

# First Detection of *Arsenophonus* in Potato Crop in Switzerland: A Threat for the Processing Industry?

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Received: 28 August 2024 / Accepted: 19 December 2024 © The Author(s) 2025

#### **Abstract**

The phloem-restricted phytopathogenic bacterium Candidatus Arsenophonus phytopathogenicus (Ap) causes the "syndrome basses richesses" (SBR), which has recently emerged as a major burden for sugar beets in several countries of Western Europe. Here, we report the first identification of Ap and its planthopper vector Pentastiridius leporinus in potato fields in Switzerland in 2023. The bacterium was detected in potato plants and tubers exhibiting phytoplasma-like symptoms and collected from cantons currently experiencing SBR outbreaks. Although our analyses indicate the absence of the Stolbur phytoplasma, the pathogenicity of Ap in potato remains unclear, a fortiori in the context of the poor phytosanitary status of crops in Switzerland in 2023. In infected tubers, we show that Ap can be detected after dormancy from the stem end to the emerging sprouts with decreasing titers. Importantly, Ap might induce threadlike sprouts and the browning of the flesh upon frying, raising strong concerns for varieties marketed for chips production. Altogether, our results align with recent studies performed in Germany, highlighting the host shift of Ap and its vector from sugar beet to potato crops. Our data should raise awareness in other European countries where the presence of the bacterium has been recently described.

**Keywords** *Cixiidae* · Gammaproteobacteria · *Pentastiridius leporinus* · Phytoplasma · Stolbur · Syndrome basses richesses

Published online: 15 January 2025



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## Introduction

The γ-proteobacterium *Candidatus Arsenophonus phytopathogenicus* (Ap) causes the "syndrome basses richesses" (SBR) on sugar beet (Gatineau et al. 2002). SBR was first reported in the East of France in the 1990s, and then in Germany and Switzerland (Gatineau et al. 2002; Behrmann et al. 2021; Mahillon et al. 2022). In the latter, the disease was first observed in 2017 in Vaud, and it has now spread to all western cantons, where it severely impacts the sugar yield (Mahillon et al. 2022). The results of the annual survey conducted in 2023 and 2024 indicate that *Arsenophonus* has now been detected in eastern Switzerland. Ap has also been documented in several countries across Central Europe in 2024, indicating that outbreaks of SBR can also be feared in these regions (Duduk et al. 2024).

Ap is transmitted by two planthoppers from the family Cixiidae, namely *Pentastiridius leporinus* and *Cixius wagneri*, the former being the most prevalent species in sugar beet fields (Bressan et al. 2008; Mahillon et al. 2022). After harvest, *P. leporinus* completes its cycle as soil-borne nymphs that feed on the roots of winter crops such as wheat (Bressan et al. 2013). Emerging adults migrate and lay eggs in nearby sugar beet fields in the following spring, with short flying distances of *ca.* 1 km (Bressan et al. 2013). An average vertical transmission rate of 30% is observed in the planthopper offspring, and naive individuals can also acquire the bacterium by feeding on infected plants (Bressan et al. 2009). This fosters the persistence of SBR in infected areas and promotes its inexorable expansion, which is further promoted by the lack of efficient control measures (Pfitzer et al. 2024).

Remarkably, Ap seems to have a broad host range, with recent reports from Germany evidencing infections of onion and potato (Behrmann et al. 2023; Therhaag et al. 2024). In the latter, Ap has been detected sometimes in association with the Stolbur phytoplasma (Sp, synon. "Ca. Phytoplasma solani"), another phloemrestricted pathogen also infecting sugar beet and sharing *P. leporinus* as vector (Gatineau et al. 2001; Behrmann et al. 2023; Rinklef et al. 2024). In sugar beet, Ap is associated with reduced sugar yield, browning of root vasculature, leaf yellowing and strong deformation of emerging leaves (Mahillon et al. 2022). Most of these symptoms are similar to those induced by Sp. Likewise, in potato, symptoms associated with Ap and Sp are similar and include wilting and yellowing. Additionally, tubers with a conspicuous rubbery texture, identified by Lindner et al. (2011) as a consequence of Sp, were also observed (Behrmann et al. 2023).

