OREGON mint
UPDATE
Spring 2016

Evaluating Green Manures for *Verticillium* Wilt Management in Central Oregon

Jeremiah Dung and Darrin Walenta, Oregon State University

Certain green manure crops such as sweet corn, sudangrass and mustards have been shown to reduce the severity of *Verticillium* wilt and improve yields in crops other than mint. Although not completely understood, the mechanisms by which green manure crops reduce *Verticillium* wilt likely varies but may be related to the production of glucosinolate-derived compounds such as isothiocyanates, increased diversity and/or activity of beneficial soilborne microbes, a change in the physical properties of the soil and/or other factors. However, removing a field from a full season of crop production in order to grow a green manure crop is not always economically feasible. In Central Oregon, short-term green manure crops that only require several weeks for growth, incorporation and decomposition may provide the benefits of a full-season green manure crop without disrupting existing cropping systems. The optimum time period for a green manure crop in Central Oregon is a 2-3 month period in late summer and early fall, before planting new mint fields in late October or early November. The objective of this project is to evaluate green manure crops for their potential to reduce *Verticillium* populations in the soil in Central Oregon cropping systems. Two experiments were performed in the 2015 season. The first study evaluated different green manure crops for their ability to reduce *Verticillium* populations in the soil under controlled conditions in a growth chamber. A second study was performed in microplots to evaluate the green manure crops for their biomass production, impact on *Verticillium* populations and reductions in disease symptoms under Central Oregon growing conditions.

Evaluation of Green Manures for *Verticillium* Control Under Controlled Conditions

A total of six green manure crops (*Brassica juncea* 'Pacific Gold' and 'Kodiak,' *Sinapis alba* 'Ida Gold,' Caliente 199 mustard blend, Caliente Nemat arugula and broccoli 'Di Cicco') were grown in pots in a growth chamber (80°F day/60°F night, 13 hour photoperiod) and moved to a greenhouse to initiate bolting. After seven weeks the green manures were chopped into approximately one inch pieces and incorporated at two rates into autoclaved soil that was artificially infested with *V. dahliae*. Green manure rates were based on the expected biomass as indicated by the companies providing the seed. Pots were watered to soil capacity and kept moist. Treatments consisting of allyl isothiocyanate at 40 gal/acre, a non-treated/non-infested control and a non-treated/infested control were also included. All treatments and rates were replicated four times. After four weeks soils were sampled and dried. A total of ten 0.1 g subsamples of soil were plated onto semi-selective medium and the number of colonies on each plate was determined after ten days of incubation.

A significant effect of green manure treatment was observed

Table 1. Effect of various green manures and allyl isothiocyanate on *V. dahliae* soil populations in the growth chamber

Treatment	Rate[1]	CFU/g soil
Non-infested/non-treated	N/A[2]	10*
Infested/non-treated	N/A	747
Sinapis alba 'Ida Gold' (1X)	3.5 tons/acre	1,242
S. alba 'Ida Gold' (2X)	7.0 tons/acre	1,217
Brassica juncea 'Pacific Gold' (1X)	4.6 tons/acre	831
B. juncea 'Pacific Gold' (2X)	9.2 tons/acre	555
B. juncea 'Kodiak' (1X)	5.1 tons/acre	796
B. juncea 'Kodiak' (2X)	10.2 tons/acre	529
Caliente 199 mustard blend (1X)	3.8 tons/acre	833
Caliente 199 mustard blend (2X)	7.6 tons/acre	881
Caliente Nemat arugula (1X)	3.4 tons/acre	163*
Caliente Nemat arugula (2X)	6.8 tons/acre	31*
Broccoli 'Di Cicco' (1X)	4.5 tons/acre	138*
Broccoli 'Di Cicco' (2X)	9.0 tons/acre	10*
Allyl isothiocyanate	40 gal/acre	24*

[1] 1X rates are based on expected biomass information from the seed suppliers
[2] N/A = not applicable
* = significantly less than the infested/non-treated control at P<0.05

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($P < 0.0001$). Although the 2X rate reduced CFUs of *V. dahliae* more than the 1X rate in five out of six green manure treatments, the effect of rate was not significant and a significant interaction between green manure and rate was not observed. Incorporation of Caliente 'Nemat' arugula and broccoli 'Di Cicco' at both rates significantly reduced *V. dahliae* CFUs compared to the infested/non-treated control (Table 1). Allyl isothiocyanate (40 gal/acre) also significantly reduced *V. dahliae* CFUs. In some cases CFUs of *V. dahliae* recovered from green manure treatments were greater than those recovered from the infested/non-treated control. This result may be due to the proliferation of *V. dahliae* on asymptomatic so-called "non-hosts" such as mustards, which has been documented in other studies. More information is needed to determine if mint strains of *V. dahliae* can reproduce on green manures, grasses or other crops that are common to Oregon cropping systems.

Table 2. Treatments and green manure seeding rates for the microplot trial at the Central Oregon Agricultural Research Center

Treatment	Seeding rate
Non-infested/non-treated	N/A[1]
Infested/non-treated	N/A
Sinapis alba 'Ida Gold'	15 lb/acre
Brassica juncea 'Pacific Gold'	15 lb/acre
B. juncea 'Kodiak'	6 lb/acre
Caliente 199 mustard blend	20 lb/acre
Caliente Nemat arugula	10 lb/acre
Broccoli 'Di Cicco'	1 lb/acre
Allyl isothiocyanate (10 gal/acre)	N/A
Allyl isothiocyanate (40 gal/acre)	N/A
Non-infested/allyl isothiocyanate (40 gal/acre)	N/A

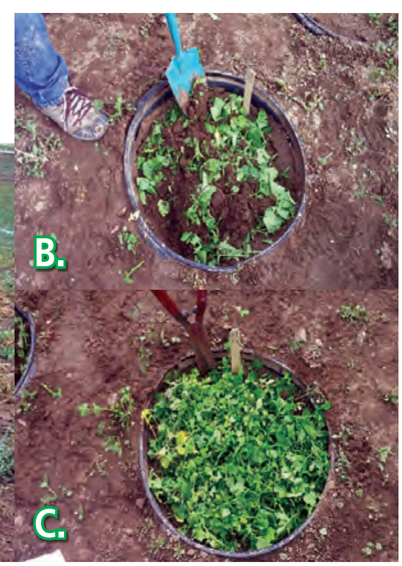
[1]N/A = not applicable

Evaluation of Green Manures for Biomass Production, *Verticillium* Control and Disease Reduction Under Central Oregon Growing Conditions

A microplot experiment was established at COARC to determine if the selected green manure crops can produce sufficient biomass if planted in late summer in Central Oregon. Round (24" diameter x 18" tall), bottomless nursery pots were placed in the ground and infested with a VCG 2B mint isolate of *V. dahliae*. Green manure crops were broadcast planted at recommended rates (Table 2) on August 11 and grown using overhead irrigation. Treatments consisting of allyl isothiocyanate at 10 and 40 gal/acre, a non-treated/non-infested control and a non-treated/infested control were included. A non-infested allyl isothiocyanate treatment (40 gal/acre) was also included to determine if allyl isothiocyanate can cause phytotoxicity in peppermint. Green manure biomass was measured on October 8 after which green manures were chopped and incorporated by hand into each microplot (Fig. 1). After four weeks microplots were planted with greenhouse-grown rhizomes of Black Mitcham peppermint. *Verticillium* wilt severity will be measured in 2016.

