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ORIGINAL PAPER

The effect of fluridone on breaking the dormancy of inflorescence shoots and the content of pigments in *Muscari armeniacum* Leichtl. ex Baker leaves*

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Abstract

The herbicide fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl(phenyl)]-4(1H)-pyridinone) affects processes related to plant growth and development. It interferes with carotenoid biosynthesis by inhibiting the activity of phytoene desaturase, which converts phytoene to phytofluene. Besides, fluridone indirectly inhibits abscisic acid (ABA) biosynthesis. The aim of the present study was to examine the effect of fluridone on plant pigments and proanthocyanidins in *Muscari armeniacum* leaves and on the dormancy of inflorescence shoots. In our experiments, uncooled *M. armeniacum* bulbs with fully differentiated adventitious roots were used. Three-week soaking of bulbs in water solutions of fluridone at concentrations of 5 and 10 mg dm^{-3} significantly inhibited the root growth of *M. armeniacum* plants. Besides, fluridone caused degradation of chlorophylls and carotenoids in the primary leaves of this species. Leaves obtained from fluridone-treated bulbs showed a pink colour due to the presence of anthocyanins, but histological analysis showed that these pigments were found only in the leaf parenchyma and not in the epidermis. Secondary leaves grew from bulbs after a long, 54-day treatment with fluridone contained almost no carotenoids and chlorophylls. This longer treatment of *M. armeniacum* bulbs also resulted in a large increase in the anthocyanin and proanthocyanidin content. The high anthocyanin content in leaves is a visible effect of fluridone-induced stress. Application of fluridone to *M. armeniacum* bulbs resulted also in breaking the dormancy of inflorescence shoots.

Keywords: anthocyanins, dormancy, fluridone, *Muscari armeniacum,* photosynthetic pigments

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INTRODUCTION

Muscari armeniacum Leichtl. ex Baker is a flowering plant in the subfamily *Scilloideae,* and is called the grape hyacinth, garden grape hyacinth or Armenian grape hyacinth (Bokov 2019). In this species, an extended period of the cold season is required to release the dormant state in preparation for stem elongation and flowering (Saniewski et al. 2016). It is interesting that leaf growth begins without cooling the bulbs, but this process is necessary for inflorescence growth and flowering (Saniewski 1978, Hanks, Jones 1987).

The effect of plant hormones, gibberellic acid (GA), benzyladenine (BA), abscisic acid (ABA), indole-3-acetic acid (IAA) and methyl jasmonate (JA-Me), on the growth of leaves and inflorescence stalk of *M. armeniacum* has been studied (Hanks, Jones 1987, Saniewski et al. 2016). GA in cooled bulbs stimulated the growth of leaves and inflorescence stalk in their early stages but the final length of these organs was similar to that in control plants. In contrast, no effect of GA on leaf growth in uncooled *M. armeniacum* bulbs was observed (Saniewski et al. 1978).

Application of IAA at the site of a removed inflorescence bud of *M. armeniacum* induced growth of the plant's inflorescence stalk (Saniewski et al. 2016). Leaf growth was induced when *M. armeniacum* bulbs were maintained in solutions of GA and BA, but ABA inhibited this growth. None of these treatments broke the dormancy of the inflorescence stalk in *M. armeniacum* bulbs (Saniewski et al. 2016). Besides, formation of new bulbs in this species was induced by BA treatment of intact bulbs (Saniewski 1978), but GA and auxins had an inhibitory effect on this process (Saniewski, Puchalski 1982).

Anthocyanins are naturally occurring plant pigments, which are accumulated in response to various environmental stresses, as has been widely reported recently (Dabravolski and Isayenkov 2023). Data obtained by Gould et al. (2000), indicate that anthocyanins in leaves are associated with photosynthesis and may serve to protect chloroplasts from high-intensity sunlight.

Proanthocyanidins (PA), also referred to as condensed tannins, are the most abundant plant-derived polyphenols. Their major function is to provide protection against biotic and abiotic stresses (Dixon et al. 2005, Smeriglio et al. 2017). They are formed by the polymerization of flavan-3-ols (Alejo-Armijo et al. 2020). Their name refers to the fact that they are converted into anthocyanidins after acid hydrolysis, which is the basis for the classical analysis of these compounds (Porter 1989).

Fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl(phenyl)]-4(1H)-pyridinone) is the active ingredient of the herbicide Sonar® which interferes with carotenoid biosynthesis, increasing the phytoene level and decreasing the β-carotene content (Doong et al. 1993, Sprecher et al. 1998, Benoit, Les 2013, Nisar et al. 2015). Carotenoids protect chlorophyll from photo-oxidation,

and therefore reduced levels of carotenoids also lower the chlorophyll level in fluridone-sensitive plant tissues (Nelson et al. 2002). Increased phytoene concentrations and decreased levels of *β*-carotene and chlorophyll are a typical response to fluridone and this has been documented in many aquatic plant species (Drexler, Flechter 1981, Netherland, Getsinger 1995). It was previously found that fluridone lowered the lycopene, *β*-carotene and lutein content in the ripening tomato fruit (Góraj-Koniarska et al. 2017, Wiczkowski et al. 2019).

Fluridone also inhibits the biosynthesis of some plant hormones (Bartels, Watson 1978, Sprecher et al. 1998). Fluridone was an effective inhibitor of ABA biosynthesis in *A. chilensis* plants (Jiang, Joyce 2003) and in wheat kernels (Rasmussen et al. 1997). Fluridone also inhibited ABA biosynthesis but increased the concentration of gibberellins in *Cistanche tubulosa* seeds, which might initiate processes associated with their germination (Chen et al. 2016). ABA may play an important role in regulating the accumulation of phenolic compounds, including anthocyanins, which has been demonstrated in plants under drought stress (Jiang, Joyce 2003, Gonzalez-Villagra et al. 2019). In the ripening fruit of figs (*Ficus carica*), application of fluridone significantly reduced anthocyanin levels in contrast to ABA (Lama et al. 2020). Besides, Karppinen et al. (2018) showed that fluridone inhibited anthocyanin accumulation in ripening blueberry (*Vaccinium myrtillus*) fruit.

According to Yamazaki et al. (1999), fluridone reduced the number of buds in the Japanese bunching onion and shallots. It was also shown that soaking bulbs of the Oriental hybrid lily (*Lilium oriental*) in fluridone prior to cooling treatment increased the height of the plants and the number of flowers (Yang et al. 2015). Besides, application of fluridone prevented aging and prolonged the life of *Hibiscus rosa-sinensis* flowers (Trivellini et al. 2011) and *Theobroma cacao* flowers (Aneja et al. 1999). By inhibiting strigolactone biosynthesis, fluridone promoted adventitious rooting in cuttings of *Pisum sativum, Plumbago auriculata* and *Jasminum polyanthum* (Rasmussen et al. 2012). However, it had no effect on the rooting of *Tradescantia fluminensis* and *Trachelospermum jasminoides* cuttings. Previously, Harvey et al. (1994) showed that fluridone stimulated the rooting of nodal segments of potato (*Solanum tuberosum*) stems grown in the dark, and that application of ABA prevented this response.

Primary roots of *Zea mays* seedlings in the presence of fluridone grew much slower than untreated ones (Ng, Moore 1985). Primary root elongation of maize seedlings at low water potential was inhibited by reducing ABA levels after fluridone treatment or in the vp5 mutant to inhibit carotenoid biosynthesis and promote shoot elongation (Saab et al. 1992, Sharp et al. 1994). In *Arabidopsis thaliana*, fluridone increases auxin levels, inhibits primary root growth and suppresses carotenoid biosynthesis (Xu et al. 2023).

A preliminary study of the effect of fluridone used for soaking *M. armeniacum* bulbs showed the formation of light purple pigmentation in the lower parts of the leaves of this plant (Marasek-Ciołakowska et al. in press).

Therefore, the aim of the current study was to investigate the effect of fluridone on the content of plant pigments and proanthocyanidins in the leaves of *M. armeniacum* and the effect on the dormancy of inflorescence shoots obtained from uncooled bulbs of this species.

MATERIALS AND METHODS

Plant material and experimental methods

Uncooled *Muscari armeniacum* bulbs with a circumference of 3-4 cm were used for the study. Fifteen bulbs per one replicate were used for each treatment. The first part of the experiment was started on 23 October 2023 by keeping the bulbs continuously in distilled water (control) or in fluridone solutions of 5 and 10 mg dm^{-3} at 17-22°C under natural light conditions in a greenhouse at the National Institute of Horticultural Research in Skierniewice, Poland. Initially, the bulbs were placed on Petri dishes with a diameter of 12 cm. As the shoots and inflorescences grew, the bulbs were transferred to 0.25 dm^3 beakers.

