Ecological costs of biotrophic versus necrotrophic pathogen resistance, the hypersensitive response and signal transduction.

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Summary

Recent advances along numerous research avenues show that plant interactions with biotrophic and necrotrophic pathogens use similar pathways with opposing effects. The hypersensitive response is associated with increased biotroph resistance but decreased necrotroph resistance. In plant/herbivore interactions, opposing effects of defenses against specialist versus generalist herbivores are controlled by plant secondary metabolites, where a metabolite that provides resistance to generalist herbivores may stimulate specialist herbivores. This multi-trophic interaction is presented as an ecological cost of plant resistance, but similar concepts are rarely applied to plant interactions with different classes of pathogens. In this review, we begin to describe how necrotrophic pathogens may place an ecological cost upon plant resistance to biotrophic pathogens. We separate these potential ecological costs into three concepts: (1) The local cost of the hypersensitive response (2) organismal cost of having machinery for a hypersensitive response and (3) antagonism between salicylate and jasmonate signaling. We describe the literature supporting these concepts and some predictions that they generate.
1. Introduction

Resource-limited environments force organisms to distribute their available resources among biological processes such as reproduction, defense, or growth [1]. Plant defense can therefore be associated with an allocation-based cost, in that resistance utilizes resources that would otherwise benefit the plants growth and/or reproduction. More generally, any situation where a given resistance trait creates detrimental consequences for fitness reveals a cost of resistance [2]. These can be separated into direct and indirect costs. Direct resistance costs are the predominant focus of plant/pathogen interaction analyses, include auto-toxicity, allocation and opportunity costs and are defined as those responses and their fitness consequences that are directly a result of the plants response to one single attacking organism [2-5]. In contrast, indirect resistance costs occur in the presence of additional species, such that resistance against one species is associated with increased sensitivity to a second attacking species [1].

Studies of secondary metabolite mediated plant/insect interactions provide valuable examples of indirect or ecological costs of resistance [1]. For instance, crucifer-produced glucosinolate secondary metabolites are effective deterrents against non-adapted lepidopteran herbivores [6-10]. Glucosinolate levels are positively associated with fitness in the presence of generalist herbivory but show direct allocation costs, in the form of negative correlations with fitness in the absence of herbivory by generalists [11,12]. Plants with high glucosinolate content also have lower fitness in environments rich in specialist lepidopterans, as glucosinolates are oviposition and feeding stimulants for these insects [13-17]. The importance of ecological costs for glucosinolate production is magnified by the observation that their impact on fitness depends upon the distribution and frequency of different glucosinolate profiles within the plant community [18]. Similar systems of ecological costs exist for most tested secondary metabolites
in plants [1]. Given the rarity of herbivore-free environments, the ratio of specialist to generalist herbivores in an environment will determine if secondary metabolites are beneficial or detrimental to the plants. This suggests that ecological costs play a stronger role than direct costs in deciding plant allocation to defense against insect herbivores.

In contrast to plant-insect interactions, modulation of plant resource allocation to pathogen resistance based on ecological costs is less studied. Plant defensive responses can be broadly divided by whether the pathogen is biotrophic (feeding on living plant tissue) or necrotrophic (feeding on dead plant tissue) [19,20]. As biotrophic pathogens require a living host, localized controlled cell death in the region of pathogen attack forms part of an effective defense strategy known as the hypersensitive response (HR). Necrotrophic pathogens are distinguished from commensals or saprophytes by their ability to actively kill host tissue; therefore programmed cell death initiated by the plant is not intuitively an effective strategy to limit pathogen growth.

The potential influence of ecological costs in plant-pathogen interactions is illustrated by the defensive tomato metabolite, α-tomatine. α-Tomatine is essential for resistance against pathogens that are susceptible to the compound [21]. However, some necrotrophic pathogens have evolved the ability to resist α-tomatine by converting tomatine to a less toxic derivative, β-tomatine, via removal of sugar moieties [21,22]. This detoxification allows the pathogen to be more virulent but does not impart complete insensitivity to the plant. The produced β-tomatine inhibits activation of the tomato hypersensitive response. β-tomatine may therefore serve as a pathogen identification signal, allowing the plant to prevent an inappropriate activation of the hypersensitive response that could aid necrotroph establishment. Repression of the hypersensitive response by tomatinase-produced β-tomatine leads to increased susceptibility to
the biotrophic pathogen *Pseudomonas syringae* p.v. *tabaci* [23]. Thus, initial direct physiological costs of tomato resistance to necrotrophic pathogens via tomatine biosynthesis are expanded by the additional ecological risk resulting from secondary effects of defense evasion by necrotrophs that increase plant susceptibility to biotrophs. This review expands on the concept that necrotrophs may impart an ecological cost on biotroph resistance.

