CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to the Plant Species

2.1.1 *Bougainvillea* spp.

Bougainvillea is a genus of native flowering plant in South America, originated from the west Brazil to southern Argentina. Different authors accept between four and eighteen species in the genus (Kent *et al.,* 2007). The name comes from Louis Antoine de Bougainville, an admiral in the French Navy who encountered the plant in Brazil, in 1768 and first described it to Europeans. Plants are thorny, woody and vines growing anywhere from 1-12 meters tall, scrambling over other plants with their hooked thorns. It is evergreen where rainfall occurs all year, or deciduous if there is a dry season. The leaves are alternate, simple ovate-acuminate, 4-13 cm long and 2-6 cm broad (Gordon, 2002). The actual flower of the plant is small and generally white, but each cluster of three flowers is surrounded by three or six bracts with the bright colors associated with the plant, including pink, magenta, purple, red, orange, white or yellow. Among the Bougainvillea plants, there are two species which are considered as horticulturally important. These are *Bougainvillea spectabilis* and *Bougainvillea glabra*. *Bougainvillea spectabilis* is a large climber with distinctive curved thorns on stems and leaves. The bracts are crinkled, fairly large, egg-shaped, and possess colors in rose, rusty-red and purple. *Bougainvillea glabra* has thinner branches that spread in many directions and have distinctive pointed triangle shaped bracts that come in range of lilacs, mauves and purples color. *Bougainvillea glabra* is sometimes referred to as paper flower because the bracts are thin and papery (Kent *et al.,* 2007; Lopez and Galetto, 2002).

	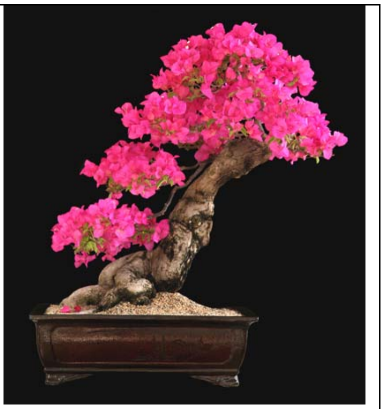
Bougainvillea glabra	*Bougainvillea spectabilis*

Figure 2.1: Bougainvillea Bonsai

2.1.2 Cultivation and Uses of Bougainvillea

Bougainvilleas are popular ornamental plant in most areas with warm climates, including Indonesia, India, Pakistan, Bangladesh, Taiwan, Vietnam, Malaysia, Philippines, Australia, Caribbean, Mexico, Panama, Zimbabwe, South Africa, the southern United States and Hawaii (Kent *et al.,* 2007). Some Bougainvillea cultivars are sterile and propagated from cuttings. Due to the aggressive growth of hardened thorns and prolific branches, this plant is ideal as a natural barrier. Bougainvillea's growth habit and beautiful showy bracts make it a popular plant for landscapes (Fatma *et al.,* 2007). It is used in mass plantings, as shrubs or bushes, and as ground cover on banks. It provides hedges, barriers, and slope coverings (Gordon, 2002). It can cover a whole hillside. Dwarf cultivars make colorful excellent ground covers. It is used as an accent plant, a specimen plant, in hanging baskets, in containers, and for bonsai. Bougainvillea is used to decorate all public, home gardens, play ground and office corridor. It is probably true to say that without bougainvillea plant, our roads, parks and private gardens would be a lot less colorful than what we see today. Recently this cultivar is vastly recommended for plantation in heavy industrial area and traffic island to absorb the pollutants and green house gases from the environment (Kulshreshtha *et al.,* 2009).

2.2 Control of Flowering Time

In general, for plants to produce flowers, require input from five major promotion pathways: long-day/photoperiod, light quality, gibberellins (GA), autonomous (maturity), and the vernalization pathways (Boss *et al.,* 2004). Bougainvillea has been reported by Gordon (2002) to be a short day plant. James and Jeffrey (1996) also showed that with progressively shorter day length, plants flowered sooner and with greater floriferousness; the critical photoperiod was about twelve hours. The idea of flowering being regulated by carbohydrate reserve is not new. A nutritional concept for vegetative and reproductive growth is regulated by carbohydrate/nitrogen ratio of the plant. Anthony *et al.* (1974) found in *Bougainvillea* sp that defoliation of young expending leaves and cytokinin applications to the shoot tips greatly enhanced flowering. The plant must integrate signals from all of these inputs (defoliation of leaves and cytokinin application) to be able to transition from a vegetative state to a flowering state. For example, in *Arabidopsis thaliana*, all five promotion pathways need to be integrated for the plant to produce flowers. However, other species are able to produce flowers after receiving input from only a single or few pathways. In a recent review by Boss *et al.* (2004), the floral pathways were divided into two groups. The first group is a pathway that enables floral transition which includes vernalization and developmental age. These are defined as repressors that function to antagonize the activation of the floral pathway integrators. The second group is a pathway that promotes the floral transition and includes photoperiod, light quality and hormones.

These pathways are defined by providing mechanisms to promote the floral transition integrators.

Previous studies investigated the use of other growth regulators on bougainvillea and these include two hydrazides (daminozide) and maleic hydrazide. Daminozide induces flowering while reducing vegetative growth. Application of malic hydrazide inhibited height and increased branching which resulted in a compact flowering plant (James and Jeffrey, 1996).

2.3 Pathways that Promote Floral Transition

The floral promotion pathways have been defined by the use of environmental and endogenous cues to promote the activation of the floral pathway integrators (Boss *et al.,* 2004). Of these, photoperiod is the well understood and recent work has shown that there is a separate pathway that links light quality with ambient temperature (Blazquez *et al.,* 2003; Cerdan and Chory, 2003). The photoperiod pathway uses both red/far red light and UV/blue absorbing phytochromes to detect light (Lin, 2000). These phytochromes act on a number of entrainment genes (Boss *et al,* 2004) that keep the plant in synchronicity with its environment. It has been suggested by Simpson and Dean (2002) that light quality also affects flowering time. This has been expressed in a ratio of red to far-red light, which can be reduced by shading. It has been shown that the photoreceptor regulates the gene phytochrome that activates flowering time (PFT) (Cerdan and Chory, 2003).

