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Australian Journal of Agricultural Research

Volume 51, 2000

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Aust. J. Agric. Res., 2000, **51**, 503–13

Susceptibility of mango (*Mangifera indica* L.) to cold-induced photoinhibition and recovery at different temperatures

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Abstract. Cold-induced photoinhibition of photosynthesis and recovery from photoinhibition in mono- and poly-embryonic mango cultivars were investigated under field and controlled temperature and light conditions. Photoinhibition, measured as a decrease in the ratio of variable to maximum chlorophyll fluorescence emission (Fv/Fm), occurred in trees growing in the field in winter and early spring. Fv/Fm ratios of all cultivars began to decrease from about 0.49 in May, reaching minimum values of about 0.33 in mid July, and then gradually increased to around 0.68 and exceeded pre-winter values by early November. A seasonal change of leaf chlorophyll content in all cultivars followed a similar pattern to the changes in Fv/Fm ratios during winter. Susceptibility to cold-induced photoinhibition and a reduction in leaf chlorophyll content were greater in poly-embryonic cultivars than in mono-embryonic cultivars. There was a positive linear relationship between the minimum air temperature the night before the measurement and the leaf Fv/Fm ratio (r^2 = 0.62–0.70). In controlled environmental experiments, poly-embryonic cultivars were also more susceptible to photoinhibition than mono-embryonic cultivars. When held at 10°C and irradiated with a photosynthetic photon flux (PPF) of 450 μmol/m^2.s, photoinhibition in detached leaves increased linearly with time. The extent of the reduction in Fv/Fm ratios induced by 6 h irradiation was 43% and 56%, respectively, in mono- and poly-embryonic cultivars. Recovery from photoinhibition in detached leaves was promoted by exposure to a PPF of 20 μmol/m^2.s for 24 h at 20°C. Negligible recovery occurred in photoinhibited leaves maintained at 10°C.

Additional keywords: chlorophyll fluorescence, chlorophyll concentration, ecotype.

Introduction

Evolution of the mango has occurred in two regions with diverse climatic conditions. The primary centre of evolution that produced mono-embryonic genotypes was the cool winter, hot monsoonal summer, northern India/Burma region. Poly-embryonic genotypes evolved later in the constantly hot, monsoonal region of south-east Asia (Mukherjee 1953). The development of diverging ecotypes indicates the potential for the production of this crop over a wide range of environments, which is validated by its successful introduction to global production in subtropical and tropical regions. However, there is scant information available on the regional adaptability of cultivars within the two ecotypes described by Whiley and Schaffer (1997), although these authors suggest the existence of inherent genetic diversity between the two populations with respect to environmental sensitivities.

Mango was introduced into Australia in the mid to late 19th century (Beal 1976) and is now commercially grown from the northern tropics through to subtropical regions of Australia (Crane *et al*. 1997). The industry is primarily based on one poly-embryonic cultivar (Kensington Pride, syn. Kensington) that is grown across all regions and is responsible for about 90% of production. When grown in the subtropics of Australia, where minimum daily winter temperatures regularly fall below 10°C, leaf yellowing asso-

10.1071/AR99076 0004-9409/00/040503

ciated with photoinhibition of the photosynthetic apparatus normally develops in Kensington. During winter in Israel, Nir *et al*. (1997) reported that daily photoinhibition of field-grown 'Sabre' mango is markedly influenced by the previous night's temperature and the severity of photoinhibition gradually increases following repetitive chilling nights. In this Mediterranean climate, low night temperatures are followed by mild sunny days, which is typical of conditions during winter in subtropical Australia. Since photoinhibition results in reduced photosynthetic efficiency (Ögren and Evans 1992; Anderson *et al*. 1997), there may be deleterious consequences for the synthesis of adequate photoassimilates required for flowering and fruit production of mango.

It has previously been reported that exposure to low temperature and high irradiance causes a reduction in both the maximum rate and quantum yield of CO_2 assimilation (Long *et al*. 1983; Powles *et al*. 1983; Nir *et al*. 1997), which can potentially reduce the carbon yield of plants. For instance, lychee (Menzel and Paxton 1985) and rambutan (Diczbalis and Menzel 1998) trees held under high light conditions at 14°C had reduced stomatal conductance of CO_2 and tree growth. Decreased vegetative growth following photoinhibition has also been reported in beans (Farage and Long 1991; Laing *et al*. 1995) and snow gum (Blennow *et al*. 1998).

Plants generally have the capacity to recover from photoinhibition of photosynthesis. Recovery from photoinhibition is temperature-dependent (Greer and Laing 1988) and can be accelerated by exposure to low photosynthetic photon flux (PPF) (Greer *et al*. 1986, 1991). These authors have proposed that slow recovery rates at low temperatures may be the reason that some species are particularly susceptible to cold-induced photoinhibition. However, there is no previous published information on the effects of light and temperature on the recovery of mango leaves from photoinhibition.

The purpose of this study was to compare the susceptibility of mango cultivars from the different ecotypes to photoinhibition. The relative susceptibility of mono- and poly-embryonic cultivars to low temperatures was also investigated as few mono-embryonic cultivars are grown in subtropical regions of Australia. In addition, the effect of PPF and temperatures on the recovery of mango leaves from photoinhibition was investigated. Together, this information may be useful for identifying cultivars that have greater cold tolerance and are thereby potentially more suited for growing in subtropical climates of Australia.

Materials and methods

Plant materials and climatic measurements

Experiments were conducted from May to December 1997 in south-eastern Queensland at the Maroochy Research Station, Nambour (26°S). Four trees each of 5 mono-embryonic cultivars (Chausa, Dashehari, Irwin, Sensation, and Tommy Atkins) and 5 poly-embryonic cultivars (Carabao, Kensington, Khieo Sawoei, Nam Dok Mai, and Nang Klang Wan) growing in the field were used in the various experiments. Trees were 5 years old, grafted to Kensington seedling rootstocks, and approximately 2 m in height with 3-m-wide canopies.

The trees were spaced 4 m apart in east–west oriented rows, with an inter-row spacing of 8 m. The north-east side of trees received full sunlight during the morning. Throughout the experimental period, the ambient air temperature was recorded every 2 h using a Tiny Talk II temperature data logger (Gemini Data Loggers, Chichester, UK). The temperature sensor was placed among leaves in a tree canopy approximately 1 m from the ground. Daily rainfall was recorded using an automatic weather station (Monitor Sensors, Caboolture, Australia).

Chlorophyll fluorescence measurements

Measurements of chlorophyll fluorescence were recorded from attached or detached mature leaves. Leaves were from the most recently grown shoots, which were *c*. 5 months old. Measurements were recorded from the adaxial surface of leaves using a portable Plant Stress Meter (BioMonitor, Umeå, Sweden) as described by Öquist and Wass (1988). The minimum fluorescence yield (Fo) was determined after a period of dark adaptation followed by the maximum fluorescence yield (Fm) during illumination for 2 s with blue light (320–550 nm) at a PPF of 600 μmol/m^2.s. The variable fluorescence (Fv) was calculated as the difference between Fm and Fo. Photoinhibition was measured by a decrease in the ratio of the variable to maximum fluorescence yield (Fv/Fm), which implies a reduction in the photochemical conversion efficiency of photosystem II (Krause 1988; Smillie *et al*. 1988).

