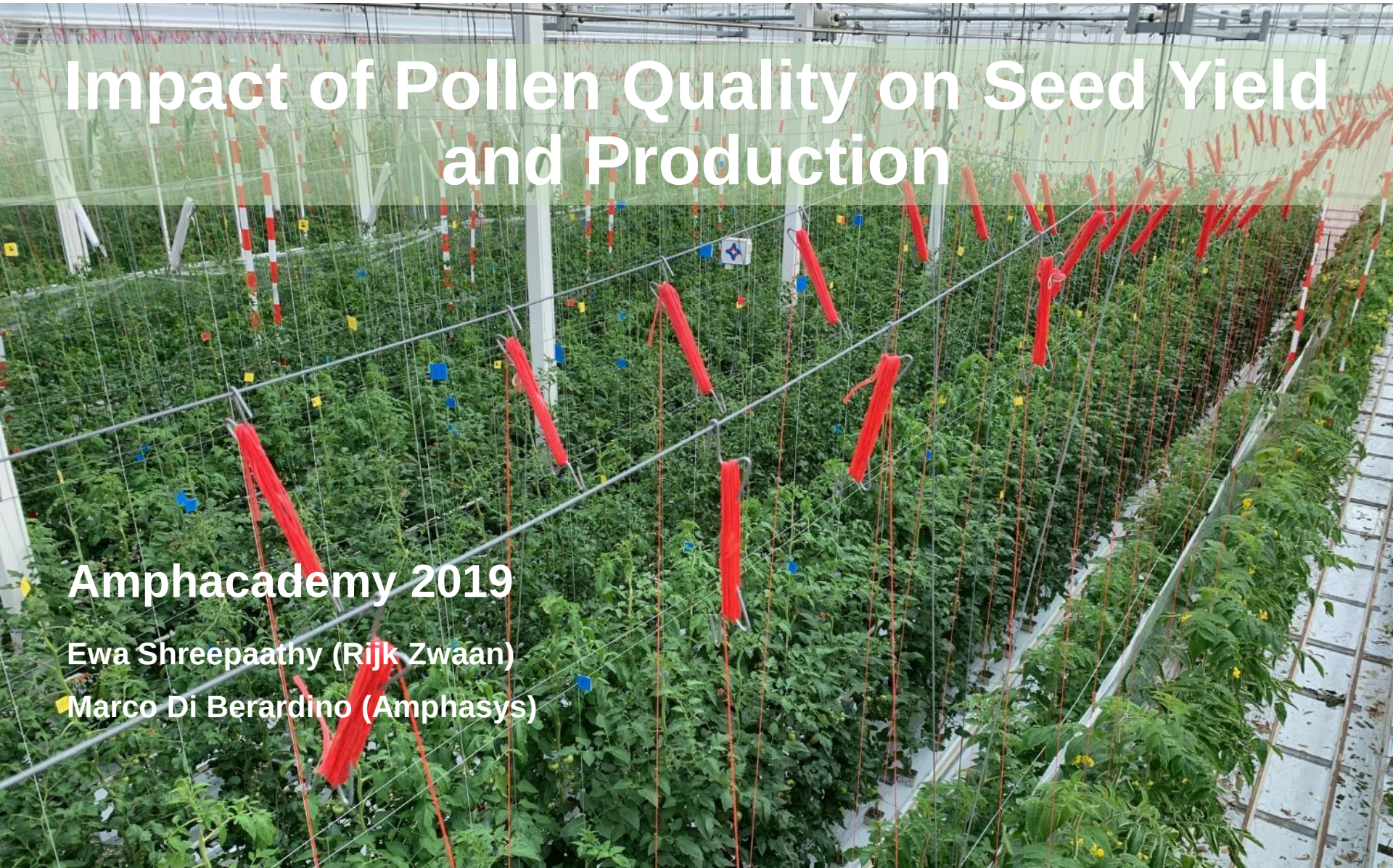
Impact of Pollen Quality on Seed Yield and Production



Amphacademy 2019

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Research Goal of the Project

Provide a robust and reliable solution, from Amphasys, to measure the quality of frozen/stored tomato pollen:

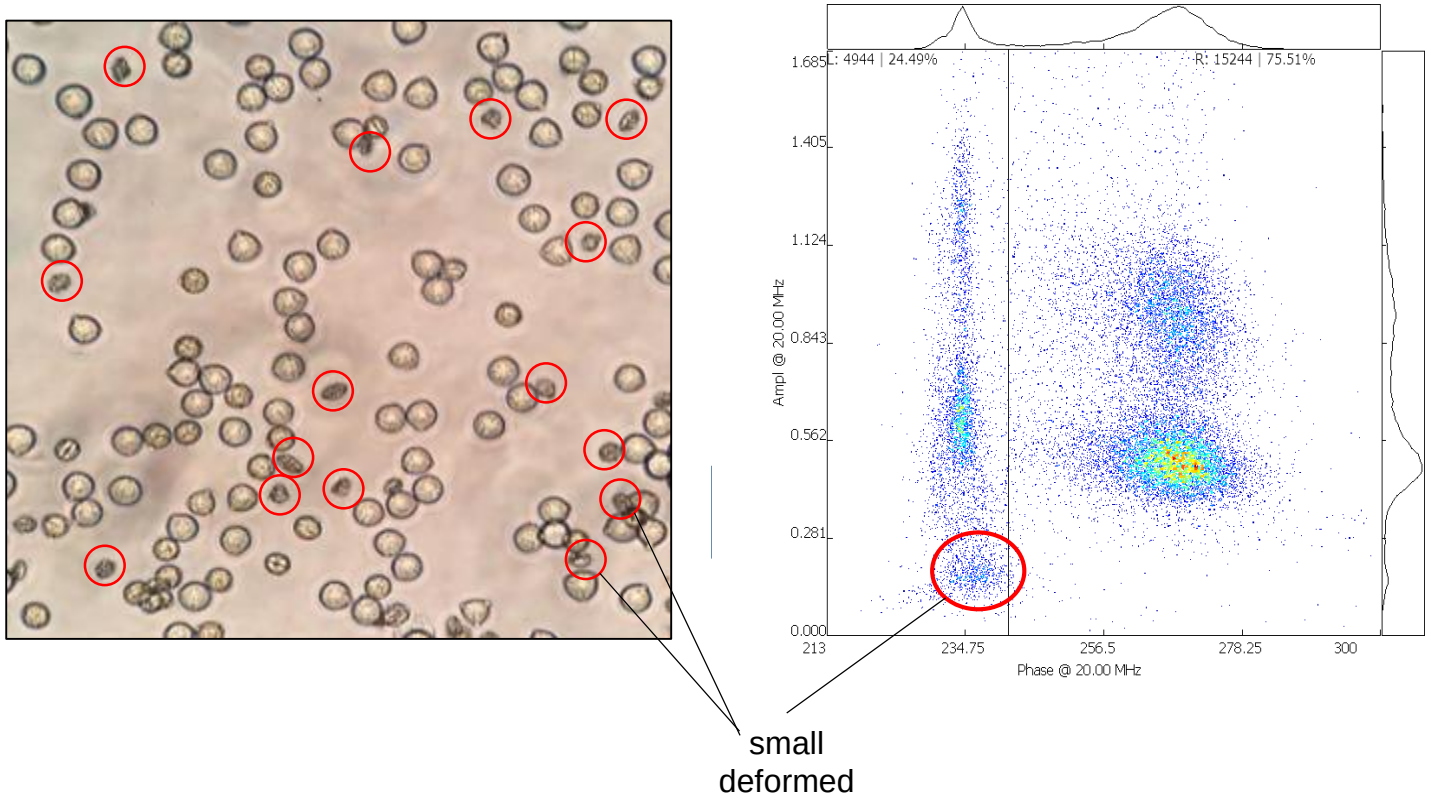
1. to establish the pollen quality needed to obtain an optimal seed set

2. to determine the variation per harvest day and per variety, and therefore to identify the gap between minimal requirement and actual pollen quality

3. determine the advantages of the Amphasys method over the germination tests

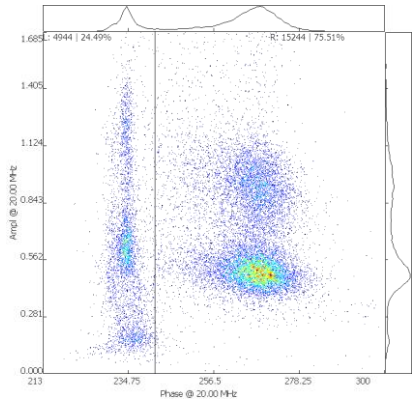
Optimization of Ampha Z32 Settings (1)

