Effects of the herbicide metsulfuron-methyl on a plant community, including seed germination success in the F1 generation

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Background

- Current European terrestrial plant risk assessment framework is based on vegetative effects (e.g. fresh weight, shoot length, emergence);
- Sub-lethal effects on non-target terrestrial plant (NTTP) growth and reproduction need to be addressed in order to adequately protect populations of NTTP[1];
- Need for evaluation if the current risk assessment scheme for NTTP is protective of sub-lethal reproductive effects on terrestrial plants;
- Charge question 2nd SETAC NTTP workshop; are vegetative growth endpoints protective of effects on sexual reproduction?[2]

[1] Arts et al., 2015. Report from the 1st SETAC NTTP workshop
[2] Arts et al., 2017. Report from 2nd SETAC NTTP workshop

Research questions

- How does the sensitivity of reproductive endpoints differ from that of vegetative endpoints?
- Is there an effect on the next generation of herbicide-exposed NTTPs?
- How do species differ in their sensitivity to the herbicide sprayed at environmentally realistic exposure rates ?
- Which knowledge, guidance and experience can be drawn from the experiment for conducting field studies and/or multispecies studies with NTTPs?

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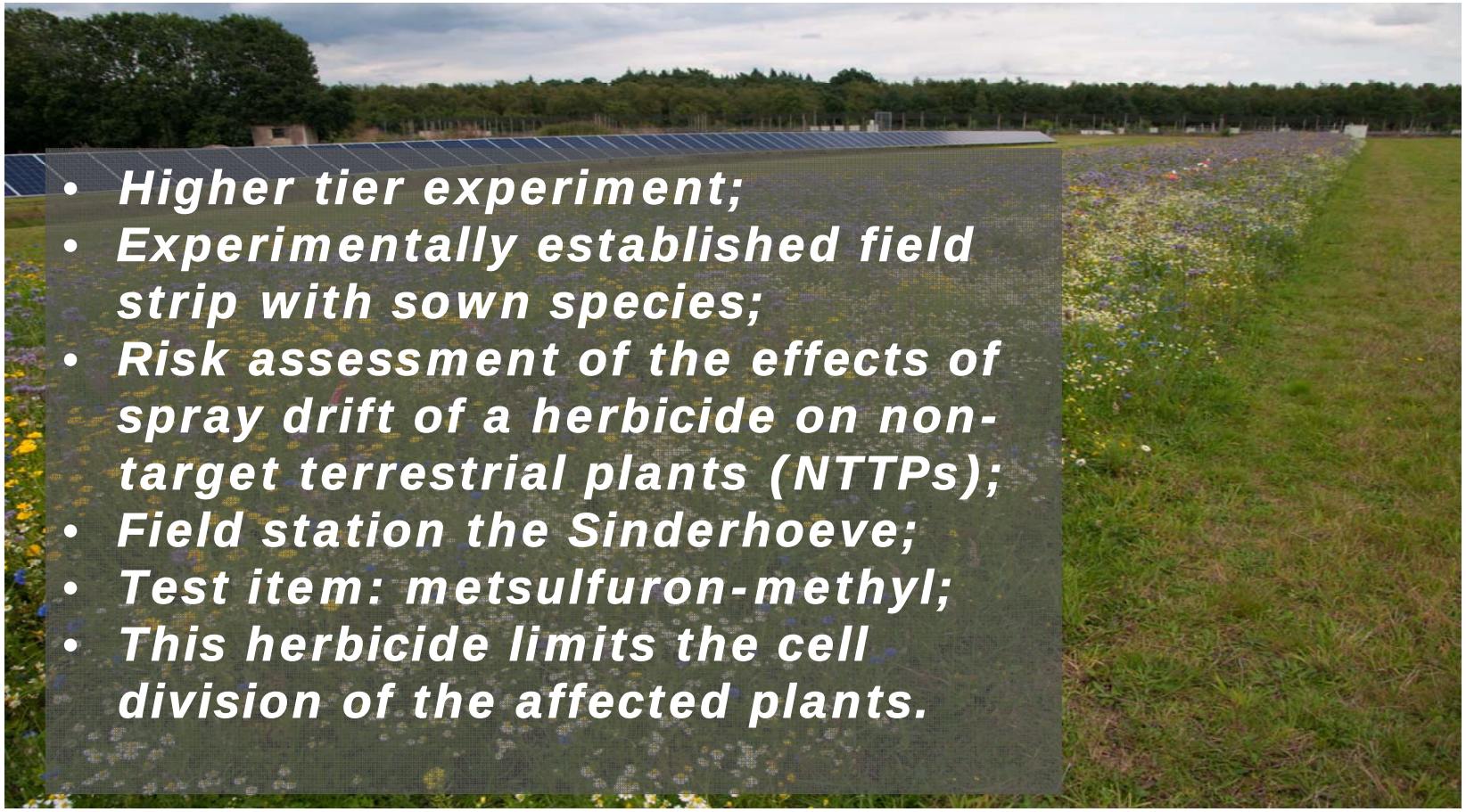
Approach



