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Nature and ecological implications of pathogen-induced systemic resistance in conifers: A novel hypothesis

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Abstract

Coniferous trees are often dominant species in both boreal and temperate forests, wherein they play critical roles in ecosystem function. In natural environments, ecosystem stability appears to be the norm, notwithstanding the co-occurrence of insect and microbial species inherently capable of killing their host trees. Adaptive plasticity of host trees involving inducible mechanisms of resistance against invading organisms is likely to play a crucial role in these interactions. We hypothesize that systemic-induced resistance represents a common and important phenomenon in coniferous trees, allowing for a balanced allocation of resources between growth and defense. Published by Elsevier Ltd.

Keywords: Genus *Pinus*; Localized resistance; Systemic resistance; Plant defense theory; Fungal pathogens; Insects

1. Introduction

Host plant-mediated interactions between microbes and insects can be significant factors affecting the survival of coniferous trees, and thus the structure and function of temperate forest ecosystems, where they are often dominant species. For example, chronic infections by root pathogenic fungi are often predisposing factors for bark beetle outbreaks. Eruptive bark beetle populations can then attack and kill virtually any host tree over extensive areas, irrespective of whether or not trees are infected with pathogens [1,2].

Even though forest pathogens and insects (and in some cases their microbial associates) pose serious threats to tree survival, the annual probability of, for example, the death of any particular tree caused directly by insect attack is relatively low. Clearly, trees have evolved effective defensive mechanisms against pathogen/insect colonization, a feature that has allowed these plants, as a group, to survive for millions of years (reviewed by Franceschi et al. [3]) and to establish stable plant communities dominated by long-lived individuals. One contributor to their persistence may be their ability to respond to fungal infections with localized defense responses that make induced tissues more resistant to a subsequent insect attack (e.g. [3–5]). On the other hand, this evidence appears to be incongruent with the observation that trees showing symptoms of pathogenic infection are usually more susceptible to pathogen and insect attack. For example, conifers visibly suffering from root disease (i.e. symptomatic trees) are more susceptible to colonization and immediate mortality caused by bark beetles [6,7]. This apparent contradiction may have more to do with spatio-temporal relationships between the attack sites of pathogens and insects than with species-specific host responses. In other words, the specific phenotype that one observes may depend on how and

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when a pathogenic infection alters whole-tree physiology in a way that affects subsequent insect behavior. We will argue that these phenomena follow general patterns that are independent of specific pathogen–tree–insect systems.

Others have produced authoritative and in depth reviews on the ecology of tripartite, angiosperm-based systems (e.g. [8–10]). This review is not meant to be a comprehensive analysis of conifer resistance to, and conifer-mediated interactions between, pathogens and insects. Rather, we will attempt to synthesize some basic, well-described phenomena into a hypothesis that will arguably have important implications for current plant defense theory. We will present a brief overview of resistance mechanisms, emphasizing the differences between constitutive and inducible, localized and systemic defense responses to pathogens that might affect insect behavior and fitness, and will speculate on the potential ecological consequences of these phenomena. We will focus primarily on relationships between two major groups of biotic stressors of conifers, fungal pathogens and bark beetles, and therefore on host responses that are expressed mainly in the phloem of stems and branches of pines.

2. Defense mechanisms

Coniferous trees have evolved both constitutive and inducible defense systems that deter or kill insects and inhibit or exclude pathogens physically and/or chemically [11–25]. Recent fossil evidence suggests that these systems have been operating for at least the past 45 million years in the Pinaceae [3,26].

3. Constitutive defenses

Constitutive defenses are present in tissue before colonization by herbivores or pathogens. The constitutive system is the first line of defense of all organisms, including plants, and it comprises a number of physical and chemical barriers. These are associated with normally occurring anatomical structures that include resin blisters or resin cells, commonly found in *Abies*, *Tsuga* and *Cedrus* as well as more morphologically complex tube-like resin ducts located in the wood and bark of *Picea*, *Pinus*, *Larix*, *Pseudotsuga* and other genera [3]. Phenolic containing phloem cells, and heavily lignified sclereids and fibers are also a natural complement in conifer tissues. Pathogens and insects that attempt to penetrate host tissue are confronted with a flow of resinous material, toxic phenolics and physical barriers posed by lignified structures, which provide immediate proximal obstacles to invasion [17,18,27–33]. Given that the focus of this paper is on inducible responses, constitutive defense mechanisms will not be reviewed further.

