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## **The Appearance of Marginal Petal Necrosis as a Result of Specific Anthocyanins' and Flavonols' Changes in *Cyclamen persicum* MILL.**

By

Katja URBANEK, Franci ŠTAMPAR and Gregor OSTERC\*)

With 1 Figure

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### **Summary**

URBANEK K., ŠTAMPAR F. & OSTERC G. 2008. The appearance of marginal petal necrosis as a result of specific anthocyanins' and flavonols' changes in *Cyclamen persicum* MILL. – *Phyton* (Horn, Austria) 48 (1): 87–97, with 1 figure.

This paper studies the occurrence of marginal petal necrosis on cyclamen flowers during the fall's unfavourable climatic conditions, in regard to changes of specific anthocyanins and flavonols. Four different cyclamen Concerto® F<sub>1</sub> cultivars were used: Pink with Eye, Deep Rose Julia, Bright Scarlet and White Apollo. They had previously shown, in practice, a different tendency to petal necrosis. The appearance of necrosis was observed from middle October to the beginning of December. Anthocyanins and flavonols were determined separately in the slips and eyes of healthy and necrotic petals, and also in the necrotic tissue. The time-causal levels of cyanidin 3-galactoside and quercetin 3-galactoside were different in different cultivars. In Pink with Eye petals, which are strong susceptible to necrosis, a significantly decrease in cyanidin 3-galactoside was noticed between weeks 41 and 48. During the same period the levels of cyanidin 3-galactoside increased in Bright Scarlet petals, where no necrosis was observed. In all cultivars, however, the content of quercetin 3-galactoside increased in different areas of both healthy and necrotic petals, at the same time.

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\*) K. URBANEK, F. ŠTAMPAR, G. OSTERC, University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Institute for Fruit Growing, Viticulture and Vegetable Growing, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia; Tel./Fax.: +386 1 423 11 61/423 10 88; e-mail: [gregor.osterc@bf.uni-lj.si](mailto:gregor.osterc@bf.uni-lj.si)

## Zusammenfassung

URBANÉK K., ŠTAMPAR F. & OSTERC G. 2008. The appearance of marginal petal necrosis as a result of specific anthocyanins' and flavonols' changes in *Cyclamen persicum* MILL. [Das Auftreten der Blütenrandnekrosen in *Cyclamen persicum* MILL. in Abhängigkeit von der Änderung spezifischer Anthocyanine und Flavonole]. – *Phyton* (Horn, Austria) 48 (1): 87–97, mit 1 Abbildung.

In der vorliegenden Studie wurde das Auftreten von Blütenrandnekrosen auf Cyclamenblüten unter ungünstigen herbstlichen klimatischen Bedingungen in Bezug auf Konzentrations-änderungen spezifischer Anthocyanine und Flavonole untersucht. Es wurden vier verschiedene Kultivare der F<sub>1</sub> Serie Concerto® Pink with Eye, Deep Rose Julia, Bright Scarlet und White Apollo geprüft, die für das Nekroseauftreten unterschiedliche Empfindlichkeit zeigten. Das Auftreten der Nekrosen wurde von Mitte Oktober bis Anfang Dezember beobachtet. Anthocyanine und Flavonole wurden in den gesunden und in den nekrotischen Blüten getrennt untersucht, jeweils im Blütenblatt-rand und in der Blütenblattbasis (Auge). Zwischen den einzelnen Sorten wurden zeitbedingte Veränderungen von Cyanidin 3-galactosid und Quercetin 3-galactosid festgestellt. Zwischen der 41. und 48. Kulturwoche wurde in den Petalen des äußerst nekrosenempfindlichen Kultivars Concerto® Pink with Eye eine signifikante Verringerung von Cyanidin 3-galactosid nachgewiesen. In den Petalen von Concerto® Bright Scarlet, an denen keine Nekrosen vorhanden waren, haben sich die Werte von Cyanidin 3-galactosid in den gleichen Kulturwochen erhöht. Dagegen war Quercetin 3-galactosid sowohl in den gesunden als auch in den nekrotischen Petalen aller vier Kultivare deutlich erhöht.

