Induced resistance in plants against insects and diseases
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Potential benefits and limitations of grapevine self protection induced by a beneficial microorganism

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Abstract: Some beneficial microorganisms can reduce disease symptoms through activation of the induced systemic resistance (ISR). Scarce knowledge is available on the efficacy, persistence and fitness cost of ISR in non-model systems. Our aim was to characterize the resistance against *Plasmopara viticola* activated in grapevine by the biocontrol agent *Trichoderma harzianum* T39. T39 activated a systemic resistance and reduced downy mildew symptoms at a level comparable to treatments with benzothiadiazole (BTH). However, if only the treated leaves were considered, T39 induced a lower protection level and a shorter persistence of the effect compared to BTH. In addition, BTH treatments entailed energy costs, which strongly reduced grapevine growth, but T39 treatments did not affect photosynthesis and plant growth. These results suggest the activation of different defense pathways in grapevine after BTH and T39 treatment.

Key words: benzothiadiazole, biological control, downy mildew, grapevine, *Plasmopara viticola, Trichoderma harzianum*, *Vitis vinifera*

Introduction

Several types of non-pathogenic microorganisms can improve the plant performance by suppressing disease symptoms through activation of the plant-mediated induced systemic resistance (ISR, Van Wees *et al*., 2008). In contrast to systemic acquired resistance (SAR), ISR is usually not associated with direct upregulation of defense genes before pathogen attack, but with the activation of a priming state (Verhagen *et al*., 2004). Primed plants display faster and stronger activation of cellular defense responses after pathogen challenge, without major fitness costs under pathogen-free conditions (Van Hulten *et al*., 2006). ISR is usually characterized by a broad-spectrum effectiveness against various types of pathogens or insects (Van der Ent *et al*., 2009) and it can be therefore, regarded as a promising biocontrol strategy for controlling crop diseases. However, the molecular mechanisms underlying the priming state are still poorly understood and scarce information is available on the efficacy, persistence and fitness cost of ISR in non-model plants.

We characterized the systemic resistance against downy mildew disease activated in susceptible grapevine by *Trichoderma harzianum* T39, which induces the ethylene/jasmonic acid (ET/JA)-dependent systemic resistance in *Arabidopsis thaliana* (Korolev *et al*., 2008). The efficacy against downy mildew and the metabolic costs for the plant of *T. harzianum* T39 treatments were compared with the application of benzothiadiazole (BTH). The characterization of biocontrol mechanisms is an important starting point to develop efficient tools for organic farming or to reduce chemical fungicides in the vineyards.

Material and methods

Plants and resistance inducers

Two-year-old plants of the susceptible *Vitis vinifera* cv. Pinot noir, grafted on Kober 5BB, were grown for three months in a greenhouse under controlled conditions (20°C, 70 ± 10% RH) in 2.5l pots. Each plant had two shoots and each shoot had 8-10 leaves.

Treatments with the resistance inducers

Water suspension of 8g/l TRICHODEX (prepared according Makhteshim Ltd., Israel), corresponding to 10^5 conidia/ml of *T. harzianum* T39, and of 0.5g/l of BTH (Bion; Syngenta, Switzerland) were used. The 4-5 basal leaves or 5-6 leaves on one side of each shoot were treated three times with the resistance inducers 1, 2 and 3 days before pathogen inoculation. Leaves that had to be kept untreated were covered with polyethylene bags and the other leaves, marked by a label on the petiole, were treated and then bags were removed. As control treatment, plants were sprayed onto all the leaves with water 6h before pathogen inoculation. Manual air sprayers were used for all treatment applying 20-40ml/plant.

Pathogen inoculation and symptoms evaluation

Plasmopara viticola sporangia suspension was prepared by washing fresh sporulating leaves with cold water and adjusting to 10^5 sporangia/ml. Abaxial leaf surfaces were sprayed with the sporangia suspension using a hand sprayer (20-40ml/plant). Inoculated plants were incubated overnight at 20°C and 95% RH and maintained under greenhouse controlled conditions for 10 days. At the 11th day disease severity was assessed as percentage of infected leaf area (sporulation) of treated (local effect) and untreated (systemic effect) leaves after incubation at 95% RH overnight.

Analysis of metabolic parameters during resistance induction

All the leaves of each plant were treated three times per week with water (untreated control) or with the resistance inducers. Plant growth was evaluated by weekly counting the number of leaves on each plant, by measuring shoots, leaves and roots fresh and dry weight at the end of the experiment. Chlorophyll content of all leaves was weakly evaluated as leaf transmittance measured by a SPADmeter (Steele *et al*., 2008).

Results and discussion

Local and systemic resistance induced by* Trichoderma harzianum *T39 or BTH

Leaf treatments with *T. harzianum* T39 or BTH increased grapevine resistance against downy mildew. In particular BTH-treated leaves showed a stronger disease reduction than the *T. harzianum* T39-treated ones (Fig. 1). Differences in the activation and persistence of resistance elicited by BTH or *T. harzianum* T39 treatments were also observed. In particular, a single preventive application of BTH was sufficient to induce a strong protection against downy mildew, and the BTH-induced resistance persisted for long time (up to 14 days). In contrast, *T. harzianum* T39-induced resistance persisted for less than 7 days after elicitation, and it had to be applied at least twice or three times (1, 2 and 3 days before inoculation) to obtain a reduction of disease severity comparable to BTH (Perazzolli *et al*., 2008).

