

## CYTOLOGICAL AND IMMUNOCYTOCHEMICAL STUDIES ON THE EFFECTS OF THE FUNGICIDE TEBUCONAZOLE ON THE INTERACTION OF WHEAT WITH STRIPE RUST

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### SUMMARY

Effects of the fungicide tebuconazole, applied as a leaf spray after inoculation of wheat leaves with stripe rust were examined by means of electron microscopy and immunogold labelling. Tebuconazole strongly inhibited the development of *Puccinia striiformis* in host leaf tissues, and also markedly altered the structures of the hyphae and haustorium. These changes included increased vacuolation, irregular thickening of cell walls, necrosis or degeneration of cytoplasm, and collapse of the hypha and haustorium. Large differences were detected in *P. striiformis*-infected wheat leaves treated with fungicide compared with the untreated control. A very thick layer of wall appositions and large encasements were usually formed in the infected fungicide-treated leaves whereas such structures were never observed in the infected wheat leaves without fungicide treatment. In addition, chitinase,  $\beta$ -1,3-glucanase and lignin were detected in wheat leaf tissues by immunogold labelling. Accumulation of the two enzymes and lignin increased slightly in infected leaves compared with uninoculated healthy leaves, while a much higher accumulation of the two enzymes and lignin was detected in infected wheat leaves after fungicide treatment. Our results suggest that tebuconazole not only inhibited development of *P. striiformis* in wheat leaves, but also enhanced structural and biochemical host defense reactions in the infected host.

*Key words:* *Puccinia striiformis*; *Triticum aestivum*; tebuconazole; chitinase;  $\beta$ -1,3-glucanase; lignin; ultrastructure; immunogold.

### INTRODUCTION

Wheat stripe rust, caused by *Puccinia striiformis* Westend f. sp. *tritici*, occurs worldwide and is a major disease in temperate regions, particularly in China. Epi-

demics of this disease often cause tremendous losses of wheat yield in China. Appropriate use of resistant wheat cultivars is the most effective and economical method of control. However, resistant is usually overcome within a few years because of appearance and development of new races of the pathogen (Li and Zeng, 2002). Thus a complementary control measure is the application of fungicides.

It has been shown that tebuconazole ((RS)-1-(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazole-1-ylmethyl)-pentan-3-ol, Folicur<sup>®</sup>) can effectively control wheat stripe rust in the field (Han *et al.*, 2003). It is a systemic fungicide of the triazole group, and the primary mode of action is the inhibition of ergosterol biosynthesis in fungi (Hewitt, 1998). Even though different triazole fungicides have a similar mechanism of action, they may show marked differences in their activity against different fungal pathogens (Buchenauer, 1987; Scheinpflug and Kuck, 1987).

Many light and electron microscope studies have been carried out on the effects of ergosterol biosynthesis-inhibiting (EBI) fungicides on plant pathogenic fungi. EBI fungicides usually cause in fungi marked morphological malformations, irregular cell wall thickening and excessive branching (Hippe, 1984; Smolka and Wolf, 1986; Heller *et al.*, 1990; Maffi *et al.*, 1995, 1998; Kang *et al.*, 1993, 1996, 2001). Kang *et al.* (1993, 1996) found that after *P. striiformis*-infected wheat leaves were treated with EBI fungicides such as triadimenol or diniconazole, the fungal hyphae and haustoria became malformed, while structural defense responses, such as formation of cell wall appositions and encasement of the haustorium, were detected in the host cells. These findings suggested that the fungicides not only affected the pathogen directly, but also indirectly by influencing host responses. Therefore, the aim of the present investigation was to document, by cytological and immunocytochemical methods, alterations of *P. striiformis* in wheat leaves and to study defense responses such as accumulation of pathogenesis-related proteins and lignin in wheat stripe rust-infected tissue after treatment with the EBI fungicide tebuconazole.

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## MATERIALS AND METHODS

**Plant cultivars, pathogen and inoculation.** The Chinese race of *P. striiformis* Westend f.sp. *tritici*, CY29, and a highly susceptible wheat (*Triticum aestivum* L.) cv. Huixianhong were used. Wheat seeds were grown in organic soil in 10-cm diameter pots in a growth chamber at 16°C and a 16 h photoperiod. Seven-day-old seedlings were inoculated. The pathogen was maintained on the susceptible wheat cv. Huixianhong. For inoculation, freshly collected urediospores were applied with a fine paintbrush to the adaxial surface of the first leaf of the seedlings. Control seedlings were treated with distilled water. Inoculated and uninoculated seedlings were kept in a humid chamber for 24 h at 15°C, then all plants were returned to the growth chamber.

