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Plant-mediated interactions between a vector and a non-vector herbivore promote the spread of a plant virus

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Herbivores that transmit plant pathogens often share hosts with non-vector herbivores. These co-occurring herbivores can affect vector fitness and behaviour through competition and by altering host plant quality. However, few studies have examined how such interactions may both directly and indirectly influence the spread of a plant pathogen. Here, we conducted field and greenhouse trials to assess whether a defoliating herbivore (*Sitona lineatus*) mediated the spread of a plant pathogen, *Pea enation mosaic virus* (PEMV), by affecting the fitness and behaviour of *Acrythosiphon pisum*, the PEMV vector. We observed higher rates of PEMV spread when infectious *A. pisum* individuals shared hosts with *S. lineatus* individuals. Using structural equation models, we showed that herbivory from *S. lineatus* increased *A. pisum* fitness, which stimulated vector movement and PEMV spread. Moreover, plant susceptibility to PEMV was indirectly enhanced by *S. lineatus*, which displaced *A. pisum* individuals to the most susceptible parts of the plant. Subsequent analyses of plant defence genes revealed considerable differences in plant phytohormones associated with anti-herbivore and anti-pathogen defence when *S. lineatus* was present. Given that vectors interact with non-vector herbivores in natural and managed ecosystems, characterizing how such interactions affect pathogens would greatly enhance our understanding of disease ecology.

1. Introduction

Most described plant viruses rely on arthropod vectors for transmission [1,2]. As vectors forage, they interact with non-vector herbivores on shared host plants [3,4]. Such interactions can affect vector movement [5–7] and host selection [8–10]. For example, chewing herbivores can alter host attractiveness to vectors, and host susceptibility to pathogens, by affecting plant defences [9,10]. Non-vector herbivores also can affect host quality, including plant nutrient composition, and subsequent behaviour of vectors [8–10]. Competitive interactions between vector and non-vector herbivores may also alter vector abundance [11–14]. Yet whether such interactions also affect pathogens has rarely been assessed outside of laboratory settings [5,6].

Plant–insect and vector–pathogen relationships can be altered by direct interactions such as herbivory, and indirectly, such as through changes to plant traits in response to herbivores. For example, non-vector herbivores may indirectly affect vectors and pathogens indirectly through 'plant-mediated effects'. Mechanistically, these indirect effects stem from induced responses to herbivory, such as when plants produce defensive responses in response to a particular herbivore that affects subsequent performance of other herbivores or pathogens [15,16]. Plant-mediated indirect effects of non-vector herbivores can exert either positive or negative effects on vectors and pathogens that often depend on the vector's feeding guild and crosstalk between hormonal signalling pathways [15,16]. Chewing herbivores, for example, can induce jasmonic acid in plants,

which may inhibit expression of defences associated with salicylic acid [15,16]. In such cases, if plant defence against vectors is regulated by salicylic acid, chewing herbivores might indirectly promote vector performance by inhibiting salicylic acid expression [9]. Yet if vector and non-vector herbivores activate similar plant pathways, prior herbivory may impede vectors by 'priming' plant defences [12]. While rarely tested, such indirect interactions may affect vector-borne pathogens as similar signalling pathways in plants may regulate both anti-herbivore and anti-pathogen defence [9,10,15,16].

Non-vector herbivores might also exert direct effect on vectors and thus indirect effects on pathogen transmission. If non-vector herbivores compete with vectors for plant resources, for example, competition may reduce vector abundance [3,4]. This direct effect of a non-vector herbivore on a vector might then indirectly affect a pathogen, as greater vector abundance typically promotes pathogen transmission [5,6]. Competition can also affect the distribution of vectors on individual plants, with weak competitors forced to avoid structures occupied by a dominant competitor [11,17]. This may affect pathogens if unique plant structures vary in susceptibility to pathogen inoculation [18–21]. Moreover, if vectors disperse away from plants occupied by non-vector herbivores [3,4], pathogen transmission could be promoted by exposing vectors to new susceptible hosts [6].

Although considerable evidence suggests that non-vector herbivores affect vectors, few studies have examined subsequent indirect effects on vector-borne pathogens [5,6]. We addressed this knowledge gap by incorporating data from a series of field and greenhouse experiments into structural equation models [22] to assess the effects of a non-vector herbivore on a vector and a plant virus. Our system included a defoliating non-vector herbivore, *Sitona lineatus*, and a plant pathogen vector, *Acyrthosiphon pisum*. We hypothesized that *S. lineatus* might affect a pathogen transmitted by *A. pisum* (*Pea enation mosaic virus*, PEMV) by influencing *A. pisum* performance, plant-to-plant movement, within-plant movement or host plant susceptibility. Our application of structural equation models allowed us to untangle the direct and indirect pathways by which *S. lineatus* affected *A. pisum* and PEMV. Complementary analyses of plant phytohormones allowed us to assess the plant-mediated mechanisms underlying these community-level patterns. Overall, our study revealed how interactions between a vector and non-vector herbivore on single host plants may affect pathogen spread at broader scales.

