WASHINGTON MINT CONVENTION

WASHINGTON MINT FLAVORS THE WORLD

Washington Mint Growers

2017 Winter Conference

PROCEEDINGS

December 5, 2017

Yakima Convention Center

Yakima, WA

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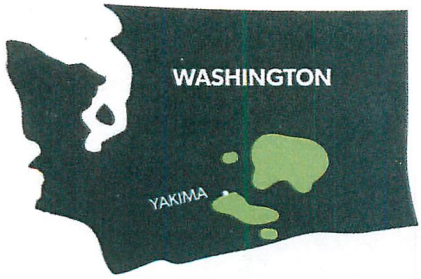
2017 Washington Mint Convention



Phil Dufault, Buyer/Fieldman
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WASHINGTON MINT CONVENTION

WASHINGTON MINT FLAVORS THE WORLD

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WASHINGTON MINT CONVENTION

WASHINGTON MINT FLAVORS THE WORLD

Yakima Convention Center, Yakima, WA
PROGRAM - Tuesday, December 5, 2017

7:00 a.m. Registration Desk Opens

Grab and Go Breakfast Sponsored by Callison's

Morning Session

8:00 a.m. **Welcome**

8:10 a.m. **Food Safety Modernization Act** – Gena Reich, WSDA Food Safety Program, Region Manager (TENTATIVE)

8:40 a.m. **Paid Sick Leave – New Law and Regulations** – Tuyen Manikhoth, Paid Sick Leave Outreach Specialist, Labor and Industries

9:10 a.m. **Agricultural Policy Update** – Madi Clark Agriculture Policy Research Director, WA Policy Center

9:40 a.m. **Financial Update** – Nancy Boettcher, Senior VP Commercial Banker, INB

10:10 a.m. ***Morning Break*** - *Coffee and Pastries Sponsored by RCB International*

Gift Card Raffle Drawing – Sponsored by Bleyhl Farm Service

10:30 a.m. **Fuel Update** –Tina Hampton, RE Powell

11:00 a.m. **AdvanceMint** - Jeremy Schifeling, Wrigley

11:20 a.m. **Up to Date Report on Activities of the Mint Industry Research Council** – Steve Salisbury, Research Director, MIRC

12:00 p.m. *Luncheon - Sponsored by Labbeemint*
Washington Mint Commission's – Annual Meeting
Mint Grower of the Year, Friend of the Industry, Lifetime Achievement Award

Afternoon Session – Researchers Session

Gift Card Raffle Drawing – Sponsored by Bleyhl Farm Service

1:30 p.m. **The Mint Varietal Improvement Project** – Mark Lange, Washington State University, Pullman, WA

1:50 p.m. **Diagnostics for Verticillium Wilt** – Dennis Johnson, Washington State University Pullman, WA

2:10 p.m. **Weed Research in Mint** – Rick Boydston, USDA-ARS, Prosser, WA

2:30 p.m. **Pyrethroids, Spider Mites, and Powdery Mildew** – Doug Walsh, Washington State University, Prosser, WA

3:00 p.m. ***Afternoon Break*** - *Mint Ice Cream Social Sponsored by Norwest Ingredients*

Gift Card Raffle Drawing – Sponsored by Bleyhl Farm Service

3:30 p.m. **Weather Forecast and Trends** –Tim Creek pacific weather consulting

4:20 p.m. **Buyers Report** – Dana Wendel, President, RCB International

5:00 p.m. **Awarding of Mint Baskets** - *Mint Baskets Sponsored by Northwest Farm Credit Services*

5:10 p.m. **Conference Adjournment**

5:15 p.m. WA Mint Growers Association Annual Meeting - All Are Invited to Attend

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Researchers Directory

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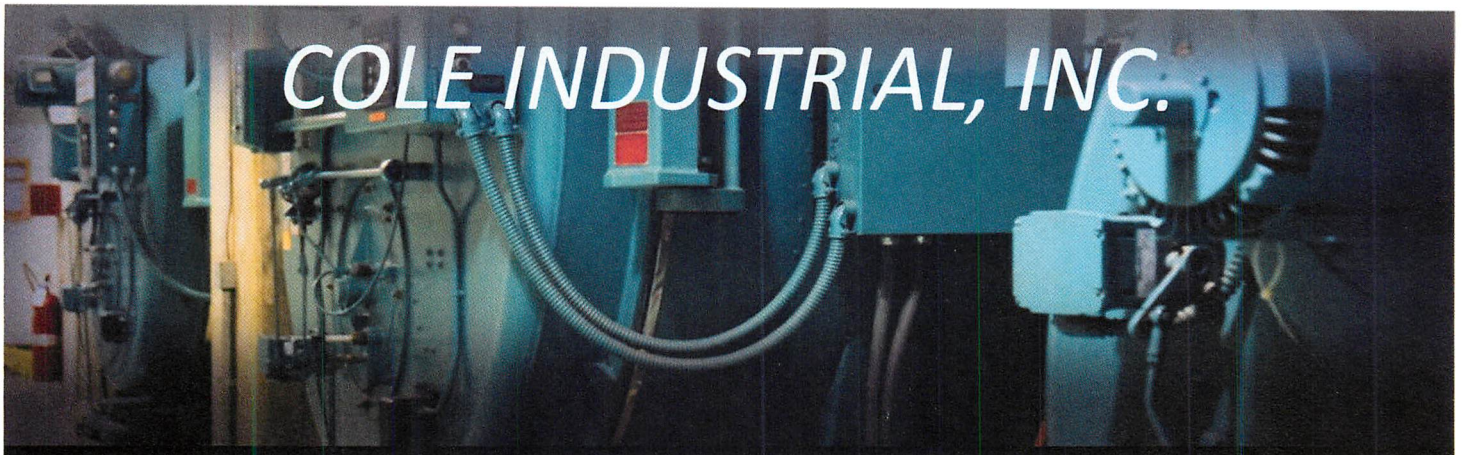

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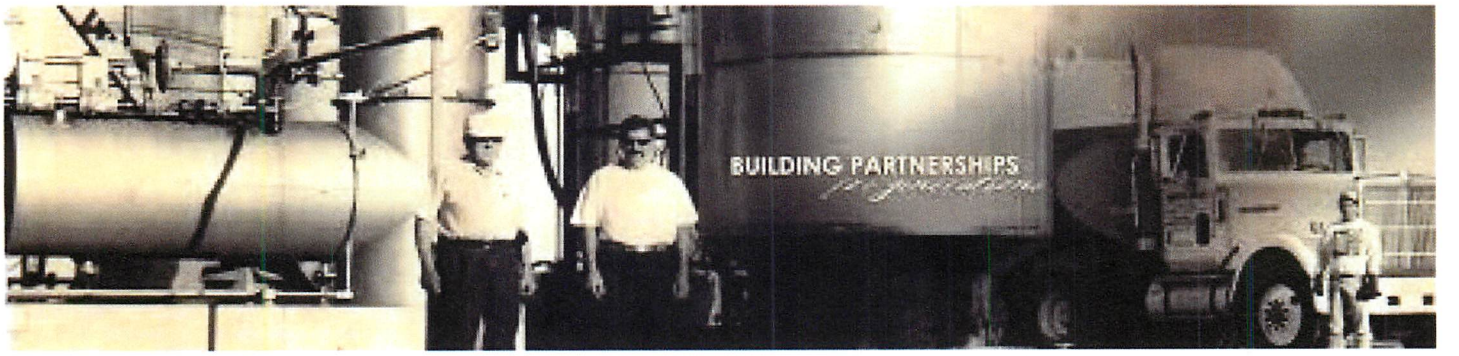

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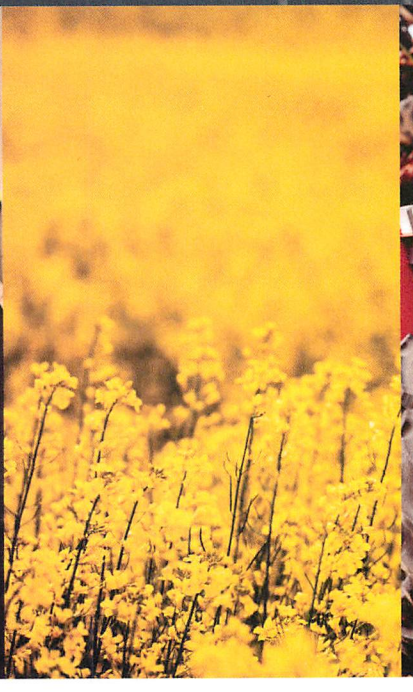

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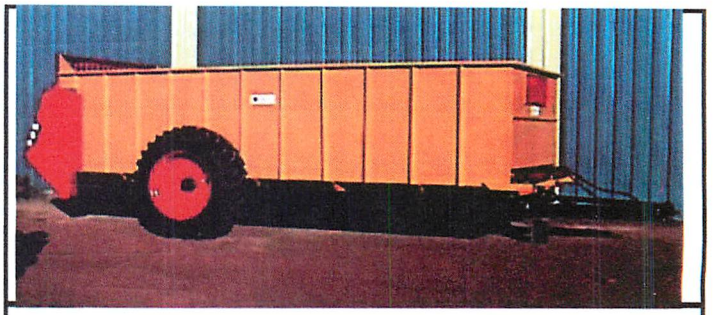

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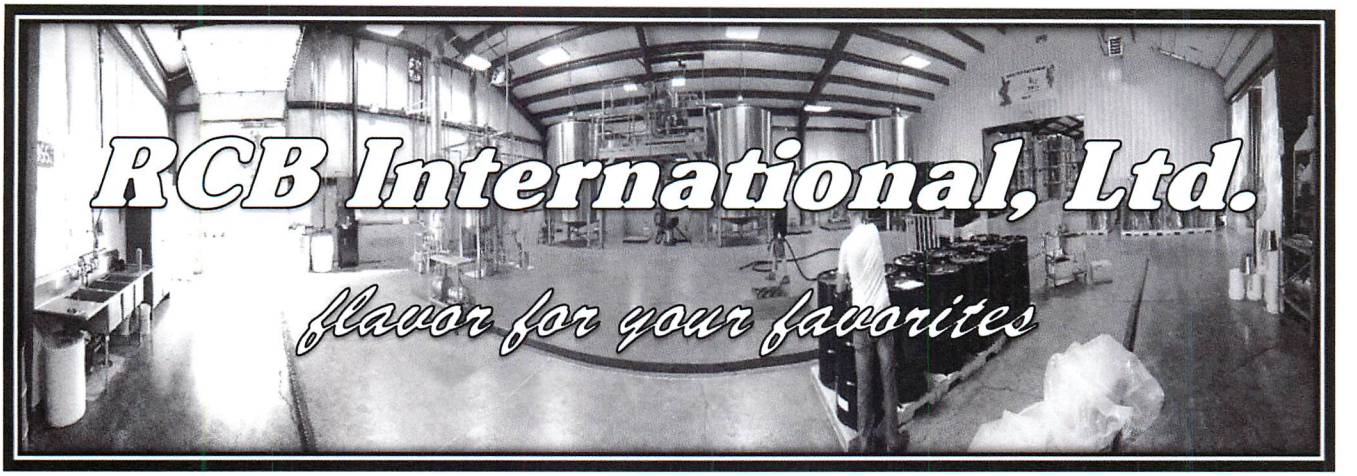

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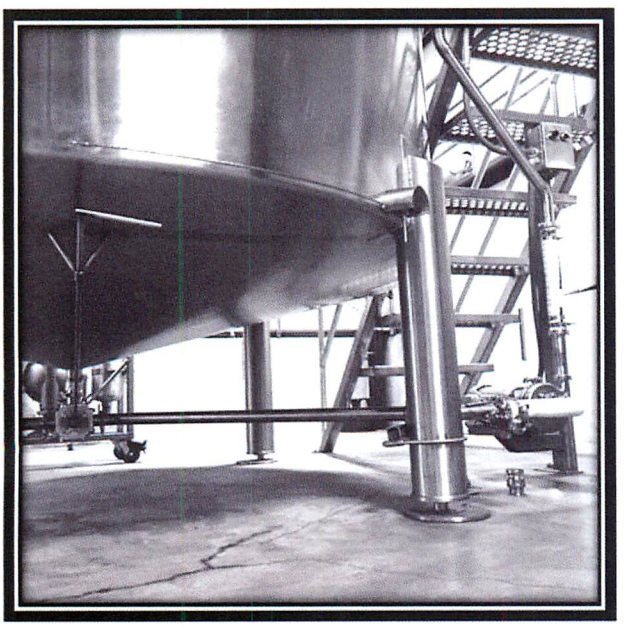

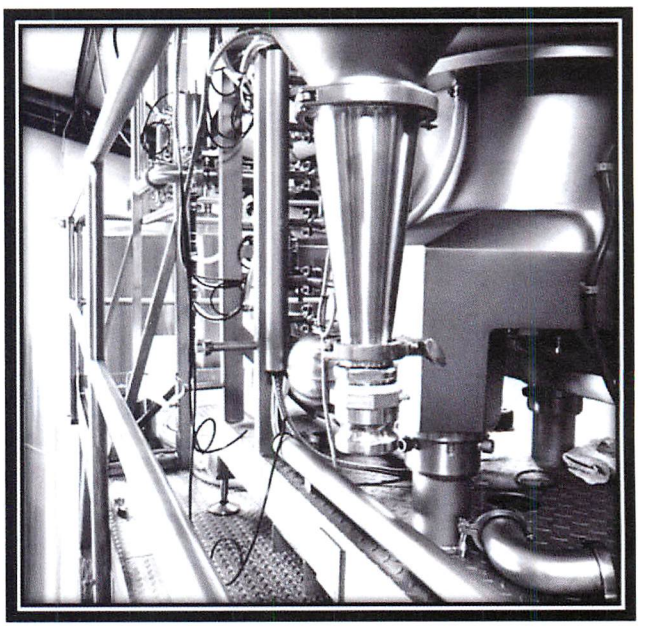

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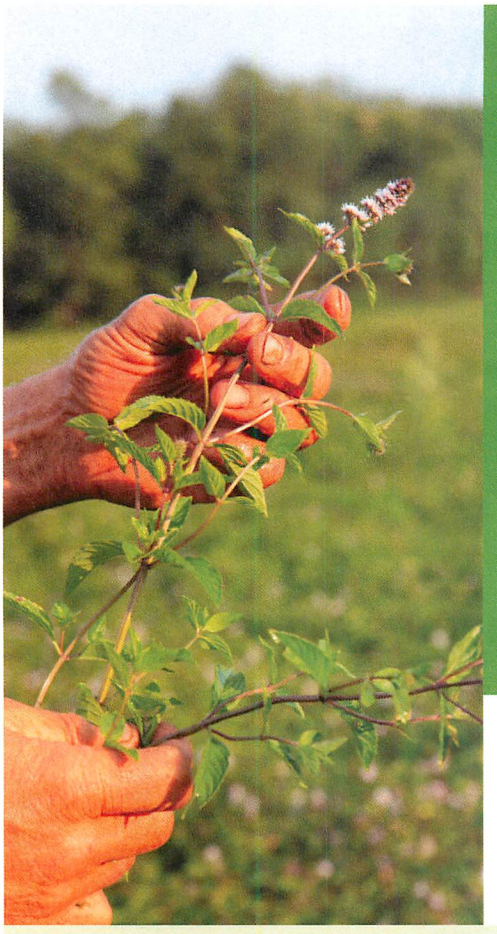

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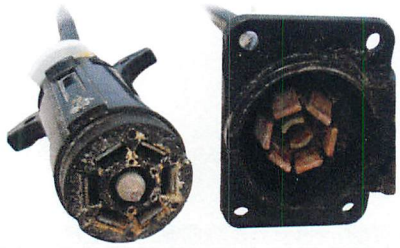