**Fungicide treatment and sampling.** The formulated product Folicur® (Bayer company, Germany), containing 25% of tebuconazole ((RS)-1-(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-yl-methyl), was used. A suspension of tebuconazole (1.25 ml/l) was prepared with sterile double-distilled water. Inoculated and uninoculated leaves were sprayed with the fungicide 3 days after inoculation. For the controls, the inoculated and uninoculated leaves were treated with sterile double-distilled water. The leaves were taken from 1 to 5 days after fungicide treatment.

**Electron microscopy.** The samples were processed for transmission electron microscopy as described by Kang (1996). The leaves were cut into small pieces and fixed with 3% (v/v) glutaraldehyde in 100 mmol/l phosphate buffer (pH 6.8) for 3-6 h at 4°C. The samples were then rinsed for 2 h with the same buffer and post-fixed with 1% (w/v) osmium tetroxide for 2 h at 4°C. The samples were rinsed thoroughly with the same buffer and dehydrated in a graded acetone series, embedded in Epon 812 and polymerized at 60°C for 24 h. Ultrathin sections were cut with a diamond knife and collected on 200-mesh copper grids. After contrasting with uranyl acetate and lead citrate, the grids were examined with a Zeiss-EM10 electron microscope at 80 kV. All experiments were duplicated.

**Immunogold labelling for lignin,  $\beta$ -1,3-glucanase and chitinase.** Rabbit polyclonal antibodies against lignin were supplied by Prof. K. Ruel (Centre de Recherches sur les Macromolécules Végétales, Université Joseph Fourier, France). The specificity of the antibodies to guaiacyl and syringyl polymers in lignin has been characterized by Joseleau and Ruel (1997) and Burlat *et al.* (1997), and they have been shown to recognize lignin epitopes in cell walls of wheat straw (Burlat *et al.*, 1997). The rabbit polyclonal antibodies against tobacco  $\beta$ -1,3-glucanase and chitinase were obtained from Prof. Frutig

(C.N.R.S., Institut de Biologie Moléculaire des Plantes, Strasbourg, France). These antibodies showed cross-reaction with  $\beta$ -1,3-glucanase and chitinase from wheat leaves by Western blotting (Siefert *et al.*, 1996). The secondary antibodies (goat anti-rabbit IgG) coupled to 15-nm gold particles were purchased from British Biocell International Ltd., Cardiff, UK. Immunogold labelling was carried out as follows: (i) incubation of ultrathin sections with blocking solution containing 1% (w/v) bovine serum albumin (BSA) in Tris-buffered saline (TBS, 10 mmol/l Tris-HCl, 150 mmol/l NaCl, pH 7.4) for 20 min; (ii) incubation of the sections with the primary antibody in the blocking solution at room temperature for 2 h (the polyclonal anti-lignin antiserum was diluted 1:600, and the anti- $\beta$ -1,3-glucanase and chitinase antibodies were diluted 1:200); (iii) washing in four 10-min baths in TBS; (iv) incubation of the sections with the secondary antibodies (diluted 1:40 in TBS) for 1 h; (v) rinsing with TBS followed by distilled water rinse. The labelling specificity was assessed by replacing the primary antibody with buffer. After immunogold labelling, the ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a Zeiss-EM10 electron microscope at 80 kV.

**Quantification of labeling.** The labelling densities for lignin,  $\beta$ -1,3-glucanase and chitinase in the non-inoculated and inoculated wheat leaves were compared by determining the number of gold particles per  $\mu\text{m}^2$  over cell wall areas on 10-15 micrographs. The difference between gold particles over cell walls of the infected and non-inoculated wheat leaves from the fungicide treatment or the untreated control was statistically analyzed by General Linear Models Procedures of SAS system (Version 6.12) (Tukey's Student Range) test.

