Evaluating Methyl Salicylate Lures on Natural Enemies, Pests and Meristem Damage in Red Maple Fields[1]

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Abstract

Red maple seedlings transplanted in spring must grow vertically quickly and insecticides are applied to prevent meristem damage. While sprays have achieved desired results, sustainable options are being sought. Methyl salicylate (MeSA) is a common herbivore-induced plant volatile that is available as a commercial lure to attract natural enemies into farms. We conducted a 2-year field study to determine if MeSA-treated plots had more natural enemies, fewer insect pests, and less meristem damage. The only natural enemy detected in statistically higher abundance within MeSA plots was the minute pirate bug (*Orius* spp.), a predator of thrips (Thripidae) and aphids (Aphidae). However, this increase in abundance was only higher during the end of the second-year trial. Abundance of aphid alates was statistically lower in MeSA blocks 2 weeks after application. No other pests were significantly lower in MeSA-treated than control plots. Meristem damage in MeSA blocks was lower overall in the first year, and for the first 3 weeks in the second-year trial. While MeSA was associated with significantly less meristem damage, the mechanism was not clear from arthropod samples and potential reasons are discussed.

Species used in this study: red maple (*Acer rubrum* L.); minute pirate bug (*Orius* spp.).

Chemicals used in this study: Methyl salicylate (Predalure).

Index words: *Acer rubrum* L., herbivore-induced plant volatile, meristem, predators, thrips, witches' broom.

Significance to the Horticulture Industry

Red maples, *Acer rubrum,* is a widely popular landscaping tree in the United States. The Willamette Valley of Oregon is home to large acreages of red maple production. A witches' broom symptom has been causing increased labor costs and insecticide applications in Willamette Valley red maple production for the past decades. The exact causal agent has not been determined; however, it is believed to be associated with an arthropod pest. While not a study designed to identify the culprit, our data provides evidence of an association between aphid abundance and instances of witches' broom symptoms. This study also provides evidence that methyl salicylate lures can help to reduce these symptoms and aphid abundance early in the season while augmenting late season populations of the predatory minute pirate bug, *Orius*. This data can help guide future work to identify the cause of witches' broom symptoms and provides evidence for the efficacy of a safer alternative to chemical sprays.

Introduction

Biological control, an important component of integrated pest management (IPM), relies on predators, parasitoids or pathogens to reduce populations of pest insects. These natural enemies can be deliberately released into a farm, or naturally occurring enemies can be encouraged by on-farm practices. Methyl salicylate (MeSA) is an herbivore-induced plant volatile that attracts a wide array of beneficial predator and parasitoid species of insects (review by Khan et al. 2008). Attraction of beneficials to synthetic MeSA lures has been documented in numerous crop systems, including but not limited to apple (*Malus*), cherry (*Prunus*), cranberry (*Vaccinium*), cotton (*Gossypium*), grapes (*Vitis*), hops (*Humulus*), soybean (*Glycine*), strawberry (*Fragaria*) and turnips (*Brassica*) (Rodriguez-Saona et al. 2011). Beyond attraction, MeSA can also increase predation rates on crop insect pests (Mallinger et al 2011, Salamanca et al. 2019) and retention rates of released predators (Kelly et al. 2014). Moreover, MeSA application has been attributed to lowering aphid damage in spruce (*Picea*) (Lee et al. 2022), and spider mite (Tetranychidae) damage to bean plants (Salamanca et al. 2018). Given the potential of MeSA in IPM, this compound is commercially sold to enhance biological control of pests in farms as a slow-release lure (Predalure™, AgBio, Westminster, CO).

In this study, we tested MeSA lures for crop protection in ornamental systems. Red maples [*Acer rubrum* L. (Sapindales: Sapindaceae)] are typically planted as seedlings with one lead stem and grown for 1-3 years in the field before being sold as bare root nursery stock. The central lead meristem is trained on a single vertical pole, and side branches are pruned to encourage apical growth. A type of deformity observed in red maple saplings is "witches' broom", characterized by a proliferation of shoots growing close together from the central lead. Witches' broom incurs significant labor costs due to retraining the lead meristem to the pole and removing excess shoots (Townsend 1989). The causes of witches' broom vary, and have been attributed to fungi, viruses, insects, and phytoplasmas in various ornamental plants (University of Maryland Extension 2023). For instance, a phytoplasma has been associated with a witches' broom

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Table 1. **Red Maple cultivars and experimental designs for both years.**

