Bulletin of Insectology ***60*** *(2): 313-314, 2007*
ISSN 1721-8861

Activity of synthetic and natural compounds for phytoplasma control

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Abstract

A sustainable and rational procedure to evaluate the activity of synthetic and natural substances towards phytoplasma agents of important tree diseases was developed with the aim of finding new strategies of control. The method of testing is based on 1) the utilization of *Catharanthus roseus* plants; 2) the artificial infection of periwinkles with two phytoplasma strains; 3) scion dipping, selected as the most suitable method of applying the substances.

A series of natural and synthetic compounds were chosen among a) new agrochemical entries, registered as plant-resistance inducers; b) secondary metabolites of fungal and plant origin; c) new and known biologically active substances never tested before for their antiphytoplasmal properties. The activity of this series of compounds on healthy and phytoplasma-infected periwinkles was evaluated on the basis of several parameters: phytotoxicity, evolution of symptoms and microscope observations. The involvement of the third component in the triangular interaction between phytoplasma, plant and compound is discussed.

Key words: Antibiotics, periwinkle, secondary metabolites.

Introduction

Phytoplasmas are widely spread plant pathogen prokaryotes affecting many plants and crops and responsible of serious diseases in orchard and vineyard trees. The complex and obligately clenched life cycle they have in the body of specific vectors (homopterous insects) and in the host phloem of the plants is far from being totally known. Symptoms can vary in severity not only in different plant species, but also in the same species either over time or season and plant age. Satisfactory management is based only on the control of vectors, due to the lack of efficient agrochemicals and the absence of resistant cultivars.

A research program to test the antiphytoplasmal activity of synthetic and natural compounds was prepared and preliminary results are reported.

Materials and methods

Catharanthus roseus (periwinkle) was chosen as a test plant, since it is herbaceous plant with shorter growth stages than a tree and it is a symptomatic host of several phytoplasma strains. The plants were grown from seeds in controlled conditions all over the year in greenhouse.

Two phytoplasma strains, belonging to chrysanthemum yellows group - 16SrI - and to elm yellows group - 16SrV -, were chosen among the most representative strains of phytoplasmal diseases (Bertaccini, 2007). They were maintained in periwinkle by grafting healthy plants.

Scion of healthy and infected periwinkles were dipped in Eppendorf vials containing treating or control solutions before grafting.

Several compounds were tested by directly diluting them in water or in dimethylsulfoxide 0.6% v/v in water at different concentrations. Molarities of the treating solutions were comprised from 1 to 10 mM. The amount of absorbed compound was calculated in µmoles per scion, by measuring the absorbed solution at known concentration.

The activity of the compounds was recorded by means of evaluation of symptoms, phytotoxicity and microscope observations of longitudinal and cross sections of plants, stained with Dienes and DAPI stains. All plants were compared with respective controls (healthy and infected plants, not treated and treated).

Results and discussion

Preliminary investigations, carried out by applying compounds in several ways (spraying, root absorption and scion dipping), and at different concentrations and number and timing of treatments, proved scion dipping to be the most suitable method of testing the substances for our experimental conditions, as it needed the lowest amount of the compound. The method of testing, based on only one treatment performed before grafting, was optimized in order to choose the most compatible concentration with the solubility of the compounds, phytotoxicity and activity. The dipping method allowed to correlate activity with the amount of the absorbed compound.

Until now, the anti-phytoplasmal activity of ten natural and synthetic compounds was tested and compared with that showed by four known antibiotics. The compounds that can be first candidates for our strategy were selected on the basis of their mechanism of action among different classes of chemicals, which could be grouped as follows: a) two compounds are referred as plant-resistance inducers, known to trigger host-defence reactions; b) five compounds are secondary metabolites of fungal and plant origin; c) three other compounds were never tested before for their antiphytoplasmal properties.

The evaluation of the sensitivity of phytoplasma strains to this series of compounds and the responses of

periwinkles to the treatments will be a part of a forthcoming paper. The rational of this program is based on the hypothesis that by inserting a third biologically active compound in the plant-pathogen system, new deepening could be added to the knowledge of the dependency of phytoplasma proliferation in the plant and to the identification of an efficient anti-phytoplasma drug in the interaction.

The use of antibiotics was suggested as model compounds to which phytoplasmas could be sensitive. Their chemical structure in relation with their activity could suggest that activity, if existing, might be related to a particular toxigenic group occurring in the molecular structure, which in turn could serve as a model for a newly constructed antiphytoplasmal compound. The four antibiotics tested until now (tetracycline, oxytetracycline, streptomycin, erythromycin A) were all capable to determine a delay in symptom appearance and phytoplasma multiplication although not active in blocking phytoplasma infection (Mcmanus *et al*., 2002), (figure 1).

The two plant resistance inducers (fosetyl Al, and chitosan low molecular weight), known to provide protection against diseases (Sticker *et al*., 1997), gave different results, being fosetil Al more efficient than chitosan. The elicitated metabolism needs more explanation, suggesting that contradictory results must be explained taking into account other variables, such as density and pH of the treating solutions.

The secondary metabolites of fungal (cercosporin, cladosporol and spirolaxin) and plant origin (pulegone and carvone) were chosen for their antibiotic activity (Assante *et al*., 2006; Bava *et al*., 2006; Daub and Chung, 2007; Rafii and Shahverdi, 2007). The trials gave some insights on which mechanism of action could positively affect the host-pathogen system: cercosporin, a photosensitive compound capable to transfer absorbed energy to oxygen with generation of toxic activated oxygen species, in turn can affect cell membrane activity; cladosporol is a β-glucan biosynthesis inhibitor; spirolaxin is a DNA topoisomerase inhibitor. The lack of conspicuous activity of these compounds suggested that neither peroxidation or β-glucan biosynthesis or β-tubulin antagonist may be involved in phytoplasma control, although the low number of μmoles absorbed from the scion for their low solubility in a harmless solvent prevented in-depth activity. Pulegone and carvone were phytotoxic and not active. All these compounds will be tested again by optimizing the method of application and absorption.

Similarly, also the discrepant results obtained by treating infected periwinkles with a mixture of riboflavin-methionine, a complex salt of potassium Al and Dienes stain (Dong and Beer, 2000; Mills *et al*., 2006) indicated that the interaction plant-phytoplasma-compound must be exploited to elucidate the physiological modifications in the phloem sap composition and in the balance of plant growth regulators. The combined reading of the results obtained revealed that the insertion of a third component in the plant-phytoplasma interaction potentially affected several processes. However, the lack of finding a totally active compound together with the difficulty of culturing these pathogens outside their host still remains a major concern of our efforts to understand for the development of phytoplasma-recovered crops.

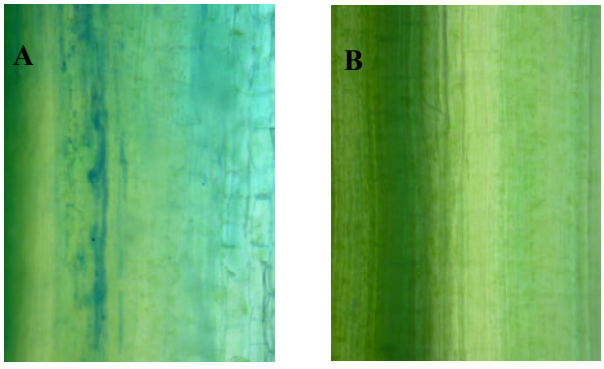

Figure 1. Microscope preparations of infected not treated (A) and tetracycline-treated (B) periwinkles (longitudinal section of the stems).
(In colour at www.bulletinofinsectology.org).

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