2. Material and methods

(a) Study system

The Palouse region of eastern Washington and northern Idaho, USA, supports a diverse assemblage of native and cultivated legumes such as vetch, clover and pea [23]. Two species of herbivores that commonly co-occur on these hosts are the pea leaf weevil (*S. lineatus*), a chewing insect, and the pea aphid (*A. pisum*), a piercing–sucking insect [24]. *Acyrthosiphon pisum* individuals can transmit the pathogen PEMV to multiple legume species [25]. This pathogen, which is obligately transmitted by aphids and consists of a symbiotic relationship between an *Enamovirus* and *Umbravirus*, is transmitted in a persistent, circulative manner [9,24,26]. As a persistent, circulative virus, once *A. pisum* acquires PEMV from a host plant, the virus passes through the gut, into the haemolymph and eventually to the salivary glands before transmission to subsequent plants can occur.

As a phloem-feeder, *A. pisum* may be strongly affected by plant-mediated indirect effects of *S. lineatus* if herbivory affects plant defences expressed in the phloem. Direct competition between *S. lineatus* and *A. pisum* could also affect aphid abundance and distribution, and such effects may indirectly affect PEMV transmission. These herbivores commonly co-occur on *Pisum sativum* and weedy legumes such as *Vicia villosa* (hairy vetch) [9,24,26,27]. *Sitona lineatus* is highly abundant in *P. sativum* fields, with data showing that nearly 90% of individual plants have feeding damage [26]. *Sitona lineatus* and *A. pisum* also commonly co-occur on weedy legumes, where they interact in ways that can affect PEMV transmission [27]. In the Palouse, *S. lineatus* has two generations a year, and overwintering adults colonize cultivated and weedy legumes in the spring [9,26]. After feeding, adults lay eggs on the soil, and subsequent larval stages feed on plant roots before pupating [26]. These individuals then emerge as second-generation adults in the mid-summer. Thus, *S. lineatus* is present during the season when *A. pisum* colonizes *P. sativum* fields (generally June through September) and transmits PEMV [9,26]. We thus hypothesized that direct and indirect interactions between *S. lineatus* and *A. pisum* on shared hosts may affect PEMV transmission.

Sitona lineatus individuals were field collected from commercial pea fields, or native legumes, immediately prior to each experiment. Colonies of *A. pisum* were started in 2012 from a field-collected population and maintained in greenhouses (16 : 8 h light : dark; 22 : 17°C light : dark) on PEMV-infected or non-infected peas (variety Banner). PEMV inoculum was obtained from an infectious *A. pisum* colony established from aphids collected from infected plants in the field near Moscow, ID, USA. The infectious *A. pisum* colonies were tested for the presence of other common viruses in the Palouse, including *Pea streak virus* and *Bean leaf roll virus*, and did not harbour these viral pathogens.

(b) Effects of *Sitona lineatus* on *Acrythosiphon pisum* and *Pea enation mosaic virus*

We conducted a two-block experiment to determine (i) whether *S. lineatus* affected the behaviour and fitness of infectious *A. pisum* on shared plants and (ii) whether *S. lineatus* affected the spread of PEMV. The first block was conducted in the field in 2015, in 60 cm^3 mesh cages at the Washington State University (WSU) Tukey Orchard in Pullman, WA, USA. Six replicates were conducted for each treatment (with or without *S. lineatus*), for a total of 12 experimental units. The second block was conducted in a greenhouse at WSU in 2017 in the same cages. This block was run to increase statistical power and to determine if trends seen in the field could be replicated under controlled greenhouse conditions. Twelve replicates were conducted for each treatment, for a total of 24 experimental units. Thus, across both of the blocks of the experiment, we had 36 total experimental units (12 in the field and 24 in the greenhouse). Greenhouse conditions were 16 : 8 h photoperiod (light : dark) with temperatures of 21–24°C during the light phase and 16–18°C during the dark phase. All experiments were conducted on pea plants grown from seed (variety Banner) in Sunshine Mix LC1 potting media (Sun Gro Horticulture, MA, USA).

