Degree of dietary specialization on furanocoumarin-containing hostplants in a newly invasive species, Depressaria depressana (Lepidoptera: Depressariidae)

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Research Article

Keywords: Herbivory, phytochemistry, coevolution, Apiaceae, Depressariidae

Posted Date: July 29th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1883087/v1

Title: Degree of dietary specialization on furanocoumarin-containing hostplants in a newly invasive species, *Depressaria depressana* (Lepidoptera: Depressariidae)

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Abstract – The genus *Depressaria* (Lepidoptera: Depressariidae) mostly comprises specialist herbivores with varying capacity for detoxification of defensive phytochemistry. *Depressaria depressana*, a Eurasian moth recently introduced into North America, is a family-level specialist of the Apiaceae ,whose hosts include more than a dozen species in multiple tribes; *Depressaria radiella* is a family specialist of Eurasian origin that feeds almost exclusively on species in the genera *Pastinaca* and *Heracleum* throughout its native and introduced range. In eastern North America it is found almost exclusively on *Pastinaca sativa*, an invasive European species, and *Heracleum maximum*, a native species. We determined whether differences in furanocoumarin metabolism exist between *D. depressana* and two isolated populations of *D. radiella,* feeding exclusively on either *P. sativa* or *H. maximum*. We also compared gravimetric estimates of efficiency to assess *D. depressana* larval performance on different diets. Both populations of *D. radiella* metabolized furanocoumarins at a greater rate than *D. depressana*. Although there was no difference in rates of metabolism of linear furanocoumarins in the two populations of *D. radiella*, individuals collected from *H. maximum* metabolized angular furanocoumarins more rapidly. The gravimetric assessments of performance revealed that *D. depressana* exhibited highest efficiencies consuming *D. carota*; moreover, this species could survive consuming fruits of *Z. aurea*, an apiaceous species native to North America. Our preliminary phylogenetic analysis, building on an earlier morphological analysis, incorporates the mitochondrial cytochrome oxidase subunit 1 data, and revealed that the presence or absence of furanocoumarins is not a strong predictor of species-level evolution in *Depressaria*.

Keywords - Herbivory, phytochemistry, coevolution, Apiaceae, Depressariidae

INTRODUCTION

Adaptive radiation is defined as the process in which ancestral species diversify into many new forms in response to environmental changes that create new habitats, make new resources available, or alter interactions with other species. This process underlies the seminal “escape and radiation” hypothesis outlined by Ehrlich and Raven (1964) to account for hostplant association patterns across a broad range of butterflies encompassing five families. In this model, Ehrlich and Raven (1964) proposed that plant lineages enter new adaptive zones within which they are free to diversify rapidly after escaping selective pressures of herbivory through novel defensive adaptations. Insects that subsequently evolve suitable counter-adaptations to these defenses are provided with a new exploitable food resource and thus similarly undergo rapid diversification upon entering an adaptive zone of their own. This “arms race” of key innovations resulting in escalating reciprocal selective pressure within plant-insect interactions is thought to be a driving force underlying the evolution of specialist feeding strategies in insects (Berenbaum et al. 1996).

In terms of degrees of specialization of herbivorous insects, Ali and Agrawal (2019) defined insects that feed on only a single plant taxon and its close relatives (often within one genus) as monophagous, on several genera (often within a single family) as oligophagous, and on more than one plant family as polyphagous, or "highly generalized." However, these authors note, “The distribution of feeding on one plant species to a diversity of plants is truly a graded continuum.” Even within groups of herbivores conventionally defined as specialists, a continuum of feeding strategies can exist, with some family-level specialists feeding on hostplants on multiple tribes within a family and other family-level specialists restricted to only one or two closely related genera. Thus, family-specialists, which feed broadly across a hostplant family, can potentially encounter a more diverse array of defense chemicals than would family-specialists restricted to a small number of closely related genera with similar phytochemical profiles, i.e., "super-specialists," and may consequently differ in the degree of specialization of their detoxification mechanisms. Diet breadth, even within a single hostplant family, may provide insights into the likelihood of success of a specialist group in invading new habitats and colonizing novel hostplants.

The insect herbivore community of species in the family Apiaceae is dominated by specialists, a pattern thought to reflect the toxicological challenges presented by the defensive chemistry of the family (Berenbaum 1990). Among the widely distributed defensive phytochemicals in the family are furanocoumarins, which owe their broad toxicity to their ability to bind to DNA in the presence of ultraviolet light and interfere with transcription

(Berenbaum 1991). Studies of the changes in furanocoumarin structure and composition throughout the evolutionary history of Apiaceae, as well as the subsequent evolution of furanocoumarin resistance in species in the genus *Papilio* (Lepidoptera: Papilionidae), provided early evidence suggestive of the mechanisms underlying the steps involved in the "escape and radiate" model (Berenbaum 1983). Of the two main structural classes of furanocoumarins, characterized by the attachment of the furan ring to the coumarin nucleus, linear furanocoumarins are widely distributed across four plant families and sporadically in seven additional families, whereas angular furanocoumarins are more derived and restricted in distribution, effectively limited to only a handful of genera in three families (Berenbaum 1991; Bruni et al. 2019).

Among Apiaceae specialists are the majority of species in the genus *Depressaria* with known feeding habits (Bucheli et al. 2010). The genus is Holarctic in distribution, with approximately 100 described species; Passoa (1995) and Bucheli et al. (2010) suggested that the Apiaceae is the ancestral host family, with the association likely originating in the Palearctic with "multiple colonization events into the Nearctic region" (Bucheli et al. 2010). Within the genus, however, there is a range of variation in the degree of specialization; whereas some species feed broadly on species in multiple tribes within the family (or in some cases, on species in the Asteraceae or Rutaceae), others are restricted to only one or two closely related genera within a single tribe (Table 1).

