IDENTIFICATION OF *FUSARIUM OXYSPORUM* F. SP. *OPUNTIARUM* ON NEW HOSTS OF THE CACTACEAE AND EUPHORBIACEAE FAMILIES

D. Bertetti, G. Ortu, M.L. Gullino, A. Garibaldi

Centre of Competence for the Innovation in the Agro-Environmental Sector (AGROINNOVA), University of Turin, Largo Braccini 2, 10095 Grugliasco, Italy.

Running title: *Fusarium oxysporum* f. sp. *opuntiarum* on succulent plants

Corresponding author: D. Bertetti

Fax number: +39.011.6709307

E-mail address: domenico.bertetti@unito.it

SUMMARY

Fusarium oxysporum has recently been detected in commercial nurseries in the Ligurian region (northern Italy) on new succulent plants belonging to the Cactaceae family (*Astrophytum myriostigma*, *Cereus marginatus* var. *cristata*, *C. peruvianus monstruosus* and *C. peruvianus florida*) and to the Euphorbiaceae family (*Euphorbia mammillaris*). The pathogen has been identified, for all the new hosts, from morphological characteristics observed *in vitro*. The identifications have been confirmed by means of ITS (Internal Transcribed Spacer) analysis and/or by Translation Elongation Factor 1α (TEF) analysis. The aim of this work was to identify the *forma specialis* of the *F. oxysporum* isolates obtained from new succulent plants. This has been investigated by means of phylogenetic analysis, based on the Translation Elongation Factor 1α gene and intergenic spacer (IGS), carried out on single-spore isolates, together with pathogenicity assays. The results of this research led to include the new isolates from succulent plants in the *F. oxysporum* f. sp. *opuntiarum*. This *forma specialis* has been identified for the first time on a new host (*Euphorbia mammillaris*) not belonging to the Cactaceae family.

Key words: ornamentals, succulent plants, soil-borne pathogens, Fusarium wilt.

INTRODUCTION

The ornamental industry is economically important throughout the world and it also represents an interesting growth opportunity for developing countries. In industrialized countries, ornamental plants are purchased throughout the year by a significant portion of the population, and moving the production of ornamental plants to developing countries would provide a remarkable source of income: in the USA, the wholesale value of floriculture crops was more than 4 billion dollars in 2014 (USDA, 2015); in Canada, the import and export value of ornamental plant products was about 418 and 312 million dollars, respectively, in 2013 (Statistical Overview of the Canadian Ornamental Industry, 2013); in Europe, the importing of live plants and floriculture products reached more than 1,500 million Euros in 2014 (European Commission, Agriculture and rural development, 2015).

The ornamental industry in Italy is important in the agricultural sector because of its favourable climatic conditions and specific economic situations that positively influence the economic returns, with a production of about 2,670 million Euros in 2011 (Schimmenti *et al.*, 2013). The value of the production of plants and flowers in the European Union was 21 billion Euros in 2013. Italy ranks second after the Netherlands and before Germany, with a share of 13.7% of the total value (Vanderelst and Zolichova, 2014). In 2013, the production of ornamental and flowering plants represented 5.4% of the total production in the agricultural sector in Italy (INEA, 2014). In 2010, a total of 4,271 farms producing ornamental and flowering plants were located in the Ligurian region, over an area of about 2,672 hectares (ISTAT, 2010). Various different new genera and species are exploited in this region, because of their commercial importance. A particular fragment of the ornamental industry is that of succulent plants, which currently show a good market potential.

The diversity of the crops and varieties, the effect of globalisation and of the intensive productions all lead to a multiplication of the number of potential pests and diseases that are able to infect new hosts. More than 120 different *formae speciales* of *F. oxysporum* have been described (Armstrong and Armstrong, 1981; O'Donnell and Cigelnik, 1999; Baayen *et al*., 2000; O'Donnell *et al*., 2009; Leslie, 2012). The detection and identification of *formae speciales*, which are classically based on pathogenicity assays (Recorbet *et al*., 2003), are at present supported by molecular diagnostic tools (Lievens *et al*., 2012). Several markers have been developed, on the basis of DNA sequences, in order to identify different *formae speciales* (Baayen *et al*., 2000; Groenewald *et al*., 2006). Genomic regions, such as the intergenic spacer region (IGS) or the Elongation factor (TEF), are useful but not enough for a correct identification (O'Donnell *et al*., 2009).

Over the last few years, Fusarium rot or wilt symptoms have appeared on five new succulent hosts grown as potted plants in commercial nurseries located in the Imperia province (Liguria region, northern Italy). The new hosts were succulent plants belonging to the Cactaceae family, that is, *Astrophytum myriostigma* (Garibaldi *et al*., 2015b), *Cereus peruvianus monstruosus* (Garibaldi *et al.*, 2011), *C. peruvianus florida* (Garibaldi *et al*., 2015a) and *C. marginatus* var. *cristata* (Garibaldi *et al*., 2014) as well as to the Euphorbiaceae family, that is, *Euphorbia mammillaris* (Garibaldi *et al*., 2015c). *Fusarium oxysporum* has been isolated and identified as the causal agent of the diseases on all the host plants by means of morphological and molecular methods.

The aim of this work was to investigate the *forma specialis* of the new isolates of *F. oxysporum* obtained from succulent plants.

