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*Original Research*

# Volatile Organic Compound Emissions by Winter Wheat Plants (*Triticum aestivum* L.) under *Fusarium* spp. Infestation and Various Abiotic Conditions

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**Abstract**

The co-occurrences of biotic and abiotic factor stresses were studied on winter wheat cv. "Tonacja." In the first experiment the controlled infestation by *Fusarium* spp. and water stress were applied, and in the second experiment *Fusarium* spp. and various light intensities were given to winter wheat plants. The objective of this study was to determine how wheat's emission of volatile organic compounds (VOCs) depends on the biotic/abiotic factors.

In the first experiment, nine VOCs were indicated as a result of *Fusarium* spp. infestation; three of the terpenoids (linalool, *β*-caryophyllene and *β*-farnesene) and six green leaf volatiles (GLVs) ((*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, 1-hexyl acetate, and (*Z*)-3-hexen-1-yl acetate). Total amount of VOCs emitted by wheat reached 4-610 $ng \cdot h^{-1}$ plant d. $wt^{-1}$. Control plants (not infested) released a significantly lower amount of volatiles than diseased. Water regimes, established as the plant available water capacity (PAWC), range from 40 to 80%, caused the increasing emission of VOCs by diseased plants. Wheat that suffered from water stress (40% PAWC) emitted the greatest amount of GLVs of all control plants. Meanwhile, diseased wheat at drought also emitted terpenoids.

In the second experiment ten compounds were indicated (linalool, *β*-caryophyllene, benzyl acetate and (*Z*)-ocimene and six GLVs listed in the first experiment) as a result of *Fusarium* infestation in various light conditions. As light intensity increased from 65 to 295 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the diseased wheat plants produced from 27 to 337 $ng \cdot h^{-1}$ plant d. $wt^{-1}$ of terpenoids and from 20 to 1,008 $ng \cdot h^{-1}$ plant d. $wt^{-1}$ of GLVs. Diseased plants subjected to the highest light intensity (295 $\mu mol \cdot m^{-2} \cdot s^{-1}$) released 45-fold more VOCs than control plants. This can suggest that stress caused by *Fusarium* spp. at higher light intensity multiplied the production of VOCs by wheat.

**Keywords:** winter wheat, *Fusarium* spp. crown rot, volatile organic compounds, water stress, light intensity

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## Introduction

Plants release into the surrounding atmosphere a vast range of volatile organic compounds (VOCs). These compounds belong to different groups of chemicals, e.g. the terpenoids include among others *β*-farnesene, *β*-caryophyllene (sesquiterpenes $C_{15}$) or linalool and (*Z*)-ocimene (monoterpenes $C_{10}$), and the green leaf volatiles (GLVs) include (*Z*)-3-hexenal or (*E*)-2-hexenal (*cis*, *trans* aldehydes $C_6$), among others. The release of VOCs by plants is frequently associated with various abiotic stress factors, ranging from high temperature and oxidative stress and high light intensity to water stress and salt stress [1-4]. The induction of VOC emissions by various stress factors can be considered part of a plant response to alleviate the negative consequences of stress. Responses to abiotic stress factors by VOC emissions have been tested on different species, both wild and cultivated genera, annual and perennial plants from mono- and dicotyledonous classes [5].

Biotic stresses also induce the emission of plant volatiles. Herbivorous insect damage to vegetative tissue causes increased release of VOCs by plants, especially GLVs and terpenoids [6]. The GLVs are aldehydes, alcohols, and esters derived from lipoxygenase cleavage of fatty acids, and their name is taken from a specific small green leaf. Undamaged plants emit small quantities of GLVs, while intensive broadcasting of these compounds begins by damaged tissues [7, 8].

