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覆盆子在亞熱帶氣候的產期調節與空心莓亞屬植物高溫光合生理

Scheduling Raspberry Production in Subtropical Climate and Photosynthetic Heat Tolerance Physiology of *Rubus subgenus Idaeobatus*

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摘要 (Chinese Abstract)

覆盆子(*Rubus idaeus* L.)為源自於溫帶地區的多年生小果類作物，近年來國內鮮果需求快速成長，多由美國空運進口供應。臺灣等亞熱帶氣候區在以往因為夏季氣溫過高與冬季低溫不足，並不適合傳統二年生枝條結果型(floricane-fruiting)品種的生產，然而當年生枝條結果型品種(primocane-fruiting)的普及化，提供了覆盆子在亞熱帶地區生產的契機。本論文嘗試利用產期調節方式解決覆盆子在亞熱帶種植時，當年生枝條開花節位數減少使得產量低落的問題，並探討臺灣原生空心莓亞屬植物與覆盆子在夏季高溫下光合作用差異的生理機制，提供未來耐熱育種篩選與栽培參考。

本論文第二章之產期調節試驗於 2016 年 10 月至 2017 年 2 月進行，嘗試利用枝條彎曲及暗中斷處理，誘導溫室內之覆盆子 'Summer Festival'植株當年生枝條之低節位萌芽與二次採收，結果顯示兩種處理方式皆無顯著效果，各處理組內標準差大。所有處理之萌芽皆集中於當年生枝條第 20-40 節位，開花數量亦最多。試驗期間因溫室相對濕度過高，導致授粉不良與真菌性病害嚴重等問題，是未來在亞熱帶環境中之溫室生產覆盆子必需要克服之處。

本文第三章之空心莓亞屬光合作用生理試驗於 2017 年 7 月進行，比較溫帶覆盆子及三種原生於台灣亞熱帶平地的空心莓亞屬植物，刺莓(*R. rosifolius*)、薄瓣懸鉤子(*R. croceacanthus*)與愷葉懸鉤子(*R. fraxinifolius*)之光合耐熱生理。試驗以不同溫度(25、30、35℃)環境，測量葉片氣體交換與葉綠素螢光之光反應曲線及二氧化碳反應曲線(A/Ci curve)後，利用 FvCB 光合生化模型，進行參數擬合，並以數值積分法量化各參數對於淨光合作用速率變化的貢獻程度。在 35℃ PPFD=1200 μmol·m^{-2}·s^{-1} 下，覆盆子的淨光合作用速率最低，且與其他三種原生種差異最大。以 FvCB 參數進行量化後可得知光合生理之擴散性因子是造成此

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差異的主要原因，氣孔導度與葉肉細胞導度分別佔總差異一半的貢獻度；生化因子如最大羧化速率與最大電子傳遞速率在所測試的 4 個物種中，皆隨溫度上升而提高，對物種間高溫下淨光合作用的差異影響不明顯。另由 35℃下，葉面溫度與氣溫之差值，也顯示高溫下原生種具有較覆盆子佳的葉片蒸散散熱機制。

關鍵字:覆盆子、產期調節、高溫逆境、光合作用、FvCB 模型

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Abstract

The red raspberry (*Rubus idaeus* L.) is a perennial berry crop originated in the temperate area. In recent years, the demand for fresh raspberry has been increasing in Taiwan and mostly supplied by import products from the USA. Traditional florican-fruiting raspberry cultivars are difficult to grow in the subtropical climate due to heat stress in summer and lack of chilling in winter. However, the new primocane-fruiting cultivars has brought up the possibility to produce raspberries in the warm climate. In this thesis, scheduling techniques to increase the number of flowering laterals on primocanes were tested. In addition, photosynthetic heat tolerance mechanisms of raspberries were investigated by comparing a primocane-fruting cultivar with three relative subtropical species native to low land Taiwan.

In chapter two, a scheduling production trial was conducted from October 2016 to February 2017. Shoot bending and night breaking treatments to induce lateral shoots on primocanes and winter harvest were tested in raspberry 'Summer Festival' grown in a greenhouse in Taipei. The results showed that neither treatments promoted flowering or lateral development. Most laterals were emerged from 20^{th} -40^{th} node on the primocanes and had the highest flower number per lateral. During the experiment period, the high relative humidity in the greenhouse resulted in poor pollination and servere fungus disease.

In chapter three, photosynthesis of the raspberry and three native subtropical species in the same subgenus *Idaeobatus* were evaluated in July 2017. Gas exchange and chlorophyll fluorescence were simultaneously measured at 25, 30, or 35℃. Light response curves and CO_2 response (A/Ci) curves were obtained and key

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photosynthetic variables were fitted with a modified FvCB model. Differences in net assimilation rate among different species and environmental factors were partitioned into the correspondence of each variable by a numerical integration method. At 35℃ under PPFD=1200 μmol·m^{-2}·s^{-1}, the raspberry had the lowest net assimilation rate. The results from partitioning indicated that diffusional factors in the FvCB model were the main contributor to the differences, and stomatal conductance and mesophyll conductance each contributed about 50% of the total difference. Biochemical factors such as the maximum value of carboxylation and electron transportation rate increased in all species as the temperature increased and their contributions to net assimilation differences were little. Temperature differences between leaf surface and the air showed that the native species had a more efficient transpiration cooling mechanism at 35℃ than the raspberry.

Key words: raspberry, scheduling technique, heat stress, photosynthesis, FvCB model

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Chapter 1 Literature review and hypothesis

1.1 Introduction

The red raspberry (*Rubus idaeus* L.) is a temperate species in the subgenus *Idaeobatus* of *Rubus*, a very diverse genus in the family *Rosaceae*. The raspberry is a popular deciduous berry crop with high commercial value and great health benefits. The demand for raspberry fruit has been growing strong worldwide and at least 50% of the production have redirected from processing to fresh fruit market (Hall and Sobey, 2013). Major production areas of raspberries concentrate in the high latitude region. The top three production countries in 2016 were Russia Federation, the United States of America, and Poland. Overall, 62.7% of the world production were harvested in Europe and 35.5% from America. In recent years, production of raspberries in the relatively low latitude area such as Mexico, Spain, and Portugal has been popular for the off-season market (FAO stats 2016). There is no commercial raspberry fruit production in Taiwan but the demand for fresh and frozen raspberry fruits is growing in the past few years and is supplied mostly by imported products from the USA with a high average price up to 600 NT/kg for fresh fruit (Customs Administration, Ministry of Finance 2016). Fresh raspberries are very perishable with an extremely short shelf life. In addition, quality of imported berries is poor due to early harvest (Sjulin and Robbins, 1987). Local raspberry production in Taiwan may have advantages on improved fresh fruit qualities and reduced carbon footprint of the berry industry in the future.

However, growing the temperate-originated raspberries in the subtropical area has several challenges. Two of the main constraints in the subtropics for most of the commercial raspberry cultivars are the hot, humid summer climate in and the insufficient winter chilling hours (Ballington and Fernandez, 2008; Hall and Kempler,

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2011).

1.2 Scheduling primocane-fruiting raspberry in subtropical lowlands

1.2.1 Biology

Raspberries are perennial woody plants with a biennial cane growth pattern and a perennial root system. New canes developing from the underground root bud or crown bud in the spring are called primocanes and the overwintered second-year-old canes are called floricanes. After the floricanes set fruits in the summer of the second year, their life cycle ends and the canopy is replaced by the new primocanes (Hudson, 1959).

Raspberry cultivars can be categorized into two major groups by their different flowering habits. The traditional cultivars were all floricane-fruiting (biennial-fruiting, summer-fruiting, or non-remontant) type that produces flowers only on the second-year-old floricanes. The initiation of flowers in the primocanes and the cessation of cane growth occur simultaneously in autumn when temperature below 15℃ and day length less than 15h (Sønsteby and Heide, 2008). The plant enters dormancy soon after the flower initiation process and chilling is necessary to break the differentiated bud on the floricane. Chilling is also necessary for new primocanes to grow from the crown or root buds. Chilling requirement for most of the floricane cultivars are usually very high (800-1500 CU)(White et al. 1999) and this characteristic makes it almost impossible to grow these old culitvars perennially in the low latitude region where lack of chilling is common in the winter.

On the other hand, primocane-fruiting (annual-fruiting, autumn-fruiting, ever-bearing, or remontant) type raspberries are cultivars that in addition to the typical

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floricane fruiting cycle, produce flowers and fruits on the upper nodes of the primocanes before entering dormancy, which brings up the possibility to grow raspberries in low latitude area with insufficient chilling hours. Flower bud initiation in primocane-fruiting raspberries is not limited by temperature or day length. Instead, these cultivars are able to flower freely when grown in conditions with 9-30℃ under 10-24 hours of day length (Carew et al. 2003, Sønsteby andHeide 2009). After flower initiation, the bud on the top position of the primocanes and the adjacent buds below (depending on environment and genotype interactions, see 1.2.2) develop into flowering laterals and bloom. The fruit, therefore, can be harvested on the primocanes before dormancy. The growth pattern of the rest of the buds on the lower position of the cane is similar to those on the floricane-fruiting type (Heide andSønsteby 2011). Primocanes of the primocane-fruiting cultivars can also grow out from the underground without any chilling. (see 1.2.2).

There are many advantages for primocane-fruiting raspberry cultivars, including autumn fruiting, new primocane development in low chilling conditions, and low chilling requirement for floricanes. In addition, it is possible to harvest only first year crop in fall on the primocanes then apply simple machine pruning instead of selective hand pruning in the winter to save the labor cost when planting primocance-fruiting raspberry (Pritts, 2008). First commercialized primocane-fruiting type cultivars was ‘Heritage’ released in New York in 1969 (Ourecky, 1969). The expansion of raspberry production in low latitude areas has been mostly based on the new primocane-fruiting type cultivars. Most of the current breeding programs have been focusing on primocane-fruiting type raspberries (Hall and Kempler 2011).

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1.2.2 Environmental factors affecting yield and harvest time in primocane-fruiting raspberries

Although primocane-fruiting raspberries are able to flower on the upper nodes of the primocanes without any chilling, yield and harvest time can still be strongly affected by insufficient chilling of root buds and plants at early vegetative stage. Without chilling at the dormant stage for root buds, final height of primocanes increased from 1.5m to 3m and days to flower delayed from 85 to 243 days in 'Heritage' raspberry (Takeda, 1993). Early primocane growth rate was slow without dormant chilling in 'Autumn Bliss' and 'Heritage' raspberries (Carew et al., 2001; Takeda, 1993). Yield on primocanes was low in primocane-fruiting raspberries 'Autumn Britten' and 'Polana' without 6 weeks of chilling at 7℃, and the harvest period was delayed (Dale et al., 2005). Floricanes on the primocane-fruiting raspberries required temperatures below 7℃ for 6-8 weeks to break endodormancy (Dale et al., 2003).

Vernalization in the early growing stage hastens transformation from vegetative stage to flowering stage in raspberries. It was reported that temperatures below 6℃ had vernalization effect on primocanes of raspberry 'Heritage' in the early stage of the growing season (Vasilakakis et al., 1980). In tissue cultured raspberry 'Heritage' plants, relative growth rate and flowering were promoted after plants received chilling treatment (Prive and Sullivan, 1991). Vernalization at the early stage also reduced the final height of the flowering primocanes of raspberry 'Autumn Bliss' (Carew et al., 2001). Young raspberry 'Polka' received vernalization advanced the plants to switch into reproductive stage, and created more but shorter nodes on primocanes than plants without vernalization. However, plants grown in a consistent temperature around 25℃ throughout the vegetative growing stage still had slightly higher total flower

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number than plants grown with 6 weeks vernalization treatments (Sønsteby and Heide, 2009). The environmental effects on flowering in primocane-fruitng cultivars varies among genotypes and is termed level of primocane-fruiting or scale of remontancy, determined by the proportion of reproductive nodes on primocanes, is an important breeding target. Raspberry 'Heritage' without chilling at the early stage developed much less fruiting laterals than plants with sufficient chilling. A breeding program in Chile is trying to create cultivars with high level of primocane-fruiting traits while insensitive to vernalization (Gambardella et al., 2016). On contrary, the yield of raspberry 'Autumn Bliss' did not affected by vernalization (Neocleous et al. 2005, Gambardella et al. 2016). Raspberry 'Erika' and 'Polka' grown at 6℃ for six weeks had lower yield than plants grown at 18℃ (Sønsteby and Heide, 2012).

The most important structural trait for high yield in primocane-fruiting raspberries grown in different temperature and day length conditions is the number of fruiting laterals on the primocanes (Sønsteby and Heide, 2012). The ideal temperature for primocane-fruiting raspberry is around 23℃. For raspberry 'Polka' and 'Erika', growing at 25℃ under 20 hours long day condition in a six-week period of vegetative stage, developed the tallest primocanes and highest yield compared with plants in other temperature and day length treatments. However, the optimum temperature for raspberry 'Autumn Treasure' is 20℃, and showed no significant difference between long (20H) and short (10H) day conditions. Temperature affected the speed of node formation in primocane-fruiting raspberries and day length only affected the length of internodes (Sønsteby and Heide, 2012). Raspberry 'Polka' grown at 24℃ with a long day condition increased yield by increasing number of laterals on primocanes while days to first anthesis was decreased (Sønsteby and Heide, 2009, 2012). Night interruption trials confirmed that the effect of 20-hour day length treatment was a true

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long day reaction rather than the effect of total radiation received by plants (Sønsteby and Heide, 2009). The supra-optimal growing temperature for the fastest vegetative growth and earliest harvest time for raspberry ‘Autumn Bliss’ was 23.4℃ (Carew and Darby, 1999).

In the subtropical lowland of Taipei, winter temperatures fluctuate and seldom drop below 7℃. Temperatures in summer, on the other hand, easily exceed 35℃, which is considered too high for raspberries. Low yield (low proportion of the fruiting laterals) may due to the results of no chilling on the root buds, no vernalization at the early stage of primocane growth , not enough chilling for buds on the floricanes to break endodormancy, and also heat stress in the growing season.

1.2.3 Scheduling techniques in raspberry production

Raspberries can be produced year-round in many areas using scheduling techniques (Oliveira et al. 2002, Dale et al. 2005).

For early harvest, protected facilities and annual long-cane production system can be used. Long-cane plants can be produced by either primocane-fruiting or floricane-fruiting type raspberry. Plants were packed up after finishing the vegetative and flower initiation stages. After receiving a period of artificial or natural chilling to meet the chilling requirement, the plants were transplanted to a warm protected facility to obtain an early harvest in spring (Carew et al. 2000, Darnell et al. 2004, Heiberg andLunde 2008, Sønsteby et al. 2013, Palonen et al. 2015, 2016). Both primocane-fruiting and floricane-fruiting type raspberries can be used in long cane production system. For primocane-fruiting cultivars, raspberry ‘Heritage’ after 1224 chilling hours and transplanted in polyethylene tunnels finished fruiting season 116 days after planting in Florida (Darnell et al., 2004). Higher yield can be obtained by

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warmer locations and later transplant dates in Puerto Rico compare to Florida (Darnell et al. 2006). Root pruning before transplant decreased final yield due to the loss of carbon hydrate reserves in the root. Therefore, it should be avoid in annual production system (Alvarado-Raya et al. 2007, Darnell et al. 2008). Sprouting raspberry 'Heritage' in a plastic greenhouse produced berries one month earlier than plants grown outdoors in northeast Japan (Imanishi 2012, Imanishi and Miyairi 2016). For floricane-fruiting cultivars, very high yield up to 3kg per cane was achieved by using greenhouse to extend vegetative growing season of the primocanes in Norway before artificial chilling (Sønsteby et al. 2013).

For scheduling the late harvest season on the primocanes of primocane-fruiting raspberries, summer pruning is a common method in southern Europe where cultivation in tunnels is the most common production system. Summer pruning after the first harvest delayed the second harvest period by 10 weeks in the early cultivar raspberry 'Autumn Bliss' and by 21 weeks in the late cultivar 'Heritage'. Pruning severity also affects the time of the second harvest. Pruning cut on the 5th or the 15th node created a 3-week interval for the second harvest in raspberry 'Autumn Bliss'. Severe pruning creates long new laterals but low yield (Oliveira et al. 1998). Late pruning dates also decreased yield because of the lower temperature and shorter day length during the lateral development period (Oliveira et al. 1996). In Portugal, year-round production system was achieved by open field production, early long-cane production and late primocane production (Oliveira et al. 2002). For subtropical areas with insufficient chilling, chemical forcing agents such as cyanamide were used for improving bud break (Snir 1986, 1988).

1.3 Photosynthesis modeling in *Idaeobatus* plants in different light and

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temperature environments

1.3.1 Photosynthesis in *Idaeobatus* plants at high temperatures

Net assimilation rate (*A*) in raspberries can be greatly limited by high temperatures and therefore, most of the commercial raspberry cultivars are recommended only for regions with mild summer. The optimal temperatures of *A* in red raspberry were around 17-21 °C (Hall and Sobey, 2013). The optimal temperature for whole plant *A* in raspberry 'Heritage' is 20℃ or 17/25℃ for air and root temperature (Percival et al. 1996). Photosynthetic rate in raspberry 'Titan' declined geometrically as the temperature increased from 15℃ to 40℃ (Fernandez and Pritts 1994). Photosynthetic rate in raspberry 'Summit' was significantly higher than raspberry 'Autumn Bliss' and 'Heritage' measured in summer, with higher transpiration rate but no difference in chlorophyll content (Percival et al. 2001). Seasonal changes in *A* of raspberry 'Autumn Bliss' grown in Canada showed nonstomatal inhibition between June and September, and stomatal conductance (*gs*) remained high at 30℃ (Privé et al. 1997). Raspberry 'Jeanne d'Orléans' showed a persistent negative response of *A*, *gs*, and mesophyll conductance (*gm*) to increasing temperatures from 20 to 35℃ (Qiu et al. 2017). Differences in leaf gas exchange parameters among siblings in a breeding program found that the decline in *A* at high temperatures (35℃) did not follow with the great differences observed in *gs* and evapotranspiration rate (*E*), which indicated that the decline in *A* might be mostly caused by non-stomatal factors (Stafne et al., 2000). However, another research by the same author comparing one blackberry and five raspberry cultivars at high temperatures showed that stomatal factors were also critical to maintain a high *A* (Stafne *et al*., 2001). Comparisons among temperate originated raspberry 'Fall Gold' and two native species from low altitude Taiwan, *R. rosifolius* and *R. croceacanthus*, showed strong correlation between *A* and *gs* at high temperatures ranging from 28℃

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to 38℃. However, the impact of biochemical factors in *FvCB* model was not quantified (Cheng, 2016).

Chlorophyll fluorescence data using *Fv/Fm* value collected in the field showed that photosystem II (*PSII*) efficiency was damaged and chlorophyll content decreased by heat stress in midday when moving raspberry plants from 20℃ to high temperatures over 27℃ (Gotame et al., 2013). High temperatures and light intensities in the afternoon decreased both *A* and *Fv/Fm* values of top leaves in raspberries (Mochizuki et al. 2010). A protocol measuring *Fv/Fm* of detached leaves for selecting heat tolerance traits has also been developed in raspberry and black raspberry (*Rubus occidentalis* L.). By measuring chlorophyll fluorescence every 20 min in 45℃ water bath heat shock, heat susceptible cultivars showed faster decline in *Fv/Fm* values than heat tolerant cultivars (Molina-Bravo et al. 2011, Bradish et al. 2016).

Due to the complicity of photosynthesis, various approaches as mentioned, has been used to assess *A* responses to high temperatures in *Idaeobatus* plants. Some emphasized the impact of the biochemical factors, while others focused on the diffusional factors. However, in these studies, physiological variables were mostly compared by significant test among treatments or cultivars. Limited information were obtained from these studies about the quantitative contribution of individual variables to the difference in *A*.

1.3.2 Farquhar, von Caemmerer and Berry (*FvCB*) photosynthesis modelling

FvCB model is a static state model to describe photosynthesis process based on photosynthetic biochemical mechanisms (Farquhar et al. 1980). Net assimilation rate in this model is limited by either Rubisco carboxylation limited state, RuBP

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regeneration limited state, or triose phosphate utilization (TPU) limited state. The different limitation states of net assimilation rate in saturated light conditions can be expressed as:

$$A_c = \frac{V_{cmax}\,(C_c - \Gamma^*)}{C_c + K_c(1+\frac{O}{K_O})} - R_d$$

$$A_j = \frac{J\,(C_c - \Gamma^*)}{4C_c + 8\Gamma^*} - R_d$$

$$A_p = 3\mathrm{TPU} - R_d$$

$$C_c = C_i - \frac{A_c}{g_m}$$

where A_c is A at Rubisco carboxylation limited state, V_{cmax} is maximum carboxylation rate, C_c and O are CO_2 and O_2 concentrations at carboxylation sites, Γ^* is CO_2 compensation point in the absence of photorespiration, K_C and K_O are catalytic constants for the carboxylation and oxygenation reactions of Rubisco, R_d is light respiration rate, A_j is A at RuBP regeneration limited state, J is electron transport rate, A_p is A at TPU limited state, C_i is intercellular CO_2 concentration, and gm is mesophyll conductance. For a modification version of the non-light saturated state (Archontoulis et al. 2012), J was revised as:

$$J = (\phi i + J_{max} - \sqrt{(\phi i + J_{max})^2 - 4\theta_j\,\phi i J_{max}})/2\theta_j$$

Where J_{max} is the maximum electron transport rate at saturating light levels, i is PPF; ϕ is the initial slope of the response of potential electron transport rate (J) to i, and θj is dimensionless convexity parameter for the response of J to i.

This model has been successfully used in the study field of ecology and agriculture. The original paper have been cited more than 6,000 times since 1980 (by Google Scholar, 2018). There are many different methods to obtain variables described in *FvCB* model. Two of the most important biochemical factors V_{cmax} and

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J_{max}, can be obtained by gas exchange measurement *in vivo* under light saturated environments with various CO_2 concentrations (*A/Ci* curve) (Dubois et al. 2007, Sharkey et al. 2007, Gu et al. 2010, Sharkey 2016) or by light response curves with a constant CO_2 concentration (Archontoulis et al. 2012). In addition, there were also *in vitro* ways to obtain V_{cmax} (Yamori et al. 2006). Despite the advantages of user-friendly and popularity, most of these methods have problems on mesophyll conductance, which was either considered as infinite (using C_i instead of C_c), fitted by only gas exchange data, or obtained by other independent time-consuming measurement such as variable *J* or online carbon isotope discrimination method (Flexas et al. 2013). In order to overcome these problems with a shorter measuring time, variables in *FvCB* model in this thesis were obtain from a new excel tool combing gas exchange and chlorophyll fluorescence (Moualeu-Ngangue et al. 2017) (detailed in 3.2.2).

1.3.3 Temperature responses of *FvCB* variables

1.3.3.1 Temperature responses of biochemical factors

Two of the most important biochemical factors in *FvCB* models are V_{cmax} and J_{max}. Temperature responses of these two variables have been reported in several species. In model plant *Nicotiana tabacum* and *Arabidopsis thaliana*, both V_{cmax} and *J* increased as temperatures increased from 25°C to 35°C (Bernacchi et al. 2001, 2002, Walker et al. 2013). In Panamanian lowland tropical tree species, values of J_{max} optimized at 34-37 °C and V_{cmax} were ~2 °C higher than J_{max} (Slot andWinter 2017). Average optimal values of J_{max} and V_{cmax} were 33°C and 40°C in data reviewed with 19 gas exchange studies on warm climate tree and crop species. On the other hand, cold climate tree species had a lower optimal temperature at 19°C and 29°C for J_{max} and V_{cmax} (Medlyn et al. 2002). Temperature responses of J_{max} and V_{cmax} can be

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affected by temperature acclimation (Hikosaka et al. 2006). In *Populus balsamifera*, populations from cool and warm regions were collected, and then grown under cool and warm environment. The results showed that V_{cmax} were mostly affected by the origin of the population. Populations from cool regions had higher V_{cmax} than populations from warm regions. By contrast, *J* value were affected more by growing temperatures. Higher *J* can be found in plants grown under cool conditions than plants grown under warm conditions. However, none reached their maximum value between17-37°C (Silim et al. 2010). Studies in *Eucalyptus* species also showed significant J_{max} and V_{cmax} differences in climate of the origin (Lin et al. 2013). The electronic transportation process is generally more sensitive to high temperatures or abiotic stress than the carboxylation process because thermal sensitivity of membrane-bound proteins in whole electron transport chain are higher due to high-temperature induced alterations in thylakoid membrane composition (Xue et al. 2016).

Rubisco kinetics including *Kc*, *Ko*, and Γ^* can be generated from *in vivo* (Bernacchi et al. 2001, Walker et al. 2013, Bellasio et al. 2016) or *in vitro* (Galmés et al., 2016) methods. For simplification, most of the research adopted temperature response data from model plants (Bernacchi et al. 2001, 2002). However, several published data have suggested that the differences in temperature responses of these variables are big enough to affect the model result (Galmés et al. 2016).

1.3.3.2 Temperature responses on stomatal conductance

Studies of direct impact of temperatures on *gs* have been limited because most of the temperature response measurements were not conducted with a consistent vapor pressure deficit (VPD). In order to maintain a consistent VPD at elevating temperatures, relative humidity have to increase correspondingly, which is rare in

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natural conditions. Temperature responses in the dark with a consistent VPD showed that stomatal conductance increased at high temperatures (Mott andPeak 2010). Stomatal conductance increased by 40% in broad leaf pine and needle leaf pine when temperature increased from 30 °C to 40 °C at VPD of 1 kPa (Urban et al. 2017). Temperature response of litchi *gs* optimized maximum value around 28°C while holding constant VPD= 0.7 KPa (Chang and Lin, 2007).

For most of the studies on temperature responses of *gs*, leaves were measured in conditions with inconsistent VPD. In raspberry, *gs* decreased as the short-term temperature increased from 20 to 35°C (Stafne et al. 2001, Qiu et al. 2017) and from 28 to 38°C (Cheng, 2016). In raspberry 'Autumn Bliss' , *gs* remained high at temperatures between 15-30°C measured in July and September (Privé et al. 1997). In rabbiteye blueberry, *gs* increased corresponding to increasing temperatures from 27 to 42°C , while *gs* decreased in southern and northern high bush cultivars at the same temperature range (Chang, 2016). In Panamanian lowland tropical tree species, *gs* decreased at high temperatures and the maximum *gs* value was observed around 30-35 °C (Slot andWinter 2017). Optimal temperature of maximum *gs* in litchi measured with VPD change as the increasing temperature was lower than measured with constant VPD (28°C) (Chang and Lin, 2007). Stomatal conductance decreased from 16-30°C in *Eustoma grandiflorum* under both consistent and variable VPD (Wang, 2015).

The mechanism of VPD effect on temperature responses of stomatal conductance has not been completely validated. Several models have been proposed to describe *gs* in plants under different environments using empirical model combined with *A* (Ball Berry, 1987), empirical data and optimal theory (Medlyn et al. 2011), or

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mechanism process (Peak and Mott, 2011).

