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Evidence for the transfer of a soil-borne contaminant from plants to ants via an aphid mediator

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Abstract. 1. Uptake of environmental contaminants by lower trophic groups can have negative effects on higher trophic groups. This study tested the ability of selenium, an environmental contaminant found in high concentrations throughout the tissues of certain accumulating plants, to be transferred to ants via aphid tissue and honeydew.

2. Plants of the selenium accumulator, *Raphanus sativus* (wild radish), were watered with three different selenium treatments (0, 0.25 or 0.5 μg Se ml^{-1}). Aphids, *Myzus persicae,* and Argentine ant colonies, *Linepithema humile,* were added to each caged plant and allowed to interact freely. Ant colonies were supplemented with one of three different food options to encourage the consumption of aphids, aphid honeydew, or aphids and honeydew.

3. The accumulation of selenium by each trophic group and a trophic transfer factor (TTF) was calculated. The TTF for plants to aphids was > 1, indicating biomagnification, whereas the TTF for aphids to worker ants was < 1, indicating only biotransfer. Accumulated levels by worker ants did not statistically differ as a result of diet.

4. The amounts of selenium acquired by ants as a factor of diet and caste were compared. Plants, aphids and worker ants accumulated selenium in a dose-dependent manner. Ant queens did not contain detectable amounts of selenium. Honeydew contained comparable amounts of selenium to plant selenium levels.

5. Access to toxic compounds via honeydew and insect protein may have negative effects on the range expansion of invasive species, such as the Argentine ant.

Key words. Aphids, Argentine ant, bioaccumulation, honeydew, pollution, selenium.

Introduction

Ants have been regarded as keystone species for their abundance and role in critical ecological processes (Pętal *et al.,* 1977; Power *et al.,* 1996; Folgarait, 1998; MacMahon *et al.,* 2000) that can impact both the surrounding flora and fauna. The involvement of ants in tritrophic plant–herbivore–ant interactions is especially evident through their mutualistic associations. One of the more common examples is the evolved obligate relationship between the acacia plant and its protective ant inhabitants, where the plant provides both food and shelter to the ants in turn for protection against herbivores (Janzen, 1966). However, there is evidence to suggest that even opportunistic protection by ants may be enough to reduce herbivory for plants that contain food rewards, such as extrafloral nectar (Heil & McKey, 2003 and references therein). The use of ants as potential biological control agents in agricultural settings has also been gaining more attention (Perfecto, 1991; Vandermeer *et al.,* 2002; Morris *et al.,* 2015). On the other hand, ants can also display an opposite effect when the herbivore, rather than the plant, provides the food reward. Such is the case with various species of ants that protect honeydew-producing hemipterans against predation by other organisms in return for their sugar-rich reward (Hölldobler & Wilson, 1990).

Despite the available evidence for the influence of ants on community dynamics involving both plants and arthropods, there is a lack of information regarding the impacts of pollution on trophic interactions. Pollution has the potential to indirectly alter food web dynamics via changes in ant species composition and diversity (Hoffmann *et al.,* 2000; Eeva *et al.,* 2004; Grześ, 2009), behaviour (Sorvari & Eeva, 2010; Barbieri *et al.,* 2013; De La Riva & Trumble, 2016) and health (Sorvari *et al.,* 2007). Previous research has also documented the accumulation of heavy metals in plants and insects collected near ant colonies (Bengtsson & Rundgren, 1984; Heikens *et al.,* 2001; Del Toro & Floyd, 2010), which serve as likely sources of contamination

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for ants, whose diets often comprise both plant resources and insects. Honeydew has also been explored as an alternative exposure route to ants. Głowacka *et al.* (1997) reported heavy metal levels present in psyllid honeydew as a potential source of contamination, but did not measure its transfer to ants. Starý and Kubizňáková (1987) measured pollutant levels in wood ants tending aphid colonies and determined that honeydew was the most likely route of metal acquisition, but they were unable to rule out other possible sources of exposure such as plant nectar, ingestion of seeds or other insect prey. Furthermore, there is no comparison available between the amounts of contaminant that might be acquired from insect prey versus honeydew.