During the experiment, morphological observations and measurements of plant growth were conducted. After 21 days, the plants were photographed and the lengths of the leaves and roots were measured. Then. samples were taken from the lowest (basal) part of the leaves, their middle and upper parts, and each part was 4-5 cm long. The samples were freeze-dried, powdered and used for analysis of plant pigments content.

The second part of the experiment started after removal of primary leaves on 15 November 15. When primary leaves were removed from the control and fluridone-treated bulbs, the subsequent secondary leaves continued to grow. After 33 days from the removal of primary leaves, the development of new leaves finished and they were collected, freeze-dried and, after grinding, used for plant pigments analysis.

Determination of plant pigments

The content of chlorophylls and total carotenoids was determined using the method described by Lichtenthaler and Wellburn (1985). Briefly, the freeze-dried and ground samples were ground in a mortar with 80% acetone/water solution. After centrifugation, the absorbance of the solutions was measured at three wavelengths: 470 nm for carotenoids, 646 nm for chlorophyll b and 663 nm for chlorophyll a. The contents of chlorophyll a, chlorophyll b and total carotenoids were calculated according to the formulas described by Lichtenthaler and Welburn (1985).

The total anthocyanin content was analyzed using the Mancinelli method (1984). Absorbance at 530 nm and 657 nm was measured in extracts obtained with acidified (1% HCl, w/v) methanol. Products of chlorophyll

degradation in acidic methanol show some absorbance at 530 nm. To compensate for absorbance resulting from chlorophyll degradation, the formula A_{530} - $0.25A_{657}$ was used. Anthocyanin content was calculated using the molar extinction coefficient of cyanidin-3-glucoside.

For the determination of proanthocyanidins (PA), a method based on measuring the difference in absorbance before and after their hydrolysis in mineral acid was used (Quettier-Deleu et al. 2000). Briefly, freeze-dried and powdered tissue was hydrolyzed with a mixture of n-butanol-conc. HCl (95:5, v/v) and a 2% (w/v) NH_4 $Fe(SO_4)_2$ ×12 H_2O in 2 M HCl at 95°C for 60 min. The absorbance at 550 nm was measured before and after hydrolysis. The values of an increase in absorbance were converted to the proanthocyanidin content using the molar absorption coefficient for cyanidin chloride. All spectrophotometric analyses were performed in triplicate using an UV-1800 UV/Vis spectrophotometer (Beijing Rayleigh Analytical Instrument Corporation, Beijing, China). The results were statistically processed using analysis of variance followed by the Duncan's multiple range test at p=0.05 (Statistica 13.1, StatSoft Inc., Tulsa, USA).

RESULTS AND DISCUSSION

Rates of the growth and development *Muscari armeniacum* plants, both primary and secondary, from the onset of the experiment on 23 December 2023 to its end on 18 January 2024, and results of chemical analyses of plant pigments are shown in Tables 1 and 2 and in Figure 1-5. Normal green leaves developed from uncooled *M. armeniacum* bulbs maintained in water (Figure 1 A). However, bulbs maintained in fluridone solutions developed pink leaves in the lower part, yellow in the middle part and green in the upper part. (Figure 1 B, C). Fig. 1 D shows bulbs without leaves. The lack of leaves occurred in both bulbs soaked in water and fluridone solution at a ratio of about 1 per 20 bulbs. Its reason is not known.

After being divided into basal (lower), middle and top (upper) parts, leaves were separately analysed. The results showed significant disappearance of chlorophylls *a* and *b* in the lower part of leaves grown from bulbs treated with fluridone. Smaller reductions in chlorophylls occurred in the middle part of the leaves, and were negligible in the upper part, regardless of the concentrations of fluridone applied (Figure 2). The level of total carotenoids was also significantly reduced in the lower part of leaves from bulbs treated with fluridone and to a lesser degree in their middle part. In the upper part of the leaves, the carotenoid content decreased only at a fluridone concentration of 10 mg dm^{-3} (Figure 2).

