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SUMMARY

The opening of gladiolus florets was accompanied by a substantial increase in fresh and dry weight and carbohydrate content of the perianth. The principal soluble carbohydrate was fructose, with substantially lower concentrations of glucose and sucrose. Although stored carbohydrate, apparently starch, disappeared during floret opening, its contribution to the total carbohydrate content of the open flower was minor. Removal of basal florets from the fresh-cut spike substantially reduced the dry weight of the opening upper young buds. During development of the inflorescence it appears that there is a transfer of carbohydrate from the senescing lower florets to those developing acropetally.

In studies of the opening of cut flowers, a diversity of behaviours has been reported. In roses, hydrolysis of petal starch occurs steadily, and the rapid opening of flowers in the early morning appears to be the result of proton extrusion and increased cell wall plasticity (Evans *et al.*, 1988). In contrast, the rapid opening of the ephemeral flowers of daylily (*Hemerocallis* sp.) appears to be driven by decreased osmotic potential resulting from rapid hydrolysis of a fructan in the petal cell (Bieleski *et al.*, 1992; Bieleski, 1993). The vase life and display quality of spike-type flowers like gladiolus is a function both of the life of the earliest-opened florets and of the opening of the many developing buds present in the freshly-harvested spike. Yamane *et al.* (1991) showed that the predominant sugars in the gladiolus perianth were fructose and glucose, and considered that 'starch in florets was the primary source of soluble carbohydrate which contribute to the early stages of flower expansion'. Their data, however, indicate that starch content fell only 2 mg per perianth between stage 1 (bud showing colour) and stage 2 (corolla exerted), while sugar content of the perianth increased by approximately 15 mg. This indicates the possibility that there is some source of carbohydrate other than starch driving the opening of gladiolus florets, for example, the presence of a different storage carbohydrate like the fructan present in daylily flowers. Yamane *et al.* (1991) also demonstrated high activity of acid invertase in opening florets, indicating the possibility that the osmotic changes required to drive cell expansion might be achieved by hydrolysis of sucrose in the buds, or sucrose translocated to the developing bud. This latter mechanism is consistent with the observation that postharvest pulsing with high concentrations of sucrose improves the opening and quality of flower buds on gladiolus spikes (Mayak *et al.*, 1973). In freesia, a closely-related spike-type flower, van Meeteren *et al.* (1995) demonstrated results quite similar to those reported by Yamane *et al.* (1991). The increase in perianth sugars could not be explained by starch

hydrolysis. They found that the sugar content of opening buds was dramatically reduced when stems were reduced in length, suggesting that the stem was a major source of sugars required for flower opening. Yamane *et al.* (1995) demonstrated export of radioactive sugars from wilting florets to younger buds, raising the possibility that dying florets might be a major source of carbohydrate for acropetal opening in spike-type flowers. We report here an evaluation of the source of the carbohydrate increase that accompanies petal expansion in gladiolus florets.

MATERIALS AND METHODS
Plant material

Flowers of gladiolus 'New Rose', 'Sierra Snow' and 'Prima Classe' were obtained from a local grower, transported to the University of California – Davis, and used in experiments within 24 h after harvest. Inflorescences were harvested with one or two open florets. They were immediately placed in water or in a vase solution containing 250 ppm HQC (8-hydroxyquinoline citrate). Cut spikes were divided into two batches: those to be sampled immediately after harvest and those to be sampled after 5 d in vase solution. Spikes put in vase solution for 5 d were placed in a controlled environment maintained at 20°C, 60% relative humidity, and a 12 h day length under cool fluorescent lighting at an intensity of 15 μmol m^{-2} sec^{-1}.

Fresh and dry weight measurements of flower buds per spike were individually sampled starting with the oldest from the bottom (open florets) to the youngest (closed buds). Dry-weight measurements were similarly done on florets from those spikes with three florets removed before being put in the vase solution for 5 d. Samples were replicated five times.