MATERIALS AND METHODS

100 **Fungal isolates.** The *F. oxysporum* isolates were obtained from diseased plants, placing
101 on Potato Dextrose Agar (PDA) and/or Komada Fusarium selective medium (Komada, 1975)
102 small pieces taken from the margin of affected tissues. To obtain pure isolates, colonies were
103 subcultured on Potato Dextrose Agar (PDA). Two *F. oxysporum* f. sp. *opuntiarum* strains
104 coming from different collections were used as reference isolates. To obtain the single-spore
105 isolates used in this work (Table 1), a fungal suspension of each isolate was prepared in
106 Potato Dextrose Broth (PDB), shaking cultures (90 r.p.m.) at 25°C for 10 days. Then, each
107 suspension was diluted to 1×10^{-8} CFU/ml. A drop from more diluted concentrations was
108 subcultured on Komada selective medium. Single germinated microconidia were selected
109 using an optical microscope.
110 **Pathogenicity essays.** The isolates listed in table 2 were artificially inoculated on
111 *Schlumbergera truncata* plants, which are notoriously susceptible to *F. oxysporum* f. sp.
112 *opuntiarum* (Lops *et al*., 2013). The plants were inoculated by wounding the stems (3
113 lesions/plant) with a sterilized needle contaminated with spores and mycelium taken from
114 pure PDA cultures of the isolates (Talgø and Stensvand, 2013). Control plants were wounded
115 with sterilized needles without any inoculum. All the plants were maintained under
116 greenhouse conditions, at temperatures ranging from 20 to 35°C and at RH ranging from 40
117 to 65%.
118 Furthermore, two *F. oxysporum* f. sp. *opuntiarum* reference isolates (Table 3) were
119 inoculated on each new host and on *S. truncata* for comparison purposes. Three plants were
120 inoculated for each isolate (3 wounds/plant), according to the method described by Talgø and
121 Stensvand, 2013. The control plants were wounded with sterilized needles without any
122 inoculum. All the plants were kept in a greenhouse, at the same environmental conditions
123 described above.

After the first symptoms of rot appeared around the needles on the inoculated plants, the severity of *Fusarium* rot was evaluated every 4-7 days by measuring the size of the rotted area and by removing the dead plants. The presence of *Fusarium* symptoms was assessed using a 0 to 100 scale, where 0 indicates the absence of rot; 25: rot diameter of 0 to 5 mm; 50: rot diameter of 5 to 10 mm; 75: rot diameter of 10 to 20 mm; 100: rot diameter of more than 20 mm. The disease index was then calculated using the following formula: $(25n_1+50n_2+75n_3+100n_4)/(n_0+n_1+n_2+n_3+n_4)$, where n_0 is the number of rotting areas that scored 0; n_1 is the number of rotting areas that scored 25; ..... n_4 is the number of rotting areas that scored 100. Finally, each tested species was classified in 5 susceptibility classes: R = Resistant (disease index 0–5); PR = Partially Resistant (disease index 6–20); AS = Averagely Susceptible (disease index 21–50); S = Susceptible (disease index 51–75); HS = Highly Susceptible (disease index 76–100) (Tables 2 and 3).

DNA extraction. DNA extraction was carried out using an E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-Tek), according to the manufacturer's instructions. Fresh mycelium was obtained for each isolate listed in table 1 in 50ml of a liquid PDB culture incubated at 25°C. The cultures were filtered after 6 days and 50µg of mycelium was transferred to a 2 ml tube containing 400 µL of lysis buffer and two tungsten beads (Qiagen Stainless Steel Beads, 5 mm). Homogenization was performed using Qiagen TissueLyser for 4 min with 30 repetitions per minute, and the obtained lysate was used for DNA extraction. The DNA concentration was measured using a NanoDrop spectrophotometer, and the extracted DNA was stored at -20°C until further use.

PCR amplification. *Elongation factor 1-α* (*EF1-α*) and intergenic spacer (IGS) regions were used for the phylogenetic analysis. *EF-1α* was amplified with the EF1/EF2 primers (Table 4) using a T100 Thermal cycler (Biorad) in a 20 µL reaction mixture containing: 10 ng of gDNA, 1 µL of 10 µM stock (final concentration 0.5 µM) of each primer, 1 unit of Taq

149 DNA polymerase (Qiagen), 2 µL of PCR buffer 10×, 1 µL of dNTPs stock (final
150 concentration 0.25 mM) and 0.8 µL of MgCl2 (final concentration 1 mM). Amplification was
151 carried out with an initial denaturing step at 94°C for 5 min, followed by 35 denaturation
152 cycles at 94°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min and final
153 extension at 72°C for 7 min. For the IGS amplification, a PCR reaction was performed in a
154 50 µL reaction mixture containing: 30 ng of gDNA, 5 µL of 10 µM stock (final concentration
155 1 µM) of primers CNL12 and CNS1 (Table 5), 3 units of Taq DNA polymerase (Qiagen), 5
156 µL of PCR buffer 10×, 5 µL of dNTPs stock (final concentration 0.25 mM) and 10 µL of 5X
157 Q solution to amplify the G-C rich regions. A negative control (no template DNA) was
158 included in all the experiments. Amplifications were checked by electrophoresis on 1%
159 agarose gel (Agarose D-1 LOW EEO, Eppendorf). After purification with a QIAquick PCR
160 purification kit (Qiagen), the PCR products were measured using a NanoDrop
161 spectrophotometer and were then sent to Macrogen for sequencing
162 (http://www.macrogen.com/eng/). *EF-1α* was sequenced in both directions, while the two
163 internal primers CNS and CRU were also used for the IGS region. The sequences were
164 deposited at GenBank and the accession numbers are listed in table 5.