Plants are able to emit VOCs as consequences of insect herbivory and also pathogen infestation. It has been previously documented that fungal or bacterial pathogen attacks may also result in VOC production [9]. Moreover, usually pathogen infestation results in a different blend of VOCs than after herbivory or mechanical injury. Leitner et al. [10] and Yi et al. [11] documented that fungal attack can enhance the release of phenylpropanoids and isoprenoids. Wang and Dorn [12] recorded that vegetative VOCs play a crucial role in plants as defense against pathogens. Thus, the essential oils can be active against the fungal pathogens both in contact and on exposure to headspace volatiles [13]. Wheat plants that were parasitized by *Fusarium* species (crown rot pathogens) released linalool, linalool oxide, and β-farnesene, and the amounts of these metabolites varied significantly with *Fusarium* species [14]. Moreover, uninfested plants subjected to infested wheat released larger amounts of secondary metabolites as compared to control plants [15]. Within plants, VOC signaling also has the potential to be detected by neighboring individuals. This phenomenon has been interpreted as a "prophylactic response toward impending herbivore attack" [16].

Winter wheat (*Triticum aestivum* L. emend Fiori et Paol.) was chosen for this investigation because it is a world-wide crop plant and is considered to have the broadest adaptation of all cereal crop species to a wide range of environments [17]. However, environmental (abiotic) conditions play a crucial role in its development and growth. Water stress and drought, are the most important limiting factors in crop productivity in semi-arid agricultural areas [18]. Under drought conditions transpiration decreases significantly. When transpiration is reduced, heat loss from leaves slows down and leaf temperature increases. Water stress on plants is increased by low humidity, high temperatures, strong winds and high light intensity [19]. In this study the water requirements of wheat were established based on the soil water content. According to Graeff and Claupein [20], plant available water content (PAWC) at the start of the experiment was about 80%, and then 60% (optimal) and 40% (drought stress) levels were subjected as experimental treatments. Light intensity is one of these factors that are responsible for the photosynthesis and the appropriate rate of dry matter production. Relatively low light intensities could also be responsible for variation of tillering when wheat plants are competing for light [21]. However, the amount of light ultimately reaching plants in natural conditions is different, depending on atmospheric transparency, cloudiness, meteorological factors, and canopy shading [22]. The range of light intensity for winter wheat can be between 100-300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in growing chamber trials [21], hence in this study the similar levels were performed.

Under natural conditions, plants rarely are subjected to single stress factors one by one, but more likely are exposed to multiple stresses simultaneously [5]. In this study the co-occurrence of biotic stress (infestation by *Fusarium* spp.) and abiotic stress factors (water or light intensity) were applied to the winter wheat in two strict laboratory experiments.

The main purpose of this study was to recognize the impact of biotic and abiotic factors on biogenic VOCs emitted by winter wheat. The hypothesis to this study presumed the additive or multiplicative effects of biotic stress caused by fungal pathogens in interaction with water or light stresses. The important issue of manipulating these factors is a pest control strategy involving VOCs.

## Materials and Methods

The study was carried out at the Plant Growth Centre at the University of Technology and Life Sciences in Bydgoszcz, Poland, and at the Chair of Environmental Chemistry and Bioanalytics at Nicolaus Copernicus University in Toruń, Poland, in 2009. The objective of the study was winter wheat *Triticum aestivum* L. emend Fiori et Paol cv. Tonacja.

### *Fusarium* Preparation

Mixed cultures of *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W. G. Smith) Sacc., and *F. graminearum* Schwabe were used for infestation of wheat. These species were found and isolated from wheat (roots and crowns) grown in crop fields at the Experimental Station in Mochełek, Bydgoszcz vicinity (53º7′ N, 18º0′ E). Isolates were deposited at the Department of Phytopathology (University of Technology and Life Sciences) collection

on potato-dextrose agar (PDA) slants. For the inoculum preparation the stored cultures were rejuvenated on PDA plates at 21-24ºC for 14 days. Equal parts of barley and wheat grain up to 250 g were mixed and placed in water in 1000 ml glass jars for 16 h. Excess water was removed and the grain was autoclaved for 30 min at 121ºC. Grain was inoculated with three 1-cm-diameter plugs of mycelium of each *Fusarium* sp. from a PDA culture and incubated for 21 days at 24ºC [23]. The colonized grain was air-dried and ground in a laboratory mill to pass through a 1-mm sieve. 'Tonacja' wheat plants were planted at 3-cm depth in pots (1210 $cm^3$) filled with sterilized soil (mixture of 40% sandy soil and 60% quartz sand) and moistened to 37.5% until seedlings emerged [24]. The dry, ground inoculum was applied as a layer placed 1 cm above the seed at a rate of 1.4 g per pot. In each treatment there were six replicates (pots) and ten seeds were sown per pot. After emergence, the plant number per pot was reduced to six to obtain the uniform size samples.