3.1. Localized induced resistance

Once the initial constitutive barriers have been compromised, subsequent resistance is thought to involve the expression of a multitude of mechanisms that are induced postpenetration [34]. Indeed, plants protect themselves facultatively by mobilizing chemical defenses shortly after attack by herbivores or pathogens by various processes, e.g. the ‘‘hypersensitive response’’ and the ‘‘localized induced resistance’’ response. In conifers, the inducible system may include secondary resin [4,17,23,28,35,36] (that may or may not be associated with traumatic resin duct formation [28,37–39]) and additional phenolics, leading to qualitative and quantitative changes in chemical composition near the colonization site [4,14,19,38,40–44]. Local changes in cell metabolism, which can occur in a matter of minutes following challenge, might be viewed as rapid deployment of defense processes that evolved to respond to the initial invasion, while changes involving cell division and differentiation (e.g. necrophylactic periderm formation, traumatic resin duct formation) are slower processes, taking days to months to complete, and are aimed at containing the invasion and enhancing the defense of the plant against further attack.

In the strictest sense, phytoalexins are low molecular weight antimicrobial compounds synthesized by plants *de novo* upon infection. They represent an extremely diverse group of secondary metabolic compounds including isoflavonoids, pterocarpans, stilbenes and saponins [34]. In some cases, accumulation of phytoalexins has been shown to be instrumental in disease resistance [45,46].

In conifers, true phytoalexins are unknown, but pathogen-induced accumulation of related constitutive antimicrobial compounds (also known as phytoanticipins) has been reported [47], including stilbenes (e.g. [48]), diterpenic resin acids (e.g. [49]), lignans (e.g. [50]) and flavonoids (e.g. [51]). The localization of some of these classes of compounds has been investigated. For example, Norway spruce (*Picea abies* (L.) Karst.) accumulates phenolics upon pathogenic infection in specialized phloem polyphenolic parenchyma cells [3]. Whether or not accumulation of these compounds in response to fungal infection is directly related to resistance remains to be demonstrated, and in many cases such accumulations may represent an incidental effect of disease rather than a true resistance mechanism (e.g. [43]).

Pathogenesis-related (PR) proteins (currently comprising 17 families—http://www.bio.uu.nl/~fytopath/PR-families.htm) also accumulate following infection, including lytic enzymes such as β-1,3-glucanases (PR-2 family) and chitinases (PR-3, -4, -8, -11 families), which are putatively involved in the degradation of fungal cell walls. They presumably impact fungal pathogens directly or through release of elicitors from host or pathogen cell walls that induce other defense responses (e.g. [52]). Evidence of accumulation of PR proteins (or their transcripts) in conifers is not as widespread as with angiospermous, herbaceous plants. However, this is probably due more to less intensive investigation in conifers than to a lack of PR-proteins in these plants. Indeed, expression of various groups of PR-proteins has been documented in conifers under various conditions: chitinases (e.g. [53–59]); β-1,3-glucanases

(e.g. [53,60]); PR-5 (thaumatin-like) group [61]; PR-9 (peroxidase) group (e.g. [58,62,63]); PR-10 (ribonuclease-type) group (e.g. [59,64,65]); and PR-12 (plant defensins) group [66].

As the name itself implies, these are all proteins whose accumulation in host tissues is associated with infection processes. However, to date, the role of PR-proteins in actual resistance to pathogens (or insects) remains unclear [67].

A pathogenic invasion may also activate traumatic resin duct formation and secondary resin production. For example, in response to fungal inoculations Norway spruce has been shown to develop large numbers of induced traumatic resin ducts in the xylem that increase the volume of resin to fend off successive trauma from either the initial cause or a secondary invader [3], as well as to cleanse and seal off the wound [5].

