Protection of Sylvester Palms (*Phoenix sylvestris*) from Texas Phoenix Palm Decline using Systemic Trunk Injections of IMA-jet, AzaSol and Arbor-OTC.

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Abstract

Texas Phoenix Palm Decline (TPPD), recently introduced to Florida is a disease lethal to a number of palms including *Phoenix* species. The causal agent, a phytoplasma belonging to the 16SrIV group is spread by an as yet unknown vector. TPPD can kill mature, susceptible palms in as little as 4 months. In this study, we investigated protection of mature Sylvester palms (*Phoenix sylvestris*) in Highlands County, FL in a nursery where TPPD was suspect. Tree protection using systemic trunk injection was studied for a 16 month period. Treatments were randomized and each was replicated six times. At 16 months, 5 of 6 of the untreated checks died, whereas only 1 of 6 of the imidacloprid plus OTC treatments died. None of the other treatments suffered losses. Tissue samples of diseased and dying palms were sent out for nested PCR to UF/IFAS-PDC, Gainesville or qPCR and HRMA to FLREC-Davie for positive identification of the presence of the phytoplasma. Our results demonstrate that the use of systemic insecticides (i.e., imidacloprid and azadirachtin) as well as the use of an antibiotic (i.e., oxytetracycline HCl) (OTC) was effective in protecting Sylvester palms. IMA-jet (5% wt. /wt. imidacloprid, Arborjet Inc. Woburn, MA) was effective applied alone, or in conjunction with OTC; AzaSol (6% wt. /wt. azadirachtin SP) and Arbor-OTC (39.6% wt. /wt. oxytetracycline HCl) were effective applied in combination. Treatments were applied either every 90 or 180 days. Based on the efficacy and residual protection observed in this study, it is our recommendation to extend the retreatment interval from 90 to 120 days to protect Sylvester palms. Further we recommend using both IMA-jet and Arbor-OTC once every 120 days to reduce the incidence of vector transmissions and to maintain plant health by suppressing *Phytoplasma palmae* infection.

Background

Texas Phoenix Palm Decline (TPPD) is caused by *Phytoplasma palmae* (a mollicute similar to bacteria, but lacking a cell wall) that is closely related to Palm Lethal Yellowing (Harrison et al., 2002). TPPD was first described in 1980 in Texas, and first reported in Hillsborough County, FL in 2006. Since 2006, TPPD has spread throughout Florida, mostly around the central part of the state. The specific vector of TPPD is unknown. TPPD is most likely vectored by piercing-sucking insects (Order Hemiptera) that are known to spread phytoplasmas to plants. Phytoplasmas infect the phloem, the vascular tissue that moves carbohydrates from leaves to the roots. As its name implies, TPPD infects *Phoenix* species. In Texas, the disease was reported to kill *Phoenix canariensis* (Canary Island date palm) and *P. dactylifera* (date palm) in as little as 4 months (McCoy et al., 1980). Currently there are 10 confirmed hosts of TPPD. In Florida, host range includes *Phoenix sylvestris* (Sylvester palm)*, P. canariensis, P. dactylifera, P. roebelinii* (pygmy date palm)*, Sabal palmetto* (sabal palm)*, Syagrus romanzoffiana* (queen palm)*, Adonidia merrillii* (Christmas palm)*, Bismarckia nobilis* (Bismark palm)*, Livistona chinensis* (Chinese fan palm)*, and Carpentaria acuminata* (Carpentaria palm) (Bahder, 2017). Symptoms include fruit drop, necrosis of inflorescence, bronzing of lower foliage which progresses upward, subsequent spear leaf collapse and

death. University of FL/IFAS Extension recommends systemic treatment with oxytetracycline hydrochloride on a 4-month schedule (therapeutically and /or preventatively) for Lethal Yellowing of Palm (LY), also incited by *Phytoplasma palmae* (Harrison and Elliott, 2015). While the two phytoplasmas are related the causal organism of LY belongs to subgroup 16SrIV-A, whereas the causal agent for TPPD belongs to subgroup 16SrIV-D. Both subgroups of phytoplasma infect *Adonidia merrillii, P. canariensis, P. dactylifera, P. sylvestris,* and *Livistona chinensis* (Bahder, 2017). In this study, in a nursery plot with positive occurrences of TPPD in Highlands County, FL, we selected mature Sylvester palms (*Phoenix sylvestris*) to evaluate the efficacy of palm injections to (a) limit vector transmission, (b) to suppress disease development, or (c) both.

