A Florigenic Effect of Sucrose in *Fuchsia hybrida* Is Blocked by Gibberellin-Induced Assimilate Competition

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The use of gas chromatography-mass spectrometry-selected ion monitoring along with a ^{13}C internal standard has allowed sensitive measurements of the sucrose (Suc) content of individual shoot apices of *Fuchsia hybrida*. With intact plants, as the photosynthetic irradiance increased, so did shoot apex Suc content, reaching saturation at about 500 μmol m^{-2} s^{-1}. These same plants flowered at the higher irradiances, remaining vegetative in 10-h short days at an irradiance of 230 μmol m^{-2} s^{-1}. The strong correlation ($r = 0.93$) in these studies between flowering and shoot apex Suc content indicates a role for Suc as a stimulus to flowering in this species. However, Suc is not the long-day (LD) "florigen" of *F. hybrida* because 2 to 4 LD given as a 14-h low-irradiance photoperiod extension (10–15 μmol m^{-2} s^{-1}) induced flowering but without increase in shoot apex Suc content. Flowering induced by either pathway, the LD- or the Suc-mediated one, was inhibited by applying gibberellin (GA) to the shoot tip. Such inhibition of flowering by GA, at least for the LD pathway, was associated with a reduced apex Suc content, enhanced elongation of subapical stem tissue, and a reduced import into the shoot apex of leaf-sourced assimilate. Thus, our findings show how GA inhibits flowering of *F. hybrida* and confirm the importance of nutrient diversion in regulating flowering.

In a series of studies with the long-day plant (LDP) *Sinapis alba* using sensitive enzymatic measurements, early increases in shoot apex Suc content were found during exposure to either a florally inductive long day (LD) of high irradiance or to a displaced short day (SD; Bodson, 1977; Bodson and Outlaw, 1985). Lejeune et al. (1993) subsequently reported an increase in LD of Suc delivery to the apex of *S. alba* based on measurements of phloem exudate in the stem below the apex. Thus, such increases in assimilate/Suc supply to the shoot apex might be important for floral induction.

Quite separate from effects of a high irradiance, for most LDP a low irradiance non-photosynthetic LD can be effective for flowering. Such a non-photosynthetic LD could still alter leaf carbon metabolism but it is unlikely that this would alter Suc supply to the shoot apex. For example, the LDP *Lolium temulentum* can flower with no increase in Suc at the shoot apex following a low-irradiance LD extension of 10 to 15 μmol m^{-2} s^{-1} (King and Evans, 1991). In contrast, when various photosynthetically effective irradiances were imposed during the LD photoperiod extension there were substantial increases in apex Suc content and parallel increases in flowering of *L. temulentum* (King and Evans, 1991). Thus, there is a clear distinction for *L. temulentum* between Suc and the LD photoperiodic florigen as conceived by Chailakhyan in 1936. Suc is not a component of the true LD "florigen(s)" of *L. temulentum* but it acts as an effective florigenic synergist.

The contrasts between the various studies with *L. temulentum* and *S. alba* are informative. More often than not, the LD exposure with *S. alba* has involved photosynthetically effective irradiances of about 150 μmol m^{-2} s^{-1} (Bodson, 1977; Bodson and Outlaw, 1985; Lejeune et al., 1993). Thus, a synergistic, irradiance-driven photosynthetic Suc response might be expected and it would act along with the low-irradiance photoperiodic LD response known for this species (Bodson et al., 1977). The more complex experiments with *S. alba* involving a displaced SD also showed an early increase in apex sugars with floral induction (Bodson, 1977; Lejeune et al., 1993).

The scenario above, with the implication of increased photosynthesis, places the increase in shoot apex sugar of *S. alba* as separate to but synergistic with an LD photoperiodic flowering response. However, there might also be a direct Suc/photosynthetic effect with *S. alba*, which flowers after 3 SD of high (e.g. 500–600 μmol m^{-2} s^{-1}) photosynthetic irradiance (Bodson et al., 1977). In contrast, *L. temulentum* does not flower in SD even with exposure for weeks to very high irradiances (1,200–1,600 μmol m^{-2} s^{-1}; King and Evans, 1991). Thus, Suc can apparently act florigenically in *S. alba* in SD or LD but, again, not as the day length-dependent florigen of Chailakhyan (1936).

To further our understanding of how apex Suc levels relate to flowering, another LD plant, *Fuchsia hybrida*, has been examined here for two reasons. First, like *S. alba*, its flowering is enhanced by in-

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creased photosynthetic irradiance. Second, applied gibberellin (GA) inhibits its LD-induced flowering (Sachs and Bretz, 1961). The simplest explanation, and one which links these two findings, is that a rise in apical Suc content is sufficient for flowering of *F. hybrida*, as in *S. alba*. GA would inhibit flowering by diverting assimilate away from the apex and into elongating stem tissue. This concept, often referred to as the nutrient diversion hypothesis (see Sachs and Hackett, 1983), fits with our recent finding that the greater the stem elongation of *F. hybrida* in response to GAs, the greater the inhibition of its flowering (King et al., 2000). Furthermore, this inhibition of flowering was lost following slight structural changes to create either an elongation-inactive GA (epimerization of the C3-hydroxyl) or an elongation-inhibiting GA (e.g. 16,17-dihydro GA5). Such evidence points to an indirect, inhibitory effect of GA on flowering due to enhanced stem growth, a hypothesis also supported by Steffen et al. (1988) from their studies of inhibitory effects of GA on flower development in *Bougainvillea* "San Diego Red."

