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ORIGINAL ARTICLE

Responses of Non-Target Arthropods to the dsRNA Bioinsecticide Calantha™ and Conventional Insecticides Targeting Colorado Potato Beetle, *Leptinotarsa Decemlineata* (Say)

Erik J. Wenninger[1] · **Samuel P. DeGrey**[1] · **Jonas Insinga**[2] · **Eric Knopf**[3] · **Andrei Alyokhin**[4] · **Ethann R. Barnes**[5] · **Ben Bradford**[6] · **Russell L. Groves**[6] · **Brian Manley**[5] · **Julia Piaskowski**[7]

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Abstract

Management of the Colorado potato beetle (*Leptinotarsa decemlineata*) is reliant on conventional insecticides that can negatively affect non-target arthropods. Calantha™ (active ingredient: ledprona) is a sprayable double-stranded RNA biopesticide specific for *L decemlineata* proteasome subunit beta 5 gene that triggers the RNA-interference pathway and is designed to have limited non-target effects. To test this hypothesis, we conducted two years of field trials in Idaho, Wisconsin, and Maine comparing arthropod responses to different insecticide regimes, with and without Calantha, targeting the Colorado potato beetle. Comparisons of arthropod abundance among treatments showed no evidence of effects of Calantha on non-target arthropods, including beneficials (predators, parasitoids), "neutrals" (i.e., non-pests), and other beetle species. Conventional insecticides generally showed more non-target effects, and responses were always stronger for arthropods from vacuum samples than pitfall samples. Insecticide programs featuring Calantha, especially in rotation with other biorational products, may reduce pests while preserving beneficial arthropods and contribute to biological control of arthropod pests in potato fields.

Resumen

El manejo del escarabajo de la papa de Colorado (*Leptinotarsa decemlineata*) depende de insecticidas convencionales que pueden afectar negativamente a los artrópodos no objetivo. CalanthaTM (ingrediente activo: ledprona) es un biopesticida de ARN bicatenario rociable específico para el gen de la subunidad beta 5 del proteasoma de *L decemlineata* que desencadena la vía de interferencia del ARN y está diseñado para tener efectos limitados no objetivo. Para probar esta hipótesis, realizamos dos años de ensayos de campo en Idaho, Wisconsin y Maine comparando las respuestas de los artrópodos a diferentes regímenes de insecticidas, con y sin Calantha, dirigidos al escarabajo de la papa de Colorado. Las comparaciones de la abundancia de artrópodos entre los tratamientos no mostraron evidencia de efectos de Calantha en artrópodos no objetivo, incluidos los benéficos (depredadores, parasitoides), los "neutros" (es decir, no plagas) y otras especies de escarabajos. Los insecticidas convencionales generalmente mostraron más efectos no objetivo, y las respuestas siempre fueron más fuertes para los artrópodos de muestras de vacío que para las muestras de trampa. Los programas de insecticidas que incluyan Calantha, especialmente en rotación con otros productosbiorracionales, pueden reducir las plagas al tiempo que preservan los artrópodos beneficiosos y contribuyen al control biológico de las plagas de artrópodos en los campos de papa.

Keywords Beneficial insects · Natural enemies · RNAi · Integrated pest management · Biological control

✉ Erik J. Wenninger
erikw@uidaho.edu

[1] Department of Entomology, Plant Pathology & Nematology, Kimberly Research & Extension Center, University of Idaho, Kimberly, ID, USA

[2] Independent researcher, Nashua, NH, USA

[3] Independent researcher, Fredericton, NB, Canada

[4] School of Biology and Ecology, University of Maine, Orono, ME, USA

[5] GreenLight Biosciences, Research Triangle Park, NC, USA

[6] Department of Entomology, University of Wisconsin-Madison, Madison, WI, USA

[7] Statistical Programs, University of Idaho, Moscow, ID, USA

Introduction

The Colorado potato beetle [Coleoptera: Chrysomelidae, *Leptinotarsa decemlineata* (Say)] is an important pest of potato that can severely defoliate plants, reducing tuber yield (Alyokhin et al. 2022a; Stieha and Poveda 2015). Severity of yield reduction is influenced by several factors including cultivar and timing of damage in relation to plant growth stage (Stieha and Poveda 2015). Management is heavily dependent on insecticides with little regard for integrated pest management (IPM); however, mounting concerns regarding the environmental, economic, and socioeconomic sustainability of this approach underscore the need for alternative approaches (Alyokhin et al. 2009, 2022).

The Colorado potato beetle is notorious for development of resistance to insecticides. To date this species has exhibited resistance to more than 50 different compounds belonging to all major insecticide classes (Alyokhin et al. 2008, 2022a; Kuhar et al. 2022). Some of the older chemical classes to which the Colorado potato beetle has developed resistance are no longer available for use on potato, but more than 30 insecticides accounting for more than 15 different Insecticide Resistance Action Committee (IRAC) groups are currently registered in the US (Alyokhin et al. 2008, 2022a). New modes of action are needed, especially those with fewer non-target effects.

Broad-spectrum insecticides that have been traditionally used against the Colorado potato beetle and other arthropod pests in potato have a strong negative effect on beneficial arthropods (Alvarez et al. 2013; Chapman 2003; Koss et al. 2005; Metcalf 1980; Radkova et al. 2017; Reed et al. 2001). Such applications have been associated with apparent pest resurgence and outbreaks of secondary pests (Metcalf 1980). In contrast, organic potato production may preserve beneficial arthropods but provide suboptimal control of pests like the Colorado potato beetle and the green peach aphid, *Myzus persicae* (Koss et al. 2005). More selective, but efficacious, modes of action have been shown to reduce these pests while also preserving beneficials (Koss et al. 2005). Thus, insecticides with fewer non-target effects should be more conducive to promoting conservation biological control of the Colorado potato beetle.

Indeed, there has been a shift in recent decades toward the development of insecticides with more targeted, narrow-spectrum, modes of action and more targeted delivery (e.g., seed versus foliar application), in part to reduce effects on non-target arthropods, including natural enemies of insect pests. However, even targeted delivery methods can still result in substantive non-target effects. For example, Douglas and Tooker (2016) found that seed-applied neonicotinoids had a negative effect on abundance of arthropod natural enemies that was similar to the effect of pyrethroids. Neonicotinoids may have even stronger negative effects on behavior of non-target arthropods than on abundance (Main et al. 2018), which might contribute to underestimates of potential deleterious effects on biological control services. Clearly more targeted active ingredients are needed if we are to effectively incorporate conservation biological control into agricultural pest management.

RNA interference (RNAi) is a process by which double stranded RNA (dsRNA) that is complementary to a target messenger RNA (mRNA) sequence is used to degrade that mRNA, thereby suppressing gene expression (i.e., production of the protein encoded by the gene is reduced) (Mishra and Jurat-Fuentes 2022). Coleopterans are generally more sensitive to dsRNA than other insect orders that have been studied (Cooper et al. 2019) and, indeed, the Colorado potato beetle has been the focus of several RNAi-based studies including more than 15 different target genes (Baum et al. 2007; Guo et al. 2018; He et al. 2020; Hussain et al. 2019; Máximo et al. 2020; Mehlhorn et al. 2020; Petek et al. 2020; Rodrigues et al. 2021; San Miguel and Scott 2016; Shen et al. 2020; Shi et al. 2016; Xu et al. 2019; Zhang et al. 2015; Zhu et al. 2011). Various dsRNA delivery methods have been shown to be effective against the Colorado potato beetle, including foliar sprays, bacterially expressed dsRNA, and transgenic potato plants.

Calantha™ (active ingredient, ledprona) is a novel dsRNA-based biopesticide that recently received Federal Sect. 3 registration by the United States Environmental Protection Agency (EPA). Calantha is the first product featuring a sprayable dsRNA that triggers the RNAi pathway in the Colorado potato beetle (Rodrigues et al. 2021). Specifically, the target gene codes for the proteasome subunit beta 5 (PSMB5), which is part of the ubiquitin/proteasome machinery that removes damaged proteins and prevents the accumulation of poly-ubiquitinated protein aggregation in cells (Hershko et al. 2000). Efficacy of Calantha against the Colorado potato beetle has been demonstrated in laboratory, greenhouse, and field studies (Pallis et al. 2022, 2023; Rodrigues et al. 2021), showing up to 100% mortality and significant reduction in defoliation compared to checks. Given the high specificity that is dictated by dsRNA sequence complementarity through RNAi, the mode of action should be extremely taxon specific. This should result in little or no non-target effects; however, this hypothesis has yet to be tested in the field for Calantha.

The primary aim of the current study was to evaluate responses of non-target arthropods to insecticide programs targeting Colorado potato beetle in potato, particularly programs featuring Calantha. In addition, we compared non-target effects among various insecticide programs, including those with conventional insecticides. Of particular interest to evaluate were effects of Calantha on beneficial arthropods

Table 1 Study site details for each trial location during 2020 and 2021

Location	Cultivar	GPS coordinates		Planting date	
		2020	2021	2020	2021
Kimberly, ID	'Russet Burbank'	42.54922, −114.34168	42.54969, −114.34169	28 April	27 April
Hancock, WI	'Goldrush'	44.11893, −89.55665	44.119, −89.5501	16 April	26 April
Presque Isle, ME	'Katahdin' (2020) 'Keuka Gold' (2021)	46.65517, −68.01168	46.65525, −68.00923	26 May	26 May

(predators and parasitoids), neutral arthropods (i.e., those not known to be pests or natural enemies of pests), and on other coleopterans given that closely related taxa would be the most likely to show effects.

Materials and Methods

Study Sites and Experimental Design

We conducted field studies at three different experimental research farms over two growing seasons using a potato cultivar appropriate for each region (Table 1). Plots at each site were arranged in a randomized complete block design with 4 replicates. Each plot was 8 rows wide by 7.6 m long. Row spacing was 91.4 cm and seed spacing was 30.5 cm. Plots were planted with a two-row small-plot planter. Blocks were separated from each other by a 1.5-m patch of bare ground. We maintained plots according to standard agricultural practices appropriate to each growing area, including cultivation, irrigation, and herbicide and fungicide applications. Any such practices were applied uniformly across all plots in a field experiment. Plots in Idaho were watered using solid set irrigation; plots in Wisconsin and Maine received no supplemental irrigation.

Insecticide Treatments

In-furrow treatments were applied using a CO_2-powered sprayer with one nozzle positioned within the furrow of each row just behind the dropping seed pieces and just in front of the discs that closed each row. In-furrow insecticide was applied with ca. 10 mL of water per row-meter. Foliar sprays were applied similarly, but with one nozzle (Teejet 8002VS flat fan nozzle) over each row and applied with 159 L of water per hectare. We applied treatments as rotations of insecticides aimed at Colorado potato beetle control, including various approaches (Table 2). Treatment 1 did not receive any insecticide applications and was used as a non-treated check ("check" is used throughout as a synonym for the term "untreated control"). Calantha alone (treatment 2) was evaluated against rotations with more broad-spectrum products (treatments 3 and 4) and a rotation of conventional insecticides that were expected to have fewer non-target effects (treatment 5). In addition, Calantha was evaluated in rotation with the insect growth regulator novaluron (treatments 6 and 7). Four of the seven treatments also included an at-plant neonicotinoid treatment given the prevalence of use of such products on commercial potato acreage in the growing areas of each state considered in this study. For all insecticides we used the highest label rate recommended for Colorado potato beetle management in potato (Table 2).

The timing and frequency of sprays was region dependent, reflecting the differences in Colorado potato beetle pressure and phenologies among the different regions. The Colorado potato beetle exhibits two generations per summer in Idaho and Wisconsin, but pressure is considerably higher in Wisconsin. Therefore, Idaho featured two sprays against the first generation and one against the second; in Wisconsin two sprays were applied to each generation (Table 2), except for treatment 2 (Calantha only), which received three sprays targeting the first generation. Only one generation is observed each year in Maine, so three applications of all products were made against that single generation.