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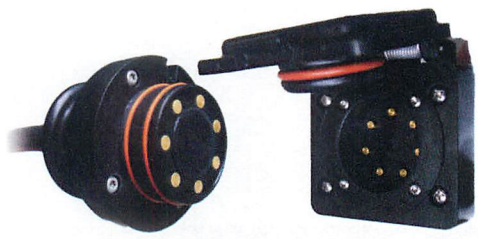

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Viking Cives Ltd	ON	Snow Equipment
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Mint Industry Research Council – Research Report, November 2017
Integrated Disease Management of Verticillium Wilt

1. Abstract

Sustainable mint production is threatened by the fungal pathogen, *Verticillium dahliae,* which causes Verticillium wilt in various crops worldwide. Management strategies that mitigate wilt are limited because *V. dahliae* survives as microsclerotia in soil for over a decade, infects several hundred crop species, and fumigants cannot be applied to perennial mint fields between growing seasons. Fast and sensitive diagnostic methods are required to detect, quantify, and differentiate strains of *V. dahliae* from soils before planting. The goal of this research was to develop and validate a quantitative polymerase chain reaction (qPCR) method to expedite accurate detection of *V. dahliae* from commercial mint fields. Soils samples were collected from 17 commercial mint fields from 2015 to 2017 in Washington and Oregon. *V. dahliae* was quantified from the soil using the traditional plating method and a qPCR assay optimized by Oregon State University (OSU). Plant-parasitic nematodes were also quantified from field soils since they can contribute to disease symptoms. Disease was assessed in mint fields and in a greenhouse where susceptible and resistant mint varieties were grown in field soils. Estimates of *V. dahliae* DNA from the qPCR method were positive correlated (please see Dung's report) with *V. dahliae* counts from the traditional plating method. The qPCR method was more sensitive than the traditional method and detected *V. dahliae* in soils where the traditional method did not. The qPCR method was reproducible across labs at OSU and Washington State University (WSU) with different personnel, machines, and reagents (see Dung's report). Wilt observed in commercial mint fields was correlated with *V. dahliae* DNA in Oregon (r_s = 0.49, P = 0.001) but not in Washington (r_s =0.14, P = 0.37). Wilt in fields that is not explained by *V. dahliae* inoculum is currently being explored and is likely due differences in strains of *V. dahliae,* nematodes, the cultivar of mint grown, and field age. Finally, the inoculum threshold of *V. dahliae* needed to cause wilt in mint is being determined by comparing *V. dahliae* DNA from inside vs. outside wilt foci in commercial fields in Washington.

2. Principal Researchers

Principal Investigators:

Dennis A. Johnson, Professor and Plant Pathologist, Department of Plant Pathology, Washington State University, P.O. Box 646430, Pullman, WA 99164-6430

Jeremiah K.S. Dung, Assistant Professor and Plant Pathologist, Central Oregon Agricultural Research Center (COARC), Oregon State University, 850 NW Dogwood Ln, Madras, OR 97741

Collaborators:

Russell E. Ingham, Professor and Nematologist, Botany and Plant Pathology, Oregon State University, 2082 Cordley Hall, Corvallis, OR 97331

Darrin L. Walenta, Associate Professor and Extension Agronomist, Union County Extension Office, Oregon State University, 10507 N McAlister Rd. #9, La Grande, OR 97850

3. Statement of Purpose

Verticillium dahliae is a perennial problem for peppermint and spearmint production. The long-lived microsclerotia of *V. dahliae* increase annually on debris of infected hosts and contribute to soilborne inoculum. Resident populations of *V. dahliae* are comprised of strains that vary in aggressiveness across dicotyledonous hosts including mint species. One strain of *V. dahliae* causes severe wilt on susceptible mint cultivars, is a member of vegetative compatibility group (VCG) 2B, and is genetically different from other strains (Douhan and Johnson 2001; Dung et al. 2013). Isolates of *V. dahliae* within the mint strain interact synergistically with the root lesion nematode, *Pratylenchus penetrans*, to cause more disease than either pathogen alone (Johnson and Santo, 2001).

Detection and differentiation of *V. dahliae* strains before planting is therefore imperative to determine which cultivar to plant and if soil fumigation is warranted. The traditional detection method for *V. dahliae* requires excessive time and resources to dry soil samples, plate samples onto semi-selective media, incubate, and count colonies. Several additional weeks are needed to differentiate strains of the pathogen.

The objective of this project was to develop fast and sensitive diagnostic method to detect, quantify, and discriminate the strain *V. dahliae* aggressive towards mint from soil. Molecular methods that utilize differences in DNA sequences are valuable alternatives to the traditional method because they are fast, sensitive to low levels of pathogen inoculum, and are reproducible across institutions. Polymerase chain reaction (PCR) uses short pieces of DNA called primers to find and anneal to a sequence of target DNA in a sample. The target sequence is then copied by a DNA polymerase enzyme which uses extra nucleotides to replicate the target sequence under favorable temperatures. The quantity of the target sequence of an organism can be estimated in real time with quantitative PCR (qPCR) where fluorescent dyes associated with the target DNA sequence are monitored and quantified by comparison to samples with known amounts of DNA.

To develop a qPCR test for *V. dahliae* we sequenced multiple genomic regions to identify sequences that differed among *V. dahliae* strains. All candidate regions sequenced did not yield differences that were consistent among strains. Primers from a published qPCR assay (Wei et al. 2015) were subsequently used together with a large scale DNA extraction method optimized by the OSU lab to detect less than one cell of *V. dahliae*/g of soil.

4. Materials and Methods

Soil samples from seventeen commercial mint fields were sampled in Washington and Oregon from 2015 to 2017. Three fields were sampled consecutively from 2015 to 2017 and three were sampled from 2016 to 2017 (figure 1). Fields were split into quarters and 30 subsamples from 9 inches into the soil profile were collected and bulked (figure 1).

From each soil sample *V. dahliae* was quantified using the traditional plating method and the qPCR method. Plant-parasitic nematodes were counted by OSU. Performance of the qPCR test was determined by comparing results from the traditional plating method with the qPCR method. The reproducibility of the qPCR method was evaluated by testing all samples from 2017 in labs at OSU and WSU with different personnel, experience levels, equipment, and reagents.

Disease pressure was assessed by planting susceptible and resistant mint cultivars in each soil and monitoring signs and symptoms of wilt in a greenhouse. Mint fields were also monitored for disease during the growing season by counting wilt foci and the number of stems/focus in each field quarter. The relationship between wilt in mint fields and *V. dahliae* DNA estimates from the qPCR was determined by comparing disease ratings from fields with *V. dahliae* DNA estimates. A predictive model is also being constructed and validated to predict wilt in fields from *V. dahliae* DNA levels, nematode counts, mint cultivar used, and the age of the field. The inoculum threshold of *V. dahliae* needed to cause disease in mint is currently being estimated by comparing *V. dahliae* DNA estimates from inside and outside of wilt foci from 5 foci/field in 3 fields in Washington.

5. Research Project Goals and Objectives

The objective of this project was to develop and validate a qPCR test to detect and quantify *V. dahliae* from commercial mint field soils.

6. Success Criteria and Timing

- Soil samples from five commercial mint fields in Washington and four commercial mint fields in Oregon were collected between the fall of 2016 and spring of 2017.
- From each soil sample:
    - *V. dahliae* was quantified using the traditional plating method.
    - DNA was extracted with the large-scaled method optimized by OSU.
    - *V. dahliae* was quantified using the qPCR method.
    - Nematodes were quantified at OSU by Russell Ingham.
    - Disease pressure was evaluated by planting susceptible scotch spearmint and resistant native spearmint in each soil sample, growing plants in a greenhouse, and monitoring symptoms and signs of Verticillium wilt.
- Lab to lab reproducibility was determined between labs at OSU and WSU.
- Disease was monitored in each commercial mint field.
- Soil samples were collected from inside and outside of wilt foci from 5 foci/field in 3 fields.

7. Data Analysis

Data from the qPCR test was analysed using QuantStudio Design and Analysis Software (version 1.4.1, Applied Biosystems). The sampling map, graphs, exploratory data analysis, and Pearson's and Spearman's (r_s) correlations were performed in R (version 3.3.3, R Foundation for Statistical Computing, Austria).

8. Results and Discussion

Inoculum of *V. dahliae* varied among fields and ranged from 0 to 135 colony-forming units (CFU)/g of soil (figure 2). Inoculum of *V. dahliae* consistently increased in 3/6 fields sampled over at least two consecutive years and remained relatively constant in the remaining fields (figure 3). Plant-parasitic nematodes also varied among fields and only root lesion nematodes were detected across most fields (figure 4-5). The bioassay is ongoing and will be completed by early 2018.

Inoculum estimates from the qPCR method were positively correlated with estimates from the traditional plating method (please see Dung's report). The qPCR method was more sensitive and detected *V. dahliae* in soils where the traditional method did not. The qPCR assay was also reproducible across labs (see Dung's report) where it was completed by different personnel using different equipment and reagents.

Wilt in mint fields also varied across fields and was correlated to *V. dahliae* inoculum in Oregon ($r_s = 0.49$, $P = 0.001$) but not in Washington ($r_s = 0.14$, $P = 0.37$) (figure 6). The presence of wilt when inoculum levels were low and no wilt when inoculum levels were high, as show in the upper left and bottom right corner of figure 6, provides observational evidence that strains of *V. dahliae* can cause differential disease. Additionally, the plateau observed in figure 6 where wilt no longer increases as a function of *V. dahliae* inoculum provides evidence of a limit of inoculum, after which disease does not increase. The variation observed around wilt that is not completely explained by *V. dahliae* inoculum is currently being modeled as a function of *V. dahliae* inoculum, plant-parasitic nematodes, mint cultivars in fields, and field age. The inoculum threshold is currently being determined by quantifying *V. dahliae* from inside and outside wilt foci using the qPCR assay and the traditional plating method.

9. Research Expenditures (MIRC and WA MINT COMMISSION FUNDS)

Salary[1]	7300
Benefits	4700
Supplies	3500
Travel	1500
TOTAL	17,000

[1]0.50 FTE for 4 months at step 32.50, Ph.D student

10. Conclusion

The qPCR method optimized by OSU provides estimates of *V. dahliae* from commercial field soils that correlate with the traditional plating method but requires less time and is more sensitive. The qPCR test is also reproducible across labs despite lab to lab differences. *V. dahliae* estimates from the qPCR method were related to wilt in commercial mint fields in Oregon but not Washington. The variation in wilt that was not

explained by *V. dahliae* inoculum may be explained by differences in strains, cultivars of mint, nematode populations, and the age of the field. A model is currently being developed to predict wilt based on the aforementioned variables. The inoculum threshold is also being determined and should provide additional information to help growers decide on pre-planting management decisions including fumigation and choice of mint cultivar. Cumulatively the qPCR assay developed and validated by the collaborative team at OSU and WSU will contribute to disease management efforts in mint production systems.

Literature Cited

Douhan, L. I., and Johnson, D. A. 2001. Vegetative compatibility and pathogenicity of *Verticillium dahliae* from spearmint and peppermint. Plant Dis. 85:297-302.

Dung, J. K. S., Schroeder, B. K., and Johnson, D. A. 2010. Evaluation of Verticillium wilt resistance in *Mentha arvensis* and *M. longifolia* genotypes. Plant Dis. 94:1255-1260.

Dung, J. K. S., Peever, T. L., and Johnson D. A. 2013. Verticillium dahliae populations from mint and potato are genetically divergent with predominant haplotypes. Phytopathology 103:445-459.

Johnson, D. A., and Santo, G. S. 2001. Development of wilt in mint in response to infection by two pathotypes of Verticillium dahliae and co-infection by Pratylenchus penetrans. Plant Dis. 85:1189-1192.

Wei, F., Fan, R., Dong, H.-T., Shang, W.-J., Xu, X.-M., Zhu, H.-Q., Yang, J.-R., and Hu, X.-P. 2015. Threshold microsclerotial inoculum for cotton Verticillium wilt determined through wet-sieving and real-time quantitative PCR. Phytopathology 105:220-229.

Figures.

Figure 1. Map of 17 fields in Washington and Oregon and sampling design used to collect soil from each field.

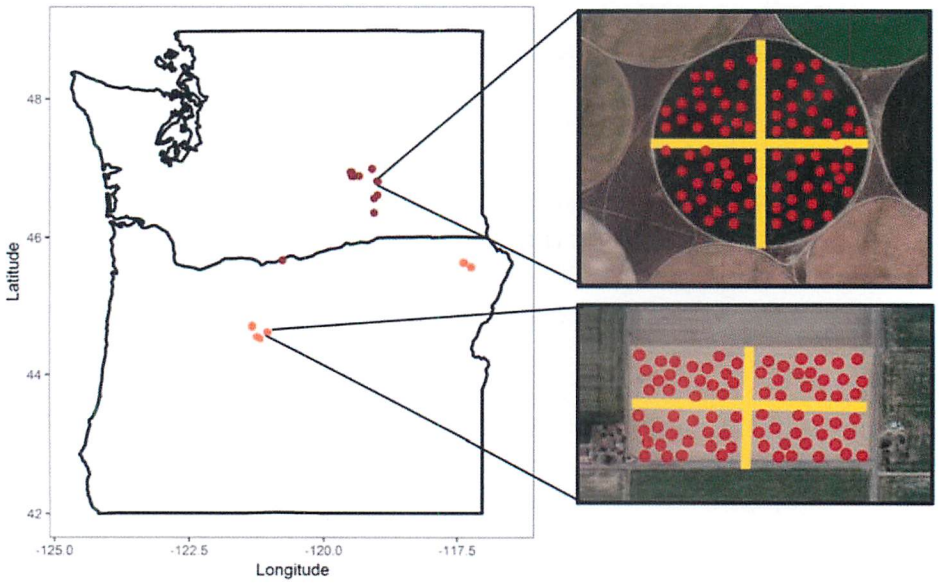


Figure 2. Inoculum density of *Vericillium dahliae* observed in commercial fields in Washington and Oregon from 2015-2017 from the traditional plating assay.

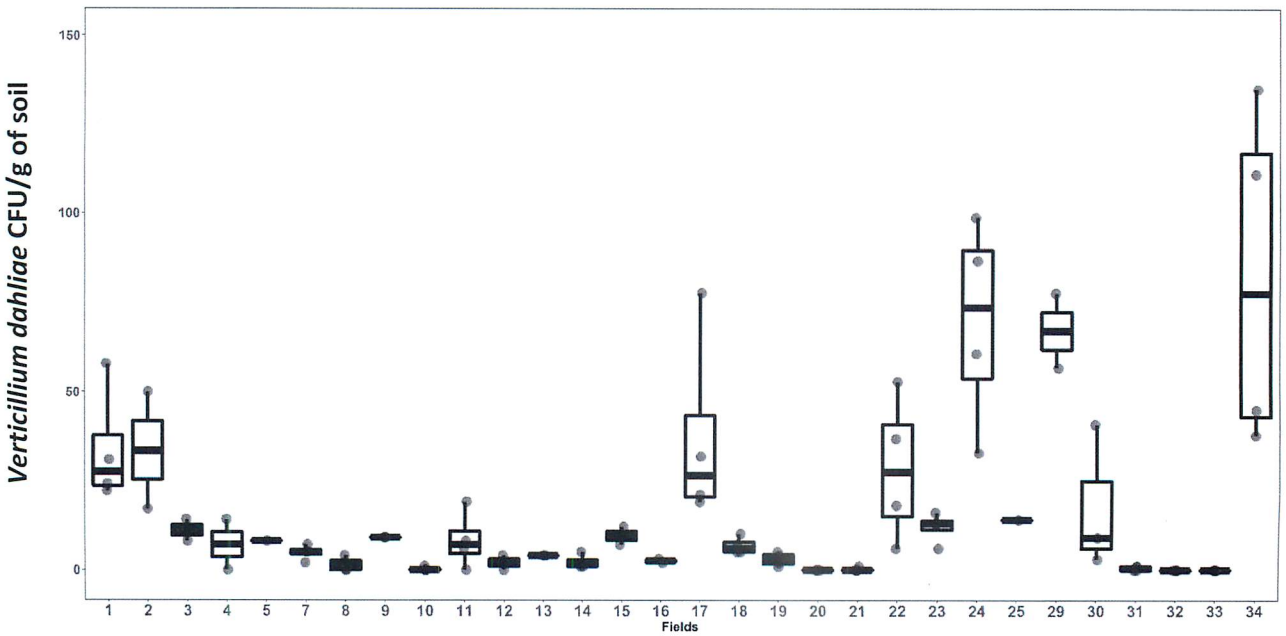


Figure 3. Inoculum density of *Verticillium dahliae* in 6 fields over time.

