Crop Protection 36 (2012) 43–48

Contents lists available at SciVerse ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Fusarium wilt of cyclamen: Pathogenicity and vegetative compatibility groups structure of the pathogen in Argentina

Gladys A. Lori [a,*], Paula M. Petiet [b], Ismael Malbrán [a], Cecilia A. Mourelos [a], Eduardo R. Wright [b], Marta C. Rivera [b]

[a] CIDEFI-CIC (Centro de Investigaciones de Fitopatología), Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Calle 60 y 119, 1900 La Plata, Prov. Buenos Aires, Argentina
[b] Fitopatología, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, 1417 Ciudad Autónoma de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 22 August 2011
Received in revised form
19 December 2011
Accepted 16 January 2012

Keywords:
Fusarium oxysporum f. sp. *cyclaminis*
Pathogenicity
Vegetative compatibility groups
Cyclamen persicum
Argentina

ABSTRACT

Cyclamen persicum is a winter flowering species, cultivated as a potted ornamental. It is an economically important crop in Argentina. *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cyclaminis* (*Foc*) is a destructive disease that limits the production of quality plants. The aims of this work were to analyze *Fusarium* isolates from cyclamen crops in Argentina for pathogenicity and vegetative compatibility groups (VCGs) structure. As a result of crop surveys, 64 isolates of *Fusarium oxysporum* were obtained from cyclamen plants with typical wilt symptoms, cultivated in 13 locations in Buenos Aires and Córdoba provinces, Argentina. Pathogenicity of all the isolates was confirmed by two different methods of inoculation. Initial symptoms usually appeared 12–15 days after inoculation for both methods. All the isolates were identified as *Foc*. VCGs structure was determined by the complementation of nitrate-nonutilizing (*nit*) mutants as a visual indicator of heterokaryon formation. All the isolates were arranged in 5 VGC groups. The largest group (VCG1) included 53 isolates (83%) obtained from all of the different production areas and most commercial crops. The other VCGs were formed by 1–4 isolates obtained from 5 commercial crops. Our results suggest the existence of a uniform population of *Foc* in Argentina, which is widespread in geographically separated areas, since some locations are distant approximately 700 km. The distribution of members of the same VCG in distant areas would demonstrate that those pathogenic isolates have a common origin, and a close genetic relationship. Since the pathogen can be spread on seed coats and in seed debris, it's introduction in Argentina with multiplication material seems possible.

1. Introduction

Cyclamen persicum (Mill.) is a winter flowering species, cultivated as a potted ornamental (Dole and Wikins, 1999). It is an economically important crop in Argentina. According to the last census, 685,600 potted cyclamen plants are produced in Buenos Aires per year (Gobierno de la provincia de Buenos Aires, 2006). As with many potted flowering plants, cyclamens are mainly cultivated in the green belts of Buenos Aires city. In spite of its high market value, its production decreased abruptly during the last decade, mainly due to diseases (Romero and Rivera, 2005; Wright et al., 2006; Rivera et al., 2009).

Fusarium wilt of cyclamen, caused by *Fusarium oxysporum* Schlechtend. Fr. f. sp. *cyclaminis* Gerlach (*Foc*), is a destructive disease that limits the production of quality plants. Losses of over 59% and 90% of cyclamen productions due to *Fusarium* wilt have been informed for Connecticut (Elmer, 2002) and Germany (Orlicz-Luthardt, 1998), respectively. In Argentina, crop losses up to 50% have been observed.

The presence of the disease has been reported in many countries including Italy (Bongini, 1940), Great Britain (Moore, 1947), Germany (Gerlach, 1954), Bulgaria (Kristova, 1958; Doganova, 1976), U.S.A. (Tompkis and Snyder, 1972), Belgium and France (Rouxel and Grouet, 1974), Brazil (Pitta and Teranishi, 1979), Australia (Beardsell and Nichols, 1981), New Zealand (Pennycook, 1989), Netherlands (Rattink, 1986; Rattink, 1990), Argentina (Palmucci and Wright, 1990), Japan (Kujima and Kumada, 1991), and Korea (Cho and Shin, 2004). The pathogen can cause sudden death in all of the plant development stages. Symptoms of the disease include yellowing areas at the base and blade of leaves that develop into wilt. However, external symptoms may not be conspicuous until plant reproductive stage, when

* Corresponding author. Tel.: +54 221 423 6758; fax: +54 221 425 2346.
E-mail address: galori@infovia.com.ar (G.A. Lori).