In both blocks, treatments were applied in single-plant cages arranged in a grid (2 × 6 in the field and 4 × 6 in the greenhouse) with 1 m spacing between cage perimeters (electronic supplementary material, figure S1). On each *P. sativum* plant, we first released 25 apterous, 4-day-old, infectious *A. pisum* individuals using a paintbrush with a 5 mm head. The *S. lineatus* treatment was then applied by placing 0 (control) or 2 (treatment) adult *S. lineatus* onto plants immediately afterwards. Twenty-four hours later, the

number of settled *A. pisum* individuals (i.e. individuals on plants) was recorded and plants were moved, along with the aphids and weevils, to the middle of an eight-plant ring of pea plants enclosed by the mesh cage in a 3 × 3 grid (electronic supplementary material, figure S2).

Six days later, the number and stage (nymph or adult) of *A. pisum* individuals infesting each plant from each cage was recorded (electronic supplementary material, figure S2). Since *A. pisum* individuals require 7–10 days to reach full size and maturity [28], the released *A. pisum* adults were distinguishable from *A. pisum* nymphs born during the experiment. At this time, terminal leaflets were clipped from each plant and tested with a commercial double-antibody sandwich (DAS) ELISA (AC Diagnostics, Fayetteville, AR, USA) for the presence of PEMV [29]. Samples were read in duplicate, and plants were diagnosed as infected if their absorbance value was greater than or equal to two times the value of the negative control [29].

(c) Examining displacement between herbivores

We conducted a series of experiments to assess mechanisms by which *S. lineatus* affected *A. pisum* and PEMV. The first determined if *S. lineatus* affected *A. pisum* feeding location within plants. This greenhouse (16 : 8 h light : dark; 22 : 17°C light : dark) assay was conducted on individually caged two-week-old pea plants that were exposed to one of two treatments: (i) *S. lineatus*—two adults released or (ii) undamaged—no adults released; eight replicates were conducted for each. After 2 days of feeding, *S. lineatus* individuals were removed from plants and we then released fifteen 4-day-old *A. pisum* onto the plants. As *S. lineatus* was removed before adding aphids, effects on *A. pisum* were driven by indirect 'plant-mediated effects'. After 24 h, we recorded the height (node) of each *A. pisum* individual. The number of *S. lineatus* feeding notches on the two leaves on each node was also recorded to assess herbivory.

(d) Feeding location and inoculation success

We next determined if *A. pisum* feeding location influenced the inoculation efficiency of PEMV with a 3 × 5 factorial greenhouse (16 : 8 h light : dark; 22 : 17°C light : dark) experiment that manipulated *A. pisum* feeding location (top, middle or bottom of plants) and density (two, four, six, eight or ten 6-day-old infectious *A. pisum*). Three replicates were conducted for each treatment combination. Cages enclosing the top of the plant contained the top two vegetative nodes, and were constructed from a mesh bag attached to a support (electronic supplementary material, figure S3). Bottom cages contained the lowest two vegetative nodes and were constructed from inverted plastic medicine cups (30 ml) with a slit to allow it to be fitted over the plant (electronic supplementary material, figure S3). Following the addition of *A. pisum*, the slit was taped and the cup was sealed with cotton balls. *Acyrthosiphon pisum* individuals applied to the middle were not caged, but were restricted from feeding at the top and bottom. Top and bottom cages were applied to each plant regardless of treatment to control for cage effects.

After an inoculation period of 2 days, *A. pisum* individuals were killed with an application of 60 ml of a 5% imidacloprid solution to the soil of each plant. After this insecticide treatment, we waited 6 days to allow viral titre to build to detectable levels, after which the aboveground portions of the plant were harvested and analysed for the presence of PEMV with ELISA.

