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Test of plant defense elicitors for arthropod pest suppression and *PR-1* gene induction in pear orchards

Robert J. Orpet[1]*, W. Rodney Cooper[2], Elizabeth H. Beers[1] & Louis B. Nottingham[1]

[1]*Department of Entomology, Washington State University Tree Fruit Research and Extension Center, 1100 N Western Ave, Wenatchee, WA, 98801, USA, and* [2]*USDA-ARS-Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA, 98951, USA*

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Abstract

Plant defense elicitors (PDEs) are chemicals that stimulate plant defenses against pathogens and herbivores. Previous work shows that PDEs acibenzolar-S-methyl (ASM) and harpinαβ protein (harpin) can induce the pathogenesis-related gene *PR-1* in plants and suppress herbivorous arthropods. In this study, we tested the potential for these PDEs to induce *PR-1* in pear, *Pyrus communis* L. (Rosaceae) orchards and suppress pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae), and spider mites, *Tetranychus* spp. (Acari: Tetranychidae). In 2017, we compared densities of each pest on mature pear trees following a single application of either an ASM product (Actigard; Syngenta), a harpin product (Employ; Plant Health Care), or no PDE treatment in four commercial and two research center orchards. In 2018, we sampled pear psylla and used qPCR to assess *PR-1* induction in pear leaf samples before and after PDE treatments at one commercial orchard. Neither PDE treatment showed evidence of pest suppression in either year, and no differences in *PR-1* expression were detected. Potted greenhouse trees treated with ASM in 2019 showed higher *PR-1* expression relative to untreated trees, verifying that our procedures can detect induction and suggesting that a single PDE application was sufficient to induce *PR-1* in potted but not mature pear trees. We conclude that plant defense elicitors may contribute to pear pest suppression in some contexts, but effects are unlikely to be strong or consistent. Our results highlight the need for field experiments to advance plant defense elicitor knowledge towards effective field applications.

Introduction

Plants often respond to pathogens and herbivory by activating structural or chemical defenses to effect long-term local or systemic resistance that can reduce future damage. Molecules that can elicit this resistance, including harpinαβ protein (harpin) and acibenzolar-S-methyl (ASM; an analog of salicylic acid), are currently registered, labeled, and marketed in the USA for disease suppression and plant growth regulation in pear, *Pyrus communis* L. (Rosaceae), and other crops. Harpin and ASM are thought to regulate the salicylic acid pathway in plants to induce pathogenesis-related (PR) genes, leading to accumulation of PR proteins associated with acquired resistance (Dong et al., 1999; Peng et al., 2003; Johnson & Temple, 2016). Co-regulation and complex interactions between defense pathways such as jasmonic acid, ethylene signaling, and active oxygen species can influence mechanisms and targets of acquired resistance, making it difficult to predict effects of plant defense elicitation on herbivores (Moran & Thompson, 2001; Dong et al., 2004; Faize et al., 2004a; Gordy et al., 2015; Cui et al., 2019). Therefore, assessing potential utility of defense elicitors for pest management requires field tests.

In pear, defense elicitors may improve management of the key pest pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae). In Washington and Oregon, which combined grow about 70% of the pears in the USA, pear psylla management commonly relies on around 15 applications of insecticides per season, diminishing the potential for biological control by natural enemies (DuPont et al., 2021). This makes it difficult to keep pear psylla below damaging levels and sometimes induces outbreaks of the spider mites *Tetranychus mcdanieli* McGregor and

*Correspondence: Robert J. Orpet, Department of Entomology, Washington State University Tree Fruit Research and Extension Center, 1100 N Western Ave, Wenatchee, WA 98801, USA.
E-mail: robert.orpet@wsu.edu

Tetranychus urticae Koch (Acari: Tetranychidae) (Riedl et al., 1981; Murray & DeFrancesco, 2014), spurring long-term interest in developing management programs that integrate tactics other than broad-spectrum pesticides (Burts, 1983; Nottingham & Beers, 2020; DuPont et al., 2021). Application of ASM and harpin, which are not expected to directly harm biological control, can reduce pear psylla populations on potted pear trees via reduced oviposition and survival of nymphs (Cooper & Horton, 2015). In unmanaged orchards with high pear psylla populations, repeated applications of ASM and harpin moderately but inconsistently reduce pear psylla (Saour et al., 2010; Cooper & Horton, 2017). Studies in other systems show plant defense elicitors may suppress herbivorous mites (Gols et al., 2003; Choh et al., 2004; Warabieda et al., 2020) without harm to predator mites (Warabieda, 2015). Furthermore, ASM and harpin application suppresses fire blight [*Erwinia amylovora* (Burrill) Winslow et al.] disease in pear (Günen et al., 2006; Johnson et al., 2016). Although ASM is currently recommended for fire blight management in Washington State (DuPont et al., 2020), potential contributions to arthropod suppression need study in commercially managed orchards.

