

Comparison of the Effect of Flurochloridone and Fluoranthene on the Root and Shoot Anatomy and Morphology of Pea Plants (*Pisum sativum*)

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ABSTRACT

Despite current efforts to minimize the impact of industry on ecosystems, the environment is polluted by a range of foreign substances, that can have a negative impact on the living organisms. Examples of widely studied substances are polycyclic aromatic hydrocarbons (PAHs) and recently, substances commonly used in conventional agriculture. In our study we focused on the morphology and anatomy of the vegetative organs of pea plants treated with the active substance of herbicides, flurochloridone (FLC), in concentration representing the residual amount in the soil (5 μM), and PAH fluoranthene (FLT) in concentration representing the middle to high environmental load (5 μM). During the long-term cultivation in nutrient solutions modified by the mentioned pollutants, the growth parameters of roots and shoots were observed in the three growth phases (4 and 8 fully developed leaves and the flowering phase). The growth parameters and observation of the morphology were supplemented with root and stem anatomical analysis using the transverse cross-sections. Both xenobiotics caused the decrease in the biomass production, while the more significant inhibition of growth, compared with control plants, was detected in FLC-treated plants, where the root system was reduced up to 75% and growth parameters of the shoots were reduced about more than 50%. The decrease in root biomass production was accompanied by changes in root branching. FLT treatment caused milder growth inhibition, it was observed about 50% reduction of the root system induced by the shortening of the main and lateral roots. Less pronounced, was also the decrease in stem length caused by FLT. Similar information was obtained about the different degrees of effect of FLC and FLT using anatomical analysis. Both studied substances increased the main root diameter accompanied with increase of the average number of the primary cortex layers. Their influence also caused the more intensive formation of exodermis. Changes in anatomical architecture were also observed in stem, where the FLC treatment changed the arrangement of the vascular bundles and decreased their average number. Our elementary morpho-anatomical study suggests that FLC despite its trace concentration could be more detrimental to plants than FLT, known for its harmful effect on living organisms, in relatively higher concentration.

Keywords: anatomy, flurochloridone, fluoranthene, pea, primary cortex, root branching, shoot, vascular bundles.

INTRODUCTION

The wide spectrum of chemical substances that can have a negative impact on individual components of ecosystems enters the environment in connection with industry and traffic expansion, such as polycyclic aromatic hydrocarbons (PAHs). Other foreign compounds are released into the environment by targeted application in conventional agriculture systems, where they are used as fertilizers or pesticides.

However, residues of their active substances or products of their transformation can also affect non-target organisms [Nikoloff et al. 2014]. Because plants are, in general, sessile organisms without the possibility of moving from the place to place, the impact of environmental pollution is often manifests itself in metabolic and morpho-anatomical changes related to stress reactions which can lead to the fatal damage of the affected plants [Hernández-Vega et al. 2017; Tomar and Jajoo 2014].

According to the report on the state of the environment by the Organisation for Economic Co-operation and Development [OECD 2018], the Czech Republic, due to its economic dependence on industry and energy dependence on coal, is among the countries with the greatest energy demand and high carbon emissions, which has a negative impact on air quality. Even though the volume of emissions released into the air is still decreasing (in the period 2008–2012 it was able to reduce greenhouse gas emissions by 30 % compared to 1990), according to the data of European Environment Agency, in 2016, almost 1.6 tons of PAHs were released into the atmosphere in the Czech Republic. PAHs belong to one of the most monitored groups of pollutants due to their carcinogenic and mutagenic effects on living organisms [Abdel-Shafy and Mansour 2016]. The problem is not only the presence of these pollutants in the air, but also the fact that, through atmospheric deposition, they can be transferred to other components of the environment – the water and soil [Arey and Atkinson 2003]. The most frequently detected molecules of PAH family during environmental pollution monitoring are benzo[*a*]pyrene and fluoranthene (FLT). In 2020 it was observed that the limit values of these two substances were exceeded in 30% resp. 10% analysed soil profile in farmland near the big rivers in the Czech Republic [CENIA 2021]. The main way of PAH uptake for plants is the atmospheric deposition via the leaf cuticle or the stomata in case of volatile PAHs. Most of the quantity of absorbed PAHs is incorporated in the lipid rich parts of plants or in the cell walls of exposed biomass [Wild et al. 2006; Desalme et al. 2011; Wiczorek et al. 2015]. The second possible way of the PAHs uptake is the root system. This uptake and subsequent translocation to the above-ground parts of exposed plants is strongly modulated by the bioavailability and the chemical structure of the specific PAH [Wang et al. 2012], mobility in the living organism, plant species [Kacálková and Tlustoš 2011] as well as the root system architecture and protein content [Zhan et al. 2013] and the species composition of the soil microbiome [Liu et al. 2018; Sun et al. 2014]. The presence of PAHs in plant's environment can adversely affect all life processes from the seed germination [Kummerová et al. 2008; Wei et al. 2014], to the modification of metabolic processes such as photosynthesis [Oguntimehin et al. 2013; Jajoo et al. 2014], which is related to the damage of biological

