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Transmission of European Stone Fruit Yellows Phytoplasma ('*Candidatus* Phytoplasma prunorum') during the propagation process

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The study focused on the incidence of 'Candidatus *Phytoplasma prunorum' transmission during the propagation process. Two common commercial propagation procedures, whip grafting in winter and budding in summer, were applied. Infected scions were grafted on certified apricot seedling rootstocks. Transmission of the pathogen was observed with both procedures. Whip grafting in winter, however, resulted in significantly lower infection rates of the progeny (0 to 15 %) than budding in summer (46 to 88 %).*
Keywords: ESFY, European Stone Fruit Yellows, whip grafting, budding, mother tree, *Prunus armeniaca*, apricot

Übertragung des Europäischen Steinobstvergilbungs-Phytoplasma ('Candidatus **Phytoplasma prunorum**') ***während der Vermehrung.*** *Ziel unserer Untersuchungen war es herauszufinden, in welchem Ausmaß das Europäische Steinobstvergilbungs-Phytoplasma* 'Candidatus *Phytoplasma prunorum' über Vermehrungsmaterial verschleppt werden kann. Dazu wurden zwei praxisübliche Vermehrungsverfahren, nämlich Kopulation im Winter und Okulation im Sommer, eingesetzt. Bei beiden Veredelungsverfahren wurde eine Verschleppung des Pathogens auf die Jungbäume nachgewiesen. Allerdings traten beträchtliche Unterschiede zwischen den beiden Verfahren auf. Bei Kopulation im Winter wurde das Pathogen im Lauf der ersten vier Jahre nach der Veredlung an 0 bis 15 % der Jungbäume nachgewiesen. Bei Okulation im Sommer lagen die Infektionsraten der Jungbäume bereits ein Jahr nach der Veredlung zwischen 46 und 88 %.*
Schlagwörter: ESFY, Europäische Steinobstvergilbung, Kopulation, Okulation, Mutterbaum, *Prunus armeniaca,* Marille

La transmission du phytoplasme à l'origine de l'European stone fruit yellows (ESFY,* 'Candidatus Phytoplasma prunorum') *au cours de la multiplication. *Nos études ont été effectuées pour déterminer dans quelle mesure l'ESFY* 'Candidatus *Phytoplasma prunorum' peut être disséminé par le matériel végétal. Dans ce but, nous avons eu recours à deux procédures de multiplication utilisées dans la pratique habituelle, soit la copulation en hiver et le greffage des yeux en été. La transmission du pathogène aux jeunes arbres a été prouvée pour les deux procédures de greffage. On a cependant constaté des différences importantes entre les deux procédures. Dans le cas de la copulation en hiver, le pathogène a été détecté sur 0 à 15 % des jeunes arbres au cours des quatre premières années après le greffage. Dans le cas du greffage des yeux en été, le taux d'infection des jeunes arbres se situait déjà entre 46 et 88 % un an après le greffage.*
Mots clés : ESFY, European stone fruit yellows, copulation, greffage des yeux, arbre-mère, *Prunus armeniaca,* abricot

Phytoplasmas are phloem limited wall-less procaryotes associated with more than 700 diseases in hundreds of plant species (Weintraub and Beanland, 2006). One of these disease agents is '*Candidatus* Phytoplasma prunorum' (16SrX). It primarily infects plants of the genus Prunus, causing European Stone Fruit Yellows (ESFY). The disease can be transmitted by the psyllid *Cacopsylla pruni* (Carraro et al., 1998) and by grafting of infected mother trees (Seemüller et al., 1998; Marcone, 2010).
During the last fifteen years ESFY has become a major concern in Austrian apricot production (Richter; 1999; Laimer da Câmara Machado et al., 2001). Knowledge about relative significance of spread via propagation material on one hand and vector transmission on the other is essential for a well adapted pest management. Therefore our study focused on the incidence of ESFY transmission during the propagation process. Two common commercial propagation procedures, whip grafting in winter and budding in summer, were investigated.

