



Non-destructive repeated phenotyping in vegetable crops using digital image analysis

Ralph van Berloo, José Guerra



Contents of talk

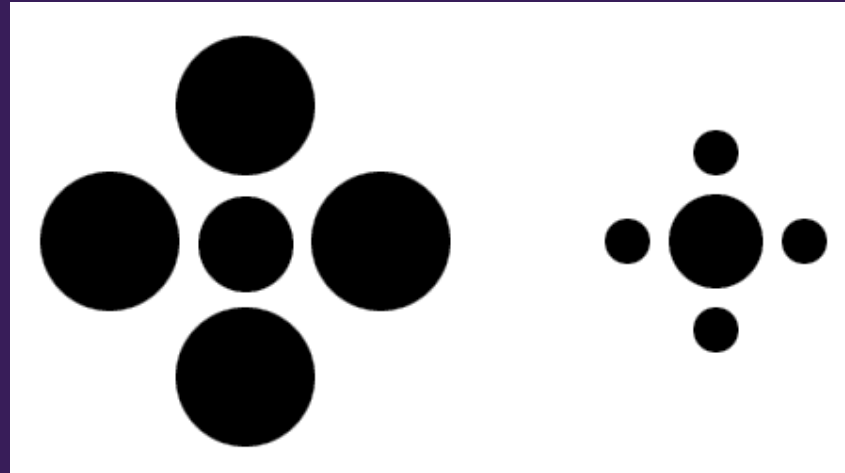
- ▶ The importance of phenotyping
- ▶ Subjective observations
- ▶ Keygene & phenotyping
- ▶ Digital image analysis and the Lemnatec system
- ▶ Examples of digital imaging of vegetable crops & challenges encountered
- ▶ Conclusions and future directions
- ▶ Acknowledgements



The importance of phenotyping

- ▶ For plant breeders (and growers and consumers) the phenotype is the final goal..
- ▶ New improved phenotypes can be created through genetic improvement (QTLs)
- ▶ Detection of QTLs requires:
 - ▶ high quality molecular markers
 - ▶ high quality phenotypes !
- ▶ But ..Phenotyping is not always straightforward
 - ▶ Genetic and environmental factors are intertwined
 - ▶ Subjective/ biased observations
 - ▶ Phenotype may depend on complex interactions of plant and (a) biotic agent (resistance, tolerance)

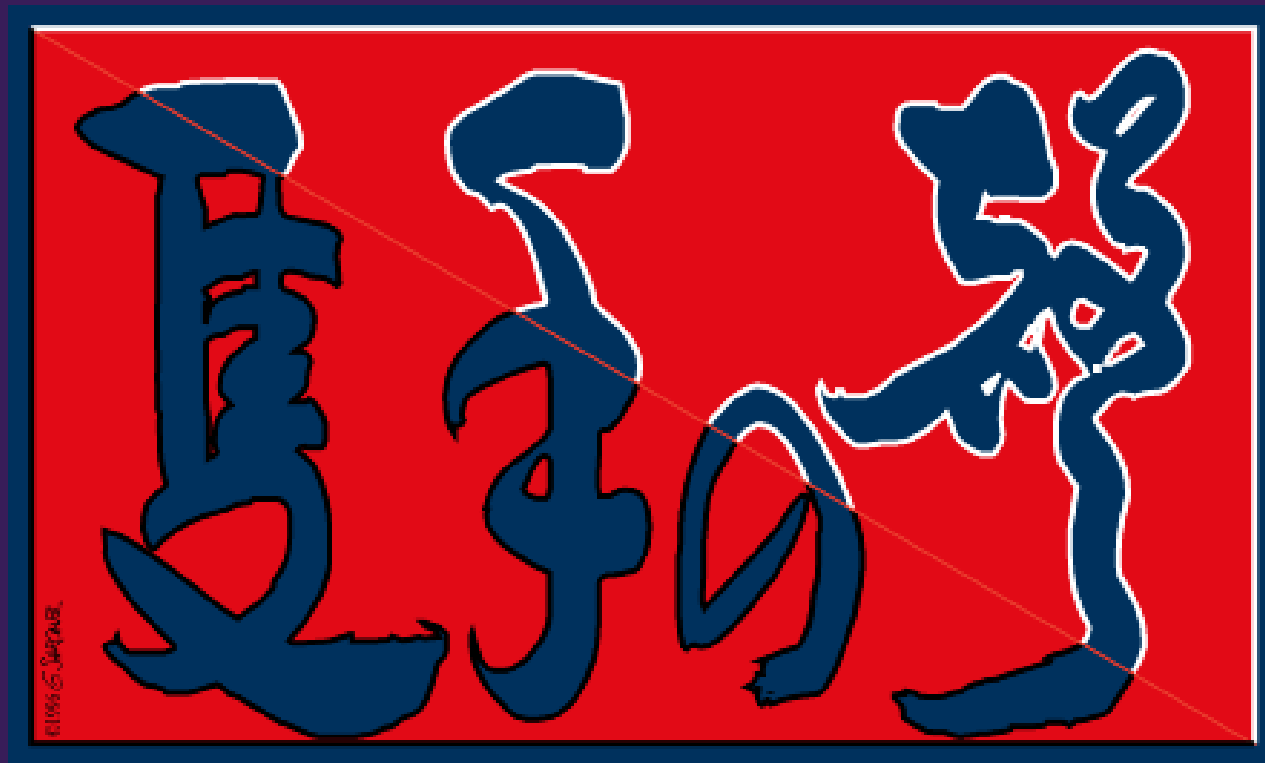
Easy to make subjective observations



- ▶ Left centre circle smaller than right one?

