

## THE EFFECT OF POLYAMINES ON PHYSIOLOGICAL PARAMETERS OF POST-HARVEST QUALITY OF CUT STEMS OF *Rosa* ‘Red Berlin’

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**Abstract:** The effect of conditioning of *Rosa* ‘Red Berlin’ stems in solutions of polyamines: spermidine at a concentration of 1.5 and 3 mmol dm<sup>-3</sup>, spermine at a concentration of 1 and 2 mmol dm<sup>-3</sup> as well as putrescine at 2 and 4 mmol dm<sup>-3</sup>, on their post-harvest quality were investigated. Advancing senescence of the stems of *Rosa* ‘Red Berlin’ resulted in their reduced post-harvest quality, which is evidenced by changes in the values of the plant physiological parameters. Conditioning of the rose stems in the solution of polyamines did not significantly affected the vase life. The most stabilising effect on cytoplasmic membranes of leaf cells had spermine at a concentration of 1 mmol dm<sup>-3</sup>. But the same polyamine applied at a twice higher concentration caused a decrease in the value of E<sub>L</sub> in petals. The highest values of leaf relative water content were found after conditioning of the rose stems in the solution of putrescine at a concentration of 2 mmol dm<sup>-3</sup>. The highest contents of photosynthetic pigments were noted after the application of spermidine at both concentrations. Conditioning of the rose stems in the solutions of each polyamine increased anthocyanin content in petals and had effect of the values on all analysing physiological parameters in leaves and petals.

**Key words:** anthocyanins, chlorophyll, carotenoids, polyamines, *Rosa*, RWC, electrolyte leakage.

### INTRODUCTION

A growing interest in research related to innovative techniques designed to enhance the quality of florist material is now observed worldwide. Plants available for sale must be of highest quality; this can be assessed visually by evaluating the shape, dimension, and colour of cut flowers and also by making sure that there is no mechanical damage and insect – or pathogen – induced damage [Gullino and Garibaldi 2007]. Another

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method used to check plant material quality is the determination of its post-harvest shelf life. This parameter is defined as an interaction between the plant genotype and the environmental conditions in which it is grown and stored after cutting.

Cut rose stems are a very delicate and easily damaged product. A loss of its decorative values can be observed already shortly after harvest: petal curling, desiccation and abscission, colour change, stalk withering or breaking. The quick loss of decorative values of rose stems is caused, *inter alia*, by disturbances in water transport to leaves and flower petals, which results in the appearance of symptoms evidencing increasing water stress in the stems. The appearing water stress is additionally enhanced by the process of transpiration and is also aggravated as a result of the increase of cells during the development of flower buds. Kumar et al. [2008a] suggest that there is an interaction between water transport disturbances and the content of abscisic acid and ethylene in rose petals. Studying two varieties of *Rosa hybrida*, these authors found a significant increase in the content of both phytohormones, correlated with advancing senescence of flowers and a substantial reduction in water potential in the stems. Since rose is considered to be a species sensitive to ethylene, inhibitors such as 1-MPC (1-methylcyclopropene) and STS (silver thiosulphate), are therefore frequently used for post-harvest conditioning of stems [Kumar et al. 2008b]. Due to high toxicity of the silver ion included in STS, there is a great interest in 1-MPC, which prevents the increased production of this phytohormone through permanent bonding to the ethylene receptor. Moreover, methylcyclopropenes prevent bud/flower desiccation and abscission as well as leaf yellowing already at very low concentrations [Kępczyński 2008]. The polyamine and ethylene synthesis pathways are also interrelated, which results from the competition for the common precursor, S – adenosylmethionine (SAM); hence, one synthesis pathway is stimulated and the other one inhibited [Bouchereau et al. 1999].

Polyamines are low molecular organic cations participating in many physiological and developmental processes in bacteria, animals, and plants. Putrescine, spermidine and spermine are most frequently found in higher plants. These compounds are closely related to many physiological processes, such as: fruit maturation, leaf senescence, development of reproductive organs, cell divisions and plant responses to stresses, including water and oxidative stress [Bais and Ravishankar 2002]. Polyamines also affect the blooming process through the induction of flowering [Kaur-Sawhney et al. 1988] and development of the floral organs [Kakkar and Rai 1993]. Sood and Nagar [2003, 2004] confirm changes in putrescine, spermidine and spermine content, depending on the developmental stage of *Rosa damascena* and *R. bourboniana* flowers. The participation of polyamines in the prevention of plant senescence [Niklas et al. 1998], through the inhibition of chlorophyll and protein degradation [Baraniak and Kostecka 1999], cell membrane stabilization [Martin-Tanguy 1997], and the inhibition of production of ethylene [Apelbaum et al. 1985], is also of essential importance. A study conducted on carnation stems conditioned in solutions of polyamines confirms their effect on the extension of vase life, but only when flowers were treated with polyamines at the bud stage [Upfold and Van Staden 1991].

