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.), collected from naturally infected orchards from Bursa and non-infected orchard from Hatay province of Turkey. *C. pyri* adults captured from infected orchards were directly transmitted to healthy periwinkle plants (*Catharanthus roseus*) whereas the second group firstly fed on infected pear for two weeks and then transferred to periwinkles. 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 analysed by nested PCR using P1/P7 and U3/U5 primer pairs.. Although *C. pyri* has limited host range, they were able to survive up to 20 days on periwinkles. Insects collected from Bursa province survived 16-20 days whereas second group from Hatay were survived 7-12 days on periwinkles. Symptoms consist of a yellowing or clearing of the veins in newly infected leaves and shortening of the internodes of the main stem. They also remain stunted and flowers were small. According to the RFLP analysis of Bursa samples, the experimental infection rate of periwinkle plants and psyllids was 33.3 % and 16.6 %, respectively. No infected periwinkle was found in second group but psyllids 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 as vector 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 Bursa province of Turkey in 2005 and then 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 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 part of Europe *Cacopsylla pyri* (L.) has been found as main vector (Carraro et al., 2001; Garcia-Chapa et al., 2005). Transmission of PD by *C. pyri* has already 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 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) and gave 3 to 4 generations a year (Kovanci et al., 2000). Naturally infected psyllids, captured from infected pear orchards, were already reported (Ulubas Serce et al., 2006) but its capability to transmit Ca. *P. pyri* has not been investigated yet.

Present paper describes experimental transmission possibility of Ca. *P. pyri* using *C. pyri* which were collected from naturally infected orchards, to periwinkle plants.

Materials and methods

Field studies: In December 2007 two commercial plots of pear cv. 'Deveci' located in Bursa (B1 and B2) and one plot of cv. 'Santa Maria' in Antakya (A) provinces of Turkey were selected. PD symptoms and presence of C. *pyri* had been previously recorded in plot B1 and B2 but no PD symptoms were observed in plot A despite previous report on presence of the disease in that province (Sertkaya et al., 2005). The incidence of the disease in three plots was evaluated and 10 % of the total pear trees were randomly selected and tested by nested PCR. The psyllids were also captured in December by shaking insects onto an underlying net. Twenty individual insects from each plot were also 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 then 3 groups of psyllids, each of 5 individuals, were transferred to healthy periwinkle seedlings. Psyllids captured from plot A were firstly fed on PD infected pear plant for 2 weeks and then transferred to periwinkles. All test plants were covered individually with a plastic-screen cage (Figure 1). Another group of three healthy periwinkle plants were used as negative controls. Longevity of the insects and symptom expression were observed and died psyllids were immediately analyzed 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 with the universal primers P1/P7 (Lee ve ark., 1992). FU5/rU3 amplicons of nested PCR were digested with *Ssp*I and *RsaI* at 37 °C fallowing 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 were kindly supplied by Dr. Foissac-INRA, France and used as positive controls.

Results

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

Experimental transmission of PD by *C. pyri* on periwinkle plants: Although *C. pyri* has 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 were survived 7-12 days on periwinkles. First symptoms were observed 4 months after exposure on test plants. Symptoms consist of a yellowing or clearing of the veins in newly infected leaves and shortening of the internodes of the main stem. They also remain stunted and flowers were small (Figure 2). Two periwinkle plants from plot B1 and B2 showed phytoplasma-like symptoms whereas no symptomatic plant was found in plot A. According to the RFLP analysis of Bursa samples, infection rate of periwinkle plants and psyllids was 33.3 % and 16.6 %, respectively. No infected periwinkle was found in second group but psyllids were 33.3 % infected.

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

Location and number of test plants		Survival (days)
Bursa 1 (B1)	B1-1	19
	B1-2	16
	B1-3	20
Bursa 2 (B2)	B2-1	12
	B2-2	12
	B2-3	17
Antakya (A)	A1	7
	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: Using the primer pair FU5/rU3, PD-phytoplasma DNA was amplified from 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 same restriction profiles, by which three different phytoplasmas could be, distinguished (Figure 3).

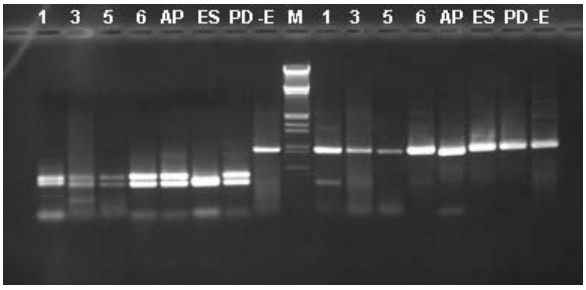

Fig. 3 Restriction products of Ca. *Phytoplasma pyri* DNA after digestion with *Ssp*I and *RsaI*, respectively. 1, 3, 5 and 6 represent infected periwinkle plants. Positive controls: AP (apple proliferation), ES (European stone fruit yellows), PD (pear decline). –E: negative control without enzyme.

Discussion

Pear decline is a destructive disease that occurs in Europe, North America and wherever the domestic European pear (*Pyrus communis* L.) is grown (Davies et al., 1992; Garcia-Chapa et al., 2003). In the last 5 to 6 years rapid spread of PD disease in Bursa province of Turkey represents a serious outbreak with high level of infection. Previous studies in this province showed that out of the 116 tested pear samples, 52.58 % were found 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 same RFLP pattern for pear, psyllid and periwinkle confirm that *C. pyri* is an active vector of PD agent in that province. However in plot A no naturally infected psyllids were found and PD was only detected in one insect but none of the periwinkle plants. This data showed us that *C. pyri,* collected from plot A may be also potential vector candidate for this province because it can acquire phytoplasma from the infected pear tree but not able to transmit to periwinkle for this experiment. It might be due to using limited number of insects and periwinkles. Since *C. roseus* does not seem to be a good host for pear decline transmission, it also might be necessary to use pear seedlings for transmission experiments (Avinent et al., 1997). Because of PD is difficult to reproduce experimentally with other psyllid species (Davies et al., 1992), new laboratory transmission trials with insects fed on infected trees should be performed in future.

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