Standardizing Pathogenicity Assays for Fusarium Wilt Pathogens of Ornamental Palms

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Abstract

Standardized protocols for determining pathogenicity of *Fusarium oxysporum* ff. spp. *canariensis* and *palmarum*, the cause of Fusarium wilt of ornamental palms, were developed using small palm plants with a minimum of three to four seedling leaves. For both protocols, a standard amount of inoculum (25 ml of 10⁶ spores/ml) was pipetted onto and between the leaf bases of each plant, with excess material running down onto the roots and collecting in the container. After 3 days, the palm plants were transplanted into 450-ml containers filled with pine bark/sedge peat/sand potting mix. The protocol for *F. oxysporum* f. sp. *canariensis* differed from the protocol of *F. oxysporum* f. sp. *palmarum* by requiring that the lower 20% of

roots be cut prior to inoculation and having the assay run for 6 months versus 3 months. These two assays were used to evaluate pathogenicity of multiple isolates of each pathogen. All 15 isolates of *F. oxysporum* f. sp. *palmarum* were pathogenic, whereas only 7 of 13 *F. oxysporum* f. sp. *canariensis* isolates were pathogenic. These assays were also used to determine susceptibility of other palm species to these pathogens. *Washingtonia filifera*, *Butia odorata*, *Phoenix dactylifera*, and *P. reclinata* appeared susceptible to *F. oxysporum* f. sp. *palmarum*, at least in the seedling stage. Other inoculation techniques are described that may be useful for evaluating Fusarium wilt disease management methods.

One of the most variable aspects of plant pathology research is pathogenicity testing. For any given crop, it is a challenge to develop an inoculation protocol to deliver consistent disease pressure that will produce repeatable results. This can be especially difficult when working with diseases of mature trees, because finding enough trees of the same size for large-scale experiments can be expensive and require considerable space in a greenhouse or field nursery. To circumvent the problem, plant pathologists often develop pathogenicity assays with seedlings or small trees where results correlate with the symptoms observed in large, mature trees and where testing can be done in a manageable and controlled research facility. Ornamental tree nurseries are not usually willing to allow researchers to use their field nurseries for research for fear that the pathogen may infect their material. Moreover, using established trees in the landscape is not feasible because the researcher will have minimal control, if any, over the geographical terrain and microenvironmental conditions which can vary over large landscape areas. For palms, seedlings are preferred to larger material for safety reasons, because some palm species have spines (e.g., genera *Phoenix* and *Washingtonia*) on their petioles that can cause puncture wounds that may lead to plant-thorn synovitis (Kratz et al. 2003; Stevens et al. 2000).

For almost four decades in the United States, Fusarium wilt in palms was restricted to *Phoenix canariensis* (Canary Island date palm), which is caused by *Fusarium oxysporum* f. sp. *canariensis*. This disease occurs worldwide (Elena 2005; Palmucci 2006; Summerell et al. 2001). It was first detected in the continental United States in California in 1976 (Feather et al. 1989), followed by Florida in 1994 (Plyler et al. 1999). This disease has continued to spread in the United States to Louisiana, Nevada, South Carolina, and Texas (Elliott et al. 2011; Singh et al. 2011; Summerell et al. 2001). In 2010, a new palm Fusarium wilt pathogen was confirmed in Florida (Elliott et al. 2010). This fungus was given the forma specialis name of “*palmarum*” because it had at least two common palm hosts, *Washingtonia robusta* and *Syagrus romanzoffiana* (i.e., it was not restricted to just one host species as is usually the case for Fusarium wilt pathogens). *F. oxysporum* f. sp. *palmarum* has spread

throughout Florida and to other palm species, including *P. canariensis* (Elliott 2011) and *× Butyagrus nabonnandi* (Elliott et al. 2017).

A review of the literature revealed at least five different methods for pathogenicity testing of *F. oxysporum* f. sp. *canariensis* on *P. canariensis* (Elena 2005; Feather 1982; Mercier and Louvet 1973; Priest and Letham 1996; Singh et al. 2011). Although all of the methods worked, some were more simple to conduct than others. However, at least three of these methods produced mixed results for pathogenicity testing of *F. oxysporum* f. sp. *canariensis* on *P. dactylifera* (Elena 2005; Feather et al. 1989; Mercier and Louvet 1973; Priest and Letham 1996).

For *F. oxysporum* f. sp. *palmarum*, three different techniques were evaluated when the pathogen was initially described in Florida (Elliott et al. 2010). One of these techniques (seedlings inoculated with spore suspension) was used again in determining pathogenicity of an isolate obtained from symptomatic *W. robusta* in Texas (Giesbrecht et al. 2013) and for confirming pathogenicity on new palm hosts (Elliott 2011; Elliott et al. 2017).

Fusarium wilt caused by *F. oxysporum* ff. spp. *canariensis* and *palmarum* is a lethal disease of ornamental palms. Although it normally occurs as a canopy infection in the landscape, if the pathogens become incorporated into the soil, infection of the roots will occur in susceptible palm species (Elliott et al. 2010; Summerell et al. 2001), a phenomenon observed in date palm groves infested with *F. oxysporum* f. sp. *albedinis* (Louvet and Toutain 1981) and oil palm plantations infested with *F. oxysporum* f. sp. *elaeidis* (Colhoun 1981). This root infection aspect of the disease on ornamental palms has led to the recommendations of not planting a Fusarium wilt-susceptible palm back into the same location where the previous palm died from Fusarium wilt. The question that then arises from a landscape owner is: “What palm would be acceptable to replant in this location?” The other question by landscape owners concerns methods and materials that could be used to prevent the disease or mitigate damage if a susceptible palm becomes symptomatic. Although nursery growers have the options of fumigation or sterilization, such options cannot be used in landscapes. Both of these questions could be initially investigated using a standardized plant-pathogen assay.

Because it is likely that these two formae speciales of *F. oxysporum* associated with ornamental palms will continue to spread, it would be useful to have a standardized procedure for testing pathogenicity and evaluation of potential management protocols. For example, the European and Mediterranean Plant Protection Organization (EPPO) has a standard protocol for diagnosing and determining pathogenicity of *F. oxysporum* f. sp. *albedinis*, an A2 regulatory pathogen, which causes Fusarium wilt of *P. dactylifera* (EPPO 2003).

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Therefore, the objectives of this research were to (i) develop a standard pathogenicity assay for *F. oxysporum* f. sp. *palmarum*, (ii) develop a standard pathogenicity assay for *F. oxysporum* f. sp. *canariensis*, (iii) determine potential susceptibility of other palm species to these two *Fusarium* wilt pathogens, and (iv) develop a method for evaluating prevention or mitigation of *Fusarium* wilt using *F. oxysporum* f. sp. *palmarum* as the model pathogen.

