



A PROPOSED RING-TEST PROTOCOL FOR THE EMERGENT MACROPHYTE, *GLYCERIA MAXIMA*, IN A WATER-SEDIMENT SYSTEM

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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. $EC_{50} > 1$ 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.

Objectives

The current ring-test with the emergent reed sweet grass (*Glyceria maxima*) is designed to establish the following test parameters:

1. Test duration: a.o. time needed to achieve doubling in control biomass;
2. Characterization of variability e.g. for measurement endpoints (Coefficient of Variation $CV < 35\%$);
3. Identification of appropriate validity criteria.

Approach

OECD TG 239 has been adapted for *Glyceria maxima* (reed sweet grass) and the modified protocol is being ring-tested in several laboratories in the EU and the US. This poster presents preliminary results of the 1st ring-test using isoproturon as a test compound.

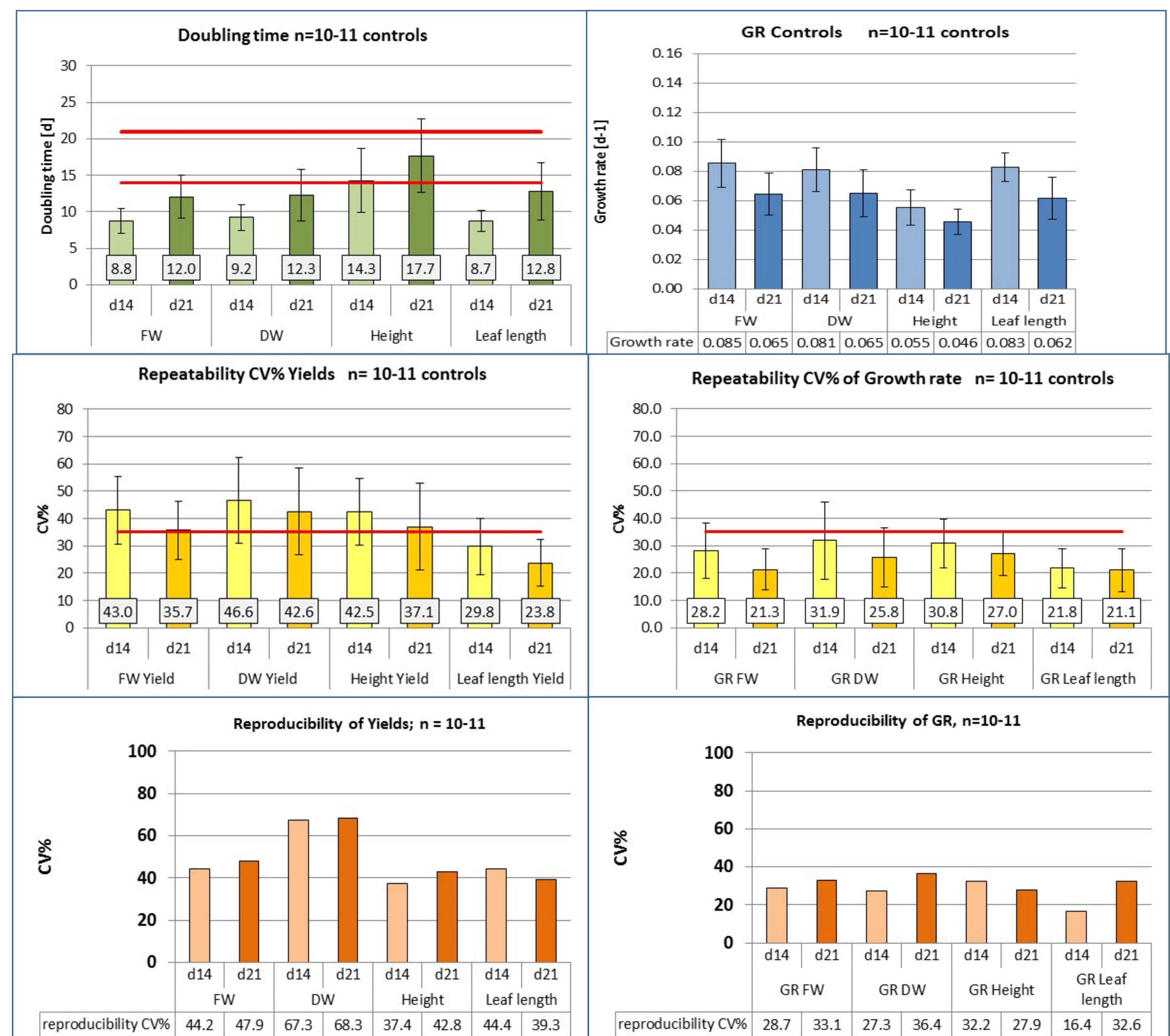
Method



Table 1: Ring-Test Protocol for *Glyceria maxima*

Test compound	Isoproturon technical
Test system	Plant pots / beakers in glass test vessels;
Plant propagation	Individual shoots with rhizome sections are cut from stock plants and transplanted into fresh sediment to produce test plants (see Figure)
Sediment	Artificial sediment supplemented with nutrients as described in OECD TG 239
Media	Smart and Barko
Application	Isoproturon was dissolved in Smart and Barko media and added to the water column
Test design	<ul style="list-style-type: none">• Untreated control with 6 replicate test vessels• Five test concentrations each with 4 replicate test vessels• Each replicate test vessel contains 1 plant pot of 1 shoot at test initiation
Test conditions	22 ± 2°C with 16 hour day-length of 180 (± 20) $\mu E\ m^{-2}\ s^{-1}$
Test duration	21-day exposure phase with biological assessments at 14 and 21 days
Biological assessments	Shoot number, shoot height (SH), total leaf length (TLL), shoot fresh weight (FW) and shoot dry weight (DW)
Environmental assessments	pH and DO recorded on Days 0, 7, 14 and 21 Water temperature measured daily
Endpoints	Yield and growth rate EC_{50} values based on FW, DW, SH and TLL

Preliminary results: growth in controls



Conclusions

- Doubling of endpoints in controls was achieved both in day 14- and in day 21-tests, except for height;
- CV% of yields are larger compared to CV% of growth rates; Yields: 24%-47%; Growth rate 21%-32%;
- CV% at day 14 are larger compared to day 21;
- Total Leaf Length (TLL) shows the lowest CV%;
- For yield, only TLL has a CV below 35% (both at d14 and at d21);
- For growth rate, most endpoints have a CV below 35% (both at d14 and at d21);
- Growth rate at day 21 is lower compared to growth rate at day 14, which indicates growth limitation after 14 days;
- No clear relationship between test duration and reproducibility;
- Reproducibility growth rate 16%-36%: OK;
- Reproducibility yield 37% - 67%: yield shows higher variability probably due to varying initial conditions, e.g. different numbers of initial shoots per pot;

Next steps

- Further analysis of variation in sensitivity of *Glyceria maxima* to the test compound;
- 2nd ringtest with Imazapyr in 2018;
- Submission of draft protocol to OECD.