1.3.3.3 Temperature responses of mesophyll conductance

Mesophyll conductance (*gm*) is the reciprocal of resistance that carbon dioxide moves from sub-stomatal cavities to the site of carboxylation inside chloroplasts. It has been ignored and thought to be 'infinite' in the past but now has been proven to be one of the main factors limiting photosynthesis rates (Flexas et al. 2012).

In general, *gm* was positively correlated to temperatures. Nevertheless, great variations on temperature responses of *gm* have been reported in different species. For species originated in the warm areas such as *Nicotiana tabacum, Gossypium hirsutum, Glycine max,* and *Oryza sativa,* a positive, linear response of *gm* to temperatures was observed between 15℃ and 40℃. For those originated in cool areas such as *Triticum aestivum, Quercus engelmannii,* and *Arabidopsis thaliana,* a relatively insensitive temperature response was observed, and the maximum *gm* values were between 25℃ to 30℃. In addition, some tropical species, e.g., *Lophostemon confertus,* have shown insensitive *gm* to increasing temperatures (von Caemmerer and Evans, 2015). *Populus balsamifera* grown under cool environment had insensitive temperature response on *gm* (Silim et al. 2010).

The mechanisms controlling *gm* has yet to be documented. Although variations on structural differences at cellular level might be the major contributor to variable *gm* among species (Tomás et al. 2013, Tosens et al. 2016, Veromann-Jürgenson et al. 2017), it seemed less possible for the cellular structure being readily responsive to a short-term environmental change (Tomás et al. 2014, Flexas andDiaz-Espejo 2015). Studies have shown that *gm* can be regulated by aquaporins (Uehlein et al. 2003,

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2012, Hanba et al. 2004, Flexas et al. 2006, Heckwolf et al. 2011, Perez-Martin et al. 2014, Ding et al. 2016), carbonic anhydrase (Price *et al.*, 1994; Gillon andYakir, 2000; Perez-Martin *et al.*, 2014), which could have a high dependency on temperatures. Abscisic acid (ABA) has also been proved to affect *gm* (Mizokami et al. 2015, Sorrentino et al. 2016, Qiu et al. 2017).

1.3.4 Partitioning variable contributions to *A* differences

Net assimilation rate as well as many of the photosynthetic variables described in *FvCB* model change simultaneously when a plant is experiencing environmental fluctuations. Quantifying the change of these variables and estimating their contributions to the overall change in *A* will be of great helpfulness on assessing the underlying photosynthetic mechanisms and provide useful information for breeding heat tolerance traits in the future. However, it is impossible to actually measure the photosynthetic limitations of all variables and currently, the only approachable way is using mathematic methods. This approach using finite change of *A* between different environments and then partitioned variable changes into percentages was first developed by Jones (1985). After several modifications, the most common method now combining with *FvCB* model is the quantitative limitation analysis (QLA), which partitions the difference in *A* between a reference point and a comparison point into contributions of stomatal limitation (S_L), mesophyll limitation (M_L), and biochemical limitation (B_L) and expresses these contributions as percentage of changes in *A* (Grassi et al., 2009; Grassi and Magnani, 2005):

$$l_s = \frac{g_t/g_s \cdot \partial A/\partial C_c}{g_t + \partial A/\partial C_c}$$

$$l_m = \frac{g_t/g_m \cdot \partial A/\partial C_c}{g_t + \partial A/\partial C_c}$$

$$l_b = \frac{g_t}{g_t + \partial A/\partial C_c}$$

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$$\frac{dA_c}{A} = S_L + M_L + B_L = \frac{dg_s}{g_s} l_s + \frac{dg_m}{g_m} l_m + \frac{dV_{cmax}}{V_{cmax}} l_b$$

where $S_L$is stomatal limitation, $M_L$is mesophyll limitation, $B_L$is biochemical limitation.

This method has been very useful for studying photosynthesis in both ecological and agricultural fields. Several examples including drought stress, salinity stress and seasonal changes in different plant materials has been evaluated with this approach (Tomás et al. 2014, Chen et al. 2015, Sperlich et al. 2015, Perdomo et al. 2016, Wang et al. 2017). A recent modification further extended this method to be not only suitable under light saturating state (Rubisco carboxylation limited state in *FvCB* model) but also in non-saturating state (RuBP regeneration limited state) (Archontoulis et al. 2012). When using the modified method for non-saturated *A* , changes in *J* instead of V_{cmax} were partitioned (Chen et al. 2014):

$$l_s = \frac{g_t/g_s \cdot \partial A_j/\partial C_c}{g_t + \partial A_j/\partial C_c}$$

$$l_m = \frac{g_t/g_m \cdot \partial A_j/\partial C_c}{g_t + \partial A_j/\partial C_c}$$

$$l_j = \frac{g_t}{g_t + \partial A_j/\partial C_c}$$

$$\frac{\partial A_j}{\partial C_c} = 12J\Gamma^*/(4C_c + 8\Gamma^*)^2$$

$$\frac{dA}{A} = S_L + M_L + B_L + L_L = \frac{dg_s}{g_s} l_s + \frac{dg_m}{g_m} l_m + \frac{J_{dB}}{J} l_j + \frac{J_{dI}}{J} l_j$$

where $L_L$is light limitation.

However, the use of partial deviation made this method difficult to combine with other variables such as the Rubisco kinetics, *θj*, *φ*, and *Rd* between different species and temperature, which may not be suitable for the aim of comparing different *Idaeobatus* plants in different light and temperature environments in this thesis. In order to overcome this problem, a recent published calculation based on numerical

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integration is preferred (Buckley and Diaz-Espejo, 2015). The direct numerical approach partitions the real differences in *A* rather than the natural logarithm of *A* in the partial deviation. Several advantages including user friendly excel tool, readily extendable into most of the variable changes in *FvCB* model, suitable for all situations and selections of reference and comparison points, and also small error in modeling (Buckley and Diaz-Espejo, 2015). It can be written in:

$$\rho_{xj} = \frac{100}{A_{ref}} \cdot \sum_{k=0}^{n-1} \left[\delta A \middle| \delta x_j\right]_{k}^{k+1}$$

$$\mathrm{A} = \mathrm{A}\left(g_s, g_b, g_m, V_{cmax}, J_{max}, V_{tpu}, R_d, K_c, \Gamma^*, \theta_j, \varphi, O, i\right)$$

where ρxj is the contribution of variable xj to the A differences between the reference point and the comparison point (%).

Although the claim of smaller error has been debating, it is still considered helpful on photosynthesis limitation analysis (Chen et al. 2018).

1.4 Objectives and Hypothesis

1.4.1 Scheduling primocane-fruiting raspberry in the subtropical climate (Chapter 2)

In chapter two, a trial using shoot bending and night break for scheduling primocane-fruiting raspberry ‘Summer Festival’ flowering in the winter was aimed to overcome the low flowering rate (low proportion of the flowering lateral nodes on primocanes) and long vegetative period possibly cause by heat stress in summer and lack of chilling in winter. Although it has been reported that primocane-fruiting raspberries ‘Summit’ were able to produces multiple harvests by pruning and growing perennially under subtropical climate (Funt, 2013). Another perennial trials for growing primocane-fruiting raspberry in subtropical (Florida) and tropical (Puerto

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Rico) climates failed to maintaining non-dormant, fruitful plants by using nitrogen fertilization, pruning, or defoliation treatments (Darnell, 2009). In the previous research, we adpoted the summer pruning techniques for late production in Portugal (Oliveira et al. 1996, 2004) and the night breaking approach (Sønsteby andHeide 2009) to induce winter harvest. However, the laterals on the lower position of the primocane of raspberry 'Summer Festival' primocane grown in Taipei failed to generate second harvest by combining pruning and night break treatments, possibly because of the late pruning time (Nov. 30th) and the intensities of pruning treatments (15^{th} node)(Chen, 2014). Shoot bending technique in this thesis allowed the upper nodes of the canes continue to develop flowering and fruiting process while breaking the paradormancy on the lower nodes of the primocanes at the same time, so it can be applied earlier than the pruning method did (Oct. 2nd).

1.4.2 Photosynthesis modeling in *Idaeobatus* plants under different light and temperature environments (Chapter 3)

There are about 40 native *Rubus* species from low to high altitude in Taiwan (Huang and Hu, 2009) and some of them may have the potential for breeding raspberry cultivars suitable for subtropical area in the future. In chapter three, heat tolerance mechanisms of selected native species in the subgenus *Idaeobatus* and raspberry 'Summer Festival' were investigated in different temperatures and light intensities using a biochemical based photosynthetic modeling approach. Gas exchange and chlorophyll fluorescence data were measured simultaneously under 25°C, 30°C, and 35°C with light response curve and *A/Ci* curve to generate variables in FvCB models including: *gs, gm,* V_{cmax}, J_{max}, *Rd,* θj, and φ (Sharkey 2016, Moualeu-Ngangue et al. 2017). Then the *A* differences between *Idaeobatus* species under different temperature and light intensities would be quantified by the numerical

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integration method(Buckley andDiaz-Espejo 2015). The detail heat adaptation mechanisms of *Idaeobatus* based on FvCB model can be identified for future breeding and other horticultural practice research.

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Chapter 2 Scheduling production of primocane-fruiting raspberries in subtropical climate

2.1 Abstract

Primocane-fruiting raspberry cultivars are capable of flowering freely on the upper nodes of the current season primocane before dormancy, bringing up the possibility to produce raspberries in the subtropical climate. However, plants often suffer from low flowering rate. In this experiment, effects of shoot bending at the 30th node position and night breaking treatments (22:00-2:00) on promoting laterals and second harvest were investigated in raspberry 'Summer Festival' grown in a greenhouse in Taipei. The results showed that all treatments failed to promote flowering rate or improve lateral development. In all treatments, laterals between 20th and 40th node on the primocanes had the highest emerged rate and flowering number per lateral. During the experiment period, plants suffered serve fungus disease and poor pollination.

Key words: raspberry, primocane-fruiting, shoot bending, night breaking.

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2.2 摘要 (Chinese abstract)

一年生枝條結實型覆盆子的當年生枝條高節位處能夠在進入休眠前開花結果，使得在亞熱帶氣候的條件下，有機會能夠生產覆盆子，然而植株當年生枝條卻常常有低開花率的問題(開花側枝節位數少)。在本章之試驗中，於 2016 年 10 月使用枝條彎曲(當年生枝條第三十節)與暗中斷處理(22:00-2:00)，嘗試促進覆盆子‘Summer Festival’ 低節位側枝的生長，並促成冬季臺北溫室內的二次採收。然而結果顯示兩種處理方式皆無法有效提高開花率與側枝生長。所有受試植株於當年生枝條第二十節至第四十節有最高的萌發率與每側枝開花數量。試驗期間，所有植株於溫室內皆受到嚴重真菌性病害，且有授粉不良的問題。

關鍵字:覆盆子、當年生枝條結果型、枝條彎曲、暗中斷

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2.3 Introduction

The raspberry (*Rubus idaeus* L.) is a common berry crop originated in the high latitude area and is recommended to grow in areas with enough winter chilling and mild summer (Hall and Sobey, 2013). Until now, there is no commercial production of raspberries in Taiwan but the demand has been increasing rapidly in recent years (Customs Administration, Ministry of Finance 2016). Although fresh raspberries are very perishable with an extremely short shelf life, worldwide demand has been strong and at least 50% of the world production have turned from proceed product to fresh market (Funt and Hall 2013). Producing fresh raspberry in Taiwan may have the potential to benefit farmers, improve fresh fruit qualities, and reduce carbon foot print of the berry industry in the future.

Primocane-fruiting raspberry cultivars initiate flowers on the top nodes of primocanes in conditions of temperatures between 9 and 30℃ and day length between 10 and 24 hours (Carew et al. 2003, Sønsteby and Heide 2009). However, insufficient winter chilling can still affect yield and flowering. Insufficient chilling during the early development stage of primocanes (Dale et al. 2005, Gambardella et al. 2016) or during the dormancy period (Takeda 1993, Carew et al. 2001) greatly affected the number of flowering laterals on the primocane, which is one of the most important architectural component affecting final yield (Sønsteby and Heide 2012). Flowering time can also delayed due to insufficient chilling (Takeda 1993) or high temperature stress over 27℃ (Gotame et al. 2013). Harvest on the second year floricanes can certainly be limited without enough chilling to break endodormancy (Dale et al. 2003).

Year-round production in raspberries can be achieved in many temperate areas

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(Oliveira et al. 2002, Dale et al. 2005). In Portugal, the second harvest from the lower position of the primocanes was achieved by pruning on the 15th node in July after the first harvest in raspberry 'Autumn Bliss' (Oliveira et al. 1996, 1998). Although it had been reported that raspberry 'Summit' planted in the subtropical area was harvested multiple times on the primocanes by repeated pruning (Funt, 2013), trials in Florida and Puerto Rico failed to maintain a non-dormant system by using nitrogen fertilization, pruning, or defoliation treatments (Darnell, 2009).

The harvest time of raspberry 'Summer Festival' grown in Taipei is about four months later than raspberry 'Autumn Bliss' grown in Portugal. It had been reported that the development of canes in some raspberry cultivars such as 'Polka' is sensitive to day length. Long day length treatments by night breaking was able to increase the number of flowers but decrease number of dormant buds at 18-24℃ (Sønsteby and Heide 2009). In a previous study, pruning and LED night breaking treatments were applied on the 15th node on the primocanes at the end of November but the results failed to promote a second harvest. The number of laterals after pruning were very small and inconsistent within treatments. In addition, most laterals developed after treatments were in a rosette status (Chen, 2014). In this experiment, instead of using pruning method to induce flowering laterals, early shoot bending in October was tested to investigate its effects on breaking paradormancy of the lower nodes on primocanes while the upper nodes of the canes continue to develop flowers and fruits.

2.4 Materials and methods

2.4.1 Plants materials

Rubus idaeus 'Summer Festival' plants in a greenhouse in National Taiwan University (25°2′ N, 121°32′ E, 15m). Plants were cultivated in 5.6-L pots using a

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mixed media of peat moss, cow waste, and commercialize medium (Gen-chi-Wan medium) at 1:1:1 ratio for over two years. Plants were well-watered and fertigated with 1000X 20-20-20 (HYPONeX) twice a week throughout the whole growing season. In February 2016, all canes were pruned to the groundline to induce new primocanes . Temperature and humidity of the greenhouse were recorded by a data logger (HOBO Pro v2 RH/TEMP, Onset, Bourne, Massachusetts, USA) (Appendix Fig.1).

2.4.2 Treatments

On October 2, 2016, plants were randomly subjected to one of the following three treatments (n=20): night breaking + shoot bending (NB+SB), night breaking alone (NB), shoot bending alone (SB), and the control (CK). Treatments were applied when the upper nodes of the primocanes were stilling flowering and fruiting . The shoot bending treatments were applied at the of the primocanes using bamboo sticks and aluminum wire to reposition the primocane above the 30^{th} node over 120 degree from the vertical (Fig. 2.1). Night breaking treatments were applied from 22:00 to 2:00 every night throughout the winter (2016.10.2~2017.2.28) using white light LED (Quan, Xin GSL-60DX, manufacture, New Taipei City) hanging about 160cm above the ground (PPFD≒60μmol·m^{-2}·s^{-1} measured at the bending site).

2.4.3. Measurements and Statistic Analysis

Flower number of each treatments were counted each 3-4 days during the trials. Lateral number, lateral length, lateral node number and flower number per lateral were measured on Feb. 28, 2017 and analyzed with two way analysis of variance (ANOVA) with mean separation by least significant difference (LSD) (CoHort. Version 6.101, Costat, Inc., Montery, CA, U.S.). Lateral distribution percentage was

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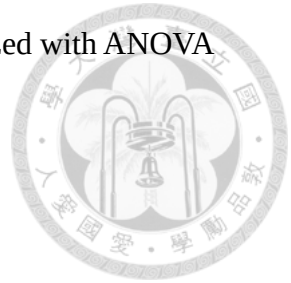
transformed with angular transformation ($y=\sin^{-1}(X)^{1/2}$), then analyzed with ANOVA and mean separation by LSD.



2.5 Results

The results showed that number of flowers were the highest in NB. (249.6 flowers/primocane) treatment, and lowest in SB (107.2 flowers/primocane) treatment. However, the inconsistency within treatments caused very high standard errors. The number of laterals on the primocanes were similar among treatments. Plants subjected to SBfailed to induce more laterals than the control. There were also no significant differences in length of laterals (about 42cm) or number of lateral nodes (about 25 nodes). NB also failed to promote shoot elongation of the laterals in winter. Although it seemed that plants subjected to NB had slightly more flowers per laterals, significance test showed no differences among treatments, possibly because of large standard errors. In all tested plants, laterals were mostly emerged from 20-40th nodes of the primocanes (Table 2.1;Fig. 2.2). Regardless of the treatments, laterals developed from the 0-20th node were longer in length and had more nodes than those from the 20-40th node. However, laterals from the 20-40th node produced more flowers. (Table 2.2).

2.6 Discussion

The comparisons of the treatments showed no significant difference on most of the observed traits (Table 2.1). Some of the biggest problems might be the large standard error of each treatments. The shoot bending treatments caused mechanical damage on the bending site in some plants and precise control of the biomechanics of the bending treatments was difficult (Han et al. 2007). Shoot bending has been used in many Rosacea crops, for example, roses for cut flower production (Kool and Lenssen

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1997), and apples in the tropics (Edwards and Notodimedjo 1987). However, in this chapter, bending did not have significant effect on promoting laterals in raspberries, and due to the difficulty of controlling mechanical damage, further adjustment on bending positions or approaches should be investigated to improve the effects of this technique.

During the experiment period, some laterals were developed from the lower nodes of the primocanes and flowered in the untreated control. This was not observed in a previous study (Chen, 2014). The laterals with the highest flowering numbers were between the 20^{th}-40^{th} nodes on the primocanes. On the upper nodes, laterals tended to be shorter with less flower number than the laterals emerged from the 20-40^{th} nodes, and on the lower nodes, laterals tended to be longer but with few flowers.. High temperatures decrease fruit size and quality in raspberries (Remberg et al. 2010). In late summer and the first harvest on the upper position of the primocane can be seriously affected by heat stress. Tipping primocanes in early summer is a common way for promoting the number of laterals , improving fruit quality and delaying the harvesting period (Oliverira et al. 2004; Strik, 2012; Zorenc et al. 2017). Instead of bending, tipping might allowed the laterals from 20^{th}-40^{th} node to develop in a cooler environment and would be helpful for commercialize the raspberry fruit production in the winter.

In this experiment, most of the flowers were pollinated by hand using a paint brush due to lack of natural pollinators in the greenhouse. However, due to high humidity caused by pad and fan cooling system, and the rainy days of winter in Taipei, fruit set was poor and therefore, fruit yield record was missing. Without pollinators in the greenhouse, flowers of raspberries often suffered excess nectar and

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fungus disease such as *Botrytis*, which cause the flower been seriously infected before. Even in some of the few flowers that did set fruits, crumbliness happened in most of them, and caused almost no fruits with commercial value in raspberry 'Summer festival'. Crumbliness of red raspberry fruit have fewer drupelets and poor drupelet adhesion when picked, it can be cause by inadequate pollination, partial self-incompatibility, low or high temperatures, or raspberry bushy dwarf virus (RBDV)(Moore and Robbins 1990, Muster 2008, Graham et al. 2015). High air humidity is one of the reasons that flowers and fruits of raspberries faced such a serious stress of fungus disease (Xu et al. 2012).

Growing raspberries in central and southern Taiwan where winter is mild and dry can be an alternative option. Also, it is important to find suitable cultivars with little chilling affection on the primocane growth habit, that insured the primocanes flowering well without chilling (Gambardella et al. 2016). Poor pollination cause by excess nectar of raspberry flower grown in the greenhouse can be improved by using bumble bees and other insects (Cane 2005, Lye et al. 2011). Botrytis infection on raspberry flowers can also be controlled by using inoculum of *Gliocladium roseum* delivered by bumblebees or honeybees (Yu and Sutton 1997).

2.7 Conclusion and future perspective

The shoot bending and night breaking treatments generated little effect on forcing the second harvest on raspberry primocanes grown in Taipei. Regardless of the treatments, poor pollination and severe fungal disease were challenges for greenhouse raspberry production in subtropical climate.. Future research should be focusing on solving these two problems and introducing more primocane-fruiting cultivars which flowering well without chilling is also important in the future. We also

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suggested that using tipping technique in early summer to promote laterals on the 20^{th}-40^{th} nodes of the primocane to develop under cooler environment might be worth trying in the future.

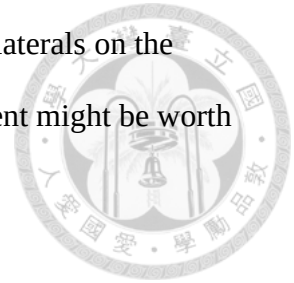

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Table 2.1 Effects of night breaking and shoot bending on inducing laterals and flowers on primocanes in ‘Summer Festival’ raspberries. Effects

Night[z] breaking (NB)	Shoot bending (SB)	Flowers per cane (no.)	Laterals (no.)	Length of laterals (cm)	Lateral nodes (no.)	Flowers per lateral (no.)	Distribution of laterals (%)		
							Nodes 0-20	Nodes 20-40	Nodes 40-
+	+	210.0±69.6 ab[y]	5.8±2.2 a	46.5±10.7 a	24.0±4.2 a	44.0±30.2 a	15.5±9.7 aB	84.5±9.7 aA	0.0±0 aC
+	-	249.6±105.7 a	6.2±1.1 a	40.2±10.9a	21.7±3.5 a	41.6±18.6 a	19.4±20.2 aB	76.6±17.2 aA	4.0±8.9 aB
-	+	107.2 ±43.0 c	6.0±1.4 a	42.1±15.8 a	24.0±6.3 a	18.6±7.6 a	22.5±14.9 aA	55.0±33.1 aA	22.5±31.2 aA
-	-	126.6±66.6 bc	4.4±1.9 a	46.6±15.5a	26.6±5.6 a	33.8±20.5 a	16.4±15.7 aB	68.2±32.4 aA	15.4±24.9 aB
Probability levels of significance by ANOVA									
Source of variation									
NB		**	NS	NS	NS	NS	NS	NS	NS
SB		NS	NS	NS	NS	NS	NS	NS	NS
NB X SB		NS	NS	NS	NS	NS	NS	NS	NS

[z] Treatments were applied on container grown raspberry bushes on Oct. 26, 2016. Measurements were taken on Feb. 28, 2017.

[y] Significant difference among treatments were indicated by lower case letters. Mean separation within columns by LSD test $P<0.05$.

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Table 2.2 Traits of laterals emerged from different position of the primocane in

Distribution[z] (node order)	Length (cm)	Length of nodes (cm)	Nodes (no.)	Flowers per lateral (no.)
0-20	77.76 a[x]	2.24 a	33.68 a	21.40 b
20-40	38.89 b	1.69 b	22.51 b	38.13 a
40-	22.06 c	1.35 c	16.41 c	10.29 c

'Summer Festival' raspberries.

[z] Treatments were applied on container grown raspberry bushes on Oct. 26, 2016. Measurements were taken on Feb. 28, 2017.

[y] Significant difference among treatments were indicated by lower case letters. Mean separation within columns by LSD test $P<0.05$

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Fig. 2.1 The bending treatments on primocane-fruiting raspberry ‘Summer Festival’ constructed by bamboo sticks and aluminum wires.

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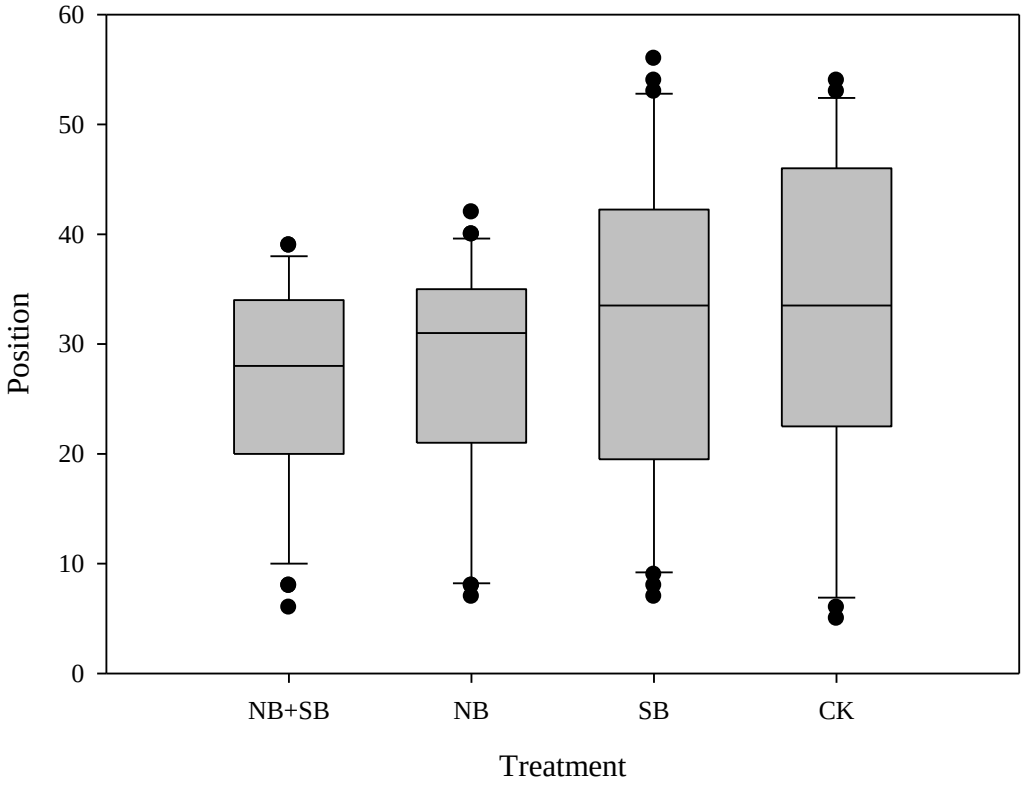

Fig. 2.2 Box plot of the lateral emerged position on the primocane under different treatments. The gray colored box indicated the quartile of the position in different treatments. Black dots indicated the value of mild outlier. Night break + shoot bending (NB+SB), night break alone (NB), shoot bending alone (SB), and the control (CK).

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Chapter 3 Modeling photosynthetic heat tolerance mechanisms in *Idaeobataus* plants in different light and temperature environments

3.1 Abstract

Photosynthetic heat tolerance mechanisms of raspberries were investigated by comparing raspberry 'Summer Festival' with three *Rubus* species native to subtropical lowland Taiwan (*R. rosifolius, R. croceacanthus,* and *R. fraxinifolius*) in the same subgenus (*Idaeobatus*). Gas exchange and chlorophyll fluorescence were measured simultaneously at 25, 30, 35℃ with varied light intensities or CO_2 concentrations. Data were fitted to a modified FvCB model and the key photosynthetic variables were extracted. The differences in net assimilation rates among species or environmental conditions were partitioned into the correspondence of each variable by numerical integration method. The results showed that at 35℃ and 1200 μmol·m^{-2}·s^{-1} PPFD, raspberry leaves had the lowest net assimilation rate in all tested speices. Diffusional factors in the FvCB model were the most important factors this differences, with stomatal conductance and mesophyll conductance shared nearly half of the correspondence. Biochemical factors, such as the maximum value of carboxylation and electron transportation rate, were increased in all species as the temperature increase. . Temperature differences between leaf surface and the air showed that the native species had significant better mechanism of cooling by transpiration under 35℃.

