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Protective effect of organic substrates against soil-borne pathogens in soilless cucumber crops

Virginie Montagne[a,b,c,d,*], Hervé Capiaux[b], Patrice Cannavo[b], Sylvain Charpentier[b], Sophie Renaud[c], Emilie Liatard[c], Claire Grosbellet[c], Thierry Lebeau[d,**]

[a] Plateforme d'Analyse Moléculaire Biodiversité-Environnement, IUT, 85035 La Roche sur Yon, France
[b] IRSTV-AGROCAMPUS OUEST, UP EPHor, 49042 Angers, France
[c] FLORENTAISE company, 44850 Saint-Mars du Désert, France
[d] UMR 6112CNRS, LPG, 44322 Nantes, France

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ABSTRACT

The development of sustainable crop protection is expected by vegetable producers and highly encouraged by authorities. For crops grown in soilless systems, vegetable fibers are relevant for both agronomical and plant protection purposes. This work examines their potential against the soil-borne pathogen *Fusarium oxysporum* f. sp. *radicis-cucumerinum*.

Wood fiber, coir fiber and peat were tested over two cucumber cropping periods. Fusarium blight symptoms were monitored on cucumber, and fungal community structure (PCR-TTGE) in substrates. Substrate sterilization and bio-augmentation with antagonistic strains were also studied; they did not modify protection. Compared to the other substrates, wood fiber increased protection at the end of the first assay, but did not during the second assay. Differences in crop season and plant density may have impacted on cucumber physiology and may have indirectly modified rhizosphere fungal community structure.

The sole determination of microbial activity in substrates is not sufficient to predict protection. Growth conditions, substrate type and the microbiome altogether impacted on the protection of cucumber.

1. Introduction

Consumers are more and more mindful of the quality of the vegetables they consume. In parallel, the impact of agricultural practices on the environment is more and more under focus. New agricultural systems, such as soilless culture, can increase productivity whatever the climate conditions, but also optimize the management of inputs (fertilizers, pesticides), within a given economic and environmental framework. They also aim at controlling diseases more efficiently (Gullino et al., 2015). Mineral soilless culture substrates, which hardly contain any microorganisms, have been widely used. Organic substrates are also in use because they appear more natural, they host microorganisms likely to be useful for plant health and thus they can supply part of plants' nutritional needs. Peat is the most commonly used material in organic substrates because it exhibits unequalled stability and physico-chemical properties (Al Naddaf et al., 2011). Composts are added to the growing media to improve microbial activity. They can substantially reduce disease severity (Khalil, 2013). However, peat bogs represent a slowly renewable pool and sanitary issues remain lingering despite the use of pesticides. Other materials (coir or wood fibers) are now being used instead of peat (Olle et al., 2012; Robin, 1997). Their physical resilience is considered as sufficient under cropping conditions, but little is currently known about their physico-chemical and microbiological properties.

Soilless culture of cucumber represents a substantial economic stake round the world (2,114,000 tons produced every year in Europe). Knowing i) how severe some pathogens can be (especially the formae speciales forms of *Fusarium oxysporum*on cucumber; Abeysinghe, 2012), ii) the restricted use of several active molecules, and iii) the producers' wish to adopt agro-ecological production practices, other means of protection need to be developed.

Phytopathogenicity is related to the microbial populations present in the culture substrates. Yet, organic matter type appears as a determining factor for the development of both

* Corresponding author at: Agrocampus Ouest–Centre d'Angers, 2 rue Le Nôtre, 49045 Angers cedex 1, France.
** Corresponding author.
E-mail addresses: montagne.v@live.fr, virginie.montagne@dijon.inra.fr (V. Montagne), thierry.lebeau@univ-nantes.fr (T. Lebeau).

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Table 1
Physico-chemical and microbial characteristics of the substrates (±: standard deviation, n = 3).

Substrate	Substrate origin	Process	C:N ratio	pH[a] (water)	Organic matter[b] (g dw kg $^{-1}$)	Dry bulk density[c] (kg dw m $^{-3}$)	Water storage capacity[c] (ml l $^{-1}$)	Microbial organic C[d] (mg dw kg^{-1})	BacterialCFUs[e] (substrate g^{-1})	Fungal CFUs (substrate g^{-1})
PiF1s	Pine 1	Screw grinding	612	4.7	996	72.4	101	457.5 (±216.8)	2.1×10^6 (±5.2×10^5)	5.7×10^7 (±2.6×10^7)
CoF	Coconut	Unknown	130	6.4	962	59.4	116.8	388.6 (±103.3)	9×10^6 (±9×10^5)	2.19×10^5 (±1.04×10^4)
SpPn	Sphagnum	Extracted	48	6.9	867	110.2	284.9	875.9 (±30.8)	9.3×10^6 (±3.16×10^6)	1.23×10^6 (±4.06×10^5)

