



Accelerating Crop Improvement
with Pollen Quality Analysis

Improved seed yield and forecasting through pollen quality monitoring

Dr. Marcel Ottiger, Executive Partner



The Challenge



Content



1. Why invest in pollen quality analysis?
2. How Impedance Flow Cytometry works
3. Examples of use cases



Why Invest in Pollen Quality Analysis?



BREEDING

Male line selection

Improved crossing efficiencies

Microspore development

To save time and money in breeding programs

PRODUCTION RESEARCH

Optimize collection, drying and storage

Set quality gates in supply chain

Optimize F to M ratio

Improved synchronization

To optimize processes for higher yields

SEED PRODUCTION

Quality control

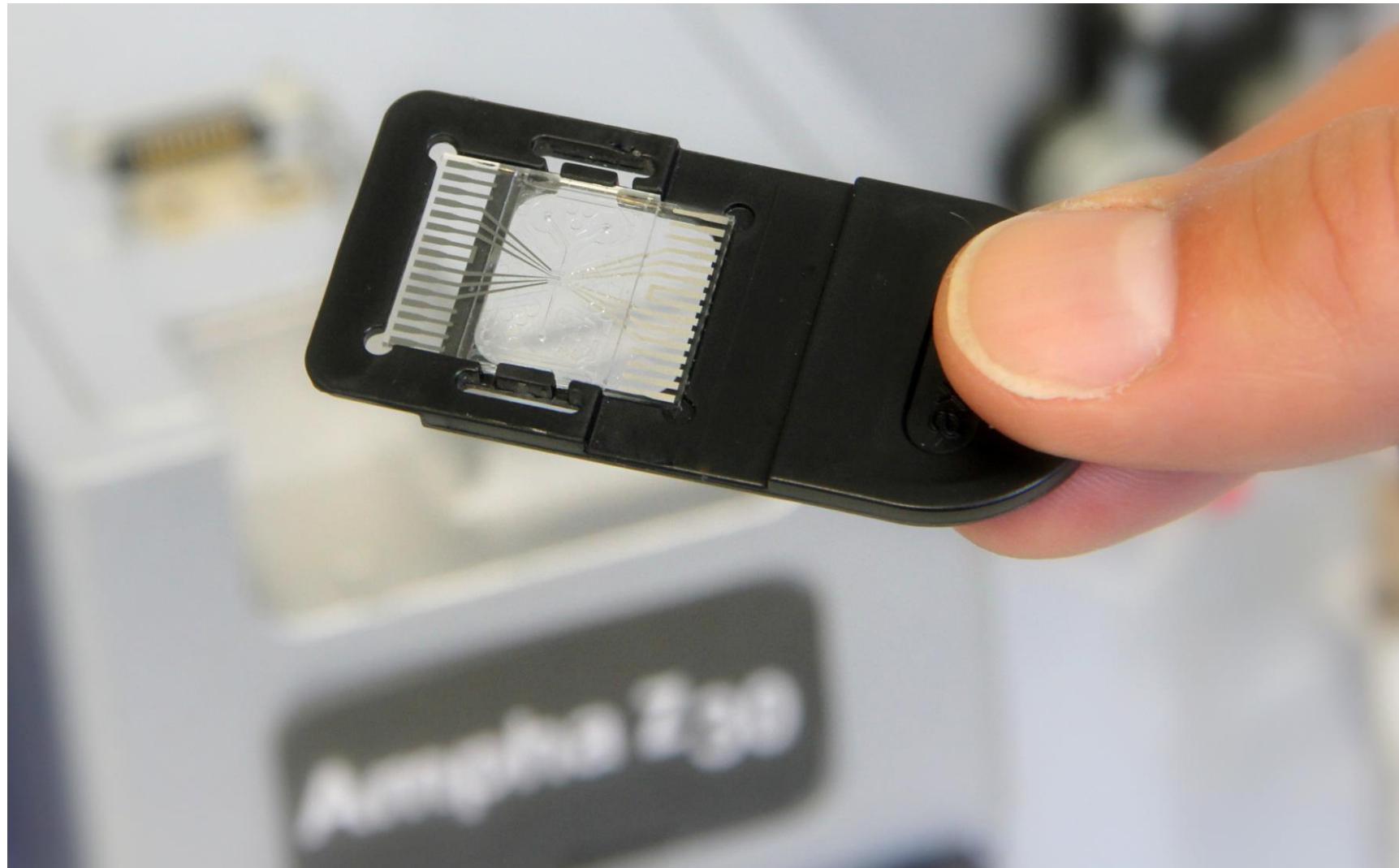
Maximize daily pollination window

Improved yield forecasting

Optimize manual pollination

To minimize costs and losses in production

How Impedance Flow Cytometry Works



Impedance Flow Cytometry (IFC)



