Glyceria maxima: Development of an OECD Test Guideline

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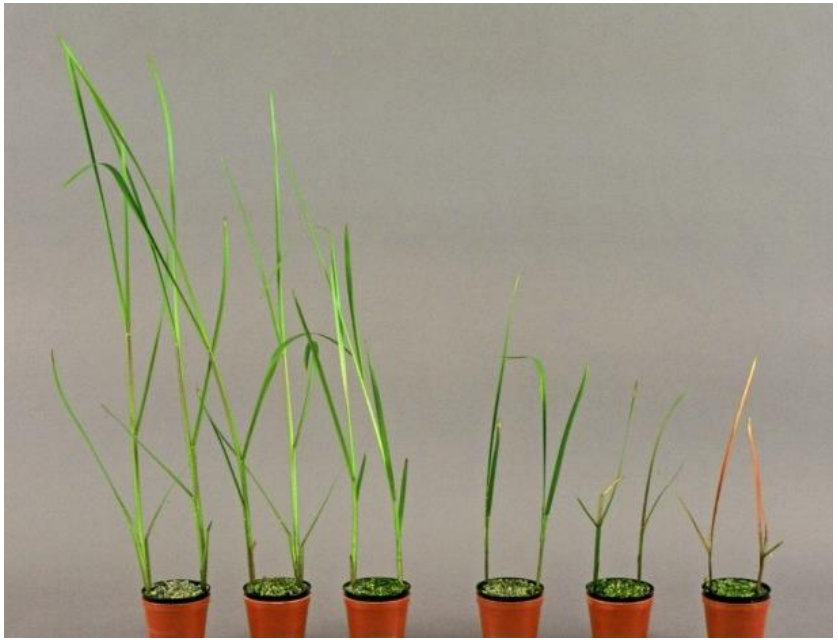


Courtesy of Syngenta

Background

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

- Emergent, rooted, rhizomatous
- Perennial reaching 2m in height

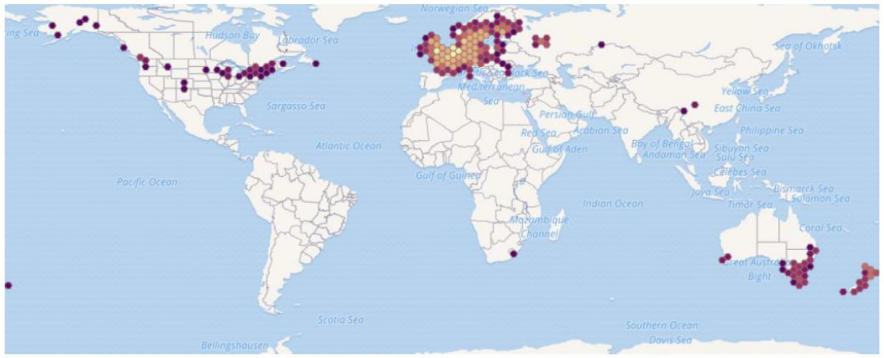
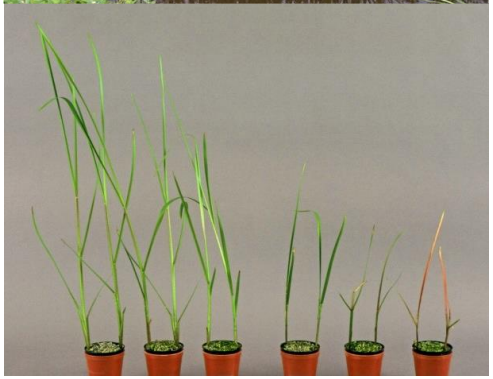


Figure 1. Known global distribution of *Glyceria maxima*. Map from GBIF Secretariat (2018).



