EFFECT OF ACCEL, SUCROSE AND SILVER THIOSULPHATE ON SUBSTRATE UTILISATION IN CUT TUBEROSE (*POLIANTHES TUBEROSA* L.) FLOWERS

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ABSTRACT

This study was undertaken to investigate the effect of Accel™, sucrose and silver thiosulphate (STS) on the dry weight, accumulation of sucrose and reducing sugars in cut tuberose (*Polianthes tuberosa* L) petals at various positions along the spike. Cut stems of tuberose were held in optimum treatments that prolonged their vase life (Hutchinson *et al*., 2003): continuous holding in 25 mg/L BA equivalent of Accel; pulsing in 20% sucrose for 24 hrs and subsequently holding in either deionised water (DIW) or in 25 mg/L BA; pulsing in 2 mM STS for 1 hr and subsequent holding in DIW. The middle and bottom florets of cut flowers held in DIW were heavier than the top florets. Pulsing tuberose cut flowers in sucrose or in STS improved the dry weights of the middle and bottom florets in the first 3 days, but up to 6 days of top florets. Florets of cut flowers pulsed in sucrose and subsequently held in Accel were heavier than those subsequently held in DIW or those held continuously in Accel. Sucrose, STS and Accel increased floret opening but had varied influence on the accumulation of sucrose and reducing sugars in petals of florets along the spike. Cut tuberose stems pulsed in sucrose and subsequently held in either DIW or 25 mg/L BA equivalent of Accel accumulated the largest amounts of sucrose and reducing sugars. Pulsing cut tuberose flowers in 10% sucrose and subsequently holding them in Accel or DIW or pulsing in STS, while having no influence on sucrose levels in bottom florets, significantly increased levels in top florets for the first 3 days before a sharp decline in petals pulsed in sucrose. The main difference was that while most of the sucrose accumulated in the middle florets, reducing sugars was concentrated on the bottom florets along the spike. Unexpectedly, pulsing stems in STS or holding them in Accel had no significant influence on levels of sucrose or reducing sugars within the 9 days of testing even though most florets had opened by this time. The results of the present study suggest that while sucrose had a direct influence on accumulating of sucrose and reducing sugars in florets, Accel and STS improved vase life and floret opening in cut tuberose stems either indirectly through substrate mobilisation and increased metabolism, or may have played another different role other than substrate mobilisation.

Key words: Accel, sucrose, silver, thiosulphate, tuberose

1.0 INTRODUCTION

Although tuberose has gained importance in Kenya as an export cut flower crop, incomplete floret opening along the spikes, premature abscission and/or abortion of

the distal florets and short vase-life of the remaining florets continue to hamper expansion of its production (Watako, 1992; HCDA, 1998). Substrate limitation during post-harvest period has been cited as one of the principal reasons for incomplete floret opening and short vase life of flowers (Halevy and Mayak, 1981). Inadequate levels of sucrose and/or reducing sugars increase competition for substrates especially in spike-type inflorescence with mature proximal florets (nearest the assimilate source) accumulating large quantities at the expense of the immature distal florets. Indeed, supplemental sucrose application increased the floret opening and vase life of spike flowers such as Brodiae (Han *et al*., 1990), Freesia (Woodson, 1987), Limonium (Doi and Reid, 1995) and Tuberose (Naidu and Reid, 1989). The mode of action of applied sugars in delaying senescence of cut stems has not been clearly outlined (Halevy and Mayak, 1981). However, absorbed sucrose is reportedly converted rapidly in petals to reducing sugars, which accumulate in the corolla of Carnation (Nichols, 1973; Acock and Nichols, 1979) and Rose (Paulin and Muloway, 1979) cut flowers.