When all leaves were removed from the control and fluridone-treated bulbs, new leaves continued to grow. They were fully green in the control

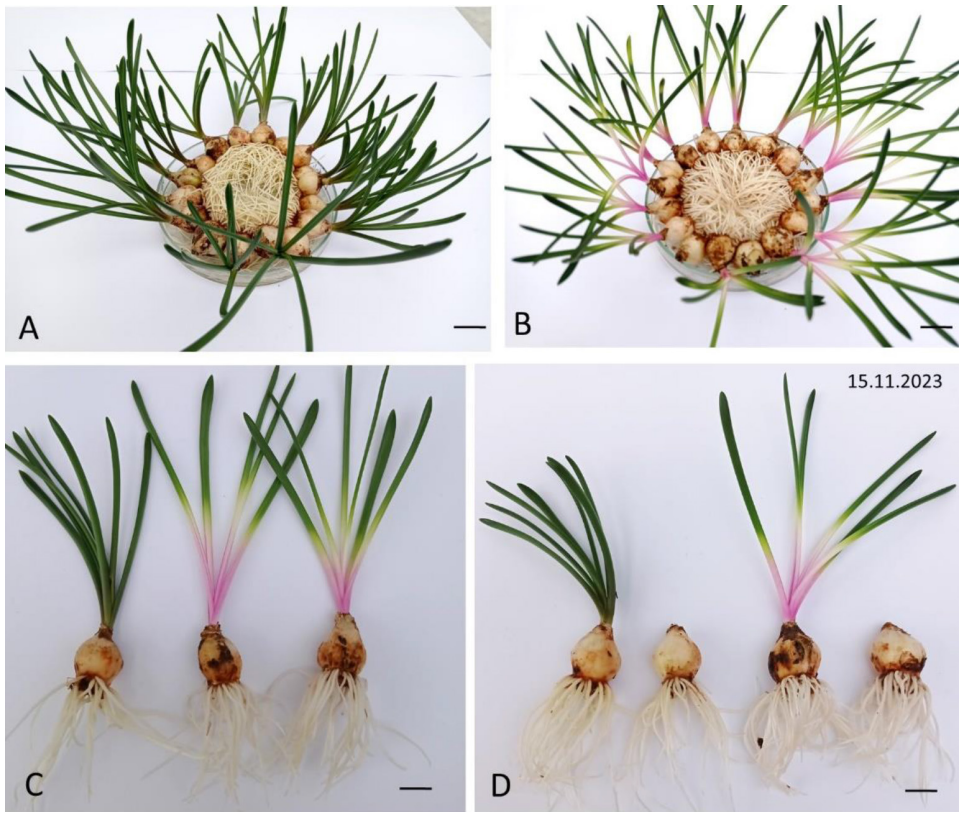

Fig. 1. The effect of soaking uncooled *Muscari armeniacum* bulbs in fluridone solution (10 mg dm^{-3}) on leaves and roots of plants: A – plants from bulbs soaked in water (control), B – plants from bulbs soaked in the fluridone solution, C – plant from bulb soaked in water (left) and two plants from bulbs soaked in the fluridone solution (right), D – no leaf development was observed in some control (left) and fluridone-treated (right) bulbs, despite root formation. Bars represent 2 cm

plants and pink in leaves from fluridone-treated bulbs (Figure 4B, D, Figure 5B, D, F, Tables 1 and 2). Analyses of the plant pigment content were conducted on samples collected on 18 December, 34 days after the removal of the original leaves. Newly growing leaves from fluridone-treated bulbs showed almost no chlorophyll content and the level of total carotenoids was decreased significantly (Figure 3). The results of our study showed that the use of fluridone to soaking bulbs reduced the level of chlorophylls in the lower parts of *M. armeniacum* leaves by about 5-6 times, about 2-3 times in the middle parts and about 30% in their upper parts.

These results in general confirm previous data obtained for other plants. It was found earlier that fluridone decreased the carotenoid and chlorophyll content in mature *Hydrilla verticilliata*, and increased the anthocyanin content in mature but did not in young *Hydrilla* (Doog et al. 1993). Later,