Subjective observations (2)

Two shades of red in this picture?





Keygene and phenotyping

- ▶ Keygene originated 20 years ago as a biotechnology startup, in which Dutch breeding companies joined forces
- ▶ Currently Keygene is owned by four large breeding companies, mainly active in vegetable breeding
- ▶ Over the years Keygene has evolved into a medium sized research company, and built up considerable expertise in the field of genotyping (AFLP) and, more recently, (applications of) genomic sequencing



Vilmorin Clause & Cie



Keygene and phenotyping (2)

- ▶ Combining (externally provided) phenotype data with in-house generated genotype data is one of the main activities of Keygene applied research
- ▶ This explains our interest in new methods to obtain objective, high quality phenotype data
- ▶ Focus lies on phenotypes derived from image analysis as a method with a lot of potential for gathering non destructive, robust, objective and high throughput observations → Lemnatec system



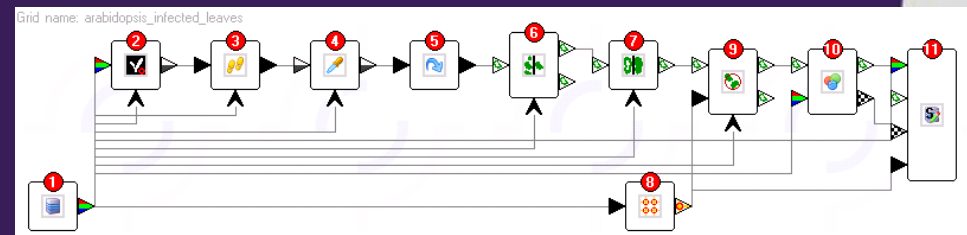
Lemnatec system - video



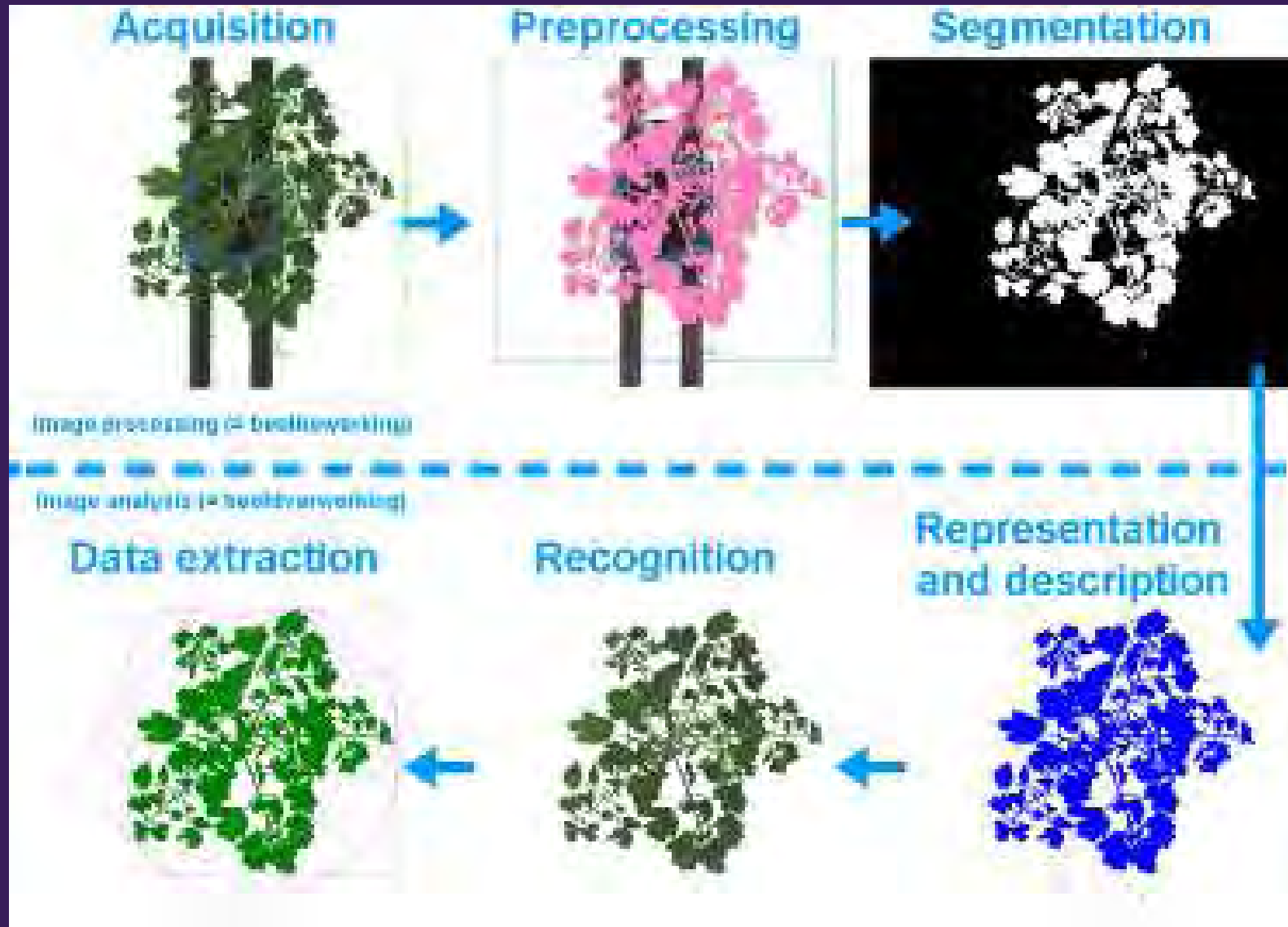
Moving field concept

