CHAPTER 4
CHANGES IN STARCH AND SUGAR STATUS OF POLLINATED *DENDROBIUM* POMPADOUR

4.1 INTRODUCTION

As was established in Chapter 3, pollination is a process that accelerates senescence and the physiological processes that senescence entails. The post pollination symptoms that occur are all events that are necessary to ensure the progression of successful ovary development. This is obviously an energy demanding process which occurs at the expense of carbohydrates present in the flower (Nichols, 1971). In fact some studies have elucidated that one of the factors determining longevity of pollinated flowers may be the status of carbohydrate reserves in the flower (Endo and Ikusima, 1992).

In order to fulfil the energy demands of the developing ovary, the recycling of nutrients and energy reserves is necessary. The broken down products of lipids and proteins serve as an alternative energy (Zhou *et al.,* 2005). Metabolic alterations have been linked to pollination induced senescence, where breakdown of complex compounds such as starch into simpler compounds appear to be the response in pollinated flowers. Considering pollination results in the eventual death of flowers, similar degradation and breakdown of cellular and structural material is expected (Attri *et al.,* 2008). Van Doorn (2004) elucidated that senescence may be a result of sugar starvation considering application of exogenous sugars have been reported numerous times to be successful in delaying senescence.

The application of exogenous sugars seems to act as a continuous supply of respirable energy which in turn eliminates the need to use up carbohydrate reserves or breakdown complex cell component. This results in the extension of vase life of flowers. The role of ethylene as the hormone responsible to signal the senescence-related events have also been established in many studies. Suppressing this signal would also mean delaying the changes that characterizes senescence. The use of ethylene inhibitors has been proven to delay vase life and improve the physiological process in pollinated flowers (Chapter 3). In some studies, the use of ethylene inhibitors was found to also affect the status of carbohydrates in flowers (Attri *et al.,* 2007). It was found that the ethylene inhibitors resulted in a more stable level of sugars in pollinated flowers as opposed to untreated flowers. Leon and Sheen (2003) postulated that there exists a cross talk between sugar and ethylene signalling which means that the intervention on one would affect the other. The exact mechanism of the interaction between ethylene and sugars however, has yet to be established concluded.

The goal of this chapter is to investigate starch and carbohydrate status in pollinated *D.* Pompadour and to evaluate whether the application of ethylene inhibitors and sugars have any effect on the status of starch and sugars in pollinated flowers.

4.2 MATERIALS & METHODS

4.2.1 Plant Material

Dendrobium Pompadour flowers were obtained from the glasshouse of University of Malaya. Flowers were hand-pollinated by placing the pollinia onto the stigma. Individual flowers were cut at the proximal end of the peduncles in water and placed in 20 ml water vials containing distilled water and treatment solutions of 4% (w/v) glucose, 2% sucrose (w/v), 0.05 mM AOA and 0.6 mM STS. For experiments that required different parts of the flower to be used, careful separation of the parts of the perianth was carried out. The flower was then separated into sepals and petals (perianth), labellum and column. A total of 15 flowers were used for the experiment.

4.2.2 Extraction of Starch and Sugars

Flowers were oven dried at 80^{o}C for 24 hours at which they have reached a constant dry weight. Dried flowers were weighed for their dry weight and used for starch and sugar extraction. Approximately 0.1g of dried flower was ground in liquid nitrogen into powder. The powder was added to 1 ml of 0.5 *N* sodium hydroxide [Sigma] and the mixture was centrifuged at 3500 x g for 20 minutes at 4 ^{0}C. The supernatant was neutralised with 0.5 *N* acetic acid and the volume made up to 20 ml ddH_2O. This solution was used in the determination of starch and sugars (Areas and Lajolo, 1980).

4.2.2.1 Starch determination

Aliquots of 0.1 ml of the solution obtained as described above (4.2.3.) were treated with 4 ml of absolute ethanol. After centrifugation at 12,000 x g for 20 minutes, the supernatant was discarded and the pellet was washed twice with 80% ethanol. 0.9 ml of 0.2M acetic acid was added and after incubation in boiling water for an hour, the solution was hydrolysed by addition of 0.1 ml of amyloglucosidase (14 Uml^{-1}).

The resulting solution was deproteinized with 1 ml of 0.6*N* perchloric acid and the glucose formed was evaluated by the glucose-oxidase-peroxidase (GOD/POD) method (Bergmeyer and Bernt, 1974) whereby 0.2 ml of the sample was added to 5 ml of Glucose Reagent and the mixture was incubated at room temperature for 40 minutes. The absorbance at 470 nm was recorded. A standard plot for starch determination was made by adding 5.0 ml of Glucose Reagent to standard glucose solution with concentrations between 0-20.0 $\mu g\ ml^{-1}$. The starch content was calculated as glucose content x 0.9 and expressed in mgg^{-1}dry weight.