In addition, gibberelic acid (GA_3) has been shown to have a dramatic effect on flowering as plants deficient in the hormone often have delays in flowering (Wilson *et al.,* 1992). Currently no genes have been isolated on GA pathways that are specific for flowering control (Jack, 2004). However, among other suggestions, it seems most feasible that GA has an effect on flowering time. This has been shown by studies relating to the gene early bolting in short days (Gomez *et al.,* 2001; Pineiro *et al.,* 2003).

2.4 Physiological Effects on Plant Growth

2.4.1 Effect of Pruning Techniques on Plant growth

Pruning has been defined as the art and science of cutting away a portion of the plant for horticultural purposes. Pruning can be used to improve tree shape, to influence its growth, flowering and fruitfulness, to improve fruit quality, to repair injury, to contain the plant and to increase light and spray penetration (Jean *et al.,* 2007). The ultimate goal of pruning is to improve light distribution so that more tree canopies would maintain high quality flowers and fruit production. In recent years, flower growers and pomologists have been interested in pruning as a mean of growth control when dwarfing rootstocks do not adequately control tree size (Pinkard, 2002). Bougainvillea plants respond well to pruning. Unless the plants are pruned regularly, it grows into a tangled mass of old and new growth and overcrowding often leads to pests and diseases. In order to prevent overcrowding, the cutting out of any unneeded shoots and all lateral shoots back to within two or three buds of the main stems are suggested. These will bear the new flowers and bracts (Pratap *et al.,* 2003). Regular pruning is necessary to shape the plant and direct its growth because the shoots often grow vigorously. Flowers are initiated on new growth, so pinching back and pruning is necessary to induce new growth. Pruning should be done after flowering, as this encourages the new growth on which next flush of flowers would occur (kent *et al.,* 2007). Numerous studies on pruning have been reported and results vary with cultivar, rootstock, geography as well as time of pruning (Lal *et al.*, 2000). Pruning reduced tree vigor because new shoots especially leaves, utilize reserve food and are not be able to replace them during season. Reduced vegetative growth as a result of pruning has been reported by many researchers (Navarretea and Jeannequin, 2000).

Angeles *et al.* (2008) concluded an experiment on pruning to control growth and maintain flowering in rose plants. They stated that pruned plants showed a maximum efficiency of photosystem (PS) of dark-adapted leaves, a higher actual quantum yield and a higher proportion of PS reaction centers that are open, but a lower non-photochemical quenching, indicating a lower energy dissipation as heat, compared to non-pruned plants. These results, related to chlorophyll *a* fluorescence, indicate that pruned plants have a higher capacity for promoting the photosynthetic light reactions than non-pruned plants. The increased nitrate reeducates activity in pruned compared to non-pruned plants, could result from a higher photosynthetic activity by leaves. Pruning promoted a large number of metabolic sinks that can cause depletion of stored carbohydrates flowing from lower plant parts to the new developing flower shoots (Salakpetch *et al.,* 1990). Low carbohydrate content was sufficient to promote the development of new flower shoots both pruned and non-pruned plants.

An offset benefit of a smaller canopy is a water consumption and improved tree water status due to lower canopy transpiration. A reduction in canopy leaf area whether naturally or artificially by pruning, would reduce water loss, raise leaf and soil water potentials (Khan *et al.,* 1998). Canopy-average leaf transpiration increased after pruning in relation to pruning intensity. The reduction of canopy was possibly due to the combination of decreased canopy light interception after pruning and the lack of photosynthesis recovery of previously shaded spur leaves after re-exposure to sunlight by pruning (Robinson *et al.,* 1992). Marini (1996) carried out an experiment on peach flower bud formation as influenced by pruning treatments. Pruning consisted of heading back vertical shoots to maintain tree height at 2.2 m and thinning to improve light penetration. Flower bud rating was higher in vertical (2.2 m) than in horizontal shoots (1.5 m) in summer-pruned trees compared to control trees.

Bougainvillea plants might be pruned at any time of the year. Bloom or flower initiation depends upon pruning and the bloom cycle followed by a rest period. A hard prune where branches are removed, is recommended when it was needed to contain growth or preparing to move indoor in winter. Soft pruning which refer to pruning at the apical part of the plant, was recommended for bougainvillea only when trying to obtain continuous blooming. A bougainvillea, like most vining-type plants, would continue to grow outward without sending out side branches from each leaf-bud point unless the stem is pinched (Gordon, 2002).

Flowering and vegetative growth might be modified by training and pruning (Johnson *et al.,* 1993). Pruning increased vegetative growth and decreased yield in the following season depending on the severity of pruning (Miller, 1987; Miller *et al.,* 2001). It also influenced the position of inflorescences with regard to vegetative growth, e.g. heading cuts might dissociate flowering from vegetative areas by enhancing vegetative growth in the outer part of the tree canopy and keeping inflorescences inside the canopy (Hoying, 1992; Calatayud *et al.,* 2002). This might have deleterious effects on flowers and fruit quality.

Different methods of pruning according to the length of the eliminated organs, their type (flowering and vegetative shoots) and the timing of cutting have been described by Pinkard (2002). However, all pruning techniques tend to reduce the excessive vegetative growth, at least in the short term. An architectural analysis of these procedures showed that the effects of pruning also vary with the architectural level at which it was done: the removal of a portion of a long shoot usually prompts a strong reiteration process, leading to vegetative shoot growth (Nunez and Crane, 1998), whereas the complete removal of 1-year-old short lateral shoots tends to stimulate growth of the remaining shoots (Lauri and Trottier, 2004) and fruit set of adjacent inflorescences (Lauri and Terouanne, 1999). These findings led to the proposal of pruning strategies based on the removal of flowering shoots at a young stage of growth, with less pruning of old branches (Lauri and Trottier, 2004; Lauri *et al.*, 2004). This procedure was called artificial extinction or, more commonly, extinction pruning. It increased leaf area of remaining shoots and light interception by the tree canopy through a decreased shoot density and a better distribution of shoots in space (Sharma and Room, 2006; Daniells *et al.,* 1994). From a physiological point of view, this procedure would then improve the physiological autonomy of the flowering shoot by enhancing carbon assimilation necessary for current fruit and bourse-shoot growth and flower initiation in the terminal bud of the bourse-shoot (Lauri *et al.*, 2004). Pruning occupy a unique position in the management of an orchard, being the most costly cultural practice and the practices most often poorly done, with dramatic influences on orchard efficiency and flower quantity. Pruning interacted with other cultural practices, such as fertilization, growth regulators and pest control (Hoying and Robinson, 2000). Enhancing of flower number and fruit quality was a sensitive indicator of the effectiveness of pruning, as canopy light distribution influenced all aspects of yield and fruit quality. Although good pruning did not guarantee success, economic success without good pruning was inconceivable (Wunsche and Lakso, 2000).

