*Bulletin of Insectology* ***60*** *(2): 319-320, 2007*
ISSN 1721-8861

# Management of pear decline caused by '*Candidatus* Phytoplasma pyri' in Hungary

**Sándor SÜLE, Gábor JENSER, Éva SZITA**
*Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary*

## Abstract

Pear decline caused by '*Candidatus* Phytoplasma pyri' is a serious disease in Hungary. The disease up to now has occurred sporadically without causing very serious damages. Recently, several 4-5 years old intensive pear orchards in Eastern-Hungary were severely infected with a disease symptomatologically similar to pear decline (PD) caused by '*Ca*. P. pyri'. The affected varieties were Abate Fetel, Williams and Conference grafted on quince C and BA29 rootstocks. To detect if '*Ca*. P. pyri' was associated with this pear disease and with its putative vector (*Cacopsylla pyri*), diseased shoots and psyllas were tested with PCR. The amplified DNA products were digested with *Ssp*I and *Sfe*I restriction enzymes. The results indicate that the likely vector of phytoplasma associated with PD in Hungary is *C. pyri*. In the last two years psyllas were collected every two weeks for PCR assays, and groups of 25 to 100 individuals were caged for 4 weeks on healthy pear seedlings. More than 40% of the investigated winterform psyllas were positive in PCR. The new first and second generations were much less infected. Shoot infections with '*Ca*. P. pyri' were detected from the end of July. To obtain commercially acceptable fruits in Hungary, pear psylla must be controlled with insecticides. For psylla control oil and Vertimec are used in Hungary. This later chemical is absorbed into the leaf tissue and kill psyllids very efficiently. In field trials, it has provided 4-5 weeks of protection under normal growing conditions. A second Vertimec application was made just prior to a late June population increase. After this spray, egg and nymph populations were back down to negligible levels. Practically, it was possible to control pear decline by controlling the vector of the disease.

**Key words:** Pear decline, '*Candidatus* Phytoplasma pyri', *Cacopsylla pyri,* disease control.

## Introduction

Pear decline is an important disease of pear all over Europe where pears are cultivated. In Hungary, up to the recent years the disease has occured rather sporadically (Del Serrone *et al.*, 1998) without causing very serious damages. However, in the last 2-3 years a very serious outbreak occurred in plants grafted on quince rootstocks earlier thought to be tolerant to the disease. The severity of pear decline depends on the rootstocks on which the cultivar is grafted (Poggi Pollini *et al.,* 1995). Pear decline is caused by '*Candidatus* Phytoplasma pyri' that is transmitted by pear psylla. 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 the main vector (Carraro *et al.,* 2001; Garcia-Chapa *et al.,* 2005). In Hungary, *C. pyri* is the predominant psylla on pear trees (Jenser, 1968), but its capability to transmit the phytoplasma has not been investigated. *C. pyri* has two adult forms: a darker winterform, and a light-brown summerform. The winterform overwinters in pear orchards and lay eggs on the pear buds just before or during bud burst. These eggs then develop into the summerform psylla. The summerform has 3-5 generations during the vegetation period. Recent studies indicate that pear decline phytoplasma is able to overwinter in the body of *C. pyri* (Carraro *et al.*, 2001; Garcia-Chapa *et al.*, 2005), and spread the disease all over the vegetative period.

The objectives of the present work are to show the results of a two-year investigation, to identify the phytoplasma in *C. pyri*, to verify the vector infectivity, and to determine the management of this disease in Hungary.

## Materials and methods

Psyllids were collected by several methods (beating, aspiration) from the end of January up to mid of November. Freshly collected or ethanol killed psyllids were homogenized in liquid nitrogen and their DNA was extracted with the CTAB method. DNA was also extracted similarly from fresh midribs of leaves with symptoms and from phloem of non-lignified branches. The extracted DNA was precipitated with ethanol and dissolved in water.