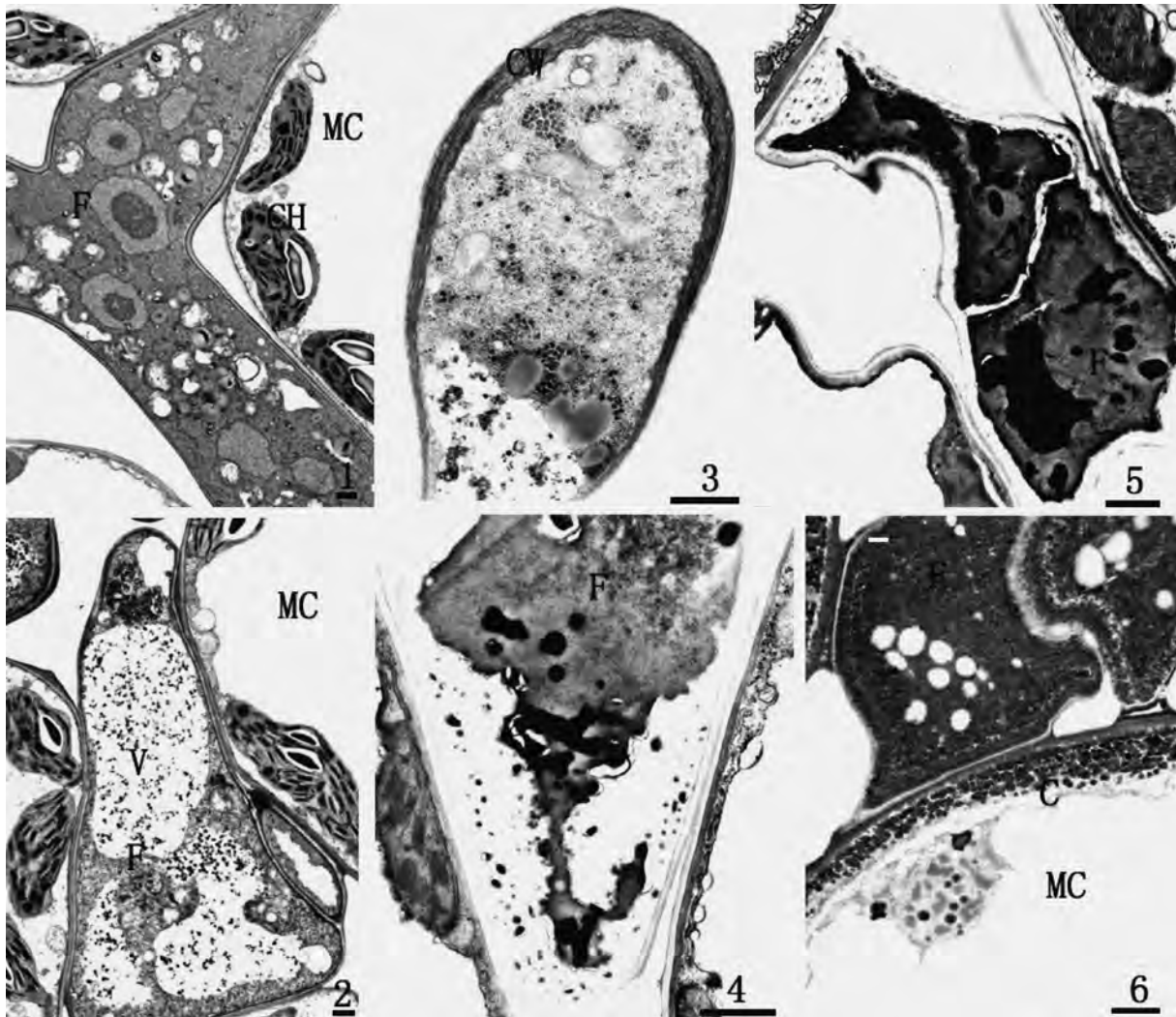
## RESULTS

When tebuconazole was applied 3 days after inoculation, further development of *P. striiformis* in the wheat leaves was inhibited. Chlorotic spots appeared earlier on the fungicide-treated leaves than on the untreated leaves. The spots did not enlarge and usually became necrotic, and no uredia or urediospores were formed on the fungicide-treated leaves.

