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The occurrence of '*Candidatus* phytoplasma rhamni' in *Rhamnus cathartica* L. without symptoms

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Abstract

Common buckthorn, *Rhamnus cathartica* L., is a small tree or shrub of Eurasian origin that has become invasive in North America. A survey was conducted during 2010 to evaluate occurrence of buckthorn witches' broom (BWB) disease symptoms induced by '*Candidatus* Phytoplasma rhamni' in the native range of *R. cathartica*. The distribution of phytoplasma-infected trees was identified over a large expanse of Europe, from south-west Switzerland to north-east Serbia. Although 25% of analyzed buckthorn trees were infected with phytoplasma (34 out of 133 plants), all plants were symptomless, indicating a commensal relationship between the phytoplasma and its plant host without negative effects which would lead to disease development.

Key words: common buckthorn, distribution, PCR, phytoplasma, *Rhamnus cathartica*, symptoms.

Introduction

Common buckthorn, *Rhamnus cathartica* L., is a shrub or small tree native to much of Europe and western Asia that has become invasive in North America. *R. cathartica* was introduced to North America as an ornamental shrub in the earlier 1800's and is now naturalized throughout the Upper Midwestern and Northeastern United States and the maritime provinces of Canada.

In 1964 research using native range surveys in biological control programs resulted in over 30 specialized arthropod species recorded on *R. cathartica* in Europe, including nine sap sucking species: six Hemiptera and three Acari (Gassmann *et al.*, 2008). Among these, three are psyllids: *Trichochermes walkeri* (Foerster) and *Trioza rhamni* (Schrank) of the family Triozidae and *Cacopsylla rhamnicola* (Scott) belonging to the family Psyllidae. Psyllids are phloem-feeding insects and have potential to acquire and transmit the phloem-limited pathogens such as phytoplasmas. This ability represents a possible threat to the native plants, if a phloem-feeding insect is introduced as a biocontrol agent in a new geographic area without previous experimentation of its capability to act as a possible vector. A new program was initiated in 2001 taking into consideration increasing concerns over potential non-target impacts of biological control agents and greater demands for high levels of agent specificity (Louda *et al.*, 1997; Pemberton, 2000).

In its native range *R. cathartica* is found to be naturally infected with a phytoplasma - a wall-less, non-culturable, phloem-limited, insect-transmitted plant pathogen of the class *Mollicutes*. A lethal witches' broom disease of *R. cathartica* induced by buckthorn witches' broom BWB phytoplasma ('*Candidatus* Phytoplasma rhamni', 16SrX-E subgroup) was observed for the first time in the Rhine Valley in south-western Germany in the 1990's (Mäurer and Seemüller, 1996). The disease is characterized by brush-like witches' brooms, leaf distortion, phloem necrosis, off-season growth and overall decline of the plant. The main goal of this study was to determine occurrence of '*Ca.* P. rhamni' in the native range of *R. cathartica*.

Materials and methods

A survey was conducted during July and August of 2010 in the buckthorn growing habitats of western and south-eastern Europe. Altogether, 26 localities in five countries were surveyed and overall 133 *R. cathartica* samples were collected (table 1). Characteristic symptoms of witches' broom, which would indicate phytoplasma presence, were not observed on any of the surveyed buckthorn sites. In some localities discrete leaf yellowing and/or small leaves were present on few trees and these were sampled separately and treated as possibly symptomatic. All other sampled *R. cathartica* trees were symptomless.

Total nucleic acids from plant midribs and petioles were extracted using a previously reported CTAB protocol (Angelini *et al.*, 2001). Phytoplasmas were detected by polymerase chain reaction (PCR) amplification of 16S rRNA gene using the universal phytoplasma and group specific primer pairs. Amplification was performed in nested PCR with P1/P7 primers followed by R16F2n/R2 universal primer pair according to Lee *et al.* (1998) or R16(X)F1/R1 primers specific for amplification of 16SrX group and related phytoplasmas (Lee *et al.*, 1995). Amplicons obtained with R16F2nR2 primers were subjected to RFLP analyzes with *Mse*I, *Alu*I and *Hpa*II endonucleases (Lee *et al.*, 1998). '*Ca.* P. rhamni' DNA isolated from naturally infected *R. cathartica* located between Neuhofen and Ludwigshafen in Rhineland-Palatinate (type locality of '*Ca.* P. rhamni'; provided by Bernd Schneider) was used as a reference positive control in all reactions.

Table 1. Geographic origin and number of *R. cathartica* samples analyzed with PCR results on '*Ca.* P. rhamni' presence.

Country	Canton/Region	PCR results positive	analyzed
Switzerland	Geneva	0	7
Switzerland	Vaud	4	5
Switzerland	Fribourg	5	5
Switzerland	Jura	5	18
Germany	Hesse	3	25
Austria	Niederösterreich	9	25
Austria	Burgenland	2	5
Serbia	South Banat	1	22
Serbia	Braničevo	1	5
Serbia	Bor	4	9
Serbia	Zaječar	0	5
Montenegro	Kolašin	0	2
TOTAL		34	133

Results

'*Ca.* P. *rhamni*' was detected in 25% of *R. cathartica* samples, at several sites in all countries surveyed, except for Montenegro (table 1). We have not observed the witches' broom disease symptoms as described by Mäurer and Seemüller (1996), at any locality, and at present we cannot associate the presence of the phytoplasma with any particular symptoms in buckthorn.

Discussion

Trees and shrubs of common buckthorn were found to be infected with '*Ca.* P. rhamni' at almost all surveyed localities confirming previous reports of host association of this phytoplasma with *R. cathartica*. However, the symptoms of witches' broom were not observed. Absence of symptoms on all phytoplasma infected trees could be an indication of commensal relationship between the phytoplasma and its plant host without negative effects which would lead to a disease development. Plants with this kind of symptomless presence of phytoplasma are considered to be a wild reservoir of the pathogen, since they are not affected by its presence, which can be the case with '*Ca.* P. rhamni' and the common buckthorn.

However, occurrence of phytoplasma-infected trees over a wide geographic area within the native range of *R. cathartica* is raising concerns about possible infection of this weedy shrub in its naturalized range in North America, which needs to be tested. Considering that this phytoplasma has been described as associated with witches' broom disease of *Rhamnus*, its influence on *R. cathartica* and other *Rhamnus* species needs to be tested in controlled conditions.

Considering possible vectors of the phytoplasma, the importance of psyllids in the phytoplasma transmission has been recognized only recently, and more comprehensive research on their role as vectors has been elucidated in the past few years (reviewed in Jarausch and Jarausch, 2010). All confirmed and recognized psyllid vectors today belong to a single genus – *Cacopsylla*, and they are transmitting apple proliferation group phytoplasmas (AP, 16SrX) on apple, stonefruit and pear trees. '*Ca.* P. rhamni' has the closest phylogenetic relatedness to the phytoplasmas of the AP group and could be expected to be transmitted by the psyllids. This enforces the need for the assessment of the potential role of *C. rhamnicola* as a vector, elucidation of '*Ca.* P. rhamni' epidemiology, as well as a host-plant specificity of '*Ca.* P. rhamni' to *R. cathartica* and its congeners.

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