To replay the film please visit
www.amphasys.com/lab-on-chip_label-free

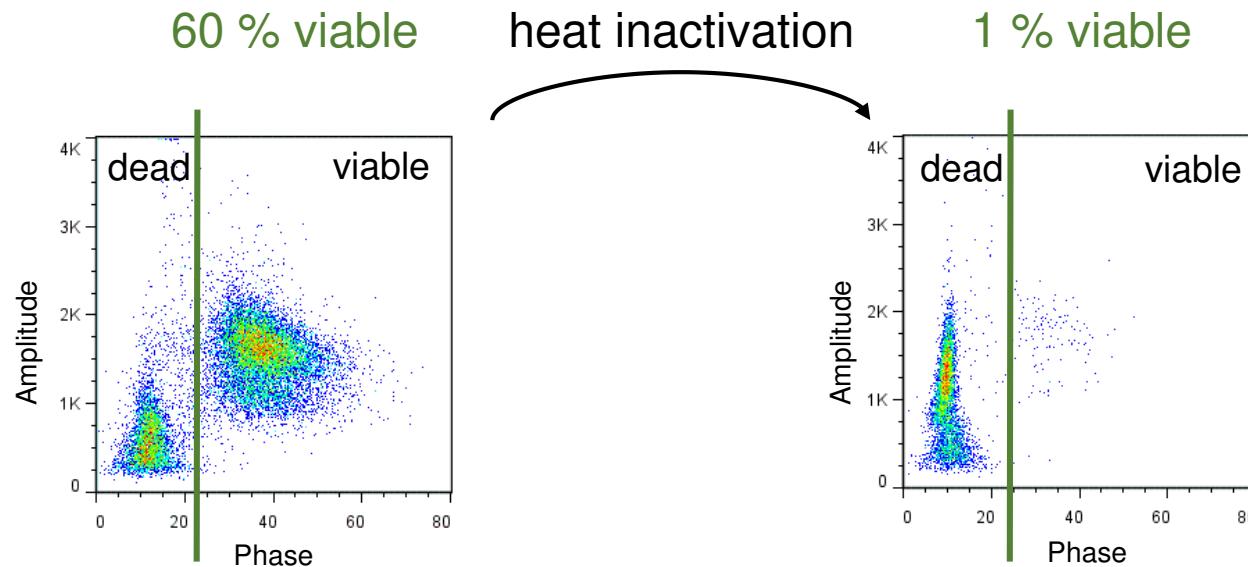


In this chip we measure changes of the electrical impedance of a fluidic medium when cells pass through the applied electric field.

Detection of Pollen Viability by IFC



- Detection, counting, and distinction of dead and viable pollen
- Fast, easy and reliable measurement, in the field or lab
- Works for all species (< 180 µm)



