



cv. Calardis Blanc



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Federal Research Centre for Cultivated Plants

*Sensor based
phenotyping for grapevine
breeding and genetic
analyses*

*Reinhard Töpfer, Rudolf Eibach, Oliver Trapp,
Katja Herzog, Florian Rist, Robert Richter,
Eva Zyprian, Anna Kicherer*



powdery mildew



downy mildew

Cultivars found in the German variety list (2015)

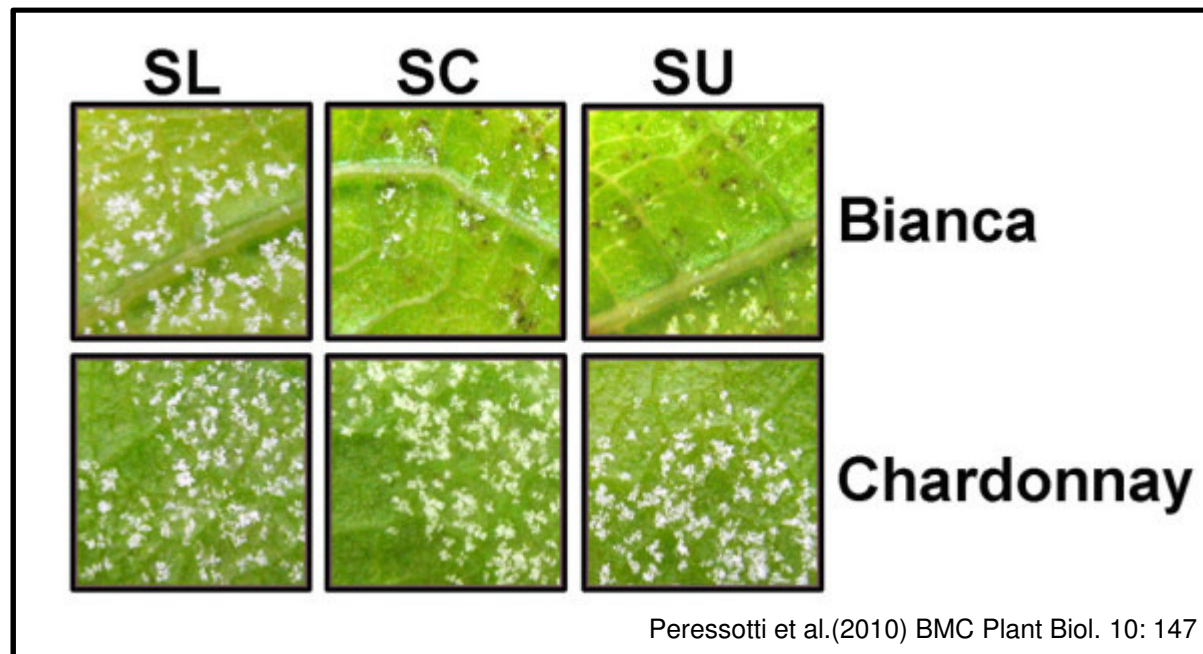
Plants are cultivated with reduced fungicide application



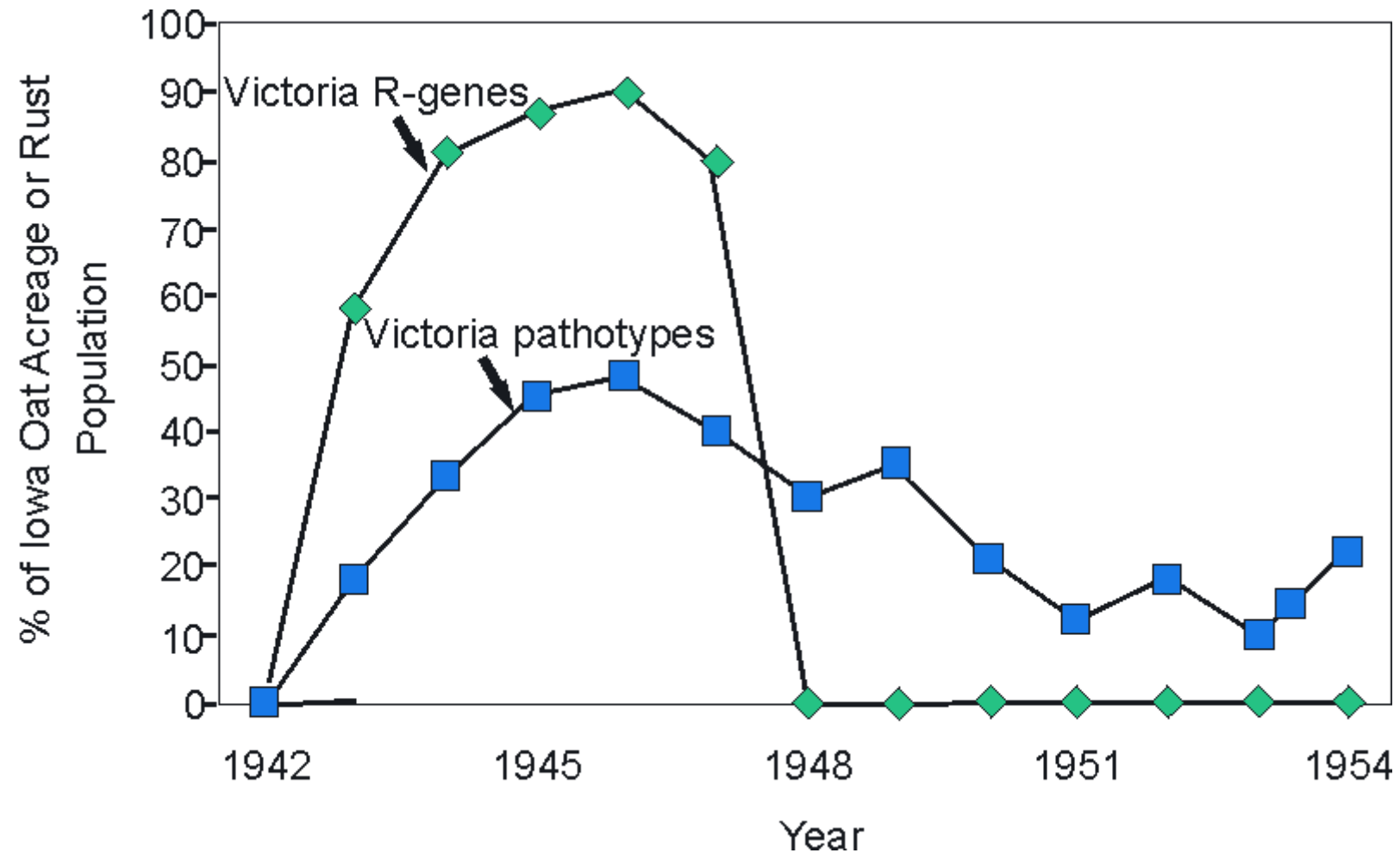
No.	cultivar	year of protection/ admission	parents	berry color	DM	PM	botrytis	Rpv 3-1	Rpv10	Ren3
1	Calvinke	2000	Regent x Regent	B	3	4	7	X		X
2	Pragya	2007	Calvinke x Regent	B	2	3	8	X		X
3	Prix	2004	Domino Super 594-95 x First Lady x Brenner	B	2	3	8	X		X
4	Regent	1994	Danae Chambourci	B	3	3	4	X		X
5	Nelae	2001	Mendling x Seyve Villard 22-121 x Müller Thurgau	B	3	3	4	X		X
6	Islewinke	1997	Nelae x Mendling x Seyve Villard 22-121 x Müller Thurgau x Müller Thurgau	B	2	3	4	X		X
7	Muscat	2003	Sola x Götter Thurgau	B	2	3	4	X		X
8	Gran	1994	Sola x Müller Thurgau	B	2	3	4	X		X
9	Pharis	1994	Sola x Müller Thurgau	B	2	4	4	X		X
10	Arbe	1994	Sola x Müller Thurgau	B	2	5	4	X		X
11	Saphir	1994	Sola x Müller Thurgau	B	2	4	3	X		X
12	Solaris	2004	Sola x Müller Thurgau	B	2	4	3	X		X
13	Cabernet Cuvée	2004	Cabernet Sauvignon x Solaris	B	2	3	4		X	X
14	Cabernet Cuvée	2004	Cabernet Sauvignon x Solaris	B	2	3	4		X	X
15	Muscat	2004	Sola x Götter Thurgau	B	2	4	3		X	X
16	Brenner	1997	Mendling x (Sola Super x St. Laurent)	B	2	3	3		X	X
17	Sola	2003	Mendling x (Sola Super x Müller Thurgau)	B	3	3	3		X	X
18	Sola x Götter Thurgau	2003	Cabernet Sauvignon x Brenner	B	2	3	3		X	X
19	Sola	2003	Cabernet Sauvignon x Brenner	B	2	3	3		X	
20	Cabernet Cuvée	2004	Cabernet Sauvignon x Brenner	B	2	3	3		X	
21	Sola	2003	Sola Super x St. Laurent	B	3	5	4		X	
22	Acqua	2007	Sola x Chenonceau	B	3	4	3			X
23	Allegro	2006	Chenonceau x Sola	B	3	4	3			X
24	Sola	2006	(Brenner x Solonstein) x Chenonceau	B	3	4	3			X
25	Brenner	2004	Regent x Müller Thurgau	B	3	4	3			X
26	Müller Thurgau	1997	Chenonceau x Müller Thurgau	B	4	5	3			X
27	Müller Thurgau	1994	Seyve Villard 2-374 x (Mendling x Moscat)	B	4	4	3			X
28	Prinzipal	2007	Müller Thurgau x Chenonceau	B	7	5	3			X
29	Saphir	2004	Amberburg x Seyve Villard 2-73	B	7	5	4			X
30	Pharis	2006	(Paragol x Herakles) x (Dachstein x Freiburg 599-54)	B	2	4	4	n.t.	n.t.	n.t.