In this study, we assessed effects of single applications of ASM or harpin on pear psylla, spider mites, and *PR-1* induction on pear trees in Washington State orchards. Previously, Cooper & Horton (2017) applied ASM or harpin to pear trees every 4 weeks in April through July in a Washington State experiment station orchard. They found modest pear psylla suppression, occurring mainly for second-generation nymphs in June to July and concluded that neither material is warranted as a standalone pear psylla management tactic. Given this, here we conducted tests mainly in commercially managed orchards to test for effects of single applications of ASM or harpin targeting second-generation pear psylla nymphs in summer in the context of full management programs. We also tested one earlier application at the start of petal fall, which aligns with the bloom period when managers in the study region sometimes consider ASM for fire blight management, generally with only one or two applications (ST DuPont, pers. comm., 2021). We assessed *PR-1* induction in mature commercially managed pear trees treated with ASM or harpin to verify defense induction in the field. The *PR-1* gene is readily induced in plants following treatment with ASM (Maxson-Stein et al., 2002; Faize et al., 2004a; Johnson & Temple, 2016) or harpin (Peng et al., 2003). Previous pathogenesis gene induction studies in pear trees treated with defense elicitors are to our knowledge limited to potted trees (Faize et al., 2004a,b, 2009). Overall, our tests were designed to test for effects of ASM and harpin on pest arthropods and *PR-1* induction under realistic field situations.

Materials and methods

2017 field experiment

Design. In 2017, effects of plant defense elicitors on densities of pear psylla immatures and spider mites were examined using a randomized complete block design with four replications of three treatments (ASM, harpin, and a non-treated control) repeated at each of six orchard sites (Table 1), creating a total of 24 replicate plots per treatment across the experiment. Two of the orchard sites were located at Washington State University research center orchards (Table 1). Two commercial orchards managed by different growers each contained two additional orchard sites (Table 1). The commercial sites and one of the research center sites used integrated pest management programs like those described by DuPont et al. (2021) entailing multiple pre-bloom sprays of kaolin clay and post-bloom broad-spectrum sprays against pear psylla, codling moth, and mites. The other research center site was not managed with insecticides or miticides during 2017. The four blocks within each orchard site contained trees of the similar age, size, and either the same cultivar or different cultivars evenly distributed among replicates (Table 1). Each of the three treatment plots within a block consisted of three adjacent trees separated from other plots by at least one buffer tree, and plots within the same block were in the same tree row.

Treatment applications. Between 27 and 31 July, ASM (Actigard; Syngenta, Wilmington, DE, USA) and harpin (Employ; Plant Health Care, Pittsburgh, PA, USA) treatments were applied at high label rates (Table 2), which varied depending on tree size and spacing (Table 1). With an expectation of weeks-long induction of plant defenses (Johnson & Temple, 2016), this application timing targeted pear psylla and mite generations occurring in late summer when they are most difficult to control in commercial orchards. ASM was applied as a soil drench because the labeled 60-day post-harvest interval for foliar application would overlap with expected harvest times for Bartlett pears. Harpin was applied as a foliar spray using a hand-pump pressurized backpack sprayer according to the label. Harpin sprays included 0.5% petroleum oil (IAP 440) to match local commercial practice of including 0.5% oil in most pear pesticide sprays for penetrant and spreading effects. To control for potential effects of oil in the harpin spray, ASM and control plots were also sprayed with 0.5% petroleum oil via backpack sprayer.

Pest monitoring. Pear psylla and spider mite densities were monitored with leaf samples the day before treatment

Table 1 Study orchard locations, characteristics, and dates of treatment applications for the 2017 pear field experiment

Location (WA, USA)	Management	Cultivar(s)	Age (years)	No. trees/ha	Application date
Rock Island	University, conventional	Bartlett	Young (10)	840	27 July
Wenatchee	University, none	Anjou	Old (49)	370	27 July
Malaga	Grower 1, conventional	Bartlett	Young (20)	620	28 July
		Anjou, Bartlett	Old (45)	450	28 July
Cashmere	Grower 2, conventional	Bartlett	Young (12)	740	31 July
		Anjou	Old (50)	450	31 July

applications followed by post-treatment visits between 31 July and 31 August (sites 1–4: three visits on 7- to 15-day intervals; sites 5 and 6: one visit 7 days post-treatment and one visit 31 days post-treatment). On each sampling visit, 50 leaves were randomly collected from the center tree of each plot, brought to the laboratory, and brushed with a leaf brushing machine (Leedom Manufacturing, Mi-Wuk Village, CA, USA) over a revolving glass plate coated with undiluted dishwashing liquid. Each plate was then inspected under a stereoscope for pear psylla eggs and immatures and spider mite eggs and motile stages.

2018 field experiment

Design. In 2018, two separate experiments were conducted to assess the effects of ASM and harpin on pear psylla and spider mites. The experiments were identical in plot design and sampling methods, with the only differences being treatment timings and sample dates. The first experiment was treated on 27 April, the second on 23 May. Both experiments had four replications of three treatments (ASM, harpin, or untreated) organized in a randomized complete block design. Plots were located within one commercially managed pear orchard near Malaga, WA, with 20-year-old Anjou trees. Each replicated plot consisted of four consecutive intra-row trees.

