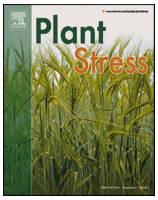
Plant Stress 17 (2025) 100963

Contents lists available at ScienceDirect

Plant Stress

journal homepage: www.sciencedirect.com/journal/plant-stress



Review

Is it time to shift towards multifactorial biotic stress in plant research?

Augusto B. Penteriche [1], Diego Z. Gallan [1], Marcio C. Silva-Filho [*]

Departmento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brazil

ARTICLE INFO

Keywords:
Plant-multitrophic interactions
Biotic stress
Holobiont

ABSTRACT

In natural and field environments, plants are simultaneously exposed to a diverse set of stressful conditions, like biotic and abiotic stressors, and must respond accordingly. These interactions result in intricate cross-talk between hormonal pathways, microbial communities, and volatile organic compounds (VOCs), shaping plant fitness and resistance mechanisms. Recent studies demonstrate that multifactorial stress combinations often trigger unpredictable transcriptional and metabolic responses, distinct from those induced by individual stressors alone. How multiple simultaneous or sequential biotic stresses affect plant fitness and defense, however, have been less reported. Climate change and extreme weather events are intensifying the likeliness that these conditions strike simultaneously or in close sequence, and increase the complexity of stress responses. Still, our knowledge on the mechanisms of plant defense to concomitant or sequential biotic stresses remains critically limited. Findings from unifactorial plant defense studies are often hindered in their precise extrapolation to complex, multifactorial natural environments. Thus, plant research urgently needs to consider multifactorial and combinatorial stressors, dynamic microbial interactions, and ecological trade-offs in plant stress adaptation. Resilient crop development and sustainable agriculture under global warming profoundly depend on our characterization of the complex mechanisms and cross-talks involved. We believe it is time for research to shift towards more complex, realistic stress scenarios to improve the applicability of findings and strengthen crop resilience amidst evolving agricultural demands.

Introduction

Driven by the increase in greenhouse gases and the consequent global warming, the intensification of severe weather events across the globe is becoming an ever-present threat caused by climate change (Ebi et al., 2021; Zandalinas et al., 2021a). Intense heat waves, prolonged drought periods, or rapid flooding are only a few of the extreme phenomena that are becoming more common annually, posing significant detrimental effects and yield losses on crops (Ebi et al., 2021; Yadav et al., 2021).

It is widely acknowledged that these weather events lead to abrupt changes in ecosystems and their interconnectedness, shaping already established communities in unforeseeable ways (Blois et al., 2013; Hoegh-Guldberg et al., 2018; Surówka et al., 2020; Trivedi et al., 2022). Particularly for crops, this implies an increased prevalence of simultaneous biotic and abiotic interactions in the field, challenging plant defense responses on multiple fronts (Hamann et al., 2021; Rivero et al., 2022; Schneider et al., 2022; Trivedi et al., 2022). Crops interact with various species of insect, bacteria, fungi, and viruses, which can occur in a simultaneous or sequential manner. If properly combined and given sufficient time, the possible number of permutations can reach astronomical magnitudes (Dietz, 2021; Suzuki et al., 2014). Although the concept of plants encountering multiple concomitant stresses in nature is not new (Chapin et al., 1987; Mooney and Winner, 1991; Stout et al., 2006), the topic has gained significant attention recently, notably by studies on simultaneous abiotic combinations (Rillig et al., 2019; Zandalinas et al., 2021b). These cumulative stress factors were shown to exert pronounced effects on soil microbial diversity and plant fitness, while stress combinations showed additive effects even when individual stress levels were insufficient to cause significant fitness loss (Rillig et al., 2019; Sinha et al., 2024; Zandalinas et al., 2021b). Furthermore, a study with 10 *Arabidopsis* ecotypes demonstrated that over half of transcriptome changes induced by dual combinations of abiotic stresses or flagellin were not predictable from individual stress responses

---

[*] Corresponding author at: Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, *Av.* Pádua Dias 11, 13418-900, Piracicaba, SP, Brazil.

E-mail addresses: abpenteriche@usp.br (A.B. Penteriche), diego.gallan@usp.br (D.Z. Gallan), mdcsilva@usp.br (M.C. Silva-Filho).

[1] These authors contributed equally

https://doi.org/10.1016/j.stress.2025.100963
Received 7 April 2025; Received in revised form 26 June 2025; Accepted 19 July 2025

(Rasmussen et al., 2013). These studies highlight the inherent limitations of single-stress models, in which mechanisms and signaling pathways identified therein may not fully represent plant defense responses under natural, complex conditions. This knowledge gap is particularly critical for understanding multiple biotic interactions in plants. Thus, we believe it is imperative that future plant stress research incorporate a broader spectrum of variables. This is particularly crucial to validate known mechanisms in natural contexts and to discover novel signaling responses that emerge only under combined stress conditions. The topic of multifactorial stress combination, including examples of synergistic and antagonistic interactions and their molecular mechanisms, has been extensively reviewed (Atkinson et al., 2014; Dietz, 2021; Nadeem et al., 2023; Rivero et al., 2022; Suzuki et al., 2014; Zandalinas et al., 2021a; Zandalinas and Mittler, 2022). Despite their excellence, most reviews primarily focused on combinations of different abiotic factors with at most a single biotic stressor. This underscores the need for more comprehensive consideration of multiple biotic stress in plant research.

Concomitant combinations of pests, pathogens and their respective microbiome modulation are ubiquitous in plants, yet our understanding of the intricate molecular response is still limited (De-la-Pena and Loyola-Vargas, 2014; Franco et al., 2017, 2021; Gallan et al., 2023; Leite-Mondin et al., 2021). Similarly to what was observed for multiple combined abiotic stresses, the complexity introduced by concomitant or sequential biotic interactions is vast, influencing plant signaling mechanisms and defense responses in ways that require further investigation (Fig. 1). A holistic view of plants and their surrounding organisms is essential to elucidate the complex interactions occurring in real environmental conditions. This review focuses on addressing this critical knowledge gap by highlighting recent examples of multifactorial biotic interactions and their impact on plant fitness, examining how multiple biotic stressors shape plant defense responses. Also, we strongly advocate that researchers in the plant stress science field prioritize the investigation of plant defense responses to multiple simultaneous stresses. For that, we propose an integrated framework for designing and performing multifactorial biotic stress research in plants. This is particularly important for crops involving complex biotic interaction networks. Moving beyond isolated, binary interactions will be crucial to better prepare plants for future challenges caused by climate change.

Stress order and intensity affect plant defense response

Agricultural ecosystems are hotspots of biotic stress interactions, where herbivory, pathogen infections, and competition with invasive species often co-occur. Unlike abiotic stresses, these interactions are characterized by co-evolutionary dynamics, as plants and their antagonists engage in a constant evolutionary arms race (Savary et al., 2019). The underlying mechanisms governing these interactions in plants often involve complex trade-offs between growth and defense, mediated by phytohormones, particularly jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) (Thaler et al., 2012). These hormones orchestrate plant defenses, with JA predominantly associated with herbivory or necrotrophic pathogen responses, while SA is often linked to biotrophic pathogens and phloem-feeding insects (Roychowdhury et al., 2025; Vos et al., 2013). However, their signaling pathways can exhibit antagonistic cross-talk, compromising the overall defense strategy when pests and pathogens co-occur (Lazebnik et al., 2014). Furthermore, ET has been implicated in complex cross-talk networks with both JA and SA (Natalini and Palma, 2023). Consequently, a system-level understanding of how plants manage such stressors is essential to uncover the molecular mechanisms involved. Advances in multi-omics approaches, especially transcriptomics, proteomics, and metabolomics, offer promising avenues for identifying key regulators of combined stress responses, and are