The aboveground biomass of the four mustard treatments (Ida Gold, Pacific Gold, Kodiak and Caliente 199) were higher than expected, ranging between 12.1 and 18.3 tons/acre; Nemat arugula produced 6.3 tons/acre of biomass (Fig. 2). However, these data were obtained under microplot conditions and do not take into account row spacings that might be used under field conditions. Regardless, these results suggest that a sufficient amount of green manure biomass can be obtained under Central Oregon growing conditions. More research is needed to determine if higher rates of mustard green manures, which appear to be obtainable based on these results, are more effective at reducing *V. dahliae* populations under controlled conditions. Although the broccoli green manure was effective at reducing *V. dahliae* CFUs in the growth chamber trial, it failed to grow in the microplots and did not produce any biomass during the trial period.

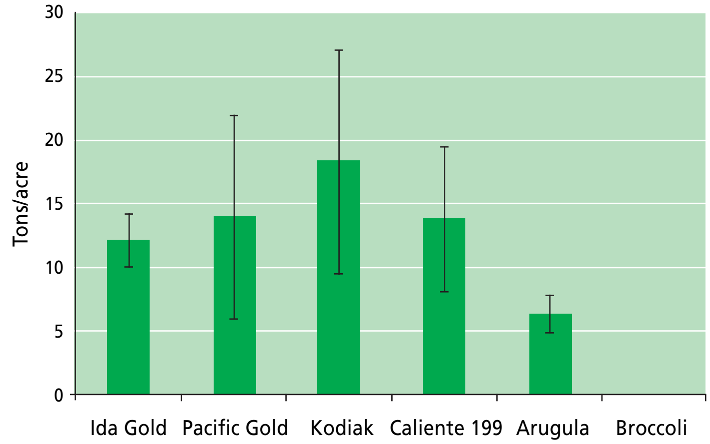
Fig. 1. (A) Green manure microplots; (B) green manure *Sinapis alba* 'Ida Gold' at the 2X rate after chopping and before incorporation; and (C) green manure Caliente 199 mustard blend at the 1X rate during incorporation.



Glucosinolates, the primary compounds responsible for disease control in mustard green manures, are at optimal levels when the plants are in the flowering stage. Among the four replications of each treatment, 75 percent of Pacific Gold microplots bolted, 100 percent of Ida Gold microplots were flowering, 50 percent of Caliente 199 microplots bolted or flowered and 75 percent of Kodiak microplots bolted or flowered (Fig 1). An earlier planting time or a later incorporation date may be required in order to reach full flowering and glucosinolate potential in Caliente 199, Kodiak or Pacific Gold mustards under Central Oregon growing conditions

Verticillium levels in each microplot are currently being enumerated and *Verticillium* wilt severity will be determined during the 2016 growing season. Since allyl isothiocyanate is potentially phytotoxic, the phytotoxicity of allyl isothiocyanate on Black Mitcham peppermint will also be evaluated during the growing season. Final data will be available in fall 2016.

Fig. 2. Green manure biomass produced between August 11 and October 8 in microplots under Central Oregon growing conditions



Mint Pest Alert Newsletter & Survey Results

Marvin Butler, Central Oregon Agricultural Research Center, COARC

Pest Control Prior to Damage

Research was conducted in multiple regions of Oregon to evaluate Coragen, a new environmentally friendly insecticide. It is applied at very low rates with low toxicity to beneficial insects and the environment to control larval pests on peppermint. Treatments were most effective when applied at peak moth flight to control eggs and first instar larvae, with application timing based on insect development models. For mint root borer in particular, this mid-growing season application timing differs from traditional fall application after larval feeding damage that began in July.

Regional Electronic Pest Alert Newsletter

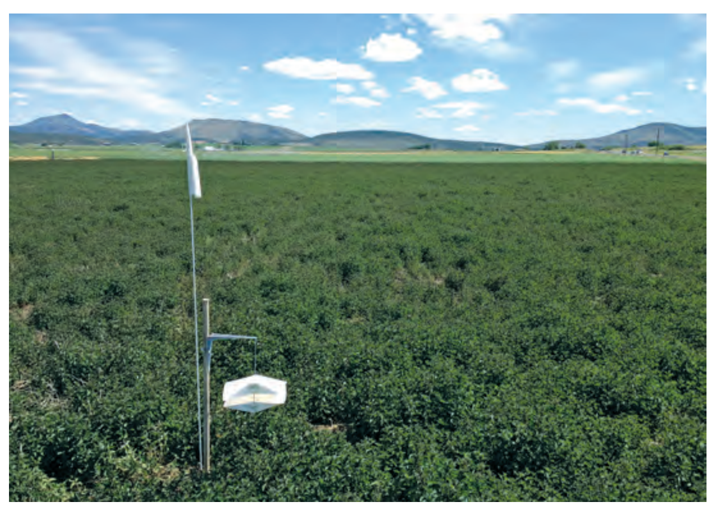
The objective of this electronic Mint Pest Alert Newsletter was to assist growers and field scouts in thinking about insecticide application timing much earlier in the season and assure that they are aware of this new insecticide option. Similar, but separate, electronic Mint Pest Alert Newsletters were developed for the three regions: Willamette Valley, northeastern Oregon and Central Oregon. Cooperators on the project are Darrin L. Walenta (Union Co.), Clare Sullivan (S. Willamette Valley), Nicole Anderson (N. Willamette Valley) and Ralph Berry, Entomology Professor Emeritus.

Positive Grower & Industry Response to Survey

An electronic survey was conducted to evaluate impact. Twenty-six percent of newsletter recipients responded to the survey, 43 percent growers and 57 percent field scouts or industry representatives. Based on a scale from 1 (uninformed) to 5 (fully informed), knowledge of degree-day insect development models increased from 3.0 to 3.9. Knowledge of the new insecticide, Coragen, increased from 3.4 to 4.1. Although 72 percent of respondents continued to use traditional insecticides during 2015 based largely on cost, 63 percent indicated their future plans included the use of Coragen. When asked whether the newsletter should continue, 75 percent of respondents said yes, 25 percent indicated maybe and 0 percent replied no.

Sign Up for Mint Pest Alert Newsletter

To add your name to the newsletter list, provide your email to Clare Sullivan at: Clare.Sullivan@oregonstate.edu, call 541-602-2009, or send to the Linn County Extension Office, 33630 McFarland Rd, Corvallis, OR 97389.



Mint root borer pheromone trap used in scouting associated with the Pest Alert Newsletter.

Weed Control in Western Oregon

Andrew Hulting, Kyle Roerig, Carol Mallory-Smith and Daniel Curtis
Department of Crop and Soil Science, Oregon State University

During the 2015 growing season trials were conducted in Lane and Polk counties of western Oregon. All studies were conducted with the cooperation of local mint growers on commercial mint fields. The objectives of our work are to evaluate herbicides for use in peppermint and continue to provide updated weed control information to peppermint growers. These trials mainly focused on the use of pyroxasulfone (Zidua), pyroxasulfone + flumioxazin (Fierce) and saflufenacil (Sharpen) among other herbicides. Many treatments discussed in this report are not registered for use in peppermint. For a current list of registered treatments refer to the Pacific Northwest Weed Management Handbook (http://pnwhandbooks.org/weed/).

Evaluation of Pyroxasulfone and Pyroxasulfone + Flumioxazin for Crop Safety and Weed Control in Peppermint

A trial was conducted in an established peppermint field south of Monroe, Oregon, in Lane County. The objective of the trial was to assess the crop safety and weed control efficacy of pyroxasulfone (Zidua). Pyroxasulfone provides pre-emergent control of many annual broadleaf and grass weeds impacting mint production in

Table 1. Peppermint injury ratings and fresh hay yield at Monroe, OR in 2015.

