Morphological and physiological adaptations in the vascular system of infected fruit trees provide tolerance against phytoplasma diseases

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Abstract

The host-pathogen combinations - Malus domestica (apple)/´Candidatus Phytoplasma mali´, Prunus persica (peach)/'Ca. P. prunorum´ and Pyrus communis (pear)/'Ca. P. pyri´ show different course of diseases although the phytoplasma strains belong to the same 16SrX group. While infected apple trees can survive for decades, peach and pear trees die within some weeks to few years period. So far, in these phytoplasma-plant interactions neither morphological nor physiological differences have been studied in the hosts. Phytoplasma-induced morphological changes of the vascular system as well as physiological changes of the phloem sap and leaf phytohormones were analysed and compared with non-infected plants. Unlike peach and pear, infected apple trees showed substantial reductions in leaf and vascular morphology, causing negative effects on phloem mass flow. In contrast, in pear mass flow and physicochemical characteristics of phloem sap increased. No changes in phytohormone levels were detected in pear but in apple and peach trees, where defence- and stress-related phytohormones increased. If compared with peach and pear trees, the data from apple suggest that the long-lasting morphological adaptations in the vascular system, which likely cause reduced sap flow, triggers the ability of apple trees to survive phytoplasma infection. Some phytohormone-mediated defences might support the tolerance.

Keywords

Callose, '*Candidatus* Phytoplasma mali', '*Candidatus*Phytoplasma prunorum', '*Candidatus* Phytoplasma pyri', mass flow, phloem, phytohormones, plant-pathogen interaction

Introduction

Phytoplasmas are very small bacteria lacking a cell wall but causing severe plant diseases to a number of important agricultural crops. They cause a bundle of symptoms in their respective host plants. '*Candidatus* Phytoplasma' species of the group 16SrX include three economically important disorders of temperate fruit trees: The diseases apple proliferation (AP), pear decline (PD) and European stone fruit yellows (ESFY), which are of high economic significance, causing crop losses just in Europe of around half a billion Euro a year (Eurostat 2009; Strauss, 2009). Like the host plants of the 16SrX phytoplasmas that all belong to the Rosaceae, the causing agents of these diseases '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma pyri' and '*Candidatus* Phytoplasma prunorum', are phylogenetically closely related and believed to be indigenous to Europe (Jarausch et al. , 2019a; Seemüller & Schneider, 2004). These phytoplasmas have small linear chromosomes and lack many genes encoding important metabolic functions, such as amino and fatty acid

synthesis (Kube et al., 2008; Oshima et al. , 2013). Therefore, they need to consume essential metabolites from their plant hosts.

Phytoplasmas are restricted to the phloem sieve elements in their host plants (Seemüller, 2002; Zimmermann et al., 2015). The phloem serves as main route for the long and short-distance transport of mainly organic compounds (Hafke et al., 2005; van Bel, 1996). Sieve elements (SEs), companion cells (CCs) and phloem parenchyma cells (PPCs) are the three phloem cell types involved also in transport of defence- and stress related signalling molecules, such as RNA, proteins, and phytohormones (e.g. Dempsey & Klessig, 2012; Furch et al., 2014; Jung et al., 2009; Park et al., 2007). The sieve element sap is an energy-rich environment, sustaining phytoplasmas with nutrients and enabling them to distribute all over the plant. Therefore, an impairment of the phloem cells and a change in the phloem sap composition is most likely.

The distribution of secondary compounds plays a crucial role in plant communication and the induction of defence mechanisms against invading pathogens and attacking herbivores. It was previously shown that phytoplasmas produce and secrete effector proteins into phloem cells that induce physiological changes in infected host plants (Sugio et al. , 2011a). A number of non-specific symptoms, such as chlorosis, leaf yellowing, premature reddening, swollen leaf-veins, leaf curl and reduced vigor might be attributed to the impairment of the vascular system and the photosynthesis apparatus (Bertamini et al., 2002; Bertamini et al. , 2004; Maust et al., 2003). Additionally, abnormal growth, stunting, growth of witches' brooms, reduced root size and dwarf fruits occur in phytoplasma infected plants indicating a disturbed hormone balance (Dermastia, 2019). Phytohormones are induced in reaction to abiotic and biotic stresses and lead to the induction of defense responses (Walling, 2000). The influence of phytoplasma infections on salicylic acid, jasmonates, auxins, abscisic acid, ethylene and cytokinine biosynthesis and pathways was recently reviewed by Dermastia (2019), illustrating the diverse and complex interactions between the specialized pathogens and their host plants.

In the case of phytoplasmas, we have to take into consideration the impact on vector insects that are crucial for the distribution of phytoplasmas. So far, all phytoplasmas of the group 16SrX causing important fruit crop diseases are vectored by jumping plant lice (Hemiptera: Psylloidea) or succinctly psyllids (Jarausch et al., 2019b). Psyllids are phloem feeders and both nymphs and adults feed on plant phloem and occasionally on xylem sap, too (Gallinger & Gross, 2018, 2020; Weintraub & Beanland, 2006). Therefore, morphological changes of the plant vascular system may affect psyllid feeding behaviour and suitability of host plants. Additionally, phloem/xylem components may influence host choice and oviposition behaviour of psyllids (Gallinger & Gross, 2018, 2020; Mayer et al., 2011). In addition, to detect appropriate host plants for feeding and reproduction, volatile signals are used by many vectoring psyllids species during migration (Gallinger et al., 2019, 2020; Gross & Mekonen, 2005; Mayer et al. , 2008a,b, 2009; Soroker et al. , 2005; Weintraub & Gross, 2013). As often plant volatile emission is regulated by phytohormones their changes in concentrations play an important role on the interplay of vector insects, plants and phytoplasmas (Gross, 2016).

Intra- and interspecific differences in the response of fruit trees to phytoplasma diseases have been observed over the last decades under both experimental and natural infection conditions (Fiore et al., 2019; Marcone & Rao, 2019). However, only few studies provide firm data on host response, host–pathogen interaction and on anatomical, physiological and molecular basis of resistance (Seemuller & Harries, 2010), which is still poorly understood (Marcone & Rao, 2019).

In the present study, we explored how infections with specific fruit tree phytoplasmas ('*Ca* .P. mali', '*Ca* . P. pyri' and '*Ca* . P. prunorum') belonging to the 16SrX group (Seemuller & Schneider, 2004), changed important morphological and physiological parameters of their respective host plants belonging to the same plant family, the Rosaceae (Potter et al., 2007). We measured typical parameters such as leaf morphology, plant vascular morphology and callose deposition, determined physical phloem parameters (mass flow velocity and volumetric flow rate, relative density and dynamic viscosity), and analysed the content of several phytohormones in leaf tissues of healthy and phytoplasma-infected plants. The importance of measured parameters for symptom manifestation as well as the impact on vector insects and phytoplasma spread is

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discussed.

