STRATEGIES FOR MANAGING COTTON ROOT ROT (CRR) DISEASE IN TEXAS WINE GRAPES

A Thesis

by

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MASTER OF SCIENCE

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ABSTRACT

Among the challenges facing the Texas winegrape industry is *Phymatotrichopsis omnivora,* the cotton root rot (CRR) pathogen. A survey instrument was created and distributed to grape growers which revealed that the disease is a serious problem in counties where acreage of new vineyards is on the increase. Grape growers expressed significant concern because there were no effective recommendations for disease control.

One control option for growers is the use of promising rootstock x scion combinations. In 2012, a root stock field trial was planted with two own-rooted grape varieties (Chardonnay, Merlot) and the same two varieties on Dog Ridge root stock. Both varieties on the root stock showed a significant statistical difference in disease development when compared to own-rooted vines. Merlot own-rooted vines were more resistant than the Chardonnay own-rooted vines.

Two experimental fungicide field trials were initiated in vineyards in the spring of 2012. In one vineyard, several fungicides were tested including flutriafol, a fungicide being used successfully to control CRR on cotton. In the commercial vineyard in Travis County, TX, flutriafol was applied at 0.26 lbs/a.i./acre (1X), 2.6 lbs/a.i./acre (10X), and 5.2 lbs/a.i./acre (20X) through a manually applied soil drench. There was a significantly lower level of disease development in the 10X plots, as compared to untreated controls. Vines treated with a 10X rate of flutriafol also showed decreased incidence of non-target foliar pathogens and possible plant health effects when compared to the other treatments.

In 2014, two new field trials were established using chemigation at an established vineyard and a newly planted vineyard. The experimental block at the newly planted vineyard consisted of 30 rows of Petite Syrah on 5BB rootstock containing 118 vines/row. Two rates of flutriafol at 0.26 lbs/a.i./acre (1X) and 0.52 lbs/a.i./acre (2X) were applied through the irrigation system with 10 plots (rows)/treatment. In the untreated plots, disease increased from 0-5.5% over a 12 week period following treatments. No disease was observed until week 11 in any of the treated plots. However, by week 12, disease incidence had increased to 2.5% and 1.4% in the 1X and 2X plots, respectively. Based on these and similar studies, a Section 24(c) special local needs registration was requested and granted for use by the grape growers to control *Phymatotrichopsis omnivora*.

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Finally, thanks to my mother, Betty Mollohan, who continued to call me on Sundays when I needed reinforcement. Also, to my son, Manuel McBride, who said "you can do it Mamma" and the rest of my wonderful family for their encouragement, patience and love. I don't think I could have made it all the way through without their support and confidence.

NOMENCLATURE

CITI	Collaborative Institutional Training Initiative
CRR	Cotton Root Rot
DNA	Deoxyribonucleic Acid
EPA	Environmental Protection Agency
FRAC	Fungicide Resistance Action Committee
IRB	Internal Review Board
lb/a.i./ac	Pounds per active ingredient per acre
ul	Microliter
ml	Milliliters
mm	Millimeters
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PGR	Plant Growth Regulator
P.o,	*Phymatotrichopsis omnivora*
ppm	Parts per million
spp.	Species
TDA	Texas Department of Agriculture
TLC	Thin Layer Chromatography
TPDDL	Texas Plant Disease Diagnostic Laboratory
TX	Texas

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1. INTRODUCTION

Texas is currently the 5th largest wine producing state in the nation. In 2013, there were 286 wineries in Texas with an economic impact of 1.8 billion dollars (2). As grapevine acreage has increased to supply the demand for wine production, there is a growing concern for the widespread and destructive presence of the cotton root rot (CRR) pathogen, *Phymatotrichopsis omnivora* (Duggar) Hennebert, (syn. *Phymatotrichum omnivorum* Duggar) (7).

Phymatotrichopsis omnivora was first described in 1880 on cotton (19, 26, 33). The pathogen is a soil-borne fungus which occurs in the high pH, calcareous soils of southwestern U.S. and northern Mexico (17, 27). Pathogen survival can last for several decades because the fungus produces sclerotia with the ability to lie dormant until encountered by the root system of a susceptible plant (1, 10, 16, 19, 34). When present, CRR causes significant economic losses on many specialty crops, such as, peanuts, pecans, peaches, apples and winegrapes (28, 34). *P. omnivora* colonizes the grapevine root system and spreads to adjacent vines through the soil, and under the right environmental conditions can lead to death of the vine. Heavy losses have occurred in grapevines at high risk sites with own-rooted *Vitis vinifera* varieties and vines grafted on susceptible rootstocks (22). There are some promising control measures with the potential to offer relief to growers, but there must be further research before recommendations can be made.

CRR has been extensively studied on other crops such as cotton, but research with grape is extremely limited. One of the possible control measures is the use of rootstocks previously thought to have resistance/tolerance to the pathogen (3, 4, 21, 22, 23). As early as 1924, J. J. Bayles (4) in his grape trials in Balmorhea, TX showed Champanel, Black Spanish, Mustang and St. George was resistant to CRR. In 1934, W. J. Bach's (3) grape trials in Weslaco, TX also had shown Champanel, Black Spanish, and Mustang, to be tolerant as well as Dogridge (*V. champini*, a central Texas natural hybrid between *V. mustangensis x V. rupestris)*, and *V. solanis*. Research was conducted by Ernest Mortensen in 1931 (22), in which 46 grape varieties were planted in Winter Haven, TX for an adaptation test. The climate was favorable for grape production; however CRR was the limiting factor. He observed most of the Texas native varieties such as Dog Ridge, Champanel, and Lukfata were survivors of CRR. He further observed that varieties of *V. champini, V. candicans, V. monticola and V. berlandieri* origin usually have good survival. He also concluded, *V. vinifera* varieties on their own roots are highly susceptible to CRR, and will have to be grafted on resistant rootstock. Rootstocks in vineyard production are an important variable for growers because they impart many influences on the vigor of a grapevine (11). Therefore, there are many choices available to growers to match rootstocks to the site, growing conditions and the variety of grape being considered. A greenhouse rootstock-screening procedure was developed to artificially inoculate containerized grapevines with the pathogen and monitor for root growth and symptom development (11, 18).

Another potential management strategy for CRR is the application of appropriate fungicides. *Phymatotrichopsis omnivora* is sensitive to triazole fungicides in the laboratory and field (13, 14, 31, 36). The use of propiconazole for grape CRR control was previously suggested based on successful studies in greenhouse tests, but no vineyard field trials have been attempted (8). Recent field research has shown when the fungicide flutriafol was applied through "t-band" drench applications in cotton, the impact of the disease was reduced (14). Due to trellises, drip irrigation, and other infrastructure issues, the equipment involved for "t-band" applications in a vineyard setting would not be appropriate. A method for the necessary soil application could be adapted to test flutriafol in grapevines.

Due to the enhanced systemic movement of triazole fungicides (31, 36), a concern for the wine industry would be whether or not the fungicide is present in the fruit at harvest. The fermentation process, taste, and quality of the wine are high priorities for the wine makers (24). Another concern with the use of triazole fungicides is potential phytotoxicity, as has been observed in cotton (8, 14). If the application of flutriafol to grapevines proves to be efficacious against *P. omnivora*, sufficient testing will be needed to register the fungicide for use on grapevines in TX. One of the criteria in obtaining final approval through the Environmental Protection Agency (EPA) will be to test the grapes growing on treated grapevines for fungicide residuals (see website Exemption from Registrations http://www.epa.gov/agriculture/lfra.html#Emergency).

The purpose of this study is to address the critical deficiencies in the understanding of CRR on grapevines by approaching the problem with the following three objectives. Objective one was to assess the impact of CRR in the winegrape industry in Texas. Objective two was to test the efficacy of potential fungicides and rootstock selections for control of CRR. Objective three was to find a cost effective way to determine if there is any detectable fungicide residual in the grape.

2. MATERIAL AND METHODS

2.1 Objective one

The first objective was to determine the impact of cotton root rot (CRR) in Texas vineyards to improve recommendations for management and inform growers of the disease risk. With final approval from the Texas A&M University Internal Review Board (IRB) (https://vpr.tamu.edu/compliance/rcc/irb) and required Collaborative Institutional Training Initiative (CITI) training (https://www.citiprogram.org/), a survey instrument was created and distributed to the Texas Wine and Grape Grower Association annual grape camp meeting. The survey included questions pertaining to acreage, variety (rootstock/scion), county, soil pH, and whether or not they have suspected or had confirmation of CRR in their vineyards (Appendix A). A history of grapevine samples submitted to the Texas Plant Disease Diagnostic Lab and confirmed positive for CRR was also included in the assessment. The data were collected and a county map of Texas was generated indicating the counties with confirmed CRR.

