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Opinion

Epichloë Fungal Endophytes and Plant Defenses: Not Just Alkaloids

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Although the role of fungal alkaloids in protecting grasses associated with *Epichloë* fungal endophytes has been extensively documented, the effects of the symbiont on the host plant's immune responses have received little attention. We propose that, in addition to producing protective alkaloids, endophytes enhance plant immunity against chewing insects by promoting endogenous defense responses mediated by the jasmonic acid (JA) pathway. We advance a model that integrates this dual effect of endophytes on plant defenses and test its predictions by means of a standard meta-analysis. This analysis supports a role of *Epichloë* endophytes in boosting JA-mediated plant defenses. We discuss the ecological scenarios where this effect of endophytes on plant defenses would be most beneficial for increasing plant fitness.

Defenses in Symbiotic Plants

Herbivory is one of the most important threats for plants, impacting net primary productivity in natural ecosystems and causing important economic losses in agriculture [1]. As a result of coevolution with a diverse group of herbivores, plants have acquired a variety of mechanisms and strategies to defend themselves [2–4]. At the same time, plants establish interactions with other organisms that have beneficial or detrimental effects on fitness, such as mutualistic fungi and bacteria, parasites, and pathogens. Although the mechanisms of plant defenses have been very well documented [2–5], it is less clear how these defenses work when plants are associated with other organisms, such as beneficial microorganisms.

The association of plants with microorganisms modulates the relationships between plants and herbivores [6–9]. It has been pointed out that symbioses with mycorrhizal fungi and certain rhizobacteria confer additional defensive mechanisms as a consequence of the modulation of the immune system of the host plants [10–14]. Specifically, these symbionts generally modulate plant hormonal pathways involved in defenses to successfully colonize the host plant and to establish the symbiosis [14,15].

The effects of vertically transmitted *Epichloë* fungal endophytes of grasses on plant defenses are not well understood. The endophytes are an active source of bioactive alkaloids that protect their host plants against herbivores [16,17]. Therefore, the grass–endophyte symbiosis is considered a case of 'defensive mutualism' [18] (Box 1). However, it is striking how little is known about the effects of *Epichloë* endophytes on the immune responses of their host plants. Only 3% of the 109 published articles dealing with the effects of fungal endophytes on plant–herbivore interactions discuss topics related to the hormonal pathways involved in plant defense (Scopus database; December 2016). Here we propose that a better understanding

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To achieve a well-established symbiosis, plants and beneficial microorganisms engage in a complex molecular dialog that affects several plant functions, including resource allocation and the expression of plant defenses.

Beneficial microorganisms such as rhizobia and mycorrhizal fungi can enhance the defenses of their host plants, thereby increasing plant resistance to a broad spectrum of attackers.

Recent studies reveal that *Epichloë* fungal endophytes can also induce changes in molecular components associated with defense signaling in the host plant. These changes could have impacts on the plant's resistance to attackers.

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Box 1. Endophyte Alkaloids

The positive effect of *Epichloë* endophytes on plant defense has been traditionally attributed to the fungal alkaloids. Alkaloids are nitrogen-rich compounds and four classes have been well characterized in endophytes: ergot alkaloids (i.e., ergopeptine and ergovaline), indole diterpenes (i.e., lolitrem B and epoxy-janthitrem), pyrrolizidines (i.e., lolines), and peramine [8,16,68–71]. All enzymes for their synthesis are encoded in the fungal genome and all of the biosynthetic routes have been mostly elucidated [16,71,72]. Alkaloid profiles depend on the endophyte species and strain and the amount of alkaloids is associated with the plant's phenological stage, plant tissue, and environmental conditions [8,73,74]. In addition, the effectiveness of alkaloid defenses against a given attacker depends on the concentration and the chemical type of alkaloid produced by the endophyte [8,16,75,76].

of the different ecological outcomes of grass–endophyte symbioses could be achieved by simultaneously considering the alkaloid-based defense mechanism and the potential effects of these endophyte symbionts on the immune responses of their hosts.

With a focus on the symbiosis between *Epichloë* fungal endophytes and grasses, we advance a model that takes into account fungal alkaloids and plant defensive hormonal pathways [mainly salicylic acid (SA) and JA]. Based on knowledge derived from other symbioses for which more information exists (e.g., plant–mycorrhizal and plant–rhizobacterial interactions), the model incorporates the potential modulation of plant defensive hormones by the fungal endophytes. The model allows us to predict whether endophyte-symbiotic plants would be defended against insects belonging to different feeding guilds and which mechanisms would explain the defense phenotype (Figure 1, Key Figure).

We took two complementary approaches to validate our conceptual framework and the predictions of our model. First, we reviewed the literature looking for evidence that *Epichloë* endophytes affect the immune system of the host plant. Second, the predictions of the model were evaluated through a standard meta-analysis of published results. The strength of the model is that it offers a robust framework to understand grass–endophyte interactions under different scenarios of herbivory.

Mechanisms of Plant Defense against Insects

Before considering the effects of *Epichloë* endophytes on plant resistance to herbivores (Box 1), it is necessary to briefly outline the functional bases of the mechanisms of plant defense (for comprehensive reviews see [2,13,19,20]) and the effects of other beneficial microorganisms on plant responses to herbivory. Defensive mechanisms are classified as constitutive, if they are switched on by default, or inducible, if they are activated on attack [2,4]. Compared with constitutive mechanisms of resistance, inducible expression is thought to have evolved as a cost-effective strategy [2,5]. Examples of inducible defenses include the plant hypersensitive reaction (HR), systemic acquired resistance (SAR), and herbivore-induced production of toxins, antinutritional compounds, and indirect defenses, such as plant volatiles. These responses are controlled by a group of hormones that includes SA, JA, and ethylene (ET) [2,3,5]. JA and SA are important factors regulating responses to insect herbivores and pathogens [5,20,21]. The predominant model establishes that SA-dependent defenses are effective against **biotrophic pathogens** (see Glossary) and **sap-sucking insect** herbivores whereas JA-dependent defenses are effective against **necrotrophic pathogens** and **chewing insects** [5,22–26]. In addition, SA and JA may have antagonistic actions in several systems [5].

