"Interim Progress Report for CDFA Agreement Number 18-0399-000-SA"
Project Title: Field testing transgenic grapevine rootstocks expressing CAP and PGIP proteins.

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Introduction

The focus of this study is to evaluate rootstock-based expression of chimeric antimicrobial proteins (CAP; Dandekar et al., 2012a) and polygalacturonase inhibitory protein (PGIP; Agüero et al., 2005, 2006) to provide trans-graft protection of the scion grapevine variety against PD. A field trial testing four lines of CAP and of PGIP-expressing rootstocks was successfully concluded recently (Dandekar et al., 2019). Transgenic lines showed reduced vine mortality and lower bacterial titer. They also had higher spring bud break, indicative of vine health. The present study builds on earlier work and incorporates advances in transformation of commercially relevant grapevine rootstocks and improvements in individual components present in the CAP and PGIP constructs. Methods to transform two commercially relevant rootstocks, 101-14 and 1103 (Christensen, 2003), have been developed (Dandekar et al., 2011; 2012b) and further improved by David Tricoli in the plant transformation facility at UC Davis. Sixty 2nd generation lines (total of 379 vines) were planted in the field in Aug 2018, as transgenic rootstocks grafted to Chardonnay as the scion. This spring (2019), the remaining 60 lines will be planted to conclude the field planting stage of this project. We will evaluate in the field effectiveness of rootstocks expressing either of these two proteins in limiting PD development in the Chardonnay scion while maintaining vine health and productivity. Elite rootstock lines identified in this project will be good candidates for commercialization.

List of objectives

The goal of this project is to field-test transgenic rootstocks expressing CAP and/or PGIP proteins to determine their ability to trans-graft protect a sensitive scion grapevine from developing and succumbing to PD.

Objective 1: Develop commercially relevant transgenic rootstock lines expressing CAP and/or PGIP
Objective 2: Field test the efficacy of commercially relevant transgenic rootstock lines expressing CAP and/or PGIP proteins to trans graft protect a sensitive grapevine cultivar from developing and spreading PD.

Description of activities conducted to accomplish each objective, and summary of accomplishments and results for each objective

Objective 1. Develop commercially relevant transgenic rootstock lines expressing CAP and/or PGIP.
This objective translates the results of two previously funded projects, 11-02040-SA and 12-0130-SA. Project 12-0130-SA, entitled "Building a next generation chimeric antimicrobial protein to provide rootstock-mediated resistance to Pierce's Disease in grapevines," led to the development of additional CAP proteins with components derived from grapevine and other proteins (Dandekar et al., 2015). Project 11-0240-SA, entitled "Engineering multi-component resistance to Pierce's Disease in California grapevine rootstocks," led to the development of a method to transform the commercially relevant rootstocks 101-14 and 1103 (Dandekar et al., 2011; 2013). David Tricoli at the plant transformation facility at UC Davis has further improved the grapevine rootstock transformation protocol and carried out all of our transformations. Several CAP vectors are being field tested in this project (Fig 1). CAP-1 is the original vector that was field tested in TS rootstocks, of which several lines showed efficacy (Dandekar et al., 2016; 2018). CAP-2 has the original components described earlier (Dandekar et al., 2012c); however, the expression of the CAP was improved by including a translational

enhancer (omega) and an efficient secretion sequence (Ramy3D), and the CAP-2 protein has an epitope tag (FLAG) to enable detection of the protein in transgenic tissues. CAP-3 to 6 are four vector constructs to test the *Vitis*-derived components. CAP-3 and CAP-4 are designed to test the *Vitis* component replacing protease from CAP-1. The CAP-3 vector, pDP13.35107, tests the VsP14a protein by itself. The VsP14a component is present in *Vitis shuttleworthii* (Vs) and has a similar function to the CAP-1 protease (Dandekar et al., 2014; Chakraborty et al., 2013). Expression of VsP14a alone confirmed its protease and antimicrobial activity against *Xf* (Dandekar et al., 2014). The fourth vector, pDP13.36122 (CAP-4), expressess VsP14a linked to CB, the antimicrobial peptide domain used successfully in CAP-1 (Dandekar et al., 2012a). CAP-5, in pDM14.0708.13 (Fig. 1), links the VsP14a to a 52-amino acid segment of the HAT protein from *Vitis vinefera* that displays a moderate clearance activity against *Xf* (Chakraborty et al. 2014b; Dandekar et al. 2013). CAP-6, in pDM14.0436.03 (Fig 1), links the VsP14a to a 20-amino acid segment of the PPC protein from *Vitis vinefera* that has very good antimicrobial activity against *Xf* (Chakraborty et al. 2014b; Dandekar et al. 2013). The final construct, CAP-7 in pDG14.01 (Fig 1), expresses PrtA, a protease with antimicrobial activity against *Xylella* in a tobacco system (Gouran et al., 2016) All seven vectors were transformed in the plant transformation facility and transgenic grapevine rootstocks have been obtained.

