Oecologia (2018) 186:703–710
https://doi.org/10.1007/s00442-018-4070-1

PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Plants eavesdrop on cues produced by snails and induce costly defenses that affect insect herbivores

John L. Orrock[1] · **Brian M. Connolly**[1,2] · **Won-Gyu Choi**[3,4] · **Peter W. Guiden**[1] · **Sarah J. Swanson**[3] · **Simon Gilroy**[3]

Received: 12 October 2017 / Accepted: 12 January 2018 / Published online: 16 January 2018

Abstract

Although induced defenses are widespread in plants, the degree to which plants respond to herbivore kairomones (incidental chemicals that herbivores produce independent of herbivory), the costs and benefits of responding to cues of herbivory risk, and the ecological consequences of induced defenses remain unclear. We demonstrate that undamaged tomatoes, *Solanum lycopersicum*, induce defenses in response to a kairomone (locomotion mucus) of snail herbivores (*Helix aspersa*). Induced defense had significant costs and benefits for plants: plants exposed to snail mucus or a standard defense elicitor (methyl jasmonate, MeJA) exhibited slower growth, but also experienced less herbivory by an insect herbivore (*Spodoptera exigua*). We also find that kairomones from molluscan herbivores lead to deleterious effects on insect herbivores mediated through changes in plant defense, i.e., mucus-induced defenses of *Solanum lycopersicum*-reduced growth of *S. exigua*. These results suggest that incidental cues of widespread generalist herbivores might be a mechanism creating variation in plant growth, plant defense, and biotic interactions.

Keywords Cue · Herbivory · Indirect effects · Induced defenses · Kairomone · Plant defense

Introduction

Plants are not passive participants in plant–herbivore interactions: many plant species exhibit induced defenses that are deployed either before, or following, the onset of herbivore attack (Karban and Baldwin 1997). Just as information regarding risk of attack is critical in animal predator–prey systems (e.g., Weissburg et al. 2014), information on potential herbivore attack is an important component of plant-induced defense (Karban et al. 2016). Plants respond to a diverse array of information regarding herbivory risk, including airborne cues (Heil and Karban 2010; Howe and Jander 2008; Karban and Baldwin 1997; Schaller 2008), trichome stimulation (Peiffer et al. 2009), herbivore saliva (Felton 2008), and herbivore oviposition (Hilker and Meiners 2010).

A novel form of information use in plants has recently been described: plants may induce defense in response to herbivore kairomones (Falk et al. 2014; Helms et al. 2013; Orrock 2013), chemical cues that are not directly associated with attack by a natural enemy (i.e., they are incidental cues created by herbivore metabolism or secreted as part of processes not related to herbivory, such as a mating pheromone). The role of kairomones has long been appreciated in plant–pathogen studies (Choi and Klessig 2016) and was recently documented for plant–parasite systems (Manosalva et al. 2015). Moreover, kairomone-mediated effects are a cornerstone of animal ecology, e.g., prey often respond to predator kairomones in ways that affect prey growth, survival, and reproduction (Kats and Dill 1998; Preisser et al. 2005). In plant–herbivore interactions, many potential kairomones exist, such as chemicals produced by herbivore

---

Communicated by Carlos L. Ballare.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00442-018-4070-1) contains supplementary material, which is available to authorized users.

✉ John L. Orrock
jorrock@wisc.edu

[1] Department of Integrative Biology, University of Wisconsin, Madison, WI 53706, USA

[2] Biology Department, Eastern Michigan University, Ypsilanti, MI 48197, USA

[3] Department of Botany, University of Wisconsin, Madison, WI 53706, USA

[4] Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV 89557, USA

excretia and frass (Kim et al. 2011), and chemicals used for herbivore communication and locomotion (Falk et al. 2014; Helms et al. 2013, 2017; Orrock 2013). Given the existence of many potential kairomones and their potential to be widespread, understanding how plants respond to herbivore kairomones may provide important insight into the ecology of plant defense.

Because plants are often attacked by multiple herbivore species, changes in plant defense caused by herbivore kairomones might alter the nature of interactions among herbivores and affect the composition of herbivore communities (Poelman et al. 2008; Stam et al. 2014). For example, Helms et al. (2013) demonstrated that changes in plant defense in response to the sex pheromone of a specialist herbivore lead to a reduction in herbivory by another specialist herbivore species. Compared to the effects observed for specialist herbivores, kairomone-mediated changes in plant defense may be particularly important for generalist herbivores, because they may be more sensitive to variation in plant defense (Ali and Agrawal 2012). Recent research illustrates that the potential exists for some plant species to initiate defense in response to kairomones of generalist herbivores (e.g., snails and slugs; Falk et al. 2014; Orrock 2013). However, whether such kairomone-mediated changes in defense alter plant susceptibility to a wider array of different generalist herbivore species remains an open question.

In this study, we coupled experimental manipulation of snail kairomones, assays of plant defensive compounds and plant performance, and assays of herbivore performance to evaluate (1) whether plants induce defense in response to kairomones of generalist snail herbivores and (2) whether kairomones from one generalist herbivore species (snails) might lead to changes in the performance of another generalist herbivore species (larval lepidopterans) due to kairomone-mediated changes in plant defense.