Limitations of Pollen Viability by Microscopy



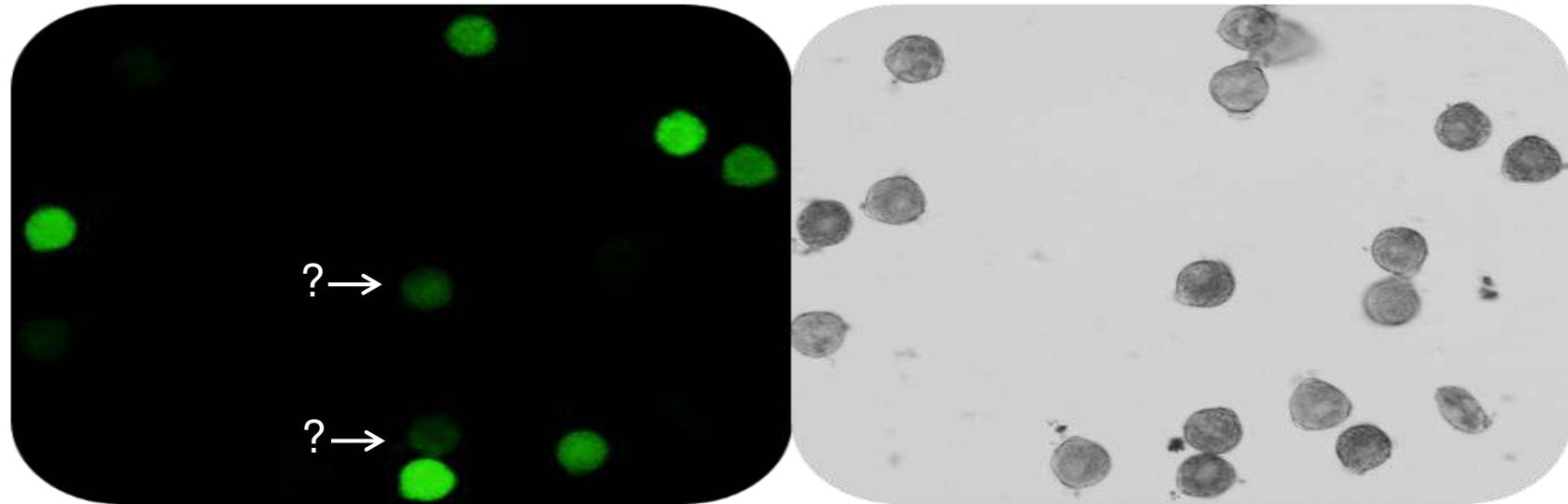
Low statistical relevance

Low throughput

Complex sample prep

Operator dependent

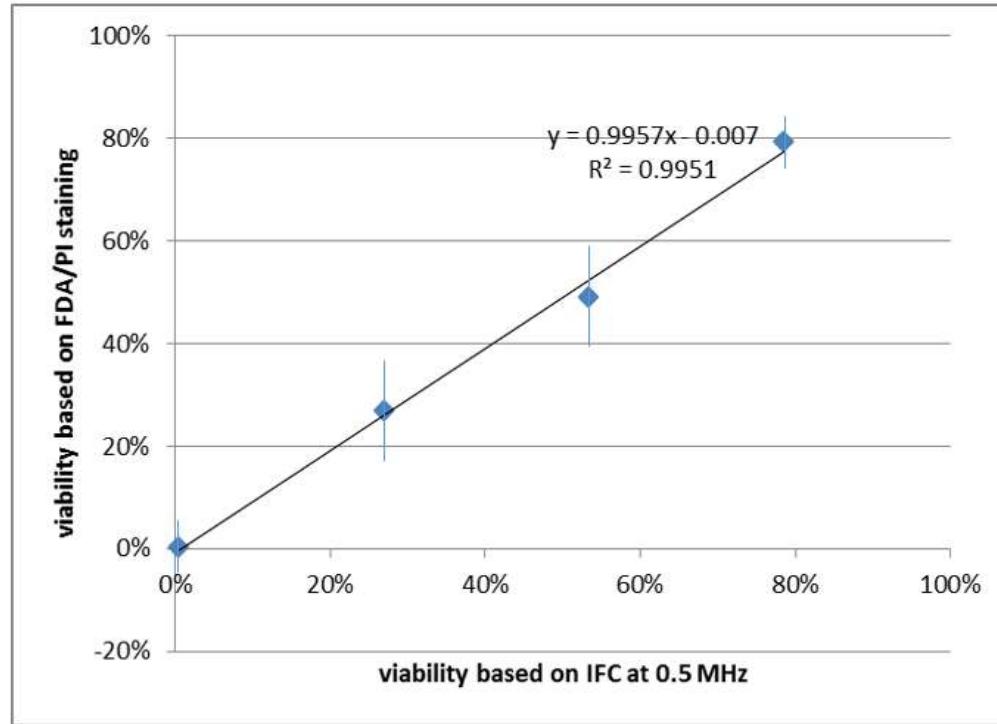
Does not work
for all species



Advantages of IFC over Microscopy



IFC Viability vs. FDA/PI staining



IFC

- > 10'000 pollen / min
- Error IFC < 1%
- Results in 1-2 minutes
- all species < 180 µm

FDA/PI staining

- ~100 pollen per assay
- Error 10-15%
- Results in 15 min.
- some species do not work

Source confidential, 2015

“Viability by IFC is faster and more precise than microscopy”

Limitations of Pollen Germination Analysis



Low statistical relevance

Low throughput

Error-prone sample preparation

Subjective scoring

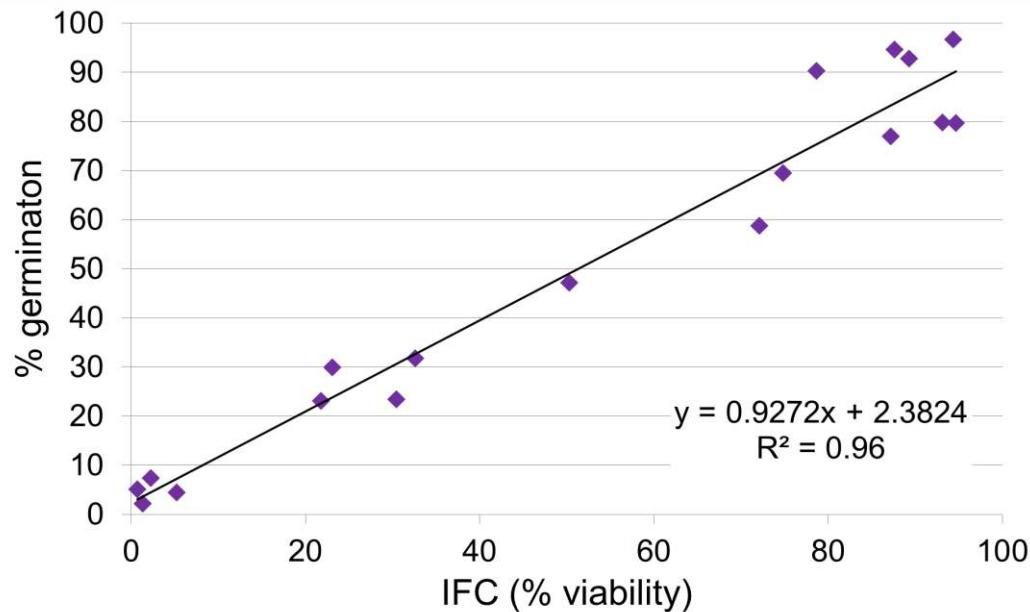
Manual procedure



Advantages of IFC over Germination Assays



IFC Viability vs. Germination



IFC

- > 10'000 pollen / min
- Error IFC < 1%
- Results in 1-2 minutes

Germination (in vitro)

- ~100 pollen per assay
- Error 10-20%
- Results in 90 min.