- *Higher tier experiment;*
- *Experimentally established field strip with sown species;*
- *Risk assessment of the effects of spray drift of a herbicide on non-target terrestrial plants (NTTPs);*
- *Field station the Sinderhoeve;*
- *Test item: metsulfuron-methyl;*
- *This herbicide limits the cell division of the affected plants.*

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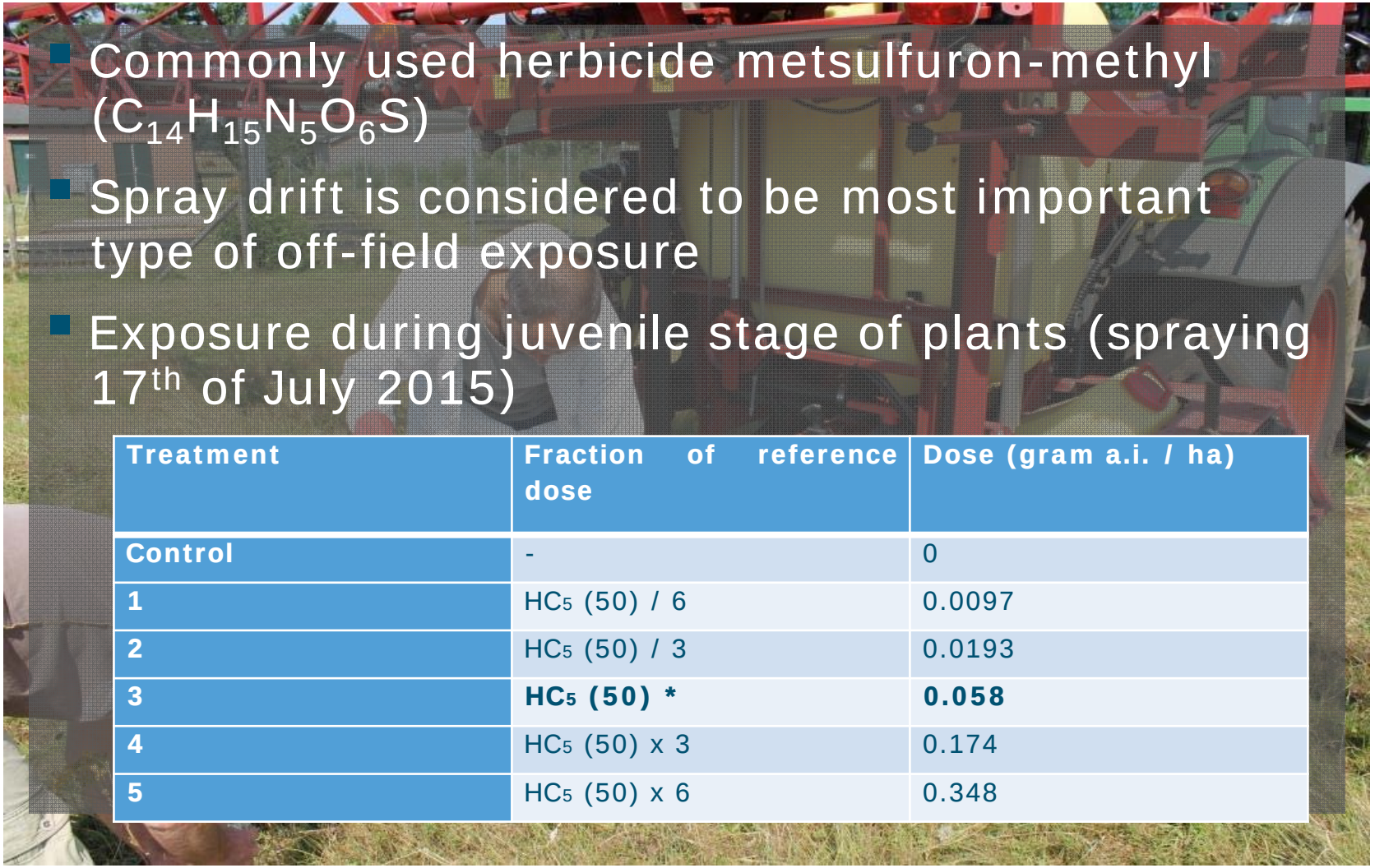
Selection of plant species

- Sown species in a mixture of perennial and annual non-crop species

Species	Family	Presence in field	Life cycle
Matricaria recutita	Asteraceae	x	Annual
Centaurea cyanus	Asteraceae	x	Annual
Sinapis alba	Brassicaceae	x	Annual
Phacelia tanacetifolia	Boraginaceae	x	Annual
Campanula persicifolia	Campanulaceae	-	Perennial
Papaver rhoeas	Culture	-	Annual
Melilotus officinalis	Fabaceae	x	Annual/Bi-annual
Consolida regalis	Ranunculaceae	-	Annual
Pastinaca sativa	Apiaceae	-	Perennial
Lupinus perennis	Fabaceae	x	Bi-annual
Ranunculus acris	Ranunculaceae	-	Perennial

