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Survey of diseases caused by *Fusarium* spp. on palm trees in the Canary Islands

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Summary. Between 2006 and 2007, palm trees growing in both gardens and public parks and natural palm groves in the Canary Islands (Spain), and showing symptoms of wilt and dieback, were surveyed. Isolates were recovered from affected tissues of the crowns, leaves and vascular fragments on potato dextrose agar (PDA). After incubation, the *Fusarium* spp. colonies recovered were single-spored. They were transferred to PDA and Spezieller Nährstoffarmer Agar (SNA) for morphological identification. Identification of *Fusarium oxysporum* f. sp. *canariensis* was confirmed by PCR with the specific primers HK66 and HK67, which amplified a fragment of 567 bp. Fusarium wilt caused by *F. oxysporum* f. sp. *canariensis* was found on 54 *Phoenix canariensis* trees growing on four islands: Gran Canaria, Fuerteventura, La Palma and Tenerife. *F. proliferatum* occurred on fifteen palms (10 *P. canariensis*, 1 *P. dactylifera*, 3 *Roystonea regia* and 1 *Veitchia joannis*) located in Gran Canaria, Fuerteventura and Tenerife. Both these *Fusarium* species were found only in diseased palms from gardens and public parks, but not in natural palm groves. The results show that Fusarium wilt of *P. canariensis* is common in the Canary Islands and for the first time report *F. proliferatum* affecting different palm species in those islands.

Key words: *Fusarium oxysporum* f. sp. *canariensis*, *Fusarium proliferatum*, Fusarium wilt, *Phoenix canariensis*.

Introduction

The most common fungal diseases of palm species in the Canary Islands are: black rot caused by *Ceratocystis paradoxa* (Dade) C. Moreau, pink rot caused by *Nalanthamala vermoesenii* (Biourge) Schroers and false smut caused by *Graphiola phoenicis* (Moug.) Poit., as well as some less common diseases caused by *Phoma* spp., *Phomopsis* spp., *Pestalotiopsis* spp. and Botryosphaeriaceae (Gallo-Llobet *et al*. 1988; Cabrera *et al*., 1990; Trujillo-García *et al*., 2002).

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The main fungal disease on Canary palm (*Phoenix canariensis*) is a Fusarium wilt caused by *Fusarium oxysporum* f. sp. *canariensis*. The most characteristic symptom of this disease is an asymmetrical wilt of the leaflets on only one side of the rachis (Figure 1A). The wilt progresses acropetally from the rachis base to the rachis apex and, only after it has progressed, the opposite side of the rachis starts to wilt as well, in this case basipetally, from the apex to the base. The first fronds affected are generally the oldest ones, placed at the base of the crown, although other patterns are also seen. The disease is usually lethal, but the time elapsed from first symptom appearance to final collapse and death of the palm tree varies (Figure 1B) (Feather *et al*., 1989; Priest and Letham, 1996; Plyler *et al*., 1999).

In recent years, an increasing number of diseased *P. canariensis* and other palm species have been seen in the Canary Islands. The aim of this

Figure 1. A. Characteristic asymmetrical wilt of the leaflets in just one side of the rachis in *Phoenix canariensis*. B. Severe wilt symptoms of a *P. canariensis* palm tree in a garden.

study was to survey diseased palms in gardens and public parks, and natural palm groves in the islands, concentrating on diseases caused by *Fusarium* spp.

Between 2006 and 2007, palms showing symptoms of wilt and dieback were surveyed in both gardens and public parks, and natural palm groves of the Canary Islands, Spain. Selected fragments from affected tissues of the crown and leaves, and vascular fragments were washed thoroughly with water, surface-disinfected for 1 min in a 1.5% sodium hypochlorite solution, and washed twice with sterile distilled water. Fragments were placed on potato dextrose agar (PDA) supplemented with 0.5 mg mL^{-1} of streptomycin sulphate. Plates were incubated at 25ºC in the dark, and fungal colonies were transferred to PDA. Colonies of *Fusarium* spp. were single-spored by dilution series, and grown on PDA and Spezieller Nährstoffarmer Agar (SNA). Two 1 cm^2 square pieces of sterile filter paper were placed on the surface of the agar for morphological identification. The PDA plates were incubated at 25ºC in the dark, and the SNA plates were incubated at 25ºC with a 12-h photoperiod. All isolates were examined after 10 days.

