Effects of the fungicide Tebuconazole with herbicidal mode of action on monocot and dicot macrophyte species

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Background

Since 2010, it is known that dicot macrophytes, such as *Myriophyllum spicatum*, can significantly be more sensitive to herbicides with special mode of actions (MoA) such as growth regulators (GR) than the standard monocot test species *Lemna* sp.. For fungicides such as tebuconazole (TBZ) with indications for a MoA as a GR (Rogach et al., 2022), we hypothesized that dicot macrophytes are more sensitive than monocots. Therefore, we performed 14 d *in-situ* macrophyte tests within a comprehensive mesocosm study at the artificial stream and pond facility of the German Environment Agency. The goal of this research was to improve the risk assessment (RA) of fungicides with herbicidal MoA for better aquatic ecosystem/macrophyte protection.

Methods

Table 1: Mesocosm set-up

Mesocosms with standing stock of macrophytes	8 naturally-like established freshwater stream-lake mesocosms (Fig. 1 A, B) planted with *Nymphoides peltata* (dicot), *Myriophyllum spicatum* (dicot) and *Potamogenton natans* (monocot) in May 2022
Nominal test concentrations	2 controls, 1 x 5, 50, 100, 1000, and 5000 µg/L TBZ (as formulation Folicur®, Bayer AG), one application at 6.9.2022 (day 0)
System conditions	Oligo- to mesotrophic nutrient level; temperature range: 15-20°C
Light conditions	6 4-channel LED luminaires (Neptun®, Roschwege) per pond (150 µmol/m2/s) at the water surface. Light duration monthly adapted to autumn conditions

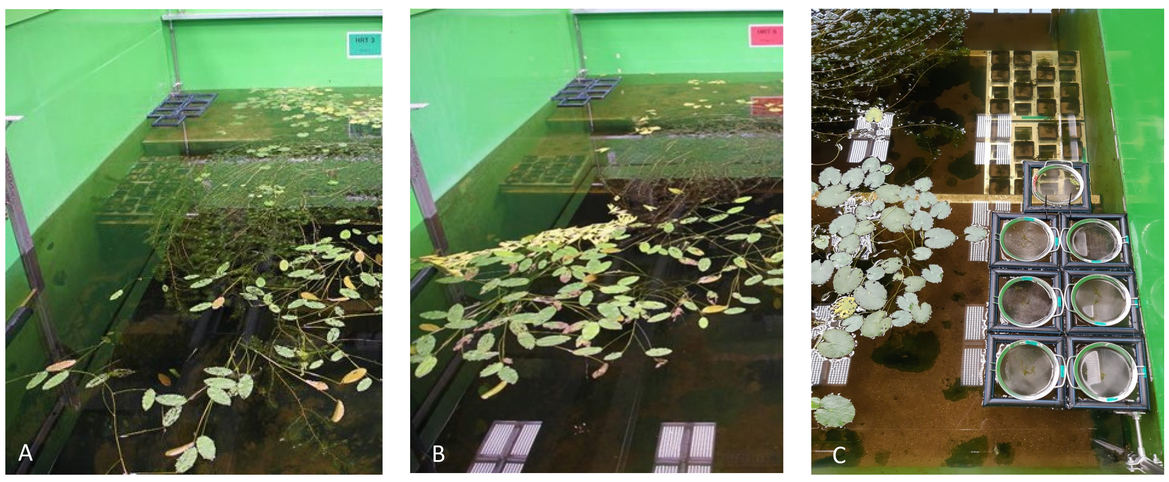

Figure 1 A-C: Control and 5000 µg/L TBZ mesocosm with *in-situ* tests (top left A,B) and naturally grown macrophyte stock (*Potamogeton natans* in front, *Myriophyllum spicatum* in middle, submerged, *Nymphoides peltata in* back) after 21 days of exposure. On the right-hand side *in-situ* tests with *Myriophyllum spicatum* (potted plants, back) and *Lemna trisulca* and *Spirodela polyriza* in floating device (front).