In the present paper the role of Suc in flowering of *F. hybrida* has been examined in three ways: (a) causality in the relationship between shoot apex Suc content and flowering has been examined by imposing various irradiances during otherwise noninductive SD, (b) changes in Suc content of the shoot apex have been measured during its transition to flowering and following inhibition of flowering by GA treatment, and (c) the distribution of ^{14}C-labeled assimilate has been examined to establish if GA action involves diversion of leaf-sourced assimilate away from the shoot apex.

RESULTS

For flowering of *F. hybrida* two inductive treatments were utilized: an LD of low irradiance or an SD at a high irradiance. As shown in Figure 1, flowering resulted after exposure to 2 or more LD, this LD involving the 10-h high light (230 μmol m^{-2} s^{-1}) of the SD followed by 14-h low irradiance (15 μmol m^{-2} s^{-1}) LD daylength extension. On return to SD the first morphological sign of the onset of flowering of axillary buds was at 8 to 10 d and they were visible to the naked eye at 21 d. In general, only two pairs of axillary buds were induced to flower by the brief LD treatment (i.e. a total of about four flowers per plant). Plants held in SD at an irradiance of 230 μmol m^{-2} s^{-1} remained vegetative for many months.

The second inductive treatment involved increase in the irradiance during the SD. As shown in Figure 1, flowering required 5 to 10 d of exposure to a high irradiance (500 μmol m^{-2} s^{-1}). Interaction of these two treatments is shown in Figure 2, where a 7-d increase in SD irradiance was combined with exposure to 4 LD. The increased photosynthetic input was clearly most important when it immediately preceded or coincided with early events of LD floral initiation of *F. hybrida*. This experiment also included control SD plants exposed to an increase in irradiance for 7 d. Their flowering response was very weak (an average of 0.5 flowers per plant; data not shown) and contrasts with the effective SD induction at high irradiance shown in Figure 1 and also later. We cannot explain why there was variability between floral

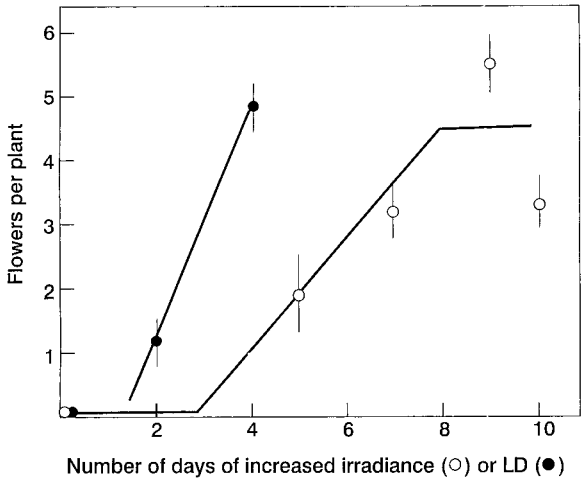


Figure 1. Induction of flowering of *F. hybrida* either by exposure to LD or by increasing the irradiance to 500 μmol m^{-2} s^{-1} in SD. Control plants maintained at an irradiance of 230 μmol m^{-2} s^{-1} in SD remained vegetative. Values are means ± SE (n = 12).

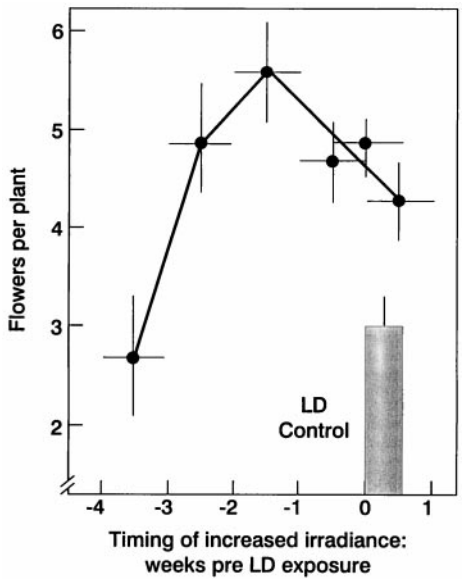


Figure 2. Flowering of *F. hybrida* in response to a 4-LD exposure combined with a 1-week increase in irradiance during the daily 10-h main light period. The 1-week increase in irradiance (from 230 to 500 μmol m^{-2} s^{-1}) was imposed at various times before, during, or after exposure of the plants to the 4 LD. The low-irradiance (230 μmol m^{-2} s^{-1}) LD control is shown as a column. Horizontal bars on the data points show the period of high-light exposure with the plot points being for the midpoint of the 7 d. Vertical bars are ±SE of the mean (n = 15).

induction across experiments and treatments but we confirmed the response in two further experiments (see Figs. 4 and 7).