Carbohydrate analysis

Samples from individual flower buds replicated five times were frozen in liquid nitrogen, lyophilized and ground to powder with mortar and pestle. Using 1 g powder of the sample, soluble sugars were extracted for

1 h with 5 ml 80% ethanol in glass tubes held at 80 °C in a water bath. The extract was centrifuged at 3000 **g** and the pellet extracted again with 5 ml 80% ethanol for a further 1 h at 80°C and again centrifuged. The supernatants were pooled and evaporated to near dryness under vacuum and re-dissolved in 3 ml glass distilled water plus 3 mg per sample of adonitol (0.1%) as internal standard. The mixture was filtered through a Biorex-5 slurry column (OH form) and washed with 3 ml of 80% ethanol. The soluble sugar contents in the filtrate were determined and quantified by High Performance Liquid Chromatography (HPLC), using an ion exchange column (BioRad Aminex HPX-87C) and refractive index monitoring of the column effluent. The alcohol-insoluble fraction remaining from ethanolic extraction was used for determination of starch. The pellet was dissolved in 4 ml 0.1N H_2SO_4 plus 1 ml adonitol (internal standard) and heated for 1 h at 100°C in a water bath, then cooled to room temperature. The mixture was centrifuged at 3000 **g** as above and the supernatant filtered through Biorex-5 slurry column and washed with 2 ml glass distilled water. The sugar contents from starch hydrolysis was determined by HPLC as described above.

Statistical analysis

Statistical analysis were performed using PC-Excel software package. The data were subjected to one-way analysis of variance (ANOVA) and means separated by LSD at $P = 5\%$.

RESULTS
Fresh weight of gladiolus florets

The fresh weight of florets on freshly harvested spikes of gladiolus 'New Rose' was highest in the fully open florets at the base of the spike, and decreased towards the tip (Figure 1). After 5 d in the vase, the fresh weight of the bottom (oldest) florets had fallen (with wilting), and that of the middle florets had increased substantially

(with bud opening). The fresh weight of the newly-opened florets was somewhat lower than that of the open florets on the freshly-harvested spikes.

Dry weight of gladiolus florets

The distribution of floret dry weight up the spike changed dramatically during the postharvest period (Figure 2). The maximum dry weight in freshly harvested spikes was in the lower (open) florets; dry weight decreased up the spike. After 5 d in the vase there was a marked change in dry-weight distribution. The dry weight of the lower florets, which were now senescent, had fallen substantially in 'New Rose' and 'Sierra Snow' (Figures 1, 2); the dry weight of the opening florets at position 6 to 13 had increased. The dry weight of the newly opened florets in these cultivars was substantially less than that of the open florets on freshly-harvested spikes. 'Prima Classe' cultivar behaved a little differently (Figure 2). There was little loss of dry weight in the senescing florets, and newly opened florets had a dry-matter content similar to that of open florets on the freshly harvested spike.