2.2 Objective two

Laboratory: This component of Objective 2 compares the efficacy of two triazole fungicides, propiconazole (Alamo ®) and flutriafol (Topguard ®), to inhibit growth of *P. omnivora in vitro* with poison plate assays. At Flat Creek Estates Vineyard a potentially positive vine showing the classic symptoms of rapid wilting and chlorotic leaves was excavated to obtain a pure culture of *P. omnivora*. The roots were microscopically inspected for the presence of typical *P. omnivora* mycelial strands (Fig. 1A). Fine tipped forceps were used to tease several sections of mycelial strands from the infected roots. The sections were further dissected into smaller sections and placed in a sterile petri dish with 15 ml sterile distilled water. The petri dishes with the segments of mycelia were periodically observed microscopically for hyphal growth in the sterile water (Fig. 1B). Difco ®Potato Dextrose Agar (PDA) plates were prepared according to manufacturer's directions. Hyphal tips were transferred to PDA and incubated at 27^{o}C. PDA plates were observed for the typical growth of *P. omnivora* (Fig. 1C). DNA extraction of mycelia from the pure culture was performed using the Qiagen ® DNeasy Blood & Tissue kit. Conventional PCR was performed according to Go Taq Colorless ® directions using Internal Transcribed Spacers (ITS1 and ITS4) primers. PCR parameters were set at 95^{o}C for 3 minutes, [95^{o}Cfor 30 seconds, 55^{o}Cfor 1 minute, 72^{o}C for 1 minute] for 40 cycles, 72^{o}C for 10 minutes, 4^{o}C until ready to load reactions into an

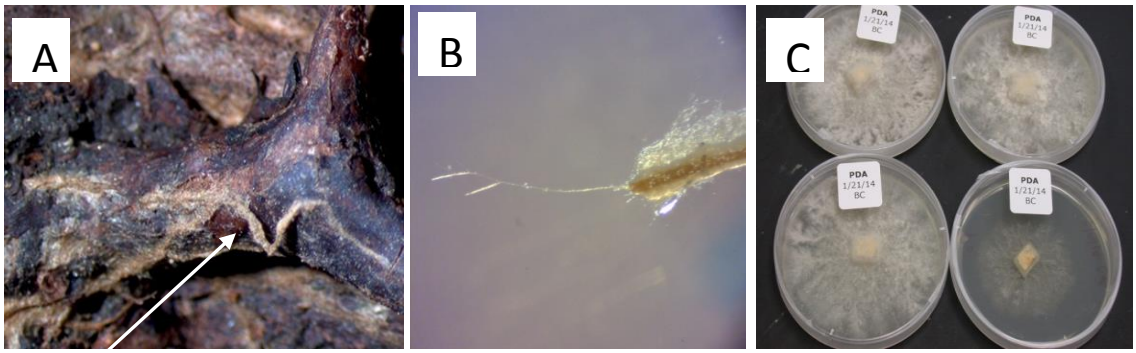

Fig 1. Characteristic signs of *P. omnivora*. A. Hyphal strands infecting grapevine root. B. Microscopic view of a mycelial filament growing from a strand. C. Culture plates of *P. omnivora*.

agarose gel PCR reactions were electrophoresed on a 1% agarose gel with Tris-Borate-EDTA (TBE) buffer. The identification of the isolate was confirmed by sequencing the approximate 700 bp PCR amplicon at the Texas A&M University Gene Technology Lab (http://www.idmb.tamu.edu/gtl/) and using a Basic Local Alignment Search Tool (BLAST) search to confirm homology (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Pure cultures were maintained on PDA for use in the poison plate assays and greenhouse mini-rhizotron experiments (described in next section). PDA was amended with the following concentrations of the two fungicides (0.1ppm, 0.05ppm, 0.02ppm, 0.01ppm, and 0.004ppm) and poured into sterile petri plates. Un-amended PDA (control) plates served as controls. A sterile cork borer (No. 2) was used to extract a mycelial plug of *P. omnivora* and then the plug was transferred to the center of the PDA plates. Every 1-2 days for 10 days the diameter was measured for growth inhibition. The percentage growth reduction was plotted against the log of the fungicide concentrations (29). The EC_{50} was calculated through the use of a web-based, Microsoft Excel routine ED50plus v1.0 (see website http://www.sciencegateway.org/protocols/cellbio/drug/hcic50.htm).

Greenhouse experiments: A mini-rhizotron prototype screening method was developed to assess the interaction of different grapevine rootstocks to *P. omnivora*. A growth box was constructed from 0.118" clear acrylic plexiglass and ¼" plywood cut into 12" wide by 24" sections. Clear tubing (¾" PVC) was used as a spacer between the plexiglass and plywood to contain the soil and grape roots while directly observing the growing root system. All three components were sandwiched together with washers,

nuts and bolts (Fig. 2A). Merlot cuttings were propagated according to standard horticultural practices for grape cultivation (21). Field soil from Flat Creek Vineyards was collected and sieved. The soil was moistened with distilled water, autoclaved for one hour and placed at room temperature for 24 hours. The soil was then autoclaved again for an additional hour and allowed to cool. Ten mini-rhizotrons were filled with the sterile soil, 10 filled with non-sterile soil, and then all were planted with the merlot rooted cuttings. When the rooted cuttings were established in the mini-rhizotrons, they were artificially inoculated with *P. omnivora* by removing the plexi-glass and placing a plug of mycelium from a PDA culture plate. The roots were observed for root growth and colonization of the fungus from April 2014 until October 2014.

Field experiments: With the information gathered from the grower survey, two grape grower cooperators with histories of CRR in high risk regions of Texas were identified.

The first site was planted in an established commercial vineyard (Flat Creek Estates, Marble Falls, TX) with a fifteen year history of CRR (Fig. 3). The existing mature, dead and declining vines were rated for symptoms of CRR and rogued to establish the experimental block in an area known for the presence of CRR. Sangiovese on SO4 rootstock vines were purchased in 2011 (Vintage Nursery, CA) and potted in 6" plastic horticulture pots with Metro-Mix 360. Vines were grown and maintained in the greenhouse during the months of June through March 2012. In the spring of 2012, the

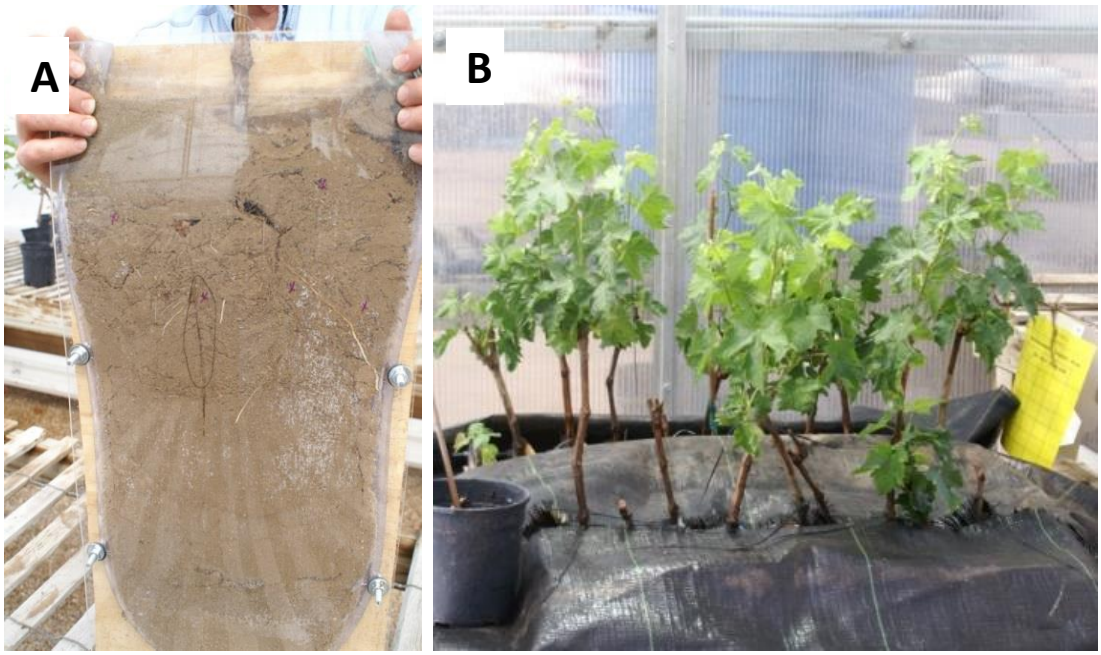

Fig 2. The mini-rhizotrons used to grow grapevines (Merlot) for visual observation of root growth. A. Root chamber with a plexi-glass front for observation of root growth. B. Covered, stacked rhizotrons with vine growth emerging from tops of the box.

Fig. 3. Vineyard and winery view of Flat Creek Estates in Travis County, TX, designated as Site Number One.

vines were planted on 8’ spacing including border vines planted between each treatment to prevent confounding treatment effects by runoff of fungicide among plots. The experimental field design was a randomized complete block with 4 treatments, 5 vines/treatment and 5 replications (Fig. 4). The four treatments included three different rates of flutriafol and untreated controls: 1X=28 fluid ounces per acre, 10X=280 fluid ounces per acre, 20X=560 fluid ounces per acre. The three different fungicide concentrations were applied via a slow-drip drench method using 5 gallon buckets with six 1/32” holes drilled in the bottoms. Furrows were made under the drip-line next to the vines with two buckets placed on either side of the vine. Half a gallon of fungicide was poured into each bucket and allowed to drain and drench the soil (Fig. 5). Immediately after treatment, the drip irrigation was turned on to help the fungicide penetrate to the surrounding root zone. No disease ratings were collected in 2012. In 2013, 2014, and 2015 vines were treated again as described above. In 2013 and 2014, disease ratings of 0-5, where 0 = dead, 1 = mostly necrotic foliage, 2 = more than half chlorotic/necrotic, wilt, 3 = less than half chlorotic, mild wilting, 4 = minimal chlorosis, 5 = healthy, were collected at the end of each growing season. Data were subjected to a Kruskal-Wallis analysis using SAS Proc NPAR1WAY, SAS version 9.4 to determine if there was statistical difference among treatments in disease ratings. Pruning weights were taken in the winters of 2014 and 2015 prior to treatment. Data were subjected to SAS Proc GLM, SAS version 9.4 to determine if there was a treatment effect of the fungicide on growth of canes. In 2015, plant health ratings were collected. Health ratings of 0-5, where 0 = dead, 1 = mostly defoliated, 2 = more than half defoliated, wilt, 3 = minimal