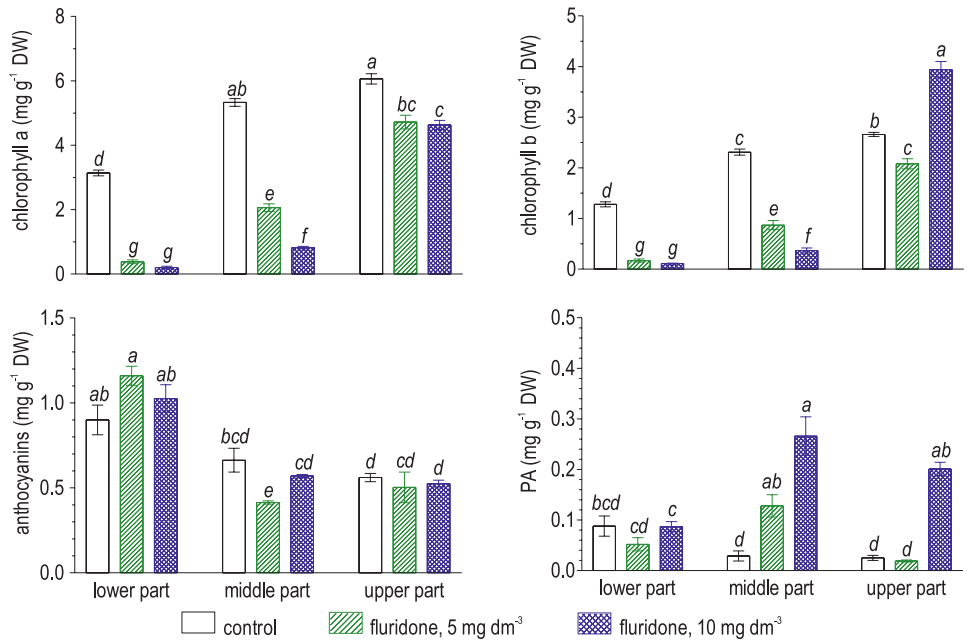

Fig. 2. Effect of three-week soaking of *M. armeniacum* bulbs in fluridone on the pigments and proanthocyanidin (PA) content in primary leaves. The bars in each graph marked with the same letter do not differ at the significance level of p=0.05 according to the Duncan's test

Zou et al. (2018) showed that fluridone strongly induced leaf bleaching in rice (*Oryza sativa*) by decreasing the content of chlorophyll and carotenoids. Recently Sun et al. (2022) found that fluridone significantly inhibited the growth and accumulation of astaxanthin, a specific carotenoid, in the red dormant stage of *Haematococcus pluvialis* algae. The decline of carotenoids and chlorophylls in *M. armeniacum* could be an evident effect of the inhibition of ABA biosynthesis (Le Bris et al. 1999, Gonzalez-Villagra et al. 2019).

Application of fluridone by soaking *M. armeniacum* bulbs for 21 days had hardly any effect on the anthocyanin content of all analyzed parts of the primary leaf (Figure 2). However, the content of proanthocyanidins was higher in the upper parts of leaves compared to the control, but the effect of fluridone was not significant in the lower and middle parts (Figure 2). After excision of primary leaves on 15 November, new sprouted leaves (secondary) were green in control plants and pink in leaves sprouted in fluridone--treated bulbs (December 18). Trace amounts of chlorophyll and a decreased level of carotenoids were measured in leaves treated with fluridone in comparison to leaves in control plants (Figure 3, Tables 1 and 2). In contrast, the content of anthocyanins and proanthocyanidins increased markedly compared to control in the secondary leaves after removing primary leaves. Previously, Gonzalez-Villagra et al. (2019) reported that fluridone treatment significantly reduced ABA and the total anthocyanin content in *Aristotelia*

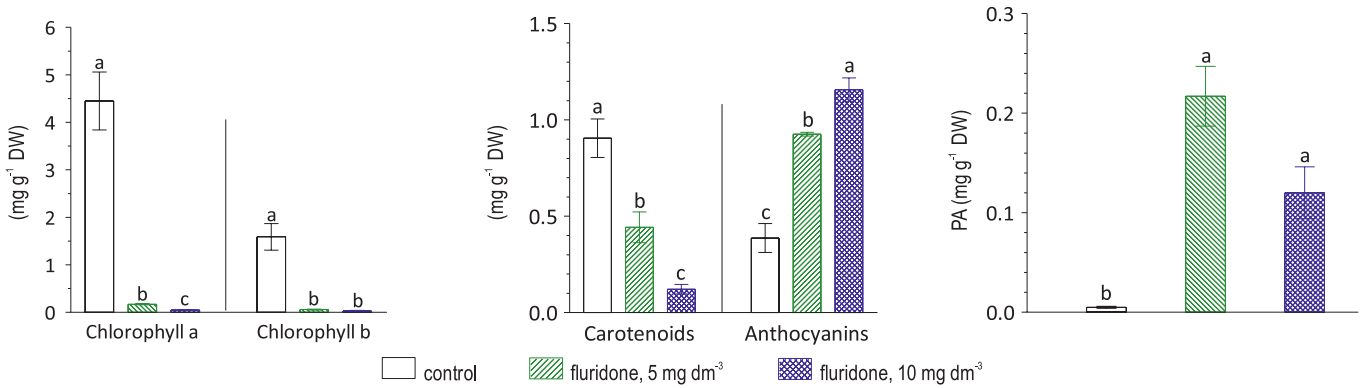

Fig. 3. Effect of 54-day soaking of bulbs in fluridone on pigment and proanthocyanidin (PA) contents in secondary leaves of *M. armeniacum*. The bars in each graph marked with the same letter do not differ at the significance level of p=0.05 according to the Duncan's test