4.2.2.2 Determination of total sugar

Total sugar content was determined using the Phenol-sulphuric method (Dubois *et al*., 1956). In this method, 0.5 ml of the sample solution (4.2.3) was added to 0.1 ml of 80% phenol, followed by the addition of 5 ml of concentrated sulphuric acid. The mixture was allowed to stand at room temperature (24 ± 2) for 10 minutes and then incubated in a 25-30 ^{0}C water bath for 20 minutes. The absorbance at 490 nm was recorded and the sugar concentration was obtained by referring to the glucose standard graph. The standard graph was obtained by adding phenol and sulphuric acid to a standard glucose solution with concentrations between 0-100 μgml^{-1}.Total sugar was expressed in mgg^{-1} dry weight.

4.2.2.3 Determination of total reducing sugar

Reducing sugars were assayed as described by Somogyi (1945). 0.5 ml of the sample solution (4.2.3) was added to 1 ml of copper reagent. The mixture was vortexed and heated in boiling water for 20 minutes (the mouths of the test tubes used were closed and the tubes were covered with aluminium foil). After that, the tubes were cooled on ice and 0.1 ml of aresenomolybdate solution was added. 5 ml of dH_2O was added and mixed thoroughly. The absorbance at 520 nm was recorded and the total reducing sugar concentration was obtained by referring to the glucose standard graph. Total reducing sugar was expressed in mg g^{-1} dry weight.

4.3 RESULTS

Figure 4.1 shows that starch content in pollinated, unpollinated and treated pollinated flowers exhibited a pattern of decline throughout the experiment. Pollinated flowers held in distilled water exhibited the most rapid decline in starch content. A small decline in starch content was observed on day 1, after which a more rapid pattern of decline was observed. At the end of the experiment, starch content in pollinated flowers held in distilled water was 0.9 mg g^{-1} compared to 3.58 mg g^{-1} at the beginning of the experiment. This translates into a massive 75% loss in starch content. On the other hand, a much less decline in starch content was observed in unpollinated flowers. A significant change was observed only on day 4 and thereafter, and by the end of the experiment 2.2 mg g^{-1} starch remained, which meant that 38.5% of starch was lost in unpollinated flowers at the end of the experiment. Pollinated flowers held in distilled water retained 25% of starch compared to 61.5% retained by unpollinated flowers.

Treatment with ethylene inhibitors and sugars proved to be effective in reducing loss of starch in pollinated flowers. 0.05 mM AOA and 0.6 mM STS were more effective compared to 4% glucose and 2% sucrose in retaining total starch at the end of the experiment. At the end of the experiment, pollinated flowers held in 4% glucose and 2% sucrose showed total starch content of 1.4 mg g^{-1} and 1.54 mg g^{-1}, a loss of 60.9% and 57% of total starch respectively. For flowers held in 0.05 mM AOA, 2.1 mg g^{-1} of starch (41.3%) was lost while flowers in 0.6 mM STS lost a total of 42.5% of starch.