membranes caused by increased formation of reactive oxygen species [Zezulka et al. 2013; Tomar and Jajoo 2014]. The effect of PAHs on plants is also manifested in growth parameters, morphological and anatomical changes, as well as on the level of cell organelles [Shen et al. 2019 a, b].

As mentioned above, the important group of foreign chemical substances in the environment also represent the chemicals used in conventional agriculture. Although the use of chemicals in agriculture is regulated by many legislative and safety restrictions (generally referred to as The Good Agricultural Practices), intensive chemical use can be a burden on the environment. For example, the mineral fertilizers could present the source of heavy metals. Persistent herbicides and their residues could stay in the soil for several months sorbed on soil particles and by the leaching from the soil they could reach ground and surface water and then affect non-target organisms [Sondhia 2014]. The monitoring carried out in 2020 in the Czech Republic revealed the presence of pesticides or products of their transformation in 91% of the analysed surface water profiles and its exceeded limit also in 26.4 % of groundwater samples [CENIA 2021]. Several active substances of herbicides target photosynthesis as the basic metabolic process in green plants. For example, photodynamic herbicides which were developed in 1980s caused the accumulation of tetrapyrrole compounds in leaves and stems [Rebeiz et al. 1984], and fluridone and flurochloridone inhibiting the carotenoids biosynthesis [Bartels and Watson 1978; Klíčová 2002], both lead to photoactive damages of leaves visible as bleaching. The abovementioned flurochloridone (FLC) is, in general, supposed to be the safe preemergent herbicide without the potential effect on the subsequent crops because FLC is not easily mobile in the soil profile and the risk of its leaching and hitting in the un-targeted area is minimal [Andr et al. 2017; Jursík et al. 2020]. On the other hand, the problem of FLC application could be the relatively high persistence of this substance, which is not easily biodegradable and could appear in the soil a few months after application [EFSA 2018]. In addition to the known negative effect of FLC on carotenoid biosynthetic pathway it was observed its negative effect on the other components of antioxidant system as glutathione and the antioxidant enzymes activity of the plants as well as animals [Shi et al. 2016; Kaya and Yigit 2019; Zhou et al. 2019]. The relatively high persistence

of FLC and its ability to be translocated via xylem to the different parts of the plant body could be the potential risk for non-targeted crops and the contamination of food chain [Shi et al. 2016; Li et al. 2021]. And even though the report of the European Food Safety Authority [EFSA 2018] mentions that FLC does not pose a risk to the environment and the food sources, when it is used following the Good Agricultural Practices, it also admits that very trace amounts of this substance have also been experimentally detected in some parts of crops. Therefore, it is appropriate to study the effect of this widely used substance on the environment.

In this solely morpho-anatomical study we observed the effect of 5 μM FLC on the growth parameters and the anatomical structure of the roots and stems of pea plants (*Pisum sativum*). This studied concentration corresponds with the thousandth concentration of the recommended application dose. The effect of the trace FLC concentration is compared with the effect of 5 μM FLT, the PAH with known negative effects on the living organisms. In the case of FLT, the studied concentration corresponds with the medium to the higher environmental load with this pollutant. This current study follows up on the previous study by Lónová et al. [2023] that focused on the effect of FLT and FLC on the photosynthetic apparatus of affected pea plants.

