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EFFECT OF CHLORSULFURON (GLEAN-75) AND SUCROSE ON SOME POST-HARVEST PHYSIOLOGICAL EVENTS IN CUT FLOWERS

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Summary. Treatment of cut flowers with cytokinins is found to be beneficial in delaying senescence processes but the response to cytokinin application varies depending on cultivar, stage of flower development and type of cytokinin. The present study was conducted in view of exploring the effect of herbicide Glean-75 [chlorsulfuron; N^1-(4-methoxy-6-methyl-*sim*-triazine-2-yl)-N^2-(2-chlorophenyl-sulphonyl)urea] with cytokinin properties on the post-harvest life of cut chrysanthemum and rose flowers and of investigating the influence of this ingredient on the activity of α-amylase and invertase in petals. The experiments were conducted with open chrysanthemum flowers, cv. Westland, cold stored buds of cv. Walusa and cut at bud stage roses, cv. Sonia. The treatments were provided by holding solutions where sucrose, Glean-75 and their combination were used. Positive effect of chlorsulfuron on the elimination of leaf yellowing in chrysanthemums was established. The appearance of leaf damage was observed on the 18th day for cv. Westland which was 10 days over control flowers kept in water. Less pronounced effect was noticed on cold stored cv. Walusa where leaf injuries appeared on the 8th day after placing in the solutions. Treatment of cut roses cv. Sonia with Glean-75 + sucrose resulted in a longer vase life, successful opening and good quality of the flowers. The dynamics of α-amylase and invertase were associated with the phases of development. Decrease of the activities in controls during the phase of senescence was shown both

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for chrysanthemums and roses. An enhancement of enzyme activities by the compounds applied was monitored to be in a correlation with extended flower keepability.

Key words: α-amylase, chrysanthemum, holding solutions, invertase, post-harvest flowers, rose

Introduction

The post-harvest life of flowers is strongly dependent on the carbohydrate status and the acceptable amounts of metabolic sugars are factors that affect the rate of senescence. The vase-life of cut chrysanthemum and rose flowers has been often extended when they have been held in vase solutions containing sucrose (Kofranek and Halevy, 1972; Ho and Nichols, 1977). The vitality of many flowers has been prolonged when cytokinins have been added to holding solutions (Mayak and Halevy, 1980; Van Staden et al., 1990). Treatments with cytokinins appear to be also beneficial for increasing the resistance to stress conditions.

It has been found that active degradation of sucrose and starch occured more intensively in stressed tissues where an enhanced induction of invertase and α-amylase has been observed (Sturm and Chtispeels, 1990; Koizuka et al., 1995). Recently it has been suggested that in stress situations cells require more sugars to fulfil the energy and carbon needs for the defensive response to stresses (Koizuka et al., 1995). Since cut flowers suffer from an energy deficiency and are susceptible to different stresses, the demand for hexoses in petals might be satisfied by the hydrolysis of disaccharides and starch. Moreover, it has been established that flower petals contained highly active invertase (Hawker et al., 1976; Halaba and Rudnicki, 1986; Borochov and Woodson, 1989). In addition, according to Hammond (1982) and Tirosh and Mayak (1988) the activity of α-amylase plays an important role in the mechanism of petal opening and regulates the appearance of senescence syndrome.

The physiological effects of purine type cytokinins as senescence retarding factors have been broadly studied in cut flowers (Eizinger, 1977; Borochov and Woodson, 1989). However, the data about metabolic events in roses, chrysanthemums and some other post-harvest flowers in response to treatments with phenylureas are scarce. Considerable delay of ageing in cut carnations has been reported by Vassilev et al. (1979) when thiourea derivatives, including *p*-phenylthioureidosalicylic acid have been applied. It has been demonstrated (Karanov and Iliev, 1985; Karanov et al., 1992) that phenylurea type cytokinins efficiently retarded the senescence of detached leaves.

The present study was conducted to explore the effect of herbicide chlorsulfuron [Glean-75; N^1-(4-methoxy-6-methyl-*sim*-triazine-2-yl)-N^2-(2-chlorophenyl-sulphonyl)urea] with cytokinin properties on post-harvest life of cut chrysanthemum and rose

flowers and to investigate the influence of this compound and sucrose on the activity of α-amylase and invertase in petals.