Table 1

Effect of fluridone on length of tprimary leaves and inflorescence shoots of *M. armeniacum*

Date of measurements	Length (cm) after starting the experiment on 23 October					
	control		fluridone 5 mg dm^{-3}		fluridone 10 mg dm^{-3}	
	leaves	stalk	leaves	stalk	leaves	stalk
15.11.2023	11.5^{a}*	-	12.7^{a}	-	11.8^{a}	-
18.12.2023	16.5^{a}	-	16.2^{a}	-	16.8^{a}	-
27.12.2023	16.8^{a}	0	18.5^{a}	0	17.3^{a}	0
03.01.2024	16.7^{a}	0	18.0^{a}	6.5^{b}	18.7^{a}	5.2^{b}
18.01.2024	16.0^{a}	0	17.8^{a}	9.5^{b}	18.3^{a}	8.5^{b}

* The means in the rows marked with the same letter do not differ at the significance level of p=0.05 according to the Duncan's test

Table 2

Effect of fluridone on length of the secondary leaves and inflorescence shoots of *M. armeniacum* after removal of leaves at the top of bulbs on 18 December

Date of measurements	Length (cm) after removal of leaves on 15 November					
	control		fluridone 5 mg dm^{-3}		fluridone 10 mg dm^{-3}	
	leaves	stalk	leaves	stalk	leaves	stalk
27.12.2023	1.2^{c}*	0	2.2^{b}	9.2^{a}	2.5^{b}	6.5^{b}
03.01.2024	2.7^{b}	0	2.6^{b}	11.1^{a}	3.1^{b}	10.4^{a}
18.01.2024	5.2^{b}	0	3.2^{b}	10.3^{a}	4.8^{ab}	11.8^{a}

* The means in the rows marked with the same letter do not differ at the significance level of p=0.05 according to the Duncan's test

chilensis plants subjected to drought stress, but ABA application restored anthocyanin levels. However, the set-up of our experiments that involved soaking *M. armeniacum* bulbs in aqueous solutions did not induce drought stress. This could mean that the effect of fluridone on anthocyanin accumulation during drought stress is different than in the conditions without this stress.

It was found earlier that dormancy in *Lilium rigidum* (annual ryegrass) seeds can be alleviated by warm stratification in the dark or by application of fluridone (Goggin et al. 2015). Besides, soaking the bulbs of Oriental hybrid lily (*Lilium oriental*) in fluridone before the chilling period increased the height of the plants and the number of flowers on the plant (Yang et al. 2015). Furthermore, dormancy in *Lilium rigidum* (annual ryegrass) seeds can be alleviated by warm stratification in the dark or by application of fluridone (Goggin et al. 2015). Also, treatment of *Setaria viridis* seeds with fluridone improved germination rates but the resulting seedlings appeared albino and failed to recover subsequently (Sebastian et al. 2014).

It seems that fluridone may be more widely used to break the dormancy of seeds and other plant organs. This has been shown in many previous

