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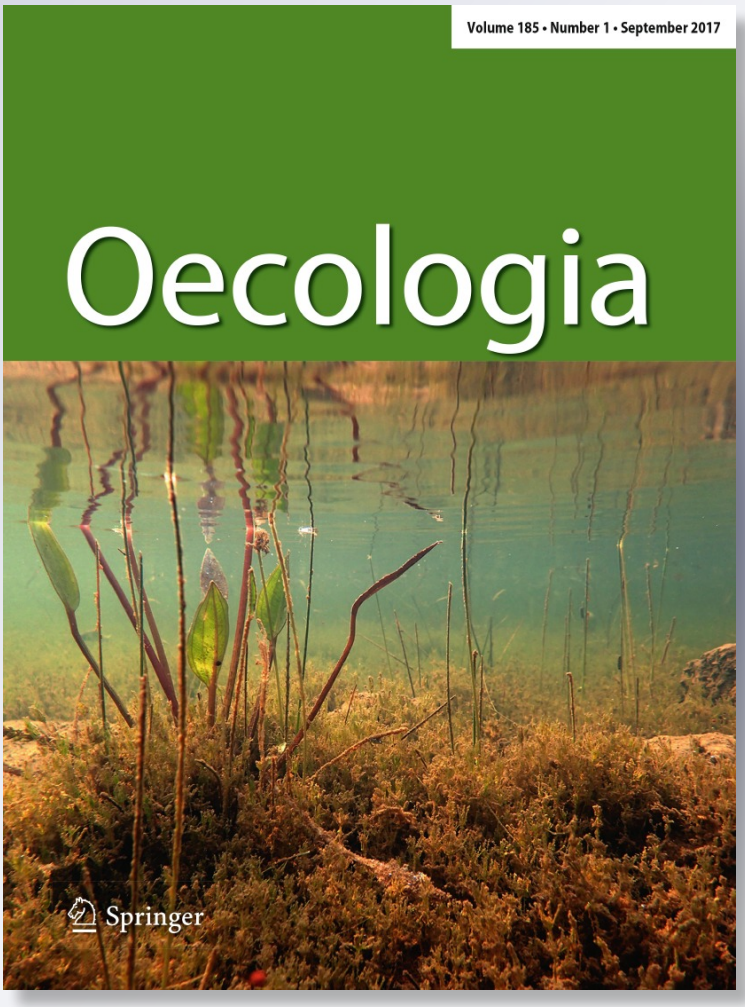

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Gypsy moth herbivory induced volatiles and reduced parasite attachment to cranberry hosts

Muvari C. Tjiurutue[1,5] · Hilary A. Sandler[2] · Monica F. Kersch-Becker[3] · Nina Theis[4] · Lynn S. Adler[1]

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Abstract Interactions between species can have cascading effects that shape subsequent interactions. For example, herbivory can induce plant defenses that affect subsequent interactions with herbivores, pathogens, mycorrhizae, and pollinators. Parasitic plants are present in most ecosystems, and play important roles in structuring communities. However, the effects of host herbivory on parasitic plants, and the potential mechanisms underlying such effects, are not well known. We conducted a greenhouse study to ask whether gypsy moth (*Lymantria dispar*) damage, host cultivar, and their interaction affected preference of the stem parasite dodder (*Cuscuta* spp.) on cranberry hosts (*Vaccinium macrocarpum*). We then assessed the mechanisms that could underlie such effects by measuring induced changes in phytohormones and secondary compounds. We found that damage by gypsy moths delayed dodder attachment by approximately 0.3 days when dodder stems were added 2 days after damage, and reduced attachment by more than 50% when dodder stems were added 1 week after host plant damage. Gypsy moth damage significantly increased jasmonic acid (JA) levels, total volatile emissions, and the flavonol, quercetin aglycone, suggesting possible mechanisms underlying variation in dodder ability to locate or attach to hosts. Dodder preference also differed between cranberry cultivars, with the highest attachment on the cultivar that had significantly lower levels of total volatile emissions and total phenolic acids, suggesting that volatile composition and phenolics may mediate dodder preference. Our results indicate that herbivory can reduce subsequent attachment by a highly damaging parasitic plant, demonstrating the potential importance of early damage for shaping subsequent species interactions.

Keywords Chemical defense · Parasitic plants · Phenolics · Phytohormones · Volatiles

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Introduction

Plants dynamically respond to their abiotic and biotic environment (Karban et al. 1999). Interactions that occur early in the growing season can affect subsequent interactions at different trophic levels by altering plant phenotypes. For example, wild radish plants (*Raphanus sativus*) previously damaged by specialist caterpillars had reduced subsequent growth of a generalist herbivore and decreased subsequent herbivory by grasshoppers (Agrawal 1999). In milkweeds (*Asclepias syriaca*), the identity of herbivores causing early-season damage can have large impacts on the performance of subsequent herbivores, indicating that there are community-wide consequences of early-season interactions (Van Zandt and Agrawal 2004). In addition to affecting

subsequent herbivores, early leaf damage can also alter interactions with pollinators, root feeders, and mycorrhizae (e.g., Erb et al. 2011; Gehring and Bennett 2009; Gilbert and Johnson 2015; Lucas-Barbosa 2016). Despite all the evidence that herbivory can shape subsequent interactions through plant-mediated changes, little is known about how herbivory affects subsequent interactions with parasitic plants.

Parasitic plants are present in most ecosystems, where they can change the outcome of competition and increase plant diversity, with consequences for animal species across multiple trophic levels. For example, in a saltmarsh community, the parasite *Cuscuta salina* preferred the competitively dominant species, resulting in its suppression and increasing local plant diversity (Pennings and Callaway 1996, 2002). In a species-rich grassland, plots with experimentally augmented *Rhinanthus minor*, a hemiparasitic plant, had reduced plant biomass but twice the abundance of invertebrates, including effects on herbivores, predators, and detritivores (Hartley et al. 2015). Although the effect of parasitic plants on plant communities via altering competitive dominance has been demonstrated in several natural systems (reviewed in Pennings and Callaway 2002), we know remarkably little about how herbivore-mediated changes in hosts affect parasite preference.

Parasite preference and performance is largely dependent on host quality. For example, parasite performance is often better on legume hosts, suggesting that nitrogen is important to performance (Matthies 1996, 1998; Seel et al. 1993), although this is not always the case (Pennings and Simpson 2008; Ren et al. 2010; Rowntree et al. 2014). Host defenses can also affect parasite performance. For example, dodder plants produced more biomass on hosts deficient in salicylic acid (SA) or insensitive to jasmonic acid (JA) (Runyon et al. 2010); these phytohormones mediate plant responses to pathogens and herbivores, but their role mediating interactions with parasitic plants is largely unknown. Although several studies have shown that host species and quality affect parasite performance, only a handful of studies have assessed parasite preference. The parasite dodder (*Cuscuta pentagona*) differentiated between preferred tomato (*Lycopersicon esculentum*) and non-preferred wheat (*Triticum aestivum*) hosts using volatiles cues emitted by the host plants (Runyon et al. 2006). Dodder (*Cuscuta* spp.) seedlings given choices between the same host species with varying nutritional content grew towards and coiled around hosts of higher nutritional content and grew away from nutritionally poor hosts (Kelly 1992).