Block	Cultivars	Plot dimension	Distance between control & MeSA	Total seedlings Control, MeSA
2022				
I	Autumn Blaze	4.6 × 88.4 m (15 × 290 ft)	189 m (621 ft)	753, 777
II	Redpointe	10.7 × 38.1 m (35 × 125 ft)	231 m (758 ft)	718, 713
III	Armstrong Gold	10.7 × 38.1 m (35 × 125 ft)	230 m (755 ft)	353, 501
IV	October Glory	7.0 × 57.9 m (23 × 190)	163 m (535 ft)	590, 680
2023				
I	Autumn Blaze	21.3 × 18.9 m (70 × 62 ft)	341 m (1,119 ft)	623, 656
II	Firefall, Celebration, Marmo, Sienna Glenn	21.3 × 18.9 m (70 × 62 ft)	247 m (791 ft)	748, 686
III	Redpointe	21.3 × 18.9 m (70 × 62 ft)	233 m (764 ft)	416, 541
IV	Autumn Blaze	21.3 × 18.9 m (70 × 62 ft)	233 m (764 ft)	748, 754

symptom in Japanese maples (*Acer palmatum* Thunb.) in China (Li et al. 2012). The potato leafhopper *Empoasca fabae* (Harris) was determined as the causative agent of witches' broom in red maples growing in the eastern U.S. and Canada (Townsend 1989). In red maple seedling fields in the Mid-Willamette Valley of Oregon, the western flower thrips, *Frankliniella occidentalis* (Pergande; Thysanoptera: Thripidae) was commonly found in damaged fields (Lee and Velasco Graham 2020). Witches' broom was observed when thrips were present and appeared to be reduced by pesticide applications targeting thrips, thus suggesting thrips to be a causative agent in the Mid-Willamette Valley (S. Doane, personal communication).

Witches' broom is currently managed in Oregon nurseries by insecticide sprays targeting thrips, including a rotation of acephate, chlorpyiphos, cyantraniliprole and spinosad (Scherr and Nackley 2023b). While insecticides are convenient, western flower thrip can develop resistance to commonly used insecticide classes, making resistance management necessary (Gao et al. 2012). To avoid resistance development, nursery managers have been releasing predatory rove beetles, *Dalotia coriaria*, and *Hypoaspis scimitus* mites to control thrip pupae on the ground (Hedstrom and Sandlin 2022), and planting cover crops (Scherr and Nackley 2023a). In a 2021 IPM Strategic Plan, nursery growers considered thrips management as a top research priority, particularly biological approaches (Hedstrom and Sandlin 2022).

We examined MeSA to enhance biological control in ornamental maple nurseries due to prior studies and grower interest. Previously, a two-year field study with MeSA in red maples was conducted to reduce aphid and two-spotted spider mite (*Tetranychus urticae* Koch) abundance (Lee et al. 2022). In that study, the authors found that aphid abundance was significantly lowered in one out of two years, and predators and parasitic wasps were more commonly trapped in plots with MeSA treatment. While not the primary focus, fewer thrips were consistently caught on sticky cards in MeSA plots than control plots both when thrip density was high in 2009, and when density was low in 2010. With meristem damage common in 2019-21, the grower was interested in testing non-insecticide alternatives such as MeSA. Thus, our objectives in 2022-23 were to: 1) check if MeSA-treated maple fields harbored more natural enemies, 2) had lower pest abundance, and 3) exhibited fewer cases of meristem damage. In this present study, we sampled the meristems to monitor insect activity and damage during rapid seedling growth in May-June; this was not examined in the 2009-10 study which focused on leaf pests in July-August (Lee et al. 2022).

Materials and Methods

In 2022, four newly planted red maple fields (blocks) in a commercial nursery were selected for trials (see Table 1 for cultivars, measurements, and plant counts). In 2023, two other newly planted fields from the same nursery were selected. Each field in 2023 had one block at opposite ends, for a total of four experimental blocks. Blocks in the same large field were at least 198 m (650 ft) apart. In both years, each block contained one MeSA-treated plot and one control plot. All plots were 0.04 ha (0.1 acre). Maple fields were managed with insecticides as the grower saw fit to produce viable crops; blocking ensured that both treatments within a block received the same chemical treatments.