Measurements of winter-induced photoinhibition

To determine whether leaves on 4 mono-embryonic (Dashehari, Irwin, Sensation, and Tommy Atkins) and 4 poly-embryonic (Kensington, Khieo Sawoei, Nam Dok Mai, and Nang Klang Wan) mango trees were photoinhibited during winter, measurements of Fv/Fm were recorded every 2 weeks from June to November on leaves from the east to north-east sides of trees. Fv/Fm measurements were repeated in the laboratory using similar aged leaves removed from adjacent branches. To ensure that uniform levels of light reached the experimental leaves, in early May prior to the start of the experiment, branches on the east to north-east side of trees were selected and tied to a horizontal position. Some side branches were removed if they shaded the restrained branches. Four branches, each approximately 1.5 m long, were tied to a horizontal position on 4 trees of each cultivar. Leaves on 2 of the restrained branches were used for Fv/Fm measurements *in situ*, and the other 2 branches provided leaves for removal to monitor Fv/Fm ratio and chlorophyll content.

Chlorophyll fluorescence measurements on attached leaves of 2 mono-embryonic (Irwin and Sensation) and 4 poly-embryonic (Kensington, Khieo Sawoei, Nam Dok Mai, and Nang Klang Wan) cultivars were recorded from 8 leaves per cultivar, 1 leaf per branch per tree. Measurements were made on the middle of the lamina of the 3rd or 4th fully mature leaf from the shoot tip. Leaves were tagged at the start of the experiment and measurements were repeated twice weekly from June to November on the same leaves. *In situ* Fv/Fm measurements were made at 09 00–10 00 hours after leaves had been dark-adapted for 40 min using a dark adaptation cuvette (BioMonitor, Umeå, Sweden).

Immediately following the field measurements, leaves from the adjacent restrained branches of 4 mono-embryonic (Dashehari, Irwin, Sensation, and Tommy Atkins) and 5 poly-embryonic (Carabao, Kensington, Khieo Sawoei, Nam Dok Mai, and Nang Klang Wan) cultivars were collected for Fv/Fm determinations in the laboratory. Harvested leaves were placed in plastic bags and the cut ends of leaf petioles were kept under distilled water to prevent moisture loss. For each cultivar, 2 leaves were harvested from each of 4 trees and Fv/Fm ratios were measured after the leaves had been dark-adapted at 20 ± 2°C for 40 min.

Determination of chlorophyll content

For each cultivar of 2 mono-embryonic (Irwin and Sensation) and of 4 poly-embryonic (Kensington, Khieo Sawoei, Nam Dok Mai, and Nang Klang Wan) mangoes, 2 fully mature leaves on 2 horizontally tied branches of each of 4 trees were collected to monitor changes in chlorophyll content at monthly intervals from June to October 1997. A sample of leaf lamina (0.25 g) was frozen in liquid nitrogen and ground in a mortar with a pestle. Ground samples were extracted in 40 mL of 99.8% methanol (HPLC grade) (Porra *et al*. 1989). The aliquots were then homogenised, filtered, and adjusted with methanol to a final volume of 50 mL. The absorption spectrum of each extract was measured at 665 and 652 nm for chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*), respectively, using a Heλios β spectrophotometer (Unicam, Cambridge, UK). The contents of Chl *a* and Chl *b* were determined by comparison against the standard curves of purified Chl *a* and Chl *b* from spinach leaves (Sigma Chemical Co., USA). Leaf chlorophyll contents (mg/g fresh weight) were calculated using the linear equation from standard curves which were: Chl a = –0.000233 + 0.03958Xa (r^2 = 0.99) and Chl b = –0.000158 + 0.023511Xb (r^2 = 0.99) where Xa and Xb were the absorbance ratios at 665 and 652 nm, respectively.

Cold-induced photoinhibtion of detached leaves

For photoinhibition and recovery experiments, fully mature leaves were collected from the east to north-east sides of trees in December between 09 00 and 10 00 hours. The leaves were kept in a darkened, insulated box and maintained at 20 ± 2°C for 40 min before being used in experiments.

To induce photoinhibition, the detached leaves were placed on a temperature gradient table and exposed to light. The gradient table was made of a slab of rolled aluminium (100 by 30 by 3 cm), with solid aluminium legs (30 by 3 cm) welded to either end. A temperature gradient was established along the table by placing one leg in a warm (29°C) water bath and the other leg in a cool (4°C) water bath. The water baths (Linder and May, Brisbane, Australia) were maintained at constant temperature which ensured a stable temperature gradient along the table with only a small variation (< ±0.6°C) detected after allowing 24 h for equilibration. Temperatures along the table were measured every hour using chromel-alumel thermocouples (LI-1000 temperature data logger; LI-COR, Lincoln, NE, USA) inserted into holes drilled at 20-cm intervals into the table. The temperature gradient table and water baths were kept in a controlled temperature laboratory at 20 ± 2°C. The table was also used in some experiments where a specific constant temperature was required. This was obtained by maintaining both water baths at the same temperature.

Immediately before being placed on the table, the petioles of leaves were re-cut and sealed with white petroleum jelly (Ponds International, Clayton, Vic., Australia) to reduce water loss. Leaves were then laid abaxial surface down on damp blotting paper placed on the table, sprayed with distilled water, and covered with thin polyethylene film (Glad Products, Rhodes, NSW, Australia) to prevent desiccation. A dark control for each leaf was obtained by screening a portion of each leaf with a strip of aluminium foil (3 cm wide) before leaves were covered with polyethylene film. To ensure even contact and prevent movement of leaves, the table was covered with a piece of clear glass (100 by 30 by 0.8 cm). The adaxial surfaces of leaves were exposed to a PPF of 450 μmol/m^2.s produced by 10 cool daylight fluorescent tubes (36 W; Philips, The Netherlands) positioned over the temperature gradient table. The variation in PPF either across or along the table did not exceed 15%.

To examine the development of photoinhibition over time, 10 fully mature leaves of 2 poly-embryonic cultivars, Kensington and Nang Klang Wan, and 2 mono-embryonic cultivars, Irwin and Sensation, were harvested from the east to north-east side of trees in December. The detached leaves were kept in darkness at 20 ± 2°C for 40 min prior to being placed on the temperature gradient table held at 10°C and exposed to a PPF of 450 mmol/m^2.s for 6 h. The Fv/Fm ratios were measured after 1, 2, 4, and 6 h of irradiation followed by 40 min of dark adaptation at 10°C.