Herbicide



- Commonly used herbicide metsulfuron-methyl ($C_{14}H_{15}N_5O_6S$)
- Spray drift is considered to be most important type of off-field exposure
- Exposure during juvenile stage of plants (spraying 17th of July 2015)

Treatment	Fraction of reference dose	Dose (gram a.i. / ha)
Control	-	0
1	HC_5 (50) / 6	0.0097
2	HC_5 (50) / 3	0.0193
3	**HC_5 (50) ***	**0.058**
4	HC_5 (50) x 3	0.174
5	HC_5 (50) x 6	0.348

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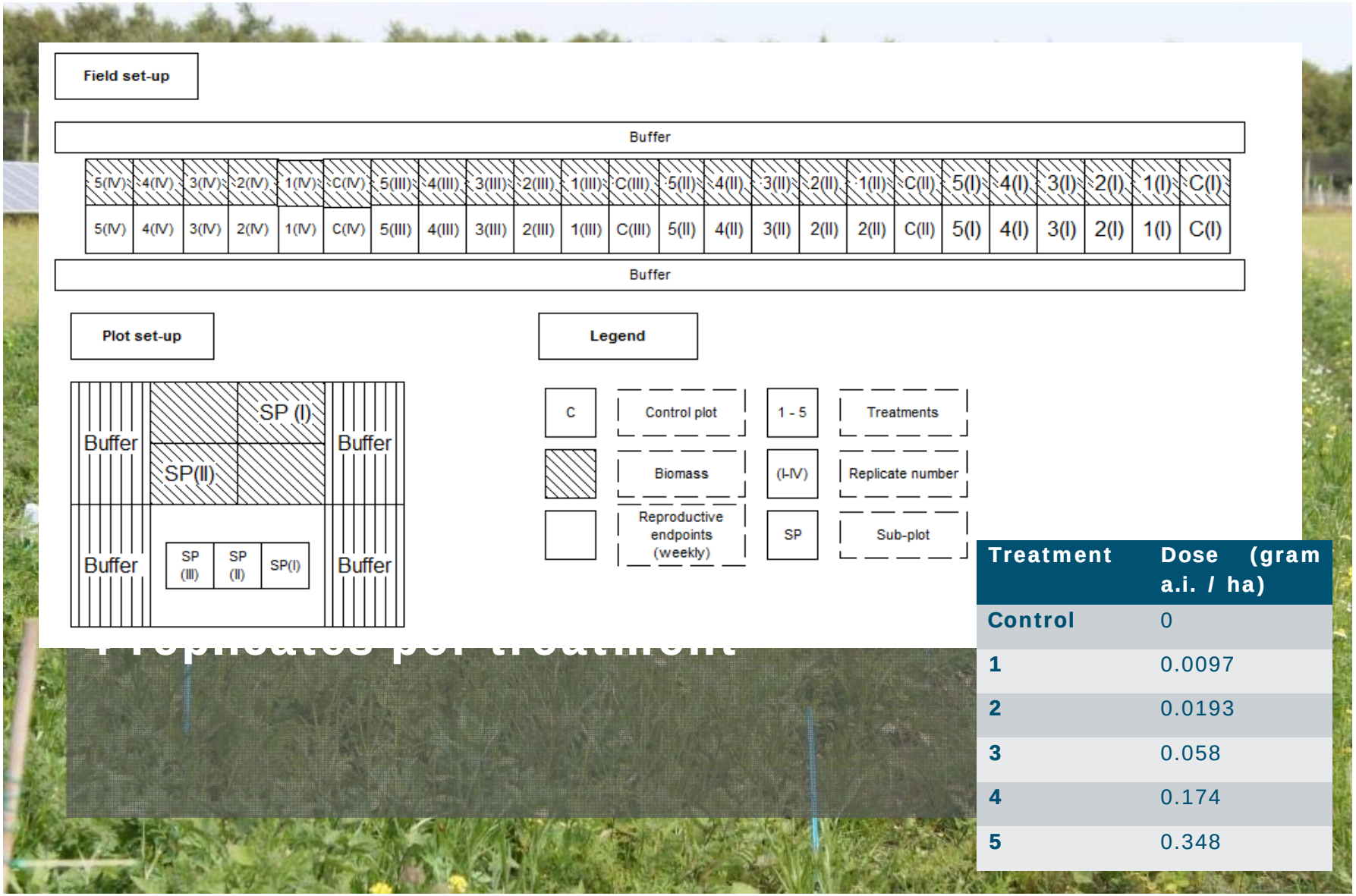
* Reference dosage, viz. the Hazardous Dosage at which 95% of the plants are potentially protected (HD_5), available from greenhouse tests (Boutin et al., 2000).

