PLANT–MICROBE–INSECT INTERACTIONS

Combined effects of arthropod herbivores and phytopathogens on plant performance

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Summary

1. Many plants are simultaneously attacked by arthropod herbivores and phytopathogens. These may affect each other directly and indirectly, enhancing or reducing the amount of plant resources they each consume. Ultimately, this may reduce or enhance plant performance relative to what should be expected from the added impacts of herbivore and pathogen when they attack alone.

2. Previous studies have suggested synergistic and antagonistic impacts on plant performance from certain combinations of arthropods and pathogens, for example, synergistic impacts from necrotrophic pathogens together with wounding arthropods because of facilitated infection and antagonistic impacts from induction of pathogen resistance by sucking herbivores.

3. We compiled published studies on the impact of plant–herbivore–pathogen interactions on plant performance and used meta-analysis to search for consistent patterns of impacts among plant, herbivore and pathogen characteristics and experimental conditions, and to test the suggested hypotheses on synergistic or antagonistic impacts.

4. None of the hypotheses based on proximate interactions between arthropods and pathogens were supported by our analysis; in contrast, the patterns we found were related to plant traits and experimental conditions.

5. Our results suggest that immediate loss of resources from interactions between arthropod herbivores and pathogens is generally moderated by compensation to an extent where there are no interactive effects on plant performance. However, as interactive impacts also differed among environments and parasite manipulation methods, this suggests that the ability of plants to compensate such losses may depend on environmental conditions and probably also overall infection load.

Key-words: additivity, antagonism, compensation, experimental conditions, herbivore–pathogen interactions, meta-analysis, resource loss, synergism

Introduction

Plants are the main primary producers in terrestrial ecosystems, and most other organisms depend on plants for energy and organic material. It is, therefore, not surprising that herbivores and phytopathogenic micro-organisms are likely to utilize the same host plants. Consequently, interactions between plants, micro-organisms and herbivores are also likely to occur. Many studies and several reviews have reported and discussed how such plant parasites affect each other's transmission, behaviour, performance and repro-

duction, and how this is modified by physiological and molecular responses by the plants (De Nooij, Biere & Linders 1992; Hatcher 1995; Paul, Hatcher & Taylor 2000; Rostas, Simon & Hilker 2003; Stout, Thaler & Thomma 2006; Tack & Dicke 2013). Much fewer studies and reviews have asked whether and how such interactions ultimately affect plant performance and fitness.

The consequences of multiple parasites on plants are likely to be complex. Even if we, for simplified illustration, consider the plant to be an inactive resource, the different kinds of parasites will compete for plant resources, which may involve monopolization and interference, and ultimately determine the amount of plant resources lost. In other cases, one parasite may facilitate access of another,

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for example, by easier entry of pathogens to plant tissue via wounds made by arthropod herbivores (Hatcher 1995).

Obviously, real plants are not inactive resources, but resist attack by physiological and chemical changes in affected and other tissues (Paul, Hatcher & Taylor 2000). Most beneficial for the plant would probably be if it could defend itself against different kinds of parasites by one common response; however, this is not always possible (Leimu & Koricheva 2006). If the plant can only defend itself against one type of parasites, other parasites may benefit from less competition for plant resources and possibly from increased nutrients from defence compounds. If the plant has to operate different defence responses against the parasites, these may be more costly in terms of energy and resources than a single defence, which may affect plant performance.

Parasites have in response to plant defences evolved counter resistances and can sometimes detoxify chemical defence compounds and modify plant defence reactions to become inactive (Jones & Dangl 2006; Thaler, Humphrey & Whiteman 2012). This may allow a second parasite to utilize the plant, even if it would not be able to do so without the first parasite (Hammond & Hardy 1988); thus, the plant will be more affected by combined attack of both than if either was on its own.

Upon attack, plants may reallocate resources to better protected and less attacked plant parts and thereby partition their resources spatially and temporally (e.g. Paul, Hatcher & Taylor 2000). This, and already stored resources, may allow the plant to compensate for losses to parasites by increased branching and growth of less attacked plant parts and by increased levels of photosynthesis (Paul, Hatcher & Taylor 2000; Fornoni 2011); the ability to do this depends on ontogenetic stage, plant architecture, ability to recruit new meristems, resource status etc.

Direct and indirect interactions between arthropod herbivores and phytopathogens may, therefore, affect plant performance in many different ways. The question we focus on here is whether general patterns can still be detected: is the combined impact of arthropod herbivores and phytopathogens on plants more severe than the sum of impacts of either parasite on its own, that is, synergistic; are the individual impacts independent and additive (our null hypothesis); or is the combined parasite impact less severe, that is, antagonistic, for certain arthropod and pathogen functional groups and for certain plant traits and environmental conditions?

Knowledge about such interactive effects is central both for basic and applied research. To understand how plant defence mechanisms evolve and function, we need to understand the selective pressures imposed by parasites on plants and whether they are determined by tight associations with a single or few species or by associations with several different parasites. In plant production, if the combined impact of herbivores and pathogens is more, or less, severe than additive, it may be useful to monitor for the *combined* occurrence of

parasites and treat both organisms to minimize their combined effect (e.g. Braun *et al.* 2009).