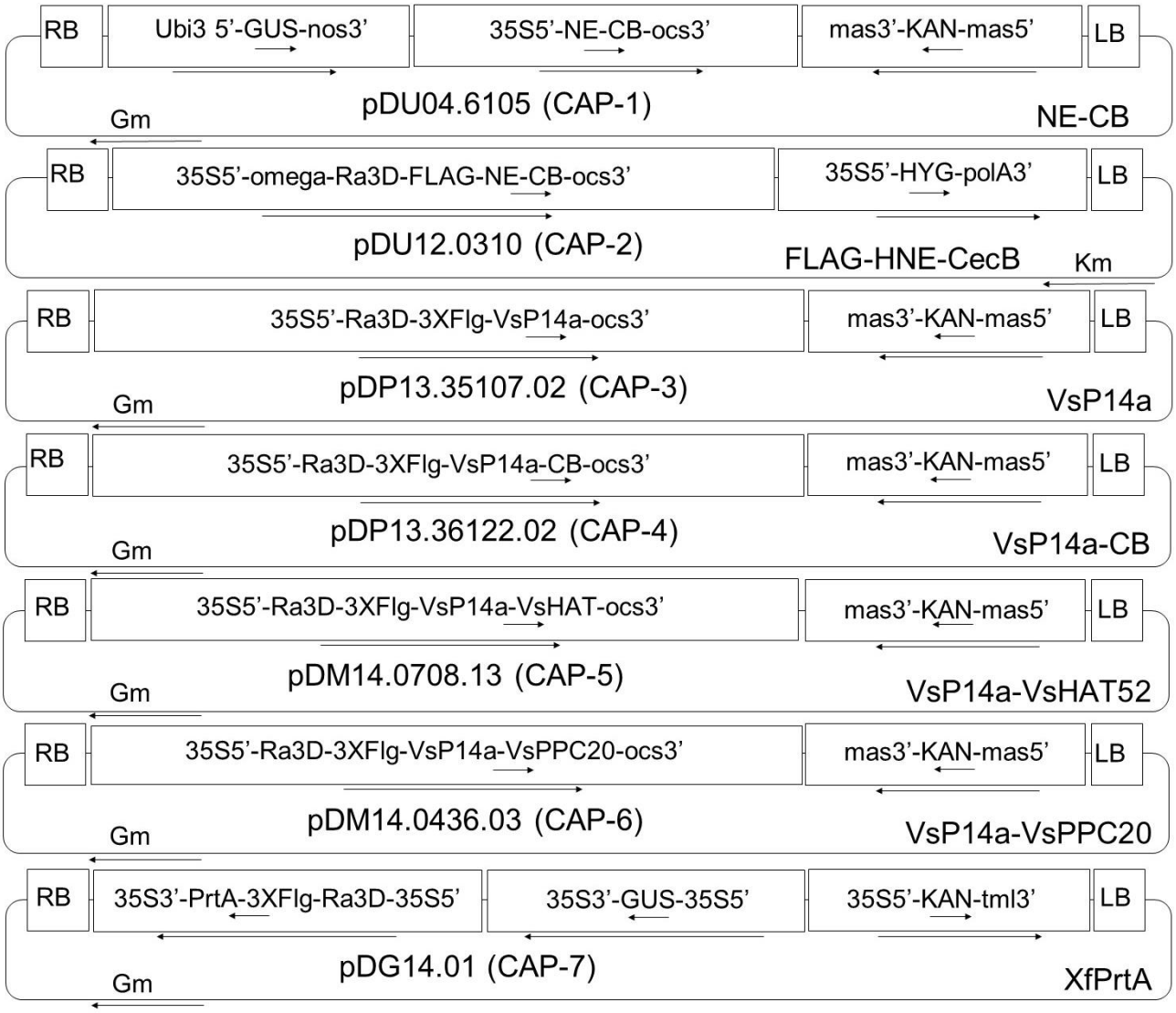

Figure 1. CAP vectors used to develop transgenic rootstocks that will be evaluated in the field.

