

2018

PRUNE RESEARCH REPORTS



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by

The California Prune Board
(The California Dried Plum Board)

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CALIFORNIA DRIED PLUM BOARD

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January 2, 2019

Dear Reader:

This report summarizes the results to date of the 2018 research projects conducted by University of California and other researchers. California Dried Plum Board (CDPB) funding for these projects totaled \$250,000.

I would like to extend my thanks to John Taylor who serves as Chairman of the CDPB Crop Management & Sustainability Research Subcommittee and continues to bring his depth of knowledge and experience to this role. Along with John's leadership, the Research Program would not function without the assistance of Franz Niederholzer, U.C. Extension Orchard Systems Farm Advisor, who serves as our University Liaison, Luke Kinney-Milliron, Orchard Systems Advisor Butte, Tehama & Glenn Counties and U.C Extension Prune Workgroup Chairman and Gary Obenauf, President of Agricultural Research Consulting, who coordinates the CDPB Research program. I would also like to take this opportunity to recognize Richard Buchner for his years of dedicated work towards improving prune orchard management and profitability.

As a critical component of this program, staff and the subcommittee establish strategic priorities and systematically assess the return on investment that the CDPB contribution delivers to prune growers and handlers. It is our intention to continually ensure that resources are directed towards projects that help growers avoid unnecessary losses and enhance profitability.

The expertise and commitment of the Research Subcommittee members listed below are appreciated. If you have any questions about individual research projects, please contact Gary Obenauf at (559) 449-9036.

Sincerely-

Donn Zea
Executive Director

CROP MANAGEMENT & SUSTAINABILITY

RESEARCH SUBCOMMITTEE MEMBERS

Bob Amarel, Jr.	Ranvir Singh
Matt Bozzo (Co-Chair)	James Strong
Concetta Cotter	John Taylor (Chair)
Matt Kelly	Joe Turkovich
Mark Kettmann	Mike Turkovich
Bob Kolberg	Michael Vasey
Dave Loquaci	Mike Verschagin
Nick Micheli	Melvin Ward
Pete Righero	David Wohletz

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Added: Whole Genome Sequencing of *Prunus domestica* cv Improved French. 18 CPB 1. Dardick, Chris and Tetyana Zhebentyayeva.

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Prune research is on the web site <http://ucanr.org/sites/driedplum> and is searchable.

California Dried Plum Board 2018 Research Proposals				
Project Number	Pages	Project Title/Project Leader	2017 Report Reference Pages	2018 CDPB Request (2016)
Varietal Improvements				
85 CPB 3	1-4	Prune Cultivar Evaluation and Development- Ted De Jong-UC Davis	8-20	\$ 90,259 \$ (123,567)
18 CPB 1	5-10	Development of molecular markers associated with Self-compatibility in dried plum (Prunus domestica)-Richard Dodd-UC Berkeley		\$ 28,898 New
09 CPB 2	11-14	Field Evaluation of Prune Rootstocks-Luke Milliron-Butte County	21-29	\$ 7,445 \$ (2,229)
16 CPB 1	15-17	Field Evaluation of Prune Rootstocks- Katherine Pope-Wolfskill	30-35	\$ 5,000 \$ (2,600)
Flower and Fruit Development				
08 CPB 2	18-19	Managing Heat at Bloom-Franz Niederholzer- UC Sutter County	36-44	\$ 15,500 \$ -
Diseases				
07 CPB 6	20-23	Epidemiology and management of brown rot and rust of prune – Development of an integrated program with new fungicides and optimal timing-Jim Adaskaveg-UC Riverside	48-58	\$ 20,000 \$ (20,000)
13 CPB 2	24-32	Diagnosis, Epidemiology and Management of Canker Diseases in Dried Plums- Themis Michailides-UC Parlier	58-72	\$ 43,065 \$ (44,304)
15 CPB 2	33-36	Investigating Incidence and Type of Wood Decay Fungi in Stone Fruit - Dave Rizzo - UC Davis	73-79	\$ 75,181 \$ (19,156)
Nematodes				
18 CPB 2	37-41	Characterizing current rootstocks of the dried plum industry for host status to plant-parasitic nematodes - Andreas Westphal - Kearney/Parlier		\$ 18,378 New
Miscellaneous				
10 CPB 2	42-44	California Dried Plum Research Reports Database-Carlos Crisosto-UC Davis	80-82	\$ 1,500 \$ (1,500)
16 CPB 4	45-48	Life Cycle Assessment (LCA) of Prune Production-Elias Marvinney-UC Davis		\$ 33,979 \$ -
Research Total				\$ 339,205 \$ (213,356)

2018 CALIFORNIA DRIED PLUM RESEARCH CONFERENCE AND WORKGROUP MEETING

(Hosted by the California Dried Plum Board - Donn Zea, Executive Director and Gary Obenauf, Research Director)

Thursday, December 13, 2018, 9:30am – 5:00 pm

and

Friday, December 14, 2018, 8:30am – 12:30 pm



MEETING LOCATION: Safe Food Alliance – Kingsburg Lab
2037 Morgan Drive
Kingsburg, CA 93631

HOTEL INFORMATION: Fairfield Inn and Suites Selma Kingsburg
216 Ventura Court
Kingsburg, CA 93631
[Visit their website here](#)

MEETING RSVPs: Please RSVP here: ucanr.edu/survey/survey.cfm?surveynumber=25748. If you have any questions reach out to Becky Poland at bpoland@cdpb.org or Luke Milliron at lmilliron@ucanr.edu.

AGENDA – DAY ONE THURSDAY, 12/13/18

9:20 – 9:30 Coffee and pastries will be provided at the meeting.

Introduction

9:30 – 9:45 Introduction to the 2018 Prune Research Conference and Workgroup with highlights of 2018 UCCE prune extension activities and plans for the year ahead. Luke Milliron, UCCE Farm Advisor Butte, Glenn and Tehama counties.

9:45 – 10:00 California Dried Plum Board Welcome and Introductory Comments.

Bloom Management Research

10:00 – 10:20 Measuring heat risk at bloom (08 CPB 2). Dr. Franz Niederholzer, UCCE Farm Advisor Sutter, Yuba and Colusa Counties.

10:20 – 10:45 Thinning for consistent fruit production (report). Franz Niederholzer UCCE Farm Advisor Sutter, Yuba and Colusa Counties.

10:45 – 11:00 Blossom Application of Growth Regulators to Improve Fruit Set (Proposal). Dr. Kelsey Galimba postdoctoral researcher in the lab of Dr. Ann Callahan USDA-ARS, Appalachian Fruit Research Station.

11:00 – 11:15 Break

- 11:15 – 11:35 Life cycle assessment: A tool for quantifying the environmental impacts of fresh and dried plum production. Dr. Elias Marvinney with Dr. Alissa Kendall, Civil and Environmental Engineering UCD and Sonja Brodt, Agricultural Sustainability Institute, UCD.
- 11:35 – 11:50 Effects of mechanical hedging and topping on yield, fruit quality, and labor costs (Proposal). Dr. Richard Rosecrance, California State University, Chico.
- 11:50 – 12:00 California Dried Plum Research Reports Database (10 CPB 2). Dr. Ted DeJong, Plant Sciences UC Davis.
- 12:00 – 12:35 Lunch – Compliments of the California Dried Plum Board.**
- 12:35 – 12:40 Discussion of UC REC charges. Dr. Ted DeJong, Plant Sciences UC Davis.
- 12:45 – 1:00 Re-evaluating our priorities: 2019 re-evaluation of CDPB Research Committee Goals and PRAC Workgroup Priorities. Franz Niederholzer UCCE Farm Advisor Sutter, Yuba and Colusa Counties.
- 1:00 – 1:15 Determining quantifiable payoffs to prune research and extension (proposal). UC Davis Ag Issues Center.

Rootstock Evaluation

- 1:15 – 1:30 Characterizing current dried plum rootstocks for host status to plant-parasitic nematodes. Dr. Andreas Westphal, Nematology, UC Kearney Ag Center Parlier.
- 1:30 – 2:00 Field Evaluation of Prune Rootstocks (09 CPB 2). Luke Milliron & Franz Niederholzer.
- 2:00 – 2:15 Field evaluation of prune rootstocks (16 CPB 1). Dr. Katherine Jarvis-Shean, UCCE Farm Advisor Yolo, Solano, and Sacramento.
- 2:15 – 2:30 Development of molecular markers associated with Self-compatibility in dried plum (*Prunus domestica*, 18 CPB 1). Dr. Angel Fernandez Marti with Dr. Richard Dodd, UC Berkeley
- 2:30 – 2:45 Break
- 2:45 – 3:15 Advances in the development of genomic tools for prune breeding (report). A chromosome scale assembly of the *Prunus domestica* genome using Hi-C and long read sequencing (presentation). Dr. Chris Dardick, USDA ARS, Appalachian Fruit Research Station.
- 3:15 – 4:45 Prune Cultivar Evaluation and Development. Prune tasting and evaluation. Sarah Castro and Dr. Ted DeJong, Plant Sciences UC Davis.
- 4:45 – 5:00 Gathering input on the next generation of field evaluation: Variety trial, rootstock trial 3.0, rootstock x spacing trial? Sarah Castro, Dr. Ted DeJong, Dr. Katherine Jarvis-Shean, Luke Milliron and Franz Niederholzer.
- 5:00 Adjourn for dinner at Fugazzi's compliments of the California Dried Plum Board! No-host bar social hour, preceding 6:00 dinner reservation.
- 1335 Draper Street, Kingsburg, CA.

AGENDA – DAY TWO
FRIDAY, 12/14/18

8:20 – 8:30 Coffee service. Continental breakfast will be provided at the hotel.

Disease Management

- 8:30 – 9:00 Hydrogen Cyanamide, a new tool for those seeking alternatives to soil fumigation (proposal). Michael McKenry, UC Emeritus.
- 9:00 – 9:20 Epidemiology and management of blossom, leaf, and fruit diseases of dried plum (07 CPB 6). Dr. Jim Adaskaveg, Professor Plant Pathology UC Riverside.
- 9:20 – 9:40 Early detection of Cytospora and other canker diseases of prune (13 CPB 2). Dr. Themis Michailides, Plant Pathology UC Kearney Ag Center Parlier.
- 9:40 – 10:10 Phellinus in prune orchards (15 CPB 2). Bob Johnson, graduate project supervised by Dr. Dave Rizzo Plant Pathology UCD.
- 10:10 – 10:25 Break
- 10:25 – 10:30 Tree Crop Intern Program Update (12 CPB 2). Dr. Franz Niederholzer, UCCE Farm Advisor Sutter, Yuba and Colusa Counties
- 10:30 – 10:35 Discussion regarding 2019 Workgroup Chair & Research Tour. Luke Milliron.
- 10:35 – 10:40 Conversions of the Prune Manual and IPFP binder. Richard Buchner.
- 10:40 – 11:00 Postharvest research update. Spencer Walse, USDA ARS Commodity Protection and Quality Research: Parlier, CA.
- 11:00 – 12:30 DFA Laboratory presentation and facility tour. Thomas M. Jones and Dr. Wiley Hall. DFA of California/Safe Food Alliance.
- 12:30 Adjourn. Lunch on your own.

2018 DRIED PLUM CULTIVAR DEVELOPMENT AND EVALUATION

T.M. DeJong and S.J. Castro

INTRODUCTION

California is the world leader in dried plum production but is almost entirely dependent on the use of a single cultivar, the 'Improved French' prune. This monoclonal situation lends itself to vulnerability to widespread disease, pest outbreaks and annual, statewide variations in yield caused by variable weather conditions that can negatively or positively affect fruit set and/or fruit retention. In addition to the risks of a monoculture system, the entire industry harvests and dehydrates the crop within a few weeks, since the entire crop has a similar developmental pattern. The development of new, acceptable or superior, dried plum cultivars will increase the efficiency of California dried plum production and give some protection against the risks involved with a monoculture. The California dried plum industry is also facing increasing marketing competition from other regions of the world and must seek ways to reduce production costs to stay competitive. Thus, the industry would also benefit from the development of new dried plum cultivars that have cost saving characteristics such as improved tree structure that would require less pruning, improved fruit dry matter content that would decrease drying costs, and increased tolerance to pests and diseases. Introducing new dried plums that differ in flavor or color could also promote a broadening of the consumer base.

The Dried Plum (*Prunus domestica*) Development and Evaluation program has enlarged its germplasm and bred new generations of progeny through traditional horticultural breeding methods since its conception in 1985. Through thirty years of evaluation and selection, the breeding program has increased the occurrence of desired characteristics in the germplasm. To insure that the germplasm and new cultivars are well adapted to California's dry, hot climate, the program evaluates elite selections at two locations; the UC Wolfskill Experimental Orchards, near Winters, in the north; and the Kearney Ag Center, near Parlier, in the southern San Joaquin Valley. The breeding program has matured and is now entering what we anticipate to be a very productive period for producing potential new cultivars that are specifically adapted for California growing conditions and markets.

In recent years we have increased our focus on tree and fruit characteristics that will be particularly helpful in reducing grower costs while improving the dried fruit products. To this end we have put a greater emphasis on evaluating tree structure and fresh fruit characteristics that may influence dry-away ratios and ease of dried fruit handling.

In several years during the last decade dried plum orchard yields have been low because of poor weather conditions for fruit set during the bloom period. The consensus is that this has been largely due to high temperatures during bloom. Since the California industry is composed of one cultivar, in some years the whole industry suffered with poor crops during the years of high temperatures during bloom. Because the time of pollination and fruit set is so critical, we have increased the evaluation of our seedlings and selections for

differences in bloom date. In doing so, new cultivars can potentially introduce greater diversity of bloom timing so that the entire Californian crop will not be dependent on the same set of weather conditions during periods critical for fruit set and retention.

PROGRAM OBJECTIVES

Objectives:

- 1.) To develop new dried plum varieties, through traditional horticultural breeding methods, with the following characteristics:
 - Fruit characteristics similar to 'Improved French' so the industry can seamlessly incorporate new cultivars into their handling and marketing systems
 - Tree characteristics that reduce labor costs involved in producing dried plums.
 - Increased fruit quality and fruit characteristics that increase efficiency and quality of drying and processing.
 - Earlier or later bloom dates and tolerance to high temperatures during bloom.
 - Earlier/later fruit maturity dates than "Improved French" dried plum.
 - Increased tolerance/resistance to disease.
 - New specialty traits; with the dried product being equal or improved in quality to "Improved French", but different in taste and/or color.
- 2.) Test and evaluate advanced selections resulting from the current breeding program at UC and grower locations in the Sacramento and San Joaquin Valleys.
- 3.) Cooperate Dr. Chris Dardick (USDA Kearneysville WV) to obtain sources of Plum Pox (Sharka) resistance that can be incorporated into the breeding program.

PROCEDURES

Breeding methods, pollination procedures, seedling cultivation, and selection evaluation were described in detail in the Dried Plum Cultivar Development and Evaluation annual report in the 2004 Prune Research Reports published by the California Dried Plum Board. A few changes have been made over the years to cut operational costs. Costs were cut by utilizing help from industry partners such as Sierra Gold Nurseries to help clean, stratify and plant seed as well as grow nursery plants prior to planting in field seedling blocks. The following is a brief description of our testing and evaluation procedures as a reference for the Results section of this report.

Levels of Testing

Field testing and evaluation of dried plum selections developed within this program are being carried out at four levels.

Level 1 testing involves evaluations made in the seedling blocks located at UC Davis. The initial fruit evaluation is made on self-rooted seedlings in high density seedling blocks. Fresh and dried fruit characteristics are evaluated at this level of testing. If a positive evaluation results, the seedling becomes a “selection” and is then considered for re-propagation in dried plum selection blocks located at the Kearney Research and Extension Center in Parlier, CA and at the Wolfskill Experimental Orchards in Winters, CA.

Level 2 testing occurs in the selection blocks at Wolfskill and Kearney. Depending on the perceived potential of the individual selection, two to four trees of any one selection are established on commercial rootstocks. This level of testing is concerned with fruit characteristics and tree growth habit. Variations in fruit size, tree vigor, maturity date and other characteristics may, and often do, occur when the selection is grafted onto a rootstock from the original seedling. Individual selections are evaluated using specific criteria that match the goals of the program. These criteria must be achieved before advancing to Level 3. Therefore, there are multiple types of Level 2 trees: those that have yet to fruit in the selection block; others that are still being evaluated and have the potential to advance to grower’s orchards and others that are kept for germplasm and breeding purposes.

Level 3 testing involves the establishment of advanced selections in grower orchards in various locations. This level involves items that have been extensively tested in the selection blocks and are ready for more in-depth evaluation. Despite this, testing at this level is still somewhat preliminary since these plantings are the first instance in which selections are established in varying soil types and in varying climatic regions. Again, depending on the perceived value of the individual item, two to one hundred trees of any one selection are established at any one location. Level 3 grower tests are established in counties throughout the Sacramento and San Joaquin Valleys where dried plums are a commercial crop. In recent years we have increased our selectivity of trees advancing to Level 3 status. The specificity of criteria for new advanced selections is quite narrow and we have chosen to not promote trees to this level until we have confidence in the desirability of their structure, production and process-ability.

Level 4 testing involves the planting of extensive test acreage, usually of a single targeted selection. The size of these Level 4 plantings depends on the apparent potential of the individual selection and the level of risk that the cooperating grower is willing to assume. Ideally these plantings would be as large as 20-40 acres. At this level, thorough tests of process-ability and acceptability in the commercial market are conducted. These tests are designed to gauge the commercial value of the item prior to formal release. The promotion of items to Level 4 is based on the industry’s input and feedback. When the California Dried Plum Board decides a selection is ready for such extensive testing, the University and breeders will develop a research agreement with the Dried Plum Board and the grower. Release of the selection for full-scale commercial production will be delayed until a decision by the Dried Plum Board is made concerning the suitability and desirability of the selection for further commercial production.

Dried Plum/Prune Testing Group

The Plum/Prune Testing Group incorporates the participation of growers and processors to evaluate and test dried plum selections for their potential as new cultivars before patenting and public release. Participation in the group involves two general meetings a year, one in the summer just before prune harvest to look at fresh fruit and tree characteristics and a second time in the fall or winter, for the evaluation and discussion of dried product characteristics. The objective is to benefit from greater grower and processor input on individual selections as well as increase grower test plot participation so that by the time a selection is identified for release, the industry is well informed about the cultivar and comfortable about committing to plant, process and sell the cultivar commercially.

The Dried Plum/Prune Testing Group is currently the primary group that will make recommendations to the California Dried Plum Board for initiating large-scale Level 4 commercial testing of new selections. The advantage for participation in this testing group is that growers and processors gain first-hand information on all new selections in the program on which to base future planting/marketing strategies, participate in test plantings, have early access to new cultivars slated for release, and help direct the breeding and evaluation program to address germplasm-based issues in the future.

RESULTS

Bloom Data

The importance of bloom data has grown in the last decade because of the changing weather patterns that California has experienced. It has become more common to have warm spells in March that often have temperatures near 80°F. If high temperatures occur when 'Improved French' is blooming the biological mechanisms for successful pollination and fertilization are negatively affected. Historically, the result has been low fruit set across the state. Variation for time of bloom is naturally found within the breeding program's germplasm. Introducing new cultivars to the California dried plum industry that have bloom times earlier or later than 'Improved French' could reduce the risk of having the entire crop reliant on good weather conditions occurring during 'Improved French' bloom. Bloom set was reduced in two of the last five years in specific areas of California due to low chill winters. The 2016 the state-wide crop was one of the lowest in years, but that conversely was not due to low chill but due to an aggressive storm near bloom. Regardless of the reason for a specific year's crop failure, the need for a spread in bloom remains essential for industry-wide success in maintaining crop security from year to year.

Bloom data, including date of full bloom (90% flowers open), amount of bloom, and the first and final day of bloom have been recorded for all the Level 2-4 selections since 2003. Table 1 shows the number of days each top selection blooms, days before or after 'Improved French' full bloom as well as the number of days in bloom, the 90% full bloom date and the average bloom date relative to 'Improved French' over the last 2-5 years when known.

Table 1. Bloom data at the Winters selection orchard for the 2018 top selections.

Item Name	2018 Full Bloom Date (90%)	Days in Bloom 2018	Days from Imp. French 2018	Average Days from Imp. French
G37S- 45	18-Mar	12	-11	-8
G43S- 15	23-Mar	17	-6	-7
H13S- 65	13-Mar	15	-16	-11
H13S- 58	26-Mar	14	-3	-4
H9S-27	20-Mar	10	-9	-7
H18N- 42	20-Mar	11	-9	-8
I2N-32	25-Mar	10	-4	*
I14N-25	28-Mar	14	-1	*
H7S-12	28-Mar	18	-1	*
J2S- 58	18-Mar	10	-11	*
H11N- 87	26-Mar	14	-3	*
Imp French	29-Mar	18		

* 2018 first year of bloom data

Level 4 Testing

As of now, there are no active Level 4 selections. We would however recommend to the industry to advance H13S- 58 to large scale testing. It has a very low dry away ratio, wide harvest window, partially dries on the tree, self-pollinates, and harvests with or after 'Improved French'. Historically, this fruit has an average dry away ratio of 2.5 but can get as low as 2.2. At the CDPB meeting in 2017, tasting participants were asked to guess the harvest dates spanning 3 weeks. Four out of 13 participants were able to guess the correct dates. The reason the tree was harvested over three different weeks was to determine the best harvest timing; the fruit can hang remarkably well on the tree, and does not soften like a typical 'Improved French'. The results from the tasting suggest that fruit quality doesn't discernably change over a wide, 3-week harvest window. As the fruit matures, it tends to dry on the tree, thus making the fruit firmer and sometimes even making the fruit pressure rise. In 2017, all fruit was dried at the UC Davis pilot plant, then run through a commercial Ashlock pitter. The pitted fruit was beautiful and seemed to be able to withstand that type of pitting process.

This year, the mature H13S-58 tree in Winters was caged again to confirm for a second time, its bloom self-compatibility. The tree did set some fruit but did not have a large crop. As many industry members are aware, bloom this year produced low crops all over the state. This happened in our orchard as well. We intend to re-cage the tree a third year, to again confirm its ability to produce self-compatible pollen. Despite this set back, our cooperating growers still plan to plant more trees of this item. Expansion plantings started in 2016, we anticipate to have at least a few bins of fruit to dry and pit in 2019 to further test this fruit's processing abilities. By 2021 and 2022, there will be enough fruit to provide a

thorough pitting evaluation with ashlock type pitters. And, by 2023, there will be enough fruit to run a thorough evaluation at the Sunsweet plant.

Level 3 Testing

Level 3 testing items are selections that are ready for small trials in grower's orchards. We have chosen only to promote selections to Level 3 status when the tree has proven to meet specific criteria over multiple years. This has limited the number of active Level 3 selections. We only plant trees in grower's orchards when we are fairly confident in their fruit and tree quality. The top selection at Level 3 is H13S- 58. Last year's Level 3 items G37S- 45, G43S-15 and H13S-65 are listed in Table 2 as well as H18N- 42.

Table 2. Level 3 selection performance for 2018 at university selection blocks. 'Days from French' refers to the difference between the 'Improved French' harvest date (8/22 at Kearney and 8/27 at Winters) and the harvest date of the selection at the same location.

Harvest date	Item Name	Location grown	Days +/- French	Weight (g/frt)	Pressure	Sugar in Brix	Dry away ratio	Dry count per lb.	Comments
8/27	H13S- 58	Winters	0	21.8	3.9	28.9	2.43	56.7	yellow fruit dries on the tree, wide harvest window. In grower trials currently. Harvests with or after Imp. French
8/22	H13S-58	Kearney	0	26.7	3.9	24.3	2.57	55.9	
8/13	G37S- 45	Winters	-14	22.8	5.4	29.0	2.35	53.4	Wide branching tree, self pollinating. Round purple fruit
8/22	G37S-45	Kearney	0	26.2	3.3	25.7	2.55	47.2	
8/20	H18N- 42	Winters	-7	25.7	3.3	32.3	2.42	46.3	Self pollinating, new promising fruit
8/7	H18N-42	Kearney	-14	27.4	3.5	24.9	2.71	48.8	
8/27	H13S- 65	Winters	0	27.5	3.5	26.0	2.87	45.9	Good tree structure, Imp. French shape fruit. Self compatible, but should be retested
8/22	H13S-65	Kearney	0	35.9	2.6	21.8	2.91	38.4	

H13S-58: This top item is an offspring of open pollinated D2N- 76, which was a former top item. As mentioned in the Level 4 section, this is a good tasting dried fruit with a low dry away ratio. It is a yellow fresh fruit that can be a little astringent if picked too early. The high sugars are usually due to the fruit starting to dry on the tree before harvest. This was its fifth year of evaluation. In 2016, it was caged, and was determined to be self-pollinating, but the self-pollination test in 2018 was inconclusive. Bloom time is typically 4 days before 'Improved French' and harvest ranges from -3 to 16 days after Imp. French.

H13S- 65 is a sibling of H13S- 58. This good looking tree was caged in 2017 and had a low fruit set. So it is likely self-pollinating, but needs to be retested. Fruit is medium-large with low dry away ratios (2.7-2.9), purple, has thick flesh and small, free pits. The tree has an upright and spreading structure that could help reduce pruning as compared to 'Improved French'. This year there was a great fruit set, and good fruit size.

G37S- 45 was grafted into the seedling blocks in 2012. This year it looked good with non-juicy fruit, is pollen self-compatible and has a small pit. It has historically had a low dry away ratio of 2.6, 2.5, and 2.0 in 2014, 2015 and 2016, respectively. It has had some mediocre dry evaluations in the past for skin peel. In 2017 the dried fruit looked good, but this year the dried fruit looked dull, but that would probably disappear during processing. We would recommend this fruit be solely for commercial processing with addition of potassium sorbate. The round, dull appearance as a natural product would not likely be accepted in the marketplace. The tree structure is wide and spreading, it was planted in a grower's orchard Fall 2018, so we will be able to begin to determine the best way to prune and manage its structure. In 2017, fruit was dried at a Sunsweet drying facility, then rehydrated and pitted in an Ashlock pitter. The dried, pitted fruit looked very similar to the 'Improved French' pitted at the same facility.

H18N-42 was spotted in the seedling block by grower cooperator Joe Turkovich in 2013 and had its second year of fruit in the selection block this year. It was caged this year and the fruit set indicates that the tree is likely self-pollinating. It should not be long pruned because it produces fruit on first year wood. The fruit tends to dry on the tree, creating a dry away ratio of 2.5 last year, 2.7 at Kearney in 2018, and 2.4 at Winters in 2018. There was a little bit of heat damage in the fruit this year, but even so it had a really good dried evaluation. This tree would be a good candidate for grower testing if there is interest by the industry. The fruit typically ripens 7 to 10 days before 'Improved French'.

G43S-15 has been a top item in previous years, it is not listed in the table because the pit size is too large to qualify as a top item. It harvests 7-13 days before Improved French. It is the product of a cross between D4N-46 and Muir Beauty. Over the years it has had low dry away ratios of 2.9, 2.8, and 2.5 in 2014, 2015, and 2016, respectively. The main concern in the past was that it may be too large for some pitting operations, this year the pit appears to be too large. So pending interest from the industry, this item will be discarded from the program.

Level 2 Testing

Level 2 testing evaluates a selection after it has been promoted from the Davis seedling blocks to the advanced selection blocks at Kearney and Wolfskill. Once the tree has matured and has started producing fruit, the whole tree and fruit characteristics are evaluated. Table 3 shows the harvest data of the top Level 2 selections this year. This is a very exciting time in our program since many of our newer Level 2 trees are starting to bear fruit in the selection block. Since 2012, the increase of selections in Winters have made for a lot of evaluations during harvest. These evaluations are important to determine if the promising characteristics observed in Level 1 seedlings transferred over to the grafted Level 2 trees in the selection block. With many of these fruit with low dry away ratios, there is a tendency for the fruit to dry on the tree, have late harvest and have dense fruit flesh. These characteristics will likely change how pressure is used as a harvest indicator in the future.

Table 3. 2018 Level 2 selection performance in University blocks. 'Days from French' refers to the difference between the Imp. French harvest date (8/27 at Winters) and the harvest date of the selection at the same location.

Harvest date	Item Name	Days +/- French	Weight (g/frt)	Pressure	Sugar in Brix	Dry away ratio	Dry count per lb.
8/13	H9S- 27	-14	21.8	6.3	20.9	2.3	52.3
9/10	H15S- 71	+13	35.6	9.7	28.3	2.7	37.8
9/4	H7S- 12	+7	25.5	5.4	32.6	2.5	48.2
8/27	I14N- 25	0	25.1	4.5	25.2	2.6	36.5
9/10	H11N- 87	+13	29.1	7.2	26.0	2.7	39.9
9/4	J2S- 58	+7	26.6	5.2	30.3	2.6	48.3
9/10	J2N-127	+13	24.5	6.4	30.0	2.4	47.3
8/20	I2N- 32	-7	20.6	4.6	25.8	2.8	61.4
9/4	H18N- 22	+7	20.8		29	2.5	56.7

H9S-27 was grafted into the selection block in 2014. This is its third year of fruit evaluation in the selection block. It has stood out because of its low dry away ratios: 2.4, 2.4 and 2.3 in 2016, 2017 and 2018, respectively. The tree produced a small amount of fruit under a cage this year, so is likely pollen self-compatible or partially self-compatible. The fruit is small, and might be too small for commercial production. Last year the average count per lb. was 52.5, this year at Kearney it was 41, but in Winters (with low fruit set) it only had a count/lb/ of 52.3 which appears comparable to 'Improved French'. The program encourages industry feedback on whether the fruit of this item is indeed too small.

H11N-87 was grafted into the selection block in January 2016 and has an upright structure. The tree is precocious and produces fruit on first year wood. Interestingly, the first-year wood that grew fruit had the ability to hold its branches upright despite the weight of the fruit. This would be a beneficial characteristic to add to our germplasm. The dried fruit looks promising with a dry away ratio of 2.7 and excellent dried taste. The fruit is also unique in the fact that it does not soften as it ripens. The fruit this year was harvested on September 10th with a pressure of 7.2. When selected from the seedling block in 2015, the pressure was 6.3 and dried to a 2.8 dry away ratio.

Items **I14N-25**, **J2N-127**, **H18N-22**, **H7S- 12** and **J2S- 58** are all Level 2 items that looked promising this year. They all have dry away ratios of 2.6 OR LESS and harvest with, or after 'Improved French'. These items typically dry slightly on the tree, so the flesh does not soften as a typical 'Improved French' fruit would. The pressures of these items ranged from 4.5 to 6.5.

Level 1 Testing

Level 1 testing evaluates the young seedling trees at Davis with fruit quality being the primary selection criteria at this level. The seedlings set medium to heavy sized crops and minimal thinning was done. Fruit samples of 115 trees were taken from the Level 1 seedling blocks for fresh evaluations. Of those, 88 samples were dried and processed for

the rehydrated in-house tasting evaluation in October. Twenty-two of the 88 items were chosen to be grafted into the selection blocks. Table 4 shows the harvest data of the top 22 Level 1 seedlings. The fruit from items selected in the last few years have substantially lower dry away ratios than we have seen in prior years. This is the result of continued development of an advanced prune germplasm collection that has enabled selection of parent genotypes to create new selections that can substantially improve fruit dry away ratios and potentially impact grower profitability.

Table 4. 2018: Harvest data for advanced selections in Level 1 seedling testing at Davis arranged by date. Improved French harvest date for that block was 9/4/18.

Harvest date	Item name	Days +/- French	Weight (g/frt)	Pressure	Sugar in Brix	Dry away ratio	Dry count per lb.
7/19	J11N-175	-44	23.8	2.4	25.6	2.87	52.7
7/19	J3N-196	-44	22.7	1.9	32.8	2.39	44.6
7/24	J4N-138	-40	25.4	4.9	26.9	2.69	52.2
7/26	I12N- 68	-37	37.5	6.5	24.7	3.17	40.9
7/31	J1S- 13	-33	30.0	4.7	22.4	3.20	53.5
7/31	J6N- 114	-33	28.2	4.9	29.3	2.76	44.4
8/8	I11N- 13	-26	25.5	5.9	24.5	2.48	48.6
8/20	J5N-150	-14	29.0	4.8	29.1	2.84	47.1
8/21	J1S- 33	-13	23.0	6.3	28.4	2.59	51.1
8/21	J2S- 20	-13	23.4	3.8	31.0	2.49	47.9
8/28	J10N-168	-6	26.9	2.5	26.0	3.05	50.5
8/28	J10S- 56	-6	32.6	3.3	24.9	2.65	42.1
8/29	J10S- 71	-5	35.9	9.2	28.8	2.61	36.7
8/30	J9N-134	-4	25.5	3.4	27.8	2.69	49.9
9/6	I17S- 42	+2				2.61	39.3
9/6	J10N-130	+2	26.0	5.3	26.4	2.87	54.1
9/6	J2N-156	+2	27.9	8.1	31.2	2.41	40.5
9/6	J2N-182	+2	27.0	4.0	28.7	2.64	46.6
9/6	J2S- 98	+2	38.4	4.3	31.3	2.55	33.3
9/6	J3N-127	+2	27.4	8.0	31.5	2.47	44.8
9/6	J3N-149	+2	25.4	6.1	32.2	2.26	44.7
9/11	I13N- 84	+7	30.9	6.7	28.1	2.66	44.1

Levels Summary

In 2011 the program was challenged to aggressively pursue reducing grower input costs by reducing the dry away ratio and reducing the costs of pruning through new cultivar development. This program has responded to the challenge and all of our top Level 2 and

Level 3 items have a dry away ratio of less than 3.0. In doing this, the program has bred new selections that could save California growers money by reducing the cost of dehydration. Item H13S-58, with its dry away ratio of 2.5 and wide harvest window, is an example of a selection that could dramatically reduce the cost of drying, however the industry will have to decide if it can handle dealing with this new potential cultivar. Extra tests need to be performed to determine the best drying times and temperature for fruit that have already lost a significant portion of their water content prior to harvest.

In regards to reduced pruning costs, we have many new items with more spur bearing tree structure and an upright growing habit. For example, H13S- 65 and H11N-87 both have upright growing habits. Additionally, most of our selections will produce fruit on first year wood. This means the trees will start to bear fruit at a younger age than 'Improved French'.

Program Inventory

All the seedling blocks are located in the UC Davis campus research orchards (Table 5). In the summer of 2017, over 1,000+ seedling trees were discarded after evaluation of the seedlings showed negative fruit or tree characteristics. This year, we will discard all of the I block and over 500 trees of the J block. Crosses were made in spring of 2016, the seeds were germinated in January and were planted in the fall at a local nursery. These second year bare root nursery trees will be planted in January of 2019. Potted seedlings from 2017 will be planted in spring of 2019 and seeds from the crosses made in 2018 are currently going through stratification for planting. The inventories of selections at each level of testing were re-inventoried and are shown in Table 6. The numbers in this table represent the number of unique selections and not the number of trees. The "breeding population" category was separated into two categories, breeding and germplasm. The breeding trees are actively being used for breeding whereas the germplasm items are old selections or cultivars collected from other programs that have negative characteristics that prevent them from currently being used in breeding. There is value in preserving them in our germplasm collection to keep the species-wide germplasm diversified. They may someday be important parents for future generations. Because of funding and space constraints we plan to discard a segment of our germplasm and breeding stock. We have decided to cut out a majority of the trees in the Kearney selection block. Due to increases in land and labor costs, only special trees and Level 3 items will be grown there.

This program usually breeds trees using a mix of hand pollinations and crosses from pollination cages. This is the second year of using only caged pollination crosses. These caged crosses create a large amount of seeds for planting in the seedling block (Table 5). These large amounts of seedlings will allow our program to be extra selective when choosing trees from the seedling block.

Table 5. Seedling block inventories for 2018 located in the Davis UC research orchards.

Block	Acres	Year Planted	Seedlings Planted at UCD	Seedlings Remaining	New Advanced Selections
J	4	2013-cont.	5,022	2,902	34
Nursery trees		2016		(1,560) ^c	
Nursery trees		2017		(3,197) ^f	
Seeds		2018		(4,626) ^e	
Totals	4		5,022	7,659 ^d	

^cnumber of seedlings in the nursery row to be planted in January.

^d not including seeds

^e seeds under stratification

^f potted seedling to be planted in Spring

Table 6. Number of unique selections in the dried plum program and their level of testing including the breeding and germplasm population.

Level of Testing	Number of Items	Number of new 2018 additions
Level 1	2,902	4,750 ^(c+f) (4,626)
Level 2	95	22
Level 3 & 4	4	1
Fresh Items	11	2
Breeding Items	82	5
Germplasm Items	80	8
Items Removed	25	

^cnumber of seedlings in the nursery row to be planted in January.

^f potted seedling to be planted in Spring

Disease Screening

This year, little disease pressure was displayed in the orchard. Therefore, no statistical data was collected on brown rot. Seedlings with visible brown rot hits were rogued from the program. There were also very few incidences of scab in our orchards this year, nonetheless, a few selections were evaluated for scab. If an item showed either scab or brown rot it was noted and the item was marked as more susceptible than the general population. Any genotypes documented as being more sensitive to scab than 'Improved French' were discarded.