The Argentine ant, *Linepithema humile*, was used in an effort to investigate the ability of an environmental contaminant to enter recently developed tritrophic relationships, following the introduction of an invasive ant species. In its native range the Argentine ant is reported to feed on both carbohydrate and protein sources (Tillberg *et al.,* 2007), but it has become a common problem in agricultural settings in its introduced ranges for its mutualistic relationships with various honeydew-producing plant pests (Bartlett, 1961; Markin, 1970; Daane *et al.,* 2007). This diet flexibility of the Argentine ant has provided it with substantial ecological advantages over obligate carnivory during range expansion (Tillberg *et al.,* 2007) and has been suggested to play a large role in its invasive success (Grover *et al.,* 2007), in locations such as the southwestern United States. The impact of pollution on this novel relationship has yet to be explored. Selenium (Se), an abundant soil-borne metalloid in the western U.S. (Brown *et al.,* 1999), was used as the target contaminant. Although naturally present in rocks and shales from the Cretaceous period, selenium can also be mobilised following human activities such as coal burning, mining, or irrigation (Eisler, 2000). Toxicity can occur in animals ingesting selenium-accumulating plants (Eisler, 1985). Previous studies investigating the concentration of selenium in accumulating plants found high concentrations in resources often consumed by ants, such as the nectar, pollen and seeds (Hladun *et al.,* 2011; Prins *et al.,* 2011; Quinn *et al.,* 2011). When given an artificial nectar source contaminated with selenium, De La Riva *et al.* (2014) found that Argentine ant workers were not deterred even by lethal concentrations in artificial nectar.

Therefore, a study was initiated with the following objectives: (i) to determine whether selenium can be transferred to ants from plants via an aphid intermediate; (ii) to determine the concentration of selenium within each trophic group (plant, aphid, ant) to determine the trophic transfer factor (TTF); (iii) to compare the difference in accumulation of selenium by different castes within the colony; and (iv) to determine whether ants might acquire different levels of selenium when ingesting protein (insect prey) as compared with carbohydrates (honeydew).

Materials and methods

Plant and insect material

Seedlings of *Raphanus sativus* (cultivar 'White Globe'; Livingston Seed Co., Columbus, OH, U.S.A.) were transplanted to small plastic pots containing UC soil mix III (Matkin & Chandler, 1957), watered three times per week and maintained in a greenhouse environment. Miracle Gro nutrient solution (Scotts Co., Marysville, OH, U.S.A.) was added to the watering regime once every other week. When plants were 6 weeks old, 36 healthy plants were transferred to 2.5 l pots to begin treatments with selenium.

Green peach aphids, *Myzus persicae*, were obtained from infested pepper plants in greenhouses located at the UCR Agricultural Operations Field Station. They were placed on caged radish plants and allowed to propagate for two to three generations before further use. A large colony of Argentine ants, *Linepithema humile*, containing queens, brood and several thousand workers, was collected in a wooded grassy area on the UCR campus (33°58′34″ N, 117°19′57″W, 312 m). Thus, the genetic variability of the ants used in these tests was as standardised as possible. Ants were then separated into 36 sub-colonies, each containing ∼300 workers, six queens and brood. Sub-colonies were placed individually in small plastic boxes (20 l × 15 W × 10 H cm) lined with liquid Teflon (PTFE TE-3859; DuPont Fluoroproducts, Wilmington, DE, U.S.A.) to prevent escape. Each box had a lid and a 1 in. breathing hole covered with mesh. A 9 mm Petri dish containing a moistened bottom layer of plaster of Paris was provided as nest material and a cotton-plugged vial of 25% sucrose, a vial of water and approximately two to three chopped cockroaches (*Gromphadorhina portentosa)* were provided as food prior to the beginning of the experiment. Ants remained in these boxes until they were introduced into arenas as described in the following sections.