In addition to the seven CAP constructs, we will evaluate five PGIP constructs (Fig 2). The PGIP-1 construct, pDU94.0928, is the original pear PGIP expressed in grapevine and shown to provide resistance/tolerance to PD (Aguero et al., 2005). PGIP-2, in pDU05.1002, encodes a pear PGIP sequence with its native signal peptide deleted and is called mPGIP, as it is similar in sequence to the mature form of PGIP found in pear tissues. PGIP-3, in pDU05.1910, contains a pear PGIP coding sequence fused to the signal peptide from the nt-protein of grapevine, which was sequenced previously (Aguero et al., 2008). PGIP-4, in pDU06.0201,

contains the mPGIP coding sequence fused to the signal peptide from the chi protein from grapevine, also sequenced previously (Aguero et al., 2008). PGIP-5, in pDA05.XSP, contains the mPGIP coding sequence fused to the signal peptide from a xylem abundant protein from cucumber and PGIP-6, in pDU05.0401, links the mPGIP sequence to the Ramy3D signal peptide from the rice alpha-amylase protein.

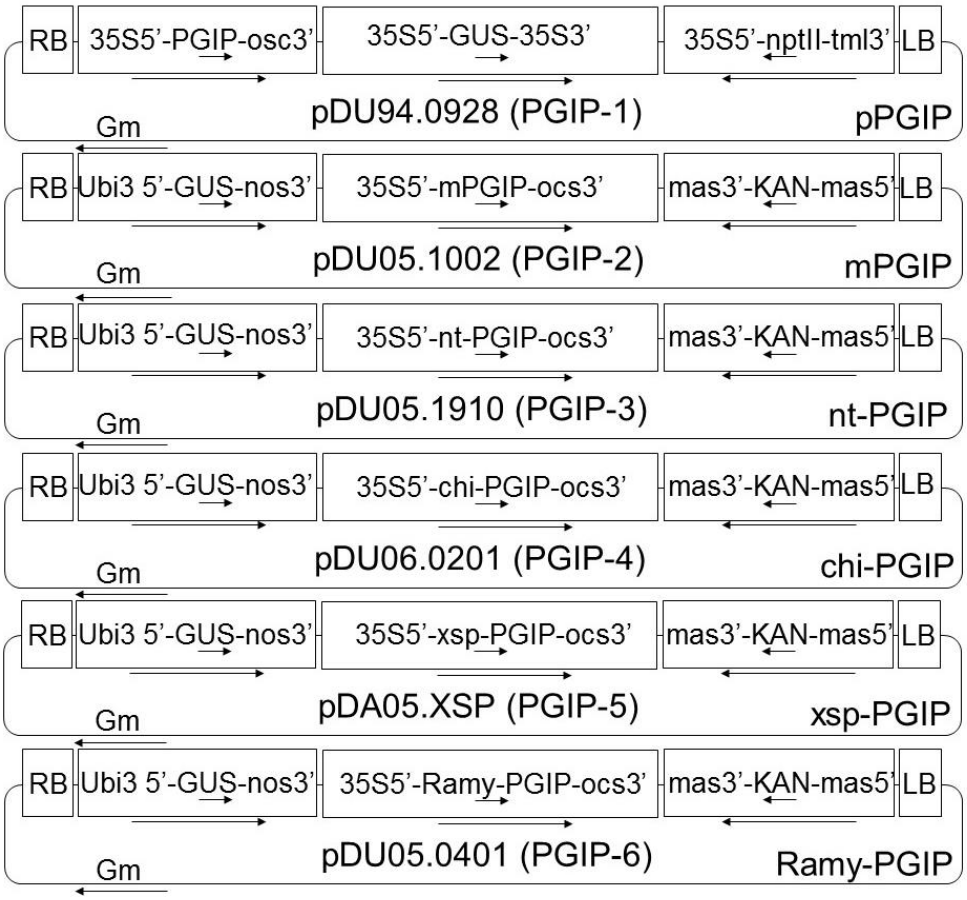

Figure 2. PGIP vectors used to develop transgenic rootstocks that will be evaluated in the field.

