

Fungicide resistance of smco mutants of *Neurospora crassa*.

M Grindle

W. Temple

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Abstract

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We are using *N. crassa* as a model organism to investigate the mode of action of agricultural fungicides and the genetic and biochemical bases for fungicide resistance. Certain aromatic hydrocarbon and dicarboximide fungicides ("AHD" fungicides) presumably have a similar mode of action because mutants are usually cross-resistant to them. For example, Vin and os mutants of *N. crassa* are resistant to the aromatic hydrocarbons dicloran, chloroneb and quintozone and the dicarboximides iprodione, procynidone and vinclozolin (Grindle and Temple, 1982 *Neurospora News*l. 29: 16-17). The fungicides are used on a wide range of crops, especially grapes, soft fruits and glasshouse plants, to combat pathogens such as species of *Botrytis*, *Rhizoctonia* and *Sclerotinia*. There is increasing concern that dicarboximide-resistant mutants of *Botrytis* *cinerea* might become a practical problem in agriculture and horticulture.

The sensitivity of Vin and os mutants to media of high osmolarity suggests that there are defects in the cell wall-plasma membrane complex of AHD resistant mutants. Many morphological mutants of *N. crassa* are believed to have abnormal cell walls or membranes, and the biochemical lesions and enzyme defects of some mutants have been determined (Scott 1976, *Ann. Rev. Microbiol.* 30: 85-104; Mshra 1977, *Adv. Genet.* 19: 341-405). Representative morphological mutants were analysed for resistance to AHD fungicides.

Each mutant was grown on dishes of Vogel's minimal medium (MM) and on MM containing 2-10 µg/ml vinclozolin to detect isolates that were more resistant than the wild type 74-OR8-1a. Colony diameters (mean of 2 measurements per colony) were noted after 18 hr and 24 hr growth, and the growth rate (mm/24 hr increase in diameter) was calculated from the growth during 6 hr. Growth rates on MM and on fungicide-supplemented MM were used to determine the amounts of fungicide that reduced colony diameters by 50% (ED₅₀) and by 95% (ED₉₅). The ED₅₀

TABLE I

Growth rate and fungicide resistance of smco mutants of *Neurospora crassa*

| locus. | Allele or isolation number | FGSC stock number | Rate of growth, mm/24 hr ^a | | | | Resistance to fungicides ^b | | | |
|-----------|-------------------------------------|-------------------------|---------------------------------------|-----|--------------------|--------------------|---------------------------------------|-------------|----------|------------|
| | | | MM | CM | MM + 2% NaCl | MM + 4% NaCl | iprodione | vinclozolin | dicloran | quintozone |
| smco-1 | Y2330 | 1363 | 58 | 12 | 14 | 12 | 0 | 0 | 0 | 0 |
| smco-2 | R2386 | 1377 | 24 | 10 | 0 | 0 | + | +++ | +++ | +++ |
| smco-4 | R2435 | 1367 | 10 | 12 | 12 | 12 | 0 | 0 | 0 | 0 |
| smco-5 | R2442 | 1361 | 50 | 60 | 58 | 58 | 0 | 0 | 0 | 0 |
| smco-6 | R2477 | 1353 | 50 | 14 | 34 | 22 | 0 | 0 | 0 | 0 |
| smco-8 | R2505 | 1404 | 38 | 8 | 5 | 0 | + | +++ | + | + |
| smco-9 | R2508 | 1405 | 56 | 12 | 18 | 12 | + | +++ | + | + |
| wild type | 74-OR8-1a | | 98 | 112 | 94 | 78 | 0 | 0 | 0 | 0 |

^aIncrease in colony diameter at 26°C, mean of at least 3 replicates; diameters measured 18 hr and 24 hr after inoculation, and growth rate calculated from the growth during 6 hr. CM = MM + 0.5% casamino acids + 0.5% yeast extract.

^b0 = sensitive (ED₅₀ < 3 µg fungicide/ml); + = low resistance (ED₅₀ > 3 < 10 µg/ml); +++ = high resistance (ED₅₀ > 50 µg/ml; ED₉₅ > 100 µg/ml). ED values are concentrations of fungicide that reduce growth on MM by 50% or 95%.

values for 74-OR8-1a were 2.8 $\mu\text{g/ml}$ dicloran and 1.3 $\mu\text{g/ml}$ vinclozolin and ED_{95} values were 7.4 $\mu\text{g/ml}$ dicloran and 3.3 $\mu\text{g/ml}$ vinclozolin. The snco mutants were analysed for growth on MM CM (i.e. MM + 0.5% casamino acids + 0.5% yeast extract), MM plus NaCl and MM plus 2-100 $\mu\text{g/ml}$ AHD fungicides to compare them with os mutants (Note: the ED_{50} and ED_{95} values for os mutants given in Neurospora Newsl. 29 were determined from growth on sorbose medium MS, i.e. NM + (1.5% sorbose + 0.2% sucrose; ED values are lower on MS than on MM).