Treatment applications. Compared with 2017, earlier spray timings were used and ASM was applied as a foliar spray instead of a soil drench. The first timing (27 April) aligned with the beginning of production of first-generation summerform pear psylla adults and the start of petal fall, which includes the period of effectiveness of ASM for fire blight management, its primary use in pome fruit (Johnson et al., 2016; DuPont et al., 2020). The second timing (23 May) aligned with the period of pear psylla oviposition. Sprays were applied with a Pak-Blast air-blast sprayer at a product rate of 140 g ha^{-1} for ASM (Actigard) and 420 g ha^{-1} for harpin (Employ). All ASM and harpin sprays included 0.5% petroleum oil as an adjuvant, so control plots were also treated with 0.5% petroleum oil.

Table 2 Application methods and rates for 2017 experiment with ASM (trade name Actigard) and harpin (trade name Employ) products on 3-tree plots

Treatment	Method	Tree age	Product application per tree
ASM	Soil drench	Young	0.36 g in 473 ml
		Old	0.50 g in 473 ml
Harpin	Foliar spray	Young	0.68 g in 1.89 l
		Old	0.94 g in 3.56 l

Pest monitoring. Pre-treatment leaf and beat tray samples were made on the two center trees of plots 1 day before applications. This was followed by biweekly sampling from 2 May to 13 June for the trees treated on 27 April, and from 1 June to 12 July for the trees treated on 23 May. Pear psylla eggs and nymphs and spider mite eggs and motile stages were monitored by the leaf brush method as in the 2017 experiment. Pear psylla adults were monitored on each visit with a beat tray tap method. Beat tray taps for each plot consisted of four taps made across the two center trees, with each tap consisting of three strikes of a stiff rubber hose to a horizontal branch held over a 45 × 45 cm cloth tray. The pear psylla adults landing on the tray were counted for each tap.

Field qPCR test. Real time qPCR was used to test for induction of plant defenses following application of ASM and harpin in the 2018 field experiments by assessing expression of *PR-1*. On some pest sampling dates (experiment 1: 27 April pre-treatment, 4 May, 22 May, 15 June; experiment 2: 22 May pre-treatment, 15 June, 12 July), five leaves were collected from each monitored plot, wrapped in aluminum foil, then dropped into a dewar of liquid nitrogen to flash freeze. Samples were then transported to the laboratory and stored at −80 °C.

In the laboratory, each sample of frozen leaves was ground under liquid nitrogen with a mortar and pestle. Total RNA was extracted from 50 to 100 mg of powdered leaf tissue per sample using a Qiagen RNeasy Mini Kit, and

RNA was converted to cDNA using a QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA, USA). Elongation factor gene (*eEF1a1*) was used as a control housekeeping gene in qPCR analysis by using the primers elong-346F: TCA GAC CCG TGA GCA TGC and elong-609R: GCC TCG AGA AGG GTG GG. These primers were designed from the PCR product produced by primers EF1-F: ATT GTG GTC ATT GGY CAY GT and EF1-R: CCT ATC TTG TAV ACA TCC TG (Faize et al., 2004a) using Primer3+ integrated into Geneious v.10.2.6. The *PR-1* gene was amplified using primers PR1-F: AGT AGG CGT TGG TCC CTT and PR1-R: GCC AAA CCA CCT GTG TAT AA (Faize et al., 2004a). Each 25 µl qPCR reaction included 1 ng of total cDNA, 250 nM of each primer, and LightCycler 480 SYBR Green 1 Master Mix (Roche, San Francisco, CA, USA). The qPCRs were performed on a Roche 480 LightCycler with 45 cycles of 95 °C for 10 s, 56 °C for 10 s, and 72 °C for 10 s, followed by melt curve analysis to confirm primer specificity. In addition, the specificity of primer pairs was confirmed before conducting qPCRs by cloning amplicons using a TOPO TA cloning kit with TOP10 *Escherichia coli* chemically competent cells (Invitrogen, Carlsbad, CA, USA) and sequencing (MC Laboratories, San Francisco, CA, USA) five selected clones following extraction using a QIAprep spin mini prep kit (Qiagen).

Potted tree qPCR test

To confirm the ability of the qPCR assay protocol described above to detect *PR-1* induction, a greenhouse experiment was conducted to assess *PR-1* gene expression following treatment with ASM. Potted 1-m-tall Bartlett pear trees grafted to OHXF-87 rootstock were given foliar treatment using a handheld atomizer with water (control) or ASM at a rate of 0.38 g Actigard 500 ml^{-1} water, based on 28.3 g (1 oz) per acre at 38 l (10 gal) per acre. Each tree received 30 ml of spray solution. The trees were arranged in five blocks, each containing one control and one treated tree. Leaves were collected from each tree 1 day before treatment, 1 week after treatment, and 3 weeks after treatment for analysis. The leaves were stored in RNAlater and kept at −80 °C until purified and converted to cDNA followed by qPCR as described above.

