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The optimum temperature window for leaf C-export in the light is narrower than that for C-fixation

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Introduction

Heat or cold "stress" can be defined as a change in temperature that exerts a negative influence on plant growth. Attempts to quantify stresses ultimately demand a measure of the plant's fitness to cope with the challenge. A change in photosynthesis (Pn) frequently reflects changes in plant growth (S17-025). Plants tolerate sustained temperature stresses differently. A rise in air temperature above the optimal for a cool-season crop, pea, may not affect the development of the more heat-tolerant pepper plant. Similarly, decreased air temperature can also induce stress conditions and tolerance to them. In the late summer and fall sustained lower ambient temperatures will inhibit Pn in many plants yet some perennials acclimate or "harden" to be able to tolerate the stresses imposed by cold and light (see Savitch *et al.* 2000 and S35-020).

Temperature affects leaf Pn, C partitioning in the leaf, allocation to and acquisition of assimilates among different sinks (Farrar 1988). In C_3 plants increasing leaf temperature above the growth temperature generally results in reduced leaf Pn (Berry and Björkman 1980). One major reason for this reduction is the enhanced oxygenase activity of Rubisco in the chloroplasts (Jordan and Ogren 1984; Brooks and Farquhar 1985). High leaf temperature can also result in other changes in chloroplast metabolism such as thermal uncoupling of thylakoids and inactivation of the photosystems (Havaux *et al.*1991; Hubbs and Roy 1993; Ghosh *et al.* 1994). However, a leaf is not a homogeneous structure consisting of one cell or tissue type. Many different physical and biochemical processes regulate organ (leaf) homeostasis. Changes in C partitioning and Pi exchange within the mesophyll tissue at elevated (Weis 1981; Kobza and Edwards 1987; Stitt and Grosse 1988) and low temperatures (Leegood and Furbank 1986; Holaday *et al*. 1992; Mitchell and Madore 1992) can affect export (Exp). The intracellular and intercellular movements of photoassimilates from the site of production to the main vascular channels can be inhibited reducing phloem loading and end product (assimilate) removal from the leaf (Webb 1970; Farrar 1988; Robards and Lucas 1990; van Bel 1993). Unfortunately, few measurements exist quantifying the extent to which changes in the temperature of a source leaf actually alter Pn and concurrent Exp of newly fixed C via phloem (Jiao and Grodzinski 1996, 1998; Leonardos *et al.* 1996). The objectives of this study were to examine the effects of sub-lethal temperature stress on net CO_2 influx (i.e., Pn) and the ability of the source leaf to sustain a high immediate rate of C Exp.

Materials and methods

In order to differentiate between the effects of temperature stress on Pn and those on Exp processes *per se*, we measured ^{14}C-Exp during the period of active $^{14}CO_2$ fixation as described previously (Jiao and Grodzinski 1996; Leonardos *et al.* 1996). Six C_3 species representing a small sampling of sucrose transporting species, *Alstroemeria sp.* cv. Jacqueline (alstroemeria), *Pisum sativum* cv. Improved Laxton's Progress (pea), *Capsicum annuum* cv. Cubico (pepper), and *Rosa hybrida* cv. Samantha (rose), and two species producing auxillary transport sugars of the raffinose series, *Cucumis sativus* cv. Revenue (cucumber) and *Salvia splendens* cv.Bonfire (salvia) were grown in a greenhouse (25±3°C day and 16±1°C night temperatures). *Triticum aestivum* L. cv. Monopol (winter wheat) were grown in controlled environment chambers under two regimes 20/16°C and 5/5°C (day/night) as described elsewhere (Savitch *et al.* 2000). Generally, the air temperature of the plant holding chamber was maintained at the growth temperature (e.g., 25°C) while that of the leaf being tested was varied between 10 and 45°C. With wheat plants which had been cold-5°C -stressed (for 12 h) or 5°C -acclimated (for 8 wks) day/night temperatures of the plant and the leaf cuvette were adjusted to match those used to challenge the plants.

