December 2018

2018-2019

(FY 2017-2018 Funded Projects)

Research Progress Reports

for the

OREGON

RASPBERRY & BLACKBERRY COMMISSION

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Oregon Raspberry and Blackberry Commission
FY 2017-18 Progress Report

Title: Development of Biologically-based RNAi insecticide to control Spotted Wing Drosophila

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Collaborators: Jana Lee and Robert R. Martin, USDA-ARS Horticultural Crops Research Unit

Specific Objectives for 2018-19

1. Inject RNAi into adult flies and monitor RNAi impacts (*i.e.* mortality) on SWD.
2. Feed RNAi selected into larvae and/or adults, and monitor RNAi impacts on SWD.

Practical and Economic Impact: The goal of this research is to develop biologically-based insecticides as a chemical insecticide alternative to control SWD in berry crops and small fruits. The results will help growers improve production and fruit quality in the crop, and prevent potential development of chemical insecticide resistance.

Procedures:

More identification of SWD RNAi targets - A feasible approach for RNAi target gene screening was to search previous targets or systems observed already from same or similar insect groups. Based on our RNAi experience, knowledge and previous RNAi reports, we selected 32 potential candidates including housekeeping genes, neuropeptide hormones, and receptors for SWD RNAi target(s). We employed a PCR-based strategy to identify homologous genes in SWD.

Design and construct each dsRNAs (= RNAi material) - Using routine molecular biology skills and software, specific primers sets target genes were designed to amplify partial lengths between 200- 400 nucleotides. Once confirmed the sequence DNA fragments were served as the templates for dsRNA synthesis using a dsRNA synthesis kit. The negative dsRNA control (dsGFP) was also constructed by the same method described above for SWD.

Evaluate RNAi impact on SWD adults by injection: DsRNAs of each target SWD gene and GFP were dissolved in RNase free water and injected into adult flies using a Nanoliter injector. After injection of 20-25 flies per treatment, phenotypic changes including mortality were observed. Once we identified best RNAi target genes, feeding assays were conducted for next step.

Evaluate RNAi impact on SWD adults by feeding: Colony reared SWD adults were collected at two days, starved for 24hr, and then exposed to dsRNA treatments, supplemented with a 10% sucrose solution, by means of a 1.5ml moisture wick. For adult feeding assays, various dsRNA concentrations determined from the injection experiment were mixed in a fly diet. Ten flies (5 males & 5 females) were exposed to the treatment for 3-7 days inside a 50ml tube. After the treatment period, flies were moved to a bioassay cage and provided a moisture wick and a 1oz diet cup. The diet cup was replaced each day for 3 days. Mortality was monitored daily and fecundity was measured by the number of eggs laid on each diet cup.

Evaluate RNAi impact on Drosophila cells with dsRNA application – As a pilot screening test, 10 different dsRNAs targeting housekeeping genes were synthesized and prepared in three different dosages (1 μg, 5 μg and 10 μg). S2 cells (*Drosophila* cells, Schneider 2 cells originated from *D. melanogaster*) were plated in 96-well plates with 20,000 cells per well. One day later the dsRNAs were treated into the wells and then the cell density was observed every day. Cell density was calculated as a percentage compared to the confluent density (100%). After three days, the cells were harvested and total RNA was extracted from each well. The inhibition of target gene expression by dsRNA treatment was measured by quantitative real-time PCR (qRT-PCR).

Results

1. Identification of additional RNAi candidate genes from SWD: We have identified 32 RNAi candidate genes, and constructed DNA templates to synthesize dsRNAs (Table 1).

Table 1. SWD genes for RNAi

RNAi target	Gene family
SWD ID1	Neurohormone
SWD ID2	Neurohormone
SWD ID3	Hormone receptor
SWD ID4	Housekeeping
SWD ID5	Housekeeping
SWD ID6	Housekeeping
SWD ID7	Housekeeping
SWD ID8	Housekeeping
SWD ID9	Housekeeping
SWD ID10	Housekeeping
SWD ID11	Housekeeping
SWD ID12	Neurohormone
SWD ID13	Hormone receptor
SWD ID14	Housekeeping
SWD ID15	Housekeeping
SWD ID16	Hormone receptor
SWD ID17	Hormone receptor
SWD ID18	Hormone receptor
SWD ID19	Hormone receptor
SWD ID20	Hormone receptor
SWD ID21	Hormone receptor
SWD ID22	Hormone receptor
SWD ID23	Hormone receptor
SWD ID24	Housekeeping
SWD ID25	Housekeeping
SWD ID26	Housekeeping
SWD ID27	Housekeeping
SWD ID28	Housekeeping
SWD ID29	Housekeeping
SWD ID30	Housekeeping
SWD ID31	Chemosensory
SWD ID32	Chemosensory
GFP	unrelated gene as a control

Housekeeping genes as constitutive genes are expressed in all cell types at a level that does not fluctuate with the cell cycle. Functional examples of housekeeping genes for RNAi targets_are related in the muscle physiology, detoxification, ATP metabolism, protein sorting and transporting, and cell membrane structure in cells. These genes have been selected for RNAi targets in many insect pests.

2. Microinjection: The system and skill is particularly important to inject a nano-litter volume (50 nL = 0.02 uL) per fly without or a minimum physical damage in the fly. About 90% of SWD adults after injected with a shame or water only were not affected and survived for two weeks monitored. This means the injection skill can be utilized to screen RNAi targets.

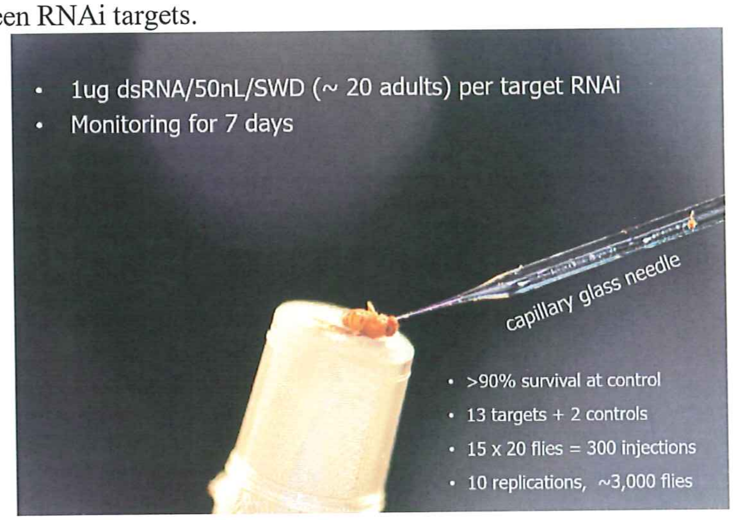

Figure 1. Photo of the nanoinjection with dsRNA into SWD adult. More than 3,000 flies have been injected to screen RNAi targets from 13 SWD genes for past 3 years.

3. Initial screening of 13 potential RNAi targets: 13 RNAi candidates had completed over 3,000 nano-injections to flies with 10 replications. We found effective phenotypic impacts, mainly mortality, from some of the RNAi injection into SWD flies (Fig. 2).

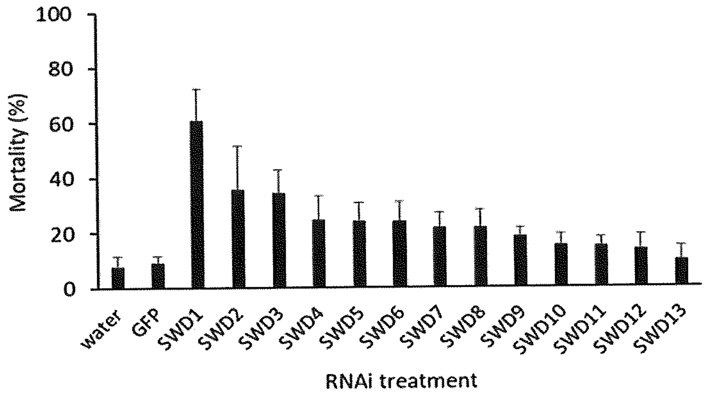

Figure 2. Survival rates of flies injected with dsRNA for 3 days. Each treatment was consisted with 20 flies, and replicated 10 times.

4. Genotypic impact of the housekeeping genes for RNAi targets: Three SWD genes (SWD1, 2, &3) were selected and investigated their gene expression levels to find whether those genes are being suppressed or not after target RNAi (dsRNA) injected into SWD. Using the quantitative gene analysis we found all three RNAi target genes have been knock downed by dsRNA introduction to SWD (Fig. 3).

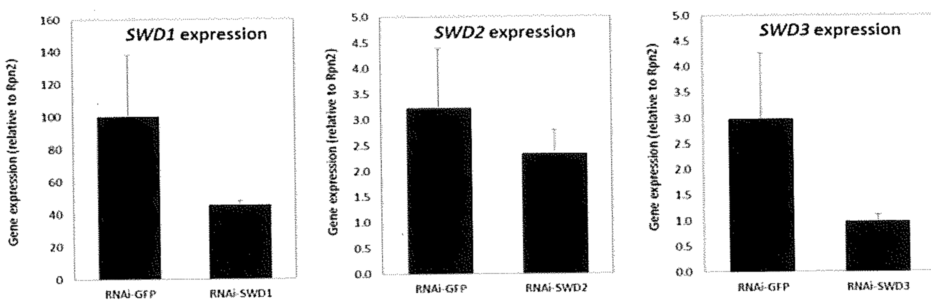

Figure 3. Knock-down of housekeeping genes expression by RNAi. The mRNA expression levels of SWD1, SWD2, and SWD3 were compared between RNAi-GFP and RNAi-target in SWD 12h after dsRNA injection of SWD1, SWD2, SWD3, and GFP.

5. Feeding assay with dsRNA mixed in the diet: Flies fed dsRNA mixed diet, and they were monitored for the RNAi impact on the fly survival rates (Fig .4). The percentages of mortality in flies fed on the diet were not significantly different between the water control and dsRNA treatment for 7 days. Various dsRNA feeding tests with diet or blueberry also showed similar results on the fly survival rates. The female fecundity has been investigated with vitellogenin receptor dsRNA fed by flies, the egg reduction was not significant compare to the control (data not shown). The outcome results indicate SWD dsRNA ingested in the flies could be degraded in the midgut or not pass through the midgut membrane.

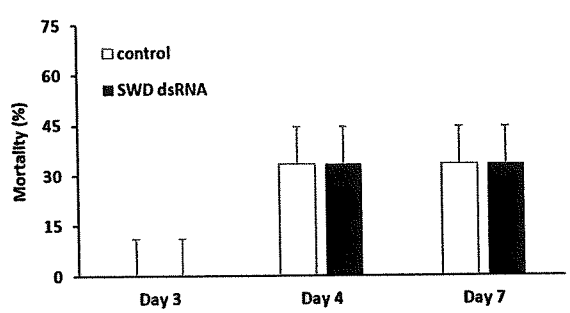

Figure 4. Survival rates of SWD fed on dsRNA mixed in the diet. Mortalities between control and treatment were not significantly different.

6. RNAi impact on *Drosophila* cells with dsRNA in vitro assay: Because the RNAi feeding impacts were not consistent with the injection results. We tried to confirm if SWD RNAi was really to knock-out of the target gene and negative impact in *Drosophila* cells. Among ten SWD RNAi showed significant decreases of cell density (Fig. 5). Their effect on cell growth inhibition was dose-dependent, and resulted in 20% reduction of cell viability (Fig. 5). This result confirmed that the expression of SWD target genes were suppressed by dsRNA. With different dosages, the lowest dosage (1 μg) was significantly reduce expression of all target genes (Fig. 6).

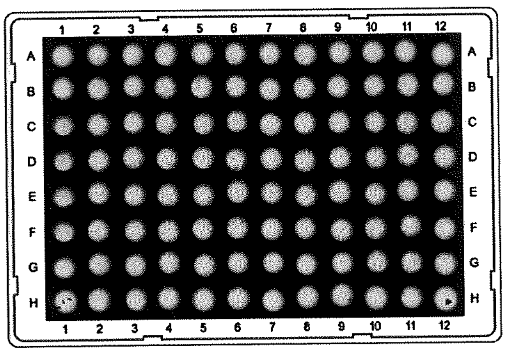

Figure 5. SWD dsRNAs were assayed in the S2 cell line (derived from Drosophila melanogaster). When the 96-well plates were about 70% covered with cells, an aliquot of dsRNAs (1, 5, or10 μg) were mixed in the cell medium. Cell viability and numbers (= coverage) and target gene expression levels.

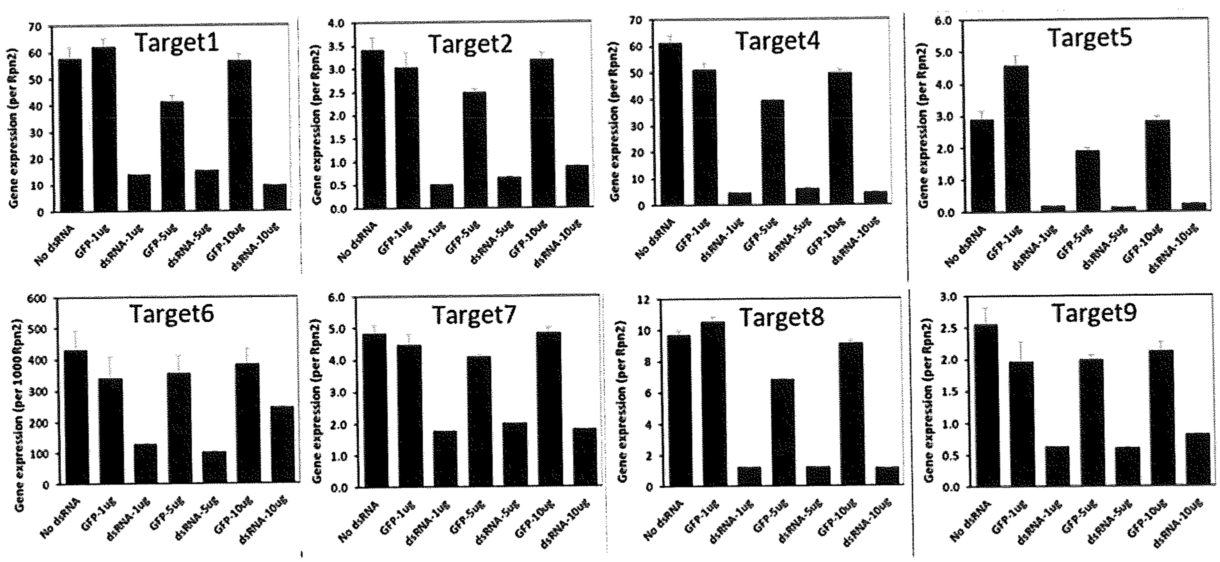

Figure 6. Suppression of target gene expression by in vitro RNAi using quantitative PCR (qPCR). *Drosophila* cells harvested day 3 after RNAi treatment were extracted for total RNAs.

7. DsRNA degradation enzymes in the Mid-gut: Oral administration (ingestion) of dsRNA would be more feasible; however, the target dsRNA must survive in the midgut and pass into the hemolymph where it can then act on the target gene. Low efficacy of RNAi impact by orally delivery could be attributed to extracellular degradation of the dsRNA in the gut lumen. In order to overcome any possible obstacle in the RNAi application to SWD, it is necessary to look into the dsRNA degrading activity in SWD digestive system. Alimentary tract of *Drosophila suzukii* is consisted with foregut, midgut, and hindgut (Fig. 7). Crop is a storage organ in the foregut section present only in adults.

We dissected the fly digestive tissues, and investigated potential enzyme(s), RNaseIII type enzyme which is to degrade dsRNA only. Surprisingly, we found activity of dsRNA degradation in the midgut, not other digestive organs. The putative dsRNA degrading activity was compared between midgut and crop of the SWD adult using their crude homogenates. When incubated with midgut homogenate (10 gut equivalent) at 37 °C for 30 min, the dsRNA was degraded, crop homogenate showed lower enzyme activity than midgut homogenate. We also found two potential dsRNases genes from our SWD midgut transcriptome (RNA-seq) data (Fig. 8). The identification and characterization of target genes are under this project, because this is a critical to develop RNAi-based application for SWD. Potential strategy will be approached to two methods: 1) deactivation of the enzyme activity or 2) novel formulation of dsRNA to protect from the enzyme activity in the midgut.

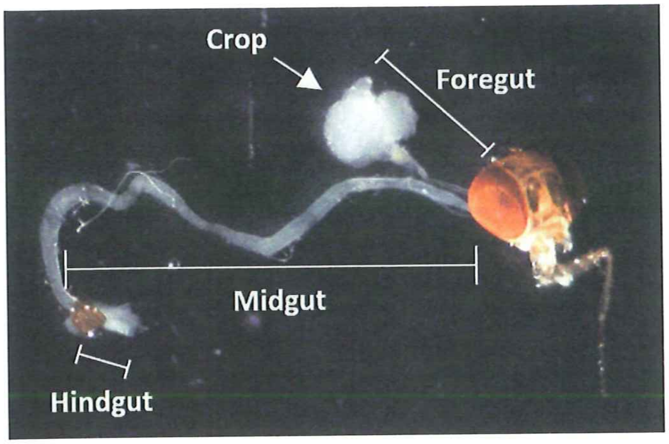

Figure 7. Alimentary tract of *Drosophila suzukii* is consisted with foregut, midgut, and hindgut. Crop is a storage organ in the foregut section present only in adults. The gut homogenate used in this study was prepared from midgut.

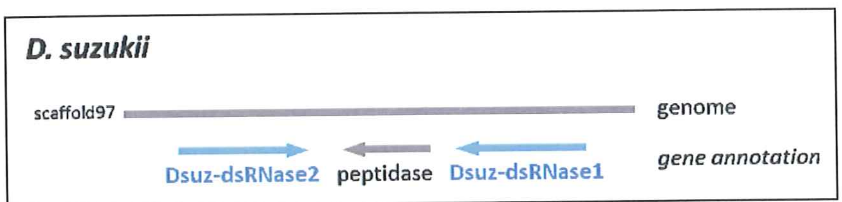

Figure 8. Two dsRNase genes, Dsuz-dsRNase1 and Dsuz-dsRNase2, found in the SWD genomic scaffold.

Conclusion and ongoing study for SWD RNAi

From the research project we identified potential RNAi targets for SWD through nanoinjection into SWD, and confirmed the RNAi impact on *Drosophila* cells from in vitro assay. However, oral administration for SWD dsRNA is limited to delivery into target cells due to a partial degradation of the dsRNA in the fly midgut. Therefore, we need to deactivate or block the enzyme genes to protect dsRNA in the midgut, that will be increase the efficacy of RNAi impact on the SWD.

Although RNAi technology is a promising tool for insect pest management, there are still technical challenges to successfully develop a next generation pesticide. Three major challenges are: 1) identifying a suitable target gene and/or physiological system; 2) developing suitable RNAi delivery into the target pest; and 3) providing cost-effective dsRNA production. We have established a bacterial-based system produced a large quantity of dsRNA for the cost-effective dsRNA production, and developed non-toxic sugar as a phagostimulant to enhance RNAi delivery into SWD.

Publications related in this project:

1. S.-J. Ahn, K. Donahue, Y. H. Koh, R. Martin, M.-Y. Choi. 2018. Microbial-based dsRNA production to develop cost-effective RNAi application for insect pest management. J. Appl. Entomol. (*submitted*).
2. M.-Y. Choi, H. Lucas, R. Sagili, D. H. Cha, J. c. Lee. 2018. Effect of erythritol on Drosophila suzukii in the presence of naturally-occurring sugar sources, and on the survival of *Apis mellifera*. J. Econ. Entomology (*in press*).
3. S. B. Tang, Lee, J. C., Jung, J. K., and Choi, M. - Y., 2017. Effect of erythritol formulation on the mortality, fecundity and physiological excretion in *Drosophila suzukii*. J Insect Physiol. 101: 178-184.

Report to the Agricultural Research Foundation
for the Oregon Raspberry and Blackberry Commission

Title: Caneberry Pesticide Registration, Tracking, and New Chemistries

Principal Investigator: Joe DeFrancesco
Oregon State University
North Willamette Research and Extension Center

Funding Period: 2017-2018

Progress:

I. We continue to keep track of pesticide issues affecting the Oregon caneberry industry. Each week, I monitor the published US Federal Register, which is the official venue for notices and actions relating to pesticide registrations at EPA, and follow-up on any issues that may affect the Oregon caneberry industry. Some new US-registered caneberry pesticides are quick to obtain an MRL in foreign markets, while others are slower and still in progress. I continue to work with the USDA-Foreign Agricultural Service and pesticide registrants to get tolerances (MRLs) established for caneberries in foreign markets.

II. The Pesticide Registration Update Chart that I develop for caneberry growers and field representatives is updated about three times a year. Growers and other industry representatives indicate this list is widely used as a reference for pest management decisions. I also develop and distribute a list of MRLs (maximum residue levels) for caneberries in the US, Canada, Japan, the EU/UK, Korea, Taiwan, and Codex (international). This helps growers and processor/packers develop a pest management spray regime based on the anticipated destination of their fruit.

III. We communicate with representatives of the caneberry industry and continue to identify and prioritize pest management gaps and needs, which may be created by the loss of currently registered pesticides. The ORBC is kept updated on important pesticide issues via grower meetings, ORBC meetings, newsletters, or personal communication

IV. ***New Pesticide Registrations - 2018:***
The residue and efficacy data we generated and submitted to EPA for review allowed the registration of the following products in caneberries:

(1) Quinstar 4L (quinclorac). A post-emergence herbicide from Albaugh that controls broadleaf weeds and some annual grass weeds. Quinstar is especially effective on field bindweed, morningglory, dandelion, sowthistle, and Canada thistle.

(2) Fusilade (fluazifop-butyl). Fusilade had been registered for many year for use in non-bearing caneberry fields. This new registration allows use in bearing fields and the label now has a 1-day PHI.

(3) Exirel (cyantraniliprole). Exirel controls many different insect pests, including SWD, adult root weevils, and leafrollers. Like its sister compound, chlorantraniliprole (Altacor), it is in IRAC #28 but is more effective and has a wider spectrum of activity. Exirel has contact activity but is most effective through ingestion of treated plant material, so good coverage is important. FMC has not yet issued a label that includes caneberries but hopes to do so by the start of the 2019 field season.

V. *Impacts and Benefits of this Project:*

The registration of safe and effective pest management solutions helps growers produce a high quality crop, remain economically viable, and enables them to be competitive in the national and international marketplace. Providing growers and the caneberry industry with current information about pest management and pesticide issues helps them be up-to-date and better informed as they make important pest management and marketing decisions that affect their operation. In addition, the registration of new chemistries, with unique modes of action, helps reduce the likelihood of the development of resistance and increase the chances of successful pest management.

PROGRESS REPORT TO OREGON RASPBERRY AND BLACKBERRY COMMISSION 2018

TITLE: Development of New Raspberry Cultivars for the Pacific Northwest

PROJECT LEADER: Patrick P. Moore, Professor
Wendy Hoashi-Erhardt, Scientific Assistant
WSU Puyallup Research and Extension Center

PROJECT STATUS: Continuing (indefinite)

FUNDING: USDA/ARS Northwest Center for Small Fruits Research
Amount Awarded $32,299 for 2018-2019 for both raspberry and strawberry breeding

Washington Red Raspberry Commission
Amount Awarded $70,000 for 2018 "Development of New Raspberry Cultivars for the Pacific Northwest"

OBJECTIVES:
Develop summer fruiting red raspberry cultivars with improved yields and fruit quality, and resistance to root rot and raspberry bushy dwarf virus. Selections adapted to machine harvesting or fresh marketing will be identified and tested further.

Release. WSU 2166 was recommended for release by the Cultivar Release Committee October 13, 2017, and a patent application has been filed. Plants should be available in 2019. WSU 2166 is an early season selection with large, firm, good flavored fruit that machine harvests very easily. It is not immune to root rot, but appears to have good levels of tolerance. WSU 2188 will be evaluated again in 2019 and if performance warrants, may be recommended for release.

Crosses/selections. Seventy-nine crosses were made in 2018 for floricane breeding with emphasis on parents that are machine harvestable and root rot resistant. Seventy-five of the 79 crosses had at least one parent that has root rot resistance in its background. All of the crosses had at least one parent with good machine harvestability. Thirty-one selections were made in 2018 from seedlings planted in 2016. Twenty-eight of the 31 selections had at least one parent that has root rot resistance in its background.

Machine Harvesting Trials. A new machine harvesting trial was planted in 2018 in Lynden with 35 WSU selections, 12 ORUS selections and 'Cascade Harvest', 'Meeker' and 'Willamette' for reference. This planting will be harvested in 2020 and 2021. The 2015 and 2016 planted machine harvesting trials were harvested in 2018 and subjectively evaluated.

Grower trials
Four WSU selections were planted in Grower Trials in 2014. All of these selections appeared very promising in small plots in previous Machine Harvesting Trials in grower fields in the Lynden area. In the Grower Trials, one grower field has a history of very high levels of root rot and WSU 1980 and WSU 2122 did not perform well on this site. WSU 2188 had some root rot damage in 2016 but appeared healthy and vigorous in 2018. WSU 2166 did not show any

damage in 2014-16, slight damage in 2017 and no damage in 2018. Three selections (WSU 1914, WSU 2010, WSU 2162) were planted in Grower Trials in 2017. These selections will be harvested in 2019 and 2020. WSU 1914 is a sib of WSU 1912 and is very root rot tolerant, but may not have enough yield. WSU 2010 has root rot resistant parents and dark fruit with good yields in the second harvest season. WSU 2162 appears to be susceptible to root rot, but will continue to be evaluated. Three additional selections (WSU 1962, WSU 2068 and WSU 2069) were planted in Grower Trials in 2018.

