TOXIC EFFECTS OF THE FUNGICIDE TEBUCONAZOLE ON THE ROOT SYSTEM OF FUSARIUM-INFECTED WHEAT PLANTS

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Abstract

The study investigates toxic effects of the fungicide tebuconazole (TEB) on *Fusarium*-infected wheat (*Triticum aestivum*) plants based on the morphological characteristics of root apices and changes in integrated parameters of redox homeostasis, including the contents of free proline and products of peroxidation of proteins (carbonylated proteins, CP) and lipids (malondialdehyde, MDA) in roots. In two-day-old wheat sprouts infected by *Fusarium graminearum*, the levels of proline, CP, and border cells of root apices are higher than in roots of uninfected sprouts by a factor of 1.4, 8.0, and 3, respectively. The triazole fungicide tebuconazole (TEB) at concentrations of 0.01, 0.10, and 1.00 μg ml^{-1} of medium causes a dose-dependent decrease in the number of border cells. The study of the effects of TEB and fusarium infection on wheat plants in a 30-day experiment shows that the effect of the fungicide TEB on redox homeostasis in wheat roots varies depending on the plant growth stage and is significantly different in ecosystems with soil and plants infected by *Fusarium* phytopathogens. The study of the morphology of root apices shows that toxic effects of TEB and fusarium infection are manifested in destructive changes in root apices and degradation of the root tip mantle.

Key words: Fusarium, tebuconazole, free proline, carbonylated proteins, malondialdehyde, border cells

1. Introduction

Fusarium infection is one of the most common diseases affecting cereal crops. This disease is caused by soil pathogenic fungi of the genus *Fusarium*. Crop losses due to fusarium infection in case of maize, wheat, and rice affected by fusarium infection are economically important, as they are major sources of plant protein and their yields constitute over 55% of the total yield of cereal crops. The crop losses may range between 5 and 30%. Many *Fusarium* species produce mycotoxins: deoxynivalenol (vomitoxin), zearalenone, and T-2 mycotoxin (Binder et al., 2007). Fusarium infection may damage the ear and result in reduced grain yield. Mycotoxin-contaminated grain is unsuitable and even unsafe food and feed. Application of fungicides decreases the incidence of fusarium infection and reduces the levels of mycotoxins in commercial grain (Schmale and Bergstrom, 2003; SANCO, 2013). Triazole fungicides now constitute 30% of the marketed fungicides. One of them is tebuconazole (TEB). TEB is an effective multifunctional systemic fungicide used to protect a number of cereal crops. TEB rapidly penetrates into the plants through both their vegetative organs and roots. However, triazole fungicides, including TEB, are phytotoxic (Ahemad and Khan, 2012a, b). The mode of action of the triazole group is to suppress ergosterol biosynthesis, preventing the formation of cell membranes, causing the death of pathogens (Lamb et al., 2001; Hartwig et al., 2012). Thus, *Fusarium*-infected crops treated with triazole fungicides are adversely affected by two factors: *Fusarium* infection and fungicide.

At the systemic level, toxic effects of triazoles lead to hormonal imbalance (Yang et al., 2014), nitrogen imbalance, lower seed germination rates, disorders of root growth and development (Serra et al., 2013, 2015), and the appearance of chromosomal abnormalities (Wandscheer et al., 2017). The fungal sterol-14-α-demethylase – the effector target of triazole fungicides in cells of mycopathogens – belongs to the evolutionarily ancient cytochrome-450(CYP)-superfamily, which has also been detected in plants and animals (Lamb et al., 2001). Phytotoxicity (and toxicity of triazole fungicides for humans and animals) has been associated with the effect of fungicides on the activity of sterol demethylases and disturbance of the sterol dependent signaling (Hartwig et al., 2012). At the systemic level, sterol dependent signaling determines the activity of such processes as proliferation, differentiation, and production of reactive oxygen species (Wassmann et al., 2001; Park et al., 2008).

The cause-effect chain "inhibition of sterol demethylases → sterol dependent signaling deficiency → inhibition of generation of reactive oxygen species" can be used as the basis for evaluating myco- and phytotoxicity of herbicides and for assessing plant resistance to mycopathogens. Regulated hyperproduction of reactive oxygen species (ROS) in response to pathogen invasion is one of the major protective responses of plants. In addition to being highly toxic, ROS trigger specific signaling systems, which cause changes in gene expression patterns and induce development of host plant resistance or sensitivity to pathogens (Frederickson and Loake, 2014; Swarupa et al., 2014).