(e) Viral titre and defence gene expression across feeding locations

We conducted another greenhouse assay (16 : 8 h light : dark; 22 : 17°C light : dark) to assess if *S. lineatus* affected the expression of genes associated with salicylic acid (*Pathogenesis-related protein 1*, PR1) [30–32] and jasmonic acid (*12-oxophytodienoate reductase 1*, OPR1) [32,33] phytohormones across plant feeding locations (top, middle, bottom). The experiment involved four treatments on two-week-old plants: (i) control—uninfectious *A. pisum* only, (ii) infectious *A. pisum* only, (iii) *S. lineatus* only, and (iv) infectious *A. pisum* + *S. lineatus*; each was replicated five times. Prior work has shown that uninfectious *A. pisum* do not affect phytohormones in this system [9]. Thus, to isolate the effects of *S. lineatus* in the context of PEMV infection, we used uninfectious *A. pisum*, rather than plants with no herbivores, as our baseline control condition. In *S. lineatus* treatments, two adults were allowed to feed for 48 h, after which they were removed. Fifteen 6-day-old adult *A. pisum* were added to each plant (either all uninfectious or infectious), with five individuals in each of the feeding locations as in the prior experiment. Aphids were allowed to feed for 48 h before being removed. After treatments were applied, individual plants were moved to bug dorms for 7 days to allow PEMV symptoms to develop [9]. Although differences in the expression of *PR1* and *OPR1* following treatments may manifest earlier than 7 days, and could vary over time, we did not take earlier samples for phytohormone measurements as this may have unintentionally affected plant subsequent development of PEMV, which cannot be reliably detected until 6–7 days post-inoculation [9]. Moreover, prior work in this system has shown that induced changes in phytohormones following attack from PEMV and *S. lineatus* can be detected 6–10 days post-inoculation [9].

After 7 days, we harvested plant tissue from each location, with the 'top' consisting of tissue from the first two nodes, 'bottom' the bottom two nodes and 'middle' the remaining nodes. Tissue samples were wrapped in aluminium foil, frozen in liquid N_2 and snap chilled in dry ice before storing in −80°C. Total RNA was extracted using a Promega SV total RNA kit following the instructions (Promega), and cDNA from 1 µg of total RNA from each sample using Bio-Rad iScript cDNA Synthesis Kit. Gene-specific primers for *PR1* (Forward [F] 5′ TGGGGCAGTGGTGACATAAC 3′; Reverse [R] 5′ TGCGCCAAACAACCTGAGTA 3′), *OPR1* (F 5′ AAGTGAATGACAGAACCGATGA 3′; R 5′ ATGGAAACCGACAGCGATT 3′), as well as a reference gene, Psβ-tubulin (F 5′ GTAACCCAAGCTTTGGTGATC 3′; R 5′ ACTGAGAGTCCTGTACTGCT 3′) were used in qRT-PCR reactions (10 µl) containing 3 µl of ddH_2O, 5 µl of iTaq Univer SYBR Green Supermix (Bio-Rad), 1 µl of specific primer mix and 1 µl of diluted (1 : 25) cDNA template. The qRT-PCR program included an initial denaturation for 3 min at 95°C followed by 40 cycles of denaturation at 95°C for 15 s, annealing for 30 s at 60°C, and extension for 30 s at 72°C. For melting curve analysis, a dissociation step cycle (55°C for 10 s, and then 0.5°C for 10 s until 95°C) was added. All reactions were in a CFX96 qRT-PCR machine using 96-well Microseal plates.

(f) Data analysis

(i) Mesocosm experiments

All statistical analyses were conducted in R v. 3.5.2 [34] using base functions unless otherwise specified. We analysed the mesocosm experiments with a series of generalized linear models, using the MASS package for model selection [35]. Response variables were: (i) proportion of infected plants (weighted binomial fit for the number of infected plants out of nine), (ii) proportion of aphids off the source plants (out of total aphids; this was an indicator of movement), and (iii) aphid per capita reproduction (number of nymphs produced relative to the number of initial adults). Experimental block (field or greenhouse) was included in all models initially but was dropped because effects on model fit were insignificant ($\Delta AIC < 2$).

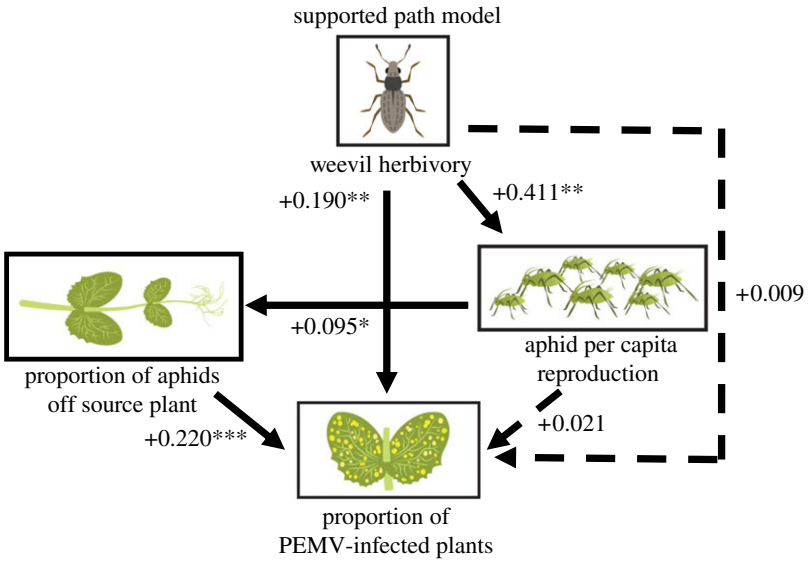

Figure 1. Accepted path model by confirmatory path analysis (Fisher's $C = 3.25$, $p = 0.52$, d.f. = 4), where 'weevil' is *S. lineatus* and 'aphid' is *A. pisum*. Values on solid lines indicate standardized β coefficients with asterisks indicating levels of statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Dotted lines are inferred strengths of indirect effects calculated by multiplying intermediate path coefficients. (Online version in colour.)