Comparing IFC to germination

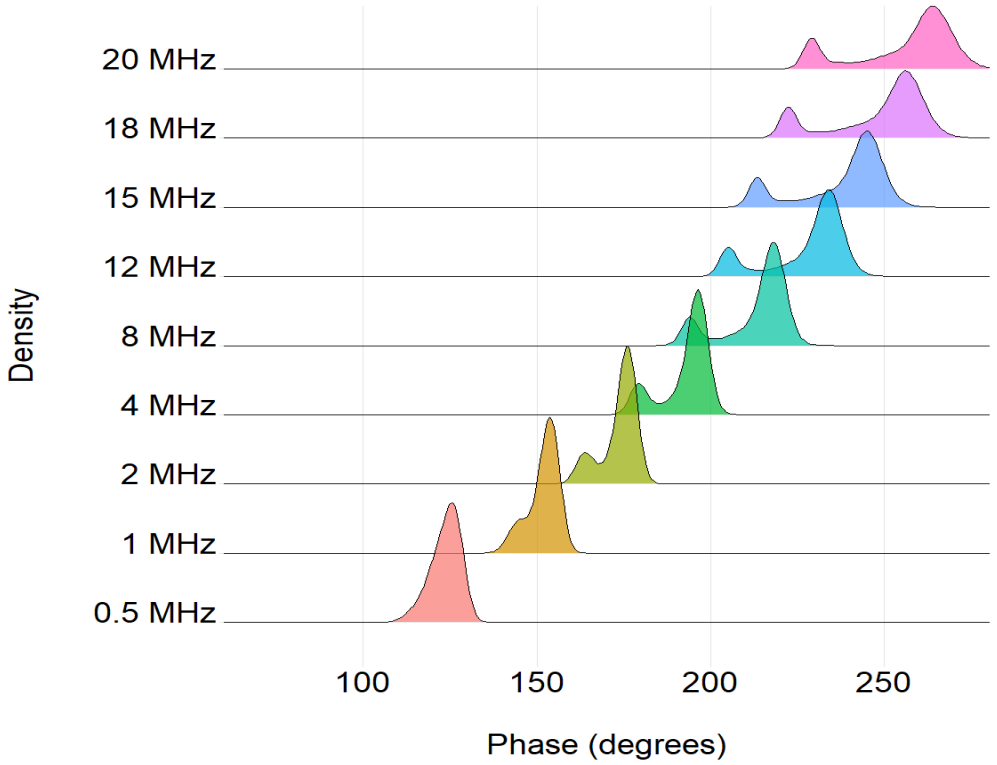


- Adjust gain and trigger level settings to reveal shrunken cells (needed to validate germination analysis).

Optimization of Ampha Z32 Settings (2)

