Neth. J. Pl. Path. 81(1975) 122–123 SHORT COMMUNICATION

Acquired resistance to carboxin in Aspergillus nidulans

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Accepted 12 December 1974

Development of resistance to carboxin (5,6-dihydro-2-methyl-1,4-oxathiine-3-carboxanil anilide) has been reported for several fungi (Georgopoulos et al., 1972; Ben-Yephet et al., 1974). Although this fungicide is predominantly active against fungi belonging to the Basidiomycetes, also some other fungi appeared sensitive, i.a. *Aspergillus nidulans* (Eidam) Wint. perfect state *Emericella nidulans* (Eidam) Wint. Aspects of acquired resistance of this fungus to carboxin were studied.

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 UV irradiated conidia between two layers of malt agar containing 100 ppm carboxin. Resistant mutants appeared with a frequency of one in 5.10^6 conidia, which had survived UV irradiation. The ED^{50} values of ten strains, which were selected for further experiments, varied from 130-195 ppm, in comparison with 9 ppm for sensitive strains. From crossings of resistant with sensitive strains it appeared that in all cases resistance was due to mutation in one gene. Diploid analysis showed that four strains had a gene for resistance on linkage group VII (Car A), five on linkage group VIII (car B) and one on linkage group III (car C). Car A appeared dominant, car B and car C semidominant. The location of car B was determined by crossing a resistant strain carrying this gene with a strain, carrying two markers on linkage group VIII: cnx B-11 (requirement for ammonium) and fpa D-43 (resistance to fluorophenyl alanine). Car B appeared located 7 units from cnx B-11 and 6 units from fpa D-43. In a similar experiment it was shown that car C was located at a distance of 18 units from phen A-2 (phenylalanine requirement).

Ragsdale and Sisler (1970) showed in experiments with *Ustilago maydis*, *Neurospora crassa* and *Saccharomyces pastorianus* that carboxin is ten times as fungitoxic on an acetate substrate as on a glucose substrate. A similar phenomenon was observed for the sensitive strains of *Aspergillus nidulans*: on the former medium carboxin was seven times as toxic as on the latter one. Surprisingly, however, seven highly resistant strains showed a ratio of 0.8–1.4 instead of 7, and three moderately resistant strains a ratio of 2.4–3.5. The highly resistant strains, therefore, do not show a significant difference in sensitivity to carboxin on the two differemt media.

Mathre (1971) and White (1971) revealed that carboxin inhibits mitochondrial respiration of *U. maydis* at or close to the site of action of succinate oxidase. Because acetate is in contrary to glucose directly used in the tricarboxylic acid cycle, the toxicity of carboxin on an acetate medium is much higher than on a glucose medium.

These observations of carboxin resistant strains support the view that carboxin acts primarily on reactions in the tricarboxylic acid cycle.

Acknowledgment

The author is indebted to Prof. Dr J. Dekker for his continuous interest and for help in preparing the manuscript.

Samenvatting

Carboxin-resistentie in Aspergillus nidulans

In tien, na UV-bestraling verkregen carboxin resistente stammen werden drie resistentiegenen Car A, car B en car C, respectievelijk gelegen op de koppelingsgroepen VII, VIII en III, aangetoond. Car B werd gelocaliseerd op koppelingsgroep VIII op een afstand van 7 eenheden van cnx B-11 en 6 eenheden van fpa D-43.

In tegenstelling tot gevoelige stammen, die op een acetaatmedium veel sterker geremd worden dan op een glucosemedium, tonen resistente stammen geen verschil in gevoeligheid op deze media. Daar acetaat in tegenstelling tot glucose rechtstreeks in de citroenzuurcyclus gebruikt wordt, ondersteunt deze waarneming de theorie van Mathre (1971) en White (1971) dat carboxin primair aangrijpt op het succinaatdehydrogenase.

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