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Role of *Cacopsylla pyri* in the epidemiology of pear decline in Spain

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Abstract

The frequency of pear decline-positive insects and transmission of pear decline (PD) phytoplasma by *Cacopsylla pyri* in Spain has been studied. Psyllids were used for experiments on phytoplasma transmission both to healthy *Pyrus communis* trees and to an artificial feeding medium. Over a period of 1 year, about 100 psyllids were collected monthly from pear trees, cv. Williams, using the beating tray method, and tested for the presence of PD phytoplasma. Results indicate that the frequency of PD positive psyllids changes through the year and that *C. pyri* transmits the pear decline associated disease agent. Phytoplasma transmission was also effective under laboratory conditions using a feeding medium. The relationship between PD positive *Cacopsylla pyri*, Pear decline phytoplasma transmission and the sex of the vector was also evaluated. Although the percentage of PD positive psyllids was similar in both genders, PD phytoplasma transmission by females was significantly higher than by males. Since the sex ratio (male/female) was less than 1:1 for most of the year, these results should be taken into consideration for controlling Pear decline in Mediterranean climates.

Introduction

Phytoplasmas, belonging to the class of Mollicutes, are associated with a variety of plant diseases. They are transmitted by grafting or by insect vectors of the Homoptera order. Phytoplasmas included in the apple proliferation group (AP) are transmitted by psyllids.

The Pear decline (PD) phytoplasma that belongs to the AP group (Seemüller et al., 1998) is transmitted by the pear psylla, and causes important loss in pear fruit production (Seemüller, 1992), particularly as PD is widespread in many pear-growing areas of Europe and North America (Avinent et al., 1997).

Cacopsylla pyricola is responsible for the spread of the PD in North America and the United Kingdom (Jensen et al., 1964; Hibino et al., 1971; Davies et al., 1992). Transmission by *C. pyri* has been demonstrated in Italy (Carraro et al., 1998) and France (Lemoine, 1984), suggesting that this species is probably the most important vector in the Mediterranean area. *Cacopsylla pyri* is also the most common psyllid in pear orchards in Spain, and in the northeast it has been identified as carrying the PD phytoplasma (Avinent et al., 1997; Batlle et al., 1999). The capability of transmission has not yet been evaluated.

Cacopsylla pyri has two morphologically distinct adult stages: the summerform and the winterform, which is darker in colour. In Spain, the insects produce 4–6 generations per year on pear trees, depending on the region and crop conditions (García de Otazo et al., 1992). Individuals of the last generation emerge in September and October. The winterform morphotype overwinters as an

adult on the same host (Conci et al., 1992) or on any other support. Activity resumes in January–February, when diapause ends and mating takes place. Females lay clusters of eggs, and the summerform morphotype appears. There are usually only a few in the first and second generations, but in the third generation there are large numbers, and they cause a huge amount of damage.

Because phytoplasmas can be persistently spread by phloem-feeding insects (Lee et al., 2000), and *C. pyri* is also the most serious pest of the pear tree in Europe (Kapatos and Stratopoulou, 1999) a great deal of effort has been made to control this vector. Nevertheless, for the appropriate control of PD disease spread, it is necessary to know factors such as the ease of acquisition of PD phytoplasma by psyllids and the ability of the insect to transmit the phytoplasma during the year. Some studies have been done with *C. pyricola* (Davies et al., 1998; Blomquist and Kirkpatrick, 2002) and *C. pyri* (Carraro et al., 2001), however, various factors influence PD phytoplasma acquisition and transmission. This includes the behaviour of the insect in particular geographical areas and ecological factors (Fletcher et al., 1998). As the behaviour of the psyllid could be different in different areas, this is consequently of great interest.

The aim of the present work was to determine the ability of *C. pyri* to transmit PD phytoplasma in Spain, and to analyse the efficiency of *C. pyri* transmission. Young pear trees and an artificial sucrose medium were used for this purpose. The seasonal variation in rates of PD phytoplasma infected psyllids was also evaluated. Finally, the relationship between PD positive insects or PD phytoplasma transmission and the sex of the vector were studied.