	Rate	Application	Injury[a]	Injury[b]	Injury[c]	Injury[d]	Oil Yield[d]
	lb ai/a	date	----------------	----------------	%	----------------	lb/a
check			0a	0b	0a	0a	76a
pyroxasulfone	0.19	2/12	0a	0b	0a	0a	70a
+ terbacil	0.5	2/24					
+ paraquat	0.5	2/24					
+ NIS	0.418	2/24					
pyroxasulfone	0.19	2/12	0a	0b	0a	0a	79a
+ pyroxasulfone	0.19	2/24					
+ terbacil	0.5	2/24					
+ paraquat	0.5	2/24					
+ NIS	0.418	2/24					
pyroxasulfone	0.19	2/24		0b	0a	0a	73a
+ terbacil	0.5	2/24					
+ paraquat	0.5	2/24					
+ NIS	0.418	2/24					
terbacil	0.5	2/24		0b	9a	0a	81a
+ paraquat	0.5	2/24					
+ NIS	0.418	2/24					
+ pyroxasulfone	0.19	3/27					
pyroxasulfone	0.38	2/24		0b	0a	0a	74a
+ terbacil	0.5	2/24					
+ paraquat	0.5	2/24					
+ NIS	0.418	2/24					
terbacil	0.5	2/24		0b	0a	0a	75a
+ paraquat	0.5	2/24					
+ NIS	0.418	2/24					
pyroxasulfone-flumioxazin	0.339	2/24		53a	8a	0a	82a
+ paraquat	0.5	2/24					
+ COC	1.67	2/24					
pyroxasulfone	0.19	2/24		0b	0a	0a	79a
+ paraquat	0.5	2/24					
+ COC	1.67	2/24					
pyroxasulfone-carfentrazone	0.2035	2/24		0b	0a	0a	81a
+ COC	1.67	2/24					
LSD P=.05			0.0	2.3	5.5	0.0	11.4
Standard Deviation			0.0	1.6	3.8	0.0	7.9

[a]Evaluated 2/24/15, [b]Evaluated 3/27/15, [c]Evaluated 5/6/15, [d]Evaluated 7/30/15

Table 2. Sharppoint fluvellin control in spearmint at Independence, OR in 2015.

	Rate	Application	Mint Injury[a]	Sharppoint fluvellin Control[b]	Mint Injury[c]	Mint oil yeild[d]
	lb ai/a	date	----------------------------	%	----------------------------	lb/a
check			0 a	0 a	0 d	59 a
terbacil + paraquat	1.2 0.75	2/20 2/20	61 c	66 a	10 cd	52 a
pyroxasulfone + flumioxazin	0.09 0.08	2/20 2/20	96 a	100 a	25 cd	50 a
flumioxazin	0.08	2/20	89 ab	93 a	13 cd	53 a
saflufenacil	0.0445	2/20	91 ab	75 a	18 cd	54 a
saflufenacil + saflufenacil	0.0223 0.0223	2/20 4/16	86 b	97 a	40 bc	45 a
thiencarbazone	0.00444	4/16		74 a	55 ab	40 a
flumetsulam	0.0665	4/16		79 a	75 a	25 b
amicarbazone	0.219	4/16		80 a	38 bc	41 a
asulam	1.5	4/16		32 b	0 d	54 a
carfentrazone	0.0156	4/16		13 b	0 d	57 a
LSD P=.05			6.7	28.6	20.5	13.4
Standard Deviation			4.5	19.8	14.2	9.2

[a]Evaluated 3/20/15, [b]Evaluated 5/12/15, [c]Evaluated 6/12/15, [d]Harvested 6/19/15

western Oregon. The trial site was free of weeds, including in the untreated check plots, so weed control ratings are not included. However, past research results have indicated 90-100 percent control of annual bluegrass, redroot pigweed, purslane speedwell, willowherb, red sorrel and St. John's wort can be achieved when pyroxasulfone is applied pre-emergent. Pyroxasulfone treatments were applied at three different timings, in combination with other herbicides and in two premixes (Fierce and Anthem Flex). None of the treatments injured the peppermint, including the 2X rate and sequential application of pyroxasulfone, with the exception of pyroxasulfone-flumioxazin (Fierce) premix (Table 1). Injury from pyroxasulfone-flumioxazin was no longer visible by harvest and did not affect yield.

Evaluation of Herbicides for Sharppoint Fluvellin Control in Spearmint

Sharppoint fluvellin is a spreading annual weed affecting mint fields, especially in the spring. This trial was conducted in a field of spearmint north of Independence in Polk County. The objectives of this trial were to evaluate herbicides that could be utilized to control sharppoint fluvellin and identify herbicides that may have adequate crop safety to be used in mint. Flumetsulam (Python) significantly reduced oil yield (Table 2). Yield in plots treated with two applications of saflufenacil (Sharpen), amicarbazone (Xonerate) and thiencarbazone (Varro) may have been reduced by the herbicide application, however, due to variability in the plots this reduction in yield may have been due to other factors. Although injury ratings coincide with reduced yield in this trial, additional work would be necessary to determine whether these compounds reduce mint yield. Saflufenacil followed by an additional application of saflufenacil or flumioxazin each provided 90 percent or greater control of sharppoint fluvellin. Pyroxasulfone-flumioxazin controlled 100 percent of sharppoint fluvellin.

Trials in Progress for 2016

In 2016 three trials are in progress. Two focus on preparing data for registration of linuron for use in peppermint. One is an MIRC funded collaboration with scientists in California, Indiana, Oregon, Washington and Wisconsin and includes several rates, tank mixes and timings of linuron. The second will provide crop safety data to support the IR-4 registration process. The rates and timings are the same as the MIRC trial, however, tank mixes are not included. In our third trial, several Group 14 (PPO inhibitor) herbicides that have been identified through our research as alternatives to paraquat (Gramoxone) that can be used to burndown annual weeds growing in dormant mint will be applied at three timings. These include flumioxazin (Chateau), carfentrazone (Aim) and saflufenacil (Sharpen). This will provide additional crop safety data that can be utilized in supporting possible registrations of carfentrazone and saflufenacil in mint and in making management timing decisions. Continued evaluation of pyroxasulfone (Zidua) will also occur at this site.

Comparative Genomics of Mint Pathogenic *Verticillium dahliae* Isolates

Kelly Vining and Jeremiah Dung, Oregon State University, Mark Lange, Washington State University

Our long-term goal is to use DNA and genome resources to enable sustainable breeding of *Verticillium* wilt-resistant mints for the Pacific Northwest growing region. We are utilizing the mint genome resources we have built over the past two years and have expanded to include the genome of *Verticillium dahliae*. One application of the Oregon Mint Commission-funded mint genome sequence involves studying which genes are switched on or off during *Verticillium* challenge. A second project, initiated in 2015, aims to detect genetic differences among Pacific Northwest mint isolates in order to gain insight into how rapidly the fungus is evolving. Here, we report on the status of our work on both projects.

Research Objectives, Mint Gene Expression:

1. Sequence the complement of genes that are expressed in *Verticillium*-wilt resistant and susceptible *Mentha longifolia* accessions with and without *Verticillium* inoculation.

2. Compare gene expression in the resistant and susceptible mints with and without *Verticillium* inoculation in order to identify all differentially expressed genes.