2. Do Necrotrophs impart an ecological cost of plant resistance to biotrophs?

Recent evidence suggests that necrotrophic pathogen-plant interactions impart an ecological cost on plant resistance to biotrophic pathogens. The ecological cost is here defined as resulting from any plant mechanisms that provide resistance to biotrophic pathogens but increase susceptibility to necrotrophic pathogens. The potential for these two pathogen classes to establish this ecological cost scenario is highlighted by the identification of plant mutants that are resistant to necrotrophs yet sensitive to biotrophs and vice versa [24-29]. Three lines of evidence support the existence of ecological costs of necrotroph versus biotroph resistance:

1) Local cost of having a hypersensitive response

2) Organismal cost of having machinery for a hypersensitive response

3) Antagonism between salicylate and jasmonate signaling.

We keep these three topics separate as molecular data suggests that they have mechanistic differences and as such are not one single biological trait. The ecological costs controlling the relationship between biotroph and necrotroph resistance in plants are an under appreciated component of how environmental complexity influences the evolution of plant/pathogen interactions.
2.1. Local cost of having a hypersensitive response

The hypersensitive response is frequently associated with resistance to biotrophic pathogens when resistance mediated by major resistance genes (R genes) or innate immunity controls the plant-biotroph interaction [20,30,31]. Further, a HR leads to direct ecological costs from the loss of photosynthetic tissue and the resulting metabolic capacity. Yet plant cell death is required for full virulence of necrotrophic pathogens [32,33]. As such, the dead cells caused by the hypersensitive response provide resistance to the invading biotroph, but also provide potential entry points for necrotrophs in the local environment (Figure 1). This creates the potential for increased sensitivity to necrotrophs in the local presence of a hypersensitive response. Because this cost is incurred only after attack by a biotrophic pathogen, it is limited both temporally and locally within the plant. This local cost was elegantly illustrated by pre-treatment of leaves with avirulent biotrophic bacteria capable of inducing the hypersensitive response four hours prior to inoculation with necrotrophic *Botrytis cinerea* [34]. *B. cinerea* placed in the center of the pre-treated zone generated spreading lesions much faster than *B. cinerea* in the absence of bacterial inoculation. Additionally, virulent bacteria incapable of generating a hypersensitive response did not have the same stimulatory activity on *B. cinerea* growth in plants [34].

In contrast, extending the time between inoculation with avirulent biotrophic bacteria and necrotrophic fungus to 48h resulted in a slight decrease in growth of the necrotroph [34]. This was originally interpreted as indicating that the presence of dead cells did not facilitate increased necrotroph virulence, yet considering the potential for ecological costs of a local hypersensitive response suggests that the discrepancy between results at 4 hours versus 48 hours may reflect plant counter-responses to hypersensitive response-induced susceptibility. Decreasing the capacity for additional programmed cell death in the presence of an active hypersensitive
response might locally enhance resistance to necrotrophs. One study found that infection with a virulent biotrophic bacterium led to increased necrotroph virulence if the necrotroph was inoculated 24 h later at a separate site on the same leaf [35]. In contrast, a hypersensitive response-inducing avirulent biotroph did not alter necrotroph virulence, and instead led to an increased plant defense response against the necrotrophic Alternaria brassicicola [35].

Further studies suggest that the timing of any hypersensitive response with regard to pathogen inoculation is of critical importance for determining the ecological risk. For instance, Arabidopsis thaliana mutants with a thinner plant cuticle have a more rapid production of anti-fungal toxins after necrotrophic fungal germination. This enhanced rate of response led to increased resistance to fungal necrotrophs even in the presence of a biotroph induced hypersensitive response [29,36,37]. Botrytis cinerea can delay this rapid plant response by producing 2-methyl-succinate, which actively delays plant defenses, possibly via stimulation of plant abscisic acid signaling networks [38,39]. These data suggest that local lesions associated with the hypersensitive response provide an opening to necrotrophic pathogens but that plant counter-mechanisms can temporally limit the necrotrophic pathogen’s opportunity.