With very few exceptions, studies of defense responses in conifers to pathogens have mainly addressed localized induced accumulation of host chemicals immediately around infection sites (e.g. [68]), with very little knowledge of the whole-plant effects of pathogenic infections. However, some recent evidence indicates that fungal pathogens can induce disease resistance in conifers throughout the plant in early stages of infection (i.e. *pre- or early-symptomatic stages*). This phenomenon, in which prior infections have been shown to induce resistance in previously non-infected parts of the plant, is called systemic induced resistance (SIR) (see [43]) and is functionally analogous to immunization. Our present understanding of the role of SIR in conifer–pathogen–insect interactions is quite limited and lags well behind what is known about this phenomenon in herbaceous angiosperms (e.g. [69]).

3.2. Systemic induced resistance

Extensive research in the last 20 years has dissected the biochemical and molecular bases of SIR, mostly in herbaceous model species, particularly tobacco and *Arabidopsis*. Many studies in model hosts have shown that, following hypersensitive cell death, SIR is mediated by, or at least associated with, the accumulation of the hydroxybenzoic acid derivative salicylic acid, the linolenic acid derivative jasmonic acid, and ethylene [67,70–72]. Other phytohormones, e.g. abscisic acid, can also play a role in SIR [73].

Although such extensive knowledge is not available for conifers, SIR phenotypes against stem and branch pathogens have been observed in pine in response to plant growth promoting rhizobacteria [74] and pathogens. For example, Bonello et al. [75] demonstrated that resistance against the pitch canker pathogen, *Fusarium circinatum* Nirenberg and O'Donnell, can be induced systemically in Monterey pine (*Pinus radiata* D. Don) in the field using mechanical inoculations with the same pathogen. Induced resistance was sustained and intensified with boost inoculations over the course of at least one and a half years. The natural occurrence of induced resistance to pitch canker has been documented in long-term monitoring plots. At these sites, a number of Monterey pines that were severely affected by pitch canker in 1996 was shown to be free of disease in 1999 [76] (Storer et al., unpublished). Furthermore, existing infections had become contained and no new infections had been recorded, which suggested that trees in remission were more resistant to the pathogen. Resistance to pitch canker was confirmed in a subset of the trees in remission by direct challenge with the pathogen [77]. In a separate study, Monterey pines in areas where pitch canker was well established were shown to be significantly more resistant than trees of this species in areas where the disease was a more recent occurrence [77]. This result suggests that exposure to the pathogen resulted in enhanced disease resistance over time.

Blodgett et al. [78] showed that SIR also occurs in Austrian pine (*Pinus nigra* Arnold). When the stems of 4–5 year-old saplings are inoculated with the necrogenic canker pathogen *Sphaeropsis sapinea* (Fr.:Fr.) Dyko & Sutton in Sutton) (syn. *Diplodia pinea*), and its less aggressive [79] sister species, *Diplodia scrobiculata* de Wet, Slippers and Wingfield [80], the whole stem becomes more resistant to subsequent inoculations with *S. sapinea*. The phenomenon is bidirectional, suggesting that molecular signals move both acropetally and basipetally in the tree to elicit the SIR response [78].

Work on the Austrian pine/*S. sapinea* model pathosystem is beginning to reveal biochemical changes that are associated with SIR in the stems. In particular, SIR may be linked, in part, to enhanced lignin deposition and accumulation of certain soluble phenolics [43,78], and induction of traumatic resin ducts and resin flow [39]. Since wounding alone can have strong regulatory effects on terpenoid biosynthesis [81], it is possible that traumatic resin composition in systemically induced trees is also altered in at least one of the three terpenoid groups, i.e. the monoterpenes, the sesquiterpenes or the diterpenes. This change may contribute to the expression of SIR because monoterpenes can differ in their activity against *S. sapinea* [82]. Thus, it is plausible that systemic effects of pathogenic infection on phenylpropanoid and terpenoid metabolisms, as well as de-differentiation of phloem and xylem tissues to form traumatic resin ducts resulting in stronger resin flow, may all contribute to the SIR phenotype in an integrated manner.

