

Detection of latent apple proliferation infection in two differently aged apple orchards in South Tyrol (northern Italy)

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Abstract

Apple trees acquiring the agent of apple proliferation, '*Candidatus Phytoplasma mali*', remain infected for their entire life span although symptom manifestation can vary considerably or the disease might never break out. Thus, symptom based assessments do not necessarily reflect the true infection rate of a particular orchard. In order to reveal the level of latent AP infections, root samples from a total of 450 trees were collected in a productive orchard and in a young orchard in South Tyrol (Italy) to be tested by real-time PCR. In the orchard in production the true infection rate of 3.8% was 2.6 times higher than the one that was revealed by symptom observation. In the young apple orchard 10.5% of the trees were infected already during the first year, most probably by the insect vector *Cacopsylla picta*. These trees remained latently infected for at least one and a half years, while two years after detection all positively tested trees of the orchard manifested disease symptoms.

Key words: '*Candidatus Phytoplasma mali*', latency, symptom expression, *Cacopsylla picta*.

Introduction

Apple proliferation (AP), a serious disease of apple trees caused by the '*Candidatus Phytoplasma mali*', has recently been spreading in many European apple growing areas. The pathogen is restricted to the phloem tissue of the plant and is transmitted through vegetative propagation and sap-sucking insect vectors (e.g. Frisinghelli *et al.*, 2000). Some of the characteristic symptoms of AP are witches' broom, elongated stipules, chlorosis and early leaf reddening, while economically relevant are diminished fruit size, quality, and overall yield. However, severity of symptom expression can vary considerably among trees, and within the same plant between different years. Although an apple tree remains AP infected for its entire life span, symptom manifestation can be remitted for up to several growing seasons, or in certain cases the disease might never break out (Seemüller *et al.*, 1984; Carraro *et al.*, 2004). Thus, symptom-based assessments do not necessarily reflect the true infection rate in a particular orchard. In order to gain an estimation of latent AP infection level in commercial orchards, we collected root samples for large-scale laboratory testing from an orchard in production and from a young orchard, both located in areas with high disease presence.

Materials and methods

The first case study involved a productive orchard in a hillside area at Ritten/Renon in the Autonomous Province of Bozen/Bolzano (South Tyrol, northern Italy) comprising 1,013 trees of the cultivar Golden Delicious clone B on rootstock M 9, which were planted in 1996 (table 1). Until sampling in summer 2003 the orchard displayed a cumulative infection rate of about 5% based on symptom monitoring since 2001. Each tree from eight adjacent rows representing one-third of the orchard was sampled by collecting at least three pencil-

thick root pieces. In total, samples from 345 trees were taken for laboratory analysis, five of them with specific AP symptoms.

The second case study was performed in a young orchard in Lana close to Meran/Merano. The orchard of Red Delicious Spur Sandidge on M 9 was established in 2005. It attracted our attention because of the noticeable occurrence of *Cacopsylla picta* (Foerster), immediately after planting. In winter 2005/2006, 105 trees representing 10% of the orchard were selected randomly for sampling. At that time no single plant of the orchard displayed AP symptoms.

Total DNA was extracted from freshly prepared phloem tissue of roots according to the protocol described in Baric *et al.* (2006). Each extracted DNA was analysed in duplicate applying a qualitative TaqMan real-time PCR approach for specific detection of '*Ca. P. mali*' (Baric and Dalla Via, 2004).

After sampling both orchards were monitored for symptom expression for two consecutive growing seasons.

Results

In the production orchard at Ritten/Renon 13 out of 345 samples tested positive for AP phytoplasma, including the five trees previously showing specific symptoms. Eight trees were considered latently infected, since the '*Ca. P. mali*' was detected in the roots, but no symptoms were observed during the preceding monitoring period. Two of the positive trees were younger plants, which were planted to replace previously diseased trees. The true infection rate in the section of the orchard under investigation amounted to 3.77% instead of 1.45%, which would have been revealed based on symptom occurrence (table 1). Two of the latently infected trees (but not the two younger trees) became symptomatic already in fall 2003 and retained specific symptoms during the entire following two-year monitoring period. Six of the AP positive trees remained asymptomatic.

Table 1. Orchards investigated in the two case studies with the latent infection rates at the time of sampling.

Orchard	Year of sampling	no. trees sampled	no. trees symptomatic	no. trees tested positive	Latent infections	
					no.	%
Ritten/Renon Golden Delicious clone B	summer 2003	345 ^C	5	13	8	2.32
Lana Red Delicious Spur Sandidge	winter 2005/2006	105 ^R	0	11	11	10.48

^CSampling of every tree from eight consecutive rows; ^RTrees for testing were selected at random.

In the young orchard in Lana, 11 out of 105 samples tested positive, resulting in an AP infection rate of 10.48% (table 1). None of the trees was symptomatic at the time of sampling. Five of the trees displayed specific AP symptoms for the first time in autumn 2006, while the remaining plants showed typical symptoms in spring 2007.

Discussion

Symptom expression of AP is not necessarily correlated with the presence of the pathogen in the plant, but it seems to depend on the distribution of the phytoplasma in the aboveground parts of the tree (Seemüller *et al.*, 1984; Carraro *et al.*, 2004). Thus, observation of symptoms will not always reveal the true disease status of an orchard, since it will fail to detect latent infections. The effective infection in the investigated section of the production orchard at Ritten/Renon was 2.6 times higher than it was assessed by symptom scoring. Furthermore, six of the eight AP positive trees remained asymptomatic even during the following two-year monitoring period. Since the visual observation of the orchard was initiated five years after its establishment, it can not be excluded that the trees displayed symptoms before, but were overseen by the farmer. However, although there are observations that symptom expression is more pronounced within the first years after infection (Seemüller *et al.*, 1984), other studies demonstrate that a high percentage of infected plants might never become symptomatic (Carraro *et al.*, 2004).

Symptom expression could depend on the condition of a plant at the time of infection and on the titer of the initial inoculum. One can speculate that an older vigorous plant becoming infected by AP could better cope with the pathogen and suppress disease symptoms more efficiently, even for longer periods of time. In contrast, a younger plant, which has not only a lower biomass but also suffers from physiological stress due to recent planting, would develop symptoms sooner and in more pronounced way. Transmission experiments in the screen house, either with insect vectors or with infected plant material, showed that young trees can exhibit symptoms after a minimum incubation period of four to six months (Carraro *et al.*, 2004; Frisinghelli *et al.*, 2000). In the field, however, the latency period might last longer, even in younger trees.

In South Tyrol *C. picta* was noted for the first time in 2004 and since then a tremendous increase of the dis-

ease incidence is being observed even in younger orchards. Farmers often report that this psyllid species is highly abundant on newly planted trees. As this was also the case in the Lana orchard, we suppose that the trees became infected in spring 2005 when *C. picta* was numerous present. A later infection is less probable due to intensive subsequent insecticide treatments. We also rule out the possibility that planting material with an infection rate of more than 10% could have been obtained from the nursery. Many outbreaks of AP in younger apple orchards in South Tyrol thus seem to be caused by infections through insect vectors immediately after planting, followed by a latency period of at least one and a half years.

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