Recovery from photoinhibition

To study recovery from photoinhibition, 48 leaves from each of 3 mono-embryonic cultivars (Irwin, Sensation, and Tommy Atkins) and 3 poly-embryonic cultivars (Kensington, Khieo Sawoei, and Nang Klang Wan) were exposed to a PPF of 450 μmol/m^2.s at 10°C for 24 h. Half of the photoinhibited leaves (24 leaves) were then kept on the gradient table at 10°C and the remaining leaves were placed on damp blotting paper in a tray and transferred to 20 ± 2°C. All leaves were sprayed with distilled water and covered with polyethylene film. At each temperature, half of each leaf was covered with aluminium foil to exclude light, and all leaves were illuminated for 24 h with a PPF of 20 μmol/m^2.s from 2 cool daylight fluorescent tubes. Measurements of Fv/Fm ratios were made after 40 min of dark adaptation on the illuminated and light-excluded areas of the same leaves at 0.6, 1.5, 3, 6, 18, and 24 h during the recovery period.

Comparison of cultivar susceptibility to photoinhibition

Relative susceptibilities of 5 mono-embryonic (Chausa, Dashehari, Irwin, Sensation, and Tommy Atkins) and 5 poly-embryonic (Carabao, Kensington, Khieo Sawoei, Nam Dok Mai, and Nang Klang Wan) cultivars to cold-induced photoinhibition were compared by measuring the Fv/Fm ratio of detached leaves held at different temperatures on the temperature gradient table following exposure to a PPF of 450 μmol/m^2.s for 4 h. For each cultivar, 30 non-cold-stressed leaves were harvested from the east to north-east side of trees and equilibrated in darkness at 20 ± 2°C for 40 min. Six leaves of the same cultivar were placed side by side across the temperature gradient table at each temperature position. The temperatures used were 8, 13, 17, 19, or 22°C. Fv/Fm ratios were determined after dark adaptation for 40 min on the gradient table. Because the space on the table was limited, the experiment was repeated 4 times for each cultivar.

Statistical analyses

Data for the development of photoinhibition with time were analysed using a 4 × 5 factorial analysis of variance (ANOVA), with 2 factors of cultivar and duration of exposure to irradiance. Changes in Fv/Fm ratio and chlorophyll content in leaves during winter were analysed separately on each sampling date using a completely randomised design to compare cultivars and to compare between the mono- and poly-embryonic groups. Data were analysed by ANOVA and regression analysis using GENSTAT 5 (Genstat 5 Committee 1993). Comparisons of different means were undertaken using the least significant difference (l.s.d.) at P = 0.05. Space on the temperature gradient table limited the number of leaves available for replication; hence, there were no statistical analyses and standard errors (s.e.) were used to show variability between means in experiments using the gradient table on cold-induced photoinhibition and recovery from photoinhibition.

Results

Climatic data

Mean monthly maximum and minimum air temperatures and rainfall at Maroochy Research Station from May to December 1997 are presented in Fig. 1. Mean minimum temperatures were below 10°C during June–August with a low of 8.5°C in August. Mean maximum temperature ranged from 21 to 30°C. There were relatively few cloudy, wet days, with mostly cloud-free, sunny weather during the winter months (data are not presented).

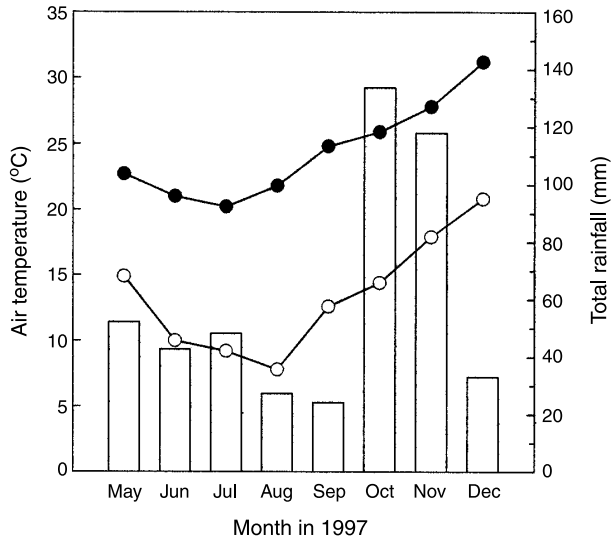

Fig. 1. Mean monthly maximum (●) and minimum (○) air temperatures and total monthly rainfall (histogram) at Maroochy Research Station, Nambour, south-east Queensland (26°S), between May and December 1997.

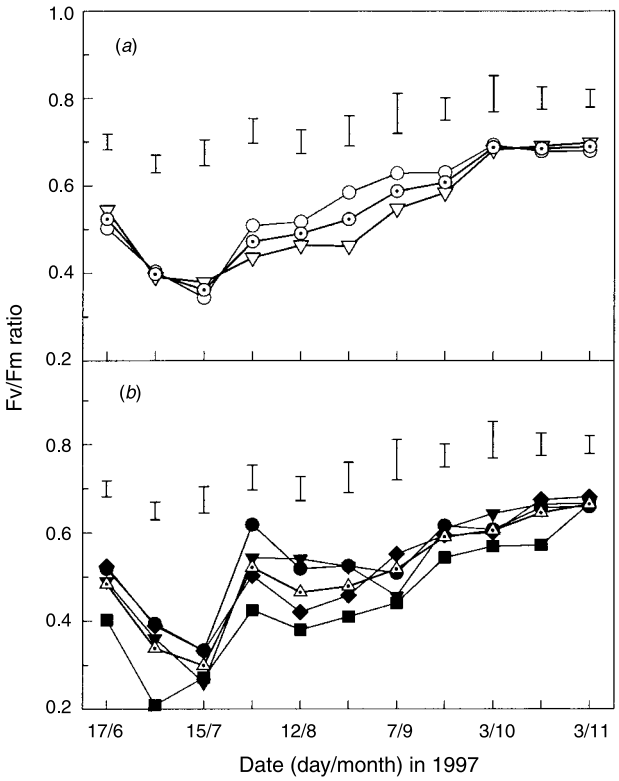

Fig. 2. Seasonally induced changes in Fv/Fm ratios of leaves on field-grown mango cultivars between May and November 1997: (*a*) mono-embryonic cultivars Irwin (○) and Sensation (▽); (*b*) poly-embryonic cultivars Kensington (◆), Khieo Sawoei (▼), Nam Dok Mai (●), and Nang Klang Wan (■). Mean Fv/Fm ratios for mono- (⊙) and poly-embryonic (△) cultivars are also presented. Leaves were dark-adapted for 40 min before the Fv/Fm ratio was determined. For each cultivar, data are mean ratios from 8 leaves on 4 trees. Vertical bars indicate the l.s.d. (P = 0.05) for the differences between cultivars at each measurement date.