The aim of the present study was to verify whether conditioning of *Rosa* ‘Red Berlin’ stems in polyamine solutions had an effect on their post-harvest quality. Vase life,

plant pigment content in leaves and petals, tissue water status and electrolyte leakage were analysed.

## MATERIALS AND METHODS

All experiments were done on cut stems of *Rosa* × *hybrida* ‘Red Berlin’ that were grown under polyhouse cultivation. All stems were harvested in the same day in morning hours, cut to a uniform length (40 cm) and placed in water in a cold storage room for 24 hours at a temperature of 4°C. Conditioning and the further stage of the experiment were carried out in a phytotron; 23/16°C day/night, relative air humidity of 60%, quantum irradiance of 190  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , with a light/dark cycle of 14/8 hours. The experiment consisted of seven treatment combinations, each of them comprising 15 rose stems. Directly after their transfer from the cold storage room, the stems were conditioned for 24 hours in aqueous solutions of spermidine (SPD) at a concentration of 1.5 and 3  $\text{mmol dm}^{-3}$ , spermine (SPM) at 1 and 2  $\text{mmol dm}^{-3}$  as well as putrescine (PUT) at 2 and 4  $\text{mmol dm}^{-3}$ . After that stems were transferred to containers with distilled water. Stems kept in distilled water, from the end of cold storage, were the control.

The flowers were examined daily and their vase life was recorded as the time to more than one-third petals wilted or bent neck. Electrolyte leakage ( $E_L$ ) from the tissues was measured following the method given by Kościelniak [1993] using a CC-317 microcomputer conductivity meter (Elmetron). Relative water content (RWC) was determined by the method of Barrs [1968], anthocyanins according to the method of Leng and Qi [2003]. The leaf content of photosynthetic pigments (chlorophyll “a”, chlorophyll “b”, and carotenoids) was determined after extraction in 80% acetone. Pigment content was calculated according to the method described by Lichtenthaler and Wellburn [1983].

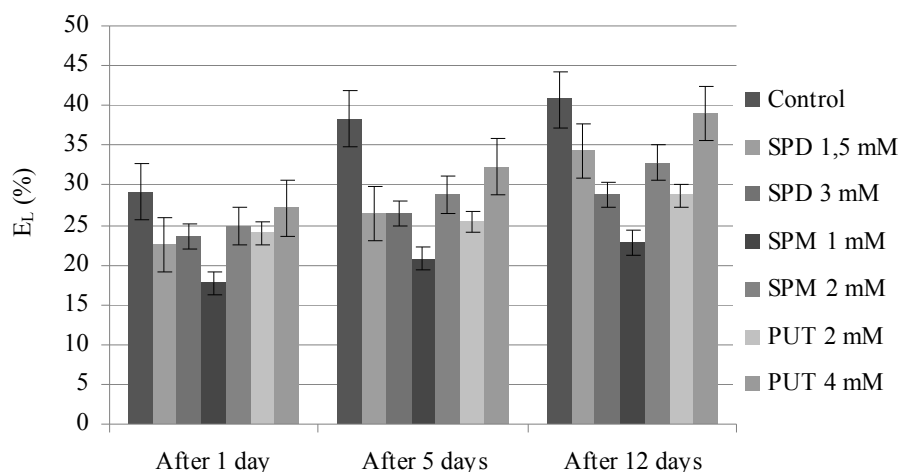
Data were taken from two independent experiments, and mean and standard error (SE) were determined. Measurements were performed in quintuplicate after 1, 5 and 12 days of keeping the rose stems in distilled water. Results were statistically analysed with SAS software package, ver. 9.1.3, while the significance of differences was assessed using Duncan’s confidence intervals at  $p = 0.05$ .