Material and methods

Plant material and grafting procedures

The study was carried out by grafting infected scions on commercial certified apricot seedling rootstocks. Seven candidate mother trees (apricot cultivar 'Klosterneuburger Marille') were selected in a commercial orchard by visual inspections and PCR analyses (three samples per tree).
At the beginning of March 2009 and at the end of February 2010 scions from three infected mother trees (referred to as mother trees 1 to 3) were collected (about 10 bud sticks per tree) and whip grafted (33 grafts from mother tree 1 and 19 from mother tree 2 in 2009; 23 grafts from mother tree 3 in 2010). Scions from four other infected mother trees (referred to as trees 4 to 7) were budded in July 2011 (14 grafts from mother tree 4, 15 from tree 5, 9 from tree 6 and 13 from tree 7). Control grafts with uninfected scions were also carried out (24 whip grafts in 2009, 26 whip grafts in 2010, 20 bud grafts in 2011).
All trees were cultivated in a screen house and individually analyzed by PCR for presence of '*Candidatus* Phytoplasma prunorum' every late summer. Due to extreme frost in winter 2012, however, only six out of the 23 young trees originating from mother tree 3 survived. PCR analyses in 2012 therefore only comprised the remaining plants.

Detection of '*Candidatus* P. prunorum'

DNA extraction from leaf and root samples was performed as described (Maixner et al., 1995). From 2009 to 2011 samples were analyzed by a single PCR. 20 µl of reaction volume contained 1 µl template preparation, 0.5 µM of primers f01/r01 (Lorenz et al., 1995), 200 µM of each dNTP, 0.5 U Taq-DNA-polymerase (Quiagen, Erlangen, Germany), 1 x reaction buffer (Quiagen). Reaction mixtures were subjected to 40 cycles with 45 seconds denaturation at 94 °C, 45 seconds annealing at 45 °C and 60 seconds extension at 72 °C. Obtained PCR fragments were analyzed by restriction fragment length polymorphism (RFLP). 10 µl of amplicon were digested with 5 U of RsaI (Promega, Madison, USA) at 37 °C for 4 hours (Seemüller and Schneider, 2004), separated on 2 % agarose gels, stained with Midori Green (Nippon Genetics, Dueren, Germany) and visualized under UV light.
In 2012 analyses were carried out both by single PCR and by a real time PCR procedure using the Sensifast NoRox kit (Bioline, London, UK) based on SYBR Green technology. Reaction preparations comprised 0.5 µl of template DNA, 0.2 µM of primers ESFYf and ESFYr (Yvon et al., 2009), 10 µl 2 x Sensifast Mix in a volume of 20 µl. Real time PCR was carried out in a RotorGene 2.0 (Corbett, Mortlake, Australia) thermocycler with a denaturation step at 94 °C for 3 minutes and 40 PCR cycles with 5 seconds denaturation at 94 °C, 10 seconds annealing at 65 °C and 10 seconds extension at 72 °C. SPSS 19.0 (SPSS Inc., Chicago, USA) was used for statistical analysis (Chi square test).

Results and discussion

Both grafting procedures led to infected progeny. PCR results for whip grafts are illustrated in Figure 1 (identical results for single and real time PCR). In case of mother tree 1 15 % of the progeny were infected with '*Candidatus* Phytoplasma prunorum', in case of mother trees 2 and 3 no transmissions were found. Real time PCR results for bud grafts are illustrated in Figure 2. Transmission rates ranged from 46 to 88 %. Differences between the two grafting procedures were statistically significant at the $p < 0{,}001$ level. Within the duration of the experiments no young tree showed

explicit disease symptoms. Analysis of control trees revealed no infections of the rootstocks.

The observed transmission rates are in accordance with previous experiments indicating that '*Candidatus* Phytoplasma prunorum' is viable and transmissible throughout the year (Seemüller et al., 1998). Ermacora et al. (2010) observed transmission rates of 35 to 61 % when infected scions were budded.

