Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

New or Unusual Disease Reports

Stem rot caused by *Fusarium oxysporum* f. sp. *opuntiarum* on *Mammillaria painteri* in Italy

Domenico BERTETTI[1,*], Pietro PENSA[2], Slavica MATIĆ[1], Maria Lodovica GULLINO[1,3], Angelo GARIBALDI[1]

[1] *Centre of Competence AGROINNOVA, University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy*
[2] *ANT-NET s.r.l., Via Livorno 60, Torino, Italy*
[3] *DiSAFA, University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy*
*Corresponding author: domenico.bertetti@unito.it

Summary. Potted plants of *Mammillaria painteri* (*Cactaceae*) showing symptoms of stem rot were collected from a nursery in Imperia province, Liguria region, Italy. Isolations from internal rotting tissues allowed gave constantly similar fungal colonies. Morphological characteristics of the isolates identified them as *Fusarium oxysporum*. Molecular analyses of the elongation factor 1α (*EF1α*) and *RPB2* genes confirmed the identification. Analysis of part of the intergenic spacer (IGS) region of the ribosomal DNA identified the pathogen as *F. oxysporum* f. sp. *opuntiarum*. In pathogenicity tests, stems of *M. painteri* plants were inoculated with representative *F. oxysporum* f. sp. *opuntiarum* isolates. Approx. 30 d after the inoculation, yellowing appeared around the inoculated wounds. The inoculated stems then rotted developing symptoms similar to those observed in greenhouse-grown plants. This is the first report of *F. oxysporum* f. sp. *opuntiarum* on *M. painteri.*

Keywords. Ornamentals, cacti, Fusarium wilt.

OPEN ACCESS

Citation: D. Bertetti, P. Pensa, S. Matić, M.L. Gullino, A. Garibaldi (2020) Stem rot caused by *Fusarium oxysporum* f. sp. *opuntiarum* on *Mammillaria painteri* in Italy. *Phytopathologia Mediterranea* 59(2): 365-369. DOI: 10.14601/Phyto-11735

Accepted: August 3, 2020

Published: August 31, 2020

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Jean-Michel Savoie, INRA Villenave d'Ornon, France.

INTRODUCTION

In the Liguria region of Italy, production of ornamental plants is continuously enriched with genera, species and cultivars, including several succulent plants belonging to *Cactaceae*. Cacti are extensively grown in specialized nurseries, with risks of propagating pathogens that can cause severe economic losses.

Fusarium oxysporum f. sp. *opuntiarum* is an important fungal pathogen of succulent host species (Gerlach, 1972; Souza de *et al*., 2010), including *Mammillaria zeilmanniana* (Alfieri *et al*., 1984; French, 1989). In Italy, this fungus has been identified on several succulent plants, including *Echinocactus grusonii* (Polizzi and Vitale, 2004), *Schlumbergera truncata* (Lops *et al*., 2013), *Astrophytum myriostigma*, *Cereus marginatus* var. *cristata*, *C. peruvianus monstruosus* and *C. peruvianus florida* (Bertetti *et al*., 2017), and, more recently, on *Sulcorebutia heliosa* (Garibaldi *et al*., 2019a) and *S. rauschii* (Gar-

ibaldi *et al*., 2019b). This pathogen was also reported on *Euphorbia mammillaris* var. *variegata* (*Euphorbiaceae*) (Garibaldi *et al*., 2015). On affected plants, *F. oxysporum* f. sp. *opuntiarum* can cause root rot, stem rot and wilting, and the mycelium of the pathogen can appear at soil level. Sporodochia producing abundant macroconidia can also be observed on affected stem tissues.

Mammillaria painteri Rose (*Cactaceae*) is a small plant native to Mexico, which produces globose stems with pale rose flowers, and is commercialized as potted plants. The aim of the present study was to identify the causal agent of disease on *M. painteri*, detected during the summer of 2018, on plants grown in a specialized cactus nursery, located in Vallecrosia (Imperia province, Liguria region of Italy).