165 **Alignment and phylogenetic analysis.** Similarity searches (BLASTN, default
166 parameters) were performed for all the obtained sequences before a phylogenetic analysis
167 was performed. The sequences were then considered for CLUSTALW multiple sequence
168 alignments using MEGA6 software set to the default parameters. The sequences were
169 corrected manually for each alignment in order to delete any external trimmer regions and
170 discard any incomplete sequences. Phylogenetic trees were constructed in MEGA6 (Tamura
171 et al. 2013) using the Neighbor joining method with 1,000 bootstrap repeats and the pairwise
172 deletion option. The evolutionary distances were computed using the Tajima-Nei method,
173 and are in the units of the number of base substitutions per site. Sequences derived from

174 different *F. oxysporum formae speciales* obtained from the GenBank database were included
175 in each analysis.
176
177 **RESULTS AND DISCUSSION**
178
179 **Fungal isolates.** The single-spore isolates selected for this work are listed in table 1.
180 **Pathogenicity essays.** After the artificial inoculation on *Schlumbergera truncata,* all the
181 isolates listed in table 2 developed necrosis around the wounds, only on the inoculated stems.
182 The necrosis then became extended as far as the stems that rotted, whereas the controls
183 remained healthy. *F. oxysporum* was consistently re-isolated from symptomatic plants for all
184 the new hosts. *S. truncata* showed high susceptibility to all the tested isolates, including the
185 *F. oxysporum* f. sp. *opuntiarum* reference isolates (Table 2).
186 Furthermore, all the artificially inoculated succulent hosts were also susceptible to various
187 degrees to the *F. oxysporum* f. sp. *opuntiarum* isolates, as reported in table 3.
188 **Molecular phylogenetic analysis of the *EF-1α* region.** Amplification of the *EF-1α* gene
189 resulted in 750bp fragments of DNA. After multi-alignment with other *formae speciales*
190 present in Genbank, the obtained sequences were used for the phylogenetic analyses. The
191 results of the analyses showed that the isolates obtained from *Astrophytum myriostigma*,
192 *Cereus peruvianus monstruosus, C. peruvianus florida*, *C. marginatus* var. *cristata* and
193 *Euphorbia mammillaris* were included in *F. oxysporum* f. sp. *opuntiarum,* with a 66 bootstrap
194 value (Fig. 1). Two different reference strains were used in this cluster: *F. oxysporum* f. sp.
195 *opuntiarum* CBS 743.79 and a strain isolated from barrel cactus (*Echinocactus grusonii*) in
196 Italy (Polizzi and Vitale, 2004). The sequences used for the phylogenetic analysis were
197 deposited at Genbank (Table 5).

198 **Molecular phylogenetic analysis of the IGS region.** As observed for the *EF 1-α*
199 analysis, all the isolates in the analysis based on the IGS sequences also grouped together
200 within the *F. oxysporum* f. sp. *opuntiarum* clade with a high bootstrap value (100) (Fig. 2). A
201 reference sequence deposited by O'Donnell et al. in 2009 was used for this phylogenetic
202 tree. A 1994 bp sequence was obtained for each isolate, and the sequences used for the
203 phylogenetic analysis were deposited at Genebank (Table 5).
204 Phylogenetic analysis permitted to include the new *F. oxysporum* isolates from succulents
205 in f. sp. *opuntiarum*, according to the results of the pathogenicity essays. These last showed
206 that all the new isolates were pathogenic on *Schlumbergera truncata*, and when inoculated,
207 provided the same effect as that caused by the tested *F. opuntiarum* reference strains. These
208 last also caused disease on all the artificially inoculated new succulent hosts.
209 *Fusarium oxysporum* has been identified as the causal agent of stem and root rot on
210 different hosts belonging to the Cactaceae family: *Echinocactus grusonii* in England
211 (Hazelgrove, 1979), *Zygocactus truncatus* in the U.S.A. (Moorman and Klemmer, 1980),
212 *Schlumbergera truncata* (Petrone *et al*., 2007) and *Hylocereus undatus* in Argentina (Wright
213 *et al*., 2007). *F. oxysporum* on Cactaceae has been identified as belonging to f. sp.
214 *opuntiarum*: on *Zygocactus* and *Rhipsalidopsis* in Germany (Gerlach, 1972), on *Opuntia*
215 *ficus-indica* in Brazil (Souza de *et al*., 2010), on *Echinocactus grusonii* (Polizzi and Vitale,
216 2004) and *Schlumbergera truncata* in Italy (Lops *et al*., 2013). Moreover, on new succulent
217 hosts belonging to Crassulaceae family (*Crassula ovata*, *Echeveria agavoides* and *E.*
218 *tolimanensis*) two new *formae speciales*, named f. sp. *crassulae* (Ortu *et al*., 2013) and f. sp.
219 *echeveriae* (Ortu *et al*., 2015; Garibaldi *et al*., 2015d), respectively, have been recently
220 identified. Up to the present, *F. oxysporum* f. sp. *opuntiarum* has only been identified on
221 Cactaceae, and it has never been detected on hosts belonging to other families: this *forma*
222 *specialis* is reported on *Euphorbia mammillaris*, Euphorbiaceae family, for the first time.

