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Using the CODIT model to explain secondary metabolites of the xylem in defence systems of temperate trees to decay fungi

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- **Background** In trees, secondary metabolites (SMs) are essential for determining the effectiveness of defence systems against fungi and why defences are sometimes breached. Using the CODIT model (Compartmentalisation of Damage/Dysfunction in Trees), we explain defence processes at the cellular level. CODIT is a highly compartmented defence system that relies on the signalling, synthesis and transport of defence compounds through a three-dimensional lattice of parenchyma against the spread of decay fungi in xylem.
- **Scope** The model conceptualises 'walls' that are preformed, formed during, and after wounding events. For sapwood, SMs range in molecular size, which directly affects performance and the response times in which they can be produced. When triggered, high molecular weight SMs like suberin and lignin are synthesised slowly

(phytoalexins), but can also be in place at the time of wounding (phytoanticipins). By contrast, low molecular phenolic compounds such as flavonoids can be manufactured *de novo* (phytoalexins) rapidly in response to fungal colonisation. *De novo* production of SMs can be regulated in response to fungal pathogenicity levels. The protective nature of heartwood is partly based on the level of accumulated antimicrobial SMs (phytoanticipins) during the transitionary stage into a normally dead substance. Effectiveness against fungal colonisation in heartwood is largely determined by the genetics of the host.

- **Conclusion** Here we review recent advances in our understanding of the role of SMs in trees in the context of CODIT, with emphasis on the relationship between defence, carbohydrate availability, and the hydraulic system.We also raise the limitations of the CODIT model and suggest its modification, encompassing other defence theory concepts. We envisage the development of a new defence system that is modular-based and incorporates all components (and organs) of the tree from micro-to-macro scales.

Key words: CODIT, decay fungi, plant defence, parenchyma, plant-fungi interactions, heartwood, induced systemic resistance, non-structural carbohydrate (NSC), sapwood, secondary metabolites (SMs), secondary xylem, systemic induced resistance.

INTRODUCTION

In terms of longevity, size, and ecological dominance, trees are among the most successful life forms to have evolved. The latter characteristics are largely a consequence of a unique ability to form highly specialised tissues, where like other organisms, trees are exposed to a wide variety of potential pathogens throughout their often very long lifespan. To successfully defend themselves, trees combine constitutive defences with short- and long-term inducible

defences. The ultimate function of these defences is to protect the nutrient- and-energy-rich phloem, the vascular cambium, and the transpiration stream in the sapwood. Defence compartments in trees can be observed at macro-and- microscopic scales. This review focusses on the molecular scale of the secondary xylem tissue in response to wood decay fungi where an expanded version of the CODIT model (Compartmentalisation of Damage/Dysfunction in Trees) is used to explain defence processes (Fig. 1).

Secondary or 'special' metabolites (SMs) are normally defined - with the exception of lignin and suberin - as low-molecular weight secondary products of plants and fungi derived from primary metabolites, and are not considered active in growth processes (Fig. 2, Scharf *et al*., 2014; Pusztahelyi *et al*., 2015). SMs are a crucial part of the tree defence system, which also includes the lignification of secondary walls (Miedes *et al*., 2014), the secretion of peptides called defensins (Terras *et al*., 1995; Lacerda *et al*., 2014), and reactive oxygen species (Inupakutika *et al*., 2016). The latter is involved in programmed cell death in plants during reaction zone formation (a key component of CODIT) against pathogenic fungi in xylem tissue (Inupakutika *et al*., 2016). Trees possess a huge diversity of SMs, where different chemical classes are constitutively present or synthesised *de novo* depending on the threat and the type of the secondary xylem tissue; for example, whether it is sapwood (active defence) or heartwood (constitutive defence). Xylem is composed of four main cell types, tracheids, vessels, fibres (the last two only found in angiosperms), and parenchyma. Parenchyma and living fibres are the only living components of functional xylem (sapwood). The key functions of xylem are water conduction (i.e. vessels and tracheids), storage of non-structural carbohydrates and water (i.e. parenchyma and living fibres), and mechanical support (i.e. tracheids and fibres). Symplast is formed by plasma continuum through cell-to-cell connections via plasmodesmata within mainly simple pits and is interwoven among conductive tissues and fibres forming two kinds, ray and axial parenchyma. Together, ray and

axial parenchyma form a three-dimensional lattice in radial and axial directions and provide a functional link between phloem and the water transport system. Since the vast majority of xylem is composed of dead cells, inducible defences rely on the living parenchyma and the three-dimensional network for signalling, synthesis, and transport of SMs (Fig. 1 and 2A, B, E). It is important to mention that there is remarkable variation among and between angiosperms and gymnosperms in the organisation of xylem and in the presence and frequency of cell types, and this should be considered when applying the CODIT model (Fig. 2).

