity of the fruit

(Boggio *et al.*, 2000). Khalid *et al.* (2012) reported that the fruit quality of 'Kinnow' mandarin was different among tree age; fruit from 18-year-old trees were higher in total sugars, reducing sugars, acidity and total soluble solids than fruit from young trees (3-years-old). Moon *et al.* (2011) reporting that fruit diameter, fruit weight, pulp weight, and pulp thickness were higher in citrus fruits harvested from the top position of canopy than in the middle or bottom ones. Apart from tree age, fruiting position in canopy also plays an important role in determining fruit quality of 'Kinnow' mandarin which is different in the internal and external tree canopy in terms of juice mass (%), rind mass (%), rind smoothness and thickness (Khalid *et al.*, 2012).

In mangosteen, the fruit quality judging based on fruit size and weigh (Chapter 1). However, contents and compositions of sugars, organic acids, and amino acids have often been regarded as the indicator of fruit quality traits (Bartolozzi *et al.*, 1997). The effect of fruiting position and tree age of mangosteen on the contents and compositions of sugars, organic acids, and amino acids is not currently known. This research was done to understand contents and compositions of them as the indicator of fruit quality. In this chapter, I will describe the contents and components of sugars, organic acids, and amino acids of mangosteen fruits as affected by different sector (position in the canopy) and tree age.

4.2 Materials and Methods

Plant materials

Mangosteen trees of 3 different ages (young, 20 years old; middle, 35 years old; and old, 50 years old; n = 5 per age) growing in a commercial orchard in Bogor, Indonesia, were studied in May 2010. The canopy of each tree was divided into 9 sectors, based on tree height (bottom, middle, and top) and width (inner, center, and outer) (refer to Fig. 2 in Chapter 1).

One fruit was harvested from each sector in mid-October, 2010 at the mature green stage (i.e., yellowish white with to light green skin color), which is the stage that is usually harvested (Palapol *et al.*, 2009). Each fruit was separated into pericarp, aril, and seed. Fresh aril was dried in an oven at 70 °C for 4 days, then ground into a powder using a mill.

Extraction of sugars, organic acids, and amino acids from the aril

Approximately 500 mg of dried aril powder was placed in a flask, and 20 mL of 80 % ethanol was added. The mixture was shaken in a water bath for 20 minute at 50 °C and filtered to extract sugars, organic acids, and amino acids. This extraction procedure was repeated 3 times, and all the filtered extracts were combined. The extracts were then dried under vacuum at 45 °C, and the residue was dissolved in 10 mL of distilled water and filtered. Subsequently, 2 mL of the filtrate was passed through a column of Amberlite CG 120 cation (H^+) ion exchange resin joined to a column of Amberlite IRA 45 anion (OH^-) ion exchange resin, and eluted with distilled water; the volume was then made up to 50 mL.

Sugar analysis

Twenty-five milliliters of the eluate was evaporated to dryness using a rotary vacuum evaporator at 45 °C, and the residue was then dissolved in 5 mL of distilled water. Following centrifugation at 7×10^3 rpm for 10 minute, the concentrations of sucrose, glucose, and fructose were determined by high performance liquid chromatography (HPLC), under the following conditions: pump, Hitachi L-6000

(Hitachi, Tokyo); column, Shodex sugar series SP0810 (Showa Denko, Tokyo); column temperature, 80 °C; mobile phase, distilled water at 1 mL·min^{-1}; and detector, Hitachi RI 7490 (Hitachi).

Organic acid analysis

The organic acid fraction was obtained by eluting the substances adsorbed onto the Amber lite IRA 45 anion (OH^-) ion exchange resin with 50 mL of 2N NH_4OH. The eluate was evaporated to dryness at 45 °C, and then dissolved in 2.5 mL of distilled water. Following centrifugation at 7×10^3 rpm for 10 minute, the concentrations of malic acid and citric acid were determined using HPLC under the following conditions: pump, Hitachi L-6000; column, 8 mm × 50 cm stainless steel, packing Hitachi resin #2618 (H^+) (Hitachi); column temperature, 80 °C; mobile phase, 0.05 % H_3PO_4 at 1.5 mL·min^{-1}; and detector, Hitachi L-4000 UV (Hitachi).

Amino acid analysis

The amino acid fraction was obtained by eluting the substances adsorbed onto the Amber lite IRA 45 cation (H^+) ion exchange resin with 50 mL of 2N NH_4OH. The eluate was evaporated to dryness at 45 °C, and then dissolved in 5 mL of distilled water. Following centrifugation at 7×10^3 rpm for 10 minute, the supernatants were analyzed using a fully automated HPLC (Hitachi, L-8500). Amino acid components were identified by comparing their retention times with those of authentic standards under the same analytical conditions, following which the concentration of each amino acid was calculated.

Statistical analysis

A two-way analysis of variance (ANOVA) was performed to test whether there were significant differences in productivity and/or fruit quality among sectors (positions in the canopy) and trees of different ages. Means were compared using Duncan's Multiple Range Test. All statistical test were carried out using SPSS version 16.0 (IBM SPSS Inc., Chicago) and differences with *p*-values less than 0.05 were considered significant.