Identification of *F. oxysporum* f. sp. *canariensis* was confirmed by PCR with the specific primers HK66 and HK67, which amplified a fragment of 567 bp (Plyler *et al.,* 1999). Fungal mycelium and conidia from pure cultures grown on PDA for 1 week at 25°C in the dark were scraped and ground to a fine powder under liquid nitrogen using a mortar and pestle. Total DNA was extracted using the E.Z.N.A. Plant Miniprep Kit (Omega Bio-tek, Norcross, GA, USA) following manufacturer's instructions. DNA was visualized on 0.7% agarose gel stained with ethidium bromide and was stored at -20°C. Each PCR reaction contained 1×PCR buffer, 2.5 mM $MgCl_2$, 200 μM each dNTP, 0.4 μM of each primer (HK66 and HK67), 1 U of DNA *Taq* polymerase (Dominion MBL, Córdoba, Spain), and 1 μL of template DNA. The PCR reaction mix was adjusted to a final volume of 25 μL with water (Chromasolv Plus, Sigma-Aldrich, Steinheim, Germany). PCR amplifications were performed on a Peltier Thermal Cycler-200 (MJ Research). The program consisted of an initial step of 30 s at 95°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 62°C

Table 1. Distribution of *Fusarium oxysporum* f. sp. *canariensis* in *Phoenix canariensis* collected from gardens and public parks in the Canary islands.

Island	Locality	No. of positive samples
Gran Canaria	Las Palmas de Gran Canaria	5
Fuerteventura	Pájara	2
La Palma	El Paso	1
	Los Cancajos	2
	Puntagorda	1
	Santa Cruz de La Palma	2
Tenerife	Bajamar	2
	El Socorro	1
	El Sauzal	1
	Garachico	1
	La Laguna	7
	Puerto de La Cruz	1
	Santa Cruz de Tenerife	23
	Santa Úrsula	5
	Valle Guerra	1
Total		54

for 1 min, and an elongation at 72°C for 2 min. A final extension was performed at 72°C for 10 min. The PCR products were visualized in 1.5% agarose gel (agarose D-1 Low EEO, Conda, Madrid, Spain). A 100-bp DNA ladder was used as a molecular weight marker (Dominion MBL).

Pathogenicity tests were conducted on *Roystonea regia* and *Veitchia joannis* with *F. proliferatum* strains obtained from these hosts. The inoculum of each strain was prepared by flooding the agar surface with 10 mL of sterile distilled water (SDW) and scraping with a spatula. The resulting spore suspension was filtered through four layers of cheesecloth. The filtrate was diluted with SDW and the conidial concentration was adjusted to 10^6 conidia mL^{-1} with a haemocytometer. Sixteen-month-old *R. regia* and *V. joannis* plants were inoculated by wounding the base of the leaflets in the crown area with a scalpel and spraying this area with approximately 50 mL of spore solution. Five plants of each species were inoculated with each isolate; the corresponding controls were sprayed with SDW. The pots were arranged in a completely randomized design and incubated in an environmentally controlled greenhouse.

Fusarium wilt caused by *F. oxysporum* f. sp. *canariensis* was found on 54 *Phoenix canariensis* trees in four islands: Gran Canaria, Fuerteventura, La Palma and Tenerife (Table 1, Figure 2). *F. proliferatum* was found on fifteen palms (10 *P. canariensis*, 1 *P. dactylifera*, 3 *R. regia* and 1 *V. joannis*) located in Gran Canaria, Fuerteventura and Tenerife (Table 2, Figure 2). Both *Fusarium* species were found only on diseased palm trees from gardens and public parks, and not in natural palm groves. Palms from palm groves yielded only *Nalanthamala vermoesenii*, *Serenomyces* spp. and anamorphs of Botryosphaeriaceae.

The results indicate that Fusarium wilt of *P. canariensis* is widespread in the Canary Islands. Fusarium wilt of *P. canariensis* caused by *F. oxysporum* f. sp. *canariensis* is one of the most damaging diseases of this palm species in the Spanish Mediterranean regions (Abad *et al.*, 2002), but so far in the Canary Islands it has been reported only on Tenerife (Plyler *et al.,* 2000).