EU Directive 1107: Annex II 8.2.6 & EFSA Aquatic GD:

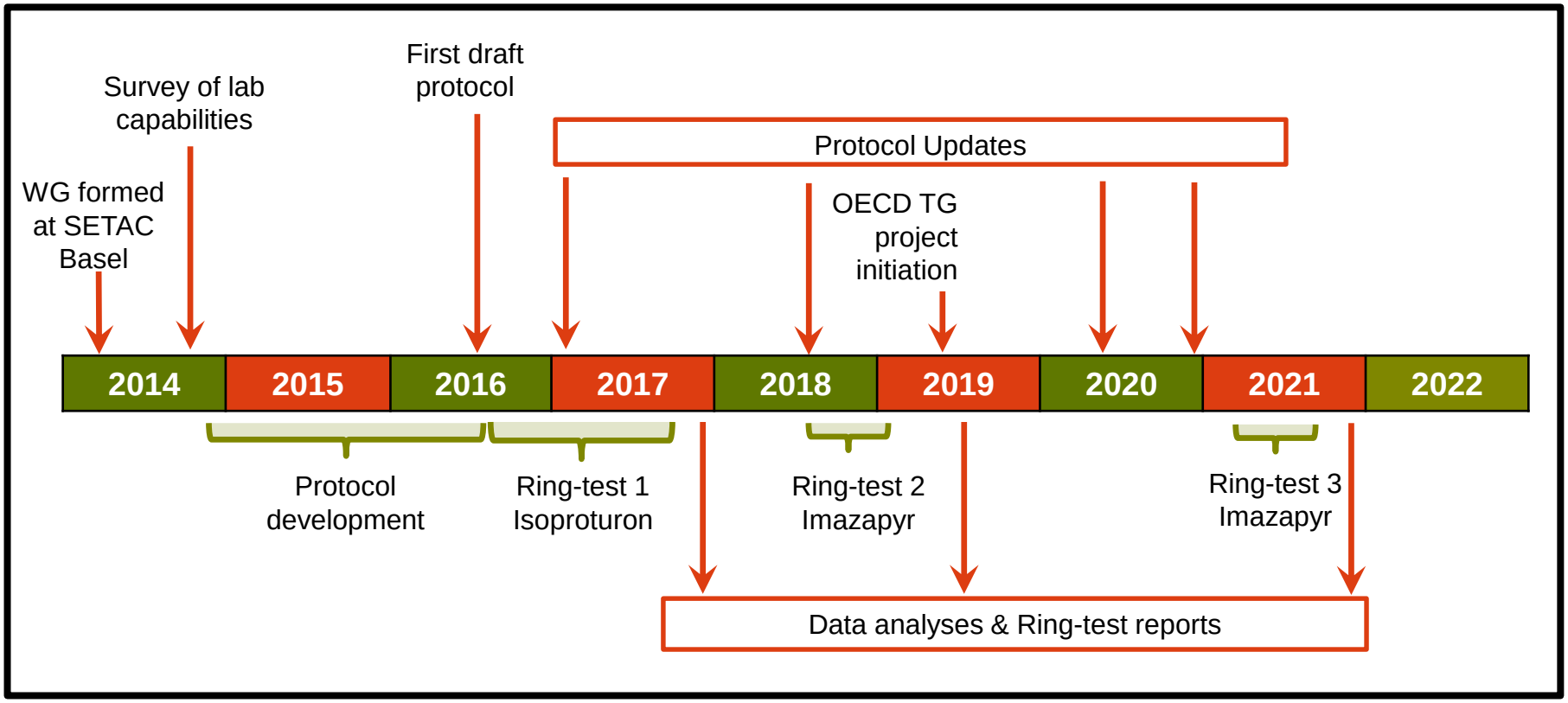
Tests with an additional macrophyte species are required when:

- *Lemna* and algae are not sensitive (EC_{50} > 1 mg/L)
- OR sediment is an important exposure route
- test species should be *Glyceria* for compounds that primarily affect monocots in terrestrial plant trials

Glyceria Work Group – Project History

Objective

- To ring-test a protocol for *Glyceria maxima* in a water-sediment system
- To deliver an OECD Test Guideline



Ring-test Objectives

1. Propagation method	• Establish a reproducible method for maintaining stock plants and propagation of test plants from rhizome sections
2. Test duration	• Ring-test 1 • Assessments at 14 and 21 days
3. Assessment parameters	• Ring-test 1: leaf length *versus* shoot height • Ring-test 2 : shoot v root fresh & dry weights
4. Understand variability	• Determine the experimental factors driving variability • Replication required to achieve acceptable control coefficients of variation of <35%

