

# Use of disease suppressiveness in the battle against potato blackleg

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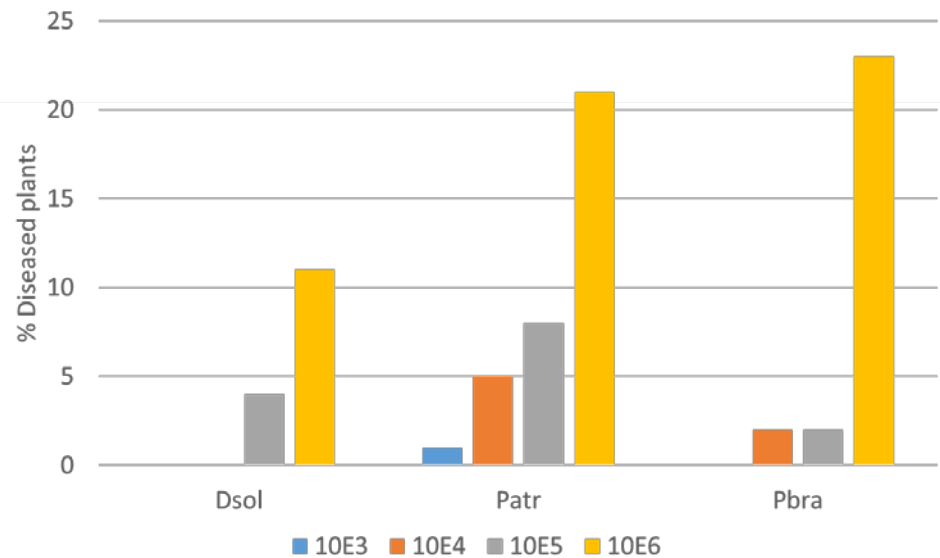
# Background study

- Bacterial diseases caused by *Dickeya* and *Pectobacterium* species are still the most important problem in seed potato cultivation
- There are no pesticides available to control the disease
- There is no (full) resistance present in commercial potato varieties
- Infections are hard to prevent
- Disease expression is difficult to predict



# Factors determining expression of potato blackleg

- Potato cultivar
- Pathogen density
- Pathogen variant
- Environmental conditions (temperature, humidity)
- Suppressiveness seed tubers??  
(Ability to prevent disease expression)



# Suppressiveness in seed lots

## ■ Research questions

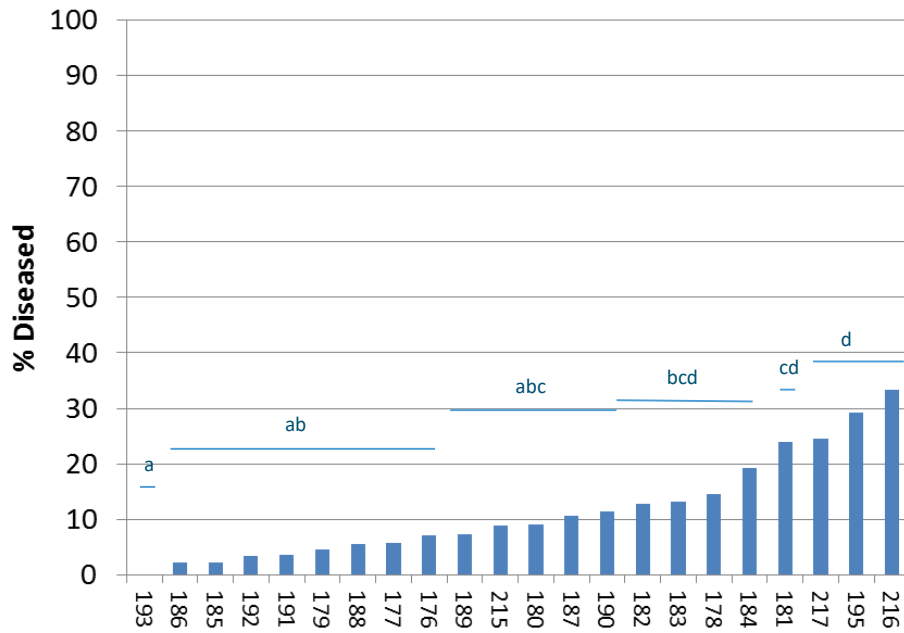
- Does suppressiveness against soft rot *Pectobacteriaceae* (SRP) exist in seed lots?
- If yes, what is the basis?
  - Physical-chemical properties?
    - Dry matter, moisture content?
    - (micro)nutrients?
  - *Metabolites*?
  - Microbiome?