The physiological mechanisms leading to tree mortality are a complex interaction between abiotic and biotic stresses (Cailleret *et al.*, 2017), and tree defence and resistance capabilities (Boddy and Rayner, 1983; Shigo, 1984; Pearce, 1996; Morris *et al*., 2016a). Only living parenchyma cells within plant tissues are responsive to stimuli through forming the active component of tree defence systems in roots, leaves, and stems. Dead parenchyma, whose extractives (released during cell death) are responsible for the dark colouration in heartwood, together with heavily lignified fibres or tracheids (forming annual rings in temperate tree species), occluded vessels from tylosis (outgrowths from the protoplast of vessel-associated parenchyma cells) and suberised periderm, form the physical and biologically inactive defence.

In wood, both active and physical/preformed defences can be explained by the CODIT model, with 'D' originally standing for 'Decay' but superseded by 'Damage or Dysfunction' in light of later findings in our understanding of tree defence processes (Shigo, 1970, 1984; Shortle, 1979; Liese and Dujesiefken, 1996). CODIT is a concept showing how trees are highly compartmented perennial organisms formed of discrete three-dimensional units that when under threat from any abiotic or biotic stress can trigger the tree into walling-off or compartmentalising damaged or decayed regions. The tree compartmentalises by the

formation of reaction zones together with the conceptual 'walls' (barriers) already present at the time of attack (Morris *et al*., 2016a). The original intention of Alex Shigo, the creator of the CODIT model in the 1970s, was to make the science of tree compartmentalisation accessible to practitioners within forestry and arboriculture, giving them a sound scientific foundation prior to carrying out work on trees (Shigo and Marx, 1977). In the 1970s, wood in living trees was considered a dead substance by practitioners and by even many wood scientists (Schenk, 2018). Therefore, the distribution of an early CODIT booklet aimed at the layman was a breakthrough in forestry and arboriculture in terms of changing our understanding of wood in living trees and our treatment of them. However, due to the model's simplicity, it was open to enormous scrutiny by the scientific community, irrespective of its original purpose. The scrutiny mostly hinged on what caused the tree to compartmentalise, whether that be decay fungi, as the original acronym suggested, or any impairment of the tree defence system that allows the entrance of air to impede the hydraulic system (Boddy and Rayner, 1983). Making a strong case for the CODIT model and the concept's usefulness in explaining defence systems in trees in relation to fungi is subject to the premise that fungi can pose a threat to tree function and that the threat is met with an appropriate response. Our modern understanding of CODIT is that it encompasses the trees' response to any desiccation-inducing threat, whether biotic or abiotic. However, a shortcoming in our understanding of CODIT lies in the uncertainty of whether the tree can recognise a biological threat from a non-biological threat and recognise different fungal classes while reacting discriminately, which is a focus in this review.

CODIT still holds huge relevance in practical tree applications as demonstrated in relatively recent texts (Gilman, 2011; Dujesiefken and Liese, 2015). Moreover, the model can also be used by researchers to advance our understanding of tree pathology, as was intended by Shigo in later works (Shigo, 1984). Another key point is that traditionally the CODIT

model only explained defence processes from a macroscopic viewpoint, with little attention given to cellular and molecular levels (Shigo and Tippett, 1981). Using a chainsaw, Shigo and co-workers made approximately 15,000 dissections of trees in the forest with a chainsaw before developing the CODIT model from his observations of large cross-sections and longitudinal slices of wood (Shigo, 1991). However, since the introduction of the CODIT model, our knowledge of tree-fungal interactions has widened, and we now have a far greater understanding of defence processes, especially at the cellular level. Moreover, the CODIT model was traditionally used to explain tree defence processes in relation to decay fungi generally confined to secondary xylem, a projection of previous studies into decay and boundary-setting concepts in trees (Hartig, 1878; Küster, 1913; Hepting, 1935). Although the concept was developed for the xylem alone, the model could be used effectively to explain all stress-related interferences within and beyond xylem (Boddy and Rayner, 1983; Liese and Dujesiefken, 1989, 1996; Morris *et al*., 2016a). Compartmentalisation has been studied extensively in bark (Biggs, 1992), but not linked to xylem to help us understand CODIT as an integrated system, when considering that both phloem and xylem are connected through ray parenchyma.

