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Original Research

Physiological Activity of Wheat cv. Tonacja Seedlings as Affected by Chemical Stress of Styrene Vapours

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Abstract

This study examines the effects of styrene vapours in a mixture with air on the physiological response and anatomical changes of winter wheat cv. "Tonacja" seedlings. Seven different concentrations of styrene vapours (71, 142, 285, 570, 1,140, 2,280, and 4,559 mg·m^{-3}) were applied to germinating seeds and growing wheat plants. At the lowest styrene concentration, a stimulating effect on the wheat plants was observed. However, styrene vapours inhibited germination and development of plants at higher concentrations, ranging from 570 to 4,559 mg·m^{-3}. Starting with a concentration of 570 mg·m^{-3}, a distinct reduction of the height of plants and the yield of dry matter was observed. Additionally, the number of stomata on the leaf surface decreases and an inhibition of assimilation and water use efficiency in the photosynthesis process were also observed in plants exposed to higher levels of styrene vapours. At the styrene concentration of 2,280 mg·m^{-3}, the number of stomata on the leaf surface decreased about 10 times to the control sample and a strong dehydration of stomatal cells occurred. Styrene at highest concentration (4,559 mg·m^{-3}) completely inhibited the germination ability of wheat seeds. These results clearly suggest that styrene vapours impaired the physiology of wheat under conditions tested.

Keywords: styrene, VOC, wheat, physiological activity, chemical stress, germination, growth, stomata

Introduction

With the development of civilization has come an increase in emissions of harmful substances to the natural environment. One of the more harmful compounds is styrene. It is used for the production of polystyrene, unsaturated polyester resins, styrene-butadiene rubber (SBR), acrylonitrile-butadiene-styrene polymers (ABS), and styrene-acrylonitrile rubbers (SAN) [1, 2]. This compound also has found application in the food industry as a flavour-aromatic additive [3]. Under natural conditions, styrene is a product of cinnamic acid decomposition [4]. Due to the universal application of styrene in different industrial branches and its volatility, many living organisms are exposed to its action [5-8]. The harmful affect of this compound for animal organisms and humans is presented in the fact sheets [9, 10] and by different authors [11-15]. In many countries, including Poland, the existing legal regulations define similar permissible concentrations of this compound both in the atmospheric air and at work stations [16, 17]. According to the first of the two above-mentioned legal acts, a permissible concentration of styrene in the atmospheric air

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should not exceed 20 μg·m^{-3} for 30 minutes, 5 μg·m^{-3} for 24 hours and 2 μg·m^{-3} for a year. According to the second act, the maximum permissible concentration of styrene at a work station is 50 mg·m^{-3} (mediana for 8 hours), which is consistent with the WHO recommendations [18], while a maximum permissible momentary concentration is 200 mg·m^{-3}. With quite high allowable concentrations at work stations, styrene concentrations in the ventilation air frequently exceeds 500 and sometimes even 1,000 mg·m^{-3} [19]. Under unfavourable conditions for spreading in the air (inversion) and low with low discharges, the concentration of styrene in the vicinity of discharges can be similar to those prevailing in a ventilation air stream. Therefore, the ecosystems situated a close distance from emission sources of examined substances, including plants, can be exposed to the effect of its high concentrations, similar to those applied in the present study. Despite a considerable number of studies dedicated to styrene environmental harmfulness, data on the effect of styrene on plants are still lacking. Few reports showing the harmfulness of this compound for vegetation cover [20] do not exhaust the subject. Further studies are necessary to determine the effect of styrene on plant physiological processes and productivity. To determine the effect of styrene on plant physiological processes and productivity, further studies are necessary.

Our study examines the physiological responses of wheat cv. "Tonacja" seedlings in the presence of varying concentrations of styrene vapours in a growth environment.