Some strains of *F. oxysporum* isolated from diseased wild *Euphorbia* spp. in European countries have been shown to be virulent when artificially inoculated for the biological control of leafy spurge (*Euphorbia esula*) (Caesar, 1996). *F. oxysporum* was also isolated from affected poinsettias (*Euphorbia pulcherrima*) (Orlikowski *et al*., 2007). Nevertheless, the *forma specialis* was never investigated in any of these cases. Usually, *formae speciales* of *F. oxysporum* are assigned on the basis of their ability to infect a specific host. However, the acquisition of a pathogenicity genes by horizontal transfer is common on *F. oxysporum* species complex (Van der Does and Rep, 2007). In addition, *F. oxysporum* genome is about 70% larger compared to *F. verticilloides* with a more large intergenic regions, as well as the possibility of rearrange his genome by the high presence of transposable element sequences. The common presence of different transposable elements (Daboussi and Capy, 2003) suggests the ability to genomic rearrangement in response to the selection pressure, as well as to the intensive cultivation of host crop plants.

Because a large number of succulent species and cultivars are grown in the same farms, there is the risk of spread of *F. oxysporum* f. sp. *opuntiarum* among the susceptible hosts listed above. In these farms of intense floriculture, strategies to avoid the diffusion of *F. oxysporum* f. sp. *opuntiarum* are particularly recommended, especially in the case of rooted cuttings production. Finally, there is the necessity to test the susceptibility to this pathogen of the most diffused succulent plants in the Italian market, specially Cactaceae and Euphorbiaceae, to provide growers with a list of resistant and/or tolerant species and cultivars.

AKNOWLEDGEMENTS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634179 "Effective Management of Pests and Harmful Alien Species - Integrated Solutions" (EMPHASIS)

REFERENCES

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.

Armstrong G.M., Armstrong J.K., 1981. *Formae speciales* and races of *Fusarium oxysporum* causing wilt diseases. In: Nelson P.E., Toussoun T.A., Cook R.J. (eds.). *Fusarium*: Diseases, Biology and Taxonomy, pp. 391-399. The Pennsylvania State University Press, University Park, USA.

Baayen R.P., O'Donnell K., Bonants P.J.M., Cigelnik E., Kroon L.P.N.M., Roebroeck J.A., Waalwijk C., 2000. Gene genealogies and AFLP analysis in the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic *formae speciales* causing wilt and rot disease. *Phytopathology* **90**: 891-900.

Caesar A.J., 1996. Identity, pathogenicity, and comparative virulence of *Fusarium* spp. related to stand declines of leafy spurge (*Euphorbia esula*) in the Northern Plains. *Plant Disease* **80**: 395-1398.

Daboussi M.J., Capy P., 2003. Transposable elements in filamentous fungi. *Annual Review of Microbiology* **57**: 275–299.

European Commission, Agriculture and rural development, 2015. http://ec.europa.eu/agriculture/fruit-and-vegetables/product-reports/flowers/statistics-2015_en.pdf

272 Garibaldi A., Pensa P., Bertetti D., Poli A., Gullino, M.L., 2011. First report of basal stem rot
273 of Apple Cactus (*Cereus peruvianus monstruosus*) caused by *Fusarium oxysporum* in Italy.
274 *Plant Disease* **95**: 877.
275 Garibaldi A., Pensa P., Bertetti D., Ortu G., Gullino M.L., 2014. First report of dry and soft
276 rot of *Cereus marginatus* var. *cristata* caused by *Fusarium oxysporum* in Italy. *Plant Disease*
277 **98**: 1441.
278 Garibaldi A., Bertetti D., Pensa P., Ortu G., Gullino M.L., 2015a. First Report of *Fusarium*
279 *oxysporum* on *Cereus peruvianus florida* in Italy. *Journal of Plant Pathology*, in press.
280 Garibaldi A., Bertetti D., Pensa P., Ortu G., Gullino M.L., 2015b. First Report of *Fusarium*
281 *oxysporum* Causing Wilt on *Astrophytum myriostigma* in Italy. *Plant Disease* **100**: 215.
282 Garibaldi A., Bertetti D., Pensa P., Ortu G., Gullino M.L., 2015c. First Report of *Fusarium*
283 *oxysporum* Causing Wilt on *Euphorbia mammillaris* var. *variegata* in Italy, *Journal of Plant*
284 *Pathology*, in press.
285 Garibaldi A., Bertetti D., Pensa P., Ortu G., Gullino M.L., 2015d. First report of *Fusarium*
286 *oxysporum* f. sp. *echeveriae* Causing Wilt on *Echeveria tolimanensis* in Italy. *Plant Disease*
287 **99**: 1448.
288 Gerlach W., 1972. *Fusarium* rot and other fungal diseases of horticulturally important cacti in
289 Germany. *Phytopathologische Zeitschrift* **74**: 197-217.
290 Groenewald S., Van Den Berg N., Marasas W.F.O., Viljoen A., 2006. The application of high
291 throughput AFLPs in assessing genetic diversity in *Fusarium oxysporum* f. sp. *cubense*.
292 *Mycological Research* **110**: 297-305.
293 Gullino M.L., Garibaldi A., 2007. Critical aspects in management of fungal diseases of
294 ornamental plants and directions in research. *Phytopathologia Mediterranea* **46**: 135-149.
295 Gullino M.L., Katan J., Garibaldi A., 2012. The genus *Fusarium* and the species that affect
296 greenhouse vegetables and ornamentals. In: Gullino M.L., Katan J., Garibaldi A. (eds.).