[Source: Arvalis, 2018](#)

“IFC is an efficient alternative to germination assays”

Pollen Viability and Germination/Vigor

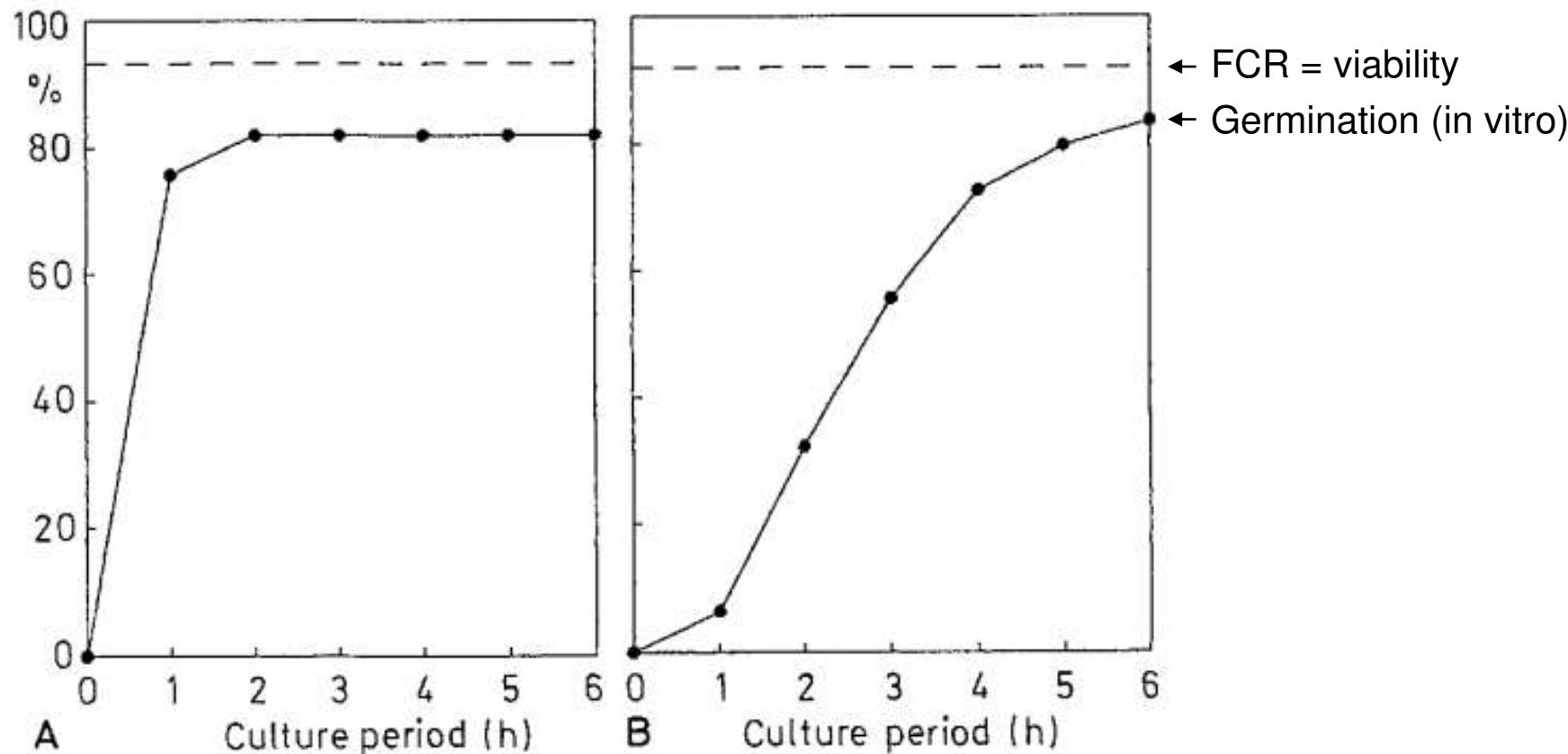
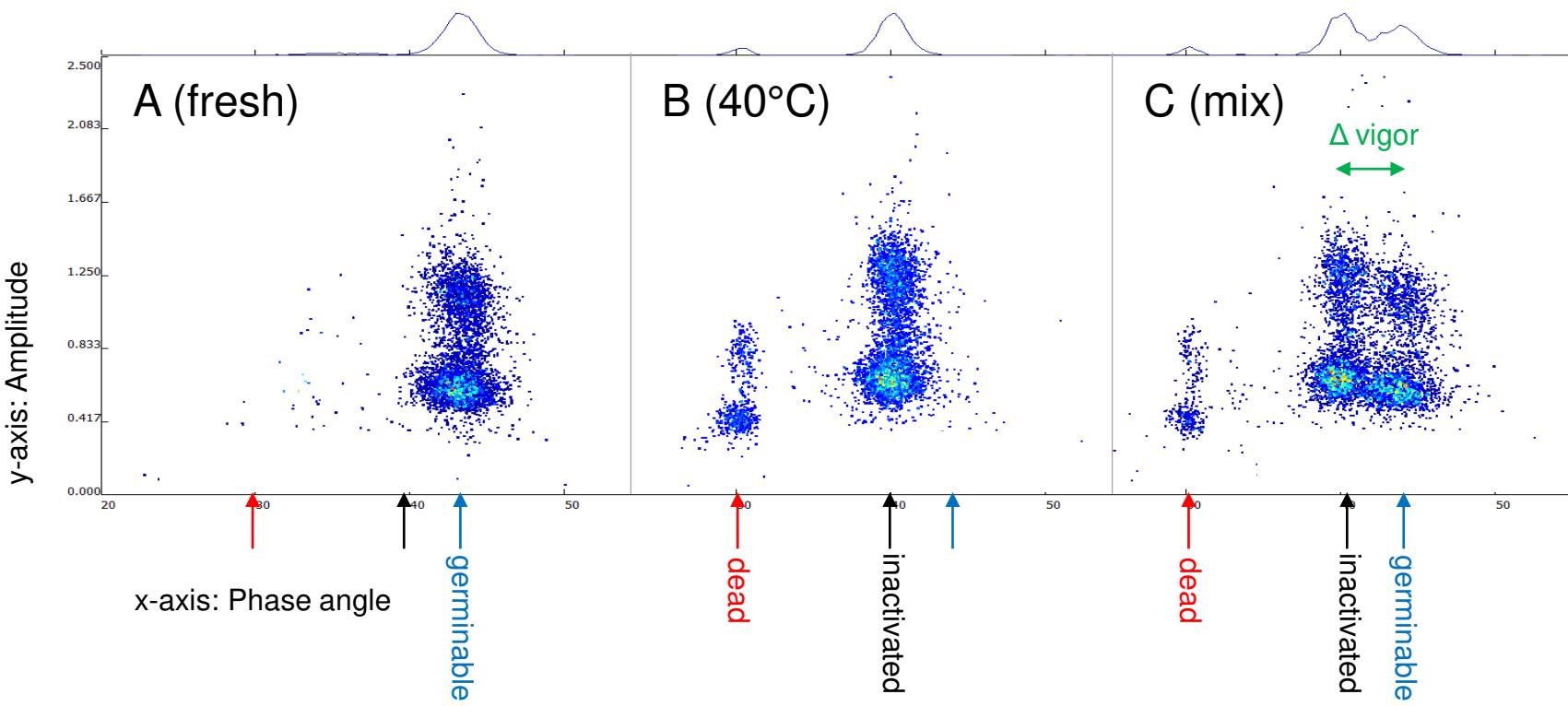