0261-2194/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2012.01.006

flower blooms and flowers wilt and the entire plants collapse. Corms remain un-rotten, showing a brown-black discoloration of the veins and surrounding parenchyma. In infected plants, wilt symptoms usually start on one side, and transverse sections of the corms show one-sided discoloration of the veins (Woudt et al., 1995).

F. oxysporum is a ubiquitous fungus that is present in natural and cultivated soils. Pathogenic and non-pathogenic strains are distinguishable only by molecular or other genetic studies such as vegetative compatibility tests (Leslie, 1996). Due to research initiated by Puhalla (1985), isolates of various *formae specialis* of *F. oxysporum* (pathogenic variants) could be characterized into vegetative compatibility groups (VCG) based on the fungus genetics instead of on host–pathogen interactions. When complemented with molecular tools, VCGs may help to analyze populations' pathology, biology, genetic diversity and race relationships (Appel and Gordon, 1994, 1996; Aloi and Baayen, 1993; Baayen et al., 1997; Kalc Wright et al., 1996; Manicom et al., 1990; Vakalounakis and Fragkiadakis, 1999). Correll (1991) found that vegetative compatibility is a useful tool to distinguish pathogens from non-pathogens, as well as to characterize the genetic diversity within a pathogenic population. For many *formae specialis* of *F. oxysporum*, pathogenicity has shown a strong correlation with VCGs. Katan et al. (1989), Elena and Tjamos (1995), and Lori et al. (1996, 2004) used this technique to separate *F. oxysporum* f. sp *dianthi* (*Fod*) from non-pathogenic *F. oxysporum* strains and to identify pathogen races. Steinberg et al. (1997) and Lori et al. (2004) reported that VCGs and genetic characterization provided the grouping of correlated strains, and that, for that purpose, VCGs are more discriminating than PCR-RFLP analysis of the IGS region.

To our knowledge, there is only one published research on the variability within *Foc* population (Woudt et al., 1995). Although many authors consider that pathogenic strains are different from non-pathogenic ones, Woudt et al. (1995) reported that non-pathogenic isolates of *F. oxysporum* associated with cyclamen were similar to the pathogenic isolates of *Foc* after the analysis of the polymorphism in the IGS region of the ribosomal DNA. However, the isolates showed differences when studied through vegetative compatibility and DNA fingerprints, and pathogenic strains could be separated from saprophytic ones by VCGs analysis. Woudt et al. (1995) detected only 3 lineages for pathogenic strains in different countries. The authors identified the presence of the same VCGs in the Netherlands, United Kingdom, U.S.A., Japan, France, Germany and Australia, thus demonstrating that variability within the pathogenic population was considerably less than within the non-pathogenic.

The genetic diversity of *F. oxysporum* isolates related with cyclamen crops has been scarcely studied in the world (Katan, 1999) and no information has been reported from Argentina, in spite of the early report of the disease in the 1990's. The knowledge of the pathogen population in cropping areas is extremely important for the development of disease management strategies. In this context, the aims of this work were to analyze *F. oxysporum* isolates obtained from cyclamen crops in Argentina for pathogenicity and VCGs structure.

2. Materials and methods

2.1. Isolation of F. oxysporum

Thirteen commercial cyclamen crops located in Ciudad Autónoma de Buenos Aires, and Buenos Aires and Córdoba provinces (Argentina) were surveyed and plants with typical wilt symptoms (Fig. 1) were sampled (Table 1). Ciudad Autónoma de Buenos Aires and Cordoba city, in Cordoba province, are distant approximately 700 km.

For isolations, pieces of corms with discolored vascular tissue (Fig. 2) were surface disinfested with 0.5% NaOCl for 1 min, rinsed in sterilized distilled water, and cultured in 2% potato dextrose agar (PDA) at 25 °C. Single-spore cultures were made from *Fusarium* isolates. Identification of *F. oxysporum* was achieved according to cultural and morphobiometrical features (Booth, 1971; Nelson et al., 1983; Leslie and Summerell, 2006). The isolates were stored in mineral oil at 4 °C until needed for further studies.

2.2. Pathogenicity tests

The pathogenic ability of each *F. oxysporum* isolate was tested by target inoculations on cyclamen plants with 5–7 leaves, of cultivar Sierra rose. Each isolate was cultivated in Petri dishes with 10 ml of PDA at 23–25 °C for 10 days. Spore suspensions were prepared by pouring sterile water into the Petri dishes and rotating them slightly to dislodge conidia, filtered through cheesecloth, and spore concentration was adjusted to 1×10^6 microconidia ml^{-1} using a hemacytometer.