## Introduction

The cyclamen (*Cyclamen persicum* MILL.) is a widely grown and increasingly produced pot plant. A continual supply means that the production-time in Central Europe is spread over the whole year from different sowing dates in the spring, through the summer growing season and on to the sales period from October to March. The main growing season for the later selling dates is, therefore, during the colder periods of the year, in late fall and winter. Due to difficult greenhouse conditions during this period, especially in regard to temperature and air humidity, growth technology is concerning itself with those pathogen and physiological diseases, which affect the selling-quality. Marginal petal necrosis is one of the main physiological disorders in cyclamen production.

Marginal necrosis often occurs on the petals and bracts of many ornamental plants independently of the senescence process or pathogen infection, causing loss of quality and ornamental value (TORRE & al. 2001). Flower necrosis usually appears on cyclamen at the upper-third of the petal, in the shape of a half-moon. Consequently, the petal's edges become dry and brown. The boundary line between the healthy and necrotic tissues is at more-intensive coloured cultivars usually dark-coloured, whereas the line is transparent for white flowers.

The main cause for the occurrence of petal necrosis on cyclamen has not been determined, as yet. Cultivar's characteristics relating to flower colour have been shown to have a great influence on the frequency of necrosis. It is well known, in practice, that red, purple and light pink cyclamen cultivars are more susceptible to marginal petal necrosis than others. External factors, such as temperature, air humidity, light, irrigation, and the application of chemicals can also affect discolouration of the petals' edges (TORRE & al. 2001). Since growth conditions and cultural methods have been reported as important factors that modify colour development in flowers and fruits (UBI 2004, ANTONEN & al. 2006), it is thus of interest to investigate the relation between the occurrence of petal necrosis and the content of anthocyanin pigments, which mainly determine the flowers' colours.

Previous studies concerning petal or bract necrosis have generally been limited to the effects of growing factors on post harvest characteristics of roses (STARKEY & PEDERSEN 1997, TORRE & al. 1999, CAPDEVILLE & al. 2005), and poinsettias (STARKEY & ANDERSSON 2000, WISSEMEIER & al. 2000). Overall, the pigmentation chemistry of these necrotic flowers has been largely unexplored.

The flower pigments of some cyclamen cultivars have been investigated previously, providing considerable information about flower colour biochemistry (WEBBY & BOASE 1999). However, cyclamen flowers vary in colour from white, through shades of pink and red to purple, with a more-intensely coloured inner-petal, termed as the "eye". The reddish colouration is mainly due to cyanidin 3-galactoside, one of the most commonly occurring anthocyanins (MARAIS & al. 2001). Flower colour is also influenced by various flavonols, usually acting as co-pigments. For these reasons, cyanidin 3-galactoside and its appurtenant flavonol, quercetin 3-galactoside, were the prime focus of our investigation.

The aim of the present study was to follow the appearance of cyclamen petal necrosis regarding any changes in cyanidin 3-galactoside and quercetin 3-galactoside levels in petals. Different cultivars of a widely grown large-flowered F<sub>1</sub> cyclamen series Concerto<sup>®</sup> were included in the experiment. In addition, we compared changes in both substances for both healthy and the necrotic petal tissues. The presented results could possibly serve as an essential key for understanding necrosis appearance on cyclamen petals.

## Material and Methods

### Plant Material

Seedlings of *Cyclamen persicum* cultivars Concerto<sup>®</sup> Pink with Eye, Deep Rose Julia, Bright Scarlet and White Apollo were imported from Syngenta seeds B.V. (Enkhuizen, The Netherlands). In total, 100 seedlings of each cultivar were used, di-

vided into four plots. According to the ROYAL HORTICULTURAL SOCIETY FLOWER COLOUR CHARTS 1986, Concerto® Pink with Eye has a light pink (RHS 56 D) outer-petal (slip) with a cyclamen purple (RHS 74 A) inner-petal (eye); Concerto® Deep Rose Julia has a Bengal rose (RHS 57 B) slip with a ruby red (RHS 61 A) eye, and the Concerto® Bright Scarlet has a Turkey red (RHS 46 C) slip with a darker red (RHS 59 A) eye. Concerto® White Apollo is a pure white cultivar (RHS 155 D).

