

Evaluating Grafting and Dodder as Non-Vector Transmission Routes of 'Candidatus Arsenophonus phytopathogenicus' in Sugar Beet

Britta Kais

Julius Kühn Institute: Julius Kuhn-Institut

Bruna Czarnobai de Jorge

Julius Kühn-Institut: Julius Kuhn-Institut

Jasminka Köhler

Julius Kühn Institute: Julius Kuhn-Institut

Karthi Balakrishnian

University of Göttingen Faculty of Agricultural Sciences: Georg-August-Universität Göttingen Fakultät für Agrarwissenschaften

Michael Rostás

University of Göttingen Faculty of Agricultural Sciences: Georg-August-Universität Göttingen Fakultät für Agrarwissenschaften

Jürgen Gross

juergen.gross@julius-kuehn.de

Julius Kühn Institute: Julius Kuhn-Institut <https://orcid.org/0000-0003-2614-9658>

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Abstract

Syndrome Basses Richesses (SBR) is a phloem-limited disease of sugar beet caused by '*Candidatus Arsenophonus phytopathogenicus*' (ARSEPH), a bacterium currently considered a facultative endosymbiont of its insect vector, *Pentastiridius leporinus*. Due to increasing disease incidence and the need for reliable vector-free resistance screening methods, this study evaluated alternative ARSEPH transmission routes using chip grafting and the parasitic plant dodder (*Cuscuta campestris*). Given that *Arsenophonus* species are unculturable and their insect vectors have complex life cycles so laboratory propagation is labor intensive, the development of vector-independent systems is desirable for controlled pathogen transmission and host–pathogen interaction studies. Chip grafting enabled successful ARSEPH transmission to sugar beet, though with low efficiency (~ 9.6%) and delayed pathogen detection. While *C. campestris* acquired and maintained high ARSEPH titers, it failed to transmit the bacterium to healthy plants, indicating limited systemic movement. In contrast, vector-mediated transmission via *P. leporinus* achieved 100% efficiency within 25 days, confirming a strong biological compatibility. These findings underscore the limitations of non-vector-based approaches for phloem-restricted pathogens and highlight the need for methodological optimization. The study provides key insights into ARSEPH's complex transmission biology and supports a functionally specialized association with its insect vector.

2. Introduction

The 'Syndrome Basses Richesses' (SBR) is a phloem-limited bacterial disease of sugar beet (*Beta vulgaris*) that causes significant yield losses by reducing sugar content in the taproot (Sémétey et al., 2007). It is associated with two uncultured bacterial pathogens '*Candidatus Arsenophonus phytopathogenicus*' (ARSEPH) and '*Candidatus Phytoplasma solani*' (PHYPSO) which are transmitted by the planthopper *Pentastiridius leporinus* (Hemiptera: Cixiidae). Previous studies have specifically linked ARSEPH to sugar content reduction and symptom development in sugar beet (Gatineau et al., 2002; Gatineau et al., 2001; Olivier Sémétey et al., 2007). This focus remains appropriate for the present study, as no evidence of PHYPSO co-infection was found in the sampled population during the 2022–2023 investigation period.

The topic is highly relevant, since in 2025, approximately 119,000 ha of sugar beet cultivation areas in Germany were affected by SBR (Wirtschaftliche Vereinigung Zucker, 2024), representing a substantial increase from the 60,000 ha recorded in 2023 equivalent to 30.7% of the total cultivation area (Hausmann et al., 2026). The agronomic and economic consequences of SBR infestation are considerable. The disease has been associated with a reduction in sugar content of up to 5%, a decline in root yield of up to 25%, and a reduction in adjusted sugar yield per hectare of up to 50% (Baden-Württemberg 2025). These effects pose a serious threat to regional sugar production and necessitate comprehensive control strategies against the planthopper, *P. leporinus*. Furthermore, both the planthopper and the phytopathogens have broadened their host plant spectrum recently and additionally

infest now potatoes and several vegetable species like carrots, onion and beet root (Witczak et al., 2025; Lang C, 2025; E. Therhaag et al., 2024; Eva Therhaag et al., 2024).

This study focuses on ARSEPH, a γ -3-proteobacterium from the family Morganellaceae, and is part of one of the most widespread genera of insect endosymbionts (Bressan, 2014; Bressan et al., 2011; M. Mahillon et al., 2025; Nadal-Jimenez et al., 2023). The bacteria inhabit a wide range of arthropods (Duron et al., 2008; M. Mahillon et al., 2025) including insects and arachnids (Jiang et al., 2022). In two independent events, members of this genus have shifted to plant-pathogenic lifestyles: ARSEPH infects sugar beet, potato and many vegetables (Behrmann et al., 2023; Duduk et al., 2024; Mahillon et al., 2025; Rinklef et al., 2024; Therhaag et al., 2024), while '*Candidatus* Phlomobacter fragariae' targets strawberry (Gross et al., 2022; Bell et al., 2025). These phloem-limited bacteria cannot be cultivated on artificial culture media and follow a biphasic lifestyle, multiplying in both plant sieve elements and hemipteran vectors (Mahillon et al., 2025; Perilla-Henao & Casteel, 2016). In the case of ARSEPH, the possible transition from an insect symbiont to a plant pathogen remains poorly understood (Bressan, 2014; Mahillon et al., 2025; Vogel et al., 2025). An endosymbiotic lifestyle in insects or plants typically leads to strong host adaptation, often accompanied by genome reduction and loss of genes essential for independent survival (Masson & Lemaitre, 2020; Vogel et al., 2025; Wernegreen, 2015). But such patterns of specialization and metabolic dependency are not restricted to mutualistic endosymbionts, they are also found in obligate plant pathogens like Mollicutes (Chen et al., 2012), reflecting a convergent evolutionary response to long-term host association. ARSEPH occupies two ecologically and physiologically distinct niches - the insect vector and the plant phloem. The molecular biology underlying its adaptation to these environments remains largely uncharacterized (Gross et al., 2022), highlighting the inherent difficulties in cultivating this bacterium under laboratory conditions.

As direct control of the vector *P. leporinus* is challenging, current management strategies focus on developing sugar beet varieties with enhanced resistance to infection. Effective evaluation of varietal resistance, however, requires a practical and reproducible method of plant inoculation that does not depend on the insect vector. A non-vector-mediated infection system would enable rapid and large-scale screening of plant material under controlled conditions, eliminating the need for time-consuming and technically demanding vector rearing. Importantly, such an approach would also prevent the accidental introduction or establishment of the vector in regions where it is not yet present, thereby reducing both ecological and phytosanitary risks.

A well-known non-vector method for transmitting pathogens from an infected to a healthy plant is grafting. The grafting technique, placing the cut end of one plant in direct contact with the tissues of another to form a single organism, has long been widely practiced. A variety of grafting techniques exist, including stem grafting or wedge grafting, tuber grafting (as in potatoes), and root grafting. Graft inoculation was the first extensively used technique for the transmission and detection of viruses, particularly in woody plants (Bhat & Rao, 2020).

In addition to the well-documented transmission of viruses *via* grafting, several bacterial phytopathogens have also been shown to be graft-transmissible. Notable examples include ‘*Candidatus Liberibacter asiaticus*’ (Hilf & Lewis, 2016), *Clavibacter michiganensis* (Xu et al., 2010), and various phytoplasma species (Görg et al., 2021; Jarausch et al., 2014; Schaper, 1982; Weintraub & Beanland, 2006). However, to the best of our knowledge, grafting experiments have not yet been conducted with the ARSEPH. Interestingly, (Dittmer et al., 2021) demonstrated that ‘*Candidatus Phlomobacter fragariae*’, a member of a distinct genus but phylogenetically related to ‘*Candidatus Arsenophonus*’, was abundant in the phloem sieve elements of strawberry stolons and was efficiently transmitted to daughter plants, which subsequently developed characteristic disease symptoms. These findings suggest that ARSEPH may also exhibit similar systemic mobility and capacity for plant-to-plant transmission *via* grafting, independently of its ancestral insect vector.