Several studies have also indicated that plant growth regulators could be involved in the assimilation of the substrates present in the cut flowers during the post harvest stages of development. Ethylene blocked the transfer of assimilates to the flower buds of several cut flowers (Mayak, 1987). Ethylene also promoted the translocation of assimilates from the petals to the ovary of glasshouse Carnation, leading to the loss of petals and the increase of the ovary's fresh and dry weights (Nichols and Ho, 1975). Indeed, ethylene antagonists such as silver thiosulphate (STS), increased the floret opening and vase life of *Lilium longiflorum* 'Enchantment' (Swart, 1986), Gypsophila (Downs *et al.*, 1988), *Alstroemeria* (Chepkairor and Waithaka, 1988) and tuberose (Watako, 1992). Cytokinins, on the other hand, improved the sink activity of the flower buds (Mayak, 1987), delayed the reduction in the dry weights (Mayak and Halevy, 1974) and counteracted the ethylene effects on senescence of rose, Lily-of-the-Nile flowers and other ornamental crops (Jeffcoat, 1977; Mor *et al.,* 1984). In our previous work, we have shown that Accel improved the vase life and post-harvest life of *Alstroemeria* (Mutui *et al.*, 2001) and tuberose (Hutchinson *et al*., 2003).

The main purpose of the present study was, therefore, to investigate the amounts of sucrose and reducing sugars accumulating along the floral spikes and the accompanying dry weights of cut tuberose stems in the presence or absence of exogenously applied sucrose and in the presence of Accel and STS.

2.0 LITERATURE REVIEW

The vase life and display quality of spike-type flowers like tuberose is a function of both the life of the earliest opened florets and of the opening of the many developing

buds present in the freshly-harvested spike. The opening of flower buds is an energy-consuming process and incomplete flower opening and short vase life have been attributed to insufficient nutrient availability (Halevy and Mayak, 1981). Yamane *et al*. (1991; 1995) showed that the predominant sugars in the perianth of gladiolus, a

spike-type flower, were fructose and sucrose. Sucrose is normally included in most preservative formulations, but other metabolic sugars like glucose and fructose are similarly effective. Sucrose has been shown to promote opening and delay of senescence of gladiolus spikes (Mayak *et al*., 1973; Waithaka *et al*., 2001), freesia, a closely related spike type flower (van Meeteren *et al*., 1995), Rose (Mayak and Halevy, 1974), Carnation (Bravdo *et al*., 1974), *Anthurium* (Paull and Goo, 1982), Lily-of-the-Nile (Mor *et al*., 1984), and Gypsophila (Downs *et al.,* 1988) cut stems. However, in *Convallaria* (Aarts, 1957), Daffodil (Nichols, 1975) and *Oncidium* orchids (Yong and Ong, 1979), exogenously applied sugar had little or no benefit and was even sometimes damaging. Controversial or inconsistent results were also obtained with Tulips (Aarts, 1957), Carnation (Systema, 1981) and Cyclamen (Aarts, 1957; Kohl, 1975). In Narcissus (Nichols, 1975) and *Alstroemeria* (Chepkairor and Waithaka, 1988), applied sugar was utilised for ovary growth. Because of these inconsistencies, there is need to carry out empirical studies for each flower crop to determine the levels of sucrose and reducing sugars in relation to post harvest physiology.

Several explanations have been put forward to delineate the benefits of increased flower opening and vase-life in the presence of sugars. Sugars are primarily respiration substrates that are translocated from the leaves to the petals of the flower (Nichols, 1976). But in Narcissus and *Alstroemeria*, flower opening and short vase-life was attributed to a rapid depletion of the available substrates and or translocation of the substrates to other stronger sinks such as the ovary (Nichols, 1976; Chepkairor and Waithaka, 1988).

The rate of substrate assimilation and/or translocation is thought to be under the control of plant hormones. Ethylene blocked the transfer of assimilates to the flower buds (Mayak, 1987) and promoted the translocation of assimilates from the petals to the ovary leading to a loss of the petals' fresh and dry weights in Carnations (Nichols and Ho, 1975). Indeed ethylene antagonists such as STS increased the floret opening and vase life of *Lilium longiflorum* (Swart, 1986), Gypsophila (Downs *et al.,* 1988), and *Alstroemeria* (Chepkairor and Waithaka, 1988).

Cytokinins, on the other hand, improved the sink activity of Rose flower buds (Mayak and Halevy, 1970; Mayak, 1987). Cytokinins also increased respiration (Shirakawa *et al*., 1964), delayed the reduction in dry weights (Mayak and Halevy, 1974), and counteracted ethylene effect of promoting cut flower senescence of rose cut flowers (Jeffcoat, 1977; Mor *et al*., 1984). To our knowledge, the influence of Accel, sucrose and STS on the distribution of sucrose and reducing sugars in Tuberose (*Polianthes tuberosa* L.) cut flowers has not been reported. The overall objective of the present study was therefore to investigate the effects of Accel, sucrose and STS on dry weights and levels of sucrose and reducing sugars in Tuberose cut flowers.