Material and methods

Location and characteristics of the pear plot used in this study

A commercial plot of pear cv. Williams, located in the northeast of Spain and grafted onto *Pyrus communis* rootstock was used. This plot, with 400 non-certified pear trees, was selected because PD disease had already been identified and there were a high number of trees with symptoms of the disease. Presence of *C. pyri* had also been previously recorded. The age of the trees was between 12 and 14 years. Insecticide treatments against the vector were applied due to pear psylla control problems.

The incidence of the disease in this plot was evaluated at the beginning of this study. In September 2001, 20 pear trees were selected randomly and analysed for the presence of PD phytoplasma (Garcia-Chapa et al., 2003b).

Transmission of PD phytoplasma by C. pyri *on pear test plants*

In September 2001, 10 groups, each of 30 *C. pyri* individuals, were collected from and transferred to 10 different 2-year-old micropopagated *Pyrus communis* OHF-333 trees. Test plants were covered individually with a plastic-screen cage. Another group of 10 healthy pear trees were used as negative controls. Psyllids were collected 30 days after inoculation and the test plants were treated with insecticides. Collected psyllids were analysed for PD phytoplasma. One year after exposure, the test plants were analysed for the presence of PD phytoplasma (Garcia-Chapa et al., 2003b).

Frequency of PD positive psyllids and transmission to feeding medium

From June 2002 to May 2003, *C. pyri* adults collected from the experimental plot were sampled monthly using the beating tray method (Burts and Retan, 1973). On each sampling date, about 100 individuals of *C. pyri* were collected, always at the same hour (10–12 h). The psyllids were placed on falcon tubes for 1 day to avoid false detection of PD phytoplasma due to residual phytoplasmas adhering to the stylet tissues. In January and March, *C. pyri* individuals were not collected because the populations were very low.

After the first day, *C. pyri* individuals were transferred individually to 1.5 ml microcentrifuge tubes utilised as insect chambers. The caps were filled with 200 μl of 5% sucrose, 0.5% sorbitol and 9.4 mg l^{-1} of NCTC 135 medium (Sigma) then sealed with Parafilm (M. Maixner, Institut für Pflanzenschutz im Weinbau, Germany, personal communication). Sucrose medium has been reported as suitable for studying the transmission of phytoplasmas by leafhoppers (Tanne et al., 2001). Each tube, containing an individual psyllid, was

kept at 23–25 °C until the insect died or for a maximum of 72 h. Time of death was recorded.

In the laboratory, the adults were split in groups by gender (Hodkinson and White, 1979), and the ratio of males/females was calculated. Males and females were separated into groups, each of 4 individuals, and kept at −20 °C for further DNA analysis.

Pear decline phytoplasma detection

DNA was extracted from insects and feeding medium in order to detect the presence of PD phytoplasma by nested-PCR. DNA extraction from insects was according to Daire et al. (1992), with slight modifications. Insects were ground in extraction buffer (100 mM Tris–HCl at pH 8.0, 2% cetyltrimethylammonium bromide (CTAB), 1.4 M NaCl, 20 mM EDTA and 0.2% 2-mercaptoethanol). The slurry was incubated for 10 min at 65 °C. After incubation, an equal volume of chloroform was added and centrifuged for 10 min at 12,000 rpm. The supernatant was collected and the nucleic acids precipitated with an equal volume of 2-propanol, following 30 min incubation at −20 °C. DNA was pelleted at 12,000 rpm for 30 min. The pellet was washed with 70% ethanol and resuspended in 50 μl of sterile water.

DNA was extracted from the feeding medium according to: the methodology of Wizard plus (Wizard plus, SV minipreps. DNA purification system, Promega), partially modified. Two hundred microliter of the feeding medium were transferred to a 1.9 ml microcentrifuge tube with 900 μl of buffer TNE (SDS 1.1%, 150 mM NaCl, 2 mM EDTA, 10 mM Tris–HCl at pH 8), 100 μl of 5 M guanidine hydrochloride and 50 μl of proteinase K (20 mg ml^{-1}). The mixture was incubated for 2 h at 58 °C and centrifuged for 10 min at 13,000 rpm. An equal volume of chloroform was added to the supernatant and centrifuged for 10 min at 13,000 rpm. Five hundred microliter of the supernatant were mixed with a DNA purification resin (Wizard minipreps, Promega) in a 1.5 ml microcentrifuge tube and mixed by inverting the tube several times. The mixture was transferred to a new spin column-vacuum adapter and a vacuum was applied to pull all the liquid through the column. One milliliter of 2-propanol was added and vacuum again applied. Finally, the spin column was transferred to another sterile 1.5 ml microcentrifuge tube and the DNA was eluted by adding 50 μl of nuclease-free water and centrifuging at 13,000 rpm for 1 min.