2.2. Organismal cost of having machinery for a hypersensitive response

The recently proposed zigzag model of the plant immune system describes the evolution of gene-for-gene resistance with regard to a “threshold for the hypersensitive response” (Figure 2A)[30]. This threshold for activation of the hypersensitive response implies two factors: 1) the plant cell must have a capacity for self-regulated and directed programmed cell death and 2) a specific signaling threshold is required to stimulate this programmed cell death (Figure 2). The very existence of plant signaling pathways that initiate a hypersensitive response generates molecular
targets that a necrotrophic pathogen may inappropriately stimulate to facilitate its invasion of the plant by stimulating the plant to kill itself. This cost does not require the actual presence of a biotrophic pathogen, merely the potential for attack by a biotrophic pathogen and is as such different from the first HR cost we proposed.

This “threshold for HR” presents a potential ecological cost of biotroph resistance engendered by increased sensitivity to necrotrophic pathogens. The specific activation threshold for signaling the hypersensitive response has the potential to modulate between the risks of plant invasion by biotrophic versus necrotrophic pathogens. As such, plants in an environment with mainly necrotrophic pathogens may face an evolutionary pressure to raise the threshold for initiation of the hypersensitive response (Figure 2B). In contrast, an environment with mainly biotrophic pathogens may introduce pressure to activate a hypersensitive response at a lower threshold.

Evidence supporting this concept shows that the virulence of necrotrophic plant pathogens is enhanced by stimulation of the hypersensitive response. Increasing the threshold for an HR by transgenically introducing anti-apoptotic proteins from animals into plants led to enhanced resistance to necrotrophic *B. cinerea* [40]. Another factor controlling the threshold for inducing a hypersensitive response is the level of reactive-oxygen species (ROS) in the plant [20,30,31]. Alterations in metabolism of reactive oxygen species due to exogenous enzyme treatment, plant mutations, or transgenic modification of fungal pathogens show that virulence of necrotrophic *B. cinerea* and *Sclerotinia sclerotorium* is positively correlated with production of reactive oxygen species, possibly via alteration of the threshold for hypersensitive response [34,41,42]. The importance of sensitivity to reactive oxygen species and the hypersensitive response threshold is also supported by the identification of a series of *Botrytis*-susceptible (bos)
mutants in *Arabidopsis thaliana* that appear to identify a signaling network involved with plant sensitivity to reactive oxygen species [43,44]. This suggests that the threshold for hypersensitive response as established by reactive oxygen signaling may balance susceptibility to biotrophic versus necrotrophic pathogens. Another mechanism to modulate the HR threshold is co-regulation of groups of R genes as recently found for the RPP5 R gene cluster in Arabidopsis that is coordinately regulated by a positive feedback loop and in response to altered miRNA metabolism [45]. The role of group R gene regulation in this model remains to be tested.

There is evidence consistent with the hypothesis that necrotrophic pathogens may enhance their virulence through active stimulation of the hypersensitive response (Figure 2B). It was initially observed that necrotrophic pathogens in the genus *Botrytis* induce cell death with molecular characteristics similar to a hypersensitive response [34,46,47]. Additional direct evidence of necrotrophic stimulation of the hypersensitive response comes from the discovery of unidentified metabolites in *B. cinerea* that induce several components of an HR, including cell death and ROS production in multiple plant species [42,48](Figure 2B). Botrydial, another *Botrytis* toxin that is required for full virulence, also causes cell death when applied to plants, but the contribution of active plant defenses to this response is currently unknown [49-52]. The production of molecular signatures of the hypersensitive response by *Botrytis elliptica* is blocked by the application of chemicals known to inhibit apoptosis in multiple systems [53]. Finally, enhanced expression of *RPW8*, a known hypersensitive response-inducing R-gene that functions in biotroph resistance, increased plant sensitivity to the necrotrophic pathogens *Botrytis cinerea* and *Alternaria brassicicola* [54]. This suggests that necrotrophic pathogens may stimulate the hypersensitive response system to enable enhanced virulence in plants.
If the potential for a hypersensitive response creates vulnerability to necrotrophic pathogens, this predicts that specific plant R genes may be targeted by necrotrophic pathogens to generate cell death and enhance virulence. A naturally variable locus, LOV1, controlling Arabidopsis thaliana susceptibility to the Cochliobolus victorae toxin victorin, was recently found to encode a coiled-coil NBS-LRR R-like gene [55,56]. In this case, the victorin toxin requires the functional R-gene to produce disease, suggesting that the R-gene function enhances pathogen access to the plant. Interestingly, this genetic interaction between toxin and R-gene utilizes the normal biological conformation of the LOV1 protein as shown by the requirement of proper disulfide bridge formation in the LOV1 protein for the victorin/R gene interaction [57]. As LOV1 is a naturally variable gene homologous to other known R-genes, LOV1 may provide resistance to a different pathogen, but from the plant’s perspective the victorin toxin inappropriately stimulates this R-gene to suppress resistance to Cochliobolus victorae. Identification of a pathogen whose interaction with LOV1 leads to hypersensitive-response mediated plant resistance would validate this hypothesis. It will be intriguing to test how frequently necrotroph-produced toxins interact with R-genes to provide susceptibility. The existence of a defense system against biotrophic pathogens that includes a hypersensitive response may provide opportunities for necrotrophic pathogens to successfully invade the plant. As such, necrotrophic pathogens may establish an ecological cost of the hypersensitive response and the ratio of necrotrophic to biotrophic pathogens may place evolutionary pressure on the “threshold for a hypersensitive response” in the zigzag model (Figure 2).