Fresh weight/dry weight ratio

The ratio of fresh to dry weight of the florets on a freshly harvested spike of the New Rose cultivar declined slowly and steadily up the spike (Figure 3). After 5 d in the vase, there was a marked change in the ratio. The senescent flowers had a low fresh weight/dry weight ratio, whereas the newly opened florets between positions 6 and 14 had increased the fresh weight/dry weight ratio in the harvested spikes. In the youngest buds measured (buds 17 to 20), the ratio was the same in buds of fresh spikes, and in those that had been in the vase for 5 d (Figure 3).
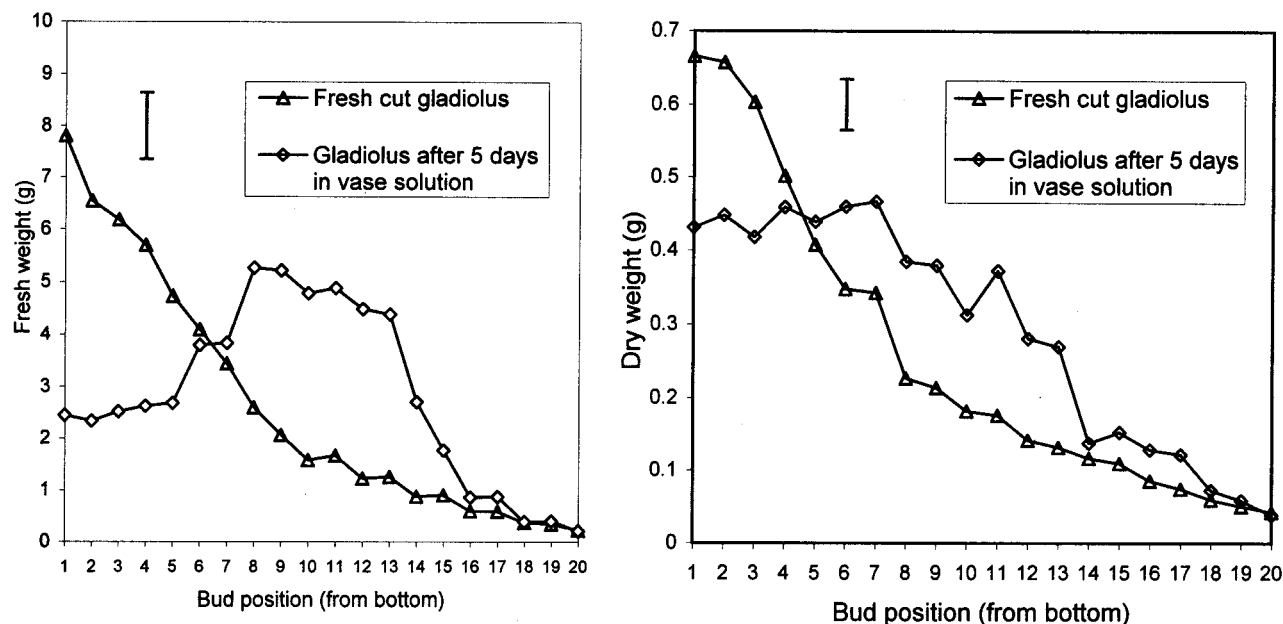

Fig. 1
Fresh and dry weights of florets of gladiolus 'New Rose' from freshly harvested spikes and spikes held in vase solution for 5 d. Vertical bars represent differences between means using LSD ($P = 0.05$).
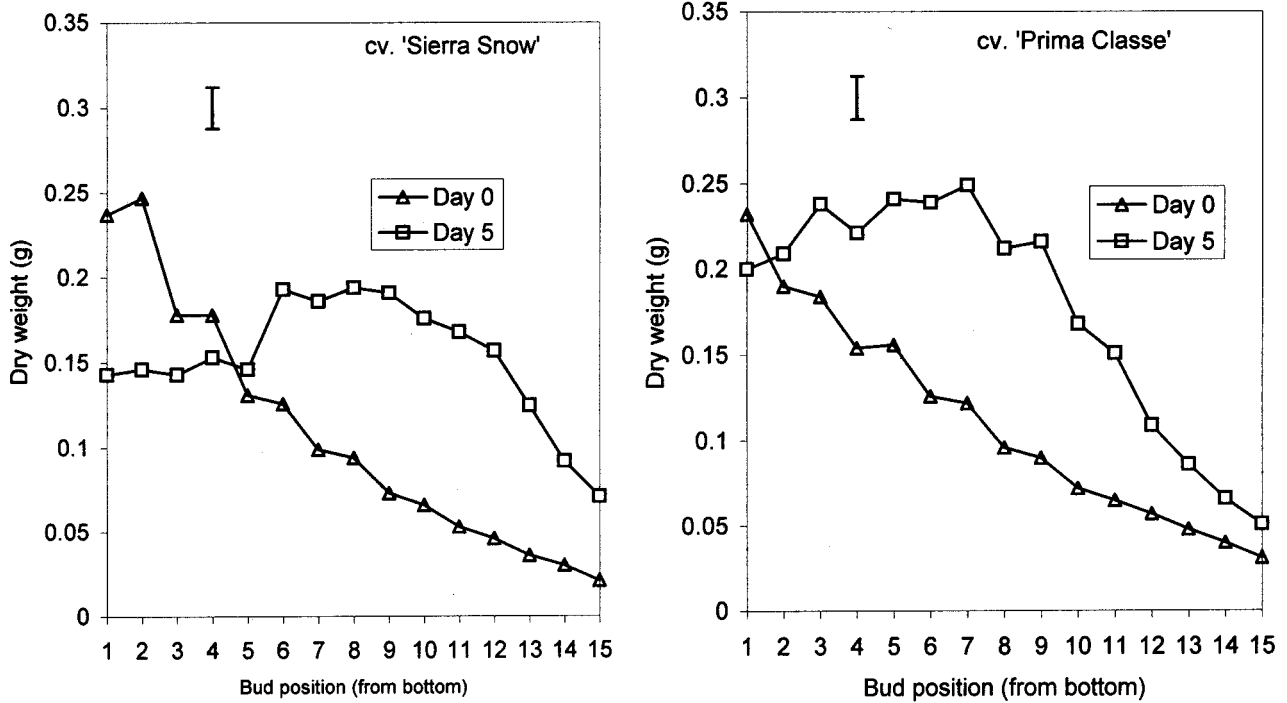
FIG. 2

