Determine the Etiological Agent for New Disease Affecting *Syagrus romanzoffiana* (Queen Palm) in Landscapes and Nurseries

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Abstract

The fungus causing a new lethal disease of *Syagrus romanzoffiana* (queen palm) is *Fusarium oxysporum*. Pathogenicity was determined using both seedling and juvenile palms inoculated with *F. oxysporum*, *F. proliferatum* and *F. semitectum* isolates obtained from symptomatic queen palms. Morphological characteristics were used to identify these fungal species. Species confirmation was accomplished by sequencing specific portions of the translation elongation factor 1-alpha gene and comparing sequences obtained with those in the FUSARIUM-ID and NCBI databases. The sequences from *F. oxysporum* did not match any known formae speciales. A similar *F. oxysporum* was subsequently isolated from symptomatic *Washingtonia robusta* (Mexican fan palm) and confirmed to be the same fungus and a pathogen of this palm species.

Objectives and Methods

- **Identify the *Fusarium* species isolated from symptomatic queen palms (and subsequently Mexican fan palms).**

- Isolates

Fusarium isolates were obtained from queen palms exhibiting symptoms of the new lethal disease, primarily from landscape sites across southern Florida but also from a container nursery (Martin County) and a field nursery (Lee County). Late in the study, *Fusarium* was isolated from Mexican fan palms with symptoms similar to those of queen palms. These palms were obtained from landscapes and a field nursery (Lee County). All isolates were single-spored prior to morphological and molecular characterization to ensure that isolates were not mixtures of *Fusarium* species.

- Morphology

Single-spored isolates were transferred to potato dextrose agar (PDA) and water carnation leaf agar and maintained at a constant 26°C with 12 hour light to ascertain colony and spore morphology. Fungal spore morphological characteristics examined and documented included shape, size and cell number of macroconidia, mesoconidia and microconidia (if present), presence and color of sporodochia, presence of microconidia in chains or false heads, type of phialide, and presence and characteristics of chlamydospores.

- Molecular analysis

Single-spored isolates were transferred to potato dextrose broth and grown for 4 days without shaking. Mycelia was collected and used to obtain the DNA template for a PCR assay using a set of nucleotide primers that amplify a specific portion of the translation elongation factor 1-alpha gene (tef-1α). The resulting amplified product was purified and sequenced. Resulting sequences were entered into a BLAST program, which compares your nucleotide sequence

against nucleotide sequences in the database. The databases used were FUSARIUM-ID (Pennsylvania State University) and National Center for Biotechnology Information (NCBI).

- **Determine if a *Fusarium* species is the etiological agent of the new lethal disease affecting queen palms in Florida (and subsequently Mexican fan palms).**

- Experiment 1

Four *F. oxysporum*, three *F. proliferatum* and two *F. semitectum* isolates were used for a pathogenicity experiment using juvenile queen palms. Isolates were grown on PDA and asexual spores harvested after 2 weeks. Inoculum was standardized at ~10^6 spores per ml.

Queen palms used for the experiment had 4-5 leaves, with at least one juvenile leaf, and were growing in 3-gallon containers in full sun. At 8-10 cm above the soil, a 4-mm hole was drilled. Using a syringe, 2 ml of the spore suspension was injected into this hole. On the second youngest fully expanded leaf, a 5-cm slit was cut with a sterile blade and 1 ml of the spore suspension was dribbled into the slit. Control treatments used sterile water rather than a spore suspension. Palms were encased in clear bags and placed in deep shade for 3 days. Bags were then removed and palms placed back into full sun with daily overhead irrigation. There were four replicate plants for each isolate and control treatments.

When palms became symptomatic, petiole tissue was sampled for a pathogen. If *Fusarium* was isolated, the culture was processed and subjected to molecular analysis as described previously.

