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ARTICLE

Study of Inhibitory Effects and Action Mechanism of the Novel Fungicide Pyrimorph against *Phytophthora capsici*†

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The antifungal activity of the novel fungicide pyrimorph, (*E*)-3-[(2-chloropyridine-4-y1)-3-(4-*tert*-butylpheny1)acryloyl]morpholin, against *Phytophthora capsici* was investigated in vitro. Pyrimorph inhibited different stages in the life cycle of *P. capsici* including mycelial growth, sporangium production, zoospore release, and cystospore germination with EC_{50} values of 1.84, 0.17, 4.92, and 0.09 μg mL^{-1}, respectively. The effect of pyrimorph on mycelial growth was reduced by the addition of different concentrations of ATP, which suggested that the action mechanism of pyrimorph was connected with impairment of the energy generation system. Meanwhile, pyrimorph exhibited certain inhibition on metabolic approaches of Embden−Meyerhof−Parnas (EMP), tricarboxylic acid cycle (TCA), and hexosemonophosphate (HMP) by measuring the oxygen consumption of pyrimorph combining with three representative inhibitors to the metabolic approaches. The results indicated that pyrimorph could inhibit the approach of HMP significantly. Morphological and ultrastructural studies showed that pyrimorph caused excessive septation and swelling of hyphae, distortion and disruption of most vacuoles, thickening and development a multilayer cell wall, and accumulation of dense bodies. These results suggested pyrimorph exhibited multiple modes of action including impairment of the energy generation system and effect on cell wall biosynthesis directly or indirectly.

KEYWORDS: Fungicide; pyrimorph; *Phytophthora capsici*; inhibitory effect; morphology; ultrastructure

INTRODUCTION

Phytophthora blight of pepper (*Capsicum annuum* L.), caused by *Phytophthora capsici* Leonian, is one of the most destructive diseases of pepper production in the world (*1*, *2*). This pathogen causes root, stem, and crown rots, as well as fruit and foliar blight from splash dispersal of propagules from soil, which have been responsible for major loss of pepper production, but control methods have been limited because the oospores of *P. capsici* can endure desiccation, cold temperatures, and other extreme environmental conditions and can survive in the soil in the absence of a host plant for several years (*3*). Chemical control is still the main measure to control the disease (*4*) besides biological control (*2*, *5*) and induced resistance (*6*); due to resistance and chemical residue of fungicides (*7*), it is necessary to develop new kinds of fungicides with high efficacy and safety to human health and the environment.

Pyrimorph, (*E*)-3-[(2-chloropyridine-4-yl)-3-(4-*tert*-butylphenyl)acryloyl]morpholine, was a novel fungicide synthesized by Qin et al. in 2003 (*8*) (**Figure 1**). Pyrimorph exhibits excellent activity against oomycetes, such as *Phytophthora infestans*, *P. capsici*, and *Pseudoperonospora cubensis* (*9*, *10*). Pyrimorph also showed strong preventive activity, persistence activity, and sporulation inhibition activity against late blight of tomato caused by *P. infestans* (Montagne) de Bary (*9*, *10*) in glasshouse studies. However, there are no data concerning the antifungal activity in vitro and the mode of action of pyrimorph against *P. capsici*.

This paper describes the antifungal activity of pyrimorph against *P. capsici* in vitro. The fungicidal properties of pyrimorph on different stages in the life cycle of *P. capsici* are presented. Moreover, the effects of pyrimorph on respiration of mycelia and on hyphal morphology and ultrastructure were evaluated to obtain much information about the mode of action. The commercial fungicides of dimethomorph and azoxystrobin were chosen as reference agents.

MATERIALS AND METHODS

Chemicals and Reagents. Pyrimorph (with a purity of >95%) was provided by the China Agricultural University. Dimethomorph (with a purity of 95%) was purchased from Anhui Fengle Agrochemical Co., Ltd. (Anhui, China). Azoxystrobin (with a purity of 95%) was provided by Syngenta Co., Ltd. (Beijing, China). Adenosine triphosphate (ATP) was purchased from Sigma Chemical Co. (St. Louis, MO). Stock fungicide solutions were prepared by dissolving pyrimorph, dimethomorph, and azoxystrobin in acetone and diluted with distilled water or culture medium to make the volume of acetone concentration <1% of the test solution

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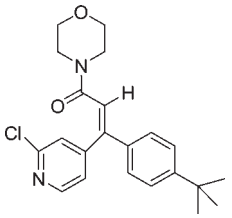

Figure 1. Structure of pyrimorph.

bioactivity in vitro. All other chemicals and reagents were of analytical reagent grade and were used without further purification. All aqueous solutions were prepared using distilled water.

