Cranberry Resistance to Dodder Parasitism: Induced Chemical Defenses and Behavior of a Parasitic Plant

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Abstract Parasitic plants are common in many ecosystems, where they can structure community interactions and cause major economic damage. For example, parasitic dodder (*Cuscuta* spp.) can cause up to 80–100 % yield loss in heavily infested cranberry (*Vaccinium macrocarpon*) patches. Despite their ecological and economic importance, remarkably little is known about how parasitic plants affect, or are affected by, host chemistry. To examine chemically-mediated interactions between dodder and its cranberry host, we conducted a greenhouse experiment asking whether: (1) dodder performance varies with cranberry cultivar; (2) cultivars differ in levels of phytohormones, volatiles, or phenolics, and whether such variation correlates with dodder parasitism; (3) dodder parasitism induced changes in phytohormones, volatiles, or phenolics, and whether the level of inducible response varied among cultivars. We used five cranberry cultivars to assess host attractiveness to dodder and dodder performance. Dodder performance did not differ across cultivars, but there were marginally significant differences in host attractiveness to dodder, with fewer dodder attaching to Early Black than to any other cultivar. Dodder parasitism induced higher levels of salicylic acid (SA) across cultivars. Cultivars differed in overall levels of flavonols and volatile profiles, but not phenolic acids or proanthocyanidins, and dodder attachment induced changes in several flavonols and volatiles. While cultivars differed slightly in resistance to dodder attachment, we did not find evidence of chemical defenses that mediate these interactions. However, induction of several defenses indicates that parasitism alters traits that could influence subsequent interactions with other species, thus shaping community dynamics.

Keywords Parasitism · Plant-plant interactions · Induced responses · Phytohormones · Volatiles · Flavonols

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Introduction

Plants face a wide range of antagonistic interactions, including competition from other plants and consumption by herbivores. Although plants usually interact with other plants as competitors, many ecosystems also include parasitic plants that may play a key role in structuring community interactions (Pennings and Callaway 2002). In managed ecosystems, parasitic plants can cause major economic damage (Smith et al. 2013). Despite the importance of parasitic plants in both natural and agricultural settings, the role of plant chemical defenses that mediate resistance to parasitism, and the effect of parasitic plants on induced host responses are still unexplored.

The effect of different types of herbivory on induction of phytohormones is well established, but the extent to which

plant-parasitic plant interactions are similar to those of plant-herbivore interactions is largely unknown. In general, piercing and sucking herbivores (e.g., aphids and leafhoppers) induce salicylic acid (SA) mediated responses similar to those induced by biotrophic pathogens (Glazebrook 2005; Walling 2000), while chewing insects and necrotrophic pathogens generally induce jasmonic acid (JA) mediated defense responses (Glazebrook 2005; Walling 2000). However, both JA and SA have been shown to crosstalk, and the classification of JA as an herbivore defense response and SA as a pathogen defense response is not mutually exclusive (Thaler et al. 2012).

Several previous studies have examined induced chemical defenses in response to parasitic plants. The stem parasite, *Cuscuta pentagona*, had little effect on JA and SA accumulation upon first attachment to 10-d-old tomato plants (*Lycopersicon esculentum*), and did not induce a hypersensitive-like response (Runyon et al. 2010). However, older tomato plants responded to a second dodder attachment by activating both JA- and SA- signaling pathways and by inducing a strong hypersensitive-like response (Runyon et al. 2010). The hypersensitive-like response has been reported in dodder-resistant tomato cultivars in response to attachment by *C. reflexa* (Sahm et al. 1995), suggesting it may play a role in host plant resistance to dodder. In the *Striga* system, which has been extensively studied, parasitism by *S. hermonthica* induces genes involved in SA defense responses in the most resistant sorghum cultivar, suggesting that SA-induction may mediate interactions between the host and parasite (Smith et al. 2009). Additionally, in non-host species, resistance to *Striga asiatica* typically involves browning and necrosis of the root cortical cells of the host accompanied by cell wall thickening (Hood et al. 1998). However, more work is neededed in other systems to determine the generality of host induced responses and mechanisms of resistance to parasitic plants.

Cuscuta species may exhibit 'foraging behavior,' and discriminate among hosts based on quality. *Cuscuta pentagona* seedlings show directed growth towards tomato seedlings compared to artificial tomato plants, and toward extracted tomato plant volatiles in the absence of other cues (Runyon et al. 2006), suggesting that dodder uses volatiles to find host plants. *Cuscuta europaea* also exhibit directed growth towards hawthorn (*Crataegus monoguna*) hosts with high nutritional content, and grow away from hosts with low nutritional content (Kelly 1992), but the mechanism by which parasites distinguish between hosts is unknown. Understanding the mechanism of dodder preference and host resistance will broaden our understanding of cues used in plant foraging, and the roles that plant defenses play in mediating interactions with a range of antagonists.

Cranberry (Ericaceae*: Vaccinium macrocarpon*) is a temperate, perennial vine common in North American wetlands (Rodriguez-Saona et al. 2011) and native in Massachusetts. With sale values of $99.8 million in 2012, cranberry production was the second largest of all agricultural commodities in Massachusetts (National Agricultural Statistics Service 2011). Cultivated cranberry is genetically similar to native wild genotypes, making research with agricultural cultivars relevant to understanding ecological interactions in native systems (Rodriguez-Saona et al. 2011). There is some evidence for differences in host resistance across cultivars; e.g., chemical defenses and gypsy moth (*Lymantria dispar*) performance differ across cranberry cultivars (Rodriguez-Saona et al. 2011). Gypsy moth performed best on the highest yielding variety, NJS98-23, and on its parental variety, Ben Lear. The NJS98-23 cultivar had lower concentrations of JA and of induced volatile sesquiterpenes compared to ancestral cultivars, suggesting that high yielding cultivars may be susceptible to herbivore damage due to reduced chemical defenses (Rodriguez-Saona et al. 2011).

Dodder (*Cuscuta* sp.) is a generalist host stem parasite that infests and causes extensive damage each year to a wide range of agricultural crops including tomato (*Solanum lycopersicum*), alfalfa (*Medicago sativa*), potato (*Solanum tuberosum*), soybean (*Glycine max*), and onion (*Allium cepa*) (Runyon et al. 2008). Dodder can cause up to 80–100 % yield loss in heavily infested cranberry patches (Devlin and Deubert 1980). Its management is difficult because seeds can remain dormant for several years underground, and the close association of dodder and its host necessitates highly specific pesticides that target the parasite without killing the crop (Goldwasser et al. 2012). Currently, effective management of dodder requires integrating various methods, including killing current plants with herbicides, preventing seed production, and restraining the growth of new seedlings (Sandler and Ghantous 2014). Given the economic costs of dodder as a cranberry pest, it is important to assess variation in cultivar resistance to dodder and evaluate the potential role of chemical defenses and induced responses that mediate resistance. Such information could be used to target traits for developing resistant cranberry cultivars, offering producers an alternative management strategy for dodder control (Sandler 2010).