Photoinhibition in the field

During winter (June–July), Fv/Fm ratios of leaves from all cultivars measured in the field decreased (Fig. 2). Changes in Fv/Fm ratios of leaves on the mono-embryonic cultivars Irwin and Sensation were similar except on 17 June when Irwin was significantly lower than Sensation, and 29 July and 24 August when Sensation was significantly lower than Irwin (Fig. 2*a*). In contrast, there were generally significant differences between Fv/Fm ratios of the 4 poly-embryonic cultivars (Fig. 2*b*). In particular, Nang Klang Wan generally had lower Fv/Fm ratios than the other poly-embryonic cultivars. Mean Fv/Fm ratios for mono-embryonic cultivars were significantly higher than those of poly-embryonic cultivars. From 17 June to 15 July, the mean Fv/Fm ratios for mono- and poly-embryonic cultivars decreased from 0.524 and 0.484 to 0.362 and 0.299, respectively. After this, the mean Fv/Fv ratios of 2 cultivar groups gradually recovered and were 0.688 and 0.668, respectively, on 3 November. Associated with the low Fv/Fm ratios, lamina necrosis was observed on all 4 Nang Klang Wan trees.

Fv/Fm ratios of detached leaves of mono- and poly-embryonic cultivars followed a similar pattern to leaves measured on trees (Fig. 3). For both ecotypes, mean Fv/Fm ratios gradually declined from 0.656 (mono-) and 0.649 (poly-) in early May to 0.492 (mono-) and 0.389 (poly-) on 1 July, and thereafter increased to 0.763 (mono-) and 0.726 (poly-) by 3 November. For the most part, Fv/Fm ratios of the 4 mono-embryonic cultivars (Dashehari, Irwin, Sensation, and Tommy Atkins) were similar, declining in winter and increasing in spring. However, Dashehari had a slower recovery than other cultivars as temperatures increased during spring (Fig. 3*a*). The Fv/Fm ratios of detached leaves of the 5 poly-embryonic cultivars (Carabao, Kensington, Khieo Sawoei, Nam Dok Mai, and Nang Klang Wan) followed a similar pattern to the mono-embryonic cultivars with Carabao least affected and Nam Klang Wan most affected by cool temperatures (Fig. 3*b*). The mean Fv/Fm ratios of mono-embryonic cultivars (Fig. 3*a*) were significantly higher than those of poly-embryonic cultivars (Fig. 3*b*) on all assessment dates except 9 May and 29 July. Additionally, the results in Fig. 4*a* indicate that for both cultivar groups there was a linear relationship (r^2 = 0.68–0.70) between the mean Fv/Fm ratios and the minimum temperature of the night before the Fv/Fm measurement. However, there was no significant difference between the slopes and intercepts of the regression lines of the 2 cultivar groups (Fig. 4*a*).

Changes in chlorophyll concentration

Chlorophyll concentration in leaves of all cultivars decreased during winter with most cultivars reaching the

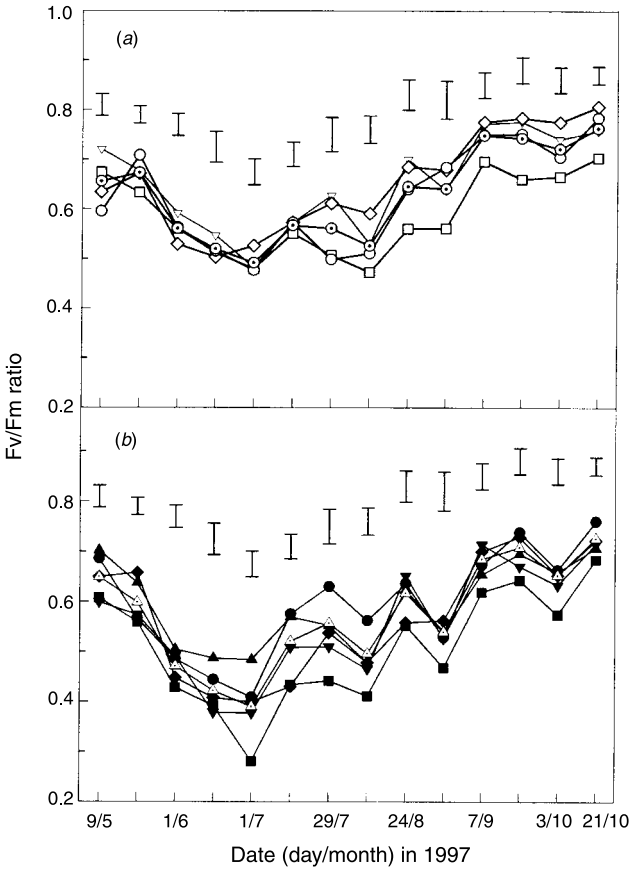

Fig. 3. Seasonally induced changes in Fv/Fm ratios of detached leaves of (*a*) the mono-embryonic cultivars Dashehari (□), Irwin (○), Sensation (▽), and Tommy Atkins (◇); and (*b*) the poly-embryonic cultivars Carabao (▲), Kensington (◆), Khieo Sawoei (▼), Nam Dok Mai (●), and Nang Klang Wan (■). Mean Fv/Fm ratios for mono- (⊙) and poly-embryonic (△) cultivars are also presented. Detached leaves were equilibrated at 22°C for 40 min before Fv/Fm ratios were determined. Data shown are mean ratios from 8 leaves sampled from 4 trees. Vertical bars indicate the l.s.d. (P = 0.05) for the differences between cultivars at each measurement date.

lowest concentration in mid-July (Fig. 5). Chlorophyll concentration then gradually increased apart from a decline in Irwin leaves at the end of October. Leaves of Sensation (mono-) had the highest chlorophyll concentration, whereas Nang Klang Wan (poly-) leaves had the lowest content during winter. At each assessment date, there was no significant difference in the Chl *a* : Chl *b* ratio between cultivars (data are not presented). There was a positive linear correlation between the minimum temperature the night before measurement and the mean Chl *a* concentration of the 2 ecotypes (Fig. 4*b*). However, whereas the correlation was high for poly-embryonic cultivars (r^2 = 0.88), it was low for mono-embryonic cultivars (r^2 = 0.44).

Development of photoinhibition

The relationship between duration of irradiation and photoinhibition of mono- and poly-embryonic cultivars is shown in Fig. 6. Prior to irradiation the Fv/Fm ratio for freshly harvested leaves of Sensation, Irwin, Kensington, and Nang Klang Wan was 0.784, 0.782, 0.737, and 0.749, respectively. The Fv/Fm of these cultivars slightly declined to 0.744, 0.731, 0.694, and 0.719 for dark controls after 6 h of exposure (data are not presented). When leaves held at 10°C were exposed to a PPF of 450 μmol/m^2.s over a period of 1–6 h, the extent of photoinhibition in all cultivars was linearly correlated with the duration of irradiation. The regressions for all 4 cultivars are significant and highly correlated (r^2 = 0.92–0.98); however, there were no differences between the slopes of these 4 regression lines. Over the duration of irradiation, there were significant differences in Fv/Fm ratios between cultivars. After 6 h of irradiation, Fv/Fm ratios of Sensation, Irwin, Nang Klang Wan, and Kensington were reduced by 40, 46, 54, and 58%, respectively. Mean ratios of Fv/Fm were reduced by 56% for the poly-embryonic cultivars and by 43% for the mono-embryonic cultivars.