Materials and Methods

Fungal isolates and inoculum preparation. All *F. oxysporum* ff. spp. *canariensis* and *palmarum* isolates used in this study are listed in Table 1. Single-spored isolates were stored as colonized agar plugs in sterile deionized water (SDW) in cryogenic vials sealed with Parafilm and maintained at room temperature. As needed, a plug was transferred from storage to one-fifth-strength potato dextrose agar (1/5 PDA) and incubated at 26°C with 12 h of light.

To prepare inoculum, fungal isolates were transferred from the 1/5 PDA plate to full-strength PDA and incubated at 26°C with 12 h of light. After at least 2 weeks of growth, approximately 7 ml of SDW was added to each plate and the agar surface was gently rubbed with a sterile bent glass rod to loosen asexual spores (mostly microconidia but also macroconidia) from the mycelia. This suspension was poured into a sterile bottle and the plate was rinsed with approximately 10 ml of SDW into the bottle. The suspension was filtered through four layers of sterile cheesecloth to remove mycelia and then diluted with SDW to obtain 10^6 spores/ml. Although the number of spores produced varied for each isolate, they all yielded a minimum of 25 ml of 10^6 spores/ml per PDA plate, which became the standard amount of inoculum applied to each plant.

Standard inoculation method for pathogenicity assay. Palm seedlings were obtained in deep cell plug trays from various nurseries in Florida and Texas. Exact source depended on availability when the experiments were initiated. Only seedlings with a minimum of three to four seedling leaves were used. The inoculation method used is

similar to the standard pathogenicity assay described for *F. oxysporum* f. sp. *albedinis* (EPPO 2003). Palm seedlings were removed from plug trays and potting mix was shaken or hand removed from the roots. Then, for each inoculum treatment, five replicate palm plants were placed in a 1- to 3-liter container. Size of the container depended on the size of the palm seedling, because a three-to-four-seedling-leaf palm will vary in size based on the palm species. The standard spore suspension (25 ml of 10^6 spores/ml) was used per palm, with the suspension pipetted down among the leaf bases, with excess material running down onto the roots and collecting in the container. The control treatment for each experiment was always SDW at 25 ml/palm. The top of the container was covered with a clear polyethylene bag, with the leaves left exposed.

After 3 days in the dark, palm plants were transplanted into 450-ml containers filled with a pine bark/sedge peat/sand (5:4:1) potting mix amended with dolomite and micronutrients. Osmocote fertilizer was applied to the potting mix surface according to directions, which were based on container size, duration of experiment, and expected mean temperature.

Palm growth conditions and dry weights. After transplanting, plants were placed on benches in a structure with shade-cloth walls and a clear polycarbonate sheet roof, where they were irrigated daily. Because the experiments were conducted in southeastern Florida, no attempt was made to regulate temperature unless the temperature was likely to drop below 10°C, at which time vents were closed and side-wall curtains were dropped ahead of the temperature decline to maintain ambient heat in the structure. Palm plants were examined weekly. Duration of each experiment varied as noted below. At the termination of each experiment (except for experiment 1), control-treatment palm plants and any plants surviving fungal inoculation were harvested by cutting plants at the leaf base–root interface and drying at 60°C for at least 5 days prior to weighing. Palm plants that died prior to termination of an experiment were given the dry weight value of 0. Dry weights were not obtained for experiment 1.

Experiment 1. Using the standard inoculation method, 22 palm species used in the Florida landscape were evaluated to determine their susceptibility to *F. oxysporum* f. sp. *palmarum* (Table 2). This

Table 1. Isolates of *Fusarium oxysporum* f. sp. *palmarum* and f. sp. *canariensis* used in this study, with location (county and state) and palm host from which the isolate was originally obtained

Isolate	County, state	Palm host
<i>F. oxysporum</i> f. sp. <i>palmarum</i>		
PLM-119C	Martin, FL	<i>Syagrus romanzoffiana</i>
PLM-140B	Broward, FL	<i>S. romanzoffiana</i>
PLM-153B	Manatee, FL	<i>S. romanzoffiana</i>
PLM-249A	Lee, FL	<i>Washingtonia robusta</i>
PLM-320B	Pinellas, FL	× <i>Butyagrus nabonnandi</i>
PLM-510A	Orange, FL	<i>Phoenix canariensis</i>
PLM-560A	St. Lucie, FL	<i>W. robusta</i>
PLM-596A	Brevard, FL	<i>W. robusta</i>
PLM-619A	Orange, FL	<i>Bismarckia nobilis</i>
PLM-676A	Broward, FL	<i>P. canariensis</i>
PLM-729A	Sarasota, FL	<i>S. romanzoffiana</i>
PLM-741A	Miami-Dade, FL	<i>S. romanzoffiana</i>
PLM-791A	Palm Beach, FL	<i>P. canariensis</i>
PLM-853A	Orange, FL	× <i>Butyagrus nabonnandi</i>
PLM-920	Harris, TX	<i>W. robusta</i>
<i>F. oxysporum</i> f. sp. <i>canariensis</i>		
PLM-183C	Miami-Dade, FL	<i>P. canariensis</i>
PLM-221B	Manatee, FL	<i>P. canariensis</i>
PLM-224A	Manatee, FL	<i>Phoenix sylvestris</i>
PLM-385B	Travis, TX	<i>P. canariensis</i>
PLM-386B	Sarasota, FL	<i>P. canariensis</i>
PLM-387B	Manatee, FL	<i>P. reclinata</i>
PLM-511A	Charleston, SC	<i>P. canariensis</i>
PLM-588A	Sarasota, FL	<i>P. canariensis</i>
PLM-605A	Duval, FL	<i>P. canariensis</i>
PLM-696A	Palm Beach, FL	<i>P. reclinata</i>
PLM-706A	Marin, CA	<i>P. canariensis</i>
PLM-724A	Duval, FL	<i>P. canariensis</i>
PLM-754A	Palm Beach, FL	<i>P. canariensis</i>

Table 2. Palm species evaluated for susceptibility to *Fusarium oxysporum* f. sp. *palmarum*