Here, we report the first detection of Ap in potato in Switzerland, six years after its first detection in sugar beet in the same area. Given the extensive damage associated with SBR, the discovery of Ap in potatoes triggered considerable concern among growers and seed producers. In addition, representatives of the industrial processing sector questioned the surprising correlation between the dynamic progression of SBR in sugar beet crops in western Switzerland and increasing issues in chips processing. Indeed, tuber batches of certain varieties from the same region have been associated with unusual browning that appear upon frying. In this context, we evaluated the distribution of the bacteria in potato tubers following dormancy, and we obtained preliminary data on tubers quality and processing.



# **Material and Methods**

#### **Insect and Plant Material**

Six *P. leporinus* adults were collected in a potato field in July 2023 in Moudon (Vaud) using a SH 86 suction device (Stihl). The insect were stored in ethanol at -20 °C until further use.

For seven potato fields, pooled samples were taken from fresh leaves, petioles, stems and roots of individual symptomatic plants. In parallel, pools of 5-10 rubbery tubers from a further 10 sites were collected to complete the survey (Table 1). Samples were stored at 4  $^{\circ}$ C until further use.

In order to investigate the influence of Ap on chips processing, tubers from lots of three varieties were collected in a field in Cuarnens (Vaud). This field was chosen since it experienced important SBR outbreaks in previous years. None of the collected tubers showed internal signs of browning before processing.

**Table 1** Detection of Ap in potato samples from Swiss fields in 2023

Sample type	Location (Canton)	Detection rate		Genbank
		PCR	qPCR	Accession number
Pooled tissues	Messen (SO)	0/2	0/2	-
	Büren (BE)	0/2	2/2	-
	Combremont (VD)	0/1	1/1	-
	Lucens (VD)	0/1	1/1	-
	Combremont le Petit (VD)	1/1	1/1	PQ219974
	Lossy (FR)	0/1	0/1	-
	Vesin (FR)	0/2	0/2	-
	Total	1/10	5/10	-
Pooled tubers	Bercher (VD)	3/4	3/4	PQ219975
	Grossaffoltern (BE)	1/1	1/1	-
	Ins (BE)	1/1	1/1	-
	Kallnach (BE)	2/2	2/2	-
	Kappelen (BE)	2/2	2/2	PQ219976
	La Rippe (VD)	0/4	1/4	-
	Ponthaux (FR)	1/2	2/2	-
	Trey (VD)	1/2	1/2	PQ219977
	Walperswil (BE)	1/1	1/1	-
	Wileroltigen (BE)	0/1	1/1	-
	Total	12/20	15/20	-

BE, Bern; FR, Fribourg; VD, Vaud; SO, Solothurn



# **Symptom Development after Dormancy**

Tubers were stored at 8 °C for 5 months to break dormancy and then tested for the presence of Ap. For each variety, ten positive tubers with symptomatic or asymptomatic shoots were grown 10 weeks in greenhouse conditions to assess the presence of Ap in leaves and stems of the daughter plants. Four symptomatic positive tubers and two asymptomatic negative tubers were planted for 10 weeks in large pots to assess tuber production yield.

#### **DNA Extraction**

All DNA extractions were conducted as previously described (Mahillon et al. 2022). Briefly, plant material ( $\it ca. 0.5~g$ ) or whole insect bodies were ground in 3% CTAB extraction buffer using either a Homex 6 homogenizer (Bioreba, Switzerland) or pestles, respectively. The homogenates were clarified by low-speed centrifugation, and supernatants were collected and incubated for 30 min at 65 °C in the presence of  $\beta$ -mercaptoethanol. DNA was then extracted using chloroform: isoamyl alcohol and precipitated with isopropanol. Pellets were resuspended in 100- $\mu$ L nuclease-free water and stored at – 20 °C until further use.

#### **PCR Detection**

For Ap, PCR and qPCR protocols have been recently described (Mahillon et al. 2022). The plant COX gene was used to normalize data (Weller et al. 2000). In order to detect Sp, the qPCR for universal detection of phytoplasmas was used (Hodgetts et al. 2009) along with the 18S rRNA developed by Oberhänsli et al. (2011). For the identification of planthopper species, a recently described PCR method was followed (Pfitzer et al. 2022).

## Sequence Analysis

For Ap-positive samples from each locations, PCR amplicons of the partial *Spo* operon were sent to Fasteris (Switzerland) for Sanger sequencing. The obtained sequences were aligned with representative sequences from other *Arsenophonus* strains (Table S1) using Muscle (Edgar 2004). A maximum-likelihood phylogenetic tree was then constructed using ModelFinder (Kalyaanamoorthy et al. 2017) and IQ-tree (Nguyen et al. 2015) in combination with ultrafast bootstrap (Hoang et al. 2018). The tree was then curated on ITol (Letunic and Bork 2019).