Plum Pox Virus (PPV)

This program is taking action in preparation for when Plum Pox Virus might come to California. As mentioned in previous reports, we have been incorporating Stanley and Jojo genetics into the germplasm. We have also been contributing to Dr. Chris Dardick's (formerly Dr. Ralph Scorza's) research on fast track genetically modified plum pox resistant plums.

In 2016, the program initiated the importation of hypersensitive cultivars from the German breeding program of Dr. Neumuller. These items have the potential to either be good California cultivars or good breeding sources for hypersensitive resistance. The trees are scheduled to come out of quarantine in Winter 2019-2020. We also have recently acquired trees of the 'Docera 6' rootstock that has hypersensitive resistance to PPV. We hope to plant 'Improved French' and top items from the program on this rootstock to begin determining it's adaptability to California conditions.

Dried Plum/Prune Testing Group Evaluations

The Dried Plum/Prune Testing Group met in August this year at the Wolfskill Experimental Orchards to discuss strategies for testing and to tour the program's orchard. The group looked at fresh fruit and tree characteristics of top selections and discussed their potential as cultivars. Starting in 2011, the November meeting was moved to combine with the Dried Plum Research and Workgroup meeting in December. This was done to help reduce travel for those located far from Davis. The workgroup evaluated our top selections and the results of this tasting are located at the end of this document (Table 8). Table 7 provides details on the fresh and dried characteristics of each of the selections chosen for the December taste testing.

Table 7. Summary table of all the items tasted at the 2018 CDPB meeting in December. All items were grown in the selection blocks using various rootstocks and different harvest dates.

Taste #	Harvest date	Item Name	Location grown	Days +/- French	Weight (g/frt)	Pressure	Sugar in Brix	Dry away ratio	Dry count per lb.
1	8/13	G37S- 45	Winters	-14	22.8	5.4	29.0	2.35	53.4
2	8/22	H13S-58	Kearney	0	26.7	3.9	24.3	2.57	55.9
3	8/27	H13S- 65	Winters	0	27.5	3.5	26.0	2.87	45.9
4	8/27	I14N- 25	Winters	0	25.1	4.5	25.2	2.62	36.5
5	8/27	J2N-127	Winters	0	25.5	6.5	27.1	2.99	54.0
6	9/4	H7S- 12	Winters	+7	25.5	5.4	32.6	2.56	48.2
7	9/10	H15S- 71	Winters	+14	35.6	9.7	28.3	2.79	37.8
8	8/7	H18N-42	Kearney	-13	27.4	3.5	24.9	2.71	48.8
9	8/7	H9S-27	Kearney	-13	27.8	4.9	26	2.41	41.4
10	8/27	IMP FRENCH	Winters		22.0	3.0	27.2	2.74	56.0

Table 8. Results from the industry tasting conducted by members at the California Dried Plum Board annual meeting in December. Table listed by tasting number, comments are a compilation of responses. Ranking is 1-5, 1 being poorly rated and 5 being the best.

	Overall Taste	Skin Quality	Flesh texture	General Appearance	n	Comments
G37S- 45	2.8	3.4	2.9	2.7	13	good flavor, dark flesh, bad, small too dry
H13S-58	3.1	3.3	3.2	3.8	13	nice yellow flesh, floral, bland but good
H13S- 65	2.7	3.2	3.1	3.6	13	tart, chewy, not enough flesh, good
I14N- 25	3.3	3.7	3.1	3.5	13	cling, dull skin, balanced flavor, dense, gooey, thick skin
J2N-127	3.6	3.2	3.2	3.1	13	distinct taste, sweet, molasses taste, good texture
H7S- 12	2.6	3.0	2.5	2.7	13	dry, tough, speckled, closest flavor to French
H15S- 71	3.5	3.2	3.2	3.2	13	melty
H18N-42	2.7	3.4	3.6	3.0	13	tastes like fruit leather, pit seems to crack, good flesh, mixed color, my favorite
H9S-27	2.8	3.6	3.4	2.6	13	too much skin, not bad
Improved French	3.2	3.2	3.2	3.3	12	bland but not good, too thick skin, crunch, not very tasty

DONATIONS

We would like to thank Sierra Gold Nursery, for the donation of nursery care of the program's seedlings and donation of shade cloth for pollination isolation cages. Their generosity helps support UC research and the California dried plum industry's goal in developing new dried plum cultivars for California.

Project Title: DEVELOPMENT OF MOLECULAR MARKERS ASSOCIATED WITH SELF-COMPATIBILITY IN DRIED PLUM (*PRUNUS DOMESTICA*)

Angel Fernandez i Marti, Sarah Castro, Ted DeJong and Richard Dodd

ABSTRACT

Information on the *S*-genotype of European plum cultivars is rather limited. Until now, no cross-incompatibility groups have been determined and knowledge about the number of plum *S*-alleles is uncertain. Based on early controlled self- and cross-pollination experiments in other *Prunus* species, “fully” self-compatible, “partially” self-compatible and self-incompatible cultivars were described. Since self-incompatibility is general in plum, cross-pollination is essential for most cultivars. Male sterility may also occur in some domestic plum cultivars and this further complicates the optimal orchard design. Because there are no available specific markers of plum *S*-alleles in comparison with other *Prunus* species, primers designed for the conservative regions of *S*-RNase (pistil determinant) and F-box (pollen determinant) genes will be used for gaining information about hexaploid plums.

OBJECTIVES

This project aims to develop short term and long-term solutions to sexual compatibility problems to address the needs of specialty crop growers and to advance knowledge on dried plum breeding, genetics and genomics. The proposed project evaluates and advances classical novel horticultural approaches that have never been addressed in domestic plum, such as self-incompatibility that will help the dried plum industry. This project will address long-term industry needs by enabling and accelerating the discovery of natural sources of self-compatibility by direct DNA analysis. It will allow the rapid implementation of improved orchard management, as well as development of molecular tools that will permit the evaluation of allelic diversity at the *S* locus and the identification of self-compatible cultivars of domestic plum

PROCEDURE

To accomplish this project, several approaches have been undertaken:

a) Phenotyping for SI/SC in dried plum:

In the first year of analysis, ten flowers from the selections H13S-58, G3N16, G16N19, G43N1, D18-50, S3-55, G5N35, G9S-27, G37S-45, Sutter, Muir Beauty and Improved French were collected from the field (Davis and Winters), emasculated and self-pollinated in the lab. Microscopic observation of pollen tube growth was performed 96h after pollinations.

A second phenotyping approach was undertaken in the field in order to assess the level of SC by evaluating fruit set on enclosed branches. A branch with a minimum of 100 flowers for each selection was bagged and/or caged before blooming in Davis and Winters. Fruit set was evaluated three months after bagging by counting the total number of fruits and ranking according to Grasselly et al (1981), who classified the self-compatibility behavior in several *Prunus* species as follow:

- a) < 2% = SI
- b) between 2 and 10 % = partially SC
- c) > 10% = highly SC



Figure 1: Bagged branches and caged tree of several dried plum cultivars past April 2018.

b) Genotyping the *S*-alleles in dried plum: Total DNA was isolated from young leaves using a Genomic DNA Purification Kit (Fermentas, USA). The quantification and quality evaluation of DNA was performed by a spectrophotometer NanoDrop 1000 (NanoDrop products, USA). Initially, *S*-genotyping of domestic plum cultivars was performed using pairs of consensus primers that have shown good transferability among other *Prunus* species and shown high diversity within the first and fifth introns of *S*-RNase gene (PaConsI, PaConsII, EMPC5-consRD, PruT2 and PruC2). Amplification products were separated on 1% TAE agarose gel in 1X TAE buffer and stained with ethidium bromide.

c) Cloning the *S*-alleles in dried plum: Once the cultivars were PCR-genotyped, the putative SC allele from Improved French and Sutter were cloned and sequenced. Prior to cloning, the band size corresponding to the target allele was purified using the Wizard Plus Miniprep DNA Purification System (Promega, CA, USA) and quantified on 1.5% agarose gel using standard 1 kb DNA ladder (Invitrogen, CA, USA). The purified PCR products were cloned into the vector pCR2.1 using the TA Cloning Kit (Invitrogen). Plasmids were isolated using the QIA prep Spin Miniprep Kit (Qiagen, Hilden, Germany). For the SC allele, at least three plasmids from different PCRs were sequenced from both ends following the methodology described by Fernandez i Marti et al (2009, 2010, 2011 and 2014).

RESULTS AND DISCUSSION

i) Phenotyping for SI/SC in dried plum:

Preliminary phenotypic results are as follows:

H13S-58	G3N16	G16N19	G43N1	D18-50	S3-55	G5N35	G95-27	G37-S-45	Sutter	Muir beauty	Improved French
SI	Highly SC	SI	Partially SC	Highly SC	Highly SC	Highly SC					

Out of the twelve genotypes, G3N16, Sutter, Muir beauty and Improved French showed a high SC phenotype, since in at least 6 out of the 10 flowers their pollen tubes reached the ovary. In the partially SC cultivars (G43N1, D18-50, S3-55, G5N35, G95-27 AND G37-S-45), the number of pollen tubes reaching the base of the style were no higher than three. However, in the SI cultivars, no pollen tubes were observed at the bottom part of the pistil and they stopped their growth in the first third of the pistil.

The results obtained in the lab were consistent with the ones carried out in the field under enclosed branches. For the SI cultivars, we did not observed any fruit set after being bagged in the field. In the partially SC cultivars, we obtained an average of 7% of fruit set after bagging, whereas in the highly SC cultivars, the number of fruits were in all cases higher than 10%.

Although these results are very promising, phenotypic data obtained last spring with the two different approaches has to be confirmed in the coming blooming season in order to have at least two-three consistent years of data.

ii) Genotyping and cloning for the SC allele in dried plum:

The DNA analyses aimed to identify markers associated with self-fertility in dried plum. For it, we used the same 12 cultivars as previously and were genotyped by using conserved primers of the *S*-RNase locus region developed in other *Prunus* species. Out of the six primers tested, the combination of PruC2/PruC5 and PasPcons-F1/PaC1cons-R1 were the most successful and showed the best DNA amplification.

Our first genotype screening showed a potential DNA band that might be related to the SC allele in Improved French. The size of this band was at the same location as the other highly SC cultivar, Sutter. We cloned and sequenced this gel-band in both cultivars in order to obtain the whole DNA sequence of this putative SC allele. After cloning and sequencing three different colonies for each cultivar, the nucleotide alignment showed a match of 100 % in both genotypes. We then proceeded to design specific primers for SC (figure 2) to be used later in the other cultivars for a marker-assisted selection (MAS) genotypic analysis.

Sutter	ATTTGAGATAAGGAAAGTGGTACGTATGGTTTTTATTTCCACATACTCTTTAGCATTTA
Improved_French	ATTTGAGATAAGGAAAGTGGTACGTATGGTTTTTATTTCCACATACTCTTTAGCATTTA

Sutter	CTTTTAGAAAAATTAGACCGCTCAGAAAATCTTCCACGTACATCAAATTAAAGTC
Improved_French	CTTTTAGAAAAATTAGACCGCTCAGAAAATCTTCCACGTACATCAAATTAAAGTC

Sutter	CGTATAATAGTCAGGTTAGTTAGAAAAATAATCATATCCAGAAATGAAAATCTCCCTT
Improved_French	CGTATAATAGTCAGGTTAGTTAGAAAAATAATCATATCCAGAAATGAAAATCTCCCTT

Sutter	GCTTGGTGTCTCAGTACCCCTCAATTGCGATCTGAACCTGGAGATATCTGGCCCGACGTGG
Improved_French	GCTTGGTGTCTCAGTACCCCTCAATTGCGATCTGAACCTGGAGATATCTGGCCCGACGTGG

Sutter	TAGCCGCACTGATATAAACTTTGGGAAGGAGAAATGGAACAACATCGTAGATCTCCG
Improved_French	TAGCCGCACTGATATAAACTTTGGGAAGGAGAAATGGAACAACATCGTAGATCTCCG

Sutter	AGCAAACACTCAACCAAATGCAATACTTCGAGCGATCCACGAAATGTGGAACCTCCACA
Improved_French	AGCAAACACTCAACCAAATGCAATACTTCGAGCGATCCACGAAATGTGGAACCTCCACA

Sutter	ATATTACAGAGATCCTTAAAAACGCTTCAATCGTACCACATCCGACACAGACATGGAAT
Improved_French	ATATTACAGAGATCCTTAAAAACGCTTCAATCGTACCACATCCGACACAGACATGGAAT

Sutter	ACTCGGACATAGTAGCACCCATTAAAGCAGCAACTAAAAGAACACCTCTCCTCGTTGCA
Improved_French	ACTCGGACATAGTAGCACCCATTAAAGCAGCAACTAAAAGAACACCTCTCCTCGTTGCA

Sutter	AAACTCTCCAGCTCAGCCTAAGAGACATTCAGCACAGACTAAGAGCGGGCCGAAGCCTC
Improved_French	AAACTCTCCAGCTCAGCCTAAGAGACATTCAGCACAGACTAAGAGCGGGCCGAAGCCTC

Sutter	AGTTGTTACATGAAGTGGT
Improved_French	AGTTGTTACATGAAGTGGT

Figure 2: DNA sequence of the cultivars Improved French and Sutter. Red arrows represent the genomic region of the *S*-RNase gene where we have designed specific markers for the SC trait.

Although the results are very promising and we believe we have targeted the right SC allele, additional analyses need to be conducted in 2019 in order to confirm this hypothesis.

In addition, we are planning to clone and sequence next year the rest of SI alleles present in the other cultivars in order to determine the cross-incompatibility groups.

ADDED: 1/30/2019- Report for Project Year: 2018

Project Leaders: Dr. Chris Dardick and Dr. Tetyana Zhebentyayeva

Location: USDA ARS Appalachian Fruit Research Station Kearneysville, WV

and Clemson University Genomics & Computational Biology Laboratory, Clemson, SC 29634;

Cooperating Personnel: Dr. Chris Saski, Clemson University Genomics & Computational Biology Laboratory, Clemson, SC

Project Title: **Genomic profiling and development of a comprehensive catalogue of plum germplasm using Genotyping-By-Sequencing (GBS)**

Keywords: *Prunus domestica*, Genotyping-By-Sequencing, genomic profiling, molecular markers

Commodity(s) European (*Prunus domestica*) plum.

Note- the information provided in this report along with datasets, figures, and references is available under the following reference:

Zhebentyayeva T, Shankar V, Scorza R, Callahan A, Ravelonandro M, Castro S, DeJong T, Saski CA, Dardick C. (2019) Genetic characterization of worldwide *Prunus domestica* (plum) germplasm using sequence-based genotyping. *Hortic Res.* 6:12.

Summary:

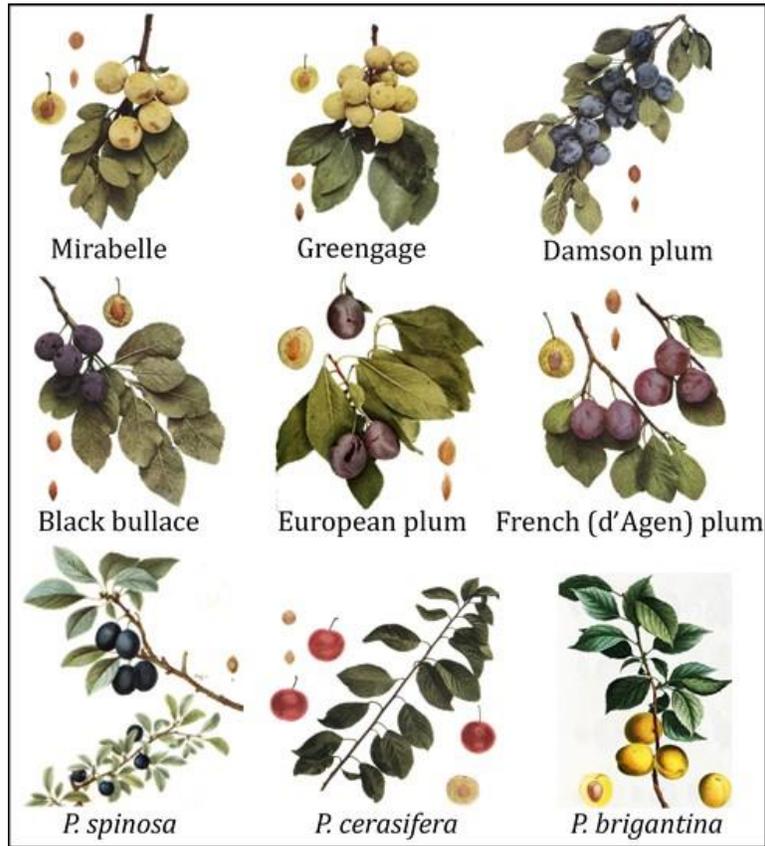
European plum (*Prunus domestica*) is a polyploid (hexaploidy) fruit tree species cultivated around the world primarily for production of prunes. Despite its agricultural importance and long history of cultivation, many questions remain about the origin of this species, the relationships among its many pomological types, and its underlying genetics. Here, we used a sequence-based genotyping approach to characterize worldwide plum germplasm including the potential progenitor wild plum species. Analysis of 405 DNA samples established a set of four distinct clades consistent with the pomological groups Greengages, Mirabelles, European plums, and d’Agen (French) prune plums. Overall, there was relatively low genetic diversity across all cultivated plums suggesting they have been largely inbred and derived from a limited number of founders. The results support *P. domestica* having originated as an interspecific hybrid of a diploid *P. cerasifera* and a tetraploid *P. spinosa* that itself may have been an interspecific hybrid of *P. cerasifera* and an unknown Eurasian plum species. The low genetic diversity and lack of true wild-types coupled with the known cultivation history of Eurasian plums imply that *P. domestica* may have been a product of inter-specific cross breeding and artificial selection by early agrarian Eurasian societies.

Background:

European plum *Prunus domestica* L. is a polymorphic allopolyploid (hexaploid) species ($2n=6x=48$) commercially grown worldwide for a variety of uses including fresh fruit, prunes, distilling, and as processed additive ingredients. This plum species, commonly referred to as ‘European plums’ or ‘prune plums’, is distinct from the large round diploid ‘Japanese plums’ (*Prunus salicina*) which are widely grown for fresh market consumption. *P. domestica* plums are critical components of human diets as prunes have been shown to have a broad range of health promoting activities including protection against cardiovascular disease, diabetes, digestive disorders, and osteoporosis. Historical evidence suggests *P. domestica* and other Eurasian plum species including *Prunus cerasifera* (‘Cherry plum’) and *Prunus*

spinosa ('Sloe' or 'Blackthorn' plum) were important to the development of early European agrarian societies.

It is now well accepted, that *P. domestica* originated from the Middle East, from the area south of the Caucasus situated between the Black Sea and the Caspian Sea (now Georgia, Armenia, Azerbaijan and the northern plateau of Iran). Vavilov (1930) placed the center of its origin in the region south of Caucasian Mountains through the Caspian Sea, in the area overlapping the distribution of *P. cerasifera* L. and *P. spinosa* L.; which were likewise concurrently distributed across Europe for cultivation, consumption, and use as rootstocks. The absence of missing botanical links, widespread feral growth and the apparent lack of natural stands in forests, and human movement of both cultivated and wild type plums across the continent has made determining the origin of *P. domestica* extremely difficult.



Consequently, the origin of hexaploid *P. domestica* plum has been a matter of debate for nearly a century. A major complication is the wide range of intraspecific variations and transitional forms. Traditionally, plum cultivars have been divided into a number of pomological groups; small fruited mirabelle plums, damsons, small wild plums or bullaces, greengages, prune plums, and large-fruited European plums (Figure 1). However, clear delineations among these groups are extremely difficult due to the inherent phenotypic variability across the germplasm.

Molecular phylogenetic analyses have more recently confirmed the close relationships among *P. cerasifera*, *P. insititia*, *P. domestica* L., *P. spinosa* L., and the 'Marmot' plum *Prunus brigantina* and dated their divergence from a common Eurasian ancestor to the Oligocene (31Myr) (14). It is now generally accepted that *P. domestica* is an interspecific hybrid of *P. cerasifera* and *P. spinosa*. However, defining the precise genetic history of these events has been complicated by the fact that *P. cerasifera* and *P. spinosa* are found as both diploids and polyploid individuals, sometimes having morphologies very similar to *P. domestica*. In fact, hybridization experiments and restriction site analysis of Ribosomal RNA genes indicated that *P. spinosa* itself may be an inter-specific hybrid having *P. cerasifera* as one of the parents. Crane and Lawrence (1934) and Rybin (1936) performed direct interspecific hybridization experiments between *P. cerasifera* (2x), and *P. spinosa* (4x) and found that, while the vast majority of the resulting offspring were infertile, a very small number of fertile hybrids that were morphologically similar to *P. domestica* could be obtained. Still, many questions remain unresolved as to the origins of the various pomological plum groups and whether they represent independent inter-specific hybridization events or arose from the same or limited number of events.

In the present study, we generated a set of sequence-based SNP markers densely distributed across the *Prunus* genome in order to: (1) investigate genetic relationships among cultivated plums from different pomological groups that originated under the influence of different ecological factors and were propagated in different geographical regions; (2) estimate the extent of variation in plum germplasm, between and within pomological groups and identify a set of diagnostic molecular markers; and (3) estimate the potential contributions of other plum species *P. cerasifera* and *P. spinosa* to the nuclear genome of hexaploid *P. domestica*. The resulting phylogenetic relationships along with their implications for the origins, history, and future of plum cultivation are discussed.

Results

SNP discovery

In total, we sequenced 405 DNA samples representing different pomological groups of plum and other Eurasian *Prunus* species including potential progenitors of hexaploid plum, *P. cerasifera* and *P. spinosa* (Table 1, Table S1).

Table 1. Plum pomological groups and species in this study.

<i>plum group/species</i>	code	<i>accessions</i>
European plums	EUR	195
Greengages	GRG	46
Mirabelles	MIR	15
Prunes	DAP	107
<i>P. insititia</i>	Pi	9
<i>P. spinosa</i>	Psp	20
<i>P. cerasifera</i>	Pc	10
<i>P. brigantina</i>	Pbr	2*
<i>P. simonii</i>	Psi	1
total		405

* including *P. brigantina* x *P. cerasifera* hybrid

Genetic diversity and phylogenetic analysis

We investigated genetic relationships among all 405 plum accessions by performing a hierarchical clustering analysis (between-group average linkage method, or UPGMA) on a dissimilarity matrix. In total, 103,382 polymorphic SNPs out of 129,110 sites were used for phylogenetic classification. The inter-plate control with biological and technical replicates included in the analysis were used to assess dendrogram quality. Results showed robust and consistent clustering of identical samples and an overall absence of a strong lane or location effects on genetic signals.

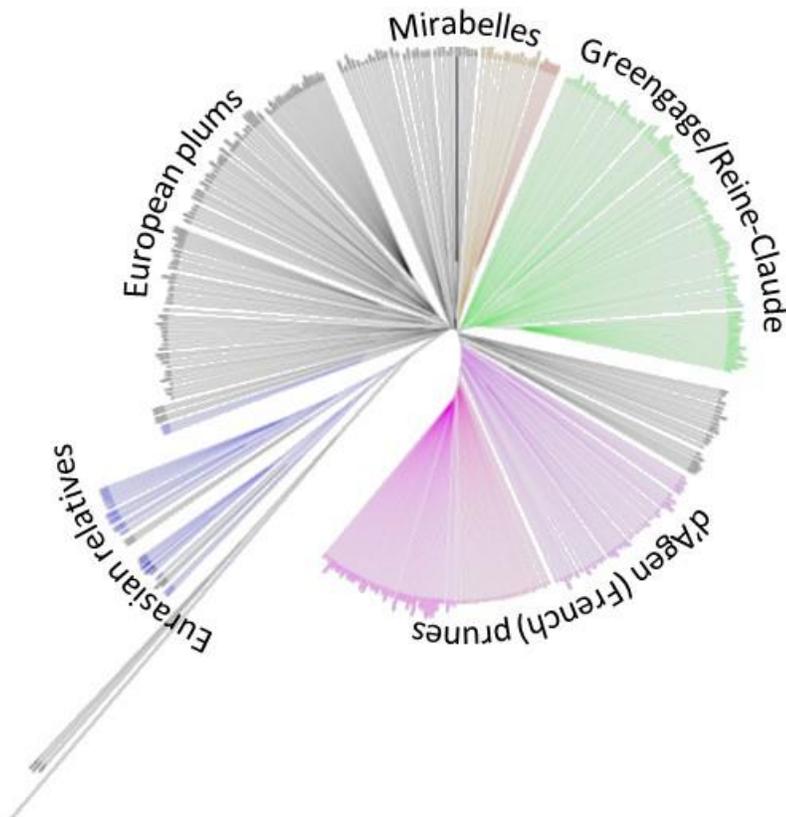


Figure 1. Phylogeny of *P. domestica* and related species. Dendrogram was generated using UPGMA clustering method on dissimilarity matrix computed for 405 accessions and 103,382 nuclear markers. A high-resolution version of the dendrogram is available as Figure S1. Branches are shown in ascending order based on pairwise distances. Different *Prunus* species and pomological groups are labeled and defined by colors – *P. cerasifera* and tetraploid *P. spinosa* (blue), European plums (black), mirabelles (brown and amber), greengages (green and bold green) and d’Agen prunes (fuchsia and lilac). More intensive colors are given to founding clonal varieties with typical morphological traits.

***Prunus domestica* germplasm structure**

Domesticated hexaploid plums were grouped into 4 distinct clades that comprised some of the known pomological groups – European plum (EUP), Mirabelles (MIR), Greengages (GRG), and d’Agen prune plums (DAP) (Figure 3). However, genetic differentiation between groups was relatively low. Differentiation of other pomological groups including *P. domestica* ssp. *insititia*, ‘Damsons’, and ‘Bullaces’ was not supported as such identified individuals did not form discrete clades.

Among the 4 identified clades, subclades consisting of technical replicates, biological replicates and clonal varieties could be distinguished in all pomological groups. Overall, varieties with known parentage clustered as expected within clades supporting the accuracy of the phylogenetic classification. Results from individual *P. domestica* clades are given below:

D’Agen prune plums (DAP): The DAP plum clade (144 accessions) was composed of a set of d’Agen prunes from different geographical regions, progeny from self-pollination of ‘Improved French’, cultivars with known d’Agen types in their pedigree and a few cultivars of European origin with unknown

pedigree. Using Geneious software, we explored genetic distances among prune varieties known to have different pedigrees and calculated a cutoff value (0.363) for discriminating clonal material and replicates from siblings in our study. All 45 individuals named as d'Agen types including technical replicates were within this threshold. Subclades comprised of 26 individuals from self-pollination of the commercial variety 'Improved French' and known hybrids of d'Agen clones as one of the parents collapsed at the thresholds of branch length 0.373 and 0.392, respectively, while advanced generations of hybrids with d'Agen in the pedigree collapsed at a threshold of 0.454.

European plums (EUP): European plums include many old English and Eastern European cultivars. The EUP clade showed the highest level of genetic diversity. Two clonal clades were identified among them. This included the 'Pozegaca' or 'Questche' types which are round, small fruited plums commonly referred to as 'german prunes'. The second clonal clade consisted of a small set of French varieties with mixed but often related names (37). This group includes the so-called 'Italian' plums which produce large, oblong shaped fruit.

Mirabelles (MIR): Mirabelle plums are small, typically red or yellow in color, and slightly oval in shape. They are often found growing feral and commonly used for fresh consumption and mostly served as jams or jellies. A small clonal group consisting of 5 different types were identified along with a set of 13 cultivars and germplasm sources that are potentially seedlings. A number of other German and French cultivars also grouped within this clade such as 'Ruth Gerstetter' and two cultivars named as 'Reine Claude' types suggesting they have MIR parentage.

Greengages: Greengage types are named after Sir William Gage who supposedly imported these small, often green-fruited types from France in the early 1700s (38). Many of the cultivars carry their French name 'Reine Claude' in honor of Queen Claude who ruled France in the early 1500s. Several groups of clonal material consisting largely of material named as 'Reine Claude' types were identified indicating that much of the commercial 'Greengage' germplasm is clonal.

Eurasian plums and P. domestica ancestry:

Diploid accessions, *P. simonii* and the interspecific hybrid rootstock *P. cerasifera* x *P. munsoniana* (Marianna 2634) formed a distinct out-group; highlighting the close genetic similarity among Eurasian plums of different ploidy level - hexaploid *P. domestica*, tetraploid *P. spinosa*, diploid *P. cerasifera* and *P. brigantina*. Diploid *Prunus* species and most *P. spinosa* accessions (17 out of 20) were clearly separated from cultivated hexaploid plums with a few exceptions. Three *P. spinosa* genotypes from Portugal were grouped with autochthonous *P. domestica* forms 'Wegierka wiedenska' and *P. insititia* used as rootstock in commercial nurseries. Some hexaploid *P. domestica* varieties including 'Saint Julien', 'Krikon' plum and 'Mirabelle sans nom' (i.e. unknown) appear to be more distantly related to commercial types and grouped with non-domesticated Eurasian *Prunus* species.

Conclusions:

Differentiation of P. domestica pomological groups

Based on the analysis of worldwide germplasm presented here, we report genetic evidence that at least some pomological groups of *P. domestica* have distinctive genotypic signatures that can be used for assignment of unknown accessions. Two pomological groups, DAP and GRG, were clearly separated from each other and from the rest of plum germplasm.

Based on the uneven distribution of SNPs across the genome, we hypothesize that these genomic regions may hold important genes for fruit type or other agronomic traits distinct in DAP and GRG groups. Historically, these DAP and GRG cultivars were selected for different fruit usage such as drying vs fresh consumption. In many cases significant SNPs important for classification of groups, were proximal to orthologues of proteins with key roles in sugar metabolism and transport. These SNPs may

serve as good markers for screening hybrid material in breeding programs aimed at dried or fresh types of fruits.

Despite the ability to clearly resolve some plum pomological groups, the results revealed relatively small genetic distances between them, many of which consisted of clonally selected and synonymous cultivars. This conclusion was made possible by the inclusion of siblings derived from a self-pollinated 'Improved French' individual which allowed us to calculate cutoffs to identify clonal vs. inbred clades. These results imply that the overall breeding history of plum was derived from a limited set of founders and consisted primarily of self-pollination and/or hybridization among selected siblings. It also underscores the finding that nearly all commercially grown DAP varieties from France, USA, Argentina, and Australia were found to be clonal, establishing that the plum industry worldwide is predominantly a monoculture.

The sequence-based genotyping method used here has limitations caused by the lack of a plum reference assembly, and the reliance on the *P. persica* genome for genotyping hexaploid accessions of *P. domestica* sequences. Consequently, we likely missed an opportunity for discovery of polymorphic markers derived from intergenic regions, as well as SNPs that can discriminate between subgenomes (homoeoSNPs). Despite this limitation, the results of our analyses were highly consistent with known genetic relationships.

Relationship of Prunus domestica to other Eurasian plums

Questions regarding the origins of *P. domestica* have been studied and debated for over a century. The wide range of phenotypic diversity observed in *P. domestica* and its Eurasian relatives has made resolving these questions particularly difficult. A handful of competing theories have been proposed that are not all mutually exclusive: [1] *P. domestica* is entirely of *P. cerasifera* origin and represents an autopolyploid event of this species, [2] *P. domestica* is a hexaploid of *P. spinosa* which is a discrete species, [3] *P. domestica* is an interspecific hybrid between a diploid *P. cerasifera* and a tetraploid *P. spinosa*, and [4] *P. domestica* is a hybrid of interspecific hybrids having a hexaploid chromosome complement composed of *P. cerasifera*, *P. spinosa*, and possibly contributions from other Eurasian plum species. Unfortunately, the results presented here do not definitively resolve these possibilities. Consistent with the prevailing theory proposed by early researchers our data support model [4] whereby one subgenome was contributed by diploid *P. cerasifera* and the other from a tetraploid *P. spinosa* that itself is an interspecific hybrid between diploid *P. cerasifera* and a second, as of yet, unknown *Prunus* species such as *Prunus ramburii* Boiss as suggested by Reales et al. 2010. The endemic species *P. ramburii* was not present in our study but close grouping of *P. brigantina* and its hybrid *P. brigantina* x *P. cerasifera* with *P. spinosa* accessions is in agreement with results reported by these authors and Shi et al. 2013. The relatively close genetic distances among the germplasm we found here supports the hypothesis by Eryomine, 1990 that some or all of the interspecific hybridization events that gave rise to *P. domestica* did not occur naturally but were artificially selected for by early Eurasian societies. Such a scenario is supported by the lack of wild *P. domestica*, the low genetic diversity across the germplasm reflecting a more recent origin, and the low pollination and fertility rates among experimental interspecific hybrids which would presumably have had tremendous difficulty naturally establishing themselves. In addition, historical evidence suggesting widespread simultaneous cultivation of *P. domestica*, *P. cerasifera* and *P. spinosa* is consistent with the current wild Eurasian germplasm that appears to be comprised by feral populations with relatively low genetic diversity. Whole genome sequencing, accompanied with additional genotyping using a broader range of Eurasian plum germplasm will be necessary to fully resolve these possibilities.

Added: 1/30/2019- Report for project year 2018 - Whole Genome Sequencing of *Prunus domestica* cv Improved French

Project Leaders: Dr. Chris Dardick and Dr. Tetyana Zhebentyayeva

Location: USDA ARS Appalachian Fruit Research Station Kearneysville, WV

and Clemson University Genomics & Computational Biology Laboratory, Clemson, SC 29634;

Cooperating Personnel: Dr. Chris Sasaki, Clemson University Genomics & Computational Biology Laboratory, Clemson, SC

Keywords: *Prunus domestica*, Genome sequencing.

Commodity(s) European (*Prunus domestica*) plum.

Summary:

Prunus domestica is a hexaploid plum species that is commercially grown worldwide for prune production. Recent genomic evidence suggests that *P. domestica* resulted from an interspecific hybridization event between *Prunus cerasifera*, *Prunus spinosa*, and possibly another unknown *Prunus* species. Currently, breeding efforts for *P. domestica* are hampered by a lack of genetic knowledge regarding its origins and segregation patterns. Likewise, this knowledge gap also limits the potential for the use of modern molecular breeding tools to enhance seedling selection. Our previous efforts funded through the CDPB were largely unsuccessful due to the complex nature of the genome. Here, we contracted with NRGene to perform sequencing and phased assembly of the *P. domestica* genome cultivar 'Improved French' to help address key genomic questions and enable molecular assisted breeding. Results showed that the entire genome was approximately 1.4Gb in size and is 98% complete. Gene annotation using approximately 10 billion *P. domestica* RNAseq reads revealed the presence of approximately 130,000 genes - about 5 times the number present in the peach genome. The results indicate that the phased genome assembly was able to discriminate 5 of the 6 *P. domestica* chromosome copies, suggesting one pair may be highly homozygous. The genome sequence and annotation has been made available to the research community via the Genome Database for Rosaceae: <https://www.rosaceae.org/analysis/303>.

Background:

European plum (*Prunus domestica*) is a hexaploid fruit tree species used predominately for drying as prunes. Currently, almost all known commercial production is based on 'French' cultivars which were recently shown to have been clonally selected. Thus, the worldwide industry is largely a monoculture. The development of molecular markers and genomic selection technologies would greatly aid breeding new commercial French prune cultivars that would help to diversify the germplasm and lead to superior fruit qualities for growers and consumers. Current evidence suggests that *P. domestica* originated as an interspecific hybrid of a diploid *P. cerasifera* and a tetraploid *P. spinosa* that itself may have been an interspecific hybrid of *P. cerasifera* and an unknown Eurasian plum species. This complexity of the *P. domestica* genome has made the sequencing and assembly of the genome extremely challenging.

The availability of an assembled genome sequence would help address questions regarding the *P. domestica* genome structure and genetic segregation as well as provide a basis for genetic mapping and molecular marker development. Such resources are critical to enabling molecular breeding strategies which could profoundly benefit prune cultivar selection. Here we contracted with NRGene to utilize their proprietary sequencing/assembly pipeline to generate a phased *P. domestica* genome. The advantage of a phased genome is that it would provide separate sequences for each of the *P. domestica* subgenomes – presumably having originated from different wild species. Such a genome assembly would reveal the precise nature of the genome and its origins as well as the ability to

perform genomic selection on seedlings for the integration of desirable genomic regions or specific combinations thereof. Details regarding the methods and results follow.

Materials and methods:

DNA Extraction

High quality genomic DNA was extracted from leaves of the *P. domestica* cultivar ‘Improved French’ by the Clemson University Genomics Computational Laboratory (CUGCL) using an in house high molecular weight DNA extraction method. The tissue source was ~30 grams of young leaf tissue derived from greenhouse grown ‘Improved French’ grafted shoots that were dark treated for 3 days. A total of 100ug of DNA was obtained and evaluated for integrity with Pulsed Field Gel Electrophoresis (PFGE) and quantitated with a dsDNA dye binding assay (Qubit) (Figure 1).