Selection Trial Puyallup. The 2015 and 2016 replicated plantings at Puyallup were hand harvested in 2018. In the 2015 selection trial, WSU 2001 and WSU 2088 had the highest yield in 2017 and Cascade Harvest and WSU 2088 had the highest yield in 2018. WSU 2088, WSU 2001 and 'Cascade Harvest' the highest two year total yield (**Table 1**). In the 2016 selection trial, WSU 2087, WSU 2130 and WSU 2088 had the highest yields (**Table 2**). However, problems with the irrigation system in 2018 may have resulted in reduced yields.

Table 1 2017/18 harvest of 2015 planting, Puyallup, WA.

	Yield (t/a)		Fruit rot (%)		Fruit weight (g)		Fruit firmness (g)		Midpoint of Harvest	
	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017
WSU 2088	4.26 ab	9.12 ab	23.5% ab	4.3% b	3.43 ab	3.36 a	365 a	181 a	7/11 a	7/16
WSU 2001	2.73 c	9.96 a	30.3% b	7.9% b	3.52 a	3.52 a	275 b	132 b	7/11 a	7/16
C Harvest	5.12 a	7.19 bc	18.3% ab	14.6% ab	3.66 a	3.69 a	238 bc	108 bc	7/3 b	7/11
Meeker	4.06 a-c	7.11 bc	18.2% ab	7.2% b	2.89 bc	2.89 b	232 bc	86 cd	7/8 a	7/9
WSU 2133	3.58 bc	7.26 bc	11.3% b	5.7% b	2.64 c	2.27 c	207 c	73 d	7/3 b	7/12
WSU 2299	3.58 bc	7.14 bc	16.6% ab	9.7% ab	2.70 c	2.38 c	154 d	60 d	6/29 b	7/9
Willamette	3.49 bc	5.65 c	16.3% b	7.9% b	3.22 a-c	3.33 ab	227 c	112 bc	7/1 b	7/6
	3.83	7.63	19.2%	8.2%	3.15	3.05	243	107	7/5	7/11

Table 2018 harvest 2016 planting

	Yield (t/a)		Fruit rot (%)		Fruit weight (g)		Fruit firmness (g)		Midpoint of harvest	
WSU 2087	4.83	a	25%	ab	3.00	ab	315	a	7/1	bc
WSU 2130	4.55	ab	14%	c	2.73	ab	247	b-d	6/27	d
WSU 2088	4.02	a-c	22%	a-c	2.77	ab	295	ab	7/4	ab
C.Harvest	3.38	a-d	18%	a-c	3.51	a	234	c-e	6/30	cd
Willamette	2.37	a-d	14%	c	2.44	ab	192	e	6/27	d
WSU 2191	2.31	a-d	14%	c	2.07	b	212	de	6/29	cd
WSU 2162	2.01	b-d	18%	a-c	2.56	ab	185	e	7/5	a
Meeker*	1.79		30%		2.52		171		6/29	
WSU 1962	1.71	cd	17%	bc	2.92	ab	208	de	7/4	ab
WSU 2195	1.05	d	27%	a	2.95	ab	271	a-c	7/7	a
	2.80		20%		2.75		233		7/2	

* only two replications of Meeker harvested in 2018

Publications/Presentations

North Willamette Horticultural Society, Canby, OR. January 11, 2018
Strawberry and Raspberry Cultivar Development at Washington State University. LMHIA, Abbotsford, BC. January 25, 2018
Machine Harvesting Field Day Lynden, WA July 12, 2018

Summary

This project will develop new raspberry cultivars using conventional breeding methods. Controlled pollinations will be made, seedlings grown, selections made among the seedlings and these selections evaluated. The primary goal of the program is to develop new summer fruiting red raspberry cultivars with improved yields and fruit quality, and resistance to root rot. Selections adapted to machine harvesting or fresh marketing will be identified and tested further. The most promising selections will be tested in grower trials and evaluated for possible release.

Several raspberry selections tested in machine harvesting trials appear very promising: machine harvesting well, productive, with good fruit integrity, good flavor and some with probable root rot tolerance. WSU 2166 was recommended for release by the Cultivar Release Committee and a patent application has been filed. Plants should be available in 2019.

The Northwest Berry Foundation
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www.nwberryfoundation.org

Small Fruit Update Progress Report

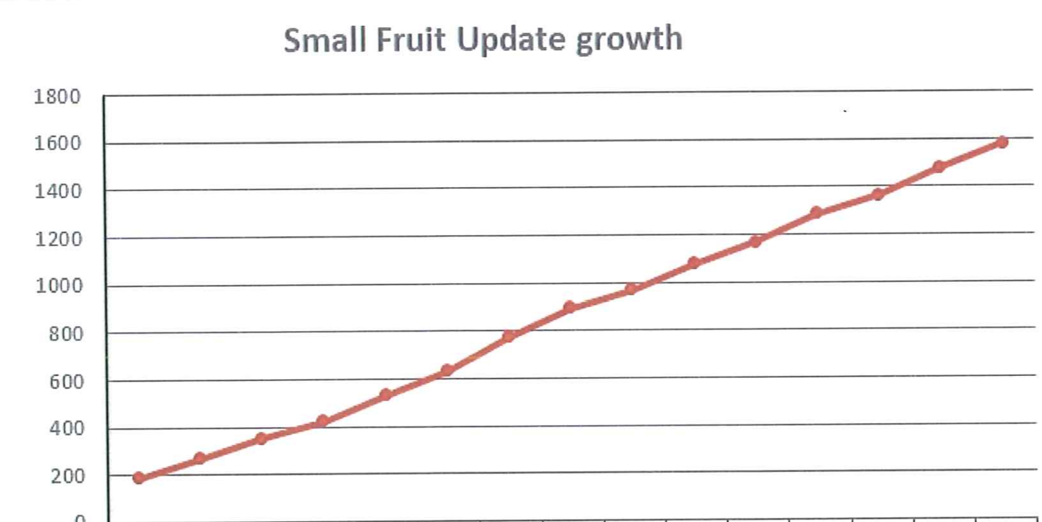
As of December 2018

Objectives:

- Increase industry communication.
- Increase grower knowledge of IPM strategies.
- Accelerate the dissemination of pesticide information. such as label changes to growers.
- Facilitate real time pest alerts to growers throughout the growing season.
- Inform industry personnel of upcoming meetings as well as other relevant commission news such as elections, seat vacancies and/or legislative activities.

Overview

Peerbolt Crop Management has been providing a weekly emailed Small Fruit Update (SFU) to an increasing number of growers, industry personnel, and researchers since February 2000. Three years ago, the SFU was taken over by Northwest Berry Foundation. At the time of this report, the email list grew by 100 addresses (from 1480 addresses in 2017, to 1580 addresses in 2018). As several recipients regularly pass it on to others, we estimate the total number receiving the Update to be well over 2,000 people.

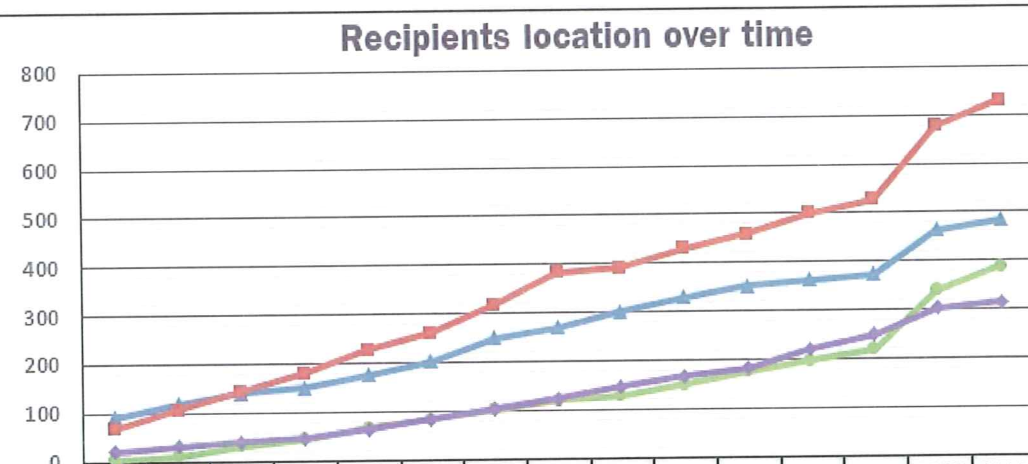


	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Subscribers	186	269	353	422	532	631	772	896	969	1076	1169	1286	1365	1480	1580

2018 Profile of the Small Fruit Update

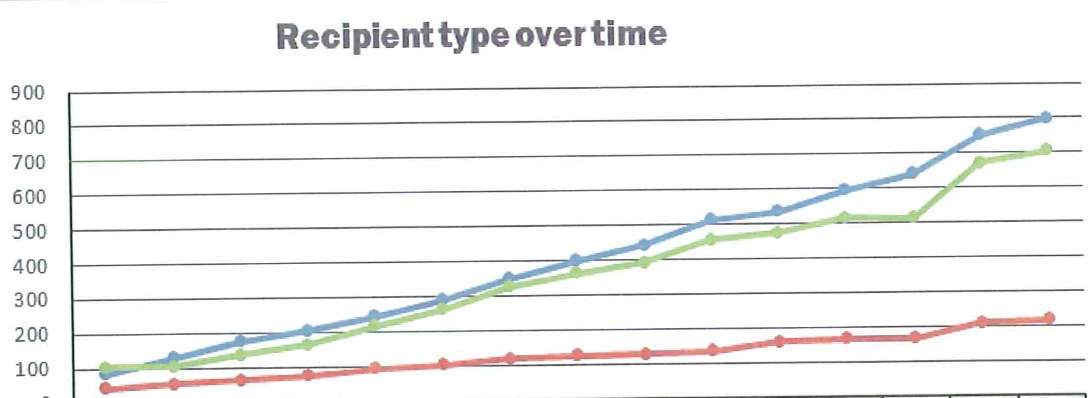
The following charts illustrate the profile of the Small Fruit Update recipients in our database as of the date of this report.

We make every effort to provide you with accurate information. We don't mandate those who sign up for the Update to give us any information beyond their email address, name, address, and phone number. We also request that growers note what crops they grow. Sometimes they do, and sometimes they do not. This means that our annual demographic reports often change previous report's numbers. Also note that each year we lose a certain number of recipients. Some drop out because of a job change, but there are always a few dropped simply because their email address no longer works, and we are unable to rectify the situation after attempting to contact them. However, you can see that even with these individuals dropped, the overall trend for the SFU is an *increase* in recipients across all locations.



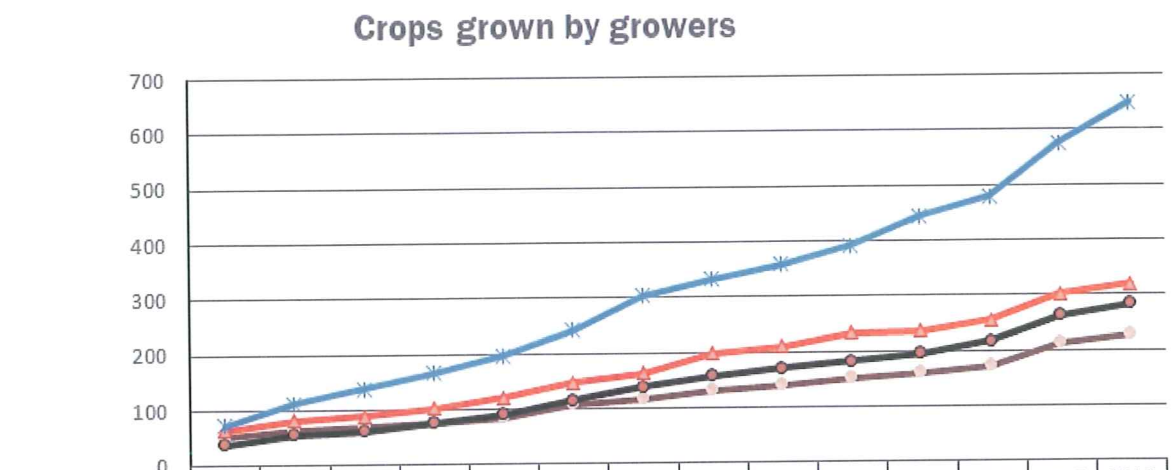
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Washington	91	119	140	150	175	203	249	269	301	329	352	363	373	463	484
Oregon	69	106	143	180	227	261	318	383	392	430	458	501	528	678	730
Out of Area	4	12	31	45	67	84	101	121	129	150	176	197	219	339	388
BC	22	32	39	47	63	83	104	123	147	167	182	220	250	302	314

Since the beginning of the year, there has been a subscriber increase of 12 recipients in BC, 52 in Oregon and 21 in Washington. The remaining recipients are located throughout the U.S., Canada, and the rest of the world. That segment increased by 49 subscribers. We screen new subscribers from potentially competitive markets and only add them after staff discussion.



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Growers	83	130	175	205	244	289	349	399	443	513	536	596	641	751	799
Researchers	42	53	63	74	92	103	118	125	127	134	158	166	165	210	217
Industy	104	104	135	163	217	262	325	362	391	455	474	516	513	671	705

The "Growers" category increased by 6% -- individual subscribers going from 751 in 2017 to 799 in 2018.
The "Researchers" category includes anyone associated with USDA, ARS, a college, or university, as well as state or federal departments of agriculture, and others who work for public agencies. Over the past year, researchers receiving the Small Fruit Update increased by 7 individuals. The category "Industry" includes suppliers, newspaper reporters, propagators, processors, nurseries, fruit buyers, manufacturers, sales reps, and even bankers. This year the number of industry recipients increased by 34 individuals.



	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Strawberry	52	64	70	75	85	108	117	131	141	152	161	172	213	228
Raspberry	65	82	90	103	121	147	163	198	210	233	237	255	302	320
Blackberry	39	56	62	76	91	116	139	158	171	183	196	217	262	283
Blueberry	73	113	139	167	197	242	303	333	358	392	444	480	576	648

Our signup form encourages those wanting the Update to give us demographic information. The crop data reflects the fact that some growers do not indicate what crop they grow, and many growers are harvesting more than one small fruit.

In general, the trend over the past 10 years is that strawberry. Blackberry, and raspberry recipients have grown at near parallel rates. Blueberry producers have been rising steadily. In 2018, the number of recipients identifying themselves as strawberry growers increased by 15, raspberry growers increased by 18, and blackberry growers increased by 21. Blueberry growers increased by 72.

As noted at the start of this report the Small Fruit Update continues to expand its recipient list and the quality and quantity of the information provided. In 2004 our list comprised the addresses of 186 individuals. We have added 1,394 addresses since that time. All this is due to your continued support.

Progress Report to the Oregon Raspberry & Blackberry Commission

November 30, 2018

Project Title: Coordinated Regional on-farm Trials of Advanced Blackberry & Raspberry Selections

Principal Investigator:
Thomas Peerbolt –Executive Director, Northwest Berry Foundation
--Senior Consultant, Peerbolt Crop Management
Co PIs
Chad E. Finn – USDA-ARS-HCRU, Corvallis, OR
Patrick Moore – Washington State University, Puyallup, WA

Justification

The Northwest blackberry and raspberry breeding programs have been a cornerstone of the industry's success. Their ability to produce cultivars of commercial value is crucial to continued success. Global competition is increasing and public funding for these programs at our land grant institutions is under increasing budget constraints. Accelerating the commercialization of the cultivars produced by these programs is of great economic value to the northwest caneberry industry.

Previous objectives (2013-2018) now completed

First funded in 2013 it has taken these five growing seasons to complete the establishment phase and evaluation stages of this pilot project. There is now in place a viable onfarm testing program for advanced caneberry selections. The elements that are now in place:

- Communication links and agreed upon timelines with the three Northwest public caneberry breeding programs (USDA/ARS & OSU; WSU; and the British Columbia Program) for deciding which advanced selections should be included in the trials each year.
- A set of protocols with the wholesale commercial propagators (North American Plants, Northwest Plants and Sakuma/NorCal) to be able to supply viable, quality plant material to the growers at the appropriate time and in the needed quantities.
- Informal protocols for spreading out regional costs between the three Northwest industry commissions/councils (ORBC, the Washington Red Raspberry Commission & the B.C. Raspberry Development Council) for onfarm trials throughout the western Oregon, Washington and British Columbia.
- A network of cooperating growers.
- Realistic cost estimates for viable budget projections and fiscal planning.
- An information dissemination network that includes use of the Small Fruit Update newsletter, grower meeting presentations, one-to-one grower communication and production of information factsheets.

Previous objectives (2013-2018) needing further investment

- Onfarm trial site evaluation protocols.
- The format and forms needed for site visits has been developed.
- Not enough resources have been allocated during the season. That will be addressed in the 2019 proposal by increasing the time committed to the site visits.

Yearly Calendar of On-Farm Caneberry Trials

Mid-November: Propagator and wholesale nursery meeting.

- Decide on selections for following season in collaboration with plant breeders & nurseries.
- Edit list of promising candidate selections for trials 2-3 years in the future.
- Coordinate with wholesale nurseries to decide on plant source and date needed to deliver on farms.

December- March: Winter meetings, production of factsheets, submit reports and funding proposals, web postings.

- Disseminate information to stakeholders through newsletters, meeting presentations, factsheets and websites.
- Coordinate with on farm trials in Washington and British Columbia.
- Collect stakeholder feedback on selections, independent selection trials and commercially planted cultivars.
- Recruit grower cooperators for the coming season.

April-May: Getting new trials planted. First check on ongoing trials.

- Coordinate deliveries with propagators and growers.
- Expedite memorandums of understanding paperwork for growers.
- Evaluate trials in the ground for winter damage, cane vigor, bud break, and any other pest symptoms that might be visible in the early season. (Could be either site visit or a phone interview with grower.)

June-August: Harvest Season

- Site visits during harvest to evaluate: Fruit quality; yield potential; machine harvestability; fruit disease susceptibility…
- Second site visit during third to fourth week of harvest to evaluate: late season fruit quality; revise yield potential; machine harvestability; length of harvest; disease harvestability, etc.
- Visit trials in Washington and British Columbia at least once during the season.

August-October: Post harvest

- Phone interviews with growers for comments on train-ability, pruning methods, etc.
- Determine which plantings should be removed and/or continued.

Compilation Blackberry and Black Raspberry Selections/Cultivars included in ORBC-funded onfarm trials to date

Blackberries

- **ORUS 2635-1 (Erect-thorny)** Still being evaluated. Primarily to see if upright growth habit could make it more economic to prune and tie. Most likely not acceptable for main processing uses.
- **ORUS 3172-1** Ripens two weeks later than Marion. Discarded. Fruit is too soft for machine harvesting.
- **ORUS 3447-1 (Columbia Star)** Excellent fruit quality. Planted acreage quickly increasing.
- **ORUS 3447-2 (Columbia Giant)** Very large fruit. Is quickly finding a fresh market niche. Possible that we could trial it in large trials to see if it could be useful for processing uses.
- **ORUS 3448-2 (Columbia Sunrise)** Ripens 10 days before Black Diamond. Fruit size is a bit smaller than Marion. Has excellent potential early season harvest window. Overall yield potential is still being evaluated.
- **ORUS 3453-2 (Hall's Beauty)** Good fruit size and yield. Timing is similar to Marion. Not sure of niche in the processed market yet. Still being evaluated.
- **ORUS 2707-1 (Marion timing)** Discarded. Fruit too soft for machine harvesting.

- **ORUS 1324-1 (Newberry)** Niche market potential. Thorniness, fruit color and fruit flavor profile make it unacceptable for present processing uses.
- **ORUS 1939-4 (Thorny-Fresh Market)** Has good fresh market potential uses in California and elsewhere. Could provide royalty income to support breeding program without directly competing with our major markets.
- **ORUS 1793-1 (Thorny-Fresh Market)** Potential Advantage for Oregon industry: Has good fresh market potential uses in California and elsewhere. Could provide royalty income to support breeding program without directly competing with our major markets.
- **ORUS 2816-4 (Thornless-Fresh Market)** Very late-three weeks after Marion. Fresh market potential.
- **ORUS 2635-1 (Thorny-High yielding)** While thorny it still produces 50-70% more than Marion. Good for fresh. Might be worth trialing for processing even though thorny.

Black Raspberries

- **ORUS 3735-3** Potential for larger fruit and higher yields than Munger. Plants used for trials had crumbly fruit—likely propagation problem. Will replant in 2017 with new planting material.
- **ORUS 3013-1** In some early trials had double the yields of Munger. Will get new fruit this season.
- **ORUS 3217-1** In some early trials had double the yields of Munger. Will get new fruit this season.
- **ORUS 3409-1** May have verticillium resistance. Probable fresh market niche. Fruits on primocanes and floricanes.

Oregon Raspberry and Blackberry Commission

Progress Report for 2017 Project entitled:

Fungicide Resistance Profiles of *Botrytis* Isolates Collected from Raspberry and Blackberry in Oregon.

Principle Investigator: Virginia Stockwell, USDA-ARS Horticultural Crops Research Unit, Corvallis, Oregon

Objective: The objective of this project was to assess the sensitivity of *Botrytis* isolates from caneberries in Oregon in 2014 and 2015 to fungicides.

Project duration: 2/1/2017 to 1/31/2018

Accomplishments:
The research project provides important information about the fungicide resistance profiles of isolates of *Botrytis* from Oregon caneberries during the 2014, 2015, and 2016 growing seasons.

We documented that boscalid (FRAC 7) resistance was most common among isolates of *Botrytis* with resistance to the tested fungicides.

We documented multi-fungicide resistance (e.g. boscalid and cyprodinil [FRAC 9] or boscalid, cyprodinil and fenhexamid [FRAC 17]) in some of the isolates from each year.

The fungicide resistance profiles of *Botrytis* on Oregon caneberries may provide useful information to growers in the selection of chemical control programs for management of gray mold.

Materials and Methods:
Isolate sources: Dr. Lisa Jones, a former postdoctoral scholar at Oregon State University, amassed a collection of *Botrytis* isolates from caneberries in Oregon in 2014 and 2015. Lisa gave this collection to our laboratory for curation when she left OSU. In 2016, we sampled one raspberry field and eight blackberry fields in Oregon and isolated *Botrytis* from asymptomatic fruits.

We tested *Botrytis* isolates collected from caneberries in 2014, 2015, and 2016 for sensitivity to boscalid, cyprodinil, fenhexamid, fludioxonil, and iprodione. We tested 43 *Botrytis* isolates from six locations in 2014; 167 *Botrytis* isolates from 36 locations in 2015; and 168 *Botrytis* isolates from nine locations in 2016.

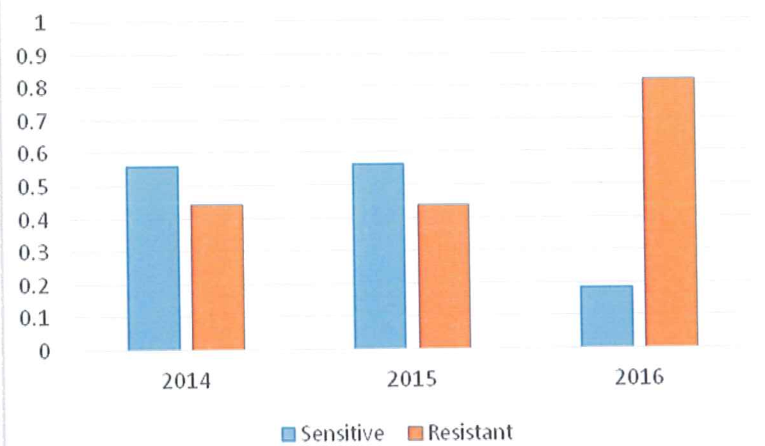
A standard radial growth assay on culture media with and without fungicides was used to measure resistance to each fungicide (boscalid, cyprodinil, fenhexamid, fludioxonil, or iprodione). Botrytis isolates were retrieved out of storage and grown on potato dextrose agar (PDA). Uniform-sized agar plugs with actively-growing *Botrytis* were transferred onto fresh media and media amended with a specific discriminatory dose of a fungicide (a discriminatory dose permits resistant isolates to grow and inhibits growth of sensitive isolates.

The inoculated media in petri dishes were incubated in sealed containers at room temperature. The radius of growth of each isolate on control media and media amended with a fungicide was measured daily for at least four days. The ratio of growth of an isolate on media without fungicides to growth on a medium with fungicides was calculated. Isolates whose growth on media with fungicides was similar to growth on control media without fungicides was scored as resistant. Isolates that do not grow or grew poorly (less than 50% of the growth of the control) on a medium containing a fungicide was scored as sensitive to the fungicide.

Results:
As shown in Figure 1, each year, between 40 to 80% of isolates of *Botrytis* were resistant to at least one fungicide.

