Phytochemical defences and performance of specialist and generalist herbivores: a meta-analysis

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Abstract. 1. Phytochemical coevolution theory, a long-standing paradigm in plant−insect interactions, predicts that specialist herbivores are less negatively affected by the allelochemicals of their host plants than are generalist herbivores. Although this theory is prevalent in plant−insect science, it is not always supported by empirical studies measuring the performance of specialist and generalist insects in response to allelochemicals.

2. The present study aimed to investigate: (i) whether there a difference between specialist and generalist performance in response to allelochemicals and (ii) whether the effect of allelochemicals on specialists and generalists depend upon allelochemical class or insect order.

3. A meta-analysis was conducted incorporating 76 effect sizes drawn from studies that directly compared the performance of specialist and generalist insects in response to treatment and control diets. Most of the effect sizes were related to the performance metric growth, the insect order Lepidoptera, and the allelochemical class nitrogen-containing compounds.

4. As predicted by phytochemical coevolution theory, specialist insects responded less negatively to allelochemicals of their hosts than generalist insects in terms of growth. There were no significant differences in terms of fecundity or survival, or among allelochemical classes or insect orders.

5. These results support the prediction of phytochemical coevolution theory that specialist insects respond less negatively to allelochemicals of their hosts than generalists, although only in terms of growth.

Key words. Allelochemicals, diet breadth, generalists, phytochemical coevolution theory, specialists.

Introduction

Insect herbivores can be broadly classified as 'generalist' and 'specialist' feeders, defined by the relative extent of their diet breadth (Dethier, 1954). Commonly, insects limited to one or few closely-related plant species containing particular allelochemicals are defined as dietary specialists. Dietary generalist insects accept a variety of distantly related plant hosts producing a diversity of allelochemicals (Fraenkel, 1959). On the spectrum of host plant breadth, most herbivorous insects lie somewhere between broad generalism and narrow specialism (Loxdale & Harvey, 2016).

Many influential plant defence hypotheses are based on the premise that specialist insects are better adapted to the allelochemicals of their host plants than generalists (Ehrlich & Raven, 1964; Scriber & Feeny, 1979). In particular, phytochemical coevolution theory is based on the evolutionary interactions of herbivorous insects and their hosts. First, herbivores exert selective pressure on plants to produce allelochemicals. In return, plants exert selective pressure on herbivores to evolve tolerance and/or detoxification mechanisms (Feeny, 1976; Rhoades & Cates, 1976; Petschenka & Agrawal, 2015). This can lead to fitness advantages for specialists over generalists using the same host plants, in terms of growth, survival, and/or higher trophic level interactions (Cornell & Hawkins, 2003). For example, although both oligophagous common crow caterpillars and highly specialised monarch caterpillars feed on milkweed plants, monarchs are able to sequester the allelochemicals

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of their host plants, whereas common crow caterpillars cannot (Petschenka & Agrawal, 2015). The ability to sequester confers anti-predator defence to the specialist insect, whereas the generalist acquires no such fitness advantage (Brower & Moffitt, 1974; Dyer, 1995).

Empirical evidence for phytochemical coevolution theory has been to some extent system-dependent, with evidence in both support and conflict with its predictions. In one well-studied system, specialist lepidopteran herbivores have developed physiological and behavioural mechanisms to detoxify an array of linear furanocoumarins produced by host plants in the Umbelliferae (Apiaceae). Generalised feeders are unable to cope with these compounds even at much lower concentrations (Berenbaum, 1983). Nonetheless, certain linear furanocoumarins have detrimental effects even on highly specialised feeders (Berenbaum *et al.*, 1989). Similarly, defensive iridoid glycosides in narrowleaf plantain tend to positively or neutrally impact the survival, growth, and fecundity of specialist lepidopteran herbivores (Bowers & Puttick, 1988; Harvey *et al.*, 2005; Reudler *et al.*, 2011), yet negatively impact immune response in these same feeders (Richards *et al.*, 2012). A meta-analysis examining patterns of insect performance across studies published prior to 2005 found no significant difference in survival, growth or fecundity between specialist and generalist insects in response to woody or herbaceous plant allelochemicals (Smilanich *et al.*, 2016).

Drawing broad conclusions from the literature is complicated by variation in experimental design and ambient conditions among studies. When conducting a review or meta-analysis, we are often comparing the performance of specialist and generalist insects that were subjected to quite different treatments and controls. Moreover, ambient conditions such as temperature, humidity, and seasonality certainly differ among studies, all of which can considerably alter the outcome of a feeding trial. Experiments that simultaneously compare the performance of specialist and generalist insects in response to identical treatment and control diets are ideal for a review or meta-analysis on this topic. Additionally, the requirement of a control diet, which is not always present in plant–insect studies, establishes a baseline that may cut down noise between the specialist and generalist insect species used.