Effects of Beneficial Microorganisms on Plant Immunity

The association between plants and beneficial microorganisms can have important consequences on the plant immune system [13,27,28]. Certain species of rhizobacteria and mycorrhizal fungi induce special types of systemic defenses, which are termed induced systemic resistance (ISR) and mycorrhiza-induced resistance (MIR), respectively [12–14,29–31]. Essentially, ISR and MIR are distinguished from other induced resistance mechanisms because

Glossary

Biotrophic microorganisms: microorganisms feeding on living plant tissues. Generally, they live between plant cells, using only a group of cells to obtain the nutrients by means of specialized structures called haustoria.

Chewing insects: known as mandibulates, these insects possess mandibles (or jaws) equipped with tooth-like ridges that serve to cut and grind plant tissues. Chewing insects include herbivore species belonging to the orders Lepidoptera (larvae of months and butterflies), Orthoptera (grasshoppers), and Coleoptera (beetles).

Necrotrophic microorganisms: microorganisms that kill plant tissues, by means of enzymes or toxins that cause the disintegration of plant cells, and feed on the released nutrients.

Sap-sucking insects: insects possessing tubular mouthparts called haustella that serve to pierce plant tissues and imbibe plant fluids in the form of phloem, xylem, and the contents of mesophyll cells. Sap suckers include species belonging to the order Hemiptera, such as aphids and whiteflies.

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Key Figure

Schematic Representation Showing the Combined Effects of *Epichloë* Endophyte-Produced Defenses (Alkaloids) and the Plant's Own Defenses on Plant Resistance to Insects Belonging to Two Different Feeding Guilds
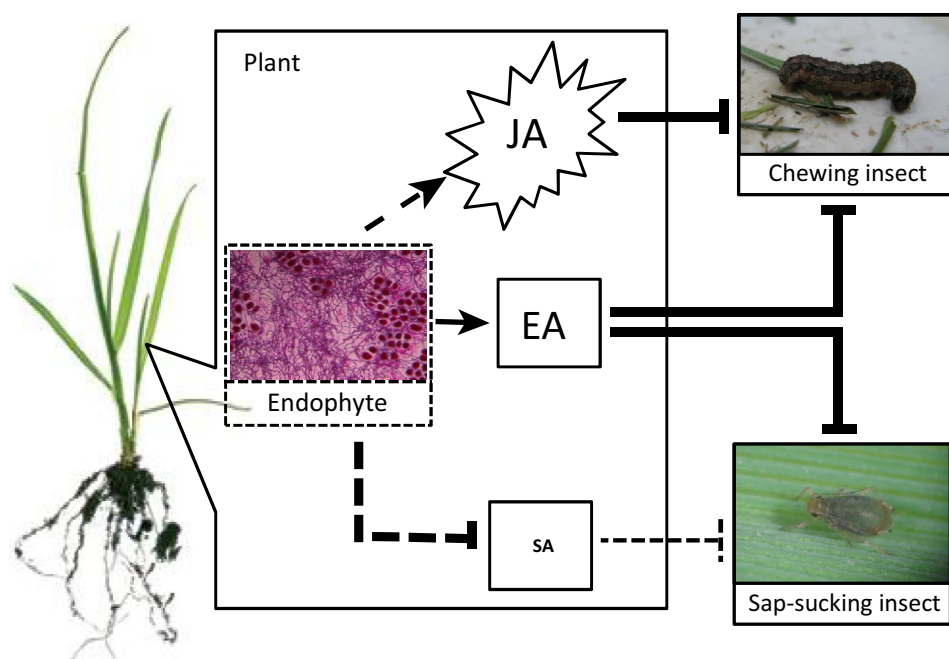
Trends in Plant Science

Figure 1. In the case represented in the figure, the endophyte generates a profile of effective alkaloids (EAs) against both chewing and sap-sucking insect species. Plant defenses, mediated by the jasmonic acid (JA) and salicylic acid (SA) pathways, are effective only against chewing insects because, according to the model, defenses against sap-sucking insects are dependent on SA and the SA pathway is repressed by the endophyte. The model predicts that plant resistance to sap-sucking insects is solely dependent on the effectiveness of the alkaloids produced by the fungus, whereas resistance to chewing insects is mediated by both EA-based and JA-dependent defenses. Arrows indicate positive regulation and truncated connectors indicate inhibition or negative regulation. Unbroken lines indicate functional connections that are well documented in the literature; broken lines denote assumptions of the model that require further testing by direct experimentation. Image of endophyte fungus was provided by I. Hernández.

symbiotic plants have a 'primed state' of defenses generally associated with the JA pathway. Primed plants display faster, earlier, stronger, and/or more sustained expression of defenses on pathogen or insect attack [7,13,32]. The mechanism behind 'priming' is not completely clear, but it might involve accumulation of transcription factors and signaling proteins in cells. These factors remain inactive in enemy-free environments but when a potential attacker is detected they promote the rapid activation of a plant response [13,19,32,33].