For this work, we are using two *Mentha longifolia* accessions from South Africa: wilt-resistant CMEN 585 and wilt-susceptible CMEN 584. CMEN 585 is the accession used to build the *Mentha* reference genome. To study gene expression, a mint isolate of *Verticillium dahliae* was used to inoculate rooted cuttings, which were then incubated in a growth chamber. RNA (the "message" molecules produced when genes are expressed) was extracted from roots and stems of inoculated and control plants at two time points (10 days and 20 days post-inoculation) and ultimately sequenced at OSU on two different DNA sequencing instruments from the company Illumina: the HiSeq2000 and the HiSeq3000.

With an enormous quantity of high-quality sequence data in hand, we have begun to look at gene expression differences among the samples. Genes that are switched on in one condition and switched off in another condition are said to be "differentially expressed." We are looking at pairwise comparisons between resistant and susceptible accessions between control and inoculated treatments and between 10-day and 20-day post-inoculation time points. We last reported preliminary results from roots ten days post-inoculation. We have now completed this part of the analysis for roots as well as for stems of all the mint varieties. Results for the 10-day post-inoculation time point are shown in Table 1. Consistent with previous results, greater numbers of genes were differentially expressed between the different mints than were differentially expressed between control and inoculated plants of any particular type. In stems numbers of differentially-expressed genes are substantially higher than in roots in all mints. Now, we are comparing the sets of genes with either increased or diminished activity in response to *Verticillium* inoculation. In roots, only one to four genes with differential expression due to inoculation are shared among any of the mint varieties. In stems, however, 100-

Table 1. Gene expression differences in mint roots and stems ten days after *Verticillium* inoculation.

Condition 1	Condition 2	No. differentially-expressed genes	Higher in Condition 1	Higher in Condition 2
CMEN 585 roots - control	CMEN 585 roots - Verticillium-inoculated	145	93	52
CMEN 584 roots - control	CMEN 584 roots - Verticillium-inoculated	72	57	15
CMEN 585 roots - control	CMEN 584 roots - control	5,222	3,244	1,978
CMEN 585 roots - Verticillium-inoculated	CMEN 584 roots - Verticillium-inoculated	6,412	3,716	2,696
Black Mitcham roots - control	Black Mitcham roots - Verticillium-inoculated	142	99	43
Native spearmint roots - control	Native spearmint roots - Verticillium-inoculated	112	29	83
Black Mitcham roots - control	Native spearmint roots - control	4,326	2,489	1,837
Black Mitcham roots - Verticillium-inoculated	Native spearmint roots - Verticillium-inoculated	3,716	1,919	1,797
CMEN 585 stems - control	CMEN 585 stems - Verticillium-inoculated	1,177	568	609
CMEN 584 stems - control	CMEN 584 stems - Verticillium-inoculated	175	60	115
CMEN 585 stems - control	CMEN 584 stems - control	8,088	4,754	3,334
CMEN 585 stems - Verticillium-inoculated	CMEN 584 stems - Verticillium-inoculated	5,501	3,404	2,097
Black Mitcham stems - control	Black Mitcham stems - Verticillium-inoculated	212	80	132
Native spearmint stems - control	Native spearmint stems - Verticillium-inoculated	310	172	138
Black Mitcham stems - control	Native spearmint stems - control	3,520	1,970	1,550
Black Mitcham stems - Verticillium-inoculated	Native spearmint stems - Verticillium-inoculated	5,011	2,698	2,313

200 differentially-regulated genes are in common between inoculated mints. Our search for genes involved in wilt resistance response is now narrowing to focus on these genes, especially those in stems, where there are more apparent gene expression changes.

Research Objectives, *Verticillium* Diversity:

1. Collect 25 new *Verticillium dahliae* isolates from infested mint field sites in Oregon that represent the local diversity of the species.

2. Sequence genomic DNA from 50 mint *V. dahliae* isolates, including newly-collected and archival samples, as well as from 50 *V. dahliae* isolates from other plant hosts.

3. Compare genome sequence data from all isolates to identify polymorphisms that distinguish mint-pathogenic *V. dahliae* from 50 isolates pathogenic on other plant species.

Our effort to survey the genetic diversity of the fungal pathogen in Oregon and the broader Pacific Northwest is now in its second year. During summer of 2015, Co-PI Vining collected peppermint samples from 23 field sites around the Willamette Valley. Co-PI Dung collected peppermint samples from six fields in Central Oregon and obtained samples from one field in Union County (collected by Darrin Walenta). Most of the sampled plants displayed mild to moderate symptoms of *Verticillium* wilt such as chlorosis and crescent leaves. Co-PI Dung then plated surface-sterilized stems onto selective NP-10 medium and recovered 62 *V. dahliae* isolates. DNA was obtained from 51 of these isolates as well as 39 isolates that were collected between 1996 and 2014 (29 from peppermint, five from scotch spearmint and five from native spearmint). With DNA from an additional 63 isolates from non-mint hosts, we now have 153 samples ready for sequencing, with the sequencing run to initiate on March 14, 2016. Nitrate-nonutilizing mutants of these isolates were generated and vegetative compatibility group (VCG) testing is currently in progress. Results have been obtained for 40 of the mint isolates and all but two isolates belong to VCG 2B, consistent with the mint pathotype of *V. dahliae*. One isolate paired with both VCG 2B and VCG 4A, while the other isolate belongs to VCG 4A/B.

Effects of Applying Varying Amounts of Mint Slugs on Established Mint in Northeast Oregon (Second year)

Bryon Quebbeman, Quebbeman's Crop Monitoring, La Grande, Oregon

Mint slugs are a good source of clean nutrients and organic matter. Cover over the mint plants during a cold dry winter is speculated to help protect plants from desiccation and becoming weakened. This research was instigated to investigate if there were any negative or positive results of applying fresh mint slugs to live mint fields after harvest. In addition, different application times and rates of mint slugs were tested.

Objective

Measure any effect on weed control, pests, disease and plant health from applying different amounts of mint slugs, at different times, to established mint in the La Grande area.

Materials and Methods

Four experiments were established in four grower peppermint fields in the La Grande area. The mint slugs were applied after the 2013 harvest. In addition, more mint slugs were applied in the fall of 2014 to different plots, which are next to the original plots. The plots for both years were established in the fall of 2013 and were replicated four times in a randomized block design. Plot size was 18' x 20'. One of the original four experiments was flooded out in the winter of 2013/14 so the experiment was reestablished in the fall of 2014. This flooded out experiment only has one year of data. In addition, a fifth experiment was started in November of 2014.

This fifth experiment only had a high and low rate of slugs applied on the late application date of November. The following treatments were applied:

1. Untreated check (no mint slugs)
2. Early application of mint slugs at a low rate (1 inch) September 2 and 19, 2013
3. Early application of mint slugs at a high rate (2 inches) September 2 and 19, 2013
4. Late application of mint slugs at a low rate (0.5 inch) November 8 and 12, 2013
5. Late application of mint slugs at a high rate (1 inch) November 8 and 12, 2013
6. Early application of mint slugs at a low rate (0.5 inch) September 6 and 10, 2014
7. Early application of mint slugs at a high rate (1 inch) September 6 and 10, 2014
8. Late application of mint slugs at a low rate (0.5 inch) October 31 and November 6, 2014
9. Late application of mint slugs at a high rate (1 inch) October 31 and November 6, 2014

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Mint slugs were applied by measuring the volume of mint slugs needed to cover the entire plot area with the correct rate. The mint slugs were spread evenly by hand.

Dry hay weights were determined by cutting all the mint from a 50 square feet area and then weighting the hay after it was air dried.

No mint oil yields were taken.

RESULTS

Table 1 - Average dry weight of mint hay from 50 sq. ft.