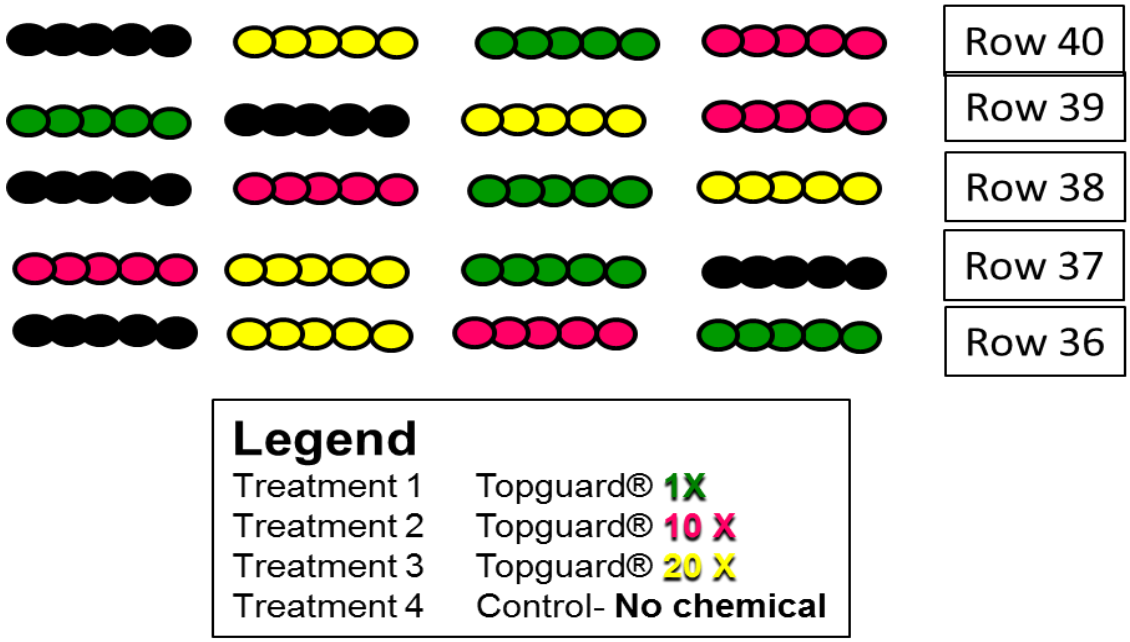

Fig. 4. A map depicting the treatment plots at Site Number One (Flat Creek Estates, TX).

Fig. 5. Application of flutriafol using a slow-drip drench method with modified, 5 gal. buckets. A. Close up of buckets with six 1/32 in. holes in bottoms. B. Filling the buckets with 0.5 gal. of fungicide solution.

defoliation and chlorosis, 4 = minimal chlorosis and leaf spots, 5 = no foliar symptoms were used as the basis for quantifying vine health. Data were again subjected to a Kruskal-Wallis analysis using SAS Proc NPAR1WAY, SAS version 9.4 to determine if there was statistical difference between treatments.

Site number two was a newly established experimental vineyard in a high risk area for the presence of *P. omnivora* (Frio Canyon Vineyard, Leakey, TX) (Fig. 6A). Merlot was grafted onto 5BB rootstock in 2010 and allowed to grow in the greenhouse until planted in May of 2011. The experimental design for the fungicide trial was a randomized complete block with 8 treatments, 5 vines/treatment and 6 replications (Fig 7). In March 2012, the vines were treated with a pre-selected multiclass of fungicides applied via the slow-drip drench method (described previously) (Table 1). A border vine was planted between each treatment along the row. No disease ratings were taken in 2012. In 2013 the vines were treated again as previously described above. Vines were rated for disease development in 2013 at the end of the growing season as described for the first site. Data were subjected to a Kruskal-Wallis analysis using SAS Proc NPAR1WAY, SAS version 9.4 to compare any statistical differences between treatments.

The Frio Canyon Vineyard also included a rootstock trial. The experimental design consisted of Chardonnay and Merlot on own roots and each variety grafted on *Vitis champinii* (Dog Ridge) rootstock under natural disease pressure. The experiment consisted of a completely randomized block design within two vineyard rows. Each

Fig. 6. Site locations for: A. Leakey Experimental Vineyard (Site Number Two, Real County, TX), and B. Hoover Valley Vineyard (Site Number Three, Burnet County, TX).

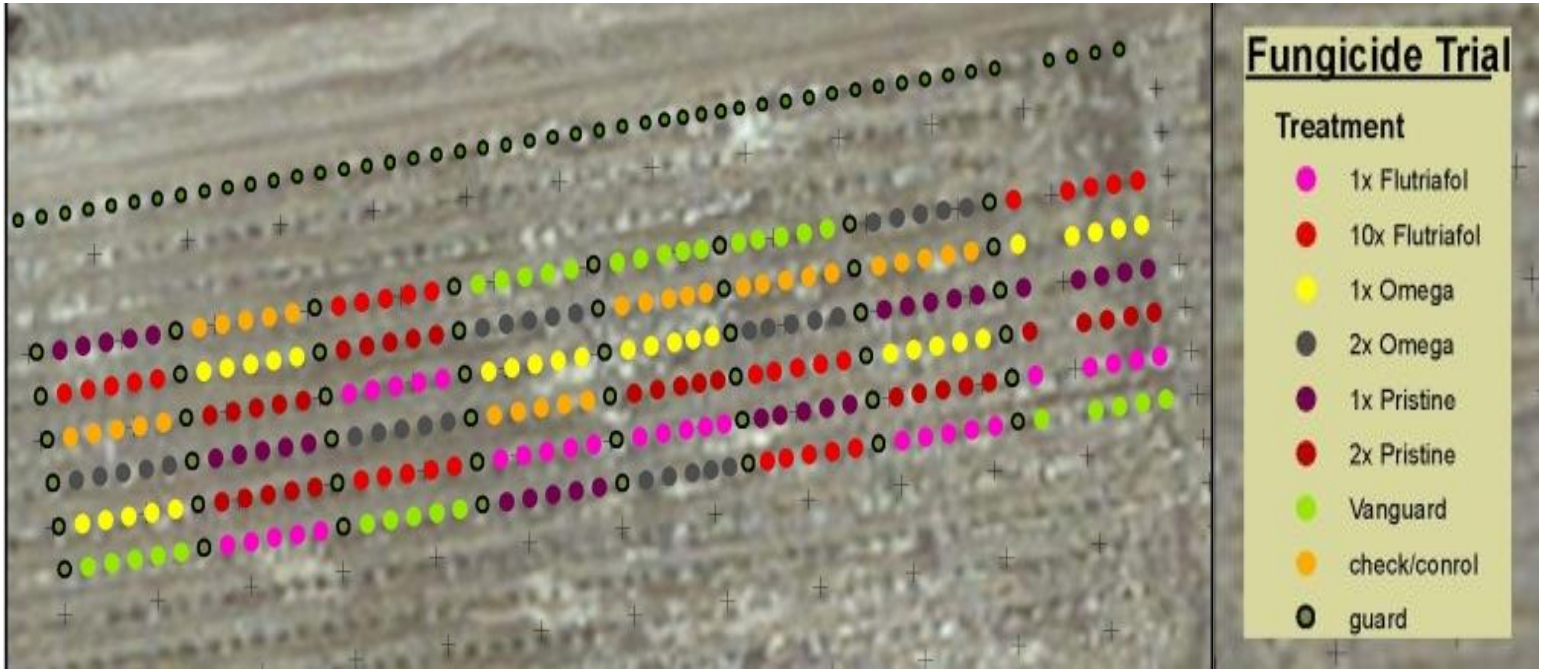

Fig. 7. Map depicting the treatment plots at the Leakey Experimental Vineyard (Site Number Three).

Table 1. Fungicides and their characteristics used to treat winegrapes at the experimental vineyard in Leakey, TX.

Chemical Trade name	Chemical Company	Active Ingredient	Class of Fungicide Mode of action	Registered for Grapes	Rate per acre	Maximum per year
Topguard®	FMC (formally Cheminova)	Flutriofol (11.8%)	Group 3 Sterol inhibitor	NO	16-32 oz.	28 oz./acre No more than 3 apps/year
Pristine®	BASF	Pyraclostrobin (12.8%) Boscalid (25.2%)	Group 7,11 Carboximide, Strobilurin	YES	8-12.5 oz.	69 oz./acre No more than 5 apps/year
Omega®	Syngenta	Fluazinam (40%)	Group 29	NO	1.25 pints/acre	6 pints
Vanguard®	Syngenta	Cyprodinil (75%)	Group 9 Anilinopyrimidine	YES	10oz/acre	30 oz./acre

plots had 3 vines repeated ten times per variety. Vines were surveyed for CRR symptom development and in 2015 disease ratings as previously described were collected. Data were subjected to a Kruskal-Wallis analysis using SAS Proc NPAR1WAY, SAS version 9.4 to compare any statistical differences between rootstocks.

In 2014, an additional third site, Hoover Valley Vineyards (Fig. 6B), was treated with two different concentrations of flutriafol, 1X = 0.26 lb a.i. /acre, and 2X = 0.52 lb a.i./acre via chemigation. The field trial consisted of 30 rows, 118 vines/row, 10 plots (rows)/treatment planted with Petit Syrah on 5BB rootstock in 2012 (Fig. 8). Disease progress was recorded for 7 weeks with ratings of dead vine (red dots), symptomatic vine for CRR (black dots), and healthy (green dots). A disease progress curve was created from these data with the x axis being the observation date and the y axis being numbers of dead vines infected with *P. omnivora*.