Fig. 1 A and B. *Nicotiana*: FCR and in vitro germination responses of fresh pollen (A) and pollen stored for 20 days under laboratory conditions (B)

Source: Shivanna et al, 1991

Pollen Activity and Vigor by IFC



Heat inactivation of pollen germination (tomato): AmphaSoft plots of fresh (A), 40°C -inactivated (B), and a mixed (C) pollen population. The red, black and blue arrows point at the dead, inactivated and fresh pollen populations.

Source: Heidmann et al. 2016

Examples of Use Cases



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1. Male Line Selection



Goal:

Selection of good pollinators for hybrid wheat breeding

- ▶ How much pollen is produced and is it viable?

Measuring:

Viability of ~ 2000 samples a season, measured directly in the field



“Over 100 samples could be processed this way in less than four hours in the field. The use of the Ampha Z32 in the field also guaranteed immediate measurement after collection, free of aging effect on the wheat.”

→ Download Customer Application Review

Dr. Michael Schmolke
Lead Discovery Breeder
Bayer CropScience

2. Improved Crossing Efficiencies



Goal:

Improving crossing success of inbreed depressed lines
by optimizing pollen collection

Measuring:

Viability of ~ 400 samples a season



“By use of the Ampha Z32 in various plant crosses, we could improve these efficiencies by 30%.”

ENZA ZADEN



Joep Lambalk
R&D Director

3. Optimize Collection, Treatment and Storage



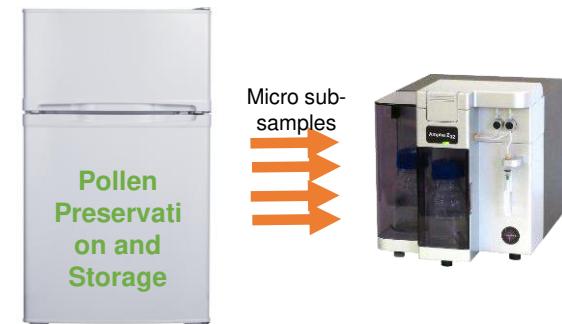
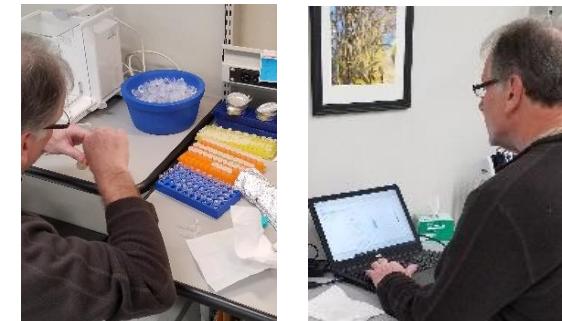
Goals:

1. Start with high pollen quality
 - ▶ Optimize timing of harvest with onsite measurements for highest pollen viability
2. Optimized collection and storage protocols
 - ▶ Iterate collection, treatment and storage conditions to determine optimal conditions
3. Assure sufficient viability before pollination
 - ▶ Ampha Z32 provides immediate feedback

