Mycobiota of the date palm phylloplane: description and interactions

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Summary We have analysed the mycobiota of date palm (*Phoenix dactylifer*a, L.) leaves using several techniques. Profusely sporulating fungi (*Penicillium* spp. and *Cladosporium* spp.) developed when plating leaf fragments and leaf washings. *Fusarium oxysporum*, was particularly abundant in leaves infested with the red scale insect *Phoenicococcus marlatti* Cockerell, 1899, but an undescribed *Lecanicillium* cf. *psalliotae* was also found. Dual and overlay cultures showed interactions between palm pathogens, entomopathogenic and saprotrophic fungi. The most significant was the strong inhibition of the palm pathogen *Penicillium vermoesenii* caused by the entomopathogen *Beauveria bassiana*. No symptoms developed when *F. oxysporum* isolated from scale insects or the entomopathogens *B. bassiana* or *Lecanicillium dimorphum* were wound-inoculated on *P. dactylifer*a petioles.

Key words *Phoenix dactylifera*, *Phoenicococcus marlatti*, *Lecanicillium*, *Beauveria bassiana*, *Fusarium oxysporum*, Entomopathogenic fungi, Scale insect, *Penicillium vermoesenii,* Biological control, Plant pathology

Micobiota de la filoplana de palmera datilera: descripción e interacciones

Resumen Se ha analizado la micobiota presente en las hojas de la palmera datilera (*Phoenix dactylifer*a, L.) utilizando diferentes técnicas. Tras lavar y cultivar en placas fragmentos de hoja de palmera se observó una alta esporulación de varios hongos (*Penicillium* spp. y *Cladosporium* spp.). *Fusarium oxysporum* fue particularmente abundante en hojas infestadas por la cochinilla roja (*Phoenicococcus marlatti* Cockerell, 1899). Además se detectó un hongo entomopatógeno no descrito: *Lecanicillium* cf. *psalliotae*. Los cultivos duales y las técnicas de sobreposición de membranas mostraron interacciones entre hongos patógenos de la palmera, entomopatógenos y saprótrofos. Lo más significativo fue la fuerte inhibición del hongo patógeno de palmeras *Penicillium vermoesenii* producida por el entomopatógeno *Beauveria bassiana*. Ni el hongo *F. oxysporum*, aislado de la cochinilla roja, ni los entomopatógenos *B. bassiana* o *Lecanicillium dimorphum*, causaron síntomas cuando fueron inoculados en heridas de pecíolos de *P. dactylifer*a.

Palabras clave *Phoenix dactylifera*, *Phoenicococcus marlatti*, *Lecanicillium*, *Beauveria bassiana*, *Fusarium oxysporum*, Hongos entomopatógenos, Cochinilla, *Penicillium vermoesenii*, Control biológico, Patología vegetal

Introduction

Palms are affected by several fungal diseases [2,11] and insect pests [10]. Date palms (*Phoenix dactylifera*, L.) in SE Spain are severely affected by the red scale insect (*Phoenicococcus marlatti* Cockerell, 1899) [18]. This pest is difficult to control with agrochemicals since it develops on meristematic tissue and young leaves. Pink bud rot, caused by *Penicillium vermoesenii* Biourge, affects several palm species including *P. dactylifera* [2]. *Penicillium vermoesenii* is an opportunistic fungal pathogen which may affect apical meristems of palms and kill them [20].

The introduction of biocontrol fungi in the palm phylloplane to control pests and diseases seems, therefore, desirable. Any such introduction should consider potential interactions with naturally occurring microorganisms on palm leaves. Non-pathogenic *Fusarium* spp. are commonly found colonising scale insects saprotrophically [1]. Palm leaves also harbour a wide endophytic and saprotrophic mycobiota [15,23].

Fungal endophytes may be beneficial in preventing disease by induction of host defence mechanisms [26] or by directly affecting plant pathogens [3]. On the other

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hand, palm leaf-colonising fungi may be detrimental by affecting the performance of introduced biocontrol fungi.

