

# Asymmetric effects of a leaf-chewing herbivore on aphid population growth

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**Abstract.** 1. Plant responses to herbivory are often specific to the feeding guild of the attacking herbivore. These phytochemical responses to herbivore damage can affect herbivore performance and activity. Comprehensive studies on the ecological consequences of multi-herbivore plant interactions are key to understanding plant–herbivore community dynamics.

2. This study examined how feeding damage by co-occurring herbivores from separate feeding guilds, *Myzus persicae* (Sulzer), a sucking herbivore, and *Leptinotarsa decemlineata* (Say), a chewing herbivore, alter plant chemistry and indirectly affect herbivore performance. Performance was measured when each insect fed on plants individually, sequentially, or simultaneously in laboratory and field experiments. Phytohormone and glycoalkaloid content were measured for each feeding sequence to evaluate plant responses to herbivory by each guild. Mid-season and end-of-season tuber yield were evaluated in the field study.

3. Damage by *L. decemlineata* negatively impacted *M. persicae* performance in both laboratory and field settings. Damage by *M. persicae* did not affect *L. decemlineata* performance in laboratory assays. However, *L. decemlineata* performance was positively affected by *M. persicae* herbivory in the field, but this effect was temporary. Although phytohormones and plant defences varied across treatments, they provide little resolution on interaction outcomes.

4. These results confirm that the presence of multiple feeding guilds on a single plant can affect these chewing and sucking herbivores differentially, but given the variability in our phytochemical analyses compared with other studies, the mechanism remains unclear. The study's findings show that aphids are negatively affected by chewing herbivores across systems, while aphids temporarily affected beetles positively.

**Key words.** aphids, beetles, dual herbivory, feeding guild interactions, induced plant resistance, jasmonic acid, plant-mediated interactions, salicylic acid.

## Introduction

Herbivores can interact in a variety of ways, both directly (e.g. competition for shared resources) and indirectly (e.g. plant-mediated responses) (Denno *et al.*, 1995; Lynch *et al.*, 2006). One way that indirect interactions can arise is through feeding induced changes in plant chemistry (Levin, 1976; Faeth, 1986; Rausher *et al.*, 1993; Agrawal, 1998, 1999; Lynch *et al.*, 2006). Herbivore-inducible defences can be deployed locally, at

the site of herbivore damage, or systemically in undamaged plant tissues (Karban & Baldwin, 1997) with immediate and/or persisting effects (Thaler *et al.*, 2001; Kaplan *et al.*, 2008). Therefore, herbivores that share a particular host plant can interact indirectly even when they are spatially or temporally distant (Brunissen *et al.*, 2009), and the co-occurrence or sequential occurrence of multiple herbivores could have varied effects on herbivore performance (Kaplan *et al.*, 2009; Soler *et al.*, 2012; Ali & Agrawal, 2014).

Plant responses to biotic and abiotic stresses are dynamic, and plants have evolved multiple defence strategies in response to diverse herbivore attacks. Herbivores sharing physiological and morphological characteristics, such as mouthpart structures,

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salivary components, feeding location or frequency, and activity period are grouped into feeding guilds based on these traits, or combinations of these traits (Heidel & Baldwin, 2004; Novotny *et al.*, 2010). Recognition of an attacking herbivore's feeding guild enables plants to allocate resources efficiently and tailor defence responses specific to the attacking herbivore (Hlywka *et al.*, 1994; Walling, 2000; Rodriguez-Saona *et al.*, 2005; Mewis *et al.*, 2006; Howe & Jander, 2008; Erb *et al.*, 2012; Soler *et al.*, 2012). In contrast, herbivore manipulation of plant defences can suppress plant responses, allowing the herbivore to feed without inhibition (Thompson & Goggin, 2006; Zarate *et al.*, 2007; Weech *et al.*, 2008; Diezel *et al.*, 2009; Chung *et al.*, 2013; Züst & Agrawal, 2016).

Feeding guild specific plant responses occur in several systems and there is evidence that plant responses to one herbivore differ from those against multiple attackers (Stout *et al.*, 1998; Heidel & Baldwin, 2004; Mewis *et al.*, 2006; Rodriguez-Saona *et al.*, 2010; Sotelo *et al.*, 2014). The sequence of herbivore damage can also have lasting effects, where damage by a first herbivore can impact subsequent herbivory (Danoff-Burg & Bird, 2002; Erb *et al.*, 2011; Faeth, 1986; Gómez *et al.*, 2012; Kroes *et al.*, 2015; Lu *et al.*, 2016; Lynch *et al.*, 2006; Preisser & Elkinton, 2008; Poelman *et al.*, 2008; Poelman *et al.*, 2010; Soler *et al.*, 2012; Underwood *et al.* 2000; Viswanathan *et al.*, 2007; Wang *et al.*, 2014), yet few studies have focused on reciprocal interactions (but see Ali & Agrawal, 2014; Bezemer *et al.*, 2003; Wang *et al.*, 2014). Many insect herbivores have multiple generations throughout a season and are not restricted to a single sequence of arrival on a shared host plant. Testing reciprocal interactions across multiple systems and under laboratory and field conditions will allow us to better understand how herbivore performance is affected when herbivores from separate feeding guilds co-occur on host plants, and what their combined impact may be on host-plant chemistry and fitness.

Colorado potato beetles (CPBs), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), and green peach aphids (GPAs), *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), are two pests that commonly occur on potato, *Solanum tuberosum* L. (Solanaceae). Colorado potato beetles are foliar-feeding herbivores with chewing mouthparts, while GPAs are phloem-feeding herbivores with piercing-sucking mouthparts. Both pests can cause significant damage to potato crops by reducing plant quality and tuber yield, and in the case of GPAs, by transmitting pathogens (Alyokhin *et al.*, 2013; Saguez *et al.*, 2013).

These herbivores represent morphologically diverse feeding guilds and interact with host plant hormones and associated defences differently. Jasmonic acid (JA) plays a central role in the regulation of wound responses that are often associated with damage by chewing herbivores, such as CPBs, while salicylic acid (SA) signalling is generally activated in response to pathogens and phloem-feeding insects (Thompson & Goggin, 2006; Wu & Baldwin, 2009). However, these pathways serve multiple functions and are not always restricted to a single feeding guild (Paul *et al.*, 2000; Walling, 2000; Bezemer & Van Dam, 2005). Green peach aphids have also been shown to induce JA-associated gene expression in *Arabidopsis thaliana* (Moran & Thompson, 2001; Moran *et al.*, 2002; De Vos *et al.*, 2005), *S. tuberosum* (Gosset *et al.*, 2009), and closely related plants,

such as tomato (Martinez de Ilarduya *et al.*, 2003; Kaloshian, 2004), and CPBs can also induce SA-signalling (Chung *et al.*, 2013). To further complicate these interactions, crosstalk between phytohormone pathways alters defence expression, where induction of one pathway may suppress the other, leading to reduced expression of the defensive traits associated with the suppressed pathway (Kessler & Baldwin, 2002; Zarate *et al.*, 2007; Thaler *et al.*, 2012). Therefore, induction of a pathway by a herbivore from one feeding guild could provide disparate fitness benefits or consequences to a herbivore from a separate guild.