# **Frying Assay**

For the frying assay, tubers were first sampled at the stem end part and tested by qPCR in order to confirm infection by Ap. Five pre-chips were then obtained from longitudinal cuttings using a classical cooking device. The cuts were briefly dried on clean tissue paper and then fried for 3 min at 170 °C in commercial sunflower oil.



The produced chips were briefly dried and photographed. Chips were grouped into three categories according to the intensity of browning symptoms: "no", "mild" or "severe" browning. In comparison to the classification used by the potato industry to assess tuber quality (Mini et al. 2004), the "severe browning" category corresponds to classes 1-5, the "mild browning" corresponds to class 6, and the "no browning" corresponds to classes 7-10.

## Results

# **Identification of Ap in Swiss Potato Fields**

In July 2023, during routine phytosanitary inspections in Moudon (Vaud), adult specimens of *P. leporinus* were observed for the first time in potato fields. For six specimens from one field, species identification was confirmed using a PCR method that can discriminate *P. leporinus* from closely related planthoppers (Pfitzer et al. 2022). Importantly, all collected insects tested positive for Ap by PCR.

In September of the same year, a surprisingly high level of symptoms commonly associated with Sp was observed on plants from potato fields in four cantons of western Switzerland (Table 1). The symptoms included aerial tubers, wilting and rubbery tubers. Rubbery tubers displayed no indications of shriveling and could only be differentiated from other tubers through an examination of their consistency. Pooled samples of various plant tissues were collected and subsequently tested for Ap and Sp (Table 1). While there was no detection of the phytoplasma, one and five out of ten samples tested positive for Ap using PCR and qPCR, respectively. In parallel, rubbery tubers were also sampled from ten locations and were submitted to similar analyses. Here again, there was no detection of Sp, while Ap was evidenced with high frequency (60-75%) by PCR and qPCR analyses. Altogether, these data indicated the presence of Ap in potato field in a least three cantons of Switzerland.

In order to further confirm the aforementioned results, the PCR amplicons from four plant samples (Table 1) and one insect sample (PQ219973) were sequenced. All 625-bp amplicons were almost identical (1-2 single nucleotide polymorphisms) to the reference Ap sequence (FM992680.1), and accordingly cluster together in a Maximum likelihood phylogenetic tree including other *Arsenophonus* strains (Fig. 1).

## Plant Symptoms

The detection of Ap in rubbery tubers prompted us to further analyse these tissues. Therefore, we obtained three lots of tubers of the Agria, Bintje and Venezia varieties from a single SBR-infected field in Cuarnens (Vaud). Although these tubers showed no sign of disease, a preliminary qPCR screening on 30 randomly chosen tubers revealed 30, 27 and 29 Ap-positive samples for Agria, Bintje and Venezia, respectively, whereas Sp was not detected. After a dormant period of five months, some tubers exhibited threadlike sprouts, which are usually associated with the presence of Sp (Wright et al.



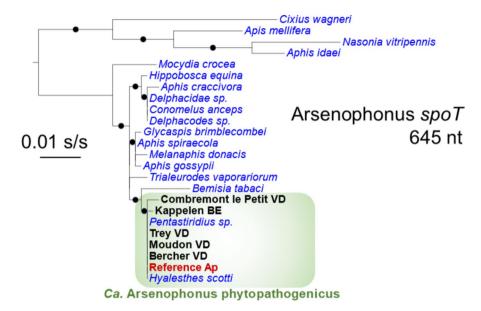


Fig. 1 Maximum-likelihood phylogenetic tree for the spoT genes of Arsenophonus strains. The tree was built using the substitution model HKY+F+G4. Names in blue and italic represent insect host species. Names in black refer to the Swiss potato or insect samples. The reference sequence of Ap is highlighted in red. The scale is given in substitution per site (s/s). Black circles on branches indicate > 60% bootstrap support (BS)

1981). The distribution of threadlike sprouts was variable and there was no relationship with the varieties Agria, Bintje or Venezia. They all showed tubers with exclusively symptomatic, partially symptomatic, or completely asymptomatic sprouts (Fig. 2A).