Materials and methods

We used tomato (*Solanum lycopersicum*) as the focal plant species, snail mucus collected from *Helix aspersa* (also called *Cornu aspersum*) as the herbivore kairomone, and used the generalist lepidopteran herbivore *Spodoptera exigua* to examine changes in plant defense on rates of herbivory and herbivore performance. *Solanum lycopersicum* is ideal for our study, because it is known to induce chemical defenses that affect herbivore performance and feeding, such as polyphenol oxidase (PPO), peroxidase (POD), and lipoxygenase (LOX; Duffey and Felton 1991; Kennedy 2003; Thaler et al. 1996), and *S. lycopersicum* also responds to kairomones related to other trophic interactions (i.e., parasitic nematodes; Manosalva et al. 2015). However, it is unknown whether *S. lycopersicum* responds to herbivore kairomones. *Helix aspersa* is a widely distributed generalist mollusk herbivore found in temperate North America, South America, Europe, Africa, and Australia and is a recognized pest of tomatoes and many other crops (Barker 2002). Herbivorous snails can have significant, deleterious effects on plant survival (Cleland et al. 2006; Hulme 1996; Motheral and Orrock 2010; Strauss et al. 2009). Importantly, locomotion by terrestrial mollusks requires mucus, and snail mucus alone is sufficient to induce plant defense, i.e., no physical contact is necessary between plants and snails (Meldau et al. 2015; Orrock 2013). Although the previous work has shown that plants in the Brassicaceae will induce defenses in response to snail and slug mucus (Meldau et al. 2015; Orrock 2013), it is not known whether *S. lycopersicum* responds to mucus and whether defenses induced in response to snail mucus have effects on generalist insect herbivores. We used *Spodoptera exigua* to assess whether defenses induced in response to *H. aspersa* kairomone application lead to changes in plant palatability. *Spodoptera exigua* often attacks *S. lycopersicum* in natural settings (Kennedy 2003) and experiences reduced growth and survival on *S. lycopersicum* that has induced defenses (Kennedy 2003; Thaler et al. 1996, 1999; Underwood 2010).

Five seeds of *S. lycopersicum*, variety “Large Red Cherry” (Plantation products LLC., Norton, MA, USA), were sown on 25 June 2015 into 30 individual 6 × 6 × 5.5 cm pots containing peat moss–vermiculite mix (Sun Gro Horticulture, Agawam, MA, USA). Pots were placed a growth chamber maintained at 23 °C with a 12:12 light:dark photoperiod. Pots were checked daily and watered if necessary. The first seedling to emerge was used in the experiment; subsequent germinants were removed.

Mucus collection and treatment application

The process of collecting snail mucus occurred over 2 days. On 15 June 2015, stock populations of *H. aspersa* were placed on a diet consisting of only Brassicaceae. The day before the mucus-treatment application (5 July 2015 and 7 July 2015, respectively) twenty-four (24) snails were selected and held without food for 12 h to minimize the presence of non-mucus components during the mucus-collection period. Snails were then weighed and placed in independent circular plastic dishes with lids (11 cm diameter × 4.5 cm deep). Snails were housed for 20–22 h in their individual dishes alongside control dishes (i.e., dishes that did not receive snails). After this period, snails were removed from the cups, so that mucus left behind on the inside of the cups (from snail locomotion while in the cup) could be collected. Mucus was collected by adding 30 mL of Millipore-filtered reverse-osmosis water to each plastic dish; control dishes were identically treated. We focus on collecting only water-soluble components of mucus, because these are the components that rainwater is likely to move through the soil into the rooting zone of plants in natural situations, where snails move around on the ground near plants. Lidded dishes were stirred for 20 min at 200 RPM on an orbital shaker to allow the components of the mucus to form a solution. Three aliquots of 10 mL each were collected from each dish for the application of mucus or control treatments.

Plants were randomly assigned to one of three treatments: a water control, an MeJA-spray treatment, or a snail-mucus soil application. Treatments were first applied on 6 July 2015 (i.e., 11 days after sowing) and then applied again on 8 July 2015. Plants assigned to receive the MeJA treatment were sprayed with 1 mM MeJA (0.125% v/v) Triton-X100 solution (Farmer and Ryan 1990). Control plants and plants assigned to snail-mucus treatment received only a solution of 0.125% (v/v) Triton-X100 without MeJA. During treatment application, plants were removed from the growth chamber and placed in individual circular plastic dishes (11 cm diameter × 4.5 cm deep) to contain water seepage and snail-mucus soil application treatments. To prohibit cross contamination of the MeJA-spray treatments, each plant and plastic dish was then covered with a randomly assigned rigid green plastic tube (37 cm tall; diameter ranged from 13 to 15.5 cm), so that the end of the cylinder was flush with the bench top and encased both the potted plant and the plastic dish; the open end of the cylinder was sealed with a layer of plastic wrap held in place by a rubber band. Each cylinder was separated by at least 20 cm. Plant placement was randomly assigned to positions across the bench top. For each spray treatment, the plastic film was removed from the top of the cylinder, the nozzle of a spray bottle was inserted into the opening (~ 22 cm from the top of the plant), and four sprays (12.00 ± 0.06 mL, mean ± SE, $n = 30$) were applied to each plant. Immediately after spraying, the plastic covering was replaced over the top of the cylinder. To guard against cross contamination, only one plant was sprayed at a time, such that all other plants were within their own sealed cylinders. Plants were left covered for a minimum of 1.5 h.

