

PROOF Induced Suppressiveness to *Fusarium oxysporum* f.sp. *radicis lycopersici* in Rockwool Substrate Used in Closed Soilless Systems

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Tomatoes grown in soilless systems can be seriously damaged by *Fusarium oxysporum* Schlecht f.sp. *radicis lycopersici* (Forl) causing Fusarium crown and root rot (FCRR). FCRR suppression can be achieved through the use of chemicals, selected substrates, composts and artificially introduced antagonistic microorganisms. This study evaluated the natural capacity of a used rockwool to suppress FCRR infections. New and used rockwool, sampled from closed soilless systems, was either autoclaved or not, either artificially inoculated with Forl or not and, finally, sown with tomato seeds cv. 'Cuore di Bue'. The effects of autoclaved/non-autoclaved and used/new rockwool on FCRR incidence were assessed by evaluating the symptoms of crown rot on the root – shoot transition zone of tomato seedlings. Non-autoclaved and inoculated used rockwool significantly reduced FCRR incidence when compared with non-autoclaved and inoculated new rockwool. Autoclaved and inoculated used rockwool did not suppress FCRR, similarly to new and inoculated rockwool. These findings are in accordance with other research that, on a cucumber/*Pythium* host/pathogen complex in a closed rockwool soilless system, demonstrated the key role of resident microflora in suppressing the root rot disease.

KEY WORDS: Fusarium crown and root rot; tomato; disease suppressiveness.

INTRODUCTION

Fusarium oxysporum Schlecht f.sp. *radicis lycopersici* Jarvis and Shoemaker, the causal agent of Fusarium crown and root rot (FCRR) of tomato (*Lycopersicon esculentum* Mill.), is an important soilborne disease causing high yield losses in fields and commercial greenhouses. FCRR is considered a key soilborne disease of tomatoes in commercial greenhouses in northern (35), central and southern Italy (4). It acts as a polycyclic soilborne pathogen under field conditions (28) and infections occur either *via* soil infestation and subsequent root infection or by direct infection of the foliage (29). Moreover, airborne conidia produced by this pathogen (31), which may infect the plants and, particularly, recolonize disinfested soil/substrates, are one potential means of long-range dissemination (30).

Among the control strategies, soilless cultivation enables us to avoid the use of soil fumigants in greenhouse tomatoes (9,18). Unfortunately, FCRR can infect and spread

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very quickly in open soilless systems (19) as well as in closed ones (10,23), probably because soilless systems contain less diverse (15) and probably smaller populations of microorganisms (38) than soil (conventional culture).

Occurrence of suppressiveness to plant disease agents in soils has been thoroughly investigated (2,6,11,16,39), whereas suppression of disease in a soilless culture system, whether naturally (26) or artificially induced (3,7,8), is a new research area.

In a soilless crop a natural disease decline has been described in a nutrient film technique (NFT) system and on rockwool. In both cases the adoption, respectively, of a reused nutrient solution and of used rockwool slabs, permitted the gradual reduction of disease incidence and severity across three or more subsequent cropping cycles of *Fusarium wilt* on carnation and of *Pythium aphanidermatum* on cucumber (25). Suppressiveness can be transferred into a closed soilless system through the nutrient solution and/or the substrate itself. Suppression of *P. aphanidermatum* in cucumber can be restored in steamed substrate or in a new one either through the contact between used and steamed/new slabs or through the nutrient solution itself drained from used slabs and used to irrigate the steamed/new ones (26). Other research has shown the important role of recirculation of nutrient solution compared with the adoption of run-to-waste systems: in a NFT system with *Phytophthora cryptogea* in tomato, a closed system showed less disease than a parallel run-to-waste (25), but, unfortunately, the role of the microflora was not explained. Similarly, infestation with *Pythium* spp. on a tomato crop was less severe on rockwool than in run-to-waste systems (37). Moreover, the use of a nutrient solution drained from a long-running closed soilless system aimed at transferring disease suppressiveness to *P. cryptogea* on soilless-grown gerbera has been demonstrated to be an inefficient strategy (40), indicating the possible role of additional factors other than the microflora. Nevertheless, several examples demonstrate the conductivity of soilless systems to several soilborne diseases and, particularly, to several zoosporic soilborne pathogens (34). Moreover, Mihuta-Grimm *et al.* (19) reported an increase in severity of FCRR in soilless tomatoes at the seedling stage and other authors described severe outbreaks of FCRR in rockwool systems in Japan (14).

