# Draft OECD TG for the emergent macrophyte, *Glyceria maxima,* in a water-sediment system
# Results of a ring-test with Imazapyr

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## Introduction

- Regulation in the EU requires data for *Lemna* for all herbicidal compounds and data for an additional monocot or dicot species for some herbicidal compounds.

- The dicot species, *Myriophyllum spicatum*, and the monocot species, *Glyceria maxima* have been identified as suitable additional test species.

- OECD TG 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 in several labs.

- In 2016-17, 13 labs participated in a ring-test with the herbicide isoproturon (IPU), generating 10 to 11 control datasets and 5 to 8 datasets for the effects of isoproturon at 14 and 21 days.

- Based on these data, the test duration was set to 14 days and the protocol was revised as highlighted in Table 1. The revised protocol was used to test the herbicide, imazapyr (IMA), in 11 labs between August and October 2018.

## Objectives of Imazapyr Ring-Test

1. To assess the potential inclusion of root endpoints
2. To improve growth rates by modification of environmental conditions
3. To reduce control coefficients of variation by improving uniformity of starting plant material
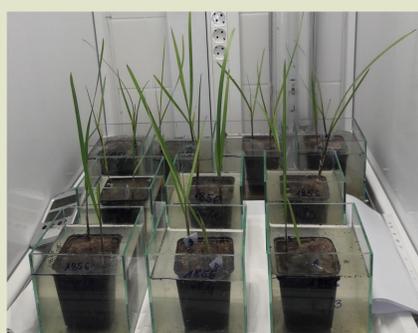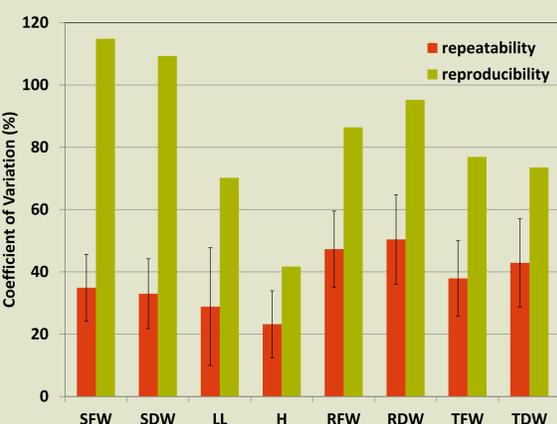


Fig 2: Variability in control plants at test initiation (n=9-11)

## Key Results

### Growth and variability of control plants

- Repeatability CoVs for control (or representative) plants at test initiation, control yields & control growth rates were typically <40% for shoot variables but > 40% for root variables (Figs 2 & 3).
- Yield CoVs are higher than growth rate CoVs (Fig 3).
- Shoots & leaves typically doubled in weight & length within 14 days , whereas roots frequently failed to double in weight within 14 days (data not shown).

### Sensitivity to Imazapyr

- $EC_{50}$ values were calculated for 80, 52 and 25 % of data sets for LL, shoot weights & root weights, respectively.
- NOECs were determined for the majority of data sets. For root variables, only effects >40% could typically be detected (Fig 4).
- Therefore, root variables are less reliable than shoot variables, due to high variability.
- Mean $EC_{50}$ values were estimated for SFW, SDW & LL but the dataset was too small to draw conclusions regarding the relative sensitivity of shoot parameters.

| | Imazapyr $EC_{50}$ (µg ai/L; 95% CI) | | |
|---|---|---|---|
| **Assessment parameter** | **SFW** | **SDW** | **LL** |
| **Growth rate** | 165.6 (43 -592 ) | 227.3 (68-760) | 105.4 (45-249) |
| **Yield** | n.d. | n.d. | 64.4 (29-142) |

### Comparison between Isoproturon and Imazapyr ring-test data

- Control yields for SFW, SDW & LL are lower in the IMA than the IPU ring-test, due to smaller starting material, whereas control growth rates for all shoot parameters were similar in both ring-tests (data not shown).
- Repeatability CoVs were similar or slightly improved in the IMA relative to the IPU ring test but reproducibility CoVs were typically worse in the IMA ring-test. These changes could be related to increasing lab experience over time confounded by a change in lab participation between the two ring-tests (Fig 3).

## Conclusion

- Root endpoints were more variable & less robust than shoot endpoints.
- Control growth rates of 0.08 / d were achieved for shoot parameters.
- Intra-laboratory variability must be reduced to meet validity criterion of <35% for control CoVs.

Fig 1: Project Schedule

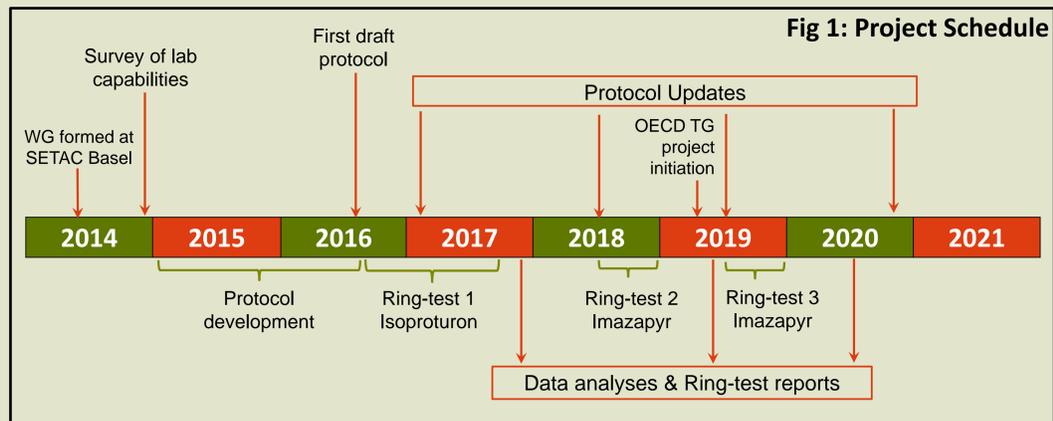| 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 |