Durability of resistances

*Plasmopara
viticola*
(downy mildew)
6 dpi

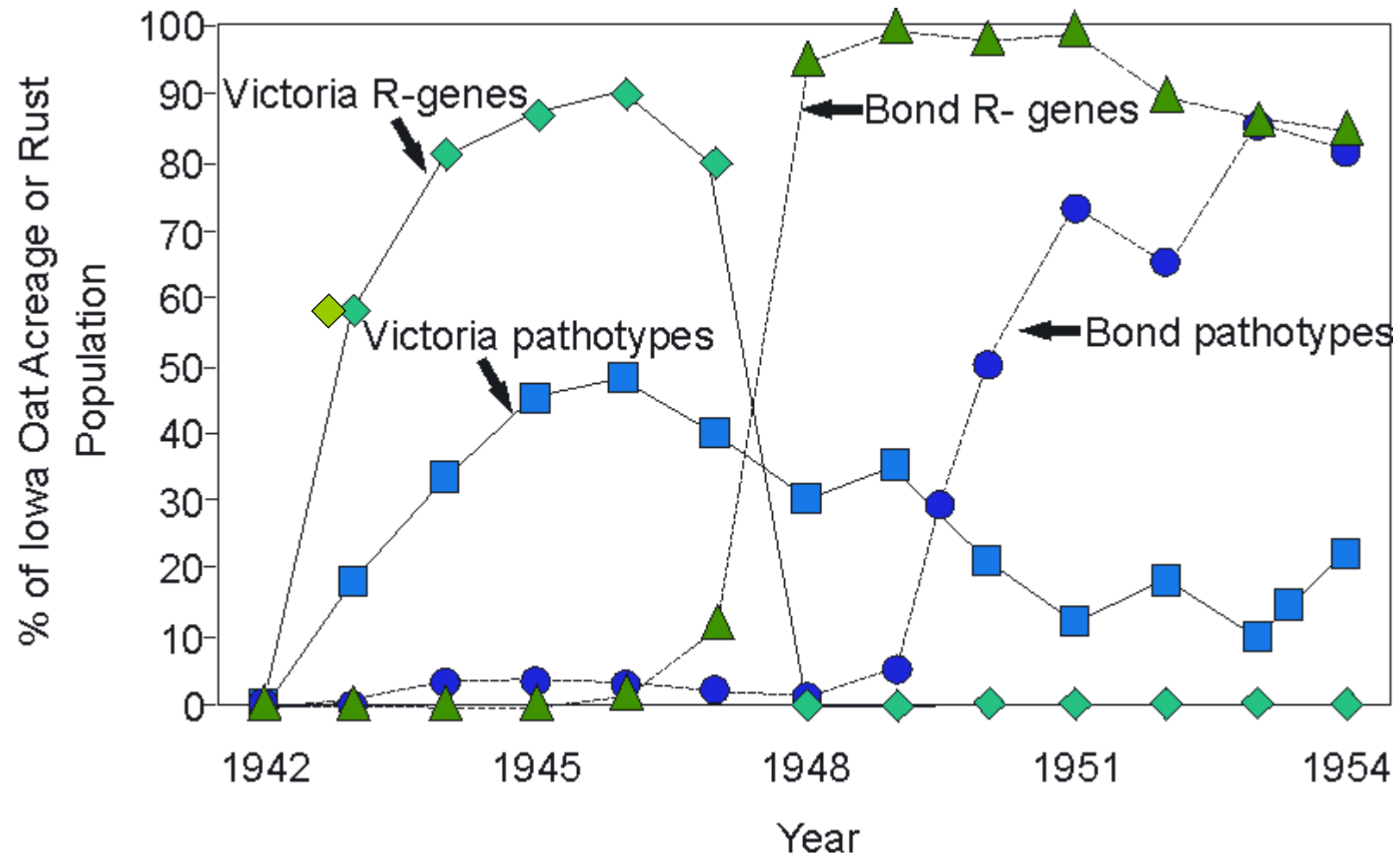


Durability of resistances – Classical Boom-and-Bust Cycle



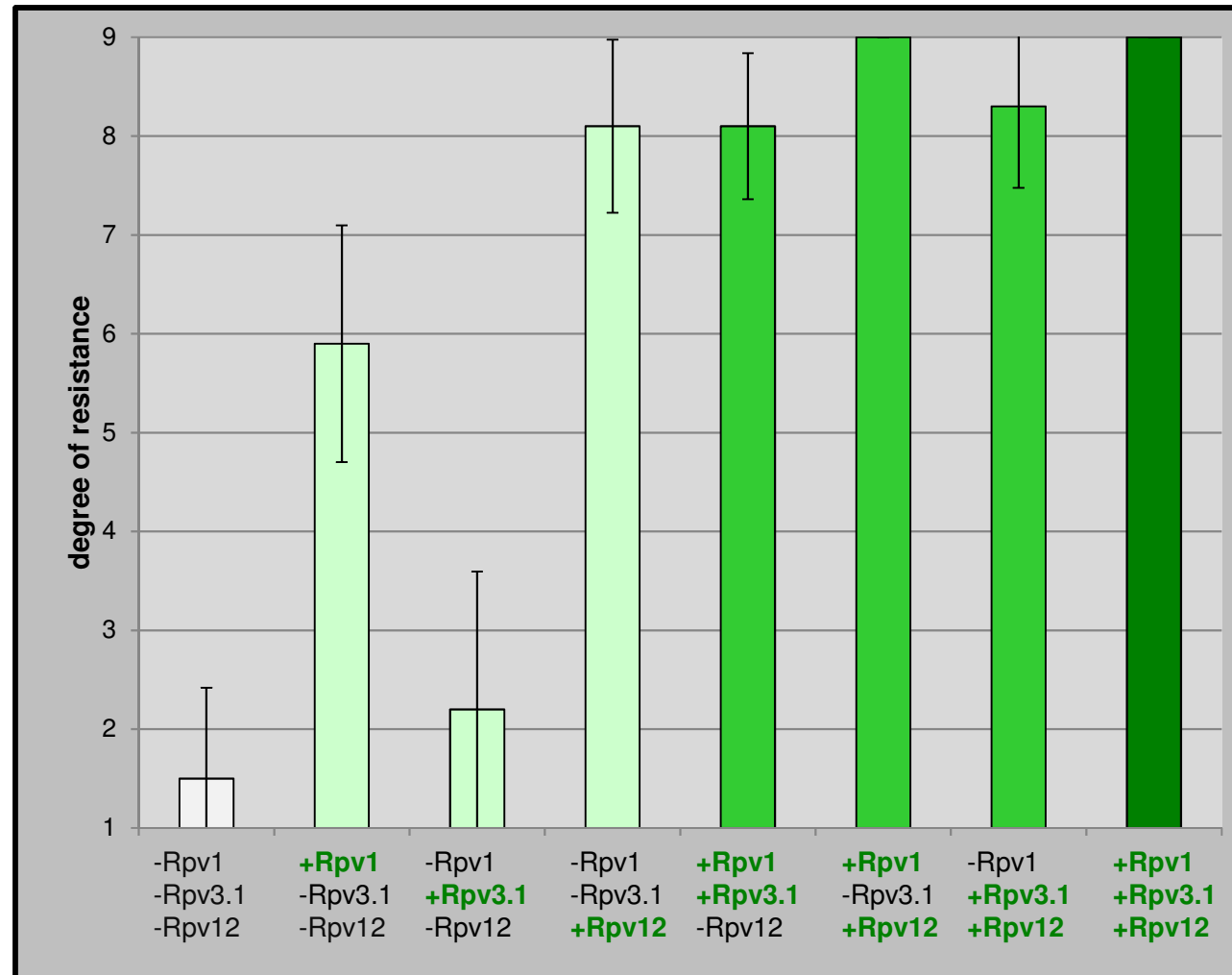
(nach: Mundt et al. 2014, modifiziert; Originaldaten von Browning und Frey 1969)

Durability of resistances – Classical Boom-and-Bust Cycle



(Form: Mundt et al. 2014, modified; original data from Browning and Frey 1969)

Breeders answer: select for stacked R-loci (**downy mildew**)



Durability of resistances - Occurrence of Fungicide Resistances

(according to HG Hewitt (1998) Fungicides in Crop Protection, modified by Deising et al.)

Class of Fungicides	First occurrence of resistance	Years prior to occurrence of resistance	Pathogen
Organic mercury	1964	40	<i>Pyrenophora avenae</i>
Benzimidazole	1970	2	<i>Venturia inaequalis</i> , <i>Botrytis cinerea</i>
Phenylamide	1980	2	<i>Phytophthora infestans</i> , <i>Plasmopara viticola</i>
Dicarboximide	1982	5	<i>Botrytis cinerea</i>
DIMs	1982	4	<i>Blumeria graminis</i>
Carboxanilide	1986	14	<i>Ustilago nuda</i>
Morpholine	1994	34	<i>Blumeria graminis</i>
Strobilurine	1998	2	<i>Blumeria graminis</i> f.sp. <i>tritici</i>

Anti Resistance Strategy 2017 Against Fungicides

	Category	Commercial Product	Active Compound	Group of Active Compound
	Peronospora - Fungizide			
1	A	Universalis	Azoxystrobin + Folpet	
2	A	Equation Pro	Famoxadone +	Oxazolidinedione +
3	B		Cymoxanil	Azetamide
4	A	Galactico	Famoxadone +	Oxazolidinedione +
5	B		Cymoxanil + Folpet	Azetamide
6	B	Aktuan	Cymoxanil + Dithianon	Azetamide
7	C	Forum Gold	Dimetomorph + Dithianon	Morpholine (CAA)
8	C	Forum Star	Dimetomorph + Folpet	
9	C	Vino Star		
10	C	Melody Combi	Iprovalicarb + Folpet	Valinamide (CAA)
11	C	Vincare	Benthiavalicarb + Folpet	
12	C	Pergado	Mandipropamid + Folpet	Mandelamide (CAA)
13	C	Orvego	Dimetomorph +	CAA +
14	S		Initium (Ametoctradin)	Pyrimidylamine
15	D	Fantic F *	Benalaxyl-M + Folpet	Phenylamide
16	D	Ridomil Gold Combi *	Metalaxyl-M + Folpet	
17	D	Ridomil Gold MZ *	Metalaxyl-M + Mancozeb	
18	E	Electis	Zoxamide + Mancozeb	Benzamide
19	F	Mildicut	Cyazofamid	Sulfonamide
20	F	Sanvino	Amisulbrom + Folpet	
21	P	Profler	Fluopicolide + AI-Fosethyl	Acylpicolide
22	S	Enervin	Initium (Ametoctradin) + Metiram	Pyrimidylamine

Maximum 3 applications per season for all fungicides labeled by the same letter and color; Category D: only one application per year

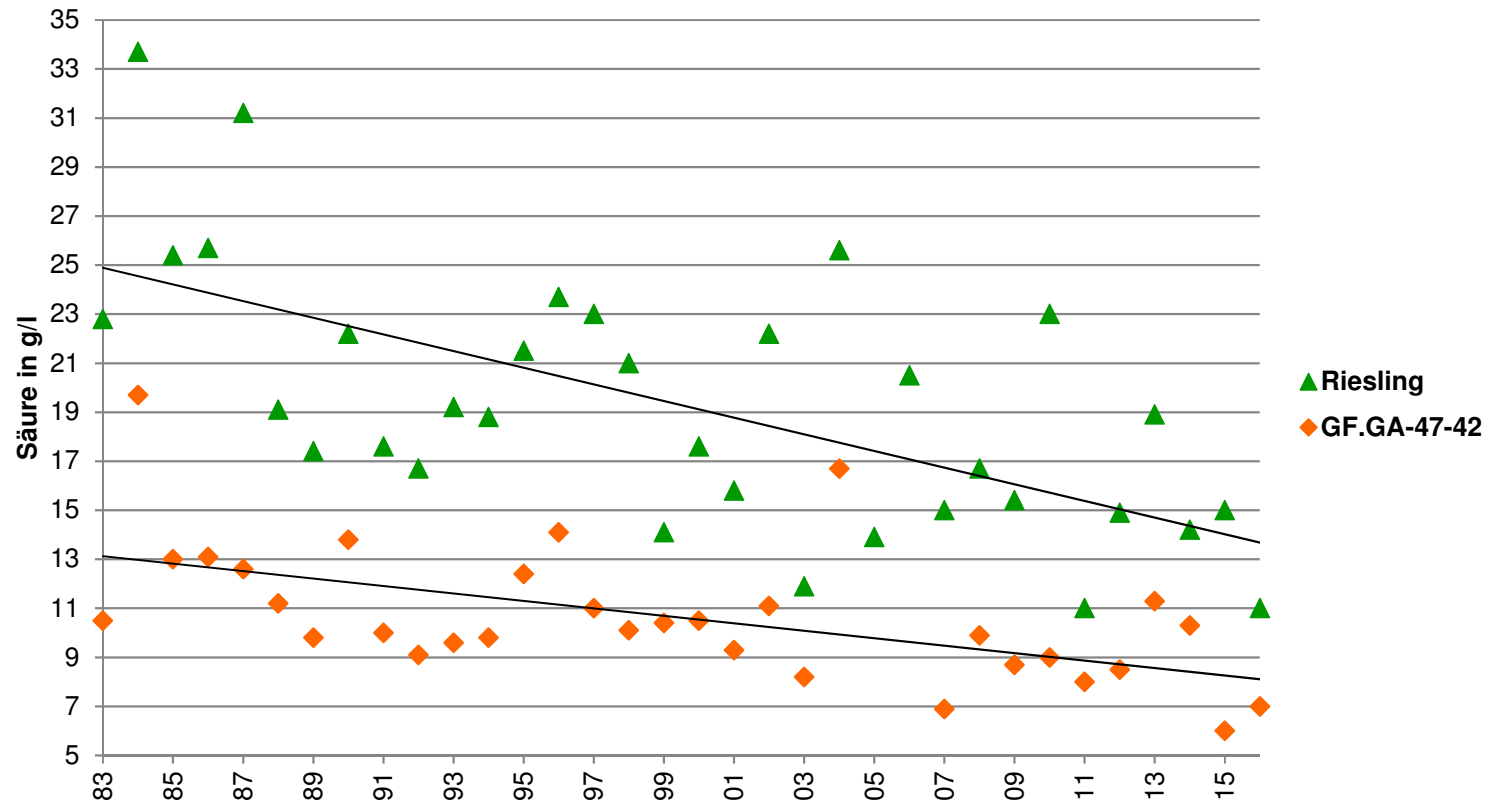
Anti Resistance Strategy 2018 Against Fungicides