297 Fusarium wilts of greenhouse vegetable and ornamental crops, pp. 5-9. APS Press, St. Paul,
298 MN, USA.
299 Hazelgrove A.J., 1979. A dry rot disease of *Echinocactus grusonii* caused by *Fusarium*
300 *oxysporum* Schlect. *National Cactus and Succulent Journal* **34**: 37-39.
301 INEA, 2014. L'agricoltura italiana conta 2014, Ministero delle Politiche Agricole Alimentari
302 e Forestali, Roma.
303 ISTAT, 2010. In: L'Agricoltura in Liguria. Rapporto sui dati provvisori del 6° Censimento
304 generale dell'Agricoltura in Liguria. 2011. Regione Liguria, Collana Convergenze &
305 Divergenze **15**.
306 Komada H., 1975. Development of a selective medium for quantitative isolation of *Fusarium*
307 *oxysporum* from natural soils. *Review of Plant Protection Research* **8**: 114-125.
308 Leslie J.F., 2012. Genetics and *Fusarium oxysporum.* In: Gullino M.L., Katan J., Garibaldi A.
309 (eds.). Fusarium wilts of greenhouse vegetable and ornamental crops, pp. 39-47. APS Press,
310 St. Paul, MN, USA.
311 Lievens B., Hanssen I.M., Rep M., 2012. Recent developments in the detection and
312 identification of *formae speciales* and races of *Fusarium oxysporum*: from pathogenicity
313 testing to molecular diagnostics. In: Gullino M.L., Katan J., Garibaldi A. (eds.). Fusarium
314 wilts of greenhouse vegetable and ornamental crops, pp. 47-55. APS Press, St. Paul, MN,
315 USA.
316 Lops F., Cibelli F., Raimondo M.L., Carlucci A., 2013. First report of stem wilt and root rot
317 of *Schlumbergera truncata* caused by *Fusarium oxysporum* f. sp. *opuntiarum* in southern
318 Italy. *Plant Disease* **97**: 846.
319 Moorman G.W., Klemmer R.A., 1980. *Fusarium oxysporum* causes basal stem rot of
320 *Zygocactus truncatus*. *Plant Disease* **64**: 1118-1119.

O'Donnell K., Kistler H.C., Cigelnik E., Ploetz R.C., 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences of the United States of America* **95**: 2044-2049.

O'Donnell K., Cigelnik E., 1999. A DNA sequence-based phylogenetic structure for the *Fusarium oxysporum* complex. *Phytoparasitica* **27**: 69-70.

O'Donnell K., Gueidan C., Sink S., Johnston P.R., Crous P.W., Glenn A., Riley R., Zitomer N.C., Colyer P., Waalwijk C., van der Lee T., Moretti A., Kang S., Kim H.S., Geiser D.M., Juba J.H., Baayen R.P., Cromey M.G., Bithell S., Sutton D.A., Skovgaard K.R., Kistler P., Elliott H.C., Davis M., Sarver M.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.

Orlikowski L.B., Skrzypczak C., Valiuskaite A., Sroczyn´ski M., 2007. *Fusarium* rot - new disease of poinsettia in Poland. *Progress in Plant Protection* **47**: 212-215.

Ortu G., Bertetti D., Gullino M.L., Garibaldi A., 2013. A new *forma specialis* of *Fusarium oxysporum* on *Crassula ovata*. *Journal of Plant Pathology* **95**: 33-39.

Ortu G., Bertetti D., Gullino M.L., Garibaldi A., 2015. *Fusarium oxysporum* f. sp. *echeveriae*, a novel *forma specialis* causing crown and stem rot of *Echeveria agavoides*. *Phytopathologia Mediterranea* **54**: 64-75.

Petrone M.E., Lori G., Wright E.R., Rivera M.C., 2007. First report of stem and root rot of *Schlumbergera truncata* caused by *Fusarium oxysporum* in Argentina. *Journal of Plant Pathology* **89** (3, Supplement): S70.

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.

345 Recorbet G., Steinberg C., Olivain C., Edel V., Trouvelot S., Dumas-Gaudot E., Gianinazzi
346 S., Alabouvette C., 2003. Wanted: pathogenesis-related marker molecules for *Fusarium*
347 *oxysporum*. *New Phytologist* **159**: 73-92.
348 Schimmenti E., Galati A., Borsellino V., Ievoli C., Lupi C., Tinervia S., 2013. Behaviour of
349 consumers of conventional and organic flowers and ornamental plants in Italy. *Hort. Sci.*
350 *(Prague)* **40**: 162-171.
351 Souza de A.E.F., Nascimento L.C., Araújo E., Lopes E.B., Souto F. M., 2010. Occurrence
352 and identification of the etiologic agents of plant diseases in cactus (*Opuntia ficus-indica*
353 Mill.) in the semi-arid region of Paraiba. *Biotemas* **23** (3):11-20.
354 Statistical Overview of the Canadian Ornamental Industry, 2013. http://www.agr.gc.ca/
355 Tamura K., Stecher G., Peterson D., Filipski A., Kumar S., 2013. MEGA6: Molecular
356 Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.* **30**: 2725-2729.
357 Talgø, V., and Stensvand, A. 2013. A simple and effective inoculation method for
358 *Phytophthora* and fungal species on woody plants. *OEPP/EPPO Bulletin* **43** (2): 276-279.
359 USDA, 2015. Floriculture Crops, 06.04.2015.
360 http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1072
361 Van der Does H.C., Rep M., 2007. Virulence Genes and the Evolution of Host Specificity in
362 Plant-Pathogenic Fungi. *Molecular Plant-Microbe Interactions* **20** (10): 1175-1182.
363 Vanderelst D., Zolichova L., 2015. Live plants and products of floriculture sector in the EU.
364 DG Agriculture and Rural Development (DG AGRI) Document.
365 http://ec.europa.eu/agriculture/fruit-and-vegetables/product-reports/flowers/market-analysis-
366 2015_en.pdf
367 Wright E.R., Rivera M.C., Ghirlanda A., Lori G.A., 2007. Basal rot of *Hylocereus undatus*
368 caused by *Fusarium oxysporum* in Buenos Aires, Argentina. *Plant Disease* **91**: 323.
369