## RESULTS AND DISCUSSION

It has been reported that polyamines have the stimulated effects on the preservation of carnation [Luo at all. 2003; Bagni and Tassoni 2006], lilies [Geng at all. 2009] and gerbera [Bagni and Tassoni 2006]. In this study polyamines did not significantly affect the longevity of *Rosa* ‘Red Berlin’ (tab. 1). Also Nada at all. [2004] reported that cut flowers of *Rose* ‘Noblesse’ kept in spermidine withered earlier than those in distilled water. Additionally Rubinowska at all. [2012] confirmed no effect of spermine on prolonging the vase life of *Weigela florida* ‘Variegata Nana’.

Table 1. Effect of conditioning of *Rosa* 'Red Berlin' in the solutions of spermidine, spermine and putrescine on their post-harvest vase lifeTabela 1. Wpływ kondycjonowania pędów *Rosa* 'Red Berlin' w roztworach spermidyny, sperminy i putrescyny na ich trwałość pozbiorną

Conditioning substance Substancja kondycjonująca	Vase life (days) Trwałość (dni)
Control – Kontrola	8.60 a*
SPD 1.5 mmol · dm <sup>-3</sup>	9.39 a
SPD 3 mmol · dm <sup>-3</sup>	9.21 a
SPM 1 mmol dm <sup>-3</sup>	9.27 a
SPM 2 mmol dm <sup>-3</sup>	9.83 a
PUT 2 mmol dm <sup>-3</sup>	8.70 a
PUT 4 mmol dm <sup>-3</sup>	8.76 a

\* Means followed by the same letter within a column do not differ significantly at P<sub>0,05</sub>\* Średnie wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy P<sub>0,05</sub>Fig. 1. Effect of conditioning of *Rosa* 'Red Berlin' stems in polyamine solutions of varying concentrations on the value of electrolyte leakage ( $E_L$ ) in leaves. Vertical bars show standard errors of means ( $n = 5$ )Rys. 1. Wpływ kondycjonowania pędów *Rosa* 'Red Berlin' w roztworach poliamin o różnym stężeniu, na wartość wypływu elektrolitów ( $E_L$ ) w liściach. Pionowe linie obrazują błąd standardowy ( $n = 5$ )

Due to its location in the plant cell, the plasmalemma is the place where a plant's response to the effect of various adverse factors occurs earliest. During senescence, there also occur biochemical and biophysical changes in the cell membranes [Leurentz et al. 2002]. One of the first symptom of destabilisation of these structures is increased elec-

trolyte leakage ( $E_L$ ) from cells [Kacperska 1996]. Analysing the value of  $E_L$  under the conditions of the present experiment, the greatest disintegration of the cytoplasmic membranes was found on day 12 of the experiment, both in the leaves and petals of *Rosa* 'Red Berlin' (fig. 1, 2). Pogroszewska et al. [2009] also found an increase in electrolyte leakage from the petals of peony flowers, correlated with advancing senescence.

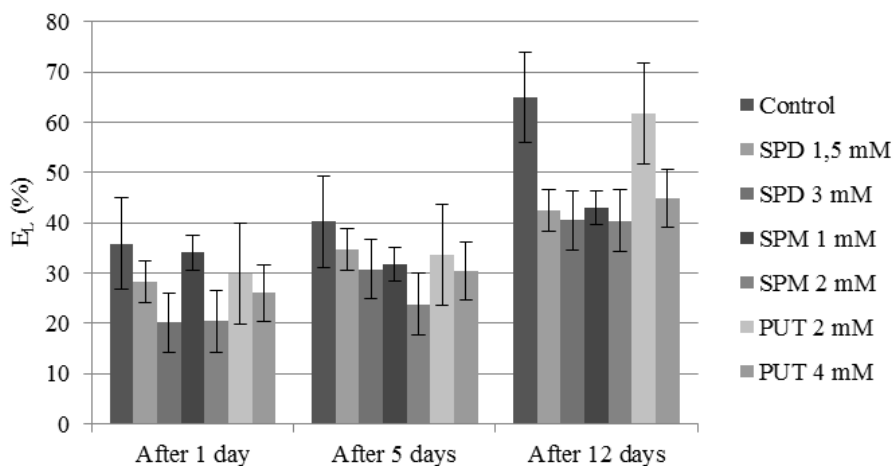


Fig. 2. Effect of conditioning of *Rosa* 'Red Berlin' stems in polyamine solutions of varying concentrations on the value of electrolyte leakage ( $E_L$ ) in petals. Vertical bars show standard errors of means ( $n = 5$ )