Two components of plant defense remain largely unexplored in the study of SIR in conifers: necrophylactic periderm formation and expression of PR and other proteins. The role of the former in limiting invasion of pathogens and insects in trees systemically primed by induction with pathogens is unknown. On the other hand, a recent study by Wang et al. [83] has begun addressing the systemic aspects of host–pathogen interactions in Austrian pine using a proteomics approach. No known PR-proteins were detected in the stem phloem of trees systemically primed with *S. sapinea* and *D. scrobiculata* among the differentially expressed proteins, perhaps indicating that in this system a challenge inoculation is necessary to initiate a PR-protein response. However, Wang et al. showed that

small heat shock proteins, among other proteins, accumulated differentially and systemically in pathogen-induced trees, suggesting a supporting role (as chaperones—e.g. [84]) for these proteins in the eventual expression of the SIR phenotype. No other similar cases of potential "pathogen-induced priming" of protein-based defense responses in conifers have been reported.

4. Ecological consequences of SIR

4.1. Ontogenetic disease resistance

One interesting implication of SIR in conifers is that it may provide an alternative explanation for what is known as ontogenetic disease resistance (ODR) (also known as age-related resistance [85]). ODR refers to resistance to a pathogen that changes with the developmental stage of the host, with resistance usually increasing with age. For example, ODR to white pine blister rust (WPBR), caused by the exotic and invasive pathogen *Cronartium ribicola* J.C. Fisch, has been reported in five-needled pines. ODR appears to be a significant factor in development of this disease, at least in sugar pine (*Pinus lambertiana* Dougl.). In particular, older trees may suffer little damage from WPBR, whereas young trees tend to be severely affected and are often killed [86]. To the best of our knowledge, no specific physiological, molecular or anatomical studies have been conducted to characterize this phenomenon. Nonetheless, this age-dependent expression of resistance has generically been attributed to developmental changes in the host. An alternative explanation is that some trees challenged with the pathogen manifest SIR and this, rather than ODR, is responsible for lesser impacts of the disease on older trees. In fact, ODR to WPBR observed in sugar pine may reflect the cumulative induction of resistance not only by the rust pathogen but also by other microbes, such as endophytes and mycorrhizal fungi. Ontogenetic resistance against insects has also been documented, for example in ponderosa pine against the tip moth *Rhyacionia neomexicana* (Dyar) (Lepidoptera: Tortricidae) [87], and might also be the result of cross-induction of resistance over the life of the tree/tissues by resident microorganisms.

An SIR basis for ODR is an intriguing concept, particularly in light of the fact that induced resistance (and more generally plant responses to stress) appears to have epigenetic components, making it durable and also heritable [88–90]. Such transgenerational adaptive plasticity could be advantageous for the host tree species by generating seedlings that are primed to respond more forcefully to pathogens and insects. However, at present there is no experimental support for this concept in trees.

4.2. Cross-induction of systemic resistance between pathogens and insects

An example of cross-induction of resistance in trees was provided by McNee et al. [91], who demonstrated that *Heterobasidion annosum* (Fries) Brefeld induced decreased feeding by the bark beetle *Ips paraconfusus* Lanier on pre-symptomatic ponderosa pine (*Pinus ponderosa* Lawson) in areas away from the site of infection. *H. annosum* is an important agent of root and butt rot of conifers [92] that is often involved in predisposition of conifers to eventual bark beetle-caused mortality [6]. However, this study demonstrated that systemic resistance against a bark beetle induced by a root pathogen in pre-symptomatic trees may be operating in this system. This phenomenon was associated with biochemical changes in the phloem of similarly inoculated trees [93], but the exact biochemical and/or anatomical mechanisms underlying this cross-induction of resistance remain unknown.

Based on the evidence provided above as well as studies in angiospermous tree/folivore systems (e.g. [94,95]), it is clear that trees possess defense traits that are locally and systemically inducible by pathogens and that can potentially affect insect behavior. However, such host-mediated interactions between pathogens and insects are not often studied in an integrated manner [10]. In the context of host-mediated interactions between root pathogens and tree-killing bark beetles, for example, it appears that such an integrated approach might provide significant insight into factors that can either enhance or diminish stability in conifer-dominated ecosystems. An improved mechanistic understanding of these associations could contribute to development of better management strategies, the practical value of which could be considerable, given that an estimated 6 million m^3 of timber are lost every year to root disease-bark beetle complexes in the western US alone [6].