## Disease and Rating

The first assessment of plants' response to tested *Fusarium* spp. infection was done after germination at the BBCH 11-12 (BBCH is the identification key for the cereal development, scale from 00 to 99, obligated in the EU [25]. The percentage of seedlings damping off before germination and after germination was calculated. Isolations on PDA medium of the casual agent of the disease were made. The second examination was carried out at BBCH 36. Disease severity on stems and roots was estimated for all plants. The following scale was used:

0º – for roots and crowns of healthy plants without symptoms; for roots and crowns of plants with symptoms:
1º – up to 10% parasitized
2º – 11 to 30% parasitized
3º – 31 to 60% parasitized
4º – above 60% parasitized roots and crowns

Infection degrees were converted into per cent disease indexes (DI) following the Townsend-Heuberger formula [15]. Isolations of the pathogens from infected stems and roots tissue on PDA medium were made for confirmation of the identity of *Fusarium* spp.

## *Fusarium* spp. Infestation and Water Stress for Winter Wheat – First Experiment

The trial was conducted in a greenhouse supplemented with a drip irrigation system. Two-layer containers of so-called Koch's pots were supported by a pipe system that allowed maintaining the plant available water capacity (PAWC) in the range 40 to 80%. The supplemental light was 5 klx (photon flux density of 165 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and ambient humidity 75-85%. The photoperiod was 16L:8D, day temperature was 6±0.5ºC, and the night temperature was 2±0.5ºC. Plants were grown with six individuals per pot fertilized twice a week with 25:12:12 mg of N:P:K at 100 ml in aqueous solution as part of the watering. Fertilizing commenced when the plants reached the third leaf stage. The plants were allowed to grow for 45-50 days, thus all individuals used for volatiles collection were at BBCH 35. At this stage there are five large leaves projecting upward from the area of the elongating stem. When plants started BBCH 36, after volatiles collection, they were removed from pots and their fresh and dry weights, number of tillers, and DI were measured.

There were two experimental factors in this trial:

A. *Fusarium* spp. infestation versus control (no infestation) for wheat.
B. The plant available water content (PAWC) as three levels: 40, 60, and 80% for wheat.

## *Fusarium* spp. Infestation and Light Intensity for Winter Wheat – Second Experiment

The experimental pots with *Fusarium*/control wheat at BBCH 13 stage were placed in a growth room at 6±0.5ºC day temperature and 3±0.5ºC night temperature, with a 16L: 8D photoperiod. Light was supplied by Philips TLD 36W/54 fluorescent tubes at photon flux density in three separated cabinets at 65, 165, and 295.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The ambient humidity was 80% and the PAWC was maintained at 60%. The application of fertilizers was similar to that in the 1st experiment. Winter wheat plants were grown with six individuals per pot until they reached BBCH stage 35-36 (50 days from emergence to the volatile collection). Plants were removed after volatiles collection and the measurements were taken as described in the first experiment.

There were two factors:

A. *Fusarium* spp. infestation versus control (no infestation) for wheat.
B. The light intensity (LI) as three levels: 65, 165, and 295 $\mu mol \cdot m^{-2} \cdot s^{-1}$. These photon flux densities respond to the klx: 1.4, 5, and 8.

## Volatile Collection System

Pots with wheat were subjected to the volatile collection laboratory when plants reached BBCH 35. Volatiles were collected from wheat plants enclosed to Nalophan (polyethylene terephtalate), odor- and taste-free cooking bags made of a plastic film resistant in the temperature range -60ºC to +220ºC (Charles Frères-Saint Etienne-France). Whole stems from experimental plants were subjected to the volatile collection (non-destructive) system and the Nalophan bag filled with stem was tightly closed using tape. The apparatus allowed for the collection of odors from 4 plants at the same time. A volatile collector trap (6.35 mm OD (outside diameter), 76 mm long glass tube; Analytical Research Systems, Inc., Gainesville, Florida, USA) containing 30 mg of Super-Q (Alltech Associates, Inc., Deerfield, Illinois, USA) adsorbent was inserted into each of 4 Tygon tubes (connection between airflow meter and collector trap). Purified (with charcoal), humidified air was delivered at a rate of 1.0 $l \cdot min^{-1}$ over the plants, and a vacuum pump sucked 20% less (0.8 $l \cdot min^{-1}$) to avoid collecting