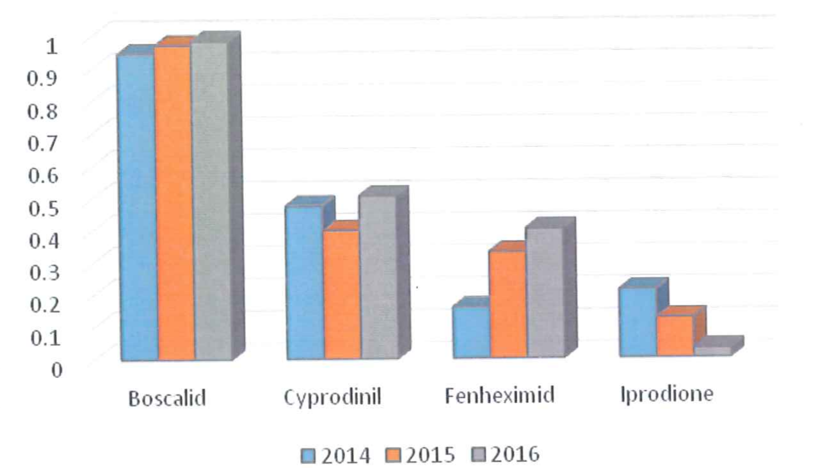
We speculate that the large increase in percent of isolates with fungicide resistance in 2016 compared to 2014 and 2015 reflects the fields that were sampled. In 2014 and 2015, Dr. Jones sampled a mixture of fields that were not treated with fungicides and also some fields that were conventionally managed. In 2016, the *Botrytis* samples tested for fungicide resistance were obtained from fruit from conventionally managed caneberry fields.

Figure 1. Proportion of *Botrytis* isolates sensitive or resistant to tested fungicides



Considering only isolates that were resistant to at least one fungicide, we examined which type of fungicide resistance was most common. Figure 2, illustrates that boscalid (FRAC 7) resistance was most common. Each year, over 95% of the fungicide-resistant isolates of *Botrytis* were resistant to boscalid. Tolerance to cyprodinil (FRAC 9) varied between 40 to 50% of the isolates from 2014 to 2016. The percent of isolates with resistance to fenhexamid (FRAC 17) increased from 15% in 2014 to 40% in 2016. We speculate that the increase in the number of isolates with resistance to fenhexamid merely reflects sample size or the selection of fields sampled in a particular year. Iprodione (FRAC 2) resistance decreased from 20% of the

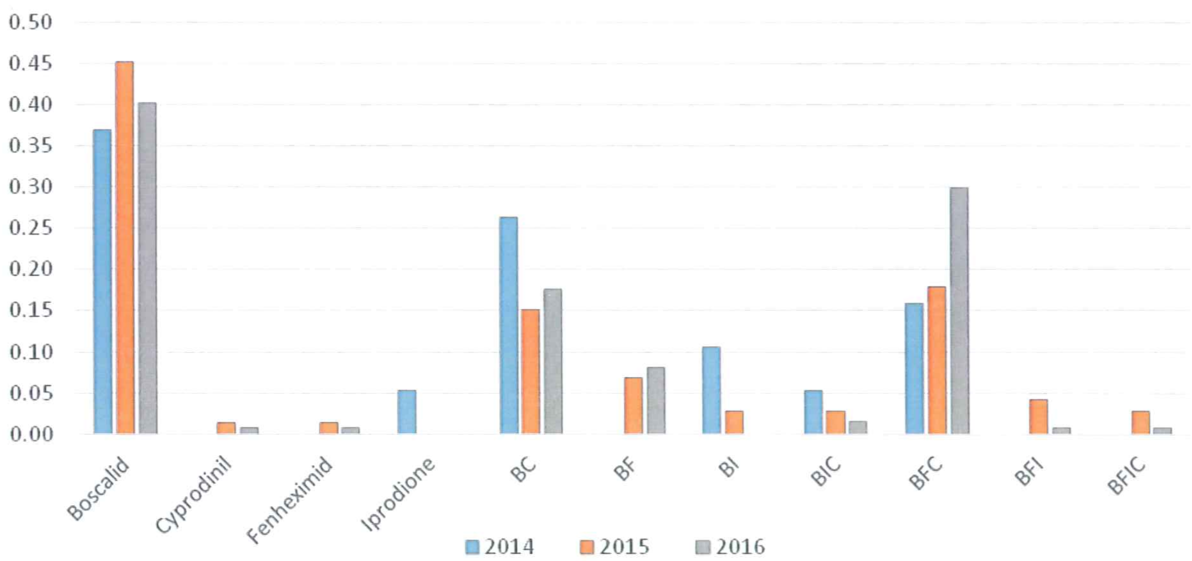
Figure 2. Proportion of fungicide-resistant isolate of *Botrytis* with resistance to various fungicides



isolates in 2014 to 3% in 2016. Finally, resistance to fludioxonil (FRAC 12) was not detected in the Botrytis isolates from 2014 to 2016.

Single isolates of Botrytis with resistance to more than one fungicide (also called multi-fungicide resistance) were detected each year. Figure 3 (below) provides the incidence of isolates resistant to a single fungicide each year (left-most four bar groups) and also the incidence of isolates resistant to two or more fungicides (bar groups labeled by first letter of the fungicides to which the isolates are resistant). Given the high incidence of boscalid resistance, it is not surprising that the multi-fungicide resistant isolates were resistant to boscalid. Tolerance of cyprodinil and boscalid (BC) was detected each year, as well as tolerance of cyprodinil, fenhexamid, and boscalid (BFC). The emergence of multi-fungicide resistance in *Botrytis* may limit the array of effective fungicides for gray mold control if these isolates persist. These isolates may be difficult to control, especially in years with weather conditions that are conducive for the disease during bloom and near or during harvest.

Figure 3. Proportion of fungicide-resistant isolates of *Botrytis* with resistance to only a single fungicide or to multiple fungicides



<u>TITLE</u>: Evaluating the Effects of Extracts from Oregon Blackberries & Black Raspberries to Kill Relevant Strains of *Helicobacter Pylori*

<u>Principal Investigator:</u> Gary Stoner, Montana State University,
Department of Immunology & Infectious Disease, Bozeman, MT

<u>Cooperators</u>: Candace Goodman, Montana State University
Department of Chemistry & Biochemistry, Bozeman MT

Diane Bimczok, Montana State University
Department of Immunology & Infectious Disease, Bozeman MT

<u>Objectives:</u>

1) Evaluate the effects of harvesting region and storage conditions on the phytochemical (anthocyanin and ellagic acid) composition of blackberries and black raspberries and develop berry extracts that are well standardized.

2) Determine the ability of standardized extracts of Oregon blackberries and black raspberries to kill relevant strains of *H. pylori.* Given the causative relationship between *H. pylori* infection and gastric cancer, proving black raspberry and/or blackberry extracts as effective antimicrobial agents against *H. pylori* is the first step in demonstrating the ability of black raspberries and/or blackberries to treat and prevent gastric cancer.

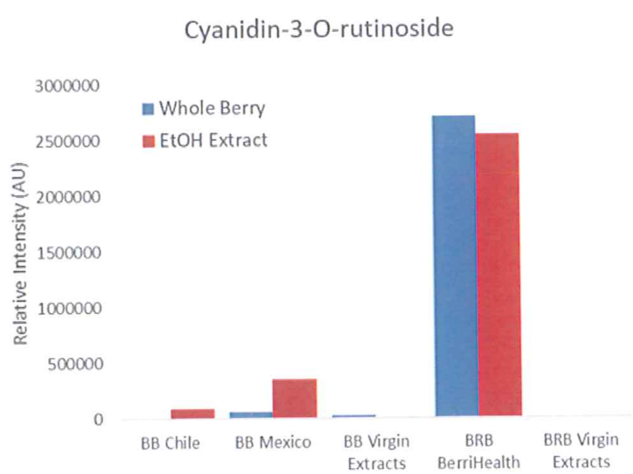
<u>Progress:</u>

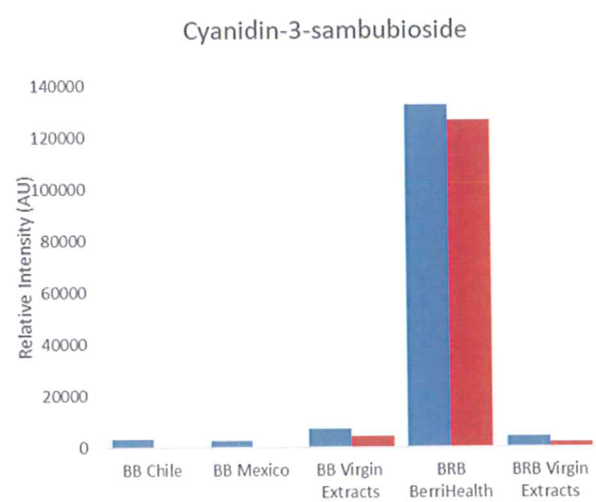
Specific Aim 1. Eight berry samples (2 black raspberry, BRB, and 3 blackberry, BB) were either purchased as a freeze-dried powder or processed into a freeze-dried powder from frozen whole berries purchased at a local food market (these will be referred to as whole berry, WB). Nonpolar compounds were extracted using 3 x 100 mL hexane per 10 g powder. Suspensions were filtered between each extraction, and the hexane filtrate discarded. Anthocyanins and all water-soluble compounds were extracted using ethanol:water (3 x 100 mL 80:20 ethanol:water per 10g berry). This extract was dried to a syrup under reduced pressure at 30°C then lyophilized (referred to as ethanol extract, E) to yield between 1-4 g per 20 g powder. A portion of the dried samples (both whole berry and ethanol extract) underwent comparative analysis of the anthocyanin composition (cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, cyanidin-3-sambubioside) via LC-MS (Figures 1 & 2). Samples were prepared by dissolving the lyophilized extract in 80:20 (Water:ACN). The solution was injected into LC-MS system (Agilent 6538 UHD- QTOF equipped with Agilent 1290 infinity UPLC). Upon extracting the chromatograms based on the reported m/z, calculations were preformed based integrating the peak to obtain the area. HPLC was performed on the extracts as well and showed possible degradation products at shorter elution times. (Figure 3). This will need to be verified in future experiments.

Table 1: Berry Extract Yield

Sample	Berry Origin (Country/ State)	Mass Whole Powder	Mass of dried Ethanol Extract	Percent Mass
BerriHealth (BH) BRB	Oregon	20.345 g	3.486 g	17.1%
Virgin Extract (VE) BRB	"across the globe"	20.332 g	1.184 g	5.8%
Virgin Extract (VE) BB	"across the globe"	20.451 g	2.791 g	13.6%
Insert Brand Name (MX) BB	Mexico	19.971 g	ND*	ND*
Western Family (CH) BB	Chile	20.059 g	ND*	ND*

ND*--Not Determined





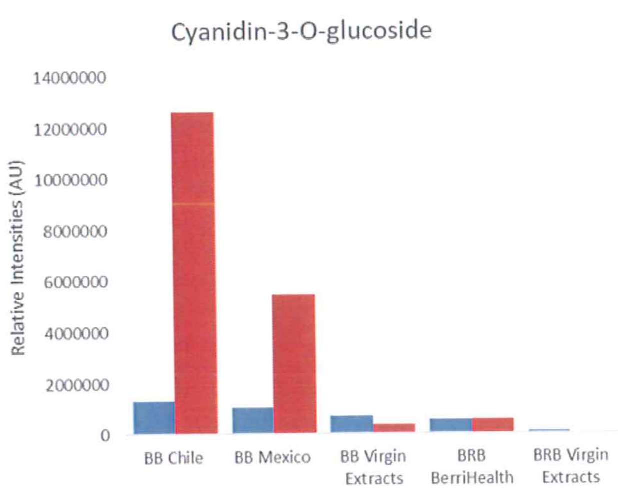
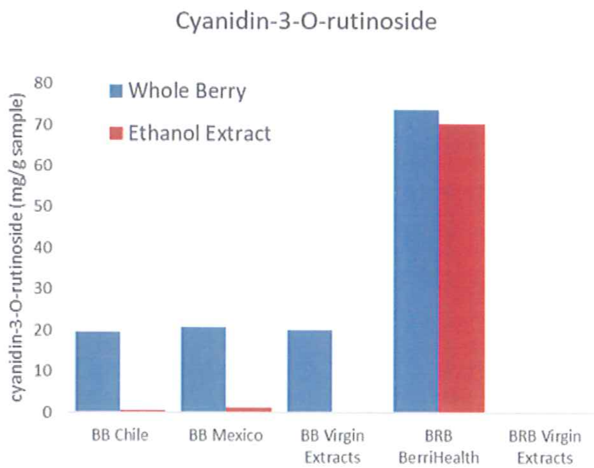
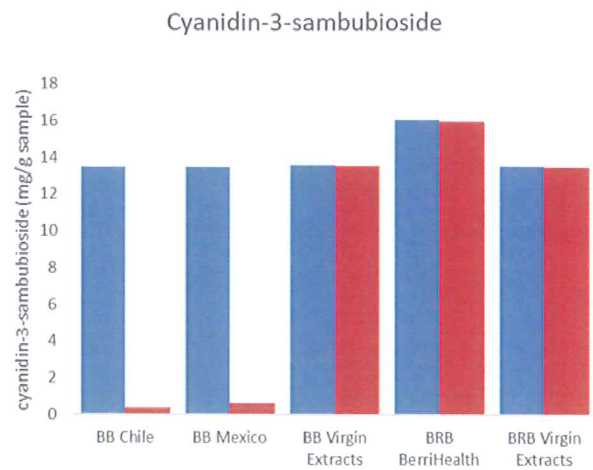


Figure 1: LC-MS Relative Intensity of Whole Berry (blue) and Ethanol Extract (red) Powders.





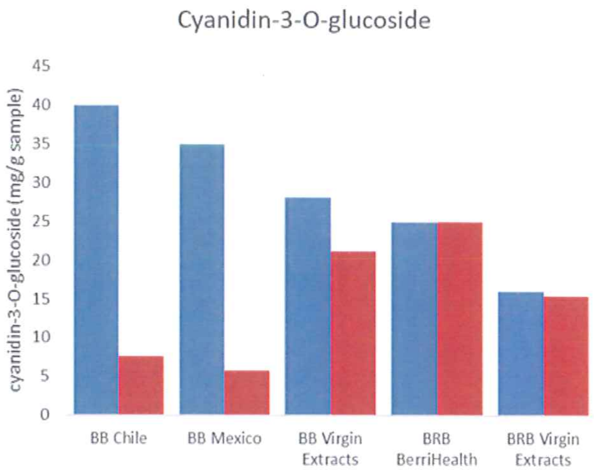


Figure 2: Quantity of Anthocyanins (mg anthocyanin/g berry) in Whole Berry (blue) and Ethanol Extract (red) Powders.

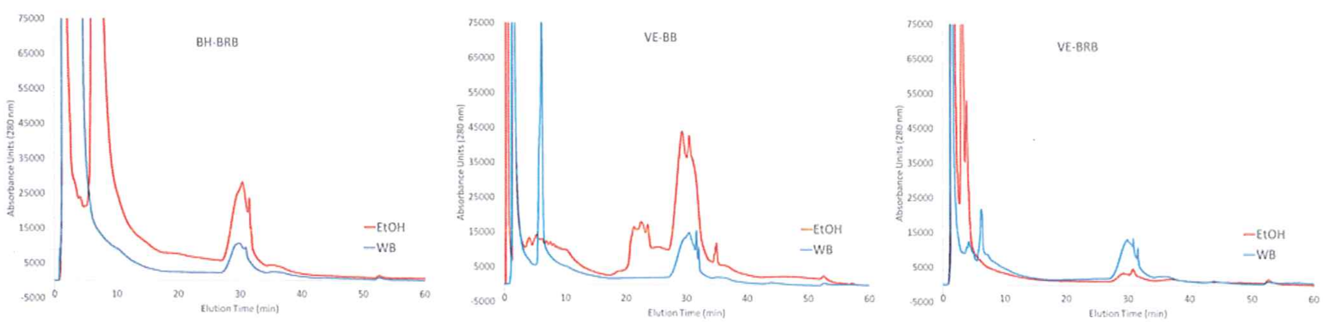


Figure 3: HPLC chromatograms of Whole berry (WB, blue) and Ethanol Extracts (EtOH, red) for BerriHealth (BH) BRB, and Virgin Extracts (VE) BRB and BB Powders.

The BerriHealth black raspberry ethanol extract was stored at -80°C and anthocyanins quantified. Significant loss of all compounds were observed (Figure 4). A laboratory accident prevented the remaining samples to be tested. New samples were prepared and have been stored at -20°C for 2 months. These will be tested again in June 2019.

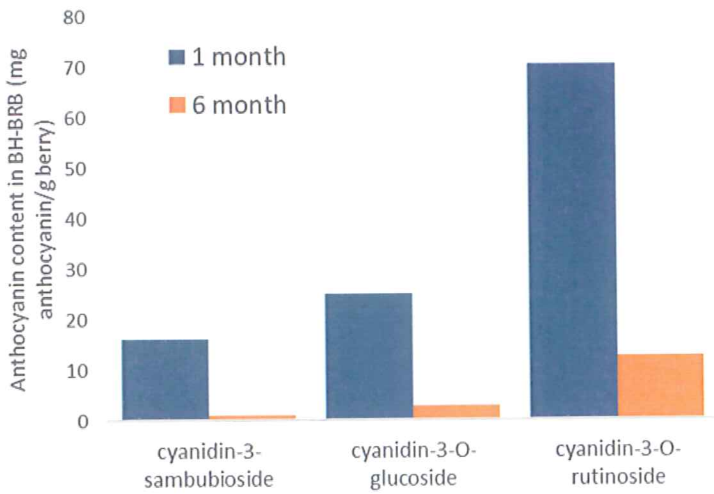


Figure 3: Quantity of Anthocyanins (mg anthocyanin/g berry) in freeze-dried Ethanol Extract stored at -20°C for 1 month (dark blue) and 6 months (orange).

Specific Aim 2. A second objective is to determine the ability of the WB and E powders for both BRB and BB obtained kill *Helicobacter pylorus (H. pylori),* the most significant causative agent for stomach cancer. *H. pylori* infection results in the development of an inflammatory condition in the stomach termed gastritis that can result in the formation of ulcers and ultimately, stomach cancer. Each extract was tested at five different concentrations for two *H. pylori* strains. Strain 60190 (ATCC 43503, *vacA s1a/m1*, $cagA^{+}$) is a commonly used laboratory strain known to excrete two toxins associated with peptic ulcers and development of gastric cancer: cytotoxin-associated gene A (*cagA*), and cell vacuolation toxin (*vacA*). Strain PMSS1 is a modified strain suitable for mouse infection that will be used in future *in-vivo* experiments. These strains were grown microanaerobically for 72 hours on Iso-Sensitest agar (Thermo Scientific) supplemented with 10% sheep blood to produce isolated colonies. Colonies were then transferred to 10 mL Brucella Broth supplemented with 10% fetal bovine serum and incubated overnight, microanaerobically. Cell suspensions were centrifuged then washed and resuspended in IF-10a media to an $OD_{600} = 0.6$. This cell suspension was used to inoculate berry-containing media. Cell metabolic activity (and thus cell growth) was tracked using a modified tetrazolium dye provided by Biolog, Inc. Prepared plates were incubated for 48 hours at 37°C. A typical plate configuration is shown in Figure 4 (image shows plate following a 48 hour incubation). To determine if exposure to the berries resulted in the bacterium becoming dormant or eradicating the bacterium, samples were plated on blood agar and incubated microaeaerobically for 72 hours. For both *H. pylori* strains, colonies were observed for all samples containing less than 5% berry. These results suggest the berries must be administered at concentrations ≥ 5% to be an effective antimicrobial.

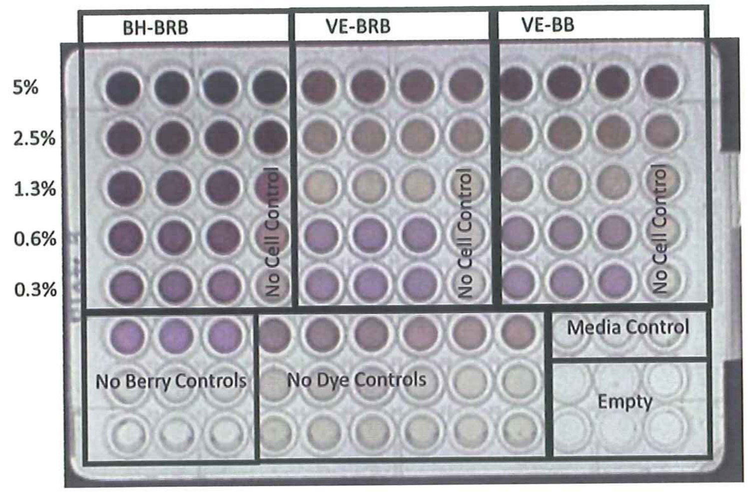


Figure 4: Representative image of plate setup for growth experiments. Three berry types could be tested in a single plate at concentrations of 5% w/w berry

The BerriHealth Black Raspberry extracts (BH-BRB) were the most effective at preventing bacterial growth (Figure 5, red) with the ethanol extract being more effective than the whole berry.

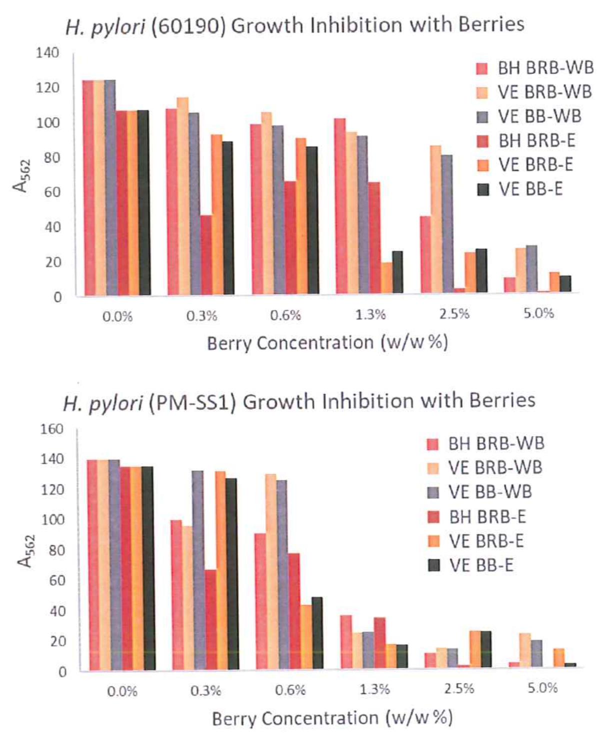


Figure 5: Growth inhibition of H. pylori (strains 60190, left, and PM-SS1, right) by Whole Berry (WB) and Ethanol Extracts (E) of BerriHealth (BH) BRB and Virgin Extracts (VE) BRB and BB samples.

SUMMARY:

Gastric cancer is the fifth most common cancer worldwide, and the second leading cause of cancer related deaths. Higher incidence rates are correlated with infection with the bacterium *H. pylori* as well as certain dietary factors. Elimination of *H. pylori* infection can prevent transformation of milder gastric diseases to gastric carcinoma, and this study preliminarily shows BB and BRB to be effective at eliminating *H. pylori* infection regardless of the berry origin. Frozen whole blackberries appear to maintain higher levels of anthocyanins compared to frozen powdered berries. Extraction of anthocyanins with ethanol promotes degradation, which we believe can be resolved using an acidified extraction procedure. Further degradation of the anthocyanins in the ethanol extracts was observed for the sample stored at -20°C. This was not observed for whole berry powder stored at -20°C.

Progress Report to the Agricultural Research Foundation
2018-19

Title: Cooperative breeding program - Caneberries

Principal investigators: Bernadine Strik, Professor, Horticulture
Berry Production System Research Leader, NWREC
Chad Finn, USDA/ARS Geneticist

Pat Jones & Amanda Vance Faculty Research Assistants NWREC
Mary Peterson, USDA/ARS Technicians

Cooperators: Pat Moore, WSU, Puyallup
Michael Dossett; Agriculture and Agri-Foods Canada
Brian Yorgey, OSU, Dept. Food Science & Tech.
Bob Martin, USDA-ARS
Enfield Farms/Northwest Plants
North American Plant Co.
Northwest Plants
Oregon Raspberry and Blackberry Commission
USDA-ARS Northwest Center for Small Fruit Research
Oregon and Washington berry growers

Objectives:

- To develop new blackberry cultivars for the Pacific Northwest that are high yielding, thornless, winter tolerant, adapted to mechanical harvesting, and that have excellent fruit quality. While the primary emphasis is on blackberries with excellent processed fruit quality, high quality fresh market cultivars will be pursued as well.
- To develop raspberry cultivars for the Pacific Northwest in cooperation with Agriculture and Agri-Foods Canada and Washington State University that are high-yielding, machine harvestable, disease/virus resistant and that have superior processed fruit quality. While the priority will be on the processed market, fresh market cultivars will be pursued as well.
- To evaluate black raspberry selections and cultivars for their adaptation to the Pacific Northwest and to develop selections that combine similar processed fruit quality to 'Munger' with greater yields and plant longevity (disease tolerance).
- To collect, evaluate and incorporate new *Rubus* germplasm into the breeding program.