Trmt.	Treatments	Mean lbs of dry mint hay per 50 sq. ft.		
	Application time and amount of slugs (inches)	Experiment 1	Experiment 3	Experiment 4
1	UTC	6.9 bc	6.3	7.1
2	Early, low rate, 1 in.*	6.6 b	6.4	7.6
3	Early, high rate, 2 in.*	2.2 a	6.4	7.9
4	Late, low rate, ½ in.*	7.5 bc	7.0	7.9
5	Late, high rate, 1 in.*	7.5 bc	7.0	7.2
6	Early, low rate, ½ in. **	7.5 bc	7.1	8.4
7	Early, high rate, 1 in. **	6.8 bc	7.2	7.8
8	Late, low rate, ½ in. **	7.0 bc	6.7	8.1
9	Late, high rate, 1 in.**	8.4 c	6.8	7.7
	LSD	1.6	NS	NS

LSD Sample means were compared with Fisher's Protected LSD (p=0.05).
*Treatments applied fall 2013 **Treatments applied fall 2014

Table 2 Average dry weight of mint hay from 50 sq. ft. Sampled between August 3 and August 8, 2015

Trmt.	Treatments	Mean lbs of dry mint hay per 50 sq. ft.	
		Experiment 2	Experiment 5
1	UTC	10.4	2.8 a
2	Early, low rate, 1/2 in.*	8.5	-----
3	Early, high rate, 1 in.*	9.0	-----
4	Late, low rate, ½ in.*	9.5	4.8 b
5	Late, high rate, 1 in.*	9.8	5.6 b
LSD		NS	1.7

LSD Sample means were compared with Fisher's Protected LSD (p=0.05).
*Treatments all applied fall 2014

Experiment One

The results in the summer of 2014 found that both of the early treatments of mint slugs were detrimental to the mint stand and growth, however, the late applications significantly increased the dry hay weight in 2014. (Data not shown.)

It is speculated that the extra nutrients from the late mint slug applications increased the mint's growth in the 2014 season.

In 2015, the early high rate applied in the fall of 2013 had significantly less hay than any of the other treatments (Table 1). This is due to a thin mint stand caused by the high rate of mint slugs, which smothered the mint in 2014. Treatment nine, of Experiment One (late high rate applied in fall of 2014) did not have significantly more hay than the untreated check, but numerically treatment nine had the greatest dry hay weight of any treatment.

In the fall of 2014, after the second set of mint slug applications had been made, the grower had composted dairy manure spread on this field. Unfortunately, the compost was accidently spread over the mint slug plots. It is likely that the extra nutrients from the dairy compost masked some of nutrient benefits of the mint slugs.

In 2015, visual observations in May through June generally found that the mint in the plots with mint slugs applied in the fall of 2014 had a slightly darker green color to them than did the other treatments with the mint slugs applied in the fall of 2013 or the untreated plots. It was surprising that the mint slugs caused any difference in the mint growth, considering that the dairy compost as well as the grower's fertilizer was applied to these treatments. The darker green color faded by harvest time.

There was no noticeable difference in the amount of bloom between treatments at harvest time.

Experiment Two

This experiment was flooded in the winter of 2013/14 and so there was no data from it in 2014. Mint slugs were applied in the fall of 2014 to a new area of the same field.

In addition, Experiment Two had an increase in visible crop injury in this trial from the 2014 application of the mint slugs. The high rate of mint slugs for both early and late applications caused the most foliar damage.

The soil in Experiment Two caused some of the mint foliage to turn yellow and later turn necrotic. This foliar damage occurred outside of the mint slug plots also, but the mint slugs increased the severity of foliar damage that was already occurring. This foliar damage started in April and continued through July. The damage stopped by mid-May and by the end of May the mint was starting to outgrow the damage. By the first of August there was no visible foliar damage.

As can be seen from Table 2, the hay weights of the mint slug treatments of Experiment Two are not significantly different from the UTC but they are numerically lower. The lowest hay weights were from the early applications of slugs. The mint appeared to fully recover by harvest time and there were no visible differences in mint color or the amount of bloom, although the hay weights indicate there was some lasting effect on the mint from the foliar damage that occurred earlier in the season.

Experiment Three

This experiment had some stand thinning from the early high rate that was applied in 2013. By early August of 2015 the mint in these damaged plots had mostly filled back in.

When the first observation was made in May of 2015, the late treatments that were applied in the fall of 2014 had thinner stands of mint, with the highest late rate being the thinnest stand. Some of the treatments that were applied in the fall of 2013 had a darker green color than the 2014 applied treatments or the UTC. These darker green plots were not consistent across replications. This plot area was on a slight ridge and this changed topography and soil type and affected the plant growth slightly.

By late June of 2015 the late slug applications that were applied in the fall of 2014 were still slightly thinner, but by harvest time in early August there were no significant differences in the appearance of the mint. Numerically all of the slug treatments did have more dry hay than the UTC, indicating there was a slight trend towards the mint slugs increasing the mint growth.

Experiment Four

There were no significant differences of the dry hay weights between any treatments and the UTC (Table 1). However, dry hay weights were all numerically higher than the UTC. This shows a trend for the slug treatments to have increased mint growth compared to the UTC.

From May through most of June, most of the slug treatments from both years produced darker green mint and caused the mint stands to be slightly thicker.

At harvest time there was no visible differences between the amount of bloom between the treatments and the UTC.

Experiment Five

This experiment was an extra trial that was done as an after thought. Only the late applications in the fall of 2014 were applied. This experiment was located in a ten-year-old field which had a very weak stand in the fall of 2014.

The results in Experiment Five were striking as both late applications of the mint slugs significantly increased the mint dry hay weight compared to the UTC (Table 2). Although the total dry hay weight was low compared to the other experiments, the late mint slug applications in Experiment Five nearly doubled the dry hay weights. The mint stand was still thin even with the mint slugs helping to thicken the stand. There were no differences in the amount of bloom on the mint between any of the treatments.

This experiment clearly showed that fall applied mint slugs can help a weak stand to better survive the winter and spring and produce more mint hay.

CONCLUSIONS

There were no increased problems from insects, mites, weeds or diseases caused by the mint slugs.

Mint slugs that were applied soon after harvest sometimes caused a temporary reduction of mint growth.

Applying two inches of mint slugs soon after harvest killed the mint in one experiment and damaged the mint stand in a second experiment. Healthy mint was able to withstand two inches of mint slugs early without a significant amount of crop injury.

There were no significant differences in hay weights, between one-half and one-inch rates of late applied mint slugs.

In general, the late application (late October-November) of one-half to one-inch of mint slugs did not cause any lasting detrimental effects to the mint.

If the mint stand is weak, the best time to apply the mint slugs is late October through November.

Mint slugs should not be applied in the amounts listed previously on soils that are prone to have salt or sodium problems as the nutrients in the mint slugs may add to the existing problems.

In old and/or weaker mint fields or in fields with poorer soil fertility the late application of one-half to one-inch of mint slugs can have some positive effects by slightly increasing dry hay weight and reducing winter kill.

Although no soil samples were taken, it could be concluded that the addition of mint slugs could reduce the need for fertilizer to be applied to the mint. No oil yields were taken so no conclusions about effects on oil yields could be made, however, if a weak thin stand is thickened up, it stands to reason that the oil yield would be increased.

Crop Tolerance and Efficacy of Zidua (Pyroxasulfone) on Dormant Mint in Northeast Oregon

Bryon Quebbeman, Quebbeman's Crop Monitoring, La Grande, Oregon

Zidua herbicide may soon be labeled for use on mint. Its effectiveness and crop safety on mint grown in Northeast Oregon had not been tested before these experiments were conducted.