Arthropod Sampling

We sampled arthropods every two weeks from each plot using two approaches: vacuum sampling and pitfall trapping. Vacuum samples were collected via the intake tube of a gasoline-powered leaf blower. A cloth insect net was secured to the opening of the tube with rubber bands and the intake tube was brushed across the foliage of the center two rows of each plot over a 60-second sampling period. Collected arthropods were then transferred to a plastic zipper-lock bag and stored in a −20℃ freezer. They were then transferred to glass vials with 70% ethanol. Pitfall traps, one per plot, were established and maintained within one of the middle rows in each plot. Pitfall traps in Idaho were as described by Wenninger et al. (2020). In Wisconsin and Maine, a pitfall trap consisted of a plastic cup (532 ml; 9 cm opening diameter) that was buried with the rim flush with the soil surface and a rain shield made of a clear plastic plate (17.5 cm in diameter) supported 3 cm above the ground by three legs made of bent iron wire. All pitfall traps contained ca. 90 ml of a 27.5–50% propylene glycol

Table 2 Insecticide rotation treatments evaluated in this study

Treatment	At-plant			1st generation[a]			2nd generation[b]		
	Active ingredient	Trade name	Application rate[c]	Active ingredient	Trade name	Application rate[c]	Active ingredient	Trade name	Application rate[c]
1	—	—	—	—	—	—	—	—	—
2	—	—	—	ledprona	Calantha[d]	9.4	ledprona[d]	Calantha	9.4
3	thiamethoxam	Platinum	140.0	abamectin	Agri-Mek	21.5	esfenvalerate	Asana	55.6
4	thiamethoxam	Platinum	140.0	thiamethoxam + lambda-cyhalothrin	Endigo	46.6 + 4.7	abamectin	Agri-Mek	21.5
5	thiamethoxam	Platinum	140.0	Spinosad	Blackhawk	88.0	rynaxypyr	Coragen	73.0
6	—	—	—	Novaluron	Rimon	58.0	ledprona[d]	Calantha	9.4
7	thiamethoxam	Platinum	140.0	ledprona	Calantha[d]	9.4	novaluron	Rimon	58.0

[a]Two sprays were applied for all treatments in Idaho; in Wisconsin, two sprays were applied for all treatments except for Calantha which was sprayed three times; in Maine, all treatments were sprayed three times

[b]One spray was applied in Idaho; two sprays were applied in Wisconsin; no sprays were applied in Maine

[c]g a.i. per hectare

[d]Calantha sprays included the adjuvant Bondmax at 0.25% v/v

solution and a few drops of dish detergent. Pitfall trapped arthropods were taken to the lab and transferred to glass vials with 70% ethanol.

All arthropods were identified to the lowest taxon possible based on gross morphology using published resources. Identifications allowed for assignment of each individual into an ecological guild based on their relevance to agriculture and/or human society: beneficial, pest, or "neutral" (i.e., not known to directly affect crops or pests). Beneficial arthropods included predatory species, herbivores that feed on weeds (including seed-feeding carabid beetles), parasitoids that attack pests, and pollinators. The pest group included all pests of potatoes as described by Alyokhin et al. (2022b), as well as any other taxon known to be an agricultural pest. We also included taxa that are broadly known to be harmful to humans such as black flies (Simuliidae), but did not include minor nuisance pests, such as most vinegar flies (*Drosophila* spp.) or earwigs (*Forficula auricularia* (L.). The neutral category was composed of the remainder of taxa which were neither beneficial nor pests, and included a wide range of species including saprophages, necrophages, aquatic insects, soil detritivores, and phytophagous species that feed on plants not directly relevant to agriculture.

Data Analyses

Abundance (i.e., the total number of individuals within each taxon in each sample) was the primary response variable in analyses. Data were analyzed separately for each category (beneficial, neutral, pest, beetles, or total arthropods), year (2020 or 2021), and sampling type (pitfall or vacuum sample). On the last date in 2020 in Wisconsin (8 August), many plots (especially in the non-treated check) were so heavily defoliated from Colorado potato beetle feeding that there was essentially no leafy matter remaining; these plots were not sampled at this time (Figure S1). For this reason, this date was removed from all vacuum and pitfall datasets from that year in Wisconsin. Since our interest in this experiment was to model non-target effects on taxa other than Colorado potato beetle, any observations of Colorado potato beetle were removed from the data for analysis. Preliminary analyses included evaluating responses of arthropod richness to insecticide treatments. Because responses were similar to those for abundance and provided limited additional illustrative value, they are not considered further here.

All statistical analyses were performed in R (version 4.3.1, R Core Team 2023). The effect of pesticide treatment regime on arthropod abundance was evaluated using generalized linear mixed models fit using the "glmmTMB" package (Brooks et al. 2017). Date, treatment (insecticide program), and date × treatment interaction were modeled as fixed effects, and block as a random effect. A first-order autocorrelation covariance structure (In glmmTMB – ar1(date + 0|Plot) was also introduced to account for autocorrelation among plots since they were repeatedly sampled through the growing season. Models were generally fit using a Poisson error distribution with a log-link function, although for a few cases a generalized Poisson error distribution was used instead to permit model convergence. Model fit was assessed by examining the residual plots, checking the log likelihood, and when possible, conducting log-likelihood ratio tests and checking that the chosen model converged without warnings or errors. Zero-inflated models (In glmmTMBziformula = ~ 1, family = poisson()) were used when necessary for models with many zeroes. In the case of extreme zero-inflation (i.e., when the percentage of zeroes in a dataset was higher than 30% and an entire treatment combination consisted solely of zeros), we fit aggregate models (see below). Assumption checking of residuals was performed using the "DHARMa" package (Hartig 2017). The two main fixed effects (date and treatment) were tested for multicollinearity by using the "performance" package (Lüdecke et al. 2021). The variance inflation factor (VIF) was less than 5 for all full (non-aggregate) models, indicating low multicollinearity. Inference on models was done by conducting chi-square tests for ANOVA using type III sum of squares in the "car" package. For models that showed a significant treatment or interaction effect, estimates were extracted from models and post hoc comparisons were conducted comparing all treatment to the non-treated check using the "emmeans" package, which calculates estimated marginal means. Estimated marginal means represent the predicted average score of each group after adjusting for any differences on the covariate; they are in the same units as the dependent variable (in our case, counts of arthropods). Suspected outliers were evaluated using the testResiduals() function in "DHARMa" and by removing suspect outliers and refitting models to determine the magnitude of a single outlier on model terms. Only two models were affected by such influential outliers (see Results).

To better understand the cumulative effects of insecticide treatment on arthropod abundance across the whole season and to assess posthoc differences between the check and each treatment for the main effect of insecticide treatment, we also summed all insect counts across dates and fit models to the resulting aggregated data, referred to here as "aggregate models." Since aggregated data were summed across dates, models fit on those data sets included only treatment as a fixed effect, block as a random effect, and did not include an extra covariance parameter. Methods for fitting and assumption checking for aggregate models were otherwise the same as for the full abundance models, except that the aggregate models mostly used a generalized Poisson error distribution and occasionally a regular Poisson distribution where needed for model convergence.

Results

Sampling Date Effects

For nearly all models, arthropod abundance differed significantly over time (i.e., there was a significant response to the main effect of date). However, this result was of less interest to us than the treatment effect and the interaction between date and treatment, which are highlighted here.

Table 3 Total abundance of arthropods collected from each site by collection method, year, and ecological guild

Ecological guild	Vacuum		Pitfall		Total
	2020	2021	2020	2021	
Idaho					
Beneficials	2,736	2,177	1,122	1,159	7,194
Neutrals	3,991	5,018	412	434	9,855
Pests	2,383	1,646	299	86	4,414
Beetles	87	47	435	469	1,038
Total	9,197	8,888	2,268	2,148	22,501
Wisconsin					
Beneficials	496	1,031	859	1,049	3,435
Neutrals	3,537	7,665	2,415	3,935	17,552
Pests	4,813	7,233	1,623	1,610	15,279
Beetles	109	530	677	686	2,002
Total	8,955	16,459	5,574	7,280	38,268
Maine					
Beneficials	526	113	7,449	3,858	11,946
Neutrals	1,004	243	13,077	2,548	16,872
Pests	1,811	384	1,710	299	4,204
Beetles	216	11	6,958	2,973	10,158
Total	3,557	751	29,194	9,678	43,180

Idaho Overview

In Idaho, we captured more arthropods in vacuum samples than pitfall traps for all groups besides beetles, in which the prevalence of carabid and staphylinid beetles, together comprising 86% of pitfall beetles, contributed to higher beetle captures in pitfall traps (Table 3; supplementary data). The neutral taxa were the most abundant group in vacuum samples for both years (Table 3), about 45% of which were drosophilid and chironomid flies (supplementary data). In pitfall traps, however, beneficial arthropods were the largest category in both years, reflecting high counts of common ground-dwelling predators like spiders (33% of pitfall samples) and beetles (26% of pitfall samples) (supplementary data). Relative abundance between years varied by collection method and guild, though counts were generally similar between the two years for the same guild and sampling method (Table 3). For the beneficial taxa, 81% were predators, 19% were parasitoids; beneficial herbivores and pollinators constituted less than 0.01% of this group (supplementary data).

Idaho Beneficials

Abundance of beneficial arthropods from vacuum samples differed significantly among treatments only in 2021 and by the interaction term only in 2020 (Table 4; Fig. 1a-b). In 2020, beneficial abundance in treatment 4 was significantly

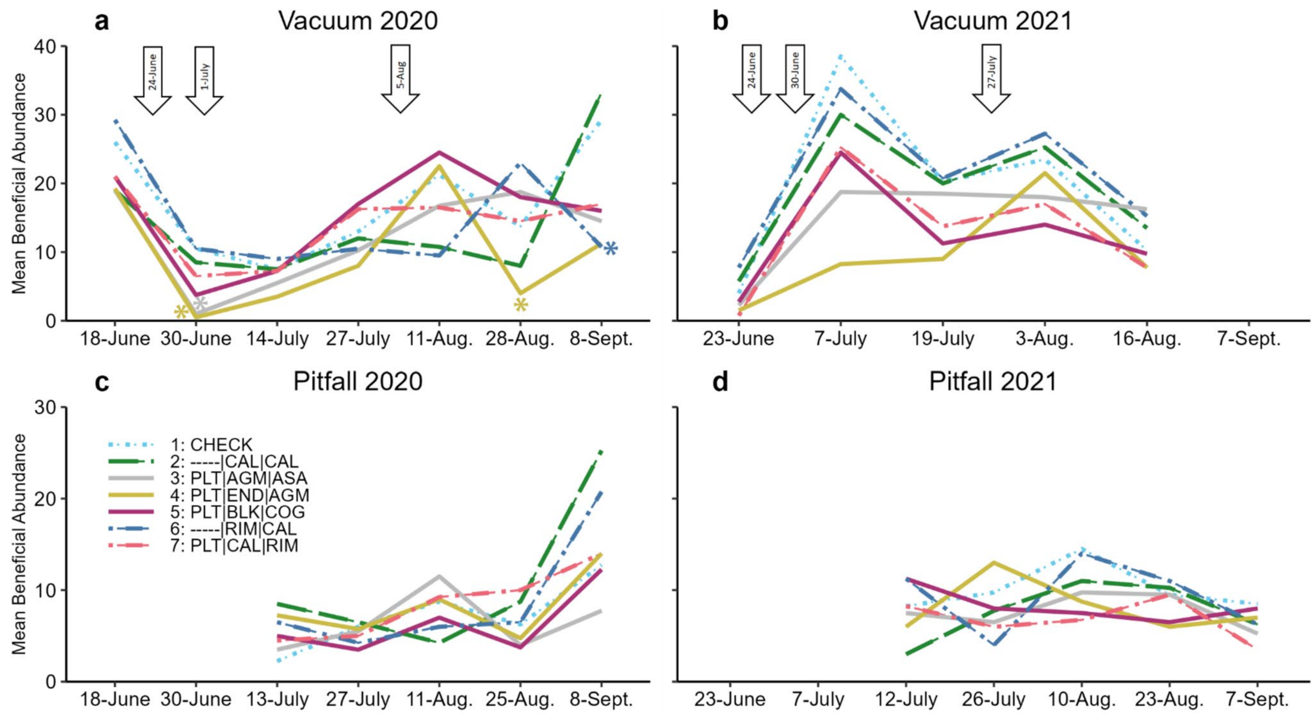

Fig. 1 Mean abundance per plot of beneficial arthropods in Idaho compared among treatments over time from **a** vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

lower than the check on three dates (once in late June, following the first foliar spray, as well as on the last two sample dates) (Fig. 1a). Treatment 6 also differed from the check on the last sample date (Fig. 1a). In 2021, beneficial abundance was significantly lower in treatments 4 and 7 relative to the check (Table S2; Fig. 1b). Abundance of beneficial arthropods collected in pitfall traps in Idaho did not differ among treatments nor by the interaction term in either year (Table 4; Fig. 1c-d; Table S2).

Idaho Neutrals

In 2020, abundance of neutral arthropods from vacuum samples differed significantly by the interaction term, but not among treatments (Table 4). Treatments 2, 4, and 5 each were lower than the check on one date and treatment 3 was lower than the check on two dates (Fig. 2a). These differences occurred primarily for samples that followed the first and second foliar sprays. For treatment 2, the difference occurred approximately 30 days after the last foliar spray and is likely not biologically meaningful. In 2021, abundance in vacuum samples did not differ among treatments, nor by the interaction term (Table 4; Fig. 2b). The interaction term was significant for neutral arthropods collected in pitfall traps in 2020 (Table 4). Treatments 4, 5, and 6 showed significantly fewer captures of neutral arthropods relative to the non-treated check on the first sample date after the last foliar spray (Fig. 2c). In 2021, neutral abundance from pitfall traps in Idaho did not differ among treatments nor by the interaction term (Table 4; Fig. 2d).

