Neth. J. Pl. Path. 82 (1976) 85–87 SHORT COMMUNICATION

The likelihood of development of resistance to systemic fungicides which inhibit ergosterol biosynthesis

A. FUCHS[1] and C. A. DRANDAREVSKI[2]

[1] Laboratory of Phytopathology, Agricultural University, Wageningen, the Netherlands
[2] Celamerck GmbH & Co., KG, Ingelheim, Germany

Accepted 11 December 1975

Recently, various systemic fungicides, viz. triarimol and triforine (Sisler and co-workers, see Ref.), S-1358 (Kato et al., 1975) and triadimefon (Buchenauer, 1975), have been shown to interfere with ergosterol biosynthesis. Of these, triforine in particular has been studied; it acts against several powdery mildew and rust diseases, and a number of foliar diseases caused by non-obligate pathogens (cf. Fuchs and Drandarevski, 1973).

In some triforine-sensitive non-obligate plant pathogens transient resistance to triforine was readily obtained by successive transfer in the presence of increasing concentrations of the fungicide. However, tolerance was lost again upon subculturing in the absence of triforine (Fuchs and Viets-Verweij, 1975).

Triarimol-resistant mutants of several fungi were either isolated from colonies which developed in the presence of high concentrations of triarimol (*Cladosporium cucumerinum*, Sherald et al., 1973; *Aspergillus fumigatus*, Sherald and Sisler, 1975) or were produced by UV irradiation and selection in the presence of either triarimol (*Ustilago maydis*, Sherald et al., 1973; *C. cucumerinum*, *Verticillium albo-atrum*, Fuchs and Viets-Verweij, 1975; *Aspergillus nidulans*, De Waard and Sisler, 1976) or triforine (*C. cucumerinum*, Fuchs and Viets-Verweij, 1975). In all instances this form of resistance persisted through numerous transfers in the absence of the toxicants, and was, therefore, called permanent resistance (Fuchs and Viets-Verweij, 1975). All mutants of *C. cucumerinum* tested showed cross-resistance to triforine and S-1358 (Sherald et al., 1973; Sherald and Sisler, 1975), although the levels of resistance did not parallel each other in all strains (cf. Fuchs and Viets-Verweij, 1975). Mutants of *A. fumigatus* and *A. nidulans* were also resistant to triforine and fenarimol, respectively, the latter being a newly developed fungicide related to triarimol (see De Waard and Sisler, 1976).

As has been shown by Sherald and Sisler (1975) triarimol markedly inhibited sporulation in both wild type and triarimol/triforine-resistant strains of *A. fumigatus*, at concentrations which were not inhibitory to mycelial growth; similar observations were made for *A. nidulans* by De Waard and Sisler (1976). Triforine, however, did not inhibit sporulation in these strains. According to Sherald and Sisler (1975) the ergosterol content of a triarimol/triforine-resistant mutant of *A. fumigatus* was not severely affected by triarimol, while that of the wild type was distinctly reduced. Therefore, at first glance lack of sporulation does not seem to be correlated with abnormal or decreased ergosterol biosynthesis; unfortunately, effects on sterol

synthesis were studied at a concentration (5 μg/ml), at which growth and sporulation of both wild type and mutant strains were affected in the same way.

Some mutant strains of *A. nidulans*, isolated by De Waard and Sisler (1976), are characterized by a highly reduced ability to produce spores. On routine subculturing of various mutant *C. cucumerinum* strains we observed, that, despite profuse sporulation, new growth of many of them was either moderate or severely retarded; of the ca 125 strains isolated in this way 9 even succumbed entirely. Since in each case numerous spores must have been transferred, many or even all of them were apparently inviable. In many fungi, ergosterol appears to play a very important role in the sporulation process (cf. Weete, 1974). Therefore, reduced ability of mutants to produce spores, or at least viable spores, might indicate that ergosterol biosynthesis is adversely affected. If in the fungal species mentioned ergosterol is necessary for the production of viable spores, mutations affecting the ergosterol biosynthetic pathway would hamper formation of viable spores or at least retard spore maturation. The observation that ergosterol entirely annulled this retardation in a UV-irradiated triarimol/triforine-resistant strain of *C. cucumerinum* (OWTa 4; cf. Fuchs and Viets-Verweij, 1975) seems to confirm this assumption.

The results of the above-mentioned in vitro experiments are confirmed by several observations we made in in vivo experiments. Spore formation was considerably reduced after treatment of scab mycelium on apple leaves with triforine; moreover, inhibition of sporulation involved loss of germination capacity of the few conidia formed. After treatment of apple powdery mildew sporulation was also inhibited (Drandarevski and Schicke, 1975). Attempts to obtain triforine-tolerant bean rust by successive transfers of uredospores to bean plants treated with sublethal concentrations of triforine did not yield a positive result: invariably, there was a decline in infection and spore production rate and after a few passages no more uredospores were produced. Apparently, triforine seriously affected sporulation. From pathogenicity tests at identical spore densities it was concluded, that *C. cucumerinum* strains with a high level of triarimol/triforine resistance were usually less virulent than the parent strain (Fuchs and Viets-Verweij, 1975). It should be realized, however, that not only diminished virulence as such but also a low percentage of viable spores could have been responsible for the observed low degree of infection.