In lepidopterans specialized to some extent on apiaceous hostplants, furanocoumarins are metabolized largely via cytochrome P450 monooxygenases (Berenbaum et al. 1986; Mao et al. 2006; Calla et al. 2020). A long-studied Apiaceae-specialist is the parsnip webworm, *Depressaria radiella* (formerly *D. pastinacella*), a Eurasian species that was first introduced to North America in the mid-19th century (Zangerl and Berenbaum 2003). As a specialist with few hostplants other than species in the closely allied genera *Pastinaca* and *Heracleum, D. radiella* displays phenotype matching with its principal eastern North American hostplant *Pastinaca sativa* (wild parsnip), whereby population-level profiles of furanocoumarin detoxification activity reflect the population-level profile of furanocoumarin defensive chemistry of the immature fruits of its hostplants (Berenbaum and Zangerl 1992). Since its introduction to North America, *D. radiella* has colonized the native species *Heracleum maximum* (cow parsnip) (Zangerl and Berenbaum 2003), a congener of several European hostplant species, including *H. sphondylium* and *H. sibiricum* (Palm 1989). Relative to *P. sativa*, and to most other apiaceous species in other genera, *H. maximum* produces a broad spectrum of furanocoumarins, including six angular furanocoumarins (Berenbaum 1981; O'Neill et al. 2013; Dhar et al. 2014). In one central Illinois population, levels of sphondin, an angular furanocoumarin

commonly produced by apiaceous species, in *H. maximum* were 29-fold higher than levels in nearby populations of *P. sativa* (Berenbaum and Zangerl 1991). Although a comparison of performance of *D. radiella* on these two hostplants revealed overall higher survival of larvae on *P. sativa* irrespective of their natal hostplant, Berenbaum and Zangerl (1991) found that larvae from *H. maximum* populations exhibited more rapid development and higher pupal weights on diets containing fruits of *H. maximum* compared to individuals collected from *P. sativa* and raised on the same diet (Berenbaum and Zangerl 1991), suggestive of some degree of local adaptation.

Less is known about the furanocoumarin detoxification capacity of a *D. radiella* congener, the recently invasive *Depressaria depressana*. This exotic species, Eurasian in origin, was first discovered in North America in Quebec, Canada, in 2008 (Landry et al. 2013) and reported in United States in 2016 (Harrison et al. 2016). *D. depressana* consumes flowers and immature fruits of *P. sativa* and *Daucus carota* (wild carrot) in North America (Harrison et al. 2016) and in its native range is associated with at least a dozen apiaceous genera in a diversity of tribes (Table 1). While *P. sativa* produces high amounts of furanocoumarins, particularly the linear furanocoumarin xanthotoxin (Zangerl and Berenbaum 2003), *D. carota* fruits contain linear but not angular furanocoumarins (Khalil et al. 2018), which do not exhibit the same phototoxic activity characteristic of linear furanocoumarins. Although *D. depressana* has been reported to feed on "cow parsnip" in Europe (Ovsyannikova and Grichanov 2021), it has not yet been reported to feed on any *Heracleum* species in North America. Taken together, these components of the ecology of *D. depressana* suggest that its capacity for metabolizing furanocoumarins may be limited relative to *D. radiella*.

Thus, the recent appearance of *D. depressana* in central Illinois provided a unique opportunity to test the hypothesis that the detoxification capacity of a family-specialist herbivore is likely to be limited in comparison to the detoxification capacity of a super-specialist herbivore relative to the principal defensive chemicals across their range of hostplants. A multifaceted approach, with direct experimental tests of the metabolic activity and development of *D. depressana* interpreted in the context of the evolutionary history of *Depressaria* species and their hostplants, can shed light on the evolutionary history of association with plants containing furanocoumarins as well as provide a preliminary basis for evaluating the likelihood of this species to colonize native North American plant species, an important issue for evaluating potential adverse ecological impacts of invasive herbivores. To achieve these goals, we conducted midgut enzymatic assays to compare the efficiency with which furanocoumarins are metabolized by these two *Depressaria* species, assessed the ability of *D. depressana* to utilize hostplants varying in

furanocoumarin content and composition, including a native species (*Zizia aurea*, golden alexanders), and reconstructed the phylogeny of *Depressaria* in the context of acquisition of furanocoumarin-containing hostplants.

Bucheli et al. (2010) presented the most comprehensive species-level *Depressaria* phylogeny to date. This phylogeny was constructed using a parsimony analysis on a dataset of 47 morphological characters. Hostplant and geographic associations were mapped onto the consensus tree. Hostplant family was used as a proxy for presence of furanocoumarins in hostplants. On the basis of coevolutionary theories proposed by Ehrlich and Raven (1964) and Berenbaum (1983), Bucheli et al. (2010) predicted that more derived species of *Depressaria* would show increasingly specialized feeding on hostplant families possessing furanocoumarins but could find no evidence for that pattern in their analysis. Using COI barcodes with morphological data, as well as incorporating a more comprehensive review of hostplant associations, I revisited this phylogenetic analysis with special consideration of the phylogenetic placement of *D. depressana*.

METHODS AND MATERIALS

Three experiments were performed to evaluate the ecological capacity of two species of *Depressaria* to detoxify furanocoumarins and utilize species containing these chemicals as hosts within the context of the evolutionary history of the acquisition of furanocoumarin-containing hostplants within the genus. In Experiment 1, we compared the rate of midgut enzymatic metabolism of two furanocoumarins, representing linear and angular configurations, between *D. depressana* and *D. radiella* sampled from two populations differing in hostplant availability. In Experiment 2, we examined hostplant suitability by calculating gravimetric estimates of performance of *D. depressana* consuming tissues of actual or potential hostplants in comparison with those consuming a semi-defined artificial diet. In Experiment 3, we performed a phylogenetic analysis to infer changes in ancestral hostplant associations throughout the evolutionary history of the genus *Depressaria*.

Experimental Animals. Depressaria radiella were collected as eggs from the underside of hostplant leaves and reared to ultimate instar under laboratory conditions prior to experimentation. Eggs from a source population with *P. sativa* as the sole available hostplant were collected at Phillips Tract (40.12966, -88.14390), an oldfield maintained by University of Illinois Urbana-Champaign (UIUC) in central Champaign County. Populations of *D. radiella* have been observed at this location at least since 1974 (Thompson and Price 1977). Eggs from a source population with *H. maximum* as the sole available hostplant were collected at Hart Woods (40.22880, -88.35639), a

densely forested woodlot in northwest Champaign County also managed by UIUC. Populations of *D. radiella* have been recorded at this location since 1984 (Berenbaum and Zangerl 1991).