Progress:

The USDA-ARS breeding program in cooperation with Oregon State University and the Pacific Northwest industry continues to develop red and black raspberry, blackberry, and strawberry cultivars that meet the industry stated objectives. A primary objective for the Oregon caneberry industry has been the development of thornless blackberry cultivars with outstanding flavor/processing characteristics that can be machine harvested for processing and ideally are a bit firmer and more winter tolerant than 'Marion'. 'Black Diamond' has been the most widely planted cultivar from this effort and has been the #1 for plant sales for several years. In addition, while thorny, 'Obsidian', 'Metolius', 'Newberry', and 'Onyx', have been released to provide different options for the blackberry fresh market. 'Columbia Star' since its release has been 2nd

only to 'Black Diamond' for sales. 'Columbia Sunrise', the earliest ripening thornless blackberry we are aware of was released in 2016. In 2017, the trailing blackberry 'Hall's Beauty' and the semi-erect blackberries 'Eclipse' and 'Galaxy' were released. They will be working their way into the marketplace over the next few years. We released 'Twilight' (ORUS 4370-1), a semi-erect, in 2018. We have been active in testing WSU and AgCanada raspberry selections to assess what is appropriate for Oregon and we were partners in the new release 'WSU2166' and the recent release of 'Cascade Harvest' a couple years ago. We have several selections in machine harvest trials in northern Washington and a few of these are promising. The relatively recent primocane fruiting release 'Vintage' is performing well for some growers and 'Kokanee' was released in 2016. We identified several black raspberry selections for processing that we are moving to the nurseries with the goal of having quantities available for commercial trial soon.

In 2018 we evaluated about 5,000 blackberry and red and black raspberry seedlings. We made 45 red raspberry (22 floricane, 23 primocane), 39 black raspberry, and 37 blackberry (17 trailing, 12 semi-erect and 8 primocane) selections. Below are the highlights of the genotypes at various stages of evaluation.

Blackberry

Cultivar Releases

'Hall's Beauty' trailing blackberry released and patent application filed in 2017

In grower trial observations, 'Hall's Beauty' plantings subject to flooding when rivers run high seemed to suffer **no problems with root rot.**

'Eclipse' and 'Galaxy' semi-erect blackberries released and patent application filed.

These are the 1st semi-erect releases developed within our program. They are a cross of ('Navaho' x ORUS 1122-1 ['Olallie' x ORUS 728-3]) x 'Triple Crown' and so are ¾ eastern blackberry germplasm and ¼ western. Earlier ripening than 'Triple Crown' and 'Chester Thornless'. They are high yielding but less than 'Chester Thornless'. The fruit are medium size, black, firm, with tough skin and have better flavor than 'Chester Thornless' and have no bitter flavors. Comparing the two; 'Eclipse' is slightly earlier and the fruit are smaller, firmer, and more uniform than for 'Galaxy'. Expect these to grow wherever eastern blackberries like 'Triple Crown', 'Navaho', and 'Chester Thornless;' can be grown. In California's central valley, 'Eclipse' was more erect and vigorous than 'Galaxy'. Both have done well in fresh market storage trials.

'Twilight' (ORUS 4370-1) semi-erect blackberry released and patent application filed in 2018

'Twilight' is an early ripening (10d<'Triple Crown' but later than 'Eclipse' and 'Galaxy') thornless blackberry that is ¾ eastern blackberry and ¼ western blackberry. It has outstanding fruit quality, particularly skin toughness, and a pleasant firmness along with large attractive fruit. Yield was comparable to 'Chester Thornless' in 2/3 years tested. Can be stored fresh for a couple weeks with excellent quality.

To be released

ORUS 4535-1 is a dwarf, thornless blackberry for homeowner market. While a floricane type, it has short internodes and its 0.60-0.75 m (2-2.5 ft.) long canes will cascade out of containers. The fruit quality is fine but unremarkable. A patent application will be applied in spring 2019.

Grower trials

In addition to the above, the following have been/are being propagated for grower trials

- ORUS 4024-3 **has 'Willamette' red raspberry as a grandparent.** Very attractive glossy red fruit that look like a 'Tayberry'. Picks easily and may even be machine harvestable. Wonderful flavor and commercial growers want it after 1st look.
- ORUS 4057-3. Thornless that produces high yields of high quality fruit 7-10 d ahead of 'Black Diamond' and ahead of Metolius/Obsidian in some seasons.
- ORUS 4222-1 is thornless and very high yielding, comparable to 'Black Diamond', with fruit size comparable to 'Marion'. Excellent quality for processing
- **ORUS 4670-1** is a new thornless semi-erect selection that I would release immediately if not for the fact that it is based on one year's observation and yield in replicated trial! Excellent eating quality for a semi-erect, much better than 'Chester Thornless' and remarkably in the first harvest season it **had significantly greater yield than 'Chester Thornless'.** It ripens about 1 week ahead of 'Chester Thornless'
- **ORUS 4902-1** trailing selection has the **rare combination of extremely firm with outstanding flavor**. Thornless but will not see yield in trial until 2019.

2014 Trailing Blackberry Planting (Tables BLK1 and BLK7)

- Confirmed that 'Columbia Star' and 'Hall's Beauty' are as high yielding as 'Black Diamond' in our trials.

2015 Trailing Planting (Tables BLK2 and BLK7)

- Nothing appears better than current standards in 1st harvest season

2016 Trailing Planting (Tables BLK3 and BLK7)

- ORUS 4663-1 was particularly impressive. It is thornless and from the cross 'Columbia Giant' x 'Obsidian' and it has large fruit size and very good yields in the Marion/Columbia Star season. Growers visiting the plot gravitated to this one.

2014 and 2015 Semi-erect trials (Tables BLK4, BLK5 and BLK7)

- ORUS 4670-1 is the most exciting semi-erect blackberry we have ever had! It has the potential to challenge the yield of 'Chester Thornless', and in the first year had significantly greater yields, with a much higher quality and larger fruit. While we need to see yields beyond the first year. We are cleaning this one up for grower trial in grower fields.

2014 Planted Primocane-fruiting trials (Table BLK6 and BLK7)

- **ORUS 4545-2** looked promising for trial due to medium berry size, comparable or greater yield higher than 'Prime-Ark® 45' and it was about 1 week ahead of 'Prime-Ark® 45'. Fruit size is less than 'Prime-Ark® 45', but is still a very respectable 5.7 g.
- While lower yielding than the standards, ORUS 4546-1 was firm with superior fruit quality.
- In trying to assess commercial viability of our selections I had a colleague who has looked at a lot of PF blackberries for a late season assessment: "ORUS 4545-1 is earlier and appears to have good yield relative to 'Prime-Ark® 45' but I'm concerned about small fruit and an apparent susceptibility to heat leading to poor set. ORUS 4545-2 looks better with regard to fruit quality and I like its more compact habit. Not sure how much earlier it is. Of the bunch my favorite is ORUS 4546-1 which I think has good size and a good growth habit and fruit presentation. I think this one shows promise. Would like to see yield and season though."

2016 Planted Primocane-fruiting trials (Table BLK6 and BLK7)

- Nothing appears better than current standards in 1st harvest season

2017 Planted Primocane-fruiting trials

- While we did not harvest this trial for yield and so will not report on it until next year, ORUS 4999-2 clearly stood out. The plant had been identified in the seedling field as one to be propagated for Grower Trial. In our observation plot, the plants were vigorous, began to ripen fruit in mid-August, much earlier (8/13) than 'Prime-Ark® 45' (9/4). The fruit were4 large, firm, tough-skinned, and had a good sweet flavor.

Winter hardiness and machine harvestability evaluation

Since 2001, hundreds of our blackberry selections have been planted at Enfield Farms (Lynden Wash.), which sits on the Canadian border, to evaluate winter hardiness and machine harvestability in a commercial setting. Most but not all selections have been machine harvestable. 'Columbia Sunrise' and Hall's Beauty', 'Eclipse', 'Galaxy' and 'Twilight' and ORUS 4670-1were scored as similar to, or much better than, 'Marion' for cold hardiness in comparable years in Lynden.

Red Raspberry

Being propagated for Grower Trial

- ORUS 4373-1, **Floricane** processed. Good yield. Good fruit quality. Excellent root rot resistance at WSU-Puyallup. Fair yield in MH trial in Washington
- ORUS 4600-3, **Floricane** processed. Promising in MH at NWREC. Very high quality. Very good yield.
- ORUS 4607-2, **Floricane** processed. Promising in MH Trial at Enfield. Excellent quality. Main concern is whether fruit get crumbly too quickly.
- ORUS 4089-2, **Primocane or floricane fresh**. Looked very good in Lynden and at NWREC. Bright firm and attractive as PF
- ORUS 4291-1, **Primocane**, fresh. Very early! 18-21 d < 'Heritage'; Concerned with yield, may be good enough for an early cultivar but not incredible.
- ORUS 4487-1, **Primocane, fresh**. Very early! 10d < 'Heritage'; Concerned with yield, may be good enough for an early cultivar but not incredible.
- ORUS 4716-1, **Primocane fresh**. Impressive quality and yield

2015 Floricane Fruiting Trial (Tables RY1 and 7)

- All harvested with Littau harvester
- Although yields were statistically similar ORUS 4607-2 and ORUS 4600-3 looked very promising for yield and machine harvestability at NWREC. In addition they looked good in machine harvest trials in Lynden, WA (see comments below and table RY3).

2016 Floricane Fruiting Trial (Tables RY2)

Floricane fruiting raspberry genotypes at OSU-NWREC planted in 2016, would normally be harvested in 2018, two years after planting, however, due to rose stem girdler damage in 2017, we cut floricanes to the ground and had no crop in 2018. They look fine for harvest in 2019.

WRRC supported machine harvest trials planted in 2016 and 2017 (Table RY3)

- While ORUS 4462-2 was only moderate yielding in 1st year, it was the highest yielding selection in 2018 with good fruit size and firmness similar to 'Meeker' and less than 'Wakefield'.
- ORUS 4607-2 in its first year looked comparable to 'Wakefield' and 'Cascade Harvest' with a fairly large berry and fruit firmness similar to or slightly better than 'Meeker'. Several selections had first year yield similar to 'Meeker' and greater than 'Wake®field', we will see if this holds up in the second harvest. Most are firmer than 'Meeker' and less firm than and 'Wake®Field'.

2015 Primocane Fruiting Trial (Tables RY4 and RY8)

- While ORUS 4291-1 was lower yielding than 'Heritage', it had much larger fruit and ripened over 3 weeks earlier.

2016 Primocane Fruiting Trial (Tables RY5 and RY8)

- This trial was very problematic in 2017 due to rose stem girdler. Usually we would have data for two years at this stage but data was not collected in 2017 and only 2018 yield was analyzed.
- ORUS 4864-1 looked good for yield and fruit quality. Since it is 2 weeks earlier than 'Heritage' it is interesting but yields were not amazing, perhaps due to the heat.
- ORUS 4723-2 had good yields of firm, bright easy to pick fruit.
- While there were things to like about the 'Imara', 'Kweli', and 'Kwanza', in their 1st harvest year it was hard to believe they will do well commercially in the NW. 'Imara' has a "greasy" texture, is crumbly, and is very dark. 'Kweli' fruit look a lot like 'Heritage', they are dark, crumbly, and bland. 'Kwanza' was large and bright but very crumbly and is very hard to pick when light colored.

2017 Primocane Fruiting Trial (Tables RY6 and RY8)

- 'Lagorai Plus' was described by its Netherland developers as floricane cultivar but had a large primocane crop. We had planted it with our floricanes but went ahead and harvested the primocane crop. While we were not that impressed with its performance in the field, it was high yielding with good sized (4 g) berries.
- ORUS 4716-1, despite not statistically being any different from 'Heritage' for yield or size, was impressive. The plants were strong and healthy and the fruit are better suited to the fresh market than 'Heritage' as they seem to be larger (maybe larger in early season), are much lighter colored and visually more attractive. They could easily be picked very firm and pink.

Evaluation of Root Rot resistance at WSU

Pat Moore at WSU has been screening raspberries in root rot trials. Based on his results he identified a range of responses to root rot. While many would appear to be susceptible, it was exciting to see some at the high end of the graph. The results:

- Probably better than 'Meeker': ORUS 4373-1
- Probably comparable to 'Meeker': ORUS 4482-3
- Probably comparable to or worse than 'Meeker': Kokanee, Lewis, Vintage, ORUS 3234-1, ORUS 4090-2, ORUS 4097-1, ORUS 4283-1, ORUS 4289-1, ORUS 4462-2, ORUS 4465-2, and ORUS 4619-1.

Black Raspberry

Developing the Genomic Infrastructure for Breeding Improved Black Raspberries (Bushakra, Bassil, Dossett, Ju. Lee, Weber, Scheerens, Fernandez, Weiland, Ja. Lee, Finn)
Project number 2072-21220-002-04R

While this project is completed, we are now further refining the markers for aphid resistance and are using the markers to screen seedlings for aphid resitance. We now have selections that have mulitple sources of aphid resistance.

Grower Trial

- ORUS 3735-3 has been discarded as every plant that came from the nursery was crumbly.
- ORUS 3013-1 Processing. High yields of fruit that appear to machine harvest well. Not the long-lived replacement we want for 'Munger' but may be better for the short-run.
- ORUS 3021-1 Processing. Larger than 'Munger'. Similar yield but may be more durable. Machine harvests
- ORUS 3032-3 Processing or fresh. Great size and fruit quality. Comparable yield to 'Munger'. Machine harvests.
- ORUS 3038-1. Processing. High yields of very tasty fruit. May have root rot problem.
- ORUS 3217-1. Processing. High yields of fruit that appear to machine harvest well. 'Munger' size not sure color is dark enough. Not the long-lived replacement we want for 'Munger' but may be better for the short-run.
- ORUS 3381-3 Fresh. While would work for processing, it is as late as 'MacBlack' with better fruit size and quality. Yield comparable to or slightly less than 'Munger' but starts ripening 12 d later
- ORUS 3409-1 produces a nice floricane and primocane crop. Excellent root rot tolerance in WSU-Puyallup trials.
- ORUS 4412-2 Processing. Excellent yield and fruit quality. Machines well.
- ORUS 4499-1 Processing. Excellent yield and fruit quality. Machines well. Excellent root rot tolerance in WSU-Puyallup trials

2014 Planted Trials (Tables BLKRY1 and BLKRY4)
- Harvested with Littau machine harvester
- ORUS 4154-1, ORUS 4412-2, ORUS 4499-1, and ORUS 4410-1 had high yields, relatively large fruit, machine harvested well and had excellent fruit quality. In addition:
  - ORUS 4154-1 has Ag4 aphid resistance
  - ORUS 4412-2 has looked outstanding for fruit quality as a puree (better than 'Munger')
  - ORUS 4499-1 has stood up extremely well to root rot in WSU root rot trials
  - ORUS 4410-1 has looked outstanding for fruit quality as a puree (better than' 'Munger') and may have verticillium tolerance.
- ORUS 3381-3 has looked great as a late complement or replacement for 'MacBlack'. While ORUS 3381-3's performance in rep looks similar to 'MacBlack's in a single plot, our experience is that yield in rep trials is usually 65-75% of what we get for the same genotype in single plots assuming healthy plants. ORUS 3381-3 is a better looking genotype than 'MacBlack'.

2015 Planted Trials (Tables BLKRY2 and BLKRY4).
- Harvested with Littau machine harvester.

- A few had yields comparable or higher than 'Munger' with similar berry size and excellent fruit quality. Very exciting but need to see how they stand up.
- ORUS 4583-1 stood out for yield and ORUS 4559-1 for overall quality as well as machine harvestability.

2016 Planted Trials (Tables BLKRY2 and BLKRY4).

- Hand harvested all as observation plots. Rose stem girdler in 2017 caused plant vigor and size to be compromised. Should be fine in 2019.
- ORUS 4585-1, ORUS 4304-192 and ORUS 4311-1 all had sufficient quality and yield to justify targeting for full rep trial.

Table BLK1. Fruit size and yield in 2016-18 for trailing blackberry genotypes at OSU-NWREC. Planted in 2014. All thornless except Marion.

Genotype	Berry size (g) 2016-18	Yield ($tons \cdot a^{-1}$) 2016	2017	2018	2016-18
2016	6.3 b				7.12 b
2017	7.3 a				6.49 c
2018	6.3 b				8.20 a
Replicated					
Columbia Star	**6.9 a**	**7.24 a**	**6.92 a**	**9.82 a**	**7.99 a**
Black Diamond	**6.4 b**	**6.73 a**	**6.32 a**	**8.22 a**	**7.09 a**
Hall's Beauty	**6.6 ab**	**7.40 a**	**6.21 a**	**6.54 a**	**6.72 a**
Nonreplicated					
Marion	**5.7**	**4.64**	**5.57**	**7.14**	**5.78**

[z] Mean separation within columns by LSD, $p \leq 0.05$.

Table BLK2. Fruit size and yield in 2017-18 for thornless trailing blackberry genotypes and 'Marion' at OSU-NWREC[z]. Planted in 2015; single plot harvested.

Genotype	Berry size (g) 2017-18	Yield ($tons \cdot a^{-1}$) 2017	2018	2017-18
Columbia Star	**7.4**	**7.02**	**8.83**	**7.93**
Black Diamond	**6.7**	**3.80**	**10.59**	**7.19**
Marion	**5.5**	**4.58**	**7.92**	**6.25**
ORUS 4532-2	5.2	5.47	6.60	6.04
ORUS 4200-1	5.7	2.53	5.79	4.16

[z] Mean separation within columns by LSD, $p \leq 0.05$.

Table BLK3. Fruit size and yield in 2018 for trailing blackberry genotypes at OSU-NWREC. Planted in 2016

Genotype	Thornless or thorny? Type	Berry size (g)[z]	Yield ($tons \cdot a^{-1}$)
Replicated			
Columbia Star	**Thls**	**7.1 a**	**4.28 a**
Marion	**Thorny**	**5.3 c**	**3.59 a**
Black Diamond	**Thls**	**6.4 ab**	**2.64 ab**
ORUS 4200-1	Thls	5.4 c	2.49 ab
ORUS 4441-1	Thls	5.6 bc	0.95 b
Nonreplicated			
ORUS 4530-1	Thls	7.8	6.95
Columbia Giant	**Thls**	**12.9**	**6.23**
ORUS 4535-2	Thls	5.8	6.15
ORUS 4534-1	Thls	4.8	6.06
ORUS 4537-2	Tay-type Thorny	5.2	5.17
ORUS 4663-3	Thls	8.5	4.51
ORUS 4524-1	Thls	6.5	3.83
ORUS 4663-4	Thls	12.1	3.49
ORUS 4650-1	Thls	4.1	2.35
ORUS 4230-3	Thls Semi-Dwarf	6.9	2.07
ORUS 4659-1	Thls	8.8	1.45
ORUS 4535-1	Thls Full Dwarf	3.1	0.65

[z] Mean separation within columns by LSD, $p \leq 0.05$.

Table BLK4. Fruit size and yield in 2016-2018 for semi-erect, thornless blackberry genotypes in trial at OSU-NWREC[z]. Planted in 2014.

Genotype	Berry size (g)[z] 2016-18	Yield(tons·a^{-1}) 2016	2017	2018	2016-18
Nonreplicated					
Chester Thornless	**5.6**	**8.43**	**11.16**	**16.27**	**11.95**
Triple Crown	**8.8**	**7.27**	**11.68**	**11.53**	**10.16**
ORUS 4453-1	7.2	4.64	8.28	7.65	6.86
ORUS 4453-2	6.2	4.91	5.34	8.32	6.19

Table BLK5. Fruit size and yield in 2018 for thornless semi-erect blackberry genotypes in replicated trial at OSU-NWREC planted in 2016.

Genotype	Berry size (g)	Yield(tons·a^{-1})
ORUS 4670-1	6.3 a	7.72 a
Chester Thornless	**5.4 b**	**5.34 b**

Table BLK 6. Primocane fruiting genotypes planted in **nonreplicated**, observation plots in 2014 or 2016. Harvest stopped in early October each year. All are thorny.

Genotype	Berry size (g)[z]	Yield(tons·a^{-1}) 2015	2016	2017	2018	2015-18
2014 Planted						
ORUS 4545-2	5.7	2.58	5.48	6.97	8.62	5.91
Prime-Ark®45	**10.4**	**1.69**	**3.60**	**5.78**	**7.50**	**4.64**
ORUS 4545-1	6.7	2.79	1.94	4.41	4.50	3.41
ORUS 4546-1	6.7	1.28	1.89	3.45	3.83	2.61
2016 Planted						
Prime-Ark®45	**7.2**				**3.75**	
ORUS 4801-1	6.3				1.84	
ORUS 4545-4	4.1				1.39	
ORUS 4802-1	5.9				1.15	
ORUS 4805-3	5.6				0.84	
ORUS 4805-2	4.0				0.62	
Prime-Ark®Freedom	**5.3**				**0.53**	

Table BLK7. Ripening season, date at which each genotype's yield passed the given percentage, for blackberry genotypes at OSU-NWREC.

Genotype	Type	Year planted	Harvest season 5%	50%	95%	No. yrs. in mean	Rep/ Obsv
ORUS 4530-1	Tr	2016	14-Jun	19-Jun	3-Jul	1	Obsv
Columbia Sunrise	**Tr**	**2016**	**16-Jun**	**19-Jun**	**3-Jul**	**1**	**Rep**
ORUS 4518-2	Tr	2016	19-Jun	19-Jun	19-Jun	1	Obsv
ORUS 4650-1	Tr	2016	19-Jun	26-Jun	3-Jul	1	Obsv
ORUS 4537-2	Tr	2016	19-Jun	26-Jun	10-Jul	1	Obsv
ORUS 4663-3	Tr	2016	19-Jun	26-Jun	10-Jul	1	Obsv
ORUS 4663-4	Tr	2016	26-Jun	26-Jun	10-Jul	1	Obsv
Hall's Beauty	**Tr**	**2014**	**25-Jun**	**29-Jun**	**11-Jul**	**3**	**Obsv**
Columbia Star	**Tr**	**2014**	**20-Jun**	**1-Jul**	**8-Jul**	**3**	**Obsv**
Marion	**Tr**	**2014**	**25-Jun**	**1-Jul**	**13-Jul**	**3**	**Obsv**
Black Diamond	**Tr**	**2016**	**19-Jun**	**3-Jul**	**10-Jul**	**1**	**Rep**
Columbia Star	**Tr**	**2016**	**19-Jun**	**3-Jul**	**10-Jul**	**1**	**Rep**
Hall's Beauty	**Tr**	**2016**	**19-Jun**	**3-Jul**	**10-Jul**	**1**	**Rep**
ORUS 4663-1	Tr	2016	19-Jun	3-Jul	10-Jul	1	Rep
ORUS 4057-3	Tr	2016	19-Jun	3-Jul	24-Jul	1	Rep
ORUS 4535-1	Tr	2016	26-Jun	3-Jul	3-Jul	1	Obsv
Marion	**Tr**	**2016**	**26-Jun**	**3-Jul**	**10-Jul**	**1**	**Rep**
ORUS 4524-1	Tr	2016	26-Jun	3-Jul	10-Jul	1	Obsv
ORUS 4534-1	Tr	2016	26-Jun	3-Jul	10-Jul	1	Obsv
ORUS 4535-2	Tr	2016	26-Jun	3-Jul	10-Jul	1	Obsv
ORUS 4659-1	Tr	2016	26-Jun	3-Jul	10-Jul	1	Obsv
Columbia Giant	**Tr**	**2016**	**26-Jun**	**3-Jul**	**17-Jul**	**1**	**Obsv**
Black Diamond	**Tr**	**2014**	**20-Jun**	**4-Jul**	**18-Jul**	**3**	**Obsv**
Columbia Star	**Tr**	**2015**	**24-Jun**	**4-Jul**	**14-Jul**	**2**	**Rep**
ORUS 4532-2	Tr	2015	24-Jun	4-Jul	21-Jul	2	Obsv
Black Diamond	**Tr**	**2015**	**24-Jun**	**7-Jul**	**17-Jul**	**2**	**Rep**
Marion	**Tr**	**2015**	**30-Jun**	**7-Jul**	**21-Jul**	**2**	**Rep**
ORUS 4230-3	Tr	2016	26-Jun	10-Jul	17-Jul	1	Obsv
ORUS 4453-2	SE	2014	8-Jul	13-Jul	25-Jul	3	Obsv
ORUS 4207-2	Tr	2015	7-Jul	17-Jul	24-Jul	2	Rep
ORUS 4453-1	SE	2014	8-Jul	20-Jul	3-Aug	3	Obsv
ORUS 4200-1	Tr	2015	14-Jul	21-Jul	31-Jul	2	Rep
ORUS 4670-1	SE	2016	17-Jul	31-Jul	15-Aug	1	Rep
Triple Crown	**SE**	**2014**	**18-Jul**	**3-Aug**	**12-Aug**	**3**	**Obsv**
Chester Thornless	**SE**	**2016**	**24-Jul**	**7-Aug**	**15-Aug**	**1**	**Rep**
Chester Thornless	**SE**	**2014**	**25-Jul**	**10-Aug**	**29-Aug**	**3**	**Obsv**

Table BLK7. Cont.