The priming of defenses and other modifications of the plant immune system are a consequence of the continuous molecular dialog between plants and microorganisms during colonization and the establishment of symbiosis [14,15]. At colonization, plants recognize a given microorganism, either a rhizobacterium or a mycorrhizal fungus, as a biotrophic organism that

activates the SA pathway and the microorganism responds by repressing this pathway by means of specific effectors to establish an interaction with the future host [14,31,34,35]. As a consequence of this initial communication between partners, a well-established symbiosis typically shows a repressed SA pathway, enhanced levels of JA and JA precursors, and upregulated JA-responsive genes [13,14,31,36]. The increased level of JA seems to be involved in regulation of the development and functioning of the symbiont in mycorrhizal plants [14,31,37]. Proteins that repress the SA pathway have been identified. For example, the mycorrhizal fungus *Gigaspora margarita* expresses the Ca^{2+}/calmodulin kinase DMl3, which suppresses genes related to SA in *Medicago truncatula* [38]. Nod factors, which are required for the establishment of symbiosis between *Medicago sativa* and the rhizobacterium *Rhizobium meliloti*, are also known to suppress the SA pathway [39].

This fine-tuned regulation of the SA and JA signaling pathways by beneficial microorganisms could explain their effects on plant resistance to herbivorous insects. Thus, while symbiotic plants are usually well defended against chewing insects, they are susceptible to sap-sucking insects [40], presumably because the JA pathway is primed whereas the SA pathway is repressed [30,31,35,41,42]. This is also consistent with the observation that mycorrhization tends to increase plant resistance to necrotrophic pathogens while reducing defenses against biotrophic microorganisms [10,31].

Epichloë-Driven Changes in Host Plant Immune System

Whereas our understanding of the impacts of rhizobacteria or mycorrhizal fungi on plant defenses has increased rapidly, virtually nothing is known on the effects of *Epichloë* endophytes on the immune responses of their hosts. As found in symbioses between plants and other beneficial microorganisms, the communication of endophytes and grasses leads to transcriptional reprogramming of the plant. A recent study with the *Lolium perenne–Epichloë festucae* var. *lolii* system showed that 38% of the host genes were differentially expressed between endophyte-symbiotic and non-symbiotic plants [43]. Differentially regulated genes included GO categories related to primary and secondary metabolism, response to biotic and abiotic stress factors, and hormonal responses, with a general downregulation of genes related to SA biosynthesis and signaling [43]. As found in previous studies on the symbioses between plants and other biotrophic microorganisms, the downregulation of SA may be the result of active suppression of plant defense by *Epichloë* endophytes [14,44,45]. This could be achieved by means of a salicylate hydroxylase synthesized by the endophyte that has been found to be upregulated in the *Festuca rubra–E. festucae* symbiotic system [46] (but see [47]). The SA hydroxylase catalyzes the transformation of salicylate into the inactive product catechol [46]. Several PR genes were down-regulated in the *L. perenne–E. festucae* var. *lolii* symbiosis [43] and the *PR-10* gene was downregulated in the *Schedonorus arundinaceus–Epichloë coenophiala* symbiosis [48]. In addition to the changes in the SA pathway, another study suggested that the presence of the endophyte could also directly affect the JA pathway. Plants of tall fescue (*S. arundinaceus*) symbiotic with *E. coenophiala* showed increased expression of the *TFF41* plant gene. The TFF41 protein has high similarity with the ω-3 FAD enzymes of potato and parsley, which increase the abundance of trienoic fatty acids that are precursors of JA [48]. Nonetheless, whether upregulation of the *TFF41* gene leads to a higher level of JA is currently unknown.

Indirect evidence also supports the idea that *Epichloë* endophytes suppress SA-mediated immune responses. For example, endophyte-symbiotic plants were found to be more susceptible to a group of biotrophic pathogens, such as *Puccinia graminis*, *Blumeria graminis*, *Typhula ishikariensis*, and *Claviceps purpurea*, than their non-symbiotic counterparts [49–54]. Moreover, evidence is emerging that this apparent downregulation of the SA pathway may be accompanied by an enhanced JA response. The endophyte *E. festucae* var. *lolii* strain AR1 had

a negative effect on the performance of the chewing insect *Heteronychus arator* that was apparently independent of the fungal alkaloids [55]. Recent studies have revealed the molecules that could explain this alkaloid-independent resistance. For instance, metabolomic studies showed that endophyte-symbiotic *L. perenne* plants had augmented levels of non-alkaloid metabolites such as phenylpropanoids [56,57]. More recently, another study with the same symbiotic system showed upregulation of several genes involved in the phenylpropanoid pathway [43]. In addition, higher levels of phenolic compounds were detected in various plant tissues of the *S. arundinaceus–E. coenophiala* and *L. perenne–E. festucae* var. *lolii* symbioses [58,59]. Whether this positive effect of endophytes on the accumulation of phenolic compounds is dependent on activation of the JA pathway remains to be elucidated.

Toward an Integrative Model of Defenses in Plants Symbiotic with *Epichloë* Endophytes

We propose that *Epichloë* fungal endophytes increase plant resistance to herbivory via two different mechanisms: production of fungal alkaloids and promotion of the JA pathway in the host plant. While the JA-dependent defenses would be effective against a broad spectrum of chewing insects, alkaloid-based defenses would be more specific, depending on the alkaloid type and the identity of the attacker (Box 1). As described for other symbiotic interactions, the promotion of the JA pathway would be a consequence of the manipulation on the plant immune system by the endophyte during the molecular dialog that leads to the establishment of the mutualistic relationship. The conceptual framework is depicted graphically in Figure 1. The model predicts scenarios of resistance or susceptibility of plants in symbiosis with *Epichloë* endophytes, depending on the endophyte species and the feeding guild of the insect herbivore.