Another well-established non-vector method for plant pathogen transmission involves the use of *Cuscuta* spp. (dodder). All species in the genus *Cuscuta* (Convolvulaceae) are holoparasites found both in temperate and tropical regions. They lack roots and have thread-like stems with scale-like leaves and minimal content of chlorophyll (Machado & Zetsche, 1990), relying entirely on host plants for water and nutrients (Jeschke et al., 1994). Soon after germination, dodder seedlings form haustoria, specialized organs that penetrate host tissues and establish vascular connections (Vaughn, 2003), including confirmed xylem and phloem connectivity (Birschwilks et al., 2006). Cytoplasmic continuity *via* plasmodesmata has also been reported (Vaughn, 2003), with haustorial hyphae forming plasmodesmata between dodder and host cortex cells.

Dodder is also known to transmit plant viruses between simultaneously parasitized hosts (Bennet, 1940; Hosford, 1967; Johnson, 1941; Mikona & Jelkmann, 2010) and was historically used for diagnostics and studying mycoplasma-like organisms (Siller et al., 1987), which are now classified as phytoplasmas (Lee et al., 1998; Namba, 2019). In laboratory settings, phytoplasmas, phloem-inhabiting bacterial pathogens, can be experimentally transferred from infected to healthy plants *via* dodder (Carraro et al., 1988; Davis RE, 1991; Khanchezar et al., 2012; Schneider et al., 2014). These pathogens enter through the haustorium, replicate within dodder tissues, and spread to the recipient plant.

Based on the phloem-restricted lifestyle of ‘*Candidatus Arsenophonus phytopathogenicus*’ (ARSEPH) and its phylogenetic relatedness to other graft- and dodder-transmissible bacterial pathogens, we hypothesize that ARSEPH can be systemically transmitted between sugar beet plants *via* both grafting and parasitism by dodder (*Cuscuta campestris*). Demonstrating such non-vector-mediated transmission would not only suggest intrinsic phloem mobility of the bacterium but also establish foundational methodologies for vector-independent inoculation systems to facilitate resistance screening under controlled conditions.

3. Materials and Methods

3.1 Plant Material

Eight different sugar beet varieties (*Beta vulgaris* subsp. *vulgaris*, Altissima group) were used for both the vector-free and vector transmission trials. Half of the plants were classified as 'Candidatus Arsenophonus phytopathogenicus' (ARSEPH)-sensitive, while the other half were categorized as ARSEPH-tolerant. The varieties were provided by four different seed companies in cooperation with the GFPi (Gemeinschaft zur Förderung von Pflanzeninnovation e. V.).

For the vector-free transmission trial, fifteen plants of each variety were potted in 3-liter pots filled with a clay-containing potting substrate (Klasmann Deilmann, Geeste, Germany) and cultivated in a greenhouse under controlled conditions: a temperature of $24 \pm 1^\circ\text{C}$, relative humidity of 55–75%, and a minimum PPFD of approximately $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ corresponding to a DLI of approximately $9.6 \text{ mol m}^{-2} \text{day}^{-1}$ under a 12 h photoperiod. Fourteen plants of each variety were grafted with ARSEPH-infected plant material, while one plant per variety was grafted with healthy plant material as negative control. The infected plant material was obtained from a diverse set of 33 sugar beet plants, which had been inoculated with ARSEPH by feeding activity of *P. leporinus*. The inoculum originated from rearing facilities of the University Göttingen and the JKI Dossenheim. Before grafting, the infected plants were individually tested for ARSEPH in root tissue using qPCR following the protocol of Zübert & Kube (2021). The sampling material was consistently taken from the beet crown in sugar beet plants. To facilitate access to this tissue, the young plants were cultivated in a manner that exposed the upper part of the storage root, commonly referred to as the beet crown.

3.2 DNA isolation

DNA from plant material was extracted following a modified Doyle protocol, as described by Görg et al. (2021). For sugar beet samples, 50 mg of beetroot tissue was transferred into a microcentrifuge tube and homogenized using two stainless steel beads (hardened, non-rusting, 3.5 mm diameter; Kugel-Winnie, Bamberg, Germany) in a Fast Prep® sample preparation system (MP Biomedicals, Irvine, CA, USA) for 60 seconds at speed level 4.0. Subsequently, 500 μl of Doyle buffer + 2% β -mercaptoethanol was added, and DNA extraction proceeded according to the protocol. The DNA pellet was resuspended in 50 μl of Tris-EDTA buffer (pH 8.0). Tissue from non-insect-exposed sugar beets served as a negative control, while ARSEPH-infected sugar beet material was used as a positive control to detect the γ -proteobacterium.

3.3 Real-Time PCR Assay

To detect ARSEPH in beet plants, TaqMan based real-time PCR assays were conducted using DNA eluates obtained from plant tissue extraction. The assay included both pathogen- and plant-specific primers (Eurofins Genomics Europe Shared Services GmbH, Germany) as described by Zübert & Kube (2021), allowing for simultaneous detection and internal control verification.

Each reaction (total volume: 10 μl) consisted of 5 μl of qPCR PROBE Master Mix (primaQUANT Probe Advanced; Steinbrenner Laborsysteme GmbH, Wiesenbach, Germany), 0.3 μl of each pathogen-specific forward and reverse primer (10 μM), 0.3 μl of the labeled probe (6-FAM/BHQ-1, 10 μM), 0.1 μl of each

plant-specific forward and reverse primers (10 μ M), the labeled plant-specific TaqMan probe (Cy5/TAMRA, 10 μ M), 2.8 μ l of nuclease-free HPLC-grade water, and 1 μ l of DNA template.

The quantification and evaluation of the TaqMan qPCR assays were performed using synthetic double-stranded DNA fragments (gBlocks, Integrated DNA Technologies, Leuven, Belgium). A standard curve was generated to determine the detection limit (10^8 - 10^1 ARSEPH), using 125 bp gBlocks as described in Zübert & Kube (2021).

3.4 Insect collection and rearing

Planthoppers of the species *P. leporinus* used in the transmission trial were field-collected on June 22, 2022, using sweeping nets in a sugar beet field near Worms (Ilbersheim, coordinates: 49°43'29.1"N 8°23'20.1"E). The insects were stored separately in insect cages (BugDorm-4 Insect Rearing Cage, 47.5 x 47.5 x 47.5cm, NHBS GmbH, Bonn, Germany) for transport and identified based on morphological traits, according to Biedermann & Niedringhaus (2009). The planthoppers were kept at the JKI Dossenheim rearing facility under controlled conditions following established protocols (Behrmann et al., 2022; Pfitzer et al., 2022) until the trial was conducted. Only *P. leporinus* specimens were used for the transmission trial.

3.5 Grafting-Trial

To investigate the distribution of ARSEPH within infected leaves, three different sections (lower petiole near the beet crown, middle petiole, and upper petiole) were sampled from three leaves of five individual plants. A root sample from each plant served as a positive control and tested positive for ARSEPH in all cases. Based on these results, we selected the lower section for grafting, despite similar bacterial loads to the middle section, because it yielded a higher rate of positive samples. (see Fig. 3).