	Category	Commercial Product	Active Compound	Group of Active Compound
	Peronospora - Fungizide			
1	B	Aktuan	Cymoxanil + Dithianon	Azetamide
2	B	Moximate 725 WG	Cymoxanil + Mancozeb	Azetamide
3	C	Ampexio	Mandipropamid +	CAA +
4	E		Zoxamide	Benazamide
5	C	Forum Gold	Dimetomorph + Dithianon	Morpholine (CAA)
6	C	Forum Star	Dimetomorph + Folpet	
7	C	Vino Star		
8	C	Melody Combi	Iprovalicarb + Folpet	Valinamide (CAA)
9	C	Vincare	Benthiavalicarb + Folpet	
10	C	Orvego	Dimetomorph +	CAA +
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20	S	Enervin	Initium (Ametoctradin) + Metiram	Pyrimidylamine

Maximum 3 applications per season for all fungicides labeled by the same letter and color; Category D: only one application per year

Climate Change: Decrease of Acidity

Acidity of Grapevine Must on September 20th



Trend:

Riesling:	11,2 g/l in 33 years	(0,34 g/l per year)
Gf.Ga-47-42:	5,0 g/l in 33 years	(0,15 g/l per year)

Breeders answer: select for late ripening

	bud burst													ripening																											
Variety	on average	April												September												October												on average			
		10	11	12	13	14	15	16	17	18	19	20	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10								
Felicia	16.04.																																			21.09.					
Calardis blanc	16.04.																																			09.10.					
Müller - Thurgau	13.04.																																			21.09.					
Riesling	14.04.																																			08.10.					

A new cultivar needs

- resistance ...
 - downy mildew
 - powdery mildew
 - Botrytis
 - Phylloxera
 - other pests & diseases
 - abiotic stress factors
- vigor
- yield
- wood maturation
- phenological adaptation
- quality
- ...
- a name

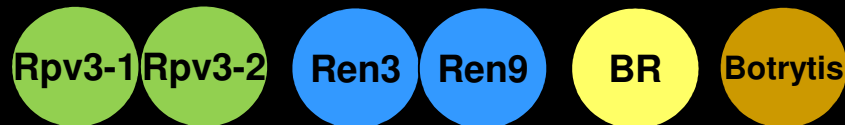


A new cultivar is
always a
compromise on
the timeline



→ Oliver Trapp, Poster 151

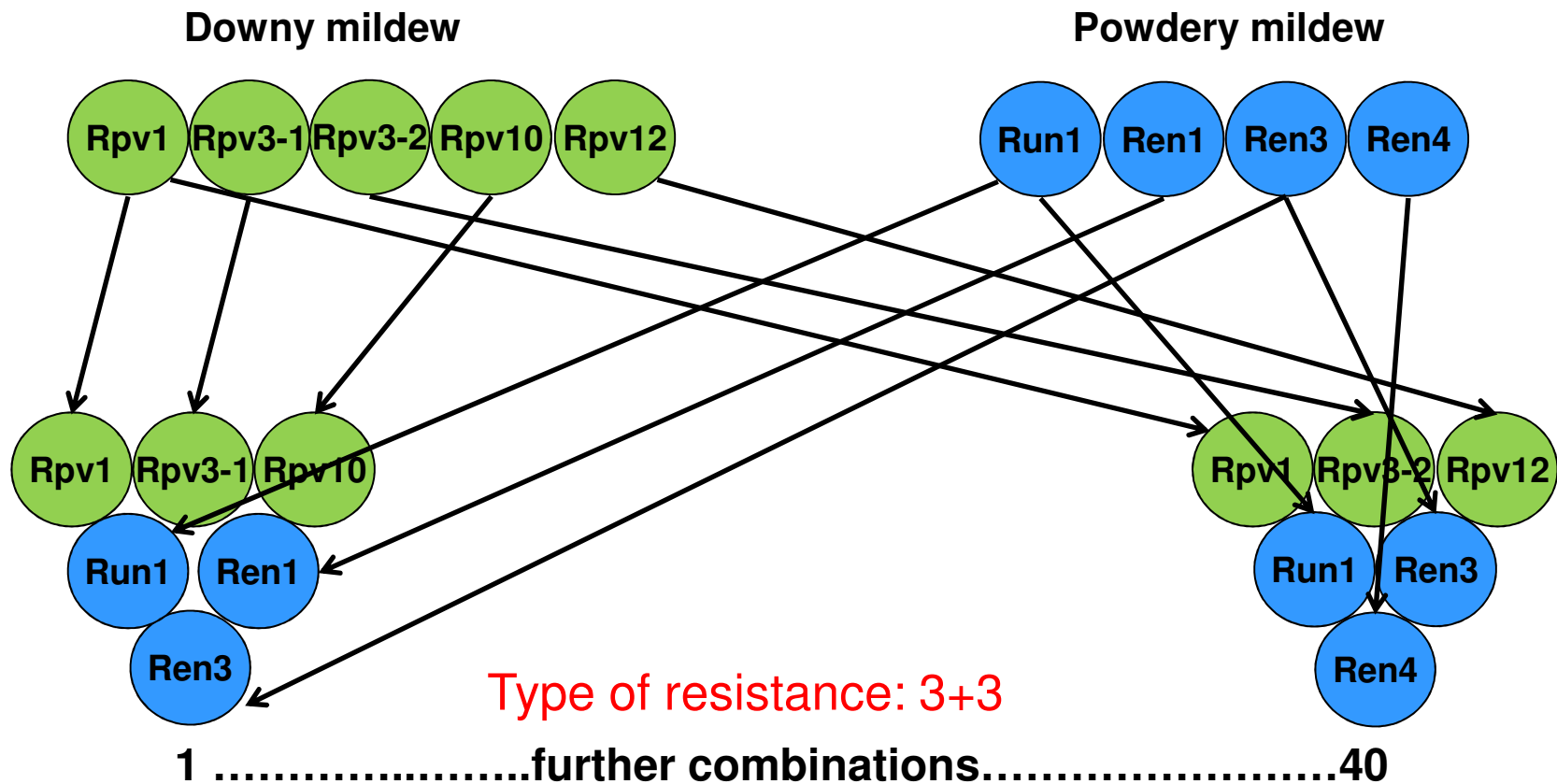
A new cultivar is
always a
compromise on
the timeline



Calardis Blanc

upright growth, late ripening,
small berries, loose cluster
wine style: decent aromas of Muskateller

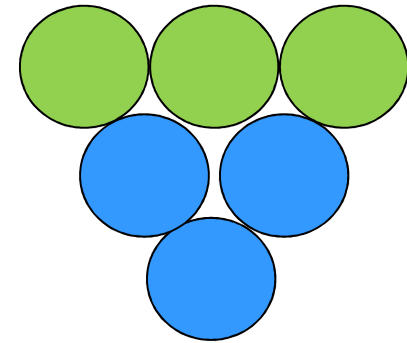
Stacking of resistances: Possibilities in elite genetic background



Limitations in Grapevine Breeding



- Combination of resistance and quality
 - Combinations with other traits
- Seedling numbers need to be high



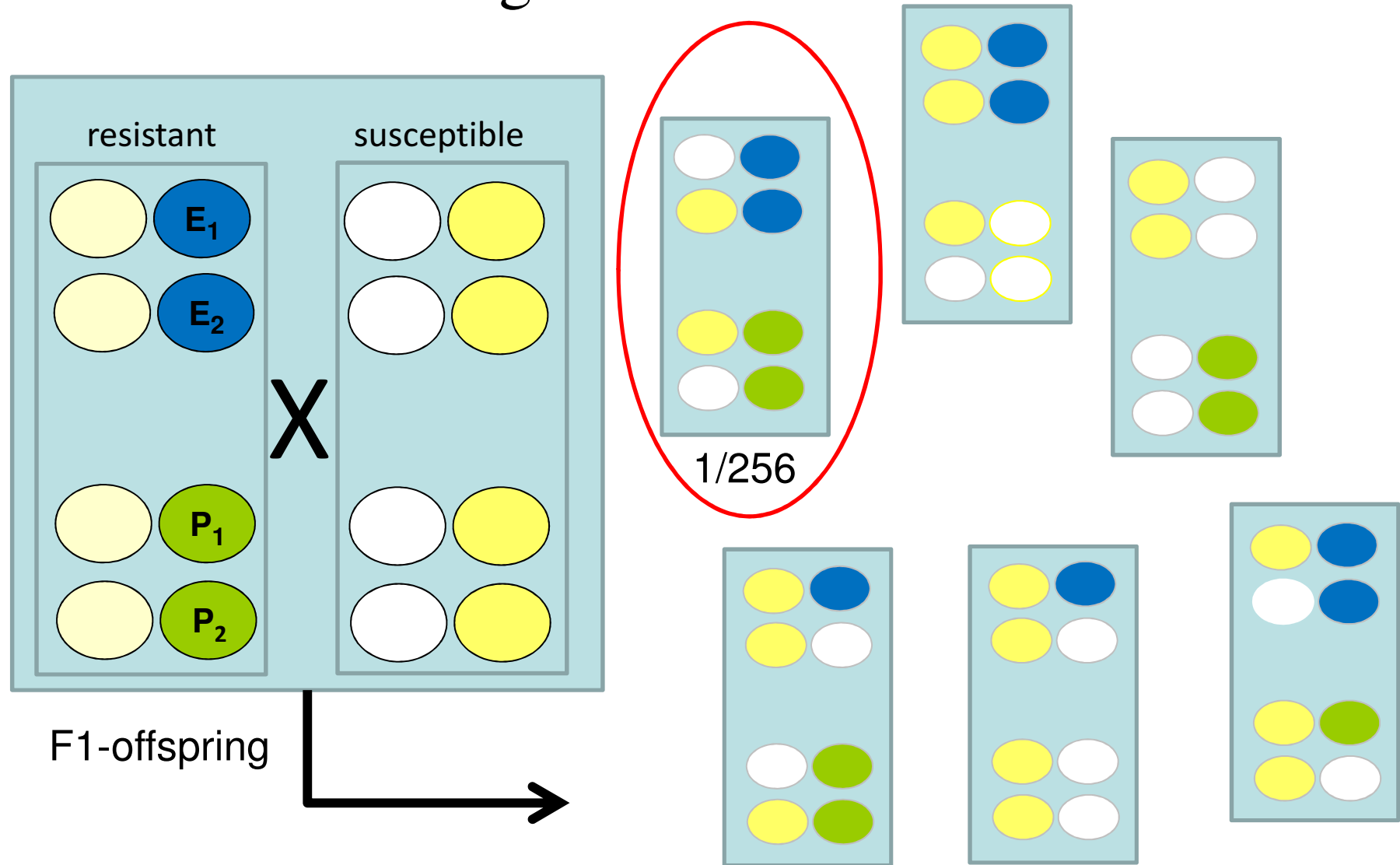
- Limitation in space in greenhouse and field
- Limiting number of markers for MAS
- Limiting phenotypic possibilities

