Potato varietal susceptibility to wireworms (*Agriotes lineatus*) in relation to their sugar and glycoalkaloid profiles

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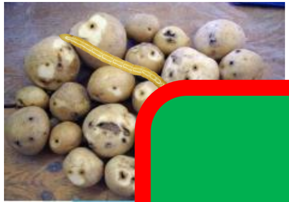
Pesticides use and restrictions in crop production (1930s to present day situation)

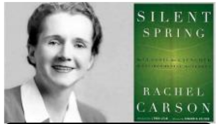
«*Wireworm: the most damaging pest of field crops* »

Balachowsky (1935)



193

1939 - 46: Dr Paul Müller
=> the Nobel Price for

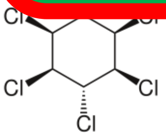


1960 - 62

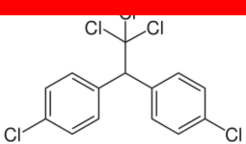
Sept. 1987 : The Montréal Protocol

May 2001: The Stockholm Convention

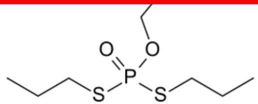
How can we sustainably deal with potato wireworm problems in a pesticide free future?



Lindane
(Gamaline: Ɣ-HCH)
1998



DDT
2004



Ethoprophos
(MOCAP)
2019

European Green Deal

Ecological Transition

2030 target

2020 … => ???

1

Know your enemy

Life cycle of potato pests wireworms species of the genus *Agriotes*

Mating and fertilisation: March to May

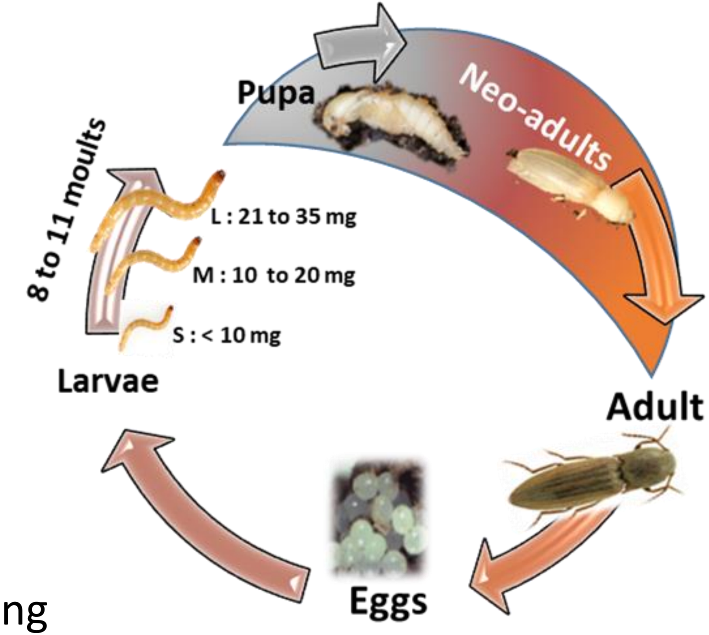
Oviposition : Mid-april to June

Eclosion of larvae: May to June (followed by several larval stages)

Pupation : June/ July

Neonate adults: July / August => overwintering under soil surface

Adults: => overwintering and emergence in spring



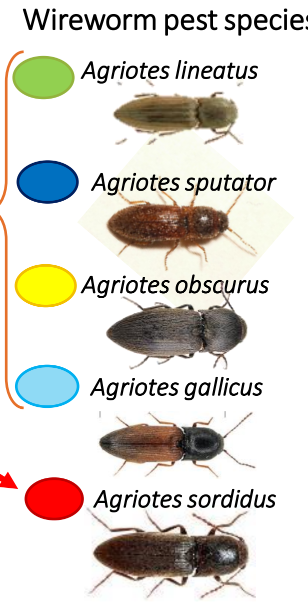
Duration: 12 months to 5 years

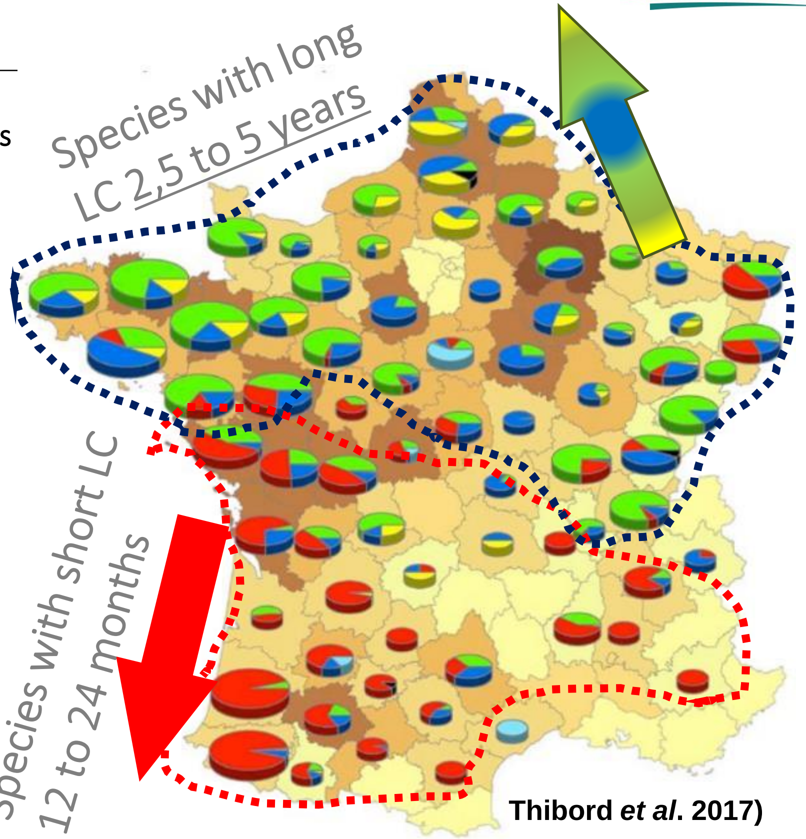
- **Depending on species and**
- **Climatic conditions**

Five main potato pest species on mainland France – 2005 à 2014

All crops : 80% maize fields

- ✓ **<u>974 field sampled:</u>** 2005 to 2014
- ✓ 4 species with long lifecycle (2,5 à 5 years)
- ✓ 1 species with short LC (12 to 24 months)



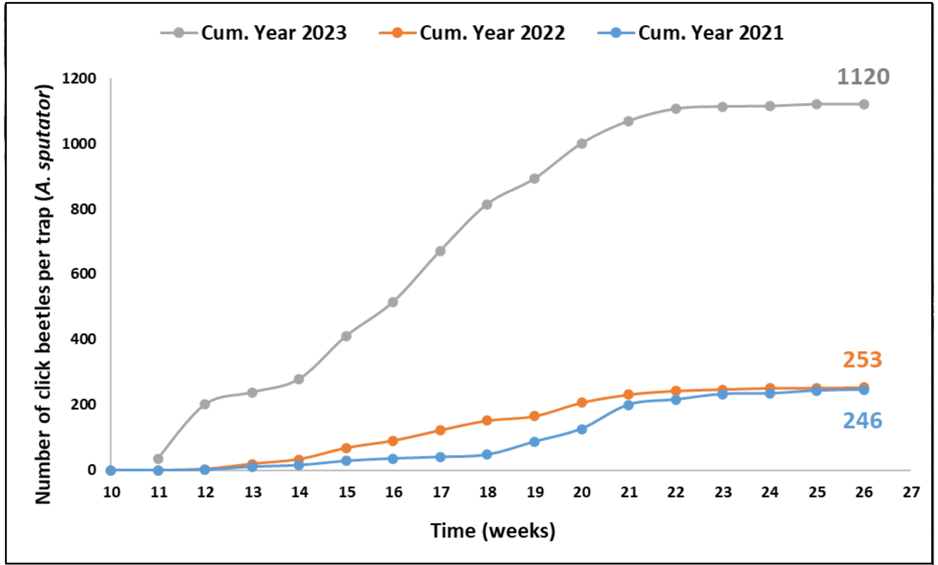


Thibord *et al*. 2017)

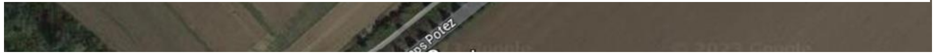
(3 larves min /soil sample) 11 351 larvea of *Agriotes sp.* (5 principal species)

Monitoring flight activity for *Agriotes sputator* (Achicourt, France 62)

- **2023 :** third year into the study of adult flights activity
- Same positioning of **forage (refuge) traps**
- Data for 2023 (Weeks **10 to 26**)
- The kinetics indicate an outbreak of adult flight in 2023 **(+867 compared with previous years)**
- **Early flight activity in 2023**
  - Early March 2023
- ***A warning sign for growers ??***



- **10,842 (9 traps)** adult *Agriotes sputator* captured till date
- An average of **1120** per forage trap (thus an increase of +867)



2

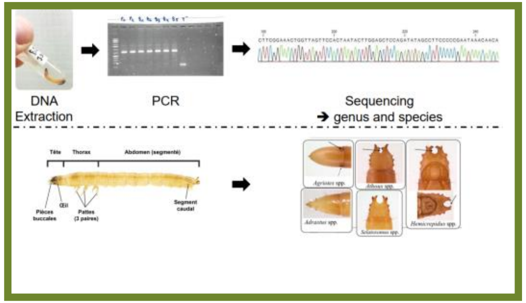
Adapt your strategy

Research work on alternative solutions to wireworm prblems in potato production


Cultures for wireworms

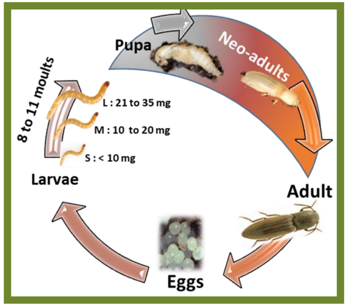

Screening biocontrol products


Cover crop and feeding studies



Species identification


Varietal susceptibility



Wireworms biology and life cycle

Objectives :

- Better understand the pest,
- Target the period/ and life stage most adapted to employed control options…
- Implement adapted IPM approaches

Varietal assessments for wireworms damage

Aim: select for tuber susceptibility to wireworms pest species

Potato variety List

- Charlotte
- Spunta
- Cara
- Monalisa

- VAR-1
- VAR-2
- VAR-3
- VAR-4
- VAR-5

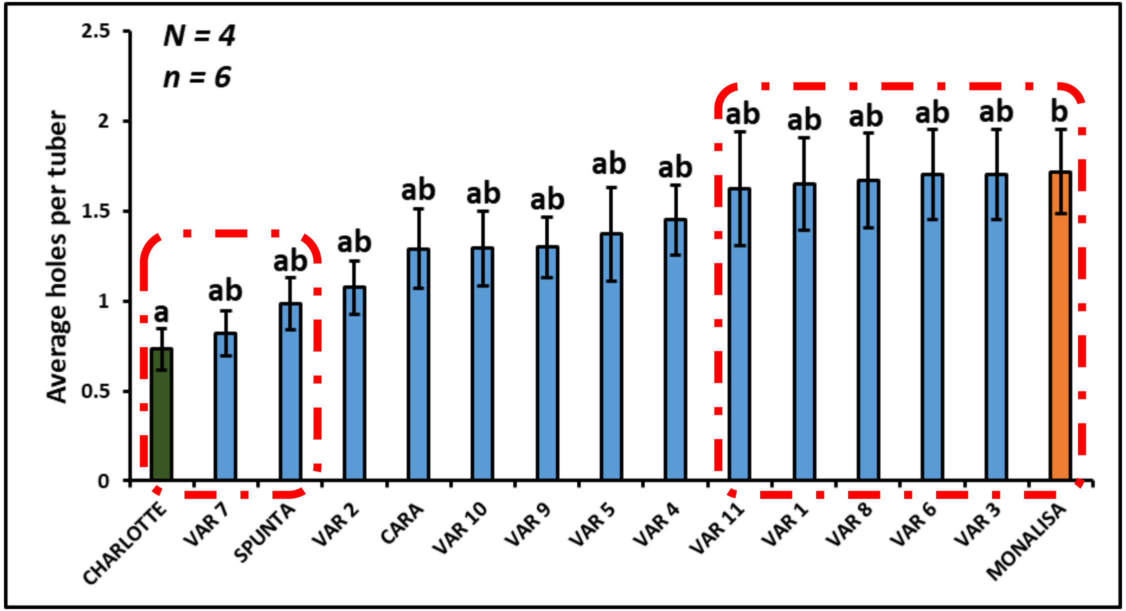
- VAR-6
- VAR-7
- VAR-8
- VAR-9
- VAR-10
- VAR-11