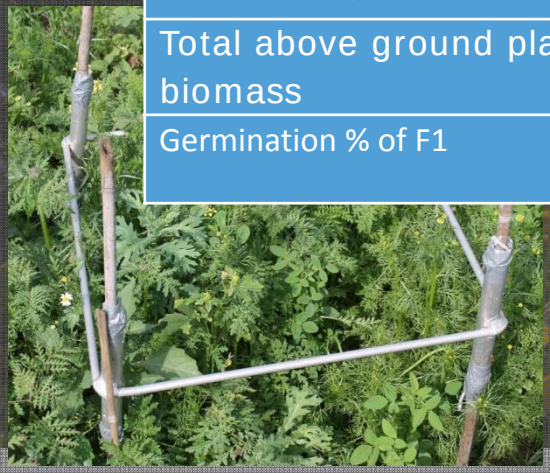
Experimental design



Treatment	Dose (gram a.i. / ha)
Control	0
1	0.0097
2	0.0193
3	0.058
4	0.174
5	0.348

Plant endpoints

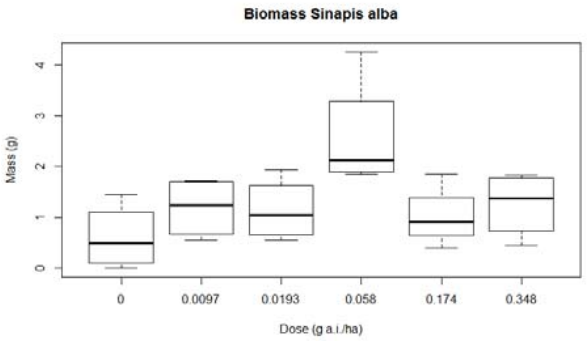
Plant Endpoints	Weekly over period of 4 months	Final sampling
Number of individuals	x	
Number of flowering individuals	x	
Plant cover estimation	x	
Fruit/seed production	x	
Total above ground plant biomass		x
Germination % of F1		Following final sampling

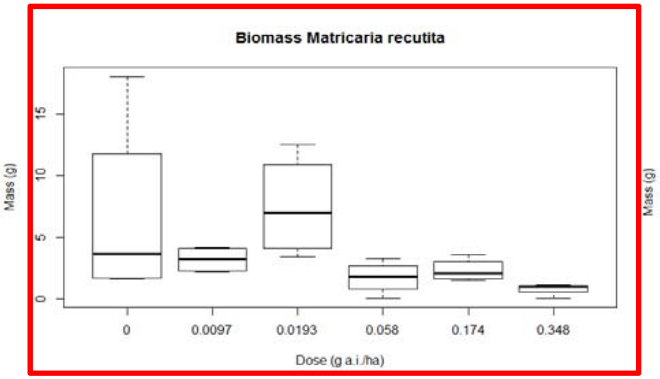


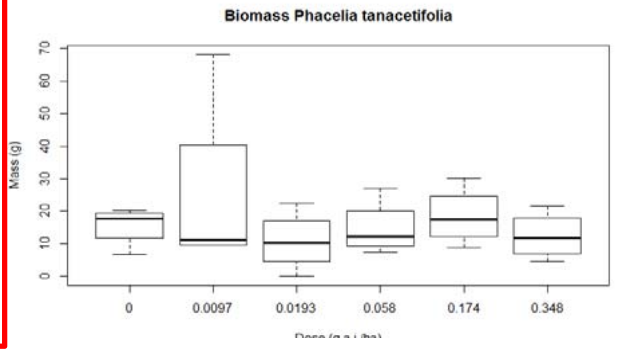
Handling of samples

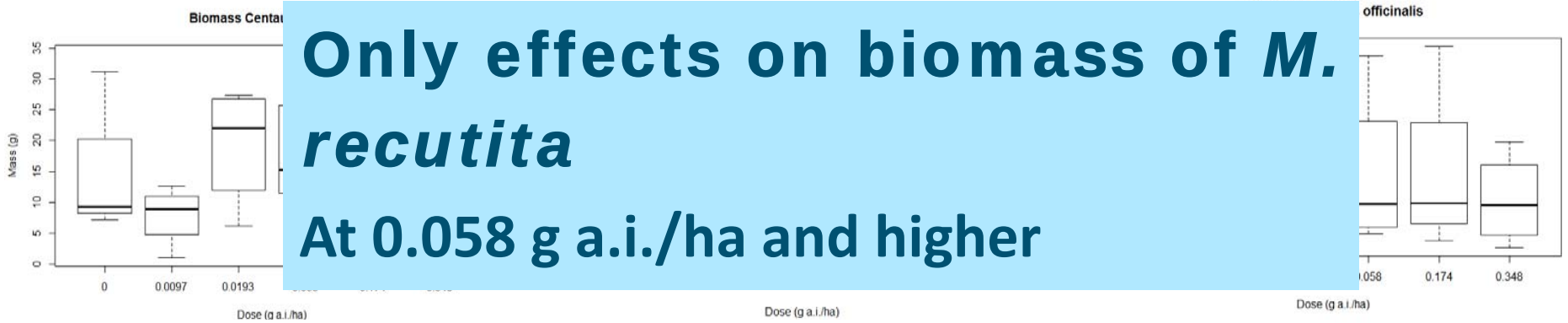
- Drying of plant material (biomass) in lab of UNIFARM
- Extraction of seeds from fruits in lab of UNIFARM
- Seed quality test in seed germination experiment in climate chamber