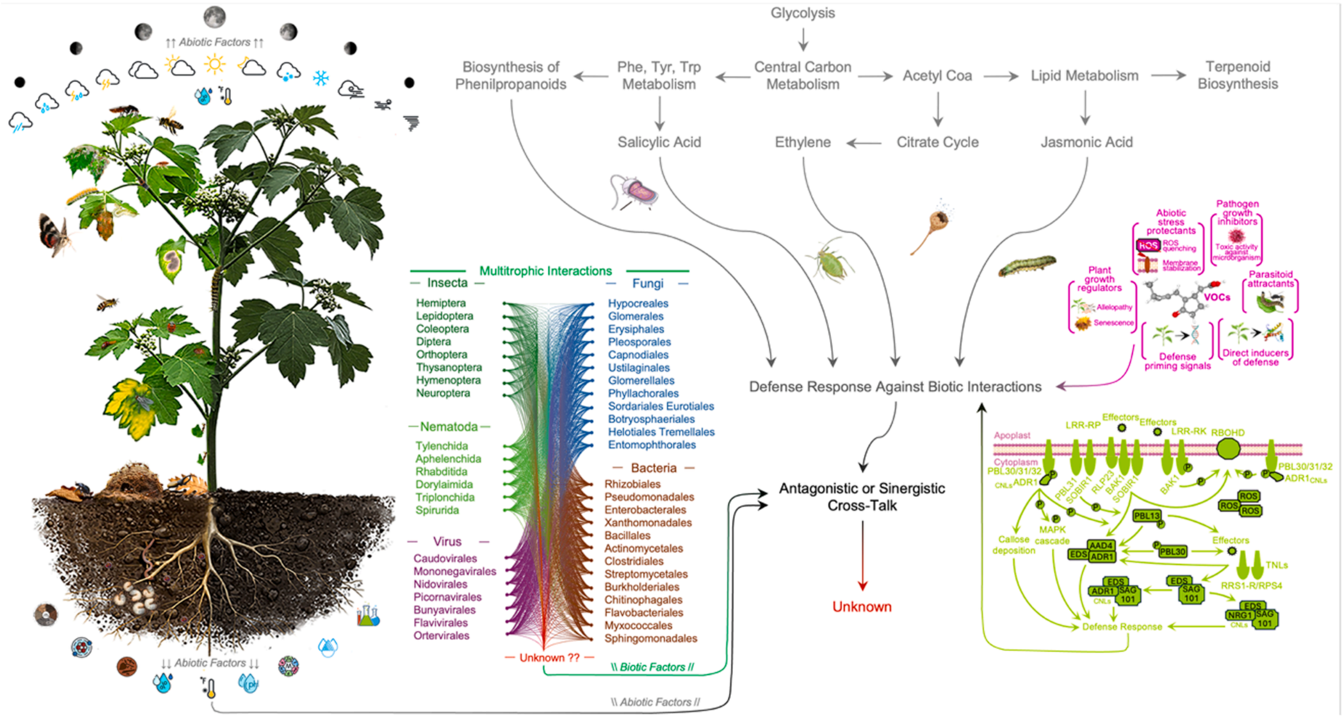


Fig. 1. Schematic representation of the complexity of biotic and abiotic interactions influencing plant physiology and metabolic processes. Biotic factors that interact with plants, including insects, nematodes, fungi, bacteria, and viruses, may form mutualistic, pathogenic, or complex trophic network relationships involving organisms from many different families. Biotic signaling mechanisms include the activation of PRRs (Pattern Recognition Receptors) and NLRs (Nucleotide-binding Leucine-rich Repeat Receptors) which are essential for defense against pests and pathogens. Complex molecular cross-talks between immunity hormone signaling pathways in multitrophic interactions are still poorly understood. Volatile organic compounds (VOCs) mediate inter- and intraspecific communication, influencing plant-microorganism and plant-insect interactions. Abiotic stresses can further affect plant responses to these organisms and alter biochemical processes in plant metabolism and defense. The understanding on how each stress impact plants molecular response to other concomitant interactions is paramount for the development of more resilient crops prepared to withstand future challenges.

being increasingly used to understand them in plants (Dangi et al., 2018; Schneider et al., 2022). Despite these advances, the question of how plants will respond to multiple biotic stressors striking at the same time or in sequence remains in debate.

In multifactorial biotic stresses, the type of defense response induced appears to depend on the order and intensity that each stressor interacts with the plant. For example, a study showed that *Eurydema oleracea* herbivory in *Arabidopsis* causes the induction of the SA pathway as a strategy to antagonize JA signaling that would deter its feeding (Costarelli et al., 2020). However, when plants were pre-inoculated with *Botrytis cinerea*, a necrotrophic pathogen, a JA-dependent response was triggered, which consequently decreased leaf damage caused by subsequent herbivory. Yet, this effect was not seen when the pre-inoculation was carried out with the biotrophic pathogen *Golovinomyces orontii*, indicating that the pre-induction of JA was beneficial for the plant against a following insect interaction (Ederli et al., 2021). Similarly, pre-inoculation of *Arabidopsis* plants with *B. cinerea* delayed caterpillar-induced transcriptome changes caused by subsequent herbivory of *Pieres rapae*, in early time points of 3 h, although changes soon converged to an insect response (Davila Olivas et al., 2016). Conversely, *P. rapae* herbivory on *Arabidopsis* induced a JA-response that proved ineffective against the necrotrophic fungi *Alternaria brassicola*, but bolstered local defense against bacterial pathogens *Pseudomonas syringae* pv *tomato* and *Xanthomonas campestris* pv *armoriciae* (De Vos et al., 2006). Nevertheless, a transcriptomics study in *Brassica nigra* showed that *Pieres brassicae* herbivory dominated the transcriptome response even when plants were pre-treated with *X. campestris* pv. *raphanin*, egg extracts, or aphids, while larvae gained more weight from plants pre-treated with pathogen than egg extracts (Bonnet et al., 2017). Authors hypothesize that the high induction of SA under pathogen treatment might have led to decreased JA response due to antagonism, and thus decreased defense against the larvae. Regardless, eggs or egg extracts of *P. brassicae* deposited on *Arabidopsis* leaves were able to induce systemic acquired resistance, via SA, that reduced growth of bacterial pathogen *Pseudomonas syringae* and inhibited *B. cinerea* and *Hyaloperonospora arabidopsidis* infection (Alfonso et al., 2021; Hilfiker et al., 2014). Although the defense response against *B. cinerea* is expected to be more JA-dependent, it is suggested that SA might play a partial role in the defense against this pathogen (Alfonso et al., 2021). This intricate response network highlights that much still needs to be learned about hormone cross-talk in multifactorial biotic stresses. These findings still need to be validated in more realistic field trials, with crops attacked by these pests and pathogens, while enduring natural field variations and interactions. The aforementioned studies make evident that the order and intensity that each stress interacts with the plant host are crucial in defining its defense response, but it remains to be seen if this is always the case.

Antagonistic multitrophic interactions were also reported in crops. In wheat, infection by powdery mildew *Blumeria graminis* reduced subsequent *Sitobion avenae* aphid performance while attracting its natural enemy, *Aphidius gifuensis*, likely due to SA pathway gene induction (Kang et al., 2018). However, *S. avenae* infestation, followed by *Fusarium graminearum* infection, led to increased disease severity, mycotoxin accumulation, and the suppression of pathogenesis-related genes compared to those not pre-treated with the aphid (De Zutter et al., 2016). Similarly, *F. graminearum* was shown to benefit from concomitant interaction with *S. avenae*, also exhibiting faster disease progression and mycotoxin accumulation when both treatments were combined (Drakulic et al., 2015). Most likely, the hormonal cross-talk of SA and JA is involved, meaning, in practical terms, that the underlying mechanisms governing this host-pathogen interaction in the field are directly influenced by the presence or absence of the aphid. In a similar manner, herbivory by *Diatraea saccharalis* in sugarcane leads to strong upregulation of pathogenesis-related proteins that have no effect against the insect, but rather act against phytopathogenic fungi that commonly occur after mechanical damage (Franco et al., 2014; Medeiros et al., 2012). These examples underscore why studies seeking to understand plant defense to pests and pathogens should consider their other biological interactors, which might be present in natural environments, and have co-evolved complex traits and responses. The classification of the diverse set of strategies that plants adopt for multiple biotic stresses arriving simultaneously or sequentially has already been proposed for herbivores, but is still lacking for the other multitude of organisms that commonly interact with plants (de Bobadilla et al., 2022).

Not only insects modulate plant pathogen responses, but co-infection by multiple pathogens also influences their reciprocal disease severity and progression (Tollenaere et al., 2016). While one might assume that the co-occurrence of two detrimental biotic stresses (e.g., pest + pathogen, or pathogen + pathogen) invariably increase plant damage, this is not always true. In potato, combined infection by *Alternaria solani* and *Phytophthora infestans* resulted in an increased severity effect only for *A. solani*, both in plant and in field trials (Brouwer et al., 2023). Authors hypothesize that the necrotrophic lifestyle of *A. solani* could lead to the death of cells needed for the early biotrophic phase of *P. infestans*, thus hindering the start of the infection. Another possibility is that molecules secreted by *A. solani* could lead to cell death of *P. infestans*, thereby preventing its survival (Brouwer et al., 2023). On the other hand, *P. infestans* secretes effectors that could be hampering potato's immune response or leading to increased SA production, thus favoring subsequent infection by *A. solani* (Brouwer et al., 2023). In this case, the order of infection does matter and has a direct impact on disease dynamics in the field. A summary of multifactorial stress interactions and their key findings is presented in Table 1.

To date, few studies have investigated how plants respond to multiple combined biotic stresses, and many open questions remain. Will plants always prioritize the most intense stress (e.g., herbivory), or does order matter most? What are the molecular cues that cause plants to shift defense pathways for one stressor in detriment of the other, and how can we manipulate them to breed better crops? If SA and JA are not always the sole messengers, as indicated by the previous examples, which other hormones and signaling molecules are involved in combined biotic interactions? Most importantly, how applicable are the conclusions drawn from single interaction studies, performed under controlled conditions, to the actual plant response in natural environments and crop fields? If we aim to better translate basic research into applied technology, more effort must be dedicated to understand multifactorial stresses in plant research.

