European Journal of Plant Pathology (2005) 112: 123–131
DOI: 10.1007/s10658-005-2552-6
© Springer 2005

Identification, incidence and characterization of *Fusarium proliferatum* on ornamental palms in Spain

J. Armengol[1], A. Moretti[2], G. Perrone[2], A. Vicent[1], J.A. Bengoechea[1] and J. García-Jiménez[1]
[1]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain; (Phone: +34-96-3879254; Fax: +34-96-3879269; E-mail: jarmengo@eaf.upv.es);* [2]*Institute of Sciences of Food Production, C.N.R., Via Amendola, 122/0, 70126 Bari, Italy*

Accepted 11 February 2005

Key words: Chamaerops, *Liseola* section, mycotoxins, *Phoenix*, *Trachycarpus*, *Washingtonia*

Abstract

During a survey conducted from 1998 to 2002, *Fusarium proliferatum* was found associated with young and adult palms belonging to the genera *Chamaerops*, *Phoenix*, *Trachycarpus* and *Washingtonia* showing symptoms of wilt and dieback. The fertility and toxicological profile of 36 strains representing different locations and hosts were studied. All of them except two, which were infertile, belonged to mating population D. Both mating types (MATD-1 and MATD-2) were isolated from the same host species, showing a high potential of genetic recombination in the field. Additionally, eight strains were fertile once crossed as female. Toxin analysis showed differences in the ability of strains to produce fumonisin B_1, moniliformin, beauvericin, fusaric acid and fusaproliferin. Only 17 of them produced all the toxins analyzed. Pathogenicity tests were conducted on *Phoenix dactylifera* and *P. canariensis* using nine *F. proliferatum* Spanish strains and two reference strains from Saudi Arabia. Eight months after inoculation all strains caused disease, with palms showing lesions on the bases of leaves and development of wilt symptoms similar to those originally observed in affected plants. This is the first report on the occurrence of *F. proliferatum* on *P. dactylifera* in Spain and also the first report of this pathogen on *C. humilis*, *P. canariensis*, *P. reclinata*, *T. fortunei*, *W. filifera* and *W. robusta*.

Introduction

Palms are one of the most important ornamental crops in Spain, which annually produces approximately 2 million plants with a considerable export to European markets. *Phoenix canariensis* (1.2 million plants) is the predominant species, followed by other species such as *P. dactylifera*, *P. reclinata*, *Washingtonia filifera*, *W. robusta*, *Chamaerops humilis* and *Trachycarpus fortunei*. During a survey conducted from 1998 to 2002, a high number of young and adult palms belonging to the genera *Chamaerops*, *Phoenix*, *Trachycarpus* and *Washingtonia* showing symptoms of wilt and dieback were detected. Isolations from the crown area, the bases of leaves and vascular fragments of affected plants yielded primarily *Fusarium* strains. While in some locations *Fusarium* strains obtained from *P. canariensis* were identified as *F. oxysporum* f.sp. *canariensis* (Olmo et al., 2001; Abad et al., 2002) based on specific molecular techniques described by Fernandez et al. (1998) and Plyler et al. (1999), most of the strains were identified as *F. proliferatum*.

Fusarium proliferatum has recently been described as the causal agent of wilt and dieback of date palms in Arabia Saudi (Abdalla et al., 2000) and as the agent of blight on majesty palm

(Ravenea rivularis) in Italy (Polizzi and Vitale, 2003). This fungus is a well-known worldwide pathogen of several plant crops, including maize (Logrieco et al., 1995), rice (Desjardins et al., 1997) and asparagus (Elmer, 1990). It is a toxigenic species, producing a broad range of toxins, such as fumonisin B_1 (FB_1; Nelson et al., 1992), moniliformin (MON; Marasas et al., 1984) beauvericin (BEA; Logrieco et al., 1995), fusaric acid (FA; Bacon et al., 1996), and fusaproliferin (FUP; Ritieni et al., 1995). Some of these toxins have a well-known phytotoxic activity; these include FA, which is implicated in the pathogenesis of tomato-wilt symptoms (Gaumann, 1957), MON, which is toxic toward tobacco plants (Cole et al., 1973), and FB_1, which has shown to be phytotoxic to maize and tomato (Lamprecht et al., 1994). Recently, or moreover, BEA was shown by Paciolla et al. (2005) to possess a phytotoxic activity as it caused a severe reduction in tomato protoplast viability.