Two inoculation methods were used:

a) Immersion of cyclamen roots in conidial suspensions

For each treatment, the roots of six potted plants were washed under running tap water. Root tips were cut off and the roots were immersed in 250 mL glass beakers containing 100 mL of spore suspensions or sterilized distilled water (control) during 20 min. The plants were transplanted into pots containing an autoclaved substratum composed by perlite and peat (1:2 by vol.) pH 6.5.

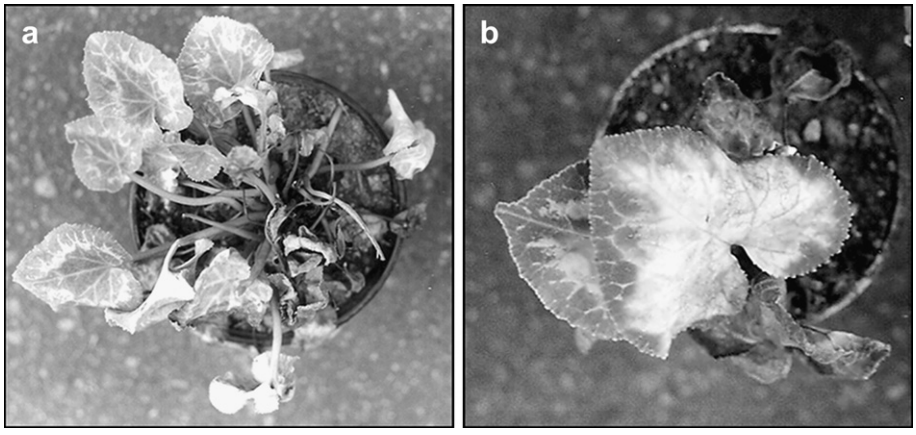

Fig. 1. a. Cyclamen plant with typical one-side wilt symptoms, b. leaf yellowing that initiates at the base of the leaves.

Table 1
Vegetative compatibility groups structure of isolates of *Fusarium oxysporum* obtained from cyclamen commercial crops located in Ciudad Autónoma de Buenos Aires, and Buenos Aires and Córdoba provinces (Argentina).

VCG	Department (Province)	Commercial crops (no.)	*F. oxysporum* isolates (no.)
1	Tres de Febrero (Buenos Aires)	2	24
	Ciudad Autónoma de Buenos Aires	1	5
	Malvinas Argentinas (Buenos Aires)	2	7
	Moreno (Buenos Aires)	2	6
	José C. Paz (Buenos Aires)	2	7
	Junín (Buenos Aires)	1	3
	Córdoba (Córdoba)	1	1
2	Ciudad Autónoma de Buenos Aires	1	2
	Moreno (Buenos Aires)	1	2
3	Córdoba (Córdoba)	1	3
4	Moreno (Buenos Aires)	1	3
5	Ciudad Autónoma de Buenos Aires	1	1

b) Irrigation of cyclamen plants with conidial suspensions

For each treatment, six plants were transplanted into pots that contained the described substrate, and two complete leaves were detached per plant, simulating wounds caused by crop labors. Each plant was irrigated with 10 mL of the spore suspensions or distilled sterilized water (control).

As additional control treatments, plant sets were inoculated with a non-pathogenic isolate of *F. oxysporum* (*Fox* 25) and a different *formae specialis*, *F. oxysporum* f. sp. *dianthi* (*Fod* 99 VCG 0021, race 2) in both inoculation experiments.

Inoculated and control plants were enclosed in moistened polyethylene bags and placed in a climatic chamber at 22–24 °C under fluorescent light (12 h photoperiod). The bags were removed 48 h after inoculation. The plants remained in the chamber, and symptoms were evaluated every 3 days, taking into account loss of leaf turgor, leaf yellowing and necrosis, and plant death due to vein necrosis. At the end of the experiment each plant was uprooted and the corms cut transversally to evaluate the presence of discolored vessels. Pathogenic isolates were obtained by reisolation from infected corms.