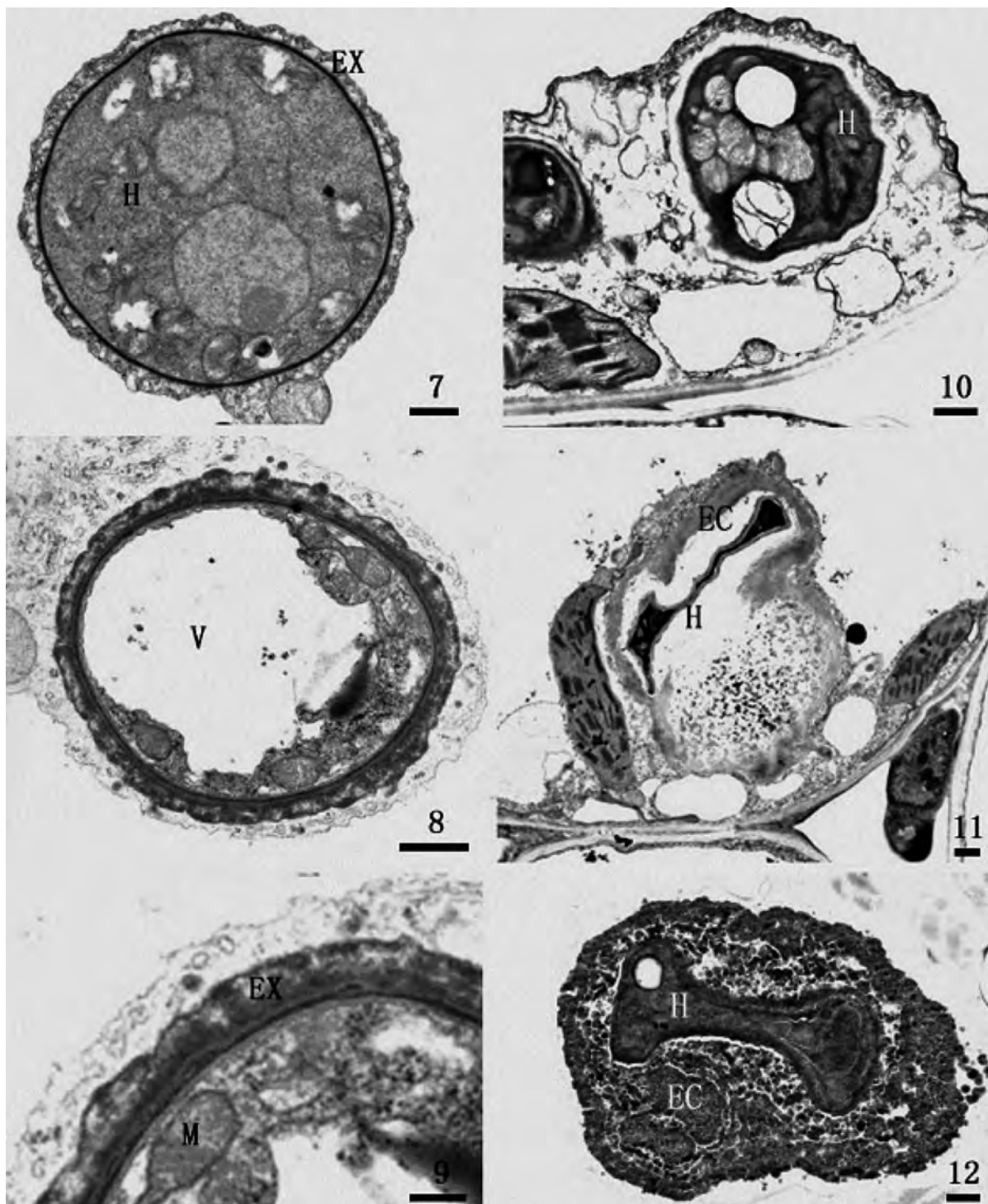
**Cytological changes of the wheat - stripe rust host pathogen complex after treatment with the fungicide tebuconazole.** Electron microscopical investigation showed no marked changes in the uninoculated wheat leaves compared to untreated healthy wheat leaves after treatment with the fungicide. In *Puccinia striiformis*-infected wheat leaves without fungicide treatment, there was extensive colonization of host tissues 3 to 5 days after inoculation. The hyphae ramified between host cells,

and numerous haustoria were formed within them. The hyphal cells showed dense cytoplasm with numerous mitochondria, nuclei and small vacuoles (Fig. 1). The hyphal cell walls and septa appeared as clear, distinguishable layers of uniform thickness (not shown). In the infected wheat leaves 1 day after fungicide treatment, size of vacuoles markedly increased in the hyphal cells (Fig. 2). The cell walls of the hyphae became considerably thicker 2 days after fungicide treatment compared with hyphal cell walls of the untreated control (Fig. 3). Some areas of the cell walls showed extremely irregular thickenings, particularly on the hyphal apical region (Fig. 3

and 4), and some electron-dense vesicles of different size and shape were always detected in the irregularly thickened walls (Fig. 4). The hyphal cytoplasm and organelles usually became necrotic or degenerated, and the hyphal cells were collapsed 4 days after fungicide treatment (Fig. 4, 5 and 6). Some changes were also detected in the host cells at this time. A significant reaction in the host cells was the formation of thick wall appositions between the host cell wall and plasmalemma in the area near the hyphal cell (Fig. 6). However, such a thick layer of wall appositions was never found in *Puccinia striiformis*-infected wheat leaves not treated with the fungicide.



**Fig. 1-6.** Transmission electron micrographs of hyphae of *Puccinia striiformis* in wheat leaves untreated (1) and treated (2-6) with the fungicide tebuconazole. Fig. 1. Hyphal cell in an untreated wheat leaf 5 days after inoculation (dai), show normal ultrastructure. Fig. 2. Hyphal cell in a wheat leaf, 1 day after fungicide treatment (dat), showing large vacuoles in the cytoplasm. Fig. 3. Hypha in a wheat leaf, 2 dat, showing thickened hyphal cell wall especially in the tip region. Fig. 4. Hypha in a wheat leaf, 4 dat, showing hyphal cell wall irregularly thickened, with the hyphal cytoplasm becoming necrotic. Many vesicles of different size and shape appeared in the thickened wall. Fig. 5. Hyphae in a wheat leaf, 4 dat, showing necrotic hyphal cytoplasm with the hyphal cell walls irregularly thickened. Fig. 6. Hypha and host cell in a wheat leaf, 4 dat, showing necrotic hyphal cytoplasm, with a thick layer of wall apposition formed in host cells between cell wall and plasmalemma adjacent to the hyphae. (All bars = 1µm). F = Fungal cell; CW = Hyphal cell wall; C = Cell wall apposition; CH = Chloroplast; MC = Mesophyll cell; V = Vacuole.