Following each of the two MeJA/control treatments, plants assigned the snail-mucus treatment had 10 mL aliquots of snail-mucus solution (snail mucus and reverse-osmosis water) poured directly onto the soil surface in a circular area approximately 2 cm away from the main stem of the plant. Control plants and MeJA-treated plants received only reverse-osmosis water. This method of soil-based application was used to mimic the natural situation, where snails traveling near a plant would leave a mucus trail that would then be washed down into the soil by precipitation. We opted to use this approach, instead of other approaches (e.g., directly applying mucus to leaves of plants), because our experiment sought to examine whether plants might use snail kairomones to pre-empt an attack before it was underway. Once snails are crawling on a plant, attack is much more likely, and the movement of snails along a leaf (or researchers simulating a snail by brushing mucus onto a leaf) is also likely to damage trichomes, which may also initiate the induction of defense. Our approach of using a water–mucus solution provides a more direct test of whether plants use snail kairomones (alone) to induce defense, without other potential factors (e.g., physical damage, trichome stimulation) that are already known to lead to changes in defense (Kim et al. 2011). Following snail-mucus application, plants were left within their cylindrical containers for approximately 1 h and then returned to the growth chamber.

Herbivory trials

On 9 July 2015, 14 days after sowing, the number of fully expanded leaves was counted on each plant. Plants were then harvested at ground level and the total aboveground biomass was determined. A randomly selected leaflet was cut from each plant, weighed, and measured for the herbivory trial. Each excised leaf was placed in a 100 × 15 mm Petri dish on top of blotter paper (Anchor Paper Co., St. Paul, MN, USA) saturated with reverse-osmosis water to help maintain leaf tissue turgor during the herbivory trial. A single second-instar *S. exigua* was weighed and placed in each Petri dishes containing a single leaflet; dishes were sealed with Parafilm to prevent leaf desiccation and herbivore escape. After 24 h, the proportion of leaf consumed was visually estimated by two observers. Observers were blind to treatments, were stratified across all treatments, and randomly assigned to individual leaves (see Supplemental Materials for additional information). Each *S. exigua* was then removed and weighed; *S. exigua* growth rate was calculated as the difference in pre- and post-trial mass divided by the average of pre- and post-trial mass (Waldbauer 1968); a negative value indicates a loss in mass. Because leaves were placed on blotters to maintain natural leaf turgor during herbivory trials, individual leaves were damp, and post-trial leaf mass was not a reliable metric for the amount of plant material consumed (e.g., leaves actually gained 1.89 ± 0.55 g during the trial, despite the removal amount of leaf material from many leaves). As such, to estimate the quantity of leaf material consumed, we multiplied initial leaf mass by the proportion of the leaf consumed; our results suggest that this metric effectively captured variation in leaf tissue loss (Supplemental Materials).

Crude proteins were extracted from liquid nitrogen snap-frozen, weighed cotyledon leaves (~ 50 mg) excised immediately before plant harvesting. This tissue was assayed for activity of common defensive enzymes (Duffey and Felton 1991; Thaler et al. 1996): polyphenol oxidase

(PPO), peroxidase (POD), and lipoxygenase (LOX); see Supplemental Materials for details.

Statistical analyses

We used multivariate analysis of variance (MANOVA) to evaluate differences in plant defensive enzymes, followed by univariate analysis of variance (ANOVA) for each enzyme (Scheiner and Gurevitch 2001). ANOVA was also used to evaluate whether final plant biomass, *S. exigua* growth rate, and amount of leaf consumed differed in response to the treatments.

Results

We found differences in defense enzyme activities due to exposure to snail mucus, MeJA, or control treatments (Wilks' Lambda = 0.426, $F_{6,48} = 4.26$, $P = 0.002$). Subsequent univariate analyses demonstrated that treatments led to significant differences in LOX ($F_{2,26} = 4.86$, $P = 0.017$), with significantly greater LOX in MeJA ($t_{26} = -2.55$, $P = 0.017$) and mucus ($t_{26} = -2.80$, $P = 0.009$) treatments relative to the control (Fig. 1), but no difference between MeJA and mucus ($t_{26} = -0.32$, $P = 0.751$). POD was also significantly different among treatments ($F_{2,26} = 7.11$, $P = 0.003$), with significantly greater POD in MeJA treatment relative to the control ($t_{26} = -3.75$, $P < 0.001$) and mucus ($t_{27} = -2.19$, $P = 0.038$) treatments, but no difference

