REVIEW

Tougher and thornier: general patterns in the induction of physical defence traits

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Summary

1. Most plants rely at least in part on physical defence traits to deter herbivores, and yet, very little is known about the inducibility of these traits.

2. A meta-analysis was conducted on data extracted from 57 publications from 1982 to 2014, including 53 species in 20 plant families in which the induction of physical defence traits was examined. Log response ratio effect sizes were calculated for 112 separate responses and analysed to characterize general patterns and test whether induction differed between physical and chemical defence traits (in the same study), among defence traits, and in response to the damage type (artificial, herbivore, jasmonates).

3. Physical defence traits are generally inducible, showing a mean increase of 52% in damaged compared to control plants. The magnitude of increase did not differ between physical and chemical defence traits measured within the same experiments, and nutrients showed no overall response. Physical defence traits varied significantly in their responses, with non-glandular trichomes showing the greatest magnitude of increase, and no induction detected for leaf toughness. Responses induced by real herbivores or by jasmonate application were significantly greater than responses elicited by mechanical damage alone. This suggests that specificity in physical trait induction in response to different kinds of damage may occur.

4. Induction of physical defence traits is common and widespread, but the ecological and evolutionary consequences of this response remain unknown. Future studies examining genetic variation in the inducibility of physical defence traits and their fitness consequences for plants would be particularly informative.

Key-words: defence syndrome, herbivory, induced defence, jasmonates, leaf toughness, meta-analysis, spinescence, structural defence

Introduction

Induced responses to herbivory are widespread, having been documented in hundreds of plant species (Karban & Baldwin 1997; Karban 2011). Although plants may respond to herbivory by increasing both the levels of secondary compounds and densities or sizes of physical defence traits, the vast majority of studies have focused exclusively on chemical induction. Yet, most plants rely at least in part on physical defences, which include external structures that deter herbivores such as trichomes, prickles, spines and thorns, as well as cellular features that contribute to plant toughness such as lignification, silica and calcium oxalate crystals (Hanley *et al.* 2007). As a result of the emphasis on chemical induction, we have a poor understanding of the general patterns and key sources of variation in the induction of physical defence traits. Furthermore, increasing evidence suggests that plants are not constrained in their simultaneous expression of physical and chemical defence traits, potentially giving rise to 'defence syndromes' (Koricheva, Nykanen & Gianoli 2004; Agrawal & Fishbein 2006). However, very few studies have tested whether induction leads to simultaneous increases in both chemical and physical defence traits, limiting our test of the syndrome concept.

Physical defence traits are common and found in plants across all geographic regions. Because physical defences vary drastically in size, they may function in defence against a variety of herbivores. While thorns and spines reduce herbivory by large mammalian herbivores (Milewski, Young & Madden 1991; Young & Okello 1998), trichomes and crystals specifically deter insect herbivores

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(Agrawal 1998, 1999; Mathur *et al.* 2011). Tough leaves can protect against a range of herbivores and may be the most important defence in tropical trees (Coley 1983; Coley & Barone 1996; Westbrook *et al.* 2011). Like secondary chemicals, physical defence traits are inducible by herbivory, but while general patterns in chemical induction have been the focus of dozens of reviews and books, physical defence induction has only been reviewed three times, and none of these used a quantitative approach (Myers & Bazely 1991; Karban & Baldwin 1997; Hanley *et al.* 2007). Because chemical and physical defences differ in their rates of production, their capacities to be remobilized and recycled, and the specificities of their effects, their induction by herbivores is likely to be very different.

Physical defence traits are anatomical plant features that arise during plant development. As structural modifications to leaves, physical defence traits may require relatively 'cheap' investments of carbon as cellulose or lignin (Westbrook *et al.* 2011). However, once these structures have been produced, they cannot be broken down to allow building blocks to be recycled or remobilized. Thus, overall construction costs may be high, and studies have shown that high levels of physical defence traits reduce fitness, particularly in the absence of herbivores (Mutikainen & Walls 1995; Agrawal 2000; Gómez & Zamora 2002; Sletvold *et al.* 2010). Because induction may benefit plants via cost savings by allowing them to allocate resources to growth and storage when defence is not needed (Cipollini & Heil 2010), plants may benefit from the inducibility of physical defences. On the other hand, because plasticity in physical defence traits requires the production of new structures (e.g. leaves with higher trichome densities), there is an inherent time lag to their induction by herbivores (Traw & Dawson 2002; Valkama *et al.* 2005), possibly reducing their benefit in ecological time. Thus, although plants may benefit from the inducibility of physical defence traits via cost savings, their high construction costs and time lag in development lead to the prediction that physical trait induction is likely to be less common and of weaker magnitude than the induction of chemical defence traits.