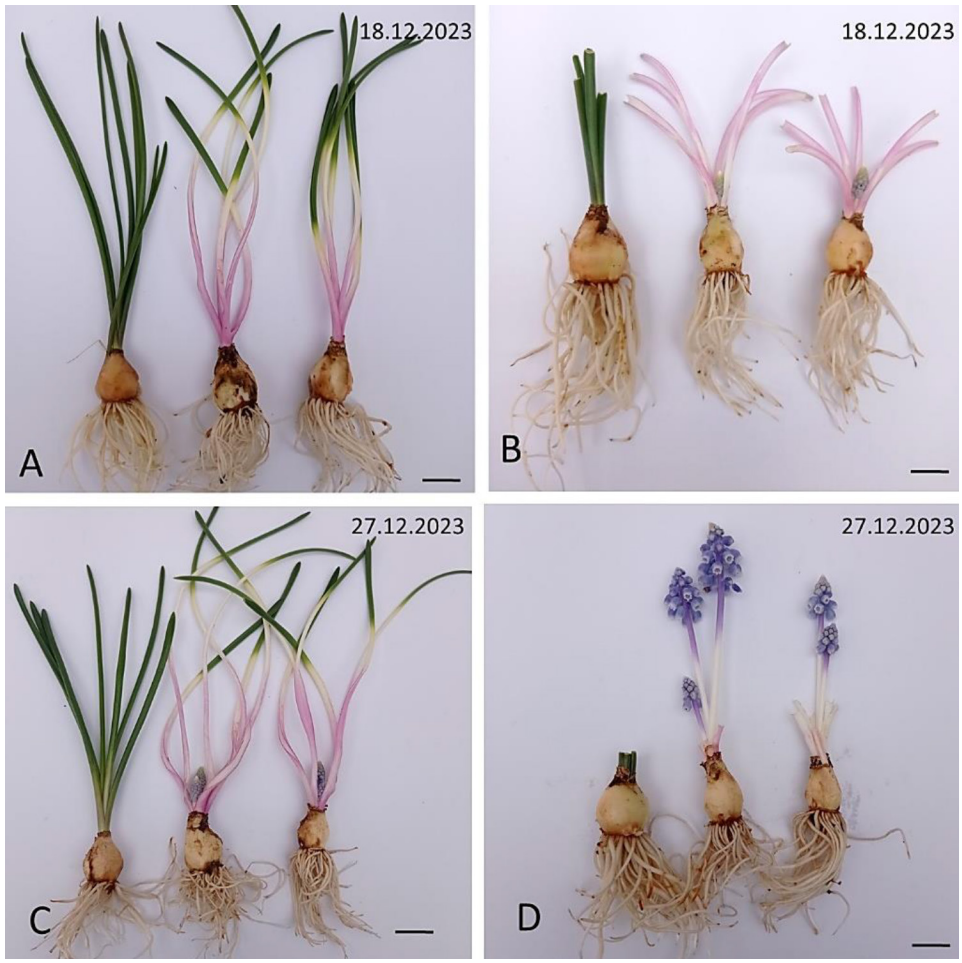

Fig. 4. Effect of fluridone on breaking the dormancy of inflorescence shoots in intact plants (A, C) and after excision of leaves on 15.11.2023 (B, D). Plants from bulbs soaked in water (left) and those soaked in the aqueous solution of fluridone 5 and 10 mg dm^{-3} (right) after 55 days of treatment (A, B) and 64 days of treatment (C, D). Bars represents 2 cm

studies. Fluridone, by inhibiting of ABA biosynthesis, effectively promoted the germination of *Gladiolus hybridus* cormels (Wu et al. 2015).

In isolated rose buds, fluridone-treatment caused a significant decrease in the endogenous ABA content and prevented the dormancy phenomenon (Le Bris et al. 1999). Also, the ABA-treated strawberry (accession Yellow Wonder) plants significantly suppressed the number of crown branches, whereas plants treated with fluridone showed an increased number of crown branches and number of leaves (Sun et al. 2024). Moreover, fluridone decreased the mesocotyl length of *Oryza sativa* (Watanabe et al. 2001), decreased the growth rate of *Vicia faba* (Popova 1995), and affected later root initiation as well as its elongation in tomato (Hooker, Thorpe 1998).

