CYTOLOGICAL EFFECT OF TILT 250 EC IN THE SUCCESSIVE PHASES OF THE TRITICALE RUST DISEASE PROCESS CAUSED BY *PUCCINIA RECONDITA* F. SP. *TRITICI*

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ABSTRACT

The disease process caused by rust (*Puccinia recondita* f. sp. *tritici*) in *Triticale* (*Triticale*-Wittmack cv. Bolero) and the effect of the application of the propiconazole – Tilt 250 EC are described. In plants not protected chemically one could observe the whole pathogen developmental cycle ending with the appearance of uredinia with urediniospores. The highest pathogen susceptibility to chemical agent was observed at the time of inoculation, during incubation and at the beginning of the actual disease. By inhibiting the ergosterol biosynthesis in the fungus cells the preparation (propiconazole) inhibited the development of the intra- and extracellular mycelium. The mycelium degeneration manifested itself by the irregular growth of intercellular hyphae, perforation of septa, homogenization of protoplasts and cell collapse. The thickening of the cell wall, modification of the periahaustorial space and protoplast obliteration were observed in the haustoria.

KEY WORDS: *Puccinia recondita*, *Triticale*, propiconazole, ultrastructure, pathogenesis.

INTRODUCTION

Rust fungi are among the most important cereal pathogens. *Triticale*, similarly as wheat is known to the host plant especially for the wheat rust (*Puccinia recondita* f. sp. *tritici*) (Zamorski et al. 1995). The aim of the present research was to analyse the morphological and cytological changes of both the host plant and pathogen and to establish the highest susceptibility and the way reaction of the pathogenic factor to the action of chemical agent.

MATERIAL AND METHODS

The experiment was performed in greenhouse conditions. *Triticale* seedlings at three leaf stages were subjected to inoculation. The inoculum was applied by hand on the previously moistened leaves (Stubbs et al. 1986). After 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 spraying was performed 3 days prior to inoculation, directly before the inoculation, at the stage of disease incubation (2 and 4 days after the inoculation), at the phase of the actual disease (after the appearance of discolourations and at the stage of full rust sporification). Urediniospores originated from wheat comprised the inoculum *Puccinia recondita* f. sp. *tritici* for *Triticale*. Each combination included 50 plants sprayed at a particular date. Two weeks after the inoculation, 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 uredinia). 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 with the Reichert ultramicrotome into sections of 10 µm in thick. The sections were stained with uranyl acetate and lead citrate and examined in the transmission electron microscope JEM 100C.

RESULTS AND DISCUSSION

The efficiency of chemical control of rust depends on the developmental phase of the disease in which the fungicide was used. Spraying applied before the inoculation resulted only in decreasing the degree of infection. 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 uredinia. No symptoms were observed on plants sprayed on the day of inoculation and during the period of disease incubation. It attests to the highest

pathogen susceptibility to the propikonazole action at those phases of the disease. On the plants sprayed after the appearance of the first discolourations (the beginning phase of the actual disease) only dead uredinia of characteristic brownish colour could be observed. The infection of triticale plants sprayed during the phase of full rust sporification did not differ from the infection of plants in the control combination (not sprayed). The vitality of urediniospores was examined in the inoculation tests. Symptoms of infection were observed only on plants inoculated with urediniospores from the normal coloured uredinia. The results of inoculation with brownish urediniospores were negative. 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 (Zamorski et al. 1988). These observations were confirmed by microscopic examinations. In the control plants (not protected chemically) the whole developmental cycle of rust could be observed ending with the formation of uredinia with urediniospores. Both the germination of spores and penetration into the plant followed an undisturbed course because on the 3rd day after the inoculation numerous intercellular hyphae and haustorium mother

Figs 1-2. Intercellular hyphae (ih) in the intercellular spaces (is) of the triticale mesophyll on the 3rd and 5th day after the inoculation.