Materials and Methods

Materials and treatments

Cut rose and chrysanthemum flowers were used in the experiments. Rose flowers (*Rosa thea hybrida* L.*),* cv. Sonia were harvested at stage 1 of development and the stems were dipped in solution for blossoming. Stages of flower development were determined according to the scale of Khayat an Zieslin (1989) as follows, stage 1 – petal colour of flower buds visiblle, sepals at vertical position; stage 2 – petal colour visiblle, petals partially open, sepals – separated from the vertical position; stage 3 – open flowers, sepals horizontally oriented; stage 4 – open flowers, most of the petals are horizontally oriented, styles visible.

The flowers of chrysanthemum (*Chrysanthemum morifolium* L.), cv. Westland, were harvested open at normal commercial maturity and placed in holding solutions. The flowers of cv. Walusa were cut off at bud stage (diameter of apical bud 15 mm) and stored dry packed in chamber at 3 °C for 15 days. Before storage the flowers were pulse treated for 20 hours by placing in a solution containing 10% sucrose + 30 mg/l silver nitrate + 100 mg/l BA. After storage the flowers were recut and placed in solutions for blossoming and testing their vase-life. Both chrysanthemum and rose cut flowers were kept at air temperature 21 °C, RH 60% and light intensity 15 $\mu mol.m^{-2}.s^{-1}$.

The harvested flowers were continously treated with water solutions of chlorsulfuron (10^{-6}M), sucrose (rose, cv. Sonia – 2%; chrysanthemum, cv. Westland – 3%; chrysanthemum, cv. Walusa – 5%) and with a combination of these two ingredients.

The applied concentrations of sucrose for roses and chrysanthemums differed due to the difference in the requirements of various cut flower species for the amount of carbohydrates necessary to keep the vitality of post-harvest flowers (Ho and Nichols, 1972; Kofranek and Halevy, 1972). The chrysanthemum cultivars studied were harvested at different phases of flower development – bud stage for cv. Walusa and fully open flower for cv. Westland. This was the reason for using higher concentration of sucrose for the stored flowers of cv. Walusa thus providing sufficient nutrient supply and flower blossoming in the post-storage period. For cv. Westland the purpose was to extend the logevity of flowers having already reached full ornamentality where lower concentration of sucrose was preferable.

Enzyme assays

The enzyme actitvity of invertase (EC 3.2.1.26) was determined following the Somogy–Nelson method (1944, 1952). The extract was prepared from 0.5 g of pet-

als with phosphocitrate buffer (pH 5.5) in cold room and the homogenate was centrifuged twice at 1700 g. Combined supernatant was assayed for invertase activity. Standard reaction time was 30 min at 37 °C and the reaction terminated by Somogyi reagent. The amount of reducing sugars generated during the reaction was quantified by the addition of Nelson's reagent. The specific activity was defined as mg reducing sugars (glucose equivalents) per mg protein.

The actitvity of α-amylase (EC 3.2.1.1.) was estimated according to modified method of Plummer (1988). The extract was prepared from 0.1 g of petals with K-phosphate buffer (pH 7.4), centrifuged at 90 g and the supernatant was used for determination of enzyme activity. The reaction mixture contained 1% starch as substrate and was incubated at 37 °C for 3 min. The method is based on the interaction of 3,5 dinitrosalicylic acid with reducing sugars over boiling water and the amount of the resulting substance was measured colorimetrically. Specific activity was expressed in mg maltose per mg protein.

Protein content was determined by the method of Lowry et al. (1951) using bovine serum albumine as a standard.

The dynamics of invertase and α-amylase was studied in petals and followed from placing the flowers in holding solutions till the end of their vase-life.

Results and Discussion

Chrysanthemum flowers are normally harvested when fully open. The problems during their vase-life are due mainly to an early appearance of leaf yellowing while the flowers are staying still fresh. Similar damages occur after long cold storage of chrysanthemums cut at bud stage. Purine cytokinins are most commonly used for post-harvest application but they are not always effective in preventing leaf yellowing and some other deteriorations related to cut flower longevity and decorativity. Phenylurea cytokinins are known as being easier to convert, possessing lower toxicity and some of them show higher activity at lower concentration in comparison with purine cytokinins (Karanov et al., 1992).

