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TESTING NEW FUNGICIDES TO REDUCE APPLE SCAB

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Abstract

The reduction of mycelium growth of scab *Venturia inaequalis* strains on malt agar amended with pyrimethanil, a pyrimidinamine, was reduced on a lower level (ED_{50} 0.28 - 33.8 µg a.i./ml) compared with other side specific compounds such as Demethylation inhibitor fungicides. Good disease control was obtained in apple seedling tests with 2 strains which showed low and high sensitivity *in vitro*. The results are discussed in relation to the test method and the mode of action of pyrimethanil.

The fungicidal activity of the new Demethylation inhibitor fungicide fluquinconazole against *V. inaequalis* strains (44) was compared with flusilazole and fenarimol in mycelium-growth tests. Positive cross resistance for all less sensitive strains was found. Fluquinconazole was the most active compound.

1. Introduction

Demethylation inhibitor (DMI) fungicides are represented by pyridine, pyrimidine, triazole and imidazole as essential groups and are considered to have moderate risk in the development of fungicide resistance (Koeller, 1994). Nevertheless, resistance to various DMI has been reported for many fungi causing important diseases like *Erisyphe graminis, Pseudocercosporella herpotrichoides, Uncinula necator* and *Venturia inaequalis* (Hermann et al., 1989; Leroux, 1991; Thind et al., 1986). A possibility to extend their efficacy is to better understand cross resistance, search more effective DMI fungicides and to alternate their application with fungicides having different modes of action. Only a few side specific compounds are permitted by the government to control apple scab in Integrated fruit production in Germany. This justifies research seeking new suitable compounds, such as pyrimethanil (pyrimidinamine) or fluquinconazole (DMI fungicide).

2. Material and methods

Seventytwo monoconidial strains of *V. inaequalis*, previously characterised by Parisi et al. (1994) were used for the experiments. All strains were conserved in tubes containing malt-agar at 4°C. The sensitivity to pyrimethanil, fluquinconazole, flusilazole, penconazole and pyrifenox was determined by measuring radial mycelium growth on unamended and amended malt agar with different fungicide concentrations. Colony diameters were measured in 2 perpendicular directions after 4 wks of growth in the dark at 17°C ± 1°C. The ED_{50} and ED_{90} values were

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calculated by regressing the relative growth (colony diameter on amended medium divided by th diameter on unamended medium * 100) against the log of the fungicide concentration.

The protective and curative action of pyrimethanil was tested on 3-wks-old apple seedling from a cross between scab susceptible cvs. „Golden Delicious" and „Granny Smith" treated witl 200 µg a.i./ml. The seedlings had two to three fully expanded leaves when inoculated. Th treatment was given 48 h before or after inoculation with a conidial suspension of 1-2*10 conidia/ml. Two batches of 73 plants were used for each variant.

3. Results

3.1 Sensitivity of *Venturia inaequalis* strains to pyrimethanil

The sensitivity to pyrimethanil was tested on 39 strains of *V. inaequalis*. The variability of ED_{50} was 0.29 - 33.8 µg a.i./ml and for ED_{90} 0.84 - 110 µg a.i./ml. In order to prove the results *in vivo* we chose a sensitive (302) and a less sensitive (636) strain for an apple seedling test. No visible lesions were observed on the leaves 17 days after inoculation (table 1).

3.2 *Venturia inaequalis* sensitivity to fluquinconazole, fenarimol and flusilazole

The ED_{50} and ED_{90} values obtained from *V. inaequalis* strains exposed to fluquinconazole, flusilazole and fenarimol are listed in table 2. The lowest ED_{50} value for the least sensitive strain was found for fluquinconazole (1.69 µg a.i./ml). Nine strains had ED_{50} values below 0.01 µg a.i./ml for fluquinconazole und only five for flusilazole. Positive cross resistance was found between all fungicides.