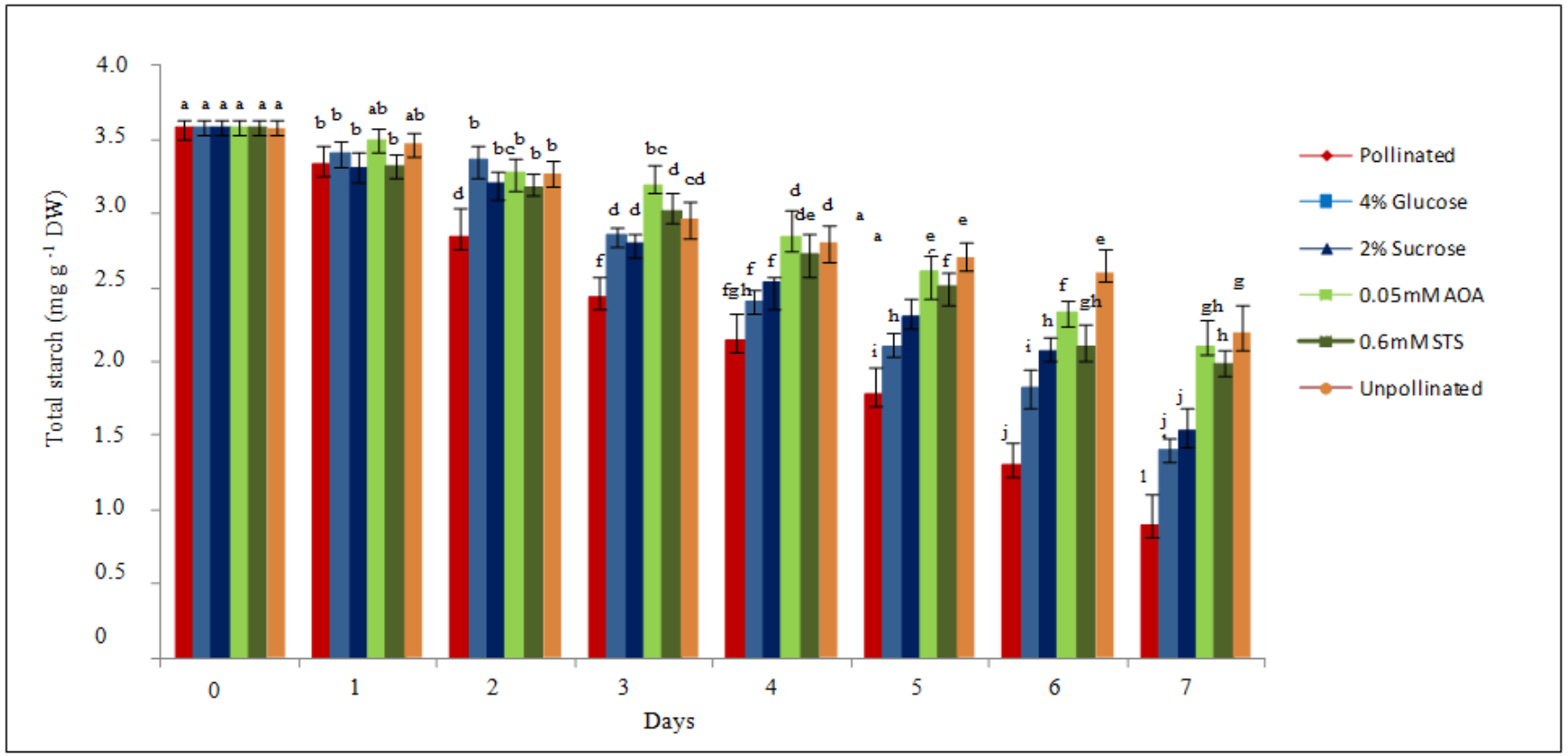

Figure 4.1: Starch content in pollinated, unpollinated and treated *D.* Pompadour flowers. Each value is the mean ± SE (n = 15), and values with different letter(s) are significantly different according to the Duncan's Multiple Range Test ($P < 0.05$).

In contrast to total starch content, where a decline of starch was observed in all flowers, total sugars in pollinated, unpollinated and treated flowers showed a pattern of increase throughout the experiment. The most rapid increase in total sugars was observed in pollinated flowers. Total sugar remained unchanged for the first 2 days, then started to increase thereafter with the most pronounced increase observed on day 7. By then, total sugar was 3.78 mg g^{-1} compared to 1.92mg g^{-1} at the beginning of the experiment. This is an approximate 2 fold increase in total sugar. On the other hand, a more fluctuating pattern was observed in unpollinated flowers. By the end of the experiment, total sugar in unpollinated flowers was 2.25 mg g^{-1}.

In flowers treated with 0.05 mM AOA and 0.6 mM STS, total sugar remained unchanged until day 5, after which a gradual increase was observed. By the end of the experiment, total sugar content was 2.8 and 2.68 mg g^{-1} for 0.05 mM AOA and 0.6 mM STS respectively. Total sugar content in 4% glucose and 2% sucrose remained unchanged until day 3, increased to 3.2 mg g^{-1} for 4% glucose and 2.8 mg g^{-1} for 2% sucrose at the end of the experiment.