Materials and methods

Plant material and phytoplasma inoculation

Apple trees (*Malus domestica*) cv. 'Gala Royal' were grown on clonal rootstock cv. 'M9' (non-infected control, n=14), pear trees (*Pyrus communis* L.) cv. 'Williams Christ' were grown on cv. 'Kirchensaller Mostbirne' rootstocks (non-infected control, n=5), and peach trees (*Prunus persica* (L.) cv. 'South Haven' were grown on peach seedlings cv. 'Montclar' (non-infected control, n=4). Plants were inoculated by grafting of two buds from trees infected with the respective phytoplasmas. Apple trees were infected with a virulent accession (3/6, n=13) in 2017 (Seemuller et al., 2010, 2011, 2013). Pear trees were infected with '*Ca.* P. pyri' (PD-W, n=5) in 2012. Peach trees were infected with '*Ca.* P. prunorum' (ESFYQ06, n=4) in 2017. Experiments with pear and peach trees were conducted in 2018. Apple trees were investigated in 2019. All plants were grown under natural conditions in an insect safe environment.

DNA extraction of phytoplasmas

DNA from leaves and phloem scrabbings was isolated using a CTAB (cetyltrimethylammonium bromide-extraction method modified from Doyle & Doyle (1990). Due to irregular distribution of '*Ca* . P. pyri' in the top (Seemuller et al. , 1984), in part infection status of pear trees was confirmed by extraction of phloem scrabbings from shoots of PD inoculated trees, in addition to extraction of leaf tissue from mass flow measurements. Leaves and phloem scrabbings were ground in preheated extraction buffer (60 degC, 2.5% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0, 1% (w/v) polyvinylpyrrolidone 40, 0.2% (v/v) 2-mercaptoethanol (with a tissue/buffer ratio 1:10; 0.1 g of tissue in 1 ml buffer) using a homogenizer (BIOREBA AG, Reinach, Switzerland) in extraction bags (BIOREBA AG). Homogenate (1 ml) was transferred into a microcentrifuge tube and incubated at 60 degC for 30 min. An equal volume of chloroform was added, the tube was briefly vortexed and shook for 5 min at room temperature. After a centrifugation step (10000 *g* , 6 min at room temperature, Heraeus Fresco 17 Microcentrifuge, Thermo Fisher Scientific, Dreieich, Germany) the aqueous phase was transferred into a new centrifuge tube. For precipitation of nucleic acids an equal volume of isopropanol was added, the tube was inverted and incubated at 4 °C overnight. Precipitate was recovered by centrifugation at 10000 *g* for 10 min at room temperature. Supernatant was discarded and the nucleic acid pellet was washed with 70% ethanol (centrifugation step at room temperature, 10000 *g* , 10 min), air dried and resuspended in 50 µl high-performance liquid chromatography (HPLC) water (VWR International GmbH, Bruchsal, Germany). Unless explicitly stated elsewhere, laboratory chemicals were purchased from Carl Roth GmbH (Karlsruhe), Bernd Kraft GmbH (Duisburg) and Sigma-Aldrich Chemie GmbH (Taufkirchen), Germany, respectively.

Real-time PCR

Quantitative PCR (qPCR) was performed with the Bio-Rad CFX96 Thermal Cycler (Bio-Rad Laboratories GmbH, Munich, Germany) using primer pair and probe of a TaqMan assay developed by Christensen et al. (2004) for the generic detection of phytoplasmas. The amplification of a part of the 16S rDNA gene was performed in 25 µl reactions containing 1 µl of DNA extraction, 0.625 U of FastGene Taq DNA Polymerase (Nippon Genetics Europe GmbH, Düren, Germany) with provided 10 x reaction buffer A (with 1.5 mM $MgCl_2$), 0.5 µl of dNTPs (10 mM each, Steinbrenner Laborsysteme GmbH, Wiesenbach, Germany), 1 µl of each primer (10 µM, Eurofins Genomics Germany GmbH, Ebersberg, Germany), 0.5 µl of TaqMan probe (10 µM, Eurofins Genomics Germany GmbH), and HPLC water (VWR International GmbH). Amplification parameters were 15 min at 95 °C followed by 46 cycles at 95 °C for 15 s and 60 °C for 1 min. Data analysis was performed with the BioRad CFX Manager 3.0 software (Bio-Rad Laboratories GmbH, Munich, Germany).

Calculation of phytoplasma titer

Phytoplasma concentration (number of copies of phytoplasma 16S rDNA gene per µl) was calculated automatically from the quantification cycle (C_q) values by the use of a cloned 16S rDNA gene standard ranging

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from 10^1 to 10^9copies in qPCR with the internal manufacturer's software (referred to as estimated qPCR concentration). Samples with C_q values higher than 30 were considered as tested negative (see Table S1; value of control DNA from healthy trees maintained under insect-proof conditions). Calculation of phytoplasma cells per gram wet weight of extracted leaf tissue and phloem scrabbings was performed by multiplying the assessed qPCR concentration by the applied volume of extraction buffer (Y) and the volume of HPLC water used for DNA resuspension, by dividing by the number of 16S rDNA operons (2) and the wet weight of extracted leaf tissue (X):

$$\text{Phytoplasma titer } \left[\frac{\text{cells}}{g}\right] = qPCR \text{ concentration } \left[\frac{\varsigma o \pi \iota \varepsilon \varsigma}{\mu\lambda}\right] * (Y * 50 * \frac{1}{2} * \frac{1}{X}) \left[\frac{\mu\lambda}{}\right.$$

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Experimental set-ups

From each tree, two (pear and peach) or four (apple) mature leaves were selected. The mass flow measurements were done *in vivo* . Thereafter the leaves were cropped, length and width of the leaf lamina were measured, cross sections were done to analyse the mass flow rate, the middle part of the midrib was fixed in a fixative and the rest of each leaf was used for phytoplasma titer determination. Leaves for phytohormone measurements were separately collected, immediately frozen in liquid nitrogen and stored at -20 °C until used.

Determination of symptoms and leaf morphology

Next to the fundamental observation and assessment of the known symptoms following a phytoplasma infection the impact on the morphology of apple, pear and peach leaves was investigated with the determination of the length and maximum width of the leaf lamina. Photographs (Canon EOS 760D, Canon Deutschland GmbH, Krefeld, Germany) were taken to visualize characteristic symptoms at whole plant and leaf level.