Experimental design

Each of the 36 potted radish plants was placed in a separate plastic container (31 cm long × 26 cm wide × 10 cm high; Figure S1) and arranged randomly in a greenhouse at the UCR Agricultural Operations Field Station. A 1.27 cm layer of plaster of Paris was poured over the soil at the base of each plant (this was necessary to prevent nesting of ants in the soil following their later introduction). Plants were then randomly assigned one of three selenium treatments (0, 0.25 or 0.5 μg Se ml^{-1}) for a total of 12 plants per treatment. Selenium treatments were chosen to be ecologically relevant and < 1 μg Se ml^{-1} to prevent plants being repellent to aphids (Hladun *et al.,* 2013b).

Treatment solutions were prepared by dissolving sodium selenate powder (Na_2SeO_4, 98% purity; Sigma-Aldrich, St Louis, MO, U.S.A.) in double-distilled water to yield the target concentration. The plants were treated three times per week for the remainder of the experiment by pouring 500 ml of the solution into the plastic containers and allowing the soil to draw up the liquid from the bottom of each pot.

Two days after the initial selenium treatments and addition of plaster, any developing flowers or buds were removed by snipping at the peduncle to ensure that nectar would not later be available to ants. Green peach aphids were then added by cutting leaf sections from the untreated radish plants used to rear the aphid colonies and laying them on the foliage of each of the

36 plants. Each leaf section added contained ~100 aphids. The potted radish plants were then bagged (Figure S2) in order to cage the aphids on each plant.

After 1 week of allowing aphids to acclimate and feed on the radish plants, the 36 sub-colonies of Argentine ants were brought to the greenhouse and paired with a plant. Ants from individual sub-colonies were allowed to enter a caged arena to access the aphids via a plastic tube (30.5 cm length, 1.9 cm diameter) that ran from one of the plastic nest boxes to one potted plant. Glue was added around the holes of the box and the pot at the tube connection sites to ensure the ants would not escape. All nest boxes were also placed in a slightly larger plastic container and moist soil was added around the smaller box. This was done to maintain a cool humid climate for the ants within the greenhouse (Figure S3).

Each of the 36 ant sub-colonies was then assigned one of three supplemental food options (Table 1) added to their colony boxes, in order to manipulate their preference for harvesting honeydew versus eating aphids as a protein source. For the first food option, colonies given a 15 ml vial of water only were predicted to gather honeydew as well as live aphids for protein in order to feed their brood. In the second case, colonies provided with protein (fresh, dry ice-killed aphids that fed on control plants without selenium) were expected to preferentially gather only honeydew from the live aphids in the test arena. Because there was no way to prevent Argentine ants from feeding on honeydew in the presence of live aphids, we did not allow the remaining 12 ant colonies access to living aphids on the plant. Instead, in the third case, they were provided with carbohydrates (25% sucrose) and leaves containing ~25 freshly killed aphids directly from their paired plant. Because consistently removing large numbers of aphids from those plants would have resulted in the population crashing, leaving no live aphids to analyse later, we waited an additional week for this group before providing ants with the killed aphids from their paired plant. However, this delay did allow the ants to acclimate to the feeding system by first providing them with uncontaminated killed aphids. Overall these methods resulted in a total of four replicate systems (plant–aphid–ant interactions) per selenium treatment–food option combination.

Honeydew collection

Collecting honeydew from aphids in the arena would have added another complication to the setup, so we prepared a group of separate plants and aphids for the task. Twelve radish plants were grown in plastic pots (10.16 cm wide × 10.16 cm long × 8.9 cm high) using soil, water and fertiliser methods described earlier. At approximately 6 weeks of age, these plants were randomly separated in space, placed in small plastic boxes (20 cm long × 15 cm wide × 10 cm high) and given one of three selenium treatments as before (0, 0.25 or 0.5 μg Se ml^{-1}). Treatments were administered by pouring 150 ml of the target treatment solution in the plastic box and allowing the soil to draw up liquid from the bottom of the potted plant. Treatments were administered three times per week.