The possibility to adopt soilless crops as an alternative to conventional growing systems in greenhouse tomatoes, would limit dependency on chemical soil fumigants of intensive vegetable production. In view of this, the objective of the present work was to evaluate the natural capacity of a previously used substrate to become suppressive against FCRR infections, thereby limiting the crop losses due to this widespread disease.

MATERIALS AND METHODS

Substrate samples Tomato (*Lycopersicon esculentum* Mill.) plants were grown in two closed soilless systems in experimental greenhouses of the University of Pisa during four different cropping cycles. Tomato plants were transplanted in rockwool slabs (Grodan Expert, Roermond, the Netherlands) and the nutrient solution (NS) was delivered via drip irrigation at two different levels of electrical conductivity (EC). Soilless tomatoes were grown in the absence of FCRR infection and plants were periodically assessed in order to verify the unexpected infections. In Table 1, substrate samples obtained from soilless tomato crops are characterized on the basis of the tomato variety grown in the substrate, the composition of the nutrient solution (NS1a; NS1b), the electrical conductivity of the nutrient solution (EC1, EC2), and the growing period before sampling.

Samples were collected separately from four tomato rows representing four separate

replicates. Each sample consisted of four rockwool slabs. All samples were maintained separately in sterile plastic bags and brought within 24 h to the laboratory of Albenga (Centro Regionale di Sperimentazione ed Assistenza Agricola, CeRSAA), where they were maintained at 4°C until utilization. In addition, four never used rockwool slabs were collected and maintained separately to be used as the untreated control during the subsequent steps.

Inoculum experiments In all experiments a mixture of two isolates of *Fusarium oxysporum* f.sp. *radicis lycopersici* (FCRR Sicily farm LL; FCRR Sicily farm D) was used. These isolates, obtained from soilless-grown tomatoes and tested for their pathogenicity on tomato seedlings (12,32), were grown in 1000-ml Erlenmeyer flasks containing 300 ml of casein liquid medium: 1 g l⁻¹ granulated yeast extract, 2 g l⁻¹ casein hydrolysate, 1.5 g l⁻¹ potassium dihydrogen phosphate, 1 g l⁻¹ magnesium sulphate, 5 g l⁻¹ glucose anhydrous, 10 ml l⁻¹ AZ solution (273.3 mg l⁻¹ of LiCl; 55.3 mg l⁻¹ of CuSO₄·5H₂O; 55.3 mg l⁻¹ of ZnSO₄; 610.0 mg l⁻¹ of H₃BO₃; 55.3 mg l⁻¹ of Al₂(SO₄)₃; 27.7 mg l⁻¹ of SnCl₂·2H₂O; 390.0 mg l⁻¹ of MnCl₂·4H₂O; 55.3 mg l⁻¹ of NiSO₄·6H₂O; 55.3 mg l⁻¹ of Co(NO₃)₂·6H₂O; 55.3 mg l⁻¹ of TiO₂; 27.7 mg l⁻¹ of KI; 27.7 mg l⁻¹ of KBr) autoclaved for 20 min at 120°C. Flasks were incubated on a rotary shaker at 20°C and under constant light. After 12–15 days, fungal liquid cultures were aseptically removed from the flasks, homogenized with a rotary hand blender, roughly filtered to avoid the presence of mycelium mats and finally centrifuged at 11,000 g for 20 min. After centrifugation, the supernatant was removed and the pellet was re-suspended in sterile distilled water and brought to a final propagules (microconidia, macroconidia, chlamydospores) concentration of 10⁴ CFU ml⁻¹.

Sample preparation and artificial inoculation Samples of used and never-used rockwool were collected and cut in 1 x 1 cm pieces after removal of the plastic and eventually moistened with sterile deionized water. After sampling, half of each sample was autoclaved (20 min at 120°C). Autoclaved and non-autoclaved samples were used to fill sterile petri plates (14-cm-diam) with a layer ~1 cm and kept in a dark growth chamber where the air temperature was maintained at 20±2°C and the relative humidity (r.h.) ranged between 90% and 95%. For each petri plate the weight of rockwool and perlite was approximately 18 g and 23 g, respectively. After one day some of the petri plates were uncovered and surface sprayed with 3 ml of FCRR cell suspension (3x10⁴ CFU per plate). All petri plates were maintained covered under the same climatic conditions as described above (20±2°C, 90–95% r.h., in the dark).