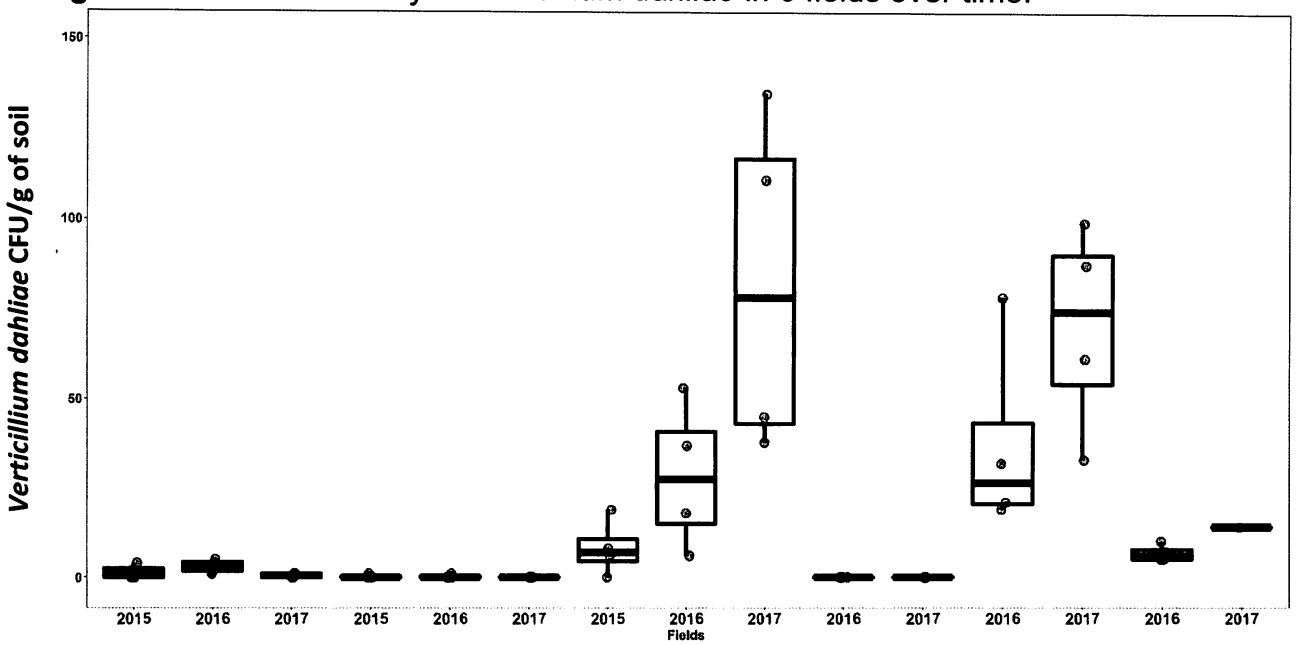


Figure 4. Occurrence of root lesion (A), pin (B), ring (C), and root-knot nematodes/250 g of dry soil (D) detected from commercial fields in Washington and Oregon from 2014-2017.

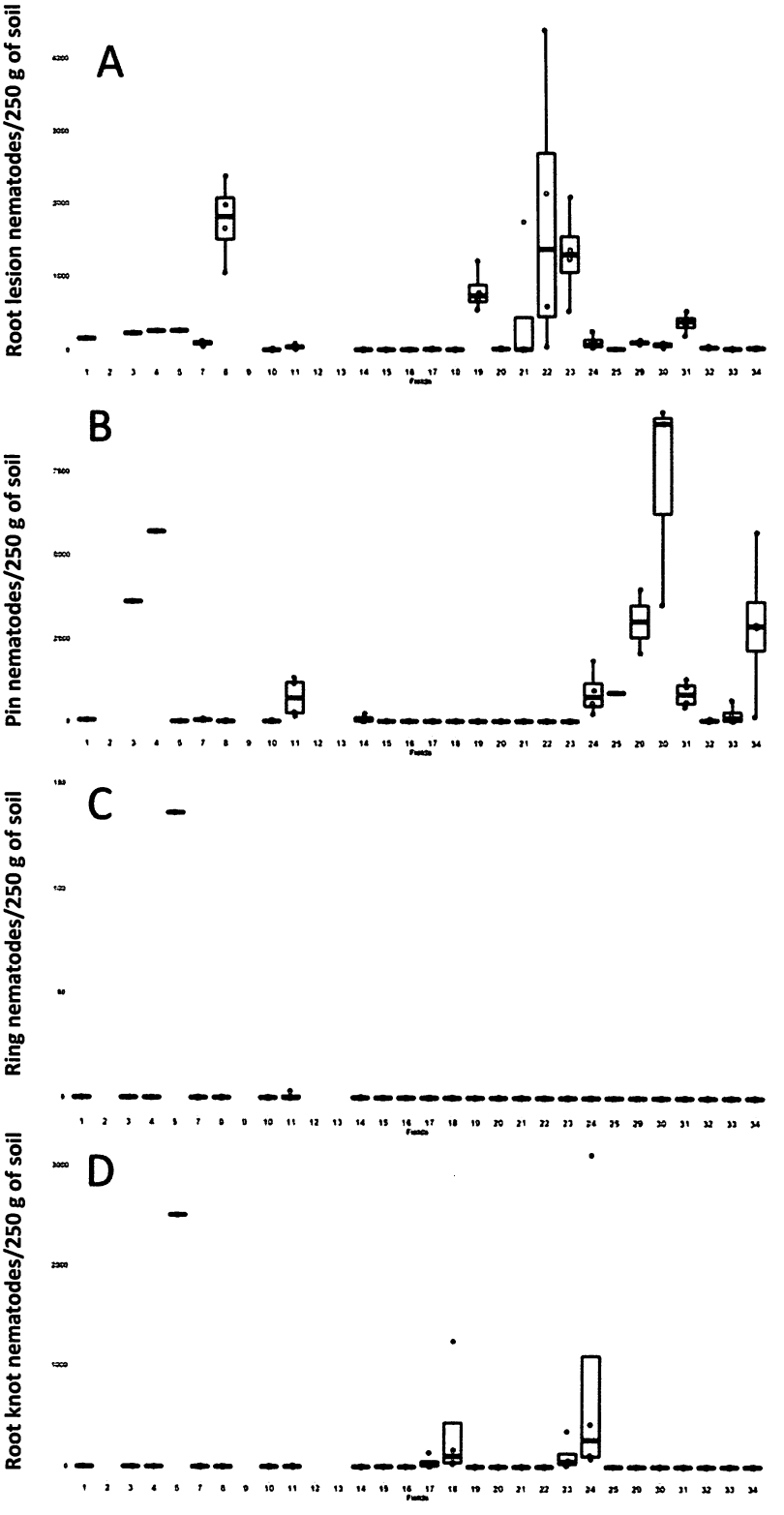


Figure 5. Occurrence of stubby-root (A), stunt (B), cyst (C), and non-parasitic nematodes/250 g of dry soil (D) detected from commercial fields in Washington and Oregon from 2014-2017.

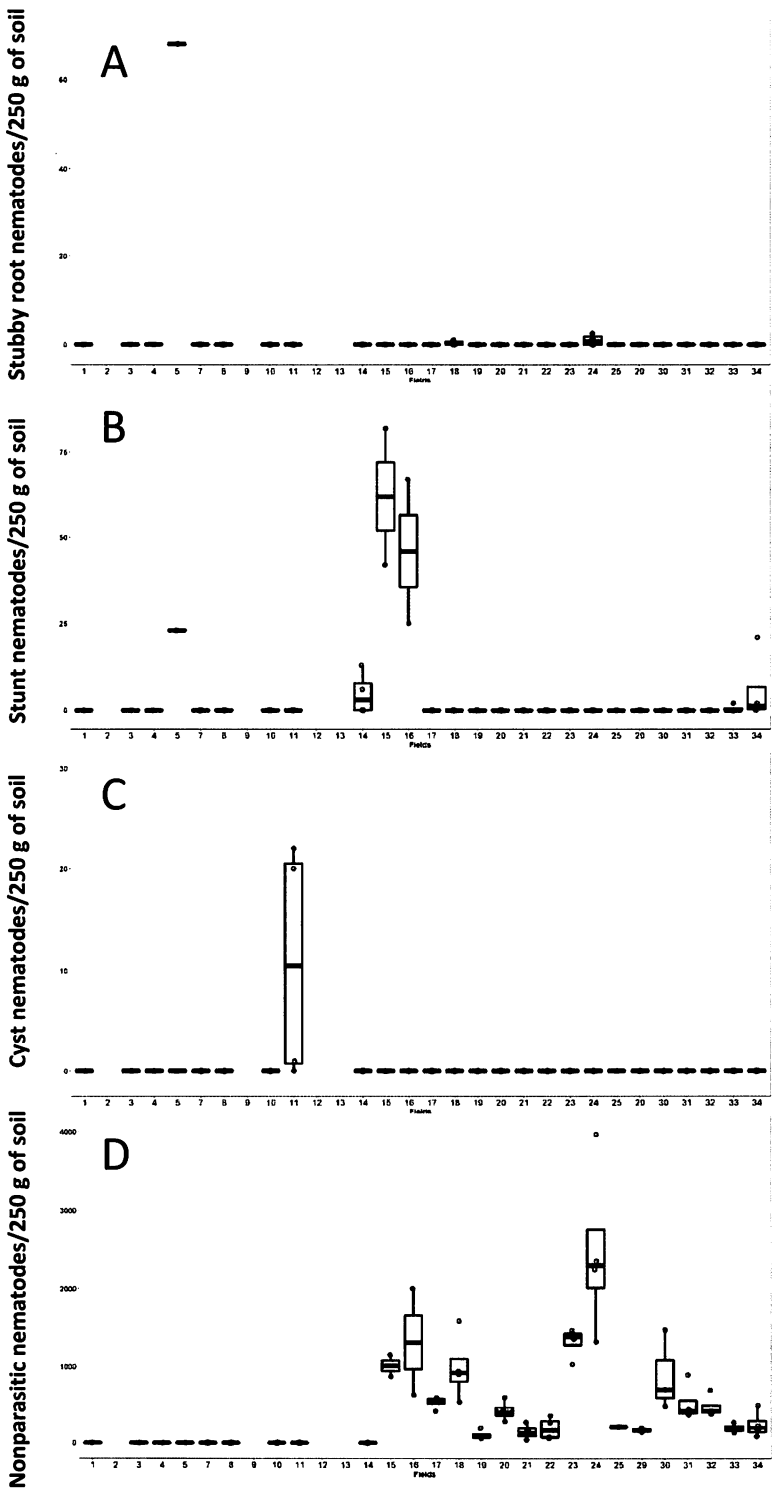
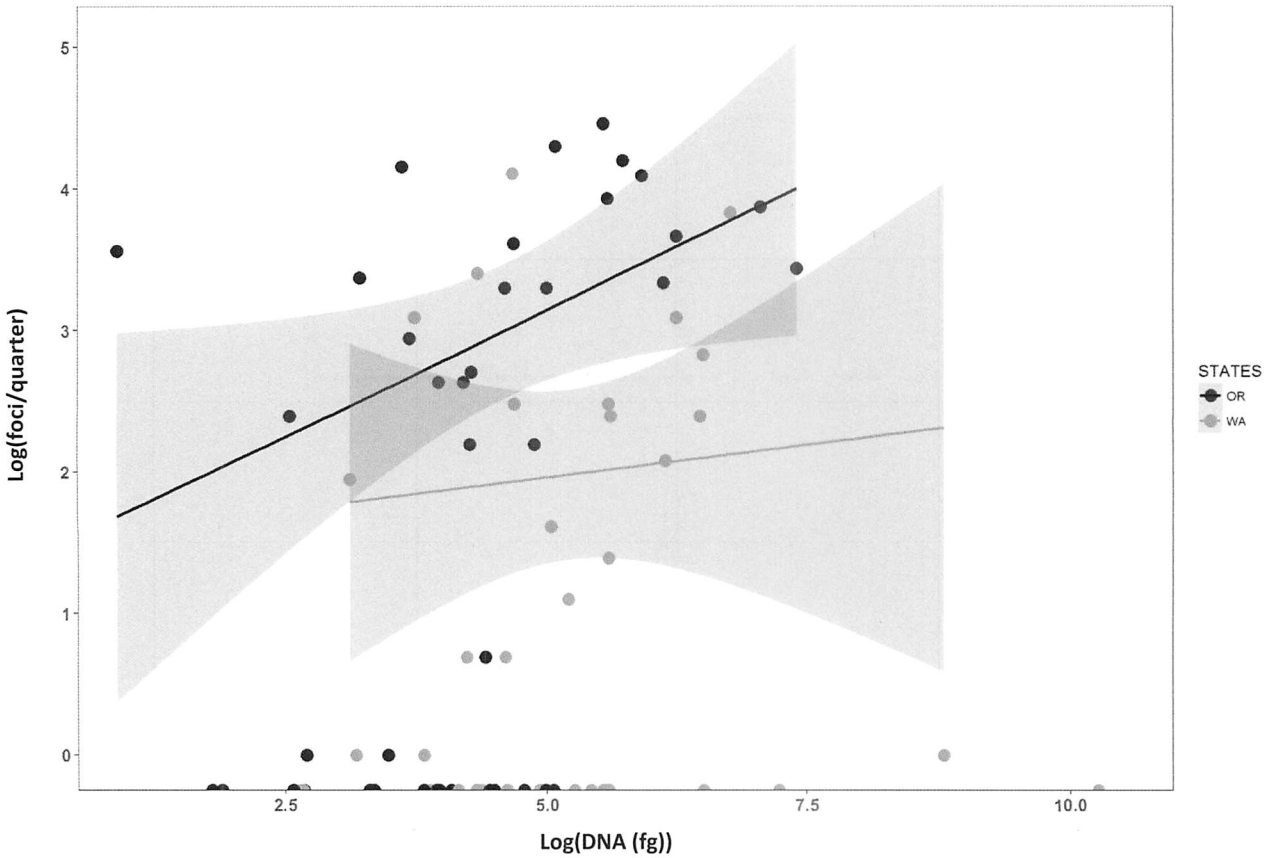


Figure 6. Relationship between the number of wilt foci/field quarter and *Verticillium dahliae* DNA.



2017 Final Report - Weed Research in Mint

Rick Boydston, Weed Scientist, USDA-ARS, Prosser, WA
Ray Baker, Agricultural Research Technologist III, WSU-Prosser, WA

Statement of purpose. Determine the selectivity and efficacy of new herbicides in mint and develop effective management programs for emerging weed problems.