Methods

On May 18, 2016, the following treatments were established: A. Arbor-OTC (39.6% wt. /wt. oxytetracycline hydrochloride, SP Arborjet Inc. Woburn, MA), B. IMA-jet (5% wt. /wt. imidacloprid, SL Arborjet Inc.), C. Arbor-OTC plus IMA-jet, D. Arbor-OTC, IMA-jet, Palm-jet Mg (1-2-2 plus magnesium, Arborjet Inc.) and PHOSPHO-jet (45.8% wt. /wt. Mono- and di-potassium salts of phosphorous acid, Arborjet Inc.) (OIPP Program), E. Arbor-OTC, AzaSol (6% wt. /wt. azadirachtin SP, Arborjet Inc.), Palm-jet Mg and PHOSPHO-jet (OAPP Program), and F. untreated checks. Mature Sylvester palms were selected for study and each treatment was randomized within the plot and replicated 6 times. Arbor-OTC and AzaSol were administered by trunk injection once every 90 days, whereas all the other injection treatments were administered once every 180 days. Arbor-OTC was applied at the rate of 2.8 g in 25 mls distilled water per palm. IMA-jet was applied at 15 mls neat (equivalent to 0.75 g active) per palm. To prepare the AzaSol solution, a 21.25 g packet was dissolved in 104 milliliters of de-ionized water; 21 milliliters was applied to each of 6 palms (equivalent to 0.21 g active) receiving Treatment E. The entire solution was used after mixing. Palm-jet Mg and PHOSPHO-jet were applied at 10 mls (the latter, equivalent to 4.58 g active) per palm. Trunk injections were made using the QUIK-jet Air (Arborjet Inc., Woburn, MA) and Arborplug™. A single injection site was used to make the applications. A 9 mm drill hole was made into the trunk 10 cm deep into un-lignified tissue. A No. 4 Arborplug was inserted into the trunk tissues using a set tool and mallet. The Arborplug is configured with an internal septum which when pierced by the injector needle prevents liquid backflow, and introduces the injected liquid into the conducting vascular (xylem) tissues. Evaluations of palm health (percent canopy decline) and percent mortality were conducted every 90 days from study initiation through September 28, 2017 (16 months). Vascular samples were taken from the trunks of suspect palms using a cordless drill when palms showed symptoms consistent with TPPD. Drill bits were cleaned and sanitized with isopropyl alcohol between palms. The drilled sawdust tissues was placed in clean sealable plastic bags, and were sent to University of Florida – Plant Diagnostic Center, Gainesville for nested PCR or to UFL, Davie for qPCR with HRMA (High Resolution Melt Analysis) for identification of the presence of *Phytoplasma palmae* (Bahder et al., 2017). Statistical analyses were conducted using Mini-tab version 17, State College, PA. One way ANOVA and T-tests were conducted and statistical significance was accepted at 95% CI and at $p = 0.05$.