Seven days after inoculation, all inoculated and non-inoculated petri plates were uncovered and 20 seeds of tomato cv. ‘Cuore di Bue’ (Selezione Albenga) were placed on the surface of the substrate of each plate. The substrate was moistened with sterile deionized water. Finally, all petri plates were covered with transparent plastic film, maintained at 20±2°C, 90–95% r.h., and 12:12 L:D (600 watt m⁻²).

Assessments and statistical analysis Seeds and germinating plants were constantly monitored for 15–40 days after sowing by assessing the number of germinating seeds and the presence of FCRR infection. FCRR infection was detected on the basis of the presence or absence of brown lesions in the root/shoot transition zone (12). The data collected during the tests (percentage of germinated seeds; percentage of germinated and infected seeds) were statistically analyzed using SPSS 12.0.1 Windows software. In order

to compare the data collected from four samples, the Kruskal-Wallis non-parametric test was performed, considering the tomato cultivar and the growing period before sampling as grouping factors. Because the null hypothesis (all populations have identical distribution functions) was always rejected ($P < 0.05$) for the percentage of germinated seeds variable, and considering the obvious correlation between the percentage of germinated seeds and the percentage of germinated and infected seeds, the four samples could not be considered as four different blocks of the same experiment and, consequently, the data collected from the four samples were analyzed separately. Prior to analysis, data expressed as percentages were arcsine transformed to homogenize variances. Then an ANOVA was performed on the data and when significant differences were detected ($P < 0.05$), treatment means were compared *via* the Tukey HSD (Honestly Significantly Different) test.

RESULTS

The evaluation of tomato seeds germination did not show a consistent effect of the inoculation and/or of the sterilizing process and/or the NS EC (Table 2). In some cases no differences were observed at all (sample 4) and in others the influence was significant but not constant (samples 1, 2 and 3).

TABLE 1. Relevant data about rockwool samples rev. tables 2.8.06

Sample no.	Closed soilless system no.	Tomato cultivar	Nutrient solution ^z	EC (mS cm ⁻¹)	Growing period before sampling (months)
1	1	Gimar F ₁	NS1a	3y	3
1	1	Gimar F ₁	NS1b	10x	3
2	1	Gimar F ₁	NS1a	3	5
2	1	Gimar F ₁	NS1b	10	5
3	2	Gimar F ₁	NS1a	3	5
3	2	Gimar F ₁	NS1b	10	5
4	2	Daniela F ₁ and Naomi F ₁	NS1a	3	4
4	2	Daniela F ₁ and Naomi F ₁	NS1b	10	4

^zNS 1a: 182 ppm NO₃; 46.5 ppm PO₄; 313 ppm K; 24 ppm Mg; 200 ppm Ca; 230 ppm Na; 354 ppm Cl; 2.74 ppm Fe; 0.45 ppm B; 0.32 ppm Cu; 0.44 ppm Zn; 0.58 ppm Mn; 0.10 ppm Mo.

NS 1b: 182 ppm NO₃; 46.5 ppm PO₄; 313 ppm K; 24 ppm Mg; 200 ppm Ca; 1380 ppm Na; 2127 ppm Cl; 2.74 ppm Fe; 0.45 ppm B; 0.32 ppm Cu; 0.44 ppm Zn; 0.58 ppm Mn; 0.10 ppm Mo.

^yEC1.

^xEC2.

On used and new rockwool samples, when not artificially inoculated, germinating tomato seeds never showed FCRR symptoms (samples 1, 3 and 4), thus confirming the absence of FCRR inoculum in the sampled rockwool, as previously suggested by the absence of symptomatic plants during the growing phase in experimental soilless systems (Table 3). Seeds sown in never-used rockwool showed a high incidence of brown lesions in the root/shoot transition zone when inoculated both with and without sterilization. Used rockwool artificially inoculated, but non-autoclaved, significantly reduced FCRR incidence when compared with never-used rockwool artificially inoculated and non-autoclaved (samples 1, 2, 3). Few differences were observed within the two levels of EC; only sample 4, obtained from soilless system fertilized with the NS at the lowest EC, did not differ