Spore germination is rather often considered to be insensitive to both triarimol and triforine, although contrary results have frequently been obtained (cf. Fuchs et al., 1971). In many sensitive fungi, mycelial growth is effectively inhibited. The above observations suggest that also interference with spore formation, maturation and viability might play an important role in the activity of systemic fungicides which inhibit ergosterol biosynthesis. If ergosterol is really vital for fungal reproduction, any interference with its synthesis should seriously affect the fungus' chances of survival by reducing its inoculum. Resistance to a systemic fungicide which inhibits ergosterol synthesis might have the same epidemiological effect: it might greatly reduce the fungus' possibility to survive.

In conclusion, the available evidence seems to make development of resistance to fungicides which inhibit ergosterol synthesis under practical conditions rather unlikely. In fact, unlike many other systemic fungicides (cf. Dekker, 1976), the practical application of, for instance, triforine has not so far led to any confirmed case of resistance.

Note added in proof

After our manuscript had been submitted for publication, an article by J. M. T. Hamilton-Miller, entitled 'Fungal sterols and the mode of action of the polyene antibiotics' (Adv. appl. Microbiol. 17 (1974): 109–134), came to our attention. It stated that 'from a clinical point of view, the problem of polyene resistance (in yeasts and fungi) is nonexistent'.

Samenvatting

De waarschijnlijkheid van resistentie-ontwikkeling tegen ergosterol-biosynthese-remmende systemische fungiciden

Op grond van literatuurgegevens en nog niet gepubliceerde eigen experimentele resultaten wordt de veronderstelling geuit, dat resistentie-ontwikkeling tegen ergosterol-biosyntheseremmers in schimmels onder praktijkomstandigheden onwaarschijnlijk is.

References

Buchenauer, H., 1975. Systemisch-fungizide Wirkung und Wirkungsmechanismus von Triadimefon (MEB 6447). Mitt. biol. Bundesanst. Ld- u. Forstw., Berlin-Dahlem 165: 154–155.

Dekker, J., 1976. Resistance. In: Marsh, R. W. (Ed.), Systemic fungicides (2nd Edition), Chapter 8. Longman, London.

Drandarevski, C. A. & Schicke, P., 1975. Spore formation and germination of apple scab and powdery mildew following curative treatment with triforine. Rep. Inf. VIII Int. Plant Prot. Congress Moscow, Sect. III Chemical Control 211–213.

Fuchs, A., Doma, S. & Vörös, J., 1971. Laboratory and greenhouse evaluation of a new systemic fungicide, N,N′-bis-(1-formamido-2,2,2-trichloroethyl)-piperazine (CELA W 524). Neth. J. Pl. Path. 77: 42–54.

Fuchs, A. & Drandarevski, C. A., 1973. Wirkungsbreite und Wirkungsgrad von Triforine in vitro und in vivo. Z. PflKrh. PflSch. 80: 403–417.

Fuchs, A. & Viets-Verweij, M., 1975. Permanent and transient resistance to triarimol and triforine in some phytopathogenic fungi. Meded. Fak. Landb.wetensch. Gent 40: 699–706.

Kato, T., Tanaka, S., Ueda, M. & Kawase, Y., 1975. Inhibition of sterol biosynthesis in *Monilinia fructigena* by the fungicide, S-1358. Agr. biol. Chem. 39: 169–174.

Ragsdale, N. N., 1975. Specific effects of triarimol on sterol biosynthesis in *Ustilago maydis*. Biochim. Biophys. Acta 380: 81–96.

Ragsdale, N. N. & Sisler, H. D., 1972. Inhibition of ergosterol synthesis in *Ustilago maydis* by the fungicide triarimol. Biochem. Biophys. Res. Comm. 46: 2048–2053.

Ragsdale, N. N. & Sisler, H. D., 1973. Mode of action of trairimol in *Ustilago maydis*. Pestic. Biochem. Physiol. 3: 20–29.

Sherald, J. L., Ragsdale, N. N. & Sisler, H. D., 1973. Similarities between the systemic fungicides triforine and triarimol. Pestic. Sci. 4: 719–727.

Sherald, J. L. & Sisler, H. D., 1975. Antifungal mode of action of triforine. Pestic. Biochem. Physiol. 5: 477–488.

Waard, M. A. de & Sisler, H. D., 1976. Resistance to fenarimol in *Aspergillus nidulans*. Meded. Fak. Landb. wetensch. Gent 41: in press.

Weete, J. D., 1974. Fungal lipid biochemistry. Monographs in lipid research, Vol. 1. Plenum Press, New York, London.

Addresses

A. Fuchs: Laboratorium voor Fytopathologie, Landbouwhogeschool, Binnenhaven 9, Wageningen, the Netherlands.

C. A. Drandarevski: Celamerck GmbH & Co., KG, Ingelheim, Germany.