Results

Trunk tissue samples were taken on four occasions, from August 30, 2016 to October 02, 2017 from the nursery plot on which we established our study. Three of the four samples sent were consistent with TPPD infection, these tested positive for *Phytoplasma palmae* (Table 1). Nested PCR conducted by PDC-Gainesville did not distinguish between phytoplasmas belonging to subgroups 16SrIV-A and 16SrIV-D, Lethal Yellows and TPPD, respectively. The most recent sample was sent to UF-Davie for qPCR with HRMA. The HRMA procedure is used to distinguish between the subgroups. Samples (No. 81415 and 25745) occurred within the nursery block among the study trees. No. 81415 had 50% canopy decline symptoms on August 30, 2016, but tested negative for *P. palmae*. Similarly, No. 25745 had canopy symptoms consistent with lower leaf bronzing and did test positive for *P. palmae*. On the same date, No. 81429 an untreated check died and tested positive for *P. palmae.* No. 81401 was sampled when canopy decline symptoms increased from 20 to 33% (from 07/24/17 to 09/28/17) and likewise tested positive. The earliest positive detection of *P. palmae* occurred approximately 6 ½ months into the study.

Table 1 Date drilled trunk samples were taken from symptomatic Sylvester palms in the study plot, their ID No. and PP test results.

Date	Tag No.	Result
October 02, 2017	81401	Positive for *P. palmae*
December 07, 2016	81429	Positive for *P. palmae*
December 07, 2016	25745	Positive for *P. palmae*
August 30, 2016	81415	Negative for *P. palmae*

As of September 28, 2017, 16 months following the initiation of this study 83.3 ± 17 SE of the untreated checks died compared to a single Arbor-OTC and IMA-jet treatment (16.7 ± 17 SE mortality); the other treatments of IMA-jet alone, OTC alone, the OIPP and OAPP program treatments suffered 0% mortality (Figure 1). Two sample T-test comparing means of the untreated check mortality and the OTC/IMA was statistically significant at $p = 0.018$.

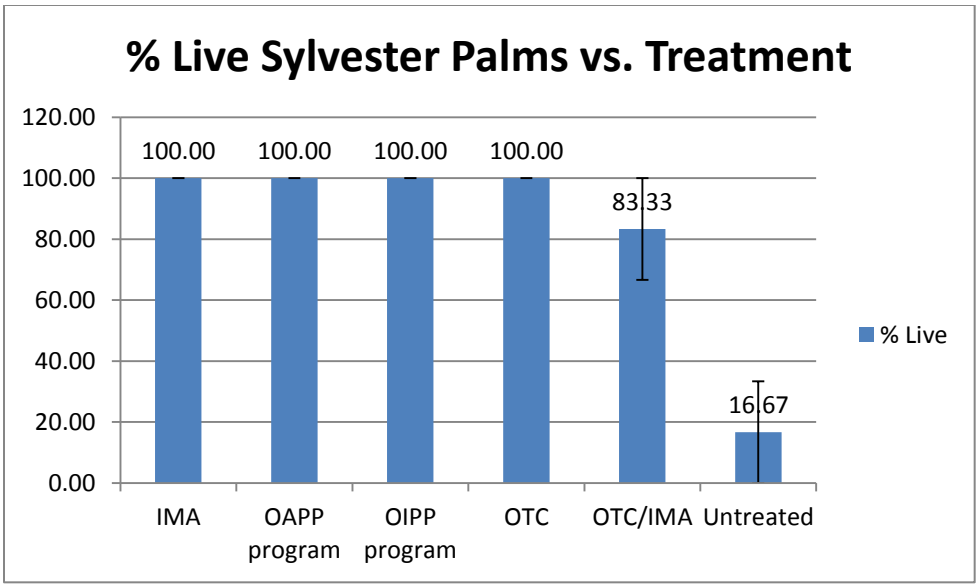

Figure 1. Percent live Sylvester palms among treatments after 16 months, where injections of IMA-jet, Arbor-OTC, AzaSol and Arbor-OTC protected palms when applied at program intervals, compared to only 16.7% survival in the untreated palms.