17 week-old seedlings were planted in July (week 25) of 2005 in 13 cm plastic pots in a commercially growing media Fruhstorfer Erde® Type Cyclamen (Hawita Gruppe GmbH, Vechta, Germany). The plants were grown in a glass greenhouse in Maribor, Slovenia, using standard cultural practices. The night temperature was set at 10 °C; the day temperature depended on the weather conditions. The temperature and relative air humidity data were collected daily, using a bimetal thermograph and a hair hygograph. The plants were fertigated with 15N-10K-15P fertilizer (Flory® 3, Euflor GmbH, München, Germany) between weeks 30 and 33 and 8N-16K-24P fertilizer (Flory® 4, Euflor GmbH, München, Germany) from week 34 to the end of the experiment.

### Marginal Petal Necrosis and Sample Preparation

The occurrence of petal necroses was observed during the fall period from middle October (week 41) to the beginning of December (week 48). The number of necrotic flowers was counted on four sampling dates during weeks 41, 43, 46 and 48. Healthy and necrotic flowers were collected, at the same time. In the cases of healthy petals, five flowers were always collected, whereas the amount of necrotic material depended on the number of necrotic flowers at a particular sampling date. The petals were divided into three separate areas: the outer-petal (slip), the inner-petal (eye) and the marginal necrotic tissue. The divided petal parts were frozen in liquid nitrogen and stored at -20 °C until anthocyanin and flavonol analyses.

### Extraction and HPLC Analysis

The petals, previously frozen in liquid nitrogen, were grounded in a mortar using a pestle. Precisely 0.5 g was weighed into a test tube and then extracted with 5 ml of methanol containing 1 % (v/v) of HCl (GRIESBACH & al. 1999, FARZAD & al. 2002) in an ultrasonic bath for 2 hours. The extracted samples were centrifuged at 8000 g (Eppendorf centrifuge 5810 R, Hamburg, Germany) for 4 min at 4 °C and filtered through a polyamide Chromafil filter with a 45-µm pore diameter (Macherey-Nagel, Düren, Germany). The samples were stored at -20 °C until used for HPLC analyses.

Analytical high-performance liquid chromatography (HPLC) was performed using a Surveyor HPLC system (Thermo Finnigan, San Jose, USA) coupled with a diode array detector. The system was controlled using the ChromQuest 4.0 chromatography workstation software system. Separations were carried-out using a Gemini 3u C18 110A column (150 × 4.60 mm) from Phenomenex (Torrence, USA). Elution (0.8 ml min<sup>-1</sup>, 30 °C) was performed using a solvent system comprising solvent A (1.5 % H<sub>3</sub>PO<sub>4</sub>) and solvent B (HOAc-CH<sub>3</sub>CN-H<sub>3</sub>PO<sub>4</sub>-H<sub>2</sub>O, 20: 24: 1.5: 54.5) mixed using a linear gradient starting with 80 % A, decreasing to 33 % A at 30 min, 10 % A at 33 min, and 0 % A at 39.3 min (MITCHELL & al. 1998). Anthocyanins were detected at 530 nm and flavonols at 352 nm. Flavonols were determined in all four cultivars, while anthocyanins only in Concerto® Pink with Eye, Deep Rose Julia, and Bright Scarlet.

The cyanidin 3-galactoside and quercetin 3-galactoside were identified by comparing their UV-vis spectra with those obtained from standards (Fluka Chemie GmbH, Buchs, Switzerland) in combination with retention times, and also by the addition of standard solutions. Quantification was achieved according to the concentrations of a corresponding external standard.

### Statistical Analysis

Statistical analyses were carried-out using the Statgraphics plus 4.0 program (Manugistics, Inc., Rockville, USA), with the one-way analysis of variance (ANOVA). Statistically significant differences between sampling dates were tested with the Least Significant Difference (LSD) multiple-range test at the 0.95 confidence level.

## Results

### Greenhouse Air Temperature and Relative Air Humidity

Due to unstable autumn weather conditions the greenhouse temperature and relative air humidity varied through the observing period. Until week 43 the predominantly sunny weather stimulated high day greenhouse temperatures, in average reaching up to 25 °C. The night temperature was mainly higher than the set temperature 10 °C. Consequently, the day relative air humidity values markedly decreased (up to 40 %), while the night relative air humidity stayed stable near 90 %.

From week 44 to the end of experiment the average day greenhouse temperature fell under 15 °C. Due to the cold weather in week 48 we had to decrease the night temperature on 8 °C. The day temperature in week 48 rarely exceeded 10 °C, while the day relative air humidity reached the unfavourable night values (90 %).