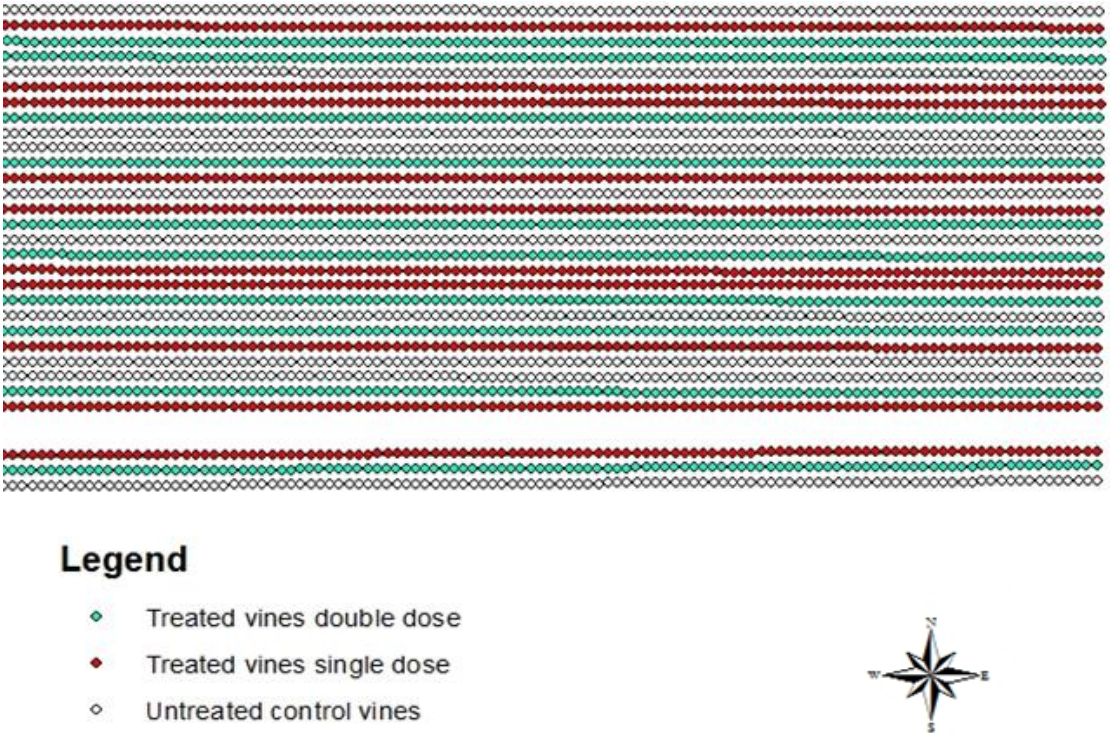

Fig. 8. A map depicting the treatment plots at Hoover Valley Vineyards.

2.3 Objective three

The bioassay for detecting residual fungicide in grapes was based on organic extracts from treated and untreated vines processed on thin layer chromatography (TLC) plates. The bioassay organism used as the indicator on the developed plates was a *Cladosporium spp*. The *Cladosporium spp.* was isolated from a spinach sample submitted to the TPDDL. A pure culture was obtained by needle transfer of spores to PDA plates and a subsequent hyphal tip transfer to additional PDA. The pure culture was maintained on PDA plates until harvested for spraying on the developed plates.

In June 2014, grapes were collected from Flat Creek Estates six weeks after fungicide application. Grapes were also collected at the end of the growing season in August from Flat Creek Estates and Hoover Valley Vineyards. Grape samples (20 gm) were weighed out and crushed with a glass rod in a 500ml Erlenmeyer flask. Ethyl acetate (200 ml) was added to the crushed grape samples. Flasks were shaken at 125rpm on ice for 2 hours. The ethyl acetate sample was transferred to a 500ml round bottomed evaporation flask. The round bottomed flask was then attached to a rotary evaporator and flash evaporated until approximately 5ml remained. Five ml of acetone was added to the extracts with the entire solution transferred to a screw-top vial and placed in the refrigerator until ready to load onto a TLC plate (15, 25). TLC plates were heat activated at 100^0C for 1 hour and allowed to cool prior to spotting the plate with the extracts.

To calibrate the system and determine end points for detection, serial dilutions of flutriafol (10, 5, 1, 0.5, 0.1, 0.05, 0.01 ppm) were originally spotted on a TLC plate.

Additional TLC plates were spotted with 10µl of the grape extractions, 10µl flutriafol in acetone (fungicide standard), and 10µl of acetone to serve as positive and negative controls. The TLC plates were then placed into a developing tank with 80ml of a carrier solvent consisting of toluene:chloroform:acetone (40:25:35) for approximately twenty to twenty five minutes. The plates were allowed to dry at room temperature for 10 minutes. Malt extract broth (10 ml) with tween 80 was added to a month-old *Cladosporium spp.* culture plate. A bent glass rod was used to gently scrape the surface of the culture to suspend the spores in the broth. The spore suspension was poured into a 50ml conical tube and an additional 15ml of malt extract broth was added. The suspension of *Cladosporium spp.* spores was counted with a hemacytometer and a final concentration of $5.0x10^5$ spores/ml was prepared. . The spore suspension was placed in an aerosol spray bottle and over-sprayed evenly on the TLC plate without significant run-off. A PDA plate was also sprayed to insure the viability of the spores. The TLC plates were placed into a plastic box with the bottom lined with moist paper towels. Plates were incubated for approximately 4 days and then observed for zones of inhibition. Plates with higher concentrations of flutriafol (1,000, 500, 400, 300, 200, and 100 ppm) were also included in the calibration process.

3. RESULTS

3.1 Objective one

Approximately 120 grape growers at the annual TWGGA grape camp meeting were invited to participate in a survey to determine the impact of CRR in Texas vineyards. Thirty six growers completed the survey. Those thirty-six growers reported growing 26 different varieties of grapes. The top six varieties, in terms of numbers of growers (n), were Tempranillo (n = 7), Syrah (n = 7), Black Spanish (n = 6), Sangiovese (n = 6), Blanc du Bois (n = 5), and Cabernet sauvignon (n = 5). The responses of the growers had numerous inconsistencies, so that precise numbers of acres in vineyard size and other details such as rootstock/scion combinations were difficult to assess. According to the responses, the size of the plantings ranged from a few vines up to 480 acres. Eight growers reported their vineyard was affected by CRR (Appendix A). Seven respondents indicated CRR was diagnosed either by TPDDL or in the field. The seven vineyards were in five counties: three in Gillespie, two in Blanco, and one in each of Travis, Austin, and Victoria. A map was created with the counties reported from the survey along with the vineyards confirmed with CRR who submitted samples to the TPDDL (Fig. 9). The growers also reported the varieties (rootstock/scion) planted in their vineyards and the number of vines affected by CRR (data not shown).

3.2 Objective two

Laboratory: Poison plate assays were used to test the efficacy of the two triazole fungicides, propiconazole (Alamo®) and flutriafol (Topguard®). The isolate

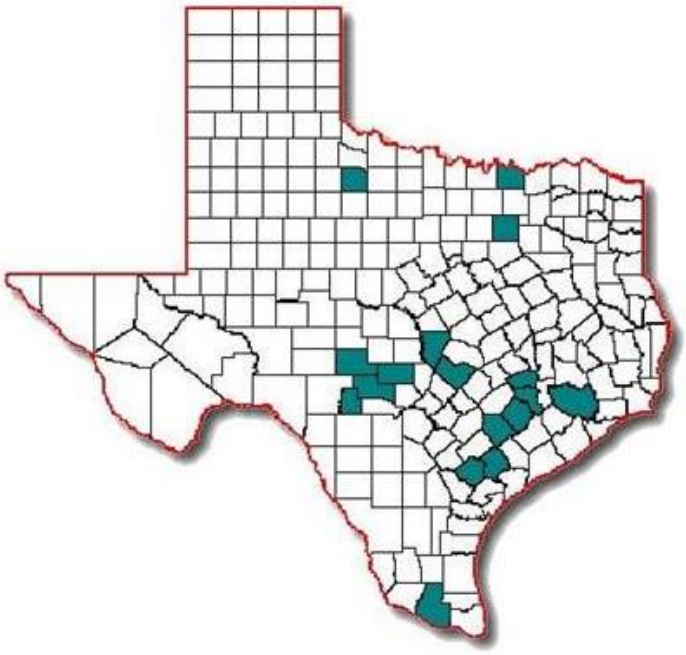

Fig 9. Map of Texas counties confirmed with CRR in vineyards. Counties indicated in blue: Knox, Kerr, Hidalgo, Grayson, Travis, Harris, Dallas, Austin, Lavaca, Goliad, Real, Gillespie, Burnet, Washington, Colorado, and Victoria.

of *P. omnivora* used in this investigation was confirmed to 98% homology with the BLAST data base. This analysis gave confidence the isolate was *P. omnivora*. Subsequently, this study demonstrated *in vitro* sensitivity levels of *P. omnivora* toward flutriafol and propiconazole.

The average diameter of growth and the percentage growth reduction is presented in Table 2. And these values are plotted against the log of the fungicide concentrations (Fig. 10) (29). A convenient interactive Microsoft Excel tool to calculate the precise EC_{50} value (50% growth reduction) was used: (http://www.sciencegateway.org/protocols/cellbio/drug/hcic50.htm). The EC_{50} values for *P. omnivora* were calculated as 0.007 ppm for propiconazole and 0.025 ppm for flutriafol.

Greenhouse: Twenty mini-rhizotrons were built and planted with Merlot cuttings in sterile field soil. Ten were artificially inoculated with a plug of *P .omnivora* and observed for colonization of the roots. The vines were able to grow in the mini-rhizotrons. However, inoculation of the roots did not result in colonization of the fungus or death of the vines.