To examine chemically mediated interactions between dodder and its cranberry host, we conducted greenhouse experiments to ask the following questions:

1. Does host attractiveness to dodder and dodder performance vary with cranberry cultivar? 2. Do cultivars vary in levels of phytohormones, volatiles, or phenolics, and does such variation correlate with host attractiveness to dodder and dodder performance? 3. Does dodder parasitism

induce chemical changes in phytohormones, volatiles, or phenolics, and does the level of inducible response vary among cultivars ?

Methods and Materials

Cranberry Cultivars and Propagation We used five cranberry (*V. macrocarpon*, Ericaceae) cultivars: Crimson Queen, Mullica Queen, Stevens, Howes and Early Black; details on genetic background of cranberry cultivars are in Supplementary Material S1.

Cranberry vines were collected from the University of Massachusetts Cranberry Station in East Wareham, MA, USA over a period of 2 d in early October 2010. Vines were cut into 7.6 cm sections and sown in 72 plug trays filled with a 3:1 sand: peat soil mixture. Cultivar identities were confirmed via DNA finger printing using SCAR markers (Rodriguez-Saona et al. 2011). Roots were well established by November 2010, and cuttings were moved in December into cold storage at 5 °C and 78 % humidity. Cuttings were taken out of cold storage in mid-March 2011 after experiencing more than 2500 dark chilling hours, transported to University of Massachusetts Amherst, and placed in the greenhouse with natural lighting. One week later, upright cuttings were repotted into 10 cm plastic round pots in 3:1 sand: peat moss mixture. Plants were watered twice daily by hand. Approximately 1.5 g of 14-14-14 Osmocote fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) were added to each pot on 16 April 2011.

Experimental Design We conducted two parallel experiments simultaneously in the same greenhouse. Both used 4 uprights of the same cultivar per pot, and pot was considered the unit of replication. Blocks in each experiment, containing one pot per cultivar per treatment, were randomly rotated regularly to reduce variation due to greenhouse lighting. The first experiment assessed host attractiveness to dodder and dodder performance on each cranberry variety. This experiment used 20 replicates per 5 cultivars, all with germinated dodder seeds, for a total of 100 replicate pots in 20 blocks. The second experiment measured traits related to chemical defense (phytohormones, volatiles, and phenolics) in each cultivar, and asked whether dodder parasitism induces changes in these compounds. The induction experiment involved 20 replicate pots per 5 cultivars x 2 treatments (with or without dodder)=200 pots total. Twenty blocks, each containing one pot of each cultivar-by-treatment combination, were established by grouping plants by height.

Dodder Treatments Dodder seeds, *Cuscuta spp.* (Convolvulaceae) were collected on 28 September 2008 from Swan Holt, a commercial cranberry bog in Carver, MA, USA.

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 (K Ghantous, University of Massachusetts Cranberry Experiment Station, pers. comm.) (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). Seeds then were placed on a fine mesh strainer, rinsed, and placed in Petri dishes lined with 90 mm moistened filter paper and sealed with Parafilm. Petri dishes were placed in an incubator at 23 °C until the seed germinated, approximately 2 d later. Over a period of 3 wk as seeds germinated, each pot received one seedling per upright for both the performance and induction experiments. Dodder was added to all pots receiving dodder within a block on the same day. Seedlings were placed about 1 cm away from the base of each upright (vertical stem) by using fine tweezers. We measured the length of each cranberry upright on the day dodder was added for potential use as a covariate in analyses. Uprights were monitored daily, and first attachment (coiling around stems) of dodder was recorded for each pot to determine dates to measure induced responses.

Host Attractiveness and Dodder Performance Experiment To measure host attractiveness to dodder, days to first attachment, and total number of attached dodder per pot was recorded. After at least 3 wk of attachment, we measured dodder performance as the number of coils, haustorial attachments, and dodder mass. First, the total number of coils per pot was determined. Next, dodder was removed from uprights with tweezers, and total number of haustorial attachments was counted. Dodder vines were dried at 45° C for 1 wk and weighed to assess total dry mass of dodder per pot. Number of coils, number of haustoria, and dodder weight per pot were divided by the number of dodder attached per pot to obtain a mean value per dodder per pot for each response.

Induction Experiment: Chemical Responses

Phytohormones We measured leaf JA, SA, and abscisic acid (ABA) phytohormones from parasitized and non-parasitized cranberry cultivars. We used one experiment to measure phenolics and volatiles (below) that was conducted simultaneously with the host attractiveness and dodder performance experiment. Due to freezer failure and loss of original samples, a separate experiment with identical design was used to measure phytohormones in April 2012. Phytohormone analysis was performed on a randomly selected subsample of 80 pots (8 pots per cultivar per treatment x 5 cultivars x 2 treatments) from the original 100 pots. Leaves of parasitized plants were

collected 1–2 d after attachment together with the corresponding control plant in that block, placed in separate 5 ml cryovials, and immediately frozen in liquid nitrogen before storage at -80 °C. Phytohormone extraction and analysis were based on Thaler et al. (2010). Briefly, 200–300 mg of frozen leaf tissue were transferred into a 2 ml screw cap tube containing pre-weighed 0.9 g Silica beads (BioSpec, Bartelsville, OK, USA), and leaves were crushed into small particles inside the tubes. We added 100 μl of d4-SA and d5-JA (800 pg ml-1 each) as internal standards (CDN Isotopes, Point-Claire, Canada) with 1 ml extraction buffer (iso-propanol:water:hydrochloric acid 2:1:0.005 by vol), and homogenized the tissue in a FastPrep homogenizer (MP Biomedicals, Solon, OH, USA) at 6 m/s for 45 sec. We centrifuged the samples at 4 °C for 20 min at 20,800 x g (14,000 rpm), then carefully transferred the supernatant of each sample into a fresh 2 ml tube, added 1 ml of dichloromethane, and vortexed for 30 min. We centrifuged the samples at 4 °C for 20 min at $12,000 \times g$ for 2 min for phase separation. We then removed the aqueous (top) and middle layer completely and discarded it before evaporation of the remaining samples overnight under a fume hood. Samples were re-dissolved in 200 ml methanol and filtered through a 0.45 μm syringe filter (13 mm diam) into 2 ml HPLC vials with insert, and 15 μl of the remaining solvent were analyzed on a triple-quadrupole LC-MS/MS system (Quantum Access; Thermo Scientific, Waltham, MA, USA). A C18 reversed-phase HPLC column (Gemini-NX, 3 μ, 150×2.00 mm; Phenomenex, Torrance, CA, USA) was used to separate compounds using a solution of 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (solvent B) at a flow rate of 300 μl/min. Separation of compounds was performed using a gradient of increasing solvent B content. The initial gradient of solvent B was maintained at 10 % for 2 min and increased linearly to 100 % at 20 min. Phytohormones were analyzed by using negative electrospray ionization (spray voltage: 3.5 kV; sheath gas: 15; auxiliary gas: 15; capillary temperature: 350 °C), collision-induced dissociation (argon CID gas pressure 1.3 mTorr [1.3 micron Hg], CID energy 16 V), and by selected reaction monitoring (SRM) of compound-specific parent/product ion transitions: SA $137 \rightarrow 93$; d4-SA $141 \rightarrow 97$; JA $209 \rightarrow 59$; d5-JA $214 \rightarrow 62$ (Thaler et al. 2010).