Steroidal glycoalkaloids, such as  $\alpha$ -solanine, are feeding deterrents that contribute to plant resistance against CPBs and GPAs (Hare, 1987; Fragoyiannis *et al.*, 1998). These compounds are constitutively expressed, but can also be induced in response to JA accumulation (Abdelkareem 2017; Thagun *et al.*, 2016). Under heavy infestation, GPA reduces glycoalkaloid concentrations in potato (Fragoyiannis *et al.*, 2001) while severe defoliation by CPBs results in higher glycoalkaloid levels (Hlywka *et al.*, 1994). Knowledge of these interactions, thus far, is based on how each herbivore interacts with potato plants individually. Elevated glycoalkaloids in response to CPB damage could have fitness consequences for GPAs. In contrast, a reduction of  $\alpha$ -solanine after GPA feeding may enhance CPB fitness. Additionally, it is still unknown how glycoalkaloid levels will be affected by simultaneous damage from these two herbivores.

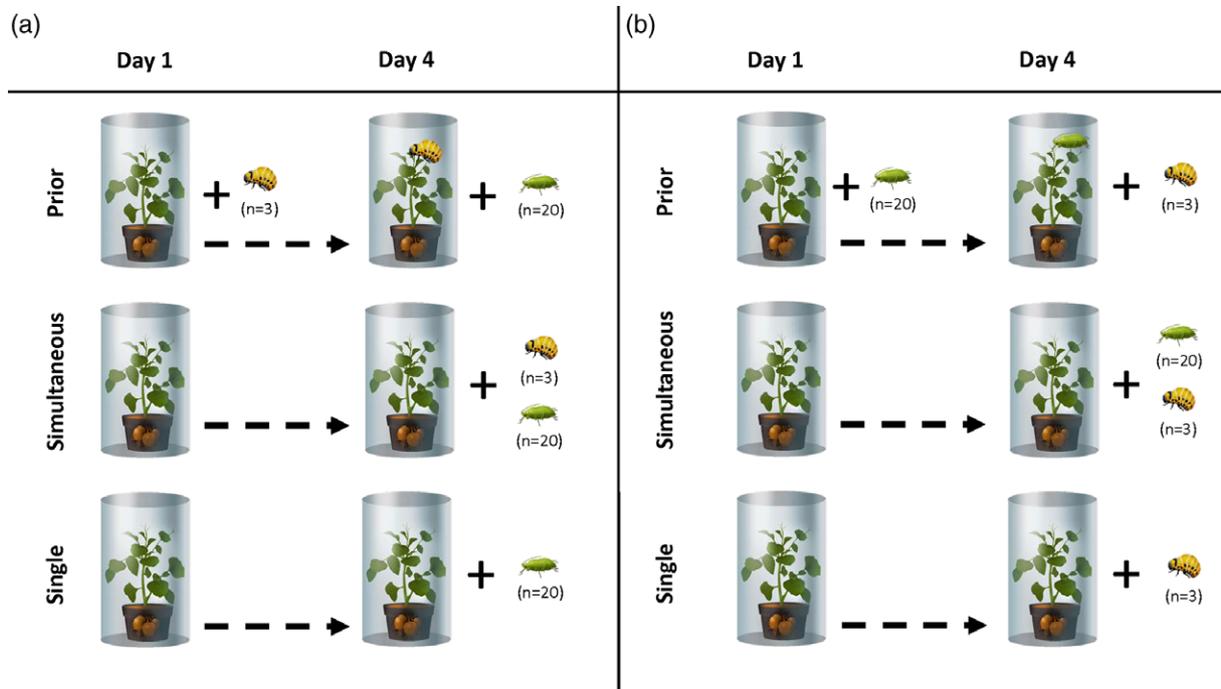
To study reciprocal interactions, we conducted laboratory and field assays to investigate how plant responses to herbivores from one feeding guild affect the performance of simultaneously or sequentially arriving herbivores from a different feeding guild. We measured plant phytohormones and chemical defence responses to elucidate the underlying mechanisms involved in interactions between CPBs and GPAs. Plant yield was assessed in the field study to evaluate the impact of dual herbivory on plant performance.

## Materials and methods

### Laboratory experiments

*Insects and plant material.* Adult and nymph aphids and second-instar CPB larvae were used for laboratory bioassays. Both colonies were maintained on *S. tuberosum* cv. Atlantic at 24–25 °C, 48–52% RH, LD 16:8 h. Colorado potato beetle larvae were collected from multiple egg clutches that hatched on the same day and apterous aphids were randomly selected from the laboratory colony.

Experiments were carried out using 4- to 5-week old *S. tuberosum* cv. Atlantic plants that were propagated from vegetative seed produced by the Montcalm Research Center (Stanton, Michigan). Plants were grown in 10-cm-diameter plastic pots with a perlite soil mix (Suremix Perlite, Michigan Grower Products Inc., Galesburg, Michigan). All plants were grown in a growth chamber maintained at 25–28 °C, 55–58% RH, under LD 16:8 h and fertilised weekly with a water-soluble 20-20-20 (N-P-K) fertiliser (J.R. Peters Inc., Allentown, Pennsylvania) at 250 ppm. Potato plants were placed in individual



**Fig. 1.** Timeline of herbivore additions for green peach aphid (GPA) (a) and Colorado potato beetles (CPB) (b) performance assays. Insects were added in three combinations: prior – with prior herbivore damage from the opposite feeding guild; simultaneous – at the same time as herbivores from the opposite feeding guild; and single – only GPAs or CPBs were added depending on the target herbivore in each assay. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

cages constructed of clear-acetate sheets (0.005-inch thickness, Grafix!, Cleveland, Ohio) to form cylinders (diameter 11.5 cm, height 30 cm). Cages were fitted with fine mesh lids to allow ventilation and watering while preventing herbivore movement between plants. Caged plants were arranged in a completely randomised design within a single growth chamber. The GPA and CPB performance assays were conducted separately under the same laboratory conditions between April and July 2015. All above-ground plant tissue was weighed and plant tissue was sampled at the end of each trial to evaluate phytohormone and glycoalkaloid content.

*Myzus persicae* performance. To evaluate plant response and herbivore performance, aphids were added to plants that were randomly assigned one of the following four treatments (Fig. 1a): (i) prior herbivory: three second-instar CPB larvae were added to 10 plants on day 1, and 20 aphids were added to the same plants on day 4; (ii) simultaneous herbivory: three second-instar CPBs and 20 aphids were added to 10 previously undamaged plants on day 4; (iii) single herbivory (i.e. aphids feeding alone): 20 aphids were added to 10 previously undamaged plants on day 4; and (iv) undamaged controls: 10 plants were left undamaged for the duration of the experiment.

During the first 3 days, plants were checked daily and dead larvae were removed and replaced. To prevent complete defoliation, larvae were removed from the plants 6 days after their initial application. Aphid density per plant was recorded every 4 days for 16 days. Herbivore-free control plants were handled

in the same manner as all other treatments throughout the experiment (i.e. removed from the cage and replaced on each aphid counting day).