Pathogens and Cultures. The test strain *P. capsici* collected from infected pepper plants (*Capsicum frutescens*) in the field in Yinchuan, Ningxia, China, was isolated, purified, and maintained on carrot agar medium (CaA) (carrot infusion from 200 g L^{-1}, 20 g L^{-1} agar) in the dark at 25 °C.

Effect of Pyrimorph on Mycelial Radial Growth. Mycelial disks (5 mm in diameter) of test fungi grown on CaA plates were cut from the margins of the colony and placed on CaA plates containing different concentrations of pyrimorph. Stock solution of pyrimorph was diluted with sterile molten CaA to obtain the desired pyrimorph concentrations. After incubation at 25 °C for 5 days, mycelial radial growth was measured and activity was expressed with EC_{50} (the concentration inhibiting mycelial growth by 50%) and EC_{90} (the concentration inhibiting mycelial growth by 90%) values. This experiment was conducted twice with four replicates. Percent inhibition was calculated as equal to the (colony diameter of control − colony diameter of treatment)/(colony diameter of control − mycelial disks diameter) × 100.

In addition, ATP (an energy supplier) was added to CaA plates together with pyrimorph to obtain more information about the mode of action of pyrimorph according to the method described by Mitani et al. (*11*) and Xu et al. (*12*).

Effect of Pyrimorph on Sporangia Formation, Zoospore Release, and Cystospore Germination of *P. capsici*. Five mycelial disks (5 mm in diameter) of *P. capsici* were cut from the edge of actively growing culture and placed in glass dishes (9 cm in diameter) containing 10 mL of sterile distilled water and different volumes of pyrimorph stock solution, making the designed concentrations of pyrimorph. The control treatment consisted of five disks (5 mm in diameter) of *P. capsici* placed in glass dishes (9 cm in diameter) containing only 10 mL of sterile distilled water. Three replicates were conducted for each dilution and incubated for 60 h at 25 °C with continuous light. Sporangia formation was determined using a light microscope (Nikon, Japan) by checking three fields in each disk.

Effects of pyrimorph on zoospore release and cystospore germination were tested by using the method of Andrieu et al. (*13*). A sporangia suspension (1×10^4 sporangia mL^{-1}) of *P. capsici* was obtained by rinsing mature mycelia with sterile distilled water. Pyrimorph was added to the suspension at the start of incubation for zoospore release test. For the cystospore germination test, pyrimorph was added to the sporangia suspension 240 min after incubation. The same amount of sterile distilled water was added to the sporangia suspension for the control treatment. After incubation at 4 °C for 60 min and at 25 °C for 120 min, approximately 250 sporangia were observed with a light microscope, and the inhibition rate of zoospore release of *P. capsici* was calculated. Cystospore germination was assessed after 24 h of incubation at 25 °C. Zoospore release was calculated as a percent of empty sporangia relative to the total number of sporangia (empty, full, and germinated). Cystospore germination was calculated as a percent of germinated cystospore relative to the total number of cystospores. All of the experiments were conducted using five doses of pyrimorph, which were repeated twice with three replicates per treatment.