Five Predalure (AgBio, Westminster, CO) 5g lures were hung above seedlings, spread equidistantly in the treatment plots, for a rate of 123 lures/ha or 50 lures/acre. In 2022, lures were placed on May 16 and replaced on July 13, and in 2023, lures were placed on May 16 and replaced on July 5. Lures were replaced after ~8-9 weeks since they were exposed to full sun. A side experiment showed that lures (n=10) consistently volatilized 0.49 ±0.02 g per week when hung in the full sun in July-August, equivalent to 4.4 g over 9 weeks (A. Butcher, unpublished data).

To monitor damage, all seedlings within each plot were counted weekly for meristem damage and compared to the total number of seedlings counted (Fig. 1). Meristems were classified as damaged if they had evidence of insect chewing or exhibited witches' broom (Fig. 1). Mechanically damaged leaders that were bent or snapped by farm equipment were not included.

To check for colonizing arthropods, we examined 40 random seedlings per plot. The leader was gently tapped three times over a whiteboard and the number of thrips and other key arthropods were counted. Additionally, we visually scanned each seedling by turning three leaves (top, middle, lower height) and counting foliar arthropods. The meristem taps and leaf turns are referred to as 'foliage check' from here on out. The following pest taxa were counted: aphid (Aphidae), caterpillar (Lepidoptera), cucumber beetle (Coleoptera:

Fig. 1. Red maple seedling with damaged meristem and witches' broom (left) and normal meristem (right).

Chrysomelidae, *Diabrotica undecempunctata* Mannerheim), lygus (Hemiptera: Miridae, *Lygus* sp.), psyllid (Hemiptera: Psyllidae), thrips (Thysanoptera: Thripidae), whitefly (Hemiptera: Aleyrodidae), and natural enemy taxa: whirligig mites (Trombidiformes: Anystidae, *Anystis* sp.), green lacewing egg (Neuroptera: Chrysopidae), ladybug (Coleoptera: Coccindellidae), minute pirate bug (Hemiptera: Anthocoridae, *Orius* sp.), parasitoid (parasitic Hymenoptera), aphid mummy (Hymenoptera: Braconidae), spider (Aranae), and syrphid fly egg (Diptera: Syrphidae). Abundant taxa were analyzed separately. If numbers were too low for analysis, less common pests were summed for an 'other pest' count, and all predators and parasitoids were summed for a total natural enemy count.

Because insects may not be present at the time of foliage checks, we also monitored pests and natural enemies in each plot using sticky traps (three cards per plot). Double-sided white sticky cards 20 by 2 cm (8 × 5 in) in size (Great Lakes IPM, Vestaburg, MI) were staked at canopy height, replaced and moved in position each week. Sticky traps were sorted in the laboratory to count these pest taxa: aphid, cucumber beetle, leafhopper, lygus, psyllid, and thrips, and natural enemy taxa: green lacewing, ladybug, *Orius*, parasitoid, rove beetle (Coleoptera: Staphylinidae), spider, and syrphid fly. Abundant taxa were analyzed separately.

Foliage check, sticky card and meristem sampling occurred weekly when meristems were most susceptible to damage, from week 0 to 6 on May 16 to June 29, 2022, and from week 0 to 7 on May 16 to July 5, 2023. Later in the season, sampling occurred every two weeks on July 13 (week 8) and July 27, 2022 (week 10), and July 19 (week 9) and July 31, 2023 (week 11). Final damage assessments were made on July 13, 2022, and July 5, 2023. Damage assessment was terminated once trees had grown to a height of ∼2 m that made visual scans difficult.

Statistics. For foliage check data, the total number of insects per taxa were summed among the 40 plants checked per plot each week, with tap and scan counts combined. Common insects were compared at week 0 to check for baseline differences before MeSA lures could have an effect. The total number of insects were compared with treatment as a fixed effect, block as a random effect with a Poisson, negative binomial, or lognormal distribution in a generalized linear mixed model (GLMM) in Proc glimmix SAS 9.4 (SAS 2016). Foliage check data were compared for the remaining weeks when MeSA lures may impact insect abundance. Each common taxon was compared with treatment, week and treatment*week as fixed effects, and block and treatment*block (repeated measure) as random effects using the best fit distribution in a GLMM.

Sticky cards were analyzed by taxa with a similar model as described for foliage check data. The number of days elapsed between sticky card collections was used as an offset. No separate baseline comparison was made because the first sticky card assessments reflected trapping from week 0 to 1.