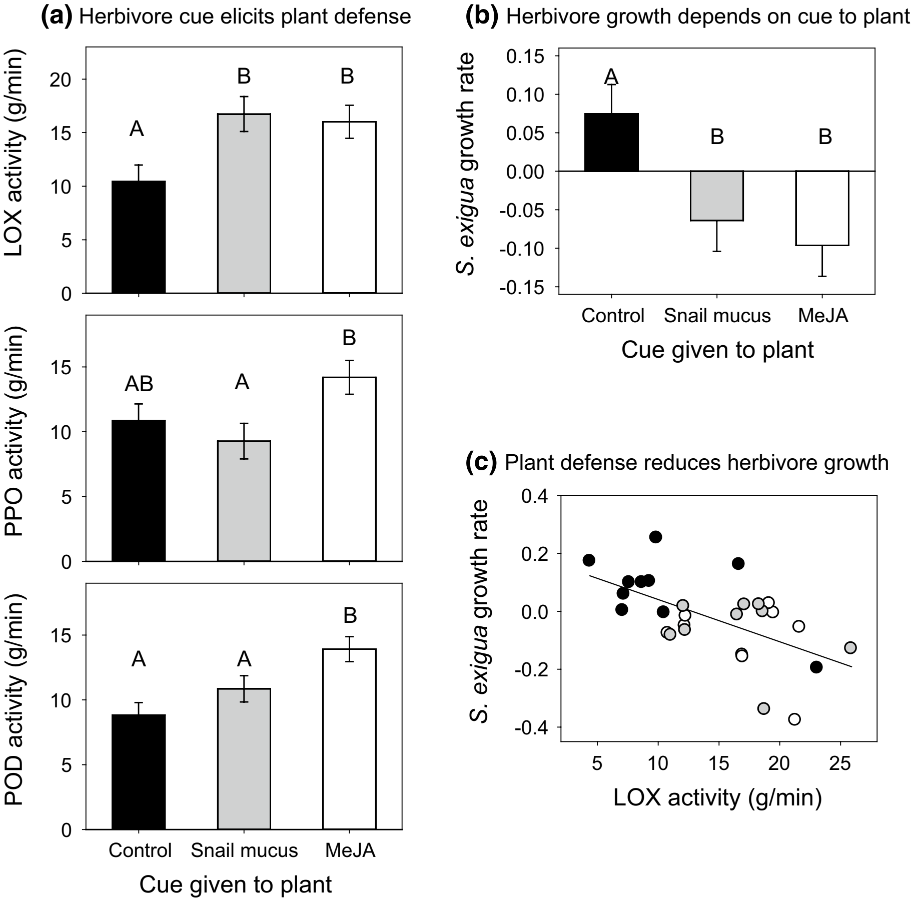

Fig. 1 A. Variation in three defensive compounds of *S. lycopersicum* in response to a control treatment, mucus from snails, and standard elicitor of plant defense (MeJA). Within a panel, bars that do not share a letter are significantly different ($P < 0.05$). B. Induced defenses in response to cues of herbivory risk (i.e., MeJA or snail kairomones) lead to significant reductions in growth of a generalist herbivore (*S. exigua*). Bars that do not share a letter are significantly different ($P < 0.05$). C. Differences in herbivore growth rate were significantly related to plant production of the defensive enzyme lipoxygenase (LOX; $r^2 = 0.34$, $F_{1,26} = 13.24$, $P = 0.001$), suggesting a relationship between plant allocation to defense and herbivore growth rate. Fills indicate the treatment associated with that data point, using the same color scheme as the top panel: black dots represent control plants, gray dots represent plants that received the snail-mucus treatment, and clear dots represent plants that received the MeJA treatment

between the control and mucus treatments ($t_{27} = -1.46$, $P = 0.156$). Levels of PPO also differed among treatments ($F_{2,26} = 3.58$, $P = 0.042$), with significantly greater PPO in MeJA-treated plants compared to mucus-treated plants ($t_{26} = 2.60$, $P = 0.015$), a trend of greater PPO than control plants ($t_{26} = -1.82$, $P = 0.081$), and no difference between mucus-treated plants and controls ($t_{26} = 0.83$, $P = 0.413$).