MATERIALS AND METHODS

Isolation and morphological characterization of the pathogen

Twenty 2-year-old potted plants of *M. painteri* with stem rot symptoms were collected for isolation of the possible causal agent of the disease. Small pieces of symptomatic stems were disinfected in sodium hypochlorite (1%) for 2 min, then washed in sterile water. Several stem pieces (approx. 3 × 3 × 3 mm) were taken from the borders of internal rotting tissues and plated onto potato dextrose agar (PDA) (Merck KGaA), and incubated at 25°C. Resulting colonies were transferred onto carnation leaf-piece agar (CLA) (Fisher *et al*., 1982), and incubated at 25°C. The morphological identification of the isolates was carried out according to colour, shape and pigmentation of the mycelia grown on PDA, and characteristics of microconidia, macroconidia and chlamydospores observed on CLA, observed using an optical microscope (Nikon Eclipse 55i). Since all the isolates were similar, one was selected for a pathogenicity test and for molecular characterization.

Molecular characterization

DNA of the isolate (coded DB18AGO01) was extracted using the E.Z.N.A. fungal DNA Mini Kit (Omega Bio-Tek) from mycelium of the fungus grown on PDA. For the molecular analyses, the following primers were used: EF1/EF2 (O'Donnell *et al*., 1998) for the elongation factor 1α gene (*EF1α*), 5F2/7CR (O'Donnell *et al*., 2007) for the *RPB2* gene encoding DNA-directed RNA polymerase II second largest subunit, and CNS1/CNL12 (Appel and Gordon, 1995) for the intergenic spacer (IGS) region of the ribosomal DNA. The resulting amplicons were sequenced, obtaining three sequences that were analyzed with the BLASTn (Altschul *et al*., 1997) to define similarities with the sequences listed in GenBank. Maximum Likelihood (ML) phylogenetic analyses were performed on IGS sequences, including the corresponding sequences of ten reference strains of *F. oxysporum* f. sp. *opuntiarum*. The *Fusarium proliferatum* (31X4) sequence was used as an outgroup.

Pathogenicity test

The isolate DB18AGO01, preserved in the Agroinnova collection (University of Torino, Italy), was used in the pathogenicity test. The isolate was tested on three 18-month-old healthy potted plants of *M. painteri*, using the method of Talgø and Stensvand (2013). Host stems were wounded (three lesions/stem) with previously sterilized needles. The inoculum consisted in a culture of the fungus grown on PDA for 5 d. Tufts of mycelium were taken from this culture and used to contaminate the tips of needles that were introduced into the lesions on stems. Three control plants were treated with needles without the inoculum. All plants were grown in a greenhouse, at 21 to 30°C.

RESULTS AND DISCUSSION

The initial symptoms on affected *M. painteri* plants were chlorosis and yellowing of stems that were followed by the browning of tissues. The exterior of stems later become blackish (Figure 1a), while the internal stem tissues were rotted (Figure 1b). The disease affected about 80% of 1,000 plants *M. painteri* in the nursery.

The isolates on PDA produced pale pink colonies generating pale pink pigments in the agar. On CLA, colonies produced microconidia, macroconidia in pale orange sporodochia, and chlamydospores. The unicellular, oval to elliptical microconidia were supported by short monophialides (Figure 1c), and measured 4.4–8.6 × 1.3–3.4 (mean = 6.0 × 2.3) µm (n = 50). The slightly falcate macroconidia had foot-shaped basal cells and short apical cells (Figure 1d), three (rarely four) septa, and measured 26.5-44.6 × 3.0–4.5 (mean = 33.5 × 3.6) µm (n = 50). The rough walled chlamydospores were intercalary or terminal, single or in pairs or chains (Figure 1e), and measured 6.2–12.3 (mean = 8.7) µm (n = 50). These morphological characteristics are typical of *Fusarium oxysporum* (Leslie and Summerell, 2006).

The morphological identification was confirmed by the molecular analyses that obtained three sequences