In the present study, we conducted a meta-analysis of papers fitting the criteria outlined above. Specifically, we addressed three questions. First, is there a difference between specialist and generalist performance in response to allelochemicals of their host plants? This question addresses a core prediction of phytochemical coevolution theory: specialists will out-perform generalists in terms of growth, survival, and/or fecundity. Second, does the effect of allelochemicals on specialist and generalist performance depend upon allelochemical class? We predicted that some allelochemicals might exert stronger effects on generalists than specialists, whereas others may not, with some differences as a result of the speed of the allelochemical effects and degree of toxicity. Finally, does the effect of allelochemicals on specialist and generalist performance depend upon insect order? We predicted that the effects of allelochemicals would differ among insect orders as a result of differences in feeding mode. Specifically, we predicted that differences between specialist and generalist insects would be more apparent in leaf-chewing Lepidoptera and Coleoptera than in sap-sucking Hemiptera. Leaf-chewing can elicit a stronger allelochemical response from the host plant than sap-sucking (Peterson *et al.*, 2016). This may result in larger differences in the selective pressure between generalist and specialist chewing herbivores than between generalist and specialist sap-suckers.

Materials and methods

Database compilation

In July 2017, we searched for primary studies measuring specialist and generalist insect herbivore performance in response to allelochemicals. Web of Science was used to search for the set of terms: generalist*, specialist*, herbiv*, insect*, plant*, defen*, diet breadth*, physical, mechanical, trichome, tough*. The latter four terms were added so as to include studies focusing on physical plant defences. However, because a low number of studies meeting our inclusion criteria were returned (four), these publications were later excluded from the data set. In total, 423 publications were returned. Subsequent to our database search, we searched the literature cited sections of all papers that had been deemed relevant in search of titles that could be pertinent to the present study. We considered an additional 30 publications returned by this process.

To be included in this meta-analysis, we established specific criteria: (i) the publication comprised a primary study, not a review or meta-analysis; (ii) only insect herbivores were used because we were interested in testing phytochemical coevolution theory as it pertains specifically to insects; (iii) insect performance was measured as growth, fecundity or survival. Studies that measured only insect preference or abundance were not retained. Although it is often the case that preference or abundance are influenced by metabolic effects of plant defences on insects, this is not always a reliable indicator of insect performance in response to plant defences (Dethier, 1954); (iv) performance of both a specialist and a generalist insect was measured, such that a direct comparison could be made between the two. In all cases, we accepted the authors' assignment of 'specialist' and 'generalist' to each insect species used. There were no discrepancies regarding any particular insect species within or among the publications included in our final data set, except within a single study: Ali and Agrawal (2016) defined *Tetraopes tetraophthalmus* and *Tetraopes texanus* depending upon which host plant was being consumed in a given treatment (i.e. each insect species was considered a specialist when feeding on its own natural host plant and a generalist when feeding on the other's; (v) both insect types were subjected to the same treatment(s) and control(s), with each treatment containing a higher amount of allelochemicals than the control. In some cases, a true control diet containing no allelochemicals was not possible as a result of the feeding stimulant requirements of insects or because leaf tissue was used as both the treatment and control diet; and (vi) finally, all statistics needed for the effect size calculation were reported in the text or in a figure or table. These included mean, error, and sample size for a trait measurement for both

the control and treatment groups of the specialist and generalist insect.

In total, 45 papers were retained based on these criteria. Details for each publication are presented in Table 1. Publication dates ranged from 1983 to 2017, with 84% of papers published in 2005 or later. Our final dataset contained performance metrics related to growth (total weight gain, development time, approximate digestibility, efficiency of conversion of ingested food, efficiency of conversion of digested food), fecundity (eggs per female per day, pupal weight), and percentage survival.

We grouped the allelochemicals in our data set into four classes: nitrogen-containing compounds (alkaloids, amides, benzoxazinoids, cyanogenic glycosides, and glucosinolates), phenolics (flavonoids, phenolic acids, phenolic glycosides, and tannins), jasmonates (jasmonic acids), and terpenoids (cardenolides, diterpenes, diterpene glycosides, iridoid glycosides, and sesquiterpenes). Given our data set, we used broad classes to obtain sufficient representation of each class. Although there is considerable overlap in mode of action among these classes, as well as variation within classes in effects on particular herbivores, some generalisations can be made about the mode of action of each class. In terms of plant defence, nitrogen-containing compounds are often potently toxic and fast-acting to insect herbivores. Phenolics, when involved in plant defence, tend to act as weak toxins and digestibility-reducers (Palo & Robbins, 1991). Jasmonates are involved in signal transduction; in plant defence, they indirectly affect insect herbivores via activation or repression of genes that produce allelochemicals (Creelman & Mullet, 1997). Finally, terpenoids with direct allelochemical effects on insect herbivores typically act as toxins or growth inhibitors in insect herbivores (Gershenzon & Dudareva, 2007). Although all of these classes play multiple roles in plants, each allelochemical in our data set has known plant defence activity.

