

Proposed Ring-test & Protocol for *Glyceria maxima* in a Water-sediment System

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Courtesy of Alterra

Outline



- Background
 - Why test *Glyceria* ? i.e.EU data requirements for aquatic plants
 - What is *Glyceria* ?
- Test protocol
 - Plant propagation
 - Example test design & results
- Next steps
 - Ring-test participants
 - Progress to date



Courtesy of Alterra

Why test *Glyceria* ?



EU Directive 1107: Annex II 8.2.6

- Additional testing may be required
 - depending on the active substance mode of action
 - or due to clear indications of higher toxicity to dicotyledonous (e.g. auxin inhibitor, broad leaf herbicides) or other monocotyledonous (e.g. grass herbicides) plant species from efficacy or testing with terrestrial non-target plants.
 - dicotyledonous species, e.g. *Myriophyllum spicatum*/*M. aquaticum*
 - monocotyledonous species, e.g. aquatic grass *Glyceria maxima*

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 with upright stems reaching 2 m in height
- Static or slow-flowing water up to 1 m deep
- Favours open or lightly shaded, nutrient-rich water ranging from neutral to alkaline pH
- Native to Europe, Siberia, Western Asia, China, Australia, New Zealand, Canada but invasive weed species in US



Use as a test species in ecotox tests

- Protocol developed for CRD (1997 to 2000)
- Sensitive to grass-selective herbicides
- Frequently included in mesocosm studies
- Limited publications in public domain

Propagation of *Glyceria maxima* for use in tests



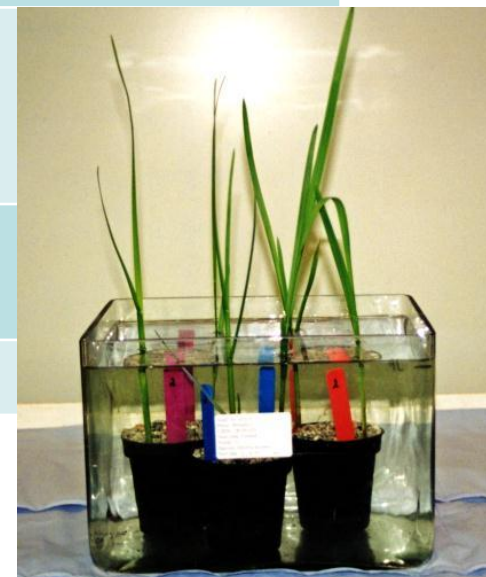
Plant source	Advantages	Disadvantages
Vegetative propagation	<ul style="list-style-type: none">• Reduces genetic variation• Available year round	<ul style="list-style-type: none">• Labour intensive• Glasshouse space• Continued maintenance by regular transplanting
Seedlings – bought in from nurseries	<ul style="list-style-type: none">• Less resource intensive	<ul style="list-style-type: none">• Genetic variability• Seasonal availability• may require transplanting
Germination from seed	<ul style="list-style-type: none">• Avoids issues of buying plants in countries where sale is prohibited eg US	<ul style="list-style-type: none">• Germination may be erratic and lengthy• Genetic variability



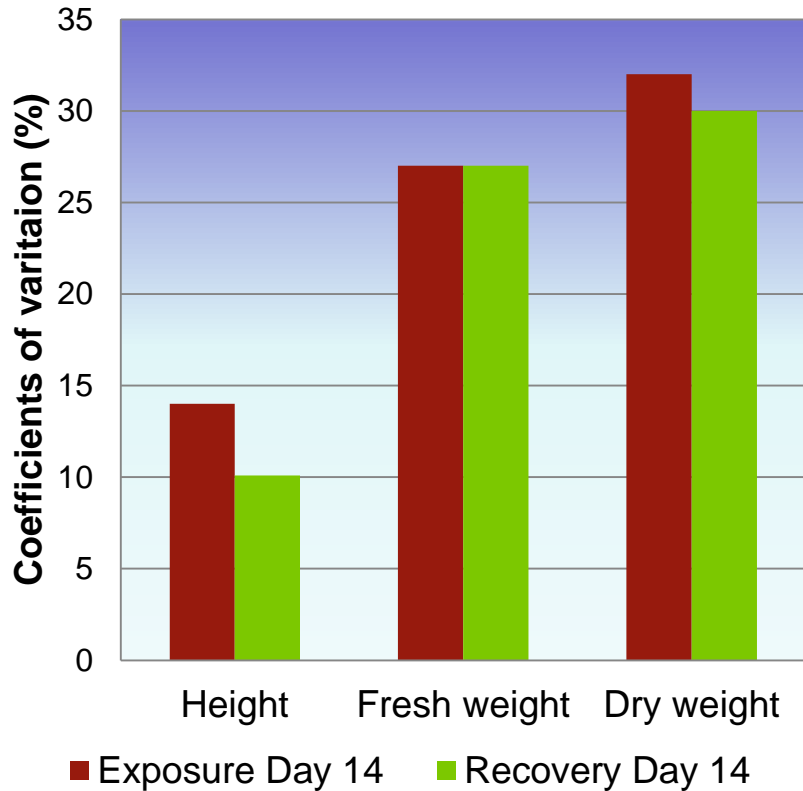
Example Protocol used to test ACCase herbicide