2.3. Vegetative compatibility testing

Sixty-four isolates of *F. oxysporum* were used for compatibility tests. Vegetative compatibility groups (VCGs) were determined through the complementation of nitrate-nonutilizing (*nit*) mutants as a visual indicator of heterokaryon formation. Mutants were generated for each isolate on potato-sucrose chlorate medium (KPS) and minimal chlorate medium (KMM) with 1.5% potassium chlorate. When isolates failed to form mutants on these media, chlorate concentration was increased to 3%. The fast-growing chlorate-resistant sectors originating from the initially restricted colony were transferred to minimal medium (MM) containing nitrate as the sole nitrogen source (Puhalla, 1985). Colonies that appeared thin and expansive without any aerial mycelium were considered to be *nit* mutants, and classified as *nit*1, *nit*3 or NitM based on their phenotype on media containing one of three different nitrogen sources (nitrate, nitrite, and hypoxanthine) (Correll et al, 1987). At least one *nit*1 or *nit*3 mutant and one NitM mutant were obtained from each isolate and used for complementation tests. Pairings were made on MM in 9 cm Petri dishes. Three mutants were inoculated on each plate, forming a triangle configuration, and the plates were incubated at 23–25 °C in the dark and scored for complementation 7 and 14 days after incubation. Different mutants derived from the same strain were paired with one another to test for self-incompatibility (Correll et al., 1989). Vegetative compatibility was determined by pairing complementary *nit* mutants derived from all 64 isolates in all pairwise combinations. To be used as controls, *nit* mutants from a non-pathogenic isolate (*F. oxysporum*, *Fox* 25) and a different *formae specialis* (*Fod* 99 VCG 0021, race 2) were obtained and paired with *nits* recovered from each cyclamen isolate. When two mutants formed a visible and robust heterokaryon indicated by the presence of dense aerial mycelium, the corresponding isolates were placed in the same VCG (Lori et al., 2004). All pairings were repeated twice.

3. Results

3.1. Isolation of F. oxysporum

A total of 64 isolates were identified as *F. oxysporum*. The number of obtained isolates varied among surveyed commercial crops (Table 1).

3.2. Pathogenicity tests

No differences were detected in the results of the two inoculation methods. Symptoms developed very quickly. Initial disease symptoms (loss of leaf turgor and yellowing at the base of the

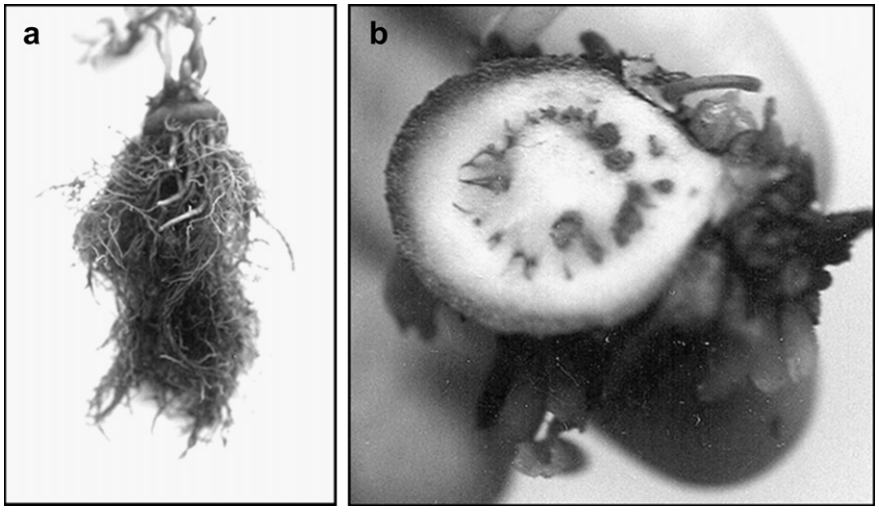

Fig. 2. a. Infected cyclamen corm and roots with no external symptoms, b. discolored corm vascular tissues.

lamina; mainly on one side of the plant) appeared 12–15 days from inoculation. Yellowing initiated in petiole insertion, and developed towards the leaf blade, finally covering it. Leaf margins turned necrotic. Plant death occurred mostly 21 days from inoculation, except for isolates 41, 42, 43 and 48 (day 24) and isolates 45, 46, 47 and 50 (on day 27). All the control plants, as well as the plants inoculated with non-pathogenic *Fox 25* and the pathogenic *Fod* VCG 0021 isolate 99, remained healthy.

All of the *F. oxysporum* isolates were pathogenic and induced typical wilt symptoms. They were re-isolated from symptomatic organs, thus fulfilling Koch's postulates. These results allow the confirmation of the presence of *F. oxysporum* f. sp. *cyclaminis* in the surveyed crops.

3.3. Vegetative compatibility testing

Isolates of *F. oxysporum* readily formed chlorate-resistant sectors on KPS and KMM. *Nit* mutants emerged from restricted growth on chlorate media after 6–14 days. The average number of sectors on KPS and KMM per isolate ranged from 0.2 to 1.4 and 1.3 to 3.3 respectively. All the isolates developed two or more phenotypically different nit mutants on KPS or KMM. The *nit*1 phenotype was the most frequently recovered (55% of the mutants), followed by NitM (29%) and *nit*3 (11%). *Nit*1 and NitM mutants were easily obtained from all 64 isolates. For each pair of isolates, compatibility reaction was evidenced by heterocaryon formation (Fig. 3) between *nit*1 or *nit*3 and NitM, thus indicating that they belong to the same VCG. Strong heterokaryons were formed in a few days for all the confrontations (Fig 3). During VCG testing, there was no evidence of self-incompatibility.