1 hectare
(5000 plants)
per year

Mainly
markers for
PM and DM

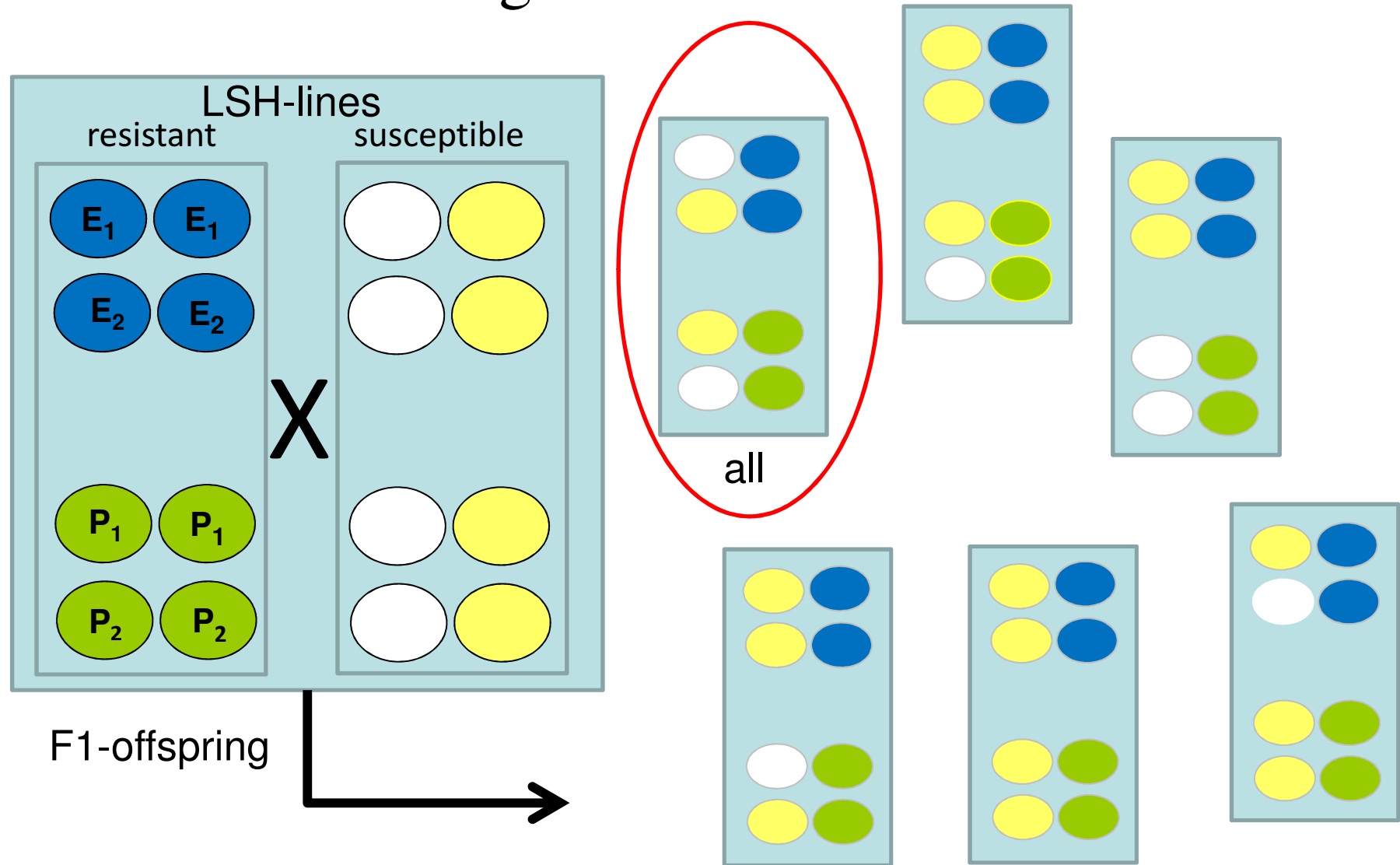
Tools for fast
trait
evaluation

Demand in seedlings – idealized crossing scheme



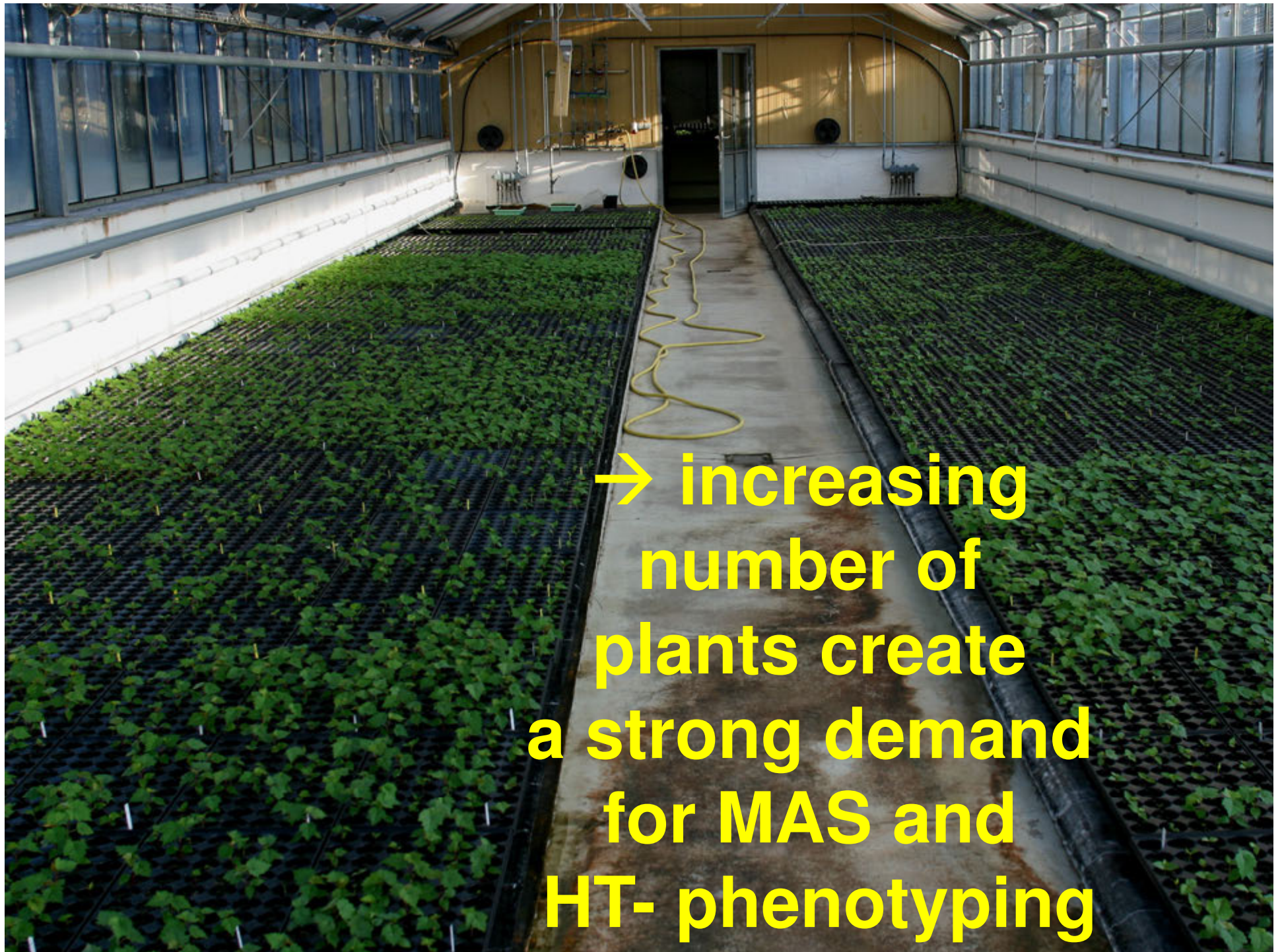
i.e. 25,600 seedlings to get 100 desired plants for further selection

Demand in seedlings – idealized crossing scheme



i.e. one can produce thousands of desired plants for further selection www.jki.bund.de





**→ increasing
number of
plants create
a strong demand
for MAS and
HT- phenotyping**

Demand in phenotyping

e.g. JKI in Siebeldingen

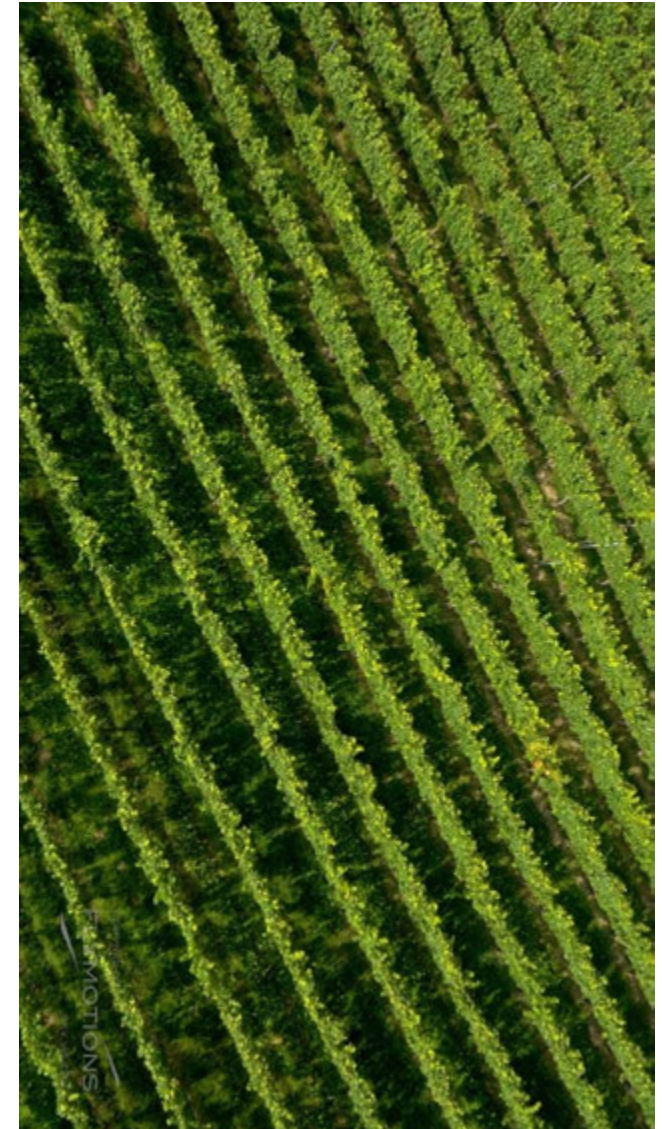
- Breeding (~ 6 ha)
- Breeding Research (~ 0.5 ha)
- Genetic Repository (~ 3 ha)



Challenges in Grapevine Phenotyping



- Plants need to be screened (for several traits) from the side not from top.