Idaho Pests

In 2020, abundance of pestiferous arthropods from vacuum samples differed significantly by the interaction term, but not by the main effect of Treatment (Table 4). Treatments 4 and 5 showed higher pest abundance than the check in early August, following the final foliar spray; treatment 4 also showed lower pest abundance than the check during late July and treatment 3 showed lower pest abundance than the check on the last sample date (Fig. 3a). The most abundant pests collected in August samples included *Lygus* bugs (60% of August samples) and potato-colonizing aphids (22% of August samples) (supplementary data). In 2021, pest abundance differed significantly among treatments and by the interaction term (Table 4; Table S4). Overall pest abundance was significantly lower than the check in treatments 3, 4, 5, and 7 (all treatments featuring an at-planting neonicotinoid; Table S4). Pest abundance in treatment 4 was lower than the check on all dates over the season except for the first sample

Table 4 Generalized linear mixed models evaluating treatment responses of different ecological guilds collected from vacuum and pitfall samples in Idaho in 2020 and 2021

Source of Variation	Vacuum						Pitfall					
	2020			2021			2020			2021		
	df	χ^2	*P*-value	df	χ^2	*P*-value	df	χ^2	*P*-value	df	χ^2	*P*-value
Beneficial abundance												
Date	6	22.1	0.001	4	45.4	< 0.001	4	13.7	0.008	4	2.4	0.672
Treatment	6	3.0	0.809	6	22.5	< 0.001	6	9.4	0.153	6	9.3	0.157
Date × Treatment	36	76.5	< 0.001	24	33.8	0.089	24	30.0	0.184	24	33.0	0.104
Neutral abundance												
Date	6	62.0	< 0.001	4	35.8	< 0.001	4	26.1	< 0.001	4	4.1	0.393
Treatment	6	11.3	0.079	6	7.99	0.239	6	11.0	0.089	6	8.2	0.222
Date × Treatment	36	113.4	< 0.001	24	25.3	0.388	24	51.1	0.001	24	29.8	0.193
Pest abundance												
Date	6	30.1	< 0.001	4	20.1	< 0.001	—	—	—	—	—	—
Treatment	6	0.26	1.00	6	19.3	< 0.001	—	—	—	—	—	—
Date × Treatment	36	77.9	< 0.001	24	51.9	< 0.001	—	—	—	—	—	—
Beetle abundance												
Date	—	—	—	—	—	—	4	2.9	0.578	4	10.4	0.035
Treatment	—	—	—	—	—	—	6	9.2	0.162	6	8.9	0.182
Date × Treatment	—	—	—	—	—	—	24	22.9	0.526	24	37.7	0.038
Total												
Date	6	46.1	< 0.001	4	32.1	< 0.001	4	20.8	< 0.001	4	3.06	0.548
Treatment	6	2.51	0.867	6	5.37	0.497	6	16.9	< 0.001	6	7.75	0.257
Date × Treatment	36	117	< 0.001	24	38.0	0.035	24	51.7	< 0.001	24	21.7	0.600

In several cases, full models could not be run due to low captures; see aggregate models (Table S1)

date (before sprays were applied); pest abundance was lower than the check on individual dates for treatments 3, 5, and 7 (Fig. 3b). Abundance of pests collected in pitfall traps in Idaho could only be compared among treatments (see Idaho Aggregate Models, below).

Idaho Beetles

Low overall captures meant that vacuum-sampled beetles could only be compared using aggregate models (see Idaho Aggregate Models, below). For pitfall traps in Idaho, beetle abundance did not differ by treatment nor the interaction term in 2020 (Table 4; Fig. 4c). In 2021 the interaction term was significant, but no differences were observed between any treatment versus the non-treated check on any sample date (Table 4; Fig. 4d).

Idaho Total Arthropods

In both years, overall arthropod abundance from vacuum samples differed significantly by the interaction term but not among treatments (Table 4; Fig. 5a-b). Abundance was significantly lower than the check in treatments 3 and 4 on the second sample date in both years (Fig. 5a-b). Abundance was also lower in treatment 3 relative to the check on the last sample date in 2020 (Fig. 5a). These patterns largely reflected treatment differences observed in beneficials, neutrals, and pests. Total arthropod abundance in pitfall traps differed among treatments and by the interaction term in 2020, but no effects were significant in 2021 (Table 4; Fig. 5c-d; Table S6). In 2020, abundance was significantly higher than the check in treatment 4 on the first sample date and lower in treatment 6 on the middle sample date (Fig. 5c).

Idaho Aggregate Models

Aggregate models for beneficial arthropods showed significant differences among treatments in vacuum samples in both 2020 and 2021 (Table S1). Similar to the full model, abundance of beneficial arthropods was lower than the check in treatment 4 in 2020 and in treatments 4, 5, and 7 in 2021 (Table S2). Abundance of pitfall-trapped beneficial arthropods did not differ among treatments either year (Table S1).

For neutral arthropods in Idaho, abundance in vacuum samples differed among treatments only in 2021 (Table S1), which differed slightly from results in full models. Abundance was higher in treatments 3, 4, and 7 relative to the check (Table S3). Abundance of neutral arthropods from pitfall traps did not differ among treatments either year (Table S1).

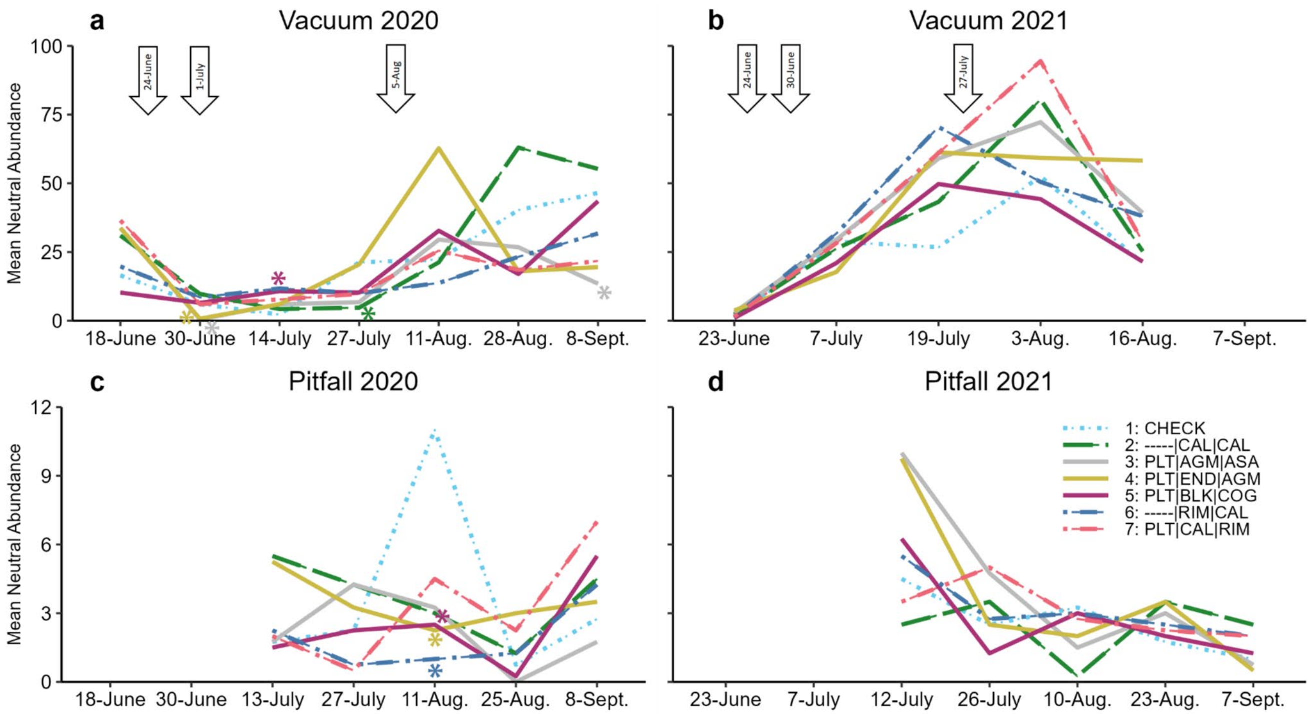

Fig. 2 Mean abundance per plot of neutral arthropods in Idaho compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

Aggregate models of pests collected in vacuum samples in Idaho were remarkably similar to the treatment effects in full models; no treatment effect was observed in 2020, but in 2021 the three treatments with at-plant insecticide showed fewer pests than the check (Tables S1, S4). For pitfall-trapped pests, the treatment effect was significant in 2020, but not 2021 (Table S1); however, no treatments in 2020 differed significantly from the non-treated check (Fig. 3c-d; Table S3).

For beetle captures in vacuum samples, a significant treatment effect was observed only in 2020, but there were no differences relative to the non-treated check (Table S1, Table S5; Fig. 4a-b). No significant treatment effect was observed for aggregate models of pitfall-trapped beetles (Table S1).

Aggregate models for total captures in Idaho showed a significant treatment effect only for vacuum samples in 2021 (Table S1); however, no significant differences were observed between each treatment and the check (Table S6).

Wisconsin Overview

In Wisconsin, overall captures in vacuum samples were higher than in pitfall traps for both years, though captures of beneficials and beetles were greater in pitfall traps (Table 3). The neutral taxa was the most abundant group for both years and sampling methods, except for vacuum samples in 2020 in which pests were the largest group (Table 3); this exception was driven by extremely high captures of flea beetles (*Epitrix* sp.), including the potato flea beetle, *Epitrix cucumeris* (Harris), which together accounted for 57% of pest counts, and 25% of the total counts (supplementary data). For all analyses except for the pest pitfall captures, more arthropods were captured in 2021 than in 2020 (Table 3). For the beneficial taxa, 86% were predators, 12% parasitoids, 0.01% pollinators, and < 0.01% were beneficial phytophages (supplementary data).

Wisconsin Beneficials

Beneficial abundance in vacuum samples differed significantly by the interaction term in both years, but not by treatment (Table 5; Fig. 6). Early during the season, beneficial abundance was significantly lower in treatments 3 and 4 relative to the check in 2020 (Fig. 6a) and significantly lower in treatments 3 and 5 relative to the check in 2021 (Fig. 6b). Later during the growing season when defoliation in the non-treated check plots was severe

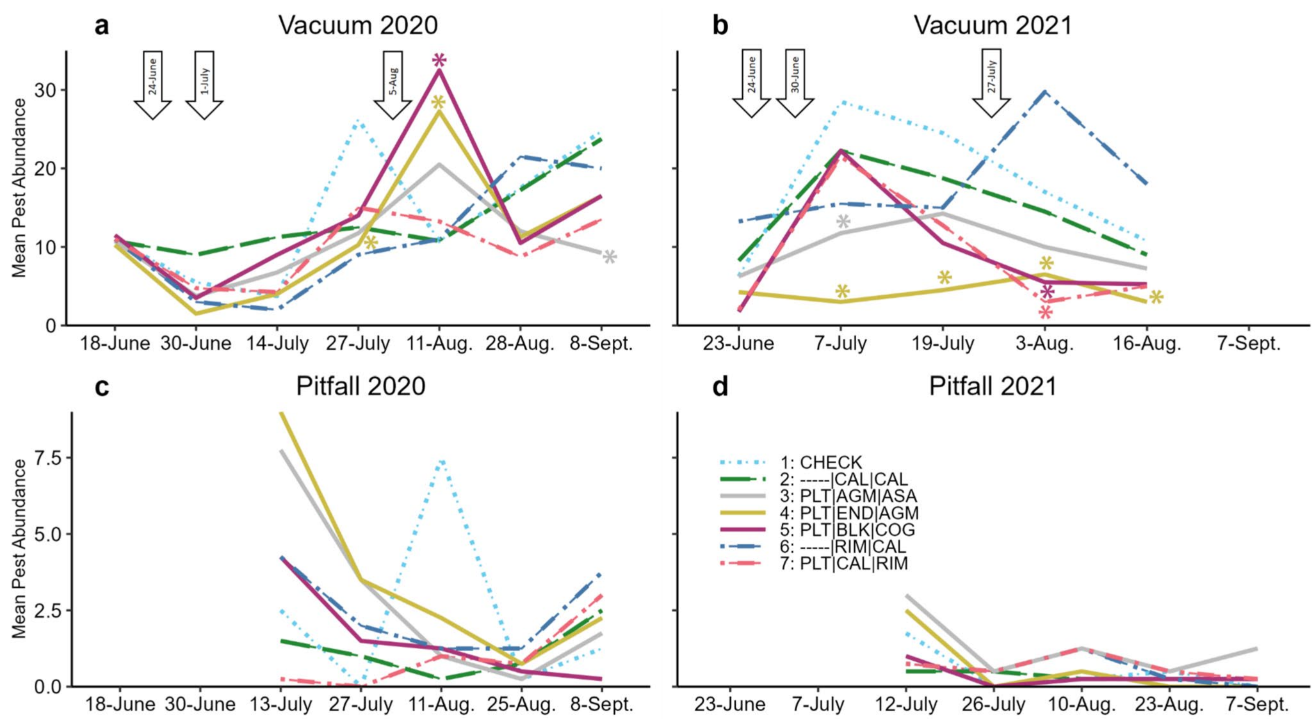

Fig. 3 Mean abundance per plot of pest arthropods in Idaho compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

(Figure S1), beneficial abundance was significantly lower in the check relative to some of the other treatments in both years. In particular, beneficial abundance in 2020 was higher on the last two sample dates for treatment 6 and on one date each for treatments 7 and 2 (Fig. 6a). In late July 2021, beneficial abundance was significantly higher than the check for all treatments except treatment 3 (Fig. 6b); these differences occurred about one week following the last foliar application. Abundance of pitfall-trapped beneficial arthropods in Wisconsin differed among treatments in 2020 (Table 5; Fig. 6c); however, no significant differences were observed between the check and other treatments (Table S7). We did not observe a significant treatment or interaction effect in 2021 (Table 5; Fig. 6d).