Table 2: Experimental set-up

Species	*In-situ* tests / standing stock of macrophytes	Endpoints	Duration (days)
Myriophyllum spicatum	10 10-cm shoots in 10 plant pots with sand/natural sediment mixture fixed in stainless steel racks (Fig. 1 C)	main shoot length (MSL), total shoot length (TSL), number of whirls (NL), dry weight (DW), relative growth rates (RGR)	14, 35
Lemna trisulca	40 leaves of ca. similar size per replicate (6 replicates per mesocosm, Fig. 1 C)	number of leaves, DW	14
Spirodela polyrhiza	3-5 colonies (1 replicate per mesocosm) in floating baskets;	number of leaves, DW	14
Nymphoides peltata	standing stock in mesocosms (Fig. A, B)	number of floating leaves	35
Potamogeton natans	standing stock in mesocosms (Fig. A, B)	number of floating leaves	35

Results

Table 3: EC_{10} and EC_{50} values for different macrophytes species (confidence interval CI). EC_x plus CI within (marked in green) and out of (marked in orange) tested concentrations

Macrophyte	Test duration	Endpoint	EC_{10} (CI) (µg/L)	EC_{50} (CI) (µg/L)
***M. spicatum*, dicot**	14 d	TSL RGR	71 (29-114)	333 (248-418)
M. spicatum	14 d	TSL	31 (-2-64)	412 (248-576)
M. spicatum	14 d	MSL	73 (31-116)	320 (240-401)
M. spicatum	14 d	NL	124 (63-186)	711 (574-847)
M. spicatum	35 d	TSL RGR	89 (55-123)	512 (436-588)
M. spicatum	35 d	TSL	50 (27-73)	340 (270-411)
M. spicatum	35 d	MSL RGR	75 (47-102)	402 (342-463)
M. spicatum	35 d	NL RGR	200 (125-275)	778 (653-801)
N. peltata, dicot	35 d	NL	29 (-36-94)	424 (6-842)
P. natans, monocot	35 d	NL	>5000	>5000
S. polyrhiza, monocot	14 d	NL RGR	410 (-371-1191)	1660 (-140-3459)
L. trisulca, monocot	14 d	NL RGR	140 (-56-336)	9598 (-8616-27813)

- TBZ is very stable (Fig. 2).
- In this study, *M. spicatum* showed the lowest, trustworthy EC_{10} and EC_{50} values based on measured TBZ start concentrations, of which TSL was the most sensitive endpoint. The dicot *N. peltata* also showed clear effects in the same concentration range (Table 3).
- The monocots *S. polyrhiza* and *L. trisulca* were less sensitive than the tested dicot species (Table 3). However, in first-tier lab test *Lemna gibba* is still most sensitive with EC_{50} (7d, RGR) = 144 µg/L (EFSA, 2014) in comparison with *M. spicatum* in this study.

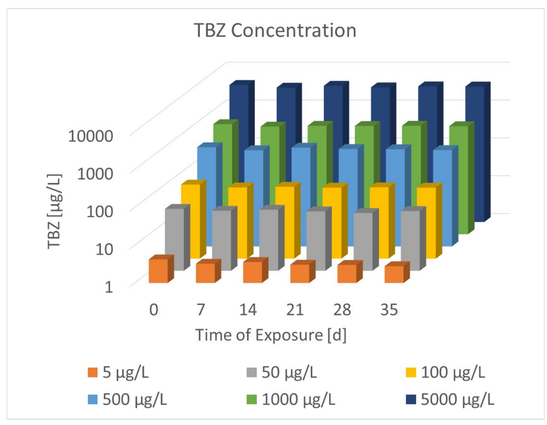

Figure 2: TBZ concentrations in water of the treated mesocosms from day 0 to 35 (measured start concentration: 4.2, 46.3, 93.2, 418.2, 856.4, and 4038.7 µg/L).

Figure 3: Effects of TBZ on growth forms *of M. spicatum* 134 days after TBZ exposure in mesocosms (left: new tips in control; right: new tips in 1000 µg/L treatment)

Conclusions

- In the mesocosm study, dicots were most sensitive. This is consistent with the MoA of TBZ and could be an indication that laboratory tests with *Myriophyllum spicatum* may also be more sensitive than those with *Lemna* sp..
- Currently, a first-tier test with *Myriophyllum spicatum* is missing in RA but should have been performed given the MoA of TBZ.
- EC_x values for monocot species were indicative due to low RGRs as a result of the low nutrient status of the mesocosms and the autumn season.

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