3.0 MATERIALS AND METHODS

The tuberose cut flowers used were obtained from Cianda Flowers, a commercial flower farm in Kiambu District, which is situated at 2300 m above sea level and around 10° South of the Equator. Tuberose inflorescences were harvested at the commercial stage of harvest with one floret open and brought to the laboratory within 2 hours. In the laboratory the flowers were re-cut under water to 60 cm in length and the lower 10 cm portion of the stem defoliated. The treatments were evaluated under cool white fluorescent lamps (4160 J/sec) at temperature of 23 ± 1^0C and RH 70±10%.

The optimum treatments that previously prolonged vase life and improved water relations of tuberose cut flower stems (Hutchinson *et al*., 2003) were used in this experiment for the determination of dry weights and levels of sucrose and reducing sugars. The treatments were as follows:

1. BA: Ten cut flowers were placed directly in 25 mg/L BA equivalent of Accel™ (Abbott Laboratories, North Chicago, USA), the optimum concentration for prolonged vase life (Hutchinson *et al*., 2003). Accel is a liquid concentrate containing 20 g a.i / L Benzyl aminopurine (BA) and 2.0 g a.i / L GA_{4+7} (Abbott Laboratories, North Chicago, USA).
2. Suc: Ten cut flower stems were first pulsed in 10% sucrose for 24 hrs before transfer to DIW holding solution.
3. SucBA: Ten cut flower stems were first pulsed in 10% sucrose for 24 hrs before transfer to holding solutions containing 25 mg/L BA equivalent of Accel at the same concentrations of BA equivalent.
4. STS: Ten cut flower stems were pulsed for 1 hr in 2.0 mM STS anionic complex and then transferred in DIW. Silver thiosulphate complex was prepared according to Gorin *et al.* (1985).
5. De-ionised water (DIW) was used as control treatment.

3.1 Sugar Determinations

Five grams of petal tissue from the lowest and the middle florets and 2.5 grams of the petal tissue from the topmost florets/buds were extracted and weighed after 3, 6, 9 and 12 days in vase solution. The samples were kept on ice during all stages of extraction. After weighing, the samples were homogenised twice with 5 ml of distilled water to ensure maximum recovery of soluble sugars. The samples were the centrifuged at 10,000 rpm for 15 minutes, filtered and the filtrate used for sugar determinations.

3.1.1 Sucrose

Sucrose was determined calorimetrically using the anthrone test (Harper *et al.*, 1979). Anthrone test solution was prepared by dissolving 1 gram of anthrone in 1 litre of 78% sulphuric acid. Sample extracts were diluted in water in the ratio 1:300, and from this dilution, 0.5 ml was used as test samples. 0.5 ml of standards at 0, 0.02, 0.04, 0.06, 0.08 and 0.1mg/ml sucrose concentrations were prepared. To both the test samples and standard, 0.5 ml of 30% potassium hydroxide was added and the solutions were heated to boiling point for 10 minutes. Five mls of the anthrone

test solution test solution was then added and the resulting solution mixed well using a vortex stirrer. At this point, the solutions produced a bright green colour and after cooling to room temperature, the optical density was determined at 620 nm wavelength. The amount of sucrose in the test samples was then estimated by the use of a standard curve and expressed as mg sucrose per unit weight of flower petal tissue.

3.1.2 Reducing Sugars

The amounts of reducing sugar were determined calorimetrically following procedures developed by Sumner (1921) (cited by Amuyunzu, 1994). This involved a colour reagent prepared by dissolving 20 grams of potassium sodium tartrate-4- hydrate and 0.5 grams of 3, 5- dinitrosalicyclic acid in 10 ml of 2N sodium hydroxide and 110 mL of distilled water to make a total volume of 120 mls. The colour reagent was kept in the dark at all times.