The role of the microbiome in multifactorial interactions

Recently, it has been proposed that plants and their associated microbiome form a single entity known as the plant holobiont, which is evolutionary influenced as a whole (Trivedi et al., 2022; Vandenkoornhuyse et al., 2015). Growing evidence also suggests that belowground communities significantly affect aboveground plant phenotypes. Notably, root-associated microbiota serves as a first line of defense against many pathogens, mediating plant immunity, fitness, and growth under various stress scenarios (Liu et al., 2020; Teixeira et al., 2019; Trivedi et al., 2022, 2020). In this context, hormonal cross-talk is especially important. Plants evolved mechanisms to recruit rhizobacteria reported to suppress disease progression and insect colonization, primarily by activating the JA pathway (Carvalhais et al., 2017). Other microbes also induce systemic resistance through SA-dependent responses, priming plants against future biotic stressors (Teixeira et al., 2019). In the case of multifactorial biotic interactions, studies have demonstrated how microbial consortia can suppress pathogens or exacerbate their effects, depending on environmental conditions (Carrion et al., 2019; Trivedi et al., 2022). This is particularly critical given the climate change scenario, as many abiotic stresses, such as drought, lead to abscisic acid (ABA) signaling and repression of JA and ET, thereby hindering plant defense responses (Anderson et al., 2004; Prasch and Sonnewald, 2015). However, how the plant holobiont is

Table 1
Summary of key findings on the impact of stress order and intensity in multi-factorial biotic interactions.

Interacting Organisms	Order/Intensity	Key Findings	References
Eurydema oleracea (herbivore) on *Arabidopsis*	*E. oleracea* herbivory	Induction of the SA pathway that antagonize JA signaling	(Davila Olivas et al., 2016)
Botrytis cinerea (necrotroph) + *E. oleracea* on *Arabidopsis*	Pre-inoculation with *B. cinerea* followed by *E. oleracea* herbivory	Prior activation of JA-dependent response decreased leaf damage caused by subsequent herbivory	(De Vos et al., 2006)
Golovinomyces orontii (biotroph) + *E. oleracea* on *Arabidopsis*	Pre-inoculation with *G. orontii* followed by *E. oleracea* herbivory	Activation of SA pathway did not alter subsequent herbivory	(De Vos et al., 2006)
Botrytis cinerea + *Pieres rapae* (herbivore) on *Arabidopsis*	Pre-inoculation with *B. cinerea* followed by *P. rapae* herbivory (early time points of 3 h)	Delayed caterpillar-induced transcriptomic changes	(Bonnet et al., 2017)
P. rapae + *Alternaria brassicola* (necrotroph) on *Arabidopsis*	Herbivory by *P. rapae* followed by *A. brassicola* infection	JA-induced response was ineffective against the subsequent necrotrophic fungus	(Hilfiker et al., 2014)
P. rapae + *Pseudomonas syringae pv tomato / X. campestris pv armoriciae* (hemibiotroph) on *Arabidopsis*	*P. rapae* herbivory followed by pathogen infection	The JA-induced response increased local defense against bacterial pathogens	(Hilfiker et al., 2014)
Pieres brassicae (herbivore) + *Brevicoryne brassicae* (aphid) + *X. campestris* pv. *raphani* (hemibiotroph) + egg extracts on *Brassica nigra*	Herbivory by *P. brassicae* on plants pre-treated with *X. campestris pv. raphanin*, egg extracts, or aphids	*P. brassicae* herbivory dominated the transcriptomic response in all pre-treatment combinations. Different marker genes in each combination suggest SA/JA cross-talk	(Alfonso et al., 2021)
Eggs / egg extracts of *P. brassicae* on *Arabidopsis* + *Pseudonomas syringae* (hemibiotroph) + *Botrytis cinerea* (necrotoph) + *Hyaloperonospora arabidopsidis* (oomycete)	Egg or egg extract treatment followed by pathogen infection	Induction of systemic acquired resistance (SAR) via SA, which reduced *Pseudomonas syringae* growth and inhibited *B. cinerea* and *Hyaloperonospora arabidopsidis* infection	(De Zutter et al., 2016; Kang et al., 2018)
Blumeria graminis (powdery mildew) + *Sitobion avenae* (aphid) on wheat	Infection by powdery mildew *B. graminis* followed by *S. avenae* infestation	*B. graminis* reduced aphid performance and attracted its natural enemy (*Aphidius gifuensis*), likely due to SA pathway induction	(Drakulic et al., 2015)
S. avenae + *Fusarium graminearum* (hemibiotroph) on wheat	*S. avenae* infestation followed by *F. graminearum* infection	Increased disease severity, mycotoxin accumulation, and suppression of pathogenesis-related genes compared to plants not pre-treated with the aphid	(Medeiros et al., 2012)
Diatraea saccharalis (herbivore) + *Colletotrichum falcatum* (hemibiotroph) + *Fusarium verticillioides* (hemibiotroph) on sugarcane	Herbivory by *D. saccharalis*	Strong upregulation of pathogenesis-related proteins that have no effect against the insect, but show antifungal activities against phytopathogenic fungi	(de Bobadilla et al., 2022; Tollenaere et al., 2016)
Alternaria solani + *Phytophthora infestans* (necrotroph) on potato	Co-infection by *A. solani* and *P. infestans*	Increased disease severity only for *A. solani*	(Trivedi et al., 2020)

modulated in response to concomitant biotic interactors, like multiple pathogens or insects, is still scarcely comprehended. Nevertheless, recent studies are beginning to shed light on these complex interactions, revealing the dynamic role of the microbiome in mediating plant responses to combined biotic challenges. Knowing which microbes are beneficial or detrimental in specific biotic interactions could be key to develop sustainable agricultural practices (Arif et al., 2020).

For instance, the interplay between soil microbiomes and multiple aboveground stressors has been investigated in a multifactorial study with oak seedlings (*Quercus robur*) (van Dijk, L.J. et al., 2022). Plants were exposed to single, dual, or triple sequential or concomitant stresses involving powdery mildew (*Erysiphe alphitoides*), aphids (*Tuberculatus annulatus*), and caterpillars (*Phalera bucephala*), while growing in soils with distinct natural microbiomes. The specific soil microbiome and the combination of stresses significantly affected the severity of powdery mildew disease. Further, these combinations mediated the impact on aphid population size (van Dijk, L.J. et al., 2022). Surprisingly, the combined treatment of *E. alphitoides* and *T. annulatus* on *Q. robur* plants did not alter the plants metabolome significantly, whereas single stressors did (van Dijk, L.J.A. et al., 2022). It is, thus, suggested that a potential negative feedback mechanism might be involved when plants face concomitant aphid and mildew interaction (van Dijk, L.J.A. et al., 2022). Similar principles of plant–microbiome–multitrophic interaction have also been observed in crop species. In *Brassica oleracea*, different insect herbivory treatments, targeting either shoots or roots, distinctly altered the soil microbiome composition, and influenced later plant growth. When subsequent plants were grown in these differentially conditioned soils, and then exposed to root-feeding *Delia radicum* larvae, plant growth and resistance were affected by the prior insect–microbiome interactions. While all soil conditioning treatments generally led to reduced growth of plants, those conditioned by prior *Plutella xylostella* shoot herbivory mitigated this negative effect, resulting in comparatively better growth than plants in other conditioned soils (Friman et al., 2021). Together, these systems demonstrate how soil microbiome composition and history can modulate plant phenotypes and responses to multiple, simultaneous above- and belowground biotic stressors. Thus, considering legacy effects and current microbiome composition is crucial to understand plant resilience in complex biotic environments.

Beyond roots and soil, aboveground structures also harbor a specialized microbiome, which was shown to be dynamically modulated in maize silks in response to ear infection by Fusarium species (Adams et al., 2024; Khalaf et al., 2021). Infection by pathogenic *Fusarium* spp., including *F. verticillioides* and *F. graminearum*, is a major concern due to kernel contamination, Fusarium Ear Rot, and mycotoxin accumulation (Munkvold, 2003). Interestingly, the general silk microbiome of 14 genotypes of healthy maize plants was similar, although some annual fluctuations occurred (Khalaf et al., 2021). However, infection by *F. graminearum* lead to a twofold increase in 16S read counts and a substantial reduction in microbial taxa, indicating a selection for a core silk microbiome during pathogen challenge (Khalaf et al., 2021). Nonetheless, environmental conditions drove the microbial diversity of maize kernels, where a prevalence of *Fusarium* spp. was identified in both healthy and *F. verticillioides* infected plants (Adams et al., 2024). Authors hypothesize that some *Fusarium* species, such as *F. subglutinans*, might compete with *F. verticillioides* and thus hinder disease progression, while most other identified *Fusarium* species could be synergistic (Adams et al., 2024). While disease resistance is often attributed to host genes alone, there is growing evidence for a significant role of host-mediated microbiome assembly in disease scenarios. For example, stalk rot resistant maize varieties can recruit core *Bacillus* strains from roots to stalks, contributing to defense against *F. graminearum* (Xia et al., 2024). Notably, a *Bacillus* synthetic community, SC-III, led to increased resistance due to activation of maize secondary metabolite pathways linked to defense, rather than through the production of direct antimicrobial peptides (Xia et al., 2024). This suggests that, in addition to classical resistance genes and molecules, the composition and function of the plant microbiome are essential components of biotic stress responses, and should be further considered in biotic stress research.