Lemnatec features

- ▶ Hardware: greenhouse conveyor belts and photography chamber
 - ▶ Plant randomization
 - ▶ Take observations at will
 - ▶ Observations from several angles + top view
 - ▶ Optional filters/ sensors in non-visible spectrum (NIR/ water content & Fluorescence)
 - ▶ Plant weight + precise water dosage
- ▶ Software: Lemnatec software environment
 - ▶ Contains building blocks for image processing & conversion to numeric data, allows you to build custom analysis pipelines



Example of image processing & analysis steps



derivation of a 'digital trait'



green pixels front / side view

< Variables >



Green pixels top view

$$Digital_biomass = \sum Pixels_{front} + \sum Pixels_{side} + Log\left(\frac{\sum Pixels_{top}}{3}\right)$$

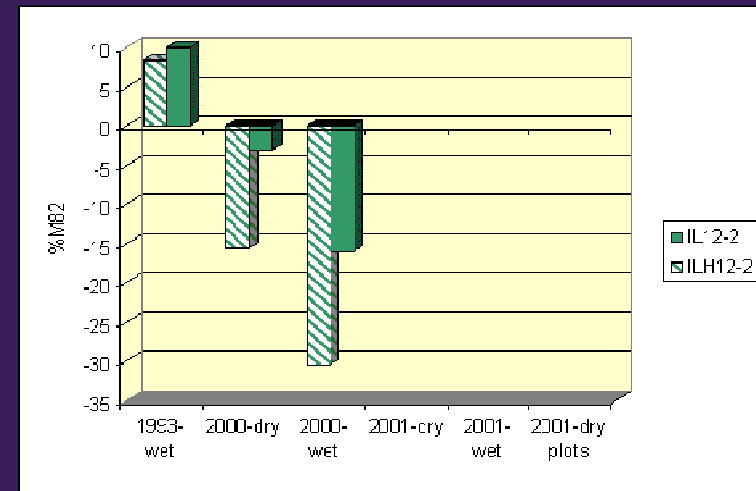
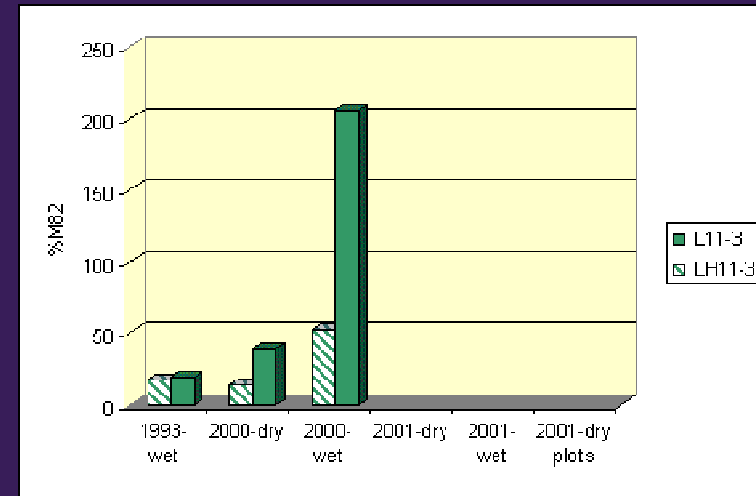
Destructive verification experiments show that this digital trait has **80% correlation** with green mass

Validation using existing data

- ▶ Zamir M82 tomato introgression lines; trait = plant weight
- ▶ Comparison of two introgression lines and the reference parental line M82