Fusarium proliferatum belongs to *Liseola* section of the Fusarium genus (Nelson et al., 1983) and its teleomorph, *Gibberella intermedia*, belongs to the *G. fujikuroi* complex, composed of at least nine reproductively isolated biological species (mating populations [MPs]), designated of letters A–I. Separate *Gibberella* species names have been assigned to all but one of these mating populations (Samuels et al., 2001; Zeller et al., 2003) and numerous additional *Fusarium* anamorphs within the *Liseola* and *Elegans* sections have been defined on the basis of morphology and sequence differences (Nirenberg and O'Donnell, 1998; O'Donnell et al., 1998; O'Donnell et al., 2000; Marasas et al., 2001), showing undoubtedly that additional biologically significant entities remain to be identified and described. Therefore, it is important to assess the biological species for strains identified as *Liseola* and *Elegans* section members, in order to characterize the phytopathological and toxigenic risks that could affect the host plants.

The objectives of this study were: (i) to determine the occurrence of *F. proliferatum* on ornamental palms in Spain; (ii) to investigate the toxicological profile of the strains; (iii) to study their possible fertility and assign them to a specific MP; (iv) to evaluate their pathogenicity to *P. dactylifera* and *P. canariensis* under greenhouse conditions.

Materials and methods

Fungal isolation and identification

Affected tissues of the crown, leaves and vascular fragments of symptomatic plants of different ornamental palm species from different locations (Table 1) were surface-sterilized for 1–2 min in a sodium hypochlorite solution (1.5% available chlorine), washed twice with sterile distilled water (SDW) and placed on PDA supplemented with 0.5 mg ml^{-1} of streptomycin sulphate (PDAS). Plates were incubated in the dark at 25–27 °C for 3–4 days. After incubation, the *Fusarium* spp. colonies were single spored and transferred to potato dextrose agar (PDA) and Spezieller Nährstoffarmer Agar (SNA) and incubated at 25 °C for 10 days for identification according to the taxonomic system of Nelson et al. (1983). To preserve the cultures, mycelia and conidia from wild strains grown on carnation leaf agar (CLA) were transferred aseptically into 1 ml sterile 18% glycerol and frozen at −75 °C. The isolates were deposited in the collection of the Institute of Sciences of Food Production (ITEM), Bari, Italy.

Fertility tests

Tester strains for mating population tests were received from J.F. Leslie, Kansas State University. Strains were crossed on carrot agar as male parents with tester strains of mating population A–G as described by Klittich and Leslie (1988). Mated cultures were considered inter-fertile if perithecia formed within six weeks. All strains were crossed twice with both testers from each mating population. Fertile strains were tested for assessing female fertility as described by Leslie (1995).

In vitro *toxin production*

Single conidium strains of *F. proliferatum* were cultured on 100 g of autoclaved yellow maize kernels that were adjusted to about 45% moisture in 500 ml Erlenmeyer flasks. Kernels were inoculated with 2 ml of an aqueous suspension containing approximately 10^7 conidia ml^{-1}. Cultures were incubated at 25 °C for 4 weeks. Each strain was cultured in three replicate flasks. Harvested cultures were dried in a fan oven at 60 °C for 48 h, finely ground, and stored at 4 °C. Control kernels

Table 1. Origin, host and mating type of *Fusarium proliferatum* strains obtained from ornamental palms