Field experiments: At site number one, disease rating data were collected in 2013 and 2014 and subjected to a Kruskal-Wallis analysis (9, 12). Kruskal-Wallis is a non-parametrical statistical method used when data are categorical, but the analysis does not identify which treatment is significantly different. Therefore, a pairwise testing method was performed comparing the control. No difference was detected between

Table 2. Growth of *P. omnivora* (mm) after 10 days on PDA amended with 6 levels of the fungicides propiconazole and flutriafol.

	Fungicide Levels					
	0 ppm	.004 ppm	.01 ppm	.02 ppm	.05 ppm	.10 ppm
			Propiconazole			
Diameter 1	85	51.5	31.5	26.75	17.25	14.25
Diameter 2	85	51.5	32.75	27.25	18	14.75
Avg. Dia.	85	51.5	32.125	27	17.625	14.5
% of Growth	100	60.6	37.8	31.8	20.7	17
% Reduction		39.4	62.2	68.2	79.3	83
			Flutriafol			
Diameter 1	62.25	56.75	45.25	35.75	21.5	13
Diameter 2	63.5	56.5	45	37.25	20.75	14.75
Avg. Dia.	62.875	56.625	45.125	36.5	21.125	13.875
% of Growth	100	90	71.8	58	33.6	22.1
% reduction		10	28.2	42	66.4	77.9

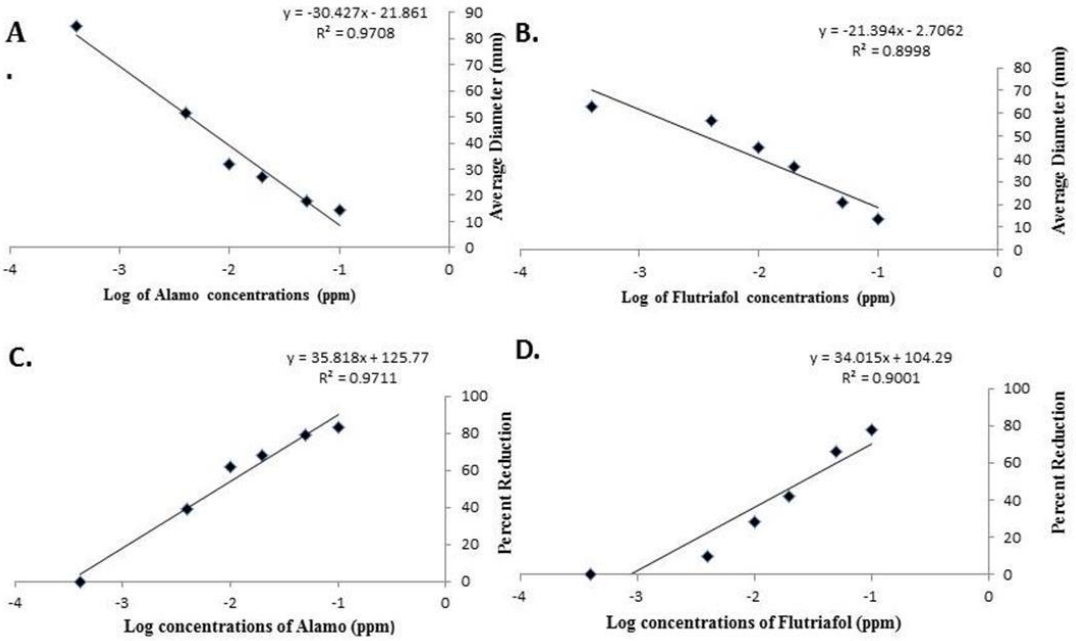

Fig 10. Results of growth of *P. omnivora* on fungicide-amended agar petri plates. A. Diameter growth plotted against the log of Alamo® concentrations. B. Diameter growth plotted against the log of flutriafol concentrations. C. Percent growth reduction plotted against the log of Alamo® concentrations. D. Percent growth reduction plotted against the log of Flutriafol concentrations.

disease development in the control and the 1X rate. However, there were statistical differences when comparing disease Wallis each treatment individually against development in the control group to the 10X and 20X rates (Table 3). Because of the late season field observations of increased vigor seen in 2013, pruning weight data were collected during December – January of 2014 and 2015 and subjected to SAS Proc GLM, SAS version 9.4. There were no statistical differences in the weights of cuttings from vines among treatments. However, positive trends in the pruning weights for each year were observed suggesting a treatment effect on vine vigor. For example, in both years, the mean weights of the control groups were less than the 1X treatment groups. Also, the 1X treatment groups were less than the higher rate treatments in each year (Table 4). In 2015, due to the observed non-target foliar pathogen suppression, plant health ratings collected and the data were subjected to a Kruskal-Wallis analysis. When comparing the control individually against the 1X, 10X and 20X rates, there were statistical differences in the health ratings (Table 5).

At site number two, vines were rated for disease development in 2013 and data were subjected to a Kruskal-Wallis analysis. Statistical differences in the disease ratings (Pr >Chi 0.0024) were found between the 10X rate and the untreated vines (data not shown). In 2013, field observations mimicked site number one with an increase in vigor. Pruning weight data were collected were in the winters of 2014 and 2015 and subjected to SAS Proc GLM, SAS version 9.4. In 2014 there were no statistical differences among treatments. In 2015, the 10X rate was significantly higher than the 1X and control treatments (Table 6).

Table 3. Kruskal-Wallis inclusive and pairwise comparisons (Proc NPAR1WAY, SAS 9.4) of disease ratings[a] for plots treated with 3 rates of flutriafol growing in Flat Creek Estates (site one) during 2013 and 2014.

Treatment	Mean	DF	Chi-square	Pr>Chi
2013				
Inclusive	2.48	3	23.4612	0.0001
1X vs. Control	2.68	1	2.5930	0.1073
10X vs. Control	3.76	1	18.4616	0.001[b]
20X vs. Control	3.88	1	7.8662	0.005[b]
2014				
Inclusive	2.68	3	44.5390	0.0001
1X vs. Control	3.12	1	0.8036	0.3700
10X vs. Control	4.00	1	20.7541	0.001[b]
20X vs. Control	3.56	1	24.2016	0.001[b]

[a] Disease ratings range from 0 (dead) to 5 (healthy) – see text for further description of the rating system.
[b] Value indicates significant difference at p =0.01.

Table 4. Mean pruning weights for 3 flutriafol treatments and control plots during 2014 and 2015 in grapevines growing at Flat Creek Estates.

Treatment	N	Mean
2014		
2 (10X)	25	0.2210a
4 (20X)	25	0.1852a
1 (1X)	25	0.1736a
3 (Ctl)	25	0.1480a
2015		
2 (10X)	25	0.4912a
4 (20X)	25	0.4760a
1 (1X)	25	0.3916a
3 (Ctl)	25	0.3384a

[a] Means for each treatment within a column followed by same letters are not statistically different (Tukey's Test, P = 0.05, PROC GLM, SAS 9.4).

Table 5 . Kruskal-Wallis inclusive and pairwise comparisons (Proc NPAR1WAY, SAS 9.4) of plant health ratings[a] for plots treated with 3 rates of flutriafol growing in Flat Creek Estates (site one) during 2015.

2015	Mean	DF	Chi-square	Pr>Chi
Kruskal-Wallis	2.88	3	11.3271	0.0101
1X vs. Control	4.08	1	10.2374	0.0014[b]
10X vs. Control	3.92	1	8.0660	0.0045[b]
20X vs. Control	3.40	1	1.6054	0.005[b]

[a] Plant health ratings range from 0 (dead) to 5 (healthy) – see text for further description of the rating system.
[b] Value indicates significant difference at p =0.01.

In 2015, statistical differences were found between Dog ridge rootstock with the different scions and own-rooted varieties. There was also a statistical difference between the two own-rooted varieties (Table 7).

In 2014, the newly planted vineyard, site number three was treated with two rates of flutriafol (1X and 2X) via chemigation and disease development was recorded for 7 weeks. The disease progressed in the untreated plots until August 29, 2014 when a few vines in the 1X and 2X plots died from CRR (Fig. 11). The rapid rate in disease progress in the untreated plots can clearly be seen in Fig.12. Recorded mortality showed that the fungicide was able to control CRR until the end of the growing season in August, 2014. Again, the increase in disease following August can be seen in the disease progress in the treated (1X and 2X) plots (Fig. 7).

Table 6. Mean pruning weights (kg.) for 2014 and 2015 in plots treated with 4 fungicides[a] at 2 rates and untreated control plots in an experimental vineyard in Leakey, TX.

Treatment	N	Mean
2014		
2 (10X Fl)	30	0.2433a
3 (Om 1X)	30	0.2450a
1 (1X Fl)	30	0.2343a
7 (Vn 1X)	30	0.2340a
5 (Pr 1X)	30	0.2170a
4 (Om 2X)	30	0.2130a
6 (Pr 2X)	30	0.2023a
8 (Ctl)	30	0.1917a
2015		
2 (10X Fl)	30	0.2417a
1 (1X Fl)	30	0.1767b
8 (Ctl)	30	0.1623b

[a] The four fungicides are designated as follows: F = Flutriafol, Om = Omega®, Vn = Vanguard®, Pr = Pristine®.

[b] Means for each treatment within a column followed by same letters are not statistically different (Tukey's Test, P = 0.05, PROC GLM, SAS 9.4).

Table 7. Kruskal-Wallis inclusive and pairwise comparisons (Proc NPAR1WAY, SAS 9.4) of disease ratings[a] for the grapevine 4 rootstocks[b] at Leakey, TX.