Predicted Response of Endophyte-Symbiotic Plants to Sap-Sucking Insects

Epichloë endophytes may permanently depress the SA pathway, which would result in potential susceptibility of plants to sap-sucking insects. However, if a given symbiont produces effective alkaloids (EAs) against a given species of sap-sucking insect, the model predicts a resistant phenotype mediated by alkaloids (Figure 1). By contrast, if the symbiont does not produce alkaloids that are effective against sap-sucking insects [i.e., it produces ineffective alkaloids (IAs)], the model predicts that symbiotic plants will be susceptible to insects of that feeding guild.

Predicted Response of Endophyte-Symbiotic Plants to Chewing Insects

Epichloë endophytes may permanently prime the JA pathway in host plants, which would result in increased resistance to chewing insects. Thus, if the endophyte is a source of EAs against one particular species of chewing insect, the model predicts plant resistance mediated by both alkaloids and JA-enhanced defenses (Figure 1). By contrast, if the symbiont produces IAs for a given chewing insect, the model predicts a response of resistance mediated only by JA-dependent defenses.

Searching for Evidence of *Epichloë* Endophyte-Mediated Induction of JA Defenses in Host Plants: A Meta-Analysis

To test the model predictions (Figure 1), we reviewed published results, which were synthesized and quantitatively analyzed through a standard meta-analysis [see the supplemental information online for details of the data selection criteria, the meta-analysis methods, and the complete list of selected articles and experiments (Table S1)].

We first determined which types of alkaloids are effective (EAs) or ineffective (IAs) as defenses for each of the insect species included in the reviewed studies (Table 1). Alkaloids were considered EAs if the available evidence showed that they had a negative effect on the

Table 1. Alkaloid Effectiveness for Each Insect Species Used in the Study[a]

Insect species	Feeding guild	Ergot	Indole diterpene	Loline	Peramine	Refs
Rhopalosiphum padi	Sap-sucking	IA	ND	EA	IA	[60,68,77–79]
Schizaphis graminum	Sap-sucking	IA	ND	EA	EA	[61,79,80]
Diuraphis noxia	Sap-sucking	EA	ND	EA	ND	[81]
Balanococcus poae	Sap-sucking	EA	ND	ND	EA	[82]
Agrotis ipsilon	Chewing	EA	ND	EA	EA	[83,84]
Heteronychus arator	Chewing	EA	IA	IA	IA	[85]
Listronotus bonariensis	Chewing	ND	EA	EA	EA	[86–89]
Spodoptera frugiperda	Chewing	EA	EA	EA	ND	[90–92]
Wiseana cervinata	Chewing	ND	EA	ND	ND	[93]

[a]EA, effective alkaloid; IA, ineffective alkaloid; ND, not determined.

performance of a given species of herbivore or IAs if the evidence showed a neutral (or even positive) effect on the insect's performance. This information was obtained from experiments comparing effects between wild-type and mutant endophytes displaying well-characterized defects in alkaloid pathways or between fungal strains with contrasting alkaloid profiles and from bioassays using artificial diets with various fungal alkaloids (see references in Table 1). With this information we evaluated whether each of the symbionts included in Table S1 had EAs for the specific insect attacker tested in the study.

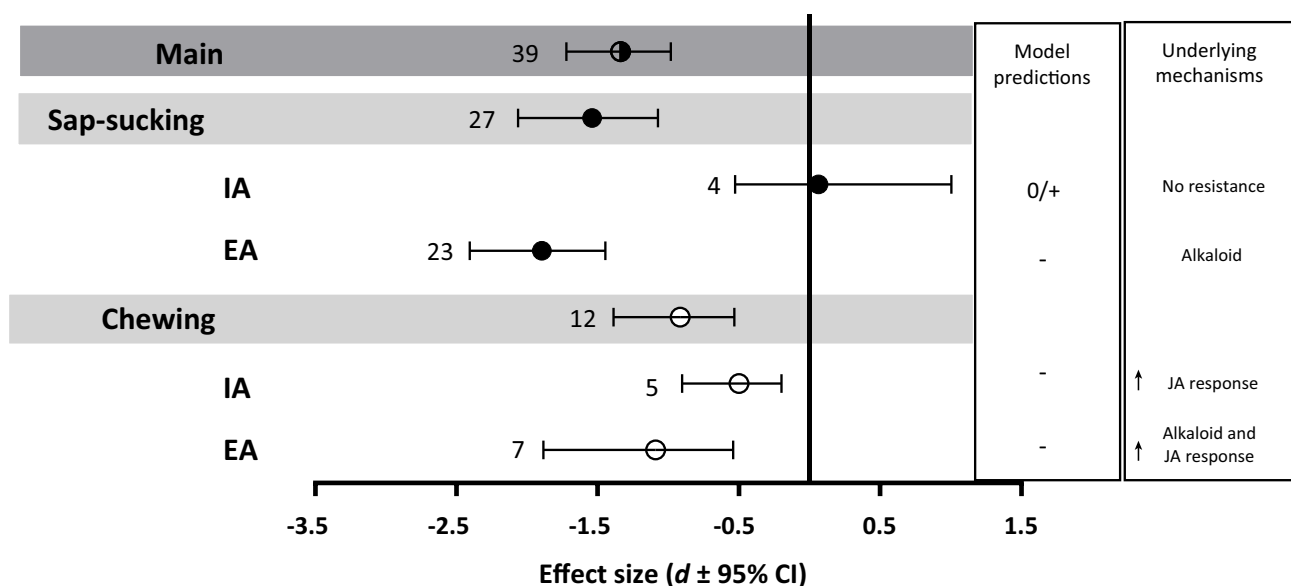### *Epichloë* Endophyte Effects on Insect Herbivores

We performed three different meta-analyses using the same database (Table S1). The first was conducted to test the overall impact of the presence of the endophyte on insect performance. In the second analysis, we estimated the effect of the endophyte on each insect feeding guild (i.e., sap suckers and chewers). In the third analysis, we compared the effects of the endophyte on insect performance within each feeding guild taking into account the effectiveness of the alkaloids (IAs and EAs). A negative effect size indicates a negative effect of the endophyte on insect performance while a positive effect size means the opposite (see the supplemental information online for details).