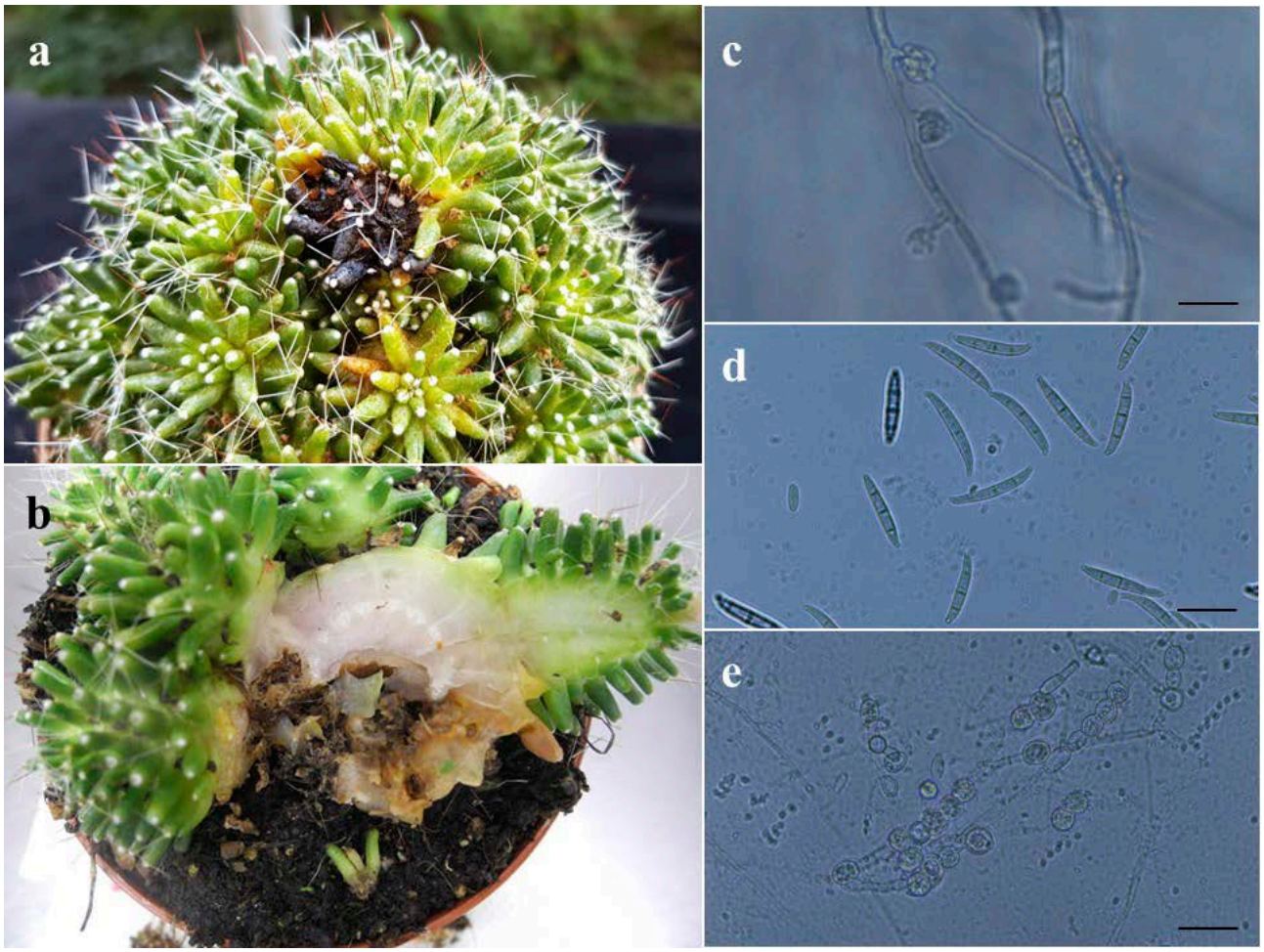

Figure 1. Disease symptoms caused by *Fusarium oxysporum* f. sp. *opuntiarum* on stems of *Mammillaria painteri* (a), and rot of the internal tissues (b). Microconidia (c), macroconidia (d) and chlamydospores (e) of the pathogen. Scale bars = 20 µm.