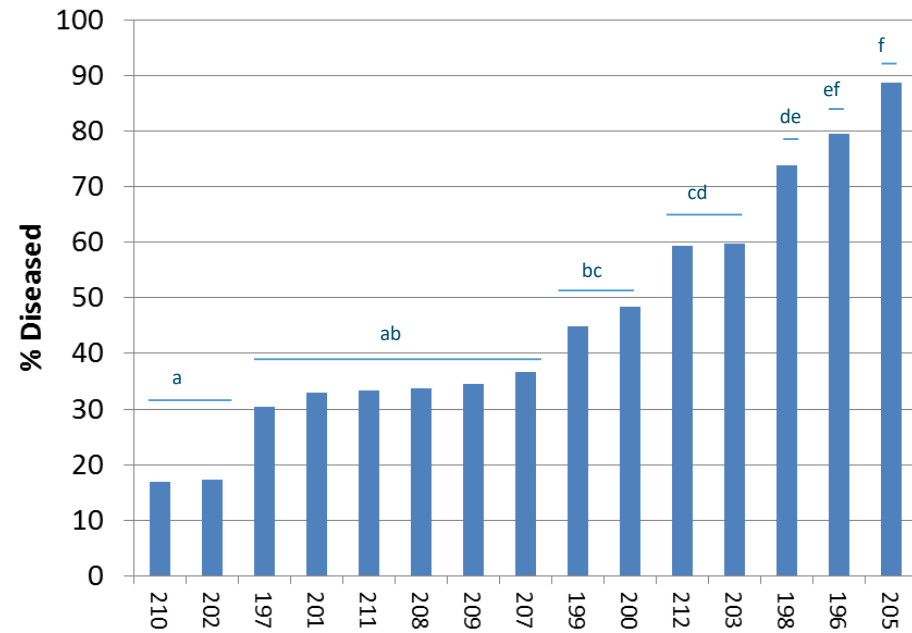
# Set up experiment

- Seed lots of two cultivars (ca. 20 per cultivar per year) collected from different locations and growers in the NL
  - Tested for a low level of SRP's
  - Analysed for dry weight content and minerals
  - Inoculated with  $10^6$  cfu/ml of *D. solani* or not inoculated
  - 100 tuber per treatment were planted in a (randomized block design) in a sandy soil in the North of the NL

# % diseased plants



Cultivar Spunta



Cultivar Kondor

# Does suppressiveness against SRP exist?

## ■ Seed lots

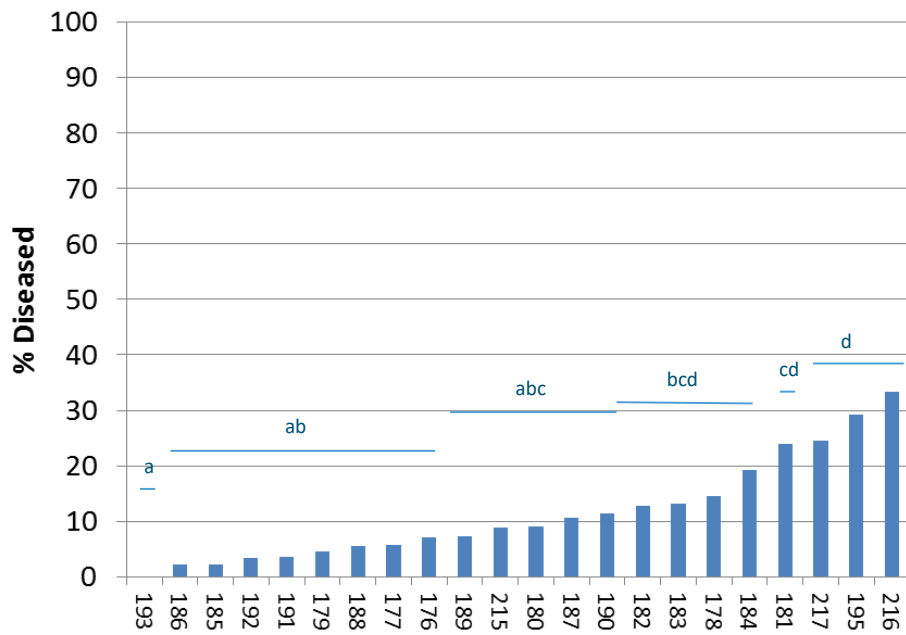
- of same cultivar  
(same genetic background)
- with the same infection level  
(*Dickeya solani*, 10E6 cells/ml)
- planted in the same field  
(same environmental conditions)

can remarkably differ in disease incidence!

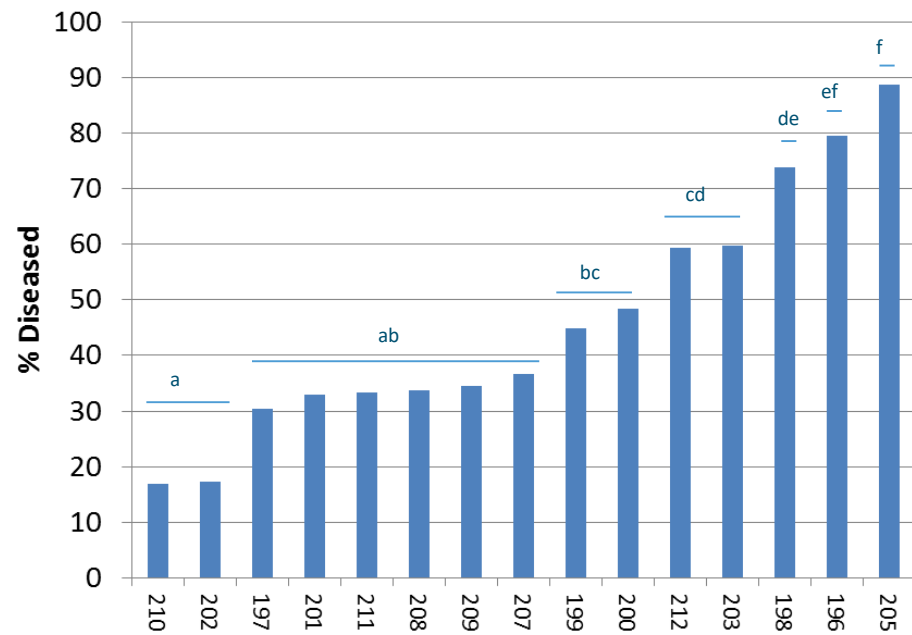
**Yes, suppressiveness against SRP exists**