### Marginal Petal Necrosis

The number of necrotic flowers increased throughout the observed period in all cultivars with necrotic symptoms, Concerto® Pink with Eye, Deep Rose Julia, and White Apollo (Fig. 1). Increase in the occurrence of flower necroses was most pronounced in Pink with Eye. The number of pink necrotic flowers significantly increased in week 48, reaching an almost six-time higher value, 35.5, than the 6.3 at the beginning of the observation. In contrast, the number of necrotic flowers in White Apollo continuously increased from weeks 41 to 46, but slightly decreased on the last sampling date. In Deep Rose Julia, variation in the number of necrotic flowers showed no significant changes, although the smallest amount of necrotic flowers was also observed in week 41. In Bright Scarlet, we determined only one necrotic flower in week 43.

Besides an increase in the number of necrotic flowers, Fig. 1 also shows the differences in necrosis appearance between different Concerto® cyclamen cultivars. A markedly larger number of necrotic flowers was



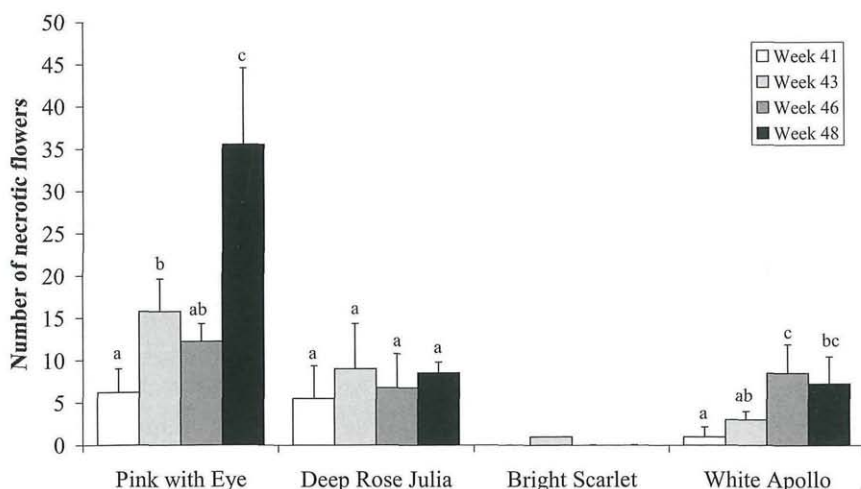


Fig. 1. The number of necrotic flowers of cultivars Concerto® Pink with Eye, Deep Rose Julia, Bright Scarlet and White Apollo at different sampling dates. Bars represent  $\pm$  SD. Means with different letters are statistically different for each cultivar ( $P = 0.05$ ; LSD test).

observed in cultivar Pink with Eye, followed by Deep Rose Julia, and White Apollo, whereas the flowers of Bright Scarlet showed a negligible amount of damage or discolouration of the petals' edges.

#### Changes in Cyanidin 3-Galactoside and Quercetin 3-Galactoside

The content of cyanidin 3-galactoside in Concerto® Pink with Eye decreased during the experiment in all parts of healthy and necrotic flowers (Table 1). In healthy petals, the cyanidin 3-galactoside content slightly decreased from weeks 41 to 46, until reaching the significantly lowest values in week 48. In necrotic tissue, similarly, the significantly highest content of cyanidin 3-galactoside ( $276.1 \mu\text{g g}^{-1}$  FW) was measured in week 41. After a strong decrease in week 43 ( $84.9 \mu\text{g g}^{-1}$  FW), it gradually increased till the end of the experiment, however, keeping much lower values than in week 41 ( $152.6 \mu\text{g g}^{-1}$  FW).

In the slips and eyes of healthy Deep Rose Julia petals, the content of cyanidin 3-galactoside remained at almost the same level throughout the whole observation period (Table 1). In contrast, the cyanidin 3-galactoside content in the slips and eyes of necrotic petals and the necrotic tissue decreased in week 43, but increased again in week 48, keeping lower values than at the first sampling date.

The results when determining the pigments in Bright Scarlet were only present in healthy petals due to the absence of marginal petal ne-

crosses. Compared to Pink with Eye and Deep Rose Julia, the content of cyanidin 3-galactoside in the slip of Bright Scarlet significantly increased during the experiment (Table 1). In the eye, however, the cyanidin 3-galactoside content gradually increased from weeks 41 to 46, but significantly decreased at the last sampling date.