In this paper we have studied the mycobiota of healthy and *Phoenococcoccus marlatti* infested date palm leaves. We have also investigated the interactions between important fungal palm pathogens, entomopathogenic fungi and a *Fusarium oxysporum* Schlecht, isolate found colonising *P. marlatti* colonies. This fungus, as well as the entomopathogens *Beauveria bassiana* (Bals.) Vuill or *Lecanicillium dimorphum* (Chen) Zare & Gams, were inoculated on *P. dactylifer*a leaves to discard their role as opportunistic pathogens.

Materials and methods

Fungal isolation from palm leaves

Twelve 15-year-old specimens of *P. dactylifera* (2-3 m high), from either Las Bayas, Algorós (Elche, SE Spain) or the gardens of the University of Alicante (SE Spain) were used for the experiments. Sampling was repeated twice taking each time one leaf per plant. For most experiments photosynthetically active (non-senescent) leaves were sampled from palms and leaf blades from leaflets selected at random and were cut into 1 cm^2 fragments. Alternatively, leaves naturally infested by *P. marlatti* were also used.

To analyze the mycobiota present on leaf fragments [18 fragments per site and part of the leaf (leaf blade or petiole) per three replicates] we adopted the following methods: 1) Palm leaf plating. Leaf fragments were surface-sterilised for 1 min in 1% sodium hypoclorite and then rinsed (three times for 1 min each) in sterile distilled water. Fragments were plated on corn meal agar (CMA, Difco) plates for scoring fungal presence. Three replicate plates were carried out. 2) Palm leaf dilution plating. Three grams of leaf fragments (18 fragments per site and part of the leaf) were shaken in 6 ml of 0.02% sterile Tween 20 (Sigma) for 30 min. The resulting liquid was used for serial dilutions (10^{-1}-10^{-4}) in sterile distilled water which were then plated on CMA (three replicate plates per concentration and sample). Plates were checked after 4 days for fungal colonies. Three colonies of each isolate were selected at random and the fungi present were identified using the appropriate media and taxonomic literature [7,9,13,14,19,22,24,28]. 3) *P. marlatti* female plating. To study the mycobiota of female scale insects from palms, ca. 200 females from 18 infested leaves were dissected and plated (nine per plate) directly on 1% water agar with antibiotics (penicillin and streptomycin, 50 µg/ml each) for detecting fungal presence. Further 100 females were first surface-sterilised as described above, plated on the agar medium and scored for fungal development too.

Fungal interactions

Dual cultures. Pairs of fungi to be tested were inoculated on 9 cm diam. Petri dishes with either CMA or potato dextrose agar (PDA, Oxoid). The inocula were placed 4.5 cm apart and 2 cm from the edge of the plate. The radii of the colonies were measured until colonies contacted each other. Plates with only one fungus were set as controls. Nine replicates of each dual culture and of controls were carried out. All fungi used belong to our laboratory collection and are kept on CMA at 4 °C in the dark.

Fungi used were: *Fusarium oxysporum* strain no. 34 (from the wax layer of *P. marlatti* females), *Fusarium redolens* Wr. strain no. 36 (isolated from *P. dactylifera* with disease symptoms), *Beauveria bassiana* strain no. 119 (from the coleopteran *Langia* sp. Moore, 1872), *Lecanicillium dimorphum* strain no. 198 (from the scale insect *Saissetia oleae*), *Penicillium vermoesenii* strain no. 113 (from an infected *Washingtonia filifera* (Linden) Wendland plant). Fungal combinations for dual cultures were as follows: (1) all dual combinations between *Fusarium oxysporum*, *Fusarium redolens* and *Penicillium vermoesenii*, (2) all dual combinations between the entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium dimorphum* vs. the group of palm fungi *Fusarium oxysporum*, *Fusarium redolens* and *Penicillium vermoesenii*.