370 Table 1. The *Fusarium oxysporum* single-isolates used in this study.

Strain	**Host plant**	**Place**
DB13GIU05-22M	*Cereus marginatus*	Italy
DB13GIU06-26M	*Cereus marginatus*	Italy
DB210211-18M	*Cereus peruvianus monstruosus*	Italy
DB220211-21M	*Cereus peruvianus monstruosus*	Italy
DB14OTT05-M1	*Astrophytum myriostigma*	Italy
DB14OTT07-M1	*Astrophytum myriostigma*	Italy
DB14NOV08-M1	*Cereus peruvianus florida*	Italy
DB14NOV09-M1	*Cereus peruvianus florida*	Italy
DB14OTT16-M1	*Euphorbia mammillaris*	Italy
DB14OTT17-M1	*Euphorbia mammillaris*	Italy
Polizzi-31M (*F. oxysporum* f. sp. *opuntiarum* reference strain)	*Echinocactus grusonii*	Italy
CBS 743.79 (*F. oxysporum* f. sp. *opuntiarum* reference strain)	*Zygocactus truncatus*	Germany

371

372

373

374

375

376

377

Table 2. Pathogenicity test carried out on *Schlumbergera truncata* plants artificially inoculated with *Fusarium oxysporum* isolates obtained from succulent plants.

***Fusarium oxysporum* tested isolates**	**Susceptibility of *Schlumbergera truncata***
Controls	R*
DB13GIU05-22M (from *Cereus marginatus*)	HS
DB13GIU06-26M (from *Cereus marginatus*)	HS
DB210211-18M (from *Cereus peruvianus monstruosus*)	HS
DB220211-21M (from *Cereus peruvianus monstruosus*)	HS
DB14OTT07-M1 (from *Astrophytum myriostigma*)	HS
DB14NOV09-M1 (from *Cereus peruvianus florida*)	HS
DB14OTT16-M1 (from *Euphorbia mammillaris*)	HS
Fusarium oxysporum f. sp. *opuntiarum* Polizzi-31M	HS
Fusarium oxysporum f. sp. *opuntiarum* CBS 743.79	HS

*R = Resistant (disease index 0–5); PR = Partially Resistant (disease index 6–20); AS = Averagely Susceptible (disease index 21–50); S = Susceptible (disease index 51–75); HS = Highly Susceptible (disease index 76–100).

391 Table 3. Susceptibility of succulent species artificially inoculated with *Fusarium oxysporum*

392 f. sp. *opuntiarum* isolates.

Tested species	***Fusarium oxysporum* f. sp. *opuntiarum* tested isolates**		
	Controls	**Polizzi-31M**	**CBS 743.79**
Astrophytum myriostigma	R*	HS	HS
Cereus marginatus	R	AS	AS
Cereus peruvianus Monstruosus	R	AS	S
Cereus peruvianus florida	R	HS	HS
Euphorbia mammillaris	R	AS	AS

393 *R = Resistant (disease index 0–5); PR = Partially Resistant (disease index 6–20); AS =

394 Averagely Susceptible (disease index 21–50); S = Susceptible (disease index 51–75); HS =

395 Highly Susceptible (disease index 76–100).

396

397

398

399 Table 4. Primers used to amplify polygalacturonase genes.

Gene	**Primer**	**Nucleotide Sequences (5'→3')**	**Source**
EF-1α	Ef1	ATGGGTAAGGAAGACAAGAC	O'Donnell *et al*., 1998
	Ef2	GGAAGTACCAGTGATCATGTT	
IGS	CNS1	CCAGAGTGCCGATACCGATT	Appel and Gordon, 1995
	CNL12	GCTTAGYGAACAKGGAGTG	

400

401

402

403

Table 5. Accession numbers of the *EF-1α* and IGS sequences obtained from *Fusarium oxysporum* and deposited in the GenBank database.

Isolates	**Accession numbers in GenBank**	
	EF-1α	**IGS**
DB13GIU05-22M	KU575888	KU575870
DB13GIU06-26M	KU575889	KU575871
DB210211-18M	KU575886	KU575872
DB220211-21M	KU575887	KU575873
DB14OTT05-M1	KU575882	KU575876
DB14OTT07-M1	KT183483	KU575877
DB14NOV08-M1	KU575885	KU575878
DB14NOV09-M1	KT183484	KU575879
DB14OTT16-M1	KT183485	KU575880
DB14OTT17-M1	KU575883	KU575881
Fusarium oxysporum f. sp. *opuntiarum* Polizzi-31M	KU575890	KU575868
Fusarium oxysporum f. sp. *opuntiarum* CBS 743.79	KU575891	KU575869

418 **FIGURE LEGENDS**

419

420 Figure 1. Phylogenetic tree based on the *EF-1α* gene sequences, built by means of Mega5
421 software with the Neighbor joining method, using default parameters, and a standard
422 bootstrapping with 1,000 replicates.

423

424 Figure 2. Phylogenetic tree based on the IGS sequences, built by means of Mega5 software
425 with the Neighbor joining method, using default parameters, and a standard bootstrapping
426 with 1,000 replicates.