Several hypotheses and patterns have been suggested for the combined parasite impact on plants. Synergistic impacts have been suggested for necrotrophic stalk and root rots (Hatcher 1995) and facultative parasitic pathogens (Turner *et al.* 2010) that benefit from insect damage to infect and colonize plants. Synergistic impacts may also arise when biotrophic pathogens or sucking arthropods interact with chewing and boring arthropods or necrotrophic pathogens, as these two groups induce different hormone-based defence signals [salicylic acid (SA) and jasmonic acid (JA), respectively] that are (sometimes) antagonistic to each other (Stout, Thaler & Thomma 2006; Thaler, Humphrey & Whiteman 2012). Thus, if defence is triggered against one type of parasite, defence against the other is repressed; however, it is still not known how consistent such antagonism is in most plant systems (Thaler, Humphrey & Whiteman 2012).

Antagonistic impacts have been suggested to arise from inhibition of insect development by biotrophic fungi (Hatcher 1995) and from induction of resistance by sucking herbivores, which is also effective against pathogens (Stout, Thaler & Thomma 2006). This could be caused by induction of the SA-signalling system by one parasite, which incidentally primes SA-dependent defence against the other parasite, too.

Finally, we may expect the combined impact of arthropods and pathogens to be more severe and synergistic if plants are more severely stressed by parasites, that is, a positive correlation between the overall impacts of parasites and their interactive effect.

To analyse whether there are consistent patterns in the combined impacts of arthropods and pathogens on plant performance and fitness and whether previously suggested patterns and hypotheses are supported by presently available data, we compiled as many published studies on interactive impacts as possible. Summary statistics from these studies were analysed with meta-analysis to test whether combined impacts differ between classes of plant traits measured, plant life histories, wild and domestic plants, generalist and specialist herbivores, arthropod and pathogen feeding guilds, different classes of pathogens and experimental methods and environments. We further tested whether combined impacts were indeed synergistic and more severe for combinations of (i) wounding (chewing and boring) herbivores *and* necrotrophic pathogens; (ii) sucking arthropods or biotrophic pathogens *and* wounding arthropods or necrotrophic pathogens; and whether combined impacts were antagonistic and less severe for (iii) biotrophic pathogens; combinations of and (iv) sucking herbivores *and* necrotrophic pathogens, or wounding herbivores *and* biotrophic pathogens. Finally, we discuss the implications of our findings for plant performance under multi-enemy conditions.

Materials and methods

DATA COLLECTION

To be accepted for the meta-analysis, studies had to (i) include all of the four treatment combinations: no parasites (control), arthropod alone, pathogen alone, and both parasites; in some studies, these treatments were made by spraying with pesticides, in others by adding parasites; as pathogens, we included fungi, oomycetes, bacteria and viruses; and (ii) measure a plant trait related to performance and fitness, with estimates of average and preferentially some measure of variation.

Studies were identified from (i) keyword searches in Web of Science (ISI) electronic bibliographic database, using mainly combinations of (herbivore* or arthropod* or insect*), (pathogen* or disease*), and (pest* and interact*), in addition to searches with weaker search terms; (ii) reference lists in articles found by (i) and in previous reviews of this field; and (iii) citations back to the articles found by (i) and (ii), using Web of Science (ISI). Reported averages, standard errors (or equivalent estimates) and sample sizes were extracted from text and tables, if available, or else extracted from figures using the software WebPlotDigitizer v2.4 (http://arohatgi.info/WebPlotDigitizer/).

In most studies, more than one plant trait was measured in the same experiment (see Table S1), and among those, we selected the traits with highest expected correlation with plant fitness (e.g. total biomass, yield, etc.) in addition to traits with weaker correlation with fitness. If the same experimental set-up was used for two otherwise independent experiments, for example, in two different years, results from both were included; if an experiment lasted for several years, we estimated an average over years, or if the interaction differed qualitatively over time, we included more than one estimate. If the study included different severities of parasite exposure, we only included the most severe in addition to the control.

Descriptive factors

For each study and trait (*record*), we noted the following plant characteristics: taxonomic family, species, the class of trait measured (size or number of plant parts, total biomass including above- or below-ground, survival rate/population growth or reproduction), plant life history (annual, perennial), and whether the plant is wild or domestic; arthropod characteristics: order, family, species, specialization (generalist, specialist), feeding guild (chewing, sucking, galling, boring, mining); plant pathogen characteristics: type of pathogen (fungus, oomycete, bacteria, virus), taxon, species, life form (necrotroph, biotroph, hemibiotroph); experimental characteristics: environment (climate chamber, greenhouse, field, natural unmanaged conditions), method of parasite control (added experimentally, removed by pesticide spraying, added and sprayed), measurement unit (populations, individuals) and sequence of parasite (arthropod or pathogen first). In some analyses, factor levels were pooled to increase sample sizes; for the last factor (sequence), we found so few results that it was not possible to include these in a full analysis.

STATISTICAL ANALYSIS

For each record, we estimated the impact of arthropod herbivore, pathogen and their interaction on plant performance, using log response ratios as described in Morris *et al.* (2007). Thus, L_a, L_m and L_{am} describe the individual effects of arthropods and microbial pathogens and their interaction, respectively. A negative value of L_a or L_m signifies that plant performance is lower upon attack by the arthropod or microbe (a or m) than without the parasite; the actual decrease in performance is found through back-trans-

formation as $1 - e^{L_a}$ and $1 - e^{L_m}$, respectively. The value of L_{am} was coded to be negative if the combined impact of the arthropod and the pathogen was more severe than the sum of impacts of either parasite in isolation (synergistic), zero if the combined impact was additive and positive if the combined impact was less severe than additive (antagonistic).