The experiments showed that vase-life of chrysanthemums cv. Westland lasted 5 days longer than the control when the flowers were kept in sucrose solution and 8 days more than the control when treated with a combination of sucrose + chlorsulfuron (Table 1). Appearance of leaf yellowing was delayed in both cases being better expressed in response to the application of sucrose + chlorsulfuron holding solution. The 10 days cold storage of chrysanthemum buds cv. Walusa reduced their longevity in water with 9 days as compared to freshly cut ones (data not shown). Placing the buds of cv. Walusa in tested holding solutions resulted in successful opening better prononced in the solution of sucrose + chlorsulfuron. However, the effect of the treatments on vase-life duration was less expressed than that on cv. Westland. Significant

Table 1. Effect of sucrose and chlorsulfuron on post-harvest life of cut rose and chrysanthemum flowers. Stages of flower development of roses are determined according to the scale of Khayat an Zieslin (1989). (±) = standard deviation from duplicated measurements from each replication (n = 7)

Treatments	First signs of wilting	Vase-life	Leaf yellowing
	(day)	(days)	(day)
	Chrysanthemum, cv. Westland		
Control–water	3.1 ± 0.21	10.0 ± 0.14	8.0 ± 0.20
Sucrose 3%	11.0 ± 0.26	15.0 ± 0.42	12.0 ± 0.21
Chlorsulfuron 10^{-6}M	2.9 ± 0.21	9.0 ± 0.07	8.0 ± 0.35
Sucrose 3%+chlorsulfuron 10^{-6}M	16.3 ± 0.14	18.0 ± 0.35	18.4 ± 0.42
	Chrysanthemum, cv. Walusa		
Control–water	3.8 ± 0.14	5.8 ± 0.17	4.9 ± 0.15
Sucrose 5%	5.0 ± 0.15	7.1 ± 0.26	5.0 ± 0.58
Chlorsulfuron 10^{-6}M	4.9 ± 0.21	6.0 ± 0.14	4.7 ± 0.14
Sucrose 5%+chlorsulfuron 10^{-6}M	7.8 ± 0.26	8.3 ± 0.07	8.0 ± 0.28
	Rose, cv. Sonia		Flower stage at the end of vase-life
Control–water	2.9 ± 0.14	4.1 ± 0.21	1–2
Sucrose 2%	4.0 ± 0.26	5.0 ± 0.20	2–3
Chlorsulfuron 10^{-6}M	2.7 ± 0.09	4.0 ± 0.14	3
Sucrose 2%+chlorsulfuron 10^{-6}M	6.3 ± 0.21	7.1 ± 0.12	4

retention of leaf yellowing was observed when sucrose + chlorsulfuron was used. The application of the same solution caused 3 days extention of Sonia roses flower longevity over the control. Treatment with sucrose + chlorsulfuron provided complete development of rose buds to stage 4, while sucrose feeding did not allow the buds to develop after stage 2-3. Although the effect of sucrose was less obvious than that of sucrose + chlorsulfuron the surose feeding improved cut flower keepability of roses and allowed good development of the buds and longer vase-life of studied chrysanthemum cultivars. Summarising the results obtained we could conclude that most effective dalay of senescence in both chrysanthemum cultivars and Sonia roses was produced in response to the treatment with sucrose + chlorsulfuron. No positive influence of chlorsulfuron applied without sucrose was observed. Therefore this type of treatment was exluded in the studies on invertase and α-amylase activity.

The activity of invertase and α-amylase was investigated in petals and the dynamics was followed from the time of placing the flowers in solutions till the end of their vase-life.

The activity of α-amylase of the rose petals in the control showed a slight increase on day 1 followed by a gradual decline toward the end of vase-life (Fig. 1). Treatment with solutions containing sucrose or sucrose + chlorsulfuron caused an enhancement of enzyme activity being better expressed in respond to sucrose + chlor-

sulfuron application. A strong stimulation of α-amylase activity was evident on day 3 in the solution with sucrose + chlorsulfuron where a development of buds from stage 2 to stage 3 appeared. The activity of invertase (Fig. 2) pronounced an acceleration after day 1 reaching a maximum on day 3 and this was expressed in the same way in case the rose flowers were kept in water, sucrose or sucrose + chlorsulfuron solutions. In stage 4 until wilting invertase activity remained higher only if the flowers were treated with sucrose + chlorsulfuron.