The growth of *S. exigua* also differed among treatments ($F_{2,25} = 5.46$, $P = 0.011$; Fig. 1b). *Spodoptera exigua* growth was greater on control plants compared to MeJA-treated plants ($t_{25} = 3.09$, $P = 0.005$) and mucus-treated plants ($t_{25} = 2.05$, $P = 0.019$). There was no difference in *S. exigua* growth between MeJA and mucus treatments ($t_{25} = 0.57$, $P = 0.574$). We found a significant negative relationship between LOX and *S. exigua* growth rate (Fig. 1C; $r^2 = 0.34$, $F_{1,26} = 13.24$, $P = 0.001$), but no relationship between *S. exigua* growth rate and PPO ($r^2 = 0.02$, $F_{1,26} = 0.42$, $P = 0.521$) or POD ($r^2 = 0.04$, $F_{1,26} = 0.98$, $P = 0.332$). The amount of leaf consumed was also affected by treatment (Fig. S1; $F_{2,25} = 5.84$, $P = 0.008$): consumption was greatest for plants receiving the control treatment compared to MeJA-treated plants ($t_{25} = 3.36$, $P = 0.003$) or mucus-treated plants ($t_{25} = 2.14$, $P = 0.043$; Fig. S1) and did not differ between mucus- and MeJA-treated plants ($t_{25} = -1.20$, $P = 0.243$). The amount plant biomass consumed by herbivores was negatively related to LOX activity (Fig. S1; $r^2 = 0.19$, $F_{1,26} = 6.19$, $P = 0.019$) but not PPO activity ($r^2 = 0.05$, $F_{1,26} = 1.35$, $P = 0.256$) or POD activity ($r^2 = 0.09$, $F_{1,26} = 2.58$, $P = 0.120$).

Data collected prior to the herbivory trial demonstrates that aboveground plant biomass prior to herbivory trials also differed among risk treatments (Fig. 2; $F_{2,26} = 4.78$, $P = 0.017$), with plants of significantly greater mass in the control treatment relative to the MeJA treatment ($t_{26} = 2.81$, $P = 0.009$) and mucus treatment ($t_{26} = 2.49$, $P = 0.019$), but no difference in plant mass between the MeJA and mucus treatments ($t_{26} = -0.24$, $P = 0.811$). Similar patterns were found for the effect of treatment on leaf biomass and the number of leaves (see Supplemental Materials). We found a significant negative relationship between LOX and plant biomass (Fig. 2; $r^2 = 0.26$, $F_{1,27} = 9.63$, $P = 0.001$), and no relationship between plant biomass and PPO ($r^2 = 0.07$, $F_{1,27} = 1.91$, $P = 0.179$) or plant biomass and POD ($r^2 = 0.02$, $F_{1,27} = 0.49$, $P = 0.491$).

Discussion

Plants may use diverse types of information from their environment to tailor investment in defense to the risk of damage by their attackers (Karban et al. 2016; Orrock et al. 2015). Our findings contribute to that base of knowledge by demonstrating that an herbivore kairomone, the mucus of a generalist snail herbivore, leads to changes in plant defense (Fig. 1), and that these kairomone-induced changes in defense alter plant quality for a different herbivore species (*S. exigua*). As such, our findings suggest that plant use of novel cue types (i.e., kairomones) may help explain some of the considerable variation in plant defense often observed in field settings, as well as inform the potential for kairomone-mediated interactions among herbivores. We discuss each of these implications below.

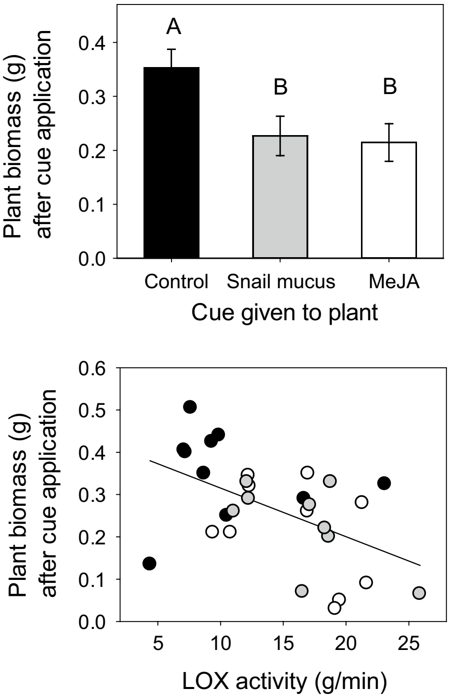

Fig. 2 Cues indicative of herbivory risk caused significant reductions in plant biomass (top panel); bars that do not share a letter are significantly different ($P < 0.05$). Plant growth was significantly related to plant investment in defense (bottom panel; $r^2 = 0.26$, $F_{1,27} = 9.63$, $P = 0.001$), suggesting the existence of a tradeoff between plant allocation to defense at the expense of growth. Fills in the bottom panel indicate the treatment associated with each data point, using the same color scheme as the top panel: black dots represent control plants, gray dots represent plants that received the snail-mucus treatment, and clear dots represent plants that received the MeJA treatment

Kairomones and variation in plant defense

A primary goal of plant–herbivore ecology is to understand the substantial variation in plant defense observed in nature. In showing that kairomones of a widespread herbivore are sufficient to generate significant investment in defense (Fig. 1), our results suggest that variation in plant defense, and associated differences in plant growth (Fig. 2) may in part reflect variation in the exposure of plants to kairomones that may be indicative of potential snail attack. Because mollusks can have strong deleterious effects on a wide variety of plants (Cleland et al. 2006; Hulme 1996; Motheral and Orrock 2010; Strauss et al. 2009), it is possible that use of mollusk kairomones is a widespread phenomenon in terrestrial plants. This hypothesis is supported by recent research demonstrating that the mucus of eight different mollusk species produces molecular responses in *A. thaliana* (Meldau et al. 2015), suggesting that mollusk kairomones may be of widespread, but under-appreciated importance as a form of risk information in plant defense.