4.3 Results

a) Sugar and organic acid composition in the aril

For all sectors and tree ages, the most abundant sugar found in the aril was glucose, followed by fructose and sucrose (Table 9). The concentration of glucose, fructose, and sucrose ranged from 60.7 to 83.5 $mg.g^{-1}DW$, 38.9 to 48.5 $mg.g^{-1}DW$, and 26.0 to 34.6 $mg.g^{-1}DW$, respectively. Fruits harvested from Sectors 2, 3, and 5 contained greater concentrations of glucose and, consequently, higher total sugar contents, but these differences were not significant. Both total sugar content and the concentration of glucose and fructose significantly increased with tree age; in contrast, sucrose concentration was the highest in fruits harvested from middle-aged trees.

Malic acid was the most abundant organic acid in fruits from all sectors and trees of all ages (Table 9). The organic acid content of fruit varied greatly among sectors, ranging from 5.2 to 8.7 $mg.g^{-1}DW$ for malic acid and 1.3 to 3.3 $mg.g^{-1}DW$ for citric acid. Fruits harvested from Sector 6 contained significantly higher levels of both malic acid and citric acid than fruits from other sectors, and thus also had the highest total acid content. Middle-aged trees also produced fruits with the highest concentrations of malic acid, citric acid, and total acid. There was wide variation in the sugar/acid ratio among sectors, ranging from 11.5 to 33.0. This ratio was the highest in Sectors 4, 8, and 5, and the lowest in Sector 6, although none of these differences were significant. The sugar/acid ratio was significantly higher in fruits harvested from old trees than in those harvested from middle-aged and young trees.

Table 9. Sugar and organic acid content and composition in mangosteen fruits harvested in mature green stage as affected by different sectors and tree ages

Sector / tree age	Sugar content ($mg.g^{-1}DW$)				Organic acid content ($mg.g^{-1}DW$)			Sugar/ acid ratio
	Glucose	Fructose	Sucrose	Total	Malic acid	Citric acid	Total	
Sector								
1	71.7 ab [z]	43.9	34.6	150.1	6.8 ab	1.8 b	8.6 ab	23.7
2	83.5 a	48.5	27.9	159.9	6.9 ab	1.9 b	8.8 ab	25.6
3	81.8 ab	48.4	30.3	160.5	6.4 ab	1.8 b	8.2 ab	23.7
4	77.8 ab	47.7	26.0	151.5	6.2 ab	1.9 b	8.1 ab	33.0
5	80.2 ab	48.3	29.2	157.7	5.2 b	1.9 b	7.1 b	30.0
6	60.7 b	47.0	32.8	140.5	8.7 a	3.3 a	12.0 a	11.5
7	72.1 ab	45.2	28.1	145.4	6.7 ab	1.9 b	8.6 ab	20.4
8	62.1 ab	38.9	31.8	132.8	5.3 b	1.3 b	6.6 b	30.1
9 [y]								
Tree age								
Young	67.3 b	38.5 b	23.7 b	129.5 b	5.8 b	1.5 b	7.3 b	18.4 b
Middle	71.9 ab	44.0 ab	35.0 a	150.9 ab	8.3 a	2.3 a	10.6 a	16.5 b
Old	83.2 a	52.2 a	29.1 a	164.5 a	5.1 b	1.7 ab	6.8 b	37.0 a

[z] Value with different letters in each column indicate significant difference by *Duncan's Multiple Range Test, $p<0.05$.*

[y] No fruit.

b) Amino acid composition in the aril

In total, 18 amino acids were detected in the aril of mangosteen fruits, including γ–aminobutyric acid (GABA), alanine (ALA), isoleucine (ILE), valine (VAL), and glycine (GLY) (Table 10). For all sectors and tree ages, GABA and ALA were the most abundant amino acids in fruit, while only small amounts of the other amino acids were detected. The content of GABA was significantly higher in Sector 4 than in Sector 7. Total amino acid content was also the highest in Sector 4 and the lowest in Sector 7, but this difference was not significant. The concentration of GABA, ALA, and SER, and the concentration of other amino acids significantly decreased with tree age, consequently, total amino acid content was also higher in fruits harvested from young trees.

4.4 Discussion

It is well known that the content and composition of sugars, organic acids, and amino acids in fruit largely affect fruit quality, particularly with regard to sensory aspects (Jia *et al.,* 2000). In this experiment, the most abundant sugar was glucose, followed, in order, by fructose and sucrose in fruits harvested from all sectors and trees of all ages. This is in agreement with previous findings (Nakasone and Paull, 1998; Osman and Milan, 2006). There was no significant difference in total sugar content among sectors, but the concentration of total sugar, glucose, and fructose significantly increased with tree age. Although it is well known that sugars and acid levels are stable in fruits harvested from mature trees, the reason for this difference in sugar content among trees of different ages is not yet known.