Material and Methods

The experiment was carried out in 25 l phytotron chambers (9 for each concentration) with a controlled photoperiod (day/night 12/12) and styrene-saturated atmosphere, in a design ensuring the limited exchange of air with atmosphere. The relative humidity was 80%, while the light intensity during a day was 200-300 μmol·m^{-2}·s^{-1}. In each chamber, except the one intended as a control, a glass bottle with 25 ml of styrene solution in silicon oil with a designed concentration was placed. In the gas area of phytotrones, an equilibrium between the liquid and the styrene vapours was stabilized. Assuming the equilibrium of styrene vapour pressure at 20ºC as being equal to 666.5 Pa [3], the concentration of styrene in the air filling the chamber amounted, respectively, to about 71, 142, 285, 570, 1,140, 2,280, and 4,559 mg·m^{-3}. The concentration of styrene stabilized after about 6 hours as ascertained by gas chromatographic analyses, similar to Wieczorek [21]. The determinations were made on CSRS's Chrom 4-type chromatograph equipped with an FID-type detector and a steel column. The length of the column was 1.5 m and its diameter was 3 mm. It was filled with SE-30 (5%) on W-HP grade, 60-80 mesh, Chromosorb base. Column temperature was 110ºC and the carrier gas was nitrogen delivered at a rate of 40 cm^3·min. The samples of the gases under study were taken by means of a Hamilton's gastight syringe and next dosed onto the chromatograph. Detection limit of the compound was approx. 0.5-1 mg·m^{-3}. Deviations in the concentrations from the expected values ranged +10-20 [21].

Afterward, pots with seeds of "Tonacja" winter wheat sown into the soil (10 seeds per pot) were placed in the chambers in three replicas. The soil used in the experiment was collected from the humus horizon at a depth of 0-10 cm. It was a black earth with the granulometric composition of light loamy clay and pH$_{H_2O}$ 7.0. The seeds and the seedlings developing from them were exposed to continuous effect of the air containing styrene at the concentrations given above for a time period of three weeks. Three series of the height and dry matter yield measurements were made on the seedlings, as well as their physiological responses to stress induced by the presence of styrene. These measurements were carried out in three-day intervals, i.e. on day 12, 15 and 18 (counting on the day of sowing). On day 18 of the experiment, the number of stomata was counted. The experiment has been repeated three times.

The physiological response of plants and the gaseous exchange parameters such as intensity of CO_2 assimilation (A - μmol·m^{-2}·s^{-1}) and transpiration (E, mmol·m^{-2}·s^{-1}), was examined by measuring with a portable infrared gas analyzer LCA-4 (ADC Bioscentific Ltd. Hoddesdon, Great Britain). The measuring camera covered a leaf fragment of 0.5 cm^2. Also, the stomatal index (number of stomata per 1 mm^2) was determined by means of a CX21 SD/SF series microscope (Olympus Optical Co. Ltd, Tokyo, Japan). Photographs were taken with an Olympus digital camera C5050Z (Olympus Optical Co. Ltd, Tokyo, Japan). Biometric parameters, i.e. dry matter and plant height, were determined by means of gravimetric method (g) and a measuring rule (cm), respectively. The water-use efficiency (WUE, μmol CO_2·mmol^{-1}H_2O) was calculated as the ratio of CO_2 assimilation to transpiration.

The results illustrating the length of seedlings and the yield of dry matter were subjected to statistical analysis, using the analysis of covariance with time being an accompanying variable. When processing the results referring to the number of stomata, one-factor analysis of variance was used, in which the factor was air styrene concentration. The homogenous groups of means were constructed by means of Tukey's procedure, at significance level $\alpha = 0.05$.

In the case of assimilation and photosynthetic water use efficiency, the Kruskal-Wallis test was applied as a nonparametric equivalent of one-factor analysis of variance. Two-sided comparisons of means were subsequently performed. Such a procedure was applied due to the impossibility of adopting the assumption about variance homogeneity for the values of these traits.

Results and Discussion

Volatile organic compounds (VOC), into which styrene is included among others, can induce stress in plants [22]. This problem is subject to a small number of studies dedicated most frequently to the inhibiting effect of VOC on the elongation growth of plants [23]. Practically, there are no studies that directly refer to the effect of styrene on plants; more often, there are only descriptions of the studies con-

Table 1. Effect of styrene vapours on germination of wheat seeds.