Herbivory can induce changes in plant quality and chemistry, including volatiles (Karban and Baldwin 1997; Stam et al. 2014). Herbivore-induced plant volatiles could attract parasites, if they use the volatiles for host finding, or deter parasites if the volatiles indicate a poor-quality host due to damage (Kelly 1992; Runyon et al. 2006). Furthermore, herbivory may activate both the SA and JA signaling pathways, which can affect parasite performance (Runyon et al. 2010). Induced chemical defenses could be detrimental to parasites if the compounds are toxic to the parasite, but may benefit parasites if secondary metabolites reduce herbivory on parasites. For example, the hemiparasite *Castillja indivisa* experienced reduced herbivory when attached to a high-alkaloid compared to a low-alkaloid host, which resulted in higher parasite seed set (Adler et al. 2001).

To our knowledge, no study has examined how insect herbivory affects subsequent parasitic plant host preference. Because parasitic plants can alter community structure and productivity (Hartley et al. 2015) and cause major losses in agroecosystems (Devlin and Deubert 1980), understanding how host-mediated interactions affect parasitic plants has important implications in both natural and agricultural systems. To examine the effects of herbivory on host parasitism, we conducted a greenhouse study to ask how herbivory affects subsequent host preference and assessed the role of phytohormones, volatiles, and phenolic induction as potential underlying mechanisms.

Materials and methods

Study system

We used the generalist gypsy moth (*Lymantria dispar*: Erebidae), a destructive pest of cranberry (*Vaccinium macrocarpon*: Ericaceae) (Franklin 1950), to assess the effects of herbivory on plant chemistry and dodder preference. Gypsy moth is a destructive pest of Northeastern forests that was accidentally introduced in the late 1800s in the US (Elkinton and Liebhold 1990) and is present in both wild and cultivated cranberry. Dodder is also a serious pest of cranberries (Devlin and Deubert 1980) and a native component of cranberry bogs (Dawson et al. 1984). Gypsy moth appears on cranberry bogs before dodder seedling emergence (H. Sandler, personal observation), making the question of how gypsy moth damage affects dodder–cranberry interactions both ecologically and economically relevant.

Cranberry cultivars are genetically similar to their wild progenitors, making work with cultivars relevant to understanding ecological interactions in native systems (Rodriguez-Saona et al. 2011). Cranberry cultivars vary in their secondary chemistry, including phenolic and volatile profiles (Rodriguez-Saona et al. 2011; Tjiurutue et al. 2016). Some newly bred cranberry cultivars seem to be more susceptible to dodder parasitism (Tjiurutue et al. 2016) or to damage by gypsy moth larvae, suggesting that defense chemistry may be compromised as a result of breeding for

traits such as higher anthocyanin production, bigger berries, and higher yields (Rodriguez-Saona et al. 2011).

We used three cranberry cultivars, Howes, Stevens, and Mullica Queen. Howes was selected from a wild planting in Bassett Swamp in East Dennis, MA in 1842 by Elias Howes; cuttings from that bog were then sold to others. Since then, Howes has become the standard late-season variety in the Eastern United States. Its origins as a native genotype make our work ecologically relevant to native cranberry bogs (Chandler and Demoranville 1958). Stevens is an older hybrid that was selected for its high productivity, fair coloring, and good fruit rot resistance (Dana 1983), while Mullica Queen is a newer hybrid bred for its high anthocyanin production, high stolon vigor, and fruit rot resistance (Vorsa 2010). We hypothesized that cultivars might respond differently to herbivory, affecting future interactions with other species, including dodder.

Plant propagation

Cranberry stem cuttings were collected from the University of Massachusetts Cranberry Research Station in East Wareham, Massachusetts on 23 Jun 2011. Stem cuttings of ~8-cm sections were sown in 72-plug trays in a 3:1 sand:peat soil mixture on 27 Jun 2011. Once the stem cuttings were well established, they were moved into cold storage at 4 °C and 78% relative humidity. Established stems were repotted in November 2011 into 10-cm pots each containing four stems and moved into the greenhouse. Approximately 1.5 g of 14-14-14 Osmocote fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) was added to each pot in Nov 2011.

Dodder seeds were collected on 28 Sep 2008 from a commercial cranberry bog in Carver, Massachusetts and stored in a glass vial. Identification of *Cuscuta* species can be challenging; PCR of DNA from dodder collected from several sites in this region indicated that plants were mostly *C. gronovii*, but with some *C. campestris* and possibly *C. compacta* co-occurring (Ghantous et al. 2012).

Seeds were scarified in batches of 100 (0.01 g) in a 2-ml microcentrifuge tube for approximately 3 min using a small Dremel tool (Ghantous and Sandler 2012). The seeds were then rinsed with water using a fine-mesh strainer, placed in Petri dishes lined with 90-mm moistened filter paper, and sealed with Parafilm. Petri dishes were then placed in an incubator at 23 °C. Seed germination began approximately 2 days later.

Gypsy moth larvae were obtained from U.S. Department of Agriculture (USDA), APHIS, Otis Air Force Base, Buzzards Bay, MA, USA and were reared on an artificial wheat germ diet at 24–25 °C, 70–75% RH, and 16:8-h light–dark cycles (Bell et al. 1981). Larvae were kept at 25 °C in the laboratory until used.

Experimental design

We conducted two greenhouse experiments in parallel, manipulating gypsy moth damage on three cranberry cultivars. In the *Preference experiment*, we damaged cranberry plants with gypsy moths and then measured subsequent dodder preference as the response. In the *Induction experiment,* we damaged cranberry plants with gypsy moths and assessed induced effects of herbivory on cranberry chemistry by measuring plant phytohormones, volatiles, and phenolics. These responses were measured in separate experiments to avoid influencing dodder responses through destructive sampling to measure chemical defenses.

For each experiment, we had 25 replicate pots × 3 cultivars × 2 treatments (gypsy moth damaged or control) for a total of 150 pots per experiment. The two experiments were carried out simultaneously and pots were placed into blocks with one replicate of each treatment in each block, for a total of 25 blocks for each experiment. Each pot contained four uprights (vertical stems, which can be vegetative or reproductive) of each cultivar, and pot was the unit of replication for both experiments. Uprights were typically 8–10 cm long and had approximately 50 leaves. Plants in the damage treatment received eight third-instar gypsy moth caterpillars in a single mesh bag that enclosed all four uprights. Caterpillars were left to feed on the plants for 2 days before removal; control plants had mesh bags without caterpillars that were added and removed at the same time as the damage treatments. Due to availability, caterpillars were not put on all plants on the same day; treatments were spaced out over 6 days (14 through 19 Dec 2011), but all plants in the same block received treatments (bags with or without caterpillars) on the same day. In both experiments, we measured cranberry damage visually as the proportion of leaves damaged. The amount of damage did not differ between cranberry cultivars ($F < 2.5$, $P > 0.09$).

Preference experiment responses

After 2 days of caterpillar damage, which is sufficient to induce chemical responses in cranberries (Rodriguez-Saona et al. 2011), all caterpillars and bags were removed. Two days later, 96 of the plants (16 blocks; each control and corresponding damaged treatment pot within a block) received dodder seedlings, with one seedling added per upright (i.e., four per pot), to assess short-term effects of gypsy moth damage on parasite preference. The other 54 plants (9 blocks) received dodder seedlings 1 week after damage to assess longer term effects. We measured the length of each cranberry stem on the day dodder was added to include as a covariate in the analyses. Seedlings were placed approximately 1 cm from each stem on the soil surface using tweezers. We monitored uprights twice daily (around 10:00 and

15:00) and recorded the date of attachment over a period of 2 weeks. We scored attachment as signs of visible swelling on dodder stems coiled around hosts, indicating the initiation of haustoria. Dodder preference was measured as the total number of dodder stems attached per pot and the average days to attachment within pot.