Photosynthetic input could be expected to regulate flowering via change in Suc supply to the shoot apex. In preliminary studies of apex Suc a diurnal cycle was evident (Fig. 3) with the maximum content occurring during the light period as was expected based on our earlier findings with *L. temulentum* (King and Evans, 1991). Apices subsequently have been harvested over the period that Suc levels were at their peak.

The relationship between flowering and irradiance of the SD is illustrated in Figure 4 and this data confirms that flowering of *F. hybrida* can be induced directly by increasing the photosynthetically effective radiation as was also evident in Figure 1. Furthermore, shoot apex sugar content increased in parallel.

Apex Suc increase approached saturation at an irradiance of >500 μmol m^{-2} s^{-1} and at 350 μmol m^{-2} s^{-1} in a second experiment (not shown). Flowering response was also saturated over this same range in the two experiments. In contrast, saturation of photosynthesis for the youngest fully expanded leaf occurred above 800 μmol m^{-2} s^{-1} (data not shown). For the apex Suc increase and for flowering, such saturation at lower irradiances was expected given our similar findings for *L. temulentum* (King and Evans, 1991). There apparently is a restriction at the shoot apex to carbon inflow, a resistance that is not overcome by large increases in carbon supply from the leaf.

GA treatment both inhibits flowering of *F. hybrida* and promotes stem elongation (e.g. Sachs and Bretz, 1961; King et al., 2000). As shown in Figure 5, with increasing GA dosage shoot apex Suc content decreased progressively and stem elongation increased for these vegetative, SD-grown plants.

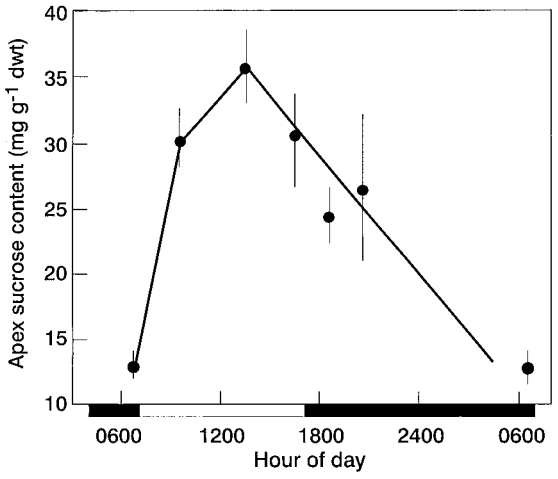


Figure 3. Effect of time of day on the Suc content of the shoot apex of *F. hybrida* plants growing vegetatively in SD. The black and white boxes, respectively, indicate the daily cycle of dark and light. Bars are ±SE of the mean (n = 5–9).

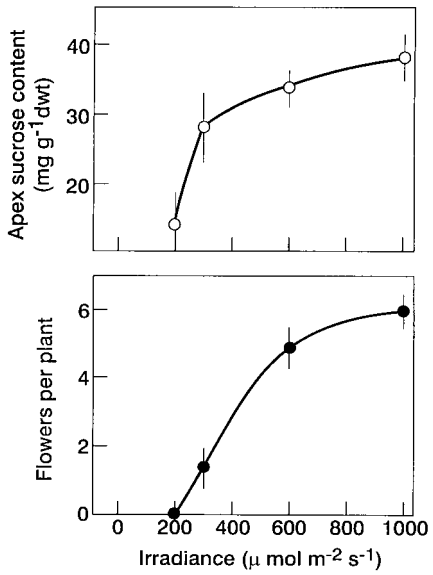


Figure 4. Effect of increasing the irradiance in SD on shoot apex Suc content and on flowering. Apices (n = 10) were harvested for Suc measurements 10 d after commencing the treatment and flowering (n = 20) was determined after 3 weeks. Values are means ± SE. The correlation coefficient between Suc content and flowering was r = 0.93.

To define when GA acted during floral initiation, flowering was induced either by exposure to 4 LD or to an increase in SD irradiance. Early applications of GA-inhibited flowering (Fig. 6) but this inhibition was lost by delaying the GA application until 6 d or more after the beginning of an inductive treatment. Morphological signs of floral development were first evident 7 to 10 d after the start of a 4-LD exposure of plants (data not shown). Thus, only early events of floral evocation and early initiation are inhibited by GA.