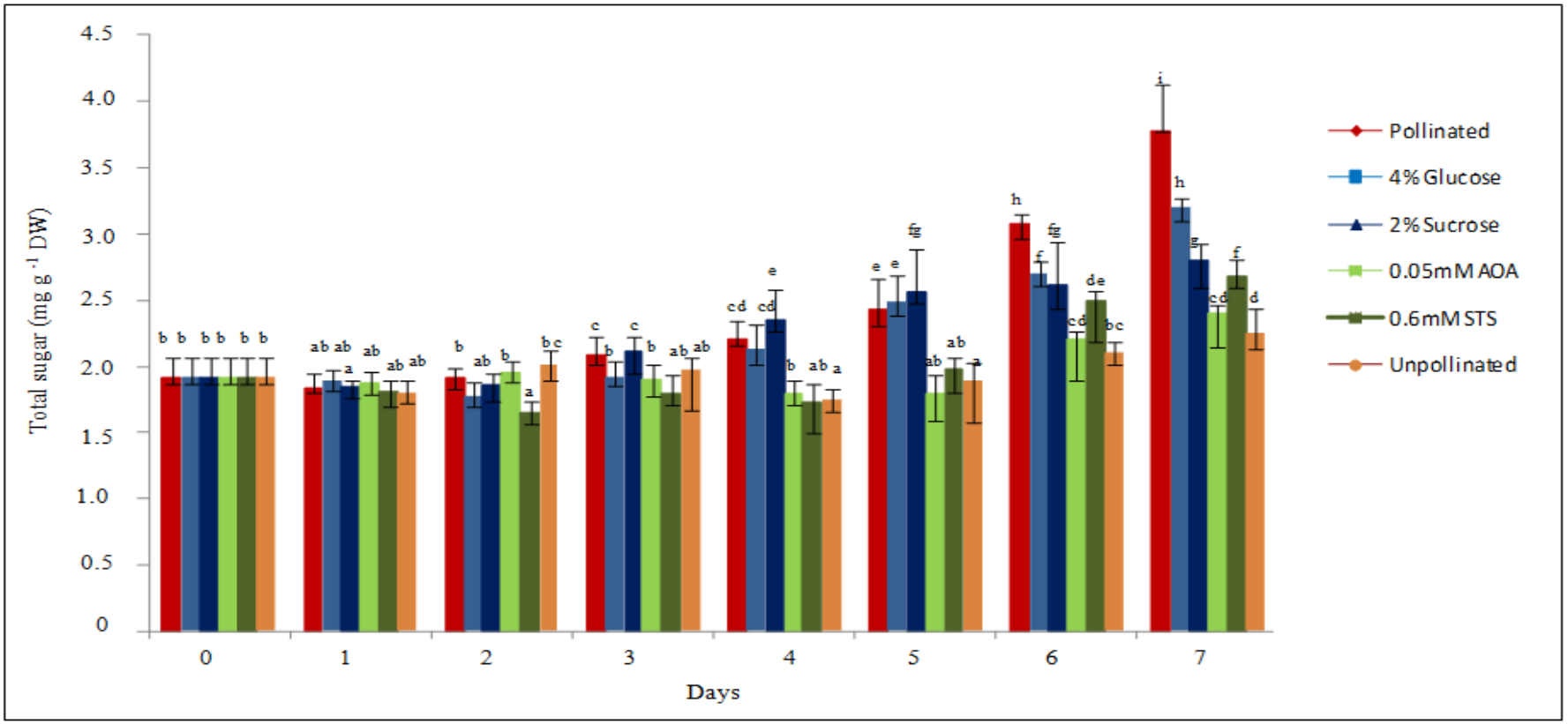

Figure 4.2 Total sugar content in pollinated, unpollinated and treated *D.* Pompadour flowers. Each value is the mean ± SE (n = 15), and values with different letter(s) are significantly different according to the Duncan's Multiple Range Test ($P < 0.05$).

Reducing sugar content in pollinated, unpollinated and treated flowers showed a similar trend with total sugar, increasing throughout the experiment. In pollinated flowers, increase in total reducing sugar was observed as early as day 1and continued to gradually increase until the end of the experiment. By the end of the experiment total reducing sugar content in pollinated flowers held in distilled water was 3.2mg g^{-1} compared to 1.22 mg g^{-1} in the end of the experiment. This translates into a 2.6 fold increase in reducing sugar content. In unpollinated flowers, total reducing sugars remained unchanged until day 6 when an increase was observed. On day 7, reducing sugar content in unpolinated flowers was 1.68mg g^{-1}.

In pollinated flowers held in treatment solutions, reducing sugar content showed similar trends to that of unpollinated flowers. Pollinated flowers held in 0.05 mM AOA and 0.6 mM STS showed no significant change in reducing sugar until day 6 after pollination. On day 7 an increase was observed for flowers in both 0.05 mM AOA and 0.6 mM STS treatment solutions which were 2.25 and 2.05 mg g^{-1}. Reducing sugar content in flowers held in 4% glucose and 2% sucrose was higher compared to flowers held in 0.05 mM AOA and 0.6 mM STS. On day 7, flowers held in 4% glucose and 2% sucrose had reducing sugar content of 2.7 and 2.65 mg g^{-1} respectively.