Key words: raspberry, heat stress, photosynthesis, FvCB model, numerical integration method

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3.2 摘要

本章節透過比較覆盆子‘Summer Festival’和原生於台灣亞熱帶平地的空心莓亞屬植物，刺莓(*R. rosifolius*)、薄瓣懸鉤子(*R. croceacanthus*)與愷葉懸鉤子(*R. fraxinifolius*)試圖了解空心莓亞屬植物的高溫光合生理機制。在不同溫度(25、30、35℃)下同時測量葉片氣體交換與葉綠素螢光之光反應曲線及二氧化碳反應曲線(A/Ci curve)後，使用光合生化模型進行參數擬合(FvCB model)，並以數值積分法量化各參數對於淨光合作用速率變化的貢獻程度。結果顯示在 35℃ PPFD=1200 μmol·m^{-2}·s^{-1} 下覆盆子的淨光合作用速率最低，且與其他三種原生種差異最大，此差異隨著光強度下降而逐漸縮小。FvCB 參數經量化計算後發現擴散性因子是造成此差異的主要原因，氣孔導度與葉肉細胞導度約各佔總差異一半的貢獻度，生化因子如最大羧化速率與最大電子傳遞速率在所有植物中皆隨溫度上升而提高。葉面溫度與氣溫差值也顯示在 35℃下原生種具有顯著較佳的葉片蒸散散熱機制。

關鍵字:覆盆子、熱逆境、光合作用、FvCB 模型、數值積分法

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3.3 Introduction

Some tropics-originated species in *Idaeobatus*, a subgenus of *Rubus*, are capable of maintaining a consistent high net carbohydrate assimilation rate (*A*) at high temperatures while the temperate-originated raspberry *(R. idaeus* L.) in the same subgenus fails to. High temperature is one of the key factors limiting raspberry production in areas with warm summer. Previously, species differentials in photosynthesis at high temperatures were often assessed using statistics significance tests with single variables obtained from either gas exchange measurements (Fernandez and Pritts 1994, Percival et al. 1996, 2001, Privé et al. 1997, Stafne et al. 2000, 2001, Qiu et al. 2017), values of *Fv/Fm* from chlorophyll fluorescence measurements (Mochizuki et al. 2010, Molina-Bravo et al. 2011, Gotame et al. 2013, Bradish et al. 2016), or variables from Farquar, von Cammerer and Berry (*FvCB*) biochemical model (Cheng, 2016). However, none of the mentioned approaches quantified the impact of each variable, i.e. the contribution of individual variable to change in *A*, and thus limited information being generated to interpret the underlying mechanism of heat adaptation in different species.

In this chapter, to assess the photosynthetic heat adaptation mechanism of *Idaeobatus* species, leaf gas exchange and chlorophyll fluorescence at various intercellular CO_2 concentrations (Ci) and photosynthetic photon flux densities (PPFD) were simultaneously measured on 'Summer Festival' raspberry and three species native to Taiwan, *R. fraxinifolius, R. rosifolius,* and *R. croceacanthus*. Data were then fitted with Farquhar, von Caemmerer and Berry (*FvCB*) biochemical model to generate the following variables: mesophyll conductance (*gm*), maximum carboxylation rate (V_{cmax}), maximum electron transport rate (J_{max}), initial slope of J versus light (φ), and convexity factor (θj) (Sharkey 2016, Moualeu-Ngangue et al.

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2017). The contribution of each variables to the differences in *A* among tested species or Environ. conditions was quantified by using a numerical integration approach (Buckley and Diaz-Espejo 2015a).

3.4 Materials and methods

3.4.1 Plants materials

'Summer Festival' red raspberry was selected as the heat sensitive sample in subgenus *Idaeobatus* originated from temperate area. *R. fraxinifolius, R. rosifolius,* and *R. croceacanthus* collected from the subtropical lowland Taiwan were selected as the heat tolerance species originated from lower latitude area. *R. fraxinifolius* and *R. croceacanthus* was collected from the suburb of Taipei (121E, 25N), and *R. rosifolius* was collected from Tainan (121E, 22N). All plants were cultivated in 5.6-L pots with a commercial potting mix and placed. in a greenhouse at the Agricultural Experiment Farm in National Taiwan University for over two years. Plants were all well-watered and fertigated with 1000X 20-20-20 (HYPONeX) twice a week throughout the whole growing season. On Feb. 2017, all canes were pruned to the groundline to induce new primocanes. Temperature and humidity of the greenhouse was recorded by a data logger (HOBO Pro v2 RH/TEMP, Onset, Bourne, Mass., USA) (Appendix Fig.1).

3.4.2 Gas exchange and chlorophyll fluorescence measurement

Gas exchange and chlorophyll fluorescence were simultaneously measured using an open gas exchange system (LI-6400, Li-Cor, Lincoln, Nebr, USA) equipped with an auxiliary leaf chamber fluorometer (LI6400-40) . Plants were moved from the greenhouse into the laboratory 1 d before measurement for acclimation to 25, 30, or 35℃ . Relative humidity of the leaf chamber was adjusted to 50-60% for all

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temperature treatments by desiccant scrolls on LI-6400 (leaf vapor pressure deficit =1.1-1.3, 1.6-1.8, and 2.3-2.5 kPa for 25, 30, and 35℃ respectively). Light intensity was controlled by LEDs built in LI6400-40 (Red: Blue =9:1). Measurements were made in July, 2017 between 7:30 and 12:00 in the morning to prevent errors caused by midday depression or circadian rhythm. All measurements were taken on the second fully expanded leaf of the primocanes (middle of the trifoliate leaf) with no disease spot or mechanical damage.

Before measurements, leaves were acclimated in the leaf cuvette for at least 30 min with the following conditions: PPFD=1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, CO_2 at 400 ppm, flow rate at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and temperature at the target treatment. *A/Ci* curves were then measured in the following conditions: CO_2= 400, 0, 40, 80, 120, 200, 300, 400, 500, 650, 850, 1050, 1300, 1500 ppm, stability wait time = 4-6min for each point, using the auto program function "Flr A-Ci Curve" built in OPEN 5.3.2 of LI-6400. Light response curves were measured in the following conditions: PPFD=1200, 800, 500, 200, 150, 100, 75, 50, 30, 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, stability wait time = 5-7 min for each point, using the auto program function "Flr Light Curve". For each species and temperature treatment, *A/Ci* and light response curve were measured in three replicates (n=3). Potential leaks by leaf cuvette foam that might affect the gas exchange results were calibrated using the mathematic calculation reported by Boesgaard et al. (2013).

3.4.3 *FvCB* model fitting

FvCB model is a static model based on biochemical processes to describe photosynthesis (Farquhar et al. 1980). The photosynthesis process can be limited by either Rubisco carboxylation (*Ac*), RuBP regeneration (*Aj*), or triose phosphate utilization (*Ap*). Data collected from the *A/Ci* measurement including *A*, *Ci*, and

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φ_{PSII} (1- *Fs* (steady state *F*)/*Fm'* (maximal *F*, light adapted)) were input to an Excel SOLVER base modeling tool to generate mesophyll conductance (*gm*), maximum carboxylation rate (V_{cmax}), and day respiration rate (*Rd*) according to a modified *FvCB* model (Moualeu-Ngangue et al. 2017):

$$A_c = \frac{V_{cmax}\,(C_c - \Gamma^*)}{C_c + K_c(1+\frac{O}{K_o})} - R_d$$

$$A_j = \frac{J\,(C_c - \Gamma^*)}{4C_c + 8\Gamma^*} - R_d$$

$$A_p = 3\mathrm{TPU} - R_d$$

$$C_c = C_i - \frac{A_c}{g_m}$$

$$J_F = \alpha \times \beta \times \mathrm{I_{inc}} \times \varphi_{\mathrm{PSII}}$$

$$g_m = \frac{A(\tau\, I_{inc}\, \varphi_{PSII} - 4(A+R_d)}{\tau\, I_{inc}\, \varphi_{PSII}(C_i - \Gamma^*) - 4(C_i + 2\Gamma^*)(A+R_d)}$$

Where C_c and *O* are the concentrations of CO_2 and O_2 , respectively, at carboxylation sites, Γ^* is the CO_2 compensation point in the absence of photorespiration, K_C and K_O are the catalytic constants for the carboxylation and oxygenation reactions of Rubisco, respectively; J_F is the actual electron transport rate α is the fraction of incoming light absorbed by the photosystems β is the partitioning fraction of photons between PSI and PSII; I_{inc} is the *PPFD* on the leaf.

Data collected from the light response curve measurement (*A*, *Ci*) and variables (*Rd*, *gm*) generated from *A/Ci* curve fitting process (Moualeu-Ngangue et al. 2017) were input into an Excel SOLVER based modeling tool to generate maximum electron transport rate (J_{max}), initial slope of *J* versus light (φ), and convexity factor (θj) according to Sharkey (2016):

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$$J = (\phi i + J_{max} - \sqrt{(\phi i + J_{max})^2 - 4\theta_j \, \phi i J_{max}})/2\theta_j$$

For all model fitting processes, Rubisco kinetics (*Kc*, *Ko*, and *Γ**) estimated in tobacco plants from a previous research (Walker et al. 2013) were used in this study (Table 1).

3.4.4 Partitioning contributions of individual variable to change in net assimilation

Variables generated from *FvCB* modeling tools were averaged (n=3) and then used as the input data for partitioning variable contributions tonet assimilation differences, therefore, no standard error in the partitioning result. Stomatal conductance and mesophyll conductance were based on the average of light response curve measurements and the model fitting results (n=3) in CO_2 = 400 ppm respectively. The partitioning tool is using numerical method based on EXCEL VBA (Buckley and Diaz-Espejo, 2015). Which can be written in:

$$\rho_{xj} = \frac{100}{A_{ref}} \cdot \sum_{k=0}^{n-1} \left[\delta A \middle| \delta x_j\right]_k^{k+1}$$

$$\mathrm{A} = \mathrm{A}\left(g_s, g_b, g_m, V_{cmax}, J_{max}, V_{tpu}, R_d, K_c, \Gamma^*, \theta_j, \varphi, O, i\right)$$

where ρ_{xj} is the percentage contribution of variable *xj* to the *A* differences between the reference point and the comparison point.

The contributions of these variables can be pooled into three groups: 1) biochemical factor, *PPFD* ρ[BIO], sum of ρ[V_{cmax}], ρ[J_{max}] , ρ[R_d] , ρ[K_c], ρ[K_o],

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ρ[Γ*], ρ[θj] and ρ[φ]; 2) diffusional factor, ρ[DIFF], sum of ρ[g_s] and ρ[g_m]; 3) light intensity ρ[i]. The effects of boundary resistance (g_b) and oxygen concentration (O) was negligible because both were maintained consistently in the leaf cuvette during all measurements. TPU rates were also negligible because A_p is considered to happen only at low temperatures and very high CO_2 concentrations (Busch and Sage 2016).

At any given environmental condition, Differences in photosynthetic variables among species were compared using *Rubus idaeus* as the reference species. Within species, changes in variables among different environmental conditions were compared using 25℃ and PPFD=1200μmol·m^{-2}·s^{-1} as the reference.

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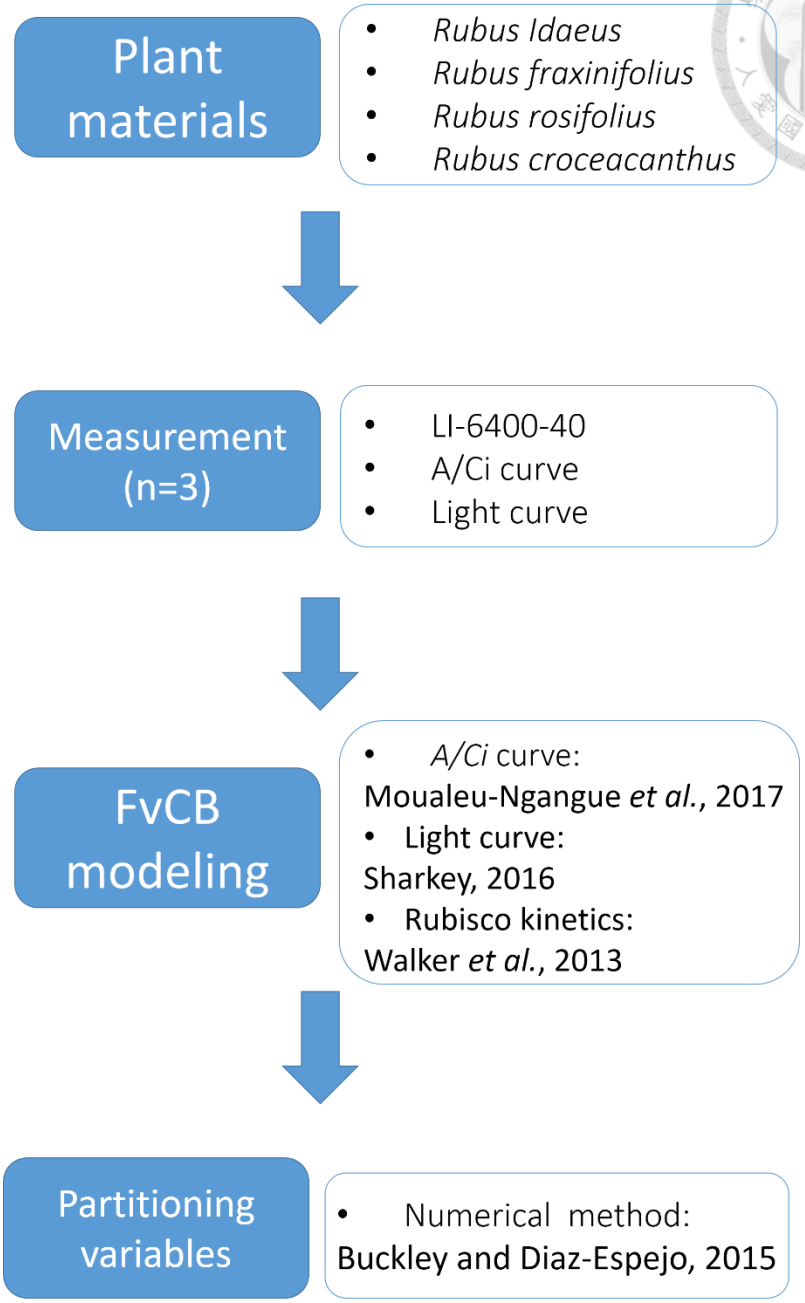
3.4.5 Experiment design



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3.5 Results

3.5.1 Gas exchange measurements at various temperature, CO_2 level, and light intensity conditions

The result of *A* in light response curve measurements at different temperature treatments were shown in Fig.3.1 and Table 3.4. At 25℃, *A* values were 11.2, 13.9, 11.1, 12.8 μmol·m^{-2}·s^{-1} for *R. idaeus, R. fraxinifolius, R. rosifolius, R.croceacanthus,* respectively, in saturated PPFD (1200 μmol·m^{-2}·s^{-1}). The *A* values of *R. idaeus* and *R. rosifolius*, measured in PPFD > 500μmol·m^{-2}·s^{-1}, were similar to each other and were 20% lower than those of *R. fraxinifolius* and *R. croceacanthus*. No significant difference in *A* among species were measured in low PPFD (<150μmol·m^{-2}·s^{-1}). At 30℃ and PPFD of 1200μmol·m^{-2}·s^{-1}, *A* were 11.2, 13.2, 14.8, 12.1μmol·m^{-2}·s^{-1} for *R. idaeus, R. fraxinifolius, R. rosifolius, R.croceacanthus,* respectively. In PPFD > 500μmol·m^{-2}·s^{-1}, *A* of *R. idaeus* was 33%, 18%, and 8% lower than those of *R. rosifolius*, *R. fraxinifolius* and *R. croceacanthus* respectively. No significant difference in *A* among species was measured in PPFD < 150μmol·m^{-2}·s^{-1}. At 35℃ and PPFD = 1200μmol·m^{-2}·s^{-1}, values of *A* were 7.1, 11.9, 14.6, 10.0 μmol·m^{-2}·s^{-1} for *R. idaeus, R. fraxinifolius, R. rosifolius, R.croceacanthus* respectively. In PPFD>500 μmol·m^{-2}·s^{-1}, *A* of *R. rosifolius*, *R. fraxinifolius* and *R. croceacanthu* was about 105%, 68%, and 40% higher than *A* of *R. idaeus* respectively. Significant differences in *A* between raspberry and the native species in low light intensities (PPFD=100μmol·m^{-2}·s^{-1}) were also recorded. The 3D mesh figure (Fig. 3.6) showed that in conditions of light intensity higher than 500 μmol·m^{-2}·s^{-1} PPFD, increasing temperature from 25℃ to 35 ℃ resulted in a greater *A* decrease in *R. idaeus* than in *R. fraxinifolius* and *R. croceacanthus*. On the other hand, increasing temperature in the same range caused an increase in *A* in *R. rosifolius*

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For stomatal conductance (Fig. 3.2 ; Table 3.5), at 25℃ and PPFD = 1200μmol·m^{-2}·s^{-1}, values of *gs* were 0.21, 0.28, 0.21, and 0.21 mol·m^{-2}·s^{-1} for *R. idaeus, R. fraxinifolius, R. rosifolius,* and *R.croceacanthus* respectively. *R. fraxinifolius* had significant high *gs* through all the light intensities. At 30℃ and PPFD = 1200μmol·m^{-2}·s^{-1}, values of *gs* were 0.18, 0.28, 0.29, and 0.21 mol·m^{-2}·s^{-1} for *R. idaeus, R. fraxinifolius, R. rosifolius,* and *R.croceacanthus* respectively. *R. idaeus* had the lowest *gs* in light intensities >500μmol·m^{-2}·s^{-1} PPFD. At 35℃ and PPFD = 1200μmol·m^{-2}·s^{-1}, values of *gs* were 0.08, 0.19, 0.22, and 0.18 mol·m^{-2}·s^{-1} for *R. idaeus, R. fraxinifolius, R. rosifolius,* and *R.croceacanthus,* respectively. *R. idaeus* had the lowest *gs* among tested species regardless of light intensity. *R. rosifolius* had the highest *gs* in PPFD>800μmol·m^{-2}·s^{-1} but were lower than *R. fraxinifolius* and *R. croceacanthus* under PPFD<500μmol·m^{-2}·s^{-1}. While *R. idaeus* have persistent negative response of *gs* as temperature rise from 25 to 35℃, *R. fraxinifolius* and *R. croceacanthus* remains consistent *gs* from 25 to 30 ℃ and slightly decrease from 30 to 35℃. *R. rosifolius* have the highest peak of *gs* at 30℃, but no significant difference between 25 and 35℃.

At 25℃ and PPFD = 1200μmol·m^{-2}·s^{-1}, transpiration rates (*E*)were 2.5, 3.2, 2.5, and 2.4 mmol·m^{-2}·s^{-1} for *R. idaeus, R. fraxinifolius, R. rosifolius,* and *R.croceacanthus,* respectively (Fig. 3.3 ; Table 3.4). In high PPFD, *R. fraxinifolius* had significant higher *E* than the other three species.. At 30℃ and PPFD = 1200μmol·m^{-2}·s^{-1}, values of *E* were 3.1, 4.7, 5.0, and 3.6 mmol·m^{-2}·s^{-1} for *R. idaeus, R. fraxinifolius, R. rosifolius,* and *R.croceacanthus,* respectively. *R. rosifolius* and *R. fraxinifolius* had significant higher *E* in high PPFD, while *R. idaeus* had the lowest. *R. fraxinifolius* had significant higher *E* among species regardless of light intensities. At 35℃ and PPFD = 1200μmol·m^{-2}·s^{-1}, values of *E* were 2.0, 4.4, 5.4, and 4.2 mol·m^{-2}·s^{-}

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[1] for *R. idaeus, R. fraxinifolius, R. rosifolius,* and *R.croceacanthus,* respectively. *R. rosifolius* had highest *E* in PPFD=1200μmol·m^{-2}·s^{-1}. However, in PPFD<200μmol·m^{-2}·s^{-1}, *E* of *R. rosifolius* was lower than those of *R. fraxinifolius* and *R. croceacanthus*. *R. idaeus* had the lowest *E* in all light intensities. In PPFD=1200μmol·m^{-2}·s^{-1}, *E* of *R. idaeus* was at 30℃ but lowest at 35℃. *R. rosifoilus* and *R. fraxinifolius* had higher *E* at 30 and 35℃ than at 25℃, *R. croceacanthus* had persistent positive response of *E* to increasing temperature from 25 to 35℃.

The difference between measured air temperature and leaf temperature showed that at 25℃ and 30℃, there were no significant difference among species regardless of light intensity. (Fig. 3.4). However, at 35℃, *R. idaeus* showed a significant smaller cooling capability than the other native species. In PPFD = 1200μmol·m^{-2}·s^{-1}, *R. idaeus* had a slightly higher leaf temperature than air temperature. Leaf temperatures were 3℃, 1.8℃, and 1℃ lower than air temperature in *R. rosifolius, R. croceacanthus,* and *R. fraxinifolius,* respectively. These temperature differences become smaller as PPFD were lower.

3.5.2 FvCB variables at different temperatures

The original data of *A/Ci* curve for modeling were given in Fig. 3.8., 3.9, and 3.10. The modeling results showed that V_{cmax} , $J_{max,}$ *Rd,* and *gm* were significant differences at different temperatures and among different species. On the other hand, differences in φ and Θj were detected only among species not among temperatures. Species and temperature interaction was found in V_{cmax} , $J_{max,}$, *Rd*, *gm,* and φ but not in Θj (Table 3.2).

V_{cmax} generated from the modeling tools showed that all four species had positive

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response to elevating temperature from 25℃ to 35℃ (Fig. 3.5 C). *R. croceacanthus* had the highest V_{cmax} at 25℃ but lowest V_{cmax} at 35℃. No significant differences at 25℃ among the other stree native species. Significant difference between *R. idaeus* and *R. rosifolius* were detected at 30℃, and between *R. fraxinifolius* and *R. croceacanthus* at 35℃ (Table 3.2).

$J_{max,}$ generated from the modeling tools showed that all four species did not reach their maximum value in the range of temperature treatments. *R. rosifolius* had the lowest $J_{max,}$ at 25℃but highest $J_{max,}$ at 30 and 35℃. *R. croceacanthus* had the highest $J_{max,}$ at 25℃ but lowest *J* at 30 and 35℃. *R. fraxinifolius* had slightly higher $J_{max,}$ than *R. idaeus* at 25℃, and significant higher *J* at 30 and 35℃. (Table 3.2).

R_d had no significant difference among four species at 25℃. *R. croceacanthus* had the lowest R_d at 30℃ and *R. rosifolius* had the highest R_d at 35℃ (Table 3.2).

Significant differences in *gm* were detectec among species at all temperature treatments. *R. rosifolius* had the lowest *gm* at 25℃ (0.091mol·m^{-2}·s^{-1}) but highest *gm* (0.201 mol·m^{-2}·s^{-1}) at 30 and 35℃ (0.425 mol·m^{-2}·s^{-1}) (Table3.2). Dramatic increases in *gm* were measured as temperature increased (Fig. 3.5). *R. fraxinifolius* and *R. croceacanthus* had the highest *gm* among species at 25℃ (Table 3.2) and had a positive correlation of *gm* to elevating temperatures from 25 and 35℃ (Fig. 3.5). *R. idaeus* had the lowest *gm* at 35℃ (Table3.2) and slightly increased *gm* from 25℃ to 30℃, then decreased from 30℃ to 35℃ (Fig. 3.5).

No significant differences in φ at 25℃ among species. Highest φ was detected in *R. idaeus* and *R. rosifolius* at 30 and 35℃, respectively. Temperature treatments had

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no significant difference in *R. idaeus* and *R. fraxinifolius*. However, *φ* of *R. rosifolius* was positively correlated with elevating temperatures from 25 to 35℃. In *R. croceacanthus*, *φ* was highest at 25℃. Values of *Θj* were significant different at 25 and 30℃ among species but were affected little by temperature treatments.

3.5.3 Partitioning variables contributions to *A* differences

3.5.3.1 Comparisons among species in conditions with given temperature and light intensity

3.5.3.1.1 *R. idaeus* versus *R. fraxinifolius*

Partitioning results between *R. idaeus* and *R. fraxinifolius* at 25℃ showed that the total variable differences (*ρ[TOT]*) were less than 20 % in PPFD=200, 500, 800, or 1200μmol·m^{-2}·s^{-1} (Fig. 3.10, 3.11). Diffusional factors (*ρ[DIFF]*) had 12.1 % contribution to *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to 4.4% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}, with *ρ[gs]* decreased from 4.4% to 1.8% and *ρ[gm]* from 7.7% to 2.7%. Biochemical factors (ρ[BIO]) had 5.4% contribution to *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, increased to 12.2% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[Vm] was 4.5% in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to 0 in PPFD less than 500 μmol·m^{-2}·s^{-1}. ρ[Jm] was 4.7% in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to 2.7% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[Rd] was less than 2%. *ρ*[Θj] was 6% in PPFD=1200μmol·m^{-2}·s^{-1}, increased to 16.6% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[φ] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to -9% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}.

At 30℃, the total variable differences (*ρ[TOT]*) were around 16 % in PPFD=500, 800, and 1200μmol·m^{-2}·s^{-1}, and decreased to less than 2% in PPFD<200μmol·m^{-2}·s^{-1}. Diffusional factors (*ρ[DIFF]*) had 11.7 % contribution to *A*

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differences in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to 5% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}, with *ρ[gs]* decreased from 8% to 3.6% and *ρ[gm]* from 3.7% to 1.2% . Biochemical factors (ρ[BIO]) had 5.4% contribution to *A* differences in PPFD=1200μmol·m^{-2}·s^{-1} and decreased to -3.2% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[Vm] was 6% in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to 0 if PPFD less than 200 μmol·m^{-2}·s^{-1}. ρ[Jm] was 0% in PPFD=1200μmol·m^{-2}·s^{-1} and increased to 1.7% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[Rd] was less than 1.5%. ρ[Θj] was 0% in PPFD=1200μmol·m^{-2}·s^{-1} and increased to 6.8% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[φ] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}and decreased to -10.5% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}.

At 35℃ the total variable differences (*ρ[TOT]*) were around 68 % in PPFD=1200μmol·m^{-2}·s^{-1} and decreased to 38.5% in PPFD= 200μmol·m^{-2}·s^{-1}. Diffusional factors (*ρ[DIFF]*) had 75.3 % contribution to *A* differences in PPFD=1200μmol·m^{-2}·s^{-1} and decreased to 34.9% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}, with *ρ[gs]* decreased from 38.2% to 19.3% and *ρ[gm]* from 37.1% to 15.6%. Biochemical factors (ρ[BIO]) had -6.4% contribution to *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, peaked 8.2% in PPFD= 500 μmol·m^{-2}·s^{-1}. ρ[Vm] was -6.3% in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to 0 in PPFD less than 500 μmol·m^{-2}·s^{-1}. ρ[Jm] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}, maximized at 8.2% in PPFD= 500 μmol·m^{-2}·s^{-1}. ρ[Rd] was less than 0.5%. P[Θj] was 0% in PPFD=1200μmol·m^{-2}·s^{-1} and decreased to -4.7% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[φ] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}, increased to -4.3% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}.