Concentration of styrene ($mg \cdot m^{-3}$)	Day of germination	Germination %
0	6	100
71	6	100
142	6	100
285	8	90
570	8	80
1140	8	70
2280	10	50
$LSD_{0.05}$		18.3

Table 2. Effect of styrene vapours on assimilation of CO_2 (A), water use efficiency (WUE) and on the number of stomata per 1 mm^2 of epidermis of wheat seedling.

Concentration of styrene ($mg \cdot m^{-3}$)	Assimilation* of CO_2 ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	WUE* ($\mu mol\ CO_2 \cdot mmol^{-1}\ H_2O$)	Number of stomata per 1 mm^2 on 18th day
0	$2.75^{1\ 2}$	$11.82^{1\ 2}$	153
71	$4.49^{3\ 4\ 5}$	$15.82^{3\ 4\ 5}$	157
142	2.11	$11.97^{6\ 7}$	157
285	1.92	9.02	142
570	1.33^{5}	1.55^{5}	83
1,140	$0.53^{2\ 4}$	$1.16^{2\ 4\ 7}$	38
2,280	$0.54^{1\ 3}$	$1.06^{1\ 3\ 6}$	13
			$LSD_{0.05}$=36

Values marked with the same upper index differ significantly according to Kruskal-Wallis test.

*The average values for 12, 15, and 18th days of measurement.

cerning the effect on plants caused by substances structurally similar to styrene, such as benzene, aniline, toluene, etc. [24-26]. For example, the exposure of a pine tree to aniline at a concentration ranging 400-10,000 $mg \cdot m^{-3}$ induced necrosis of its needles [27]. On the other hand, toluene in a concentration of 6,000-12,000 $mg \cdot m^{-3}$, induced the darkening of leaves and a decrease in turgor and numerous chloroses in tomato, barley, and carrot [28]. Similar symptoms of toxic effects on plants were observed by Miller et al. [29] in relation to benzene at a concentration of 10,000 $mg \cdot m^{-3}$.

Germination Seeds

The study presented in this paper showed that styrene contained in the air has a distinct effect on the germination time of wheat seeds (Table 1). The seeds, in the control object and at styrene concentrations of 71 and 142 $mg \cdot m^{-3}$ in the camera air, germinated after 6 days. Those at concentrations ranging 285 to 1140 $mg \cdot m^{-3}$ germinated after 8 days, while the seeds at a concentration of 2,280 $mg \cdot m^{-3}$ germinated 10 days after sowing. At the highest styrene concentration in the air (4,559 $mg \cdot m^{-3}$), the wheat seeds did not germinate at all. This shows a clearly inhibiting effect of styrene on wheat germination at larger concentrations. It was also determined that the wheat seedlings growing in the atmosphere, in which styrene concentrations ranged from 570 to 2,280 $mg \cdot m^{-3}$, were characterized by clearly slower elongation growth in relation to the control samples and showed certain changes in their anatomical structure. Plant reaction in the presence of VOCs in the atmosphere could show by inhibiting the germination process or inhibiting or stimulating growth of over-ground plant parts and roots [30]. Most often this type of reaction is caused by allelopathic substance. It has been found that this substance in small concentrations shows a stimulating influence and, in contrast to high concentrations, is inhibiting [31]. A similar reaction was observed on wheat plants in the presence of styrene. For instance, volatile monoterpens present in etheric oils show particular activity in blocking mitosis process and cell elongation. This type of activity shows cyneol, which causes deformation of plant cells [32-37].