TABLE 2. Effect of new (never used) or used, autoclaved or non-autoclaved, inoculated with FCRR or non-inoculated rockwool, on germination percentage of tomato seeds (cv. Cuore di Bue)

Rockwool	Autoclaved	Inoculated	Sample 1	Sample 2	Sample 3	Sample 4
New	no	no	98.8a ^z	93.8ab	86.2ab	77.5a
Used (EC 1) ^y	no	no	96.7ab	87.8abc	93.8a	78.8a
Used (EC 2) ^x	no	no	90.0b	93.4ab	71.3c	72.5a
New	yes	no	98.8a	83.8bc	82.5abc	68.8a
Used (EC 1)	yes	no	93.3ab	93.1ab	89.4ab	88.8a
Used (EC 2)	yes	no	92.5ab	78.4c	80.0bc	65.6a
New	no	yes	90.0b	98.8a	92.5ab	80.0a
Used (EC 1)	no	yes	95.4ab	90.3abc	95.3a	77.5a
Used (EC 2)	no	yes	90.0b	94.4ab	79.7bc	72.5a
New	yes	yes	90.0b	91.3abc	90.0ab	85.0a
Used (EC 1)	yes	yes	92.1ab	90.0abc	89.4ab	84.4a
Used (EC 2)	yes	yes	93.3ab	92.8ab	85.3ab	70.6a

^zWithin columns, values followed by a common letter do not differ significantly ($P=0.05$) according to Tukey's HSD test.

^yEC 1 = 3 mS cm⁻¹.

^xEC 2 = 10 mS cm⁻¹.

TABLE 3. Effect of new (never used) or used rockwool, autoclaved or non-autoclaved, inoculated with FCRR or non-inoculated, on percentage of infected germinated tomato seeds (cv. Cuore di Bue)

Rockwool	Autoclaved	Inoculated	Sample 1	Sample 2	Sample 3	Sample 4	Average
New	no	no	0a ^z	0a	0a	0a	0a
Used (EC 1) ^y	no	no	0a	0a	0a	0.0a	0a
Used (EC 2) ^x	no	no	0a	0a	0a	0.0a	0a
New	yes	no	0a	0a	0a	0.0a	0a
Used (EC 1)	yes	no	0a	0a	0a	0.0a	0a
Used (EC 2)	yes	no	0a	0a	0a	0.0a	0a
New	no	yes	48.3bc	15.2c	49.8cd	54.7b	42.0c
Used (EC 1)	no	yes	3.4a	9.2abc	0a	20.1ab	8.2ab
Used (EC 2)	no	yes	4.9a	1.1a	0a	2.4a	2.1ab
New	yes	yes	68.6c	14.6bc	51.8d	35.4ab	42.6c
Used (EC 1)	yes	yes	26.9ab	12.0abc	27.6bc	56.7b	30.8bc
Used (EC 2)	yes	yes	47.7bc	15.5c	8.4ab	4.9a	19.1abc

^zWithin columns, values followed by a common letter do not differ significantly ($P=0.05$) according to Tukey's HSD test.

^yEC 1 = 3 mS cm⁻¹.

^xEC 2 = 10 mS cm⁻¹.

in terms of FCRR incidence from never-used rockwool artificially inoculated and non-autoclaved.

When used rockwool samples were autoclaved prior to artificial inoculation, the FCRR incidence was similar to the incidence recorded on never-used and inoculated rockwool (samples 1, 2, 3, 4) (Table 2). The incidence of FCRR in inoculated and autoclaved used rockwool, both inoculated and non-inoculated, appeared to be independent of the level of EC of the nutrient solution. Only in sample 4, rockwool samples – autoclaved and subsequently artificially inoculated – significantly reduced the incidence of FCRR when sampled from a soilless system in which the EC of NS was highest.

DISCUSSION

It is accepted that the use of hydroponic cultivation systems in greenhouses is generally more favorable to diseases, particularly in closed soilless systems and/or by recycling soilless substrates possibly infected from preceding crops (34). Chemical and non-chemical disease control strategies effective to limit crop losses in soilless tomatoes are available, but pragmatic evaluations of benefits and constraints have to be considered. Experimental research showed the possibility to suppress FCRR incidence with benomyl in rockwool soilless tomatoes (19) and the same fungicide was effective against Fusarium wilt of tomato (33). Nevertheless, fungicides must be registered according to the current European regulations and their use could be limited during harvest.