PCR amplification

A nested PCR was carried out. The first amplification was with the universal primers P1/P7 (Deng and Hiruki, 1991; Smart et al., 1996) located at the 16S rDNA and 23S rDNA gene, respectively. In the second amplification the specific primer pair f01/r01 was used (Lorenz et al. 1995). These primers amplify the 16S rDNA gene from DNA of all phytoplasma belonging to the Apple proliferation group, producing a fragment of about 1050 bp in length. Conditions of amplification are those described by Garcia-Chapa et al. (2003b).

Amplification products were electrophoresed in a 1.5% D-1 agarose gel (Pronadisa, Madrid, Spain) according to standard procedures. DNA was stained with ethidium bromide and exposed to UV light.

Characterisation of the phytoplasma

DNA extracted from feeding medium was tested by RFLP analysis. Amplification with the primers FU5/rU3 (Lorenz et al., 1995) was carried out using the PCR products from P1/P7 amplification (Garcia-Chapa et al., 2003a). Final FU5/rU3 amplicons (10 μl) were digested with *Tru*9I restriction endonuclease following the manufacturer's instructions (MBI Fermentas, Germany). Final f01/r01 amplicons were digested with *Ssp* I and *Rsa* I at 37 °C. Five microliter of digests were run in 3% MetaPhor agarose gel (BMA, Rockland, USA) and visualised with ethidium bromide staining. Profiles obtained were compared with those established by Seemüller et al. (1998).

Data analyses

Data obtained from PD positive psyllids in male and female groups over the year, were analysed using the Systat 5.0 statistical program (Systat for Windows, Inc. Evanston, Illinois, USA). Infection rates of sampled groups were analysed by the Mantel–Haenszel Chi-square analysis using $K \times 2 \times 2$ tables, to asses whether PD positive *C. pyri* depends on sex category or sampling time variables.

Frequencies of PD infection in sampled groups further classified according to gender or sampling time, were compared by Chi-square analysis using 2×2 and $K \times 2$ contingency tables respectively. The adjusted test of Bonferroni was used to compare the infection of every month (df = 1).

The P value (Hughes et al., 2001), $P = 1 - (1-X)^{1/n}$ (where P is the proportion of PD positive insects, X the proportion of PD positive groups of insects and 'n' the number of insects per group) was used to estimate the number of insects (males or females) that carried PD phytoplasma within each sampling period. The binomial distribution is assumed to provide an appropriate description of the frequency of infected psyllids per group randomly allocated in the laboratory. Data obtained by linear regression were used to study the correlation between percentages of total insects carrying PD phytoplasma and PD positive psyllids by gender. Regression slopes were also compared using specific procedures included in the Statgraphics Plus program (Manugistics, Inc., Maryland, USA).

The relationship between PD positive psyllids and PD phytoplasma transmission to sucrose medium, depending on sex, was evaluated by Chi-square analysis. An estimation of the number of PD positive psyllids was also calculated in this case, excluding psyllids that died on the first day in microcentrifuge tubes.

Results

Incidence of PD phytoplasma in the pear plot studied

In the commercial plot of pear tree cv. Williams, analyses of the 20 trees evaluated by nested PCR showed that the incidence of PD infected trees was 80%.

Transmission of PD phytoplasma by C. pyri *on pear test plants*

Results of PCR analyses indicate that all psyllid groups used in the transmission experiment were PD phytoplasma positive. All the trees exposed to *C. pyri* (10 trees) were also PD infected 1 year after exposure. No amplification was obtained from healthy pear tree DNA (Figure 1).