Polymerase chain reaction (PCR) experiments were carried out on the nucleic acid samples from psyllids and plants. The reaction mixtures for direct PCR (20 µl) contained 1 µl of nucleic acid, 0.5 µM of primers, and 10 µl 2X master mix of *Taq* polymerase (Fermentas, Vilnius, Lithuania). Pear decline (PD)-specific primers fPD/rO1 were used as described (Lorenz *et al.,* 1995). When negative results were obtained, the PCR was repeated with P1/P7 primers and nested PCRs were carried out with fPD/rO1. Thermocycling parameters consisted of 1 cycle of 2 min at 94°C followed by 35 cycles of 94°C for 1 min, 53°C for 1 min and 72°C for 1 min. At the end 1 cycle of 72°C for 10 min. Amplification products were separated in 1% agarose gels and stained with ethidium bromide. To identify '*Ca.* P. pyri' 10 µl fPD/rO1 amplicons were digested with 3U *Ssp*I and *Sfe*I restriction enzymes. To detect the infectivity of psyllids, one year old pear seedlings were used. 25 adults of *C. pyri* collected from heavily infected trees were caged on two seedlings every month. The results of infections were checked with PCR assays carried out 6-12 months after that psyllids were caged with plants.

## Results

The first two year experiments indicated that both pear plants and the vector of the disease *Cacopsylla pyri* are infected with '*Ca.* P. pyri'. Symptoms were observed in the spring when leaves remained small and pale green; there were little or no shoot growth and no fruit production. In the summer and autumn, the affected trees developed a premature red colour and their leaves curled down at the main vein. A necrotic line was frequently visible in the bark at the stock/scion union. In the last 2-3 years heavy psylla (*C. pyri*) infestations have been recorded in these orchards. The winterform of psyllas started to move in the middle of February followed by 3-4 summerform generations up to September, when the new winterforms appeared again. The overwintered adults were able to transfer of the phytoplasma to plants.

The comparative study using living and ethanol killed insects showed that good quality DNA for PCR could be isolated from both of them. The use of ethanol may simplify field collection of psylla and make unnecessary to use ice during transportation to the lab. PCR analysis of psylla in different stages showed that the winterforms were heavily infected. Restriction enzyme analysis of the fPD/rO1 product showed that only '*Ca*. P. pyri' could be found in the investigated psylla. PCR analyis of samples from diseased plants was successful from the end of July. After this time both shoots and roots were detectably infected.

## Discussion

To obtain commercially acceptable fruits in Hungary, pear psylla must be controlled with insecticides. The phytoplasma and psylla infection are seemingly well correlated. In the new intensively-planted orchards pear psyllla is so well established that virtually all growers must apply chemical treatments for its control in order to avoid fruit or tree damage caused by psylla and phytoplasma. For psylla control, in the investigated commercial orchard, oil and Vertimec were used. In field trials, Vertimec has provided 4-5 weeks of protection under normal growing conditions. A second Vertimec application at the end of June just prior to a new population increase strongly reduced egg number and nymph populations.

Interestingly in most of the traditional pear orchards where trees were grafted on wild pear rootstocks (*Pyrus communis*) the disease caused by phytoplasma was much less important than in intensively-planted orchards using quince rootstocks, inspite the fact that pear psylla could be found in those orchards as well. This may indicate that the outbreak of the new pear decline epidemic disease in intensive orchards is probably related to the presence of phytoplasma infection in the propagation material.

## Acknowledgements

This study was funded by NKFP 4/012/04 and GVOP 3.1.1.-2004-05-0008/3.0 of the Ministry of Economy of Hungary.

## References

CARRARO L., LOI N., ERMACORA P., 2001.- The life cycle of pear decline phytoplasma in the vector *Cacopsylla pyri*.- *Journal of Plant Pathology,* 83 (2): 87-90.

DEL SERRONE P., LA STARZA S., KRYSTAI L., KOLBER M., BARBA M., 1998.- Occurrence of apple proliferation and pear decline phytoplasmas in diseased pear trees in Hungary.- *Journal of Plant Pathology,* 80 (1): 53-58.

GARCIA-CHAPA M., SABATÉ J., LAVINA A., BATLLE A., 2005.- A role of *Cacopsylla pyri* in the epidemiology of pear decline in Spain.- *European Journal of Plant Pathology*, 111: 9-17.

JENSER G., 1968.- Frequent appearance of *Psylla pyri* in pear orchards.- *Növényvédelem*, 4 (2): 93-96.

LORENZ K. H., SCHNEIDER B., AHRENS U., SEEMÜLLER E., 1995.- Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and non-ribosomal DNA.- *Phytopathology,* 85: 771-776.

POGGI POLLINI C., BISSANI R., GIUNCHEDI L., 1995.- Overwintering of pear decline agent in some quince rootstocks.- *Acta Horticulturae,* 386: 496-499.

**Corresponding author:** Sándor SÜLE (ssule@nki.hu), Plant Protection Institute, Hungarian Academy of Sciences, 1525 Budapest, P.O. Box 102, Hungary.