Variation of α-amylase activity in the petals of control cut chrysanthemums was found (Fig. 3 and 4). The activity showed an initial increase for cv. Walusa (Fig. 3)

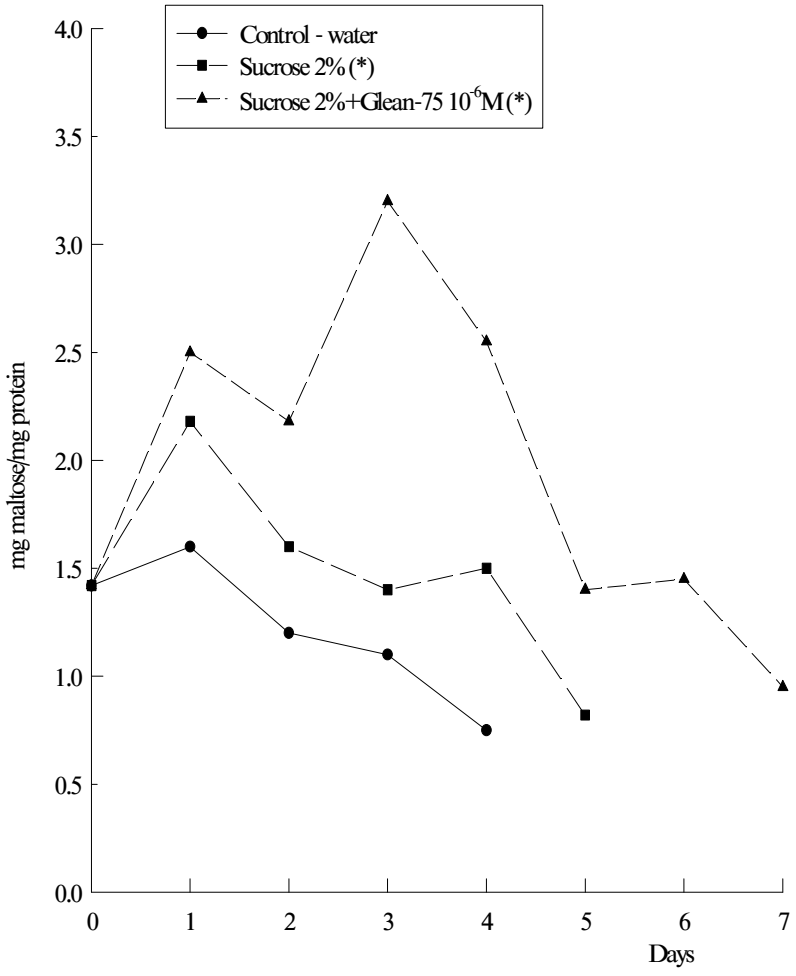

Fig. 1. Effect of Glean-75 and sucrose on the dynamics of α-amylase in petals of cut rose flowers (*Rosa thea hybrida* L.), cv. Sonia. Results show the means of three replications. Significant differences (P=0.01) from the control are indicated by *

and a decrease for cv. Westland regardless of the holding solution used. In cv. Westland α-amylase activity stimulation was observed on day 3 (Fig. 4). In both chrysanthemum cultivars an enchancement of enzyme activity was noticed in association with the appearance of the first signs of petal wilting. The activity of α-amylase was stimulated by both sucrose and sucrose + chlorsulfuron treatments but in case of sucrose + chlorsulfuron application, vase-life lasted longer and α-amylase activity stayed higher. Similar patern of enzyme dynamics to that of α-amylase was found for invertase activity in petals of cv. Westland (Fig. 5). Contrary to α-amylase in chrysanthemum petals and similar to the activity of invertase in roses the invertase activity