- Experiment 2

Five isolates, three of *F. oxysporum* (including one from Mexican fan palm), one of *F. proliferatum* and one of *F. semitectum*, were used to produce inoculum as described previously. Seedling queen palms (first three leaves) growing in conetainers (cell-packs) were used for this experiment. Since the same *F. oxysporum* had recently been isolated from Mexican fan palms, seedlings of this palm were included also in this experiment. Conetainers were separated into individual cones, and cones grouped together (five replicate plants per isolate and water control treatment) per treatment per palm species. A 40-ml spore suspension was pipeted onto each plant base and surrounding potting mix. Grouped cones were placed in clear bags for 24 hours. After an additional 24 hours, seedlings were transplanted into 1-liter pots containing a standard potting mix. Plants were placed in a shadehouse and irrigated every day. The palms were not injured prior to inoculation.

When palms became symptomatic, petiole tissue was sampled for a pathogen. If *Fusarium* was isolated, the culture was processed and subjected to molecular analysis as described previously.

Results

- **Identify the *Fusarium* species isolated from symptomatic queen palms (and subsequently Mexican fan palms).**

Approximately 75 *Fusarium* isolates obtained from symptomatic queen palms have been characterized morphologically, with 40 of these isolates also subjected to molecular analysis. Based on morphological characterization, the isolates fell into three major groups:

1) *F. semitectum*: no microconidia; no chlamydospores; mesoconidia and macroconidia present

2) *F. proliferatum*: no chlamydospores; macroconidia are longer than other two groups and fairly straight; microconidia present in both false heads and long chains with only one type of conidial shape (oval with flat base)

3) *F. oxysporum*: chlamydospores present; macroconidia are fat with pointed apical cell; microconidia present in false heads only with range of conidial shapes and sizes

Approximately two-thirds of the isolates have been identified as *Fusarium oxysporum*, with the rest identified as *F. proliferatum* and *F. semitectum*. Morphological identification matched closely with the sequencing results. However, while the *F. oxysporum* isolates did fall within molecular characterization as *F. oxysporum*, they did not match any of the *F. oxysporum* formae speciales in either the FUSARIUM-ID or NCBI databases. Consultations with Fusarium experts at Pennsylvania State University and USDA, using additional information not yet in the FUSARIUM-ID database, confirmed my results.

All *Fusarium* isolates obtained from the Mexican fan palms (ten total) were *F. oxysporum* and matched (morphologically and genetically) the *F. oxysporum* isolates from queen palms.