Table 1: List of transgenic lines in the greenhouse generated from the vectors shown in Figures 1 and 2.

No	Construct	Binary vector	Transgene	Number of novel lines			No of events
				101-14	1103	TS	
1	CAP-1	pDU04.6105	NE-CB	6			6
2	CAP-2	pDU12.031	NNE-CB		9		9
3	CAP-3	pDP13.35107	VsP14a	24	1		25
4	CAP-4	pDP13.36122	VsP14a-CB	24	1		25
5	CAP-5	pDM14.0708	VsP14a-VvHAT52	3	4		7
6	CAP-6	pDM14.0436	VsP14a-VvPPC20	7	4		11
7	CAP-7	pDG14.02	XfPrtA	9		14	23
8	PGIP-2	pDU05.1002	mPGIP			4	4
9	PGIP-3	pDU05.1910	nt-PGIP			4	4
10	PGIP-4	pDU06.0201	chi-PGIP			4	4
11	PGIP-5	pDA05.XSP	xsp-PGIP			4	4
12	PGIP-6	pDU05.0401	Ramy-PGIP			4	4
13	WT			1	1	1	3
				Total number of lines			129

All seven CAP and five PGIP constructs have been transformed successfully into grapevine by the plant transformation facility and transgenic plants have been steadily appearing; it takes 18 months to get back transformed plantlets. This task has been completed and 129 lines have been obtained. It is difficult to

transform 101-14 lines and more so for 1103 (Table 1). Two types of analysis are carried out with each transformed line as it emerges from the plant transformation facility to confirm that they are transgenic and that they express the protein. DNA and proteins are extracted from leaves obtained from each plantlet. The extracted DNA is used to confirm integration of T-DNA through positive PCR amplification of the Kan/Hyg gene present in all of our CAP and PGIP vector constructs as described (Dandekar et al. 2012a). The extracted proteins are seperated on a gel and a western blot analysis is carried out using antibodies raised against FLAG, an epitope present in CAP proteins 2 to 7, but not CAP-1. In some cases, we also isolate RNA to confirm expression of the CAP transgene, first as a positive PCR product using cDNA copied from the RNA and then to quantify the amount of expression using qRT-PCR as described (Dandekar et al. 2012a). Lines with no PCR product for the Kan/Hyg genes are not propagated and are discarded.

Table 2: List of constructs and lines that were planted in the field in August 2018.

No	Construct	Binary vector	Transgene	Number of novel lines			Plants in Field
				101-14	1103	TS	
1	CAP-1	pDU04.6105	NE-CB	6			47
2	CAP-2	pDU12.031	NNE-CB		9		53
3	CAP-3	pDP13.35107	VsP14a	7			42
4	CAP-4	pDP13.36122	VsP14a-CB	3			12
5	CAP-5	pDM14.0708	VsP14a-VvHAT52	1	3		24
6	CAP-6	pDM14.0436	VsP14a-VvPPC20	7	4		63
7	PGIP-2	pDU05.1002	mPGIP			4	24
8	PGIP-3	pDU05.1910	nt-PGIP			4	24
9	PGIP-4	pDU06.0201	chi-PGIP			4	24
10	PGIP-5	pDA05.XSP	xsp-PGIP			4	24
11	PGIP-6	pDU05.0401	Ramy-PGIP			4	24
12	WT			1	1	1	18
	Total number of lines and plants			60			379

Objective 2: Field test the efficacy of commercially relevant transgenic rootstock lines expressing CAP and/or PGIP proteins to trans graft-protect a sensitive grapevine cultivar from developing and spreading PD.