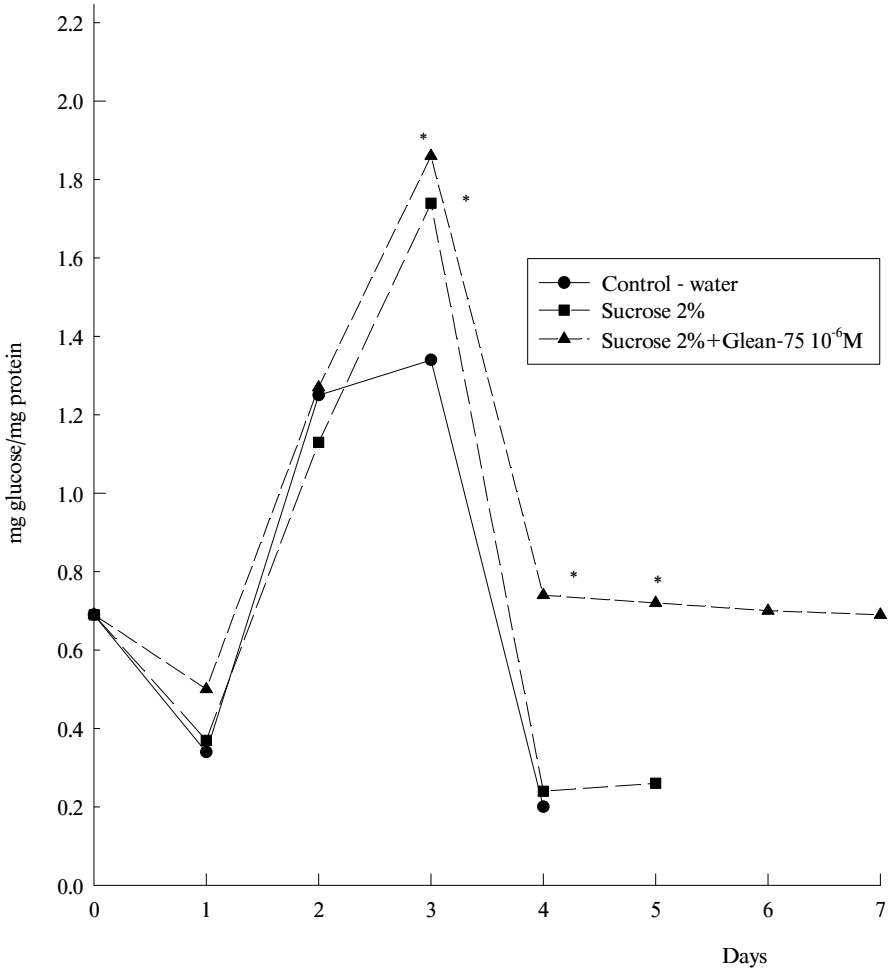

Fig. 2. Effect of Glean-75 and sucrose on the activity of invertase in petals of cut rose flowers (*Rosa thea hybrida* L.), cv. Sonia. Results show the means of three replications. Significant differences (P=0.01) from the control are indicated by *

in the petals of cut chrysanthemum flowers cv. Westland declined in the phase of petal senescence.

Data about the effects of Glean-75 and sucrose on post-harvest life and on the activity of α-amylase and invertase in petals of cut rose and chrysanthemum flowers are reported in this paper. Chemicals of the group of purine cytokinins have been widely tested, mostly in aspect of vase-life prolongation (Halevy and Mayak, 1979, 1981; Nowak and Rudnicki, 1990). Studies concerning the changes of investigated physiological parameters including that of hydrolitic enzymes included in carbohydrate turnover in response to treatments with phenylureas (Glean-75) are still limited in references. Results of our experiments showed that the treatments with chlorsulfuron + sucrose allowed the cold stored cut chrysanthemum, cv. Walusa buds, and fresh harvested buds of roses cv. Sonia to develop into fully open flowers of good quality. Application of sucrose and chlorsulfuron + sucrose retarded the leaf yellowing of chrysanthemums cv. Westland but the same effect was not observed on cv. Walusa.