3.5.3.1.2 *R. idaeus* versus *R. rosifolius*

At 25℃ the total variable differences (*ρ[TOT]*) were around -12 % under

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PPFD=200, 500, 800, 1200μmol·m^{-2}·s^{-1} (Fig. 3.10, 3.11). Diffusional factors (*ρ[DIFF]*) had -4 % contribution to *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to -6.9% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}, with *ρ[gs]* decreased from 0.2% to -0.91% and *ρ[gm]* from -4.7% to -2.4% respectively. Biochemical factors (ρ[BIO]) had -7.1% contribution on *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, peak=-9.5% in PPFD=800μmol·m^{-2}·s^{-1} increased to -6.9% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[Vm] was -8.8% in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to -7.4 if PPFD less than 800 μmol·m^{-2}·s^{-1}, ρ[Jm] was 4.7% in PPFD=1200μmol·m^{-2}·s^{-1}, peak=-11.4 in PPFD=800μmol·m^{-2}·s^{-1} decreased to -7.5% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[Rd] was less than 2%. P[Θj] was 0.86% in PPFD=1200μmol·m^{-2}·s^{-1} and increased to 4.69% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[φ] was -0.46% in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to -6.9% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}.

At 30℃ showed that the total variable differences (*ρ[TOT]*) were around 27 % under PPFD=800 and 1200μmol·m^{-2}·s^{-1} but decreased to 15.3% and 2.3% in PPFD=500 and 200μmol·m^{-2}·s^{-1}, respectively. Diffusional factors (*ρ[DIFF]*) had 17.6 % contribution to *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to 4.4% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}, with *ρ[gs]* decreased from 9.4% to 1.5% and *ρ[gm]* from 8.2% to 2.9% respectively. Biochemical factors (ρ[BIO]) had 9.6% contribution to *A* differences in PPFD=1200μmol·m^{-2}·s^{-1} and decreased to -2.2% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[Vm] was 9.7% in PPFD=1200μmol·m^{-2}·s^{-1}, decreased to 0 in PPFD less than 200 μmol·m^{-2}·s^{-1}. ρ[Jm] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}, increased to 2% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[Rd] was less than 0.2%. P[θj] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}, increase to -2.4% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}. ρ[φ] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}and decreased to 12.9% as PPFD decreased to 200 μmol·m^{-2}·s^{-1}.

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At 35℃ showed that the total variable differences (*ρ[TOT]*) were around 102.3 % under PPFD=1200μmol·m^{-2}·s^{-1}, but decrease to 41.2% in PPFD= 200μmol·m^{-2}·s^{-1}. Diffusional factors (*ρ[DIFF]*) had 98.7 % contribution on *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to 33.1% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}, with *ρ[gs]* decreased from 51.2% to 15.3% and *ρ[gm]* from 47.4% to 17.8% respectively. Biochemical factors (ρ[BIO]) had 3.5% contribution on *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, peak= 18.1% in PPFD= 500 μmol·m^{-2}·s^{-1}. ρ[Vm] was 8.1% in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to 0 in PPFD< 500 μmol·m^{-2}·s^{-1}, ρ[Jm] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}, peak= 21.5% in PPFD= 500 μmol·m^{-2}·s^{-1}. ρ[Rd] was less than 6%. P[θj] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to -2.4% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}, ρ[φ] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}, increase to -12.8% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}.

3.5.3.1.3 *R. idaeus* versus *R. croceacanthus* (Fig. 3.10, 3.11)

At 25℃ showed that the total variable differences (*ρ[TOT]*) were around 19% in PPFD=1200μmol·m^{-2}·s^{-1} and decrease to 15.9% in PPFD= 200 μmol·m^{-2}·s^{-1}. Diffusional factors (*ρ[DIFF]*) have 7% contribution on *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to 2.2% in PPFD= 200 μmol·m^{-2}·s^{-1}, with *ρ[gs]* decreased from 0.05% to -0.28% and *ρ[gm]* from -6.9% to 2.5% respectively. Biochemical factors (ρ[BIO]) have 12.1% contribution on *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, peak=14% in PPFD=500μmol·m^{-2}·s^{-1} increase to -13.7 in PPFD= 200 μmol·m^{-2}·s^{-1}. ρ[Vm] was 10.4% in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to 0 if PPFD less than 800 μmol·m^{-2}·s^{-1}, ρ[Jm] was 0.6% in PPFD=1200μmol·m^{-2}·s^{-1}, peak= 7.8 in PPFD=800μmol·m^{-2}·s^{-1} decrease to 4.6% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}. ρ[Rd] was less than 2%. P[Θj] was 0.2% in PPFD=1200μmol·m^{-2}·s^{-1}, increase to 10.5% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}, ρ[φ] was -0.02% in

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PPFD=1200μmol·m^{-2}·s^{-1}, decrease to -3.4% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}.

At 30℃ showed that the total variable differences (*ρ[TOT]*) were around 3.5% in PPFD=1200μmol·m^{-2}·s^{-1}, however decrease to -6.3% in PPFD =200μmol·m^{-2}·s^{-1}. Diffusional factors (*ρ[DIFF]*) have 4.7 % contribution on *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to 2.9% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}, with *ρ[gs]* from 2.8% to 2.1% and *ρ[gm]* from 1.9% to 0.8% respectively. Biochemical factors (ρ[BIO]) have -1.1% contribution on *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to -9.3% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}. ρ[Vm] was 2.2% in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to 0 if PPFD less than 500 μmol·m^{-2}·s^{-1}, ρ[Jm] was -6.3% in PPFD=1200μmol·m^{-2}·s^{-1},peak=-12.5 in PPFD=500μmol·m^{-2}·s^{-1}, decrease to -6.6% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}. ρ[Rd] was less than 3%. P[Θj] was 1.6% in PPFD=1200μmol·m^{-2}·s^{-1}, increase to 16.2% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}, ρ[φ] was -0.4% in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to -22% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}.

At 35℃ showed that the total variable differences (*ρ[TOT]*) were around 37 % under PPFD=500, 800, 1200μmol·m^{-2}·s^{-1}, decrease to 25.5% when as decrease to PPFD= 200μmol·m^{-2}·s^{-1}. Diffusional factors (*ρ[DIFF]*) have 62.8% contribution on *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to 34.2% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}, with *ρ[gs]* from 32.1% to 20.5% and *ρ[gm]* from 30.7% to 13.7% respectively. Biochemical factors (ρ[BIO]) have -25.5% contribution on *A* differences in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to -8.7% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}. ρ[Vm] was -26.2% in PPFD=1200μmol·m^{-2}·s^{-1}, decrease to 0 if PPFD less than 500 μmol·m^{-2}·s^{-1}, ρ[Jm] was -% in PPFD=1200μmol·m^{-2}·s^{-1}, peak= 21.5% in PPFD= 500 μmol·m^{-2}·s^{-1}. ρ[Rd] was less than 6%. P[Θj] was 0% in PPFD=1200μmol·m^{-2}·s^{-1},

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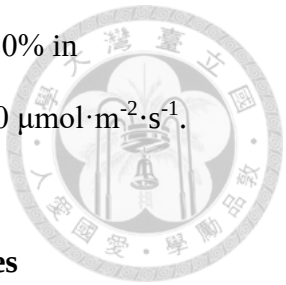
decrease to -0.45% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}, ρ[φ] was 0% in PPFD=1200μmol·m^{-2}·s^{-1}, increase to -7.2% as PPFD decrease to 200 μmol·m^{-2}·s^{-1}.



3.5.3.2 Temperature and light intensity comparison within species

3.5.3.2.1 *R. idaeus* (Fig. 3.15, 3.19)

At 25℃, ρ [TOT] decrease to -59.8% as PPFD decrease from 1200 to 100μmol·m^{-2}·s^{-1} with ρ[DIFF]= -5.08%, and ρ[PPFD]= -54.7% respectively. At 30℃, ρ [TOT]=1.5 % in PPFD=1200 μmol·m^{-2}·s^{-1}, decrease to -59.6% as PPFD decrease to 100μmol·m^{-2}·s^{-1}, with ρ[DIFF], ρ[BIO], and ρ[PPFD] decrease from 2.8%, -1.7%, 0% to -4.2%, -0.09%, -59.6% respectively. ρ[gs] and ρ[gm] were -2.8% and 5.6% to -8.7 % and 5.4% respectively. ρ[Vm] and ρ[Jm] were 17.7% and 0% to 11.4% and 3.1% respectively as PPFD decrease to 100μmol·m^{-2}·s^{-1}.ρ[Rd], ρ[Θj], and ρ[φ] were less than 1%. Rubisco kinetics combined (ρ[Kc]+ρ[Ko]+ρ[Γ*]) were around -17%. At 35 ℃, ρ [TOT]= -24.6 in PPFD=1200 μmol·m^{-2}·s^{-1}, decrease to -71.2% as PPFD decrease to 100μmol·m^{-2}·s^{-1}, with ρ[DIFF], ρ[BIO], and ρ[PPFD] decrease from -31.4%, 6.8%, 0% to -31.2%, 3.2%, -43.6% respectively. ρ[gs] and ρ[gm] were -23.4% and -8% to -25.7% and -5.5% respectively. ρ[Vm] and ρ[Jm] were 38% and 0% to 15 and 10.8% respectively as PPFD decrease to 100μmol·m^{-2}·s^{-1}.ρ[Rd], and ρ[φ] were less than 2%. ρ[Θj] was 0% in PPFD=1200μmol·m^{-2}·s^{-1} increase to 4.1% as PPFD decrease to 100μmol·m^{-2}·s^{-1}. Rubisco kinetics combined (ρ[Kc]+ρ[Ko]+ρ[Γ*]) were around -30%.

3.5.3.2.2 *R. fraxinifolius* (Fig. 3.16, 3.20)

At 25℃, ρ[TOT] decrease to -64.2% as PPFD decrease from 1200 to 100μmol·m^{-2}·s^{-1} with ρ[DIFF]=-4.4%, and ρ[PPFD]=-57.8% respectively. At 30℃, ρ [TOT]=0.75 in PPFD=1200 μmol·m^{-2}·s^{-1}, decrease to -69% as PPFD decrease to

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100μmol·m^{-2}·s^{-1}, with ρ[DIFF], ρ[BIO], and ρ[PPFD] decrease from 1.75%, -1%, 0% to -3.2%, -1.8%, -64% respectively. ρ [gs] and ρ[gm] were -0.1 and 1.9 to -4.7 and 1.4 respectively. ρ[Vm] and ρ[Jm] were 19.8% and 0% to 12.4 and 3.47% respectively as PPFD decrease to 100μmol·m^{-2}·s^{-1}.ρ[Rd], ρ[Θj], and ρ[φ] were less than 2%. Rubisco kinetics combined (ρ[Kc]+ρ[Ko]+ρ[Γ*]) were around -18%. At 35 ℃, ρ [TOT]= 8.4 in PPFD=1200 μmol·m^{-2}·s^{-1}, decrease to -69.5% as PPFD decrease to 100μmol·m^{-2}·s^{-1}, with ρ[DIFF], ρ[BIO], and ρ[PPFD] decrease from 5%, 3.4%, 0% to -5%, 0.41%, -64.8.6% respectively. ρ[gs] and ρ[gm] were -8.1 and 13 to -15.6 and 10 respectively. ρ[Vm] and ρ[Jm] were 46% and 0% to 29.4 and 5.8% respectively as PPFD decrease to 100μmol·m^{-2}·s^{-1}.ρ[Rd], ρ[φ], and ρ[Θj] were less than 2%. Rubisco kinetics combined (ρ[Kc]+ρ[Ko]+ρ[Γ*]) were around -40%.

3.5.3.2.3 *R. rosifolius* (Fig. 3.17, 3.21).

At 25℃, ρ[TOT] decrease to -59.2% as PPFD decrease from 1200 to 100μmol·m^{-2}·s^{-1} with ρ[DIFF]=-5.5%, and ρ[PPFD]= -53.5% respectively. At 30℃, ρ [TOT]=45.3 in PPFD=1200 μmol·m^{-2}·s^{-1}, decrease to -56.5% as PPFD decrease to 100μmol·m^{-2}·s^{-1}, with ρ[DIFF], ρ[BIO], and ρ[PPFD] decrease from 22.1%, 23.1%, 0% to 5.1%, 21.4%, -83% respectively. P[gs] and ρ[gm] were 4.3 and 17.8 to -7.3 and 12.5 respectively. ρ[Vm] and ρ[Jm] were 11.6% and 31.7% to 0% and 34.5% respectively as PPFD decrease to 100μmol·m^{-2}·s^{-1}.ρ[Rd], ρ[Θj], and ρ[φ] were less than 2%. Rubisco kinetics combined (ρ[Kc]+ρ[Ko]+ρ[Γ*]) were around -16%. At 35 ℃, ρ [TOT]= 72.6 in PPFD=1200 μmol·m^{-2}·s^{-1}, decrease to -92.2% as PPFD decrease to 100μmol·m^{-2}·s^{-1}, with ρ[DIFF], ρ[BIO], and ρ[PPFD] decrease from 32.5%, 39.6%, 0% to -6.2%, 36.1%, -92.18% respectively. ρ[gs] and ρ[gm] were 1.3 and 31.2 to -28.6 and 22.4 respectively. ρ[Vm] and ρ[Jm] were 41.1% and 50/9% to 0 and 65.3% respectively as PPFD decrease to 100μmol·m^{-2}·s^{-1}.ρ[Rd] were around

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4.7. ρ[φ] and ρ[Θj] were from 1.1 and 0.74 to 4 and 4.9 respectively. Rubisco kinetics combined (ρ[Kc]+ρ[Ko]+ρ[Γ*]) were around -41%.

3.5.3.2.4 *R. croceacanthus* (Fig. 3.18, 3.22).

At 25℃, ρ[TOT] decrease to -62.9% as PPFD decrease from 1200 to 100μmol·m^{-2}·s^{-1} with ρ[DIFF]=-4.4%, and ρ[PPFD]= -58.4% respectively. At 30℃, ρ[TOT]=-12.2 in PPFD=1200 μmol·m^{-2}·s^{-1}, decrease to -72.4% as PPFD decrease to 100μmol·m^{-2}·s^{-1}, with ρ[DIFF], ρ[BIO], and ρ[PPFD] decrease from 0.76%, -12.9%, 0% to -69.8%, -10.8%, -59% respectively. P[gs] and ρ[gm] were -0.02 and 0.78 to -3.3 and 0.65 respectively. ρ[Vm] were 0%. ρ[Jm] were -3.14% to -2.3% as PPFD decrease to 100μmol·m^{-2}·s^{-1}.ρ[Rd] were less than 0.5%. ρ[Θj] and ρ[φ] were 2.7 and -0.73 to 5.5 and -3.4 respectively. Rubisco kinetics combined (ρ[Kc]+ρ[Ko]+ρ[Γ*]) were around -11%. At 35℃, ρ[TOT]= -13.1 in PPFD=1200 μmol·m^{-2}·s^{-1}, decrease to -73% as PPFD decrease to 100μmol·m^{-2}·s^{-1}, with ρ[DIFF], ρ[BIO], and ρ[PPFD] decrease from 7.24%, -20.5%, 0% to 1.31%, -18.5%, -56.12% respectively. ρ[gs] and ρ[gm] were -2.5 and 9.7 to -6.6 and 8 respectively. ρ[Vm] and ρ[Jm] were 16% and 0.147% to 9 and 3.1% respectively as PPFD decrease to 100μmol·m^{-2}·s^{-1}.ρ[Rd] were around 1%. ρ[φ] and ρ[Θj] were from 0.01 and -0.005 to 1 and -1.4 respectively. Rubisco kinetics combined (ρ[Kc]+ρ[Ko]+ρ[Γ*]) were around -35%.

3.5.3.3 Reliability

A close correlation (R^2=0.94) between the total partitioning variable differences of *A* (ρ[TOT]) and measured averages of *A* differences from the light curve response measurements was found (Fig. 3.23). There was no significant systematic error in different light intensities and temperature treatments (Fig. 3.24).

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3.6 Discussion

3.6.1 Net assimilation rate

Net assimilation rates showed that the three native subtropical *Idaeobatus* species had higher heat tolerance level than the raspberry (Fig. 3.1; Table 3.3). Similar conclusions have been reported previously (Stafne et al. 2000, Molina-Bravo et al. 2011). Some native *Rubus* species in Taiwan e.g. *R. parvifolius* and *R. coreanus* would be good candidate for heat tolerance breeding materials . Light saturated *A* of *R. rosifolius* collected from Tainan was low at 25℃ but increased significantly at 30℃ and 35℃, indicating its superior heat tolerance capability. *A* of *R. fraxinifolius* and *R. croceacanthus* decreased from 30 to 35℃ but still remained much higher than *A* of the raspberries. (Fig. 3.6).. In this experiment, *A* of *R. idaeus* only slightly decreased from 25℃ to 30℃. The result was different from those reported previously (Fernandez and Pritts 1994, Percival et al. 1996, Stafne et al. 2000, 2001, Qiu et al. 2017). The raspberry plants used in this experiment had been grown in a greenhouse with much warmer temperature then the temperate open field. The acclimation effect of growing temperature may contribute to the less severe decline. Temperature acclimation have been proved to affect the optimum temperature of *A* in many species (Hikosaka et al. 2006, Yamori et al. 2014).

3.6.2 Stomatal conductance and transpiration rate

Stomatal conductance and transpiration rate were significant lower in temperate raspberry at 35℃ than the native *Idaeobatus* species (Table 3.5; Fig. 3.2). The cooling effect via transpiration through the leaves of these species wer also significanty different at 35℃, where *R. idaeus* had the least and *R. rosifolius* had the highest temperature differences between leaf and air especially in high light intensities. The photosynthesis rates at 35℃ were higher as the temperature difference between leaf

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and air, *E*, and *gs* were bigger in all tested species. This makes the *A* differences even bigger if the measurement temperature were set at the same air temperature but not leaf temperature in this thesis. The cooling effect of the plants via transpiration is a key trait for selecting heat tolerance in maize, rice, cotton, wheat, tomato, and radish (Lu et al. 1998, Chen et al. 2014, Xiong et al. 2014, Zhou et al. 2015, Naveed et al. 2016). Previous studies comparing *Rubus* genotypes suggested that *gs* was highly correlated to *A* at high temperatures (Stafne *et al.*, 2001; Qiu *et al.*, 2017; Cheng, 2016). A study comparing wild *Rubus* species also showed that mean aboveground relative growth rate was also well correlated to the midday *gs* value (Caplan and Yeakley 2013). Stomatal responses to VPD can be explained by passive regulation by leaf hydration and biochemical regulation by the ABA (Mcadam and Brodribb 2015). Another report showed that although *gs* in raspberry had slightly higher value under low VPD with 30℃ and 35℃ than under high VPD , there were still negative temperature response and no significant differences between VPD treatments, hydraulic conductance increased at high temperature (Qiu et al. 2017). The process-based (mechanistic) and goal- directed (optimality) models for *gs* control in different environments ar require future development (Buckley 2017).

The results of *R. idaeus* in this chapter have improved *gs* response between 25℃ to 30℃ than the previous research done in temperature area. Similar results have been reported in Arabidopsis, in which plants exposed to high temperature paradoxically displayed increases in transpiration and leaf cooling capacity(Crawford et al. 2012). Temperature differences between leaf and air and stomatal conductance may be important traits for selecting heat tolerance plants in the future by using convenient thermal image and porometer,

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3.6.3 Mesophyll conductance

In this experiment, temperature responses of *gm* in *Idaeobatus* plants can be categorized into two groups according to the origin of the speices. Subtropical species have great positive temperature response as the temperature rise form 25℃ to 35℃. On the other hand, temperate originated *R. idaeus* had relative stable temperature response and negative *gm* response at 30℃ to 35℃. Previous studies also showed relations between the origin of the species and the temperature response of *gm*. Temperate originated plants have a relatively stable temperature response for *gm* (von Caemmerer and Evans, 2015).

Negative *gm* responses at high temperature are rare. The only research about temperature responses of *gm* focusing on raspberries have just been published recently (Qiu et al. 2017). In this report, a persistent negative temperature response of *gm* decrease from 0.14 (20℃) to 0.06 (35℃) in raspberry ‘Jeanne d’Orléans’ was observed. . Qiu et al (2017) and other studies (Mizokami et al. 2015, Sorrentino et al. 2016, Qiu et al. 2017). concluded that the temperature response of *gm* was mainly caused by ABA accumulation and the ABA response of *gm* can be separate from drought conditions.

In addition to ABA, short-term responses of *gm* have been reported to be regulated by aquaporins (Uehlein et al. 2003, 2012, Hanba et al. 2004, Flexas et al. 2006, Heckwolf et al. 2011, Perez-Martin et al. 2014, Ding et al. 2016), carbonic anhydrase (Price *et al.*, 1994; Gillon andYakir, 2000; Perez-Martin *et al.*, 2014) and these factors which could have high dependency on temperature. Some research adding variable chloroplast surface area facing intercellular airspace per unit leaf area (*Sc)* with aquaporin factors in the temperature response model of *gm* can describe the

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negative *gm* response at high temperatures more precisely in the future (Flexas and Diaz-Espejo 2015).

The difference in growing temperatures may also be an important factor affecting *gm* temperature responses. Temperature acclimation of *gm* has been proved in spinach (Yamori et al. 2006), in which plants grown at 15/10℃ had significant lower *gm* than those at 30/25℃ ..

3.6.4 Biochemical factors

There were several significant differences in V_{cmax} and J_{max} between *R. idaeus* and the subtropical *Idaeobatus* native species at all temperature treatments (Table 3.2). The overall patterns to temperature response were similar to each other and none reached their maximum (Fig. 3.5 C,D). Therefore, the *A* limitation in these *Idaeobatus* plants at 35℃ may not limited by biochemical factors. The temperature responses of V_{cmax} and J_{max} in *R. idaeus* were similar to a previous research (Qiu *et al.*, 2017; Cheng, 2016). Optimal temperatures for V_{cmax} and J_{max} were 30-50℃ and 20-38℃, respectively, in 36 species (Kattge et al. 2007). Usually J_{max} were more sensitive than V_{cmax} at high temperatures because the thylakoid membrane complex affect the electron transportation (Sage and Kubien 2007). In this experiment, *R. idaeus* did not have the lowest V_{cmax} at 35℃. The results of FvCB biochemical factors showed that biochemical factors were not the key factors affecting *A* differences between *R. idaeus* and *R. corceacanthus*.. Significant differences in V_{cmax} and J_{max} at 25℃ can be found among species of *Eucalyptus* from different origins. Species originated from the warmer area have higher values but no significant differences in temperature response parameters (Lin et al. 2013). In this chapter, the highest V_{cmax} and J_{max} values at 25℃ were found in *R. corceacanthus* but highest value at 35℃ was

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found in *R. rosifolius*. The raspberry did not show lowest V_{cmax} and J_{max} value from 25℃ to 35℃. Therefore the correlations between V_{cmax} and J_{max} value at 25℃ with the originated area temperature may be highly dependent on the species choose in the research. Acclimation of growing temperature differences can also affect the activation energy of V_{cmax} which made a different temperature response (Hikosaka et al. 2006, Lin et al. 2013).

3.6.5 Partitioning variables contribution of net assimilation differences

The comparisons within species in different environments (Fig. 3.15-3.22) showed that *A* at 35℃ were mostly limited by diffusional factors in *R. idaeus*. Negative diffusional factors in *R. idaeus* were mostly cause by *gs* rather *gm*. In native species, *gs* had slightly negative effects but *gm* had greater positive effects on *A* differences.

Biochemical factors have slightly positive effect on raspberries at 35℃ but a negative effect in native *R. corceacanthus*. V_{cmax} had positive effect in PPFD>500μmol·m^{-2}·s^{-1} in *R. idaeus, R. corceacanthus,* and *R. fraxinifolius.* The effect of J_{max} was small though all the PPFD treatments. In *R. rosifoilus,* J_{max} was the more important biochemical factor than V_{cmax} because *A* was mostly limited by RuBP regeneration rate (non-saturated). Using data from various environment conditions and dependent significant tests, the optimal temperature of *A* was dependent more on stomatal reaction rather than biochemical factors (Lin et al. 2012). Temperature optimum of *A* had a more similar range to the optimum range of *gs* rather than V_{cmax} and J_{max} in lowland tropical tree species and indirect effects of temperature through VPD were stronger limitations than biochemical factors at high temperatures (Slot and Winter 2017). V_{cmax} and *J* optimal were at 39℃ and 36℃, respectively, in grapevine ‘Semillon’ but optimal light saturated *A* was observed at

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30℃, and stomatal limitation increased from 15% to 35% as the temperature elevated from 20℃ to 40℃ (Greer and Weedon 2012). Temperature responses in litchi showed that over 28℃, *A* was mostly limited by *gs* but rather biochemical limitations (Chang and Lin 2007).

The comparisons between species (Fig. 3.10, 3.11) showed that although there were several significant differences in these factors at all temperature treatments between *R. idaeus* and the native species, the contributions of *A* differences were relatively small. Diffusional factors dominated the *A* differences at 35℃ with *gs* and *gm* shared equally. ρ[DIFF] decreased as the PPFD decreased. ρ[BIO] was higher in *R. fraxinifolius* and *R. rosifolius* in PPFD=500μmol·m^{-2}·s^{-1} but J_{max}, V_{cmax} had bigger negative effects in *R. croceacanthus* in PPFD>500μmol·m^{-2}·s^{-1}. The 3D mesh figure showed that ρ[TOT] among native species (Fig. 3.12) were very similar to ρ[DIFF](Fig. 3.13) . These results demonstrated a more detailed quantitative mechanism for contributions of FvCB variables to differences in environmental responses among species. .

The protocol measuring detached leaves *Fv/Fm* value in hot water bathing treatment for selecting heat tolerance red raspberry and black raspberry have been developed (Molina-Bravo et al. 2011, Bradish et al. 2016). However, a previous research showed that this approach was not suitable for *Idaeobatus* species because some apparently heat tolerance species such as *R. rosifolius* showed great decreases in *Fv/Fm* than heat sensitive species in the hot water bathing process, possibly caused by the structural traits of the leaves (Cheng, 2016). In this chapter biochemical limitations at high temperatures were relatively minor for *Idaeobatus* according to FvCB model. Species comparisons also showed that it is the diffusional factor

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differences that makes the native species having higher *A* at high temperatures. Because the *gm* measuring method were still time consuming and complicated, for selecting heat tolerance *Idaeobatus* plants with high *A*, it may be more convenient to measure stomatal behavior or temperature differences between leaf and air at high temperature using porometers or thermal image analyzers.