Data analysis

2017 pest monitoring. As plant responses to defense elicitors may vary over the course of weeks (Johnson & Temple, 2016) and with site-specific factors such as tree age and size (Table 1), each response variable from 2017 (pear psylla eggs, pear psylla nymphs, and spider mites) was analyzed with a generalized linear mixed model including fixed effects of treatment, sample week (as a categorical factor), orchard site, and the interactions of treatment with week and treatment with orchard site. A significant interaction between treatment with week would indicate that differences in response variables between treatment plots varied between pre- or post-treatment or depended on the week since treatment. A significant interaction between treatment with orchard site would indicate the effects of treatments differed between orchard sites. A random intercept was included for plots (to account for repeated measures on the same plot in different weeks) and blocks (to account for spatial dependence within the randomized complete block design) within each orchard site.

Models were constructed using the 'glmmTMB' function of the 'glmmTMB' package in R. Based on inspection of scaled residual plots generated with the 'simulatedResiduals' function of the 'DHARMa' package, response variables were analyzed with a negative binomial response distribution ('nbinom1' parameterization for pear psylla eggs and nymphs and 'nbinom2' for mites). Significance of each fixed effect was assessed with a type II Wald's χ^2 test (Bolker et al., 2009) using the 'Anova' function of the 'car' package. Due to very low numbers of spider mites found at orchard blocks 1 and 2 (two mites at site 1 and three at site 2 across all samples), these two sites were excluded from spider mite analysis.

2018 pest monitoring. Response variables from 2018 (pear psylla eggs per leaf, pear psylla nymphs per leaf, and pear psylla adults) were also analyzed with generalized linear mixed models. Based on inspection of scaled residual plots, all were analyzed with a lognormal distribution by using log (x + 0.01) transformation for eggs and nymphs per leaf (these were analyzed per leaf due to variation in the number of leaves per sample) and log(x + 1) transformation for adults. Each spray timing experiment was analyzed separately. The fixed effects were treatment, week (as a categorical factor), and the interaction of treatment with week. A random intercept was included for blocks and plots. Packages, diagnostics, and tests of significance were as described above for 2017 data. For post-hoc analysis of models with significant treatment by date interaction terms, a separate model was constructed for each week, with a response variable and other explanatory variables as above (excluding week), followed by Tukey tests using the 'emmeans' function of the 'emmeans' package in R.

2018 field and potted tree qPCR tests. Data from qPCR for RNA analysis from the 2018 field experiment and potted tree qPCR test were analyzed using the $-\Delta\Delta C_T$ method (Livak & Schmittgen, 2001) to quantify *PR-1* gene expression relative to the elongation factor housekeeping gene in treated relative to untreated control trees. Relative

expression ($-\Delta\Delta C_T$) of *PR-1* was analyzed using generalized linear mixed models with normal distribution, a random intercept for block, and fixed effect of week to assess whether relative expression changed across weeks. If there was a significant effect of week in any model, we conducted a post-hoc Dunnett's test to assess whether relative expression significantly differed between the pre-application and any post-application week. Packages, diagnostics, and tests of significance were as described above for 2017 and 2018 field data.

Results

2017 experiment

Densities of pear psylla eggs and nymphs (Figure 1) and spider mites (Figure 2) varied significantly between orchards and observation weeks, but there was no significant effect of treatments or interactions between treatment with orchard or week (Table 3). Thus, there was no evidence that ASM or harpin affected pest abundance in any of the six study orchards.