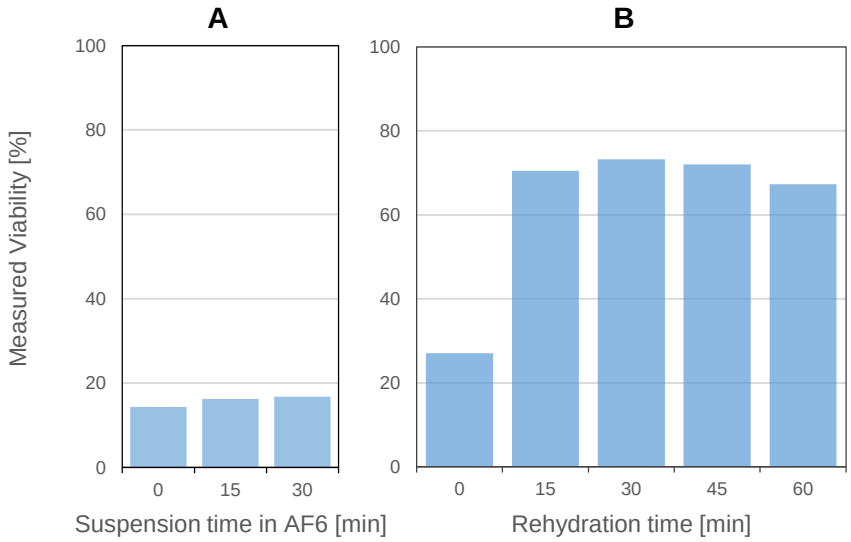


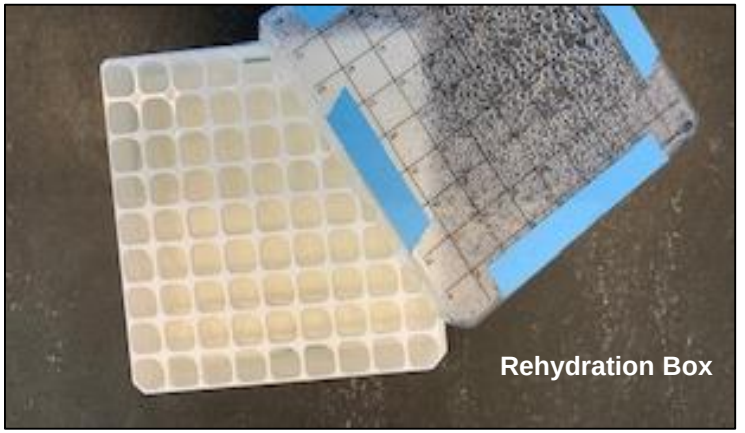
Frequency optimization



- Best dead/viable-separation at frequencies > 12 MHz
- Settings for protocol: 2/18 MHz

Optimization of Sample Preparation

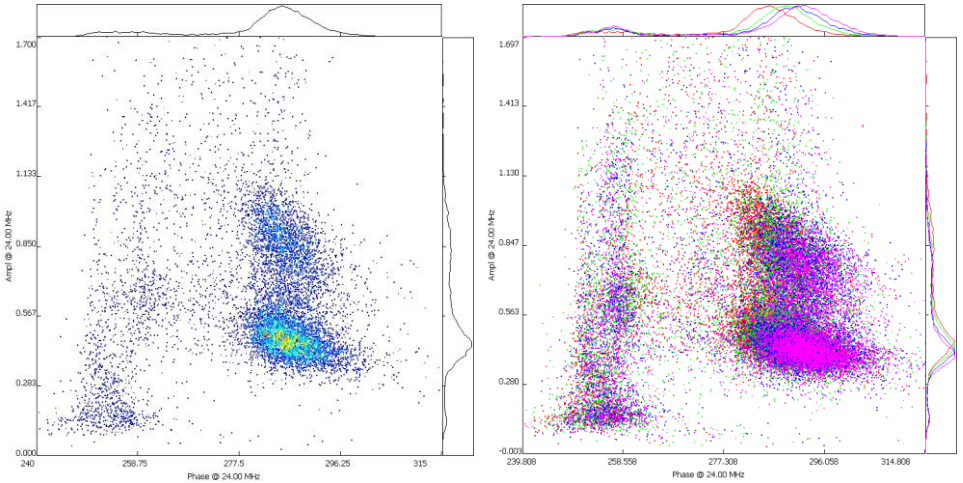




Rehydration process:

- Rehydration does not happen when suspended in AF6 (A)
- Optimal: 30 min in rehydration box (B)
- Process is reversible
- It is needed to pre-condition 5 min in AF6 before analysis

