

FUSARIUM ROOT AND STEM ROT OF GREENHOUSE CUCUMBER: AERIAL DISPERSAL OF INOCULUM

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Abstract

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Fusarium root and stem rot is one of the most damaging diseases of greenhouse cucumber. This study reveals development and spread of airborne inoculum of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* the causal agent of the disease. Infective propagules of the pathogen were trapped from the air and recovered from greenhouse interior structures and equipment, including concrete floors, paths and stumps, iron scaffolding components, glass walls, roof surfaces, plastic pots and planters.

The results suggest possible reinfestation of freshly steamed or fumigated soils by airborne propagules of the pathogen.

Key words: *Fusarium oxysporum* f.sp. *radicis-cucumerinum*, root and stem rot, cucumber, airborne disease spread, epidemiology

Introduction

Fusarium root and stem rot of cucumbers caused by *Fusarium oxysporum* Schlechtend.: Fr. f.sp. *radicis-cucumerinum* Vakalounakis (FORC) is a relatively new disease first reported in Greece by Vakalounakis (1996) who described it in detail. The disease has also been reported from Canada, China, France, Israel, The Netherlands, Spain and United States (Punja and Parker, 2000; Cercauskas et al., 2001; Moreno et al., 2001; Rose and Punja, 2004; Pavlou and Vakalounakis, 2005). In Bulgaria, the disease was first recorded in 1996, later progressed rapidly and has now occurred in most cucumber-growing greenhouses causing severe crop losses (Vatchev, 2007). Although several control methods have shown efficacy against Fusarium root and stem rot (Kannangara et al., 2000; Rose et al., 2003; Pavlou and Vakalounakis, 2005; Vatchev and Maneva, 2012), little is known about the epidemiology of the disease in greenhouse cucumbers. Symptoms of Fusarium root and stem rot include large basal stem lesions on which abundant sporulation is often observed, particularly under very humid conditions, consisting of pale salmon-pink masses of *Fusarium oxysporum* macro- and microconidia (Vakalounakis, 1996; Rose et al., 2003). Sporulating conidial layers produced on the stems and

spread of spores as airborne inoculum has been reported for several formae specialis of *Fusarium oxysporum*, such as *F. oxysporum* Schlechtend.: Fr. f.sp. *radicis-lycopersici* Jarvis & Shoemaker which causes crown and root rot of tomato (Rowe et al., 1977), as well as *F. oxysporum* Schlechtend.: Fr. f.sp. *basilici*, the causal agent of wilt and crown rot of sweet basil (Gamliel et al., 1996) and *F. oxysporum* Schlechtend.: Fr. f.sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans., the vascular wilt pathogen of tomato (Katan et al., 1997). Rekah et al. (2000) clearly showed that propagules of *F. oxysporum* disseminated by air may infect both soil and plant foliage and cause typical disease symptoms on tomato and basil plants.

The purpose of the current study was to determine if *F. oxysporum* f.sp. *radicis-cucumerinum* is capable of producing infectious airborne propagules which have the potential to cause the disease on cucumber plants under greenhouse conditions.

Materials and Methods

Culture media

Fusarium selective medium IV (Komada's medium, FSM) (Dhingra and Sinclair, 1995) and acidified potato dextrose agar (APDA) (Rose et al., 2003) were used for trapping the airborne

propagules of *Fusarium oxysporum* from air and for detection of the pathogen on pots, planters and structures inside the greenhouses. Oat meal agar (OA) was used to grow inoculum of *Fusarium* isolates for plant inoculations and pathogenicity tests.

Reference strains

Were used two pathogenic strains of *F. oxysporum* f.sp. *radicis-cucumerinum* PPI codes: FORC₁ and FORC₃, previously isolated from diseased cucumber plants and available at culture collections of the Plant Pathology Dept., as references to support the identification of *Fusarium oxysporum* isolates and in pathogenicity tests. The same strains were also used for inoculating cucumber seedlings in the procedure for simulating a sporulation on stems.

Greenhouse assessments

Examinations were carried out to evaluate the presence of *Fusarium oxysporum* f.sp. *radicis-cucumerinum* propagules in the air, in experimental glass-covered greenhouse at Plant Protection Institute (PPI) in two successive years (Table 1) and in two commercial glasshouses with year-round cucumber production and high incidence of *Fusarium* root and stem rot. The glasshouse in Dabene, Karlovo region was based on straw-bale culture where straw bales were placed directly on the soil ground. In the other glasshouse situated in Kresna, Blagoevgrad region cucumbers were planted in plastic containers filled with mix of 50% sphagnum peat, 25% perlite and 25% coir (coconut fiber, soilless culture). The two commercial glasshouses were visited for examinations during the main production period, May and June 2007.