Our finding that plants responded with increased LOX, but no measurable changes in PPO or POD, following exposure to snail kairomones suggests that cues from snails may generate variation in plant populations by prompting a specific defensive response. Such responses have been documented in tomato in the context of several other herbivore species. Consumption of tomato leaf tissue by noctuid larvae, such as *Helicoverpa zea*, induces strong systemic upregulation of PPO activity, but does not typically influence POD or LOX activity levels (Duffey and Felton 1991). In contrast, leaf colonization by the mite *Aculops lycopersici* leads to a different pattern of induction among these three enzymes: LOX is upregulated, POD activity is marginally increased, and PPO activity unaltered (Duffey and Felton 1991). In the context of the increase in LOX, we observed following snail kairomone application (Fig. 1); LOX might be increased, because it is both a defensive enzyme and is involved in signaling and initiating additional defenses via the octadecanoid pathway (Koiwa et al. 1997). For example, LOX production is a precursor to the production of green-leaf volatiles, chemicals that are associated with reduced attack of plants by *H. aspersa* (Shannon et al. 2016).

Our results show that cues associated with a possible attack can cause plants to induce costly defenses, even in the absence of any actual attack: snail kairomones led to reduced plant growth (Fig. 2) and plant growth was significantly correlated with production of LOX (Fig. 2). The previous studies have demonstrated how cue-mediated changes in plant morphology and chemistry can affect plant allocation to growth (Huot et al. 2014; van Hulten et al. 2006), plant reproduction (van Hulten et al. 2006), and plant survival (Huot et al. 2014). Indeed, wild plant species with lower constitutive but higher inducible defenses (i.e., those that rely more heavily on information and risk cues) exhibit greater competitive ability (Kempel et al. 2011), presumably because these plants are less likely to pay the costs of defense if defense is not needed (Orrock et al. 2015). Our findings suggest that mollusk kairomones may have significant effects on plant growth, just as kairomones of predation risk have been found to have significant effects on growth, reproduction, and survival of animal prey (Preisser et al. 2005). In finding that herbivore kairomones alone can generate significant effects on plant growth, our work also demonstrates that variation in risk information may yield variation in plant performance, just as variation in predation risk and the 'ecology of fear' has helped inform animal ecology (Lima and Dill 1990; Preisser et al. 2005).

Kairomones and interactions among herbivores

Although herbivore-mediated changes in plants can alter plant–herbivore interactions (Ohgushi 2005; Stam et al. 2014), the role of induced defenses in affecting arthropod communities is only beginning to be appreciated (Poelman et al. 2008; Stam et al. 2014). Changes in defense in response to actual feeding by one herbivore species have been documented to have effects on other herbivores, e.g., feeding by *Helicoperva zea* leads to changes in tomato that reduce subsequent attack by *S. exigua*, aphids, and mites, in addition to a pathogen (Stout et al. 1998). Our results demonstrate that actual attack by an herbivore is not needed to trigger changes in plant defense that can alter plant palatability for other herbivores: snail kairomones alone are capable of mediating changes in plant quality that may affect the dynamics of other generalist herbivore species, such as *S. exigua* (Fig. 1). In addition, given the similarity in the defensive chemistry profile of tomato following snail kairomone application (Fig. 1) and infestation by mites (Duffey and Felton 1991), it is plausible that snail kairomones may influence attack by arthropod herbivores other than *S. exigua*. Similarly, nematodes produce a pheromone that also induces the innate immunity system of the plant leading to broad spectrum defense against both nematodes and microbial pathogens (Manosalva et al. 2015).

Our results also demonstrate the importance of mobile cues for inducing plant defenses that affect other herbivores: plants in our experiment were never contacted or consumed by snails. Instead, kairomones from snail mucus were likely detected by roots as the water–mucus solution moved down into the soil (mimicking the situation, where mucus would naturally be carried into the soil by precipitation). It is not clear whether plants are capable of recognizing unique, mollusk-related compounds, or whether plants are responding to plant compounds that have been sequestered and are then secreted in mucus, such as damage-associated molecular patterns (DAMPs; Choi and Klessig 2016), or defensive phytohormones (such as JA) obtained from consumption of previously damaged plants. Of these explanations, it seems most likely that plants are responding to some unique component of snail mucus or to DAMPS, as the phytohormones abscisic acid and JA were absent from the mucus of 13 different species of mollusk herbivore (Kästner et al. 2014), although salicylic acid was found in the mucus of one of the mollusk species (*Deroceras reticulatum*). In our study, snails were never fed tomato plants (Supplemental Materials), but DAMPs present in the plants fed to snails might have been present in the snail mucus and capable of generating induced defenses in tomato; snail mucus contains a variety of proteins and compounds that could serve as kairomones (for an overview and links to additional references, see Greistorfer et al. 2017). However, it is unknown, to our knowledge, whether these consist of DAMPs from previously consumed plants. Snail mucus may also provide a substrate for differential growth of microbes (Pitt et al. 2015), although it is unknown whether these microbes are more or less likely to be plant pathogens. Future studies empirically evaluating the role of mollusk diet and mucus composition on levels of plant-induced defenses are necessary to fully understand the mechanism, whereby snail mucus alters plant defenses in ways that alter growth of insect herbivores (Fig. 1).