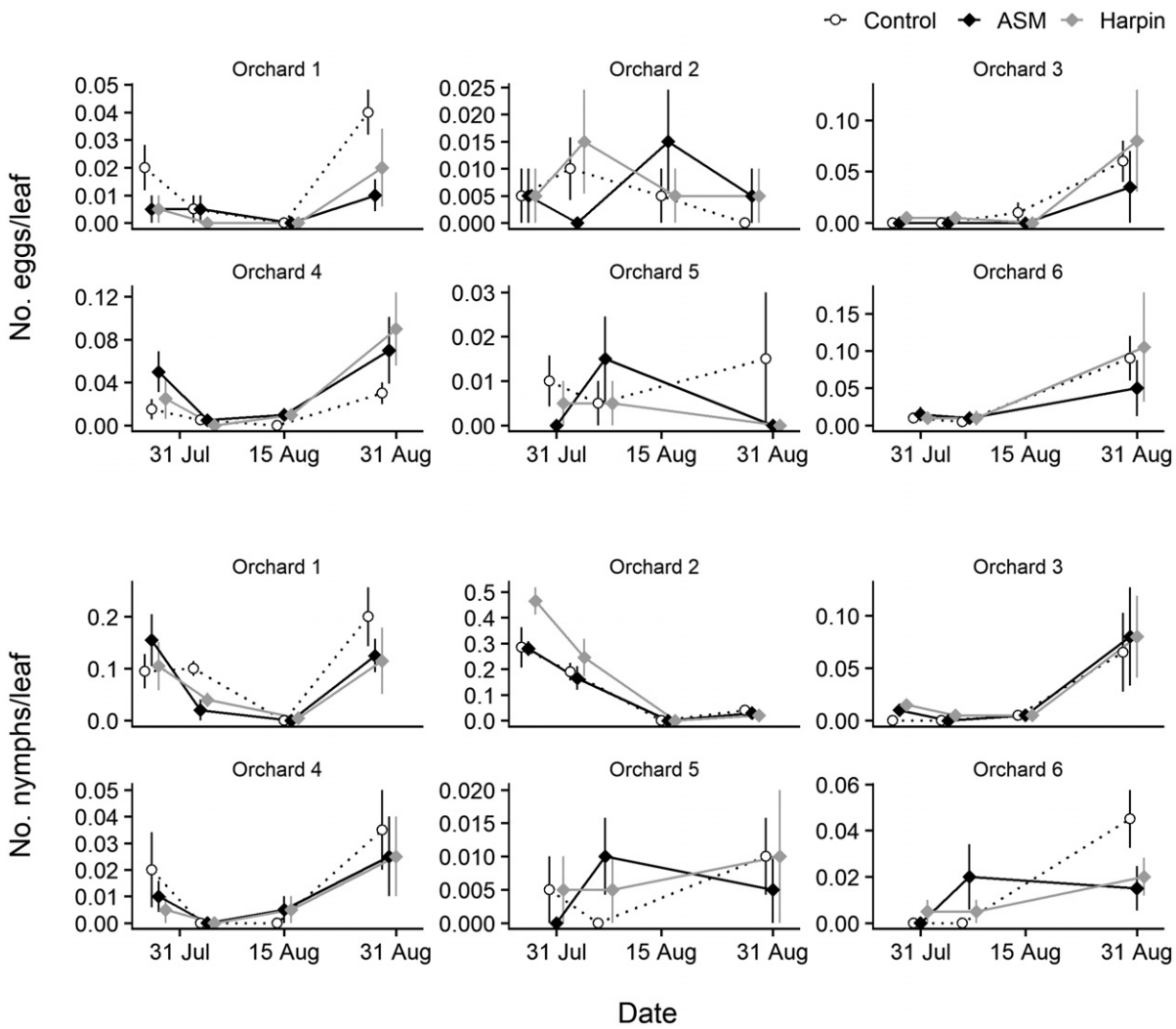

Figure 1 Mean (± SEM) pear psylla egg and nymph counts on brushed leaves from plant defense elicitor spray plots across six pear orchard blocks in 2017 (three treatments: ASM, harpin, and non-treated control). Points show mean counts among 50-leaf samples (n = 4 samples per treatment in a randomized complete block design within each of the six orchard blocks). Treatments were applied between 27 and 31 July, and the earliest sampling date for each orchard was a pre-treatment count. The points are offset horizontally by treatment within each sampling day to prevent overplotting. Y-axis scales differ between panels to emphasize visualization of population dynamics within orchard blocks.

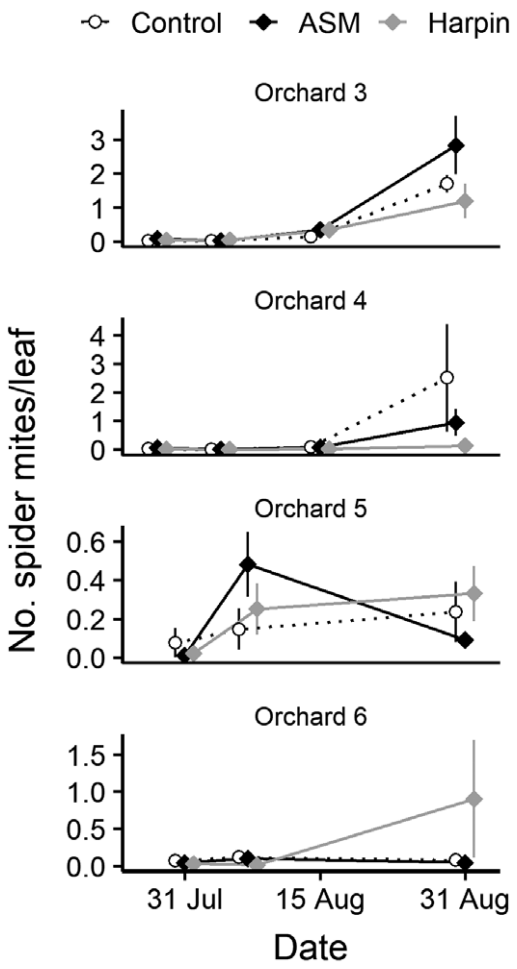

Figure 2 Mean (± SEM) spider mite counts on brushed leaves from plant defense elicitor spray plots across four pear orchard blocks in 2017 (three treatments: ASM, harpin, and non-treated control). Points show mean counts among 50-leaf samples (n = 4 samples per treatment in a randomized complete block design within each of the four orchard sites); blocks 1 and 2 not shown due to very low counts. Treatments were applied between 27 and 31 July, and the earliest sampling date for each orchard was a pre-treatment count. The points are offset horizontally by treatment within each sampling day to prevent overplotting. Y-axis scales differ between panels to emphasize visualization of population dynamics within orchard blocks.