Pruning was a management technique for some insect or disease problems. For example, removing the older wood in a lilac reduces the incidence of oyster shell scale and borers. Thinning a shrub to increase air circulation reduces the incidence of powdery mildew and leaf spot diseases (Whiting and Carol, 2007). The local control field at the wound released dormant growing points, initiated adventitious buds, and demanded food supplies (Allan *et al.,* 2006; Maricruz *et al.,* 2008).

2.4.2 Impact of Phloemic Stress on Plant Life

Many techniques have been used in making dwarfed trees. Among these, partially ringed bark strip is an important one. Phloemic stress or ringing as represented by partial ringing is a horticultural practice used to manipulate tree growth, development, and fruit growth in a variety of fruit species (Vaio *et al.,* 2001). Small, compact, dwarfed or size controlled fruit trees provide for easier pruning, thinning, spraying, harvesting, high production of high-grade fruit and lower cost of production. The primary factor limiting the use of size-controlled rootstocks in stone fruit production is the lack of suitable rootstocks with a wide range of compatibility among cultivars (Jong *et al.,* 2001). Jose (1997) found that girdling treatments caused lower vegetative growth in relation to the control in mango trees. Onguso *et al.* (2004) reported that the increase of trunk circumference above the girdle might be caused by swelling of the trunk due to the accumulation of carbohydrates. They also stated that girdling blocked the translocation of sucrose from leaf to root through the phloem bundles. The block decreased the starch content in the root and accumulated sucrose in the leaf. However, complete girdling of the stems killed the plants and partial girdling weakened the plants.

Bark-ringing increase cone initiation (*Picea sitchensis*) by 4 fold comparing with control (without ringing) and complete bark-ringing showed an additive effect with GA_3 on the number of female cones formed, but a negative interaction on the number of male cones induced. Ringing promoted almost male cone production when used alone. The partial girdling was effective in reducing shoot growth and improving fruit quality. The total soluble solids content of fruit in treatment (phloemic stress) trees was higher than that of control (Hossain and Fusao, 2008). Hossain (2006) also reported that bark ringing tended to dwarf the tree, induced flower bud formation, and promoted fruiting in peach trees. Whilst, Arakawa *et al.* (1997) reported that bark-ringing reduced chlorophyll concentrations in the upper leaves. But flowering in the same season was significantly increased in apple.

Salleo *et al.* (1996) suggested that girdling interfered with auxin for refilling embolisms. Phloem girdles block downward movement of carbohydrate and auxin (Wilson and Gartner, 2002) and they also reported that branch elongation, stem elongation and total wood growth were lower in girdled trees than un-girdled trees. Yamanishi *et al.* (1995) evaluated that effect of trunk strangulation (strangulated using 1.6, 2 and 2.6 mm steel wires) on the growth and fruit quality in 7-year-old pummelo trees. They reported that shoot number, total shoot length, and trunk circumference were lower in all girdled (strangulated with steel wire) trees than control. Net photosynthesis in leaves were found to be lower in girdled trees than in the control. Carbohydrate (sugar and starch) and specific leaf weight were higher and nitrogen was lower in leaves of girdled trees than in the control.

Flower bud number was higher while leaf number was lower in girdled apple trees than in control. Initially preset set was higher in girdled trees than control and 30-50 days after anthesis there was no significant difference. It was also observed that transverse fruit diameter, total sugar and acid content were higher in girdled trees than the control (Arakawa *et al.*, 1997).

Johnson (1998) reported that photosynthetate produced in the leaves are partially and completely stopped from reaching the roots after girdling. The root system gradually declined to the point where it could no longer support the above ground parts. Arakawa *et al.* (1997) and Elfving *et al.* (1991) reported that calcium concentration in apple was reduced by girdling and mechanical ringing. Onguso *et al.* (2004) reported that the increase of trunk's circumference above the ringing might be caused by swelling of the trunk with accumulation of carbohydrates. They also found sugars and starch were higher in upper part than in lower part of ringing. Davie *et al.* (1995) reported that N, P and Ca levels were lower in leaves in the girdled branches compared with un-girdled branches. Johnson (1998) reported that complete girdling was almost always fatal, even though death might take months to 2-3 years but the roots were still passing up water and nutrients through the undamaged wood in shrub trees. Arakawa *et al.* (1997) reported that 90% girdling in apple trees can reduce the growth. Moreover, Hossain *et al.* (2004) reported that N and Ca content were higher in partially and completely ringed trees than un-ringed trees in the case of roots. P, K and Mg content were also a little higher in partially and completely ringed trees than in un-ringed trees, in roots but there was no difference between ringed and un-ringed trees.

2.5 Impact of Light Intensity on Plant Life

Light intensity is one of the most important plant growth requirements (Tetsuro *et al.,* 2009). The process of photosynthetic light energy is used to produce Adenosine Triphosphate (ATP), Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and oxygen in the light reaction and subsequently, in the light-independent or dark reactions, carbon is fixed into carbohydrates. Under high irradiance, however, the photosynthetic apparatus can absorb excessive light energy, that result in the inactivation or impairment of the chlorophyll containing reaction centers of the chloroplasts (Bertaminia *et al.,* 2006). As a consequence, photosynthetic activity is depressed by photoinhibition (Osmond, 1994). In contrast, under low irradiance, insufficient ATP is produced to allow for carbon fixation and carbohydrate biosynthesis. This leads to reduce plant growth. In addition, Von and Deng (1996) have reported that light intensity and wavelength composition (light quality) are important factors in determining the speed of cell growth of pigment accumulation, and of plastid differentiation. Von and Deng (1996) concluded that in a given species, the specific effects of light could differ drastically from one organ or cell type to the other and even between neighboring cells. Light response is also known to exhibit fundamental changes in sensitivity as development processes. Meanwhile, stomata differential is promoted by light in both the growths arrested hypocotyls and the expanding cotyledon (Albrecht and Xing, 1996).