Over the course of evolution, pathogenic fungi have developed scavenging systems that allow them to neutralize cytotoxic effects of the oxidative burst of the host plant. Pathogenic fungi use ROS generated in cells of their host plant to regulate expression of their own genes that control cell differentiation and hyphal growth in plant tissues (Takemoto et al., 2007). Thus, ROS signaling simultaneously determines 1) activation of plant defense response against invasion of mycopathogens, 2) stimulation of growth and differentiation of the mycopathogen in plant tissues after invasion, and 3) myco- and phytotoxicity of fungicides. In order to produce consistently high crop yields, ROS-dependent readjustment of these three systems should lead to the most complete suppression of mycoinfection with the minimal phytotoxic effects.

In addition to peroxidation products, there is another important indicator of the state of the plant root system: a free amino acid proline, which is an integrated indicator of the activity of root antioxidant and defense systems. Proline is a low-molecular-weight scavenger of free radicals (Signorelli et al., 2014), which also increases gene expression in antioxidant enzymes (de Carvalho et al., 2013). Activation of the synthesis of proteins with high proline contents is an important factor in the functioning of mechanisms of root defense against pathogen invasion (Cecchini et al., 2011; Plancot et al., 2013; Qamar et al., 2015).

Pathogen invasion occurs through the plant roots, and, therefore, the state of the roots of infected plants can be characterized by a system of border cells (Berrocal-Lobo and Molina, 2008). Border cells constitute a specific population of metabolically active cells localized in the root apex and playing a fundamental role in root interactions with symbiotic and pathogenic organisms of the rhizosphere (Gunawardena and Hawes, 2002; Bais et al., 2006; Wen et al., 2007, 2009; Cannesan et al., 2011, 2012). The gel mantle is an excretory product of border cells, which encloses them (Cannesan et al., 2011, 2012; Hawes, 2012). Invasion of root pathogens elicits production of border cells and increases their secretory activity – as a defense response (Plancot et al., 2013). Thus, the number of border cells can be regarded as an integrated indicator of the activity of defense systems in pathogen-infected roots.

The present study investigated toxic effects of the fungicide tebuconazole in *Fusarium*-infected wheat (*Triticum aestivum*) stands by examining the state of the root apices and changes in integrated parameters of redox homeostasis, including free proline content and contents of protein and lipid peroxidation in roots.

2. Materials and methods

2.1. Materials

Fungicide: tebuconazole (TEB) is a multifunctional systemic fungicide, which is effective against a very wide range of fungal diseases of cereal crops. The chemical formula of TEB is $C_{16}H_{22}ClN_3O$. Molar mass (g mol^{-1}): 307.82. Solubility in water: 36 mg L^{-1} at 20°C. Melting point is 104.7°C. The substance is not hydrolyzed at pH of between 4 and 9; it is stable upon exposure to light and elevated temperature. The time of degradation in soil is 177 days. A

