

### **Title of Report**

Interim Progress Report for CDFA Agreement Number 12-0468-SA

### **Title of Project**

Continuation of the Field Evaluation of New Strategies  
for the Management of Pierce's Disease of Grapevine.

### **Principal Investigators**

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### **Time Period Covered by the Report**

07/01/2014 to present

### **Introduction**

*Xylella fastidiosa* (*Xf*) is a Gram negative, xylem-limited, insect-vectorized bacterium and is the causal agent of Pierce's Disease (PD) of grapevine (Hopkins and Purcell, 2002). Current PD management strategies primarily involve vector management through the use of insecticides. Several alternative strategies are currently being evaluated in field trials. One of the field trial is located at the Department of Agricultural Operations (AgOps) at UC Riverside. The experimental grapevines grown at UCR are subjected to natural *Xf* insect vector populations (the glassy-winged sharpshooter, GWSS). The strategies developed by principal investigators Dandekar, Lindow, Gilchrist, Powell and Kirkpatrick/Hopkins that are currently being evaluated include various transgenic grape and grape rootstocks expressing genes from different constructs as well as the use of non-virulent *Xf* strain as a biocontrol agent (see PIs reports for more information). Our goal is to maintain the vines growing at AgOps and record data on insect vector and disease pressure, and PD incidence and severity in order to identify the most effective control strategy moving forward.

### **List of Objectives**

1. Maintain grapevines and research plots.
2. Monitor sharpshooter populations and disease pressure.
3. Record Pierce's Disease severity

**Description of Activities Conducted to Accomplish each Objective, and Summary of Accomplishments and Results for each Objective.**

**Objective 1:** Maintain grapevines and research plots.

Field activities since July of 2014 are reported in **Table 1**. Water, soil, and tissue samples from each experimental plot were sent to the 'Fruit Growers Lab, Inc.' for analyses in 2014 (**Table 2, 3, 4**). The 'Ever Green Nematode Testing Lab, Inc.' also performed nematode analysis from the soil samples. For the irrigation water, no obvious problem was noticed besides a slightly alkaline pH (**Table 2**). The samples from shallow (0-25cm) and deep (25-50 cm) soils around grapevine roots as well as background soil from middle row also showed that soils were slightly alkaline. This condition is likely affecting Cation Exchange Capacity (CEC) as higher pH decreases cations availability (**Table 3**). Overall, deep soil seems to be more deficient in Magnesium and Zinc. In addition, boron and nitrate-nitrogen and phosphorous availability were limited in deep and shallow soils. Some of these carried over to tissue analyses whereby nitrogen, and sometimes phosphorous and zinc were lower than the optimum range (**Table 4**). However, toxic levels of boron were recorded in vines from all experimental plots. Mineral nutrients imbalance was also previously reported in grapevines and host plants infected with PD (Lu et al, 2003; De La Fuente et al, 2013), but never for boron (B). Those deficiency or toxicity levels may have confounded PD symptoms, as older leaves with B toxicity can appear scorched. Thus improper disease severity rating may have resulted from it. Vine tissue analyses will be repeated in the spring of 2015 to confirm some of these preliminary data and vines will be fertilized accordingly to mitigate the deficiencies and toxicities observed. Nematode analysis showed that *Tylenchulus semipenetrans* was present in Dandekar's plot (2254 nematode per Kg of soil) and those they may have stressed the vines and caused them to decline (Verdejo-Lucas and Mckenry, 2004). Interestingly, these nematodes were only found in Dandekar's block. Abiotic stress such as heavy crop load that was only observed on some vines in the Hopkins/Kirkpatrick plot may also have stressed the vines and caused them to decline (**Fig.1B**).