Considerable advances have been made over the past two decades in understanding the mechanisms underlying plant chemical induction by herbivores. For example, it is clear that plants have different induced responses to different herbivores due to jasmonate-mediated responses to elicitors from the herbivores (Erb, Meldau & Howe 2012; Heil *et al.* 2012). Such specificity in herbivore-induced responses allows plants to induce changes not only in the amounts but also in the profiles of their secondary chemicals (Lehrman *et al.* 2013; Moreira *et al.* 2013). In contrast, almost nothing is known about specificity in the induction of physical defence traits, and many studies simulate herbivory using artificial damage without the application of herbivore elicitors or jasmonates (Gibson, Bazely & Shore 1993; Abdala-Roberts & Parra-Tabla 2005; Valkama *et al.* 2005; Holeski 2007; Liu, Cai & Li 2010).

Nonetheless, there are some examples of specificity in the induction of physical defence traits. For example, glandular trichome induction differed not only in magnitude but also in timing of the response to three different insect herbivores feeding on *Brassica nigra* (Traw & Dawson 2002). While studies examining induction by multiple herbivores are rare, specificity may be revealed if induction by jasmonates or real herbivores is greater than induction by mechanical damage alone.

The goal of this study is to comprehensively synthesize research on the induction of physical defence traits using meta-analysis in order to reveal general patterns, test whether physical defence induction may contribute to an overall induced defence syndrome, and to identify gaps in our knowledge (Koricheva & Gurevitch 2014). In particular, the following questions were examined:

1. *Is the magnitude of induction similar for physical vs. chemical defence traits?* It is predicted that physical and chemical defence traits will both be inducible, consistent with previous synthetic work revealing a lack of trade-offs between these defence types, but that the magnitude of physical trait induction will be weaker than that of chemical induction due to high construction costs and time lag.

2. *Are physical defence traits induced by damage, and are the patterns of induction similar across different traits (trichomes, thorns, spines, prickles, and leaf toughness)?* All traits are predicted to be inducible, but the magnitude of induction is predicted to be greater for leaf toughness and trichomes than the other traits because of greater construction costs associated with spines, thorns and prickles.

3. *Is there specificity in the induction of physical defence traits by herbivores compared to mechanical damage?* The induction of physical defence traits is predicted to be specific, with weaker induction caused by mechanical damage than by jasmonate application or real herbivory.

4. *Does physical defence induction vary geographically?* Tropical plants are predicted to be more inducible than temperate regions consistent with the importance of physical defences and high levels of herbivory in tropical forests (Coley & Barone 1996; Boege 2004).

Materials and methods

COMPILATION OF THE DATA BASE

Searches for studies to include in the meta-analysis were made by searching Web of Science using combinations of induction keywords ('induced defen?e', 'induc* AND herbivor*', 'induction', 'induced resistance') with physical defence keywords ('plant defen?e', 'spine', 'thorn', 'plant prickle', 'trichome', 'leaf toughness', 'spinescence'). Studies were also identified by perusing the bibliographies of all retrieved studies and by performing cited literature searches on key papers (Hanley *et al.* 2007). To be included in the final data set, studies had to meet the following criteria: (i) physical defence traits were measured in damaged and properly controlled undamaged plants. (ii) Comparisons were generally made between different

plants in the damaged and control groups, but in a few cases, before vs. after damage comparisons were included. (iii) The experimental design was robust, avoiding such common pitfalls as pseudoreplication and confounding explanatory variables (e.g. different geographic areas for damaged and control plants). (iv) The data needed for calculation of effect size (sample sizes, means, standard deviations) were reported in the paper, in either numerical or graphical form. (v) When available, data on the induction of chemical and nutrient traits were extracted from the focal studies in order to compare physical vs. chemical induction. Separate searches of chemical induction were not performed, so these data were only included when reported in studies also reporting physical trait induction.