*Leptinotarsa decemlineata* performance. A similar bioassay was designed to evaluate plant responses and CPB growth in the presence and absence of GPA damage. The CPB performance assay consisted of the following treatments (Fig. 1b): (i) prior herbivory: 20 aphids were added to 10 plants on day 1, and three second-instar CPB larvae were added to the same plants on day 4; (ii) simultaneous herbivory: 20 aphids and three second-instar CPB larvae were added to 10 previously undamaged plants on day 4; (iii) single herbivory (i.e. larvae feeding alone): three second-instar CPB larvae were added to 10 previously undamaged plants on day 4; (iv) undamaged controls: 10 plants were left undamaged for the duration of the experiment. The herbivores were then left to feed for an additional 5 days, after which larvae were removed and weighed.

Due to their rapid reproductive strategy and cryptic nature, aphids were not removed at any point during these assays. Removing such a large number would be difficult without inflicting unintentional mechanical damage to the plants, which could interfere with defence responses and larval feeding (Haukioja & Niemelä, 1977; Baldwin, 1990; Karban & Baldwin, 1997). Additionally, there is a high risk of missing a single aphid during removal, which could then be left to reproduce parthenogenically (Saguez *et al.*, 2013), further obscuring results.

### Field experiment

**Field site and insects.** We conducted a field experiment to test whether the interactions observed in the laboratory persist under varying environmental conditions and at later, agroecologically relevant plant stages. All experiments were carried out at the Montcalm Research Farm (Stanton, Michigan) during May–September 2015. Seed potatoes (*Solanum tuberosum* cv. Atlantic) were planted in early May 2015. When plants were 6 weeks old, just prior to when naturally occurring GPA and CPB populations arrived, 70 exclusion cages were randomly deployed throughout the field. Cages were constructed using four pieces of plastic pipe (PVC, diameter 2.54 cm) driven into the soil around a single plant to form a 1 m<sup>3</sup> cage. Two flexible wires were crossed over the top of the pipes to support a fine mesh fabric (white polyester, 680 µm mesh aperture; Megaview, Taichung, Taiwan) tent with a drawstring opening at the top. Loop stakes were used to secure the cages in place and the sides of the cages were buried 15 cm below the soil surface to prevent arthropod movement. Neighbouring plants were removed to ensure that only one plant was contained within each cage. Mixed-instar apterous GPA and newly emerged first-instar CPB larvae were collected from multiple egg clutches from the same colonies as described earlier. The number of herbivores added to the plants and the length of herbivory differed from those outlined in the laboratory due to fluctuating environmental conditions and because plants maintained in the field were naturally larger in size than laboratory-grown potato plants, and therefore warranted higher pest pressure.

**Myzus persicae performance.** The same treatments as used in the laboratory were applied to field plants ( $n = 10$  for each herbivore treatment,  $n = 7$  for undamaged control plants) with modifications to the number of herbivores and timing of damage. Five days after the cages were deployed in the field, 20 first-instar CPBs per plant were applied to 10 plants to induce prior herbivore damage. Dead larvae were replaced 2 days later so that each plant had at least 15 larvae. Five days later, 50 GPAs were added to each plant with prior CPB damage. On the fifth day, 50 GPAs were also added simultaneously with 20 CPB larvae to 10 previously undamaged plants to simulate simultaneous damage and to 10 previously undamaged plants where they fed without CPB damage. Aphid densities were counted approximately every 5 days (contingent on weather conditions), for a total of five counts. Plants were 10 weeks old by the final counting date. After the aphids were counted, foliar tissue was collected from a subsample of five plants from each treatment and three control plants for glycoalkaloid and phytohormone analysis, and then the whole plant was harvested to evaluate mid-season yield. The remaining plants were left caged in the field for the end-of-season yield evaluation.

**Leptinotarsa decemlineata performance.** The same treatments as described in the CPB laboratory performance assay were used to assess performance in the field ( $n = 10$  for each treatment) with modifications in the number and timing of herbivore damage. Five days after the cages were deployed in

the field, 50 GPAs per plant were added to 10 plants to induce prior damage. Aphids were left to feed for 5 days, after which 20 CPB larvae per plant were added. On the same day, 20 larvae and 50 aphids per plant were applied to 10 additional undamaged plants to induce simultaneous damage and 20 CPB larvae per plant were added to 10 previously undamaged plants to feed alone. Dead larvae were replaced 2 days later so that each plant had a minimum of 15 larvae. Herbivores were left to feed for an additional 5 days, after which larvae were then removed and weighed. Plants were 8 weeks old at the end of the performance assay. Foliar tissue was sampled for glycoalkaloid and phytohormone analysis from a subset of five plants from each treatment plus three control plants, and then the whole plant was harvested to evaluate mid-season yield. A second performance evaluation was conducted and larvae were removed from the remaining plants 7 days later and weighed in the laboratory to determine whether the initial CPB performance results were consistent over time. The remaining plants were left in the field until the end of season for additional yield comparisons.

### Glycoalkaloid analysis

Foliar tissue (100 mg) was excised at the end of each laboratory bioassay from the youngest undamaged fully expanded leaf. Tissue was frozen in liquid nitrogen and placed in cold storage (−80 °C). Foliar tissue was sampled in the field by removing an upper lateral stem which was then sealed in a plastic storage bag and placed in a cooler with dry ice. Once a sample was collected from all plants, the coolers were transported back to the laboratory. Samples were removed individually from the cooler and 100 mg of tissue were excised for  $\alpha$ -solanine (glycoalkaloid) analysis. The samples were immediately flash-frozen in liquid nitrogen and placed in cold storage (−80 °C). Frozen tissue was transferred into 2-ml screw cap tubes containing 900 mg zirconia/silica beads (BioSpec, Bartlesville, Oklahoma) and 1 ml of extraction solvent (water, methanol and acetic acid, 49:49:2 v/v/v). Samples were homogenised on a FastPrep homogeniser (MP Biomedicals, Solon, Ohio) at 6 m s<sup>−1</sup> for 45 s for two cycles. The samples were then treated in a hot water bath at 60 °C for 30 min, then centrifuged at 18,900 g for 20 min. The supernatant was transferred to 2-ml glass vials and stored at −20 °C.

All samples were analysed at the Michigan State University Mass Spectrometry Core Facility (East Lansing, Michigan) using a Waters Quattro Micro triple quadrupole liquid chromatography-mass spectroscopy (LC–MS) device interfaced to a Shimadzu high-performance liquid chromatography apparatus. Chromatography was performed using a Supelco Ascentis Express C18 column (2.1 mm x 100 mm, 2.7 µm particle size) (Bellefonte, Pennsylvania) with the column oven set to 30 °C. Initial conditions were 90% solvent A (water +0.1% formic acid, v/v)/10% solvent B (acetonitrile) at a flow rate of 0.3 ml min<sup>−1</sup>, followed by a linear gradient to 5% A:95% B at 2 min, hold at 5% A:95% B to 3 min, return to 90% A:10% B at 3.01 min, and then hold at 90% A:10% B until 5 min. Compounds were ionised by electrospray ionization

in positive-ion mode, and mass spectra were acquired using multiple reaction monitoring (MRM). The capillary voltage, extractor voltage, and radiofrequency lens settings were 3.6 kV, 3 V, and 0.1 V, respectively. Cone gas and desolvation gas flow rates were 0 and 800 litres h<sup>-1</sup>, and the source and desolvation temperatures were 150 and 350 °C, respectively. The source cone potentials and collision energies for  $\alpha$ -solanine were 60 and 80 V, respectively. The precursor and product ion masses used for the MRM transitions were 868.45 > 398.35.