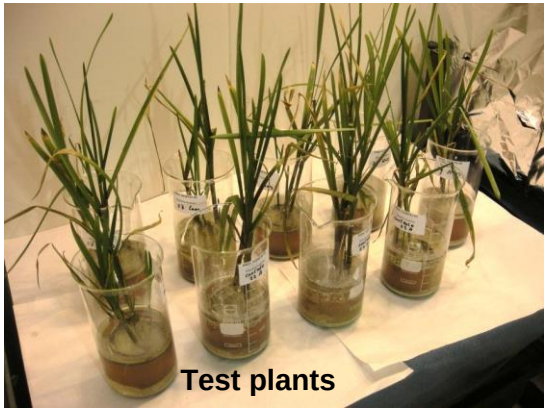
Key features of the protocol

Test parameter	Ring-test 1: Isoproturon	Ring-test 2: Imazapyr
Establishment phase	3 days	1 day
Exposure phase	14 and 21 days	14 days
Test vessel	Plant pots or beakers	Plant pots with holes
Starting material	1-3 shoot per pot	1 shoot per pot
Water depth over sediment	3 cm	5 cm
Experimental design	6 control reps & 4 reps of 5 concentrations	6 control reps & 4 reps of 6 concentrations
Assessment parameters	Shoot height, Leaf length (LL), Shoot FW, Shoot DW	Leaf length (LL), Shoot FW, Shoot DW Root FW, Root DW
Test substance analyses	None	0, 7 and 14 days
Temperature	22 ± 2°C	23 ± 2°C
Number of participants	**13 labs**	**11 labs**

Objective 1: Propagation

Seedlings grown from seed

Courtesy of BASF, WER, Syngenta



Objective 2: Test duration

Ring-test 1

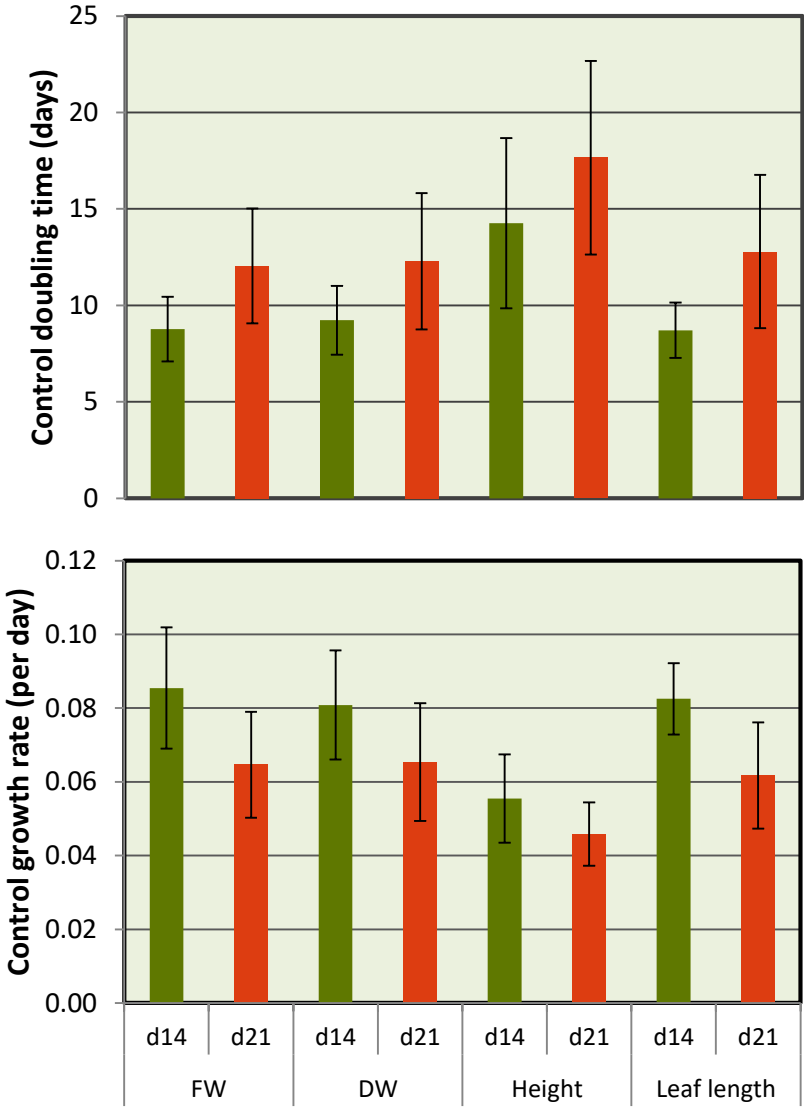
- Assessments of SFW, SDW, SH and LL were made at 14 and 21 days

