CYTOLOGICAL CHANGES OBSERVED IN THE SUCCESSIVE PHASES OF THE WHEAT RUST CAUSED BY *PUCCINIA RECONDITA* F. SP. *TRITICI* AFTER THE TREATMENT WITH TILT 250EC

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ABSTRACT

The disease process of wheat (*Triticum aestivum*) cv. Parada caused by rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici*) and the effect of applying the fungicide Tilt 250EC are described. The application of spraying at the time of inoculation, during incubation and at the beginning of the actual disease is most effective, thus attests to the highest pathogen susceptibility to that chemical agent at these phases of the disease. Tilt 250EC (propiconazole) inhibits the ergosterol biosynthesis in the fungus cells. Application of the preparation caused the inhibition of the development and necrosis of the intra- and extracellular mycelium. Observed were: irregular growth of intercellular hyphae, perforation of septas, homogenization of protoplasts and collapsing of cells. In the haustoria observed were: the thickening of the cell wall, modification of the periaustorial space, protoplast degeneration and finally the haustorium obliteration.

KEY WORDS: *Puccinia recondita*, *Triticum aestivum*, fungicide, ultrastructure, pathogenesis.

INTRODUCTION

Among many diseases observed in wheat, the most dangerous are those caused by rusts. Hence the necessity for chemical protection of plants against that disease. Tilt 250 EC (propiconazole) is one of the fungicides with quite a wide spectrum of action. As has already been demonstrated, it is characterized by a high efficiency in controlling diseases of different plant species caused, above all, by rust fungi (Zamorski et al. 1988). The aim of the present research was to analyse the morphological and cytological changes of both the host plant and pathogen (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici*) in various phases of the development, after the application of the fungicide, and to determine the moment of highest susceptibility of the pathogenic factor to the action of the chemical agent.

MATERIAL AND METHODS

The experiment was performed in greenhouse conditions. Wheat seedlings cv. Parada, at three leaf stages, were subjected to inoculation. The inoculum was applied on the previously moistened leaves. After the inoculation the plants were covered with plastic bags for 48 hours, in order to ensure maximum humidity during the infection. At all stages the plants were sprayed once with Tilt 250EC at concentration of 0.03%.

The first spraying was performed 3 days prior to inoculation, the second directly before inoculation, the third and fourth at the stage of disease incubation (2 and 4 days after the inoculation), the fifth at the phase of actual disease (after the appearance of the first discolourations, but before the formation of uredia), the sixth at the phase of actual disease (after the appearance of first uredia) and the seventh at the phase of actual disease (at the phase of full rust sporification). Urediospores originated from wheat comprised the inoculum *Puccinia recondita* f. sp. *tritici* for wheat. Each combination included 50 plants sprayed at a particular date. Two weeks after inoculation evaluation of the plant infection was performed. Each leaf was evaluated for symptoms of infection. A six-point scale (0-5) was used to express the increasing infection (0 – healthy leaf, 5 – the whole inoculated leaf surface covered with uredia). The average infection of the particular plants was calculated using the Townsend-Heuberger formula. The total infection of each combination was an arithmetical mean. Statistical analysis was not necessary for the interpretation of results. The material for microscopic examination was collected 3, 5, 6, 9 and 12 days after inoculation. On the last day the samples were collected from both the control plant and all the plants sprayed at successive dates.

Small fragments of leaves were fixed in paraformaldehyde dehydrated in alcohol of increasing concentrations, acetone and propylene oxide and embedded in Epon 812. Then they were cut on the Reichert ultramicrotome into sections, about 10 µm in thick. The sections were stained with uranyl acetate

and lead citrate, and were examined in the transmission electron microscope JEM 100C. The material was also shadowed with silver and examined in the scanning electron microscope.

RESULTS

The infection of wheat plants, sprayed with Tilt 250EC 3 days prior to inoculation, was lower as compared with the infection of unsprayed plants. No symptoms of disease were observed on the first (oldest) leaf of each plant of that combination. On the second leaf one could observe not only discolourations but also uredia. There were no symptoms on the plants sprayed on the day of inoculation and at two successive dates (the phase of disease incubation). On the plants sprayed after the appearance of the first discolourations (the beginning phase of the actual disease) only dead uredia could be observed (distinguished by the brownish colouring). The infection of wheat plants sprayed during the phase of full rust sporulation did not differ from the infection of plants in the control combination. The vitality of urediospores obtained from brown and normal (orange) uredia from wheat was examined in inoculation tests. Symptoms of infection were observed only on plants inoculated with urediospores from the normal coloured uredia. The results of inoculation with brownish urediospores were negative.