Table 10. Amino acid composition of the aril in mangosteen fruits harvested in mature green stage as affected by different sectors and tree ages

Sector / tree age	Amino acid composition [z] (nmol.g^{-1}DW)											
	GABA	ALA	ILE	VAL	GLY	PHE	LEU	TYR	SER	ARG	Other	Total
Sector												
1	461.4 ab [y]	326.2	112.6	59.6	35.8	32.3	33.9 ab	29.4 ab	22.4	17.6	68.7	1199.9
2	451.9 ab	275.4	69.5	56.3	36.1	31.0	28.7 ab	23.7 ab	21.3	11.3	74.2	1077.4
3	415.3 ab	260.4	58.8	47.7	28.9	27.1	24.3 b	18.5 b	25.5	10.4	70.0	990.8
4	596.3 a	479.6	134.5	81.7	37.4	48.6	47.5 ab	33.2 ab	21.4	23.5	77.9	1583.4
5	509.8 ab	433.0	89.0	83.6	81.7	42.2	53.7 a	38.0 ab	43.4	15.2	70.5	1455.8
6	433.1 ab	375.6	78.3	81.3	30.6	42.7	38.9 ab	53.0 a	17.9	18.4	67.9	1237.6
7	338.3 b	278.6	62.8	51.9	21.5	27.1	25.9 b	33.0 ab	25.5	12.6	49.4	926.4
8	409.8 ab	378.1	80.6	56.2	27.9	30.9	28.1 ab	26.6 ab	32.4	13.8	64.3	1148.4
9 [x]												
Tree age												
Young	572.9 a	472.1 a	62.1	71.2	42.2	40.0	38.5	34.6	55.4 a	16.1	114.0 a	1519.0 a
Middle	486.0 ab	344.7 b	97.1	65.0	26.4	34.5	35.6	34.2	24.3 b	17.2	64.0 b	1228.9 ab
Old	391.6 b	294.9 b	93.4	60.2	46.9	33.3	33.9	25.1	14.6 b	14.2	52.1 b	1059.3 b

Note: [z] GABA, γ–aminobutyric acid; ALA, alanine; ILE, isoleucine; VAL, valine; GLY, glycine; PHE, phenylalanine; LEU, leucine; TYR, tyrosine; SER, serine; ARG, arginine; Other, lysine (LYS) + threonine (THR) + glutamic acid (GLU) + cysteine (CYS) + metionine (MET) + histidine (HIS) + aspartic acid (ASP) + asparagine (ASN).

[y] Value with different letters in each column indicate significant difference by Duncan's Multiple Range Test, $p<0.05$.

[x] No fruit.

Osman and Milan (2006) has determined only citric acid as organic acid in mangosteen fruit. In this experiment, however, the most abundant organic acid was malic acid, followed by citric acid for all sectors. Reasons for different results between two experiments are not known. Arslan and Özcan (2011) reported that the malic acid concentration increases during the storage period, contrary to the citric acid concentration. The organic acid content of fruit was similar among sectors, with the exception of Sector 6, which had significantly higher concentrations. The malic/citric ratio decreased with tree age. Therefore, the sugar/acid ratio varied greatly among sectors and tree ages, ranging from 11.5 to 33.0 in Sectors 6 and 4, respectively, and from 16.5 in middle-aged trees to 37.0 in old trees. Anabesa (1992) previously reported that mangosteen fruits harvested at 113 days after flowering would have already developed the desired flavor as a result of the chemical changes that accompany maturation and the ripening process, such as a decrease in organic acids and an increase in sugars. Palapol *et al.* (2009) found that total soluble solids (TSS) in mangosteen fruit gradually increased during maturation, whereas the titratable acidity (TA) decreased with color development of the skin surface. TSS content has been found to range from 13.0 to 15.2 % in immature fruits, increasing to 18.3 to 19.0 % in fully mature fruits (Nakasone and Paull, 1998; Osman and Milan, 2006). The results obtained here for mature green stage fruit match those previously reported by Omran and Semiah (2006), who also found that large fruits contain higher TA and lower TSS than small fruits.

For all sector and tree age, GABA and ALA were the most abundant amino acids in mangosteen fruit. In contrast, Zhang *et al.* (2010) found that aspartic acid (ASP), asparagines (ASN), glutamic acid (GLU), proline (PRO), threonine (THR), and γ–aminobutyric acid (GABA) were the main amino acids in mature “Honeycrisp” apple fruits. It is common for the main amino acids in fruit to differ among species, as they are influenced by several factors, including practical and climatic differences, cultivar, growth stage, harvest time, and storage and ripening conditions (Clark *et al.,* 1992). In this experiment the total content and concentration of each amino acid decreased with tree age, with the exception of ILE and GLY. Jia *et al.* (2000) showed that the amino acid content of peach fruit significantly increased with increasing levels of nitrogen application, because nitrogen is an essential element in amino acids. We have also previously found that higher nitrogen levels (on a dry weight basis) are found in vegetative shoots in branches with fruits, dormant buds, and vegetative shoots, and also with dormant buds and vegetative shoots (Setiawan *et al.,* 2012).

4.5 Summary

Total sugar content of the aril was also higher in fruits harvested from old and middle-aged trees. Regardless of sector, the most abundant sugar in the aril was glucose, followed by fructose and sucrose. There was no significant difference in total sugar content among sectors, but glucose concentration was higher in fruits harvested from Sectors 2, 3, 5, and 4. For all sectors, the main organic acids found in the aril were malic acid and citric acid, and acid content was the highest in fruits harvested from middle-aged trees. For all sectors and tree ages, the main amino acids in the aril were γ-aminobutyric acid (GABA) and alanine. Sector 4 fruits contained significantly higher concentrations of GABA than did Sector 7 fruits. Total amino acid content and the concentration of several individual amino acids decreased with tree age.