Results (n = 10 to 11)

- Control plants achieved >2-fold increase in FW, DW & 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 slower growth rate between days 14 & 21
  - trend may be caused by nutrient limitations

Conclusion

- 14-day test is sufficient to achieve adequate growth



Objective 3: Assessment parameters

Ring-test 1

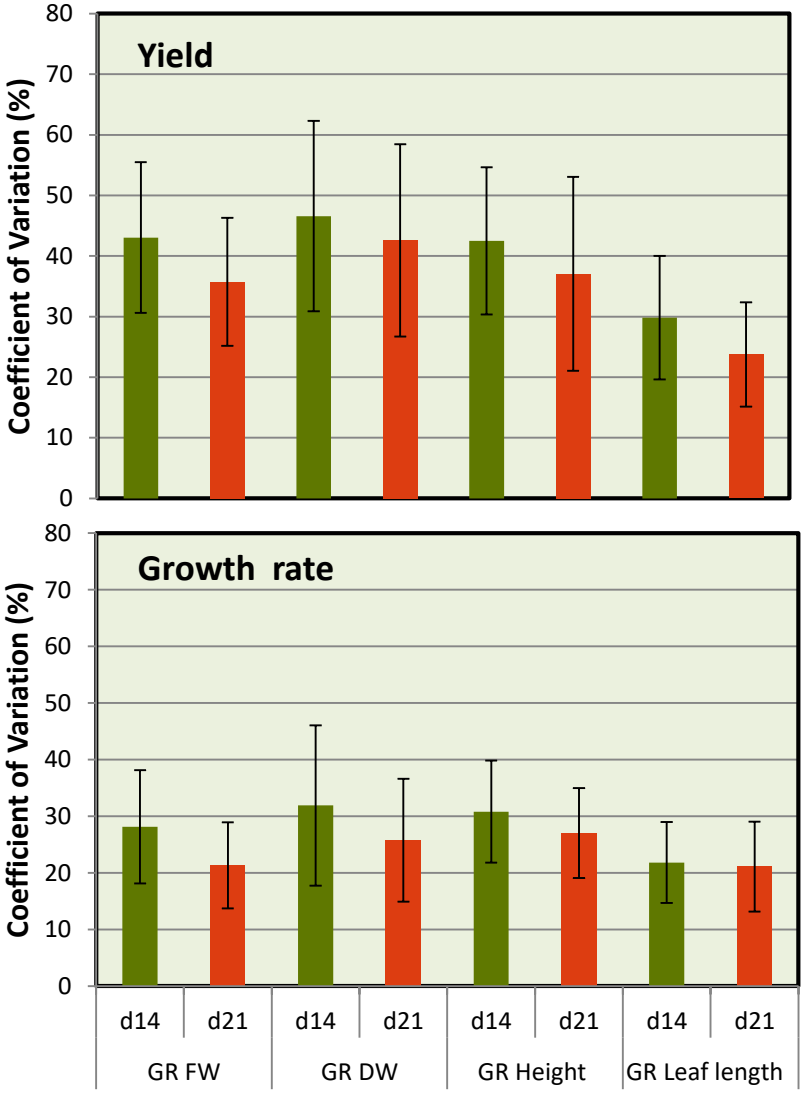
- Assessments were made of SFW, SDW, SH and LL

Results (n = 10 to 11)

- Yield CoVs are higher than growth rate CoVs.
- For growth rate, CoVs for most endpoints were <35%
- For yield, only LL has a CoV of <35%
- High CoVs are typically correlated with larger plant size and high variability at test initiation.