Materials and Methods. Materials and methods are included individually in each trial.

Objectives.

1. Identify and evaluate new herbicides for use in mint crops including saflufenacil, pyridate, and linuron.
2. Develop control methods for cinquefoil (*Potentilla supina ssp. paradoxa*) in mint production.

Success Criteria and Timing. All trials were initiated, data collected and analyzed, and reports submitted according to research proposal.

Data Analysis. Treatments in field trials were arranged as randomized complete blocks with 4 replications. Analysis of variance (AOV) was conducted and treatment means separated using the least significant difference test at the P=0.05% level.

Results and Discussion. Results of individual studies are listed below in the discussions of individual trials.

Research expenditures. MIRC funding is being utilized for technical support salary (Ray Baker), for incidental supplies (bags, ethanol, tags, etc.) and for travel to MIRC meetings.

Conclusion. Conclusions of individual studies are listed under each study subheading if studies are completed.

Multi-state pyridate trial in Scotch spearmint.

Six herbicide treatments were tested in Scotch spearmint at Wyckoff Farms near Paterson, WA. The soil was a sandy loam. The entire trial was treated with pendimethalin 1 lb ai/a, terbacil, 0.5 lb ai/a, clopyralid 0.125 lb ai/a, and paraquat 0.75 lb ai/a on Feb. 28, 2017 when mint was dormant to control winter annual weeds that were present and to control weeds other than kochia preemergence.

POST herbicides were applied May 2, 2017 with a bicycle sprayer calibrated to deliver 25 GPA and treatments were replicated four times in a RCB design. Scotch spearmint was 2 to 4 inches tall and kochia was 0.5 to 3 inches tall when POST herbicides were applied. Individual plots were 10 by 25 feet.

Scotch spearmint was harvested from a 3.25 ft swath from the center of each plot June 30, 2017 with a sickle bar mower. Hay weights (weeds included), were recorded. Steam distillation of hay will follow to determine oil yields. The grower harvested the remainder of the plots with a commercial windrower on July 4, 2017.

The trial was repeated on the same plots on the second growth of Scotch spearmint following the first harvest. Redroot pigweed and any surviving kochia were the primary broadleaf weeds present in the second regrowth of the spearmint. The clopyralid treatment, which doesn't control redroot pigweed, was substituted with sulfentrazone (Spartan) applied July 11, 2017 prior to any Scotch spearmint regrowth. This use timing of Spartan is not labeled in mint, but has given good pigweed control with minimal mint injury in previous testing. All other POST treatments were applied July 17 when new redroot pigweed seedlings began to emerge.

Scotch spearmint was harvested a second time on Sept. 13, 2017 following the same harvest and oil distillation procedures as used in the first harvest of mint. Oil obtained from steam distillation was saved and transferred to Callison Company for oil quality and component analysis.

Results.
Kochia was controlled 95 to 99% May 11, 2017, 9 days after the POST applications (DAT), with treatments of pyridate, bentazon, and bromoxynil and by May 22, 2017 kochia control was 99 to 100% with the three herbicides (Table 1). Terbacil and MCPB only controlled kochia 66% and 68%, respectively at on May 22 and control further declined on June 5, 2017. Clopyralid did not control kochia. Many small redroot pigweed were starting to emerge at 9 DAT and by June 5, in most plots. Redroot pigweed control was best in pyridate treated plots. The control of newly emerging pigweed with pyridate applied before the majority of pigweed emergence seemed unusual as pyridate is not known to have much soil residual activity.

No significant spearmint injury was observed with terbacil, bentazon, and pyridate (Table 1). Bromoxynil caused some chlorosis of the spearmint, clopyralid caused some leaf cupping, and MCPB stunted the mint growth at 9 DAT and mint injury persisted through the June 5 evaluation. Since clopyralid was applied as a maintenance treatment to all plots in February to control prickly lettuce, the clopyralid treatment received a total of two applications resulting in more injury than would typically be seen with a single application of clopyralid.

Hay yields included all weeds that were present and there was no statistical difference in hay yields among treatments. However, the pyridate treated plots averaged the lowest hay yields (10.9 ton fresh hay/acre) probably due to fewer weeds present in the hay. Clopyralid treated plots and weedy checks averaged 14.0 and 12.9 ton fresh hay/acre probably due to the excessive amounts of kochia and pigweed included in the hay. Oil yield was greatest with pyridate, bentazon, and terbacil treatments (50 to 57 lbs/A) and least in weedy checks and bromoxynil treatments averaging 32 and 37 lb/A, respectively (Table 1).

On July 25, 2017, 8 DAT of second POST applications, kochia was controlled 96% to 99% with treatments of pyridate, bentazon, and bromoxynil (Table 2). Kochia control with these three treatments remained good until harvest. Terbacil, MCPB, and sulfentrazone, did not control kochia that had been cut off during the first harvest and was regrowing (Table 2).

A new flush of redroot pigweed emerged after the first harvest of the mint in addition to the regrowth of any existing pigweed that was cut off during the first harvest. At all times after treatment, redroot pigweed control was greatest with pyridate (Table 2). Spartan controlled new pigweed seedlings well, but failed to control many of the larger pigweed that had been cut off and were regrowing. Spartan treatments also tended to have more of the larger regrowing pigweed due to the poor control of pigweed with clopyralid, which was applied to these plots during the first crop of mint. All other treatments only marginally stunted the growth of pigweed or failed to control pigweed.

No significant spearmint injury was observed with terbacil, bentazon, and pyridate (Table 3). Bromoxynil and sulfentrazone caused some chlorosis of the spearmint and MCPB stunted the mint growth at 8 DAT. Mint injury persisted for a couple of weeks after application, but by August 14, 2017 (4 WAT), injury was very minimal with all herbicide treatments.

Kochia and/or redroot pigweed completely overran most plots except those treated with pyridate. Hay yields included any weeds that were present and the greatest hay yields were in nontreated checks, which consisted almost entirely of kochia and redroot pigweed (Table 3). Pyridate treated plots averaged the lowest hay yield (4.8 ton fresh hay/acre) due to fewer weeds present in the hay. All other herbicide treated plots ranged from 6.5 to 9.2 tons fresh hay/acre and much of the hay consisted of weeds.

Scotch spearmint oil yield was greatest during the second cutting with pyridate, averaging 29.2 lbs/acre (Table 3). Bentazon treated plots averaged 10.3 lbs oil/acre and all other treatments yielded less than 5.7 lbs oil/acre. No oil was obtained from nontreated check plots as the hay consisted primarily of kochia and redroot pigweed.

Oil samples were graded for color, odor, and purchase grade by Callison Company. After filtering, no oil was recovered from nontreated checks and bromoxynil treated plots. Oil was only recovered from 3 of 4 replications of bentazon, terbacil, and sulfentrazone treatments due to excessive weed competition in certain plots. Oil from individual reps of MCPB, terbacil, and sulfentrazone treatments was combined in order to obtain enough sample to evaluate. No statistical analysis was possible due to the need to composite the oil samples from individual replications of most treatments in order to obtain enough sample to test.

Oil color graded average for all treatments except bentazon which graded poor (Table 4). Oil from pyridate treated plots had superior odor and purchase grade than all other treatments and had less weedy notes (Table 4).

The twelve most important and prevalent oil components were analyzed from the oil samples. l-carvone and l-limonene were the most prevalent components. Similar to results from a 2016 trial, pyridate treated plots tended to have lower myrcene and limonene levels, and greater l-carvone levels than treatments which contained more pigweed and kochia escapes (terbacil, MCPB, sulfentrazone) (Table 4). Oil from Basagran treated plots had a similar composition as oil from pyridate treated plots. Pyridate treated plots also averaged higher 1,8 cineole and less l-methone, l-menthol, and viridifloral than oil from all other treatments (Table 4).

Table 1. Kochia and redroot pigweed control and Scotch spearmint injury, hay, and oil yield following six POST herbicide treatments applied May 2, 2017 near Paterson, WA. Redroot pigweed emergence occurred primarily after herbicides were applied.

Treatment (May 2, 2017)	Rate	5-22-17 Kochia Control[a]	6-5-17 Kochia Control	6-5-17 Redroot Pigweed Control	5-22-17 Scotch Spearmint Injury	6-5-17 Scotch Spearmint Injury	6-30-17 Spearmint Hay Yield[b]	6-30-17 Spearmint Oil Yield
	Lb ai/a	%	%	%	%	%	Ton/A	Lb/A
Nontreated	--	0 c	0 c	0 e	0 c	0 b	12.9	32 c
Pyridate (Tough) + COC	0.94	100 a	100 a	92 a	0 c	0 b	10.9	57 a
Bentazon (Basagran) + COC	1	99 a	99 a	33 cd	0 c	0 b	12.0	56 a
Terbacil (Sinbar) + COC	0.5	66 b	56 b	69 ab	0 c	0 b	12.2	50 a
Bromoxynil (Buctril) + NIS	0.375	100 a	99 a	0 e	9 b	9 a	11.5	37 bc
MCPB (Thistrol) + NIS	0.5	68 b	58 b	15 de	12 ab	7 a	12.2	48 ab
Clopyralid (Stinger)	0.19	0 c	0 c	48 bc	15 a	9 a	14.0	48 ab

[a]Means within a column followed by the same letter are not significantly different according to LSD test at P=0.05.

[b]Hay yields included Scotch spearmint and weeds.

Table 2. Kochia and redroot pigweed control and Scotch spearmint injury following the second set of herbicide treatments applied July 11 (sulfentrazone) and July 17, 2017 near Paterson, WA following the first harvest of mint. Kochia were plants that survived the initial herbicide treatments (and harvest) and redroot pigweed that was a mix of newly emerged seedlings and plants that survived the initial treatments (and harvest).

		Kochia Control			Redroot Pigweed Control		
Treatment (July 11 or 17, 2017)	Rate	7-25-17 8 DAT	7-31-17 2 WAT	8-14-17 4 WAT	7-25-17 8 DAT	7-31-17 2 WAT	8-14-17 4 WAT
	Lb ai/a	----------%----------			----------%----------		
Nontreated	--	0 d	0 d	0 e	0 e	0 d	0 d
Pyridate (Tough) + COC	0.94	99 a	99 a	99 a	92 a	97 a	96 a
Bentazon (Basagran) + COC	1	96 a	98 a	96 ab	33 cd	66 c	63 c
Terbacil (Sinbar) + COC	0.5	91 ab	89 ab	88 bc	69 ab	65 c	56 c
Bromoxynil (Buctril) + NIS	0.375	99 a	100 a	100 a	0 e	69 bc	66 bc
MCPB (Thistrol) + NIS	0.5	73 c	64 c	54 d	15 de	71 bc	66 bc
Sulfentrazone (Spartan)	0.14	84 b	81 b	71 cd	48 bc	87 ab	82 ab

Means within a column followed by the same letter are not significantly different according to LSD test at P=0.05. Columns without letters indicate the treatment (herbicide) effect was not statistically significant.

Table 3. Scotch spearmint injury, hay, and oil yield following the second set of herbicide treatments applied July 11 (sulfentrazone) and July 17, 2017 near Paterson, WA. Spearmint was harvested the second time on Sept. 13, 2017.

Treatment (July 11 or 17, 2017)	Rate	7-25-17 Scotch Spearmint Injury[a]	8-14-17 Scotch Spearmint Injury	9-13-17 Spearmint Hay Yield[b]	9-13-17 Spearmint Oil Yield
	Lb ai/a	%	%	Ton/A	Lb/A
Nontreated	--	0 c	0 b	11.8 a	0 c
Pyridate (Tough) + COC	0.94	0 c	0 b	4.8 b	29.2 a
Bentazon (Basagran) + COC	1	0 c	0 b	7.5 bc	10.3 b
Terbacil (Sinbar) + COC	0.5	0 c	0 b	8.1 bc	5.3 bc
Bromoxynil (Buctril) + NIS	0.375	9 b	4 a	6.5 cd	2.0 c
MCPB (Thistrol) + NIS	0.5	12 ab	2 ab	9.2 b	2.7 c
Sulfentrazone (Spartan)	0.14	15 a	0 b	8.6 b	5.7 bc

[a]Means within a column followed by the same letter are not significantly different according to LSD test at P=0.05.

[b]Hay yields included Scotch spearmint and weeds.

Table 4. Twelve oil components and evaluator notes on oil color, odor, purchase grade, and other notes from Scotch spearmint oil collected from second cutting in 2017 near Paterson, WA.

Oil component	MCPB	Sinbar	Spartan	Basagran	Tough
		---------- (%) ----------			
myrcene	1.49	1.61	1.50	1.41	1.37
l-limonene	23.64	23.30	23.56	21.84	21.92
1,8 cineole	0.93	1.16	1.19	1.19	1.22
3-octanol	1.87	2.01	2.16	2.17	2.08
l-menthone	1.41	1.23	1.14	1.10	1.08
l-dihydrocarvone	0.73	1.05	1.11	0.96	1.06
l-menthol	1.94	0.34	0.49	0.33	0.24
l-dihydrovarcyl acetate	0.04	0.05	0.04	0.04	0.04
l-carvone	52.35	56.57	56.55	59.25	59.47
Cis-carvyl acetate	0.11	0.13	0.13	0.13	0.11
Cis-jasmone	0.20	0.20	0.17	0.19	0.21
viridiflorol	0.13	0.08	0.07	0.06	0.04
Evaluator Rating & Notes					
Color[a]	3	3	3	4	3
Odor[b]	4+	4+	4+	4+	3.2
Purchase grade[c]	4	4	4	4	3.5
Notes	Weedy	Weedy	Weedy	Weedy in 50% of samples	Trace weedy 25% of samples

*No samples of oil obtained from nontreated weedy checks and Buctril treated plots due to excessive weed competition.
[a]Color scale 1-6 with 1 being exceptional (white/clear) and 6 being poor (orange/brown).
[b]Odor scale 1-6 with 1 being exceptional (true to area, perfect assay, no weeds) and 6 being poor (weedy odors, etc.).
[c]Purchase grade rating scale 1-5 with 1 being excellent and 5 being unsatisfactory (do not purchase).

Saflufenacil and linuron dormant treatments.
Twelve herbicide treatments were applied PRE to native spearmint March 7, 2017 (Table 5). Either paraquat (Gramoxone) or saflufenacil (Sharpen) were included with most treatments to control winter annual weeds that were present. Linuron (Lorox), terbacil (Sinbar), pendimethalin (Prowl H2O), pyroxasulfone (Zidua), flumioxazin (Chateau), sulfentrazone (Spartan), clomazone (Command) were included in various tank mixes (Table 2). Herbicides were applied with a bicycle sprayer calibrated to deliver 25 GPA through six, 8002 XR flat fan spray tips. Individual plots were 10 by 25 feet and treatments were replicated four times in a RCB design. A nontreated check was included as a comparison. Native spearmint injury and weed control were rated March 17, March 28, April 7, April 21, and May 19, 2017.

Native spearmint was harvested July 5, 2017 by sickle bar cutting a 3.25 x 20 ft strip from the center of each plot. Fresh hay was weighed to determine hay yield and a 21 lb subsample of hay was collected in burlap bags, air dried, and steam distilled to determine oil yield.

Results.
Native spearmint injury following herbicides applied March 7, 2017 was greatest with Command plus Lorox, averaging 18% injury with symptoms of leaf bleaching (chlorosis). All other herbicide treatments resulted in less than 3% injury (Table 5). Injury from the Command plus Lorox tank mix was only 4% by April 21, 2017 and was absent in May.