The 64 isolates obtained from symptomatic plants fell into 5 VCGs arbitrarily named 1–5 (Table 1). Of these, the largest group was VCG1, which included 53 isolates obtained from 11 commercial crops in Buenos Aires and Córdoba.

The other VCGs were formed by 1–4 isolates obtained from 5 commercial crops. VCG2, VCG3, VCG 4 and VCG5 were composed by 4, 3, 3 and 1 isolates, respectively, recovered from crops located in Buenos Aires and Ciudad Autónoma de Buenos Aires, Córdoba, Buenos Aires, and Ciudad Autónoma de Buenos Aires, respectively. Only 3 of the commercial crops considered contained isolates belonging to more than 1 of the VCG groups.

The non-pathogenic control isolate, as well as *Fod*, did not form complementary heterocaryons with the any of the cyclamen strains.

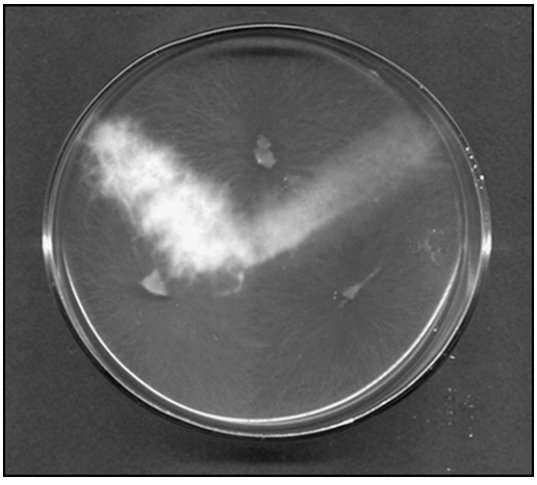

Fig. 3. Robust mycelium growth on minimal medium indicates complementation between isolates of *F. oxysporum* f. sp. *cyclaminis*.

4. Discussion

This is the first study of pathogenicity of Argentinean *Foc* isolates on young cyclamen plants and their genetic relatedness using VCGs. The pathogen infects cyclamen plants at any growth stage, particularly in conductive environments. The disease was found in 100% of the surveyed crops, demonstrating its importance in cyclamen production in Argentina, in agreement with reports from other countries (Bongini, 1940; Moore, 1947; Gerlach, 1954; Kristova, 1958; Tompkins and Snyder, 1972; Rouxel and Grouet, 1974; Doganova, 1976; Pitta and Teranishi, 1979; Beardsell and Nichols, 1981; Garibaldi, 1981; Rattink, 1986, 1990; Kujima and Kumada, 1991). As isolations were performed from plants evidencing initial wilt symptoms, only pathogenic isolates were collected.

Similarly to Woudt et al. (1995) all the inoculated plants in our assays died within 3 weeks from inoculation. The two inoculation methods employed in this work did not differ in their efficiency, and reproduced the symptoms observed in natural infections.

Using *nit* mutants of *F. oxysporum* in complementary tests, 5 VCGs could be identified among the 64 isolates recovered from 13 cyclamen commercial crops in Ciudad Autónoma de Buenos Aires, Buenos Aires province (the major Argentinean growing area) and Córdoba province. The largest VCG comprised 83% of the total isolates (53 isolates) of *Foc*, and was distributed through all of the surveyed departments. Isolates belonging to VCGs 2, 3, 4 and 5 were represented much less frequently, suggesting that VCG1 might be the dominant type in local cyclamen crops. The presence of the same VCG in different cyclamen crops indicates that this pathotype is being selectively maintained (Lamondia and Elmer, 1989).

Our results suggest the existence of a uniform population of *Foc* in Argentina, which is widespread in geographically separated areas, since distance between locations may be up to 700 km. This uniformity could be explained by the fact that all the Argentinean isolates are clonal derivates of a single introduced isolate, which subsequently proliferated due to improper management practices. Similar results were reported in Israel (Katan et al., 1989), Greece (Elena and Tjamos, 1995), Australia (Kalc Wright et al., 1996) and Argentina (Lori et al., 2004) for the race 2 of *Fod*.