Overlay cultures. We also used overlay cultures to analyse fungal interactions as follows: the first fungus was grown on a sterile dialysis membrane (Sigma) placed on the medium. After one week growth, the membrane was removed and the second fungus was inoculated in the same place as the first [25,31]. For dual cultures we used either CMA or PDA in overlay cultures. Fungal combinations for overlay cultures were as follows: *F. oxysporum*, *P. vermoesenii* or *F. redolens* acting on *B. bassiana* or *L. dimorphum* and *B. bassiana* or *L. dimorphum* acting on *P. vermoesenii*, *F. redolens* or *F. oxysporum.*

Fungal inoculations on palm leaves

We tested for the possible opportunistic pathogenicity on palms of both entomopathogenic fungi and fungi isolated from the palm phylloplane by means of an artificial inoculation of external palm leaves.

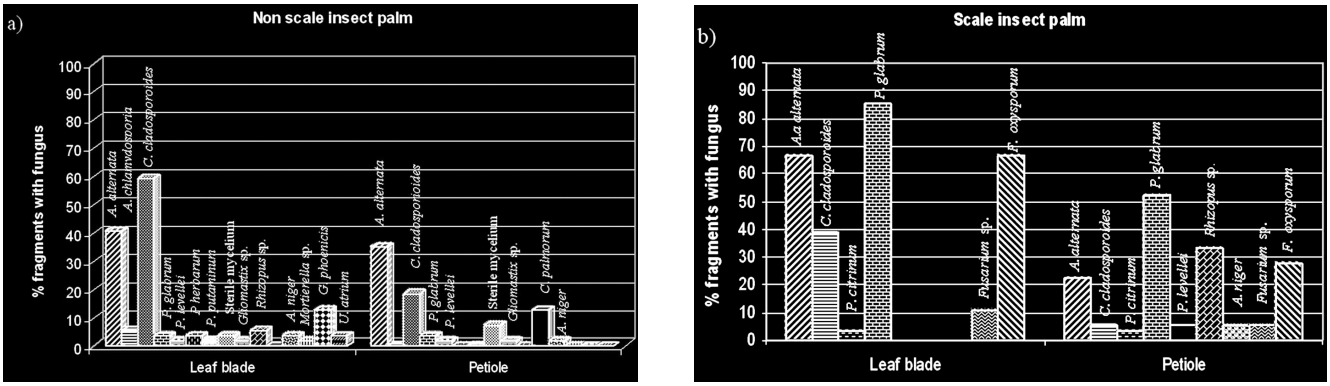

Figure 1. Mycobiota of date palm phylloplane determined by fragment plating. a) Leaf fragments from palms without *P. marlatti*. b) Fragments from palms naturally infested with *P. marlatti*. Data are the mean of 108 fragments from the leaf blade and 108 fragments from the petiole from two independent experiments (54 fragments each: 18 fragments x 3 replicates) pooled together.

Table 1. Fungal colonisation of *P. dactylifera* phylloplane determined by leaf washing dilution plating.

Fungi	Fungal colonisation (CFU· g^{-1} LEAF) LW=4.5 g			
	P. marlatti absent		*P. marlatti* present	
	Leaf blade	Petiole	Leaf blade	Petiole
A. alternata	11700 (15.0%)	3700 (17.9%)	0	100 (1.8%)
C. cladosporioides	8100 (10.7%)	10200 (49.5%)	700 (6.7%)	0
F. oxysporum	1200 (1.6%)	0	3900 (37.1%)	4400 (79.6%)
P. glabrum	19300 (25.6%)	5500 (26.7%)	0	0
P. citrinum	0	0	5700 (54.3%)	1000 (18.1%)
Phoma leveillei	3100 (4.1%)	0	200 (1.9%)	0
L.c.f. psalliotae	0	0	4 (0.04%)	28 (0.5%)
Sterile mycelium	32000 (42.4%)	1200 (5.8%)	0	0

Values in parentheses represent the percentage of fungus colonisation per g plant material. LW = leaf weight. Data are the mean of 108 fragments from the leaf blade and 108 fragments from the petiole from two independent experiments (54 fragments each: 18 fragments x 3 replicates) pooled together.