Induction experiment responses

Phytohormones

We measured phytohormones from control and caterpillar-damaged plants for each cultivar on a subsample of 60 pots (10 replicates per treatment × 3 cultivars × 2 treatments), pooling tissue from all uprights within a pot. Leaves of both control plants and damaged replicates were placed in separate 5-mL cryovials (Fisher Scientific, Fair Lawn, NJ, USA) and immediately frozen in liquid nitrogen before storage at −80 °C. Details of the phytohormone analysis protocol are presented in Online Resource 1.

Volatiles

After 2 days of feeding, caterpillars were removed and volatiles were sampled. Damage and control pots of each cultivar within a block were sampled on the same day, for an ultimate total of 18 replicate pots × 3 cultivar × 2 treatments. We sampled volatiles, pooling all uprights within each replicate pot, over a period of 1 week in December. Before sampling, we recorded the total number of leaves and the number of damaged leaves per pot to include as covariates.

Volatiles were sampled using dynamic headspace sampling for 4 h between 11:15 and 15:15. All four uprights per replicate pot were enclosed within a polyethylene bag (Toppits, Cofresco Frischhalteprodukte Gmbh & Co. Kg, Minden, Germany) following protocols detailed in Online Resource 1. Compounds were identified using GC–MS analysis by matching GC retention times to previously used standards, Kovats retention indices and to the NIST 2008 Mass Spectral Library (Theis et al. 2009). Quantification of compounds was obtained by dividing the peak area of the mass ion of each scent compound by the peak area of the mass ion of the internal standard and by the product of both mass of the internal standard and a coefficient that corrected for the response of the GC–MS to the specific scent compound (Theis et al. 2009).

Phenolics

Hereafter, we use the term 'phenolics' to include the subcategories of flavonols (quercetin glycosides), phenolic acids (total chlorogenic acids), and proanthocyanidins (total individual oligomers and polymers). We measured leaf phenolics from control and caterpillar-damaged replicates for each cultivar, pooling tissue from all uprights within a pot. This analysis was performed on a subsample of 30 pots (5 samples per cultivar × 3 cultivars × 2 treatments) that were not used for phytohormones but included samples that were used for volatile analysis. Leaves of both control plants and damaged plants were collected on the same day that the caterpillars were removed from the plants and placed in separate 5-mL cryovials (Fisher Scientific, Fair Lawn, NJ, USA) and immediately frozen in liquid nitrogen before storage at −80 °C. Detailed method of extraction and purification of leaf samples can be found in Vvedenskaya et al. (2004), and a protocol summary is provided in Online Resource 1.

Statistical methods

General approach

We tested all responses using MANOVA unless otherwise stated. All models included damage treatment, cultivar and their interactions as fixed effects, and block as a random effect. Significant MANOVAs were followed by mixed model ANOVAs (with LME function). All responses for dodder preference, phytohormones, phenolics, and volatiles were tested for normality using a Shapiro–Wilks test prior to running models and transformed where necessary. We used Tukey's HSD test ($\alpha = 0.05$) for post hoc comparison of differences between cultivars. We used R version 3.2.1 for Mac (R Core Team 2014) for all statistical analysis.

Preference experiment

To test for effects of damage treatment and cultivar on the number of dodder stems that attached and average days to attachment to cranberry hosts, we used separate ANOVAs including number of dodder stems attached and days to attachment as responses, and whether dodder was added 2 or 7 days after damage as an additional fixed factor (referred to as 'days post-damage' hereafter). Upon finding a significant interaction between damage and the days post-damage that dodder was applied (see "Results"), we analyzed preference separately for measurements 2 or 7 days post-damage and we only report these results. Plant height was initially included in the models but was removed because it was not statistically significant.

Induction experiment: phytohormones

We did not use MANOVA to analyze phytohormones because five outliers were deleted that violated assumptions of MANOVA. Deleting these outliers for one response

would have removed them from the entire analysis because MANOVA excludes replicates with any missing responses. The deleted outliers included two for SA [both damage/Stevens, 4 standard deviations (SD) and 3 SD above mean] and three for JA [control/Stevens (3 SD above mean), control/Howes (5 SD above mean) and damage/Stevens (4 SD above mean)]. Additionally, we log-transformed JA, SA, and ABA concentrations to improve normality. We ran separate mixed-effect model ANOVAs with SA, JA, and ABA as dependent responses. We initially included days between damage and sample collection as a covariate but removed it from analyses because it was not statistically significant.

Induction experiment: volatiles

All volatile emissions were calculated as an hourly rate scaled by the wet mass of the sample, with the units ng g^{-1}FW h^{-1}. Seventeen of the original 108 samples were compromised due to evaporation in the freezer and were removed from the dataset before analysis; these samples were relatively evenly divided between the damage ($n = 10$) and the control ($n = 7$) treatments. We log-transformed volatile classes to improve normality whenever necessary. Volatiles were grouped based on their biosynthetic origin into monoterpenoids, homoterpenoids, sesquiterpenoids, esters, and then by functional group: aromatics, alkanes, aldehydes/alcohols/fatty acids and unknowns that were analyzed as responses with permutational multivariate analysis of variance (PERMANOVA, R vegan package), using distance matrices (adonis) and the Bray–Curtis dissimilarity coefficient. We used Monte Carlo permutations (999) to test the significance of the results on quantitative data. PERMANOVA is a non-parametric test that is more robust to zero-inflated data and to violations of normality assumptions than MANOVA (Anderson 2001). The models included cultivar and damage treatment as independent variables, block as a random factor and difference in days to volatile collection as a fixed factor. We also analyzed changes in volatile composition based on individual volatiles rather than summed groups using a separate PERMANOVA. However, we found similar results, in terms of the effects of damage, cultivar and their interaction, when we analyzed the data using individual volatiles rather than summed groups, and so we do not report the individual volatile analysis. Finally, we analyzed the classes and total volatile emissions as well as each individual compound using separate ANOVAs (with LME function). Because gypsy moth damage reduced the fresh weight of cranberry on average by 26% (mean ± SE; control: 1.06 ± 0.04 g FW; damaged: 0.78 + 0.03 g FW) and dodder might perceive volatiles at the level of pot rather than gram, volatile groups were also analyzed by ng h^{-1}.

Induction experiment: phenolics

We analyzed phenolics as three major groups: flavonols, phenolic acids, and proanthocyanidins. Responses for the MANOVA included the following flavonols: quercetin-3-galactoside, quercetin-3-xyloside, quercetin-3-rhamnoside, quercetin-3-arabinopyranoside, quercetin-3-arabinofuranoside, and quercetin aglycone. We ran mixed-effect model ANOVAs (with LME function) with each of the flavonols plus total flavonols (all flavonols combined) as separate dependent variables. Because proanthocyanidins (total individual oligomers and polymers) and phenolic acids (total chlorogenic acids) were single responses, we ran separate ANOVAs for each. Plant height and difference in days to collection were initially included as covariates but removed from the models because they were not statistically significant.

Results

Dodder preference

The effect of days post-damage on the number of attached dodder stems and the average days to attachment varied by damage treatment ($F > 4.82$, $P < 0.03$ for both responses). Therefore, we analyzed preference separately for dodder applied 2 and 7 days post-damage.

Two days post-damage

The number of attached dodder stems varied by cranberry cultivar ($F_{2,75} = 3.88$, $P = 0.025$), but there was no significant effect of damage treatment or the damage treatment-by-cultivar interaction ($F < 0.38$, $P > 0.68$ for both). Stevens had 32–37% more attached dodder stems than Mullica Queen and Howes (mean ± 1 SE: Howes = 1.21 ± 0.17; Mullica Queen = 1.12 ± 0.17; Stevens = 1.78 ± 0.20). However, gypsy moth damage delayed attachment by ca. 8 h ($F_{1,50} = 12.66$, $P = 0.001$; Fig. 1a). There was no effect of cultivar or damage-by-cultivar interaction on time to attachment ($F_{2,50} < 1.96$, $P > 0.15$ for both).