Populations of *D. depressana* were first recorded at Phillips Tract in 2015, with larvae observed feeding on umbels of *P. sativa* and *Daucus carota* (Harrison et al. 2016). In 2019, another population of *D. depressana* was discovered infesting umbels of *D. carota* in a roadside lot in southeast Urbana in 2019 (*personal observation*) (40.08227, -88.19002). Multiple instars of *Depressaria depressana* were collected from the umbels of either *P. sativa* at Phillips Tract or *D. carota* at the southeast Urbana site and reared through to adulthood and allowed to mate and lay eggs in the laboratory. Ultimate instar *D. depressana* larvae of the subsequent generation were used for assays.

For both species, larvae were housed in 1 oz. (28 mL) Solo® cups lined with 5 g of semi-defined artificial diet (hereafter, artificial diet) (Nitao and Berenbaum 1988) and were allowed to feed *ad libitum*. Laboratory colonies were maintained at 27 $\pm$ 4ºC and exposed to a 16:8 L:D photoperiod. All wild parsnip, cow parsnip, and wild carrot fruit samples were collected from field sites from which larvae were collected. Fruits of *Zizia aurea* were collected from a wildflower garden across from Morrill Hall on the UIUC campus (40.1087, -88.2244).

Chemicals. The furanocoumarins used in this experiment had the purities ≥ 98% –xanthotoxin was purchased from Sigma-Aldrich (St. Louis, MO), sphondin was purchased from INDOFINE Chemical (Hillsborough, NJ), and bergapten was purchased from TCI America (Portland, OR). Ingredients for the semi-defined artificial diet used to rear *D. radiella* and *D. depressana* were purchased from Sigma-Aldrich (St. Louis, MO), with the exception of wheat germ (Continental Mills, Seattle, WA) and wheat germ oil (Viobin, Monticello, IL), which were purchased locally. All chemicals used for the midgut bioassay were obtained from Sigma-Aldrich (St. Louis, MO). HPLC-grade and higher-grade solvents, including cyclohexane and 1-butanol, from Alfa Aesar (Ward Hill, MA), and diethylether from Fisher Chemical (Pittsburgh, PA) were used to make the mobile phase for analysis by high-pressure liquid chromatography.

Experiment 1: Comparative furanocoumarin metabolism assays

Depressaria depressana and individuals from both populations of *D. radiella* were sampled three days after their ultimate larval molt. Tissues were processed following the protocol outlined by Berenbaum and Zangerl (2006). Midguts were dissected on ice in a dissection buffer containing phenylmethylsulfonylfluoride added to

Crankshaw's solution (Crankshaw et al. 1979). They were then homogenized in a glass tube with a glass pestle in 100 µL grinding buffer, containing dissection buffer and additional leupeptin. Homogenates were then added to a 1.5 mL microcentrifuge tube along with glycerol and stored at -80ºC for later analysis.

Once the homogenates of all populations were collected, they were thawed on ice and transferred to a 15 mL Falcon conical centrifuge tube (Thermo Fischer Scientific, Waltham, MA) containing 300 µL reaction mixture of 0.1 M, pH 7.8, phosphate buffer, with an NADPH-regenerating system and the substrate, either 10 mM xanthotoxin or 10 mM sphondin, was added. Tubes were then transferred to a 30ºC water bath for 25 min, after which time 400 µL of ice-cold ethyl acetate, containing 10 mM bergapten as the internal standard, was used to stop the reactions and to extract the furanocoumarins. The tubes were then vortexed immediately and centrifuged and 200 µL of the supernatant in the top layer of ethyl acetate were removed for analysis by high-pressure liquid chromatography (HPLC) to quantify unmetabolized furanocoumarins. A 4.6 x 250 mm Waters Analytical column (Part #PSS830115. Waters, Milford, MA) was used in conjunction with a Waters Millipore 712 WISP (Waters, Milford, MA), a Waters 501 HPLC Pump (Waters, Milford, MA), and a Waters 490E Programmable Multiwavelength Detector (Waters, Milford, MA). The furanocoumarins were eluted at a flow rate of 1.5 mL/min with a mobile phase consisted of 80% cyclohexane, 18% diethyl ether, and 2% 1-butanol, and detection was at 254 nm. Chromatogram peak integration analysis was performed using software developed in-house through LabVIEW (National Instruments, Austin, TX). Assumptions of normality and variance homogeneity were tested via Shapiro-Wilk test and Bartlett respectively. A one-way analysis of variance (ANOVA) paired with post-hoc Tukey's HSD test was performed in RStudio version 1.1.419 (Boston, MA) to test for significant difference among diet treatments.

Experiment 2: Gravimetric feeding efficiency assays

Depressaria depressana larvae were reared to ultimate instar, whereupon 80 individuals were collected and deprived of food for 24 h to ensure the complete evacuation of gut contents before obtaining the fresh weight. Twenty individuals per treatment were transferred into separate 28 mL (1 oz.) Solo© cups containing experimental diet. The five experimental diets for this analysis included approximately 3.0 g of artificial diet, 3.0 g of 0.3% xanthotoxin in artificial diet, 1.5 g *D. carota* immature seeds, 1.5 g *P. sativa* immature seeds, and 1.5 g *Z. aurea* immature seeds. A concentration of 0.3% xanthotoxin was used because this level represents the average xanthotoxin content in *P. sativa* seeds in central Illinois (Berenbaum and Zangerl 2006). *Zizia aurea* was selected as