2018 experiment

Pest monitoring. Densities of pear psylla eggs, nymphs, and adults (Figure 3) varied significantly between weeks in both spray timing experiments, but there was no significant effect of treatment on any response variable in either spray timing experiment (Table 4). There were no significant week*treatment interactions for eggs or adults, but there was for nymphs (Table 4). Based on post-hoc Tukey tests conducted for each sampling day, the interaction reflected marginally more nymphs in ASM vs. harpin plots on 2 May (1 week post-treatment) during the first experiment ($t = -2.94$, d.f. = 7, $P = 0.051$) and significantly more nymphs in harpin plots on the 22 May (pre-treatment) sample compared with both control ($t = -1.45$, d.f. = 7, $P = 0.011$) and ASM ($t = -1.31$, d.f. = 7, $P = 0.018$) plots during the second experiment (Figure 3). No other intra-week pairwise comparisons on nymphs per leaf were statistically significant (all $P>0.09$). Overall, the results show no clear evidence that plant defense elicitors reduced densities of any pear psylla life stage. Effects on spider mites could not be assessed in the 2018 experiment because none were found in our samples.

Table 3 Summary of 2017 field experiment [six pear orchard sites; four blocks each with three treatments per orchard site (ASM, harpin, and non-treated control); four sampling weeks per site including one pre-treatment count] generalized linear mixed model main effects on response variables counted on 50-leaf samples; random intercepts for plots and blocks were included in the model

		Wald's χ^2 test		
Response variable	Effect	χ^2	d.f.	P
Pear psylla eggs	Treatment	0.4	2	0.81
	Week	48.0	3	<0.0001
	Orchard	28.6	5	<0.0001
	Treatment*week	3.5	6	0.74
	Treatment*orchard	14.4	10	0.20
Pear psylla nymphs	Treatment	1.7	2	0.44
	Week	48.6	3	<0.0001
	Orchard	135.0	5	<0.0001
	Treatment*week	6.2	6	0.40
	Treatment*orchard	5.4	10	0.86
Spider mites	Treatment	4.6	2	0.10
	Week	92.2	3	<0.0001
	Orchard	7.0	3	0.07
	Treatment*week	4.9	6	0.55
	Treatment*orchard	12.0	6	0.62

Field qPCR test. There was no significant effect of week on relative *PR-1* expression for ASM or harpin for either the 27 April spray timing (ASM: $\chi^2 = 4.2$, $P = 0.24$; harpin: $\chi^2 = 2.1$, $P = 0.53$, both d.f. = 3) or the 23 May spray timing (ASM: $\chi^2 = 4.6$, $P = 0.10$; harpin: $\chi^2 = 0.6$, $P = 0.73$, both d.f. = 2; Figure 4). Thus, there was no evidence of plant defense induction from treatments in the 2018 experiment.

Potted tree qPCR test

In the potted tree qPCR test, there was an effect of week on relative *PR-1* expression in ASM-treated trees relative to

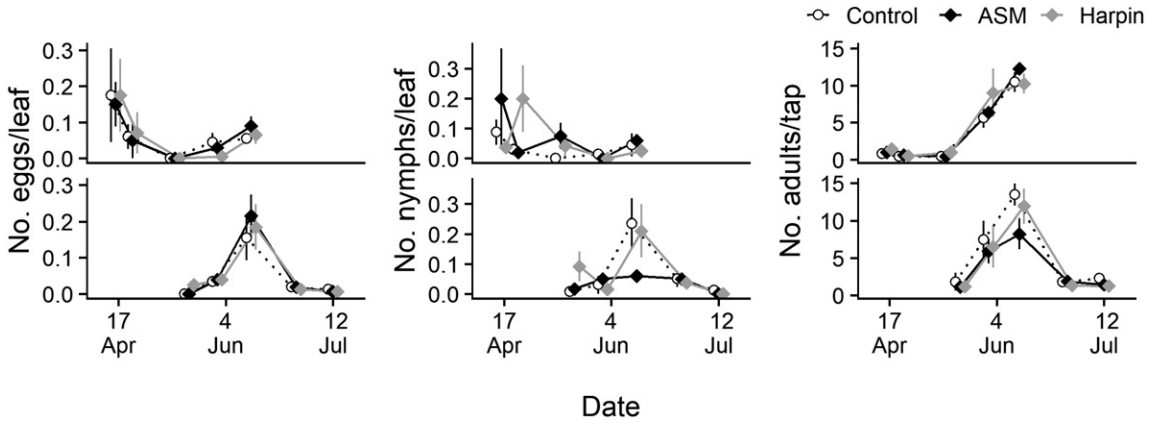

Figure 3 Mean (± SEM) pear psylla egg and nymph abundance on brushed leaves, and adults from beat tray taps from plant defense elicitor spray plots at one orchard in 2018 for each of two spray timings, 27 April (upper panels) and 23 May (lower panels) (three treatments: ASM, harpin, and non-treated control). Points show mean counts among 20- to 50-leaf samples (n = 4 samples per treatment). The points are offset horizontally by treatment within each sampling day to prevent overplotting. The earliest sampling date for each subpanel was a pre-treatment count.