This interdisciplinary review has a wood structure-function premise that focusses on our current knowledge of SMs in wood defence using the CODIT model (Fig. 1, 2). We integrate our current understanding of tissue and cellular processes with the concept and reality of compartmentalisation, one of the foundational concepts derived from forest pathology in the 20th Century (Manion, 2003). How trees' cells and tissues communicate through chemical signalling during defence is still largely unknown compared to model herbaceous annuals and perennials such as *Arabidopsis thaliana*. The latter is mostly due to the complex structure of long-lived and highly compartmented woody perennials, the difficulty in carrying out experiments, and the general lack of clonal trees for reliable and

consistent experiments. We discuss both the explicit and implicit properties of the CODIT model and its applicability to the level of microscopy in light of current biological knowledge. In the context of CODIT, we examine the tradeoffs between constitutive and inducible defences against decay fungi, alongside the strategies of fungi to overcome host defences. Also, for the purpose of this paper, SMs are everything outside of what are strictly classified as primary metabolites, with the latter including nucleic acids, proteins, and polysaccharides.

XYLEM STRUCTURE-FUNCTION AND CONSTITUTIVE VERSUS INDUCED DEFENCES

Xylem and the CODIT model

According to the CODIT model, sapwood is made up of four protective boundary-setting conceptual walls, which range from weak to strong in ascending order. Walls 1-3 form discreet anatomical units that are constitutively formed, while wall 4, the 'so-called' barrier zone is different in that it is produced after wounding, is usually enforced by suberin, and is formed by the cambium or cambial derivatives (Fig. 1). Suberin is a complex of suberin polyphenolics (SPP) and suberin polyaliphatics (SPA) that are cross-linked by glycerol, embedded with soluble waxes, and laminated to the interior side of the plant cell walls (Bernards, 2002). Although suberin likely evolved primarily as a gas and watertight layer between cells, it is also highly resistant to decay fungi, with only a few fungal species able to degrade the substance, e.g. *Heterobasidion annosum* and *Armillaria mellea* (Swift, 1965; Biggs, 1987; Boddy and Rayner, 1983). Wall 1 is formed of tracheary elements (conductive cells of either angiosperms or conifers) and the adjacent living axial parenchyma contact cells that can plug vessels by tyloses or production of e.g. polyphenols to impede bi-directional fungal spread along the stem above and below the wound (Morris *et al*., 2018a), and via stem-branch attachments. Wall 2 comprises the latewood of annual rings of temperate tree

species, and ray parenchyma present intermittently along a growth ring. For example, for wall 2 in *Tilia platyphyllos*, highly lignified fibres, often accompanied by marginal bands of axial parenchyma formed between ray units, are only separated by intermittent ray parenchyma, which prevents radial spread of fungi (both inward and outward). Wall 3 describes ray parenchyma that inhibits lateral fungal spread, with ray units ranging in height and width from short, uni-seriate to high, multi-seriate rays (> 10-seriate in some species, and > 1mm in height; IAWA Committee, 1989). Finally, wall 4 or the barrier zone is a region composed of usually multiple layers of enlarged ray and axial parenchyma cells (up to 30 cell layers wide; Pearce, 1996) that prevents fungal decay spread into new xylem laid down to the barrier zone's exterior.

All these conceptual walls are designed to be chemically bolstered by phytoalexins from ray and axial parenchyma in response to wounds to greater or lesser degrees, with non-structural carbohydrate (NSC) reserves providing the source for their production (Swarwick, 1926; Wargo, 1977a; Kemp and Burden, 1986). The synthesis of phytoalexins in response to pathogenic fungi likely depend on: (1) the structural integration and strength of the walls (reaction zones and barrier zone), (2) the fitness of the tree at the time of infection (water status and sufficient NSC reserves; Thalmann and Santelia, 2017), (3) the stage of cambial activity during the year, (4) the level of connectivity between axial and ray parenchyma within sapwood and their link to the secondary phloem, and (5) the pathogenic potential of the fungus. All factors related to tree fitness depend on the tree's genetic constitution (CODIT walls and phytoanticipins) and its relative plasticity (phytoalexins) when under attack, with tree species having contrasting defence strategies depending on survival priorities. For instance, research has shown tradeoffs between bark and xylem with having strong constitutive and induced defences in both tissues highly unlikely (Biggs, 1986; Romero and Bolker, 2008). Also in xylem, the lack of suberin in the reaction zone but its

abundant presence in the barrier zone, as observed in *Tilia platyphyllos*, implies a tradeoff between walls 1-3 and wall 4 (Fig. 2I; Baum and Schwarze, 2002). In secondary xylem, response to wounding almost always comes at a severe cost, the death of wood parenchyma and the cessation of water transport in conducting conduits during reaction zone formation (Shigo and Marx, 1977; Schwarze *et al*., 2000a; Oliva *et al*., 2012). However, in bark, living cells are not always sacrificed following wounding, as observed in some conifer species, where localised accumulation of methyl jasmonate-induced ethylene resulted in polyphenol production in the polyphenolic parenchyma cells along with enhanced sclereid lignification, but apparently without cell death (Hudgins and Franceschi, 2004). Tradeoffs in carbon allocation between different components of the tree are likely to be the case across species with further research necessary to confirm this.