Fig. 1 and 2. Cell nucleus (n) and mitochondria (m) are visible in the hypha cells and in older cells also the vacuoles (v). Parietally situated chloroplasts (ch), nucleus (n) and mitochondria (m) are visible in the parenchymal cells (mc).

Fig. 2 presents the organelles of the infected cell visible around the haustorium (h). Bars represent – 2 μ m.

Figs 3-4. Haustoria (h) in the parenchymal cells (mc) of the triticale leaf on the 5th day after inoculation.

Fig. 3 presents a fragment of the haustorium mother cell (hmc), neck (arrow) and haustorium (h). The haustorium contains one nucleus (n) with nucleolus and mitochondria (m). Cell nucleus (n) of the infected cell visible next to the haustorium.

Fig. 4. Numerous channels of rough endoplasmic reticulum (er) visible around the haustoria (h) surrounded with periaustorial space. A fragment of the intercellular hypha (ih) visible in the intercellular space. Bars represent – 2 μ m.

cells could be observed in the mesophyll intercellular space. On the 5th day after the inoculation numerous haustoria were also visible. The growth of the rust mycelium hyphae is at its highest thus the dikaryotic cells of that zone contained protoplast with numerous ribosomes, endoplasmic reticulum, mitochondria, lipid bodies and small vacuoles. The cells of the older hyphae were filled with a large, central vacuole and the parietal protoplast contained not numerous organelles (Fig. 1 and 2). The haustorium mother cells responsible for penetrating the cell wall of the host cell after forming the haustorium looked similarly. The mature haustorium was filled with protoplast containing numerous ribosomes, mitochondria and

centrally situated nuclei (Fig. 3 and 4). The pathogen presence in the cell (isolated from the host protoplast with the continuous plasmolemma) caused some changes in the spatial organization of the infected cell. Usually the ER, mitochondria and cell nucleus were gathered around the haustorium. The periaustorial space (the zone between the haustorium cell wall and the host plasmolemma) was filled with delicate, fibrous material. The presence of glycoproteins and polysaccharides in that zone testifies to the transportation of nutrients from the host cell to the pathogen (Chong et al. 1986). On the 6th day after the inoculation the hyphae formed a compact mycelium being the base of uredinium. The sporogenic cells

Figs 5-6. Changes in the intercellular mycelium ultrastructure caused by the chemical agent applied during the period of the disease incubation.

Fig. 5 and 6 present the degenerating intercellular hyphae (ih) in which the protoplast homogenization takes place simultaneously with the cell shrinkage. Lipid bodies (lb) are visible in the hypha cells.

Fig. 5. The arrow shows the local thickenings of the cell wall of a plant cell preventing the pathogen penetration. Bars represent – 2 μ m.

contained very numerous lipid droplets. On the 9th day after the inoculation they formed the urediniospores. The full development of the triticale rust in the described conditions suggests the lack of any defensive reaction on the part of the host. Such situations are common and described in literature (Brzezicka-Szymczyk et al. 1988, Harder 1986, Chong et al. 1989, Lennox et al. 1989, Wood et al. 1986). Most plants can defend themselves against a pathogen in a natural way. This may be connected with the lack of proper conditions necessary for the development of the infectious agent or with the presence of such substances which inhibit its development. It is shown by lack of the plant's reaction to toxins or its ability of toxin degradation. Those plants can contain fungis-

tatic substances. A proper early identification of the pathogen allows the plant to mobilize the defense mechanisms which are able to make the pathogen harmless (Mansfield 1990, Yamamoto 1995). The recognition of pathogen at the cell wall surface or during its penetration can produce a local thickening of the cell wall, its inlay with lignin, strengthening with callose deposits and phenols (Perumalla et al. 1989, Marten et al. 1988, Esquerre-Tugaye et al. 1979). A later recognition of the pathogen at the plasmalemma surface can trigger the production of fitoalexins (Yoshikawa et al. 1987) or enzymes dissolving the fungus cell wall and also cause the delayed cell wall reaction (Heath et al. 1988, Jacobs 1989).