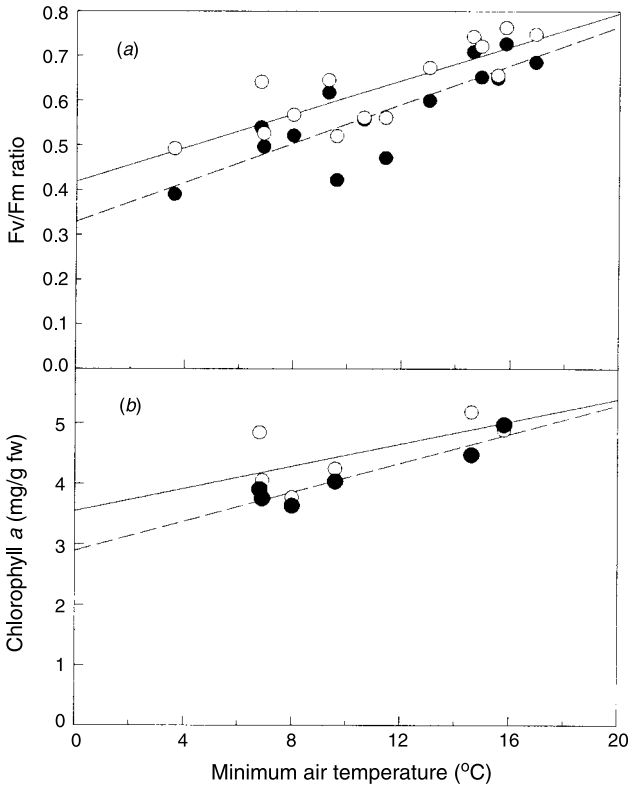

Fig. 4. The relationship between minimum air temperature (T_{min}) and (*a*) Fv/Fm and (*b*) content of chlorophyll *a* during May– November 1997. Air temperatures were recorded during the night before leaves were detached for measurements of Fv/Fm and chlorophyll content. The regression line for chlorophyll fluorescence of the mono-embryonic cultivars (–○–) is represented by Fv/Fm = 0.3264 + 0.0208 T_{min} (r^2 = 0.62; P < 0.01) and for poly-embryonic cultivars (- -●- -) by Fv/Fm = 0.2718 + 0.0225 T_{min} (r^2 = 0.70; P < 0.01). The regression line for chlorophyll *a* (Chl *a*) for mono-embryonic cultivars (–○–) is represented by Chl *a* = 3.5462 + 0.00921 T_{min} (r^2 = 0.44; P = 0.15) and for poly-embryonic cultivars (- -●- -) by Chl *a* = 2.890 + 0.1201 T_{min} (r^2 = 0.88; P < 0.01).

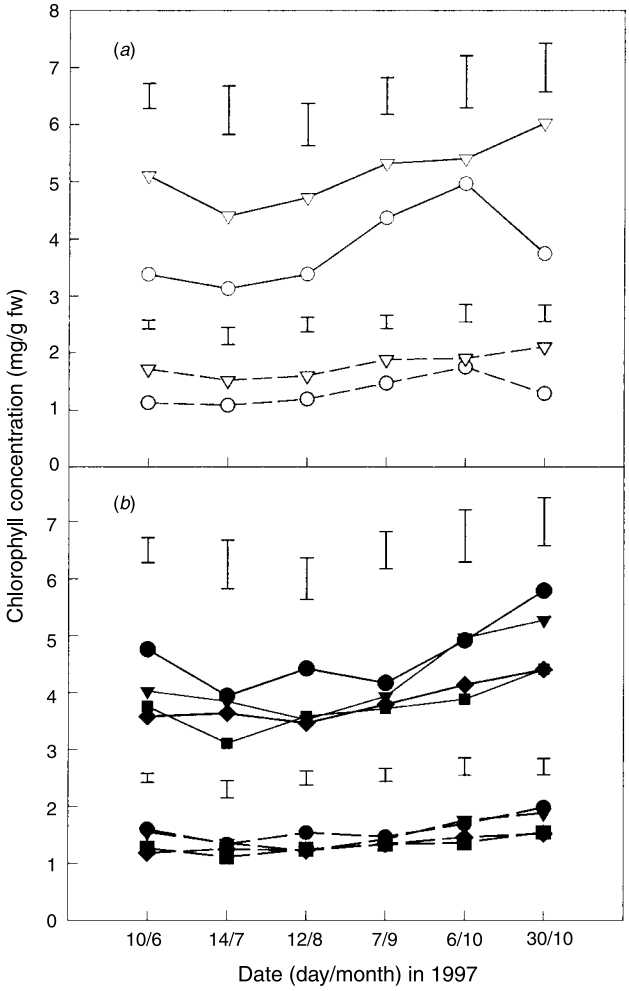

Fig. 5. Seasonally induced changes in chlorophyll *a* (—) and chlorophyll *b* (- - - -) content from June to October 1997 for leaves of (*a*) the mono-embryonic cultivars Irwin (○) and Sensation (▽) and (*b*) the poly-embryonic cultivars Kensington (◆), Khieo Sawoei (▼), Nam Dok Mai (●), and Nang Klang Wan (■). For each cultivar, data are mean ratios from 8 leaves on 4 trees. Vertical bars indicate the l.s.d. (P = 0.05) for the differences between cultivars at each measurement date.

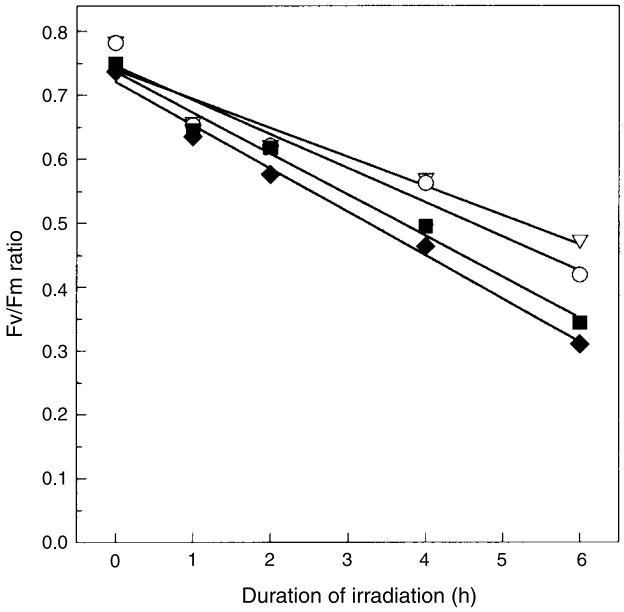

Fig. 6. Effect of the length of irradiation time on Fv/Fm ratios of detached leaves of 2 mono-embryonic cultivars, Irwin (○) and Sensation (▽), and 2 poly-embryonic cultivars, Kensington (◆) and Nang Klang Wan (■). Leaves were held at 10°C and irradiated with a PPF of 450 μmol/m^2.s for 0, 1, 2, 4, and 6 h. Initial Fv/Fm ratios were measured before the start of the irradiation period. The relationships between Fv/Fm (y) and period of irradiation (h) for leaves of Sensation (SST), Irwin (IW), Kensington (KST), and Nang Klang Wan (NKW) are represented by a simple linear regression model of y (SST) = 0.7397 – 0.0454 h (r^2 = 0.92; P < 0.01); y (IW) = 0.7459 – 0.0533 h (r^2 = 0.94; P < 0.01); y (KST) = 0.6741 – 0.0606 h (r^2 = 0.97; P < 0.01); and y (NKW) = 0.7160 – 0.0699 h (r^2 = 0.98; P < 0.01), respectively. Data are the means of 10 leaves per cultivar.