Jarausch et al. (1999) reported that the colonization of the trees was systemic from July until leaf fall. These authors also detected phytoplasmas in off-season grown leaves during winter until March, but rarely in normally grown leaves in April and May. So the differences in transmission rates observed in our experiments might be a result of this seasonal distribution of the pathogen within the trees.

Most nurseries propagate apricots by budding in summer. Based on our results we conclude that laxness or gaps in maintenance and testing of mother trees can lead to significant infestation of new plantations. During our study we observed no clear visual symptoms on PCR positive young trees. So delayed symptom development might sometimes conceal real causes for disease outbreaks. In a study identifying risk factors for ESFY in France (Thébaud et al., 2006) a nursery effect was assessed as a factor, although not as a major one.The results presented in this paper show that ESFY transmission via propagation material might be epidemiologically relevant. In order to elucidate its real role in Austrian apricot production further studies, e. g. random test of commercially available young trees, are necessary.

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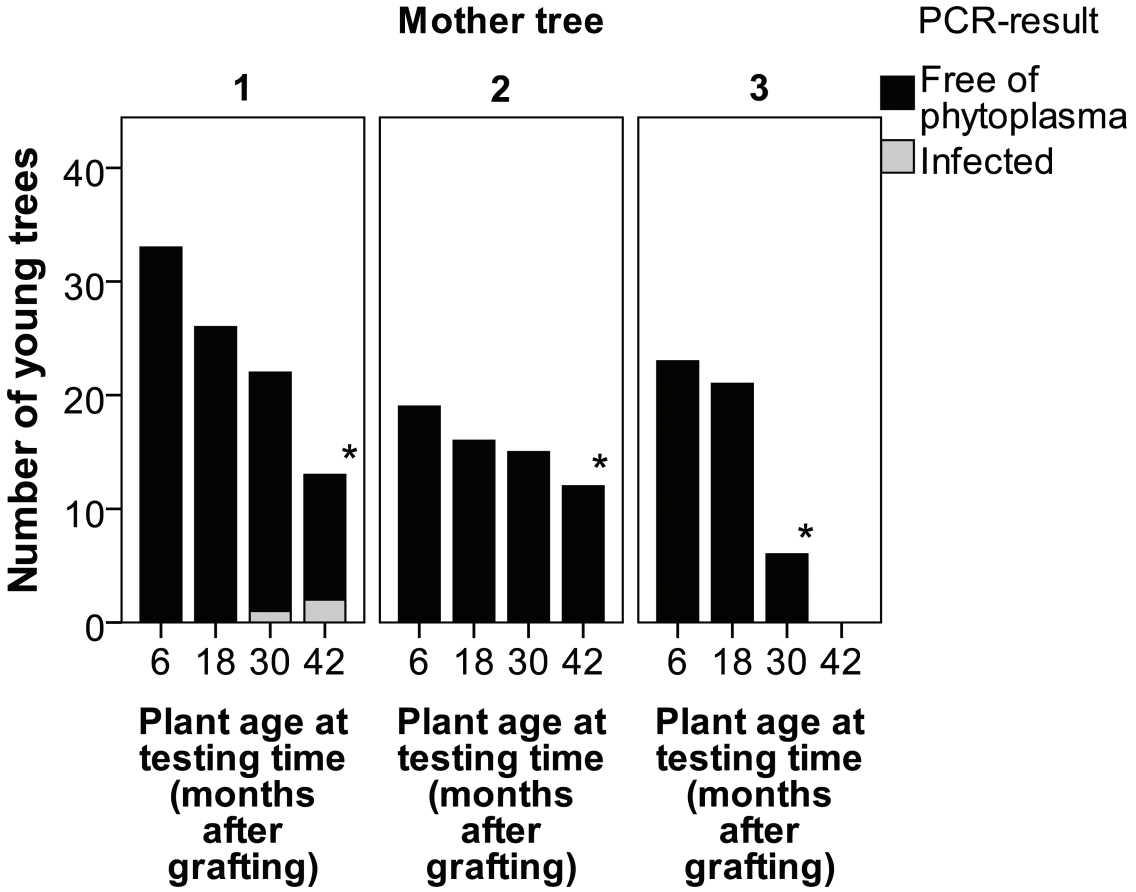