The final data set included 57 publications from 1982 to 2014, with data from 53 species in 20 plant families (Appendix S1, Supporting information). A subset of publications reported the induction of secondary compounds (glucosinolates, sesquiterpene lactones, phenolics, tannins) and nutrients (nitrogen, water) in addition to physical defence traits, and in these cases, data from all three classes of traits were included in the meta-analysis. Multiple effect sizes were included from a single study when multiple species or traits were simultaneously examined; no more than five effect sizes were extracted from a single study, and most had one or two effect sizes (Appendix S1). Studies reporting the induction of secondary compound and nutrients, but not physical defence traits, were not included. The total data set included 112 response ratios, including $n = 87$ for physical defence traits, $n = 16$ for secondary chemistry and $n = 9$ for nutrients (Table S1, Supporting information).

META-ANALYSIS

The meta-analysis was conducted using the 'metafor' package in R version 3.1.2 (Viechtbauer 2010). The effect size examined was the log response ratio of the trait mean for damaged plants (M_D) over control plants (M_C): $\ln(M_D/M_C)$, and sample variances were estimated using the escalc() function. Positive values indicate the induction of higher levels of defence traits in damaged compared to undamaged control plants. Negative values would occur when induction leads to decreases in traits following herbivory. When the means and standard deviations needed for calculation of the log response ratio were presented in graphs, they were extracted digitally using TECHDIG 2.0 software (Jones 2007).

The data were initially analysed with a random effects model to test whether the overall mean response ratio was significantly different from zero (the null hypothesis that damaged and control plants do not differ in defence traits). Subsequent analyses were done with mixed effects models using the rma.mv() function. Fixed modifiers were tested independently to identify key sources of variation in the induced responses, including (i) defence type (physical vs. chemical vs. nutrient); (ii) physical defence trait, excluding chemical and nutrient data (trichomes vs. prickles vs. spines vs. thorns vs. leaf toughness); (iii) trichome type (glandular vs. nonglandular); (iv) damage type (mechanical vs. herbivore vs. jasmonates); (v) herbivore type, excluding mechanical and jasmonate studies (insect vs. mammal); and (vi) biogeographical region (tropical vs. subtropical vs. temperate vs. boreal). Two random factors were included in each model to account for potential non-independence due to study design and phylogenetics. Because multiple effect sizes were extracted from a publication when multiple species or defence traits were examined, 'Publication' was included as a random factor. In addition, to account for the likelihood that related species show similar responses, 'Genus' was included as a random factor in the models.

To interpret significant variation due to modifiers, model-generated group means and 95% confidence intervals are presented. Mean group effect sizes are considered significant when the

confidence intervals do not overlap with zero. Log response ratios are back-transformed to % difference between damaged and control plants to better interpret significant effects using the equation $100-100e^{(\text{response ratio})}$.

Publication bias can mislead a meta-analysis, particularly if non-significant results are not published (Csada, James & Espie 1996). To investigate whether this data set was likely influenced by publication bias, three approaches were taken. First, a weighted histogram was examined to assess whether effect sizes were normally distributed around zero. Secondly, a funnel plot was constructed, and funnel plot asymmetry was tested using the rank correlation test (Begg & Mazumdar 1994). Finally, because it seemed plausible that negative or null induction effects would be reported for physical defence traits when they were found simultaneously with significant induction of chemical traits (but not when detected by themselves), a heterogeneity test was conducted to compare effect sizes of physical defence traits when reported in combination with chemical or nutrient traits vs. when reported alone. If publication bias occurs in this way, effect sizes should be significantly higher for physical defence traits published without chemical traits than when published with chemical traits.

Results

Overall, defence traits were significantly higher in damaged compared to control plants, induced by an average of 42·5% (95% CI 29·1–57·2; $Z = 7·0671$, $P < 0·0001$). Significant variation was detected for the induction of chemical vs. physical vs. nutritive traits ($Q_M = 292·1397$, d.f. = 2, $P < 0·0001$). Induction led to significant increases of similar magnitude in both physical defence traits and secondary chemistry, but failed to affect nutrient concentrations (Fig. 1).