Genotype	Type	Year planted	Harvest season 5%	50%	95%	No. yrs. in mean	Rep/ Obsv
Primocane fruiting blackberries							
ORUS 4545-4	PF	2016	21-Aug	28-Aug	4-Sep	1	Obsv
ORUS 4805-2	PF	2016	21-Aug	28-Aug	4-Sep	1	Obsv
ORUS 4805-3	PF	2016	21-Aug	28-Aug	4-Sep	1	Obsv
Prime-Ark®Freedom	**PF**	**2016**	**21-Aug**	**28-Aug**	**11-Sep**	**1**	**Obsv**
ORUS 4802-1	PF	2016	21-Aug	28-Aug	18-Sep	1	Obsv
ORUS 4801-1	PF	2016	21-Aug	4-Sep	26-Sep	1	Obsv
ORUS 4545-2	PF	2014	22-Aug	6-Sep	25-Sep	4	Obsv
ORUS 4545-1	PF	2014	26-Aug	9-Sep	27-Sep	4	Obsv
Prime-Ark®45	**PF**	**2016**	**21-Aug**	**11-Sep**	**26-Sep**	**1**	**Obsv**
Prime-Ark®45	**PF**	**2014**	**24-Aug**	**11-Sep**	**26-Sep**	**4**	**Obsv**
ORUS 4546-1	PF	2014	24-Aug	14-Sep	27-Sep	4	Obsv

[y] Tr=Trailing; SE= Semi-erect; PF= Erect primocane fruiting. Where fraction of species (*R. georgicus*) listed the remainder is cultivated germplasm.
[x] Stopped harvest of PF blackberries 10/10/2016, 10/10/17, 10/1/2018.

Table RY1. Mean yield and berry size in 2017-18 for floricane fruiting raspberry genotypes at OSU-NWREC planted in 2015. Harvested with a Littau (Stayton, OR) machine in 2017-18.

	Berry size (g)	Yield (tons·a^{-1})		
Genotype	2017-18^{z}	2017	2018	2017-18
2016	4.5 a			3.84 a
2017	3.8 a			4.26 a
2018	3.4 a			4.16 a
Replicated				
ORUS 4607-2	3.7 ab	5.33 a	4.83 a	5.08 a
ORUS 4600-2	4.0 a	4.46 a	5.26 a	4.86 a
ORUS 4600-3	3.3 c	4.21 a	4.57 a	4.39 a
ORUS 4603-1	3.5 bc	3.81 a	4.28 a	4.05 ab
Meeker	3.3 c	3.99 a	3.86 ab	3.93 ab
ORUS 4603-2	3.6 b	3.73 a	2.15 b	2.94 b
Nonreplicated				
ORUS 4611-1	4.2	4.12	2.17	3.14

^{z} Mean separation within columns by LSD, $p \leq 0.05$.

Table RY2. Floricane fruiting raspberry genotypes at OSU-NWREC planted in 2016- Would normally be harvested in 2018, two years after harvest, however, due to rose stem girdler damage in 2017, we cut floricanes to the ground and had no crop in 2018. They look fine for harvest in 2019

Genotype	Berry size (g)^{z}	Yield (tons·a^{-1})
Replicated		
Nonreplicated		

^{z} Mean separation within columns by LSD, $p \leq 0.05$.

Table RY3. Performance of ORUS selections in machine harvest trials in Lynden, Washington at commercial grower fields. Planted in 2016 and 2017.

Genotype	Total yield (tons/acre)			Berry weight (g)	Firmness (g/mm)			Brix (%)			Acidity (%)	pH
	2017	2018	2017-18	2017-18	2017	2018	2017-18	2017	2018	2017-18	2017-18	2017-18
Grower 1 2016 planted												
Wake®field	**6.10**	**7.80**	**6.95**	**3.4**	**49.91**	**35.78**	**42.85**	**8.6**	**11.6**	**10.1**	**2.34**	**3.18**
Squamish	-	**6.45**	**6.45**	**4.3**	-	**30.00**	**30.00**	-	**10.7**	**10.7**	**1.74**	**3.32**
Meeker	**4.20**	**7.22**	**5.71**	**3.0**	**37.87**	**13.73**	**25.80**	**10.6**	**11.2**	**10.9**	**1.82**	**3.40**
Cascade Harvest	**3.20**	**7.06**	**5.13**	**5.2**	**34.07**	**24.72**	**29.40**	**9.8**	**11.5**	**10.7**	**1.48**	**3.44**
ORUS 4482-3	2.40	7.67	5.03	5.1	36.10	24.15	30.13	8.8	10.1	9.5	1.78	3.36
ORUS 4089-2	2.60	7.00	4.80	3.3	32.10	23.71	27.91	9.9	10.4	10.2	1.48	3.39
ORUS 4462-2	1.40	8.05	4.72	5.0	30.38	28.44	29.41	8.5	10.9	9.7	1.31	3.55
ORUS 3702-3	3.00	5.48	4.24	5.1	22.26	10.70	16.48	10.2	10.5	10.4	1.43	3.46
ORUS 4373-1	3.00	5.35	4.17	4.5	37.58	28.50	33.04	9.6	9.7	9.7	1.55	3.41

Table RY3. (Cont.)

Genotype	Total yield (tons/acre)	Berry weight (g)	Firmness (g/mm)	Brix (%)	Acidity (%)	pH
Grower 1 2017 planted						
Meeker	**7.91**	**3.85**	**17.65**	**11.6**	**1.4**	**3.53**
ORUS 4371-4	7.46	5.83	25.97	11.3	1.9	3.75
ORUS 4851-1	7.46	6.54	22.80	10.6	1.4	3.42
Cascade Harvest	6.74	5.86	21.38	10.1	1.1	3.64
ORUS 4607-2	6.50	4.86	21.50	10.8	1.8	3.30
ORUS 4465-3	4.98	4.71	17.52	10.1	1.4	3.48
Wake®field	**3.90**	**4.10**	**33.86**	**10.7**	**2.3**	**3.21**
Squamish	**3.72**	**4.67**	**24.54**	**11.0**	**1.8**	**3.27**
Rudiberry	**2.57**	**4.75**	**26.45**	**10.7**	**1.8**	**3.32**

Table RY4. Mean yield and berry size in 2016-2018 for primocane fruiting raspberry genotypes at OSU-NWREC planted in 2015.

Genotype	Berry size (g) 2016-2018	Yield (tons·acre^{-1}) 2016	2017	2018	2016-2018
Non replicated					
Heritage	**1.9**	**1.77**	**5.08**	**5.22**	**4.02**
Kokanee	**3.0**	**2.65**	**1.85**	**2.94**	**2.48**
ORUS 4291-1	2.7	1.96	1.33	2.63	1.97
Vintage	**3.0**	**1.99**	**1.15**	**2.44**	**1.86**
BP1 (Amira)	3.5	1.32	1.58	1.54	1.48

Table RY5. Mean yield and berry size in 2018 for primocane fruiting red raspberry genotypes at OSU-NWREC planted in 2016. Rose stem girdler wiped out 1st harvest in 2017.

Genotype	Berry size (g)	Yield (tons·a^{-1})
Replicated		
Heritage	**1.9 b**	**2.74 a**
ORUS 4864-1	2.7 a	1.92 a
Vintage	**2.5 a**	**1.89 a**
Nonreplicated		
ORUS 4858-2	3.1	4.59
ORUS 4874-1	2.9	4.50
Imara	**3.4**	**4.17**
Kweli	**2.9**	**3.71**
ORUS 4494-3	4.0	3.71
ORUS 4873-1	2.4	3.42
ORUS 4858-3	2.9	3.23
ORUS 4723-2	4.1	2.79
ORUS 4872-1	1.9	2.65
Kokanee	**2.7**	**2.57**
ORUS 4722-1	3.9	1.90
ORUS 4722-2	3.6	1.87
Kwanza	**3.8**	**1.32**
ORUS 4856-1	2.6	0.86

Mean separation within columns by LSD, $p \leq 0.05$.

Table RY6. Mean yield and berry size in 2018 for primocane fruiting red raspberry genotypes at OSU-NWREC planted in 2017.

Genotype	Berry size (g)	Yield (tons·a^{-1})
Replicated		
Lagorai Plus	**4.2 a**	**3.61 a**
ORUS 4716-1	2.8 b	2.65 b
Heritage	**2.0 b**	**1.86 b**
Non replicated		
ORUS 4990-1	3.5	2.19
ORUS 4988-4	2.4	1.72
ORUS 5005-1	3.6	1.70
ORUS 4988-5	2.5	1.47
Amaranta	**3.0**	**1.35**
ORUS 5005-3	3.6	1.29
ORUS 4981-2	2.5	0.91
ORUS 4989-1	4.7	0.89
ORUS 4857-1	2.0	0.82
ORUS 4289-4	1.9	0.74
ORUS 4291-1	2.1	0.73
ORUS 4988-2	3.0	0.47
ORUS 5004-3	3.6	0.42
ORUS 5004-2	2.6	0.22
ORUS 5004-5	2.9	0.18

Mean separation within columns by LSD, $p \leq 00.05$.

Table RY7. Ripening season for floricane fruiting red raspberry genotypes at OSU-NWREC. Planted in 2015 and harvested 2017-18.

Genotype	Year planted	Harvest season 5%	50%	95%	No. years in mean	Rep/ Obsv
ORUS 4611-1	2015	17-Jun	29-Jun	7-Jul	2	Obsv.
ORUS 4607-2	2015	20-Jun	2-Jul	14-Jul	2	Rep
ORUS 4603-2	2015	23-Jun	4-Jul	14-Jul	2	Rep
ORUS 4600-3	2015	24-Jun	4-Jul	16-Jul	2	Rep
ORUS 4600-2	2015	27-Jun	4-Jul	16-Jul	2	Rep
Meeker	**2015**	**26-Jun**	**5-Jul**	**16-Jul**	**2**	**Rep**
ORUS 4603-1	2015	26-Jun	5-Jul	16-Jul	2	Rep

Table RY8. Ripening season for primocane fruiting red raspberry genotypes at OSU-NWREC. Planted in 2016, 2016, or 2017 and harvested 2015-18.

Genotype	Year planted	Harvest season 5%	50%	95%	No. years in mean	Rep/ Obsv
ORUS 4988-2	2017	17-Jul	24-Jul	24-Jul	1	Obsv.
ORUS 4988-1	2017	17-Jul	24-Jul	14-Aug	1	Rep
ORUS 4291-1	2017	24-Jul	31-Jul	21-Aug	1	Obsv.
ORUS 4291-1	2015	4-Aug	5-Aug	19-Aug	3	Obsv.
ORUS 4988-3	2017	17-Jul	7-Aug	14-Aug	1	Rep
Amaranta	**2017**	**17-Jul**	**7-Aug**	**28-Aug**	**1**	**Obsv.**
ORUS 4864-1	2016	24-Jul	7-Aug	21-Aug	1	Rep
ORUS 5005-3	2017	31-Jul	7-Aug	28-Aug	1	Obsv.
ORUS 4981-2	2017	31-Jul	7-Aug	4-Sep	1	Obsv.
ORUS 4289-4	2017	31-Jul	14-Aug	14-Aug	1	Obsv.
ORUS 4858-3	2016	31-Jul	14-Aug	28-Aug	1	Obsv.
ORUS 4873-1	2016	31-Jul	14-Aug	28-Aug	1	Obsv.
ORUS 4988-5	2017	31-Jul	14-Aug	4-Sep	1	Obsv.
ORUS 4872-1	2016	31-Jul	14-Aug	18-Sep	1	Obsv.
ORUS 4988-4	2017	7-Aug	14-Aug	21-Aug	1	Obsv.
Lagorai Plus	**2017**	**7-Aug**	**14-Aug**	**28-Aug**	**1**	**Rep**
ORUS 5005-2	2017	7-Aug	14-Aug	28-Aug	1	Rep
ORUS 5005-1	2017	7-Aug	14-Aug	4-Sep	1	Obsv.
BP-1 (Amara)	**2015**	**27-Jul**	**15-Aug**	**29-Aug**	**3**	**Obsv.**
ORUS 4858-2	2016	31-Jul	21-Aug	4-Sep	1	Obsv.
Vintage	**2016**	**31-Jul**	**21-Aug**	**4-Sep**	**1**	**Rep**
Heritage	**2016**	**7-Aug**	**21-Aug**	**4-Sep**	**1**	**Rep**
Imara	**2016**	**7-Aug**	**21-Aug**	**11-Sep**	**1**	**Obsv.**
ORUS 4494-3	2016	7-Aug	21-Aug	11-Sep	1	Obsv.
ORUS 5004-2	2017	14-Aug	21-Aug	21-Aug	1	Obsv.
ORUS 4289-3	2016	14-Aug	21-Aug	4-Sep	1	Obsv.
Heritage	**2017**	**14-Aug**	**21-Aug**	**11-Sep**	**1**	**Rep**
ORUS 4856-1	2016	14-Aug	21-Aug	11-Sep	1	Obsv.
Vintage	**2015**	**5-Aug**	**22-Aug**	**5-Sep**	**3**	**Rep**
Kokanee	**2015**	**8-Aug**	**22-Aug**	**12-Sep**	**3**	**Rep**
Heritage	**2015**	**12-Aug**	**24-Aug**	**7-Sep**	**3**	**Rep**
Kweli	**2016**	**7-Aug**	**28-Aug**	**11-Sep**	**1**	**Obsv.**
Kokanee	**2016**	**7-Aug**	**28-Aug**	**18-Sep**	**1**	**Obsv.**
ORUS 4857-1	2017	14-Aug	28-Aug	4-Sep	1	Obsv.
Kwanza	**2016**	**14-Aug**	**28-Aug**	**11-Sep**	**1**	**Obsv.**
ORUS 4716-1	2017	14-Aug	28-Aug	11-Sep	1	Rep
ORUS 4723-2	2016	14-Aug	28-Aug	18-Sep	1	Obsv.
ORUS 4874-1	2016	14-Aug	28-Aug	18-Sep	1	Obsv.
ORUS 4990-1	2017	14-Aug	4-Sep	26-Sep	1	Obsv.
ORUS 4722-1	2016	28-Aug	18-Sep	26-Sep	1	Obsv.
ORUS 4722-2	2016	28-Aug	18-Sep	26-Sep	1	Obsv.
ORUS 4989-1	2017	11-Sep	18-Sep	26-Sep	1	Obsv.
ORUS 5004-3	2017	11-Sep	18-Sep	26-Sep	1	Obsv.
ORUS 4861-1	2016	18-Sep	18-Sep	26-Sep	1	Obsv.
ORUS 5004-5	2017	18-Sep	26-Sep	26-Sep	1	Obsv.

Table BLKRY1. Mean yield and berry size in 2016-2018 for black raspberry genotypes at OSU-NWREC planted replicated trial in 2014. Harvested with Littau Harvester (Stayton, OR).

Genotype	Berry size (g) 2016-18	Yield ($tons \cdot a^{-1}$) 2016	2017	2018	2016-18
2016	1.7 a				3.77 a
2017	1.5 b				2.37 c
2018	1.5 b				2.03 b
Replicated					
Munger	**1.4 d**	**4.40 a**	**2.12 a-c**	**3.20 a**	**3.24 a**
ORUS 4154-1	1.6 bc	3.81 ab	2.45 a	2.76 ab	3.01 ab
ORUS 4412-2	1.7 a	4.56 a	1.87 a-c	2.26 b-d	2.90 a-c
ORUS 4499-1	1.5 cd	3.94 ab	2.41 a	2.25 b-d	2.87 a-c
ORUS 4410-1	1.5 d	4.39 a	1.64 bc	2.28 b-d	2.77 a-d
ORUS 4399-1	1.7 ab	3.41 ab	2.22 ab	2.54 a-c	2.72 b-d
ORUS 4395-1	1.6 a-c	3.34 ab	1.49 c	2.55 ab	2.46 c-e
ORUS 3381-3	1.7 a-c	3.06 b	2.21 ab	1.82 cd	2.36 de
ORUS 3902-2	1.2 e	3.03 b	1.88 a-c	1.66 d	2.19 e
Nonreplicated					
ORUS 4411-3	1.4	5.79	1.53	2.83	3.66
ORUS 4412-4	1.7	4.66	2.23	2.36	3.45
ORUS 4411-2	1.3	4.71	1.87	1.92	2.83
MacBlack	**1.6**	**3.16**	**1.68**	**1.28**	**2.42**

Mean separation within columns by LSD, $p \leq 0.05$.

Table BLKRY2. Yield and berry size in 2017-2018 for black raspberry genotypes planted in replicated trial and single observation plots in 2015 at the OSU-NWREC. Harvested with Littau Harvester (Stayton, OR).

	Berry size (g)	Yield (tons·a^{-1})		
	2017-18	2017	2018	2017-18
2017	1.2 a			2.48 a
2018	1.3 b			2.27 a
Replicated				
ORUS 4583-1	1.2 bc	3.18 a	2.65 a	2.92 a
ORUS 4559-1	1.2 d	2.71 ab	2.15 a	2.43 ab
ORUS 4583-2	1.1 d	2.60 b	2.25 a	2.43 ab
Munger	**1.4 ab**	**2.57 bc**	**2.24 a**	**2.40 ab**
ORUS 4396-2	1.5 a	2.01 c-e	2.47 a	2.24 b
ORUS 4553-1	1.4 a	1.97 de	2.42 a	2.20 b
ORUS 4155-2	1.3 bc	2.30 b-d	1.68 a	1.99 b
Nonreplicated				
ORUS 4304-128	1.4	2.11	1.64	1.87
ORUS 4159-2	1.5	-	1.85	1.85
ORUS 4304-12	1.3	1.88	1.21	1.54
ORUS 4562-1	0.9	2.51	0.52	1.52
ORUS 4304-179	1.5	1.58	1.44	1.51
ORUS 4412-5	1.7	2.16	0.51	1.33
ORUS 4550-1	1.0	2.02	0.63	1.32
ORUS 4549-1	1.2	1.79	0.80	1.29
ORUS 4587-1	0.9	1.79	0.53	1.16

Mean separation within columns by LSD, $p \leq 0.05$.

Table BLKRY3. Yield and berry size in 2018 for black raspberry genotypes harvested from a single plot planted in 2016 at the OSU-NWREC. Harvested by hand.

Genotype	Berry size (g)	Yield (tons·a^{-1})
ORUS 4585-1	1.1	3.28
ORUS 4304-192	1.0	2.95
ORUS 4311-1	1.0	2.84
Munger	**1.3**	**2.62**
ORUS 4686-3	1.0	2.56
ORUS 4305-51	1.3	2.46
ORUS 4304-5	1.2	2.43
ORUS 4305-44	1.6	2.40
Niwot	**0.9**	**1.78**
ORUS 4686-2	1.2	1.61
ORUS 4305-74	1.3	1.13

Table BLKRY4. Ripening season for black raspberry genotypes at OSU-NWREC. Planted in 2014-16 and harvested 2016-18.

Genotype	Year planted	Harvest season 5%	50%	95%	No. years in mean	Rep/ Obsv
ORUS 4686-3	2016	18-Jun	18-Jun	26-Jun	1	Obsv
ORUS 4305-74	2016	18-Jun	18-Jun	3-Jul	1	Obsv
Munger	**2014**	**13-Jun**	**25-Jun**	**3-Jul**	**3**	**Rep**
ORUS 4395-1	2014	19-Jun	25-Jun	3-Jul	3	Rep
ORUS 4412-4	2014	19-Jun	25-Jun	3-Jul	3	Obsv
ORUS 4499-1	2014	19-Jun	25-Jun	3-Jul	3	Rep
ORUS 3902-2	2014	19-Jun	25-Jun	4-Jul	3	Rep
Niwot (Floricane)	**2016**	**18-Jun**	**26-Jun**	**26-Jun**	**1**	**Obsv**
ORUS 4311-1	2016	18-Jun	26-Jun	26-Jun	1	Obsv
ORUS 4686-2	2016	18-Jun	26-Jun	26-Jun	1	Obsv
Munger	**2016**	**18-Jun**	**26-Jun**	**3-Jul**	**1**	**Obsv**
ORUS 4304-192	2016	18-Jun	26-Jun	3-Jul	1	Obsv
ORUS 4304-5	2016	18-Jun	26-Jun	3-Jul	1	Obsv
ORUS 4305-44	2016	18-Jun	26-Jun	3-Jul	1	Obsv
ORUS 4305-51	2016	18-Jun	26-Jun	3-Jul	1	Obsv
ORUS 4585-1	2016	18-Jun	26-Jun	3-Jul	1	Obsv
ORUS 4411-2	2014	19-Jun	26-Jun	3-Jul	3	Obsv
ORUS 4154-1	2014	19-Jun	26-Jun	4-Jul	3	Rep
ORUS 4399-1	2014	19-Jun	26-Jun	4-Jul	3	Rep
ORUS 4410-1	2014	19-Jun	26-Jun	4-Jul	3	Rep
ORUS 4412-5	2015	23-Jun	26-Jun	30-Jun	2	Obsv
ORUS 4411-3	2014	19-Jun	27-Jun	3-Jul	3	Obsv
ORUS 4412-2	2014	21-Jun	27-Jun	4-Jul	3	Rep
ORUS 4159-2	2015	25-Jun	28-Jun	9-Jul	1	Obsv
Munger	**2015**	**13-Jun**	**30-Jun**	**7-Jul**	**2**	**Rep**
ORUS 4155-2	2015	23-Jun	30-Jun	9-Jul	2	Rep
ORUS 4304-12	2015	23-Jun	30-Jun	9-Jul	2	Obsv
ORUS 4304-128	2015	23-Jun	30-Jun	9-Jul	2	Obsv
ORUS 4559-1	2015	23-Jun	30-Jun	9-Jul	2	Rep
ORUS 4583-1	2015	23-Jun	30-Jun	9-Jul	2	Rep
ORUS 4583-2	2015	23-Jun	30-Jun	9-Jul	2	Rep
ORUS 4553-1	2015	25-Jun	30-Jun	9-Jul	2	Rep
ORUS 4587-1	2015	25-Jun	30-Jun	9-Jul	2	Obsv
ORUS 4304-179	2015	23-Jun	2-Jul	9-Jul	2	Obsv
ORUS 4396-2	2015	25-Jun	2-Jul	9-Jul	2	Rep
ORUS 4550-1	2015	25-Jun	2-Jul	9-Jul	2	Obsv
ORUS 4562-1	2015	25-Jun	2-Jul	9-Jul	2	Obsv
ORUS 3381-3	2014	26-Jun	3-Jul	15-Jul	3	Rep
Mac Black	**2014**	**28-Jun**	**8-Jul**	**15-Jul**	**3**	**Obsv**

Project title: Understanding and enhancing biocontrol of SWD with native parasitoid releases

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Relationship to ORBC 2017 Research Priorities for 2018-2019 FY:
"Management and biology of spotted-wing drosophila".

Progress: In order to optimize mass releases and success in biocontrol against spotted-wing drosophila (SWD, *Drosophila suzukii*), we studied how released parasitoids could be impacted by different water management regimes in production systems. Most organisms must ingest water to compensate for dehydration. Longevity measurements indicated that *P. vindemiae* benefits from drinking water and from host-feeding on the water-rich hemolymph of SWD pupae. After exposing wasps to different water regimens, we observed increased host-feeding in water-deprived wasps despite honey availability. This resulted in higher SWD mortality because the host-feeding process killed the pupae, and because wasps that engaged in greater host-feeding and parasitized more hosts. The host-feeding time of water-deprived wasps doubled compared to individuals that had available water. Host-feeding did not affect parasitoid offspring mortality. We conclude that *P. vindemiae* benefits from ingesting water. We however also found that this may apply to how growers should consider common management practices such as irrigation. Situations where less water is available, such as drip irrigation may significantly increase biocontrol of SWD by the resident parasitoids. This is because under dry conditions, we believe that these parasitoids will host-feeds on SWD pupae as a water-intake strategy. This strategy enhances parasitoid survival and reproduction, with positive consequences to its host-killing capacity and potential as a biocontrol agent.
Both short- (30 minutes) and long-term (entire lifespan) provision of host, water + honey, or honey increased parasitoid survival compared to water and fasting. Long-term provision of water + honey caused the highest parasitoid survival, independent of sex and host availability. Long-term provision of honey supported the highest fecundity (432 offspring/wasp/life) and host-feeding (157 SWD pupae/wasp/life) rates, and the lowest sex ratios (0.50/wasp/life), independent of water availability. However, when we controlled for diet-related survival differences and calculated the daily performance, such honey effects were absent, and water deprivation resulted in the highest fecundity (23 offspring/wasp/day) and host-feeding (3.5 SWD pupae/wasp/day), independent of honey availability. Neither water nor honey contributed to parasitoid emergence rate (0.97). These findings show that short- and long-term water, sugar, and host availability affect the survival of *P. vindemiae*, and that water plays a direct role on parasitism capacity and host-feeding, while sugar indirectly affects parasitism capacity, host-feeding, and sex ratio by regulating female lifespan.

1. **Summary of achievements (2018):**
    - We investigated the impact of nutritional regimens on the biology of the resident parasitoid *Pachycrepoideus vindemiae* on spotted-wing drosophila (SWD).
    - Both short- and long-term availability of water, sugar, and host increased parasitoid survival.
    - Water deprivation directly elevated fecundity and host-feeding.
    - Sugar provision indirectly increased fecundity, host-feeding, and decreased sex ratio by extending parasitoid survival.
    - *P. vindemiae* has a high biocontrol potential against SWD, which can be maximized by manipulating nutrient availability.