In bougainvillea, light intensity, day length and temperature are major factors influencing the rate of development and therefore, initiation of flowers. Kent *et al.* (1997) reported that bougainvillea would flower sooner and more profusely with high light intensities. They also reported that minimal 8-11 hours day length with temperatures above 58-64° F was suitable for flowering. Generally, shade inhibit flowering in bougainvillea. In low light environments, bracts have a lighter color than those grown in full sunshine.

Maria and Domenico (2006) found that the plants was grown with a continuous photon flow rate of 125 mmol/m^{2}s, provided by Cool White (CW), or by Gro-Lux and Gro-Lux wide spectrum agricultural lamps (GRO and GRO/WS). There were no differences in cell concentration and growth rates between GRO and GRO/WS-grown cultures, but biomass was significantly higher with GRO lamps.

Nicolas ***et al.*** (2008) reported that daily averages of canopy conductance and photosynthesis were not affected by shading; however, the daily transpiration was reduced in shaded plants, which displayed an increase in water use efficiency compared to the exposed trees. The decoupling coefficient was higher in the shaded trees, indicating that the transpiration of lemon trees was efficiently controlled by stomata in exposed plants, while the transpiration rate was mainly influenced by radiation in the plants growing under the net. This influence was more pronounced in the afternoon, when the whole tree transpiration was largely dominated by equilibrium transpiration in the plants under netting, and the relationship between transpiration and radiation showed a steeper slope in shaded trees.

Of the experimental treatments, whole-plant exposure to upwardly directed light was the most effective treatment for elongating internodes and petioles and in stimulating changes in leaf angle. These results were helpful in developing future plant growing systems where the light environment was manipulated to regulate plant development (Heather *et al.,* 1997).

Under low light levels, sucrose and protein allocation to apical shoots in plants with low biomass ratios was suggested a preferential distribution of resources directed to feed the high metabolic demands of apical shoots as well as to stimulate the shoot recruitment (Irene *et al.,* 2007). Thus, the acclimatory response of plants especially under low light conditions, seemed to be modulated by clonal traits, that is, the dominance of apical shoot over nearest ones and clonal integration of resources among different modules or plant parts.

Guo and Marinus, (1999) showed that the deciduous species *Q. aliena* var. *acuteserrata* had many characteristics in common with less shade tolerant (or competitive) plants and the evergreen species *C. multinervis* had many characteristics of shade-tolerant plants. The shade-tolerant plants had a positive effect between 23% and 38% shade cover and yield was then maintained up to 48%. Production might decrease under shade cover 50%. The role of ecological features associated with shade on yields and availability of natural resources obtained from coffee systems were discussed (Lorena *et al.,* 2000; Tetsuro *et al.,* 2009). The effect of three shade levels on morphology, growth and productivity of 'Grand Nain' bananas during their first and second production cycles was studied in the Jordan Valley (Israeli *et al.,* 1995). The resultant photosynthetic photon flux density (PPFD) was reduced to 80%, 60% or 30% of the un-shaded control. Although only the heaviest shade affected plant vegetative growth in the first cycle, bunch weight was reduced by 7% and 32% under medium and heavy shade, respectively. A highly significant effect on vegetative growth and production was observed during the second cycle. Flowering date was delayed by 6 days, 9 days and 15 days, and bunch weight was reduced by 8%, 21% and 55% under light, medium and heavy shade, respectively. Yield was reduced by all levels of shade. Shading reduced the rate of leaf emergence, leaf and foliage area, plant height and pseudostem circumference. The leaves had thinner laminae, with a reduced number of stomata and higher chlorophyll content (Israeli *et al.,* 1995).

Yajuan *et al.* (2009) showed that plant attained greatest leaf size when cultivated with 67% shade. Leaves of seedlings grown with 90% shade were the smallest. Leaf color of plants grown under full sunlight and 50% shade was yellowish-green. Full sunlight and 50% shade treatments resulted in significant reduction of electron transport rates (ETR) and increased nonphotochemical quenching of chlorophyll fluorescence (NPQ). Chlorophyll *a*, chlorophyll *b* and total chlorophyll content increased and chlorophyll *a*/*b* values decreased with increasing shading. Researchers developed a sub-individual-based simulation model, cast at the level of interacting plant components. The model explicitly simulated growth, development and competition for light at the level of leaves and branches (Ben and Bullock, 2007).

The growth versus irradiance response of the sea grass *Zostera noltii* from Cadiz Bay Natural Park (southwestern Spain) was characterized. Plants were exposed along 14 days to different light treatments (1%, 7%, 42% and 100% surface irradiance, SI), using shade screens in an outdoor mesocosm. Growth at 100% SI (1.6 mg) was lower than that at

42% SI (2.4 mg), suggesting photoinhibition. Light availability affected the pattern of plant development and the overall plant growth. The possibility of a light threshold for rhizome branching could explain the seasonality of shoot recruitment, as well as the observed decrease in shoot density along depth (or light) gradients in sea-grass meadows. Carbon demands at low irradiances were partially met by mobilization of carbohydrate reserves (sucrose in belowground and starch in aboveground parts). Plant nitrogen content decreased with increasing light, especially in belowground parts, reaching critical levels for growth (Peralta *et al.,* 2002).

Nazrul *et al.* (2005) examined the effects of different photoperiod and light intensity on floral initiation, development and subsequent growth of *Eustoma grandiflorum*. Both the photoperiod and daily light integral strongly influenced the subsequent growth after initiation. Short day delayed the time to visible bud (VB), increased the number of nodes to first open flower, number of branches, stem diameter and shoot dry weight compared to long day. High light promoted flowering and increased several shoot characteristics and flowering compared to low light.

The effect of pre and post-anthesis shading on pericarp development, cotyledon cell number and seed growth dynamic of fruits from three positions in the capitulum (peripheral, mid and central) of two sunflower genotypes were studied by Lindstrom *et al.* (2006) at two locations. Both shading treatments reduced pericarp weight, fruit volume and total yield per plant. Plants shaded during pre-anthesis maintained the number of filled fruits but reduced their individual weight and cotyledon cell number in the three positions on the capitulum.