Test system	<ul style="list-style-type: none">• Plant pots / beakers in glass test vessels• Sandy loam soil with added fertiliser (Osmocote®)• Deionised water
Application method	<ul style="list-style-type: none">• Via water phase
Test design	<ul style="list-style-type: none">• Untreated control with 3 replicate test vessels• 5 concentrations, each with 2 replicate test vessels• Each replicate test vessel contained 3 pots.
Test conditions	<ul style="list-style-type: none">• $15 \pm 2^{\circ}\text{C}$ with 16-h day length
Test duration	<ul style="list-style-type: none">• Establishment phase of 7 days• Exposure phase of 14 days• Recovery phase of 14 days in fresh water
Biological assessments	<ul style="list-style-type: none">• Shoot Height (SH), Shoot FW & DW• Beginning, end of exposure & recovery phases
Endpoints	<ul style="list-style-type: none">• EC_{50} & NOEC for yield (Y)

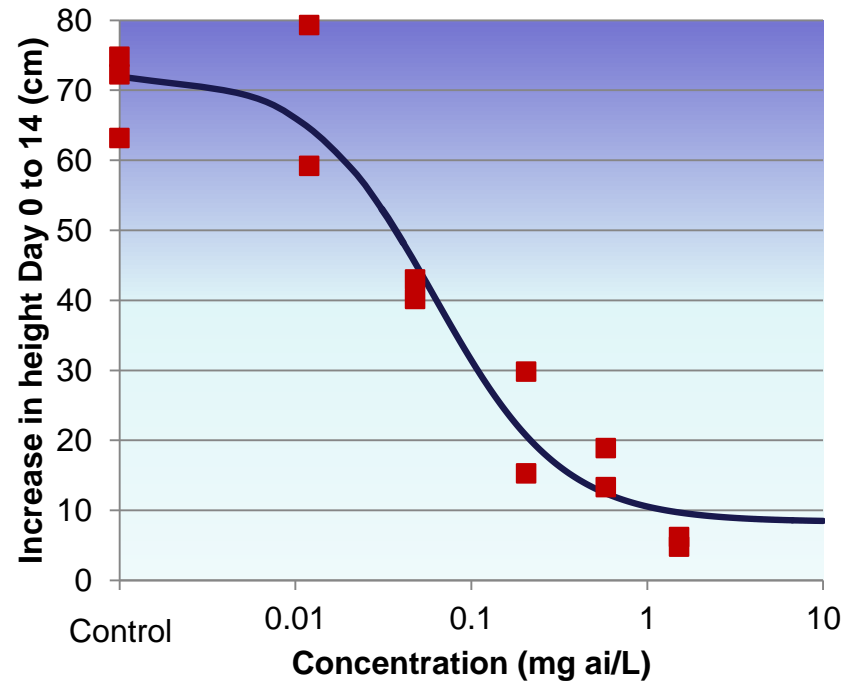
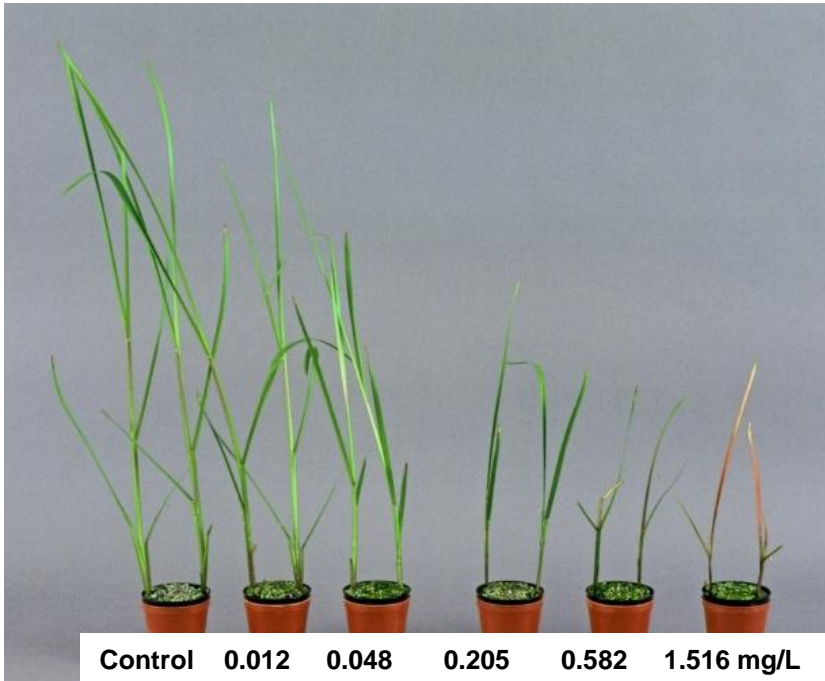