Volatiles We sampled volatiles over a period of 2 wk in June, sampling each replicate 1–3 d after dodder's first attachment. We chose 1–3 d post-attachment with the intention of covering peak induction, since gypsy moth damage induce chemical changes after 2 d of damage (Rogriquez-Saona et al. 2011). Parasitized and non-parasitized pots of each cultivar within a

block were sampled on the same day. We sampled 200 pots in total (20 pots per cultivar per treatment x 5 cultivars x 2 treatments). We collected volatiles using dynamic headspace sampling for 4 h between 11:15 and 15:15 each day spanning a period of 2 wk as dodder attached to plants. One or more uprights were enclosed in polyethylene bags (Toppits, Cofresco Frischhalteprodukte Gmbh & Co. Kg, Minden, Germany). Only parasitized uprights were sampled in treatment pots, and we excluded dodder tissue by sampling new upright growth above parasitism sites. All living uprights with new growth were sampled in control pots. Thus, we sampled between 1 and 4 uprights together per pot, and considered pot as the unit of replication. A cartridge packed with 100 mg Porapak (Waters Corporation, Milford, MA USA) was inserted carefully into the top opening of the polyethylene bag, and ambient air was pulled by vacuum pump at a flow rate of ca. 200 ml/min (Air Check 52 or Air Check 2000 diaphragm pump, SKC, Eighty Four, PA, USA). A small inlet hole at the bottom of the bags allowed airflow. We collected ambient air at each sampling date for subtraction purposes. Cranberry flower fragrance and dodder fragrance also were collected for subtraction purposes, and we recorded the number of flowers on each upright if present. After sampling, cartridges were wrapped in aluminum foil, placed in a cooler, and eluted with 3 ml n-hexane into 4 ml vials, and stored in a refrigerator at -20 °C. Cartridges were cleaned with 10 ml acetone followed with 5 ml *n*-hexane (Fisher Scientific brand, Fair Lawn, NJ, USA), and stored in aluminum foil in polyethylene bags in the refrigerator between uses.

An internal standard (IS) of 3 μl of anisole was added to each sample before reducing the volume to 75 μl under a constant flow of nitrogen gas. We analyzed samples using combined capillary gas chromatography-mass spectrometry (GC-MS), with an Agilent GC 6890 equipped with a Mass Selective Detector 5973 (Agilent Technologies, Santa Clara, CA, USA). The GC was injected with 1 μl of each sample onto a non-polar column (ZB-5 ms, 30 m × 0.25 mm × 0.25 μm; Zebron, Phenomenex), at an initial temperature of 50 °C held for 2 min and then increased 10° C per min until temperature reached 275° C and held there for 3.5 min. Compounds were identified by matching GC retention times to previously used standards and to the Wiley Mass Spectral Library (Theis et al. 2009). Compounds were quantified by dividing the peak area of the mass ion of each scent compound by the peak area of the mass of 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). Compound identity was determined by

running standards, mass spectral libraries and published Kovats indices.

Phenolics Here and hence, we use the term 'phenolics' to include the sub-categories of flavonols (quercetin glycosides), phenolic acids (total chlorogenic acids), and proanthocyanidins (total individual oligomers and polymers). We measured leaf phenolics from parasitized and non-parasitized cranberry plants for each cultivar. This analysis was performed on a randomly selected subsample of 60 pots from the original experiment (6 samples per cultivar per treatment x 5 cultivars x 2 treatments) after volatile sampling. Leaves of both parasitized and non-parasitized control plants were placed in separate 5 ml cryovials (Fisher Scientific, cat. No.12-567-502, Fair Lawn, NJ, USA) and were immediately frozen in liquid nitrogen before storage at −80 °C. For extraction, leaves were crushed with liquid nitrogen using a mortar and pestle. Extraction and purification of leaf samples were carried out as described in Vvedenskaya et al. (2004). Briefly, approximately 0.25 g of leaf powder were placed into a 2 ml centrifuge tube, and 0.7 ml of a mixture of 80 % HPLC acetone, 0.1 % HPLC acetic acid and 19.9 % distilled water (by volume) was added to the tube. Samples were vortexed for 2 min, sonicated for 15 min, and then centrifuged at 12,000 rpm for 15 min at 4° C. The supernatant from each sample was transferred into a new centrifuge tube and the extraction was repeated using 0.5 ml of the acetone-acid-water solution. The supernatants were combined and filtered by using Spin-X microcentrifugal filters at 5000 rpm for 0.5 min, and the filtered samples were dried using a speed vacuum concentrator. Each sample was mixed with 1.2 ml of solvent B (20 % water adjusted to pH 3.5 using formic acid, 20 % methanol, and 60 % acetonitrile) and vortexed until the pellet was completely broken, followed by sonication for 20 min, centrifugation at 12,000 rpm for 1 min, and filtration using spin filters as described above. Complete analytical detection of phenolic acids and flavonol glycosides was achieved using HPLC (Waters, Milford, MA, USA) with a C18 Luna column (4.6 X 150 mm; particle size 5 μm; Phenomenex, Torrance, CA, USA) (Wilson et al. 2008). Forty μl of each filtered extract were injected, and compound separation was achieved using binary solvent system of solvent A (10 % methanol in water adjusted to pH 3.5 using formic acid) and solvent B (20 % water adjusted to pH 3.5 using formic acid, 20 % methanol and 60 % acetonitrile) with a linear gradient of 0 to 27 % B from 0 to 5 min; 27 to 40 % B from 5 to 27 min; isocratic elution of 40 % B from 27 to 30 min; linear gradient of 40 to 50 % B from 30 to 35 min; 50 to 90 % B from

35 to 40 min; 90 to 0 % B from 40 to 45 min and isocratic elution of 0 % B from 45 to 55 min at a flow rate of 1 ml/min for a final run time of 55 min. Equilibrium at 100 % A was performed for 5 min before and after each injection. Phenolic acids and flavonol glycosides were detected at 320 nm and 366 nm, respectively, in a photodiode array (PDA) detector. Identification of phenolic acids and flavonols was achieved by comparing their retention times and absorbance spectra to previously published data and authentic standards (Ranger et al. 2007; Vvedenskaya et al. 2004; Wilson et al. 2008).