To assess whether the factors to be tested were confounded, counts of records within each factor level were cross-tabulated for each pair of factors and their distributions evaluated by chi-square test. Very confounded factors were combined and tested additionally as one (see Results).

Single studies often reported results for more than one plant trait. Previous meta-analyses have accommodated this by either retaining only a single effect measurement from each study, performing separate meta-analyses on subsets of data based on different effect measures or resampling among multiple results from single studies, including only a single measurement in each resample (e.g. Morris *et al.* 2007). Here, to retain as large a sample size as possible and to compare interaction impacts among the different classes of plant traits (size or number of plant parts, whole plant biomass, survival or population growth, reproduction), all effect measurements were retained and included in the same analysis. Still, the trait class was included as an additional factor in parallel meta-analyses to account for the expected effect of this factor and minimize the possible effects of within-study interdependence. As has been found in other studies (Turner *et al.* 2010), interactions were consistently significant for this factor. Our results were relatively robust whether accounting for classes of plant traits or not in the analyses.

From a subset of the retrieved records (32%), measures of variation were either not reported or irretrievable. To keep all records in the weighted meta-analyses, the missing variances were imputed by drawing randomly with replacement from the collection of reported variances within each factor level. Each model analysis was repeated 1000 times, each based on resampled variances, and values for each test statistic derived as the average across resamplings. This so-called multiple imputation is the only available method that accounts for the uncertainty from the missing standard deviations and was recommended over a wide range of other imputation methods in the methodological review by Wiebe *et al.* (2006) and shown to provide accurate results by Furukawa *et al.* (2006). This imputation approach was computationally incompatible with a sensitivity analysis of meta-estimates such as bootstrapping (e.g. a leave-one-out procedure). On the other hand, our approach provided percentages of runs that supported the test or not, and in our analyses, these percentages reflected *P*-values (average across runs) well, supporting that results were robust.

Overall meta-estimates of arthropod and pathogen impacts were derived from random effects models, considering the impacts calculated from each record as i in $1, ..., m$ independent and approximately unbiased samples, θ_i, from an asymptotically normally distributed population of effects described as $\hat{\theta}_i = \hat{\theta} + u_i + \varepsilon_i$, where $\varepsilon \sim N(0, s_i^2)$ and s_i^2 are the assumed known variances of $\hat{\theta}_i$, $\mathrm{Var}(\theta_i) = \mathrm{Var}(C_i)$, as devised above. Then, $\hat{\theta}$ denotes the central meta-effect, $u_i \sim N(0, \tau^2)$, where τ^2 is the random variation between experimental effects, known as residual heterogeneity. To estimate $\hat{\theta}$, the original effect estimates were first weighted with the inverse of their respective variances, divided by the sum of all inverse variances (Hedges & Olkin 1985).

The potential importance of the extracted characteristics of plants, parasites and experimental conditions was tested by including them as explanatory factors in the meta-analysis model, leading to a mixed effects meta-regression model of the form $\hat{\theta}_i = \beta_0 + \beta_1 X_1 + \cdots + \beta_q X_q + u_i + \varepsilon_i$, where β_0 is the overall level of meta-estimates, $\beta_1, ..., \beta_q$ are meta-regression coefficients, $X_1, ..., X_q$ are vectors of other experimental factors or observations for each of j in q covariates (including dummy variables to encode the effects of grouping factors), and u_i and ε_i are defined

previously. The significance of relationships between covariates and effect sizes was tested against the null hypothesis $\beta_j = 0$. Models with and without a given factor were compared by Wald tests to evaluate whether inclusion of that factor improves model explanatory power. A random effects (*REML*) meta-analysis framework was used.

To ensure that meta-estimates were independent of the phylogenetic relatedness of the plants, a phylogenetic meta-analysis was further undertaken, as formulated by Adams (2008) and Lajeunesse (2009). Here, information on the proportion of shared to average branch lengths of the analysed plant families was obtained from the Angiosperm super tree (Davies *et al*. 2004), using the online tool Phylomatic (www.phylodiversity.net/phylomatic). Results were generally consistent whether correcting for phylogenetic relatedness or not; hence, in the following, we present the results from the phylogenetic meta-analyses. All analyses were coded and calculated in the R environment (version 2.14.0, R Development Core Team 2012) with origin in the implementation of the *metaphor* package (Viechtbauer 2010).

Results

RETRIEVED STUDIES

A total of 35 studies were found, from which 132 records were extracted for the meta-analysis (see Table S1 in Supporting Information). The studies were published in 27 different journals, with the highest numbers in Biological Control (5), Journal of Economic Entomology (5) and Phytopathology (4). They were published between 1969 and 2011, with 19 studies published after 1995 and 9 after 2006, the publication years of the reviews by Hatcher (1995) and Stout, Thaler & Thomma (2006), respectively.

OVERALL IMPACTS ON PLANTS

The combined impacts of herbivore–pathogen interactions on plant performance were on average close to zero ($L_{am} = -0.03$, with a median of 0.00), indicating that impacts were additive in the majority of studies; see Fig. 1a-e. In contrast, the impacts of only arthropods or only pathogens were overall negative and significantly different from zero ($L_a = -0.26$ and $L_m = -0.25$, corresponding to $1\text{-}exp(L_a) = 23\%$ and $1\text{-}exp(L_m) = 22\%$ reduction in plant performance, respectively; see Fig. 1a and Table S2). Residual heterogeneity (variation between rather than within studies) accounted for more than 98% of the total variation for combined as well as separate impacts of herbivores and pathogens, suggesting that these were highly dependent on numerous characteristics of the plants, arthropods and microbes and on the experimental conditions in each experiment.

