Experimental transmission trials by *Cacopsylla pyri*, collected from Pear Decline infected orchards in Turkey

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Abstract

A study was carried out on the experimental transmission efficiency of the Pear Decline (PD) phytoplasma by *Cacopsylla pyri* (L.) C. pyri were collected from naturally infected orchards in Bursa province (Plots B1 and B2) and a non-infected orchard in the Hatay-Antakya province (Plot A) of Turkey. *C. pyri* adults captured from infected orchards were placed directly onto healthy periwinkle plants (*Catharanthus roseus*), whereas the *C. pyri* from plot A were allowed to feed first on infected pear for two weeks, then transferred to healthy periwinkle plants. Groups of five psyllids per plant were used for transmissison tests and the study was replicated three times. The presence of 'Candidatus *Phytoplasma pyri*' in psyllids and *C. roseus* plants was checked by nested PCR using P1/P7 and U3/U5 primer pairs. Although *C. pyri* have a limited host range they were able to survive up to 20 days on periwinkle plants. Insects collected from Bursa province survived 16-20 days whereas insects from Antakya survived 7-12 days on periwinkle plants. Symptoms consisted of yellowing or clearing of the veins in newly infected leaves, and shortening of the internodes of the main stem. The infected plants remained stunted and with small flowers. Results based on the RFLP analysis of infected plants exposed to psyllids from plot B1and B2 indicated that the experimental infection rate of periwinkle plants and psyllids was 33.3% and 16.6%, respectively. No infected periwinkle was found in plants exposed to psyllids from plot A, but the psyllids used for experimental transmission experiments were 33.3 % infected. Transmission trials under controlled conditions showed the capability of *C. pyri* to transmit PD from infected pears to healthy periwinkles and confirmed their potential as vectors of *Ca*. P. pyri in Turkey.

Keywords: Candidatus *Phtoplasma pyri*, pear psyllid, transmission efficiency

Introduction

Pear decline caused by 'Candidatus *Phytoplasma pyri*' (Seemüller and Schneider, 2004) is widespread in many pear-growing countries including Turkey. The first suspicious and common symptoms of PD was observed on cv. 'Deveci' in the Bursa province of Turkey in 2005. The disease was confirmed by PCR and RFLP analyses (Ulubas Serce et al. 2006). This phytoplasma belongs to the Apple proliferation group (16SrX) (Seemüller et al. 1998), and is transmitted by pear psyllids (*Cacopsylla pyricola, C. pyrisuga, C. pyri*). In North America and England the known vector is *Cacopsylla pyricola* (Foerster) but in other parts of Europe *Cacopsylla pyri* (L.) has been found to be the main vector (Carraro et al., 2001; Garcia-Chapa et al., 2005). Transmission of PD by *C. pyri* has been demonstrated in Italy (Carraro et al., 1998) and France (Lemoine, 1984), suggesting that this psyllid is probably the most important vector in the Mediterranean area. Although transmission capability has not yet been evaluated, *C. pyri* is also the most common psyllid in pear orchards in Spain (Garcia-Chapa et al., 2005). In Turkey *C. pyri* is the predominant psylla on pear trees (Gençer, 1999) producing 3 to 4 generations a year (Kovanci et al., 2000). Naturally infected psyllids captured from infected pear orchards have been reported (Ulubas Serce et al., 2006) but its capability to transmit Ca. *P. pyri* has not been demonstrated. The present paper describes experimental transmission of *Ca*. P. pyri to periwinkle plants by naturally infected *C. pyri* collected from infected orchards.

Materials and methods

Field studies: In December 2007 two commercial plots of pear cv. 'Deveci' (B1 and B2) located in Bursa province and one plot of cv. 'Santa Maria' (A) in the Antakya province of Turkey were selected. PD symptoms and the presence of *C. pyri* had been previously recorded in plot B1 and B2, but no PD symptoms were observed in plot A despite a previous report on the presence of the disease in that province (Sertkaya et al., 2005). The incidence of the disease in these three plots was evaluated and 10% of the pear trees were randomly selected and tested by nested PCR. The psyllids were collected in December by shaking insects onto an underlying net. Twenty individual insects from each plot were analyzed for the presence of PD .

Experimental transmission of PD by *C. pyri* on periwinkle plants: All the transmission experiments were carried out in an environmentally controlled growth room at 25±1°C, with supplementary light and 16-h days. In December 2007, adult *C. pyri* were captured and 3 groups of psyllids, each consisting of 5 individuals, were transferred to healthy

periwinkle seedlings. Psyllids captured from plot A were first fed on PD infected pear plant for 2 weeks and then transferred to healthy periwinkle plants. All test plants were covered individually with a plastic-screen cage (Fig. 1). Another group of three healthy periwinkle plants was used as negative control. Survival of the insects and symptom expression were monitored and dead psyllids were analyzed immediately for the presence of PD phytoplasma (Garcia-Chapa et al., 2003).