To examine changes over time in shoot apex Suc content and stem elongation, GA was applied either to noninduced plants or at the start of floral induction for plants exposed to 4 LD. As shown in Figure 7, within 1 d of GA treatment there was a brief but significant increase in apex Suc content for both vegetative and florally induced apices. Suc content subsequently dropped to a value below the untreated control and this drop was correlated with the onset of detectable GA-induced elongation of the stem 0.5 to 1 cm below the apex (Fig. 7). Over time, the GA treatment maintained rapid stem elongation and shoot apex Suc levels remained low.

With non-GA-treated flowering plants, apex Suc content first decreased and then increased after the LD was terminated (Fig. 7). The changes in stem growth were the inverse of those for apex Suc content with at first rapid elongation during the 4 LD and a drop in Suc but afterward an almost instantaneous return to the slower rate of elongation of SD plants

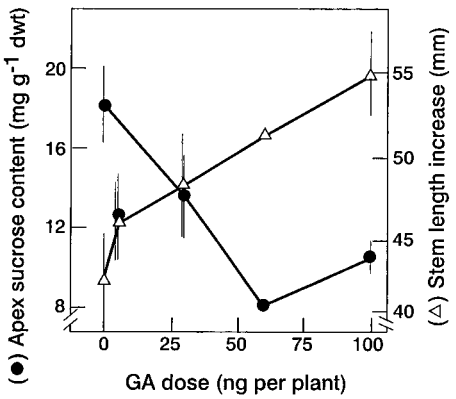


Figure 5. Relationship between GA_3 dose, stem elongation, and shoot apex Suc content for plants of *F. hybrida* growing vegetatively in SD. For Suc measurements, apex samples (six–eight per treatment) were taken 4 d after the GA_3 treatment. Stem lengths (n = 13) for each treatment are given as an increment over the starting value. Plants were measured daily but lengths are only shown after 7 d. Values are means ± SE.

(see inset Fig. 7) and a rise in Suc content. Such stem growth responses are most simply interpreted as an LD effect involving a temporary increase in endogenous GA content as is well-known for other species (compare with spinach; Zeevaart, 1971). With GA treatment coupled with LD, the changes in Suc content and stem elongation were more extreme. The more prolonged growth stimulation due to applied GA apparently inhibited flowering by keeping apex Suc levels low for much longer.

Examination of the supply of ^{14}C-labeled photosynthetic assimilate from the leaf to the apex confirmed that the effects of GA on apex Suc were linked to assimilate import. Six days after GA treatment, import from the leaf into the apex of ^{14}C-labeled assimilate was reduced relative to control plants as shown in Table I for a 10-min feed followed by a 6.75-h export period. This response fits with the decrease seen in apex Suc content (Fig. 7) and the increase in stem growth (Fig. 7; Table I). A similar trend was found in a repeat experiment (not shown) and there was a significant increase in assimilate import to the apex 1 d after GA application, which fits with the increase seen in apex Suc at this early time (Fig. 7).

DISCUSSION

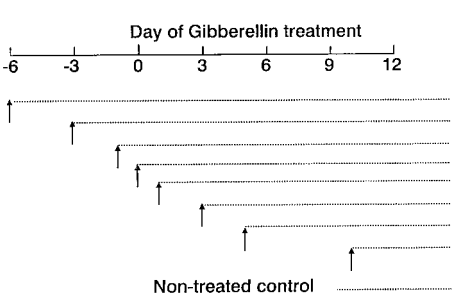
By combining the sensitivity and specificity of gas chromatography (GC)-mass spectrometry (MS)-selected ion monitoring (SIM) with accuracy due to the addition of a ^{13}C-Suc internal standard, we have developed a robust technique for measuring the Suc content of single, 2-μg dry weight, shoot apices of *F. hybrida*. Apex Suc increased with increased photosynthetic input; hence, these measurements are sensitive. They are also physiologically meaningful because, when photosynthetic input was increased, we found that flowering increased in parallel with apex Suc increase (Fig. 4). We consequently have been able to examine the role of apex Suc in environmental and hormonal regulation of flowering of *F. hybrida*.

Environmental Regulation of Flowering and of Suc Supply to the Shoot Apex

Here, by manipulating photoinductive conditions, we have shown that Suc is florigenic in *F. hybrida* but it is not a stimulus specific to LD photoperiodic exposures. Increases in photosynthetic input in non-photoinductive SD led to increased apex Suc content and, in parallel, to flowering of *F. hybrida* (Figs. 1 and 4). In contrast, exposure to low-irradiance inductive LD photoperiods resulted in flowering but without any increase in apex Suc content (Fig. 7). Thus, for *F. hybrida*, evidence of a relationship between flowering and apex Suc depends on whether a photosynthetic or a photoperiodic, non-photosynthetic LD irradiance has been used.

The low-irradiance florally effective LD photoperiod extensions contributed to a 6% increase at the most in photosynthesis and did not increase apex Suc, as we also reported previously for *L. temulentum* (King and Evans, 1991). In contrast, induction of flowering in the absence of LD required a greater than 200% increase in irradiance (Figs. 1 and 4). It is clear that photosynthetically produced Suc may cause flowering of *F. hybrida* but separately from (Fig. 1 and 4) yet additively with (Fig. 2), a low-irradiance LD photoperiodic treatment.