Effects of *Epichloë* Endophytes on Plant Resistance to Insects

There was an overall negative effect of the presence of an endophyte symbiont on the performance of grass insect herbivores (main effect size = −1.35, 95% CI = −1.75 to −1.01) with no differences between insect feeding guilds (Q_b = 2.87, P = 0.09, df = 1) (Figure 2) (sap suckers: effect size = −1.54, 95% CI = −2.08 to −1.07; chewers: effect size = −0.95, 95% CI = −1.43 to −0.55). The Rosenthal fail-safe number was higher than the reference number [feeding guild: 1109.5 > 5 * (39) + 10] indicating that the statistical inference was robust (see the supplemental information online for more details).

When the analysis was limited to sap-sucking insects, the observed endophyte effect was strongly dependent on whether the alkaloids produced by the fungus were effective against the specific attacker tested in the study (Q_b = 13.46, P < 0.001, df = 1) (Figure 2) (sap suckers/EA: effect size = −1.89, 95% CI = −2.40 to −1.44; sap suckers/IA: effect size = 0.06, 95% CI = −0.53 to 1.01). The Rosenthal fail-safe number was higher than the reference [alkaloid effectiveness for sap suckers: 648.1 > 5 * (26) + 10].

When the analysis was restricted to studies that tested the performance of chewing insects, the observed effect of the endophyte was always negative on the herbivore regardless of the

Figure 2. Effect of *Epichloë* Fungal Endophytes on Herbivore Performance (Relative Effects of Endophyte-Symbiotic versus Non-symbiotic Plants). The overall main effect is broken down into sap-sucking and chewing insects, which in turn are split into two groups depending on the nature of the alkaloids provided by the endophyte [i.e., effective alkaloids (EAs) or ineffective alkaloids (IAs)]. The box on the left indicates the predictions from the model on insect performance: 0, neutral; +, positive; −, negative. The box on the right indicates the proposed mechanism of defense. The analyses were performed using the experiments summarized in Table S1 in the supplemental information online.

alkaloid category (i.e., IA or EA) ($Q_b = 1.46$, $P = 0.23$, df = 1) (Figure 2) (chewers/EA: effect size = −1.10, 95% CI = −1.86 to −0.52; chewers/IA: effect size = −0.56, 95% CI = −1.03 to −0.22). The Rosenthal fail-safe number was higher than the reference [alkaloid effectiveness for chewers: 72.7 > 5 * (12) + 10].

Concluding Remarks and Future Perspectives

Our meta-analysis supports the idea that *Epichloë* fungal endophytes improve antiherbivore defenses of their grass hosts via alkaloid-dependent and -independent mechanisms. According to our results, if the fungus produces IAs against a specific attacker, it will still have a protective effect increasing plant resistance against chewing insects. We suggest that this pattern of resistance, inferred from published ecological studies, could be explained by the proposed effect of fungal endophytes on the plant immune system, which involves repression of SA signaling and promotion of JA responses.

The molecular interaction between fungal effectors and the plant immune system would give the host a versatile array of defensive mechanisms to cope with herbivory. Activation or priming of the JA pathway would increase the plant's defense against chewing insects independently of the supply of fungal alkaloids. The relative importance of the JA pathway, in terms of resistance, would be inversely proportional to the defense provided by alkaloids. If the endophyte produces ineffective alkaloids against the attacker, the JA response would be essential for the host plant. However, even if the endophyte produces EAs, their effectiveness may be constrained in tissues where these metabolites are expressed in low concentrations (such as leaf blades and roots) [12,60,61]. Thus, the enhanced JA defense pathway could be critical to protect plant parts that are essential for resprouting and hence to tolerate herbivory. In addition, since alkaloid production may be constrained in nitrogen-deficient soils [62], promotion of the plant's own defenses could become highly important under natural conditions, particularly for plants growing in poor soils.

The interaction with *Epichloë* fungal endophytes could also have negative consequences for plant immunity and fitness. Thus, given that repression of the SA pathway could be essential to maintain the symbiotic relationship, plants may become more susceptible to biotrophic pathogens, as shown in previous studies [49–54]. Therefore, there may be ecological tradeoffs associated with this symbiosis that could explain the variation in infection rates under field conditions [63,64]. In addition, any biotic or abiotic factor that activates the SA pathway is likely to have a negative effect on the endophyte and its antiherbivore function (Figure 1). For example, activation of SA pathway by sap-sucking herbivores or biotrophic pathogens could negatively affect the endophyte and the level of antiherbivore resistance conferred by the symbiont. Similarly, exposure to oxidant agents such as ozone, a tropospheric pollutant that reportedly enhances plant resistance to biotrophic pathogens and sap-sucking herbivores by activating the SA signaling pathway [65], could have a negative impact on grass interactions with fungal endophytes. In agreement with this hypothesis, it was recently shown that acute exposure of *Lolium multiflorum* plants to ozone resulted in a reduction of the effectiveness of the defensive mutualism provided by the endophyte *Epichloë occultans* [66].