Set up :

- Varietal selection based on farmers info.
- 15 varieties assessed in field and glasshouse
- Source: Bretagne Plant Innovation
- Wireworm species: *Agriotes lineatus*
- Origin: field plots post-potato harvest (Brittany, Fr)

Varietal assessment: field experiments

- ✓ **2021 & 2022:** 4 experiments **(N)** in Brittany, Fr
- ✓ **15 potato varieties studied**
- ✓ Randomised blocks with **6 reps (n)**
- ✓ **Assessments:** 100 tubers/rep. per var. at harvest
- ✓ **Average N° of wireworms holes per tuber**



- **Charlotte:** **Var-7** and **Spunta** suffered less wireworms damage
- **Monalisa:** **Var-1, 3, 6, 8, 11** & suffered more damaged by wireworms

Varietal preference assessments : Glasshouse experiments

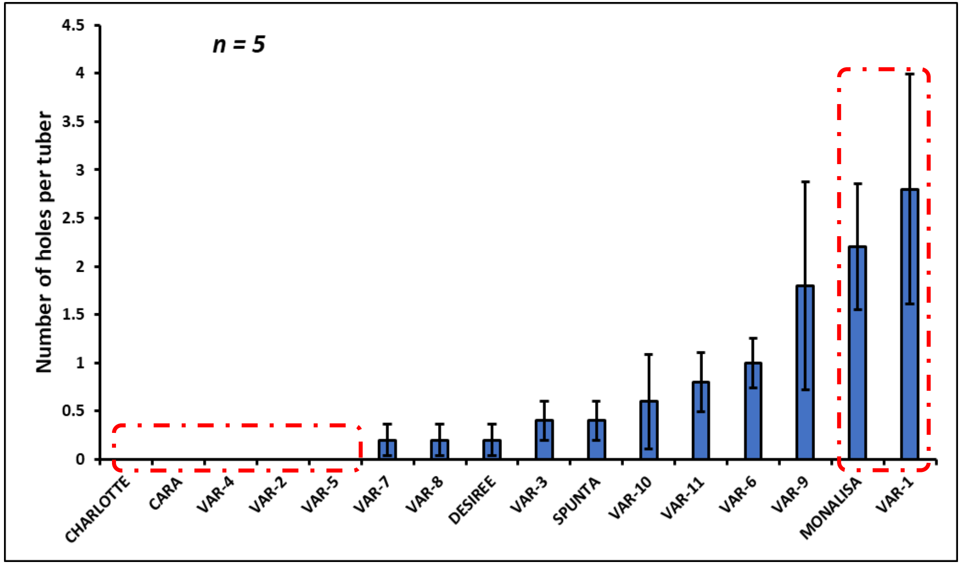
Tow Experiments in 2021: choice test

- Parent tuber exposure to wireworms
- 15 tubers / box arranged in circles
- 1 tuber per Variety, 5 Replicates
- 20 wireworms per box
- 14 days incubation in compost at 20°C in the dark

Inoculation of larvae (20/pot) at incubation of 15 potato Varieties

14 days

Assessments for tuber damage



Surface bites or no holes on:

- **Charlotte, Cara**
- **Var-2, 4 & 5**

Badly damaged:

- **Monalisa,**
- **Var-1 & 9**

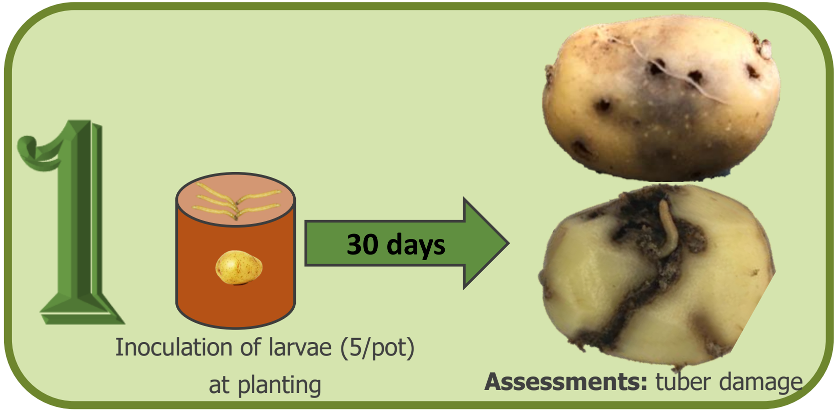
Varietal palatability assessments : Glasshouse experiments

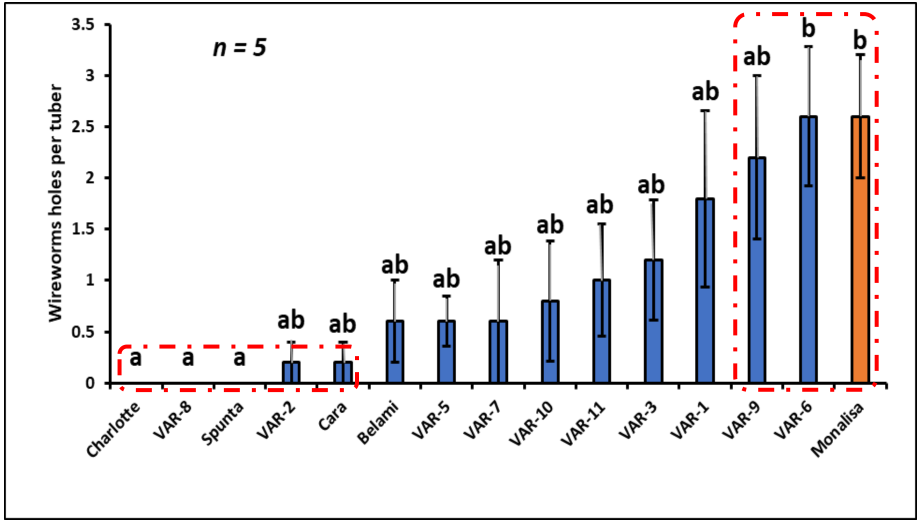
Results: wireworms holes per parent tuber

Designs N°1:

1) Parent tuber exposure to wireworms

➢ Off-spring tubers exposure to wireworms





Very little damage on:

- Charlotte, Spunta,
- Cara, VAR-2 & 8

Badly damaged:

- Monalisa,
- VAR-1, 6 & 9

Varietal palatability test : Glasshouse experiments

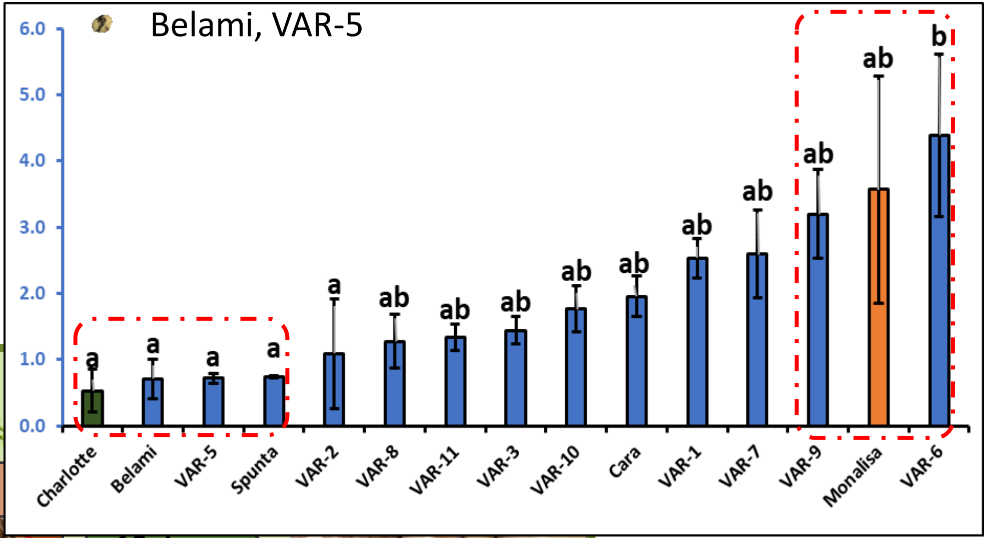
Results: wireworms holes per offspring tuber

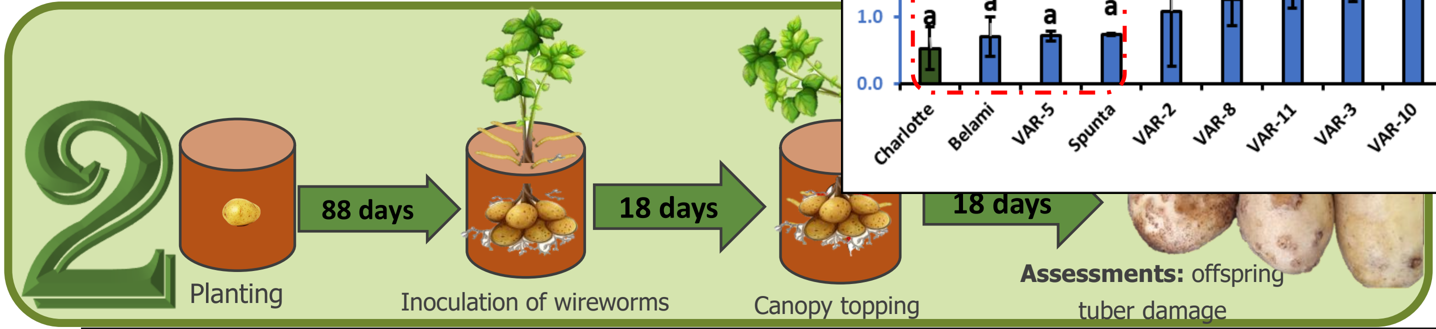
Designs N°2:

- ➢ Parent tuber exposure to wireworms
- 2) Off-spring tubers exposure to wireworms

Very little damage on:

- Charlotte, Spunta
- Belami, VAR-5





Badly damaged:

- Monalisa,
- VAR-6 & 9

Total sugar and glycoalkaloid levels in tubbers

What attributes favours susceptibility to wireworms pest species

Sugars

1) Glucose
2) Fructose
3) Sucrose

SOP-No. 569:2021-08 (HPAEC-PAD)