Table 2. Assessment of interactions of fungal palm pathogens, saprotrophs and entomopathogenic fungi on two solid growth media using dual cultures.

Interacting fungi (F1/F2)	Growth medium	% Control growth of F1
F. oxysporum / P. vermoesenii	CMA	95.14*
F. oxysporum / P. vermoesenii	PDA	92.48
F. oxysporum / F. redolens	CMA	98.54
F. oxysporum / F. redolens	PDA	98.26
F. redolens / P. vermoesenii	CMA	92.23*
F. redolens / P. vermoesenii	PDA	104.28
F. redolens / F. oxysporum	CMA	101.94
F. redolens / F. oxysporum	PDA	100
P. vermoesenii / F. oxysporum	CMA	120.24*
P. vermoesenii / F. oxysporum	PDA	92.34
P. vermoesenii / F. redolens	CMA	124.54**
P. vermoesenii / F. redolens	PDA	89.73
B. bassiana / F. oxysporum	CMA	86.60
B. bassiana / F. oxysporum	PDA	93.63
B. bassiana / F. redolens	CMA	73.30**
B. bassiana / F. redolens	PDA	90.90
B. bassiana / P. vermoesenii	CMA	60.0**
B. bassiana / P. vermoesenii	PDA	72.72**
L. dimorphum / F. oxysporum	CMA	93.63
L. dimorphum / F. oxysporum	PDA	111.5
L. dimorphum / F. redolens	CMA	84.54
L. dimorphum / F. redolens	PDA	115.04*
L. dimorphum / P. vermoesenii	CMA	84.54
L. dimorphum / P. vermoesenii	PDA	73.45**
F. oxysporum / B. bassiana	CMA	58.15**
F. oxysporum / B. bassiana	PDA	45.85**
F. oxysporum / L. dimorphum	CMA	60.52**
F. oxysporum / L. dimorphum	PDA	54.72**
F. redolens / B. bassiana	CMA	80.0**
F. redolens / B. bassiana	PDA	67.50**
F. redolens / L. dimorphum	CMA	82.86*
F. redolens / L. dimorphum	PDA	86.11
P. vermoesenii / B. bassiana	CMA	96.60*
P. vermoesenii / B. bassiana	PDA	46.61**
P. vermoesenii / L. dimorphum	CMA	90.0
P. vermoesenii / L. dimorphum	PDA	47.70**

*: significant (ANOVA or U Mann Whitney Tests, $p = 0.05 = \alpha$) growth inhibition of F1 vs F2
**: significant (ANOVA or U Mann Whitney Tests, $p < 0.05 = \alpha$) growth inhibition of F1 vs F2

Healthy (asymptomatic) external leaves of palm trees were selected for fungal inoculations. On each leaf petiole, a wound about 2 cm long and 3 mm deep was axenically made. These were then inoculated with 1000 µl of a 10^6 conidia/ml suspension in sterile distilled water with 0.002% sterile Tween 20 of each test fungus using an automatic pipette. Nine petioles (three per palm) were inoculated with each fungus tested. Typical symptoms of palm rot pathogens include both necrotic and hydropic zones up and down the inoculation zone [20]. After inoculation these symptoms were scored at 4- or 7-day intervals.

Data analyses

For the analysis of dual culture data and for the overlay technique a Kolmogorov-Smirnov test was used to prove whether data fit a normal distribution. Data came from 10 days inoculation in case of the overlay technique. Dual culture data came from the day when fungal contact was established (depending of the pair of fungi). For positive cases an ANOVA test was performed; otherwise, a Kruskal-Wallis test was carried out to detect interspecific variations. U Mann-Whitney test was performed for comparing populations [32].