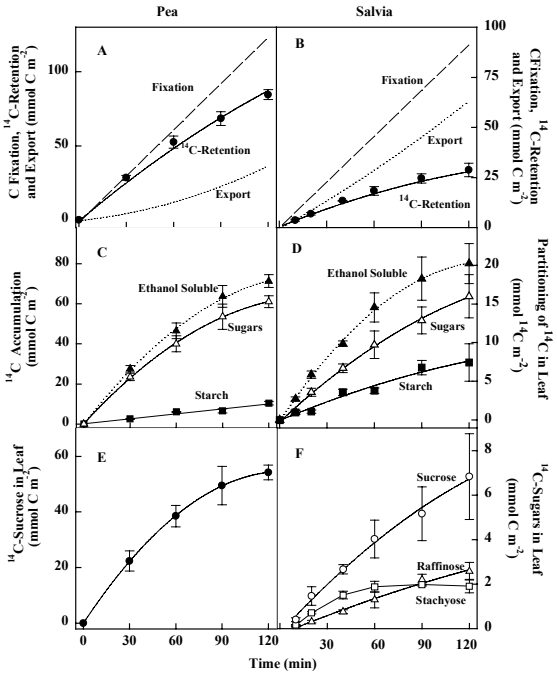

Figure 1. Total Pn, Exp and partitioning in pea (A) and salvia (B) during 2h-feedings under ambient CO_2 and 25°C. Cumulative net C "Fixation" (dashed line) was calculated from IRGA data, whereas "^{14}C-Retention" in the leaf was measured both non-destructively by monitoring ^{14}C continuously (solid line) and in a parallel set of leaves by destructive analysis (•). "Exp" (dotted line) during steady-state $^{14}CO_2$ feeding was estimated as the difference between total "Fixation" (dashed line) and "^{14}C-Retention" in the leaf (solid line). Each point is the average of at least 4 leaves on 4 different plants and error bar represents the SE of the mean.

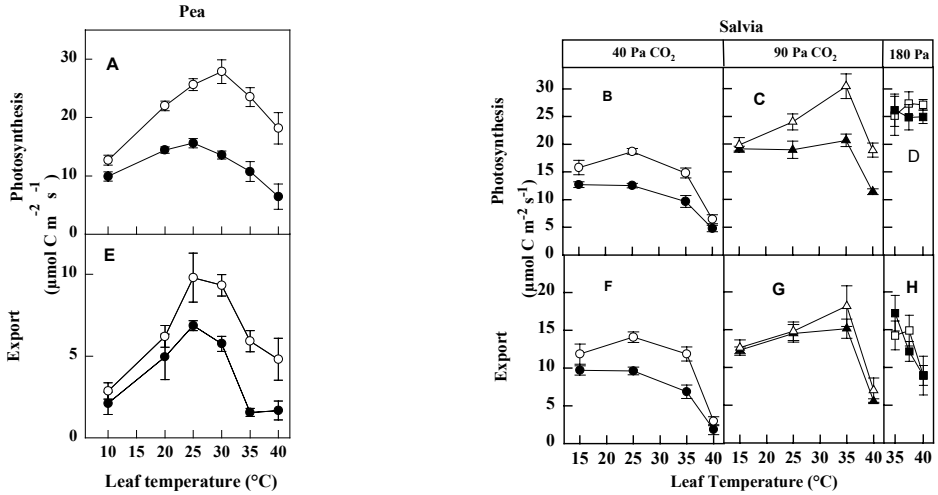

Figure 2. Effects of altering leaf temperature on Pn and immediate Exp in pea (A and E) and salvia (B,C,D and F,G,H). In A and B pea leaflets were assayed under ambient (37Pa CO_2, 21kPa O_2; closed circles) and enriched CO_2 conditions (125Pa CO_2, 21kPa O_2: open circles) to suppress photorespiration. Salvia leaves were at 40Pa (B,F), 90Pa (C,G) and 180Pa CO_2 (F,H) and at either 21kPa O_2 (closed symbols) and 2kPa O_2 (open symbols).