MATERIALS AND METHODS

Cultivation of plants

As the experimental plant was used pea (*Pisum sativum* L. var. Oskar, SEMO a.s., CZ) cultivated in unmodified or modified Richter's nutrient solution [Richter 1926]. Pea seeds were imbibed in water overnight and then transferred to wet perlite. After four days of germination, the nine seedlings were placed in hydroponic cultivation vessels (volume 2.5 L) for each cultivation variant – Richter's nutrient solution for control plants and for two experimental variants treated with FLC and/or FLT. The xenobiotics were added to the nutrient solutions before the stage of the first fully expanded leaf [BBCH 10, Weber and Bleiholder 1990; Feller et al. 1995], FLT (Sigma-Aldrich, 83.3 mM stock solution at the ethanol) in final concentration of 5 μM or FLC (stock solution RACER EC 25, ADAMA CZ s.r.o., CZ) in

final concentration 5 μM . The plants were cultivated in cultivation chamber under controlled conditions – photoperiod 18/6 h, temperature 22/16 °C (day/night), 70% relative humidity and 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD.

Measurement of growth parameters

Growth parameters were evaluated in roots and stems resp. shoots (stems including leaves). In both organs, fresh weight, immediately after sampling, and dry matter weight, after drying to constant weight at 80 °C, were measured. The measured dry matter weights were then used for calculation of R/S ratio according to the formula $R/S = \text{root dry matter weight} / \text{shoot dry matter weight}$. The other growth parameters were specific to each organ. These parameters were also measured in the roots: main root length (from apex to cotyledons, including hypocotyl), the length of the unbranched zone of the main root (from apex to the nearest lateral root), the total root length using square grid method [Tennant 1975] and the number of lateral roots. In the shoots, in addition to the abovementioned weight parameters, the following parameters were also measured: length of the stem (from base to apex, including epicotyl) and number of internodes (resp. leaves).

Anatomical preparations

Samples for anatomical studies were collected from the fifth node of the stem and from the root zone of the roots of the pea plants in the eight fully developed leaves. The segments taken from fresh plants were fixed in FAA fixative (ethanol : acetic acid: 40% formaldehyde: H_2O , 9: 1: 1: 9). After washing off the fixative solution, the samples were gradually dehydrated in ethanol solutions (10, 30, 50, 70, 90, 96, 100 %) and then gradually transferred to xylene (ethanol: xylene 3: 1, 1: 3, 100% xylene) and paraffin (Paraplast Plus, Leica, DE). Paraffin blocks with samples were cross-sectioned using a Leica RM2255 rotary microtome (Leica microsystems, DE), thickness of the stem cut was 16 μm and thickness of root cross-sections was 12 μm . The cross-sections of stems were stained with Safranin O + Fast Green FCF [Ruzin 1999]. Cross-sections of roots were stained with 0,0025% solution of toluidine blue [Soukup 2014]. Stained preparations were mounted on glass slides with Solakryl (Chemapol, Neratovice, CZ). Cross-sections

were observed under the light microscope Olympus IX70 (Olympus Corporation, J), microphotographs were taken using Canon EOS 1300D digital camera (Canon Inc., J) and Quick Photo Micro 3.2. software (Promicra, CZ). Anatomical analyses based on the thickness of the different tissue layers were measured using ImageJ (National Institute of Health, USA). The diameter of root and stem cross-sections was measured twice in every sample, in roots in x- and y- axis planes and in the stems as junction between the midpoints of opposite sides of stem. The number of the primary cortex layers and the thickness of the primary cortex were determined for both organs as the area included exodermis (hypodermis), middle cortex and endodermis (in root). In stem, these two parameters were observed in the areas between the vascular bundles.

Sampling and statistical analyses

Samples were collected in the growth phase of four and eight fully developed leaves and the flowering phase [BBCH 14, BBCH 18, BBCH 6x; Weber and Bleiholder 1990; Feller et al. 1995]. Samples from minimally five plants were collected for every variant. Samples for the anatomical studies were taken from the fifth internode of stem and/or the differentiation zone of root both

in the eight fully developed leaves growth phase. Data are presented as mean values (\pm standard errors). The significance of the differences in presented values between control and treated plants was evaluated using one-way analysis of variance (ANOVA) and Tukey's test, the P-value ≤ 0.05 was considered statistically significant. Statistical analysis was done with Excel (Microsoft) using the script written in Python programming language. The graphic presentation of the results was created in Excel (Microsoft) using standard tools.

