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

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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 for substances 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.
- A Test Guideline for *Myriophyllum* in a water-sediment test system was approved by OECD (OECD Test Guideline 239, April 2014).
- OECD TG 239 has been adapted for *Glyceria maxima* (reed sweet grass) and the modified protocol is now being ring-tested in several laboratories in the EU and the US.
  - Ring-test with isoproturon – Autumn 2016 to Spring 2017
  - OECD Test Guideline project submission – Autumn 2017
  - Ring-test with imazapyr – Autumn 2017 to Spring 2018 (to be confirmed)

Primary Objectives of Isoproturon Ring-Test

The current ring-test is designed to establish the following test parameters:

1. Propagation method, i.e. seedlings versus vegetative-propagated rhizome sections with shoots
2. Test duration, i.e. the duration of time required to achieve a doubling in control biomass
3. Test design, i.e. replication required to achieve acceptable control coefficients of variation of <35%

Thirteen labs have confirmed their intention to test isoproturon and, to date, 5 labs have provided either data for isoproturon or control data from other studies, which are suitable for defining control parameters.

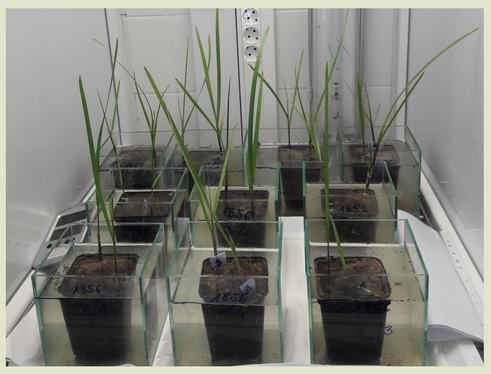

Fig 1: Propagation of test plants from glasshouse-grown stock cultures

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 (Figure 1)
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	• 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), leaf length (LL), 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 LL

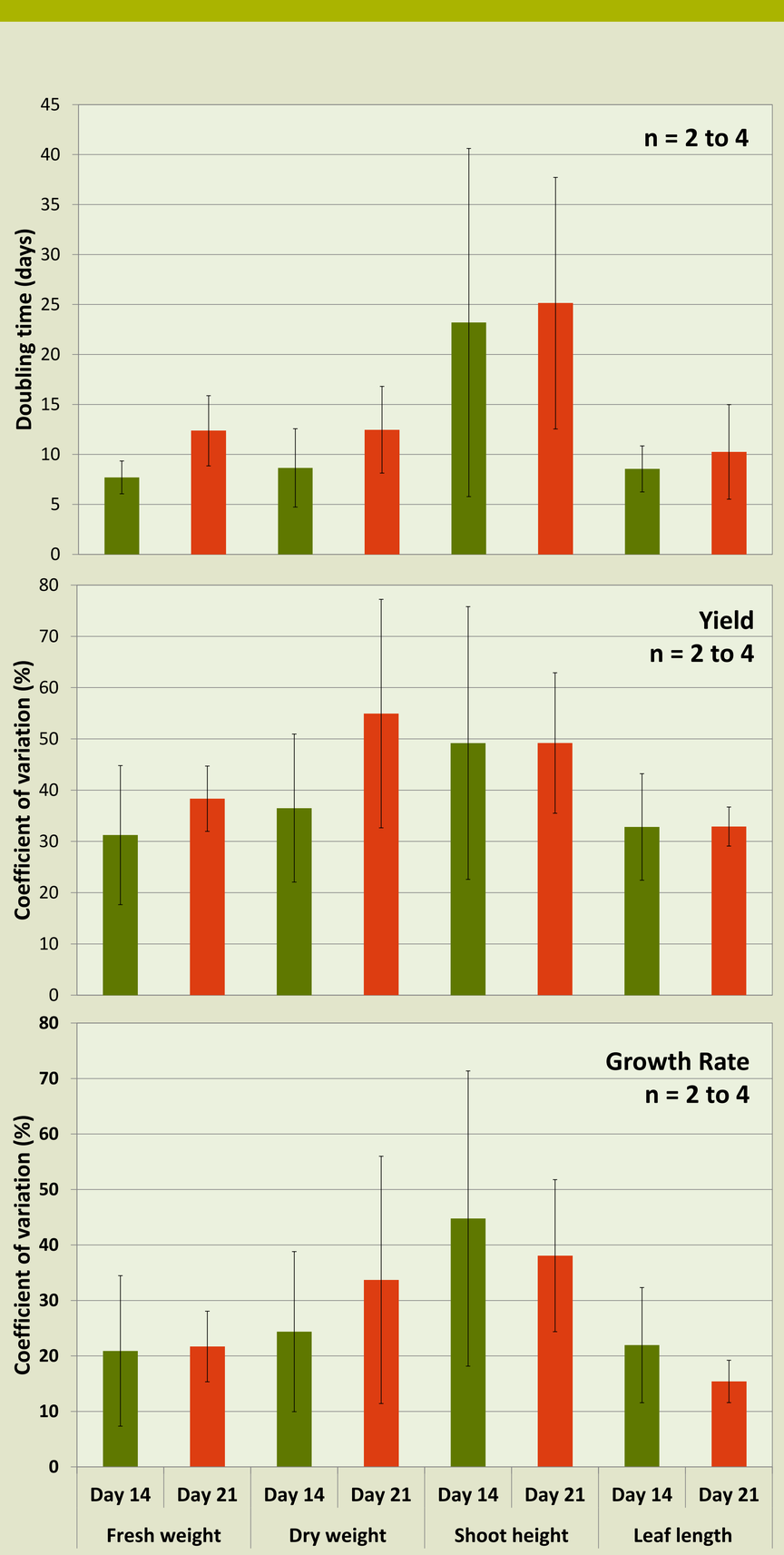

Fig 2: (a) Doubling time in control plants; (b) variability in control plants based on yield; (c) variability in control plants based on growth rate.

Key Results

Propagation method

- Labs provided information regarding their experiences with propagation and their preferred method.
- Vegetative propagation was selected as the preferred propagation method by the majority of labs.

Growth of control plants (Figure 2a)

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

Variability in control plants (Figures 2b and 2c)

- Mean control CoVs for yield were 35 - 55% based on DW and SH and 30 - 40% based on FW and LL.
- Mean control CoVs for growth rate were 35 - 45% based on SH and below 35% based on FW, DW and LL.
- High CoVs are typically correlated with larger plant size and high variability at test initiation.

Sensitivity to Isoproturon

- 14-d EC_{50} estimates range from 0.26 to 7.2 mg ai/L (n=2).
- Yield EC_{50}s were lower than growth rate EC_{50}s while FW & DW parameters tend to be more sensitive than SH & LL.
- Recommended concentrations for future testing are: 30, 100, 300, 1000 and 3000 µg ai/L.

Key points to be revised in protocol

- Vegetative propagation will be advocated and more stringent recommendations will be made regarding the size of plants at test initiation.
- Test duration will be set according to doubling time in controls and impact on coefficients of variation.
- Validity criteria will be set regarding maximum doubling time and acceptable control coefficients of variation.
- The inclusion of some growth parameters, (eg leaf length) may be revised depending on ease of measurement and sensitivity to test substance effects.

Next Steps

- Continued evaluation of isoproturon data (Summer 2017).
- Protocol update and circulation to all participants (Autumn 2017).

Acknowledgements

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- Test items are supplied by Bayer AG (isoproturon) and BASF (imazapyr).
- Photographs were provided by Smithers Viscient (US) and Toxi-Coop (HU).
- Statistical advice is provided by ToxRat GmbH (DE).

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