Pre-conditioning in AF6 buffer

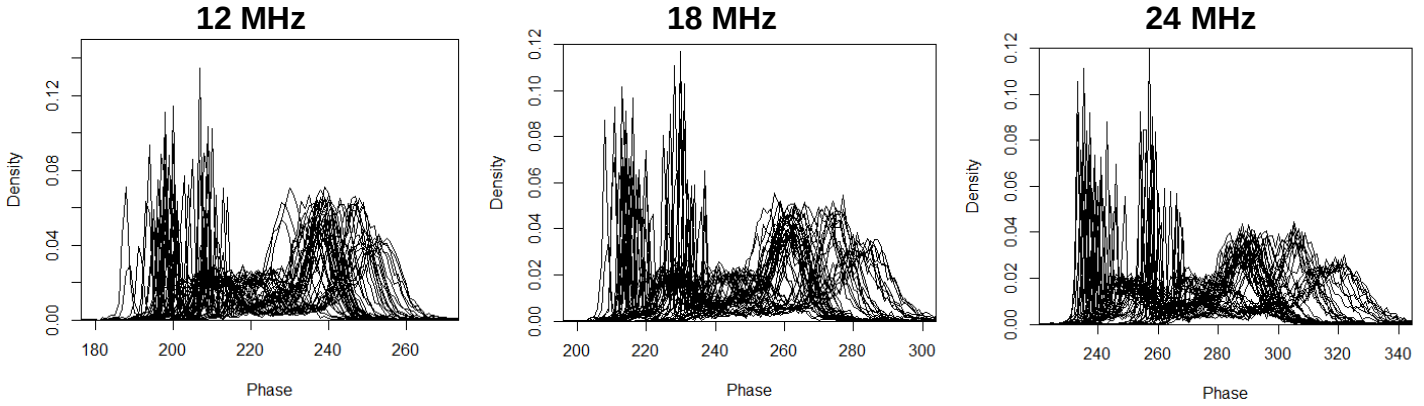


Autocalibration Feature

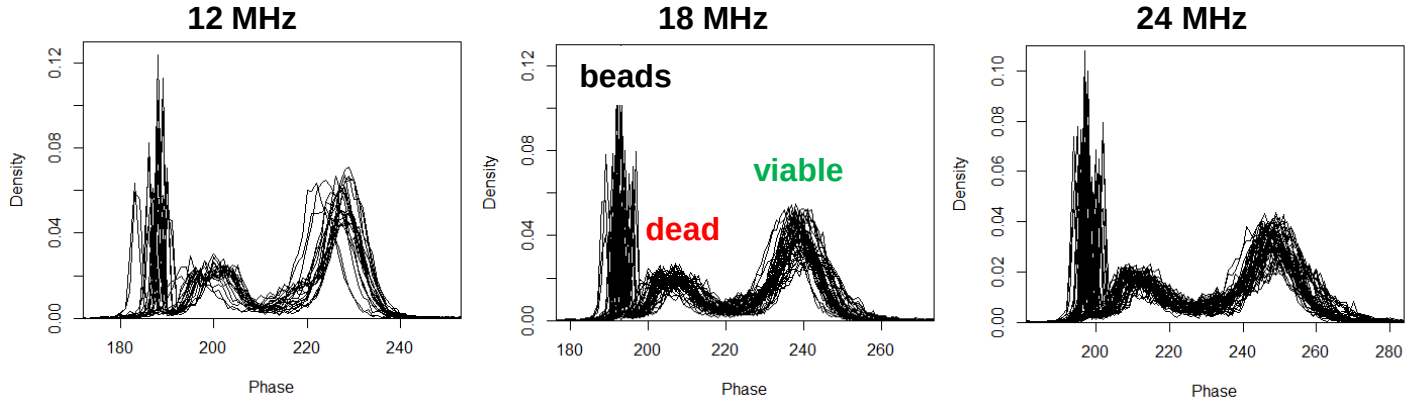
Sample: Tomato pollen (same line) in AF6 + 30 µm beads

- Measurements with 10 different chips, 4 different frequencies and 2 different instruments
- Overlay of 50 measurements