106 commercial formulation Raxil Ultra (Bayer Crop Science, Russia), with tebuconazole (TEB) as the active ingredient
107 was used.
108 Wheat: experiments were performed in communities of soft spring wheat cv. Altaiskaya 70.
109 *2.2. Wheat cultivation*
110 Fusarium-infected and uninfected wheat seeds were used. Toxic effects of tebuconazole and fusarium infection
111 were studied in experiments with two-week-old wheat sprouts and in the long-duration experiment with fusarium-
112 infected wheat stands in the laboratory soil system. Wheat sprouts were grown as follows: the seeds were washed in
113 running water for 5-6 h and soaked in distilled water for 24 h at room temperature. Germinated seeds were placed into
114 Petri dishes containing 7 ml of distilled water, 50 seeds per dish. Tebuconazole solutions of concentrations 0.01, 0.10,
115 and 1.00 $\mu g.ml^{-1}$ were added to the treatments – 7 ml per dish. The seeds were sprouted at room temperature under
116 continuous light.
117 In the other experiment, wheat plants were grown in laboratory soil microecosystems. Soil microecosystems
118 were prepared as follows. The agrogenically-transformed soil (collected at the village of Minino, the Krasnoyarsk
119 Territory, Siberia, Russia) was placed into 500-cm^3 plastic containers (500 g soil per container). Wheat seeds were
120 sown into the soil, at a planting density of 100.45 g seeds per 1 m^2. Plants were grown in a Conviron A1000 growth
121 chamber (Canada) for 30 days under stable conditions: at an irradiation of 100-300 $\mu mol.\ m^{-2}\ s^{-1}$, under the 12L:12D
122 photoperiod, at a temperature of 18-25°C and humidity of 65%; conditions of the experiment and soil ecosystems are
123 described in detail elsewhere (Volova et al., 2017). In this experiment, we used infected wheat seeds. The experiment
124 consisted of two treatments and control: in control, infected wheat seeds were sown into the soil, with no fungicide
125 applied; in Treatment 1, seeds and the commercial formulation Raxil were buried in soil simultaneously, with Raxil
126 applied at a concentration corresponding to 3 µg TEB/g soil; and in Treatment 2, seeds were soaked in a Raxil solution
127 for 10 min before sowing, with no more TEB added to the soil.
128 *2.3. A biochemical study*
129 Toxic effects of TEB were evaluated by measuring changes in the integrated parameters of redox homeostasis:
130 the contents of proline, malondialdehyde, and carbonylated proteins in roots of two-day-old wheat sprouts and wheat
131 plants grown in soil-based systems – at Days 10, 20, and 30 of the experiment. Root samples were prepared by cutting
132 1-cm-long terminal portions of the roots with apices. Then, the root biomass was homogenized in a 0.05 M Tris-HCl
133 buffer solution, pH=7.4, in a hand-held homogenizer, at T=4°C. To remove coarse debris, the homogenates were
134 centrifuged at 5000 *g*, for 45 min, at T=4°C. The supernatant fluid was collected and used to determine the contents of
135 carbonylated proteins – by the method of Carty et al. (Carty et al., 2000), malondialdehyde – by the method of Bailly et

al. (Baily et al., 1996), and proline – by the method of Bates et al. (Bates et al., 1973). The contents of carbonylated proteins, malondialdehyde, and proline were calculated per 1 mg of root homogenate protein.

2.4. A morphological study of root apices

Prior to microscopic analysis, root apices were fixed in 2.5% glutaric aldehyde in 0.1 M phosphate buffer, pH=7.2. The root apices were rinsed in distilled water to remove the fixative and stained with 0.01% methylene blue. Using a light microscope, we counted the number of free border cells that had detached from the surface of the root and measured the size of the gel mantle (whose color had changed to blue due to the presence of a large amount of polysaccharides). Sixty to seventy root apices were analyzed in each treatment and in the control.

2.5. A study of the contamination of seeds and soil by phytopathogenic fungi

Intrinsic contamination of wheat seeds with phytopathogens was determined by sprouting the seeds in Petri dishes on sterile nutrient medium MEA (Russian Federal Standard 12044-93). In the experiment with wheat stands, the number of phytopathogenic fungi, including *F. moniliforme*, in soil was counted at Days 10, 20, and 30 of the experiment. Counting of the total microscopic fungi was performed by plating soil suspension onto Petri dishes with malt extract agar, which was supplemented with chloramphenicol (100 μg L^{-1} of the medium) to suppress cell growth. All platings were performed in triplicate from 10^2-10^5 dilutions of soil suspension. The dishes were incubated at a temperature of 25°C for 7-10 days. Microscopic analysis of the colonies was done using an AxioStar microscope (Carl Zeiss). Microscopic fungi were identified by their cultural and morphological properties, with identification guides (Sutton et al., 2001; Watanabe, 2002).

2.6. Statistical analysis

Statistical analysis of results was performed using the standard software package of Microsoft Excel, STATISTICA 8. Arithmetic means and standard deviations were determined using Student's t test. Results are given as X±m.

3. Results and Discussion

3.1. The effect of phytopathogenic infection on wheat seed germination and biochemical parameters and morphology of roots of sprouts

Phytosanitary analysis of wheat seeds grown on the nutrient medium showed the presence of infections caused by the fungi of the genera *Fusarium* Link, *Alternaria* Nees, and *Bipolaris* Shoem. Wheat seeds infected by plant pathogens constituted 9.5±1.2%, 5.6±0.2% of which (over 50%) were infected by *Fusarium* species. Thus, natural infections of the seeds were caused not only by the predominant *Fusarium* species, but also by the phytopathogenic microscopic fungi that developed when the seeds containing internal infection were germinated.