Seven days post-damage

Damage reduced the number of dodder stems that attached by ~50% ($F_{1,39} = 8.59$, $P = 0.006$; Fig. 1b), but cultivar and the damage-by-cultivar interaction did not affect attachment ($F < 0.69$, $P > 0.51$ for both). There was no effect of damage, cultivar or their interaction on days to attachment ($F < 0.69$, $P > 0.15$ for all).

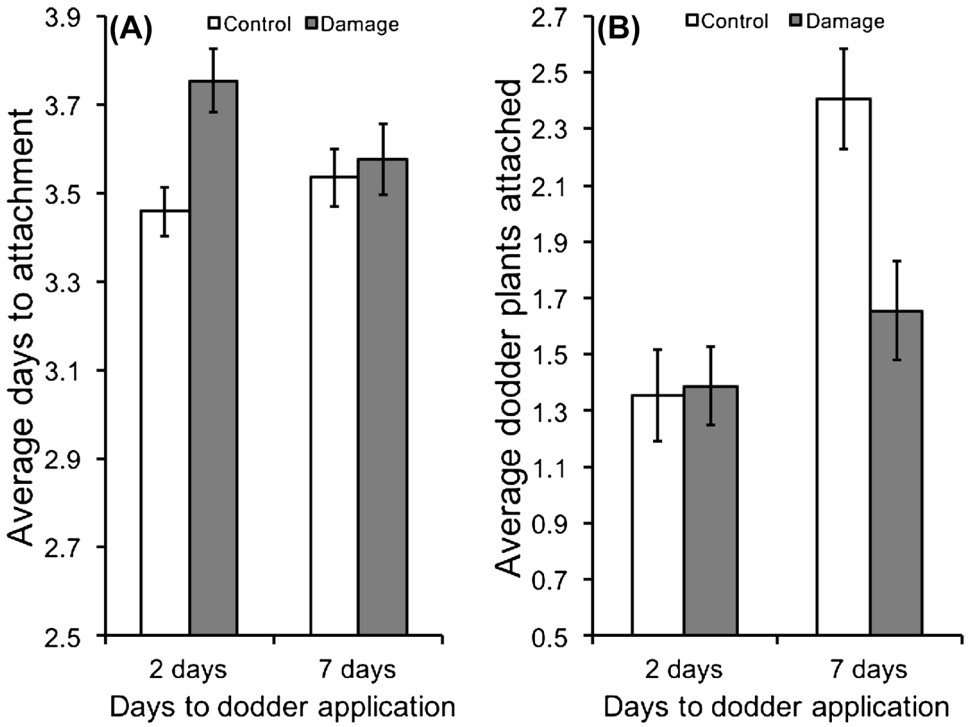

Fig. 1 Effects of gypsy moth damage (*Lymantria dispar*) on subsequent dodder (*Cuscuta*) preference measured as the **a** average days to attachment and **b** total number of dodder stems attached for 2 and 7 days post-damage, respectively. Responses 2 and 7 days post-damage were analyzed with separate ANOVAs. Damage delayed attachment to plants 2 days post-damage ($F_{1,50} = 12.66$, $P = 0.001$) and reduced dodder attachment 7 days post-damage ($F_{1,39} = 8.59$, $P = 0.006$). *Bars* are means ± 1 standard error

Induction experiment

Phytohormones

Caterpillar damage increased JA levels by nearly 50% compared to the controls (mean ± 1 SE: control = 1982 ng gFW^{-1} ± 326; damage = 2940 ng gFW^{-1} ± 356; Table 1; see Online Resource 2 for means and SE of all phytohormones within cultivar and damage treatment), but there was no effect of cultivar or the damage-by-cultivar interaction (Table 1). For SA, there was no main effect of damage treatment or cultivar, but the effect of damage differed with cultivar (Table 1). Further analysis within cultivar showed that gypsy moth damage doubled SA levels compared to controls in Howes (mean ± 1 SE: control = 19.23 ng gFW^{-1} ± 3.47; damage = 38.16 ng gFW^{-1} ± 5.91) but had no effect in the other cultivars. ABA was not affected by any factor (Table 1).

Volatiles

Damage induced changes in the composition of volatile groups (PERMANOVA, SS = 1.18, $F_{1,80} = 62.76$ $P = 0.001$) and volatile composition differed between cultivars (PERMANOVA, SS = 2.80, $F_{2,80} = 4.56$, $P = 0.003$). However, cultivars did not differ in their response to damage (cultivar × damage interaction: SS = 0.05, $F_{2,80} = 1.38$, $P = 0.23$). Results were qualitatively the same on a per-pot basis, i.e., volatile composition differed with damage treatment and cultivar, but not their interaction (PERMANOVA, damage: SS = 0.63, $F_{1,80} = 3.9$, $P = 0.019$; cultivar: SS = 1.08, $F_{2,80} = 3.37$, $P = 0.007$, interaction: SS = 0.6, $F_{2,80} = 1.87$, $P = 0.08$). On a per gram basis, damage also increased total volatile emissions by 113% compared to controls (Table 1; Fig. 2a) and cultivars differed in total volatile emissions (Table 1; Fig. 2a). Stevens had significantly lower volatiles than Howes and Mullica Queen, and also the most dodder attachments (Fig. 2a). Subsequent ANOVAs (Table 1) showed that damage increased levels of monoterpenoids, homoterpenoids, sesquiterpenoids, esters, aromatics, and alkanes and that cultivars differed in the aldehydes/alcohols/fatty acids group as well as homoterpenoids and esters (Fig. 2; see Online Resource 2 for means and SE of all major volatile categories within cultivar and damage treatment). The effect of damage on sesquiterpenoids was particularly strong, increasing them 44-fold relative to control plants. Results were qualitatively similar when volatiles were analyzed per pot rather than per g, except that damage no longer significantly affected the emission

Table 1 *F* values from mixed-effect model ANOVAs testing the effects of herbivore damage and cultivar on cranberry chemistry with block as a random factor

Response	Damage	Cultivar	Damage × cultivar	Error *df*
Phytohormones				
Jasmonic acid	**4.18***	0.78	0.77	39
Salicylic acid	0.74	1.01	**4.85***	40
Abscisic acid	0.81	1.71	0.52	40
Volatiles				
Monoterpenes	**50.45*****	1.34	1.68	68
Homoterpenes	**106.18*****	**8.73****	0.07	68
Sesquiterpenes	**219.67*****	0.80	0.41	68
Esters	**9.51***,§	**5.27***	2.44	68
Aromatics	**202.44*****	0.47	0.26	68
Alkanes	**4.06***,§	0.19	0.18	68
Alcohols, aldehydes, fatty acids	0.13	**10.75****	1.27	68
Unknowns	1.52	0.84	0.29	68
Total	**10.83****,§	**6.17****	2.00	68
Flavonols				
Quercetin-3-galactoside	0.40	**8.11****	1.67	20
Quercetin-3-arabinopyaranoside	0.16	**15.75****	1.43	20
Quercetin-3-arabinofuranoside	0.15	**4.71***	1.80	20
Quercetin-3-xyloside	3.15	**8.00****	0.51	20
Quercetin-3-rhamnoside	0.057	**7.42****	2.25	20
Quercetin aglycone	**4.61***	**4.39***	0.34	20
Total Flavonols	0.26	**8.12****	1.99	20
Phenolic acids	0.80	**27.76*****	0.012	19
Proanthocyanidins	0.78	0.89	1.74	20
Phenolic acids	**8.33***	**19.81****	2.68	9

For all analyses, the numerator degrees of freedom (*df*) is 2 for cultivar, 1 for dodder treatment and 2 for their interaction; error *df* is listed for each analysis. Volatile analysis is reported for volatiles measure in units of ng g^{-1}FW h^{-1}. Results were qualitatively the same when analyzed in units of ng h^{-1} (i.e., per pot rather than per g FW), except for the effect of damage on alkanes, esters, and total volatiles, indicated by §

Bold values indicate significant effects at $P < 0.05$

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

of esters, alkanes or total volatiles (Table 1). Separate ANOVAs for individual compounds, rather than summed groups, showed that both damage and cultivar affected many compounds (Table 2).