Simulation of sporulation on the stems of cucumber plants in experimental glasshouse

Cucumber seedlings, cv. Kalunga F₁ and Gergana (Long English type) were grown in 2.5-liter plastic pots filled with

sterilized soil collected from the experimental field of the PPI. At two-true-leaf stage, approximately 2-3 weeks after sowing cucumber seedlings were reduced to one per pot and inoculated with one of the two reference strains. Inocula were prepared after 7-day incubation of pure cultures on OA in 90 mm Petri plates at 27°C in the dark. Plates were flooded with 10 ml of sterile distilled water (SDW) and mycelia and spores were scraped off using inoculating loop. Suspension was filtered through a two layers of cheesecloth to reduce mycelial fragments and diluted with SDW to 1.5-2.5 x 10⁵ conidia ml⁻¹. Each plant was inoculated by pouring 25 ml of inoculum suspension in each of four 3-cm-deep holes around each plant at a distance of 5 cm (Katan et al., 1997). Inoculated plants were maintained in the glasshouse at 17.5 to 29.5°C and examined periodically for symptom development. The appearance of sporulating layers of *Fusarium oxysporum* on the stems was confirmed by direct observations under magnifying glass and a light microscope. Noninoculated control plants were maintained under the same conditions.

Propagules on the greenhouse components and equipment

The presence of *Fusarium oxysporum* propagules on internal greenhouse structures (concrete floors, paths and stumps, iron posts, glass walls and roofs, heating pipes) and equipment including plastic pots and planters was examined following the procedure described by Gamliel et al. (1996). Surfaces were tested at distances and heights ranging from 20 to 200 cm from basal stems of diseased plants showing or not showing formation of sporulating layers. A wet sterile swab was rubbed over the tested surface so as to cover a 5-cm² area and blotted onto six points or rolled over FSM or APDA plates. The developed *Fusarium oxysporum* colonies were counted and results were presented as number per plate. Representative colonies were subcultured on APDA or OA for further identification and pathogenicity testing.

Table 1
Airborne propagules of *Fusarium oxysporum* and *Fusarium oxysporum* f.sp. *radicis-cucumerinum* in three cucumber greenhouses

Greenhouse	Sampling date	Colonies per plate	Pathogenicity test	
			Isolates tested (no.)	Pathogenic isolates (no.)
PPI	07 June	0.3	6	2
	08 May	0.4	8	2
	08 June	0.15	3	3
	08 July	0.35	7	3
	07 April	0.03	1	0
Kresna	07 June	3.35	10	6
	07 June	0.7	7	3

Trapping of airborne propagules

To trap airborne propagules of *Fusarium oxysporum* Petri plates containing FSM or APDA were placed randomly at heights ranging from 20 to 200 cm above the ground. Twenty to 50 plated per 0.2 ha were exposed open to the air for 2 h, then returned to the laboratory and incubated for 5-7 days at 27°C in the dark. Colonies with morphological characteristics of *Fusarium oxysporum* were subcultured on APDA or OA for further identification and pathogenicity testing.

Identification of isolates

Colonies of *F. oxysporum* were identified on the basis of morphological and microscopical characteristics of mycelia and reproductive structures using the taxonomic references of Booth (1971, 1977) and Nelson et al. (1983). All pathogenic isolates were identified as *F. oxysporum* f.sp. *radicis-cucumerinum* by their cultural characteristics and pathogenicity tests as described by Vakalounakis (1996) and Vakalounakis and Chalkias (2004). The pathogenicity test was considered positive when 60% or more of the inoculated cucumber seedlings showed typical disease symptoms (Vatchev and Maneva, 2012).

Pathogenicity tests

Cucumber seedlings (cv. Gergana, Long English type) at two-true-leaf stage were used in all pathogenicity tests. Pathogenicity of 85 representative *F. oxysporum* isolates was tested using the root dip inoculation method as described by

Vakalounakis and Chalkias (2004). The pathogen was successfully reisolated from root and stem tissues of symptomatic cucumber plants.

Results and Discussions

F. oxysporum f.sp. *radicis-cucumerinum* sporulation of on cucumber plants and aerial spread of inoculum

One out of 10 to one out of 12 artificially inoculated cucumber plants developed visible crown and stem necrotic lesions on which conidial formation was detected. In the two inspected commercial greenhouses sporulation of *F. oxysporum* on stem surfaces was observed on a few diseases cucumber plants. Light microscopic observations of individual stem pieces revealed the presence of both macro- and microconidia of *F. oxysporum*. Previous study confirmed the ability of this inoculum to reproduce the disease on cucumber seedlings (Vatchev, 2007).