Respiration Measurement. Mycelial plugs of *P. capsici* from 5-day-old colony margins were transferred to conical flasks containing 150 mL of carrot liquid medium for shake culture (25 °C, 150 r/min). After 5 days, mycelia were washed three times with 50 mM potassium phosphate buffer (pH 7.2) and resuspended in 0.1 M phosphate buffer (pH 7.2) containing 2% glucose (50 mg of fresh weight of mycelia mL^{-1}) (*14*). Pyrimorph was added to *P. capsici* mycelial suspension at a concentration of 50 μg mL^{-1}. Three kinds of respiratory inhibitor, malonic acid, iodoacetic acid, and sodium phosphate, were also added to the mycelial suspension at the concentration of 1000 μg mL^{-1}, respectively. All measurements were carried out at 25 ± 1 °C. The ratio of the mycelial oxygen consumption was tested by oxygraphy (DW1, Hansatech Instruments Ltd., Norfolk, U.K.), and the inhibition of respiration was calculated using the following formula: $I_R = (R_0 - R_1)/R_0 \times 100$, where I_R is the inhibition of respiration (%); R_0 and R_1 are the ratios of mycelial oxygen uptake pre- and post-addition of inhibitors (nmol of O_2/min/mg of mycelia). $R_R = (R_1 - R_1')/R_1 \times 100$, where R_R is the the superpose rate of representative respiratory inhibitor with pyrimorph (%), R_1 is the ratio of mycelial oxygen uptake adding pyrimorph (nmol of O_2/min/mg of mycelia); and R_1' is the the ratio of mycelial oxygen uptake adding pyrimorph and representative respiratory inhibitor (nmol of O_2/min/mg of mycelia).

Table 1. Effect of Pyrimorph on Mycelial Growth of *Phytophthora capsici*

concn of pyrimorph (μg/mL)	concn of ATP[a] (μg/mL)	% inhibition ± SD of mycelial growth[b]
0.6	0.0	9.0 ± 0.6
1.0	0.0	28.0 ± 1.8
2.0	0.0	57.0 ± 8.1
5.0	0.0	81.0 ± 8.7
	1.0	75.0 ± 3.7
	5.0	72.0 ± 0.5
10.0	0.0	98.0 ± 1.0
	1.0	78.1 ± 0.3
	5.0	77.0 ± 0.3

[a] ATP was added to CaA plates together with pyrimorph to give final concentrations of 1 and 5 μg mL^{-1}. Mycelial radial growth was measured after incubation for 5 days. [b] Mean and standard deviations (SD) of inhibition of mycelial growth (n = 4).

Electron Microscopy. *Scanning Electron Microscopy* (*SEM*). *P. capsici* mycelial tips (5 mm) of an actively growing colony on CaA medium amended with 0, 5, and 10 μg mL^{-1} pyrimorph were cut from the edge of the colony cultured for 72 h. The tips were treated with 4% glutaraldehyde at 4 °C, followed by rinsing with 0.1 M phosphate buffer (pH 7.3) and fixed with 1% w/v osmium tetraoxide solution. Rinsed with 0.1 M phosphate buffer three times, the mycelial tips were dehydrated using a series of acetone solutions in the order of concentration 30, 50, 70, 80, and 90% anhydrous acetone. The processes of drying at critical point, mounting, and gold spraying were completed at last and examined in a scanning electron microscope (S-3400N, Hitachi, Nissei Sanyo, Japan) with an accelerating voltage of 18−20 kV (*15*).

Transmission Electron Microscopy (*TEM*). The mycelial tips were prepared according to the method given above. After dehydrating and embedding in Epon 112, thin sections were cut and double-stained with uranyl acetate and lead citrate. The grids were examined with a JEOL-1230 (JEOL, Tokyo, Japan) transmission electron microscope.

Statistical Analysis. All of the values of EC_{50} and EC_{90} were calculated using Data Processing System (DPS 10.15, Hangzhou, China). The data of superpose rate of respiration for each treatment were compared to each other by one-way ANOVA at $\alpha = 0.05$ using SPSS software (10.0.1, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Effect of Pyrimorph on Different Stages in the Life Cycle of *P. capsici*. Pyrimorph inhibited mycelial growth of *P. capsici* as shown in **Table 1**. Pyrimorph at 0.6, 1.0, and 10.0 μg mL^{-1} exhibited 9.0, 28.0, and 98% inhibition, respectively. The EC_{50} and EC_{90} values of pyrimorph against *P. capsici* were 1.84 and 5.71 μg mL^{-1} ($Y = 4.31 + 2.61X$; Y is the inhibition rate (%) and X is the $\log_{10}$ concentration (μg mL^{-1}); $r = 0.99$).