Phenolics

Flavonols differed across cultivars (MANOVA, Pillai's trace = 1.38, $F_{10,42} = 9.36$, $P < 0.0001$) but were not affected by damage or the cultivar-by-damage interaction (MANOVA; Pillai's trace < 0.15, $F < 0.72$, $P > 0.60$ for both). In univariate ANOVAs, all six flavonols and total flavonols differed across cultivars (Table 1; Fig. 3a–c; see Online Resource 2 for means and SE of phenolics within cultivar and damage treatment). We note that damage significantly increased levels of quercetin aglycone (mean ± 1 SE: control = 0.0073 mg gFW^{-1} ± 0.00043; damage = 0.0083 mg gFW^{-1} ± 0.00042; Table 1) and marginally decreased levels of quercetin-3-xyloside (mean ± 1 SE: control = 0.019 mg gFW^{-1} g ± 0.0051; damage = 0.014 mg gFW^{-1} g ± 0.0061; Table 1), even though the MANOVA found no main effects of damage.

Phenolic acids differed between cultivars (Fig. 3d) but were not affected by damage or the damage-by-cultivar interaction (Table 1). Proanthocyanidins were not affected by cultivar, damage or the damage-by-cultivar interaction (Table 1).

Discussion

Herbivory has a wide range of effects on subsequent interactions on shared hosts (Erb et al. 2011; Gehring and Bennett 2009; Gilbert and Johnson 2015; Lucas-Barbosa 2016; Lucas-Barbosa et al. 2011), and our results demonstrate that herbivory can also reduce subsequent attachment by

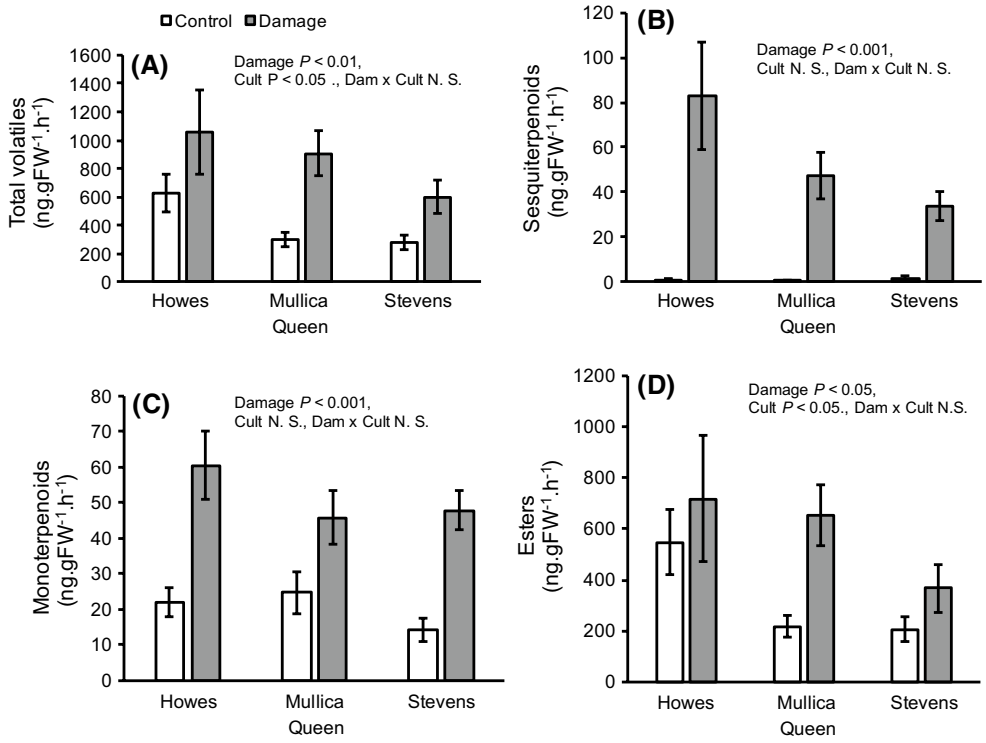

Fig. 2 Effect of damage and cranberry cultivar for overall **a** total amount of volatile emissions, **b** sesquiterpenoids, **c** monoterpenoids, and **d** esters. All volatiles are expressed in units of ng per g fresh weight (FW) per hour. Responses were analyzed with ANOVA. *Cult* cultivar, *dam* damage. *Bars* are means ± 1 standard error, and $n = 47$ for control and 44 for damage treatments

dodder, a parasitic plant. We also found that gypsy moth damage induced a wide range of chemical changes in hosts, suggesting potential mechanisms of dodder repellence that could be tested with future experimental approaches. The effects of damage intensified with the time post-damage. When dodder was added 2 days after gypsy moth damage, damage delayed attachment time by approximately 0.3 days, but the number of dodder attached was not affected. When dodder was added 7 days after damage, damage did not affect time to attachment, but the number of dodder that attached was reduced by about 50% compared to undamaged hosts. This suggests that even a week after damage, herbivory can reduce dodder preference or ability to attach to hosts.

Because dodder can have profound impacts on host fitness (e.g., Devlin and Deubert 1980), our results suggest that herbivory, by deterring dodder, may have unexpected benefits for hosts. If the cost of early leaf damage is low relative to the impact of subsequent parasitism, then initial damage could benefit hosts by increasing resistance to a highly damaging parasite. Similarly, early leaf damage to *Oenothera biennis* increased plant fitness by inducing floral defenses that deterred seed predators (McArt et al. 2013). Although we did not evaluate the effect of host damage on parasite performance, another study found that leaf defoliation of the host plant *Poa annua* reduced subsequent performance of the hemiparasite *Rhinanthus serotinus* (Puustinen and Salonen 1999). One caveat is that reduced parasite deterrence or performance in a greenhouse study may not ultimately translate to reduced parasitism in the field; future field manipulations are necessary to evaluate the ultimate costs and benefits of early-season damage via changes in parasitism.