We used confirmatory path analysis to evaluate a putative interaction network among *S. lineatus*, *A. pisum* reproduction, *A. pisum* movement and the proportion of plants infected with PEMV using the piecewiseSEM package [22]. Path analysis allowed us to estimate the relative strength of direct and plant-mediated effects of *S. lineatus* on *A. pisum* and PEMV. We included all significant effects from GLMs on the proportion of infected plants, proportion of *A. pisum* off the source plant (our metric of *A. pisum* plant-to-plant movement) and *A. pisum* reproduction. A path model can be accepted when no significant direct effects are missing, and thus connections were added iteratively until a path model was accepted ($p > 0.05$) [22].

(ii) Competitive displacement

Analyses of the displacement experiment involved generalized linear models in R [34] (logit regression). These models tested whether *S. lineatus* feeding affected the within-plant distribution of *A. pisum*. The first model tested if the proportion of *S. lineatus* feeding notches (relative to total notches on plants) was related to the height of the plant (node number). The second model tested if the proportion of *A. pisum* at a node (relative to total *A. pisum* on the plant) was related to the number of *S. lineatus* feeding notches on leaves at the same node.

(iii) Feeding location and inoculation success

For the inoculation experiment, we used logistic regression to model plant infection status as a function of *A. pisum* abundance and feeding location, which was treated as an ordinal variable (bottom, 1; middle, 2; top, 3). As there was no significant interaction (abundance × location) effect ($\alpha = 0.10$), we only included the two main effects in the final model.

(iv) Defence gene analysis

The relative transcript abundance of *PR1*, a gene representative of salicylic acid hormone, and *OPR1*, a gene representative of jasmonic acid, were calculated using the ΔCt method, ($2^{-\Delta Ct}$) using Psβ-tubulin as a reference gene [36,37]. The control with non-infectious aphids and no weevils set baseline gene expression levels (no change in expression = 0). We then evaluated results for ΔCt using MANOVA for *PR1* and *OPR1* expression levels.

3. Results

(a) Direct and indirect effects of *Sitona lineatus* on *Acrythosiphon pisum* and *Pea enation mosaic virus*

The presence of *S. lineatus* increased the proportion of PEMV-infected plants compared to mesocosms without *S. lineatus* (figure 1). Confirmatory path analysis revealed the direct and indirect pathways by which *S. lineatus* mediated the proportion of plants infected with PEMV (figure 1; electronic supplemental material, table S1). Specifically, herbivory by *S. lineatus* increased aphid per capita reproduction ($\beta_{std} = 0.41$, $p = 0.001$), which stimulated a greater proportion of aphids to move off the source plant ($\beta_{std} = +0.096$, $p = 0.031$), which correlated with a greater proportion of PEMV-infected plants ($\beta_{std} = +0.22$, $p = 0.010$). *Sitona lineatus* also directly increased the proportion of PEMV-infected plants directly in ways that were not explained by aphid reproduction or movement ($\beta_{std} = +0.19$, $p = 0.002$) (figure 1).

(b) Competitive displacement between *Sitona lineatus* and *Acrythosiphon pisum*

As *S. lineatus* affected *A. pisum* and PEMV, we assessed underlying mechanisms. First, we assessed whether *S. lineatus* affected within-plant distribution of *A. pisum* on *P. sativum* plants. We found a potentially biologically relevant negative relationship between the number of *S. lineatus* feeding notches and the plant node ($z = -1.75$, $p = 0.080$) that suggests weevils prefer to feed close to the ground (figure 2*a*). We found a significant negative relationship between the proportion of *A. pisum* at a given node and the number of *S. lineatus* feeding notches at the same node ($z = -3.26$, $p = 0.001$), which indicated *A. pisum* avoiding feeding on parts of the plant that were damaged by herbivory from *S. lineatus* individuals (figure 2*b*).