Data extraction and effect size calculation

We extracted the mean, error, and sample size from each study for calculation of effect sizes. When mean and error were reported in figures or tables, we extracted them using IMAGEJ (NIH, Bethesda, MD, USA) (Schneider *et al.*, 2012). When standard error was reported, we converted this to standard deviation prior to effect size calculation. One or two effect sizes were extracted from most studies, although up to five effect sizes were extracted from a single publication in some cases. We did this because many studies measured different performance metrics, such as both growth and fecundity, for which separate effect sizes were calculated. We accounted for non-independence of these effect sizes in our models as described in the statistical analyses.

Although most studies included only one specialist and one generalist species per experiment, it was also necessary to extract multiple effect sizes from a single study when multiple species of specialist or generalist insects were used. For example, in one study, there was a specialist–generalist pair of the order Lepidoptera, as well as a pair of the order Coleoptera. In cases such as these, where multiple specialists or generalists belonging to different orders or families were used, we

paired insects by the lowest level of taxonomy possible. In a few studies, there were uneven numbers of specialist and generalist species used. For example, in one experiment, the researchers used four specialist species but only one generalist species, all belonging to the order Lepidoptera and the family Nymphalidae. In a situation like this, we calculated a mean for all of the specialists used in the study, which was used for effect size calculation. This was carried out for four out of 45 studies.

For our effect size, we used a modified version of the log response ratio (LRR; Hedges *et al.*, 1999). LRR is normally a simple ratio of the treatment group response to the control group response: ln (mean response of treatment group/mean response of control group). Because we made direct comparisons between specialist and generalist insects in each study, each of which were subjected to treatment and control conditions, our effect size reflected the difference in LRR between the specialist and generalist insect: ln (mean response of specialist treatment group/mean response of specialist control group) − ln (mean response of generalist treatment group/mean response of generalist control group). Because this effect size is not built into the statistical software we used, we calculated the effect size(s) for each publication using EXCEL (Microsoft Corp., Redmond, WA, USA) prior to incorporating them into models.

For all measures of performance, a positive difference in effect size indicates that the specialist performed better than the generalist, whereas a negative effect size difference indicates that the generalist out-performed the specialist. For development time, for which a lower value would normally indicate improved performance (i.e. more rapid development), we reversed the sign so that the direction of all effect size differences would be consistent (as in Smilanich *et al.*, 2016).

In total, 76 effect sizes were calculated. Most effect sizes were related to herbivore growth ($n = 61$). There were far fewer effect sizes related to fecundity ($n = 8$) and survival ($n = 7$) in our dataset. Effect sizes were heavily skewed towards the insect order Lepidoptera ($n = 66$), with Coleoptera ($n = 8$) and Hemiptera ($n = 2$) being used much less often. Nitrogen-containing compounds ($n = 37$) were the most common allelochemical class in our dataset, followed by phenolics ($n = 24$), terpenoids ($n = 9$), and jasmonates ($n = 6$).

Statistical analysis

We analysed our data using R software (R Core Team, 2014). Standard meta-analytic mixed effects models were generated using the rma.mv function within the metafor package (Viechtbauer, 2010). We built three models to test the null hypothesis that there is no difference between specialist and generalist performance in response to allelochemicals (Question 1). The first model included all effect sizes related to growth; the second to fecundity; and the third to survival. Because each effect size represented the difference in performance between a specialist and generalist insect, diet breadth was inherently a fixed effect in all three models. We incorporated one random factor into each model to account for cases where multiple effect sizes were extracted from a single publication (Barton, 2016).

To determine whether the moderators 'allelochemical class' or 'insect order' affected the differences in effect sizes between

Table 1. Publications included in this meta-analysis, organised by insect order.