Red-light irradiation effectively inhibited the decrease in chlorophyll levels of the leaves that unfolded prior to storage (old leaves, strawberry plants). However, the decrease in both reducing sugar and sucrose levels of the roots, crown and old leaves was not inhibited by red-light irradiation, and the values were the same as those for plants in continuous darkness during the entire experimental period. Leaves that were newly grown in continuous darkness had smaller areas, lower chlorophyll levels and lower photosynthetic rates compared to those grown under red-light irradiation when measured at the end of storage (Takashi *et al.,* 1997).

Seeding of ash (*Quercus robur L.)*, beech (*Fagus sylvatica L.),* oak (*Quercus robur L.*) and sycamore (*Acer pseudoplatanus*) trees were grown for four years under shade netting which gave approximately 30%, 60%, 80% and 100% of full sunlight; plants were maintained weed free, fertilized, and irrigated as necessary. The greatest total numbers of shoots were clipped from beech and oak but the greatest total dry weight was from ash, both numbers of shoots and dry weights clipped at each harvest declined during each growing season and as shade increased. Stem diameter and increment were also reduced by shade. Overall growth of these clipped seedlings was adversely affected by 60-80% shade, oak and sycamore appearing to be the most affected (Ralph, 1999).

2.6 Behaviors of Stomatal Conductance

Stomata are the primary structures that exchange water and CO_2 between plants and the atmosphere. Therefore, stomatal conductance is an important factor in the cycling and balancing of water, CO_2 and energy between plants and the atmosphere. Stomatal conductance is the measure of the rate of passage of carbon dioxide or water vapors through the stomata of leaf. Stomata are small pores on the top and bottom of a leaf that are responsible for taking in carbon dioxide and expelling moisture from and to the outside air, respectively (Ainsworth and Rogers, 2007; Konrad *et al.,* 2008).

The degree of opening of stomata, as measured by the stomatal conductance is a vital parameter that determines the rates of photosynthesis and transpiration by all types of plants including those found in natural forests. A characteristic feature of natural forests is the heterogeneity and variability of the environmental conditions experienced by the plants, both spatially and temporary. The way that different species co-existing within a forest respond to the above variation and heterogeneity of the environment determines their survival and the degree of dominance that they would achieve in the succession process (Atsushi and Tsuneo, 2008; Ueno and Seiwa, 2003).

Ability of the stomata to respond rapidly to variations in environmental factors such as light intensity, temperature, humidity and wind make them ideal organs though which forest plants respond and adjust their functioning to rapid fluctuations in the environment. This ability is especially crucial for survival and growth of natural forest plant species because key physiological process such as photosynthesis of these species occur mostly under rapid-fluctuating transient environmental conditions rather than stable, steady state conditions (Guangxiu *et al.,* 2009; Katul *et al.,* 2003).

The primary determinant of stomatal movements at the cellular level is the water content of guard cell. Guard cell water content is determined by water status of leaf which is in turn determined by transpiration stream through the soil-plant-atmosphere continuum. Hence, it is hypothesized that leaf water status, measured as the leaf water potential, may play a crucial role in determining stomatal conductance in a variable environment (Royer, 2001; Lamaud *et al.,* 2009; Guangxiu *et al.,* 2009).

Therefore, it is important to investigate the environmental factors that determine the stomatal conductance and leaf water potential of different tree species found in natural forests (Jessen *et al.,* 2007). There is a significant inter-species variation in how different plant species respond to ever the same perturbation in the external environment. For example, the response to an increase in the incident light intensity may be substantially-positive for stomatal conductance in pioneer and early successional species. On the other hand, the corresponding response may be insignificant in late successional and climax species (Susana *et al.,* 2007; Atsushi and Tsuneo, 2008).

2.7 Effects of Potassium on Plant Growth

A source of potassium in the soil is organic matter and within the soil microbial population. This soil source of potassium provides little of the potassium needed for plant growth. Potassium uptake is most rapid on warm, moist soils that are well aerated and have a slightly acidic to neutral pH. As soil temperature increases, plant metabolic activity increases which increases root growth and root activity. Warmer soil temperatures also increase the diffusion rate of potassium in the soil solution which increases potassium uptake by the root system (Basile *et al.,* 2003; Bednarz and Oosterhuis, 1999).

Potassium deficiency was negatively affected by tree light interception. Defoliation after mechanical harvesting indicated that potassium deficiency accelerated premature leaf senescence and premature leaf abscission. The CO_2 exchange rates were curvilinearly related to leaf potassium concentration during mid-summer after most vegetative shoot growth was completed. Large variations in leaf potassium concentration were found in the non-fertilized trees. Indeed, most of the response curve of leaf CO_2 exchange rate to leaf potassium concentration could be described by analyzing the leaves (Bednarz and Oosterhuis, 1999; Bednarz *et al.,* 1998).

The plant nutrient element potassium plays a key rule for regulating the stomatal behaviors as well as plant physiology. Therefore, this issue discusses the importance of potassium as a key plant nutrient and problems associated with excess and/or deficiencies of potassium in the plant. Many plant physiologists consider potassium as a second to nitrogen in plant tissue levels with ranges of 1% to 3% by weight. As a trivia, potassium is the only essential plant nutrient that is not a constituent of any plant part (Sringarm *et al.,* 2009). Potassium is a key nutrient in the plants tolerance to stresses such as cold/hot temperatures, drought, wear and pest problems. Potassium acts as catalysts for many of the enzymatic processes in the plant that are necessary for plant growth to take place. Another key role of potassium is the regulation of water use in the plant (osmoregulation). This osmoregulation process affects water transport in the xylem, maintains high daily cell turgor pressure which affects water tolerance, affects cell elongation for growth and most importantly it regulates the opening and closing of the stomates which affect transpirational cooling and carbon dioxide uptake for photosynthesis (Cheng and Fuchigami, 2000).

Leaf photosynthetic rate has been shown to vary with potassium status in several herbaceous crop species and woody ornamental species (Egilla and Davies, 1995). Although the relationships between leaf nitrogen and phosphorus concentration and leaf photosynthetic responses have been studied extensively in deciduous fruit tree species, the relationship between leaf potassium concentration and leaf photosynthesis has received less attention (Minoru *et al.,* 1998; Osaki *et al.,* 1993).

