Testing the emergent macrophyte, *Glyceria maxima,* in a water-sediment system
Results of a ring-test with Isoproturon

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Introduction

- Regulation in the EU requires data for a rooted macrophyte species for some herbicidal compounds. Specifically, the EFSA Guidance Document on tiered risk assessment for aquatic organisms indicates that data for a rooted macrophyte species may be required where:
  - terrestrial plant data indicates a high selectivity for monocot or dicot species.
  - standard *Lemna* and algae test species are not sensitive to the mode of action (e.g. EC_{50} > 1 mg ai/L).
  - partitioning to sediment is a concern.
- The dicot species, *Myriophyllum spicatum*, and the monocot species, *Glyceria maxima* have been identified as alternative test species in light of prior experience and known sensitivity to some chemistries.
- OECD Test Guideline 239 for *Myriophyllum* in a water-sediment test system has been adapted for *Glyceria maxima* (reed sweet grass) and the modified protocol has been ring-tested using the protocol in Table 1 in several laboratories in the EU and the US.

Objectives of Isoproturon Ring-Test

The ring-test was designed to establish the following test parameters:

1. Test duration, i.e. the duration of time required to achieve a doubling in control biomass
2. Test design, i.e. replication required to achieve acceptable control coefficients of variation

Thirteen laboratories participated in the ring test, generating between 10 to 11 control datasets and 5 to 8 datasets for estimation of effects of isoproturon at 14 and 21 days.

Fig 1: Project Schedule

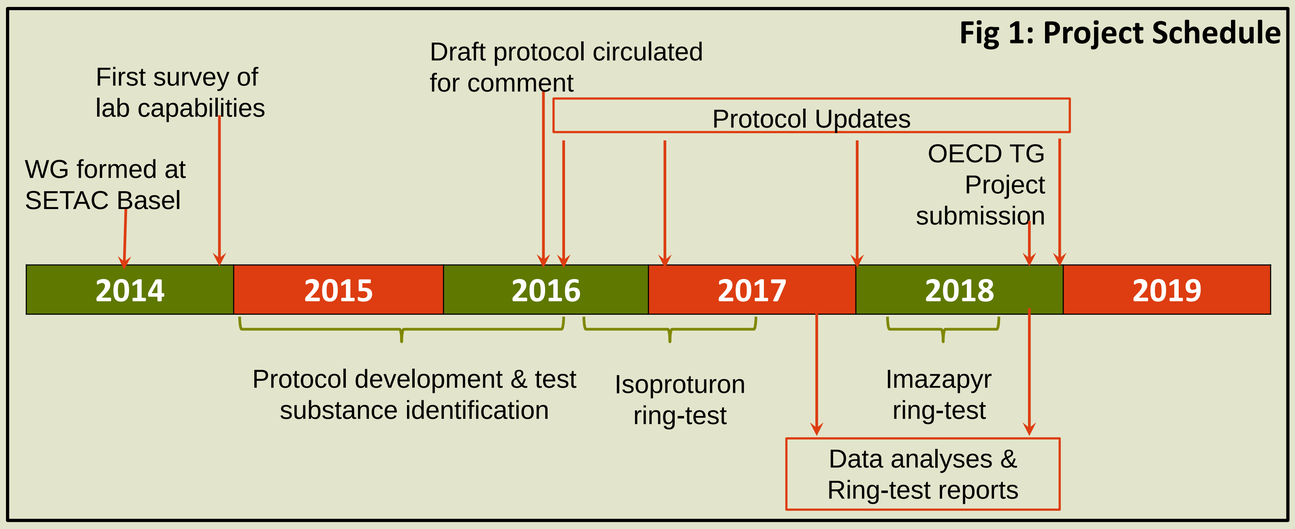


Table 1: Ring-Test Protocol for Isoproturon Technical Material

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 (Figure 2)
Sediment / Media	Artificial sediment supplemented with nutrients as described in OECD TG 239, overlaid with Smart & Barko
Application	Isoproturon was dissolved in Smart & Barko media & added to the water column
Test design	• 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) µE 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 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

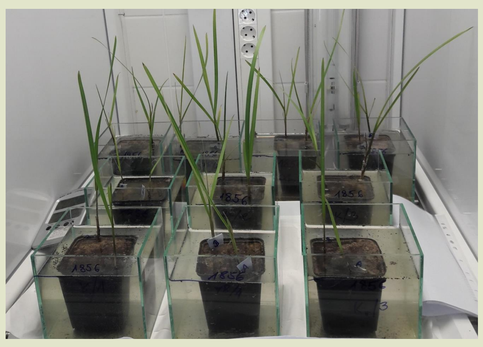


Fig 2: Propagation of test plants

Key Results

Growth of control plants (Figure 3)

- Control plants achieved >2-fold increase in FW, DW and TLL within the minimum 14-day test duration.
- Doubling time for all growth parameters increased with increasing test duration due to a slower growth rate between days 14 and 21. This trend may be caused by nutrient limitations.