Results

Leaf fragment plating

Results of fungal isolations from leaf fragments derived from palms at the three sites sampled were pooled to better represent the mycobiota of palm leaves. Most (90-100%) surface-sterilised *P. dactylifera* leaf fragments rendered fungal colonies when plated. *Cladosporium cladosporioides* (Fries) de Vries and *Alternaria alternata* (Fries) Keissler were the most abundant species on fragments without scale insects (Figure 1a). On leaves infested with *P. marlatti* scale insects, *Penicillium glabrum* (Wehmer) Westling, *Fusarium oxysporum* and *A. alternata* were the most frequent. Fungal diversity was higher for leaves without *P. marlatti* than for infested leaves. However, on infested leaves fungal incidence was higher (Figure 1b).

Leaf washing dilution plating

Leaf washing dilutions of healthy *P. dactylifera* leaves showed the same fungal species as leaf fragment platings, except for *F. oxysporum* (Table 1). The most abundant fungi by this technique were *F. oxysporum* followed by *Penicillium citrinum* Thom. and *Cladosporium cladosporioides*. The mycobiota of petioles slightly varied than that of leaf blades. A higher frequency of *F. oxysporum* and *P. citrinum* was also noticeable in leaves infested with *P. marlatti* as compared to healthy ones. The presence of *Lecanicillium* cf. *psalliotae* was also unique to *P. marlatti* infested leaves.

Phoenicococcus marlatti *female plating*

Two hundred *P. marlatti* females were plated directly on CMA and 60.6% displayed *Penicillium citrinum*, 35.2% displayed both *P. citrinum* and *F. oxysporum* and the rest were fungus-free. One hundred scale insects were plated after surface sterilization with sodium hypochlorite and 90% only displayed *F. oxysporum* (the rest were fungus-free).

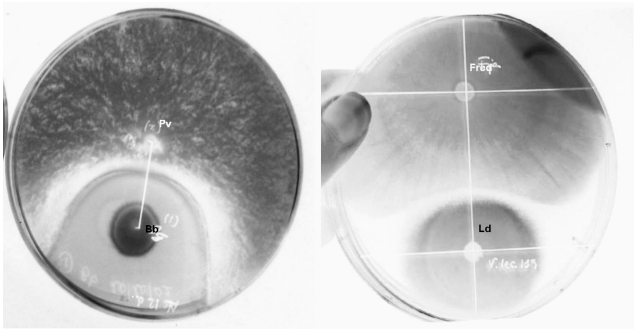

Figure 2. Inhibition of phytopathogenic fungi *P. vermoesenii* (Pv) (left) and *F. redolens* (Fred) (right) by the entomopathogenic *B. bassiana* (Bb) and *L. dimorphum* (Ld) in dual cultures.

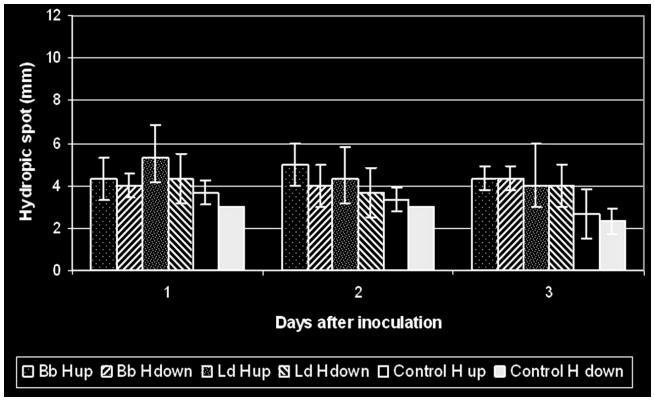

Figure 3. Symptoms on *P. dactylifera* petioles after in vivo inoculations with the entomopathogenic fungi *B. bassiana* and *L. dimorphum*. Hup: upwards hydropic spot referred to the position of the inoculation point; Hdown: downwards hydropic spot; Bb: *B. bassiana*; Pv: *P. vermoesenii.*

Interactions between phylloplane fungi and entomopathogenic fungi

We tested possible interactions between fungi isolated from the palm phylloplane (pathogens or saprotrophs) and entomopathogenic fungi with the aim of selecting pathogens of *P. marlatti*. The results of the interaction tests using dual cultures are shown in table 2.