Woudt et al. (1995) found only three lineages for pathogenic strains of *Foc* in different countries and the same VCGs (0151, 0152 and 0153) were identified in the Netherlands, United Kingdom, U.S.A., Japan, France, Germany and Australia, thus demonstrating that variability within the pathogenic population was considerably less than within the non-pathogenic one. Of 53 pathogenic strains examined by Woudt et al. (1995), 37 clearly belonged to VCG 0151, and another four were assigned to the same VCG on the basis of weak complementation or absent complementation plus DNA fingerprinting. In this way the authors found that 77% of the strains belonged to the widely distributed VCG 0151. Similarly, 83% of the pathogenic isolates covered by this work shared a single VCG.

The origin of VCG 0151 and other *Foc* VCGs detected by Woudt et al. (1995) is most likely to be Europe. Algeria and Tunisia are possible centers of origin of *C. persicum*, and the first ornamental cyclamens are thought to have appeared in European gardens. So, the origin of the host and the widely distributed pathogen VCGs could coincide. Taking into account that cyclamen seeds are mostly imported from Europe, VCG1 (the most frequently found in Argentina) may have been originated in the same area.

The low population diversity found in this work is probably due to the isolation of pathogenic strains only. Diversity would probably be considerably higher if the non-pathogenic population of *F. oxysporum* associated with cyclamen was taken into account, as reported by Correll et al. (1986), Gordon and Okamoto (1992) and

Katan et al. (1989, 1994). In Argentina, diversity of non-pathogenic population of *F. oxysporum* recovered from soils associated to carnation in Buenos Aires was found to be high (Lori et al., 2004).

Pathogenicity tests are one of the methods used for differentiation of *F. oxysporum* isolates. However, their requirements of young plants, high costs and time demand make it difficult to carry them out to test great numbers of isolates. These drawbacks may be solved by the usage of vegetative compatibility (VCG). In this work grouping of pathogenic isolates was achieved by combining pathogenicity tests and VCG.

Vegetative compatibility groups have been widely used as a powerful tool to subdivide populations of plant pathogenic fungi and assess relationships among individuals (Leslie, 1993), and to establish genetic frameworks for assisting in the interpretation of data on molecular diversity (Correll et al., 2000). Furthermore, the use of VCGs may be particularly helpful in characterizing population diversity in many cyclamen growing areas of the world, including Argentina, where cost and laboratory facilities may limit the number of isolates that can be analyzed by molecular methods (Correll et al., 2000).

Late and sudden symptom evidence for cyclamen wilt makes it difficult to implement control measures. Pathogen dispersal through infested potting mix may have been the way in which the disease was spread in all cyclamen cropping areas in Argentina, i.e. from plug growers. Since the pathogen can be spread on seed coats and in seed debris (Tompkins and Snyder, 1972) its introduction in Argentina with imported multiplication material seems possible.

This research constitutes the basis for future studies that may include the comparison of Argentinean isolates with international VCG tester strains. Future deep studies on the pathogenic population of *Foc* would determine the possible presence of new pathotypes, not yet identified in other parts of the world, or known ones, that could have been introduced into our country together with plant propagation materials, that can not be identified by classic phytopathological techniques.

Differences in pathotypes distribution, if found, should determine the implementation of quarantines to avoid the entrance of pathotypes not identified in our country. A broader study that includes the collection and evaluation of non-pathogenic *F. oxysporum* isolates from cyclamen crops could be of particular interest for the biological control of cyclamen wilt disease.

Acknowledgments

We thank cyclamen growers for providing diseased samples for pathogen isolation and healthy plants for pathogenicity tests. This work was financially supported by the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT 2007-468 and the Universidad de Buenos Aires (UBA) UBACyT G012. G. A. Lori is member of the CICPBA Researcher's Career. I. Malbrán is a grant holder of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and C.A. Mourelos is a grant holder of the ANPCyT.

References

Aloi, C., Baayen, R.P., 1993. Examination of the relationship between vegetative compatibility groups and races in *Fusarium oxysporum* f. sp. *dianthi*. Plant Pathol. 42, 839–850.

Appel, D.J., Gordon, T.R., 1994. Local and regional variation in population of Fusarium oxysporum from agricultural field soils. Phytopathology 84, 786–791.

Appel, D.J., Gordon, T.R., 1996. Relationship among pathogenic and non pathogenic isolates of *Fusarium oxysporum* based on the partial sequence of the intergenic spacer region of the ribosomal DNA. Mol. Plant Microbe Interact. 9, 125–138.