Two-month-old sugar beet plants were inoculated *via* chip grafting by inserting approximately 1 cm-long segments of infected petioles into the stems of recipient plants. To perform the graft, a small incision was made at the center of the stem, into which the infected petiole segment was inserted (see Fig. 1) and then secured in place using buddy tape (Aglis BuddyTape, Aglis Corporation, Japan). Each plant was graft-inoculated at five evenly spaced leaves near the center of the rosette or at the crown region. To ensure phloem sap flow through the graft sites, all ungrafted leaves were removed. To minimize systematic bias, each graft site received infected tissue from a different donor plant and was grafted by a different individual. Following the grafting procedure, plants were regularly monitored for ARSEPH infection over a six months period. Regular qPCR tests were performed on beet tissue, following the protocol of Zübert & Kube (2021). The first test was conducted 10 weeks after grafting, followed by subsequent tests every four weeks.

3.6 Vector Transmission Trial

For the vector transmission trial with *P. leporinus*, one ARSEPH-sensitive and one ARSEPH-tolerant sugar beet variety from each company were placed together in a cage (BugDorm-4 Insect Rearing Cage, 47.5 x

47.5 x 47.5cm, NHBS GmbH, Bonn, Germany), and 33 planthoppers were added to each cage. The experiment lasted for 25 consecutive days and was conducted in the same climate chamber as the insect rearing to maintain consistent conditions. After the inoculation period, all plants were transferred to an insect-proof greenhouse. Six weeks post-inoculation, all plants were tested individually for the presence of ARSEPH by qPCR tests on beet tissue, following the protocol of Zübert & Kube (2021).

Seedlings from two ARSEPH-sensitive sugar beet genotypes were grown in 3 L pots and maintained in an insect-proof greenhouse. Five sugar beet plants were inoculated with approximately 200 *P. leporinus* nymphs for 28 to 113 days, while one additional plant was exposed to 44 adult *P. leporinus* for 14 days. Since only infection status was relevant for subsequent analyses, the duration of vector exposure was not standardized. All inoculated plants tested positive for ARSEPH prior to the experiments.

3.7 Experimental transmission trial via dodder

In this study, dodder (*Cuscuta campestris*) was used in all transmission trials to investigate a possible transmission of ARSEPH bacterium from an infected to a healthy sugar beet plant. Based on findings by (Příbylová & Špak, 2013), who noted species-specific transmission efficiencies, *C. campestris* was selected due to its natural occurrence in sugar beet fields. According to Carraro et al. (1991), who showed higher transmission rates when dodder grew from the infected to the healthy plant, the experimental setup was designed accordingly. All *C. campestris* plants were grown from seed and initially established on three healthy sugar beet plants of two sensitive genotypes (genotype A and genotype B). After six weeks, the dodder plants have been well established on the three healthy sugar beet plants of both genotypes.

To enable the transfer of the proteobacterium, a bridging strategy was employed. Vigorous stolon ends were selected and gently wrapped around the petioles of infected donor plants to establish a parasitic connection (see Fig. 1, red square). As new growth emerged from the initial stolon, it was regularly redirected to maintain stable connection with the infected tissue. This ensured sustained interaction between the dodder and the host vascular system. The dodder bridges remained intact for the duration of their viability and stayed in place until the parasite naturally senesced.

All donor and recipient plants were maintained under insect-proof greenhouse conditions. One month after establishing dodder bridges on the infected plants, three healthy sugar beet plants were positioned adjacent to each infected plant (see Fig. 2). Dodder strands were then guided from the infected plants on to the healthy recipient plants to establish parasitic connection. The presence of ARSEPH in the dodder tissue was confirmed by qPCR after 63 days for genotype A and 34 days for genotype B. Recipient sugar beet plants were periodically screened for ARSEPH using qPCR beginning one month after the dodder bridge had been established.

3.8 Statistics

To assess the dynamics of ARSEPH transmission following grafting, quantitative PCR (qPCR) results were analyzed based on the $\log_{10}$ -transformed starting quantity values (Sq). Non-detectable samples ($Cq > 35$) were considered below the detection threshold. Values returning NA were assigned a value of 1 ($\log_{10} = 0$) for visualization purposes, but were excluded from statistical comparisons to avoid distortion of the variance structure. Statistical analysis focused exclusively on detectable ($Cq \leq 35$) values. A one-way analysis of variance (ANOVA) was performed to evaluate the effect of time after grafting on the accumulation of ARSEPH ($\log_{10}$ Sq), with time treated as a categorical factor.

To assess the effect of tissue type, genotype, and time on bacterial load (measured as Sq), we used a linear model (LM) with a log-transformed response variable $\log(Sq)$ to account for skewness in the data distribution. The model included the fixed effects of tissue type (type: Donor Plant, Parasitic *Cuscuta*, Recipient Plant), genotype (variety: Genotype sensitive A or B), sampling time (date: week 0, 1, 2, 3, 4, 5, 6 and 7), and all two- and three-way interactions between these factors.

For statistical modeling, only positive (measured) Sq mean values were used; substituted values (e.g., those resulting from non-detects in qPCR) were excluded from the linear model. Model residuals were checked using the DHARMA package, and no significant violations of model assumptions were observed.

Post hoc comparisons of genotypes within each tissue type and time point were conducted using estimated marginal means (emmeans), with p-values adjusted for multiple testing. All statistical analyses were performed in R (Version "Mariposa Orchid" 2025.05.1 + 513; (R. Core Team, 2025) using RStudio (R. Team, 2025). Unless otherwise stated, all figures were generated using the ggplot2 package (Wickham, 2016). Statistical models were evaluated using the lm function (stats) for linear models, DHARMA for residual diagnostics (Hartig, 2022), and emmeans for post hoc comparisons (Lenth, 2023). For graphical representation, Sq values equal to zero - corresponding to undetectable signals - were substituted with a value of 1 to allow log transformation. These substituted points were visually distinguished in the plots using a different shape and transparency to clearly differentiate them from directly measured values.

4 Results

4.1 Preliminary Test for the Grafting Trial

Figure 3 shows an analysis of pathogen load in different sections of the plant petiole, based on log-transformed Sq values ($\log_{10}(Sq)$) and corresponding Cq values from qPCR measurements. The left side presents a schematic division of the petiole into three segments: upper, middle, and lower. On the right, a boxplot illustrates the distribution of $\log_{10}(SQ)$ values across five plants. The grouping into 0%, 35%, and 70% refers to the proportion of petiole samples that tested positive for the pathogen in the upper, middle, and lower petiole sections, respectively. In relation to the bacterial load (Sq), the ANOVA revealed a significant effect of plant identity ($F_4 = 11.59$, $p = 0.001$), but no significant differences among petiole

positions, middle and lower ($F_2 = 0.6159$, $p = 0.45$), and no interaction between plant and position ($p = 0.34$). Although the lower petiole segment showed a tendency toward higher pathogen loads and the highest proportion of positive samples (70%), differences among petiole positions were not statistically significant. To maximize the probability of collecting infected tissue for grafting, the lower petiole segment was selected, as approximately 70% of samples from this region tested positive.

4.2 Vector-free transmission of ARSEPH *via* grafting

In total, 120 sugar beet plants, each bearing five leaves, were chip-grafted, resulting in 600 individual grafts. All plants were monitored for ARSEPH infection at four-week intervals over a six-month period using quantitative PCR (qPCR). The first infections were detected after 18 weeks (see Fig. 4). By then 12 plants (11.5%) exhibited Cq values below 35, indicating successful infection. One month later (22 weeks), only seven plants tested positive, reducing the infection rate to 6.7%. After 26 weeks, nine plants showed clear signs of infection ($Cq < 35$), and one plant displayed a weaker signal with a Cq value close to the detection threshold. The experiment concluded with a total of ten infected plants, corresponding to a low overall success rate of 9.6%.