Microscopic analyses of theplant vascular morphology and callose deposition

Cross sections of the midribs were done in the middle (halfway from the base to the tip) of each leaf. Therefore, pieces of about 1 x 1 cm were fixed in 2.5% (w/v) glutaraldehyde, 2% (v/v) paraformaldehyde in 0.1 M sodium-potassium phosphate buffer (pH 7.4, Merck KGaA, Darmstadt, Germany). Sections were cut at a thickness of 20 µm with a cryostat (Leica JUNG CM3000, Leica Microsystems, Wetzlar, Germany) at a chamber temperature of -26 °C and a specimen head temperature of -23 °C. Pieces were bound to a specimen disc by embedding them in plant tissue freezing medium (Jung, Leica Microsystems, Wetzlar, Germany) and frozen at the quick freeze shelf for 10 min prior sectioning. Each cross section was stained for at least 30 min with 0.1% aniline blue solution (Sigma Aldrich, St. Louis, Missouri, USA) to visualize callose deposition at sieve plates.

Each cross section was imaged using an AXIO Imager.M2 (Zeiss Microscopy GmbH, Jena, Germany) equipped with a 10x objective (N-Achroplan 10x/0.3) and a 40x objective (W N-Achroplan 40x/0.75). The bright field and fluorescence images were recorded with a colour camera (AXIOCAM 503 colour Zeiss, Jena, Germany) by use of a DAPI (EM 445/50 nm) filter. Each digital image of infected and healthy cultivars was analysed with the determination of (1) the diameter of midribs, (2) the area of the vascular bundle, (3) the xylem area, (4) the phloem area and (5) the area of 10 sieve elements per section using the ZEN®software (Zeiss, Jena, Germany). The digital images were processed with the ZEN® software and edited with Adobe® PhotoShop to optimize brightness, contrast and colouring. The intensity of aniline blue fluorescence was measured using the ZEN® software by analysing the whole phloem area as region of interest (ROI) and ROIs of healthy and infected plants were comparatively evaluated.

Determination of the phloem mass flow velocity

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The phloem mass flow rate was measured with the phloem mobile fluorochrome 5,6-carboxyfluorescein diacetate (CFDA) dye (ThermoFisher Scientific, Waltham, Massachusetts, USA). CFDA permeates the plasma membrane in the non-fluorescent acetate form and is cleaved by cytosolic enzymes producing membrane impermeant fluorescent carboxyfluorescein (CF) (handbook from Molecular Probes, Eugene, OR, USA). CF is trapped inside SEs transported by mass flow in the sieve tubes. A stock solution was prepared by solubilisation of 1 mg CFDA in 1 ml DMSO. A working solution of 1 µl stock solution in 1 ml buffer solution (containing 2 mol m^{-3} KCl, 1 mol $m^{-3}CaCl_2$, 1 mol $m^{-3}MgCl_2$, 50 mol m^{-3} mannitol, and 2.5 mol m^{-3} MES/NaOH buffer, pH 5.7) was applied at a cut leaf tip. After an inoculation period of 1 to 2 h at room temperature each leaf was removed from the plant. Immediately cross sections of the mid ribs were made by hand with a sharp and fresh razor blade in one centimeter intervals from the basal side of the leaf. Sections were covered with distilled H_2O, a cover glass and examined for appearance of fluorescence emitted from CF (emission 510-580 nm) with an inverted fluorescence microscope (AxioVert S100, Carl Zeiss, Jena, Germany). The transport velocity was calculated by dividing the measured distance the CF moved in the sieve elements from the application side towards the leaf base with the exact inoculation time (from dipping one leaf tip into CFDA to removing of the specific leaf from the plant).

Calculation of the volumetric flow rate

The volumetric flow rate (J_V) was calculated by multiplying the measured phloem mass flow velocity (V_a) with the median area of ten measured sieve elements ($\tilde{A}_{SE}$):

$$J_v\ [\frac{cm^3}{h}] = V_a\ [\frac{\mathrm{cm}}{h}] *\ \tilde{A}_{\mathrm{SE}}\ [cm^2]$$

Determination of phytohormones

From each tree four leaves were harvested and immediately frozen in liquid nitrogen and stored at -20 °C. The leaves of each tree were pooled and 250 mg (per sample and two samples for each tree) were homogenized using a Geno/Grinder® (Spex SamplePrep, Stanmore, UK) at 1100 rpm for 1 min and extracted in 1.5 ml methanol containing 60 ng D4-SA (Santa Cruz Biotechnology, USA), 60 ng D6-JA (HPC Standards GmbH, Germany), 60 ng D6-ABA (Santa Cruz Biotechnology, USA), 12 ng D6-JA-Ile (HPC Standards GmbH), and D5-indolacetic acid (D5-IAA, OlChemIm s.r.o., Olomouc, Czech Republic) as internal standards. Samples were agitated on a horizontal shaker at room temperature for 10 min. The homogenate was mixed for 30 min and centrifuged at 13,000 rpm for 20 min at 4 °C and the supernatant was collected. The homogenate was re-extracted with 500µl methanol, mixed and centrifuged and the supernatants were pooled. The combined extracts were evaporated under reduced pressure at 30 °C and dissolved in 500µl methanol.

Phytohormone analysis was performed by LC-MS/MS as in Heyer et al. (2018) on an Agilent 1260 series HPLC system (Agilent Technologies) with the modification that a tandem mass spectrometer QTRAP 6500 (SCIEX, Darmstadt, Germany) was used. Details of the instrument parameters and response factors for quantification can be found in Table S2.

Indolacetic acid was quantified using the same LC-MS/MS system with the same chromatographic conditions but using positive mode ionization with an ion spray voltage at 5500 eV. Multiple reaction monitoring (MRM) was used to monitor analyte parent ion - product ion fragmentations as follows: m/z 176 -130 (collision energy (CE) 19 V; declustering potential (DP) 31 V) for indolacetic acid (IAA); m/z 181 -133 + m/z 181 -134 + m/z 181 -135 (CE 19 V; DP 31 V) for D5-indolacetic acid.

Collection of phloem sap

Phloem saps were sampled applying centrifugation technique according to Hijaz and Killiny (2014). Briefly, the bark from young flush of *M. domestica* , *P. communis* and *P. persica* trees was manually removed with a clean scalpel and sliced into 2 cm pieces. After removing the bottom of a 0.5 ml Eppendorf tube, the tube was immersed in a second, larger tube (1.5 ml). Bark pieces were placed into the 0.5 ml tubes and

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centrifuged at 12.000 rpm at 4 °C for 10 min. The extracted phloem sap was collected and the refractive index, the density and the viscosity was determined.

Determination of the refractive index

The refractive index of the phloem sap was determined with a handheld refractometer (type 45-81; Bellingham + Stanley Ltd., Tunbridge Wells, UK) and specified as °Brix. The refractometer was standardized for sucrose.