One mechanism by which microbes influence plant defense responses is through the induction of defense pathways, known as induced systemic resistance (ISR) (Sugio et al., 2015). By priming plant defenses, bacteria and fungi can prepare plants for impending attacks, leading to higher expression of defense-related genes. Overall, this induction also helps plants defend against insect herbivores, although the degree of specialization of the herbivore seems to be relevant. For example, *Arabidopsis* plants primed in the roots by non-pathogenic *Pseudomonas fluorescens* led to decreased weight gain of generalist *Spodoptera exigua*, but not against specialist *P. rapae* (Van Oosten et al., 2008). Interestingly, three *Arabidopsis* accessions, primed in the leaves with bacterial suspensions of two pathogenic (*P. syringae* DC3000 and *X. campestris*) and one non-pathogenic (*Bacillus cereus*) bacteria, also exhibited increased performance under constant herbivory by a generalist fungus gnat compared to non-inoculated plants (Saleem et al., 2017). Although spraying a pathogenic bacterial suspension might typically lead to disease and decreased plant growth, the low inoculum concentration in two-week-old seedlings likely facilitated the incorporation of these bacteria into the plant's microbiome community (Saleem et al., 2017). On the other hand, phloem-feeding or chewing herbivory can also shape root-associated microbial diversity independently, adding further complexity to microbiome modulation in plants under stress (Xing et al., 2024). The holobiont concept need to be further considered to fully comprehend pest and disease dynamics in the field. This is particularly critical given the limited availability of studies that integrate multiple concomitant or sequential biotic treatments on plant-associated microbiome modulation.

Nonetheless, the insect microbiome also plays an important role in determining plant-insect biotic interactions. The gut microbiota of many insect pests provides nutrients or detoxifies plant metabolites, thereby allowing insects to live and reproduce on plants (Guillen Sanchez, 2021). For instance, over the course of four generations, the generalist pest *Trichoplusia ni* selected for gut genera that either degrade glucosinolate compounds, when fed in *Arabidopsis*, or that degrade alkaloids, when fed in *Solanum lycopersicum* (Leite-Mondin et al., 2021). Similarly, a *Pseudomonas fulva* strain, identified in the gut of specialist *Bombyx mori*, was shown to detoxify the DNJ metabolite from mulberry, and confer this tolerance to non-specialist Lepidopterans upon inoculation (Zhang et al., 2024). Hence, identifying plant defense genes and pathways induced by insects requires careful consideration, as the resulting products could be degraded by the gut microbiome, rendering them ineffective in real environments. On top of that, microbial exchange and community modulation between plants and herbivores commonly occur. Plant-associated microbes, including endophytes, influence the composition of the insect gut microbiome and can be transferred between hosts, often conferring adaptive advantages to the herbivore, such as detoxifying plant defenses (Pirttila et al., 2023). Additionally, insect herbivory was shown to significantly reshape the plant leaf microbiome, amplifying specific bacterial taxa, such as putatively phytopathogenic *Pseudomonas syringae*, while reducing overall diversity (Humphrey and Whiteman, 2020). This reciprocal microbial interchange can also facilitate invasive insect species' adaptation to new hosts and environments (Zhang et al., 2024). Given the effects of climate change in fostering new biotic interactions, the presence of microbes that confer tolerance to toxic compound and facilitate insect adaptation requires thorough consideration. Incorporating more variables into biotic stress studies is thus imperative to assess the real-world impact of research discoveries.

The chemical ecology behind multitrophic interactions

Volatile organic compounds (VOCs) play a fundamental role in the chemical ecology that mediates trophic interactions between plants, herbivores, and microorganisms (Takabayashi, 2022; Weisskopf et al., 2021). Specifically, VOCs serve as essential defense and communication mechanisms between plants and their pests and pathogens. Under herbivore attack, plants release specific VOCs that can attract natural enemies or alert neighboring plants to anticipate their defensive responses (Franco et al., 2014; Medeiros et al., 2012). Similarly, pathogen recognition induces antimicrobial VOCs that can act directly to reduce pathogen growth, or indirectly by activating plants defense (Abbas et al., 2022; Duc et al., 2022). Root-emitted volatiles mediate attractive or repellent interactions between plant roots and nematodes, and are crucial for shaping belowground interactions, by suppressing fungal pathogens or enhancing beneficial mycorrhiza (Massalha et al., 2017; Wenke et al., 2010). Moreover, seagrasses can release volatile cues that influence microbial colonization and herbivore behavior in aquatic ecosystems (Akakabe and Kajiwara, 2009; Saha and Fink, 2022). These findings reinforce the ecological importance of VOCs as key regulators of multitrophic interactions across diverse environments. By altering their VOC profiles, plants can reshape multitrophic networks, influencing herbivore behavior and microbial community structure.

Microbes also release a plethora of VOCs that can have distinct effects in plant physiology, insect behavior, and microbe-microbe communication (Schulz-Bohm et al., 2017). Microbial VOCs (mVOCs) can directly alter host plant metabolism, promoting growth, alteration in hormonal pathways or even priming for pathogen defense (Gamez-Arcas et al., 2022; Weisskopf et al., 2021). Interestingly, Arabidopsis plants could not distinguish between pathogenic or non-pathogenic mVOC profiles, while both promoted growth compared to control. This effect also led to increased susceptibility to subsequent leaf chewing herbivory, highlighting a complex trade-off between growth and defense (Moisan et al., 2019). Some species of *Trichoderma*, known for their biocontrol activity, can emit mVOCs that inhibit pathogen growth and induce systemic resistance in plants (Contreras-Cornejo et al., 2016). Similar interactions have been observed in mosses, where VOCs mediate the suppression of fungal colonization and prevent microbial overgrowth in high-humidity environments (Briard et al., 2016; Vicherova et al., 2020). However, our understanding of VOC modulation in multifactorial biotic interactions is still scarce, and few solid examples illustrate this complex mechanism.

In an extensively studied case in natural ecosystems, the bark beetle *Ips typographus* causes severe damage to spruce forests, mainly due to frequent outbreaks and its interaction with phytopathogenic symbiont fungi (Six and Wingfield, 2011). Different genera of bark beetle symbionts are pathogens of spruce trees and benefit from their association with *I. typographus* (DiGuistini et al., 2011; Zhao et al., 2019). On the other hand, these fungi aid bark beetles most likely by exhausting tree defenses (Six and Wingfield, 2011). Interestingly, immature adult beetles are attracted for media colonized by their symbiotic fungi over other saprophytic species, and volatiles released by symbionts can be detected by beetle antenna (Kandasamy et al., 2019). Strikingly, fungal symbionts were able to convert spruce tree monoterpenes to a blend of volatiles that attracted *I. typographus* and stimulated tunneling (Kandasamy et al., 2023). Moreover, bark beetles' attacks were shown to alter the VOC profile of native spruce trees at outbreak areas, correlating with increased insect abundance (Ghimire et al., 2016). Although *I. typographus* preferred symbiont infected over fungus-free bark trees regardless, this preference was independent of native or naïve host and symbiont species (Tanin et al., 2021). This is particularly alarming, as it suggests that different geographical *Ips* species could easily adapt to new hosts and environments, even from different continents.

Similar insect behavior modulation by microbial VOCs have also been reported in crop multitrophic interactions. In sugarcane, pathogenic *F. verticillioides* and *Colletotrichum falcatum* produce volatiles that increase *D. saccharalis* attraction, both in larvae and adults (Franco et al., 2021). Interestingly, adult moths without the fungi prefer to oviposit in infected over healthy sugarcane plants. However, when insects were previously contaminated with either *F. verticillioides* or *C. falcatum*, moths were more attracted to healthy plants, possibly increasing pathogen dissemination in the field (Franco et al., 2021, 2022).

In a broader ecological context, VOCs emitted by plant-pathogen interactions have also been shown to influence insect pollinators, as floral scent alterations caused by pathogen infections can reduce pollination success (Cellini et al., 2019). Furthermore, VOCs are not only used defensively but can also serve as cues for parasitic plants such as *Cuscuta*, which detects volatile signals from host plants to guide its haustorial growth and attachment (Runyon, 2008). VOCs can also mediate indirect interactions by altering the composition of rhizosphere microbiota, promoting microbial communities that are more resilient against infections (Kang et al., 2021). Interestingly, field inoculation of *Trichoderma harzianum* in maize was recently shown to regulate the aboveground community of interacting arthropods. By inducing JA defense and volatile compounds, *T. harzianum* inoculation led to an increase in chewing herbivores and their predators, while reducing pierce-sucking insects (Contreras-Cornejo et al., 2021).

Thus, elucidating the role of VOCs in plant biotic interactions can open new perspectives for agricultural biotechnology, enabling the exploitation of these natural metabolites as tools for integrated pest and disease management. However, the understanding of what a given volatile means in terms of biological significance must consider the multiple organisms that interact with it, in natural or field conditions. Therefore, characterizing VOC emissions in plants subjected to multiple concomitant or sequential stresses can provide valuable insights into their molecular communication. Understanding their evolutionary role in nature is crucial to develop sustainable agricultural management strategies that exploit VOC-mediated regulation of plant biotic interactions.

Transitioning from binary to multifactorial stresses in plant research

In crop fields and natural ecosystems, plants are constantly exposed to a multitude of concomitant or sequential biotic interactions. While our understanding of plant responses to individual pests and pathogens has advanced considerably, research that evaluates plant performance under multiple, combined stress variables remains limited. Plant stress research has largely relied on reductionist models and highly controlled growth conditions (e.g., growth chambers, single biotic interactors, undefined microbiome), which hardly reflects native and field environments. Consequentially, many laboratory findings have often failed to be effectively employed in the field (Atkinson et al., 2014; Mittler, 2006). Although the isolation of variables was crucial for dissecting fundamental immune signaling networks, hormonal pathways, and defense mechanisms, it is evident that more complex stress combinations must be investigated. Current and future agricultural and ecological challenges, intensified by climate change, demand a shift towards more realistic, multifactorial stress scenarios in plant stress research.

