Analysis of the acquisition and multiplication efficiency of different strains of Ca. *Phytoplasma mali* by the vector *Cacopsylla picta*

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Abstract

Based on previous observations during long-term acquisition and transmission trials, studies were carried out under standardized conditions in order to analyse the acquisition and multiplication efficiencies of different strains of Candidatus *Phytoplasma mali* by different developmental stages of *Cacopsylla picta*. The acquisition of Ca. *P. mali* from micropropagated plants infected with different strains was tested for nymphs, larval stages and new adults of *C. picta*. When born on infected plants a nearly 100% acquisition was achieved for all strains of Ca. *P. mali* by *C. picta*. Differences in acquisition efficiency were observed for new generation adults which acquired the phytoplasma as imagines. The multiplication efficiency of the different Ca. *P. mali* strains inside the insects was analysed by quantitative real-time PCR. Significant differences in the capacity of the different strains to colonise the insect were found. Despite high acquisition rates only few subsequent transmission events to healthy test plants could be recorded.

Introduction

For several years now increased efforts have been undertaken to characterize different strains of Ca. *P. mali* by molecular means and to elucidate the impact of strain variability on the plant-pathogen interaction. Using PCR-RFLP analysis of an important number of isolates of Ca. *P. mali* from different European apple growing regions three dominant subtypes could be described: AP, AT-1 and AT-2 (Jarausch et al., 2000). Recently, the complete genome of Ca. *P. mali* strain AT has been sequenced (Kube et al., 2008). For a couple of years, strains with different biological properties (virulent/avirulent) have been differentiated in plants (Seemüller & Schneider, 2007) and an increasing genetic variability was currently discovered by SSCP analysis of the *hfl*B gene within strains or isolates of Ca. *P. mali* (Schneider et al., 2009). However, the biological meaning of the strain variability and especially its role in the pathogen-vector-interaction are still unknown.

Ca. *P. mali* is transmitted under natural conditions by two psyllid species: *Cacopsylla picta* (Frisinghelli et al., 2000; Jarausch et al., 2003) and *Cacopsylla melanoneura* (Tedeschi et al., 2002). In most apple growing regions *C. picta* is the most efficient vector (Jarausch et al., 2007). *C. melanoneura* is even regarded as non-vector in Germany (Mayer et al., 2009).

This study was therefore initiated to obtain the first indications on the influence of the different strains of Ca. *P. mali* on its interaction with the insect vector *C. picta*.

Materials and methods

All studies were conducted with homogeneous micropropageted plant material transferred *ex vitro* into the greenhouse. Healthy plants of *M.* x *domestica* cultivar 'Golden Delicious' as well as 'Golden Delicious' plants infected with Ca. *P. mali* strains PM4, PM5, PM6, PM 7, PM 9 and PM19 were used. Micropropagation, *in vitro* rooting and acclimatisation was done as previously described (Jarausch et al., 1996; Bisognin et al., 2008; Ciccotti et al., 2008). All *ex vitro* plants used were of similar age and stage. Ca. *P. mali* strains were those described by Schneider et al. (2009).

Insect material originated from rearings of *C. picta* on healthy or infected plants. All trials were conducted in greenhouse chambers or climatic cabinets under controlled conditions. Transmission trials with new adults were conducted according to Jarausch et al. (2004). The acquisition efficiency of different strains of Ca. *P. mali* by its vector *C. picta* was analysed in the following versions:

- acquisition feeding of fully developed new imagines from healthy rearings.
- acquisition feeding of larval stages obtained from healthy rearings.
- acquisition feeding during the complete larval development until emergence of new adults (born on infected plants).

Total DNA from individual insects as well as from plants was extracted using the CTAB method described in Jarausch et al. (2004). PCR amplification of phytoplasma 16S rDNA and of a non-ribosomal fragment was carried out following the protocols reported in Jarausch et al. (1994; 2004). Samples with positive signals were quantified for the phytoplasma titer by real-time PCR using the SYBR green method (Jarausch et al., 2004b).

Statistical analysis was done with R statistical software (R development core team, 2009) applying non-parametric tests to the data.

Results and discussion

The acquisition efficiency of the different developmental stages of *Cacopsylla picta* was highest when the individuals were born on infected plants thus reaching an acquisition rate of about 100 % for almost all strains of Ca. *P. mali.* In this variant the specimens could acquire the agent from the inoculum source plant during their whole larval cycle. When the insects were only allowed to acquire the agent as new adult a consistent percentage of about 10 % of the specimens became infected in 2006 and 2007, respectively. This indicates that the fully developed individuals of the new generation do not feed constantly on apple anymore but already prepare for migration to their overwintering host plants. The acquisition of Ca. *P. mali* was also less efficient by individuals which were transferred to infected plants as larval stages (L1-L5). These stages yielded intermediate acquisition rates. Although the larval stages should still feed intensively they might be affected by the technical transfer conditions where the stylet can be damaged.

A detailed analysis of the acquisition capacity of new imagines of *C. picta* showed that strain PM 6 was acquired most efficiently after an acquisition period of 7 days compared to all other strains tested. Interestingly, regardless of the strain, no significant increase of the phytoplasma concentration - as determined by quantitative real-time PCR in single individuals - could be observed after an acquisition feeding of 7 days and subsequent latency period compared to individuals tested after 2 or 4 days acquisition only. This indicates that no significant multiplication occurred in new adults during the latency period and might explain the low transmission rate to healthy plants. However, apart from the main portion of individuals which showed statistically no significant multiplication some individuals reached a high phytoplasma concentration and could be evaluated as potentially infectious.

By analysing the multiplication efficiency of the different strains in the new adults a clear trend became obvious: PM6 strain had a significantly higher multiplicationefficiency when compared to PM5 while the other strains reacted intermediately. Amazingly, the same trend was observed when analyzing the multiplication efficiency of the different strains inside individuals which were born on infected plants. In this case a significant difference in multiplication efficiency was obtained in the order PM6>PM4>PM5. Due to the more important number of individuals tested this result strongly confirms that the PM6 strain of Ca. *P. mali* was the most efficiently acquired, multiplied and transmitted by all developmental stages of *C. picta.* Surprisingly, the analysis of the phytoplasma concentration in the inoculum plants gave exactly the opposite trend with preference for PM4 and low multiplication of PM6 (Bisognin et al., 2008). These results indicate that there is a difference in the multiplication capacity of the various strains of Ca. *P. mali* within the insect and plant milieu.

Conclusions

The study showed that acquisition and transmission with new generation adults of *C. picta* is not efficient in the same season. The acquisition efficiency was highest when individuals were born on infected plants but the subsequent transmission was not efficient. The most reliable and only measurable parameter is the multiplication efficiency once the phytoplasma has been acquired. All data together reveal significant differences among the Ca. *P. mali* strains in colonising their insect vector. The multiplication efficiency of the strains in insects is different from that in plants which may indicate the existence of various interaction mechanisms.

Literature

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