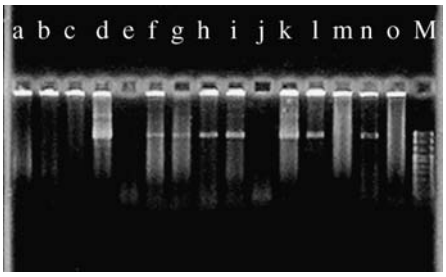

Figure 1. Nested-PCR amplification of ribosomal DNA from some Pear decline inoculated and non-inoculated pear trees, using the universal phytoplasma pair P1/P7 followed by the primers f01/r01, which are group-specific for the apple proliferation cluster. Lanes a, b, c, e, j, m, o.: DNA samples from non-inoculated pear trees; lanes d, f, g, h, i, k, l, n: DNA samples from inoculated pear trees; M, pUC mix marker 8.

Natural infection of C. pyri *in the infected plot*

The percentage of both groups and individuals of *C. pyri* infected by PD during the year are shown in Table 1. Psyllids were infected all through the year but percentages varied depending on the season. The highest percentages of PD infected individual psyllids occurred in September and October (100% and 41.14% respectively), and the lowest levels were found in February (6.63%). In the other months, percentage infection was between 16% and 25%. The Mantel–Haenszel Chi-square analysis performed showed that there was no interaction between the three variables 'time', 'infection' and 'sex' ($P = 0.551$). Therefore, a $k \times 2$ contingency table was applied, showing that the association between sampling time and infection was highly significant ($\chi^2 = 42{,}24$; df = 9; $P < 0.001$).

The Bonferroni adjusted test showed that the percentage of groups of psyllids infected during September was significantly different in comparison with consecutive months such as November and December ($\chi^2 = 15.65$; $P < 0.0001$), February ($\chi^2 = 28.93$; $P < 0.0001$), April ($\chi^2 = 14.72$; $P = 0.0001$), May ($\chi^2 = 13.12$; $P = 0.0003$) and June ($\chi^2 = 12.41$; $P = 0.0004$). Differences between February and October were also found ($\chi^2 = 20.43$; $P < 0.0001$).

Sex ratio of C. pyri *adult population*

The ratio of males to females in the pear psylla population over 1 year period is shown in

Table 1. Monthly detection of Pear decline phytoplasma in about 100 *Cacopsylla pyri* insects collected from a pear orchard cv. Williams

Month	No of female groups infected by PD phytopl/groups of females	No of females infected by PD according to the *P* value*	No of male groups infected by PD phytopl/groups of males	No of males infected by PD according to the *P* value*	Total number of groups (total number individual psyllids)	% of groups infected by PD phytopl	% of infected psyllids (total) according to the *P* value*
June	11/20 (55%)	14.5 (18%)	4/5 (80%)	6.6 (33%)	25 (100)	60	20.5
July	14/19 (73.7%)	21.6 (28.4%)	4/7 (57.1%)	5.3 (18.9%)	26 (104)	69.2	25.5
August	9/18 (50%)	11.5 (16%)	6/8 (75%)	9.4 (29.4%)	26 (104)	57.7	19.3
September	16/16 (100%)	64 (100%)	8/8 (100%)	32 (100%)	24 (96)	100	100
October	13/14 (92.9%)	27 (48.2%)	9/11 (81.8%)	15.3 (34.7%)	25 (100)	84.6	41.1
November	5/11 (45.5%)	6.2 (14.1%)	8/14 (57.1%)	10.7 (19.1%)	25 (100)	50	16.8
Dec–Jan	6/12 (50%)	7.6 (15.8%)	7/14 (50%)	8.9 (15.9%)	26 (104)	50	15.9
February	5/21 (23.8%)	5.5 (6.5%)	1/4 (25%)	1.1 (6.9%)	25 (100)	24	6.63
April	7/15 (46.7%)	8.7 (14.5%)	6/10 (60%)	8.2 (20.5%)	25 (100)	52	16.76
May	9/16 (56.3%)	11.9 (18.6%)	5/9 (55.6%)	6.6 (18.3%)	25 (100)	56	18.56

Psyllids were divided in groups of 4 individual males or females and analysed for the presence of PD phytoplasma by nested PCR with universal primers P1/P7 and specific primers f01/r01 from June 2002 to May 2003. * *P* value (Hughes et al., 2001) was used to estimate the number of insects (males or females) infected by Pear decline during the year.