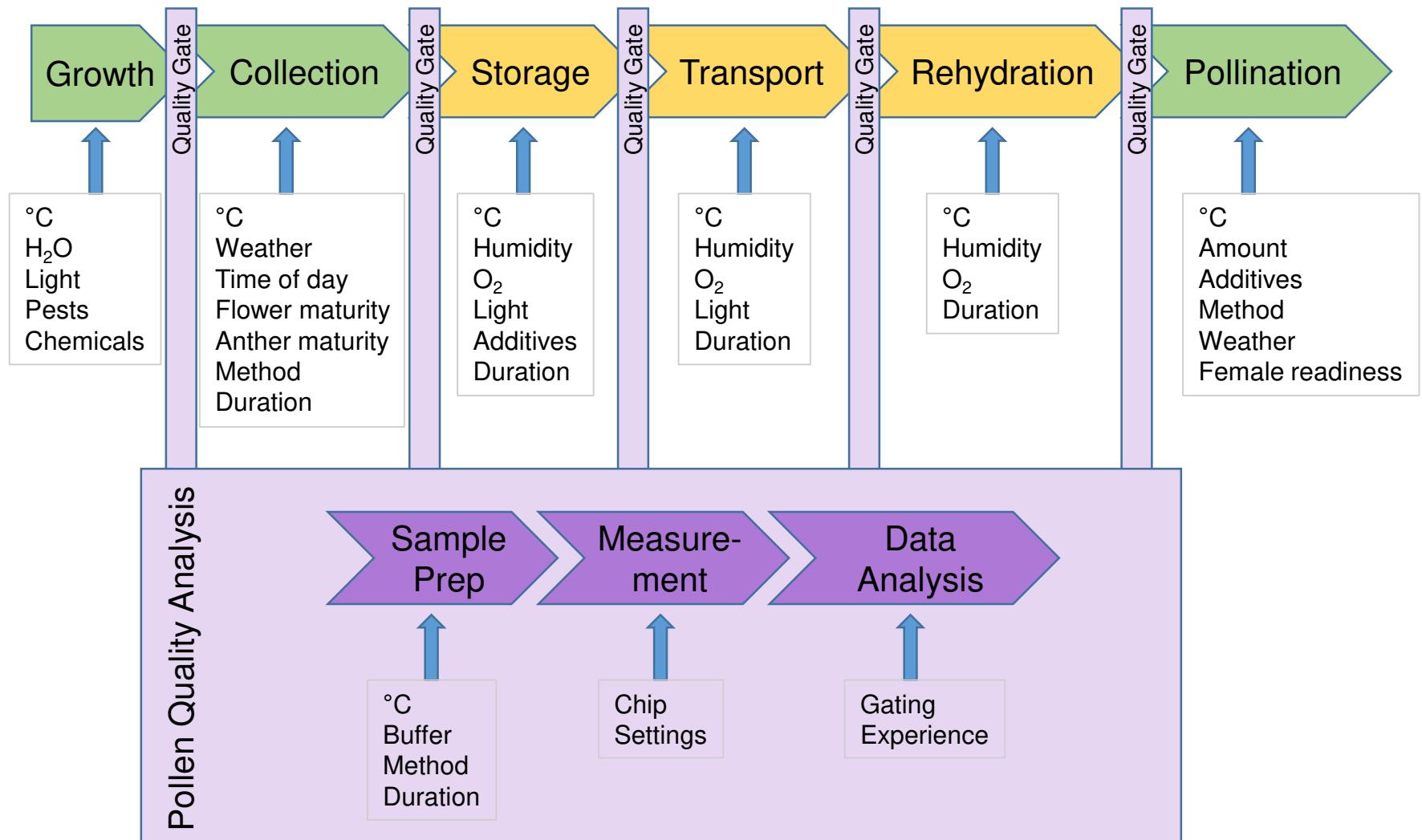
"We use the instrument to check pollen quality before pollination, to improve pollen preservation and for rescue pollinations when natural pollination was not sufficient to get a good seed yield"