Other LD species that show photosynthetically driven flowering in SD include *S. alba* (Bodson et al., 1977) and Arabidopsis (Bagnall, 1992; Koornneef et



	Flowers per plant	
	Long Day induction by 4LD	Short day induction by increased light
	1.4±0.3	-
	1.3±0.4	-
	0.5±0.3	-
	0	0
	-	0.2±0.1
	0.3±0.2	-
	2.5±0.3	1.4±0.5
	3.7±0.2	1.9±0.6
	4.5±0.3	1.4±0.5

Figure 6. Effect of GA_3 application before, during, or after induction of flowering of *F. hybrida* by exposure to 4 LD or to 10 d at high irradiance in SD. The high irradiance was 500 μmol m^{-2} s^{-1}, control plants receiving 230 μmol m^{-2} s^{-1}. GA_3 was applied at 200 ng/plant in the experiment involving LD-induced flowering and at 100 ng/plant where high irradiance SD were used to induce flowering. Control plants were induced to flower but not treated with GA. Values are means ± SE. (n = 12–20).

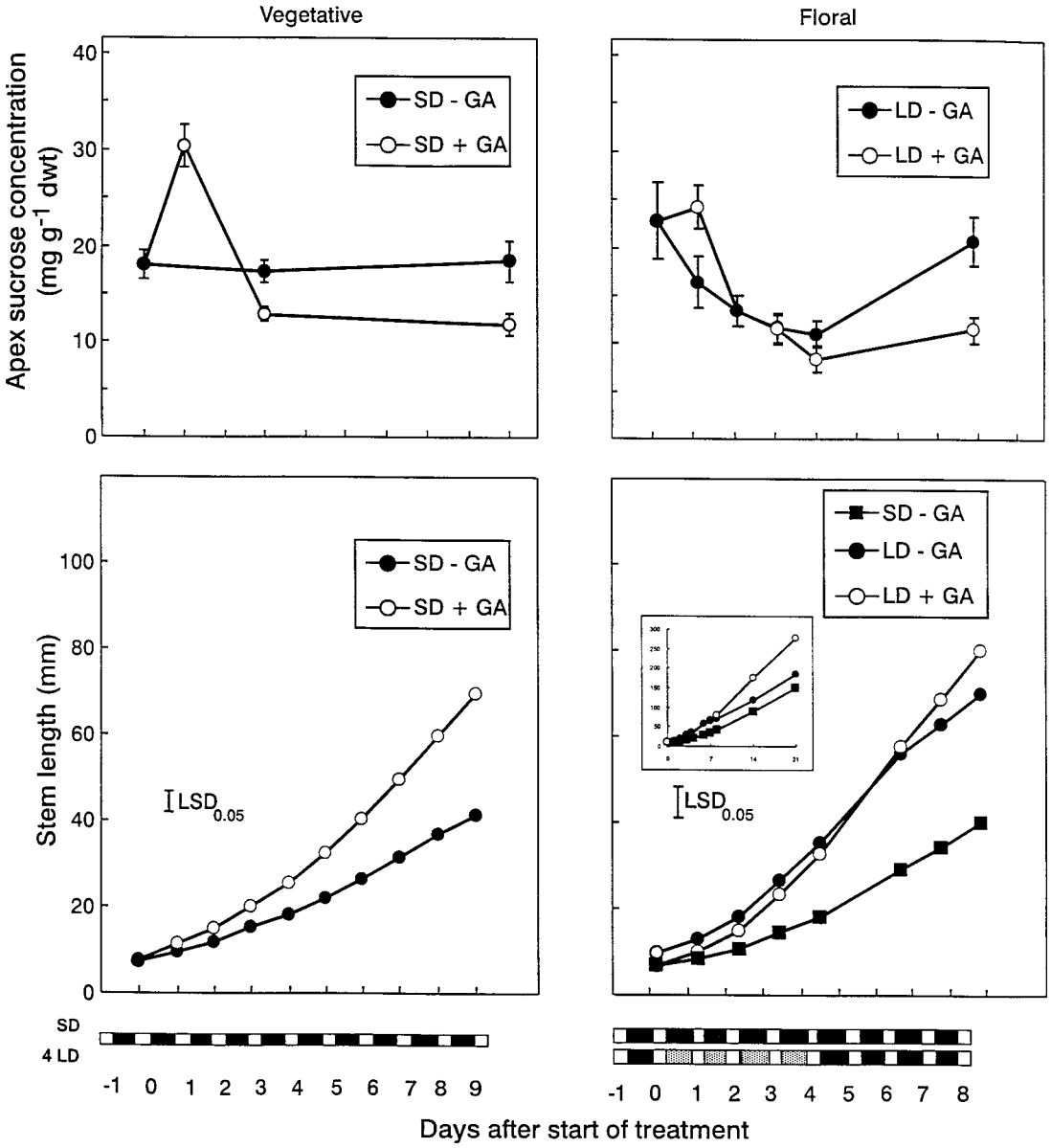


Figure 7. Change over time following GA treatment in shoot apex Suc content (top) and stem elongation (bottom) of vegetative or florally induced plants of *F. hybrida*. The synthetic GA 2,2-dimethyl-GA_4 was applied at a dose of 200 ng/plant to the vegetative plants in SD and at 400 ng/plant to florally induced plants. The daily light (white box), dark (black box), and LD treatment (hatched box) is shown on the abscissa. The inset shows further measurements of stem elongation out to 21 d by which time the stem length of the GA-treated LD plants was 275 mm. Errors are shown as ± SE or as the least significant difference ($P = 0.05$).

al., 1998). Furthermore, with both the latter species, supplying Suc promoted flowering to the same extent as achieved by increasing irradiance (Deltour, 1967; King and Bagnall, 1996). In contrast, for flowering of *L. temulentum* Suc is necessary but alone an increase in apical Suc is not sufficient. For example, Suc supplied to excised, cultured apices of *L. temulentum* was not sufficient to induce flowering, but if apices were excised when the LD photoperiodic floral stimulus had reached the apex (after 36 h), then a strong Suc response was obtained (McDaniel et al., 1991). Similar response was evident with intact plants in that substantial increases in the irradiance were not effective for flowering in SD but were additive to LD induction (King and Evans, 1991). As an aside, as for *F. hybrida* (Fig. 4), these earlier studies with *L. temulentum* showed that apex Suc as measured was a valid estimate of that pool of Suc of physiological importance for flowering.

One apparent contradiction between *F. hybrida* and *L. temulentum* on the one hand and *S. alba* on the other is that flowering of *S. alba* was associated with

Table I. *Effect of prior GA treatment (GA_3 400 ng/plant) on the import of ^{14}C-labeled assimilate into the shoot apex of F. hybrida over a period of 6.75 h*

The leaf was exposed to $^{14}CO_2$ for 10 min beginning 1.5 h after the start of the daily main-light period for untreated control plants or for plants treated 1 or 6 d earlier with GA. Apices from control and GA-treated plants were harvested at the same time. Stem elongation was followed daily and the data shown were from measurements 5 d after the start of GA treatment. Values are means ± SE for $n = 20$.