RESULTS

Morphological assessment – growth parameters

The morphological assessment of the effect of FLC and FLT on pea plants was based on the growth parameters listed in Table 1. From the evaluated parameters, it is evident that the negative effect of 5 μM FLC manifested itself on the reduction of the root biomass and the above-ground biomass that was observable throughout the cultivation period. The reduction in root biomass was reflected in the decrease in the fresh weight of the root system connected with strong decrease of the total length of root system. This



Figure 1. The habit of experimental pea plants with root system in the middle of the cultivation time: (a) control plant, (b) FLT-treated plant, (c) FLC-treated plant. The yellow arrow represents length of 3 cm

Table 1. The growth parameters of the roots and shoots

Growth parameter	Growth phase	Variant		
		Control	FLT	FLC
Length of main root [cm]	4leaves	12.20 ± 1.06 a	8.27 ± 1.01 a	9.53 ± 0.77 a
	8leaves	11.87 ± 1.67 a	12.00 ± 0.12 a	9.33 ± 0.32 a
	flowering	13.77 ± 0.42 a	7.93 ± 1.80 a, b	7.33 ± 1.63 b
Length of the unbranched zone of the main root [cm]	4leaves	3.80 ± 0.81 a	2.53 ± 0.90 a	2.97 ± 0.07 a
	8leaves	3.00 ± 0.45 a	2.3 ± 0.29 a	1.77 ± 0.52 a
	flowering	2.07 ± 0.34 a	2.4 ± 0.91 a	2.20 ± 0.61 a
Length of main root and unbranched zone ratio [cm/cm]	4leaves	0.31 ± 0.04 a	0.3 ± 0.09 a	0.32 ± 0.03 a
	8leaves	0.25 ± 0.003 a	0.19 ± 0.02 a	0.19 ± 0.06 a
	flowering	0.15 ± 0.02 a	0.33 ± 0.11 a	0.32 ± 0.80 a
Total root length [cm]	8leaves	233.33 ± 16.03 a	115.47 ± 21.49 b	56.63 ± 4.37 c
Number of lateral roots	8leaves	45.33 ± 2.09 a	42.34 ± 0.67 a, b	38 ± 10 b
Fresh weight of root [g]	4leaves	1.31 ± 0.04 a	1.19 ± 0.82 a	0.44 ± 0.30 b
	8leaves	2.09 ± 0.15 a	0.93 ± 0.06 b	0.61 ± 0.17 b
	flowering	3.28 ± 0.33 a	1.79 ± 0.10 b	0.49 ± 0.07 c
Fresh weight of shoot [g]	4leaves	1.83 ± 0.12 a	1.48 ± 0.10 a	0.82 ± 0.02 b
	8leaves	7.03 ± 0.40 a	3.90 ± 0.13 b	3.03 ± 0.71 b
	flowering	10.62 ± 0.69 a	4.96 ± 0.53 b	3.16 ± 0.47 b
Length of stem [cm]	4leaves	13.07 ± 1.07 a	10.83 ± 0.23 a, b	8.57 ± 0.09 b
	8leaves	41.90 ± 1.23 a	32.20 ± 0.64 a, b	22.33 ± 3.71 b
	flowering	53.83 ± 0.96 a	34.17 ± 3.30 b	24.43 ± 2.39 b
Root/shoot ratio [g DW/g DW]	4leaves	0.393 ± 0.002 a, b	0.422 ± 0.012 a	0.352 ± 0.015 b
	8leaves	0.165 ± 0.009 a	0.154 ± 0.009 a	0.134 ± 0.005 a
	flowering	0.176 ± 0.008 a	0.191 ± 0.018 a	0.162 ± 0.014 a

Note: Average values ± standard errors (±SE), the values with different letter scripts are statistically different ($P \leq 0.05$).

parameter was four times lower than in control plants and twice lower compared to the plants of FLT variant. The decrease in total length of root system is primarily related to the reduction of the number of lateral roots and their significantly shorter length, which is evident from the Figure 1. The decrease in the length of the main root and the fresh weight of root system of plants affected with FLC was also caused by partial rotting of its apical and differentiation zone in the end of cultivation, in flowering phase. Although no difference in the number of internodes (resp. leaves) was found between the experimental variants and the control plants during cultivation time, it means that the individual growth phases occurred in the experimental plants at the same time intervals, it was observed that the application of xenobiotics affected the above-ground biomass production. The parameters measured in stems (shoots) were significantly reduced in plants affected with FLC. The fresh weight of shoot was significantly reduced by more than half during the cultivation, in flowering growth stage by more

than two-thirds compared to control plants. This decrease in the shoot biomass was caused by the shorter stem length, which was reduced to the approximately one half of the stem length of control plants (Table 1) and the reduction of the leaf area (data not presented).