(c) Feeding location and inoculation success

The second mechanistic experiment assessed whether the feeding location of *A. pisum* individuals affected PEMV transmission. This experiment revealed that inoculation success

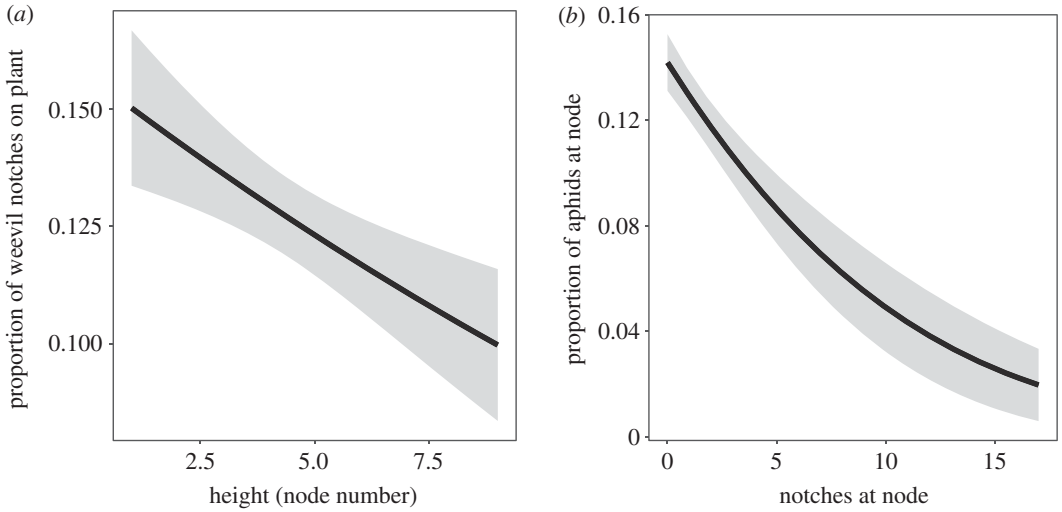

Figure 2. (*a*) Distribution of *S. lineatus* (weevil) feeding notches on *P. sativum* plants and (*b*) distribution of *A. pisum* (aphid) across plant nodes in relation to *S. lineatus* feeding notches. As *A. pisum* avoids *S. lineatus* feeding sites, *A. pisum* adults skew more towards higher vegetative nodes when *S. lineatus* is present. In each panel, values shown represent the best-fit trend lines from the logit regression models, along with the 95% confidence bands around the regression lines.

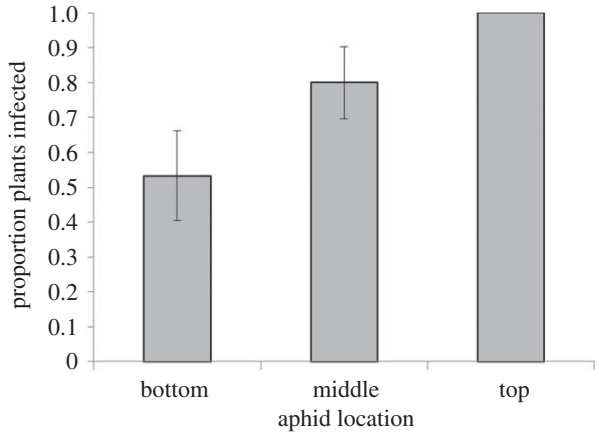

Figure 3. Influence of *A. pisum* (aphid) feeding location on the probability of successful inoculation of plants with PEMV. Values shown represent the means and standard errors for each feeding location. Values shown at each location represent means across all levels of *A. pisum* density.

significantly increased when *A. pisum* fed higher on the plant ($Z = 2.68$, $p = 0.007$; figure 3), regardless of the abundance of inoculating *A. pisum* individuals ($Z = 0.86$, $p = 0.390$). When *A. pisum* individuals were restricted to the top two nodes of the plant, inoculation success was 100%, compared to 53% when they were restricted to the bottom two nodes (figure 3).

(d) Defence gene expression

The expression of defence genes in *P. sativum* plants was modified by *S. lineatus* herbivory and was dependent on the location where *A. pisum* fed on the plant (MANOVA, Pillai = 0.85, $p = 0.003$; figure 4; electronic supplementary material, table S2). The SA-mediated *PR1* gene was not induced by *S. lineatus* herbivory, but when *S. lineatus* was present on the same plants as infectious *A. pisum*, the expression of *PR1* was significantly higher in the 'bottom' compared with the 'top' of plants. By contrast, in the absence of *S. lineatus*, *PR1* expression was highest in the 'middle' of plants (figure 4; electronic supplementary material, table S2). The JA-mediated *OPR1* gene was affected by *S. lineatus* but not by *A. pisum* or PEMV; *OPR1* levels were highest in the 'bottom' of the plant when *S. lineatus* was present, regardless of whether *A. pisum* was also present (figure 4; electronic supplementary material, table S2).