**Table 1:** Field activities for all grapevine experimental plots located at AgOps, UC Riverside.

Date	Activity
<b>2014</b>	
July 2	Traps collected, sharpshooters censused, new traps deployed
July 11	Rodent control
July 12	Grape tissue sampling for analysis - 'Fruit Growers Lab'
July 17	Fungicide application (rally + stylet oil) for powdery mildew control
	Weed control
August 7	Traps collected, sharpshooters censused, new traps deployed
August 8	Pruning and burying grape cuttings
	Fungicide application (stylet oil) for powdery mildew control
August 21	Pruning and burying grape cuttings
August 22	Soil and root sampling for nematode count – 'Ever Green Nematodes testing Lab'
	Soil sampling for analysis - 'Fruit Growers Lab'
August 12-26	Weeding and vine training
August 26	Weed control
September 4	Traps collected, sharpshooters censused, new traps deployed
September 17	Water sampling from drip irrigation for analysis – 'Fruit Growers Lab'
September 22	Pierce's Disease severity rating
September 23	Weed control
September 29	Pierce's Disease severity rating
October 6	Pierce's Disease severity rating
	Sampling petioles for <i>X. fastidiosa</i> detection by qPCR
<b>2015</b>	
February	Grapevine pruning



**Figure 1:** (A) Grapevine cv. 'Pinot' improperly trained also showing powdery mildew symptoms. (B) Over cropped grapevine cv. 'Pinot' showing signs of stress.

**Table 2:** Grape irrigation suitability analysis for 2014, Fruit Growers Laboratory, Inc.  
 Values highlighted in red represent higher level than the optimal requirements.

Test Description	Results	
	mg/L	Meq/L
<b>Cations</b>		
Calcium	50	2.5
Magnesium	10	0.82
Potassium	3	0.077
Sodium	40	1.7
<b>Anions</b>		
Carbonate	< 10	0
Bicarbonate	170	2.8
Sulfate	57	1.2
Chloride	29	0.82
Nitrate	16.1	0.26
Nitrate Nitrogen	3.6	
Fluoride	0.5	0.026
<b>Minor Elements</b>		
Boron	0.1	
Copper	0.01	
Iron	0.04	
Manganese	0.04	
Zinc	< 0.02	
TDS by Summation	376	
<b>Other</b>		
pH	7.6	
E.C.	0.513 dS/m	
SAR	1.4	
<b>Crop Suitability</b>		
No amendments	Fairly good	
With amendments	Good	
<b>Amendments</b>		
Gypsum requirement	0.2 Tons/AF	
Sulfuric acid (98%)	9.8 oz/1000Gal	
Leaching requirement	3.3 %	

**Table 3:** Soil analysis for 2014, Fruit Growers Laboratory, Inc. Soil samples representing shallow soil (SS-R) and deep soil (DS-R) of grapevine roots as well as background soil from middle row (BS), were collected from each experimental block. Values highlighted in yellow and red represent lower and higher levels than the optimal requirements, respectively.