2.8 Bougainvillea Plant is Ethylene Sensitive

Traditionally, flowers (like fruits) are categorized as being climacteric or non-climacteric. In climacteric or ethylene sensitive flowers such as carnations, Gypsophila and orchids, senescence is accompanied by a sudden, transient increase in ethylene production and respiration while treatment of non-senescent flowers with ethylene rapidly induces petal senescence. In non-climacteric flowers such as gladiolus, tulip and iris, generally, no increases in ethylene production and respiration are apparent during flower senescence, and exogenous ethylene has a little or no effect on petal senescence (Serek *et al.,* 2006). Bougainvillea has sensitivity to ethylene, with symptoms such as flower, bud and leaf abscission, bud abortion, rapid flower senescence and short vase life or longevity (Chang and Chen, 2001). Knowledge about ethylene sensitivity of flower species is necessary to predict the effects of e.g. mixed storage and transport of flowers with fruit species, to predict the usefulness of anti-ethylene treatments and to direct breeding programs towards better flower vase life. With respect to petal senescence, sensitivity to ethylene was found to be roughly determined at the plant family level (Woltering and Van Doorn, 1988).

Depending on the species, ethylene, either applied or endogenously produced, may induce various processes (Woltering and Van Doorn, 1988). In many flowers, ethylene hastens senescence of petals that initially stay attached to the flower. In other flowers, ethylene may induce abscission of fully turgescent, non-senescent petals or of whole corollas. In contrast to petal senescence, which is also found in ethylene insensitive species, ethylene insensitive abscission of petals seems extremely rare in plant kingdom (Serek and Reid, 2000).

The above examples clearly underline that the distinction between ethylene sensitive and insensitive senescence needs to be handled with care for better flower vase life. Controlling ethylene effects through interference with ethylene perception may therefore be very beneficial in a variety of cases, both in “ethylene sensitive” and “ethylene insensitive” flowers. In ethylene sensitive species ethylene generally causes petal senescence and/or abscission of petals or whole flowers; in insensitive species ethylene may still have undesirable effects on e.g. proper flower opening (Van Doorn, 2001; Serek *et al.,* 1994).

2.9 Flower Longevity and Vase Life

The flower shelf life or longevity in general varies greatly among different species. The shelf life and commercial value of many potted plants are seriously affected when subjected to dark transport or low-light indoor conditions, which often induces flower and leaf abscission (Tjosvold *et al.,* 1994). In the case of bougainvillea, same results were observed when placed in long periods of cloudy or rainy weather or dim indoor lighting (Chang and Chen, 2001). Wein and Turner (1989) pointed out that dim lighting induced ethylene evolution of bell pepper, thereby causing flower bud abscission. Ethylene also induced flower and bud abscission in some flower crops, such as *Impatiens hawkeri* (Dostal *et al.,* 1991). Some flowers are generally treated with NAA to prevent lower senescence. Cameron and Reid (1983) reported that NAA could reduce flower abscission in zygocactus, slipperwort, and bougainvillea when these potted plants are subjected to environmental stress. Cushman and Pemberton (1994), and Tjosvold *et al.* (1994) showed that spraying STS on miniature roses before transportation could prolong flower longevity. Experiments on *Easter cactus* (Han and Boyle, 1996) showed that STS was more effective when applied during early flower development. The auxin, 1-naphthaleneacetic acid (NAA), has been shown to prevent flower bud abscission in roses (Halevy and Kofranek, 1976) and peppers (Wein and Zhang, 1991). For bougainvillea, Hackett *et al.* (1972) indicated that the drop of mature flower-bracts could be delayed 2-3 weeks by applying NAA at the time of bract opening. They concluded that the effects of NAA were almost always positive at different flower-bract maturity stages.

2.10 Flower Senescence

Senescence is distinct from ageing. Senescence may be simply defined as those changes that lead eventually to the death of an organism or some part of it. The term senescence and aging should not be confused. Aging refers to processes accruing maturity with the passage of time (Ferrante *et al.,* 2002). In the case of orchid flowers, this would include changes in all segments, essential and non essential and occurs gradually as a degradative process. While senescence is the phase (the final phase) of the aging process that lead to death (Ichimura, 1998.). Senescence can also be defined as the deteriorative processes those natural causes of death. Hence, a flower lifespan and post production quality is determined by senescence, a genetically controlled stage of flower development that culminates in the wilting or abscission of the corolla (Ichimura and Goto, 2002.). From their findings, one could deduce that by slowing down the senescence process, it would delay the wilting process and therefore prolong the longevity of the flower. Senescence would be a highly regulated death, comparing with ageing process, ageing depends on the general failure of the flower where senescence depends on the deteriorate process of the flower due to specific signals (Redman *et al.,* 2002). For cut flower industry, one could first distinguish between the two distinct stages in the physiology of the flower. The first growth would be flower growth and development of the plant to full opening. The second stage would be of maturation, senescence and wilting (Kende and Baumgartner, 1974). On the other hand, Elgar *et al.* (2003) defines senescence in second stage which is also the final phase in the ontogeny of the organ, in which a series of normally irreversible events is initiated that it is the end of flower life (Chooruut and Kanlayanarat, 2002; Ting *et al.,* 2008).

Li *et al.* (2007) suggested that the following events are to take place during senescence of flower. At first, changes happen in membranes, including changes in micro-viscosity and phase-transition temperature. This is followed by a rise in ethylene production and subsequent loss of membrane permeability reflected in increased ion and water leakage (Nair *et al.,* 2002). As a result of excessive water loss, the flower weight was reduced and wilting symptoms appear as the tissues desiccate.

The abscission point of a flower organ showed the first sign of wilting or fading to the total death of flower petal (Kosugi *et al.,* 2000). Usually the first sign of senescence are indicated by severe loss of flower color which signals the ends of bench-life.

2.11 Hormonal Effects on Bougainvillea

2.11.1 Effects of GA_3 Hormone

The transition from vegetative to reproductive growth is a critical event in the life cycle of plants. Plant hormones play an integral role in controlling the growth, development, metabolism and morphogenesis of higher plants (Claus, 2008). Auxins, gibberellins, cytokinins, ethylene and abscisic acid are well known plant hormones. However, growth hormones differ from each others considerably in their mode of actions (Goro *et al.,* 2001; Andrea *et al.,* 2004).