4.3. A new synthesis

SIR may not contribute to a tree's defense if the inducing event is quick and severe enough. Expression of SIR may also be contingent on which parts of a tree are affected by pathogens and insects. For example, systemic-induced susceptibility (SIS) was observed in cases where Austrian and Italian stone pines were induced on the stem but challenged on the shoots, providing evidence for organ-dependent expression of SIR and SIS [78] (Bonello et al., unpublished results). Thus, the manifestation of SIR may be contingent on the type of damage to which a tree is subjected and the circumstances under which it occurs.

However, we propose that SIR is a critical component of the dynamic interplay between trees, pathogens and phloem-feeding insects, i.e. organisms affecting root systems, stems and branches. Within this specific interaction domain, SIR can be sustained or transiently expressed, depending on the damage level resulting from the induction event (Fig. 1).

Besides providing a framework for understanding specific pathogen–tree–insect associations, the "SIR hypothesis" offers a foundation for expansion of current plant defense theory, specifically those hypotheses addressing environmental effects on expression of host defense

such as the growth/differentiation balance hypothesis (GDBH) [96,97]. The domain of GDBH focuses primarily on plastic responses of constitutive secondary metabolism to variation in resource availability [98], and does not address more or less rapid induced responses such as SIR to pathogen infection. For example, GDBH predicts a nonlinear, parabolic response of constitutive secondary metabolism across a resource gradient (Fig. 2a) [97]. Rapidly growing plants in resource-rich environments are predicted to have low secondary metabolite concentrations due to a resource-based trade-off between primary and secondary metabolic pathways. However, secondary metabolism is predicted to increase under moderate water or nutrient limitation, as growth is more sensitive to resource limitation than is carbon assimilation. Consequently, substrate available for secondary metabolism may increase. However, in extremely resource-limited environments, carbon assimilation will also decrease, and secondary metabolism is predicted to be low due to energy and substrate constraints on biosynthesis.

Induced accumulation of secondary metabolites in stems of trees is energetically expensive, rapidly depleting local carbohydrate reserves, and so must be supported by photosynthate translocated to the site of *de novo* biosynthesis [99,100]. Furthermore, increasing evidence has emerged to suggest that the energetic costs of induced responses can result in tradeoffs with growth or reproduction (e.g. [101–103]). Although relatively few studies have examined environmental effects on expression of induced resistance, evidence is also emerging that the strength of induced responses can be impacted by resource availability. For example, Dietrich et al. [104,105] shown that the strength of constitutive and induced protein-based responses to pathogens in *Arabidopsis* is modulated by nitrogen availability, while induced secondary metabolite production in stems of conifers was found to be weakened by drought stress [106–109]. Similarly, Frischknecht et al. [110] observed that wound-induced alkaloid accumulation was decreased substantially in drought stressed plants. They also found that while moderate defoliation induced high levels of alkaloids, severe defoliation greatly reduced the induced response. Collectively, these studies suggest that severe carbon stress resulting from limitations in photosynthetic function (e.g. nitrogen limitation, drought stress) or destruction of photosynthetic tissue (e.g. defoliation) may constrain biosynthesis of induced as well as constitutive defenses, thus providing a conceptual basis for extending predictions of the growth/differentiation balance hypothesis from constitutive to induced secondary metabolism.

We postulate that the time course of pathogen infection may have a quadratic effect on the strength of SIR that is similar to spatial variation in constitutive secondary metabolism generated by resource availability (Fig. 2b). In the earliest stages of pathogen infection, SIR responses are predicted to rapidly and systemically increase