The most important growth inhibition was found for *P. vermoesenii*, which was reduced by ca. 54% on PDA by *B. bassiana* (Figure 2) and by ca. 46% by *L. dimorphum*. The entomopathogen *L. dimorphum* also reduced the incidence of *Fusarium redolens* (Figure 2). *Fusarium oxysporum* was inhibited by *B. bassiana* and *L. dimorphum* by 50-60% on PDA. Moreover, *B. bassiana* and *L. dimorphum* were inhibited by *P. vermoesenii* on PDA by ca. 30%. The results of fungal interactions using overlay cultures are presented in table 3. The most significant feature found was the growth inhibition of *P. vermoesenii* by *B. bassiana*. This accounted for 63% of the control growth of *P. vermoesenii* on PDA. The effect was statistically significant on both CMA and PDA ($p = 0.05$).

Pathogenicity of antagonistic fungi and saprotrophs from palm phylloplane in palm

This study could not find statistical differences in symptoms on palm petioles 12 or 24 days after inoculation with entomopathogenic fungi with respect to controls ($p > 0.05$). Four days after inoculation slight differences in *L. dimorphum* treatments were found ($p < 0.05$) for the "up and down" (upwards and downwards from inoculation point) vertical hydropic spot measurements (Figure 3). When phytopathogenic fungi were inoculated, *P. vermoesenii* showed statistical differences with respect to controls (Figures 4 and 5) at 17 and 24 days ($p < 0.05$, for upwards and downwards hydropic spot measurements). *Fusarium redolens* inoculations did not differ from controls.

Discussion

We have recently found [5] that the red scale insect defensive barriers (wax layer and cuticle) and associated saprotrophic mycobiota may interfere with potential biocontrol agents such as entomopathogenic fungi. Knowledge of the palm mycobiota as well as of the interactions between selected components, including biological agents and palm fungal pathogens, therefore seems to be a desirable prerequisite for a successful biocontrol of palm pests and diseases.

Our main finding regarding the palm mycobiota was that healthy palm leaves show higher fungal species diversity than leaves infested with scale insects. The main fungi found on date palm leaves were *Penicillium* spp., *Cladosporium cladosporioides*, *Fusarium oxysporum* and *Alternaria alternata*. After leaf dilution plating, *Lecanicillium* cf. *psalliotae*, *Phoma* sp. and sterile mycelia could also be found. *L. psalliotae* has been described as a mycoparasitic, nematophagous and entomopathogenic fungus [13]. *Fusarium oxysporum* was more abundant in plant material when scale insects were present on the leaf surface. The differences found agree with the study of the mycobiota of red scale insect females themselves. *Phoenicococcus marlatti* females were mostly colonised by *F. oxysporum* (89% of the surface-sterilised insects, and ca. 79% of those

Table 3. Assessment of fungal palm pathogens, saprotrophs and entomopathogenic fungi interactions on agar growth media using overlay cultures (growing F2 first on a membrane and measuring F1).