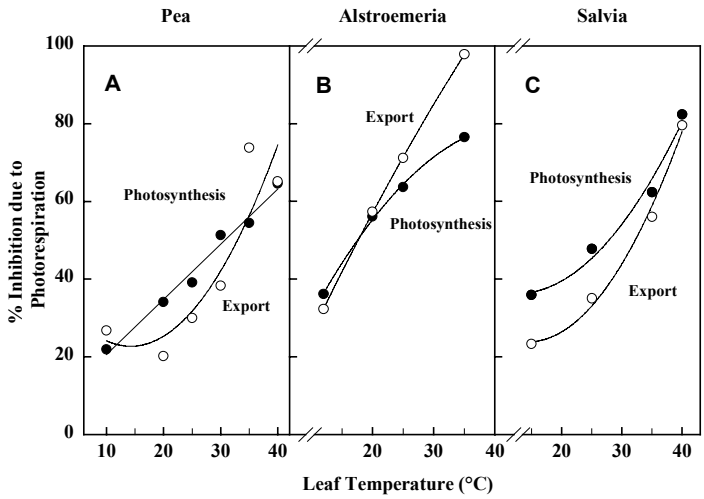

Figure 3. Effect of temperature on the percentage inhibition of Pn (•) and Exp (°) due to photorespiration in pea (A), alstroemeria (B) and salvia (C). The % inhibition due to photorespiration was estimated in a manner similar to that of Kozba and Edwards (1987). The rate of Pn or Exp at each temperature obtained under non-photorespiratory conditions was subtracted from the rate under photorespiratory conditions, divided by the rate at non-photorespiratory conditions times 100.

Results

Exp often decreases more than Pn: Figure 1 shows typical estimates of Pn and concurrent Exp in pea (A) and salvia (B). Immediate Exp rates were normally estimated during the 90 to 120 min period after $^{14}CO_2$ labeling began, since isotopic equilibrium of key translocate pools and the $^{14}CO_2/^{12}CO_2$ air stream were established (Fig. 1 C, D, E and F). Fig. 2 shows that Pn at ambient CO_2 and O_2 levels was generally highest near the day-time growth temperature (approx. 25°C). At higher temperatures, Pn decreased due to increased photorespiration, but high CO_2 and/or low O_2 increased Pn and Exp. In roses and cucumber the patterns were similar to those of pea and salvia except that the temperature windows were several °C wider

(i.e., higher) than shown in Fig 2. In alstroemeria Pn and Exp were markedly reduced above 25°C even under non-photorespiratory conditions. Both pea and alstroemeria tolerate "cool" temperatures, while pepper grows well at warmer temperatures. Pepper Pn was maximal between 30 and 35°C, slightly above the growth temperature (i.e., 25°C). At 40°C Pn was still almost 85% of that at 35°C. Pn decreased by 60% at 43°C but at high CO_2 and low O_2 was similar to that at 35°C. Even so Exp in peppers decreased proportionally more between 40 and 48°C than did Pn (data not shown). As illustrated in Fig. 2D and H when photorespiration was severely depressed at 180Pa CO_2 Pn rates were maintained extending the upper portion of the temperature window but concurrent Exp rates still "crashed". Fig. 3 provides a qualitative picture of the importance of photorespiration in controlling net C-fluxes through the leaf (i.e, fixation to export) in response to temperature. In all C_3 species (e.g. Fig 3), suppressing photorespiration at higher temperatures increased Pn and Exp. Exp appeared to be more sensitive to high temperature than did Pn.