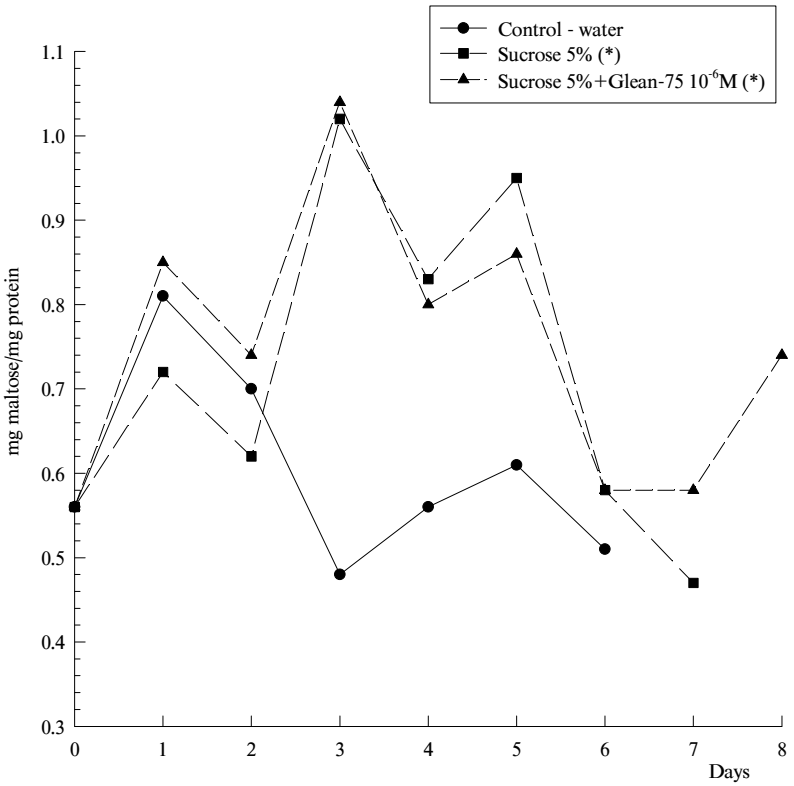

Fig. 3. Effect of Glean-75 and sucrose on the dynamics of α-amylase in petals of cut chrysanthemum flowers (*Chrysanthemum morifolium*), cv. Walusa. Results show the means of three replications. Significant differences (P = 0.01) from the control are indicated by *

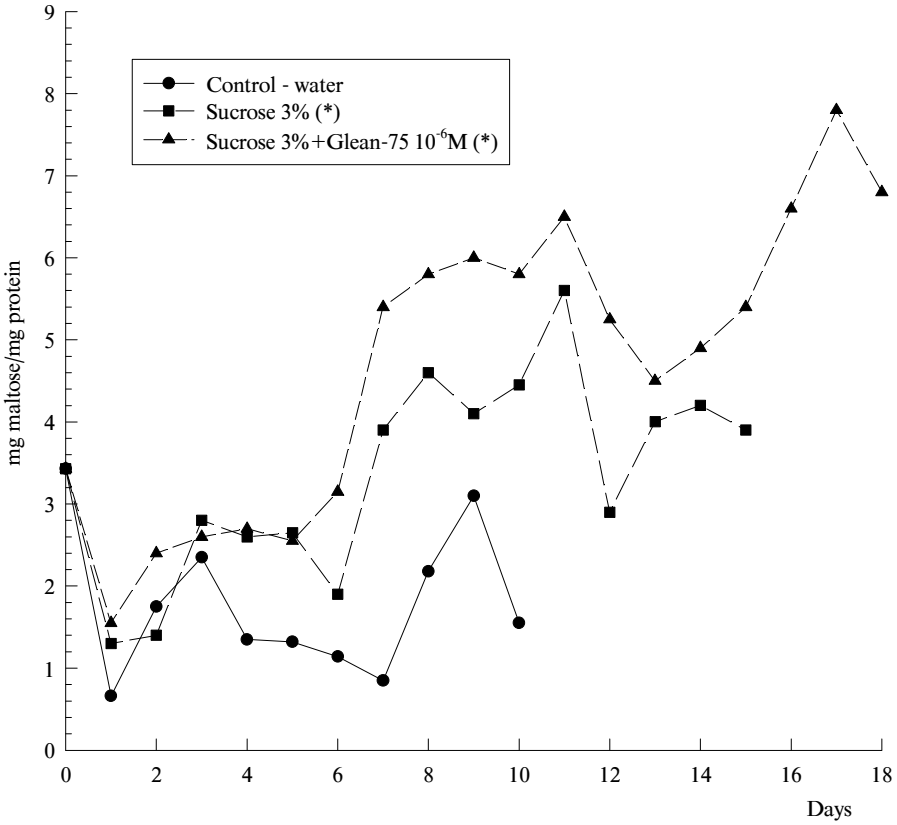

Fig. 4. Effect of Glean-75 and sucrose on the dynamics of α-amylase in petals of cut chrysanthemum flowers (*Chrysanthemum morifolium*), cv. Westland. Results show the means of three replications. Significant differences (P=0.01) from the control are indicated by *