The main early season weed was common chickweed. All treatments containing Gramoxone controlled chickweed well (>93%) (Table 6). Treatments containing Sharpen (without Gramoxone) did not control common chickweed well in March and early April. However, the tank mix of Sharpen with Sinbar eventually controlled common chickweed in May, whereas tank mixes of Sharpen with Prowl or Zidua did not (Table 6).

There were lesser amounts of Shepherd's purse and downy brome present and the number of plants per plot was recorded on May 19, 2017. Among Sharpen and Sharpen tank mix treatments, only the tank mix with Sinbar totally controlled shepherd's purse and downy brome (Table 6). Three-way tank mixes of Gramoxone with Lorox combined with Chateau, Sinbar, or Command all eliminated downy brome. Three-way tank mixes of Gramoxone with Lorox combined with Sinbar, Command, or Spartan eliminated shepherd's purse (Table 6).

Plots were harvested July 5, 2017 and the native spearmint averaged 76 lbs/A oil and 13.6 tons hay/A (fresh weight) (Table 5). There were no statistically significant differences among herbicide treatments for spearmint oil or hay yield. However, the high rate of saflufenacil (0.089 lb ai/a) was among the lowest average oil yields, which causes some concern and additional studies may be warranted. Due to the low amount of weed pressure in the study, there was likely minimal effect of weeds on oil or hay yields.

Table 5. Native spearmint injury following twelve PRE herbicide treatments applied March 7, 2017 at WSU-Roza station near Prosser, WA.

	Treatment	Rate	Native Spearmint Injury			Spearmint Hay Yield	Spearmint Oil Yield
			April 7	April 21	May 19	July 5	July 5
		(lbs ai/a)	----------% ----------			Ton/A	Lb/A
1.	Nontreated		0 c	0 c	0	11.7	75
2.	Sharpen	0.044	0 c	0 c	0	12.6	69
3.	Sharpen	0.088	0 c	0 c	0	13.0	61
4.	Sharpen + Sinbar	0.044 + 0.5	1 bc	0 c	0	15.7	86
5.	Sharpen + Prowl H_2O	0.044 + 1.5	0 c	0 c	0	13.0	63
6.	Sharpen + Zidua	0.044 + 0.19	0 c	0 c	0	15.0	83
7.	Lorox + Chateau + Gramoxone	1 + 0.125 + 0.5	1 bc	0 c	0	14.2	79
8.	Lorox + Gramoxone	1 + 0.5	0.5 c	0 c	0	12.7	71
9.	Lorox +Sinbar + Gramoxone	1 + 0.5 + 0.5	0.5 c	0 c	0	13.1	78
10.	Lorox + Command + Gramoxone	1 + 0.35 + 0.5	18 a	4 a	0	13.1	85
11.	Lorox + Spartan + Gramoxone	1 + 0.19 + 0.5	0 c	0 c	0	12.1	71
12.	Lorox + Chateau + Gramoxone	1 + 0.125 + 0.5	0 c	0 c	0	15.4	81
13.	Lorox + Zidua + Gramoxone	1 + 0.19 + 0.5	3 b	1 b	0	14.7	80

Numbers within a column followed by the same letter are not significantly different according to LSD test at P=0.05.
Columns without letters indicate the herbicide treatment effect was not statistically significant.
Treatments 2-6 included MSO at 1% and AMS at 2% (v/v) spray solution.
Treatments 7-13 included COC at 1% (v/v) spray solution.

Table 6. Weed control following PRE and POST herbicide treatments applied Feb. 24, 2016 and April 14, 2016 to peppermint planted the previous fall at Paterson, WA.

	Treatment	Rate	Common Chickweed May 19	Shepherd's Purse May 19	Downy Brome May 19
		(lbs ai/a)	% Control	No./plot	No./plot
1.	Nontreated		0 d	1.8	0.5
2.	Sharpen	0.044	0 d	2.5	1.3
3.	Sharpen	0.088	15 c	1.3	1.3
4.	Sharpen + Sinbar	0.044 + 0.5	100 a	0	0
5.	Sharpen + Prowl H_2O	0.044 + 1.5	18 c	0.5	0.8
6.	Sharpen + Zidua	0.044 + 0.19	45 b	1.3	1.5
7.	Lorox + Chateau + Gramoxone	1 + 0.125 + 0.5	100 a	0.3	0
8.	Lorox + Gramoxone	1 + 0.5	100 a	1.0	0.8
9.	Lorox +Sinbar + Gramoxone	1 + 0.5 + 0.5	100 a	0	0
10.	Lorox + Command + Gramoxone	1 + 0.35 + 0.5	100 a	0	0
11.	Lorox + Spartan + Gramoxone	1 + 0.19 + 0.5	98 a	0	1.3
12.	Lorox + Chateau + Gramoxone	1 + 0.125 + 0.5	94 a	0.5	0
13.	Lorox + Zidua + Gramoxone	1 + 0.19 + 0.5	96 a	0.8	0.3

Numbers within a column followed by the same letter are not significantly different according to LSD test at P=0.05.
Treatments 2-6 included MSO at 1% and AMS at 2% (v/v) spray solution.
Treatments 7-13 included COC at 1% (v/v) spray solution.

Saflufenacil applied between cutting on double cut peppermint.

Peppermint tolerance to saflufenacil (Sharpen) applied between cuttings was tested in a field trial at the WSU-Roza station. The soil was a Warden loamy sand. Saflufenacil was applied at 0.044 and 0.089 lb ai/a plus MSO at 1% (v/v) to double cut peppermint July 18, 2018 following the first cutting and prior to irrigation of mint. Herbicides were applied with a bicycle sprayer calibrated to deliver 25 GPA through six, 8002 XR flat fan spray tips. Individual plots were 10 by 20 feet and treatments were replicated four times in a RCB design. A treatment of sulfentrazone (Spartan) at 0.13 lb ai/a with COC and a nontreated check were included as a comparison.

Injury and weed control were evaluated at various times after application. Peppermint was harvested Oct.4, 2017 by sickle bar cutting a 3.25 x 20 ft strip from the center of each plot. Fresh hay was weighed to determine hay yield and a 21 lb subsample of hay was collected in burlap bags, air dried, and steam distilled to determine oil yield.

Results.
Peppermint was not injured by any herbicide treatment on all rating dates. Redroot pigweed began to emerge in late July about 7 to 10 days after the first irrigation following the herbicide application. Redroot pigweed control was 100% with sulfentrazone, 98% with 0.088 lb ai/a saflufenacil, and 93% with 0.044 lb ai/a saflufenacil at 2 WAT (Table 7). At 3 WAT, redroot pigweed control was still excellent with sulfentrazone and the high rate of saflufenacil, but many pigweed began to infest the plots treated with the lower rate (0.044 lb ai/a) of saflufenacil. By late September, pigweed control averaged only 35% with the lower rate of saflufenacil, 82% with the high rate of saflufenacil, and 99% with sulfentrazone.

Peppermint oil and hay yield averaged 41 lbs/a and 9.8 T/a, respectively, and was not significantly different among treatments.

Table 7. Redroot pigweed control and peppermint hay and oil yield following saflufenacil or sulfentrazone applied July 17, 2017 to double cut peppermint at WSU-Roza station near Prosser, WA.

	Treatment	Rate	Redroot Pigweed Control				Mint Yield	
			July 31[a]	Aug. 7	Aug. 15	Sept. 27	Hay	Oil
		(lbs ai/a)	----------% ----------				T/A	Lbs/A
1.	Nontreated	--	0 c	0 c	0 c	0 d	8.5	41
2.	Sharpen + MSO[b]	0.044	93 b	78 b	81 b	35 c	12.2	36
3.	Sharpen + MSO	0.088	98 a	96 a	92 a	82 b	8.4	40
4.	Spartan + COC[c]	0.13	100 a	100 a	100 a	99 a	10.1	47

[a]Numbers within a column followed by the same letter are not significantly different according to LSD test at P=0.05.
[b]Methylated seed oil (MSO) and ammonium sulfate (AMS) included at 1% and at 2% (v/v) spray solution, respectively, in treatments 2 &3.
[c]Crop oil concentrate (COC) included at 1% (v/v) spray solution.

Cinquefoil control.
Potentilla supina (cinquefoil, formerly *Potentilla paradoxa)*, has become more prevalent in wet areas of mint fields in the last several years. Commercial peppermint fields north of Mesa, WA and near Othello had the weed present in 2016 and 2017. Cinquefoil appears to emerge in late summer or fall and can overwinter giving rise to larger sized plants that are not controlled well with dormant season applications of Gramoxone and other mint herbicides. Cinquefoil can act as an annual or short lived perennial. It prefers moist soil and tends to establish first in wet areas of fields. It blooms (yellow flower) from June through August.

Cinquefoil control following six POST applied herbicide treatments was evaluated (Table 8). Cinquefoil seed was planted in potting soil mix in 6-inch pots and when seedlings emerged pots were thinned to 1 plant per pot and placed outdoors at the WSU-Prosser research station. In trial 1, herbicides were applied May 22, 2017 when

seedlings were 5-6 inches diameter. A nontreated check was included for comparison. Herbicides were applied with a bicycle CO_2 sprayer calibrated to deliver 20 GPA through three, 8002 XR flat fan spray tips. Treatments were replicated 6 times in a RCB design. Cinquefoil control was visually estimated on May 25, 2017 (3 DAT), May 30 (8 DAT) and June 5, 2017 (2 WAT).

In trial 2, herbicides were applied June 13, 2017 when plants were 5.5-7.5 inches diameter averaging 38 leaves. Seven herbicide treatments were tested in trial 2 (Table 9).

Results, trial 1.
Cinquefoil control at 10 DAT was 100% with paraquat which is a faster acting herbicide than other herbicides tested (Table 8). At 10 DAT, pyridate + MCPB and saflufenacil + MCPB controlled cinquefoil 56% and 57%, respectively. Some suppression of cinquefoil was noted with all herbicide treatments. At 21 DAT, all treatments reduced top fresh weight of cinquefoil compared to nontreated checks with paraquat providing the greatest reduction.

Table 8. Bushy cinquefoil (*Potentilla supina*) control following POST applied herbicides at WSU-Prosser. Treatments applied May 22, 2017 when cinquefoil was 5 to 6 inches diameter.

	Treatment	Rate	Cinquefoil Control June 1	Cinquefoil Fr Wt. June 12
		(Lb ai/a)	(%)	(g)
1	Nontreated	--	0 d	12.8 a
2	Saflufenacil (Sharpen) + MCPB (Thistrol) + MSO	0.044 + 0.5	57 b	6.5 c
3	Paraquat (Gramoxone) + NIS	0.75	100 a	0.6 d
4	Pyridate (Tough) + MCPB (Thistrol) + MSO	0.95 + 0.5	56 b	7.2 bc
5	Saflufenacil (Sharpen) + MSO	0.044	38 c	8.1 bc
6	Carfentrazone (Aim) + NIS	0.031	22 c	9.0 bc
7	Saflufenacil (Sharpen) + Carfentrazone (Aim) + MSO	0.044 + 0.031	34 c	9.4 b

Numbers within a column followed by the same letter are not significantly different according to LSD test at P=0.05.

Results, trial 2.
Cinquefoil control on June 27, 2017 (2 WAT) was 99% with paraquat at 0.75 lb ai/a (Table 9). Saflufenacil plus MCPB controlled cinquefoil 79% at 2 WAT and all other treatments resulted in less than 50% control. These results suggest that paraquat could be useful at maximum labeled rates to control emerged cinquefoil. Field trials when mint is dormant, either in late fall, winter or late winter, should be conducted on the weed when temperatures are cooler and light intensity is lower than tested in this trial to determine if paraquat is effective under those conditions.

Table 9. Bushy cinquefoil (*Potentilla supina*) control following POST applied herbicides at WSU-Prosser. Treatments applied June 13, 2017 when cinquefoil was 5.5 to 7.5 inches diameter.

	Treatment	Rate Lb ai/a	Cinquefoil Control[a] June 20	June 27
1	Nontreated	--	0 f	0 f
2	Saflufenacil (Sharpen) + MCPB (Thistrol) + MSO	0.044 + 0.5	90 a	79 b
3	Paraquat (Gramoxone) + NIS	0.75	97 a	99 a
4	Pyridate (Tough) + MCPB (Thistrol) + MSO	0.95 + 0.5	66 bc	49 c
5	Saflufenacil (Sharpen) + MSO	0.044	60 cd	23 e
6	Carfentrazone (Aim) + NIS	0.031	40 e	23 e
7	Saflufenacil (Sharpen) + Carfentrazone (Aim) + MSO	0.044 + 0.031	71 b	35 d
8	Bromoxynil + MCPB + MSO	0.35 + 0.5	55 d	44 cd

[a]Numbers within a column followed by the same letter are not significantly different according to LSD test at P=0.05.

Marsh yellow cress control.

Marsh yellow cress (*Rorippa palustris*) has become more prevalent in wet areas of mint fields in the last several years in the Columbia Basin. It appears to be in similar wet areas that cinquefoil has been reported and plants that are established are not controlled well with dormant season applications of Gramoxone and other mint herbicides.

March cress control with seven POST applied herbicide treatments was evaluated in three greenhouse trials (Tables 9 and 10). Cress seed was planted in potting soil mix in 4-inch pots and thinned to one plant per pot after emergence. In trial 1, herbicides were applied when seedlings were 10-13 cm diameter with 12-14 leaves. In trial 2, seedlings were 18-23 cm diameter with 12-17 leaves and in trial 3 plants were 20-25 cm diameter with 16-18 leaves. A nontreated check was included for comparison in each trial. Herbicides were applied with a pneumatic powered bench sprayer calibrated to deliver 25 GPA through a single, 80015 E flat fan spray tip. Treatments were replicated 6 times in a RCB design. Cress control was visually estimated weekly after application and plant dry weight determined at 3 weeks after treatment (WAT).

ResultsTrial 1.

Saflufenacil controlled marsh yellow cress 99-100% at 3 WAT at both rates (Table 10). Pyridate plus MCPB controlled marsh yellow cress 96%. Other herbicide treatments only controlled marsh yellow cress ranging from 56 to 81% at 3 WAT except for the combination of saflufenacil with MCPB which totally controlled the weed similar to saflufenacil alone. Paraquat killed leaves exposed to the herbicide at the time of application, but the center meristematic area always greened back up and plants regrew. Saflufenacil tended to turn centers of the rosettes white (bleached) and plants seldom were able to regrow.

ResultsTrials 2 and 3.
Saflufenacil at 0.022 and 0.044 lb ai/a controlled marsh yellow cress 97 to 100% in trial 2 and 80 to 97% in trial 3, respectively (Table 11). In trial 3, plants were larger and more difficult to kill with the herbicide treatments tested. The lower saflufenacil rate of 0.011 lb ai/a was less effective than the two higher rates. When saflufenacil was tank mixed with paraquat, control was 90-95% in trial 2 and 35-64% in trial 3. There was no advantage of including paraquat with saflufenacil and on the larger plants, saflufenacil alone performed better than the tank mix with paraquat. Paraquat alone at 0.5 or 0.75 initially killed most exposed leaves, but plants regrew from the center meristematic region. Paraquat applied at the higher rate of 0.75 lb ai/a tended to control plants better than the 0.5 lb ai/a rate. Saflufencil could offer growers a new tool to control marsh yellow cress if labeled for use in mint.

Table 10. Marsh yellow cress response to POST herbicide treatments in trial 1.