To investigate the temporal dynamics of signal emergence in grafted plants, we quantified signal accumulation at various time points post-grafting using quantitative PCR. Signal quantities were $\log_{10}$ -transformed ($\text{Log}_{10}(\text{SQ})$) and assessed alongside corresponding quantification cycle (Cq) values between 10 and 26 weeks after grafting, aiming to determine when systemic movement of the signal becomes detectable.

As illustrated in Fig. 4, no detectable signal was observed during the early time points (10 and 14 weeks post-grafting), with all data points falling below the detectability threshold (dashed line at $\text{Log}_{10}(\text{SQ}) = 2$) and associated Cq values clustering above 35. However, starting at 18 weeks post-grafting, signal intensity began to rise noticeably. At this stage, a subset of 11 plants surpassed the detection threshold, with median $\text{Log}_{10}(\text{SQ})$ values increasing sharply above 3. This trend was reduced at 22 weeks and was intensified at 26 weeks, when seven and ten plants, respectively, exhibited detectable signals, with Cq values consistently below 35. All negative controls remained consistently below the detection limit throughout the experiment (see blue dots in Fig. 3). An analysis of variance (ANOVA) based on the log-transformed Sq values showed that the factor 'time after grafting' had no significant effect on bacterial load ($F(8,8) = 0.34$, $p = 0.925$).

Figure 4. Temporal dynamics of ARSEPH accumulation in grafted plants. Boxplots display $\log_{10}$ -transformed ARSEPH quantities (Sq, left y-axis) in plants over time (x-axis, weeks after grafting). Individual data points above the detectability threshold ($Cq \leq 33$) are shown in black; negative controls (NC) are shown in blue, non-detectable samples are represented by red "o" symbols and were assigned a value of 1 for visualization purposes. The horizontal dashed line indicates the detection threshold ($\text{Log}_{10}(\text{Sq})$ corresponding to $Cq = 35$). The right y-axis (in reverse order) shows the corresponding Cq values for reference.

4. 3 Experimental Transmission Trial

4. 3 Experimental Transmission Trial *via Cuscuta campestris*

To assess the spatial and temporal distribution of ARSEPH within the host–parasite system, we performed quantitative PCR (qPCR) analyses at eight time points. (Fig. 6).

Initial qPCR analyses focused on the donor plants and revealed high Sq values at the onset of the trial (T0), with both sugar beet genotypes A and B exhibiting mean $\text{Log}_{10}(\text{Sq})$ values exceeding 4.5. These values correspond to Cq values well below 30, indicating sufficiently high bacterial titers to initiate the trial. Over time, a decline in bacterial load was observed in both genotypes, with genotype B showing a more pronounced reduction, reaching near-threshold levels by T4 ($\text{Log}_{10}(\text{Sq}) \sim 3.5$; $\text{Cq} \sim 26$).

In the donor-associated *Cuscuta campestris* tissues (parasitic *Cuscuta*, Fig. 6, T1), Sq levels remained high — particularly after 63 days for genotype A and 34 days for genotype B (T1) - demonstrating efficient bacterial uptake from the infected donor plant. Median $\text{Log}_{10}(\text{Sq})$ values in genotype A exceed 6, indicative of high bacterial concentrations. While slightly lower levels were observed in genotype B, the values remained well above the threshold for reliable detection.

In *C. campestris* plant parts attached to healthy recipient plants (parasitic *Cuscuta*, Fig. 6 $\geq$ T2), bacterial titers decreased over time. The genotype-specific differences observed in donor tissues persisted: genotype A maintained elevated Sq levels from T1 to T4 (median $\text{Log}_{10}(\text{Sq})$ between 4.5 and 6; $\text{Cq} < 27$), whereas genotype B exhibited a gradual decline. By T7, one probe of *C. campestris* from genotype B approached the detection threshold ($\text{Cq} \sim 35$), suggesting reduced persistence or transport deficiency.

With the exception of four outliers, ARSEPH was largely undetectable in the recipient plants throughout the experiment. For both genotypes, $\text{Log}_{10}(\text{Sq})$ values remained consistently below 1, corresponding to Cq values above 35 and, in some cases, not amplifiable (indicated by crosses in Fig. 6).

To statistically evaluate these patterns, a linear model fitted to log-transformed Sq values (log Sq MW) revealed significant main effects of tissue type (ANOVA: $F(3, 47) = 12.70$, $p < 0.001$), genotype ($F(1, 47) = 14.26$, $p < 0.001$), and time ($F(4, 47) = 19.30$, $p < 0.001$). Interactions between tissue type and genotype ($p = 0.09$) and between tissue type and time ($p = 0.059$) both were not significant.

Post hoc comparisons using estimated marginal means (emmeans) indicated that genotype A exhibited significantly higher bacterial loads in *C. campestris* at timepoints 3 and 7 ($p = 0.0001$ and $p = 0.0082$, respectively), whereas genotype B showed significantly higher loads in recipient plant tissues at timepoint 3 ($p = 0.0022$). While additional trends were observed, they did not reach statistical significance.

In conclusion, the results demonstrate that ARSEPH can be taken up by *C. campestris* from an infected donor plant within one month and subsequently translocated to newly formed stolons within another month. However, there is no evidence for systemic transmission of the bacterium from *C. campestris* to the healthy recipient plant under the conditions tested.

Quantitative PCR analysis of ARSEPH was conducted at eight time points (T0–T7) across three tissue types: donor plant (infected sugar beet, T0; T4)), parasitic *Cuscuta* (*C. campestris* on infected T1; and healthy plants T2-T7), and recipient plant (healthy sugar beet plants, T3-T6). Results for two sensitive genotypes (A, blue; B, orange) are shown as $\text{Log}_{10}(\text{Sq})$ on the left y-axis, with corresponding Cq values on the right y-axis (inverted scale). Circles represent individual measurements; “X” symbols denote substituted zero values (NA = not amplifiable, set to 1 for log transformation), threshold > 35 (dashed lines), n = 3.

4.4 Vector Transmission Trial

For the vector transmission trial, one ARSEPH-sensitive (1-4S) and one ARSEPH-tolerant (1-4T) variety from the same company were placed together in a single cage with 33 planthoppers for a period of 25 days. Six weeks post-inoculation, all plants showed Cq values below 30 (see Table 1). The supposedly tolerant varieties tended to exhibit slightly higher Cq values. This experiment was conducted once as a demonstrative case to illustrate the contrast with non-vector-based transmission routes.

Table 1 Comparison of Cq values across eight genotypes, each comprising one ARSEPH-sensitive (1-4S) and one presumed ARSEPH-tolerant (1-4T) genotype, after 25 d.								
Genotype	1T	1S	2T	2S	3T	3S	4T	4S
Cq -value	24.50	22.81	28.12	24.71	23.31	23.69	24.19	19.65

5. Discussion

5.1 Vector-free transmission of ARSEPH via grafting

In this study, we employed chip grafting to facilitate the transmission of ‘*Candidatus Arsenophonus phytopathogenicus*’ (ARSEPH) vector-independent in sugar beet. To the best of our knowledge, this represents the first systematic large-scale attempt at chip grafting in sugar beet. Moreover, this is the first documented attempt to transmit ARSEPH from infected to healthy plants by *Cuscuta campestris*.

Overall, our findings confirm that ARSEPH can be transmitted *via* chip grafting, although the success rate was low. The low transmission efficiency is likely attributable to a combination of biological and technical factors, particularly the bacterium’s strict confinement to the phloem. Comparable challenges have been reported for other phloem-limited pathogens, such as phytoplasmas, ‘*Candidatus Liberibacter*’, and *Phlomobacter* (Bendix & Lewis, 2018; Bové & Garnier, 2003; Haider et al., 2024; Lewis et al., 2022).