Figure 2. For 10 months of the year (January and March psyllids were not sampled), the sex ratio was calculated, and in six of them (June, July, August, September, February and May) the relationship of males to females was less than 0.6:1. In October and April, coinciding with two peaks in the adult population, this ratio was 0.78: 1 and 0.66:1 respectively and during the winter period (November–December) the ratio was above 1:1 (1.27:1 and 1.16:1 respectively).

Relationship between sex category and PD infection in adult C. pyri

Table 1 shows the proportion of groups (males and females) and the percentages of individual *C. pyri* (males and females) infected by the phytoplasma over the year. Percentages obtained were very similar for both genders; and the $k \times 2$ contingency table showed that there was no association between sex category and PD infection ($P = 0.42$). Moreover, using the estimated number of positive psyllids from the *P* value, the linear regression analysis of infection for both genders showed that there is a good fit for both of them ($R^2 = 0.99$; $P < 0.0001$ for females and $R^2 = 0.96$; $P < 0.0001$ for males) with slopes between both linear regression lines not significantly different ($P = 0.32$).

Transmission of PD phytoplasma to artificial feeding medium depending on sex category

Results of transmission to feeding medium are shown in Table 2. With the exception of February, *Cacopsylla pyri* was capable of transmitting the PD phytoplasma to artificial medium all the year. PD phytoplasma transmission to feeding medium by male and female differed significantly ($\chi^2 = 5.14$; df = 1; $P = 0.02$).

Characterisation of the phytoplasma

The results of PCR-RFLP analyses from DNA extracted from feeding medium are shown in Figure 3. Patterns obtained from RFLP of amplified sequences were indistinguishable from each other. All profiles belonged to the AP group (Figure 3A) and the restriction profile was identical to that of the PD phytoplasma strain and different from the patterns associated with AP (Figure 3B) and

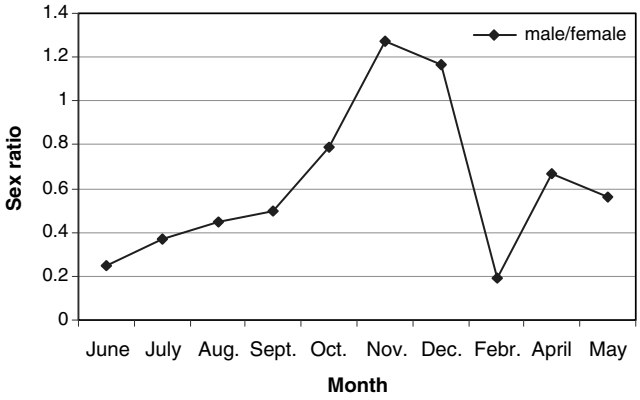

Figure 2. Monthly evolution of the sex ratio (male/female) from an adult *Cacopsylla pyri* population measured from June 2002 to May 2003, using the beating tray method (Burts and Retan, 1973).

European Stone Fruit Yellows phytoplasma (ESFY) (Figure 3C).

Discussion

In north-eastern Spain, *C. pyri* is a vector of PD phytoplasma as found in previous studies in Italy and France (Lemoine, 1984; Carraro et al., 1998). Since this pear psyllid is the most important one in Spain (Portillo et al., 1993), as in other Mediterranean countries (Lemoine, 1984; Stratopoulou and Kapatos, 1995; Carraro et al., 2001), *C. pyri* could also be the main vector of PD associated disease agent.