ATP could accelerate the mycelial growth of *P. capsici* when different concentrations of ATP (1 and 5 μg mL^{-1}) were added into the CaA plates. In the presence of ATP, the inhibition of mycelial growth on CaA plates containing different concentrations of pyrimorph (5.0 and 10.0 μg mL^{-1}) was reduced, but the trend of inhibition was not reduced markedly. Pyrimorph at 10.0 μg mL^{-1} exhibited 98.2% inhibition on mycelial growth, and this effect was reduced to 78.1% when 1.0 μg mL^{-1} ATP was present.

Pyrimorph strongly inhibited sporangia formation with EC_{50} and EC_{90} values of 0.17 and 3.21 μg mL^{-1} ($Y = 5.77 + 1.00X$; $r = 0.95$), respectively. After incubation for 60 h, there was ca. 130 (±10) zoosporangia formed around the margin of one mycelial disk in sterile distilled water. In the presence of 10 μg mL^{-1} pyrimorph, the formation of sporangia was completely inhibited, and pyrimorph at 2 μg mL^{-1} exhibited 85% inhibition (**Table 2**).

Pyrimorph was inefficient in inhibiting zoospore release compared with the inhibition of pyrimorph on the different stages in the life cycle of *P. capsici*, with EC_{50} and EC_{90} values of 4.92 and 45.66 μg mL^{-1} ($Y = 4.08 + 1.32X$; $r = 0.97$), respectively (**Table 3**).

Cystospore germination was the stage most sensitive to pyrimorph. The EC_{50} and EC_{90} values of pyrimorph on cystopore germination were 0.09 and 8.16 μg mL^{-1} ($Y = 5.68 + 0.66X$; $r = 0.96$), respectively. After the sporangia suspension was incubated with pyrimorph for 24 h, cystospore germination in control was ca. 90%, whereas inhibition of cystospore germination by pyrimorph was 54% at 0.1 μg mL^{-1} and 84% at 2 μg mL^{-1} (**Table 2**).

Table 2. Effect of Pyrimorph on Sporangia Formation and Cystospore Germation of *Phytophthora capsici*[a]

concn of pyrimorph (μg mL^{-1})	% inhibition ± SD of sporangia formation[b]	% inhibition ± SD of cystospore germation[c]
0.1	52 ± 2.8	54 ± 2.9
0.2	57 ± 2.8	56 ± 2.4
0.5	66 ± 2.0	68 ± 1.6
1.0	75 ± 1.5	71 ± 0.9
2.0	85 ± 0.9	84 ± 2.1

[a] Inhibition of sporangia formation was observed after incubation mycelia disks with pyrimorph for 60 h. Pyrimorph was applied after 240 min of incubation of sporangia. Inhibition of cystospore germination was observed after incubation with pyrimorph for 24 h after incubation with pyrimorph at 4 °C for 60 min and at 25 °C for 120 min. [b] Mean and SD of inhibition of sporangia formation ($n = 3$). [c] Mean and SD of inhibition of cystospore germination ($n = 3$).

Table 3. Effect of Pyrimorph on Zoospore Release of *Phytophthora capsici*[a]

concn of pyrimorph (μg mL^{-1})	% inhibition ± SD of zoospore release[b]
6	58 ± 0.7
12	65 ± 1.2
25	80 ± 2.0
50	94 ± 0.2
100	95 ± 0.3

[a] Pyrimorph was applied at the start of incubation of sporangia. Inhibition of zoospore release was observed after incubation with pyrimorph at 4 °C for 60 min and at 25 °C for 120 min. [b] Mean and SD of inhibition of zoospore release ($n = 3$).

Pyrimorph inhibited different stages in the life cycle of *P. capsici* including mycelial growth, sporangia formation, zoospore release, and cystospore germination. It is well-known that zoospore release is affected by the inhibition of the energy supply (*16*). Energy generation inhibitors such as azoxystrobin, fluazinam, cyazofamid, and famoxadone (*11*, *13*, *17*, *18*) inhibit all stages in the life of *P. capsici* and show extremely remarkable inhibition on zoospore motility and zoospore release. On the other hand, nonrespiratory inhibitors cymoxanil, dimethomorph, and metalaxyl (*17*−*20*) exhibit low or no inhibition of zoospore motility and zoospore release of *P. capsici*. It was shown that the effect of pyrimorph on zoospore release from sporangia of *P. capsici* was in the middle inhibition degree, which lies between energy generation inhibitors and nonrespiratory inhibitors in our study. Pyrimorph can be regarded as a cinnamic acid derivative that was similar to another oomycete fungicide of dimethomorph in chemical structure. Presumably, a similar mechanism of action might exist between the two fungicides. Moreover, the pyridine ring, which exists in the chemical structure of pyrimorph, was reported to be the dominant factor contributing to high antifungal activity in the broad-spectrum fungicide fluazinam, owing to the electronic property of the pyridine ring and the hydrophobicity of substituents on the pyridine ring (*21*). Meanwhile, the benzene ring in fluazinam was also a crucial factor contributing to high antifungal activity (*21*, *22*). Comprehensive consideration of the reasons mentioned above can give us a reasonable interpretation for why the inhibition of pyrimorph against zoospore release of *P. capsici* was lower than that of fluazinam and higher than that of dimethomorph.