One key technical consideration is that ARSEPH resides exclusively within the sieve elements of the phloem, which means successful transmission *via* chip grafting depends on the precise alignment of phloem tissues between donor and recipient plants. Even slight misalignment can prevent bacterial transfer. Similar observations have been made in phytoplasma grafting studies, which emphasize the

need for exact vascular contact (Ahrens & Seemuller, 1992; Jarausch et al., 1999; Melnyk & Meyerowitz, 2015).

Another important factor is the unknown bacterial load in the donor tissue. The inoculum used in this study originated from rearing facilities at the University of Göttingen and the Julius Kühn Institute in Dossenheim. Prior to grafting, infected plants were individually tested for ARSEPH in the beet crown. However, it became evident that infection in the root tissue does not necessarily indicate the presence of the bacterium in the leaves. Preliminary testing confirmed the relatively irregular distribution of ARSEPH in leaf tissue and highlighted its uneven presence in above-ground plant parts, a pattern also observed in phytoplasma infections (Bendix & Lewis, 2018).

Such variability likely results in grafted tissues receiving a bacterial load insufficient to establish a successful infection. This situation is further complicated by the passive transport of the bacterium in the phloem stream. In graft-based studies on phytoplasmas, pathogens rely entirely upon phloem flow to reach distal tissues (Carminati et al., 2021). Likewise, ARSEPH moves basipetally through the phloem following vector-mediated transmission by *P. leporinus* (Haider et al., 2024), and source-sink dynamics may divert sap away from the graft site, preventing bacterial colonization. Although we removed all other leaves to encourage nutrient flow through the graft site, misdirection cannot be ruled out.

The survival of the bacterium during and after the grafting process is also a concern. Within the genus *Arsenophonus*, several lineages exhibit extensive genome reduction, resulting in the complete absence or substantial diminution of genes essential for stress responses - such as those involved in DNA repair, osmoregulation, or oxidative stress defense. Therefore, some *Arsenophonus* strains demonstrate pronounced sensitivity to oxidative stress when cultured or maintained at low densities (McCutcheon & Moran, 2011; Pontes et al., 2008; Siozios et al., 2024; Tran-Nguyen et al., 2008). Grafting-related factors such as dehydration or oxidative damage can result in bacterial death before colonization occurs. Moreover, the larger wound created by grafting may provoke a stronger plant immune response compared to the minimal damage inflicted by insect vectors, further inhibiting bacterial establishment (Bendix & Lewis, 2018; Haider et al., 2024).

In addition to these transmission-related challenges, detection of ARSEPH within the plant is itself prone to variability. False-negative results may occur if the bacterial concentration falls below the qPCR detection threshold. To better understand the spatial distribution of ARSEPH, it is essential to consider the transport routes of phloem sap. After vector-mediated transmission by *Pentastiridius leporinus*, ARSEPH is known to move basipetally - from the leaves downward toward the beet root - and is entirely reliant on phloem flow due to its non-motile nature.

Each sugar beet leaf is connected to specific cambial rings in the root via dedicated vascular bundles (Artschwager, 1926; Hoffmann, 2010; Stieber & Beringer, 1984; Zamski & Azenkot, 1981). These leaf traces pass through the hypocotyl region, linking the leaf vasculature with corresponding vascular bundles in the root (Milford & Thorne, 1973). During this process, vascular bundles from different leaves intersect, forming a highly complex and interconnected network. Because each leaf is associated with a

specific cambial ring that extends through the hypocotyl, the crown region of the beet exhibits a high density of conducting vessels. This anatomical feature increases the likelihood that the sampled cambial tissue contains a direct connection to the vascular bundles of the grafted leaf. Conversely, this complexity also illustrates how unpredictable the detection of phloem-restricted bacteria can be, particularly if the storage parenchyma associated with the cambium has not been successfully colonized, thereby further diluting the detection of ARSEPH.

5.2 Experimental Transmission Trial *via Cuscuta campestris*

This study provides novel insights into the transmission biology of ARSEPH and clarifies the role of the parasitic plant dodder (*C. campestris*) in its potential function as transmission tool for this phytopathogen. Our data demonstrate that *C. campestris* can acquire ARSEPH from infected donor plants, as shown by consistently high bacterial titers in attached dodder. However, no transmission to recipient plants was observed across multiple sampling points in two susceptible sugar beet genotypes. These results indicate that *C. campestris* does not act as a competent vector for this phloem-limited bacterium.

This distinction between acquisition and transmission underscores the complexity of pathogen movement through parasitic interfaces. While *Cuscuta* species are known for their ability to form haustorial connections that facilitate the bidirectional flow of water, nutrients, RNAs, proteins, and even microbes (Anderson et al., 2021; Dawson et al., 1994; Lachner et al., 2020; Liu et al., 2020; Radouane et al., 2024), successful pathogen transfer to a new host requires several additional steps. These include pathogen survival within the parasitic tissue, replication, and subsequent movement into the phloem of the recipient plant (Savov et al., 2024).

Previous studies have demonstrated that parasitic plants such as *Cuscuta* spp. (dodder) can serve as conduits for the horizontal transmission of various plant viruses and phytoplasmas. For example, *C. campestris* has been associated with the transmission of *Beet curly top virus* and *Tomato spotted wilt virus* (Savov et al., 2024; Teofanova et al., 2022), and several phytoplasmas have been successfully transmitted through dodder haustoria (Marcone et al., 1999; Přibylková & Špak, 2013; Schneider et al., 2014). However, this capacity appears to be highly pathogen-specific. In the case of ARSEPH, transmission *via* dodder has not been reported. This likely reflects its highly specialized lifestyle and strict reliance on its insect vector, the planthopper *P. leporinus* (Bressan et al., 2009; Gatineau et al., 2002). The life cycle of phloem-limited pathogens (e.g. 'Ca. Phytoplasma', 'Ca. Liberibacter') depend on specific interactions within their hemipteran insect vectors that enable acquisition, passage through the gut and salivary glands, and eventual transmission into plant phloem tissues (Gross et al., 2022; Haider et al., 2024)

Such conditions are not present in *Cuscuta* spp., as this plant lacks the anatomical and biochemical properties necessary for the development and transmission of this bacterium. Even though *Cuscuta* spp. establishes haustorial connections with its hosts, this does not guarantee the efficient transmission of

all endophytic organisms. Studies showed that the overlap of endophytic fungal communities between *C. reflexa* and its host plants ranged only from 25–37% (Suryanarayanan et al., 2000), and *C. campestris* altered the endophytic microbiome of *Helianthus annuus* without substantial microbial overlap (Avila et al., 2020). While these findings suggest that microbial exchange between *Cuscuta* spp. and its hosts is limited, another dimension of host-microbe interaction is exemplified by Vogel et al. (2025), who highlighted the genomic adaptability of ARSEPH to both its insect vector *P. leporinus* and various host plants. They describe numerous effector proteins encoded in the ARSEPH genome and plasmids. These are highly specific and presumably helpful in establishing themselves in the host plant, but presumably not in non-host plants such as *Cuscuta*. Our findings confirm that while ARSEPH can be acquired by *Cuscuta* spp., its viability post-acquisition remains uncertain, as no transmission to recipient plants was observed. This suggests an incompatibility, potentially due to inactivation by the parasite's antimicrobial compounds, as *Cuscuta* spp. are known to be rich in bioactive metabolites with strong *in vitro* antibacterial activity (Ahmad et al., 2017). Beyond microbial viability, this raises broader questions about the directional selectivity and regulatory control of molecular exchange between *Cuscuta* spp. and its hosts. Although bidirectional biomolecule transport has been established, the underlying mechanisms remain elusive (Balios et al., 2024).