# Consequences for management and research (including breeding)



Cultivar Spunta

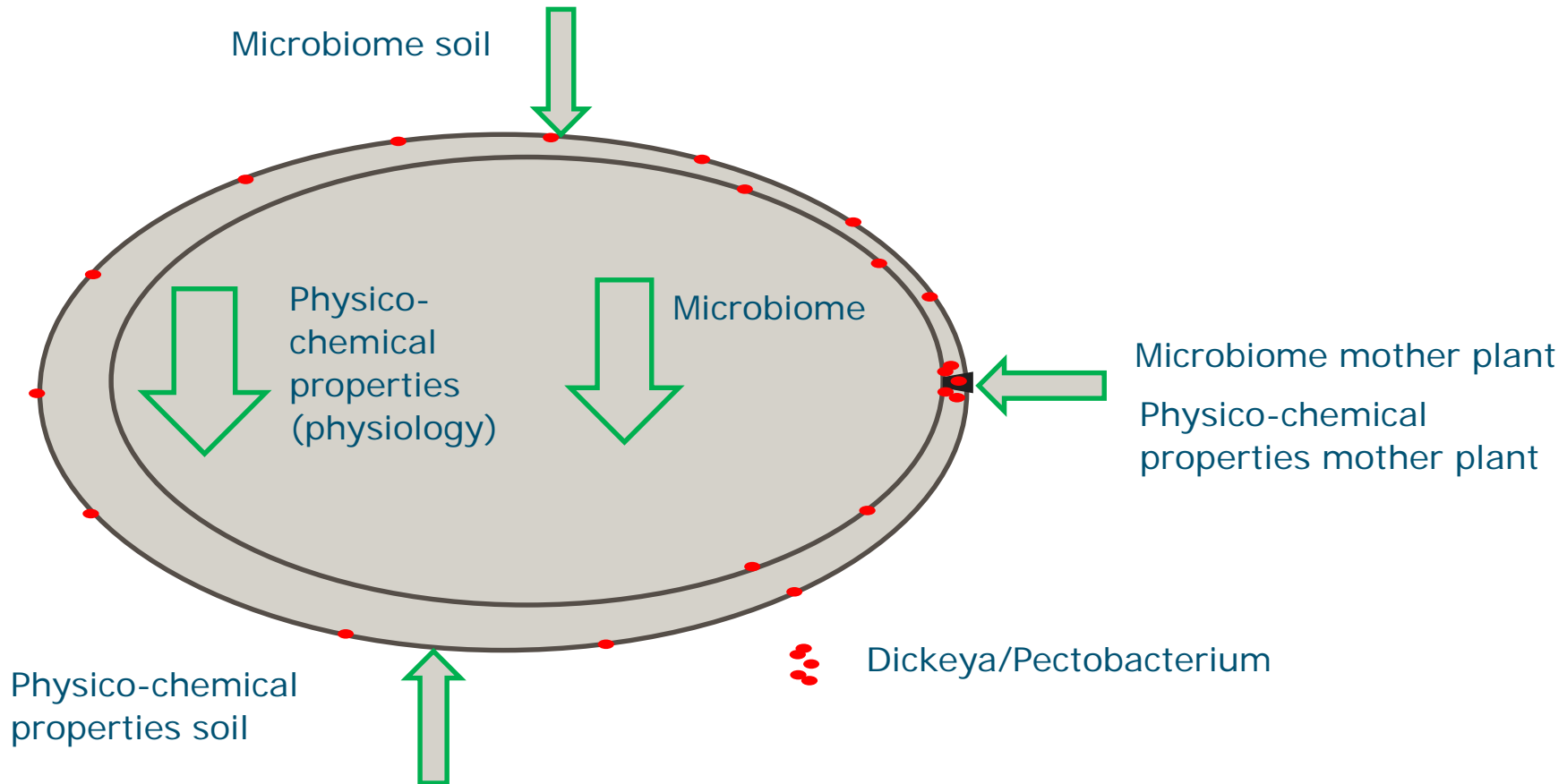
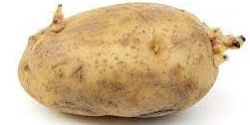


Cultivar Kondor

Use a minimum number of seed lots in your studies



# Background suppressiveness



Microbiome = the micro-organisms living in a particular environment

# Role of minerals/dry weight

Infection prevalence ~ Year + Cultivar + DS + Na + Ca + P + Mn + Cu + B + N  
 Intercept = KONDOR in 2015, when all minerals and dry weight have average values

term	p.value		prevalence	net_effects
(Intercept)	0.000196	***	39	0
Year2016	0.000119	***	28	-11
CultivarSPUNTA	5.80E-58	***	12	-27
DS	7.76E-07	***	36	-3
Na	3.20E-07	***	40	1
Ca	2.78E-06	***	42	3
P	0.0063	**	38	-1
Mn	0.074895	.	40	1
Cu	0.063117	.	38	-1
B	0.003335	**	37	-2
N	9.33E-13	***	43	4