2015	Mean	DF	Chi-square	Pr>Chi
Kruskal-Wallis		3	72.7341	0.0001
MD vs. M	2.48	1	28.4037	0.0001[c]
CD vs. C	2.68	1	44.0656	0.0001[c]
CD vs. MD	3.76	1	0.0046	0.9460
C vs. M	3.88	1	3.9757	0.0462[c]

[a] Disease ratings range from 0 (dead) to 5 (healthy) – see text for further description of the rating system.
[b] Rootstocks are designated as follows: M = Merlot own rooted, C = Chardonnay own rooted, MD = Merlot on Dogridge, and CD = Chardonnay on Dogridge.
[c] Value indicates significant difference at p = 0.05.

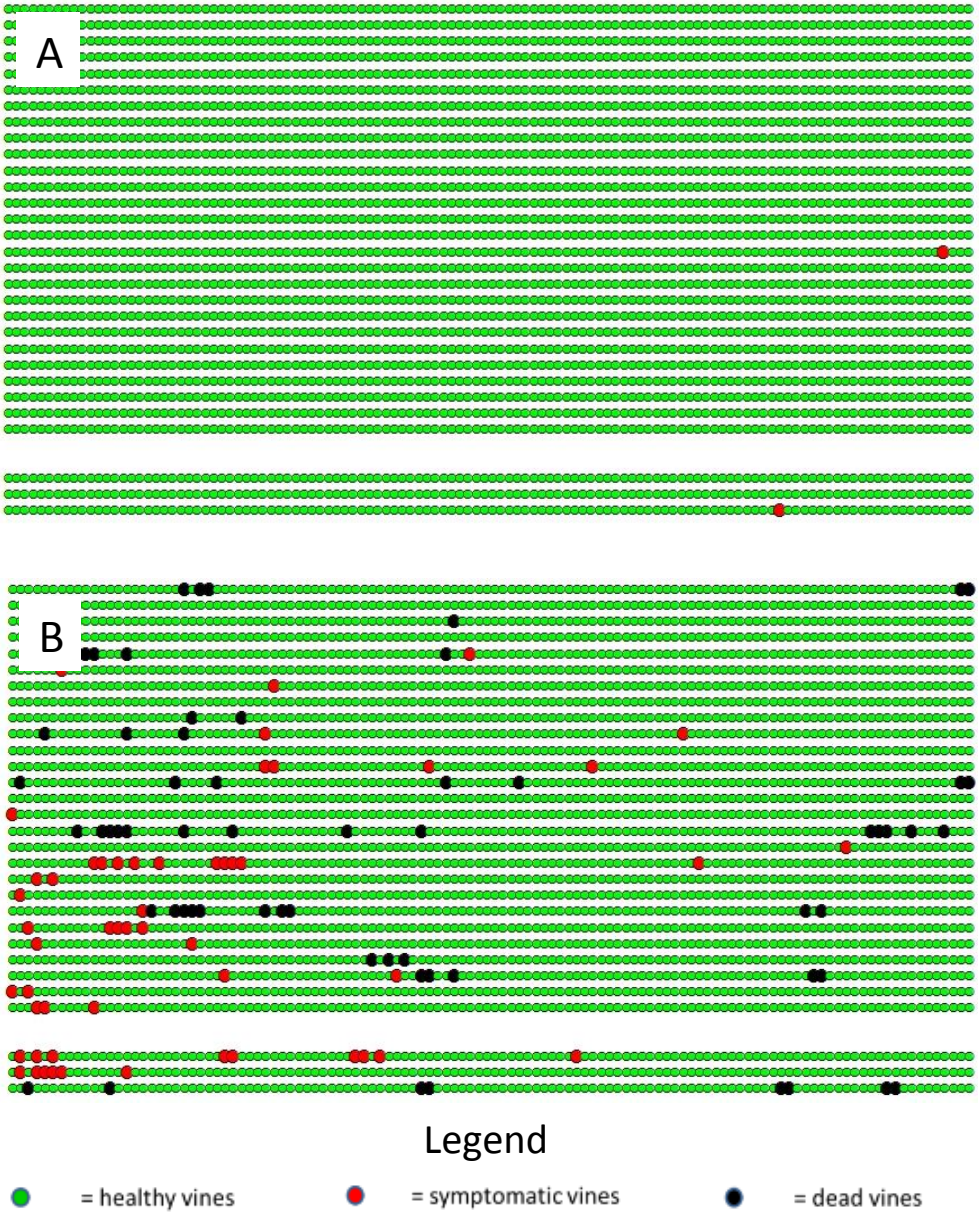

Fig 11. Disease development in the experimental plot at Hoover Valley Vineyard. **A**, June 13, 2014, **B,** September 8, 2014. (Green dots represent healthy vines, the black dots are diseased vines, and the red dots are dead vines).

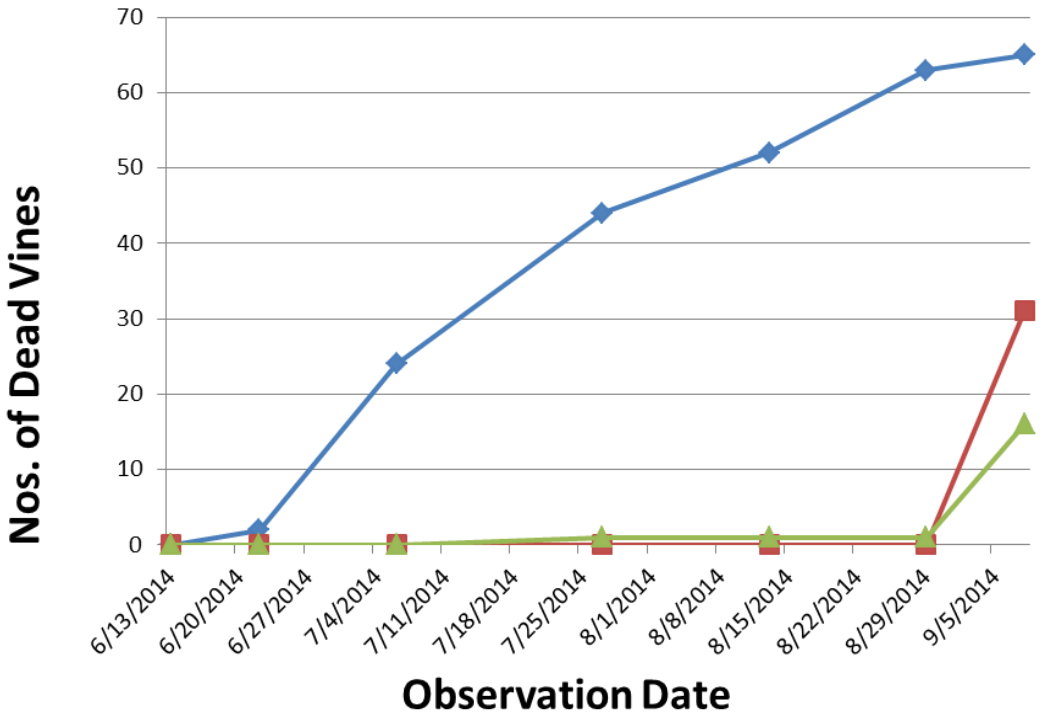

Fig 12. Disease progress curves for treated (1X and 2X) and untreated (none) plots of vineyard rows at Hoover Valley Vineyards.

3.3 Objective three

Originally, tests were performed to determine the detection limits of the bioassay. These tests revealed that the bioassay was not sufficiently sensitive to detect flutriafol at rates less than 10 ppm (data not shown). In subsequent tests, rates and volumes were increased. In those experiments zones of inhibition could be seen at the following concentrations: 1000ppm, 500ppm, 400ppm, 300ppm (Fig. 13). The rate of 300 ppm was the lowest detectable level. The faint zone of inhibition can be seen in Fig. 13. A. in lane number 7 at the position on the TLC plate where flutriafol migrates. The grape extracts (50ul) were spotted on the heat activated TLC plates and over-sprayed with the *Cladosporium spp.* spore suspension resulting in no zones of inhibition at the position where flutriafol would be expected (Fig. 8). There were, however, zones of inhibition in the grape extracts indicating an unknown fungitoxic compound was present and migrating beyond the zones of either fungicide.