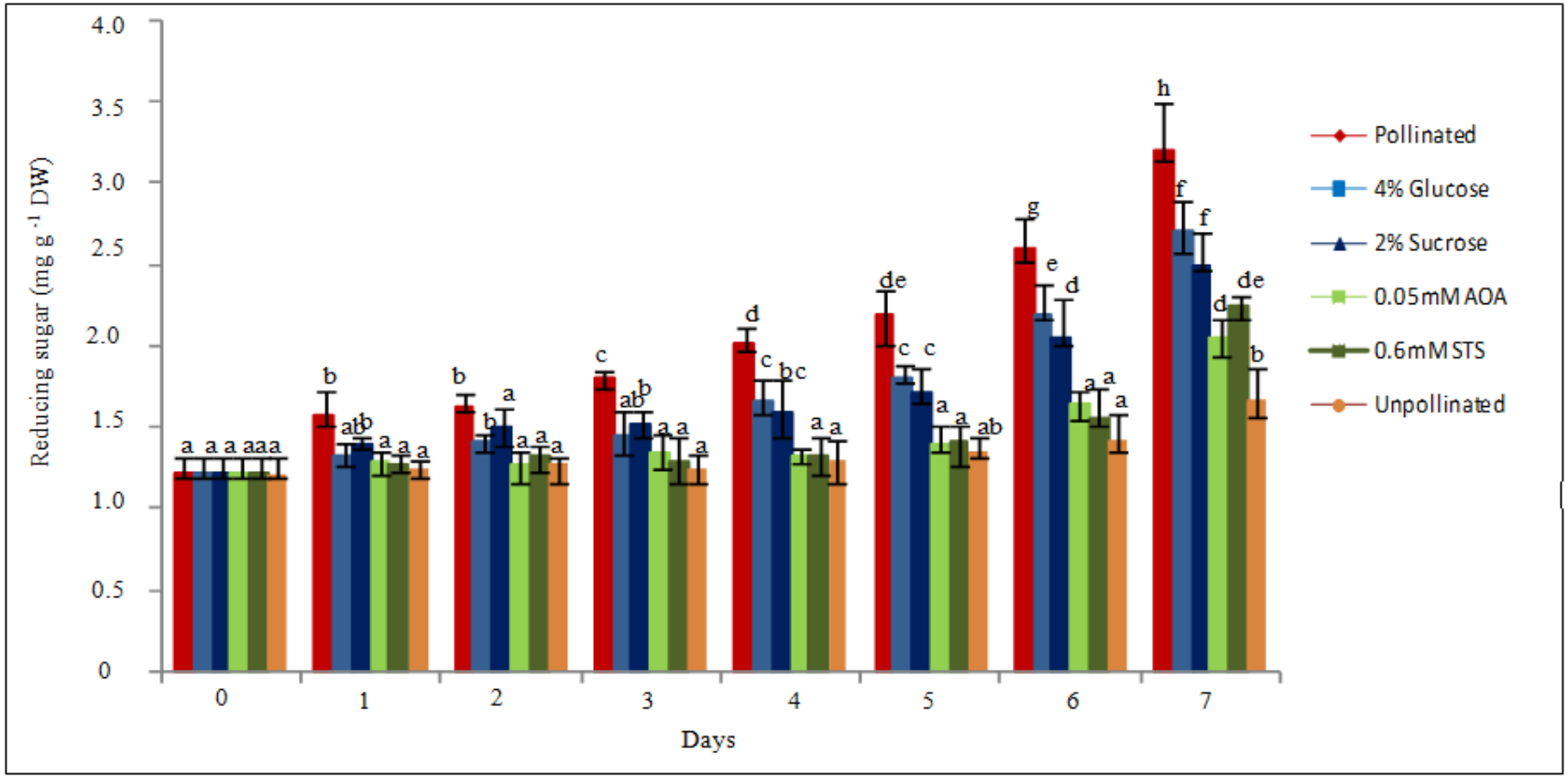

Figure 4.3 Reducing sugar content in pollinated, unpollinated and treated *D.* Pompadour flowers. Each value is the mean ± SE *n* = 15), and values with different letter(s) are significantly different according to the Duncan's Multiple Range Test ($P < 0.05$).

Non- reducing sugar content in pollinated flowers fluctuated throughout the experiment. Initially a decrease was observed until day 3. Thereafter the content in pollinated flowers gradually increased and reached 0.59 mg $^{g-1}$ at the end of the experiment compared to 0.70 mg g $^{-1}$ on day 0. In unpollinated flowers, non-reducing sugar content was generally higher compared to pollinated ones. However at the end of the experiment, the level of non-reducing sugar in unpollinated flowers was no different than those pollinated.

Content of non-reducing sugars in treated flowers also fluctuated throughout the experiment. By the end of the experiment, flowers in all four treatment solutions had non-reducing sugar lower than both pollinated and unpollinated flowers. .

