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Biological and integrated control of plant pathogens
IOBC-WPRS Bulletin Vol. 115, 2016
pp. 117-121

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Marc Bardin, Lea Dantony, Magali Duffaud, Laurent Neu, Michel Pascal, Claire Troulet, Philippe Nicot
INRA, UR407, Plant Pathology Unit, Domaine St Maurice, 84140 Montfavet, France
e-mail: marc.bardin@avignon.inra.fr

Abstract: Various biological methods, mainly based on the utilization of antagonistic microorganisms or plant extracts, have been studied to control powdery mildews. The hyperparasite fungus *Ampelomyces quisqualis* (AQ-10, De Sangosse) is registered in many countries to control powdery mildew on various crops, including *Oidium neolycopersici* on tomato in France. A plant extract from orange (Prev-Am, Vivagro) is registered to control powdery mildew and whitefly on various crops but its effect on tomato powdery mildew is not clearly established. Spray of soluble carbohydrates on leaves of various plant species proved to have an effect against some plant pests but their effect against plant pathogens is not well known, except for trehalose against powdery mildew of wheat.

The objectives of this study were (i) to test the effect of spraying low concentration of sugars (glucose, fructose, sucrose, trehalose) on leaves of tomato against *O. neolycopersici* in controlled conditions and (2) to evaluate the effect of sucrose, AQ-10 and Prev-Am separately and in combinations in semi-commercial tunnel conditions.

Results revealed that spray of sugars on tomato leaves have no significant effect against powdery mildew in controlled conditions. In tunnels, the plant extract Prev-am showed a significant effect against *Oidium neolycopersici* on tomato. Combination of products did not enhance the efficacy of a single product. The relevance of these results to ensure the control of powdery mildew on tomato without fungicides will be discussed.

Key words: *Oidium neolycopersici*, biocontrol, tomato

Introduction

Powdery mildew caused by *Oidium neolycopersici* is among the principal diseases encountered on tomato crops grown in greenhouse conditions. Various biological methods, mainly based on the utilisation of plant extracts or antagonistic microorganisms, have been evaluated for the control of powdery mildews. Among them, a plant extract from the giant knotweed *Reynoutria sachalinensis* was shown to have a high efficiency against powdery mildews on various crops including tomato (Trottin-Caudal *et al*., 2003). The hyperparasitic fungus *Ampelomyces quisqualis* is registered in many countries to control the disease on various crops, including *O. neolycopersici* on tomato in France (http://e-phy.agriculture.gouv.fr/). A plant extract from orange (PrevAm) is also registered in France to control powdery mildews and whitefly on various crops (http://e-phy.agriculture.gouv.fr/) but its effect on tomato powdery mildew is not clearly established. Finally, spray of soluble carbohydrates on leaves of various plant species proved to have an effect against various bioagressors (Derridj *et al*., 2011), including trehalose against wheat powdery mildew (Renard-Merlier *et al*.,

2007), but their effect against *O. neolycopersici* is not well known. Except for the plant extract from *Reynoutria sachalinensis*, none of these products taken separately do show a completely satisfactory efficiency. Their complementarities in culture conditions deserve to be evaluated in order to develop a reliable non chemical method to control the disease.

The objectives of this study were then (i) to test the effect of spraying low concentrations of sugars (glucose, fructose, sucrose, trehalose) on leaves of tomato against *O. neolycopersici* in controlled conditions and (2) to evaluate the effect against *O. neolycopersici* of sucrose, AQ-10 and PrevAm separately and in combinations in semi-commercial tunnel conditions.

Material and methods

Preparation of powdery mildew inoculum and of biocontrol agents

Inoculum of *O. neolycopersici* was produced on fresh tomato plants (4-6 weeks old) in controlled conditions (21 °C, 14 hours light). After 2 weeks, conidia were harvested, diluted in sterile distilled water with tween80. The conidial suspension was adjusted at 10^4spores/ml and used extemporaneously.

In controlled conditions, glucose, fructose and sucrose were tested at 1, 10 or 100 mg/l and trehalose was tested at 100mg/l only. In the tunnels, sucrose alone was sprayed on the plants at 100mg/l. The commercial biocontrol agent AQ10, based on *A. quisqualis*, was provided by De Sangosse (France) as a formulated wettable powder. It was used as recommended by the manufacturer at the dose of 0.07 kg/ha. PrevAm was provided by Vivagro (France) as a formulated liquid product. It was used as recommended by the manufacturer at a dose of 2 l/ha. As a control, we used the formulated plant extract 'Milsana', which was kindly provided by Biofa (Germany) and sprayed on the plants at a dose of 0.3% (V/V) as recommended by the manufacturer for greenhouse tomato production. All these products were used extemporaneously.