To advance the field and enhance the applicability of laboratory findings, we propose an integrated framework for designing and executing multifactorial biotic stress research in plants (Fig. 2). First, the foundation of any such study should be in the ecological and field contextualization of a given plant model. This requires identifying co-occurring pests, pathogens, and other interacting organisms specific to the target plant species, cropping system, or geographical location. Proper interactor identification relies on thorough investigation of biotic stress literature of a given plant species, ideally complemented by consultation with farmers for cropping systems, and ecologists for natural ecosystems. However, the limited availability of multifactorial stress research represents a bottleneck, as documented synergistic, additive, or antagonistic interactions for specific organisms may be absent. Nevertheless, this also reflects an open field for elucidating new mechanisms and cross-talks in plant defense response.

Second, experimental design must incorporate at least two co-occurring interactions in ecologically relevant concomitant or sequential patterns. This requires specifically defining the order, timing, and intensity of each desired stress to capture dynamic outcomes. We advocate for the implementation of factorial designs that move beyond simplified pairwise interactions, integrating combinations of herbivores (chewing insects and/or phloem-feeders) and pathogens (necrotrophic and/or biotrophic) in both simultaneous and sequential applications. Additionally, experiment design should always include single-stress and unstressed control treatments. Ideally, soil and plant microbiome, and its modulation upon stress, should be characterized to reduce inter-experiment variation, which could be further mitigated by using synthetic microbial communities in sterilized soil. Furthermore, a comprehensive understanding of plant-environment communication requires deeper exploration of the chemical ecology governing these multitrophic interactions. Particularly, characterizing plant and microbial VOCs during multifactorial stress combinations can be promising for the identification of candidate molecules for field application. Inter- and intra-kingdom communication signals, alongside non-volatile compounds and root exudates that influence above and belowground interactions are key targets.

Phenotypic evaluation of plant fitness under different stress combination is paramount when considering novel stress combinations in model pathosystems. For instance, plant growth parameters, disease progression and insect damage are important measurements, that can be compared between individual and combined stresses, to identify unique phenotypes. System biology approaches using multi-omics are essential tools for generating hypotheses related to dynamic and systemic metabolic alterations in combined stress, and should be employed thoroughly. Key candidate genes and pathways identified therein require further functional validation, which can be performed by evaluating overexpression and knockout lines against concomitant or simultaneous stress scenarios. Candidate metabolites can be directly tested if synthetic compounds are available, either by eliciting plants defense response, or directly modulating disease progression or insect behavior. Finally, findings validated in controlled or semi-controlled conditions must be readily assayed for efficacy in natural and field environments. Depending on the outcome, successful traits can be effectively added to breeding programs, or new hypothesis should be generated if flaws in experiment design are identified.

In conclusion, the traditional binary approach in plant stress research, while fundamental, is insufficient to address the complex ecological realities and agricultural challenges of a changing world. The integrated framework proposed herein offers a structured pathway to transition from binary to multifactorial experimental designs. By systematically incorporating ecological networks, factorial experimental setups, and field validation pipelines, the intricate defense responses that govern plant performance in natural environments can be further elucidated. Integrating the exponential complexity of multifactorial stress into experimental frameworks represents a critical shift for the field, and is crucial for developing multi-resilient crops and preserving natural ecosystems in the face of climate change.

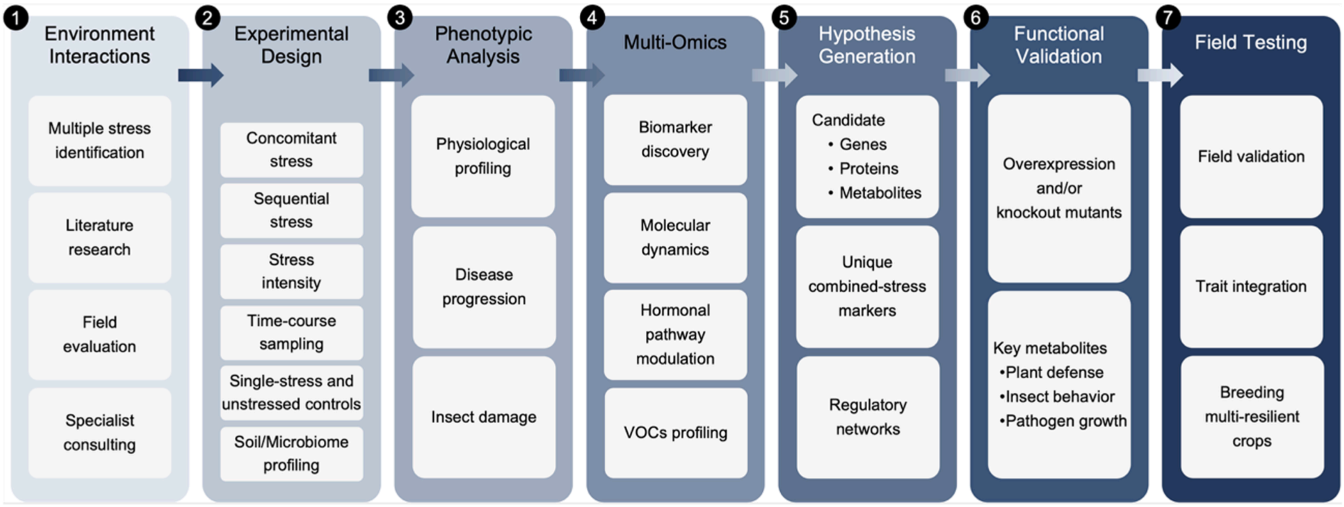


Fig. 2. Integrative framework for multifactorial biotic stress research in plants. The scheme details an integrated methodology for incorporating multiple variables in biotic stress research, and is divided in seven sequential stages: (1) Environment Interactions and the ecological context need to be considered to identify co-occurring stresses for a given plant model, and should be done through literature research and expert consultation. (2) Experimental Design need to integrate concomitant and sequential stress scenarios, defining stress order and intensity to be applied, and ideally with time-course sampling. Single-stress and unstressed controls are necessary to identify unique responses and reduce bias. The microbiome characterization is crucial to understand its dynamics, and reduce inter-experiment variability. (3) Phenotypic Analysis, especially in novel stress combinations, should focus on evaluating plant growth parameters, disease progression, and insect damage under multiple stress scenarios to identify synergistic or antagonistic interactions; (4) Multi-Omics approaches are essential tools for biomarker discovery, hormonal pathway cross-talk, VOC profiling and unique molecular dynamics caused by multifactorial stresses. (5) Hypothesis Generation is the fundamental step where candidate molecules and unique combined-stress markers are linked to regulatory networks and the respective phenotype. (6) Functional Validation should evaluate overexpression and knockout lines against multiple simultaneous or sequential biotic interactions to validate hypotheses. Key metabolites, as VOCs, can be directly tested on their role eliciting plant defense, modulating insect behavior, or directly acting on pathogen growth. (7) Field Testing represents the final step in integrating laboratory findings into traits that can breed multi-resilient crops.

Author information

These authors contributed equally to this review: Augusto B. Penteriche and Diego Z. Gallan.

CRediT authorship contribution statement

Augusto B. Penteriche: Writing – original draft, Methodology, Conceptualization. **Diego Z. Gallan:** Writing – original draft, Methodology, Investigation, Formal analysis. **Marcio C. Silva-Filho:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marcio C. Silva-Filho reports financial support and administrative support were provided by State of Sao Paulo Research Foundation. Marcio C. Silva-Filho reports a relationship with State of Sao Paulo Research Foundation that includes: funding grants. Not applicable. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grants 2019/15488–5 and 2022/09473–8 to MCSF, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Brasil (CAPES) – Finance Code 001. DZG is the recipient of FAPESP fellowships 2021/06565–6 and 2023/01945–0 and ABP is recipient of a CAPES fellowship; MCSF is also research fellow of CNPq.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2025.100963.

Data availability

No data was used for the research described in the article.

References

Abbas, F., O'Neill Rothenberg, D., Zhou, Y., Ke, Y., Wang, H.C., 2022. Volatile organic compounds as mediators of plant communication and adaptation to climate change. Physiol. Plant 174 (6), e13840. https://doi.org/10.1111/ppl.13840.

Adams, A., Landry, D., Sykes, V., Rickman, T., Cham, A.K., Timling, A., Kelly, H., McBeath, J.H., Olukolu, B.A., 2024. Maize kernel-associated metagenomes reveal potential microbe–microbe interactions that underlie fusarium ear Rot disease. Phytobiomes. J. 8 (3), 327–339.

Akakabe, Y., Kajiwara, T., 2009. Bioactive volatile compounds from marine algae: feeding attractants. In: Nineteenth International Seaweed Symposium: Proceedings of the 19th International Seaweed Symposium, held in Kobe, Japan, 26-31 March 2007. Springer, pp. 211–214.

Alfonso, E., Stahl, E., Glauser, G., Bellani, E., Raaymakers, T.M., Van den Ackerveken, G., Zeier, J., Reymond, P., 2021. Insect eggs trigger systemic acquired resistance against a fungal and an oomycete pathogen. New. Phytol. 232 (6), 2491–2505. https://doi.org/10.1111/nph.17732.

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R., Kazan, K., 2004. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. Plant Cell 16 (12), 3460–3479. https://doi.org/10.1105/tpc.104.025833.