Similar to previous work (Rodriguez-Saona et al. 2011), we found that gypsy moth damage induced JA production. The effect of damage on SA was more complex, with damage inducing higher SA in one cultivar but not others. The role of the JA signaling pathway in mediating plant defenses against chewing insect herbivores and the SA-signaling pathway in mediating defenses against phloem-feeding herbivores and pathogens has been well established (Walling 2000), but their roles mediating interactions with parasitic plants are less clear. *Cuscuta pentagona* produced more biomass on both JA-insensitive and SA-deficient tomato hosts, suggesting that both SA and JA responses may be important in deterring parasitism (Runyon et al. 2010). Furthermore, both JA and SA have been shown to be involved in the hypersensitive response (HR), which is an effective defense against *C. reflexa* (Goldwasser et al. 2001; Ihl et al. 1988; Runyon

Table 2 *F* values from mixed-effect model ANOVAs testing effects of damage treatment and cultivar on cranberry individual volatile composition, with block as random factor

Response	Kovats index	Damage	Cultivar	Damage × cultivar
1-Hexanol	868	0.0006	**9.41****	0.10
Alpha-pinene	936	**11.06****	1.66	0.44
Camphene	954	1.45	2.97	0.79
Sabinene	976	**5.16***	**3.34***	**0.02***
Beta-pinene	982	**4.07***	1.02	0.09
6-Methyl-5-heptene-2-one	984	**8.84****	0.35	0.93
Beta-myrcene	989	**8.35****	0.79	0.74
1,3,5-Trimethylbenzene	996	0.70	0.59	0.46
Decane	1000	2.03	0.03	0.40
Cis-3-hexenyl acetate	1004	**7.02***	**4.20***	0.50
Hexyl acetate	1010	3.07	0.48	0.12
Limonene	1033	**4.09***	**4.81***	0.18
Phenylacetaldehyde	1049	**35.77*****	**3.26***	2.29
Cis-linalool oxide	1075	**49.23*****	1.51	1.19
Linalool	1101	**53.95*****	1.11	2.16
Nonanal	1105	**5.08***	0.73	0.84
(*E*)-4,8-Dimethyl-1,3,7-nonatriene (DMNT)	1114	**21.41*****	**5.30****	**5.34****
Benzeneacetonitrile	1142	**21.41*****	**5.30****	0.29
Cis-3-hexenyl butyrate	1184	3.02	**4.88****	0.07
Methyl salicylate	1199	**4.92***	**4.63***	0.23
Dodecane	1200	**4.82***	0.01	1.29
Alpha-terpineol	1201	**18.61*****	1.26	0.31
Decanal	1207	**5.49***	0.07	0.96
Unknown (m/z 59,79,93)	1219	2.92	0.15	0.11
(*Z*)-3-Hexenyl-2-methylbutanoate	1231	1.52	**5.28****	2.29
Unknown (m/z 79,107,135)	1247	**6.59***	0.89	1.06
Indole	1299	**54.04*****	0.29	0.26
Tridecane	1300	1.05	0.11	2.40
Hexenyl hexanoate	1385	**8.77****	3.05	2.87
Alpha-copaene	1387	**64.04*****	0.66	1.29
Beta-cubebene	1398	**44.94*****	1.53	1.26
Tetradecane	1400	0.13	0.05	**3.42***
Beta-caryophyllene	1436	**38.83*****	2.94	**3.40***
Neryl acetone	1450	**16.49****	0.97	1.51
Alpha-humulene	1473	**32.38*****	3.00	**3.63***
Germacrene D	1497	**31.46*****	2.84	**2.95**[a]
Pentadecane	1501	0.27	0.10	0.96
(*E*, *E*) Alpha-farnesene	1507	**12.14****	0.90	0.50
Gamma-cadinene	1517	**8.03****	3.64	**4.08***
Delta-cadinene	1529	**43.00*****	1.60	2.06
Isopropyl myristate	1822	1.63	1.44	0.47
Cyclohexadecane	1883	0.05	0.05	2.65
Hexadecanoic acid	2011	2.07	0.81	0.24

For all analyses, numerator *df* is 2 for cultivar, 1 for damage treatment, and 2 for their interaction, and the error *df* is 68

Bold values indicate significant effects at $P < 0.05$

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

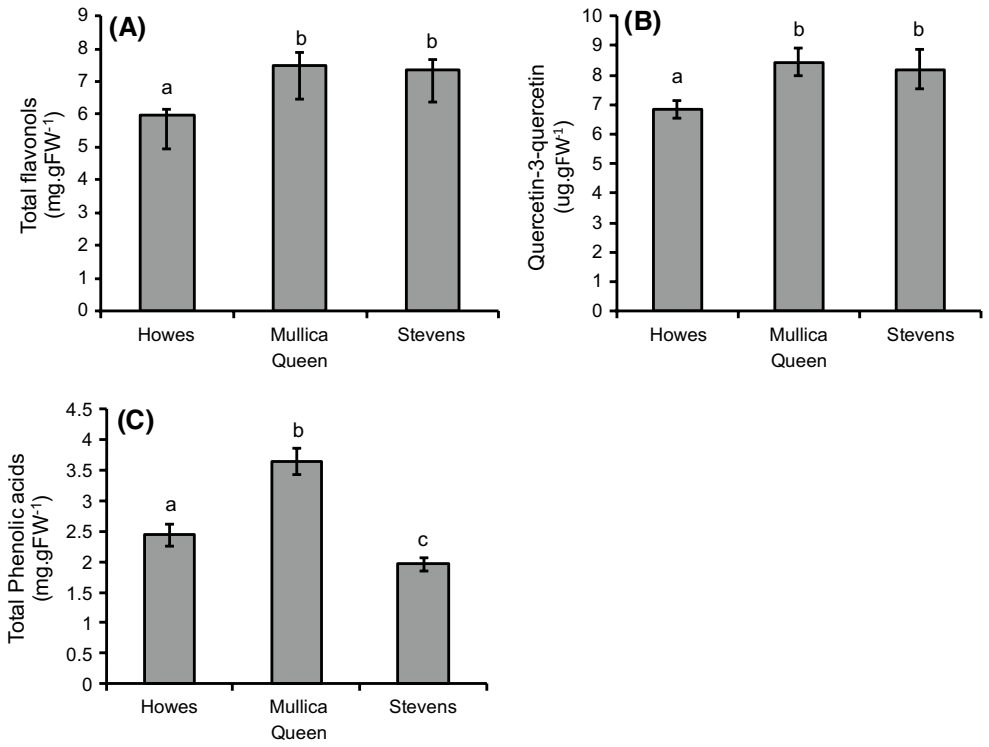

Fig. 3 Differences between cranberry cultivars in **a** total amount of flavonols, and two example flavonols, **b** quercetin aglycone, and **c** total phenolic acids. Responses were analyzed with mixed-effect ANCOVAs and depicted means include plants from both damaged and undamaged treatments. *Bars* are means ± 1 standard error, *different letters* above *bars* indicate significantly different means using Tukey's HSD test ($P < 0.05$), and $n = 15$ per treatment

et al. 2010). Because gypsy moth damage increased JA and reduced parasite attachment across cultivars, our results suggest that JA signaling may be involved in deterring dodder via changes in induced direct and indirect defenses, such as volatiles.

Herbivory doubled host volatile emissions (Table 1; Fig. 2a) and altered volatile composition, with particularly strong increases in sesquiterpenoid emissions (Fig. 2b). Previous work has shown that dodder can orient to hosts and away from non-hosts based solely on volatiles (Runyon et al. 2006). Furthermore, volatile components of the preferred host included alpha-pinene, limonene, and beta-myrcene (Runyon et al. 2006), all of which were induced by damage in our study (Table 2). Thus, it seems reasonable to suggest that induction of volatiles following damage played a central role influencing dodder preference in this study. Herbivore-induced plant volatiles are known to mediate how both plants and insects perceive and respond to their environment (De Moraes et al. 1998; Dicke 2016; Karban et al. 2014). While manipulation of volatiles is necessary to conclusively demonstrate their role in dodder preference following host damage, our results combined with previous work (Runyon et al. 2006) suggest that dodder can distinguish between damaged hosts and undamaged hosts using volatile cues. One caveat is that we measured induced volatiles and other compounds 2 days post-damage, while we saw the biggest effects of damage on dodder attachment 7 days post-damage. Future work can determine how the volatile profile changes over time and whether damaged plants are deterrent due to greater overall volatile production, or whether dodder is particularly sensitive to altered blends of volatiles following damage.