EFFECTS OF PLANT CHARACTERISTICS

The combined impact of arthropods and pathogens differed significantly among different classes of plant traits measured, that is, size or number of plant parts, total biomass including above- or below-ground, survival/population growth or reproduction (Fig. 1a; Table S1), with a more severe and negative (synergistic) impact on size and number of plant parts than on whole plants and population level. The combined impact on plant parts was 19% more severe than if impacts were additive. In contrast, the combined impact on biomass was slightly positive (antagonistic; Fig. 1a). Model fit was significantly improved ($P = 0.01$) by including class of plant traits as an explanatory factor. In contrast to this, the impacts of arthropods or pathogens, only, did not differ among classes of plant traits.

The combined impact of parasites was significantly less severe for annual plants than for perennial, with L_{am} being significantly different from zero (Fig. 1b); the combined impact on annuals was 17% less severe than if additive. The model fit was significantly improved by including the interactive effect ($P = 0.02$). Annual and perennial plants were not affected differently by arthropods or by pathogens when tested on their own.

There was a tendency that the combined impact of parasites was more severe for wild than for domestic plants; however, neither was significantly different from zero. Wild and domestic plants were not affected differently by arthropods or by pathogens, either.

Wild plants in the data set are predominantly perennial (90%) and have been measured more frequently for size of plant parts than domestic plants. To analyse this confounding in more detail, we combined plant life history and domestication status into a four-level composite factor (combined factor 1 in Table S2). Perennial wild plants were more severely (synergistically) affected by combined parasites than annual domestic plants; however, this result disappeared if plant trait class was included as a covariate.

EFFECTS OF ARTHROPOD AND PATHOGEN CHARACTERISTICS

The combined impacts of parasites did not differ between specialist and generalist herbivores or among herbivore feeding guilds (Table S2). In contrast, specialist herbivores alone had a significantly more negative impact on plants than generalists (29% and 14% decrease in plant performance, respectively), and chewing herbivores alone had a more negative impact than boring arthropods (29% and 10% decrease).

We found only one study with oomycetes, one with viruses and two with bacteria; these were, therefore, merged into one class ('others') previous to analysis. There was no significant difference between fungi and others for neither L_m nor L_{am}. Neither did we find a difference in combined impacts (L_{am}) among biotrophic, necrotrophic and hemibiotrophic pathogens. In contrast, biotrophic and necrotrophic pathogens impacted plant performance more severely than hemibiotrophic pathogens (25%, 27%, and 9% decrease, respectively) when they occurred without arthropods.

To explore whether combinations of arthropod feeding guilds and pathogen life forms differed in interactive effects
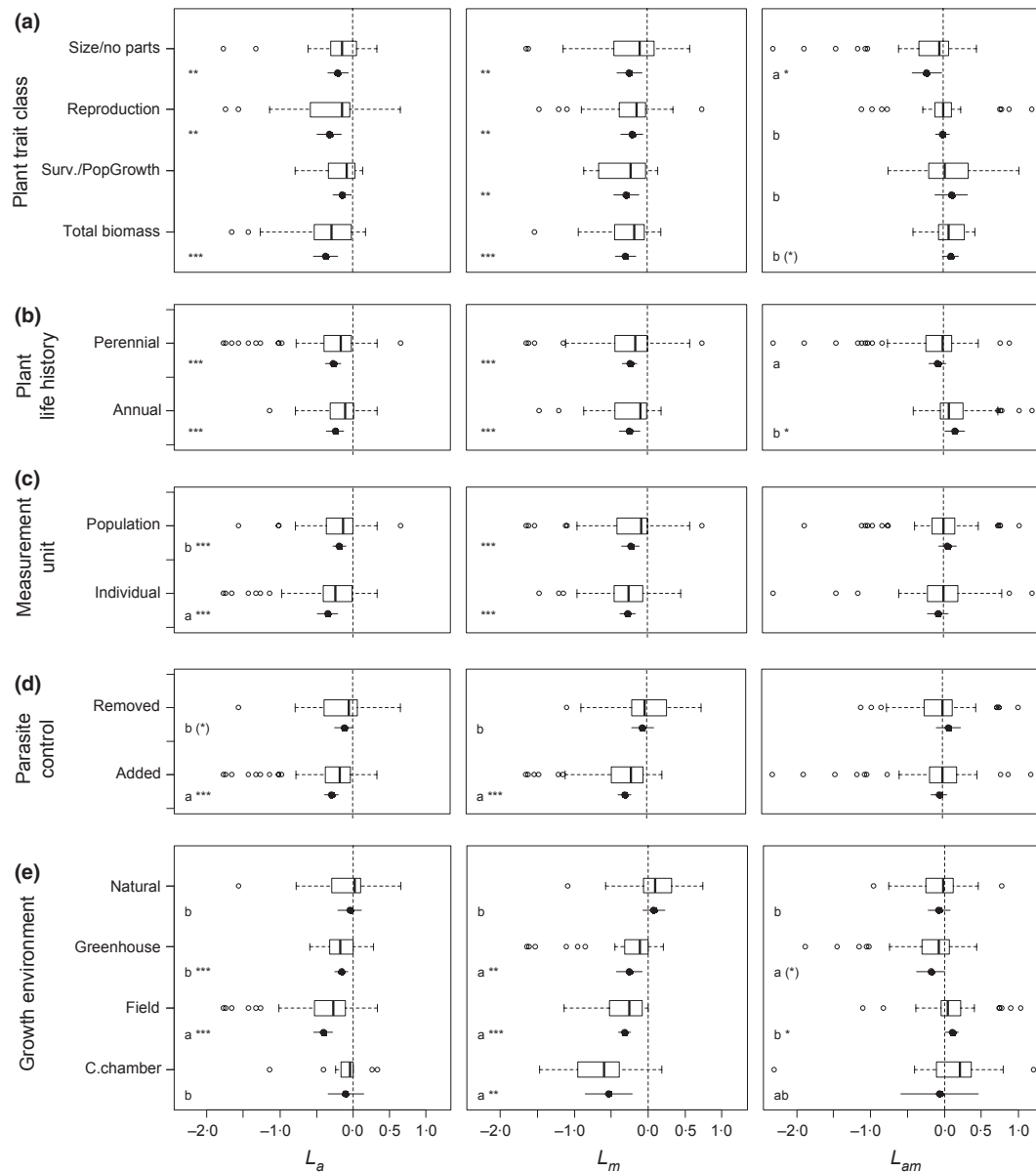
Fig. 1. Impacts of arthropods (L_a), pathogens (L_m) and their combination (L_{am}) on plant performance, shown for factors with significant tests of interaction impacts (more details in Table S2). Distributions of impact values are shown as box plots for each factor level, with means (middle vertical line), quartiles (box ends), range of moderate data points (within 4 times the interquartile range; whiskers) and extreme data points (open circles). Meta-estimates (filled circles) are shown with confidence intervals (horizontal lines), each a mean of 1000 imputations of missing variances (see text). Significant meta-estimates are indicated by asterisks ((*):$P < 0.1$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$), and factor levels that differ significantly are indicated by different letters.