A closer examination of induced responses among different kinds of physical defence traits (trichomes, prickles, thorns, spines, leaf toughness) revealed additional significant variation ($Q_M = 36·0575$, d.f. = 4, $P < 0·0001$). Significant positive induction was detected for trichomes, prickles, thorns and spines, although trichomes showed the
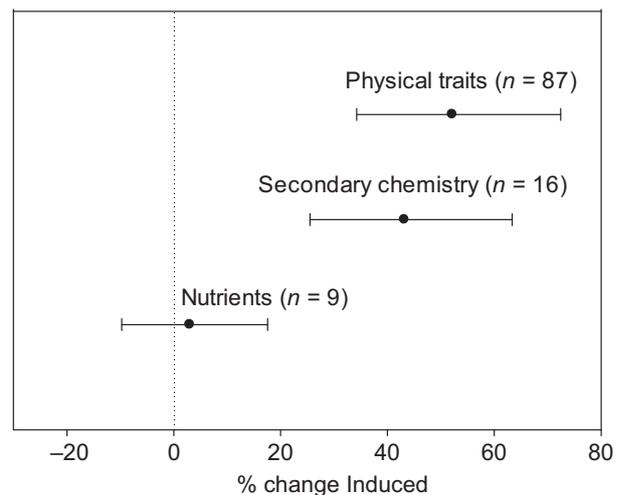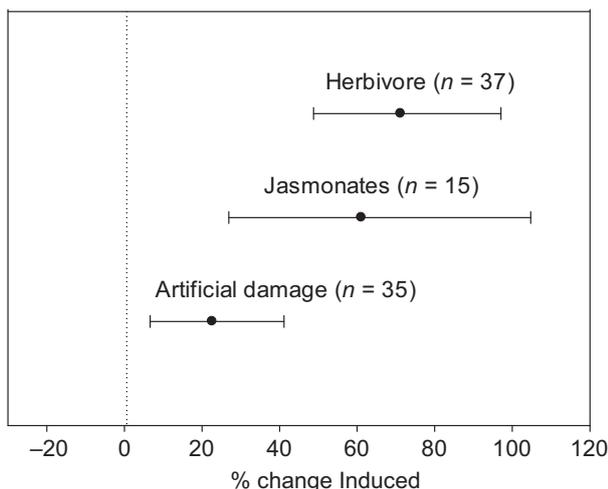

Fig. 1. Per cent changes with bias-corrected 95% confidence intervals in classes of plant defence traits induced by herbivory or simulated herbivory, calculated from log response ratios.

greatest magnitude of induction (Fig. 2). Furthermore, non-glandular trichomes (mean 82·7%, 95% CI 39·8–138·8) were significantly more inducible than glandular trichomes (mean 41·2%, 95% CI 5·0–89·8; $Q_M = 36·0575$, d.f. = 4, $P < 0·0001$). In contrast, no induction was detected for leaf toughness (Fig. 2).

Significant variation was detected among studies that used different methods of damage to induce physical defence traits ($Q_M = 36·1996$, d.f. = 2, $P < 0·0001$). Although artificial damage (simulated herbivory) led to significant increases in physical defence traits, the magnitude of induction was significantly less than plant-induced responses to real herbivores and jasmonate application (Fig. 3). There was no detectable difference between studies on insect vs. mammalian herbivores ($Q_M = 0·1952$, d.f. = 1, $P = 0·3586$).
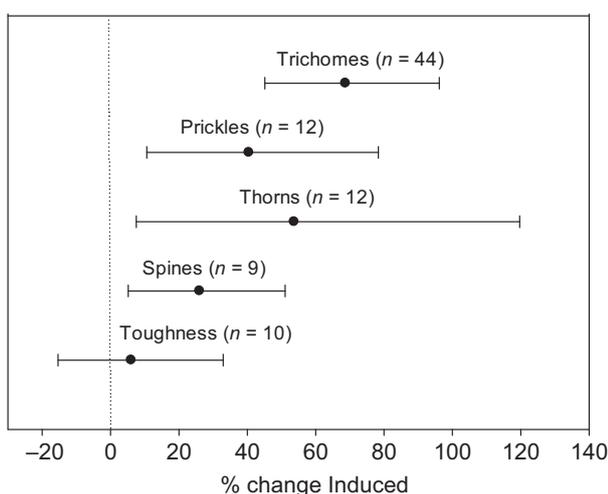
Fig. 2. Per cent changes with bias-corrected 95% confidence intervals in physical defence traits induced by herbivory or simulated herbivory, calculated from log response ratios.