Author(s) and year	Specialist insect family	Generalist insect family	Plant family	Chemical class	Measured	Effect
Coleoptera						
Ali and Agrawal (2016)	Cerambycidae	Cerambycidae	Asclepiadaceae	Terpenoid	% survival	1.438
Alouw and Miller (2015)	Chrysomelidae	Chrysomelidae	Poaceae	Nitrogen	Weight gain	0.369
					Weight gain	0.419
					% survival	−0.157
Buhl *et al.* (2015)	Chrysomelidae	Erebidae	Salicaceae	Phenolic	Dev. time	−0.054
					Weight gain	0.008
					No. eggs	0.596
Hemiptera						
Tariq *et al.* (2013)	Aphididae	Aphididae	Brassicaceae	Nitrogen	Dev. time	0.038
					No. eggs	0.071
Lepidoptera						
Ahn *et al.* (2011)	Noctuidae	Noctuidae	Solanaceae	Nitrogen	Dev. time	0.048
					Weight gain	0.247
					Pupal mass	0.259
Arany *et al.* (2008)	Plutellidae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	1.793
Bandoly *et al.* (2016)	Sphingidae	Noctuidae	Solanaceae	Phenolic	% survival	−0.456
Barth and Jander (2016)	Plutellidae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	−0.543
Berenbaum (1983)	Papilionidae	Papilionidae	Apiaceae, Oleraceae	Phenolic	AD	−0.177
					ECD	0.031
					ECI	−0.248
					RGR	−0.276
					Pupal mass	0.156
Bi *et al.* (1997)	Sphingidae	Noctuidae	Solanaceae	Phenolic	Weight gain	0.322
Bodenhausen and Reymond (2007)	Pieridae	Noctuidae	Brassicaceae	Jasmonate	Weight gain	0.582
Bowers & Puttick (1988)	Nymphalidae	Erebidae	Artficial diet	Terpenoid	Weight gain	0.831
					Weight gain	0.452
Bruessow *et al.* (2010)	Pieridae	Noctuidae	Brassicaceae	Phenolic	Weight gain	−0.493
Dyer *et al.* (2003)	Geometridae	Noctuidae	Piperaceae	Nitrogen	Dev. time	0.072
Ferreira *et al.* (1997)	Crambidae	Noctuidae	Artficial diet	Nitrogen	Dev. time	−0.122
					Weight gain	−0.126
					% survival	0.129
Ferreira *et al.* (1997)	Crambidae	Noctuidae	Artficial diet	Phenolic	Dev. time	−0.065
					Weight gain	−0.089
					% survival	0.067
Fortuna *et al.* (2014)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	% survival	3.285
Gols *et al.* (2008a)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Dev. time	0.438
					Pupal mass	0.287
Gols *et al.* (2008b)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Dev. time	0.082
Huang *et al.* (2010)	Nolidae	Limacodidae	Euphorbiaceae	Phenolic	Dev. time	−0.007
					Weight gain	−0.129
Irwin *et al.* (2003)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	0.292
Kaplan *et al.* (2014)	Sphingidae	Noctuidae	Solanaceae	Jasmonate	Weight gain	0.254
					ECD	0.325
					ECI	−0.044
Kaur *et al.* (2010)	Sphingidae	Noctuidae	Solanaceae	Phenolic	Weight gain	< 0.001
Kelly and Bowers (2016)	Nymphalidae	Erebidae	Plantaginaceae	Terpenoid	Weight gain	1.424
					% Survival	0.398
Kos *et al.* (2012)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Dev. time	0.248
Lampert and Bowers (2015)	Sphingidae	Sphingidae	Bignoniaceae	Terpenoid	Dev. time	0.333
					Weight gain	−0.248
Li *et al.* (2000)	Plutellidae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	1.694
					RGR	0.047
Mao *et al.* (2017)	Plutellidae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	−0.248
Mathur *et al.* (2014)	Plutellidae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	−1.451
Ogran *et al.* (2016)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	1.680
Poelman *et al.* (2008a)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	0.277

Table 1. Continued

Author(s) and year	Specialist insect family	Generalist insect family	Plant family	Chemical class	Measured	Effect
Poelman *et al.* (2008b)	Plutellidae	Noctuidae	Brassicaceae	Nitrogen	Dev. time	0.240
					Weight gain	1.507
					Pupal mass	−0.004
Rasmann *et al.* (2015)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	0.079
Rohr *et al.* (2011)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	−0.140
Roslin and Salminen (2008)	Noctuidae	Noctuidae	Fagaceae	Phenolic	Weight gain	0.752
					AD	−0.407
					ECI	1.653
					ECD	1.922
Rostas (2007)	Noctuidae	Noctuidae	Poaceae	Nitrogen	RGR	< 0.001
Santolamazza-Carbone *et al.* (2016)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Dev. time	−0.354
					Weight gain	0.503
					Pupal mass	−0.217
Schlaeppi *et al.* (2008)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	1.489
Schweizer *et al.* (2013)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	0.847
Schweizer *et al.* (2017)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	2.066
van Leur *et al.* (2008)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	No. eggs	−0.120
van Oosten *et al.* (2008)	Pieridae	Noctuidae	Brassicaceae	Terpenoid	Weight gain	2.673
van Oosten *et al.* (2008)	Pieridae	Noctuidae	Brassicaceae	Phenolic	Weight gain	0.913
Woldemariam *et al.* (2012)	Sphingidae	Noctuidae	Solanaceae	Jasmonate	Weight gain	0.145
Zheng *et al.* (2011)	Pieridae	Noctuidae	Brassicaceae	Nitrogen	Weight gain	0.302
Coleoptera and Lepidoptera						
Hull-Sanders *et al.* (2007)	Chrysomelidae	Noctuidae	Asteraceae	Terpenoid	Weight gain	−0.464