3.6.6 Methodology

3.6.6.1 Measurement

Chlorophyll fluorescence measurements in this chapter were made with a single strong flash light controlled by the OPEN 5.3.2 system program built in LI-6400. However, it can be improved using multiple flash method by updating new version of OPEN program to get a less error results (Loriaux et al. 2013). The *A/Ci* curve measurement is a very time consuming process. New gas exchange system and model to shorten the time consumed is necessary (Stinziano et al., 2017). Several data with big differences between ρ[TOT] and measured average *A* differences were found in comparisons within *R. rosifolius* at 35℃. This may be the result of A/Ci curve measurements not light saturated (Fig. 3.1C; 3.9D), leading to an overestimated V_{cmax} and p[BIO].

3.6.6.2 *FvCB* modeling

For simplification, the day respiration rates (*Rd*) used in this chapter were generated from the *A/Ci* model fitting method combined with chlorophyll fluorescence (Moualeu-Ngangue et al. 2017), which may be considered unreliable. The accuracy of this value can be improved via low O_2 light response curve with chlorophyll fluorescence measurement to minimize the effect of photorespiration (Yin et al. 2009).

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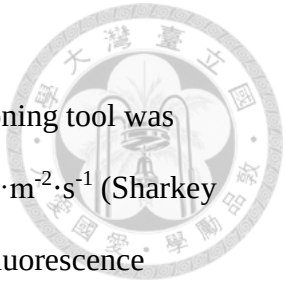

The maximum electron transport rate (J_{max}) using in the partitioning tool was derived from the light curve model fitting method in CO_2=400 μmol·m^{-2}·s^{-1} (Sharkey 2016). The modeling *J* generated from A/Ci curve and chlorophyll fluorescence (Moualeu-Ngangue et al. 2017) were recommended to be annotated as J_{1200} or J_{high} in order to distinguish the electron transport rate modeled under certain PPFD from the theoretic maximum electron transport rate (Buckley and Diaz-Espejo 2015b). Theoretically, J_{1200} should be lower than $J_{max.}$ However, the average differences between J_{max} and J_{1200} in this chapter were only 8.95μmol·m^{-2}·s^{-1} (4.47%). This may explain some of the error between the real *A* differences and the modeling total *A* differences at lower light intensities, where *A* in non-saturated state were considered as RuBP regeneration limited state in FvCB model (Archontoulis et al. 2012).

Mesophyll conductance (gm) in this thesis were generated by using A/Ci curve and chlorophyll fluorescence modeling (Moualeu-Ngangue et al. 2017). The model fitting method were sometimes considered as not very reliable. It can be improve by using variable J or online isotope method (Flexas et al. 2013).

For the temperature response of Rubisco kinetics (*Kc* and *Ko*), most of the previous research in *FvCB* modeling were using *in vivo* data from tobacco leaves as the reference (Bernacchi et al. 2001, 2002). In this chapter, to simplified the measurement process and the comparisons data , all the plants fitting in *FvCB* model were also using *in vivo* tobacco data collected with the consideration of mesophyll conductance (Walker et al. 2013). Recent studies have found that Rubisco kinetics in temperature response data were big enough to affect the modeling results of *FvCB* especially at high temperatures (Walker et al. 2013, Galmés et al. 2016). Rubisco

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kinetics can also be affected by different growing temperatures. Our results of *FvCB* variables and the partitioning results might be slightly different, if using a specific determined Rubisco kinetics temperature response of each different plant materials. Differences in V_{cmax} between raspberry and the native species were not big and none of them reached maximum at 25-35℃. The contribution of biochemical factors to differences in *A* at Rubisco limited state between species and environmental factors were relatively small compared with diffusional factors. Therefore, using Rubisco kinetics data from the model plants is adopted in this thesis.

Temperature responses of rubisco kinetics can be determined by comparing wild plants with genetic modified plants having low Rubisco content *in vivo* (Bernacchi et al. 2001, 2002, Walker et al. 2013). However, this method might not be approachable with other non-model plants. Alternatively, determining Rubisco kinetics in *Idaeobatus* plants may be obtained by using the model fitting method with additional measurements in low O_2 conditions or *in vitro* measurements (Bellasio et al. 2016, Galmés et al. 2016).

3.6.6.3 Partitioning variables

In this research, some errors appeared between the actual differences in *A* and the partitioning results of total differences. This is possibly the result of the assumptions in the modeling process that *gm* did not affected by light intensity and *Ci.* Studies focusing on short term light responses in *gm* were still rare. Some found small differences (Flexas et al. 2007, Yin et al. 2009, Théroux-Rancourt and Gilbert 2017), while others did not (Tazoe et al. 2009). The effect of *Ci* on *gm* was considered in the model fitting process (Flexas et al. 2007, Moualeu-Ngangue et al. 2017) but not in the partitioning steps.

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3.7 Conclusion and future perspective

The difference in leaf *A* between temperate originated raspberries and subtropical originated native *Idaeobatus* species in conditions of high temperatures and light intensities was mostly caused by diffusional factors, in which *gs* and *gm* shared nearly the same correspondence. *A* of raspberry 'Summer Festival' grown in subtropical summer remained high at 30℃ but decreased dramatically at 35℃.

For selecting heat tolerance *Idaeobatus* plants in a raspberry breeding program, instead of measuring chlorophyll fluorescence value (*Fv/Fm*) on detached leaves after heat shock water bathing suggested by the previous research (Molina-Bravo et al. 2011, Bradish et al. 2016), diffusional traits based on results of partitioning variable differences in *A* is suggested and can be achieved with . porometers or thermal imaging techniques (Costa et al. 2013; Humplik et al. 2015). However, the efficiency of this method should be tested in the future on a larger scale, such as in a true hybrid progenies from raspberry and the native species.

Future research on the photosynthetic modeling aspect can be focusing on horticultural practices to improve *A* at high temperatures in raspberries, such as partial shade or water fogging devices to decrease temperature and VPD in the greenhouse. The study of detail biochemical photosynthetic mechanisms in this chapter were only based on FvCB comparisons between young full expanded leaves in summer mornings, it would be interesting to quantified the *A* differences by diurnal, seasonal (acclimation), and architectural perspective by functional structural plant model (FSPM) in the future.

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Table 3.1 Rubisco kinetics value of tabaco (*Nicotiana tabacum*) used for model fitting (Walker et al. 2013). Parameter= exp (c-$\frac{\Delta Ha}{RT_k}$), where c is a scaling constant, ΔHa is the energy of activation, R is the molar gas constant (8.314 $JK^{-1}mol^{-1}$) and T_k is the leaf temperature in Kelvin.

	Value at 25°C	c	ΔHa
*Γ** (Pa)	4.00	20.07	46.3
Kc (Pa)	21.50	26.95	58.3
Ko (kPa)	31.50	18.21	37.5

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Table 3.2 FvCB variables generated by Moualeu-Ngangue *et al*.(2017) and Sharkey *et al*. (2016) at different temperatures .

Temperature	Species	*Vcmax*	*Jmax*	*Rd*	*gm*	*Φ*	*Θ*
25℃	*R. idaeus*	58.31±1.02 b C[x]	97.42±2.96 b C	1.83±0.15 a A	0.116±0.01 b B	0.47±0.04 a A	0.64±0.06 c A
	R. fraxinifolius	61.84±4.23 b C	103.21±6.50 ab C	1.66±0.16 a AB	0.154±0.02 a B	0.40±0.03 a A	0.86±0.03 a A
	R. rosifolius	59.00±2.32 b C	82.99±0.78 c C	1.63±0.11 a C	0.091±0.01 c C	0.41±0.02 a C	0.72±0.08 bc A
	R. croceacanthus	68.02±2.45 a C	107.18±4.36 a B	1.64±0.07 a A	0.149±0.002 a B	0.44±0.04 a A	0.80±0.05 ab B
30℃	*R. idaeus*	75.07±3.10 c B	128.36±5.71 b B	1.89±0.12 a A	0.144±0.01 b A	0.49±0.01 a A	0.72±0.07 c A
	R. fraxinifolius	81.27±0.76 ab B	135.19±2.37 a B	2.01±0.15 a A	0.167±0.01 b B	0.42±0.03 b A	0.82±0.02 b A
	R. rosifolius	84.70±1.90 a B	135.77±0.13 a B	1.91±0.08 a B	0.201±0.03 a B	0.46±0.03 ab B	0.74±0.04 bc A
	R. croceacanthus	78.61±1.31 bc B	102.77±2.43 c B	1.56±0.22 b A	0.158±0.01 b B	0.35±0.01 c B	0.93±0.03 a A
35℃	*R. idaeus*	123.37±9.66 ab A	145.81±12.92 c A	1.53±0.02 b B	0.088±0.01 c C	0.43±0.04 bc A	0.86±0.19 a A
	R. fraxinifolius	113.86±8.30 b A	177.39±9.12 b A	1.56±0.28 b B	0.297±0.03 b A	0.45±0.04 ab A	0.78±0.13 a A
	R. rosifolius	134.40±5.00 a A	203.13±8.63 a A	2.34±0.19 a A	0.425±0.02 a A	0.50±0.00 a A	0.82±0.02 a A
	R. croceacanthus	87.04±5.10 c A	130.41±4.08 c A	1.41±0.17 b A	0.260±0.03 b A	0.40±0.01 c AB	0.85±0.06 a AB
Species		***[y]	***	***	***	***	*
Temperature		***	***	*	***	ns	ns
Species X Temperature		***	***	***	***	***	ns

[x] Significant difference among species within temperature were indicated by lower case letters. [x] Significant difference among temperature within species were indicated by lower case letters. Mean separation within columns, species and temperature by LSD test $P<0.05$.

[y] '*', '**', '***' indicated LSD test $P<0.05$, 0.01, 0.001, respectively.

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Table 3.3 Net assimilation rate (μmol· m^{-2} ·s^{-1}) of *Idaeobatus* plants at different temperatures and PPFD treatments.

Species	Temperature	PPF(μmol·m^{-2}·s^{-1})				
		1200	800	500	200	100
R. idaeus	25℃	11.22±0.56 b A[x]	10.82±0.46 c A	10.28±0.36 b A	7.61±0.34 b A	4.50±0.34 ab A
	30℃	11.15.±0.21 d A	11.02±0.10 d A	10.13±0.18 c A	7.14±0.47 c A	3.91±0.27 b A
	35℃	7.13±0.58 d B	7.25±0.71d B	6.82±0.41 c B	4.93±0.36 b B	2.81±0.35b B
R. fraxinifolius	25℃	13.09±0.06 a A	13.12±0.30 a A	12.49±0.06 a A	8.90±0.29 a A	4.81±0.39 a A
	30℃	13.19±0.12 b A	12.89±0.11 b A	11.96±0.12 b B	7.90±0.10 ab B	4.09±0.27 ab B
	35℃	11.89±0.21 b B	11.45±0.21 b B	10.69±0.31 b C	6.94±0.24 a C	3.75±0.05 a B
R. rosifolius	25℃	11.05±0.25 b B	10.92±0.40 c B	10.15±0.36 b C	7.44±0.31 b B	4.25±0.16 b A
	30℃	14.79±0.15 a A	14.43±0.20 a A	12.86±0.31 a B	8.07±0.19 a C	4.25±0.19 a A
	35℃	14.56±0.64 a A	13.49±0.75 a A	12.02±0.52 a A	6.80±0.38 a A	3.56±0.03 a B
R. croceacanthus	25℃	12.79±0.06 a A	12.79±0.06 b A	12.05±0.12 a A	8.57±0.41 a A	4.85±0.16 a A
	30℃	12.12±0.44 c A	12.09±0.40 c A	11.62±0.36 b A	7.48±0.02 bc B	3.69±0.06 ab B
	35℃	9.99±0.22 c B	9.97±0.28 c B	9.55±0.19 b B	6.60±0.24 a B	3.49±0.21 a B
Species		***[y]	***	***	***	**
Temperature		***	***	***	***	***
Species X Temperature		***	***	***	***	**

[x] Significant difference among species within temperature were indicated by lower case letters. [x] Significant difference among temperature within species were indicated by lower case letters. Mean separation within columns, species and temperature by LSD test $P<0.05$.

[y] '*', '**', '***' indicated LSD test $P<0.05$, 0.01, 0.001 respectively.

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Table 3.4 Transpiration rate (mmol· m^{-2} ·s^{-1}) of *Idaeobatus* plants under different temperature and PPFD treatments.

Species	Temperature	PPF(μmol·m^{-2}·s^{-1})				
		1200	800	500	200	100
R. idaeus	25℃	2.50±0.19 b B[x]	2.56±0.15 b A	2.40±0.05 b B	2.05±0.06 b B	1.55±0.10 b B
	30℃	3.09±0.09 c A	2.93±0.04 c A	2.78±0.05 c A	2.42±0.15b A	2.00±0.21 b A
	35℃	2.04±0.21 c C	2.09±0.34 b B	1.93±0.17 c C	1.52±0.21d C	1.17±0.13 c C
R. fraxinifolius	25℃	3.24±0.03 a B	3.33±0.18 a C	3.08±0.08 a C	2.71±0.12 a B	2.07±0.15 a B
	30℃	4.68±0.11 a A	4.39±0.09 a A	4.15±0.22 a A	3.61±0.58 a A	3.07±0.48 a A
	35℃	4.36±0.31 ab A	4.06±0.17a B	3.69±0.06 ab B	2.99±0.50 b AB	2.32±0.40 b B
R. rosifolius	25℃	2.53±0.14 b B	2.50±0.17 b B	2.17±0.39 b B	1.76±0.20 c B	1.32±0.30 b B
	30℃	4.98±0.23 a A	4.56±0.09 a A	4.09±0.28 a A	2.83±0.52 b A	2.04±0.20 b A
	35℃	5.39±0.92 a A	4.59±0.74 a A	3.78±0.45 b A	2.43±0.29 c AB	1.56±0.08 c B
R. croceacanthus	25℃	2.42±0.09 b C	2.37±0.07 b C	2.20±0.09 b C	1.98±0.06 bc C	1.63±0.12 b C
	30℃	3.58±0.19 b B	3.41±0.14 b B	3.42±0.15 b B	2.93±0.17 ab B	2.44±0.10 b B
	35℃	4.35±0.13 b A	4.37±0.22a A	4.23±0.12 a A	3.87±0.24 a A	3.12±0.38 a A
Species		***[y]	***	***	***	***
Temperature		***	***	***	***	***
Species X Temperature		***	***	***	***	***

[x] Significant difference among species within temperature were indicated by lower case letters. [x] Significant difference among temperature within species were indicated by lower case letters. Mean separation within columns, species and temperature by LSD test $P<0.05$.

[y] '*', '**', '***' indicated LSD test $P<0.05$, 0.01, 0.001 respectively.

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Table 3.5 Stomatal conductance (mol· m^{-2} ·s^{-1}) of *Idaeobatus* plants under different temperature and PPFD treatments.

Species	Temperature	PPF(μmol·m^{-2}·s^{-1})				
		1200	800	500	200	100
R. idaeus	25℃	0.21±0.02 b A[x]	0.21±0.02 b A	0.20±0.01 b A	0.17±0.00 b A	0.13±0.01 b A
	30℃	0.18±0.01 d B	0.17±0.00 c B	0.16±0.00 c B	0.14±0.01b B	0.12±0.01 b A
	35℃	0.08±0.01 c C	0.08±0.01 b C	0.07±0.01 c C	0.06±0.01d C	0.04±0.01 c B
R. fraxinifolius	25℃	0.28±0.01 a A	0.28±0.02 a A	0.26±0.02 a A	0.23±0.01 a A	0.18±0.01 a A
	30℃	0.28±0.00 b A	0.26±0.01 a A	0.24±0.02 a A	0.21±0.04 a A	0.18±0.03 a A
	35℃	0.19±0.01 abB	0.18±0.01 a B	0.16±0.01 ab B	0.13±0.02 b B	0.10±0.02 b B
R. rosifolius	25℃	0.21±0.02 b B	0.20±0.01 b B	0.18±0.03 b B	0.15±0.02 c A	0.11±0.02 b A
	30℃	0.29±0.01 a A	0.27±0.00 a A	0.23±0.02 a A	0.16±0.03 b A	0.12±0.01 b A
	35℃	0.22±0.04 a B	0.19±0.03 a B	0.15±0.02 b B	0.10±0.01 c B	0.06±0.00 c B
R. croceacanthus	25℃	0.21±0.01 b A	0.20±0.01 b A	0.19±0.00 b B	0.17±0.01 bc A	0.13±0.01 b A
	30℃	0.21±0.01 c A	0.21±0.01 b A	0.20±0.01 b A	0.18±0.01 ab A	0.15±0.00 b A
	35℃	0.18±0.01 b B	0.18±0.01a B	0.18±0.01 a B	0.16±0.01 a A	0.13±0.02 a A
Species		***[y]	***	***	***	***
Temperature		***	***	***	***	***
Species X Temperature		***	***	***	***	***

[x] Significant difference among species within temperature were indicated by lower case letters. [x] Significant difference among temperature within species were indicated by lower case letters. Mean separation within columns, species and temperature by LSD test $P<0.05$.

[y] '*', '**', '***' indicated LSD test $P<0.05$, 0.01, 0.001 respectively.

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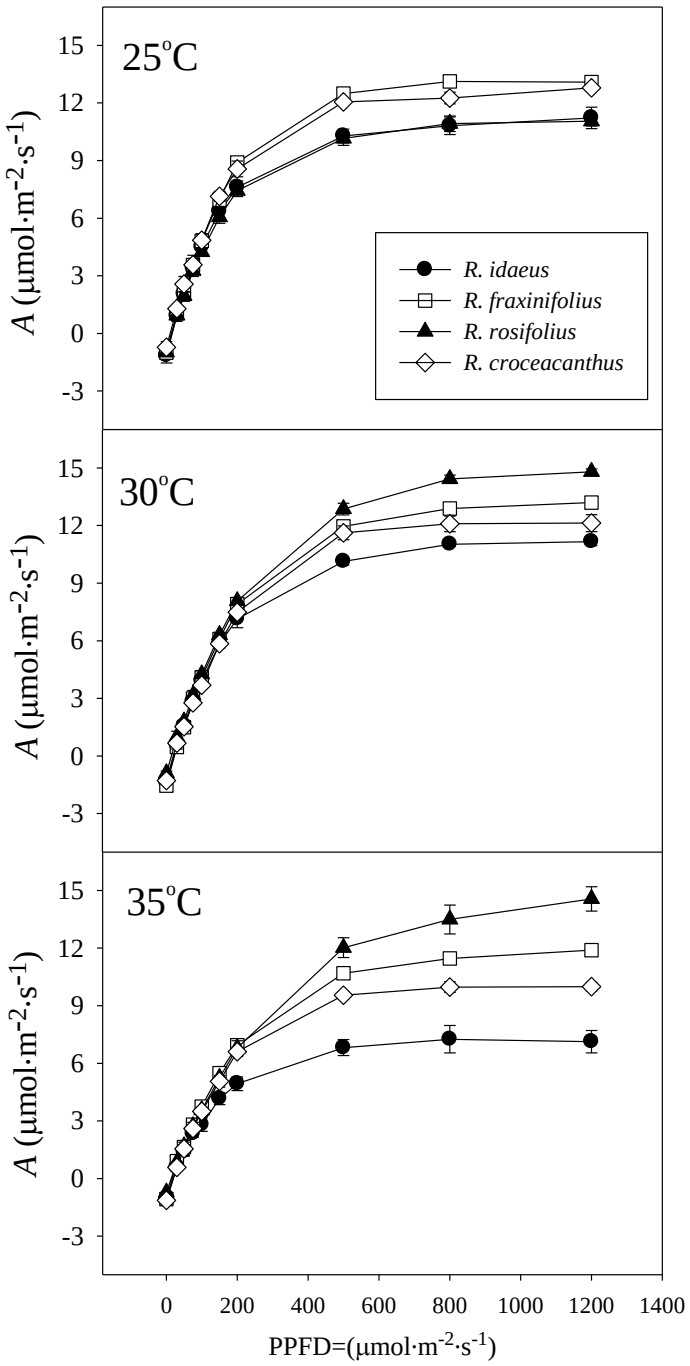

Fig. 3.1 Light response curve of net assimilation rate (μmol· m^{-2} ·s^{-1}) in *Idaeobatus* plants under different temperature and PPFD treatments.

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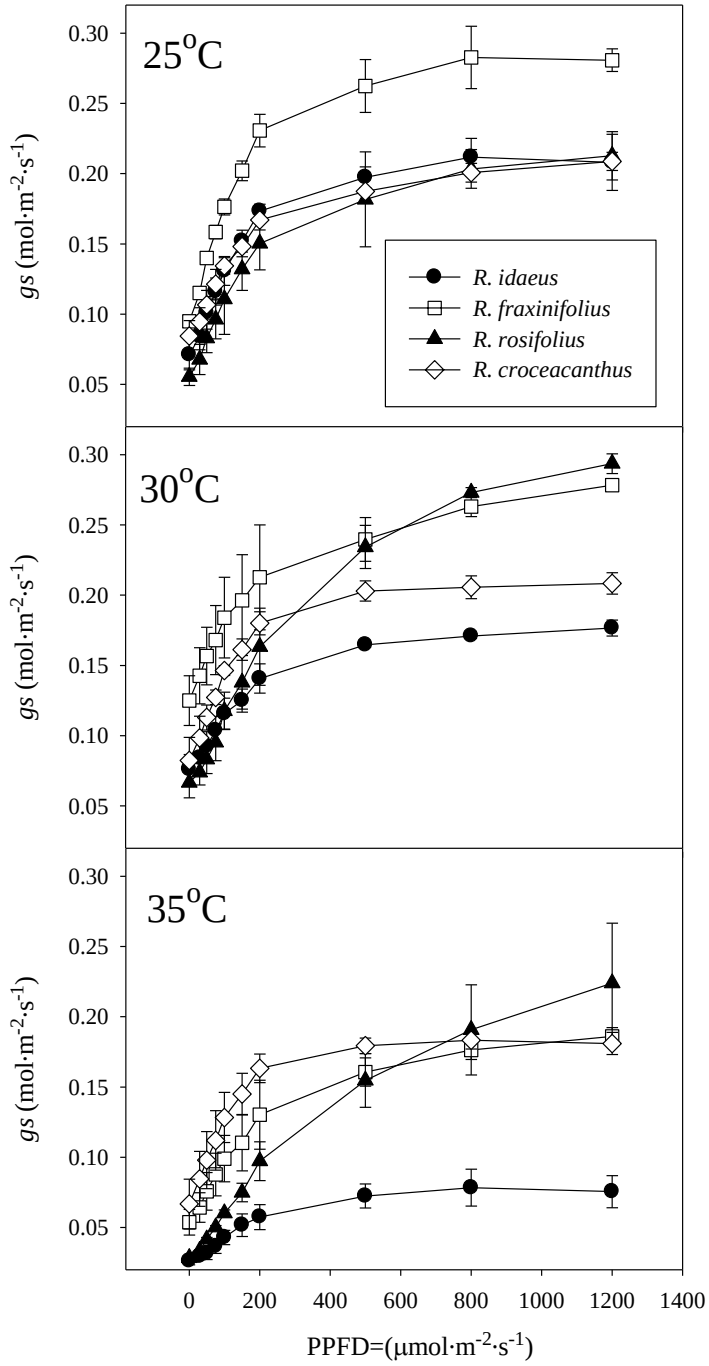

Fig. 3.2 Light response curve of stomatal conductance (mol· m^{-2} ·s^{-1}) in *Idaeobatus* plants under different temperature and PPFD treatments.

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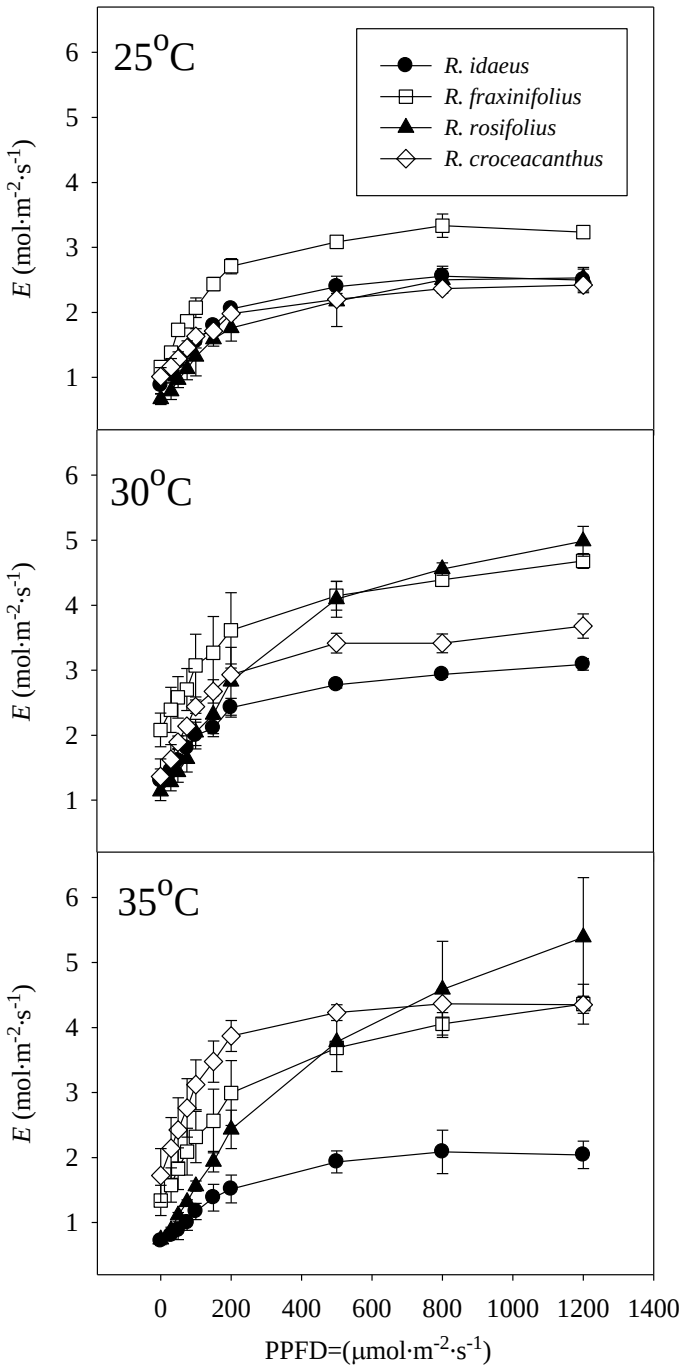

Fig. 3.3 Light response curve of transpiration rate (mol· m^{-2} ·s^{-1}) in *Idaeobatus* plants under different temperature and PPFD treatments.