Strain ITEM[a]	Year	Origin	Host	Mating type
4284	1999	Elche (Alicante)	*Phoenix canariensis*	MATD-2
4285	1999	Elche (Alicante)	*Phoenix canariensis*	MATD-2
4286	1999	Elche (Alicante)	*Phoenix canariensis*	MATD-1
4287	1999	Picassent (Valencia)	*Phoenix canariensis*	MATD-2
4288	1999	Picassent (Valencia)	*Phoenix canariensis*	MATD-2*
4289	1999	Picassent (Valencia)	*Phoenix canariensis*	MATD-2
4290	1999	Picassent (Valencia)	*Phoenix canariensis*	MATD-2
4291	1999	Elche (Alicante)	*Phoenix reclinata*	MATD-2
4292	1999	Elche (Alicante)	*Washingtonia* sp.	MATD-2
4293	1999	Elche (Alicante)	*Chamaerops humilis*	MATD-2
4294	1999	Elche (Alicante)	*Chamaerops humilis*	MATD-2*
4295	1999	Elche (Alicante)	*Chamaerops humilis*	MATD-2
4296	1999	Crevillente (Alicante)	*Phoenix dactylifera*	MATD-2
4297	1999	Crevillente (Alicante)	*Phoenix dactylifera*	MATD-2*
4298	1999	Crevillente (Alicante)	*Phoenix dactylifera*	MATD-1
4299	1999	Alboraya (Valencia)	*Phoenix canariensis*	MATD-1
4300	1999	Alboraya (Valencia)	*Phoenix canariensis*	MATD-1*
4301	1999	Alboraya (Valencia)	*Phoenix canariensis*	MATD-1
4302	1999	Elche (Alicante)	*Phoenix dactylifera*	Infertile
4303	1999	Elche (Alicante)	*Phoenix dactylifera*	MATD-1*
4304	1999	Elche (Alicante)	*Phoenix canariensis*	MATD-2
4305	1999	Elche (Alicante)	*Phoenix canariensis*	MATD-2
4306	1998	Cambrils (Tarragona)	*Washingtonia filifera*	MATD-2*
4307	2000	Barcelona	*Phoenix* sp.	MATD-1
4308	2001	Torrent (Valencia)	*Trachycarpus fortunei*	MATD-1
4309	2001	Torrent (Valencia)	*Trachycarpus fortunei*	MATD-1
4310	2001	Torrent (Valencia)	*Trachycarpus fortunei*	Infertile
6151	2002	Alicante (Alicante)	*Washingtonia filifera*	MATD-1*
6152	2002	Alicante (Alicante)	*Washingtonia filifera*	MATD-1
6153	2002	Alicante (Alicante)	*Washingtonia filifera*	MATD-1*
6154	2002	Alicante (Alicante)	*Washingtonia filifera*	MATD-1
6155	2002	Alicante (Alicante)	*Washingtonia filifera*	MATD-1
6156	2002	Alicante (Alicante)	*Washingtonia filifera*	MATD-1
6157	2002	Alicante (Alicante)	*Washingtonia robusta*	MATD-1
6158	2002	Alicante (Alicante)	*Washingtonia robusta*	MATD-1
6159	2002	Alicante (Alicante)	*Washingtonia robusta*	MATD-1

[a] = Collection of the Institute of Sciences of Food Production (ITEM), Bari, Italy; * = Female fertile.

were treated the same way, except that they were not inoculated.

Toxin analysis

For BEA and FUP extraction, 10 g of each culture were homogenized by shaking in 100 ml of methanol (99.5%) for 1 h. Samples were then filtered through Whatman no. 4 filter paper and methanol was removed under reduced pressure. Each extract was eluted in 1 ml of methanol and filtered through an Acrodisk filter (pore size, 0.22 μm), before high performance thin layer chromatography (HPTLC) analysis, performed for BEA according to the procedure described by Logrieco et al. (1993) and for FUP following the procedure described by Logrieco et al. (1996). FA extraction and analysis were performed as previously described (Abdalla et al., 2000). FB_1 extraction and analysis were performed by HPTLC according to the procedure described by Munkvold et al. (1998) and MON extraction and analysis as described by Bottalico et al. (1982).

Pathogenicity tests

Pathogenicity tests were conducted on *P. dactylifera* and *P. canariensis* using nine Spanish *F. proliferatum* strains representing different locations

and hosts (Table 2). Additionally, two strains from Saudi Arabia (ITEM 2336 and ITEM 2339) (Abdalla et al., 2000) were also used as reference isolates. Isolates were grown on SNA for 14 days at 25–27 °C prior to inoculation. The inoculum was prepared for each strain by flooding the agar surface with 10 ml SDW and scraping with a spatula. The resulting spore suspension was filtered through four layers of cheesecloth. The filtrate was diluted with SDW and conidial concentration was adjusted with a haemocytometer to 10^6 conidia ml^{-1}. Seeds of *P. dactylifera* and *P. canariensis* were provided by a commercial producer. They were surface-disinfested for 10 min in a sodium hypochlorite solution (1.5% available chlorine), soaked in tap water for 24 h, and then sown in plastic pots (17 cm diam) filled with a sterilized mixture of equal portions (v/v) of soil, sand and peat moss. One seedling was grown in each pot. Plants were maintained in a temperature-controlled greenhouse (25–30 °C). Nine-month-old plants were inoculated as described by Abdalla et al. (2000) by injecting 3 ml of spore suspension into the crown using a hypodermic needle and syringe. After inoculation, all plants were covered separately with plastic bags for 48 h to maintain high humidity. Five plants of each species were inoculated with each isolate, and corresponding controls were injected with SDW. The pots were arranged in a complete randomized design. The pathogenicity test was conducted twice. Pathogenicity was evaluated 8 months after inoculation and disease reaction was rated as: 0 = healthy; 1 = small lesions (length of the lesions from 1 to 2 cm) developed on the bases of leaves, no wilting; 2 = large lesions (length of the lesions from 2 to 7 cm) developed, some leaves wilted; 3 = severely wilted leaves or plant death. A disease severity index (DSI) was calculated as the mean for five plants of each species. At the end of the pathogenicity test the recovery of the fungus was also studied. DSI data were analyzed using the software Statgraphics Plus 5.1 (Manugistics, Rockville, MD). Analysis of variance (ANOVA) was conducted on DSI data to determine the overall effects of isolate, host and isolate *x* host interaction. Comparisons of isolates for each host were made using Duncan's multiple range test ($P < 0.05$).