#### Phytohormone analysis

An additional 100 mg of fresh tissue was removed from the same leaf, or an adjacent leaf on the same lateral stem, as those used for glycoalkaloid analysis. Leaf tissue was frozen in liquid nitrogen and placed in cold storage (−80 °C) for phytohormone analysis. Foliar tissue was sampled in the field and transported back to the laboratory for processing in the same manner as described earlier in the glycoalkaloid sampling procedure. Jasmonic acid and SA were extracted from the frozen tissue using the same protocol as described earlier for glycoalkaloid analysis, with the following modification to the extraction procedure. Frozen plant tissue was transferred into 2-ml screw cap vials and homogenised with 1 ml extraction solvent (water, methanol and formic acid, 49:49:2 v/v/v). Samples were homogenised, heated, and centrifuged as described earlier. The supernatant was transferred to 2-ml glass vials and stored at −20 °C and analysed at Michigan State University Mass Spectrometry Core Facility.

Extracts containing SA, glycosylated salicylic acid (SAG), JA, and jasmonoyl-isoleucine (JA-Ile) were analysed using a Waters Quattro Premier triple quadrupole LC-MS device interfaced to a Waters Acquity ultraperformance LC apparatus. Chromatography was performed using a Supelco Ascentis Express C18 column (2.1 mm x 100 mm, 2.7  $\mu$ m particle size) with column oven set to 50 °C. Initial conditions were 99% solvent A (water + 0.1% formic acid, v/v)/1% solvent B (acetonitrile) at a flow rate of 0.4 ml min<sup>-1</sup> for 0.5 min, followed by a linear gradient to 70% A:30% B at 1 min, then to 10% A:90% B at 3.5 min, hold at 10% A:90% B to 4.5 min, return to 99% A:1% B at 4.51 min, and then hold at 99% A:1% B until 5 min. Compounds were ionised by electrospray ionization in negative-ion mode, and mass spectra were acquired using MRM. The capillary voltage, extractor voltage, and radiofrequency lens setting were 3 kV, 3 V, and 0 V, respectively. Cone gas and desolvation gas flow rates were 50 and 700 litres h<sup>-1</sup>, and the source and desolvation temperatures were 120 and 350 °C, respectively. The source cone potentials and collision energies, respectively, were as follows: for SA, d4-SA, and JA, 28 and 16 V; for SAG, 15 and 15 V; for d5-JA and JA-Ile, 34 and 10 V. The precursor and product ion masses used for the MRM transitions were 137 > 93 (SA), 140.8 > 96.7 (d4-SA), 209.1 > 59 (JA), 214.1 > 62 (d5-JA), 299 > 137 (SAG), 322.2 > 130.1 (JA-Ile).

#### Yield

For mid-season yield, half of the plants from each treatment were sampled at the end of each bioassay ( $n = 5$  for each

treatment,  $n = 3$  for controls). This reduced overall sample sizes for mid- and end-of-season yield analyses but allowed us to see whether differences in plant fitness could be observed earlier in the season. All remaining plants in the field ( $n = 5$  for each treatment,  $n = 4$  for controls) were harvested at the end of the growing season, 18 weeks later. Above- and below-ground plant material was bagged separately and transported back to the laboratory to be weighed. Whole-plant weight was recorded for mid-season yield calculations, but not for end-of-season calculations because tubers are usually harvested only after above-ground biomass senesces. Total tuber weight and average tuber weight per plant were recorded for both mid-season and end-of-season yield calculations. The simultaneously infested plants from the *M. persicae* performance trial were removed from final yield analysis after several cages were contaminated by field herbivores.

#### Statistical analyses

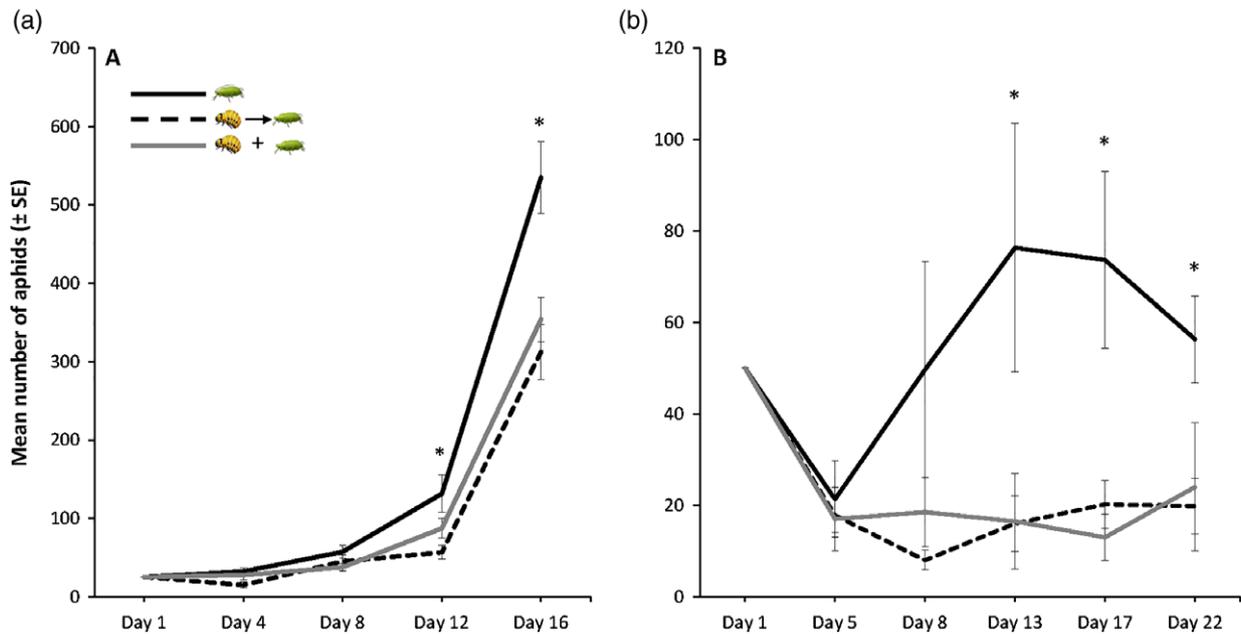
All statistical analyses for laboratory and field trials were completed using JMP (Version 12.1, SAS Institute Inc., Cary, North Carolina). A repeated-measures model on aphid numbers over time was used to test the effects of prior and simultaneous CPB feeding on GPA performance. A one-way ANOVA was used to analyse the impact of GPA feeding on CPB larval weight and to compare the effect of herbivore feeding sequence on JA, SA, JA-Ile, SAG, and  $\alpha$ -solanine, as well as mid-season and end-of-season yield. A *post hoc* Tukey honestly significant difference test was used to determine significant differences among treatments ( $P < 0.05$ ). Data were log- or square-root-transformed as needed to meet assumptions of homogeneity of variance and normality of residuals. Differences in degrees of freedom between treatments and response variables are due to differential recovery of bioassay insects or loss of plant material.