The standards consisted of glucose solutions of concentrations 0, 0.33, 0.67, 1.00, 1.33, 1.67 and 2.00 mg/ml. The test sample was diluted in distilled water in the ratio 1:25. This was followed by adding 2.4 ml of the coloured reagent to test tubes containing 0.6 ml of both the standard and test samples and then heating to boiling point for 5 minutes. At this point the solutions produced an orange colour. After the solutions had cooled to room temperature, 3 ml distilled water was added. The optical density was then measured in a spectrophotometer at 550 nm and by use of a standard curve the amount of reducing sugars was determined and expressed as mg of reducing sugar per unit weight of petal tissue.

3.2 Experimental Design and Data Analysis

Experiments were arranged in a completely randomised design (CRD; Steel and Torrie, 1980). Treatments were replicated 4 times and experiments repeated once. The results presented are an average from the two experiments. Determinations of dry weights, sucrose and reducing sugars of petals were carried out at 0, 3, 6, 9 and 12 days after harvest. Analysis of variance was by the General Linear Model Procedure of SAS (SAS Institute Inc. 1995). Means were compared using Tukey's method at 5% level of probability.

4.0 RESULTS AND DISCUSSION

Tuberose cut flowers bear a large number of florets, which develop sequentially from the proximal to the distal end of the inflorescence. The main challenge to expansion of production of tuberose has been the short vase life characterised by incomplete floret opening along the spike, premature abscission and/or abortion and the short vase life of the remaining florets. The tuberose cut flowers used in the present study were harvested when the lowest floret opened. Tuberose cut stems held in DIW had a vase life of 13 days with an average 63% of the florets along the spike opening (Hutchinson *et al*., 2003). Most of the florets failed to open at this stage of development. Incomplete floret opening and short vase life, especially in spike-type inflorescences, has been attributed to possible substrate limitation (Woodson, 1987; Naidu and Reid, 1989; Han *et al*., 1990; Doi and Reid, 1995). Placing the cut stems in either a 10% sucrose or STS pulse or holding solution of

Accel, significantly improved vase life and floret opening in Tuberose (Hutchinson *et al.*, 2003). In addition, sucrose, STS and Accel hastened the opening of the middle florets to between 3-6 days and the topmost florets to around the 9th day.

The addition of Accel, sucrose and STS to the vase solution improved the dry weights of the florets up to 3 days in vase solution (6 days in topmost florets) after which there was a decline (Figure 1). In all treatments, the lowest florets had higher dry weights than their middle and topmost counterparts, respectively. Depending on the position of the florets along the spike, dry weights decreased over time or increased during the first 3-6 days of their vase lives before decreasing. Pulsing tuberose cut flowers in sucrose or in STS improved the dry weights of the middle and bottom florets in the first 3 days but up to 6 days of top florets. Of the treatments tested, a combined sucrose pulse with a BA holding solution, was superior in the increase in dry weight. Overall, improved dry weights for florets suggest an increase of substrate accumulation in florets in the first few days in vase solution.

Sucrose has been reported to influence vase life of various cut flowers by acting as a respiratory substrate, improving water balance and/or osmotic potential of carnation flowers (Halevy and Mayak, 1981). In previous studies, sucrose improved the vase life of Gypsophila (Farnharm, 1975), tuberose (Naidu and Reid 1989; Watako 1992; Hutchinson *et al*., 2003), and hybrid *Limonium* (Doi and Reid, 1995). Usually, substrate limitations increase competition for substrates by florets along the spike, so that mature, proximal florets accumulate the highest amounts of sugars at the expense of the immature distal (topmost) florets (Yamane *et al*., 1995). Pulsing tuberose cut flowers in 10% sucrose with subsequent holding in DIW or BA in the form of Accel resulted in the highest accumulation of sucrose (Figure 2). Indeed, it has been reported that supplemental sucrose application increased floret opening and vase life of spike flowers such as *Broadiae* (Han *et al*., 1990), Freesia (Woodson, 1987), *Limonium* (Doi and Reid, 1995 and tuberose (Naidu and Reid, 1989). In the present study, the middle florets accumulated highest amounts of sucrose followed by the topmost florets while the bottom florets or buds accumulated the lowest (Figure 2), ranging from around 1 to 8-14 and 4-6 mg/g, respectively. The lower than expected levels of sucrose in the bottom florets suggest its rapid conversion to reducing sugars to facilitate floret opening at this position. The extra sucrose could have been translocated to successive florets once the lowest have accumulated adequate amounts necessary for opening, resulting in higher levels in middle and very little in bottom florets at the time of determination (Figure 2). The maintenance of the pool of dry matter and respiration substrates (Rogers, 1973) in the petals is crucial for delayed senescence.