4. Discussion

Monoconidial Isolates of *V. inaequalis* were tested with pyrimethanil and showed a relatively high sensitivity ratio (ED_{50} of the most resistant strain devided by the ED_{50} of the most sensitive strain) of 116. This is consistent with the results of Birchmore et al. (1996), who tested the sensitivity of *Botrytis cinerea* isolates never exposed to pyrimethanil. The authors explain their data by the mode of action of the fungicide: the decrease of virulence755 after penetration into the host (Daniels & Lucas, 1995) and the ability to inhibit the secetion of enzymes involved in pathogenetic processes (Milling & Daniels, 1996). Milling and Richardson (1995) demonstrated with *B. cinerea* that the fungicide was more active on a media where the fungus needs extracellular enzyme activity. It is assumed that a nutrient rich medium does not give an objective estimation of the sensitivity of the *B. cinerea* strains to pyrimethanil. This study was carried out before the publication of this data on the mode of action of the fungicide; we tested the *V. inaequalis* strains on malt-agar medium. So, our data concerning the variability of ED_{50} and ED_{90} values may be related to the test procedure.

The excellent disease control obtained in the apple seedling tests underlines the hypothesis that mycelium growth determination on amended, nutrient rich malt agar is not convenient to estimate the real sensitivity of *V. inaequalis* to pyrimethanil.

The fungicidal activity of fluquinconazole on *V. inaequalis* strains was compared with flusilazole and fenarimole. We assume the new fungicide is the most active because we found nine strains having ED_{50}`s lower than 0.01 µg a.i./ml (only five strains for flusilazole) and because the highest ED_{50} value (1.69 µg a.i./ml) found for this fungicide was inferior to the corresponding value of flusilazole (5.27 µg a.i./ml).

Positive cross resistance was found between fluquinconazole and flusilazole and fenarimol. These findings are consistent with the results of Thind et al. (1986), indicating positive cross resistance for seven *V. inaequalis* strains tested with eight DMI fungicides. The practical consequence of our findings on cross resistance between fluquinconazole and the other fungicides may suggest that resistant strains to current DMI fungicides may be controlled better by the new compound. But no experimental field data is available to support this hypothesis. It should also be considered that fungicides are usually sprayed with different concentrations in the field.

5. References

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Table 1 - Sensitivity of the scab strains 302 and 636 to pyrimethanil after preventive or curative treatment of apple seedlings, 17 days after inoculation [% infected leaves]

strain	protective untreated	pyrimethanil	curative untreated	pyrimethanil
302	47.9	0	58.2	0
636	32.9	0	48.6	0

Table 2 - ED_{50} and ED_{90} values of *V. inaequalis* strains exposed to fluquinconazole, flusilazole and fenarimol

Strain	Fluquinconazole	Flusilazole	[a]Fenarimol
483	0.16	0,19	0.75
488	0.08	0.16	0.48
491	<0.01	0.03	0.14
495	0.05	0.1	0.09
496	0.12	0.18	0.51
498	1.52	5.27	0.18
501	0.06	0.14	0.19
503	0.46	0.75	2.25
504	0.53	0.35	1.69
518	0.31	0.52	1.6
524	0.15	0.33	0.56
531	0.3	0.67	0.99
533	0.37	0.81	2.48
552	1.46	1.58	5.23
559	0.8	0.76	1.51
598	0.04	0.02	0.24
736	0.01	<0.01	0.14
481	1.22	2.25	2.72
532	0.39	0.65	4.88
537	0.15	0.76	1.31
538	0.25	0.51	0.5
550	0.28	0.56	1.39
555	0.49	0.65	2.54
557	0.97	0.78	2.2
566	0.44	0.48	2.67
567	<0.01	<0.01	0.04
570	<0.01	0.04	0.27
581	<0.01	<0.01	0.25
740	<0.01	<0.01	0.19
755	<0.01	<0.01	0.12

Strain	Fluquincoazole	Flusilazole	[a]Fenarimol
769	<0.01	0.01	0.05
794	0.06	0.12	0.24
508	0.05	037	1.09
514	0.04	025	1.55

Strain	Fluquinconazole	Flusilazole	[a]Fenarimol
528	0.34	043	1.78
544	0.03	011	1.1
551	0.06	01	0.93
556	0.33	146	3.16
560	1.69	344	8.42
568	0.03	003	0.16
726	0.02	001	0.24
731	0.03	003	0.18
747	0.05	003	0.68
766	0.01	001	0.1

[a]Data from Parisi et al. (1993)