Chapter 5

Effect of Sector on Translocation and Distribution of ^{13}C-photosyntates in Branches

5.1 Introduction

The quality of fruit produced by mangosteen trees varies markedly among individual trees, and according to season and growing conditions (Chapter 1, 2, and 4; Apiratikorn *et al.,* 2012; Malip and Masri, 2006; Nakasone and Paull, 1998). The commercial quality of mangosteen fruit is evaluated according to the following criteria: size and weight (Chapter 1; Diczbalis, 2009), scarring of skin surface due to thrips infestation or other damage (Affandi and Emilda, 2010), and sugar and acid content of the aril (Chapter 4; Chaisrichonlathan and Noomhorm, 2011). Some researchers report that the highest quality mangosteen fruit is borne on branches close to the trunk, or on internal branches that are not exposed to direct sunlight (Nakasone and Paull, 1998; Setiawan and Poerwanto, 2008; Yaacob and Subhadrabandhu, 1995). We have previously reported that the quality of mangosteen fruit varies substantially among bearing positions in tree canopy (Setiawan *et al.,* 2006). Moreover, we found that the quality of mangosteen fruit borne on Sectors 1, 2, 4, and 5 was higher than that of fruit in Sectors 3, 6, 7, and 8, in terms of size, occurrence of scars, and sugar content of the aril (Chapter 1 and 4).

For many species of fruit trees, size and sugar content of fruit are largely affected by partitioning of photosynthates (Kubota *et al.,* 1990; Teng *et al.,* 1999; Yamamoto, 2001). Numerous studies have examined the translocation and distribution of ^{13}C-photosynthates in temperate fruit trees, including peach, grape, and pear (Kubota *et al.*, 1990; Ono *et al.*, 2000; Teng *et al.*, 1998). Carbon assimilation and allocation in different plant organs varies with plant species, phenology, and growing conditions (Ge *et al.*, 2012). An important outcome of understanding photosynthate allocation within fruit tree species would be improved pruning practices that could lead to increased fruit yield (Yamamoto, 2001). Judging from the facts mentioned above, different fruit quality of mangosteen among sectors may be caused by different allocation of photosynthates. In this chapter, I investigated the partitioning of ^{13}C-photosynthates in branches of mangosteen varied by sector (position in the canopy), and how this partitioning related to fruit quality.

5.2 Materials and Methods

Plant Materials

Our experiment was conducted on three 25-year-old mangosteen trees grown in a commercial orchard in Bogor, Indonesia. The canopy of each tree was divided into 9 sectors based on tree height (bottom, middle, and top) and width (inner, center, and outer) (refer to Fig. 2 in Chapter 1). A lateral branch of moderate size (ca. 120 cm in length) bearing approximately 5 fruits was selected from each sector for $^{13}CO_2$ feeding. The experimental was set up a randomized complete block design (RCBD) in three replications with one tree per replicate.

Feeding and Analysis of ^{13}C

Pulse-labeling was conducted by enclosing each branch in a transparent plastic bag (120 × 120 cm), and exposing it to $^{13}CO_2$ for 1 h. The $^{13}CO_2$ label was made by reacting 1.5 g $Ba^{13}CO_3$ (99 atom % ^{13}C) with 50 % lactic acid. $^{13}CO_2$ feeding was repeated 2 times for each branch and was carried out on the mornings of December 12 or 13, 2003, which corresponded to approximately 1 month before harvesting stage. At 0 and 48 h after $^{13}CO_2$ feeding, 2 leaves and 1 fruit were collected from each branch and washed with tap water, and the fruits were separated into pericarp and aril. At 96 h after feeding, all lateral branches fed with $^{13}CO_2$ were removed from the secondary scaffold limb and were separated into stem, leaf, and fruit (receptacle and pedicel, pericarp, and aril). All samples were dried in an oven at 70 °C for 4 days, and ground to a fine powder with a mill. Concentration of ^{13}C in each sample was determined using CN Corder (MT-700MC; Yanaco, Tokyo), attached mass spectrometry (MSI-150; Yanaco). ^{13}C atom % excess was calculated using a previously published method (Kubota *et al.,* 1990). Translocation and distribution of ^{13}C-photosynthates to lateral branches and fruits were compared among sectors.

Statistical Analysis

A one-way analysis of variance was performed to examine whether there were significant differences in photosynthate allocation among sectors. Means were compared using Duncan's multiple range test. All statistical tests were carried out using SPSS version 16.0 (IBM SPSS Inc., Chicago) and differences with p-values less than 0.05 were considered significant.

5.3 Results

Changes in ^{13}C concentration of leaf, pericarp, and aril in each sector of mangosteen are shown in Fig. 19. At the end of $^{13}CO_2$ feeding, leaves in Sectors 4 and 5 contained more labeled ^{13}C than leaves of other sectors. This may suggest that photosynthetic activity of leaves in Sectors 4 and 5 is higher than that of other sectors; however, we do not have an explanation for this phenomenon. In all sectors, ^{13}C

concentration in leaves decreased rapidly within 24 h after feeding, followed by a gradual decrease. Leaves in middle and top positions of canopy (e.g., Sectors 5 to 9) showed a larger decline of ^{13}C concentration than did leaves bottom one (e.g., Sectors 1 to 3). At 0 and 48 h after feeding, ^{13}C atom % excess in leaves was higher in Sectors 4 and 5 than in leaves of other sectors. ^{13}C concentration in the aril was higher than that of the pericarp in Sectors 1, 2, 3, 4, and 6, while in Sectors 5, 7, 8, and 9 the opposite was true.