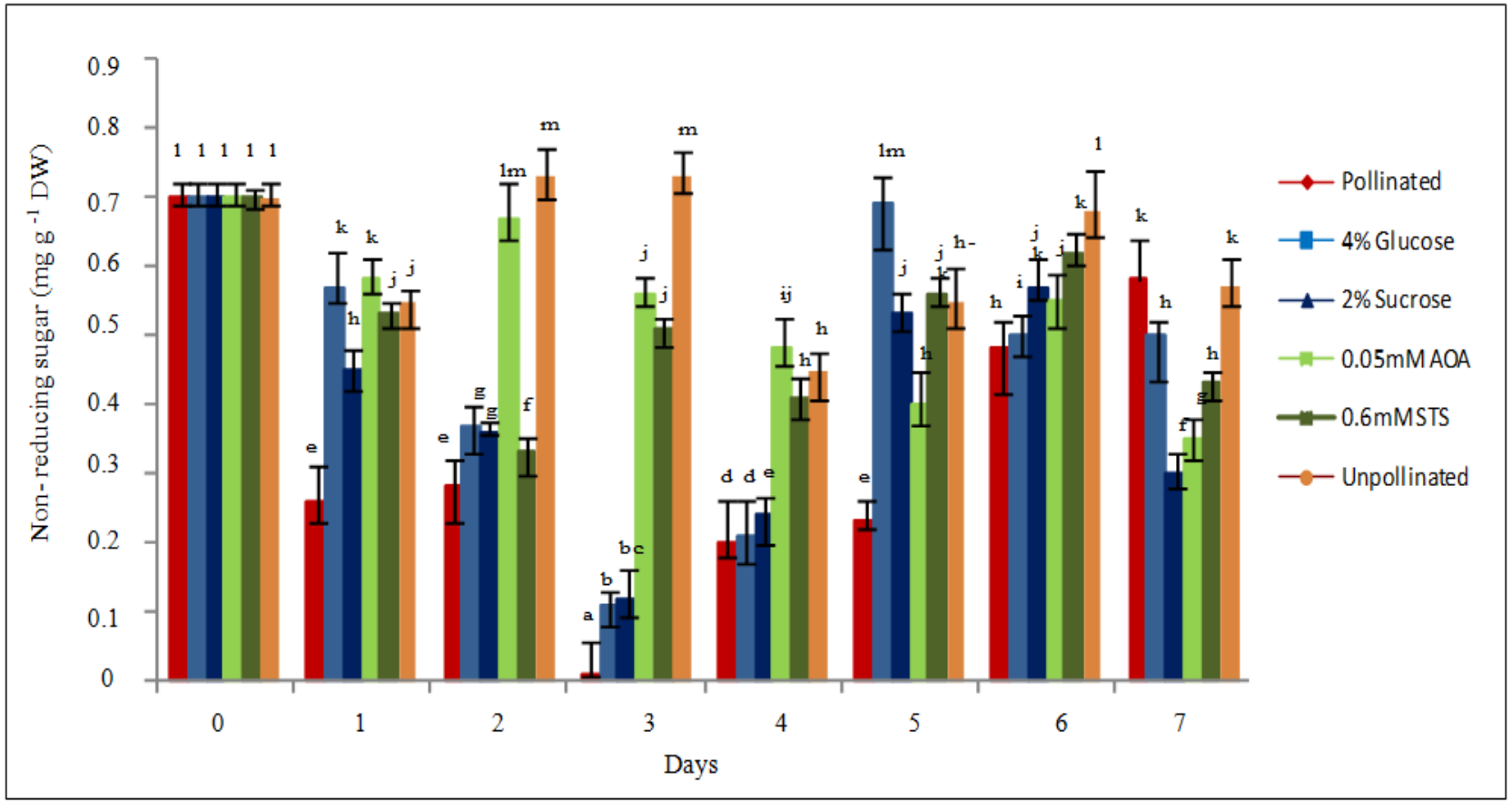

Figure 4.4 Non reducing sugar content in pollinated, unpollinated and treated *D.* Pompadour flowers. Each value is the mean ± SE (n = 15), and values with different letter(s) are significantly different according to the Duncan's Multiple Range Test ($P < 0.05$).

All segments sampled in the pollinated flowers had higher content of both total sugars and reducing sugars compared to the unpollinated and treated flowers (Table 4.1). The highest amounts for the sugars were present in the column, followed by the perianth and the labellum. In unpollinated flowers, increase in sugars was also observed in all parts with the exception of total sugars in the perianth. The increase however, was much less compared to pollinated flowers.

Floral segments of flowers treated with 0.05 mM AOA and 0.6 mM STS showed levels of total sugar and reducing sugar that were similar to unpollinated flowers. However, a significant difference was observed in the amount of total sugars in the column of flowers treated with 0.6 mM STS. Generally, the levels of sugars in the perianth and labellum of flowers treated with 2% sucrose and 4% glucose were also similar to unpollinated flowers. In contrast, columns of flowers for both treatments showed content of total sugars and reducing sugars that were significantly higher than unpollinated flowers.

Table 4.1: Total sugar content in perianth, labellum and column of pollinated, unpollinated and treated *D.* Pompadour flowers. Each value is the mean ± SE (n = 15), and values with different letter(s) are significantly different according to the Duncan's Multiple Range Test ($P < 0.05$).

		Total sugars (mg g^{-1}DW)			Reducing sugars (mg g^{-1} DW)		
Day	Treatment	Perianth	Labellum	Column	Perianth	Labellum	Column
0	All flowers	0.83^{a}	0.49^{a}	0.60^{a}	0.46^{a}	0.19^{a}	0.57^{a}
7	Unpollinated	0.95^{ab}	0.60^{b}	0.70^{b}	0.61^{b}	0.34^{b}	0.88^{b}
	Pollinated	1.39^{c}	0.69^{bc}	1.70^{ef}	0.98^{d}	0.57^{d}	1.65^{e}
	4% glucose	1.11^{b}	0.55^{a}	1.54^{e}	0.79^{c}	0.48^{bc}	1.43^{d}
	2% sucrose	0.98^{ab}	0.52^{a}	1.30^{d}	0.60^{b}	0.49^{bc}	1.41^{d}
	0.05 mM AOA	1.04^{ab}	0.61^{b}	0.75^{b}	0.77^{c}	0.37^{b}	0.91^{b}
	0.6 mM STS	1.08^{b}	0.67^{b}	0.93^{c}	0.72^{bc}	0.42^{b}	1.11^{bc}

4.4 DISCUSSION

Senescence is a highly regulated process that requires the utilization of energy in the form of respirable substrates (Biswal and Biswal, 1999). However, when substrates are limited metabolic changes that ensure a continuous supply of energy become necessary. A degenerative process is part of the general response that occurs during flower senescence (van Doorn and Woltering, 1994). Processes which occur during senescence involve hydrolytic reactions that break down complex molecules to simpler ones.

In this study, starch hydrolysis was found to occur most rapidly in pollinated flowers compared to unpollinated ones. Starch content is inversely proportional to the rate of senescence. A significant decrease in starch coincided with perianth closure of pollinated *D.* Pompadour on day 2 (as established in chapter 3). Thereafter, starch continued to rapidly decrease parallel with the rapid deterioration of the flower. In contrast, lost in starch was much less in unpollinated flowers. This is also reflective of the morphology of the flower that remains fresh and open throughout the experiment. The results in this chapter corroborate findings by Attri *et al.,* (2008) that reported an increase in both alpha and beta amylases in pollinated *Cymbidium* flower, indicating increased starch hydrolysis. In *D. crumenatum,* starch levels continued to decrease throughout floral development. Upon senescence, more than 50% of starch was lost (Yap *et al.,* 2008).