198 a treatment because this species is an umbellifer native to North America with low furanocoumarin production
199 (Berenbaum 1981) and therefore represents a potential novel hostplant for *D. depressana*. After a 24 h feeding
200 period, the larvae, frass, and remaining diet were separated into labeled 1 oz. (28 mL) cups and relocated to a drying
201 oven set to 60ºC for 5 days. The contents of each cup were weighed after the drying period to obtain the post-
202 experiment dry weights. Pre-experiment dry weights, representing the initial dry weight, were calculated using a
203 separate group of 10 larvae for each diet and calculated by dividing fresh weight by dry weight and averaging the
204 measurements within each treatment. Four measures of efficiency were calculated as outlined by Waldbauer et al.
205 (1968): Efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), relative
206 growth rate (RGR), and relative consumption rate (RCR).[1] Data analysis was performed in SPSS version 27 (IBM,
207 Armonk, NY). In each test, the response variable was square-root transformed to meet assumptions of normality,
208 which were tested on residuals via Shapiro-Wilk normality test. This was necessary as initial larval weight and
209 absolute consumption varied across samples used in this experiment. A Bartlett test was implemented to test
210 assumptions of equal variance. One-way ANOVA and ANCOVA analyses were performed to test for the
211 significances of differences ($\alpha = 0.05$).
212
213 *Experiment 3: Depressaria phylogeny and hostplant ancestral state reconstruction*
214 Barcode analysis was incorporated into the morphology-based phylogeny of Bucheli et al. (2010). The COI
215 analysis incorporated 60 total samples, including 53 *Depressaria* individuals representing 41 total species within the
216 ingroup, and 7 *Agonopterix* individuals, representing 5 total species, as outgroups (BOLD IDs and Accession
217 Numbers in Supplementary Table 1). These taxa were selected based on the availability of COI barcodes on the
218 BOLD database (Ratnasingham and Hebert 2007) and availability of reliable hostplant records (Table 1). Reliability
219 of hostplant record information was assessed with reference to reports in the published literature. Mitochondrial COI
220 was incorporated into this analysis as it is particularly useful at providing resolution for more derived clades of
221 *Depressaria*, an issue encountered by Bucheli et al. (2010). COI barcodes were aligned in Geneious (2021.1.1) using
222 the MAFFT version 7.450 alignment algorithm (Katoh and Standley 2008) with default parameters. The COI dataset
223 was then partitioned by codon position to account for variable mutation rates at each position. A dataset of 33

[1] ECI = biomass gain/diet ingested. Biomass gain represents the converted dry weight of the larva at 0 h minus the dry weight of the larva at 24 h. Diet ingested represents the diet dry weights at 0 h minus diet dry weights at 24 h. ECD = biomass gain/(consumption – frass). Consumption represents converted dry weights of the diet at 0 h minus the dry weights of the food at 24 h. RGR = biomass gain/larval mass. RCR = consumption/larval mass. Larval mass represents the converted dry weights of the larvae at 0 h.

morphological characters was obtained from Bucheli et al. (2010) (Table 2) and incorporated into the phylogenetic reconstruction. Fourteen morphological characters from the original dataset of 47 available traits (Bucheli et al. 2010) were not used in this analysis as they were not taxonomically informative within our sample set of 60 taxa.

Maximum likelihood (ML) analysis was performed with RAxML version 8.2.12 (Stamakis 2014). PartitionFinder (Lanfear et al. 2012) was used to select GTRGAMMA as the best fitting nucleotide substitution model under AIC and the Markov k (Mk) model for morphological character evolution. A general parametric constraint was implemented to establish *Agonopterix* and *Depressaria* as monophyletic. Support values were obtained via 1000 bootstrap replicates.

Bayesian phylogenetic (BP) analysis was performed using BEAST version 2.6.0 (Bouckaert et al. 2019). Site model averaging was implemented using the bModelTest package (Bouckaert and Drummond 2017) for each codon position and morphological characters. A relaxed clock log normal model was applied with a 0.0115 clock rate for COI nucleotide mutation rate (Brower 1994) with rates for morphological characters left as estimated with no specified clock. As in the maximum likelihood analysis, monophyletic constraint priors were applied to *Agonopterix* and *Depressaria*. The BP analysis ran for 150 million Markov chain Monte Carlo (MCMC) generations beginning from a random tree with every chain sampling every 5,000th cycle. Tracer version 1.7.2 (Rambaut et al. 2018) was used to ensure effective sample size (ESS) values for all parameters were greater than 200. A maximum clade credibility tree was generated in TreeAnnotator (Bouckaert et al. 2019) with median node heights and 10% burn-in.

The BP tree was loaded into RASP (Yu et al. 2020) for a multistate reconstruction of ancestral hostplant associations. Hostplant families were coded as traits in RASP as either Asteraceae (A), Apiaceae (B), Fabaceae (C), Caprifoliaceae (D), Rutaceae (E), Betulaceae (F), Salicaeae (G), or a combination of these for species that feed upon multiple hostplant families. Trait evolution was evaluated using Bayesian Binary MCMC. The resulting tree was edited in Adobe Illustrator version 25.4.1 (Adobe Inc. 2021) where maximum likelihood bootstrap support values were added.

RESULTS

Experiment 1: Gravimetric feeding efficiency assays

Diet type differentially affected absolute weight gain in *D. depressana* larvae, with *D. carota* resulting in a greater increase in larval mass relative to all other diet types (*ANOVA*, Tukey's HSD, $P < 0.05$) (Fig. 2a). Diet type also affected absolute consumption, with the three fruit tissue diets resulting in significantly lower overall consumption relative to the 0.3% xanthotoxin artificial diet and unsupplemented artificial diet being intermediate in terms of consumption (*ANOVA*, Tukey's HSD, $P < 0.05$). The ANCOVA analysis for ECI revealed that both diet ($F = 6.672$, $df = 4, 94$, $P < 0.05$) and rate of food ingestion ($F = 11.880$, $df = 4, 94$, $P < 0.05$) affected larval mass. Similarly, the ECD ANCOVA analysis determined larval mass increase was significantly affected by diet ($F = 10.513$, $df = 4, 94$, $P < 0.05$) and amount of digested food ($F = 6.604$, $df = 4, 94$, $P < 0.05$). ANCOVA analysis for relative growth rate (RGR) revealed that diet affected larval weight gain ($F = 4.291$, $df = 4, 94$, $P < 0.05$), but that initial weight did not ($F < 0.01$, $df = 4, 94$, $P = 0.996$). Lastly, ANCOVA analysis for relative consumption rate (RCR) found that diet affected rates of larval consumption ($F = 4.261$, $df = 4, 94$, $P < 0.05$), but that initial weight did not ($F = 0.244$, $df = 4, 94$, $P = 0.622$).