Table 2. Disease severity in *Phoenix canariensis* and *P. dactylifera* inoculated with several *Fusarium proliferatum* strains, 8 months after inoculation

Strain ITEM*	DSI[a]	
	P. canariensis	*P. dactylifera*
4284	1.4[b] bc[c]	1.8 bcde
4288	2.1 d	2.4 e
4291	1.5 bc	1.7 bcd
4294	1.6 bcd	2.3 cd
4296	1,5 bc	1.6 bc
4299	1.2 b	1.4 bc
4302	1.2 b	1.2 b
4306	1.6 bcd	1.2 b
4307	1.5 bc	1.7 bcd
2336	1.6 bcd	1.4 bc
2339	1.8 cd	2.0 cde
Control	0.0 a	0.0 a

[a]0, healthy; 1, small lesions (length of the lesions from 1 to 2 cm) developed on the bases of leaves; 2, large lesions (length of the lesions from 2 to 7 cm) developed, some leaves wilted; 3, Severely wilted leaves or plant death. [b] Mean of 10 plants; [c]Numbers in a column followed by the same letter are not significantly different at ($P < 0.05$) according to Duncan's multiple range test. * Collection of the Institute of Sciences of Food Production (ITEM), Bari, Italy.

Results

Fungal isolation and identification

Fusarium proliferatum was consistently isolated from symptomatic plants of *P. canariensis*, *P. dactylifera*, *P. reclinata*, *W. filifera*, *W. robusta*, *C. humilis* and *T. fortunei* at different locations. According to these origins, thirty-six strains were selected for this study (Table 1).

Fertility tests

All the strains analyzed belonged to mating population D with the exception of two strains isolated from *P. dactylifera* in Elche (Alicante) and *T. fortunei* in Torrent (Valencia). Among these strains, isolated from different palm species, both mating types (18 strains MATD-1 and 16 strains MATD-2, respectively) occurred on the same host species, showing a high potential of genetic recombination in the field (Table 1). Female fertility was also evaluated for all fertile strains, showing that eight strains were fertile once crossed as female (Table 1).

Table 3. Mycotoxin production of *Fusarium proliferatum* strains from ornamental palms

Strain	Mycotoxin production (mg/kg)[a]				
ITEM*	FB_1[b]	BEA	FUP	MON	FA
4284	250	270	150	350	110
4285	3500	135	100	80	125
4286	3000	235	200	250	480
4287	2000	ND	135	ND	240
4288	3000	70	70	190	280
4298	ND	ND	70	300	530
4290	2000	200	170	60	140
4291	2000	100	70	ND	90
4292	1500	70	70	470	ND
4293	1500	100	135	50	1100
4294	1250	70	70	290	ND
4295	2000	100	115	480	700
4296	1750	50	100	250	ND
4297	3000	ND	100	140	ND
4298	ND	800	100	400	250
4299	675	ND	85	210	800
4300	3000	70	200	380	1400
4301	50	335	100	70	560
4302	ND	ND	270	120	ND
4303	3000	70	135	60	430
4304	ND	85	70	ND	ND
4305	ND	200	100	530	340
4306	2500	100	70	420	70
4307	2500	85	80	330	60
4308	3500	300	120	160	ND
4309	1600	70	90	200	500
4310	2000	150	210	550	ND
6151	1800	ND	110	40	1000
6152	ND	200	70	360	ND
6153	1200	ND	220	210	ND
6154	1000	50	200	180	180
6155	850	170	180	190	330
6156	1100	280	80	480	ND
6157	2300	140	50	270	240
6158	1500	100	80	ND	ND
6159	1750	60	50	290	390

[a]Strains grown on autoclaved maize kernels in the dark at 25 °C for 4 weeks; FB_1 = fumonisin B_1; BEA = Beauvericin; FUP = Fusaproliferin; MON = moniliformin; FA = fusaric acid; ND = not detected; [b] Detection limits: FB_1 = 25 ppm; BEA = 10 ppm; FUP = 10 ppm; FA = 0.01 ppm; MON = 30 ppm; *Collection of the Institute of Sciences of Food Production (ITEM), Bari, Italy.