Figs 7-8. Changes in the haustorium ultrastructure caused by the chemical agent applied during the period of the disease incubation.

Fig. 7 and 8 show the degenerating haustoria (h) surrounded and constricted with homogenous material (asterisks). Bars represent 2 μ m.

In cases when the plant does not possess any defense mechanisms it is necessary to apply chemical agents. A direct action of fungicides on pathogenic fungi affects energetic processes, biosynthesis of new cell material or functioning of the plasmatic membranes. The azole fungicides including Tilt 250 EC owe their fungicidal action to the ability to inhibit ergosterol biosynthesis in the fungus cells. Ergosterol is the main sterol compound playing an important role in the structure and function of the plasma membranes. It is produced 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, Górska-Poczopko et al. 1988). In the performed experiment the treatment with Tilt did not prevent the germination of spores, but it always destroyed the mycelium at the current

stage of its development. The procedures carried out at the time of inoculation and during the period of incubation destroyed extra- and intracellular hyphae thus preventing the formation of uredinia. The cells of the older hyphae reacted to propikonazole with irregular growth, perforation of septas, disintegration of plasma membranes, swelling of some organelles, mainly the mitochondria. The cells of physiologically active hyphae were undergoing intensive necrotization during which the protoplast was transformed into homogenous substance with numerous large lipid granules. The shrinkage of cells usually led to the fragmentation of the hypha (Fig. 5, 6). As a result of shrinkage and protoplast degeneration the periaustorial space increased in the haustoria. It was filled with homogeneous material tightly surrounding the haustorium. Numerous vesicles situated around the haustoria probably transported the material filling up the periaustorial space

from the protoplast of the infected cell. Finally the haustoria became obliterated and ceased functioning (Fig. 7, 8). It suggests that the application of fungicide allows the plant to activate the apoplast system isolating pathogen in the cell by providing the haustorium with callose or even earlier by stopping the penetrating hypha in the cell wall (Heath et al. 1988, Pring 1994, Brzezicka-Szymczyk et al. 1995). The infected cells of the mesophyll in chemically unprotected plants usually wasted away. That process was most often connected with lysis of the cell walls leading to the breakdown of cells. Properly applied chemical treatment helped the infected cell to control the pathogen and to regenerate the protoplast as a result of the starvation of a cell and its return to full physiological efficiency.

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CYTOLOGICZNY EFEKT DZIAŁANIA TILTU 250 EC W KOLEJNYCH FAZACH PROCESU CHOROBOWEGO RDZY BRUNATNEJ PSZENŻYTA POWODOWANEGO PRZEZ *Puccinia recondita* F. SP. *TRITICI*

STRESZCZENIE

Opisano proces chorobowy pszenżyta (*Triticale*-Wittmack odm. Bolero) wywołany rdzą brunatną (*Puccinia recondita* f. sp. *tritici*) i efekt zastosowanego fungicydu – Tilt 250 EC. W roślinach nie chronionych chemicznie obserwowano pełny cykl rozwojowy patogena kończący się wytworzeniem urediniów i uredinio-sporami. Największa wrażliwość patogena na środek chemiczny wystąpiła w momencie inokulacji, w fazie inkubacji i na początku choroby właściwej. Preparat (propikonazol) hamując biosyntezę ergosterolu w komórkach grzybów powstrzymywał rozwój grzybnii zewnętrznej i wewnętrzkomórkowej. Degeneracja grzybnii objawiała się nieregularnym wzrostem strzępek międzykomórkowych, perforacją sept, homogenizacją protoplastów i zapadaniem się komórek. W ssawkach obserwowano pogrubienie ściany komórkowej, modyfikację przestrzeni wokółhaustorialnej i obumieranie protoplastów.

SŁOWA KLUCZOWE: *Puccinia recondita*, *Triticale*, propikonazol, ultrastruktura, patogenezą.