Experiment 2: Comparative furanocoumarin metabolism assays

Furanocoumarin metabolism is reported as mean percentage decrease of the substrate, either xanthotoxin or sphondin, in the midgut extracts of the same individuals between 0 mins and 25 mins of the bioassay. Metabolism of xanthotoxin (Fig. 3a) did not differ between individuals from populations of *D. radiella* associated exclusively with either *P. sativa* and *H. maximum*, with a mean decrease of 39.3% and 38.9% in initial xanthotoxin content, respectively (*ANOVA*, Tukey HSD, $P < 0.05$). *D. radiella* larvae from both populations, however, exhibited significantly higher xanthotoxin metabolism than *D. depressana* (*ANOVA*, Tukey HSD, $P < 0.05$), which metabolized only 19.6% of the initial xanthotoxin content (Fig. 3b). *D. radiella* from *H. maximum* populations metabolized 39.0% of the initial sphondin substrate, which was significantly greater than did larvae from *P. sativa* populations, which metabolized 27.9% (*ANOVA*, Tukey HSD, $P < 0.05$). As with xanthotoxin, both populations of *D. radiella* metabolized sphondin more efficiently than *D. depressana* larvae, which metabolized 11.3% of the initial substrate (*ANOVA*, Tukey HSD, $P < 0.05$).

Experiment 3: Depressaria phylogeny and hostplant ancestral state reconstruction

279 The COI dataset comprised 658 aligned base pairs. The combined maximum likelihood (ML) and Bayesian
280 phylogenetic (BP) analyses on the COI barcode and morphology dataset are shown in Fig. 4, with corresponding
281 posterior probabilities and bootstrap support values. There were 58 internal nodes, of which 33 display posterior
282 probabilities of 0.9 or higher and 23 with bootstrap support values about 70%. In general, nodes with high support
283 values across both measures of support tended to be more derived, except when separating individuals of the same
284 species. Of the 58 nodes, ~19% displayed incongruence between the ML and BP analyses. Each tree is given
285 separately in Fig. 3.
286 The ancestral hostplants of *Depressaria* and *Agonopterix* were estimated for all 58 nodes (Fig. 4). The
287 probability of feeding on Apiaceae as compared to other hostplant families was 90.62% for the *Depressaria* and
288 *Agonopterix* most recent common ancestor (MRCA). The most recent common ancestor to the *Agonopterix* clade
289 was predicted to use as hostplants species within the Apiaceae with a 77.11% probability, whereas the most recent
290 common ancestor to the *Depressaria* had a 74.73% probability of utilizing apiaceous plants as hosts. *Depressaria*
291 *radiella* belongs to the clade containing *D. libanotidella*, *D. pimpinellae*, and *D. velox,* and its most recent common
292 ancestor had a 95.68% probability of feeding upon apiaceous hosts. The most recent common ancestor of the clade
293 containing *D. depressana* and *D. chaerophylli* was predicted to utilize Apiaceae with a 99.70% probability.
294
295 DISCUSSION
296 Dietary xanthotoxin causes a decrease in the efficiency with which *D. depressana* can convert ingested and
297 digested food to biomass (Figure 2). Ultimately, the presence of xanthotoxin, both in *P. sativa* and in artificial diet,
298 results in reduced growth rate. Carroll (2003) observed a similar reduction in growth rate in *D. radiella* in response
299 to dietary xanthotoxin and found that these effects can be ameliorated by presence of lutein, a carotenoid possessing
300 antioxidant properties. Previous studies report that *D. radiella* larvae do not discriminate against diet containing
301 xanthotoxin when presented with an alternative diet lacking xanthotoxin (Berenbaum and Zangerl 1992) and even
302 increase their rate of consumption (Berenbaum et al. 1989), unlike other Lepidoptera for which xanthotoxin acts as a
303 feeding deterrent (Berenbaum 1995). Additionally, *a priori* exposure to furanocoumarins in the source population
304 influenced the capacity of *D. radiella* to metabolize furanocoumarins (Berenbaum and Zangerl 1998). *D. depressana*
305 larvae in this analysis were sourced from a population feeding exclusively on *D. carota*, lacking nearby *P. sativa*
306 that could potentially exert selective pressure for xanthotoxin detoxification as alternative hosts. The increase in

efficiency of conversion of ingested and digested *D. carota*, and subsequent increased growth rate, even relative to the artificial diet not supplemented with xanthotoxin, suggests the presence of beneficial dietary components that promote development. While *D. depressana* displayed significantly reduced performance on *Z. aurea* fruits relative to performance on *D. carota* fruits, conversion efficiencies and growth rate were not reduced relative to *P. sativa*, suggesting that *D. depressana* could potentially establish populations on *Z. aurea* as an alternate host in areas of invasion, with potential consequences for the survival of vulnerable or imperiled *Z. aurea* populations.

That larvae from both populations of *D. radiella* displayed markedly higher xanthotoxin and sphondin metabolism than did *D. depressana* larvae is consistent with the expectation that family-specialists display lower levels of resistance to relatively rare defense chemicals compared to family-superspecialists. With respect to *D. radiella*, differences in furanocoumarin metabolism across geographically separated populations feeding on *P. sativa* varying in chemical profiles have been well documented (Zangerl and Berenbaum 2003; Berenbaum and Zangerl 2006). Proximity to *H. maximum* alters the intensity of selection and therefore precision of the phenotype match between the detoxification profile of *D. radiella* larvae and the furanocoumarin defense profile of associated *P. sativa* populations (Zangerl and Berenbaum 2003). Immature seeds of *H. maximum* can contain up to 11 times the total amount of furancoumarins than do immature seeds of *P. sativa*; as well, the furanocoumarins of cow parsnip contain angular furanocoumarins in higher proportions (Berenbaum and Zangerl 1991). As such, *D. radiella* collected from *H. maximum* appear to have responded to selective pressure to adapt to higher concentrations of sphondin, as evidenced by the HPLC results, providing preliminary evidence for local host-races of *D. radiella*.