Δετερμινατιον οφ τηε δενσιτψ οφ ασϛυλαρ σαπϛ (ρ)

The density of the phloem sap was measured using 0.5 µl glass capillaries (CAMAG®, Muttenz, Switzerland) allowing the distinct determination of bulk and volume. The bulk of empty and filled capillaries was separately measured (Analytical Balance, Sartorius Weighing Technology GmbH, Göttingen, Germany) and subtracted to determine the pure mass of the phloem sap. Simultaneously, the corresponding volume inside the capillaries was calculated and the density was converted to gram per litre (g l^{-1}).

Δετερμινατιον οφ τηε δψναμιϛ ισϛοσιτψ οφ ασϛυλαρ σαπϛ (η)

The measuring instruction of the dynamic viscosity was described in Adam et al. (2009). The dynamic viscosity of the phloem sap was quantified with 0.5 µl glass capillaries (CAMAG®, Muttenz, Switzerland). The calibration required the determination of the specific viscometer constant ($\varkappa$ = mPa l g^{-1}) by using H_2O as reference solution with known dynamic viscosity (η_0 = 1.0087 mPa s) and density (ρ_0 = 997.9 g l^{-1}) for 20 °C. Following the measurement of elapsed time (t_0), the specific viscometer constant was calculated:

$$\kappa \; = \; \frac{\eta_0}{\rho_0 \; t_0} \; (\frac{\text{mPa l}}{g})$$

The dynamic viscosity of the phloem sap was calculated after the measurement of elapsed time (t) and determination of density (ρ):

$\eta = \varkappa * \rho * t$ (mPa s)

Statistics

All statistical analyses were performed using R version 3.5.1 (R Core Team, 2019). The data visualization was done with the package 'ggplot2' (Wickham, 2016).

Mass flow, volumetric flow rate, morphology data and functional/physiological parameters: Linear mixed effect (LMM) or generalized linear mixed models (GLMM) were used to determine the effect of phytoplasma infection on phloem mass flow, volumetric flow rates and morphological and functional parameters (phloem sap viscosity and density) in *M. domestica* , *P. persica* and *P. communis* leaves. To account for non-independent errors, which may occur due to repeated measurements at each tree, trees were specified as a random factor in all models. Models with different error distributions and link-functions were compared by AICc (Akaike information criterion with correction for small sample size) with the *AICctab* function from the 'bbmle' package (Bolker & R Development Core Team, 2017). Models with the lowest AICc values were used if model assumptions were valid. LMMs were fitted with the *lmer* function from the 'lme4' package (Bates et al. , 2015), and Typ III analysis of variance (ANOVA) with Satterthwaite's method, which was calculated with the*anova* function from the 'lmerTest' package (Kuznetsova et al. , 2017). GLMMs were fitted with the *glmer* function from the 'lme4' package, and Typ II analysis of variance was calculated with the *Anova* function from the 'car' package (Fox & Weisberg, 2019) to determine treatment effects. Used error distribution, link-function and ANOVA results were specified in the Tables S3-S5 in the Supporting Information.

Phytohormone data and Brix values: Linear models were fitted to determine the influence of phytoplasma infections on the concentration of phytohormones and the relative density of phloem sap in *M. domestica* , *P. persica* and *P. communis* plants. In case of non-normality of the residuals the data was log, square

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root or box-cox transformed as specified in the Table S6. Variance heterogeneity was detected in abscisic acid content in samples from *P. communis* . In this case the generalized least squares method (GLS) was applied with the *gls* function from the 'nlme' package (Pinheiro et al., 2019). The different variance in the treatments was incorporated into the model with the varIdent variance structure. Treatment effects were calculated by Typ I analysis of variance.

Callose deposition: Linear models were fitted with the GLS method, to model the different variance structures of the data with the varIdent function. Treatment effects were calculated by Typ I analysis of variance and were reported in Table S7.

General procedure: For all models, the estimated marginal means (EMMs) and corresponding 95% confidence intervals were calculated and used to determine differences between treatment levels with the 'emmeans' package (Lenth, 2019). All model assumptions were validated graphically as recommended by (Zuur et al. , 2009).

Results

Phytoplasma infection affects the leaf and vascular morphology.

We first investigated and compared the effects of phytoplasma infection on the leaf and vascular morphology.

The phytoplasma infections resulted in already known different visible disease symptoms: witches' broom and enlarged stipules in apple trees, premature foliar reddening in pear trees and chlorosis and suberization in peach trees (Figure 1). The symptoms indicated impairments in the leaf development that was closer analysed with a comparison of the leaf lamina, midrib sizes and their ratios among infected and healthy plants. It was found that leaves of AP-infected apple trees were significantly ($p<0.05$) smaller (length -17% and width -22%) and the diameter of midribs were significantly reduced (-27%) compared to those of healthy plants (Table 1). The phytoplasma infection in apple trees did not affect the leaf size ratio and the midrib ratio (Figure 1a). In pear, basing upon a significant increase of the leaf width (+8.5%), a significant decrease of the leaf size ratio of nearly 9% was observed, but no changes for the midrib ratio were found (Figure 1b). In contrast to apple and pear plants, phytoplasma infected peach trees exhibited a significant rise of the leaf size ratio of +13% and the midrib ratio of +16% (Figure 1c). No significant changes were found for leaf length, width and midrib diameter (Table 1). All the morphological results demonstrated the heterogeneity of the symptoms and indicated differences in the individual host-pathogen interactions.

The specific impact of the phytoplasma infection on the vascular morphology was investigated by analysing the areas of vascular bundle, xylem, phloem and SEs as well as the ratios of xylem to phloem and SE to phloem (Figures 2-4). For apple, the phytoplasma infection exhibited significantly ($p<0.05$) degraded areas of the vascular bundle (-39.1%), xylem (-49.8%), phloem and SE (-33.7%) in comparison to healthy plants whereas the ratio of SE to the phloem was not affected (Figure 2b). Phytoplasma infected pear trees did not show any changes (Figure 3) whereas in peach trees infected with ESFY, the mean sieve element area (-26%) and the ratio of SE to phloem (-46.9%) decreased significantly (Figure 4b). Confirming the heterogeneity of the morphological results (Figure 1), different disease patterns were also found on the cellular level of the vascular system for apple, pear and peach (Figures 2-4).

The translocation situation and phytohormone distribution are different in the individual host-pathogen-systems.

We examined the consequences of the morphological changes (Figures 2-4) on the physiological situation within the sieve elements.