The application of 5 μM FLT to the cultivation nutrient solution affected especially the fresh weight of roots and total length of root system, which is mainly related to the shortening of the main and lateral roots, because the average number of lateral roots was comparable with control plants. In latest monitored growth phase of the flowering, the decrease in the main root length could relate to the rotting of its apical part similar to the plants of FLC variant. The reduction of the root system was not as significant as in the case of FLC treatment despite this application of FLT led to about 50% reduction of root biomass compared to control plants. Similar results were obtained during the evaluation of the shoot growth parameters. FLT treatment caused the decrease in stem length of approximately 35% and reduced the

fresh weight of above-ground biomass about one half compared to control plants in the end of the cultivation. Measurement of the growth parameters (Table 1) shows that both xenobiotics in applied concentrations caused the inhibition in the biomass production. The analysis of the R/S ratio, which was comparable between all experimental variants (including control) during the cultivation period, proves the similar negative impact of both xenobiotic on the roots and shoots. The morphology of the whole experimental plants is presented in the Figure 1.

Anatomical assessment

Anatomical structure was observed on the cross-sections of the stems and roots which are presented in Figure 2.

The samples for stem anatomical preparations were taken from the fifth internodes of the experimental pea plants. Despite this fact, the differences in the anatomical architecture between cross-sections prepared from the stems of the individual experimental variants were observed (Figure 2: Sa – Sc). From the microscopic observation of stem cross-sections of control plants, it was clearly visible the typical anatomical structure of the

pea stem with the open collateral wedge-shaped vascular bundles arranged in the one ring (Figure 2: Sa). The altered stem anatomical structure was observed in the FLC-treated plants (Figure 2: Sc), where the presence of the cortical vascular bundles was found. Although the presence of these vascular bundles outside of the stele is typical for the lower internodes, their presence in the stems of FLC-treated plants was observed in every analysed sample. This phenomenon was also observed in some of the stem cross-sections of FLT-treated plants, but very rarely. Moreover, in this variant the cortical vascular bundles were almost the part of the vascular bundles in the stele (Figure 2: Sb), so the anatomical structure of FLT-treated stems was closer to the control plants (Figure 2: Sa) with vascular bundles arranged in the one ring in eustele. Despite this fact that the FLC-treated plants had vascular bundles localized in two areas in the stem, their average number was significantly lower than in the stems of FLT-treated plants or control plants, almost about 25% (Figure 3 d). The stem diameter and the thickness of primary cortex (Figure 3 a, b) of FLC treated plants were lower compared with control, although the number of the tissue layers in cortex was higher (Figure 3c). It follows that the FLC treatment caused

