

Sensitivity to inhibitors of sterol biosynthesis in isolates of *Venturia inaequalis* from Italian and Dutch orchards

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Fungicides which inhibit the biosynthesis of sterols (SBIs) are increasingly used for control of apple scab, caused by *Venturia inaequalis*. It is known that intensive use of fungicides with specific action may lead to development of fungicide resistance and subsequent failure of disease control, as has been shown for benomyl and dodine. Some fungicides appeared to have a higher risk than others to encounter fungicide resistance; it has been suggested that SBIs should be classified as compounds with a relatively low risk (Dekker, 1982). Although in view of this a sudden development of resistance does not seem likely, the possibility remains that a gradual reduction of sensitivity will occur under continuous pressure by SBIs. In fact this has already been reported from Germany (Stanis and Jones, 1985) and France (Thind et al., 1986). This paper reports about the sensitivity of *V. inaequalis* isolates from SBI-treated orchards in Italy and the Netherlands.

Fungicides. The fungicides applied in the orchards were: Baycor 25% WP (a.i. biter-tanol), Rubigan 6% WP (a.i. fenarimol) and Topas 2.5% WP (a.i. penconazole). The fungicides used in the sensitivity tests in the laboratory were technically pure biter-tanol, fenarimol and penconazole, obtained from Bayer AG (Leverkusen, Fed. Rep. Germany), Eli Lilly (Research Centre Ltd, Surrey, England) and Ciba Geigy AG (Basle, Switzerland), respectively; solutions of these fungicides were made in 1% methanol.

Origin of isolates. Isolates of *V. inaequalis* were obtained from the following commercial or experimental orchards (N = Netherlands, I = Italy).

- NW-C Experimental orchard at Wageningen, the Netherlands, never treated with SBIs; cv. Golden Delicious.
- NG-Bay Experimental orchard at Geldermalsen, the Netherlands, which had received 14 sprays with Baycor 25 WP in 1985; cv. Goudreinette. Control still satisfactory in 1986.
- NE-Bay Commercial orchards at Eck and Wiel, the Netherlands, which had been sprayed with Baycor 25 WP in 1984 (6 ×), 1985 (6 ×) and 1986 (5 ×); cv. Golden Delicious. Control unsatisfactory in 1986.

- IB-C Experimental orchard at Bologna, Italy, never treated with SBIs; cv. Imperatore.
- IB-Bay Experimental orchard at Bologna, Italy, which had received 5-8 sprays per year with Baycor 25 WP in 1983, 1984, 1985 and 1986; cv. Stark Delicious. Control unsatisfactory in 1986.
- IB-Rub Experimental orchard at Bologna, Italy, which had received 5-8 sprays with Rubigan 6 WP in 1985 and 1986; cv. Imperatore. Control unsatisfactory in 1986.
- IR-Rub Experimental orchard at Fiesso Umbertiano, Italy, which had received 5 sprays with Rubigan 6 WP from 1979 to 1986; cv. Starking. Control unsatisfactory in 1986.
- IB-Top Experimental orchard at Bologna, Italy which had received 7-8 treatments in 1984, 1985 and 1986 with topas 2,5 WP; cv. Imperatore. Control unsatisfactory in 1986.

Sampling and isolation. Samples of 30-40 scab infected leaves were collected in June and July 1986, brought to the laboratory in plastic bags, and kept under refrigeration until they were processed as described by Gilpatrick (1982). Conidia were harvested from lesions by rinsing lesions with sterile water and the suspension was diluted to 1000 conidia per ml; 0.2 ml of the suspension was streaked on water agar in a Petri dish. After 1-3 days of incubation at 18 °C, pieces of agar, containing one germinated spore, were selected under a dissecting microscope, cut out and placed on PDA, containing 50 µg oxytetracycline ml⁻¹ to prevent bacterial growth. Colonies present after three weeks were used for the experiments. Initially 40 isolates were prepared from each orchard, of which 12 were finally used in the tests.

Testing of sensitivity. Agar discs, 3 mm diameter, were punched from the colonies and placed on agar containing per ml 0.03, 0.075, 0.10, 0.20, 0.40 or 0.60 γg/ml of the fungicides. Usually six discs were placed in one Petri dish; the experiments were carried out in triplicate. After three weeks of incubation at 20 °C, the diameter of the colonies was measured and expressed in percentage of the control. The ED₅₀ and ED₉₅ values were calculated after transformation to probits.

Results. The results are presented in Table 1. The ED₅₀ and ED₉₅ values of biter-tanol for *V. inaequalis* isolates from an experimental orchard in Italy, which had received 5-8 sprays with Baycor, yearly from 1983 to 1986 (IB-Bay), were 4-5 times higher than those of isolates from orchards not treated with SBI fungicides (IB-C). A significant increase of these values was also found for isolates from Topas- and Rubigan-treated orchards in Italy (IB-top and IF-Rub). No significant shift in sensitivity to biter-tanol was found in Baycor-treated orchards in the Netherlands. In the latter case unsatisfactory control could, therefore, not be attributed to development of fungicide resistance.

The detection of less sensitive strains of *V. inaequalis* in the Italian orchards underlines the warning by Stanis and Jones (1985), that potential problems in practice may arise from the intensive use of the C-14 demethylation group of sterol-inhibiting fungicides.

Table 1. Sensitivity to SBI fungicides of *V. inaequalis*, isolated from orchards in the Netherlands and Italy, treated with fungicides. C = control, untreated.

Isolates ¹	Bitertanol		Fenarimol		Penconazole	
	ED ₅₀	ED ₉₅	ED ₅₀	ED ₉₅	ED ₅₀	ED ₉₅
NW-C	0.039a ²	0.27a				
NG-Bay	0.041a	0.22a				
NF-Bay	0.048a	0.31a				
IB-C	0.035a	0.30a	0.033a	0.22a	0.022a	0.24a
IB-Bay	0.120b	1.63b				
IB-Rub			0.045a	0.34a		
IF-Rub			0.058b	0.60b		
IB-Top					0.106b	0.45b

¹ Explanation of codes: see under 'Origin of isolates'.

² Values with the same letter within one column are not significantly different at $p = 0.05$, based on Student's t-test.

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Samenvatting

Gevoeligheid voor sterol-biosyntheseremmers in isolaten van Venturia inaequalis uit boomgaarden in Italië en Nederland

Een geringe, maar significante reductie in gevoeligheid van *Venturia inaequalis* voor fungiciden die tot de sterol-biosyntheseremmers behoren werd vastgesteld bij isolaten uit proefboomgaarden in Italië, die gedurende enkele jaren met deze middelen behandeld waren. Bij isolaten uit enkele commerciële boomgaarden in Nederland werd nog geen significante verschuiving in gevoeligheid waargenomen.

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