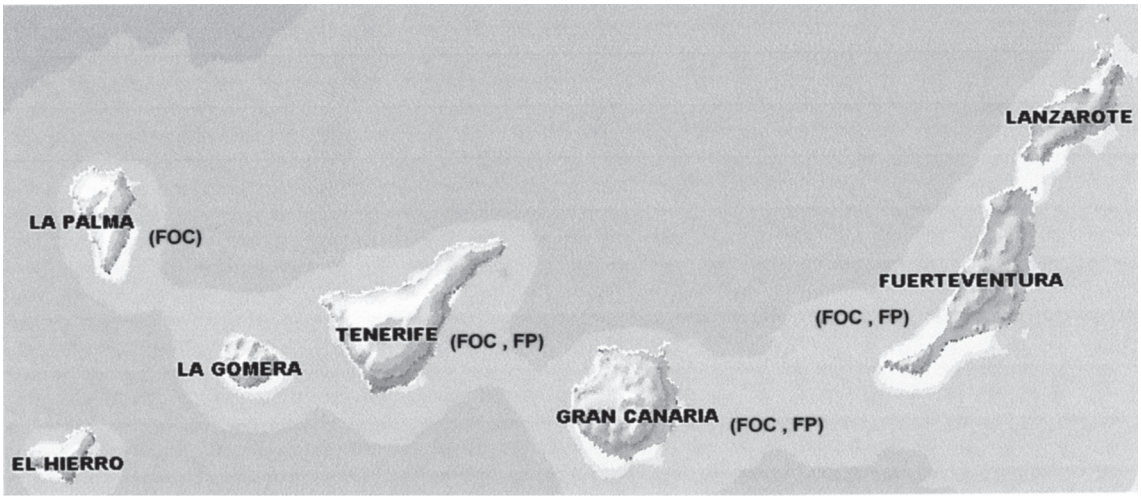

Figure 2. Map of the Canary Islands showing the distribution of *Fusarium oxysporum* f. sp. *canariensis* (FOC) and *F. proliferatum* (FP).

This work for the first time in the Canary Islands reports *F. proliferatum* on different palm species. *F. proliferatum* was described as the causal agent of wilt and dieback of date palms in Saudi Arabia (Abdalla *et al*., 2000) and was subsequently reported to cause blight on majesty palm (*Ravenea rivularis*) in Italy (Polizzi and Vitale, 2003). More recently, Armengol *et al*. (2005), reported that *F. proliferatum* caused wilt and dieback on *P. canariensis*, *P. dactylifera*, *P. reclinata*, *Chamaerops humilis*, *Trachicarpus fortunei*, *Washingtonia filifera* and *W. robusta* in Spain. In our survey, *R. regia* and *V. joannis* plants inoculated with *F. proliferatum* showed lesions at the base of the leaves and developed wilt symptoms 7 to 8 months after inoculation, confirming Koch's postulates. Consequently, these results extend the host range of *F. proliferatum* on palms to *R. regia* and *V. joannis*.

More attention should be given to *Fusarium* diseases in the Canary Islands, and particularly to the increasing importance of *F. oxysporum* f. sp. *canariensis*, whose occurrence in gardens and public parks threatens natural groves of *P. canariensis*, which are characteristic of the landscape of these

Table 2. Distribution of *Fusarium proliferatum* in different palm species collected from gardens and public parks in the Canary islands.

Island	Locality	No. of positive samples	Palm species
Gran Canaria	Las Palmas de Gran Canaria	1	*Phoenix canariensis*
Fuerteventura	Pájara	1	*P. canariensis*
Tenerife	Icod de los Vinos	1	*Roystonea regia*
	La Laguna	4	*P. canariensis*
	Puerto de La Cruz	4	*P. canariensis*
		1	*P. dactylifera*
	Santa Cruz de Tenerife	1	*R. regia*
		1	*Veitchia joannis*
	Santa Úrsula	1	*R. regia*

islands. The management of Fusarium wilt of *P. canariensis* is based on disease prevention. In this sense, further characterization of *F. oxysporum* f. sp. *canariensis* obtained from the different islands using vegetative compatibility groups (VCGs) and molecular markers will lead to a better understanding of the epidemiology of the disease.

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