Tests in controlled conditions

The effect of sugars on the susceptibility of tomato cv. Monalbo to *O. neolycopersici* was evaluated by spraying either glucose, fructose, sucrose or trehalose over three leaves per plant, 7 or 3 days before or concomitantly with pathogen inoculation. Powdery mildew was sprayed as a spore suspension on the whole plant. Milsana was applied 48 hours before inoculation of powdery mildew. Five plants per replicate were evaluated for each treatment under study and the whole set of experiments was repeated two times. Plants were randomly distributed in controlled growth chambers with climatic conditions conducive to the development of *O. neolycopersici*. For powdery mildew, disease severity (number of colonies/ leaf area) was estimated 14 days after inoculation.

Semi-commercial tunnel conditions

Compatibility of the three biological methods was evaluated in two semi-commercial tunnels (128 m^2 each) on tomato cv. Marmande between April and June 2013. Powdery mildew was sprayed as a spore suspension (10^4 sp/ml, 5l per tunnel) on the whole plant, 5 weeks after their plantation in the tunnels. The first symptoms of powdery mildew appeared approximately 2 weeks after inoculation. Sucrose was sprayed on the plants once a week for 6 weeks, starting one month after plantation. AQ10 and PrevAm were sprayed twice, one week after the apparition of symptoms of powdery mildew and the week after. Milsana was applied once, 48 hours before inoculation of powdery mildew.

Each tunnel was divided in nine plots corresponding to the nine treatments realized: each product sprayed separately or in combination and two control plots (no treatment and Milsana). Each plot was composed of 16 plants. The number of powdery mildew colonies was counted on one leaf per plant, four and five weeks after inoculation.

The data were analyzed using Statistica software (Statsoft, Tulsa, OK, USA). The protective effect of a treatment was expressed as the percentage of reduction in number of pustules on treated plants relative to the control plants.

Results and discussion

Effect of sugars against O. neolycopersici in controlled conditions

Sugars applied as foliar spray on tomato cv. Monalbo had no inhibitory effect on the development of *O. neloycopersici*. On the contrary, a slightly stimulatory effect was observed on the development of the pathogen. However, this trend was not always significant and repeatable. Timing of application or concentration of sugars has no effect on the inhibitory effect of treatments. As an example, the results obtained with the four sugars tested at the dose of 100 mg/ml are presented in Figure 1.

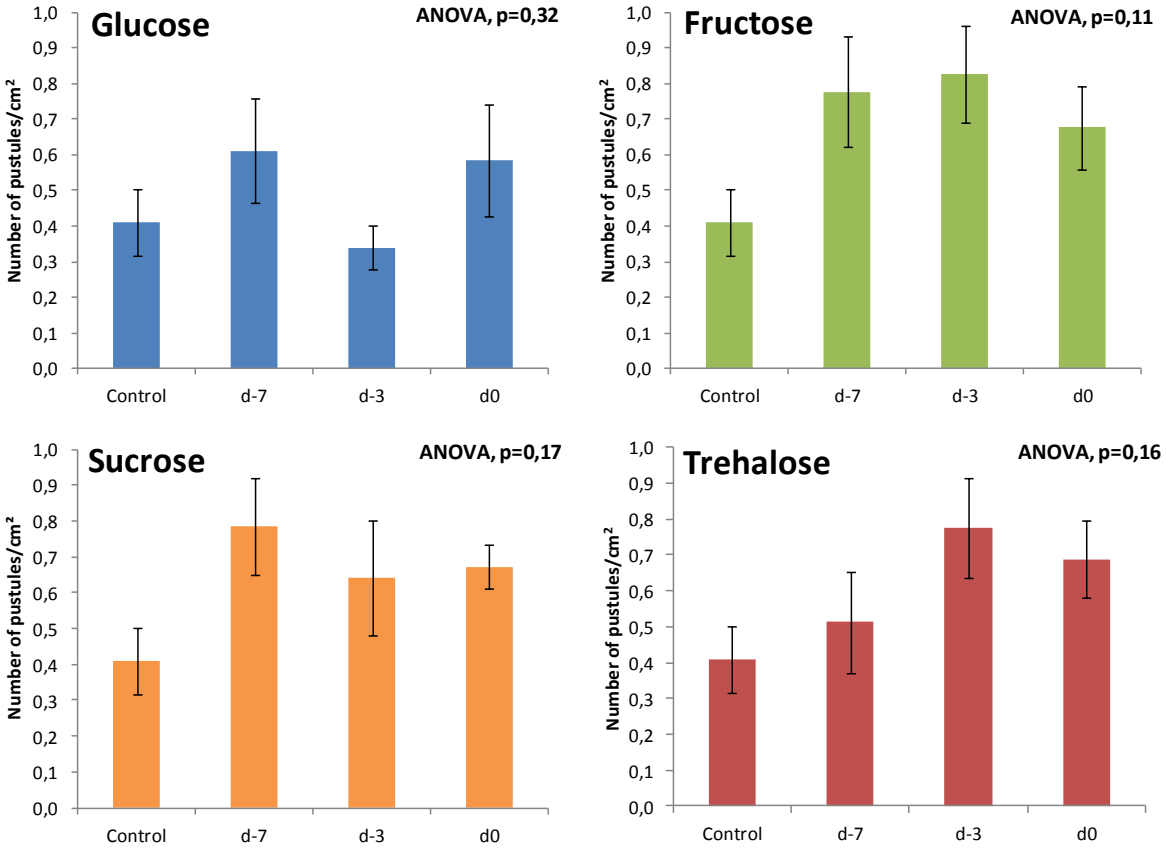

Figure 1. Effect of sugar sprays at 100 mg/l on the development of *O. neolycopersici* on tomato leaves in controlled condition (assessed 2 weeks after inoculation). d-7, d-3 and d0 correspond to a treatment 7, 3 days before or concomitantly with pathogen inoculation.