	Treatment	Rate	Control 3 WAT	Dry wt. 3 WAT
		(Lb ai/a)	(%)	(g)
1	Nontreated	--	0 d	1.63 a
2	Saflufenacil +MSO	0.022	99 a	0.24 d
3	Saflufenacil + MSO	0.044	100 a	0.28 d
4	Paraquat + NIS	0.75	81 b	0.29 cd
5	Pyridate + COC	0.95	60 c	0.45 c
6	MCPB	0.5	56 c	0.68 b
7	Pyridate + MCPB	0.95 + 0.5	96 a	0.20 d
8	Saflufenacil + MCPB	0.044 + 0.5	100 a	0.21 d

Numbers within a column followed by the same letter are not significantly different according to LSD test at P=0.05.

Table11. Marsh yellow cress response to POST herbicide treatments in trials 2 and 3.

			Trial 2		Trial 3	
	Treatment	Rate Lb ai/a	Control 3 WAT	Dry wt 3 WAT	Control 3 WAT	Dry wt 3 WAT
			(%)	(g)	(%)	(g)
1	Nontreated	--	0 d	3.3 a	0 g	3.9 a
2	Saflufenacil + MSO	0.011	83 b	0.8 bcd	53 cd	2.1 bc
3	Saflufenacil + MSO	0.022	97 ab	0.7 bcd	80 b	2.0 bc
4	Saflufenacil + MSO	0.044	100 a	0.7 bcd	97 a	1.9 bcd
5	Paraquat + MSO	0.5	56 c	0.9 bc	19 f	2.2 b
6	Paraquat + MSO	0.75	65 c	1.0 b	43 de	1.9 bcd
7	Saflufenacil + paraquat + MSO	0.011 + 0.5	90 ab	0.5 cd	35 e	1.6 cd
8	Saflufenacil + paraquat + MSO	0.022 + 0.5	95 ab	0.4 d	64 c	1.4 d

Numbers within a column followed by the same letter are not significantly different according to LSD test at P=0.05.

Soil residual activity of pyridate (Tough) on pigweed (Powell amaranth).

Pyridate is normally used as a POST applied herbicide and there are no reports of pyridate having soil residual activity. In several recent field studies in mint, less redroot pigweed seemed to emerge in plots following pyridate applications and control tended to extend longer than other POST applied herbicides such as bentazon or bromoxynil. Therefore, we initiated a greenhouse trial to determine if pyridate has some PRE activity on pigweed.

Powell amaranth PRE control with pyridate was evaluated in two greenhouse trials (Table 11). One hundred Powell amaranth seed were planted 0.3 mm deep in a sandy loam soil mix in 8 x 8 inch flats, watered, and herbicides applied after planting.

Herbicides were applied with a pneumatic powered bench sprayer calibrated to deliver 25 GPA through a single, 80015 E flat fan spray tip. Treatments were replicated 4 times in a CRD design. A nontreated check was included for comparison. In trial 1, pyridate was applied at 0.45 and 0.9 lb ai/a and compared with saflufenacil at 0.022 lb ai/a, which has known PRE activity on pigweed. In trial 2, and additional rate of 1.35 lb ai/a pyridate was included.

Live pigweed seedlings were recorded at 1 and 2 weeks after treatment (WAT). In trials 1 and 2, all pigweed seedlings were removed after the 2 WAT counts, and pigweed was reseeded, covered with 1 cm of untreated soil, and live seedlings recorded at 1 and 2 weeks after reseeding. At the time of this report, 3 and 4 WAT seedling counts for trial 2 were not available so only the 1 and 2 WAT data are shown.

Results.

Some limited PRE activity of pyridate on Powell amaranth was evident in trial 1 and activity was greater with the 0.9 lb ai/a rate compared to the 0.45 lb ai/a rate (Table 12). In pyridate treated flats, some seedlings emerged, but died shortly after, typical of other photosynthesis inhibitor herbicides. When applied PRE, pyridate did not control pigweed as well as saflufenacil at 0.022 lb ai/a. Upon replanting pigweed seed after recording the 2 WAT data, additional pigweed seedlings emerged in all treatments at 3 and 4 WAT (Table 12). All herbicide treatments failed to provide PRE control of pigweed at 3 and 4 WAT. When reseeding at 2 WAT, new pigweed was placed on the soil surface and covered with 1 cm of untreated soil. Previously applied herbicides may have been leached deeper or degraded and were no longer effective in controlling newly seeded pigweed.

In trial 2, pyridate again showed PRE activity on Powel amaranth and activity increased as rate increased from 0.45 to 1.35 lb ai/a at 1 WAT (Table 13). Many of the emerged pigweed seedlings counted at week 1 died by the second counting at week 2. These results indicate that pyridate does have some limited PRE activity on pigweed. The

amount of PRE activity and length of soil residual would likely be influenced by soil type (OM, pH, CEC), soil temperature, and moisture.

Table 12. Powell amaranth (pigweed) PRE control at 1, 2, 3, and 4 weeks after pyridate application in greenhouse trial 1.

	Treatment	Rate	Live Pigweed Seedlings[a] 1 WAT	2 WAT	3 WAT[b]	4 WAT
		(lbs ai/a)	----------no./pot ----------			
1.	Nontreated	--	46 a	47 a	24 a	23 a
2.	Pyridate	0.45	32 b	27 b	31 a	36 a
3.	Pyridate	0.9	23 c	14 c	43 a	40 a
4.	Saflufenacil	0.022	1 d	1 d	23 a	25 a

[a]Numbers within a column followed by the same letter are not significantly different according to LSD test at P=0.05.
[b]All seedlings were removed after 14 day count and flats were reseeded with pigweed and covered with 1 cm of untreated soil.

Table 13. Powell amaranth (pigweed) PRE control at 1 and 2 weeks after pyridate application in greenhouse trial 2.

	Treatment	Rate	1 WAT	2 WAT
		(lbs ai/a)	----no./pot----	
1.	Nontreated	--	40 a	41 a
2.	Pyridate	0.45	31 ab	9 b
3.	Pyridate	0.9	29 b	3 b
4.	Pyridate	1.35	14 c	2 b
5	Saflufenacil	0.022	0 d	0 b

Progress report for:

Identifying the Most Effective and Least Disruptive Pyrethroid Insecticide for Potential Registration on Mint.

Submitted to the Mint Industry Research Council

August 15, 2017

1. Abstract: There has been considerable long-term controversy regarding registering a pyrethroid insecticide on US mint. There are a substantial number of pyrethroids available that could potentially be registered on mint. We are conducting plot work to evaluate which of the most recently developed pyrethroids controls the range of pests that infest peppermint, while simultaneously quantifying the disruption these insecticides cause to the beneficial arthropods and how much their application to peppermint may contribute to the outbreak of secondary pests, particularly spider mites. Plots have been established at a commercial peppermint field near Mabton, WA. Candidate pyrethroids under evaluation include zeta-cypermethrin, bifenthrin, lambda-cyhalothrin, and beta-cyfluthrin. The performance of these pyrethroids is being compared to control plots that have not be treated with a pyrethroid. The first scheduled application was applied on June 13, 2017 and a second is planned for early September.

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3. Statement of Purpose:

Washington State is the nation's #1 peppermint and spearmint producing state and spider mites are the most economically damaging arthropod pest in the peppermint cropping systems. Other pests of concern include mint root borer, aphids, and caterpillar pests including cutworms and armyworms. Traditionally, the pests beyond spider mites have been controlled with organophosphate insecticides including chlorpyrifos and acephate. Other insecticides have been registered over the past several years including thiamethoxam for aphid pests and chlorantraniprole for root borer and other caterpillar pests. These insecticides tend to be fairly selective and have not gained complete grower acceptance leading to sporadic use by growers. Pyrethroid insecticides are fast-acting, broad-spectrum insecticides that typically have a relatively short pre-harvest interval. There has been substantial controversy in the past regarding the registration of pyrethroids on mint due to the fact that pyrethroid application to peppermint research plots and many other crops have been documented to promote the outbreak of secondary pests, most notably spider mites. The phenomena of "flaring" mite populations and mite outbreaks have been attributed to biological disruption. First, pyrethroids in general are toxic to the beneficial predatory arthropods that feed on spider mites. Second, spider mites exposed to pyrethroid residues tend to become

irritated. They will often redistribute themselves within the plant canopy, typically to new growth if it has grown out since a pyrethroid application. Irritated mites tend to feed more and consequently lay more eggs as a result of this increased feeding. Spider mite feeding can negatively impact peppermint plant performance, causing leaves to yellow, bronze, dry, and fall under heavy infestations. Spider mite infestations can reduce mint oil yields and oil quality.

We are comparing the direct and residual effects of 4 different pyrethroid insecticides on the pest and beneficial arthropods that persist in peppermint fields. This is being completed in an established peppermint field near Paterson, WA. Concurrent laboratory studies focusing on the effects of pyrethroid residues to pest and beneficial mites on peppermint leaves are being conducted using field collected leaves on a timed basis and observing the behavior and biology of spider and predatory mites exposed to field aged residues of pyrethroids on peppermint foliage.

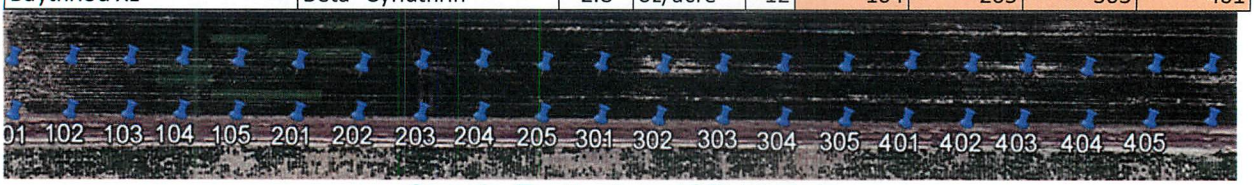
4. Materials and Methods:

Objective 1. Conduct a qualitative and quantitative assessment on the effect of applying pyrethroids to large (by research standards) plots of peppermint on the abundance of pest and beneficial arthropods.

In May 2017 a field plot was established in grower collaborator peppermint field located near Mabton, WA. Four pyrethroid insecticides were selected for use in this study and 4 replicates of 45 by 45 feet (2025ft^2) were established for each treatment. The treatments are detailed below.

Table 1.

Sprays on Mint 2017								
Hand boom sprays		**Formulation**						
Product	**Chemical name**	**Rate/acre**		**REI**	**Plots #**			
Control		-	Fl oz/acre		103	203	305	402
Brigade 2EC	Bifenthrin	6.4	Fl oz/acre	12	102	201	302	403
Mustang	Zeta- cypermethrin	4.3	Fl oz/acre	12	101	203	301	404
Warrior II	Lambda-Cyhalothrin	1.92	Fl oz/acre	24	105	204	304	405
Baythriod XL	Beta- Cyfluthrin	2.8	oz/acre	12	104	205	303	401



Google Earth view of field plots.

The first of two scheduled insecticide applications was sprayed on the plots on June 13th. The insect abundance in the plots was evaluated pretreatment on 2, 7, and 13 June and 7 and 22 days post treatment on 20 June, and 5 July, respectively by insect sweep net. In this method five 180° sweeps are taken per replicate plot. The contents of the net were transferred to a 2 liter paper bag sealed with a clip, placed in a blue-ice

cooled ice chest and transported to the laboratory at WSU IAREC. In the laboratory the insects collected were sorted and quantified. Descripte statistics in the form of mean number of arthropods captured in the sweepnet by insecticide treatment is detailed in table 2.

Table 2. Mean number of arthropods by grouping captured in sweepnet samples by date.

		Flies	Caterpillars	Moths	Aphids	Spiders	BEB	MPB	Damsel	Wasps	Lygus	Thrips	Leafhoppers	Leaf beetles
17-May	beta-cyfluthrin	1.5	0	1	0	0.75	0	0.25	0.25	1.5	0.5	0.75	11.5	0
17-May	bifenthrin	2	0.5	1	0.25	0	0.5	1	0.75	2	0.5	3.25	10	0
17-May	control	1.75	0.25	0.5	0	1.75	0	0.25	0.25	1	0.25	3.25	13.5	0
17-May	lambda-cyhalothrin	1.5	0	0.25	0	0.25	0	0.25	0	1.25	0.75	7	11.75	0
17-May	zeta-cypermethrin	1.25	0.5	0.5	0.25	0	0	0	0	1.25	0.25	3.5	6.25	0
2-Jun	beta-cyfluthrin	3.25	0.5	2.5	0	1.25	0.5	0.75	0.5	2	1.25	4.25	21.75	0
2-Jun	bifenthrin	3	0.5	1	0.25	1	0.5	0	0.75	2.5	1.75	4.25	27.25	0
2-Jun	control	1.25	0.25	2.75	0.25	2.5	0.5	0.5	1	1.25	1.5	4.75	39.25	0
2-Jun	lambda-cyhalothrin	4.25	0.25	2	0.25	1.25	0.25	0.5	0.5	1.75	2.25	3.5	31.25	0
2-Jun	zeta-cypermethrin	2.5	0.75	1.25	0.5	0.5	0.5	0.25	0.75	0.75	1	6.5	25.75	0
7-Jun	beta-cyfluthrin	1.5	4	0.25	1.25	0.75	2.25	0	0.75	0.5	1.5	1.25	42	0
7-Jun	bifenthrin	0.75	3.75	0.25	0.25	1	1	0	1.25	1.25	2	3.75	45	0
7-Jun	control	2.75	5	0.25	1.25	0.75	0.75	0	1.5	1	0.75	1.75	48.75	0
7-Jun	lambda-cyhalothrin	1.75	4.75	0.5	0.5	0.75	1.5	0	0.5	1.75	1.25	3	56.25	0
7-Jun	zeta-cypermethrin	2.5	3	1.5	0.75	0.5	1.25	0	1	1.5	3.25	3	41.5	0
12-Jun	beta-cyfluthrin	1.5	4.25	0	0	0.5	0.5	0	0.5	0.25	1.5	20.25	36.75	0
12-Jun	bifenthrin	1	4.5	1	0	0	0.25	0	0.75	0	1.5	29.25	43.75	0
12-Jun	control	0.75	5.5	0.5	0	0.25	0.5	0	1.5	1.25	2.5	21.75	35.75	0
12-Jun	lambda-cyhalothrin	0.75	5.25	0.5	0	0.25	0	0	1.5	0.25	2.25	15	34.5	0
12-Jun	zeta-cypermethrin	2	5.25	0.25	0	0	0.5	0	1	0	1.5	41.75	52.5	0
14-Jun	beta-cyfluthrin	1.25	10.5	0	0	1	0	0	0	0.25	0.25	0	2	0
14-Jun	bifenthrin	0.25	7.5	0	0.25	0.75	0	0	0	0	0	0.75	0	0
14-Jun	control	0.5	6	0	0.5	1.25	0	0	0	0.25	1.25	0	18.5	0
14-Jun	lambda-cyhalothrin	1.25	7.25	0	0.25	0.25	0	0	0	0.25	0	0.5	7	0
14-Jun	zeta-cypermethrin	0.75	6.5	0	0	0.25	0	0	0	0.25	0	1	3.75	0
20-Jun	beta-cyfluthrin	0.25	4.5	0	0	0	0.25	0	0	0	0	15.25	14.5	53
20-Jun	bifenthrin	1.25	2.25	0	0.25	0	0.25	0	0	0.75	0	14.25	3.5	42.25
20-Jun	control	0.75	10.25	0.25	0	0.25	1.25	0	0.75	0	0.5	19.25	23.75	10.25
20-Jun	lambda-cyhalothrin	1	3.75	0	0	0.25	0.25	0	0	0	0.5	15	18	50.5
20-Jun	zeta-cypermethrin	1.25	3	0	0	0.25	0.75	0	0	0	0.25	13	10.5	22.25
5-Jul	beta-cyfluthrin	1.75	0	5.5	0	0.5	1	2	0	3.75	0.5	5.25	19.5	0
5-Jul	bifenthrin	3.75	0	4.25	0	0	1	0.25	0.25	4	1.5	11.25	11	0.5
5-Jul	control	4.5	0	3.25	0	0	1	0.75	0	4.5	1.5	12	8.25	0.5
5-Jul	lambda-cyhalothrin	2.5	0	2	0	0	1	1.25	0	3	1.25	9.25	14.75	0
5-Jul	zeta-cypermethrin	3.5	0	2.75	0	0.5	0.75	1.5	0	2.5	2	4.25	14.25	0.75

Spider mite abundance pretreatment has been post treatment has been quantified by removing 60 leaflets per replicate plot (20 low, 20 in the middle and 20 high in the canopy) bagging them, and placing them in in a blue-ice cooled ice chest for transport to the laboratory at WSU IAREC. In the laboratory the leaflets are scanned under a dissection microscope and the abundance of pest and beneficial present on the leaflets are quantified. Eggs and other relevant organisms are quantified too.The mean abundance of two spotted mites and eggs are detailed in Table 3. Mite abundance was low in these plots during the sample period.