Glycoalkaloids

1) α-chaconine
2) α-Solanine

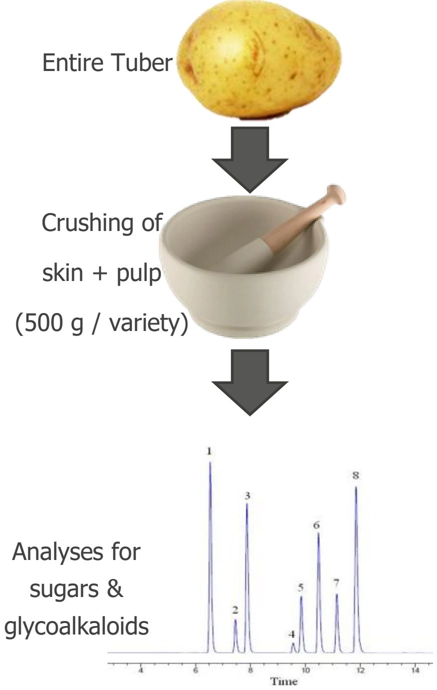
SOP-498: 2016-09

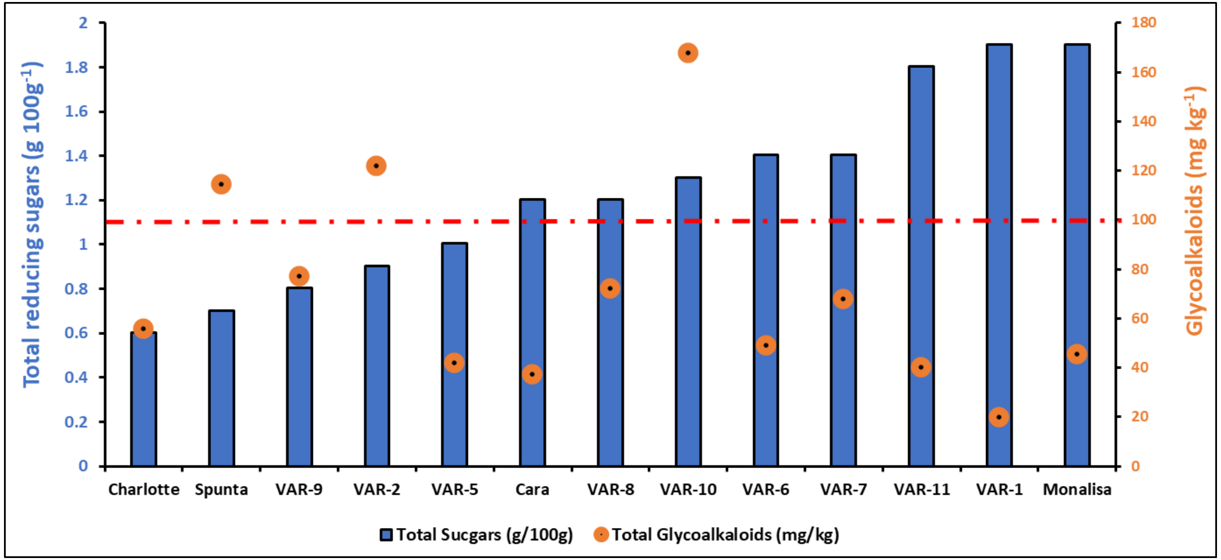
GALAB Laboratories

Total sugar and glycoalkaloid levels in tubbers

Aim: understand the tuber attribute that favours wireworms damage

Extraction and Analysis:





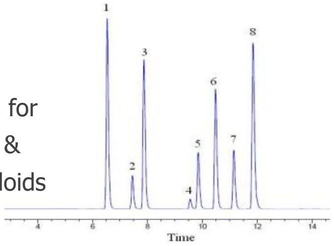
- **Charlotte, Spunta** with low sugar contents suffered less wireworms damage
- **Monalisa, VAR-1** & **11** with high sugar levels seemed prefered by wireworms
- Combined **high sugar** with **low glycoalkaloids** seems to favour wireworm damage

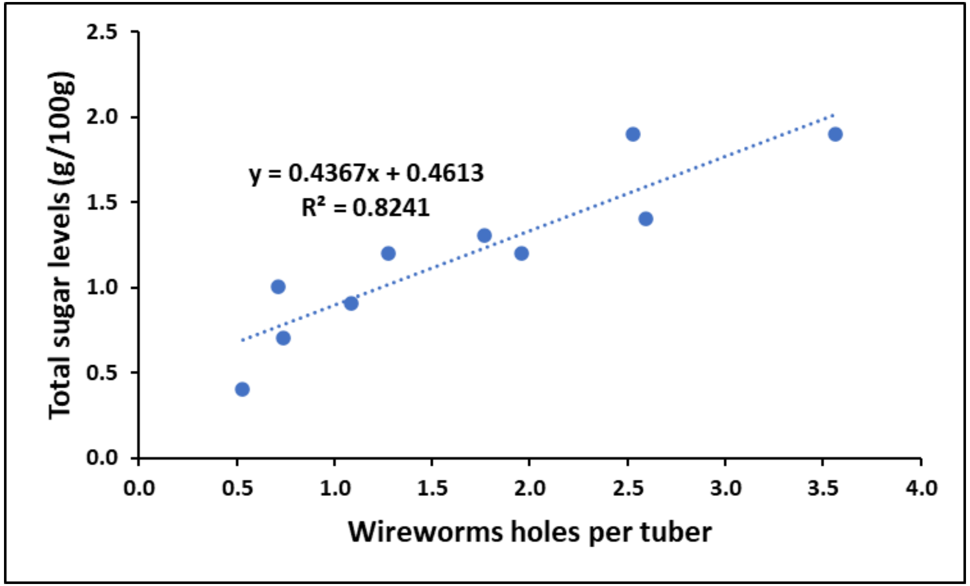
Results: Corelations between tuber damage and sugar contents

Entire Tuber

Crushing of skin + pulp (500 g / variety)

Analyses for sugars & glycoalkaloids





- Positive relationship between tuber damage and sugar contents (*R^2 = 0.824*)
- A very weak negative relationship observed between glycoalkaloid contents and tuber damage (*R^2 = 0.086*) in line with (*Johnson et al., 2008*)

Take home message

- None of the 15 varieties was resistant to wireworms *Agriotes* spp.
- Sugar levels in tubers correlates positively with wireworms damage
- High sugar levels / low glycoalkaloids linked to wireworms damage
- Other important attributes / compounds may be involved ?

Future directions

- **Understanding the genetic basis of palatability to wireworms *Agriotes* spp.**
- **Damage intensity of different ww pest species on less palatable varieties**
- **Integration of palatability into IPM for wireworms pest species**
- **Targeted breeding programs for potatoes with low sugars?**

Ackwoledgements : The French research network on wireworms pest species

TAUPIC project

FN3PT/inov3PT, ITA
Project leader
Chef de projet : Yves Le Hingrat
Expert technique : Michel Malet

Avec la contribution financière du compte d'affectation spéciale développement agricole et rural CASDAR
MINISTÈRE DE L'AGRICULTURE ET DE LA SOUVERAINETÉ ALIMENTAIRE
Liberté Égalité Fraternité

InnoPlant

INRAE-UMR IGEPP
équipe EGI

FREDON Hauts-de-France

Service providers : Université de Liège-Gembloux Agro Bio Tech (recherche de COV) ; Midi Agro Consultant; Astria Sud Garonne (essais)

Other partners (without funding) : opérateurs sollicités pour enquêtes; semenciers (plantes de service : Jouffray Drillaud) ; obtenteurs de variétés ; sociétés de biocontrôle

Regional organisations of Potato Seed Producers

bretagne Plants POMME DE TERRE — Comité Centre & Sud — Comité Nord Plants de Pommes de Terre

Arvalis-institut du végétal

Acknowledgments:

Jérémy CIGNA

Molecular diagnostics

Bruno NGALA

Florian MANCEAU

Experiments under controlled conditions

Philippe DOLO

Antoine LE ROUX

Field experiments

***A. liniatus* Field pop**

Seeds for pot experimens

Studying Colorado Potato Beetle resistance in wild Solanum species

Lotte Caarls

Wageningen University & Research – Plant Breeding

September 6, 2023

CPB: biggest defoliator of potato

- Colorado potato beetle feeding causes yield reduction

NIEUWE OOGST NIEUWS VEEHOUDERIJ AKKER- & TUINBOUW REGIO VIDEO PODCAST

AKKERBOUW FRUITTEELT GROENTETEELT SIERTEELT

Coloradokever duikt op door zachte winter en laat pootseizoen

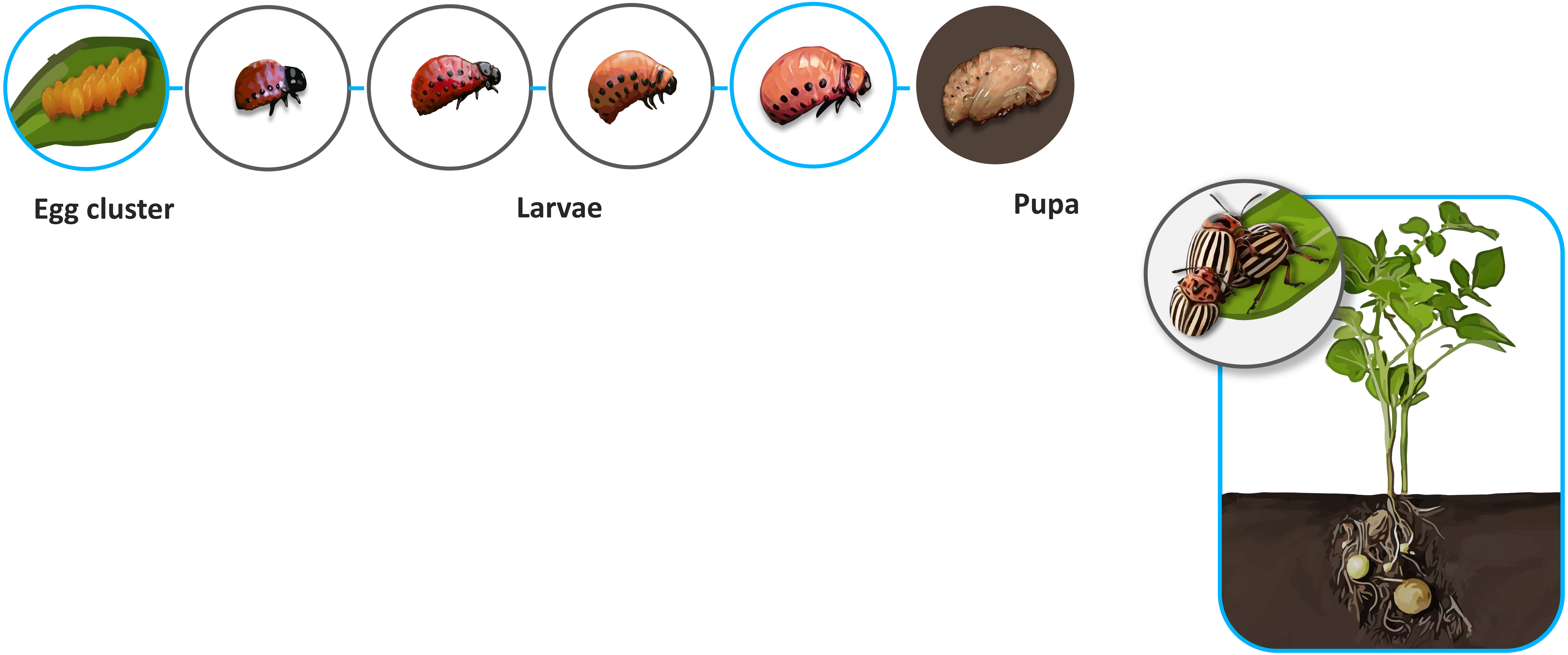
NIEUWS AKKERBOUW JOB HIDDINK 26 JUN 2023 OM 15:50UUR

Veel aardappelpercelen in vooral Noordoost-Nederland worden getroffen door de coloradokever. Vanwege de zachte winter en late start van het pootseizoen meldt de aardappelkever zich vaker dan voorgaande jaren. Maar als telers op tijd zijn met hun bespuitingen, is de plaag volgens Delphy beheersbaar.