166 Germination rate of uninfected *Triticum aestivum* seeds reached 90±3%. The roots of two-day-old sprouts
167 contained 1.03±0.09 nM carbonylated proteins (CP)/mg protein, 10.59±0.26 μg proline/mg protein, and 0.300±0.035
168 nM malondialdehyde (MDA)/mg protein. MDA, as a product of peroxidation of membrane lipids, can be involved in
169 regulation of activity of cell membranes (via rearranging of the lipid bilayer and changing of the activity of membrane-
170 bound proteins) (Ansari et al., 2015; Antosik et al., 2015). The levels of CP, MDA, and proline revealed in the
171 experiment characterize redox homeostasis in normally developing roots of uninfected *Triticum aestivum* sprouts.

172 Germination rate of infected wheat seeds was lower (77±7%). The CP content in roots was 8 times higher than
173 in the roots of uninfected sprouts (Fig. 1). The level of proline in the roots of infected sprouts was 1.4 times higher than
174 in the roots of uninfected sprouts. These results are consistent with the notion of pathogen invasion inducing activation
175 of the system for production of free radicals as a major defense mechanism of a plant cell exposed to biotic and abiotic
176 stresses (Sham et al., 2014; Chanclud and Morel, 2016). An increase in the activity of protein peroxidation results from
177 the regulated activation of generation of ROS in plant tissues as a response to invasion of pathogenic fungi; it is
178 necessary for inducing ROS-dependent signaling of defense systems (Frederickson and Loake, 2014; Swarupa et al.,
179 2014). The level of MDA in the roots of infected sprouts was not significantly different from the MDA level in
180 uninfected roots (Fig. 1). That may be attributed to the involvement of MDA in oxidative modification of proteins
181 (Augustyniak et al., 2015). Thus, proportions of CP, MDA, and proline in roots of infected sprouts differed from those
182 in roots of uninfected sprouts, suggesting a transition of the redox systems to another level of homeostasis.

183 In the experiment with the fungicide tebuconazole (TEB) added to the culture medium at concentrations of
184 0.01, 0.10, and 1.00 $\mu g.ml^{-1}$, germination rate of the infected wheat seeds was similar to that of the infected seeds in the
185 experiment without TEB addition (75±8%). None of the TEB concentrations tested in this study affected CP, MDA, and
186 proline levels in the roots of infected sprouts (Table).

187 Morphological dissimilarities between the root apices of uninfected and infected sprouts, with pronounced
188 differences in the contents of carbonylated proteins, are shown in Figure 2. The root apex of an uninfected sprout is
189 ensheathed in a small gel mantle containing border cells (BC) that have detached from the surface of the apex (Fig. 2a);
190 there are 15±3 border cells/apex. The infected sprouts contain considerably more BC (47±7 cells/apex). Thus, ROS
191 signaling may be involved in activation of BC production in response to invasion of pathogenic fungi. As the number of
192 BC in the root apices of infected sprouts increased, the size of the gel mantle increased, too (Fig. 2b, c, d). The
193 scattering of the border cells around the root apex corresponds to the size of the gel mantle. In the root apices of
194 infected sprouts, the border cells inside the gel mantle may form large aggregates (Fig. 2b), and layers of border cells
195 may peel off the lateral surface of the root apex (Fig. 2d).

196 Formation of the gel mantle is caused by activation of the excretory function of border cells, which defends the
197 root apex against pathogen invasion (the root apex is the most "protected" part of the root). The effectiveness of defense
198 is determined by not only an increase in the size of the gel mantle but also changes in its composition (Baetz and
199 Martinoia, 2014). In infected roots, border cells secrete xylogalacturonans, which are resistant to the effects of
200 pectolytic enzymes of pathogenic fungi, and arabinogalactan proteins (Cannesan et al., 2011, 2012).

201 Supplementation of the culture medium with the fungicide tebuconazole caused a dose-dependent decrease in
202 the number of border cells in the root apex (Fig. 3a). At TEB concentrations of 0.01 and 0.10 $\mu g.ml^{-1}$, the number of
203 border cells decreased by a factor of 2, and at TEB concentration of 1.00 $\mu g.ml^{-1}$, the number of BC dropped by a factor
204 of 4.7 compared with the test where no TEB was applied to the soil.