Other research demonstrated that slabs of bark of *Chamaecyparis obtusa* suppress FCRR in soilless systems (41) and that raw material as well as bark byproducts would represent a useful alternative to other more popular substrates such as rockwool, perlite, peat and pumice stone, but the commercial availability and the risks of release of toxic compounds might limit their wide adoption (21,22).

More recently, the use of three composts produced by mixing and composting three carbonaceous amendments (orange peels, grape marc, wheat straw) with fresh cow manure suppressed FCRR in soilless tomatoes compared with a standard peat substrates (27) and, in this case, in accordance with previous research (24), the beneficial effect of compost in reducing disease symptoms was associated with induced plant resistance to fungal colonization.

Several studies demonstrated that suppressiveness of soilborne disease in soilless growth media can be induced by introducing microbial antagonists previously isolated from suppressive soils and/or soilless media (7, 13, 17, 31), this being a feasible and effective practice also against FCRR (3,8,20). Nevertheless, the use of antagonistic microorganisms is subject to several pragmatic considerations related to the possibility of producing the microbial antagonists on a large scale, to formulate them with an acceptable shelf life and at an acceptable cost and, finally, to register them for agricultural uses (5).

From this perspective, the exploitation of other mechanisms of disease suppression effective in controlling FCRR in soilless tomatoes – not mediated by the use of chemicals, selected substrates, composts, antagonistic microorganisms artificially introduced – would represent a more feasible and transferable possibility. Our results appear to agree with previous experiments carried out to test the suppressiveness of recycled and non-autoclaved rockwool to *P. aphanidermatum* (26). Those experiments showed that the microflora in used rockwool plays a key role in suppressing *Pythium* disease in cucumber and, similarly to our experiments, suppressiveness was detected in rockwool batches that were free of *Pythium* symptoms in the preceding cucumber crop (26). Moreover, data recorded from never-used rockwool, both autoclaved and non-autoclaved, confirm the observations of Mihuta-Grimm *et al.* (19) regarding the conduciveness of sterile and never-used rockwool to FCRR infections.

Data collected by testing the suppressiveness of rockwool slabs sampled from soilless systems irrigated with nutrient solution at low or high EC do not enable us to correlate the effects on the disease severity with the EC levels except in one case (sample 4) and with autoclaved rockwool, where enhancement of the disease suppressiveness due to a higher concentration of Na and Cl has been observed.

Although FCRR has been well studied in soil systems, little work has been done in soilless tomatoes. Recent research reported the increase of FCRR disease severity in conventional soil tomatoes when irrigated with saline water and demonstrated that FCRR growth and conidial germination are not adversely affected by water salinity at 100 mM NaCl, indicating that the pathogen tolerates high salinity levels (36). Our results represent the first report of FCRR suppression induced in closed soilless systems and further tests have to be carried out in order to investigate the role of microflora and growing conditions (EC, NaCl).

In conclusion, the possibility to suppress FCRR in rockwool soilless-grown tomatoes might represent a strategy to manage this disease without applying chemical control methods. The strategy would be expected to be easily integrated along with other non-chemical control methods, such as slow sand filtration, a technique effective to limit the spread of several zoosporic and non-zoosporic soilborne diseases throughout closed systems (38) without affecting the non-pathogenic microflora resident in the soilless system (40). Moreover, FCRR suppression, naturally induced after a growing cycle of 3 to 5 months, would have positive effects for open and, particularly, closed soilless tomatoes. Previous experience demonstrated the possibility to exploit disease decline phenomena in order to control soilborne diseases in soilless crops (25,26), but several constraints were identified as barriers to transfer this technique into practice, first of all being the need to reuse the soilless substrate after heavy disease attacks without substrate disinfection (25). On the contrary, because our results demonstrated the natural occurrence of FCRR suppression in used rockwool sampled from healthy soilless tomato crops, an easier transfer and adaptation of this technique into practice might be expected and further experimentally tested. Finally, negative effects on physical properties of reused rockwool, crop growth and marketable yield are, probably, remote risks: 2-year-old reused rockwool slabs were used successfully to grow sweet pepper (1) and the continuous crop growing during 2 years could be considered as current practice in rockwool crops in Italy.

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