**Fig. 7-12.** Transmission electron micrographs of haustoria of *P. striiformis* in wheat leaves untreated (7) and treated (8-12) with fungicide. Fig. 7. Haustorium 5 days after infection. The haustorial body is spherical with a narrow extrahaustorial matrix (bar = 0.5  $\mu\text{m}$ ). Fig. 8. Haustorium, 2 days after treatment. The haustorium wall is irregularly thickened (arrow), with a large vacuole formed in the haustorial cytoplasm (bar = 0.5  $\mu\text{m}$ ). Fig. 9. Enlargement of Fig. 8. The extrahaustorial matrix is enlarged and contains electron-dense material (bar = 0.2  $\mu\text{m}$ ). Fig. 10. Haustorium 2 days after treatment, showing angular shape, dense cytoplasm and large vacuoles (bar = 0.5  $\mu\text{m}$ ). Fig. 11. The haustorium, 4 days after treatment, has collapsed and is encased by electron-dense material (arrow) (bar = 0.5  $\mu\text{m}$ ). Fig. 12. The haustorium 4 days after treatment, has collapsed, become necrotic, and is completely encased (bar = 0.5  $\mu\text{m}$ ). EC = Encasement of haustoria; H = Haustorium; EX = Extrahaustorial matrix; V = Vacuole. M = Mitochondrion.

The structure of the haustorium of *P. striiformis*, formed inside the cells of wheat leaves, typically consisted of a haustorial neck and haustorial body as earlier described (Kang *et al.*, 2002). The haustorial bodies were spherical to elongated shape, surrounded by the host cell cytoplasm (Fig. 7). In the infected host tissues treated with the fungicide, large vacuoles often appeared in the haustorial bodies, and organelles in the haustorial cytoplasm became disorganized (Fig. 8). The extrahaustorial matrix between haustorial wall and host plasmalemma was enlarged and contained electron-dense material (Fig. 8 and 9). Some haustorial bodies with dense cytoplasm and large vacuoles were angular in shape (Fig. 10). Another significant reaction detected in the host cells was the deposition of electron-dense material around haustorial bodies, resulting in pronounced formation of a large encasement of the haustoria in the infected host cells (Fig. 11 and 12). The encased haustorial bodies usually collapsed and became necrotic while the host cells showed no marked effect. However, such encasement of haustoria was never detected in the *P. striiformis*-infected wheat leaves without fungicide treatment.

**Immunogold localization of lignin in the wheat-stripe rust host-pathogen complex treated with the fungicide tebuconazole.** Following incubation of the ultrathin sections of wheat leaves from all treatments with anti-lignin antiserum and the secondary antibody, clear labelling occurred over the host cell walls in all treatments, while other structures, such as cytoplasm, plasma membrane, organelles and hyphal cells, were free of labelling (Fig. 13 and 14). The density of gold particles over the cell walls in uninoculated leaf tissues showed almost no difference between the fungicide treatment and non-fungicide treatment (Table 1). The density of gold particles over cell walls of the infected, untreated wheat leaves was slightly but not significantly higher than that over uninoculated leaves. However, there was

a significant increase in labelling over host cell walls of infected leaves treated with fungicide compared to the uninoculated wheat leaves (treated with fungicide or untreated) and the untreated *Puccinia striiformis*-infected wheat leaves (Table 1, Fig. 13 and 14). For labelling specificity assessment, the incubation of sections with the secondary antibody alone yielded no labelling.