with, respectively, 676 (*EF1α*), 975 (*RPB2*), and 914 (IGS) base pairs. These sequences were deposited in GenBank (accession numbers, respectively, MT450439, MT450441, MT450440). The BLASTn analysis of these sequences showed 100% similarity with *Fusarium oxysporum* strain CBS 133.023 (accession no. KF255547) in the *RPB2* portion. Furthermore, 100% similarity was obtained with the reference strain of *F. oxysporum* f. sp. *opuntiarum* NRRL28368 (O'Donnell *et al*., 2009) in the *EF1α* portion (accession no. AF246871), and IGS region (accession no. FJ985530). Phylogenetic analysis of IGS sequences was performed, showing that the DB18AGO01 isolate grouped together with the reference NRRL_28368 strain and other strains of *F. oxysporum* f. sp. *opuntiarum* from different plant hosts (Figure 2) (Bertetti *et al*., 2017; Garibaldi *et al*., 2019a; Garibaldi *et al*., 2019b). Within the main cluster, three different phylogenetic subgroups were observed. The first subgroup comprised the DB18AGO01 isolate from *M. painteri*, and strains originating from *Disco placentiformis*, *Cereus peruvianus florida*, *C. marginatus*, *Sulcorebutia rauschii*, *S. heliosa*, and *Euphorbia mammillaris*. The second subgroup included strains from *C. peruvianus monstruosus, Zygocactus truncatus,* and the *F. oxysporum* f. sp. *opuntiarum* reference strain from *Echinocactus grusonii*. The DB14OTT05 M1 strain from *Astrophytum myriostigma* represented the third subgroup. These subgroups may indicate the presence of different physiological races within *F. oxysporum* f. sp. *opuntiarum*, which will require further molecular studies for adequate differentiation. Therefore, the fungus isolated from *M. painteri* was added in the *forma specialis opuntiarum* of *F. oxysporum*.

In the pathogenicity test, the first symptoms consisting of yellowing around the inoculated wounds appeared approx. 30 d after inoculation. As the disease progressed, stems became blackish around the

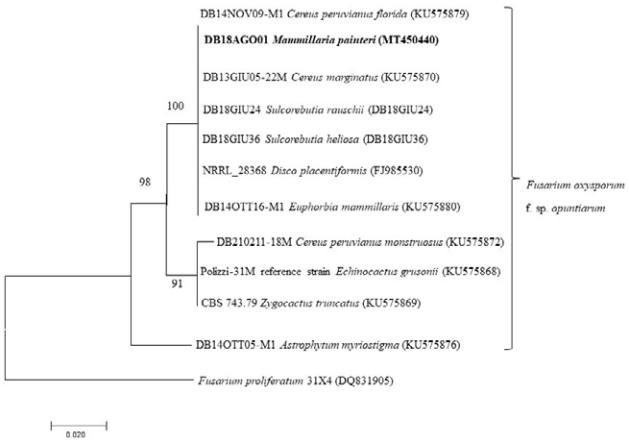

Figure 2. Phylogenetic analysis of a *Fusarium oxysporum* f. sp. *opuntiarum* isolate on the basis of intergenic spacer (IGS) sequences, inferred from maximum likelihood analysis. The values at the dendrogram nodes are bootstrap support values based on 1000 replicates. The strain DB18AGO01 from *Mammillaria painteri* is shown in bold font. The strain 31X4 of *Fusarium proliferatum* was used as an outgroup.

wounds, and the internal stem tissues rotted. Affected plants died. *Fusarium oxysporum* was constantly re-isolated from inoculated plants, whereas control plants remained symptomless. Most of the isolates reported in Figure 2 (DB14NOV09-M1, DB13GIU05-22M, DB14OTT16-M1, DB210211-18M, CBS 743.79, Polizzi-31M and DB14OTT05-M1) were previously inoculated onto *Schlumbergera truncata*, a species very susceptible to *F. oxysporum* f. sp. *opuntiarum*, and they all showed the same high virulence towards this host. Moreover, *C. peruvianus florida*, *C. marginatus*, *E. mammillaris*, *C. peruvianus monstruosus* and *A. myriostigma* were more or less susceptible to the *F. oxysporum* f. sp. *opuntiarum* reference isolates (CBS 743.79 and Polizzi-31M) (Bertetti *et al*., 2017). Further cross-pathogenicity assays should be performed. including to additional hosts of *F. oxysporum* f. sp. *opuntiarum*, in order to establish the host range of the DB18AGO01 isolate from *M. painteri*, and to investigate the occurrence of physiological races of *F. oxysporum* f. sp. *opuntiarum*.

Morphological and molecular identifications were in agreement, and Koch's postulates were satisfied, demonstrating that *F. oxysporum* f. sp. *opuntiarum* was the causal agent of the disease observed on *M. painteri*. This is the first report of this pathogen on *M. painteri*. Stem rot could cause significant economic losses in *M. painteri* cultivated in Italy. Commercial growing of succulent plants is increasing in Italy, and the evaluation of their susceptibility to *F. oxysporum* from *M. painteri* may provide useful information to avoid the spread of this pathogen.