Day of $^{14}CO_2$ after GA Treatment	Shoot Apex ^{14}C Radioactivity	Stem Length Increment
	dpm mg^{-1} dry wt: % of control	*mm*
Control (no GA)	100 ± 7	17 ± 1
GA plus 1 d	95 ± 7	—
GA plus 6 d	81 ± 7[a]	22 ± 2[a]

[a] Significant difference from control for $P = 0.05$.

increase in apex Suc with LD or displaced SD treatments (Bodson, 1977; Bodson and Outlaw, 1985). However, these responses with *S. alba* require further examination. In particular, high irradiances were generally imposed during the inductive LD or displaced SD treatments, thus making it impossible to separate photosynthetic change in shoot apex sugars from any truly photoperiodic response. In their presentations these authors also claim that floral induction by exposure to a displaced SD should avoid photosynthetic effects. However, as applied to *S. alba*, the near-proximity of the 2 SD introduces a durational photosynthetic effect due to the 16 h of light given over 22 h. In addition, a potential LD photoperiod is imposed so that the treatment might be better described as a displaced LD.

GA Inhibition of Flowering and the Relationship with Suc Supply to the Shoot Apex

Inhibition of flowering by GA has been reported previously for two herbaceous LDP, *F. hybrida* (Sachs and Bretz, 1961) and *Pisum sativum* (Barber et al., 1958; Reid et al., 1977). GA may also inhibit flowering of SD species including *Bougainvillea* "San Diego Red" (Hackett and Sachs, 1967) and *Pharbitis nil* (see King et al., 2000) and it inhibits flowering of woody species including citrus (Monselise and Goldschmidt, 1982). As a corollary, lowering endogenous GA levels may promote flowering of woody species as has now been demonstrated very clearly with one *Eucalyptus nitens* (Moncur and Hasan, 1994).

Mechanistically, our findings of GA inhibition of flowering of *F. hybrida* are most simply interpreted in terms of assimilate diversion, as originally proposed by Sachs and Hackett (1983). Because of the importance of assimilate/Suc input for flowering of *F. hybrida* (see above), competition for assimilate between the shoot apex and the rest of the plant should result from the promotion of stem elongation by GA treatment. This proposal fits with our observation that when flowering was inhibited by GA treatment (Fig. 6), Suc content of the apex was reduced (Figs. 5 and 7) and there was apparently reduced import of assimilate into the shoot apex (Table I). Such inhibition of flowering only occurred if the GA was growth active; simple growth-inactive GA structural variants were inactive for inhibition of flowering (King et al., 2000). Overall, our findings that GA causes a diversion of assimilate/Suc from the apex and inhibits flowering are unique for two reasons. First, we have focused on changes at the shoot apex over the time that enhanced stem growth is first becoming evident (Fig. 7). Second, these changes have been documented during the brief (4–5 d), early period of development when floral initiation is inhibited by GA (Sachs and Bretz, 1961; Fig. 6).