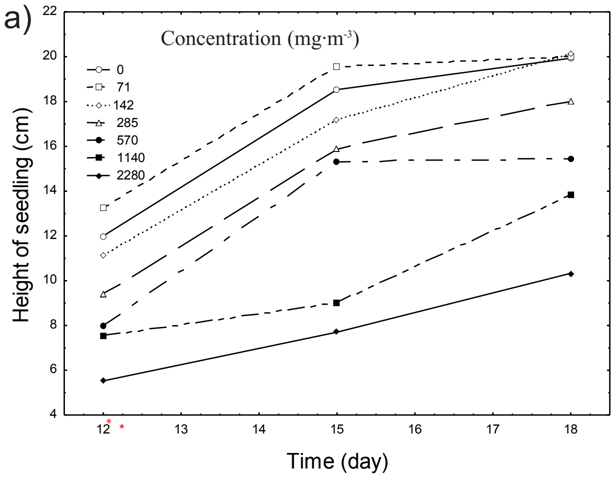
For example, at a styrene concentration of 2,280 $mg \cdot m^{-3}$, wheat leaf blades were wrinkled, fragile, breakable and showed features of xeromorphic plants; their leaves were strongly hardened and covered by a thick cuticule and wax, while their epidermis was characterized by a smaller number of stomata (Table 2).

Growth of the Wheat Seedlings

The effect of styrene in the air within the analyzed concentration range on the growth and development of the wheat seedlings is presented in Fig. 1a as a dependence of stem length on time. Growth of plants between day 12 and 15 was intense, both in the control object and within the concentration range of 71 to 570 mg styrene in m^3 of air. It was less intense in successive days. Different growth dynamics were observed at two successive concentrations, i.e. 1,140 and 2,280 $mg \cdot m^{-3}$. At a concentration of 1,140 $mg \cdot m^{-3}$, the growth of wheat plants within the period of 12-15 days was slow; it was more intense between days 15 and 18. On the other hand, for the concentration range of 2,280 $mg \cdot m^{-3}$, the growth of wheat plants was almost linear (Fig. 1a).

On a diagram in Fig. 2, dependence of the dry matter increase with time is presented for respective concentrations. The dynamics of wheat seedling dry matter increase in all styrene concentrations assumed a character similar to the linear one.

Analysis also showed that the observed effect of styrene within the examined concentration range on above-ground plant parts and wheat dry matter yield was significant. The analysis of covariance was applied here, with styrene concentration being an independent variable (qualitative predicator) and time being an accompanying variable (continuous predicator). Such a procedure allowed eliminating the effect of time, insignificant from the point of view of our experiment, on stem length and dry matter yield values.



*) The age of seedlings depended on the styrene concentrations and is the difference between the day of measurement and the day germination (Table 1).

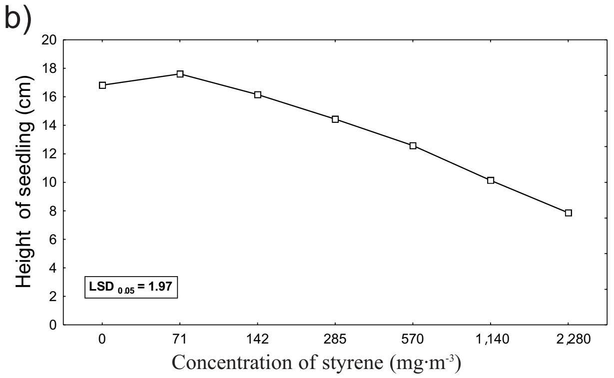


Fig. 1. The effect of different styrene concentrations on seedling height (a) and dependence of seedling height (average values for days 12, 15, and 18 of measurement) on styrene concentration (b).

This analysis was necessary to confirm the significant effect of time on stem length. The constructed covariance models proved to be highly significant, while the value of coefficient of determination for stem length was $R^2=0.962$ and for the yield of dry matter $R^2=0.972$. This demonstrates that differences in the length of plants and the yield of dry matter were analyzed, induced first of all by the action of styrene at different concentrations. The results in Figs. 1b and 2b are a graphical illustration presenting curves with a weakly marked maximum at a concentration of 71 mg·m^{-3}. Starting with the styrene concentration of 71 to 142 mg·m^{-3}, the values of the above-mentioned parameters approximated the control. A similar response was observed for dry matter. It may be concluded that styrene at a concentration of 71 mg·m^{-3} slightly stimulated the growth of seedlings, while only with further increases in its concentration was a regular decrease in the height of plants and their yield induced. Starting with a concentration of 285 mg·m^{-3}, this decrease was already distinct, and at concentrations of 1,140 and 2,280 mg·m^{-3} the height of seedlings and the yield of dry matter were approximately two and five times smaller, respectively.