Arif, I., Batool, M., Schenk, P.M., 2020. Plant microbiome engineering: expected benefits for improved crop growth and resilience. Trends Biotechnol. 38 (12), 1385–1396. https://doi.org/10.1016/j.tibtech.2020.04.015.

Atkinson, N.J., Jain, R., Urwin, P.E., 2014. The Response of Plants to Simultaneous Biotic and Abiotic stress, Combined stresses in plants: Physiological, molecular, and Biochemical Aspects. Springer, pp. 181–201.

Blois, J.L., Zarnetske, P.L., Fitzpatrick, M.C., Finnegan, S., 2013. Climate change and the past, present, and future of biotic interactions. Science 341 (6145), 499–504. https://doi.org/10.1126/science.1237184.

Bonnet, C., Lassueur, S., Ponzio, C., Gols, R., Dicke, M., Reymond, P., 2017. Combined biotic stresses trigger similar transcriptomic responses but contrasting resistance against a chewing herbivore in brassica nigra. BMC. Plant Biol. 17 (1), 127. https://doi.org/10.1186/s12870-017-1074-7.

Briard, B., Heddergott, C., Latge, J.P., 2016. Volatile compounds emitted by Pseudomonas aeruginosa stimulate growth of the fungal pathogen Aspergillus fumigatus. mBio 7 (2), e00219. https://doi.org/10.1128/mBio.00219-16.

Brouwer, S.M., Wolters, P.J., Andreasson, E., Liljeroth, E., Vleeshouwers, V.G., Grenville-Briggs, L.J., 2023. Double trouble: co-infection of potato with the causal agents of late and early blight. Plant Pathol. 72 (8), 1365–1379.

Carrion, V.J., Perez-Jaramillo, J., Cordovez, V., Tracanna, V., de Hollander, M., Ruiz-Buck, D., Mendes, L.W., van Ijcken, W.F.J., Gomez-Exposito, R., Elsayed, S.S., Mohanraju, P., Arifah, A., van der Oost, J., Paulson, J.N., Mendes, R., van Wezel, G. P., Medema, M.H., Raaijmakers, J.M., 2019. Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. Science 366 (6465), 606–612. https://doi.org/10.1126/science.aaw9285.

Carvalhais, L.C., Schenk, P.M., Dennis, P.G., 2017. Jasmonic acid signalling and the plant holobiont. Curr. Opin. Microbiol. 37, 42–47. https://doi.org/10.1016/j.mib.2017.03.009.

Cellini, A., Giacomuzzi, V., Donati, I., Farneti, B., Rodriguez-Estrada, M.T., Savioli, S., Angeli, S., Spinelli, F., 2019. Pathogen-induced changes in floral scent may increase honeybee-mediated dispersal of Erwinia amylovora. ISMe J. 13 (4), 847–859. https://doi.org/10.1038/s41396-018-0319-2.

Chapin, F.S., Bloom, A.J., Field, C.B., Waring, R.H., 1987. Plant responses to multiple environmental factors. Bioscience 37 (1), 49–57.

Contreras-Cornejo, H.A., Macias-Rodriguez, L., del-Val, E., Larsen, J., 2016. Ecological functions of trichoderma spp. And their secondary metabolites in the rhizosphere: interactions with plants. FEMS. Microbiol. Ecol. 92 (4), fiw036. https://doi.org/10.1093/femsec/fiw036.

Contreras-Cornejo, H.A., Viveros-Bremauntz, F., Del-Val, E., Macías-Rodríguez, L., López-Carmona, D.A., Alarcón, A., González-Esquivel, C.E., Larsen, J., 2021. Alterations of foliar arthropod communities in a maize agroecosystem induced by the root-associated fungus trichoderma harzianum. J. Pest. Sci. 94, 363–374.

Costarelli, A., Bianchet, C., Ederli, L., Salerno, G., Piersanti, S., Rebora, M., Pasqualini, S., 2020. Salicylic acid induced by herbivore feeding antagonizes jasmonic acid mediated plant defenses against insect attack. Plant Signal. Behav. 15 (1), 1704517. https://doi.org/10.1080/15592324.2019.1704517.

Dangi, A.K., Sharma, B., Khangwal, I., Shukla, P., 2018. Combinatorial interactions of biotic and abiotic stresses in plants and their molecular mechanisms: systems biology approach. Mol. Biotechnol. 60 (8), 636–650. https://doi.org/10.1007/s12033-018-0100-9.

Davila Olivas, N.H., Coolen, S., Huang, P., Severing, E., van Verk, M.C., Hickman, R., Wittenberg, A.H., de Vos, M., Prins, M., van Loon, J.J., Aarts, M.G., van Wees, S.C., Pieterse, C.M., Dicke, M., 2016. Effect of prior drought and pathogen stress on Arabidopsis transcriptome changes to caterpillar herbivory. New. Phytol. 210 (4), 1344–1356. https://doi.org/10.1111/nph.13847.

de Bobadilla, M.F., Vitiello, A., Erb, M., Poelman, E.H., 2022. Plant defense strategies against attack by multiple herbivores. Trends. Plant Sci. 27 (6), 528–535.

De Vos, M., Van Zaanen, W., Koornneef, A., Korzelius, J.P., Dicke, M., Van Loon, L.C., Pieterse, C.M., 2006. Herbivore-induced resistance against microbial pathogens in Arabidopsis. Plant Physiol. 142 (1), 352–363. https://doi.org/10.1104/pp.106.083907.

De Zutter, N., Audenaert, K., Arroyo-Manzanares, N., De Boevre, M., Van Poucke, C., De Saeger, S., Haesaert, G., Smagghe, G., 2016. Aphids transform and detoxify the mycotoxin deoxynivalenol via a type II biotransformation mechanism yet unknown in animals. Sci. Rep. 6 (1), 38640. https://doi.org/10.1038/srep38640.

De-la-Pena, C., Loyola-Vargas, V.M., 2014. Biotic interactions in the rhizosphere: a diverse cooperative enterprise for plant productivity. Plant Physiol. 166 (2), 701–719. https://doi.org/10.1104/pp.114.241810.

Dietz, K.-J., 2021. Synergism and antagonism in plant acclimation to abiotic stress combinations. Turk J Bot. 45 (7), 587–600.

DiGuistini, S., Wang, Y., Liao, N.Y., Taylor, G., Tanguay, P., Feau, N., Henrissat, B., Chan, S.K., Hesse-Orce, U., Alamouti, S.M., Tsui, C.K., Docking, R.T., Levasseur, A., Haridas, S., Robertson, G., Birol, I., Holt, R.A., Marra, M.A., Hamelin, R.C., Hirst, M., Jones, S.J., Bohlmann, J., Breuil, C., 2011. Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont Grosmannia clavigera, a lodgepole pine pathogen. Proc. Natl. Acad. Sci. u S. a 108 (6), 2504–2509. https://doi.org/10.1073/pnas.1011289108.

Drakulic, J., Caulfield, J., Woodcock, C., Jones, S.P., Linforth, R., Bruce, T.J., Ray, R.V., 2015. Sharing a host plant (Wheat [Triticum aestivum]) increases the fitness of fusarium graminearum and the severity of fusarium head blight but reduces the fitness of grain aphids (Sitobion avenae). Appl. Env. Microbiol. 81 (10), 3492–3501. https://doi.org/10.1128/AEM.00226-15.

Duc, N.H., Vo, H.T.N., van Doan, C., Hamow, K.A., Le, K.H., Posta, K., 2022. Volatile organic compounds shape belowground plant-fungi interactions. Front. Plant Sci. 13, 1046685. https://doi.org/10.3389/fpls.2022.1046685.

Ebi, K.L., Vanos, J., Baldwin, J.W., Bell, J.E., Hondula, D.M., Errett, N.A., Hayes, K., Reid, C.E., Saha, S., Spector, J., Berry, P., 2021. Extreme Weather and Climate Change: population health and health system implications. Annu. Rev. Public Health 42 (1), 293–315. https://doi.org/10.1146/annurev-publhealth-012420-105026.

Ederli, L., Salerno, G., Quaglia, M., 2021. In the tripartite combination Botrytis cinerea-Arabidopsis-Eurydema oleracea, the fungal pathogen alters the plant-insect interaction via jasmonic acid signalling activation and inducible plant-emitted volatiles. J. Plant Res. 134 (3), 523–533. https://doi.org/10.1007/s10265-021-01273-9.

Franco, F.P., Moura, D.S., Vivanco, J.M., Silva-Filho, M.C., 2017. Plant-insect-pathogen interactions: a naturally complex menage a trois. Curr. Opin. Microbiol. 37, 54–60. https://doi.org/10.1016/j.mib.2017.04.007.

Franco, F.P., Santiago, A.C., Henrique-Silva, F., de Castro, P.A., Goldman, G.H., Moura, D.S., Silva-Filho, M.C., 2014. The sugarcane defense protein SUGARWIN2 causes cell death in Colletotrichum falcatum but not in non-pathogenic fungi. PLoS. One 9 (3), e91159. https://doi.org/10.1371/journal.pone.0091159.

Franco, F.P., Tuler, A.C., Gallan, D.Z., Goncalves, F.G., Favaris, A.P., Penaflor, M., Leal, W.S., Moura, D.S., Bento, J.M.S., Silva-Filho, M.C., 2021. Fungal phytopathogen modulates plant and insect responses to promote its dissemination. ISMe J. 15 (12), 3522–3533. https://doi.org/10.1038/s41396-021-01010-z.