Phylogenetic position of several taxa differed between our analysis and that of Bucheli et al. (2010). Most notable is the placement of *D. dictamnella.* This Old World species, mostly concentrated in Portugal and Spain, has as its primary host *Dictamnus albus* (Krone 1911), a rutaceous species that produces the linear furanocoumarins xanthotoxin and bergapten, as well as trace amounts of psoralen (Zobel and Brown 1990). This pattern suggests that adaptation to furanocoumarins is a basal trait in the evolution of *Depressaria* or perhaps even earlier in ancestral lineages predating the *Depressaria* and *Agonopterix* split. The phylogenetic position of *D. depressana* also differs greatly between our analysis and the analysis of Bucheli et al. (2010). Bucheli et al. (2010) placed *D. depressana* as sister to the remaining unresolved clade termed the *artemisiae* group (*D. chaerophylli*, *D. atrostrigella*, *D. artemisiae*, *D. absynthiella*, and *D. heydenii*), which is characterized as containing Old World species with a bisetose SV seta condition on the A1 segment; by contrast, our analysis positions *D. depressana* as sister to *D.*

335 *chaerophylli*, though support is relatively low (pb = 0.8, bs = 38) (Fig. 4). The remaining species of the *artemisiae*
336 group, save for *D. atrostrigella* (omitted from our study), are repositioned within a more derived clade sister to *D.*
337 *veneficella*, *D. discipunctella*, and *D. silesiaca,* whose hostplants include members of the Asteraceae and Apiaceae.
338 Further, our analysis shows that this new clade is more closely related to the group Bucheli et al. (2010) termed the
339 *pastinacella* group (*D. badiella*, *D. bupleurella*, *D. libanotidella*, *D. pimpinellae*, *D. velox*, and *D. radiella*), which
340 are Old World species that feed on a combination of Apiaceae and Asteraceae and also possess bisetose SV setae.
341 With reasonably high support, our analysis further resolves the relationships within the clade containing *D. radiella*
342 with the group containing *D. badiella* and *D. bupleurella* as sister to the remaining taxa and therein *D. radiella* as
343 sister to *D. libanotidella*, *D. pimpinellae*, and *D. velox*. Additional differences between phylogenies include the
344 dissolution of the *discipunctella* group, and the placement of the *betina* group as sister to *D. albipunctella* within the
345 *douglasella* group. The clade containing the *betina* and *douglasella* groups comprise both New and Old World
346 species that utilize as hostplants species in the Apiaceae and Asteraceae. Despite the many limitations of this
347 study—use of only mitochondrial COI in a molecular dataset to supplement the morphological data, incomplete
348 hostplant records, and differential coverage of furanocoumarin distributions in the literature, our analysis generally
349 shows strong support for intra-genus relationships.
350 As for testing the escape-and-radiate hypothesis, definitive conclusions are more difficult to draw. Bucheli
351 et al. (2010) evaluated *Depressaria* as a test of the escape-and-radiate hypothesis across several different axes:
352 furanocoumarin specialization, plant tissue specialization (flowers versus leaves), and habitat specialization (woody
353 versus herbaceous). They reached the conclusion that association with furanocoumarin-producing hostplants (with
354 plant family serving as a proxy) is a weak predictor of species-level evolution within *Depressaria*, as this trait
355 predates the genus with feeding on plants in the Asteraceae (assumed to lack furanocoumarins) being the result of
356 multiple independent instances of convergent evolution. However, because broad-scale heterogeneity of
357 furanocoumarin production exists within plant families, even the Apiaceae, and that plant species that produce
358 furanocoumarins do so in varying amounts, proportions, and chemical complexity, plant family is not a precise
359 proxy for plant chemistry (furanocoumarins are not reported to occur in some apiaceous tribes and Kinghorn et al.
360 (2017) report finding furanocoumarins in at least two asteraceous genera). Bauri et al. (2016) reported finding a
361 photobiologically active furanocoumarin from *Artemisia reticulata*, a species in an asteraceous genus that is a
362 hostplant for multiple *Depressaria* species. A further analysis of hostplant chemical profiles is warranted. Natural

product discovery is proceeding apace; according to Sarker et al. (2017), "Well over 400 coumarin isolations were reported in the years 2014 and 2015," so absence of records of furanocoumarins in any particular plant species may well represent absence of investigative effort rather than biological reality.

Another obstacle to drawing any definitive conclusion about testing the escape-and-radiate hypothesis is, as mentioned, the incomplete nature of host records for many species in this group. (1964). Within the clade contain *D. radiella*, only one species, *D. pimpinellae*, utilizes a host plant species known to produce angular furanocoumarins (Fig. 4), *Pimpinella* spp. However, *D. pimpinellae* is most closely related to *D. velox*, and that group is sister to *D. libanotidella*; although neither of these species are reported to utilize plants that produce angular furanocoumarins (Table 1), hostplant records are rare and almost certainly incomplete. Angular furanocoumarin detoxification appears to be a trait independently evolved in several lineages in both *Depressaria* and *Agonopterix*, as is the case with *A. angelicella* (Table 1). This contrasts with apparent evolutionary trends among species within the genus *Papilio* (Lepidoptera: Papilionidae), which are a better fit for the escape-and-radiate model with respect to angular furanocoumarin detoxification (Sperling and Feeny 1995). Sperling and Feeny (1995) found that evolution of angular furanocoumarin tolerance likely led to the diversification of the *P. machaon* group as this trait would have led to wider hostplant availability.

Many studies examining plant-insect interactions were founded on the premise that specialist herbivores are better adapted to overcome the defensive chemistry of their hostplants and will thus outperform generalists (Scriber and Feeny 1979). However, recent studies call this long-maintained assumption into question because they often fail to take into consideration confounding ecological factors such as feeding guild, plant response, behavioral idiosyncrasies, and the effect of trophic-level interactions (Ali and Agrawal 2012; Smilanich et al 2016; Loxdale and Harvey 2016). Additionally, the terms ‘generalist’ and ‘specialist’ may also oversimplify a continuous spectrum of insect diet breadth (Loxdale and Harvey 2016). Rothwell and Holeski (2020) conducted a meta-analysis incorporating 45 studies and found evidence that specialist insects do outperform generalists in terms of growth, but not fecundity or survival. However, the need remains for further consideration of insects occupying the middle portion of the diet breadth spectrum.