2. Results

2.1. Importance of water on the attack of *P. vindemiae* on SWD

To determine whether *P. vindemiae* ingest free water and its potential impact on their life-history traits, we exposed adult female wasps to (1) water, (2) honey, (3) water + honey (independent sources), or (4) fasting (no water, no honey), in the presence and absence of fresh hosts (SWD pupae), for their entire life. Honey was included in the experimental design as a low-water energy source (Solayman Md. et al. 2015) to control for energy-deprivation. In host absence, water + honey extended female longevity more than either nutrient separately, significantly surpassing water by more than 6-fold and honey by more than 2-fold (Fig. 1a), giving the first demonstration that that host-deprived females of *P. vindemiae* seek and ingest free water. In host presence, no significant difference was observed among the water, honey, and water + honey treatments, but the latter was the only regimen that significantly increased longevity relative to fasting (Fig. 1a), indicating that host-provided females of *P. vindemiae* also seek and drink free water. In honey-fed wasps (honey, and water + honey), the presence of hosts dramatically shortened female longevity relative to host absence, exposing a trade-off between longevity and reproduction in sugar-rich environments. The opposite effect was observed in the honey-deprived individuals (water alone and fasting), demonstrating that females of *P. vindemiae* host-feed on pupae of SWD (Fig. 1a). A single host-feeding bout significantly increased female longevity by 1.3 days relatively to water-fed wasps (P<0.0001, Fig. 1b).

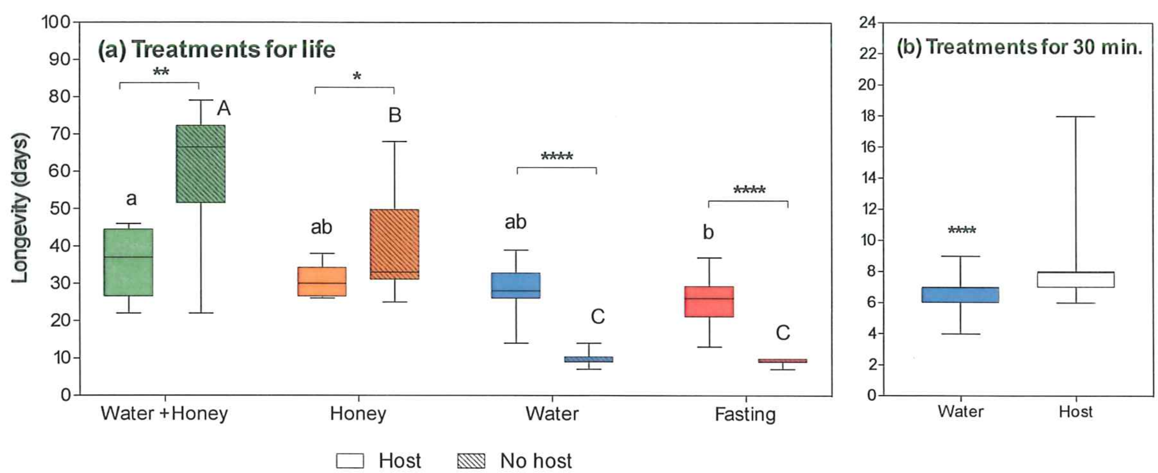


Figure 1. Effects of water, honey, and hosts on the longevity of females of *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae) reared on pupae of spotted-wing drosophila (*Drosophila suzukii*). (a) Uninterrupted provision of treatments, N=8-14 mated individual wasps. (b) 30-minute provision of treatments, N=70-94. Distinct lowercase and uppercase letters indicate significant differences among water/honey regimens within host-provided and host-deprived females, respectively (Kruskal-Wallis followed by Dunn's method). *P<0.05, **P<0.01, ****P<0.0001 according to either unpaired two-tailed t-test or Mann-Whitney U test.

Mean male longevity in fasting, water, honey, and water + honey was 10.4±0.5, 11±0.9, 24.5±3.1, and 55.8±5.3 days, respectively. In the presence of hosts, mean female longevity was 25.4±1.6, 28.2±1.8, 30.7±0.9, and 35.6±2 days, respectively. When hosts were absent, female longevity averaged 9.1±0.3, 9.8±0.2, 40.4±3.4, and 59.1±5.2, respectively. The availability of water + honey resulted in significantly higher survival curves than honey, water, and fasting, independent of sex and host availability. In host absence, both male and female *P. vindemiae* receiving honey displayed the second highest survival curve, followed by water and fasting, which did not significantly differ from each other (Table 1, Fig. 2ab). In host presence for female *P. vindemiae*, no significant differences in survival were found among the water, honey, and fasting treatments (Table 1, Fig. 2b). Considering each diet for female *P. vindemiae*, the survival curves for host presence and host absence significantly differed from each other. In honey and water + honey, the presence of hosts resulted in significantly lower survival curves than in their absence. Conversely, when considering water and fasting, the presence of hosts resulted in significantly higher survival curves compared to host absence (Table 1, Fig. 2b).

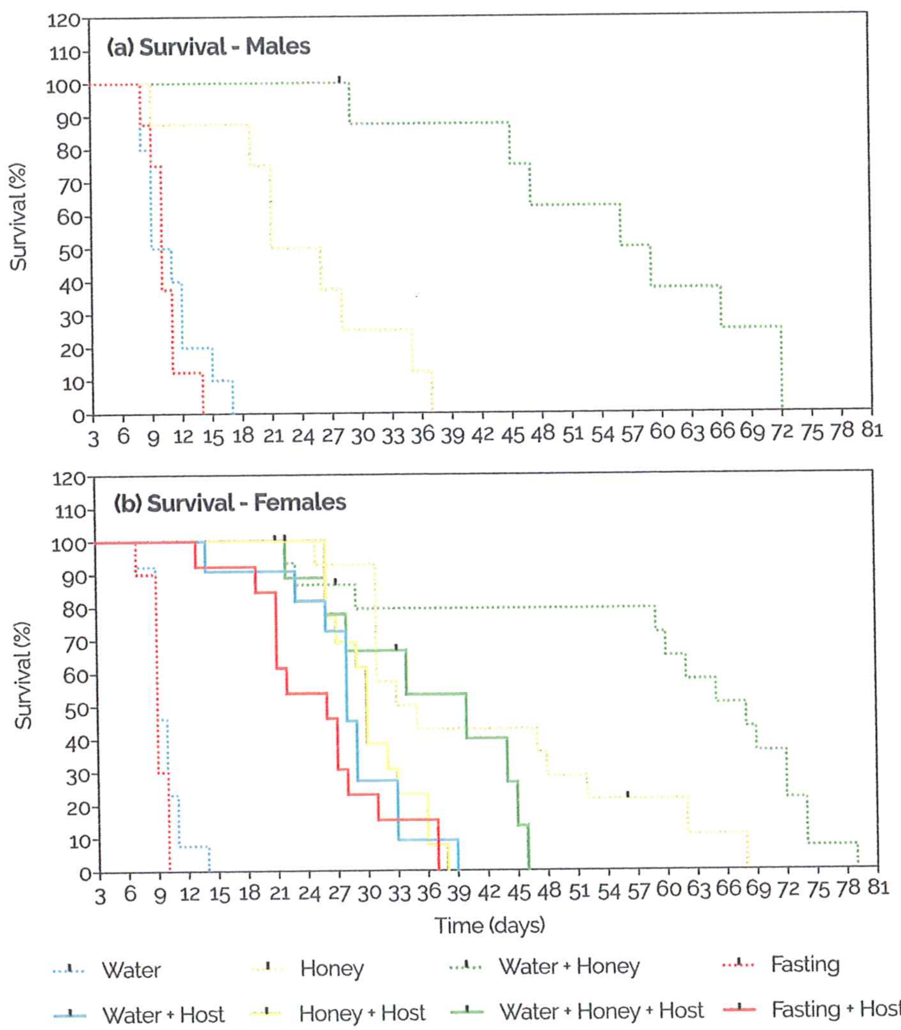


Figure 2. Effects of long-term availability of water, honey, and host on the survival curves of male (a) and female (b) *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae) reared on pupae of spotted-wing drosophila (*Drosophila suzukii*). Curves were estimated according to the Kaplan-Meier method. Dark dots represent censored data (either because wasp escaped or was accidentally killed when food, water, or hosts were being replaced). Comparisons among curves (long-rank Matel-Cox tests) are shown in Table 1. N=10-14 mated individual wasps.

Females fed water, water + honey, host, honey, and fasted for 30 minutes only survived for 6.6±0.11, 8.7±0.16, 7.9±0.18, 7.8±0.27, and 6.4±0.16 days respectively. Water + honey caused the highest survival curve, followed by honey and host (which did not significantly differ from each other), and by water and fasting (no significant differences between curves) (Table 1, Fig. 3).

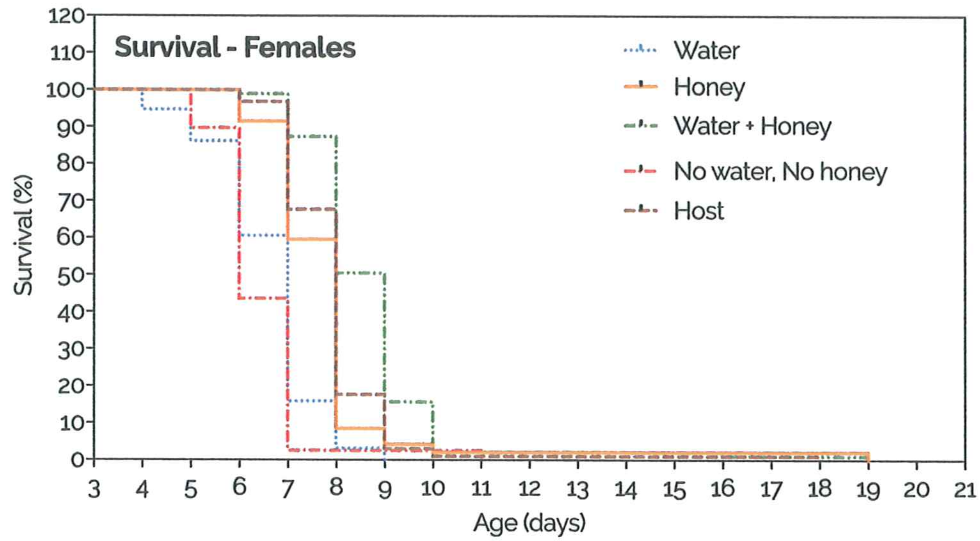


Figure 3. Effects of short-term availability (30 minutes) of water, honey, and host on survival curves of females of *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae) reared on pupae of spotted-wing drosophila (SWD, *Drosophila suzukii*). Curves were estimated according to the Kaplan-Meier method. Comparisons among curves (long-rank Matel-Cox tests) are shown in Table 1. N=39-96 mated individual wasps.

Table 1. Survival curve analysis (long-rank Mantel-Cox test) to evaluate the impact of short- long-term availability of water, honey, water + honey, and fasted (no honey, no water) on males and females of *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae), in the presence and absence of hosts (pupae of spotted-wing drosophila, *Drosophila suzukii*). N=8-14 (Assay 1) or 39-95 (Assay 2) mated individual wasps.

Contrast	χ^2	DF	P-value
Assay 1 – Long-term availability			
Males			
Honey *vs.* Water + Honey	15.08	1	0.0001***
Honey *vs.* Water	13.02	1	0.0003***
Honey *vs.* Fasting	10.94	1	0.0009***
Water *vs.* Water + Honey	19.89	1	<0.0001****
Water *vs.* Fasting	0.5688	1	0.4507 NS
Fasting *vs.* Water + Honey	18.15	1	<0.0001****
Females (no host)			
Honey *vs.* Water + Honey	7.577	1	0.0059**
Honey *vs.* Water	30.35		<0.0001****
Honey *vs.* Fasting	26.01	1	<0.0001****
Water *vs.* Water + Honey	33.73	1	<0.0001****
Water *vs.* Fasting	1.724	1	0.1892 NS
Fasting *vs.* Water + Honey	28.92	1	<0.0001****
Females + Host			
Honey *vs.* Water + Honey	4.71	1	0.0300*
Honey *vs.* Water	0.26	1	0.6101 NS
Honey *vs.* Fasting	2.283	1	0.1308 NS
Water *vs.* Water + Honey	5.731	1	0.0167*
Water *vs.* Fasting	1.345	1	0.2461 NS
Fasting *vs.* Water + Honey	7.742	1	0.0054**
Honey			
Females (no host) *vs.* Females + Host	7.167	1	0.0074**
Water			
Females (no host) *vs.* Females + Host	23.99	1	<0.0001****
Water + Honey			
Females (no host) *vs.* Females + Host	10.57	1	0.0107*
Fasting			
Females (no host) *vs.* Females + Host	24.55	1	<0.0001****
Assay 2 – Short-term availability			
Water + Honey *vs.* Water	107.2	1	<0.0001****
Water + Honey *vs.* Honey	22.61	1	<0.0001****
Water + Honey *vs.* Host	23.94	1	<0.0001****
Water + Honey *vs.* Fasting	92.14	1	<0.0001****
Water *vs.* Honey	118.6	2	<0.0001****
Water *vs.* Host	59.88	1	<0.0001****
Water *vs.* Fasting	2.49	1	0.1148 NS
Honey *vs.* Host	1.077	1	0.2994 NS
Honey *vs.* Fasting	32.32	1	<0.0001****
Host *vs.* Fasting	60.89	1	<0.0001****

*P≤0.05, **P≤0.01, ***P≤0.001, ****P<0.0001, NS= non-significant difference.

To elucidate whether water-deprivation increases host-feeding in *P. vindemiae* females, we measured the emergence rate of adult SWD from pupae exposed to wasps under the four water/honey regimens previously described. We observed a strong reduction of SWD emergence in water-deprived wasps relative to their water-fed counterparts, irrespective of honey availability (P<0.0001, Fig. 4a). Further analysis showed that such reduction was caused by clear increments in both parasitism capacity (P=0.0073, Fig. 4b) and host-feeding (P=0.0207, Fig. 4c) of water-deprived wasps. No significant effect of water or honey was found on parasitoid offspring mortality (Fig. 4d).

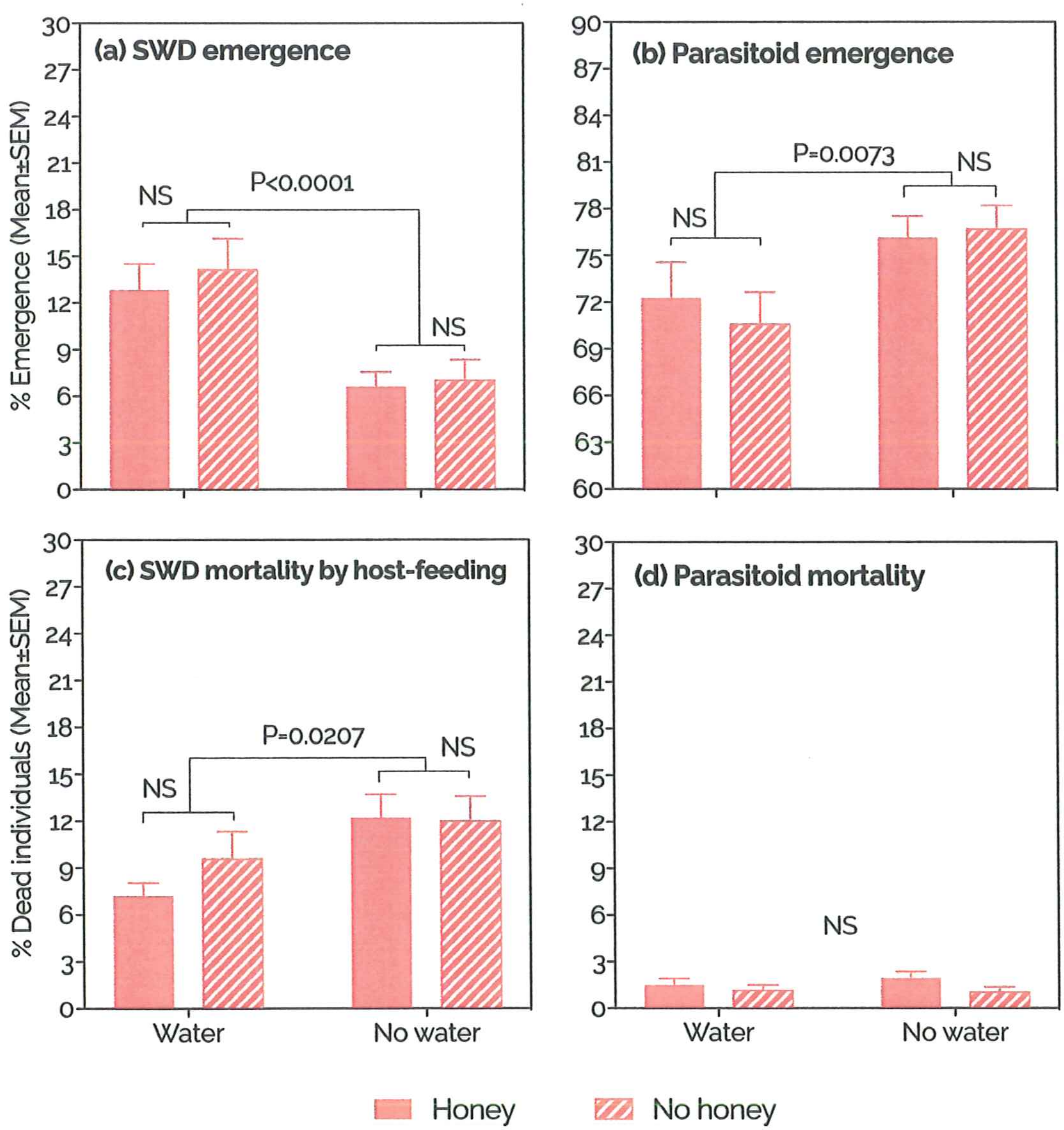


Figure 4. Effects of water and honey on life-history traits of *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae) reared on pupae of spotted-wing drosophila (SWD, *Drosophila suzukii*). (a) SWD emergence – percent flies that emerged as adults. (b) Parasitoid emergence – percent SWD pupal cases that gave rise to adult parasitoids. (c) SWD mortality caused by host-feeding – percent dead SWD pupae (excluding parasitism) minus natural death. (d) Parasitoid mortality – percent SWD pupal cases containing a dead parasitoid. P-values were calculated by Two-Way ANOVA (N=10-14 mated individual wasps); NS= no significant effect.

The parasitism rates of female *P. vindemiae* on SWD pupae were highest at 3-12 days old (the first 10 days of the study). After that time, parasitism rates consistently declined for all diet treatments. A stronger decline was observed in the water and fasting diets compared to the other treatments (Fig. 5ab). The parasitism capacity of *P. vindemiae* females was completely exhausted at ages *ca.* 26 (fasting), 31 (water), 35 (honey), and 40 (water + honey). The likelihood of wasps to remain alive at each of those ages was 48, 28, 40, and 40%, respectively, declining to 0% 3-11 days later. Daily fecundity (wasps aged 4-9 days old) was significantly affected by water availability (P=0.0045, Table 2), with water deprivation (honey and fasting) causing significantly higher offspring counts than water provision (water and water + honey) (Fig. 6a). Water availability, however, did not significantly affect total fecundity (entire lifespan) (P=0.3076, Table 2, Fig. 6b). Conversely, honey availability did not significantly affect daily fecundity of same-age individuals (P=0.2784, Table 2, Fig. 6a), but it strongly impacted the total fecundity (P<0.0001, Table 2, Fig. 6b), with both honey-provided diets (honey and water + honey) producing more offspring than the honey-deprived diets (water and fasting).

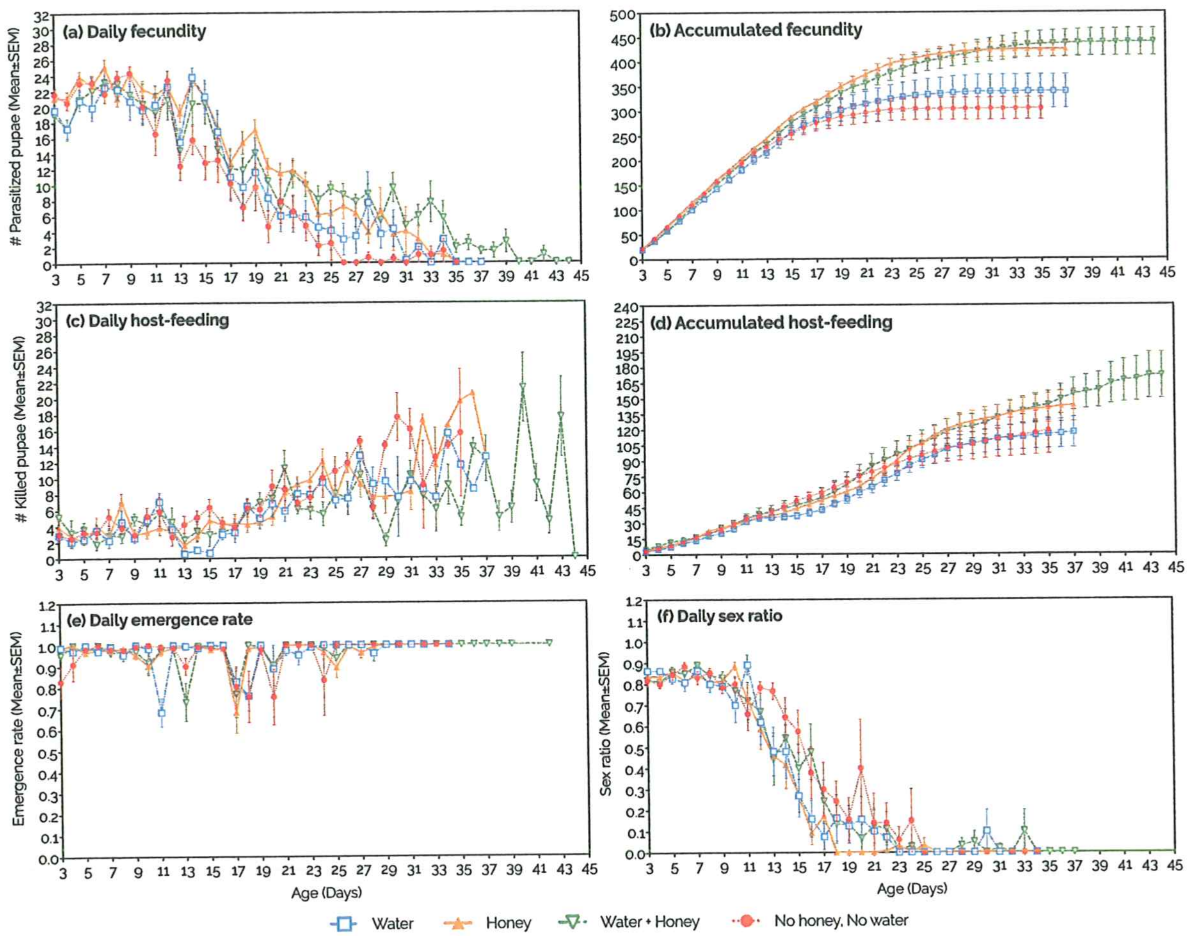


Figure 5. Effects of long-term availability of water and honey on life-history traits of females of *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae) reared on pupae of spotted-wing drosophila (SWD, *Drosophila suzukii*). Traits examined include daily fecundity (a), cumulative fecundity (b), daily host-feeding (c), cumulative host-feeding (d), daily parasitoid emergence rate (e) and daily sex ratio (f). N=10-14 mated individual wasps.

Table 2. Parametric Two-Way ANOVA to evaluate the effects of long-term availability of water and honey on several life-history traits of *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae) reared on pupae of spotted-wing drosophila (*Drosophila suzukii*). N=8-14 mated individual wasps.

Life-history trait	Age range	Source of variation	DFn, DFd	F	P-value
Fecundity	4-9 days old	Water	1, 44	8.969	0.0045**
		Honey	1, 44	1.205	0.2784 NS
		Interaction	1, 44	0.02397	0.8777 NS
	Entire lifespan	Water	1, 41	1.068	0.3076 NS
		Honey	1, 41	19.22	<0.0001****
		Interaction	1, 41	0.2097	0.6494 NS
Host-feeding	4-9 days old	Water	1, 41	5.748	0.0211*
		Honey	1, 41	0.0007962	0.9776 NS
		Interaction	1, 41	0.4119	0.5246 NS
	Entire lifespan	Water	1, 41	0.2126	0.6472 NS
		Honey	1, 41	4.547	0.0390*
		Interaction	1, 41	0.3465	0.5593 NS
Emergence rate	4-9 days old	Water	1, 40	0.1835	0.6707 NS
		Honey	1, 40	0.7755	0.3838 NS
		Interaction	1, 40	0.1153	0.7360 NS
	Entire lifespan	Water	1, 44	0.5582	0.4589 NS
		Honey	1, 44	1.295	0.2613 NS
		Interaction	1, 44	3.218	0.0797 NS
Sex ratio	4-9 days old	Water	1, 44	0.8284	0.3677 NS
		Honey	1, 44	0.755	0.3896 NS
		Interaction	1, 44	0.563	0.4571 NS
	Entire lifespan	Water	1, 41	1.558	0.2191 NS
		Honey	1, 41	19.01	<0.0001****
		Interaction	1, 41	3.724	0.0606 NS

*P≤0.05, **P≤0.01, ****P<0.0001, NS=non-significant effect.