## Table 1: Ring-Test Protocol for Imazapyr (bold: updated in test protocol)

| Test system | Plant pots in glass test vessels; **pots must have holes in base** |
|---|---|
| Plant propagation | Individual shoots with rhizome sections are cut from stock plants & transplanted into fresh sediment to produce test plants (Fig 2); **initial plant size defined as 1-2 leaves unfolded with 2-4 cm of rhizome.** |
| Sediment / Media | Artificial sediment supplemented with nutrients as described in OECD TG 239, overlaid with Smart & Barko; **water depth specified as 3cm above sediment surface** |
| Application | **Imazapyr** was dissolved in Smart & Barko media & added to the water column |
| Test design | Untreated control with 6 replicate test vessels; 6 test concentrations each with 4 replicate test vessels; each replicate test vessel contains 1 plant pot of **1 shoot** at test initiation |
| Test conditions | Temperature increased to **23 ± 2°C** with 16 h day-length at 180 (± 20) µE m$^{-2}$ s$^{-1}$ |
| Test duration | **1-d establishment phase with 14-d exposure phase** |
| Biological assessments | Total leaf length (TLL), shoot fresh weight (SFW) & dry weight (SDW), **root fresh weight (RFW) & dry weight (RDW), total fresh weight (TFW) & dry weight (TDW)** |
| Analytical assessments | **Mandatory measurement of imazapyr concentrations on days 0, 7 & 14** |
| Environmental assessments | pH & DO recorded on Days 0, 7 & 14; Water temperature measured daily |
| Endpoints | Yield & growth rate $EC_{50}$ and NOEC values based on TLL, SFW, SDW, RFW, RDW, TFW, TDW |
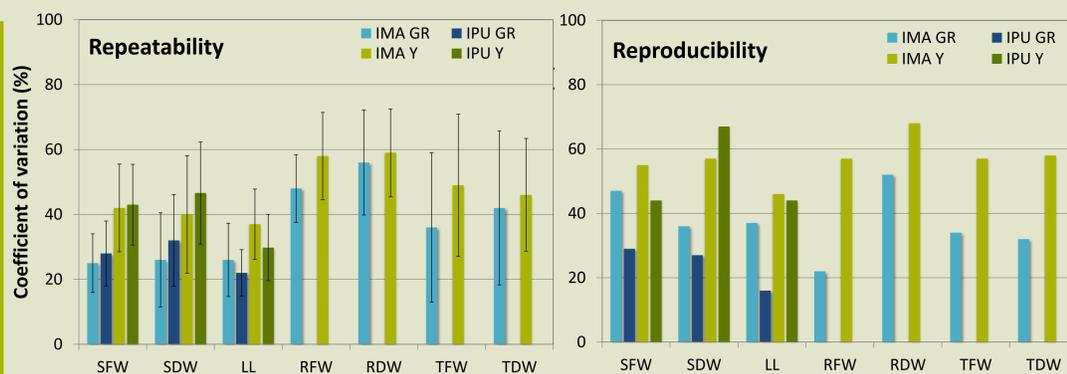


Fig 3: Repeatability (within lab) & reproducibility (between lab) CoVs in control plants based on yields (green) & growth rates (blue) from IMA (light shades) & IPU ring-tests (dark shades)

## Key points for future protocol updates

- Test duration will remain at 14 days
- Root assessments will not be included due to high variability and unreliable endpoints
- Establishment phase will be 3 days to reduce incidence of unhealthy control plants
- Further standardisation of starting plant material is necessary, e.g. initial height & leaf length
- Replication and test design will be revisited

## Next Steps

- The protocol has been submitted to OECD and accepted as a Test Guideline project
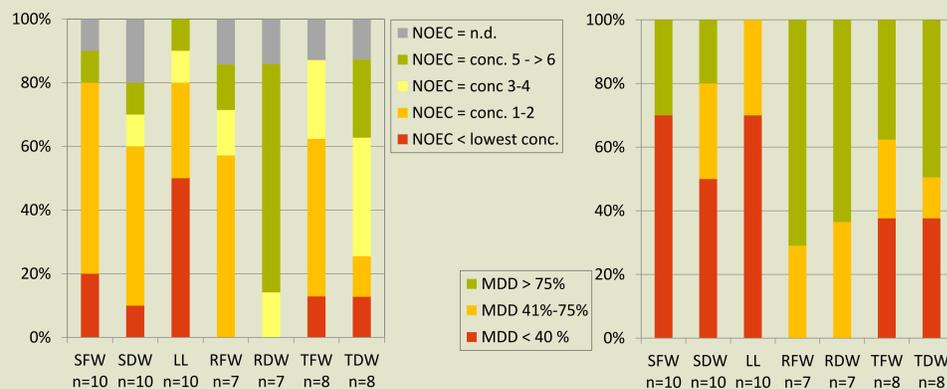- A third ring-test is being planned for 2019 to 2020



Fig 4: Percentage of growth rate data sets falling into defined NOEC & Minimum Detectable Difference (MDD) categories