on plant performance, we coded two composite factors that combine biotrophic, necrotrophic and hemibiotrophic pathogens (i) with wounding (chewing and boring) and non-wounding (galling and sucking) arthropods (combined factor 2 in Table S2); and (ii) with specialist and generalist arthropods (combined factor 3).

Necrotrophic pathogens together with non-wounding arthropods had a somewhat more severe and synergistic impact on plant performance than necrotroph pathogens together with wounding arthropods (12% more severe and 17% less severe than additive, respectively; this difference

was, however, only significant at the $P < 0.1$ level); other combinations were intermediate to these. Necrotrophic pathogens together with specialist arthropods had a significantly more severe combined impact on plants than biotrophs together with specialists (11% more severe and 11% less severe than additive, respectively). These results were found for both analyses with and without inclusion of plant trait class as a covariate; however, model fit was not significantly improved by inclusion of either of the two composite factors (combined factor 2: $P < 0.1$; combined factor 3: $P < 0.05$).

Plants measured as individuals were slightly more affected by combinations of parasites than plants measured as populations (Fig. 1c; different at the $P < 0.1$ level); neither differed significantly from being additive, though.

Two different experimental methods have been used to compare the presence with absence of parasites: experimentally added parasites compared with none, and exposure to natural infection compared with controls where parasites have been removed by pesticide spraying (very few studies have combined these approaches). The combined impact of parasites was slightly more severe in experiments with addition than in experiments with removal (Fig. 1d; $P < 0.1$ for test of pairwise significances). Single impacts of herbivores or pathogens alone were more negative when added than when removed by pesticides for the control; however, this effect was only marginally significant for herbivores.

The combined impact of herbivores and pathogens was significantly more severe and synergistic for plants in greenhouses than in fields and under natural conditions (Fig. 1e; 17% more severe, and 11% less severe (fields) than additive, respectively). However, plants in fields and natural conditions are more often treated by spraying, and we, therefore, combined parasite control method and environment into a composite factor (combined factor 4 in Table S2). The combined parasite impact was significantly more severe for plants indoors (greenhouse and climate chamber) with added parasites than for field plants with added parasites and outdoor plants with spraying (15% more severe, 3% and 14% less severe than additive, respectively). Experimental methods and environments also differ between domestic and wild plants, and we, therefore, analysed a composite factor combining these three factors (combined factor 5). As above, indoor plants with added parasites were more seriously affected by parasites in combination than outdoor plants, except domestic plants in fields with spraying that did not differ from either groups. Inclusion of the two composite factors significantly improved model fit ($P = 0.002$ and 0.007, respectively).

The effects of arthropods alone, or pathogens alone, also differed significantly among experimental methods and environment. Plants in field experiments with added arthropods were more severely affected than indoor plants with added arthropods and outdoor plants treated by insecticide spraying (35%, 14% and 12% decrease relative to control, respectively; see Table S2). Plants in fields and indoors with added pathogens were more severely affected than outdoor plants treated by fungicide spraying (27% and 6% decrease, respectively).

The estimates of L_a and L_m were significantly positively correlated in the full data set (Spearman rank correlation: $r = 0.40$, $P < 0.001$), whereas correlations between each of these and L_{am} were negative ($r = -0.312$ and -0.256, respectively; $P < 0.01$), as was the correlation between ($L_a + L_m$) and L_{am} ($r = -0.31$; $P < 0.001$). When analysed within each of the four plant trait classes, all correlations between L_a and L_m were positive, all correlations with L_{am} were negative, and approximately half of the correlation coefficients were significant.