Positive differences in effect size estimates indicate that specialists out-performed generalists and negative differences indicate the opposite.
Dev., development; No., number; AD, approximate digestibility; ECD/ECI, efficiency of conversion of digested/ingested food; RGR, relative growth rate.

specialists and generalists, we built an analysis of variance model to test main and interactive effects of each (Question 2). The allelochemical classes in our model included nitrogen-containing compounds, phenolics, terpenoids, and jasmonates. Insect orders included Lepidoptera, Hemiptera, and Coleoptera. We partitioned effect sizes into growth, fecundity, and survival as described above. We used Shapiro–Wilk and Breusch–Pagan tests to assess the assumptions of normally and equally distributed error terms (Shapiro & Wilk, 1965; Breusch & Pagan, 1979). Although we did detect non-normality in effect sizes related to growth ($P < 0.001$), we did not detect heteroskedasticity ($P = 0.824$). Because analysis of variance is robust to assumptions of normality, we did not apply any transformations to our data.

Finally, we evaluated the level of homogeneity and publication bias in our database. In the context of meta-analysis, homogeneity refers to little variation of results among the studies included in the analysis. We tested for homogeneity using the test statistic I^2. Publication bias can result from a disproportionately small number of publications reporting negative or unexpected results; in this case, results where generalists out-performed specialists. The most common tests for publication bias indicate how many negative results would need to be added to the database to change the outcome of statistical analyses. We used two methods to detect publication bias: a funnel plot and Rosenberg's *n* (Rosenberg, 2005).

Results

As predicted by phytochemical coevolution theory, performance was significantly higher for specialists than for generalist insects in response to allelochemicals (Fig. 1 and Table 2), indicating that specialists responded less negatively to allelochemicals than generalists. This effect was driven more by differences in growth between specialists and generalists than by differences in fecundity or survival (Fig. 1 and Table 3). The effect was not dependent upon allelochemical class or insect order (Table 3).

By contrast with phytochemical coevolution theory, statistically significant differences were not detected regarding fecundity or survival of specialist versus generalists (Fig. 1 and Table 3). We cannot state whether or not this was a true biological pattern or an artefact of small sample size (O'Keefe, 2007). Because there was no overall difference, we did not test for the fixed effects of allelochemical class or insect order in relation to these performance metrics as we did for growth.

There was significant heterogeneity in effect sizes related to growth ($I^2 = 84.48$, d.f. = 61, $P < 0.001$), survival ($I^2 = 66.68$, d.f. = 6, $P = 0.006$), and fecundity ($I^2 = 63.48$, d.f. = 7, $P = 0.008$). Funnel plots and fail-safe *n* tests did not reveal evidence of publication bias in effect sizes related to growth (Rosenberg's $n = 15212$, $P < 0.001$), fecundity (Rosenberg's $n = 14$, $P = 0.001$) or survival (Rosenberg's $n = 3195$,
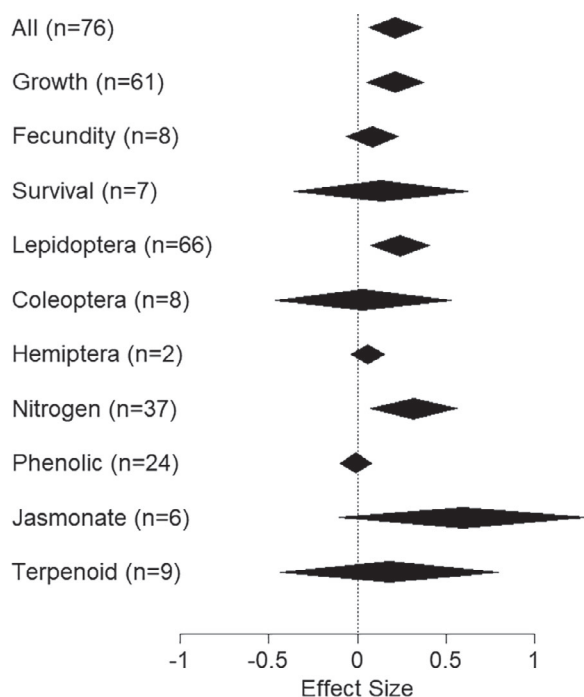
Fig. 1. Mean difference in effect sizes between specialist and generalist insects related to all performance metrics, insect orders, and chemical classes. Diamond widths represent 95% confidence intervals. *n*, number of effect size differences included in the mean. Positive effect size estimates indicate that specialists out-performed generalists and negative effect size estimates indicate the opposite.

