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Lack of cross resistance to benomyl and thiabendazole in some strains of Aspergillus nidulans

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Development of resistance in fungal plant pathogens to benzimidazole derivatives, as has been reported frequently in recent years, endangers the future of these compounds for plant disease control. Laboratory studies of u.v.-induced resistance in fungi to these compounds may provide a better understanding of this phenomenon. Using the non-pathogen *Aspergillus nidulans* (perfect state *Emericella nidulans*) as a test organism, Hastie and Georgopoulos (1971) found two genes for benomyl resistance, once conferring relatively high resistance, located on chromosome VIII (ben A-1 locus). and the other low resistance, located on chromosome II (ben B-2 locus). All strains showed cross resistance to benomyl and thiabendazole. This paper reports about resistance on the ben A-1 locus and the rare occurrence of benomyl resistant strains which are sensitive to thiabendazole, and thiabendazole resistant strains which are even more sensitive to benomyl than wild type strains.

Strains of *Aspergillus nidulans* with markers on different chromosomes were obtained from Dr. A. J. Clutterbuck, Institute of Genetics, University of Glasgow, U.K. The genetic methods were those of Pontecorvo et al. (1953). Resistant mutants were selected by plating u.v. irradiated conidia on malt agar medium containing benomyl or thiabendazole. From 14.10^8 conidia of which 6% survived after u.v. irradiation, 211 colonies were obtained on a medium containing 3 ppm benomyl, of which 37 showed sporulation. Of these nine highly resistant strains were selected for further study. The ED_{50} of these strains varied from 7–20 ppm, but most of them continued to grow even at 100 ppm of benomyl, while growth of the wild type strains is completely inhibited at 1 ppm benomyl. Cross resistance between benomyl and thiabendazole appeared to be the rule. One of the benomyl resistant strains, surprisingly, did not show cross resistance to thiabendazole. A second group of 100 benomyl resistant isolates, obtained in the same way as described above, did, however, not include colonies without cross resistance to thiabendazole. Benomyl resistance without concomitant thiabendazole resistance thus does not seem to occur frequently. The reverse, thiabendazole resistance without benomyl resistance, was encountered in several isolates. Out of 203 thiabendazole resistant colonies developing from u.v.-irradiated conidia on agar containing 25 ppm thiabendazole, 8 isolates appeared even more sensitive to benomyl than the wild types strain. Characteristic examples of three types of mutants are shown in Fig. 1. For most isolates, however, a close correlation exists between the degree of resistance to benomyl and to thiabendazole as was shown in an experiment with 50 mutants, isolated from thiabendazole containing medium (Fig. 2).

Fig. 1. Cross resistance and lack of cross resistance in strains of *Aspergillus nidulans*, isolated from medium containing benomyl (A5, A8, A13) or thiabendazole (186, 186y, R); w = wild type strain. Colonies replicated on medium containing increasing concentrations of benomyl or thiabendazole (ppm).