Table 1. The content of cyanidin 3-galactoside ( $\mu\text{g g}^{-1}$  FW) in different petal areas of cultivars Concerto<sup>®</sup> Pink with Eye, Deep Rose Julia and Bright Scarlet at different sampling dates.

Cultivar	Petal area*	Week 41	Week 43	Week 46	Week 48
Pink with Eye	HS	71.0 $\pm$ 20.6 <sup>#</sup> a	72.8 $\pm$ 18.9 a	72.0 $\pm$ 17.0 a	41.6 $\pm$ 7.8 b
	HE	135.5 $\pm$ 19.2 a	124.7 $\pm$ 18.5 a	121.5 $\pm$ 18.5 a	84.6 $\pm$ 8.5 b
	NS	63.4 $\pm$ 12.5 b	44.5 $\pm$ 7.6 a	43.0 $\pm$ 4.6 a	50.3 $\pm$ 7.6 a
	NE	110.8 $\pm$ 10.6 a	110.6 $\pm$ 13.8 a	102.6 $\pm$ 3.2 ab	93.6 $\pm$ 12.2 b
	N	276.1 $\pm$ 108.5 b	84.9 $\pm$ 54.1 a	120.0 $\pm$ 66.9 a	152.6 $\pm$ 85.5 a
Deep Rose Julia	HS	1888.2 $\pm$ 290.4	1971.6 $\pm$ 33.0	2075.9 $\pm$ 373.7	1734.8 $\pm$ 206.7
	HE	1273.2 $\pm$ 194.4	1364.3 $\pm$ 44.7	1533.1 $\pm$ 130.9	1423.1 $\pm$ 166.2
	NS	2435.7 $\pm$ 233.4	1776.4 $\pm$ 208.3	ND <sup>§</sup>	2124.2 $\pm$ 330.6
	NE	1729.7 $\pm$ 344.6	1236.2 $\pm$ 207.3	ND	1522.9 $\pm$ 28.0
	N	11872.2 $\pm$ 1088.7	7899.8 $\pm$ 4043.2	ND	10189.7 $\pm$ 1635.9
Bright Scarlet	HS	2.4 $\pm$ 0.8 a	2.6 $\pm$ 0.4 a	3.1 $\pm$ 1.4 ab	4.8 $\pm$ 1.8 b
	HE	0.6 $\pm$ 0.6 a	1.7 $\pm$ 0.9 bc	2.5 $\pm$ 0.5 c	1.2 $\pm$ 0.2 ab

\* Petal areas: HS: slip from a healthy petal; HE: eye from a healthy petal; NS: slip from a necrotic petal; NE: eye from a necrotic petal; N: necrotic tissue; <sup>#</sup> Data are reported as means  $\pm$  SD. Means in a row followed by different letters are significantly different for each petal area ( $P = 0.05$ ; LSD test); <sup>§</sup> ND: not determined because of deficiency of plant material.

While the cyanidin 3-galactoside content generally showed a decreasing tendency during the observation period, the content of quercetin 3-galactoside increased in most cultivars at the same time, with the exception of the pink necrotic tissue.

In the healthy petals of Pink with Eye, the quercetin 3-galactoside content showed the lowest values at the first and the largest values at the last sampling dates (Table 2). The necrotic tissue, however, contained the highest amount of quercetin 3-galactoside at the beginning of observation (592.3  $\mu\text{g g}^{-1}$  FW), later the flavonol content strongly decreased in week 43, reaching the lowest value at the end of the experiment (335.4  $\mu\text{g g}^{-1}$  FW).

In Deep Rose Julia and Bright Scarlet, an increasing pattern of quercetin 3-galactoside was determined (Table 2). In both cultivars, the values significantly decreased in week 43 and gradually increased at the last two sampling dates. In the necrotic tissue of Deep Rose Julia, the quercetin 3-galactoside content in week 48 (51.4  $\mu\text{g g}^{-1}$  FW) almost reached the same amount as at the beginning of observation (53.6  $\mu\text{g g}^{-1}$  FW) (Table 2).

Table 2. The content of quercetin 3-galactoside ( $\mu\text{g g}^{-1}$  FW) in different petal areas of cultivars Concerto® Pink with Eye, Deep Rose Julia, White Apollo and Bright Scarlet at different sampling dates.