We have yet to explain the early transient (24-h) increase in apex Suc content following GA application to the shoot tip (Fig. 7). However, the increase might result if GA can mobilize assimilate to the apex, but such action is then rapidly masked due to increased stem elongation after 2 to 3 d. In other species a GA-regulated increase in Suc import to the apex may not be so transient. For example, in a red clover (*Trifolium pratense*) mutant requiring GA for its flowering, GA treatment appears to increase apex Suc levels (Jones, 1990).

The contrast between GA action on *F. hybrida* and that of *L. temulentum* is dramatic. For this latter species, GAs or their derivatives promote flowering but may variously promote or inhibit stem elongation, or be growth neutral (Evans et al., 1994). Therefore, it is clear that unlike *F. hybrida*, for flowering of *L. temulentum* there is no GA-induced inhibition of flowering due to growth effects. By default, in species such as *L. temulentum* where GA predominantly promotes flowering, a quite different mechanism of its action must be argued. It is possible that for *L. temulentum* this GA regulation involves activation of the myb transcription factor LtGAMYB (see Gocal et al., 1999).

How Suc regulates flowering of *F. hybrida* is not clear. Where applied Suc can induce flowering as for *S. alba* (Deltour, 1967) or promote it as in *L. temulentum* (McDaniel et al., 1991); then the Suc itself is active. On the other hand, because florigen(s) can be cotransported with assimilate in some species (e.g. *P. nil*, King et al., 1968; *Perilla frutescens*, King and

Zeevaart, 1973) then changes in the import of assimilate will change the delivery of known hormones and florigenic compounds. Similar "cotransport" effects may also result from changes in the sourcing of assimilate where the total supply to the apex remains constant, as shown in studies of labeled assimilate supply to the apex from matched but induced or noninduced cotyledons of *P. nil* (Ogawa and King, 1979). It is clear that further effort is needed to resolve the extent to which applied GA inhibits LD-induced flowering of *F. hybrida* by diversion of assimilate only or also by diverting any cotransported LD florigen.

MATERIALS AND METHODS

Clonally propagated plants of *Fuchsia hybrida* cv Lord Byron were grown in the Canberra phytotron (Morse and Evans, 1962) in 8-cm cylindrical plastic pots in a 1:1 mixture of perlite:vermiculite and irrigated twice daily with nutrient solution in the morning and water in the afternoon. They were grown until they were 10 to 12 cm high in shuttered cabinets under natural light in SD of 10 h at 24°C during the day and 19°C at night. Then they were transferred to artificially lit cabinets at the same temperatures and with 10-h daily illumination at a photosynthetic flux density of 220 to 230 μmol m^{-2} s^{-1} from a combination of metal halide and quartz halide lamps. Plants were kept under these conditions for 3 to 4 weeks. For LD treatment in the artificially lit cabinets the main daylight period was extended for 14 h with low irradiances from incandescent lamps (15 μmol m^{-2} s^{-1}). Experimental treatments were usually started when plants were about 25 cm high. After 4 LD the plants were returned to the regular SD light regime. In one series of experiments the irradiance during the regular 10 h of the SD light period was doubled for 1 week beginning at various times before, during, or after the 4-LD exposure. Three weeks after any florally inductive treatment, flowering was assessed on 14 to 18 plants. Values are presented as the average ± SE of the mean. To simplify presentation, errors are sometimes shown as least significant differences.

GAs (supplied by L.N. Mander, Australian National University, Canberra) were applied in 10% (v/v) ethanol as a 10-μL drop to the shoot tip. The control received 10 μL of 10% (v/v) ethanol. All chemical applications were made 2 to 3 h before the end of a daily light period. The GA treatment preceded the LD exposure unless specified otherwise. At the time of GA treatment and for controls treated with 10% (v/v) ethanol, the pair of leaves about two-thirds fully expanded was marked. Stem elongation subsequently was measured for the young expanding internodes above this leaf pair.

Suc Extraction and Quantitation

Shoot apices, including one or two just-visible primordia, were excised under a dissecting microscope, transferred onto a piece of aluminum foil, laid in planchets, and oven dried (62°C) for at least 24 h. The dry weight of each apex (generally between 2–8 μg) was determined using a Sartorius Supermicro S4 balance (Sartorius GmbH, Goettingen, Germany). After weighing they were transferred to clean tapered glass autosampler inserts. To each sample a fixed amount of ^{13}C-Suc internal standard was added plus 100 μL of 80% (v/v) methanol (HPLC grade + double distilled water). The inserts were capped in tapered 1.5-mL microcentrifuge tubes and shaken overnight on an orbital shaker to extract Suc from the apex. The methanol was then evaporated under vacuum (Speed Vac SC 110, Savant Instruments Inc., Holbrook, NY).