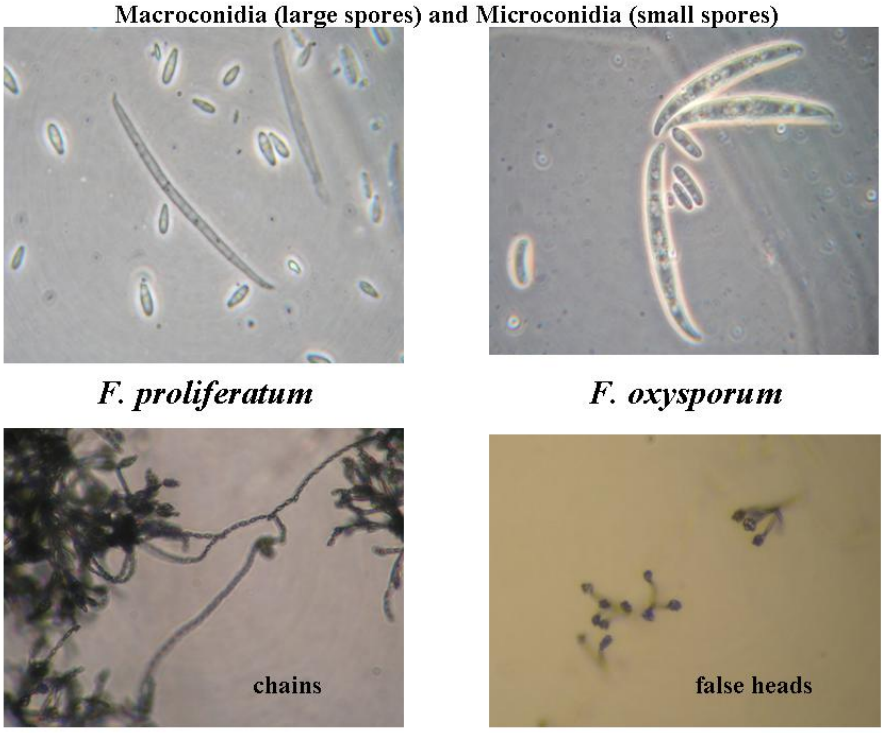

Figure 1. Comparison of macroconidia (larger spores in each top photo) and microconidia (smaller spores in each top photo) of *Fusarium proliferatum* and *Fusarium oxysporum*, and illustration of chains of microconidia associated with *F. proliferatum* only (bottom left) and false heads of microconidia associated with both species (bottom right).

- **Determine if a *Fusarium* species is the etiological agent of the new lethal disease affecting queen palms in Florida (and subsequently Mexican fan palms).**

In Experiment 1, 12 months after inoculation with the *Fusarium* isolates, 21 of 44 palms have died. Palms have died in all treatments, including the control. If a palm died, the fungi subsequently isolated were either *Fusarium oxysporum* only (16 of 21) or a mixture of *Macrophoma*-types (3 of 21), *Colletotrichum* (2 of 21), or *Pestalotiopsis* (1 of 21). In other words, even palms inoculated with other *Fusarium* species or sterile water were most often killed by *F. oxysporum*. Initial symptoms observed were the same as found on mature queen palms – more older leaves had died than would be normal for natural senescence and leaves initially expressed a one-sided death of leaflets. Symptomatic palms usually died within one month of symptom onset. None of the palms died from a bud rot, despite the method of inoculation. Identification of the *Fusarium* isolates was determined using the molecular analysis described.

In Experiment 2, after 4 months, only the seedlings (100%) inoculated with *F. oxysporum* have died, both queen palms and Mexican fan palms. Seedlings inoculated with the other *Fusarium* species or with sterile water remain healthy thus far. Interestingly, the Mexican fan palms died within 4 weeks of inoculation, a full 2 months prior to any symptom development on queen palms. As determined with molecular analysis, only *F. oxysporum* was isolated from infected leaf base tissue. Although roots came in contact with the inoculum, root infection was minimal.

