NEW INSIGHTS ON THE MODE OF ACTION OF THIOPHANATE-METHYL

Tim HIRSCHFELD, Frank ELLNER, Herbert BUSCHHAUS, Monika GOßMANN

NIPPON SODA CO.,LTD.

Julius Kühn-Institut
Federal Research Centre for Cultivated Plants

INTRODUCTION

Thiophanate-methyl (TM) belongs to the benzimidazole fungicides and has been approved for the indication Fusarium head blight in wheat and triticale since 2009. The primary effect of TM is caused by the transformation product methyl-benzimiazole-2yl-carbamate (MBC), which is a β-tubulin inhibitor and disturbs the cell division. In field trials, TM reduced the deoxynivalenol (DON) contents in wheat kernels more efficiently than Fusarium infestation. TM reduced the biosynthesis of mycotoxins by about 80-95% in mycotoxin-producing *Fusarium* spp. *in vitro* although the fungal growth hardly decreased. Respiration of *Fusarium* spp. being subject to TM and MBC was also reduced without a comparable inhibition of growth. Therefore, we investigated the effect of TM and MBC on the activity of the respiration enzyme cytochrome c oxidase (COX).

MATERIAL and METHODS

Isolates of *F. culmorum* and *F. verticillioides* were cultivated on Slight-Nutrient-Agar from permanent culture in soil. Pieces of the SNA were used for the incubation of the liquid Bilay`s medium. The fungi incubated for another 3-5 days before the mycelium was filtered and a mitochondrial preparation made. After that we measured the activity of the COX in the mitochondrial preparations spectrophotometrically. The assay is based on the light absorption at 550 nm of cytochrome c (cyt c), which change depending on the oxidation state. Reoxidation of a reduced cyt c due to COX in the sample leads to a decrease in light absorption, the slope of which reveals the activity of the enzyme. We investigated the effect of TM and MBC at levels of 10mg/l and 1mg/l, respectively, on the activity of the COX subject to incubation times of 0, 5 and 10 minutes.

RESULTS and DISCUSSION

The activity of the COX of *F. culmorum* decreased under the influence of TM by about 40% for all incubation times referring to the untreated controls (Fig. A, Tab. 1). MBC inhibited the activity of the enzyme by about 23-33%, increasing with longer incubation times. TM reduced the COX activity of *F. verticillioides* by about 44-59%, depending on the incubation time (Fig. B, Tab. 2). As for incubation times of 0 and 5 minutes, the COX activity increased by about 12-15% while it was limited by about 18% after 10 minutes of incubation under the influence of MBC.

Fig. A

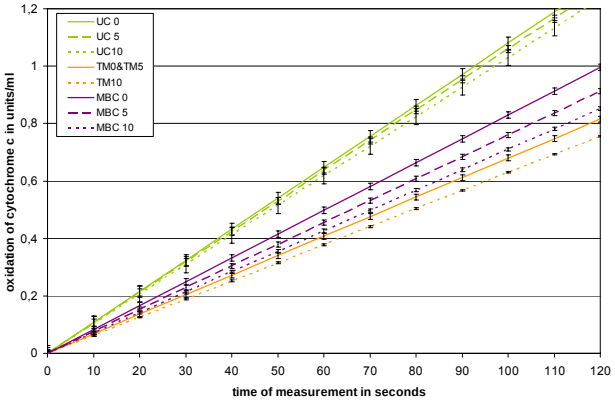

Fig. B

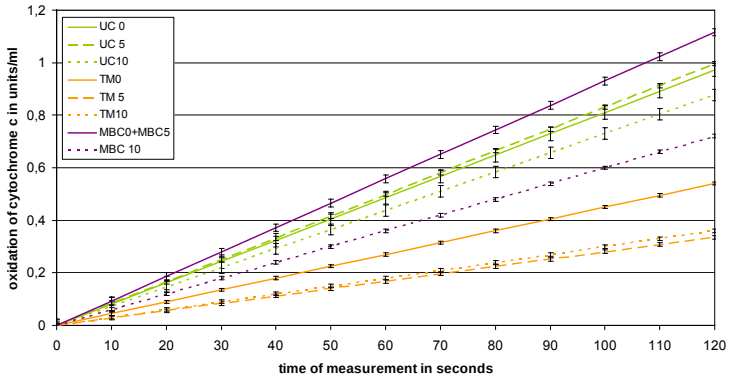

Fig. A&B: Activity of the Cytochrome c oxidase in a mitochondrial preparation of *F. culmorum* (Fig. A) and *F. verticillioides* (Fig.B) under the influence of TM(10µg/ml) and MBC (1µg/ml)

Tab. 1: Cytochrome c oxidase activity of *F. culmorum* subject to TM (10µg/ml) and MBC (1µg/ml) at certain incubation times

Incubation time (min)	UC (units/ml)	TM (%)	MBC (%)
0	0.108	-37	-23
5	0.106	-36	-28
10	0.103	-39	-33

Tab. 2: Cytochrome c oxidase activity of *F. verticillioides* subject to TM (10µg/ml) and MBC(1µg/ml) at certain incubation times

Incubation time (min)	UC (units/ml)	TM (%)	MBC (%)
0	0.081	-44	+15
5	0.083	-46	+12
10	0.073	-59	-18

CONCLUSION

Present data suggest that there may be an additional mode of action of TM in Fusarium spp. directly affecting the energy supply due to an inhibition of the respiration, which will result among others in a reduced mycotoxin formation.

Institute for Ecochemistry, Plant Analysis and Stored Product Protection
Königin-Luise-Str. 19, D-14195 Berlin-Dahlem, Germany

www.jki.bund.de