Fig. 1: Transmission of '*Candidatus* Phytoplasma prunorum' by whip grafting in winter; PCR-analyses of the resulting progeny 6-42 months after grafting

* Due to heavy winter frost in 2012 only 6 out of 23 trees survived.

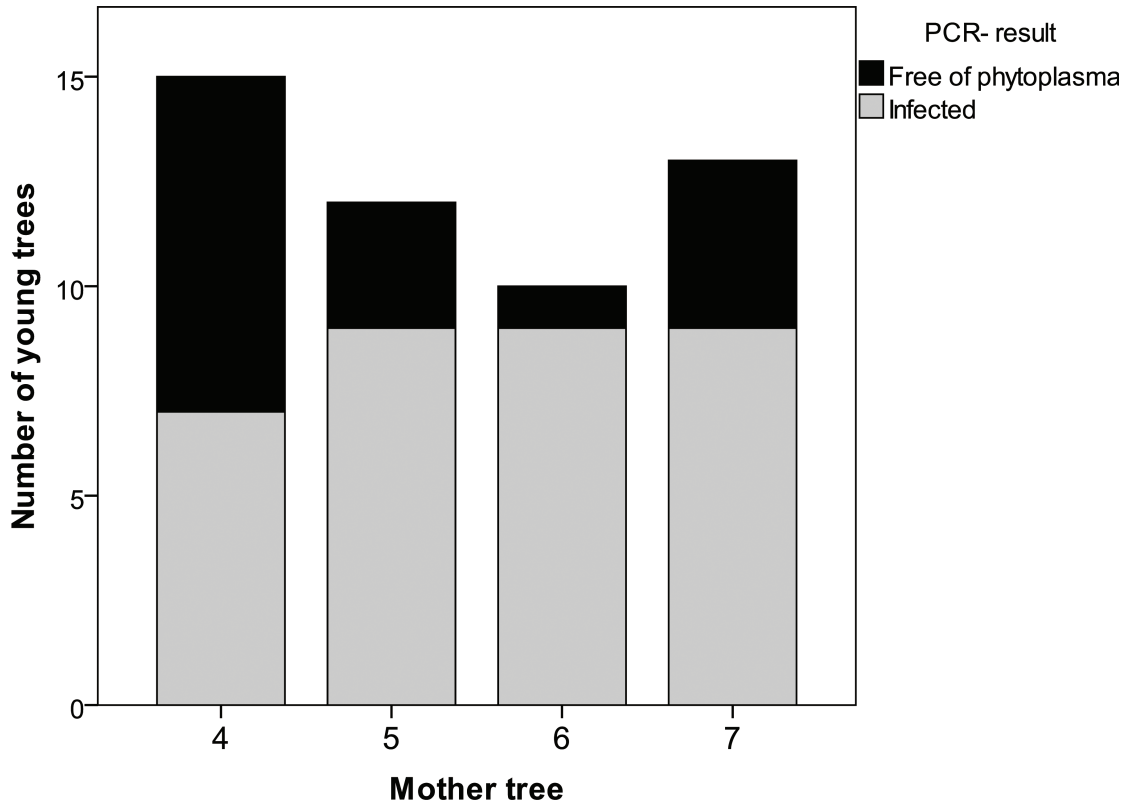

Fig. 2: Transmission of 'Candidatus Phytoplasma prunorum' by budding in summer; real time PCR analyses of the resulting progeny 12 months after grafting

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