Traits of interest

Breeding

- Screening of seedlings → once a year
- Resistance, i.e. downy and powdery mildew
 - Yield
 - Phenology, ripening
 - Wine Quality Parameters

→ **Seedling selection**

Greenhouse



Field



Wine taste



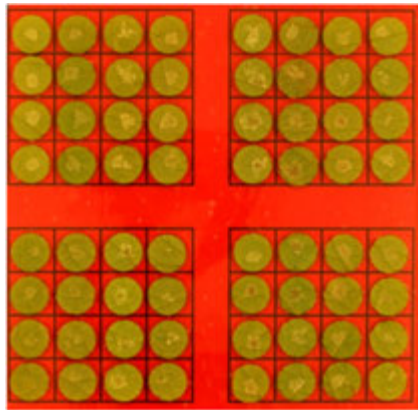
Traits of interest

Breeding Research/Genetic Resources

- E.g. Mapping populations → several times during the year
- Aim: Identification of new trait-related Loci
 - E.g. resistance, root and bunch architecture

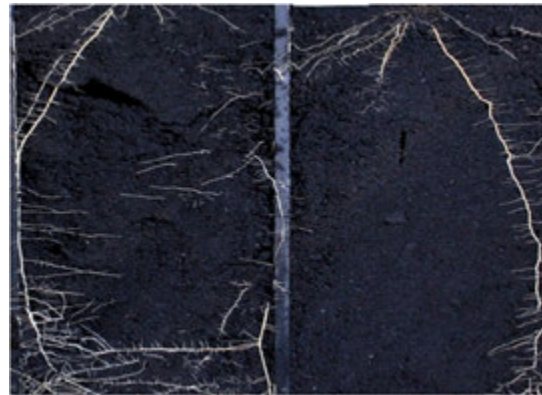
→ **Development of molecular markers for MAS**

Lab



Plasmopara viticola
on leaf disks

Greenhouse



Root architecture in
Rhizotrones

Field



Bunch architecture

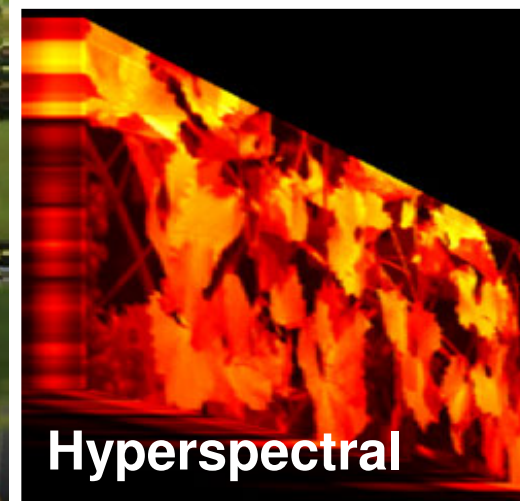
Aim

- Objective and precise phenotypic data
- Reduced error variation, retro perspective analysis
- High throughput (field) phenotyping
- Increased efficiency of grapevine breeding

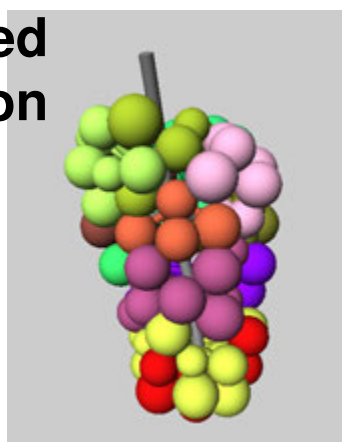


Automated acquisition of the grapevine phenotype

Various Sensors Options



**Computer-based
3D reconstruction**



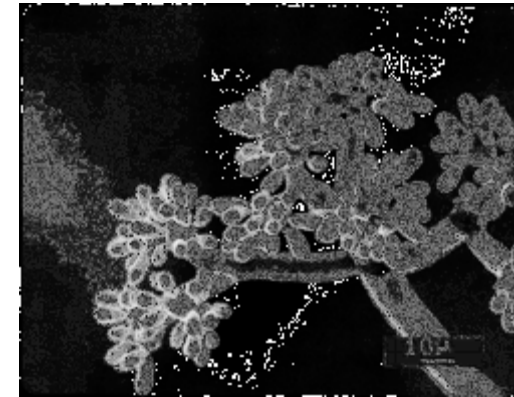
Phenotyping
for traits for
bunch rot
resilience



The gray mold disease



Source: JKI



- Botrytis cinerea* ➡ Compact bunches: high damage potential
- ➡ No active defense response in *Vitis vinifera*
- ➡ Breeding: Focus on physical barriers
 - ➡ e.g. loose bunch architecture

Phenotyping of bunch architecture as a trait



- **Bunch Traits**

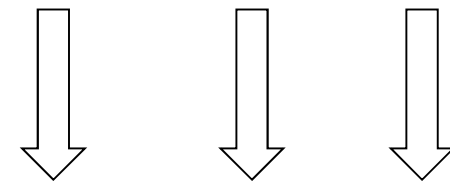
- length and width
- number of berries

- **Berry Traits**

- diameter and volume

- **Stem Traits**

- number and length of internodes
- length of pedicels



Density

OIV 204: 1 - 3 - 5 - 7 - 9

loose

dense

Labor intense approach for objective phenotyping



• **Bunch Traits**

- length and width
- number of berries

• **Berry Traits**

- diameter and volume

• **Stem Traits**

- number and length of internodes
- length of pedicels

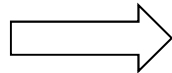
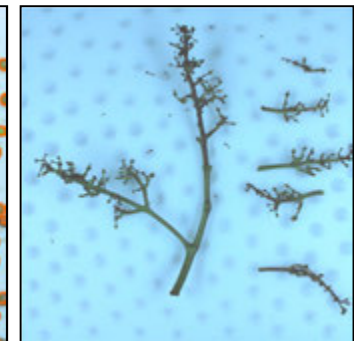
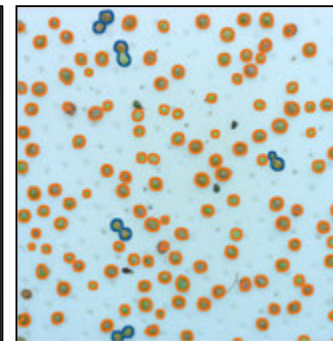
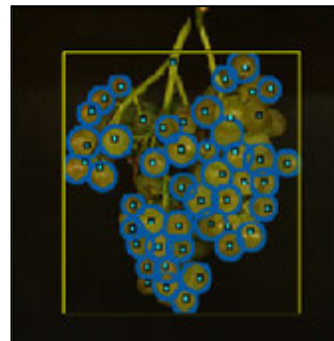


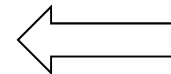
Image based Methods

- Cluster-Analysis-Tool (**CAT**)
- Berry-Analysis-Tool (**BAT**)
- ImageJ



Workflow

- Harvesting
- Image of the bunch
- Destemming
- Berries on plate
- Image of the berries
- Image of the stem skeleton

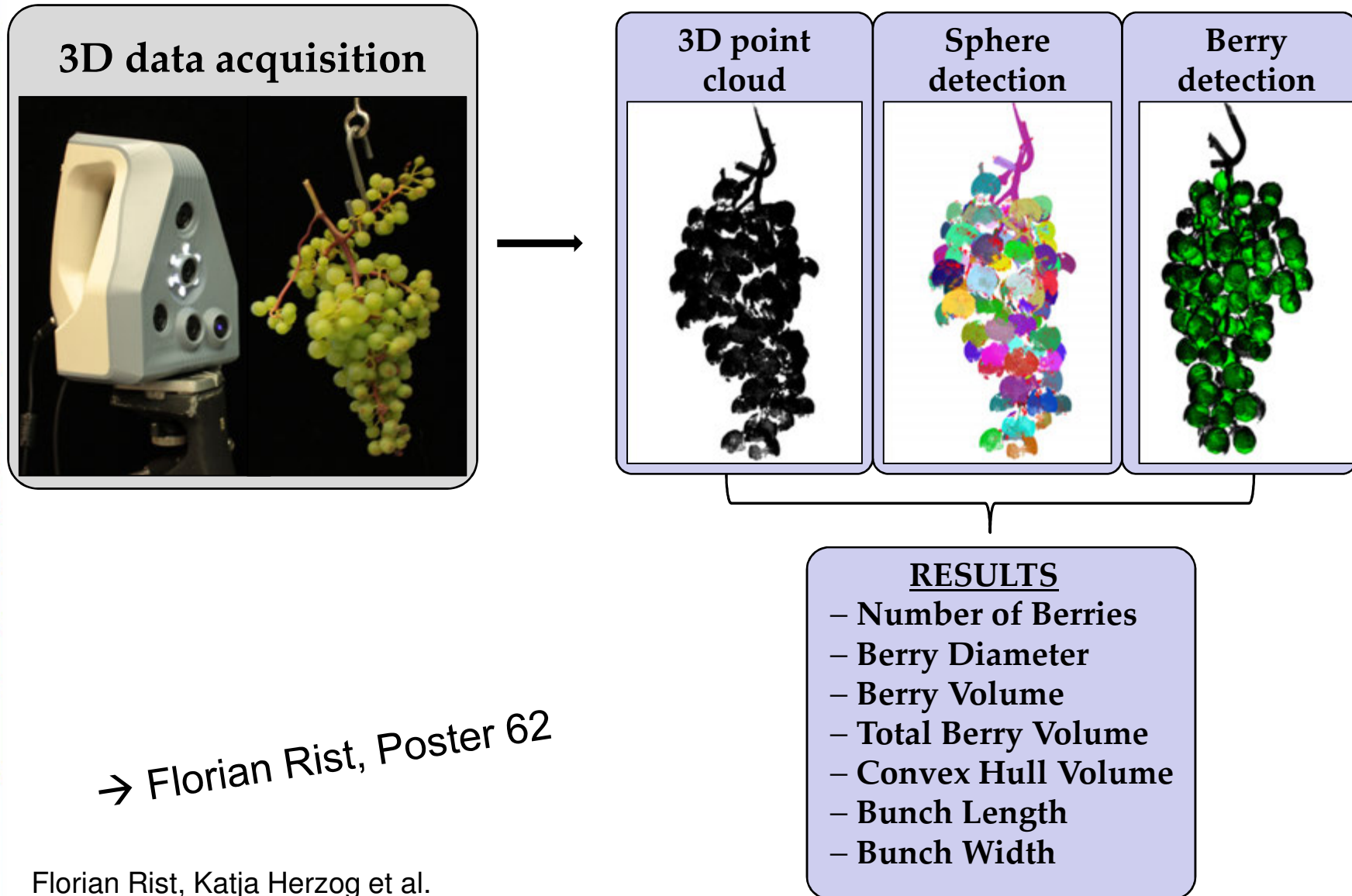


time intense

labor intense

determined by on-going season

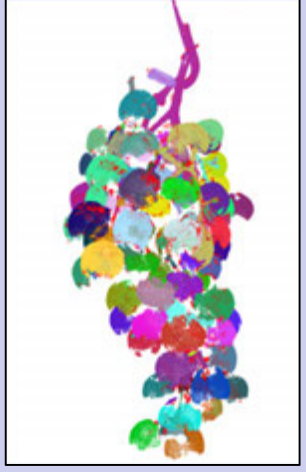
Establishment of a 3D-based high throughput phenotyping pipeline



3D point cloud

A visualization of the 3D point cloud data, showing a dense collection of black points forming the shape of a grape bunch.