Objective

Determine the crop tolerance of dormant, established mint to different rates of Zidua and application dates to the standard herbicides. Also determine the amount of weed control on any existing weed populations.

Results

No significant crop injury was seen from any Zidua treatment in either experiment. There was some temporary stunting of the mint from the standard treatment, which contained Sinbar, Chateau and Gramoxone in both experiments (Tables 1 and 2). The Zidua treatments provided no significant control of yellow mustard (Tables 1 and 2).

The Zidua treatments did have some effect on prickly lettuce, but did not provide adequate control, even at the 2X rate (Tables 1 and 2).

In Experiment One all of the Zidua treatments provided a significant amount of control of kochia and provided the same level of control of kochia, as did the standard treatment. Although the control of the kochia was statistically significant and may have appeared to be good (72 to 94 percent control), the kochia population was so heavy that even 94 percent control would have been inadequate weed control (Table 1).

There was no significant amount of precipitation after the treatments were applied until the growers irrigated the fields. This lack of precipitation may have had a negative effect on the herbicides' effectiveness or on the amount of crop tolerance.

There were no significant differences in oil yields between any of the treatments and the untreated check (Table 3).

In Experiment One, some of the mint in the plot area died in March/April from a soil condition and strawberry root weevil damage. This left some treatments with little to no mint in them. Extra samples were taken in other replications to make up for the plots that had thin mint stands.

In Experiment Two there was also some leaf burning that occurred in April. This damage was throughout the field and was not associated with the herbicide treatments. It appeared that a heavy infestation of spider mites in April caused leaf damage to the smallest mint plants. The mint fully outgrew this damage with no visible affects.

Summary:

The unusually dry spring likely caused all the herbicides to provide less weed control. In addition, the Zidua treatments may have been safer on the mint due to the lack of precipitation to activate the Zidua.

There was a slight amount of crop injury from the standard herbicide treatment. It is speculated that the Gramoxone (paraquat) in this standard mix burned some of the emerging mint buds and slightly stunted the mint. The damage from the standard treatment did not affect the oil yields.

Table 1. Effectiveness and crop tolerance of Zidua (pyroxasulfone) on established mint in the La Grande area (Experiment One, 6th year field)

Treatment	Rate lb ai/a	Applied	Yellow Mustard [2]	Prickly Lettuce [2]	Kochia [2]	Injury [1]	Injury [2]
			-------------- % Control		-------------------	----------%----------	
UTC			0 a	0 a	0 a	0 a	0
Zidua	0.16	2/23/15	3 a	9 a	86 bc	0 a	0
Zidua	0.32 (2x)	2/23/15	4 a	16 ab	94 c	0 a	0
Zidua	0.16	3/16/15	0 a	38 b	72 b	---	0
Sinbar + Chateau + Gramoxone	0.4 0.128 0.5	2/23/15	100 b	100 c	88 bc	7 b	0
LSD			4	24	18	2	
CV			20	71	26	148	

LSD Sample means were compared with Fisher's Protected LSD (p=0.05).

1 Evaluated 3/16/2015

2 Evaluated 5/23/2015

Table 2. Effectiveness and crop tolerance of Zidua (pyroxasulfone) on established mint in the La Grande area (Experiment Two, 5th year field)

Treatment	Rate lb ai/a	Applied	Yellow Mustard [2]	Prickly Lettuce [2]	Injury [1]	Injury [2]
			--------% Control--------		-------------%-----------	
UTC			0 a	0 a	0 a	0
Zidua	0.16	2/23/15	0 a	63 b	0 a	0
Zidua	0.32 (2x)	2/23/15	13 ab	88 c	0 a	0
Zidua	0.16	3/16/15	22 b	50 b	---	0
Sinbar + Chateau + Gramoxone	0.4 0.128 0.5	2/23/15	100 c	100 c	5 b	0
LSD			18	15	1	
CV			64	24	90	

LSD Sample means were compared with Fisher's Protected LSD (p=0.05).
1 Evaluated 3/16/2015
2 Evaluated 5/14/2015

Table 3. Mean oil yields from Zidua (pyroxasulfone) on established mint in the La Grande area (Experiments One and Two, 2015)

Treatment	lb ai/a	Rate Applied	Experiment One	Experiment Two
			------ Lbs /ac of oil -------	
UTC			48.4	73.7
Zidua	0.16	2/23/15	52.5	72.6
Zidua	0.32 (2x)	2/23/15	46.7	78.0
Zidua	0.16	3/16/15	56.8	79.9
Sinbar + Chateau + Gramoxone	0.4 +0.128 0.5	2/23/15	56.1	73.9
LSD			NS	NS
CV			20.3	12.2

LSD Sample means were compared with Fisher's Protected LSD (p=0.05).

No herbicide treatment had a significantly different oil yield compared to the untreated check in either experiment.

All the Zidua treatments provided little to no control of yellow mustard and minimal control of prickly lettuce.

Zidua provided good control of kochia at both rates, as did the standard treatment of Sinbar, Chateau and Gramoxone in Experiment One, however, due to a very heavy kochia population, the control would have still been inadequate if the weeds had not been hand rogued.

No other weeds were present in adequate amounts to make any evaluations on weed control.

Another year of data would be helpful to determine the tolerance of the peppermint to Zidua and its effectiveness on the weeds when it is activated by rainfall.

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Mint Varietal Improvement Project at WSU

Mark Lange, Washington State University,
Kelly Vining, Oregon State University and Brian Dilkes, Purdue University