Recovery and acclimation to temperature stresses: There are other indications that Exp processes might be more sensitive to temperature than Pn. When leaves were treated for several hrs at warm temperatures (e.g., peas @ 38°C; roses @ 45°C; or salvia @ 40°C, peppers @ 46°C) Pn and Exp were reduced by 60 to 80% of their maximum. When re-cooled, Exp rates generally recovered to a lesser extent and not as rapidly as Pn. For example, in cucumber (Table I) within 2 h Pn had recovered, but Exp remained low compared to controls for almost a day. Concomitantly, elevated temperatures reduced ^{14}C-partitioning into storage pools (e.g., starch), but increased partitioning into sugars and other ethanol soluble assimilates (data not shown). In cucumber and salvia, raffinose and sucrose were stored but stachyose did not accumulate. Slightly more ^{14}C was also lost via dark respiration when immediate Exp was reduced. In roses we noted that a decrease in day-time Exp induced by high temperature stress (40°C) did not result in an increase in Exp at night.

Table I. Recovery of heat stress induced inhibition of Pn and Exp in *C. sativus.* Leaves were assayed at 35 and 40 °C and exposed to both ambient CO_2 (40 Pa) and O_2 (21 kPa), and high CO_2 (180 Pa) and low O_2 (2 kPa). In addition, leaves which were pre-incubated at 40°C for 40 min and subsequently cooled to 35°C for 2 h at 40 Pa CO_2, or for 30 min at 180 Pa CO_2 prior to measurements of Pn and Exp during a 2-h steady-state $^{14}CO_2$ feeding (i.e., 40/35 °C values).

Temp.	% Inhibition of Pn		% Inhibition of Exp	
(°C)	36PaCO_2/21kPaO_2	180PaCO_2/2kPaO_2	36PaCO_2/21kPaO_2	180PaCO_2/2kPaO_2
35	0	0	0	0
40	61	2	78	42
40/35	11	-1	41	24

Cool air temperatures also induce stress conditions and tolerance to them (i.e., acclimation). For example, sustained lower temperatures will inhibit primary Pn and Exp processes in winter wheat (Fig. 4). Winter wheat were acclimated (grown) at 20 or 5°C, 250 µmol m^{-2} s^{-1} PPFD and ambient CO_2 and assayed under these growth conditions as well as at high light and high CO_2 conditions. In addition, plants grown at 20°C were cold-stressed for 12h at 5°C prior to measurements at 5°C. Whole-plant Pn was similar in 20°C, 5°C-stressed and 5°C-acclimated plants at the growth conditions (data not shown). However, 5°C-stressed plants had lower whole-plant Pn at high light and high CO_2 than did the two acclimated controls which were similar to each other. Diurnal patterns of leaf (organ) Pn and ^{14}C Exp were

determined (Fig. 4). At the growth conditions, the 20°C and 5°C-acclimated leaves had similar Pn, but daytime Exp from 5°C-acclimated leaves was 45% lower. Pn and Exp remained steady during the day in both cases. In contrast, Pn and Exp of leaves of 5°C-stressed plants were reduced significantly. Pn of 5°C-stressed plants declined during the photoperiod from 60 to 20% of the 20°C controls. In 5°C-stressed plants Exp remained constant during the photoperiod but was inhibited proportionally more than Pn was. When challenged with high light and enriched CO_2 conditions these stressed leaves did not increase their Pn or Exp whereas both acclimated controls demonstrated enhanced daily Pn and Exp which reflected whole-plant growth patterns (data not shown). Taken together, our data show that following an initial inhibition of Pn and Exp due to cold stress, acclimation to low temperatures (autumn conditions) occurs resulting in sustained Exp (Fig.4B) that supports growth and storage of reserves in the crown and roots which must over-winter.