Results – Vegetative endpoints: biomass



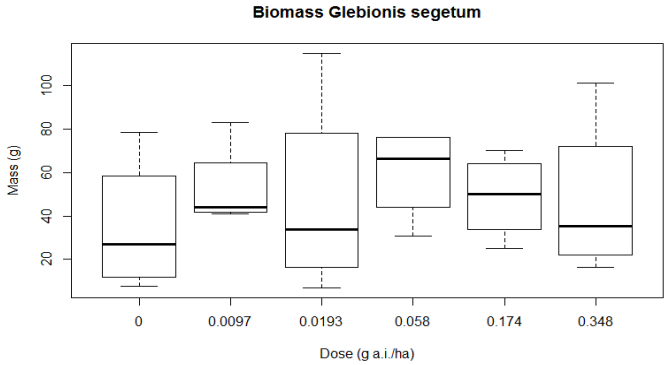


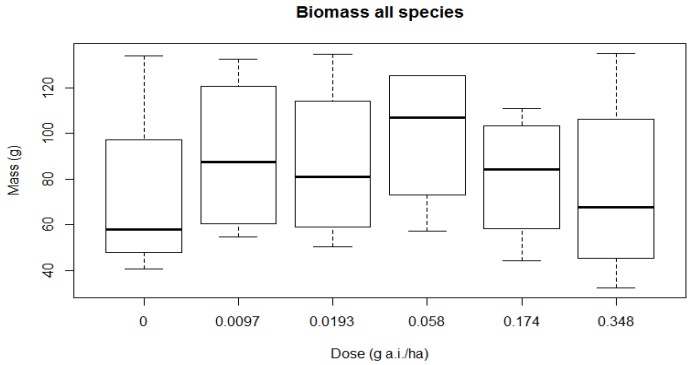




Only effects on biomass of *M. recutita*

At 0.058 g a.i./ha and higher





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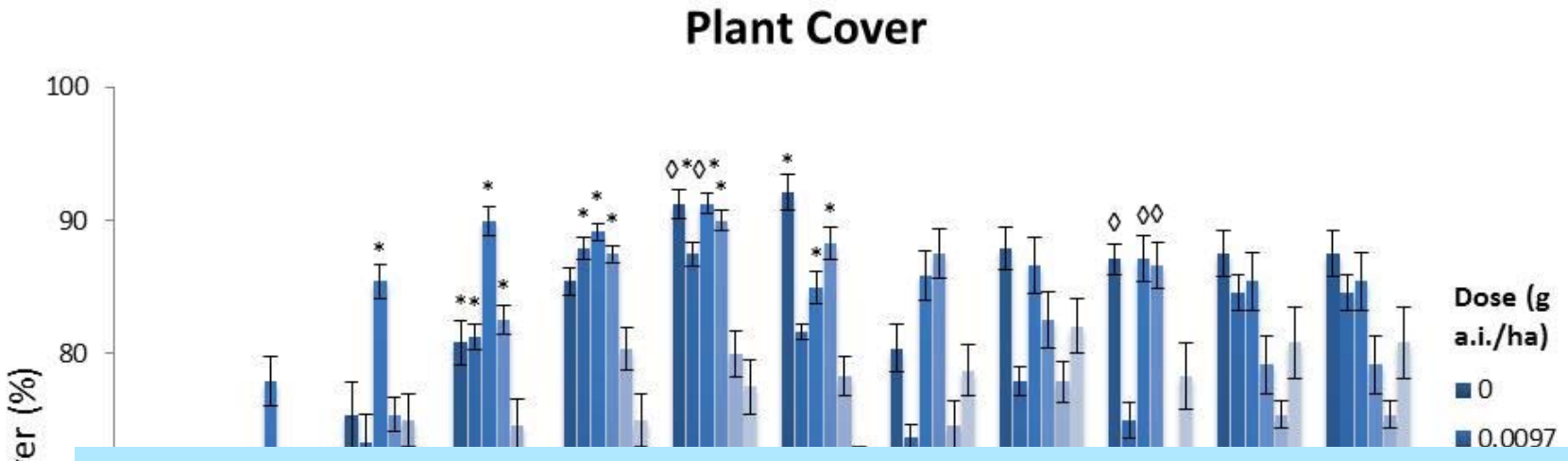
Results – Vegetative endpoints: plant cover



Significant differences in plant cover mainly in the first 7 weeks after spraying

Field dosages of 0.174 and 0.348 g a.i./ha differed significantly in the endpoint "plant cover" compared to lower dosages and controls.