Gaseous Exchange and Number of Stomata

These results are well-correlated with the measurements of gaseous exchange parameters discussed below.

The statistically processed results referring to the effect of styrene on photosynthesis rate and photosynthetic water use efficiency are presented in Fig. 3. The general picture of these relationships was similar to that observed in the case of dependence of seedling height and their dry matter yield on styrene concentration (Figs. 1b and 2).

Styrene at a concentration of 71 mg·m^{-3} clearly intensified CO_2 assimilation by leaves, both in relation to the control samples and for plants growing in its presence at the remaining higher concentrations (Fig. 3a). In the literature, information is found about a positive, even a causative, function of stress through so-called activation of new behaviours by plants [38]. A similar issue is raised by Machado [39] and Hoberg [40]. Further increase of styrene concentrations induced a decrease in CO_2 assimilation, while starting with a concentration of 570 mg·m^{-3} it was significantly lower than in control samples.

The analysis of two-sided comparisons of styrene concentrations (Table 2) showed that the values of assimilation and photosynthetic water use efficiency (WUE) corresponding to these concentrations differed significantly.

As reported by Grantz and Assman [41], the efficiency of water use in photosynthesis is a measure of plant sensitivity to stress factors. Photosynthetic water use efficiency in the wheat seedlings that grew under control conditions and in the atmosphere saturated with styrene vapours within the concentration range of 71 to 570 mg·m^{-3} was significantly higher than in those growing in chambers, where styrene concentrations amounted to 2,280 mg·m^{-3}. This parameter for the wheat seedlings growing in the atmosphere saturated with styrene at 140 mg·m^{-3} was also significantly higher than that recorded for the styrene concentration of 1,140 mg·m^{-3}. The maximum value of photosynthetic water use efficiency obtained for the seedlings at the lowest applied concentration of styrene (71 mg·m^{-3}) was

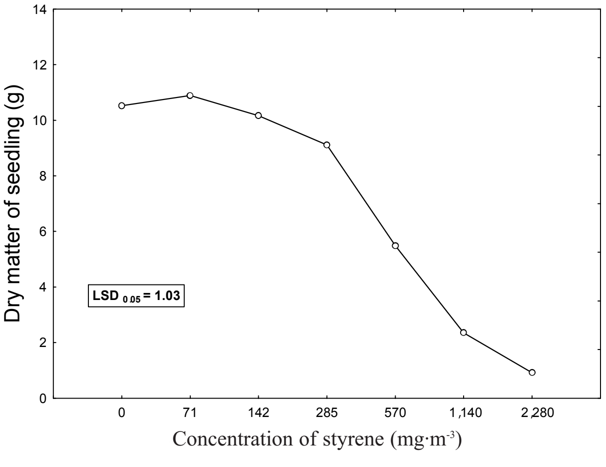


Fig. 2. The dependence of seedling dry matter (average values for days 12, 15, and 18 of measurement) with styrene concentration.