During flower development, GA_3 was found to be essential for the development of stamens and petals (Claus, 2008, Saifuddin *et al.,* 2009). High concentration of GAs showed a positive role on flower formation in olive during induction and initiation period. In addition, the application of gibberellic acid (GA_3) has the potential to control growth and flowering and induce earliness of meristem. In strawberry, GA_3 application increased petiole length and leaf area. It reduced the time needed for inflorescence emergence, accelerated flowering and increased the number of flower buds and open flowers in most growing conditions (Khan and Chaudhry, 2006; Sharma and Room, 2009).

Application of GA_3 promotes flowering in a range of plant species. Many species that flower early in response to GA_3 also flower early in response to long days or vernalization, raising the possibility that GA_3 may be involved in these responses (Mariko *et al.,* 2001). This response to exogenous GA_3 is paralleled to the effect of photoperiod and vernalization on GA_3 metabolism. Brooking and Cohen (2002) observed that high gibberellin doses (1000 mg l^{-1} GA_3) induced more rapid floral initiation than lower doses (10 mg l^{-1} GA_3). In addition to their role in floral initiation, gibberellins played a role in floral development. Uptake of gibberellin was shown to be primarily through the buds on the upper surface of the tuber. The proportion of shoots that initiated inflorescences increased with an increase in both gibberellin concentration and treatment duration. Increased gibberellin dose also increased in the proportion of initiated flowers that subsequently emerged and the proportion of axillary buds that initiated flowers. Plants treated with high gibberellin doses also flowered earlier and with a lower leaf number (Xingjun *et al.,* 2003).

Ogale *et al.* (2000) inferred that GA_3 spraying changed the mode of action, by increasing the flower size to varying degrees (20- 40%) in all *Portulaca grandiflora* cultivars. As the percentage of changed flower colors was altered in the subsequent clonal generation vis-à-vis the effect also diminished with the passage of time.

Hye and William (2009) reported that GA_{4+7} were a potentially useful compound in horticultural practice to enhance postproduction quality of many cultivars of pot tulips. It enhanced the quality of pot tulips by improving flower longevity and reducing leaf yellowing, although proper concentrations should be determined for each tulip cultivar. GA plus BA also had a strong effect on enhancing flower longevity when sprayed at the mature (fully colored) bud, and a lesser effect when applied to immature (green) buds (Hye and William, 2008).

The flower is a heterogonous organ, composed of floral parts each of which may be at a different physiological development stage. Gibberellic acid is an endogenous phytohormone present in different concentrations in different floral parts and developmental stages. Gibberellic acid (GA_3) significantly increased the number of disc florets open because GA_3 decreased the dry matter in the flower heads and stems. Gibberellins are reported to increase hydrolysis of starch and sucrose into glucose and fructose which were utilized by flowers for disc floret opening, hence a reduction in dry matter contents in the flower heads stems (Fernfindez *et al.,* 1997). The increased reducing sugars in the flower heads and stems of gerbera flowers may increase the osmotic potential of the flower head and stems, thus improving their ability to absorb water and maintain their turgidity, which may explain the increase in water contents of gerbera cut flower heads and stems. Maintenance of turgidity is an important in extension of longevity of gerbera cut flowers. The final stages of flower development are characterized by a decline in the content of carbohydrates and dry weight of petals (Chert *et al.,* 1994; Xingjun *et al.,* 2003).

2.11.2 Ethylene Effects on Flowers Longevity

Ethylene is the simplest organic compound. Its structural simplicity and the fact that it is gaseous in nature and make it a unique plant hormone (Arshad and Frakenberger, 2002). The effect of ethylene on plant growth and others cell activity was noted as early as 1858 by the behavior of plants exposed to illuminating gas. Nevertheless, the Russian scientist identified the active growth-regulating component of the illuminating gas as ethylene. In presence of ethylene, etiolated pea plants exhibit inhibition of elongation, an increase in diameter, and horizontal growth of shoots. Wei and Chi (2009) reported that gases released from oranges caused premature ripening after that many evidence has cleared the idea that ethylene was produced autocatalytically. Its production is regulated by a number of developmental factors. Ethylene production is induced by many physiological and chemical stressess at germination, ripening of fruits, senescence of leaf and flowers (Kazuo *et al.,* 1998).

Besides acting as a plant hormone influencing many aspects of plant growth and development, it is also one of the natural occuring plant growth regulatory substances (PGRS) or famously known as phytohormones (Bleecker and Kende, 2000). This simplest carbon atom compound is a powerful regulator of plant metabolism, acting, and interacting with other plant hormones in trace amounts. Ethylene is the key phytohormone in promoting senescence in cut flowers and causes fruit ripening. This directly reduces quality and display life of flowers.

The effect of ethylene could be traced back in scientific literature as early as nineteen century when a toxic illuminating gas was said to have caused radial swelling of the stem, inhibiting of the stem elongation and absence of normal geotropic response in etiolated pea seeding grown in a laborary (Sunjoo and Woo, 2007)

Ethylene is a natural plant product that induces senescence in plants. This hormone is known to play a key role in regulating the biochemical and anatomical changes that constitute the post-pollination syndrome in Phalaeopsis (Beatriz and James, 2008; Serek and Reid, 2000). It acts by inhibiting respiration, decreasing cell wall material and lipids, and increasing sugar levels of the plants. In general, it appears to hasten senescence plant organs and accelerate the abscission process (Redman *et al.,* 2002; Reid *et al.,* 2002; Xu and Hanson, 2000).

Investigation by other researchers also proved that ethylene production increased during flower senescence and ethylene accelerates flower senescence (Celikel *et al.,* 2002). It is devided into three phases which corresponds to the three phases of senescence. The first phases is the pre-climacteric phase, the concentration of ethyene metabolized by the tissues is extremely a little and the respiration rate is a stable (Kosugi *et al.,* 2000). Ethylene production occurus in all plant organs including flower but the magnitude of its production varies and depends of growth and development process. Recent scientific process has made clear the understanding of biosynthetic pathway and enzymes involment in ethylene production (Kenza *et al.,* 2000).

This autocatalytic effect of ethylene could be very pronouced and lead to loss of quality during transporation and storage (Van Door, 2001). Among those are as follows: 1) stimulation of ripening in fruits and vegetables, 2) leaf abscission, 3) fading in flowers, 4) leaf yellowing 5) flower wilting and 6) leaf epinasty (Yamane *et al.,* 2004).