# Microbiome analysis

Sampling plant material



Homogenization



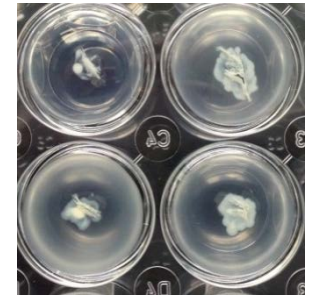
Enrichment on plates



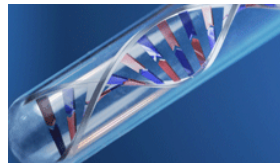
Pure culture



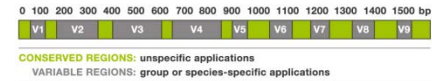
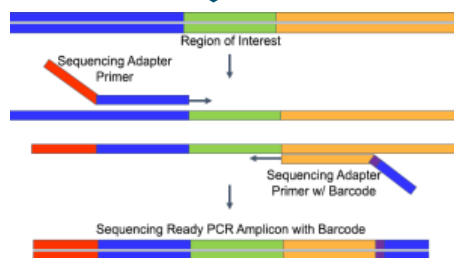
Antibiosis against SRE



DNA extraction



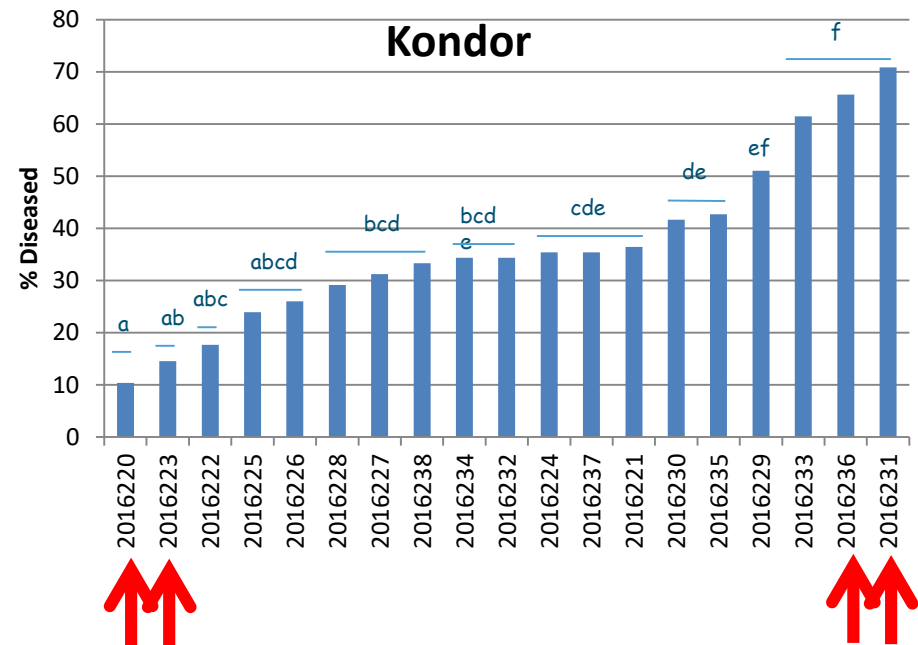
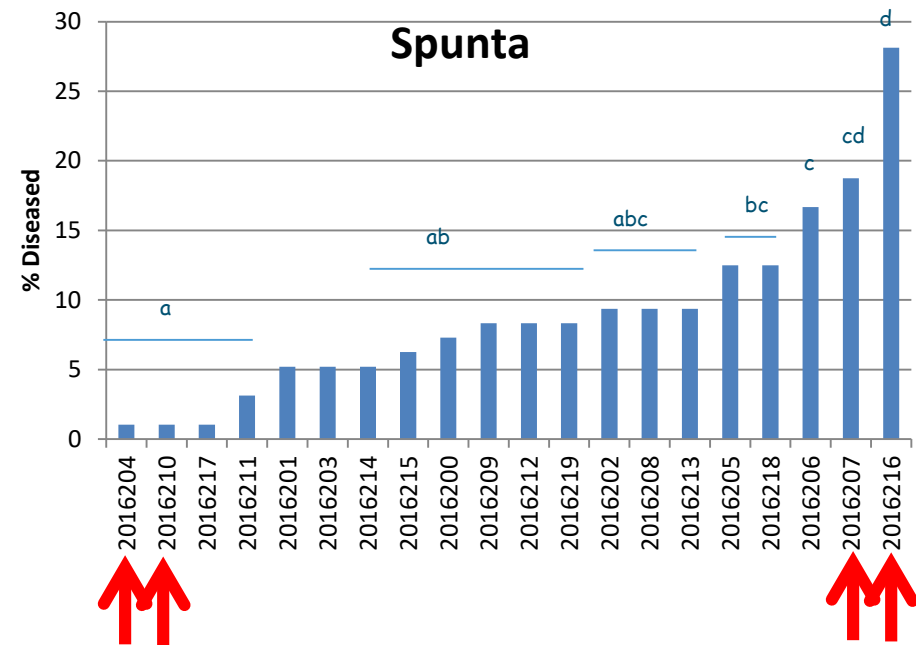
16S amplicon sequencing (15 million reads)