The proportion of damaged meristems was compared at week 0 baseline with treatment as a fixed effect, and block as a random effect using a binomial distribution in a GLMM. The proportion damaged from the remaining weeks was tested with treatment, week, and treatment*week as fixed effects, and block and treatment*block (repeated measure) as random effects with a binomial distribution in a GLMM. To check for associations with commonly collected insects, the proportion of damaged meristems was regressed with aphid or thrip counts from foliage check or sticky card data from each plot in Proc reg in SAS 9.4. Regressions were run with meristem and insect samples taken during the same week (simultaneous), with insects collected the prior week and insects collected two weeks prior in case symptoms took time to develop. A total of 12 regressions were run per year.

Results and Discussion

Natural enemies. In 2022, there was no significant increase of any of the natural enemies detected in foliage checks or on sticky cards in MeSA-treated plots compared to control plots based (Table 2, 3). In 2023, treatment differences were detected on sticky cards, but not from foliage checks (Table 2, 3). While foliage check data more accurately reflect natural enemy activity (Kaplan 2012), the number of observations was low, making it hard to detect treatment effects in commercial fields. Sticky cards collected higher numbers of arthropods, enabling comparisons between treatments of 6-7 taxa per year (Table 2,3). More minute pirate bug, a thrips predator, were captured in MeSA-treated than control plots on weeks 9 and 11 on sticky cards (Fig. 2a). Similarly in other studies, *Orius* was also trapped more on sticky cards in MeSA treatments in cotton (Yu et al. 2008), grapes (James and Price 2004), hops (James 2003) and strawberry (Lee 2010), or directly observed more often on MeSA-baited bean plants (Salamanca et al. 2018). Besides minute pirate bugs, we did not observe significant differences in the abundance of any other natural enemy taxa between treatments.

Pests. In 2022, there were no differences in thrips and aphid abundance between treatments based on leaf turns and sticky cards (Table 2). In 2023, there was about 59% fewer winged aphids detected on sticky cards during week 2 in MeSA plots than in control ones (Fig. 2b). An application of insecticide was applied in week 3 which may have impacted the ability to detect further significant differences as no other weeks showed any significant differences.

Table 2. **Average natural enemy, pest and meristem damage in MeSA-treated and control red maple plots per week.**

Data type	Time	Control	MeSA
		Ave±SE	Ave±SE
Natural enemy 2022 Foliage check			
Ladybug	wk 1-10	1.84±0.46	1.59±0.35
Parasitoid	wk 1-10	0.75±0.29	0.75±0.17
Spider	wk 1-10	0.63±0.19	0.78±0.38
Natural enemy 2022 Sticky card			
Green lacewing	wk 1-8	0.30±0.08	0.33±0.07
Ladybug	wk 1-8	0.20±0.05	0.32±0.07
Minute pirate bug	wk 1-8	0.69±0.19	0.57±0.12
Parasitoid	wk 1-8	4.60±0.31	4.21±0.28
Spider	wk 1-8	1.35±0.18	1.07±0.20
Syrphid	wk 1-8	4.15±1.13	5.37±1.34
Natural enemy 2023 Foliage check			
Natural enemy (sum)[z]	wk 0	11±2.55	11±2.48
	wk 1-11	6.11±0.87	6.33±0.98
Natural enemy 2023 Sticky card			
Green lacewing	wk 1-11	0.20±0.059	0.41±0.088
Ladybug	wk 1-11	0.36±0.073	0.56±0.084
Minute pirate bug	wk 1-11	0.08±0.042	0.35±0.117
Parasitoid	wk 1-11	3.82±0.32	4.19±0.31
Rove beetle	wk 1-11	0.29±0.187	0.21±0.065
Spider	wk 1-11	2.81±0.64	1.77±0.30
Syrphid	wk 1-11	0.08±0.030	0.17±0.057
Pest 2022 Foliage check			
Thrips	wk 0	0.25±0.25	0.75±0.75
	wk 1-10	18.1±4.8	14.25±3.95
Aphid	wk 0	32±10.22	12.25±3.25
	wk 1-10	11.53± 4.59	13.09±6.05
Pest 2022 Sticky card			
Thrips	wk 1-8	245.12±40.45	253.21±41.79
Aphid	wk 1-8	12.32±1.01	12.92±1.23
Pest 2023 Foliage check			
Thrips	Wk 0	8.75±3.07	8.75±2.25
	Wk 1-11	14.4±3.25	17.9±4.55
Aphid	Wk 0	25.8±19.5	3.75±0.85
	Wk 1-11	5.97±1.69	3.25±0.53
Other pests[y]	Wk 0	2.25±1.32	1±0
	Wk 1-11	3.42±0.86	2.58±0.55
Pest 2023 Sticky card			
Thrips	Wk 1-11	266.9±46.6	278.9±39.3
Aphid	Wk 1-11	4.22±0.65	3.27±0.41
Cucumber beetles	Wk 1-11	0.19±0.063	0.20±0.061
Leafhopper	Wk 1-11	0.18±0.045	0.26±0.053
Lygus	Wk 1-11	0.32±0.118	0.25±0.120
Psyllid	Wk 1-11	0.32±0.075	0.39±0.085
% Seedlings with damaged meristems			
2022	wk 0	0.7±0.26%	0.7±0.23%
	wk 1-8	2.0±0.4%	1.2±0.1%
2023	wk 0	0.67%±0.15	0.39%±0.19
	wk 1-7	2.6%±0.3	1.98%±0.29