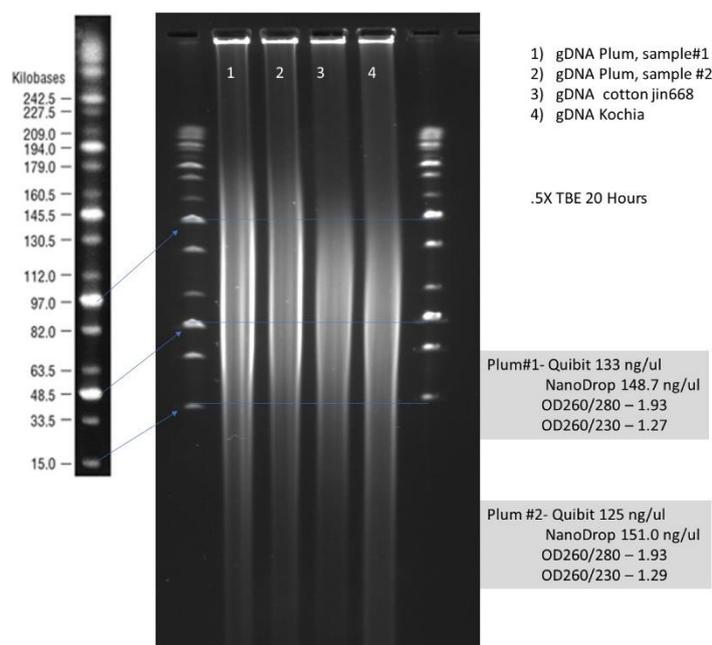


Figure 1: gDNA quality size and quality assessment.

Sequencing and assembly

The gDNA samples were used for library preparation and sequenced using 2nd generation sequencing technology with total depth (coverage) of 210X using Illumina™ technologies. A combination of Pair-End (PE) and Mate-Pair (MP) libraries of different sizes were generated. In addition, 3rd generation sequencing libraries using Chromium 10X technology were prepared and sequenced using Illumina™ machines, creating additional sequencing data with depth (coverage) of 55X. A complete list of the libraries used and coverage is provided in Table 1. The sequencing data was processed and assembled using DeNovoMAGIC™ assembler application version 3.0.

Table 1.0- Sequencing strategy description

Library type	Insert size	Reads	Number of Libraries produced	Approximate depth (coverage)
PCR-free PE library (PE250X2)	450-470bp	250bpX2	1	X84
PCR-free PE library (PE150X2)	700- 800bp	150bpX2	1	X44
MP (Nextera™ MP Gel Plus)	2-4kbp	150bpX2	1	X29
MP (Nextera™ MP Gel Plus)	5-7kbp	150bpX2	1	X24
MP (Nextera™ MP Gel Plus)	8-10kbp	150bpX2	1	X29
10X genomics™ Chromium™	N/A	150bpX2	1	X55

Results

The Phased assembled scaffolds statistics are given in Table 2.0. Approximately 500 Mbp of unplaced small scaffolds below 2kbp were excluded that showed redundancy and/or incompatibility with other scaffolds. The statistics of the unplaced filtered scaffolds are shown in table 2.1.

Table 2.0 Scaffolds statistics summary	
	Phased
Total scaffolds	27,870
Assembly size	1,399,321,220
Gaps size	27,033,790
Gaps %	1.93
N50	1,627,960
N50 #sequences	238
N90	128,064
N90 #sequences	1,152
MAX	8,330,377

Table 2.1 Unplaced Scaffolds statistics summary	
Total scaffolds	1,223,718
Assembly size	501,562,711
Gaps size	13,889
Gaps %	0.003
N50	496
N50 #sequences	319,837
N90	208
N90 #sequences	911,665
MAX	1,999

The contigs composing the scaffolds are gapless, non-chimeric sequences. These sequences are indicated to contain error rate of 1 in 3Mbp. Contigs' statistical analysis summary is presented in table 2.2.

Table 2.2 Contigs statistics summary	
	Phased
Total scaffolds	96,980
Assembly size	1,368,997,486
N50	33,016
N50 #sequences	12,095
N90	6,468
N90 #sequences	45,839
MAX	317,873

The integrity of the assembly was verified using several quality-assurance procedures including the independent BUSCO benchmark (<http://busco.ezlab.org/>) which is used to specifically indicating the genic region integrity, ploidy and zygosity characteristics of the assembled genome. The BUSCO statistics are presented in table 3.1 and the BUSCO copy number analysis in shown in table 3.2

Table 3.1- BUSCO statistics summary	
	Phased
Complete BUSCOs	1385 (96.2%)
Complete BUSCOs - Single-Copy	67 (4.7%)
Complete BUSCOs - Duplicated	1318 (91.5%)
Fragmented BUSCOs	9 (0.6%)
Missing BUSCOs	46 (3.2%)
Total BUSCO groups searched	1440

Table 3.2- BUSCO copy number analysis summary	
Number of Gene copies	Number of genes identified Phased
1	76
2	95
3	210
4	347
5	636
>=6	30

A subset of 2,747 scaffolds >10Kb in length were selected for genome annotation. These scaffolds represent approximately 95% of the entire genome sequence. These were annotated using GenSAS (www.gensas.org). The genome sequence was masked using RepeatMasker (other dicots) and RepeatModeler. A set of approximately 10 billion raw RNAseq reads (150bp paired-end) derived from vegetative bud and leaf tissues at various stages of development were used for gene prediction. This RNAseq set was created using the translome profiling technique where epitope tagged ribosomes are immunopurified to enrich for actively translating mRNAs. This technique enriches the mRNA fraction for fully spliced transcripts (Collum et al. 2019). The gene models were predicted using BRAKER2 which was trained with a BAM file derived from the aligned RNA-seq reads created with HISAT2. 130,866 predicted genes were identified across the 2,747 scaffold subset. This result indicates an average of 4.87 copies per gene represented within the phased genome scaffolds.

Conclusions

The finding that only 5 of the 6 chromosome copies could be delineated was surprising. We hypothesize that this is likely due to the presence of a relatively homozygous diploid subgenome that could not be phased. One possibility is that the origin of the *P. domestica* genome involved an unreduced gamete from a diploid ancestor.

The resulting phased genome assembly is the first for *P. domestica* and will provide a critical tool for understanding the genetics and breeding of this species. The annotated phased genome should allow for the identification of the progenitor species as well as the separation of the scaffolds into assigned subgenomes. In addition, it will aid in the development of molecular markers for breeding and the mapping of segregating agronomic traits to linked genomic regions. Future efforts will focus on assembly improvements including larger scaffolds, the assignment of scaffolds at the chromosomal scale, and synteny analyses to other *Prunus* genomes. The genome including all annotations is being made publicly available on the Genome Database for Rosaceae for use by the worldwide prune research community.

FIELD EVALUATION OF PRUNE ROOTSTOCKS AT GROWER TRIALS, 2018

Luke Milliron, Franz Niederholzer, Richard Buchner, Joe Connell, Allan Fulton, Mark Gilles, Ted DeJong, and Sarah Castro

ABSTRACT

The California prune industry has historically utilized five rootstocks, Myrobalan seedling, Myrobalan 29C, Marianna 2624, Lovell peach and M40. The last statewide organized prune rootstock effort was the “M” series rootstock plots planted in 1987 (Vina Monastery 3/20/87). Since the conclusion of that experiment, many more potential rootstocks for prune have been identified. HBOK 50, Krymsk1, Krymsk 86, Citation, Rootpac-R, Viking, Atlas, and others.

Two replicated rootstock experiments in grower orchards have been planted in Northern California. One in Butte County planted 4/28/11 and a second in Yuba County planted 6/3/11. All trees were nursery grafted to the ‘Improved French’ variety. There are 14 rootstocks planted in a replicated and randomized scheme at both locations, although Rootpac-R is only at the Yuba location, and Empyrean 2 is only at the Butte location. In 2016, 2017, and 2018 bloom timing and density were visually rated at the Butte County location, with notable differences in full bloom timing between rootstocks noted. A low bloom density in 2018 at the Butte County location, following a heavy crop in 2017 helps explain low yields at the Butte location in 2018. While low yields at the Yuba County location in 2017 may be in-part due to saturated soils at many locations in the region at bloom, which was linked to poor set in different locations around the Sutter/Yuba region. The two trials were mechanically harvested in 2017 and 2018, with notable differences in dry weight per tree, dry ratio, and dry fruit size at the two sites between both rootstocks and years.

OBJECTIVES

- 1) Evaluate 15 rootstocks potential for use in California Prune production.
- 2) Evaluate trunk cross sectional area (TCSA), bloom date and bloom conditions, dry weight per tree, dry ratio, and dry fruit size.

PROCEDURES

Butte County Location

The Butte County location was planted 4/28/11. The wet winter delayed soil preparation resulting in the late planting date. The Butte County soil survey lists the soil as Farwell clay adobe alternating with a lighter textured soil described as Nord loam. Nord loam is noted for its higher pH, low nutrient status and a greater association with replant disease. Test trees followed almonds on Lovell peach rootstock with no soil treatments prior to planting. Lesion nematodes were isolated from soil samples.

The layout is a randomized complete block design with 14 treatments and 5 replicates. There are 6 trees per plot in the original design. Trees were headed at 40 inches on 5/10/2011 and the test planting is drip irrigated. The HBOK 50 rootstock came as potted trees and were delivered 5/4/11 and planted by 5/10/11. The HBOK 50 rootstock produced small bush like trees and did not have sufficient trunk growth to head the first year and were left alone. Viking and Atlas were not available in 2011 and were added to the experiment in 2012 and are consequently one year younger. Viking and Atlas were propagated by Dave Wilson nursery, HBOK 50 from Duarte nursery and the remaining trees were propagated by Fowler nursery.

Tree mortality was high during the 2011 season. Missing tree locations were site fumigated with 0.5 pound of chloropicrin on 11/15/11 and replanted 2/10/12. Viking and Atlas were also planted 2/10/12. Many of the Rootpac-R trees did not survive the initial planting, and replacement trees were not available. On 2/10/12 the few remaining Rootpac-R were extracted at Butte and replanted in the Yuba plot. The goal was to have one complete set of Rootpac-R at one location. Both the Butte and Yuba locations have mixed tree ages because of the high initial tree mortality. Fumigated replant trees grew well, and growth caught up with trees planted the first year.

Trunk measurements (11/7/16 and 3/9/18) comprise of scion circumference measured 12 inches above the graft union. Trunk circumference is used to calculate trunk cross sectional area in cm². Bloom dates and flower development were visually rated seven times (2 to 3-day intervals) starting March 20 and finishing April 4th. Bloom charts for 2016, 2017 and 2018 are included for comparison. Leaves were sampled from each replicated treatment rootstock at the Butte location on 8/3/18 and sent to Dellavalle Laboratory (Fresno, CA) for analysis (N, P, K, Zn, Mn, B, Mg, Fe, Cu, and Ca).

The 2018 harvest was the second whole plot mechanical harvest for the Butte location. Load cells were installed on the harvester forks so entire green weight per plot could be measured. As green fruit entered the bin, a 5 to 6-pound random subsample was collected. Harvest subsamples were field weighed and transported to Sunsweet for commercial drying. Subsample weights and fruit counts were used to calculate dry ratio, dry yield per tree, and fruit size distribution using an A, B, C, D and “under” stainless steel screen sorter. The original experimental design featured 6 trees per individual rootstock. Tree death per plot often resulted in less than the original 6 trees per plot. Consequently, whole plot yield is divided by the number of surviving trees per plot and reported as fruit load or yield per tree. Butte trees were not fruit thinned to manage crop load and the experiment was harvested 8/31/2018.

Yuba County Location

The Yuba County location was planted 6/3/11. This was a replant site, with prune following prune. Telone[®] fumigation occurred in the early spring. The wet winter and late fumigation delayed soil preparation and subsequently delayed planting. Similar to Butte, the plot is a randomized complete block design with 14 treatments and 5 replicates. There are 6 trees per plot in the original design. Rootstocks are the same as the Butte plot with the exception of Rootpac-R which was transplanted from Butte to Yuba and Empyrean 2 which did not survive in the Yuba location. Tree mortality was high during the first growing season. The soil is described as Kilga clay loam. In 2012, Atlas and

Viking rooted trees were planted, and missing trees were replanted. In March 2014, French on Fortuna, WRM2 or AP45 trees were planted as replicated observations in the spaces designated for Empyrean 2 in the experimental design. The Yuba experiment is complete and trees are growing well with the exception of canker disease (bacterial and *cytospora*) in some of the varieties. Test trees are micro sprinkler irrigated. Leaves were sampled from each replicated treatment rootstock at the Yuba County location on 8/16/18 and sent to Dellavalle Laboratory (Fresno, CA) for analysis (N, P, K, Zn, Mn, B, Mg, Fe, Cu, and Ca). The plot had its second commercial harvest on 9/6/18, and harvest methodology matched the Butte County location.

RESULTS AND CONCLUSIONS

Butte County Location

Bloom conditions and bloom dates for 2016, 2017, and 2018 for the Butte County rootstock experiment are shown in figures 2, 3, and 4 respectively. For 2016 (figure 2) full bloom for the controls occurred about March 10 indicated as “0” with Krymsk 86, Marianna 58, Krymsk 1 and HBOK 50 being the last to full bloom, “+4” days after the control rootstocks. Early bloom conditions and bee activity were less than ideal in 2016 due to cold and rainy conditions resulting in poor cropping on the early bloom and relatively good fruit set on the rootstocks that imparted later bloom. For 2017 (figure 3), full bloom for the controls occurred about March 19 as the “0” date. Different from 2016 was the spread on the late blooming group: Krymsk 86 “+3” days after control, Marianna 58 “+2” days after control, Krymsk 1 “+1” day after control and HBOK 50 same as control rootstocks (“0”). Bloom conditions in 2017 were very good and all rootstocks set a good crop. Late bloom conditions in 2018 (figure 4), saw full bloom on March 30th or 31st for most rootstocks (“0” and “+1” dates, respectively). In the late blooming group, Marianna 30, Marianna 58, and Citation bloomed “+2” two days, and Krymsk 86 and Krymsk 1 were “+3” days.

A poor crop in 2018 was likely due to low bloom density (figure 5). On a subjective rating of bloom density (scale of 1-5, with 5 being the greatest density), 2018 had lower bloom density across nearly every rootstock compared to 2016 and 2017. In both 2016 and 2017, bloom was rated an average of 3.8/5, compared to just 2.4/5 in 2018. With additional years of bloom density assessment at the site, consistency of bloom from one year to the next and tendencies toward alternate bloom/bearing may be indicated by these ratings.

Tree size (trunk cross sectional area cm²), dry ratio (dry weight to fresh weight), dry yield (lbs./tree) and percent A screen fruit are shown in Figure 6 and 7 for 2017 and 2018. Rootstocks that impart smaller canopy size (Krymsk 1, HBOK 50, M58 and Empyrean 2) generally had lower yield (dry pounds) per tree and larger fruit size. Rootstocks that impart large canopy size (Lovell, Viking, Atlas and Myro 29C) had more dry pounds per tree and smaller fruit size. In 2017 TCSA explained 92% of the differences in yield between rootstocks at the Butte County location. Rootstocks that impart smaller canopy size and yield were expected to have lower dry away ratios (dry wt: fresh wt) and more vigorous rootstock treatments having higher dry away ratios, however this was not the case in 2017.

Tree size (trunk cross sectional area cm²) significantly increased (p-value = 0.0002 < 0.05) at the Butte trial from an average of 77.45 cm² in November 2016 to an average of 85.41 cm² in March

2018. Following lower bloom density ratings, mean rootstock yield (least square mean of five replicates in dry lbs/tree) ranged from just 4.4 - 23.7 in 2018, compared to 17.5 - 61.6 lbs/tree in 2017. At the low 2018 yield levels, rootstocks did not statistically differentiate as clearly as 2017. However, M30, M40, and M29C significantly out yielded HBOK 50 (dry lbs/tree). Although TCSA and yield per tree were significantly correlated (p -value = 0.0007 < 0.05), TCSA only explained 17% of the variation in yield in 2018. At the low yield levels and large fruit size across rootstocks, yield (dry lbs/tree) was insignificantly correlated with % A sized fruit (R^2 = 0.004, p -value = 0.6214 > 0.05). By a narrow margin, yield was also insignificantly correlated with dry away ratio (R^2 = 0.058, p -value = 0.0542 > 0.05).

Yuba County Location

Yuba County rootstock trial harvest and trunk cross sectional area (TCSA) data for 2017 and 2018 seasons are found in Figure 8 and 9, respectively. With dry away ratios between 2.3-2.6 for all rootstocks in 2017, fruit size was large for all rootstocks (data not presented). Percent A and B sized fruit were between 70-100% by weight for test samples (10 individual rootstock replicates comprising 13% of total samples). As in the Butte County location, larger trees (bigger TCSA) generally produced more fruit in 2017, with TCSA explaining 54% of the differences in yield between the rootstocks. We suspect that differences in soil between the Yuba and Butte sites explain the differences in tree size and yield in 2017, particularly the potential for saturated soils in Yuba County at bloom, which has been linked to poor set in different locations around the Sutter/Yuba region.

Unlike the Butte County location, the Yuba County location trended towards a more productive year in 2018, with mean (least square mean of five replicates) yield per tree numerically ranging from 14.4 - 37.6 dry lbs/tree to, compared to 3.78 - 10.46 in 2017. Like the Butte County location, with a high % A screen across rootstocks, % A screen was not significantly correlated with yield (dry lbs/tree) in 2018 (R^2 = 0.019, p -value = 0.2575 > 0.05). However, yield (dry lbs/tree) and dry away ratio were significantly correlated (R^2 = 0.081, p -value = 0.0184 < 0.05) in 2018. The Butte and Yuba County locations had opposite boom-bust years in both 2017 and 2018, possibly due to different bloom conditions, as well as some level of alternate bearing at each site. Although 2019 was intended to be the final year of the investigation, we will continue to follow yield at least through 2020 to establish clearer trends at each site, as well as the differences and commonalities between the two sites.

2018 Leaf Sampling at Butte and Yuba County Locations

The leaf sampling at both sites in August 2018 revealed many differences between the nutritional status of rootstocks at each site and some commonalities between sites. Total leaf percent nitrogen had statistical separation between varieties at both locations and like many mineral nutrients analyzed had similarities between rootstocks at both sites. However, these differences were over a relatively narrow range of leaf N, 2.69% (Atlas) to 2.36% (Citation) at Butte, and at Yuba 2.34% (M40) to 2.10% with Myroblan seedling. Although there were no statistical differences between rootstock phosphorous levels in Butte, there were differences in Yuba, with total leaf % P of 0.19% for HBOK, down to 0.09% for Krymsk 1. Again, for potassium there were no differences at the Butte location, while at the Yuba location % K ranged from 2.68 with Krymsk 86, down to 1.03 with M58. There were no differences in zinc at the Butte location, but at the Yuba location, M29C

at 30 mg/kg Zn, was significantly greater than Roopac-R at 17. Finally, there were no differences in iron at the Butte location, while Krymsk 1 and M58 had significantly higher Fe % than Viking at the Yuba location.

There were differences between rootstock leaf nutritional content at both sites for manganese, boron, magnesium, copper, and calcium. At the Butte location, manganese ranged numerically from 108 mg/kg with Krymsk 1 to 43 mg/kg with Atlas. At the Yuba location, manganese ranged from 78 mg/kg with M58 to 22 with HBOK 50. For total boron (mg/kg), Myroblan seedling, M40, Citation, and M58 had amongst the lowest values at Butte, while M58 and Rootpac-R were amongst the lowest at Yuba. Myroblan seedling had the highest magnesium levels at both sites, while M2624 was the lowest at Butte, and Krymsk 1 was the lowest at Yuba. Emyrean 2 had significantly higher copper levels than Atlas in Butte, while in Yuba, Myroblan seedling (9 mg/kg) had the highest levels and Viking the lowest (6 mg/kg). For calcium Emyrean 2, Myroblan, and Krymsk 1 had the highest levels at Butte. Finally, at the Yuba location Myroblan seedling, M58, and K86 had amongst the highest calcium levels, while Krymsk 1, HBOK 50, and Rootpac-R had the lowest levels.

In general differences were more relatively muted at the Butte County trial location in 2018, where yields were low across rootstocks. The rootstocks imparting smaller trunk size and crop load, often had amongst the highest nutrient levels (e.g. HBOK 50, Emyrean 2, Krymsk 1, and M58), particularly for micronutrients. This may in part be due to the lower crop nutritional demand of smaller trees and smaller crop load. Given the muted differences at the Butte location, more targeted nutritional analysis in 2019 could be beneficial in beginning to determine relationships between nutritional status and yield. At the unreplicated prune rootstock trial at Wolfskill Experimental Orchards (Winters, CA), yield was correlated with potassium in 2017, but not in 2018 when yields were lower.

ACKNOWLEDGEMENTS

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BUDGET SUMMARY

A. Breakdown of expenditures

Salaries & Benefits	\$ 0
Supplies & Expenses (Leaf Analysis)	\$ 5,615
Equipment	\$ 0
Travel	<u>\$ 0</u>
Total to Date	\$ 5,615

B. A portion of the grant that was requested for salaries and benefits in 2018 (\$1,790) was transferred to supplies on 7/30/18 to allow for leaf sampling at both sites. The current remaining balance for 2018 is estimated at \$1,830. Some of the \$1,000 travel budget will be used by Milliron and Buchner to pay for their costs of attending the 2018 Prune Research Conference. Remaining 2018 funds will pay for part of more focused leaf analysis (N, P, K) at both sites in 2019.

Rootstock	Pedigree (scientific)	Pedigree (Common)	Interest to CA
Atlas	P. persica (Nemaguard) x (Prunus dulcis x Prunus blierianna)	Nemagaurd x (almond x (apricot x plum))	Bac canker resistant?
Viking	P.persica x (P. amygdalus x P. blireiana (P.ceresifera x P.Mume)	Nemagaurd x (almond x (apricot x plum))	Bac canker resistant?
Citation	Prunus salicina x Prunus persica	Red Beaut plum x peach	
Empyrean 2	Prunus domestica	European prune (OP seedling of 'Imperial Epineuse')	small tree
HBOK 50	Prunus persica	Harrow Blood X Okinawa	nematode resistant?
Krymsk 1	Prunus tomentosa x Prunus cerasifera	Plum x plum	grown in Europe
Krymsk 86	Prunus cerasifera x Prunus persica	Plum/peach hybrid	anchorage
M30	Prunus cerasifera x Prunus munsoniana	Plum x wild plum	
M40	Prunus cerasifera x Prunus munsoniana	Plum x wild plum	less suckering
M58	Prunus cerasifera x Prunus munsoniana	Plum x wild plum	smaller tree?
Myrobalan seedling	Prunus cerasifera	Myrobalan seedlings	control
Rootpack R	Prunus cerasifera x prunus dulcis	Plum/almond hybrid	smaller tree?
Lovell	Prunus persica	peach seedling	control
M2624	Prunus cerasifera x Prunus munsoniana	Plum x wild plum	control
Myro 29C	Prunus cerasifera	Myrobalan clone	control

Figure 1. Scientific and common pedigree for test plantings at the Butte and Yuba prune rootstock experiments.

Figure 5. The density of 'Improved French' bloom is rated annually for each rootstock on a subjective scale of 1 to 5 with a rating of 5 being the heaviest bloom.

2017 Butte Rootstock Experiment Harvest Comparisons

Rootstock	2017 TCSA (cm ²)	Dry Away Ratio (dry wt: fresh wt)	Dry Yield (lbs./tree)	% A Screen
M29C	111.55 i	3.27 abcd	61.6 e	31.4 abc
Atlas	101.38 hi	3.61 f	48.9 d	26.2 ab
Viking	97.39 ghi	3.40 de	49.4 de	29.2 abc
M30	92.45 gh	3.38 cde	40.7 cd	33.7 abcd
Lovell	89.17 fgh	3.56 ef	41.4 cd	20.3 a
M40	84.69 efg	3.10 a	40.5 cd	39.9 bdc
M2624	75.22 def	3.33 bcd	41.4 cd	41.2 cd
Mryo seedling	73.37 cde	3.19 abc	35.9 bc	59.8 ef
Krymsk 86	73.19 cde	3.31 bcd	36.1 bc	48.0 de
Citation	66.52 bcd	3.4 de	27.9 ab	56.0 ef
Empyrean 2	58.92 abc	3.31 bcd	20.6 a	66.9 fg
M58	56.34 ab	3.22 abcd	23.7 ab	68.5 fg
HBOK50	56.07 ab	3.35 bcd	27.5 ab	41.1 cd
Krymsk 1	46.01 a	3.17 ab	17.5 a	75.8 g

Figure 6. 2017 trunk size (trunk cross sectional area in cm²) and yield characteristics for the Butte Co. rootstock experiment harvested 8/29/17. Values are treatment means for the five replicates. Values followed by the same letters are not significantly different at 95% LSD, with letter order denoting lowest to highest. The numerically highest and lowest values are highlighted in each column.

2018 Butte Rootstock Experiment Harvest Comparisons

Rootstock	2018 TCSA (cm ²)	Dry Away Ratio (dry wt: fresh wt)	Dry Yield (lbs./tree)	% A Screen
M29C	119.00 a	2.75 d	21.7 ab	92.8 ab
Atlas	109.52 ab	2.81 bcd	13.0 abc	90.3 ab
Viking	106.32 abc	2.84 bcd	13.3 abc	91.3 ab
M30	101.06 abc	2.80 bcd	21.2 ab	93.2 ab
Lovell	98.08 abc	2.86 bcd	9.1 bc	85.9 ab
M40	94.12 abcd	2.71 d	23.7 a	88.7 ab
Mryo seedling	83.52 bcde	2.72 d	13.3 abc	95.8 a
Krymsk 86	82.95 bcde	3.01 ab	11.6 abc	83.6 ab
M2624	82.15 bcde	2.74 d	16.7 abc	94.1 a
Citation	76.38 cde	2.96 abc	17.6 abc	90.1 ab
HBOK50	63.60 e	2.78 cd	4.4 c	92.5 ab

M58	63.19 e	2.80 cd	13.8 abc	90.8 ab
Empyrean 2	62.78 de	3.16 a	14.2 abc	79.2 b
Krymsk 1	51.97 e	2.77 cd	14.8 abc	90.5 ab

Figure 7. 2018 trunk size (trunk cross sectional area in cm²) and yield characteristics for the Butte Co. rootstock experiment harvested 8/31/18. Values are least square means for the five replicates. Values followed by the same letters are not significantly different at 95% using Tukey's HSD, with letter order denoting highest to lowest. The numerically highest and lowest values are highlighted in each column.

2017 Yuba Rootstock Experiment Harvest Comparisons

Rootstock	Dec. 2016 TCSA (cm ²)	Dry Away Ratio (dry wt: fresh wt)	Dry Yield (lbs./tree)
Viking	66.85 d	2.45 abcd	9.51 ab
Atlas	65.87 d	2.43 abcd	9.03 ab
M30	63.53 d	2.43 abcd	5.90 ab
HBOK50	63.09 cd	2.60 bcd	10.46 b
Rootpac-R	61.84 cd	2.33 a	8.26 ab
Lovell	60.62 bcd	2.55 bcd	8.52 ab
Krymsk 86	60.23 bcd	2.58 cd	9.95 b
M29C	58.44 bcd	2.30 a	9.16 b
M40	53.77 bcd	2.39 abc	6.17 ab
M2624	47.97 abc	2.37 abc	4.93 ab
Myro seedling	48.37 abc	2.35 ab	3.78 a
Citation	46.94 ab	2.43 abcd	8.22 ab
M58	39.91 a	2.61 d	4.74 a
Krymsk 1	39.03 a	2.26 a	5.13 ab

Figure 8. 2017 trunk size (trunk cross sectional area in cm²) and yield characteristics for the Yuba Rootstock experiment harvested 9/1/17. Values are treatment means for the five replicates. Values followed by the same letters are not significantly different at 95% using Tukey's HSD, with letter order denoting lowest to highest. The numerically highest and lowest values are highlighted in each column.

2018 Yuba Rootstock Experiment Harvest Comparisons

Rootstock	March 2018 TCSA (cm ²)	Dry Away Ratio (dry wt:fresh wt)	Dry Yield (lbs./tree)	% A Screen
Viking	84.24 a	2.75 ab	37.4 a	84.9% ab
Atlas	82.36 a	2.75 ab	34.8 a	82.3% ab
M30	72.45 abcd	2.83 ab	26.1 ab	83.3% ab
HBOK50	82.63 ab	2.83 ab	29.0 ab	86.2% ab
Rootpac-R	68.38 bcde	2.91 ab	29.4 ab	57.6% c
Lovell	75.18 abcd	2.80 ab	34.5 a	81.8% ab
Krymsk 86	80.98 ab	2.77 ab	37.6 a	71.7% abc
M29C	76.82 abc	2.64 b	29.5 ab	81.2% ab
M40	70.36 abcde	2.65 b	23.4 ab	91.5% a
M2624	63.56 cdef	2.69 ab	28.9 ab	86.0% ab
Myro seedling	61.79 def	2.80 ab	21.3 ab	90.5% a
Citation	56.58 ef	4.73 a	28.6 ab	63.7% bc
M58	50.88 fg	2.97 ab	16.5 b	67.7% bc
Krymsk 1	41.90 g	3.10 ab	14.4 b	50.9% c

Figure 9. 2018 trunk size (trunk cross sectional area in cm²) and yield characteristics for the Yuba Rootstock experiment harvested 9/6/18. Values are treatment means for the five replicates. Values followed by the same letters are not significantly different at 95% using Tukey's HSD, with letter order denoting highest to lowest. The numerically highest and lowest values are highlighted in each column.

2018 Butte and Yuba Rootstock Experiment Leaf Sampling

Butte (N)	
Root	% N
Atlas	2.69 a
M40	2.62 ab
2624	2.57 abc
M29C	2.56 abc
K86	2.56 abc
K1	2.54 abc
Emp	2.54 abc
HBOK	2.52 abc
Viking	2.47 abc
Lovell	2.46 bc
M58	2.43 bc
Myro	2.42 bc
M30	2.39 bc
Cit	2.36 c

Yuba (N)	
Root	% N
M40	2.34 a
M30	2.33 ab
M29C	2.30 ab
K86	2.28 abc
Root-R	2.27 abc
2624	2.26 abcd
Atlas	2.26 abcd
K1	2.24 abcd
HBOK	2.23 abcd
Cit	2.22 abcd
M58	2.18 abcd
Viking	2.16 bcd
Lovell	2.13 cd
Myro	2.10 d

Butte (P)	
Root	% P
HBOK	0.22 a
Atlas	0.21 a
Emp	0.21 a
2624	0.20 a
M29C	0.20 a
Viking	0.18 a
M40	0.17 a
Lovell	0.16 a
M30	0.16 a
K1	0.16 a
M58	0.15 a
K86	0.15 a
Myro	0.15 a
Cit	0.14 a

Yuba (P)	
Root	% P
HBOK	0.19 a
Atlas	0.17 ab
Lovell	0.17 abc
K86	0.15 abcd
M30	0.14 bcde
Viking	0.14 bcd
M40	0.14 bcde
2624	0.13 bcde
Myro	0.13 cde
M29C	0.13 cde
Cit	0.13 cde
Root-R	0.12 de
M58	0.11 de
K1	0.09 e

Butte (K)	
Root	% K
K86	3.17 a
M30	3.13 a
2624	3.10 a
Viking	3.03 a
Emp	3.02 a
Cit	2.99 a
M29C	2.98 a
Atlas	2.94 a
M40	2.90 a
K1	2.79 a
Lovell	2.77 a
HBOK	2.77 a
Myro	2.72 a
M58	2.63 a

Yuba (K)	
Root	% K
K86	2.68 a
Viking	2.14 ab
Atlas	2.07 ab
Cit	2.00 bc
Lovell	1.89 bc
HBOK	1.88 bcd
M30	1.78 bcd
M40	1.77 bcd
M29C	1.60 bcde
2624	1.55 bcde
Root-R	1.43 cde
Myro	1.40 cde
K1	1.20 de
M58	1.03 e

Butte (Zn)	
Root	mg/kg Zn
Emp	43 a
M40	33 a
K1	29 a
Atlas	26 a
Viking	21 a
Cit	21 a
M29C	20 a
2624	20 a
HBOK	19 a
M30	19 a
K86	18 a
Myro	18 a
Lovell	17 a
M58	17 a

Yuba (Zn)	
Root	mg/kg Zn
M29C	30 a
Cit	23 ab
K86	23 ab
K1	22 ab
M40	22 ab
M30	22 ab
Myro	21 ab
Atlas	21 ab
M58	21 ab
Lovell	20 ab
Viking	20 ab
2624	19 ab
HBOK	19 ab
Root-R	17 b

Butte (Mn)	
Root	mg/kg Mn
K1	108 ab
2624	106 a
Emp	99 ab
Myro	99 ab
HBOK	97 ab
M30	93 abc
M40	85 abcd
M58	83 abcde
M29C	75 abcde
Cit	64 abcde
K86	62 bcde
Viking	53 cde
Lovell	48 de
Atlas	43 e

Yuba (Mn)	
Root	mg/kg Mn
M58	78 a
2624	65 ab
Myro	61 ab
M30	56 abc
K1	54 bcd
M40	52 bcd
M29C	49 bcd
Root-R	37 cde
Cit	34 cde
K86	32 de
Atlas	26 e
Lovell	25 e
Viking	23 e
HBOK	22 e

Butte (B)	
Root	mg/kg B
Atlas	61 a
Viking	53 ab
2624	52 ab
K86	50 ab
HBOK	50 ab
Emp	49 ab
M30	49 ab
K1	49 ab
M29C	48 ab
Lovell	48 ab
Myro	46 b
M40	44 b
Cit	44 b
M58	41 b

Yuba (B)	
Root	mg/kg B
Atlas	39 a
K86	38 ab
M40	36 abc
M30	36 abc
K1	36 abc
Viking	35 abc
2624	34 abc
HBOK	34 abc
Myro	34 abc
M29C	34 abc
Lovell	33 abc
Cit	32 abc
Root-R	31 bc
M58	29 c

Butte (Mg)	
Root	% Mg
Myro	1.00 a
Emp	0.98 a
Lovell	0.95 ab
M58	0.93 ab
HBOK	0.93 ab
M30	0.84 ab
Atlas	0.83 ab
Cit	0.81 ab
K1	0.81 ab
K86	0.80 ab
Viking	0.80 ab
M29C	0.80 ab
M40	0.79 ab
2624	0.74 b

Yuba (Mg)	
Root	% Mg
Myro	1.07 a
M58	1.05 a
M29C	0.88 b
Cit	0.84 bc
M30	0.83 bcd
Atlas	0.83 bc
Lovell	0.83 bc
M40	0.81 bcd
K86	0.79 bcd
2624	0.77 bcde
Root-R	0.77 bcde
HBOK	0.69 cde
Viking	0.66 de
K1	0.62 e

Butte (Fe)	
Root	mg/kg Fe
K1	168 a
Emp	142 a
K86	137 a
Cit	136 a
M30	135 a
M29C	135 a
M40	135 a
2624	134 a
Myro	131 a
Viking	126 a
HBOK	117 a
Lovell	116 a
Atlas	115 a
M58	111 a

Yuba (Fe)	
Root	mg/kg Fe
K1	210 a
M58	208 a
M30	180 ab
Lovell	171 ab
M29C	166 ab
Myro	166 ab
2624	163 ab
Root-R	160 ab
Atlas	158 ab
Cit	157 ab
K86	154 ab
M40	145 ab
HBOK	139 ab
Viking	134 b

Butte (Cu)		Yuba (Cu)		Butte		Yuba	
	mg/kg Cu		mg/kg Cu	Roots	% Ca	Roots	% Ca
Emp	18 a	Myro	9 a	Emp	3.01 a	Myro	3.03 a
M58	17 ab	M58	8 ab	Myro	2.68 ab	M58	2.65 ab
K1	16 abc	M30	8 abc	K1	2.37 ab	K86	2.50 bc
Myro	15 abc	M40	8 abc	Viking	2.34 b	Atlas	2.49 bc
M40	14 abc	M29C	8 abcd	Lovell	2.30 b	Cit	2.49 bc
Cit	14 abc	K86	8 abcd	Cit	2.30 b	M29C	2.43 bc
2624	14 abc	2624	8 abcde	M58	2.29 b	M30	2.40 bc
M30	14 abc	Cit	7 bcde	M29C	2.27 b	Viking	2.35 bc
Lovell	14 bc	K1	7 bcde	Atlas	2.22 b	2624	2.35 bc
K86	14 bc	HBOK	7 bcde	HBOK	2.20 b	Lovell	2.34 bc
M29C	13 bc	Atlas	7 cde	K86	2.17 b	M40	2.33 bc
Viking	13 bc	Lovell	7 cde	2624	2.16 b	Root-R	2.26 bcd
HBOK	13 bc	Root-R	6 de	M30	2.16 b	HBOK	2.05 cd
Atlas	13 c	Viking	6 e	M40	2.14 b	K1	1.86 d

Figure 10. Leaves were sampled from each replicated treatment rootstock at the Butte (8/3/18) and Yuba (8/16/18) sites and sent to Dellavalle Laboratory (Fresno, CA) for analysis. Values are least square means of total percent nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg) and, total mg/kg zinc (Zn), manganese (Mn), boron (B), and iron (Fe). Values followed by the same letters are not significantly different at 95% using Tukey's HSD.