Identification of individual oligomeric proanthocyanidins was obtained using a Dionex (Sunnyvale, CA, USA) HPLC apparatus equipped with a G-40 gradient pump, model 100 PDA detector, model AS50 autosampler/thermal compartment, and model ED50 detector. Separation of compounds was obtained by injecting 20 μl of each filtered sample onto A Develosil® diol column (250 X 4.6 mm internal diam; particle size 5 μm; Phenomenex, Torrance, CA, USA) at 25° C with a binary solvent system of solvent A (acetonitrile:acetic acid:10 mM ammonium acetate, 98:1:1 by volume) and solvent B (methanol:10 mM ammonium acetate:acetic acid, 95:3:2 by volume) with linear gradient of 0 to 10 % B from 0 to 5 min; 10 to 12 % B from 5 to 8 min; 12 to 13 % B from 8 to 10 min; 13 to 20 % B from 10 to 15 min; 20 to 40 % B from 15 to 35 min; isocratic elution of 40 % B from 35 to 40 min; linear gradient of 40 to 0 % B from 40 to 45 min and isocratic elution of 0 % B from 45 to 50 min at a flow rate of 1 ml for a total run time of 50 min. Proanthocyanidins were detected at 280 nm in PDA detector, and identified based on peak retention times and absorbance spectra (Wilson et al. 2008).

Statistical Analysis

Host Attractiveness and Performance Experiment We used R. Studio (version 0.98.507, RStudio, Inc.) to carry out all statistical analyses. We analyzed host attractiveness as days to first attachment using analysis of variance (ANOVA), and analyzed the total number of dodder attached per pot using generalized linear mixed models (GLMMs) with a quasibinomial distribution and logit link function to correct for overdispersion. We measured dodder performance as mean number of coils per dodder, mean number of haustoria per dodder, and mean dry weight per dodder, all log-transformed to improve normality. We tested for effects of cultivar on these three measures of performance using MANOVA. For all analyses, the model included cultivar as a fixed factor and block as a random factor, using linear mixed effects models (LME) where appropriate. Host height initially was included as a covariate but dropped because it was not

significant. Significant MANOVA results were followed with separate ANOVAs for each response variable. We used Tukey's Studentized Range test ($\alpha = 0.05$) for *post-hoc* tests of differences between cultivars.

Induction Experiment: General Approach Models (GLMMs) for all chemical responses included dodder treatment, cultivar, and their interactions as fixed effects and block as a random variable. In all analyses host height was included initially as a covariate, but removed because it was never significant. All chemical responses were tested for normality and log-transformed when appropriate. All significant MANOVAs were followed by separate ANOVAs, and we used Tukey's Studentized Range test ($\alpha = 0.05$) for *post-hoc* comparisons between cultivars and treatments.

To determine whether induced responses were stronger with more dodder plants attached and whether cultivars differed in the strength of induction in response to multiple attachments, we ran a separate ANCOVA or MANCOVA for each chemical response category using only dodder treated plants, including cultivar as a fixed effect, block as random factor, the number of dodder seedlings attached as a covariate, and the number of dodder seedlings attached x cultivar interaction. We also included the number of days between dodder attachment and sampling as a covariate. However, there was never a significant relationship between number of dodder attached and the strength of induction, and so we did not report these analyses.

Phytohormones Independent ANOVAs were used for SA, JA, and ABA; each response was log-transformed prior to analysis. We did not use MANOVA because 7 strong outliers that violated normality assumptions (all 3 SD above the mean) were deleted for JA (3 dodder with Howes, one Early Black and one Crimson Queen; 2 control Howes and one control Mullica Queen), of which a subset of three were also outliers for ABA (one control Mullica Queen, one control Howes, and one parasitized Early Black). Including all phytohormones in one MANOVA would have removed those 7 replicates from all analyses.

Volatiles All volatile emissions were calculated as an hourly emission rate scaled by the wet mass of the sample, with the units ng/g wet mass/hour. We log-transformed all volatile classes to improve normality. Volatiles were grouped based on their biosynthetic origin into sesquiterpenoids, homoterpenoids, monoterpenoids, esters, fatty acids, alkanes, and unknowns that were analyzed as responses with MANOVA. We also analyzed individual volatiles as volatile composition with a separate MANOVA, and analyzed total terpenoids (sum of sesquiterpenoids, homoterpenoids, and monoterpenoids) and total volatile emissions using separate ANOVAs. We deleted two outliers from the analysis of

alkanes (one Mullica Queen control plant and one Early Black dodder treatment, both 3 SD above the mean) and one outlier from unknowns (the same deleted for alkanes from Mullica Queen) because they violated normality assumptions.

We also used a permutational multivariate analysis of variance (PERMANOVA), a non-parametric test that is more robust to violations of normality assumptions than MANOVA (Anderson 2001) to test for differences in volatile composition, including cultivar and dodder treatment as independent variables, block as a random factor, height as a covariate, and all individual volatiles or group of volatiles described earlier as responses. However, this analysis gave results that were similar to the MANOVA and so we did not report it. Finally, we calculated volatile diversity for all individual compounds, and calculated diversity within volatile groups and then averaged across groups for each replicate, using the Shannon-Weiner diversity index and evenness using Evar (Smith and Wilson 1996). Separate ANOVAs were employed to test measures of volatile diversity and evenness, including dodder treatment and cultivar as independent variables.

Phenolics We analyzed phenolics in 3 major groups consisting of flavonols, total phenolic acids, and proanthocyanidins. Flavonols included quercetin-3-galactoside, quercetin-3-xyloside, quercetin-3-arabinopyranoside, quercetin-3-arabinofuranoside, quercetin-3-rhamnoside, and quercetin aglycone. They were analyzed using MANOVA. Total flavonols were analyzed separately with ANOVA. Phenolic acids were calculated from total chlorogenic acids, and total proanthocyanidins were calculated from individual proanthocyanidin oligomers and polymers. Phenolic acids and total proanthocyanidins each comprised single categories and were analyzed using separate ANOVAs. We log-transformed proanthocyanidins and all individual flavonols to improve normality; total flavonols and phenolic acids were untransformed.

