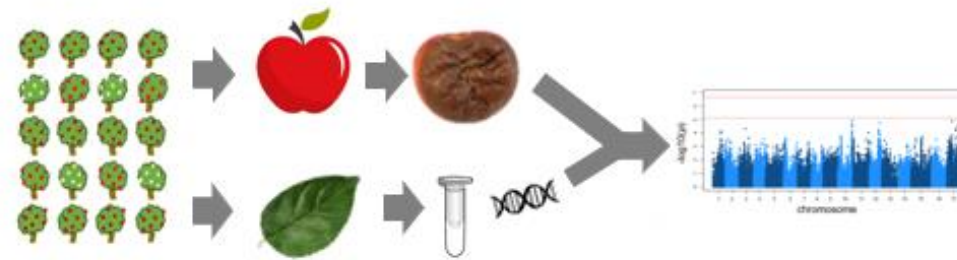
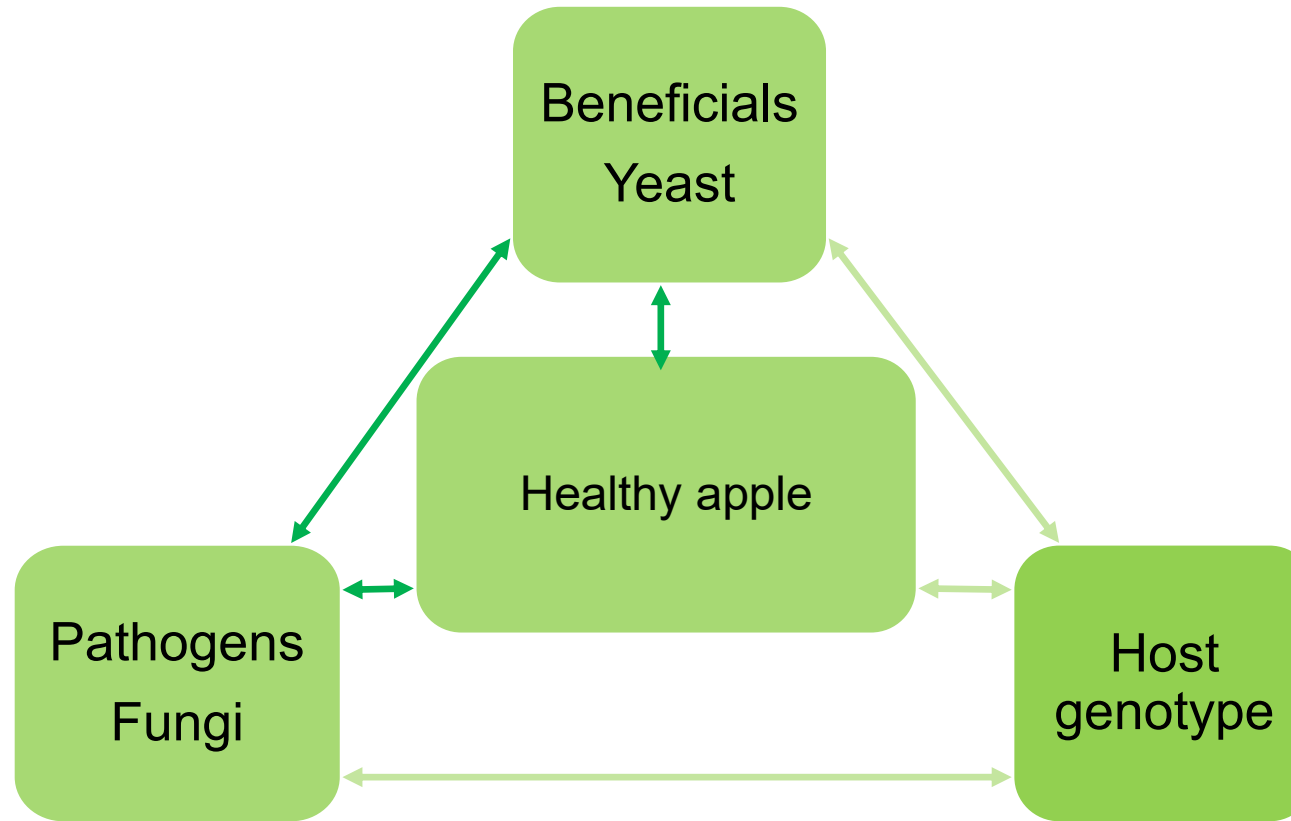