Dry weights of florets of gladiolus 'Sierra Snow' and 'Prima Classe' from freshly harvested spikes and spikes held in vase solution for 5 d. Vertical bars represent differences between means using LSD ($P = 0.05$).

Sugar content

Florets at all stages of development contained three soluble sugars, primarily fructose, with a smaller quantity of glucose, and a much smaller concentration of sucrose (Figure 4). The sugar content of the florets changed markedly during the postharvest life of the spikes, mirroring the changes in flower fresh and dry weights. In freshly harvested spikes, sugars were high in the basal florets, and fell in the younger florets (Figure 4a). After 5 d in the vase solution (250 ppm HQC), the sugar content of the older (senescent) florets had fallen dramatically (Figure 4b) whereas that of the newly open florets had increased markedly, even in the youngest florets measured. The total sugar content of open flowers on the freshly harvested spikes was about half that of open florets on spikes that had been in the vase for 5 d.

Storage carbohydrate

Florets and buds of gladiolus spikes contained only small amounts of acid-labile alcohol-insoluble carbohydrate (Figure 5). The hydrolysis products of the storage carbohydrate were largely fructose with variable proportions of glucose. Open florets and mature buds on freshly harvested spikes of gladiolus contained small amounts of storage carbohydrate (Figure 5a), and the upper young florets contained even less. In the senescent florets of spikes held in vase solution containing 250 ppm HQC for 5 d, the storage carbohydrate content had fallen somewhat (Figure 5b). In contrast, it had risen markedly in the younger florets on the spike, where glucose formed a larger proportion of the acid hydrolysis products.

Effects of floret removal

Removal of the open and opening lower florets on the spike at harvest markedly changed the dry-matter content of the newly opening florets (Figure 6). After 5 d in a 250 ppm HQC vase solution, the dry matter in buds of control spikes had fallen in the senescent florets, and risen substantially in the opening florets. The dry-matter content of opening florets on the spikes where three open basal florets had been removed at the start of the experiment was consistently significantly less than that of those on control spikes (Figure 6).