2.3. Salicylate versus jasmonate signaling pathway variation
Dependency of the hypersensitive response on salicylate signaling may couple with antagonism between the salicylate and jasmonate signaling networks to create ecological cost trade-offs between plant resistance to biotrophic and necrotrophic pathogens [24,25,27,58,59]. Salicylate-mediated defenses are commonly observed to play a major role in regulating resistance against biotrophic pathogens while jasmonate-mediated defenses control resistance against necrotrophic pathogens [20]. Infection by virulent hemibiotrophic Pseudomonas syringae leads to a localized repression of jasmonate signaling. This repression, in Arabidopsis thaliana mediated via induction of the salicylate signaling pathway through the NPR1 gene product, leads to locally decreased resistance to the necrotroph Alternaria brassicicola [35]. As such, stimulation of salicylate-mediated defenses by a biotrophic pathogen leads to direct repression of the jasmonate pathway and its mechanisms of resistance to necrotrophs in Arabidopsis thaliana. This is similar to the proposed tradeoff in plant/pathogen versus plant/herbivore resistance for salicylate versus jasmonate signaling networks [60-62]. However, while plants heavily rely upon jasmonate signaling in responding to both necrotrophic pathogens and herbivores, there are numerous transcriptional responses that are jasmonate dependent yet differ between these two responses. This suggests that the mechanisms of any salicylate/jasmonate tradeoff may differ when considering the different attacking organisms. As such, we do not attempt to implicitly suggest that salicylate/jasmonate trade-offs are identically affected by herbivores or necrotrophic pathogens.

In apparent contradiction of a simple model of jasmonate-mediated resistance to necrotrophic pathogens, a JA signaling mutant defective in the MYC2 transcription factor (jin1), were more resistant to necrotrophic pathogens than wild-type plants [63,64]. However, this gene mediates cross-talk between salicylate and jasmonate signaling such that jin1 mutants, while
abolishing a number of jasmonate-dependent responses, actually increase certain jasmonate-mediated pathogen responses, such as expression of the PDF1.2 gene and genes associated with production of anti-microbial defense metabolites such as indole glucosinolates and the phytoalexin camalexin [65-67]. This suggests that NPR1, the salicylate signaling regulator, and MYC2 may both play a central role in controlling the ecological risk balance between salicylate and jasmonate signaling.