Although the presented model is indirectly supported in both qualitative and quantitative terms (Figure 2), our study also reveals important gaps in our understanding of the interactions between plants and *Epichloë* fungal endophytes (see Outstanding Questions). More experiments are needed to address specific questions related to the interplay between endophytes and plant defense hormones [47,67]. For example, the use of JA and SA markers, plants mutant for the JA and SA pathways, or endophytes mutant for alkaloids would be interesting approaches to test the potential modulation by the fungus of the immune responses of the host plant. Moreover, the use of more biotic or abiotic factors that regulate specific hormonal pathways in the host will give us further ideas about the interaction between the host immune system and the defenses provided by the fungal endophytes.

Our study represents a first attempt to understand the multiple effects of *Epichloë* fungal endophytes on plant resistance to herbivory and highlights how little is known about grass–endophyte symbiosis at the mechanistic level.

Acknowledgments

Supplemental Information

Supplemental information associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tplants.2017.08.005.

References

1. Oerke, E.C. (2006) Crop losses to pests. *J. Agric. Sci.* 144, 31–43
2. Karban, R. and Baldwin, I. (1997) *Induced Responses to Herbivory,* University of Chicago Press
3. Ballaré, C.L. (2014) Light regulation of plant defense. *Annu. Rev. Plant Biol.* 65, 335–363
4. Schoonhoven, L.M. *et al.* (2005) *Insect–Plant Biology,* Oxford University Press
5. Thaler, J.S. *et al.* (2012) Evolution of jasmonate and salicylate signal crosstalk. *Trends Plant Sci.* 17, 260–270
6. Koricheva, J. *et al.* (2009) Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. *Ecology* 90, 2088–2097
7. Pineda, A. *et al.* (2013) Beneficial microbes in a changing environment: are they always helping plants to deal with insects? *Funct. Ecol.* 27, 574–586
8. Saikkonen, K. *et al.* (2013) Chemical ecology mediated by fungal endophytes in grasses. *J. Chem. Ecol.* 39, 962–968
9. Zhu, F. *et al.* (2014) Insect herbivore-associated organisms affect plant responses to herbivory. *New Phytol.* 204, 315–321
10. Pozo, M.J. and Azcón-Aguilar, C. (2007) Unraveling mycorrhiza-induced resistance. *Curr. Opin. Plant Biol.* 10, 393–398
11. Porras-Alfaro, A. and Bayman, P. (2011) Hidden fungi, emergent properties: endophytes and microbiomes. *Annu. Rev. Phytopathol.* 49, 291–315
12. Cameron, D.D. *et al.* (2013) Mycorrhiza-induced resistance: more than the sum of its parts? *Trends Plant Sci.* 18, 539–545
13. Pieterse, C.M.J. *et al.* (2014) Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* 52, 347–375

14. Pozo, M.J. *et al.* (2015) Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. *New Phytol.* 205, 1431–1436

15. Gutjahr, C. (2014) Phytohormone signaling in arbuscular mycorrhiza development. *Curr. Opin. Plant Biol.* 20, 26–34

16. Schardl, C.L. *et al.* (2013) Currencies of mutualisms: sources of alkaloid genes in vertically transmitted epichloae. *Toxins (Basel)* 5, 1064–1088

17. Schardl, C.L. *et al.* (2004) Symbioses of grasses with seedborne fungal endophytes. *Annu. Rev. Plant Biol.* 55, 315–340

18. Clay, K. (1988) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology* 69, 10–16

19. Conrath, U. (2011) Molecular aspects of defence priming. *Trends Plant Sci.* 16, 524–531

20. Vos, I.A. *et al.* (2013) Costs and benefits of hormone-regulated plant defences. *Plant Pathol.* 62, 43–55

21. Bari, R. and Jones, J.G. (2009) Role of plant hormones in plant defence responses. *Plant Mol. Biol.* 69, 473–488

22. Kunkel, B.N. and Brooks, D.M. (2002) Cross talk between signaling pathways in pathogen defense. *Curr. Opin. Plant Biol.* 5, 325–331

23. Glazebrook, J. (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43, 205–227

24. Schwartzberg, E.G. and Tumlinson, J.H. (2014) Aphid honeydew alters plant defence responses. *Funct. Ecol.* 28, 386–394

25. Schweiger, R. *et al.* (2014) Interactions between the jasmonic and salicylic acid pathway modulate the plant metabolome and affect herbivores of different feeding types. *Plant Cell Environ.* 37, 1574–1585

26. Ballaré, C.L. *et al.* (2012) Canopy light and plant health. *Plant Physiol.* 160, 145–155

27. Balmer, A. *et al.* (2015) The "prime-ome": towards a holistic approach to priming. *Trends Plant Sci.* 20, 443–452

28. Leuchtmann, A. *et al.* (1994) Sexual compatibility and taxonomy of a new species of *Epichloë* symbiotic with fine fescue grasses. *Mycologia* 86, 802–812

29. Verhagen, B.W.M. *et al.* (2004) The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Mol. Plant Microbe Interact.* 17, 895–908

30. Van der Ent, S. *et al.* (2009) Priming of plant innate immunity by rhizobacteria and β-aminobutyric acid: differences and similarities in regulation. *New Phytol.* 183, 419–431

31. Jung, S. *et al.* (2012) Mycorrhiza-induced resistance and priming of plant defenses. *J. Chem. Ecol.* 38, 651–664

32. Martinez-Medina, A. *et al.* (2016) Recognizing plant defense priming. *Trends Plant Sci.* 21, 818–822

33. Frost, C.J. *et al.* (2008) Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiol.* 146, 818–824

34. Paszkowski, U. (2006) Mutualism and parasitism: the yin and yang of plant symbioses. *Curr. Opin. Plant Biol.* 9, 364–370

35. Zamioudis, C. and Pieterse, C.M.J. (2011) Modulation of host immunity by beneficial microbes. *Mol. Plant Microbe Interact.* 25, 139–150