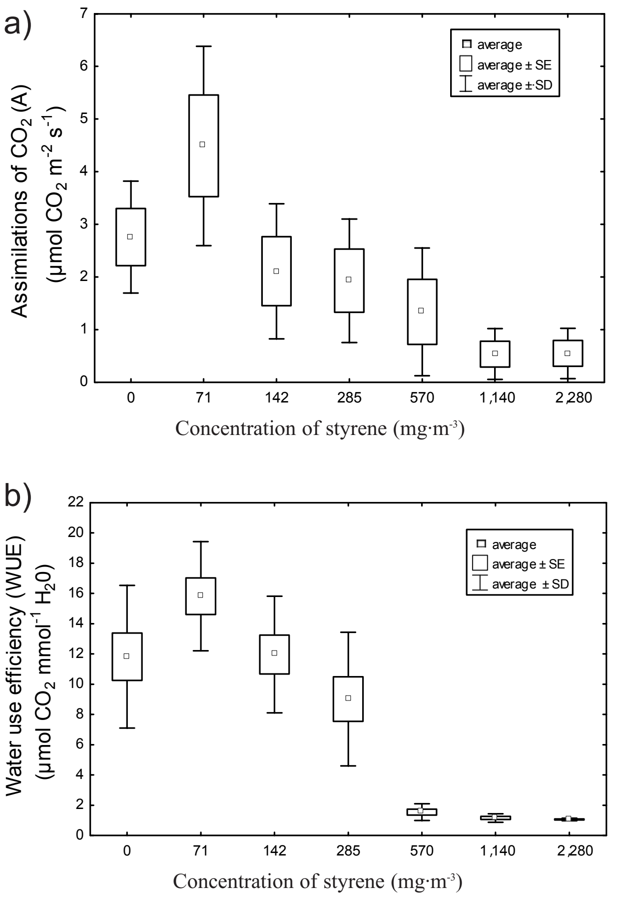
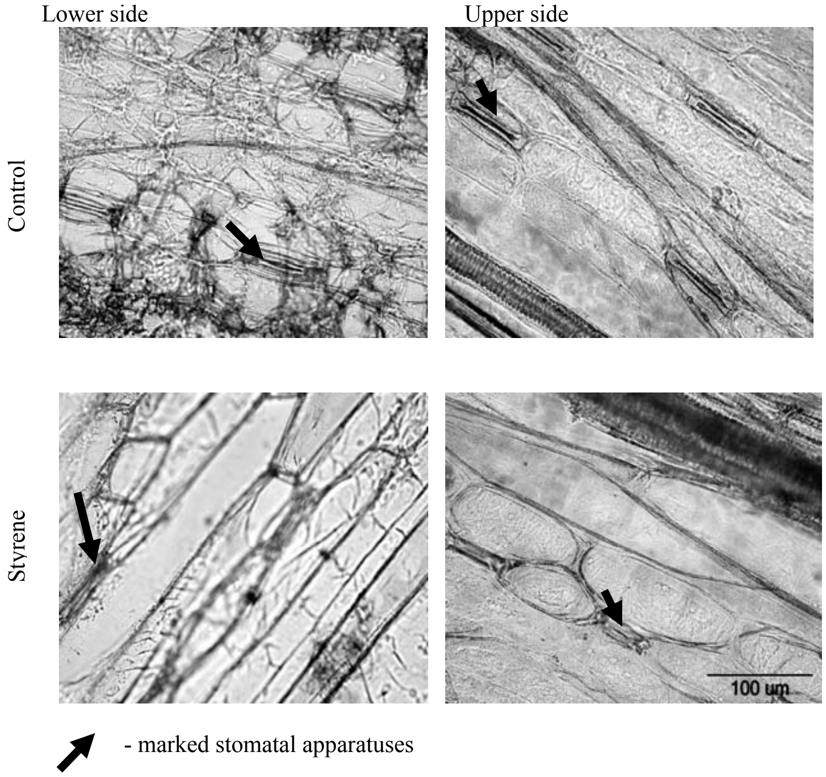


Fig. 3. The effects of different styrene concentrations in the atmospheric air on the intensity of assimilations of CO_2 (A) (a) and the water use efficiency (WUE) (b) in photosynthesis (average values for days 12, 15, and 18 of measurement).

significantly different than those recorded for the plants growing in the atmosphere saturated with styrene vapours at a concentration of just 570 mg·m^{-3}.