Toxin analysis

Toxin analysis reported in Table 3 showed that 30 strains of *F. proliferatum* produced FB_1 in a range from 50 to 3500 $\mu g\ g^{-1}$, 29 strains produced BEA in a range from 50 to 800 $\mu g\ g^{-1}$, all 36 strains produced FUP (range from 70 to 220 $\mu g\ g^{-1}$), 32 strains produced MON (range from 60 to 550 $\mu g\ g^{-1}$) and 25 strains produced FA with a range from 60 to 1100 $\mu g\ g^{-1}$. Among the strains tested, only 17 strains produced all the toxins analyzed (Table 3).

Pathogenicity tests

Eight months after inoculation, plants showed lesions on the bases of leaves and developed wilt symptoms similar to those originally observed in affected plants. Analysis of variance revealed that DSI data between both experiments were not statistically different ($P = 0.71$). Therefore, data from both experiments were combined and presented here. There were no significant differences between *P. dactylifera* and *P. canariensis*

($P = 0.089$), but the DSI was affected significantly ($P < 0.05$) by the isolates (Table 2). All isolates used in this test were pathogenic to palm species including those from Saudi Arabia. The DSI ranged from 1.2 to 2.1 for *P. canariensis*, and from 1.2 to 2.4 for *P. dactylifera* with a complete gradation among the isolates. *Fusarium proliferatum* was re-isolated from affected plants confirming Koch's postulates.

Discussion

Fusarium proliferatum was the predominant species isolated from visibly diseased young and adult plants of palms of our survey. Although the wilt symptoms were similar to Fusarium wilt of Canary Island date palm caused by *F. oxysporum* f.sp. *canariensis* (Mercier and Louvet, 1973) and Bayoud disease, the vascular wilt of date-palm trees caused by *F. oxysporum* f.sp. *albedinis* and widely distributed in North Africa (Louvet and Toutain, 1981), the first was found only on *P. canariensis* in a few locations (Olmo et al., 2001; Abad et al., 2002) and the latter has never been reported in palm trees in Spain. As far as we are aware, this is the first report of the occurrence of *F. proliferatum* on *P. canariensis*, *P. reclinata*, *W. filifera*, *W. robusta*, *T. fortunei*, and *C. humilis*. It is also the first report on the occurrence of *F. proliferatum* on *P. dactylifera* in Spain.

The pathogenicity tests on *P. dactylifera* and *P. canariensis* seedlings showed that *F. proliferatum* should be regarded as a potentially dangerous pathogen of ornamental palms in Spain. This agrees with symptoms on date-palm trees (*P. dactylifera*) recently described in Saudi Arabia, caused by this fungus (Abdalla et al., 2000), where strains were also identified as mating population D of the *G. fujikuroi* complex (Leslie, 1995); Polizzi and Vitale (2003) also identified *F. proliferatum* as the agent of blight on majesty palm in Italy. However, in this latter case, besides morphological identification, no further confirmation of species identity was provided and the authors considered *F. proliferatum* an occasional pathogen of majesty palm in nurseries, and a potential cause of loss in the cultivation of the majesty palm in the field. On the contrary, our data indicates that *F. proliferatum* could be considered as an important pathogen of palms in nurseries since this fungus was consistently isolated from different diseased palm species and geographical origins.

In order to confirm the identity of the *F. proliferatum* strains, we assessed their ability to produce fertile perithecia from *in vitro* crosses. Since both mating types occurred among the population isolated from palms and, for *P. dactylifera*, *P. canariensis*, and *W. filifera*, also from the same host species, the high ratio of *in vitro* fertility among the tested strains suggests that a potential for a high recombination in the palm fields and greenhouse exists. This is important because a high frequency of genetic recombination in the field could improve the genetic pool available for the pathogenic population of *F. proliferatum* and for its toxigenicity. This risk is confirmed by the frequency of female fertility within the population of *F. proliferatum* reported here. A high frequency of hermaphrodites in field populations is considered as a selective advantage every time sexual reproduction occurs (Leslie and Klein, 1996). Thus, the occurrence of female fertile strains within the population of *F. proliferatum* from palms in Spain would suggest a high possibility of sexual reproduction in the palm fields and/or greenhouses in Spain. In this respect, fertile perithecia of *F. proliferatum* were detected on seedlings of *C. humilis* in one of the nurseries surveyed (data not shown).