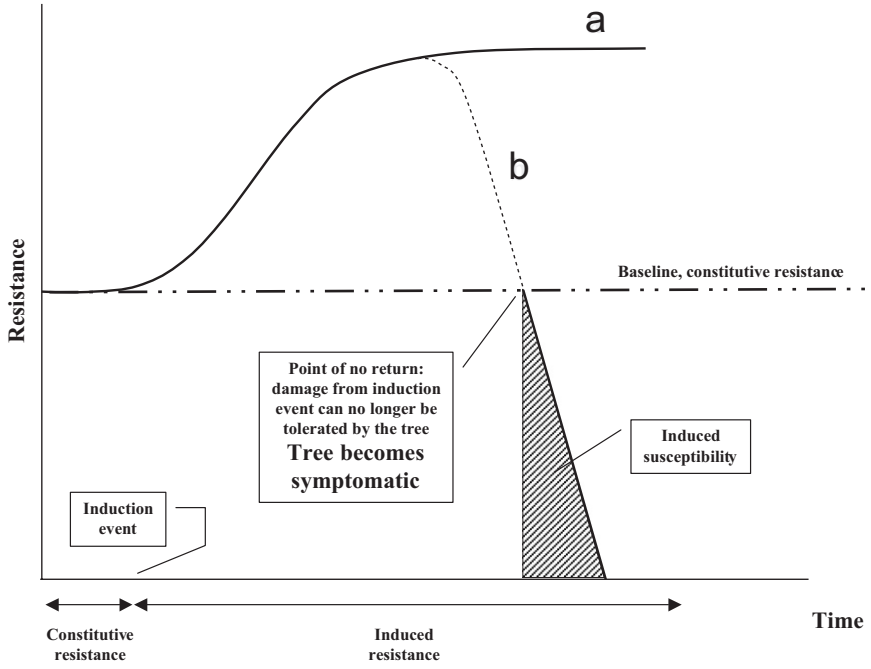

Fig. 1. SIR hypothesis. Diagram representing the systemic induced resistance (SIR) hypothesis, illustrating the interplay between SIR and induced susceptibility in trees against microbes and herbivores. A baseline level of constitutive resistance is present in all trees, but an induction event is predicted to induce SIR against both microbes and insects. SIR is predicted to remain sustained for extended periods of time (a) (e.g. [75]), unless the induction event results in severe impairment of the tree's defensive machinery, with subsequent collapse of tree resistance and expression of systemic induced susceptibility (SIS) (b). An example of this would be pines infected with a root pathogen. Initially, i.e. in pre-symptomatic stages, the pines would be more resistant to bark beetle attack and infection by bark beetle-associated fungi. If the pine becomes symptomatic, then resistance begins to decline and results in the often observed increased susceptibility of symptomatic, root diseased pines to bark beetle infestation. Response scales are arbitrary.

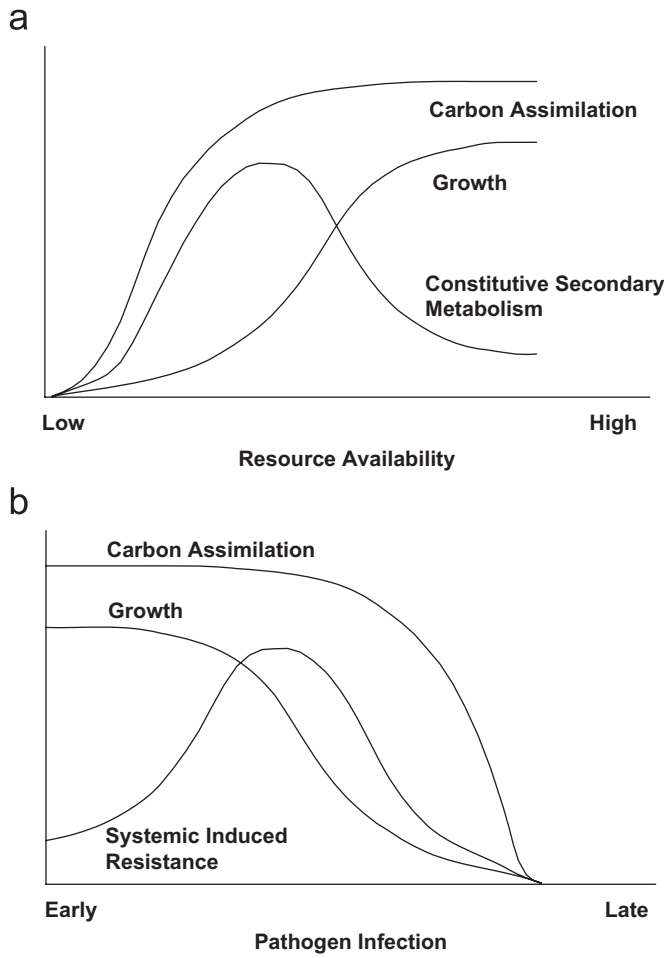

Fig. 2. Postulated nonlinear effects of resource availability (a) [97] and pathogen infection (b) on constitutive secondary metabolism and systemic induced resistance (SIR), respectively. The quadratic response for SIR in (b) is somewhat different from that shown in Fig. 1 to highlight the similarities with the model described in (a).