Effect of low temperature on susceptibility to photoinhibition

In Fig. 7 the relative susceptibilities of 10 mango cultivars to cold-induced photoinhibition at temperatures between 8 and 22°C with a PPF of 450 μmol/m^2.s for 4 h are compared. Decreasing the temperature resulted in a reduction in Fv/Fm ratios of both mono- and poly-embryonic cultivars (Fig. 7*a*, *b*). For mono-embryonic cultivars, Dashehari was the most sensitive to cold-induced photoinhibition with a Fv/Fm ratio of 0.384 at 8°C. Kensington recorded the lowest Fv/Fm ratio for poly-embryonic cultivars between 13 and 22°C. The relationship between temperature and mean Fv/Fm was non-linear for cultivars from both ecotypes (Fig. 7*c*). Photoinhibition, as indicated by the decrease in Fv/Fm ratio, was collectively greater in poly-embryonic cultivars than in mono-embryonic cultivars. For example, when the temperature decreased from 22 to 8°C, the mean Fv/Fm ratios for mono- and poly-embryonic cultivars declined 28 and 32%, respectively. However, the difference between mono- and poly-embryonic cultivars was not significant.

Recovery from photoinhibition

The mean Fv/Fm ratios for mono- and poly-embryonic cultivars before exposure to cold temperature were 0.758 ± 0.004 and 0.775 ± 0.005, respectively (data are not presented). Following exposure to 10°C at a PPF of 450 μmol/m^2.s for 24 h, Fv/Fm ratios of all cultivars declined with Kensington reaching the lowest Fv/Fm ratio of 0.068. At the end of cold-induced photoinhibition, the mean Fv/Fm ratios of mono- and poly-embryonic cultivars were 0.148 and 0.183, respectively. The recovery from photoinhibition was both temperature- and light-dependent with a similar dependency shown in all cultivars (Fig. 8). Whether in the presence or absence of light, photoinhibited leaves held at 10°C (Fig. 8*a*) had lower recovery capacity than leaves maintained at 20°C

(Fig. 8*b*). At 20°C, exposure to a PPF of 20 μmol/m^2.s during the recovery period enhanced the Fv/Fm ratio, but no positive effect from light was apparent at 10°C; in fact recovery of some cultivars was slightly inhibited by light. With the exception of Tommy Atkins, Fv/Fm ratios rapidly increased during the first 1–2 h of recovery; thereafter, the rate of recovery was slower at 20°C with little improvement at 10°C.

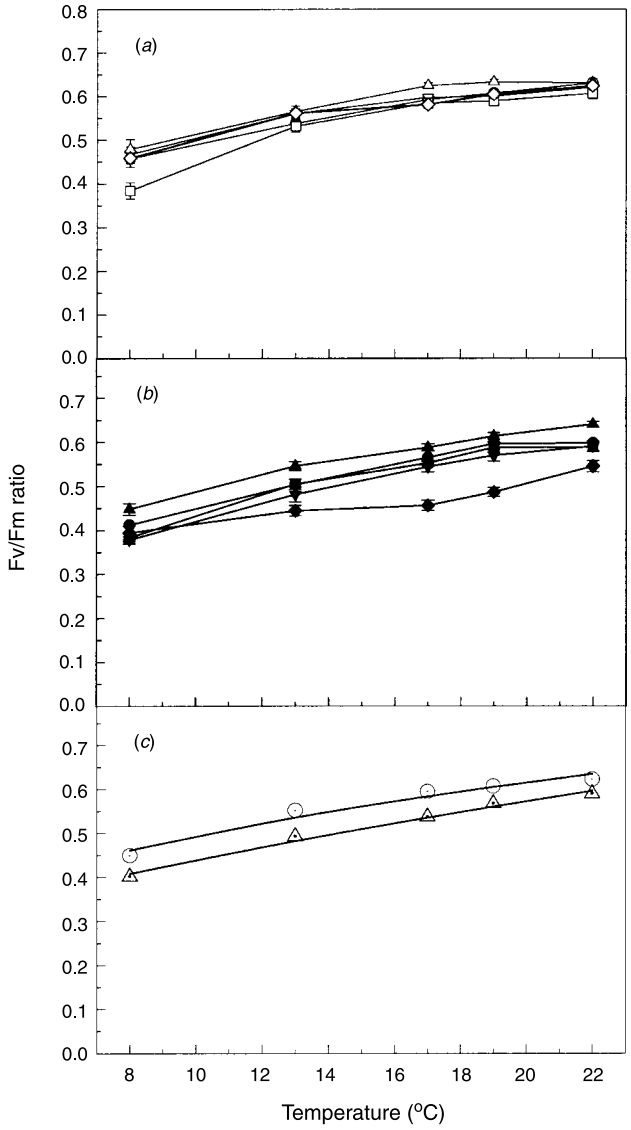

Fig. 7. Susceptibility to cold-induced photoinhibition in detached leaves of (*a*) mono-embryonic cultivars Chausa (△), Dashehari (□), Irwin (○), Sensation (▽), and Tommy Atkins (◇); and (*b*) poly-embryonic cultivars Carabao (▲), Kensington (◆), Khieo Sawoei (▼), Nam Dok Mai (●), and Nang Klang Wan (■). A non-linear regression model (*c*) for the relationship between Fv/Fm (*y*) and temperature (*t*) for mono-embryonic cultivars (⊙) is represented by $y = 0.124 + 0.143t^{(0.411)}$ ($r^2 = 0.96$; $P = 0.04$) and for poly-embryonic cultivars (◬) by $y = 0.225 + 0.043t^{(0.698)}$ ($r^2 = 0.99$; $P < 0.01$). Leaves were irradiated at 8, 13, 17, 19, or 22°C with a PPF of 450 μmol/m^2.s for 4 h. Fv/Fm ratios were determined from leaves dark-adapted for 40 min at the different equilibration temperatures. Data in (*a*) and (*b*) are the means ± s.e. of 24 leaves per cultivar. In many instances, vertical s.e. bars are obscured by symbols.