## Results

#### *Myzus persicae* performance and plant response

**Laboratory.** Green peach aphid performance was over 48% lower on plants with both prior and simultaneous CPB feeding and was effectively reduced over time (Fig. 2a) (treatment:  $F_{2,18} = 13.55$ ,  $P < 0.001$ ; time:  $F_{4,15} = 76.9$ ,  $P < 0.0001$ ; time  $\times$  treatment interaction:  $F_{8,30} = 4.01$ ,  $P = 0.002$ ). Although populations in all treatments continued to grow, this trend persisted, and after 16 d, GPA populations remained over 43% lower on CPB-damaged plants than on plants that were damaged by GPAs alone. Above-ground biomass was measured in a preliminary assay, with no differences detected (Fig. S1A) ( $F_{3,36} = 1.92$ ,  $P = 0.143$ ) suggesting that GPA performance was not affected by leaf limitation.

Concentrations of  $\alpha$ -solanine did not differ among treatments (Fig. 3a) ( $F_{3,32} = 1.67$ ,  $P = 0.192$ ). However, we found significant differences in JA across treatments ( $F_{3,21} = 5.04$ ,  $P = 0.009$ ), with *post hoc* Tukey analysis showing that levels of JA were not significantly different between undamaged



**Fig. 2.** Green peach aphid (GPA) population growth in the laboratory (a) and in the field (b) when feeding on *Solanum tuberosum* alone (solid black line), with prior Colorado potato beetle (CPB) damage (dashed black line), or with simultaneous CPB damage (solid grey line). Asterisks above error bars indicate days when GPA numbers were significantly different (Tukey honestly significant difference,  $\alpha = 0.05$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

plants and herbivore-damaged plants, but mean JA levels were 42% higher in plants that were damaged simultaneously by CPBs and GPAs than in plants where GPAs fed alone or with prior CPB damage (Fig. 4a). Differences were also detected in JA-ile across treatments ( $F_{3,24} = 3.78$ ,  $P = 0.02$ ) with a 60% higher accumulation in samples collected from plants that were simultaneously damaged by CPBs and GPAs compared with samples from undamaged controls (Fig. 4b). Salicylic acid accumulation was lowest in undamaged plants, but did not significantly differ from plants damaged by GPA alone or after prior CPB damage, yet mean SA levels from simultaneously damaged plants were significantly higher than in control samples with an approximately 85% difference observed ( $F_{3,25} = 3.86$ ,  $P = 0.02$ ) (Fig. 4c). Herbivore feeding sequence had no effect on SAG content in plants (Fig. 4d) ( $F_{3,25} = 0.69$ ,  $P = 0.56$ ).

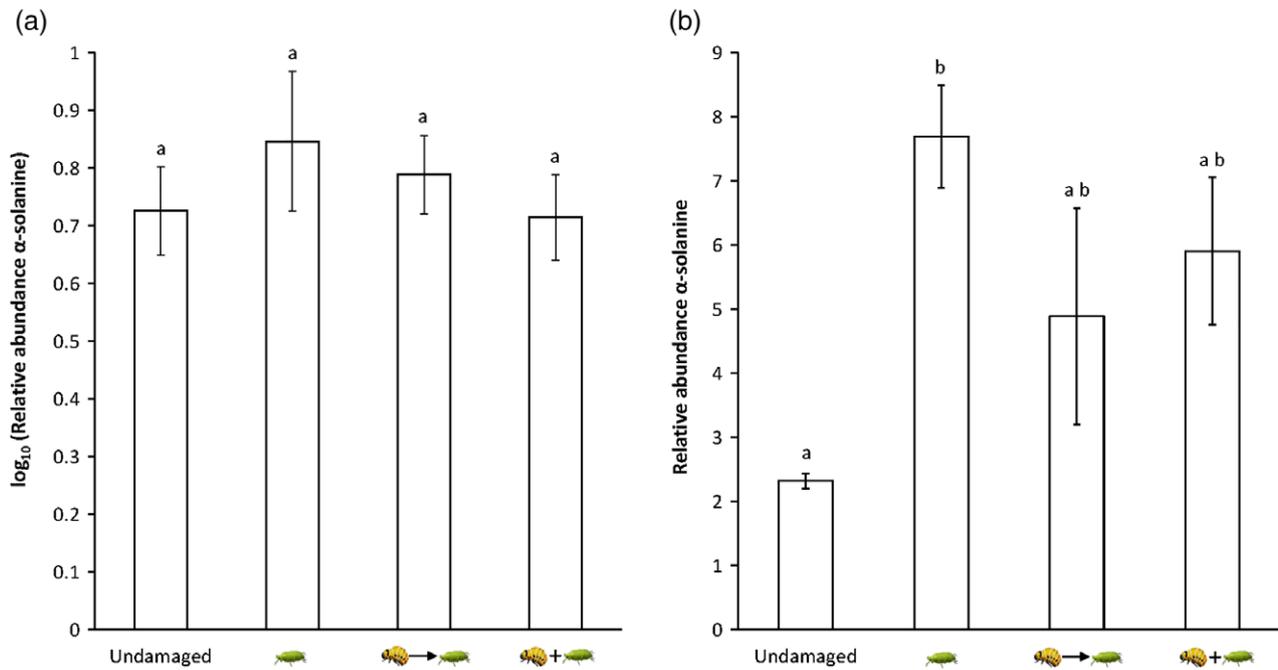
**Field.** Similar to our laboratory bioassays, GPA population growth was not initially impacted by the presence of CPBs. However, after 8 days, aphid numbers were on average over twice as high on plants without CPB damage than on plants with simultaneous damage, and over six times higher compared with plants with prior CPB damage (Fig. 2b) (treatment:  $F_{2,7} = 6.32$ ,  $P = 0.027$ , time:  $F_{5,3} = 18.93$ ,  $P = 0.018$ , treatment  $\times$  time interaction:  $F_{6,10} = 1.95$ ,  $P = 0.213$ ). Despite a nearly three-fold increase in  $\alpha$ -solanine after GPA damage, these results were marginally significant, probably due to low replication and the number of treatments (Fig. 3b) ( $F_{3,17} = 3.07$ ,  $P = 0.0622$ ). However, analysis of just aphid-treated plants compared with control plants resulted in significant differences in solanine levels ( $t_7 = -5.02$ ,  $P = 0.0024$ ). There were no detectable differences

in JA ( $F_{3,9} = 1.58$ ,  $P = 0.262$ ), JA-ile ( $F_{3,9} = 0.226$ ,  $P = 0.876$ ), SA ( $F_{3,11} = 0.872$ ,  $P = 0.485$ ), or SAG ( $F_{3,9} = 1.15$ ,  $P = 0.382$ ).

Mid-season yield showed marginal variation depending on the sequence of herbivore damage (Fig. S2A) ( $F_{3,9} = 3.72$ ,  $P = 0.055$ ), with tubers from undamaged plants weighing over 68% less than plants that were damaged by GPA alone or plants that were damaged previously and sequentially damaged by CPBs. There were no differences observed between the number of tubers ( $F_{3,14} = 0.486$ ,  $P = 0.698$ ) or the maximum tuber weight ( $F_{3,14} = 0.98$ ,  $P = 0.431$ ) among treatments. The effect of herbivore feeding sequence on yield was weakened by the end of the season, at which point mean tuber weight was consistent across all treatments (Fig. S2B) ( $F_{2,13} = 1.15$ ,  $P = 0.344$ ), indicating that herbivore damage did not affect seasonal yield. Again, there was no difference between the number of tubers ( $F_{3,16} = 0.9$ ,  $P = 0.463$ ) or the maximum tuber weight ( $F_{3,16} = 0.434$ ,  $P = 0.732$ ) for each plant between treatments.