Discussion

Our analysis clearly shows that the combined impact on plant performance by arthropod herbivores and phytopathogenic micro-organisms is on average additive, that is, close to the sum of impacts of either parasite when it attacks the plant on its own. This has also been found in other reviews on plant impacts of multiple enemies (Hatcher 1995; Hatcher & Paul 2001; Morris *et al.* 2007; Rayamajhi *et al.* 2010). However, there was a substantial variation, with some studies finding significantly more severe (synergistic) impacts by herbivores and pathogens in combination, and other studies significantly less severe (antagonistic) impacts (see Fig. 1a-e). Our analysis found that part of this variation was explained by which plant traits were measured under what experimental conditions, but only weakly by traits related to the herbivores or pathogens. This is surprising, as most of the hypotheses that have been suggested for synergistic and antagonistic impacts on plants relate to differences in arthropod and pathogen feeding biology (Hatcher 1995; Rostas, Simon & Hilker 2003; Stout, Thaler & Thomma 2006; Turner *et al.* 2010).

Hatcher (1995) suggested that impacts on plant performance may be synergistic for necrotrophic stalk and root rot that benefit from entry through wounds created by co-occurring insects. However, in our analysis, necrotrophic pathogens attacking together with wounding (boring and chewing) arthropods had a somewhat less severe impact on plant performance than necrotrophs together with non-wounding arthropods. Turner *et al.* (2010) suggested that facultative parasitic pathogens in combination with wounding insects may impact plants synergistically. In our analysis, the impact of hemibiotrophic pathogens in combination with wounding arthropods did not differ from other combinations of pathogens and arthropods.

Antagonistic impacts on plant performance were suggested by Hatcher (1995) for biotrophic fungi that inhibit insect development. However, this was supported neither by the review of Rostas, Simon & Hilker (2003), nor by our results. Stout, Thaler & Thomma (2006) suggested that sucking herbivores appear to induce resistance to pathogens in many plants, which could possibly lead to less negative impacts on plant performance. However, the combined impact of sucking arthropods and pathogens in our analysis did not differ significantly from the combined impact of other arthropod feeding guilds.

Necrotrophic pathogens and wounding arthropods both induce plant defences via the JA signalling pathway (Kunkel & Brooks 2002; Glazebrook 2005; Thaler, Humphrey & Whiteman 2012). One such parasite may, therefore, possibly also prime defence against another, which could lead to less severe, antagonistic, impacts on plants. Indeed, the least severe combined impact on plant performance was found for necrotroph pathogens in combination with wounding arthropods (17% less severe than additive, in contrast to other combinations of pathogen life forms and arthropod feeding guilds that had additive or synergistic interactive impacts). However, we should be cautious with concluding on this result, as (i) model fit was not significantly improved by including the combinations of arthropod feeding guilds and pathogen life forms in the analysis; and (ii) the dichotomy and antagonism JA and SA signalling and defences are oversimplified (Thaler, Agrawal & Halitschke 2010; Thaler, Humphrey & Whiteman 2012). If the hypothesis was correct, we should perhaps expect to see the same for biotroph pathogens in combination with sucking arthropods, as they both trigger plant defence signalling via the SA pathway (Stout, Thaler & Thomma 2006). However, the impact of biotrophs in combination with sucking herbivores was not different from that of other combinations of life forms and feeding guilds.

OBSERVED PATTERNS OF COMBINED IMPACTS

The combined impact of arthropods and pathogens was synergistic for size and number of plant parts, such as number of shoots, tubers, plant height, etc., in contrast to the impact on population growth and reproduction that was additive and on whole plant biomass that was antagonistic. These results were supported by a significantly better model fit when plant trait class (size or number of plant parts, total biomass including above- or belowground, survival rate/population growth or reproduction) was included as a factor, and by a significant effect of plant trait class as a factor. This suggests that whole plant biomass and reproduction are in general less severely affected by direct and indirect interactions among arthropods and pathogens than plant parts, probably due to compensation by reallocation from other plant modules less affected by parasites, and by changes in photosynthesis and metabolism (Nunez-Farfan, Fornoni & Luis Valverde 2007; Fornoni 2011); a similar conclusion was drawn by Stout, Thaler & Thomma (2006) for a subset of the studies included here. At the level of survival/population growth, compensation may additionally occur via complementation of weak plants by neighbours.

The combined impact of arthropods and pathogens also differed among different experimental environments and methods. Plants growing indoor were more severely affected by synergistic interactions than outdoor plants, as also found by Morris *et al.* (2007) for a broader group of plant enemies. When combining growth environment and parasite control method in the analysis, indoor plants with experimentally added parasites were more negatively affected by dual parasites than outdoor plants. This was unexpected, as outdoor plants are more exposed to sub-optimal environmental conditions and stress, which may minimize resources available for compensatory responses (Sciegienka, Keren & Menalled 2011). A possible explanation could be that the effective parasite load on plants in manipulated indoor experiments is higher than in less controlled outdoor experiments, where parasites are also exposed to naturally occurring predators and parasitoids. This was, however, not supported by our analysis, where plants in field experiments suffered more severely from arthropod herbivores (lower L_a) than plants in indoor experiments; no difference was found for pathogens. Other explanations for the more synergistic impacts in indoor experiments could be that these are often shorter than outdoor experiments, leaving less time for plants to build up a full defence response and to compensate losses (Rostas, Simon & Hilker 2003), that sprayed controls in outdoor experiments may not really be without parasite damage, and that arthropods and pathogens in outdoor experiments with larger plants possibly partition their use of plant parts more (Dickson & Mitchell 2010), which could reduce synergistic impacts. None of these explanations fit with the lower estimated L_a in outdoor experiments, however.