16S amplicon sequencing

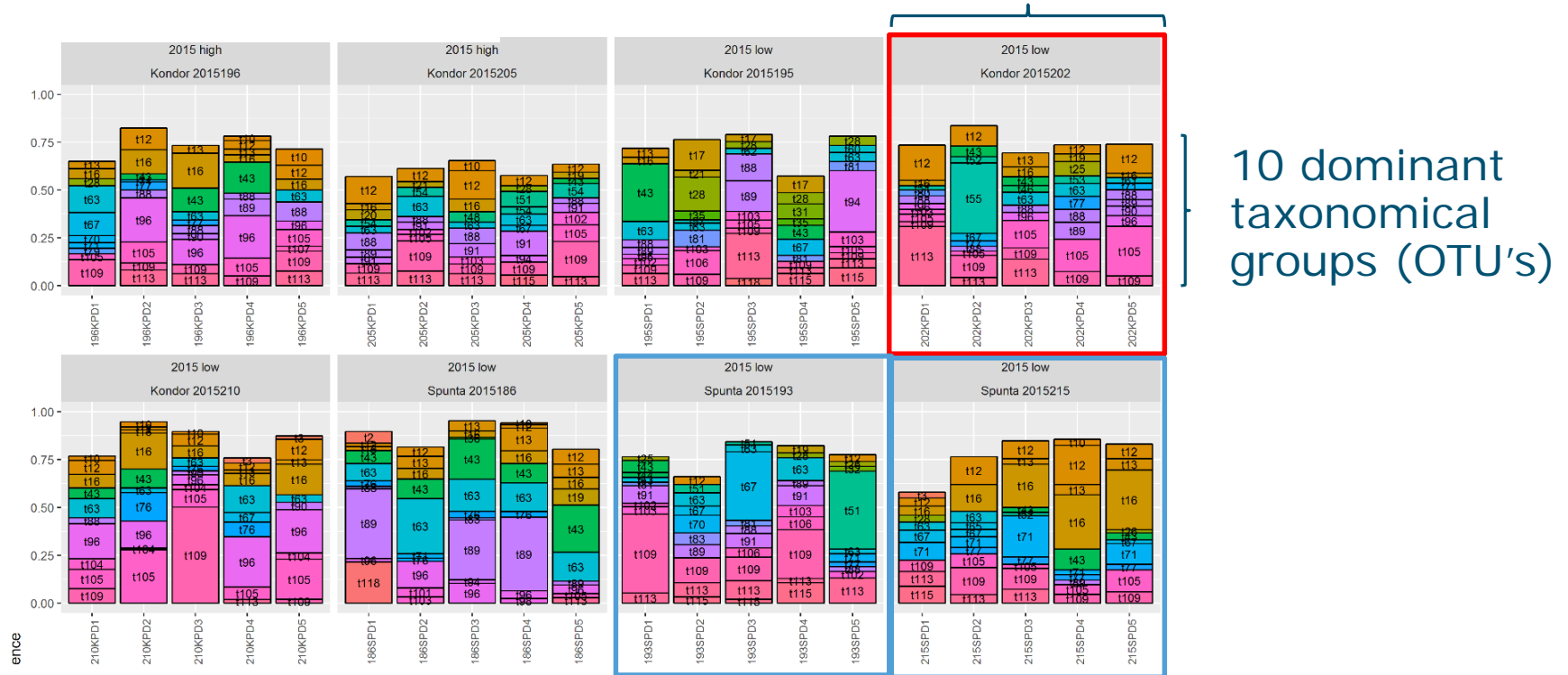
Link data

# Selection of seed lots for microbiome analysis



# Results microbiome analysis

5 tubers/lot

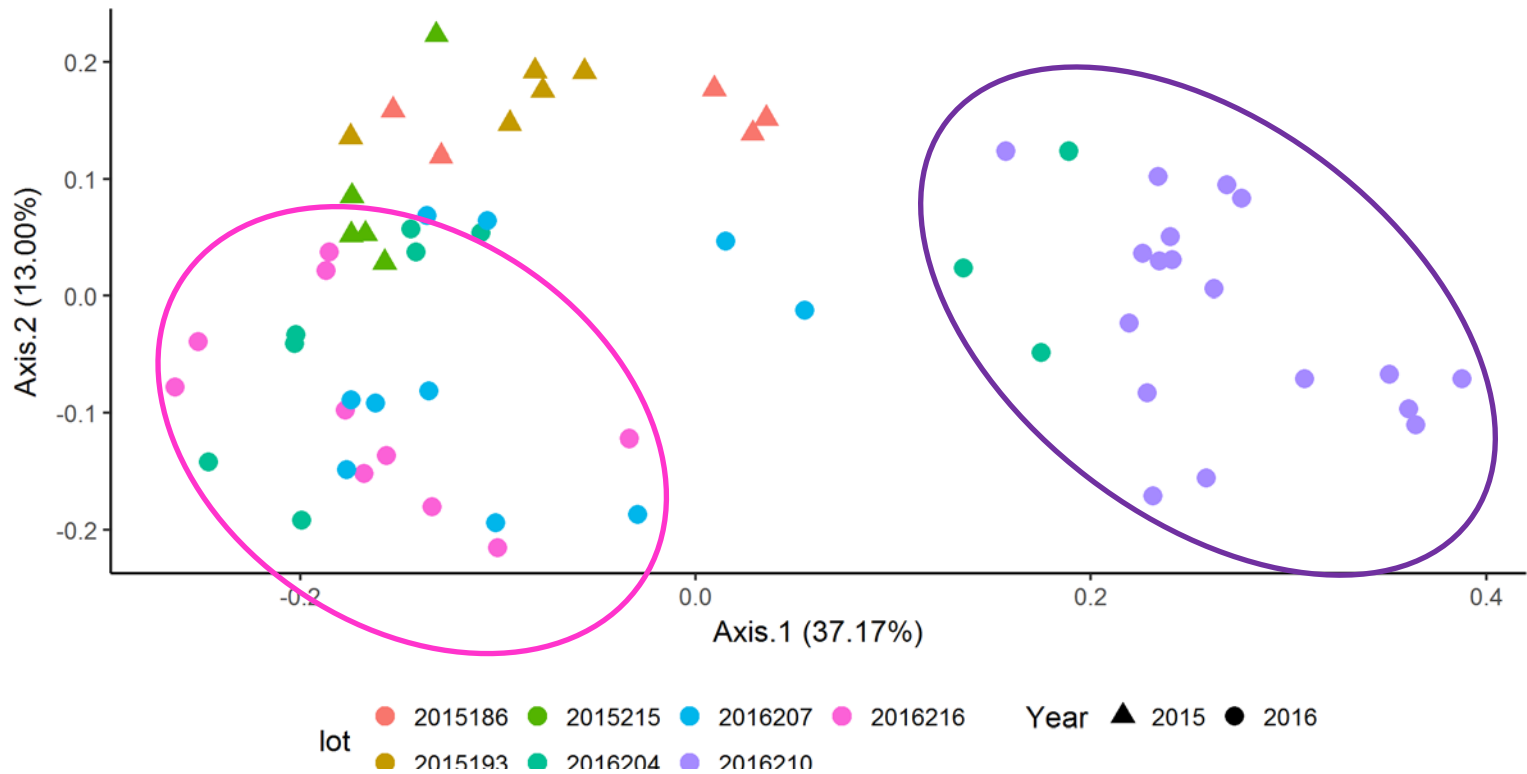