Gypsy moth damage did not generally change phenolic concentrations, corroborating previous findings in which exogenous JA application did not alter phenolics in cranberry (Rodriguez-Saona et al. 2011). However, although the overall MANOVA was not significant, damage did induce higher levels of the flavonol quercetin aglycone (Table 1). Quercetin glycosides have been implicated in mediating plant defenses against chewing herbivores (Beninger and AbouZaid 1997; Rodriguez-Saona et al. 2011). However, these compounds cannot be the only mechanism mediating resistance to dodder, since the cultivars Howes and Mullica Queen, which had the fewest attached dodder stems, had variable amounts of flavonols (Fig. 3a–c) compared to Stevens. Similarly, in a previous study in this system, we did not find any evidence implicating flavonols as the mechanism of resistance to dodder parasitism although

dodder induced many changes in flavonols (Tjiurutue et al. 2016). These findings suggest that induced flavonol responses in cranberries evolved for other functions, such as defenses against insect herbivores, rather than against dodder parasitism.

We also found evidence for genetic variation in dodder resistance, volatile emission and defense production demonstrated by differences between cultivars. Cultivars differed in their volatile profile and in total volatile emissions (Tables 1, 2; Fig. 2). Interestingly, Stevens had the highest dodder attachment after 2 days, but the lowest total volatile emission (Fig. 2). This contrasts with the damage result, where damage both increased total volatile emissions and reduced attachment. These contrasting relationships between total volatile emission and attachment suggest that total emissions are not the main cue promoting dodder attachment. Alternatively, just as previous work found that some volatiles are attractive to dodder (Runyon et al. 2006), including some of the volatiles induced in our study, it is also possible that specific volatile components or blends attract or deter attachment. For example, Howes and Mullica Queen had significantly higher levels of *cis*-3-hexenyl butyrate compared to Stevens (Table 2). In a previous study, caterpillar-induced nocturnal plant volatiles including cis-3-hexenyl butyrate repelled conspecific female moths, *Heliothis virescens* (De Moraes et al. 2001). We speculate that volatiles such as cis-3-hexenyl butyrate, or another compound, or mixtures of compounds could repel dodder from attaching. Therefore, volatile composition as well as total emission could play key roles against dodder parasitism. Manipulative experiments with single compounds and blends at multiple concentrations are needed to elucidate which host-emitted components drive dodder attraction or repellence.

Cultivars also differed in phenolic acids that could mediate interactions with dodder. Stevens, which had the highest dodder attachment after 2 days of damage, also had significantly lower concentrations of phenolic acids compared to Howes and Mullica Queen. This suggests that phenolic acids could be a defense mechanism against dodder in cranberries. The role of phenolic acids against herbivore defense is well established, and phenolics are found in phloem and have been previously implicated in parasitic plant defense (Pérez-de-Luque et al. 2008; Yoder and Scholes 2010). Host plant resistance against parasite establishment in the cortex and endodermis of hosts has been linked to accumulation of phenolic acids, which could be toxic to the parasite (Pérez-de-Luque et al. 2008; Yoder and Scholes 2010). Additionally, phenolic compounds may be toxic or act as deterrents to herbivores, reducing digestibility or palatability of leaf tissues, which leads to reduced insect growth (Lattanzio et al. 2006). Thus, it is plausible that phenolic acids could mediate defenses against dodder in cranberry, but this mechanism seems more likely to affect processes post-attachment unless such compounds accumulate and are perceived at the stem surface. However, *C. subinclusa* responses to the host *Malosma laurina* were influenced by host surface bark flavonoids (Kelly 1990), suggesting that in some cases these compounds could affect pre- as well as post-attachment processes.

Although cultivars differed in production and composition of both volatiles and phenolics, it is interesting that cultivars did not differ in their induced responses to gypsy moth damage, indicated by non-significant cultivar-by-damage interaction terms. Consistent with these findings, our previous study showed that cranberry cultivars did not respond differently to dodder parasitism (Tjiurutue et al. 2016). In other systems, plant genotypes often respond differently to the same herbivores (e.g., Gouinguene et al. 2001; Uesugi et al. 2013). By responding differently to the same herbivore, genotypes may support different species, leading to different species composition in the community (Stam et al. 2014). The lack of genetic variation in induced responses suggests that cranberry may not pose a 'moving target' for antagonists (Adler and Karban 1994), but rather a more predictable chemical landscape that may support a less diverse community, or be easier for antagonists to overcome.

In conclusion, we found that damage to cranberry hosts reduced parasite attachment by nearly 50% 1 week after damage, which could provide an important benefit of early leaf herbivory given the highly damaging effects of this parasite. Consistent with cascading effects of early damage on a wide range of other interactions (McArt et al. 2013; Poelman et al. 2010; Van Zandt and Agrawal 2004), our results suggest that herbivore damage could confer an indirect benefit by reducing attachment of a highly damaging parasitic plant. Understanding how parasites and other species interact through a shared host will give a clearer understanding of the underlying mechanisms that influence parasite preference and performance, which can shape community composition at multiple trophic levels (Hartley et al. 2015).

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Author contribution statement MCT, HAS, and LSA conceived and designed the experiments. MCT, MFK, and NT performed the experiments. MCT and NT analyzed the data. MCT and LSA wrote the manuscript; all authors provided editorial advice.

Compliance with ethical standards

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

Data availability The data for this article are publicly available in Dryad: doi:10.5061/dryad.bj3tt.

References

Adler FR, Karban R (1994) Defended fortresses or moving targets? Another model of inducible defenses inspired by military metaphors. Am Nat 144:813–832

Adler LS, Karban R, Strauss SY (2001) Direct and indirect effects of alkaloids on plant fitness via herbivory and pollination. Ecology 82:2032–2044

Agrawal AA (1999) Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. Ecology 80:1713–1723

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46. doi:10.1111/j.1442-9993.2001.01070

Bell R, Owens C, Shapiro M, Tardif J (1981) Development of mass rearing technology. The gypsy moth: research toward integrated pest management, vol 1584. USDA, Washington, DC, pp 599–633

Beninger CW, AbouZaid MM (1997) Flavonol glycosides from four pine species that inhibit early instar gypsy moth (Lepidoptera: Lymantriidae) development. Biochem Syst Ecol 25:505–512. doi:10.1016/s0305-1978(97)00034-3

Chandler FB, Demoranville IE (1958) Cranberry varieties of North America. Bulletin—Massachusetts agricultural experiment station no. 513

Dana MN (1983) Cranberry cultivar list (*Vaccinium macrocarpon*). Fruit Var J 37:88–95

Dawson JH, Musselman J, Wolswinkel P, Dorr I (1984) Biology and control of *Cuscuta*. Weed Sci 6:265–317

De Moraes CM, Lewis WJ, Pare PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. Nature 393:570–573

De Moraes CM, Mescher MC, Tumlinson JH (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature 410:577–580

Devlin RM, Deubert KH (1980) Control of swamp dodder (*Cuscuta gronovii*) on cranberry bogs with butralin. Proc Northeast Weed Sci Soc 11:112–113

Dicke M (2016) Plant phenotypic plasticity in the phytobiome: a volatile issue. Curr Opin Plant Biol 32:17–23. doi:10.1016/j.pbi.2016.05.004

Elkinton JS, Liebhold AM (1990) Population dynamics of gypsy moth in North America. Annu Rev Entomol 35:571–596

Erb M, Robert CAM, Hibbard BE, Turlings TCJ (2011) Sequence of arrival determines plant-mediated interactions between herbivores. J Ecol 99:7–15. doi:10.1111/j.1365-2745.2010.01757.x

Franklin HJ (1950) Cranberry insects in Massachusetts. Bulletin no. 445, Part 1. MA Agricultural Experiment Station, Amherst, MA