Genetic loci controlling susceptibility to bull's eye rot and its link to apple ripening



Andreas Bühlmann
Agroscope

«Biotic» status of an apple



Can we optimize host breeding through identification of resistance Loci and application of MAS

«Apfelzukunft dank Züchtung»

Teilprojekt 1

Genomic selection



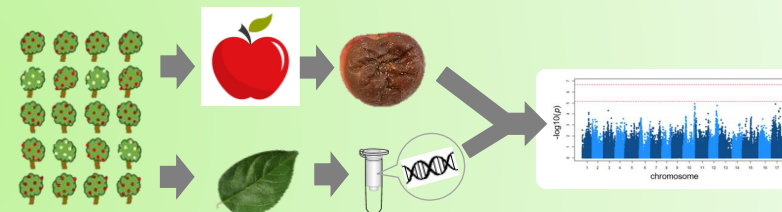
Teilprojekt 2

Combine Fast Track breeding and genomic selection



Teilprojekt 3

Genome wide association study for Lenticel Rot Resistance



WP3

Genome wide association study for Lenticel Rot Resistance



1) Inoculation protocol

Development and validation

2) Phenotyping

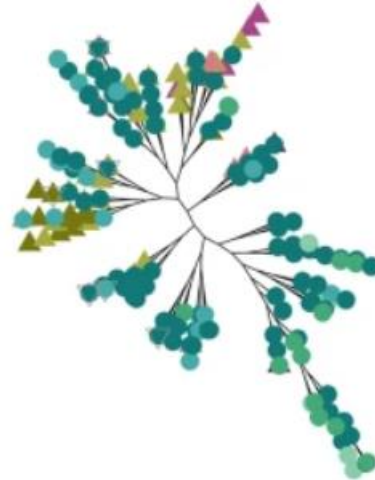
Phenotype REFPOP at
least 2 seasons

3) GWAS

Analyse associations, develop
markers if applicable

Inoculation protocol: the fruit

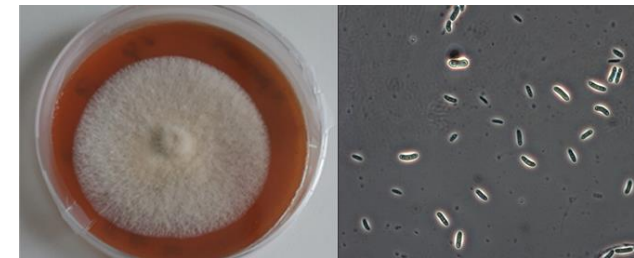
- Refpop- a population of ~250 accessions
- Planted at 6 locations accross Europe
- Genotyped with 480k SNP chip
- Study genotype x phenotype x environment



Jung M, et al. (2020) The apple REFPOP—a reference population for genomics-assisted breeding in apple. Horticulture Research 7:189

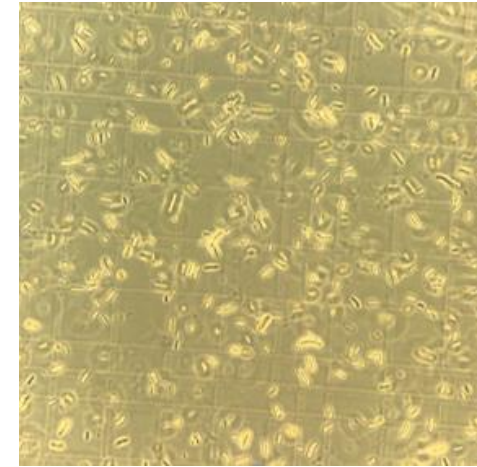
Inoculation protocol: the pathogen

- *Phlyctema vagabunda* syn *N. alba*
 - 5 strains tested, 1 selected for reproducibility of infection and handling
- *Neofabraea perennans*
 - 3 strains tested, 1 selected for reproducibility of infection, and handling



Inoculation protocol: reproducible infection

- Order new strains yearly from strain collection
- Control stock regularly
- Control growth rates
- Renew inoculations spores monthly
- Store spores at 0-4°C



Solved 

Inoculation protocol: contamination

- Decontaminate inoculation area EtOH 70 %
- Close wound with Parafilm
- Adapt relative humidity
- Disinfect bins between uses