Discussion

Exposure to high PPF and low temperatures (<10°C) can cause photoinhibitory damage to the photosystem II of tropical species (Powles 1984; Smillie *et al*. 1988), resulting in a reduction in quantum yield and capacity of photoassimilation (Demmig and Björkman 1987). In addition, high light and chilling temperatures can cause photo-oxidative destruction of photosynthetic pigments that show as yellowing and necrosis of the lamina (van Hasselt and van Berlo 1980). In our study, low night temperatures during winter induced photoinhibition in mango leaves, and this was demonstrated by a significant reduction in Fv/Fm from June to July (Figs 2 and 3). Whiley and Schaffer (1997) and Whiley *et al*. (1999) have also reported a reduction in Fv/Fm values of mango leaves when exposed to chilling temperatures and high PPF with an associated reduction in photoassimilation (from 15.2 to 10.6 μmol CO_2/m^2.s) and quantum efficiency (from 0.042 to 0.025 μmol CO_2/μmol quanta). The results of the present study show that leaves of poly-embryonic mango cultivars were generally more susceptible to cold-induced photoinhibition than leaves of mono-embryonic cultivars, both in the field and under controlled conditions. At the extreme, Nang Klang Wan developed severe leaf chlorosis during midwinter, indicating a high sensitivity to photo-oxidation of chlorophyll. Nang Klang Wan is a poly-embryonic cultivar that originated in Thailand (Kusumo *et al*. 1984); thus, it is consistent with its evolutionary history in that it is expected to show greater sensitivity to low temperatures. Our results support the recent conclusions of Whiley and Schaffer (1997) who suggested that the physiological responses of mango cultivars to environmental variables are related to their evolutionary centres of origin. The chlorophyll concentration of mango leaves in all cultivars was lowest during midwinter. These results are supported by Searle *et al*. (1995) who studied pheno-physiological changes in Kensington mango growing in subtropical Queensland and reported similar reductions in leaf chlorophyll content during winter. Haldimann *et al*. (1996) also found that maize grown at 15°C led to a 50% decrease in chlorophyll concentration when compared with growth at 25°C. Similarly, for bananas grown in the subtropics of south-eastern Queensland, Damasco *et al*. (1997) reported both a loss of leaf greenness and a decline in Fv/Fm of leaves directly exposed to sunlight during winter. The high correlation between minimum night temperature and Fv/Fm ratio or Chl *a* concentration in our study suggests that an increased Fv/Fm ratio or chlorophyll concentration of leaves during spring is partially due to higher temperatures.

Measurements of Fv/Fm showed a lower value for attached leaves than detached leaves from the same field

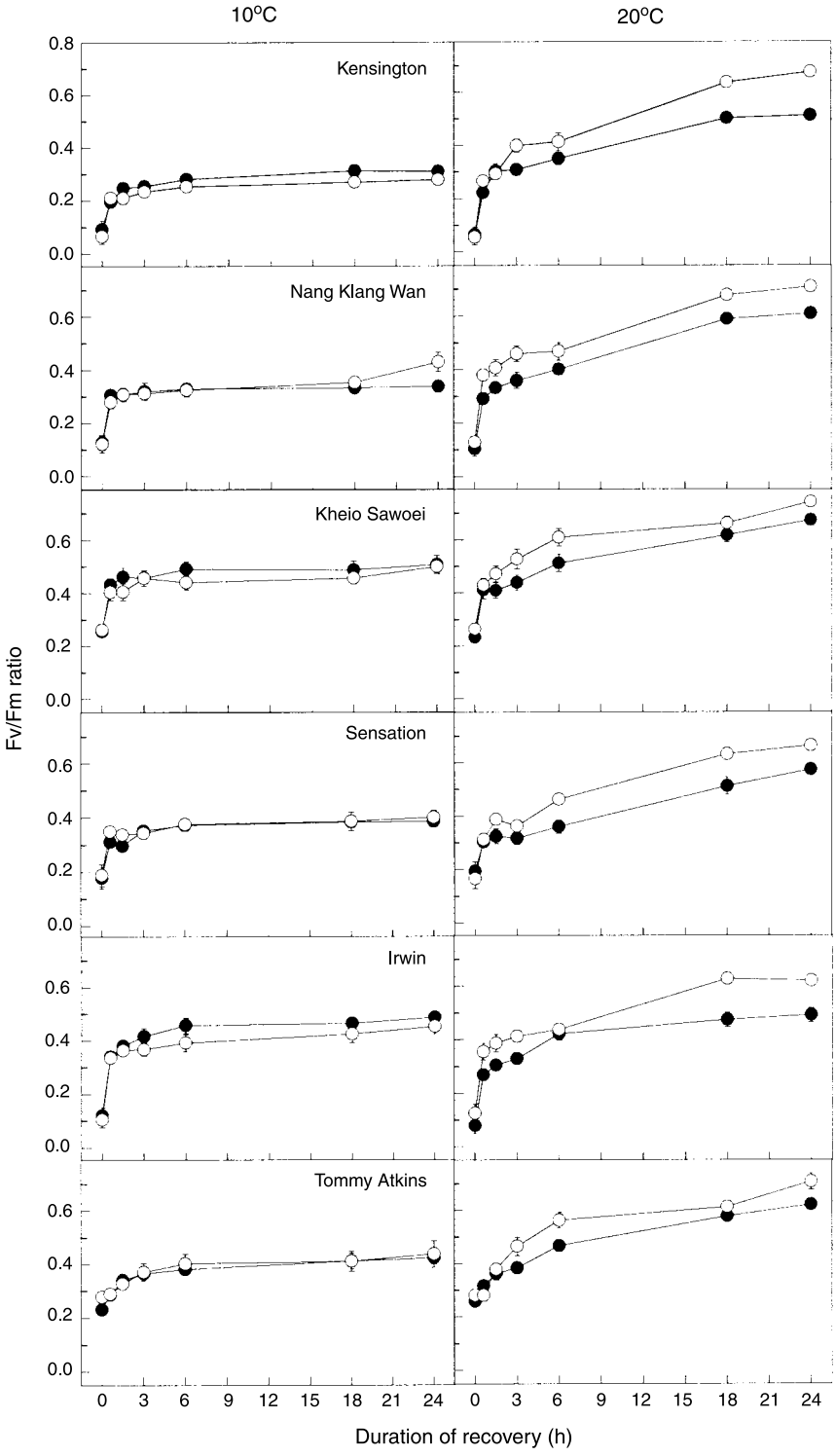

Fig. 8. Recovery from photoinhibition in detached leaves of 3 mono-embryonic cultivars, Sensation, Irwin, and Tommy Atkins, and 3 poly-embryonic cultivars, Kensington, Nang Klang Wan, and Khieo Sawoei, held at 10°C and 20°C at a low PPF of 20 μmol/m^2.s (○) or in darkness (●) for 24 h. Detached leaves were initially photoinhibited by maintaining leaves at 10°C and irradiating at a PPF of 450 μmol/m^2.s for 24 h. Values shown are the means ± s.e. of 12 leaves per cultivar. In many instances, vertical s.e. bars are obscured by symbols.

trees (Figs 2 and 3). These differences are probably due to detached leaves having a greater capacity for recovery during equilibration at a constant 20 ± 2°C compared with equilibration using a dark-acclimation cuvette under ambient field conditions (<10°C). This is supported by our results that show a greater capacity for recovery of Fv/Fm at higher temperatures (Fig. 8).