Fig. 3. Per cent changes with bias-corrected 95% confidence intervals in physical defence traits induced by real herbivores, jasmonate application or artificial damage to simulate herbivory, calculated from log response ratios.

Geographic regions were not found to vary in the induction of physical defences ($Q_M = 5·1823$, d.f. = 3, $P = 0·1589$). However, because studies on trichome induction were conducted predominantly in the temperate region (29/44 total trichome studies; see Table S1), while thorn induction was concentrated in tropical areas (7/12), there is a lack of independence between traits and geographic areas ($\chi^2 = 38·3$, d.f. = 12, $P < 0·0001$). While these trait biases might reflect real biogeographical distributions in trait expressions (e.g. thorns might be more prevalent in the tropics than in temperate and boreal areas), it makes it difficult to separate biogeographical patterns in induction from differences among plant defence traits.

Evidence for publication bias was detected, suggesting that positive induction effects are published more frequently than null or negative effects. The weighted histogram revealed relatively few studies showing no induction or an induced decrease in defence traits (Fig. 4a), and the funnel plot was skewed (Fig. 4b) consistent with a significant correlation between effect size and standard error ($t = 2·4838$, d.f. = 10, $P = 0·0145$). However, the mean effect sizes for the induction of physical defence traits was not significantly greater when published together with chemical traits than when published alone ($Q_M = 0·0162$, d.f. = 1, $P = 0·8987$), indicating that the inclusion of chemical traits does not change the likelihood of publishing physical trait induction results.

Discussion

This meta-analysis reveals that physical defence traits are generally inducible and that they are induced to similar magnitudes as chemical defence traits. This indicates that despite the disadvantages of a time lag in the development of tissues with higher densities of physical defence traits and the lack of remobilization of resources used in their construction, herbivore- and damage-mediated plasticity in trichomes, prickles, spines and thorns appear to be common and widespread. However, interesting variation was detected among the different physical defence traits. Trichomes are generally induced to a greater magnitude than spines, prickles or thorns, and non-glandular trichomes are more inducible than glandular trichomes. In contrast, leaf toughness showed no response to damage or herbivory, a surprising result given that leaf toughness is driven largely by high cellulose content and laminar density (Westbrook *et al.* 2011; Kitajima *et al.* 2012), which are relatively inexpensive for plants to produce. It may be that leaf toughness is largely controlled by eco-physiological needs such as water balance, preventing plasticity in response to herbivory. However, given the small sample size of studies testing herbivore induction of leaf toughness (*n* = 10), additional studies are needed to confirm this result.