Table1. Effect of plant-available water content (PAWC) and *Fusarium* crown rot disease on winter wheat plants and VOC production.

| Characteristics | PAWC (%) | | | | | |
|---|---|---|---|---|---|---|
| | 40 | | 60 | | 80 | |
| | Diseased wheat | Control | Diseased wheat | Control | Diseased wheat | Control |
| Number of tillers | 1.9±0.2* aA# | 2.2±0.2 bX | 3.3±0.4 aB | 4.1±0.5 bY | 4.0±0.4 aC | 4.4±0.3 bY |
| Plant dry weight (g) | 1.53±0.21 aA | 1.70±0.40 bX | 2.71±0.53 aB | 3.13±0.62 bY | 3.12±0.41 aC | 3.83±0.36 bZ |
| DI (%) | 45.0±3.5 A | 0.0 | 50.0±5.0 A | 0.0 | 70.0±6.5 B | 0.0 |
| Volatiles production ($ng \cdot h^{-1}$ plant $d.wt^{-1}$) | 139.3±6.4 aA | 65.9±2.8 bX | 435.6±10.5 aB | 4.09±0.4 bY | 610.4±21.2 aC | 4.31±0.5 bY |
| Terpenoids | 53.9±6.5 bA | 0.38±0.2 aX | 165.1±8.5 bB | 0.03±0.0 aX | 233.6±12.5 bC | 0.06±0.0 aX |
| Linalool | 23.6±7.5 | 0.17±0.0 | 71.8±10.5 | 0.03±0.0 | 97.2±10.0 | 0.01±0.0 |
| *β*-caryophyllene | 23.8±5.6 | 0.17±0.0 | 71.8±9.4 | 0.00 | 102.3±16.4 | 0.02±0.0 |
| *β*-farnesene | 6.55±1.5 | 0.04±0.0 | 21.5±5.4 | 0.00 | 34.1±6.8 | 0.03±0.1 |
| GLVs | 85.4±7.5 aA | 65.5±2.0 aY | 270.5±12.5 bB | 4.06±0.4 aX | 376.8±18.9 bB | 4.25±0.5 aX |
| (*Z*)-3-hexenal | 20.6±6.8 | 23.6±2.2 | 62.3±6.8 | 1.12±0.0 | 84.2±12.5 | 0.89±0.4 |
| (*E*)-2-hexenal | 13.8±4.5 | 13.9±3.4 | 47.3±5.0 | 0.66±0.1 | 61.9±6.8 | 0.74±0.3 |
| (*Z*)-3-hexen-1-ol | 13.8±5.1 | 6.62±1.3 | 45.9±3.5 | 0.63±0.1 | 62.8±10.5 | 0.74±0.3 |
| (*E*)-2-hexen-1-ol | 10.0±2.1 | 4.03±0.6 | 29.4±4.1 | 0.71±0.2 | 44.9±14.5 | 0.57±0.3 |
| 1-hexyl acetate | 6.81±1.4 | 2.99±0.5 | 23.9±2.4 | 0.24±0.0 | 41.8±12.5 | 0.38±0.2 |
| (*Z*)-3-hexen-1-yl acetate | 20.4±5.5 | 14.4±2.4 | 61.7±10.6 | 0.70±0.4 | 81.2±20.4 | 0.93±0.3 |

*Mean±standard error, n=36
#a,b – differences between diseased and control plants within PAWC levels
A,B,C – differences between PAWC levels within diseased plants
X,Y,Z –differences between PAWC levels within control plants

odors from any gap of the system. The volatile collection sequence (three-h duration) was performed at room temperature.