concentrations of compounds involved in defense against pathogens and insects. However, if the inducing pathogen is able to grow despite the deployment of localized defensive responses, the infection will progress, and the plant will become increasingly stressed by the resulting resource limitations (e.g. reduction of nutrient and water absorption in root-diseased trees). In severely diseased plants, carbon assimilation will decline, and SIR is predicted to weaken as it transitions from an adaptive to an energy-constrained response. Eventually the trees would become symptomatic and systemically susceptible to subsequent attacks by pathogens and insects.

We propose that SIR, as defined herein, is a common and important phenomenon in coniferous trees, one that provides a relatively rapid and effective mechanism for limiting the invasive potential of microbes and insects, at least in stems and branches. Whereas more specific forms of genetic resistance to insect pests and pathogens may also be operative, the long generation times of most conifers suggest that centuries are likely required to achieve significant shifts in the relative frequencies of susceptible and resistant genotypes within a population. In contrast, insects and microbes that exploit coniferous hosts have generation times measured in weeks or months. This difference in life spans implies that other adaptive mechanisms must underlie the stability of forested ecosystems dominated by long-lived conifers. We submit that SIR, inducible by pathogens and effective against both pathogens and insects [111], is one of these mechanisms. For example, rapid- and delayed-induced resistance are thought to exert stabilizing and delayed density-dependent effects, respectively, on herbivore population density [112]. Therefore, any effects of previous pathogen infection on expression of induced resistance may influence the trajectory of insect outbreaks. Elucidation of SIR-based phenomena will advance plant defense theory while increasing understanding of bottom-up regulation of herbivore populations and insect outbreaks in conifers.

5. Conclusions

A mechanistic understanding of physiological, biochemical and molecular processes that influence the outcome of host-mediated interactions, both direct and indirect, between pathogens and insects can contribute to a richer understanding of ecosystem function and the ongoing maturation of plant defense theory. We hypothesize that *SIR is a significant factor in pine stem and branch resistance to pathogen and lethal insect attack*. If this hypothesis is supported, we would expect that the normal state of a tree in a forest is resistance (to pathogens and insects) resulting from a combination of genetic (i.e. constitutive) resistance and resistance induced by chronic, low-level "irritations" caused by microbes (i.e. infections that do not immediately result in symptoms). One way to test predictions of the SIR hypothesis might be by inducing trees with root pathogens and challenging them with pathogens and/or insects on the stem or branches at a later stage. A similar approach was used in a previous field study in which inoculation of large (i.e. >35-year-old) ponderosa pines with *H. annosum* showed systemic effects on both host phloem chemistry and resistance to insect feeding [91,93]. However, working with trees along a temporal axis as modeled in Fig. 1, under field conditions, is very challenging. Induction of SIR requires the inoculation of large numbers of trees at or below ground level in a forest setting and a follow-up period of many years, perhaps up to 10, depending on the root pathogen used, the inherent susceptibility of specific host populations to specific strains of a root pathogen, specific site conditions, etc. In contrast, if space could be substituted for time, it might be feasible to test some predictions of the SIR hypothesis much more efficiently. An arena in which such testing might be possible is offered by well-defined pocket mortality syndromes. One such syndrome is produced by the red pine [*Pinus resinosa* (Aitman)]/*Leptographium terebrantis* Barras & Perry pathosystem in the Great Lakes region [113,114]. In principle, this system is suited to testing the SIR hypothesis