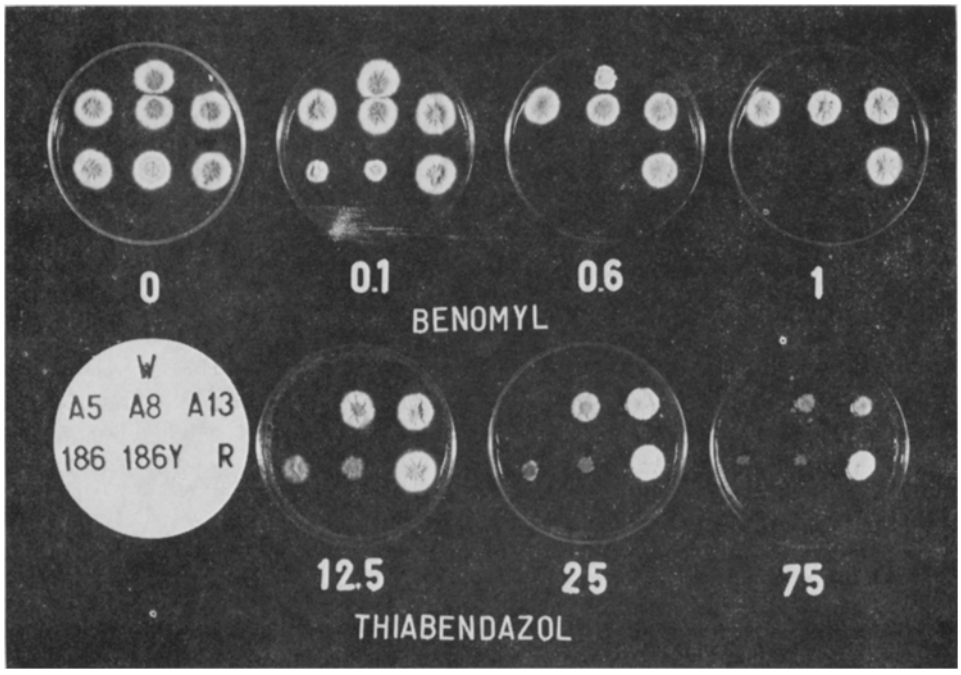

Fig. 1. Kruisresistentie en ontbreken van kruisresistentie in stammen van Aspergillus nidulans, geïsoleerd van een medium dat benomyl (A5, A8, A13) of thiabendazol (186, 186y, R) bevat; w = wild type, gevoelig. Replica's van kolonies op medium dat toenemende concentraties benomyl en thiabendazol bevat (ppm).

The location of the gene for benomyl resistance was investigated by mitotic analysis using the master strain technique of McCully and Forbes (1965). Heterozygous diploids were made of benomyl resistant mutants and either master strain 001 and 002, which together carry markers on all eight chromosomes. By examining the independent haploid segregants, obtained by plating conidia of the diploid on benomyl containing medium (Hastie, 1970), it appeared that the marker ribo B-2 (riboflavin requirement) on chromosome VIII had not segregated in the resistant haploids. Subsequent crossing of resistant isolates with strains possessing ten known markers on chromosome VIII showed, that this gene for benomyl resistance is located 34 units from ts-D 15, marking a specific temperature sensitivity, and 5 units from orn-B 7, marking ornithine deficiency.

Crossing of benomyl- and thiabendazole resistant strains, independently of the medium from which they were isolated, always gave 100% resistant progeny. A crossing of a benomyl resistant strain with one of the strains isolated from the thiabendazole containing medium and showing extra sensitivity to benomyl, yielded a thiabendazole resistant progeny, of which about 50% was resistant to benomyl and 50% extra sensitive to benomyl. This indicates that all mutants arose due to mutation in

Fig. 2. Correlation between thiabendazole (25 ppm) resistance and benomyl (4 ppm) resistance in strains of *Aspergillus nidulans*, isolated from a medium containing 25 ppm thiabendazole; some strains lack cross resistance to benomyl.

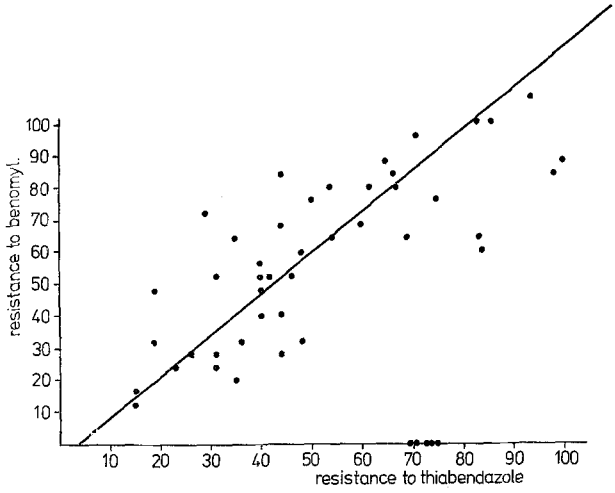

Fig. 2. Correlatie tussen thiabendazol (25 ppm) resistentie en benomyl (4 ppm) resistentie in stammen van Aspergillus nidulans, die geïsoleerd zijn van een medium dat 25 ppm thiabendazol bevat; enkele stammen vertonen geen kruisresistentie met benomyl.

one and the same gene. Usually this results in resistance to both benomyl and thiabendazole, but apparently a mutation in this gene may in rare cases also lead to a) resistance to benomyl but not to thiabendazole and b) resistance to thiabendazole concomitant with increased sensitivity to benomyl. The biochemical aspects of this phenomenon will be published elsewhere by the second author.

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Samenvatting

Ontbreken van kruisresistentie tegen benomyl en thiabendazol in enkele stammen van Aspergillus nidulans

Verworven resistentie in *Aspergillus nidulans* tegen benomyl en thiabendazol bleek te berusten op één gen, gelocaliseerd op chromosoom VIII, op een afstand van 34 eenheden van ts-D 15 en 5 eenheden van orn-B 7. Hoewel kruisresistentie de regel is, blijkt dat sommige mutanten tegen slechts één van beide fungiciden resistent zijn, en dat resistentie tegen thiabendazol zelfs gepaard kan gaan met extra gevoeligheid voor benomyl (Fig. 1 en 2).

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