Increasing evidence indicates that plant defence consists of suites of traits that are expressed simultaneously and may covary, giving rise to 'defence syndromes'
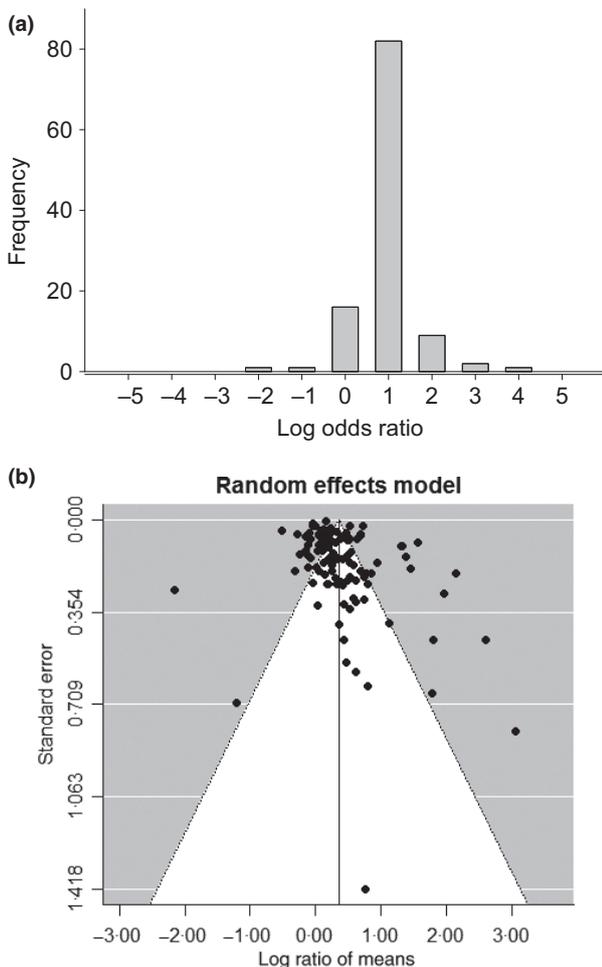
Fig. 4. Evidence for publication bias. (a) Histogram of log odds ratio showing high frequencies of positive effect sizes, and (b) funnel plot with significant asymmetry.

(Agrawal & Fishbein 2006; Hanley *et al.* 2007). However, most studies characterizing suites of defence traits consider only the constitutive expression patterns (Koricheva, Nykanen & Gianoli 2004; Moles *et al.* 2013), and very few examine the simultaneous induction of multiple defence traits as a test for induced defence syndromes. For example, only 17 publications were identified that analyse the induction of physical and either chemical or nutrient traits (Table S1), and only a single paper that examined all three (Traw & Dawson 2002). From this small pool, it looks like damage induces increases in physical and chemical defence traits to a comparable magnitude while having no general effect on nutrient concentrations. These synchronous induced changes would result in particularly unpalatable plants that are better defended in terms of both their chemical and physical traits. Clearly, more studies are needed to determine whether these induction patterns represent general induction syndromes and under what circumstances induction leads to more complex patterns among traits. For example, physical trait induction is plastic in response to abiotic factors such as nutrient (Gibson,

Bazely & Shore 1993) and water availability (Gonzales *et al.* 2008), and it is likely that these factors differentially alter the induced patterns of physical, chemical and nutritive traits. For the purposes of this meta-analysis, induction in 'control' nutrient and water conditions were included in order to avoid confounding induction with resource availability.

Specificity is now recognized to be a key aspect of plant chemical induction (Agrawal & Heil 2012). Elicitor-driven responses allow plants to vary the induced chemicals quantitatively as well as qualitatively, leading to different amounts and profiles of plant secondary compounds following herbivory (Lehrman *et al.* 2013; Moreira *et al.* 2013). A consequence of the discovery that herbivore elicitors drive plant chemical induction has been a shift in the experimental methodology of these studies to exclude 'herbivory' treatments that involve only mechanical damage to simulate herbivory. Instead, it is common for mechanical damage to be paired with jasmonate applications to activate the defence signalling pathway when real herbivores are unavailable or difficult to manipulate. Here, physical defence induction was also shown to be dependent on the jasmonate signal transduction pathway, as evidenced by weaker induction in response to mechanical damage alone compared to induction by herbivores or jasmonate application. While few studies further examined the mechanism by which jasmonates signal increases in physical defence traits, in *Arabidopsis thaliana*, it has been shown that the induction of greater trichome densities in leaf primordia is the result of long-distance signalling initiated by a jasmonate burst in the mature leaves where damage occurred (Yoshida *et al.* 2009).

Determining that jasmonates play a role in the induction of physical defence traits does not prove that their induction is specific like that of chemical traits, but it does suggest that it is feasible. Only a few studies have examined induction by multiple herbivores in a controlled setting. For example, induction of trichomes in *B. nigra* varied significantly in magnitude and timing in response to three different insect species (Traw & Dawson 2002). Additional studies in the field measuring induction by naturally occurring herbivory undoubtedly include responses to multiple species (Rohner & Ward 1997; Milewski & Madden 2006; Mizumachi *et al.* 2012; Frederickson *et al.* 2013), but without controlling herbivore identities, it is impossible to assess specificity.