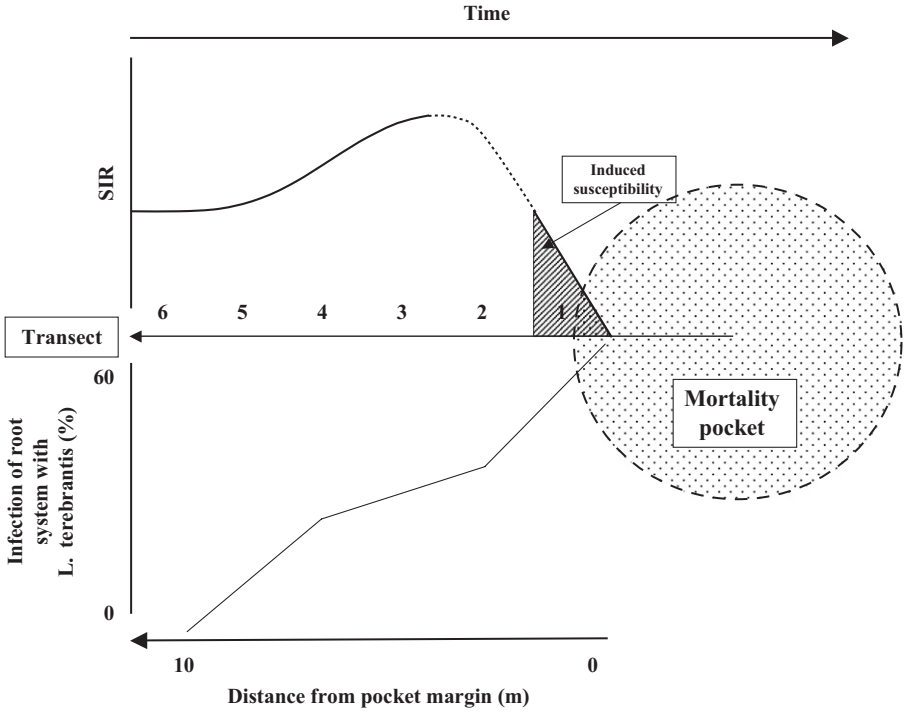

Fig. 3. Schematic representation of a potential test of the SIR hypothesis. The SIR model (upper half of the diagram) is superimposed on a red pine mortality pocket. The transect shows six sampling points moving away from the pocket margin. The lower half of the diagram illustrates the known rate of root infection by *Leptographium terebrantis* [114,115]. Positioning of transect and relationship between actual root infection rates and resistance to pathogen and insect attack are speculative and intended for illustrative purposes only.

in the field because it allows for straight substitution of space for time (Fig. 3). Moving from the outside into a mortality pocket is equivalent to moving forward in time between a root disease-free state to a heavily infected state, all the way to mortality when the pocket edge is reached. Based on the SIR hypothesis and our knowledge of root infection rates in this system [114,115], a constitutive level of resistance to pathogen and insect attack is predicted at least 10 m away from the pocket margin, with *Leptographium*-induced SIR increasing to a maximum somewhere between this outer area and the pocket margin, and decreasing sharply to induced susceptibility levels as the pocket margin is approached. Such varying levels of SIR could be measured in the stem against both pathogens (e.g. *L. terebrantis* itself) and insects (e.g. *Ips pini* (Say), an important bark beetle in this system [113,114]). Concurrently, variables such as strength of defense responses (e.g. PR-protein accumulation, phenolic and terpenoid metabolism, anatomical responses, etc.) as well as physiological traits related to substrate availability (e.g. soil fertility), effects of varying soil fertility on the host (e.g. tissue nitrogen content, C:N ratios), photosynthetic rates, and stomatal conductance, etc., could be measured to integrate the SIR hypothesis with the GDBH.

While this review focuses specifically on pathogens as SIR-inducing agents, the reciprocal process might also be operative, one in which insect feeding induces SIR to insects and pathogens. At present the evidence for such reciprocal induction in trees is lacking. Thus, SIR should be investigated in all possible directions to be able to assess its ecological significance. Indeed, the SIR hypothesis could also be tested in other symbioses, since pathogenicity is just one case in an interactive continuum between plants and microbes. In this sense, testing of the SIR hypothesis with pathogens would provide the foundation for work in other systems, e.g. mutualistic associations such as endophytism and mycorrhization, for which some evidence exists for systemic induction of disease resistance in plants (e.g. [116,117]).

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