Moreover, our results suggest that ARSEPH does not replicate efficiently or at all within *C. campestris* tissues, as indicated by the decline in bacterial titers over time. This finding is supported by earlier work showing that even when *Cuscuta* spp. acquires pathogens, further transmission depends on the pathogen's compatibility with the parasite's internal physiology (Gal-On et al., 2009; Savov et al., 2024). A similar pattern has been documented in phytoplasma transmission, where the efficiency varies significantly depending on both the pathogen and the *Cuscuta* species involved (Marcone et al., 1999; Savov et al., 2024).

Finally, these findings emphasize that while *C. campestris* can function as a conduit for pathogen acquisition, it does not support the transmission of this phloem-limited bacterium. The transmission of ARSEPH appears to be tightly restricted to its insect vector, reflecting a high level of specialization. This highlights the importance of pathogen–vector compatibility in disease ecology and points to the need for further research into the molecular barriers that prevent transmission through alternative biological pathways.

5.3 Vector Transmission Trial

Unlike the low transmission efficiency observed in grafting and *Cuscuta*-mediated experiments, vector transmission by *Pentastiridius leporinus* resulted in 100% infection after just 25 days. This underscores the high efficiency of *P. leporinus* as vector of ARSEPH. The bacterium is well adapted to its insect host, establishing persistent infections in the salivary glands and reproductive organs, enabling both horizontal and vertical transmission (Bressan et al., 2009; Gatineau et al., 2002; Haider et al., 2024; Vogel et al., 2025).

Although ARSEPH is classified as a plant pathogen, it like other Arsenophonus-like bacteria exhibits several traits typical of insect symbionts, like tissue specificity for reproductive organs, high prevalence, vertical transmission, absence of harmful effects on the host, and a life cycle tightly integrated with the host organism (Bressan et al., 2009; Gross et al., 2022). These characteristics likely contribute to the highly efficient transmission observed *via* its insect vector, *P. leporinus*, in our study. In contrast, alternative transmission routes, such as grafting or *via Cuscuta*, proved unsuccessful. This failure likely reflects the absence of key molecular and physiological cues required to initiate and sustain colonization through these artificial interfaces, mechanisms that are inherently present in the natural, vector-mediated transmission pathways (Chiel et al., 2009; Chrostek et al., 2017). Thus, while ARSEPH successfully colonizes a broad range of crop species in the field, such colonization appears to depend on the specific context provided by its insect vector.

Overall, our results underscore the indispensable role of *P. leporinus* in the epidemiology of ARSEPH and emphasize the significance of vector specificity in pathogen life cycles. Understanding these dynamics is essential for accurate risk modeling and targeted control strategies in affected cropping systems.

6. Conclusion & Outlook

This study provides the first systematic evaluation of non-vector transmission routes for ‘*Candidatus Arsenophonus phytopathogenicus*’ (ARSEPH) in sugar beet via chip grafting and the parasitic dodder *Cuscuta campestris*. Chip grafting enabled limited ARSEPH transmission under controlled conditions, confirming basic feasibility. In contrast, no evidence for transmission via *C. campestris* was found, despite successful bacterial acquisition by the parasite. Compared to these artificial approaches, vector-mediated transmission by *Pentastiridius leporinus* was highly efficient and remains the most reliable method for systemic infection. These results highlight the current limitations of non-vector transmission systems for ARSEPH and underline the need for methodological refinement before broader application in resistance screening.

Further work should focus on improving chip grafting efficiency through standardized inoculum quality, optimized tissue selection, and refined grafting protocols. Clarifying the phloem distribution dynamics of ARSEPH in donor plants and the conditions required for successful colonization will support the development of reliable, vector-independent inoculation methods for resistance screening in sugar beet.

Declarations

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

Britta Kais and Jürgen Gross conceived the study and developed the experimental design. Britta Kais and Jasminka Köhler conducted the experiments and collected the data. Karthi Balakrishnan and Michael Rostás contributed to the experimental design and interpretation of the results. Bruna Czarnobai de Jorge performed the data analysis, and Britta Kais wrote the first draft of the manuscript. All authors contributed to the revision of the manuscript, read, and approved the final version.

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding authors upon reasonable request.

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Figures



Figure 1

Chip grafting procedure for *Candidatus Arsenophonus phytopathogenicus* (ARSEPH) transmission in sugar beet. (A) An incision is made in the stem of the recipient plant. (B) A 1 cm segment of infected petiole is prepared and inserted into the incision. (C) The grafted site is secured using buddy tape. Grafting was performed on five leaves near the center of the rosette or crown. All non-grafted leaves were removed to maintain phloem sap flow through the graft sites.

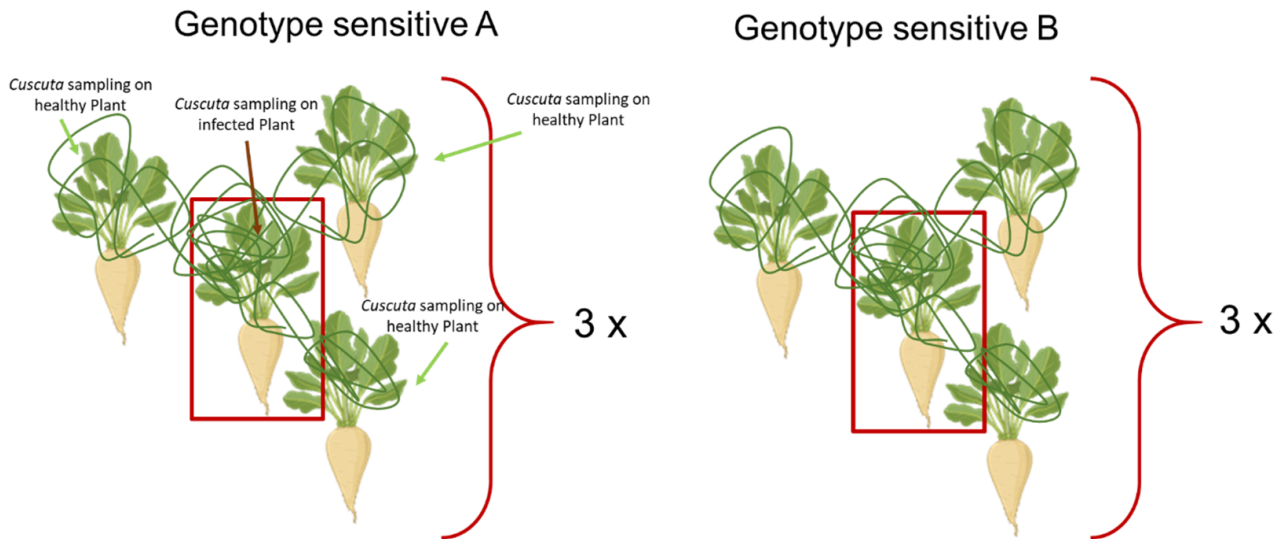


Figure 2

Experimental design. Transmission of '*Candidatus Arsenophonus phytopathogenicus*' by dodder (*Cuscuta campestris*) from an infected (red square) sugar beet plant (*Beta vulgaris*) to healthy sugar beet plants of genotype A (left) and genotype B (right), $n=3$. Created with BioRender.com.