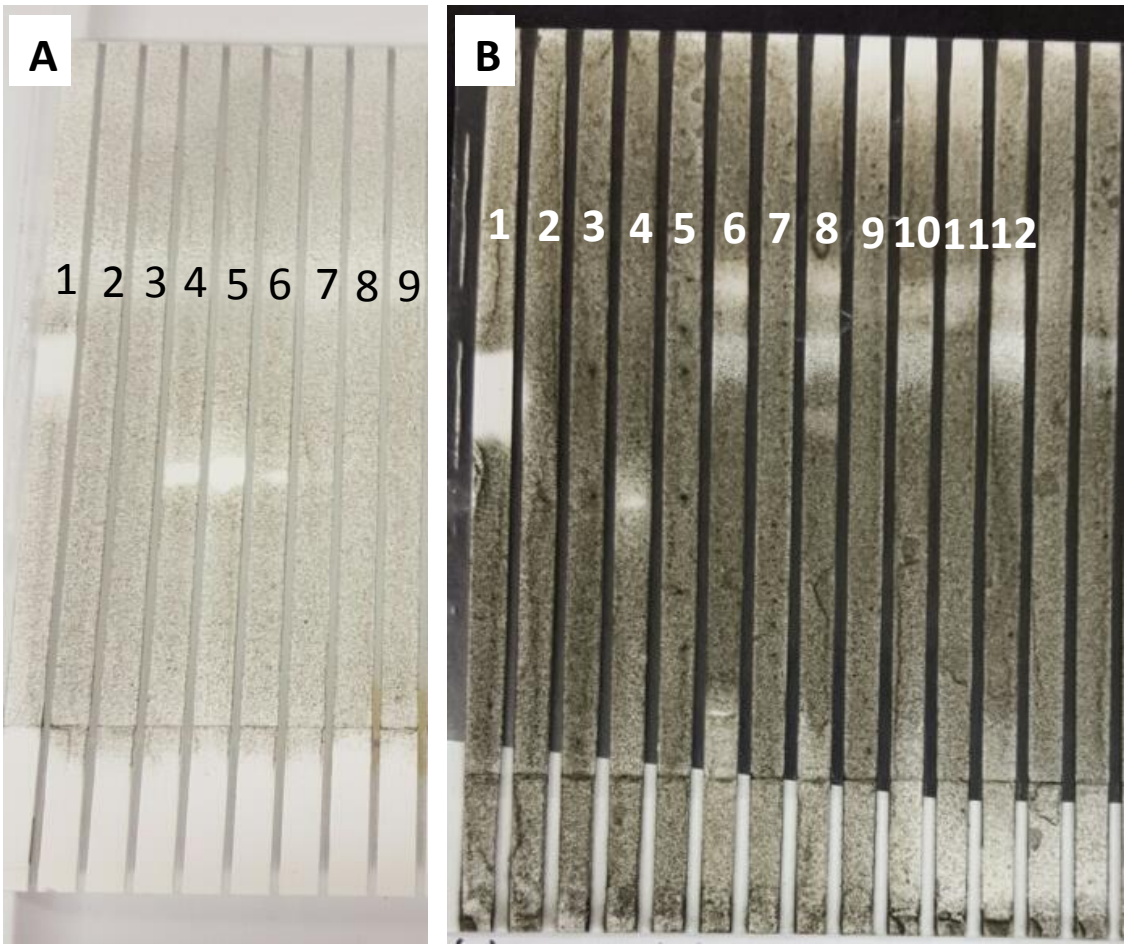

Fig 13. Bioassay TLC plates developed to detect fungicides. A. Trial run to demonstrate detection limits. Numbered lanes contain the following solutions; 1=propiconazole at 1000 ppm, 2 and 3 = acetone, 4 = flutriafol at 1000 ppm, 5 = 500 ppm flutriafol, 6 = 400 ppm, 7 = 300, 8 = 200 ppm, and lane 9 = 100 ppm. B. Results of testing grape extracts from site 1. Numbered lanes contain the following solutions; 1 = propiconazole at 1000 ppm, 2 = acetone, 3 = blank, 4 = 500 ppm flutriafol, 5 = blank, 6 = untreated grape extract, 7 = blank, 8 = 1X rate extract, 9 = blank, 10 = 10X extract, 11 blank, 12 = 20X extract.

4. SUMMARY AND DISCUSSION

Since Pammel (26) first described *Phymatotrichopsis omnivora* over a century ago, many attempts have been made to find a control for cotton root rot in cotton and other specialty crops in TX. Attempts such as lowering the pH of the soil, adding ammonium nitrate, incorporating green manure, applying soil fumigants or application of fungicides available in the past failed to adequately control CRR (8, 13, 14, 20, 32, 36). Despite the disappointments with control attempts in the past, plant pathologists continued to try developing management strategies for this recalcitrant disease. This frustration is not limited to just the cotton industry, but has also been a hindrance in the winegrape industry. Winegrapes have been grown in Texas since the 17th century by Spanish padres who brought cuttings from the old world to produce sacramental wine for their new founded missions (28). From 1931 to 1936, Mortensen (22) concluded that Phymatotrichum root rot was the major limiting factor in grapevine performance after evaluating table grape cultivars at the Crystal City, Texas Agricultural Experiment Station. With the phenomenal expansion of winegrape production in Texas during the last decade, CRR has again become a major detriment to of the wine and grape industry. This concern was the driving factor in the inception of this project, and the development of an immediate relief to growers was the overall goal of the project.

The first objective in this study was an attempt to understand more completely the concerns of grape growers with respect to *P. omnivora* in their vineyards. A survey instrument was created and distributed to grape growers, which indicated the desire

among grape growers for reasonable management strategies to control CRR in their vineyards (Appendix B). In addition to CRR, early and late frosts, other diseases such a Pierce's disease, insects, hail, poor site selection, nutrient variability and even the availability of a trained workforce make winegrape growing in TX difficult. The addition of CRR to these limitations may discourage new growers as well as limit expansion of existing vineyards. The economic impact of the Texas wine industry has increased dramatically in recent years. To achieve the recent initiative for increasing wine production in TX, a control for CRR is pertinent in areas where vineyards are at risk for CRR. The survey was also useful in identifying the cooperators for the experimental field plots. The commercial vineyard (site number one) grower had been experiencing devastating losses of vines due to CRR for 15 years. He had attempted to reduce the pH of the soil with phosphoric acid chemigation as well as incorporating green manure for control measures, but to no avail. The other cooperator (site number three) became discouraged as his young vineyard was losing second leaf vines due to CRR at alarming rates. He was inspired to cooperate with the probability of obtaining control through the use of flutriafol. As a result of the field testing and development of a potential solution to the CRR problem in winegrapes, this survey also provided an important benchmark for future surveys regarding diseases of winegrapes in TX.

Two different approaches to possible CRR control were studied. The first approach was the use of a triazole fungicide, Topguard® with the active ingredient,1H-1,2,4-Triazole-1-ethanol, alpha-(2-fluorophenyl)-alpha-(4-fluorophenyl) (generic name flutriafol). Flutriafol is in the FRAC chemical class Group 3 (see website

www.frac.info). Group 3 fungicides include the triazoles, which achieve control by inhibiting ergosterol production in fungi. Flutriafol is a de-methylation inhibitor in the ergosterol biosynthesis pathway. The second approach was the use of rootstocks to convey resistance to otherwise susceptible, yet desirable, varieties of winegrapes. A field evaluation was conducted with Chardonnay and Merlot varieties grafted on Dog ridge rootstock and, own-rooted vines in a field at high risk of CRR (site number two). In addition to field testing, experiments were conducted in rhizotrons in order to facilitate rootstock trials under controlled, greenhouse conditions. These two approaches indicated that the best CRR control was achieved through the applications of Topguard® when applied to grapevines either via the soil drench bucket method or through chemigation. The higher concentrations of the flutriafol used in site number one (2.6 lbs/a.i./acre (10X), and 5.2 lbs/a.i./acre (20X) and site number two (2.6 lbs/a.i./acre (10X), were applied due to concerns from previous data indicating pronounced PGR and phytotoxicity effects of triazole fungicides such as propiconazole, myclobutanil, and flutriafol (8, 14, 31). In previous greenhouse studies, triazole fungicides have been shown to reduce grapevine shoot elongation, rate of growth, number and length of nodes, leaf area, number of leaves, area per leaf, and lateral roots (8). In the current study, another potential PGR effect was discovered through field observations and subsequent plant health ratings at site number one. The application of the 2.6 lbs/a.i./acre (10X) rate of flutriafol controlled non-target foliar disease organisms such as *Erysiphe necator* (powdery mildew) and *Guignardia bidwellii* (black rot) and thus improved the plant health of the vines. This could be explained by the systemic

movement of flutriafol following uptake from the grapevine roots into the xylem. The control of these non-target pathogens may be due to the direct, fungicidal effect of the systemic flutriafol. Or, the fungicide may be stimulating an SAR response by the vine, thus influencing development of these foliar pathogens. Also, at sites one and two, field observations and pruning weights showed an increase in vine vigor, possibly due to the PGR effect of the flutriafol. Grape growers do not always desire an increase in vine vigor due to canopy shading and the influence on grape quality. Additional research needs to be conducted to explain the effects flutriafol has on growth and response within the vines.

At site three, application of two rates of flutriafol at 0.26 lbs/a.i./acre (1X) and 0.52 lbs/a.i./acre (2X) were applied through the irrigation system in 2014. These rates of flutriafol are more practical relative to the current accepted rate for use on cotton. Site three proved to have the best experimental results due to the high natural disease pressure and the disease progress within the untreated plots. However, by the end of the growing season, CRR began showing up in the treated plots. One possibility is the degradation of the fungicide in the soil. Another possibility could be the soil structure/type in which the fungicide was applied. Site three is a sandy loam soil with good drainage, and the flutriafol may be leached out of the root zones of the vines. Previous studies found that flutriafol is relatively persistent in soil when compared to other triazole fungicides. Flutriafol was also found in those previous studies to be more readily leached into lower soil layers due to the polar nature of the molecule (5, 37). Based on these and similar studies a Section 24(c) special local needs registration was

requested and granted by the Texas Department of Agriculture for use by the grape growers to control *P. omnivora* (see Appendix C).

A concern in the winemaking industry is the presence of fungicide residues in grapes. Fungicides can have an influence on fermentation and the organoleptic characteristics of wine, not to mention the health and toxicological effects on the consumer (24). The winemakers and consumers would have a reason to be concerned if, or for how long, the flutriafol remained in the grapes. In this current study, attempts were made to develop a cost effective TLC bioassay for detecting flutriafol in the grapes. Unfortunately, the sensitivity of this bioassay was only able to detect 300 ppm. As was shown in the poison plate assays, *P. omnivora* was sensitive at levels far below 300 ppm. There were also other compounds in the grape extracts detected from an unknown source. There are obviously limitations to using a TLC bioassay. For instance natural chemical compounds in the grape skins or the possible application of fungicides to target other pathogens could complicate the detection of the flutriafol. There are more sophisticated methods such as High Pressure Liquid Chromatography for detecting fungicides in fresh produce and other food crops but they can be costly (15).