In addition to the local effect, the systemic activation of grapevine resistance was also observed in untreated leaves of elicited plants (Fig. 1). The systemic effect of BTH and *T. harzianum* T39 were comparable, both with basal or lateral treatments. The leaves treated with the inducers had the highest levels of resistance, without BTH and *T. harzianum* T39 affecting the *P. viticola* sporangia germination (Perazzolli *et al.*, 2008). The systemic resistance activation indicates that a plant-mediated resistance induction was the main process involved in the disease reduction.

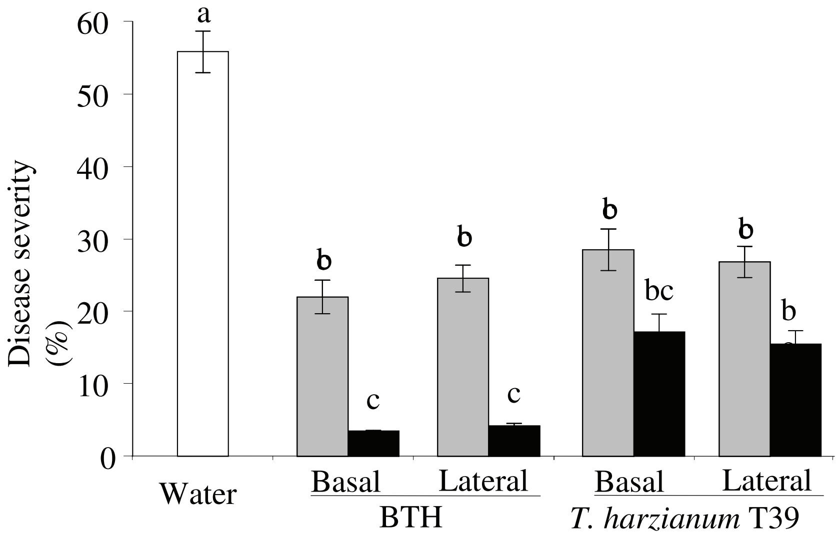

Figure 1. Effect of *Trichoderma harzianum* T39 and benzothiazole (BTH) treatments on downy mildew severity on grapevine leaves. Basal leaves (basal) or the leaves of one side of each shoot (lateral) were treated with the resistance inducers and downy mildew severity was analyzed locally (black columns) and systemically (grey columns) and compared with water treated plants (white column). For each treatment the mean severity and standard error of nine replicates are reported; different letters indicate significant differences (Tukey's test, α = 0.05).

Metabolic costs of grapevine resistance induced by* Trichoderma harzianum *T39 or BTH

In absence of pathogen infection, *T. harzianum*T39 treatments had no effect on plant growth, whereas BTH treatments strongly affected grapevine growth. In particular, the repeated application of *T. harzianum*T39 did not reduce the number of leaves (Fig. 2) and fresh weight of leaves, shoots and roots (data not shown). In contrast, the shoot growth (Fig. 2) and the leaves chlorophyll content of BTH-treated plants was significantly lower than untreated control plants (data not shown), indicating that BTH could entail energy cost to the allocation of resources into defense processes or it could be toxic to the plant after recurring applications.

The differences of resistance activation and the effects on growth parameters suggested the activation of different defense pathways after BTH and *T. harzianum* T39 treatments. In particular, the absence of evident metabolic costs in the *T. harzianum* T39-treated plants suggested the activation of a priming state in grapevine. BTH is more active against downy mildew than *T. harzianum* T39, but it may pose risks for grape quality. More knowledge of the mechanisms of defense activation is necessary in order to optimize the induction of the *T. harzianum* T39-elicited plant self-protection, which at the moment is too low for an application under commercial field conditions.

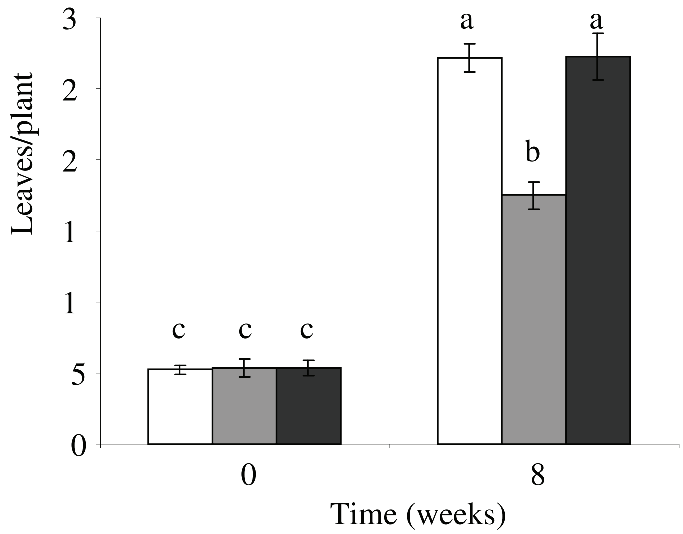

Figure 2. Effect of repeated water (white columns), benzothiazole (grey columns) or *Trichoderma harzianum* T39 (black columns) treatments on grapevine growth, measured as number of leaves/plant. For each treatment the mean severity and standard error of ten replicates are reported; different letters indicate significant differences (Tukey's test, α = 0.05).

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