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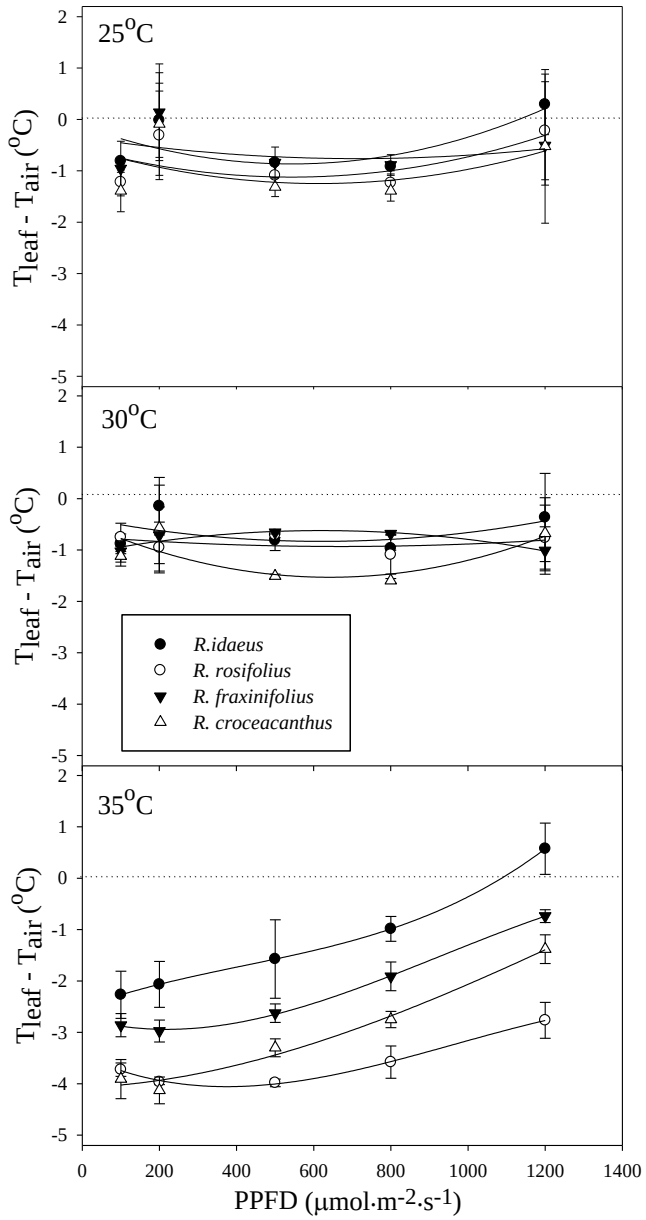

Fig. 3.4 Light response curve of temperature difference between leaf and air (Tl_{eaf} – T_{air}, ℃) in *Idaeobatus* plants under different temperature and PPFD treatments.

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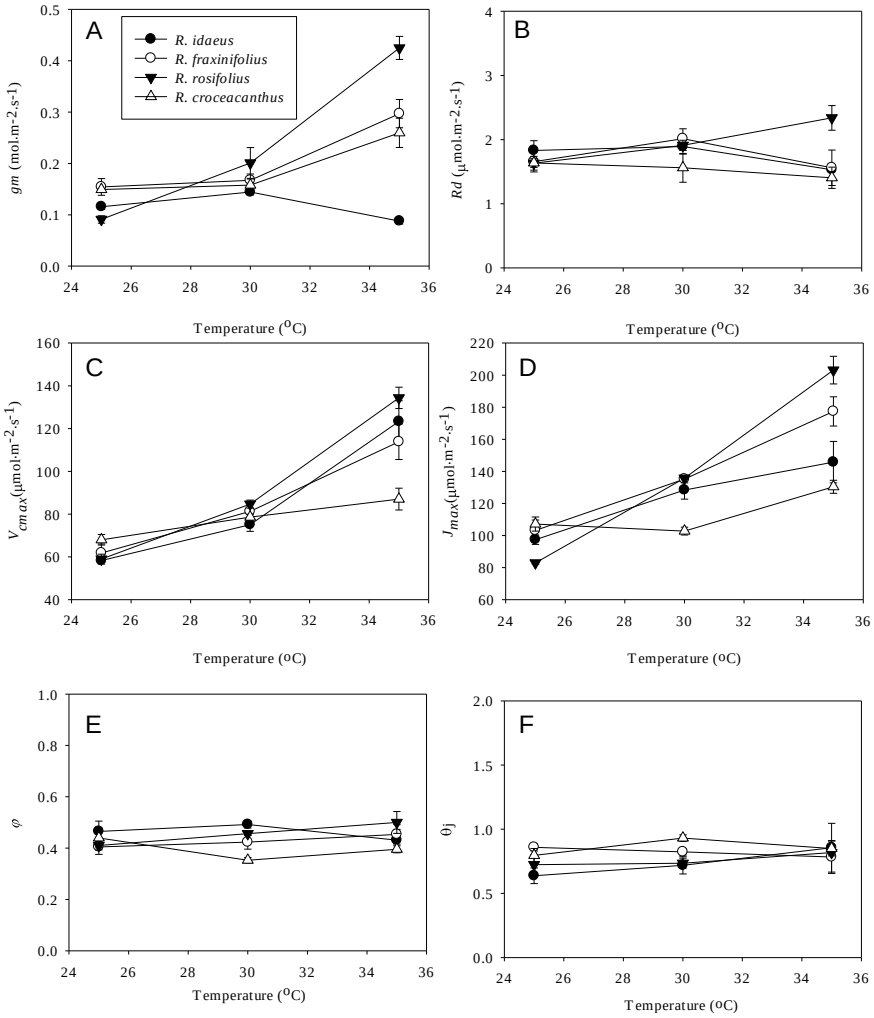

Fig. 3.5 Temperature response curve of FvCB variables in Idaeobatus plants under different temperature (A=gm, B= Rd, C= Vcmax, D= J, E=φ, F=θj)

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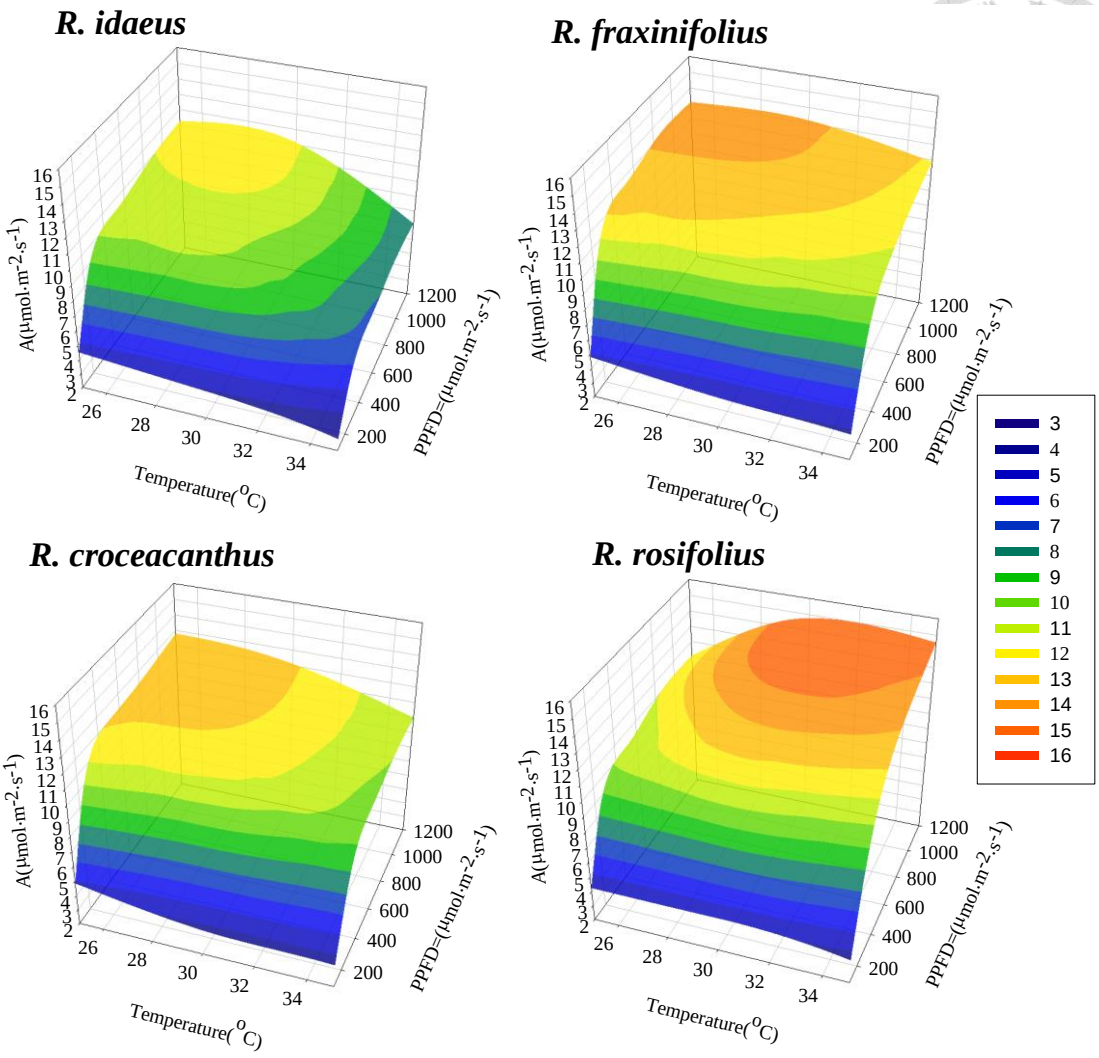

Fig. 3.6 Light and temperature response curve of net assimilation rate (μmol. m-2 .s-1) in Idaeobatus plants under different temperature and PPFD treatments.

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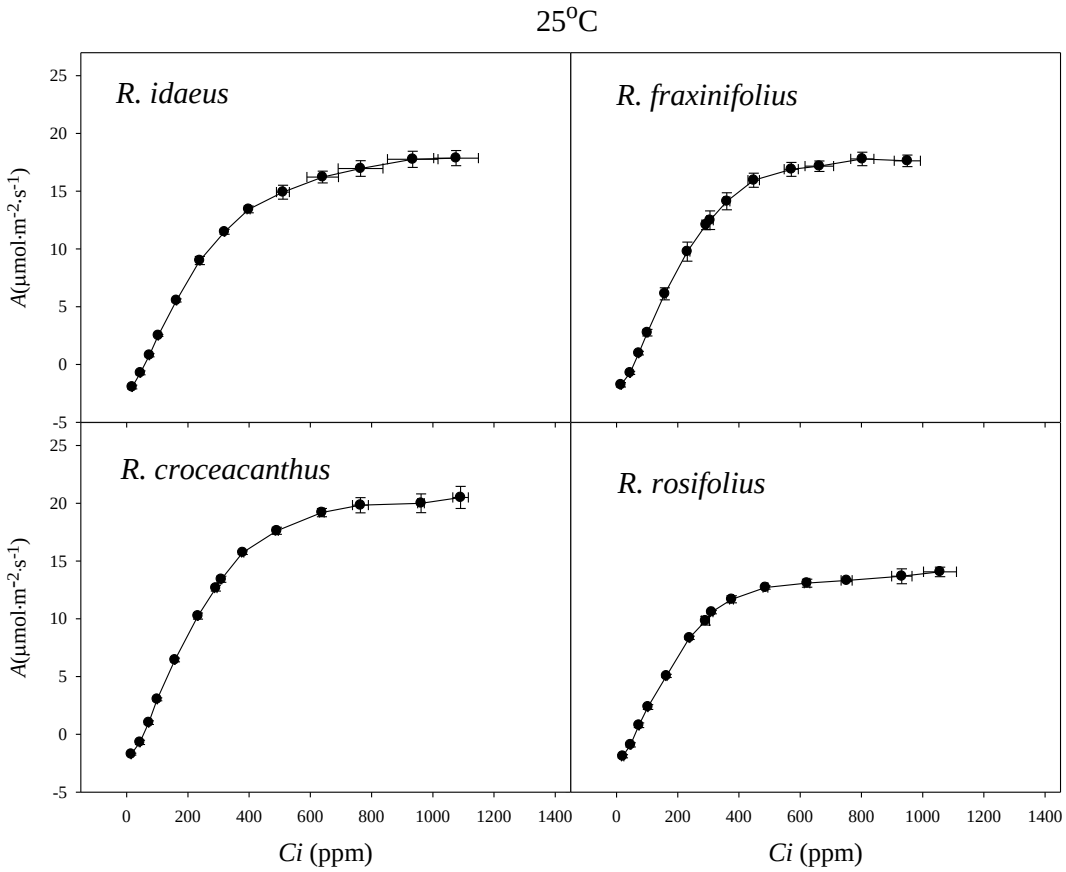

Fig. 3.7 CO_2 response curve (*A/Ci*)of net assimilation rate (μmol· m^{-2} ·s^{-1}) in *Idaeobatus* plants under 25℃.

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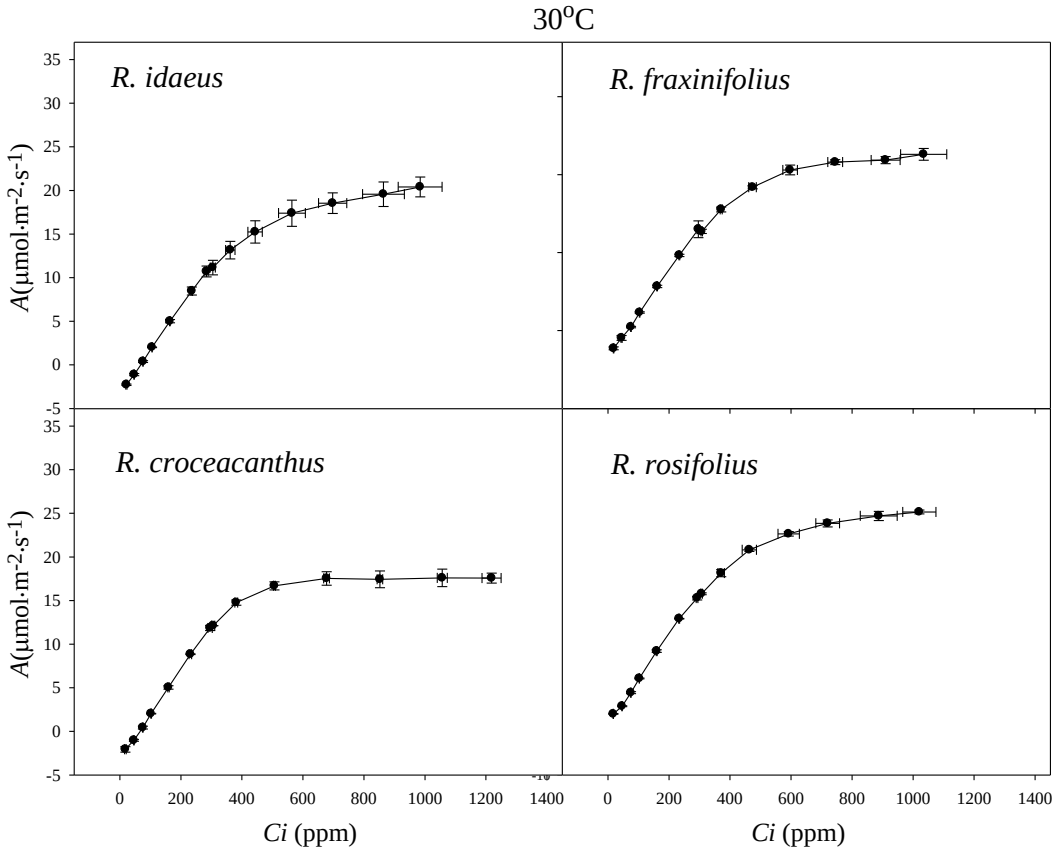

Fig. 3.8 CO_2 response curve (*A/Ci*)of net assimilation rate (μmol· m^{-2} ·s^{-1}) in *Idaeobatus* plants under 30℃.

doi:10.6342/NTU201801406

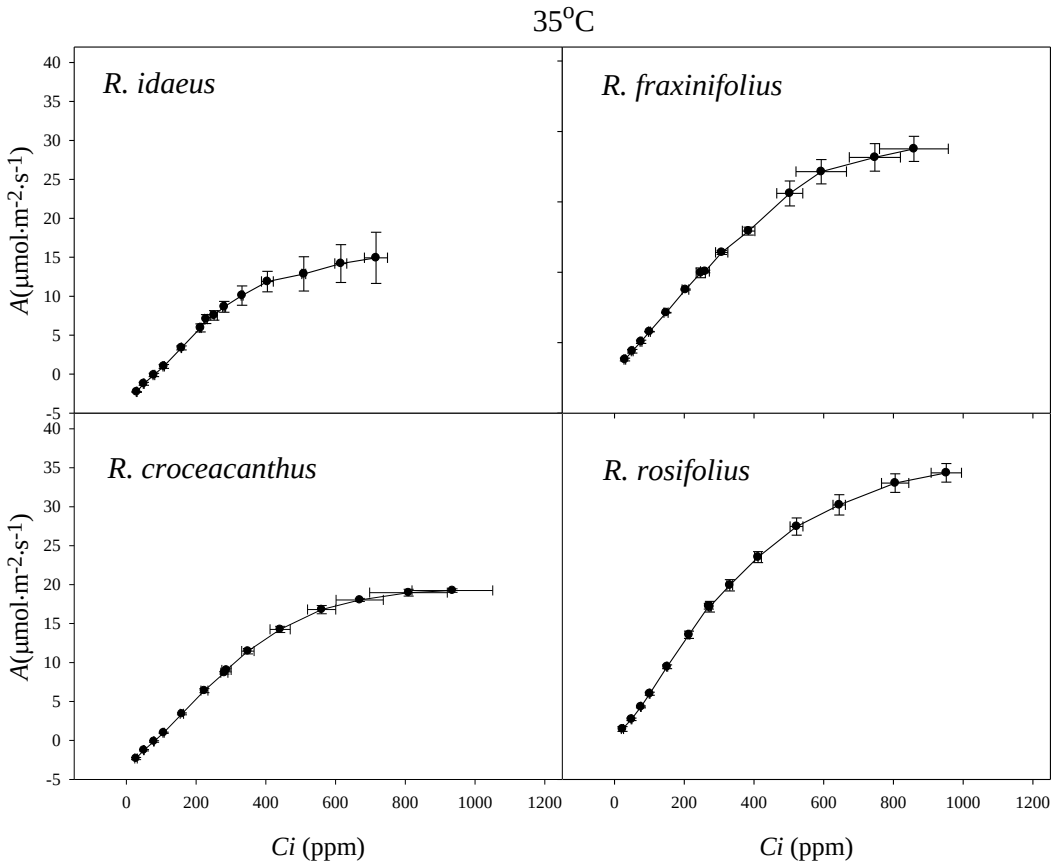

Fig. 3.9 CO_2 response curve (*A/Ci*)of net assimilation rate (μmol· m^{-2} ·s^{-1}) in *Idaeobatus* plants under 35℃.

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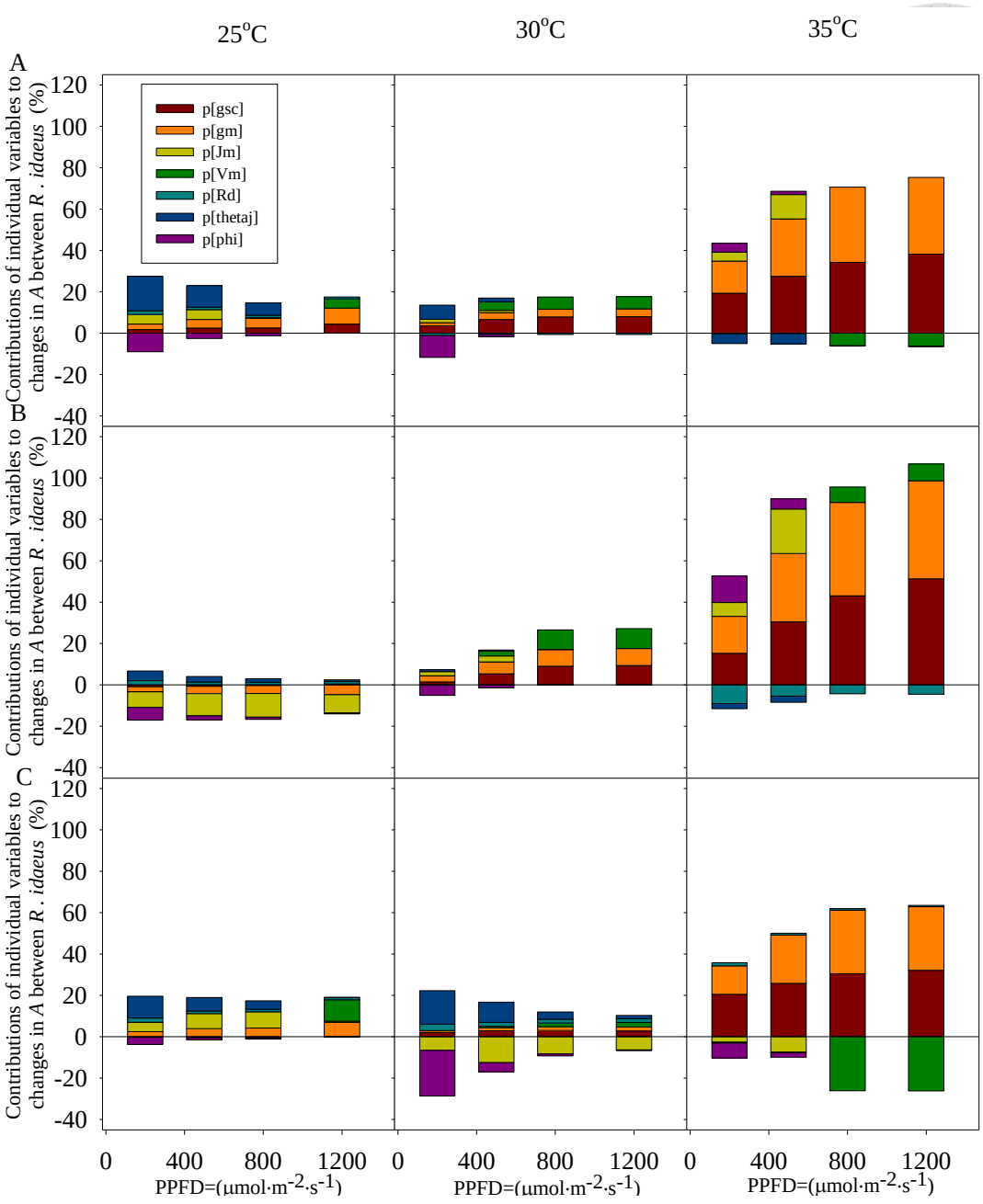

Fig. 3.10 Species comparisons partitioning under different temperature and PPFD treatments using *R. idaeus* as reference point (A= *R. fraxinifolius*, B=*R. rosifolius*, C= *R. croceacanthus*)

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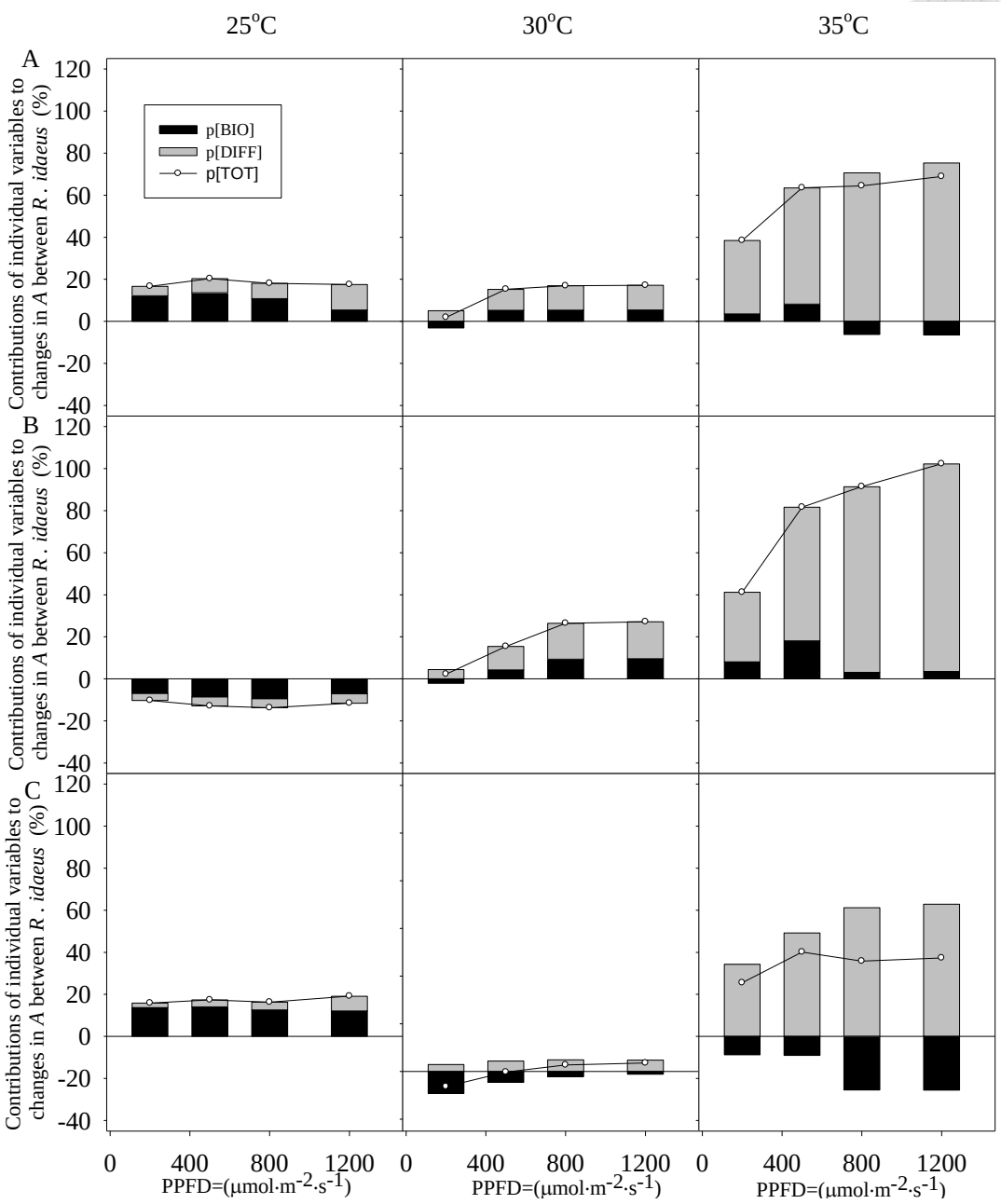

Fig. 3.11 Species comparisons partitioning under different temperature and PPFD treatments using *R. idaeus* as reference point ($\rho[BIO] = \rho[V_{cmax}] + \rho[J_{max}] + \rho[R_d] + \rho[\theta j] + \rho[\varphi]$, $\rho[DIFF] = \rho[g_s] + \rho[g_m]$)(A= *R. fraxinifolius*, B=*R. rosifolius*, C= *R. croceacanthus*).