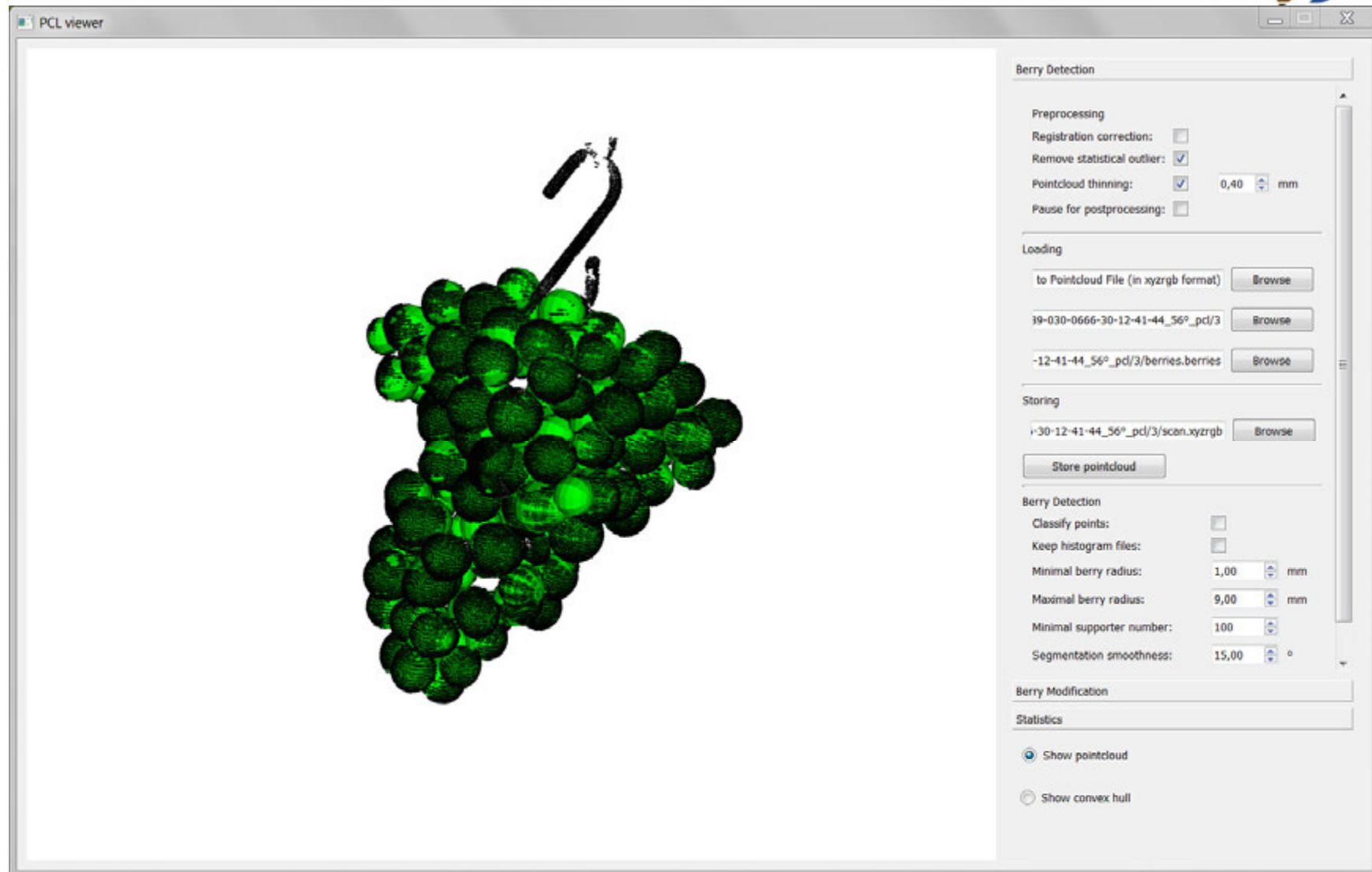
Sphere detection

A visualization showing the 3D point cloud with individual spheres (representing berries) detected and colored in various colors (red, blue, green, yellow, etc.).

Berry detection

A visualization showing the 3D point cloud with individual berries detected and colored in green.

Easy to handle user interface



Validation of the sensor

- Validation on 4 varieties, BBCH87



Dornfelder



Pinot Noir



Calardis Blanc



Riesling

- Application on segregating population GF.GA-47-42 X Villard Blanc, BBCH89
- highly variable in morphotype



Class 1



Class 3



Class 5



Class 7

Artec Spider is applicable for analysis of grape cluster determining parameters



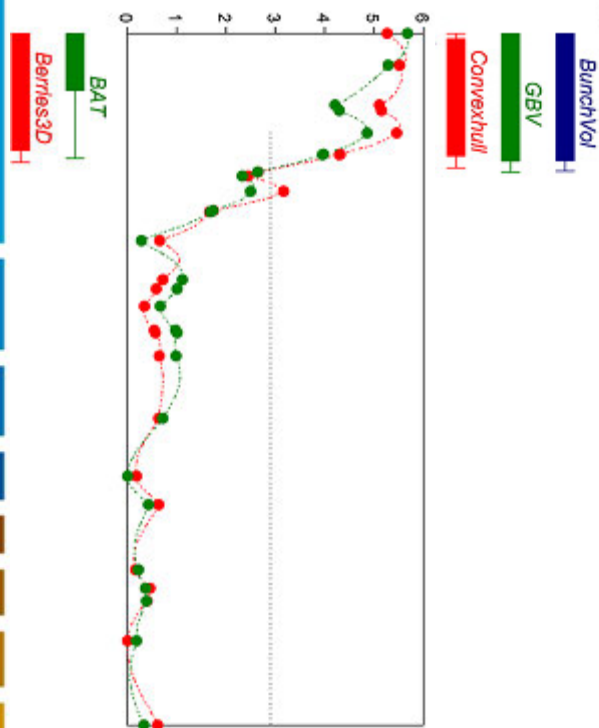
Trait	4 Varieties	GF.GA-47-42 X Villard Blanc	P-value
	r^2	r^2	
Number of Berries	0.94	0.91	<.0001
Berry Diameter [mm]	0.93	0.89	<.0001
Berry Volume [ml]	0.93	0.91	<.0001
Total Berry Volume [ml]	0.83	0.91	<.0001
Convex Hull/Bunch Volume [ml]	-	0.85	<.0001
Grape Width [mm]	0.54	-	<.0001
Grape Length [mm]	0.85	0.75	<.0001
	n = 75	n = 802	

- ⇒ High correlation of berry parameters
- ⇒ Lower value for width
- ⇒ Massive time saving compared to 2D (> 10x)

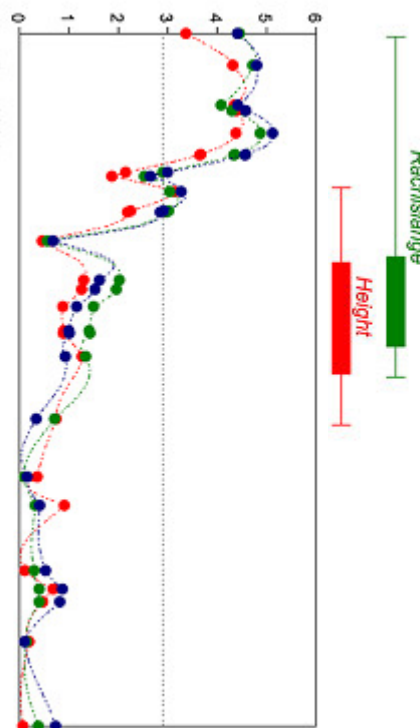
3D bunch trait data show similar QTL regions compared to reference data



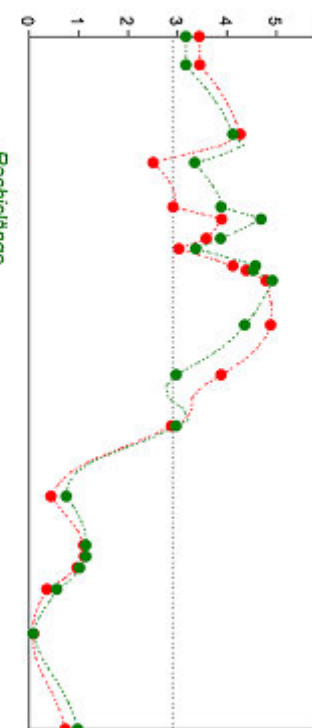
Number of Berries
Chr 18



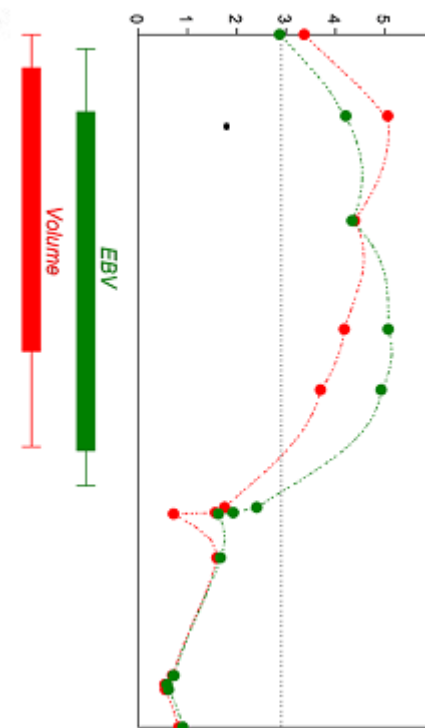
Total Volume
Chr 18



Grape Length/
Rhachis Length
Chr 9



Berry Volume
Chr 17



green reference data

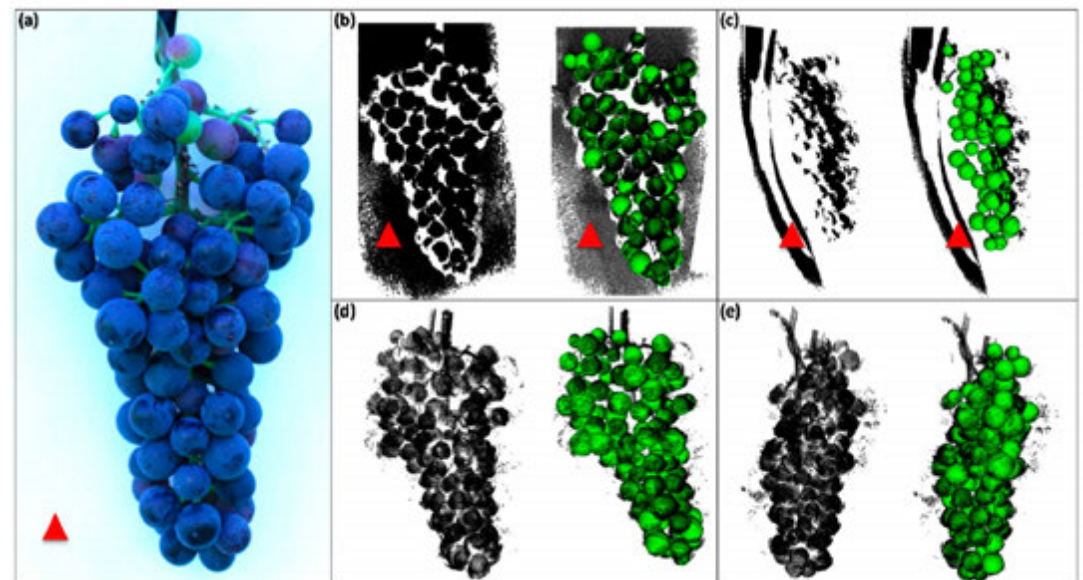
red / blue 3D data

➡ Massive time saving compared to 2D (up to **10x**)

Extended application in the field

For a more intense mapping/association approach: extensive phenotyping

- ⇒ Increasing number of plants
- ⇒ Multi cultivar screening
- ⇒ **non invasive**
phenotyping