Aphids were introduced a few days later by placing leaf sections containing ~100 uncontaminated aphids on each of the 12 fresh plants. A square piece of foil paper (~12.5 × 12.5 cm) was placed at the base of each plant to collect droplets of honeydew. The foil was collected 2 weeks later, brought back to the laboratory and placed in a −60 °C freezer overnight. Then, each piece of foil was inspected for moulted aphid exuviae. Frozen honeydew allowed for easier removal of the aphid exuviae through the use of a fine paintbrush. Once the foil was cleared of debris, the total wet weight of the honeydew from each piece of foil was obtained using a microbalance. The honeydew was then removed from the foil by taking smaller sections of each foil sample and rinsing them in warm, double-distilled water in a glass funnel. A 1.5 ml microcentrifuge tube was placed at the end of the funnel to collect the rinse. A glass rod also proved helpful in rubbing the honeydew free of the foil during each rinsate. Each foil sample was rinsed using a total of ~1.5 ml of water. The microcentrifuge tubes containing the rinsate honeydew samples were placed in a −40 °C freezer until further use.

Selenium analysis

At 2 weeks after the addition of ants, the experiment was terminated and all plant, aphid and ant material was then sacrificed. Ant nest boxes were placed in a −40 °C freezer. All live aphids were removed from each bagged plant and placed in collection vials. A portion of the plant foliage was taken from each plant and was rinsed off in double-distilled water to remove any remaining debris or aphids. Foliage samples were standardised by utilising fully expanded leaves from the centre of plant, and then placed in separate collection vials. Both aphid and plant samples were then stored in a −40 °C freezer. Ants were removed from the nest boxes and the workers and queens were placed in separate collection vials, and then placed back in the freezer. We were unable to obtain enough ant brood mass for analysis. All frozen plant and insect tissues were freeze-dried (Labconco Corp., Kansas City, Missouri) at −40 °C and −25 psi for 72 and 48 h, respectively. Dried samples were weighed on a microbalance prior to microwave digestion. All plant, insect and honeydew samples were digested with 5 ml concentrated HNO_3 for 20 min, at 1200 W in a microwave oven (CEM Corp., Matthews, NC, U.S.A.). Quantification of selenium concentration was carried out by diluting a portion of the digestate (0.25–1 ml) in a 6 M-HCl matrix, heating for 20 min in a 90 °C water bath, and analysing each sample with hydride generation atomic absorption spectroscopy. Standard reference material for insects (oyster tissue, NIST 1566B) and plants (wheat durum, NIST 8436), selenium spikes and blanks (H_2O) were used to verify recovery. Selenium recoveries in reference material were over 90%.

Data analysis

Statistical analysis was carried out using R v.3.2.2 (2015, The R Foundation for Statistical Computing). Concentration data for plants, aphids and ants could not be normalised with transformation. Therefore, a non-parametric Kruskal–Wallis test with

Table 1. Items listed under 'supplement' are those that were provided to ant colonies in their nest boxes, to manipulate their preference for feeding on the items listed under 'expectation' in the caged arena.

Supplement	Expectation	Total diet
Water only	Contaminated aphid tissue + contaminated honeydew	Water + contaminated aphids + contaminated honeydew
Protein (uncontaminated aphids)	Contaminated honeydew	Uncontaminated aphids + contaminated honeydew
Carbohydrates (25% sucrose)	Contaminated aphids	Sucrose + contaminated aphids

Items listed in bold font under 'total diet' were the sources of selenium acquisition by foraging ants.

a *post hoc* Dunn test was used to compare concentrations accumulated by each group across selenium treatments. To determine which fixed factor (selenium treatment versus food option) influenced the concentration of selenium accumulated by ants, we conducted a two-way ANOVA following a normality check using the Shapiro–Wilk test. Concentration data contained zeros, but was transformed to meet the normality requirements by using the formula $y = \log(x + 1)$, where x represents the original value. Multiple comparisons of honeydew concentrations across selenium treatments were conducted using a Welch test for normal data with unequal variance. Separation of means was conducted with the *post hoc* Games and Howell test for normal data with unequal variance.

We determined the TFF for each trophic exchange by calculating the ratio of selenium concentration in the organism to its food item (DeForest *et al.,* 2007). For example, the selenium concentration in the aphids was compared with the concentration in the plants, and the selenium concentration in the ants was compared with the concentration in the aphids.