Flower petal tissues contain high invertase activity (Hawker *et al*., 1976) and absorbed sucrose is reportedly converted rapidly to reducing sugars (Acock and Nichols, 1979; Paulin and Muloway, 1979; Stommel and Simon, 1990; Waithaka *et al*., 2001; Yamane *et al*., 1991; 1995). In all treatments, the bottom florets accumulated highest levels of reducing sugars followed by the middle and topmost florets (Figure 3). Cut stems pulsed in 10% sucrose and held in either DIW or Accel, showed a concentration of reducing sugars in the lowest florets

early in the vase life but by day 6, most of the reducing sugars were concentrated at the middle florets (Figure 3). These differences were clear on day 3 of their vase life (Figure 3). Exogenous sugar treatments have been reported to delay the onset of autocatalytic ethylene production (Dilley and Carpenter, 1975) and flowers supplemented with sucrose senescenced less rapidly when treated with ethylene than untreated flowers (Mayak and Dilley, 1976). This sucrose effect on ethylene production probably explains why pulsed tuberose cut flowers had a lower rate of depletion of substrates and consequently longer vase life. What was not clear from the present study was the accumulation of reducing sugars in the topmost florets of DIW-held cut flowers (Figure 3), yet these florets did not open until around day 12 (Hutchinson *et al*., 2003).

The role of ethylene and cytokinins in substrate utilisation and general post harvest physiology of tuberose cut stems was not clear from the present study. Ethylene has been reported to either block transfer of assimilates to flower buds of several cut flowers (Mayak, 1987), or promote translocation of the same from the petals to the ovary, leading to loss of petals and an increase of fresh and dry weights of ovaries (Nichols and Ho, 1975). Silver thiosulphate improved vase life and floret opening of cut Tuberose stems (Hutchinson *et al*., 2003), *Lilium longiflorum* 'Enchantment' (Swart, 1986), Gypsophila (Downs *et al*., 1988) and *Alstroemeria* (Chepkairor and Waithaka, 1988). However, cut flowers pulsed in STS and subsequently held in DIW and those held in Accel had highest dry weights but contained the least amounts of reducing sugars (Figure 3). Silver thiosulphate could, therefore, be acting as a strong biocide, improving water balance and improved vase life and not necessary via ethylene activity. The findings of the present study differ from those obtained in Carnations (Paulin and Muloway, 1979), *Luecospermum* (Napier *et al*., 1986) and Roses (Mayak and Halevy, 1974) where BA-treated flowers slowed down rate of depletion of assimilates. The BA effect
was thought to result from strengthening of the sink activity of the petals, as was observed in Iris (De Munk and Gijzenberg, 1977) and Roses (Halevy, 1987).

The limitations of substrates may not totally explain the short vase life and other post harvest problems in flowers. At petal wilting in Carnations (Nichols, 1973), Roses (Ho and Nichols, 1977) and Narcissus (Nichols, 1973), a substantial amount of reducing sugar remained while sucrose almost disappeared. In addition to the depletion of assimilates from the petals through respiration, a reduction in the amount of assimilates from the petals to the ovary was demonstrated in senescing Orchids (Hsiang, 1951) and Carnations (Nichols, 1976; Nichols and Ho, 1975). The translocation, especially in Carnation flowers, was promoted by pollination and ethylene (Nichols and Ho, 1975). Another possibility is that STS and BA could have reduced respiration rate of the flowers thus enabling respiration to proceed for a longer period of time resulting in a longer vase life. Indeed, the respiration rate of Carnations was reduced by a BA treatment through a reduction in glycolysis (Paulin and Muloway, 1979). Likewise, BA treatment reduced the respiration rate of *Anthurium* (Shirakawa *et al*., 1964), Carnations and Chrysanthemums (Maclean and Dedolph, 1962) and Daffodils (Ballantyne, 1966).