4. Discussion

Our study shows that non-vector herbivores can have strong direct and indirect effects on the spread of a vector-borne plant pathogen. Vector fitness and movement are key factors that affect the spread of vector-borne pathogens [38–40]. We thus hypothesized that *S. lineatus* might influence PEMV by affecting the fitness, behaviour or distribution of *A. pisum*, the PEMV vector or by affecting host plant susceptibility to the PEMV pathogen. This was supported in that A. pisum per capita reproduction was greater on plants in the presence of *S. lineatus*, which appeared to cause crowding that stimulated a greater proportion of *A. pisum* to move to new host plants, promoting the spread of PEMV. At the same time, *S. lineatus* displaced *A. pisum* to the most susceptible locations on individual plants, which promoted PEMV transmission. Overall, our study provides strong evidence that interactions between vector and non-vector herbivores can mediate the dynamics of vector-borne plant pathogens.

While previous studies have similarly shown that non-vector herbivores might affect a plant pathogen vector [9,12,13,26], our novel application of structural equation models to a plant pathosystem allowed us to elucidate the direct and indirect pathways underlying these effects. Our structural equation modelling approach suggested that the most pronounced effect of *S. lineatus* on PEMV appeared to be driven by displacement of *A. pisum* to younger plant tissue higher on individual plants, and this tissue was more susceptible to PEMV inoculation. This was revealed by higher parameter values associated with the direct pathway from *S. lineatus* to PEMV than the indirect pathways (figure 1). Greater susceptibility to infection in younger leaves is reported in plant pathosystems [18–21], a phenomenon that has also been reported in peas and is associated with PEMV management [41]. Herbivory by *S. lineatus* can also suppress anti-pathogen defences in this system [9], which may also provide additional context for the strong direct effects observed in the structural equation models.

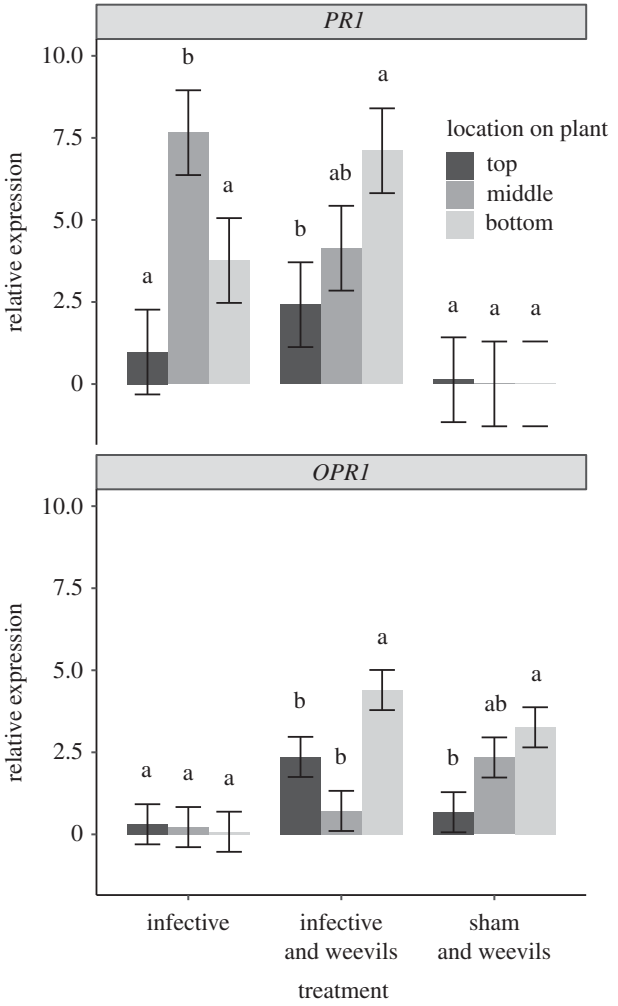

Figure 4. Effects of *S. lineatus* (weevil) and infectious *A. pisum* (infective) feeding on the relative expression of the JA-mediated gene *OPR1* and the SA-mediated gene *PR1*. Bar height indicates average response to treatments measured as fold change in gene expression ($2^{-\Delta\Delta Ct}$). All bars are relative to control (no aphids or weevils), which was set to a value of 0 for the ease of comparison. Error bars indicate standard error of the mean (s.e.m.). Bars not connected by the same letter within each gene are significantly different (Tukey's HSD).

