Effect of Fluazinam on Infection Process of *Alternaria alternata* Japanese Pear Pathotype

Shigeru MITANI, Kaori OHHASHI, Tomona YAMAGUCHI and Terumasa KOMYOJI

Central Research Institute, Agrochemicals Development Division, Ishihara Sanga Kaisha, Ltd., Nishishibukawa, Kusatsu 525, Japan
(Received June 5, 1995; Accepted October 16, 1995)

INTRODUCTION

Fluazinam (3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α, α, α-trifluoro-2, 6-dinitro-p-toluidine, Frownicide, IKF-1216) is a new preventive fungicide, providing a broad spectrum of disease control. One of the major targets of fluazinam is *Alternaria alternata* Japanese pear pathotype, a pathogen of black spot disease of Japanese pear. The black spot disease is the most economically important disease of Japanese pear orchards, and several products have been introduced as effective fungicides, including fluazinam. Fluazinam is the newest among these fungicides. Besides the main target *A. alternata*, it has an excellent controlling effect against disease of Japanese pear caused by *Venturia nashicola* and *Physalospora piricola* at low dosage.

We previously reported effects of this fungicide on the infection process of *Botrytis cinerea*, *Colletotrichum lagenarium*, *Phytophthora infestans* and *Pyricularia oryzae*. In this study, we examined the effects of fluazinam on the infection process of *A. alternata* Japanese pear pathotype. The differences between the effect of this fungicide to *A. alternata* and to other plant pathogenic fungi are described.

MATERIALS AND METHODS

Fluazinam was synthesized by Ishihara Sanga Kaisha, Ltd. (50% wettable powder). *A. alternata* Japanese pear pathotype isolate No. 15A was kindly provided from Professor Kohmoto, Tottori University, Japan.

Effects of fluazinam on the spore germination, appressorial formation and penetration hyphae formation were observed under a light and fluorescence microscope. A length of germ tubes was measured by video micrometer (OLYMPUS VM-30). Fluazinam at final concentrations of 0.1 to 100 ppm was mixed with spore suspensions (5×10⁵ spores/ml). The suspension (80 μl) was placed onto a cellophane membrane, and incubated at 25°C for 24 hr on the cellophane membrane on slide glass in a moist chamber.

Effect of fluazinam on germinated-spore was also examined. Spores were first germinated on cellophane membrane as the method described above, and just before or after appressoria formed (6 or 12 hr after incubation started) fluazinam was added.

RESULTS AND DISCUSSION

Fluazinam inhibited 94.1% of spore germination at 100 ppm, and as low as 1 ppm of fluazinam prevented spore germination (Table 1). EC₅₀ of fluazinam on spore germination was 3.2 ppm. Fluazinam also inhibited appressorial and penetration hyphae formations (Table 1), and EC₅₀ was less than 0.1 ppm in both cases. An inhibition of germ-tube length is below 100 μm (O), 101-400 μm (H), 401-700 μm (a), more than 700 μm (O).

![Fig. 1 Effect of fluazinam on germ-tube length of Alternaria alternata Japanese pear pathotype.](image)

Table 1 Effect of fluazinam on Alternaria alternata Japanese pear pathotype.

<table>
<thead>
<tr>
<th>Fluazinam (ppm)</th>
<th>Spore germination(a)</th>
<th>Appressorial formation</th>
<th>Penetration hyphae formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>94.1 (5.7)</td>
<td>100 (0)</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>68.4 (30.4)</td>
<td>100 (0)</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>36.3 (61.3)</td>
<td>73.4 (16.1)</td>
<td>100 (0)</td>
</tr>
<tr>
<td>0.1</td>
<td>1.3 (95.0)</td>
<td>57.1 (26.0)</td>
<td>100 (0)</td>
</tr>
<tr>
<td>0</td>
<td>—</td>
<td>(96.3)</td>
<td>(60.6)</td>
</tr>
</tbody>
</table>

(a) Spores were incubated on cellophane membrane at 25°C for 24 hr. Approximately 400 spores were observed in each sample.

(b) The figures in parenthesis are the rates of spore germination, appressorial formation and penetration hyphae formation.

![Graph](image)
Elongation was also observed (Fig. 1). The elongation of germ tubes inhibited 61.1% by the addition of 0.1 ppm fluazinam, and length of more than 95% germ tubes were suppressed to less than 100 μm by addition of 10 ppm fluazinam.

Effect of fluazinam on germinated-spores was also examined. When fluazinam was added at 6 hr (just before appressoria formed) or 12 hr (after appressorial formation but before penetration hyphae formation) on germinated-spores, appressorial and penetration hyphae formation were significantly inhibited.

When fluazinam was treated, unusual swelling of germ tubes was observed (Fig. 2A). When Calcofluor White (fluorescent probe for chitin components) was applied on the fluazinam treated spores, swelling portions strongly responded and irregularly distributed on the mycelial wall of germ tubes (Fig. 2C and D). The fluorescence of the untreated germ tubes were weak and uniformly distributed (Fig. 2E). Many fungicides including ergosterol biosynthesis inhibitors (EBIs), polyoxins, dicarboximides, benzimidazoles, pyridylcarbamates are known to alter the cellular morphology of plant pathogenic fungi. Among them, EBIs and polyoxins are known to induce the alternation on the cell wall composition. Because of similar phenomena was observed after treatment of fluazinam, the abnormal structures of the pathogen by the treatment of fluazinam may be correlated with irregular deposition of chitin. This type of morphological change was observed on some of germ-tubes which was treated with fluazinam. However this change was not observed on all germ-tubes. Moreover, a constant result was not obtained in the frequency of this abnormality. These facts indicate that this morphological change is not the primary mode of action in the inhibition of infection process of A. alternata.

Among many Alternaria diseases, some A. alternata have been known to produce host-specific toxins (HST), which is determinant factor of the pathogenesis, during spore germination. However effect of fluazinam on HST (AK-toxin) synthesis also examined by the bioassay based on the toxicity of spore germination fluids according to the methods reported, it did not significantly reduce AK-toxin production.

From the results of this study, fluazinam inhibited all infection process of A. alternata from spore germination to
penetration hyphae formation except for AK-toxin productivity, but it acted characteristically rather in the process of germ tube elongation to penetration hyphae formation than in spore germination.

At this point, the effect of fluazinam on the infection process between A. alternata and other plant pathogenic fungi was somewhat different. In previous study, Komyoji et al. who studied effects of this fungicide on the infection process of B. cinerea, C. lagenarium, P. infestans and P. oryzae, reported that spore germination and infection structure formations were inhibited at the same degree at same dosage. 2)

ACKNOWLEDGMENTS

We wish to thank Associate Prof. Dr. Kazuya Akimitsu, Kagawa University for his critical reading of the manuscript.

REFERENCES