2018 FIELD EVALUATION OF PRUNE ROOTSTOCKS AT WOLFSKILL

Katherine Jarvis-Shean, Richard Buchner, Franz Niederholzer, Ted DeJong, Sarah Castro and Carolyn DeBuse

PROBLEM AND ITS SIGNIFICANCE

The California Prune Industry has historically utilized five rootstocks, Myrobalan seedling, Myro 29C, Marianna 2624, Lovell Peach and some M40. The last statewide organized prune rootstock effort was the “M” series rootstock plots planted in 1987 (Vina Monastery 3/20/87). Since the conclusion of that experiment many more potential rootstocks for prune have been identified.

Three trials were planted in 2011 - two replicated experiments and one non-replicated observation experiment. Maintenance for the replicated trials is paid for by grower trial hosts. The non-replicated trial is at Wolfskill and requires funding for on-going management.

OBJECTIVES

Evaluate promising rootstocks potentially valuable for California Prune production.

PLANS AND PROCEDURES

A satellite experiment of prune rootstocks was planted at the UC Wolfskill experimental orchard in Winters, California. The plot contains 15 experimental rootstocks and 3 standard rootstocks (Marianna 2624, Lovell, and Myro 29C) nursery budded to ‘Improved French’ (Table 1). This experiment provides an initial evaluation of possible rootstocks that have previously not been tried with prune or have had very little field testing.

The experiment is planted with at least 5 trees of each rootstock and is non-replicated, which limits statistical analysis. The goal was to get a first look at how these rootstocks performed with ‘Improved French’ scions and identify any defects before commercial planting. ‘Improved French’ on its own root differs from the others in that trees were grown in the nursery for two years. Own rooted trees do have a graft union because ‘Improved French’ was budded on top. Trees were planted 17 feet across the row and 14 feet down the row, which would result in approximately 183 trees per acre.

The Wolfskill site was previously planted to peaches, removed in 2008 and the field left fallow for 3 years with annual winter wheat. The Yolo County soil survey describes the soil as Yolo loam. Nematode samples were taken at four locations within the field at approximately an 18 inch depth, and combined for nematode evaluation (8/29/11). One liter of soil contained, 50 Lesion (*Pratylenchus sp.*), 50 Pin (*Pratylenchus sp.*), and 30 Dagger (*Xiphinema americanum*). There were not enough nematodes to identify the species of either Lesion or Pin nematodes.

The majority of the trees were planted on January 19, 2011. Bare-root trees were planted directly after transportation from the nurseries sawdust box. HBOK 32 and HBOK 10 were potted trees planted on April 25, 2011. At the time of planting, trees were headed at 36 inches. Trees that had not reached heading height were left alone and allowed to grow through 2011 then headed at 36 inches in the following dormant season.

Trees were harvested August 28, 2018, when an aggregate sample of fruit from throughout the block indicated pressure was below 3 bars. The weight of the fruit from five adjacent trees (except Puente, n=4) was taken. A 4 lb sample was taken and dried to adjust total field fresh weight to estimated dry weight. This same sample was separated by size class after drying. Ten fruit were sampled, in addition to the 4 lb sample, for pressure and Brix. Figures are not given for Krymsk 2 or Krymsk 99 because it was judged by the group at the August 2017 prune breeding tasting meeting that these trees were too small and unhealthy looking to merit further tracking.

RESULTS AND DISCUSSION

Because this trial is not replicated, mean separation, also referred to as ANOVA, has not been conducted. Though we cannot say statically how rootstocks differ or rank, we can make initial observations. Averages given are for five trees.

Yield per tree varied widely by rootstock, ranging from 5.3 to 35.9 lbs per tree (Table 2, Figure 1). Imperial California, Controller 9, HBOK 27 and Speaker per in the lowest third of the yield spectrum for the year. Figure 2 demonstrates that this difference cannot be ascribed (or at least not solely) to these rootstock creating smaller trees, because many trees of comparable trunk circumference yielded much higher (Figure 2). Emphyrean 1, Emphyrean 3 and WRM 2 are the only rootstocks with trees that were in the top third of the yield spectrum besides the standard industry rootstocks of M2624 & Myro 29C. Yield in 2017 was not significant correlated with 2018 yield ($p=0.1599$). In other words, trees that were high yielding last year were not necessarily high yielding this year, and vice-versa. Nor did all high yielding trees last year collapse and perform poorly this year.

Yields in 2018 were only 11-60% of 2017 yields, depending on rootstock. Fruit set was quite light in 2018, and thus a larger percentage of fruit were in the Class A and B size categories, relative to 2017 (Figure 3). Emphyrean 1, Emphyrean 3, HBOK 32 and Own Root had notably lower percentages of Class A fruit, relative to the industry standard rootstocks and other novel rootstocks in the trial. This is worth some consideration and additional monitoring, particularly given how favorable to large fruit the light set was this year.

The high fruit set in 2017 and low set this year provides an opportunity to contrast tree potassium status (as measured by leaf percent potassium) in high and low demand years. Figure 4 shows 2017 and 2018 leaf potassium level compared with this 2018 yields. Yield in 2018 is significantly correlated with leaf potassium in 2017 ($p=0.03279$), but only weakly with 2018 ($p=0.06073$), indicating that potassium stress in 2017 maybe have impacted return bloom, or that factors that covaried with low potassium level (other high yield stresses).

Figure 5 compares 2017 and 2018 leaf percent potassium as a scatterplot. Interestingly, there is a significant but not tight linear relationship along the whole spectrum of potassium status ($p=0.01033$, Adjusted $R^2=0.3411$). It looks like trees that were good at taking up potassium when demand was high are also good at doing so when demand is low (i.e. they have relatively high leaf percent K in both cases). Trees that were not good at taking up sufficient potassium when demand was high (were low in leaf K in 2017) were inconsistent in their uptake ability when demand was low (some were high and some were medium in K in 2018).

CONCLUSION

The fact that 2017 yields were not significantly correlated with 2018 yields shows that many more years of data will be necessary to detect trends in which trees will be consistently high or low yielding. The small size of fruit in Empyrean 1, Empyrean 3, HBOK 32 and Own Root, despite the low set this year, always warrant additional years of monitoring. More years of data will be necessary before judging whether any of the rootstocks being tested should be tested in replicated trials to assess their long-term potential for the industry.

Table 1. Rootstock name and pedigree.

Rootstock	Species/ Hybrid Pedigree
Controller 9 (P30-135)	P. salicina x P. persica
Empyrean 1 (Barrier)	Peach x Chinese wild peach
Empyrean 3 (Tetra)	P. domestica
Fortuna	P. cerasifera x P. persica
HBOK 10 (Controller 8)	Harrow Blood x Okinawa
HBOK 27	Harrow Blood x Okinawa
HBOK 32 (Controller 7)	Harrow Blood x Okinawa
Imperial California	Plum R/S Italian Origin
Ishtara (Ferciana)	Peach/Plum hybrid
Krymsk 2	P. incanus x P. tomentosa
Krymsk 99	Plum/Peach hybrid
Lovell	Peach seedling
M2624	Marianna 2624
Myro 29C	Myrobalan
Own Rooted French	Own Rooted
Puente (Adara)	P. cerasifera
Speaker (Spicer)	Plum/Peach hybrid
WRM 2	Red leaf myroblan type

Table 2. Yield, fruit quality and size and tree size measurements by rootstock in 2018.

Rootstock	Lbs/ Tree	Pressure	Brix	Class size (% of weight in subsample)					Trunk Circum, Inches @ 12". 04/08/18
				A	B	C	D	Und er	
Controller 9	13.7	3.0	25.6	87	10	2	1	0	11.1
Empyrean 1	32.4	4.1	22.5	69	29	1	1	1	14.6
Empyrean 3	31.1	3.1	27.8	82	12	4	2	0	16.7
Fortuna	24.9	2.5	26.8	68	22	8	1	1	21.7
HBOK 10	24.0	4.8	23.5	47	29	19	5	0	15.1
HBOK 27	14.7	3.8	26.7	95	4	1	1	0	19.3
HBOK 32	21.0	4.0	25	79	16	1	2	1	14.4
Imperial CA	5.3	3.7	25.5	92	6	0	1	1	14.4
Ishtara	16.6	3.9	26	60	31	9	0	0	14.8
Lovell	19.5	3.8	26.8	90	4	4	0	2	15.2
M2624	35.9	3.3	23.3	100	0	0	0	0	14.6
Myro 29C	27.1	2.7	24.9	93	5	2	0	0	16.6
Own Root	19.5	3.7	26.8	88	9	2	2	0	15.7
Puente	20.6	2.4	26.4	87	10	1	1	0	17.8
Speaker	14.8	3.7	25.5	54	23	17	5	0	15.2
WRM 2	27.0	3.4	26.6	80	14	4	2	0	18.1

Figure 1. Yield by rootstock, 2017 & 2018 (ordered by 2018 yield).

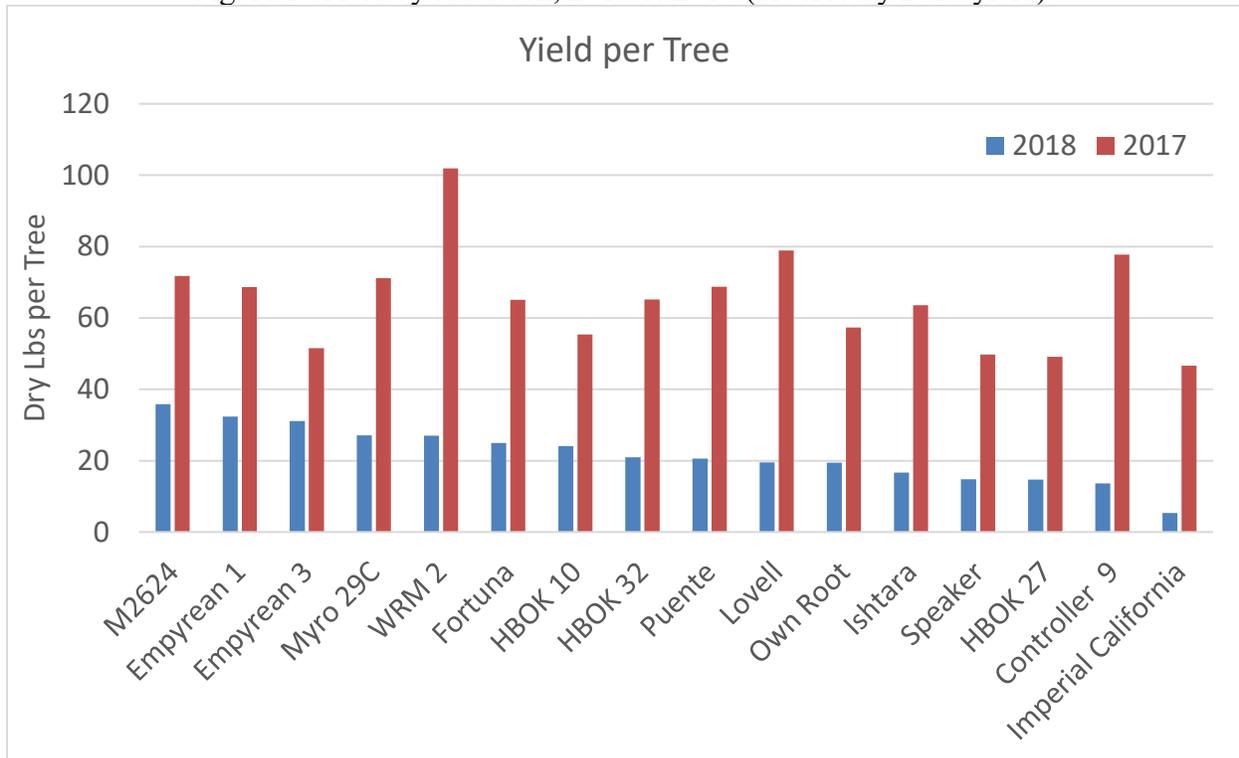


Figure 2. Yield in 2018 compared with trunk circumference in the spring of 2018.

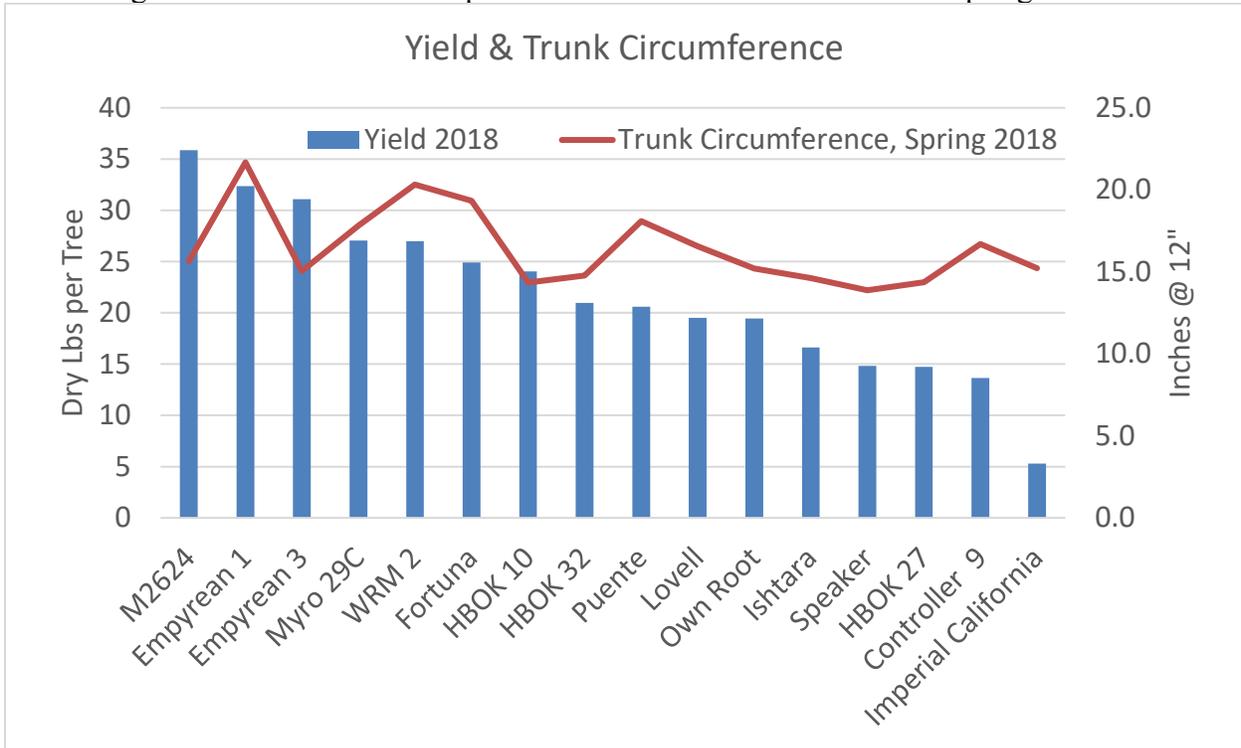


Figure 3. Size Class of Fruit by Rootstock, 2018 (ordered by 2018 yield).

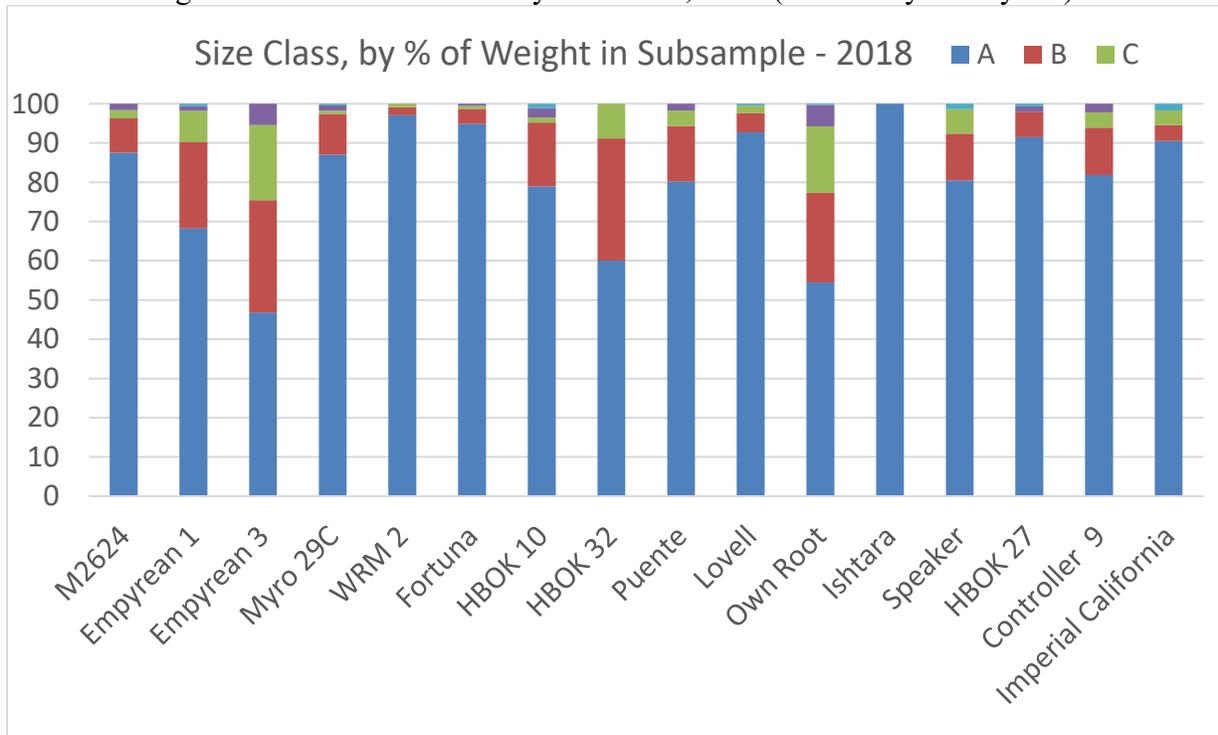


Figure 4. Yield and leaf potassium by rootstock.

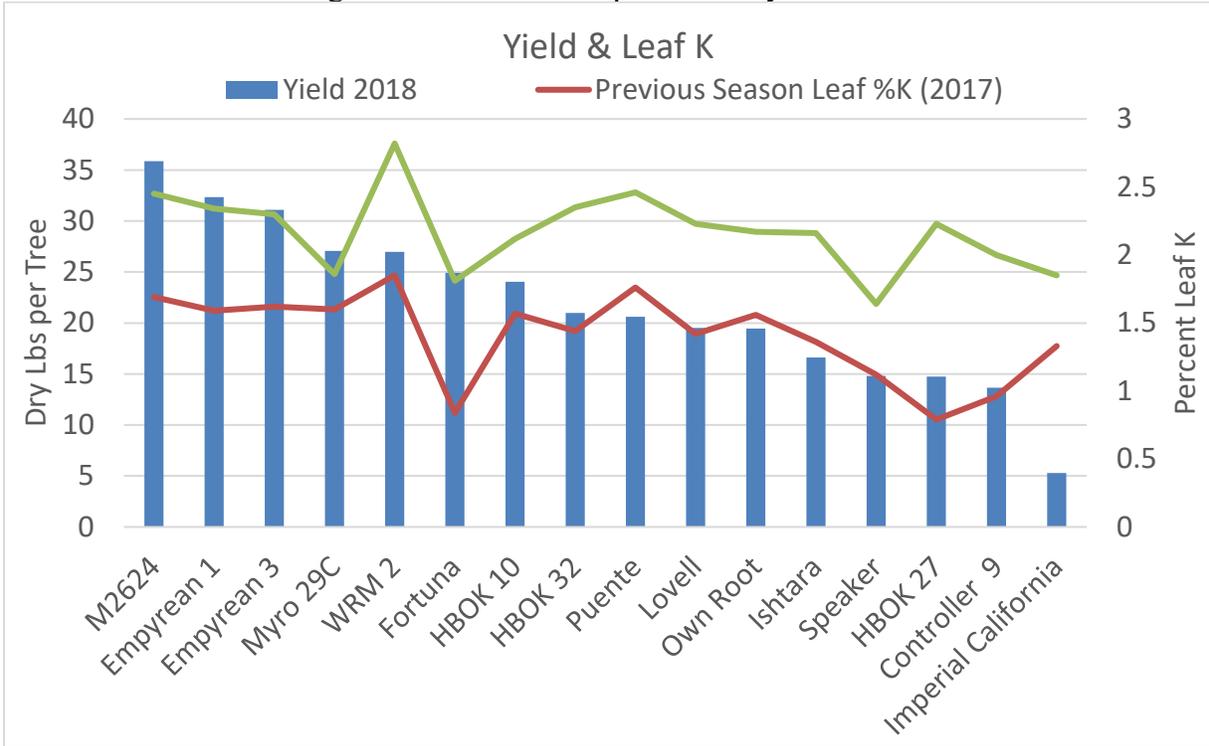
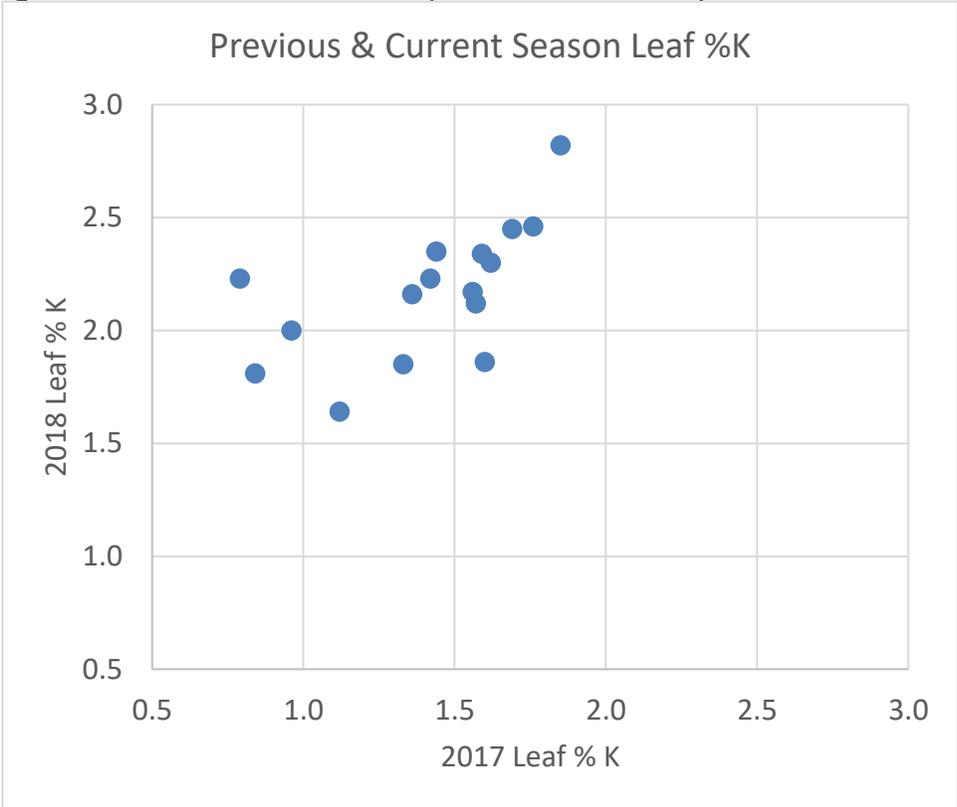


Figure 5. The correlation between previous and current potassium tree status.



MANAGING HEAT AT BLOOM IN 'FRENCH' PRUNE, 2018

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PROBLEM AND ITS SIGNIFICANCE

Excessive heat or extended wet, cool weather at bloom is linked to significantly reduced prune production in key California growing regions in five of the last fourteen years (2004, 2005, 2007, 2014, and 2016). Total grower economic losses in Sutter and Yuba Counties – with 40% of the prune acres in the state -- were in the range of \$240 million for 2004, 2005, and 2007, based on county ag commissioners' data. Overall economic damage to the regional economy was probably 1.5x that loss -- \$360 million. As the probability of heat in March appears to be increasing (Rick Snyder, retired UCCE microclimate specialist, personal communication), California prune growers must develop management strategies to mitigate heat damage at bloom to remain economically viable, while remaining aware of crop risk due to unusually cool bloom weather,

Recent research results show that temperatures $>75^{\circ}\text{F}$ begin to negatively affect pollen tube growth rate and viability, but research has not identified temperature thresholds for actual crop damage.

OBJECTIVES

- Determine bloom-time temperature thresholds above and below which crop damage occurs and bloom patterns that present crop risk.

PROCEDURES

Madera, Sutter, Glenn, Solano/Yolo and Tehama Counties:

Bloom timing and temperature have been monitored since 2010 along most of the length of the major fruit growing regions of the Sacramento Valley, home to 85% of the bearing acres in California. In 2018, a study site in Madera County in the San Joaquin Valley was added. Additional sites in Tehama County (3), Glenn County (3), Sutter County (2) and Solano County (1) were monitored for bloom timing and orchard weather (temperature and percent relative humidity).

Combined temperature and relative humidity sensors housed in radiation shields were placed in

between trees down the tree row at 6-8' above the orchard floor within the study orchard. Sensors were not placed in tree canopies. Temperatures and relative humidity in each block were continually recorded during bloom at all sites. Average hourly temperatures are reported, not maximum temperature for the day.

Bloom progression was measured by counting open flowers on 2-5 short branches (roughly 100 flowers, each) at approximately 6' height around 3 trees in each orchard. Initial set was measured in May.

RESULTS AND DISCUSSION

Weather during the 2018 prune bloom in the Sacramento Valley began cool and wet but ended alarmingly warm (max temperatures = 83-86°F on March 29). Very similar temperatures were recorded in Tehama, Glenn, Sutter, Solano, and Madera Counties for the same time period. Bloom lasted for 9-14 days, depending on location. Fruit set ranged from 25-41%. Reports from field observers noted the light bloom present in many orchards up and down the state.

CONCLUSIONS

Excellent fruit set (25-50% of flowers set fruit) occurred despite the very high temperatures at full bloom – the same temperature range that has produced crop failures in previous years. It appears that the extended bloom, during which early opening flowers were pollinized and fertilized under moderate temperatures, before the heat at full bloom (Mar 28-30), allowed the high levels of fruit set measured. In 2005, when extreme heat at bloom produced very poor set (5%) in prune orchards, bloom was rapid (5 days from first flower to full bloom) so that flowers did not have time to be fertilized and set fruit before the damaging extreme heat occurred (Figure 1).

The reduction in prune crop production in California predicted for 2018 (80,000 tons compared to the 104,000 tons in 2017 crop) does not appear to be due to poor set resulting from extreme bloom weather. The light bloom reported in many orchards in the Sacramento Valley may be a key factor in the lighter crop in 2018.

Weather/climate conditions that promote flash bloom and increase the risk of crop disaster appear, from our weather and bloom monitoring, to be 1) consistent chill accumulation (Dynamic Model chilling units) and 2) rapid warming that continues through bloom. Weather/climate conditions that promote extended bloom are 1) inconsistent chilling, especially in January and/or February followed by initially cool and/or moderate bloom weather. Examples of chilling unit accumulation for crop disasters (2004 and 2005) or good fruit set conditions despite heat at full bloom (2018) appear in Figure 3. For more information on the Dynamic Model and chill portions, see http://fruitsandnuts.ucdavis.edu/Weather_Services/chilling_accumulation_models/ and pages accessible from there.

Financial value of this research: Prune crop loss in the Sacramento Valley in 2016 was at best estimate, at least 1.5 dry tons/acre across 90% of the acres in the region (37,000 acres using 2015 crop report data). At \$2000/dried ton, that loss = \$111M in farm gate value before the multiplier effect on local economies. This research, developing information to allow growers to more accurately predict crop risk at bloom, will help growers use management tools to minimize damage from unseasonable weather at bloom.

Table 1. Prune bloom timings, full bloom (80% open flowers) date and average prune fruit set (May) for individual orchards plus maximum orchard temperatures (hourly average of measurements taken every 5 minutes) during bloom in Tehama, Glenn, Sutter, Solano and Madera Counties, 2018. Maximum orchard temperatures on the day of full bloom in each orchard appear in **BOLD** font. Background fill indicates the length of bloom. Bold and italics indicate date of first bloom. Where temperature is not bolded on the 15th, flowers open before that date. Data logger troubles produced no accurate temp measurements for two sites (one Tehama and one Glenn sites).

-----March-----																	
County	General Location	15 th	16 th	17 th	18 th	19 th	20 th	21 st	22 nd	23 rd	24 th	25 th	26 th	27 th	28 th	29 th	% set
Tehama	Red Bluff								53	68	57	60	67	79	83	84	19
Tehama	S. Red Bluff								62	58	58	59	66	78	82	84	41
Tehama	Los Molinos																24
Glenn	Capay 1																51
Glenn	Capay 2	49	58	58	59	66	57	62	68	58	58	60	67	77	81	83	41
Glenn	Road S		58	57	60	65	57	61	71	63	61	62	67	77	82	84	41
Sutter (+oil)	SW Yuba City	52	58	59	60	60	66	65	64	61	60	61	68	79	84	86	28
Sutter(-oil)	Dingville (10 m S. YC)	N.D.	57	58	59	65	59	64	66	60	58	60	66	77	81	84	41
Solano	Wolfskill	64	56	55	57	63	56	65	64	61	58	60	66	78	83	85	25
Madera	S Madera	59	56	60	60	68	58	63	68	62	64	60	64	70	76	80	41

Figure 1. Average, hourly temperatures (deg F, blue diamonds) and bloom progression, Solano County, 2018 (red squares) as well as bloom progression relative to full bloom from Sutter Co, 2005 (purple diamonds). Fruit set was 25% in 2018 and 5% in 2005. Blue bars indicate periods of rain in 2018.

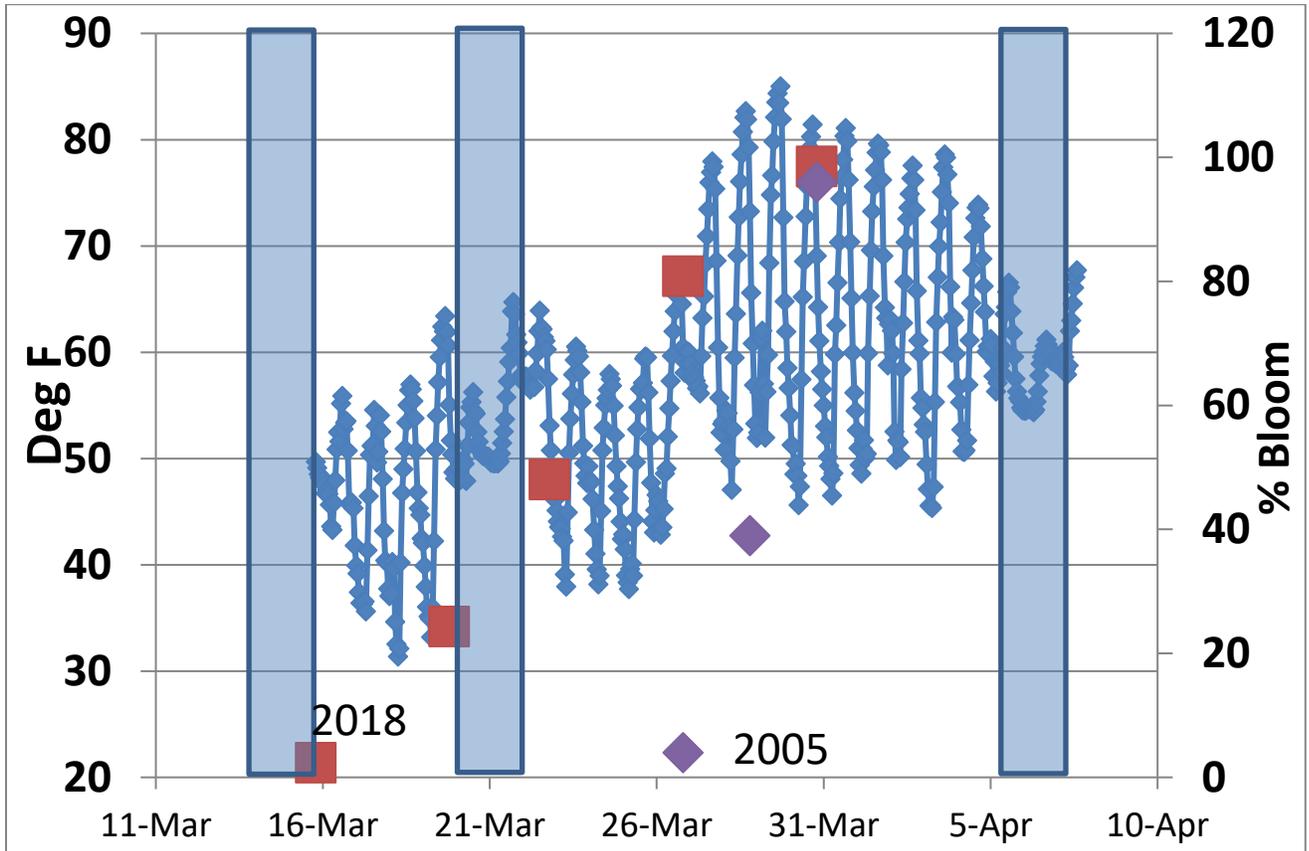
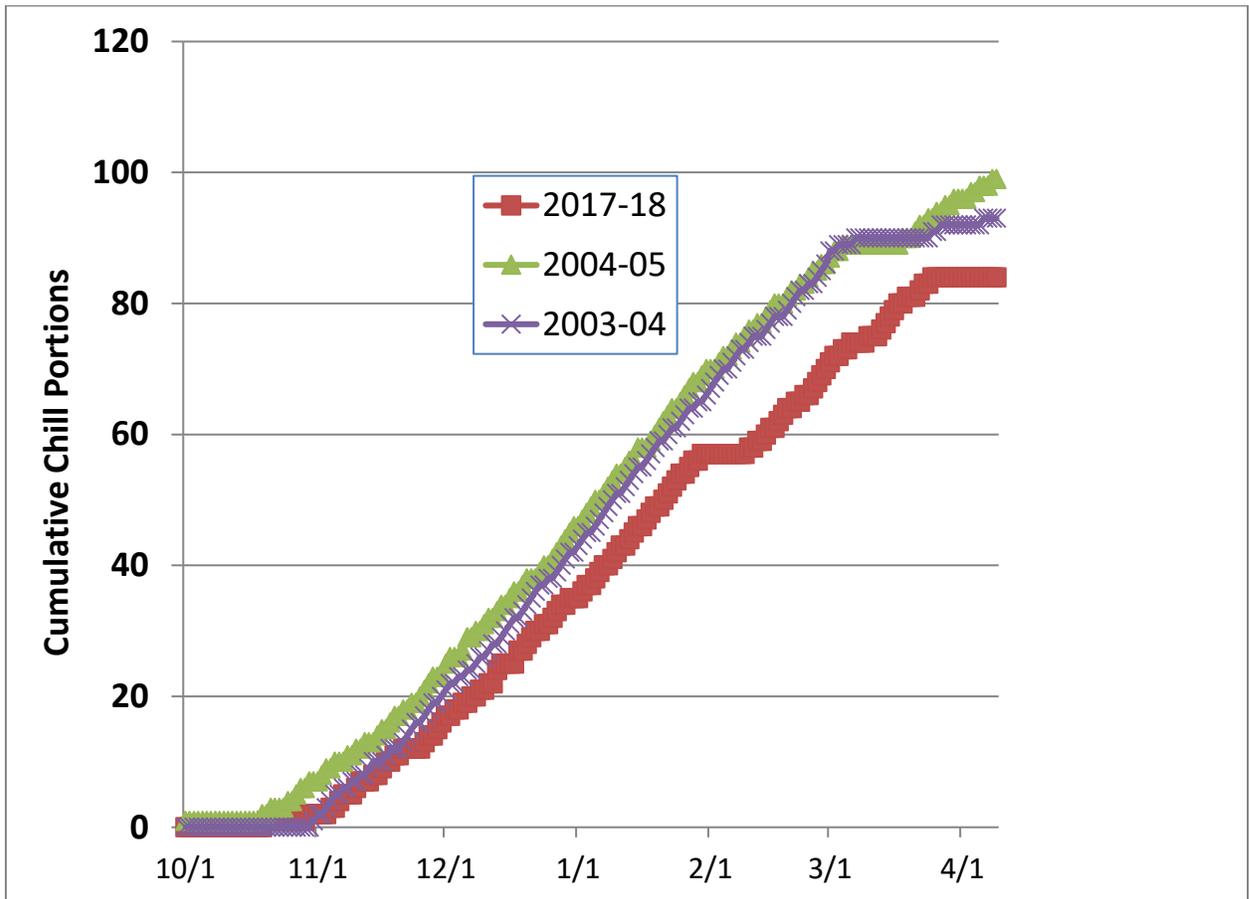


Figure 2. Beta bloom weather model for estimating prune fruit crop set conditions.