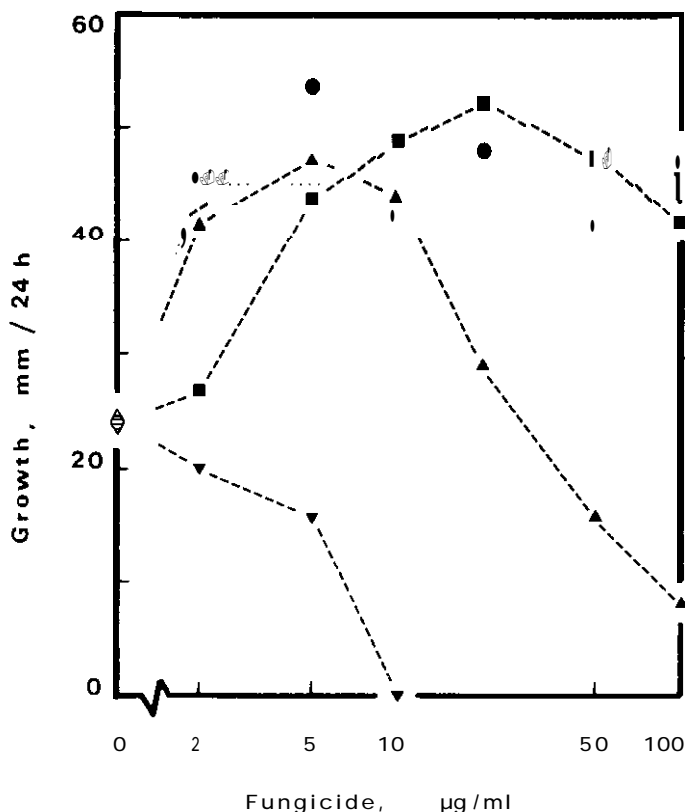


Figure 1. -- Effect of fungicides on growth of *N. crassa* mutant snco-2. Each point is the mean growth rate (increase in colony diameter at 26°C) of at least 3 replicates on MM containing dicloran ▲; iprodione ▼; vinclozolin ■; or quintozone ●

(see Mishra 1977) and fungicide resistance: cr-2 (defective adenyl cyclase), csp-1 (defective cell wall autolysing enzyme), col-2 and fr (defective glucose-6-phosphate dehydrogenase), cot-3 (defective 6-phosphogluconate dehydrogenase) and rg-1 (defective phosphoglucomutase) are not resistant to AHD fungicides. The primary biochemical lesions in AHD-resistant mutants and the mode of action of AHD fungicides have yet to be elucidated.

The following mutants were sensitive as sensitive as the wild type to dicloran and vinclozolin and were not analysed further: locus cl (isolation number CL11); col-2 (Y5331); col-3 (Y5296); cot-2 (R1006t); cr-1 (B123); cr-2 (R2445); csp-1 (UCLA37); do (DS5-51); fr (B110); ain (637/3.4); gran (B42); le-1 (c-MB); pat (no number); rg-1 (B53, 8187 and R2357); ro-1 (B4); ro-6 (R2431); ro-10 (AR7); sh (R2371); sn (C136); spco-5 (R2450); spco-9 (R2480); sp (1405); ti (8233).

Three snco mutants resembled some os mutants in their resistance to fungicides and sensitivity to CM and MM containing NaCl (Table I). The snco-2 mutant often grew better, and its morphology became more normal, on fungicide-supplemented MM (Figure 1). Some of our Vin mutants grow better on low levels of fungicide (i.e. about 1-3 $\mu\text{g/ml}$) than on MM but they differ from snco-2 in morphology and growth rate on MM

We showed previously (Neurospora Newsl. 29) that resistance to AHD fungicides can result from mutations in four genes: os-1, os-4, os-5 on L.G. I and os-2 on L.G. IV. This report implicates three additional genes in AHD-resistance: snco-2 on L.G. III and snco-8 and snco-9 on L.G. IV (snco-8 and snco-9 may be closely linked to os-2 see Perkins et al. 1982. Microbial. Rev. 46: 426, 470). Resistance of some mutants to the fungicides may be related to changes in cell wall composition: os-1 mutants have abnormal amounts of hexosamines and enlarged pores in their cell walls (Trevithick and Metzenberg 1966, J. Bacteriol. 92: 1016-1020); snco-8 and snco-9 have abnormal amounts of cell wall peptides (Wrathall and Tatum 1974. Biochem Genet. 12: 59-78) and snco-9 might be defective in glucan biosynthesis (Abramsky and Tatum 1976, Biochim Biophys. Acta 421: 106-114). However, most of the mutants with abnormal amounts of cell wall peptides (cot-2; cr-2; do; fr; gran; le-1; ro-1; spco-5; sp; ti see Wrathall and Tatum 1974) and a mutant with abnormal hexosamine content (do see Edson and Brody 1976, J. Bacteriol. 126: 799-805) are not resistant to fungicides. There is no correlation between known enzyme defects in morphological mutants

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--- Department of Genetics, The University, Sheffield S10 2TN, England.