→ Download Amphacademy lecture

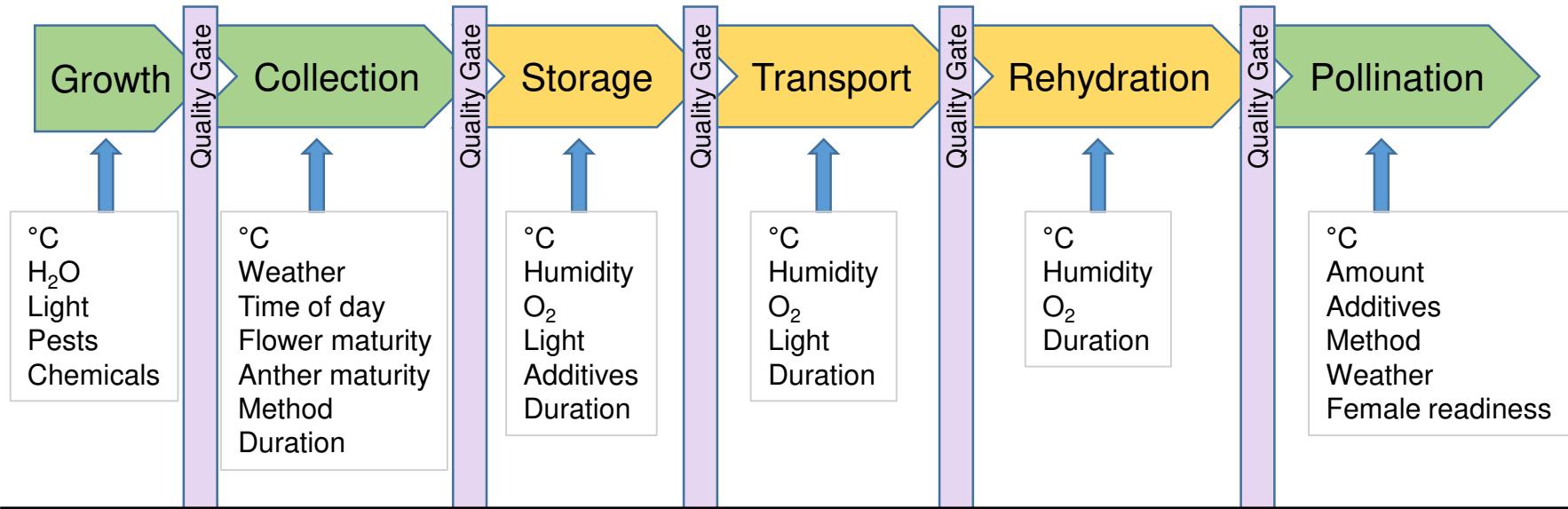
Todd Krone
CEO Power Pollen



4. Set Quality Gates in Supply Chain



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“One of its biggest advantages lies in the continuous monitoring of pollen quality directly at the production site providing better control over the production chain.

It thereby helps to prevent production losses due to pollination with inferior pollen. Control over pollen quality is even more important when working with contractors.”

ENZA ZADEN

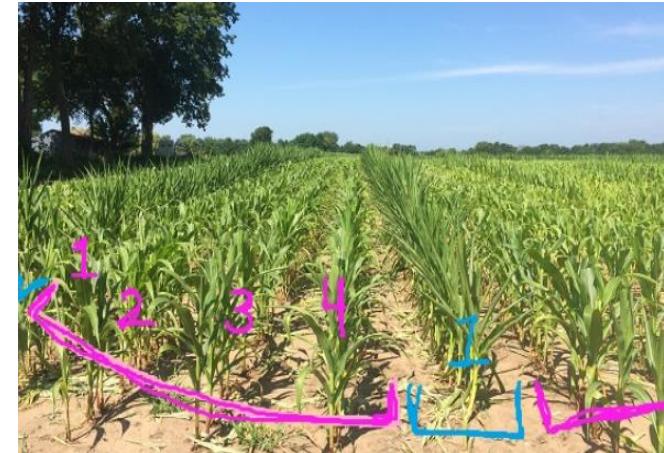


Joep Lambalk
R&D Director

5. Optimizing Female to Male (F:M) Ratio

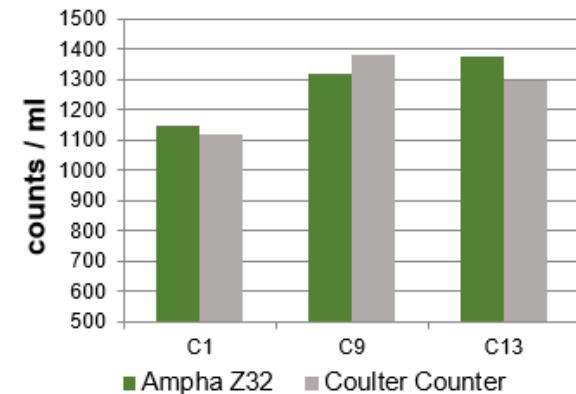


Optimization of the F:M ratio allows for significant cost saving in row crop hybrid seed production



Pollen count is traditionally done by pollen deposition analysis and counting in a Coulter Counter.

- ▶ Now you can also count on the Ampha Z32
- ▶ In addition, with the Ampha Z32 you also get viability measurements



[Source: Arvalis, 2018](#)