Conclusions

- LL provides a more robust measure than SH
- Stricter recommendations on plant size at test initiation
- Modifications to test design are necessary



Objective 3: Assessment parameters

Ring-test 2

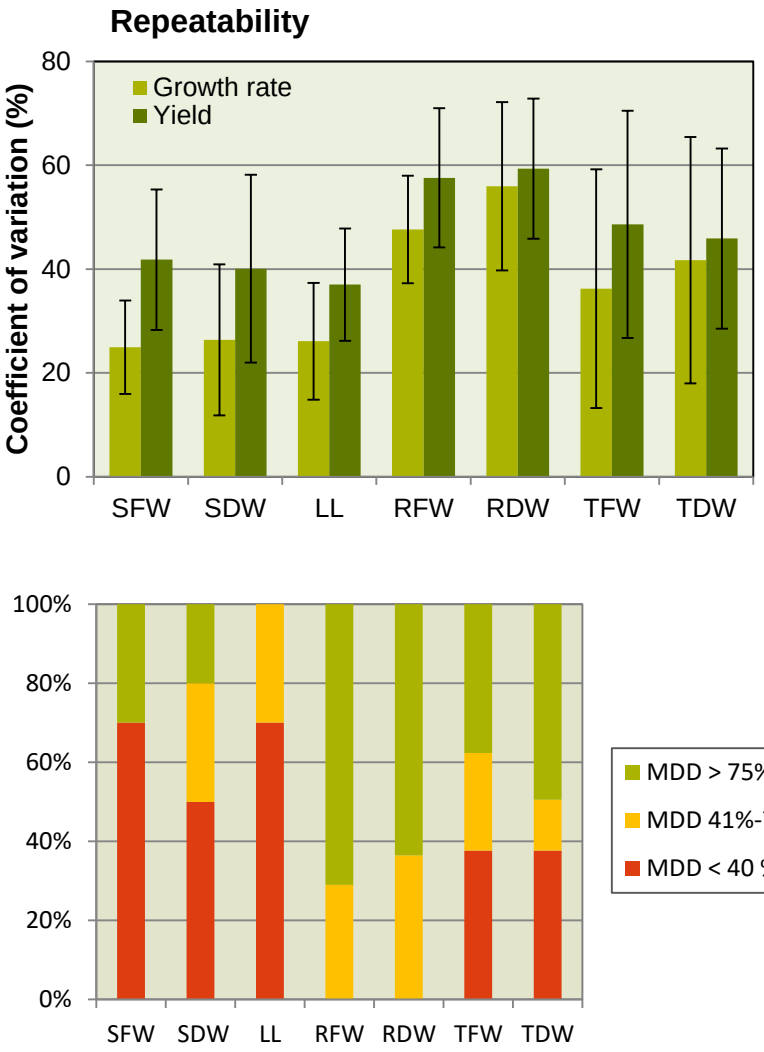
- Assessments of Shoot & root FW & DW were made at 0 and 14 days
- Comparison of control CoVs
- Ability to detect effects of imazapyr (i.e. minimum detectable differences, MDDs)

Results (n = 10 to 11)

- Shoots & leaves typically doubled in weight & length within 14 days, whereas roots frequently failed to double in weight (data not shown).
- Repeatability CoVs for control (or representative) plants at test initiation, control yields & control growth rates were <40% for shoots but >40% for roots
- For root variables, only effects >40% could typically be detected