Fig. 1 Test plants covered individually with a plastic-screen cage (on left), *Cacopsylla pyri* feeding on periwinkle plant (on right).

Testing for the presence of phytoplasmas in test plants and in psyllids: All test plants and individual psyllids were tested by nested PCR. The first amplification was done with the universal primers P1/P7 (Lee et al., 1992). FU5/rU3 amplicons from the nested PCR were digested with *Ssp*I and *RsaI* at 37°C following the manufacturer's instructions (MBI Fermentas, Germany). Digested products were analyzed by electrophoresis using 2% agarose gel, and stained with ethidium bromide. DNA bands were photographed under UV light. PD, Apple proliferation (AP) and European Stone Fruit Yellows (ESFY) infected periwinkle plants used as positive controls were kindly supplied by Dr. Foissac, INRA, France.

Results

Field studies: PCR analyses of trees from the two commercial plots of pear trees cv. 'Deveci' in Bursa (B1 and B2) showed the incidence of PD infected trees with 60% and 65%, respectively. Whereas no infection was detected in plants from plot A. Analyses of 20 field collected psyllids evaluated by nested PCR showed that 2 and 5 psyllids from plot B1 and B2, respectively, were infected by PD. No infected psyllid was found in plot A from the province of Antakya.

Experimental transmission of PD by *C. pyri* on periwinkle plants: Although *C. pyri* have a limited host range, they were able to survive up to 20 days on periwinkles (Table 1). Insects collected from Bursa province survived 16-20 days whereas insects from Antakya survived 7-12 days on periwinkles. The initial symptoms were observed 4 months after exposure of infected psyllids to test plants. Symptoms consisted of yellowing or clearing of the veins in newly infected leaves, and shortening of the internodes of the main stem. The plants remained stunted and flowers were small (Fig.2). Two periwinkle plants exposed to psyllids from plot B1 and B2 showed phytoplasma-like symptoms. No symptomatic plants were obtained using psyllids from plot A. According to the RFLP analysis of the Bursa samples the infection rate of periwinkle plants and psyllids was 33.3% and 16.6%, respectively. No infected periwinkle plants were found among those exposed to psyllids from plot A but the psyllids were 33.3 % infected having acquired the PD phytoplasma after being allowed to feed on infected pear.

Tab. 1 Survival of *Cacopsylla pyri* L. on periwinkle plants

Location and number pf test plants		Survival (days)
	B1-1	19
Bursa 1 (B1	B1-2	16
	B1-3	20
	B2-1	12
Bursa 2 (B2)	B2-2	12
	B2-3	17
	A1	7
Antakya (A)	A2	12
	A3	7

Fig. 2 Symptoms of stunting, shortening of the internodes of the main stem and small flowers of experimentally infected periwinkle (on right) and healthy control (on left) .

Testing for the presence of phytoplasmas in test plants and in psyllids: The primer pair FU5/rU3 was validated by amplification of DNA from the positive controls - that is PD, AP and ESFY infected periwinkle plants as well as from test plants and individual psyllids used in the trials. After digestion with *Ssp*I and *RsaI*, the restriction products obtained from all samples showed the appropriate restriction profiles by which the three different phytoplasmas could be distinguished (Fig. 3).

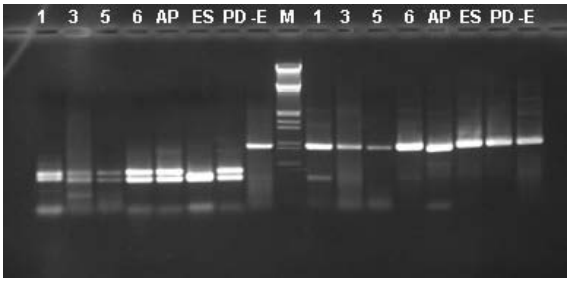

Fig. 3 Restriction products of Ca. *Phytoplasma pyri* DNA after digestion with *Ssp*I and *RsaI*, respectively. 1, 3, 5 and 6 represent infected periwinkle plants. Positive controls: AP (apple proliferation), ES (European stone fruit yellows), PD (pear decline). –E: negative control without enzyme.

Discussion

Pear decline is a destructive disease that occurs in Europe, North America and wherever domestic European pear (*Pyrus communis* L.) is grown (Davies et al., 1992; Garcia-Chapa et al., 2003). In the last 5 to 6 years the rapid spread of PD disease in the Bursa province of Turkey represents a serious outbreak with high levels of infection. Previous studies in this province showed that out of the 116 pear samples tested 52.58% were found to be infected by PD (Gazel et al. 2007). In this study similar results were obtained and 60 to 65% infection rate was recorded in randomly tested pear trees from which psyllids were captured for transmission trials. The psyllids, collected from two different infected orchards of Bursa province were also found infected by PD (7 infected out of 40). Two periwinkle plants out of 6 were experimentally infected by *C. pyri* collected from Bursa province where PD is very common. According to these results, the detection of the same RFLP pattern for pear, psyllid and periwinkle confirm that *C. pyri* is a potential vector of the PD agent in that province. However in plot A no naturally infected psyllids were found. After being allowed to feed on infected pear and based on experimental transmission trials PD was detected in one insect, but in none of the periwinkle plants. This data showed that *C. pyri*, collected from plot A may be a potential vector candidate for this province. It can acquire phytoplasma from infected pear trees, but was not able to transmit to periwinkle in this experiment. This might be due to the use of a limited number of insects and periwinkles. *C. roseus* does not appear to be a good host for pear decline transmission. It might be necessary to use pear seedlings for more reliable transmission

