Biological and integrated control of plant pathogens
IOBC-WPRS Bulletin Vol. 117, 2016
pp. 238-241

Identification and characterization of volatile organic compounds active against barley pathogens

Caroline De Clerck[1], Amine Kaddes[1], Marie Fiers[1], Lucie Jallais[1], Olivier Parisi[1], Marie-Laure Fauconnier[2], Sébastien Massart[1], M. Haissam Jijakli[1]
[1]Gembloux Agro-bio Tech, Integrated and Urban Plant Pathology Laboratory, Gembloux, Belgium; [2]Gembloux Agro-bio Tech, Unit of General and Organic Chemistry, Gembloux, Belgium
e-mail: caroline.declerck@ulg.ac.be

Abstract: Barley is threatened by various edaphic fungal diseases. Today, since most of the chemicals used for crop protection are being forbidden, there is a growing need of sustainable ways to control these diseases. In this paper, the volatile interactions that take place below ground between barley roots and two pathogenic fungi, *Cochliobolus sativus* and *Fusarium culmorum* were investigated and the effect of fungal volatiles on barley growth and the effect of barley root volatiles on fungal growth were evaluated by cultivating both organisms in a shared atmosphere without any physical contact. We show that the blend of VOCs emitted by infected barley roots decreased *C. sativus* growth by 13 to 17% while having no significant effect on *F. culmorum*. Methyl acrylate and methyl propionate were afterwards identified as the two molecules of the blend responsible for the growth reduction observed. The efficiency of these organic esters on a large panel of pathogens was tested and complete growth inhibition was obtained for five of them. Our results open promising perspectives concerning the biological control of edaphic diseases.

Key words: barley, volatile organic compounds, *C. sativus*, *F. culmorum*

Introduction

Barley has high agronomical significance (123 million tons produced in the world in 2010) and is sensitive to many diseases (FAOstat, 2012). In particular, common root rot caused by *Fusarium culmorum* and *Cochliobolus sativus* is a common barley foliar pathogen worldwide. The disease is particularly aggressive under conditions of high relative humidity and temperature, where yield losses can rise up to 33% (Karov *et al*., 2009). Today, following consumers pressure for an eco-aware and health friendly agriculture, the amount of chemicals pesticides available for cereal protection is decreasing (Hossard *et al*., 2014). There is thus a growing need for new sustainable ways to control cereal diseases.

Plants are able to interact with their environment by emitting volatile organic compounds. Since the 1980's, the effects of VOCs on their biological environment and their potential as bio-pesticides have been on particular interest for the scientific community (Dudareva *et al*., 2006; Morath *et al*., 2012). However, most studies focused on aerial emission and studies of below-ground emissions of VOCs only started recently.

The objective of this paper is to show what kind of volatile interactions can take place between barley and the pathogenic fungi *C. sativus* and *F. culmorum*. The effects of the total VOCs emission from healthy and infected barley root on fungi, and *vice versa* will be assessed. Finally, VOCs with potential fungicidal effects will be identified in the blend of molecules emitted by infected barley roots.

Material and methods

Fungal strains

F. culmorum (MUCL 28166) and *C. sativus* (MUCL 46854) strains were provided by the Belgian Co-ordinated Collection of Microorganisms (BCCM – MUCL) (Louvain-la-Neuve, Belgium). They were stored on PDA medium (Merck KGaA, Darmstadt, Germany) at 23 °C and by cryopreservation at -80 °C.

In vitro co-culture of fungi and barley

In order to investigate the effect of fungal VOCs on barley and *vice versa*, barley and fungi were grown in different compartments of a co-culture device, sharing the same atmosphere. VOCs were thus the only way the two organisms could communicate. Moreover, barley leaves were removed from the co-culture device in order to only take into account the VOCs emitted by the roots (Figure 1).