With the addition of different concentrations of ATP (an energy supplier) to CaA plates containing pyrimorph, the inhibitions of mycelia growth of pyrimorph against *P. capsici* were all decreased, but the extent of decrease was not very obvious. The results suggested that ATP was a factor but not the most important factor to mycelial growth.

Effect of Pyrimorph on Mycelial Respiration. Effects of pyrimorph, dimethomorph, and azoxystrobin on oxygen consumption of *P. capsici* mycelia are shown in **Table 4**. Pyrimorph at 100 μg mL^{-1} exhibited 40.95% inhibition of oxygen consumption of intact mycelia. When three kinds of representative inhibitors of metabolic approach of TAC, EMP, and HMP, as malonic acid, iodoacetic acid, and sodium phosphate, were added to the

Table 4. Respiratory Inhibition of Intact Mycelia of *P. capsici* by Pyrimorph

	μmol of O_2 g^{-1} min^{-1}			
inhibitor	R_0	R_1	inhibition rate (%)	superpose rate[a] (%)
malonic acid	15.43 ± 0.31	6.17 ± 0.30	60.00 ± 1.95	
iodoacetic acid	15.43 ± 0.31	4.52 ± 0.42	70.69 ± 2.75	
sodium phosphate	15.43 ± 0.31	5.76 ± 0.02	62.66 ± 0.13	
pyrimorph[b]	15.43 ± 0.31	9.11 ± 0.48	40.95 ± 3.14	
pyrimorph + malonic acid	15.43 ± 0.31	7.44 ± 0.25	51.80 ± 1.63	18.31 ± 2.08b
pyrimorph + iodoacetic acid	15.43 ± 0.31	6.14 ± 0.49	60.20 ± 3.15	32.61 ± 3.66a
pyrimorph + sodium phosphate	15.43 ± 0.31	8.64 ± 0.49	44.03 ± 3.21	5.22 ± 0.51c
dimethomorph[c]	15.43 ± 0.31	12.43 ± 0.42	19.43 ± 2.70	
dimethomorph + malonic acid	15.43 ± 0.31	6.23 ± 0.77	59.60 ± 4.98	49.91 ± 5.17a
dimethomorph + iodoacetic acid	15.43 ± 0.31	6.01 ± 0.22	61.07 ± 1.44	51.62 ± 3.04a
dimethomorph + sodium phosphate	15.43 ± 0.31	6.94 ± 0.55	54.99 ± 3.54	44.06 ± 5.45a
azoxystrobin[d]	15.43 ± 0.31	4.37 ± 0.02	71.65 ± 0.13	
azoxystrobin + malonic acid	15.43 ± 0.31	3.98 ± 0.06	74.21 ± 0.37	9.02 ± 1.20b
azoxystrobin + iodoacetic acid	15.43 ± 0.31	2.04 ± 0.04	86.76 ± 0.26	53.27 ± 0.82a
azoxystrobin + sodium phosphate	15.43 ± 0.31	2.13 ± 015	86.21 ± 0.94	51.36 ± 3.26a

[a] Different letters (a−c) indicate significant difference according to Duncun's multiple-range test ($P = 0.5$). [b] The concentration of pyrimorph was 100 μg mL^{-1}. [c] The concentration of dimethomorph was 100 μg mL^{-1}. [d] The concentration of azoxystrobin was 10 μg mL^{-1}.