Canopy decline symptoms were recorded at three periods over the 16 month study, which were on May 18, 2016, July 24, 2017 and September 28, 2017, and presented in Table 2. Change in canopy condition (percent decline) is presented in Table 3. From May 18, 2016 to July 24, 2017, we observed 50.6 ± 9.7 SE in decline in the untreated checks which was statistically different from the treated palms which ranged from -11.9 ± 5.4 to 11.6 ± 13.6 SE. From May 18, 2016 to September 28, 2017, we observed 49.5 ± 10.6 SE decline in the untreated checks which was statistically different from the treated palms which ranged from 2.69 ± 3.8 to 18.8 ± 11.8 SE for the treated palms. Canopy condition deteriorated from July 24 to September 28, 2017 evaluations primarily due to the effects of Hurricane Irma, which occurred on September 8 of the same year. Figure 2a – f present images of the trees and canopy condition typical of the treatments at 16 months.

Table 2 Percent canopy decline (arcsine transformed data) on May 18, 2016, July 24, 2017 and September 28, 2017 by treatment

Date	Treatment	N	Mean*	±SE Mean
May 18, 2016	IMA-jet	6	15.90a	3.51
	OAPP	6	21.87a	1.64
	OIPP	6	17.88a	4.16
	OTC	6	22.46a	4.92
	OTC/IMA	6	16.40a	1.69
	Untreated	6	29.94a	6.95
July 24, 2017	IMA-jet	6	18.61a	4.24
	OAPP	6	15.17a	3.29
	OIPP	6	15.80a	3.63
	OTC	6	10.57a	4.88
	OTC/IMA	6	28.0a	12.8
	Untreated	6	80.54b	9.47
September 28, 2017	IMA-jet	6	25.73a	1.82
	OAPP	6	25.15a	1.64
	OIPP	6	29.88a	5.26
	OTC	6	25.15a	1.64
	OTC/IMA	6	35.2a	11.0
	Untreated	6	79.4b	10.6

*treatments within a column with the same letter are not significantly different at $\alpha = 0.05$

Table 3 Changes in canopy condition (arcsine transformed) from May 18, 2016 to July 24, 2017 and from May 18, 2016 to September 28, 2017. Negative (-) change indicates an improvement in canopy condition.

Time Period	Treatment	N	Mean*	±SE Mean
05/18/16 to 07/24/17	IMA-jet	6	2.71a	2.62
	OAPP	6	-6.70a	3.11
	OIPP	6	-2.08a	2.37
	OTC	6	-11.89a	5.38
	OTC/IMA	6	11.6a	13.6
	Untreated	6	50.60b	9.71
05/18/16 to 09/28/17	IMA-jet	6	9.83a	4.44
	OAPP	6	3.28a	1.63
	OIPP	6	12.0a	4.13
	OTC	6	2.69a	3.75
	OTC/IMA	6	18.8a	11.8
	Untreated	6	49.5b	10.6

*treatments within a column with the same letter are not significantly different at $\alpha = 0.05$