For all wheat plants (the first experiment), four diseased plants and four control plants (uninfested) were collected for each treatment of PAWC. For the second experiment the same number of replicates was conducted for each treatment of LI. Individuals were randomly chosen from the different pots. Additionally, five blanks (odors gathered from empty Nalophan bags) were collected (data not presented due to lack of VOCs in chromatograms).

## Analytical Methods

Volatiles were eluted from the Super-Q in each volatile collection trap with 225 µl of hexane, followed by adding 7 ng of decane as an internal standard. Previous experiments showed that this quantity of hexane was sufficient to extract all trapped volatiles (unpublished data). Individual samples (1 µl) were injected and analyzed by coupled gas chromatography-mass spectrometry (GC/MS). The GC/MS Auto System XL/Turbomass (Perkin Elmer Shelton, CT, USA) fitted with a 30 m Rtx-5MS capillary column (0.25 mm ID, 0.25 µm film thickness; Restek, USA). The temperature program increased from 40ºC to 200ºC at 5ºC/min. The identification of volatiles was verified with authentic standards purchased from commercial sources that had the same GC retention times and mass spectra (NIST, Wiley, Gatesburg, USA). Peaks were integrated directly from the GC-chromatogram.

The results were calculated to the ng per 1 h per stem using the following equation:

$$x = \frac{S_c \times IS}{3 \text{ x } S_{IS}}$$

$S_c$ – area of compound,
*IS* – internal standard – 7 ng,
$S_{IS}$ – area of the internal standard.

Since the stems were not equal in size, the amount of volatiles per one stem was calculated by the plant dry weight (g) and expressed as $ng \cdot h^{-1}$ plant $d.wt^{-1}$.

## Statistical Analyses

Both experiments were carried out in a randomized block design, with six replications (pots) of each combined treatment (2x3=6) containing six plants per replicate. Arcsin transformation was performed on percentage data for disease indexes before analysis of variance. The nor-

Table 2. Effect of light intensity (LI) and *Fusarium* crown rot disease on winter wheat plants and VOC production.

| Characteristics | LI (μmol·m$^{-2}$·s$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 65 | | 165 | | 295 | |
| | Diseased wheat | Control | Diseased wheat | Control | Diseased wheat | Control |
| Number of tillers | 2.2± 0.3* aA# | 2.8±0.2 bX | 3.7±0.4 aB | 4.7±0.3 bY | 4.0±0.3 aB | 4.9±0.5 bY |
| Plant dry weight (g) | 0.77±0.08 aA | 1.76±0.61 bX | 3.44±0.85 aB | 5.64±0.87 bZ | 3.44±0.46 aB | 4.90±0.74 bY |
| DI (%) | 83.1±5.0 C | 0.0 | 47.1±4.5 A | 0.0 | 59.5±7.5 B | 0.0 |
| Volatiles production (ng·h$^{-1}$ plant d.wt$^{-1}$) | 46.8±2.1 bA | 10.9±0.8 aY | 614.0±8.9 bB | 1.49±0.8 aX | 1344.3±10.8 bC | 29.5±1.3 aZ |
| Terpenoids | 26.8±1.5 A | 0.00 | 176.5±3.4 B | 0.00 | 336.5±8.6 C | 0.00 |
| Linalool | 10.0±2.3 | 0.00 | 75.4±2.5 | 0.00 | 127.4±15.5 | 0.00 |
| *β*-caryophyllene | 0.86±0.1 | 0.00 | 7.3±0.4 | 0.00 | 16.9±0.8 | 0.00 |
| Benzyl acetate | 8.86±0.1 | 0.00 | 61.4±5.6 | 0.00 | 118.9±10.4 | 0.00 |
| (*Z*)-ocimene | 7.1±0.9 | 0.00 | 32.4±1.1 | 0.00 | 73.3±5.2 | 0.00 |
| GLVs | 20.0±3.2 aA | 10.9±0.8 aY | 437.5±10.5 bB | 1.49±0.08 aX | 1007.8±16.4 bC | 29.5±1.3 aZ |
| (*Z*)-3-hexenal | 9.8±3.0 | 5.4±0.6 | 184.7±18.4 | 0.26±0.03 | 433.3±22.4 | 11.2±1.0 |
| (*E*)-2-hexenal | 0.00 | 0.00 | 17.5±4.6 | 0.08±0.01 | 36.5±6.5 | 3.20±1.2 |
| (*Z*)-3-hexen-1-ol | 0.00 | 0.00 | 44.0±6.8 | 0.13±0.02 | 103.6±14.2 | 1.50±0.3 |
| (*E*)-2-hexen-1-ol | 0.00 | 0.00 | 5.1±0.9 | 0.02±0.01 | 10.8±5.6 | 1.50±0.1 |
| 1-hexyl acetate | 0.00 | 0.00 | 4.6±0.8 | 0.25±0.01 | 10.1±5.4 | 2.30±0.8 |
| (*Z*)-3-hexen-1-yl | 10.2±3.4 | 5.5±0.9 | 181.6±10.8 | 0.75±0.5 | 413.5±12.5 | 9.80±2.1 |