Interacting fungi (F1/F2)	Cultura medium	% Control growth of F1
B. bassiana / *F. oxysporum*	CMA	82.9*
B. bassiana / *F. oxysporum*	PDA	63.3**
B. bassiana / *F. redolens*	CMA	240.0**
B. bassiana / *F. redolens*	PDA	39.8**
B. bassiana / *P. vermoesenii*	CMA	76.0**
B. bassiana / *P. vermoesenii*	PDA	73.0**
L. dimorphum / *F. oxysporum*	CMA	88.2*
L. dimorphum / *F. oxysporum*	PDA	85.0**
L. dimorphum / *F. redolens*	CMA	250.0**
L. dimorphum / *F. redolens*	PDA	80.0*
L. dimorphum / *P. vermoesenii*	CMA	95.0
L. dimorphum / *P. vermoesenii*	PDA	77.6**
P. vermoesenii / *B. bassiana*	CMA	33.7**
P. vermoesenii / *B. bassiana*	PDA	17.2**
P. vermoesenii / *L. dimorphum*	CMA	124.0**
P. vermoesenii / *L. dimorphum*	PDA	60.0**
F. oxysporum / *B. bassiana*	CMA	100.0
F. oxysporum / *B. bassiana*	PDA	66.0**
F. oxysporum / *L. dimorphum*	CMA	100.0
F. oxysporum / *L. dimorphum*	PDA	74.0**
F. redolens / *B. bassiana*	CMA	100.0
F. redolens / *B. bassiana*	PDA	77.6**
F. redolens / *L. dimorphum*	CMA	100.0
F. redolens / *L. dimorphum*	PDA	81.0*
P. vermoesenii / *B. bassiana*	CMA	33.7**
P. vermoesenii / *B. bassiana*	PDA	17.2**
P. vermoesenii / *L. dimorphum*	CMA	124.0**
P. vermoesenii / *L. dimorphum*	PDA	10.0**

CMA: corn meal agar; PDA: potato dextrose agar.
*: significant (ANOVA or U Mann Whitney Tests, $p = 0.05 = \alpha$) growth inhibition of F1 vs F2
**: significant (ANOVA or U Mann Whitney Tests, $p < 0,05 = \alpha$) growth inhibition of F1 vs F2

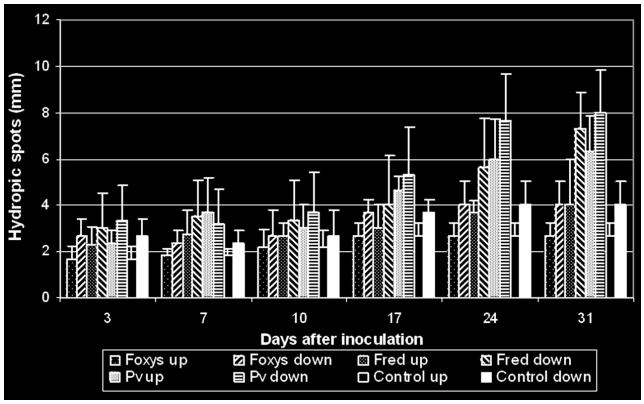

Figure 4. Symptoms on *P. dactylifera* petioles after *in vivo* inoculations with the phytopathogenic fungi *F. oxysporum*, *F. redolens* and *P. vermoesenii*. Up: upwards hydropic spot; Down: downwards hydropic spot; Foxys: *F. oxysporum*; Pv: *P. vermoesenii.*

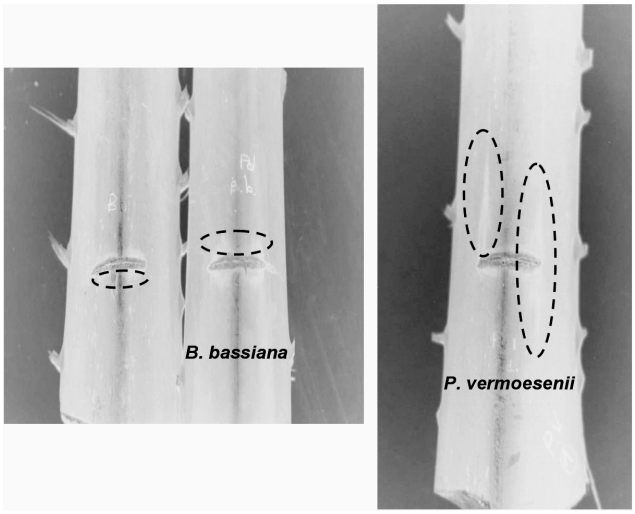

Figure 5: Symptoms after 12 days on *P. dactylifera* petioles after *B. bassiana* (left) or *P. vermoesenii* (right) inoculations. Note the difference in spot length.