205 As the BC number in the root apex decreased, the gel mantle grew smaller, too. At the highest tebuconazole
206 concentration in the medium (1.00 $\mu g.ml^{-1}$), the majority of root apices were "bare", with no noticeable gel mantle and
207 free BC (Fig. 3b). The uninfected sprouts had no "bare" root apices. The "bareness" of the apices could be caused by
208 fungicide phytotoxicity. As TEB diffuses through the gel mantle into the root apex, it may inhibit BC production
209 through the sterol dependent signaling, which is involved in regulation of cell proliferation activity (Roy et al., 2011).
210 The dramatic decrease in the number of free BC – major producers of molecular components of the gel mantle – results
211 in the occurrence of "bare" apices. This most probably weakens the defense systems of plant tissues subjected to
212 invasion of phytopathogens.

213 Thus, in contrast to fusarium infection, the fungicide tebuconazole at concentrations used in the experiments
214 did not affect the content of carbonylated proteins in the roots of infected sprouts but caused a dramatic decrease in the
215 number of border cells and the size of the gel mantle, which eventually disappeared completely. These results, as well
216 as detection of products of peroxidation of proteins and lipids, suggest that border cell population can be regarded as
217 one of the effector targets of the fungicide tebuconazole, which can be used to evaluate the phytotoxicity of fungicides.

218 *3.2. Morpho-biochemical parameters of the root system of Fusarium-infected wheat grown in laboratory*
219 *experiments with variously applied tebuconazole*

220 In this experiment, we used wheat seeds initially infected by phytopathogenic fungi (as in the previous
221 experiment). In addition to that, analysis of the microbial composition of soil showed that microscopic fungi were
222 mainly represented by *Penicillium* species (58-65%); fungi of the genera *Fusarium, Trichoderma*, and *Aspergillus*
223 constituted 8-11% of the fungal population in soil samples. *Fusarium* species isolated from the initial soil samples were
224 represented by *F. solani* and *F. lateritium*. No *F. moniliforme* was detected in the initial microbial community.
225 *Fusarium* species in the initial soil constituted 3.1×10^{3} $CFU\cdot g^{-1}$. Over the course of the experiment, *Fusarium* counts in

226 the soil supplemented with tebuconazole decreased by a factor of 1.7 compared with their counts in the initial soil and
227 by a factor of 2.3 compared to the control (soil with no TEB supplementation).

228 Figure 4 shows the contents of free proline and products of peroxidation of proteins (carbonylated proteins,
229 CP) and lipids (malondialdehyde, MDA) in wheat roots at different time points of the 30-day experiment.

230 At Day 10, the contents of proline, MDA, and CP in the control did not differ significantly between the three
231 groups, reaching 0.189-0.232 10^{-6} $M.mg^{-1}$ protein, 0.265-0.41 10^{-9} $M.mg^{-1}$ protein, 0.586-0.78 10^{-8} $M.mg^{-1}$ protein,
232 respectively; these values were 2-3 times higher than those in the experiment with uninfected seeds. Thus, at that time
233 point (Day 10), application of Raxil to soil and pretreatment of the seeds did not produce any significant effect on the
234 contents of proline, CP, and MDA in wheat roots as compared to the control (Fig. 4a). The proportions of proline,
235 MDA, and CP revealed in the experiment characterize the level of redox homeostasis resulting from the interaction
236 between the host plant and pathogen under the experimental conditions.

237 At Day 20, the contents of proline and MDA in roots of the control plants increased dramatically (by a factor
238 of 19 and by a factor of 8.5) compared to Day 10 while CP decreased slightly (by a factor of 1.8) (Fig. 4b). These
239 changes may be associated with the tillering stage – underground branching of the stem and development of the
240 secondary root system, which occurred between Days 10 and 20 of wheat plant growth. As root biomass grew rapidly,
241 the rates of cell proliferation and cell wall synthesis increased, requiring considerable energy expenditure. The reason
242 for the dramatic increase in proline content is that proline is a proteinogenic amino acid involved in synthesis of
243 arabinogalactans – glycoproteins forming cell wall matrix. Arabinogalactans are also excreted to the rhizosphere (Gong
244 et al., 2012; Nguema-Ona et al., 2013; Kishor et al., 2015). Since large amounts of proline are used in biogenesis of cell
245 walls and synthesis of root exudates, it is synthesized in larger quantities during rapid growth of root biomass. In
246 addition, proline metabolism in mitochondria is accompanied by synthesis of FADH2 and NADH (Deuschle et al.,
247 2001), which supply electrons to the mitochondrial respiratory chain. Oxidative phosphorylation causes generation of
248 ATP molecules. The high demand of the rapidly growing root system for energy equivalents during the tillering stage
249 causes a sharp rise in proline content in wheat roots. On the other hand, the high level of proline and active oxidation of
250 proline in mitochondria increase not only the activity of oxidative phosphorylation but also production of free radicals,
251 which is related to this process (Kishor et al., 2005). The increase in the MDA level in wheat roots at Day 20 may be
252 caused by the high rate of oxidative phosphorylation.