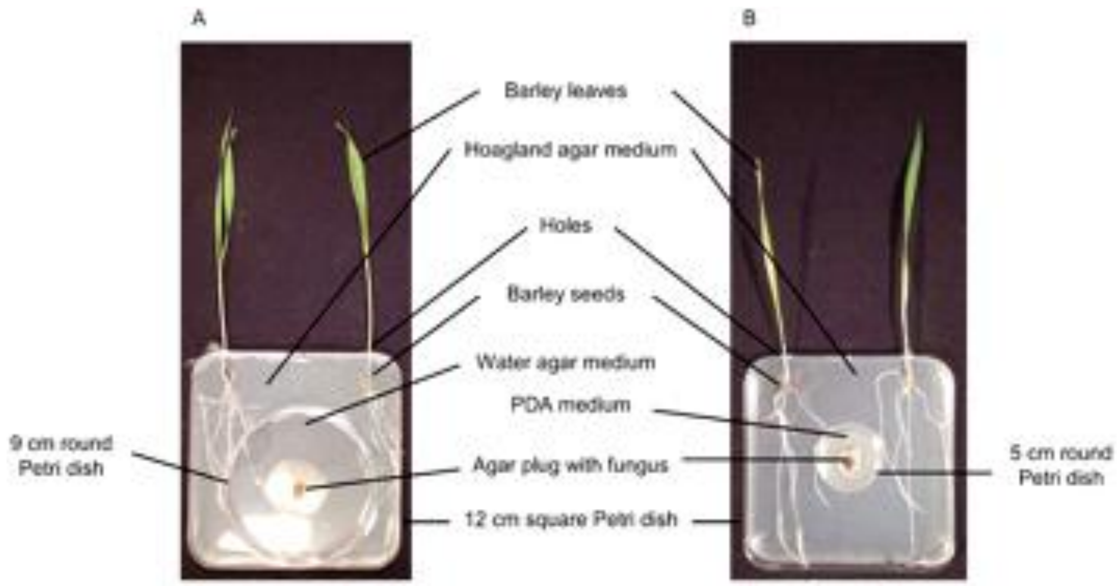

Figure1: Experimental devices for *in vitro* co-culture for the study of the effects of VOCs from non-infected or infected barley roots on pathogenic fungi (A) and for the study of the effects of fungal VOCs on barley (B) (Fiers *et al*., 2013).

Evaluation of the effect of selected VOCs on pathogen growth

Each molecule was mixed with 40 ml of water agar (1% agar (Difco, France)) then poured in cell culture flasks of 600 ml (VWR, Belgium). After medium solidification, a 70 mm disk of an active culture of the pathogen was placed in the center of medium. The cell culture flasks were placed in a growth chamber under LED light (94 mmol photons/m^2/s) with a 16 h L: 8 h D photoperiod at 22 °C. The radial growth of the fungus was measured each 24 h with a graduated ruler until 240 h.

Results and discussion

Our results show that the VOCs emitted by *C. sativus* decreased barley leaf surface by 19%. This allows us to conclude that fungi can affect plant growth by emitting VOCs and suggest that pathogenic fungi interact with plants through volatiles. This phenomenon could be part of the "pathogenic strategy" of fungi. VOCs emitted by pathogenic fungi weakening plants before any physical contact between the two organisms.

More interestingly, we saw that the diameter of *F. culmorum* grown with non-infected barley roots was slightly larger than when it was grown with infected roots. However, significant differences were observed only punctually. Therefore, it is difficult to conclude that the VOCs released by infected barley roots had a long-term effect on *F. culmorum* growth. However, the diameter of *C. sativus* was significantly smaller (more than 13% 168 hours after infection (hai) and up to 17% 192 hai) when the fungus shared the atmosphere of *F. culmorum*-infected barley roots. Similar results have been obtained when the fungus shared an atmosphere containing VOCs from *C. sativus*-infected roots (13% of growth diminution 192 hai).

In order to determine which VOC of the blend emitted by infected barley roots were responsible for this growth reduction, the separate effect of the major compounds emitted during the interaction were tested. Methyl propionate (MP) and methyl acrylate (MA) were identified as the most efficient compounds responsible for the pathogens' growth inhibition.

The effect of MA was particularly important, going up to 87 and 91% of growth inhibition for *F. culmorum* and *C. sativus*, respectively. For MP also growth inhibition was important, going up to 81 and 91% for *F. culmorum* and *C. sativus*, respectively. The efficiency of these organic esters was then tested on seven pathogenic fungi and two bacteria. Complete growth inhibition was obtained for five of the tested fungi. The effect of the molecules on bacteria was more contrasted but still statistically highly significant.

To go further, we have investigated the mode of action of these molecules, which seem to act on cells membranes and to destabilize ion channels and proton pumps.

In conclusion, the effects of MA and MP observed *in vitro* are promising and the studied esters could be regarded as an interesting and innovative starting point in the development of a sustainable way to control barley's diseases.

References

Dudareva, N., Negre, F., Nagegowda, D. A. & Orlova, I. 2006: Plant volatiles: recent advances and future perspectives. Critical Reviews in Plant Sciences 25(5): 417-440.

FAOSTAT website. Available at: http://faostat.fao.org/. Accessed 2012 September 10.

Fiers, M., Lognay, G., Fauconnier, M.-L. & Jijakli, M. H. 2013: Volatile compound-mediated interactions between barley and pathogenic fungi in the soil. PLoS ONE 8(6) doi: 10.1371/journal.pone.0066805.

Hossard, L., Philibert, A., Bertrand, M., Colnenne-David, C., Debaeke, P., Munier-Jolain, N., Jeuffroy, M. H., Richard, G. & Makowski, D. 2014: Effects of halving pesticide use on wheat production. Scientific Reports 4: 4405.

Karov, I. K., Mitrevand, S. & Kostadinovska, E. D. 2009: *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*): Causer of barley leaf lesions and root rot in Macedonia. Zbornik Matice Srpske za Prirodne Nauke. doi: 10.2298/zmspn0916167k.

Morath, S. U., Hung, R. & Bennett, J. W. 2012: Fungal volatile organic compounds: A review with emphasis on their biotechnological potential. Fungal Biology Reviews 26(2-3): 73-83.