Results: Performance of control plants



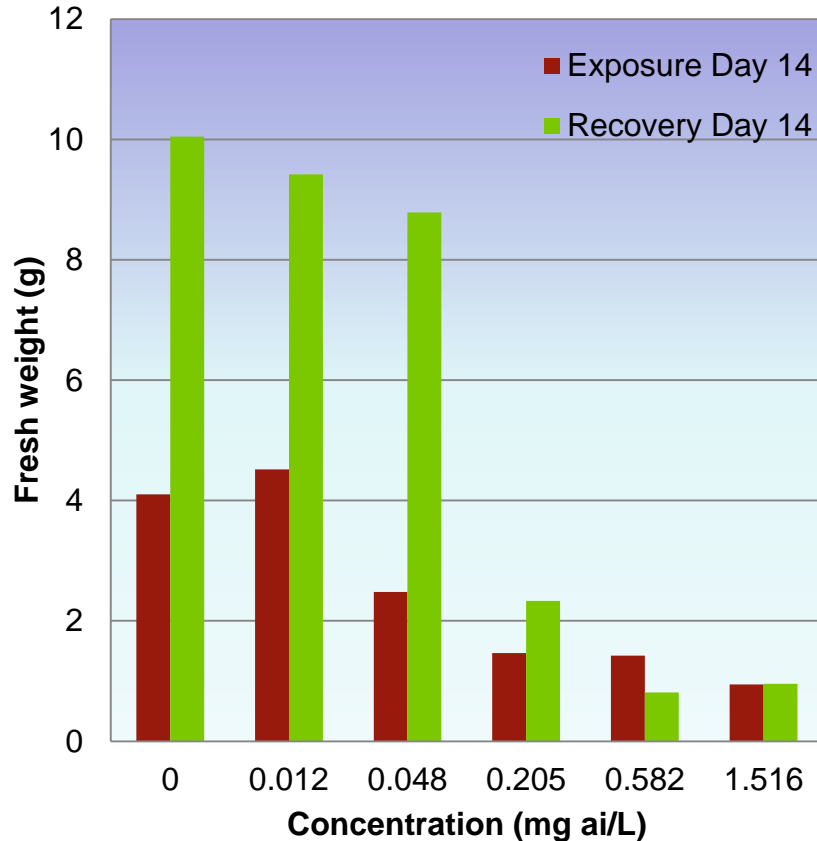
- Doubling of Height, FW and DW within 14-day exposure phase
- Coefficients of variation
 - Height < 15%
 - FW & DW 25 to 32%

Results: Exposure Day 14



- NOEC of 12 $\mu\text{g ai/L}$
- EC_{50} values for SH, FW and DW in the range of 43 to 62 $\mu\text{g ai/L}$
- No obvious trend in sensitivity of endpoints

Results: Recovery Day 14



- recovery at 48 $\mu\text{g ai/L}$
- limited growth during recovery phase at 205 $\mu\text{g ai/L}$
- continued inhibition at 0.582 & 1.516 mg ai/L

Conclusions re test system

- Adequate growth in control plants
- reasonable CVs for measured parameters
- Demonstrated sensitivity to test substance
- Assessment of potential for recovery

Progress so far



- Presentation at SETAC Europe in Basel, May 2014
 - 13 laboratories expressed interest
 - Questionnaire circulated Sept 2014
 - to establish level of experience
 - to establish propagation methods
 - Wide range of experience
 - No labs growing/maintaining indoor stock plants
 - Ring-testing over winter 2014 is not feasible
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|------------------------------------|
| • Alterra |
| • BASF, Limburgerhof |
| • Bayer CropScience |
| • Centre Ecotox, Eawag/EPFL |
| • Eurofins |
| • IBACON |
| • Mesocosm GmbH |
| • Rheinland Pfalz AgroScience GmbH |
| • Smithers Viscient |
| • Syntech Research |
| • Toxi-Coop Research Services |
| • WIL Research |
| • Wildlife International |



Next Steps

- Updated protocol to be circulated Jan 2015
- Laboratories advised to work on culture methods in preparation for participation in a pre-ring-test in Spring/Summer 2015
 - Exact timing will be determined by plant availability
- Identify compounds for use in a ring-test
 - 3,5-dichlorophenol (for consistency with *Lemna* / *Myriophyllum* ring-tests)
 - PSII inhibitor
 - Grass herbicide
- Ring-test in 2015 - 2016