* Mean±standard error, n=36
#a,b – differences between diseased and control plants within LI level
A,B,C – differences between LI levels within diseased plants
X,Y,Z –differences between LI levels within control plants

mality of distribution was checked using Shapiro-Wilk's W test. Data were analyzed using two-way ANOVA in randomized block followed my means separation using Tukey's HSD test at P≤0.05 (PROC GLM, SAS® 9.1.3).

## Results

### Response of Winter Wheat to PAWC and *Fusarium* spp. Infestation

The assessment of the impact of soil water content occurred on the wheat at BBCH 36, when the plants reached the sixth visible node, before flag leaf developed and its interaction with *Fusarium* spp. inoculation (Table 1). The analysis of variance indicated high significant differences between PAWC treatments displayed both for the number of tillers and the dry biomass of wheat individuals. The values of these two characteristics indicated increasing trends with the increased PAWC range. Wheat plants grown at PAWC 40% produced viewer tillers (43 and 50% less than at 60 and 80% PAWC) as well as less dry biomass (45 and 53% less, respectively) (Table 1). As each water treatment had matching *Fusarium* diseased-control healthy plants, the measurements of the number of tillers and the dry biomass of wheat could be compared as interaction for the matched *Fusarium* treatment-control plants using HSD Tukey's test (Table 1). *Fusarium* spp. caused a significant reduction in the number of tillers and also reduced the biomass of the whole plant. It was associated with the incidence/severity of the crown rot disease caused by three pathogenic isolates belong to *F. culmorum*, *F. avenaceum*, and *F. graminearum* used for the mix inoculation of soil. The DI reached 70% for the plants cultivated at 80% PAWC, while less necrosis was observed on the plants cultivated at 40 and 60% PAWC, DI reached 45 and 50%, respectively (Table 1).

Nine VOCs were indicated by GS-MS that wheat plants emitted in this trial; three of the terpenoids (linalool, *β*-caryophyllene and *β*-farnesene) and six belong to GLVs ((*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, 1-hexyl acetate, and (*Z*)-3-hexen-1-yl acetate). The total amount of VOCs emitted by wheat, measured in ng per 1 h per plant d. wt, reached from 4.09 to 610.4 (Table 1). For the control (healthy) wheat plants the significantly greater amount of VOCs (65.9 ng·h$^{-1}$ plant d.wt$^{-1}$) was indi-

cated only at 40% PAWC. Meanwhile, for the diseased plants the production of VOCs increased from 139.3 to 610.4 ng·h$^{-1}$ plant d.wt$^{-1}$, along with elevated water content. Generally, diseased plants released greater amounts of VOCs at each water treatment, then the control ones; two-fold at 40% and over one hundred repeatedly at 60 and 80% PAWC (Table 1). The spectrum of VOCs emitted by host plants of *Fusarium* spp. included both the terpenoids and the GLVs, with the constant ratio between them equling 38:62. This means that 1 ng of terpenoids was covered by 1.6 ng of GLVs. Among the terpenoids, the linalool and *β*-caryophyllene prevailed, meanwhile (*Z*)-3-hexenal and (*Z*)-3-hexen-1-yl acetate dominated within the group of VOCs. While the plants suffered from water stress only they did not released the terpenoids, and in the spectrum of GLVs the *cis*- and *trans*-hexenal and *cis*-hexenyl acetate prevailed. Healthy plants not subjected to abiotic stress (growing at 60 or 80% PAWC) produced small amounts of VOCs (4.09 and 4.31 ng·h$^{-1}$ plant d.wt$^{-1}$) with similar ratios of particular compounds (Table 1).