[z]Counts were pooled due to low counts. Natural enemy counts in 2023 foliage check include *Anystis* mites, green lacewing eggs, ladybugs, *Orius*, parasitoids, spiders and syrphid eggs.

[y]Other pests in 2023 foliage check include caterpillars, cucumber beetles, lygus, psyllids and whiteflies.

A prior 2009-10 study with MeSA in different red maple seedling fields found higher natural enemy abundance, and consistently fewer thrips and aphids in MeSA than control plots (Lee et al. 2022). This could be due to different insect communities at the different farms, seasonality, or lure changes. The past study was initiated in mid-July, and MeSA might work more favorably as the canopy grew and became more hospitable for natural enemies. This current study was initiated in May to prevent damage to meristems when seedlings were small with little canopy to shade the lures. In a side study, 5 g lures hung in the full sun volatilized 0.49 +0.023 g per week, which was 0.137 g higher than lures hung in partial shade (A. Butcher, unpublished data, 10 replicates per shade/sun). Also, due to product availability, 5 g lures were used here in 2022-2023 whereas 2 g lures were available in 2009-2010. The volatilization and higher dosage in the current study may have attracted natural enemies over a greater area. The distances between

Table 3. Generalized linear mixed model outcomes comparing MeSA-treated with control red maple plots.

Data type	Time	Effect	df	F	P
Natural enemy 2022 Foliage check					
Ladybug	wk 1-10	Treatment	1, 3	8.83	0.059
		Week	7, 42	23.79	<.0001
		Treatment*wk	7, 42	21.16	<.0001
Parasitoid	wk 1-10	Treatment	1, 3	2.94	0.185
		Week	7, 42	6.46	<.0001
		Treatment*wk	7, 42	4.47	0.0009
Spider	wk 1-10	Treatment	1, 3	7.98	0.0664
		Week	7, 42	16.1	<.0001
		Treatment*wk	7, 42	17.78	<.0001
Natural enemy 2022 Sticky card					
Green Lacewing	wk 1-8	Treatment	1, 3	0.05	0.8376
		Week	6, 148	1.67	0.1311
		Treatment*wk	6, 148	0.1	0.996
Ladybug	wk 1-8	Treatment	1, 3	0.36	0.5891
		Week	6, 148	0.86	0.5277
		Treatment*wk	6, 148	0.06	0.9989
Orius	wk 1-8	Treatment	1, 3	0.07	0.8125
		Week	6, 148	5.88	<.0001
		Treatment*wk	6, 148	0.1	0.9965
Parasitoid	wk 1-8	Treatment	1, 3	0.85	0.4243
		Week	6, 148	4.85	0.0002
		Treatment*wk	6,148	1.84	0.0942
Spider	wk 1-8	Treatment	1, 3	0.76	0.447
		Week	6, 148	7.22	<.0001
		Treatment*wk	6, 148	0.29	0.94
Syrphid	wk 1-8	Treatment	1, 3	1.14	0.3647
		Week	6, 148	164.2	<.0001
		Treatment*wk	6, 148	0.21	0.9734
Natural enemy 2023 Foliage check					
Natural Enemy[z]	wk 0	Treatment	1, 3	0	1
	wk 1-11	Treatment	1, 3	0.54	0.5165
		Week	8, 48	12.56	<.0001
		Treatment*wk	8, 48	0.73	0.6685
Natural enemy 2023 Sticky card					
Green Lacewing	wk 1-11	Treatment	1, 3	5.31	0.1045
		Week	8, 192	1.4	0.1993
		Treatment*wk	8, 192	0.67	0.7137
Ladybug	wk 1-11	Treatment	1, 3	1.15	0.3619
		Week	8, 192	1.12	0.3517
		Treatment*wk	8, 192	0.26	0.9765
Orius	wk 1-11	Treatment	1, 3	1.71	0.2824
		Week	8, 192	2.93	0.0041
		Treatment*wk	8, 192	3.66	0.0005
Parasitoid	wk 1-11	Treatment	1, 3	0.13	0.7425
		Week	8, 192	9.43	<.0001
		Treatment*wk	8, 192	1.43	0.1844
Rove Beetle	wk 1-11	Treatment	1, 3	0.05	0.844
		Week	8, 192	1.25	0.2697
		Treatment*wk	8, 192	0.8	0.6019
Spider	wk 1-11	Treatment	1, 3	0.04	0.8482
		Week	8, 192	15.17	<.0001
		Treatment*wk	8, 192	1.04	0.4087
Syrphid	wk 1-11	Treatment	1, 3	1.2	0.3533
		Week	8, 192	6.17	<.0001
		Treatment*wk	8, 192	0.86	0.555
Pest 2022 Foliage check					
Thrip	wk 0	Treatment	1, 3	0.91	0.4116
	wk 1-10	Treatment	1, 3	3.07	0.1779
		Week	7, 42	5.05	0.0003
		Treatment*wk	7, 42	1.63	0.1532
Aphid	wk 0	Treatment	1, 3	10.59	0.0473
	wk 1-10	Treatment	1, 3	3.76	0.1478
		Week	7, 42	211.7	<.0001
		Treatment*wk	7, 42	24.96	<.0001