Our results indicate that development of photoinhibition in mango leaves was dependent on both temperature and light (Figs 6 and 7). Photoinhibition of mango leaves increased non-linearly with decreasing temperature but linearly with the length of the irradiation period. Similar results have been reported for bean (Greer *et al*. 1986), kiwifruit (Greer and Laing 1988), and a range of chilling-tolerant and -sensitive plants, e.g. broad bean, pea, cucumber, and sesame (Hetherington *et al*. 1989). These authors suggest that low temperatures generally slow down the enzymatic reactions of electron transport and carbon metabolism. Additionally, differences in Fv/Fm of freshly collected leaves (Fig. 6) indicate the differences of prevailing photoinhibition on leaves of each cultivar.

When detached leaves of mono- and poly-embryonic cultivars were exposed to temperatures from 8 to 22°C and a PPF of 450 μmol/m^2.s, Fv/Fm ratios of cultivars from both ecotypes decreased with temperature (Fig. 7). However, the reduction in Fv/Fm ratios at each temperature was different among cultivars and between ecotypes. This difference in cultivar susceptibility to cold-induced photoinhibition is an important factor to consider in the selection of suitable mango cultivars for subtropical conditions. Selection of cold-tolerant cultivars using chlorophyll fluorescence has already been used for other species, e.g. potato (Sundbom *et al*. 1982) and banana (Damasco *et al*. 1997).

The reduction in Fv/Fm ratio of Kensington leaves at 22°C from 0.545 ± 0.012 to 0.375 ± 0.015 after photoinhibition induced in the laboratory at 8°C for 4 h is consistent with the measurements on trees in the field. Field measurements showed that Fv/Fm ratios in leaves of this cultivar declined to 0.333 ± 0.023 in mid-July (<10°C) and increased to 0.681 ± 0.007 by November (Fig. 3). Our data for this cultivar are supported by Whiley *et al*. (1999) who reported Fv/Fm ratios in winter of 0.45 ± 0.03, which increased to 0.80 ± 0.03 in summer. Searle *et al*. (1995) also reported that the Fv/Fm of Kensington leaves in subtropical south-eastern Queensland declined to about 0.50 in winter.

The recovery experiments in this study, together with previously reported results (Lasley *et al*. 1979; Greer and Laing 1988; Haldimann *et al*. 1996), clearly show that recovery of leaves of mango and other species from photoinhibition is directly dependent on both temperature and light. After an initial recovery, all 6 mango cultivars continued to recover at a warm temperature (20°C) but very slowly at a chilling temperature (10°C), whether in the presence or absence of light (Fig. 8). A similar temperature-dependency, which showed as a higher capacity for recovery at 20°C and slower recovery at 10°C, was reported for maize (Greer and Hardacre 1989). Greer *et al*. (1986) also reported that recovery for bean leaves held at 30°C was higher than when leaves were held below 15°C and that no recovery occurred at 10°C.

Photoinhibited leaves were able to recover immediately after light stress was removed. Under the same controlled environments, all cultivars had similar temperature-dependency during recovery (Fig. 8). The faster rate of recovery of most cultivars during the first 2 h is consistent with that reported for maize (Haldimann *et al*. 1996). It was suggested by Leitsch *et al.* (1994) that the fast phase of recovery is independent of D_1 protein turnover. More recent evidence (Thiele *et al*. 1996) indicated that the fast recovery phase (at ≥20°C) is related to epoxidation of zeaxanthin in the xanthophyll cycle.

Repetitive field measurements of Fv/Fm demonstrated that leaves maintain photosynthetic proficiency during winter in subtropical south-eastern Queensland. The increased Fv/Fm of leaves that recovered from photoinhibition in darkness at mild temperatures (10–20°C) (Fig. 8) suggests that overnight recovery can occur providing temperatures do not fall below 10°C. If recovery does not occur during the night and leaves are again exposed to photoinhibitory conditions in the morning then chronic photoinhibition is likely to develop. Although light and low temperature can result in photooxidative destruction of chlorophyll, this generally does not occur unless photoinhibitory conditions are particularly severe (high light or chilling temperatures; van Hasselt and van Berlo 1980), and not until after permanent damage to the photosynthetic system has taken place (Long *et al.* 1983). In our studies with mango, chronic photoinhibition developed during winter and was accompanied by a decrease in leaf chlorophyll (Fig. 5).

Despite heavy flowering, fruit set and yield of Kensington in subtropical regions is unreliable (Whiley *et al*. 1988). Due to the high flowering intensity of mango trees, great demand is placed on stored energy reserves, which are exhausted by the completion of anthesis (Searle *et al.* 1995). Hence, currently produced assimilates from leaves are required for fruit set and growth. Damage to the photosynthetic mechanism through cold-induced photoinhibition will limit the supply of photosynthates for fruit retention and subsequent ontogeny. Searle *et al*. (1995) reported that the number of fruit set and retained per inflorescence in the mono-embryonic mango cultivar Irwin was almost 50% greater than that in Kensington. They suggested that this is related to the higher photoassimilation rate of the mono-embryonic cultivar due to the faster recovery of its photosynthetic apparatus following cold-temperature exposure during winter. This conclusion is consistent with our findings that in the winter and spring months Irwin maintained higher Fv/Fm ratios and leaf chlorophyll concentration than Kensington. This result has important implications with respect to the selection of culti-

vars for production in the subtropics of Australia. The greater cold sensitivity of poly-embryonic cultivars in relation to reproductive biology has previously been reported by Sukhvibul *et al.* (1999*a*, 1999*b*) and the additional evidence of greater reduction in physiological leaf function emphasises the need to avoid growing cultivars from this ecotype in subtropical regions.

We conclude that cold-induced photoinhibition of leaves of field-grown mango trees occurs during winter in subtropical climates. However, damage is not irreparable and recovery occurs when temperatures increase. The susceptibility to photoinhibition and the ability of leaves to recover differed both between mango ecotypes and among cultivars. These results indicate that measurements of Fv/Fm can be used to screen cultivars or new mango hybrids for increased low-temperature tolerance. This will be particularly useful if this trait is linked to a more robust anthesis event under subtropical temperatures.

Acknowledgments

The authors thank V. J. Doogan for assistance with statistical analyses. The authors are also grateful to Dr C. Searle for helpful advice in the measurements of chlorophyll fluorescence and to Dr R. M. Smillie for critical reading of the manuscript. Financial support for this research was provided by the Queensland Horticultural Institute, the Australian Centre for International Agricultural Research, and the School of Land and Food, The University of Queensland.

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Manuscript received 14 May 1999, accepted 24 December 1999