In this experiment, a greater amount of ^{13}C-photosynthates was translocated into fruits (pericarp and aril) in Sectors 8, 5, 9, 7 and 4 than in Sectors 2 and 1 (Table 11). This may suggest that translocation of photosynthate is reduced in lower, inner position of mangosteen tree, possibly because of shading or competition with another strong sink organs such as the shoot apex.

Compared to other organs and tissues, receptacles and pedicels of mangosteen fruit displayed low ^{13}C-photosynthate distribution ratios (Table 12). Receptacles and pedicels from Sectors 1 and 2 produced the highest distribution ratio of ^{13}C-photosynthates for these tissues; this was consistent with the greater aril size in Sectors 1 and 2.

At 96 h after $^{13}CO_2$ feeding, the highest distribution ratio of ^{13}C-photosynthates was observed in stems, followed in descending order by pericarp, leaf, and aril, for all sectors (Table 12). Although the distribution ratio in arils was small (<5 % for all sectors), the highest ratio was observed in Sector 1, followed in order by Sectors 4, 2, 5, and 7, with minimal $^{13}CO_2$ translocated to arils in Sectors 3, 9, 8, and 6 (Table 12 and Fig. 20). The results presented here suggest that higher quality of mangosteen fruit in Sectors 1, 2, 4, and 5 could be due to greater allocation of photosynthate into fruits.

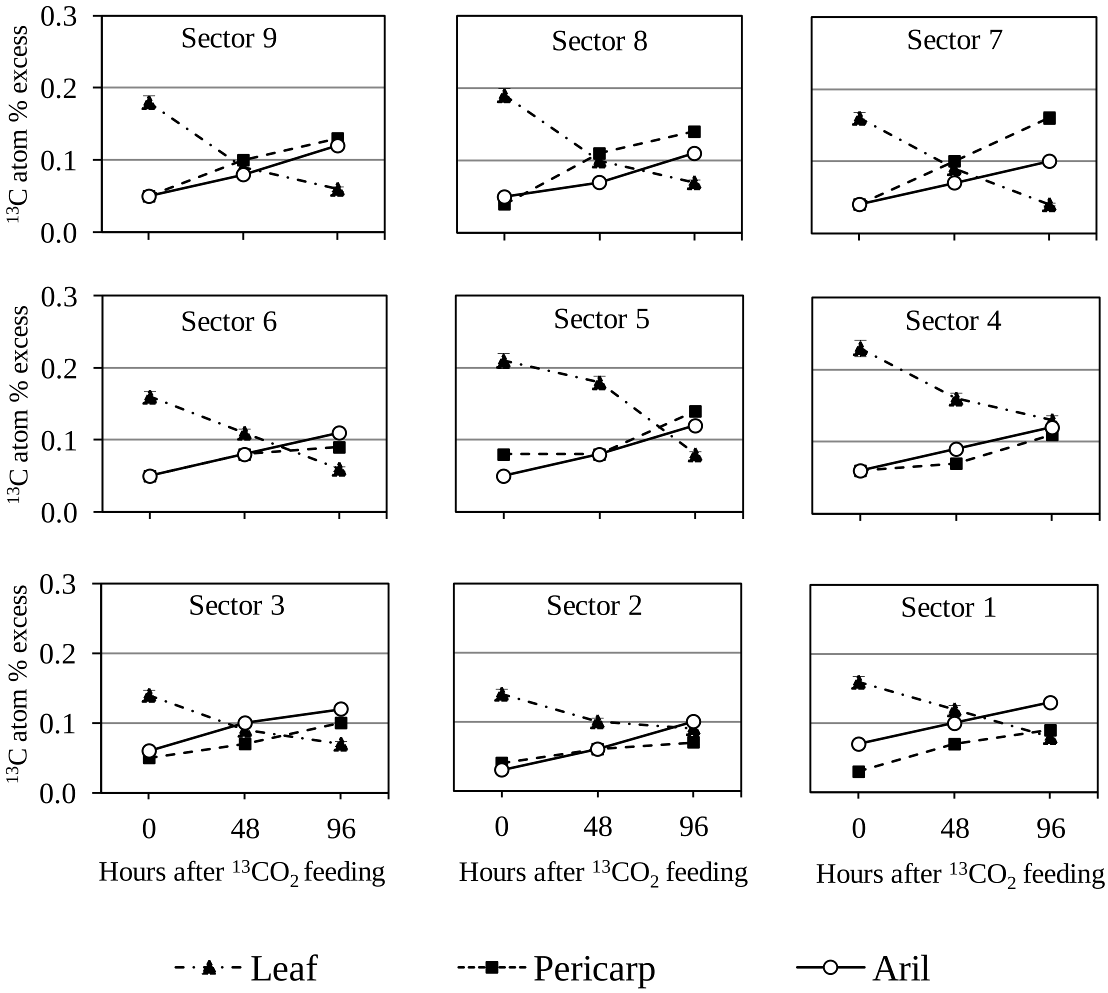

Fig. 19. Changes in ^{13}C concentration of leaves, pericarp, and aril of mangosteen as affected by different sectors (positions in the canopy).
Error bars indicate ± SE (n = 3).