The growth of petal tissues needs a large amount of osmotic and energic substrata, which could be a reason for starch degradation and a possible explanation for the rise of α-amylase activity accompaining bud opening and the increase of flower diameter found in our experiments. It has been established that in rose petals, hydrolisis of starch provides a source for reducing the sugars necessary for growth and development of harvested flower buds (Evans and Reid, 1988). Induction of α-amylase activity has been observed as a result of sucrose starvation in suspension-cultured rice cells (Sung et al., 1994) where the α-amylase activity in the cells cultured in the absence of sucrose increased, reached a maximum on day 3 and declined subsequently. Sucrose feeding of cut rose and chrysanthemum flowers caused an acceleration of enzyme activity in the petals of the three cultivars studied and it stayed higher than controls until the end of vase-life. A high α-amylase activity in the phases of senescence was established when chlorsulfuron + sucrose was applied and this was associated with improved flower quality.

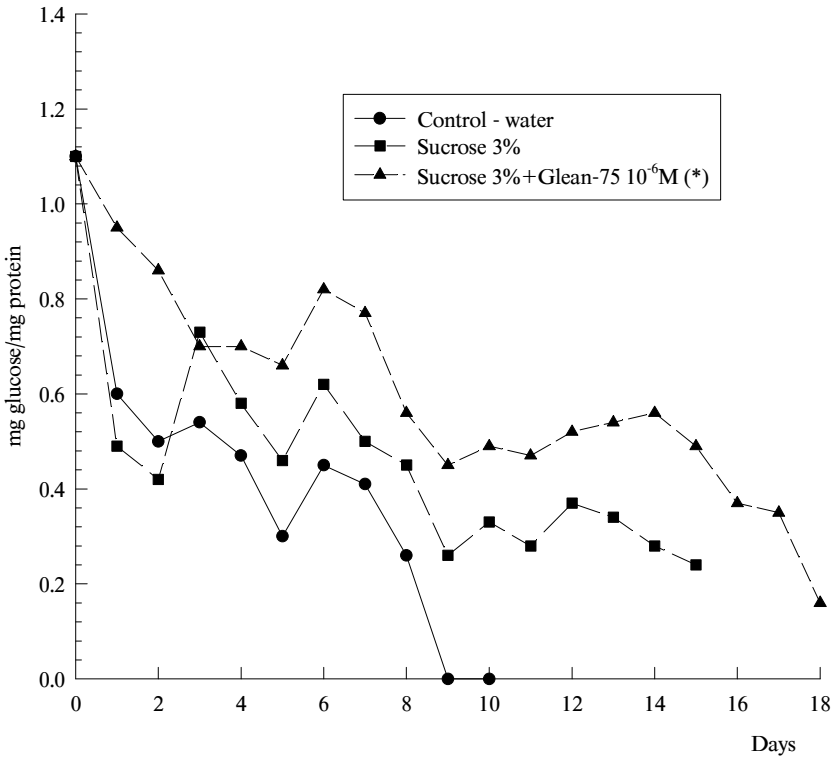

Fig. 5. Effect of Glean-75 and sucrose on the dynamics of invertase in petals of cut chrysanthemum flowers (*Chrysanthemum morifolium*), cv. Westland. Results show the means of three replications. Significant differences (P=0.01) from the control are indicated by *

A stimulation of invertase or longer kept activity was monitored when rose flowers cv. Sonia and chrysanthemums cv. Westland were placed in solutions of either sucrose or chlorsulfuron + sucrose. Acceleration of invertase activity in control flowers appears with the first signs of wilting as indicated by references from other authors (Paulin, 1986) when active degradation of sucrose in senescing tissues provides metabolic substrata for the other parts of the flower thus selfregulating the process of survival. The activity of invertase has been found to be regulated by formation of an invertase inhibitor in flower tissues of a number of flower species and the synthesis of this inhibitor has been shown to play a role in further induction of senescence processes (Halaba and Rudnicki, 1985). Since it was found that sucrose was a mobile sugar in cut flowers Nichols and Ho (1975) suggested that the invertase inhibitor might suppress sucrose hydrolysis thus helping the supply of other flower organs with energy sources. It is reported that treatments with cytokinin and cytokinin-like substances and sucrose stimulated the activity of invertase even during the process of senescence (Lukaszewska, 1986; Paulin, 1986).