DISCUSSION

This study clearly demonstrates that the opening of gladiolus florets requires the import of carbohydrate. During opening, the fresh and dry weight of the
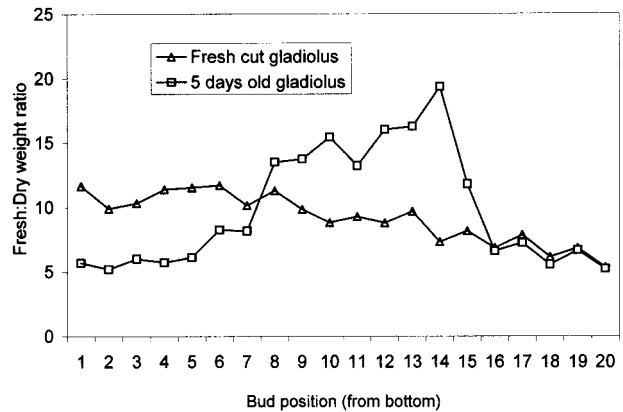

FIG. 3

Fresh to dry weight ratios of florets of gladiolus 'New Rose' from freshly harvested spikes and spikes held in vase solution for 5 d.
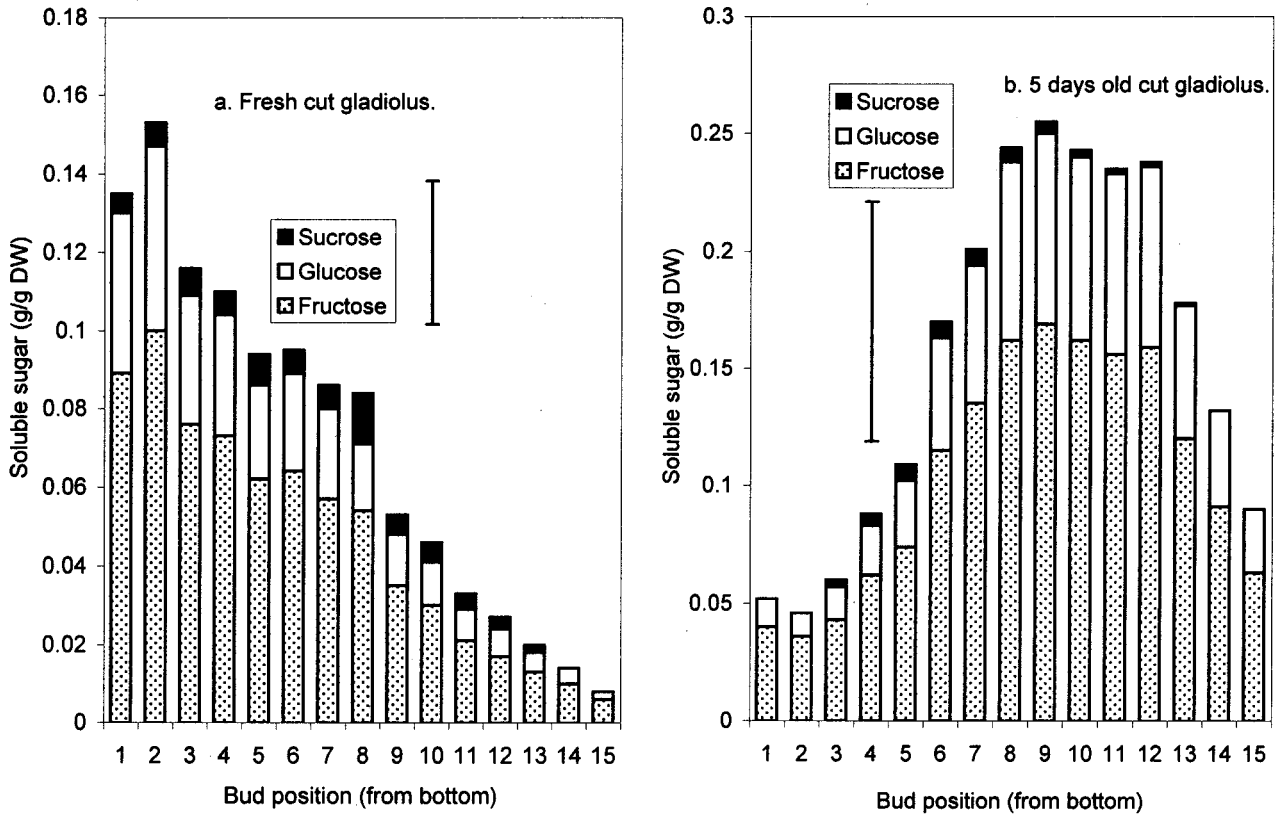
Fig. 4
Soluble sugars in florets of gladiolus 'Sierra Snow' from freshly harvested spikes and spikes held in vase solution for 5 d. Vertical bars represent differences between means using LSD ($P = 0.05$).