Wisconsin Neutrals

Abundance of neutral arthropods from vacuum samples in Wisconsin differed among treatments in both years (Table 5; Fig. 7). Overall abundance of neutrals was significantly higher in treatment 6 relative to the check in both years and in 2021 was also higher in treatment 2 (Table S8). The interaction effect was also significant both years (Table 5; Fig. 7). Abundance of neutrals was lower than the check in treatments 3, 4, and 5 about a week after the last spray against the first generation in 2020 (Fig. 7a). For the last two sample dates in 2020, abundance of neutral arthropods was relatively low across all plots but was significantly higher in treatment 6 relative to the check on one date, prior to the final sprays (Fig. 7a). Several significant differences were observed among treatments over the 2021 season (Fig. 7b). Most strikingly, treatments 3, 4, and 5 showed lower neutral captures relative to the check on the first two sample dates and higher neutral captures relative to the check on the last sample date. Treatment 7 showed a similar pattern, though only for one date in June and one date in July. Several other treatments showed higher captures of neutral arthropods relative to the check in late July (treatments 2, 4, and 6) and/or late August (treatment 6). Abundance of pitfall-trapped neutral arthropods differed among treatments in 2020 (Table 5); overall abundance was higher in the check relative to all other treatments except treatment 2 (Fig. 7c; Table S8). We did not observe a significant treatment or interaction effect in 2021 (Table 5; Fig. 7d).

Wisconsin Pests

Abundance of pests (other than the Colorado potato beetle) from Wisconsin vacuum samples differed significantly among treatments only in 2020 and by the interaction term

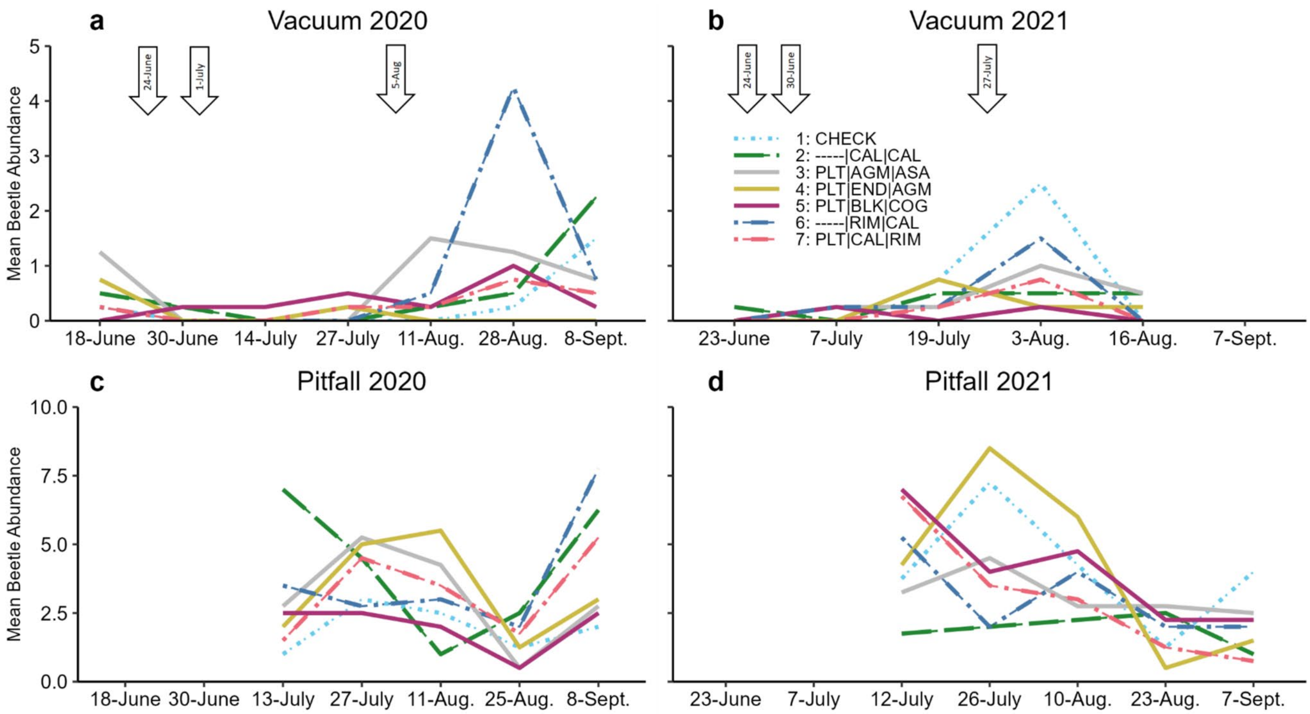

Fig. 4 Mean abundance per plot of beetles in Idaho compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

in both years (Table 5). In 2020, overall pest abundance was lower than the check in treatments 4 and 5 and higher than the check in treatments 2 and 6 (Table S9). Following the second spray in 2020, pest abundance was significantly lower than the check in all the treatments featuring an at-plant insecticide (treatments 3, 4, 5, and 7; Fig. 8a). Potato flea beetles comprised a preponderance of the pests driving these patterns (62% of captures in treatments 1, 2, and 6 on the first date; supplementary data). The only other instance in 2020 showing a significant difference from the check was in early July when treatment 2 showed higher pest abundance (Fig. 8a). In 2021, patterns were less clear; abundance was lower than the check in only two instances early in the season and higher than the check for three treatments in late July; the most striking pattern was on the last sample date when an outbreak of flea beetles in the check plots (flea beetles comprised 72% of the captures for this date across all treatments; supplementary data) resulted in significantly higher pests relative to all other treatments (Fig. 8d). Abundance of pitfall-trapped pests in Wisconsin differed among treatments in 2020, but not 2021 (Table 5; Fig. 8c-d; Table S9). In 2020, abundance was lower than the check in treatments 4, 5, and 7 (Fig. 8c; Table S9).

Wisconsin Beetles

Low captures of beetles in vacuum samples in both years in Wisconsin mandated models that only included treatment effects (see Wisconsin Aggregate Models, below). For pitfall traps in Wisconsin, beetle abundance differed among treatments in 2020, but neither the treatment nor interaction term were significant in 2021 (Table 5). In 2020, no significant pairwise differences were observed between the check and the other treatments (Fig. 9c; Table S10).

Wisconsin Total Arthropods

In both years, overall arthropod abundance from vacuum samples differed significantly among treatments and by the interaction term (Table 5; Fig. 10a-b). For both years, early season abundance was significantly lower than the check in all treatments featuring at-plant insecticide (Fig. 10a-b). In 2020, total arthropod abundance late season–when defoliation from the Colorado potato beetle was increasing in check plots (Figure S1)–was significantly higher than the check in the two treatments lacking at-plant insecticide (Fig. 10a). In late July 2021, all treatments showed higher total abundance relative to the check, but by late August when flea beetle abundance spiked, this pattern was reversed (Fig. 10b);

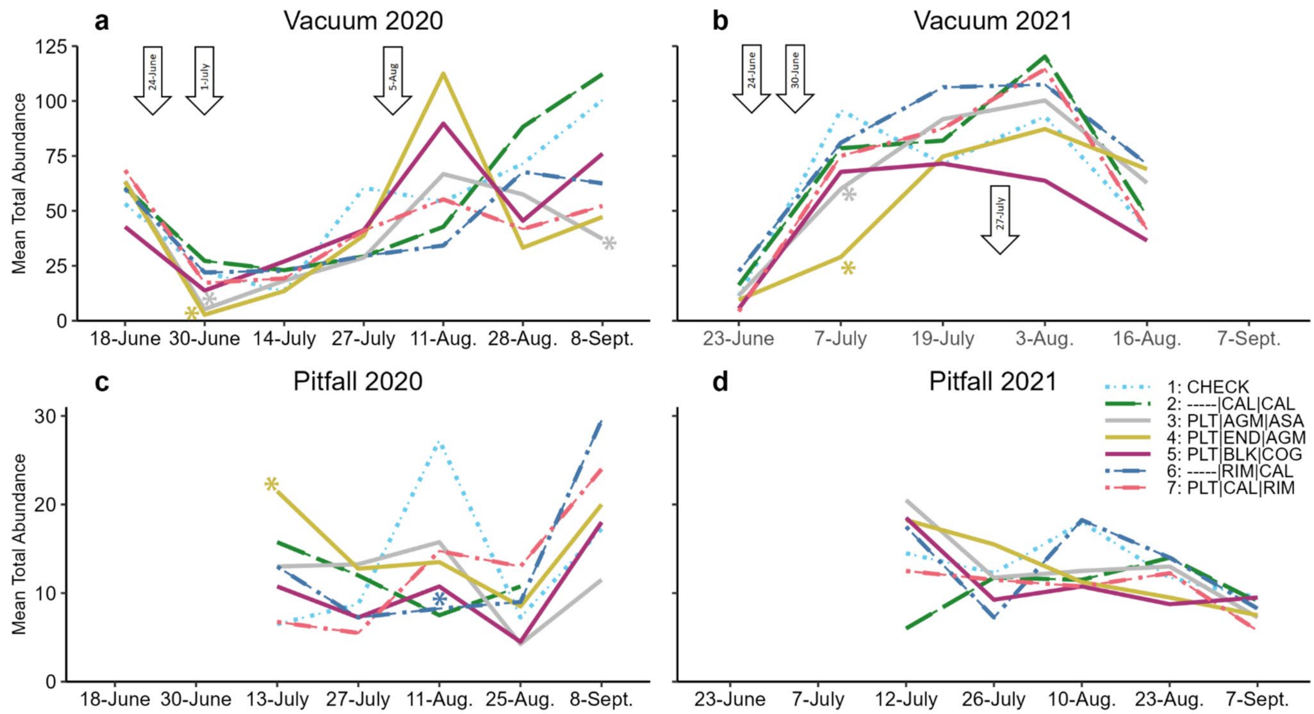

Fig. 5 Mean abundance per plot of total arthropods in Idaho compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

this was more than 30 days after the last foliar spray and likely not a direct result of treatments. Seasonal patterns in total arthropod abundance primarily reflected treatment differences observed in beneficials and pests. Pairwise treatment comparisons in 2020 showed higher total abundance relative to the check in treatments 2 and 6 and lower total abundance in treatments 3 and 4 (Table S11). In 2021, treatments 3, 4, 5, and 7 (all treatments including at-plant insecticide) showed lower total abundance relative to the check (Table S11). Total arthropod abundance in pitfall traps differed among treatments in 2020, but not by the interaction term, nor by treatment or interaction in 2021 (Table 5; Fig. 10c-d; Table S11). In 2020, total abundance in pitfalls was significantly lower in treatments 4, 5, and 7 relative to the check (Table S11).

Wisconsin Aggregate Models

Aggregate models showed significant differences among treatments in vacuum samples in both 2020 and 2021 (Table S1). In 2020, abundance of beneficials was lower than the check in treatments 3, 4, and 6, but in 2021 no treatment differed from the check (Table S7). Abundance of beneficial arthropods from pitfall traps differed among treatments in 2020 but not 2021 (Table S1); however, no treatment differed significantly from the check (Table S7).

Neutral arthropods from vacuum samples in Wisconsin were significantly less abundant than the check in treatments 3, 4, and 5 during both years and also less abundant in treatment 7 in 2021 (Tables S1; Table S8). Abundance of neutral arthropods from pitfall traps was significantly lower in each treatment relative to the check in 2020, but did not differ among treatments in 2021 (Table S1; Table S8).

For pests captured in vacuum samples, we observed a significant effect for both years in aggregate models in Wisconsin (Table S1). Lower abundance was found in treatments 3, 4, 5, and 7 relative to the check in 2020 and in all treatments relative to the check in 2021 (Table S9). In 2020 pitfall samples, the treatment effect was significant (Table S1); fewer pests were captured in treatment 5 relative to the check (Table S9). In 2021 pitfall samples, a single plot had extremely high captures of the scarab beetle, *Strigoderma arboricola*, resulting in an influential outlier. In the refitted model without this outlier, the treatment effect was not significant (Table S9).