Current Research Progress and Future Plans

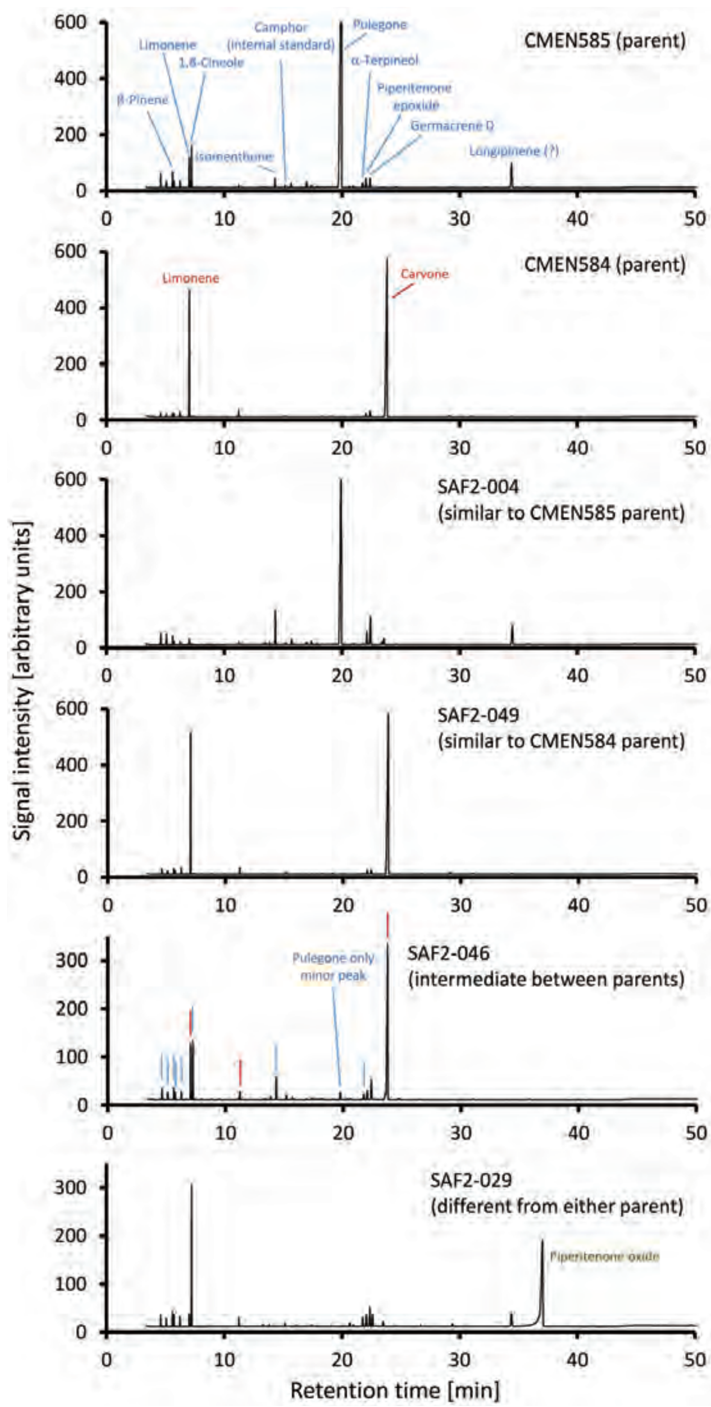
Oil compositional analysis of a segregation horse mint population. Horse mint (*Mentha longifolia*) has been chosen as a model species for the genus *Mentha* because of its relatively simple diploid genome and remarkable within-species chemotypic (oil characteristics) diversity. Kelly Vining (OSU) generated an *M. longifolia* population derived from a cross between the South African accessions CMEN 585 (wilt-resistant) and CMEN 584 (wilt-susceptible). We hypothesize that this population segregates for oil characteristics and, to test this hypothesis, are currently analyzing all members for their oil composition. We obtained the population from OSU and grew all 94 members to maturity (10 percent flowering) in WSU greenhouses. Leaves were collected and distilled and oils analyzed by GC/MS (triplicate distillations and oil analyses in early, mid and late summer to control for technical variation). When various controls are included, we processed roughly 330 samples. The project was completed in October 2015.

Outcomes

A total of ~150 peaks were quantified. Oil from the CMEN 585 parent contained pulegone as the most abundant component (Fig. 1). Other prominent constituents were limonene, 1,8-cineole and a late eluting peak tentatively annotated as longipinene. Less abundant components were ß-pinene, isomenthone, a-terpineol, piperitonene epoxide and germacrene D. The CMEN 484 parent produces an oil with carvone and limonene as major components, whereas all other peaks are fairly minor (Fig. 1). Of all samples, 20-25 percent had an oil with characteristics similar to those of the CMEN 585 parent (example: SAF2-004 in Fig. 1), 20-25 percent resembled the CMEN 584 parent (example: SAF2-049 in Fig. 1), about 50 percent had characteristics of both parents (example: SAF2-046 in Fig. 1; however, pulegone was only a minor peak) and about five percent had an oil that did not resemble that of any parent (example: SAF2-029 in Fig. 1; contains piperitenone oxide as principal constituent).

Oil compositional analysis of diverse mint accessions. The National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon maintains one of the most comprehensive collections of mint accessions worldwide. An analysis of these accessions allows us to evaluate the existing chemical diversity in the genus *Mentha.* Ultimately, after integrating sequence data from our collaborators, we will use this information to select candidate accessions for crosses

Fig. 1. Characteristic GC-FID chromatograms of essential oil distilled from members of a segregating population generated by crossing the *Mentha longifolia* accessions CMEN 585 and CMEN 584.



between a horse mint (*M. longifolia*) and apple mint (*M. suaveolens*) parent, a first step toward re-creating peppermint from its presumed ancestors. We have collected material from all available accessions (~60) and transferred it to WSU (August 5-9, 2015). Replicated data sets have been obtained for oils from 28 accessions (Fig. 2). We will continue these analyses for the remainder of 2015 and parts of Q1, 2016. Note that no funds will be requested to complete these analyses in early 2016.

Our data obtained with various mint accessions indicates that there is considerable oil compositional diversity across the family and even within a single genus. During the next funding period we will focus on integrating these oil data with *Verticillium* wilt resistance and ploidy information (to be generated by collaborators at OSU). We will also collect gene expression data for glandular trichomes (anatomical structures responsible for oil synthesis) to evaluate which gene and gene variants correlate with favorable oil characteristics. We will then make decisions about which accessions will be used for crosses, a critical first step toward breeding resources for the continual improvement of mint.

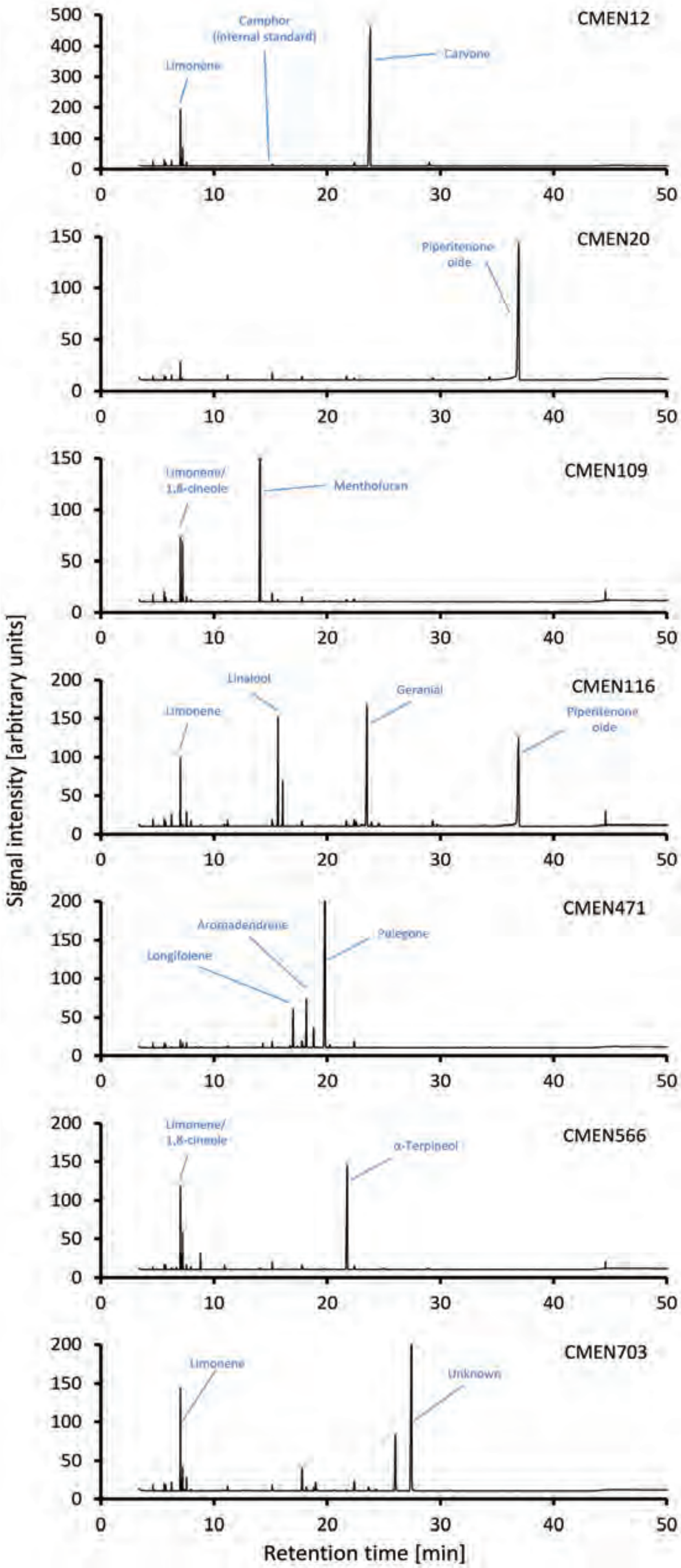


Fig. 2. Characteristic GC-FID chromatograms of essential oil distilled from diverse mint accessions obtained from the National Clonal Germplasm Center. Acronyms: CMEN 12, *Mentha suaveolens* (Turkish seed lot in Netherlands); CMEN 20, *Mentha longifolia* (wild collection in Syria); CMEN 109, *Mentha aquatica* (seedlot in Germany); CMEN 116, *Mentha aquatica* var. citrata (European stock); CMEN 471, *Mentha aquatica* (likely hybrid); CMEN 566, *Mentha aquatica* (wild collection in Netherlands); and CMEN 703, *Mentha longifolia* (wild collection in Armenia).