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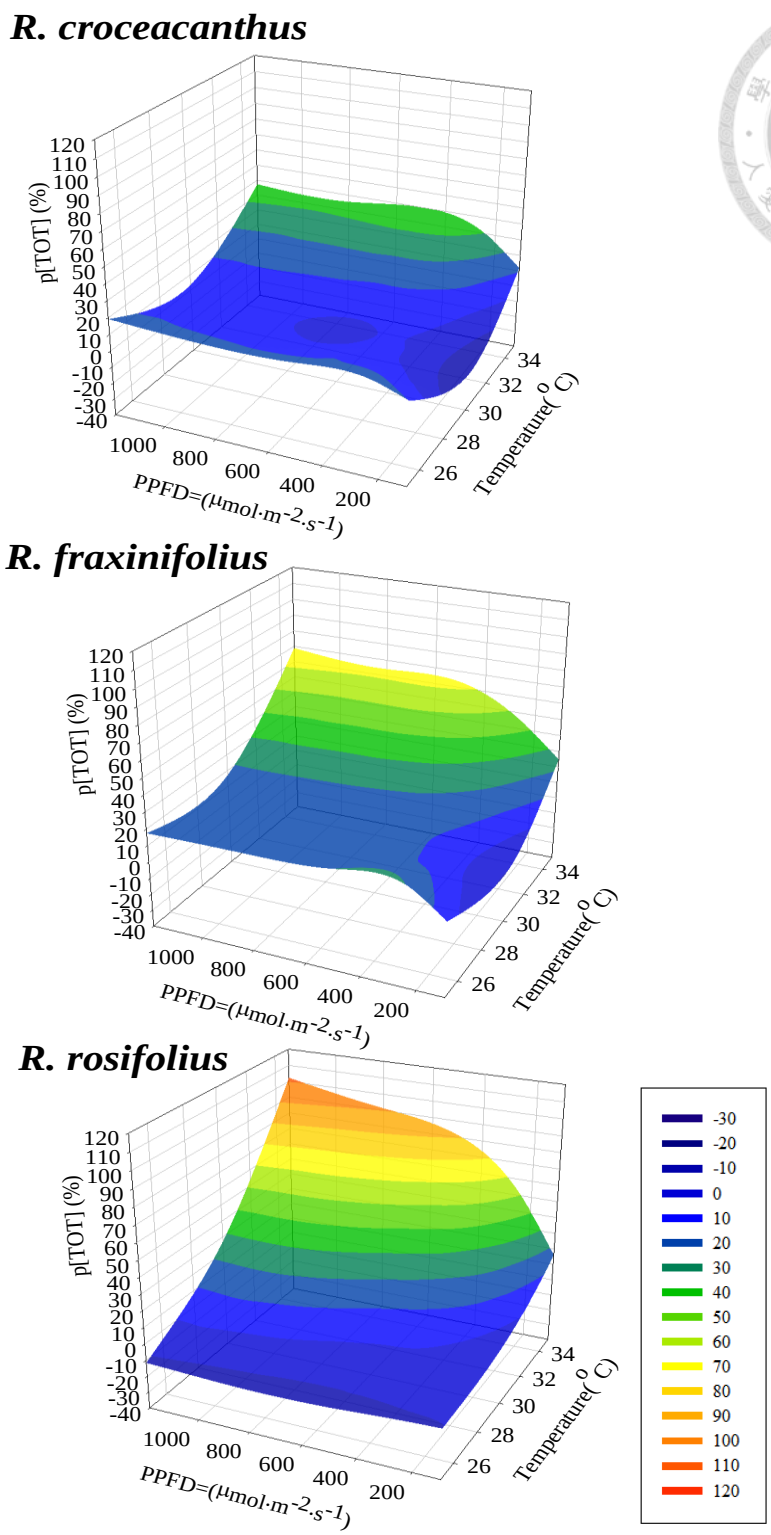

Fig. 3.12 Partitioning the *A* differences of all variables ($\rho[TOT] = \rho[V_{cmax}] + \rho[J_{max}] + \rho[R_d] + \rho[\theta j] + \rho[\varphi] + \rho[g_s] + \rho[g_m]$) versus *R. idaeus* under different light intensities and temperature in %.

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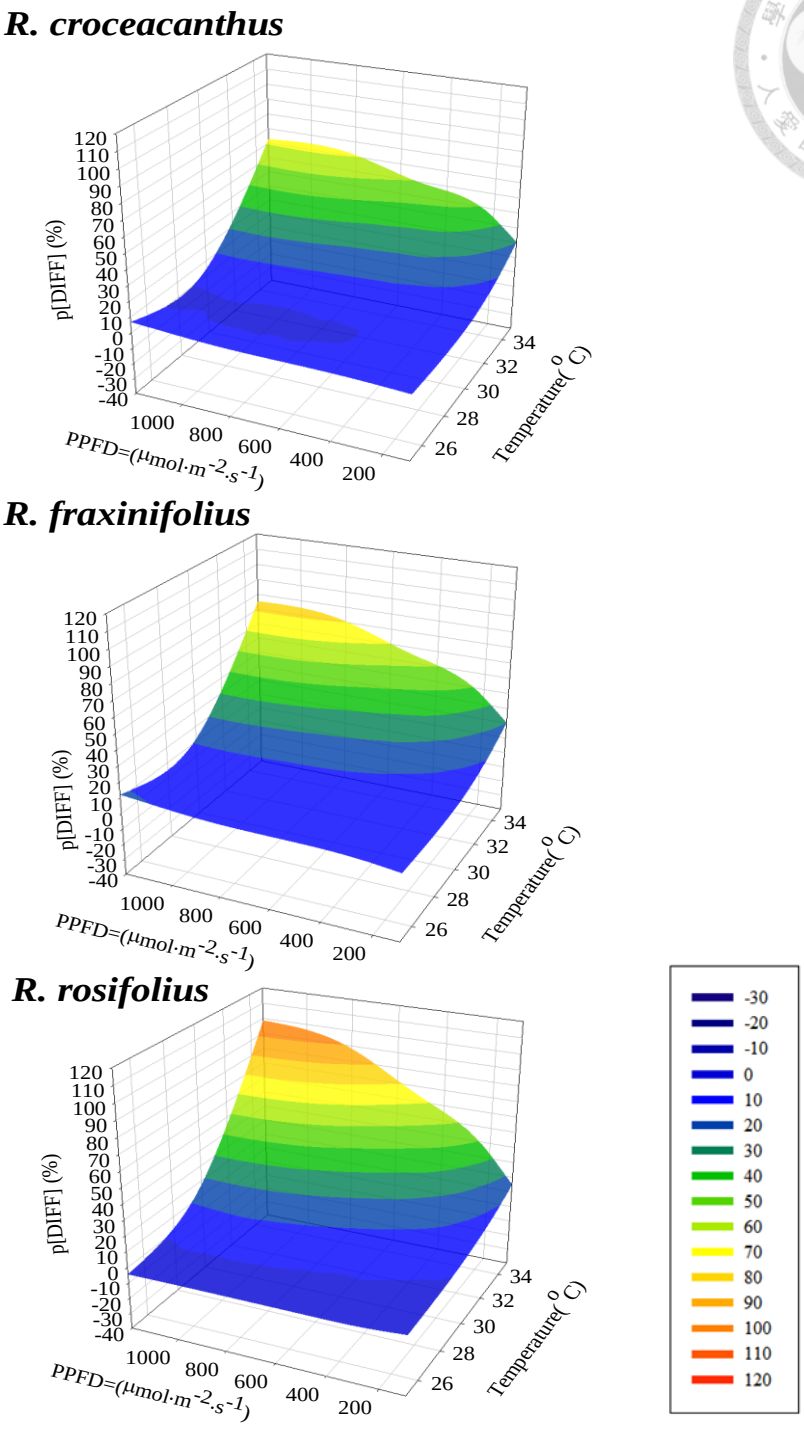

Fig. 3.13 Partitioning the *A* differences of diffusional factors ($\rho[DIFF] = \rho[g_s] + \rho[g_m]$) versus *R. idaeus* via excel tool from Buckley and Diaz-Espejo (2015) under different light intensities and temperature in %.

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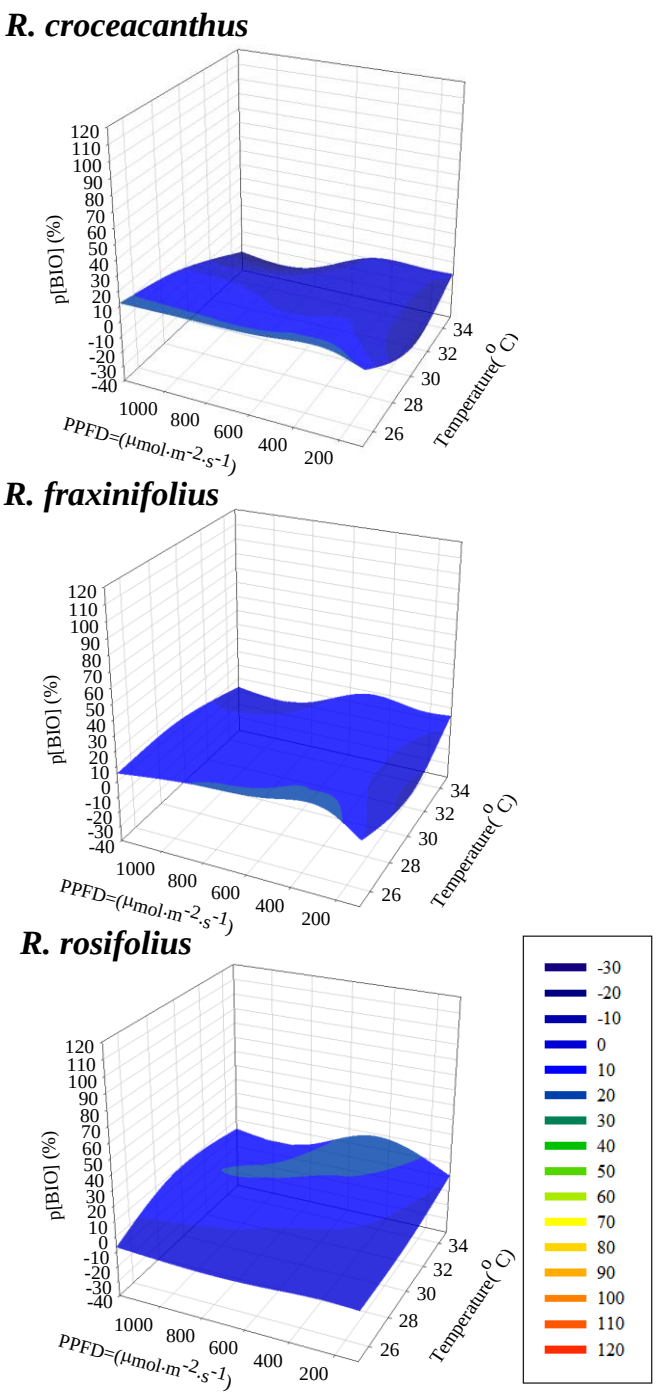

Fig. 3.14 Partitioning the *A* differences of biochemical factors (ρ[BIO] = ρ[V_{cmax}] + ρ[J_{max}] + ρ[R_d] + + ρ[θj] + ρ[φ]) versus *R. idaeus* under different light intensities and temperature in %.

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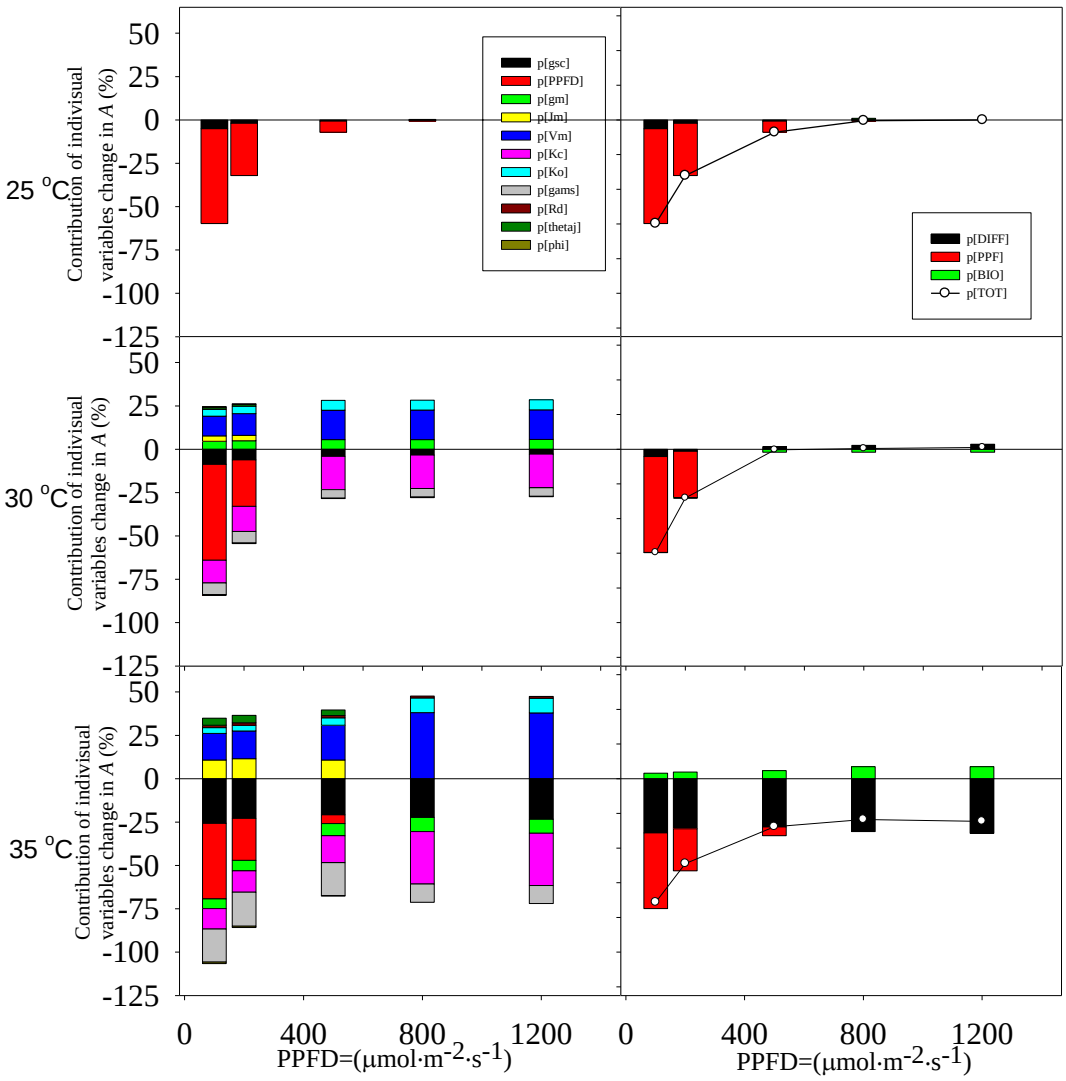

Fig. 3.15 Partitioning individual variables change in *A* (%) at different temperature and PPFD treatment in *R. idaeus*. (25°C PPFD=1200μmol· m^{-2} · s^{-1} as reference point)

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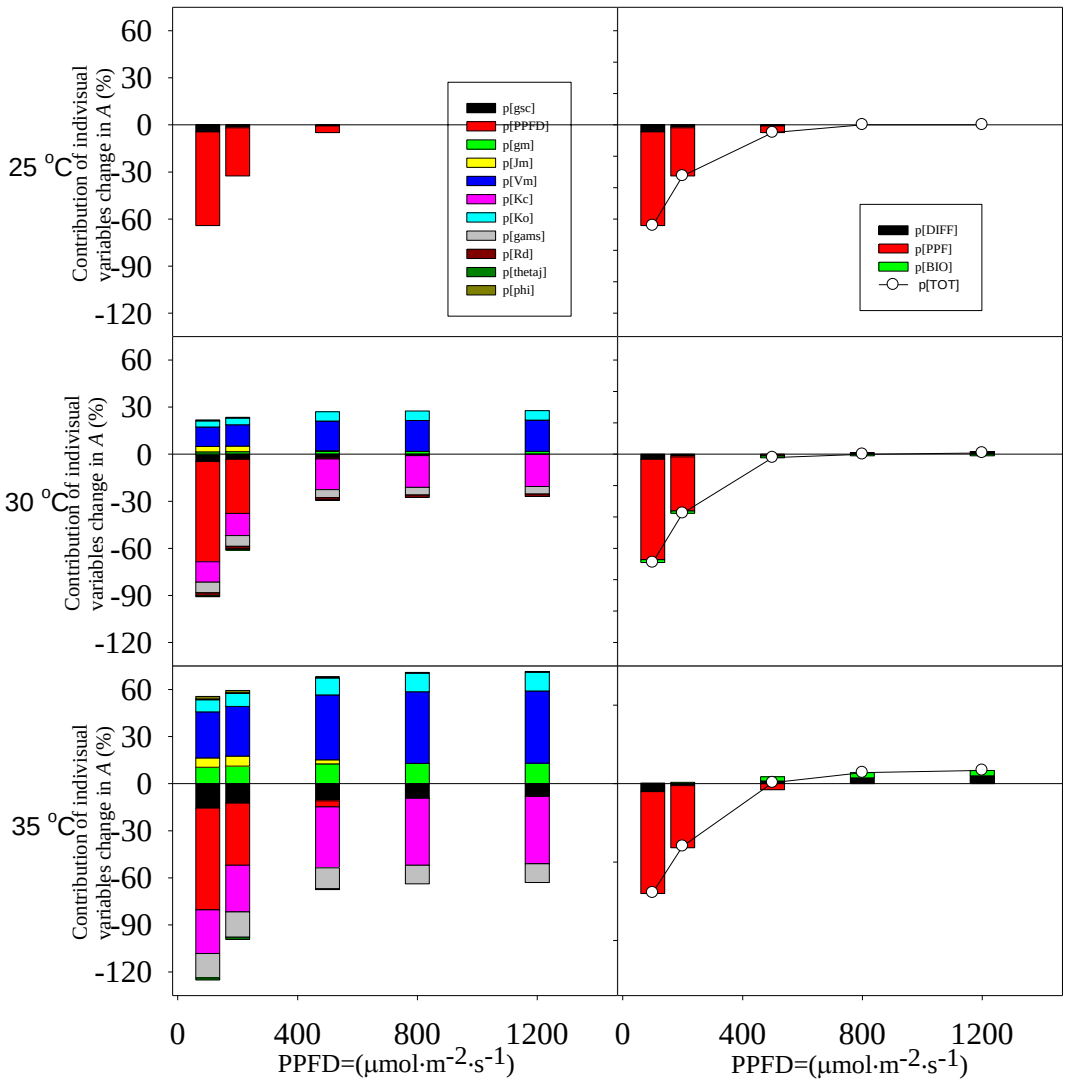

Fig. 3.16 Partitioning individual variables change in *A*(%) at different temperature and PPFD treatment in *R. fraxinifolius*. (25°C PPFD=1200μmol· m^{-2} ·s^{-1} as reference point)

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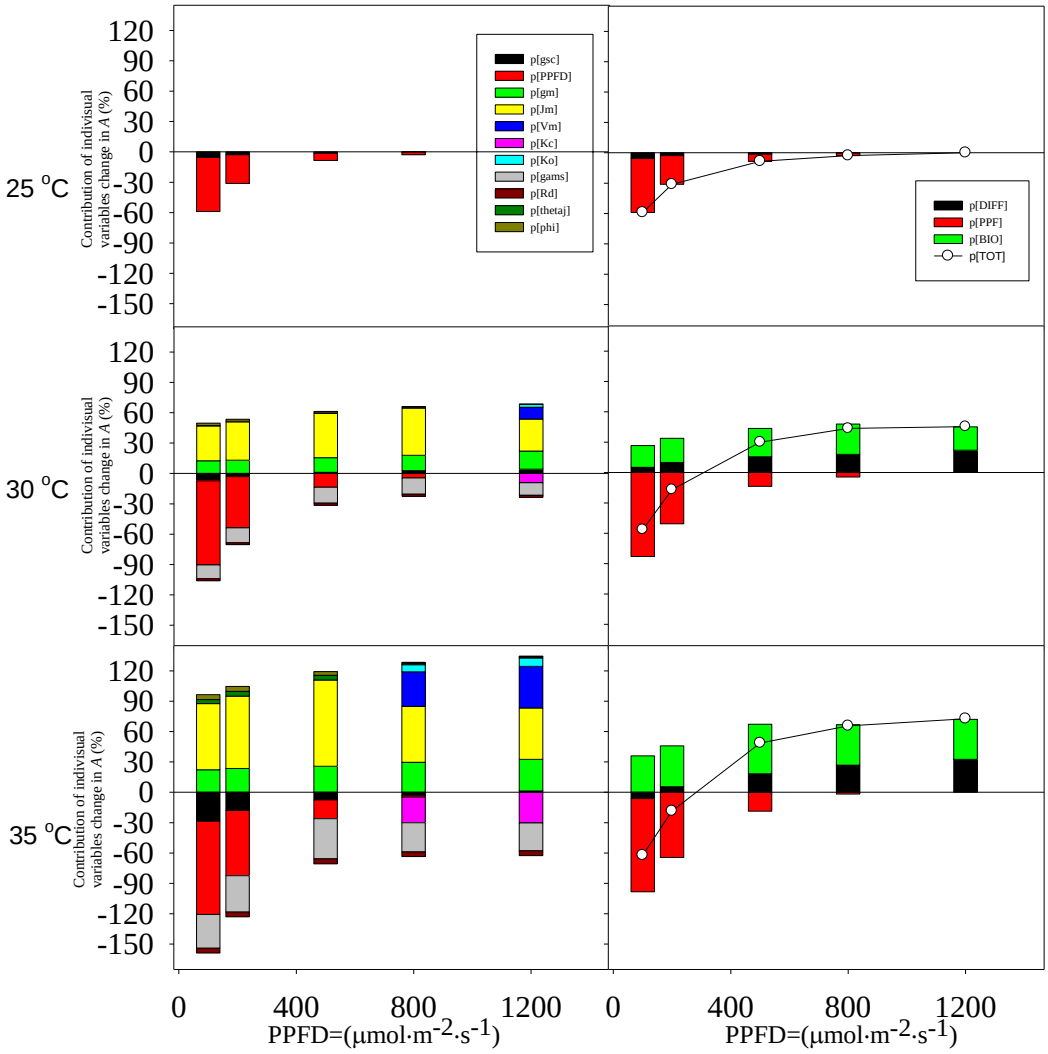

Fig. 3.17 Partitioning individual variables change in *A*(%) at different temperature and PPFD treatment in *R. rosifolius*. (25°C PPFD=1200μmol· m^{-2} ·s^{-1} as reference point)

doi:10.6342/NTU201801406

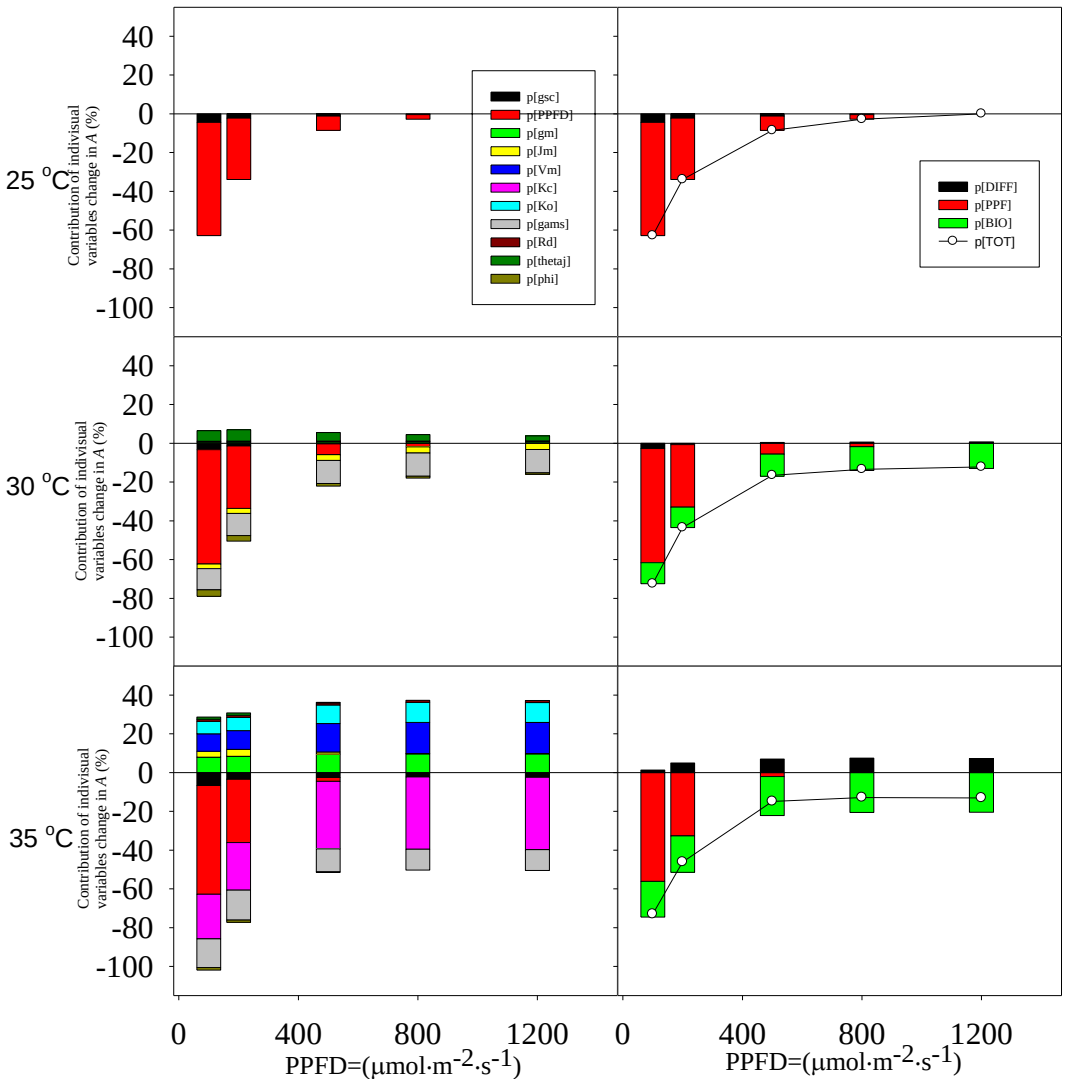

Fig. 3.18 Partitioning individual variables change in *A*(%) at different temperature and PPFD treatment in *R. croceacanthus*. (25°C PPFD=1200 μmol· m^{-2} ·s^{-1} as reference point)

doi:10.6342/NTU201801406

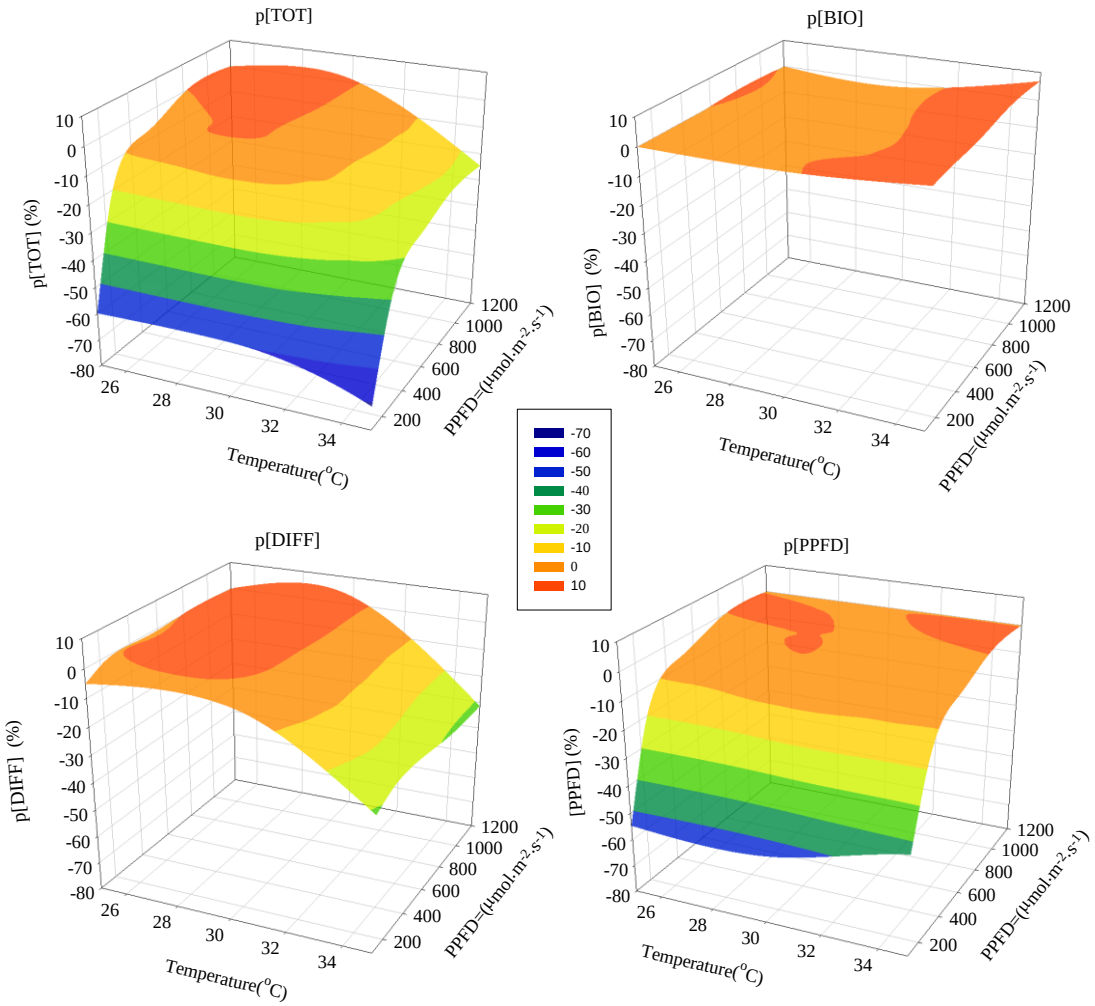

Fig. 3.19 Partitioning different variables change in *A*(%) at different temperature and PPFD treatment in *R. idaeus*. ρ[BIO] = ρ[V_{cmax}] + ρ[J_{max}] + ρ[R_d] + ρ[K_c]+ ρ[K_o] + ρ[Γ*] + ρ[θj] + ρ[φ], ρ[DIFF]= ρ[g_s] + ρ[g_m], and ρ[PPFD]. (25°C PPFD=1200μmol· m^{-2} ·s^{-1} as reference point)