Mating population D of *G. fujikuroi* has been reported as occurring on several host plants (Leslie, 1995), including asparagus (Elmer, 1995) and rice (Desjardins et al., 1997), where the most common MP is C. *Fusarium proliferatum* is considered as the anamorph of both C and D MPs, that differ significantly in toxin production (Moretti et al., 1996; Desjardins et al., 1997). This is the first report on the occurrence of *F. proliferatum* on several genera of palms; therefore, it was of relevance to assess that MP D was the biological species of this group of isolates, this being, consequently, the first report of MP D on several genera of palms. Moreover, it is interesting to observe that *F. proliferatum* strains from palms belong to MP D together with many other populations of this species isolated from a very broad range of plant hosts, on which they show a high level of pathogenicity (e.g., maize, rice, sorghum, tomato and asparagus; Elmer et al., 1995; Leslie et al., 1995; Logrieco et al., 1995; Desjardins et al., 1997; Moretti et al., 1998). Among the toxins studied here, some of them have well known

phytotoxic activity, including FA, implicated in the pathogenesis of tomato wilt symptoms (Gauman, 1957); MON, toxic towards tobacco plants (Cole et al., 1973); FB_1, shown to be phytotoxic to maize and tomato (Lamprecht et al., 1994); and BEA that affects the viability of tomato protoplasts (Paciolla et al., 2005). All of these secondary metabolites have phytotoxic activity and all tested strains proved to be producers; therefore, we suspect their involvement in the expression of symptoms on palm plants. However, before drawing any conclusions about the role played by these mycotoxins in palm wilt, it would be necessary to study the *in vivo* toxin production of the strains in pathogenicity tests and compare the results with tests using pure toxins, also evaluating possible additive and/or synergistic effects of the toxins on the plants.

In addition to the phytotoxic effects, all five toxins analyzed have mycotoxic activity. In particular, FB_1 was linked to human oesophageal cancer (Rheeder et al., 1992) and is involved in several mycotoxicoses (Kellerman et al., 1990; Ross et al., 1990); FA affected both brain and pineal neurotransmitters of rats (Porter et al., 1995); MON was reported to cause haematological disorders, myocardial hypertrophy, and mortality in farm animals (Ledoux et al., 1995; Harvey et al., 1997); BEA was toxic to several human cell lines and induces apoptosis in mammalian cells (Logrieco et al., 2002); and FUP showed cytotoxic activity and caused apoptosis on human B-lymphocytes (Logrieco et al., 1996; Di Paola et al., 1998) and teratogenic effects on chicken embryos (Ritieni et al., 1997). It is of concern that all strains of *F. proliferatum* we studied can produce at least 2 of these toxins and that 17 strains were able to produce all of them. *Fusarium proliferatum* strains were also isolated from leaves of palm seedlings and trees. In preliminary experiments on the virulence of *F. proliferatum* as a pathogen causing date-fruit rot, *F. proliferatum* was able to cause fruit rot at the Khalal and the Rutab stages in Saudi Arabia (M. Y. Abdalla, Plant Protection Department, College of Agriculture and Veterinary Medicine, Buraidah, Saudi Arabia; personal communication). Similar results were also obtained by Elarosi (1989), working with *F. lateritium* and *F. verticillioides* (= *F. moniliforme*) at the eastern province of Saudi Arabia. This could be of concern because of the possible contamination of date palm fruits. Therefore, the pathogenic and toxigenic data reported here warrant further investigations on the occurrence of *F. proliferatum* strains and some of their metabolites worldwide to evaluate the risk of consumption of contaminated date-palm fruit.

Finally, more attention should be given to the identification of *F. proliferatum*, which has morphological traits similar to *F. oxysporum*, using tools other than morphological identification (e.g. molecular approaches and fertility tests) and further investigation is needed on the distribution of *F. proliferatum* in other geographical areas where palms are cultivated. The pathogenicity tests suggested the isolates have varying ability to produce disease. As a consequence further research is required to accurately establish the role of this fungus as a pathogen of the different genera of palms.

Acknowledgements

We thank A. Ritieni, Napoli University, for fusaric acid analysis, and J. F. Leslie, Kansas State University, for providing tester strains for mating population analysis.

References

Abad P, Armengol J, Olmo D and García-Jiménez J (2002) Detección mediante PCR de *Fusarium oxysporum* f.sp. *canariensis* en *palmera canaria*. Phytoma-España 138: 94–96

Abdalla MY, Al-Rokibah A, Moretti A and Mulè G (2000) Pathogenicity of toxigenic *Fusarium proliferatum* from date palm in Saudi Arabia. Plant Disease 84: 321–324

Bacon CW, Porter JK, Norred WP and Leslie JF (1996) Production of fusaric acid by *Fusarium*. Applied and Environmental Microbiology 62: 4039–4043

Bottalico A, Visconti A and Solfrizzo M (1982) Production of moniliformin by *Fusarium* species in Italy. Phytopathologia Mediterranea 21: 105–106

Cole RJ, Kirskey JW, Cutler HG, Doupnik BL and Peckam JC (1973) Toxin from *Fusarium moniliforme*: effects on plants and animals. Science 179: 1324–1326