Our hypothesis that antagonism between salicylate and jasmonate signaling creates ecological costs of resistance predicts the existence of naturally variable genetic loci controlling salicylate or jasmonate signaling that have opposing effects on these two networks. This is supported by studies of natural variation in Arabidopsis thaliana showing that the Cvi-0 accession of Arabidopsis is hyper-responsive to jasmonic acid while hypo-responsive to salicylic acid [68-70]. This demonstrates the existence of genetic polymorphisms with broad effects on defense-related gene expression networks [70]. Polymorphisms at specific loci frequently exerted opposing effects on salicylate- versus jasmonate-mediated gene expression. For instance, several QTL identified in the Arabidopsis Bay × Sha recombinant inbred line population are associated with elevated gene expression of salicylate-regulated transcripts and decreased expression of jasmonate-regulated transcripts [71,72]. These expression polymorphisms are predicted to have opposing effects on biotrophic and necrotrophic pathogens and engender associated ecological costs dependent on the pathogen community. Interestingly, two QTL with opposing effects on salicylate versus jasmonate signaling networks co-localize with PRP-PS1 and -PS4, QTL controlling resistance to a virulent hemibiotrophic pathogen Pseudomonas syringae pv. tomato DC3000 [73]. It remains to be tested if these QTL for salicylate and
jasmonate gene expression and biotroph resistance have the same molecular bases, and also control plant susceptibility to necrotrophic pathogens.

The proposed existence of ecological costs of resistance mediated by antagonistic salicylate and jasmonate signaling predicts that salicylate signaling would be favored in environments containing predominantly biotrophic pathogens, while an abundance of necrotrophic pathogens would favor jasmonate signaling. The study of natural qualitative polymorphisms controlling resistance has focused on gene-for-gene resistance mechanisms rather than quantitative polymorphisms affecting underlying signal regulation. This signaling-mediated trade-off between plant resistance to biotrophic versus necrotrophic pathogens is similar to the ecological-cost relationship between salicylate and jasmonate signaling postulated to exist for plant-pathogen versus plant-insect interactions [74-78].

3. Concluding Remarks
Recent literature supports the potential for three distinct aspects of plant resistance to biotrophic pathogens to generate ecological costs. These include two different components of the hypersensitive response, one the act of having a hypersensitive response in response to a biotrophic pathogen leading to a local increase in necrotroph susceptibility; and two the potential for hypersensitive response signaling pathways to act as targets for necrotrophic virulence mechanisms. Finally, antagonistic interaction between salicylate- and jasmonate-mediated signaling may support a balance between ecological costs of resistance to biotrophic versus necrotrophic pathogens. This 3-way model predicts that plants evolved in a pathogen environment biased towards biotroph resistance may have lost the resistance to necrotrophs, and vice versa. Recent dramatic shifts of environment are most likely to have occurred due to human-
mediated redistribution of plants or plant domestication, for instance the transfer of wild lettuces (Lactuca spp.) from a dry natural environment to wetter cropping systems is likely to have increased exposure of these plants to necrotrophic pathogens such as Botrytis cinerea. As such, testing broad spectrum pathogen resistance of domestic plants versus their wild relatives may identify these biased selection regimes. Alternatively, some environments may be dominated by one or the other pathogen class or alternatively environments may cycle between predominantly biotrophic and necrotrophic pathogens lending these systems to studying these proposed ecological costs. Finally, this ecological cost model raises important questions about the potential for the introduction of long lasting pathogen resistance into crop plants, such as how the potential pathogen community encountered by the crop should influence breeding strategies.

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Figure Legends

Figure 1. Local Costs of the Hypersensitive Response

A model representing the potential for local costs of a hypersensitive response. Grey shows the extent of the actual hypersensitive response induced by a biotrophic pathogen. Spores deposited by the necrotrophic pathogen (Orange) are able to grow in the lesion formed by the hypersensitive response. Other defenses stimulated by the hypersensitive response may inhibit necrotroph growth adjacent to the hypersensitive lesion.

Figure 2. Opportunity costs of a hypersensitive response

A. The proposed zig-zag model of plant disease resistance as previously described by Jones and Dangl [28]. Abbreviations are as previously classified in the published model. PTI is pathogen triggered immunity. ETS is effector-triggered susceptibility. ETI is effector-triggered immunity.

B. The proposed ecological costs of having a “threshold for HR” with regards to necrotroph invasion. Toxin shows the potential direct stimulation of an HR by necrotrophic toxins. The arrow increasing the “threshold for HR” indicates the potential pressure upon plants to require a sufficiently high threshold as to limit necrotrophs capacity to induce the HR via toxins.
Literature Cited


Figure 2

(A) Amplitude of Defence Response

High | PTI | ETS | ETI | ETS | ETI
Low

Threshold for HR
Threshold for effective resistance

(B) Amplitude of Defence Response

High | PTI | ETS
Low

Threshold for HR
Threshold for effective resistance

Toxin | PAMP

Necrotrophic Pressure