Macroscopic observations were confirmed by microscopic examinations. In plants not protected chemically the whole developmental cycle of rust could be observed, ending with the formation of uredia with urediospores on the 9th day following the inoculation. The first stages of infection (germination of spores, penetration into the plant) followed a normal course because on the 3rd day after the inoculation some intercellular hyphae forming the haustorium mother cells on the surface of the parenchymal cells could be observed in the intercellular space. On the 5th day after the inoculation inter- and intracellular hyphae were observed (Figs 1-6). The apical cells of the hyphae were filled with dense protoplast containing nuclei, elongated mitochondria, endoplasmic reticulum, ribosomes, lipid bodies and small vacuoles. In the older cells the protoplast was lighter, lacking the typical cellular structures, which had been replaced by large vacuoles (Fig. 1 and 2). The haustorium mother cells responsible for penetrating the cell wall of the host cell after forming a haustorium were taken up by a large vacuole appearing in the place of protoplast shifting to the haustorium (Fig. 1).

The mature haustorium, seen in Fig. 2 and Fig. 4, 5 and 6, was filled with dark protoplast rich in ribosomes. The mitochondria were usually situated parietally, while the nuclei were in the centre of the cells. The periaustorial space was filled with some delicate fibrous material. On the 6th day after the inoculation the hyphae formed a compact mycelium forming the base of uredium (Fig. 7, 8). Apart from the typical protoplast components, the small sporogenic cells also contained numerous lipid droplets. Fig. 7 and 9 present a mature uredium with generously sculptured urediospores placed on the stalks (pediceles).

Treatment with Tilt did not prevent the germination of spores, but it always destroyed the mycelium at the current stage of its development (Figs 10-18). The procedures carried out during the time of inoculation and during the period of incubation are considered to be the most effective. They inhibited the development of both the intercellular hyphae and haustoria. Fig. 11 and 12 present cells of the old hyphae, the large vacuoles of which were filled with fine fibrous material. The

cytoplasm containing few swollen mitochondria comprised a narrow parietal layer. Small osmophilic granules were situated in the plasmolemma. Those hyphae had very irregular shapes and had grown randomly into one another. This way they had formed a disordered mycelium with perforated, incomplete septas. Metabolically active hyphae (as confirmed by the look of protoplast containing typical cellular structures) were undergoing intensive necrotization. The protoplast which originated from a fusion of numerous small ones was transformed into homogeneous substance in which large lipid granules were visible. That picture was only temporary because there was, at the same time, an intensive shrinkage of cells leading to the breakdown of the hypha. Figs 16-18 presents the successive stages of haustorium degeneration. In Fig. 16 the enlarged space between the cell wall and the haustorium plasmolemma can be seen. It is filled with some delicate fibrous material arranged parallel to the haustorium surface.

The haustorium protoplast remained unchanged. The material gathered in the periaustorial space is more homogeneous than that observed in the infected cells of the control plants. Fig. 16 shows the haustorium 3 days after spraying. The changes which the haustorium had undergone are very clear. They are connected with the progressing protoplast homogenization (in which only the lipid bodies are visible as darker than the background), increase in the haustorium parietal area and further modification of the periaustorial zone. In that zone, there appeared a narrow, dark, homogeneous layer adhering to the haustorium outer surface. Material was delivered to that layer in numerous vesicles shifting from ER of the plant cell. The remaining external layer of the periaustorial zone was filled with a light colourless substance. Finally the haustoria became obliterated and ceased functioning (Fig. 18).

DISCUSSION

Plants' contact with pathogen does not always lead to the disease process. This may be due to the lack of proper conditions necessary for the development of the infectious agent or it may result from the presence of the substances which inhibit the pathogen development. It is shown by lack of the plant's reaction to toxins or its ability of toxin degradation. Those plants can contain fungistatic substances or have barriers which prevent the pathogen to reach the cells and tissues of the plant. There is an exchange of signals between the plant and the pathogen which are or are not recognized by both parties. A proper early identification of pathogenic factors allows the plant to mobilize the defense system which makes the pathogen harmless (Mansfield 1990, Yamamoto 1995). Plants possess a number of mechanisms which can be turned on at the time of pathogen recognition already on the surface of the cell wall or during its penetration. It can produce a local thickening of the cell wall, its inlay with lignin, strengthening with callose (Perumalla et al. 1989) and phenols (Marten et al. 1988) deposits and the accumulation of specific glycoproteins (Esquerre-Tugaye 1978).