Gehring C, Bennett A (2009) Mycorrhizal fungal-plant-insect interactions: the importance of a community approach. Environ Entomol 38:93–102. doi:10.1603/022.038.0111

Ghantous KM, Sandler HA (2012) Mechanical scarification of dodder seeds with a handheld rotary tool. Weed Technol 26:485–489. doi:10.1614/wt-d-11-00077.1

Ghantous KM, Stefanovic S, Sandler HA (2012) Initial investigations into dodder species variation in Southeastern Massachusetts. In: Proceedings of *n*ortheastern weed science society, vol. 66, Philadelphia, p 60

Gilbert L, Johnson D (2015) Plant-mediated 'apparent effects' between mycorrhiza and insect herbivores. Curr Opin Plant Biol 26:100–105. doi:10.1016/j.pbi.2015.06.008

Goldwasser Y, Lanini WT, Wrobel RL (2001) Tolerance of tomato varieties to *Lespedeza* dodder. Weed Sci 49:520–523

Gouinguene S, Degen T, Turlings TCJ (2001) Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). Chemoecology 11:9–16. doi:10.1007/pl00001832

Hartley SE, Green JP, Massey FP, Press MCP, Stewart AJA, John EA (2015) Hemiparasitic plant impacts animal and plant communities across four trophic levels. Ecology 96:2408–2416. doi:10.1890/14-1244.1

Ihl B, Tutakhil N, Hagen A, Jacob F (1988) Studies on Roxb. 7. defense mechanisms of *Lycopersicon esculentum* Mill. Flora 181:383–393

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

Karban R, Agrawal AA, Thaler JS, Adler LS (1999) Induced plant responses and information content about risk of herbivory. Trends Ecol Evol 14:443–447

Karban R, Yang LH, Edwards KF (2014) Volatile communication between plants that affects herbivory: a meta-analysis. Ecol Lett 17:44–52. doi:10.1111/ele.12205

Kelly CK (1990) Plant foraging: a marginal value model and coiling response in *Cuscuta subinclusa*. Ecology 71:1916–1925

Kelly CK (1992) Resource choice in *Cuscuta europaea*. Ecology 89:12194–12197

Lattanzio V, Lattanzio VMT, Cardinali A (2006) Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochem Adv Res 661:23–67

Lucas-Barbosa D (2016) Integrating studies on plant-pollinator and plant-herbivore interactions. Trends Plant Sci 21:125–133. doi:10.1016/j.tplants.2015.10.013

Lucas-Barbosa D, van Loon JJA, Dicke M (2011) The effects of herbivore-induced plant volatiles on interactions between plants and flower-visiting insects. Phytochemistry 72:1647–1654. doi:10.1016/j.phytochem.2011.03.013

Matthies D (1996) Interactions between the root hemiparasite *Melampyrum arvense* and mixtures of host plants: heterotrophic benefit and parasite-mediated competition. Oikos 75:118–124. doi:10.2307/3546328

Matthies D (1998) Influence of the host on growth and biomass allocation in the two facultative root hemiparasites *Odontites vulgaris* and *Euphrasia minima*. Flora 193:187–193

McArt SH, Halitschke R, Salminen J-P, Thaler JS (2013) Leaf herbivory increases plant fitness via induced resistance to seed predators. Ecology 94:966–975. doi:10.1890/12-1664.1

Pennings SC, Callaway RM (1996) Impact of a parasitic plant on the structure and dynamics of salt marsh vegetation. Ecology 77:1410–1419

Pennings SC, Callaway RM (2002) Parasitic plants: parallels and contrasts with herbivores. Oecologia 131:479–489

Pennings SC, Simpson JC (2008) Like herbivores, parasitic plants are limited by host nitrogen content. Plant Ecol 196:245–250. doi:10.1007/s11258-007-9348-z

Pérez-de-Luque A, Moreno MT, Rubiales D (2008) Host plant resistance against broomrapes (*Orobanche* spp.): defence reactions and mechanisms of resistance. Ann Appl Biol 152:131–141

Poelman EH, Van Loon JJA, Van Dam NM, Vet LEM, Dicke M (2010) Herbivore-induced plant responses in *Brassica oleracea* prevail over effects of constitutive resistance and result in enhanced herbivore attack. Ecol Entomol 35:240–247. doi:10.1111/j.1365-2311.2010.01179.x

Puustinen S, Salonen V (1999) The effect of host defoliation on hemiparasitic-host interactions between *Rhinanthus serotinus* and two *Poa* species. Can J Bot Rev Can Bot 77:523–530

R Core Team (2014) R: a language and environment for statistical computing R Foundation for Statistical Computing. Austria, Vienna

Ren YQ, Guan KY, Li AR, Hu XJ, Zhang L (2010) Host dependence and preference of the root hemiparasite, *Pedicularis cephalantha* Franch. (Orobanchaceae). Folia Geobot 45:443–455. doi:10.1007/s12224-010-9081-6

Rodriguez-Saona CR et al (2011) Tracing the history of plant traits under domestication in cranberries: potential consequences on anti-herbivore defences. J Exp Bot 62:2633–2644

Rowntree JK, Barham DF, Stewart AJA, Hartley SE (2014) The effect of multiple host species on a keystone parasitic plant and its aphid herbivores. Funct Ecol 28:829–836. doi:10.1111/1365-2435.12281

Runyon JB, Mescher MC, De Moraes CM (2006) Volatile chemical cues guide host location and host selection by parasitic plants. Science 313:1964–1967

Runyon JB, Mescher MC, Felton GW, De Moraes CM (2010) Parasitism by *Cuscuta pentagona* sequentially induces JA and SA defense pathways in tomato. Plant Cell Environ 33:290–303

Seel WE, Cooper RE, Press MC (1993) Growth, gas-exchange and water-use efficiency of the facultative hemiparasite rhinanthus-minor associated with hosts differing in foliar nitrogen concentration. Physiol Plant 89:64–70

Stam JM et al (2014) Plant interactions with multiple insect herbivores: from community to genes. Annu Rev Plant Biol 65(65):689–713. doi:10.1146/annurev-arplant-050213-035937

Theis N, Kesler KE, Adler LS (2009) Leaf herbivory increases floral fragrance in male but not female *Cucurbita pepo* subsp. *texana* (Cucurbitaceae) flowers. Am J Bot. 95(5):897-903

Tjiurutue MC, Sandler HA, Kersch-Becker MF, Theis N, Adler LS (2016) Cranberry resistance to dodder parasitism: induced chemical defenses and behavior of a parasitic plant. J Chem Ecol 42:95–106. doi:10.1007/s10886-016-0671-5

Uesugi A, Poelman E, Kessler A (2013) A test of genotypic variation in specificity of herbivore-induced responses in *Solidago altissima* L. (Asteraceae). Oecologia 173:1387–1396

Van Zandt PA, Agrawal AA (2004) Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). Ecology 85:2616–2629

Vorsa N (2010) Cranberry, in register of new fruit and nut cultivars, (List 45). In: Clark JR, Finn CE (Eds) HortScience 45:536–562

Vvedenskaya IO, Rosen RT, Guido JE, Russell DJ, Mills KA, Vorsa N (2004) Characterization of flavonols in cranberry (*Vaccinium macrocarpon*) powder. J Agric Food Chem 52:188–195. doi:10.1021/jf034970s

Walling L (2000) The myriad plant responses to herbivores. J Plant Growth Regul 19:195

Yoder JI, Scholes JD (2010) Host plant resistance to parasitic weeds; recent progress and bottlenecks. Curr Opin Plant Biol 13:478–484