Palm species	Common name	Susceptibility ^a
<i>Adonidia merrellii</i>	Christmas or Manila palm	–
<i>Allooptera arenaria</i>	Seashore palm	–
<i>Arenga engleri</i>	Dwarf sugar palm	–
<i>Beccariophoenix alfredii</i>	High plateau coconut palm	–
<i>Butia odorata</i>	Pindo or Jelly palm	+
× <i>Butyagrus nabonnandi</i>	Mule palm	+
<i>Chamaerops humilis</i>	European fan palm	–
<i>Dypsis decaryi</i>	Triangle palm	–
<i>D. lutescens</i>	Areca or Golden cane palm	–
<i>Kentiopsis oliviformis</i>	none	–
<i>Livistona chinensis</i>	Chinese fan palm	–
<i>Phoenix canariensis</i>	Canary Island date palm	+
<i>P. dactylifera</i>	Date palm	+
<i>P. reclinata</i>	Senegal date palm	+
<i>P. roebelenii</i>	Pygmy date palm	–
<i>P. sylvestris</i>	Wild date palm	+
<i>Ravenea rivularis</i>	Majesty palm	–
<i>Rhapis excelsa</i>	Lady palm	–
<i>Roystonea regia</i>	Royal palm	–
<i>Syagrus romanzoffiana</i>	Queen palm	+
<i>Thrinax radiata</i>	Florida thatch palm	–
<i>Washingtonia filifera</i>	California or Desert fan palm	+
<i>W. robusta</i>	Mexican fan palm	+
<i>Wodyetia bifurcata</i>	Foxtail palm	–

^a Symbols: + indicates that at least one of five replicate palm plants per fungal treatment died or severely declined compared with the water only (control) treatment and – indicates that none of the five replicate palm plants per fungal treatment died or appeared different from the control treatment.

experiment was conducted in three repetitions. Each repetition included seven or eight different palm species, plus *W. robusta* as the susceptible control. Two *F. oxysporum* f. sp. *palmarum* isolates, PLM-140B and PLM-249A (treatments), were used for each repetition, plus the control (SDW only) treatment. There were five replicate palm plants per treatment–palm species. The experiment was terminated after 6 months. For each palm species, it was noted whether a fungal inoculated palm died or declined compared with their respective control treatment.

Experiment 2. *W. filifera*, *Butia odorata*, and $\times$ *B. nabonnandi* (a sterile, interspecific cross between *S. romanzoffiana* and *B. odorata*) were examined more closely for susceptibility to *F. oxysporum* f. sp. *palmarum*. *S. romanzoffiana* and *W. robusta* were included as the known susceptible controls. The *F. oxysporum* f. sp. *palmarum* isolates used were PLM-140B, PLM-249A, and PLM-320B (treatments), which represented all three haplotypes of this pathogen (Elliott et al. 2010). There were five replicate palm plants per treatment–palm species. Plants were examined weekly, and the experiment was terminated after 6 months. This experiment was repeated twice.

Experiment 3. The cross-pathogenicity of *F. oxysporum* ff. spp. *canariensis* and *palmarum* on *P. canariensis*, *P. dactylifera*, *P. reclinata*, *P. sylvestris*, *S. romanzoffiana*, and *W. robusta* was examined in this experiment. Two *F. oxysporum* f. sp. *palmarum* isolates (PLM-140B and PLM-249A) and three *F. oxysporum* f. sp. *canariensis* isolates (PLM-224A, PLM-386B, and PLM-387B) were used as treatments. There were five replicate palm plants per treatment–palm species. Plants were examined weekly, and the experiment was terminated after 6 months. This experiment was repeated twice, except that *S. romanzoffiana* was not included in the second repetition.

Experiment 4. The results of experiment 3 with *F. oxysporum* f. sp. *canariensis* were not as expected. Thus, two additional inoculation methods were compared with the standard inoculation method for the pathogenicity assay. The second inoculation method was similar to the standard method, except that the lowest 20% of the roots were cut prior to inoculation with the individual fungal suspensions or SDW. For the third inoculation method, palm plants were transplanted as described previously. Then, for each palm, a shallow (approximately 3 mm deep), 1.25-cm-long slit was made with a sterile scalpel on the adaxial side of the two lowest leaf petioles. Approximately 12 ml of the spore suspension or SDW was dribbled slowly into the slits. The slits were then wrapped with Parafilm. The same three *F. oxysporum* f. sp. *canariensis* isolates were used as in experiment 3 but only *P. canariensis* was evaluated. There were five replicate palm plants per fungal treatment or control–inoculation method. Plants were examined weekly, and the experiment was terminated after 6 months.

Experiment 5. Because the cutting of the lowest 20% of the roots in experiment 4 appeared to provide consistent pathogenicity results for *P. canariensis* across all three *F. oxysporum* f. sp. *canariensis* isolates, this method was used for a duplication of experiment 3. In other words, all palm species had 20% of the lowest roots removed prior to inoculation with individual fungal spore suspensions of *F. oxysporum* ff. spp. *canariensis* and *palmarum* or SDW. Palm plants were examined weekly, and the experiment was terminated after 6 months. This experiment was repeated twice, except that *P. reclinata* was not included in the second repetition.

Experiment 6. To determine the consistency of the two inoculation methods—with or without cutting the lower roots—for pathogenicity assays, an experiment was established as follows. The method that appeared to be successful for *F. oxysporum* f. sp. *palmarum* did not include the cutting of roots. This method, as described initially, was used on *W. robusta*, with 15 different *F. oxysporum* f. sp. *palmarum* isolates plus a SDW control. The method that appeared to be successful for *F. oxysporum* f. sp. *canariensis* did include cutting the lower 20% of the roots. This method, as described in experiments 4 and 5, was used on *P. canariensis*, with 13 different *F. oxysporum* f. sp. *canariensis* isolates plus a SDW control. There were five replicate palm plants per fungal– and control treatments–palm species. Plants were examined weekly. The experiment with *W. robusta* was terminated after 3 months; the experiment with *P. canariensis* was terminated after 6 months.

Experiment 7. Although the level of disease obtained with *F. oxysporum* f. sp. *palmarum* using the standard inoculation method was useful for examining pathogenicity of *F. oxysporum* f. sp. *palmarum* isolates, a less severe method is needed for evaluating chemical and biological management methods. Therefore, another inoculation method was examined. Seedlings of *W. robusta* were transplanted as described previously and then allowed to grow undisturbed in a covered shadehouse until the palm plants had established with two new, fully emerged leaves. At this point, three different root disturbance techniques and four different levels of inoculum, using only isolate PLM-249A, were used for treatments. The three root disturbance techniques were (i) no disturbance, (ii) the top potting-mix layer (approximately 625 mm deep) was scraped to the edges of the pot with a sterile metal tool to disturb the fine roots growing in this top layer, and (iii) the top layer was scraped away as above plus roots were cut vertically in the pot at three places using pruners. The level of inoculum used was either 0, 5, 15, or 25 ml of the 10^6 spores/ml suspension and was applied among the leaf bases, with excess percolating into the potting mix. For root disturbance techniques (ii) and (iii), the inoculum was applied after the roots were disturbed and then the top layer was scraped back over the roots. There were five replicate palm plants per root disturbance technique–inoculum level. Plants were examined weekly, and the experiment was terminated after 6 months. This experiment was repeated three times.