Bloom Temperatures (±1 day around 80% bloom)	Details	Outcome
<60°F	If cool conditions extend for 7-10 days before FB	Poor fruit set conditions
<60°F	If early bloom temperatures are above 60°F	Good fruit set conditions
60-81°F		Good fruit set conditions
>81°F	If "flash bloom: 5 or less days between first flower and full bloom	Poor fruit set conditions
>81°F	If bloom starts cool and is extended (7-14 days)	Good fruit set conditions

Figure 3. Chilling accumulation (Dynamic Model chill portions) from Winters CIMIS station for 1) prune crop disaster years with good-excellent chilling followed by warm weather and flash bloom (2003-04 and 2004-05) and 2) good prune set conditions at bloom with inconsistent chilling beginning in late January and warm temperatures at full bloom. Flat sections of the chilling graphs are produced by warm weather with high temperatures at least in the upper 60's/70's.



BLOOM AND POSTBLOOM PRUNE MANAGEMENT PRACTICES FOR MORE CONSISTENT PRODUCTION OF HIGH QUALITY PRUNES.

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PROBLEM AND ITS SIGNIFICANCE

Inconsistent cropping (yield/acre) is a major challenge facing the dried plum industry in California. This is a year to year phenomenon, linked, primarily, with weather conditions at bloom. Hot or cold weather (above 80°F or below 60°F) coincided with or caused state-wide crop failure in 2004 and 2016 and regional failures in 2005, 2007, 2014 and 2015. However, in most years, warm to moderate temperatures at bloom (daytime highs of 60-80°F) produced excessive fruit set resulting in over production of small, lower quality fruit -- unless growers shaker-thin at reference date. Over cropping in one year will reduce bloom the next year, further reducing production the following year if poor bloom conditions exist. Prune growers are, literally, faced with boom or bust production every year (Figure 1).

There are few options for growers between bloom and reference date to manage cropload for more consistent production. Under tree sprinkler irrigation can decrease daytime orchard temperatures by 1-2°F and so may help growers avoid losses under conditions just above the damage threshold (83-84°F). However, no materials or practices have been proven to avoid crop loss at bloom when higher temperatures (>84°F) or extended wet/cold conditions occur. Shaker thinning at reference date provides inexpensive and effective crop management, but this practice limits production potential in an orchard by disproportionately removing larger fruit, potentially damaging older trees and occurs after 4-6 weeks of fruit development, wasting resources that could be used by sound fruit. The earliest possible thinning – bloom thinning – has been shown, in peach, to deliver larger fruit size and total crop at harvest compared to later thinning. The same results should follow in prune. Field research with GA-3 showed some promise in reducing flower number the year after application, but research results are not consistent and this material is not currently labeled for prunes for this purpose. Flower thinning by hand is not feasible and chemical blossom thinning with caustic materials remains largely untested in prunes. Prune production in California should benefit from cropload management practices that either improve or reduce fruit set at bloom and/or reduce excessive fruit set within 3-4 weeks of full bloom.

Objectives:

- Evaluate materials and practices intended to improve or reduce fruit set depending on the bloom temperature in the orchard to allow consistent production of large, high quality prunes.

PROCEDURES

Spray treatments were applied in a mature (12th leaf), high production (4-5 dry ton/acre, 32%

PAR interception) site in south Sutter County – the same orchard used for this study in 2015, 2016 and 2017. The orchard is planted 16' x 20' on M29C rootstock and irrigated with microsprinklers.

In March, 2018, a randomized complete block design experiment was established using trees with known cropload/size history – 2017 study trees. Individual trees were blocked by 2017 % yield of A+B fruit size per tree using the hypothesis that return bloom would be positively related to relative percentage of large fruit production the previous year, with Block 1 having the highest percentage of A+B fruit and Block 4 the lowest. With extra trees needed in the experiment, Block 5 trees were selected from trees outside the experiment in 2017, which were untreated in that year and, thus, assumed to have lower %A+B fruit – similar to Block 4.

Thirteen treatments in five blocks, one treatment rep per block, were applied between March 24 and April 14. Both thinning and “sticking” materials were applied. For “Sticking” – improving set – a treatment of ReTain[®] (AVG, which inhibits for formation of a precursor of ethylene) and Abound[®] (a FRAC Group 11 fungicide reported to improve plant health) was applied at 80% full bloom. Five treatments of a caustic fertilizer -- potassium thiosulfate (KTS) -- were applied at different concentrations (1, 1.5 or 2% v/v) at different timings (25% and 80% bloom or once at 25% or 80% bloom). Lime sulfur treatments (2.5% v/v) with either fish oil (2% v/v) or vegetable crop oil (2% v/v) as an adjuvant were applied at 80% bloom. Two ethephon treatments (75 ppm or 150 ppm) were applied on March 30 at 100% full bloom and, to separate trees, at 15 days after full bloom (DAFB). No shaker thinning treatments were applied to study trees this year. Treatment materials, rates, and timings appear in Table 1.

Treatments were applied using a gas-powered, backpack sprayer (Stihl[®] SR420; Stihl USA, Virginia Beach, VA) at a volume equivalent to 200 gallons per acre. Temperature and relative humidity were tracked using a Hobo data logger system (Onset Computer Co, Boston, MA). The first KTS treatments were applied between 9:15 and 11:15 AM on March 23. Temperatures were between 47-56°F during this application and RH from 70-61%. The second KTS treatments and the lime sulfur treatments were applied four days later on March 28 between 8AM and 12:30 PM. Temperatures ranged from 55-75°F on that morning with RH from 73-45%. The full bloom ethephon applications went out on March 30 between 10-11 AM when the temperatures ranged from 66-71°F. The final ethephon sprays were applied on April 14 between 8-9 AM when the temperatures ranged from 54-60°F and RH = 80-70%. Average hourly temperatures and percent relative humidity for each application date appear in the Appendix.

Two approaches were used to assess the influence of spray treatments on crop set and yield. The first used local measurements at multiple sites within individual trees. Measurements included flower number per shoot, bloom development over time, and % fruit set per shoot to look at local effect of thinning. The second approach used whole tree yield and fruit size estimate to measure the impact of thinning treatments on commercial production.

Local measurements: Beginning on March 19, swelling buds were counted for 1-2' back along a spur about 4-6' off the orchard floor at the outer edge of the canopy at 3-6 locations around individual trees. Flowers and fruit on multiple branches were counted on 28 trees across 11 treatments. Trees used in these measurements were not selected based on blocking factor used in

the whole tree treatment assessment (see below). The number of open flowers was measured on March 19, 20, 21, 23, 26, and 28. The total number of flowers plus the number of double and/or triple flowers emerging from the same bud were also measured for each shoot. Fruit present on the same length of shoot were counted on May 17 and % set calculated from that value divided by total number of flowers at bloom. Mean treatment differences were tested using Statgraphics Centurion XVII (Statpoint Technologies, Inc., Warrenton, VA) software package using the General Linear Model procedure with mean separation tested by Duncan's MRT method. Individual shoots were treated as experimental units (reps) as % fruit set was not significantly different between trees of the same spray treatment or by location around each tree (data not presented).

The influence of spray thinning on return bloom was assessed by counting flowers on 3-5 shoots from trees with unthinned or thinned with 2.5% Lime sulfur and 2.0% v/v Fish Oil at 80% bloom in 2017. Branches were tagged ahead of bloom and all open flowers counted at several intervals. Just prior to harvest, the entire shoot was cut from the tree and all leaves, fruit and current season growth removed. The length of wood present at bloom on each shoot was then measured by hand and bloom data presented on a flower/cm of shoot basis.

Whole tree measurements: Control tree fruit internal pressure averaged 4 lbs and fruit flesh soluble solids average 22% on August 24. All treatment trees were harvested on August 27. Individual tree dry fruit yields were determined by shaking each tree on to a commercial harvester, clearing the belts into large plastic bins without running over a fruit sizer, and weighing each tarred bin on a field scale. Following commercial drying of a four pound subsample, the fresh:dry weight ratio (dry away) was used to calculate dry fruit yield per tree. Fruit sizes in the subsample were determined by individually weighing each fruit. Fruit count per pound and the number of fruit per tree were determined from the number of fruit per subsample and the total weight of that sample. Treatment differences in dry fruit yield per tree (multiplied by 136 trees per acre to provide yield in tons/acre), large fruit (A+B screen), dry away and fruit per tree were tested using Statgraphics Centurion XVII (Statpoint Technologies, Inc., Warrenton, VA) software package using the General Linear Model procedure with mean separation tested by Duncan's MRT method.

Table 1. Treatments and timing applied in 2018.

Materials	Treatment rates	Treatment stages	First spray	Second Spray
Potassium thiosulfate (KTS)	1% v/v	25 & 80% bloom	24-Mar	28-Mar
Potassium thiosulfate (KTS)	1.5% v/v	25 & 80% bloom	24-Mar	28-Mar
Potassium thiosulfate (KTS)	2% v/v	25 & 80% bloom	24-Mar	28-Mar
Potassium thiosulfate (KTS)	1.5% v/v	25% bloom	24-Mar	--
Potassium thiosulfate (KTS)	1.5% v/v	80% bloom	--	28-Mar
Unthinned control	--	--	--	--
ReTain® + Abound®	333 g/a + 12.8 oz/a	80% bloom	--	28-Mar
Lime-sulfur + Fish oil	2.5% v/v & 2% v/v	80% bloom	--	28-Mar
Lime-sulfur + Veg crop oil	2.5% v/v & 2% v/v	80% bloom	--	28-Mar

ethephon	150 ppm	100% bloom	--	30-Mar
ethephon	75 ppm	100% bloom	--	30-Mar
ethephon	150 ppm	15 DAFB	--	14-Apr
ethephon	75 ppm	15 DAFB	--	14-Apr

RESULTS AND DISCUSSION

Local measurements: Return bloom, measured as flowers/cm of shoot, was significantly improved by thinning the previous year (Table 2).

All thinning treatments significantly reduced fruit set on individual shoots (Table 3), with double applications of KTS (1, 1.5 or 2.0% v/v at 25 and 80% bloom) producing mean set levels of <10%, while flowers on unthinned trees set at an average rate of 41%. Treating with ReTain[®] + Abound[®] did not influence set (Table 3).

Whole tree measurements: Blocking by previous year fruit size (%A+B fruit) was significant different for fruit/tree, dry away and dry fruit/acre, but not for yield of A+B screen fruit/acre (Table 4). The block with significantly higher values for fruit/tree, yield and dry away was Block 1, which included treatment trees with the highest ratio of A+B screen yield:total yield in 2017 (Table 4), compared to other blocks in the study.

Despite the significant block effect (Table 4), and the large treatments differences in fruit set on shoots (Table 3), statistical differences between thinning treatments were limited (Table 5). Tree sizes, and hence cropload potential, varied within each block and treatment group, and this many explain the limited treatment differences. In addition, bloom timing was not uniform between and within treatments, so trees with early bloom may not have been equally affected by bloom thinning treatments applied only at full bloom.

The 150 ppm ethephon treatment at 15 DAFB produced significant scaffold gumming and should be avoided in future trials. In addition, this treatment produced the lowest yield/acre.

The Vegetable oil concentrate (VOC) adjuvant used with Lime sulfur produced more thinning (Table 3) than the Fish oil, although numerical yield differences between the treatments were not statistically significant (Table 5). The use of VOC instead of Fish oil, at the same rate of 2.0% v/v, should be approached very carefully.

CONCLUSIONS

The 2018 season in this study orchard was a light cropping year with fruit per tree numbers running around 1500-5000 compared to the heavy crop year of 2018, where fruit/tree values ranged from 5,000-10,000.

Unthinned trees in 2017 produced more tonnage and less large fruit with total yield averaged 1.0-1.5 tons/acre large (A+B screen) fruit out of a total crop of 5.4 dry ton/acre compared to 3.5 tons/acre of A+B screen fruit out of a total crop of 4.3 dry tons/acre for unthinned trees in 2018 (Table 5).

The clear cut shoot thinning results and the less clear whole tree results indicate the challenges facing growers (and researchers) trying to manage variable bloom with one or two thinning sprays.

Additional take home conclusions:

- Despite very low % set values for all three KTS treatments with 2 applications, total yields for these treatments ranged from 2.5-3.3 dry tons per acre with a high percentage of large fruit.
- Ethephon at 75 ppm appears to not have significantly reduced fruit set and yield, but 150 ppm at full bloom appears to have promise as a thinner. Both total yield and large fruit yield, though not statistically significant, showed a trend towards strong production of large fruit (Table 5).
- Ethephon at 150 ppm postbloom (15 DAFB in this study) produced alarming gumming from the scaffolds and will not be repeated.

Figure 1. Average fruit set (± 1 standard error) in selected Tehama and Sutter/Yuba county prune orchards (n=2-4) from 2009-2018.

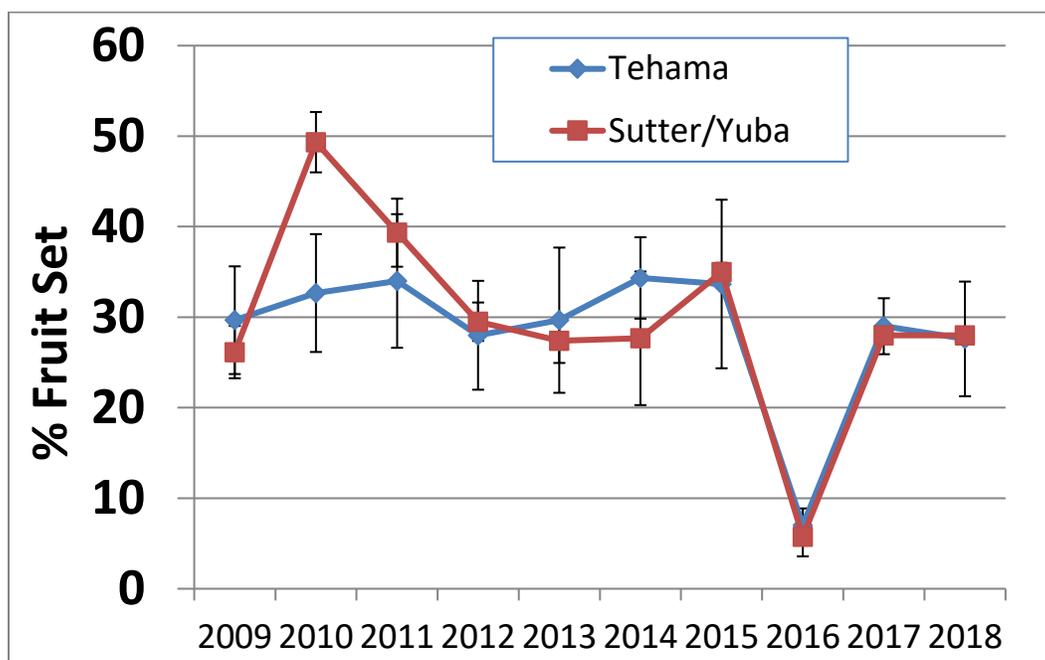


Table 2. Bloom per cm of shoot, 2018, trees thinned or unthinned in 2017.

Treatment in 2017	Bloom Density, 2018 (flowers/cm)
Untreated Control	0.18 a
Lime sulfur + Fish oil	0.36 b

Table 3. Percent fruit set on individual shoots treated as part of whole tree spraying.

Treatment	Trees measured (shoots sampled)	% fruit set
2% KTS (25 & 80% bloom)	3 (9)	4.6 a
1.5% KTS (25 & 80% bloom)	2 (7)	6.7 ab
Lime sulfur + VOC	3 (8)	7.7 ab
1.0% KTS (25 & 80% bloom)	2 (8)	9.2 abc
150 ppm Ethephon (100% FB)	3 (10)	15.7 bcd
150 ppm Ethephon (15 DAFB)	2 (6)	20.4 cde
Lime sulfur + fish oil	3 (11)	22.1 de
1.5% KTS (25% bloom)	2 (7)	23.2 de
1.5% KTS (80% bloom)	2 (7)	26.7 e
ReTain [®] + Abound [®]	3 (13)	40.8 f
Control	3 (10)	41.0 f

Table 4. Block values for fruit/tree, dry tons/acre, A+B screen fruit and dry away. 2018. Block 1

Block and blocking factor (A+B dw/acre:total dw/acre)	Fruit/tree	Dry fruit (tons/acre)	A+B screen fruit (tons/acre)	Dry Away (fresh:dry wt)
Block 1 (>0.74)	4,051 a	4.08 a	2.61 a	3.27 a
Block 2 (0.35-0.69)	3,236 ab	3.62 ab	2.78 a	3.03 b
Block 3 (0.16-0.31)	2,313 bc	3.05 ab	2.71 a	2.94 b
Block 4 (0.06-0.16)	1,796 c	2.45 b	2.23 a	2.99 b
Block 5 (added in '18)	2,652 bc	3.37 ab	2.79 a	3.01 b

Table 5. Fruit count/tree, dry fruit yield/acre, large fruit (A+B screen) yield /acre and dry away (fresh:dry fruit wt) by treatment. 2018. Values in columns followed by the same letter are not statistically different with 95% confidence using Duncan's RMT.

Treatment (with spray timing)	Fruit/tree	Dry fruit (tons/acre)	A+B screen fruit (tons/acre)	Dry Away (fresh:dry wt)
150 ppm Ethephon (15 DAFB)	1352 a	1.81 a	1.72 ab	2.94 a
2% KTS (25 & 80% bloom)	1469 ab	2.46 ab	2.42 ab	2.90 a
1.5% KTS (25 & 80% bloom)	1698 ab	2.71 ab	2.65 ab	3.03 a
Lime sulfur + fish oil	2082 abc	2.94 ab	2.71 ab	2.91 a
Lime sulfur + VOC	2286 abc	2.27 ab	1.42 ab	2.95 a
1.0% KTS (25 & 80% bloom)	2352 abc	3.34 ab	3.19 ab	3.01 a
150 ppm Ethephon (100% FB)	2499 abc	3.32 ab	3.02 ab	3.06 a
1.5% KTS (80% bloom)	2785 abc	3.25 ab	2.50 ab	3.11 a
75 ppm Ethephon (100% FB)	3180 abc	3.84 ab	3.28 ab	3.05 a
Control	3612 abc	4.31 b	3.56 b	3.16 a
ReTain [®] + Abound [®]	4142 abc	3.35 ab	1.31 a	3.19 a
75 ppm Ethephon (15 DAFB)	4251 bc	4.62 b	3.36 ab	3.10 a
1.5% KTS (25% bloom)	4818 c	4.75 b	2.86 ab	3.18 a

Appendix:

Hourly averaged weather data from the application dates, 2018. Thinning treatment spray timings appear with grey background.

Time of day	Deg F 3/24	RH 3/24	Deg F 3/28	RH 3/28	Deg F 3/30	RH 3/30	Deg F 4/14	RH 4/14
0:00	49.5	75.5	49.7	79.9	54.7	84.9	46.9	89.5
100	48.3	79.0	49.1	82.8	51.2	88.0	46.2	89.9
200	47.0	83.3	47.1	85.4	50.2	88.4	47.7	89.8
300	46.2	84.8	46.0	87.2	48.3	88.3	48.5	86.3
400	45.5	87.3	45.0	87.7	46.6	89.4	46.8	87.1
500	43.8	88.8	44.1	88.8	45.7	89.6	45.0	88.8
600	42.6	89.7	44.8	88.4	45.3	90.0	42.9	89.5
700	43.5	90.1	46.7	86.5	46.9	90.4	46.8	90.1
800	45.4	89.5	54.9	72.9	53.3	87.1	54.3	80.2
900	49.3	82.6	58.6	66.3	61.5	72.4	59.9	71.1
1000	53.2	70.9	63.1	58.9	66.4	63.0	63.2	66.8
1100	54.9	63.5	67.5	54.5	71.5	53.7	67.8	63.2
1200	56.5	59.8	71.9	47.1	75.3	49.5	71.6	57.9
1300	57.5	56.4	75.3	44.6	76.8	47.9	73.9	49.0
1400	57.2	54.7	78.5	42.7	78.9	45.0	75.4	45.1
1500	57.3	52.3	81.0	37.3	79.8	42.1	75.8	43.2
1600	57.7	49.4	81.2	37.2	79.6	41.0	75.4	44.2
1700	56.9	47.0	80.1	36.8	77.8	45.1	74.3	48.3
1800	53.5	52.4	77.4	38.1	73.1	59.4	71.9	51.9
1900	51.1	57.5	67.8	54.1	65.7	74.8	68.3	59.1
2000	49.1	61.2	61.2	68.4	60.8	84.1	66.4	58.2
2100	46.5	70.2	60.8	71.4	58.2	87.0	63.7	65.1
2200	44.7	73.5	57.4	76.2	57.3	88.0	61.0	72.5
2300	41.6	79.3	57.4	77.2	58.5	83.2	59.0	72.3

Annual Report - 2018

Prepared for the Dried Plum Board of California

Title: Epidemiology and management of blossom, leaf, and fruit diseases of prune
Status: 4th Year
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SUMMARY OF RESEARCH ACCOMPLISHMENTS IN 2018

1. **Bacterial canker caused by *Pseudomonas syringae* pv. *syringae*.** Branches of French prune trees were inoculated and treated with selected bactericides in the dormant period, but no disease developed in the 2017 (winter) – 2018 (spring) season. Current efficacy data on prunes allows for kasugamycin (trade name Kasumin) to be nominated into the IR-4 program. Full registration has been approved by EPA and CDPR on pome fruits (apples and pears), cherry, and walnut in California; registration on peach and almond is pending. Oxytetracycline that is currently registered on peach in the United States but not in CA can also be submitted to the IR-4 program for registration on prune.
2. **Brown rot blossom blight.** Fungicides were evaluated for their pre- and post-infection activity against *M. fructicola*. Single active ingredient fungicides (Rhyme, UC-1, Fontelis, pyraziflumid) and premixtures (Luna Experience, Quadris Top, Merivon, EXP-AD, EXP-AF, UC-2) all significantly reduced blossom blight from the non-treated control. Quadris Top, EXP-AD and EXP-AF had the least post-infection activity; and Quadris Top and EXP-AF were less effective pre-infection (protective) treatments compared to the other fungicides evaluated.
3. **Fruit brown rot.** In 7-day PHI applications at 130 gal/A, propiconazole (e.g., Bumper) mixed with summer oil resulted in significantly less brown rot after wound-inoculation than when the fungicide was mixed with Nu-Film 17. In previous studies, only oil but not non-ionic surfactants improved decay control. UC-2 mixed with oil or Nu-Film 17 at 8 or 32 oz/A were similar in effectiveness. No differences were observed between propiconazole or UC-2 mixtures with either adjuvant on non-wounded fruit.

In another study with registered and new fungicides in combination with 1% summer oil applied 15 days before harvest, fungicides containing a DMI compound (e.g., Luna Experience, Fervent, Quadris Top), as well as some experimentals (UC-1, UC-2, V-424) significantly reduced the incidence of brown rot from that of the control after wound-inoculation. Luna Experience, Fervent, UC-1, UC-2, and V-424 were most effective, Luna Sensation and EXP-AD were intermediate in effectiveness, and the SDHI compounds Fontelis and pyraziflumid, as well as Merivon and EXP-AF did not reduce decay compared to the control. These latter fungicides are contact materials and do not penetrate into the fruit. In contrast, all fungicides evaluated, with the exception of V-449, significantly reduced brown rot on non-wounded fruit.
4. **Rust.** In a late-season study on the management of rust, all fungicides significantly reduced rust from the non-treated control. Very low disease incidence and severity ratings were observed after treatment with Rhyme, V-424, Luna Experience, Quadris Top, Merivon, UC-2, and Fervent. Ph-D, Fontelis, pyraziflumid, Luna Sensation, EXP-AD, EXP-AF, UC-1, and V-449 were the least effective. UC-1 and EXP-AD contain DMI fungicides, but their concentrations are perhaps too low and higher rates may be needed.
5. **In vitro sensitivity of *M. fructicola* to DMI fungicides.** All 33 isolates of *M. fructicola* were determined to be highly sensitive to mefentrifluconazole, metconazole, propiconazole, tebuconazole, and tetraconazole. Mefentrifluconazole and metconazole had the lowest average EC₅₀ values at 0.6 and 2 ppb. The results indicate activity differences among DMI fungicides.

INTRODUCTION

Brown rot caused by *Monilinia* species is the most important blossom and preharvest fruit disease of

prune in California. In many growing areas of the state, *M. laxa* is the primary pathogen on blossoms, whereas *M. fructicola* is the main pathogen on fruit. Still, both species can be found causing blossom blight and fruit rot depending on microenvironments and the geographical production areas in California. Currently, properly timed fungicide treatments are the most effective method to control this disease. Highly effective fungicides of different classes have been identified and registered for the prune industry. The currently registered, single-site mode of action fungicides include FRAC codes 1, 2, 3, 7, 9, 11, 17, 19, and P06 (formerly 33) (Table 1). Pre-mixtures contain two active ingredients and are highly effective, consistent, and provide resistance management. Registered pre-mixtures include: FRAC 3/9, 3/11, 7/11, and 3/7. The experimental single active ingredients pyraziflumid, UC-1, V-424, and V-449, as well as the pre-mixtures UC-2, EXP-AD, -AF, and Fervent are also planned for registration. Pre-mixtures are ideal for California production where overall a minimal number of applications is needed for disease management as compared to other stone fruit production areas in the United States.

We also continued our evaluations of natural products (e.g., polyoxin-D) and biocontrols (e.g., Botector, Stargus). Fracture was not evaluated this year due to delays in receiving an OMRI-approved formulation. Results obtained in 2013-2017 demonstrated good to intermediate brown rot blossom blight control. Additionally, the bio-fungicide Ph-D/Oso showed intermediate efficacy against blossom blight and brown rot of fruit. The National Organic Standards Board and the Organic Materials Review Institute (OMRI) have certified the polyoxin-D formulation in Oso for organic production of stone fruit including prune. Thus, this and other potential products (i.e., natamycin) could be critical developments for the organic production segment of the dried plum industry, as well as to conventional growers because preharvest rotation programs need to be designed that prevent the overuse of any one fungicide mode of action (FRAC code).

In this report, laboratory and field studies provide efficacy data on the protective and locally systemic action of compounds. This information helps growers and PCAs in selecting materials and treatment timing to optimize management programs. Efficacy of many newer registered fungicides, especially pre-mixtures, is extremely high because of their post-infection activity (i.e., 'kick-back action'). Based on this, a delayed bloom application program was developed for managing brown rot blossom blight. In this program, a single fungicide can be applied at the phenological stage of 30-40% full bloom when environmental conditions are not favorable for disease. Under high disease pressure, however, a standard two-spray bloom program should be followed using protective or locally systemic fungicides. Characterization of the local systemic (translaminar) action of fungicides can also be useful for preharvest treatments when unexpected rains delay fungicide applications for 1-2 days. Under this scenario, fungicides with post-infection activity are needed and have performed well in our trials. Having several highly effective fungicides belonging to different FRAC Codes and characterized for their pre- and post-infection activity for managing diseases of prune allows for effective management of brown rot diseases, allows for customized rotations, and reduces the risk of selecting for resistance. The overall objective is to rotate products with different FRAC Codes and using any one code only once or twice per season. Rotations of pre-mixtures that alternate at least one of the FRAC Codes in the mixture are part of resistance management. Baseline sensitivities for new fungicides are being established to serve as references in resistance monitoring and to detect possible cross-resistance patterns among fungicides. In 2018, we focused on several DMI fungicides, and we evaluated their activity against *M. fructicola*.

In our fungicide field programs, we are also demonstrating how to improve the efficacy of preharvest fungicide treatments. The addition of a summer spray-oil significantly increases the efficacy of most fungicides in reducing brown rot. We continued this effort in comparative evaluations of a summer oil and different rates of a sticker (e.g., Nu-Film 17). We also demonstrated that preharvest fungicide applications at higher spray volumes (i.e., 160 vs. 80 gal/A) in most cases significantly improved fungicide efficacy on fruit developing in clusters inside the tree canopy.

In some years with spring and summer rainfall, early-season (e.g., early summer) epidemics of prune rust caused by the fungus *Tranzschelia discolor* can cause defoliation and subsequent direct (e.g., sunburn) and indirect (e.g., re-foliation of trees and reduced bloom in the subsequent season) crop losses. In the last few years, we have identified new effective materials in FRAC codes 3, 7, 11, and 19, as well as pre-mixtures 3/11, 3/7, 3/19, and 7/11. Fungicides and integrated approaches need to be evaluated in season-long disease management programs that take into account the control of multiple diseases such as brown rot and prune rust.

Another disease that we are studying is bacterial blast of blossoms and bacterial canker of woody tissues of prune and other stone fruit crops caused by *Pseudomonas syringae* pv. *syringae* and other pathovars. Bacterial blast and canker are associated with nematode root damage and cold, wet environments. Copper treatments have been used with inconsistent results for years. Copper can be phytotoxic to blossoms, and we have shown that pathogen populations have developed copper resistance. We will continue experiments to validate that the new antibiotic kasugamycin is effective on prune. Kasugamycin received full registration in California for managing fire blight on pome fruits (apple and pear), bacterial blast and canker on cherry, and blight on walnut. Unfortunately, no disease was detected in our field inoculation studies last year due to a warm December, and we will repeat our efforts in the 2018/19 season. Our studies could potentially lead to a major advancement for the dried plum industry.

Objectives

1. Evaluate the efficacy of new fungicides (e.g., V-449, V-424, pydiflumetofen, UC-1), pre-mixtures (UC-2, EXP-AD, -AF, Fervent), and biocontrols (Botector, Fracture) representing different modes of action for brown rot blossom blight and brown rot fruit rot in laboratory and field trials, as well as rust in field trials.
 - a. Pre- and post-infection activity of selected fungicides against brown rot blossom blight and fruit rot.
 - b. Evaluation of preharvest fungicides in combination with selected spray adjuvants
 - c. Evaluation of fungicide efficacy against prune rust.
2. Continue to develop baseline sensitivity data for new fungicides (e.g., UC-1, and other DMI fungicides).
3. Evaluate the efficacy of new products against bacterial blast and bacterial canker in flower and twig inoculation studies, respectively.
 - a. New bactericides – ZTD and SDH for bacterial blast and canker
 - b. Antibiotics – kasugamycin and other antibiotics.
 - c. Biologicals/natural products: *Bacillus*-containing products-Stargus, and Botector-*Aureobasidium pullulans*). An OMRI-approved Fracture formulation was not available in 2018.

MATERIALS AND METHODS

Evaluation of treatments for control of bacterial canker. In winter of 2017/18, the bark of 2-year-old twigs of French prune trees was puncture-wounded using a 12-gauge needle (3 wounds per twig). Wounds were sprayed with bactericides to run-off using a hand sprayer, allowed to air-dry, and spray-inoculated with a copper-resistant strain of *Pseudomonas syringae* pv. *syringae* (2×10^8 cfu/ml). Treatments included ChampION⁺⁺, copper mixed with ZTD or pre-mixed with a copper activity-enhancing compound (i.e., DAS-2), ZTD by itself, and Kasumin. In March, inoculated branches were sampled and evaluated for the severity of canker formation.

Evaluation of fungicides for management of blossom blight and preharvest fruit decay. Field and laboratory studies were conducted on the management of brown rot blossom blight and bacterial blossom blast, as well as fruit decay in 2018. Laboratory experiments were conducted to evaluate the pre- and post-infection activity of fungicides against brown rot blossom blight. Blossoms were collected at white bud, allowed to open in the laboratory, and treated in the laboratory using a hand sprayer. After 4 h, blossoms were inoculated with a spore suspension of *M. fructicola* (20 K/ml) until water droplets formed on anther filaments. For post-infection activity, blossoms were inoculated, incubated at 22 C, and treated after 20 h. Blossoms were evaluated for stamen infections after 4-5 days of incubation at 20 C >95% relative humidity. Disease incidence was evaluated as the number of stamens infected divided by the total number of stamens per blossom. Three replications of 8 blossoms were used for each treatment and data were analyzed using analysis of variance and mean separation procedures (SAS 9.4).

For blossom blight field trials in a commercial orchard, four single tree replications were sprayed with biologicals (Botector and Stargus), single ai's (UC-1, Rhyme, Fontelis, and Pyraziflumid), or pre-mixtures (Luna Experience, Luna Sensation, Merivon, Quadris Top, Fervent, UC-2, EXP-AD, and EXP-AF). A non-ionic surfactant was used for all conventional fungicide treatments, and treatments were applied at 100 gal/A. Disease was evaluated in late April 2018.

Field trials to evaluate preharvest fungicide applications for control of fruit brown rot were done in a commercial orchard in Yuba Co. with a 15-day PHI application on 7-24-2018 (using the same fungicides as described above except the biologicals that were substituted with V-424 and V-449). Treatments were applied using an air-blast sprayer calibrated at 130 gal/A. In the commercial orchard, each fungicide was applied with 1% of a spray oil (i.e., Omni Oil). A second trial was done in an experimental orchard at UC Davis with 7-day PHI treatments using Bumper and UC-2 in combination with a summer oil (e.g., Omni Supreme Spray Oil) or a sticker (e.g., Nu-Film 17). Single fruit (24 fruit from each replication) were collected and wound-inoculated (5×10^4 conidia/ml) or non-wound-inoculated (3×10^5 conidia/ml) with conidia of *M. fructicola*. After inoculation, fruit were incubated for 7-10 days at 20 C. Data were analyzed using analysis of variance and mean separation procedures of SAS 9.4.

Evaluation of fungicides for management of prune rust. A field trial was established in a commercial orchard in Yuba Co. to evaluate the efficacy of new fungicides (using the same fungicides as for the preharvest treatments). Fungicides were applied on 7-24-2018 (as a preharvest application for management of fruit brown rot) and on 9-11-2018 with no adjuvants specifically for fall season rust management. Disease was evaluated on 10-19-2018. Disease incidence and severity was determined for four quadrants of each tree using a scale from 0 (= no disease) to 4 (>75% leaf area infected). Data were analyzed using analysis of variance and mean separation procedures of SAS 9.4.

In vitro sensitivity of *M. fructicola* to new and previously registered DMI fungicides. Thirty-three isolates of *M. fructicola* were used to determine inhibitory concentrations of five DMI fungicides for mycelial growth using the spiral gradient dilution method. In the assay, conidial suspensions were streaked along fungicide concentration gradients and mycelial growth was measured after 3 days. EC₅₀ values were calculated as described previously, and data were summarized in histograms and Tables.

RESULTS AND DISCUSSION

Evaluation of fungicides for management canker. Field studies were conducted on the management of bacterial canker, however, only very low levels of disease developed at our trial sites. Wound-inoculations were done in December 2017 when temperatures were unusually warm, and there was low rainfall. The disease has been correlated with low temperatures and wet conditions. This could possibly explain why the disease was not established in this trial. Colder weather was observed in late February and early March but temperatures at field plots at UC Davis were warm during the bloom period with minimum/maximum temperatures between 37.5/56.9°F and 51.5/80.3°F and average temperatures 48.7 to 65.6°F during bloom March 19 to April 1. Some rain occurred during the white tip stage, however, during most of the full bloom period there were only trace amounts of precipitation.

Based on recent registrations of kasugamycin by EPA and CDPH specifically requested by us to find alternatives for managing bacterial plant diseases, Kasumin was registered in 2018 for managing fire blight on pome fruits (apples and pears), bacterial blast and canker on cherry, and walnut blight in California. Additionally, registrations of this bactericide on peach and almond have been submitted to EPA and CDPH. With widespread copper resistance in the bacterial pathogen *P. syringae* pv. *syringae*, new effective treatments are needed to manage bacterial canker and blast of *Prunus* spp. These are important diseases of stone fruit crops that can impact production in seasons with favorable environmental conditions and can also have long-term effects on tree health. In our studies over the years on various crops, Kasumin was the most effective and consistent treatment against both phases of the disease. Oxytetracycline was evaluated previously and also was identified as a promising bactericide against *P. syringae*. Registrants of both antibiotics are supportive of a registration on additional stone fruit crops. Oxytetracycline (i.e., Fireline, Mycoshield) and kasugamycin (i.e., Kasumin) may be submitted into the IR-4 program in the 2019 Food Use Workshop for federal registration on prune provided there is industry support (i.e., the Dried Plum Board). Over the years of our evaluations, Blossom Protect also reduced blossom blast.

Evaluation of fungicides for management of brown rot blossom blight. In laboratory studies, fungicides were evaluated for their pre- and post-infection activity against *M. fructicola*. Single active ingredient fungicides (Rhyme, UC-1, Fontelis, pyraziflumid) and pre-mixtures (Luna Experience, Quadris Top, Merivon, EXP-AD, EXP-AF, UC-2) all significantly reduced blossom blight from the non-treated control

(Fig. 1). Quadris Top, EXP-AD and EXP-AF had the least post-infection activity; whereas Quadris Top and EXP-AF were less effective pre-infection (protective) treatments compared to the other fungicides evaluated.