Artec Spider is usable for field application



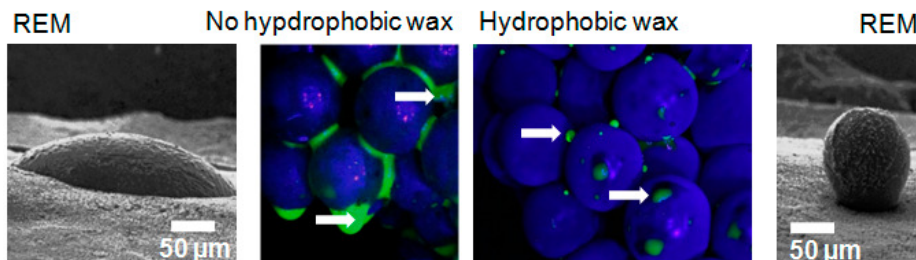
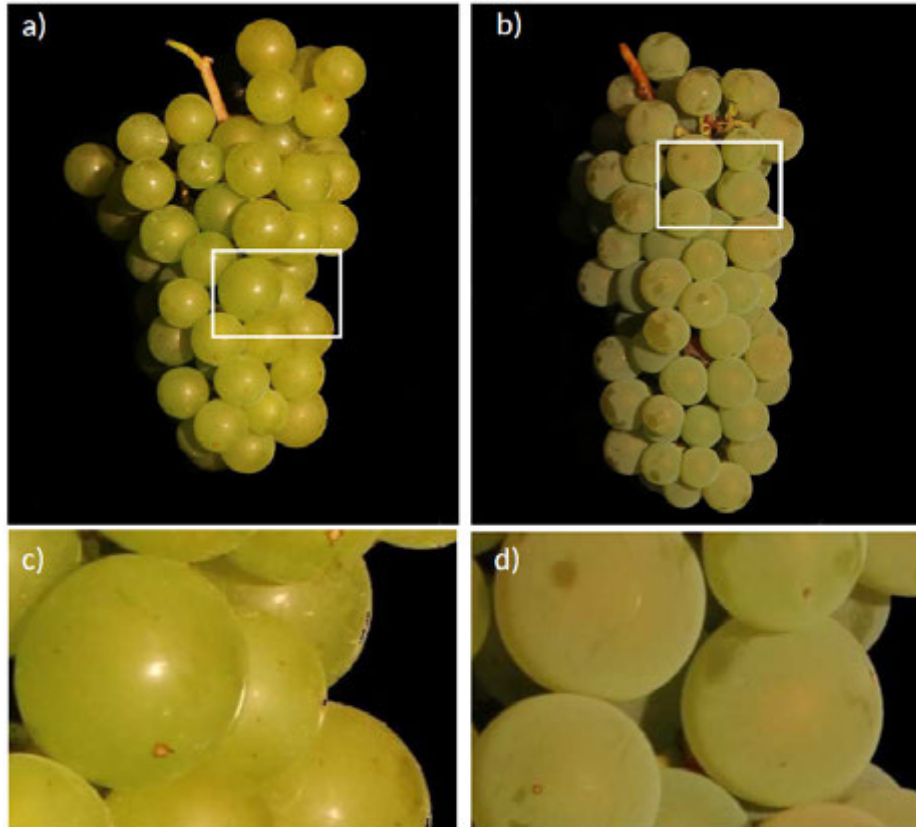
- Use of Artec Spider directly in the field
- 47 clusters of 4 varieties (Riesling, Calardis Blanc, Pinot Noir, Dornfelder)
- Analysis of variance of `field-`, `front-`, 360°-Scan

	Number of Berries	Berry Diameter [mm]	Berry Volume [ml]	Total Volume [ml]	Convex Hull	Grape Width [mm]	Grape Length [mm]
360°	137 ^A	12.8 ^A	1.2 ^A	152.6 ^A	574.8 ^A	106.4 ^A	159.1 ^A
front	66 ^B	13.1 ^A	1.3 ^A	79.7 ^B	290 ^B	90.5 ^B	151.9 ^A
field	66 ^B	13.3 ^A	1.3 ^A	84.9 ^B	409.2 ^C	109.6 ^A	155.8 ^A

⇒ Partial point clouds sufficient for detection of berry diameter, volume and cluster length

⇒ Fluctuations for width and convex hull

BERRY SURFACE AND *BOTRYTIS* BUNCH ROT



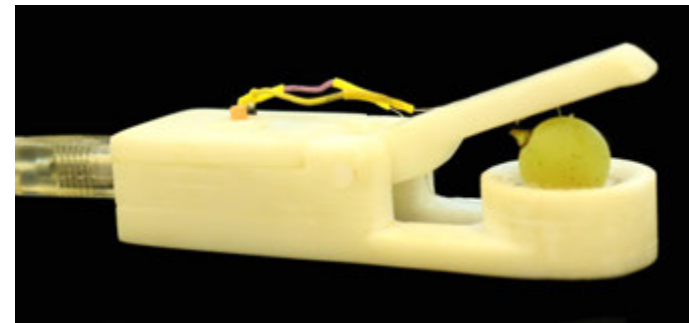
- Berry surface = cuticle and epicuticular wax layer

↓ assumption

Thickness, uniformity and intactness of the cuticle and epicuticular wax is correlated to resilience towards *Botrytis* bunch rot

OBJECTIVE PHENOTYPING DUE TO IMPEDANCE

- Measurement of electrical impedance described as indicator for thickness and permeability of cuticle/epic. wax
- Improved, simple-to-handle sensor
 - 450 berries per hour
 - One point measurements/ berry
 - Inexpensive sensor technique
 - Small memory usage



Herzog et al. 2015

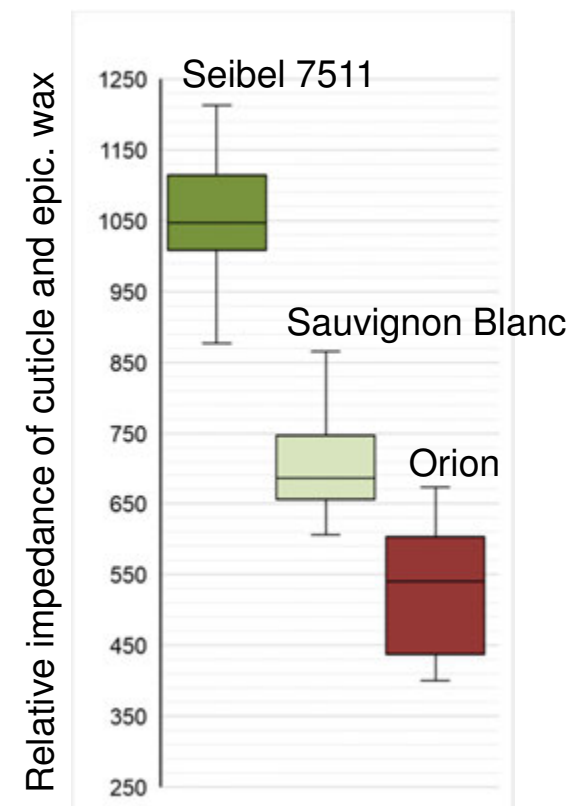
→ Katja Herzog, Poster 87

REDUCED RISK FOR *BOTRYTIS* BUNCH ROT



Impedance of cuticles

Botrytis bunch rot



23.8 19.4 17.1

Brix when up to 5% of berries were infested with *Botrytis* bunch rot

Actors to dissect Botrytis Resilience



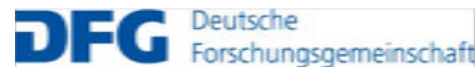
IRZ

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University of Bonn
Computer Science 4

Volker Steinhage
Jenny Mack



Development of field phenotyping platforms



FIELD PHENOTYPING PLATFORM



Multi-sensor field phenotyping platform: **PHENObot***

Automated data recording on
single vine level:

- Georeference with high precision
- PHENObot stops at each vine
- Plant ID is connected with the image



* Kicherer, Herzog, Pflanz, Wieland, Rüger, Kecke, Kuhlmann, Töpfer (2015) **Sensors**, 15(3), 4823-4836.

SENSORS FOR OBJECTIVE FIELD PHENOTYPING



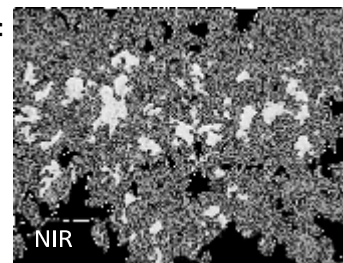
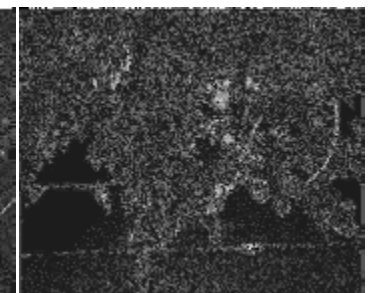
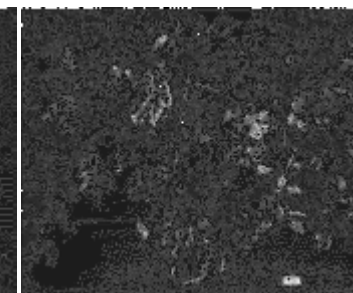
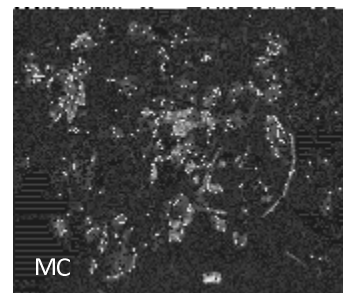
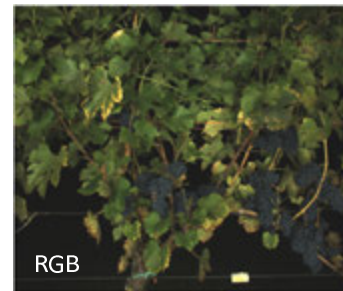
Multi-sensor field phenotyping platform: **PHENObot***



Kicherer *et al.* 2015a, *Sensors*

Synchronic
image
acquisition
15 s / vine
20 images/min

Plant ID
=
allocation of
single vine



* Kicherer, Herzog, Pflanz, Wieland, Rüger, Kecke, Kuhlmann, Töpfer (2015) ***Sensors***, 15(3), 4823-4836.