36. Wasternack, C. and Hause, B. (2013) Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Ann. Bot.* 111, 1021–1058

37. Hause, B. *et al.* (2002) Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots. *Plant Physiol.* 130, 1213–1220

38. Siciliano, V. *et al.* (2007) Transcriptome analysis of arbuscular mycorrhizal roots during development of the prepenetration apparatus. *Plant Physiol.* 144, 1455–1466

39. Martínez-Abarca, F. *et al.* (1998) Involvement of salicylic acid in the establishment of the *Rhizobium meliloti*–alfalfa symbiosis. *Mol. Plant Microbe Interact.* 11, 153–155

40. Hartley, S.E. and Gange, A.C. (2008) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. *Annu. Rev. Entomol.* 54, 323–342

41. Gols, R. (2014) Direct and indirect chemical defences against insects in a multitrophic framework. *Plant Cell Environ.* 37, 1741–1752

42. Pineda, A. *et al.* (2010) Helping plants to deal with insects: the role of beneficial soil-borne microbes. *Trends Plant Sci.* 15, 507–514

43. Dupont, P. *et al.* (2015) Fungal endophyte infection of ryegrass reprograms host metabolism and alters development. *New Phytol.* 208, 1227–1240

44. Stacey, G. *et al.* (2006) Effects of endogenous salicylic acid on nodulation in the model legumes *Lotus japonicus* and *Medicago truncatula*. *Plant Physiol.* 141, 1473–1481

45. Yasuda, M. *et al.* (2016) Effector-triggered immunity determines host genotype-specific incompatibility in legume–*Rhizobium* symbiosis. *Plant Cell Physiol.* 57, 1791–1800

46. Ambrose, K.V. and Belanger, F.C. (2012) SOLiD-SAGE of endophyte-infected red fescue reveals numerous effects on host transcriptome and an abundance of highly expressed fungal secreted proteins. *PLoS One* 7, e53214

47. Ambrose, K.V. *et al.* (2015) Functional characterization of salicylate hydroxylase from the fungal endophyte *Epichloë festucae*. *Sci. Rep.* 5, 10939

48. Johnson, L.J. *et al.* (2003) Identification of differentially expressed genes in the mutualistic association of tall fescue with *Neotyphodium coenophialum*. *Physiol. Mol. Plant Pathol.* 63, 305–317

49. Krauss, J. *et al.* (2007) Fungal grass endophytes, grass cultivars, nitrogen deposition and the associations with colonizing insects. In *Proceedings of the 6th International Symposium on Fungal Endophytes of Grasses* (Popay, A.J. and Thom, E.R., eds), pp. 53–57, New Zealand Grassland Association

50. Wäli, P.R. *et al.* (2006) Susceptibility of endophyte-infected grasses to winter pathogens (snow molds). *Can. J. Bot.* 84, 1043–1051

51. Sabzalian, M. *et al.* (2012) Reaction to powdery mildew fungus, *Blumeria graminis* in endophyte-infected and endophyte-free tall and meadow fescues. *Australas. Plant Pathol.* 41, 565–572

52. Welty, R.E. *et al.* (1991) Reaction of tall fescue infected and noninfected by *Acremonium coenophialum* to *Puccinia graminis* subsp. *graminicola*. *Plant Dis.* 75, 883–886

53. Welty, R.E. *et al.* (1993) Response of field-grown tall fescue infected by *Acremonium coenophialum* to *Puccinia graminis* subsp. *graminicola*. *Plant Dis.* 77, 574–575

54. Pańka, D. *et al.* (2011) Effect of *Neotyphodium uncinatum* endophyte on meadow fescue yielding, health status and ergovaline production in host-plants. *J. Plant Prot. Res.* 51, 362–370

55. Popay, A.J. and Baltus, J.G. (2001) Black beetle damage to perennial ryegrass infected with AR1 endophyte. *Proc. N. Z. Grassl. Assoc.* 63, 267–271

56. Rasmussen, S. *et al.* (2009) Metabolomics analysis of the *Lolium perenne*–*Neotyphodium lolii* symbiosis: more than just alkaloids? *Phytochem. Rev.* 8, 535–550

57. Rasmussen, S. *et al.* (2008) Plant–endophyte–herbivore interactions: more than just alkaloids? *Plant Signal. Behav.* 3, 974–977

58. Malinowski, D. *et al.* (1998) Evidence for chemical changes on the root surface of tall fescue in response to infection with the fungal endophyte *Neotyphodium coenophialum*. *Plant Soil* 205, 1–12

59. Pańka, D. *et al.* (2013) Production of phenolics and the emission of volatile organic compounds by perennial ryegrass (*Lolium perenne* L.)/*Neotyphodium lolii* association as a response to infection by *Fusarium poae*. *J. Plant Physiol.* 170, 1010–1019

60. Hunt, M.G. and Newman, J.A. (2005) Reduced herbivore resistance from a novel grass–endophyte association. *J. Appl. Ecol.* 42, 762–769

61. Siegel, M.R. *et al.* (1990) Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. *J. Chem. Ecol.* 16, 3301–3315

62. Faeth, S.H. and Fagan, W.F. (2002) Fungal endophytes: common host plant symbionts but uncommon mutualists. *Integr. Comp. Biol.* 42, 360–368

63. Semmartin, M. *et al.* (2015) Broad-scale variation of fungal-endophyte incidence in temperate grasses. *J. Ecol.* 103, 184–190

64. Gundel, P.E. *et al.* (2011) Incorporating the process of vertical transmission into understanding of host–symbiont dynamics. *Oikos* 120, 1121–1128

65. Kangasjarvi, J. *et al.* (2005) Signalling and cell death in ozone-exposed plants. *Plant Cell Environ.* 28, 1021–1036