Table 3. Two-spotted mite and egg abundance per leaf on 3 sample dates. 13 June was right before the insecticide applications were applied and 20 June and 5 July were 7 and 22 days after application

	Mites per leaf 13-Jun		Mites per leaf 20-Jun		Mites per leaf 5-Jul	
Chemical	Mean	SD	Mean	SD	Mean	SD
beta-cyflu	0.0667	0.3117	0.1667	1.0442	0.65	1.7254
bifenthrin	0.0167	0.1291	0.0333	0.181	0.7333	3.8173
lambda-cyh	0.1167	0.3724	0.0833	0.4235	0.3333	0.7287
untreated	0.0667	0.3117	0.95	5.7148	0.2333	0.9632
zeta-cyper	0.0833	0.334	0.1833	0.6507	1.05	3.1699

	Eggs per leaf 13-Jun		Eggs per leaf 20-Jun		Eggs per leaf 5-Jul	
Chemical	Mean	SD	Mean	SD	Mean	SD
beta-cyflu	0.4667	2.5276	0.7333	3.9867	1.5833	4.9721
bifenthrin	0.0333	0.2582	0.1	0.5734	0.5667	1.9861
lambda-cyh	1.1667	4.2353	0.5333	2.7584	2.1333	6.5392
untreated	0.3167	1.97	0.85	2.962	0.9833	3.0945
zeta-cyper	0.6	3.4257	0.9167	3.6233	1.4167	4.3347

The "other" beneficial insects in the plant canopy are being evaluated by yellow sticky cards. In this insect sampling technique 3" by 5" yellow colored plastic cards with a sticky glue are clipped to a wooden stake at plant canopy height and flying and jumping insects are captured and stuck when they contact the sticky card. These traps were placed in the plots in our experimental mint field for the weeks prior to 13 June (Pretreatment) and 20 June and 5 July following treatment. These sticky cards have been collected and evaluated pretreatment on 12 June and 7 and 22 days post treatment on 20 June and 5 July, respectively.

Table 4. Beneficial Insects (± standard deviation) captured on yellow sticky cards for the 7 day periods ending June 12 (pretreatment), June 20, and July 5 from pyrethroid treated and untreated plots.

6/12/2017	Untreated		beta-cyfluthrin		bifenthrin		lamba-cyhalothrin		Zeta-cypermethrir	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lacewings	0.50	1.00	1.75	0.96	1.60	2.30	1.00	1.00	1.75	2.36
Dance flies	14.75	3.10	16.75	7.68	12.20	2.17	7.00	5.20	8.50	2.65
Stethorus	1.75	2.87	3.00	3.46	0.20	0.45	4.33	2.52	1.00	0.82
Tachinids	1.25	0.50	2.75	1.26	2.20	2.28	2.67	2.08	1.00	1.15
MPB	0.00	0.00	2.00	2.45	2.20	2.59	1.00	1.73	2.00	3.37

6/20/2017	Untreated		beta-cyfluthrin		bifenthrin		lamba-cyhalothrin		Zeta-cypermethrir	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lacewings	1.00	0.82	0.25	0.50	0.20	0.45	0.67	1.15	1.25	1.89
Dance flies	10.75	5.91	4.25	3.20	3.00	1.87	5.33	4.62	5.75	2.99
Stethorus	0.00	0.00	0.25	0.50	0.20	0.45	0.33	0.58	0.50	0.58
Tachinids	2.25	0.96	1.25	0.96	2.60	2.70	1.33	1.53	4.25	3.20
MPB	4.75	4.86	1.75	2.87	3.60	2.70	0.33	0.58	5.00	2.58

7/5/2017	Untreated		beta-cyfluthrin		bifenthrin		lamba-cyhalothrin		Zeta-cypermethrir	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lacewings	0.00	0.00	0.00	0.00	0.40	0.89	0.00	0.00	0.00	0.00
Dance flies	14.75	19.23	4.50	3.70	5.80	4.49	1.33	0.58	2.75	0.96
Stethorus	0.25	0.50	0.25	0.50	0.00	0.00	0.00	0.00	0.00	0.00
Tachinids	0.75	0.96	0.25	0.50	0.20	0.45	1.00	1.00	0.75	1.50
MPB	1.75	2.87	1.75	2.06	1.00	0.71	4.00	6.93	3.50	3.70

The pyrethroid applications did negatively affect the abundance of these beneficial arthropods in the treated plots compared to the untreated control. However, the economic significance of these beneficial arthropods in the peppermint agroecosystem in not quantified at this point.

Objective 2. Evaluate the residual activity of field aged pyrethroids on the behavior and biology of spider and predatory mites.

Two major biological effects of exposure by mites to pyrethroids are repellency and repulsiveness. Mites in general are irritated by pyrethroids. We have developed a bioassay technique to test the repellency of these pyrethroids to spider mites in which we place the leaflets left over from objective 1 above in petri dishes with water soaked cotton. When working with two-spotted spider mites we place 5 adult females onto 1 leaflet per height within each replicate treatment for a total of 15 mites. For predatory mites we place 6 *G. occidentalis* on 3 leaflets from the lower, middle and upper part of the plant for a total of 2 G. occidentalis per leaflet from each treatment for total of 72 G. occidentalis treatment. G. occidentalis are more prone to death by drowning over staying on a pyrethroid treated leaf even in the presence of abundant food in the form of spider mite eggs. We completed these bioassays on two-spotted spider mites on leaves collected1 day, 7 days, and 22 days after the plots were treated with the pyrethroid insecticides. *G. occidentalis* 22 days after. Our 1 DAT bioasssays are somewhat confusing, but our 7 and 22 DAT bioassay results directly parallel our results from 2016.

Table 5. Repellency activity of pyrethroid insecticides to spidermites as measured by abandonment

Product	Days after treatment	Left arena out of 15	Percent left	Egg laid
Baythriod XL	1 DAT	1.5	10	36
Brigade 2EC	1 DAT	2	13.33	26
Mustang	1 DAT	1.75	11.66	35
Warrior II	1 DAT	1.25	8.33	32
Untreated	1 DAT	0.75	5	45
Baythriod XL	7 DAT	10	70	52
Brigade 2EC	7 DAT	9.25	61.68	30
Mustang	7 DAT	10.25	68.33	59
Warrior II	7 DAT	5	33.35	35
Untreated	7 DAT	1.5	10	51
Baythriod XL	22 DAT	3.75	25	21
Brigade 2EC	22 DAT	8.25	55	27
Mustang	22 DAT	7.75	51.66	28
Warrior II	22 DAT	3	20	23
Untreated	22 DAT	1	6.6	47

Table 6. Repellency activity of pyrethroid insecticides to predatory mites 31 days after application.

Product	Left arena out of 6	Percent left
Baythriod XL	2	11.11
Brigade 2EC	1.25	6.94
Mustang	4.50	25
Warrior II	2.5	13.89
Untreated	4.25	23.61

Our results indicate that the repellency of the pyrethroid insecticides to predatory is negligible when field residue are permitted to age for a month (Table6).

5. Research Goals and Objectives:

1. Conduct a qualitative and quantitative assessment on the effect of applying pyrethroids to large (by research standards) plots of peppermint on the abundance of pest and beneficial arthropods.

We have made substantive progress in 2017 and will complete these studies in as the season progresses.

2. Evaluate the residual activity of field aged pyrethroids on the behavior and biology of spider and predatory mites.

Our bioassay technique has proved robust when we are working with pest *Tetranychus urticae* spider mites. Our bioassay technique with predatory mites produced better results than we did in 2016.

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6. Success Criteria and Timing.

Objective 1 is progressing and will be completed this fall. Objective 2 is progressing well

7. Data Analysis.

All data collected was entered into MS Excel 2013 and tabulated. Analysis of Variance was conducted on the data obtained in Objective 1 and 2. Descriptive statistics including treatment means and the standard error for those means are detailed in the data tables in this report.

8. Results and Discussion:

Our results to date are detailed above. Further discussion will be provided in the fall 2017 progress report that will be submitted in November 2017. Based on our 2 years of data and support from Syngenta we will move forward with the submission of a pesticide clearance request to IR-4

9. Research Expenditures: Between 2016 and 2017 the MIRC has allocated $41,028 to this project. To date $23,321.79 has been expended.

Salaries- $10,742.92
Wages- $ 6,470.37
Benefits $ 5,275.11

Consumable goods: $633.39

There are $8,280.72 in salary encumbrances on the budget for fall 2017 leaving a true balance of $7,438.12.

10. Conclusion:
We are making progress on our objectives and will submit a complete report this fall for the MIRC winter meeting and Washington Mint Commission December meeting.

Progress report for:

Quantifying Acaracide Resistance in Spider Mite Populations Infesting Peppermint

Submitted to the Mint Industry Research Council

August 15, 2017

1. Abstract: Two-spotted spider mites *Tetranychus urticae* Koch (Acari: Tetranychidae) are renowned for developing resistance to commonly used insecticides/miticides. Over the past several years, using the latest molecular-level research into mechanisms of pesticide resistance, we have developed and validated rapid and robust molecular methods for screening *T. urticae* populations for resistance to the contact miticides bifenazate and bifenthrin and the ovicidal acaricides hexythiozox, etoxazole, and clofentazine. We have worked extensively on abamectin as well, but the direct mechanism (or specfic molecular markers) associated with increased tolerance/ resistance to abamectin has eluded us. We are presently investigating markers associated with resistance to fenpyroximate and spirodiclofen. In 2017 we have completed RNA sequencing of spider mites from hop yards and we will do the same with mites from mint fields this fall. This will give us a powerful tool in determining the mechanisms spider mites use towards resisting poisoning by acaricides. Peppermint is the first crop beyond hops where we have expanded our studies. Presently we have evaluated the phenotypic response from *10 T. urticae* populations infesting peppermint fields from locations in Granger, Mabton, Othello, Prosser, and Toppenish, WA. Our studies this summer have included laboratory bioassays on the response of these 10 peppermint field-collected *T. urticae* populations to the acaricides spirodiclofen, hexythiozox, abamectin, bifenthrin, fenpyroximate, and propargite. In fall 2017 we will complete studies on the molecular genetics of the *T. urticae* populations to discern the resistance status of these mite populations that are infesting peppermint to these commercial acaricides.

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3. Statement of Purpose:

Washington State is the nation's #1 peppermint and spearmint producing state and spider mites are among the most economically damaging arthropod pest in the peppermint cropping system. Unfortunately, feeding by arthropod pests including spider mites has resulted in millions of dollars in decreased crop value. Biological regulation of pest spider mites continues to be an appreciated component of integrated spider mite management (IPM) in peppermint production. Unfortunately, pesticide intercession is required to prevent economic loss or to reduce risk of loss to an acceptable level. We

are focusing on providing the information required to make recommendations for the evolving peppermint integrated mite management program by developing a snapshot regarding the susceptibility of spider mites present in peppermint fields to commonly used commercially available acaricides. This summer we have empirically evaluated acaricide efficacy against 10 populations of *T. urticae* collected from hop yards. We are developing cost effective, robust, and rapid mechanisms for qualifying and quantifying acaricide resistance in pest spider mite populations infesting peppermint fields.

Spider mite feeding reduces photosynthesis and decreases plant growth and overall mint oil quality and quantity produced. Efficacy studies, resistance management, and impacts on non-target species are mainstays of any IPM program including our program in mint.

To date we have developed robust molecular markers associated with spider mite resistance bifenazate and bifenthrin and explained several mechanisms associated with abamectin resistance (PIraneo et al. 2015, Morales et al. 2016). Similar work has been completed on the ovicidal acaricides hexythiozox, etoxazole, and clofentazine (Adesanya 2017). Presently we are focused on spiromesifen and fenpyroximate. Spiromesifen is a newer, selective, non-systemic acaricide that belongs to the group of spirocyclic tetronic acid derivatives (keto-enols) (Van Leeuwen *et al.* 2009). Spiromesifen targets lipid biosynthesis through inhibiting the Acetyl-coenzyme A carboxylase (ACCase). This mode of action is relatively new for miticides. Spiromesifen is active against all developmental stages of spider mite, including eggs. Importantly, spiromesifen acts on female mites by decreasing fecundity and fertility after tarsal contact (Nauen 2005). Fenpyroximate is a Mitochondrial Electron Transport Inhibitor (METI), inhibiting complex I of the respiratory chain (Lummen 2007; Van Leeuwen *et al.* 2010). Fenpyroximate has been used broadly to control two-spotted spider mites and citrus red mites due to its high efficiency on all life stages of herbivorous mites and relative safety to beneficial insects (Van Leeuwen *et al.* 2009). Unfortunately, development of resistance to fenpyroximate is reported in many populations (Sato *et al.* 2004; Van Leeuwen *et al.* 2009; Van Pottelberge *et al.* 2009a). Both spiromesifen and fenpyroximate could be important tools in a peppermint IPM program.

Building on the foundational work begun by He et al. (2009) on genetic mechanisms of abamectin resistance in *T. cinnabarinus*, by Kwon et al. (2010) on genetic mechanisms of pyrethroids resistance in *T. urticae,* and by Khajehali et al. (2010) on genetic mechanisms of organophosphate resistance in *T. urticae,* we began our studies of resistance mechanisms in 2012. Incorporating our own findings with emerging research (Demaeght et al. 2013, Karatolos et al. 2012, Morales et al. 2016, Pavlidi et al. 2015, Riga et al. 2014), we have made substantial progress toward development of techniques that will pre-screen spider mites for the presence or absence of resistance genes. Such technology, applied in the field, will benefit mint growers by indicating population susceptibilities within individual mint fields, paving the way for the adoption of less disruptive, more selective pesticides for control of the key direct pests of peppermint and the reduction of instances of field failures of specific miticides as experienced by some growers with propargite, abamectin and bifenazate in past years.

4. Materials and Methods:

Objective 1. Field test candidate miticides for their efficacy against spider mites. Field plots were not established in 2017. We focused our efforts on conducting laboratory bioassays and in the process were able to cover a much greater geography than we could via a single field trial.

Objective 2. Evaluate the impact of candidate pesticides on beneficial mites. Predatory mite colonies have been established and will continue to be maintained at the Environmental and Agricultural Entomology Laboratory in Prosser and the WSU Insectary in Pullman. This past season we have focused on the the acaricides etoxazole, clofentazine, and hexythiozox. Beneficial mites have been topically exposed in a Potter spray tower to dilute sprays of each selected chemistry at decreasing concentrations.