Variability in control plants (Figure 3)

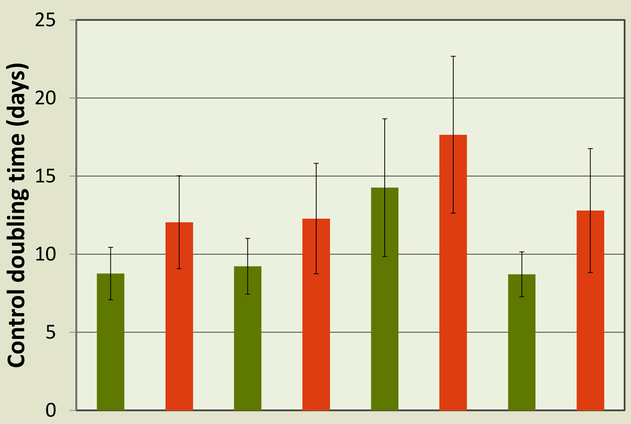
- Yield CoVs are higher than growth rate CoVs.
- For growth rate, CoVs for most endpoints were <35% while for yield only TLL has a CoV of <35%.
- Day 14 repeatability CoVs are typically higher than Day 21 values while a clear relationship was not observed between test duration and reproducibility.
- High CoVs are typically correlated with larger plant size and high variability at test initiation.

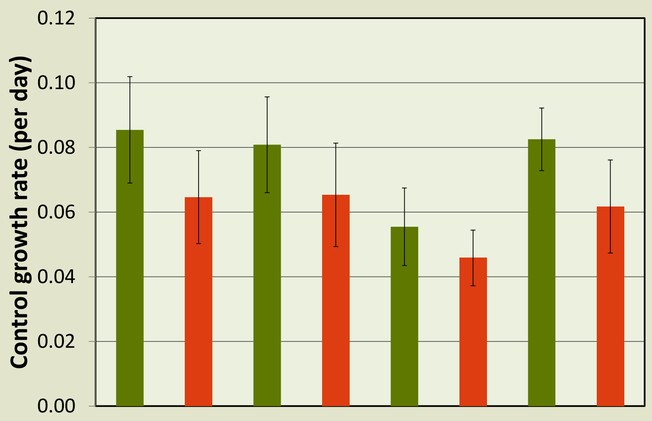
Sensitivity to Isoproturon (Figure 4)

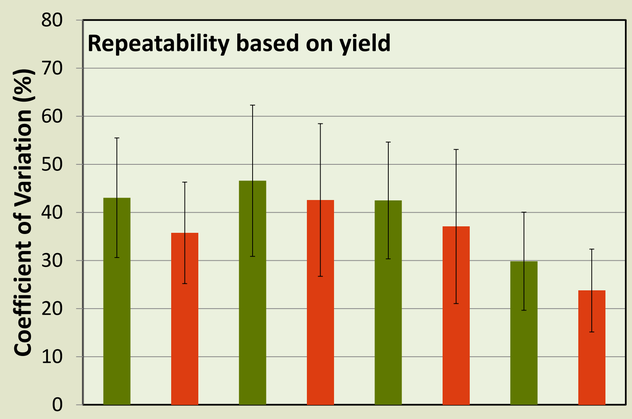
- Mean EC_{50} values based on different growth variables were not significantly different.
- Yield EC_{50}s tended to be lower than growth rate EC_{50}s.