Conclusions

Our findings demonstrate how kairomones of a widespread, generalist herbivore generate a defensive response in plants that have not been attacked and that this response affects plant chemistry, plant growth, and growth of a different herbivore species. These results add to an increasingly large body of work documenting the many cues that will trigger induced plant defenses (Karban et al. 2016; Kim et al. 2011; Mescher and De Moraes 2015). An important question for future studies is to determine how plants integrate the many sources of information regarding attack in their environment to optimize investment in defense. Our results suggest that plants prioritize risk information as might be predicted by error management theory (Orrock et al. 2015), whereby information that is likely linked with greater imminent risk triggers a stronger defense: plants in our experiment produced stronger, more consistent defenses when presented with a cue that actual herbivory has occurred nearby (i.e., MeJA) than when presented with a cue that an herbivore is near (i.e., snail mucus). Fully evaluating the utility of theory for predicting information use by plants will require additional studies that perform a factorial manipulation of different cues, and will potentially utilize experimental treatments that modify the costs and benefits of responding to particular cues. Since kairomones from herbivores (Fig. 1), microbial pathogens (Choi and Klessig 2016), and parasites (Manosalva et al. 2015) all have the ability to change plant defense in ways that affect other herbivores, pathogens, and parasites, future experiments that examine the ecology of information use by plants may also provide insight into the dynamics of consumer communities. Moreover, although our results demonstrate that kairomones can lead to significant changes in plant defense (Fig. 1), an interesting (and untested) possibility is that kairomones also affected priming, where plants do not exhibit obvious changes in defense in response to a current cue, but exhibit a stronger or more-rapid response to subsequent cue of herbivory (Kim et al. 2011). For example, although snail mucus did not lead to significant changes in PPO or POD, plants exposed to mucus might still have been primed to more rapidly respond to future cues of herbivory with PPO or POD production. Studies examining the role of kairomones in priming defenses and situations with escalating cues of imminent plant-fitness loss (e.g., experiments combining kairomones and actual herbivory) are necessary to provide a more comprehensive understanding of the role of kairomones and other sources of information in mediating plant defense.

Acknowledgements Jessica Casper provided outstanding assistance with herbivory trials; the Damschen lab kindly provided their microbalance. This study was supported by the UW Graduate School Fall Competition Grant to JO and SG and NSF Grant MCB-1329723 to SG and WC. JO was supported by the Vilas Fellowship during a portion of this work. The Biology Department at Virginia Commonwealth University hosted JO while working on this manuscript.

Author contribution statement JO conceived the study; JO, BC, and SG designed the study; BC, PG, WC, and SS collected the data; and JO analyzed the data and drafted the manuscript; all authors contributed to revising the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Ali JG, Agrawal AA (2012) Specialist versus generalist insect herbivores and plant defense. Trends Plant Sci 17:293–302

Barker GM (ed) (2002) Molluscs as crop pests. CABI, Wallingford

Choi HW, Klessig DF (2016) DAMPs, MAMPs, and NAMPs in plant innate immunity. BMC Plant Biol 16:232

Cleland EE, Peters HA, Mooney HA, Field CB (2006) Gastropod herbivory in response to elevated CO2 and N addition impacts plant community composition. Ecology 87:686–694

Duffey SS, Felton GW (1991) Enzymatic antinutritive defenses of the tomato plant against insects. In: Hedin PA (ed) Naturally occurring pest bioregulators. American Chemical Society, Washington, DC, pp 167–197

Falk K et al (2014) The role of glucosinolates and the jasmonic acid pathway in resistance of *Arabidopsis thaliana* against molluscan herbivores. Mol Ecol 23:1188–1203

Farmer EE, Ryan CA (1990) Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc Natl Acad Sci USA 87:7713–7716

Felton GW (2008) Caterpillar secretions and induced plant responses. In: Schaller A (ed) Induced plant resistance to herbivory. Springer, Stuttgart, pp 369–387

Greistorfer S et al (2017) Snail mucus—glandular origin and composition in *Helix pomatia*. Zoology 122:126–138

Heil M, Karban R (2010) Explaining evolution of plant communication by airborne signals. Trends Ecol Evol 25:137–144

Helms AM, De Moraes CM, Tooker JF, Mescher MC (2013) Exposure of *Solidago altissima* plants to volatile emissions of an insect antagonist (*Eurosta solidaginis*) deters subsequent herbivory. Proc Natl Acad Sci USA 110:199–204

Helms AM et al (2017) Identification of an insect-produced olfactory cue that primes plant defenses. Nature Comm 8:337

Hilker M, Meiners T (2010) How do plants "notice" attack by herbivorous arthropods? Biol Rev 85:267–280