Objective 3. Develop a robust bioassay method for evaluating residual efficacy of miticides including propargite, spiromesifen, and fenpyroximate. In 2017 we have completed bioassays on *T. urticae* egg and adult life stages with the acaricides spirodiclfen, hexythiozox, abamectin, bifenthrin, fenpyroximate and propargite. This exceeds the amount of work we proposed to do in our fall 2016 proposal to the MIRC. To ascertain the efficacy of the ovicidal acaricide hexythiozox, we placed 10 female *T. urticae* adults on the upper surface of a lima bean leaf disk (22mm in diameter) atop wet cotton inside a sterile petri dish. These adult females were permitted to lay eggs for 24h before being removed. The eggs on leaf discs were placed in a Potter spray tower where they will they were exposed to varying concentrations hexythiozox to achieve a dose-mortality response. We observed these eggs daily for approximately a week to compare the nuber that hatch compared to eggs that were not treated with hexythiozox. To ascertain adulticidal activity, we will use 15-20 gravid female adults, following the same protocol except that the adult females will be sprayed immediately after being placed on fresh leaf disks.

Objective 4. *Develop baseline dose response curves of spider mite populations susceptible to propargite, spiromesifen and fenpyroximate.* The results from Objective 3 have permited us to quantify bioassay results for each life stage and each acaricide. The baseline dose response will be used to calculate discriminating doses for rapid screening of resistance phenotypes among *T. urticae* populations in mint fields. Abbot's formula (1925) was used to calculate the LC10, LC50, and LC90 (lethal concentrations that kill 10%, 50% and 90% of test spider mite population respectively) with probit analysis (Polo probit 2014).

Objective 5. Establish mite colonies and "breed" resistance to candidate acaricides into the mite population through constant and consistent exposure. Over the past several years, we have established resistant mite colonies from mites originally collected from hop yards through constant and consistent exposure of the mite population to candidate miticides. Our collection efforts emphasized yards known to have been sprayed heavily

to increase the likelihood that resistance genes would be present. Up to now, we have colonies highly resistant to abamectin, bifenazate and bifenthrin. In 2016, we also established *T. urticae* colonies that are now 200-fold more resistant to ovicidal miticides (clofentezine, hexythiazox and etoxazole) than acaricide-naïve populations. In 2017, we have initiated similar selection studies with emphasis on propargite, spiromesifen and fenpyroximate with mites collected from peppermint fields.

Objective 6. Develop discriminating doses of candidate miticides that can be used to rapidly identify the prevalence of tolerance or resistance in a spider mite population. We are calculating critical dose ranges for propargite, spiromesifen, and fenpyroximate based on the results we are obtaining from objectives 4 and 5. The bioassays will provide a fast and inexpensive way to detect miticide-resistant spider mite populations, and will allow us to measure the lethal effects of discriminating doses of miticides against field-collected mites. For our purposes, the default discriminating dose will be the minimum dose required to prevent the hatching of nymphs or the survival of adults in our laboratory miticide-naïve colony, generated by the baseline LC90 concentrations developed for propargite, spiromesifen, and fenpyroximate in objective 4. Data obtained from objectives 4 and 5 will be the key to determining our developed discriminating dose. If the concentrations required to achieve the LC90 in the populations bred for resistance (Objective 5) are greater by an order of magnitude (as can be observed in field resistance), the discriminating dose value will be increased accordingly to further differentiate a susceptible mite population from a tolerant or resistant mite population.

Objective 7. Test selected field populations of spider mites from a representative sample of peppermint fields and compare their dose response curves to mite populations as in Objectives 4, 5, and 6. The discriminating doses are being evaluated against field populations as a final check against our laboratory results in this summer. We are requesting direct spray records from the individual growers for the peppermint fields from which we field-collected spider mites. We planned on testing at least 5 relevant populations of spider mites and to date we have tested 10 populations.

Objective 8. Expand robust molecular diagnostics to predict (multiple) acaricide resistance in the field. Our preliminary work funded by the Hop Research Council allowed us to sequence the putative molecule markers for bifenthrin, bifenazate, ovicidal miticides, and abamectin. We first focused on the target-site insensitivity mechanism. We investigated resistance ratios and distribution of multiple resistance-associated mutations in field collected *T. urticae* samples. Our research revealed that a mutation in the cytochrome b gene (G126S) in 35% of the tested *T. urticae* populations and a mutation in the voltage-gated sodium channel gene (F1538I) in 66.7% of the populations may contribute resistance to bifenazate and bifenthrin, respectively (Piraneo et al. 2015). We also found one field population with the mutation I1017F in the chitin synthase gene that contributes resistance to ovicidal miticides etoxazole, clofentezine, and hexythiazox (Adesanya et al. 2016). No mutations were detected in Glutamate-gated chloride channel subunits tested, suggesting target-site insensitivity may not be important in *T. urticae* populations' resistance to abamectin in Yakima Valley hopyards (Piraneo et al. 2015). Besides the target-site insensitivity mechanism,

other mechanisms such as enhanced metabolic detoxification by cytochrome P450s, GSTs, or esterases also play important roles in pesticide resistance (Liu et al. 2006). In 2016, we cloned promoter regions of abamectin resistance-associated gene CYP392D8 from the susceptible and abamectin-resistant strains. We found differences in the promoter region between the susceptible and resistant populations, which can be used for abamectin resistance monitoring in the field.

In the summer of 2017, we will collect 15-20 field samples of T. urticae from peppermint field. With these field-collected mite samples, we will expand our molecular diagnostics studies in 2018 to predict multiple acaricide (including abamectin, ovicidal miticides, spiromesifen, fenpyroximate) resistance in hop fields.

For abamectin resistance monitoring, we will use several recently identified molecular markers, including the promoter of CYP392D8 and the upregulation of CYP392A16, CYP392D10 and three GSTs, TuGSTd10, TuGSTd14, TuGSTm09. For example, the remarkably over-expression of CYP392A16 and CYP392D10 in abamectin-resistant strains collected from commercial peppermint fields when compared to the susceptible strain suggests these two P450s could serve as molecular markers for abamectin resistance monitoring (Riga et al. 2014). In addition, three GSTs, TuGSTd10, TuGSTd14, and TuGSTm09 are associated with abamectin resistance through overexpression in the resistant strains (Pavlidi et al. 2015). For the promoter of CYP392D8, we will perform polymerase chain reaction (PCR) to compare promoter sequences between the lab susceptible and field resistant populations. For the overexpression of CYP392A16, CYP392D10, TuGSTd10, TuGSTd14, and TuGSTm09, we will conduct quantitative Real-Time PCR (qRT-PCR) to evaluate relative transcriptional expressions of these genes among the lab susceptible and field mite populations. The most stable reference genes, CycA and RP49, will be used to normalize expression of genes (Morales et al. 2016).

There is no mutation on the target ACCase gene identified from a spiromesifen-resistant T. urticae strain suggesting the absence of target-site insensitivity mechanism in this strain (Demaeght et al. 2013). However, the mutation E645K was associated with low level of spiromesifen resistance in greenhouse white fly *Trialeurodes vaporariorum* (Karatolos et al. 2012). In this proposal, we will investigate the presence or absence of this mutation in the susceptible and field mite populations collected from peppermint fields. Previous biochemistry and synergist studies showed that metabolic resistance through P450 monooxygenases and esterases contributes to the high level of spiromesifen resistance (Van Pottelberge et al. 2009b). Recent studies revealed that the upregulation of two P450s, CYP392E7 and CYP392E10 (Demaeght et al. 2013) and an esterase, CCE04 (Demaeght 2015) are associated with spiromesifen resistance in T. urticae. In 2017, we will evaluate the relative expression of CYP392E7, CYP392E10 and CCE04 in the susceptible and field-collected mite populations with qRT-PCR.

Although previous synergist experiments suggested the possible involvement of cytochrome P450 monooxygenase in fenpyroximate resistance (Stumpf and Nauen 2001; Van Pottelberge et al. 2009b), the genes associated with fenpyroximate resistance have not been discovered yet. We hypothesize that the evolution of high resistance to fenpyroximate in T. urticae is caused by the overexpression of detoxification genes and/or mutations on the target site protein. In this proposal, we will use RNA sequencing (RNAseq) to reveal the mechanisms underlying fenpyroximate

resistance. RNAseq is a recently developed deep-sequencing technology to investigate the entire transcriptome in a high throughput and quantitative manner (Wang et al. 2009). Most recently, with the support by the Hop Research Council, we have taken advantage of this technology to decipher the transcriptomes responsible for abamectin, bifenthrin, and bifenazate resistance with the susceptible and three lab-selected resistant populations (Morales et al. 2016). The RNAseq analysis of these data is in progress. In 2017, we will use the same strategy to reveal the transcriptomes responsible for *T. urticae* resistance to fenpyroximate, spiromesifen, and three ovicidal miticides etoxazole, clofentezine, and hexythiazox. Currently, etoxazole-, clofentezine-, and hexythiazox-resistant populations selected from a field-collected population (Olsen Bros, Prosser, WA) have been established in the lab. This summer, we also initiated selection for fenpyroximate and spiromesifen. Once the resistant ratios reach 1,000-fold (compared to pesticide-naïve mites), we will comprehensively sequence the transcriptomes of these populations using high throughput sequencing and carry out downstream analysis by RNA-seq. The "susceptible reference strain" for this experiment is the original field-collected population (Olsen) that was used for resistance selection. Our proposed research will identify mechanisms and resistance-associated genes for candidate miticides that have limited research information available. These up-regulated genes and mutations can be used as markers to precisely predict the miticide resistance in T. urticae populations in peppermint fields.

5. Research Goals and Objectives:

1. Field test candidate miticides for their efficacy against spider mites.
2. Evaluate the impact of candidate pesticides on beneficial mites.
3. Develop a robust bioassay method for evaluating residual efficacy of miticides including propargite, spiromesifen, and fenpyroximate.
4. Develop baseline dose response curves of spider mite populations susceptible to propargite, spiromesifen, and fenpyroximate. (Please note we have accomplished this with bifenthrin, bifenazate, abamectin, hexythiozox, clofentazine, and etoxazole.)
5. Establish mite colonies and "breed" resistance to candidate acaricides into the mite population through constant and consistent exposure to propargite, spiromesifen, and fenpyroximate. (Please note we have accomplished this with bifenthrin, bifenazate, abamectin, hexythiozox, clofentazine, and etoxazole.)
6. Develop discriminating doses of candidate miticides that can be used to rapidly identify the prevalence of tolerance or resistance in a spider mite population.
7. Test selected field populations of spider mites from a representative sample of mint fields and compare their dose response curves to mite populations as in Objectives 4, 5, and 6 above.
8. Expand robust molecular diagnostics to predict (multiple) acaricide resistance in the field.

6. Success Criteria and Timing.

Objective 1 is progressing and will be completed this fall. Objective 2 is progressing well

7. Data Analysis.

All data collected was entered into MS Excel 2013 and tabulated. PoloProbit is used to quantify acaricide toxicity and the Descriptive statistics including treatment means and the standard error for those means are detailed in the data tables in this report.

8. Results and Discussion:

1. Field test candidate miticides for their efficacy against spider mites.
Not completed. We put greater emphasis on the laboratory bioassays.

2. Evaluate the impact of candidate pesticides on beneficial mites.
We have completed bioassays with clofentazine, hexythiozox, and etoxazole. These ovicidal acaricides are harmless to adult predatory mites. However the eggs laid on treated leaf discs had reduced hatching rates compared to eggs laid on the untreated control leaf discs.

3. Develop a robust bioassay method for evaluating residual efficacy of miticides including propargite, spiromesifen, and fenpyroximate.
We have developed robust repeatable methods for bioassay. They are discussed above.

4. Develop baseline dose response curves of spider mite populations susceptible to propargite, spiromesifen, and fenpyroximate. (Please note we have accomplished this with bifenthrin, bifenazate, abamectin, hexythiozox, clofentazine, and etoxazole.)
We have already conducted the bioassay for the baseline dose response for spirodoclofen and fenpyroximate. Base-line dose response for propargite using acaricide-naïve spider mite strain will soon be carried out. The results of the analysis will be presented in our fall 2017 report.

5. Establish mite colonies and "breed" resistance to candidate acaricides into the mite population through constant and consistent exposure to propargite, spiromesifen, and fenpyroximate. (Please note we have accomplished this with bifenthrin, bifenazate, abamectin, hexythiozox, clofentazine, and etoxazole.)
In addition to the previous acaricide-resistant spider mite colonies that we have in the lab (i.e. abamectin, hexythiazox, clofentezine, etoxazole, bifenthrin, bifenazate, fenpyroximate), we are currently establishing resistant spider mite colonies for propargite and acaricides including spiromesafen and fenpyroximate that inhibit mitochondrial electron transport.

6. Develop discriminating doses of candidate miticides that can be used to rapidly identify the prevalence of tolerance or resistance in a spider mite population.
This objective is in progress and we will report on it in our fall 2017 report.

7. Test selected field populations of spider mites from a representative sample of mint fields and compare their dose response curves to mite populations as in Objectives f, g, and h above.

We have collected 10 spider mite populations from peppermint farms from different localities around central Washington (Mabton, Granger, Toppenish, Othello, Prosser). These populations are currently being treated to a dose-response bioassay to an array of acaricides including bifenthrin, abamectin, hexythiazox, fenpyroximate, spirodoclofen and propargite.

Population	Collection da	Latitude	Longitude	DNA	RNA	Spirodoclofe	Hexythiazox	Abamectin	Bifenthrin	Fenpyroxima	Propargite
Granger 1	21-Jun	N 46 20.189	W 120 12.595	Collected	Collected	Done	Done	Done	Done	Done	Done
Granger 2	23-Jun	N46 21.214	W 120 16.350	Collected	Collected	Done	Done	Done	Done	Done	Done
Granger 3	23-Jun	N46 20.771	W120 16.427	Collected	Collected	Done	Done	Done	Done	Done	Done
Mabton 1	6-Jun	N46 13.219	W 120 0.10	Collected	Collected		Done	Done	Done	Done	Done
Mabton 2	7-Jun	N 46 12.189	W 120 6.51	Collected	Collected		Done	Done	Done	Done	Done
Othello 1	10-Jul	N 46 52.26	W 119 2.923	Collected	Collected			Done	Done	Done	
Othello 2	10-Jul	N 46 50.28	W119 4.821	Collected	Collected			Done	Done	Done	
Othello 3	10-Jul	N 46 50.9	W 119 5.53	Collected	Collected			Done	Done	Done	
Prosser	11-Jun	N 46 17.336	W 119 4.795	Collected	Collected	Done		Done	Done	Done	
Toppenish	11-Jul	N 46 20.736	W 120 23.834	Collected	Collected			Done	Done	Done	

8. Expand robust molecular diagnostics to predict (multiple) acaricide resistance in the field.

We are currently extracting genomic DNA and RNA from each of the 10 spider mite populations. An array of target site mutations associated with acaricide-resistance will be screen using diagnostic PCR. Relative gene expression of metabolic genes (cytochrome P450s, glutathione-S-transferase sand carboxylesterases) that are known to be involved in acaricide resistance in the two-spotted spider mites will also be evaluated using qRT-PCR. We also plan to conduct the transcriptome wide search of genes that confer resistance to peppermint allelochemicals and also acaricides by using spider mites that well adapted to peppermints.

9. Research Expenditures: In 2017 the MIRC allocated $11,501 to this project. To date $4,687.20 has been expended.

Salaries- $3,780.00
Wages- $ 0
Benefits $ 907.20
Consumable goods: $12.00

There are $3,301.00 in salary and employee benefit encumbrances on the budget for fall 2017 leaving a true balance of $7,438.12 and we anticipate expending $1800 for sequencing in fall 2017 leaving us with a balance of $1,70.80.

10. Conclusion:

We are making progress on our objectives and will submit a complete report this fall for the MIRC winter meeting and Washington Mint Commission December meeting.

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