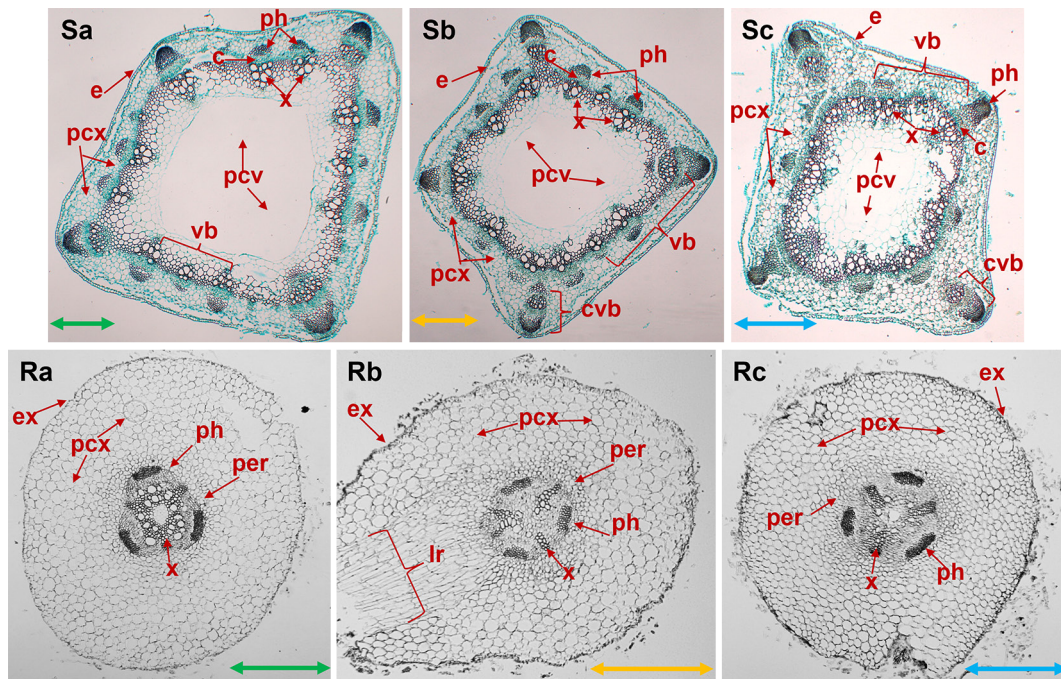


Figure 2. The transverse cross-sections of stem (Sa–Sc) and main root (Ra–Rc): Upper line: Sa – control, Sb – FLT-treated plant, Sc – FLC-treated plants. Bottom line: Ra – control, Rb – Flt-treated plant, Rc – FLC-treated plant. The coloured arrows represent distance 500 μ M. The red labels mark the individual parts of the organs: c = cambium, cvb = cortical vascular bundles, e = epidermis, ex = exodermis, lr = lateral root, pcv = pith cavity, pcx = primary cortex, per = pericycle, ph = phloem, vb = vascular bundles, x = xylem

the primary cortex cells size reduction. By the comparison of the anatomical structure of stems of the FLT-treated plants and control plants it was found only the one significant difference in the values of stem diameter, which was significantly higher about 36 % after FLT treatment.

The evaluation of root anatomical structure was based on the observation of the transverse cross-sections of the main root (Figure 2: Ra – Rc). No visible differences in the root anatomy of the experimental plants were found by microscopic observation. The roots of every cultivation variant had structure typical for pea roots – external tissue called exodermis covered the multilayer cortex and stele with vascular strands including the xylem and phloem in triarch arrangement. The differences between the individual cultivation variants were observed by the measurement of the size parameters of the individual root

tissue layers (Figure 4). The significantly smallest root diameter (Figure 4a) was observed in the FLT-treated plants which was almost about 19% decreased compared to control or FLC-treated plants. The measurement of the primary cortex thickness (Figure 4b) yielded no differences between the experimental variants including control plants, but the differences were found in the average numbers of the primary cortex layers (Figure 4c). Although, as was mentioned above, no differences were observed in root diameter or the cortex thickness between control and FLC-treated plants, it was found that the root primary cortex of FLC-treated plants contained in average of one tissue layer more than control plants. It could indicate that the cortex cells became smaller under the influence of this substance. No significant differences were observed between the FLT treated plants and the control plants. From the pictures