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Ann Rev Plant Bio 59:41–66

Hulme PE (1996) Herbivores and the performance of grassland plants: a comparison of arthropod, mollusc and rodent herbivory. J Ecol 84:43–51

Huot B, Yao J, Montgomery BL, He SY (2014) Growth-defense tradeoffs in plants: a balancing act to optimize fitness. Mol Plant 7:1267–1287

Karban R, Baldwin IT (1997) Induced responses to herbivory. University of Chicago Press, Chicago

Karban R, Orrock JL, Preisser EL, Sih A (2016) A comparison of plants and animals in their responses to risk of consumption. Curr Opin Plant Biol 32:1–8

Kästner J, von Knorre D, Himanshu H, Erb M, Baldwin IT, Meldau S (2014) Salicylic acid, a plant defense hormone, is specifically excreted by a molluscan herbivore. PLoS One 9:e86500

Kats LB, Dill LM (1998) The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience 5:361–394

Kempel A, Schädler M, Chrobrock T, Fischer M, van Kleunen M (2011) Tradeoffs associated with constitutive and induced plant resistance against herbivory. Proc Natl Acad Sci USA 108:5685–5689

Kennedy GG (2003) Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. Ann Rev Entomol 48:51–72

Kim J, Quaghebeur H, Felton GW (2011) Reiterative and interruptive signalling in induced plant resistance to chewing insects. Phytochemistry 72:1624–1634

Koiwa H, Bressan RA, Hasegawa PM (1997) Regulation of protease inhibitors and plant defense. Trends Plant Sci 2:379–384

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation—a review and prospectus. Can J Zool 68:619–640

Manosalva P et al (2015) Conserved nematode signalling molecules elicit plant defenses and pathogen resistance. Nat Comm 6:7795

Meldau S, Kästner J, Von Knorre D, Baldwin IT (2015) Salicylic acid-dependent gene expression is activated by locomotion mucus of different molluscan herbivores. Comm Integ Bio 9:e28728

Mescher MC, De Moraes CM (2015) Role of plant sensory perception in plant-animal interactions. J Exp Bot 66:425–433

Motheral SM, Orrock JL (2010) Gastropod herbivore preference for seedlings of two native and two exotic grass species. Am Midl Nat 163:106–114

Ohgushi T (2005) Indirect interaction webs: herbivore-induced effects through trait change in plants. Ann Rev Ecol Evol Syst 36:81–105

Orrock JL (2013) Exposure of unwounded plants to chemical cues associated with herbivores leads to exposure-dependent changes in subsequent herbivore attack. PLoS One 8:e79900

Orrock JL et al (2015) Error management in plant allocation to herbivore defense. Trends Ecol Evol 30:441–445

Peiffer M, Tooker JF, Luthe DS, Felton GW (2009) Plants on early alert: glandular trichomes as sensors for insect herbivores. New Phytol 184:644–656

Pitt SJ, Graham MA, Dedi CG, Taylor-Harris PM, Gunn A (2015) Antimicrobial properties of mucus from the brown garden snail *Helix aspersa*. Br J Biomed Sci 72:174–181

Poelman EH, van Loon JAJ, Dicke M (2008) Consequences of variation in plant defense for biodiversity at higher trophic levels. Trends Plant Sci 13:534–541

Preisser EL, Bolnick DI, Benard MF (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology 86:501–509

Schaller A (ed) (2008) Induced plant resistance to herbivory. Springer, Stuttgart

Scheiner SM, Gurevitch J (2001) Design and analysis of ecological experiments, 2nd edn. Oxford University Press, New York

Shannon RWR, Félix A-E, Poppy GM, Newland PL, van Dam NM, Hanley ME (2016) Something in the air? The impact of volatiles on mollusc attack of oilseed rape seedlings. Ann Bot 117:1073–1082

Stam JM et al (2014) Plant interactions with multiple insect herbivores: from community to genes. Ann Rev Plant Bio 65:689–713

Stout MJ, Workman KV, Bostock RM, Duffey SS (1998) Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. Oecologia 113:74–81

Strauss SY et al (2009) Cryptic seedling herbivory by nocturnal introduced generalists impacts survival, performance of native and exotic plants. Ecology 90:419–429

Thaler JS (1999) Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature 399:686–688

Thaler JS, Stout MJ, Karban R, Duffey SS (1996) Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. J Chem Ecol 22:1767–1781

Underwood N (2010) Density dependence in insect performance within individual plants: induced resistance to *Spodoptera exigua* in tomato. Oikos 119:1993–1999

van Hulten M, Pelser M, van Loon LC, Pieterse CMJ, Ton J (2006) Costs and benefits of priming for defense in *Arabidopsis*. Proc Natl Acad Sci USA 103:5602–5607

Waldbauer GG (1968) The consumption and utilization of food by insects. Adv Insect Phys 5:229–289

Weissburg M, Smee DL, Ferner MC (2014) The sensory ecology of nonconsumptive predator effects. Am Nat 184:141–157