Table 11. Translocation of ^{13}C-photosynthates in mangosteen branches as affected by different sectors (position in the canopy)

Organ / Tissue	Sector	C (%)	Dry weight (g)	^{13}C % of total amount of ^{13}C applied	Amount of ^{13}C (mg)
Aril	1	27.9	3.6	0.129	1.3
	2	28.6	2.5	0.105	0.8
	3	29.2	3.5	0.118	1.2
	4	31.7	4.5	0.122	1.8
	5	30.3	4.5	0.118	1.6
	6	32.2	2.5	0.110	0.9
	7	33.4	3.7	0.103	1.3
	8	28.3	4.4	0.113	1.4
	9	28.8	4.2	0.118	1.4
Pericarp	1	25.6	30.6	0.093	7.3
	2	26.3	36.3	0.069	6.6
	3	26.3	57.3	0.097	14.6
	4	24.4	61.4	0.110	16.4
	5	24.9	51.3	0.139	17.8
	6	25.3	59.3	0.095	14.2
	7	24.4	45.7	0.156	17.4
	8	25.3	55.8	0.138	19.5
	9	23.1	58.3	0.130	17.4
Receptacle and pedicel	1	29.0	3.2	0.091	0.9
	2	28.2	3.0	0.071	0.6
	3	29.2	5.2	0.060	0.9
	4	28.7	3.8	0.063	0.7
	5	28.4	3.8	0.040	0.4
	6	27.5	4.3	0.040	0.5
	7	29.2	3.8	0.064	0.7
	8	29.3	4.1	0.064	0.8
	9	29.4	5.0	0.061	0.9
Leaf	1	29.6	21.7	0.076	4.9
	2	29.7	18.5	0.085	4.7
	3	29.9	19.8	0.065	3.9
	4	28.3	21.4	0.132	8.0
	5	28.7	19.2	0.079	4.3
	6	28.8	22.5	0.055	3.6
	7	28.4	21.2	0.037	2.2
	8	28.8	22.5	0.070	4.5
	9	28.3	21.9	0.059	3.7
Stem	1	29.5	55.5	0.093	15.2
	2	30.7	45.3	0.097	13.5
	3	30.4	98.6	0.101	30.2
	4	30.2	67.3	0.126	25.5
	5	29.8	65.7	0.138	27.1
	6	30.4	74.6	0.068	15.4
	7	30.1	57.0	0.128	21.9
	8	29.9	86.5	0.119	30.9
	9	29.4	99.4	0.131	38.4

Note: Samples were taken at 96 h after $^{13}CO_2$ feeding.

Table 12. Distribution ratio of ^{13}C-photosynthates in mangosteen branches as affected by different sectors (positions in the canopy)

Sector	Distribution ratio of ^{13}C-photosynthates (%)					
	Aril	Pericarp	Receptacle and pedicel	Leaf	Stem	Total
1	4.4 a [z]	24.7 d	2.9 a	16.7 a	51.3 bcd	100
2	3.0 ab	25.3 d	2.3 b	18.0 a	51.5 bcd	100
3	2.4 b	28.8 cd	1.8 bc	7.7 bc	59.4 ab	100
4	3.4 ab	31.4 bc	1.3 cd	15.3 a	48.6 cd	100
5	3.2 ab	34.7 b	0.8 d	8.5 bc	52.8 bcd	100
6	2.5 b	41.5 a	1.4 c	10.4 b	44.2 d	100
7	3.0 ab	40.1 a	1.6 c	5.2 c	50.1 cd	100
8	2.5 b	34.2 b	1.3 cd	8.0 bc	54.0 bc	100
9	2.3 b	28.3 cd	1.5 c	5.9 c	62.0 a	100

[z] value with different letters in each column indicate significant difference among sectors by *Duncan's Multiple Range Test, p<0.05.*

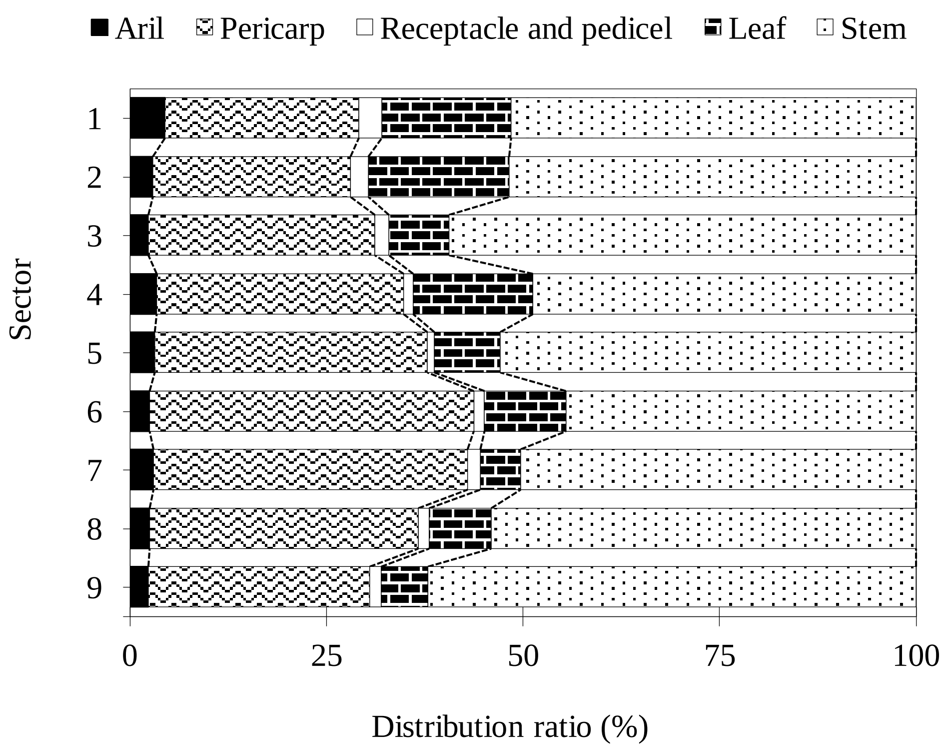

Fig. 20. Distribution ratio of ^{13}C-photosynthates in mangosteen branches based on Table 12.