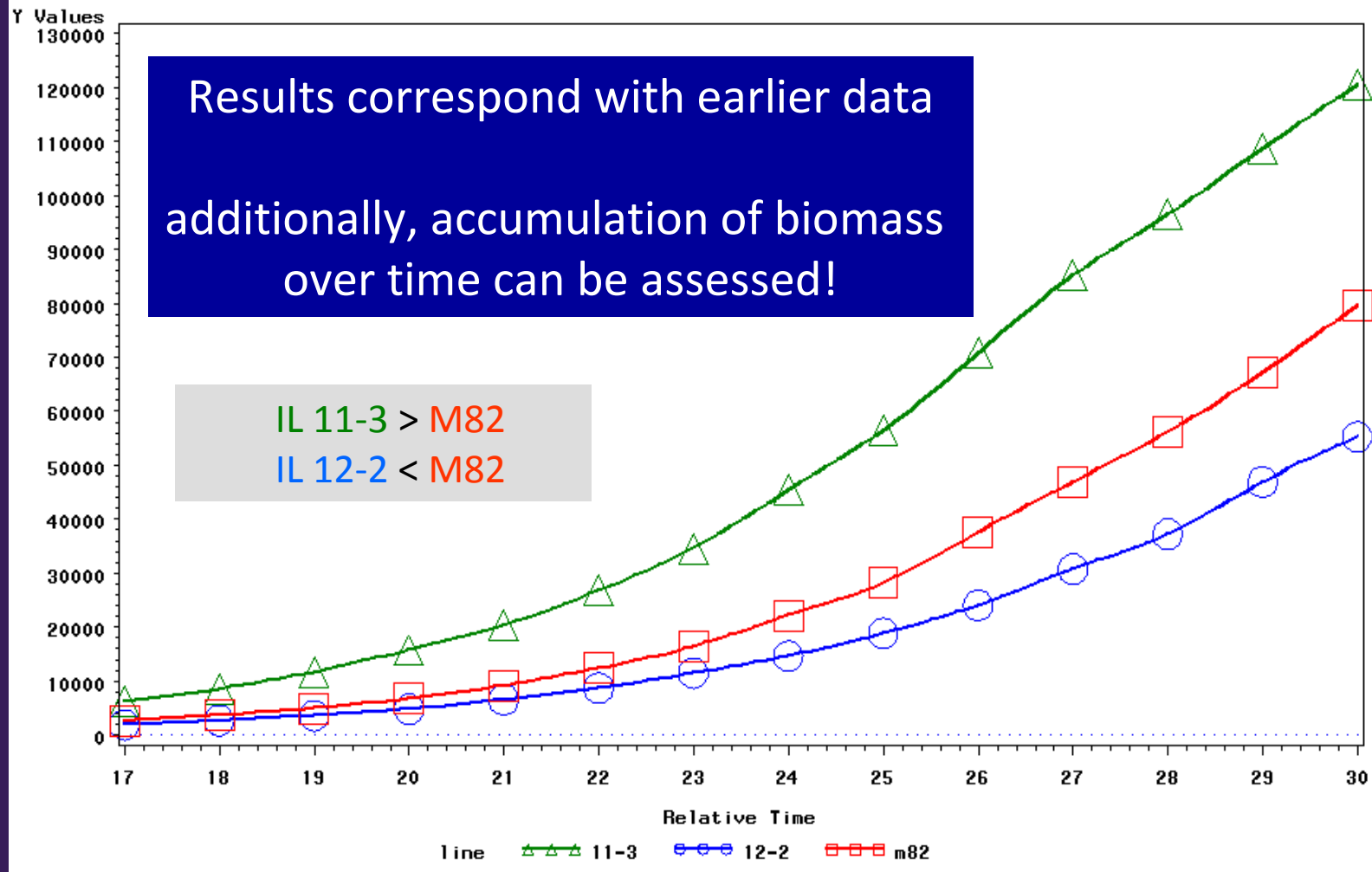
▶ IL 11-3 > M82

▶ IL 12-2 < M82



Lemnatec results

Sample Data Plot: are vs. Time



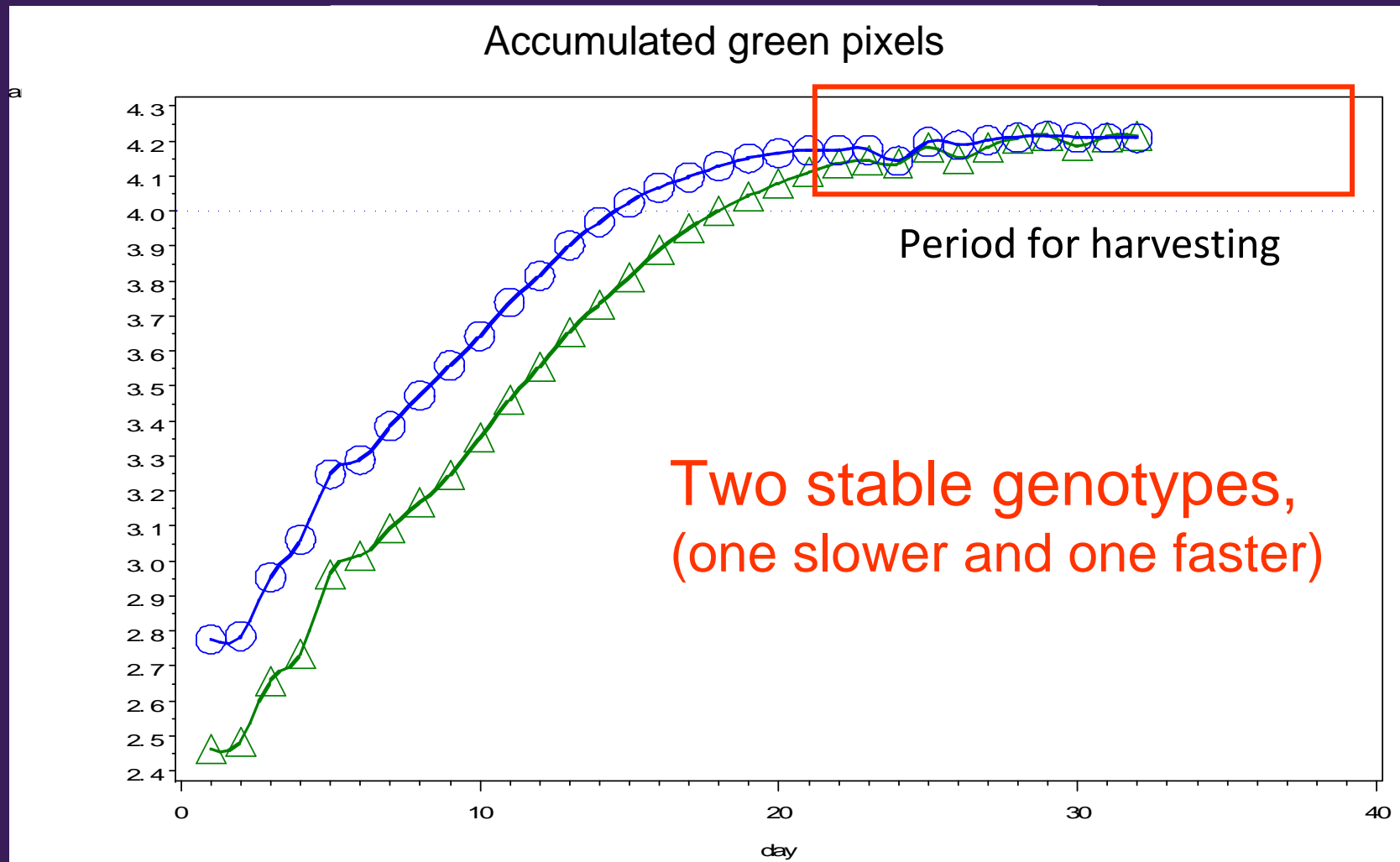
Example in spinach: leaf mass development