**Immunogold localization of chitinase and  $\beta$ -1,3-glucanase in the wheat - stripe rust host-pathogen complex treated with the fungicide tebuconazole.** Incubation of ultrathin sections of wheat leaves from the different treatments with anti-chitinase antiserum or anti- $\beta$ -1,3-glucanase antiserum, and the secondary antibody resulted in labelling of chitinase and  $\beta$ -1,3-glucanase in wheat leaf tissues (Fig. 15-18) as earlier described (Kang *et al.*, 2003). Labelling for chitinase and  $\beta$ -1,3-glucanase in wheat leaf tissues was mainly detected over cell walls, whereas the cytoplasm and organelles such as nuclei, mitochondria and chloroplasts showed very little label. Statistical analysis revealed that there was no difference in the labelling densities of both enzymes in uninoculated leaf tissues between the fungicide treatment and non-fungicide treatment (Table 1). However, a slight increase in labelling densities for chitinase and  $\beta$ -1,3-glucanase was found over cell walls of untreated *Puccinia striiformis*-infected leaf tissues, which was not significantly different, compared to the uninoculated healthy wheat leaf tissues (Table 1). On the other hand, the labelling densities of chitinase and  $\beta$ -1,3-glucanase in the *P. striiformis*-infected wheat leaves treated with fungicide increased significantly compared to the other treatments (Table 1, Fig. 15-18). The cell walls of the hyphae in infected and fungicide-treated leaf tissues usually showed more labelling of chitinase and  $\beta$ -1,3-glucanase than in infected leaf tissues without fungicide treatment (Fig. 15-18). The control sections incubated only with the secondary antibody yielded no labelling.

**Table 1.** Labelling of lignin, chitinase and  $\beta$ -1,3-glucanase over cell walls of wheat leaves from different treatments, 5 days after inoculation (= 2 days after treatment respectively)\*

Treatment	Lignin	Chitinase	$\beta$ -1,3-glucanase
<u>Uninoculated healthy leaves</u>			
Untreated	24.66 $\pm$ 3.24 b	6.24 $\pm$ 1.42 b	7.14 $\pm$ 1.62 b
Treated with tebuconazole	24.83 $\pm$ 2.82 b	6.45 $\pm$ 1.26 b	7.26 $\pm$ 1.80 b
<u><i>Puccinia striiformis</i>-infected leaves</u>			
Untreated	25.20 $\pm$ 2.64 b	6.84 $\pm$ 1.84 b	7.44 $\pm$ 1.48 b
Treated with tebuconazole	38.84 $\pm$ 3.66 a	16.42 $\pm$ 2.60 a	18.78 $\pm$ 2.82 a

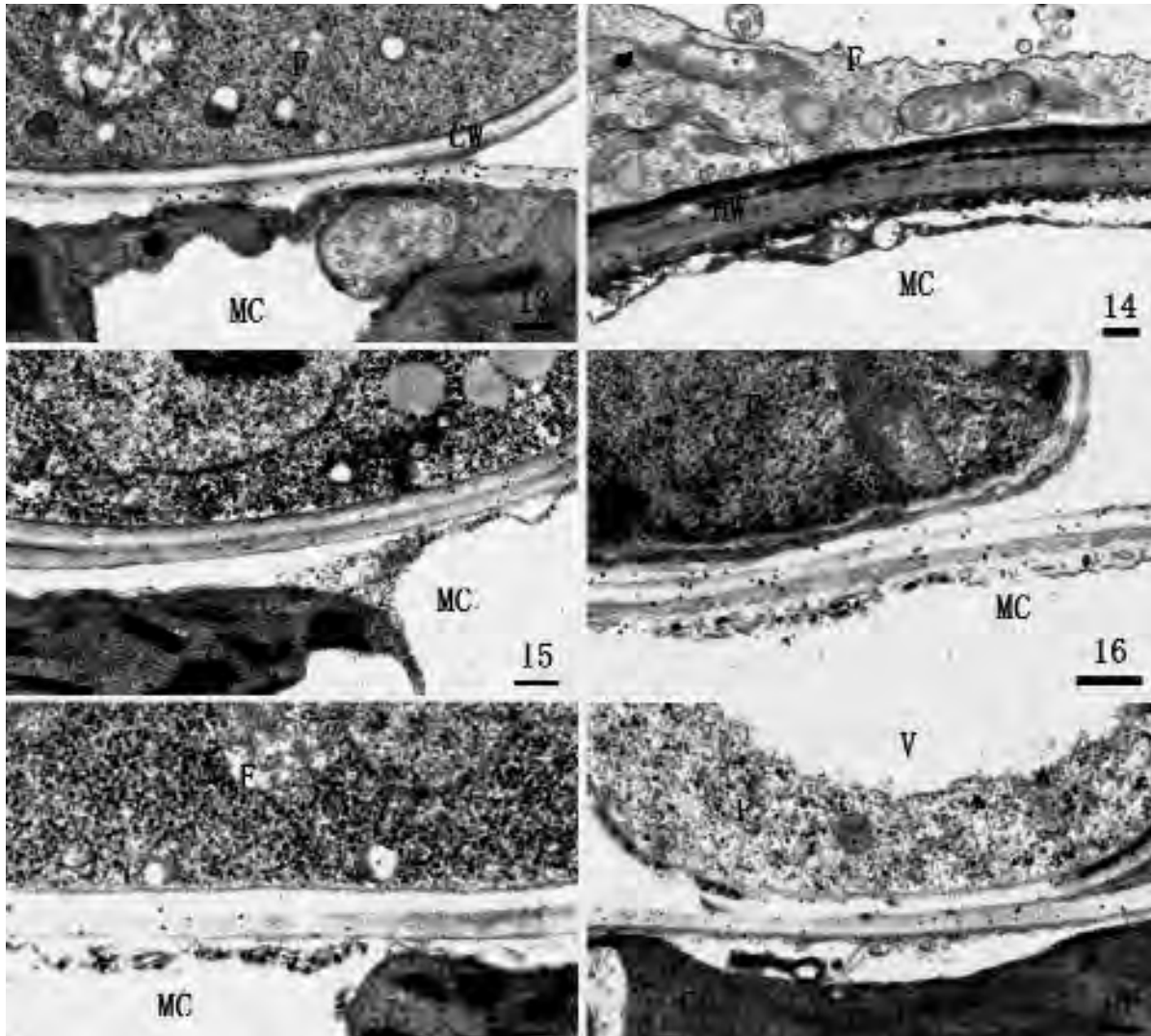
\* Labelling of lignin, chitinase and  $\beta$ -1,3-glucanase over the host cell walls was expressed by the number of gold particles per  $\mu\text{m}^2$  and different letters in the same column indicate a significant difference at the 0.05 level using the GLM procedure of SAS system (Version 6.12): test (Tukey's Student Range).