Rys. 2. Wpływ kondycjonowania pędów *Rosa* 'Red Berlin' w roztworach poliamin o różnym stężeniu, na wartość wypływu elektrolitów ( $E_L$ ) w płatkach. Pionowe linie obrazują błąd standardowy ( $n = 5$ )

As reported by Kumar et al. [2008a], senescence of cut rose stems was accompanied by a reduction in cytoplasmic membrane fluidity, which was caused by a decrease in the ratio of sterols to phospholipids. The quantitative ratio of saturated to unsaturated fatty acids also changes, and a consequence of these changes was the increased permeability. A significant effect of polyamine on the value of  $E_L$  in leaves and petals of *Rosa* 'Red Berlin' was also shown under the conditions of the present experiment (fig. 1, 2). The greatest disintegration of the membranes, was noted in the control plants, maintained in distilled water directly after cutting and cold storage. The most beneficial effect on the stability of leaf cell membranes was found for spermine at 1 mmol dm<sup>-3</sup>, and the value of  $E_L$  decreased by 39.3, 45.9 and 44.0%, respectively. In the petal cells the least damage to the cell membranes was found for SPD at 3 mmol dm<sup>-3</sup>, during the first measurement and SPM at 2 mmol dm<sup>-3</sup> during next measurements. The value of  $E_L$  decreased by 43.8, 41.0 and 37.9% respectively. A decrease in electrolyte leakage resulting from exogenous putrescine was also found by Rubinowska and Michałek [2009] who investigated the effect of PUT on senescence of sunflower plants. Pandey et al. [2000] explain the positive impact of polyamines on plant senescence by their ability to bind with

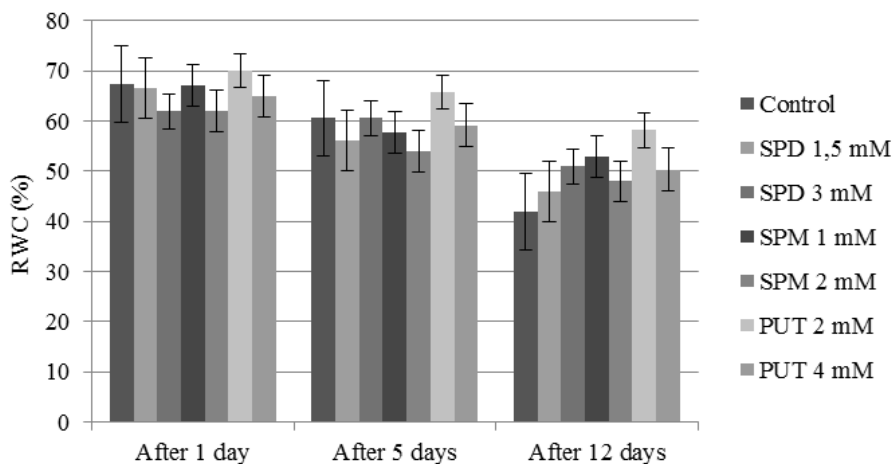


Fig. 3. Effect of conditioning of *Rosa* 'Red Berlin' stems in polyamine solutions of varying concentrations on the value of relative water content (RWC) in leaves. Vertical bars show standard errors of means ( $n = 5$ )

Rys. 3. Wpływ kondycjonowania pędów *Rosa* 'Red Berlin' w roztworach poliamin o różnym stężeniu, na wartość względną zawartości wody (RWC) w liściach. Pionowe linie obrazują błąd standardowy ( $n = 5$ )