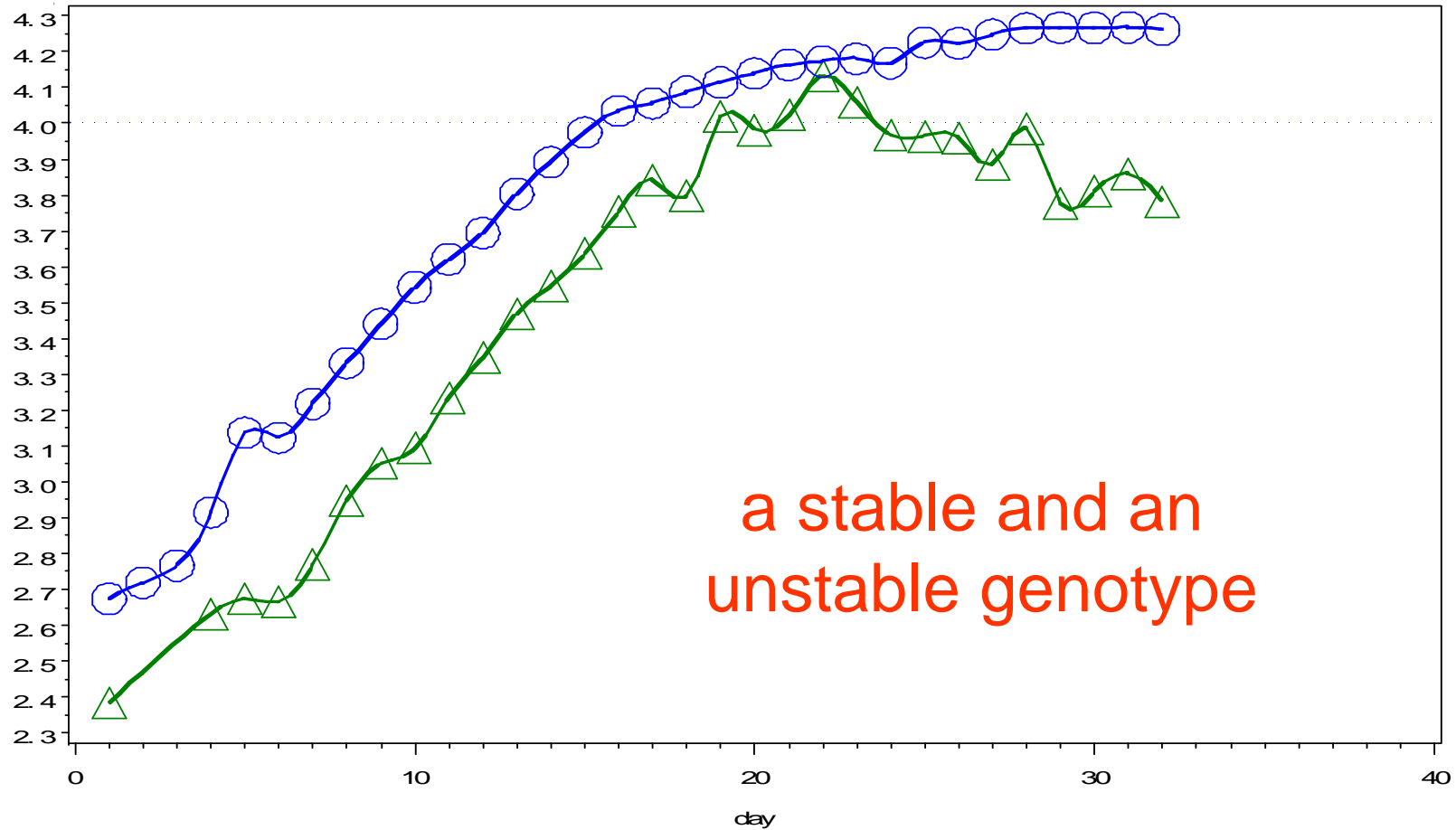
Time →



Spinach biomass stability



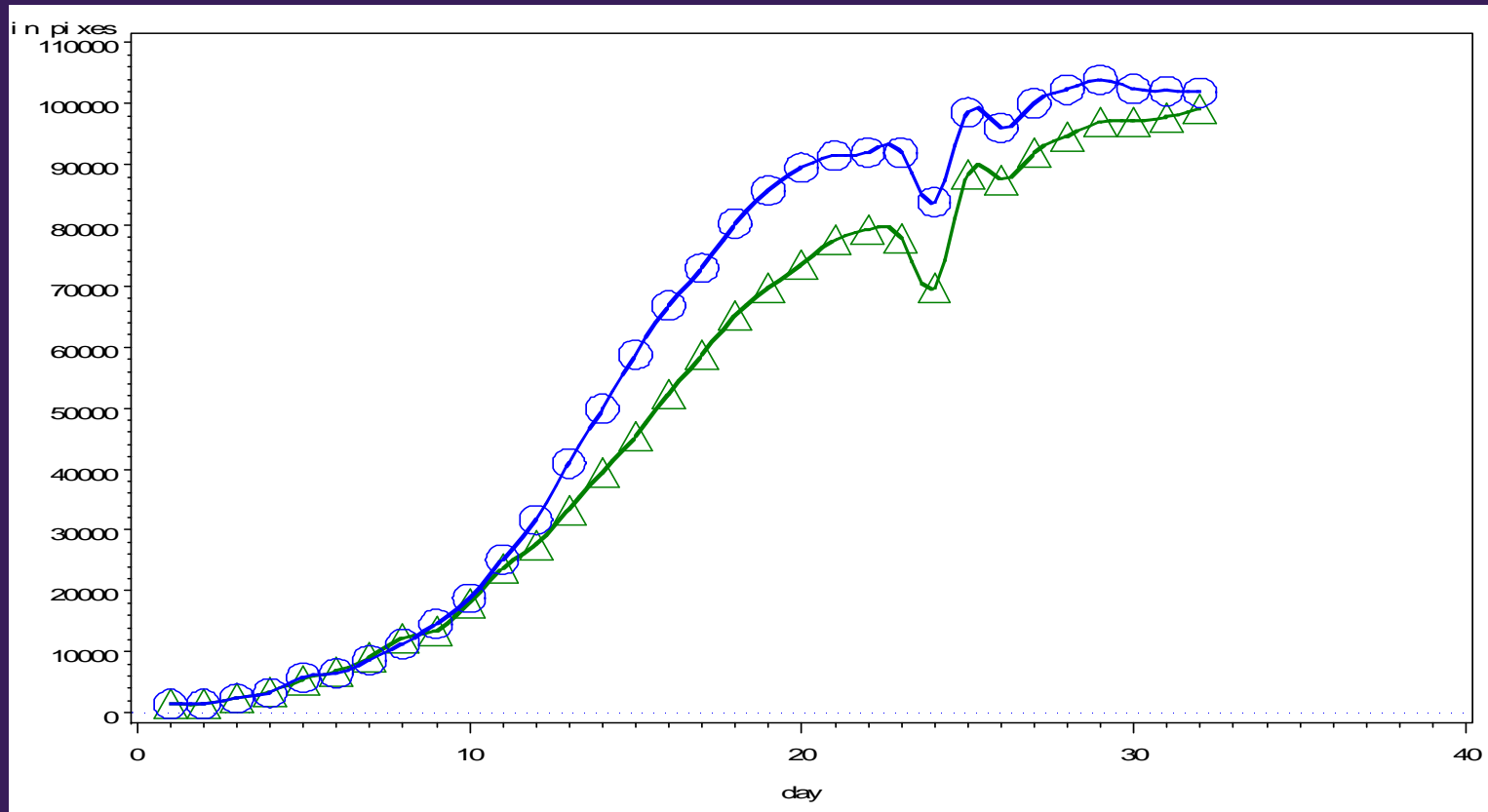
Accumulated green pixels



a stable and an
unstable genotype

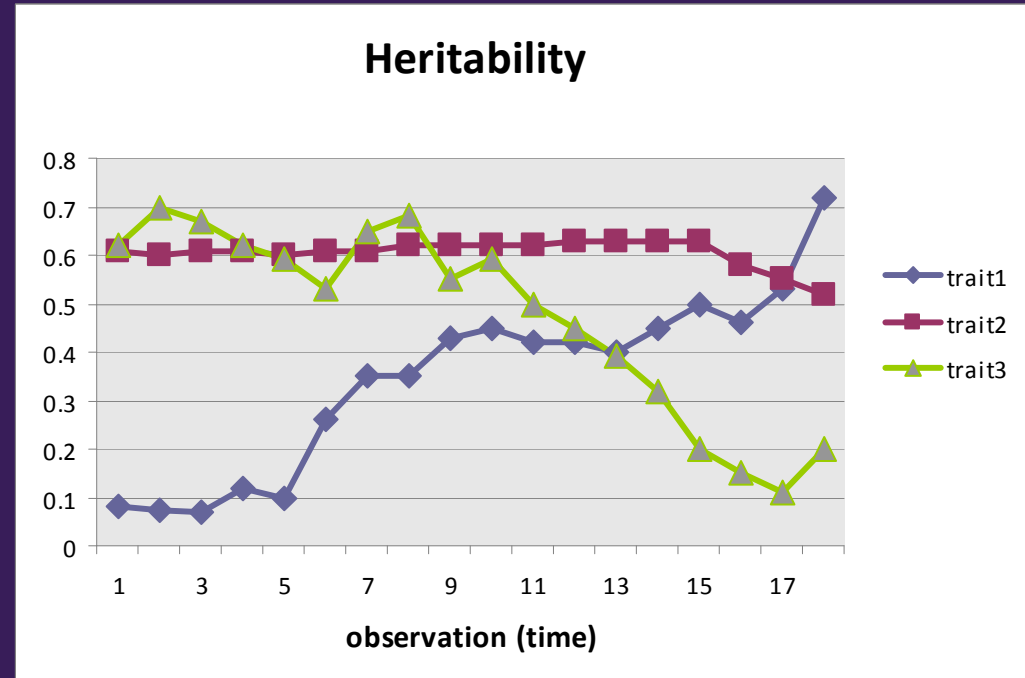
Surprising result..