A field trial was conducted in a commercial prune orchard in the spring of 2018, however, disease development on untreated control trees was very low with only 2 spur infections observed on two of the four untreated control trees in the entire plot. Currently registered fungicides with high pre- and post-infection activity for brown rot include single active ingredients such as the FRAC 2 dicarboximide Rovral (-oil) and generics; the FRAC 3 DMIs Tilt (and generics), Indar, Rhyme, Tebucon (and other generics), and Quash; the FRAC 7 SDHI Fontelis; the FRAC 9 anilinopyrimidines (APs) Vanguard and Scala; and the FRAC 17 hydroxyanilide Elevate. Pre-mixtures include the FRAC 7/11 Pristine, Merivon, and Luna Sensation; the FRAC 3/11 Quilt Xcel and Quadris Top, the FG 3/9 Inspire Super, and the FRAC 3/7 Luna Experience (Table 1). The pre-mixtures provide consistent, broad-spectrum high efficacy with built-in resistance management. Experimental materials evaluated are shown in Table 2.

Table 1. Efficacy of fungicides and pre-mixtures against major diseases of prunes (dried plum)

Fungicide	Resistance risk (FRAC#)	Brown rot		Rust
		Blossom	Fruit	
Bumper/Tilt	high (3)	++++	++++	+++
Elite/Tebucon/Teb/Toledo	high (3)	++++	++++	+++
Fontelis	high (3)	++++	+++	+++
Indar	high (3)	++++	++++	+++
Inspire Super	high (3/9)	++++	++++	+++
Luna Experience	medium (3/7)	++++	++++	++++
Luna Sensation	medium (7/11)	++++	++++	ND
Merivon	medium (7/11)	++++	++++	ND
Pristine	medium (7/11)	++++	++++	ND
Quash	high (3)	++++	++++	+++
Quadris Top	medium (3/11)	++++	++++	++++
Quilt Xcel/Avaris 2XS	medium (3/11)	++++	++++	++++
Rovral + oil	low (2)	++++	NR	NR
Scala	high (9)	++++	+++	ND
Topsin-M /T-Methyl/Incognito/Cercobin + oil	high (1)	++++	++++	---
Vanguard	high (9)	++++	+++	ND
Elevate	high (17)	+++	+++	---
Rhyme	high (3)	+++	+++	+++
Rovral/Iprodione /Nevado	low (2)	+++	NR	NR
Topsin-M/T-Methyl/Incognito	high (1)	+++	+/-	---
Abound	high (11)	++	+	+++
Botran	medium (14)	++	++	ND
Bravo/Chlorothalonil/Echo/Equus	low (M5)	++	++	---
Captan	low (M4)	++	++	---
Ph-D	high (19)	++	++	ND
Gem	high (11)	++	+	+++
Rally	high (3)	++	++	---
Sulfur	low (M2)	+/-	+/-	++

Rating: ++++= excellent and consistent, +++= good and reliable, += moderate and variable, ++ limited and erratic, +/- = often ineffective, --- = ineffective, ? = insufficient data or unknown, NR=not registered after bloom, and ND=no data

Table 2. Efficacy of experimental fungicides against major diseases of prune (dried plum)

Fungicides	Resistance risk (FRAC#)	Brown rot		Rust
		Blossom	Fruit	
Fervent	medium (3/7)	++++	++++	++++
Pyraziflumid	high (7)	++++	++++	++
EXP-AD	medium (3/7)	+++	+++	+++
EXP-AF	medium (7/12)	+++	+++	+++
UC-1	high (3)	++++	++++	++++
UC-2	medium (3/?)	++++	++++	++++
V-424	high (3)	++++	++++	++++
V-449	high (7)	---	---	---

Rating: ++++= excellent and consistent, +++= good and reliable, ++= moderate and variable, += limited and erratic, +/- = often ineffective, ---- = ineffective, ? = insufficient data or unknown, NR=not registered after bloom, and ND=no data

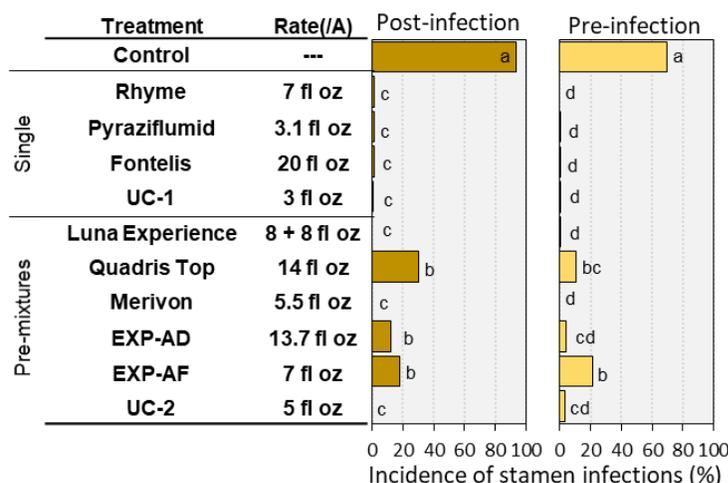
Evaluation of fungicides for management of fruit brown rot. We previously demonstrated that the efficacy of preharvest fungicide applications to prevent losses from fruit brown rot is considerably improved when used in combination with an agricultural spray oil. Additionally, applications at an increased volume of 130 gal/A generally provide better protection of fruit inside clusters. Because some growers are reluctant to use a spray oil that removes the bloom on the fruit, we evaluated if a sticker surfactant might provide the same efficacy, and this was done with two fungicides. The efficacy of additional fungicides was compared with using a 1% summer oil (i.e., Omni Supreme Spray Oil) mixture.

In comparing oil and a sticker additive, applications were done 7 days PHI at 130 gal/A. Treatments of propiconazole (e.g., Bumper) mixed with summer oil had a significantly lower incidence of brown rot on wound inoculated fruit than when the fungicide was mixed with Nu-Film 17 (**Fig. 2**). Previous studies found that only the addition of oil but not of non-ionic surfactants improved decay control. UC-2 mixed with oil or Nu-Film 17 at 8 or 32 oz/A were similar in their effectiveness in preventing brown rot of wound-inoculated fruit. No differences were observed between propiconazole or UC-2 mixtures with either adjuvant on non-wounded fruit. Thus, the sticker provided similar efficacy to the summer oil depending on the fungicide used. UC-2 has a DMI fungicide and is an SC formulation. Thus, this is the first time that an SC formulation mixed with an adjuvant other than oil performed similar to a DMI mixed with oil in preventing brown rot of fruit.

In another study with registered and new fungicides in combination with 1% summer oil (Omni Supreme Spray Oil) applied 15 days before harvest at 130 gal/A, fungicides containing a DMI compound (e.g., Luna Experience, Fervent, Quadris Top), as well as some experimentals (UC-1, UC-2, V-424 - all containing a DMI fungicide) significantly reduced the incidence of brown rot from that of the control after wound-inoculation (**Fig. 3**). Luna Experience, Fervent, UC-1, UC-2, and V-424 were most effective. Luna Sensation and EXP-AD were intermediate in their effectiveness; and the SDHI compounds Fontelis and pyraziflumid, as well as Merivon and EXP-AF did not reduce decay compared to the non-treated control. These latter fungicides are contact materials and do not penetrate into the fruit. In contrast, all fungicides evaluated with the exception of V-449 significantly reduced brown rot on non-wounded fruit. This demonstrates that these fungicides have protective activity.

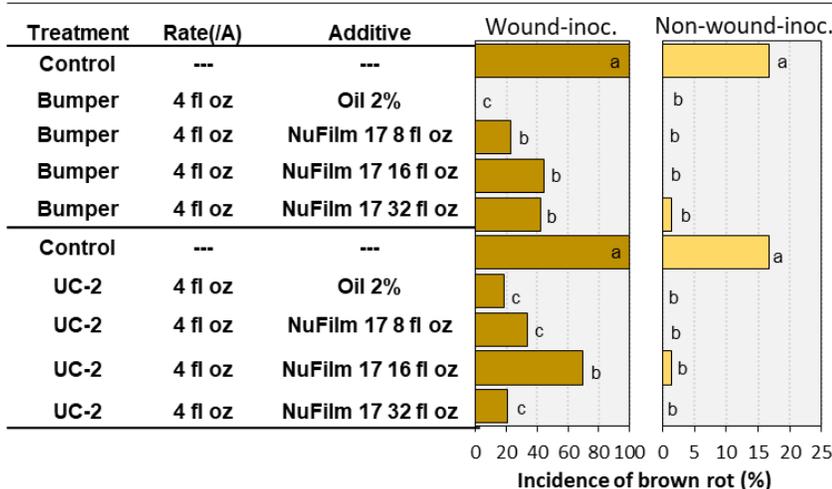
Several fungicides with high efficacy are currently available to the industry to protect fruit from brown rot decay even when applied 15 to 7 days before harvest. The highest treatment efficacy is obtained when fungicide-oil mixtures are applied at higher volumes (e.g., 130 gal/A). Spray oil provides improved coverage of fruit (acting as a spreader on waxy fruit surfaces) and likely also improves penetration of some fungicides into the fruit. Not all fungicides, however, may be compatible with oils. Using a sticker like Nu-Film 17 may also provide high efficacy by allowing the fungicides to stay on the waxy bloom. Additional research is needed with other fungicides and stickers. Still, it is important to prevent fruit injuries during and after harvest. To reduce brown rot of mechanically harvested fruit in bins, fruit should be processed for drying within 48 h of harvest.

Fig. 1. Efficacy of pre- and post-infection treatments for management of brown rot blossom blight of French prune in the laboratory 2018



For evaluation of the pre-infection activity, closed blossoms were collected in the field on 3-19-18, allowed to open, and treated in the laboratory using a hand sprayer. After 4 h, blossoms were inoculated with a spore suspension of *M. fructicola* (20 K/ml). For post-infection activity, blossoms were inoculated, incubated at 22 C, and treated after 20 h. Blossoms were evaluated for stamen infections after 4-5 days of incubation at 20 C.

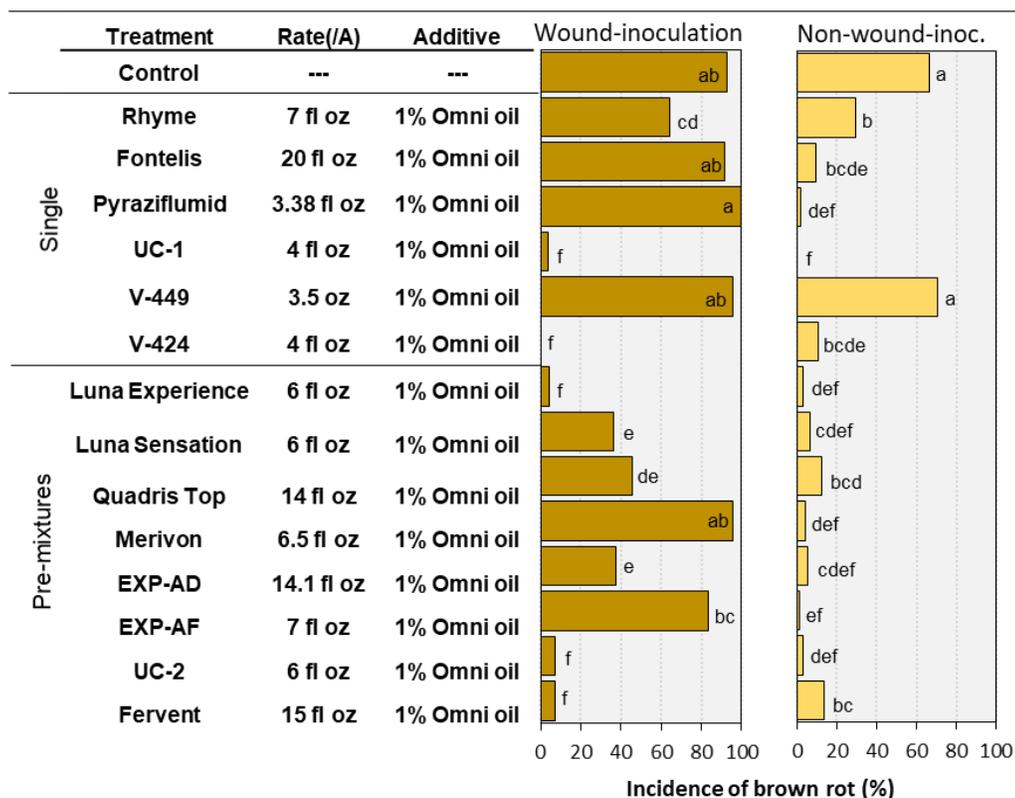
Fig. 2. Efficacy of 7-day preharvest fungicide treatments for management of postharvest brown rot of French prune – UC Davis 2018



Treatments were applied in combination with adjuvants on 8-13-18. From each tree, 8 random fruit were harvested and wound-inoculated with conidia of *M. fructicola* (50,000 spores/ml) or non-wound-inoculated with 300,000 spores/ml. Fruit were incubated for 7 days at 20 C.

Evaluation of fungicides for management of prune rust. The severity of rust was high in the fall of 2018. In a late-season study, two applications of selected fungicides (the first application was part of the pre-harvest brown rot fruit decay study and the second one was applied after harvest on 9/11/2018) all significantly reduced the severity of rust developing in the tree canopy as compared to the non-sprayed control trees (Fig. 4). Very low disease incidence and severity ratings were observed after treatment with Rhyme, Luna Experience, Quadris Top, Merivon, V-424, UC-2, and Fervent. Ph-D, Fontelis, pyraziflumid, Luna Sensation, EXP-AD, EXP-AF, UC-1, and V-449 were the least effective treatments. UC-1 and EXP-AD contain DMI fungicides, but their concentrations are perhaps too low and higher rates may be needed.

Fig. 3. Efficacy of 15-day preharvest fungicide treatments for management of postharvest brown rot of French prune – Yuba Co. 2018

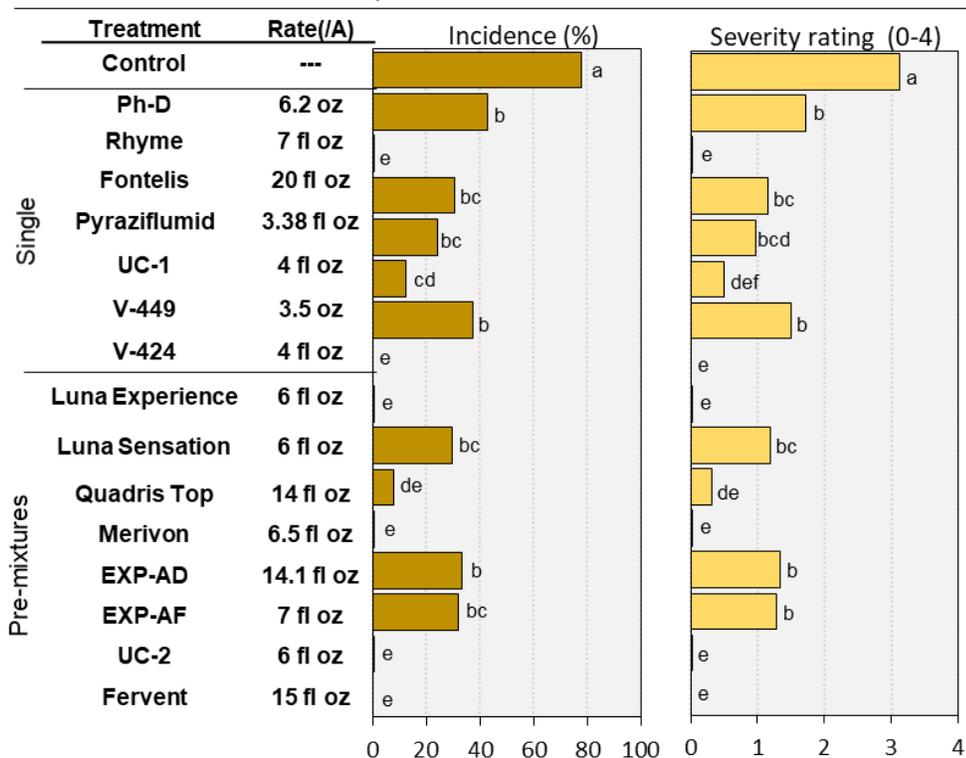


Treatments were applied in combination with adjuvants on 8-13-18. From each tree, 8 random fruit were harvested and wound-inoculated with conidia of *M. fructicola* (50,000 spores/ml) or non-wound-inoculated with 300,000 spores/ml. Fruit were incubated for 7 days at 20 C.

These results indicate that effective treatments for prune rust are available and that the disease can be managed. Over the years, treatments that include FRAC 3 or 11 have been the most effective. Polyoxin-D (Ph-D) also showed efficacy against rust (Fig. 4). This compound formulated as Oso is approved by the NOSB as an organic fungicide. It also has efficacy against brown rot blossom blight and fruit rot (previous reports). Because it is a broad-spectrum fungicide, the potential for integrating this FRAC 19 fungicide into a management program has great potential for organic prune production. Still, prune rust occurs sporadically, and protective treatments are generally not warranted. Fungicide treatments, however, should still be very effective if applied when the very first rust lesions are detected in an orchard during regular scouting and monitoring of orchards during April through July. A single pre- or postharvest application to trees in August may also have benefits in reducing rust during the fall and subsequent growing season.

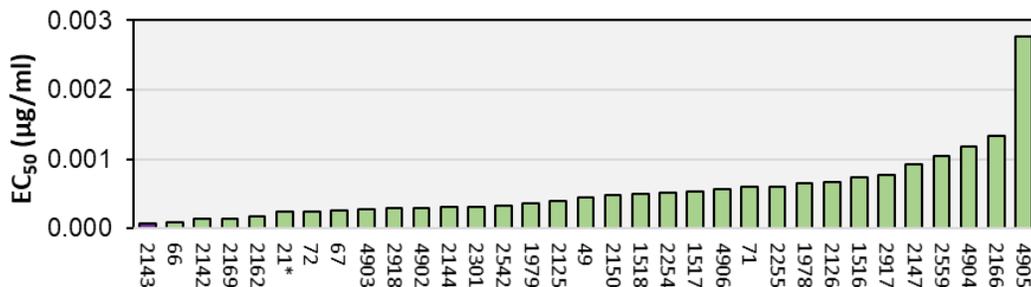
In vitro sensitivity of *M. fructicola* to new and previously registered DMI fungicides. Ranges and average EC₅₀ values for inhibition of mycelial growth of 33 isolates of *M. fructicola* by mefenftrifluconazole (UC-1), metconazole (Quash), propiconazole (Tilt/Bumper), tebuconazole (Teb, Tebucon), and tetraconazole (Mettle) all belonging to the triazole sub-group of DMIs are presented in Fig. 5. All isolates of *M. fructicola* were determined to be highly sensitive to the five fungicides with similar low ranges and average inhibitory concentrations (Fig. 5). Mefenftrifluconazole and metconazole had the lowest average EC₅₀ values at 0.6 and 2.6 ppb. The results indicate activity differences among DMI fungicides. The practical implication is that rotating between different DMIs may have benefits in preventing resistance to any one material due to the multiple, combinatorial point-mutations needed to occur for selection of resistant isolates.

Fig. 4. Efficacy of fungicide applications for management of rust of French prune – Yuba Co. 2018



Treatments were applied on 9-11-18. Two disease readings were done on each of the four quadrants of each tree with 20 leaves each on 10-19-18 using a scale from 0 (= no disease), 1 = 1-5 lesions, 2 = 6-15 lesions, 3 = 16-25 lesions, 4 = >25 lesions/leaf.

Fig. 5. Baseline sensitivities of 33 isolates of *Monilinia fructicola* to mefentrifluconazole



In vitro sensitivities were determined using the spiral gradient dilution method.

Fungicide	Range (µg/ml)	Average (µg/ml)
Mefentrifluconazole	0.0001 – 0.0028	0.0006
Metconazole	0.0006 – 0.006	0.0026
Propiconazole	0.009 – 0.045	0.013
Tebuconazole	0.005 – 0.017	0.010
Tetraconazole	0.0054 – 0.038	0.017

In vitro sensitivities of *M. fructicola* isolates to selected DMI fungicides

DIAGNOSIS, ETIOLOGY, EPIDEMIOLOGY, AND MANAGEMENT OF CANKER DISEASES IN DRIED PLUMS

Themis J. Michailides, Yong Luo, Franz Niederholzer, Dan Felts, Ryan Puckett

and

Richard Buchner, Elizabeth Fichtner, and Daniella Lightle

Objectives:

- 1) To continue the experiments to quantify inoculum densities in rainwater and latent infections of canker-causing pathogens.
- 2) To determine the dynamics or possible accumulation of latent infection of canker-causing pathogens in pruning wounds.
- 3) To conduct a survey of nursery trees and track the dynamics of latent infections of canker-causing pathogens from nurseries to recently planted orchards.
- 4) To study the impacts of water stress and nutrient (nitrogen) levels on canker development and dynamics of latent infection.

PROCEDURES

1) To continue the experiments to quantify inoculum densities in rainwater and latent infections of canker-causing pathogens.

The quantification of canker-causing pathogens in rainwater was continued in 2018 in the three dried plum orchards in Yuba County to study the dynamics of the inoculum in the rain. Since the dry condition in 2017-2018 winter, the rain samples were collected only from January 24 to April 9 of 2018. The samples were processed using the *q*PCR system that was developed in our laboratory. The specific primers of each of the six pathogens, *Cytospora* spp., *Phomopsis* spp., *Botryosphaeria dothidea*, *Lasiodiplodia* spp., *Neofusicoccum* spp., and *Diplodia* spp. (Luo et al., 2017) were used to determine the inoculum density in each orchard in terms of number of spores of corresponding pathogen group per milliliter (ml) of rainwater.

To avoid duplication of the studies done in previous years, we only collect shoot samples in October, at the end of the growing season to determine the latent infections of the 6 canker-causing pathogens. In each of above three dried plum orchard, 32 shoot samples were randomly collected and processed by using the *q*PCR assay to quantify the latent infection level, in terms of Incidence (I) and Molecular Severity (MS), for each of the six pathogen groups.

2) To determine the dynamics or possible accumulation of latent infection of canker-causing pathogens in pruning wounds.

In this objective, we needed to test our hypothesis on the role of new infection and latent infection of pruning wounds on canker development. A dried plum orchard showing severe canker disease in Yuba County was selected. On February 15, 2018, pruning wounds were generated in about 50 trees. Pruners were sterilized by a spray of 75% alcohol before use on each pruning cut. A stub of 1 inch of the shoot was left to use for subsequent sampling. About 250

wounds on 2 - 4-year-old shoots were marked immediately after pruning. The half number of wounds were wrapped with pieces of parafilm immediately after pruning to protect new infection, while another half number of wounds were left exposed to natural infection (no parafilm wrapping). Stub samplings were performed in April, July and October of 2018, and will be continued in January of 2019.

For each sampling, 24 wound-wrapped and 24 wound-unwrapped pruned stubs were collected from the part nearest the pruning wound (about 2 inch long). The wound part of each shoot sample was processed to extract DNA and to run the qPCR assay as described above to target the six canker-causing pathogen groups. The incidence (I) and molecular severity (MS) for each sampling period of each treatment were obtained.

3) To conduct a survey of nursery trees and track the dynamics of latent infections of canker-causing pathogens from nurseries to recently planted orchards.

In 2018, we identified 3 nurseries with our confidential codes as PNP, PNB and PNT, respectively. In each nursery, we sampled young shoots from 32 potted trees or bareroot trees. The shoot samples were surface sterilized, air dried and ground to extract DNA. The DNA primers to target the six canker-causing pathogens and the qPCR assay were used again to obtain the incidence of latent infection for each sampled nursery.

4) To study the impacts of water stress on canker development and dynamics of latent infection.

We had initiated an experiment at KARE in 2017 where a total of 100 potted young French prune trees were prepared. The trees were divided into four groups, each irrigated with 25%, 50%, 75% and 100% water requirements, respectively. The inoculation was performed in October 2017 with a mycelium plug of *Cytospora* spp. on a wound made with a cork borer in each tree. A piece of parafilm was used to wrap the inoculation site, and maintained it until disease recording. For each irrigation regime, four control trees will serve as the non-inoculated control. The four irrigation treatments were performed by adjusting daily irrigation levels as 100, 75, 50 and 25%, using the 100% treatment as a standard reference. The treatments were started in March of 2018 and continued until winter in rainy season.

The first shoot sampling was performed in October of 2017 immediately after inoculation to determine the initial incidence of latent infection before irrigation treatment as described below. After irrigation treatments in 2018, shoot samples were collected from each tree of each treatment on June 28 (3 months after water treatments) and September 28 of 2018 for each irrigation treatment. The qPCR assay described above was used to determine the incidence of latent infection from 24 shoot samples for each treatment for each of the 6 canker-causing pathogens. The disease recording for the inoculation sites were performed in October of 2018, and the maximum length of symptomatic canker in cm on each inoculation site was recorded. Comparison in canker length among 4 irrigation treatments was performed using the ANOVA program of SAS (SAS Institute, Cary, NC).

RESULTS AND CONCLUSIONS

1) To continue the experiments to quantify inoculum densities in rainwater and latent infections of canker-causing pathogens.

We did not find spores of *Phomopsis* spp. and *Diplodia* spp. from the rain samples collected from January to April of 2018, and very low level of spore density of *B. dothidea* was determined. *Cytospora* spp. was found for all the three orchards with various spore densities in rainwater, indicating the major pathogen of sampled dried plum orchards among 6 canker-causing pathogen groups. *Neofusicoccum* spp. was also determined from all the three orchards with relatively low densities compared to those of *Cytospora* spp. (Figure 1). *Lasiodiplodia* spp. was determined in the Orchard 1 and found at a very low density in Orchard 3, but not found in the Orchard 2 (Figure 1).

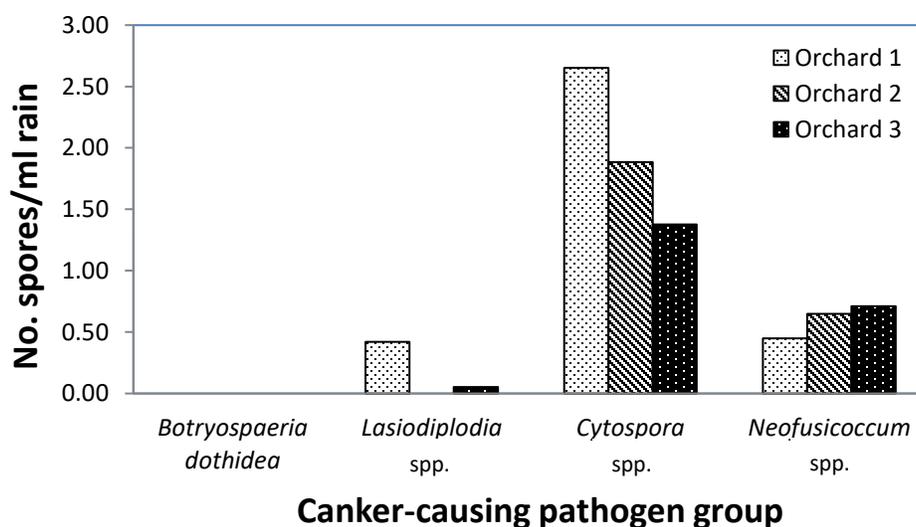


Figure 1. Spore density (number of spores per ml) in rainwater of 4 canker-causing pathogen groups collected from three dried plum orchards in Yuba County. The rainwater samples were collected from January to April of 2018.

The result demonstrated that spores of *Cytospora* spp. existed in rain in spring that likely came from the pycnidia of infected trees in the orchards. These spores may serve as initial inoculum causing new infections of canker epidemics in spring. This year's result confirmed our conclusions from multi-year experiments on the importance of *Cytospora* spp. as inoculum source in rainwater for disease development.

Although *Neofusicoccum* spp. showed low levels of spore density in rainwater, its importance as inoculum of initial infections should not be ignored, particularly since in previous research we isolated this pathogen from active cankers developed in pruning wounds of dried plum. The 2018 results demonstrate the possible risk of this pathogen population for disease development in the future.

The shoot samples collected from the 3 orchards are still in process to determine the latent infection levels for the 6 canker-causing pathogens.

2) To determine the dynamics or possible accumulation of latent infection of canker-causing pathogens in pruning wounds.

Three consequential samplings showed that the incidences of *Cytospora* spp. on unwrapped wounds were from 90 to 100%, while those from wrapped wounds were from 10 to 20% (Figure 2). The result demonstrated that although the wounds were wrapped to avoid new infections, *Cytospora* spp. can still be detected in these wounds. Thus, these infections must be from the latency phase of the pathogen found inside plant tissue before the growing season. However, new natural infections significantly and continuously occurred on wounds. Our results demonstrated that both, new natural infections and latent infections inside plant tissue, could contribute on canker development, especially starting in pruning wounds. However, the difference in their contribution depends on the inoculum density or disease pressure of the specific orchard. The sampled orchard showed a high level of spore density in rainwater as the Orchard 1 in Figure 1, confirming the existence of *Cytospora* spp. as inoculum source for natural infections on pruning wounds.

In comparison, other canker causing pathogen groups were also detected, but they showed various levels of incidences that are comparatively inconsistent among the three samplings (Figure 2). This result partially confirmed the effects of initial inoculum of different canker-causing pathogens in the Orchard 1 (Figure 1).

The range of molecular severity (MS) for different canker-causing pathogens remained from 1.0 to 5.0 (Figure 2). For *Cytospora* spp., the averages MS of unwrapped wounds for the last two samples were significantly higher than those of wrapped wounds. This may indicate that at least some of these wounds could be caused by both natural and latent infections which enhanced the infection level.

The canker lengths were recorded on October 9, 2018 for both wrapped and unwrapped wounds. The 22 wound samples were randomly selected for each treatment to record the canker length in cm. Statistical analysis showed the average canker lengths on unwrapped and wrapped wounds were 2.07 and 1.0 cm with standard deviations of 1.09 and 1.08, respectively. The analysis demonstrated that the average canker length on unwrapped wounds were significantly ($P < 0.01$) longer than that on wrapped wounds.

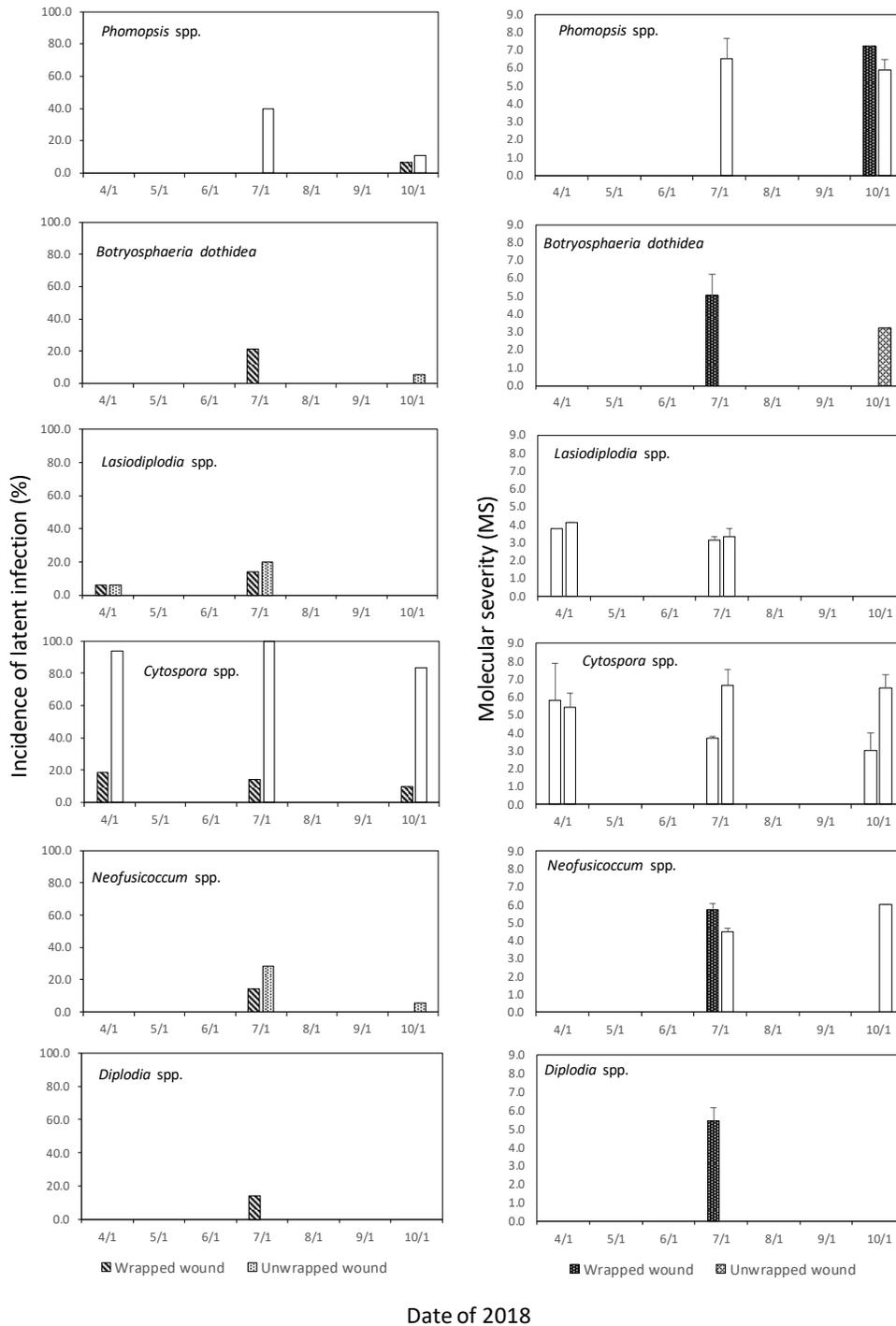


Figure 2. Incidence of latent infection and molecular severity (MS) of pruning wounds of shoots quantified using the *q*PCR assay. The experiment was performed in a canker-severe dried plum orchard in Yuba County with severe canker where two treatments were used, a) wounds wrapped and b) wounds without wrapping. Samples were periodically collected and processed to determine infection level for each of the 6 canker-causing pathogen groups.

3) To conduct a survey of nursery trees and track the dynamics of latent infections of canker-causing pathogens from nurseries to recently planted orchards.

The results of incidence of latent infections on sampled shoots from three nurseries showed that *Lasiodiplodia* spp. existed in young trees in all the three nurseries (Figure 3). *Cytospora* spp. was detected in two nurseries (PNP and PNB). *Neofusicoccum* spp. was also found in three nurseries, while the incidence in the nursery PNP was higher than those in the other two nurseries. *Phomopsis* spp. and *Diplodia* spp. were not detected in all three nurseries, indicating much less risk and potential for canker development in trees of nurseries.

In summary, our results confirmed that the nursery’s young plants could carry some canker-causing pathogens (latent infections) at different levels, depending on situation of specific nurseries. The chance of nursery trees carrying pathogens to newly planted orchards exists. Similar conclusions were drawn in almonds, and these common problematic situations in trees of nurseries should be further investigated to develop management procedures at an early stage of fruit trees life.

We need to continue this project to track the development of latent infections from nursery plants to newly planted orchards. Further intensive studies are needed.

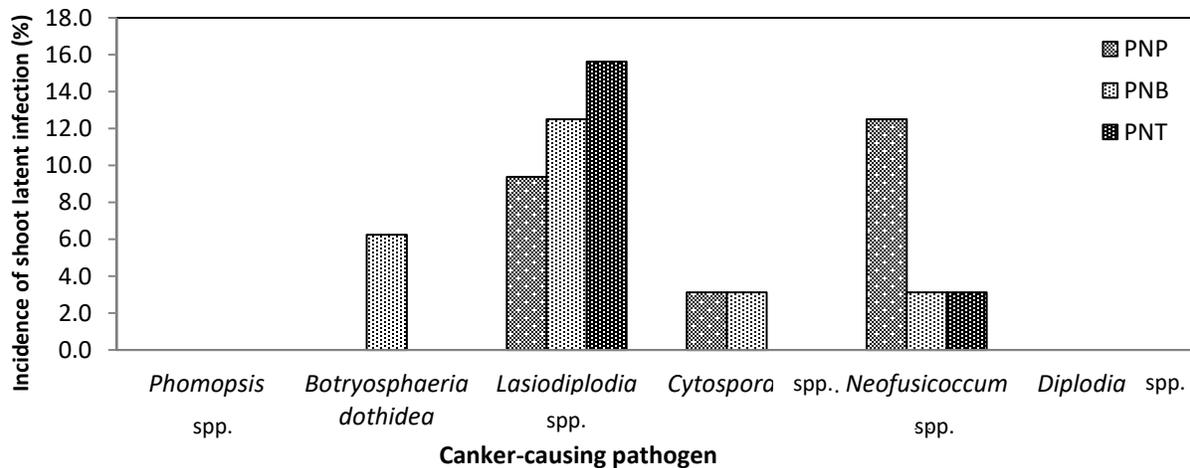


Figure 3. Incidence of latent infection in dried plum shoots by 6 canker-causing pathogen groups for 3 anonymous nurseries (PNP, PNB and PNT). The *q*PCR assay was used to obtain the latent infection data from 32 shoot samples from each nursery.

4) To study the impacts of water stress and nutrient (nitrogen) levels on canker development and dynamics of latent infection.