427

428

429

430

431

432

433

434

435

436

437

438

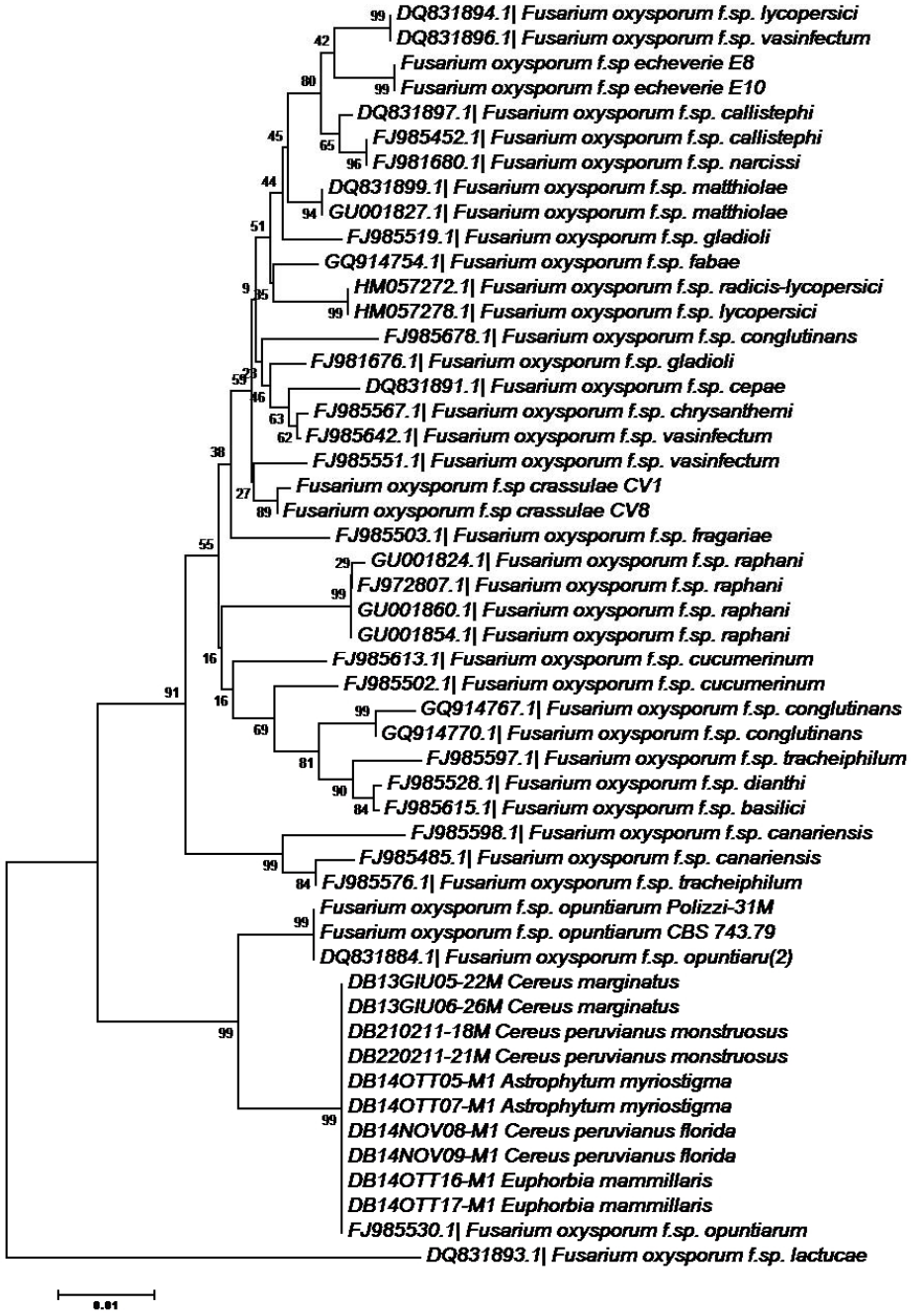
439

440

441

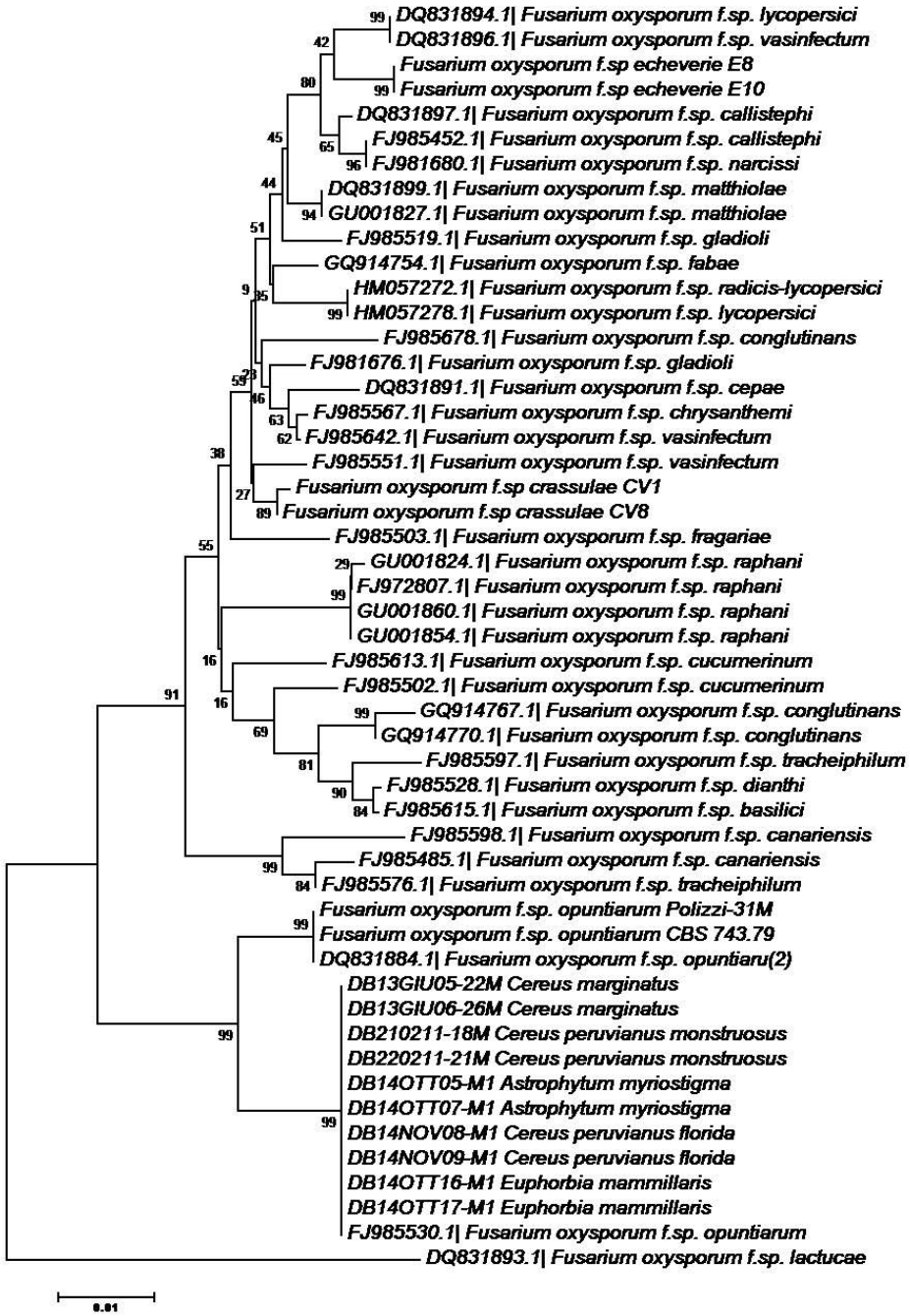
442

443 **Figure 1.**



444

Figure 2.


DQ831894.1| Fusarium oxysporum f.sp. lycopersici
DQ831896.1| Fusarium oxysporum f.sp. vasinfectum
Fusarium oxysporum f.sp echeverie E8
Fusarium oxysporum f.sp echeverie E10
DQ831897.1| Fusarium oxysporum f.sp. callistephi
FJ985452.1| Fusarium oxysporum f.sp. callistephi
FJ981680.1| Fusarium oxysporum f.sp. narcissi
DQ831899.1| Fusarium oxysporum f.sp. matthiolae
GU001827.1| Fusarium oxysporum f.sp. matthiolae
FJ985519.1| Fusarium oxysporum f.sp. gladioli
GQ914754.1| Fusarium oxysporum f.sp. fabae
HM057272.1| Fusarium oxysporum f.sp. radicis-lycopersici
HM057278.1| Fusarium oxysporum f.sp. lycopersici
FJ985678.1| Fusarium oxysporum f.sp. conglutinans
FJ981676.1| Fusarium oxysporum f.sp. gladioli
DQ831891.1| Fusarium oxysporum f.sp. cepae
FJ985567.1| Fusarium oxysporum f.sp. chrysanthemi
FJ985642.1| Fusarium oxysporum f.sp. vasinfectum
FJ985551.1| Fusarium oxysporum f.sp. vasinfectum
Fusarium oxysporum f.sp crassulae CV1
Fusarium oxysporum f.sp crassulae CV8
FJ985503.1| Fusarium oxysporum f.sp. fragariae
GU001824.1| Fusarium oxysporum f.sp. raphani