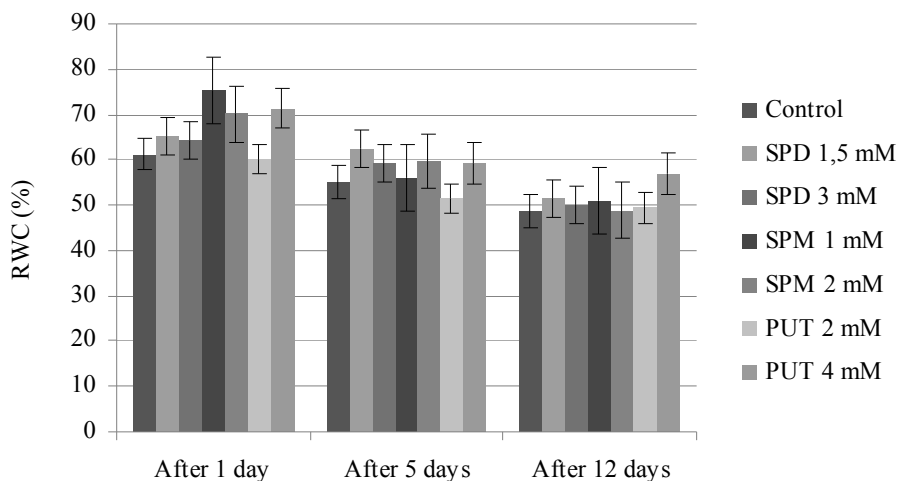


Fig. 4. Effect of conditioning of *Rosa* 'Red Berlin' stems in polyamine solutions of varying concentrations on the value of relative water content (RWC) in petals. Vertical bars show standard errors of means ( $n = 5$ )

Rys. 4. Wpływ kondycjonowania pędów *Rosa* 'Red Berlin' w roztworach poliamin o różnym stężeniu, na wartość względną zawartości wody (RWC) w płatkach. Pionowe linie obrazują błąd standardowy ( $n = 5$ )

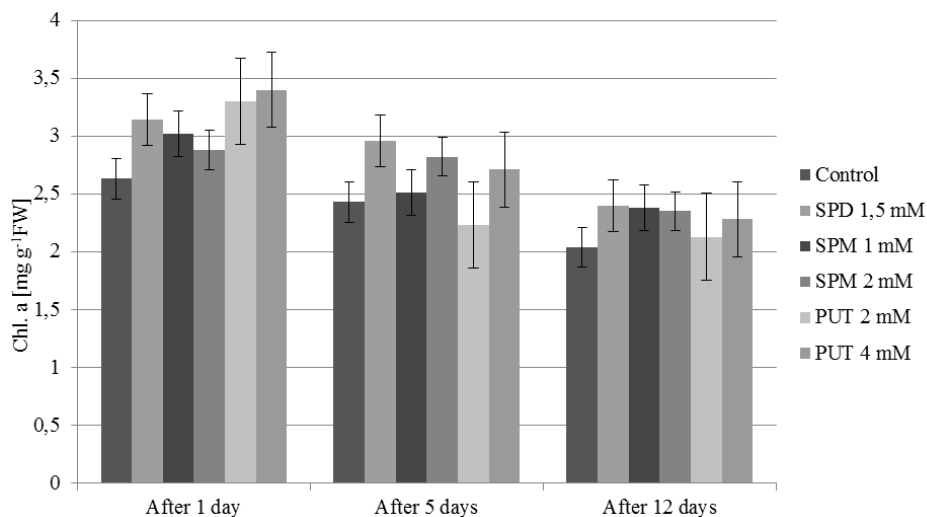


Fig. 5. Effect of conditioning of *Rosa* 'Red Berlin' stems in polyamine solutions of varying concentrations on the content of chlorophyll a in leaves. Vertical bars show standard errors of means (n = 5)

Rys. 5. Wpływ kondycjonowania pędów *Rosa* 'Red Berlin' w roztworach poliamin o różnym stężeniu, na zawartość chlorofilu a w liściach. Pionowe linie obrazują błąd standardowy (n = 5)