Solved 

Phenotyping

- **Per year:**
- **REFPOP:** n = 233 genotypes, 32 double
- 5 fruits per genotype, 5 wounds per apple
 - 1.325 apples
 - 6.625 inoculations
- ~ 10% contamination rate / „150 apples“



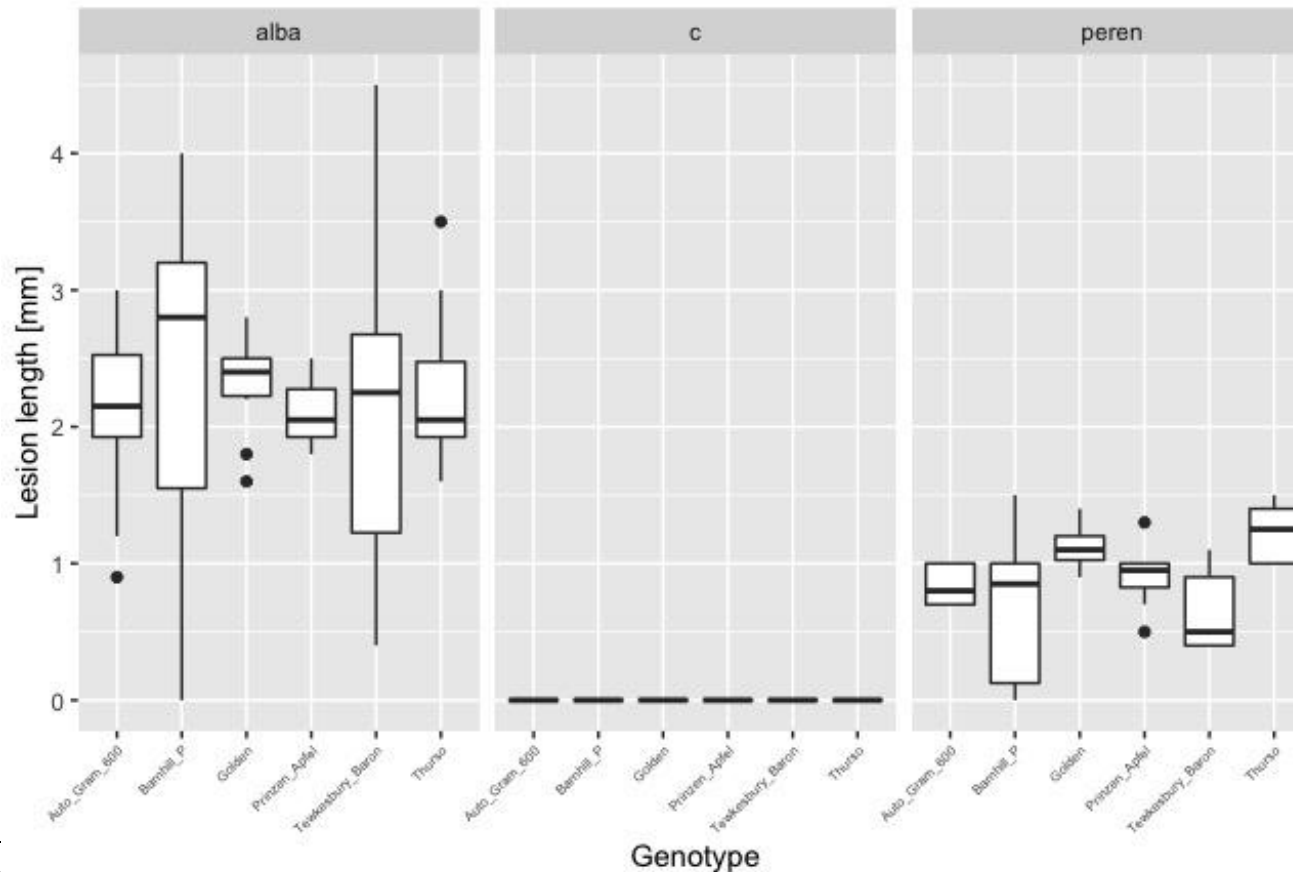
Storage, 20°C, 70% rH
Duration: 21 d

Phenotyping: Results

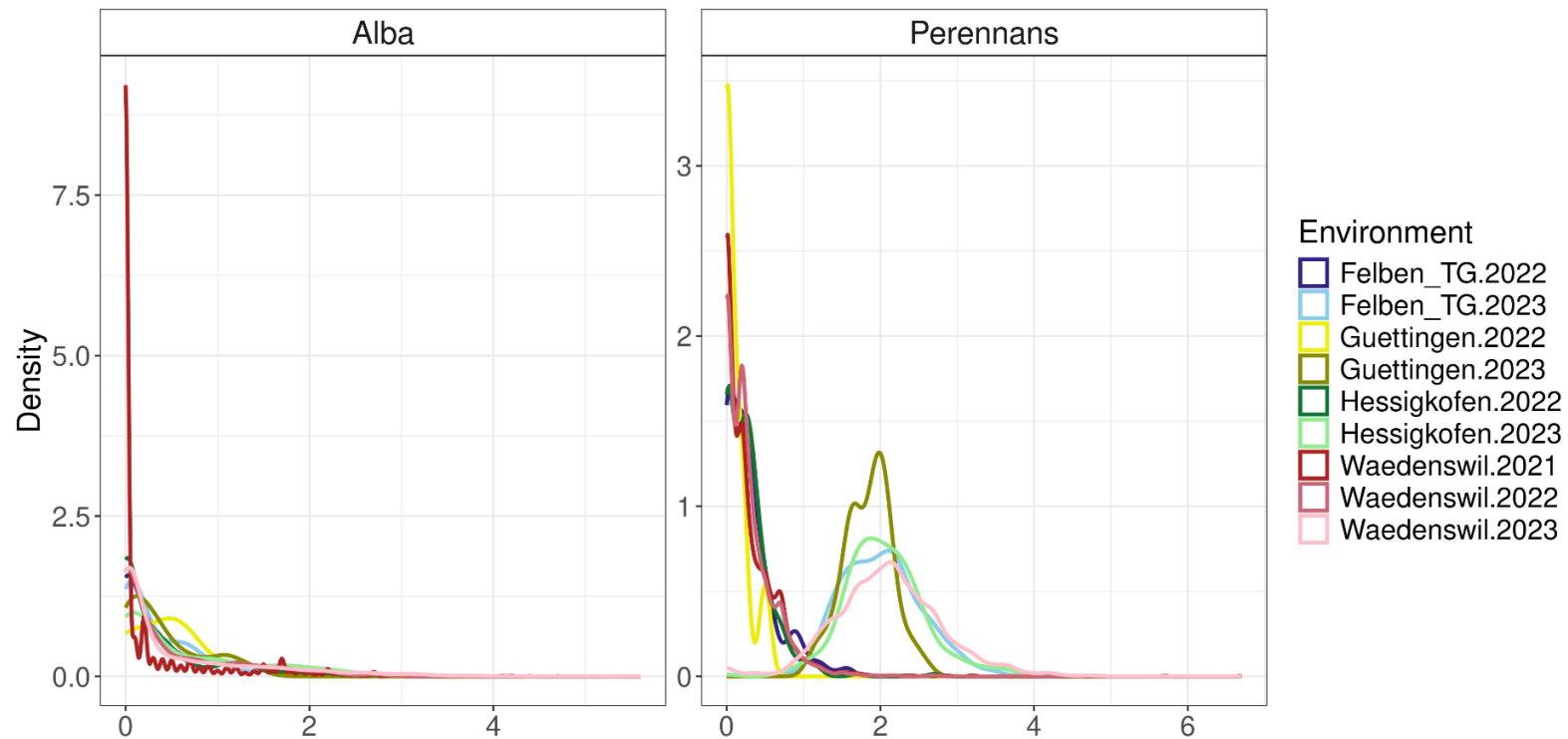