Statistical analysis. For experiments 2 through 6, the General Linear Models (GLM) procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC) was used. Means were compared using Dunnett's test (Dunnett 1955), with all means within a species compared against the control treatment for that species. Mean, standard error, and Dunnett's test were calculated using SAS 9.4. For experiment 7, data from all three repetitions were combined and a two-way analysis of variance (ANOVA) was applied to the data (PROC GLM in SAS 9.4).

Results

A preliminary experiment using the standard inoculation method, with *W. robusta* and *S. romanzoffiana* as the hosts and *F. oxysporum* f. sp. *palmarum* isolates PLM-140B and PLM-249B as the pathogens, was conducted prior to initiating any of the other experiments. All five replicate plants of each palm species inoculated with either pathogen were killed within 3 months, whereas all control plants (SDW only) were healthy (data not shown).

Experiment 1. Based on the preliminary experiment results, the standard inoculation method was used to evaluate *F. oxysporum* f. sp. *palmarum* susceptibility of 22 palm species used in the Florida landscape. Other than *S. romanzoffiana* and *W. robusta*, of which all five replicate plants of each species died after inoculation with PLM-140B and PLM-249A, the following were the only palm species where at least one of five replicate plants died or severely declined due to inoculation, compared with their respective controls: *B. odorata*, $\times$ *B. nabonnandi*, *P. canariensis*, *P. dactylifera*, *P. reclinata*, *P. sylvestris*, and *W. filifera* (Table 2). All the other palm species remained healthy after inoculation with both isolates of *F. oxysporum* f. sp. *palmarum*. Of note is the fact that *P. roebelenii* was not affected by this pathogen, even though the other four *Phoenix* spp. tested were affected. To be certain, *P. roebelenii* was evaluated a second time and the results were the same (data not shown).

Experiment 2. Previous studies combined with the results of experiment 1 prompted a further examination of *F. oxysporum* f. sp. *palmarum* haplotype susceptibility of *W. filifera*, *B. odorata*, and $\times$ *B. nabonnandi* using the standard inoculation method for the pathogenicity assay (Elliott et al. 2017; Giesbrecht et al. 2013). In both repetitions of the experiment, all three palm species, as well as *W. robusta* and *S. romanzoffiana*, had dry leaf weights that were significantly lower from the controls (Table 3). For all palm species, no matter which fungal isolate was used, either all five replicate palm plants died or the mean dry leaf weight was reduced by 75% or greater (Table 3).

Experiment 3. In this cross-pathogenicity experiment, all *W. robusta* and *S. romanzoffiana* plants inoculated with *F. oxysporum* f. sp. *palmarum* died but none died when inoculated with *F. oxysporum* f. sp. *canariensis* (Table 4). In the first repetition using *F. oxysporum*

f. sp. *palmarum*, all inoculated *P. dactylifera* plants died, and *P. reclinata* dry leaf weight was significantly reduced by isolate PLM-140B. In the second repetition, *P. reclinata* dry leaf weight was significantly reduced by both isolates but only isolate PLM-249A significantly reduced the dry leaf weight of *P. dactylifera*.

In contrast, only the *F. oxysporum* f. sp. *canariensis* isolate PLM-387B, which was originally isolated from *P. reclinata*, significantly affected dry leaf weights of *P. canariensis* (both repetitions), *P. reclinata* (both repetitions), and *P. sylvestris* (only one repetition) (Table 4). The other two isolates had no significant effects on any of the other plant species tested.

Experiment 4. It had been expected that all of the *F. oxysporum* ff. spp. *palmarum* and *canariensis* isolates would be pathogenic on

P. canariensis. However, in experiment 3, this palm species was only affected by one *F. oxysporum* f. sp. *canariensis* isolate. These conflicting results suggested that the standard inoculation method for the pathogenicity assay for *F. oxysporum* f. sp. *palmarum* may not be applicable for *F. oxysporum* f. sp. *canariensis*. Therefore, an experiment was conducted to examine additional inoculation methods for *F. oxysporum* f. sp. *canariensis* on *P. canariensis*. The two new inoculation methods—removal of 20% of the lower roots prior to inoculation and inoculation via a petiole incision—were compared against the standard inoculation method for the *F. oxysporum* f. sp. *palmarum* pathogenicity assay. Cutting the lower 20% of the roots prior to inoculation provided consistent and significant decrease in dry leaf weight compared with the control treatment plants and the

Table 3. Leaf dry weight of palm species inoculated with three haplotypes of *Fusarium oxysporum* f. sp. *palmarum* compared with control (water only) plants of each species in two repetitions of the experiment

Treatment ^b	Leaf dry weight (g) ^a				
	Wr	Wf	Sr	Bo	Bn
Trial 1					
Control	37.7 ± 2.0	47.9 ± 4.0	22.3 ± 2.9	31.5 ± 2.5	20.5 ± 1.9
PLM-140B	0*	0*	0*	6.7* ± 4.1	0*
PLM-246A	0*	0*	3.3* ± 3.3	2.4* ± 2.4	0*
PLM-320B	8.8* ± 8.8	0*	1.1* ± 1.1	6.9* ± 0.7	0*
MSD	16.6	7.2	8.4	9.9	3.5
Trial 2					
Control	54.6 ± 1.8	23.0 ± 3.1	17.8 ± 0.6	29.3 ± 3.1	18.1 ± 1.7
PLM-140B	0*	0*	5.1* ± 3.2	7.7* ± 5.0	2.4* ± 2.4
PLM-246A	0*	1.7* ± 1.7	0*	7.9* ± 3.7	0*
PLM-320B	0*	0*	4.3* ± 2.8	0*	2.6* ± 2.6
MSD	3.3	6.6	7.8	12.7	7.2

^a Mean dry weight of five replicate plants ± standard error. Note: If the standard error is the same as the dry weight mean, this indicates that only one of five replicate plants was still living at the time the experiment was terminated. Plants that had died by the time the experiment was terminated were given a dry weight value of "0". Within each column, a fungal treatment mean value followed by an asterisk (*) indicates that the mean is significantly different ($P = 0.05$) from the control (water only) mean using Dunnett's *t* test. Palm species abbreviations: Wr = *Washingtonia robusta*, Wf = *W. filifera*, Sr = *Syagrus romanoffiana*, Bo = *Butia odorata*, and Bn = *× Butyagrus nabonnandi*.

^b MSD = minimum significant difference.