plated directly). The frequency of *Penicillium citrinum* was reduced on *P. marlatti* females surface-sterilised with sodium hypochlorite. *Fusarium* spp. are known to be usually associated with scale insects [1]. In this respect we have found that the red scale insect (*P. marlatti*) has a close association with *Fusarium oxysporum* [4]. Some *Fusarium* species are even insect pathogens, such as *Fusarium larvarum* (isolated from almond scale insect), and was used by Cozzi et al. [12] as a biological control agent of *Saissetia oleae* Olivier, 1791 in South Italy.

Since previous work [5] suggested interference between *Fusarium* spp. colonizing red scale insects and entomopathogenic fungi inoculated in laboratory bioassays, we studied the interactions between selected components of the palm leaf mycobiota, palm fungus pathogens and saprotrophs and entomopathogenic fungi. The most striking feature was the strong inhibitions found in dual cultures. This phenomenon was previously reported by Benhamou & Broder [8] for *Verticillium lecanii* and the post-harvest pathogen *Penicillium digitatum*. The importance of our finding is that *B. bassiana*, a well-known entomopathogen [29], can also be a potential biocontrol agent for *P. vermoesenii*, the causal agent of pink bud rot disease of palms [2]. *Beauveria bassiana* also affected *F. redolens*, another important palm pathogen. *Lecanicillium dimorphum* affected these fungi but to a lesser extent. The mode of action of this inhibition could be due to toxic metabolites that are commonly produced by entomopathogenic fungi to reduce competition [27]. We detected differences in fungal interactions depending on the medium used. On PDA, *F. oxysporum*, *P. vermoesenii* and *F redolens* were more inhibited by entomopathogenic fungi (*B. bassiana* and *L. dimorphum*) than on CMA. Megarejo et al. [21], working with phylloplane biocontrol agents, have found similar results. Under low nutrient conditions (CMA) there was little inhibition of entomopathogenic fungi by phylloplane fungi such as *P. vermoesenii*. While *F. oxysporum* was early inhibited by the entomopathogens (data not shown), *P. vermoesenii* was inhibited 3-4 days after interaction or inoculation. This effect was buffered in CMA probably due to a lack of nutrients. After microscopical observation of interacting fungi (data not shown) we found no evidence of mycoparasitism. Therefore, the causes of inhibition found could be due to competition and/or antibiosis [31].

The overlay technique confirmed the results found in dual cultures. This technique is very sensitive and showed a rapid inhibition response, as reported by several authors such as Whipps [30] and Simon et al. [25]. The most important effect on growth appeared in *P. vermoesenii* after *B. bassiana* or *L. dimorphum* incubation on PDA (over 80% inhibition of *P. vermoesenii* growth). Bajan and Kmitowa [6] showed that if *B. bassiana* could settle first in an area this could prevent growth of saprotrophs. This finding could be one of the reasons for the stronger inhibition of *P. vermoesenii* growth detected in overlays than that seen in dual cultures.

The negative results of pathogenic tests with *F. oxysporum* discarded this fungus as a synergistic organism in palm dieback diseases caused by red scale insect [16]. However, *P. vermoesenii* produced symptoms on *P. dactylifera* after a period of action, as expected by us on the strength of results obtained by other authors, such as Lopez-Llorca & Orts [20]. Palm response to *P. vermoesenii* inoculation was not very high, probably due to the dry weather during the experimental period. On the other hand, the negative results for entomopathogenic fungi show that they can be used as possible biocontrol agents of insect palm pests. In fact, our preliminary results [17] show that they are endophytic in palm leaves.

These results showed that entomopathogenic fungi are versatile organisms that can compete in the date palm phylloplane. Furthermore, these fungi can be considered as biocontrol agents of palm pests and diseases. Further studies along these lines are underway in our laboratory.

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