Infection rate **98 %**

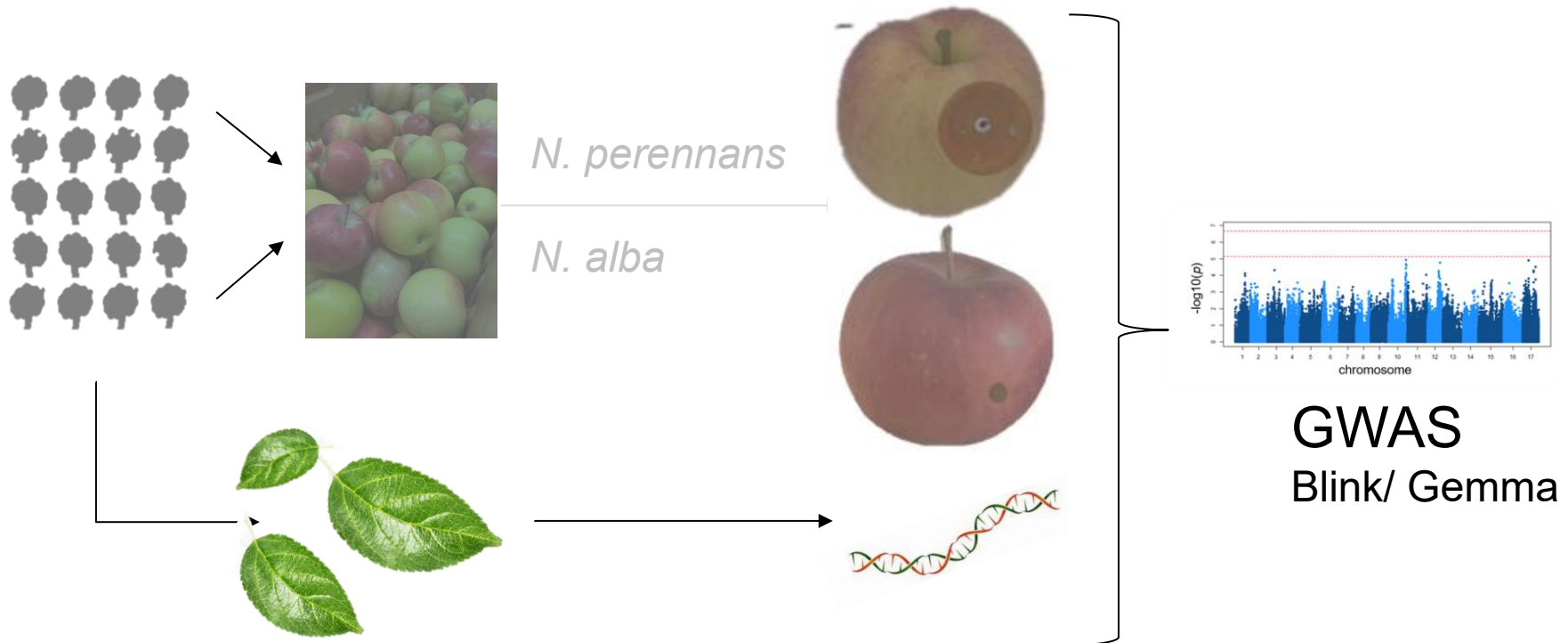
Contamination **10 %**



Phenotyping: Results

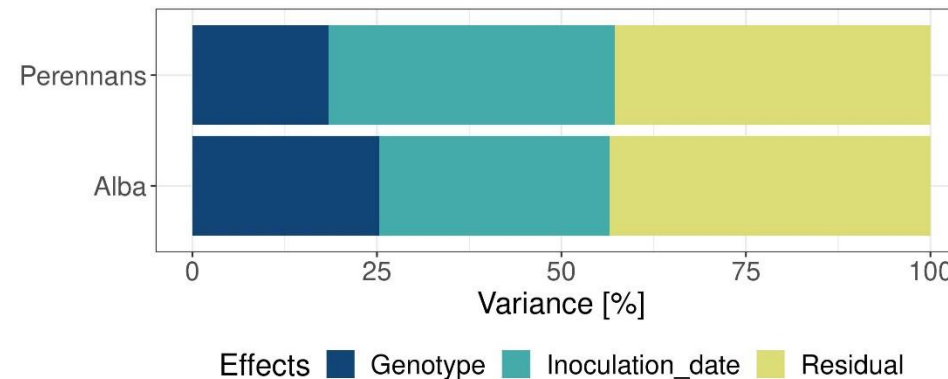


GWAS