developing florets increased substantially (Figures 1, 2) presumably reflecting cell expansion, and the necessary manufacture of new cell walls and other components of the expanding cells. Studies in other flowers have suggested that the osmotically-driven expansion of petal cells may be due to hydrolysis of storage carbohydrate in the cell as in rose (Evans and Reid, 1988) and daylily (Bieleski, 1993) or import of sugars as

in morning glory (Hanson and Kende, 1975). In gladiolus, Yamane et al. (1991) proposed that petal starch was the primary source of the sugars required for early petal expansion in gladiolus. We were unable to demonstrate substantial starch reserves in gladiolus buds, whether by enzymatic digestion of alcohol-insoluble residues (data not shown), or by acid hydro-lysis and HPLC analysis of the hydrolysed sugars. At
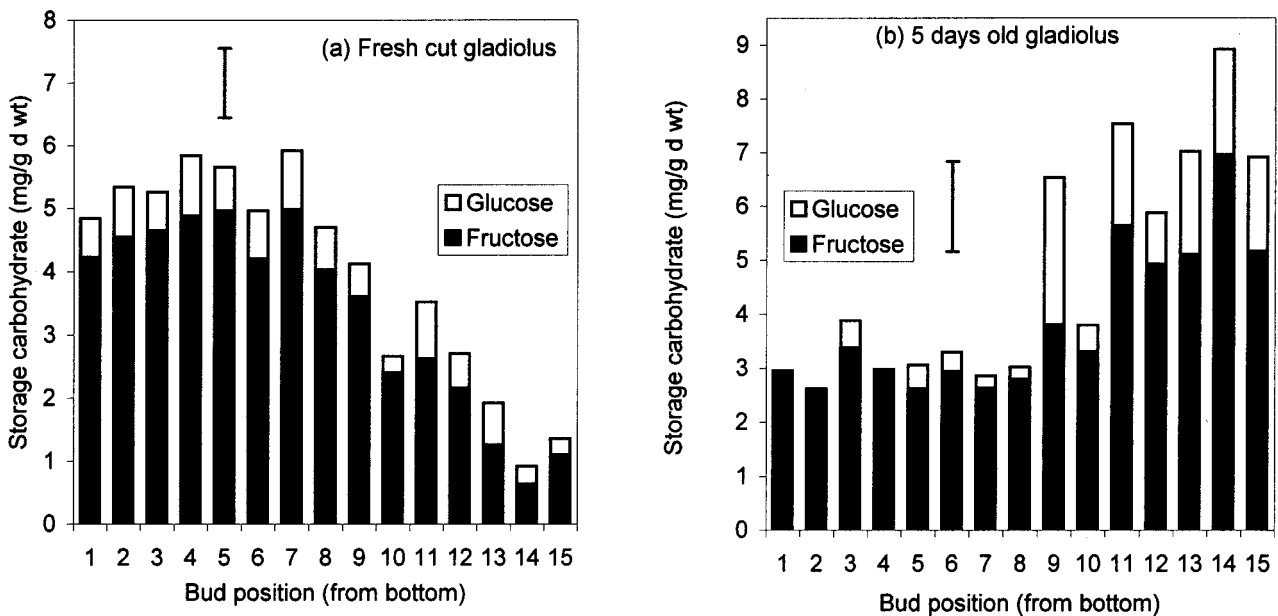