The recognition of pathogen can take place at the plasmolemma surface. The ingrowing haustorium causes the release of factors connected with the plasma membrane which can trigger the production of fitoalexins (Yoshikawa et al. 1987) or enzymes dissolving the fungus-cell wall and also cause the above mentioned modifications of the cell walls (Heath et al. 1988, Jacobs 1989, Jacobs 1989).

The lack of recognition of the signals, together with lack of the defense reaction, creates favourable conditions for the

Figs 1-3. Fragments of infected cells and mycelium on the 5th day since the inoculation.

Fig. 1. Haustorium mother cell (hmc) neck (arrows) and haustorium (h). Cell nucleus (n) and parietally situated mitochondria (m) are visible in the haustorium – 8.000x.

Fig. 2. In the enlarged fragment from the neck region one can see the collar (thick arrow) in the cell wall of the neck, next to it a dictyosome (thin arrow) – 16.000x.

Fig. 3. Intercellular hyphae (ih) younger with numerous glycogen droplets and older, lighter and strongly vacuolated. Chloroplasts with assimilative starch (ch) are visible in the parenchymal cells (mc) next to haustorium (h) – 8.000x.

Figs 4-6. Fragments of infected parenchymal cells on the 5th day since the inoculation.

Fig. 4. Haustorium – 6.400x – scanning microscope.

Fig. 5. Channels of rough endoplasmic reticulum (arrow), chloroplasts (ch) and lipid body (lp) visible around the haustoria (h). In the intercellular space there is a intercellular hypha (ih) – 8.000x.

Fig. 6. Delicate material visible in the perihyphal zone (stars). Around the haustorium (h) there are numerous channels of rough ER (arrows), dictyosome (d), chloroplasts (ch), mitochondrion (m) and lipid bodies (lb) – 16.000x.

Figs 7-9. Sporogenesis in the infected mesophyll on the 7th-9th day since the inoculation.

Fig. 7. Fragments of sporogenic mycelium filled with numerous glycogen granules (arrows), next to them fragments of the host tissue (mc) – 800x.

Fig. 8. Transverse section of the leaf blade with the uredium. Intercellular hyphae (arrows), urediospores (u) – 350x.

Fig. 9. Urediospores (u) – 1.150x – scanning microscope.

Figs 10-11. Fragments of mesophyll and mycelium after spraying on the 5th day since the inoculation.
Fig. 10. Transverse section of the leaf blade. Arrows point to inter and intracellular hyphae – 350x.
Fig. 11, 12. Degenerating intercellular hyphae (ih) – 8.000x and 13.300x.

Figs 13-15. Fragments of parenchymal cells and intercellular mycelium sprayed on the 5th day since the inoculation.

Fig. 13, 14. Degenerating mycelium cells. Next to them some parenchymal cells (mc) with strongly changed protoplast – 8,000x.

Fig. 15. A fragment of noninfected parenchymal cell (mc) with a large lipid body (lb) – 16,000x.

Figs 16-18. Fragments of infected parenchymal cells sprayed on the 5th day since the inoculation. Successive pictures presents changes taking place, both around and inside the haustorium.

Fig. 16. A thickened cell wall (hcw) and dark perihaustorial zone (thick arrows) are visible in the haustorium – 16.000x.

Fig. 17. Haustorium filled with homogeneous protoplast with very thick cell wall (hcw) and wide, modified perihaustorial space (arrows) – 13.300x.

Fig. 18. Degenerated haustorium – 5.300x.

growth and development of the pathogen and the plant becomes the host. In the described experimental system *Puccinia recondita* f. sp. *tritici*-wheat the whole developmental cycle of rust was observed. Such situations are common and described in literature (Brzezicka-Szymczyk et al. 1989, Harder 1989, Chong et al. 1985, Lennox et al. 1989, Wood et al. 1986). The pathogen presence in the cell results in the change of the spatial system of intracellular structures and the assemblage of mitochondria, ER and cell nuclei around the haustorium. The presence of glycoprotein and polysaccharides in the perihaustral space testifies to the transportation of nutrients from the host cell to the haustorium (Chong et al. 1986). A long lasting starvation of a cell most usually leads to its death. In such cases when the plant is not able to render the pathogen harmless, it is necessary to apply chemical agents.