In apple leaves the phloem mass flow velocity and the calculated volumetric flow rate decreased significantly ($p<0.05$) in infected leaves in comparison to healthy ones of - 25% and -58%, respectively (Figure 5a). In pear leaves the phloem mass flow velocity and the volumetric flow rate increased significantly for +32.6% and +46.6%, respectively (Figure 5b). In peach leaves the phloem mass flow velocity was not affected, but the volumetric flow rate decreased significantly (-30.8%; Figure 5c).

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The variating effects for the phloem mass flow indicated changes in the flow properties of the phloem sap. Thus, the dynamic viscosity, absolute and relative densities of phloem sap obtained by bark tissue centrifugation were measured (Table 2). For apple and peach, no changes were found. In contrast, the dynamic viscosity in infected pear plants was doubled (+104%) and also the relative density increased strongly (+97.7%) supporting any effects for the phloem mass flow (Figure 5b). Unfortunately, the peach plants did not deliver enough volumes of phloem sap for a complete analysis. For this reason, only the relative density could be determined without any significant changes. A comparative analysis of the phloem's -relative density among apple, pear and peach revealed significant differences, illustrating a plant specificity of the phloem sap composition regarding total sugar content. The measured/calculated phloem mass flow parameters showed again heterogeneous effects of a phytoplasma infection (Figure 5, Table 2) and confirmed the variability of previous shown anatomical/morphological results (Figures 1 to 4).

To obtain indications for the changed mass flow translocation of the individual plant-phytoplasma variations the callose deposition in the SEs was visualized and its intensity comparably analysed (Figure 6). No differences of callose depositions were found for the phytoplasma infection, in comparison to healthy apple trees (Figure 6a). In contrast to apple, a rise of callose was found in peach (+300%) and pear (+67%; Figures 6b+c) showing a stronger impact into the anatomical and physiological balance in comparison to apple trees.

The stress situation of the plants was explored with the measurement of salicylic acid (SA), jasmonic acid-isoleucine (JA-Ile), jasmonic acid (JA), abscisic acid (ABA), 12-oxo-phytodienoic acid (cis-OPDA) and indole acetic acid (IAA) (Figures 7 and S1; Table S8) in leaves. In apple, SA (+109%), ABA (+55%) and JA-Ile (+78%) increased significantly ($p<0.05$) whereas a significant decrease of cis-OPDA (-45%) and no changes of JA and IAA were observed following an infection with the virulent accession 3/6 (Figures 7a and S1). No significant changes for the several measured phytohormones could be detected in pear trees (Figure 7b). In peach trees, SA (+192%), JA-Ile (+345%) and IAA (-40%) were significantly affected whereas JA and ABA did not show any significant changes (Figures 7b and S1). Moreover, the fundamental level of SA, ABA and cis-OPDA differed among healthy apple, pear and peach plants. For example, ABA was 6-fold higher in pear and 3-fold higher in peach compared to apple.

In accordance with the morphological and functional results, the effect of phytoplasma infections on the phytohormone contents revealed different patterns among particular host-pathogen combinations.

Discussion

Although the phytoplasmas ´*Candidatus* Phytoplasma mali´, ´*Ca.* P. prunorum´ and ´*Ca.* P. pyri´ belong to the same 16SrX group their pathogenicity is quite different in their respective host plants *Malus domestica* (apple), *Prunus persica*(peach) and *Pyrus communis* (pear). Apple trees can survive a phytoplasma infection for decades whereas phytoplasma-infected peach and pear trees often die after some weeks (quick decline of pear) to a few years (Fiore et al., 2019; Marcone et al., 2010; Marcone & Rao 2019; Seemüller et al., 1986, 2018). This indicates that the *M. domestica* /´*Ca.* Phytoplasma mali´-system is more adapted and balanced in their interaction than *P. persica* /´*Ca.* P. prunorum´ and *P. communis* /´*Ca.* P. pyri´ pointing to a higher tolerance and survival rate for phytoplasma-infected apple. We collected an extensive set of data (summarized in Table 3) to elucidate anatomical and physiological responses of each plant species to a phytoplasma infection, supporting a co-evolutionary impact.

Phytoplasmas affect the vascular morphology of apple trees more than peach and pear trees.

Although apple trees are known to survive for decades with a phytoplasma infection (Seemüller et al., 2018), many significant declines were found on the level of leaf (width, length, midrib), tissue (vascular bundle, phloem and xylem) and cell (sieve elements) sizes (Figures 1 and 2). In contrast, pear and peach trees showed less significant differences between healthy and phytoplasma-infected leaves, if at all, we found significant increases for leaf size and midrib ratio for peach and leaf width for pear (Table 3). That is surprising as one might expect that plants with a higher tolerance and survival rate would show a lower rate of symptoms than plants having demonstrably a higher mortality (Marcone & Rao 2019). Additionally,

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the recovery phenomenon, describing the remission of symptoms and the disappearance of phytoplasmas in the crown, was observed for both apple and apricot trees (*Prunus armeniaca*), but not pear trees (Carraro et al., 2004; Musetti et al., 2013). Hence, the morphological and physiological changes can be considered representing the ability to handle a phytoplasma infection and might be the result of a selective adaptation.

The pear and peach phloem reactions are very sensitive to phytoplasma infections whereas apple has arranged with the infection

All observed results regarding the particular morphological (Figures 1 to 4) and functional measurements (Figure 5) illustrate well the consequences of a phytoplasma infection for a plant: They are heterogeneous and specific and depend on the individual host-pathogen interaction. One reason for the heterogeneity might be found in plant defence responses. A fundamental defence response to several (a)biotic stresses is an elevated Ca^{2+} dependent deposition of callose and was already reported for phytoplasma infections (Chen & Kim, 2009; Musetti et al., 2013). We could show that *P. communis*and *P. persica* trees reacted to phytoplasma infections with blocking of sieve plates with callose. Phytoplasma effectors may cause gating of Ca^{2+} channels leading to sieve-tube occlusion with dramatic effects on photoassimilate distribution as indicated by the reduced volumetric flow rate in *P. persica*trees. Surprisingly, the mass flow of infected *P. communis* trees was increased, by a simultaneous increase of phloem sap viscosity, which reflects an increased sugar content. The reason has to be an increased pressure gradient (˜6.5 bar) of infected trees, which drives the mass flow against the resistance. *P. communis* trees have to bring a major effort with increased energy supply that result at the end in die back. In contrast, the infection with '*Ca* . P. mali' did not lead to an increased callose deposition in apple trees. This might be due to apple cultivar, phytoplasma strain specific mechanisms or an evolutionary adaptation to the phytoplasma infection. The callose deposition in response to phytoplasma infections never results in a restriction of the bacteria and therefore is only a costly non-functional leftover of general defence mechanisms. The apple-phytoplasma interaction is maybe the oldest of our three investigated interactions where both partners coexist without destroying each other. If the callose deposition is directly or indirectly induced by phytoplasmas is an issue for prospective surveys.