In conclusion, the results of the present study indicate that addition of sucrose to tuberose pulse solution improved vase life and floret opening through improved substrate mobilisation and utilisation along the spike. Silver thiosulphate and Accel did not directly influence the substrate levels in florets along the spike over the tested period. The possibility of an indirect or delayed influence, however, cannot be ruled out.

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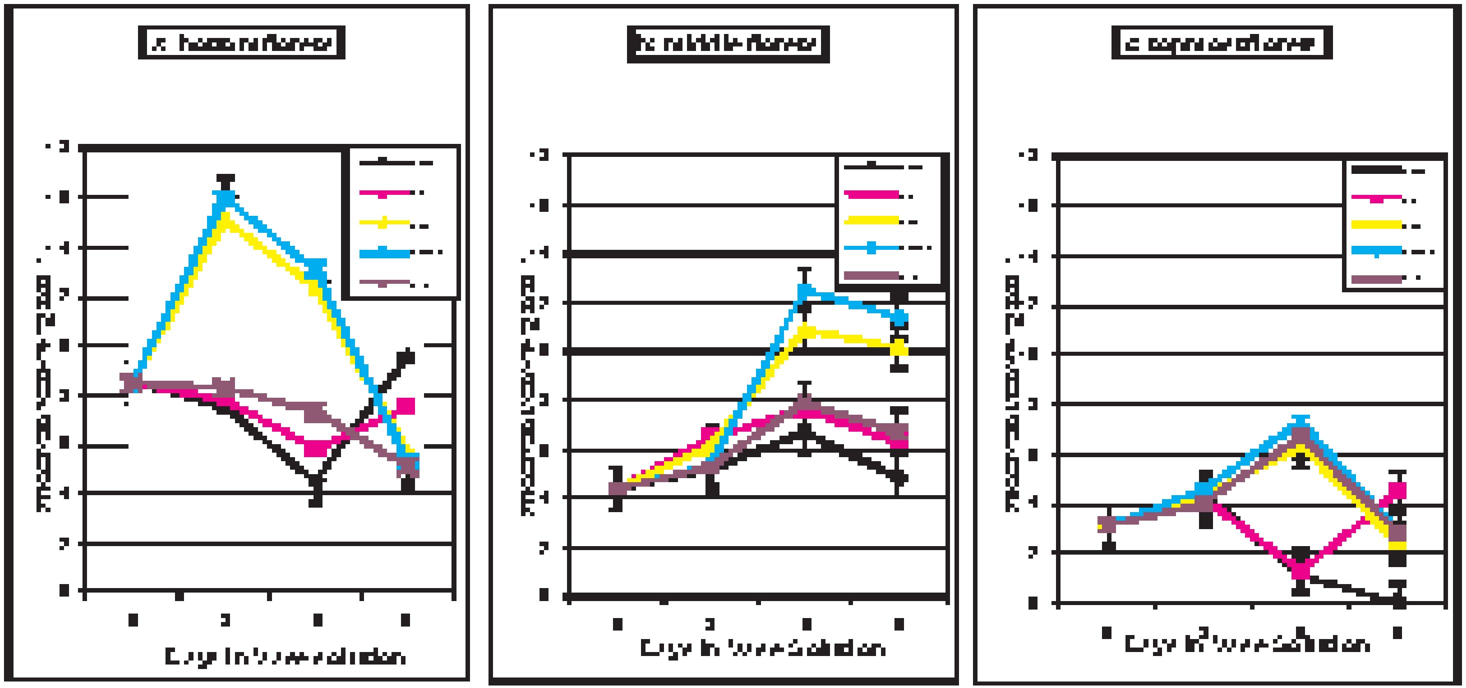

Figure 5: Effect of Accel, Sucrose and Silver thiosulphate (STS) on reducing sugars of cut Tuberose (Polianthes tuberosa L.) florets

Key: DIW = deionised water; BA = 25 mg/L BA equivalent of Accel; Suc = 24 hr pulse treatment in 20% sucrose with subsequent holding in DIW; Suc BA = 24 hr pulse treatment in 20% sucrose with subsequent holding in 25 mg/L BA; STS = pulsing for 1 hr in 2 mM STS with subsequent holding in DIW