Results

Host Attractiveness and Performance Host attractiveness, measured as the number of attached dodder stems per pot, marginally differed across cultivars ($t_{99} = 1.98$, $P = 0.051$). Although the overall cultivar effect was only marginally significant, in Tukey's *post-hoc* contrasts Early Black was least preferred by dodder, with significantly fewer attachments per upright than other cultivars (Fig. 1). Days to first attachment did not differ across cultivars ($F_{4, 76} = 0.44$, $P = 0.78$). No other measure of dodder performance (number of haustoria, number of coils, and weight per dodder) was affected by cultivar ($F_{4, 18} < 1.3$, $P > 0.3$ for all).
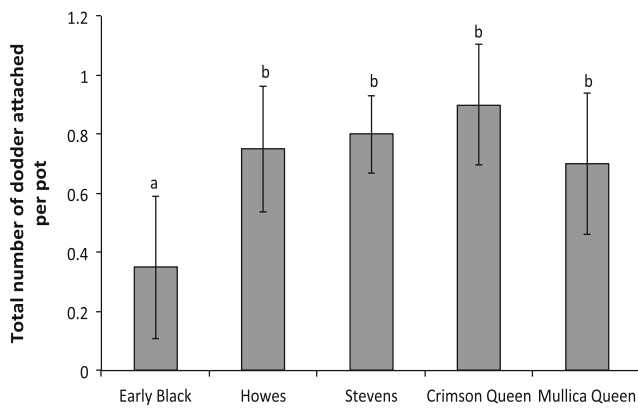
Fig. 1 Host attractiveness measured as the total number of dodder stems attached, across cranberry cultivars. Different letters above bars indicate significantly different means using Tukey's *Post-Hoc* test ($P < 0.05$). Bars are mean ±1SE

Induction Experiment

Phytohormones Dodder increased SA concentrations by approximately 50 %, but this effect was only marginally significant (Table 1; Fig. 2a). There was no dodder treatment effect on JA and ABA, and no dodder-by-cultivar interaction for any phytohormone (Table 1). All three phytohormones (SA, JA, and ABA) differed with cultivar (Table 1). *Post-hoc* tests showed that SA concentrations were highest in Stevens and lowest in Mullica Queen (Fig. 3a). Jasmonic acid was highest in Crimson Queen and Howes, and lowest in Stevens (Fig. 3b). Stevens had significantly higher levels of ABA than Mullica Queen, Crimson Queen, and Howes, with Early Black intermediate (Fig. 3c; see Supplementary Material S2 for phytohormones means across all cultivar and dodder treatments).

Volatiles Dodder parasitism did not induce changes in volatile groups (dodder treatment: Pillai's trace = 0.038, $F_{8, 164} = 0.81$, $P = 0.59$) and cultivars did not differ in their response to parasitism (cultivar x dodder interaction: Pillai's trace = 0.13, $F_{32, 668} = 0.69$, $P = 0.91$). However, cultivars differed in volatile groups (MANOVA; cultivar: Pillai's trace = 0.66, $F_{32, 668} = 4.13$, $P < 0.001$), and total volatile emissions ($F_{4, 152} = 3.89$, $P = 0.005$; Fig. 4a). Subsequent ANOVAs (Table 1) showed that cultivars differed in sesquiterpenoids (Fig. 4b), homoterpenoids (Fig. 4c), alkanes, fatty acids, and unknowns. Cultivars did not differ in emissions of monoterpenoids, terpenoids, aromatics, or esters. Similarly, when analyzing individual compounds rather than groups, dodder parasitism did not induce changes in volatile composition (dodder treatment: Pillai's trace = 0.23, $F_{47, 125} = 0.81$, $P = 0.79$), and cultivars did not differ in their response to parasitism (cultivar x dodder interaction: Pillai's trace = 0.84, $F_{188, 512} = 0.73$, $P = 0.99$). However, cultivars did differ in their volatile composition (MANOVA; cultivar: Pillai's trace = 2.14, $F_{188, 512} = 3.12$,

$P < 0.001$). Separate ANOVAs showed that cultivars differed in many individual compounds (see Supplementary Material S3).

Volatile diversity did not differ with cultivar or treatment using the Shannon-Weiner diversity index ($F < 1.4$, $P > 0.1$ for both). When calculating diversity first within volatile groups and then averaging across groups, volatile diversity differed with cultivars across groups ($F_{4, 4} = 6.41$, $P = 0.05$) and marginally with dodder treatment ($F_{4, 4} = 5.56$, $P = 0.08$). *Post-hoc* Tukey's HSD student test showed that Howes was more diverse and significantly different than Stevens. Diversity did not differ with cultivar or treatment for within groups ($F < 0.04$, $P > 0.86$ for all), and evenness did not differ with cultivar or treatment for total or grouped volatiles ($F < 1.30$, $P > 0.40$ for all).

Phenolics Overall, both cultivar and dodder treatment affected flavonol levels (MANOVA; cultivar: Pillai's trace = 0.86, $F_{28, 188} = 1.83$, $P = 0.01$; dodder treatment: Pillai's trace = 0.40, $F_{7, 44} = 4.20$, $P = 0.001$) but the cultivar-by-dodder interaction did not (Pillai's trace = 0.56, $F_{28, 188} = 1.09$, $P = 0.35$). Dodder parasitism increased the levels of two flavonols, quercetin-3-galactoside (Table 1; Fig. 2b) and quercetin-3-xyloside (mean ± SE: dodder treatment = 2.168 ± 0.396; control = 1.652 ± 0.302) by at least 250 % compared to unparasitized plants, but reduced quercetin-3-rhamnoside concentrations by approximately 38 % compared to controls (Fig. 2c). Dodder parasitism increased phenolic acid concentrations by 37 % compared to controls (Fig. 2d).

Cultivars differed significantly in five flavonols: quercetin-3-galactoside, quercetin-3-xyloside, quercetin-3-rhamnoside, quercetin-3-arabinopyranoside, and quercetin-3-arabinofuranoside (Table 1, Fig. 5). Mullica Queen generally had higher concentrations of flavonols compared to other cultivars, while Howes and Crimson Queen tended to have the lowest concentrations. Cultivars did not differ in concentrations of the flavonol quercetin aglycone, or in total proanthocyanidins or phenolic acids (Table 1).