Large variation in microbiome between tubers within a lot

Significant differences in microbiome between lots

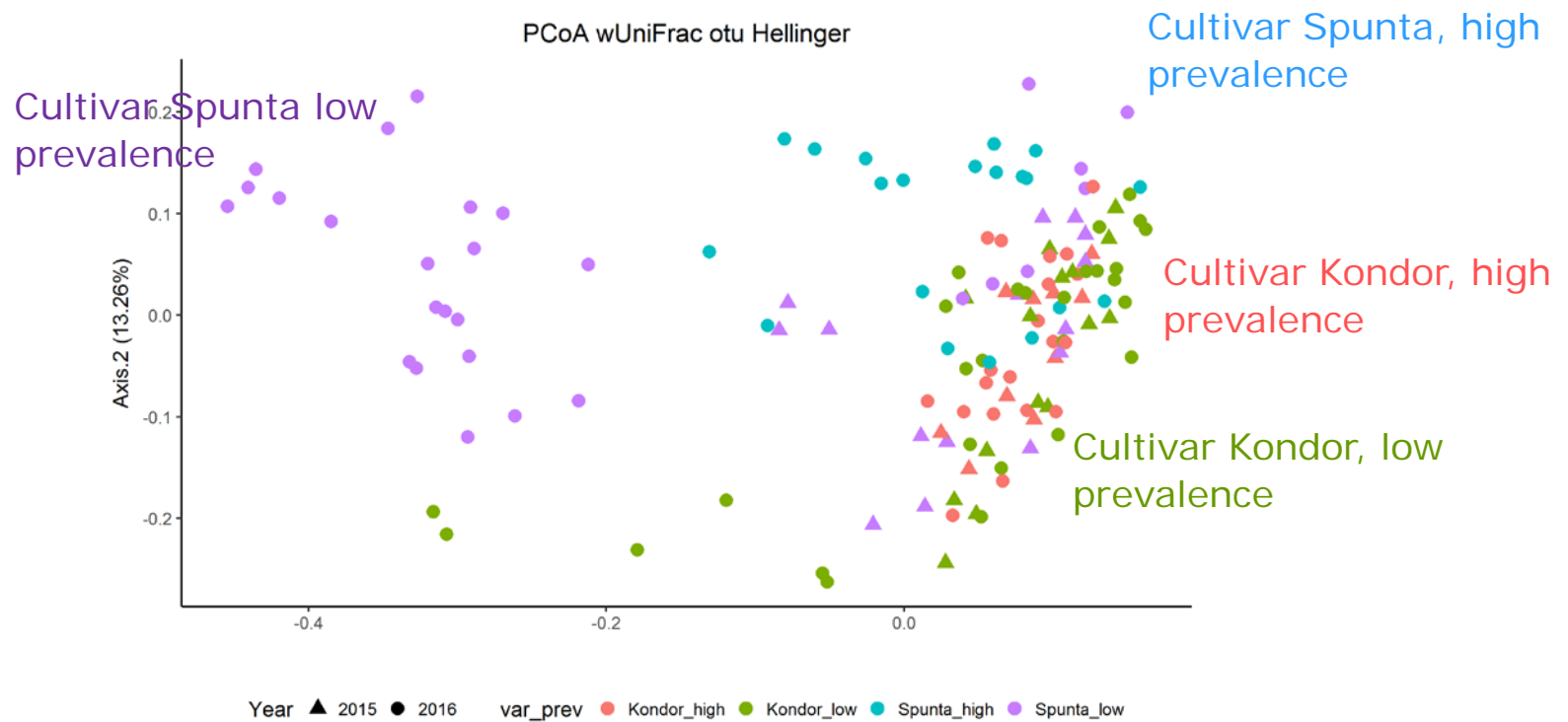
# PCoA for microbiome periderm cv. Kondor (seed lot)