Desjardins AE, Plattner RD and Nelson PE (1997) Production of fumonisin B1 and moniliformin by *Gibberella fujikuroi* from rice from various geographic areas. Applied and Environmental Microbiology 63: 1838–1842

Di Paola R, Fornelli F, Moretti A, Ritieni A, Tursi A, Caiaffa MF, Logrieco A and Macchia L (1998) Toxicity of Fusaproliferin to mammalian cells. Evidence for apoptosis-based cytotoxicity mechanism. In: First Workshop of COST Action 835. Agriculturally Important Toxigenic Fungi (pp 44). Athens, Greece

Elarosi H (1989) Studies on plants disease affecting date palm trees at the Eastern Province of Saudi Arabia. King Abdul Aziz City Science Technology 26: 79–109

Elmer WH (1990) *Fusarium proliferatum,* as casual agent in Fusarium crown and root rot of asparagus. Plant Disease 74: 938

Elmer WH (1995) A single mating population of *Gibberella fujikuroi* (*Fusarium proliferatum*) predominates in asparagus fields in Connecticut, Massachussets, and Michigan. Mycologia 87: 68–71

Fernandez D, Ouinten M, Tantaoui A, Geiger JP, Daboussi MJ and Langin T (1998) *Fot* 1 insertions in the *Fusarium oxysporum* f.sp. *albedinis* genome provide diagnostic PCR targets for detection of the date palm pathogen. Applied and Environmental Microbiology 64: 633–636

Gaumann E (1957) Fusaric acid as a wilt toxin. Phytopathology 47: 342–357

Harvey RB, Kubena RF, Rottinghaus GE, Turk JR and Buckley SA (1997) Effect of fumonisins and moniliformin from culture materials to growing swine. Cereal Research Communications 25: 415–417

Kellerman TS, Marasas WFO, Thiel PG, Gelderblom WGA, Cawood M and Coetzer JAW (1990) Leukoencelphalomalacia in two horses induced by oral dosing of fumonisin B1. Onderstepoort Journal of Veterinary Research 2: 269–275

Klittich CJR and Leslie JF (1988) Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics 118: 417–423

Lamprecht SC, Marasas WFO, Alberts JF, Cawood ME, Gelderblom WCA, Shephard GS, Thiel PG and Calitz FJ (1994) Phytotoxicity of fumonisins and TA toxin to corn and tomato. Phytopathology 84: 383–391

Ledoux DR, Bermudez AJ and Rottinghaus GE (1995) Effects of feeding *Fusarium fujikuroi* culture material, containing known levels of moniliformin, in young broiler chicks. Poultry Science 74: 297–305

Leslie JF (1995) *Gibberella fujikuroi*: available populations and variable traits. Canadian Journal of Botany 73(Suppl. 1): S282–S291

Leslie JF and Klein KK (1996) Female fertility and mating type effects on effective population size and evolution in filamentous fungi. Genetics 144: 557–567

Logrieco A, Moretti A, Ritieni A, Chelkowski J, Altomare C and Randazzo G (1993) Natural occurrence of beauvericin in preharvest *Fusarium subglutinans* infected corn ears in Poland. Journal of Agriculture and Food Chemistry 41: 2149–2152

Logrieco A, Moretti A, Ritieni A, Bottalico A and Corda P (1995) Occurrence and toxigenicity of *Fusarium proliferatum* from preharvest maize ear rot, and associated mycotoxins, in Italy. Plant Disease 79: 727–731

Logrieco A, Moretti A, Fornelli F, Fogliano V, Ritieni A, Caiaffa MF, Randazzo G, Bottalico A and Macchia L (1996) Fusaproliferin production by *Fusarium subglutinans* and its toxicity to *Artemia salina*, SF-9 insect cells, and human B lymphocytes. Applied and Environmental Microbiology 62: 3378–3384

Logrieco A, Moretti A, Ritieni A, Caiaffa MF and Macchia L (2002). Beauvericin: chemistry, biology and significance. In: Upadhyay RK (ed.) Advances in Microbial Toxin Research and its Biotechnological Exploitation (pp 23–30) Kluwer Academic Publishers, New York

Louvet J and Toutain G (1981). Bayoud, *Fusarium* wilt of date palm. In: Nelson PE, Toussoun TA and Cook RJ (eds) Fusarium: Diseases, Biology and Taxonomy (pp 13–20) Pennsylvania State University Press, University Park and London

Marasas WFO, Nelson PE and Toussoun TA (1984) Toxigenic *Fusarium* Species: Identity and Mycotoxicology, Pennsylvania State University Press, University Park