6. Quality Control Before Manual Pollination

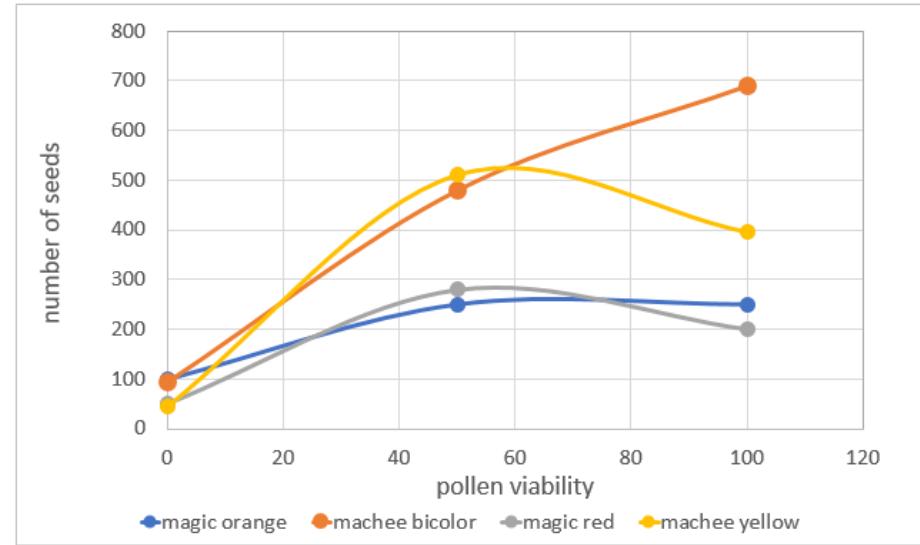


Goal:

Quality control of stored pollen to work with and cross asynchronous flowering flowers

Measuring:

- Viability and correlation to seed set for each variety



“The main advantages are time savings and understanding the minimum viability needed to get a full seed set ”

Dr. Fatma Zaki
Development Scientist Seed

7. Maximize Daily Pollination Window



Goal:

Resource allocation on a daily basis by determining:

- ▶ When males get active & pollen collection can start
- ▶ Which greenhouse has the highest pollen viability

Measuring:

Viability of tomato and pepper pollen

- ▶ ~ 40 samples a day



“The Ampha Z32 is so fast and makes it easy to take measurements, that we can inform the production team about next steps in less than 2 hours”

Karem Yazmin Luna
Quality Manager Vegetables Seed
Monsanto

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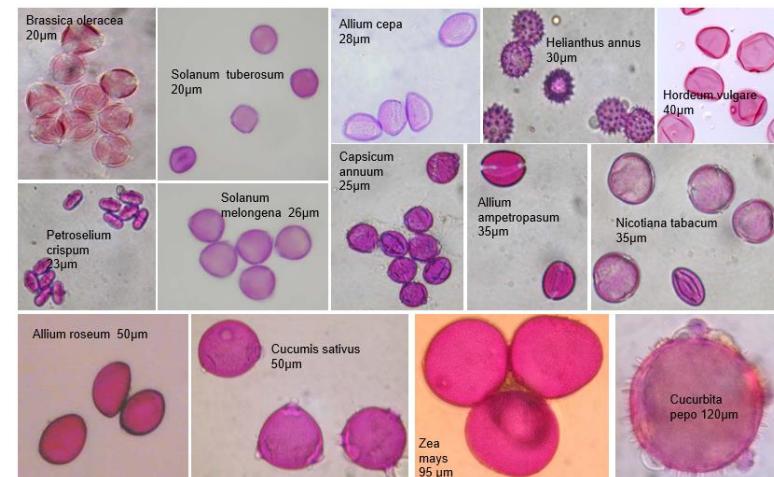
Optimize manual pollination

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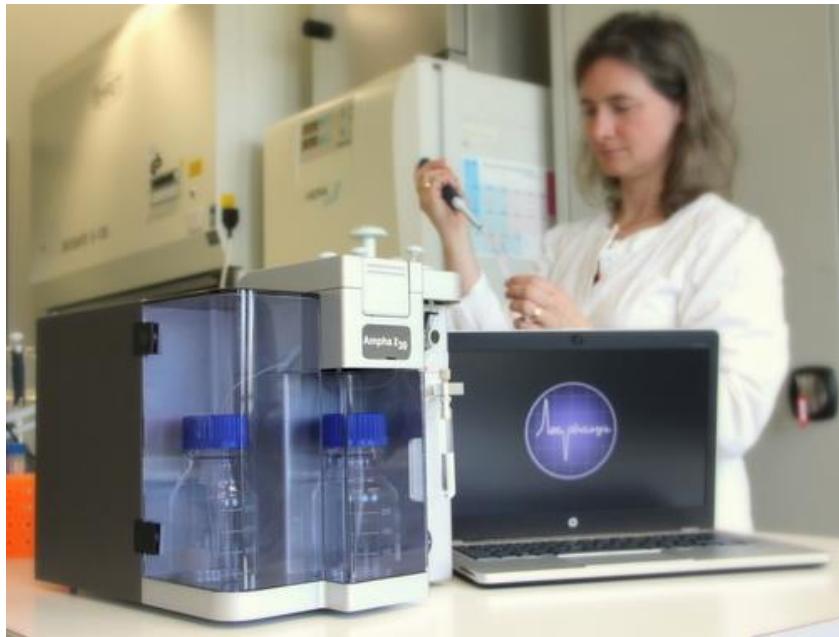
Benefits of Measuring with IFC



- Fast
 - ▶ Results in < 1 minute
- High Statistical Relevance
 - ▶ Up to 40'000 pollen / measurement
- Automation
 - ▶ up to 192 samples
- No Labeling
 - ▶ Simple sample preparation
- No Damage to Pollen
 - ▶ Microspore development
- One Analysis for all Pollen
 - ▶ Over 200 species tested



Small, Mobile System



Measure where you need it



Seed Quality starts with
Pollen Quality

Thank you!



Radboud University



Dr. Marcel Ottiger, Executive Partner

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