$P < 0.001$). For related funnel plots and correlation test statistics, see Fig. 2.

Discussion

The premise that specialist insects are better adapted to the allelochemicals of their host plants than generalist insects is at the heart of fundamental plant defence theories (Stamp, 2003; Loxdale *et al.*, 2011), although it has been increasingly debated in recent years (Ali & Agrawal, 2012; Smilanich *et al.*, 2016). Even experiments incorporating highly specialised and generalised insects are not always congruent with this prediction, as indicated by statistically significant heterogeneity among most of the publications included in our database. Testing and interpretation of this premise has been further complicated by the recognition of a more continuous spectrum of insect diet breadth, which incorporates insects that cannot be described as strictly monophagous nor highly polyphagous. This is especially problematic for the meta-analytic approach, where a database likely contains some studies that consider a certain insect species to be specialists, whereas others consider the same species generalists. Although we did not encounter this issue in our meta-analysis, we did have different degrees of differences in diet breadth between generalist/specialist pairs. For example, some studies included only 'extreme' specialists and generalists, whereas others included one or more insect species that tend closer towards the middle of the spectrum. In this meta-analysis, we used a stringent set of criteria, including generalist/specialist pairs within each study to attempt to reduce some of the noise present among insect feeding trials.

Higher performance in specialist insects

Overall, we found support for the prediction that the performance of specialist insects is less negatively affected by allelochemicals than in generalists. This result supports the key
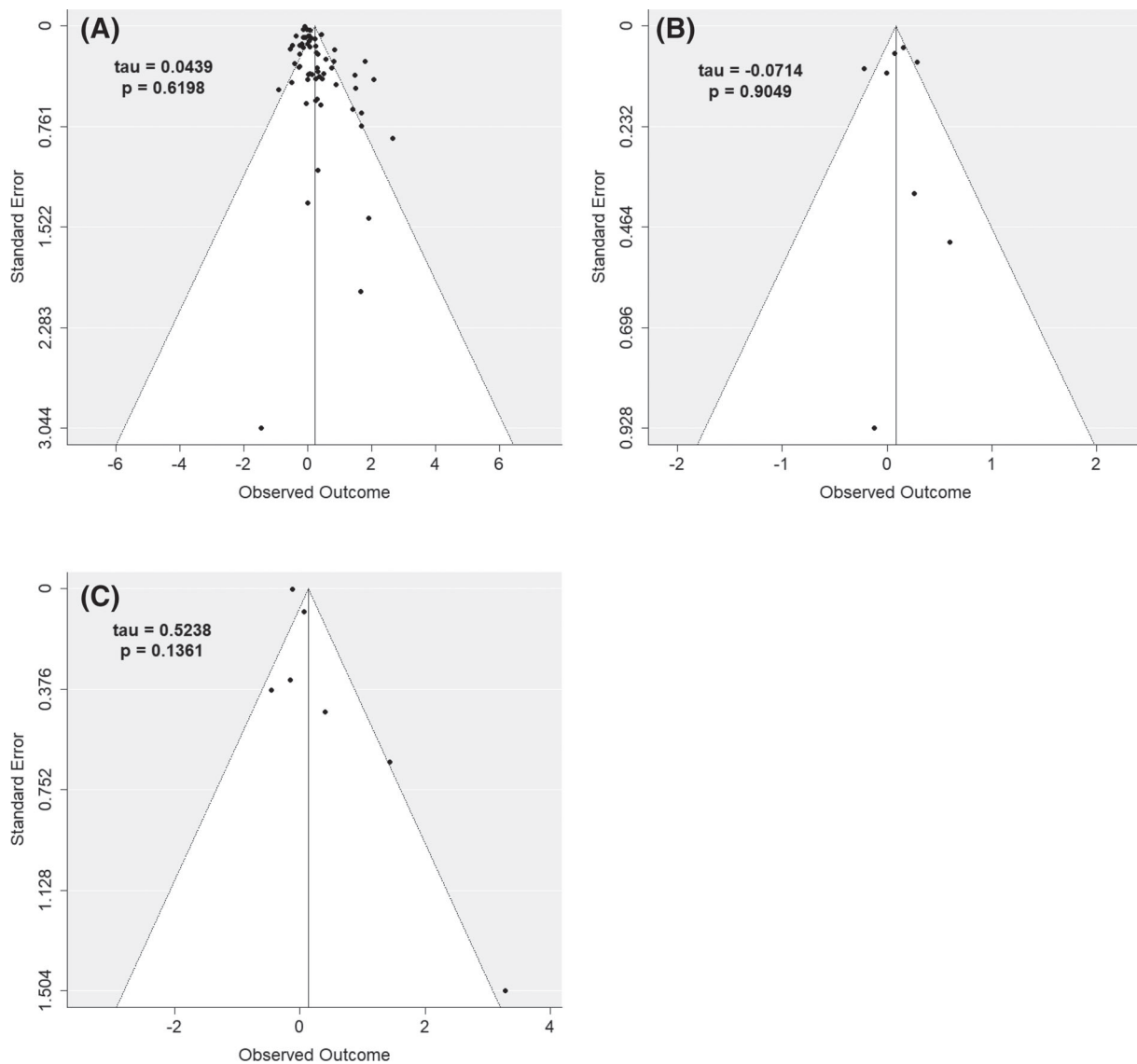
Table 2. Summary of meta-analysis results.

