



## *Glyceria* Work Group: Update

**Jo Davies (Syngenta, UK)**

**Gertie Arts (Alterra, NL)**

**Katrin Kuhl (Bayer CropScience, DE)**

**24<sup>th</sup> May 2016**



*Courtesy of Alterra*

# Outline



- **Background**

- Why test *Glyceria* ? i.e. EU data requirements for aquatic plants
- *Glyceria* Work Group

- **Pre ring-test protocol**

- Objectives
- Protocol
- Plant propagation methods



*Courtesy of Alterra*



## Why test *Glyceria* ?

### EFSA Aquatic Guidance Document

- A rooted macrophyte test is required when
  - *Lemna* and algae are not sensitive ( $EC_{50} > 1$  mg/L) OR
  - sediment is identified as important exposure route
- Test species should be *Glyceria* for compounds that primarily affect monocots in terrestrial plant trials.

### *Glyceria maxima* (Hartm.) Holmb. (Reed sweet grass)

- Monocotyledon - Poaceae
- Emergent, rooted, rhizomatous perennial



# Glyceria Work Group



## Objective

- To develop and ring-test a protocol for *Glyceria maxima* in a water-sediment system
- To produce and validate an OECD Test Guideline

## Work Group Leads

- Jo Davies (Syngenta, UK), Gertie Arts (Alterra, NL), Katrin Kuhl (Bayer CropScience, DE)

## Statistical support

- Monika Ratte (ToxRat, DE)

## Schedule

- Pre-test June to October 2016
- OECD Project Submission Autumn 2016
- Definitive ring-test – to be decided

## Participating Laboratories

- Alterra
- BASF, Limburgerhof
- Bayer CropScience
- Centre Ecotox, Eawag/EPFL
- Eurofins
- FERA
- IBACON
- IME Fraunhofer
- Mesocosm GmbH
- Rheinland Pfalz AgroScience GmbH
- Smithers Viscient
- Syntech Research
- Toxi-Coop Research Services
- WIL Research
- Wildlife International

# Pre-test Objectives & Plan



## Objectives

- To provide experience for those labs who do not routinely conduct tests
- To produce data to address the following issues:

### Test duration

- 14 or 21 days – experience varies as to time taken for doubling of shoot growth parameters
- Data will be generated at 14 & 21 days to enable selection of test duration for definitive ring-test

### Expected variability

- Proposed max CoV is 35% for control plants - data are required to validate proposal
- Experimental design (i.e. replication) needs to be optimised for the definitive ring-test – pre-test data will be used for power analyses

### Propagation methods

- Two methods of propagation – data are required to determine whether choice of method affects sensitivity but difficult for a single lab to test both methods side-by-side (**volunteers welcome!**)
- **Please notify organisers as to chosen propagation method (see next slides)**

## Plan

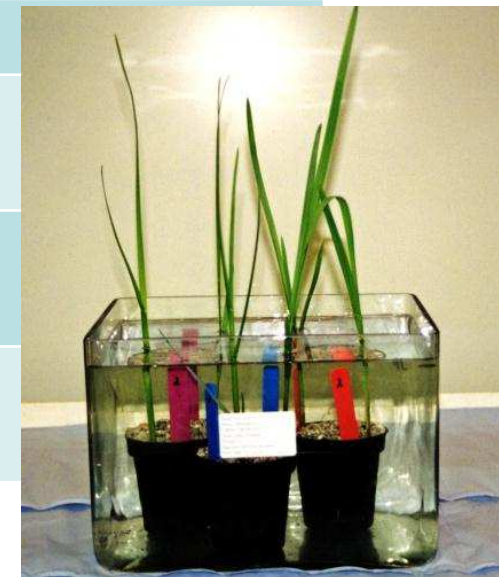
- To test 1 compound (to be confirmed) between June and September 2016
- All labs do not have to complete pre-test at exactly the same time
- Instead, emphasis should be placed on ensuring the use of healthy plant material – adequate time should be devoted to optimising propagation method