We did not detect *Phomopsis* spp., *B. dothidea*, or *Diplodia* spp. in any of the sampled shoots while *Lasiodiplodia* spp. was detected only once. Thus, we focused on *Cytospora* spp. and *Neofusicoccum* spp. *Cytospora* spp. were not detected in shoots from the first sampling before the irrigation treatments started. After the differential irrigation treatments were initiated, *Cytospora* spp. were not detected in any of the plants irrigated

with 100% irrigation treatment, while this pathogen was detected at various incidences from plants irrigated with 25%, 50%, or 75% water requirements in the two samplings (Figure 4). This result indicated that various levels of water supply below the required resulted in the development of latent infection by *Cytospora* spp.

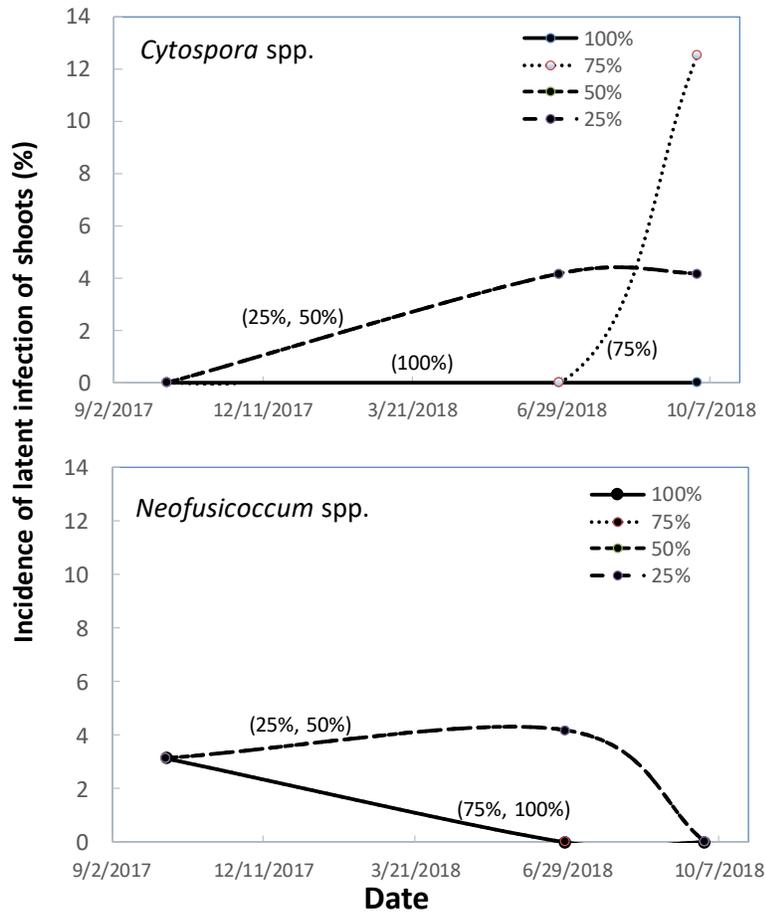


Figure 4. Dynamics of incidence of latent infection of shoots caused by *Cytospora* spp. and *Neofusicoccum* spp. for 4 levels of irrigation treatments using the qPCR assay. The first sampling was performed in October of 2017 immediately after inoculation. After irrigation treatments started in March of 2018, two more samplings were performed on 19 July and 27 October of 2018 (24 shoots were processed for each irrigation treatment).

Neofusicoccum spp. were detected from these plants before the initiation of the irrigation treatments. This pathogen was not detected from the plants with the 100% irrigation treatment on July 19 sampling, while, it was detected from the plants with 50 and 25% irrigation treatments in the second sampling (Figure 4). Thus, the experiments determined that deficient irrigation which may stress the trees could positively affect the development of latent infection by these two pathogen groups.

However, the average canker lengths at the inoculated sites among all the 4 irrigation treatments were not significantly different at $P < 0.01$. This may be because that the inoculations were performed in October of 2017 which might not be the appropriate time period for pathogen infection and development in plant tissues.

INVESTIGATING INCIDENCE AND TYPE OF WOOD DECAY FUNGI IN CALIFORNIA PRUNE ORCHARDS

Bob Johnson, Franz Neiderholzer, Luke Milliron and Dave Rizzo

Wood decay fungi reduce the structural integrity of trees, leading to wind-driven collapses and scaffold limb breakage, causing tree loss, and reduced production in the prune growing regions of California. Wood decay is caused by a wide array of fungi that colonize and digest the heartwood, and sometimes sapwood, in living trees.

Wood decay surveys in prune orchards throughout the Sacramento Valley began in 2015. A high incidence of *Phellinus tuberculosus* (formerly *P. pomaceus*) was found in every surveyed orchard older than 12 years. Most limb breakage could be attributed to extensive *Phellinus* decay. For this reason, much of our focus this year has been on understanding the epidemiology and biology of *P. tuberculosus* in prune orchards and exploring control and management strategies.

OBJECTIVES

1. Serve as a resource for growers, farm advisors, and PCAs through disease identification, orchard evaluations, and outreach.
2. Evaluate *Trichoderma* spp. applications and other possible strategies for control of *P. tuberculosus*.
3. Evaluate prune varieties susceptibility to decay.
4. Quantify the potential carbon impacts of *Phellinus* and other wood decay in prune.
5. Test and adapt new spore monitoring technology for application in prune orchards. (not an original objective)

PROCEDURES

Objective 1. Serve as a resource for growers, farm advisors, and PCAs through disease identification, orchard evaluations, and outreach.

In 2018, we gave two grower talks to prune growers and included our results in multiple almond grower talks as well. We positively identified *Phellinus* for 5 PCA/growers.

Objective 2. Evaluate *Trichoderma* spp. applications and other possible strategies for control of *P. tuberculosus*.

On several instances during our survey work *Trichoderma* spp. were isolated from *Phellinus* decay. *Trichoderma* sp. was also found colonizing a *Phellinus* fruiting body (Figure 1). The presence of *Trichoderma* spp. is intriguing, as it has been tested as a biocontrol in other cropping systems, and is often isolated from decayed tissue when no wood decay fungi can be recovered.

Additionally, in several instances, *Trichoderma* sp. contaminated *Phellinus* spp. and

Ganoderma spp. cultures in the lab rendering those cultures unrecoverable. Previously a *Trichoderma* containing product was registered in California tree crops for control of the wood decay pathogen that causes silver leaf disease.

Two isolates of *Trichoderma* (T1 and T2) from prune orchards were identified as *T. atroviride*. Laboratory experiments to test the effects of these *Trichoderma* isolates on *Phellinus* growth and mortality were carried out in plates of 2% malt extract agar (MEA). Plugs from a 14-day old culture of *P. tuberosus* were placed slightly off center on a fresh MEA plate. After 7 days, *Phellinus* cultures were inoculated with *Trichoderma* strains T1 and T2 by first inserting tip of sterilized scalpel into culture of *Trichoderma* and then into MEA opposite the *Phellinus* plug. Mock inoculations with sterile scalpel served as a control. The diameter of *Phellinus* cultures were measured 7 days after *Trichoderma* inoculation and a subculture from each was plated onto fresh MEA 7 and 14 days after inoculation. This trial was replicated, but with the addition of a combined inoculation of *Trichoderma* T1 and T2.

Similar experiments were carried out on autoclaved discs of prune wood. Wood discs with bark removed 2.5- 3cm in diameter and 1cm thick were autoclaved twice and placed in empty petri dishes. Each disc received both *Phellinus* and *Trichoderma* inoculum, applied in one of three treatment timings: Both at the same time, *Phellinus* followed by *Trichoderma* at 7 days, or *Trichoderma* followed by *Phellinus* at 7 days. Treatments included strains T1, T2 and a commercial product containing live *Trichoderma atroviride*. *Trichoderma* inoculation was carried out by pipetting 10 μ l of a spore suspension concentration $\sim 2 \times 10^7$ CFU/ml onto the wood disc. *Phellinus* was inoculated as described above. Recovery of *Phellinus* was attempted 14 days after first inoculation.

Wood block decay trials

We conducted 3 separate wood block decay trials. In each French prune branch, wood was collected and following bark removal was sectioned into 2cm long pieces. Pieces were oven-dried and mass was recorded. Eight wood pieces placed in a jar and rehydrated for 24 hours and then autoclaved twice for 30 minutes. The eight wood pieces were then placed in a moist chamber and each chamber was randomly assigned a treatment. Each treatment was replicated 5 times for a total of 40 pieces of wood. In experiments 1 and 2, chambers were incubated in the dark at room temperature for 12 weeks after the second inoculation, while experiment 3 will be terminated 16 weeks after final inoculation.

Treatments for Experiment 1 (WB1) were Vintec applied either 7 days before or 7 days after inoculation with *Phellinus*, *Phellinus* alone, and an untreated control.

In experiment 2 and 3 (WB2, WB3), each block received two treatments 14 days apart. First and second treatments were either biocontrol product, *Phellinus*, or untreated control paired to create all possible combinations. The second treatment was applied 14 days after the first treatment. At termination of each experiment exterior mycelia was removed from wood blocks and then blocks were oven dried for 48 hours at which time mass was recorded. Analysis of Variance was

performed on mass loss. A Dunnett’s multiple comparison test was done to compare each treatment to control and *Phellinus*-only treatment.

Table 1. List of biocontrol products currently under evaluation			
Product	Manufacturer	Species	Experiment
Plant Shield (PS)	BioWorks	<i>Trichoderma harzianum</i> Rifai strain T-22	WB2, WB3, T1
Root Shield (RS)	BioWorks	<i>Trichoderma harzianum</i> Rifai strain KRL-AG2	WB2, WB3, T1
Root Shield Plus (RSP)	BioWorks	<i>Trichoderma harzianum</i> Rifai strain T-22 and <i>Trichoderma virens</i> strain G-41	WB2, WB3, T1
Vintec (Vintec)	Belchim Crop Protection	<i>Trichoderma atroviride</i> strain SC1	WB1, WB2, WB3, T1
BW165N-0218 (BW165)	BioWorks	<i>Ulocladium</i> sp.	WB2, WB3, T1
BW161 (BW161)	BioWorks	<i>Trichoderma</i> sp.	WB2, WB3, T1

Tree decay trials

While we intended to put large trials out in growers’ orchards, based on the mass loss we observed in laboratory decay trials with biocontrol products alone, we decided it would be best to first test on trees of no importance. Field trials are scheduled to commence in February 2019 at Wolfskill ranch. Branches on French Prune trees of similar size (~5cm) will be selected and pruned. Following pruning, a treatment regimen similar to the wood block trials will be applied. Monitoring of disease symptoms will continue for six months at which time treated branches will be collected and analyzed for decay severity in the lab.

Objective 3. Evaluate prune varieties susceptibility to decay.

Wood block inoculation

To determine the decay rate of *P. tuberculosis* in French prune, branches with a diameter of 1.5 to 2.5 cm after bark removal were cut into 2 cm lengths. Half of the pieces were autoclaved, while the other half were surface sterilized prior to inoculation. Fresh mass was recorded for all pieces. The pieces to be autoclaved were dried and weighed prior to rehydration and autoclaving for 30 minutes twice. Seven prune varieties were included in this trial and were prepared as previously described. Non-inoculated wood pieces served as control. Each variety and a control were replicated 10 times each for an autoclaved and non-autoclaved treatment.

Each replicate was randomized and wood blocks were placed on a wire mesh inside a moist chamber. A plug of mycelia (3mm x 3mm) from 14 day old *P. tuberculosis* cultures were placed on top of each block. Moist chambers were maintained in the dark at room temperature for ten

weeks. After ten weeks, surface mycelia were removed. Wood pieces were dried at 60°C for 48 hours and dry mass recorded. Percent moisture content and initial fresh weight were used to calculate predicted initial dry weights for each wood piece. Analysis of Variance was performed on mass loss using initial dry mass as a covariable. Tukey's mean separation of mass loss was performed on prune varieties. This experiment is currently being repeated and is scheduled to be terminated in February 2019.

Sapling inoculations

We have prune rootstocks in 30 gallon pots that will be grafted with the most promising new varieties from the prune breeding program in early 2019. We will inoculate these trees with *Phellinus* in late 2019. We will continue to work with the prune breeding program to evaluate the relative susceptibility to decay of promising new prune varieties.

Objective 4. Quantify the potential carbon impacts of *Phellinus* and other wood decay in prune.

Wood-decay fungi play a crucial role in the carbon cycle of forests. However, the impact of wood decay fungi on carbon storage in orchards is largely unknown. The potential for increased regulation of greenhouse gas and pollutant emissions has led many industries to undertake life-cycle assessments; this is true of the prune industry as well. We are currently collaborating with Dr. Elias Marvinney to quantify wood decay in orchards and determine its impacts on short and long-term carbon storage. The analysis of total carbon for prune wood in different stages of decay will be performed at the UC Davis analytics lab using a standard combustion method.

The procedure for carbon quantification in a tree begins with the sectioning the above ground portion of the tree into 3 portions: fruit wood, scaffolds, and trunk. Fresh mass was recorded for each section and measurements of diameter and length of each scaffold and the trunk were taken. Wood disc were collected every 30cm from the trunk and scaffold limbs and placed in a drying oven. Once dry, mass is again recorded, and each disc is analyzed for density. Pieces of wood representing the range of densities will be submitted for total carbon analysis. Currently we have collected six trees of two different age classes and samples are being dried.

Objective 5. Test and adapt new spore monitoring technology for application in prune orchards.

We are currently collaborating with SCANIT Technologies to better understand seasonal and regional patterns of *Phellinus* and other wood-decay fungus spore inoculum. SCANIT has developed an automated airborne particle classification and monitoring system. Unlike conventional spore traps that require substantial labor and time to collect, identify, and quantify spores of interest, their system is designed to remotely classify and report, via a web-based application, inoculum levels in real-time. Particles are imaged with a patent pending light illumination techniques that force unique particle signatures. Once deployed in the field, imaged particles are compared to a cloud-based particle image library and machine learning techniques are used to identify particles. We have provided spores of *Phellinus tuberosus* and five

Ganoderma species to the company. SCANIT is currently generating baseline data to add to the particle image library.

RESULTS AND DISCUSSION

Objective 2.

Wood Block trials

In Experiment WB1 Vintec significantly reduced the ability of *Phellinus* to decay French prune when applied both before and after *Phellinus* inoculation when compared to *Phellinus* alone (Figures 1 and 2). *Phellinus* mortality was 100% for both Vintec treatments, 12 weeks after inoculation.

In Experiment WB2, all of the wood pieces inoculated with biocontrol product and *Phellinus* had significantly less mass loss than those inoculated with *Phellinus* alone, but significantly more than untreated control (Figures 3 and 4). We were unable to recover *Phellinus* from wood pieces treated with any of the biocontrol products. We did see substantial mass loss of those wood pieces that received only biocontrol products. However, this is most likely due to consumption of labile sugars and other carbohydrates, as *Trichoderma* spp. do not possess the enzymes to degrade wood. For this reason, wood blocks will be incubated for 16 weeks instead of 12 in experiment WB3 and will be terminated in January 2019.

Tree inoculations

Tree inoculations will commence in February 2019 so there are no results to report at this time.

Objective 3.

Variety Decay trials

Mass loss was greatest in Improved French variety when compared to the other prune varieties included in the trial (Figure 5). This suggests some degree of adaptation by *P. tuberosus* to more readily decay the Improved French variety. A shift to more diversity in prune varieties may benefit the industry with regards to problems caused by *Phellinus*. This experiment is currently being repeated and will be terminated in February 2019. As only preliminary and we will continue to work with breeders to further evaluate susceptibility of different varieties to *Phellinus* decay in the lab and in the field.

Objective 4.

This objective is currently underway so there are no results to report at this time.

Objective 5.

SCANIT spore traps are currently operating in the lab (Figure 6) and *Phellinus* conks are replaced once a month. We are in the process of creating reliable scans of spores (Figure 7). Once spore image and characteristics are acquired, this technology may be employed by growers to

monitor inoculum pressure and assist with pruning scheduling as to avoid times high inoculum pressure.

Figure 1. From left to right, un-inoculated control, *Trichoderma* followed by *Phellinus*, *Phellinus* followed by *Trichoderma*, *Phellinus* alone.

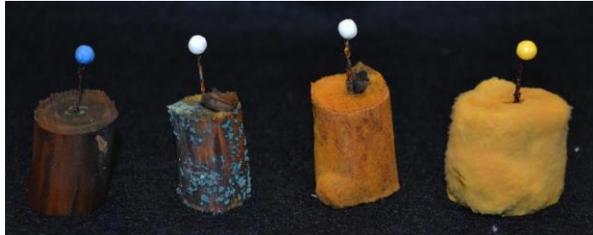


Figure 2. Results from experiment WB1. Mass loss 12 weeks after second inoculation.

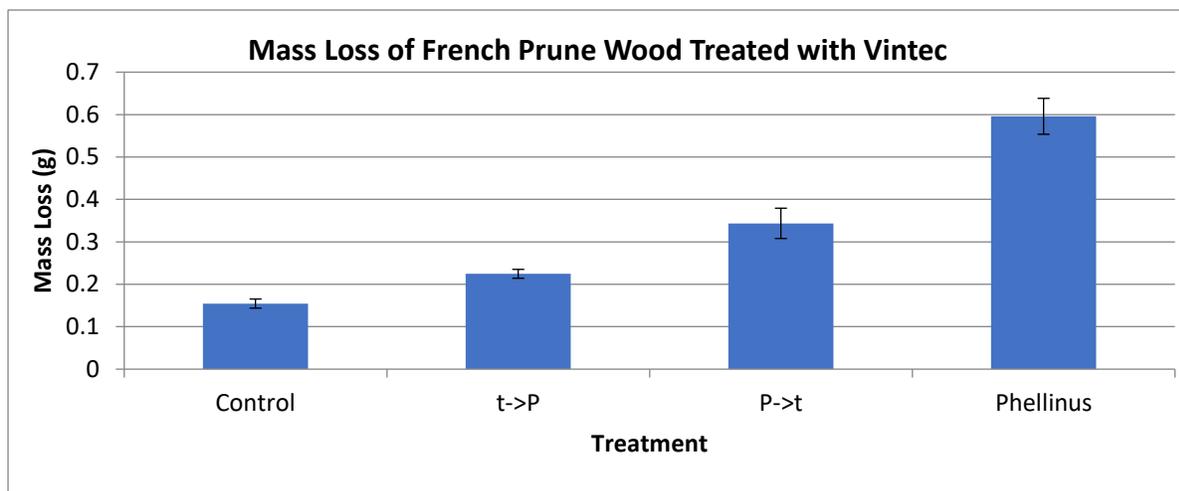


Figure 3. Representative samples of experiment WB2. From left to right. *Trichoderma* alone, *Trichoderma* followed by *Phellinus*, *Phellinus* followed by *Trichoderma*, and *Phellinus* alone.



Figure 4. Results from experiment WB2.

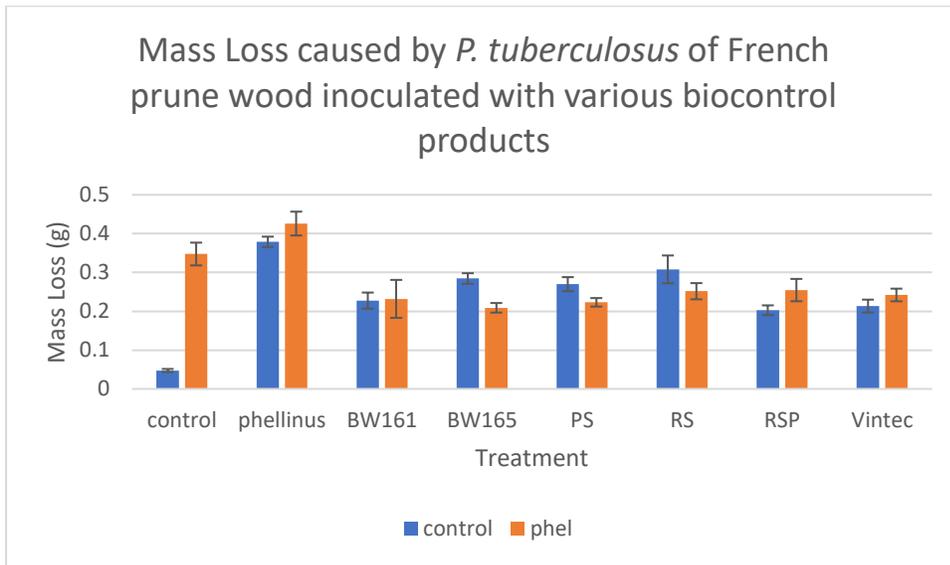


Figure 5. Mass loss caused by *P. tuberculosis* on various prune varieties after ten weeks of incubation. Tukey's mean separation groupings are presented above bars, different letters indicate significant difference at $p < .05$.

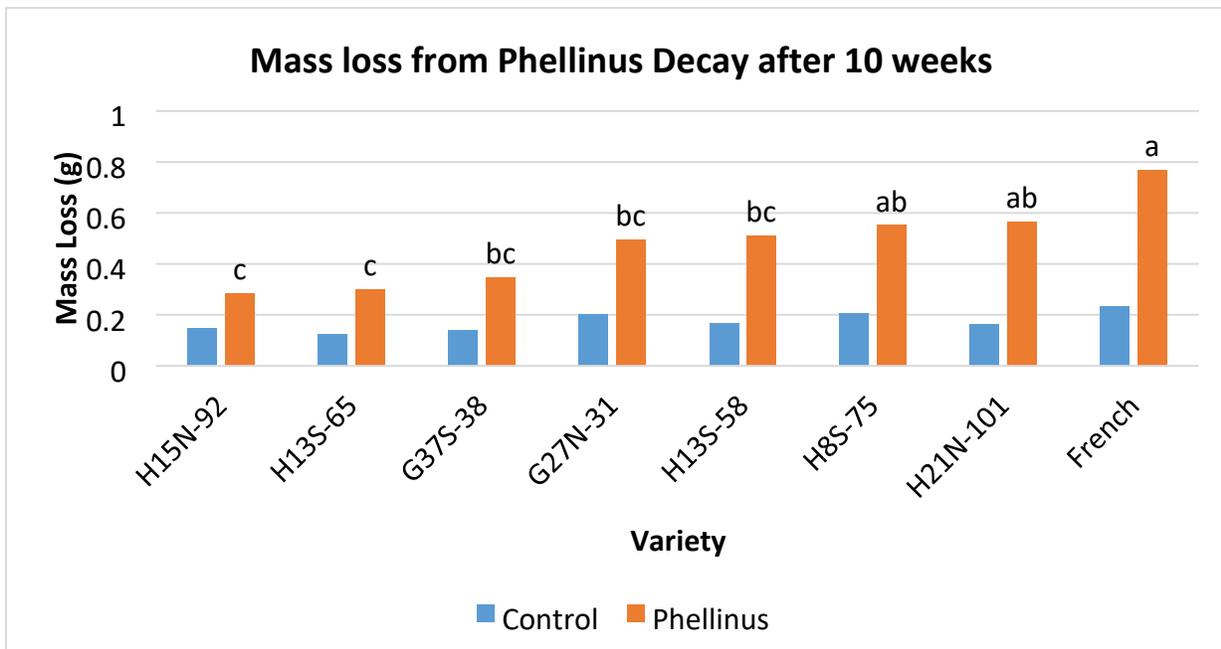
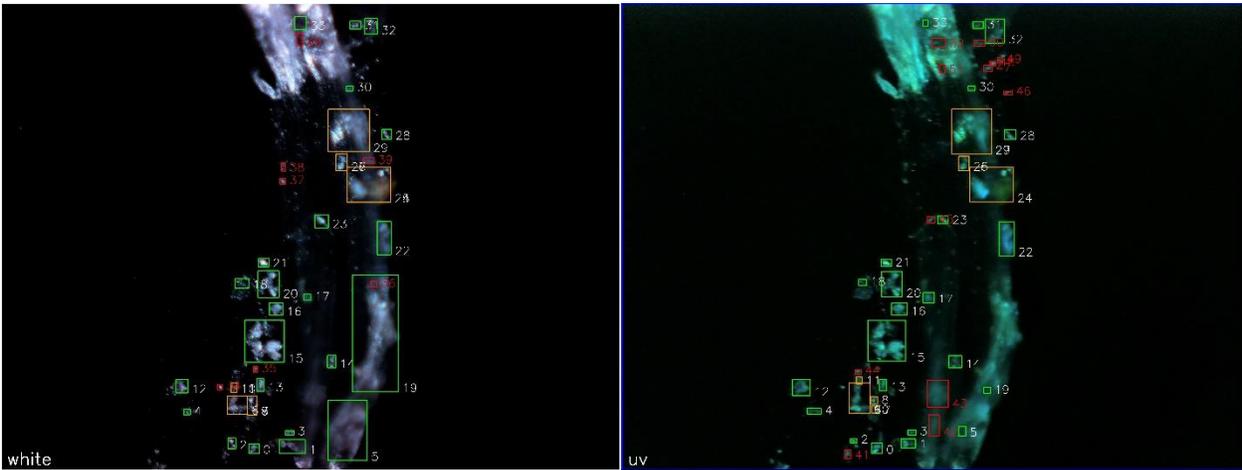


Figure 6. SCANIT Spore trap and *Phellinus* conks.



Figure 7. Early particle scans of *Phellinus tuberculatus* spores.



CHARACTERIZING CURRENT ROOTSTOCKS OF THE DRIED PLUM INDUSTRY FOR HOST STATUS TO PLANT-PARASITIC NEMATODES

Andreas Westphal, Ph.D.

ABSTRACT

Plum trees are cropped for many years, and thus can be exposed to a build-up of plant-parasitic nematodes on the roots. Such build-up and potential risk for damage depends on the host status of the rootstock genetics. Valid assessments of the host status and durability in response in regards of infection levels with these soil-dwelling pests requires multiple years of field experimentation. At the Kearney Agricultural Research and Extension Center, field plots with natural and supplemental infestations of root lesion and root-knot nematodes are used to determine the host responses of Prunus rootstocks used in the Dried Plum Industry. One planting has been made in 2018, and a second planting is planned for 2019. First-year data in 2018 illustrated low infection levels across the test set for root-knot nematodes, but trends of differences in the infection levels in root lesion nematode. Tests for responses to ring nematodes are ongoing.

OBJECTIVES

- (i) Generate information on the host plant status of the rootstocks towards root lesion, root-knot and ring nematodes.
- (ii) Test data for reproducibility by establishing replicate trials.
- (iii) Summarize and provide information on the nematode host status of currently used rootstocks for rootstock decision support.

PROCEDURE

This project was initiated in 2018. Twelve typical rootstocks used for plum: 'Bright's hybrid 5' ('BH5'), 'Citation', 'Controller 6', 'Controller 7' (= 'HBok32'), 'Controller 9', 'Ishtara', 'M40', 'M58', 'Marianna 2624', 'Myro 29C', 'Myrobalan' and 'Viking' were used along controls 'Krymsk 1', 'Lovell' and 'Nemaguard'. In spring, these genotypes were planted in pairs of two in randomized complete block design with four replications. A month after planting, each plant was inoculated with soil infested with known numbers of root lesion and root-knot nematodes. The plants were cultivated during the summer. At the beginning of the dormant season, one of each of the tree pairs was removed from soil for examination of the roots for nematode-induced galling, and for excising fibrous roots for nematode extraction. Before any plants were removed, tree height and trunk diameter were determined.

In fall 2018, the same rootstock genotypes were planted to sand tanks. After plant establishment, every plant was inoculated with ring nematodes. Measurements will be started in 2019.

RESULTS AND CONCLUSIONS

After the one vegetation period in field plots infested with root lesion and root-knot nematodes, BH5, Ishtara and Myro29C were thicker than Citation, HBok32, Krymsk 1, Lovell, and M58. Other rootstock genotypes were in between. BH5, Ishtara, M58, and Marianna 2624 were higher than Controller 6, HBok32, Lovell, and Myrobalan. These differences confirmed the hypothetical lower vigor of the dwarfing rootstocks, and illustrated the larger vigor of the resistant genotypes in this test. Only Controller 6, Controller 9 and Lovell had detectable galls on the roots. Numbers of nematodes extracted from roots were low overall. Infective juveniles of root-knot nematode were detected on the three rootstocks with galling and on Controller 7 and Viking but close to the detection level. Roots of other rootstock genotypes did not yield detectable root-knot nematode numbers in the extraction procedures. Root lesion nematodes were detected in low numbers on most rootstocks. Only BH5 and Krymsk 1 did not yield any vermiform stages of RLN. Numerical trends were not significantly different at $P = 0.05$.

In conclusion, noticeable nematode population densities were found on rootstocks typically used for plum production. While most rootstocks did not support root-knot nematodes, many of them were supporting root lesion nematodes. From our experience with other perennials, we predict that a continued evaluation is necessary to solidify the data base.

Acknowledgements

The rootstock M58 was provided from University of California, Davis.

LIFE CYCLE ASSESSMENT:
A TOOL FOR QUANTIFYING THE ENVIRONMENTAL IMPACTS OF FRESH AND
DRIED PLUM PRODUCTION

Elias Marvinney
Alissa Kendall

OBJECTIVE

Orchard production impacts have come under greater scrutiny from various California state agencies in recent years, from limitations on open burning of orchard waste to the potential carbon credit generation under California's carbon cap and trade market. In order to understand the full environmental impacts and benefits of orchard production, a life cycle assessment (LCA) or "cradle-to-grave" approach is needed. Resources and energy are consumed, and atmospheric pollutants and greenhouse gases are emitted at every stage of orchard production, from extraction of raw materials through postharvest processing and orchard end-of-life management, including synthesis of agrochemicals, delivery of materials and equipment to orchard sites, irrigation water pumping and delivery, and orchard floor soil biogeochemical processes.

Life Cycle Assessment is a comprehensive approach for quantification of this resource use and impacts throughout the full life cycle of a system or a product, such as an orchard crop. LCAs typically account for the energy and environmental impacts of all stages of a product's life cycle, such as acquisition of raw materials, the production process, handling of waste byproducts, and more. To date, this life cycle model of dried plum production considers the quantity of irrigation water used specific to orchard location, energy and fuel required for pumping water, energy required to produce, transport, and apply fertilizers and pesticides, energy needed for harvest, transport, and post-harvest processing including drying.

Orchard production systems are also responsible for substantial environmental benefits, including production of biomass co-products that can be used for renewable energy generation, temporary carbon storage in standing biomass, and long-term carbon storage in orchard floor soils. These benefits may result in significant energy or greenhouse gas credits to the orchard production system and must be considered in any accounting of orchard system impacts.

The regulatory and environmental context of California orchard production has changed significantly in recent years, with the closure of numerous biomass feedstock power plants, widespread drought conditions, and greater understanding of factors impacting total orchard biomass and carbon accumulation, including the activity of wood decay fungi such as *Phellinus* sp. and the dynamics of soil carbon in orchard systems. Data used to develop earlier versions of the prune/plum LCA model, especially regarding active biomass power plants, surface water delivery, and groundwater depth, must be continually updated to reflect such changing conditions in the Central Valley.

PROCEDURE

To date, progress towards the goal of updating and improving the California dried plum LCA model has been made in 4 main areas:

1. Updating and improving the generic orchard LCA model framework (Fig 1). The model framework has been completely overhauled in order to increase computational efficiency and flexibility in handling continual updates to orchard parameters and relevant datasets, as well as to allow reporting of a greater range of environmental impacts and chemical flows. Additionally, agronomic response sub-models have been integrated, allowing the LCA model to account for tradeoffs in biomass productivity and yield in response to changing management conditions and inputs (deficit irrigation, planting density, orchard recycling, fertilizer input, etc.). Improved modeling of irrigation energy demand based on efficiency ratings at different depths for specific pump models has been added, as well as improved estimation of soil GHG emissions in response to nutrient addition and irrigation. Currently, only almond-specific data and parameters populate this model framework, but work on inputting dried plum data is set to begin early in 2019.
2. Updated life cycle inventory (LCI) and irrigation datasets (Fig 2-3). The LCI datasets that form the basis for the LCA material and energy flow analysis have been updated with the release of a new version of GaBi Thinkstep software. Figure 2 shows differences between 2014 and 2018 datasets in select environmental flows for some inputs of significance to orchard production. Groundwater depth data from the California Department of Water Resources (DWR) has been updated to account for the effects of drought conditions in recent years (Fig 3).
3. Initiated data collection for plum tree biomass accumulation model. With cooperation from a grower in Sutter county and the Rizzo lab, samples of 3 trees each from 9 orchard blocks of different ages will be uprooted, weighed, and analyzed for density and carbon content specific to roots, trunk, scaffold and fruit wood. Thus far 2 orchard blocks have been sampled and currently being processed and analyzed. These data will be used to quantify the potential climate benefits and environmental impacts of carbon storage in standing biomass as well as end-of-life biomass handling options including open burn, bioenergy, and whole orchard recycling.
4. Completed intermodal freight transport model for orchard to international entry port section of dried plum distribution chain (Fig 5-7). Figure 5 shows the conceptual diagram of the multi-modal freight transport system as approached in this project. Transit distance and mass modeling has been completed for the following stages: orchard to postharvest (dryer/ packer) by truck, postharvest to port of departure for export by truck, and US port to international ports of entry among the top California dried plum importing nations (Fig 6). Once work is completed on estimates for transport to local, regional, national, and international markets, a complete assessment of “food miles” for global dried plum consumers will be available, accounting for the reduced mass, increased shelf-life and correspondingly reduced emissions for bulk freight transport produced by drying the product for export.

FIGURES

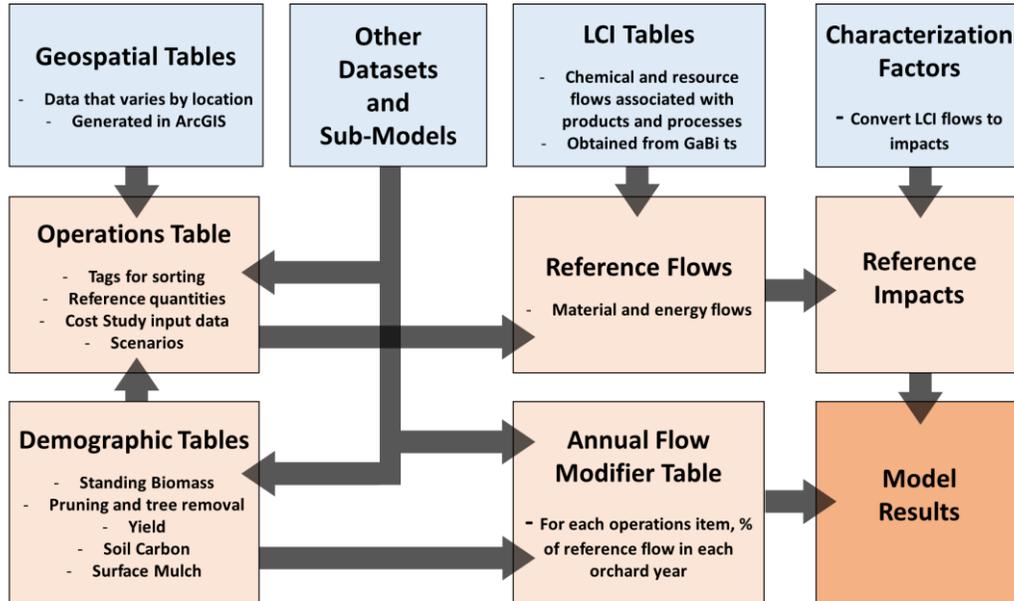


Figure 1. Organization and data flow in the updated generic orchard LCA model framework.

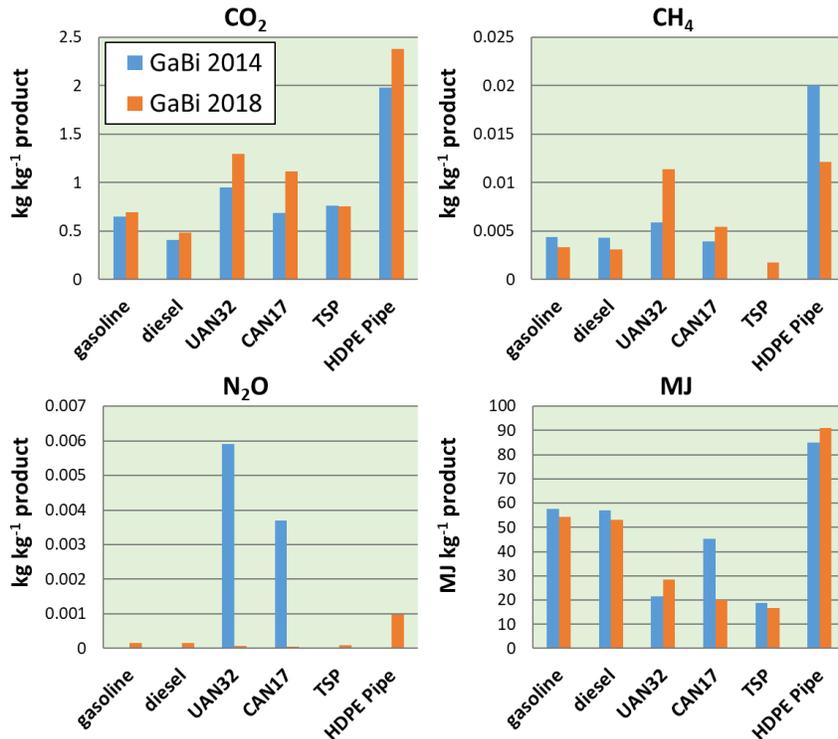


Figure 2. Changes in select impacts for select orchard inputs with updated life cycle inventory (LCI) datasets obtained from Ecoinvent and Professional databases via GaBi Thinkstep software.

