

Testing the emergent macrophyte, *Glyceria maxima*, in a water-sediment system: progress towards development of an OECD Test Guideline

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Background

Regulation in the EU requires data for a sediment - rooted macrophyte species for herbicidal compounds if: 1. terrestrial plant data indicate a high selectivity for monocot or dicot species; 2. standard Lemna and algae test species are not sensitive to the mode of action (e.g. EC50 > 1 mg ai/L); 3. partitioning to sediment is a concern. A test with *Myriophyllum spicatum* has been developed (OECD TG 239). However, a test with a rooted, emergent and monocot macrophyte is missing.

Objective

The 1st ring test with the emergent reed sweet grass (*Glyceria maxima*) demonstrated that the test system is able to detect toxic effects; propagation from rhizomes rather than seed is preferable; a test duration of 14 days is sufficient; increased standardization of test conditions is needed to reduce variability and increase growth; total leaf length was more reliable than total shoot height. The performance of the test system was further investigated in a 2nd ring test with the herbicide imazapyr between August and October 2018, including an extra root endpoint.

Results

Generation of young shoots

Young shoots are needed to do a proper testing. Stock populations were installed in greenhouses and outside in mesocosms by laboratories.



Figure 1. Young *Glyceria* shoots are obtained by pruning.

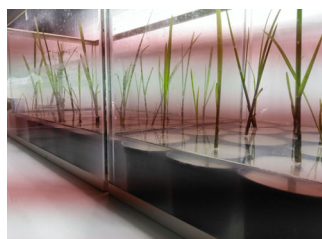


Figure 2. Test set-up was changed to one shoot per pot.

Table 1. Experimental conditions

Table 1: Ring-Test Protocol for <i>Glyceria maxima</i>	
Test compound	Imazapyr
Test system	Plant pots / beakers in glass test vessels; 1 shoot/pot
Plant propagation	Young shoots from shoot propagation and pruning
Sediment	Artificial sediment supplemented with nutrients (OECD TG 239)
Media	Smart and Barko
Test design	6 control replicates and 4 replicates of 6 concentrations
Test conditions	23 ± 2°C; temp was increased to improve growth rates
Test duration	14 days
Biological assessments	Total leaf length (TLL), shoot fresh weight (FW), shoot dry weight (DW), root fresh weight (RFW) and root dry weight (RDW)

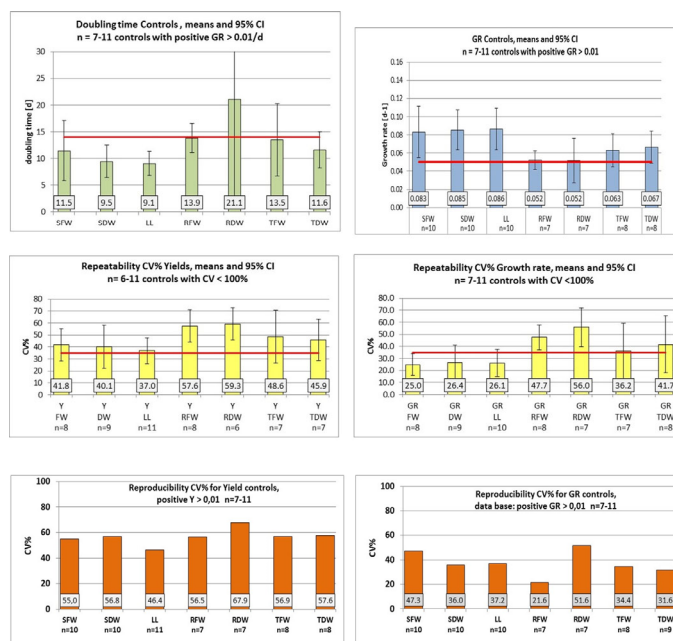


Figure 3. Results of *Glyceria maxima* testing in controls (red line represent the validity criteria, i.e. maximum CV of 35 %; maximum doubling time of 14 days; minimum growth rate).

Conclusions

- Roots are not adequate endpoints:
 - Doubling of endpoints in controls was not achieved for root endpoints;
 - Growth rate in controls was too low for root endpoints;
 - CVs highest for root endpoints (MDDs in most cases > 75 %);
- Compared to ring test 1 (Isoproturon), reproducibility was worse in ring test 2 (Imazapyr) while repeatability was usually maintained or slightly improved. Possible reasons: no specification of initial height of test plants. As a result, the mean initial heights of test plants varied between 6.4 cm and 32.5 cm; probably also establishment phase of one day was too short;
- Shoot weight and leaf length are the most suitable variables for assessing toxicity to *Glyceria maxima*;
- Next steps: standardization of start populations for the test; therefore a workshop is foreseen in 2020.

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