# Key Features of Pre-test Protocol



- Adapted from OECD TG 239 : *Myriophyllum* in a water-sediment system

<b>Test system</b>	<ul style="list-style-type: none"> <li>Plant pots / beakers in glass test vessels</li> <li>Artificial sediment (OECD 219) with added N &amp; P</li> <li>Smart &amp; Barko medium at pH 7.5</li> </ul>
<b>Application method</b>	<ul style="list-style-type: none"> <li>Via water phase</li> </ul>
<b>Draft test design (to be decided)</b>	<ul style="list-style-type: none"> <li>Untreated control with 6 replicate test vessels</li> <li>5 concentrations, each with 4 replicate test vessels</li> <li>Each replicate test vessel contains &gt;1 pot of one or two shoots</li> </ul>
<b>Test conditions</b>	<ul style="list-style-type: none"> <li>22 ± 2°C with 16/8 hour photoperiod</li> </ul>
<b>Test duration</b>	<ul style="list-style-type: none"> <li>Establishment phase of 7 days</li> <li>Exposure phase of 14 - 21 days</li> </ul>
<b>Biological assessments</b>	<ul style="list-style-type: none"> <li>Shoot Height (SH)</li> <li>Shoot FW &amp; DW at beginning &amp; end of test</li> </ul>
<b>Endpoints</b>	<ul style="list-style-type: none"> <li>EC<sub>50</sub> &amp; NOEC for yield (Y) &amp; growth rate (Gr)</li> <li>Based on SH, DW, FW</li> </ul>





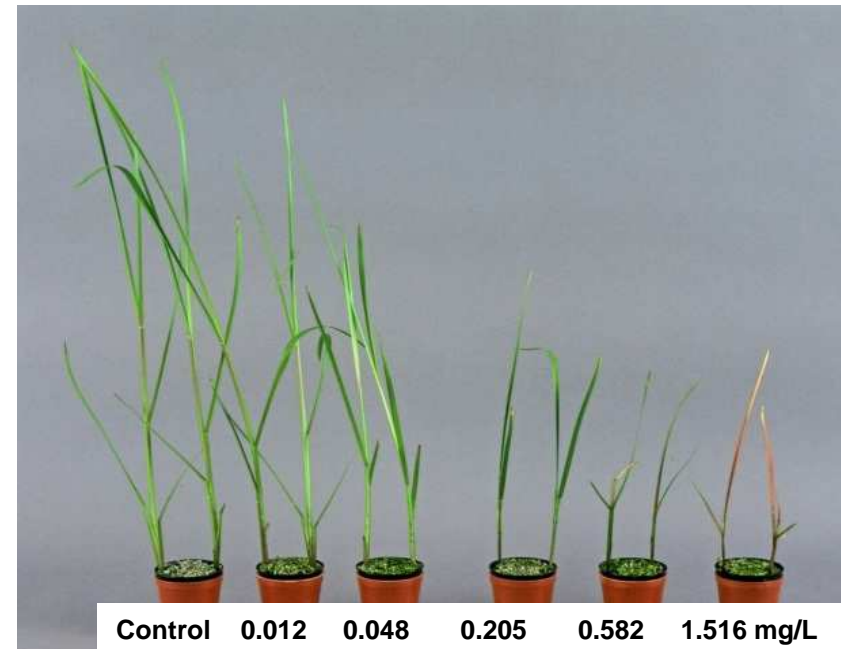
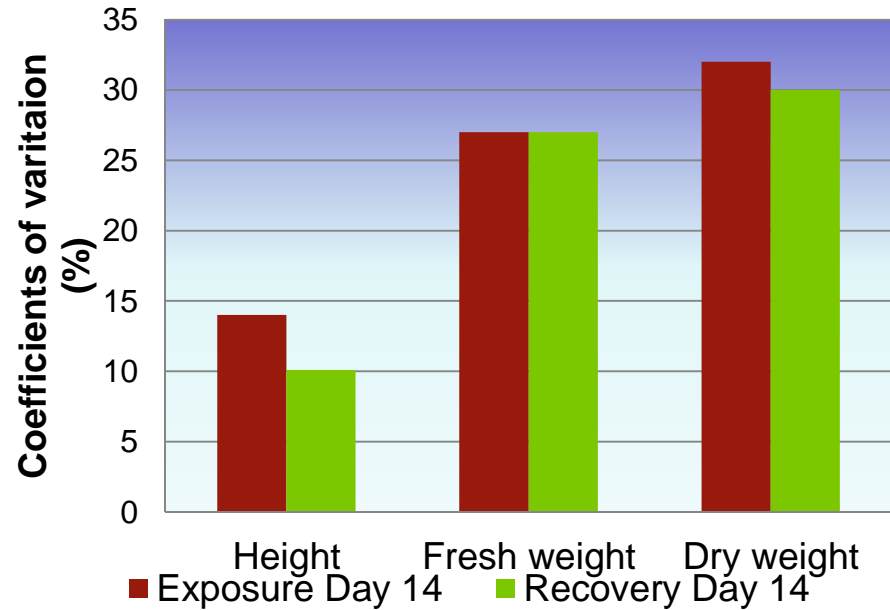
# Example results