Ethylene is involved in the senescence of many ornamental flowers, including bougainvillea which shows a climacteric-like rise in ethylene production before the abscission of bracts, and bracts are abscised by exposure to exogenous ethylene (Ichimura *et al.,* 2000). The vase life of bougainvillea flowers could be extended by silver thiosulfate (STS), an inhibitor of ethylene action (Uda *et al.,* 1997; Chang and Chen, 2001). Their findings suggested that ethylene regulated sepal abscission in bougainvillea. Abscission is a typical ethylene response that is perceived by ethylene receptors and is influenced by mutations in ethylene receptor genes (Patterson and Bleecker, 2004). One of the most important traits of ornamental plants is flower longevity, and the lengthening of longevity is an ongoing target for flower breeders (Onozaki *et al.,* 2001).

Ethylene is produced by many plants that have been studied and essentially by all parts of the plant at different levels (Antunes and Sfakiotakis, 2002.). Ethylene biosynthesis is regulated by developmental process as well as by numerous external stresses. This increased ethylene production might be due to higher amounts of mRNAs which are responsible to produce enzymes and hence the ethylene synthesis (Kim *et al.,* 1999). Ethylene is synthesized from methionine via S-adenosy-L methionine (AdoMet) and 1-aminocyclopropen-1-carboxylic acid (ACC) and is known to regulate various physiological processes during plant growth and development, including flower development and senescence (Ben *et al.,* 1999). The biosynthesis of ethylene involves three enzymes, namely, SAM synthesis, ACC synthesis and ACC oxidase. Therefore, the rate of ethylene production is determined by the rate of ACC synthesis.

2.11.3 Synthetic Auxin- NAA Works as Ethylene Inhibitor

A range of methods and chemicals has been documented for preventing the deteriorative effect of ethylene on postharvest characteristics of ornamental crops. Interfering with the plant's response to ethylene can in principle be achieved by: 1) inhibition of the plant's own ethylene production; 2) blocking the binding of ethylene to its receptor; 3) by blocking the plant's reaction to the binding of ethylene to the receptor. Several ethylene antagonists have been discovered during the last 30 years and some of them have successfully been used by the floral industry to block ethylene responses considering toxicity level of these chemical. Common used chemical is silver thiosulfate (STS) but its toxicity is high. Now, another chemical, naphthalene acetic acid (NAA) is mostly used due to its low toxicity level (Dimitrios *et al.,* 2008; Chang and Chen, 2001).

This colorless solid compound (NAA) is soluble in organic solvents. It features a carboxyl ethyl group linked to the "1-position" of naphthalene. NAA is a plant hormone in the auxin family and is an ingredient in many commercial plant rooting horticultural products. It is a rooting agent and used for the vegetative propagation of plants from stem and leaf cutting. The toxicity believed to be low up to date and its degradation behavior is still not clear; however EPA regulations decided a Reregistration Eligibility Decision and Amendment in December 2007 to establish new tolerance limits for this pesticide in fruits (Dimitrios *et al.,* 2008). In order to get appropriate biological effects, this compound must be applied at the concentrations of 20–100 mg/ml using spraying solution (Fernandez *et al.,* 2005). However, the same reports provided the information that NAA is extremely hazardous in skin contact; the amount of tissue damage depends on length of contact and eye contact can result in corneal damage or blindness (http://www.sciencelab.com). It is therefore important to develop simple and effective methods to monitor the level of NAA in foods, fruits and vegetables (Zhen *et al.,* 2008).

Synthetic auxins, like naphthaleneacetic acid (NAA) are used extensively to promote root formation in stem and leaf cuttings (Gregory *et al.,* 2001). Gardeners often spray NAA on tomato plants to increase the number of fruits on each plant. When NAA is sprayed on young fruits such as apple and olive trees, some of the fruits drop off so that the remaining fruits grow larger (Brent *et al.,* 1995). NAA is sprayed directly on maturing fruits apples, pears and citrus fruits several weeks before they are ready to be picked. NAA prevents the fruits from dropping off the trees before they are mature. The fact that NAA can have opposite effects, causing fruit to drop or preventing fruit from dropping, illustrates an important point. The effects of this hormone on a plant often depend on the stage of the plant's development (Normanly, 1997; Black *et al.,* 1995).

NAA is used to prevent the undesirable sprouting of stems from the base of ornamental trees. As previously discussed, stems contain a lateral bud at the base of each leaf. In many stems, these buds fail to sprout as long as the plant's shoot tip is still intact.

The inhibition of lateral buds by the presence of the shoot tip is called apical dominance. If the shoot tip of a plant is removed, the lateral buds begin to grow. If NAA is applied to the cut tip of the stem, the lateral buds remain dormant. This adaptation is manipulated to cultivate beautiful ornamental trees. NAA is used commercially to prevent buds from sprouting on potatoes during storage (Chang and Chen, 2001). Studies of the effects of NAA on stomata have a long history. The subject did not assume prominence in the minds of stomata physiologists until recently. The effects on the stomata were direct or indirect was not established in most of experiment. Transpiration was usually used to indicate stomatal opening, and the effects of the NAA on photosynthesis or respiration, or both, could have altered the CO_2 concentration in leaf to bring about a change in stomatal aperture. Synthetic auxin, NAA, can stimulate respiration.

At low concentrations, NAA can inhibit ethylene formation and the transport of precursor in plants (Zhen *et al.,* 2008). High concentration can induce femaleness of flowers in some species. It inhibits abscission prior to formation of abscission layer and thus inhibits senescence of leaves. NAA plays a minor role in the initiation of flowering. It can delay the senescence of flowers in low concentrations (Chang and Chen, 2001). The spectacular effects of STS in overcoming the deleterious effects of ethylene in potted flowering plants (Cameron and Reid, 1983) have led to widespread commercial use of this chemical (Veen, 1983). Because STS contains silver, which is seen as a potential environmental pollutant, its use for potted flowering plants has been restricted in some countries (Serek and Reid, 2000). Researchers have therefore been searching for alternative strategies for preventing ethylene damage in potted plants, including using ethylene biosynthesis and binding inhibitors (Serek *et al.,* 1994; Sisler and Serek, 2003).