Table 3. Continued.

Data type	Time	Effect	df	F	P
Pest 2022 Sticky card					
Thrip	wk 1-8	Treatment	1, 3	0.2	0.6831
		Week	6, 148	6825.8	<.0001
		Treatment*wk	6, 148	8.96	<.0001
Aphid	wk 1-8	Treatment	1, 3	0.02	0.8936
		Week	6, 148	66.07	<.0001
		Treatment*wk	6, 148	2.01	0.0683
Pest 2023 Foliage check					
Thrip	Wk 0	Treatment	1, 3	1.03	0.3856
	Wk 1-11	Treatment	1, 33	0.59	0.4461
		Week	8, 33	6.73	<.0001
		Treatment*wk	7, 33	0.51	0.8216
Aphid	Wk 0	Treatment	1, 3	2.43	0.2171
	Wk 1-11	Treatment	1, 3	0.13	0.7411
		Week	8, 48	3.05	0.0074
		Treatment*wk	8, 48	0.91	0.5191
Other pests[y]	Wk 0	Treatment	1, 3	1.82	0.27
	Wk 1-11	Treatment	1, 3	0.03	0.8667
		Week	8, 48	5.05	0.0001
		Treatment*wk	8, 48	0.96	0.4756
Pest 2023 Sticky card					
Thrip	Wk 1-11	Treatment	1, 3	0.24	0.6603
		Week	8, 192	69.55	<.0001
		Treatment*wk	8, 192	1.51	0.1543
Aphid	Wk 1-11	Treatment	1, 3	0.55	0.5134
		Week	8, 192	37.07	<.0001
		Treatment*wk	8, 192	2.59	0.0103
Cucumber	Wk 1-11	Treatment	1, 3	0.01	0.9376
Beetles		Week	8, 192	1.36	0.217
		Treatment*wk	8, 192	0	1
Leafhopper	Wk 1-11	Treatment	1, 3	0.29	0.6258
		Week	8, 192	1.18	0.3151
		Treatment*wk	8, 192	0.21	0.9888
Lygus	Wk 1-11	Treatment	1, 3	0.06	0.8253
		Week	8, 192	0.18	0.994
		Treatment*wk	8, 192	0.92	0.499
Psyllid	Wk 1-11	Treatment	1, 3	0.5	0.5311
		Week	8, 192	36.45	<.0001
		Treatment*wk	8, 192	0.96	0.4694
% Seedlings with damaged meristems					
2022	wk 0	Treatment	1,3	0.14	0.7338
	wk 1-8	Treatment	1,3	14.73	0.0312
		Week	6,36	8.87	<.0001
		Treatment*wk	6,36	1.58	0.1821
2023	wk 0	Treatment	1, 3	2.06	0.2465
	wk 1-7	Treatment	1, 3	1.77	0.2752
		Week	6, 35	3.06	0.0164
		Treatment*wk	6, 35	6.48	0.0001

[z]Counts were pooled due to low counts. Natural enemy counts in 2023 visual samples include *Anystis* mites, green lacewing eggs, ladybugs, *Orius*, parasitoids, spiders and syrphid eggs.