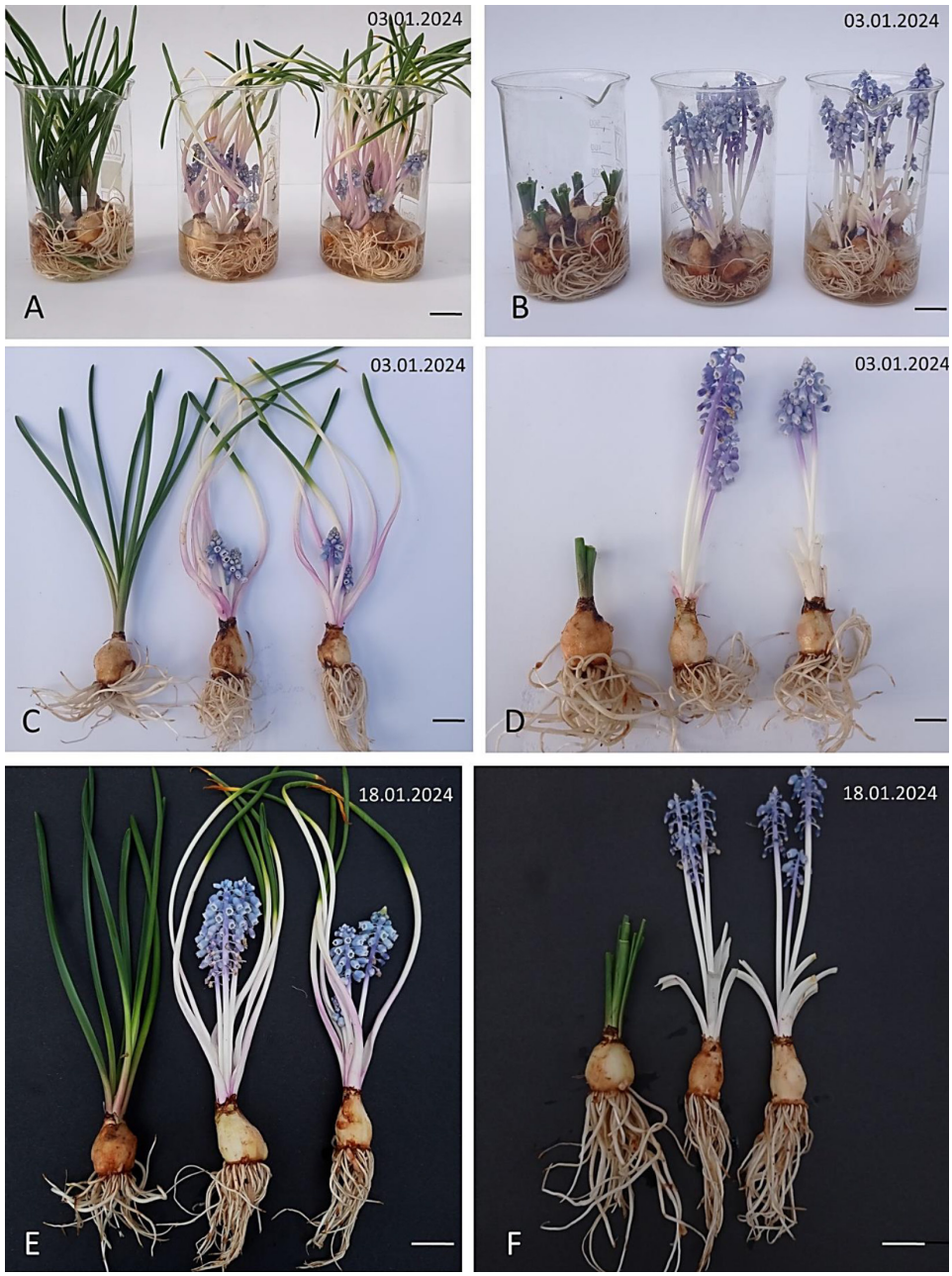

Fig. 5. Effect of fluridone on breaking the dormancy of inflorescence shoots in intact plants (A, C, E) and after excision of leaves on 18.12.2023 (B, D, F). Plants from bulbs soaked in water (left) and those soaked in solution of fluridone 5 and 10 mg dm^{-3} (right) after 71 days of treatment (A, B, C, D) and 86 days of treatment (E, F). Bars represents 2 cm

The results of our studies on *M. armeniacum* indicated that the dormant state of inflorescence shoots is interrupted by the action of fluridone, thus confirming previously published data.

CONCLUSIONS

A three-week soaking of *Muscari armeniacum* bulbs in fluridone solutions significantly inhibited root growth and caused degradation of chlorophylls and carotenoids in the leaves of this species. Longer, 54-day treatment of bulbs with fluridone caused further degradation of chlorophylls and carotenoids and, additionally, a large increase in the anthocyanin and proanthocyanidin content. The high anthocyanin content is an apparent effect of fluridone-induced stress. Histological analysis showed that anthocyanins were present only in the leaf parenchyma and not in the epidermis. Application of fluridone to uncooled *M. armeniacum* bulbs also broke the dormancy of inflorescence shoots.

Author contributions

A.M.-C. – investigation, funding acquisition, J.M. – investigation, M.S. – investigation, methodology, supervision, writing – original draft preparation, J.G.-K. – investigation; project administration, M.H. – visualization, software, writing – original draft preparation, writing – review & editing. All authors have read and agreed to the published version of the manuscript

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Conflicts of interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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