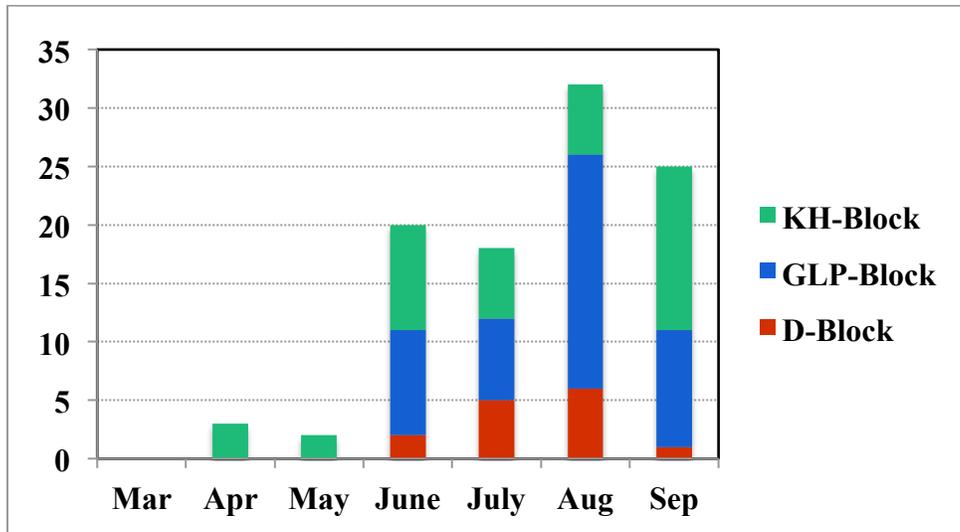
Test Description	Block								
	Dandekar			Kirkpatrick / Hopkins			Lindow/Gilchrist/Powell		
	BS	SS-R	DS-R	BS	SS-R	DS-R	BS	SS-R	DS-R
<b>Primary Nutrients</b>									
Nitrate-Nitrogen (ppm)	11.8	1.5	2.5	7.4	1.9	1.5	111	1.4	1.4
Phosphorus-P <sub>2</sub> O <sub>5</sub> (ppm)	48.1	25.2	45.8	20.6	25.2	18.3	38.9	20.6	29.8
Potassium-K <sub>2</sub> O <i>Exch</i> (ppm)	157	84	133	72	84	60	145	72	169
Potassium-K <sub>2</sub> O <i>Sol</i> (meq/L)	1.06	0.304	0.496	0.112	0.103	0.041	2.29	0.394	0.618
<b>Secondary Nutrients</b>									
Calcium <i>Exch</i> (ppm)	560	620	640	1120	1480	1140	560	580	660
Calcium <i>Sol</i> (meq/L)	3.06	2.33	1.88	3.42	5.78	3.34	26.5	4.28	1.95
Magnesium <i>Exch</i> (ppm)	78	92	84	132	174	139	79	85	96
Magnesium <i>Sol</i> (meq/L)	0.933	0.892	0.611	0.927	1.7	0.98	7.71	1.61	0.625
Sodium <i>Exch</i> (ppm)	20	40	30	40	80	50	30	30	50
Sodium <i>Sol</i> (meq/L)	0.678	4.48	2.57	2.4	7.72	4.09	5.37	4.66	3.89
Sulfate (meq/L)	0.627	3.01	1.86	1.25	7.69	3.65	6.17	2.31	2.95
<b>Micronutrients</b>									
Zinc (ppm)	0.8	1	0.8	0.5	0.8	0.3	0.6	0.5	0.3
Manganese (ppm)	6.4	4.1	3.2	4.2	5.4	4.1	5.3	5.3	4.8
Iron (ppm)	17.5	15.3	14.1	11.2	11.3	15	15.5	9.5	10.2
Copper (ppm)	0.6	0.7	0.6	0.7	0.9	0.5	0.6	0.6	0.5
Boron (ppm)	0.26	0.17	0.18	0.3	0.22	0.15	0.243	0.13	0.13
Chloride (meq/L)	0.45	1.65	0.69	0.68	4.59	2.6	4.04	1.54	1.72
CEC (meq/100g)	3.77	4.24	4.3	7.02	9.38	7.19	3.87	3.9	4.64
<b>% Base Saturation</b>									
CEC – Calcium (%)	74.3	73.1	74.4	79.8	78.8	79.3	72.4	74.4	71.1
CEC – Magnesium (%)	17	17.9	16	15.5	15.2	15.9	16.8	17.9	17
CEC – Potassium (%)	8.78	4.43	6.28	2.35	1.96	1.61	7.78	4.1	7.59
CEC – Sodium (%)	0.1	4.41	3.28	2.32	3.93	3.31	2.97	3.54	4.22
CEC – Hydrogen (%)	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<b>Others</b>									
pH	6.67	7.46	7.35	7.52	7.57	7.69	6.74	6.85	7.34
Soil Salinity (dS/m)	0.62	0.76	0.54	0.69	1.48	0.84	4.18	0.98	0.73
SAR	0.5	3.5	2.3	1.6	4	2.8	1.3	2.7	3.4
Limestone (%)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Moisture (%)	2.7	8.1	6.5	2.8	11.4	6.5	3.5	6.2	3.7
Saturation (%)	27.2	21.6	23.4	24.5	30.7	28.6	25.2	25	26.2