#### *Leptinotarsa decemlineata* performance

**Laboratory.** There was no indication that GPA feeding affected CPB larval growth ( $F_{2,27} = 1.56$ ,  $P = 0.228$ ) (Fig. 5a). Mean concentrations of  $\alpha$ -solanine did not differ between undamaged plants and plants that were damaged by CPB alone or with simultaneous GPA and CPB damage, but were 47% lower when CPB fed after prior GPA damage ( $F_{3,29} = 2.97$ ,  $P = 0.048$ ) (Fig. 6a). Mean JA levels were lowest in undamaged plants, sequentially damaged plants, and simultaneously damaged plants, but were 62% higher than controls when CPB



**Fig. 3.** Impact of green peach aphid (GPA) feeding on  $\alpha$ -solanine concentration (mean  $\pm$  SE) from foliar extracts of *Solanum tuberosum* plants in the laboratory (a) and in the field (b) after damage from GPAs alone, with prior Colorado potato beetle (CPB) damage, or feeding simultaneously with CPB compared with undamaged controls. Means followed by same letter are not statistically significant (Tukey honestly significant difference,  $\alpha = 0.05$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

fed alone (Fig. 7a) ( $F_{3,20} = 4.03$ ,  $P = 0.022$ ). There was no effect on JA-ile (Fig. 7b) ( $F_{3,29} = 1.62$ ,  $P = 0.207$ ), SA (Fig. 7c) ( $F_{3,24} = 0.106$ ,  $P = 0.956$ ), or SAG (Fig. 7d) ( $F_{3,23} = 0.871$ ,  $P = 0.47$ ). Above-ground biomass at the end of the bioassay was similar across all treatments (Fig. S1B) ( $F_{3,36} = 0.256$ ,  $P = 0.857$ ).

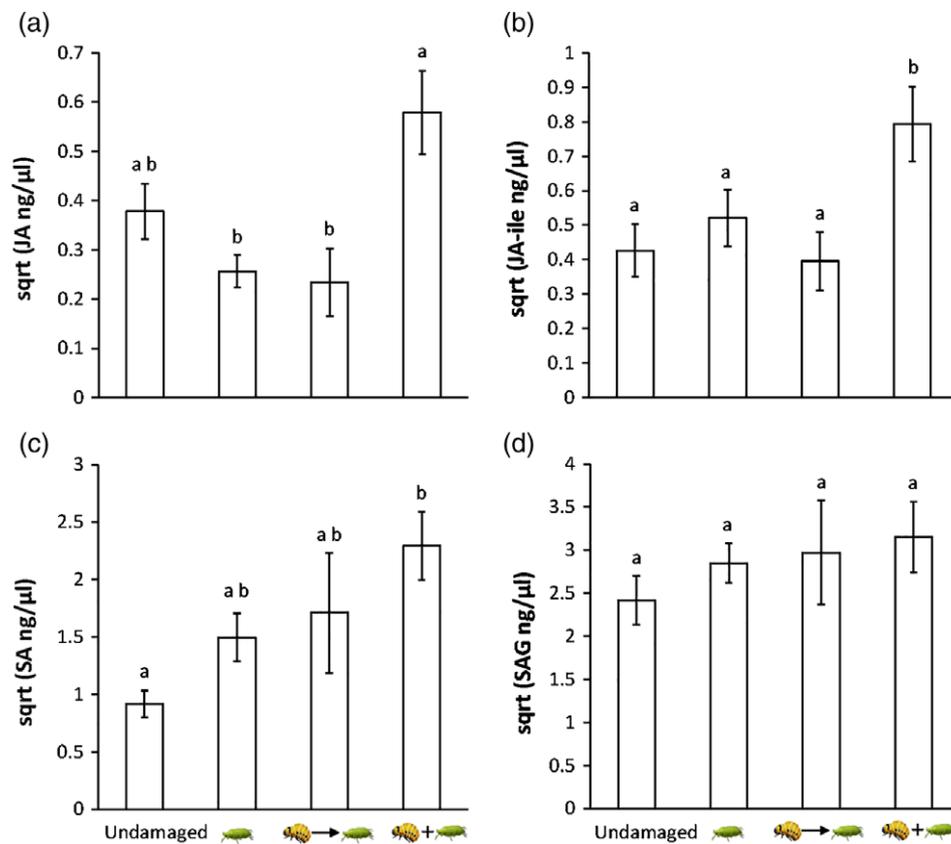
**Field.** In the field, the effect of GPA feeding on CPB performance varied compared with laboratory assays. On the first sampling date, CPB larvae that were feeding alone weighed over 1.5 times less than larvae feeding after prior GPA damage, but there was no difference in larval weight between CPB feeding alone and CPB feeding with GPA simultaneously (Fig. 5b) ( $F_{2,8} = 5.41$ ,  $P = 0.03$ ), and differences in larval weight were not detected 1 week later, on the second sampling date (Fig. 5c) ( $F_{2,12} = 2.11$ ,  $P = 0.16$ ). There was no evidence that feeding sequence affected  $\alpha$ -solanine (Fig. 6b) ( $F_{3,9} = 0.837$ ,  $P = 0.507$ ), JA ( $F_{3,10} = 1.14$ ,  $P = 0.379$ ), JA-ile ( $F_{3,9} = 0.343$ ,  $P = 0.795$ ), SA ( $F_{3,10} = 1.97$ ,  $P = 0.183$ ), or SAG ( $F_{3,10} = 0.55$ ,  $P = 0.66$ ).

There were no differences detected between mean tuber weight across herbivore damaged treatments and undamaged controls at the mid-season sampling date (Fig. S3A) ( $F_{3,10} = 2.53$ ,  $P = 0.116$ ). Similarly, end-of-season tuber weight did not differ between treatments (Fig. S3B) ( $F_{3,14} = 1.18$ ,  $P = 0.352$ ). There was no difference between the number of tubers ( $F_{3,14} = 1.24$ ,  $P = 0.332$ ) or the maximum tuber weight ( $F_{3,14} = 1.46$ ,  $P = 0.269$ ) for each plant between treatments.

## Discussion

Induced plant resistance can have cascading effects throughout communities when induction by one herbivore species affects how subsequently feeding herbivores interact with their host plant (Van Zandt & Agrawal, 2004; Viswanathan *et al.*, 2007; Poelman *et al.*, 2008; Erb *et al.*, 2011; Soler *et al.*, 2012; Uesugi *et al.*, 2013; Ali & Agrawal, 2014; Poelman, 2015; Li *et al.*, 2016; Lu *et al.*, 2016). Here, we tested whether differential plant responses to a chewing and a sucking herbivore could mediate interactions between these distinct guilds. Green peach aphid performance was negatively affected by chewing damage caused by CPBs, but this effect was not reciprocal. Colorado potato beetle performance was not affected by the presence or absence of GPAs in the laboratory, and only temporarily enhanced in the field. Overall, trends in herbivore performance were paralleled in the laboratory and field, although pest pressure may not have been sufficient to affect potato yield. Sample sizes in the field were also split in order to acquire both mid- and end-of-season yield, which reduced our ability to detect an effect. We therefore interpret these results cautiously.