FJ972807.1| Fusarium oxysporum f.sp. raphani
GU001860.1| Fusarium oxysporum f.sp. raphani
GU001854.1| Fusarium oxysporum f.sp. raphani
FJ985613.1| Fusarium oxysporum f.sp. cucumerinum
FJ985502.1| Fusarium oxysporum f.sp. cucumerinum
GQ914767.1| Fusarium oxysporum f.sp. conglutinans
GQ914770.1| Fusarium oxysporum f.sp. conglutinans
FJ985597.1| Fusarium oxysporum f.sp. tracheiphilum
FJ985528.1| Fusarium oxysporum f.sp. dianthi
FJ985615.1| Fusarium oxysporum f.sp. basilici
FJ985598.1| Fusarium oxysporum f.sp. canariensis
FJ985485.1| Fusarium oxysporum f.sp. canariensis
FJ985576.1| Fusarium oxysporum f.sp. tracheiphilum
Fusarium oxysporum f.sp. opuntiarum Polizzi-31M
Fusarium oxysporum f.sp. opuntiarum CBS 743.79
DQ831884.1| Fusarium oxysporum f.sp. opuntiaru(2)
DB13GIU05-22M Cereus marginatus
DB13GIU06-26M Cereus marginatus
DB210211-18M Cereus peruvianus monstruosus
DB220211-21M Cereus peruvianus monstruosus
DB14OTT05-M1 Astrophytum myriostigma
DB14OTT07-M1 Astrophytum myriostigma
DB14NOV08-M1 Cereus peruvianus florida
DB14NOV09-M1 Cereus peruvianus florida
DB14OTT16-M1 Euphorbia mammillaris
DB14OTT17-M1 Euphorbia mammillaris
FJ985530.1| Fusarium oxysporum f.sp. opuntiarum
DQ831893.1| Fusarium oxysporum f.sp. lactucae
0.01