## Test Design

- 3 replicates of 6 plants for control, 2 replicates of 6 plants for each treatment
- 14-d exposure phase with 14-d recovery phase

## Test system performance

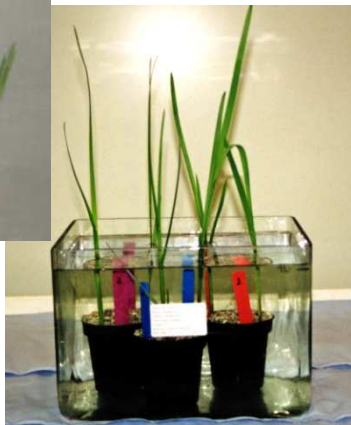
- Adequate growth in control plants
  - Doubling of Height, FW and DW within 14-d exposure phase
- Reasonable CoVs for measured parameters
  - Height < 15%
  - FW & DW 25 to 32%
- Demonstrated sensitivity to test substance
- Assessment of potential for recovery



# Propagation methods – vegetative propagation



**Key issue** ~ Impact of propagation method on relative sensitivity and variability



Initial plant material obtained as :

- mature plants from field
- mature plants from another lab
- seedlings from aquatic plant supplier

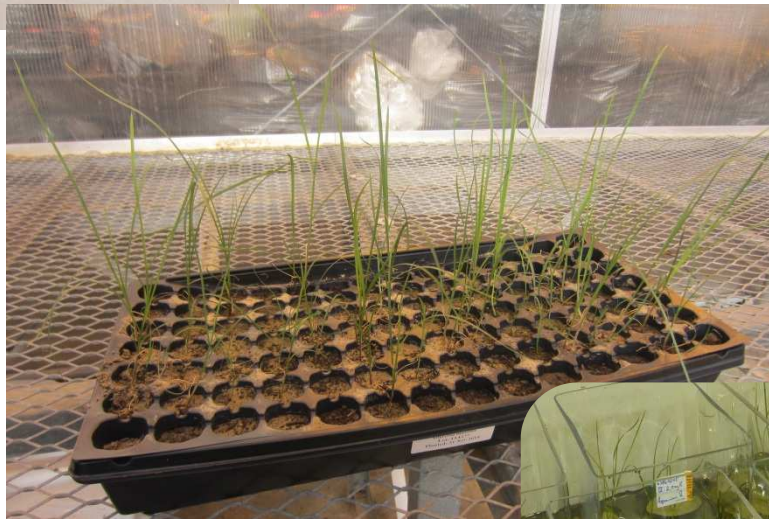
Test material propagated by transplanting rhizome + shoot sections

- Reduced genetic variability
- More robust plants
- Available year round if maintaining own stock
- Resource intensive - glasshouse space
- Continued maintenance by regular transplanting
- *Currently, preferred method of propagation*

*Photographs courtesy of Smithers Viscient & Syngenta*



# Propagation methods – germination from seed & use of seedlings



Initial plant material obtained as :

- seed from seed supplier or from field
- seedlings from aquatic plant supplier

Test material propagated by transplanting seedlings

- Germination from seed avoids issues of buying plants in countries where sale is prohibited eg some US States
- Germination may be erratic and lengthy
- Greater genetic variability
- Seedlings not available for purchase year round

*Photographs courtesy of Smithers Viscient, IBACON & Syngenta*

## Next Steps



### Protocol

- Protocol was circulated to all interested parties in early May
- Comments (in the commenting form) required by end of May
- Please advise ring-test organisers of your choice of plant propagation method asap
  - Organisers need to take statistical advice as to how to make a robust comparison between propagation methods

### Test substances for pre-test

- To be decided by ring-test organisers asap (most likely to be 3,5 DCP)

All comments / queries to: [jo.davies@syngenta.com](mailto:jo.davies@syngenta.com)

**Thank you for your attention**