▲ © Marcel Berendsen

Nieuwe oogst; 26 June 2023

Colorado potato beetle life cycle



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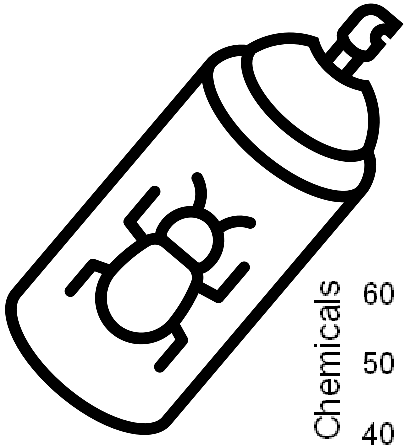
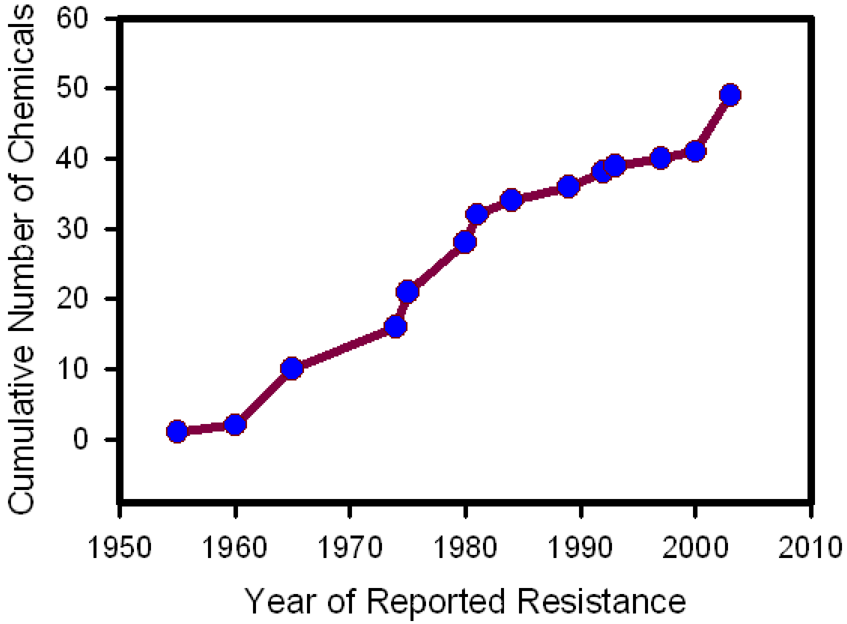
CPB: biggest defoliator of potato

- Colorado potato beetle feeding causes yield reduction
- Control with pesticides
- Alternative: host plant resistance





Resistance of CPB to chemicals (source: APRD)

Wild relatives of potato are a source of insect resistance

- Some wild species have been reported to be insect resistant
- Two main resistance mechanisms known:
  - Glycoalkaloids
  - Glandular trichomes

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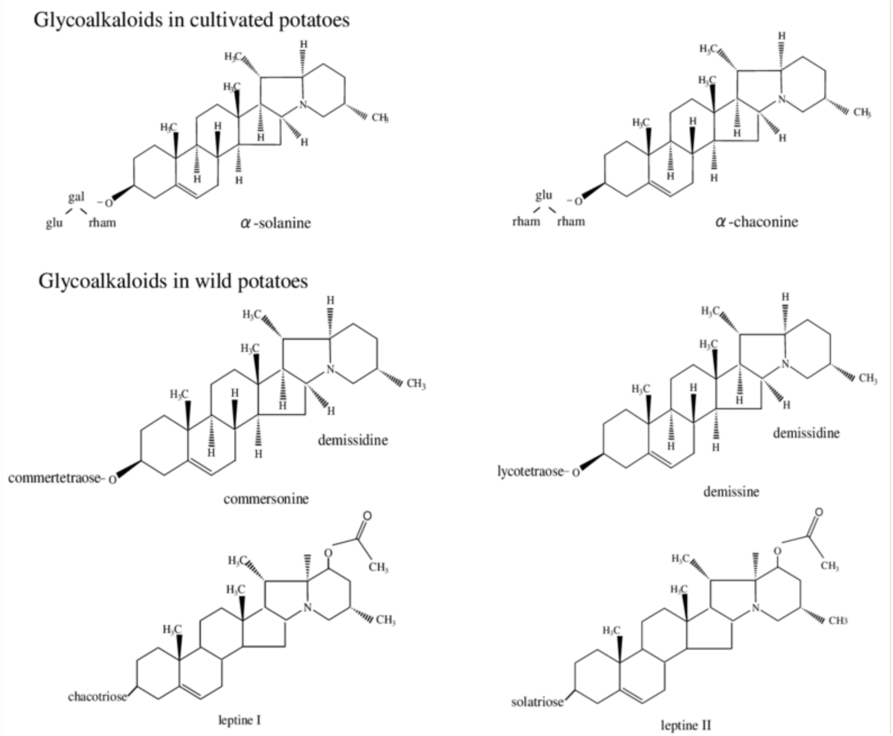
Resistance mechanism: Glycoalkaloids

- All Solanum species contain glycoalkaloids
- Many have insecticidal and antimicrobial activity

Resistance mechanism: Glycoalkaloids

- All Solanum species contain glycoalkaloids
- Many have insecticidal and antimicrobial activity
- High total levels do not confer resistance
- Resistance associated with dehydrocommersonine from S. *oplocense* and leptines from *S. chacoense*



Munir et al 2013

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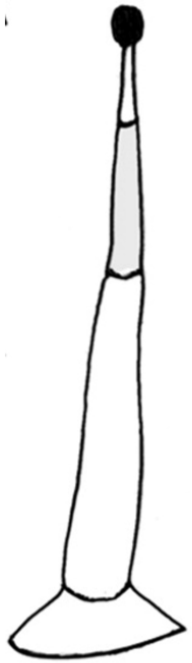


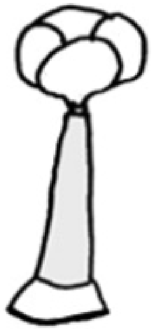
Resistance mechanism: glandular trichomes

Acyl sugars: trapping insects

Repelling pheromones

Ruptures upon contact

Exudate is polymerized (PPO)



Known from:

S. berthaultii, S. tarijense, S. neocardenasii

Search for more CPB resistance in wild potato

- Aims: identify resistance in wild species and understand resistance mechanism
- Selected species based on potential resistant sources from literature
- Focus on diploid species
- Use collection in Plant Breeding department of *in vitro* kept accessions

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Colorado potato beetle assay

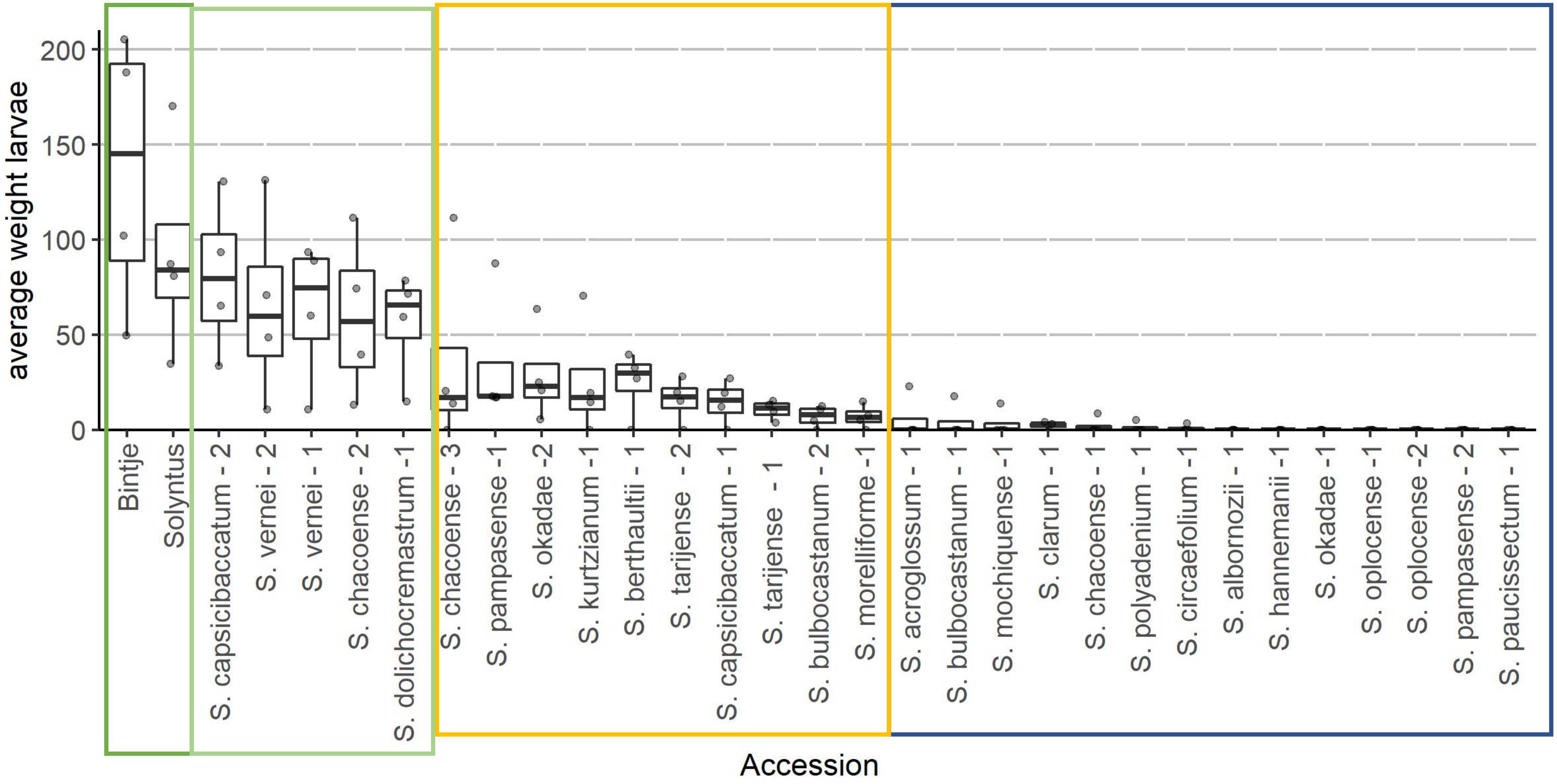
- Survival and growth of larvae
- Six week old plants
- Start 1-day old in clipcage
- Weigh after 9 days

Susceptible

Intermediate resistant

Resistant

CPB assay: weight larvae on different accessions



CPB assay: weight larvae on different accessions

- Found 14 accessions with high resistance - no survival larvae
- Low survival and low weight: 7 accessions
- High survival, but lower weight than S: 3 accessions
- Susceptible: 6 accessions

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Selection of 9 sources of resistance

species	EBN	R level	R mechanism (from literature)
S. okadae	EBN2	R	Glycoalkaloids tomatine
S. chacoense	EBN2	R	Glycoalkaloids Leptine
S. hannemanii	EBN2	R	Unknown
S. pampasense	EBN2	R	Unknown
S. tarijense	EBN2	MR	Glandular trichomes
S. kurtizanum	EBN2	MR	Unknown
S. berthaultii	EBN2	MR	Glandular trichomes
S. mochiquense	EBN1	R	Unknown
S. capsicibaccatum	EBN1	R	Unknown

- Made crosses were possible to generate populations
- Studied trichomes and measured glycoalkaloids