COMPENSATION AND RESOURCES

Arthropod herbivores and phytopathogens clearly affect each other in positive and negative ways when utilizing the same host tissues, as has been shown in many studies (see reviews by Hatcher 1995; Rostas, Simon & Hilker 2003; Stout, Thaler & Thomma 2006; Tack & Dicke 2013). But even at this local level, it has been hard to find consistent patterns across different arthropod and pathogen characteristics (see e.g. Stout, Thaler & Thomma 2006; Tack & Dicke 2013). Our results indicate that it may be even more difficult to find patterns in the impacts on plants from such interactions, due to the ability of plants to compensate for loss of resources to parasites. Thus, Fournier *et al.* (2006) suggested that many observed additive interaction impacts on plants may be due to compensatory modification of an originally synergistic interaction impact of parasites.

This implies that the impact of plant–arthropod–pathogen interactions should be considered within a broader frame of plant compensation and its dependency on plant and environmental resources (Nunez-Farfan, Fornoni & Luis Valverde 2007). As illustrated in Fig. 2, plant resource losses may be nonlinearly related to parasite load even for single parasites; and when two different kinds of parasites are present, there are additional possibilities for synergistic, additive and antagonistic interactions among them (Fournier *et al.* 2006). The ability of the plant to compensate additional losses may depend on the severity of already incurred losses to parasites (indicated by dashed
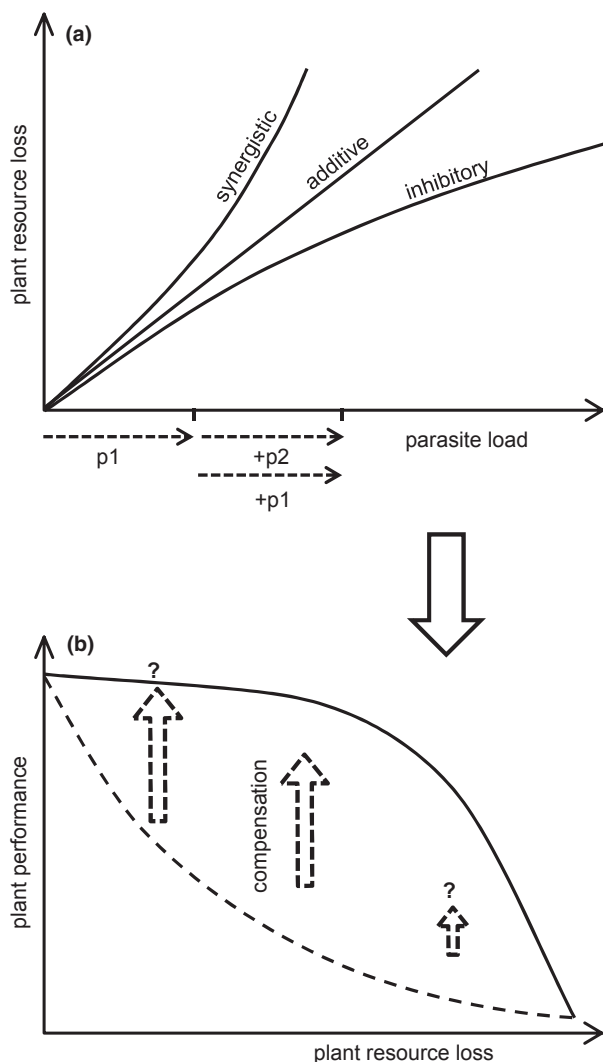
Fig. 2. Conceptual scenario for (a) losses of plant resources to parasites and (b) resulting effects on plant performance. Non-additive losses can result from combinations of different types of parasites (p1 + p2) or from increased load of the same parasite (p1 + p1). The impact on plant performance (exemplified by solid line in panel (b) is determined both by resource losses to parasites (exemplified by the broken line) and by the plants ability to compensate for that resource loss (indicated by arrows). Compensation may thus depend on already incurred resource losses to parasites and the general resource level of the plant; however, this relationship is uncertain and disputed, as indicated by question marks and different size of arrows in (b).

arrows and question marks in Fig. 2); an additional loss of resources may be easily compensated by plants with a low overall parasite load, whereas an additional loss may be difficult or impossible to compensate for plants that have already lost a large share of resources (Fournier *et al.* 2006; Fornoni 2011). In the extreme case, severely stressed plants may die from even a small extra loss. If this is true, we might expect an accelerating relationship between total parasite load and the interaction impact, that is, a positive correlation between the additive impact of herbivores and pathogens ($L_a + L_m$) and the interaction impact, L_{am}.

However, we found a weak but significant negative correlation between single parasite impacts and their interaction impact, contrary to the hypothesis above. This may be due to few studies with sufficiently severe impacts of parasites. Alternatively, the hypothesis is too simple or merely wrong, as has been proposed for the analogous hypothesis that plant compensation is more efficient in low-stress and resource-rich environments (Wise & Abrahamson 2005, 2007). Limited resources are allocated not only to compensation but also to increased defence (Nunez-Farfan, Fornoni & Luis Valverde 2007), and severely affected plants may be less attractive for additional parasites because of limited surface to infect, exhausted storage resources and nutrients, increased plant defences, etc., which could lead to antagonistic interactions among the parasites and thereby a relatively decreased loss of plant resources.