PCoA wUniFrac otu Hellinger



Significant differences in microbiome between lots

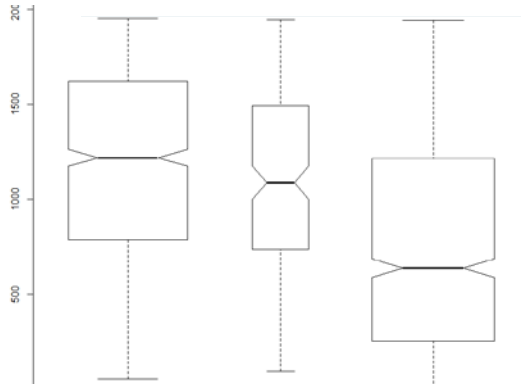
# PCoA for periderm of all seed lots (preference groups)



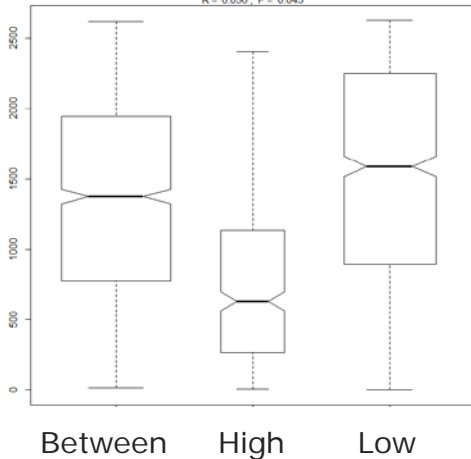


# Proportion of variance explained by the prevalence and lot – per variety

Anosim prevalence



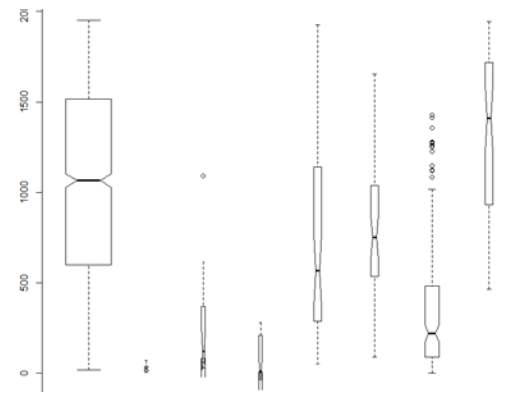
ANOSIM prevalence periderm Kondor wUniFrac Hellinger  
R = 0.056, P = 0.043



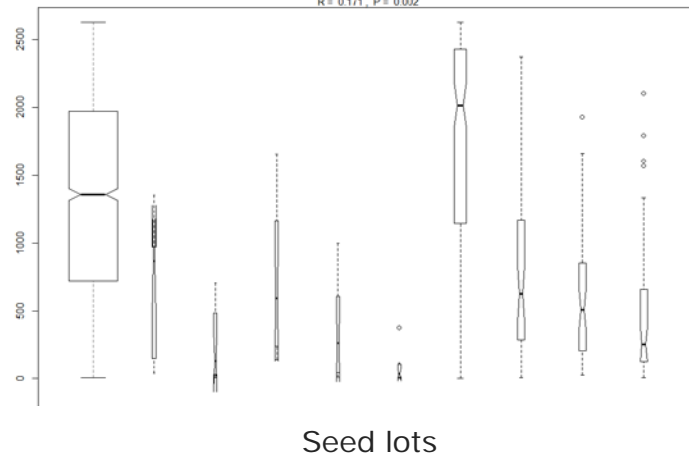
**Spunta** R2 Pr(>F)

Prevalence	0.0992	0.001 ***
lot	0.2534	0.001 ***

Anosim prevalence



ANOSIM lot periderm Kondor wUniFrac Hellinger  
R = 0.171, P = 0.002



**Kondor** R2 Pr(>F)

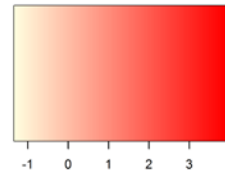
prevalence	0.0444	0.001 ***
lot	0.24586	0.001 ***

The microbiome of the tuber tissue is to some extent correlated with the infection prevalence  
The microbiome differ largely per seed lot