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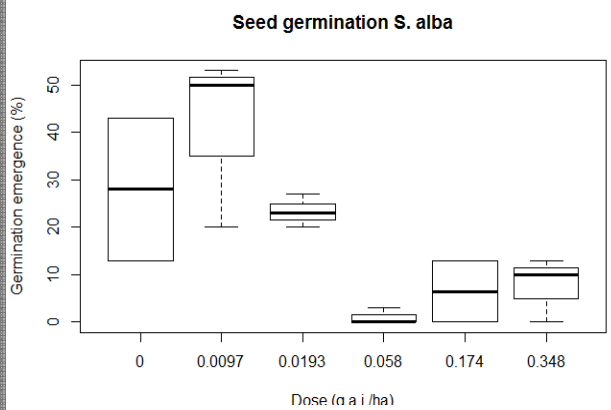
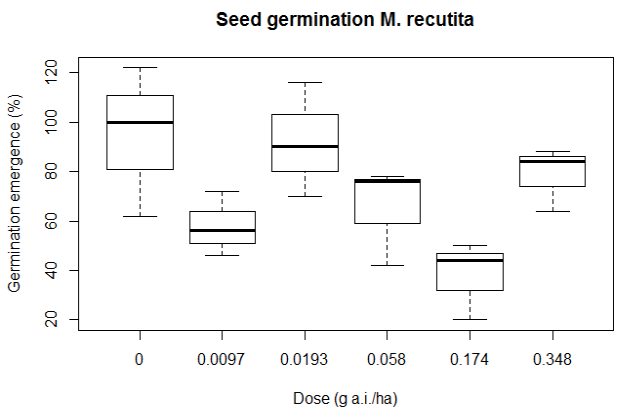
Results: Reproductive endpoints

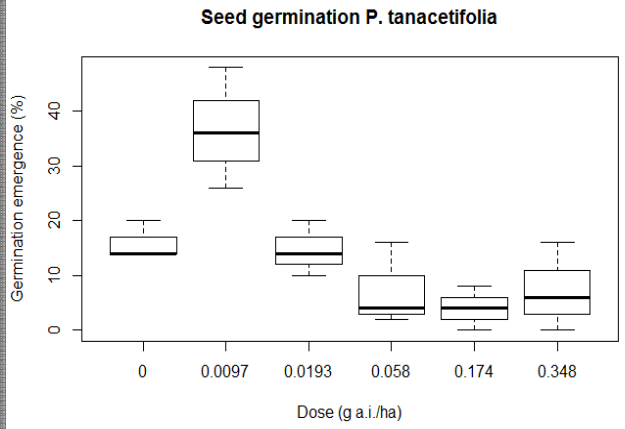
- Endpoints “mass per seed” and “number of seeds per fruit” most sensitive;
- No statistically significant differences;
- Control highest values of these endpoints in comparison with the plots treated with herbicide;
- *S. alba* in particular showed a high sensitivity of the “mass per seed” endpoint;
- *C. cyanus* and *M. reticutata* seemed to be mainly affected in their “number of seeds per fruit”.

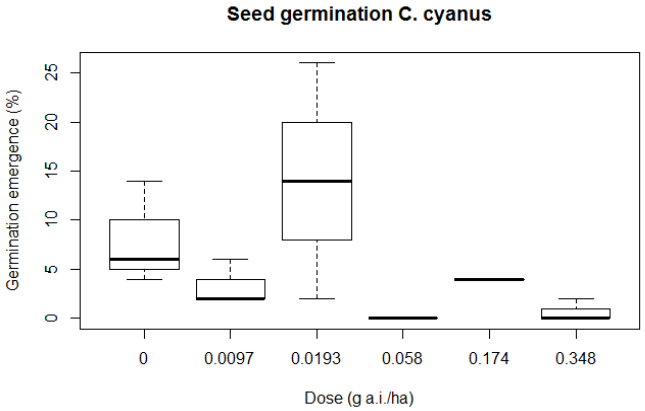
Results: Germination Experiment

- *M. recutitia* had highest germination (71.1%), second *S. alba* (18%), third *P. tanacetifolia* (14.3%) and last *C. cyanus* (5%).
- Significant effects ($p<0.05$; Kruskall Wallis test) in seed emergence for 3 species.
- Hormesis seemed to occur as well