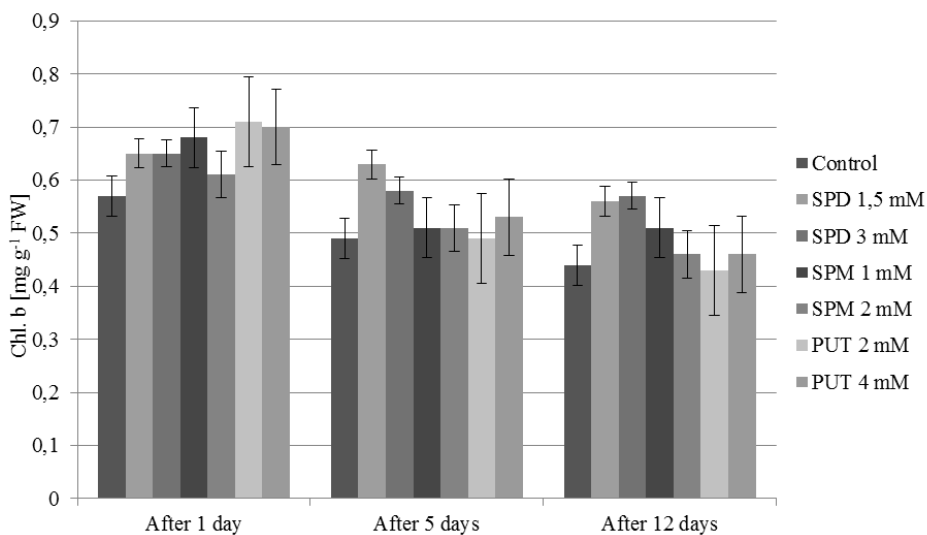


Fig. 6. Effect of conditioning of *Rosa* 'Red Berlin' stems in polyamine solutions of varying concentrations on the content of chlorophyll b in leaves. Vertical bars show standard errors of means (n = 5)

Rys. 6. Wpływ kondycjonowania pędów *Rosa* 'Red Berlin' w roztworach poliamin o różnym stężeniu, na zawartość chlorofilu b w liściach. Pionowe linie obrazują błąd standardowy (n = 5)

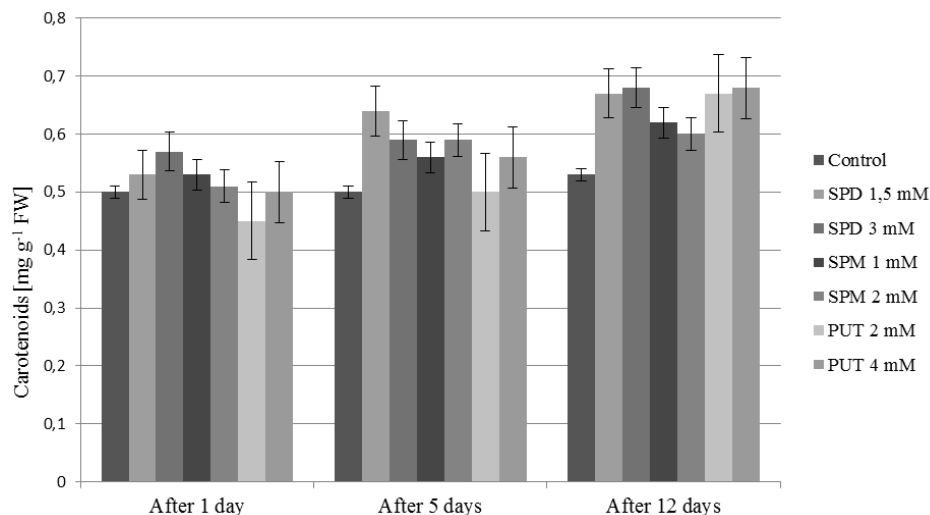


Fig. 7. Effect of conditioning of *Rosa* 'Red Berlin' stems in polyamine solutions of varying concentrations on the content of carotenoids in leaves. Vertical bars show standard errors of means (n = 5)

Rys. 7. Wpływ kondycjonowania pędów *Rosa* 'Red Berlin' w roztworach poliamin o różnym stężeniu, na zawartość karotenoidów w liściach. Pionowe linie obrazują błąd standardowy (n = 5)

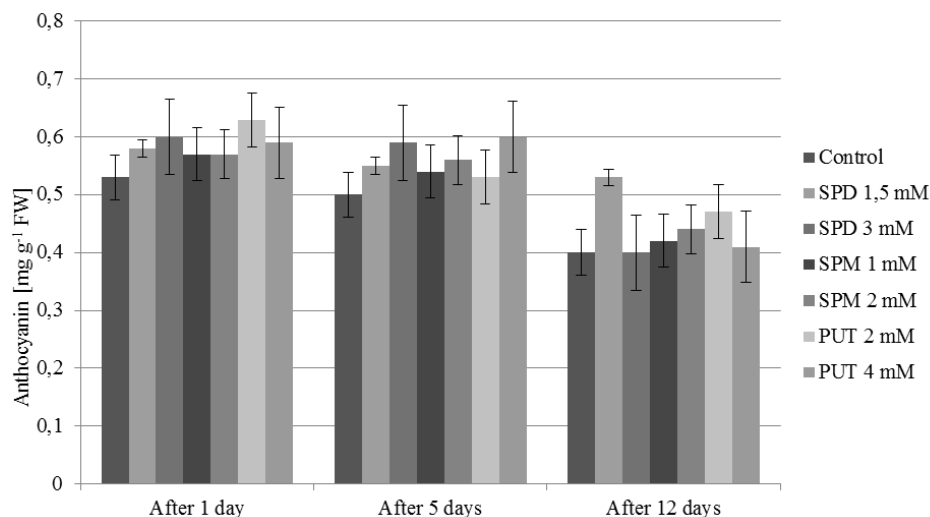


Fig. 8. Effect of conditioning of *Rosa* 'Red Berlin' stems in polyamine solutions of varying concentrations on the content of anthocyanin in petals. Vertical bars show standard errors of means (n = 5)