Franco, F.P., Tuler, A.C., Gallan, D.Z., Goncalves, F.G., Favaris, A.P., Penaflor, M., Leal, W.S., Moura, D.S., Bento, J.M.S., Silva-Filho, M.C., 2022. Colletotrichum falcatum modulates the olfactory behavior of the sugarcane borer, favoring pathogen infection. FEMS. Microbiol. Ecol. 98 (4). https://doi.org/10.1093/femsec/fiac035.

Friman, J., Karssemeijer, P.N., Haller, J., de Kreek, K., van Loon, J.J.A., Dicke, M., 2021. Shoot and root insect herbivory change the plant rhizosphere microbiome and affects cabbage-insect interactions through plant-soil feedback. New. Phytol. 232 (6), 2475–2490. https://doi.org/10.1111/nph.17746.

Gallan, D.Z., Henrique, M.O., Silva-Filho, M.C., 2023. The phytopathogen fusarium verticillioides modifies the intestinal morphology of the sugarcane borer. Pathogens 12 (3), 443. https://doi.org/10.3390/pathogens12030443.

Gamez-Arcas, S., Baroja-Fernandez, E., Garcia-Gomez, P., Munoz, F.J., Almagro, G., Bahaji, A., Sanchez-Lopez, A.M., Pozueta-Romero, J., 2022. Action mechanisms of small microbial volatile compounds in plants. J. Exp. Bot. 73 (2), 498–510. https://doi.org/10.1093/jxb/erab463.

Ghimire, R.P., Kivimäenpää, M., Blomqvist, M., Holopainen, T., Lyytikäinen-Saarenmaa, P., Holopainen, J.K., 2016. Effect of bark beetle (Ips typographus L.) attack on bark VOC emissions of Norway spruce (Picea abies Karst.) trees. Atmos. Env. 126, 145–152.

Guillen Sanchez, C., 2021. The Role of Banana Weevil Cosmopolites sordidus in the Epidemiology of the Panama disease Fusarium oxysporum f. sp. Cubense Race 1. Université de Montpellier.

Hamann, E., Blevins, C., Franks, S.J., Jameel, M.I., Anderson, J.T., 2021. Climate change alters plant-herbivore interactions. New. Phytol. 229 (4), 1894–1910. https://doi.org/10.1111/nph.17036.

Hilfiker, O., Groux, R., Bruessow, F., Kiefer, K., Zeier, J., Reymond, P., 2014. Insect eggs induce a systemic acquired resistance in Arabidopsis. Plant J. 80 (6), 1085–1094. https://doi.org/10.1111/tpj.12707.

Hoegh-Guldberg, O., Jacob, D., Bindi, M., Brown, S., Camilloni, I., Diedhiou, A., Djalante, R., Ebi, K., Engelbrecht, F., Guiot, J., 2018. Impacts of 1.5 C global warming on natural and human systems. Global Warming of 1.5 C.: An IPCC Special Report. IPCC Secretariat, pp. 175–311.

Humphrey, P.T., Whiteman, N.K., 2020. Insect herbivory reshapes a native leaf microbiome. Nat. Ecol. Evol. 4 (2), 221–229. https://doi.org/10.1038/s41559-019-1085-x.

Kandasamy, D., Gershenzon, J., Andersson, M.N., Hammerbacher, A., 2019. Volatile organic compounds influence the interaction of the Eurasian spruce bark beetle (Ips typographus) with its fungal symbionts. ISMe J. 13 (7), 1788–1800. https://doi.org/10.1038/s41396-019-0390-3.

Kandasamy, D., Zaman, R., Nakamura, Y., Zhao, T., Hartmann, H., Andersson, M.N., Hammerbacher, A., Gershenzon, J., 2023. Conifer-killing bark beetles locate fungal symbionts by detecting volatile fungal metabolites of host tree resin monoterpenes. PLoS. Biol. 21 (2), e3001887. https://doi.org/10.1371/journal.pbio.3001887.

Kang, S., Lumactud, R., Li, N., Bell, T.H., Kim, H.S., Park, S.Y., Lee, Y.H., 2021. Harnessing chemical ecology for environment-friendly crop protection. Phytopathology 111 (10), 1697–1710. https://doi.org/10.1094/PHYTO-01-21-0035-RVW.

Kang, Z.W., Liu, F.H., Tan, X.L., Zhang, Z.F., Zhu, J.Y., Tian, H.G., Liu, T.X., 2018. Infection of powdery mildew reduces the fitness of grain aphids (Sitobion avenae) through restricted nutrition and induced defense response in wheat. Front. Plant Sci. 9, 778. https://doi.org/10.3389/fpls.2018.00778.

Khalaf, E.M., Shrestha, A., Rinne, J., Lynch, M.D.J., Shearer, C.R., Limay-Rios, V., Reid, L.M., Raizada, M.N., 2021. Transmitting silks of maize have a complex and dynamic microbiome. Sci. Rep. 11 (1), 13215. https://doi.org/10.1038/s41598-021-92648-4.

Lazebnik, J., Frago, E., Dicke, M., van Loon, J.J., 2014. Phytohormone mediation of interactions between herbivores and plant pathogens. J. Chem. Ecol. 40 (7), 730–741. https://doi.org/10.1007/s10886-014-0480-7.

Leite-Mondin, M., DiLegge, M.J., Manter, D.K., Weir, T.L., Silva-Filho, M.C., Vivanco, J. M., 2021. The gut microbiota composition of trichoplusia ni is altered by diet and may influence its polyphagous behavior. Sci. Rep. 11 (1), 5786. https://doi.org/10.1038/s41598-021-85057-0.

Liu, H., Brettell, L.E., Qiu, Z., Singh, B.K., 2020. Microbiome-mediated stress resistance in plants. Trends. Plant Sci. 25 (8), 733–743. https://doi.org/10.1016/j.tplants.2020.03.014.

Massalha, H., Korenblum, E., Tholl, D., Aharoni, A., 2017. Small Molecules below-ground: the Role of Specialized Metabolites in the Rhizosphere. Wiley Online Library, pp. 788–807.

Medeiros, A.H., Franco, F.P., Matos, J.L., de Castro, P.A., Santos-Silva, L.K., Henrique-Silva, F., Goldman, G.H., Moura, D.S., Silva-Filho, M.C., 2012. Sugarwin: a sugarcane insect-induced gene with antipathogenic activity. Mol. Plant Microbe Interact. 25 (5), 613–624. https://doi.org/10.1094/MPMI-09-11-0254.

Mittler, R., 2006. Abiotic stress, the field environment and stress combination. Trends. Plant Sci. 11 (1), 15–19. https://doi.org/10.1016/j.tplants.2005.11.002.

Moisan, K., Cordovez, V., van de Zande, E.M., Raaijmakers, J.M., Dicke, M., Lucas-Barbosa, D., 2019. Volatiles of pathogenic and non-pathogenic soil-borne fungi affect plant development and resistance to insects. Oecologia 190 (3), 589–604. https://doi.org/10.1007/s00442-019-04433-w.

Mooney, H., Winner, W., 1991. Partitioning response of plants to stress. Response plants mult. stress. 129–141.

Munkvold, G.P., 2003. Epidemiology of Fusarium diseases and their mycotoxins in maize ears. Eur. J. Plant Pathol. 109 (7), 705–713. https://doi.org/10.1023/a:1026078324268.

Nadeem, H., Amir, K., Gupta, R., Hashem, M., Alamri, S., Siddiqui, M.A., Ahmad, F., 2023. Stress combination: when two negatives may become antagonistic, synergistic or additive for plants? Pedosphere 33 (2), 287–300.

Natalini, A., Palma, D., 2023. Ethylene and Biotic Stress in crops, The Plant Hormone Ethylene. Elsevier, pp. 221–232.

Pirttila, A.M., Brusila, V., Koskimaki, J.J., Wali, P.R., Ruotsalainen, A.L., Mutanen, M., Markkola, A.M., 2023. Exchange of microbiomes in plant-insect herbivore interactions. mBio 14 (2), e0321022. https://doi.org/10.1128/mbio.03210-22.

Prasch, C.M., Sonnewald, U., 2015. Signaling events in plants: stress factors in combination change the picture. Env. Exp. Bot 114, 4–14.

Rasmussen, S., Barah, P., Suarez-Rodriguez, M.C., Bressendorff, S., Friis, P., Costantino, P., Bones, A.M., Nielsen, H.B., Mundy, J., 2013. Transcriptome responses to combinations of stresses in Arabidopsis. Plant Physiol. 161 (4), 1783–1794. https://doi.org/10.1104/pp.112.210773.

Rillig, M.C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C.A., Buchert, S., Wulf, A., Iwasaki, A., Roy, J., Yang, G., 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. Science 366 (6467), 886–890. https://doi.org/10.1126/science.aay2832.

Rivero, R.M., Mittler, R., Blumwald, E., Zandalinas, S.I., 2022. Developing climate-resilient crops: improving plant tolerance to stress combination. Plant J. 109 (2), 373–389. https://doi.org/10.1111/tpj.15483.

Roychowdhury, R., Hada, A., Biswas, S., Mishra, S., Prusty, M.R., Das, S.P., Ray, S., Kumar, A., Sarker, U., 2025. Jasmonic acid (JA) in plant immune response: unravelling complex molecular mechanisms and networking of defence signalling against pathogens. J. Plant Growth Regul. 44 (1), 89–114.