A direct action of chemical components on pathogenic fungi affects energetic processes, biosynthesis of new cell material or functioning of the cell membranes. The azole fungicides including Tilt owe their fungicidal action to the ability to inhibit ergosterol biosynthesis in the fungus cells. Ergosterol is the main sterol compound in the fungus cells, playing an important role in the structure and function of the plasma membrane. It is made by demethylation of lanosterol carried out in a few stages. Triazoles inhibit the demethylation process. The inhibition of ergosterol synthesis and transformations results in the accumulation of fatty acids in the fungus cells (Górska-Poczopko 1987, 1988). Depending on the fungicide inactivating mechanism the picture of mycelium degradation both inter-, and intracellular varies. The most often observed changes include: irregular growth of intercellular hyphae, perforation of the septas, thickening of the cell walls and protoplast homogenization. The protoplast homogenization in the haustoria is accompanied by filling up the perihaustral zone with callose-like material and the thickening of the cell wall. As a result of those changes the hyphae and haustoria waste away (Heath et al. 1988, Pring 1994). It suggests that the application of fungicide allows the plant to activate the system isolating pathogen in the cell by providing the haustorium with callose or even earlier by forming a papilla around the penetration peg of the haustorium mother cell. The results of the present experiment allow to state that the efficiency of rust control depends strictly on the developmental phase of the disease in which the fungicide was used. Spraying performed prior to inoculation only decreased the infection degree. On the basis of microscopic examination and the fact that no disease symptoms were noted on plants sprayed with propikonazole on the day of inoculation, during the phase of incubation and at the beginning of actual illness it can be assumed that Tilt caused a total destruction of the pathogen. The results also revealed the highest susceptibility of the fungus at that developmental stage of the disease to the propikonazole action. The lack of differences in the degree of infection in plants sprayed prior to inoculation and at the time of full rust sporification can be due to the lack of contact between the fungicide and pathogen or to the lack of susceptibility of pathogen at a particular developmental stage to the action of the preparation. Similar results for Tilt were demonstrated in relation to *Puccinia horiana* (Zamorski et al. 1988).

The knowledge of propikonazole action at particular stages of the rust-disease process in wheat is of great practical significance. The presented results allow to obtain a better effectiveness of the Tilt action in rust control and to reduce application of the preparation at the phases of insusceptibility of the disease, thus to reduce the amount of fungicide used and to decrease the degree of agriculture chemization.

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ZMIANY CYTOLOGICZNE OBSERWOWANE
W KOLEJNYCH FAZACH RDZY BRUNATNEJ PSZENICY
WYWOŁANEJ PRZEZ *PUCCINIA RECONDITA* F. SP. *TRITICI*
PO ZASTOSOWANIU TILTU 250EC

STRESZCZENIE

Opisano proces chorobowy pszenicy (*Triticum aestivum* cv. Parada) wywołany rdzą brunatną (*Puccinia recondita* f. sp. *tritici*) i efekt zastosowanego fungicydu Tilt 250EC. Zastosowanie oprysku w momencie inokulacji, w czasie inkubacji i na początku choroby właściwej jest najskuteczniejsze i świadczy o największej wrażliwości patogenu na środek chemiczny w tych fazach choroby. Tilt 250EC (propikonazol) hamuje biosyntezę ergosterolu w komórkach grzybów. Pod wpływem działania preparatu następowało zahamowanie rozwoju i obumieranie grzybni zewnątrz i wewnątrzkomórkowej. Obserwowano nieregularny wzrost strzępek intercellularnych, perforację sept, homogenizację protoplastów i zapadanie się komórek. W ssawkach obserwowano pogrubienie ściany komórkowej, modyfikację przestrzeni wokółhaustorialnej, degenerację protoplastu i ostatecznie obliterację haustorium.

SŁOWA KLUCZOWE: *Puccinia recondita*, *Triticum aestivum*, fungicyd, ultrastruktura, patogenez.