In this context maintaining higher α-amylase and invertase activity might be considered as a way for ensuring the supply of energy and osmotica necessary for bud growth and as a factor contributing to the delay of the ageing.

Cut rose flowers are susceptible to water stress because the water balance of petals is easily disturbed after harvesting. Rose flowers are also often exposed to biotic stress (growth of bacteria) which causes vascular occlusion of the flower stem beneath the flower head (van Doorn, 1989). The observed stimulation of α-amylase and invertase activity by Glean-75 and sucrose could be part of an enhancement of defence mechanisms, giving an increase in energy supply under stress conditions, thus contributing to an extension in vase-life.

Since the application of Glean-75 without sucrose did not alter the vase-life of studied rose and chrysanthemum cultivars we did not investigate its effect of enzyme activity. The addition of sugars in vase solutions is essential for a good flower development and facilitates the movement and utilization of substances with a nature of cytokinins (Halevy and Mayak, 1979; Paulin, 1986; Van Staden et al., 1990). This might be a reason for the higher effect of the combination of Glean-75 with sucrose. An enchancement of α-amylase activity could be caused by sucrose. However, the concentration of sucrose added to the holding solutions was relatively low and was supposed to be utilised at the begining of senescence. Therefore, the observed higher α-amylase activity in the phase of senescence (after appearance of first wilting symptoms) in response to chlorsulfuron + sucrose treatment could be discussed as an effect of chlorsulfuron. Germicides were not included in tested solutions although they contained sucrose. The development of rose buds when treated with sucrose solution was suppressed and this might be due to growth of some microorganisms. However, the treatments with solutions containing chlorsulfuron + sucrose prolonged vase-life and promoted good bud development. Senescence and stresses (biotic and abiotic) trigger to formation of active oxygen species. Since cytokinins have been found involved in defence mechanisms acting as free radical scavengers in stress situations, we suppose that in case of applied treatments the cytokinin properties of chlorsulfuron might be a reason for antimicrobial activity and could inhibit unfavourable oxidative processes leading to earlier senescence (Alexieva, 1993).

The physiological function of the hydrolytic enzymes α-amylase and invertase has been discussed extensively in relation with the mobilisation of sucrose and reserve starch. Recently it has been established that the activity of α- and β-amylase could be enhanced by different stress factors, due to *de novo* synthesis of enzyme units (Koizuka et al., 1995). In barley and tobacco leaves an increase of α-amylase activity in response to temperature (Dreier et al., 1995), water stress (Jakobsen et al., 1986) and pathogenic infections (Heitz et al., 1991) has been assessed. Some authors have suggested that activation of α-amylase might play an important physiological role in stress-situations. Stress-induced factors can shift the metabolic activity of the plant. Possibly under stress conditions cells require more sugars and carbon to meet the needs

of stress-related defence reactions. Breakdown products of starch and sucrose hydrolysis can serve as substrates in the cells of stressed tissues (Sturm and Chrispeels, 1990). It has been postulated (Mayak et al., 1975) that the senescence of cut rose and carnation petals and the related changes in carbohydrate status are under hormonal control. Our results with roses and chrysanthemums indicate that the applied componds with physiological activity (Glean-75 and sucrose) delay senescence processes and stimulate carbohydrate metabolism in petals. The present study showed that these substances cause an increase of α-amylase activity. Similar results have been obtained previously with other cut flowers (Yakimova, 1997). Although the herbicide Glean-75 was applied at a lower concentration than that needed to give herbicidal activity, it might cause a stress-effect. As the established dynamics of α-amylase activity is correlated with a prolongation of vase-life, it could be assumed that Glean-75 stimulates defence metabolic events. In the case of Glean-75 application, the effect could be also attributed to its cytokinin activity, previously documented in different model systems (Baskakov et al., 1981; Kulaeva et al., 1982; Karanov et al., 1992).

Since the effect of stress-induced activity of hydrolyses has been reported (Dreier et al., 1995), the present results concerning the effect of applied holding solutions on α-amylase activity in the petals of cut rose and chrysanthemum flowers could be explained by the accelerated synthesis of stress-proteins playing a role in the resistance to stress-conditions. This may provide a clue to a better understanding of the role of α-amylase and invertase in the response of cut flowers to stress factors.

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