Table 4. Leaf dry weight of six palm species inoculated with two isolates of *Fusarium oxysporum* f. sp. *palmarum* and three isolates of *F. oxysporum* f. sp. *canariensis* compared with control (water only) plants of each species in two repetitions of the experiment^a

Treatment ^c	Leaf dry weight (g) ^b					
	Wr	Sr	Pc	Pd	Pr	Ps
Trial 1						
Control	26.1 ± 0.8	11.2 ± 2.1	6.9 ± 0.4	11.2 ± 2.1	7.7 ± 0.8	12.6 ± 1.6
<i>F. oxysporum</i> f. sp. <i>palmarum</i>						
PLM-140B	0*	0*	3.4 ± 1.5	0*	3.8 ± 1.0*	7.7 ± 0.7
PLM-249A	0*	0*	4.0 ± 1.3	0*	4.5 ± 1.5	8.2 ± 2.2
<i>F. oxysporum</i> f. sp. <i>canariensis</i>						
PLM-224A	26.1 ± 2.1	11.9 ± 2.3	5.2 ± 1.4	11.9 ± 2.3	5.7 ± 1.1	9.5 ± 1.6
PLM-386B	29.3 ± 2.1	16.2 ± 1.6	6.1 ± 0.6	16.2 ± 1.6	7.0 ± 0.4	10.9 ± 1.0
PLM-387B	23.4 ± 1.5	13.4 ± 3.2	2.6 ± 0.8*	13.4 ± 3.2	1.8 ± 0.8*	5.8 ± 2.2*
MSD	5.34	7.36	4.07	7.36	3.85	6.30
Trial 2						
Control	27.6 ± 1.8	...	10.5 ± 1.5	14.0 ± 0.5	16.7 ± 2.7	9.1 ± 1.0
<i>F. oxysporum</i> f. sp. <i>palmarum</i>						
PLM-140B	0*	...	5.7 ± 1.1	8.0 ± 2.4	4.2 ± 3.4*	8.2 ± 0.6
PLM-249A	0*	...	7.6 ± 1.9	0.6 ± 0.6*	7.8 ± 2.0*	6.4 ± 1.1
<i>F. oxysporum</i> f. sp. <i>canariensis</i>						
PLM-224A	29.7 ± 0.7	...	12.3 ± 0.9	10.4 ± 1.9	18.8 ± 1.9	10.2 ± 2.8
PLM-386B	26.7 ± 2.7	...	10.1 ± 1.7	12.5 ± 2.3	17.8 ± 2.0	8.8 ± 1.2
PLM-387B	22.5 ± 2.9	...	3.8 ± 1.7*	8.5 ± 1.9	2.0 ± 0.6*	7.4 ± 0.8
MSD	7.14	...	5.58	6.75	8.60	5.45

^a Palm roots were not cut prior to inoculation in this experiment.

^b Mean dry weight of five replicate plants ± standard error. Note: If the standard error is the same as the dry weight mean this indicates that only one of five replicate plants was still living at the time the experiment was terminated. Plants that had died by the time the experiment was terminated were given a value of "0". Within each column, a fungal treatment mean value followed by an asterisk (*) indicates the treatment mean is significantly different ($P = 0.05$) from the control (water only) mean using Dunnett's *t* test. Palm species abbreviations: Wr = *Washingtonia robusta*, Sr = *Syagrus romanoffiana*, Pc = *Phoenix canariensis*, Pd = *P. dactylifera*, Pr = *P. reclinata*, and Ps = *P. sylvestris*.

^c MSD = minimum significant difference.

other two methods (Table 5). The petiole incision technique appeared to be useful for only one of the three *F. oxysporum* f. sp. *canariensis* isolates. Thus, cutting the roots prior to pathogen inoculation became the new “standard” inoculation method for the *F. oxysporum* f. sp. *canariensis* pathogenicity assays for the remaining experiments with this pathogen.

Experiment 5. Based on the results of experiment 4, this new standard inoculation method for *F. oxysporum* f. sp. *canariensis* was used for a duplication of experiment 3. The same isolates and the same palm species as used in experiment 3 were used again but, this time, 20% of the lowest roots were cut from all the palm species.

Except for *P. sylvestris*, inoculation with both *F. oxysporum* f. sp. *palmarum* isolates resulted in consistently reduced dry leaf weights compared with their respective control treatments (Table 6). *P. sylvestris* was affected by PLM-249A but only in one repetition of this experiment. The increase in consistent results was observed only for *P. canariensis*.

Table 5. Leaf dry weight (grams) of *Phoenix canariensis* inoculated with three isolates of *Fusarium oxysporum* f. sp. *canariensis* using three different methods of inoculation^a

Treatment ^b	Roots not cut	Roots cut	Petiole incision
Control	6.2 ± 0.5	7.6 ± 0.5	12.5 ± 1.2
PLM-224B	4.7 ± 1.1	1.5 ± 0.9*	9.6 ± 1.3
PLM-386B	4.9 ± 1.0	1.1 ± 1.1*	12.2 ± 0.4
PLM-387B	3.4 ± 1.0	1.1 ± 0.7*	7.1 ± 0.4*
MSD	3.40	3.13	4.01

^a The three methods of inoculation included roots not cut prior to inoculation, roots cut prior to inoculation, and roots not cut but petiole incision made prior to inoculation. See text for more details on inoculation methods. Within each column, a fungal treatment mean value followed by an asterisk (*) indicates the treatment mean is significantly different ($P = 0.05$) from the control (water only) mean using Dunnett's t test. Mean is dry weight of five replicate plants ± standard error.

^b MSD = minimum significant difference.

F. oxysporum f. sp. *canariensis* isolate PLM-386B did not affect any of the plant species tested (Table 6). PLM-224A did significantly affect *P. canariensis* and *P. reclinata*. PLM-387B significantly decreased dry leaf weight of *P. canariensis*, *P. reclinata*, and *P. sylvestris* (Table 6).

Experiment 6. The lack of consistent pathogenicity results with *F. oxysporum* f. sp. *canariensis* but not f. sp. *palmarum* led to experiment 6. We wanted to know if the problem being observed was due to the inoculation technique or to a variation in the isolates of each palm pathogen. Only one primary host of each formae speciales was used in this study, which compared pathogenicity of 13 or 15 isolates of *F. oxysporum* ff. spp. *canariensis* and *palmarum*, respectively.

Using the standard *F. oxysporum* f. sp. *palmarum* pathogenicity assay (no roots cut), all 15 *F. oxysporum* f. sp. *palmarum* isolates evaluated on *W. robusta* resulted in significantly reduced dry leaf weights compared with the control treatment (Table 7). Eight isolates killed all five replicate plants and the other seven isolates reduced dry leaf weight by greater than 50% and included at least two dead replicate plants.

In contrast, using the standard *F. oxysporum* f. sp. *canariensis* pathogenicity assay (20% of roots cut) and *P. canariensis*, only 2 of the 13 *F. oxysporum* f. sp. *canariensis* isolates killed all five replicate plants (Table 7). Another five isolates significantly reduced dry leaf weight compared with the control treatment, two isolates reduced dry leaf weight but not significantly, and four had dry leaf weights nearly the same or greater than the control treatment (Table 7). These results indicated a distinct virulence difference between formae speciales.