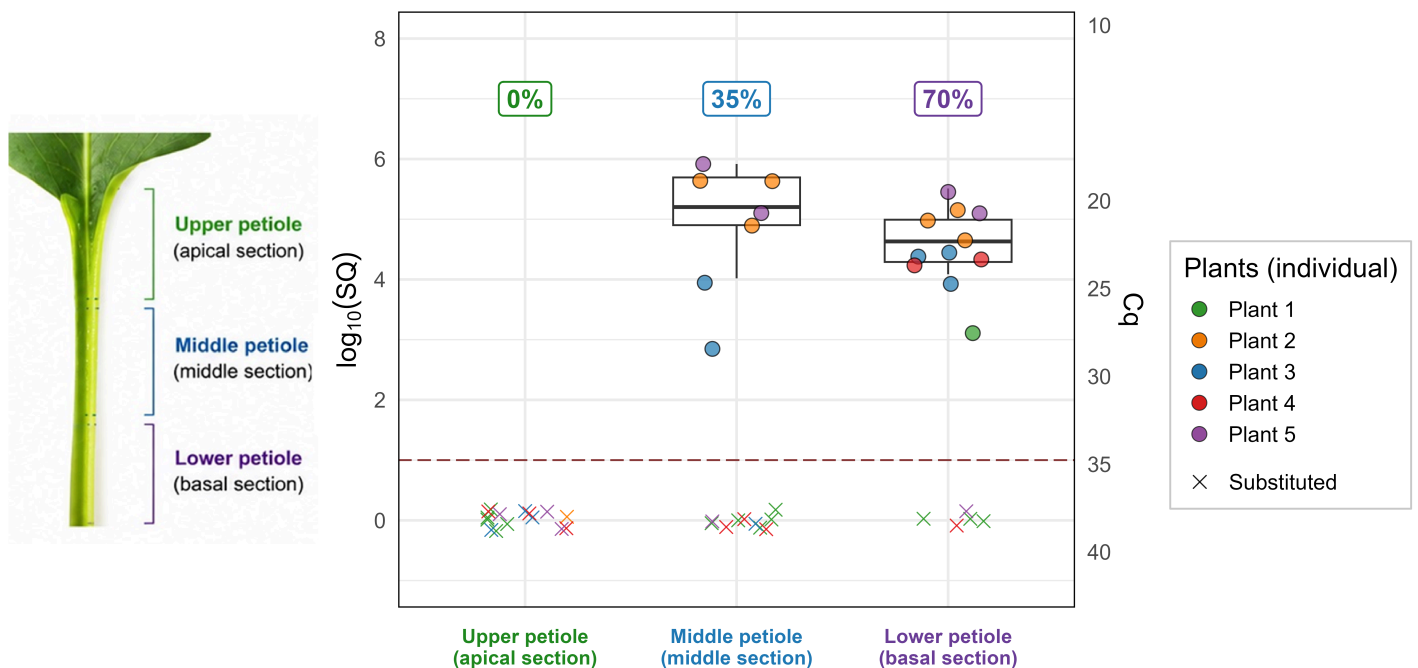


Figure 3

Boxplots display ARSEPH load ($\log_{10}(\text{SQ})$) across three petiole segments (upper, middle, lower) in five plants (colored dots) under different substitution levels (0%, 35%, 70%). qPCR results are shown as log-transformed SQ values with corresponding Cq thresholds. Non-detectable samples are represented by

red "x" symbols and were assigned a value of 1 for visualization purposes. The dashed line indicates the detection threshold ($\text{Log}_{10}(\text{Sq})$ corresponding to $\text{Cq} = 35$).

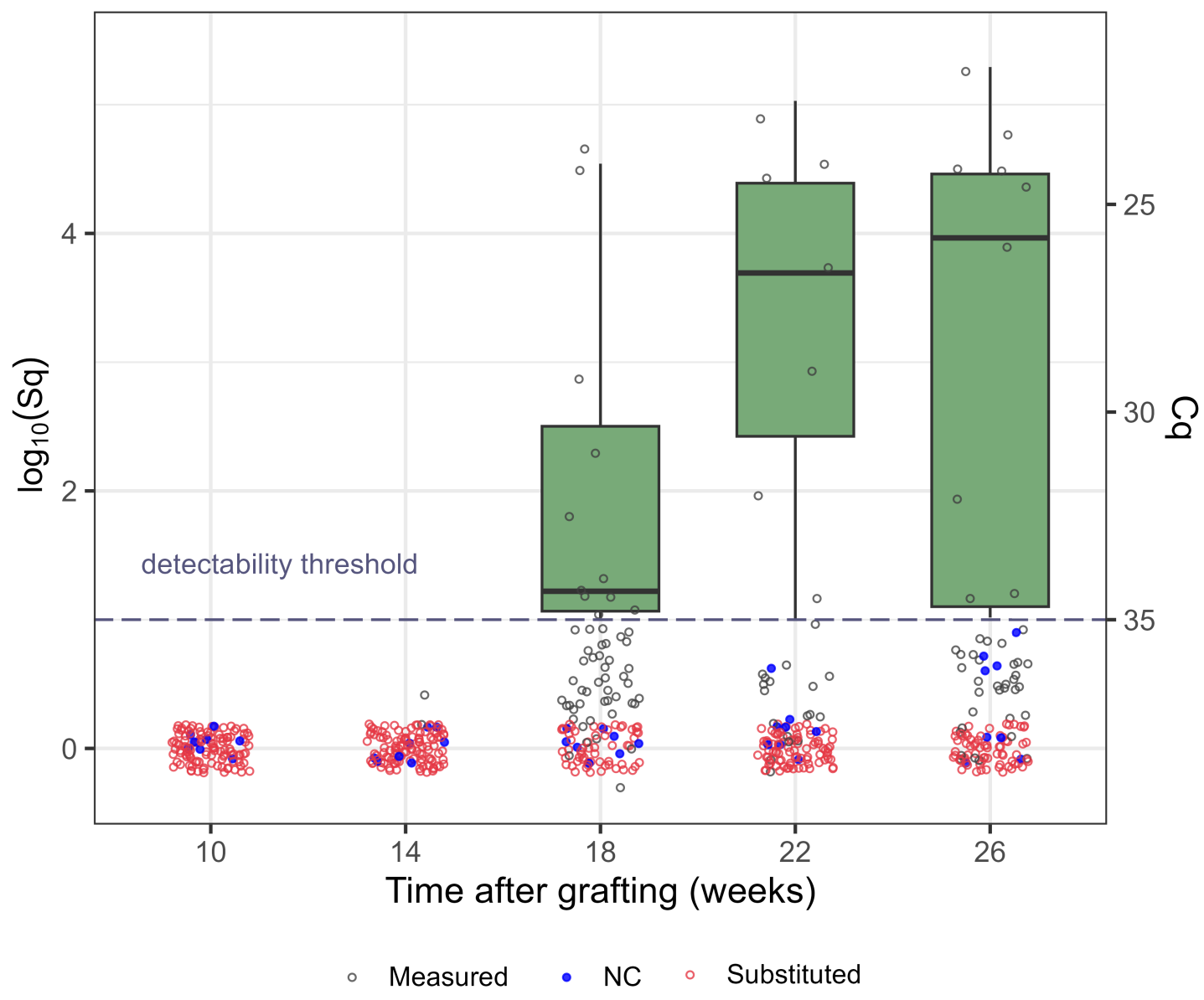


Figure 4

Temporal dynamics of ARSEPH accumulation in grafted plants. Boxplots display $\log_{10}$ -transformed ARSEPH quantities (Sq, left y-axis) in plants over time (x-axis, weeks after grafting). Individual data points above the detectability threshold ($\text{Cq} \leq 33$) are shown in black; negative controls (NC) are shown in blue, non-detectable samples are represented by red "o" symbols and were assigned a value of 1 for visualization purposes. The horizontal dashed line indicates the detection threshold ($\text{Log}_{10}(\text{Sq})$ corresponding to $\text{Cq} = 35$). The right y-axis (in reverse order) shows the corresponding Cq values for reference.