normal mode



with autocalibration

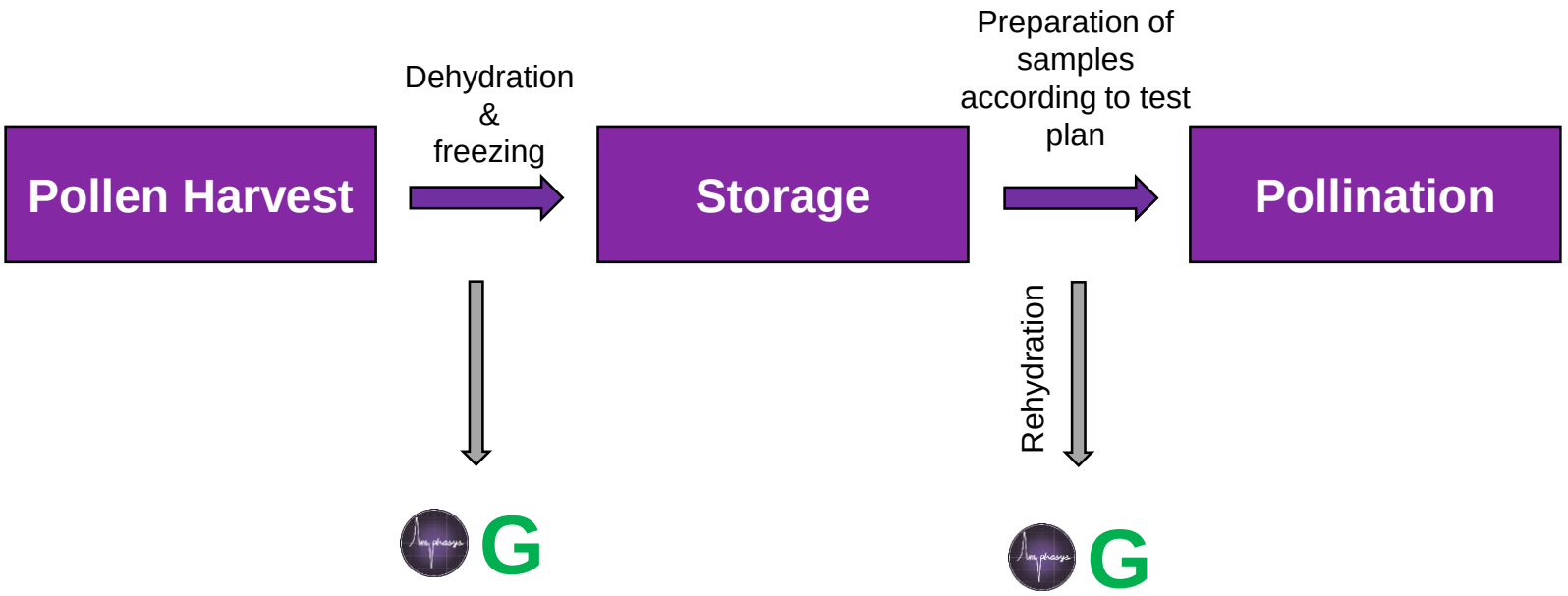


Templates possible and robust

Pollen Analysis Schedule

Determination of viability (2 replicas)

G Determination of germination (3 replicas, to increase accuracy)



Experimental Setup

Seed production

Fruit development

Fruit harvest

Seed extraction

Seed counting

Experimental Setup

12 representative varieties of RZ assortment

→ Total 812 plants (incl. females and males)

→ Greenhouse area: 500 m^2

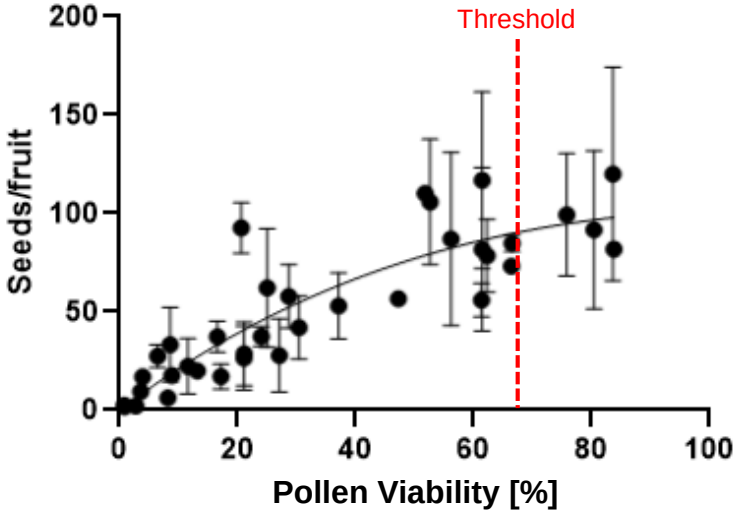
replicated randomly in space and in time (several pollination moments)