Cultivar	Petal area*	Week 41	Week 43	Week 46	Week 48
Pink with Eye	HS	159.8 $\pm$ 18.5 <sup>#</sup>	167.7 $\pm$ 35.5	150.3 $\pm$ 6.0	193.2 $\pm$ 25.4
	HE	89.7 $\pm$ 18.6	110.1 $\pm$ 2.0	81.6 $\pm$ 1.4	97.3 $\pm$ 12.4
	NS	117.1 $\pm$ 19.9 a	162.3 $\pm$ 9.2 b	135.7 $\pm$ 13.5 ab	198.8 $\pm$ 22.6 c
	NE	85.1 $\pm$ 28.5 a	89.3 $\pm$ 15.7 a	96.6 $\pm$ 26.4 a	133.6 $\pm$ 28.2 b
	N	592.3 $\pm$ 360.5	356.9 $\pm$ 262.4	389.3 $\pm$ 205.1	335.4 $\pm$ 223.8
Deep Rose Julia	HS	9.6 $\pm$ 1.5 ab	8.0 $\pm$ 1.3 a	13.4 $\pm$ 0.6 c	11.9 $\pm$ 2.4 bc
	HE	8.0 $\pm$ 1.6 ab	6.1 $\pm$ 0.5 a	8.1 $\pm$ 1.9 ab	9.2 $\pm$ 1.9 b
	NS	12.6 $\pm$ 6.2	9.9 $\pm$ 1.7	ND <sup>§</sup>	16.5 $\pm$ 2.4
	NE	7.2 $\pm$ 0.8 a	7.4 $\pm$ 0.3 a	ND	11.4 $\pm$ 1.9 b
	N	53.6 $\pm$ 9.5	43.5 $\pm$ 14.2	ND	51.4 $\pm$ 3.3
White Apollo	HS	11.2 $\pm$ 3.6	19.9 $\pm$ 7.9	10.8 $\pm$ 3.8	15.8 $\pm$ 8.1
	HE	4.5 $\pm$ 2.1 a	10.4 $\pm$ 1.5 c	6.2 $\pm$ 0.6 ab	7.3 $\pm$ 1.0 b
	NS	ND	11.4 $\pm$ 0.0	ND	24.5 $\pm$ 2.9
	NE	ND	4.8 $\pm$ 0.0	ND	14.6 $\pm$ 0.8
	N	ND	77.7 $\pm$ 0.0	ND	178.5 $\pm$ 159.5
Bright Scarlet	HS	7.9 $\pm$ 0.9	4.3 $\pm$ 1.1	8.4 $\pm$ 4.4	8.2 $\pm$ 4.3
	HE	6.2 $\pm$ 2.5	4.4 $\pm$ 1.8	7.1 $\pm$ 0.3	7.3 $\pm$ 3.4

\* Petal areas: HS: slip from a healthy petal; HE: eye from a healthy petal; NS: slip from a necrotic petal; NE: eye from a necrotic petal; N: necrotic tissue; <sup>#</sup> Data are reported as means  $\pm$  SD. Means in a row followed by different letters are significantly different for each petal area ( $P = 0.05$ ; LSD test); <sup>§</sup> ND: not determined because of deficiency of plant material.

Only the flavonol content was determined in White Apollo. The results of quercetin 3-galactoside analysis showed relatively high values compared with the red and rose cultivars (Table 2). Due to deficiency of plant material, the quercetin 3-galactoside content in necrotic flowers was measured only in weeks 43 and 48. Changes in quercetin 3-galactoside content in the healthy petals of White Apollo followed a similar pattern as that in Pink with Eye, except that the values of white necrotic tissue increased in week 48 (178.5  $\mu\text{g g}^{-1}$  FW).

## Discussion

It is well known, in practice, that necrosis appearance on cyclamen flowers becomes more frequent in the unfavourable growing conditions of the late fall. This corresponds well with the results of this study, since the number of necrotic flowers increased throughout the observed period from week 41 (middle October) to week 48 (beginning of December) in all three Concerto® cultivars with necrotic symptoms.