Table 4 Summary of 2018 field experiment [one orchard site, four blocks and three treatments (ASM, harpin, and non-treated control); five sampling weeks including one pre-treatment count; repeated for two spray timings applied in separate sets of plots] generalized linear mixed model main effects on response variables of pear psylla eggs per leaf, nymphs per leaf (20–50 leaves per sample), and the sum of adults from four taps per plot

			Wald's χ^2 test		
Spray timing	Response variable	Effect	χ^2	d.f.	P
1 (27 April)	Pear psylla eggs	Treatment	0.6	2	0.74
		Week	35.2	4	<0.0001
		Treatment*week	5.0	8	0.75
	Pear psylla nymphs	Treatment	0.7	2	0.70
		Week	34.8	4	<0.0001
		Treatment*week	16.7	8	0.03
	Pear psylla adults	Treatment	5.3	2	0.07
		Week	487.1	4	<0.0001
		Treatment*week	7.9	8	0.44
2 (23 May)	Pear psylla eggs	Treatment	0.3	2	0.84
		Week	164.7	4	<0.0001
		Treatment*week	8.7	8	0.37
	Pear psylla nymphs	Treatment	1.1	2	0.58
		Week	56.4	4	<0.0001
		Treatment*week	18.6	8	0.02
	Pear psylla adults	Treatment	4.5	2	0.11
		Week	203.7	4	<0.0001
		Treatment*week	4.5	8	0.81

control trees (χ^2 = 28.5, d.f. = 2, P<0.0001), reflecting significant *PR-1* induction 1 week post-treatment (Dunnett's test: t = 5.3, d.f. = 10, P = 0.0006) but not 3 weeks post-treatment (t = 2.1, d.f. = 10, P = 0.10; Figure 4C). Converting mean and SEM $-\Delta\Delta C_T$ values to fold-changes ($2^{-\Delta\Delta C_T}$; Livak & Schmittgen, 2001), trees selected to be treated with ASM relative to trees selected to be used as controls had a mean difference in relative *PR-1* expression (± SEM range) of 0.29-fold (0.10–0.84) pre-treatment. One week post-treatment, mean fold change (± SEM range) in ASM vs. control trees was 40.7 (11.3–146.5) and 3 weeks post-treatment it was 2.2 (0.6–8.1).

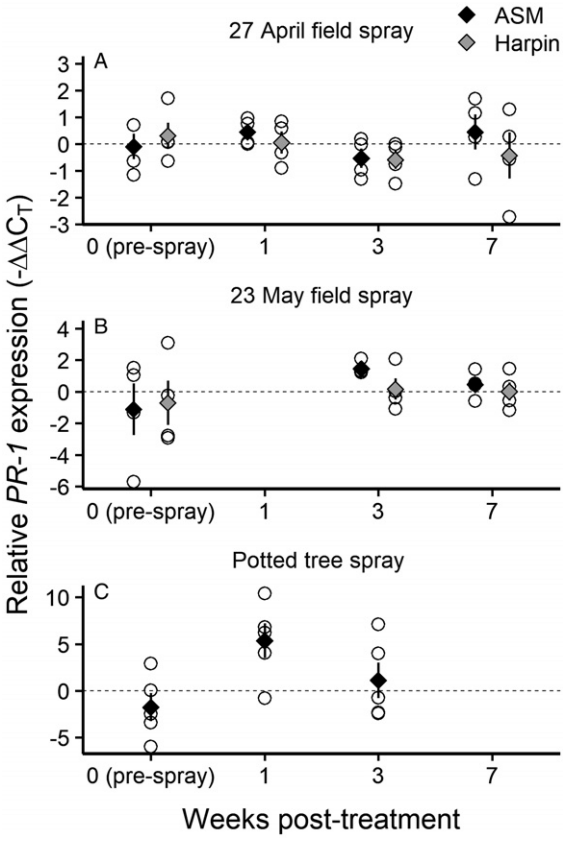

Figure 4 Expression ($-\Delta\Delta C_T$) of *PR-1* (pathogenesis-related gene 1) relative to *eEF1a1* (elongation factor) in treated (ASM and harpin) trees relative to control trees in (A, B) leaf samples from two spray timing field experiments in 2018 (n = 4 plots per treatment for each spray timing; there was no amplification for one harpin tree on 22 May for the 27 April spray timing) and (C) a potted tree test with ASM (n = 5 trees per treatment). Circles indicate individual trees and diamonds are means (± SEM).