Trapping of airborne propagules

In the trapping studies after two hours of exposition in the experimental greenhouse and in the two naturally infested commercial units, the Petri plates contained 0.03 to 0.7 colonies of *Fusarium* spp. (Table 1) of 41 *F. oxysporum* isolates tested, 19 were pathogenic for cucumber plants as evidenced by the presence of yellowish-brown root rot, crown lesions, vascular discoloration of the stem, wilted and dead plants. The results from the pathogenicity tests confirmed the iden-

Table 2
Recovery of *Fusarium oxysporum* and *Fusarium oxysporum* f.sp. *radicis-cucumerinum* from greenhouse interior structures

Greenhouse	Source	Isolates recovered (no.)	Pathogenicity test	
			Isolates tested (no.)	Pathogenic isolates (no.)
PPI	Pot walls	6	5	5
	Concrete floors	6	6	3
	Iron frames	0	0	0
	Glass walls	5	2	2
	Glass roof	3	3	0
Dabene	Iron posts	1	1	1
	Glass walls	7	3	1
	Glass roof	2	2	2
	Concrete paths	5	5	3
Kresna	Planter walls	4	4	2
	Concrete stumps	6	3	0
	Iron posts	2	2	1
	Heating pipes	4	4	3
	Glass walls	4	4	2

tity of forma specialis *radicis-cucumerinum* of the root and stem rot pathogen Vakalounakis and Chalkias (2004). Abundant masses of macroconidia of *Fusarium oxysporum* were observed on the stems of partly decomposed cucumber plants in the waste containers outside the glasshouses.

Propagules on greenhouse structures

Propagules of *F. oxysporum* were easily recovered from greenhouse components and equipment inside the greenhouses (Table 2). Were detected isolates of *F. oxysporum*, including *F. oxysporum* f.sp. *radicis-cucumerinum* on experimental plastic pots and planter beds in which infected cucumber plants were grown. The pathogen was also recovered from concrete floors, paths and stumps, iron scaffolding components, glass wall and roof surface. Of 44 *F. oxysporum* representative isolates tested for pathogenicity, the identity of 25 isolates was confirmed as forma specialis *radicis-cucumerinum*.

As had already been shown by other authors, stems of the diseased cucumber plants grown under greenhouse conditions produce masses of conidia (Vakalounakis, 1996; Vakalounakis and Chalkias, 2000) that seems to be airborne. At the present study in two greenhouses growing cucumbers commercially, infective airborne propagules of *F. oxysporum* f.sp. *radicis-cucumerinum* during two growing seasons were trapped under experimental conditions. The identity of the pathogenic isolates was confirmed as *F. oxysporum* f.sp. *radicis-cucumerinum*. Similar aerial dissemination was reported for *F. oxysporum* f.sp. *radicis-lycopersici* (Rowe et al., 1977; Rekah et al., 2000), *F. oxysporum* f.sp. *basilici* (Gamliel et al., 1996) and *F. oxysporum* f.sp. *lycopersici* and other soilborne fungal pathogens (Katan et al., 1997). The results of this study indicate that aerial dissemination of the pathogen may play an important role in the epidemiology of Fusarium crown and root rot of cucumber. The polycyclic nature of the diseases incited by various *F. oxysporum* formae specialis has already been suggested (Rekah et al., 2000), although it is not clear whether the airborne inoculum has the capacity of inducing disease on other intact plants during the same season. Most importantly, the failure of steam-sterilization treatments or chemical fumigation of greenhouse soils to control Fusarium crown and root rot of tomatoes has been attributed to recontamination of freshly-sterilized soils by airborne inoculum of *F. oxysporum* f.sp. *radicis-lycopersici* (Rowe et al., 1977; Marois et al., 1983; Jarvis, 1992; Ozbay and Newman, 2004; Vatchev, 2004). Airborne distribution of *F. oxysporum* f.sp. *radicis-cucumerinum* may have very similar implications for the success of disease management, especially when based on the application of preplant soil sterilization methods.

As emphasized by other researchers, root and crown rots and/or stem rots caused by *F. oxysporum* are “notable exceptions” within the other forms of the pathogen which cause vascular wilts in many crops (Gamliel et al., 1996; Katan et al., 1997; Rekah, 2000). Further research is needed to reveal all epidemiological aspects of this distinctive group of soilborne plant pathogens.

Conclusions

This study reveals development and spread of airborne inoculum of *Fusarium oxysporum* f.sp. *radicis-cucumerinum* the causal agent of root and stem rot of greenhouse cucumber. Propagules of the pathogen that are trapped from the air or recovered from greenhouse interior structures display typical root and stem rot symptoms on inoculated cucumber plants. It is still not clear whether the airborne inoculum has the capacity of inducing disease on intact cucumber plants under natural production conditions.

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