## DISCUSSION

Effects of the fungicide tebuconazole on the wheat – stripe rust host-pathogen complex were examined by means of electron microscopy and immunogold labelling. The results showed that inhibition of development of *Puccinia striiformis* in wheat leaf tissues treated with the fungicide was accompanied by severe morphological and structural changes in the hyphal and haustorial development. These changes included increased

vacuolation, irregular cell wall thickening and necrosis or degeneration of cytoplasm. These alterations are very similar to those reported for other plant pathogenic fungi treated with ergosterol biosynthesis-inhibiting (EBI) fungicides (Hippe, 1984; Pring, 1984; Richmond, 1984; Smolka and Wolf, 1986; Fuller et al., 1990; Heller et al., 1990; Kang et al., 1993, 1996, 2001; Maffi et al., 1995, 1998; Coutinho et al., 1995; Leinhos et al., 1997).

Morphological alterations of hyphal structures and haustoria of the stripe rust fungus in tebuconazole-



**Fig. 13-18.** Immunogold localization of lignin (Fig. 13 and 14), chitinase (Fig. 15 and 16) and  $\beta$ -1,3-glucanase (Fig. 17 and 18) in *P. striiformis*-infected wheat leaves untreated and treated with tebuconazole fungicide. Fig. 13. Labelling of lignin in infected wheat leaf tissue, 5 days after infection. The host cell wall, but not the fungal wall are labelled. Fig. 14. Labelling of lignin in infected wheat leaf tissue, 2 days after treatment. The host cell wall is more highly labelled while the hyphal wall is unlabelled. Fig. 15. Labelling of chitinase in infected wheat leaf tissue, 5 days after infection. Both the plant and the hyphal cell walls are labelled. Fig. 16. Labelling of chitinase in infected wheat leaf tissue, 2 days after treatment. A higher label density is located on both host and fungus cell walls. Fig. 17. Labelling of  $\beta$ -1,3-glucanase in infected wheat leaf tissue, 2 dat. Plant and hyphal cell walls showed labelling, while other structures were almost free of label. Fig. 18. Labelling of  $\beta$ -1,3-glucanase in infected wheat leaf tissue, 2 dat. A higher density of label occurs over host cell walls and the fungal wall, while other structures are almost free of label. (All bars = 0.5  $\mu$ m). F = Fungal cell; CW = Hyphal cell wall; HW = Host cell wall; CH = Chloroplast; MC = Mesophyll cell; V = Vacuole.

treated wheat plants may be triggered by the primary mode of action of triazole fungicides. Interference in sterol biosynthesis by inhibition of 14 $\alpha$ -demethylase results in insufficient availability of ergosterol and accumulation of 14 $\alpha$ -methyl sterols (Buchenauer, 1990). Ergosterol, an essential membrane constituent, may be responsible for maintaining membrane integrity and activity. Insufficiency ergosterol in fungal membranes severely disturbs membrane functions. Deficiency of ergosterol in the plasmalemma markedly alters activity of membrane-bound enzymes; for instance chitin synthase is activated leading to irregular thickenings and accumulation of chitin-like material in cell walls and proper synthesis of new hyphal cell walls is severely disturbed.