Rys. 8. Wpływ kondycjonowania pędów *Rosa* 'Red Berlin' w roztworach poliamin o różnym stężeniu, na zawartość karotenoidów w płatkach. Pionowe linie obrazują błąd standardowy (n = 5)



membrane phospholipids and other anion components of membranes, which results in increased stability of the structures. This was also confirmed by the observations of Roberts et al. [1986]. Authors found the binding of exogenous polyamines to the lipid membrane components, which resulted in reduced permeability of pea plants.

The level of relative water content (RWC) allows us to conclude that advancing senescence of leaves and petals of *Rosa* 'Red Berlin' was significantly correlated with changes of this parameter (fig. 3, 4). A decrease of RWC, was the evidence of transpiration advantage over the water uptake and of increasing water stress that accelerates the aging process. Studying the water relations in cut stems of 'Movie Star' rose, Lü et al. [2010] found a decrease of RWC correlated to the duration of the experiment. The results in Figure 3 indicate the highest relative water content in leaf tissues in the treatment with putrescine at 2 mmol dm<sup>-3</sup>. The value of RWC increased by 4.0, 8.6 and 39.0%, respectively. The highest hydration level in the petals was found for spermine at 1 mmol dm<sup>-3</sup> during the first measurement, spermidine at 1.5 mmol dm<sup>-3</sup> during the second measurement, and putrescine at 4 mmol dm<sup>-3</sup> during the third measurement (fig. 4). These amines increased RWC in petals by respectively, 22.8, 13.3 and 16.6%. Liu et al. [2000] and Yamaguchi et al. [2007] proposed a model describing a role of polyamines during drought stress, which is correlated with cut flowers senescence. Polyamines may modulate the activities of certain ion channels, especially Ca<sup>2+</sup> permeable channels and raise cytoplasmic Ca<sup>2+</sup> concentration. This event is known to inactivate the K<sup>+</sup> inward rectifier at the plasma membrane, which could stimulate stomatal closure. As a consequence polyamines causes an decrease in transpiration and may increase relative water content.

One of the recognised indicators of plant senescence is a decrease in chlorophyll "a" and "b" content with a simultaneous increase in leaf carotenoid content [Sood and Nagar 2003; Park et al. 2007]. The enhanced synthesis of carotenoids is also associated with their ability to scavenge free oxygen radicals (mainly due to their possession of conjugated double bonds) that are generated in increased amounts during oxidative stress accompanying plant senescence. They also react with singlet oxygen and organic radicals formed as a result of lipid peroxidation.  $\beta$ -carotene and lycopene belong to the most effective carotenoids [Sroka et al. 2005]. The analysis of photosynthetic pigment content during the experiment showed a reduction in chlorophyll "a" and "b" content with a simultaneous increase in carotenoid content, correlated with the duration of the experiment (fig. 5, 6, 7). Conditioning of the rose in the polyamines resulted in an increased content of all three photosynthetic pigments. The second measurement demonstrated the highest content of chlorophyll "a", chlorophyll "b" and carotenoids in treatment with spermidine at 1.5 mmol dm<sup>-3</sup>. The amount of pigments increased respectively by 21.81, 28.57 and 28.00%, compared to the control. On the other hand, 12 days after the experiment the highest content of pigments in the *Rosa* 'Red Berlin' stems was under the solution of spermidine at 3 mmol dm<sup>-3</sup>. The content of chlorophyll "a" increased by 29.41%, chlorophyll "b" by 29.54%, and carotenoids by 28.30%. These results were confirmed by Sood and Nagar [2003] studying the effect of polyamines on *R. damascena* and *R. burboniana* senescence. Serafini-Fraccasini et al. [2010] showed a positive influence of polyamines on *Lactuca sativa* leaf senescence. These authors report that spraying with spermine inhibited the degradation of chlorophylls, in particu-

lar chlorophyll “b”, which they explain by the ability of polyamines to bind with chloroplast proteins. Thus further enhancing the complex protein-chlorophyll stability and delaying its degradation.