When analyzing the microscopic pictures, the effect of styrene on seedling leaf morphology was also found, i.e. on the mean number of stomata per 1 mm^{-2} of epidermis and the shape of stomata. The number of stomata significantly decreased together with the increase of styrene concentration in the air, starting with the value of 570 mg·m^{-3} (Table 2). At a concentration of 2,280 mg·m^{-3}, a several times smaller number of stomata was observed than under control conditions. Such a response is shown by xerothermic plants in order to reduce the loss of water from cells; as mentioned above, the examined wheat seedlings adopted a similar defensive strategy under the effect of styrene action. Under conditions of high styrene concentration (from 570 to 2,380 mg·m^{-3}), a gradual increase was observed in the volume of epidermis cells, as well as deformation of stomata and damage of cytoplasmatic membranes. The toxic influence of styrene was particularly visible on the upper side of wheat leaves at the highest concentration of the examined compound (Fig. 4). Similar reactions were observed by [42] while testing influence of alleopathic molecules on extracellural matrix. Alleophatic molecules penetrate matrix and can destroy existing or create new structural bindings. Additionally, they can modify membranes channels. This can result in restricting the functioning of enzymes, which can influence ion transport between membranes, ion cummulation, and water balance. All of the above have an affect on cell hydration, condition of stomata, and on the photosynthesis process. It has been observed that the higher the concentration of styrene, the stronger the cell vacuolization process.



- marked stomatal apparatuses

Fig. 4. Epidermal cells with stomata – lower and upper leaf side (for 18th day of measurement).

This reaction in the presence of VOCs was observed for cucumber seedlings [43]. Hypothetically, cell vacuolization works as a cell defense system as vacuoles accumulate waste and toxic substances. A similar reaction was observed on wheat plants under the effect of ethylbenzene vaporous [44].

Summary

Based on the obtained findings and with the lack of a sufficient number of literature references, it is difficult to explain precisely the changes in the physiological activity of wheat seedlings and the stomata aperture induced by the influence of styrene vapours. Many authors [22, 45-48] have already pointed earlier to the purposefulness of carrying out studies on the phytotoxic effect of volatile organic compounds. In the opinion of McLachlan [49], volatile organic compounds are assimilated by stomata, which manifests itself in changes in the stomatal index. It is also difficult to explain the cause of cell deformation. Most likely, it occurred as a result of sorption and dissolution of styrene on cellular substances [14]. This could induce, among other things, strong swelling of proteins in the cellular structures and cytoplasm. The swelling of polymers, including biopolymers, is a phenomenon commonly known and frequently occurring in practice [50-52]. Also, phytotoxic photochemical oxidants are formed with the participation of VOCs, which induce decomposition of lipids in protein-lipid membranes [49, 53]. The observed changes could also have been induced by an indirect effect of styrene metabolites. For example, in the opinion of Franich et al. [54], styrene is metabolized by pine-tree leaves. In small amounts it does not induce any changes in the cell structure, only its excess is toxic. Without a doubt, the level of styrene toxicity depends on the plant species.

In summary, it can be stated that the effect of styrene vapours contained in the air on the germination and growth of "Tonacja" wheat depends on styrene concentrations. At low concentration values, styrene stimulates the growth and development of wheat seedlings, while at higher ones (and at those found under real conditions in close vicinity to emitters), this effect is unfavourable.

Conclusions

1. We found a stimulating effect of styrene in a concentration of 71 $mg \cdot m^{-3}$ on the growth and development of "Tonacja" seedlings.
2. Starting with a concentration of 570 $mg \cdot m^{-3}$, styrene showed a distinctly inhibiting effect on the growth and development of wheat in the juvenile period. This compound in concentrations ranging from 570 to 2,380 $mg \cdot m^{-3}$ clearly inhibited net photosynthesis and water use efficiency in the photosynthesis process, and thereby the productivity expressed as the yield of fresh and dry matter. The wheat seedlings exposed to the action of styrene in concentrations of 570-2,280 $mg \cdot m^{-3}$ were characterized by changes in their anatomical structure, with their above-ground organs showing certain features of xeromorphic plants.
3. The increase of styrene concentrations above 2,280 $mg \cdot m^{-3}$ resulted in a gradual degradation of cytoplasmatic membranes. Also, a clear dehydration of stomatal cells occurred, as well as a reduction of the number and the size of stomata. The highest applied styrene concentration of 4,559 $mg \cdot m^{-3}$ was toxic for germinating wheat seeds.

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