Vertical bars indicate standard errors

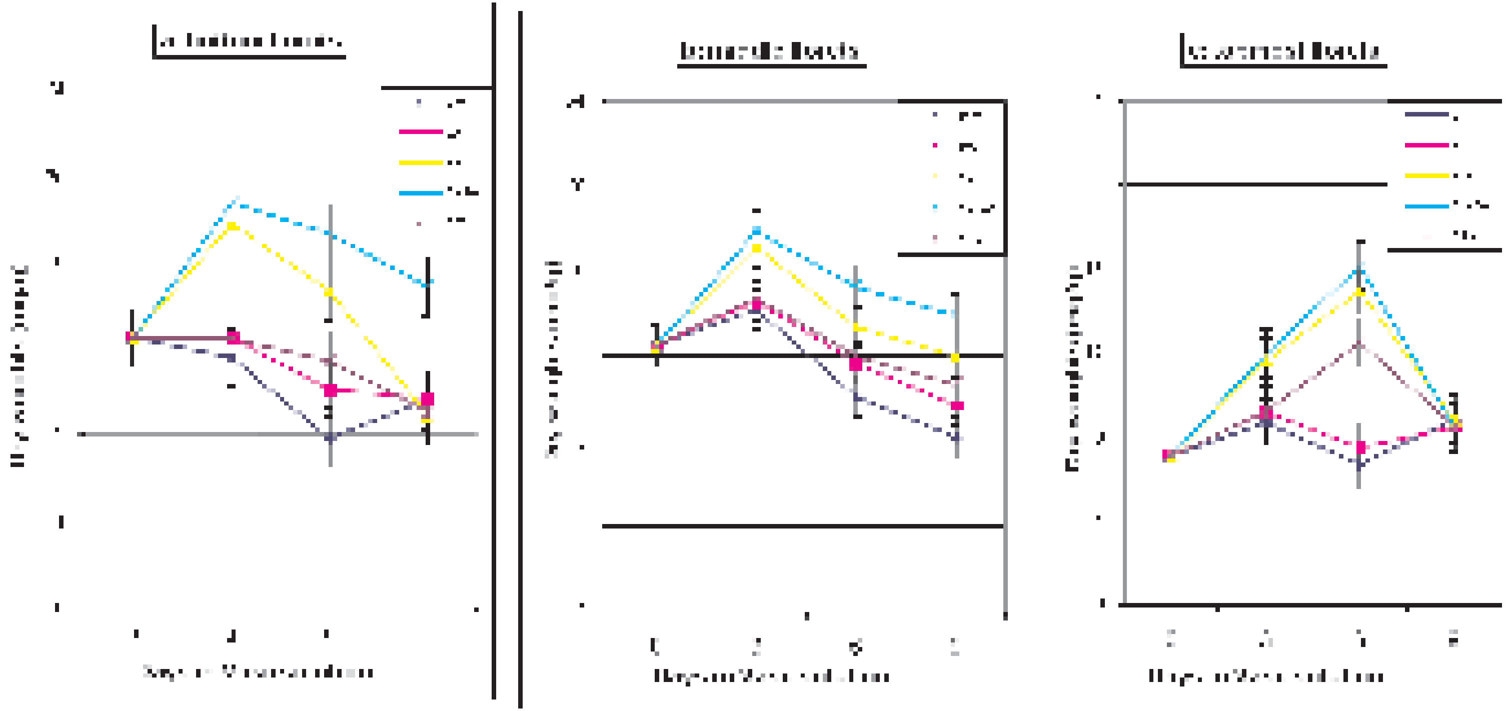

Figure [illegible] : Effect of Accel, Sucrose and Silver thiosulphate (STS) on dry weight of cut Tuberose (Polianthes tuberosa L.) florets
Key: DW = deionised water; BA = 25 mg/L BA equivalent of Accel; Suc = 24 hr pulse treatment in 20% sucrose with subsequent holding in DW; Suc BA = 24 hr pulse treatment in 20% sucrose with subsequent holding in 25 mg/L BA;
STS = pulsing for 1 hr in 2 mM STS with subsequent holding in DW
y-error bars indicate standard errors