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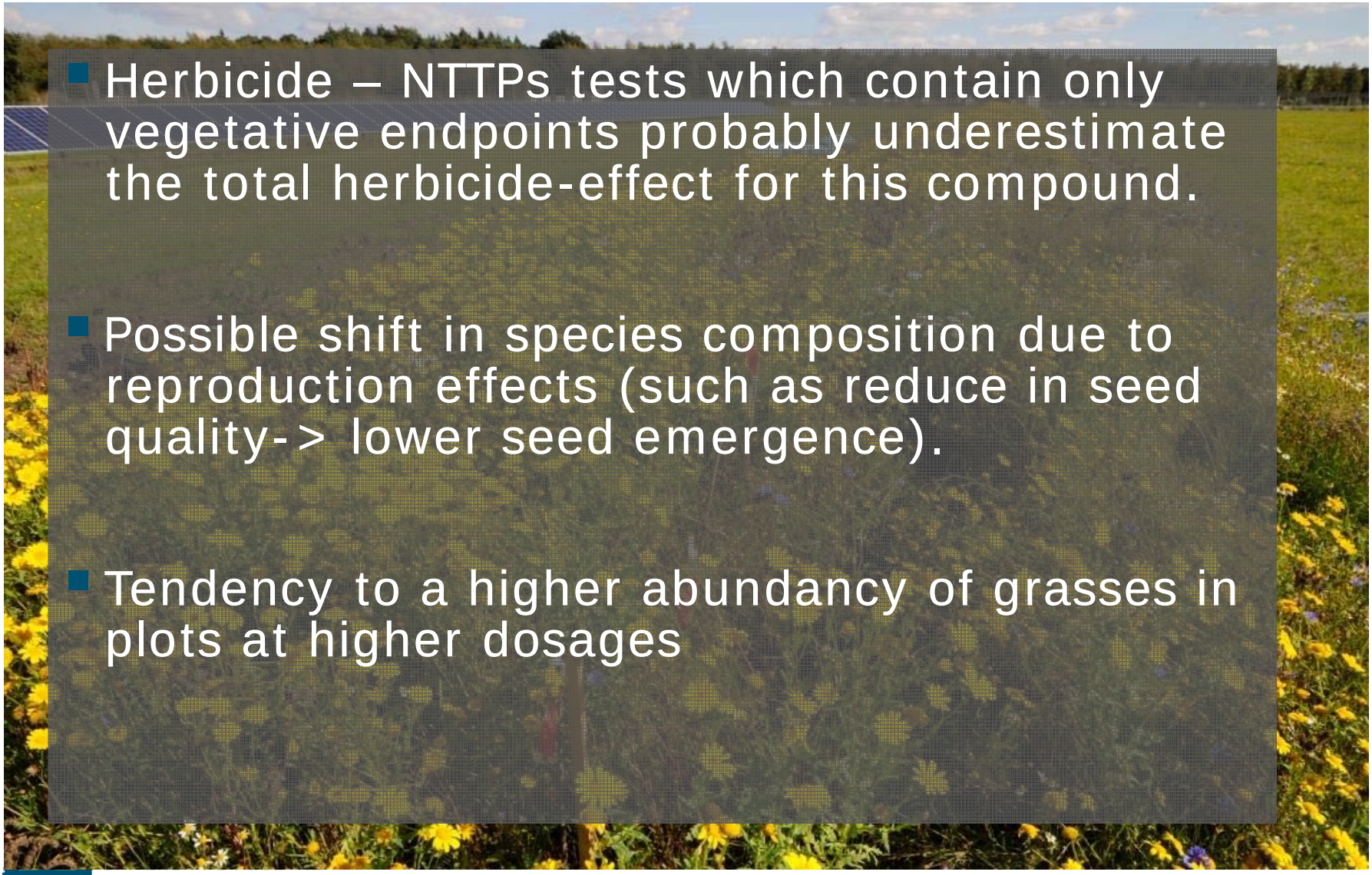
Discussion



- Herbicide – NTTPs tests which contain only vegetative endpoints probably underestimate the total herbicide-effect for this compound.
- Possible shift in species composition due to reproduction effects (such as reduce in seed quality-> lower seed emergence).
- Tendency to a higher abundancy of grasses in plots at higher dosages