Plant responses varied depending on the sequence of herbivore damage, without any clear evidence to support reliance on JA/SA signalling by plants in response to multiple herbivores in this scenario. Similar studies report that JA-related defences in response to chewing herbivores impart negative fitness consequences on sucking herbivore performance (Agrawal, 1998; Thaler *et al.*, 2001; Cooper & Goggin, 2005; Ali & Agrawal, 2014). Indeed, GPA performed worse when plants



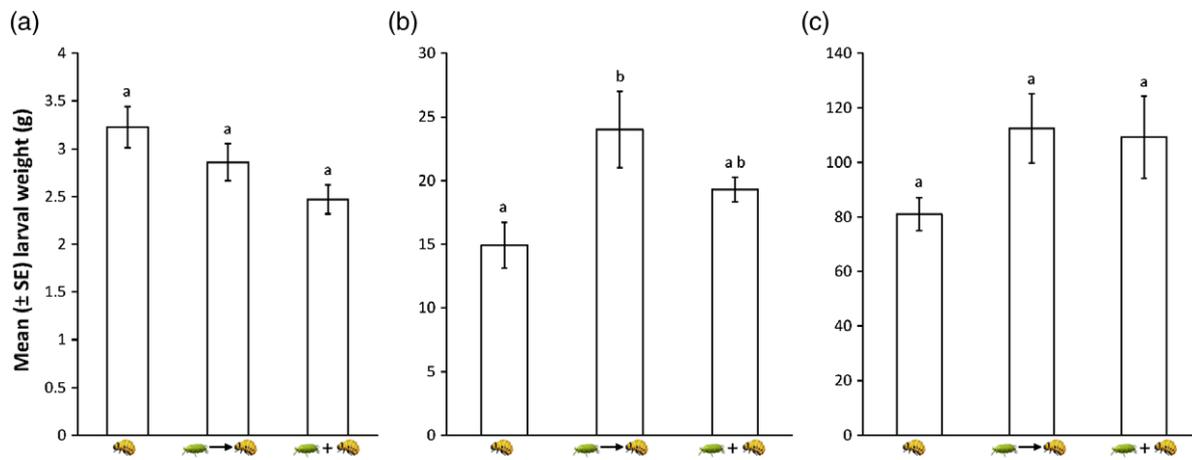
**Fig. 4.** Impact of green pea aphid (GPA) damage on mean ( $\pm$  SE) jasmonic acid (JA) (a), jasmonoyl-isoleucine (JA-ile) (b), salicylic acid (SA) (c), and glycosylated salicylic acid (SAG) (d) from foliar extracts of *Solanum tuberosum* plants feeding alone, after prior Colorado potato beetle (CPB) damage, or simultaneously with CPB damage, compared with undamaged controls. Means followed by the same letter are not statistically significant (Tukey honestly significant difference,  $\alpha = 0.05$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

were damaged simultaneously and sequentially by CPB compared with when they fed alone, but this was not linked to JA-mediated plant defences. During the GPA laboratory trial, JA levels were highest when both species were added to plants simultaneously and lowest when GPA fed alone or after prior CPB damage, while SA was highest when herbivores fed simultaneously but did not significantly differ from the other treatments. Interestingly, we found that  $\alpha$ -solanine was highest in the field when GPAs fed alone, but we were unable to detect  $\alpha$ -solanine differences in the laboratory.

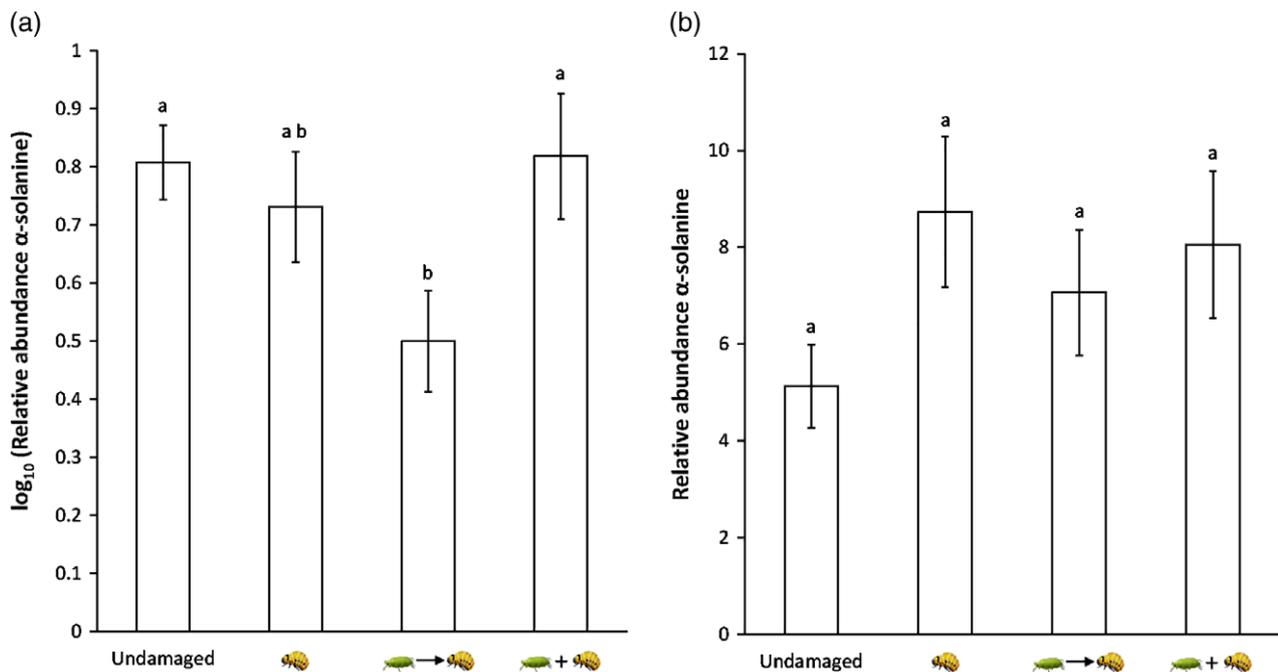
Other studies have shown that feeding damage by sucking herbivores can enhance chewing herbivore performance, often linked to the suppression of the JA pathway via induction of SA (Soler *et al.*, 2012; Ali & Agrawal, 2014), but this was not the case for our study. In the laboratory, CPB performance was not affected by GPA treatments, and performance was only temporarily enhanced when GPA fed prior to CPB in the field trial. During the CPB laboratory trials, JA was highest when CPB fed alone, but levels were similar to those of control plants in all treatments where GPAs were present. These results coincide with previous work demonstrating that chewing herbivores induce JA, while sucking herbivores suppress JA through the SA pathway (Walling, 2000; Thompson & Goggin, 2006; Howe & Jander, 2008; Wu & Baldwin, 2009). However, if JA were being

suppressed through the crosstalk mechanism, we would expect to see a corresponding increase in SA accumulation. When GPA fed prior to CPB in the laboratory, there was a reduction in  $\alpha$ -solanine even though JA and SA levels were similar among undamaged plants and plants that were damaged by GPA and CPB sequentially or simultaneously.