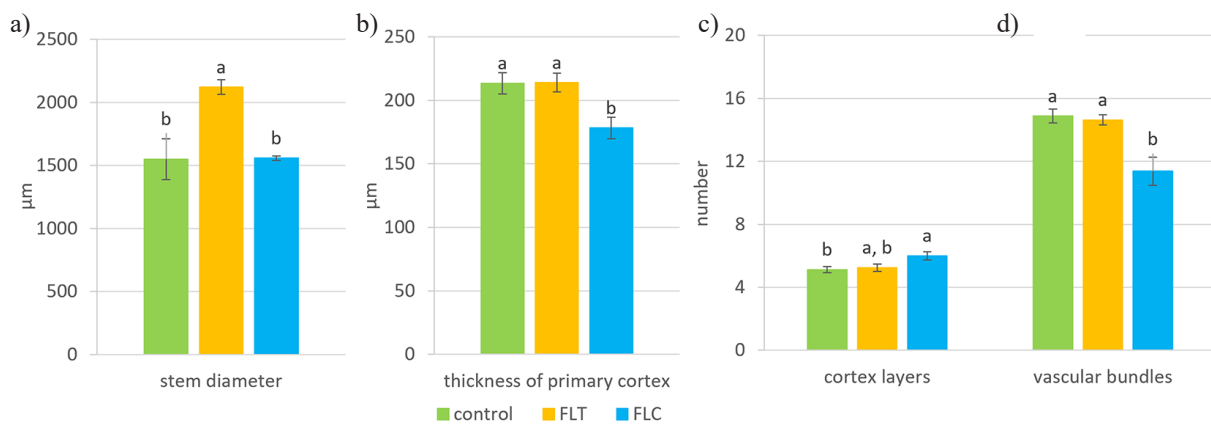


Figure 3. The parameters of stem cross-sections: (a) stem diameter [μm], (b) thickness of stem primary cortex [μm], (c) number of primary cortex layers, (d) number of vascular bundles. Height of the columns represents the average values. Error bars mean standard errors (\pm SE), columns with different letter scripts are statistically different ($P \leq 0.05$)

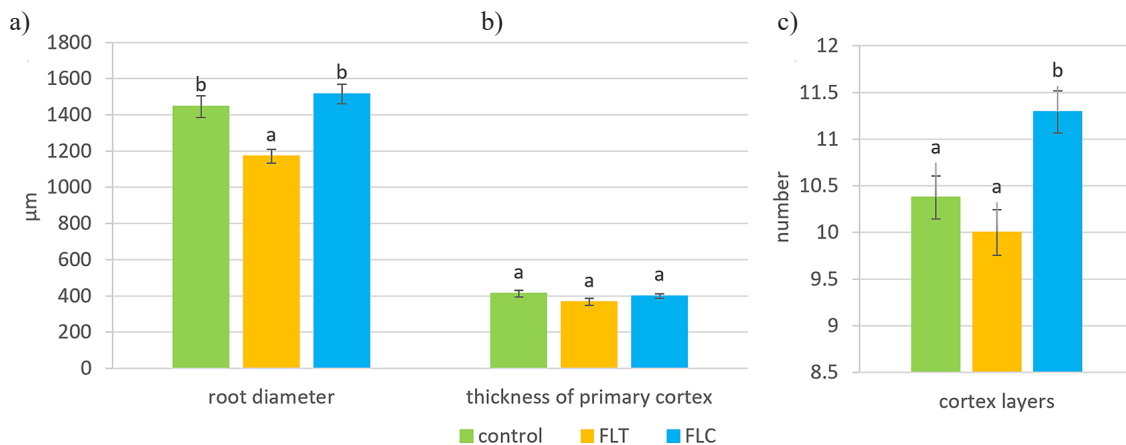


Figure 4. The parameters of root cross-sections: (a) root diameter [μm], (b) thickness of root primary cortex [μm], (c) number of primary cortex layers. Height of the columns represents the average values. Error bars mean standard errors (\pm SE), columns with different letter scripts are statistically different ($P \leq 0.05$)

of root cross-sections (Figure 2) it could be seen that the experimental plants treated by FLT and/or FLC had developed thicker layer of exodermis than control plants, however, this parameter was not statistically evaluated.

DISCUSSION

The data presented in this study show the comparison between the effect of 5 μM FLC, the active substance of some preemergent herbicide, and 5 μM FLT, the often-traced PAH, on the anatomy and morphology of the roots and shoots of treated pea plants. Although the applied FLT concentration (1 mg/L) represents the medium to higher environmental load with this pollutant, our results reveal that the impact of FLC treatment could have in some parameters more negative effect on plants even though the studied concentration of this substance represents only the residual amount of this herbicide in soil (1,6 mg/L which corresponds to one thousandth of the recommended application dose 750 g/ha [Mishra et al. 2022]). The more significant negative effect of FLC was observed in the inhibition of growth parameters of roots and stem, where the total root length was about three quarters lower than the control plants, in the case of FLT-treatment was the shortening of the root system about 50 %. This reduction in the root system was also observed in the parameter of the fresh weight. The more intensive effect of FLC on the reduction of the root system could be related to the modification of its morphology. The similar effect on the root system was also described in studies with the different concentrations of fluridone, which is the chemical and functional analogue of FLC [Hooker and Thorpe 1998]. The FLC application significantly changed the average number of lateral roots and their length, while the effect of FLT only reduced the length of lateral roots, but their number was comparable to the roots of control plants. In the study by Kummerová et al. [2013] they presented, in contrast to our results, that the application of 1 mg/L FLT led to the increase in the number of lateral roots in treated pea plants, but the total length of root system was comparable to control, which means that the lateral roots had to be shorter after FLT treatment. In the same study, they observed the significantly negative effect on the root system of pea plants after the application of the 7 mg/L FLT. However, in relation