Results

Worker ants were observed to tend aphids for their honeydew and collecting fresh-killed aphids. Brood was included to encourage the gathering of insect tissue by workers for protein. In ant colonies provided untreated sucrose and freeze-killed aphids on leaf sections directly from their paired plant, we observed the removal of those aphids from the leaf sections by workers. Those workers were also found to contain selenium in their bodies (Fig. 1a), which was presumably obtained following the ingestion of body fluids from the aphids and/or feeding on larval regurgitant of those aphids. Similarly, in colonies provided untreated aphids or water only, we observed both aphid-tending in arenas and gathering of killed aphids; workers from both of those groups were also found to contain detectable levels of selenium, indicating that ingestion of contaminated honeydew or both contaminated honeydew and selenium-laden aphids had occurred, respectively.

The concentration of selenium present in plant and insects followed a dose-dependent trend (Fig. 2), where a greater concentration of selenium added to the soil resulted in higher accumulated selenium for radish foliage (Kruskal–Wallis, $\chi^2 = 23.3$, d.f. = 2, $P < 0.0001$), aphid tissue (Kruskal–Wallis, $\chi^2 = 24.2$, d.f. = 2, $P < 0.0001$) and worker ants (Kruskal–Wallis, $\chi^2 = 19.5$, d.f. = 2, $P < 0.0001$). However, the *post hoc* Dunn tests revealed that plants and aphids only contained significantly higher concentrations of selenium when treatment groups were compared with control groups (0 vs. 0.25, $P < 0.0001$; 0 vs. 0.5, $P < 0.001$; 0.25 vs. 0.5, $P = 0.12–0.24$). In contrast, worker ants collected from arenas treated with 0.5 μg Se ml^{-1} contained greater selenium body burdens than those from the 0.25 μg Se ml^{-1} group (*post hoc* Dunn test, $P = 0.02$). The concentration of selenium found in the worker ants was not dependent on the different food options (two-way ANOVA, $F = 1.8$, d.f. = 2,28, $P = 0.18$, Fig. 1a), but, rather, on the amount of selenium entering the trophic system in response to different selenium treatments (two-way ANOVA, $F = 19.6$, d.f. = 2,28, $P < 0.0001$, Fig. 1b). Queen ants did not contain detectable amounts of selenium in any of the three treatment groups.

Table 2. Mean selenium concentrations (μg Se g^{-1}) for each trophic group, honeydew (μg Se g^{-1}) and the trophic transfer factors at each trophic step.

	Trophic transfer factor		
Initial soil treatment (μg Se g^{-1})	Aphid:plant	Ant:aphid	Ant:honeydew
0	0.12	0.00	0.00
0.25	1.19	0.04	0.04
0.5	1.23	0.08	0.11

Ratios of metal concentrations of upper trophic groups to lower trophic groups that are > 1 represent biomagnification; those < 1 represent biotransfer.

Aphid honeydew contained selenium (Fig. 3) at concentrations slightly lower than those found in aphid tissue, but comparable to the levels seen in plant tissue. Honeydew collected from plants grown in soil treated with 0.25 and 0.5 μg Se ml^{-1} contained statistically greater levels than honeydew collected from plants watered with no selenium (Welch test, $F = 21.3$, d.f. = 2, 4, $P < 0.01$; *post hoc* Games and Howell, $P < 0.05$); however, concentrations of selenium in honeydew from aphids feeding on plants grown in two treatments with selenium-amended soil were not different from each other (Games and Howell, $P = 0.66$).

The TTF of selenium from plants to aphids was < 1 in the control group, but > 1 in both selenium-treated groups, suggesting the ability of selenium to biomagnify during this first trophic step (Table 2). In contrast, the TTF from aphid tissue and aphid honeydew to ants was < 1, indicating that biotransfer of selenium had occurred without biomagnification.