### Response of Winter Wheat Plants to the LI and *Fusarium* spp. Infestation

Non-inoculated wheat, grown at 165 µmol·m$^{-2}$·s$^{-1}$, produced 4.7 tillers and 5.64 g of dry biomass (Table 2). At the same light conditions plants inoculated with *Fusarium* spp. revealed the crown rot disease index at 47%, causing their production of tillers to be about one tiller per plant less, and consequently the dry biomass was 2.2 g less. The most dramatic course of light stress was evident for minimal photon flux density, i.e. 65 µmol·m$^{-2}$·s$^{-1}$. Plants suffering from lack of light possessed only 2.2-2.8 tillers with low biomass 0.77-1.76 g. The negative effect was additionally exacerbated by *Fusarium* spp. inoculation due to the severity (83% DI). There was no difference in the number of leaves between 165 and 295 µmol·m$^{-2}$·s$^{-1}$; meanwhile, the dry biomass was significantly greater for control plants grown at 165 µmol·m$^{-2}$·s$^{-1}$ (Table 2).

Healthy wheat plants grown in medium light conditions (165 µmol·m$^{-2}$·s$^{-1}$) emitted low but detectable amounts of GLVs (1.49 ng·h$^{-1}$ plant d.wt$^{-1}$), while plants infested by *Fusarium* sp. released over 500-fold more VOCs; terpenoids – 176.5 and GLVs – 437.5 ng·h$^{-1}$ plant d.wt$^{-1}$ (Table 2). The largest emission was recorded for linalool and benzyl acetate across tested factors. Furthermore, (*Z*)-3-hexenal and (*Z*)-3-hexen-1-yl acetate were emitted in considerably larger amounts compared to other GLVs. Lack of light (65 µmol·m$^{-2}$·s$^{-1}$) induced healthy wheat to emit VOCs on a level of 10.9 ng·h$^{-1}$ plant d.wt$^{-1}$, with equal proportion of (*Z*)-3-hexenal and (*Z*)-3-hexen-1-yl acetate. Infested wheat plants released significantly greater amounts of VOCs (approx. 46.8 ng·h$^{-1}$ plant d.wt$^{-1}$), mainly linalool and benzyl acetate at lowest LI. The highest VOC emissions by wheat was recorded for infested plants simultaneously grown under higher LI. Total amount of VOCs released by infested wheat plants grown at 295 µmol·m$^{-2}$·s$^{-1}$ reached 1,344.3 ng·h$^{-1}$ plant d.wt$^{-1}$, while healthy plants emitted only 29.5 ng·h$^{-1}$ plant d.wt$^{-1}$ (Table 2). In a blend of VOCs released by wheat plants under *Fusarium* infestation and the highest LI, four terpenoids (total amount reached 24%) and six GLVs (with 35% for (*Z*)-3-hexenal and 31% for (*Z*)-3-hexen-1-yl acetate) were detected. Control wheat under the highest LI released no terpenoids, but among GLVs considerably the largest amounts across the experiment were (*Z*)-3-hexenal (38%) and (*Z*)-3-hexen-1-yl acetate (33%).

## Discussion

Wheat is one of the most important agricultural crops world-wide; however, water and light are the most limiting factors for wheat production. The soil moisture deficit (40% of soil water content) causes the slowdown of plant growing and decreases accumulation of fresh and dry biomass [26]. Water supply to the soil (ranged from 45 to 85% PAWC) has a positive effect on wheat development and final parameters of grain, i.e. productiveness, lipid contents, and starch accumulation [27]. Data presented here confirmed that 40% of PAWC caused the drought stress for wheat plants that produced a smaller number of tillers, as well as less dry biomass. Responses to mere changes in light intensity can be of great importance for wheat, whose life cycle is long, and yield is formed throughout the progression of the season [28]. In this study insufficient LI (65 µmol·m$^{-2}$·s$^{-1}$) caused stress that resulted in low production of tillers and dramatically reduced the dry biomass. It is in good agreement with Worland et al. [29], who proved that with low LI the number of days to tillering increased and the photoperiod insensitive cultivars of wheat did not tiller at all.