Baayen, R.P., Van Dreven, F., Krijger, M.C., Waalwijk, C., 1997. Genetic diversity in *Fusarium oxysporum* f. sp. *dianthi* and *Fusarium redolens* f. sp. *dianthi*. Eur. J. Plant Pathol. 103, 395–408.

Beardsell, D., Nichols, D., 1981. Capillary mat watering for indoor plants. Aust. Hort. 79, 74–76.

Bongini, V., 1940. Note fitopatologiche. Laboratorio Sperimentale Nazionale Ricerca Osservatorio Fitopatologico. Torini 18, 1–34.

Booth, C., 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey.

Correll, J.C., Klittich, C.J.R., Leslie, J.F., 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77, 1640–1646.

Correll, J.C., Klittich, C.J.R., Leslie, J.F., 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi (Fusarium moniliforme)*. Mycol. Res. 93, 21–27.

Correll, J.C., 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. Phytopathology 81, 1061–1064.

Correll, J.C., Harp, T.L., Guerber, J.C., Zeigler, R.S., Liu, B., Cartwright, R.D., Lee, F.N., 2000. Characterization of *Pyricularia grisea* in the United States using independent genetic and molecular markers. Phytopathology 90, 1396–1404.

Correll, J.C., Puhalla, J.E., Schneider, R.W., 1986. Vegetative compatibility groups among non pathogenic root-colonizing strains of *Fusarium oxysporum*. Can. J. Bot. 64, 2358–2361.

Cho, W.D., Shin, H.D. (Eds.), 2004. List of Plant Diseases in Korea, fourth ed. Korean Society of Plant Pathology, Korea.

Doganova, M., 1976. Diseases and pests of pot grown flowers. Rasitelna Zaschita 24, 5–7.

Elena, K., Tjamos, E.C., 1995. Vegetative compatibility groups of *Fusarium oxysporum* f. sp. *dianthi* from plants and the rhizosphere of carnation in Greece. Plant Pathol. 44, 148–152.

Dole, J.M., Wikins, H.F., 1999. Floriculture. Principles and Species. Prentice Hall, New Jersey.

Elmer, W.H., 2002. Influence of inoculum density of *Fusarium oxysporum* f. sp. *cyclaminis* and sodium chloride on cyclamen and the development of *Fusarium* wilt. Plant Dis. 86, 389–393.

Garibaldi, A., 1981. Le principali malattie fungine del ciclamino. In: Italian Horticultural Society (Ed.), Atti della gironata floricola su il ciclamino de vaso Fiorito, pp. 107–115. Genoa.

Gerlach, W., 1954. Untersuchungen über die Welkekrankheit des Alpenveilchens (*Fusarium oxysporum* f. sp. *cyclaminis*). Phytopathol. Z. 22, 125–176.

Gobierno de la provincia de Buenos Aires, 2006. Censo horti florícola provincia de Buenos Aires 2005. http://www.maa.gba.gov.ar/agricultura_ganaderia/archivos/resultadofinal.pdf.

Gordon, T.R., Okamoto, D., 1992. Population structure and relationship between pathogenic and nonpathogenic strains of *Fusarium oxysporum*. Phytopathology 82, 73–77.

Kalc Wright, G.F., Guest, D.Y., Wimalajeewa, D.L.S., van Heeswijk, R., 1996. Characterization of *Fusarium oxysporum* isolated from carnation in Australia based on the pathogenicity, vegetative compatibility and random amplified polymorphic DNA (RAPD) assay. Eur. J. Plant Patol. 102, 451–457.

Katan, T., 1999. Current status of vegetative compatibility groups in *Fusarium oxysporum*. Phytoparasitica 27, 51–64.

Katan, T., Hadar, E., Katan, J., 1989. Vegetative compatibility of *Fusarium oxysporum* f. sp. *dianthi* from carnation in Israel. Plant Pathol. 38, 376–381.

Katan, T., Berliner, R., Katan, J., 1994. Vegetative compatibility in populations of *Fusarium oxysporum* from wild carnation. Mycol. Res. 98, 1415–1418.

Kristova, E., 1958. New diseases of decorative planta in Bulgaria and their control. Ovorshtarst. Gradinarst 1, 38–41.

Kujima, T., Kumada, Y., 1991. Biological control of anthracnose of cyclamen and Fusarium wilt of cyclamen by inoculation with bacteria or fungi isolated from nonsymptomatic interior tissues of cyclamen. Proc. Kanto Tosan Plant Prot. Soc. 38, 123–124.

LaMondia, J.A., Elmer, W.H., 1989. Pathogenicity and vegetative compatibility among isolates of *Fusarium oxysporum* and *F. moniliforme* colonizing asparagus tissues. Can. J. Bot. 67, 2420–2424.