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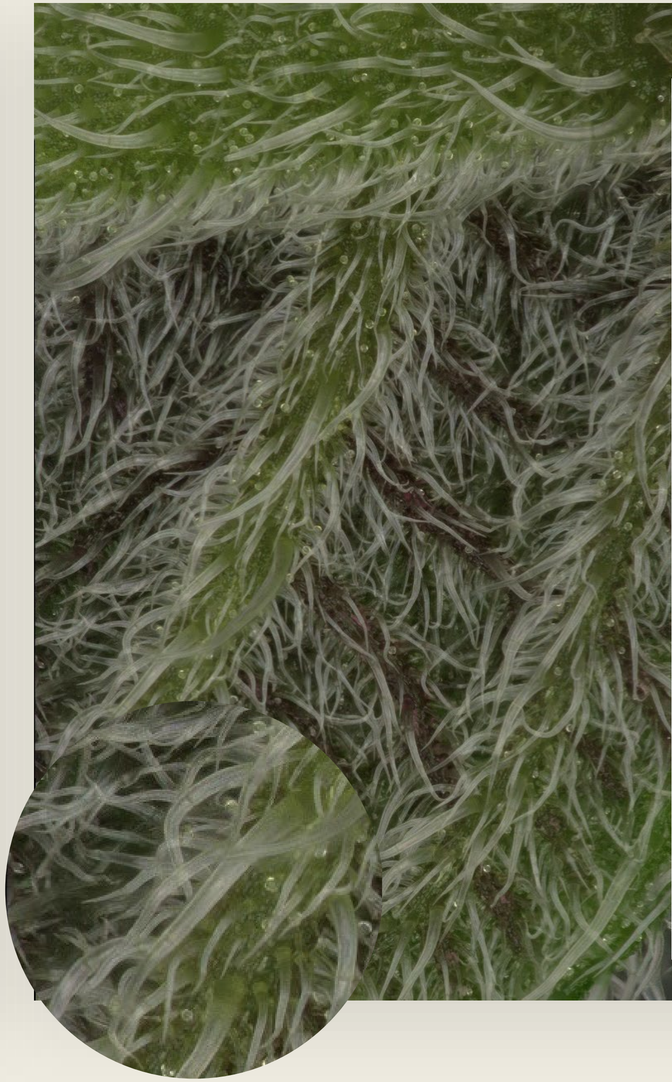
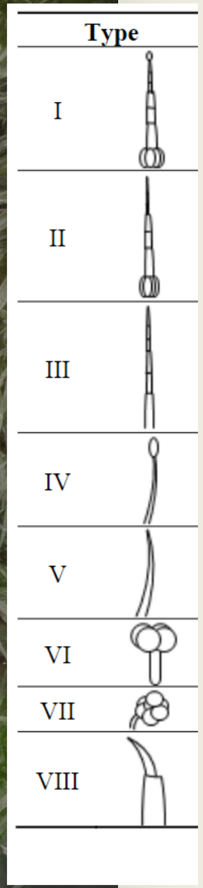
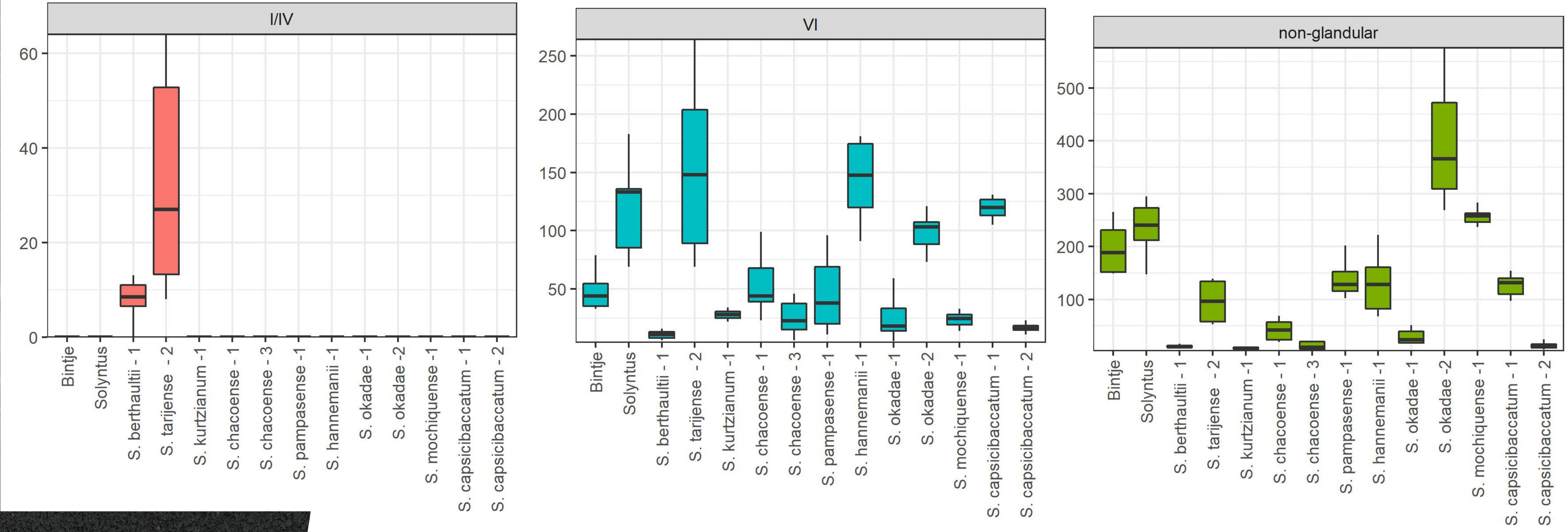
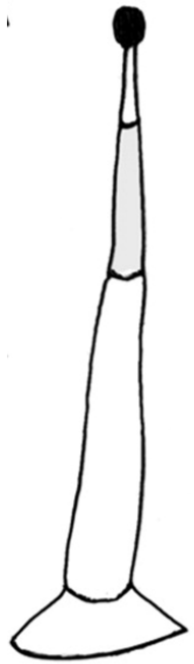
Some examples of trichomes on selected accessions



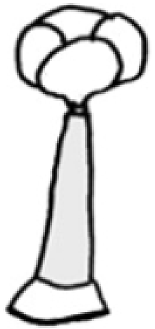


Record trichomes on selected accessions



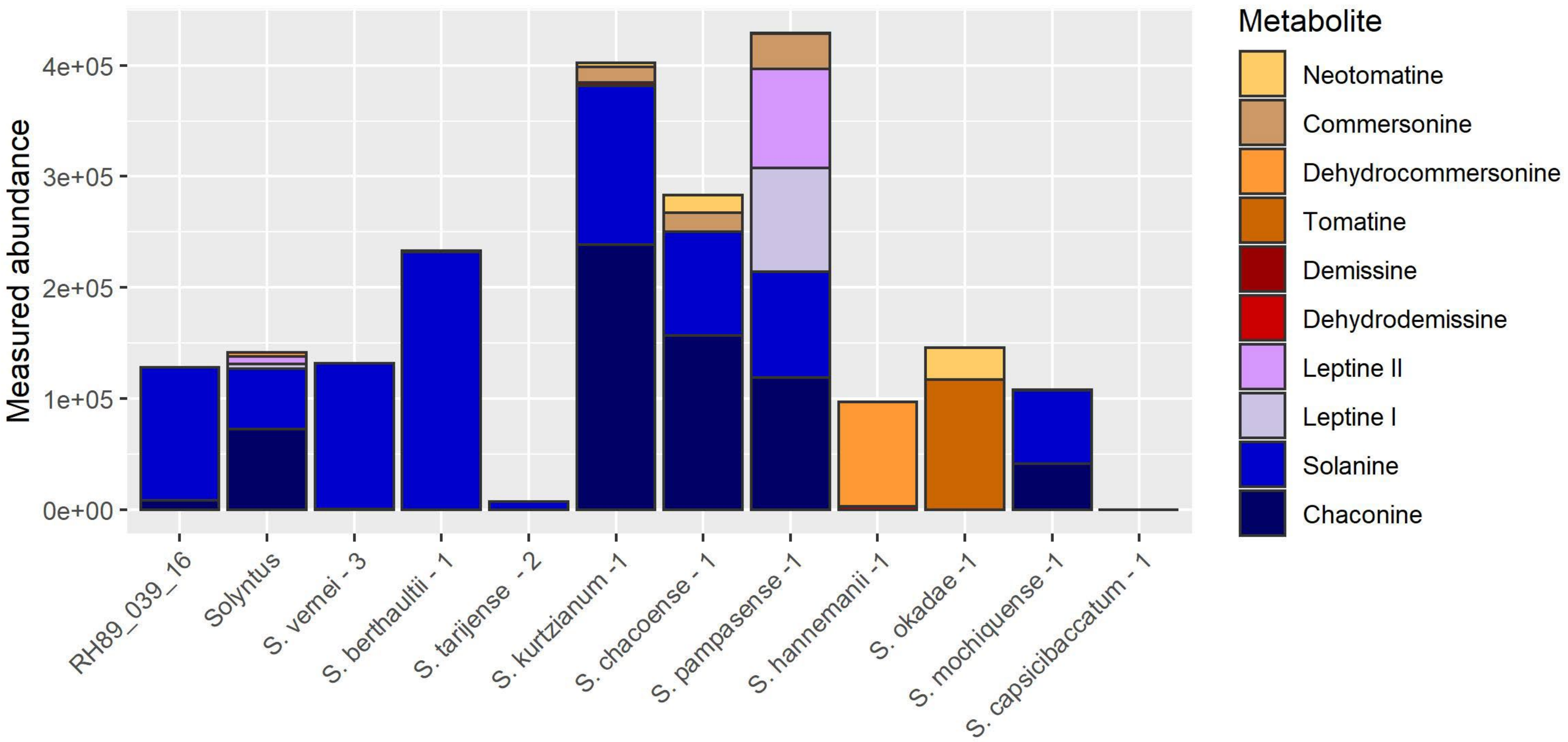


Type I and IV



TypeVI

Steroidal glycoalkaloids in selected accessions



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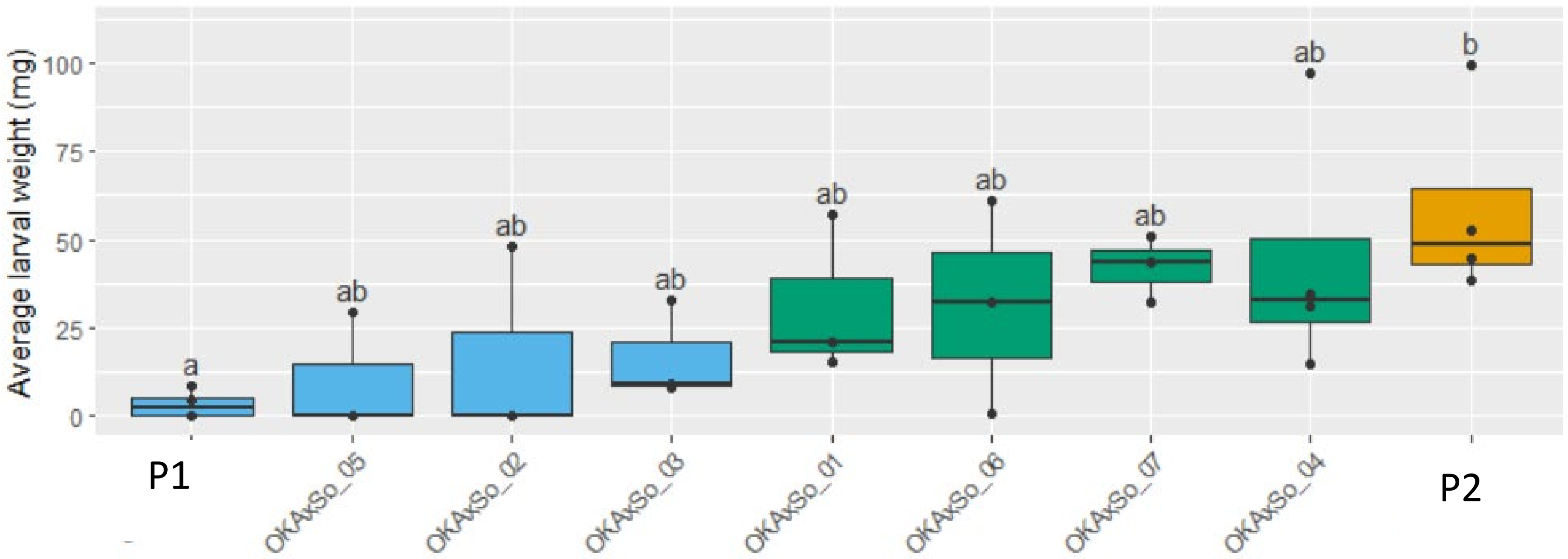
Results population *S. okadae* x Solyntus