determination system containing 100 μg mL^{-1} pyrimorph, the respiratory inhibition increased from 40.95 to 51.80, 60.20, and 44.03% and the superpose rates of consuming oxygen of mycelia treated with pyrimorph and malonic acid, iodoacetic acid, sodium phosphate were 18.31, 32.61, and 5.22%, respectively. The results show that pyrimorph presented a similar mechanism with sodium phosphate inhibiting the metabolic approach of HMP. Treatment with dimethomorph showed little influence on the mycelial respiration. From the inhibition rate and superpose rate of azoxystrobin in **Table 4**, it could be deduced that azoxystrobin strongly inhibited mycelial respiration and possessed a similar mechanism, with malonic acid inhibiting the metabolic approach of TAC.

Organisms derive useful energy and intermediates for syntheses from oxidation of compounds as a result of fermentation and respiration. The initial steps of fermentation and respiration are the same and occur under either anaerobic or aerobic conditions. The process begins with glycolysis, the stepwise conversion of glucose to pyruvic acid. After glycolysis is completed, fermentation is favored if oxygen is lacking or in short supply. Alternatively, if aerobic pathways are favored by an abundant supply of oxygen, the pyruvic acid will be utilized in aerobic respiration. The principal glycolytic pathways are the Embden−Meyerhof−Parnas (EMP) and the hexosemonophosphate (HMP) pathways, and an aerobic pathway is the tricarboxylic acid cycle (TCA) (*23*). In this research, the inhibition of oxygen consumption was increased when pyrimorph acted on mycelia together with malonic acid, iodoacetic acid, and sodium phosphate, three representative inhibitors of the metabolic approach of TCA, EMP, and HMP. The superpose rate of consuming oxygen of pyrimorph combined with sodium phosphate was the smallest, whereas the inhibition and the superpose rate of consuming oxygen of pyrimorph combined with iodoacretic acid was the opposite. These results indicated that pyrimorph probably had a similar mechanism with sodium phosphate inhibiting the metabolic approach of the HMP pathway; meanwhile, pyrimorph might have a slight or no effect on the TCA cycle and EMP pathway. In addition, azoxystrobin and dimethomorph were taken as control fungicides in this research. Azoxystrobin, a respiration inhibitor, exhibited the lowest superpose rate of consuming oxygen combined with malonic acid, indicating that pyrimorph had an utterly different mechanism from azoxystrobin. Low inhibition in mycelial respiration and high superpose rate with three inhibitors of EMP, HMP, and TCA were observed in dimethomorph, affecting mycelial respiration, indicating that pyrimorph has a different mechanism in energy consumption.

The observation above indicates that pyrimorph could affect energy generation, but disruption of energy production was a secondary effect of this fungicide.

Effect of Pyrimorph on Morphology and Ultrastructure Transformation of *P. capsici*. Pyrimorph was effective in inhibiting mycelia radial growth on CaA medium (**Table 1**). Pyrimorph at 5 and 10 μg mL^{-1} inhibited growth of *P. capsici* mycelium by 81 and 98%, respectively. Mycelium of *P. capsici* grew uniformly along the surface of culture media without pyrimorph, and the shape of the whole colony appeared to radiate from its central point. The fringe of the colony was round and regular. However, the growth of mycelium was seriously depressed when it was cultured in the media with pyrimorph. The fringe of the colony was concave−convex, irregular, and was not as smooth and regular as that of control in nonpoisonous culture media. Furthermore, the higher the concentration of pyrimorph was, the clearer this growth pattern was.

SEM observations of *P. capsici* treated with pyrimorph revealed the effects on the morphology of the hyphae (**Figure 2**).