Leslie, J.F., 1993. Fungal vegetative compatibility. Annu. Rev. Phytopatol. 31, 127–150.

Leslie, J.F., 1996. Fungal vegetative compatibility. Promises and prospect. Phytoparasitica 24, 3–6.

Leslie, J.F., Summerell, B.A., 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Ames.

Lori, G., Wolcan, S., Alippi, H., 1996. Genetic diversity of *Fusarium oxysporum* f. sp. *dianthi* in Argentina. Plant Dis. 80, 821.

Lori, G., Edel-Hermann, V., Gautheron, N., Alabouvette, C., 2004. Genetic diversity of pathogenic and nonpathogenic populations of *Fusarium oxysporum* isolated from carnation fields in Argentina. Phytopathol. 94, 661–668.

Manicom, B.Q., Bar-Joseph, M., Kotze, J.M., Becker, M.M., 1990. A restriction fragment length polymorphism probe relating vegetative compatibility groups and pathogenicity in *Fusarium oxysporum* f. sp. *dianthi*. Phytopathology 80, 336–339.

Moore, W.C., 1947. Report on Fungus, Bacterial and Other Diseases of Crops in England and Wales for the Years 1943–1946. G B. Minist. Agric. Fish. Bull. 139.

Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. *Fusarium* Species: An Illustrated Manual for Identification. The Pennsylvania State University Press University Park, Pennsylvania.

Orlicz-Luthardt, A., 1998. Studies on the resistance of cyclamen to *Fusarium* wilt. Beit.Züchtungsforschung 4, 48–49.

Palmucci, H.E., Wright, E.R., 1990. Marchitamiento del cyclamen causado por *Fusarium oxysporum* f. sp. *cyclaminis*. Rev. Fac. Agron. 11, 107–112.
Pennycook, S.R., 1989. Plant diseases recorded in New Zealand, vol. 3. Plant Diseases Division. DSIR, Auckland.
Pitta, G.P.B., Teranishi, J., 1979. Ocorrencia da murcha (*Fusarium oxysporum* Schl. f. *cyclaminis* n.f.) do cyclamen (*Cyclamen persicum* Mill.). Biológico Sao Paulo 45, 213–215.
Puhalla, J.E., 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63, 179–183.
Rattink, H., 1986. Some aspects of the etiology and epidemiology of fusarium wilt on cyclamen. Meded. Fac. Landbouwwet. Rijksuniv. Gent 51, 617–624.
Rattink, H., 1990. Epidemiology of *Fusarium* wilt in cyclamen in an ebb and flow system. Neth. J. Plant Pathol. 96, 171–177.
Rivera, M.C., López, M.V., Lopez, S.E., 2009. Mycobiota from *Cyclamen persicum* and its interaction with *Botrytis cinerea*. Mycologia 101, 173–181.
Romero, A.M., Rivera, M.C., 2005. Occurrence of corm rot of *Cyclamen persicum* caused by *Erwinia chrysanthemi* in Argentina. Aust. Plant Pathol. 34, 279–280.
Rouxel, F., Grouet, D., 1974. Premiere observation de la fusariose vasculaire du cyclamen en France. Ann. Phytopathol. 6, 475–478.
Steinberg, C., Edel, V., Gautheron, N., Abadie, C., Vallaeys, T., Alabouvette, C., 1997. Phenotypic characterization of natural population of *Fusarium oxysporum* in relation to genotypic characterization. FEMS Microbiol. Ecol. 24, 73–85.
Tompkis, C.M., Snyder, W.C., 1972. Cyclamen wilt in California and its control. Plant Dis. Rep. 56, 493–497.
Vakalounakis, D.J., Fragkiadakis, G.A., 1999. Genetic diversity of *Fusarium oxysporum* isolates from cucumber. Differentiation by pathogenicity, vegetative compatibility and RAPD fingerprinting. Phytopathology 89, 161–168.
Woudt, L.P., Neuvel, A., Sikkema, A., van Grinsven, M.Q.J.M., de Milliano, W.A.J., Campbell, C.L., Leslie, J.F., 1995. Genetic variation in *Fusarium oxysporum* from cyclamen. Phytopathology 85, 1348–1355.
Wright, E.R., Rivera, M.C., Mascarini, A., Nuñez, L.S., Gentile, C.M., 2006. Florist's cyclamen antrachnose caused by *Colletotrichum gloeosporioides* in Argentina. Aust. Plant Dis., 1–2. Notes 1.