Runyon, J.B., 2008. Chemically-mediated Interactions Between the Parasitic Plant Cuscuta pentagona, Its Hosts plants, and Insect Herbivores. The Pennsylvania State University.

Saha, M., Fink, P., 2022. Algal volatiles–the overlooked chemical language of aquatic primary producers. Biol. Rev. 97 (6), 2162–2173.

Saleem, M., Meckes, N., Pervaiz, Z.H., Traw, M.B., 2017. Microbial interactions in the phyllosphere increase plant performance under herbivore biotic stress. Front. Microbiol. 8, 41. https://doi.org/10.3389/fmicb.2017.00041.

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., Nelson, A., 2019. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3 (3), 430–439. https://doi.org/10.1038/s41559-018-0793-y.

Schneider, L., Rebetez, M., Rasmann, S., 2022. The effect of climate change on invasive crop pests across biomes. Curr. Opin. Insect Sci. 50, 100895. https://doi.org/10.1016/j.cois.2022.100895.

Schulz-Bohm, K., Martin-Sanchez, L., Garbeva, P., 2017. Microbial volatiles: small molecules with an important role in intra- and inter-Kingdom interactions. Front. Microbiol. 8, 2484. https://doi.org/10.3389/fmicb.2017.02484.

Sinha, R., Pelaez-Vico, M.A., Shostak, B., Nguyen, T.T., Pascual, L.S., Ogden, A.M., Lyu, Z., Zandalinas, S.I., Joshi, T., Fritschi, F.B., Mittler, R., 2024. The effects of multifactorial stress combination on rice and maize. Plant Physiol. 194 (3), 1358–1369. https://doi.org/10.1093/plphys/kiad557.

Six, D.L., Wingfield, M.J., 2011. The role of phytopathogenicity in bark beetle-fungus symbioses: a challenge to the classic paradigm. Annu. Rev. Entomol. 56 (1), 255–272. https://doi.org/10.1146/annurev-ento-120709-144839.

Stout, M.J., Thaler, J.S., Thomma, B.P., 2006. Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. Annu. Rev. Entomol. 51 (1), 663–689. https://doi.org/10.1146/annurev.ento.51.110104.151117.

Sugio, A., Dubreuil, G., Giron, D., Simon, J.C., 2015. Plant-insect interactions under bacterial influence: ecological implications and underlying mechanisms. J. Exp. Bot. 66 (2), 467–478. https://doi.org/10.1093/jxb/eru435.

Surówka, E., Rapacz, M., Janowiak, F., 2020. Climate change influences the interactive effects of simultaneous impact of abiotic and biotic stresses on plants. Plant Ecophysiol. Adapt. under Clim. Change: Mech. Perspect. I: Gen. Conseq. Plant Responses 1–50.

Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E., Mittler, R., 2014. Abiotic and biotic stress combinations. New. Phytol. 203 (1), 32–43. https://doi.org/10.1111/nph.12797.

Takabayashi, J., 2022. Herbivory-induced plant volatiles mediate multitrophic relationships in ecosystems. Plant Cell Physiol. 63 (10), 1344–1355. https://doi.org/10.1093/pcp/pcac107.

Tanin, S.M., Kandasamy, D., Krokene, P., 2021. Fungal interactions and host tree preferences in the Spruce bark beetle Ips typographus. Front. Microbiol. 12, 695167. https://doi.org/10.3389/fmicb.2021.695167.

Teixeira, P.J.P., Colaianni, N.R., Fitzpatrick, C.R., Dangl, J.L., 2019. Beyond pathogens: microbiota interactions with the plant immune system. Curr. Opin. Microbiol. 49, 7–17. https://doi.org/10.1016/j.mib.2019.08.003.

Thaler, J.S., Humphrey, P.T., Whiteman, N.K., 2012. Evolution of jasmonate and salicylate signal crosstalk. Trends. Plant Sci. 17 (5), 260–270. https://doi.org/10.1016/j.tplants.2012.02.010.

Tollenaere, C., Susi, H., Laine, A.L., 2016. Evolutionary and epidemiological implications of multiple infection in plants. Trends. Plant Sci. 21 (1), 80–90. https://doi.org/10.1016/j.tplants.2015.10.014.

Trivedi, P., Batista, B.D., Bazany, K.E., Singh, B.K., 2022. Plant-microbiome interactions under a changing world: responses, consequences and perspectives. New. Phytol. 234 (6), 1951–1959. https://doi.org/10.1111/nph.18016.

Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K., 2020. Plant-microbiome interactions: from community assembly to plant health. Nat. Rev. Microbiol. 18 (11), 607–621. https://doi.org/10.1038/s41579-020-0412-1.

van Dijk, L.J., Abdelfattah, A., Ehrlén, J., Tack, A.J., 2022a. Soil microbiomes drive aboveground plant–pathogen–insect interactions. Oikos. 2022 (12), e09366.

van Dijk, L.J.A., Regazzoni, E.D.E., Albrectsen, B.R., Ehrlen, J., Abdelfattah, A., Stenlund, H., Pawlowski, K., Tack, A.J.M., 2022b. Single, but not dual, attack by a biotrophic pathogen and a sap-sucking insect affects the oak leaf metabolome. Front. Plant Sci. 13, 897186. https://doi.org/10.3389/fpls.2022.897186.

Van Oosten, V.R., Bodenhausen, N., Reymond, P., Van Pelt, J.A., Van Loon, L.C., Dicke, M., Pieterse, C.M., 2008. Differential effectiveness of microbially induced resistance against herbivorous insects in Arabidopsis. Mol. Plant Microbe Interact. 21 (7), 919–930. https://doi.org/10.1094/MPMI-21-7-0919.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., Dufresne, A., 2015. The importance of the microbiome of the plant holobiont. New. Phytol. 206 (4), 1196–1206. https://doi.org/10.1111/nph.13312.

Vicherova, E., Glinwood, R., Hajek, T., Smilauer, P., Ninkovic, V., 2020. Bryophytes can recognize their neighbours through volatile organic compounds. Sci. Rep. 10 (1), 7405. https://doi.org/10.1038/s41598-020-64108-y.

Vos, I.A., Pieterse, C.M., Van Wees, S.C., 2013. Costs and benefits of hormone-regulated plant defences. Plant Pathol. 62, 43–55.

Weisskopf, L., Schulz, S., Garbeva, P., 2021. Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions. Nat. Rev. Microbiol. 19 (6), 391–404. https://doi.org/10.1038/s41579-020-00508-1.

Wenke, K., Kai, M., Piechulla, B., 2010. Belowground volatiles facilitate interactions between plant roots and soil organisms. Planta 231 (3), 499–506. https://doi.org/10.1007/s00425-009-1076-2.

Xia, X., Wei, Q., Wu, H., Chen, X., Xiao, C., Ye, Y., Liu, C., Yu, H., Guo, Y., Sun, W., Liu, W., 2024. Bacillus species are core microbiota of resistant maize cultivars that induce host metabolic defense against corn stalk rot. Microbiome 12 (1), 156. https://doi.org/10.1186/s40168-024-01887-w.

Xing, Z., Zhao, Y., Gilbert, J.A., Zhang, Z., Song, Y., Shi, Y., Ding, J., 2024. Caterpillar chewing and aphid sucking differentially shift root-associated microbial community by affecting plant performance. Entomol. Gen. 44 (5).

Yadav, A.N., Kour, D., Kaur, T., Devi, R., Yadav, A., Dikilitas, M., Abdel-Azeem, A.M., Ahluwalia, A.S., Saxena, A.K., 2021. Biodiversity, and biotechnological contribution of beneficial soil microbiomes for nutrient cycling, plant growth improvement and nutrient uptake. Biocatal. Agric. Biotechnol. 33, 102009.

Zandalinas, S.I., Fritschi, F.B., Mittler, R., 2021a. Global Warming, Climate change, and environmental pollution: recipe for a multifactorial stress combination disaster. Trends. Plant Sci. 26 (6), 588–599. https://doi.org/10.1016/j.tplants.2021.02.011.

Zandalinas, S.I., Mittler, R., 2022. Plant responses to multifactorial stress combination. New. Phytol. 234 (4), 1161–1167. https://doi.org/10.1111/nph.18087.

Zandalinas, S.I., Sengupta, S., Fritschi, F.B., Azad, R.K., Nechushtai, R., Mittler, R., 2021b. The impact of multifactorial stress combination on plant growth and survival. New. Phytol. 230 (3), 1034–1048. https://doi.org/10.1111/nph.17232.

Zhang, N., Qian, Z., He, J., Shen, X., Lei, X., Sun, C., Fan, J., Felton, G.W., Shao, Y., 2024. Gut bacteria of lepidopteran herbivores facilitate digestion of plant toxins. Proc. Natl. Acad. Sci. u S. a 121 (42), e2412165121. https://doi.org/10.1073/pnas.2412165121.

Zhao, T., Kandasamy, D., Krokene, P., Chen, J., Gershenzon, J., Hammerbacher, A., 2019. Fungal associates of the tree-killing bark beetle, Ips typographus, vary in virulence, ability to degrade conifer phenolics and influence bark beetle tunneling behavior. Fungal. Ecol. 38, 71–79.