Tolerance of *Venturia nashicola* to Thiophanate-methyl and Benomyl in Japan

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石井英夫*・山口 昭* : ナシ黒星病菌 *Venturia nashicola* のチオファネート
メチル剤およびベノミル剤に対する耐性

Abstract

In 1975, spray applications of thiophanate-methyl and benomyl failed to control scab in several Japanese pear orchards. Mycelia of *Venturia nashicola* isolates, obtained from these orchards, developed normally on PDA supplemented with 50-800 ppm of thiophanate-methyl or benomyl. Sensitive isolates did not grow even at 1 ppm. However, conidia, formed *in vitro*, germinated in suspensions of these fungicides, irrespective of tolerant or sensitive isolate. Tolerant isolates exhibited vigorous germ tube elongation, but sensitive isolates produced only short germ tubes in these suspensions. In a protection test on Japanese pear treated with 100 ppm of each fungicide, tolerant isolates developed sporulating lesions. Tolerance of *V. nashicola* to thiophanate-methyl and benomyl in the field was therefore demonstrated.

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Introduction

In Japan, pear scab, caused by *Venturia nashicola* Tanaka et Yamamoto, is one of the most important diseases of Japanese pear (*Pyrus serotina* var. *culta*). Since 1971, dimethyl [(1,2-phenylene) bis (iminocarbonothioyl)] bis [carbamate] (thiophanate-methyl) and methyl 1- (butylcarbamoyl) -2-benzimidazolecarbamate (benomyl) have been used extensively for the control of pear scab, and efficient control was achieved for three or four years. In 1975, however, spray applications of these fungicides failed to control pear scab in several orchards located in Toyama, Chiba, Mie and Tottori Prefectures.

Benzimidazole tolerance in *Venturia inaequalis* (Cooke) Winter has been reported from Australia¹³⁾, Japan^{6,9)}, U. S. A. ³⁾, West Germany⁵⁾, New Zealand¹²⁾ and Poland⁸⁾. Tolerance of *Venturia pirina* (Cooke) Aderhold to benzimidazole fungi cides has been reported in Israel¹⁰⁾.

This paper describes experiments to determine thiophanate-methyl and benomyl tolerance in *V. nashicola*. Brief results of this study have been reported^{1,2)}.

Materials and Methods

Japanese pear leaves, infected with *V. nashicola*, were collected from widely

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separated orchards in Toyama, Chiba, Mie and Tottori Prefectures. These orchards were sprayed frequently with thiophanate-methyl and/or benomyl during the past three or four years. They were heavily infected with scab during the spring and summer of 1975 in spite of several spray applications of these fungicides. Twenty-five isolates, derived from conidia on diseased portions of leaves, were tested. An isolate, "Hiratsuka No. 1", from a pear tree that had never been treated with thiophanate and benzimidazole fungicides was used as a control.

Effect of thiophanate-methyl and benomyl on mycelial growth of *V. nashicola* isolates. Mycelial disks (4 mm in diameter), previously cultured on potato dextrose agar (PDA) for 45 days at 20 C, were transferred onto PDA containing 0.19, 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400 and 800 ppm (a.i.) of commercial formulations of thiophanate-methyl or benomyl, and incubated at 20 C. After 3 weeks, the minimum inhibitory concentrations (MIC) of these fungicides to each isolate were obtained.

Effects of thiophanate-methyl and benomyl on conidial germination and germ tube length of *V. nashicola* isolates. Conidia were obtained by the following method¹¹⁾. Pieces of mycelia, previously cultured on PDA, were placed onto sterile cylinders of potato tuber tissue in test tubes, and incubated in the phytotron (Temp.: day 18 C, night 13 C, RH: 70-95 %, daylight). After 25 days, conidia were collected from the surface of the potato tissue, and the conidial suspension was obtained by filtration through tissue paper. A drop of the fungicide-conidial suspension was placed on a glass slide covered with collodion film, and incubated at 20 C in a dark moist chamber for 24 hr. The concentrations of thiophanate-methyl or benomyl tested were 1, 10, 100 and 1,000 ppm of active ingredient of each fungicide. After fixation of the suspension, conidial germination was observed and germ tube length was measured microscopically.

Isolate "No. 2-2" which showed tolerance to both fungicides in mycelial growth and sensitive isolate "Hiratsuka No. 1" were used.

Effect of thiophanate-methyl and benomyl on infectivity of *V. nashicola* isolates. Potted Japanese pear trees (cultivar Chojuro), two-year-old, were used. On the seventh day after foliation, the surface of pear leaves was sterilized with 70 % ethyl alcohol, and lightly coated with suspension of thiophanate-methyl or benomyl (100 ppm a. i.). The pear leaves were inoculated with a conidial suspension 24 hr after fungicide treatments. The inoculated trees were kept in a moist chamber (RH: 100 %) at 20 C for 72 hr, and then placed in a glasshouse. Scab development was assessed 25 days after inoculation.

Results

Effect on mycelial growth

Mycelial growth of isolate "Hiratsuka No. 1" was completely inhibited at 0.19 ppm of thiophanate-methyl or benomyl. On the other hand, 22 out of 25 isolates tested were apparently tolerant to both fungicides and grew well at the higher concentrations from 50 to 800 ppm (Fig. 1).

Effects on conidial germination and germ tube length

As shown in Fig. 2, conidia of "No. 2-2" germinated at all concentrations tested up to 1,000 ppm of thiophanate-methyl and each germination percentage was comparable to that of the control. Conidial germination of "Hiratsuka No. 1", sensitive to thiophanate-methyl, was only slightly suppressed at concentrations below 100 ppm, but no germination occurred at 1,000 ppm. In the presence of benomyl,

Fig. 1. Comparison of mycelial growth of tolerant (T) and sensitive (S) isolates of *V. nashicola* on potato dextrose agar with thiophanate-methyl. Three weeks after inoculating with 4 mm diameter mycelial disk.