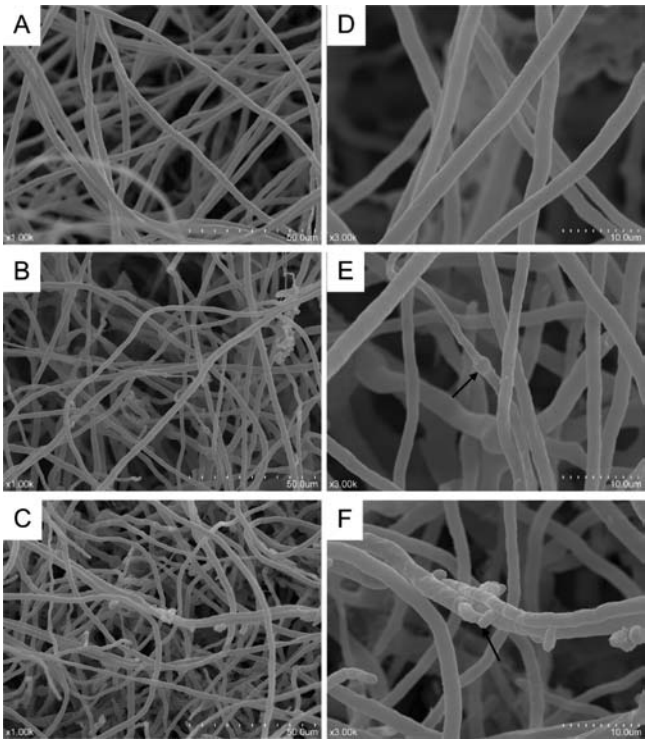

Figure 2. Scanning electron micrographs of the hyphae from the colony of *P. capsici*: (**A**−**C**) 1000×, bars, 50.0 μm; (**D**−**F**) 3000×, bars, 10.0 μm. (**A**, **D**) Sections of *P. capsici* hyphae were grown on CaA medium in the absence of pyrimorph (control). The mycelium was low density and fresh and had a fine structure. (**B**, **E**) Sections of *P. capsici* hyphae were grown on CaA medium containing 5 μg mL^{-1} pyrimorph. The mycelium was a comparatively high density colony and the mycelium swelled (arrowhead). (**C**, **F**) Sections of *P. capsici* hyphae were grown on CaA medium containing 10 μg mL^{-1} pyrimorph. The amount of ramification increased and formed irregular ramification or abnormal configuration (contracted or swelling up) on mycelium tip (arrowhead).

SEM images indicated that the mycelium grew well in nonpoisonous media, and it was of low density, had a fine structure, and was fresh (**Figure 2A**,**D**). However, in culture media with pyrimorph of 5 μg mL^{-1}, mycelium grew abnormally with comparatively high density of colony; some parts of the mycelium contracted and some parts swelled (**Figure 2B**,**E**). With an increasd concentration of pyrimorph (10 μg mL^{-1}), the tendency of the density of the colony and the distortion of mycelia were more obvious; moreover, the amount of ramification increased and mycelium formed irregular ramification or abnormal configuration (contracted or swelled up) on mycelium tip (**Figure 2C**,**F**).

P. capsici mycelial tips (5 mm) from the margins of an actively growing colony on CaA medium were examined by TEM (**Figure 3**). The control mycelia of *P. capsici* grown in the absence of pyrimorph showed many of the cytological and ultrastructural features typical of vegtative hyphae of the genus (*12*, *24*) (**Figure 3A**,**D**). Normal cell wall (CW) deposition and an undulated plasmalemma (PM) had occurred, and organelle-rich cytoplasm including vesicles (VE), vacuole (V), lipid body (L), and mitochondria (M) was observed.

In the presence of pyrimorph at 5 μg mL^{-1}, extensive cell wall proliferation was the most pronounced ultrastructural change observed in hyphae, and the organelles of vacuole, lipid body, and mitochondria had the same appearance as control hyphae, but the number of vesicles sharply decreased (**Figure 3B**,**E**). At a higher concentration of 10 μg mL^{-1}, pyrimorph caused more conspicuous cytological changes. The distortion of cell wall, the