Discussion

Does Host Attractiveness to Dodder and Dodder Performance Vary with Cranberry Cultivar?

We hypothesized that dodder attachment or performance would differ with cultivar, and that such differences would correspond with variation in chemical defense. Although only marginally significant, we found that dodder distinguished between cultivars, with greater than 50 % decrease in the number of attachments to Early Black than any other cultivar. Thus, cranberry joins a small list of other crops with varieties that differ in dodder resistance (Goldwasser et al. 2001, 2012),

Table 1 *F* values from mixed model ANOVA testing effects of dodder treatment and cultivar on cranberry chemistry, with block as a random factor

Response	Cultivar	Dodder treatment	Cultivar x Dodder	Error *df*
Phytohormones				
salicylic Acid	**2.89***	**5.31**[a]	0.51	77
sasmonic Acid	**4.56****	0.73	0.91	77
abscisic Acid	**4.37****	0.05	0.18	77
Volatiles				
monoterpenes	1.29	**3.15**[a]	0.84	152
homoterpenes	**3.39***	0.65	1.05	152
sesquiterpenes	**2.56***	0.33	0.99	152
terpenoids	1.56	2.14	0.83	152
aromatics	2.02	**2.90**[a]	0.55	152
alkanes	**4.12****	0.25	1.05	150
esters	1.49	0.15	0.70	152
fatty acids	**6.70****	0.0015	1.31	152
unknowns	6.55	0.046	0.30	151
Total	**3.89***	1.11	1.29	
Flavonols				
quercetin-3- galactoside	**2.90***	**15.26 ****	0.62	45
quercetin-3-arabinopyaranoside	**3.82****	0.74	0.08	45
quercetin-3-arabinofuranoside	**3.55***	**3.20**[a]	1.56	45
quercetin-3-xyloside	**3.64***	**7.04***	0.95	45
quercetin-3-rhamnoside	**3.19***	**9.17****	1.03	45
quercetin aglycone	1.02	2.12	**2.09**[a]	45
Total Flavonols	1.26	**3.2**[a]	0.39	45
Phenolic acids	1.78	**6.86***	1.20	45
Proanthocyanidins	0.61	2.73	1.17	45

For all analyses, the numerator *df* is 4 for cultivar, 1 for dodder treatment, and 4 for their interaction; error *df* is listed for each analysis. [a] $P < 0.08$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Bold values indicate significant effects at $P < 0.05$

although host traits responsible for resistance were not examined in their studies. In our study, although dodder attachment differed among cultivars, dodder performance post-attachment did not. This suggests that the best approaches for managing dodder may involve breeding for traits that influence attractiveness, thus preventing dodder attachment rather than traits that affect dodder performance after attachment.

Do Cultivars Vary in Levels of Phytohormones, Volatiles, or Phenolics, and Does Such Variation Correlate with Host Attractiveness to Dodder and Dodder Performance?

Cultivars differed in a wide range of chemical traits. Although closely related to wild progenitors, cranberries have been subjected to selective breeding under domestication for favorable plant traits such as high yield, vigorous growth, early-season fruit ripening, fruit color, and size, which may or may not be correlated with plant defensive traits (Rodriguez-Saona et al. 2011). Selection for these traits may have resulted in tradeoffs

with plant defense, as plants allocate more resources to fruit production and rapid growth. However, selection of some traits, such as high levels of anthocyanins favored for color intensity and antioxidant properties that can benefit human health (Blumberg et al. 2013), may have enhanced plant defense (Rodriguez-Saona et al. 2011). Thus, it might be reasonable to expect that some cranberry hybrids may have reduced plant defense as a result of selective breeding compared to their parental counterparts, and others may have enhanced defenses. We found that Crimson Queen, a hybrid resulting from a cross between Stevens and Ben Lear, had low overall levels of flavonols but higher levels of JA compared to its parental cultivar Stevens (Figs. 3 and 5). On the other hand, Mullica Queen, another recent hybrid cross, had high levels of overall flavonols but lower levels of SA compared to other cultivars (Figs. 3 and 5). Thus, recent breeding efforts have produced new hybrid cultivars that differ widely in levels of phytohormones and defensive compounds. These changes in plant defenses could affect the outcome of interactions not only with parasites, but also with herbivores and natural enemies.

Fig. 2 Effects of dodder presence using ANOVA on (**a**) salicylic acid (SA), and the flavonols (**b**) quercetin-3-galactoside and (**c**) quercetin-3-rhamnoside, as examples of induced increases and decreases following dodder parasitism, and (**d**) phenolic acid concentrations. Bars are mean ±1SE
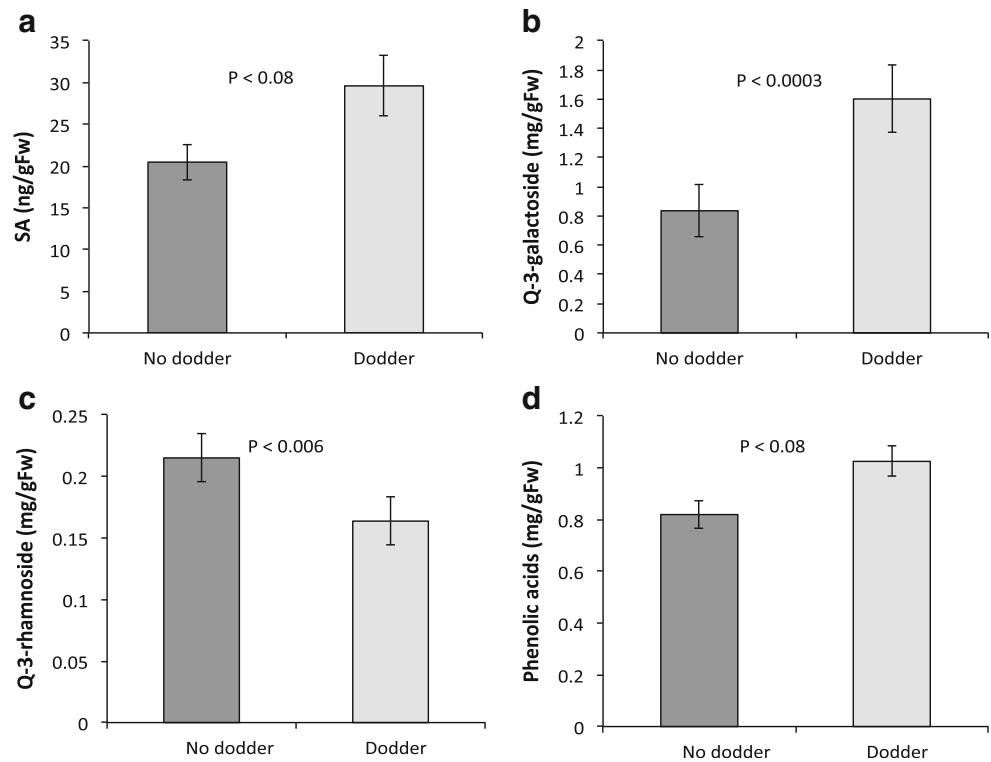
Although cultivars differed in levels of phenolics and phytohormones, we found no evidence to implicate any particular compound in dodder resistance. Early Black had lower dodder attachment than any other cultivar (Fig. 1), but no phenolic compound or phytohormone stood out as being noticeably higher or lower in Early Black compared to other cultivars (Figs. 3 and 5; data not shown for others). However, we only used leaf tissue for chemical analysis. It is possible that bark flavonoids and other secondary defenses could play a role in dodder