For GC-MS-SIM analysis the Suc was converted to its trimethylsilyl derivative in 10 μL of dry pyridine plus 10 μL of Bis(trimethylsilyl)trifluoroacetamide plus 1% (v/v) trimethyl chlorosilane (Alltech, Sydney). The inserts were sealed tightly with crimp caps, and the mixture left to react at 90°C for 90 min in an oven. For GC-MS-SIM, 1 μL of the extracted, derivatized sugar was injected into a gas chromatograph (5890 Series II, Hewlett-Packard, Palo Alto, CA) coupled to a mass selective detector (HP5971, Hewlett-Packard). The peak area of the plant ^{12}C-Suc was compared with the area of the ^{13}C-Suc internal standard. Based on a calibration curve this ratio was converted to the mass of Suc in the apex per unit dry weight. The ^{12}C-Suc base peak ion of *m/z* 361 (see Gaskin and MacMillan, 1991) was selected for Suc analysis. This *m/z* 361 ion presumably arises from cleavage of the glycosidic linkage of Suc and subsequent loss of a trimethylsilylhydroxy group from the deoxy-Glc fragment. The base peak of the ^{13}C internal standard was at *m/z* 367, which is expected because it was synthesized enzymatically from uniformly labeled ^{13}C-D-Glc (99% + purity) purchased from Cambridge Isotope Laboratories (Andover, MA). Dr. J. Lunn (Commonwealth Scientific and Industrial Research Organization) carried out the enzymic conversions that required the formation of a ^{13}C-Fru moiety from the ^{13}C-Glc prior to synthesis of ^{13}C-Suc.

The GC column was a 25-m × 0.22-mm i.d. × 0.25-μm film thickness fused silica column (BPX-5, SGE, Austin, TX). Conditions were: injector at 250°C; column temperature was held for 1.5 min at 60°C, then programmed to 200°C at 25°C min^{-1} and thereafter to 270°C at 5°C min^{-1}. The MS interface was at 290°C. The helium column carrier gas flow rate was 0.8 mL min^{-1}. The ionization potential was 70 eV.

Maximum sensitivity was not required for the GC-MS-SIM assay of Suc and the separation of ^{12}C and ^{13}C ions by 6 mass units meant that the assay was not influenced by detector tuning. The assays routinely detected as little as 0.1 ng of Suc per injection and the standard curve was linear (r = 0.999) over at least a 40-fold range. One advantage of using an internal standard was that samples once derivatized gave a constant measurement of shoot apex Suc over days when assayed again and also when the same vial was repetitively assayed (up to 10 times).

The content of Suc in an apex that we and others have reported (Bodson, 1977; King and Evans, 1991) can range from 10 to 60 mg g^{-1} dry weight depending on environ-

mental treatments. The minimum amount of ^{12}C-Suc found for the smallest apex was generally 5-fold greater than the lower limit of detection for a 1-μL injection from the 20-μL derivatization mixture. An apex alone weighed about 2 μg dry weight but, due to the difficulty of precise dissection, dry weights of apex plus leaf and stem base tissue ranged at the extreme from 2 to 25 μg. The amount of Suc per "apex" extract (50–500 ng Suc) was linearly related to dry weight (r = 0.81). The concentration of Suc apparently was similar in all "apical" tissues. Further, as shown later, when daily irradiance was increased, the content of Suc in such "apical" tissue was directly related to flowering response and so reflects change in a physiologically relevant pool of Suc.

F. hybrida flowers on axillary meristems so for Suc assay it was important to harvest the appropriate tissue. The relevant tissue in this study was the terminal meristem because all our harvests were prior to visible formation of the relevant axillary meristem.

Distribution of ^{14}C-Labeled Assimilate

The distal halves of just fully expanded leaves were exposed for 10 min in a perspex assimilation chamber to $^{14}CO_2$ generated by adding 50% (v/v) lactic acid to $Ba^{14}CO_3$ (4 mg, 52 Ci mol^{-1}). The irradiance during $^{14}CO_2$ exposure was 220 μmol m^{-2} s^{-1}. There were 20 (Experiment 1) or six (Experiment 2) replicates per treatment with up to 30 leaves fed at any one time. To make allowance for potential differences between leaves fed together, immediately after the 10-min exposure a leaf disc was cut from each leaf and later, at harvest, the area of the fed leaf was measured. The discs were dried, extracted in 80% (v/v) methanol/water and an aliquot counted in a liquid scintillation spectrometer. After 6.75 (Experiment 1) or 8 (Experiment 2) h the apical bud (apex plus a pair of primordial leaves no longer than 200 μm) was excised, dried at 62°C overnight, weighed, sugars extracted in 80% (v/v) methanol for 6 h with shaking, and extract radioactivity determined.

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