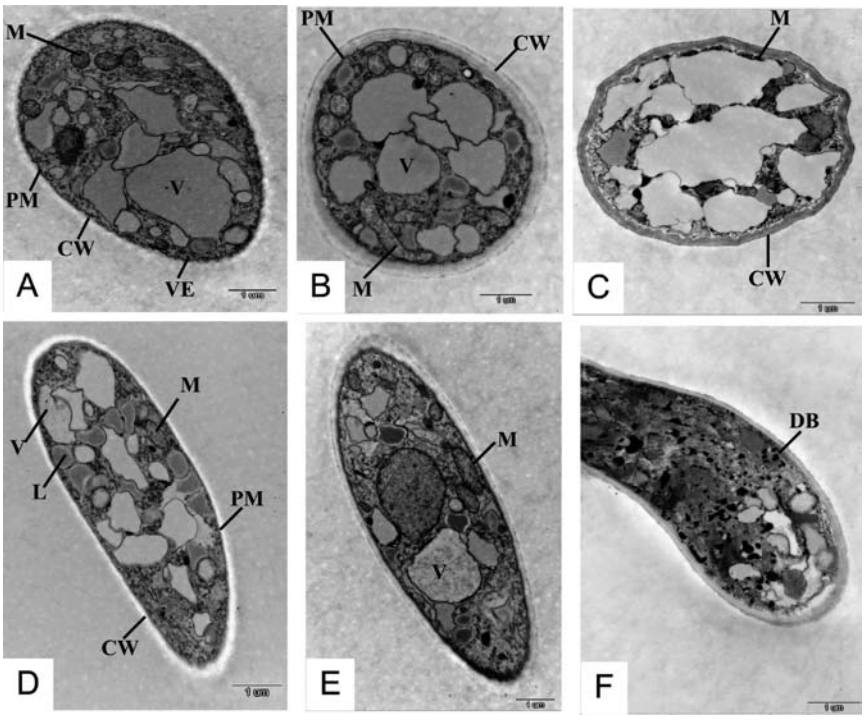

Figure 3. Transmission electron micrographs of *P. capsici* hyphae in longitudinal (**A**−**C**) and transversal (**D**−**F**). (**A**, **D**) Sections of *P. capsici* hyphae were grown on CaA medium in the absence of pyrimorph (control), showing normal cell wall (CW) and an undulated plasmalemma (PM). Many organelles were observed such as vesicles (VE), vacuole (V), lipid body (L), and mitochondria (M). (**B**, **E**) Sections of *P. capsici* hyphae were grown on CaA medium containing 5 μg mL^{-1} pyrimorph, extensive thickness of cell wall, and decreasing of vesicles. (**C**, **F**) Sections of *P. capsici* hyphae were grown on CaA medium containing 10 μg mL^{-1} pyrimorph; note the distortion of cell wall, increase of density body, and distortion and disruption of vacuoles and vesicles. Scale bar = 1 μm.

development of a multilayer cell wall, and an increase of dense bodies could be observed (**Figure 3C**,**F**). The undulated plasmalemma became unnoticeable, and vacuoles and vesicles were distorted and disrupted in the presence of pyrimorph (**Figure 3F**).

Pyrimorph had profound effects on the morphology and ultrastructure of *P. capsici*. Changes brought about by treatment with the pyrimorph included excessive septation, swelling of hyphae, and accumulation of dense bodies. The most noteworthy alteration, however, was the disruption of the endomembrane system, especially vacuoles and secretory vesicles. Aberrant morphogenesis was also manifested as cell wall thickening and in the development of multiple cell walls. The phenomena caused by pyrimorph were similar to those caused by dimethomorph, which acted on cell wall biosynthesis (*25*, *26*). Alternation of vacuoles also accorded well with the inhibition extent of pyrimorph on mycelial growth (**Table 1**). Vacuole plays an important role in maintaining the fungal turgor pressure, and it was more important for mycelial growth. Thus, this may well explain the high sensitivity of mycelial growth of *P. capsici*. Plasmalemma had an intact undulated conformation during the period of active cell growth and was disrupted or disappeared in pyrimorph-treated cells. In this study, vacuoles and vesicles related with cell wall synthesis (*27*) observed in control cells of *P. capsici* were distorted and disrupted in pyrimorph-treated cells. Hence, pyrimorph may also retard fungal growth by indirectly hindering cell wall synthesis. However, it was not confirmed whether pyrimorph disrupts the endomembrane system indirectly by binding to the cell surface or directly from inside the cell.

In conclusion, our results show that pyrimorph exhibited excellent antifungal activity against mycelial growth of *P. capsici* and that it also inhibited different stages in the life cycle of *P. capsici*. Pyrimorph probably had a mechanism similar to that of sodium phosphate, inhibiting the metabolic approach of the HMP pathway. Moreover, it was speculated that pyrimorph could inhibit the synthesis of cell wall either directly or indirectly from ultrastructural transformation of cell wall thickening and disruption of vacuoles. However, further research must be done to confirm the specific mode of action of pyrimorph.

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