**Table 4:** Grapevine leaf blades and petioles nutrient analyses from the 3 experimental blocks at AgOps, UCR. Samples were collected in July of 2014 and sent to the Fruit Growers Lab, CA. Values highlighted in yellow and red represent lower and higher levels than the optimal requirements, respectively.

Sample	Test Description	Block		
		Dandekar	Kirkpatrick Hopkins	Lindow Gilchrist Powell
Leaf blades	<b>Macronutrients</b>			
	Total Nitrogen (%)	3.3	2.62	2.88
	Phosphorus (%)	0.46	0.17	0.38
	Potassium (%)	1.37	0.47	1.78
	Calcium (%)	2.19	2.88	2.38
	Magnesium (%)	0.35	0.36	0.38
	<b>Micronutrients</b>			
	Zinc (ppm)	38.4	23.2	32.1
	Manganese (ppm)	111	100	121
	Iron (ppm)	251	290	187
	Copper (ppm)	15	8	14
	Boron (ppm)	91.3	69	102
	Sodium (%)	0.024	0.014	0.022
Petioles	<b>Macronutrients</b>			
	Total Nitrogen (%)	--	0.83	0.77
	Nitrate-Nitrogen (ppm)	--	840	710
	Phosphorus (%)	--	0.12	0.68
	Potassium (%)	--	0.98	4.30
	Calcium (%)	--	2.46	1.54
	Magnesium (%)	--	0.76	0.62
	<b>Micronutrients</b>			
	Zinc (ppm)	--	58.8	42.7
	Manganese (ppm)	--	223	218
	Iron (ppm)	--	47	72
	Copper (ppm)	--	7	7
	Boron (ppm)	--	34.2	42.9
Sodium (%)	--	0.075	0.166	

**Objective 2:** Monitor sharpshooter populations and disease pressure.

Sharpshooters were monitored at the experimental site in all three blocks in 2014 (Dandekar, Gilchrist/Lindow/Powell, and Kirkpatrick/Hopkins). For each block, six 6" x 9" double-sided yellow sticky traps were placed randomly throughout the plots. Traps were mounted on wooden stakes slightly above the vine canopy. These traps were collected every month and returned to the laboratory to identify under the stereomicroscope the number of GWSS (*Homalodisca vitripennis*). Results (**Fig.2**) showed that a low insect vector population was recorded early in the season (March to May 2014) but that that population drastically increased over the summer of 2014. This information allow for estimates of disease pressure at each experimental plot. This will be repeated in 2015.



**Figure 2:** Total number of glassy-winged sharpshooter (GWSS) insect vectors captured on yellow sticky traps from all 3 experimental blocks (D: Dandekar; KH: Kirkpatrick/Hopkins; GLP: Gilchrist/Lindow/Powell). 2014 results are based on a total of 18 traps (6 traps per block).

**Objective 3: Record Pierce's Disease severity.**

PD rating was assessed based on rating scale developed by Dr. Bruce Kirkpatrick. This PD rating scale requires that vines are cordons trained. However, because grapevines at AgOps were not always trained with cordons and were sometimes pruned improperly (**Fig.1A**), it was difficult to use that disease rating scale, so it was modified (**Table 5**). Besides improper training, powdery mildew leaf symptoms (**Fig.1B**) and boron toxicity may have confounded PD symptoms especially in the Hopkins/Kirkpatrick plot.