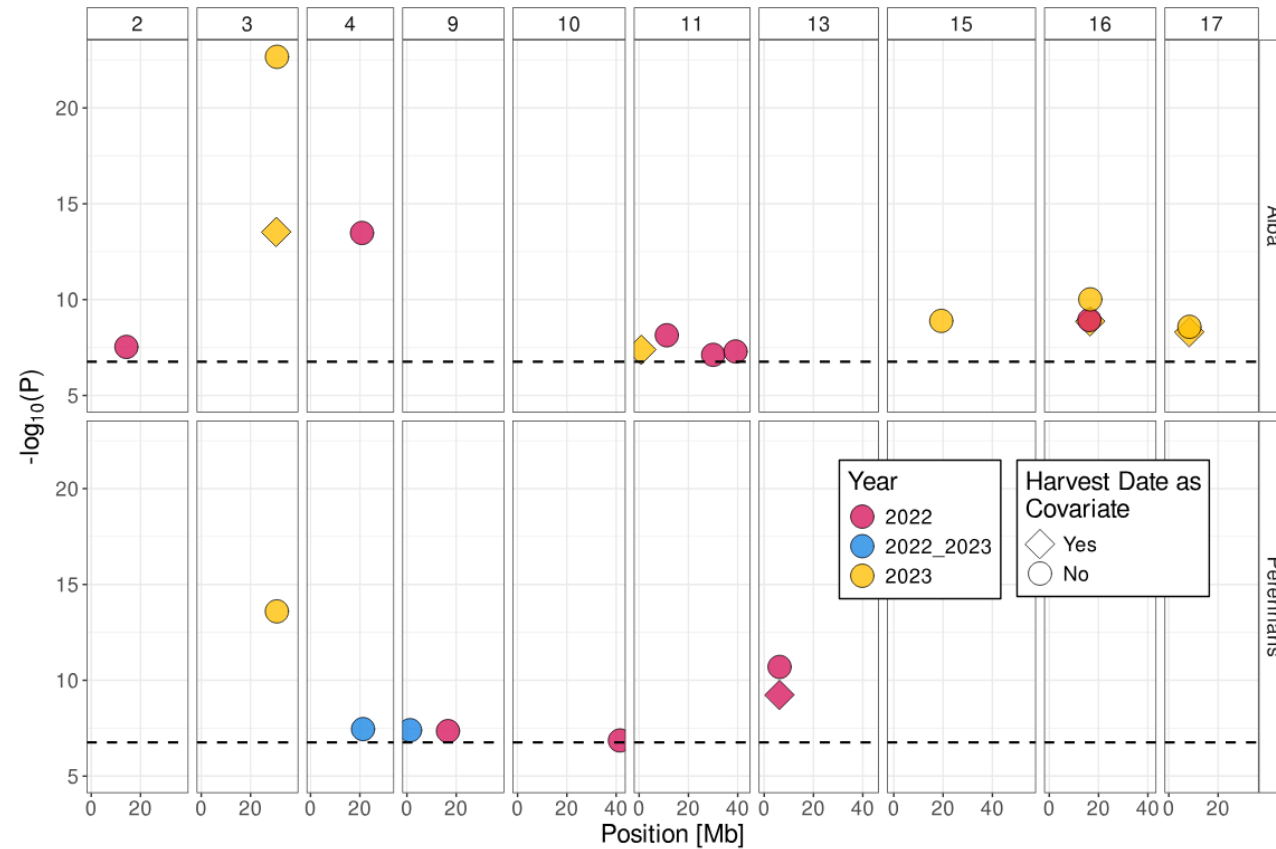


GWAS: Results BLUPs

- **Fixed effect:** Year_Location
- **Random effect:** Genotype & Inoculation date (trial effect)
- Inoculation date has large effect