Callose deposition is also a defence mechanism against phloem-feeding and is induced by phloem feeding insects (Hao et al. , 2008; Will et al., 2013). Therefore, callose concentrations are of great importance for phloem-feeding vector insects of phytoplasmas. The occlusion of sieve tubes inhibits the phloem flow and the feeding of piercing-sucking insects on the phloem tissue of host plants (Will et al. , 2009). Furthermore, the brown plant hopper *Nilaparvata lugens* is able to overcome this plant defence by activating and secreting a hydrolysing enzyme, which induces the degradation of callose in SEs (Hao et al. , 2008). Whether psyllid species transmitting AP, PD and ESFY (AP: *C. picta* ; PD: *C. pyri, C. pyrisuga* and *C. pyricola* ; ESFY: *C. pruni*) have evolved such mechanisms to overcome this particular plant defence is unknown. Nevertheless, it was shown that phloem ingestion of *C. pruni* was not influenced by phytoplasma infection of its host plants (*P. persica* and *P. insititia*), indicating that callose deposition in infected peach plants does not affect vector feeding (Gallinger & Gross, 2020).

In general, sugars (e.g. sucrose) are known to stimulate feeding of phloem-feeding insects, such as aphids (Arn & Cleere, 1971; Mittler & Dadd, 1963). Thus, the detected higher sugar concentration in infected pear phloem could increase probing and feeding behaviour of psyllids and increase the acquisition and spread of phytoplasmas in pear orchards. However, recently a detailed phloem composition analysis of*Prunus* trees revealed no major differences in the phloem metabolite composition between ESFY infected and healthy trees (Gallinger & Gross, 2020). Therefore, to study whether or not the concentration of micronutrients in the phloem of diseased apple and pear trees is affected by phytoplasma colonization is a goal of our future work.

Apple is able to restrict the phytoplasma infection via phytohormone signalling.

In apple and peach trees, SA and JA-Ile levels significantly increased in infected trees, indicating the involvement of defence pathways to phytoplasma colonization. The content of ABA in apple leaves also increased. Commonly, SA plays the central role for the interaction between biotrophic pathogens and host plants (Ma

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& Ma, 2016; Robert-Seilaniantz et al. , 2011) and an increase after *Ca.*P. mali infection was found earlier (Zimmermann et al., 2015). In contrast, the jasmonic acid pathway is induced in response to wounding, herbivore attack and necrotrophic pathogens (Heil & Ton, 2008). The development of different pathways in reaction to different threads enables plants to respond more specifically and is therefore resource-efficient. An antagonistic crosstalk between JA/ABA and SA was detected in several plant species (Flors et al. , 2005; Zimmermann et al., 2015). Not surprisingly, some bacteria species evolved the production of effector proteins that interfere with SA regulated defence responses by activating JA pathway (Chisholm et al. , 2006). This mechanism was also detected for phytoplasmas in Aster yellows-witches broom phytoplasma (AY-WB). AY-WB produces the SAP11 effector that down-regulates the plant defence response by lipoxygenase2 expression and JA production (Sugio et al. , 2011b). This down-regulation of defence mechanisms in AY-WB infected plants is advantageous to vector fitness (Sugio et al. , 2011b). Recently, an SAP11-like protein was detected in '*Ca.* P. mali' that affected JA, SA and ABA pathways (Siewert et al. , 2014; Janik et al. , 2017).

Jasmonic acid plays a central role in induced plant defence e.g. by regulating the biosynthesis of herbivore-induced plant volatiles (Heil & Ton, 2008). Moreover, exogenous application of JA can be used to elicit plant defence responses similar to those induced by biting-chewing herbivores and mites that pierce cells and consume their contents. A low-dose JA application results in a synergistic effect on gene transcription and an increased emission of a volatile compound involved in indirect defence after herbivore infestation (Menzel et al., 2014). The induction of JA defence mechanisms in apple, pear and peach in response to psyllid feeding has not been proven yet. But infestations of *Citrus* plants with Asian Citrus Psyllid (ACP, *Diaphorina citri*) lead to an upregulation of genes involved in the JA-pathway (Nehela et al. , 2018). Additionally, the infection of Citrus trees with the phloem dwelling proteobacterium *Candidatus*Liberibacter asiaticus induce the SA-pathway (Nehela et al. , 2018) and resulted in an increased emission of methyl salicylate from infected plants (Martini et al. , 2018).

Auxins (IAA and IBA) are shown to induce the recovery of periwinkle plants from '*Ca.* P. pruni' and '*Ca.* P. asteris' infections (Curković Perica, 2008), illustrating the importance of IAA in plant-pathogen interactions. Interestingly, the IAA concentration in infected *P. persica* plants was significantly lowered compared to healthy peach trees. A reduced auxin content was also detected in leaves of lime infected with '*Ca.* P. aurantifoliae' (Zafari et al. , 2012). Imbalanced auxin concentration could be responsible for abnormal growth of infected peach trees (Figure 1c).

Overall, we have to consider that measurements are always just a snapshot of time. Different results from phytohormone analysis in AP-infected apple plants are reported in the literature (Janik et al., 2017; Zimmermann et al. , 2015), indicating that reactions to phytoplasma infections depend on season, cultivar and environmental conditions. Consequently, exact phytohormone and phloem sap analysis over a longer period (season) in correlation to respective phytoplasma titers are needed to draw reliable conclusions about symptom development.

The vector of apple proliferation behaves more adapted to phytoplasma infection than the other vectors

By analysing VOCs (volatile organic components) emitted by the leaves of apple trees, it was shown that '*Ca* . P. mali' changed the odour of infected trees compared to healthy ones by inducing the production of the sesquiterpene β-caryophyllene (Mayer et al. , 2008a,b). The main vector of '*Ca* . P. mali', the apple psyllid *Cacopsylla picta* , reproduces on apple and overwinters on conifers. The adults of the new generation (emigrants) are attracted by β-caryophyllene and lured to infected apple trees (Mayer et al. , 2008b), before migrating to their overwintering host. This behaviour increases the number of psyllids, which are able to acquire '*Ca* . P. mali'. By returning in early spring to apple trees, they prefer healthy apple trees for oviposition, in order to avoid detrimental effects of the phytoplasma on the offspring development (Mayer et al., 2011). As they need to feed before oviposition, they transmit the phytoplasma to healthy apples. This is a perfect balanced transmitting system, which improves the spread of the phytoplasma without negatively impact the vector. A similar adaptation of *C. pruni* , the migrating vector of '*Ca* . P. prunorum' is not known. This species did not distinguish its host plants by odour but phloem constitution (Gallinger et al., 2019; Gallinger & Gross, 2020). As the development on *P. persica* infected by '*Ca* . P. prunorum' had no

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detrimental effects on the vector (Gallinger & Gross, 2020), there is no selection pressure on distinguishing between infected and uninfected host plants as observed in apple (Mayer et al., 2011). The two main vectors of '*Ca* . P. pyri', *C. pyri* and*C. pyricola* do not migrate between different host plant species, therefore volatile signals might be less important in host choice of this psyllid species (Jarausch et al. , 2019a).