Aggregate models for beetles in Wisconsin showed no significant differences among treatments for either year or sampling method (Table S1; Table S10).

Aggregate models for total arthropod captures in Idaho showed a significant treatment effect for both years of

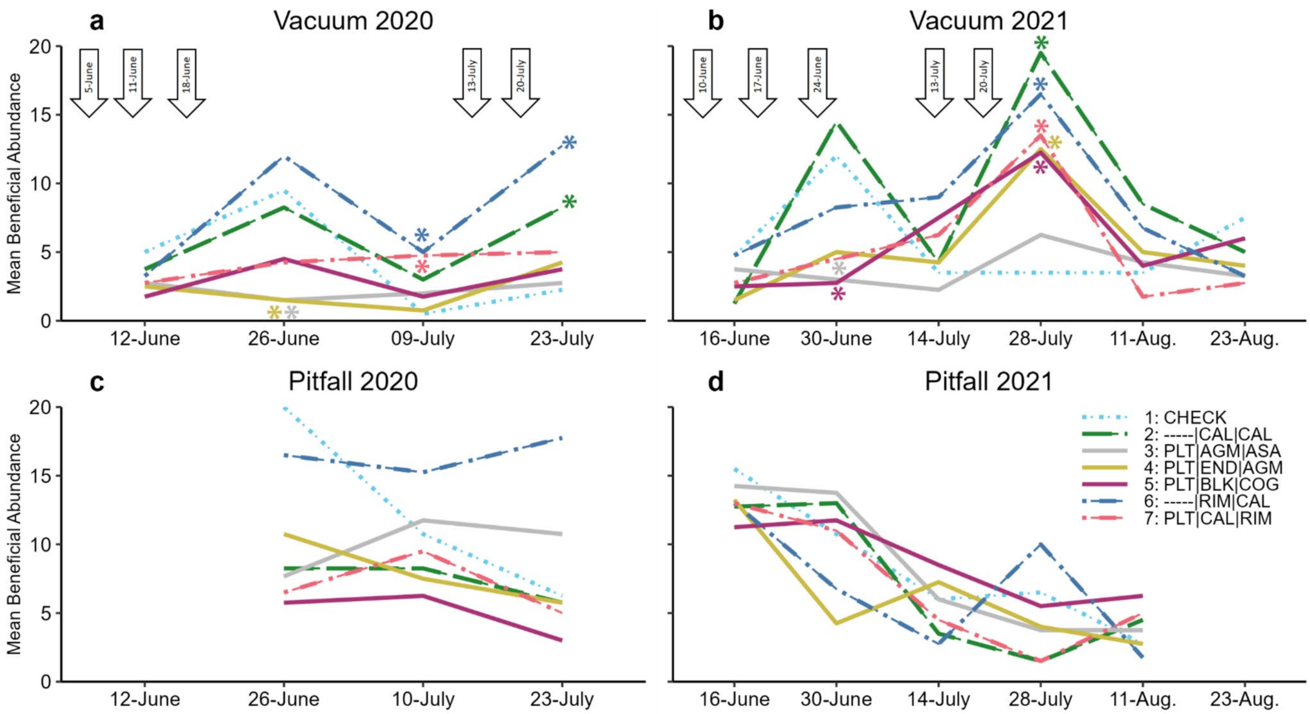

Fig. 6 Mean abundance per plot of beneficial arthropods in Wisconsin compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

vacuum samples (Table S1). In 2020, total abundance was significantly lower than the check in all treatments with at-plant insecticide (treatments 3, 4, 5, and 7; Table S11). In 2021, abundance was lower in all treatments relative to the check (Table S11). Total abundance of arthropods from pitfall traps differed among treatments in the aggregate model for 2020, but not 2021 (Table S1). Abundance was lower than the check in treatments 2, 4, 5, and 7 (Table S11).

Maine Overview

In Maine, overall captures were generally higher in pitfall traps, with the exception of the pests (Table 3). The most abundant functional group varied by year, but in 2020 neutral arthropods collected in pitfall traps showed unusually high counts (Table 3) with springtails (16%), mites (24%), and flies (44%) dominating that dataset (supplementary data). We observed greater abundance of arthropods in 2020 than 2021 for all groups and both collection methods (Table 3). For the beneficial taxa, 69% were predators, 26% were beneficial phytophagous species, 5% were parasitoids; pollinators and others made up less than 0.01% (supplementary data).

Maine Beneficials

Abundance of beneficial arthropods from vacuum samples in Maine did not differ among treatments nor by the interaction term in 2020 (Table 6; Fig. 11a; Table S12); in 2021, low captures meant that only the aggregate model could be run (see Maine Aggregate Models, below). Abundance of pitfall-trapped beneficial arthropods in Maine did not differ by the treatment nor interaction term in either year (Table 6; Fig. 11c-d; Table S12).

Maine Neutrals

Abundance of neutral arthropods from vacuum samples in Maine differed only by the interaction effect in 2020 (Table 6); treatments 5 and 6 showed higher captures than the check on the sample date between the second and third spray (Fig. 12a). In 2021, only the aggregate model could be run (see Maine Aggregate Models, below). For pitfall traps, abundance of neutral arthropods did not differ by treatment nor the interaction term in 2020, nor by the treatment effect in 2021; however, there was a significant interaction effect in 2021 (Table 6; Fig. 12c-d). Treatments 3 and 5 were each lower than the check on one date (Fig. 12d).

Table 5 Generalized linear mixed models evaluating treatment responses of different ecological guilds collected from vacuum and pitfall samples in Wisconsin in 2020 and 2021

Source of Variation	Vacuum						Pitfall					
	2020			2021			2020			2021		
	df	χ^2	*P*-value	df	χ^2	*P*-value	df	χ^2	*P*-value	df	χ^2	*P*-value
Beneficial abundance												
Date	3	19.4	< 0.001	5	16.6	0.005	2	8.9	0.012	4	8.9	0.064
Treatment	6	4.8	0.573	6	12.1	0.059	6	13.1	0.041	6	0.53	0.998
Date × Treatment	18	33.4	0.015	30	86.3	< 0.001	12	12.0	0.448	24	26.0	0.356
Neutral abundance												
Date	3	111.0	< 0.001	5	151.0	< 0.001	2	1.5	0.480	4	13.4	0.010
Treatment	6	15.5	0.017	6	29.3	< 0.001	6	19.1	0.004	6	2.0	0.918
Date × Treatment	18	62.7	< 0.001	30	127.0	< 0.001	12	14.7	0.258	24	20.6	0.662
Pest abundance												
Date	3	64.8	< 0.001	5	113.0	< 0.001	2	0.21	0.902	4	22.4	0.022
Treatment	6	107.0	< 0.001	6	11.0	0.088	6	16.4	0.012	6	8.0	0.239
Date × Treatment	18	77.6	< 0.001	30	203.0	< 0.001	12	13.3	0.349	24	22.6	0.544
Beetle abundance												
Date	—	—	—	—	—	—	2	9.4	0.009	4	6.6	0.159
Treatment	—	—	—	—	—	—	6	16.9	0.010	6	3.1	0.798
Date × Treatment	—	—	—	—	—	—	12	17.5	0.131	24	19.8	0.706
Total												
Date	3	75.7	< 0.001	5	155.0	< 0.001	2	0.04	0.982	4	14.5	0.006
Treatment	6	60.6	< 0.001	6	57.2	< 0.001	6	16.5	0.011	6	2.9	0.823
Date × Treatment	18	67.1	< 0.001	30	251	< 0.001	12	11.5	0.488	24	29.0	0.220

In several cases, full models could not be run due to low captures; see aggregate models (Table S1)

Maine Pests

In 2020 abundance of pests captured in vacuum samples in Maine did not differ by treatment nor the interaction effect (Table 6; Fig. 13a; Table S14). In 2021, a large proportion of zeros in the vacuum dataset meant that only the aggregate model could be run (see Maine Aggregate Models, below). For the pitfall traps, we did not observe a significant treatment effect for either year, but the interaction term was significant in 2021 (Table 6; Fig. 13c-d; Table S14). However, no treatment differed significantly from the check on any date (Fig. 13d).

Maine Beetles

Low captures of beetles from vacuum samples in Maine meant that only aggregate models could be evaluated in 2020 (see Maine Aggregate Models, below), and no statistical analyses could be conducted in 2021 due to extremely low counts (Table 6; Fig. 14a-b; Table S15). Beetle abundance from pitfall traps did not differ by treatment either year, but for 2021 the interaction term was significant (Table 6; Fig. 14c-d). However, we observed no pairwise differences between the check and the other treatments on any date (Fig. 14d).

Maine Total Arthropods

Total arthropod abundance from vacuum samples differed significantly among treatments only in 2021 and did not differ by the interaction term either year (Table 6; Fig. 15a-b). Total abundance in 2021 was significantly lower than the check in all treatments that included at-plant insecticide (treatments 3, 4, 5, and 7; Table S16). These patterns mirrored those observed in models of pests collected in vacuum samples. Total arthropod abundance in pitfall traps in Maine did not differ among treatments nor by the interaction term either year (Table 6; Fig. 15c-d; Table S16).

Maine Aggregate Models

An influential outlier from one plot in the beneficial vacuum data in 2020 was removed and the model was refitted. The aggregate models for beneficial arthropods from vacuum samples showed a significant difference among treatments in both years (Table S1). In 2020, treatment 6 was significantly higher than the check, and in 2021 treatment 5 was significantly lower than the check (Table S12). The pitfall trap samples did not differ among treatments either year (Table S1; Table S12).

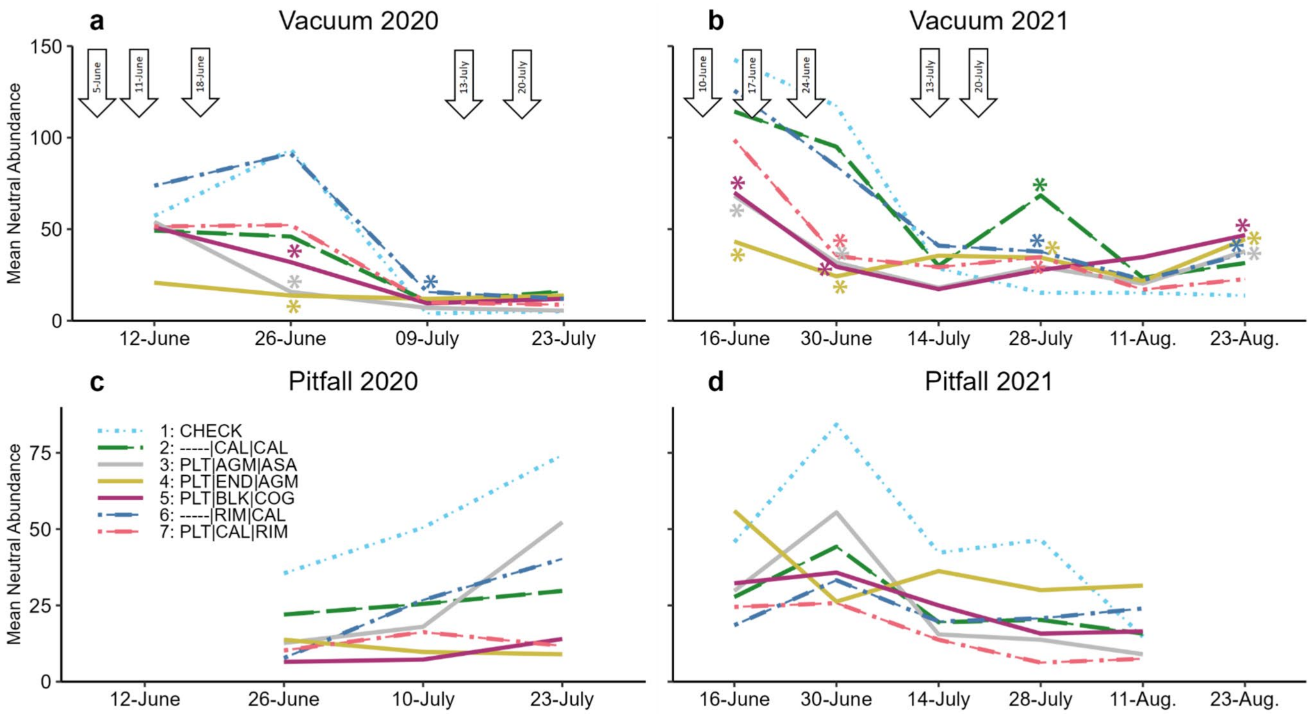

Fig. 7 Mean abundance per plot of neutral arthropods in Wisconsin compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

Abundance of neutral arthropods in vacuum samples in Maine differed among treatments only in 2021 (Table S1); however, no treatment differed significantly from the non-treated check (Table S13). Abundance of neutral arthropods from pitfall traps did not differ among treatments either year (Table S1; Table S13).