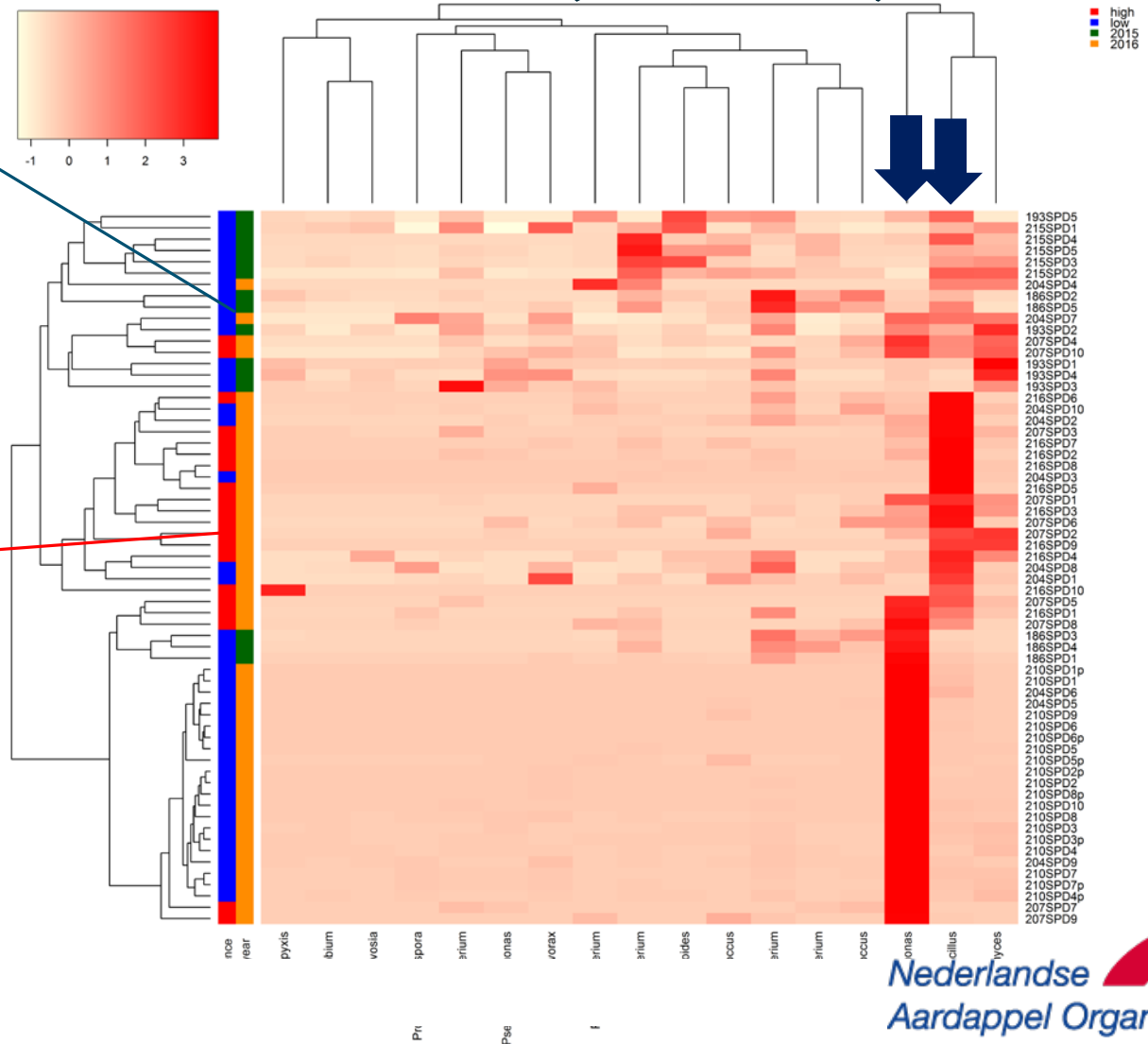
# Indicators suppressiveness cv. Spunta

Blue: tubers of seed lot with a high suppressiveness

Red: tubers of seed lots with a low suppressiveness



OTU's (Genus level)

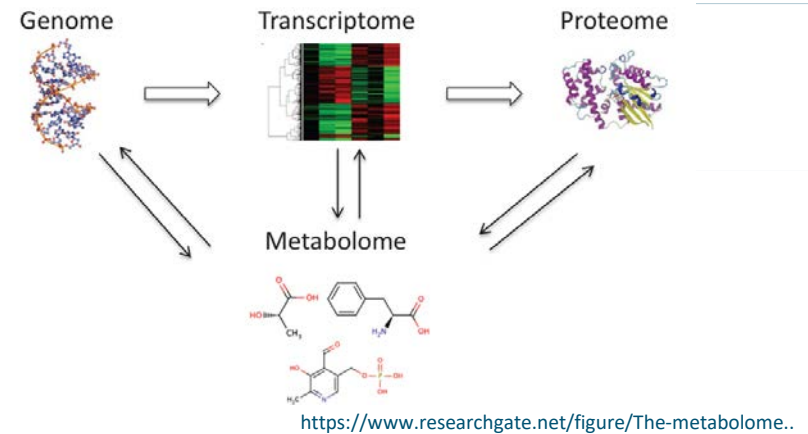


# How to use the information



- Identification of indicators for suppressiveness
- Selection of seed lots with a high suppressiveness
- Improvement of suppressiveness
  - Soil amendments
  - Tuber treatments
    - Biocontrol agents  
(selection via microbiome analysis)

# Follow up research



- Role of metabolome
- Selection of isolated bacteria related to suppressiveness
- Characterization of bacteria
  - To determine risk group (based on 16S rDNA sequences)
  - For their ability to inhibit SRP's *in planta*
- Evaluation in field experiments
  - Individual strains versus communities
  - Ability to reduce blackleg

# Acknowledgement

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Pootgoedtelersvereniging  
Anna Paulowna B.A.



**IPM POTATO GROUP**  
QUALITY IN THE BREED | QUALITY IN THE SEED