Fig. 5
Acid-labile alcohol-insoluble storage carbohydrate in florets of gladiolus 'Sierra Snow' from freshly harvested spikes and spikes held in vase solution for 5 d. Vertical bars represent differences between means using LSD ($P = 0.05$).
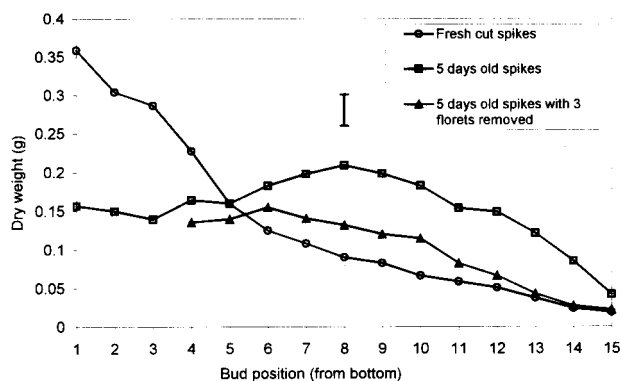
FIG. 6
Effects of floret removal on dry weight changes of florets of gladiolus 'Sierra Snow' from freshly harvested spikes; 5 d-old spikes held in vase solution and 5 d-old spikes with three basal florets removed at the start of experiment and then held in vase solution. Vertical bar represents differences between means using LSD ($P = 0.05$).

most, we found less than 0.5% of the dry weight of the buds to be an alcohol-insoluble, acid-labile carbohydrate (Figure 5) whose hydrolysis products (mostly fructose and some glucose) suggested a fructan, similar to the storage carbohydrate identified in daylily (Bieleski, 1993).

In the daylily, it was observed that the sugars and other components of the flowers were coordinately exported from the senescing corolla, thereby reducing the energetic cost of a large ephemeral flower (Lay-Yee *et al.*, 1992). A similar pattern of export was demonstrated in gladiolus by Yamane *et al.* (1995). It seems that the sugars needed for expansion of developing gladiolus buds are largely supplied by similar remobilization from senescing older florets. The reduced dry weight of the old florets (Figure 2) is accompanied by a dramatic reduction in their sugar content (Figure 4). The change in the fresh weight/dry weight ratios during 5 d in the vase (Figure 3) also indicates the changed allocation of dry matter. Interestingly, this ratio is higher in open florets on spikes held for 5 d in the vase than in open florets on fresh spikes. Presumably, this reflects reduced

expansion of petal cells in newly opening florets, perhaps as a result of water stress in the harvested spikes. Not only were these florets smaller (lower fresh and dry weights), but they also had a higher proportion of their dry matter as sugar, perhaps reflecting a greater osmotic requirement for opening.

When the bottom three florets on the spike were removed, the opening and dry weight of the younger florets were reduced (Figure 6). The total loss of carbohydrate in the three first florets on the intact spike was close to the difference between the total increase in floret dry matter in florets of control spikes and that on spikes where florets had been removed.

The small total dry-weight gain of florets on spikes where lower florets had been removed indicates that opening of gladiolus florets is largely dependent on sugars translocated from the lower and older florets to the younger ones. It is probable that stored carbohydrate in the relatively massive stem tissue can also contribute some carbohydrate to the developing buds. The ability of the stem to store carbohydrate may explain the substantial improvement in floret opening that is achieved in gladiolus by pulsing the newly harvested spikes with high concentrations of sucrose (Marousky, 1971; Bravdo *et al.*, 1974). Differences in stem carbohydrate supply may also explain the differences in dry-matter distribution in open and senescing flowers of 'Prima Classe' from that of the other cultivars studied.

Some authors have proposed the removal of the small young buds of compound inflorescences to remove putative 'sinks' for carbohydrate (Wang and Breen, 1986; van der Meulen-Muisers *et al.*, 1995). Our data show that there is little change in dry matter or fresh weight/dry weight ratio of the uppermost buds (positions 15 to 20) during the first 5 d in the vase (Figures 2, 3). It therefore seems unlikely that removing the terminal buds would improve the opening and size of florets lower on the spike.

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