Research on the mechanisms underlying induction is particularly needed for woody plants in tropical regions. Much of the early pioneering work, as well as recent work, on the induction of spinescence has been done on African *Acacia* trees (many of which have now been reorganized into different genera), and these studies have shown that trees exposed to browsing by large mammals, including gazelles, elephants and giraffes, tend to have larger and greater densities of spines, thorns and prickles, than plants protected from browsers (Young 1987; Milewski, Young & Madden 1991;

Gowda 1997; Young & Okello 1998; Gadd, Young & Palmer 2001; Young, Stanton & Christian 2003; Scogings & Macanda 2005; Milewski & Madden 2006). In many of these studies, induction is actually inferred from the relaxation of spinescence following herbivore exclusion treatments in which densities or sizes of thorns and spines decrease following herbivore removal (Young & Okello 1998; Zinn, Ward & Kirkman 2007). Although manipulative experiments in these systems are challenging, large mammals are the principal herbivores on these plants, so a better understanding of the causes and consequences of their inducibility would shed light on the evolution of spinescence in the African flora.

Controlled experiments would also shed light on the temporal dynamics of physical trait induction. While some careful analyses have been conducted to identify the timing of induction of secondary chemicals (Fuchs & Bowers 2004), these analyses are largely absent for physical trait induction. In this meta-analysis, 23 of the studies failed to include any information on the amount of time between herbivory and trait analysis, 15 studies reported induction by leaf position (i.e. omitting the amount of time for induction, but detailing the number of leaves produced before trait analysis), and the remaining studies measured induction across a range of times, from a few days to 2 years. Only a few studies have explicitly tested how physical trait induction changes over time, and all report a significant temporal aspect to induction with variable peaks and relaxation rates among species (Young, Stanton & Christian 2003; Boughton, Hoover & Felton 2005; Björkman, Dalin & Ahrné 2008). Because the timing of induction is likely to differ among physical, chemical and nutrient traits, an explicit focus on the simultaneous temporal dynamics of their induction may shed light on whether induction syndromes exist. For example, plants may respond to herbivory by immediately increasing secondary chemistry in tissues already available to herbivores while simultaneously developing new tissues that are better defended physically; this strategy would combine both short- and long-term induction with different defence traits.

This review demonstrates that the induction of physical defence traits is common, of biologically meaningful magnitudes, and shows signs of interesting complexity (e.g. jasmonate effects, and potential contribution to induction syndromes). Additional tests describing the induction of physical defence traits, particularly leaf toughness, are needed to further refine patterns in order to test ideas about ontogenetic patterns of defence, latitudinal gradients in plant–herbivore interactions and the role of invertebrate vs. vertebrate herbivory, among others. Studies detecting null or negative induced responses should be published in order to address the publication bias detected, leading to a more realistic measure of the magnitude of physical trait induction. However, even more pressing is the need to examine the ecological and evolutionary consequences of physical trait induction. Very few studies have examined the effects of physical trait induction on subsequent herbivores (but see Agrawal 1999), which is necessary to demonstrate that these responses are defensive (i.e. that they increase plant fitness compared to plants that lack the induced response). Similarly, few studies have explored whether the induction of physical defence traits is genetically variable or heritable within species. A notable exception is the excellent work done on *Mimulus guttatus* by Holeski and colleagues, in which it was shown that constitutive and induced trichome densities are genetically variable and that maternal effects on trichome induction are strong (Holeski 2007; Holeski, Chase-Alone & Kelly 2010). In contrast, in the Hawaiian prickly poppy, *Argemone glauca*, significant genetic variation within and among populations was detected for constitutive prickle density, but not for the induction of prickles, suggesting that the evolution of inducibility may be constrained in this species (Hoan, Ormond & Barton 2014). Future research focusing explicitly on these ecological and evolutionary aspects of physical trait induction will be the most fruitful for moving this field closer to that of chemical induction, thereby greatly enhancing our understanding of herbivore-induced responses in plants.

Acknowledgements

Data accessibility

All data used in this manuscript are present in the manuscript and its supporting information.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Appendix S1. Complete list of publications included in meta-analysis.

Table S1. Complete meta-data analyzed.