The percentage of pear psylla carrying the PD phytoplasma showed a seasonal trend through the year, which coincides with the seasonal detection of PD phytoplasma in pear trees (Seemüller et al.,

Table 2. Detection of Pear decline phytoplasma, where about 100 individuals of *C. pyri* were put on sucrose feeding medium in individual microcentrifuge tubes each month, from June 2002 to May 2003

Month	No. of artificial feeding medium from females infected by PD phytoplasma	No. of total females tested after removing psyllids that died the first day	No. of females infected by PD phytopl according to *P* value*	% of females that transmitted PD phytopl to artificial medium according to *P* value*	No. of artificial feeding medium from males infected by PD phyto plasma	No. of total males tested after removing psyllids that died the first day	No. of males infected by PD phytopl according to *P* value*	% of males that transmit-ted PD phytopl to artificial medium according to *P* value*	% of feeding mediums (total) infected by PD phytopl according to *P* value*
June	4	75	13.6	29.4	3	16	5.3	56.6	37.6
July	12	76	21.6	55.6	0	28	5.3	0	45.2
August	7	72	11.5	61.1	7	32	9.4	74.7	69.6
September	14	53	53	26.4	3	28	28	10.7	17.3
October	12	49	23.7	50.7	6	43	14.9	40.2	47.5
November	1	44	6.2	16.1	0	56	10.7	0	6
Dec–Jan	2	48	7.6	26.2	1	56	8.9	11.2	18.1
February	0	66	4.3	0	0	13	0.9	0	0
April	1	49	7.1	14.0	1	24	4.9	20.4	16.3
May	6	62	11.6	51.8	1	36	6.6	15.1	38.5

Presence of PD phytoplama was analysed using the nested-PCR procedure. **P*-value (Hughes et al., 2001) was used to estimate the number of insects (males or females) infected by PD phytoplasma every month. The estimation of females or males containing the PD phytoplasma was calculated discounting psyllids that died the first day.

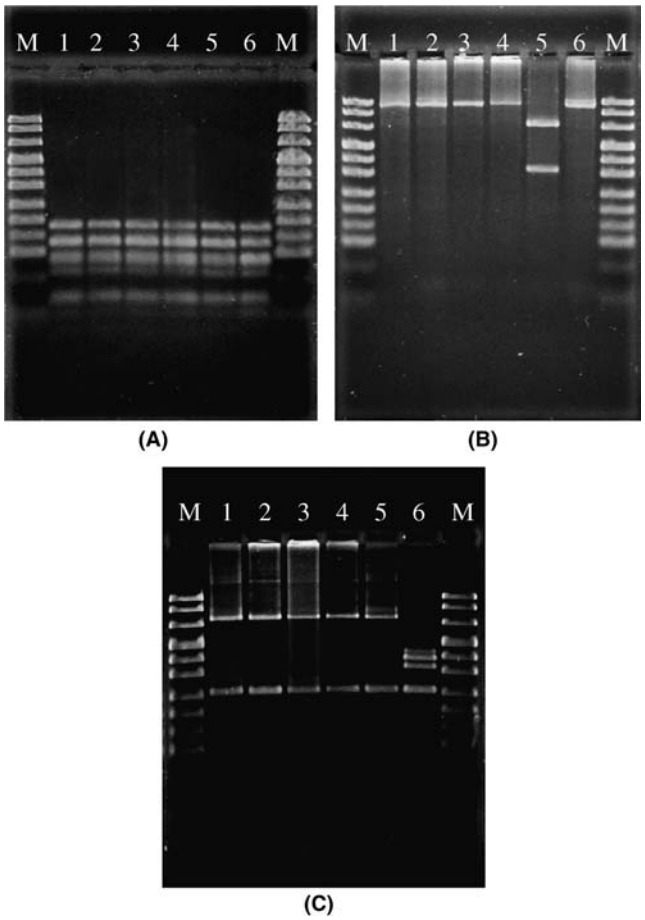

Figure 3. *Tru9*I (A), *Ssp*I (B) and *Rsa*I (C) restriction profiles of final amplimers obtained with nested-PCR of phytoplasma ribosomal DNA. A, fU5/rU3 fragments; B and C, f01/r01 fragments. Lanes 1–4: DNA extracted from sucrose artificial medium, lane 5: AP15 strain, Lane 6: ESFY strain. M: pUC mix marker, 8.