253 In the treatments with Raxil both applied to the soil and used to pretreat seeds, at Day 20, the content of proline
254 in wheat roots also increased dramatically but to levels somewhat lower than those in the control. MDA contents in
255 these treatments and in the control increased to similar levels. However, the number of the *F. graminearum* cells in soil
256 in the treatment with tebuconazole supplementation was lower than in the control by a factor of almost two. These

257 results suggest targeted effects of the fungicide on phytopathogens and lightening of the load on the defense system of
258 wheat, including changes in the level of redox homeostasis of the roots.

259 At Day 30, proline content in roots of the control wheat plants decreased considerably (by a factor of 16
260 relative to Day 20) while MDA and CP contents did not change significantly (Fig. 4). In the treatment with Raxil
261 applied to the soil, proline, MDA, and CP contents in the roots did not differ significantly from the control, suggesting
262 that phytotoxic effects of the fungicide were softened as soil contamination with phytopathogens decreased. However,
263 in the treatment with seeds pretreated with Raxil, proline, MDA, and CP contents in the roots were higher than in the
264 control by a factor of 2.2, 2.0, and 1.7, respectively. That was indicative of activation of free radical processes and
265 phytotoxic stress, as the fungicidal effect of TEB used to pretreat the seeds before sowing must have been exhausted by
266 Day 30.

267 This study showed that the effect of the fungicide TEB on redox homeostasis in wheat roots varied depending
268 on the plant growth stage and was significantly different in ecosystems with soil and plants infected by *Fusarium*
269 phytopathogens. At Day 20 of plant growth, during the tillering stage, tebuconazole produced the strongest phytotoxic
270 effect on wheat plants.

271 Morphological properties of wheat root apices in ecosystems invaded by phytopathogens, at different levels of
272 the fungicide, are shown in Figure 5. As rhizosphere population of border cells was lost when roots were pulled out of
273 the soil and then rinsed in water, only root apices were analyzed. Microscopic analysis of root apices did not show any
274 age-related morphological dissimilarities between the apices at different time points of the experiment (at Days 10, 20,
275 and 30); at the same time, morphological characteristics of root apices differed considerably between experimental
276 groups. Roots of the wheat plants grown from the seeds initially contaminated by *Fusarium* had either undamaged
277 apices (Fig. 5a) or apices with loosened cells at the tip (Fig. 5b). Most of the wheat plants grown in the soil
278 supplemented with the fungicide Raxil had root apices with root tip mantles showing obvious signs of degradation (Fig.
279 5d). We assumed that the fungicide TEB had a strong effect on the steroid metabolism of the host plant, functions of
280 cell walls (Schrick et al., 2004; Höfte, 2015), and hormonal homeostasis (Lin et al., 2015). Cell wall damage, membrane
281 system dysfunction, and disorders of hormonal homeostasis may cause a decrease in the activity of plant defense
282 systems and help the pathogens invade the root system. Damage of the root ultrastructure of *Pennisetum americanum*
283 seedlings treated with atrazine was shown by Jiang et al. (Jiang et al., 2017).

284 Pretreatment of the seeds with the fungicide also caused development of apices with clear signs of degradation
285 of the root tip mantle. The lateral surfaces of the root tip mantle were most degraded (Fig. 5c, shown by arrows). Pre-
286 emergence treatment of seeds affects the functional systems of the germinating seeds more than fungicide application to
287 soil. Penetrating through the seed coating, the fungicide can induce disorders of sterol metabolism at very early

germination stages. Sterol metabolism determines morphogenesis processes in early stages of development of the sprout (Closa et al., 52; Peng et al., 2015). Disorders of the sterol-dependent stages of morphogenesis may cause various structural and functional defects in the developing root. Thus, morphology of root apices of wheat plants reflects the effects of stresses caused by both the phytopathogen and the fungicide.