Mint Herbicide Carry Over Effect on Bluegrass Seed and Winter Wheat

Marvin Butler, Central Oregon Agricultural Research Center, COARC

Central Oregon Issues

In Central Oregon there has been grower and fieldmen concern over the last several years about herbicide injury to Kentucky bluegrass and wheat when rotating out of peppermint. Growers typically reduce Sinbar (terbacil) use in older stands to prevent future damage as they rotate out of mint. Rates are generally 1.5 lb/acre the first year, 1 lb/acre the second year, 0.5 lb/acre the third year and no Sinbar applied the fourth or final year.

To offset reduced Sinbar use in older stands various combinations of Spartan (sulfentrazone), Chateau (flumioxazin) and Command (clomazone) are used for weed control. However, as use of these products has increased rotational problems are becoming more evident. Symptoms are a generalized dying of the new crop across the entire field, rather than spotty areas of Sinbar damage as seen in the past due to soil compaction or stand die out.

Project Objectives and Methods

This project focused on these four residue herbicides applied mid-February alone and in all combinations on the final year of mint production. Treatments include Sinbar at 0.5 lb/acre and 1 lb/acre. Following harvest mint was treated with an application of Roundup and the ground lightly rotovated to minimize soil movement between plots before rotational crops Kentucky bluegrass for seed and winter wheat were planted in late August and mid-October.

Research Results

Unfortunately, there has been no discernable effect on Kentucky bluegrass or wheat based on herbicide treatments. The consensus of opinion is that perhaps the 1 lb/acre application of Sinbar this final year of production evidently does not equal what has often built up in the soil over time during normal production, despite reduced rates and non-use during the final years of production.

Mint herbicide plot area prior to treatment in late winter.

Mint Industry Pesticide Update

Steve Salisbury,
Mint Industry Research Council Research and Regulatory Coordinator

It has been busy on the pesticide front this winter and spring, and we are all anxiously watching progress on mint registrations and labels. There is headway in all phases of our pesticide pipeline projects from trial plot research, IR-4 projects and the pending Section 18 request.

As most of you are aware, a Section 18 request was pulled together for Tough herbicide (pyridate) this winter by the MIRC. The request was submitted to seven mint producing states: Oregon, Washington, Idaho, California, Wisconsin, Indiana and Michigan. After discussions and revisions with each of the states, they have submitted the requests on to the EPA for consideration. Currently, the request is being reviewed by the EPA to determine our case for urgency and the non-routine situation as well as any potential impacts the herbicide could have.

In March, Bryan Ostlund and I met with the Emergency Response Team at the EPA to discuss the situation leading us to the Section 18 request. This was a positive meeting that gave us the opportunity to explain the mint industry's significant need to regain the registered use of Tough herbicide and the tremendous efforts that have been made within the mint weed research community to discover an alternative to Tough. By the end of the meeting the EPA staff had a better understanding of our industry's weed situation, the effects of weeds on oil quality, the economic impacts and how Tough can play a great role in keeping mint growers competitive and viable in the world market.

The manufacturer and registrant of Tough is Belchim Crop Protection in Belgium, and the U.S. distributor is Engage Agro USA. Both companies have put a lot of effort into supporting the MIRC request for emergency registration. If the emergency registration is approved, they will work as quickly as possible to get product to all of the local outlets as soon as possible.

As with any Section 18 registration and label, there comes a little paperwork that all growers wishing to use the product need to participate in. That includes a "Waiver of Liability and Indemnification Agreement" that growers need to sign and have notarized prior to taking possession of any product. These agreements will be administered by the MIRC and circulated as soon as possible following an EPA approval.

In summary, the MIRC is working hard to bring Tough herbicide back to the growers. However, there are still a lot of regulatory challenges that need to be overcome before any approval will be made. This is a unique regulatory situation and we are working hard to navigate the process. If the Section 18 is not approved, then we will continue to work towards getting a full Section 3 registration.


Steve Salisbury

As for the IR-4 pipeline, there is progress being made here as well. The latest update on pyroxasulfone (Zidua herbicide, BASF) is that the residue analysis work is done and the final report will be submitted to IR-4 headquarters soon. IR-4 is projecting a submission to EPA by late summer or early fall 2016. Pyroxasulfone is a pre-emergence herbicide that will provide good to excellent control of broadleaf and grass weeds.

Sulfentrazone (Spartan herbicide, FMC) is in the IR-4 to amend the current mint label to include use in double-cut mint. It was unfortunately delayed last year. Currently, IR-4 is prepared to submit the request, however, they are waiting on FMC to provide their information for submission. A submission to EPA could occur soon once FMC is onboard with the paperwork.

The EPA should be making a decision on IR-4's submission for carfentrazone (Aim herbicide, FMC) tolerance and registration in the very near future. This will be a nice additional broadleaf contact herbicide for use in mint. However, by the time it is approved we will have missed the labeled timing for application this spring as it is projected to be a dormant application only.

The IR-4 started the residue work on linuron this spring. Since this project just got underway it is difficult to project a timeline to registration. The MIRC is also conducting several linuron performance and crop safety trials this year in the West and Midwest. This information will be useful to generate recent data and information on recommended use patterns in regional locations.

Pesticide research projects include herbicide and nematicides trials. Weed scientists continue to screen and evaluate herbicides in hopes to discover new chemistries to use in mint. Research projects also include work on nematode control materials. As you are aware, the Vydate shortage situation has hung around much longer than anticipated which continues to pose a significant challenge

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(continued from page 15)

to mint production as well as other crops. The MIRC continues to look for new materials that will perform with crop safety that could potentially lead to a new registration. At this time, trials in Southwest Idaho show some promise of potential new materials for use on mint. As soon as there is enough data generated and registrant support developed, we will pursue a new registration.

Lastly, I want to say "Thank you" to those of you who helped provide information to me for the Section 18 request. I appreciate your help and timely responses.

As further progress is made, or breaking news is available, we will circulate information as quickly as possible. As always, please contact me if you have any questions or comments (503.551.3747).

News from O.E.O.G.L.

Tim Butler, OEOGL Chairman, Aumsville, Oregon

Plans are beginning for the 2017 Annual Convention. Be sure to mark your calendars. The dates will be January 12 & 13 at the Salishan Lodge and Golf Resort, Gleneden Beach, Oregon.

If you are interested in advertising in the 2017 Meeting Program and Directory, a mailing will be made in August. If you do not receive the mailing or would like additional information on advertising, contact Kari or Sue at the League office at (503) 364-2944.

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