1984). The amount of PD positive *C. pyri* was similar from June to August. In autumn *C. pyri* can acquire the phytoplasma more easily because at this period the colonisation of phytoplasma in the tree is highest (Seemüller et al., 1984), and therefore the highest infection rate was recorded in September. The percentage of pear psylla carrying the PD phytoplasma decreased in October, possibly because the new non-infected winterforms of *C. pyri* adults were emerging. In November all summerform psyllids disappeared and since there are no cases of movement of PD phytoplasma into insect progeny (Fletcher et al., 1998), nymphs that did not feed from infected leaves in October became healthy adults. In these conditions the 16% of PD positive psyllids that appeared could be due to PD positive adult psyllids emerged during October, that acquired phytoplasma easily, and also to phytoplasma still being available in some aerial part of the pear tree. In February, when almost all winterform males have disappeared (Lyoussoufi et al., 1994), and coinciding with the lowest percentage of males collected, PD phytoplasma is not easily accessible in the aerial part of the tree as a great proportion of them overwinters in the roots (Seemüller et al., 1984; Garcia-Chapa et al., 2003b). Therefore psyllids that had not previously acquired phytoplasma were healthy. Similar patterns have been reported in other Mediterranean countries (Carraro et al., 2001) and the United Kingdom (Davies et al., 1998) but not in North America (Blomquist and Kirkpatrick, 2002). Therefore, geographical and ecological factors could be relevant in the acquisition of PD phytoplasma by the pear psylla and consequently in PD transmission.

Males and females were equally able to acquire the phytoplasma. The lack of difference in the number of PD positives could be due to both genders having preference for the same tissues and leaf parts of the tree. These results are in contrast with those obtained with Aster yellows phytoplasma infecting *Macrosteles quadrilineatus* (Beanland et al., 1999).

The feeding medium used to evaluate the transmission of PD phytoplasma is suitable for *C. pyri*. As PD phytoplasma was detected throughout the year in this medium by PCR, it is concluded that the *C. pyri* stylet is capable of traversing the parafilm and also that the sucrose medium used is appetizing for psyllids. Therefore, in contrast to the conventional method of testing plants after a long period of incubation, this method is an easy alternative to check PD phytoplasma transmission. Overwintering *C. pyri* is also able to transmit the disease during winter under laboratory conditions.

It has been found that although there is phytoplasma transmission to feeding medium during the year, rates are not very high. It has been reported that pear psylla have a low efficiency of transmission (Hibino et al., 1971). Percentage transmission to artificial medium was analysed and discussed taking into account only PD positive psyllids. In view of this, in August the highest rate of transmission was obtained. This could be due to psyllids that had acquired the phytoplasma during the previous months, being already able to transmit the phytoplasma in August. During September, all the groups of insects collected were PD positives. Nevertheless, there was no correlation with the percentage transmission, probably because there was a new emerging generation that had acquired PD but had not passed the latency period (Lefol et al., 1994; Cousin, 1995), so they were not able to transmit the phytoplasma. However, in October, the transmission rate was again high.

We have found differences between males and females in PD phytoplasma transmission. These differences could be explained by different behaviour. Females need more nutrients than males for egg production (Swenson, 1971) and are less mobile because of egg-laying (Horton et al., 1994). This behaviour was also seen under laboratory conditions, where females fed for longer periods of time on the sucrose medium in the microcentrifuge tubes, with a consequently better PD transmission. This is in agreement with reports of differences due to sex of *Macrosteles quadrilineatus* in Aster yellows phytoplasma transmission (Beanland et al., 1999).

The results of PD phytoplasma transmission by females are of some considerable interest. The sex ratio observed here in Spain, similar to that of other areas which have been studied (Priore, 1991; Lyoussoufi et al., 1994; Stratopoulou and Kapatos, 1995), indicates that there is a higher proportion of females than males. Almost all the year the sex ratio was less than 1:1, as the mortality rate of males is higher than that of females (Lyoussoufi et al., 1994; Stratopoulou and Kapatos, 1995). Only during the emergence of new pear psyllids and during winter, when the mortality rates for males and females are similar (Stratopoulou and Kapatos, 1995), was the sex ratio above 1:1. Therefore, further detailed investigation is needed on *C. pyri* females to determine how they may be involved in the spread of the disease.

It has been demonstrated that C. *pyri* is a vector of PD disease in Spain. Several factors should be taken into account when trying to control PD disease in Mediterranean climates. These include monthly detection rate of PD in pear psyllids, the efficiency of transmission depending on sex and the use of a sucrose medium as an indicator of PD phytoplasma psylla transmission.

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