Generally, abiotic stresses induce the emission of VOCs by plants and, *vice versa,* VOCs bring stress relief for plants. Many examples of such responses are given by Holopainen and Gershenzon in their review [5]. The knowledge of physical processes and biosynthesis of VOCs is helpful to understand the impact of stresses to plants. For instance, the elevated emission of VOCs at higher temperature is in part a physical process due to increase in the vapor pressure of VOCs and the higher rate of their biosynthesis [30]. It was found that increasing light intensity stimulates VOC emission in a variety of different plant types and growth forms, such as sunflower and beech [31], holm oak [32], and kudzu (*Pueraria lobata*) [33]. Appropriate LI is required for photosynthesis to dry matter production and many biogenic VOC emissions (particularly terpenoids) are light-dependent [34]. The sensitiveness of wheat to increasing LI was also confirmed in this study. At 295 µmol·m$^{-2}$·s$^{-1}$ the emission of VOCs, by control plants, was two-fold higher than at 65 µmol·m$^{-2}$·s$^{-1}$. However, the lowest LI induced wheat to emit two compounds only, i.e. (*Z*)-3-hexenal and (*Z*)-3-hexen-1-yl acetate, while the highest LI expand the spectrum of emitted GLVs.

Wheat plants that suffered from drought stress emitted 65.5 ng·h$^{-1}$ plant d.wt$^{-1}$ of GLVs, mainly (*Z*)-3-hexenal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-yl acetate and trace amounts of terpenoids in the spectrum of VOCs, while control plants not subjected to water stress produced significantly smaller amounts of VOCs.

Infestation by fungal pathogens induced the production of terpens, i.e. linalool, (*Z*)-ocimene, and *β*-farnesene [35, 36]. Lacy Costello [37] reported over 50 VOCs collected from potato piper tubers inoculated with *Phytophtora infestans* and *Fusarium coeruleum*. The application of biotic and abiotic stresses could have an additive effect on VOC emissions. Ibrahim at al. [38] described higher induction of volatiles in conventional rapeseed oil plants infested by *Plutella xylostella* grown at a high-soil nutrient level compared to infested conventional and transgenic rapeseed oil at a low-soil nutrient level. In maize, the combination of high temperatue and simulated lepidopteran herbivory resulted in greater VOC emissions than when their stress was applied alone [39].

This study confirmed the thesis about the interaction between biotic and abiotic stresses. The multiple effect of the interaction of *Fusarium* crown rot pathogens in higher LI was improved by the production 1,344.5 $ng \cdot h^{-1}$ plant $d.wt^{-1}$ of VOCs, that was the greatest amount of all in the entire study. In the case of *Fusarium* crown rot and drought stress the increase of VOCs emissions was two-fold greater than at *Fusarium* infection alone, which might suggest the additive character rather than multiplicative. However, the water content in soil had a direct effect on the extent of crown rot disease as well as on dry weight and the number of tillers. At the 80% PAWC the DI was highest and at 40% DI was lowest. This corresponded with VOC production by wheat. Presented data indicate that bigger plants will release a greater amount of VOCs regarding plant dry weight.

The percentage of individual compounds as well as the participation of whole groups of VOCs may play a significant role in the defense mechanism of wheat directed to biotic/abiotic stresses. The spectrum of VOCs emitted by wheat infected by *Fusarium* spp. included both the terpenoids and the GLVs with the constant ratio between them equal to 38:62. This means that 1 ng of terpenoids was covered by 1.6 ng of GLVs.

Further studies should be focused on transgenic plants engineered to produce modified VOC blends. This may be a new possibility in the pest control strategies involving VOCs. This study could be helpful to understand the role of plant defense mechanisms against various biotic and abiotic stresses. *Fusarium* sp. is a ubiquitous pathogen affecting many species of wild and crop plants, and the techniques of its manipulation are relatively easy. Hence, it may serve as a model pathogenic factor in future investigations.

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