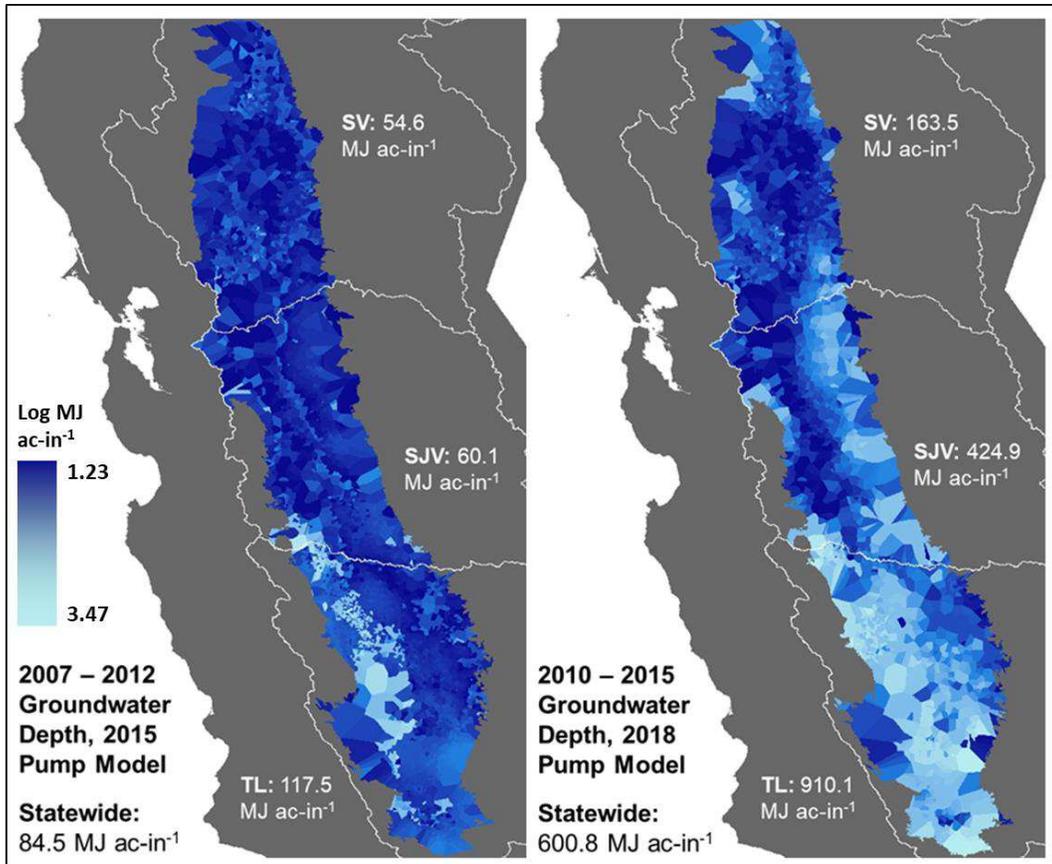


Figure 3. Change in groundwater pumping energy requirements with updates to groundwater depth data from California Department of Water Resources (DWR) and pumping efficiency and energy use modeling.

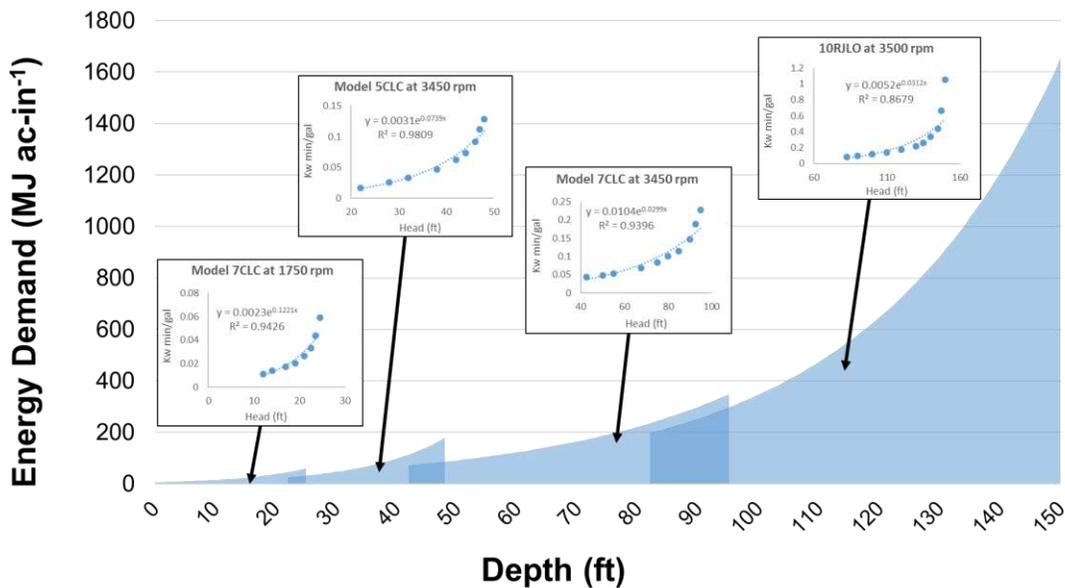


Figure 4. Updated, technology-specific, depth-sensitive irrigation pump energy demand model output.

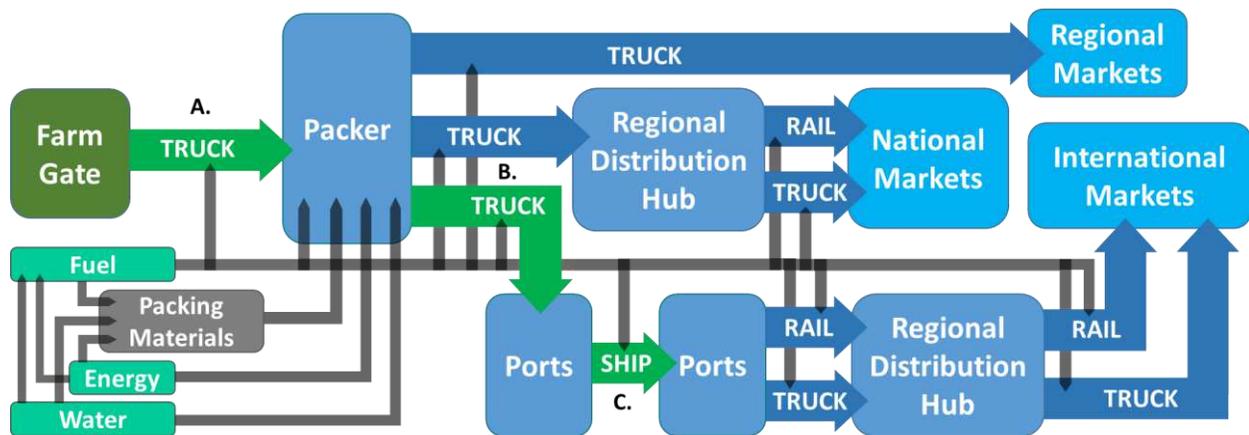


Figure 5. Flows and network nodes in global intermodal freight transport model. Sections shown in green have been quantified to date. A) Mean truck transport distance from orchards to packing operations in the three main growing regions of the Central Valley (Sacramento Valley, San Joaquin Valley, and Tulare Lake) have been calculated using data on packing operation locations obtained from the California Dried Plum Board website, and distribution of plum orchards obtained from the USDA National Agricultural Statistics Service 2017 Cropscape Data Layer (USDA 2018). B) Mean truck transport distance from packer to port have been calculated using data on global container freight shipping routes from the UCL Energy Institute (www.shipmap.org) for departure port locations, Google Earth Pro for highway routes and distances, and CDPB packer locations. C) UCL ship map data and ArcGIS data viewer were used to calculate oceanic freight transport distances.

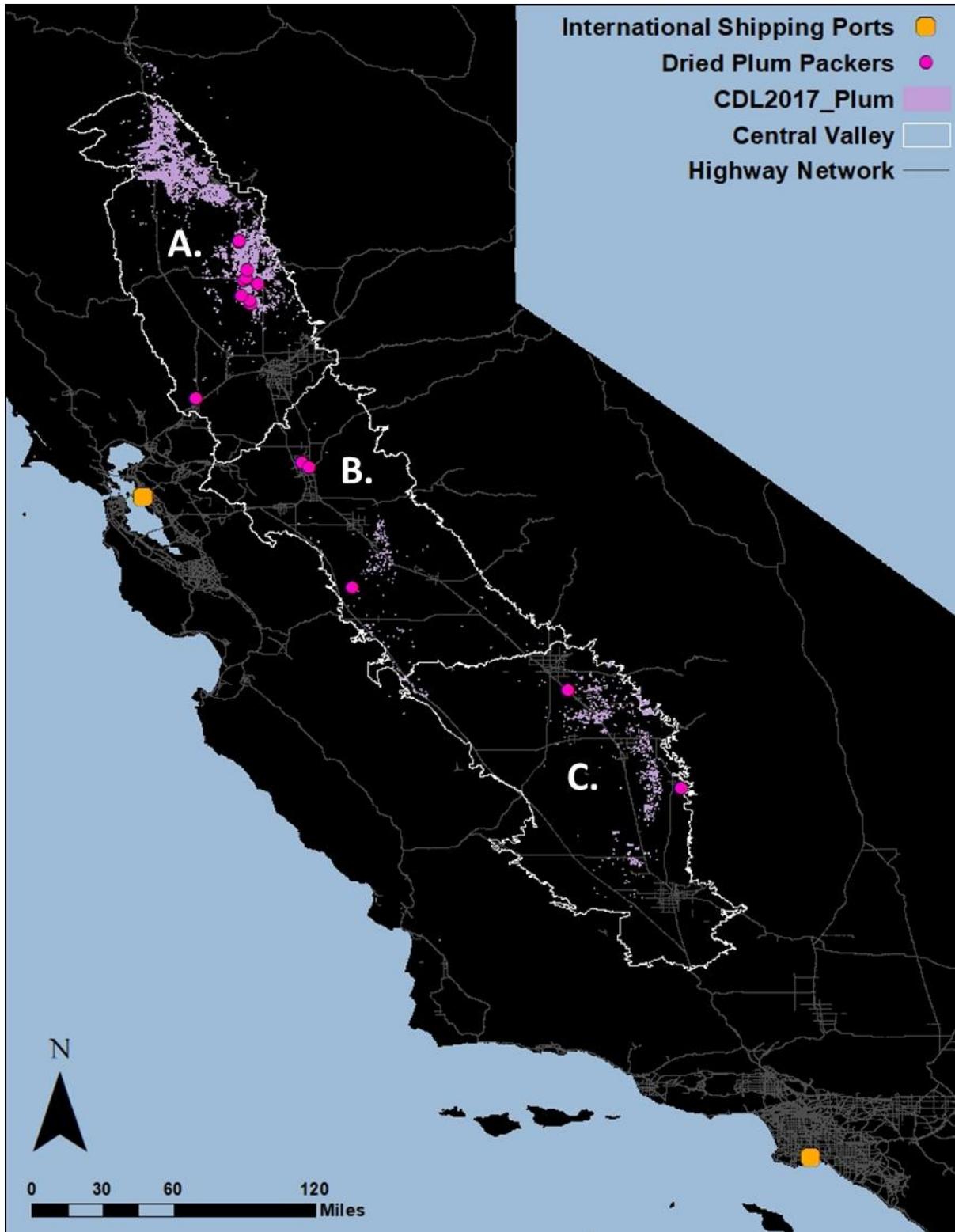


Figure 6. Geospatial data used in truck transport calculations, specific to the A) Sacramento Valley, B) San Joaquin Valley, and C) Tulare Lake growing regions.

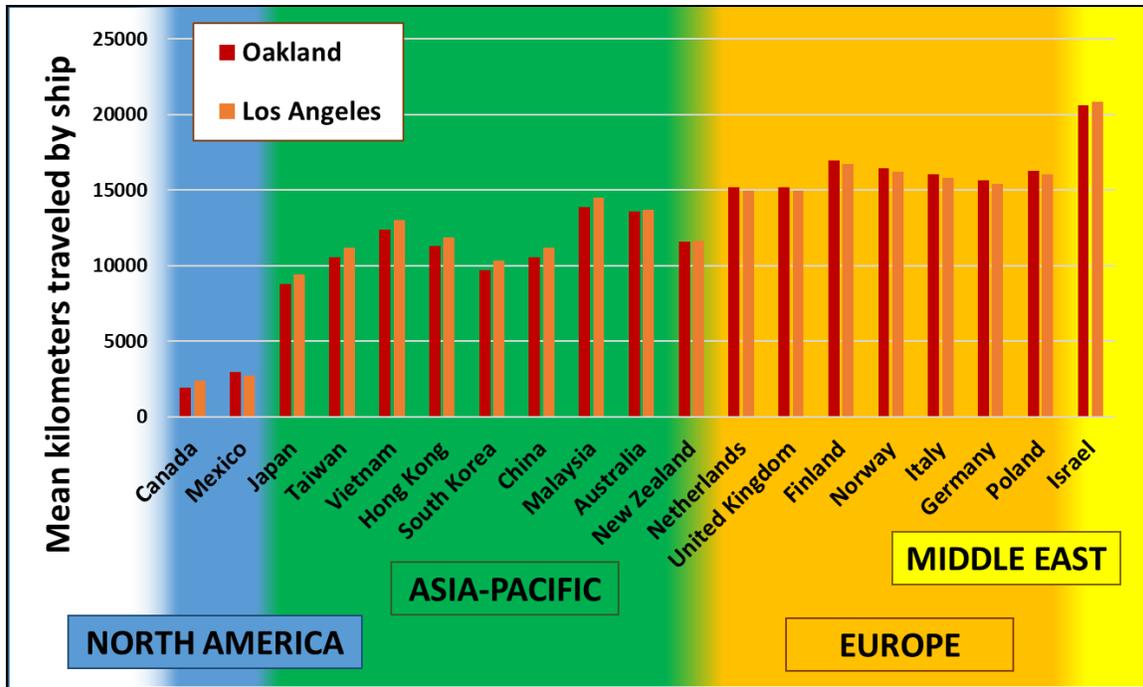


Figure 7. Mean distances traveled by oceanic container ship from Oakland and Los Angeles ports of departure to importing nations comprising 90% of California dried plum exports in a 5 year average obtained from USDA NASS crop economic data.

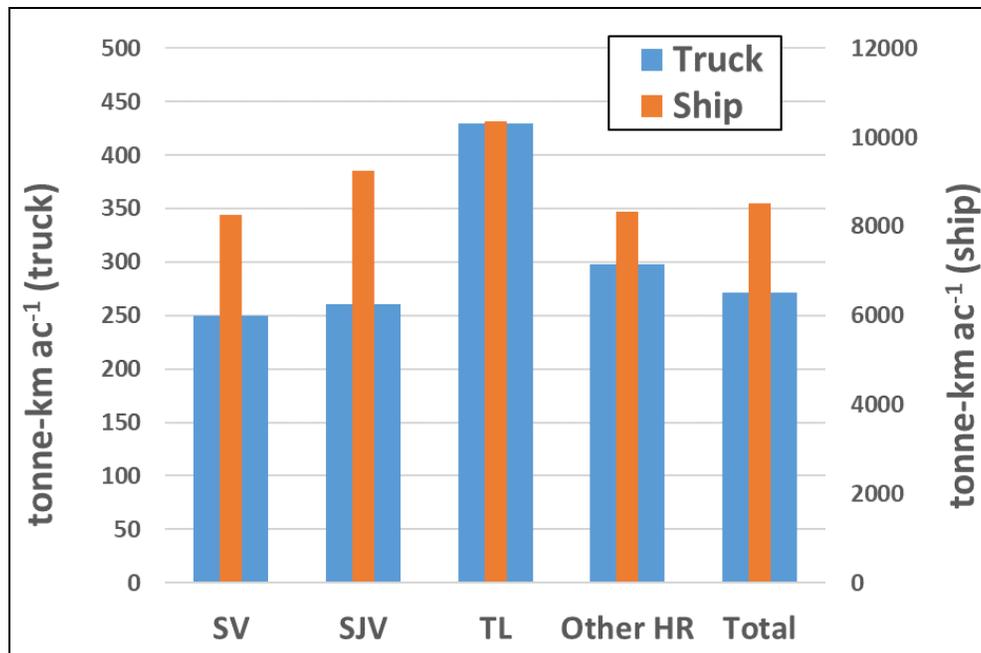


Figure 8. Total truck and ship tonne-km for freight transport impact calculation, estimated using data pictured in figures 6-7 as well as county-specific 5 year average yield values obtained from the California Crop Commission via USDA, and estimated moisture content of plums at farm gate (80%), dried for local consumption (16%), and dried for international transport (6%).

EVALUATING NATURALLY STRUCTURED WATER IN FRENCH PRUNE

Allan Fulton and Carolyn Haynes

OBJECTIVES

The objective of this trial was to evaluate the efficacy of irrigating French prune with “naturally structured water”.

Naturally structured water is an unconventional water treatment system currently being considered by some growers in the Sacramento Valley. A recent example is its consideration by a commercial nursery in the northern Sacramento Valley. Results from their small, controlled greenhouse study observed an 80 percent increase in plant blossoms of strawberry mother plants and a 20 percent increase in daughter plants. Other noted benefits included increased root volume and greener canopy color. The vendor of this water treatment system is Naturally Structured Water Systems, LLC in Redding, CA (<https://naturallystructuredwater.com/>). It is described as “a unique system utilizing natural vortices and magnetic fields to restore water to its most energized, living water condition”. One of the original icons of “structured water” systems was Viktor Schauberger (1885-1958). A suggested, independent reference for further reading on this concept and other unconventional methods of water treatment is provided by Steven Lower, retired faculty member of the Department of Chemistry, Simon Fraser University, Burnaby/Vancouver, Canada (<http://www.chem1.com/CQ/index.html> and <http://www.chem1.com/CQ/TWEbunk.html>).

PROCEDURE

Naturally Structured Water Systems, LLC provided twelve 1” diameter or slightly larger units designed to treat water in individual drip irrigation lines (figures 1a and 1b).



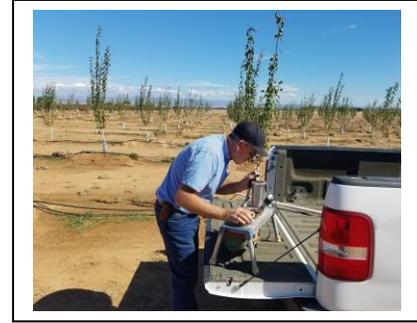
Figure 1a and 1b. Small in-line water treatment device (left) is installed between riser and tee in drip line. Larger treatment device (right) designed to treat more than 1000 gpm.

The trial was performed in a prune orchard (French variety on Myro 29C rootstock) that was planted in March 2018 near Corning, CA. One water treatment device was installed per tree row and three tree rows with these devices were grouped to form a single plot of about one acre. A randomized complete block experiment comparing four replicates of untreated and naturally structured water was performed to evaluate tree responses. All total, there were eight, one-acre plots (four without water treatment and four irrigated with structured water).

Irrigation began after the spring rainfall ended. Scheduling was relatively frequent, approximately weekly in the spring when the trees were just beginning to grow and as frequent as every other day as the trees grew in the summer. Irrigation duration did not exceed 5 hours per day using two 0.5 gph emitters near each tree to limit runoff from the raised planting mounds. Irrigation was curtailed beginning in mid-September to harden the new tree growth and lessen risk of cold injury in the fall and winter. All plots were irrigated identically. Trunk diameter and tree height were measured on ten trees in the middle row of each three-row plot receiving either untreated well water or structured water to evaluate tree vigor and growth. Measurements were taken July 2, August 7, September 6, and October 2, 2018 and on the same trees each day. Midday stem water potential (SWP) was also measured on five of these trees in each plot between noon and 1:30 p.m. on the same days to assess tree water status. All total, 80 trunk diameter, 80 tree height and 40 midday SWP measurements were taken each day to assess tree response. Photos 2a and 2b show tree size after planting in the spring and during the irrigation season. Photos 3a, 3b, and 3c illustrate the methods used to evaluate trunk diameter, tree height, and SWP. Untreated and restructured water supplies were sampled from the ends of the drip lines on September 21, 2018 from two replicates. Each drip line was flushed until there was no visible sign of suspended sediments in the flowing water and then 500 ml samples were collected, stored in an iced cooler, and shipped to a certified, commercial agricultural laboratory for analysis.



Figures 2a and 2b. Trial site in the spring after planting (left) and progress of seedling trees during the summer.



Figures 3a, 3b, and 3c. Trunk diameter was measured at 2 feet above the ground surface using a digital caliper (left). Tree height was measured manually (center) with a graduated (0.1 ft) measuring pole. Midday SWP was monitored with a pressure chamber (right).

RESULTS

Traditional agricultural water quality suitability tests were performed on the untreated and naturally structured irrigation water samples. Laboratory results suggest the main concern is relatively high bicarbonate and carbonate levels ($\text{HCO}_3 + \text{CO}_3$ or alkalinity) which also reflect high pH. Levels above 2.0 meq/l alkalinity pose increasing chances of a reaction with soluble calcium to form minerals that can potentially plug drip emitters and reduce irrigation uniformity from tree to tree. This same reaction may contribute to reduced soluble calcium levels, create more of an imbalance with sodium (Na) and magnesium (Mg), and contribute to more soil aggregate dispersion when the soils are irrigated. Soil crusts are more likely to form and slow water infiltration, cause more runoff of irrigation water, and result in less effective management of tree water stress and growth. There are also anecdotal concerns that the high alkalinity levels may interfere with efforts to manage other aspects of plant nutrition. In this instance, the alkalinity, pH, Langlier Saturation Index (L.I.), calcium (Ca), magnesium (Mg), and sodium (Na) levels all suggest that the structured water devices did not change the composition of the water when compared to the untreated water.

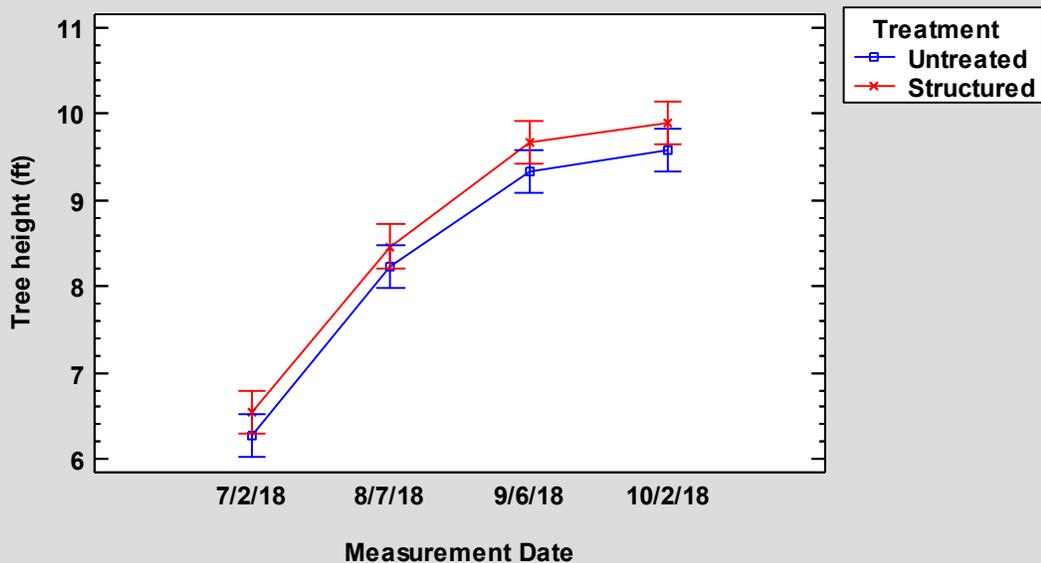
Table 1. Comparison of irrigation water quality characteristics of naturally structured and untreated irrigation water supplies.

Sample	pH	EC	Ca	Mg	Na	SAR	Cl	$\text{HCO}_3 + \text{CO}_3$	SO_4	B	$\text{NO}_3\text{-N}$	L.I.
		dS/m	meq/l				meq/l			mg/l		
Structured Rep 3	8.2	0.36	0.95	1.26	1.7	1.6	0.4	3.5	0.1	0.05	<0.1	0.4
Untreated Rep 3	8.2	0.36	1.03	1.36	1.8	1.6	0.4	3.5	0.1	0.06	<0.1	0.4
Structured Rep 4	8.2	0.36	0.96	0.96	1.7	1.6	0.4	3.6	0.1	0.05	<0.1	0.4
Untreated Rep 4	8.2	0.36	0.98	0.98	1.7	1.6	0.4	3.6	0.1	0.06	<0.1	0.4

Other suitability parameters such as excess salinity (EC), specific ion toxicities from sodium (Na), chloride (Cl), or boron (B), or high nitrate-nitrogen (NO₃-N) are not at levels of concern in this irrigation water supply. The naturally structured water system did not appear to influence them one way or another.

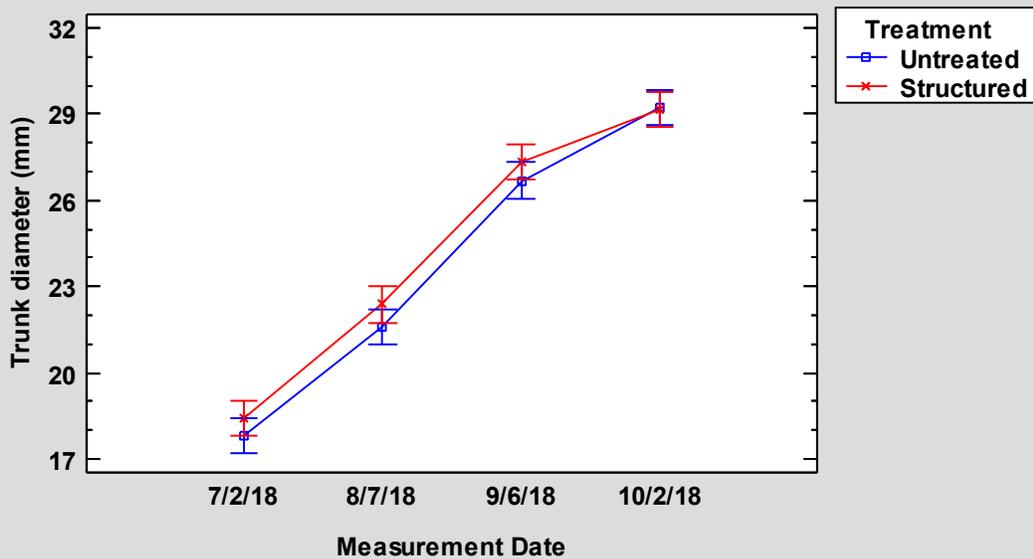
Tree height (figure 4) was measured as an indicator of tree vigor. The probability of tree height being higher in the plots irrigated with naturally structured water was significant ($p < 0.05$) for all four measurement dates. On July 2 tree height averaged 6.3 and 6.5 feet in the untreated and structured water treatments, respectively. On August 7, tree height averaged 8.2 and 8.5 feet in the untreated and structured water treatments, respectively. On September 6, tree height averaged 9.3 and 9.7 feet and on October 2 tree height averaged 9.6 and 9.9 feet in the same respective water treatments. While tree height was observed to be consistently 0.2 to 0.4 feet taller over the course of the season in the plots irrigated with the structured water, the probability of time ($p < 0.001$) and soil variability ($p < 0.01$) influencing tree height was even greater. When irrigation management effectively maintained low crop water stress at levels expected to encourage tree growth, average tree height increased 1.9 and 2.2 feet from July 2 to August 7, and from August 7 to September 6, respectively. When management curtailed the intensity of irrigation in September to harden new shoot growth in anticipation of the end of the growing season and to minimize risk of cold injury, tree height only increased an average of 0.2 feet from September 6 to October 2, 2018. Replicate effects, which likely indicate variable orchard soils, showed up to 0.8 feet difference in average tree height.

Figure 4. Mean tree height and 95 percent LSD (least significant difference) intervals. Shows interaction between water treatment and measurement date.



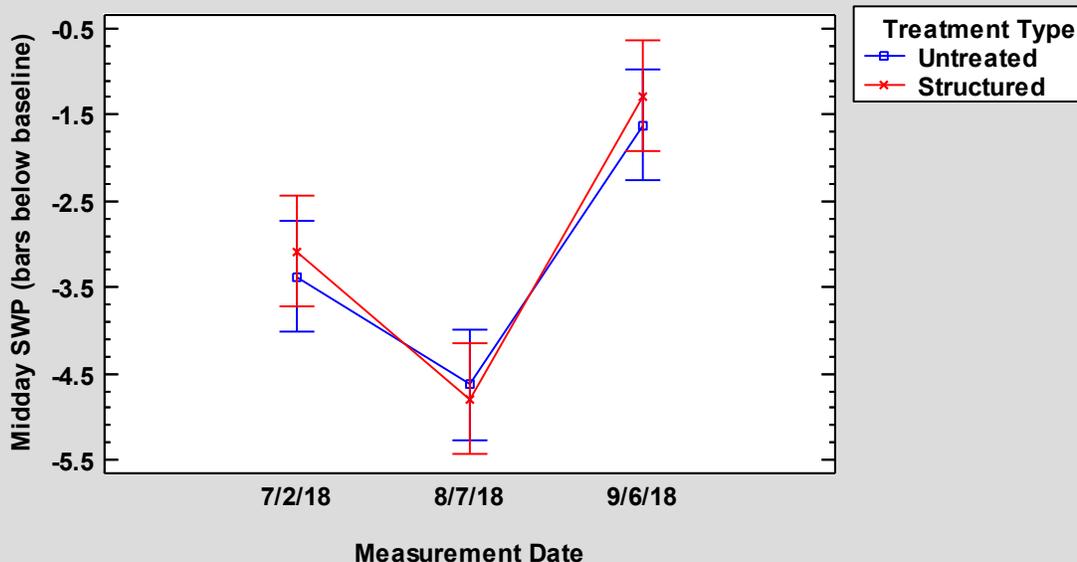
Trunk girth expressed either as diameter, circumference, or cross-sectional area is another indicator of tree vigor. Trunk girth is an important trait in terms of protecting against trunk and limb breakage during the early years of tree development and by enabling earlier tree shaking and crop harvest. Average trunk diameter (figure 5) was slightly higher for trees irrigated with structured water on three of the four measurement dates. The differences were not consistent or large enough to have a statistically significant probability of re-occurring. The differences were very small averaging less than 1 mm difference in diameter on each measurement day and there was no difference in average trunk diameter on the last measurement of the season taken October 2, 2018.

Figure 5. Mean trunk diameter and 95 percent LSD (least significant difference) intervals. Shows interaction between water treatment and measurement date.



Midday stem water potential (SWP) was measured as an indicator of tree water status. If the naturally structured water system improved water infiltration, increased plant water availability, or added root volume, presumably it would be apparent in tree water stress levels (figure 6). SWP levels ranged from about 1.5 to 4.5 bars below fully irrigated baseline levels across measurement dates. These levels represented minimal to mild crop water stress suitable for vigorous tree growth. SWP averaged only 0.2 to 0.3 bars difference in plots irrigated with untreated or structured water. Slightly lower tree stress levels were observed in the trees irrigated with structured water on July 2 and September 6 but not on August 7, 2018. These differences in SWP were too small and not consistent enough to suggest a high probability of the structured water offering an advantage over the untreated well water.

Figure 6. Mean midday stem water potential and 95 percent LSD intervals. Shows interaction between water treatment and measurement date.



CONCLUSIONS

Naturally structured water treatment systems are under consideration by some commercial farms as a feasible means of improving irrigation water quality and enhancing crop production. It is a technology that has been in existence for many decades and perhaps has undergone some changes over time, although not documented well. This field experiment was undertaken to acquire insight into this technology. Newly planted French prune trees were involved to facilitate a quick and affordable assessment of plant responses to naturally structured water treatment. We were unable to measure significant changes in irrigation water composition. The benefit of irrigating with structured water on tree growth and crop water stress levels was not conclusive. Young trees irrigated with the structured water consistently grew taller but the extent was notably less than that associated with time under good irrigation management and the effects of variable orchard soils. Structured water did not increase tree trunk diameter or reduce tree water stress. So far, prune tree response to irrigation with structured water systems in a field setting has not shown a high probability of effectiveness. Benefits have not been as impressive or visually notable as described in the greenhouse strawberry nursery study.

BUDGET SUMMARY

This was an unfunded field trial done voluntarily by the UC Cooperative Extension, Tehama County at the request of Naturally Structured Water Systems, LLC in Redding, CA. Also, special thanks to Brad and Loretta Taylor in Corning CA for giving us permission to perform this trial on their farm and collaborating with us.

2018 Prune Research Tour

Location: Sutter, Yuba, Butte, Tehama and Glenn Counties

Sponsors: California Dried Plum Board and Sunsweet Growers.

Time: 9:15 AM Tuesday, May 15 to 1:30 PM Wednesday, May 16

Accommodations: For the night of Tuesday, May 15, at **Hampton Inn & Suites Red Bluff:**
520 Adobe Rd, Red Bluff, CA 96080 (530) 529-4178

DIRECTIONS TO FIRST STOP: The first stop is the prune spray thinning experiment (same start as the 2015 tour). The orchard is south of Yuba City on the Garden Hwy (same as 99 on that stretch) we will meet there at **9:15 AM, Tuesday May 15**. The location is 1/2 mile north of 12036 Garden Hwy. That should be close to Marcuse road. There will be a yellow sign right off the Hwy on the East side marking the place to exit and Franz will leave his white county truck by the sign. Be careful slowing and exiting the Hwy. Cell numbers listed above if you need.

Day 1: Tuesday, May 15

2018 Prune Research Tour starts **9:15 AM** in Sutter County

1. **9:30 to 10:00 AM** Prune spray thinning trial after a warm bloom. Maps will be available to tour participants. – Niederholzer (UCCE)

10:00 to 10:30 AM travel to high yield and light interception Sutter County orchard

10:30 to 11:00 AM discuss light interception/yield and look at Bruce Lampinen's iPhone app – Niederholzer (UCCE)

11:00 to 11:20 AM travel to 6' x 18' planting just west of Live Oak

3. **11:20 to 11:40 AM** Discuss how this stop compares with the previous visit? app – Niederholzer (UCCE)

12:00 to 1:15 PM Lunch at Betty's in Live Oak. Order off the menu and remember that it's \$1.00 Taco Tuesday! We will be going to a Mexican restaurant for dinner as well, with American cuisine options available at both restaurants should you feel the need to mix things up.

1:15 to 2:05 drive to a hedged M&T block in western Butte County

4. **2:05 to 2:35 PM** Discuss hedging program at M&T. Compare prune growing challenges/obstacles in North versus South Sacramento Valley. Proper use of canker fungicide/protectants. – Milliron (UCCE) and Gilles (Sunsweet)

2:35 to 3:00 PM drive to Paiva Krymsk 86 block in North Chico

3:00 to 3:25 PM Evaluate young Krymsk 86 – Milliron (UCCE), Gilles (Sunsweet)

3:25 to 3:40 travel to high density Deseret prune orchard in North Chico (across the street from Butte County rootstock plot)

5. **3:40 to 4:05** evaluate high light interception hedged orchard and compare to previous plantings – Milliron (UCCE) and Gilles (Sunsweet)

4:05 to 5:20 PM drive to and check-in at Hampton Inn & Suites Red Bluff: **520 Adobe Rd, Red Bluff, CA 96080**
(530) 529-4178

5:20 to 6:50 PM Prune orchard of the future discussion over beer and wine social

7:00 PM Dinner at Casa Ramos restaurant which is a short walk from the motel (2001 Main St., Red Bluff, CA 96080)

Day 2: Wednesday, May 16

7:30 to 8:30 AM Breakfast included with the motel or Starbucks across the street

8:30 to 8:45 AM travel to first stop at Edwards Ranch **8:45 to 9:10** AM Bacterial canker challenges discussion at Edwards Ranch – Tyler Christenson

9:10 to 9:30 AM travel to second stop at Lindauer River Ranch

9:30 to 10:00 AM Nematode discussion at Lindauer River Ranch – Michael Vasey

10:00 to 10:40 AM travel to Glenn County orchard with *Cystospora* Canker

10:40 to 11:10 PM Discussion of a middle-aged orchard with productivity and lifespan reduced by canker – Lightle (UCCE)

11:10 to 11:20 travel to ENE/Nielsen Family Farms

1. 11:20 AM to 12:00 PM at ENE/Nielsen Ranch
 - a. Tour/demo of orchard shakers, what is the future of orchard shaking? – ENE/Nielsen Family Farms
 - b. Observations on recently mature Krymsk 86 – Buchner (UCCE)

12:15 PM Closing lunch at Farwood Grill, Orland