Ferreira *et al.,* (1984) reported that during senescence in *Gladiolus* (*Iridaceae)* flowers, the initially high starch concentration decreased rapidly after harvest concomitantly with an increase in amylase. The increased activities of these enzymes accelerated the breakdown of starch to simple sugars. Koizumi *et al.,* (1993) reported that active starch degradation was more pronounced in stressed tissues of *Arabidopsis* flowers as a result of enhanced induction of amylase following stress such as wounding. It was suggested that the breakdown of starch provides energy in the form of glucose for defence response. As was discussed in earlier chapters, pollination in flowers increases ethylene production as in stressed and wounded plants. As such, starch degradation in self pollinated flowers may as well be a response to the 'wounding" of the rostellum tissue or the "wounding" of the anther cap tissue. In addition to wounding of the flowers, the development and growth of the ovary as well as ovule development and seed production as a result of successful pollination would require the utilization of energy. Because of the high demand for energy the utilization of resources (such as starch) from parts of the flower which are no longer necessary makes evolutionary sense. In contrast, there is a lower demand for energy in unpollinated flowers as demonstrated by the higher content of starch in unpollinated *D.* Pompadour throughout the experiment carried out in this study.

The continued breakdown of starch following pollination coincided with an increase in total sugars and reducing sugars in *D.* Pompadour whereas non-reducing sugars were found to exhibit the opposite trend. Total sugar was measured because literature has shown opposing trends of total sugar content following pollination in flowers.

While similar trends of increased total sugars were observed in the perianth of pollinated *Cattleya multiflora* (Hsiang, 1951), and *Cattleya retusa* (Attri *et al.,* 2008), decrease of sugar content was reported in *Cymbidium* (Hsiang, 1951), *D. nobile* (Wen *et al.,* 1990) and *D. crumenatum* (Yap *et al.,* 2008). There is no agreement regarding the reasons for these differences between orchids. Nichols (1973) postulated that the content of substrates may be indicative of the potential longevity of a flower. Attri *et al.,* (2008) observed that a more rapid increase in sugar content resulted in a faster rate of senescence.

If these views are correct it would be reasonable to assume that the more stable sugars levels in unpollinated *D.* Pompadour would result in or be indicative of a longer vase life of the flowers. On the other hand the more rapid increase in sugar content reflects the increased and faster breakdown of starch and probably other larger molecules and a pronounced reduction in vase life. It has also been suggested that rapid depletion of sugars occurs in short-lived flowers. This is evident in *D. crumenatum* flowers because 50% of the decrease in sugar levels occur/red within one day following pollination and coincided with the onset of senescence (Yap *et al.*, 2008).

Views regarding the correlation between sugar status and senescence were reviewed by van Doorn (1994). He suggested that when depletion of sugars accompanies flower senescence the reductions in sugar levels cause the ageing. Thimman *et al.,* (1977) explained the increase of sugar content that accompanies leaf senescence as being a

consequence of the breakdown of complex molecules as an alternative source of energy that substitutes for a reduced sugar supply.

This is a mechanism that ensures and adequate supply of energy. In pollinated *D.* Pompadour, reduction in starch corresponded with the increase in sugars indicating breakdown of the complex molecules. This is further corroborated by a number of reports which show that an increase in sugar levels plays several roles in senescing organs (Olley *et al.,* 1996; Rolland *et al.,* 2002). Altogether it is reasonable to assume that the rapid increase in sugars and other soluble compounds is a result of breakdown of complex molecules (as evident in the reduction in starch content). The small and soluble molecules are necessary for a number of senescence–related processes such as flower closure, pigment breakdown, cell wall hydrolysis, protein synthesis and degradation, etc. Ho and Nichols (1977) hypothesized that "a change in the source-sink relationship of the flower parts contributes to the factors that determine the rate of flower senescence". Pollination–induced senescence is phenomenon that transforms the petals into an important source organ for carbohydrate for ovary growth (Mor *et al.,* 1980).

Pollination resulted in a significant increase of total and reducing sugars in all floral segments. However, it has to be noted that the greatest increase was observed in the columns. This may suggest that metabolic events are either initially activated or is most rapid in the column. It has been suggested that upon pollination, signals may emanate from the pollen or induced by metabolic changes that occur in the column

(Attri *et al.,* 2008). Reducing sugars increased about two folds in the column of pollinated flowers. This is also the case for *Cymbidium Lowianum*, where considerable increase in reducing sugars occurred in the column of following pollination.

Pollination and ethylene treatment-induced translocation of sugars from petals to ovary has been demonstrated in senescing *Arundina* orchid flowers (Chin and Hew, 1975). Furthermore, the growth of the ovary seems to be at the expense of carbohydrate reserves in wilting carnation flowers (Nichols, 1971). O'Neill and Nadeau (1997) concluded that the remobilization of substrates including sugars is critical in ensuring the success of the overall reproductive process. Ovary development (fruit formation), seed production and subsequent fertilization require both energy and nutrients. Therefore conservation of energy and materials require salvage and reutilization of substances and their transport from the sources (the perianth) to the sink (ovary). In this case, hydrolysis of large molecules and recycling and reutilization of their components takes place.

