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Aster yellows phytoplasma witches' broom (AY-WB; '*Candidatus* Phytoplasma asteris') increases survival rates of *Macrosteles quadrilineatus* and *Dalbulus maidis* on various plant species

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Abstract

Aster yellows phytoplasmas (AYPs) increase the fecundity of the leafhopper vector *Macrosteles quadrilineatus*, and expands the host range preference of the monophagous insect, *Dalbulus maidis*. The objective of this study was to assess whether aster yellows phytoplasma strain witches' broom (AY-WB) also affects survival and plant host preferences of these leafhoppers. The complete genome of AY-WB is sequenced and can be used to study the phytoplasma effect on insect-plant interactions at the molecular level. *M. quadrilineatus* is an effective vector of AY-WB to *Arabidopsis thaliana* (L.) Heynhold, *Nicotiana benthamiana* L., lettuce (*Lactuca sativa* L.), China aster (*Callistephus chinensis* Nees) and maize (*Zea mays* L.). So far, we have observed an increase in survival of *M. quadrilineatus* and *D. maidis* on AY-WB infected plants over healthy plants for all of these plant species.

Key words: Fecundity, plant host range preference, aster leafhopper, corn leafhopper, '*Candidatus* Phytoplasma asteris'.

Introduction

Aster yellows phytoplasmas (AYPs) are serious pathogens of a large range of economical important crops and ornamental plants. As a result, AYPs have been the focus of a great deal of research. Many AYPs are transmitted by polyphagous leafhoppers, including for example the aster leafhopper *Macrosteles quadrilineatus* (Forbes). This leafhopper has a host range of over 300 plant species. Of particular interest is the phenomenon of increased fecundity and plant host preferences of insect vectors in relation to AYP-infected plants. *M. quadrilineatus* has an increased fecundity on some host plant species when they are infected with AYP (Peterson, 1973; Beanland *et al*., 2000). The monophagous leafhopper *Dalbulus maidis* (Delong and Wolcott) is normally adapted to maize (*Zea mays* L.) for oviposition and feeding (Barnes, 1954). However, *D. maidis* will survive significantly longer on AYP-infected China aster (*Callistephus chinensis* Nees) and lettuce (*Lactuca sativa* L.) compared to healthy plants (Purcell, 1987).

The genome of one strain of AYP, aster yellows strain witches' broom (AY-WB), has been sequenced to completion (Bai *et al*., 2006). This will allow the investigation of host preference effects at the molecular level. This study aims to examine the change in plant host preferences of insects in relation to AY-WB-infected plants. Compared here is the survival of the polyphagous *M. quadrilineatus* and the monophagous *D. maidis* on five plant species, both healthy and infected with AY-WB. Furthermore, we started with the development of a feeding assay on *Arabidopsis thaliana* (L.) Heynhold, to monitor differences in feeding behaviour of *M. quadrilineatus* and *D. maidis*. We will use this feeding assay to quantify changes in feeding behaviour related to AY-WB-infected plants.

Materials and methods

M. quadrilineatus is an effective vector of the phytoplasma AY-WB to a wide variety of plant species (Shultz, 1973). This leafhopper was used for AY-WB inoculation of all plants used in the experiments. The plant species used in this study were *A. thaliana*, *Nicotiana benthamiana* L., lettuce (*L. sativa*), China aster (*C. chinensis*) and maize (*Z. mays*).

All plants were exposed to 10 AY-WB-infected *M. quadrilineatus* at a uniform growth stage for 7 days. An equal number of plants had the same treatment with healthy insects. Insects were then removed and plants were observed for symptom development that occurred at ~ 18 days after the first day of insect exposure. All plants were kept separately in individual plastic plant tube cages. To monitor insect survival on AY-WB-infected and healthy plants, 10 adult leafhoppers of approximately the same age of either *M. quadrilineatus* or *D. maidis* were added to the individual tube cages. Monitoring of survival was carried out over 10 days.

For the feeding assays on *A. thaliana*, 5 insects of either *M. quadrilineatus* or *D. maidis* were confined to single leaves using leaf cages. Insects were observed for 5 days. Leaves were harvested for trypan blue, DAB and aniline blue staining. Damage was quantified by counting visible damaged cell areas per unit area of leaf surface.

Results

So far we have observed an increase in survival of *M. quadrilineatus* and *D. maidis* on AY-WB infected plants over healthy plants of all five plant species used in the experiments. *D. maidis* on *N. benthamiana*, lettuce and

China aster had the most marked increases in survival on AY-WB-infected plants than on healthy plants. Three replications of each experiment have thus far been completed and three further replicates for each experiment are currently being carried out.

The feeding assays on healthy *A. thaliana* leaves have shown differences in feeding behaviour between *M. quadrilineatus* and *D. maidis*. *M. quadrilineatus* feeding sites are primarily situated on the primary and secondary veins in the centres of the leaves, whereas *D. maidis* feeding damage is clearly more abundant at the leaf periphery. *M. quadrilineatus* produced also more feeding sites compared to *D. maidis*. Finally, the majority of *M. quadrilineatus* were still alive at 5 days, whereas all the *D. maidis* leafhoppers had died.

Discussion

In this preliminary work some exciting trends are starting to appear. With respect to the survival of the leafhoppers on AY-WB infected plants verses healthy plants of the same species, both leafhopper species appear to survive in higher numbers and for longer periods of time. Furthermore, the feeding assays on healthy *A. thaliana* leaves shows that there is a clear distinction between the feeding behaviours of *M. quadrilineatus* and *D. maidis*. This assay can now be used to study changes in feeding behaviour in relation to AY-WB-infected plants. We will also conduct feeding assays on *N. benthamiana*, lettuce, and China aster, because these plants showed a marked increase in *D. maidis* survival when infected with AY-WB.

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