The reason for increased necrosis appearance on cyclamen flowers during the fall has not been determined, yet. It has been well documented in other ornamental plants, that increased air humidity and lower light intensity affect the quality parameters of flowers, due to decreased translocation of nutrients and assimilates (TORRE & al. 2001, STARKEY & ANDERSSON 2000). In our experiment, the cyclamens were mostly exposed to difficult weather conditions with low temperatures and high air humidity in week 48 (end of November), when changes in the occurrence of petal necrosis were also the most significant. However, since the number of petal necroses for rose and red flowers changed negligible in comparison to that of the pink ones, we can stress the importance of cultivar-related sensibility to changes in growing conditions.

In our previous experiment with the cyclamen series Halios® flower necroses were most frequent at the pink and salmon, but also at the red flowers (unpublished data). Since the results of this present study clearly show that the red cultivar Concerto® Bright Scarlet actually did not develop any petal necroses, the effects of cultivar characteristics are surely of great importance. The reason why some flower colours are more susceptible to petal necroses is not yet understood, but may be due to the content of flower pigments, especially anthocyanins.

In this study the occurrence of marginal petal necrosis on cyclamen flowers during the fall's climatic conditions was evaluated, in regard to any changes of cyanidin and quercetin 3-galactoside content in healthy and necrotic cyclamen petals. Generally, any changes in cyanidin 3-galactoside and quercetin 3-galactoside were similar in all petal parts of a particular cultivar, whereas the change patterns and contents of the considered pigments varied greatly between different flower colours.

The results of anthocyanin analyses in different coloured cyclamen cultivars showed that the content of cyanidin 3-galactoside in Pink with Eye and Deep Rose Julia decreased during the observation; while in Bright Scarlet it interestingly increased at the same time. Moreover, the red petals showed lower amounts of cyanidin 3-galactoside than the pink and especially the deep rose flowers, which had the highest content of the anthocyanin. Since the appearance of petal necrosis was the most frequent in Pink with Eye, not in Deep Rose Julia, it could be suggested that the occurrence of petal necrosis is more affected by the changes of an individual flower pigment than by the amount of the pigment. Accordingly, Bright Scarlet did not develop any petal necroses during the same experimental conditions because of the increasing pattern of cyanidin 3-galactoside content. Furthermore, increase in the number of marginal petal necrosis in Pink with Eye in week 48 might be due to the significant decrease in cyanidin 3-galactoside at the same sampling date.

The physiological reason for the decrease in cyanidin 3-galactoside content in pink and rose flowers is not clear. Since the healthy and necrotic

petals showed the same changes in anthocyanin contents, it could be concluded that change in floral pigments is due to the presence of one or more external factors, which affect anthocyanin biosynthesis. Light is reported to be one of the most important factors that influence the anthocyanin production in many plants (FARZAD & al. 2002, KAWABATA & al. 1999). Since we did not use additional lightening in our experiment, the plants were exposed to lower light intensity during the fall period. Furthermore, it is well known that the intensity of flower colour and the content of main anthocyanins are greatly affected by temperature (UBI 2004, MARAIS & al. 2001, NOZAKI & al. 2006). Moreover, low temperatures promote anthocyanin synthesis (OREN-SHAMIR & al. 2003). Due to a decrease in the greenhouse's temperature at the end of November (week 48), it could be assumed that the anthocyanin content would increase. However, our results clearly showed a decrease in the content of cyanidin 3-galactoside, suggesting that temperature alone does not cause changes in flower pigment values.

The increase in quercetin 3-galactoside content can also be explained by the effect of growing conditions on the metabolism of phenolic compounds. The role of flavonoids, including flavonols, in plant protection systems has been widely discussed previously (TROBEC & al. 2005, OSTERC & al. 2007). Petal necrosis as a physiological disorder represents a stress situation for the plant, especially for the flower. Accordingly, increase in quercetin 3-galactoside in cyclamen petals throughout the fall period could be a protective plant response to unfavourable environmental conditions under which anthocyanin biosynthesis is evidently reduced.

Our present results represent a further addition regarding problematic cyclamen petal necrosis to those, conducted in the past, which dealt mainly with environmental conditions. In our research the dynamics of cyanidin 3-galactoside is inversely proportional to the presence of petal necrosis, whereas the dynamic of quercetin 3-galactoside is opposite. Therefore, in the future our attention should focus on those different conditions, which are responsible for changes in the metabolic pathways of anthocyanins and flavonols.

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Autor(en)/Author(s): Urbanek Katja, Stampar Franci, Osterc Gregor

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