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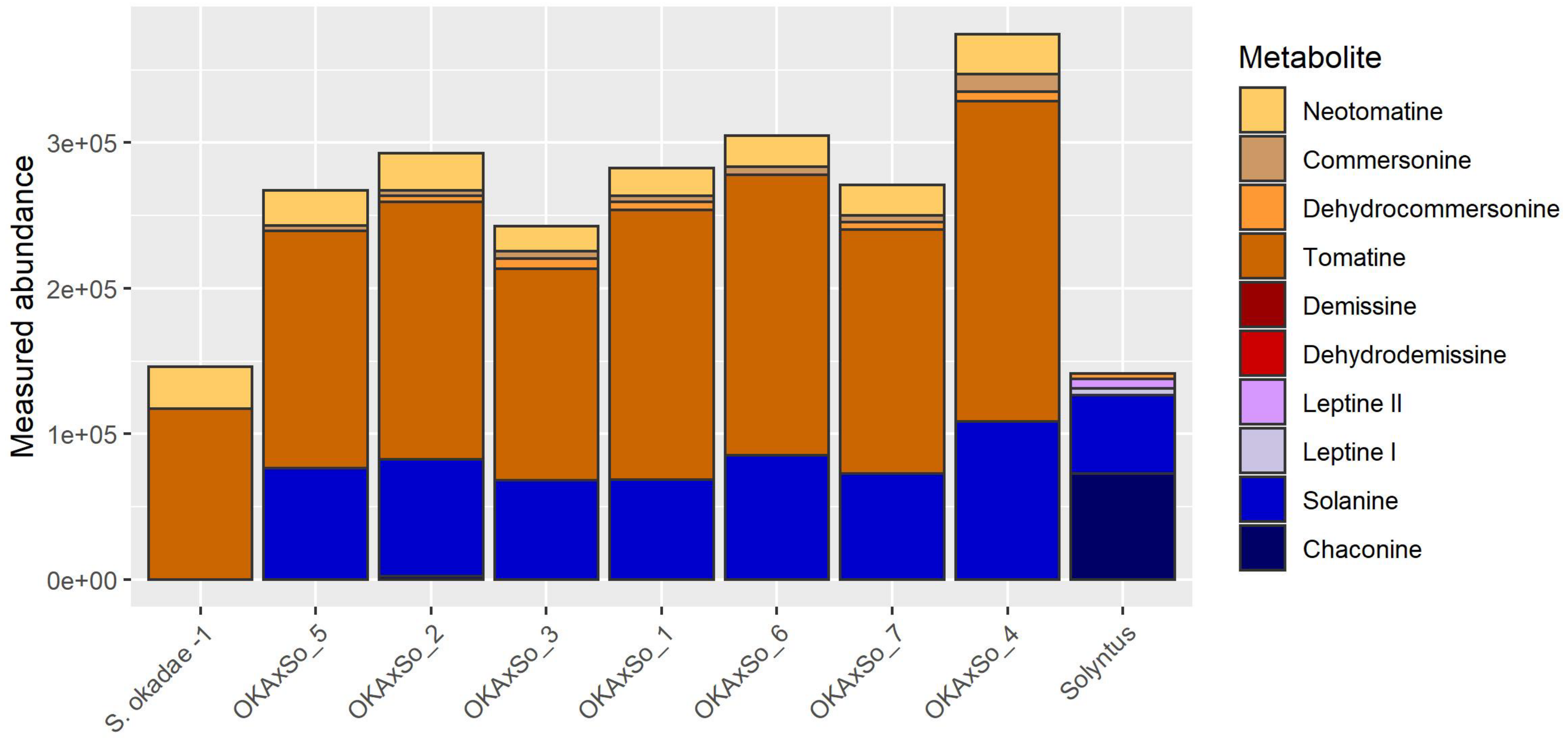
Results population *S. okadae* x Solyntus



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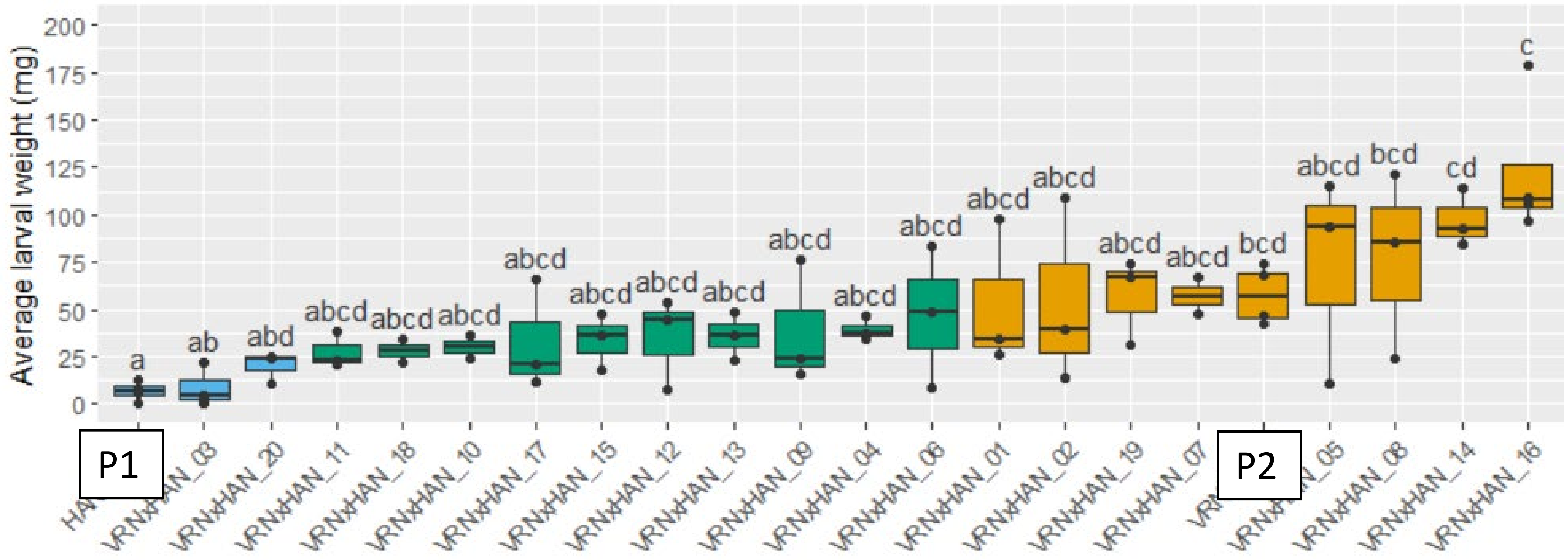
Results population *S. hannemannii* x *S. vernei*



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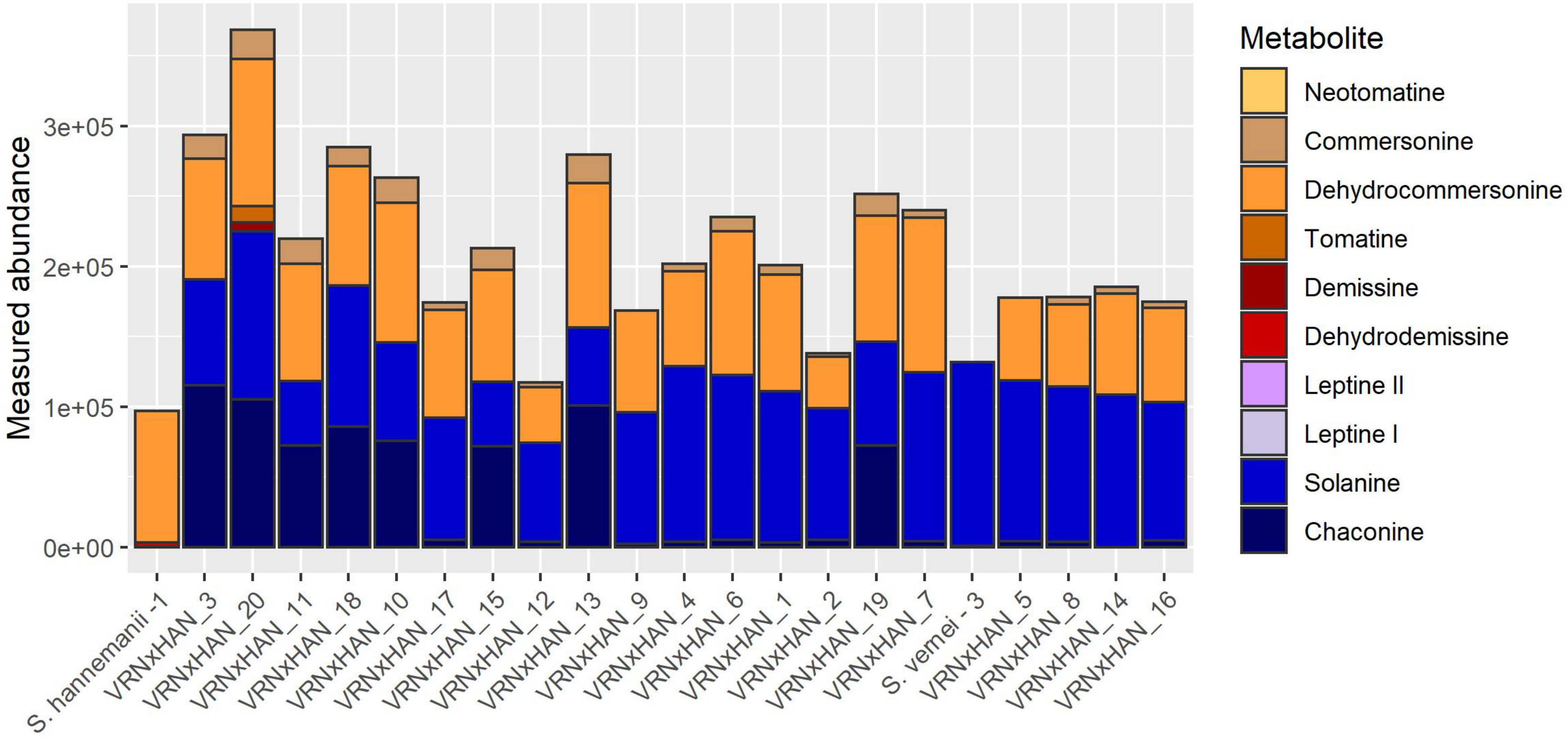
Results population *S. hannemannii* x *S. vernei*



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Conclusions

- There are many wild relatives available as sources of CPB resistance
- Different resistance mechanisms are likely present
- Tetraose glycoalkaloids seem to lead to strong resistance
- Acetylated GA may lead to medium resistance to CPB
- F1 progeny usually has combination of GA of both parents
- Role of trichomes on CPB resistance needs to be studied further
- Sources without glycoalkaloids or glandular trichomes will be studied further for novel resistances

Acknowledgements

Wageningen University & Research – Plant Breeding
Miriam Strijker
Kas Swinkels
Lize Braat
Cyril Stevens
Micha Devi
Hanneke van der Schoot
Yury Tikunov
Isolde Bertram
Jaap Wolters
Dirk Jan Huigen
Roeland Voorrips
Ben Vosman
Richard Visser

This work was funded by Europese programme OP Oost (EFRO)
HYBIO: **Hybride Aardappel voor de Biologische Teelt**

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Tillage and cover crops as strategies to control wireworms' populations before a potato crop

Dolo P. [1], Leost O. [1], Le Stum D. [1], Ropars T. [1], Le Roux A. [1], Le Moal J. [1], Barbary A.[1]