Discussion

This is the first study to demonstrate empirically the potential for ant food webs to be exposed to environmental contaminants

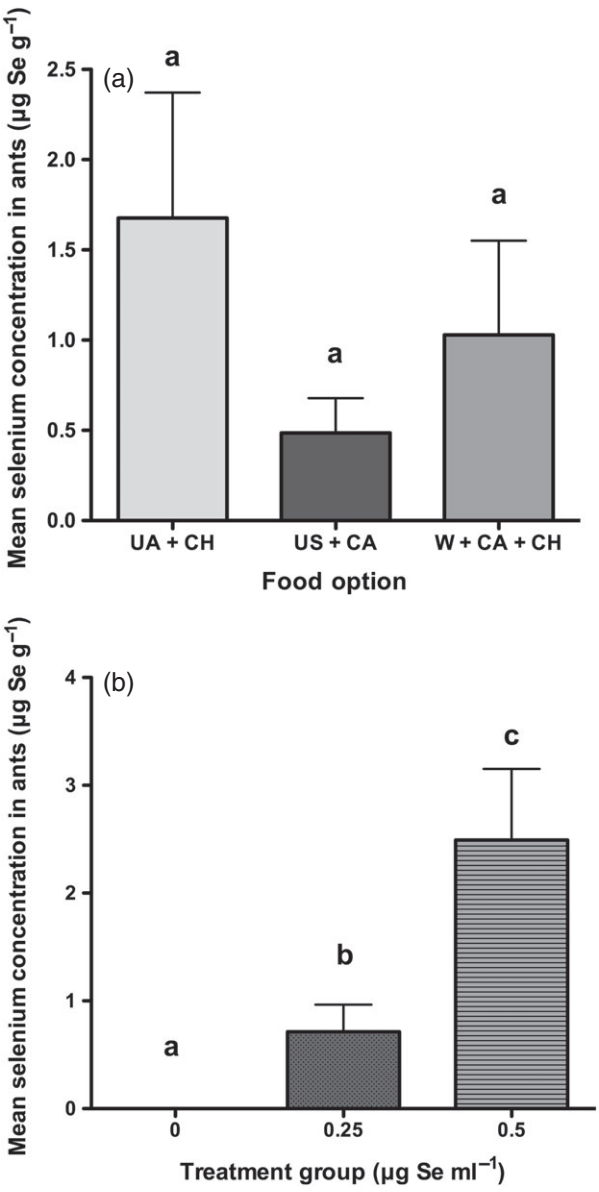

Fig. 1. (a) Mean selenium (Se) concentration in worker ants as a result of different food options: uncontaminated aphids + contaminated honeydew (UA + CH) refers to colonies fed on contaminated honeydew when provided killed, uncontaminated aphids; uncontaminated sucrose + contaminated aphids (US + CA) refers to colonies that consumed uncontaminated sucrose and freeze-killed, contaminated aphids (the only source of Se comprised aphids from contaminated plants); and water + contaminated aphids + contaminated honeydew (WC + CW) are results from colonies that consumed both aphids from contaminated plants and their contaminated honeydew when provided water only (the source of Se was provided in both aphids and honeydew). (b) Mean Se concentration in worker ants as a result of Se transferred from plants grown in soil with three concentrations of Se. Statistical differences are represented by different letters (Kruskal–Wallis test with *post hoc* Dunn test, $\alpha = 0.05$).

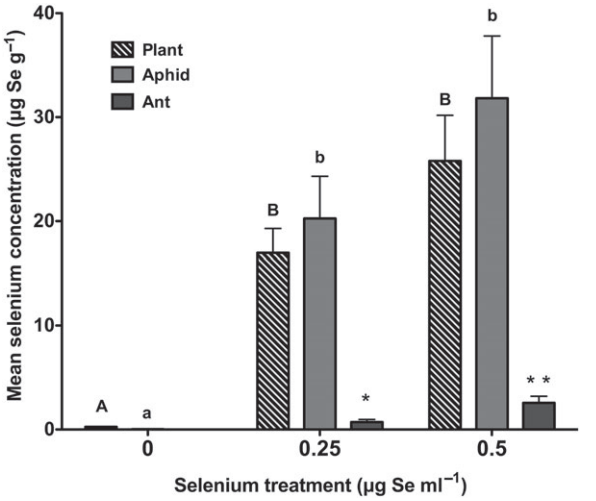

Fig. 2. Average selenium concentrations (µg Se g^{-1}) accumulated by plants, aphids, and ants following three different soil Se treatments. Different letters of the same case or those with different numbers of asterisks represent statistically significant differences (Kruskal–Wallis test with *post hoc* Dunn test, $\alpha = 0.05$). Mean concentrations in plants are compared using capital letters, mean concentrations in aphids are compared using lowercase letters, and mean concentrations in ants are compared using numbers of asterisks.