Discussion

Chemical elicitors of plant defenses are suggested to have clear potential across agricultural systems to suppress pests while limiting non-target effects (Stout et al., 2002; Rohwer & Erwin, 2008). Given the high cost and low consistency of current pesticide-based pear psylla and spider mite management, plant defense elicitors may contribute to selective approaches that suppress these pests while preserving biological control, which is critically important in North America and Europe (Trapman & Blommers, 1992; DuPont et al., 2021). Previous tests support this possibility, as both ASM and harpin treatments reduce pear psylla populations on potted pear trees (Saour et al., 2010; Cooper & Horton, 2015) and mature orchard pear trees at unmanaged research station orchards (Saour et al., 2010; Cooper & Horton, 2017). With this foundation, our experiments were designed to test ASM and harpin utility in the context of commercially realistic management programs. We found application of ASM measurably induced *PR-1* on potted pear trees, but single applications of ASM or harpin at the highest labeled rate to mature orchard trees neither increased *PR-1* expression nor suppressed pear psylla and spider mites. In comparison with previous studies in pear (Saour et al., 2010; Cooper & Horton, 2017), our findings may be explained by differences between application timing and frequency, effects of commercially realistic pest management programs in our study, or idiosyncratic factors of orchard management.

In contrast to other field studies which found pear psylla suppression in the field after foliar ASM or harpin applications (Saour et al., 2010; Cooper & Horton, 2017), we used single rather than multiple applications and tested both foliar and soil drench methods for ASM. In our study, a single application may have been insufficient to induce plant resistance and suppress arthropod pests. In support of this hypothesis, Cooper & Horton (2017) observed that pear psylla nymphs were reduced on leaves in June and July but not April and May following one application of harpin or ASM every 4 weeks starting in April in Washington State. However, this hypothesis cannot fully explain our results in comparison with past work. Saour et al. (2010) observed ca. 50% fewer European pear psylla (*Cacopsylla pyri* L.) nymphs in harpin-treated plots compared with control plots after their first spray, applied in late May in Syria, in a season-long three-spray study. In addition, although fire blight suppression in apple and pear is greater with two ASM applications, single applications also result in some suppression (Johnson et al., 2016). The previous pear psylla studies (Saour et al., 2010; Cooper & Horton, 2017) always used foliar applications of harpin and ASM. We also used foliar applications for harpin, but tested ASM with both soil drench (in 2017) and foliar (in 2018) methods. The lack of pear psylla suppression in either year suggests that differences in application methods cannot fully explain the results. Overall, multiple applications of ASM and harpin may be expected to result in more consistent effects on pear psylla in the field than single applications.

Differences between commercially managed orchards in our study compared with research station orchards in previous studies may explain why we observed no effects. Experiment station orchards used by Saour et al. (2010) and Cooper & Horton (2017) did not use any chemical pest management aside from the tested treatments on small plots. In addition, we observed a much lower maximum average infestation (ca. 0.5 nymphs per leaf among all treatment-orchard combinations) than Saour et al.

(2010) (maximum of ca. 8 nymphs per leaf), or Cooper & Horton (2017) (maximum of ca. 2.5–10 nymphs per leaf depending on the year). Suppression of pear psylla by spray programs in commercial orchards and naturally low pear psylla counts in the one unsprayed research orchard used in our study could have masked effects from our treatments, reduced pressure on trees to induce defenses, or both. The different pear cultivar and pear psylla species tested by Saour et al. (2010) in Syria – that is, Koshia pears and *C. pyri* – might also respond differently to plant defense elicitor effects than *C. pyricola* on Bartlett and Anjou pear trees tested in our study and in Cooper & Horton's (2017). Overall, a range of factors may contribute to inconsistency in plant defense elicitor effectiveness between orchards.

Plant defense chemical pathway regulation and effects on herbivores have been extensively studied in potted plants for decades (Wei et al., 1992; Inbar et al., 2001; Gols et al., 2003; Dong et al., 2004; Boughton et al., 2006; Cooper & Horton, 2015) and are clearly profoundly important in nature (Howe & Jander, 2008; Wu & Baldwin, 2010), but few defense elicitor studies have been conducted under field conditions relevant to agricultural management (Weston, 2008; Warabieda, 2015). Defense responses of plants in the field can be affected by pesticides (Ford et al., 2010), fertilization (Warabieda et al., 2020), and a range of potential factors associated with organic management (Krey et al., 2020). Moreover, multiple defense-associated chemical pathways are known to respond differently to various pests, and interact to regulate defense responses (Inbar et al., 2001; Gordy et al., 2015), making predictions of effects on any one elicitor on any one pest difficult in complex field situations where there is variation in management practices, disease prevalence, and herbivore abundance. Our findings highlight that considerable work remains to extensively integrate plant defense elicitors into arthropod pest management strategies, and we suggest that future studies under field conditions would be valuable to understand the potential contribution of these chemicals towards sustainable arthropod pest management.

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Data Availability Statement

Data available on request from the authors.

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