Marasas WFO, Rheeder JP, Lamprecht SC, Zeller KA and Leslie JF (2001) *Fusarium andiyazi* sp. nov., a new species from sorghum. Mycologia 93: 1203–1210

Mercier S and Louvet J (1973) Recherches sur les fusarioses. X. Une fusariose vasculaire (*Fusarium oxysporum*) du palmier des Canaries (*Phoenix canariensis*). Annales de Phytopathologie 5: 203–211

Moretti A, Logrieco A, Bottalico A, Ritieni A, Fogliano V and Randazzo G (1996) Diversity in beauvericin and fusaproliferin production by different populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Sydowia 48: 45–56

Moretti A, Mulè G, Perrone G and Logrieco A (1998) Genetic and toxigenic characterization of *Fusarium moniliforme* and *Fusarium proliferatum* from various plants. In Sixth International Mycological Congress (pp. 166). Jerusalem, Israel

Munkvold G, Stahr HM, Logrieco A, Moretti A and Ritieni A (1998) Occurrence of fusaproliferin and beauvericin in *Fusarium*-contaminated livestock feed in Iowa. Applied and Environmental Microbiology 64: 3923–3926

Nelson PE, Toussoun TA and Marasas WFO (1983) *Fusarium* Species: An Illustrated Manual for Identification, Pennsylvania State University Press, University Park, PA

Nelson PE, Plattner RD, Shackelford DD and Desjardins AE (1992) Fumonisin B1 production by *Fusarium* species other than *F. moniliforme* in section *Liseola* and by some related species. Applied and Environmental Microbiology 58: 985–989

Nirenberg HI and O'Donnell K (1998) New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. Mycologia 90: 434–458

O'Donnell K, Cigelnik E and Nirenberg HI (1998) Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90: 465–493

O'Donnell K, Nirenberg HI, Aoki T and Cigelnik E (2000) A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. Mycoscience 41: 61–78

Olmo D, Abad P, Armengol J, and García-Jiménez J (2001) Molecular identification of *Fusarium oxysporum* f.sp. *canariensis* on *Phoenix canariensis* in Spain. In: Proceedings of the 11th Congress of the Mediterranean Phytopathological Union (pp 168–169). Evora, Portugal

Paciolla C, Dipierro N, Mulè G, Logrieco A and Dipierro S (2005) The mycotoxins beauvericin and T-2 induce cell death and alteration to the ascorbate metabolism in tomato protoplasts. Physiological and Molecular Plant Pathology (in press)

Plyler TR, Simone GW, Fernandez D and Kistler HC (1999) Rapid detection of the *Fusarium oxysporum* lineage containing the Canary Island date palm wilt pathogen. Phytopathology 89: 407–413

Polizzi G and Vitale A. (2003) First report of Fusarium blight on majesty palm caused by *Fusarium proliferatum* in Italy. Plant Disease 87: 1149

Porter JK, Bacon CW, Wray EM and Hagler WM. (1995) Fusaric acid in *Fusarium moniliforme* cultures, corn, and feeds toxic to livestock and the neurochemical effects in the brain and pineal gland of rats. Natural Toxins 3: 91–100

Rheeder JP, Marasas WFO, Thiel PG, Sydenham EW, Shephard GS and Vanschalkwyk DJ (1992) *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in transkei. Phytopathology 82: 353–357

Ritieni A, Fogliano V, Randazzo G, Scarallo A, Logrieco A, Moretti A, Mannina L and Bottalico A (1995) Isolation and characterization of fusaproliferin, a new toxic metabolite from *Fusarium proliferatum*. Natural Toxins 3: 17–20

Ritieni A, Monti SM, Randazzo G, Logrieco A, Moretti A, Peluso G, Ferracane R and Fogliano V (1997) Teratogenic effects of fusaproliferin on chicken embryos. Journal of Agriculture and Food Chemistry 45: 3039–3043

Ross PF, Nelson PE, Richard JL, Osweiler GD, Rice LG, Plattner RD and Wilson TM (1990) Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and pulmonary edema syndrome in swine. Applied and Environmental Microbiology 56: 3225–3226

Samuels GJ, Nirenberg HI and Seifert KA (2001) Perithecial species of *Fusarium*. Summerell BA, Leslie JF, Backhouse D, Bryden WL and Burgess W (eds) Fusarium: Paul E. Nelson Memorial Symposium (pp 1–14) APS Press, St. Paul, MN

Zeller KA, Summerell BA, Bullock S and Leslie JF (2003) *Gibberella konza (Fusarium konzum)* sp. nov., a new species within the *Gibberella fujikuroi* species complex from native prairie grasses. Mycologia 95: 943–954