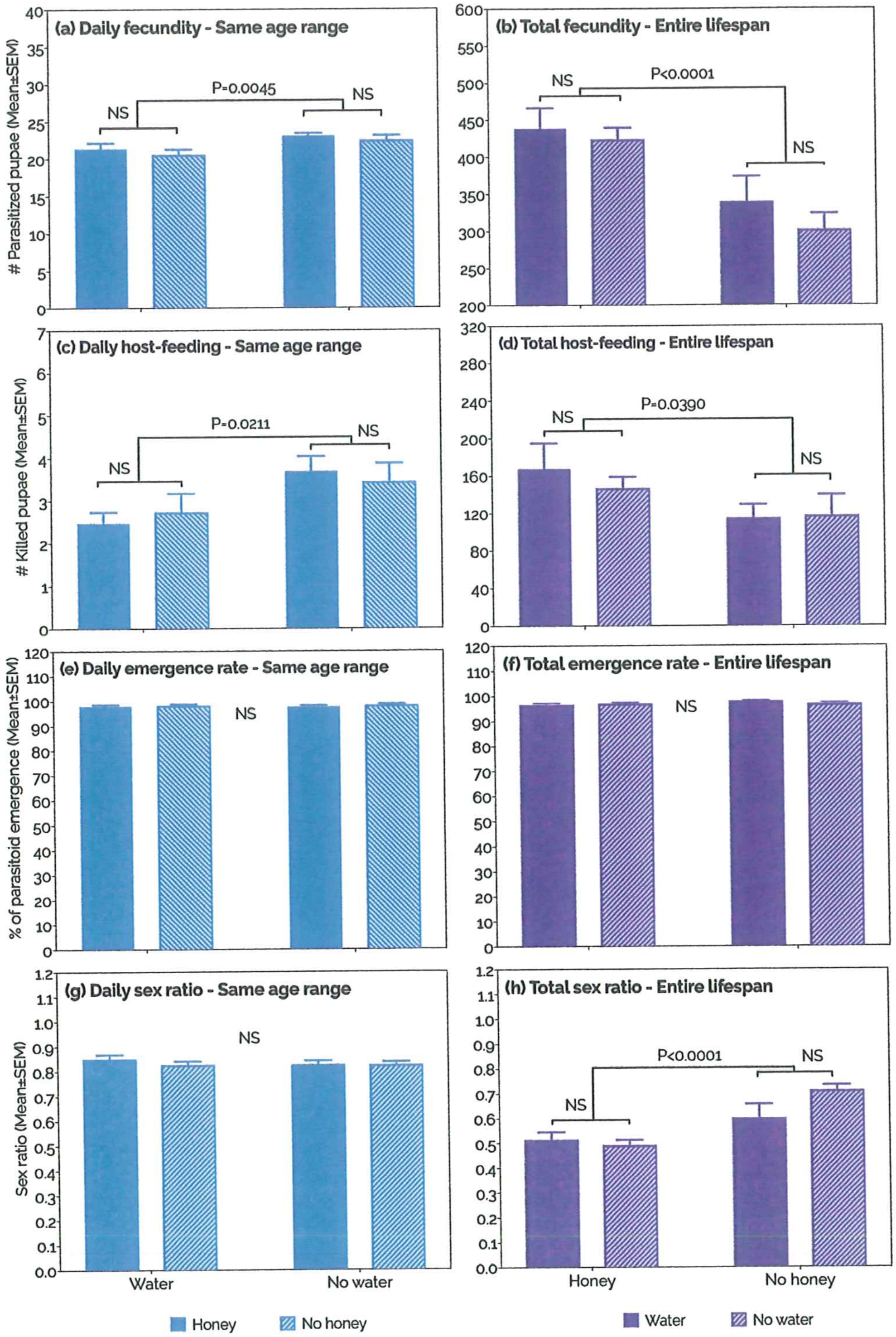


Figure 6. Effects of long-term availability of water and honey on daily (females aged 4-9 days old) and total (entire lifespan) measurements of fecundity (a, b), parasitoid emergence rate (c, d), sex ratio (e, f) and host-feeding (g, h) in *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae) reared on pupae of spotted-wing drosophila (SWD, *Drosophila suzukii*). P-values were calculated by Two-Way ANOVA; NS= no significant effect. N=10-14 mated individual wasps.

The number of SWD pupae killed by *P. vindemiae* through host-feeding increased as wasps aged independent of diet (Fig. 5cd). Daily host-feeding (wasps aged 4-9 days old) was significantly affected by water availability (P=0.0211, Table 2). Water-deprived females (honey and fasting) consistently killed more SWD pupae through host-feeding than their counterparts that had access to water (water and water + honey) (Fig. 6cd). Total host-feeding (entire lifespan) was significantly affected by honey availability (P=0.0390, Table 2), with honey-provided parasitoids (honey and water + honey) causing higher SWD mortality by host-feeding than their honey-deprived counterparts (water and fasting) (Fig. 6d).

In order to better understand how water-deprivation was affecting the host-feeding behavior of *P. vindemiae*, we filmed single water-deprived and water-fed wasps in arenas containing SWD pupae. We found no significant difference between the two groups regarding frequency of host-feeding, and 85-100% of the females practiced this behavior irrespective of the water regimen (Fig. 7a). Nonetheless, water-deprived wasps spent significantly more time host-feeding than their water-fed counterparts (P=0.0058, Fig. 7b).

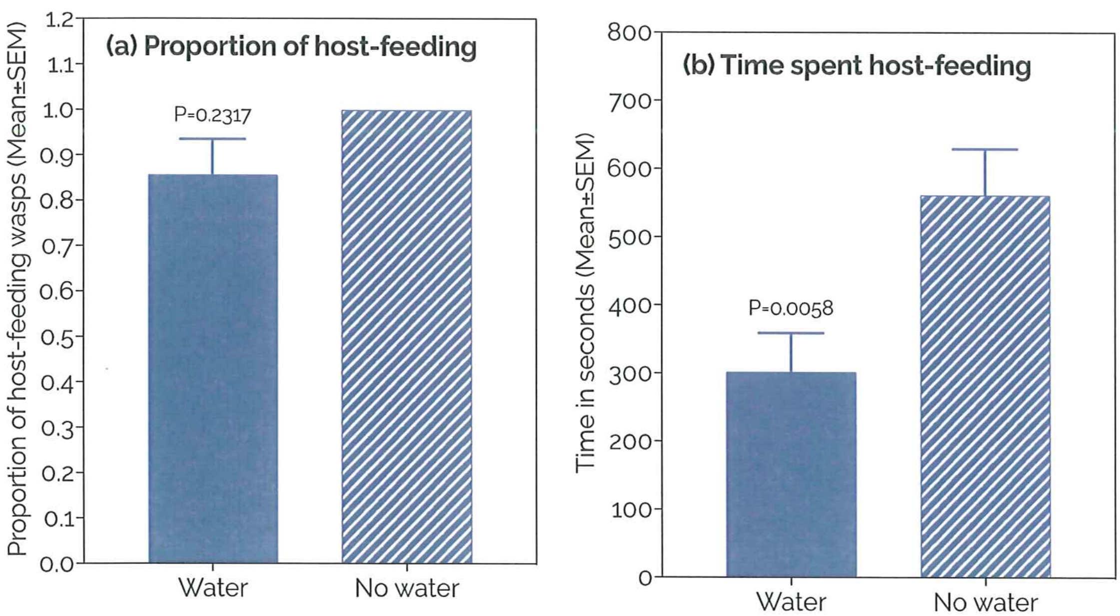


Figure 6. Effects of water on the host-feeding behavior of *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae) reared on pupae of spotted-wing drosophila (SWD, *Drosophila suzukii*). (a) Proportion of female wasps displaying host-feeding behavior, Mann-Whitney U test. (b) Length of host-feeding (seconds), unpaired two-tailed t-test. N=21-22 mated individual wasps.

Except for a few days along its total lifespan the emergence rate of *P. vindemiae* fluctuated around 100% along its total lifespan independent of diet (Fig. 5e). Neither water nor honey availability significantly affected *P. vindemiae*'s daily or total emergence rate (Fig. 6ef, Table 2).

The sex ratio (proportion of female offspring) of *P. vindemiae* was highest in young wasps, which produced *ca.* 80-90% of female offspring through the first seven days of the experiment (wasps aged 3-9 days) in each of the four treatments (Fig. 5f). From day eight on, the proportion of females consistently declined independent of diet, reaching zero between the ages of 18 and 25 days (Fig. 5f). Daily sex ratio (wasps aged 4-9 days) was neither affected by water (P=0.3677) nor honey (P=0.3896) availability (Table 2, Fig. 6g). Total sex ratio (entire lifespan) was strongly affected by honey availability (P<0.0001, Table 2). Significantly more females were produced by honey-deprived wasps (water and fasting) than by their honey-fed counterparts (honey and water + honey) (Fig. 6h).

In our initial experiment, the consumption of water + honey by host-deprived females of *P. vindemiae* increased their lifespan to a much greater extent than the sum of both nutrients offered separately, revealing a powerful synergistic effect of water + honey as a suitable nutrient regimen. Moreover, this was the only diet that extended female longevity in comparison to fasting when hosts were present. These results clearly show that females of *P. vindemiae* seek and consume free water, benefiting from it as long as a sugar source (honey) is available. While our findings strongly contrast with the literature (Heimpel et al. 1997; Olson et al. 2000; Lee and Heimpel 2008; Gómez et al. 2012; Hu et al. 2012; Zamek et al. 2013; Soyelu and Waladde 2013), such divergence is likely a result of two factors. First, these studies were often designed to assess the response of parasitoids to sugary solutions rather than water. The latter requires an experimental design that tests pure water and a negative control (total fasting) in the same study, as we did in ours. Second, relative humidity (R.H.) was often ignored in these studies. When water or an aqueous solution is offered to parasitoids, water vapor dissipates in to the immediate environment and increases the R.H. relative to a control that received no water or aqueous solution, and such R.H. divergences between treatment and control affect the parasitoid's physiology (Emana GD 2007; Tee and Lee 2015).

The fact that *P. vindemiae* females were able to feed directly on pure honey, which contains only 16-20% moisture (Solayman Md. et al. 2015), indicates that this species can feed on other viscous sugar sources such as concentrated honeydew, plant secretions, and fruit juices in the field. Moreover, *P. vindemiae*'s ability to drink free water, as shown in our study, may improve the uptake and digestion of highly viscous sugar sources such as honey (Lee and Heimpel 2008), which not only explains why water + honey caused the longest female longevities in our experiment, but also shows that they can better exploit viscous sugar sources in the field.

Although diet affected female lifespan independently of host availability, pairwise comparisons revealed a much weaker impact on host-provided individuals. Hence, to some extent, the presence of hosts stabilized female longevity relatively to host absence. Interestingly, this effect was triggered by two distinct mechanisms in a honey-dependent manner. First, individuals that consumed honey lived longer in the absence of hosts compared to when hosts were present, revealing a trade-off between longevity and reproduction in sugar-rich environments. In this scenario, while host-deprived wasps allocated all the energy obtained from honey meals exclusively to somatic maintenance, their host-provided counterparts invested it in somatic maintenance as well as in egg maturation, host foraging, and oviposition, processes with a high nutrient demand, thus explaining the reduced lifespan of females that had a chance to reproduce (Heimpel and Collier 1996; Chabi-Olaye et al. 2001; Wajnberg et al. 2012). Second, honey-starved individuals lived at least 2.5-fold longer in the presence than in the absence of hosts, providing unparalleled evidence that females of *P. vindemiae* host-feed on pupae of SWD and benefit from it to a great extent in sugar-poor environments. A bout of feeding on a single host was enough to extend female survival by more than one day. In addition, host-feeding improved female fecundity (as will be discussed below), indicating that wasps allocated nutrients obtained exclusively through host-feeding to both somatic maintenance and reproduction. This ability mitigates the parasitoids' dependency on alternative food sources such as nectar, honeydew, fruit juices, and other sugary substances, whose lack is seen as one of the causes of failure of arthropods in biological control programs (Stiling 1993; Heimpel and Jervis 2005; Winkler et al. 2006; Wäckers et al. 2008).

Given that *P. vindemiae* host-fed on SWD pupae, one may hypothesize that the wasps neither seek nor consume water and/or honey, thus explaining the much weaker impact of diet on longevity in host presence compared to host absence. However, the higher longevity observed in water + honey relative to fasting demonstrates that even when plenty of hosts are available the wasps still seek, feed, and benefit from independent water/sugar sources. This is an indication that provision of water and energy sources, although not critical for survival or reproduction of *P. vindemiae*, can contribute to its performance in the field as a biocontrol agent. In fact, exploring those sources could be a strategy to avoid the depletion of hosts since these lose quality after being fed on and will either produce smaller (thus less fit) offspring or will not be used for oviposition at all (Heimpel and Collier 1996), as was observed when *P. vindemiae* fed on pupae of *D. melanogaster* (Phillips 1993).

The higher host-feeding rates in water-deprived wasps demonstrates that such trophic behavior was employed as a strategy of water intake. Host-feeding was enhanced even in the presence of a highly energetic and phagostimulant nutrient such as honey (Wäckers et al. 2006), reinforcing that the wasps host-fed primarily for hydration rather than energetic purposes. This was confirmed in our behavioral assay, where water-deprived *P. vindemiae* females host-fed for a period twice as long as their water-fed counterparts. These are remarkable findings not only because water has rarely been reported as a critical nutrient for adult parasitoids, but especially because preying for the purpose of hydrating is not a common strategy in nature. Indeed, we are unaware of other reports on predators or parasitoids attacking, killing, and consuming prey for the purpose of quenching their thirst. It is important to note that by concluding that *P. vindemiae* females host-feed on hemolymph of SWD pupae as a strategy to ingest water we do not negate the contribution of the energetic fraction of the hemolymph. Our behavioral study revealed that most wasps practice host-feeding irrespective of water availability, suggesting that energetic needs also play a role in this trophic behavior.

The wasps that displayed enhanced host-feeding also showed the highest offspring emergence, which is a function of fecundity and offspring mortality. The latter was unaffected by the four water/honey regimens and thus cannot account for increased offspring emergence. This leaves fecundity as the cause of such an increase and hence establishes a connection among water availability, host-feeding, and fecundity. These findings seem very plausible if we consider that females of *P. vindemiae* are synovigenic and lay anhydropic eggs (Le Ralec 1995; Jervis et al. 2005), hence losing much water through oogenesis and oviposition. Because insect hemolymph is rich in water, as well as amino acids, proteins, carbohydrates, and lipids (Wyatt 1961; Heimpel and Collier 1996; Giron D. et al. 2002; Beyenbach 2016) – basically the same components that form the contents of insect eggs (Le Ralec 1995; Telfer 2009) – it is expected that increased host-feeding results in higher fecundity. By enhancing both host-feeding and fecundity, water-deprivation increased the overall host-killing capacity of *P. vindemiae*, resulting in half the SWD emergence of that observed in water-fed individuals. This has important implications to the mass rearing and use of this parasitoid in biological control programs as it demonstrates that by manipulating water availability it is possible to improve parasitoid yield and total pest-killing capacity. Moreover, the unheard strategy of drinking the host's hemolymph to hydrate is expected to be widespread in parasitic wasps as it is greatly adaptive in species whose adult females need water and food for egg maturation and whose females practice host-feeding. Both synovigeny and host-feeding are extremely common in parasitic wasps (Jervis et al. 2002; Ellers and Jervis 2004). Future studies should investigate whether pro-ovigenic species also employ this strategy, and how environmental factors such as R.H. affect it.

Even though data on survival of male hymenopteran parasitoids is scarce relative to females, there is some evidence that the wasp's sex can determine its lifespan (Maceda et al. 2003), especially when males and females differ in the way they allocate nutritional resources to survival and reproduction (Hoogendoorn et al. 2002). In our study, males and females of *P. vindemiae* were highly resilient in terms of survival. Offered both water and honey they lived for up to 72-79 days, and even those individuals that were kept in complete starvation lived for 6-10 days on average. This lifespan is long relative to other parasitoid species and to a Chinese population of *P. vindemiae* which under starvation only lived for 2.7 days (Heimpel et al. 1997; Olson et al. 2000; Hu et al. 2012; Zamek et al. 2013). It was clear that *P. vindemiae*'s survival was highly influenced by both short- and long-term availability of water and honey, especially when hosts were not available. For every diet tested, female survival was very consistent between the short- and long-term assays. Such consistency attests that feeding on water, sugars, and hosts have both immediate and cumulative benefits on the adult parasitoids. A single bout of feeding on those sources was enough to extend female lifespan by 1.2 to 2.1 days relative to starved individuals, likely more than enough time for the wasps to find a next meal. Curiously, a small fraction of those females (0.5-1%) lived for 17-19 days following a single feeding bout on water + honey, honey, or host. This is 3x longer than the majority of their counterparts that were fed the same diets, highlighting important intraspecific variability regarding nutritional requirements, which may contribute to the reestablishment of *P. vindemiae*'s populations in the field in case of extended periods of food scarcity. Additionally, by consuming water and sugars from separated sources, males and females show flexibility in relation to nutrient consumption. These findings indicate that both starvation and dehydration strongly reduce parasitoid survival, hence males and females of *P. vindemiae* must periodically search for sources of water and energy in the field. Because foraging strategies are under strong selection pressure to minimize the risks to survival and maximize the nutrient gain from feeding (Hassell and Southwood 1978), it is likely that the wasps will prefer sources where both water and sugars are combined in an ideal proportion to optimize foraging.

Females of *P. vindemiae* showed a tremendous killing potential against SWD. A single female wasp was capable of attacking between *ca.* 440 and 600 SWD pupae throughout her lifespan, depending on survival. Such high killing potential was achieved through a combination of parasitism and host-feeding (*ca.* 75% and 25%, respectively). Interestingly, long-term supply of honey led to the highest parasitism and host-feeding rates independent of water availability, showing that sugar plays a key role in the total host-killing capacity of *P. vindemiae* while water does not. Conversely, when we controlled for wasp age, no sugar impact was observed, while water affected parasitism and host-feeding as both rates were highest in water-deprived as opposed to water-fed individuals. These findings demonstrate that while water has a direct impact on *P. vindemiae*'s host-killing capacity, sugar has an indirect impact by extending wasp lifespan, thus giving sugar-fed wasps more opportunities to parasitize and host-feed. We have recently demonstrated that water availability directly affects both parasitism and host-feeding of young *P. vindemiae* (Bezerra et al., 2018), and (Phillips 1993) presented evidence that a single host-feeding bout on *D. melanogaster* increases the wasp's fecundity by 2 eggs. Nevertheless, we are unaware of other studies that provide a comprehensive description of *P. vindemiae*'s lifelong host-killing potential, or how nutritional factors like water and sugars affect such capacity.

At least one third of the females exhausted their parasitism capacity many days prior to death, independent of water and sugar availability. During the same period, host-feeding clearly rose, indicating that parasitism exhaustion was attributed to a lack of mature eggs rather than the inability to seek and drill hosts. Fasted and water-fed females were the first ones to exhaust their parasitism capacity, followed by wasps fed honey and water + honey, which is consistent with the results on survival and lifelong fecundity. Combined with the fact that honey did not affect the parasitism rates of young wasps, we infer from these findings that *P. vindemiae*'s dependence on sugar consumption increases as females age. The rise of host-feeding as females aged contributed to maintenance of a consistent host-killing potential despite the parasitoid's declining fecundity. This nearly complete asynchrony between host-feeding and fecundity may be an artifact of data collection methods, since SWD pupae that suffered both parasitism and host-feeding were counted exclusively for parasitism due to the lack of a more accurate method to measure host-feeding in parasitized SWD pupae. Phillips (1993) previously demonstrated that females of *P. vindemiae* can perform both host-feeding and parasitism on the same host without jeopardizing the survivorship or size of their offspring.

When provided with water and honey, each parasitoid was able to parasitize 438 SWD pupae throughout a 36-day lifespan, with fecundity smoothly declining as wasps aged. These results very much differ from those reported in the literature, where each honey-water-fed female of *P. vindemiae* parasitized only 78 SWD pupae and lived for only 22 days, with a sharp decline in parasitism capacity just before death (Rossi Stacconi et al. 2015). Even though the two studies maintained the wasps in very similar climatic conditions, the daily host supply regime strongly differed between the two. While we offered 30 hosts per day, the previous study offered only 5. This methodological divergence was likely the cause of the striking differences in total fecundity and pattern of parasitism exhaustion between the studies, but it does not explain the difference in lifespan. Because reproduction reduces parasitoid longevity (Heimpel and Collier 1996), and the investment in this activity was 5.5x higher in our study than in Rossi Stacconi et al. 2015, it is surprising that the former reports a much longer lifespan than the latter.

The rate of emergence of *P. vindemiae* was nearly 100%, independent of the diet offered to the adult mothers, or whether survival differences among diets were controlled for. These findings have three major implications. First, pupae of SWD can be considered highly suitable as hosts for *P. vindemiae* relative to other fly species such as *Ceratitis capitata*, *Bactrocera latifrons*, *B. cucurbitae*, and *Musca domestica*, which supported adult parasitoid emergence ranging from 35 to 85% (Pickens et al. 1975; Wang and Messing 2004; Zhao et al. 2013). Second, neither diet nor age affect the ability of female wasps to lay viable eggs as has been suggested in other studies(Olson et al. 2000, 2005; Cicero et al. 2012). And third, considering that host-feeding was directly affected by water availability while offspring emergence was not, we can infer that host-feeding of *P. vindemiae* on SWD pupae does not affect parasitoid offspring survivorship, which in turn implies that hosts that were considerably depleted by host-feeding are not used for oviposition.

Both maternal age and diet are known to affect the offspring sex ratios in parasitic wasps (King 1987). In our study, offspring sex ratio was negatively affected by the age of the mothers, independent of their diet. However, sugar-deprived mothers produced considerably more female offspring than their sugar-fed counterparts, an effect observed exclusively when we did not control for survival differences among diets (i.e., total sex ratio). Hence, it is clear that the effect of sugar on offspring sex ratio is related to the females' age instead of their diets. The mothers mated during the first 2 days following emergence and they were maintained alone beginning on day 3. Under these conditions, females lacked an opportunity to replenish their sperm storage as egg laying took place. By living longer, sugar-fed wasps laid more eggs thus having greater opportunities to deplete sperm storage, consequently producing proportionally fewer females than sugar-deprived parasitoids.

3. Peer-reviewed publications

3.1. **2018** – Bezerra Da Silva CS, Price BE, Walton VM. Thirsty parasitoids kill more: Host-feeding as a quench strategy in a parasitic wasp. Submitted to **Nature *Scientific Reports*** on September 28, 2018.

3.2. **2018 – Bezerra Da Silva C.S.**, Price BE, Soohoo-Hui A, Walton VM. Water, Sugar and Host Impact Important Life-History Traits of the Parasitoid *Pachycrepoideus vindemiae* on Spotted-Wing Drosophila (*Drosophila suzukii*). Submitted to the ***Journal of Pest Science*** on November 20, 2018.

4. Conference papers

4.1. **2018** – Bezerra Da Silva CS, Walton VM. Thirsty wasps kill more: Dehydration increases parasitism and host-feeding of *Pachycrepoideus vindemiae* on spotted-wing drosophila (*Drosophila suzukii*). *2018 ESA, ESC, and ESBC Joint Annual Meeting*, Vancouver, BC, Canada.

4.2. **2018** – Bezerra Da Silva CS, Price BE, Soohoo-Hui A, Walton VM. Two ways to kill a pest: parasitism and host-feeding of *Pachycrepoideus vindemmiae* on spotted-wing drosophila. *Orchard Pest and Disease Management Conference 2018*, Portland, OR, USA.

4.3. **2017** – Bezerra Da Silva CS, Price BE, Soohoo-Hui A, Walton VM. Effects of host availability and diet on the biology of *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae). *Entomology 2017*, Denver, CO, USA.

5. Practical and economic impact of this project

Here in the West, we are able to manipulate environmental conditions to our advantage with irrigation. Our studies demonstrate that *P. vindemiae* is long-lived and can significantly contribute towards SWD biocontrol through a combination of parasitism and host-feeding. We showed that short- and long-term availabilities of water and honey affect the survival of male and female wasps, as well as their parasitism capacity, sex ratio, and host-feeding behavior, with clear consequences for SWD mortality. By enhancing both host-feeding and fecundity, water-deprivation increased the overall host-killing capacity of *P. vindemiae*, resulting in half the SWD emergence of that observed in water-fed individuals. This has important implications to the mass rearing and use of this parasitoid in biological control programs as it demonstrates that by manipulating water availability it is possible to improve parasitoid yield and total pest-killing capacity. In the absence of water and sugars, females of *P. vindemiae* can rely exclusively on pupae of SWD to extensively extend their survival and increase their parasitism capacity. Even a single bout of feeding on hosts, sugar, and especially on both water and sugar, significantly extended the wasp's survival. Constant supplies of water and sugars in an environment without hosts can result in survival up to 72 days, allowing maintenance of parasitoid populations and resumed parasitism when hosts once again become available. But even if none of those resources are available, both males and females can survive for many days under complete starvation. Taken together, these characteristics demonstrate the high resilience and biocontrol potential of *P. vindemiae* against SWD, proving their increased likelihood of surviving unfavorable periods of water, food, and host scarcity in both the laboratory and field. These findings open many opportunities for additional studies. These include comparative studies with imported specialist parasitoids of SWD (Wang et al. 2016; Daane et al. 2016), controlled field studies to determine how nutrient supply can result in improved biocontrol (Shimoda et al. 2014; Kishinevsky et al. 2018), and studies to determine impacts of horticultural practices such as irrigation on the parasitoid efficacy. These data will also inform us to better ways to rear and release *P. vindemmiae* in the field to control SWD. We believe that the results collected from both the laboratory and field studies presented in this project can be used to effectively manage SWD populations, with practical and economic impacts on the blueberry industry.