**Table 5:** Rating scale use to score PD severity on grapevines from all experimental plots at AgOps, UCR.

PD disease Rating	Symptoms
0	None
1	One shoot/cane expresses PD symptoms
2	Two shoots/canes express PD symptoms
3	Less than 50% of the grapevine is symptomatic
4	More than 50% of the grapevine is symptomatic
5	Grapevine is dead

	V13	V12	V11	V10	V9	V8	V7	V6	V5	V4	V3	V2	V1
Row13													
Row12													
Row11													
Row10													
Row9													
Row8													
Row7													
Row6													
Row5													
Row4													
Row3													
Row2													
Row1													

Dirt Road

AVOCADOS													
Dirt Road													
Row 1													
Row 2													
Row 3													
Row 4													
Row 5													
Row 6													
Row 7													
Row 8													
Row 9													
Row 10													
Row 11													
Row 12													
Row 13													
Row 14													

Dirt Road

**Figure 3:** 2014 Pierce’s Disease severity rating (**Table 5**) for experimental plots including Hopkins/Kirkpatrick (gray), Dandekar (blue), Lindow (red), Gilchrist (green), and Powell (purple).

**Publications Produced and Pending, and Presentations Made that Relate to the Funded Project**

Rolshausen, P.E., Daugherty, M., and Mauk, P. 2014. Continuation of the Field Evaluation of New Strategies for the Management of Pierce’s Disease of Grapevine. In 2014 Pierce’s Disease Symposium Proceedings, Sacramento, CA.

### **Research Relevance Statement**

The experimental site at AgOps, UC Riverside, is the perfect site to evaluate strategies for control of Pierce's Disease, because of the natural presence the disease vector, the glassy-winged sharpshooter. Our observations and results from 2014 indicated that the management practices at the experimental site need to be modified in 2015 so one could fully assess the efficacy of each strategy. However, the symptoms and decline of the grapevines that we recorded are mostly caused by the presence of *X. fastidiosa*, although additional stressors may have caused those vines to decline faster.

### **Layperson Summary of Project Accomplishments**

Alternative strategies for control of Pierce's Diseases (PD) are currently being evaluated in the field at the Department of Agricultural Operations at the University of California, Riverside. Vines are subjected to natural disease pressure because of the presence of insect vector populations, the glassy-winged sharpshooter (GWSS). Here we present all the field activities that were done since July 2014 including irrigation water, soil, and plant tissue analyses. Based on these analyses, we identified several problems that may have limited the establishment and growth of those vines after planting. Abiotic stresses that were identified include slightly alkaline soil and water, nutrient deficiencies in the soil (i.e.; nitrogen, magnesium, boron and phosphorous), and in the plant (i.e.; nitrogen, phosphorous), as well as boron toxicities in plant tissues. Crop load was sometime an issue. Biotic stresses other than PD included nematodes and powdery mildew. As expected disease pressure increased over the summer, as indicated by an increased number of GWSS caught on yellow sticky traps. PD severity was recorded in all research plots and results are presented in this report, but will be discussed in reports by individual PIs.

### **Status of Funds**

As of January 2015, \$26,084 was available to cover 2015 and 2016 expenses related to this project.

### **Summary and Status of Intellectual Property Associated with the Project**

Nothing to report.

### **Literature Cited**

De La Fuente, L., Parker, J.K., Oliver, J.E., Granger, S., Brannen, P.M., van Santen, E., and Cobine, P.A. 2013. The bacterial pathogen *Xylella fastidiosa* affects the leaf ionome of plant hosts during infection. PLoS One, 8:article No.:e62945.

Hopkins, D. L., and A. H. Purcell. 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. Plant Disease, 86:1056-1066.

Lu, J., Xu, X., Ren, Z., Yun, H., and Liu, X. 2003. Interaction between the pathogen and host plants during the Pierce's disease development of grapevines. Hortscience, 38: 687-688.

Verdejo-Lucas, S. and Mckenry, M.V. 2004. Management of the Citrus Nematode, *Tylenchulus semipenetrans*. Journal of Nematology, 36: 424-432