Aggregate models of pests collected in vacuum samples in Maine showed a significant treatment effect both years (Table S1). In 2020, more pests were observed in treatment 2 relative to the check; in 2021, fewer pests were observed in the four treatments with at-plant insecticide (Table S14). For pitfall trap samples, the treatment effect for aggregate models was significant in 2021, but not 2020 (Table S1); however, no treatments differed significantly from the non-treated check (Table S14).

Beetle captures from vacuum samples did not differ among treatments in 2020 and too many zeros in the 2021 dataset prohibited analysis (Table S1; Table S15). No significant treatment effect was observed for aggregate models of pitfall-trapped beetles (Table S15; Table S15).

Aggregate models for total captures in Maine showed a significant treatment effect only for vacuum samples in both years (Table S1). Although no treatment differed significantly from the check in 2020, all four treatments with at-plant insecticide showed lower total arthropod abundance than the check in 2021 (Table S16). These patterns were similar to those observed for pest responses. Aggregate models for total captures in pitfall traps showed no differences among treatments (Table S1; Table S16).

Discussion

The results presented here support the hypothesis that Calantha has little or no direct effects on non-target arthropods (Table S17). Abundance of beneficial arthropods in the treatment featuring Calantha-only sprays (treatment 2) was never lower than that in check plots across both years and all three sites. Similarly, abundance of neutral arthropods was never lower in treatment 2 relative to check plots save for one sample date in Idaho (vacuum samples in 2020) and for the aggregate model of Wisconsin pitfall trap samples in 2021. For the former case, overall abundance of this group was relatively low on this date, and this may have been a spurious difference. In the latter case, abundance of neutral arthropods was lower than the check in all treatments, possibly related to higher activity density in defoliated check plots (Lang 2000). Other beetles—the group expected to be most likely affected by Calantha due to taxonomic

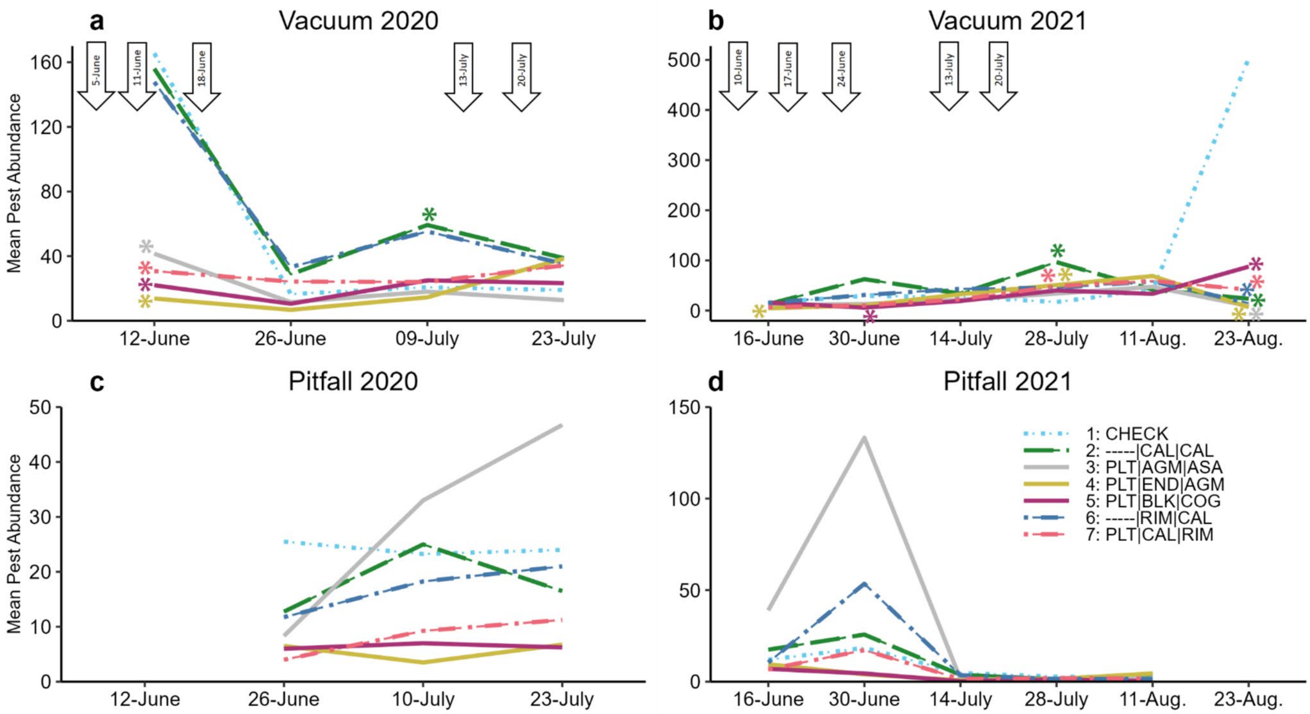

Fig. 8 Mean abundance per plot of pest arthropods in Wisconsin compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

relatedness—never exhibited any evidence of response to Calantha treatments.

This is the first field study to evaluate effects of Calantha on non-target arthropods. At least one study has shown some non-target effects of other dsRNA technologies: a dsRNA-insecticide targeting Western corn rootworm (*Diabrotica virgifera virgifera* (LeConte) exhibited some non-target effects on other beetles in the same subfamily, but not in any other tested species (Bachman et al. 2013). Most studies evaluating non-target effects of dsRNA on other species show no impact on non-target arthropods (Castellanos et al. 2022; Pampolini and Rieske 2020). Laboratory trials exposing Chrysomelinae species to Calantha directly were conducted as part of the EPA review process and showed little or no effects on those species (B. Manley, data not shown), further supporting the lack of effects on non-target beetles observed here.

The lack of adverse effects of Calantha on non-target beneficials, neutrals, and coleopterans is encouraging; however, the extremely taxon-specific effects of Calantha on the Colorado potato beetle were underscored in some cases by greater abundance of pests in the Calantha-only treatment relative to some treatments with conventional insecticides. Extremely high abundance of potato flea beetles in Wisconsin, for example, was observed in treatments that lacked an at-plant neonicotinoid insecticide. Abundance of pests was more clearly and consistently reduced in treatments that featured Calantha within a rotation of other insecticides, especially treatment 7. It is worth noting that the insecticides tested here were aimed primarily at the Colorado potato beetle, and the rotations were fixed prior to the initiation of the study. A more nimble IPM program that adjusted insecticide applications for other pests in addition to the Colorado potato beetle based on scouting could have better managed those other pests while mitigating effects on non-target arthropods.

Incorporating Calantha into an overall program targeting a diversity of arthropod pests in the potato crop would make sense from both an IPM and an insecticide resistance management standpoint. Though a new taxon-specific mode of action is a welcome addition to the tools available to manage the Colorado potato beetle, we caution that swapping out conventional insecticides with dsRNA-based bioinsecticides will not alone address insecticide resistance concerns. Indeed, development of resistance to a dsRNA targeting V-ATPaseA in the Colorado potato beetle was recently demonstrated under artificial selection conditions in the laboratory (Mishra et al. 2021). Although lab conditions can never fully replicate conditions in the field, dsRNA technology must be thoughtfully implemented as part of an IPM program if it is to remain an effective tool for potato growers.

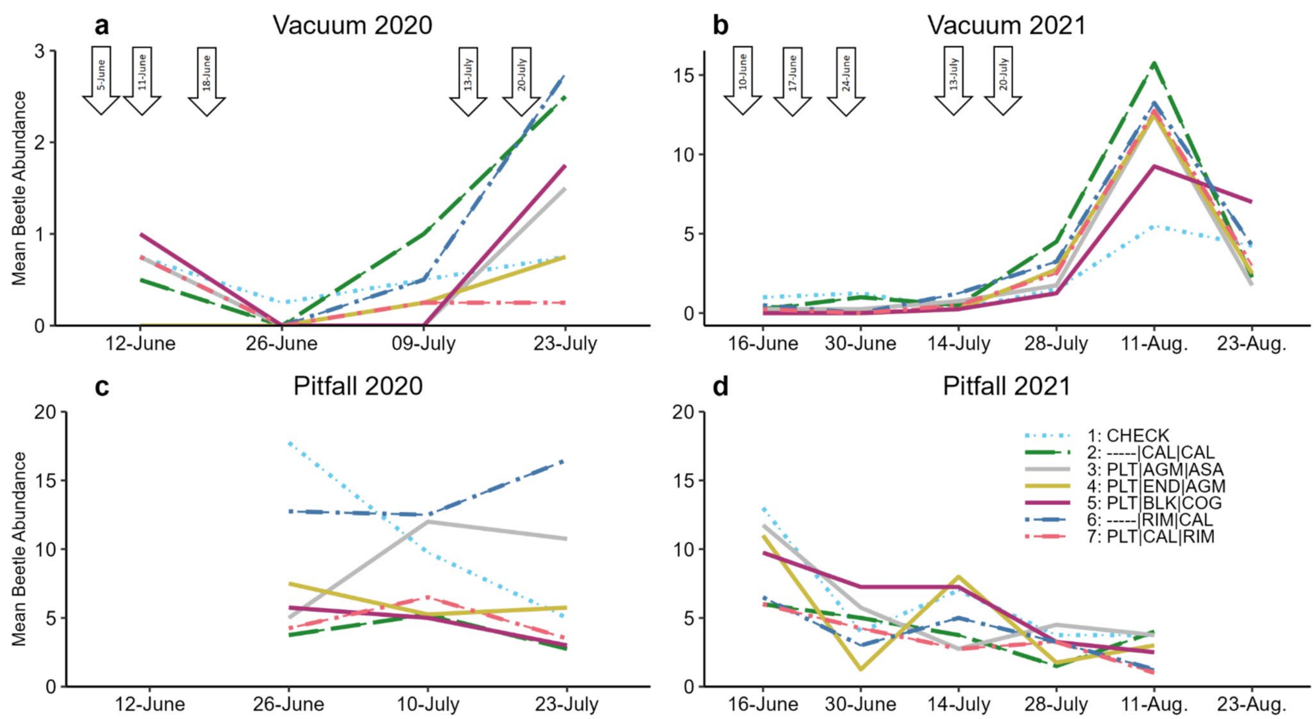

Fig. 9 Mean abundance per plot of beetles in Wisconsin compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

One of the primary benefits of using insecticides with more targeted modes of action like Calantha is the preservation of arthropod natural enemies that can contribute to conservation biological control. The role of natural enemies in contributing to biological control of insect pests in potato, or indeed in any crop, is difficult to quantify; however, a diversity of predators and parasitoids is known to attack the Colorado potato beetle with varying degrees of specificity. For example, in North America two species of tachinid flies are specialist parasitoids on the Colorado potato beetle: *Myiopharus aberrans* (Townsend) and *M. doryphorae* (Riley) (Lopez et al. 1997, Weber et al. 2022) and a species of carabid beetle, *Lebia grandis* Hentz, is both a parasitoid (as larvae) and predator (as adults) on the Colorado potato beetle as well as the false potato beetle, *L. juncta* Germar (Weber et al. 2006, 2022). In addition, predatory stink bugs, including a specialist on chrysomelid beetles, *Perillus bioculatus* (Fabr.), may strongly reduce Colorado potato beetle eggs in the field (Cloutier and Bauduin 1995; Hough-Goldstein 1998). More generalist predators, including lady beetles, predatory true bugs, predatory ground beetles, soldier beetles, and harvestmen also have been shown to prey upon the Colorado potato beetle (Szendrei et al. 2010; Alvarez et al. 2013; Weber et al. 2022). In our study, none of the natural enemy species that specialize on the Colorado potato beetle could be confirmed from our samples given the genus-level taxonomic resolution of identifications of these groups; however, any such specialists were exceedingly rare if present at all. We captured only two specimens of *Lebia* sp. in Idaho and one in Wisconsin (supplementary data); no other specimens within the genera of specialist natural enemies were collected across the study. Thus, any biological control services against the Colorado potato beetle at these sites were provided by more generalist natural enemy species.