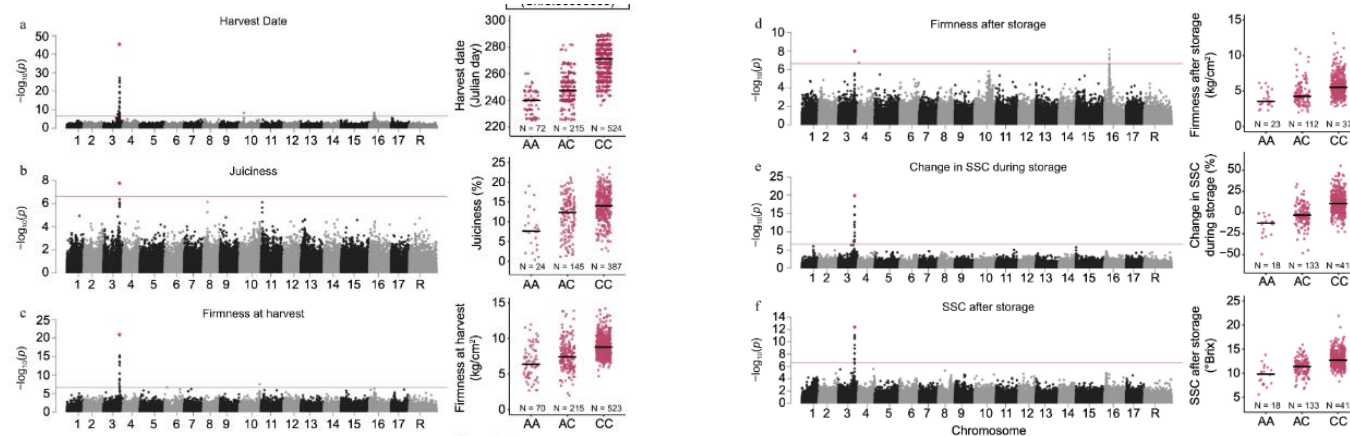


GWAS – full dataset



- Important locus on Chr3, smaller on Chr4 und Chr16

Whats on Chr3



Harvest Date, Firmness, Juicyness, SSC

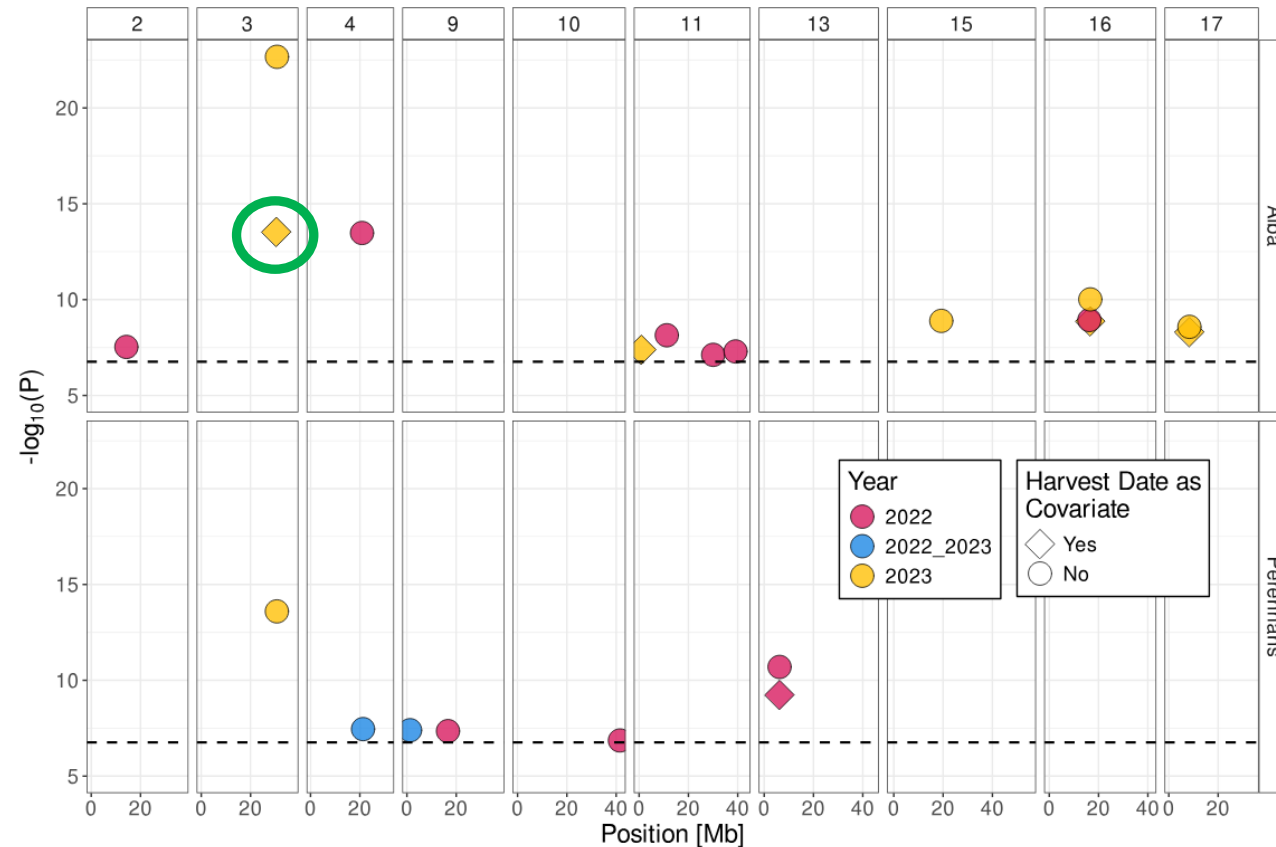
Chromosome	Class	Start	Stop	Annotation
Chr03	gene	30653663	30660855	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein
Chr03	gene	30669574	30673758	Peroxisomal membrane 22 kDa (Mpv17/PMP22) family protein
Chr03	gene	30676958	30677290	Protein of unknown function
Chr03	gene	30696191	30698216	NAC domain containing protein 2
Chr03	gene	30738599	30740479	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein

Region known, Marker exists, all modern cultivars and their parents have favourable allele,

Watts et al. 2023 Fruit research

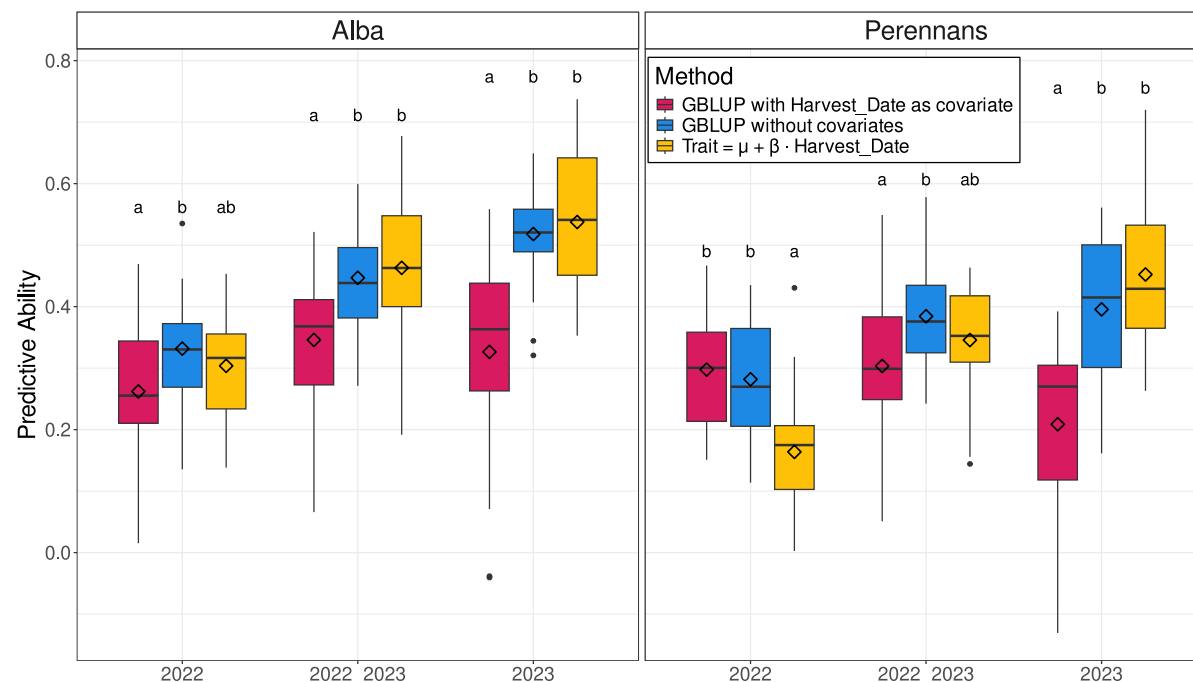
[Large-scale apple GWAS reveals NAC18.1 as a master regulator of ripening traits \(maxapress.com\)](https://maxapress.com/)

GWAS – full dataset



- BUT, Locus in model with harvest date as covariate is still significant – is there more to it?

What about genomic selection?



Model with only harvest date is good enough to predict Lenticel rot susceptibility

Summary

- Molecular markers for lenticel rot may be difficult
 - Genomic selection possible since harvest date reasonably good predictor
 - BUT Robust early varieties unlikely
 - Phenotyping our breeding program doable
-
- Further looks into the loci may be an interesting avenue
 - 20 Genotypes WGS/ PacBio – check for NAC18.1 Locus
 - Resequence NAC18.1 on a larger subsample
 - Characterise Chr4 und Chr16 loci

Thanks to

- OFAG – funding
- ETH, PomaCulta, Lubera – project partners
- REFPOP
- Andrea Knauf, Marius Hodel, Michaela Jung – data and analyses
- Andrea Patocchi, Giovanni Broggini – funding acquisition, project management
- Team Obstbau + Lager