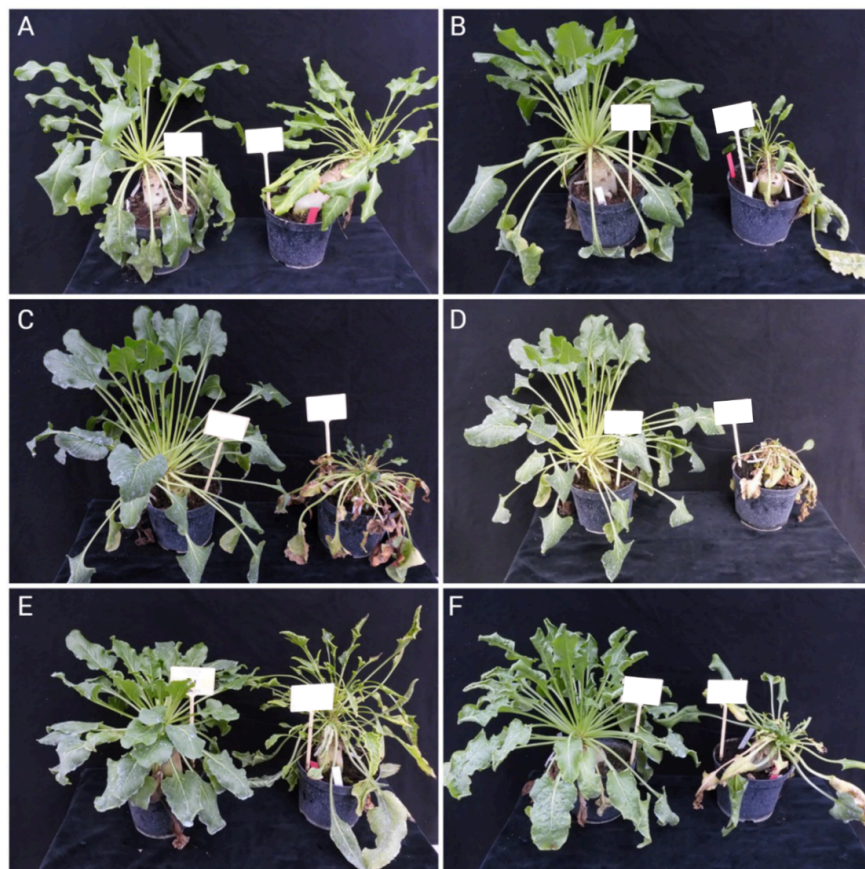


Figure 5

Healthy and SBR-affected sugar beet plants nine months after grafting with ARSEPH-infected tissue. Each panel shows a healthy control plant (left) and a symptomatic grafted plant (right) for five different cultivars: (A) 1T; (B) 1S; (C) 2T; (D) 3T; (E) 4S. The numbers indicate the breeding company, with T = tolerant and S = sensitive genotype. Infected plants exhibit typical SBR symptoms, including yellowing and bending of older leaves, regrowth of chlorotic, lanceolate, and asymmetric central leaves, as well as root deformation and reduced sugar content in the taproots. (Photos: Jasminka Köhler)

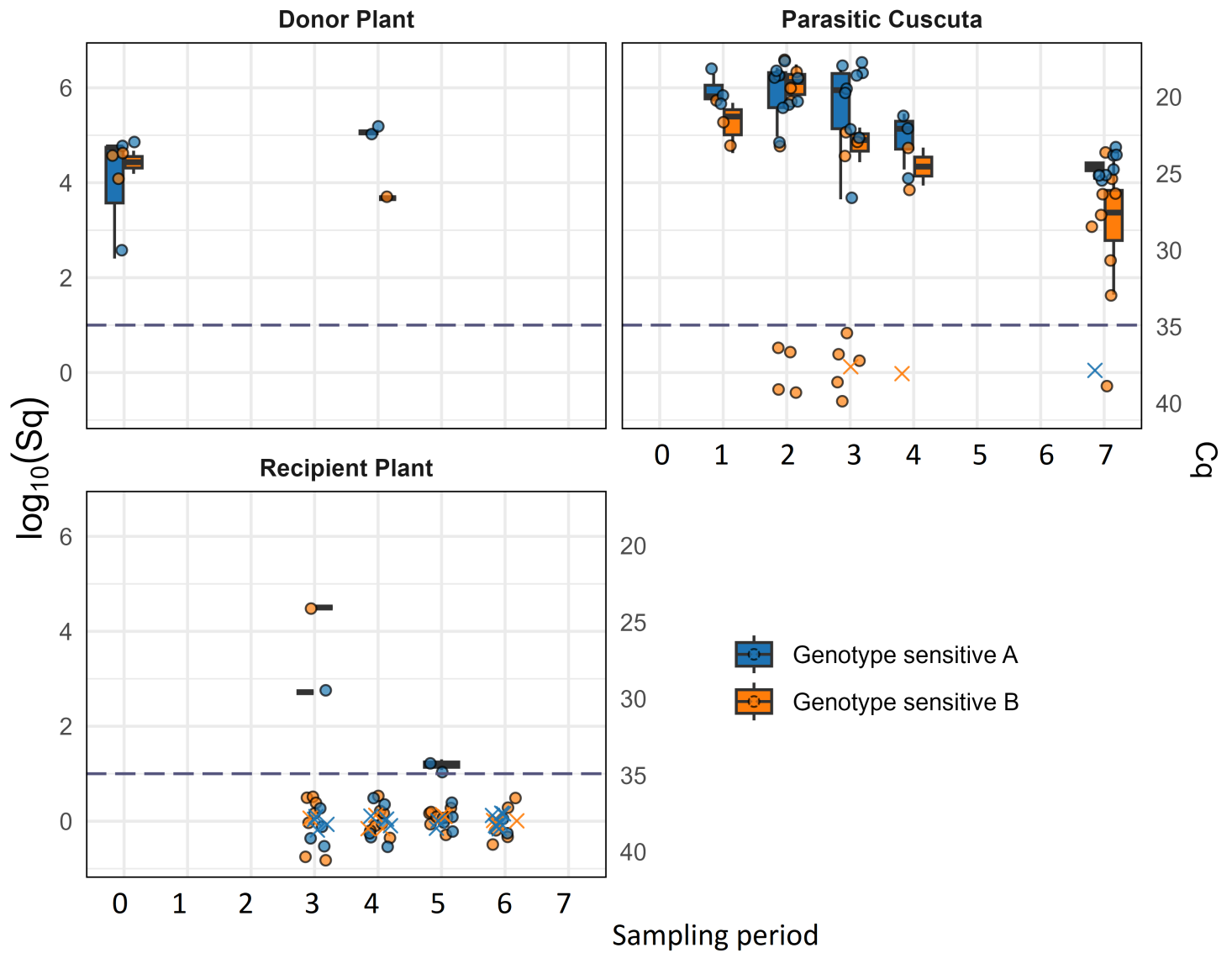


Figure 6

Temporal dynamics of ARSEPH accumulation in the host-parasite system. Boxplots display $\log_{10}$ -transformed ARSEPH quantities (Sq, left y-axis) in plants over time (x-axis, sampling period). Individual data points above the detectability threshold ($C_q \leq 33$) are shown in circles, non-detectable samples are represented by "x" symbols and were assigned a value of 1 for visualization purposes. The horizontal dashed line indicates the detection threshold ($\log_{10}(\text{Sq})$ corresponding to $C_q = 35$). The right y-axis (in reverse order) shows the corresponding C_q values for reference.