Overall effect size difference estimates, confidence intervals, and associated *P*-values of three standard meta-analytic models testing for differences in growth, fecundity, and survival between generalist and specialist insects. Positive effect size estimates indicate that specialists out-performed generalists. Negative effect size estimates would indicate the opposite.

Performance metric	Estimate	CI	P
Overall	0.214	(0.061, 0.367)	0.006
Growth	0.226	(0.065, 0.386)	0.006
Fecundity	0.084	(−0.064, 0.232)	0.266
Survival	0.134	(−0.355, 0.624)	0.591

Table 3. Summary of analysis of variance results.

F-statistics, degrees of freedom, and associated *P*-values of an analysis of variance testing for effects of chemical class (nitrogen-containing compounds, phenolics, jasmonates, and terpenoids) and insect order (Lepidoptera, Coleoptera, and Hemiptera) on differences in effect sizes between specialist and generalist insects.

Effect	F	DF	P
Chemical class	1.030	3	0.387
Insect order	0.331	2	0.720
Chemical class × Insect order	0.271	2	0.764

Fig. 2. Funnel plots for visualisation of possible publication bias. Points represent positive and negative effect sizes included in this meta-analysis related to (A) growth, (B) fecundity, and (C) survival. Test statistics shown were derived from rank correlation tests where the null hypothesis states that publication bias does not exist.

prediction of phytochemical coevolution theory, such that specialists will exhibit a less negative response to allelochemicals than generalists (Ehrlich & Raven, 1964; Scriber & Feeny, 1979; Cornell & Hawkins, 2003). This result was more strongly influenced by insect growth than fecundity or survival. There are several potential advantages of increased growth. Increased body size may correlate with greater fecundity in adulthood or decreased acceptance by predators, especially when paired with warning coloration (Mand *et al.*, 2007). Additionally, improved efficiency of conversion in specialist insects may decrease foraging time, conferring reduced predation risk and energy expenditure (Hassell & Southwood, 1978; Benrey & Denno, 1997). Although generalist insects sometimes perform equally well or better than specialist insects in response to allelochemicals, this meta-analysis demonstrates that, in terms

of growth, the central prediction of phytochemical coevolution theory is generally supported by paired feeding trial studies. Our methods differ from the generalist/specialist performance comparison in Smilanich *et al.* (2016) in two key ways: our inclusion criteria (a paired feeding trial design was required in the present study) and our date ranges of the literature [pre-2005 in Smilanich *et al.* (2016) and pre-2018 in the present study].

No differences in fecundity or survival between specialists and generalists

Growth is often used as a proxy for fecundity in insects and positive correlations between adult body size and fecundity have been shown (Briegel, 1990; Honěk, 1993; Lyimo & Takken,

1993). This relationship can be complicated by other factors, such as genetic characteristics and ambient conditions during oviposition (Sweeney & Vannote, 1978; Leather, 1988). Similarly, a correlation between growth and survival may be expected when concentrations of allelochemicals are sufficiently high to induce some mortality. However, the concentration threshold for such a relationship is unknown for many study systems.

Although growth often correlates with fecundity and survival, in the present study, we did not find significant patterns for generalist versus specialist survival and fecundity as we did for growth. This may reflect true biological patterns or may be a result of a low sample size. We had only seven effect sizes related to survival in our database, five of which favoured specialist insects. Out of the eight effect sizes related to fecundity in our database, five favoured specialists. Interestingly, all three effect sizes for fecundity that favoured generalist insects used the specialist *Pieris rapae* and the generalist *Mamestra brassicae* feeding on mustard plants (*Plutella xylostella* was used as an additional specialist in one of these studies). It is possible that this system represents an exception to phytochemical coevolution theory and drove the effect of diet breadth on fecundity in a negative direction in this analysis. Heightened performance of the generalist relative to the specialist in this system could be a result of physiological mechanisms subsequent to ingestion (Poelman *et al.*, 2008). For example, in one study in our database, the generalist *M. brassicae* appeared to effectively detoxify glucosinolates of some mustard chemotypes but not others (Van Leur *et al.*, 2008). Alternatively, our results may perhaps simply reflect an effect of allelochemicals on fitness aspects other than fecundity.