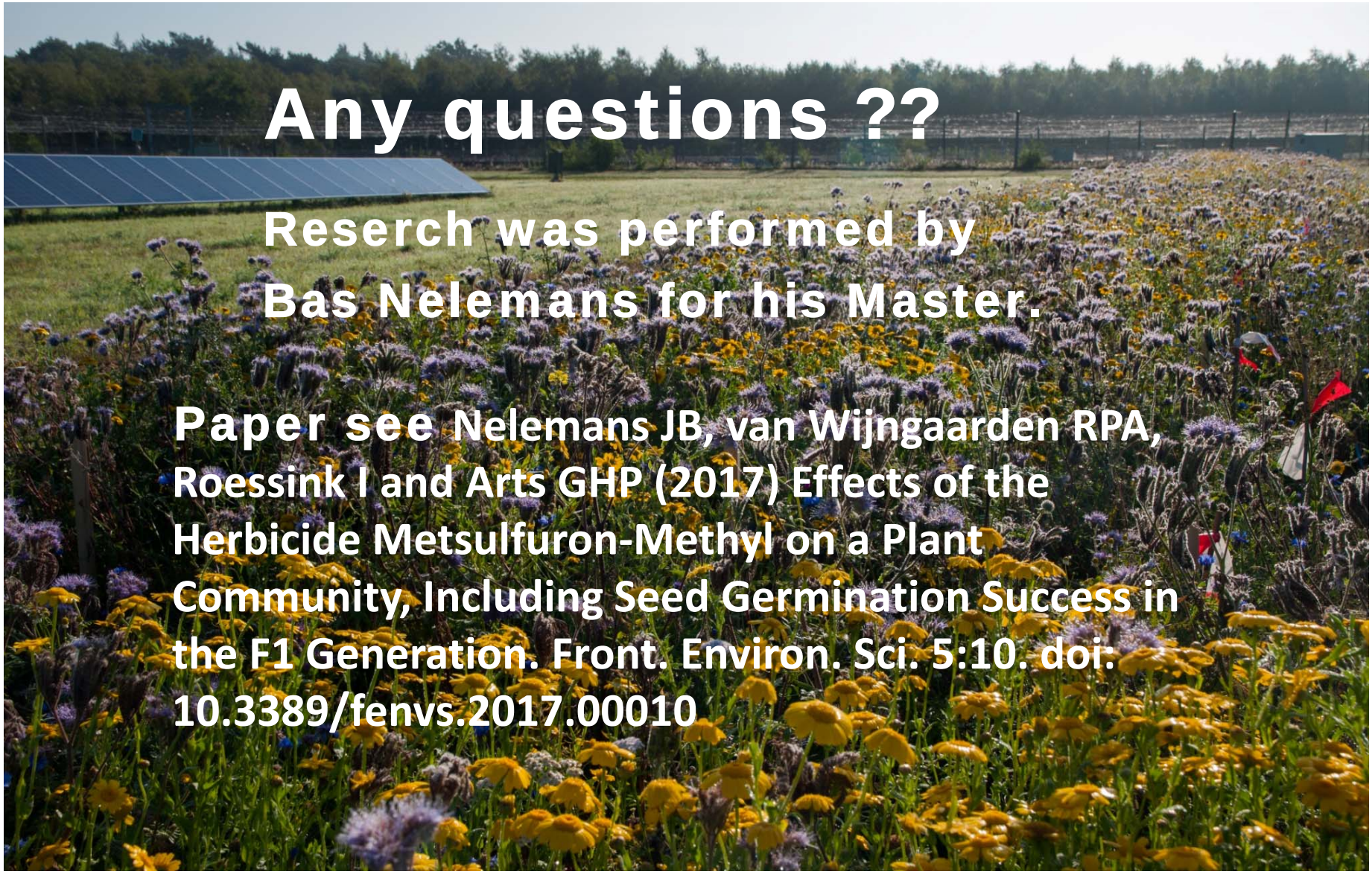
Recommendations

- Investigate **all possible confounding factors** (soil quality, seed bank, topography);
- Aim for **equal frequencies** per species;
- **At least** 2 years / growing seasons;
- Include plots with treatment: fertilizer + herbicide;
- Complete randomized block design;
- Intense monitoring in first weeks, less thereafter;
- Include reproductive endpoints !;
- Include species with good harvestable seeds (>0.15 mg per seed);

Conclusions / Summary

- How does the sensitivity of reproductive endpoints differ from that of vegetative endpoints? Reproductive endpoints, especially “mass per seed” and “number of seeds per fruit”, seemed to be more sensitive than vegetative endpoints;
- Are there any effects on the next generation of herbicide-exposed NTTP? 3 out of 4 species (*S. alba, P. tanacetifolia* and *C. cyanus)* had significantly lower seed germination rates at herbicide dosages of 0.0193 g a.i./ha and higher;
- How do species differ in their sensitivity to the herbicide?: The plant species reacted differently. *S. alba P. tanacetifolia* and *C. cyanus* sensitive in terms of reproductive endpoints and seed germination, *M. recutita s*ensitive in biomass.

Thank you


Any questions ??
Reserch was performed by
Bas Nelemans for his Master.
Paper see Nelemans JB, van Wijngaarden RPA, Roessink I and Arts GHP (2017) Effects of the Herbicide Metsulfuron-Methyl on a Plant Community, Including Seed Germination Success in the F1 Generation. Front. Environ. Sci. 5:10. doi: 10.3389/fenvs.2017.00010