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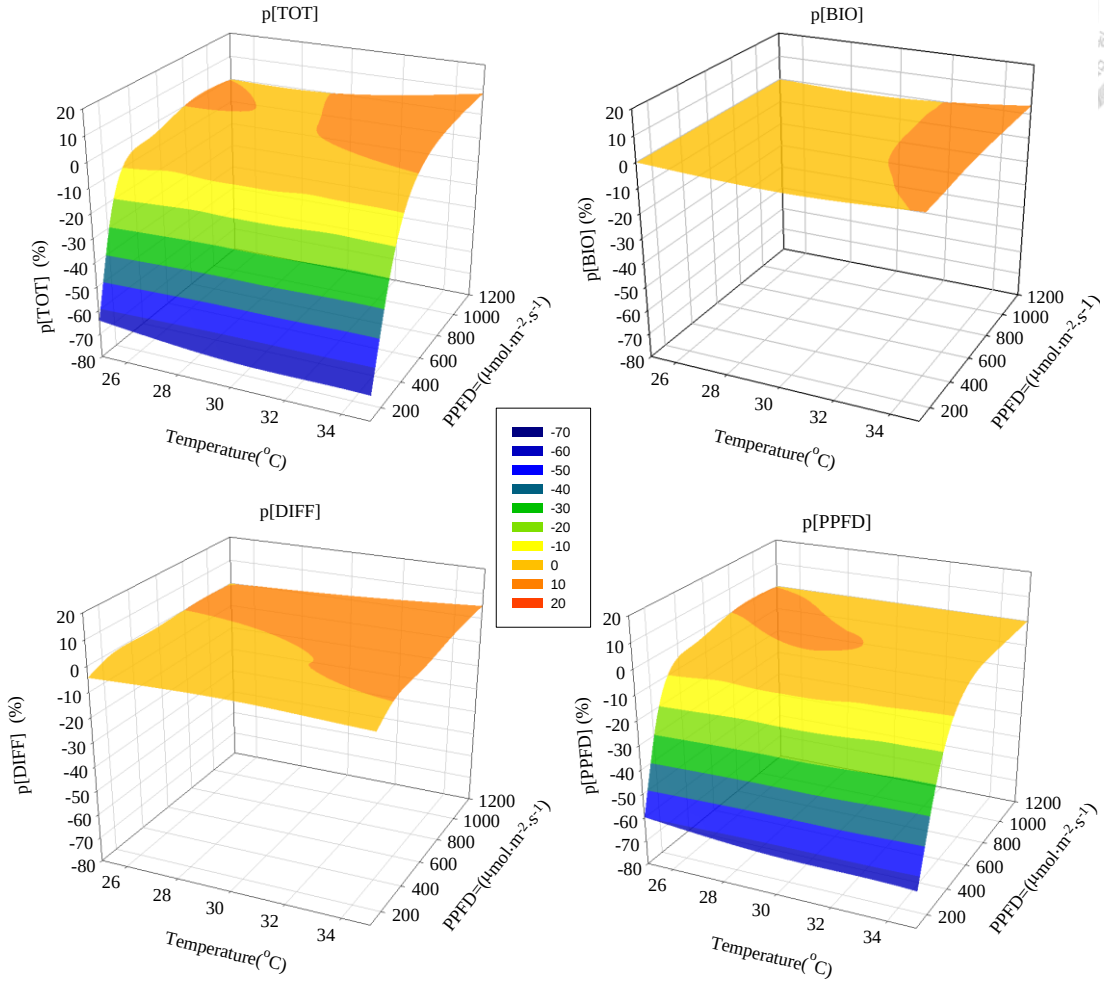

Fig. 3.20 Partitioning different variables change in A(%) at different temperature and PPFD treatment in R. fraxinifolius. ρ[BIO] = ρ[Vcmax] + ρ[Jmax] + ρ[Rd] + ρ[Kc]+ ρ[Ko] + ρ[Γ*] + ρ[θj] + ρ[φ], ρ[DIFF]= ρ[gs] + ρ[gm], and ρ[PPFD]. (25°C PPFD=1200μmol. m-2 .s-1 as reference point)

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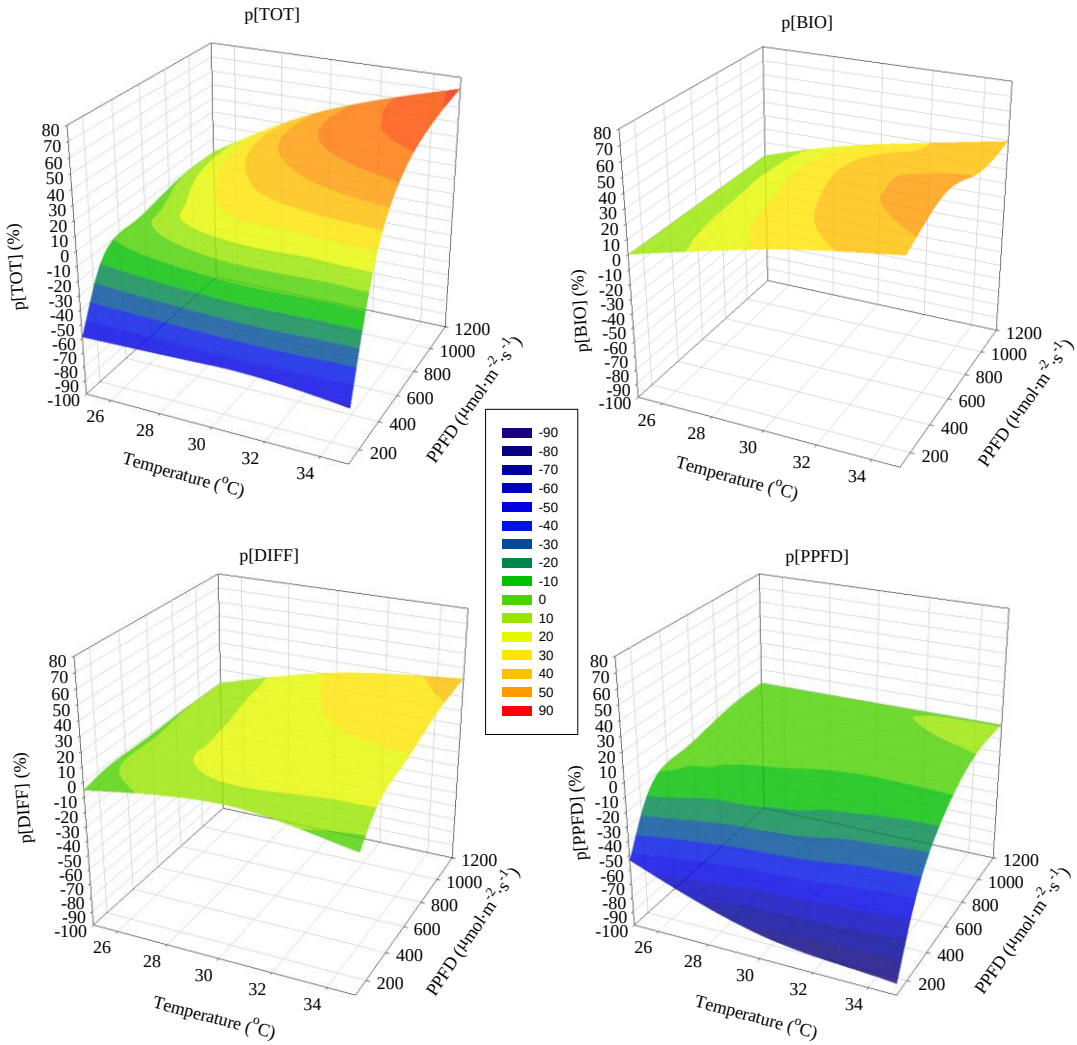

Fig. 3.21 Partitioning different variables change in *A*(%) at different temperature and PPFD treatment in *R. rosifolius*. ρ[BIO] = ρ[V_{cmax}] + ρ[J_{max}] + ρ[R_d] + ρ[K_c]+ ρ[K_o] + ρ[Γ*] + ρ[θj] + ρ[φ], ρ[DIFF]= ρ[g_s] + ρ[g_m], and ρ[PPFD]. (25°C PPFD=1200μmol· m^{-2} ·s^{-1} as reference point)

doi:10.6342/NTU201801406

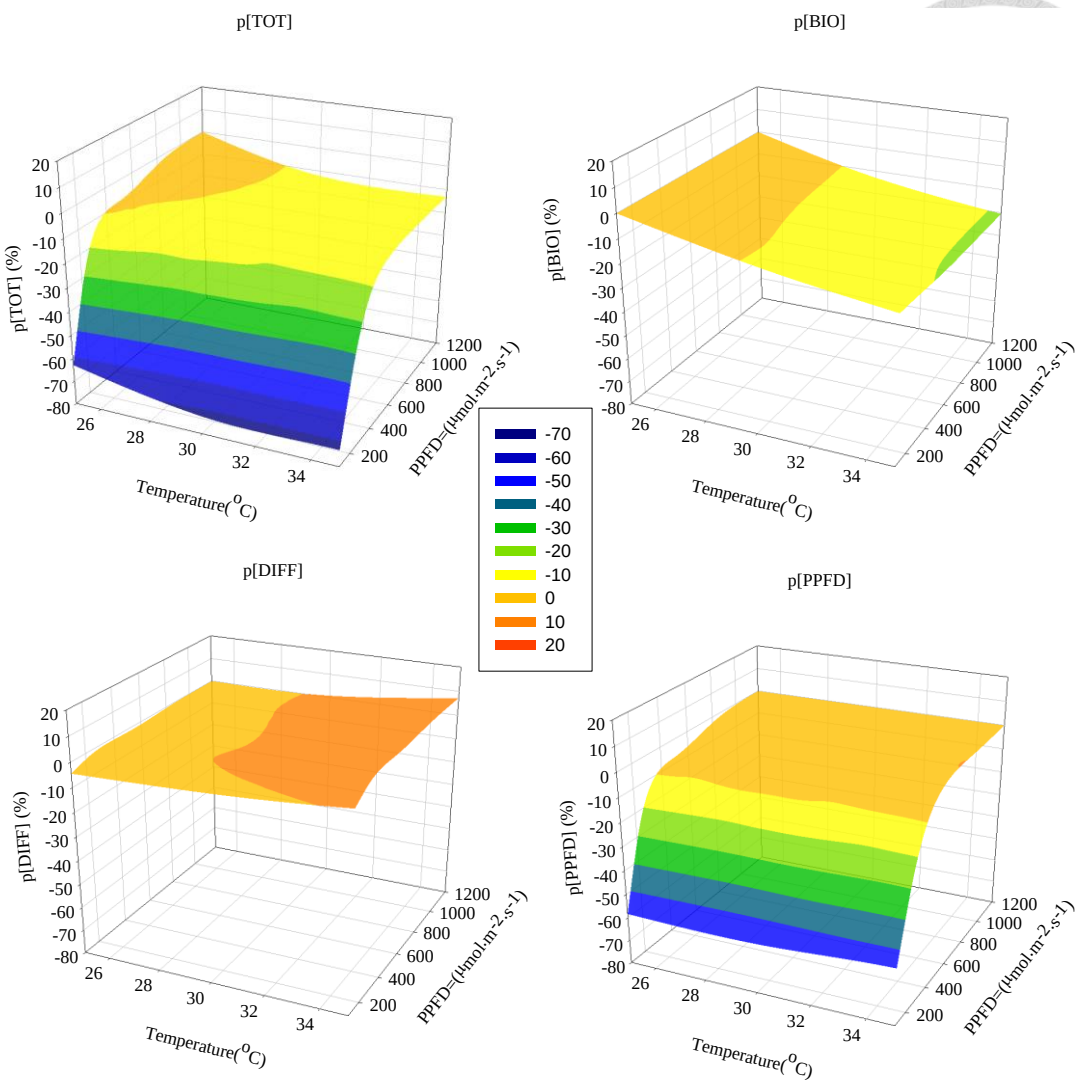

Fig. 3.22 Partitioning different variables change in *A*(%) at different temperature and PPFD treatment in *R. croceacanthus*. ρ[BIO] = ρ[V_{cmax}] + ρ[J_{max}] + ρ[R_d] + ρ[K_c]+ ρ[K_o] + ρ[Γ*] + ρ[θj] + ρ[φ], ρ[DIFF]= ρ[g_s] + ρ[g_m], and ρ[PPFD]. (25°C PPFD=1200μmol· m^{-2} ·s^{-1} as reference point)

doi:10.6342/NTU201801406

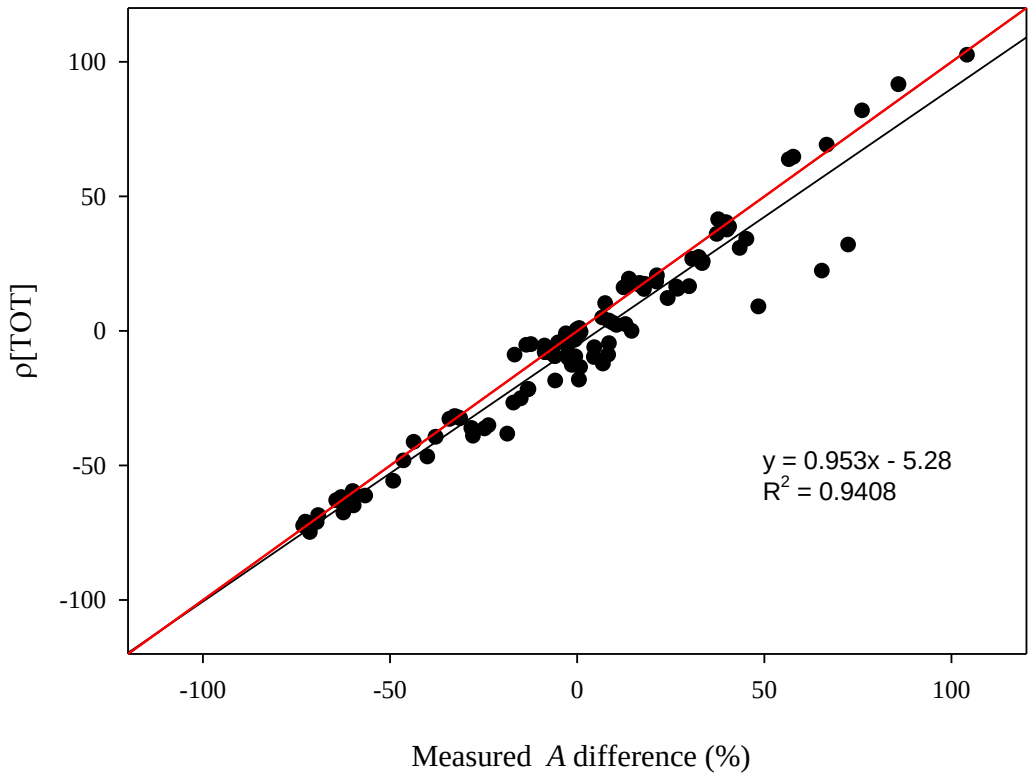

Fig. 3.23 Modelled total variable differences ρ[TOT] against average measured A differences between comparison point and reference point. (red line= 1:1. n=102)

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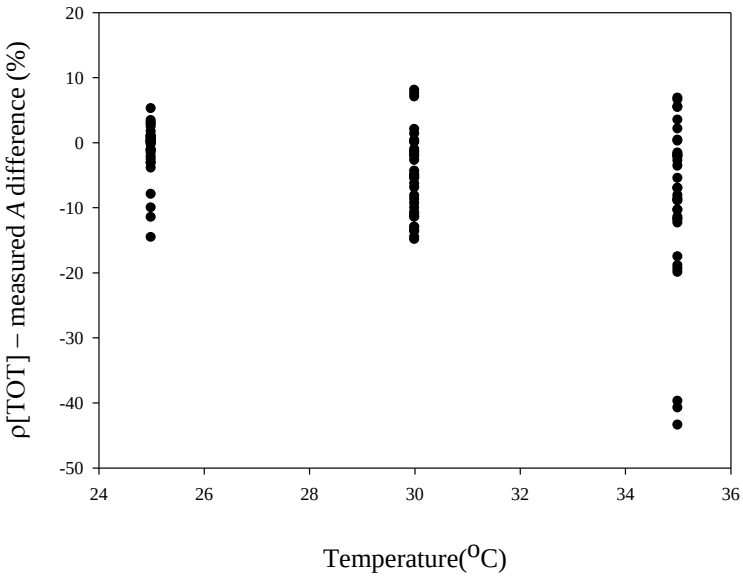

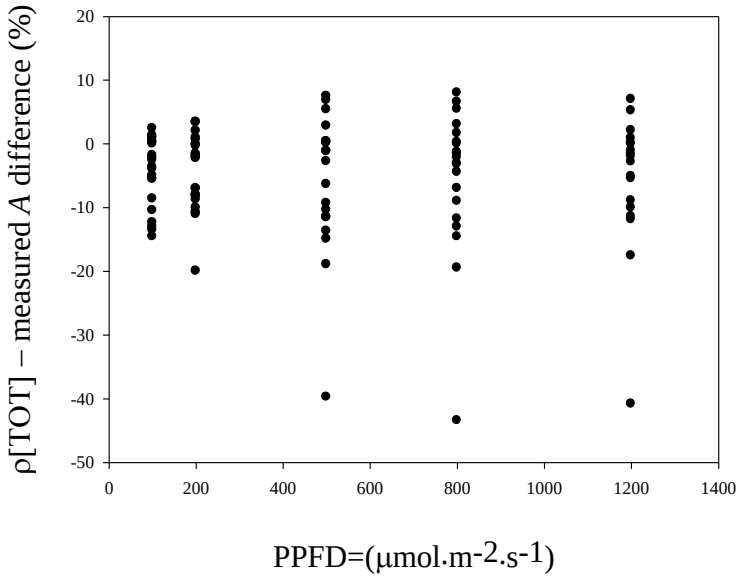

Fig. 3.25 ρ[TOT] - average measured A differences against different temperature and PPFD treatments (n=102)

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Chapter 4 General conclusion and future perspective

4.1 General conclusion and future perspective

Raspberries are recommended to grow in high latitude areas with mild summer and sufficient chilling hours in winter (Hall and Sobey, 2013). Some relatively lower latitude areas such as Portugal, Spain, and Mexico have been important off-season exporters in the past few years using primocane-fruiting cultivars (FAO, 2016). There are still limited successful examples of growing raspberries perennially in the subtropical area such as Israel, using chemical forcing agents (Snir 1986, 1988) and some trials have been done in Brazil (Moura et al. 2012, Maro et al. 2014). It has been reported that raspberry 'Summit' planted in the subtropical area could be harvested multiple times on the primocanes (Funt, 2013). However, trials in Florida and Puerto Rico failed to maintain a non-dormant system growing primocane-fruiting raspberry perennially (Darnell, 2009).

In chapter two and a previous research (Chen, 2016), raspberry trials were conducted to improve the number of flowering laterals on the primocane in a subtropical greenhouse at Taipei. In these studies, the laterals emerged from 20^{th}-40^{th} node of the primocane produced more flowers and thus a greater yield potential. To avoid heat stress in the autumn which cause poor flowering and fruit quality on the first harvest of the primocane, Tipping in the early summer to delay maturation of laterals is recommended. Retractable roof greenhouse (Dale 2012) for improved environmental control is also recommended. Alternatively, the mild and dry winter in central Taiwan would be a better option than northern Taiwan for off-season raspberry production. In this thesis, raspberry 'Summer Festival' produced poor quality fruit with crumbliness and softness. New cultivars with satisfied quality for subtropical climate is necessary. In addition, high yield on the primocanes without chilling and

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vernalization is also an important trait (Gambardella et al. 2016).

Heat tolerance raspberry cultivars are a must for commercializing raspberry production in the subtropical climate. The photosynthetic heat tolerance mechanism based on FvCB model between the temperate originated raspberry cultivar and the subtropical *Idaeobatus* species can be explained by diffusional factors including stomatal conductance and mesophyll conductance. Therefore, porometers or thermal imaging instruments can be used to assess leaf temperature responses instead of measuring chlorophyll florescence (*Fv/Fm*) on detached leaf after heat shocked treatments by water bathing (Molina-Bravo et al. 2011, Bradish et al. 2016). For future breeding programs, comparing the efficiency of these methods is necessary. Photosynthesis process at leaf level combed with FvCB model and the functional structural plant models will be more helpful for future research for modeling photosynthesis on diurnal, seasonal (acclimation), and architectural perspective. Trials using horticultural practices such as shading, fogging, mulching, to alleviate heat stress were also worth studying in the future.

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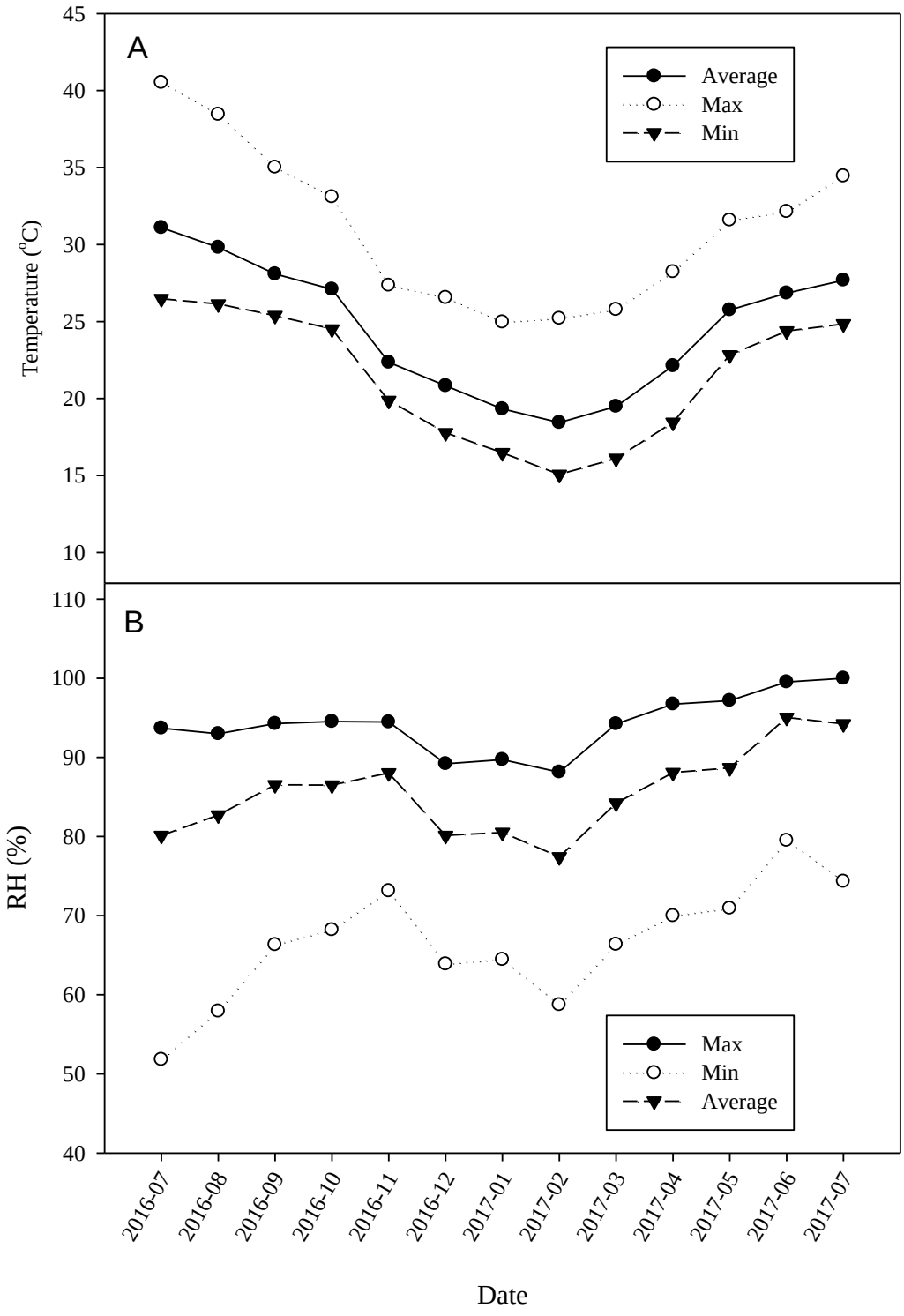
Appendix



Appendix Fig. 1. Climate data in the greenhouse of NTU. (A) Temperature (B) Relative humidity.

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