Diet breadth even within a hostplant family may provide insights into the likelihood of success of a specialist group in invading new habitats and colonizing novel hostplants. Collectively, our work suggests that *D. depressana* may not be well suited to adopt *H. maximum* as a novel host. This insect is not reported to utilize plant

391 species producing high levels of angular furanocoumarins as hosts in its native range and, according to our analysis,
392 belongs to a clade containing species that do not regularly ingest and metabolize angular furanocoumarins in their
393 diet. Moreover, because of its expanded host range, the availability of less toxic alternate hostplants may result in
394 relaxed selective pressure to colonize *H. maximum*. While *D. depressana* metabolizes sphondin to a certain degree,
395 it does so relatively inefficiently compared to *D. radiella*. Because *D. depressana* can feed and grow on *P. sativa*
396 despite metabolizing xanthotoxin at a rate much lower than that of *D. radiella*, however, direct tests of larval
397 development on *H. maximum* under controlled laboratory conditions could be clarifying. Native apiaceous plants
398 that produce low levels of linear furanocoumarins are much more likely to be colonized by *D. depressana*, as
399 evidenced by our feeding efficiency assays. While growth rate and efficiency of conversion of ingestion and
400 digestion of *Z. aurea* to larval biomass were reduced compared to *D. carota*, they did not significantly differ from *P.*
401 *sativa* or semi-defined artificial diet, suggesting that larvae may be able to utilize *Z. aurea* as an alternate host as it
402 continues to expand west across North America.

403 Increasing globalization of trade essentially guarantees that, in the future, species of Apiaceae specialists in
404 the Depressariidae will be among those with distributions that expand to new countries and new continents. In view
405 of both the economic importance of apiaceous plants as sources of herbs, spices, and vegetables and the
406 conservation concerns about many endemic species in the family, efforts to understand the nature of the
407 evolutionary association between these plants and their specialist enemies may become increasingly valuable.
408 Particularly pertinent in this regard is the impact that invasive depressariines have upon native, vulnerable
409 umbellifers. For example, three species of *Lomatium* (*L. greenmanii*, *L. erythrocarpum*, and *L. oreganum*), high
410 alpine specialists endemic to states in the Pacific Northwest in N. America, are currently under consideration for
411 increased conservation efforts and may be highly susceptible to the added selective impact of invasive depressariines
412 (Ottenlips et al. 2020). Increased attention paid to the chemical ecology of invasive species could further bolster the
413 predictive power of models attempting to evaluate the damage of invasive *Depressaria.*

414

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Acknowledgments We thank Jack Easley for assistance with field work in Illinois. We also thank Ling-Hsiu Liao and Bernarda Calla who assisted with midgut bioassay preparations.

Author Contributions The research was conceived by CAED and MRB, fieldwork was conducted by CAED, midgut bioassays and HPLC analysis was performed by CAED and WYW, gravimetric estimates of performance were calculated by CAED, phylogenetic analysis was conducted by CAED and AK, and the manuscript was drafted by CAED and revised by AK, WYW, and MRB.

Declarations

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Compliance of Ethical Standards Additional declarations for articles that report the results of studies involving humans and/or animals: Not applicable.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors have no conflicts of interest to declare.

Figures

Figure 1

Depressaria depressana feeding on native *Zizia aurea* (golden alexanders).

Figure 2

(a) Mean (± SE) rate of biomass gain, and **(b)** Mean (± SE) rate of consumption of *Depressaria depressana* after 24 hrs of feeding on either semi-defined artificial diet, 0.3% xanthotoxin in semi-defined artificial diet, *Daucus carota*, *Pastinaca sativa*, or *Zizia aurea*. Biomass gain was calculated by subtracting the converted dry weight (mg) of larvae at 0 h from larval dry weight (mg) at 24 hrs. Consumption was calculated by subtracting the converted dry weight (mg) of food at 0 h from the dry weight (mg) of food at 24 hrs. Columns marked with same uppercase letters denote no significant different (ANOVA, Tukey's HSD test, $P > 0.05$).

Figure 3

Mean (± SE) percentage decrease of (**a**) xanthotoxin and (**b**) sphondin among *Depressaria radiellia* collected from populations with wild parsnip (WP) as the sole hostplant, populations of *D. radiellia* with cow parsnip (CP) as the sole hostplant, and *Depressaria depressana* collected from wild parsnip. Columns marked with same uppercase letters denote no significant different (ANOVA, Tukey's HSD test, $P > 0.05$)."