[1] BRETAGNE PLANTS INNOVATION, 471 lieu-dit La Gare, 29460 HANVEC, FRANCE

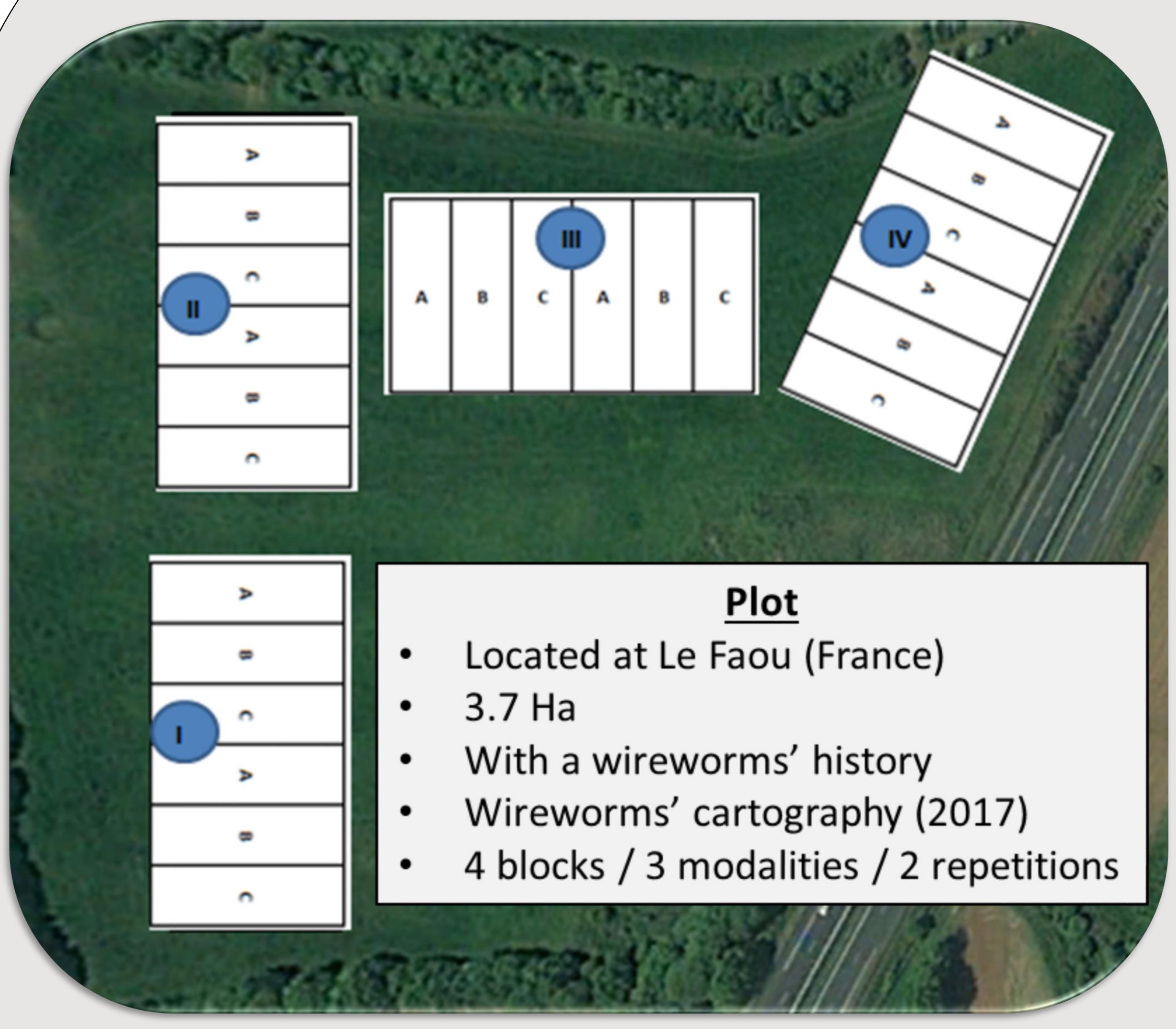
Context & Objectives

Wireworms (genus *Agriotes*) have a wide range of host crops, mainly potato. Damages due to these pests are being increasing in recent years. One explanation relies on the prohibition of Mocap 15 G (Ethoprophos), an insecticide proved to be efficient in avoiding wireworms' attacks. Even if they don't prevent potato growth, wireworms are disturbing for seed potato production as they cause bites and galleries in tubers making them unmarketable. In France, tolerance threshold for seed potato is 4% of tubers with less than ten bites (superficial and limited to 5 mm). But export customers have generally higher requirements, and it happens more frequently that seed potato lots have to be re-sorted or rejected.

This study aims at finding alternative solutions to insecticides to fight against wireworms, focussing on agronomic techniques. Thanks to a five-year cropping system, effects of tillage and cover crops on reducing wireworms' attacks on potatoes were investigated.

Material & Methods

Experimental design



Modalities

A) Intensive tillage before a late planted cover crop
B) "Classic system" with reduce tillage before an early planted cover crop
C) Tillage before sowing of a brown mustard growing as an intercrop

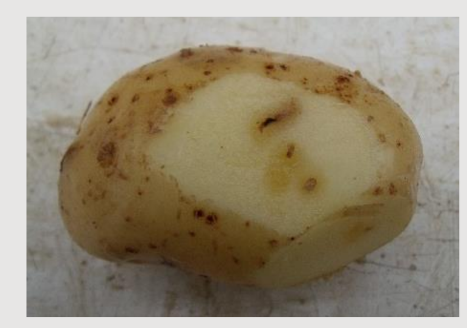
Crop rotation

	I	II	III	IV
2017	Wheat	Wheat	Wheat	Wheat
2018	Triticale	Maize	Barley	Potato
2019	Potato	Wheat	Maize	Barley
2020	Barley	Potato	Wheat	Maize
2021	Maize	Barley	Potato	Wheat
2022	Grass	Grass	Grass	Potato

Notations

- Sampling during harvest :
  - 100 tubers per sampling point
  - 10 samplings per micro-plot
  - 6000 tubers harvested on trial per year
- Ranking according to the number of bites or galleries
  1 gallery = bite > 3,5 mm (3 peels with a peeler)

Potato with bites



Potato with galleries

		Number of bites			Number of galleries		
Incidence of symptoms	Uninjured	1 to 2	3 to 5	> 5	1 to 2	3 to 5	> 5
Number of tubers	A	B	C	D	E	F	G

$$\text{Global Index (GI)} = \frac{(B \times 1{,}5) + (C \times 4) + (D \times 7) + (E \times 3) + (F \times 8) + (G \times 14)}{100}$$

- In this formula, a gallery is considered as twice more serious than a bite

$$\text{Efficiency} = \frac{\text{\% tubers with at least 1 gallery on mod. B} - \text{\% tubers with at least 1 gallery on mod. A or C}}{\text{\% tubers with at least 1 gallery on mod. B}} \times 100$$

Sampling (cartography)

Cover crop (brown mustard)

Tillage

Results

Overall notations (%) on potatoes after harvest of the five-year trial

	2018			2019			2020			2021			2022			mean		
Modality A	54,4	9,0	36,6	74,5	10,4	15,1	45,0	15,5	39,5	53,1	14,3	32,6	43,7	24,8	31,5	54,1	14,8	31,1
Modality B	33,5	9,9	56,6	64,8	15,0	20,2	18,0	15,8	66,1	40,0	13,9	46,1	18,9	23,8	57,3	35,0	15,7	49,3
Modality C	47,6	9,3	43,1	80,2	9,4	10,4	29,6	15,7	54,7	40,0	13,0	47,0	47,9	24,0	28,1	49,1	14,3	36,7

uninjured with bites with galleries

Global Index (GI) on potatoes of the five-year experiment depending on different modalities

	2018	2019	2020	2021	2022	mean
Modality A	1,96	0,74	2,46	1,84	2,26	1,85
Modality B	3,52	0,99	4,93	3,07	4,48	3,40
Modality C	2,26	0,55	3,70	2,89	1,9	2,26

Efficiency on potatoes of the five-year experiment compared to the control (modality B)

	2018	2019	2020	2021	2022	mean
Modality A	35	25	40	29	45	34,8
Modality C	23	48	17	2	51	28,2

Conclusions and discussions

- ✓ Current pratices, with a fast establishment of a cover crop and few tillage, is favourable for wireworms
- ✓ Intensive tillage shows the best results in controlling wireworm's populations. However this practice has to be considered given its impact on the soil structure and biology and its cost for growers.
- ✓ Use of brown mustard as a cover crop shows heterogeneous results from year to year. They could depend on the biomass generated by brown mustard and its biofumigant effect (glucosinolates) related to burying quality and weather conditions.

PacBio amplicon sequencing of Ry_{sto} homologues in wild potato species

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Potato virus Y (PVY) is among the top ten economically important plant viruses. It causes potato tuber necrotic ringspot disease, leading to decreased yield and tuber quality. Wild relatives of potato are valuable sources of genes involved in resistance to many pathogens that attack potatoes, including PVY. The gene Ry_{sto} derived from *Solanum stoloniferum* (Fig. 1) confers extreme resistance to PVY.

Fig. 1 *Solanum stoloniferum* donor of gene Ry_{sto} (https://ics.hutton.ac.uk/germinate-cpc/#/home)

AIM:To screen Ry_{sto} homologues and to analyze their diversity in wild relatives of potato.

PLANT MATERIAL: 298 genotypes representing 29 accessions of 26 tuber-bearing *Solanum* species, IHAR-PIB's collection.

METHOD: PacBio amplicon sequencing of the full coding sequences of Ry_{sto} homologues using barcoded primer pairs V, U and T (Fig. 2). The sequencing service was provided by the Norwegian Sequencing Centre (www.sequencing.uio.no).

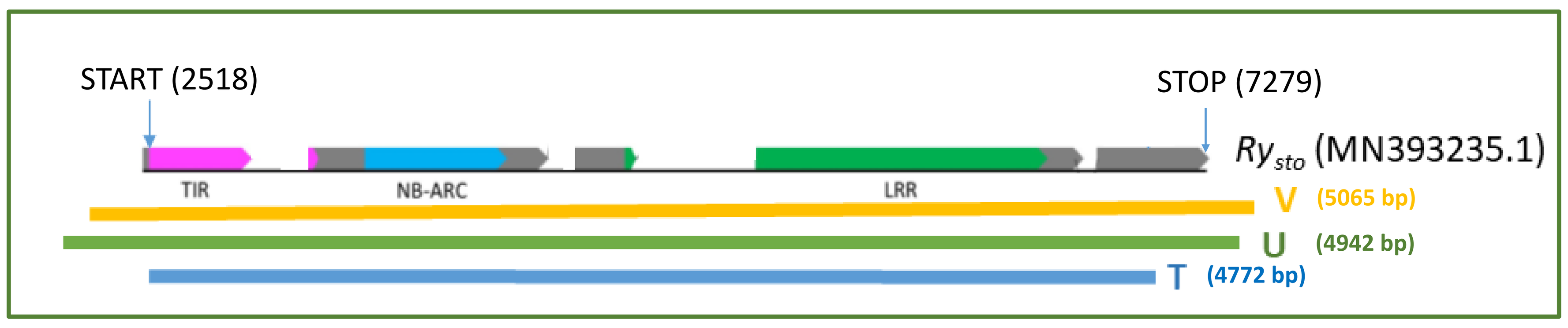

Fig. 2 PCR products obtained with primer pairs V, U and T covering Ry_{sto} gene

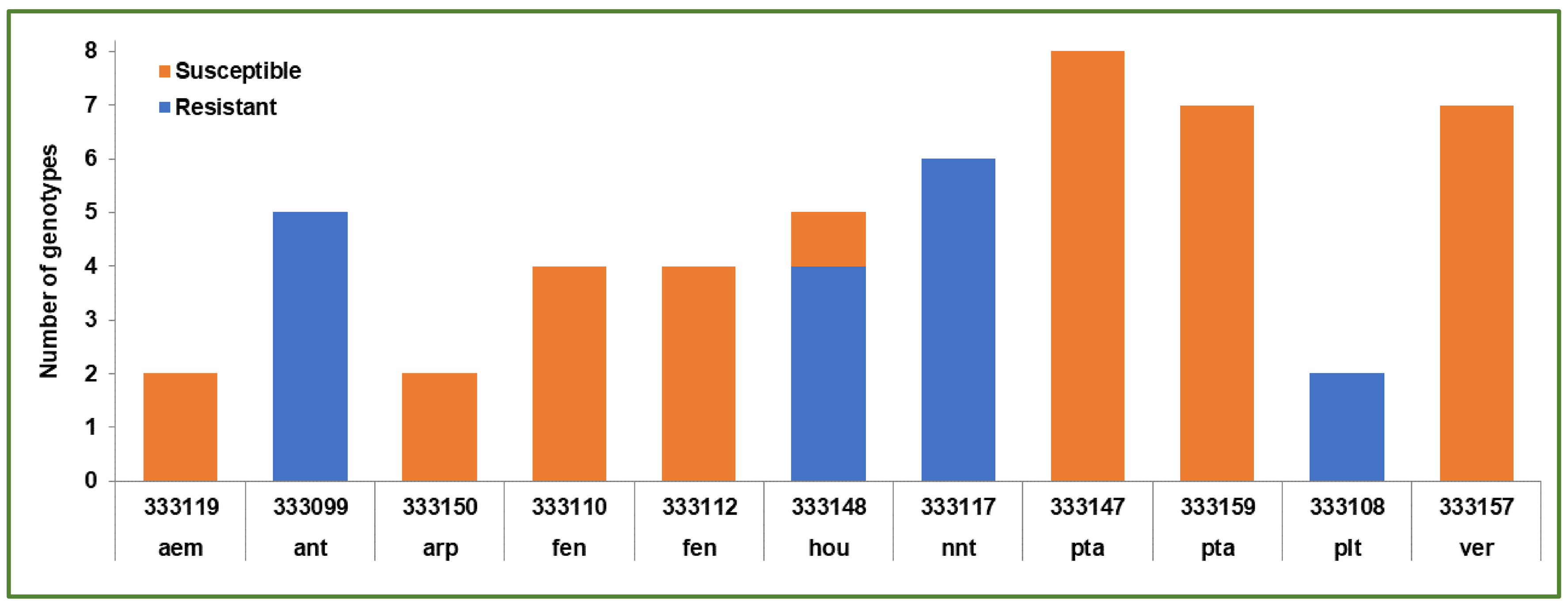

Fig. 3 Resistance of tuber-bearing *Solanum* species to potato virus Y (PVY). aem, *S. aemulans*; ant, *S. antipovichii*; arp, *S. arrac-papa*; fen, *S. fendleri*; hou, *S. hougasii*; nnt, *S. neoantipovichii*; pta, *S. papita*; plt, *S. polytrichon*; ver, *S. verrucosum*.