Experiment 7. Although death of palm plants is definitive for pathogenicity, this level of disease makes it difficult to evaluate possible chemical or biological control methods. For those types of evaluations, death of 50% or fewer plants would be preferred or, perhaps, no death at all but simply reduced growth. This was the logic behind the experiment using *W. robusta* plants that were preplanted in containers and then adding *F. oxysporum* f. sp. *palmarum* inoculum, either with or without root disturbances. The two-way ANOVA

Table 6. Leaf dry weight of six palm species inoculated with two isolates of *Fusarium oxysporum* f. sp. *palmarum* and three isolates of *F. oxysporum* f. sp. *canariensis* compared with control (water only) plants of each species in two repetitions of the experiment^a

Treatment ^c	Leaf dry weight (g) ^b					
	Wr	Sr	Pc	Pd	Pr	Ps
Trial 1						
Control	30.8 ± 1.1 ^c	12.3 ± 1.4	21.0 ± 2.3	7.1 ± 1.1	7.1 ± 0.9	13.5 ± 1.7
<i>F. oxysporum</i> f. sp. <i>palmarum</i>						
PLM-140B	0*	5.7 ± 3.1*	1.2 ± 1.2*	0.4 ± 0.4*	1.6 ± 0.6*	11.4 ± 2.4
PLM-249A	0*	4.4 ± 2.7*	0*	0.8 ± 0.7*	1.0 ± 0.6*	9.5 ± 1.4
<i>F. oxysporum</i> f. sp. <i>canariensis</i>						
PLM-224A	31.2 ± 1.8	6.7 ± 0.4	0*	8.4 ± 1.2	1.8 ± 0.5*	8.9 ± 2.9
PLM-386B	32.2 ± 0.7	7.9 ± 0.4	14.2 ± 1.1	10.5 ± 1.1	6.1 ± 0.7	15.1 ± 1.8
PLM-387B	22.5 ± 5.8	7.6 ± 2.4	0.5 ± 0.5*	9.7 ± 1.4	1.2 ± 0.6*	2.4 ± 1.8*
MSD	9.76	7.79	4.5	3.90	2.5	7.82
Trial 2						
Control	22.1 ± 1.4	15.1 ± 2.2	7.3 ± 0.6	11.7 ± 1.6	...	10.9 ± 2.0
<i>F. oxysporum</i> f. sp. <i>palmarum</i>						
PLM-140B	0*	0*	0.8 ± 0.5*	4.5 ± 1.2*	...	6.4 ± 0.9
PLM-249A	0*	0*	0*	2.4 ± 0.8*	...	3.5 ± 1.5*
<i>F. oxysporum</i> f. sp. <i>canariensis</i>						
PLM-224A	19.8 ± 0.5	11.9 ± 2.3	0.7 ± 0.5*	5.9 ± 1.6	...	6.0 ± 0.7
PLM-386B	21.8 ± 0.6	16.2 ± 1.6	5.1 ± 1.1	6.0 ± 2.3	...	6.4 ± 0.8
PLM-387B	22.1 ± 1.6	13.4 ± 3.2	0.9 ± 0.7*	7.6 ± 1.9	...	5.2 ± 1.6*
MSD	3.58	7.43	2.51	6.36	...	5.14

^a Palm roots (lower 20%) were cut prior to inoculation in this experiment.

^b Mean dry weight of five replicate plants ± standard error. Note: If the standard error is the same as the dry weight mean this indicates that only one of five replicate plants was still living at the time the experiment was terminated. Plants that had died by the time the experiment was terminated were given the dry weight value of “0”. Within each column, a fungal treatment mean value followed by an asterisk (*) indicates the treatment mean is significantly different ($P = 0.05$) from the control (water only) mean using Dunnett's t test. Palm species abbreviations: Wr = *Washingtonia robusta*, Sr = *Syagrus romanzoffiana*, Pc = *Phoenix canariensis*, Pd = *P. dactylifera*, Pr = *P. reclinata*, and Ps = *P. sylvestris*.

^c MSD = minimum significant difference.

indicated that there was no significant interaction between root disturbance technique and inoculum level ($F = 2.27$; $P = 0.1059$). However, root disturbance technique was significant ($F = 9.24$; $P = 0.0002$) and inoculum level was highly significant ($F = 86.02$; $P < 0.0001$). Addition of the highest level of inoculum (25 ml) without any root disturbance did significantly reduce dry leaf weight compared with the noninoculated plants (Fig. 1). With a minimal amount of root disturbance, the amount of inoculum could be reduced to 5 ml and essentially achieve the same level of reduced dry weight as the 25-ml inoculum without root disturbance. Cutting the roots prior to adding inoculum, no matter what level of inoculum was used, was too severe a technique for the intended purpose.

Discussion

The EPPO has developed standardized protocols for regulated pathogens, including *F. oxysporum* f. sp. *albedinis* which causes Fusarium wilt of *P. dactylifera* (EPPO 2003). The EPPO's protocol for determining pathogenicity starts by growing the fungus on PDA at 25°C for 7 days, preparing an inoculum suspension at 10^6 spores/ml of suspension, and then pipetting the suspension onto the roots of two-leaf-stage seedlings of *P. dactylifera* (the amount per palm is not

indicated). Unfortunately, no such protocol exist for *F. oxysporum* f. sp. *canariensis*, even though this fungus has been documented as a pathogen for almost as long as *F. oxysporum* f. sp. *albedinis* (Mercier and Louvet 1973). There also is no standard protocol for testing pathogenicity of *F. oxysporum* f. sp. *palmarum*, the newest Fusarium wilt pathogen of palm.

It can be argued that there is no need for pathogenicity studies for identification purposes, because *F. oxysporum* isolates can be identified via sequencing of a portion of the translation elongation factor 1- α (Geiser et al. 2004). However, simply isolating a fungus and identifying it as a pathogen does not mean the fungus is pathogenic, nor does it inform us of the pathogen's virulence level. Therefore, pathogenicity studies are still quite useful for these purposes and also for evaluating potential management methods.

Based on the experiments conducted here, the recommended protocol to determine pathogenicity of *F. oxysporum* f. sp. *palmarum* would include the following steps: (i) palm plants should have a minimum of three to four seedling leaves; (ii) palm seedlings are removed from the potting mix or soil they were growing in, and this material is shaken from the roots; (iii) for each pathogen isolate or control (water only) treatment, replicate plants are placed in containers large enough to include all roots and the lower 5 to 10 cm of leaf bases; (iv) 25 ml of a 10^6 spores/ml suspension is then pipetted onto and between the leaf bases of each palm, with excess flowing down onto the roots; (v) the container is covered with plastic, such that the leaf bases and roots are covered but not the leaf blades; (vi) plants remain in the dark or out of direct sunlight at ambient temperature for 3 days; and (vii) plants are then removed from the containers with inoculum and transplanted into containers with appropriate potting mix, fertilized, and irrigated as needed to maintain adequate soil moisture. Palm plants are monitored on a weekly basis for symptom development for a minimum of 3 months. Determining dry leaf weight may be useful for treatment comparison.