In addition to evaluating the effects of Calantha on non-target arthropods, our study allowed us to explore the effects of conventional insecticide rotations on beneficial arthropods. Not surprisingly, broad-spectrum insecticide treatments were associated with lower abundance of beneficial arthropods. In all cases (save for one date in Idaho vacuum samples) for which a significant difference was observed with respect to beneficial arthropod abundance, the only significant pairwise differences versus the check were with one or more of the treatments featuring an at-plant neonicotinoid; these reductions were most prevalent in treatments that also included broad-spectrum foliar insecticides (treatments 3 and 4). Treatments 5 and 7, which included the at-plant neonicotinoid but used more targeted foliar treatments, showed fewer instances of reduced abundance of beneficial arthropods. These results were consistent with previous

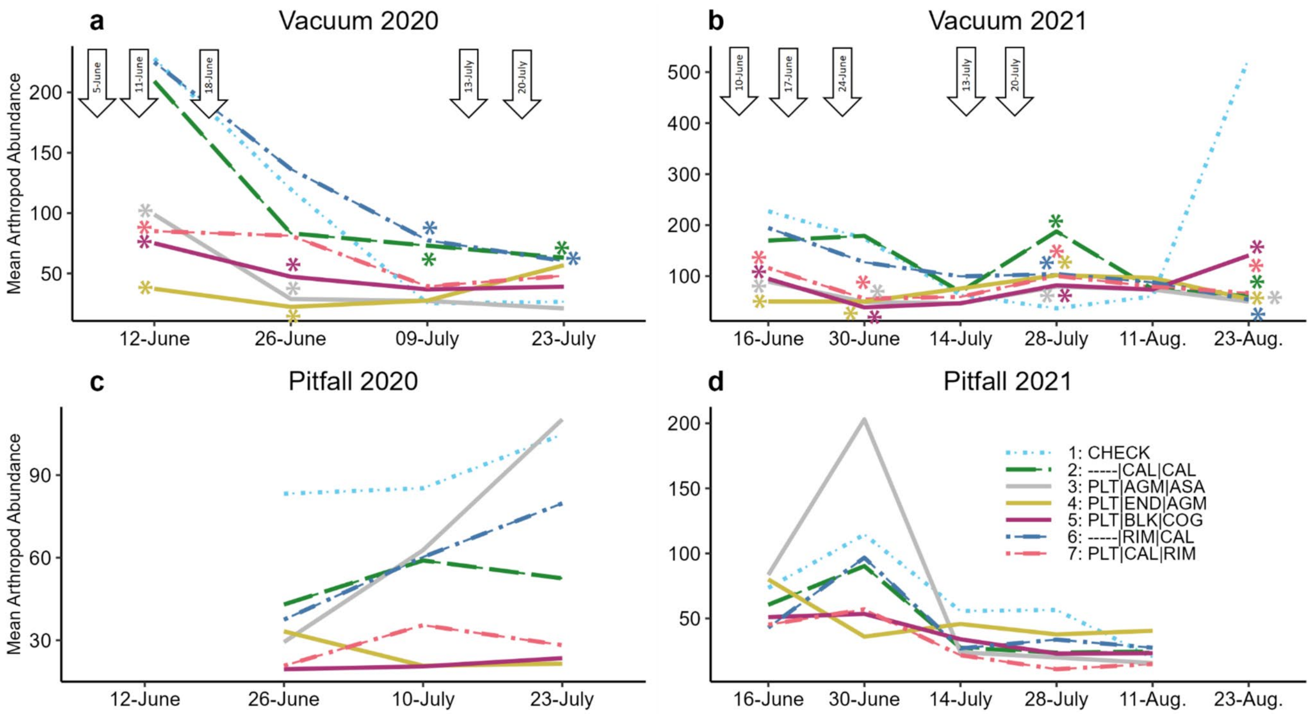

Fig. 10 Mean abundance per plot of total arthropods in Wisconsin compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

Table 6 Generalized linear mixed models evaluating treatment responses of different ecological guilds collected from vacuum and pitfall samples in Maine in 2020 and 2021

Source of Variation	Vacuum						Pitfall					
	2020			2021			2020			2021		
	df	χ^2	*P*-value	df	χ^2	*P*-value	df	χ^2	*P*-value	df	χ^2	*P*-value
Beneficial abundance												
Date	4	5.8	0.217	—	—	—	3	25.8	< 0.001	3	4.8	0.187
Treatment	6	8.4	0.212	—	—	—	6	10.1	0.122	6	3.2	0.790
Date × Treatment	24	21.6	0.601	—	—	—	18	16.7	0.545	18	21.9	0.237
Neutral abundance												
Date	4	13.5	0.009	—	—	—	3	13.8	0.003	3	13.2	0.004
Treatment	6	3.9	0.696	—	—	—	6	9.7	0.139	6	3.8	0.699
Date × Treatment	24	40.5	0.019	—	—	—	18	17.3	0.503	18	29.4	0.044
Pest abundance												
Date	4	21.6	< 0.001	—	—	—	3	14.6	0.002	3	4.7	0.194
Treatment	6	4.1	0.662	—	—	—	6	0.38	0.999	6	10.2	0.117
Date × Treatment	24	15.8	0.894	—	—	—	18	15.4	0.631	18	44.3	< 0.001
Beetle abundance												
Date	—	—	—	—	—	—	3	13.4	0.004	3	19.1	< 0.001
Treatment	—	—	—	—	—	—	6	3.2	0.786	6	5.1	0.531
Date × Treatment	—	—	—	—	—	—	18	27.9	0.063	18	29.1	0.048
Total												
Date	4	16.2	0.003	2	6.3	0.042	3	23.3	< 0.001	3	11.3	0.010
Treatment	6	8.3	0.217	6	14.1	0.029	6	8.19	0.224	6	2.1	0.915
Date × Treatment	24	29.4	0.205	12	10.7	0.551	18	13.2	0.778	18	25.6	0.111

In several cases, full models could not be run due to low captures; see aggregate models (Table S1)

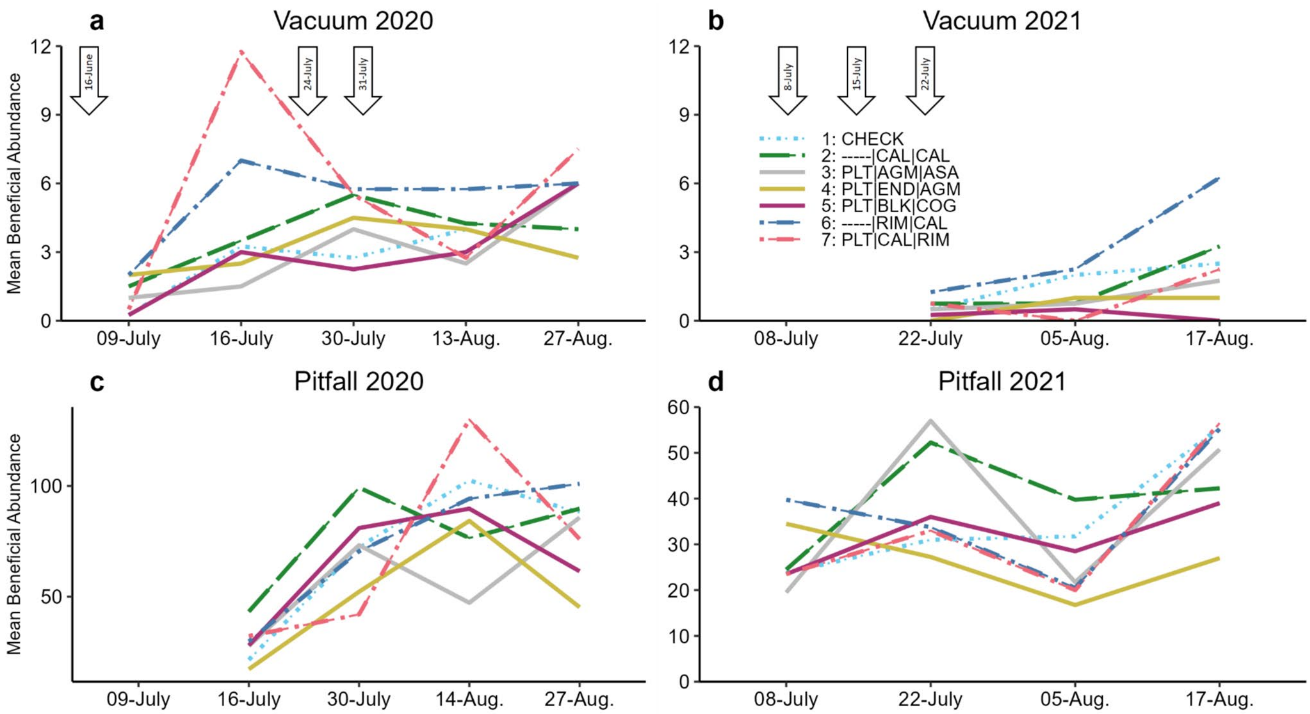

Fig. 11 Mean abundance per plot of beneficial arthropods in Maine compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

studies in potato in which broad-spectrum insecticides have been observed to reduce beneficial arthropods (Alvarez et al. 2013; Chapman 2003; Koss et al. 2005; Metcalf 1980; Radkova et al. 2017; Reed et al. 2001). Although our experimental design did not allow for direct comparison of the same foliar insecticide rotation with and without an at-plant neonicotinoid, our results support the idea that the foliar insecticides contributed substantively to these reductions in beneficial arthropods. Across locations and ecological guilds, we observed stronger responses in vacuum relative to pitfall samples. Also, the timing of reductions in beneficial abundance often, though not always, followed foliar insecticide applications. Aside from direct contact with foliar sprays, exposure pathways might have included ingestion of prey contaminated with foliar and/or at-plant insecticides (Douglas et al. 2015; Szczepaniec et al. 2011). For some natural enemies, exposure may also come from ingestion of pollen, nectar, or other plant products (Lundgren 2009; Moser and Obrycki 2009). Such routes of exposure are likely of less concern in potato given that potato flowers do not produce nectar; however, the ecotoxicology of potato pollen and the extent to which predators and parasitoids use this resource in potato crops is understudied. Though some bumble bees have been reported to visit potato flowers (Batra 1993; Buchanan et al. 2017), the lack of nectar limits the value of potato flowers as a pollinator resource. Indeed, across the three locations and two years of the current study, we collected a total of only 62 bees: 3 in Idaho, 50 in Wisconsin, and 9 in Maine (supplementary data).

Though reductions in beneficial arthropods in certain treatments almost certainly can be attributed to insecticides, other factors could affect distribution and abundance of predators and parasitoids, including distributions of prey (Wenninger et al. 2020). More mobile natural enemies may be expected to be found in higher abundance in plots with greater abundance and diversity of pests. In Wisconsin, where defoliation from Colorado potato beetles was more severe in non-treated plots, reduction of host plant quality likely caused a cascade of decreasing abundance of other herbivores and their natural enemies. Moreover, in a few cases significant treatment effects were observed at the end of the season more than one month after the last foliar spray; such differences can likely not be attributed directly to the insecticide treatments. More work would be needed to clarify the relative contributions of the many other factors influencing abundance of beneficial arthropods in this system.

Agricultural studies investigating non-target effects of insecticides understandably often focus on beneficial arthropods. For the neutral arthropods, which in large part consisted of taxa that may not interact directly with

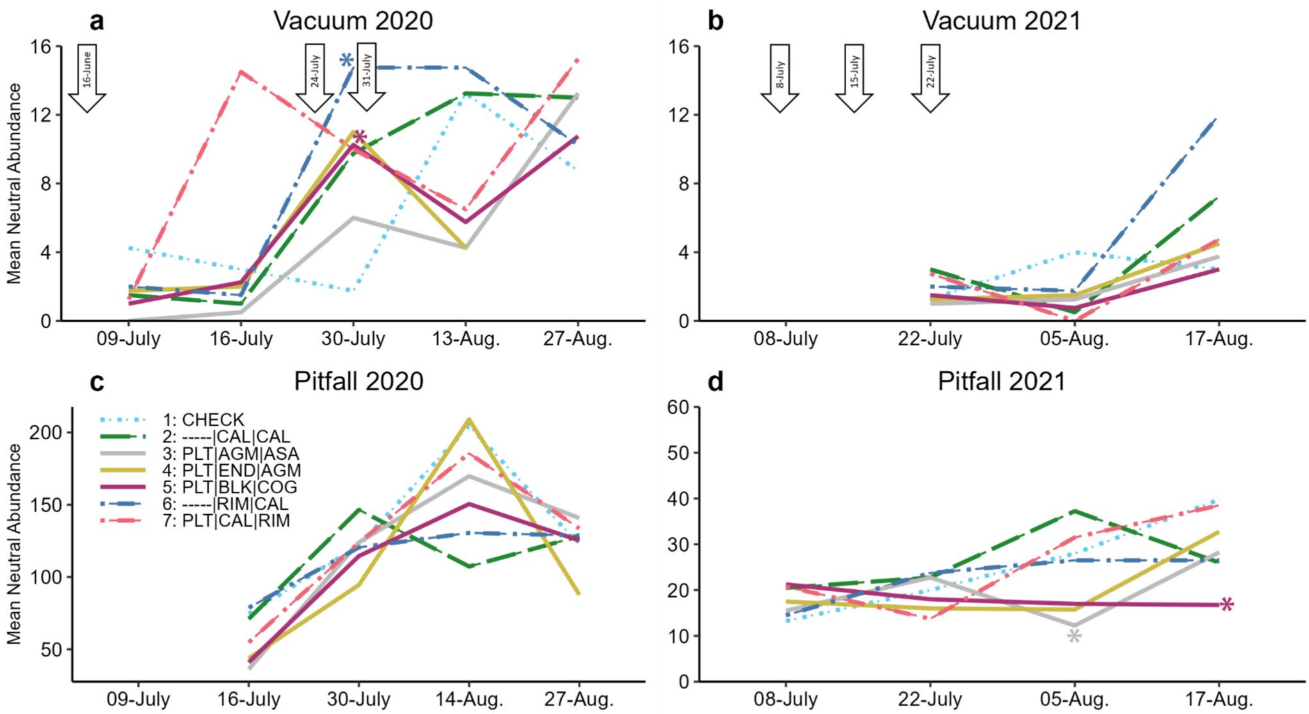

Fig. 12 Mean abundance per plot of neutral arthropods in Maine compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