66. Ueno, A.C. *et al.* (2015) Mutualism effectiveness of a fungal endophyte in an annual grass is impaired by ozone. *Funct. Ecol.* 30, 226–232

67. Simons, L. *et al.* (2008) Effects of methyl jasmonate and an endophytic fungus on plant resistance to insect herbivores. *J. Chem. Ecol.* 34, 1511–1517

68. Panaccione, D.G. *et al.* (2014) Bioactive alkaloids in vertically transmitted fungal endophytes. *Funct. Ecol.* 28, 299–314

69. Schardl, C.L. *et al.* (2012) Chemotypic diversity of epichloae, fungal symbionts of grasses. *Fungal Ecol.* 5, 331–344

70. Schardl, C.L. *et al.* (2013) The epichloae: alkaloid diversity and roles in symbiosis with grasses. *Curr. Opin. Plant Biol.* 16, 480–488

71. Young, C.A. *et al.* (2015) Genetics, genomics and evolution of ergot alkaloid diversity. *Toxins* 7, 1273–1302

72. Schardl, C.L. *et al.* (2007) Loline alkaloids: currencies of mutualism. *Phytochemistry* 68, 980–996

73. Ball, O.J. *et al.* (1995) Interrelationships between *Acremonium lolii*, peramine, and lolitrem B in perennial ryegrass. *Appl. Environ. Microbiol.* 61, 1527–1533

74. Justus, M. *et al.* (1997) Levels and tissue distribution of loline alkaloids in endophyte-infected *Festuca pratensis*. *Phytochemistry* 44, 51–57

75. Saikkonen, K. *et al.* (2010) Defensive mutualism between plants and endophytic fungi? *Fungal Divers.* 41, 101–113

76. Saikkonen, K. *et al.* (2006) Model systems in ecology: dissecting the endophyte–grass literature. *Trends Plant Sci.* 11, 428–433

77. Johnson, M.C. *et al.* (1985) Insect feeding deterrents in endophyte-infected tall fescue. *Appl. Environ. Microbiol.* 49, 568–571

78. Eichenseer, H. *et al.* (1991) Influence of endophyte infection, plant age and harvest interval on *Rhopalosiphum padi* survival and its relation to quantity of *N*-formyl and *N*-acetyl loline in tall fescue. *Entomol. Exp. Appl.* 60, 29–38

79. Wilkinson, H.H. *et al.* (2000) Contribution of fungal loline alkaloids to protection from aphids in a grass–endophyte mutualism. *Mol. Plant Microbe Interact.* 13, 1027–1033

80. Breen, J.P. (1992) Temperature and seasonal effects on expression of *Acremonium* endophyte-enhanced resistance to *Schizaphis graminum* (Homoptera: Aphididae). *Environ. Entomol.* 21, 68–74

81. Clement, S.L. *et al.* (1997) Fungal endophytes of wild barley and their effects on *Diuraphis noxia* population development. *Entomol. Exp. Appl.* 82, 275–281

82. Pennell, C.G.L. *et al.* (2005) Occurrence and impact of pasture mealybug (*Balanococcus poae*) and root aphid (*Aploneura lentisci*) on ryegrass (*Lolium* spp.) with and without infection by *Neotyphodium* fungal endophytes. *N. Z. J. Agric. Res.* 48, 329–337

83. Potter, D.A. *et al.* (2008) Contribution of ergot alkaloids to suppression of a grass-feeding caterpillar assessed with gene knock-out endophytes in perennial ryegrass. *Entomol. Exp. Appl.* 126, 138–147

84. Baldauf, M.W. *et al.* (2011) Endophyte-mediated resistance to black cutworm as a function of plant cultivar and endophyte strain in tall fescue. *Environ. Entomol.* 40, 639–647

85. Ball, O.J.P. *et al.* (1997) Ergopeptine alkaloids and *Neotyphodium lolii*-mediated resistance in perennial ryegrass against adult *Heteronychus arator* (Coleoptera: Scarabaeidae). *J. Econ. Entomol.* 90, 1382–1391

86. Tanaka, A. *et al.* (2005) A symbiosis expressed non-ribosomal peptide synthetase from a mutualistic fungal endophyte of perennial ryegrass confers protection to the symbiotum from insect herbivory. *Mol. Microbiol.* 57, 1036–1050

87. Popay, A.J. *et al.* (2009) Endophyte-infected meadow fescue and loline alkaloids affect Argentine stem weevil larvae. *N. Z. Plant Prot.* 62, 19–27

88. Rowan, D. *et al.* (1990) Effect of fungal metabolite peramine and analogs on feeding and development of argentine stem weevil (*Listronotus bonariensis*). *J. Chem. Ecol.* 16, 1683–1695

89. Prestidge, R.A. and Gallagher, R.T. (1988) Endophyte fungus confers resistance to ryegrass: argentine stem weevil larval studies. *Ecol. Entomol.* 13, 429–435

90. Ball, O.J.-P. *et al.* (2006) Importance of host plant species, *Neotyphodium* endophyte isolate, and alkaloids on feeding by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae. *J. Econ. Entomol.* 99, 1462–1473

91. Clay, K. and Cheplick, G. (1989) Effect of ergot alkaloids from fungal endophyte-infected grasses on fall armyworm (*Spodoptera frugiperda*). *J. Chem. Ecol.* 15, 169–182

92. Riedell, W.E. *et al.* (1991) Naturally-occurring and synthetic loline alkaloid derivatives: insect feeding behavior modification and toxicity. *J. Entomol. Sci.* 26, 122–129

93. Jensen, J.G. and Popay, A.J. (2004) Perennial ryegrass infected with AR37 endophyte reduces survival of porina larvae. *N. Z. Plant Prot.* 57, 323–328