For *F. oxysporum* f. sp. *canariensis*, this same protocol would be used, except that, during the second step, approximately 20% of the root system would be cut off with a sharp pruning tool. Also, the experiment should be extended to run for a minimum of 6 months, because pathogenicity of isolates appears to be inconsistent and the pathogen may not be as virulent as *F. oxysporum* f. sp. *palmarum*. Both techniques, including the size of the palm plants used, are similar to those described before for *F. oxysporum* f. sp. *albedinis* (EPPO 2003; Louvet and Toutain 1981) and *F. oxysporum* f. sp. *elaeidis* (Colhoun 1981).

In my experience, most *F. oxysporum* f. sp. *canariensis* isolates are not as virulent as *F. oxysporum* f. sp. *palmarum* isolates. Mature

Table 7. Leaf dry weight and number of dead plants of *Washingtonia robusta* due to inoculation with isolates of *Fusarium oxysporum* f. sp. *palmarum* and of *Phoenix canariensis* due to inoculation with isolates of *F. oxysporum* f. sp. *canariensis*^a

Palm species, isolate ^b	Dry weight (g) ^c	Dead plants (n)
<i>W. robusta</i> × <i>F. oxysporum</i> f. sp. <i>palmarum</i>		
Control	6.3 ± 0.8	0
PLM-119C	0*	5
PLM-140B	1.7 ± 1.7*	4
PLM-153B	0*	5
PLM-249A	1.4 ± 1.4*	4
PLM-320B	0*	5
PLM-510A	0*	5
PLM-560A	0*	5
PLM-596A	1.7 ± 1.7*	4
PLM-619A	1.0 ± 1.0*	4
PLM-676A	0.6 ± 0.6*	4
PLM-729A	0*	5
PLM-741A	1.5 ± 1.0*	3
PLM-791A	0*	5
PLM-853A	0*	5
PLM-920	2.3 ± 1.2*	2
<i>P. canariensis</i> × <i>F. oxysporum</i> f. sp. <i>canariensis</i>		
Control	7.5 ± 0.7	0
PLM-183C	0.9 ± 0.6*	3
PLM-221B	8.3 ± 1.0	0
PLM-224A	4.5 ± 2.0	2
PLM-385B	2.1 ± 1.0*	2
PLM-386B	9.0 ± 0.3	0
PLM-387B	0*	5
PLM-511A	4.0 ± 1.3	1
PLM-588A	2.4 ± 1.4*	3
PLM-605A	7.2 ± 1.4	0
PLM-696A	0.8 ± 0.6*	3
PLM-706A	2.0 ± 1.3*	3
PLM-724A	7.0 ± 0.5	0
PLM-754A	0*	5

^a Mean dry weight of five replicate plants ± standard error. Plants that had died by the time the experiment was terminated were give the dry weight value of "0". Minimum significant difference for *W. robusta* = 3.61. Minimum significant difference for *P. canariensis* = 4.18.

^b Control = water only.

^c Using Dunnett's *t* test, a fungal treatment mean value followed by an asterisk (*) indicates the mean is significantly different ($P = 0.05$) from the control (water only) mean.

Fig. 1. Leaf dry weight (grams) of *Washingtonia robusta* obtained 6 months after inoculation with *Fusarium oxysporum* f. sp. *palmarum*. Three techniques were used for inoculation with either 0, 5, 15, or 25 ml of 10^6 spores/ml of suspension. The techniques were no root disturbance (control), top 625 mm of potting mix with roots disturbed (scrape), or top 625 mm of potting mix with roots disturbed plus three vertical cuts made to the roots internal to the container (cut). Values are mean ± standard error of 15 replicate plants for each technique–inoculum amount treatment.

palm plants infected with *F. oxysporum* f. sp. *palmarum*, even *P. canariensis*, usually die within 2 to 3 months after initial symptoms are observed (Elliott et al. 2010), whereas mature plants of *P. canariensis* infected with *F. oxysporum* f. sp. *canariensis* die at a much slower pace and can take as long as 2 years (Summerell et al. 2001). This difference in virulence between the two formae speciales was demonstrated with the results of experiment 6 (Table 7). In the evaluation of 15 different *F. oxysporum* f. sp. *palmarum* isolates, either all the palm plants were killed or overall dry leaf weight was reduced by greater than 75% within 3 months. I have yet to encounter an isolate of *F. oxysporum* f. sp. *palmarum* that loses pathogenicity over time, even though some of my original isolates (e.g., PLM-140B) have been stored for over 10 years.

This is in contrast with *F. oxysporum* f. sp. *canariensis*. Until I began using the protocol where the roots were cut prior to inoculation, consistent results were elusive. Although all 15 *F. oxysporum* f. sp. *palmarum* isolates evaluated were pathogenic, not all of the 13 *F. oxysporum* f. sp. *canariensis* isolates were, even though these isolates were obtained from Fusarium wilt-symptomatic palm plants and identified as *F. oxysporum* f. sp. *canariensis* using sequencing data. Of the 13 *F. oxysporum* f. sp. *canariensis* isolates evaluated in experiment 6, only 2 killed all five replicate plants and an additional 5 had significantly reduced dry leaf weights (Table 7). In other words, just over half the isolates would be considered pathogenic. Also, palm decline occurred at a much slower pace, requiring that the experiment be run for 6 months, rather than the shorter time (3 months) required for the *F. oxysporum* f. sp. *palmarum* pathogenicity assay. The repeated use of PLM-386B in the experiments also illustrates the issue of loss of pathogenicity over time, because it was pathogenic in one experiment (Table 4) but not in later experiments (Tables 5 and 7). The same is true for PLM-511A, which had been pathogenic in a previous study (Elliott et al. 2011) but was not in the current study (Table 7).

Based on the results presented regarding potentially susceptible hosts for *F. oxysporum* f. sp. *palmarum*, palm plants that have been observed to die in the landscape also die, or their dry leaf weight is significantly reduced, in the pathogenicity assay. These would include *W. robusta*, *S. romanzoffiana*, $\times$ *B. nabonnandi*, and *P. canariensis* (Elliott 2011; Elliott et al. 2010, 2017).