via their herbivore mutualists, by successfully isolating their exposure to aphid-produced resources. We are certain that selenium was not transferred to the Argentine ants from the plants because there was no floral nectar, pollen or extrafloral nectar available. Adult ants are unable to ingest solid material, so acquisition of selenium from freeze-killed aphids could have occurred by ingesting fluids such as haemolymph or regurgitated material from larvae. No detectable amounts of selenium were measured in queens, but this is not entirely unexpected, as previous studies have also found differences in metal levels across castes. For instance, Hladun *et al.* (2013a) found that honey bee adult foragers fed selenium contained significantly greater body burdens than did larvae. Similar trends have been reported for red wood ant (*Formica* sp.) colonies residing in metal-contaminated sites where differences were found among: (i) workers versus pupae (Migula & Głowacka, 1996); (ii) workers versus pupae and newly emerged sexual progeny (Starý & Kubizňáková, 1987); and (iii) outside workers versus inside workers, pupae and queens (Maavara *et al.,* 1994). Unfortunately, we were unable to gather enough brood mass from the nests to accurately analyse the amount of selenium accumulated by the larvae and pupae. Final counts of brood were not taken after the tests ended, but it is possible that some of the brood may have died during the experiment as a result of selenium ingestion (De La Riva & Trumble, 2016). Future research is necessary to determine the reason behind this pattern of 'negative bioaccumulation' (Maavara *et al.,* 1994) from workers to brood and reproductives. It is expected that individuals that are directly exposed to the source of the pollutant, such as foragers, will contain relatively greater levels. Whether the reduction in body concentration observed

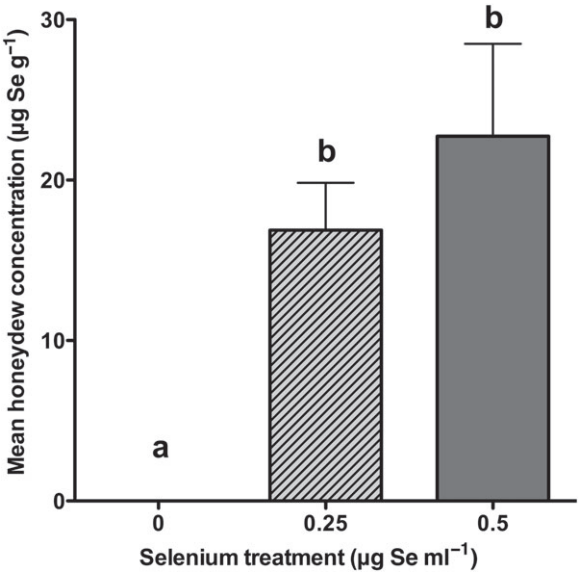

Fig. 3. Mean honeydew concentration excreted by aphids as result of selenium (Se) transferring from plants grown in soil with three concentrations of Se. Different letters indicate means that are statistically different from each other (one-way test with *post hoc* Games and Howell, $\alpha = 0.05$).

among members inside the colony is due to a dilution effect after many incidents of trophallaxis or to workers deliberately protecting other members via an evolved 'socio-biological tolerance system', as hypothesised by Maavara *et al.* (1994), is yet to be elucidated. It is possible that lower levels previously reported in pupae were due to the elimination of toxins between the larval and pupal stages by moulting of the exocuticle and/or excretion via the meconium (Dallinger, 1993; Newman & Unger, 2003). Nevertheless, this does not explain the lower levels seen in other adult members within the colony, as ants do not moult after emerging to the adult stage.