Given the ecological complexity of natural communities, plant defences are likely to be context-dependent and temporally variable (Karban & Meyers, 1989; Stout *et al.*, 1998; Dicke & Hilker, 2003; Maron *et al.*, 2014). Because plant responses are dynamic, our phytochemical results would probably be different had we sampled the plants at other time points throughout the course of these experiments (Zheng *et al.*, 2007; Schmelz *et al.*, 2009; Agrawal *et al.*, 2014). The plants in this study also differed in age depending on whether the experiment was conducted in the laboratory or in the field, which could also contribute to differences in baseline phytochemical responses (Mao *et al.*, 2017). Younger plants were used in the laboratory to accommodate both cage and growth chamber space, while older plants were used in the field because GPAs and CPBs were not detected during the first several weeks of growth at this site. Despite the fact that GPAs and CPBs were not present in the field prior to cage deployment, there was risk of plant exposure to other herbivores during the first 6 weeks.



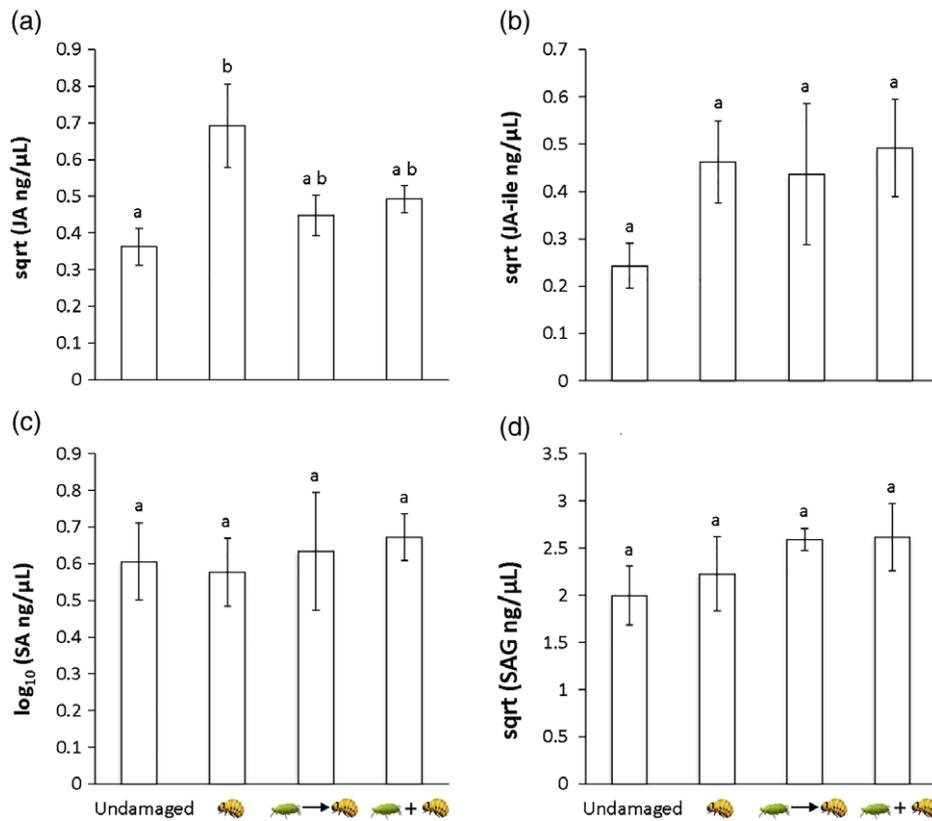
**Fig. 5.** Larval performance (mean  $\pm$  SE larval weight) of Colorado potato beetle (CPB) in the laboratory (a) and in the field at first sampling date (b) and second sampling date (c) when feeding on *Solanum tuberosum* plants alone, after prior green pea aphid (GPA) damage, or with simultaneous GPA damage. Means followed by same letter are not statistically significant (Tukey honestly significant difference,  $\alpha = 0.05$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].



**Fig. 6.** Impact of Colorado potato beetle (CPB) feeding on  $\alpha$ -solanine concentration (mean  $\pm$  SE) from foliar extracts of *Solanum tuberosum* plants in the laboratory (a) and in the field (b) after feeding alone, with prior green pea aphid (GPA) damage, or feeding simultaneously with GPA compared with undamaged controls. Means followed by same letter are not statistically significant (Tukey honestly significant difference,  $\alpha = 0.05$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

Flea beetles (Coleoptera: Chrysomelidae) were a persistent nuisance and were difficult to control throughout the duration of the experiment, which could also contribute to the overall reduced effect that we saw in plant hormone and defence comparisons in the field. However, this also lends strength to the performance results, as we generally found the same trends in herbivore performance in the field despite interference from other organisms and environmental conditions.

This study contributes to better understanding of the regulation of plant defences when faced with multiple insect attacks and the impacts on community interactions in field and laboratory settings. We demonstrate that plant responses are not fixed but that some plant–herbivore interactions remain consistent in both laboratory and field contexts. Although JA and SA were not found to contribute directly to these interactions in this study, additional research is necessary and time course assays



**Fig. 7.** Impact of Colorado potato beetle (CPB) feeding on mean ( $\pm$ SE) jasmonic acid (JA) (a), jasmonoyl-isoleucine (JA-ile) (b), salicylic acid (SA) (c), and glycosylated salicylic acid (SAG) (d), from foliar extracts of *Solanum tuberosum* plants after feeding alone, with prior green pea aphid (GPA) damage, or simultaneously with GPA compared with undamaged controls. Means followed by same letter are not statistically significant (Tukey honestly significant difference,  $\alpha = 0.05$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

of phytochemical responses may better resolve which factors facilitate the interplay between these two species. Furthermore, duration of interaction seems to be key. It will be important to design future experiments to assess whether the effect on aphids was driven by initial, single point changes or was due to an extended change in processes over time. Uncovering the underlying mechanisms and ecological consequences of plant defences to multi-herbivory is important for understanding how plants interact in natural and managed systems. Future studies investigating the effects of multi-herbivory on plant responses should provide further insights into how plant responses can regulate community dynamics across multiple trophic levels over time and under different environmental stresses (Maron *et al.*, 2014; Poelman, 2015). Expectations of signalling crosstalk may be challenged with the inclusion of multiple herbivore species and as research moves from the laboratory to more biologically relevant field-based experiments.

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### Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1** Mean plant weight at the end GPA (a) and CPB (b) bioassays. Means followed by same letter are not statistically significant (Tukey HSD,  $\alpha = 0.05$ ).

**Figure S2** Mid-season (a) and end-of-season (b) tuber yield for GPA performance. Simultaneous GPA and CPB treatment removed from final yield analysis due to field infestation by other CPB larvae. Means followed by same letter are not statistically significant (Tukey HSD,  $\alpha = 0.05$ ).

**Figure S3** Mid-season (a) and end-of-season (b) tuber yield for CPB performance in the field. Means followed by same letter

are not statistically significant (Tukey HSD,  $\alpha = 0.05$ ). Means followed by same letter are not statistically significant (Tukey HSD,  $\alpha = 0.05$ )

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