Results of biochemical and morphological investigations of wheat root apices suggest that these parameters can be used as endpoints to evaluate toxic effects of the fungicide tebuconazole. Another finding is that the effect of TEB on redox homeostasis in wheat roots depends on the plant growth stage.

4. Conclusion

We studied toxic effects of the fungicide tebuconazole and fusarium infection on wheat roots in experiments with two-day-old wheat sprouts and 30-day experiments with wheat stands based on changes in the morphology of root apices and integrated parameters of redox homeostasis: the contents of proline and products of peroxidation of proteins (carbonylated proteins) and lipids (malondialdehyde) in roots. In experiments with two-day-old wheat sprouts infected by Fusarium, the content of carbonylated proteins in the roots dramatically increased, which was accompanied by an increase in the number of border cells and the size of the gel mantle of the root apex (compared to uninfected sprouts). The fungicide tebuconazole did not influence the content of carbonylated proteins in the roots of infected sprouts at the concentrations studied, but led to a sharp decrease in the number of border cells and the size of the gel mantle (until complete disappearance) in the root. The study of the effects of TEB and fusarium infection on wheat plants in a 30-day experiment showed that the effect of the fungicide TEB on redox homeostasis in wheat roots varied depending on the plant growth stage and was significantly different in ecosystems with plants infected by fusarium infection. Results of biochemical and morphological investigations of wheat root apices suggest that these parameters can be used for evaluation of biological action of fusarium infection and fungicides.

Conflict of interest

No conflict of interest to declare.

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472 Table Contents of carbonylated proteins (CP), malondialdehyde (MDA), and proline in roots of two-day-old *T.*

473 *aestivum* sprouts infected by *F. graminearum.*

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Content in roots	Fungicide concentration in the medium, $\mu g.ml^{-1}$			
	0	0.01	0.1	1
CP ($nM.mg^{-1}$ protein)	8.31±1.22	8.98±0.96	8.91±1.10	9.05±1.53
MDA ($nM.mg^{-1}$ protein)	0.261±0.031	0.284±0.024	0.281±0.028	0.297±0.033
Proline ($\mu g.mg^{-1}$ protein)	15.05±1.85	15.61±1.01	16.82±1.65	15.51±1.32

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525 **Figure Legends**

526 Fig. 1 The contents of carbonylated proteins (nM.mg^{-1} protein), malondialdehyde (nM.mg^{-1} protein), and
527 proline (μg.mg^{-1} protein) in roots of two-day-old *T. aestivum* sprouts: along the X-axis: 1 – uninfected sprouts; 2 –
528 sprouts infected by *F. graminearum.* Asterisks denote values of 2 significantly different from values of 1, $p>0.05$.

529 Fig. 2 Morphology of the root apices of two-day-old wheat (*T. aestivum*) sprouts: a – the root apex of
530 uninfected wheat sprouts; b, c, d – root apices of *F. graminearum*-infected sprouts

531 Fig. 3 The effect of tebuconazole concentration on the number of border cells (BC) (a) and "bare" apices (b) in
532 the root apices of two-day-old wheat (*T. aestivum*) sprouts infected with *F. graminearum*. Asterisks denote values
533 significantly different from values of the test with no tebuconazole added to the medium, $p>0.05$.

534 Fig. 4 The contents of proline (10^{-6} M/mg protein), malondialdehyde (MDA 10^{-9} M.mg^{-1} protein), and
535 carbonylated proteins (CP, 10^{-8} M.mg^{-1} protein) in roots of wheat *T. aestivum* plants infected with *F. graminearum* at
536 different days of the experiment; 1 – with no fungicide application to soil (control); 2 – with Raxil Ultra added to the
537 soil; 3 – with seeds pretreated with Raxil Ultra

538 Fig. 5 Morphology of root apices of *Fusarium*-infected wheat plants: a, b – control, infected seeds, with no
539 TEB applied to the soil: a – healthy apex and b – damaged apex; c – seeds pretreated with Raxil Ultra before sowing –
540 insignificant degradation of the mantle on the tip of the apex and destroyed lateral surface (shown by arrows); d – soil
541 application of Raxil Ultra – damaged apices with loosely packed cells at the tip

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