Conclusion

In the three investigated fruit crops the infection with specific phytoplasmas induced different morphological and physiological responses in the particular host plants. As apple trees generally survive a phytoplasma infection more often and much longer than peach and pear, some unique apple-specific responses are the most interesting and indicative features that could explain how a plant might become tolerant against a phytoplasma. Based on the results obtained, the long-lasting changes in the structure of the vascular system with all physiological consequences on the sap flow found in apple trees provides a promising step towards a deeper understanding of host plant defence against phytoplasma. In spite of the growing understanding of this pathosystem, it seems clear that the complexity of these interactions is not fully elucidated yet, and many open questions remain: Does the plant perceive a phytoplasma infection at all? If so, what does the plant recognize? Is there a MAMP/DAMP/effector present that induces an increased defence response in the SEs? What are the specific events during infections in the host in terms of time, place and extent? How do the antagonists interact on the molecular level? All these questions implicate more investigation on the molecular level and strongly suggest approaches such as RNAseq and transgenic approaches.

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Legends

Fig. 1. Symptoms of phytoplasma infected apple, pear and peach trees. **(a)** The apple proliferation (AP) induced by '*Candidatus* Phytoplasma mali' led to typical disease symptoms like witches' broom, enlarged and highly serrated stipules. The leaf size ratio (length width^{-1}) and the midrib ratio (diameter midrib width^{-1}) did not show any effects.**(b)** The leaves of '*Candidatus* Phytoplasma pyri' affected trees inducing pear decline (PD) were characterized by premature foliar reddening and a significant decrease of the leaf size ratio but not by an impact to the midrib. **(c)** The leaves of '*Candidatus*Phytoplasma prunorum' infected trees inducing European stone fruit yellows (ESFY) showed chlorosis, and a significant increase of the leaf size and midrib ratio. Boxes represent the interquartile range (IQR) and whiskers extend to 1.5*IQR. Bars represent the 95% confidence intervals with the estimated marginal means obtained from mixed effect models as dots (both back transformed to the response scale). Letters indicate statistical differences between EMMs of groups at the 0.05 significance level.

Fig. 2. Analysis of morphological leaf parameters in healthy and phytoplasma infected apple trees. **(a)** The infection of apple trees with a virulent classified '*Candidatus* Phytoplasma mali' accession (3/6) was investigated with the morphology of the leaf main vein in the midrib. **(b)** The morphological analysis consisted of the vascular bundle area, the xylem area, the phloem area, the sieve element area, the xylem/phloem ratio and the sieve element/phloem ratio and showed a significant decrease for nearly all studied parameters in AP infected trees but not for the sieve element/phloem ratio. Box-whisker plots with median as lines and jittered raw values as closed circles (corresponding to each measurement). Boxes represent the interquartile range (IQR) and whiskers extend to 1.5*IQR. Bars represent the 95% confidence intervals with the estimated marginal means obtained from mixed effect models as dots (both back transformed to the response scale). Letters indicate statistical differences between EMMs of groups at the 0.05 significance level.

Fig. 3. Analysis of morphological leaf parameters in healthy and phytoplasma infected pear trees. **(a)** The infection of a pear tree with '*Candidatus* Phytoplasma pyri' inducing pear decline (PD) was studied by the morphology of the leaf main vein.**(b)** The morphological analysis consisted of the vascular bundle area, the xylem area, the phloem area, the sieve element area, the xylem/phloem ratio and the sieve element/phloem ratio and showed no significant changes. Box-whisker plots with median as lines and jittered raw values as closed circles (corresponding to each measurement). Boxes represent the interquartile range (IQR) and whiskers extend to 1.5*IQR. Bars represent the 95% confidence intervals with the estimated marginal means obtained from mixed effect models as dots (both back transformed to the response scale). Letters indicate statistical differences between EMMs of groups at the 0.05 significance level.

Fig. 4. Analysis of morphological leaf parameters in healthy and phytoplasma infected peach trees. **(a)** The infection of a peach tree with '*Candidatus* Phytoplasma prunorum' inducing European stone fruit yellows (ESFY) was studied by the morphology of the leaf main vein. **(b)** The morphological analysis consisted of the vascular bundle area, the xylem area, the phloem area, the sieve element area, the xylem/phloem ratio and the sieve element/phloem ratio and showed significant decreases for the sieve element areas and ratios of sieve element to the phloem. Box-whisker plots with median as lines and jittered raw values as closed circles (corresponding to each measurement). Boxes represent the interquartile range (IQR) and whiskers extend to 1.5*IQR. Bars represent the 95% confidence intervals with the estimated marginal means obtained from mixed effect models as dots (both back transformed to the response scale). Letters indicate statistical differences between EMMs of groups at the 0.05 significance level.

Fig. 5. Analysis of translocation in phloem sieve elements of healthy and phytoplasma infected apple, pear and peach trees. The translocation was examined with the determination of the velocity of the phloem mass flow (cm h^{-1}) using fluorescence and with the calculation of volumetric flow rates (cm$^{\mathbf{3}}$ h^{-1}) in mean single sieve elements. Both parameters were individually determined for **(a)** apple, **(b)** pear and **(c)** peach trees. Apple trees were infected with '*Candidatus* Phytoplasma mali' inducing apple proliferation (AP). Pear

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trees were infected with '*Candidatus* Phytoplasma pyri' causing pear decline (PD) and peach trees were infected with '*Candidatus* Phytoplasma prunorum' inducing European stone fruit yellows (ESFY). Infected apple trees (AP) showed a significant decrease of phloem mass flow velocity and volumetric flow rates in contrast to infected pear trees (PD) where a significant rise was observed. In infected peach trees (ESFY) the phloem mass flow velocity was not affected but the volumetric flow rate decreased significantly. Box-whisker plots with median as lines and jittered raw values as closed circles (corresponding to each measurement). Boxes represent the interquartile range (IQR) and whiskers extend to 1.5*IQR. Bars represent the 95% confidence intervals with the estimated marginal means obtained from mixed effect models as dots (both back transformed to the response scale). Letters indicate statistical differences between EMMs of groups at the 0.05 significance level.