Fig. 3 Phytohormone differences for overall levels between cranberry cultivars. **a** salicylic acid (SA), **b** jasmonic acid (JA), and **c** abscisic acid (ABA). Different letters above bars indicate significantly different means using Tukey's *Post-Hoc* test ($P < 0.05$). Bars are mean ±1SE
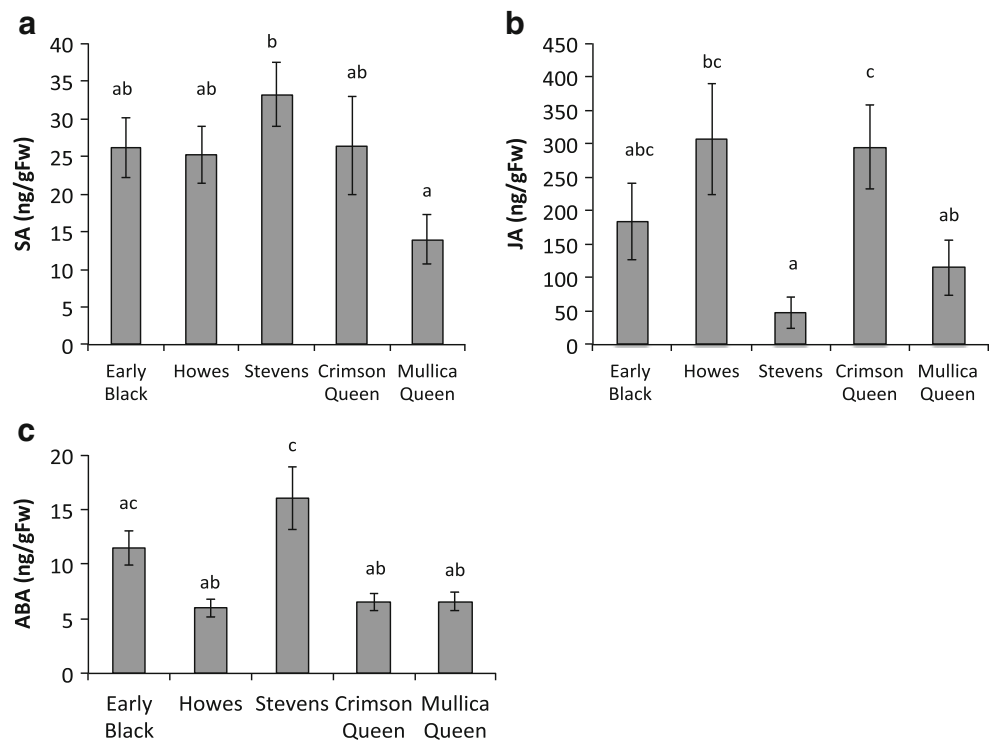
Fig. 4 Differences between cranberry cultivars for overall (**a**) total volatile emissions, (**b**) sesquiterpenes, and (**c**) homoterpenes. Different letters above bars indicate significantly different means using Tukey's *Post-Hoc* test (*P* < 0.05). Bars are mean ±1SE
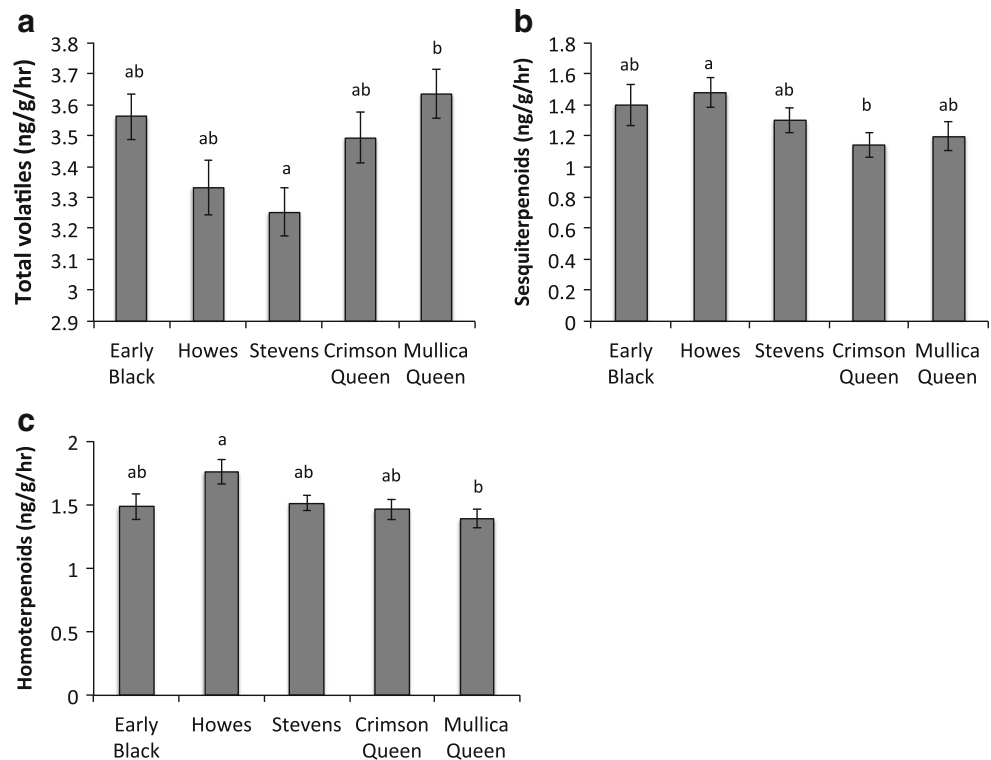
resistance. For example, Kelly (1990) reported that the dodder *C. subinclusa* can recognize host species when foraging, and may respond to the presence of flavonoid compounds from the bark of its host plant, *Malosma laurina*. Thus, host bark traits specifically, rather than leaf chemical traits, might affect host attractiveness to dodder and attachment. Future studies should