[y]Other pests in 2023 visual samples include caterpillars, cucumber beetles, lygus, psyllids and whiteflies.

control and MeSA plots ranged from 163 (year 1) to 341 m (year 2) and may not have sufficiently prevented cross-treatment movement of insects.

Meristem damage. In 2022, as much as 3.01% of seedlings were damaged in control plots, whereas 1.17% of seedlings were damaged in MeSA plots, which is a 61% reduction relative to the control [(control – MeSA)/control] (week 5 of Fig. 2c). In 2023, there was a treatment*week interaction (Table 3). This interaction could be due to the re-training of damaged meristems mid-season, thereby lowering the observed damage in control plots over time and diminishing treatment differences later in the season (Fig. 2d). MeSA-treated plots had significantly fewer trees with damaged meristems during the 2nd and 3rd weeks after lures were placed (Fig. 2d). Meristem damage in MeSA plots was 3 and 2% lower these weeks than control plots, which is a 67-68% reduction in damage relative to the control. While no economic threshold has been established, this reduction was promising so the grower started using MeSA in other production fields (J. Lee, personal communication).

Out of 12 regressions in 2022, only foliage check (leaf turn and meristem tap) counts of aphids significantly regressed with meristem damage when counts and damage assessments were taken simultaneously (Table 4). As aphid counts increased, fewer damaged seedlings were observed. While an

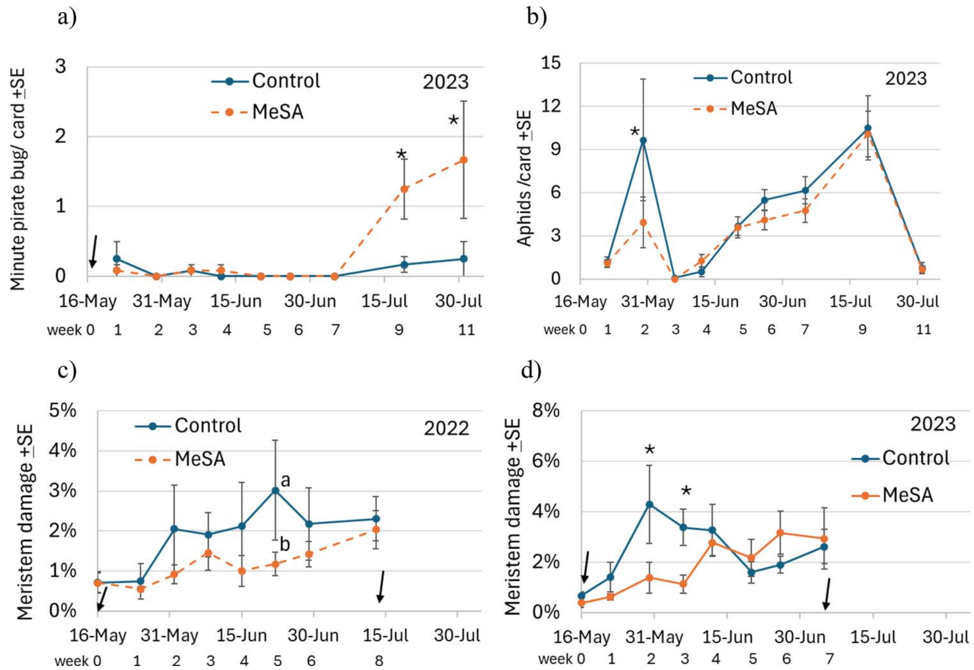

Fig. 2. Average ± standard error (SE) per week in MeSA-treated and control red maple plots for: a) minute pirate bug and b) aphid counts on sticky cards in 2023, and c) percent of trees with meristem damage in 2022 and d) in 2023. Arrows indicate when new lures were placed in plots. Different letters denote overall treatment differences, and asterisks denote treatment differences on specific dates by generalized linear mixed model, p < 0.05.

unexpected outcome, this was a weak relationship with a 1% slight slope (% damaged = 0.01*aphids + 1.72, $r^2 = 0.106$) with possibly little biological significance. Pest counts from one or two weeks prior (ie. pests from week 1 were regressed with seedling damage in week 2, pests from week 2 with damage in week 3, etc.) did not regress with meristem damage in 2022. In 2023, sticky card counts of aphids or thrips taken simultaneously with meristem monitoring and aphid counts taken two weeks prior significantly regressed with meristem damage (Table 4). In these cases, as aphid or thrips counts increased, more damaged seedlings were observed (% damaged = 0.06*aphid + 1.74 $r^2 = 0.141$; % damaged = 0.0005*thrips + 2.6, $r^2 = 0.111$; % damaged = 0.068*aphid 2 weeks ago + 1.96, $r^2 = 0.273$). The two significant regressions with aphids in 2023 suggest that aphids are either directly or indirectly associated with meristem damage.