Fig. 6. Analysis of callose deposition in the leaf phloem tissue of healthy and phytoplasma infected apple, pear and peach trees. At cross sections of the leaf mid rip, the callose deposition in sieve elements was stained with aniline blue and detected via fluorescence microscopy (see panels on the left side). The callose fluorescence was quantified after subtracting auto-fluorescence (see panels on the right side). **(a)** In apple trees, an infection with the virulent accession (3/6) inducing apple proliferation (AP) did not show any differences in the callose deposition in comparison to healthy plants.**(b+c)** The phytoplasma infection of pear trees (PD) and peach trees (ESFY) induced a significant ($p<0.05$) increase of callose deposition in sieve elements. Box-whisker plots with median as lines and jittered raw values as closed circles (corresponding to each measurement). Boxes represent the interquartile range (IQR) and whiskers extend to 1.5*IQR. Bars represent the 95% confidence intervals with the estimated marginal means obtained from generalized least square models as dots (both back transformed to the response scale). Letters indicate statistical differences between EMMs of groups at the 0.05 significance level.

Fig. 7. The phytohormone balance in healthy and phytoplasma infected apple, pear and peach trees. The concentrations (ng g_{FM}^{-1}) of several phytohormones – salicylic acid (SA), jasmonic acid-iso leucine (JA-Ile), jasmonic acid (JA) and abscisic acid (ABA) – were measured in the leaves of healthy and phytoplasma infected **(a)** apple, **(b)** pear and**(c)** peach. For apple, a virulent accession (3/6) was considered inducing apple proliferation (AP). Pear trees showed pear decline (PD) and peach trees showed the European stone fruit yellows (ESFY). Box-whisker plots with median as lines and jittered raw values as closed circles (corresponding to each measurement). Boxes represent the interquartile range (IQR) and whiskers extend to 1.5*IQR. Bars represent the 95% confidence intervals with the estimated marginal means obtained from linear models as dots (both back transformed to the response scale). Letters indicate statistical differences between EMMs of groups at the 0.05 significance level.

Tables

Table 1 – Mean (±SD) leaf morphology parameters. Different letters indicate significant differences between phytoplasma infected and uninfected trees compared within each species. AP = apple proliferation; PD = pear decline; ESFY = European stone fruit yellowing

	Apple	Apple	Pear	Pear	Peach
parameter	Healthy	AP	Healthy	PD	Healthy
refractive index [°Brix]	9.643 (±1.200)^{a}	9.423 (±1.018)^{a}	7.250 (±1.909)^{a}	14.333 (±6.280)^{b}	11.727 (±2.494)^{a}
dynamic viscosity [mPa s]	4.078 (±0.652)^{a}	3.916 (±1.093)^{a}	1.957 (±0.368)^{a}	3.999 (±2.035)^{b}	n.d.
density [g L^{-1}]	996.5 (±94.9) ^{a}	1003.6 (±133.4) ^{a}	1001.4 (±50.8)^{a}	1058.1 (±55.7)^{a}	n.d.

Table 2 – Mean (±SD) functional/physiological parameters. Various physicochemical parameters (refractive index, dynamic viscosity and density) were determined for the centrifugates of the apple, pear and peach bark. Different letters indicate significant differences between phytoplasma infected and uninfected trees compared within each species. AP = apple proliferation; PD = pear decline; ESFY = European stone fruit yellows; mPa = milli Pascal; s = second; g = gram; L = litre

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	Apple	Apple	Pear	Pear	Peach
parameter	Healthy	AP	Healthy	PD	Healthy
refractive index [°Brix]	9.643 (±1.200)[a]	9.423 (±1.018)[a]	7.250 (±1.909)[a]	14.333 (±6.280)[b]	11.727 (±2.494)[a]
dynamic viscosity [mPa s]	4.078 (±0.652)[a]	3.916 (±1.093)[a]	1.957 (±0.368)[a]	3.999 (±2.035)[b]	n.d.
density [g L^{-1}]	996.5 (±94.9) [a]	1003.6 (±133.4) [a]	1001.4 (±50.8)[a]	1058.1 (±55.7)[a]	n.d.

Table 3 – Comparison of all morphological and physiological results of phytoplasma infected apple, pear and peach trees. All studied parameters are listed below on the left side and significant changes in comparison to healthy plants are individually given for the considered plant species – increase (+), decrease (-), no change (=) and not determined (n.d.).

	Apple	**Pear**	**Peach**
leaf size ratio [length/width]	=	-	+
midrib ratio [diameter midrib/leaf width]	=	=	+
diameter midrib [µm]	-	=	=
leaf length [cm]	-	=	=
leaf width [cm]	-	+	=
vascular bundle [μm^2]	-	=	=
xylem [μm^2]	-	=	=
phloem [μm^2]	-	=	=
sieve elements [μm^2]	-	=	-
ratio xylem/phloem	-	=	=
ratio sieve element/phloem	=	=	-
mass flow [cm h^{-1}]	-	+	=
volumetric flow rate [cm^3 h^{-1}]	-	+	-
dynamic viscosity [mPa s]	=	+	n.d.
refractive index [°Brix]	=	+	=
absolute density [g L^{-1}]	=	=	n.d.
callose intensity	=	+	+
salicylic acid [ng g_{FM}^{-1}]	+	=	+
jasmonic acid-iso leucine [ng g_{FM}^{-1}]	+	=	+
jasmonic acid [ng g_{FM}^{-1}]	=	=	=
abscisic acid [ng g_{FM}^{-1}]	+	=	=
12-oxo-phytodienoic acid [ng g_{FM}^{-1}]	-	=	=
indole acetic acid [ng g_{FM}^{-1}]	=	=	-

Supporting Information

The following Supporting Information is available for this article:

Figure S1 *The phytohormone concentrations of cis-12-oxo-phytodienoic acid (cis-OPDA) and indole-3-acetic acid (IAA) in healthy and phytoplasma infected (**a**) apple, (**b**) pear and (**c**) peach trees were measured.*

Table S1

Table S2 *Details of analysis of phytohormones by LC-MS/MS [HPLC 1260 (Agilent Technologies)-QTRAP6500 (SCIEX)] in negative ionisation mode.*

Table S3 *Specification and results of statistical models used for analysis of morphology parameters.*

Table S4 *Specification and results of statistical models used for analysis of vascular morphology.*

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Table S5 *Specification and results of statistical models used for analysis of the translocation in phloem sieve elements and physicochemical parameters.*

Table S6 *Specification and results of generalized least square models analyzing the maximum callose fluorescence.*

Table S7 *Specification and results of linear models used for analysis of phytohormone concentrations.*

Table S8 *Overview of mean phytohormone concentrations (ng g_{FM}^{-1}).*

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