Post-dormancy qPCR tests were conducted to evaluate Ap titre in four parts of the tubers: the stem end connecting the tuber to the stolon, the middle part of the tuber, the base of the sprout and the sprout itself (Fig. 2B). All tubers were positive for Ap, consistent with pre-dormancy tests. Interestingly, although there was high variability among samples and tissues, a marked decrease in bacterial titre was observed gradually from the stem end to sprouts.

Ten tubers of each variety that tested positive for Ap and exhibited symptomatic and asymptomatic sprouting were planted in order to evaluate the transmission of Ap to the aerial parts of the plant. Some stems developed normally, while others were severely stunted (Fig. 2C). Despite this phenotype, no bacteria were detected in the aerial parts of these plants. In parallel, tuber yields were compared between plants produced by asymptomatic Ap-negative tubers or symptomatic Ap-positives tubers. Notably, infected plants produced significantly smaller tubers for all three varieties (Fig. 2D).

## **Frying Assays**

Phloem-limited phytoplasmas and other bacteria can induce symptoms on potato tubers either at harvest or after baking (Ember et al. 2011; Munyaneza 2012).



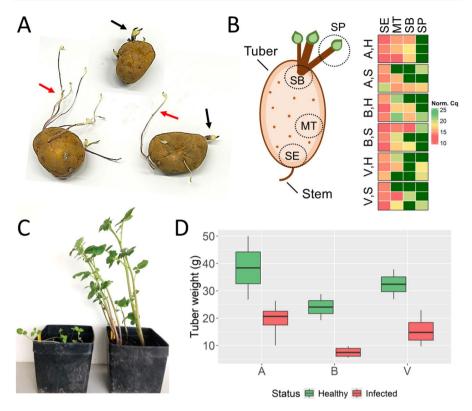


Fig. 2 (A) Tubers exhibiting normal sprouts (black arrows), deformed threadlike sprouts (red arrows) or a combination of both types. (B) Left: schematic representation of the sampling on different parts of potato tubers. Right: Bacterial titres (expressed in normalized Cq) in the different tuber parts for the three varieties. Dark green indicates the absence of qPCR signal. Tubers are grouped according to their status: healthy (H) or symptomatic (S) showing threadlike sprouts. SE: stem end; MT: middle part of the tuber; SB: sprout base; SP: sprout. (C) Plants from symptomatic (left) and asymptomatic (right) sprouts, three weeks after planting. In both cases, tubers were tested positive at the stolon end. (D) Tuber weight (in grams) obtained from healthy or infected tubers for the three varieties. A: Agria; B: Bintje and V: Venezia

We tested the latter possibility for 30 Ap-infected tubers of each of the three varieties collected at Cuarnens. We produced five chips for each tuber, and recorded the appearance of browning using a 3-level scale (Fig. 3A). The varieties Bintje and Agria have low reduced sugar content and are marketed for frying qualities. In contrast, Venezia possesses a high degree of reduced sugar content, rendering it unsuitable for the frying process. Accordingly, 75% of Venezia chips exhibited browning during frying (Fig. 3B). While Bintje maintained good aspect after frying, Agria exhibited a loss of frying qualities in the presence of the bacteria, with 70% of chips displaying browning. Interestingly, the browning pattern always first appeared on the stem end and followed a pattern along the tuber vasculature.



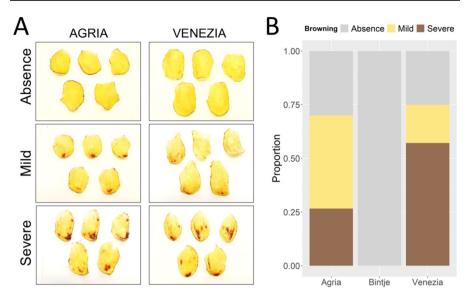


Fig. 3 Frying assay for the chips obtained from the tubers of three varieties. (A) Browning scale used to score the chips. (B) Proportion of browning for infected tubers of the three varieties

## Discussion

The climatic conditions of 2023 in Switzerland were highly unfavourable for potato crops, with reports of drought and high temperatures in many fields, resulting in highly detrimental impact on yield and quality (Federal Office for Agriculture 2024). Symptoms reported from field inspections were often unspecific, consisting of leaf and stem desiccation and aerial tubers, which can be caused by water stress, *Verticillium* wilt and phytoplasmas (Crosslin et al. 2006). In our survey of symptomatic plant tissues, half of the samples were negative for both Ap and Sp (Table 1), in line with the poor general health status of 2023. Similar observations were made in 2022 and 2023 in Germany, where the proportion of symptomatic plants neither infected by Ap nor Sp varies considerably between sites and is likely dependent on factors yet to be identified (Rinklef et al. 2024). Thus, it cannot be excluded that environmental parameters such as drought or heat, are more directly responsible for some of these symptoms in the field, which would seem to be confirmed by work currently underway in controlled conditions.