In controlled conditions, our results suggest that simple sugars sprayed on tomato plants at low doses have no protective effect against the fungus *O. neolycopersici* whatever the timing of their application.

Effect of treatments, done separately or in combinations, against O. neolycopersici in semi-commercial conditions

Whatever the treatment, *O. neolycopersici* incited symptoms on tomato leaves, suggesting that none of the products tested in this study induced a complete inhibition of disease (Figure 2). However, treatments had a significant effect on the number of powdery mildew colonies produced on the leaves regardless of the tunnels (ANOVA, $p < 0.001$ for each tunnel).

Treatments with PrevAm appeared to be the most effective against *O. neolycopersici*, regardless of the tunnels. It demonstrated that this plant extract, in addition to its anti-aphid effect, has a significant effect on powdery mildew of tomato. Therefore, this plant extract has potential to be included into an integrated protection scheme of greenhouse tomatoes.

On the contrary, sucrose and AQ10 had no effect on the development of powdery mildew. This result is quite surprising for AQ10 as, considering that treatments were applied, as previously recommended (Hofstein *et al*., 1996), before the incidence of powdery mildew had reached high levels. This lack of efficacy may be due to the fact that the epidemic of powdery mildew developed rapidly following the massive inoculation of spores of *O. neolycopersici*.

Nevertheless, the application of these two products in conjunction or in alternation with PrevAm had no negative effect on the efficacy of the orange extract. A repetition of this experiment is currently underway to confirm these results.

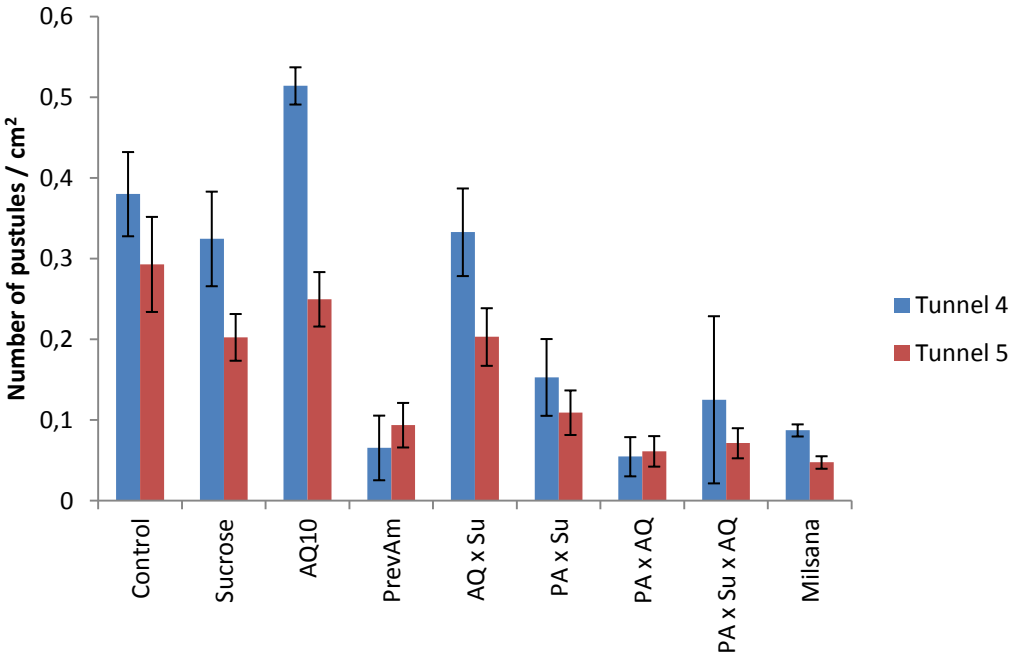

Figure 2. Effect of treatments on the development of *O. neolycopersici* on tomato leaves with each product sprayed separately or in combination (assessed 5 weeks after inoculation).

Acknowledgements

The authors thank the companies who kindly provided samples of the biocontrol products. Thanks are also due to the plant production team of the INRA Plant Pathology Unit (Joël Béraud, Michèle Leplat, Laurent Paradis, Nathalie Truglio) for their excellent technical assistance. This study was supported in part by the FP7-KBBE European Project PURE and by the Ecophyto2018-ONEMA-MAAP French National project USAGE.

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