PHENOvines

AUTOMATED PHENOTYPING – DATA MANAGEMENT



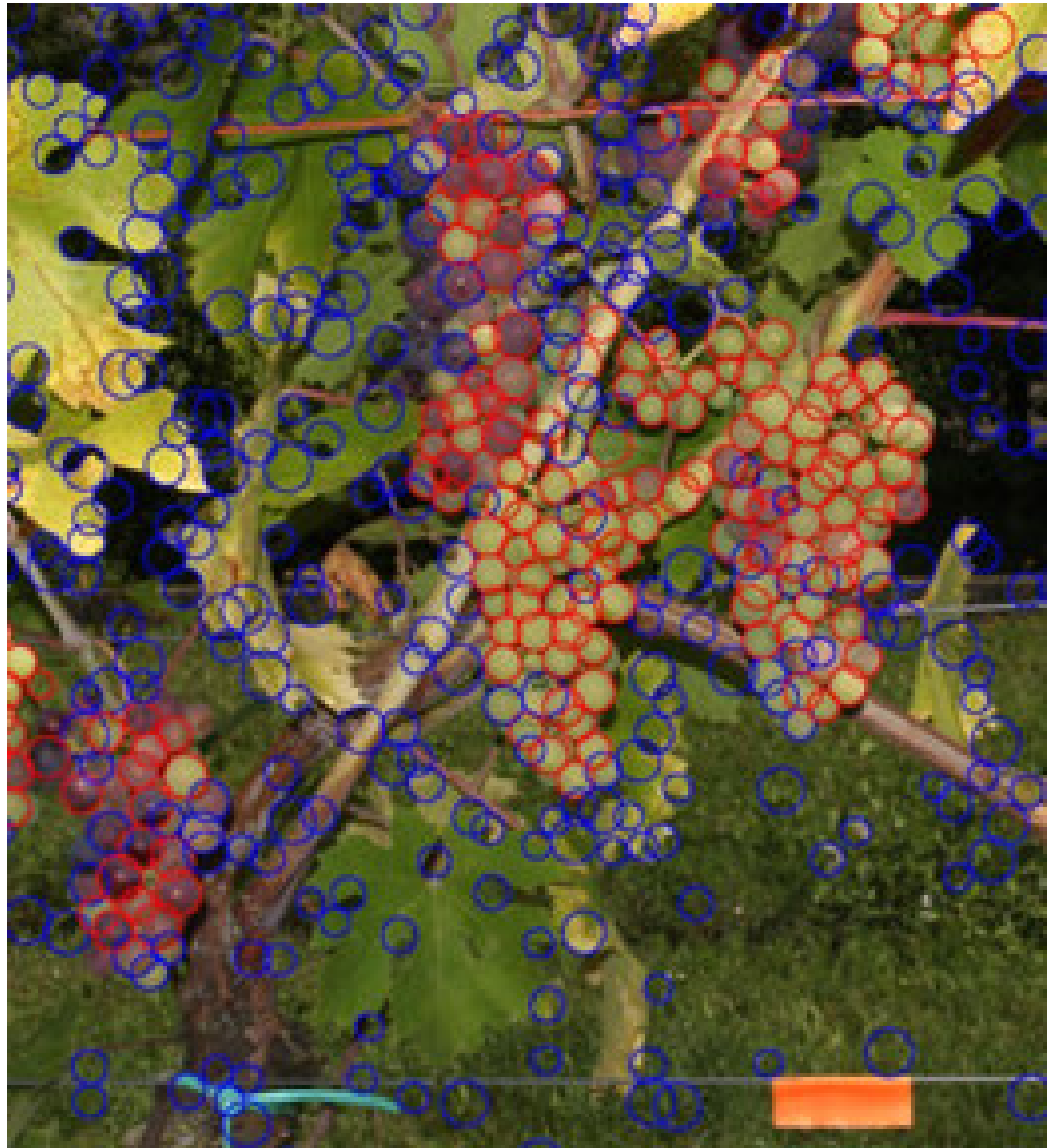
Post-processed

- Automated data management

The screenshot displays the JKI IMAGEdata web application. At the top, there is a header with the JKI logo and the text 'Julius Kühn-Institut Bundesforschungsinstitut für Kulturpflanzen'. Below the header is a navigation bar with links: Home, Import, Standorte, and Suche. The main content area is divided into two sections. On the left, there are three buttons: 'Datei(en) hochladen' (blue), 'Standorte auslesen' (green), and 'Speichern' (red). On the right, there is a section titled 'Quelle der Bilder' with four radio button options: 'Freilandbilder ohne GPS-Information', 'Freilandbilder mit POI-Datei', 'Freilandbilder Kamerasystem', and 'Laborbilder'. The 'Freilandbilder Kamerasystem' option is selected. Below this, there are input fields for 'Korrektur East-Wert' (1.5 m) and 'Korrektur North-Wert' (0.5 m). A green bar indicates 'Erfolgreiche Imports (1782)'. Below this, a table lists the imported images with columns: Bild, Dateiname, Datum, GPS-Info Bild / POI, and Standort. Each row also includes a trash icon for deletion.

Bild	Dateiname	Datum	GPS-Info Bild / POI	Standort
	F3101F81E_3456454943900.tif	12.07.2013 8:22:239	430299.967 E 5452312.049 N	09-016-003
	F3101F81E_3456454947000.tif	12.07.2013 8:22:27	430299.992 E 5452311.100 N	09-016-004
	F3101F81E_3456454950300.tif	12.07.2013 8:22:303	430300.057 E 5452310.136 N	09-016-005
	F3101F81E_3456454953200.tif	12.07.2013 8:22:166	430300.153 E 5452309.211 N	09-016-006
	F3101F81E_3456454956900.tif	12.07.2013 8:22:369	430300.259 E 5452308.119 N	09-016-006

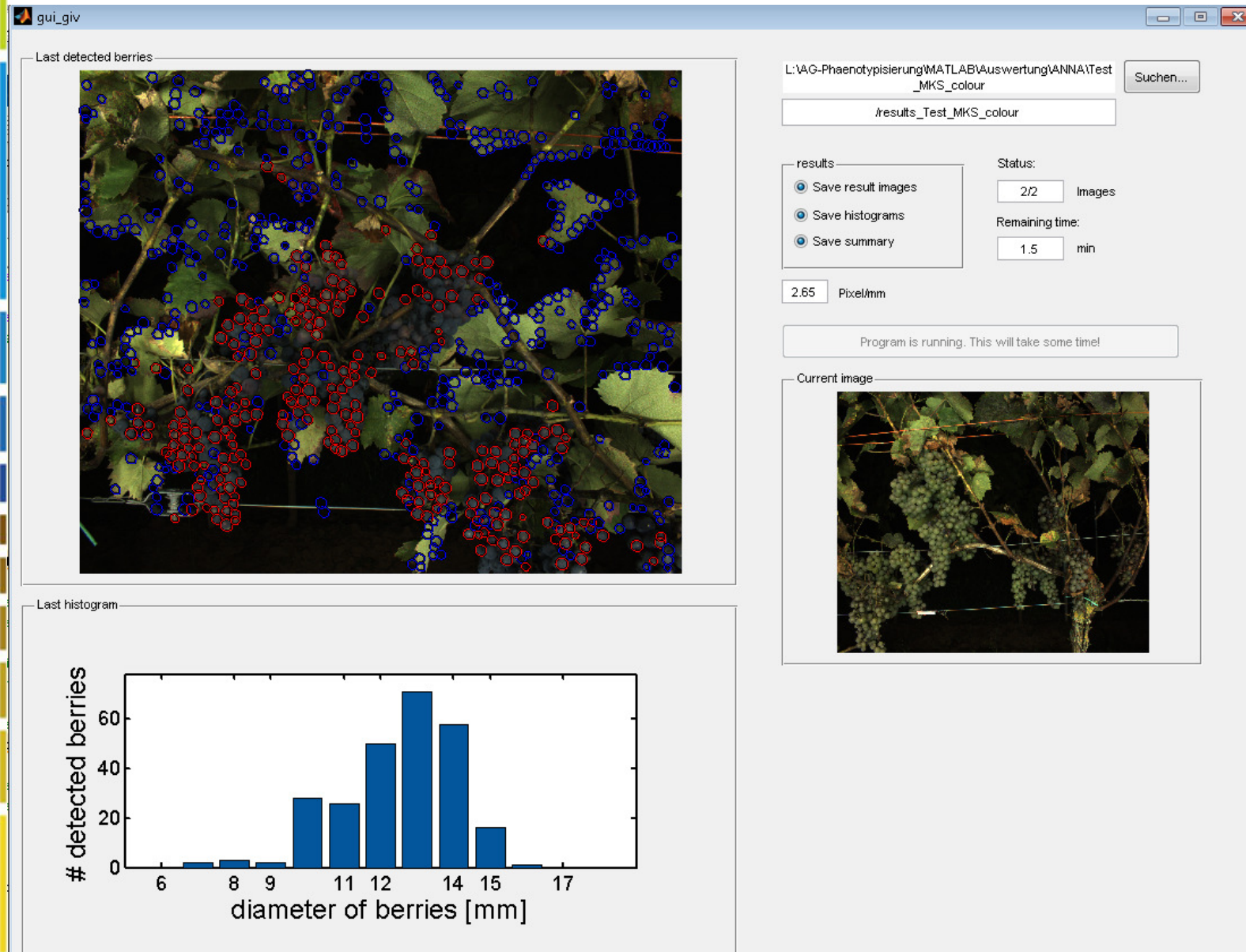
AUTOMATED PHENOTYPING – DATA ANALYSIS



Post-processed

- Automated data management and data analysis

AUTOMATED PHENOTYPING – BIVcolor



PROOF OF CONCEPT:



Acquisition of RGB images and extraction of traits



- Utilization of PHENObot and BIV_{color} in the genetic repository
- 2700 vines (~970 Accessions) within 12 hours
- berry size and -color

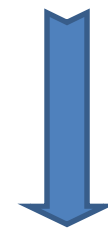
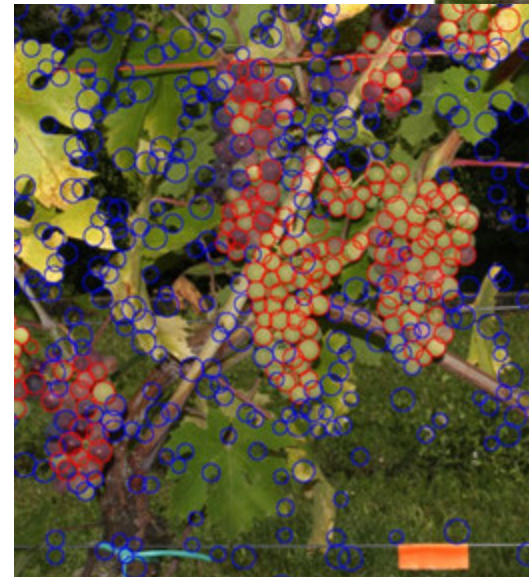
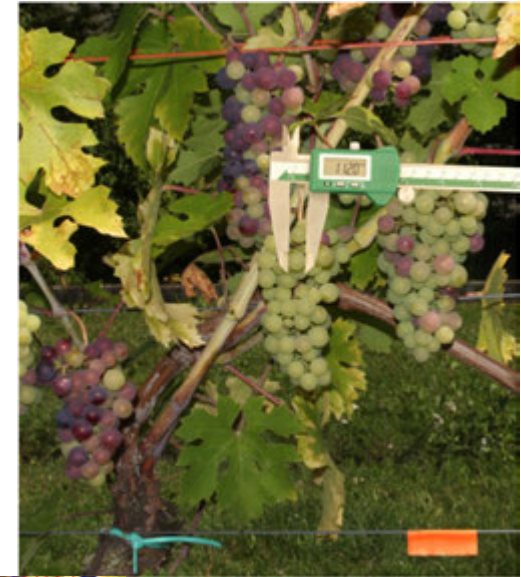


Kicherer *et al.* 2015a, Sensors

AUTOMATED PHENOTYPING

Post-processed

- Automated data management
- Automated data analysis



24 times
faster

Multi-sensor field phenotyping platform: **PHENObot***

Disadvantages:

- slow speed
- image acquisition at night to ensure standardized light conditions



* Kicherer, Herzog, Pflanz, Wieland, Rüger, Kecke, Kuhlmann, Töpfer (2015) **Sensors**, 15(3), 4823-4836.

SENSORS FOR OBJECTIVE FIELD PHENOTYPING

Improved multi-sensor field phenotyping platform:

Phenoliner*

- Automated, synchronic image capture with **high-throughput**
- Georeference with high precision



* Kicherer, Herzog, Bendel, Klück, Backhaus, Wieland, Rose, Klingbeil, Läbe, Kohl, Petry, Kuhlmann, Seiffert, Töpfer (2017) **Sensors**,

UAV FOR MANAGEMENT APPROACHES



- Inventory of vines growing in the genetic repository
 - GPS position of single vines
 - RTK-GPS for adequate precision

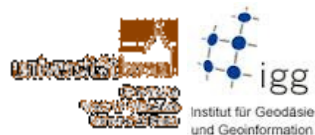
UAV FOR MANAGEMENT APPROACHES



- Inventory of vines growing in the genetic repository
 - GPS position of single vines
 - RTK-GPS for adequate precision
- Detection of missing vines → e.g. maintaining of grapevine accessions (3 plants)



MANY THANKS TO



GEFÖRDERT VOM



Bundesministerium
für Bildung
und Forschung



Federal Ministry
of Food
and Agriculture



Many Thanks to

- Breeding team
Rudi Eibach
Oliver Trapp
et al.

- *Botrytis* team
Katja Herzog
Robert Richter
Florian Rist
Eva Zyprian
et al.



Federal Ministry
of Food
and Agriculture

Calardis Blanc*

(Gf.1993-22-6)

variety protection 2018

* Naming according to:

Gailhardsswilre =

Calardiswilre =

Geilwilre =

Geilweiler =

Geilweilerhof