RESULTS:

- A total of 55 unique *Rysto-like* sequences were identified in 72 genotypes representing 12 accessions of ten tuber-bearing Solanum species and six resistant controls (potato cultivars Alicja, Bzura, Hinga, Nimfy, White Lady and breeding line PW363) (Figs. 1 and 3).
- From 1 to 13 *Rysto-like* sequences were detected per *Solanum* sp. accession, between 1 and 8 *Rysto-like* variants were detected per potato genotype. In resistant controls, the number of *Rysto-like* sequences varied from 1 to 3.
- Nucleotide identity of the obtained 55 *Rysto-like* sequences to that of the reference Ry_{sto} gene (MN393235.1, Grech-Baran et al. 2020) ranged from 89.87% to 99.98%. The *Rysto-like* sequences originated from primer pair T showed higher levels of nucleotide diversity than that originated from primer pairs V and U, with the greatest diversity found in the third intron (Fig. 4).
- In total 45 unique protein sequences were predicted (Fig. 5). Two of them, Rysto-like26 identified in potato cultivars Alicja, Bzura, White Lady and Rysto-like16 in potato clone PW363, showed 100% identity to the Ry_{sto} reference protein. Identity of the remaining 43 predicted Rysto-like proteins to the reference protein ranged from 76.41 to 99.92 %. TIR and NB-ARC domains showed the highest level of conservation, while LRR and C-JID domains were found to be most variable.

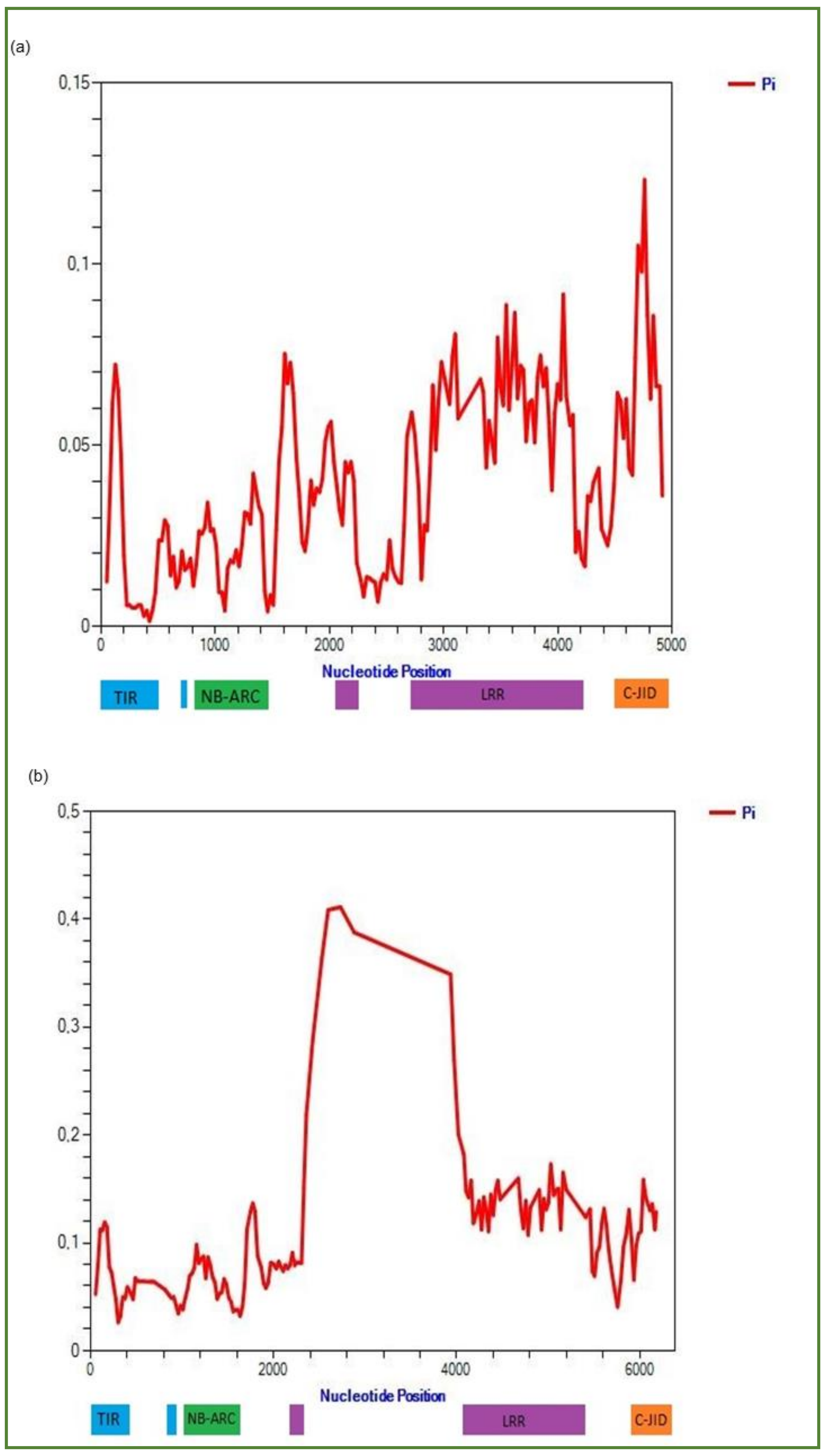

Fig. 4 Nucleotide diversity (Pi) of the Ry_{sto} homologues. Pi values range between 1 (very diverse) and 0 (conserved). (a) 25 variants obtained with U and V primers; (b) 30 variants obtained with T primer (without the ATG start codon). N-terminal domain homologous to the Drosophila Toll domain and human interleukin-1 receptor (TIR; blue); leucine-rich repeats (LRR; purple); nucleotide-binding domain (NB-ARC; green); C-terminal jelly roll/Ig-like domain (C-JID; orange).

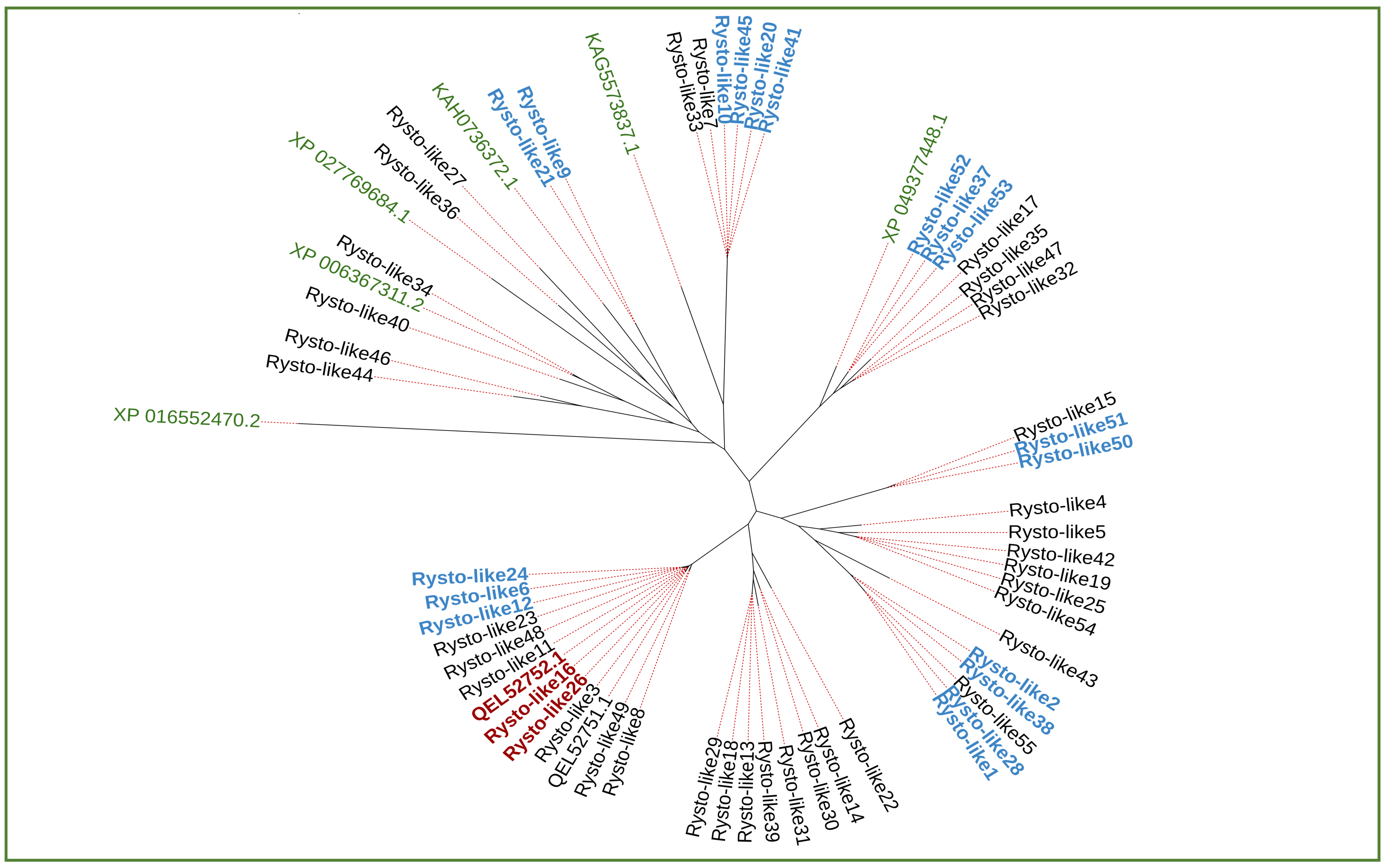

Fig.5 Phylogenetic tree of Ry_{sto} homologous proteins. Ry_{sto} protein and the obtained Rysto-like proteins with 100% identity are marked in bold (red). The Rysto-like homologous proteins with 100% identity to each other within a branch are marked in bold (blue). Other proteins from *Solanaceae* are marked with green. Protein sequences were aligned using the ClustalW program. The phylogenetic tree is constructed using FastTree2 tool used minimum-evolution subtree-pruning-regrafting (SPRs) and maximum-likelihood nearest-neighbor interchanges (NNIs). XP016552770.2 - *Capsicum annum* /disease resistance protein Roq1-like; XP006367311.2 - *S. tuberosum*/TMV resistance protein-like; XP027769684.1 - *S. pennellii*/TMV resistance protein-like; KAH0736372.1 *S. tuberosum*/hypothetical protein; KAG5573837.1 - *S. commersonii*/hypothetical protein; XP049377448.1- *S. stenotomum*/disease resistance protein Roq1-like; QEL52751.1/QEL52752.1- *S. stoloniferum* / Ry_{sto} protein – dominant/less abundant isoform.

CONCLUSION:

- This study confirmed the presence of Ry_{sto} gene in potato cultivars Alicja, Bzura, White Lady and breeding line PW363. Two variants were found in Alicja in addition to variant described by Grech-Baran et al. (2020).
- Higher levels of diversity of the *Rysto-like* sequences were found in the wild relatives of potato than in the tested potato clone and cultivars.
- Inter-species and inter-genotype variability of the *Rysto-like* sequences in the studied tuber-bearing *Solanum* species were observed, in respect to the number of variants identified, composition of the variants and content of individual variants.

References:
Grech-Baran M, Witek K, Szajko K, Witek AI, Morgiewicz K, Wasilewicz-Flis I, Jakuczun H, Marczewski W, Jones JDG, Hennig J. (2020) Extreme resistance to Potato virus Y in potato carrying the Rysto gene is mediated by a TIR-NLR immune receptor. Plant Biotechnol J. 18:655-667.

The research leading to these results has received funding from the Norwegian Financial Mechanism 2014-2021, project DivGene: UMO-2019/34/H/NZ9/00559