Table 4. Linear regression of the proportion of meristems damaged per plot by aphid or thrips counts in red maple fields.

Insect - year	Sampling	Timing	Df	F	P
Aphids 2022	Foliage check	Simultaneous	1, 62	7.34	0.0087[z]
	Foliage check	1 week prior	1, 54	2.27	0.138
	Foliage check	2 weeks prior	1, 46	0.24	0.623
	Sticky card	Simultaneous	1, 54	0.23	0.635
	Sticky card	1 week prior	1, 46	1.75	0.192
	Sticky card	2 weeks prior	1, 38	2.86	0.099
Thrips 2022	Foliage check	Simultaneous	1, 62	0.02	0.895
	Foliage check	1 week prior	1, 54	0.69	0.411
	Foliage check	2 weeks prior	1, 46	0.12	0.73
	Sticky card	Simultaneous	1, 54	3.33	0.074
	Sticky card	1 week prior	1, 46	0.11	0.739
	Sticky card	2 weeks prior	1, 38	0.01	0.960
Aphids 2023	Foliage check	Simultaneous	1, 61	2.81	0.099
	Foliage check	1 week prior	1, 53	0.39	0.535
	Foliage check	2 weeks prior	1, 45	0.01	0.909
	Sticky card	Simultaneous	1, 53	8.70	0.0047
	Sticky card	1 week prior	1, 45	1.77	0.191
	Sticky card	2 weeks prior	1, 37	13.9	0.0006
Thrips 2023	Foliage check	Simultaneous	1, 61	1.80	0.184
	Foliage check	1 week prior	1, 53	0.01	0.927
	Foliage check	2 weeks prior	1, 45	0.06	0.813
	Sticky card	Simultaneous	1, 53	6.61	0.013
	Sticky card	1 week prior	1, 45	0.69	0.412
	Sticky card	2 weeks prior	1, 37	0.41	0.528

[z]Line equation and r^2 values given in the Discussion when regression is significant.In case there was a lag between insect presence and observed damage, regressions were also run when insect counts were regressed with damage 1 or 2 weeks later in the same plot.

While meristem damage was lower in MeSA plots in both years, the mechanism for this was not clear. First, it is possible that the commonly sampled pest arthropods were not causing meristem damage. Previous field surveys of red maples done in 2020-21 which dissected meristems weekly did not reveal a causative agent of witches' broom (M. Scherr & L. Nackley, personal communication). In another study in 2020, 192 field seedlings were tracked weekly and no correlation between thrip counts and damage appeared (Lee and Velasco Graham 2020). Second, the lack of clarity could be due to an arthropod-transmitted pathogen such as with the Japanese maples (Li et al. 2012). In this case, feeding by a few individuals may cause witches' broom, and correlations between pest arthropod numbers and damage may not be observed. Since growers currently manage damage with broad-spectrum insecticide sprays, it is possible that these insecticides control another arthropod causing witches' broom or prevent an arthropod-transmitted pathogen. Third, MeSA may prime plant defensive pathways to lower meristem damage. For example, a study showed that MeSA lures primed tomato defenses and lowered caterpillar feeding and fungal growth (Rowen et al. 2017). Future studies should seek to elucidate the specific mechanisms mediating the effects of MeSA lures on meristem damage.

In summary, this study supports the growing evidence of MeSA providing crop protection. Past studies in other systems have shown that MeSA treatments can lower pest damage (Lee et al. 2022, Salamanca et al. 2018) resulting in favorable crop yields (Dong and Hwang 2017, Wang et al. 2011). As red maple nurseries establish cover crops to manage other pests or provide favorable habitats for natural enemies (Dawadi et al. 2019, Scherr and Nackley 2023a), MeSA may also be combined to attract natural enemies. In other systems, MeSA combined with floral companion plants improved the retention of natural enemies (Mercer et al. 2020, Salamanca et al. 2018).

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