Conclusion

- Root variables are less reliable than shoot variables, due to high variability



Objective 4 : Understanding variability

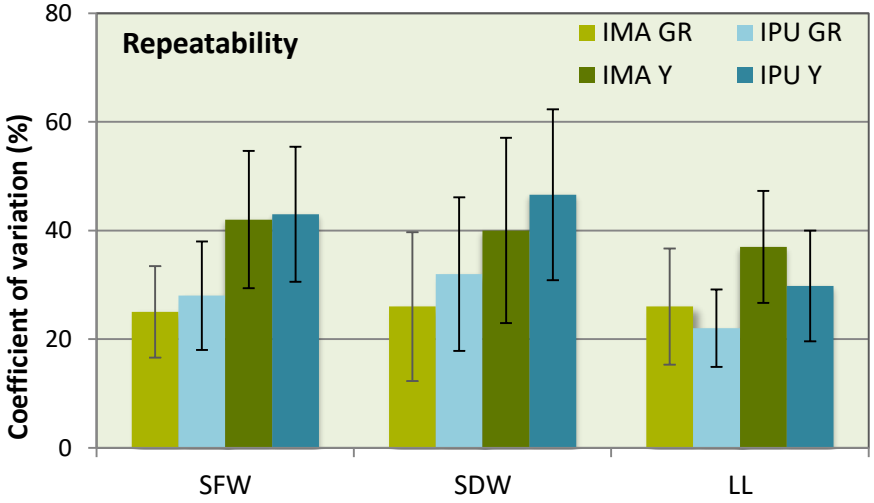
Comparison of Variability between Ring-test 1 (IPU) & Ring-test 2 (IMA)

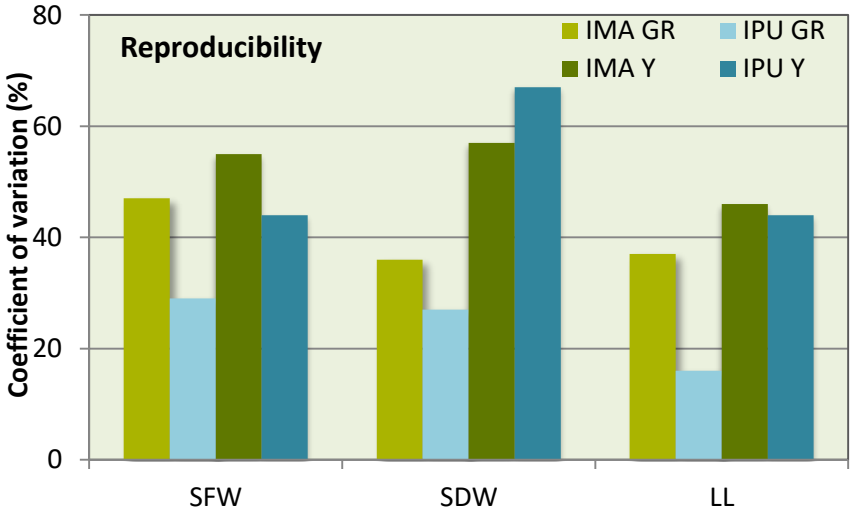
Results

- Control growth rates for all shoot parameters were similar in both ring-tests (data not shown).
- Repeatability CoVs were similar or slightly improved in Ring-test 2 but reproducibility CoVs were typically worse

Conclusion

- Intra-laboratory variability must be reduced to meet validity criterion of <35% for control CoVs





Next steps

Ring-test 3 with Imazapyr

- Rescheduled for Summer 2021
- Objective - Significantly improve CoVs
  - reducing variability in starting plant material
  - increasing standardisation of experimental conditions

Training in plant propagation and experimental techniques

- Workshop: postponed to Spring 2021
  - Hosted by Mesocosm GmbH & GG BioTech Design GmbH; Sponsored by European Crop Protection
- Online training videos
  - Request to all participants – if testing *Glyceria* in 2020, please consider sharing videos and/or photographs of work in progress
  - Details of preferred file formats and data platform will follow shortly

OECD Expert Group

- Updated version of protocol circulated for review in April 2020

Acknowledgements

Workgroup organisers

- Joanna Davies (Syngenta, UK)
- Gertie Arts (Wageningen Environmental Research, NL)
- Johanna Kubitza (BASF SE, DE)
- Rena Isemer (Bayer AG, DE)

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Test item supply

- Bayer AG (isoproturon)
- BASF (imazapyr)

Participating labs

- BASF SE (DE)
- Bayer AG (DE)
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