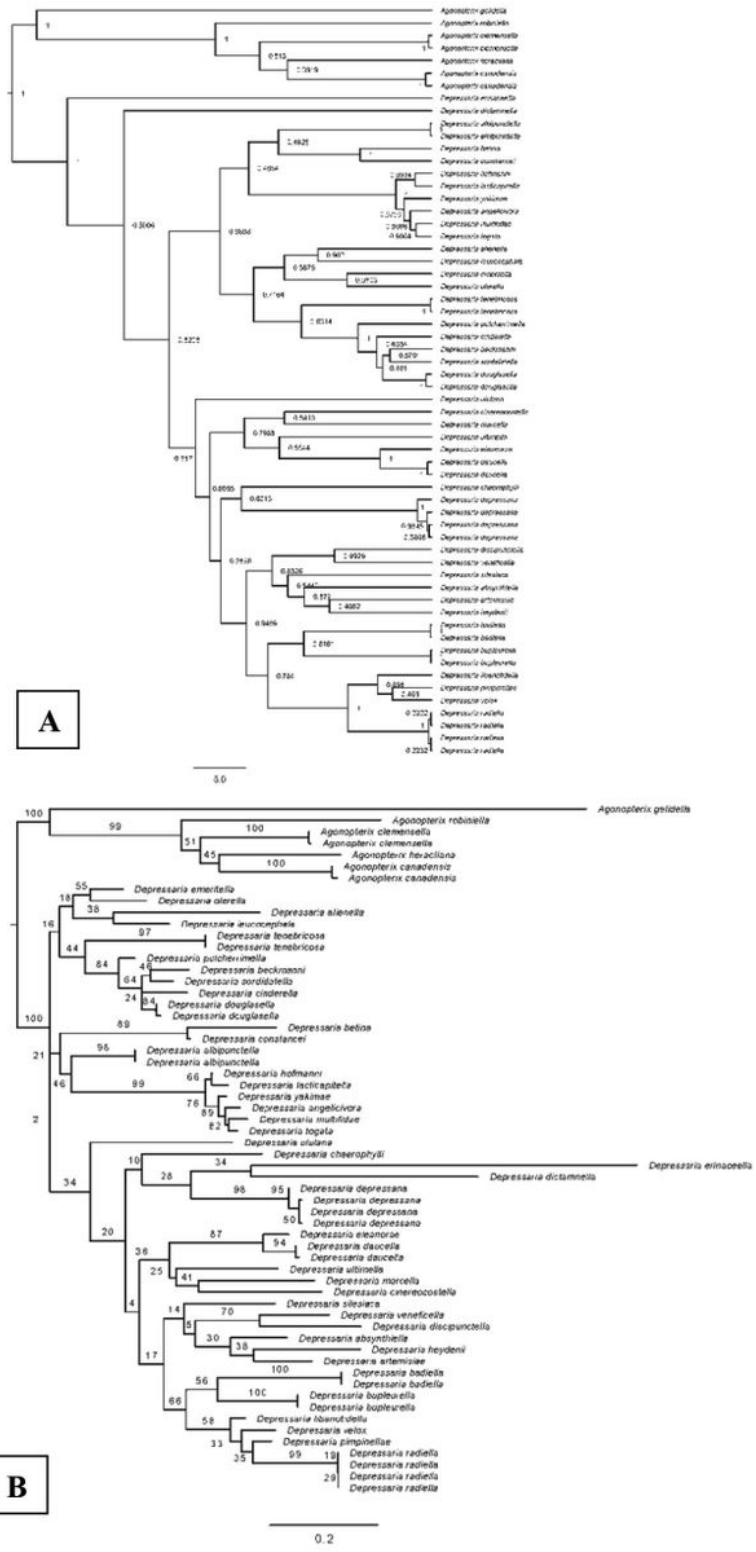

Figure 4

A) Bayesian phylogenetic (BP) analysis of COI. Numbers represent posterior probability support values for each node. B) Maximum likelihood (ML) analysis of COI. Numbers represent bootstrap support values for each node.

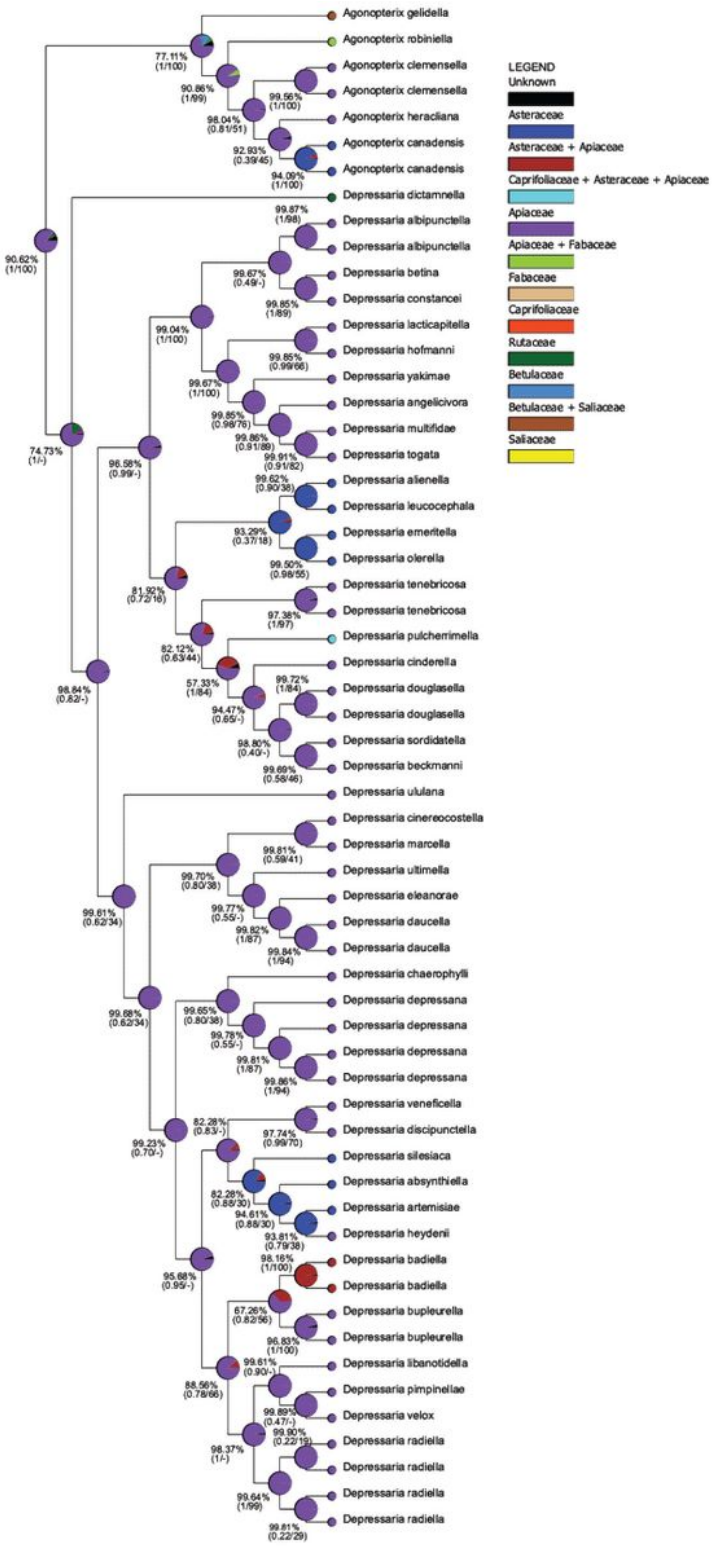

Figure 5

Cladogram resulting from Bayesian phylogenetic (BP) analysis the using COI barcode and morphology dataset retrieved from Bucheli et al. (2010) (Table 2). Pies at each node represent the probably of ancestral hostplant trait as determined from a Bayesian Binary MCMC multistate analysis. Traits were coded as (A) Asteraceae (B) Apiaceae (C) Asteraceae (C) Fabaceae (D) Caprifoliaceae (E) Rutaceae (F) Betulaceae (G) Salicaceae, or a combination of any of the above. Numbers indicate percent likelihood of

the most probable ancestral hostplant state at each node, posterior probability from the BP analysis, and bootstrap support values from the maximum likelihood (ML) analysis. Incongruent topologies between BP and ML trees are indicated with a hyphen (-) in place of bootstrap value.

Supplementary Files

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