The assay of anthocyanin content in petals of *R. ‘Red Berlin’* showed a decrease in the pigments content during senescence. Pogroszewska et al. [2009] found this correlation in their study of senescence in *Paeonia lactiflora*. The stability of anthocyanin pigments is strongly correlated with the value of vacuolar pH, which increases during flower senescence [Mazza and Miniati 1993]. Furthermore, anthocyanins are oxidized by peroxidases whose activity increases under the effect of oxidative stress, which is correlated with flowers senescence [Vaknin et al. 2005]. Conditioning of the rose in polyamines caused the inhibition of anthocyanins degradation. During the first measurement, the highest concentration of pigments was found in the treatment with putrescine at 2 mmol dm<sup>-3</sup> (an 18.13% increase, compared to the control). The measurement performed after 5 days of the experiment showed the highest pigment content after conditioning in putrescine at 4 mmol dm<sup>-3</sup>, whereas during the last assay after conditioning in spermidine at 1.5 mmol dm<sup>-3</sup> (an increase in pigment content by, respectively, 19.66 and 31.19%). Studying drought resistance of *Oryza sativa*, Farooq et al. [2009] also found an increase in anthocyanin content after exogenous application of polyamines, which they explain by the oxidative properties of the pigments.

## CONCLUSIONS

1. Advancing senescence of *Rosa ‘Red Berlin’* stems, after their cutting, resulted in their reduced post-harvest quality, conditioning in the solutions of polyamines did not significantly prolonged the vase life.

2. Conditioning of *Rosa ‘Red Berlin’* stems in the solution of spermine at a concentration of 1 mmol dm<sup>-3</sup> had a stabilising effect on the condition of the cytoplasmic membranes of leaf cells. The same polyamine applied at a twice higher concentration decreased the value of  $E_L$  in petals.

3. Conditioning of stems in the solution of putrescine at a concentration of 2 mmol dm<sup>-3</sup> resulted in the highest value of leaf relative water content.

4. Conditioning of *Rosa ‘Red Berlin’* stems in the solutions of spermidine, at both concentrations applied, inhibited degradation of the photosynthetic pigments. Furthermore, all applied polyamines caused an increase in anthocyanin content in petals.

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## **WPLYW POLIAMIN NA FIZJOLOGICZNE PARAMETRY POZBIORCZEJ JAKOŚCI CIĘTYCH PĘDÓW *Rosa* ‘Red Berlin’**

**Streszczenie:** Analizowano wpływu kondycjonowania pędów *Rosa* ‘Red Berlin’ w roztworach poliamin: spermidyny w stężeniu 1,5 i 3 mmol dm<sup>-3</sup>, sperminy w stężeniu 1 i 2 mmol dm<sup>-3</sup> oraz putrescyny w stężeniu 2 i 4 mmol dm<sup>-3</sup>, na ich jakość pozbiorną. Postępujący proces starzenia pędów *Rosa* ‘Red Berlin’, spowodował obniżenie ich jakości pozbiorną, o czym świadczą zmiany w wartościach badanych parametrów fizjologicznych roślin. Kondycjonowanie pędów róży w roztworach poliamin nie wpłynęło istotnie na wydłużenie ich okresu dekoracyjności. Najkorzystniejszy wpływ na stabilizację błon cytoplazmatycznych w komórkach liści miała spermina w stężeniu 1 mmol dm<sup>-3</sup>. Natomiast ta sama poliamina zastosowana w dwukrotnie wyższym stężeniu powodowała spadek wartości E<sub>L</sub> w płatkach. Najwyższe wartości wskaźnika względnej zawartości wody w liściach stwierdzono po kondycjonowaniu pędów w roztworze putrescyny w stężeniu 2 mmol dm<sup>-3</sup>. Najwyższe zawartości barwników asymilacyjnych zanotowano po zastosowaniu spermidyny w obydwu stężeniach. Kondycjonowanie pędów w roztworach poliamin zwiększało zawartość antocyjanów w płatkach, a także wpływało na wartości wszystkich analizowanych parametrów fizjologicznych.

**Słowa kluczowe:** antocyjany, chlorofil, karotenoidy, poliaminy, *Rosa*, RWC, wpływ elektrolitów

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