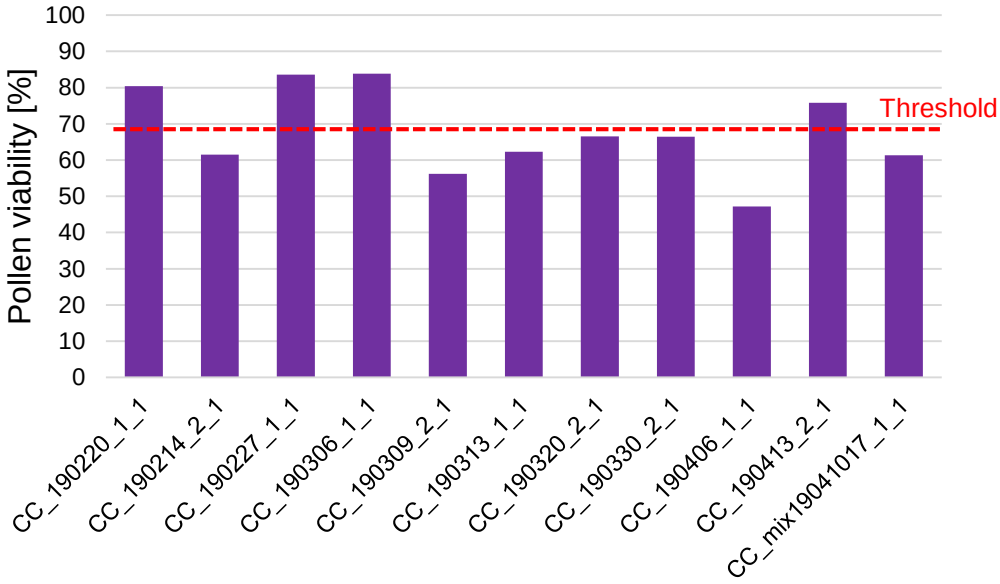
→ 2000+ pollen analyses

→ 3000+ germination assays

Pollen Viability and Seed Set

Example Line

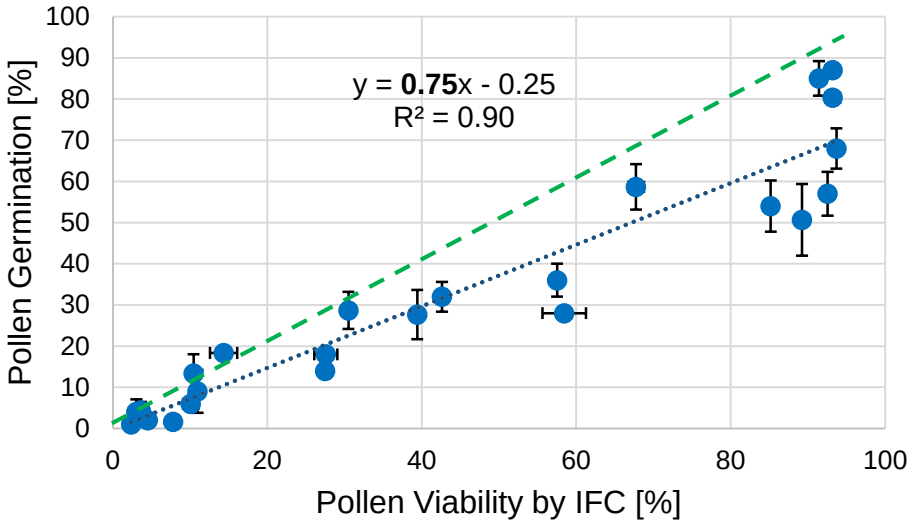




- General findings (all lines):
  - Clear correlation between pollen viability and seed set
  - Correlation between pollen viability and seed set is dependent on variety (not shown)
  - Therefore threshold depends on variety (not shown)
  - Pollen viability varies over trial period
- Findings specific for above example line:
  - Full seed set obtained with pollen viability > 70%

Pollen Viability (Amphasys) vs Germination

What	Viability with Ampha Z32	Pollen-Germination
Time to result	1-2 min	120 min
Analysis time	2 min (duplicate)	± 8 min (triplicate)
Sample size	> 10’000 cells	100 cells
Precision	< 1% SD	3 - 9 % SD
Bias	no	yes, dependent on technician
Workload	Space for other activities	Focused activity during counting



- Strong correlation between pollen viability and germination
- Germination in general with lower values than viability
- Viability measurement more precise and faster
  - One measurement is precise enough
  - Time saving for 100 analyses = 600 -700 min > 10 h!

Conclusions

- All analyzed male lines showed a clear correlation between pollen viability and seed set. Minimum pollen quality required for optimum seed set was identified for individual lines.
- Pollen viability can be influenced by the collection day, storage and variety.
- Pollen Quality Control can help in management of pollen production by:
  - Optimization of greenhouse space
  - Dilution of pollen batches to target viability
  - Re-use of stored pollen batches for dilutions
- In vitro pollen germination and Ampha Z32 were comparable for all tested lines. Advantages of Ampha Z32 over germination:
  - More precise and faster
  - Less prone to human error
  - Gives more information about cell physiology
- Data set obtained in this study indicates that application can be automated by templates.
- Ampha Z32 is therefore a reliable tool with high potential for implementation on larger scale.