The rootstock field study at site two was with 2 different varieties, Chardonnay and Merlot own-rooted and both varieties grafted onto Dog ridge rootstock. This study statistically showed what had been previously described in the literature. Mortensen (22) observed Texas native varieties such as Dog Ridge, Champanel, and Lukfata as survivors of CRR. Dr. George Ray McEachern in the Department of Horticultural Sciences, TAMU (*personal communication*) also stated Dog Ridge was the best

rootstock resistant to CRR. Dog Ridge (a natural hybrid between *V. candicans* and *V. rupestris*) is a true native Texas vine selected by T.V. Munson (23) in Bell County along what is called the Dog Ridge between Nolanville and Belton, TX. Dog Ridge has been shown to be tolerant of CRR due to its ability to regenerate roots at rates higher than the pathogen is infecting them (8). This could be explained by natural evolutionary tolerance in the native Dog Ridge grapevine which developed to adapt to the presence of *P. omnivora*. Selection pressure on plants by pathogens and the development of resistance and tolerance is a well- known premise in other plant pathogens and their hosts (1).

Another method to evaluate possible rootstock resistance or tolerance to CRR was the use of rhizotrons that enable observations of grapevine roots interacting with *P. omnivora.* Rhizotrons have been used through the years to observe *P. omnivora* infection in cotton. (16). In 2013, at an ASEV Rootstock Symposium in Monterey, CA, Dr. Andy Walker from UC Davis described a rhizotron he used in his grape rootstock breeding program (11). He was able to observe and chart root development with regards to his efforts to breed a grape rootstock resistant to pests and diseases. In the current study, a rhizotron prototype was constructed that was similar to Dr. Walker's in attempt to evaluate rootstocks with resistance or tolerance to CRR. However, the rhizotron prototypes in this study were not successful in the attempt to demonstrate susceptibility or tolerance of the own-rooted Merlot cuttings. No infections were observed in the inoculated vines. This may have been due to greenhouse conditions, watering practices, method of inoculation, amount of inoculum, or an insufficient incubation period to

observe infection. In the future, with modification to the rhizotron prototype and fine-tuning the cultural practices, this tool could prove valuable to screen potential varieties of rootstocks with regards to resistance or tolerance to CRR. Even though flutriafol was approved for use under the Topguard TERRA 24(c) exemption, the approval was only granted for the 2016 grape growing season. The exemption will be assessed from year to year as to whether it will be granted in future years. Continued rootstock screening, with the use of rhizotrons and field testing, could provide evidence for new, resistant rootstocks and give grape growers an alternative to flutriafol. Rootstocks with native Texas parentage are continually being bred in the search for superior disease resistance combined with desirable traits (11). The use of superior rootstocks would address potential environmental issues connected to the use of fungicides and alleviate the uncertainty of the temporary status granted under the 24(c) exemption.

The 24c label for use of flutriafol in winegrapes was the culmination of a highly cooperative project involving research scientists, extension specialists, growers, industry representatives and ultimately the Texas Department of Agriculture. Although not entirely efficacious, the fungicide flutriafol offers a management tool for growers until other, more sustainable approaches are developed. There is also hope for these studies for the use of flutriafol to manage CRR in other crops, such as olives, peaches, apples, and landscape ornamentals. Additionally, this fungicide should be tested against other root rot pathogens, such as *Armillaria* spp. and *Ganoderma* spp. that have proven to be impossible to manage by other means.

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APPENDIX A

TEXAS A&M UNIVERSITY

TEXAS A&M AGRILIFE EXTENSION

COLLEGE OF AGRICULTURE AND LIFE SCIENCES

Department of Plant Pathology and Microbiology

Vineyard Survey for Cotton Root Rot

General Vineyard Information:

- Acreage_____
- Rootstock/scion_____
- Age of vines (if various ages give a range)_____

Vineyard Site:

- County_____
- Soil pH_____
- Soil types with relation to drainage (circle the best fit):

  Excellent Adequate Poor Variable

Presence of Cotton Root Rot in Vineyard:

- Have you ever observed any of the following symptoms in your vineyard? (Circle all that apply)

  Leaf Scorch Sudden Death Dried leaves remaining on the vine

- Has cotton root rot been implemented/confirmed in your vineyard? **Yes No**

  If yes, how was it confirmed? **Laboratory Field observations**

- Do you believe you have lost vines in the past due to cotton root rot? **Yes No**

Please return completed form to Sheila McBride

November 2, 2015

IRB NUMBER: IRB2015-0482M
IRB APPROVAL DATE: 10/22/2015
IRB EXPIRATION DATE: 10/01/2020

APPENDIX B

Table A. Appendix B Results of Grape Grower Survey regarding Cotton Root Rot and Vineyard Characteristics.

			Vineyard Site			Observed Symptoms				
Vineyard	Acres	Vine age (years)	County	pH	Drainage	Scorch	Sudden Death	Dried eaves	Confirmed?	Interest in confirmation?
1	4	3-25	Austin	6	adequate	x	x		N	No
2	0.25	1-2	Washington		adequate				N	Not Yet
3	2	11-16	Austin	6.7	excellent		x		Lab	Yes
4	0.33	0-3	Goliad	6	adequate				N	Yes
5	1	1-3	Live Oak		adequate				N	Yes
6	1.5	.5-1.5	Fayette	7.8	adequate			x	N	Not Now
7	480	3rd Leaf	Houston		excellent				N	No
8	1	1-3	Grimes	6.4	excellent				N	Yes
9	2.1	3-5	Burleson	4.5	variable	x	x	x	N	Yes
10	5	1	Grimes	7.6	adequate	x		x	N	No
11	5	10+	Walker	6	poor				N	No
12	0.5	2-8	Harris	6.5	excellent				N	
13	1	5-34	Victoria	7-8.2	poor-adequate		x		Lab	Yes
14	1	1-5	Kimble	7.3	excellent		x		N	Yes
15	15	1-5	Kerr	>7	excellent			x	N	Yes
16	10	0-16	San Saba	8-8.3	adequate	x	x	x	N	Yes
17	13	1-5	Terry	7.3	excellent				N	
18	17	6-15	Gillespie		adequate		x	x	N	No
19	5	9-10	Gillespie	7.3	excellent		x	x		Yes
20	0.5	0-1	Gillespie	7.8-8	adequate	x			N	Yes
21	4	2,4,12	Blanco	7.5	excellent		x	x	Y	
22	0.5	3-4	Gillespie	7.8	adequate	x			Field	Yes

Table A. Continued.

			Vineyard Site			Observed Symptoms				
Vineyard	Acres	Vine age (years	County	pH	Drainage	Scorch	Sudden Death	Dried Leaves	Confirmed?	Interest in confirmation?
23	1.25	3rd Leaf	Mason	7	adequate				N	Yes
24	3	3	Lampasas	6-7	adequate	x	x		N	Yes
25	15	15	Gillespie	6.2-6.7	adequate				Field	Yes
26	2	3 or 6	Kimble	8-9	adequate	x	x		N	Yes
27	2	1	Gillespie	6-6.5	adequate					
28	10	1-13	Travis	7	variable	x	x	x	N	Yes
29	3.5	1-10	Blanco	8+	adequate	x	x		N	Yes
30	3	5-7	Gillespie		excellent			x	Lab	Yes
31	10	1-3	Sutton	8.2	adequate		x	x	N	Yes
32	1	2 mos	Llano	5.9	adequate					
33	20	10-12	Travis	7.9	excellent	x	x	x	Lab	Yes
34	1	3-4	Kerr	7.9	excellent	x	x	x	N	Yes
35	4	0-6	Blanco	7.5-8.5	excellent	x	x	x	Lab	
36	0.25		Bell Cove		excellent					

APPENDIX C

FIFRA Section 24 (c) Special Local Need
FOR DISTRIBUTION AND USE ONLY WITHIN THE STATE OF TEXAS

TOPGUARD Terra FUNGICIDE

EPA Reg. No. 67760-126
EPA SLN No. TX-150007

Active Ingredients
*Flutriafol.....42.0%
Other Ingredients...58.0%
*Topguard Terra contains 4.16 lbs ai/gal

This 24(c) label for TOPGUARD Terra Fungicide is valid until 10/31/2018, or until otherwise amended, withdrawn, canceled or suspended.

FIFRA 24 (c)
Special Local Need Label
For Control of Texas Root Rot and Cotton Root Rot by Soil Application for Grapes
EPA SLN. No. TX-150007

It is a violation of State and Federal Law to use this product in a manner inconsistent with its labeling. Persons using this product must comply with all applicable directions, restrictions, and precautions found on this labeling and that of the label of the federally registered product upon which this amendment is based.
This label and the federal label for this product must be in the possession of the user at the time of pesticide application.
Follow all applicable directions, restrictions, and precautions on this Supplemental label and the main EPA-registered label.

DIRECTIONS FOR USE

CROP	PESTS	RATE OF APPLICATION	
		Product Rate Fl. Oz per Acre*	Pounds active Ingredient per Acre
Grapes (soil applied)	Texas Root Rot Cotton Root Rot (Phymatotrichopsis omnivora)	7.6 – 15.3	0.25 – 0.5

DIRECTIONS FOR USE THROUGH DRIP IRRIGATION

APPLICATION INSTRUCTIONS
Apply at recommended use rates through above ground drip irrigation system as outlined in **USE DIRECTIONS section** of this label.

APPLICATION RATE
Apply TOPGUARD Terra in a single application at 15.3 oz per acre or in a split of two applications of 7.6 oz per acre each.

APPLICATION TIMING
Make initial application between 30 to 60 days after bud break. If a split application is desired, make second application no closer than 45 days after first application but not within 14 days of harvest. When using a split application, the second timing may be applied as a postharvest to manage late season disease.