Fig. 2. Effect of thiophanate-methyl on conidial germination of *V. nashicola* isolates.

■ No. 2-2, tolerant isolate.

□ Hiratsuka No. 1, sensitive isolate.

Fig. 3. Effect of thiophanate-methyl on germ tube length of *V. nashicola* isolates.

■ No. 2-2, tolerant isolate.

□ Hiratsuka No. 1, sensitive isolate.

similar differences in germination percentage were observed (Data not shown).

Germ tubes of "No. 2-2" developed at each thiophanate-methyl treatment including 1,000 ppm (Fig. 3), but germ tube growth of "Hiratsuka No. 1" was noticeably inhibited by thiophanate-methyl and not elongated more than 20 μ m, even at a concentration as low as 1 ppm. Similar results were obtained with benomyl treatments (Data not shown). These results demonstrate that the germ tube length rather than the germination percentage can be used to differentiate tolerant from sensitive isolates.

Table 1. Effect of thiophanate-methyl and benomyl on infectivity of *V. nashicola* isolates to Japanese pear leaves

Isolate	Scab development (%) ^{a)}		
	Thiophanate-methyl ^{b)}	Benomyl ^{b)}	Control
No. 2-2	33	0	91
a - 13	100	75	91
No. 1-1	0	0	80
Hiratsuka No. 1	0	0	100

a) $\frac{\text{Numbers of portions disease developed}}{\text{Numbers of portions inoculated}} \times 100.$

b) 100 ppm of active ingredient.

Effect on infectivity

Percentage of scab development is given in Table 1. Isolates "No. 2-2" and "a-13" which were shown to be tolerant to thiophanate-methyl and benomyl, based on mycelial growth and conidial germ tube elongation, infected fungicide-treated leaves and developed sporulating lesions. However, isolates "No. 1-1" and "Hiratsuka No. 1", sensitive to both fungicides *in vitro*, did not exhibit infectivity on fungicide-treated leaves.

Discussion

As suggested by Nishimura *et al.*⁷⁾, field tolerance of a pathogenic fungus to a specific fungicide should be demonstrated by following conditions similar to Koch's postulates: (1) demonstration of the decrease in control efficacy of the fungicide in the field, (2) isolation of the pathogen from continuously developing lesions despite treatments with the fungicide in question, (3) laboratory tests for fungicide tolerance of the isolates and reproduction of the tolerance on host plants. We feel that our experiments were able to satisfy these requirements. It seems apparent that there were isolates of *V. nashicola* tolerant to thiophanate-methyl and benomyl and that these played an important role in the failure of scab control with these fungicides.

Methyl benzimidazol-2-yl carbamate (MBC), the conversion product of thiophanate-methyl and benomyl, is generally accepted to be the actual fungitoxic compound of these fungicides⁴⁾. Consequently, the tolerant isolate was supposed to show the tolerance to MBC. In actuality, *V. nashicola*, tolerant to both fungicides, also exhibited the tolerance to MBC.

Thiophanate and benzimidazole fungicides are believed to inhibit mycelial growth rather than spore germination⁴⁾. Shabi and Ben-Yephet¹⁰⁾ reported that spores of *V. pirina* sensitive to MBC germinated on the fungicide-supplemented medium but did not develop further. In our experiments also, mycelial growth of sensitive isolates of *V. nashicola* were completely suppressed at concentration below 1 ppm of fungicides, but spore germination was not sharply inhibited.

In a protection test (Table 1), benomyl was more effective than thiophanate-methyl. This result may be explained by the slower conversion of thiophanate-methyl into MBC⁴⁾.

V. nashicola, tolerant to thiophanate-methyl and benomyl, did not show cross-tolerance to the following fungicides in mycelial growth. The tested fungicides

were *N*-(1, 1, 2, 2-tetrachloroethylthio)-4-cyclohexene-1, 2-dicarboximide (captafol), *N*-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide (captan), zinc ethylenebis (dithiocarbamate) (zineb), manganese ethylenebis (dithiocarbamate) (maneb), dizinc bis (dimethyldithiocarbamate)-ethylenebis (dithiocarbamate) (polycarbamate), 3,3'-ethylenebis (tetrahydro-4,6-dimethyl-2*H*-1,3,5-thiadiazine-2-thione) (milneb), and copper 8-quinolinolate (oxine-copper).

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和 文 摘 要

ナシ黒星病菌 *Venturia nashicola* のチオファネートメチル剤およびベノミル剤に対する耐性

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昭和50年(1975年)に、富山、千葉、三重および鳥取の各県において、ナシ黒星病に対してチオファネートメチル剤およびベノミル剤の防除効果が低下する現象がみられた。そこで、これらのナシ園から分離した菌株について、両薬剤に対する耐性の有無を検定した。

In vitro の菌糸生育試験の結果、両薬剤に耐性を示す菌株が多数検出された。胞子発芽試験の結果では、発芽管長は耐性菌株と感性菌株の間に差が認められたが、発芽率では差は認められなかった。さらに耐性菌株を、薬剤を処理したナシ生葉に接種して、薬剤防除効果の低下現象を再現することができた。

以上のことから、チオファネートメチル剤およびベノミル剤の効力低下が観察された地域では、両薬剤に耐性のナシ黒星病菌が圃場に分布し、防除効果の低下に重要な役割を果たしたものと推定される。