No effect of allelochemical class or insect order on specialist vs. generalist performance

Although higher growth in specialist than in generalist insects was not significantly affected by allelochemical class, we did observe trends in allelochemical class effect sizes. The mean difference in effect size between specialist and generalist insects associated with phenolic compounds is almost centred around zero. The effect size differences associated with terpenoids and jasmonates are not only skewed positively, but also overlap zero. However, for studies using nitrogen-containing compounds, the difference in effect size favoured specialists and did not overlap zero. It is possible that the advantage of specialist insects over generalists is more pronounced when utilising plants that produce nitrogen-containing allelochemicals than when feeding on tissues containing phenolic or terpenoid compounds. This could be a result of the typically fast-acting and highly toxic nature of certain nitrogen-containing compounds, such as alkaloids and glucosinolates (Palo & Robbins, 1991). It is also possible that our broad classifications of allelochemical class, which is necessary because of sample size limitations, obfuscated differences in effects of particular allelochemicals on generalists and specialists within some of the allelochemical classes. For example, the terpenoids in our dataset included relatively potent (e.g. cardenolides) and weak (e.g. diterpenes) allelochemicals, potentially masking the strength of the more potent allelochemicals within the class.

Similarly, although differences among insect orders were not statistically significant, some patterns were discernable. The difference in effect size distinctly favoured specialists for Lepidoptera but overlapped with zero for Coleoptera and Hemiptera. This is in contrast with our prediction that differences would arise among feeding guilds; we expected results to differ between leaf chewers (Lepidoptera and Coleoptera) and sap-suckers (Hemiptera) based on differential induction of allelochemical pathways (Mewis *et al.*, 2006) or feeding on parts of the plant that have low allelochemical concentrations (i.e. vascular tissue). Instead, our results suggest that specialism is more highly favoured for Lepidoptera than other insect orders. This may be a result of the distinct, long-term interactions of the host plant and Lepidoptera species used (Bidart-Bouzat & Kliebenstein, 2011). Long-term interactions may confer benefits other than improved allelochemical mitigation, such as modified feet facilitating walking on hairy or waxy leaf surfaces, which may indirectly improve feeding and/or assimilation efficiency (Bernays, 1998). It is also possible that effects of host specialisation were masked in some feeding guilds as a result of their relatively low representation in the present study.

Database limitations

The majority of the publications in our database used the insect order Lepidoptera and the allelochemical classes nitrogen-containing compounds or phenolics; thus, our results may not be broadly applicable to insects and allelochemicals as a whole. In addition, this limitation may have obscured patterns among allelochemical class and insect order, which were not discernable in the present study. Similarly, our database was highly skewed towards growth, with fewer studies that measured fecundity and survival. Moreover, some of the studies included in this meta-analysis introduced additional factors to feeding trials, including plant stressors such as drought and mechanical damage, which may further complicate differences among specialist and generalist insects. For example, Tariq *et al.* (2013) found that both specialist and generalist species performed poorly when consuming plants under extreme drought stress, although differences were found when drought was milder. On the other hand, certain factors present in natural conditions are reduced or eliminated in experimental feeding trial studies, such as insect food choice, plant induction of allelochemicals, and the presence of natural enemies. There were also numerous comparisons between specialist and generalist insects belonging to different families and, in one study, to different orders. Additional studies strictly testing for differences between specialist and generalist insects, using a phylogenetically controlled paired feeding trial method and including a broader array of performance metrics, insect orders, allelochemical classes, and natural bottom-up or top-down components might enable more comprehensive conclusions to be drawn.

Future work

There are many facets to phytochemical coevolution theory that remain unresolved, such as insect feeding guild,

allelochemical dosage effects or influence of higher trophic levels. Using a meta-analysis of paired feeding trial studies, we were able to uncover a difference in growth between specialist and generalist insects that has been obscured by noise among non-paired studies in previous literature reviews and meta-analyses. Thus, we propose that paired studies, fitting the criteria defined for this meta-analysis, are particularly effective for tests of plant defence hypotheses involving generalist and specialist insects. There are some factors that cannot be controlled for in such experiments, such as behavioural differences between specialist and generalist insects. However, this experimental design does allow for direct comparisons of growth, fecundity, and survival at the same time as controlling for random factors such as insect phylogeny, plant host quality or ambient conditions.

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