to the mentioned work, it should be emphasized that their experiment was already evaluated after seven days cultivation (corresponds approximately to the 4-leaf stage). This shows that not only concentration, but also duration of their action is of decisive importance for the effect of chemical substances, because the long-term application could lead to the accumulation of the xenobiotics in the roots, which intensifies their effect on the affected plants [Dupuy et al. 2016]. In addition to the morphology of the root system, the application of both substances also caused changes in its anatomical structure. Although this parameter was not statistically evaluated, it could be stated that the application of FLT and FLC induced the formation of a thicker exodermis in the main root. A thicker exodermis can probably be the result of more intense suberization of the cell walls, forming the barrier preventing the penetration of xenobiotics [Dupuy et al. 2016]. It is known that FLC and PAHs cause in plants the oxidative stress accompanied by the increased production of ROS [Liu et al. 2018b; Shen et al. 2019a]. ROS production in roots can alter the root anatomy and can cause the formation of the intercellular spaces via the programmed cell death [Svobodníková et al. 2020], which could be also initiated with the increased level of ethylene [Kummerová et al. 2010; Váňová et al. 2011]. In our study we did not observe the formation of lysigenous intercellular spaces in the roots (or stems) of experimental plants, as well as no significantly higher levels of ethylene in roots (data not presented) but about the oxidative stress could report the higher levels of malondialdehyde analysed in leaves and presented in previous study by Lónová et al. [2023]. Disruption of the root system function connected with its morpho-anatomical changes could influence the ability of the affected plants to take up the water and nutrients. Because of this fact is sometimes assumed that the symptoms, which appear in the aboveground parts of plants which are treated by some chemical substances via root system, are only the secondary manifestation of root damage. However, for FLC and PAHs in general, their xylem transport to the aboveground parts of the plants was proven [Gao and Zhu 2004; EFSA 2018], and therefore it can be assumed that these substances are able to effect directly in other parts of the plants, not only in the root system. Ratio of the dry weight of root/shoot, presented in our study shows that the production of under- and above-ground biomass is similar

within the given cultivation variant, although it is inhibited by FLC and FLT treatment compared with control plants. The strongest inhibition in the above-ground biomass production was observed in the fresh weight of the shoots of FLC-treated plants which was approximately two thirds lower than of control plants in the flowering phase. This was caused by the significant shortening of the stem and also the decrease of the leaf area (data not presented). A similar, but not so significant, reduction of shoot biomass production was also observed in FLT-treated plants. In all experimental variants including control plants, the differences in the number of internodes or the timing of initiation of the different growth phases were not observed. It could testify that both studied substances have no significant influence on the developmental processes of affected plants. On the other hand, modification of the stem anatomy caused by FLC treatment indicates that some disruption of plant development could exist. While the stems of FLT-treated plants had anatomical structure similar to control plants (except the larger stem diameter), the FLC-treated plants showed the anatomical differences in stem structure. In the fifth internodes it was observed the presence of the cortical vascular bundles in the stem corners, which is more typical for the internodes located lower in the pea stems [Aloni a Gad 1982].

CONCLUSION

The negative effect of FLC and FLT on the morpho-anatomical structure of roots and shoots of pea plants described in this study complements the known information about the negative influence of this xenobiotics on the photosynthetic apparatus and the production capacity of the affected pea plants which are accompanied by the decrease in the assimilation area, photosynthetic pigments content as well as the damage of the biomembranes [Lónová et al. 2023]. In this study applied concentration also showed that trace amount of FLC, which is considered as safe substance when used in accordance with the principles of Good Agriculture Practices, could have more negative effect as representant of PAH family FLT in the concentration which represents the middle to high contamination of environment. And even though FLC is currently studied mainly from the point of view of its effect on human health [Liu et al. 2018; Shi et al. 2016], our results and studies

working mainly with similar substance fluridone and their effect on aquatic organisms [Cozzola et al. 2022; Sun et al. 2022; Khanna et al. 2023; Park et al. 2023] show that it would be useful to focus attention also on the study of the effect of even lower concentrations of FLC on living organisms and environment.

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