- ▶ The greenhouse heating shut down during one night..



Optimizing heritability

- ▶ Repeated (digital) observations allow us to calculate the trait heritability for the same trait at different moments in time
- ▶ The best moment to gather data may differ between traits
- ▶ A more powerful genetic analysis can be achieved by phenotyping at the right moment



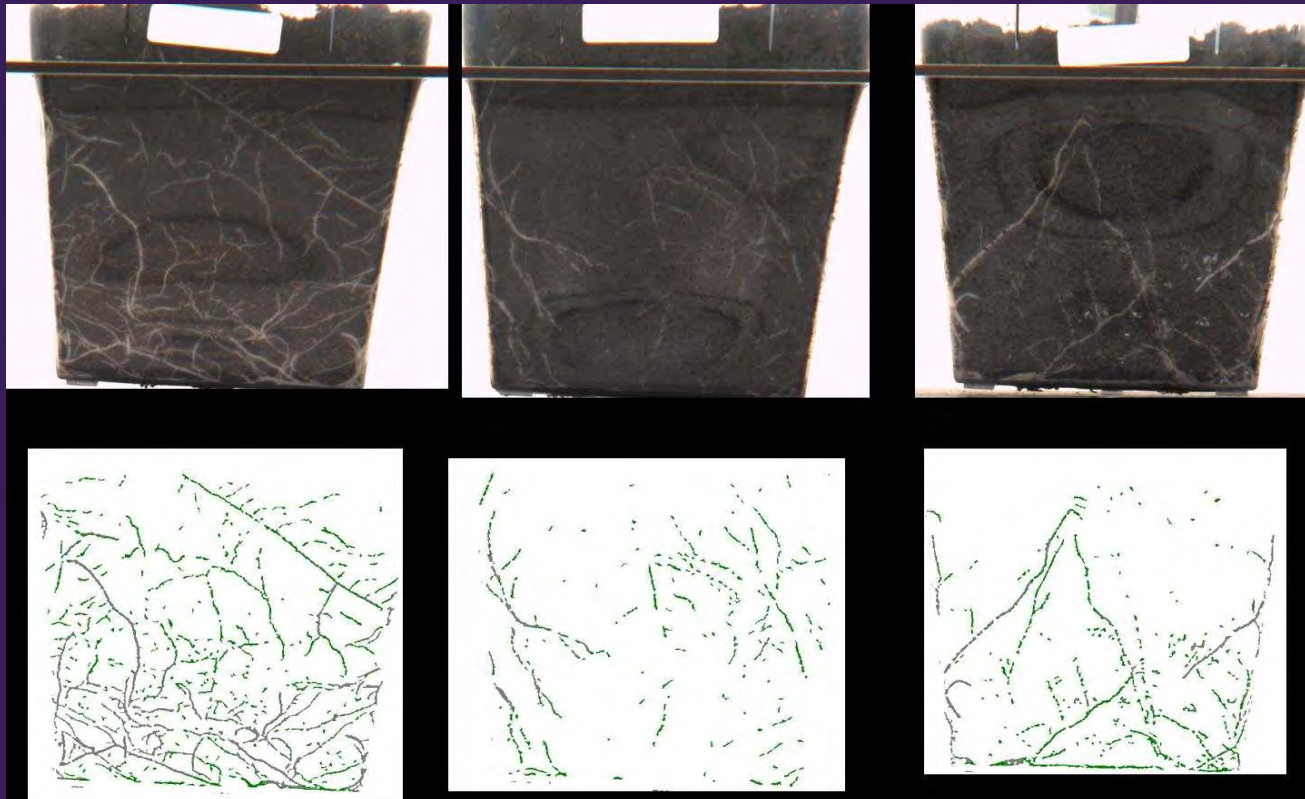
Example: Quantifying disease resistance in *Arabidopsis*