With the intergeneric cross of *S. romanzoffiana* and *Butia odorata* ($\times$ *Butyagrus nabonnandi*), it had been previously demonstrated (Elliott et al. 2017) and this study confirmed that $\times$ *B. nabonnandi* is susceptible to *F. oxysporum* f. sp. *palmarum*. However, Fusarium wilt has not been observed in *B. odorata* (M. L. Elliott, personal observation). This study indicates that it is susceptible to *F. oxysporum* f. sp. *palmarum*, at least as a seedling. Perhaps this palm species has simply escaped *F. oxysporum* f. sp. *palmarum* to date, and dying *B. odorata* should be closely examined for cause of death.

In this study, *W. filifera* was as susceptible to *F. oxysporum* f. sp. *palmarum* as *W. robusta*, confirming previous results (Giesbrecht et al. 2013). Although Fusarium wilt of *W. filifera* has not been observed in Florida, this is most likely due to this palm's scarcity in the Florida landscape. *W. filifera* does not thrive or survive in Florida as it does in California and the southwestern United States. However, the results of this study are of concern for two reasons. First, *W. filifera* is a popular palm outside of Florida. Second, the nursery industry has begun promoting an interspecific cross of *W. robusta* and *W. filifera* (*Washingtonia* $\times$ *filibusta*) for use in the Florida landscape. *W. robusta* becomes too tall in most landscapes and is very fast in its growth. By crossing with *W. filifera*, the goal is to obtain a palm with shorter stature and slower growth. However, based on the results reported herein, this cross will probably be just as susceptible to *F. oxysporum* f. sp. *palmarum* as *W. robusta*. There are no other species of *Washingtonia* (Riffle and Craft 2003).

The *Phoenix* spp. interactions with *F. oxysporum* f. sp. *palmarum* confirm that *P. canariensis* is susceptible but also indicate the possibility that *P. reclinata*, *P. dactylifera*, and, to a lesser extent, *P. sylvestris* could be susceptible. Of note, *F. oxysporum* f. sp. *palmarum* has been isolated from a Fusarium wilt-symptomatic *P. reclinata* (M. L. Elliott, personal observation) but a pathogenicity assay has not yet been completed with that isolate. The most interesting aspect of this study is that

P. dactylifera seedlings were significantly affected by *F. oxysporum* f. sp. *palmarum*, even though this palm species has not been observed to be affected in the landscape, despite the fact *P. dactylifera* is more commonly used in the landscape than *P. canariensis* or *P. reclinata* across the entire state of Florida (M. L. Elliott, personal observation).

In contrast, *F. oxysporum* f. sp. *canariensis* was only pathogenic to *P. canariensis* and *P. reclinata*, as previously confirmed in Florida (Elliott 2015), and never to *P. dactylifera*. Pathogenicity of *F. oxysporum* f. sp. *canariensis* to *P. reclinata* has been evaluated outside the United States by Priest and Letham (1996). In their study, when colonized oat grains were incorporated into the potting mix or pruned roots were immersed in a spore suspension prior to planting in containers, *P. reclinata* plants became stunted and most plants had root decay, compared with the control.

Pathogenicity of *F. oxysporum* f. sp. *canariensis* on *P. dactylifera* has been evaluated numerous times and with different techniques. In France, Mercier and Louvet (1973) used the colonized oat grain technique and determined that their *F. oxysporum* f. sp. *canariensis* isolate was actually more virulent to *P. dactylifera* than to *P. canariensis*. In California, Feather et al. (1989) conducted both crown injections and root injections of *P. dactylifera*, and both methods resulted in plants with significantly less dry weight than the controls; however, no plants died. Priest and Letham (1996) used this crown injection technique in Australia but they observed no disease development in *P. dactylifera*, even after 18 months. Most recently, Elena (2005) observed disease development in *P. dactylifera* with *F. oxysporum* f. sp. *canariensis* when roots were immersed in a spore suspension for 24 h. However, despite all of these experimental results, there was no documentation of *F. oxysporum* f. sp. *canariensis* as a pathogen of *P. dactylifera* on date plantations or landscapes anywhere in the world.

The differing results for *P. dactylifera* in research experiments probably has much to do with the genetics of this palm species. *P. dactylifera* is the only ornamental palm that has cultivars. The numerous cultivars of *P. dactylifera* are maintained by collecting basal offshoots from female plants or, more recently in the Middle East, tissue culture (Chao and Krueger 2007). However, when *P. dactylifera* is grown from seed, as was done in the experiments reported here and by other researchers (Elena 2005; Priest and Letham 1996), no cultivar name can be ascribed to it. Although the *P. dactylifera* plants used in this study were obtained from a nursery in Texas and another in Florida, the source of the seed is unknown.

An alternative explanation for the differing results for *P. dactylifera* could be maturity of the palm. This palm species could be susceptible as an immature palm (not yet fruiting) but not as a mature palm. It is quite possible that this is true for the other palm species evaluated for susceptibility to *F. oxysporum* f. sp. *palmarum*.

Although the initial screening of *P. sylvestris* indicated that it might be susceptible to *F. oxysporum* f. sp. *palmarum*, the subsequent experiments were inconclusive. For *F. oxysporum* f. sp. *canariensis*, only isolate PLM-387B significantly reduced leaf dry weight of *P. sylvestris*, whether the roots were cut prior to inoculation or not. *P. sylvestris* is a new addition to the Florida landscape but only one palm has ever been documented with Fusarium wilt. Isolate PLM-224B was isolated from that palm but pathogenicity has not been demonstrated on *P. sylvestris*, although this isolate did kill or significantly reduce leaf dry weight of *P. canariensis* and *P. reclinata* when the roots were cut prior to inoculation.

Although pathogenicity of *F. oxysporum* f. sp. *palmarum* has proven to be easy to demonstrate with the assay developed, the pathogen's virulence makes it difficult to evaluate any management methods using this standard pathogenicity assay because either all or most of the inoculated, susceptible palm plants die within 3 months. Based on the results of experiment 8, root disturbance is not required if a large enough amount of inoculum is applied. This method should be evaluated further using fungicides or other treatments for prevention or mitigation of Fusarium wilt. For example, materials could be incorporated into the potting mix when the palm seedlings are first transplanted, or materials could be applied to palm leaves or soil surface at discrete intervals prior to or after inoculation, or both.

In summary, two assays have been developed that are relatively easy to conduct and, I advocate, should become the standard for evaluating pathogenicity, and possibly virulence, of the two *Fusarium* wilt pathogens of ornamental palms: *F. oxysporum* f. sp. *palmarum* and f. sp. *canariensis*. By having a standard pathogenicity assay, comparisons can be made among isolates of these pathogens in other states and countries, without exchange of fungal isolates. These assays also appear to be valid for evaluation of isolate virulence and for determining potential susceptibility of other palm species.

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