Progress report FY 2017-2018

Title: Managing Brown Marmorated Stink Bug in caneberry using an Asian egg parasitoid

Principal Investigators: Nik Wiman and David Lowenstein

Summary: Brown Marmorated Stink Bug (BMSB) feeding threatens marketability of multiple crops including small fruits. Stink bugs can be managed through several labeled neonicotinoid and pyrethroid products as well as biological control by the egg parasitoid *Trissolcus japonicus,* known by its common name samurai wasp. Since the samurai wasp is being redistributed across Oregon, this beneficial insect has the potential to reduce BMSB populations and lower insecticide inputs needed to control the pest. Our first objective was to identify samurai wasp dispersal and parasitism in raspberry and blackberry fields. We found that wasps located and parasitized approximately 27% of BMSB egg masses placed in orchards. Though the greatest number of wasps were found 16 – 32 feet from the release point, we detected samurai wasp as far away as 164 feet from the point of release. Our second objective was to evaluate compatibility of samurai wasp with insecticides that are applied to manage SWD/BMSB and other pests in small fruits. We found the highest samurai wasp mortality from Entrust and average mortalities above 50% for three neonicotinoid and pyrethroids. Samurai wasp mortality was comparable to an untreated control after application of three compounds, the diamides Exirel and Altacor, and the bioinsecticide Grandevo. We demonstrated that samurai wasp's beneficial effects will be concentrated on field edges. Growers seeking a reduction in BMSB damage through releases of samurai wasp will need to release wasps at times that do not coincide within a week of insecticide applications to avoid non-target effects on parasitoids.

Objectives:

1) Evaluate samurai wasp dispersal in caneberry fields.

2) Investigate compatibility of samurai wasp with common insecticides.

Methods:

Objective 1) We investigated samurai wasp dispersal and host-location along 50 meter transects at raspberry fields at the North Willamette Research and Extension Center (Aurora, OR) and at the Lewis Brown Experimental Farm (Corvallis, OR). The Aurora site was treated with Mustang Maxx in 2017 and 2018 and Altacor in 2018, while the Corvallis site was unsprayed both years. We made two releases at per year at each field edge in 2017 and 2018 (Fig. 1). Separately in 2017, we released 40-50 female wasps in the field interior, to determine if dispersal varied between interior and edge locations. We placed yellow sticky cards or sentinel BMSB egg masses at 16, 32, 66, 98, 131, and 164 feet away from the release point. Sentinel egg masses are BMSB eggs reared in the laboratory that are mounted to paper and placed in the field is the standard technique for surveying egg parasitoid activity. However, it can be challenging to maintain BMSB colonies at a high enough level for consistent egg production.

Placement of unbaited yellow sticky cards has also been determined to be an effective technique for capturing adult samurai wasps that doesn't depend on egg production in BMSB colonies. After 72 hours, we collected yellow cards and egg masses. Yellow cards must be scanned for wasps; egg masses are held in the laboratory for emergence of wasps indicating that they were attacked by samurai wasp in the field. We compared the percentage of parasitized egg masses differed between distances using a generalized linear model with a binomial family.

Figure 1. (L to R) Left: A field assistant prepares to release samurai wasp at the edge of a raspberry field. Middle: The tiny wasps migrate to the leaves and developing fruit (black arrows). Right: A BMSB egg mass is attached beneath the foliage, and held with paper clips, to measure parasitism.

Objective 2) We investigated insecticide toxicity to samurai wasp in a laboratory bioassay and in the field. In the lab, we applied nine compounds at field rates (Table 1) using a Potter Spray Tower (Fig. 2). Insecticides were applied to glass plates. After drying overnight, we placed 5-7 female wasps into an arena (Munger cell) that contained both glass plates and counted the number of dead wasps at 1 and 24 hours after introducing wasps. In the field, we assessed samurai wasp survival from insecticide exposure in a hazelnut orchard. We were unable to find an experimental caneberry field that would be untreated and have mature fruit at a time when wasps were available for the project. We placed 5 samurai wasps inside a clip cage, attached it to the foliage of separate trees, and applied the same nine compounds separately. In both trials, we included an untreated control where wasps were not exposed to insecticides. We evaluated samurai wasp mortality after 24 hours and evaluated longevity and reproduction in surviving wasps. We compared wasp mortality between field and lab trials with Mann Wilcoxon tests.

Figure 2. In a lab assay, insecticides were applied by potter spray tower (left) onto glass plates. Wasps placed into glass plate arena (right) to measure lethality.

Table 1. Rate and details of insecticide applications in assays with number of replicates in lab toxicity assay.

Compound – trade name	Insecticide class	Active ingredient	Field rate	Lab rate	Replicates
Actara	4A	Thiamethoxam	4.5 fl oz / acre	4.5 fl oz / acre	12
Admire Pro	4A	Imidacloprid	2.4 fl oz / acre	2.4 fl oz / acre	19
Altacor	28	Rynaxapyr	4.5 fl oz / acre	4.5 fl oz / acre	15
Asana XL	3	Esfenvalerate	15 fl oz / acre	7.6 fl oz / acre	35
Brigade 2EC	3A	Bifenthrin	6.4 fl oz / acre	6.4 fl oz / acre	10
Entrust	5	Spinosad	10 fl oz / acre	4, 6 fl oz / acre	17
Exirel	28	Cyantraniliprole	20.5 fl oz / acre	20.5 fl oz / acre	20
Grandevo		Chromobacterium subtsugae strain PRAA4-1	3 lb / acre	3 lb / acre	17
Pyganic	3A	pyrethrins	15 fl oz / acre	15 fl oz / acre	14
Untreated control		Deionized water			35

Results:

Dispersal experiment: Of 237 egg masses placed in 2017 and 2018, samurai wasp parasitized 44. Data from interior and edge releases were pooled for analysis, as there was no difference in samurai wasp dispersal between each location. There was no significant difference in samurai wasp parasitism by distance ($\chi^2 = 2.65$, P = 0.95), although we tended to find the greatest amount of parasitized eggs up to 32 feet and at 164 feet from the release point. On yellow cards, we found no pattern of samurai wasp movement, with samurai wasps captured on cards at 16, 32, 131, and 164 m away from the point of releases. Nearly 15% of egg masses showed signs of predation from natural enemies (Fig. 3). We expected to find the greatest amount of biological control closest to field edges. Our results indicated that this is true by percentage, but wasps are also capable of moving across nearly the entire length (row total of 220 feet) of the experimental block in search of BMSB eggs.

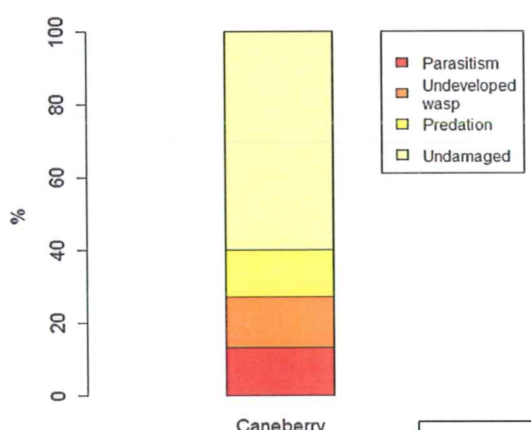


Figure 3. Classification of egg masses placed in caneberry fields, pooled across distances

Lab assay: After one hour of exposure to insecticide residue, mean *T. japonicus* mortality was above 76% in the presence of four compounds - Brigade, Pyganic, Asana, and Actara (Fig. 4). After 24 hours of exposure to insecticide residue, mean *T. japonicus* mortality was far greater (range: 80-100%) in all but two treatments (Fig. 5). *T. japonicus* mortality was significantly lower in Altacor and controls compared to other treatments ($\chi^2 = 87.3$, df = 9, P <0.001).

Field assay: The highest proportion of wasps died from application of Entrust or Brigade, and the lowest proportion of dead wasps occurred from application of Grandevo, Exirel or Altacor (Fig. 6). Wasps in the untreated control (UTC) lived an average (± SE) of 43 ± 6 days, which was not significantly different from longevity after exposure to most of the evaluated compounds (Table 2). Due to the limited number of samples, longevity was only significantly greater in Altacor and Exirel compared to Actara (F = 4.30, df = 8, 36, P = 0.001). Since only 3 treatments had ≥3 replicates of egg masses to evaluate parasitism, we have limited ability to assess sub-lethal effects of insecticides on reproduction. In these treatments, Admire Pro, Pyganic, and the untreated control, samurai wasp emergence rate was 41-51%. Field mortality was significantly lower in five insecticide treatments with at least 40% fewer dead wasps in exposure to Exirel (W = 12, P<0.001), Grandevo, (W = 28.5, P < 0.001), Pyganic (W = 7, P < 0.001), and Asana (W = 52.5, P<0.001). *T. japonicus* mortality was comparable between field and lab assays for Admire (W =151.5, P = 1), Brigade (W = 65, P = 0.17), Entrust (W = 117, P = 0.92), and the untreated control (W = 402, P = 0.43).

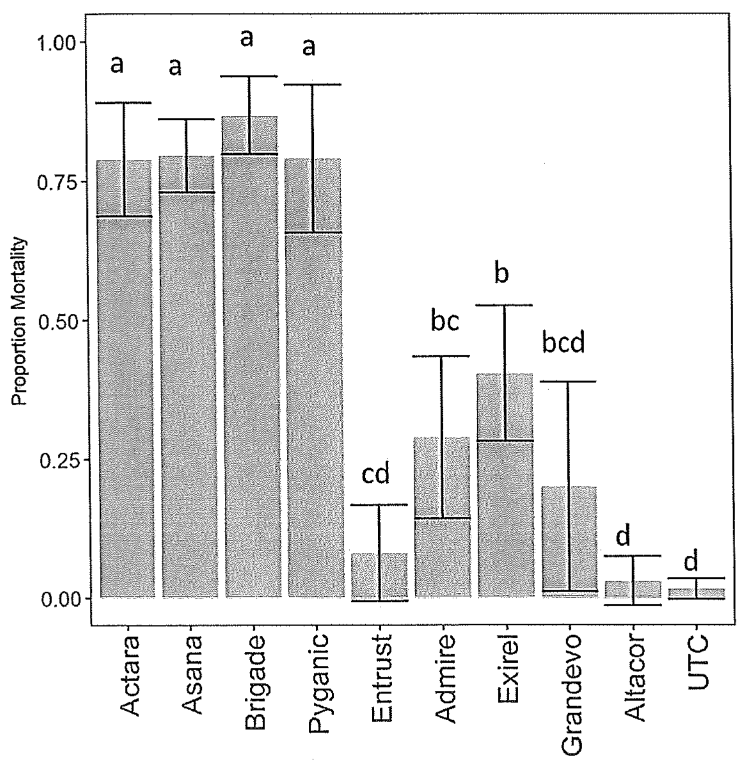


Figure 4. Proportion samurai wasp dead after **1 hour** of exposure to insecticides in lab assay. Letters above bars indicate significant differences.

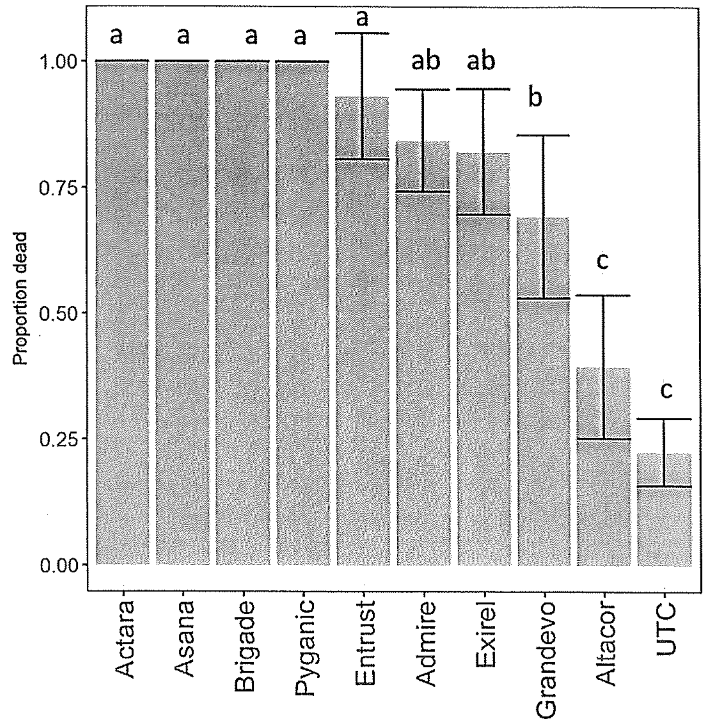


Figure 5. Proportion samurai wasp dead after **24 hours** of exposure to insecticides in lab assay. Letters above bars indicate significant differences.

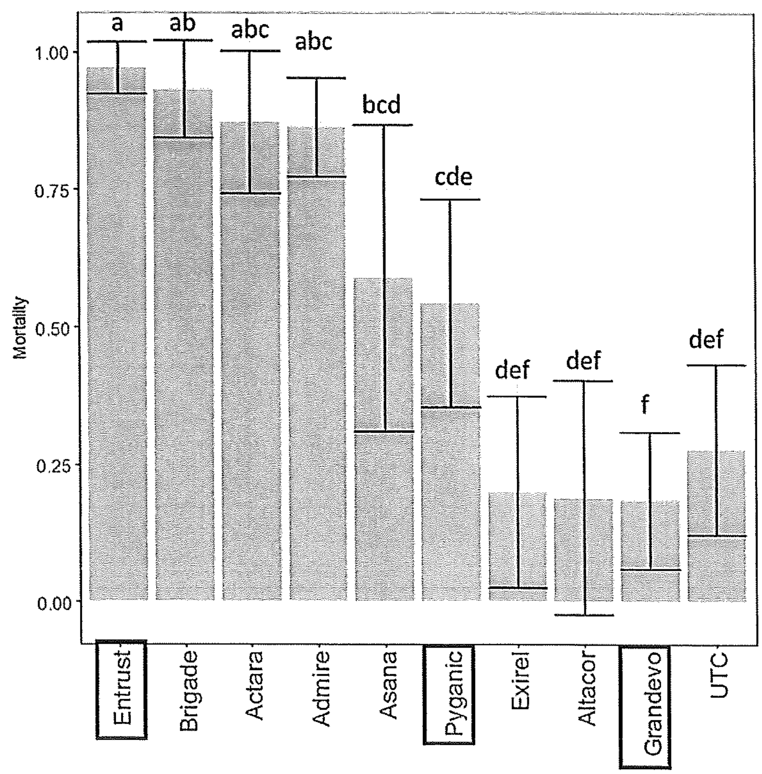


Figure 6. Proportion samurai wasp dead in field assay 24 hours after insecticide application. Organically-labeled compounds are noted by black squares around name.

Application:

Insecticides labeled for management of key arthropod pests in small fruits and orchard crops cause lethality for samurai wasp. Adult wasps fared poorly when in direct contact with insecticide residue, but a field trial demonstrated reduced mortality after application of all but thiamethoxam, bifenthrin, imidacloprid, and spinosad. Though mortality rates from these neonicotinoids and pyrethroids fit the definition of moderately harmful (80-98%) and harmful (>98%) effects to natural enemies per the International Organisation of Biological Control (Hassan 1992), adults that survived most field applications lived nearly as long as wasps from untreated controls. In particular, diamides, derived from alkaloids of the South American plant, *Ryania speciose*, show promise for limited non-target affects to *T. japonicus* and other natural enemies (Mills et al. 2016). Non-target effects of organic and conventional insecticides on *T. japonicus* will require assessment of Integrated Pest Management strategies to identify the tradeoffs between broad-spectrum insecticide application and other non-chemical controls.

Augmentative releases of *T. japonicus* are unlikely to succeed when broad-spectrum compounds are applied at times that coincide with BMSB egg-laying. Although wasps can disperse nearly 160 feet from release sites, non-target effects restricts the feasibility of using biological control in a setting with regular insecticide treatments. The use of reduced-risk compounds or releases in non-agricultural settings are expected to reduce BMSB populations and entry into caneberry fields from border habitats.

Table 2. Number of wasps surviving insecticide application in field and survivorship post-application.

Treatment	# Field replicates	# Field replicates with surviving wasps	Mean days until last survivor died ± SE
Actara	16	3	2 ± 0.6
Admire	16	9	36 ± 7.7
Altacor	11	10	71 ± 12.7
Asana	10	7	13.5 ± 6.5
Brigade	16	3	8
Entrust	14	2	0
Exirel	10	10	61 ± 7.5
Grandevo	18	17	30 ± 8.2
Pyganic	13	12	33 ± 10.6
Control	26	22	43 ± 6.1

References

Hassan, SA. 1992. Guidelines for testing the effects of pesticides on beneficial organisms: Description of test methods. IOBC/WPRS Bulletin 1992/XV/3.

Mills, N. J., E. H. Beers, P. W. Shearer, T. R. Unruh, and K. G. Amarasekare. 2016. Comparative analysis of pesticide effects on natural enemies in Western orchards: A synthesis of laboratory bioassay data. Biol Control 102: 17-25.

RESEARCH REPORT TO THE OREGON RASPBERRY AND BLACKBERRY COMMISSION AND THE AGRICULTURAL RESEARCH FOUNDATION 2017-2018

Title: **Evaluation of processing quality of advanced caneberry breeding selections**

Investigator: Brian Yorgey, Senior Faculty Research Assistant
Food Science & Technology, OSU

Cooperators: Chad Finn, USDA/ARS, Center for Small Fruits Research
Pat Moore, Washington State University

Objectives:

1. Evaluate advanced blackberry and raspberry breeding selections from NWREC and USDA for objective attributes related to processing potential
2. Process samples of advanced selections, selected field crosses, and standard varieties for display to and evaluation by breeders and the industry

Project Duration: July 1, 2017, through June 30, 2018

ORBC Funding for 2017-2018: $ 8585

Results:

2017 was one of the most messed up years I've had since I started working at OSU in 1985 – surpassed only by 2018. Even though I told our department head a year and a half ahead that I was planning to retire on April 1, 2017, and had a plan all worked out, he didn't start making anything happen until <u>after</u> I retired. This meant that I was left to run the whole processing season myself while being paid at 20% of full time salary. (This did get increased finally to 100% in August through the rest of the year.) A replacement was finally hired who started in December. However, his fiancée, who was in graduate school in our department, decided she didn't want to go on for a PhD and left after she completed her Masters degree. The replacement person quit at the beginning of June 2018.

In the 2017 season, we only processed the most important breeding selections and standard cultivars for display and evaluation. We did not do any chemistry analyses.

Caneberry varieties and selections from plots at the North Willamette Research and Extension Center were sent to the OSU Food Science Pilot Plant for processing from June 26 to September 12, 2017. The following numbers of genotypes were processed:

Blackberries – 4 processing cultivars, 6 ORUS processing selections, 4 fresh market floricane fruiting cultivars, 4 ORUS fresh market floricane fruiting selections, 1 fresh market primocane fruiting cultivar, 3 ORUS fresh market primocane fruiting selections
Red raspberries – 3 processing cultivars, 7 ORUS processing selections, 1 WSU processing selection, 4 primocane/fall fruiting cultivars, 6 ORUS primocane/fall fruiting selections
Black raspberries - 2 cultivars, 21 ORUS selections

Samples were displayed at the Research Evaluation at OSU in December, 2017, and at the ORBC Commission Research meeting – also in December, 2017.

Red Raspberry Puree Evaluation:

In March 2018, I presented purees of four advanced red raspberry selections from our breeding program along with Meeker and Cascade Premier as standards to growers, processors, and researchers in a blind evaluaton. The purees were rated on a 1 to 9 scale for overall quality, aroma, color, flavor, and bitterness. Sixty nine people participated. The results are shown in the figure following.

Results:

Overall Quality
Four selections scored in the highest tier: Meeker, Cascade Premier, ORUS 4465-3 and ORUS 4607-2. ORUS 4603-2 was rated lowest.

Aroma
Though Cascade Premier and Meeker were rated highest, three of the selections were rated statistically equal. Only ORUS 4603-2 was rated statistically lower.

Color
Cascade Premier was rated highest but all the samples were rated as statistically equivalent.

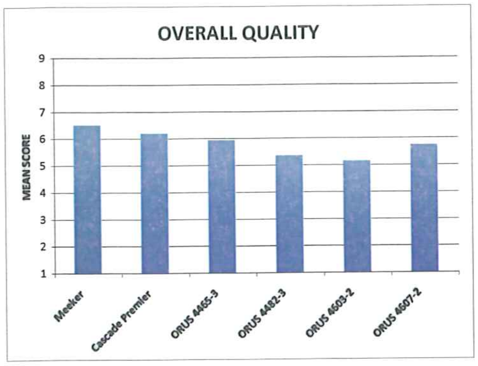
Flavor
The three highest rated selections in order were Meeker, Cascade Premier and ORUS 4465-3. ORUS 4603-2 was rated statistically lower than these.

Bitterness
The three highest rated samples were Meeker, ORUS 4465-3 and ORUS 4607-2 though only ORUS 4603-2 was rated significantly lower than these.

Discussion:

Of the four breeding selections evaluated, ORUS 4465-3 and ORUS 4607-2 appear to be the most promising at this point.

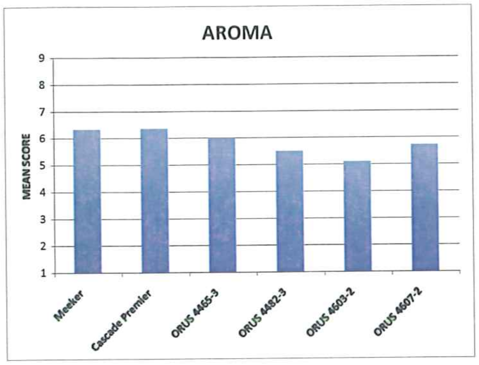
2017/2018 Red Raspberry Puree Evaluation



OVERALL QUALITY

ANOVA Mean Score
Tukey's HSD = 0.87
Values followed by the same letter are not statistically different.

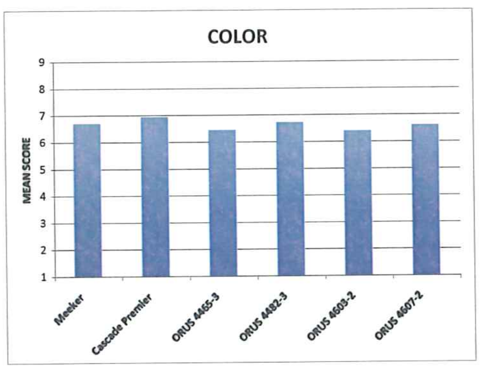
Meeker	6.53	a
Cascade Premier	6.22	ab
ORUS 4465-3	5.96	abc
ORUS 4607-2	5.76	abc
ORUS 4482-3	5.37	bc
ORUS 4603-2	5.16	c



AROMA

ANOVA Mean Score
Tukey's HSD = 0.91
Values followed by the same letter are not statistically different.

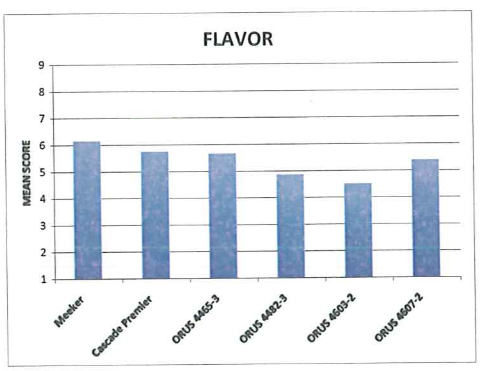
Cascade Premier	6.37	a
Meeker	6.35	a
ORUS 4465-3	6.00	ab
ORUS 4607-2	5.73	ab
ORUS 4482-3	5.51	ab
ORUS 4603-2	5.12	b



COLOR

ANOVA Mean Score
Tukey's HSD = 0.73
Values followed by the same letter are not statistically different.

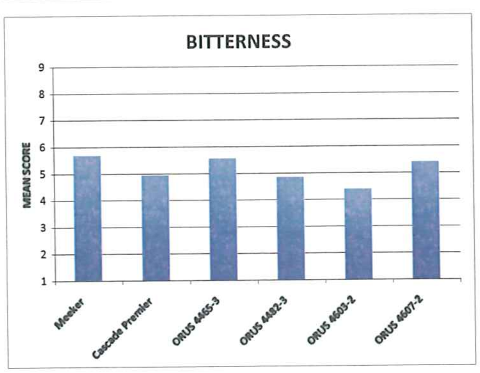
Cascade Premier	6.96	a
ORUS 4482-3	6.74	a
Meeker	6.72	a
ORUS 4607-2	6.62	a
ORUS 4465-3	6.46	a
ORUS 4603-2	6.42	a



FLAVOR

ANOVA Mean Score
Tukey's HSD = 0.98
Values followed by the same letter are not statistically different.

Meeker	6.16	a
Cascade Premier	5.76	ab
ORUS 4465-3	5.68	ab
ORUS 4607-2	5.4	bc
ORUS 4482-3	4.88	bc
ORUS 4603-2	4.52	c



BITTERNESS

ANOVA Mean Score
Tukey's HSD = 0.86
Values followed by the same letter are not statistically different.

Meeker	5.69	a
ORUS 4465-3	5.57	a
ORUS 4607-2	5.41	a
Cascade Premier	4.96	ab
ORUS 4482-3	4.86	ab
ORUS 4603-2	4.41	b