[a] NF EN 13037 (2000) standard method.
[b] NF EN 13039 (2011) standard method.
[c] NF EN 13041 (2000) standard method.
[d] NF ISO 14240-2 (2011) standard method.
[e] Estimation of the number of culturable bacteria (TSA + cycloheximide) and fungi (PDA + streptomycin and tetracycline).

phytopathogenic microorganisms and their antagonists (Domeño et al., 2011; Kleiber et al., 2012; Pérez et al., 2002)

Environment-friendly control strategies, among which microbiological control, are of growing interest for the sector. Many studies have addressed the role of microorganisms in plant protection against pathogens, including soil-borne ones (Mercier and Manker, 2005; Shanmugam and Kanoujia, 2011). The specific physico-chemical properties of organic substrates are believed to drive microbial development, and sometimes lead to a protective effect against opportunistic pathogens (Clematis et al., 2009; Martínez et al., 2013). Competition for nutrients and/or space and antibiosis phenomena occur among microorganisms (Benítez et al., 2004), but our understanding of these mechanisms still remains to be further examined. In this context, we previously compared the biochemical composition of different organic substrates, the related microbial activities, and the microbial community structures (Montagne et al., 2015). Our results confirmed that specific microbial activities and microbial population structures are related to material type (wood fiber, coir fiber, peat) and therefore to specific organic compositions. Hence it appeared interesting to investigate the involvement of specific microbial communities in the control of soil-borne pathogens of soilless crops.

The present study aims at testing the responsiveness of pine wood fiber, coir fiber and peat to the host/pathogen pair *Cucumis sativus* L.-*Fusarium oxysporum*, f. sp.*radicis-cucumerinum* (FORC), a pathogen of cucumber. Two bioassays were performed: one in summer 2014, and the other in spring 2015, under greenhouse production conditions. Plant growth, pathogen attack symptoms, and the evolution of the rhizospheric fungal community structure were monitored throughout the two bioassays.

2. Materials and methods

2.1. Organic substrates

The following substrates were studied: pine wood fiber (PiF1s), coir fiber (CoF), and peat (SpPn) (Florentaise company, France). Their physico-chemical and microbiological properties are presented in Table 1.

2.2. Plant: cropping and management

Cucumber (*Cucumis sativus* L. Galaxy F1, ENZA Zaden, Enkhuizen, The Netherlands) non-treated seeds were placed in vermiculite shelves (25 °C, 12 h photoperiod). After germination, plantlets were transferred to pots filled with 1.1 l of substrate. A drop-irrigation-fertilization system was used. The nutrient solution (pH 5.6, electrical conductivity 1.5 dS m^{-1}) was prepared from mineral fertilizers (Plant Prod 229 and 216, Fertil, Boulogne-Billancourt, France) to reach the equivalent of a 10-2-8 nitrogen-phosphorus-potassium ratio.

Crops were grown under the greenhouse, minimum temperature was 18 °C, and openings allowed for aeration when temperatures reached 24 °C. During the cultivation period, plants were propped, fruit were collected at the end of the assays, and biological protection was applied to control the development of pest insects by using the auxiliary insects *Euseius gallicus*, *Phytoseiulus persimilis,* and *Steiner nemafeltiae* (Biobest, Belgium). Climatic parameters are summarized in Table 3.

2.3. Microorganisms: growth conditions and inoculum preparation

Two antagonistic strains and one pathogenic strain were studied in the bioassays:

– the antagonistic strain *Fusarium oxysporum* (MIAE 00047, UMR1347 Agroécologie, INRA DIJON; Alabouvette et al., 1987) isolated from soil from Châteaurenard (France) in 1976 (Fo47);

– the *Trichoderma atroviridae* strain (MUCL45632, NIXE Laboratoire, Sophia Antipolis, France) (Tricho);

– the pathogenic strain *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC), first isolated from diseased plants at a producer's farm, and then identified by PCR using specific FORC primers (Lievens et al., 2008).

Microbial culture and substrate inoculation were performed as follows:

– the Fo47 strain was grown on Potato Dextrose Broth (PDB, Laboratorios CONDA, Spain) under shaking at 100 rpm at 26 °C. It was inoculated when seeds were sown (mixed with vermiculite) and when plantlets were potted, at a concentration of 10^3 conidia per ml of non-tyndallized substrate. Conidia were collected after filtration on a 48-μm nylon filter (Buisine, France), and were enumerated on a Malassez counting chamber;

– the *Trichoderma* strain MUCL45632 was first grown on wood fiber for 3 weeks to reach a high concentration, and then the inoculum was mixed with the PiF1s substrate to reach a final concentration of approximately 10^5 cells g^{-1} of non-tyndallized substrate;

– the FORC strain was grown in the same conditions as the Fo47 strain. Conidia were collected and enumerated as described above. FORC was inoculated at the surface of the pots at a concentration of 5×10^3 conidia per ml of substrate (tyndallized or not), when plantlets were at the 3–4 leaf stage.

To prevent microorganisms leaching from the substrates, in the week following inoculation plants were irrigated with the same amount of water as in the following weeks, but it was provided in smaller quantities and more frequently.