Fig. 5 Differences between cranberry cultivars for the flavonols (**a**) quercetin-3-galactoside, (**b**) quercetin-3-arabinopyranoside, (**c**) quercetin-3-rhamnoside, and (**d**) quercetin-3-xyloside. Different letters above bars indicate significantly different means using Tukey's *Post-Hoc* test (*P* < 0.05). Bars are mean ±1SE
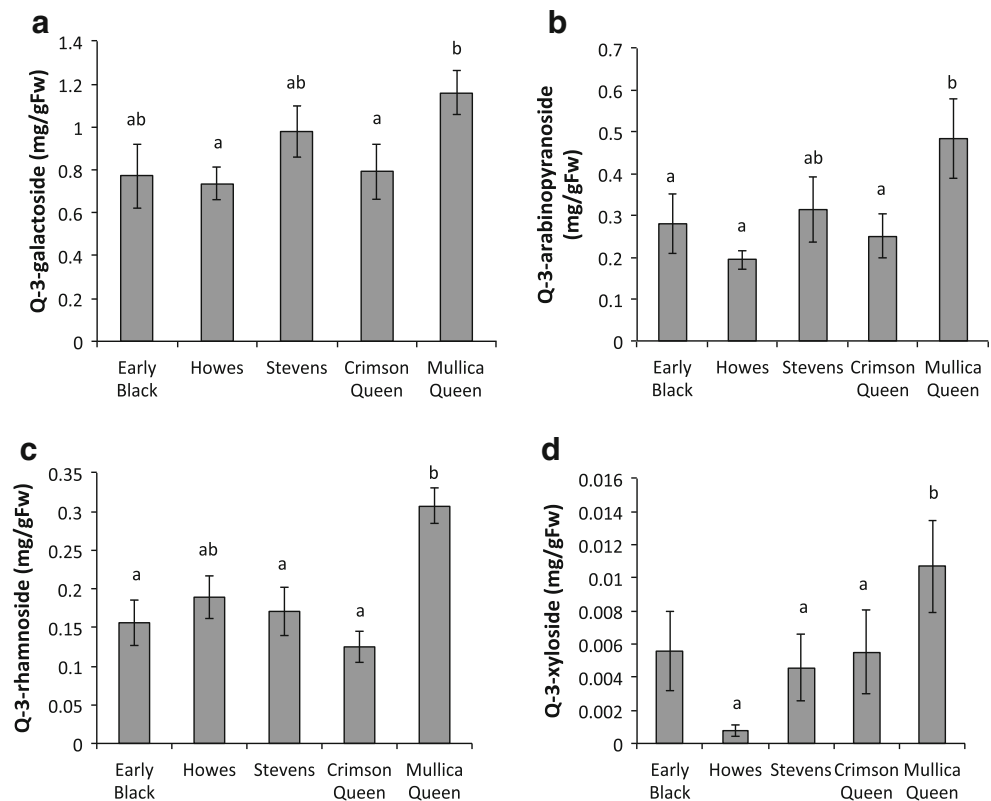
assess the role of cranberry bark host chemistry in dodder resistance.

Cultivars differed widely in both the amount and composition of volatile emissions. Runyon et al. (2006) showed that dodder explorers hosts and selects between preferred hosts (tomato) and non-preferred hosts (wheat) based on volatile cues. Furthermore, experiments with individual compounds from tomato blends showed that dodder grew towards the monoterpenes β-phellandrene, β-myrcene, and α-pinene, and one compound ((Z)-3- hexenyl acetate) caused a significant negative growth response. These results suggest that individual volatile compounds can attract or deter dodder from particular hosts. As with the phenolics, Early Black was not notably different from other cultivars in total or any particular category of volatile emissions. It also is possible that blends of volatile compounds, rather than single compounds or compound classes, mediate dodder responses to cranberry (Snoeren et al. 2010). Although volatile diversity differed with cultivar, Howes rather than Early Black had the highest diversity, suggesting that diversity *per se* does not explain differences in dodder preference. Exploring plant parasite responses to different cranberry host volatile cues as whole blends rather than individual compounds or compound classes may yield more insight into the mechanisms of dodder resistance.

Does Dodder Parasitism Induce Chemical Changes in Phytohormones, Volatiles, or Phenolics, and Does the Level of Inducible Response Vary Among Cultivars?

Dodder parasitism did not affect the concentrations of JA or ABA, but increased SA concentrations. SA is involved in defense responses induced by pathogens (Brading et al. 2000), while JA usually is involved in mediating responses to chewing herbivores (Thaler et al. 2001). However, the classification of SA as a pathogen induced-signaling pathway and JA as a chewing herbivore induced-signal pathway is not always mutually exclusive, and cross-talk can occur between the two pathways (Thaler et al. 2010). Our results suggest that dodder may induce a defense response that is similar to that induced by pathogens. The only other study to examine phytohormone induction in response to dodder parasitism found that a second dodder attachment to a 20-day-tomato plant induced both JA and SA responses, which appeared to reduce growth of the parasite (Runyon et al. 2010). In that study, both the JA and SA pathways were induced with different time courses, suggesting that the host may recruit both pathways as a defense mechanism (Runyon et al. 2010).

Dodder parasitism induced many changes in phytohormone, volatile, and flavonol levels in cranberry. However, we found no evidence suggesting that any of these compounds influenced dodder parasitism, which did not differ across cultivars, or preference. Cranberries may have a general wound

response against parasitism instead of a specific defense mechanism against plant parasites.

Despite the important roles that parasitic plants play in communities (Pennings and Callaway 2002), little is known about plant defenses against parasitic plants and how these defenses affect other host-plant interactions. Runyon et al. (2008) found that parasitized tomato plants by *C. pentagona* produced one-third lower JA levels in response to insect feeding by the beet armyworm (*Spodoptera exigua,* Noctuidae*: Lepidoptera*) compared to unparasitized tomato plants. Additionally, parasitized tomato plants did not produce herbivore-induced volatiles after 3 days of insect feeding, and growth of the beet armyworm was slower on parasitized compared to unparasitized plants. Thus, understanding induced defenses in response to parasitic plants is important not only for understanding ecological dynamics, but also to explore the manipulation of defense pathways to control parasitic pests in agriculture. Our work and one previous study (Runyon et al. 2008) both indicate that dodder may induce a range of chemical changes in host plants that have the potential to affect herbivore preferences and shape subsequent herbivore communities, with likely consequences for crop yield.

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