The greater susceptibility of younger leaves to infection was consistent with increased expression of *PR1*, a gene associated with anti-pathogen defence [30–32]. Differential expression of *PR1* was most pronounced when *S. lineatus* was present, which may account for the positive effects of *S. lineatus* in increasing susceptibility to PEMV. We also found that expression of the *OPR1* gene, which is associated with defence against defoliating insects, was highest at the bottom of plants when *S. lineatus* was present. Our gene expression data were not consistent with the literature on crosstalk [15,16,42,43], however, as expression of *PR1* did not lead to the suppression of *OPR1*. However, as we only took *P. sativum* tissue samples 7 days after inoculation with PEMV, we may have failed to detect potential crosstalk that could have occurred at earlier time points. For example, it is possible that induced defences in response to *S. lineatus* and *A. pisum* may manifest most strongly within 48 h after feeding damage, but this response may attenuate by 7 days when we measured gene transcripts. An alternative (although not mutually exclusive) explanation is that crosstalk mechanisms may be weaker in legumes than in other more well-studied plant families such as the Solanaceae [9,15,16,42].

While direct effects of *S. lineatus* on host plant susceptibility appeared to most strongly mediate the spread of PEMV in mesocosms, indirect effects of *S. lineatus* that were mediated through effects on *A. pisum* performance and behaviour were also significant. Specifically, *A. pisum* per capita reproduction increased when *S. lineatus* was present, which may be partially explained by movement of *A. pisum* to locations on plants that had reduced anti-herbivore defences. The spike of *OPR1* at the bottom of plants may provide an explanation for why *A. pisum* individuals were displaced from those areas, since *OPR1* is associated with defence against piercing-sucking insects [32,33]. Moreover, as *S. lineatus* is a voracious feeder than consumes considerable amount of leaf tissue [9,26,27], it is also likely that movement of *A. pisum* to higher locations on plants was simply in response to the availability of resources.

In turn, greater *A. pisum* per capita reproduction led to an increased proportion of individuals off of source plants, an indicator of increased plant-to-plant movement. Prior studies show that when *A. pisum* individuals reach high densities on plants, crowding can exacerbate plant-to-plant movement or alate production [44,45]. Disease ecology models show that the prevalence of persistent pathogens can increase in response to density-dependent movement [46] or when vectors increase plant-to-plant movement in response to interactions with competitors [47]. Although our design did not allow us to directly measure carrying capacity or crowding, our results are probably explained at least in part by such a mechanism, where increased *A. pisum* reproduction in the presence of *S. lineatus* led to crowding that promoted plant-to-plant movement and subsequent PEMV transmission. Moreover, as *A. pisum* congregated on a smaller overall proportion of plants when *S. lineatus* was present, this may have exacerbated crowding compared to when aphids were more evenly distributed across plants. Regardless of whether *S. lineatus* altered *A. pisum* performance and behaviour through direct competition, indirect plant-mediated effects or both, it is clear that interactions between these herbivores affected vector movement and dynamics of PEMV transmission. We show that incorporating such data into structural equation models is a powerful statistical approach to untangle these complex pathways and test specific hypotheses by which a non-vector herbivore can affect a vector herbivore and pathogen transmission.

Our study highlights the role of interactions between vector and non-vector herbivores in mediating the spread of a vector-borne plant pathogen. In agriculture, pest management is typically based on direct damage, without considering interactions with other pests. Although *S. lineatus* typically causes little damage to plant yields [48], yield losses due to PEMV are a serious problem for legume producers [9,24]. Given that *S. lineatus* promoted PEMV spread, this species should be given greater consideration for its indirect impacts. For example, *S. lineatus* may need to be managed more aggressively when *A. pisum* and PEMV are also present in a legume field. More broadly, competition among herbivores is ubiquitous in natural and managed ecosystems, with complex herbivore assemblages competing for the same plant resources [3,4]. Among the most important agricultural crops in the world, non-vector defoliators almost always co-occur with vectors and viral pathogens they transmit [3,4].

Understanding the interplay between vector and non-vector species is thus critical for basic disease ecology and for developing effective integrated disease management strategies.

Data accessibility. All data available as part of the electronic supplementary material.

Authors' contributions. P.J.C., S.D.E., S.B. and D.W.C. conceived the ideas and designed methodology; P.J.C., S.B. and R.E.C. collected the data; P.J.C., R.E.C. and D.W.C. analysed the data; all authors contributed critically to the drafts and gave final approval for publication.

Competing interests. We declare we have no competing interests.

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