Nevertheless, the presence of rubbery tubers, which typically indicates the presence of Sp, was observed in an unusual number of post-harvest batches in several cantons of Switzerland. In our survey, Ap was detected in 75% of these soft tubers while Sp was not. We found that some infected tubers of all three varieties developed aberrant spindling sprouts, a phenomenon also commonly observed in phytoplasma-associated diseases (Wright et al. 1981). These symptomatic sprouts have continued to develop after planting, but we show that these sprouts result in stunted growth and reduced tuber yield. Importantly, we failed to detect Ap in aerial parts of the plants, either in normal-growing plants obtained from normal sprouts of infected tubers, or in reduced-growing plants obtained from symptomatic sprouts of infected tubers.



This aligns with a study in Germany showing that Ap is not transmitted vertically to seed potatoes (Rinklef et al. 2024). Seed potatoes should therefore not be a source of Ap, and it does not seem necessary to change certification schemes at this point.

The emergence of Ap in potato likely reflects a host shift of its vector *P. leporinus*. This insect is polyphagous and has been documented on several common plants in natural ecosystems (Ellis 2001). Until recently, this planthopper was considered endangered (Nickel et al. 2016), but it is currently massively present in areas affected by SBR in Germany and Switzerland. The slow migration of *P. leporinus* induces the spread of Ap (Bressan et al. 2010), which now appears to pose a threat not only to sugar beets, but also to onions and potatoes. It seems therefore urgent to evaluate the potential host range of Ap and its vector.

The distribution of phloem pathogen in plant tissues is often heterogeneous (Christensen et al. 2004), and this is also observed for viral pathogens in potato tubers (Schumpp et al. 2021). Likewise, we found a similar heterogeneous distribution of Ap in infected tubers. Interestingly, the stem end represents the tissue where bacterial titres are the highest, making it the optimal location for diagnostic sampling. There is no notable increase in bacterial titre before and after tuber dormancy, indicating little to no multiplication of the bacteria.

The industrial sector has expressed concerns regarding the potential correlation between the presence of Ap in production areas experiencing quality issues during frying. In response to these concerns, we analysed tubers of three varieties collected in a single field with previous SBR outbreaks. Our results indicate a probable varietal influence when faced with the presence of Ap, with Agria being potentially more prone to browning. Interestingly, this variety is also described as susceptible to Sp, whereas Bintje is both resilient to browning upon Ap infection and tolerant to Sp. Further varietal evaluations are now being carried out on a range of varieties used by the processing industry.

# **Conclusion**

The detection of Ap in multiple potato fields in Switzerland in 2023 provides compelling evidence that this pathogen has experienced a host shift, from sugar beet to potato, reflecting the situation recently reported in Germany. The impact of Ap on potato crop remains however unclear. The development of symptoms may be contingent upon environmental factors, and may be facilitated by the heat and drought that were particularly pronounced in Switzerland in 2023. In light of the global climate crisis, it is imperative to ascertain the conditions that might influence the emergence of symptoms in varieties of industrial and commercial significance. In the absence of vertical transmission, including Ap in seed certification scheme does not appear to be a necessary measure.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11540-024-09840-y.

**Acknowledgements** We would like to thank André Zimmermann (DGAV, Switzerland) for providing the potato samples from Cuarnens, Marc Passerat for taking care of the plants in the greenhouse tests and Basile Cornamusaz (CBS, Switzerland) for collecting samples in the field.



**Author Contribution** MM, CD, and OS designed the study and analyzed the data. MM, FB, ND, JB, and CD conducted the experiments. MM and OS wrote the original manuscript, which was reviewed and corrected by CD.

**Funding** Open access funding provided by Agroscope. This research was funded by the Swiss Federal Office for Agriculture to OS (grant 477 2020/33/LES-Z II).

**Data Availability** Sequence data are available on Genbank under the references given in Tab. 1 and Suppl. Tab. S. and other supporting data are included in the figures of the article.

#### Declarations

Ethics Approval Not applicable.

Consent to Participate All authors consent.

**Competing Interests** The authors declare no competing interests.

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