This objective field-tests all seven CAP and five PGIP lines listed in the last column of Table 1. Table 2 shows the lines that were propagated last fall (2017) to create mother plants that were transferred to the lath house. Foundation Plant Materials and Service (FPMS) helped to create the grafted plants for field planting. First cuttings were harvested from mother plants in the lath house after the plants went dormant. In spring 2018, these cuttings were rooted to make plants that were later budded with the scion variety Chardonnay, creating vines that were planted in the field. FPMS successfully propagated and grafted 70% of our lines (Table 2); the remaining 30% were propagated and bud-grafted by us. On the map of the field planting of 379 plants, there are currently 11 gaps that remain; these plants are being generated and will be planted in spring 2019 (Table 3). On August 1, 2018, we planted the first batch of plants grafted by FPMS, which constituted 70% of the planting. On August 19, 2018, we planted the remaining 30% that were grafted by us. We are currently maintaining stock or back-up plants of all plants listed in Table 2, so we can replace any plants lost in the field. We are currently evaluating 66 remaining lines that have emerged from the transformation facility and are transiting from the lab to the greenhouse. These vines are being tested for protein expression and for the presence of the selectable marker gene. The remaining lines will be propagated to create grafted plants for field introduction next year.

Table 3: Field-planting map of the 60 lines shown in Table 2. The row number appears on the top and the vine number on the side

R/V	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
30	P4.78	C2.6	C1.182	P6.33	P2.29	C5.6	P6.32	C3.3	C1.183	P3.08	P5.23	P6.33	C6.2
29	P4.78	C2.6	C1.182	P6.33	P2.29	C5.6	P6.32	C3.3	C1.183	P3.08	P5.23	P6.33	C6.2

28	P4.78	C2.6	C1.182	P6.33	P2.29	C5.6	P6.32	C3.3	C1.183	P3.08	P5.23	P6.33	C6.2
27	C3.3	C1.186	C5.3	P5.21	C6.9	C3.1	C2.5	C2.2	P2.22	C5.5	C3.5	C2.4	P3.27
26	C3.3	C1.186	C5.3	P5.21	C6.9	C3.1	C2.5	C2.2	P2.22	C5.5	C3.5	C2.4	P3.27
25	C3.3	C1.186	C5.3	P5.21	C6.9	C3.1	C2.5	C2.2	P2.22	C5.5	C3.5	C2.4	P3.27
24	C6.12	P4.7	P3.16	P2.27	M-WT	C5.6	C6.4	C6.1	P4.7	P6.4	C5.1	****	C6.11
23	C6.12	P4.7	P3.16	P2.27	M-WT	C5.6	C6.4	C6.1	P4.7	P6.4	C5.1	****	C6.11
22	C6.12	P4.7	P3.16	P2.27	M-WT	C5.6	C6.4	C6.1	P4.7	P6.4	C5.1	C3.4	C6.11
21	C1.187	C6.8	C1.184	P3.07	C2.7	C6.1	****	P4.56	****	C1.185	C3.6	P4.77	P5.21
20	C1.187	C6.8	C1.184	P3.07	C2.7	C6.1	C2.9	P4.56	C6.9	C1.185	C3.6	P4.77	P5.21
19	C1.187	C6.8	C1.184	P3.07	C2.7	C6.1	C2.6	P4.56	C6.9	C1.185	C3.6	P4.77	P5.21
18	C6.3	P5.27	P2.35	C6.3	P3.08	P6.32	****	C1.182	C6.7	P4.78	C6.12	C6.8	C2.6
17	C6.3	P5.27	P2.35	C6.3	P3.08	P6.32	C1.187	C1.182	C6.7	P4.78	C6.12	C6.8	C2.6
16	C6.3	P5.27	P2.35	C6.3	P3.08	P6.32	C1.187	C1.182	C6.7	P4.78	C6.12	C6.8	C2.6
15	C6.6	C6.4	C2.8	C1.186	C2.2	P2.22	P3.27	P2.35	T-WT	C6.3	M-WT	C6.1	P5.02
14	C6.6	C6.4	C2.8	C1.186	C2.2	P2.22	P3.27	P2.35	T-WT	C6.3	M-WT	C6.1	P5.02
13	C6.6	C6.4	C2.8	C1.186	C2.2	P2.22	P3.27	P2.35	T-WT	C6.3	M-WT	C6.1	P5.02
12	C6.1	C2.1	C1.185	C4.3	C3.3	C2.3	P-WT	P2.27	C1.184	P-WT	C1.186	C3.1	C2.3
11	C6.1	C2,1	C1.185	C4.3	C3.3	C2.3	P-WT	P2.27	C1.184	P-WT	C1.186	C3.1	C2.3
10	C6.1	C2.1	C1.185	C4.3	C3.3	C2.3	P-WT	P2.27	C1.184	P-WT	C1.186	C3.1	C2.3
09	P4.77	T-WT	P6.4	P5.23	C3.7	C4.2	P6.3	P6.3	P2.29	****	C5.6	****	****
08	P4.77	T-WT	P6.4	P5.23	C3.7	C4.2	P6.3	P6.3	P2.29	****	C5.6	****	****
07	P4.77	T-WT	P6.4	P5.23	C3.7	C4.2	P6.3	P6.3	P2.29	C3.3	C5.6	C6.6	P3.7
06	C2.9	C2.5	C3.6	C1.182	C3.5	C3.4	C3.2	C4.3	C2.8	P3.16	P3.07	C5.3	C3.2
05	C2.9	C2.5	C3.6	C1.182	C3.5	C3.4	C3.2	C4.3	C2.8	P3.16	P3.07	C5.3	C3.2
04	C2.9	C2.5	C3.6	C1.182	C3.5	C3.4	C3.2	C4.3	C2.8	P3.16	P3.07	C5.3	C3.2
03	C6.2	C5.1	C1.183	C2.4	P4.56	C6.11	P5.02	P5.27	C1.182	C2.7	C4.2	C2.1	C1.186
02	C6.2	C5.1	C1.183	C2.4	P4.56	C6.11	P5.02	P5.27	C1.182	C2.7	C4.2	C2.1	C1.186
01	C6.2	C5.1	C1.183	C2.4	P4.56	C6.11	P5.02	P5.27	C1.182	C2.7	C4.2	C2.1	C1.186