Carbohydrates, in particular sucrose and glucose, play crucial roles in plant metabolism. Besides being an energy source they can have osmo-regulatory functions, act as signalling molecules involved in crucial processes in plants and are precursors of a multitude of other compounds. An apparent paradox in the status of reducing and non reducing sugars during flower senescence has not been solved. In flowers such as daylilies decrease in non reducing sugar concomitant with an increase in reducing

sugars was apparent in senesced flowers (Bieleski *et al.,* 1995; Gluzar *et al.,* 2005). In contrast, no significant reduction in non- reducing sugar was observed in *Ranunculus Asiatic* flowers (Shahri and Tahir, 2011) while flowers of *Aeridus multiflora* showed a decrease in reducing sugars (Attri *et al.,* 2007). Results in this chapter showed that reducing sugar levels increased in pollinated *D.* Pompadour flowers while non-reducing sugar content fluctuated. Reducing sugars seem to play an important role in pollinated *D.* Pompadour flowers. This is in line with findings by Bieleski (2000) in which glucose was found to be of considerable importance for senescing daylily flowers. Increased invertase activity was observed in pollinated *Aerides multiflora* and *Rhynchostylis retusa* orchid flowers (Attri *et al.,* 2008).Higher levels of invertase activity in the ovary of pollinated carnations were reported to maintain a sucrose gradient as the ovary becomes the carbohydrate sink in pollinated flowers (Nichols, 1977).

In *Rhynchostylis retusa* flowers, total soluble sugars were found to be at lower levels in the ovary initially. Their levels increased following pollination. Sugar concentrations increased sequentially /starting with the column and proceeding to the ovary (Attri *et al.,* 2008). The rate of substrate translocation is thought to be under the control of ethylene (Nichols, 1977; Hew *et al.,* 1989; Attri *et al.*, 2008; Bai *et al.,* 2010). In carnations, ethylene was found to promote substrate translocation from the petals to the ovary (Ho and Nichols, 1977). Ethylene-treated carnations exhibited a rapid remobilization of carbohydrates from the perianth to ovaries.

Remobilization of simple sugars coincided with climacteric ethylene production. The sugar-ethylene relationship was confirmed when lower amounts of sugars and decreased enzyme activities were detected in flowers treated with ethylene inhibitors (Mor *et al.,* 1980). An interaction may therefore exist between ethylene and carbohydrate metabolism during senescence, presumably via the down-regulation of enzymes. The results from this study concur with the aforementioned reports. Treatment of pollinated *D.* Pompadour with ethylene inhibitors, AOA and STS resulted in a greater retention of starch and sugar levels following pollination. In fact, the patterns of starch and sugar levels were similar to unpollinated flowers. The changes in sugar status correspond to the physiological changes flowers treated with AOA and STS as established in Chapter 3, where the slower breakdown of starch and stable levels of sugar seem to reflect the fresh state of the flower.

Furthermore, as ethylene has been proven to promote ovary growth (Nichols, 1977; Zhang and O'Neill, 1993; Ketsa *et al.,* 2004), obstruction of the ethylene signal would reduce, inhibit or prevent ovary growth. This in turn maintains existing metabolic activities in the petals without alterations to carbohydrate metabolism. Mor *et al.,* (1980) suggested that during pollination-induced senescence, ovaries and petals compete for a common supply of carbohydrate, where more often than not the ovary prevails. STS pre treatment on pollinated flowers was found to increase sink strength of the petals allowing for the petals to compete more effectively with the ovary. In senescing carnations, STS treatment was also found to suppress ovary growth while maintaining petals as active sink (Cook and Staden, 1982).

When senescence in flowers is regulated by ethylene, exogenous sugars considerably delay the large increase in ethylene production and the time to visible senescence (van Doorn, 2004). High ABA production in plants has been found to block ethylene sensitivity and sugars have been found to cause low ABA production. This in turn indirectly interferes with the ethylene signaling network. (Leon and Sheen, 2003). Furthermore, during senescence of carnation flowers, cDNA micro analysis results showed that the expression of group of genes that were suppressed by exogenous sugars was also suppressed by STS, which blocks ethylene receptor perception (Hoeberichts *et al.*, 2007). In that same study, the author suggested that a cross talk may occur between sugar and ethylene signaling. Treating flowers with sucrose was found to suppress the upregulation of the EIL3 gene, a gene involved in the regulation of ethylene.

Providing a source of carbohydrate in the form of sugar also means that starch need not be broken down. Ho and Nichols (1977) found that treating carnations with sucrose prevented the loss of starch reserves. In sugar-fed carnations, starch retention lasted two days after which rapid loss in starch occurred coinciding with increase in total and reducing sugars (Mayak and Dilley, 1976). Similarly, in this study, *D.* Pompadour flowers treated with sugars also retained starch at the initial stages following pollination. Reduction in starch levels was inversely proportional with total sugar content.

In conclusion, pollination induced senescence results in a rapid breakdown of starch which is accompanied by a corresponding increase in total sugars and reducing sugars. Treatment with ethylene AOA, STS, glucose and sucrose suppress the breakdown of starch which results in a more stable retention of sugars upon pollination.