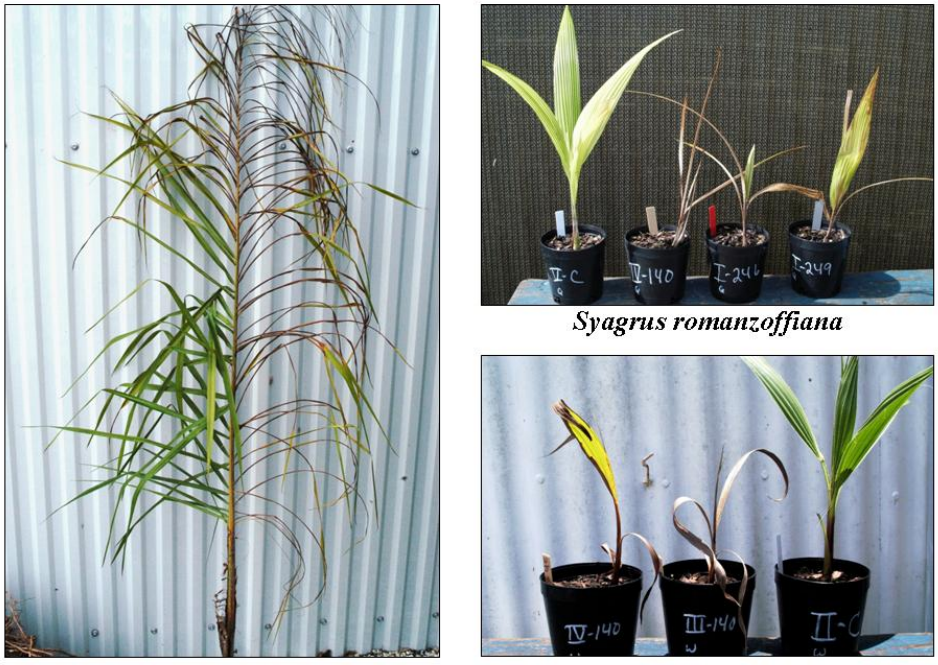

Figure 2. Left photo illustrates typical one-sided leaf necrosis associated with Fusarium decline of queen palms (from Experiment 1). The right photos illustrate susceptibility of both queen palms and Mexican fan palms to *Fusarium oxysporum* (Experiment 2). The larger, healthy green plants are the water control treatments.

Conclusions

Morphological and molecular analysis identified the *Fusarium* species isolated from queen palms as *F. proliferatum*, *F. semitectum* and *F. oxysporum*. The vast majority of the isolates collected from symptomatic queen palms were *F. oxysporum*. The *F. oxysporum* isolates obtained from symptomatic Mexican fan palms were an exact match to those from queen palms. However, the molecular data did not clearly identify the formae speciales of the *F. oxysporum*. Formae speciales are essentially subspecies of *F. oxysporum* based on plant host. In other words, a match was not found in either the FUSARIUM-ID or NCBI databases, or in the databases not yet open to the public. Since these databases contain a world-wide collection of *F. oxysporum*, this would indicate that a unique formae speciales probably exists in Florida.

The pathogenicity studies completed to date strongly indicate that *Fusarium oxysporum* is the pathogen causing the lethal disease observed on queen palms, and now Mexican fan palms. The results from the experiment using juvenile queen palms (Experiment 1) was surprising in that the *F. oxysporum* isolates appeared to be promiscuous. Palms inoculated with the other *Fusarium* species or with sterile water developed the same symptoms as those inoculated with *F. oxysporum*, and *F. oxysporum* was isolated from these palms. All palms in this experiment had been placed under the same overhead irrigation head. This would indicate that the fungus can spread by either air movement or splashing water, and does not necessarily have to be spread with infested pruning tools as is the case with Fusarium wilt of Canary Island date palms. The quick death of the Mexican fan palm seedlings in Experiment 2 was also a surprise. Most *F. oxysporum* formae speciales are relatively host specific. The only commonality between queen palms and Mexican fan palms is that they belong to the same family. Queen palms are pinnate-leaf palms originating from sub-tropical areas of Brazil, and Mexican fan palms are fan-leaf palms originating from desert areas of Mexico.

Recommendations

- This *F. oxysporum* needs to be identified. Truly new pathogens are unusual, so it should be determined if this is a new pathogen and, if not new, determine its origin. This can be accomplished by sequencing the nuclear ribosomal intergenic spacer region of this fungus.
- A faster method of identifying this particular *F. oxysporum* is necessary as all *F. oxysporum* are similar in culture. Development of a PCR assay, as was done for *F. oxysporum* f. sp. *canariensis*, would speed confirmation of this pathogen from host material.
- The surprising development regarding susceptibility of Mexican fan palms to this fungus indicates a need to determine if other palm species are susceptible to this fungus. A seedling assay would provide a first step in this process, beginning with the ten most commonly grown palms in the state.
- *F. oxysporum* is considered a soilborne pathogen. Although the fungus is currently observed causing a lethal disease of foliar origin, it is likely that the fungus can become incorporated into the soil to cause a true vascular wilt. For the field nursery industry, it is critical to know if the fungus can be killed with fumigation. Otherwise, the pathogen could render land blocks unsuitable for growing queen palms and Mexican fan palms, and other palm species if the host range is determined to be quite expansive.