Fig 3: View of field planting August 2018 (left), and dormant plant pre-pruning February 2019 (right).

Table 4: Lines currently in the lab and greenhouse thate are being evaluated and propagated for field introduction in 2019.

No	Construct	Binary vector	Transgene	Number of novel lines			Plants
				101-14	1103	TS	
1	CAP-3	pDP13.35107	VsP14a	17	1		108
2	CAP-4	pDP13.36122	VsP14a-CB	21	1		132
3	CAP-5	pDM14.0708	VsP14a-VvHAT52	2	1		18
4	CAP-7	pDG14.02	XfPrtA	9		14	138
				66			396

Fig 4: Plants lines being grafted with Chardonnay buds and replicated for 2019 planting.

Publications produced and pending, and presentations made related to the funded project.

Dandekar, A.M., A. Jacobson, A.M. Ibáñez, H. Gouran, D.L. Dolan, C.B. Agüero, S.L. Uratsu, R. Just and P. Zaini. 2019. Trans-graft protection against Pierce's disease mediated by transgenic grapevine rootstocks. Front. Plt. Sci. Feb 6;10:84. doi: 10.3389/fpls.2019.00084.

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Research relevance statement, indicating how this research will contribute toward finding solutions to Pierce's disease in California.

This proposal is a continuation of a project to test expression of a chimeric anti-microbial protein (CAP) and polygalacturonase inhibitory protein (PGIP) as a means to provide resistance to Pierce's disease (PD). Commercially relevant rootstocks expressing these proteins have been planted to initiate a second round of testing that builds on the success observed with the field-testing of TS rootstock lines expressing either CAP or PGIP. The expected outcome of this study is the identification of elite rootstock lines that can be commercialized to provide trans graft resistance to Pierce's Disease.

Layperson summary of project accomplishments.

This project continues the field evaluation of new rootstocks to provide protection against PD to the grafted scion variety. One hundred and twenty new rootstock lines were created and half was planted last summer. The remaining half will be planted this year. Once planting and training are completed, the vines will be challenged by infection with *Xf* to identify rootstock lines that protect the scion from developing PD while maintaining their productivity. Elite rootstock lines identified in this project will be good candidates for commercialization.

Status of funds.

We have expended all the funds available for the period July 1, 2018 to Feb. 28, 2019. Remaining funds will be spent in the period March 1, 2019 to June 30 2019.

Summary and status of intellectual property associated with the project.

An invention disclosure will be made for a plant patent once an elite transgenic rootstock line demonstrates excellent field efficacy in protecting a grafted sensitive scion from coming down with PD.

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