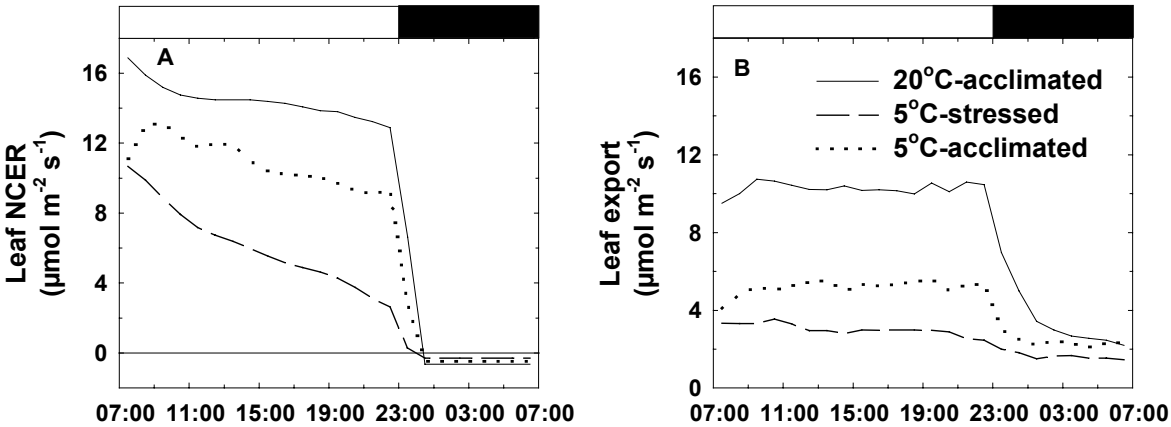

Fig. 4. Daily pattern of leaf Pn, dark respiration and ^{14}C-Exp during full-day $^{14}CO_2$ feeding to 20°C, 5°C-stressed and 5°C-acclimated wheat plants, under 1000 µmol m^{-2} s^{-1} PPFD and ambient CO_2

Discussion

The two primary functions of a "source" leaf are to fix energy during Pn and transport that chemical energy to the rest of the plant (Exp). Quantitatively, most leaves Exp the bulk of newly fixed C in the light (Geiger and Servaites 1994; Jiao and Grodzinski 1998; Jiao *et al.*1999; S17-025). The full daytime ^{14}C-feedings and the daily Exp patterns of the 20°C, 5°C-stressed and 5°C-acclimated wheat plants in Fig. 4B show that in each test more ^{14}C-assimilates were exported in the light during Pn than in the dark. It appears clear that "feed-forward" regulation of Exp exists in the light, as Pn and assimilate production are driven and regulated by light, CO_2, O_2, and temperature. Immediate Exp measurements of about 50 species representing a range of C_3, C_4 and C_3-C_4 intermediate photosynthetic types confirm that immediate Exp is highly correlated (r >0.9) with Pn at the growth conditions (Grodzinski *et al.*1998; Leonardos and Grodzinski 2000). When source leaves of several C_3 species (e.g., alstroemeria, cucumber, peas, pepper, roses and salvia) were exposed to air temperatures that were warmer than their growth regime (e.g., >10 to15°C) Pn was generally altered proportionally less than Exp. Significantly, using our steady-state labeling procedure it was shown that leaf warming resulted in a reduction in immediate Exp (Fig.2H) without inhibition of the photosystems and/or C-fixation processes *per se* (Fig.2D). These data challenge the concept that membrane disruption leading to a loss of the photosystem activity or inhibition of enzymes of C-fixation in chloroplasts are the major limiting events resulting in decreased

sugar export from source leaves during episodes of heat stress (see **Introduction**). Membrane processes associated with intra- and intercellular assimilate transfers (e.g., phloem loading), blockage of plasmadesmata as might occur from callose formation, and protein changes in the phloem could contribute to reduced Exp during Pn and induce a feed-back situation. An earlier study showed that the symplastic loaders (i.e., cucumber and salvia) generally had higher immediate Exp (relative to their Pn) than did many C_3 species (Grodzinski *et al.* 1998). However, collectively the data from this study indicate that the temperature windows for Exp relative to Pn in the two symplastic loaders, salvia and cucumber, were similar to those of species loading and exporting sucrose only. For example, the temperature for Exp from pea, alstroemeria, roses and peppers were also narrower than that for their Pn. In both agricultural and natural settings plants are frequently exposed to changes in air temperature that can become stressful in a few minutes. Differentiation of the processes in the source leaf that influence Exp versus those that influence Pn *per se* deserve further evaluation.

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