5.4 Discussion

^{13}C concentration in fruit (pericarp and aril) increased with time after ^{13}C feeding, but there was no significant difference among sectors. Our results coincide with the findings for peach and grape plants, in which ^{13}C concentration decreases in leaves and increases in fruits with time after $^{13}CO_2$ labeling (Kubota *et al.*, 1990; Ono *et al.*, 2000). It is apparent that ^{13}C-photosynthates exported from leaves of mangosteen were translocated into fruits. Teng *et al.* (1999) reported that the highest concentration of ^{13}C in Japanese pear 1 week after labeling was found in folded leaves, followed in descending order by the upper parts of extension shoots, unfolded leaves, fruits, and lower parts of extension shoots.

Goenaga and Rivera (2005) reported that mangosteen tree grown under various levels of shade was produced higher amount of dry matter as compared to plants grown under full sunlight. We have previously reported that carbon levels (in terms of dry weight) in leaves of old mangosteen trees increased at 1 month before harvesting (Setiawan *et al.*, 2012). Moon *et al.* (2011) have reported that the diameter and weight of fruit and weight and thickness of pulp in citrus were higher in fruits harvested from top position of canopy than in the middle and bottom ones.

Zhang *et al.* (2005*a*) reported that the application of growth hormone GA_{3+4} to Japanese pear during the period of rapid fruit growth resulted in a marked increase in pedicel diameter and fruit size at harvest. These researchers maintained that fruit growth in Japanese pear is dependent upon the supply of assimilates from leaves (leaf area and photosynthetic ability) and the sink capacity of fruit (number, size, and duration of growth) (Zhang *et al.*, 2005*b*).

The partitioning calculated from ^{13}C measurements indicates the ratio of carbon translocated to each organ to total carbon fixed by photosynthesis (Sasaki *et al.*, 2005). This is in contrast to the results observed in peach (Kubota *et al.,* 1990) and grape (Ono *et al.*, 2000) plants, in which more than half of the measured photosynthate was allocated to fruits or berries. These differences in experimental results may be due to the different xylem volumes of stems, limbs, and trunks in tropical and temperate fruit trees. We previously found that the quality of mangosteen fruit in terms of size and sugar content was higher in Sectors 1, 2, 4, and 5 than in Sectors 3, 6, 7, and 8 (Chapter 1 and 4). The results presented here suggest that higher quality of mangosteen fruit in Sectors 1, 2, 4, and 5 could be due to greater allocation of photosynthate into fruits. It has been reported that the highest quality fruits of mangosteen are produced on branches close to the trunk or hidden below the canopy (Nakasone and Paull, 1998; Yaacob and Subhadrabandhu, 1995).

Few experiments characteristic the interrelationship between growth and shade of mangosteen have been reported in the literature. The results of this study demonstrate that in bottom and middle positions of canopy, and also in inner and central positions of canopy were high in distribution ratio of leaves. However, in contrast to that leaves, the partitioning ratio declined significantly for stems with increases in shade levels. Outer position of canopy such as Sectors 9 and 3 was highest than inner one. Goenaga and Rivera (2005) reported that mangosteen tree under shade levels treatments was allocated the greatest percentage of their total dry matter to leaves.

Amano *et al.* (1998) showed that shoot elongation during growth of kiwi fruit adversely affects the distribution of photosynthate into fruit. Exported assimilates are partitioned among sink organs, including shoots, stems, and fruits, and can be accumulated in the aril as sugar, starch, or proteins (Gifford and Evans, 1981). Teng *et al.* (1998) reported that ^{13}C-photosynthates in Japanese pear were exported from spur leaves within 24 h of feeding, but that translocation into fruit decreased with age of spur leaves.

5.5 Summary

Regardless of sector, ^{13}C concentration in leaves of mangosteen decreased with time after feeding, whereas ^{13}C concentration increased in the pericarp and aril of mangosteen fruit, demonstrating translocation of photosynthate into the fruits from leaves. Translocation of ^{13}C-photosynthates into fruit was high in Sectors 4 and 5, and in top position (Sectors 7 to 9). At 96 h after $^{13}CO_2$ feeding, the highest distribution ratio of ^{13}C-photosynthates was observed in stems, followed in decreasing order by pericarp, leaf, aril, and receptacle and pedicel. Although the distribution ratio was low overall, the ratio in arils was highest in fruits from Sectors 1, 4, 2, 5, and 7; this finding was consistent with those of previous studies that found superior quality of fruit produced in these sectors.

Conclusions and Proposal to Improve the Productivity and Fruit Quality of Mangosteen Crops

The objectives of this study were to: (1) investigate the effect of sector (position in the canopy) and tree age on the productivity and fruit quality of mangosteen; (2) clarify the meteorological conditions of mangosteen orchards; (3) monitor seasonal changes in carbon and nitrogen contents, the C-N ratio, SPAD value, and chlorophyll content of leaves; (4) determine the quantity and component of sugars, organic acids, and amino acids of fruit (aril) in relation to sector and tree age; and (5) evaluate the partitioning of ^{13}C-photosynthates in branches of different sectors in relation to fruit quality. For the surveys performed in this study, the tree canopy was divided into 9 sectors on the basis of height (bottom, middle, and top) and width (inner, center, and outer).