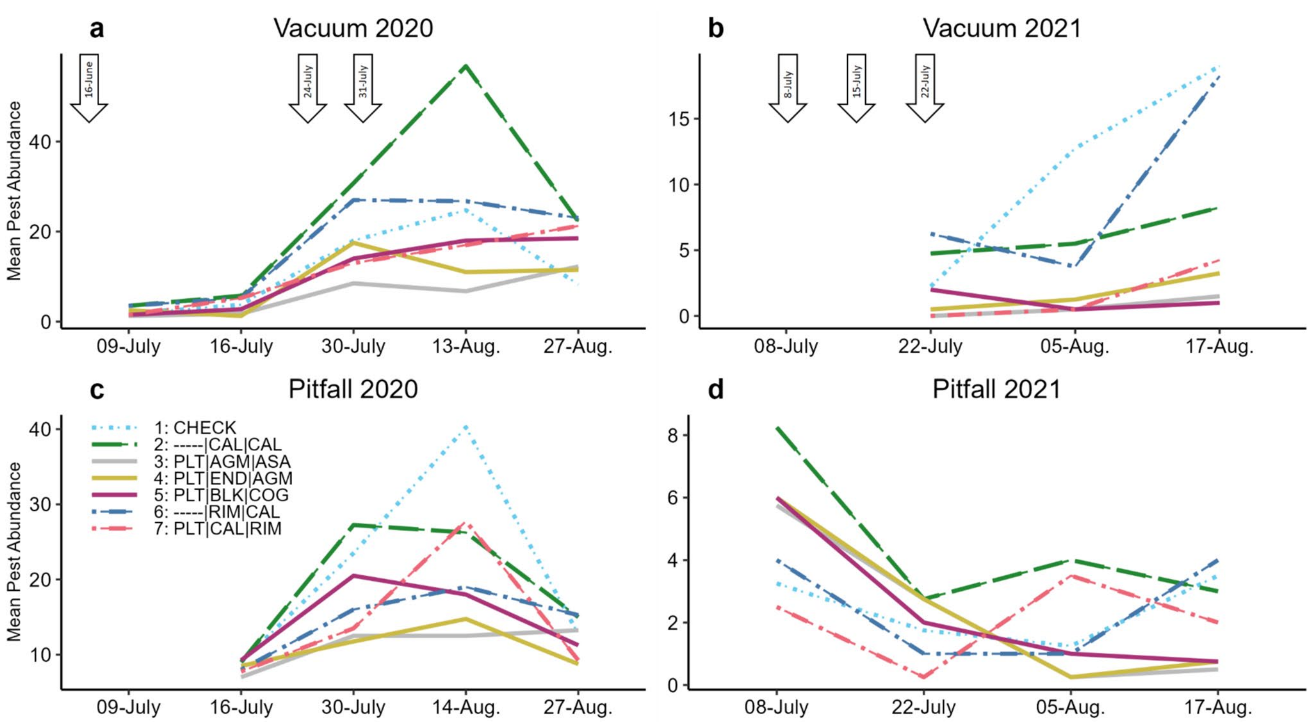

Fig. 13 Mean abundance per plot of pest arthropods in Maine compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

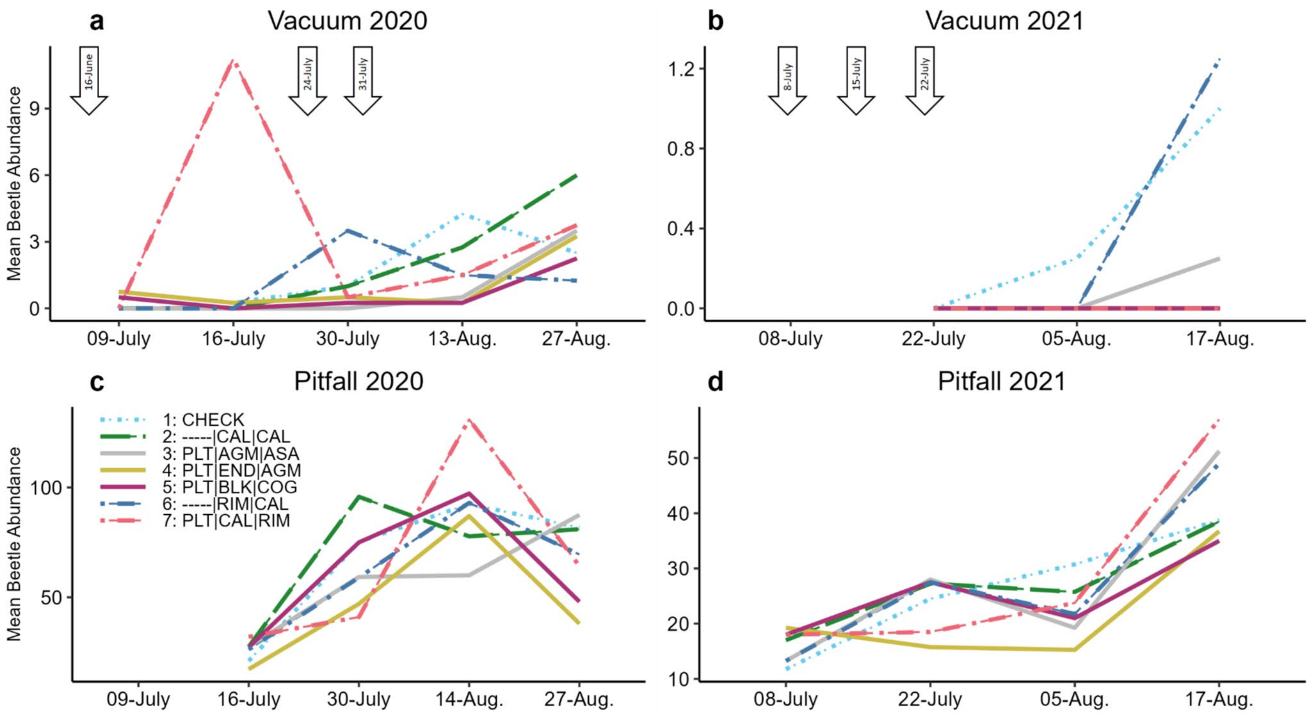

Fig. 14 Mean abundance per plot of beetles in Maine compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

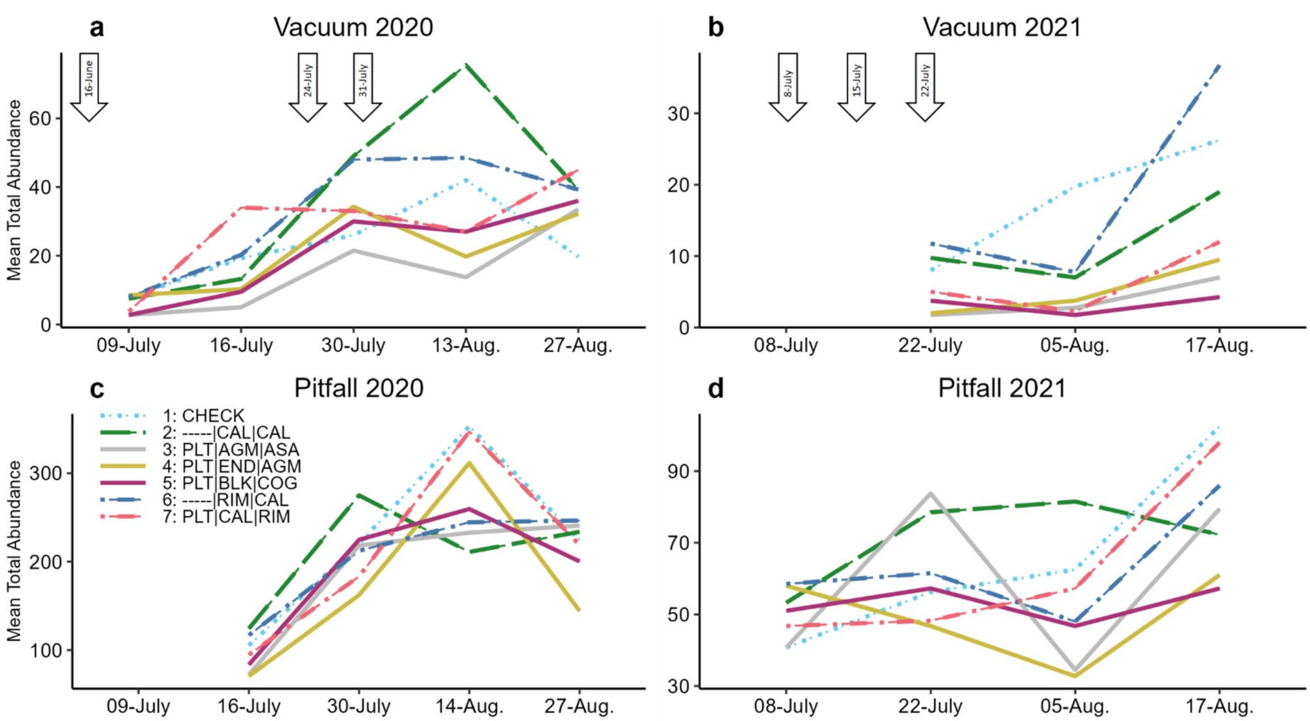

Fig. 15 Mean abundance per plot of total arthropods in Maine compared among treatments over time from (**a**) vacuum samples in 2020, **b** vacuum samples in 2021, **c** pitfall traps in 2020, **d** pitfall traps in 2021. Color-coded asterisks indicate significant differences between a treatment and the non-treated check on that date. Treatment trade names are abbreviated (see Table 2 for full treatment details). Arrows indicate spray dates

the potato crop—including aquatic insects, detritivores, saprophages, and certain members of the soil biota—responses to treatments were generally similar to those of the pest and beneficial taxa from a given year and collection method. About half of all models for neutral arthropods from pitfall samples did not show significant treatment nor date × treatment interactions, and those that did rarely showed significant differences relative to the non-treated check; the sole exception was from Wisconsin in 2020. This may indicate that the ground-dwelling taxa captured in pitfall traps had less exposure to the foliar insecticides than did the arthropods in the foliage. Indeed, vacuum-sampled arthropods often showed reduced abundance in broad-spectrum insecticide treatments, though the patterns were somewhat complicated; for instance, in Wisconsin in 2021, neutral arthropods were less abundant than the check in the broad-spectrum insecticide treatments early in the season and more abundant later in the season. Although the neutral taxa may not be directly relevant to agriculture, many species play broader ecological roles that are important for other industries or the environment at large; for example, chironomids are an important part of aquatic food webs (Armitage 1995; Nath et al. 2021). Thus, the reduction of neutral arthropods by broad-spectrum insecticides is still concerning and worth noting here and further underscores the importance of developing insecticides with reduced off-target effects.

Our results demonstrate the value of incorporating Calantha into a rotation of insecticides targeting specific arthropod pests in potato production while reducing non-target effects and promoting biodiversity in this agroecological system. Further, this new taxon-specific mode of action coupled with an IPM approach to management of the Colorado potato beetle can help to prevent or delay the development of resistance to Calantha and other insecticides. Incorporation of Calantha into potato pest management programs should contribute to preservation of beneficial arthropods which should support conservation biological control. In addition to preserving beneficials, our results show no evidence for effects of Calantha on neutral or coleopteran arthropods, further supporting its value as a tool for managing the Colorado potato beetle while supporting biodiversity in potato production, an agricultural system with increasing focus on integrated approaches to pest management with minimal off-target effects.

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Declarations.

Author Contributions All authors contributed to the study conception, design, and analysis. Data collection and collation was performed by EJW, SPD, AA, BB, and RLG. Arthropod identification was performed by JI and EK. Data analyses were led by SPD, JP, and EJW. The first draft of the manuscript was written by EJW and SPD; all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The experiments described comply with the current laws of the United States.

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Data Availability Raw data are available in Supplementary Material.

Declarations

Competing Interests EJW, JI, EK, AA, and RLG received funding from GreenLight Biosciences to conduct this study. ERB and BM were employed by GreenLight Biosciences while this study was conducted.

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