Hyphal cell walls of rust fungi in host tissues contain chitosan (mainly in the outer layers) and chitin (especially in the inner layers) as well as  $\beta$ -1,3-glucans (Deising and Siegrist, 1995; Chong *et al.*, 1985). Thus, it may be suggested that, following treatment of stripe rust-infected wheat plants with triazole fungicides, chitin synthase will become overactive in young developing hyphae. Consequently, because of defective cell wall synthesis, chitin and chitooligosaccharides, oligo- and polymers of chitosan as well as  $\beta$ -linked oligoglucosides may accumulate. All these compounds may act as inducers of resistance (Boller, 1995; Hadwiger *et al.*, 1986; Hahn, 1996; Umemoto *et al.*, 1997).

In addition, it may be assumed that, due to the severe effects of triazole fungicides on the development of hyphal and haustorial structures of rust fungi, more substances may be released contributing to triggering of defense reactions in infected tissues.

In the present study, we found great differences in structural alterations in *Puccinia striiformis*-infected wheat leaves between the fungicide treatment and the untreated control. In the untreated control, a common host response was the deposition of electron-dense material in some of the infected host cells at the penetration site in form of a collar around the haustorial neck, and the collar usually did not extend beyond the joint region between haustorial neck and haustorial body (Kang *et al.*, 2002). However, in *P. striiformis*-infected wheat leaves treated with the fungicide, significant reactions to infection were induced such as formation of cell wall appositions between the host cell wall and plasmalemma in host cells in proximity to the hyphae, and the encasement of haustoria with electron-dense material of different texture in the infected host cell. The encased haustoria usually became necrotic and collapsed, with probable loss of function.

These reactions of host cells to stripe rust infection in infected wheat leaves treated with fungicide were very similar to those found in the incompatible interaction of this host-pathogen system (Kang *et al.*, 2002). These studies indicated that structural defense reactions were induced in *P. striiformis*-infected wheat leaves after

treatment with the fungicide tebuconazole, and formation of cell wall appositions and encasement in the infected wheat leaves treated with the fungicide might constitute an additional physical barrier, which strengthens the host cell wall and contributes to restricting the pathogen attack.

Furthermore, in the present study we detected chitinase and  $\beta$ -1,3-glucanase in the wheat – stripe rust complex by means of immunogold labelling (Kang *et al.*, 2002). These two enzymes were mainly localized in the host and fungal cell walls, and labelling densities in the different treatments varied significantly. The enzyme levels in *P. striiformis*-infected wheat leaves were slightly increased compared to the uninfected control, but much higher enzyme levels were induced in inoculated wheat leaves following fungicide application. The increased activity of both enzymes may contribute to defense reactions. These enzymes show antifungal activity because they not only degrade the main cell wall components of fungi (Young and Pegg, 1982), but also produce fragments of the polymers which act in turn as elicitors of defense responses in the host (Keen and Yoshikawa, 1983).

In this study, we also found evidence for chitinase and  $\beta$ -1,3-glucanase in hyphal cells of the invading fungus, which is similar to the findings in tomato and eggplants infected by vascular wilt fungi (Benhamou *et al.*, 1989), and in wheat infected by *P. striiformis* (Kang *et al.*, 2002), *Gaeumannomyces graminis* var. *tritici* (Huang *et al.*, 2001), and *Fusarium culmorum* (Kang and Buchenauer, 2002). It indicated that the enzymes may diffuse to the hyphal surface where they might degrade the cell wall constituents of hyphae and haustoria. The release and accumulation of cell wall fractions might simultaneously contribute to activation of secondary defense responses.

In addition, the labelling pattern of lignin in wheat leaf tissues from different treatments in the present study agrees with earlier reports (Kang *et al.*, 2001). Lignin accumulation slightly increased in *Puccinia striiformis*-infected wheat leaves as compared to uninoculated healthy wheat leaves (Smart, 1991; Kang *et al.*, 2001). In contrast, a much higher accumulation of lignin was detected in *Puccinia striiformis*-infected wheat leaves after treatment with the fungicide tebuconazole, indicating that the lignin level is enhanced in the *Puccinia striiformis*-wheat system after fungicide treatment. These results are similar to the situation found for incompatible interactions where lignin content increases significantly in host plant tissues infected by avirulent pathogens (Smart, 1991; Kang *et al.*, 2001). Lignin is a complex polyphenol responsible for the thickening and strengthening of plant cell walls. A rapid lignification of plant cell walls was shown to be very effective against pathogen ingress in many host-pathogen interactions (Smart, 1991).

The present study shows that application of tebuconazole not only inhibited development of *Puccinia striiformis* in wheat leaves, but also enhanced host defense reactions including the formation of cell wall appositions, encasements of haustoria and accumulation of chitinase,  $\beta$ -1,3-glucanase and lignin in host tissue. Our results show that the fungicide itself did not directly induce such defense reactions, but may trigger host defense reactions in wheat tissue infected by *P. striiformis*. However, further studies will be needed to obtain more detail on the factors contributing to activation of secondary defense responses.

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