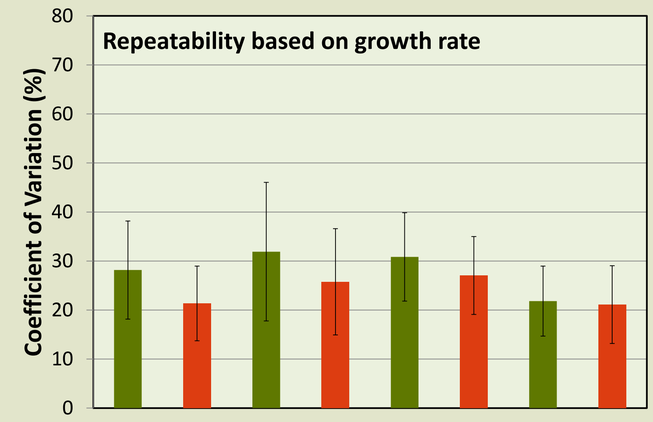
Key points to be revised in protocol

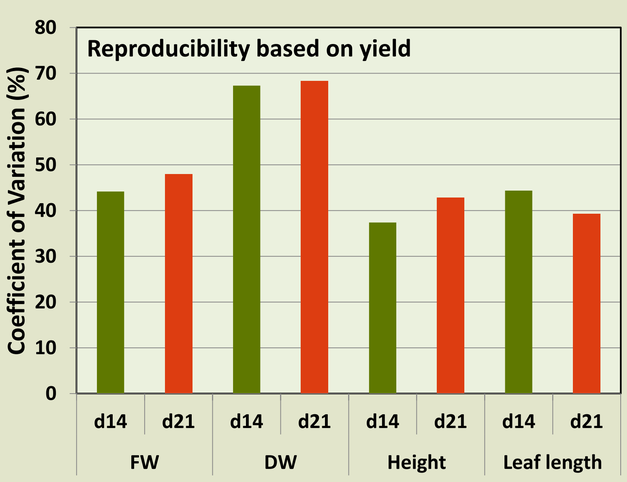
- More stringent recommendations will be made regarding the size of plants at test initiation.
- Environmental conditions may be modified to promote growth rates.
- Maximum / minimum water-sediment ratios will be defined.
- Test duration will be set according to doubling time in controls and is likely to be 14 days.
- Validity criteria are likely to set as follows (to be confirmed):
  - Maximum variability of 35% for growth rates and for yield leaf length
  - Minimum growth rates of 0.070 d^{-1} for fresh weight and leaf length











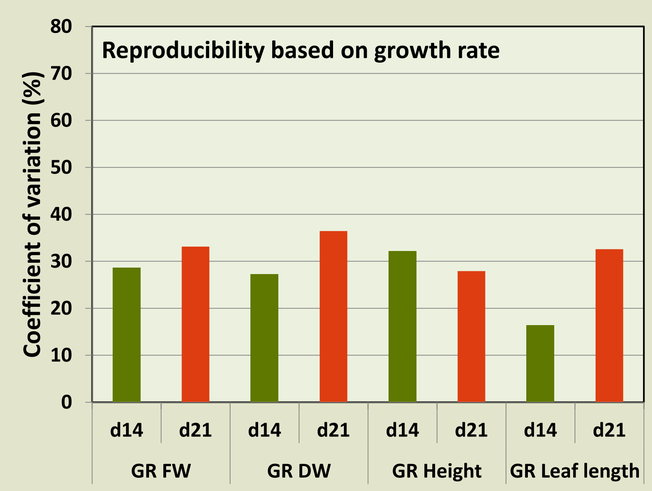
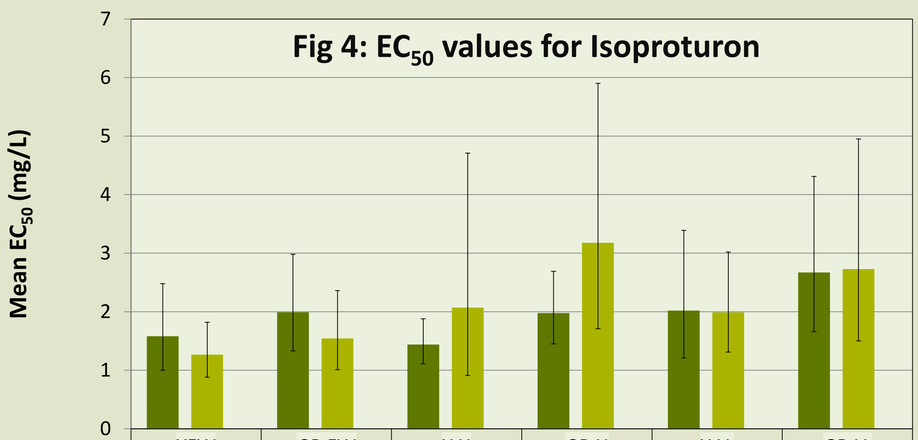


Fig 3: Doubling time, growth rate, repeatability (within lab) CoVs and reproducibility (between lab) CoVs in control plants based on yields and growth rates; n=10-11.



	YFW	GR FW	Y H	GR H	Y LL	GR LL
EC50 d14	1.58	1.99	1.44	1.98	2.02	2.67
n d14	6	6	5	5	5	5
EC50 d21	1.27	1.54	2.07	3.18	1.99	2.73
n d21	8	8	6	7	7	7

Acknowledgements

- Ring-test participants include BASF SE (DE), Bayer AG (DE), BioChem Agrar GmbH (DE), ECT Oekotoxikologie GmbH (DE), Eurofins (DE), FERA (UK), Ibacon GmbH (DE), Fraunhofer IME (DE), Mesocosm GmbH (DE), Rheinland Pfalz AgroScience GmbH (DE), Smithers Viscient (US), Toxi-Coop (HU), Wageningen Environmental Research (NL).
- Test items are supplied by Bayer AG (isoproturon) and BASF (imazapyr).

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