experiments (Avinent and Llacer, 1994). Since the transmission of PD is difficult to reproduce experimentally with other psyllid species (Davies et al., 1992), new laboratory transmission trials with Turkish pear cultivars are under investigation, using *C. pyri* fed on infected pear trees.

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Literature

Avinent, L.; Llacér, G.; 1994. Deteccion de fitoplasmas en frutales mediante la reaccion en cadena de la poimerasa (PCR). Investigacion Agraria: Produccion Proteccion Vegetales. Fuera de Serie. 2: 201-205.

Carraro, L.; Osler, R.; Loi, N.; Ermacora, P.; and Refatti, E.; 1998. Transmission of European stone fruit yellows phytoplasma by *Cacopsylla pruni*. Journal of Plant Pathology 80, 233-239.

Carraro, L.; Osler, R.; Loi, N.; Ermacora, P.; and Refatti, E.; 2001. Fruit tree phytoplasma diseases diffused in nature by psyllids. Acta Hort.550: 345-350.

Davies, D. L.; Guise, C. M.; Clark, M. F.; Adams, A. N.; 1992. Parry's disease of pears is similar to pear decline and is associated with mycoplasma-like organism transmitted by *Cacopsylla pyricola* . Plant Pthology, 41: 195-203.

Garcia-Chapa, M.; Medina, V.; Viruel, M.; Lavina, A.; Battle, A.; 2003. Seasonal detection of pear decline phytoplasma by nested-PCR in different pear cultivars. Plant Pathology, 52 (4): 513-520.

Garcia-Chapa, M.; Sabaté, J.; Lavina, A.; Battle, A.; 2005. Role of *Cacopsylla pyri* in the epidemiology of pear decline in Spain. European Journal of Plant Pathology, 111 (1): 9-17.

Gazel, M.; Ulubaş Serçe, Ç.; Çağlayan, K.; Öztürk, H..; 2007. Detection of '*Candidatus* Phytoplasma pyri' in Turkey. Bulletin of Insectology 60 (2), 125-126.

Gençer, N.S.; 1999. Bursa ilinde armutlarda zarar yapan *Cacopsylla* (Homoptera: Psyllidae) türleri üzerinde biyolojik ve ekolojik araştırmalar. U.Ü. Fen Bilimleri Enstitüsü. Bitki Koruma Anabilim Dalı Doktora Tezi. (PhD. Thesis, Unpublished).

Kovancı, B.; Gençer, N.S.; Kaya, M.; Akbudak, B.; 2000. Uludağ Üniversitesi Ziraat Fakültesi armut bahçesinde *Cacopsylla pyri* (L.) (Homoptera: Psyllidae)' nin populasyon değişimi üzerinde araştırmalar. Türk.Entomol. Derg. 24(4): 289-300.

Lee, I.M.; Davis, R.E.; Chen, T.-A.; Chiykowski, L.N.; Fletcher, J.; Hiruki, C.; Schaff, D.A.; 1992. A genotype-based system for identification and classification of mycoplasmalike organisms (MLOs) in the aster yellows MLO strain cluster. Phytopathology 82, 977-986.

Lemoine, J.; 1984. Is *Psylla pyri* a vector of pear decline in France? Bulletin-SROP. 7: 245-251.

Seemuller, E.; Marcone C.; Lauer U.; Ragazzı-No A.; Göschl M.; 1998. Current status of molecular classification of the phytoplasmas. Journal of Plant Pa-thology 80: 3-26.

Seemüller, E.; Schneider, B.; 2004. Candidatus *Phytoplasma mali*, Candidatus *Phytoplasma pyri* and Candidatus *Phytoplasma prunorum*, the causal agents of apple proliferation, per decline and European stone fruit yellows, respectively. In J. Syst and Evol. Microbiol, 54: 1217-1226.

Sertkaya G.; Martini M.; Ermacora P.; Musetti R.; Osler R.; 2005. Detection and Characterization of Phytoplasmas in Diseased Stone Fruits and Pear by PCR-RFLP Analysis in Turkey. Phytoparasitica 33:380-390.

Ulubaş Serçe Ç.; Gazel M.; Çaglayan K.; Baş M.; Son L.; 2006. Phytoplasma diseases of fruit trees in germplasm and commercial orchards in Turkey. Journal of Plant Pathology. 88(2)179-185.