Previous studies appear to agree on the ability of selenium to biotransfer across trophic groups (Vickerman & Trumble, 2003; Mathews & Fisher, 2008), but contrasting results have been reported for the ability of selenium to biomagnify (Liu *et al.,* 1987; Barwick & Maher, 2003). When comparing selenium levels across trophic groups in our study, our findings suggest that selenium was biomagnified from the soil to the plants and again between plants and herbivores. Although selenium was found in statistically greater concentrations among the aphids than in the radish foliage, lower levels of selenium were found in worker ants tending/ingesting those aphids. These findings support previous studies reporting differences in metal excretion abilities among various arthropods (Dallinger, 1993; Grześ, 2010), but are in contrast to higher metal levels seen in ants than in aphids by Starý and Kubizňáková (1987). It appears that green peach aphids are eliminating selenium in their honeydew, but also retaining substantial amounts in their tissue, presumably from the action of metal-binding proteins such as metallothioneins found in other invertebrates (Roesijadi, 1992; Amiard *et al.,* 2006). The presence of much lower selenium levels occurring in the ants suggests that the ants may be excreting selenium. This was a slightly unexpected result due to the fact that workers of the same species bioaccumulated concentrations twice as high as that provided in the sucrose diet in a previous experiment (De La Riva *et al.,* 2014), whereas in this study, ants exposed to aphids with body burdens $> 30\,\mu g\,Se\,g^{-1}$ and/or honeydew $> 30\,\mu g\,Se\,g^{-1}$ were found to contain $< 10\,\mu g\,Se\,g^{-1}$ in their own tissues (Fig. 2). However, it is important to point out that workers in that previous experiment were not exposed in the presence of brood or reproductives and it is possible that selenium in their bodies was passing through at a much lower rate than might occur when other caste members are present. Body concentrations in aphids in both selenium treatment groups averaged above $20\,\mu g\,Se\,g^{-1}$; yet, concentrations as low as $5\,\mu g\,Se\,ml^{-1}$ have been shown to negatively impact both queen fecundity and viability of developing offspring in Argentine ant colonies (De La Riva & Trumble, 2016).

The findings of this study highlight the potential for both natural and anthropogenically occurring sources of pollution in the environment to influence trophic interactions. Previous studies measuring pollutant effects on ant communities have focused on native species (Eeva *et al.,* 2004; Sorvari *et al.,* 2007; Grześ, 2009; Del Toro & Floyd, 2010; Sorvari & Eeva, 2010). This is the first study to investigate the transfer of a contaminant within a relatively newly established tritrophic system involving an invasive ant species. Argentine ants are largely omnivorous in their native ranges, but in their introduced ranges, their relative trophic position may differ with the stage of invasion as a result of food availability (Tillberg *et al.,* 2007). Nevertheless, our results demonstrate that exposure can occur by consuming both insect prey and honeydew, which is likely to influence their expansion into contaminated habitats. Toxic honeydew might also bring about negative effects for native species residing in that same habitat that also seek out honeydew in addition to plant nectar (De La Riva & Trumble, 2016). In this study diet was less of a factor than the concentration present in the source material, but future research should be conducted to determine whether this might change with time. Exposure levels have been shown to vary across species as a result of diet preferences in other studies. Mogren *et al.* (2013) found that levels of arsenic transferred to predators differed based on their feeding strategy, where preying mantids consuming entire arsenic-laden mosquitoes accumulated higher levels than spiders that consumed only internal mosquito body fluids. Species-specific accumulation patterns of heavy metals have also been described for ants residing in the same habitat (Rabitsch, 1997; De La Riva *et al.,* 2017), suggesting possible differences in both metal regulation and diet preferences. Exposure levels may also differ seasonally, depending on the source of the contaminant available in the environment, such as changes in floral nectar availability or herbivore populations. Seasonal differences may also occur within a particular population, depending on the colonies' current dietary requirements.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12415

Figure S1. Potted radish plant with a layer of plaster covering the soil surface to prevent ant nesting in the soil. The pot is placed in a plastic shoebox container where treatment solutions are added. Holes at the bottom of each black pot allow the soil and plant to take up the solution.

Figure S2. Caged radish plant. The mesh bag has a circular plastic observer window. The clear tube sticking out of the right side of the pot was later attached to a plastic ant colony box to allow ants to enter the arena through the tubing.

Figure S3. Tubing from the main plant arena is extended through the soil and attached to an entry hole at the side of the ant nest box (blue lid). The soil was added around the nest box as an insulator in the greenhouse. A larger lid was gently laid over both boxes to create darkness in the nest.

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