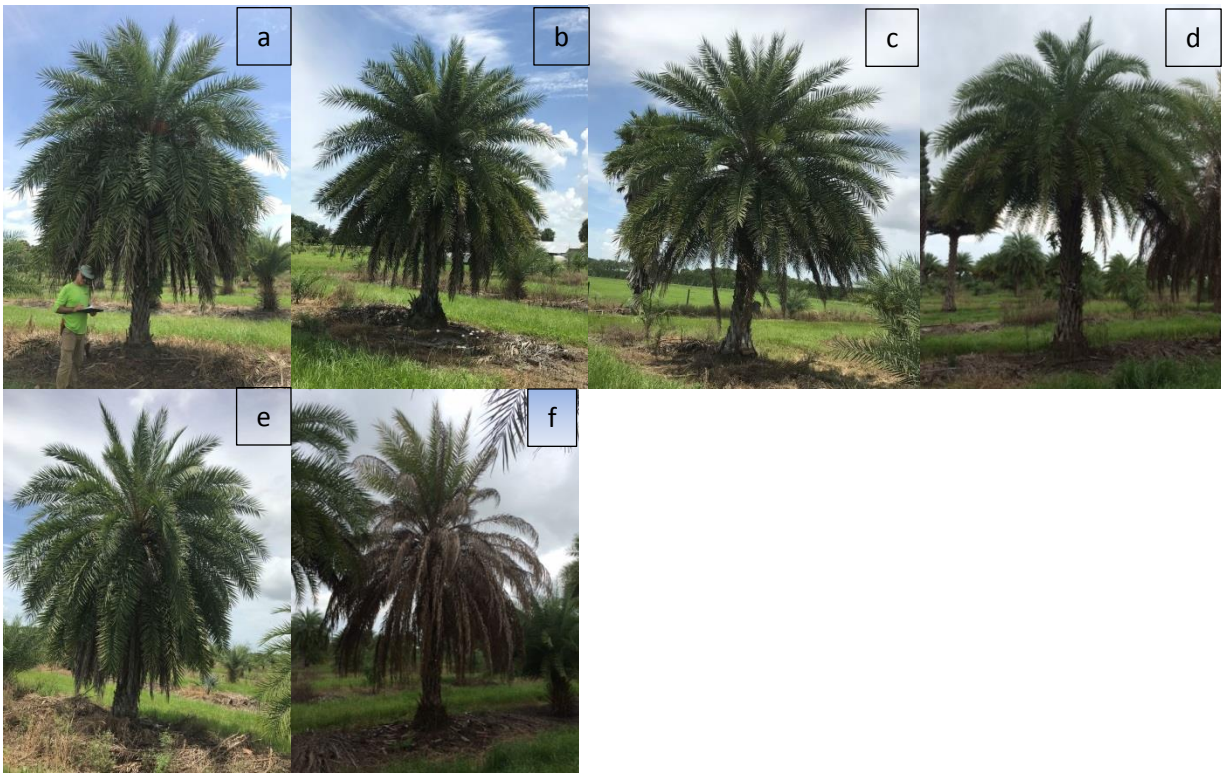

Figure 2a – f. Sylvester palms 16 months after treatment from left to right, (a) Arbor- OTC, (b) IMA-jet, (c) Arbor-OTC & IMA-jet, (d) OIPP program, (e) OAPP program and (f) untreated check. The OIPP program applied PHOSPHO-jet and Palm-jet Mg in addition to IMA-jet and Arbor-OTC. The OAPP program applied PHOSPHO-jet and Palm-jet Mg in addition to AzaSol and Arbor-OTC. Note bronzing of canopy in the untreated check, symptomatic of TPPD.

Conclusions and Discussion

Early diagnostics from UF/IFAS-PDC, Gainesville using nested PCR confirmed the presence of *P. palmae* in infected samples consistent with TPPD or LY. FLREC/IFAS-Davie confirmed TPPD (*P. palmae* subgroup 16SrIV-D) in infected samples from symptomatic Sylvester palms using qPCR and HRMA. This distinction has import from the standpoint of identification of a previously naturalized phytoplasma (LY) or a new invasive (TPPD). Based on these results, we are confident that the phytoplasma in the Sylvester block was TPPD. Moreover, these data are consistent with the known distribution of TPPD within the state (inclusive of Highlands County). IMA-jet (imidacloprid) alone or with Arbor-OTC (oxytetracycline HCl), OTC alone or with AzaSol were each successful in protecting palms by trunk injection. AzaSol and /or Arbor-OTC were applied every 90 days. IMA-jet, on the other hand was applied once every 180 days. Trunk injection of imidacloprid has the added benefit of extending the retreatment interval to once every 180 days at the rates used in this study. Based on the efficacy and residual protection observed in this study, it is our recommendation to extend the retreatment interval from 90 to 120 days to protect Sylvester palms. Further we recommend using both IMA-jet and Arbor-OTC once every 120 days to reduce the incidence of vector transmissions and to maintain plant health by suppressing *Phytoplasma palmae* infection.

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