In Chapter 1, the productivity and fruit quality of mangosteen were compared among 9 sectors and 3 tree ages. More fruit were produced in the Sectors 1, 2, 4, and 5 than in the top position (such as Sectors 7 to 9). Older trees produced significantly more fruit per tree. More shoots developed in the top and outer positions of the canopy than in the other positions. Fruits harvested from the Sectors 1, 4, 7, 5, and 2 were of higher high quality, judging from the size and frequency of scarring, which is a skin disorder caused by thrips. Therefore, further investigation is needed to control the number of vegetative shoots in the canopy to optimize fruit production.

In Chapter 2, the meteorological conditions of mangosteen in an orchard were compared with respect to sector and tree age. It was found that light intensity was significantly different among sectors; however, no significant difference was observed among tree ages. Regardless of tree age, higher light intensity was recorded in the outer position of the canopy (such as Sectors 3, 6, and 9) than in the inner position (such as Sectors 1, 4, and 7). Furthermore, higher light intensity was recorded at the top position than at the bottom position of the canopy. Phenological aspects markedly changed across years because of variability in annual rainfall. Further investigation is needed to clarify the relationship of water stress with flower production and fruit quality in mangosteen trees.

In Chapter 3, carbon and nitrogen content and the C-N ratio of mangosteen leaves were investigated on the basis of productivity and fruit quality results obtained in Section 2 of Chapter 1. In young trees, the carbon content of the leaves was higher in the top position of the canopy than in the bottom position. In comparison, middle-aged and old trees showed no significant difference in carbon content for any positions of the canopy. Regardless of month and tree age, the nitrogen content of the leaves was higher in the bottom position than in the top position of the canopy. Consequently, the C-N ratio of the leaves was higher in the top position of the canopy than in the bottom one. For young and middle-aged trees, leaf SPAD values were high in the inner position. In contrast, SPAD values were high in the outer position of old trees. Regardless of tree age, leaf SPAD values were higher in the top position than in the bottom position of the canopy. Leaf chlorophyll a content was higher in the top position of the canopy than in the bottom one; however, chlorophyll b content did not significantly differ among sectors. The chlorophyll a and chlorophyll b content of the leaves were both higher in young and middle-aged trees than in the old trees. The chlorophyll a/b ratio gradually increased with tree age. Further investigation is needed to clarify the effect of removing several branches from the canopy, as well as the relationship between light penetration and variation in C-N ratio.

In Chapter 4, the quantity and components of sugars, organic acids, and amino acids of the fruit (aril) were compared for different sectors and tree ages. Total sugar content of the aril was higher in fruit from old and middle-aged trees. Regardless of sector, glucose was the most abundant sugar in the aril, followed by fructose and sucrose. There was no significant difference in total sugar content among sectors; however, glucose concentrations were higher in fruits from Sectors 2, 3, 5, and 4. For all sectors, malic and citric acids were the main organic acids, with total acid content being higher in the fruit of middle-aged trees. Regardless of sector and tree age, γ-aminobutyric acid (GABA) and alanine were the main amino acids in the aril, with several other amino acids also being detected. Aril of the fruit from Sector 4 contained significantly higher concentrations of GABA than aril in Sector 7. Both total the amino acid content and the concentration of several individual amino acids decreased with tree age. Further investigation is needed to clarify the relationship between fertilizer use (organic versus an-organic fertilizer) and mangosteen fruit quality, particularly with respect to the type and quantity of sugars, organic acids, and amino acids in fruit.

In Chapter 5, the partitioning of ^{13}C-photosynthates in the branches of mangosteen trees was evaluated for different sectors in relation to fruit quality. After trees were fed with ^{13}C, its concentrations in the leaves decreased with time, but it increased in the pericarp and aril of the fruit. The translocation of ^{13}C-photosynthates into fruit was higher in the middle and top positions of the canopy than in other positions. At 96 h after feeding with ^{13}C, the highest distribution ratio of ^{13}C-photosynthates was observed in the stem, followed by the pericarp, leaf, aril, and receptacle and pedicel. The ^{13}C distribution ratio in the aril was generally higher in fruit from the Sectors 1, 4, 2, 5, and 7, which produced higher quality fruits. More detail investigation is needed to clarify the relationship between fruit quality and the partitioning of photosynthate in mangosteen, particularly to determine differences in sugar accumulation in the fruit of different sectors.

As a general conclusion, the bottom and middle positions of the mangosteen canopy have higher productivity (number of fruit) and fruit quality (less skin scars and bigger size of fruit) than of the top position, also the fruit productivity and fruit quality was higher in the inner positions than in the outer one. This indicates that the shade positions of canopy such as Sectors 1, 2, 4, 5, and 7 have higher quality fruit and productivity. One of main reason is supposed that the ^{13}C distribution ratio in the aril showed highest in the fruit harvested from the Sectors 1, 4, 2, 5, and 7, which produced high-quality fruit.

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(* in Japanese with English summary, **: in Indonesian with English summary, ***: in Thai with English summary)

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