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Effects of temperature and CO_2 on phytoplasma multiplication pattern in vector and plant

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Abstract

Multiplication patterns of two phytoplasmas, chrysanthemum yellows and 'flavescence dorée' were characterized over time in insect vectors and plant hosts under different climatic (temperature, T, and CO_2) conditions. Acquisition and transmission experiments were run in parallel in two phytotrons (P1 and P2). *Euscelidius variegatus* and daisy were used as chrysanthemum yellows hosts, *Scaphoideus titanus* and broad bean as 'flavescence dorée' hosts. Phytoplasma concentration was measured three times in insects and four in plants, at different days after acquisition and inoculation, respectively. Latent period in the vector was also evaluated for both phytoplasmas under the two conditions. On average, phytoplasma multiplication was faster under cooler conditions in insects (P1, 18-22°C; CO_2 400 ppm) and under warmer conditions in plants (P2, 22-26°C; CO_2 800 ppm). An influence of T and CO_2 concentrations was observed for chrysanthemum yellows latency in the vector only. Results suggest that T and CO_2 influence on phytoplasma multiplication is host-dependent.

Key words: leafhopper vectors, *Chrysanthemum carinatum*, *Vicia faba*, phytotron, global warming.

Introduction

Climate change is predicted to have a progressively negative effect on the yield of food crops. As with all species, plant pathogens will have varying responses to climate change. Whilst the life cycle of some pathogens will be limited by increasing temperatures, other climatic factors, such as increasing atmospheric CO_2, may provide more favourable conditions for pathogens. Moreover, climatic variability can affect not only the pathogen, but also plant host and insect vector, as well as the interactions between or among these organisms (Luck *et al*., 2011).

To date, no studies have linked changes of phytoplasma disease impact to changes in climatic conditions (Foissac and Wilson, 2010). Aim of this work was to monitor multiplication rate of two different phytoplasmas in plant and insect vector in two phytotrons under different temperature (T) and CO_2 levels, in order to investigate effects of global warming on phytoplasma epidemiology.

Materials and methods

Healthy colonies of *Euscelidius variegatus* Kirschbaum were reared on oats, *Avena sativa* (L.), of *Scaphoideus titanus* Ball on grapevine, *Vitis vinifera* (L.), and broad bean, *Vicia faba* (L.). Chrysanthemum yellows phytoplasma ('*Candidatus* Phytoplasma asteris', CY, 16SrI-B), isolated in the Liguria Region was maintained by insect transmission on daisy, *Chrysanthemum carinatum* Schousboe. 'Flavescence dorée' phytoplasma, FD 16SrV-C/secY-C isolated in Piemonte, was routinely maintained on broad beans by transmission with *E. variegatus*.

E. variegatus and daisy were used as host species to characterize CY multiplication pattern under different experimental conditions; *S. titanus* and broad bean were used for FD. Two phytotrons were set as described in table 1.

All acquisition and transmission experiments were performed in parallel in the two phytotrons. To identify the CY latent period and to characterize CY multiplication pattern in insect, nymphs of *E. variegatus* were isolated on infected daisy for one week of acquisition access period (AAP) and then maintained on healthy oats. Groups of ten insects randomly sampled were transferred on two daisies for 2/3 days for transmission, starting from 18 days after acquisition (dpa) until 39 dpa, for 9 successive inoculation access periods (IAP). After IAPs, transmission was evaluated by symptom observation and confirmed by PCR. To characterize CY multiplication pattern in insect, *E. variegatus* adults were sampled for DNA extraction, phytoplasma detection and quantification by real time PCR at 10, 20 and 30 dpa (about 15 insects for each sampling date from each phytotron). To characterize CY multiplication pattern in host plant, 6 daisies were inoculated in each phytotron with infective *E. variegatus*. Leaf portions were sampled for DNA extraction, phytoplasma detection and quantification at 5, 8, 13 and 15 days after inoculation (dpi), according to Saracco *et al*. (2006).

To investigate FD multiplication pattern in insects and plants, and latency, experiments were carried out similarly as detailed for CY. Ten consecutive IAPs were performed to evaluate latent period every 2/3 days, starting from 20 to 44 dpa. *S. titanus* individuals were sampled at 10, 20 and 30 to measure FD titer in insect. Six broad beans were inoculated in each phytotron and leaf portions were sampled for phytoplasma detection and quantification at 17, 23, 30 and 37 dpi.

Table 1. Conditions of the two phytotrons employed.

Phytotron	Temperature °C		CO_2 (ppm)	Relative humidity (%)	PAR* (µmol m^{-2} s^{-1}) (16 hours photoperiod)
	Min.	Max			
P1	18	22	400	85-90	0 (night) - 600 (day)
P2	22	26	800	85-90	0 (night) - 600 (day)

* PAR = Photosynthetically active radiation.

Total DNA was extracted from single insects and from 100 mg of leaf tissues and analysed by nested-PCR for the presence of CY and FD with ribosomal primers R16F2/R2 followed by group specific primers R16(I)F1/R1 or R16(V)F1/R1. For quantitative real time PCR, the procedures described in Marzachì and Bosco (2005) were followed for CY and FD quantification, respectively.

Results

Considering all *E. variegatus* sampled at different dates in the two phytotrons, CY was detected in 65 samples out of 74 (88%). No significant differences were found among the frequencies of CY-infected *E. variegatus* sampled at each date in both phytotrons. Two way ANOVA was run on CY titers in *E. variegatus* for phytotron and sampling dates. No significant difference between P1 and P2 was found, regardless sampling dates. However at 10 dpa, CY titer in P1 was significantly higher than in P2. CY titer significantly increased over time in *E. variegatus* in both phytotrons. *E. variegatus* showed a shorter latency in P2 than in P1.

All daisies exposed to CY vectors in both phytotrons were infected, and tested CY-positive in PCR since the first sampling date (5 dpi). Two way ANOVA for phytotron and sampling date was run on CY titers measured in daisies. Average titers were always higher in plants kept in P2, but the difference was significant only at 5 dpi. CY titers significantly increased over time in daisies maintained in both P1 and P2.

Considering all *S. titanus* sampled at different dates in the two phytotrons, FD was detected in 65 samples out of 114 (57%). No significant differences were found among sampling dates and phytotrons. Two way ANOVA for phytotron and sampling date was run on FD titers measured in *S. titanus*. FD titers measured in P1 were significantly higher than in P2 at any sampling date. FD titer significantly increased over time in *S. titanus* in both phytotrons. The same FD latent period in *S. titanus* was recorded in P1 and P2.

All broad beans exposed to FD vectors in both phytotrons were infected. However, in PCR assays two, four and six out of six inoculated plants tested FD-positive at 17, 23 and 30 dpi, respectively, in both phytotrons. Two way ANOVA for phytotron and sampling date was run on FD titers measured in broad beans. Average titers were higher in plants kept in P2, but this difference was significant only at 30 dpi. FD titers significantly increased over time in broad bean, in both phytotrons.

Discussion

The tested experimental conditions did not affect acquisition and transmission capabilities of both CY and FD vectors. On the contrary, higher temperature and CO_2 concentration resulted in a shorter CY latent period in *E. variegatus*. While no clear influence of environmental conditions was observed on CY titer in both plant and vector, FD multiplication in *S. titanus* was faster at lower T and CO_2 concentration. Overall, phytoplasma multiplication was faster under cooler conditions in insects and under warmer conditions in plants. We can suggest that the effect of environmental conditions on phytoplasma multiplication is host-dependent. These are the first experimental results addressing aspects of phytoplasma epidemiology under global warming scenario.

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