LITERATURE CITED

Alfieri Jr. S.A., Langdon K.R., Wehlburg C., Kimbrough J.W., 1984. Index of Plant Diseases in Florida (Revised). *Florida Department of Agriculture and Consumer Service, Division of the Plant Industry Bulletin* 11: 1–389.

Altschul S.F., Madden T.L., Schaffer A.A., Zhang Z., Miller W., Lipman D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programme. *Nucleic Acids Research* 25: 3389–3402.

Appel D.J., Gordon T.R., 1995. Intraspecific variation within populations of *Fusarium oxysporum* based on RFLP analysis of the intergenic spacer region of the rDNA. *Experimental Mycology* 19: 120–128.

Bertetti D., Ortu G., Gullino M.L., Garibaldi A., 2017. Identification of *Fusarium oxysporum* f. sp. *opuntiarum* on new hosts of the *Cactaceae* and *Euphorbiaceae* families. *Journal of Plant Pathology* 99: 347–354.

Fisher N.L., Burgess L.W., Toussoun T.A., Nelson P.E., 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* 72: 151–153.

French A.M., 1989. California plant disease host index. California Dept. of Food and Agriculture, Division of Plant Industry, Sacramento, California, USA, 394 pp.

Garibaldi A., Bertetti D., Pensa P., Ortu G., Gullino M.L., 2015. First Report of *Fusarium oxysporum* Causing Wilt on *Euphorbia mammillaris* var. *variegata* in Italy. *Journal of Plant Pathology* 97 (4 Supplement): S68.

Garibaldi A., Bertetti D., Pensa P., Matić S., Gullino M.L., 2019a. First Report of stem rot caused by *Fusarium oxysporum* f. sp. *opuntiarum* on *Sulcorebutia heliosa* in Italy. *Plant Disease* 103: 2678.

Garibaldi A., Bertetti D., Pensa P., Matić S., Gullino M.L., 2019b. First report of stem wilt caused by *Fusarium oxysporum* f. sp. *opuntiarum* on *Sulcorebutia rauschii* in Italy. *Journal of Plant Pathology* 102: 577.

Gerlach W., 1972. Fusarium rot and other fungal diseases of horticulturally important cacti in Germany. *Phytopathologische Zeitschrift* 74: 197–217.

Leslie J.F., Summerell B.A., 2006. *The Fusarium Laboratory Manual*. Blackwell Professional, Ames, Iowa, USA, 388 pp.

Lops F., Cibelli F., Raimondo M.L., Carlucci A., 2013. First report of stem wilt and root rot of *Schlumbergera truncata* caused by *Fusarium oxysporum* f. sp. *opuntiarum* in southern Italy. *Plant Disease* 97: 846.

O'Donnell K., Kistler H.C., Cigelink E., Ploetz R.C., 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Pro-*

ceedings of the National Academy of Science USA 95: 2044–2049.

O'Donnell K., Sarver B.A., Brandt M., Chang D.C., Noble-Wang J., … Ward T.J., 2007. Phylogenetic diversity and microsphere array-based genotyping of human pathogenic Fusaria, including isolates from the multistate contact lens-associated U.S. keratitis outbreaks of 2005 and 2006. *Journal of Clinical Microbiology* 45: 2235–2248.

O'Donnell K., Gueidan C., Sink S., Johnston P.R., Crous P.W., … Sarver B.A.J., 2009. A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. *Fungal Genetics and Biology* 46: 936–948.

Polizzi G., Vitale A., 2004. First report of basal stem rot of golden barrel cactus caused by *Fusarium oxysporum* f. sp. *opuntiarum* in Italy. *Plant Disease* 88: 85.

Souza de A.E.F., Nascimento L.C., Araújo E., Lopes E.B., Souto F.M., 2010. Occurrence and identification of the etiologic agents of plant diseases in cactus (*Opuntia ficus-indica* Mill.) in the semi-arid region of Paraiba. *Biotemas* 23: 11–20.

Talgø V., Stensvand A., 2013. A simple and effective inoculation method for *Phytophthora* and fungal species on woody plants. *OEPP/EPPO Bulletin* 43: 276–279.