Classification as Resistant/Susceptible, observed via digital imaging was accurate in >80% of the cases

Challenges in digital imaging

- ▶ Quantifying root growth
 - ▶ Problems with reflection, condensation, transparency of pots etc.. were solved in the end



Conclusions & future directions

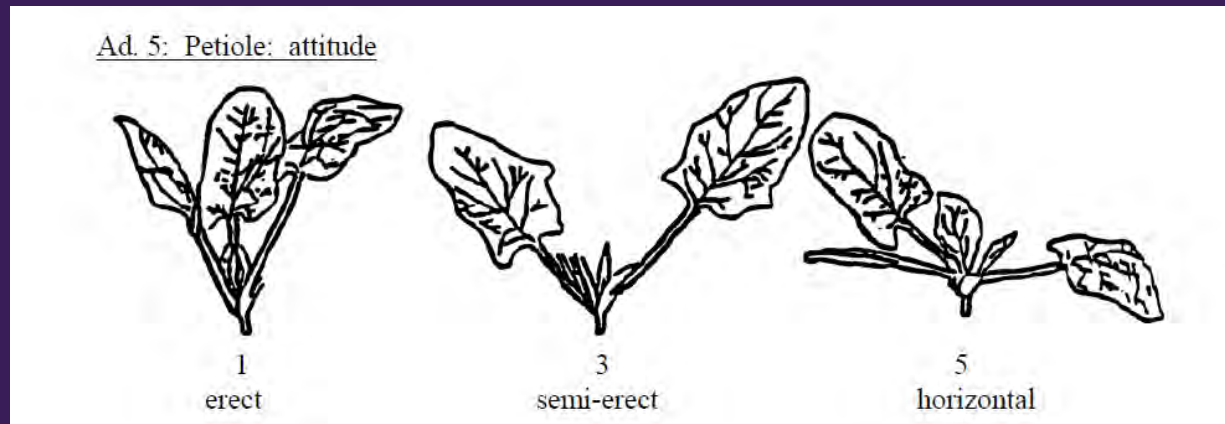
- ▶ Digital imaging has added value in many cases like:
 - ▶ Early prediction (ergo early selection!) of final trait
 - ▶ Investigating plant development
 - ▶ Heritability optimization (determine when to measure)
 - ▶ Replacement of destructive tests
 - ▶ Traits that are cumbersome to measure by hand
 - ▶ and others...

Conclusions and future directions (2)

- ▶ Keygene has used the last 1½ years to explore the Lemnatec system for phenotyping and we decided to continue on this road
- ▶ The current system was recently enlarged to a capacity of over 200 plants
- ▶ Developing analysis pipelines for plants with a complex architecture such as tomato is not straightforward!
- ▶ Expertise in image analysis is growing, including the use of third party hard and software
- ▶ New services and products in this field are to be expected in the near future

Ideas for novel possibilities

- ▶ Add more detail to existing observations?



- ▶ ‘Leaf angle’ is an important harvest related trait in spinach
- ▶ The UPOV description for spinach now has 3 classes for leaf angle
- ▶ With image analysis the true leaf angle can be estimated which allows much more detailed comparison and analysis of underlying genetic differences

Flexible field layout

- ▶ Traditionally, in field/ glasshouse experiments environmental variance is controlled by applying replications and randomization
- ▶ Systems such as Lemnatec allow us to define a different field layout every day!
- ▶ Randomization in place and time
- ▶ Could this be a way to avoid the buildup of environmental noise (related to positional effects) altogether?
- ▶ Still preliminary thoughts at the moment..

Acknowledgements

- ▶ Keygene Lemnatec team:
 - ▶ José Guerra (statistics)
 - ▶ Bas de Regt (programming)
 - ▶ Gert-Jan Speckmann (operations)
 - ▶ Kornelis Stol (ICT)
 - ▶ Harold Verstegen (management)
 - ▶ Arjen van Tunen
 - ▶ ..and others

- ▶ Lemnatec GmbH