DATA DEPENDENCY AND CONCLUSIONS

The purpose of our study was to analyse whether there are consistent patterns across arthropod herbivores and phytopathogens in how they affect plant performance when they infest the same plants. We did not detect any convincing patterns, while our results reject a number of previously suggested hypotheses on interactive impacts of certain combinations of herbivores and pathogens. Instead, our results suggest that the impact of multiple parasites on plant performance is determined more by the ability of plants to compensate resource losses, which may depend on experimental and environmental conditions of the plant and on life span of the plant. Thus, interactions between different arthropods and pathogens may easily be modified and ultimately become biologically insignificant at the whole plant or population level.

These results and conclusions are, however, based on a relatively small data set. Even with intensive searching and relatively relaxed requirements for data structure (i.e. including both measurements at the individual and population level and studies with no variance estimates, see Methods), we only found 35 studies that had investigated both the individual and interactive impacts of herbivores and pathogens on plant performance (providing 132 records in total). As a consequence, the species covered in these studies represent a very small subset of the huge diversity of phylogenetic and functional groups of herbivores, pathogens and plants.

Only 16 taxonomic plant families are represented in our study, with several economically and scientifically important families missing, such as Brassicaceae and Apiaceae. Associated with these are many specialized herbivores and pathogens, which are, therefore, not represented either. In contrast, 86 of the 132 records come from just four plant families, Asteraceae, Fabaceae, Poaceae and Polygonaceae. Among the arthropods, important herbivore taxa are missing (e.g. Hymenopteran herbivores, several families of Lepidoptera, such as Geometridae and Noctuidae) or clearly

under-represented (e.g. Thripidae), others are better represented (e.g. Curculionidae with 34 of 132 records). Some arthropod functional groups are clearly under-represented, with only four records on mining arthropods and three on galling, in contrast to 55 records on chewing and 23 on sucking arthropods. Likewise, studies with phytopathogenic vira, bacteria and oomycetes were seriously missing (we only found 1, 5 and 2 studies with these, respectively) and with them highly significant pathogen taxa like *Peronospora* and *Albugo*. Whereas the effects of oomycetes may to some extent be similar to those of fungi with the same life form (biotroph, necrotroph), the same is certainly not the case for vira and bacteria, which have a very different biology.

To avoid conclusions based on too few data points, we combined under-represented groups into larger groups, for example, short-lived plants with perennials (in contrast to annual plants). Even though we have some biological justifications for these combinations, future analyses of broader data sets may, however, find that these groupings have hidden real biological differences. Further, with the limited number of records, it has not been possible to test for higher order interactions between combinations of taxa and types, interactions that may turn out to be biologically meaningful, given more data.

Many more biological complexities than those analysed here may affect the outcome of arthropod–pathogen interactions and their impacts on plants. For example, impacts may differ depending on whether arthropods and pathogens utilize the same or different parts of the plant, especially if one attacks above-ground and another below-ground. The majority of available studies only included above-ground parts of the plant, even though below-ground allocation, herbivory and disease potentially could have influenced results. Interactive impacts may also depend on temporal differences in the order and time of arrival of the parasites; this was planned to be included in our analyses, but was not justified with the few studies available.

If we are to understand the interactive impacts of arthropods and phytopathogens on plant performance better, we need more studies with a broader representation of taxonomic and functional groups. Probably as important are studies that employ several levels of exposure to parasites, alone and in combination, in conducive and stressful environments, and which measure impacts on both proximate parts of the plants and at whole plant levels.

However, even with a better coverage and more detailed studies, we may still be left with a conceptual problem: that of finding globally predictive patterns of parasite–plant interactions in a system that is highly complex due to co-evolved relationships, food web structure, plant allocation patterns, resources, climate, etc. This may simply not be realistic. Perhaps, it is realistic only to find predictable patterns embedded in specific systematic and environmental contexts. Thus, we may find repeatable patterns of combined impacts of arthropod herbivores and pathogens

in, for example, a cabbage field, which can be extrapolated to predict yield losses also in a neighbouring cabbage field, but even this understanding is perhaps unlikely to be transferable to a wild Brassica population. However, we still need those complex studies to learn how herbivores and pathogens interact at the immediate level and how this translates into plant performance and fitness, global patterns or not.

Acknowledgements

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Supporting Information

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

Table S1. Studies included in the meta-analysis, with number of arthropod and pathogen combinations, duration of experiment, and number of data points extracted.

Table S2. Results from meta-analysis of impacts of arthropod herbivores and phytopathogens, alone and in combination, on plant performance. Results are given for each of the factors tested: N: number of records; p(model): test of whether model fit is improved by inclusion of the given factor; log- response ratios of arthropod effect (L_a), pathogen effect (L_m), and interaction effect (L_{am}); for each of L_a, L_m, and L_{am}: p($L \lessgtr 0$): probability of the regression estimate for the given factor level; exp(L): back-transformed estimate of L; level difference: pairwise significant differences among factor levels indicated by differing letters; significance level: the order of magnitude of the largest P value for pairwise level differences; Model w pl_stage: test statistics for model including plant stage as a co-factor, only shown for significant effects.