The tree's immune system

There are two known branches forming the plant immune system: the first branch is non-specific and applies to a range of fungi, herbivores, and mechanical wounds, while the second branch discriminates between general threats and virulent pathogens (Jones and Dangl, 2006). Through different response strategies depending on the level of threat, plant energy costs are balanced by administering appropriate SM doses, deploying the second level of defence (pathogen specific response) only as a last resort. However, regarding the latter, our current knowledge of pathogen specific responses in trees is limited to only a few studies making it an important avenue for research.

In general, a process known as pattern triggered immunity (Bigeard *et al*., 2015; Nasir *et al*., 2017), involves the activation of jasmonic and salicylic acid pathways, which communicate to target pathogenic fungi. While SMs from the jasmonic acid mediated pathway are effective against fungi that kill parenchyma directly (necrotrophs), salicylic acid, on the other hand, triggers the accumulation of hydrogen peroxide (H_2O_2) resulting in controlled programmed

cell death in the immediate vicinity of the pathogen (biotrophs). The hypersensitive response forms a defence against biotrophs (fungi with haustoria) by retarding fungal growth and spread to other parts of the plant (Glazebrook, 2005). All decay fungi, the subject of this paper, lie in a spectrum between saprotrophic and necrotrophic.

Sapwood as a three-dimensional structure in relation to defence

An important factor of parenchyma cells is their arrangement as a three-dimensional network (Spicer, 2014; Morris *et al*., 2018a, 2018b) with symplastic continuity throughout secondary xylem and living bark being intermeshed in an apoplastic skeleton (Carlquist, 2018). We still do not know how the level of symplastic interconnectivity plays a role in CODIT, signalling, synthesis, and transport of SMs from a systems biology standpoint. It is reasonable to suggest that the close alignment of the living cells running vertically (axial parenchyma; CODIT walls 1, 2) and radially (ray parenchyma; CODIT wall 3) and their intertwinement allows for the easy transfer of phytohormone signals from the immediate contact region of the pathogen. The former shows that reaction zones also form in advance of decay spread in some species, which is referred to as a dynamic reaction zone in conifers (Shain, 1995) and the temperate angiosperms species *T. platyphyllos* and *Eucalyptus nitens* (Barry *et al*., 2000; Baum and Schwarze, 2002). However, the signalling that leads to reaction zone development at distance from the wound or decay colony in secondary xylem is unknown (Eyles *et al*., 2010; Germain and Séguin, 2011). Also, a dynamic reaction zone may not form in response to the detection of fungal hyphae, but in response to the ingress of air that seeps through upon breakdown of the previous reaction zone. Communication between living cells of ray and axial parenchyma through small densely packed connecting pores called plasmodesmata (Morris *et al*., 2016b) could be augmented through combinations of the phytohormones jasmonic acid, salicylic acid (SMs regulated by COI1 and EIN2 genes), ethylene and abscisic acid, allowing trees to respond in advance of decay/disease spread.

Another important factor to consider is the speed at which host defence signalling macromolecules can travel, which is likely to be extremely limited in parenchyma alone; to keep pace with advancing decay or pathogen-related mycotoxins would demand a faster apoplastic route. Utilising vessels for long-distance signalling transport could meet defence demands, but this requires a depositary system from vessel-associated cells (parenchyma in contact with vessels) into vessels and possibly the retrieval of defence proteins along-route, which involves apoplastic to symplastic transport as suggested by Słupianek *et al*. (2019). The latter workers provide visual evidence of clathrin-mediated and clathrin-independent endocytosis in three temperate angiosperm tree species through loading experiments into the dead vessels using various fluorescent tracers, while also measuring a heightened metabolic activity in vessel-associated cells. Direct evidence of endocytosis helps explain long-distance transport defence signalling in plant-fungi interactions (Mbengue *et al*., 2016).

Conifers, owing to an early-derived anatomical and functional makeup, have a symplastic system that is less densely connected three-dimensionally. Less integration of symplast in conifers is a likely tradeoff with other functions, i.e. tracheids are narrow and have a torus-margo pit-membrane mechanism, making them less prone to embolism (Choat *et al*., 2012; Morris *et al*., 2016b), therefore not requiring assistance from abundant contact parenchyma cells, especially axial parenchyma (Morris *et al*., 2018b), while resin canals surrounded by epithelial cells are specialised in defence against pathogens and general damage and are a likely substitute for 'typical' axial parenchyma (Evert, 2006). Conifers are mostly composed of uni-cellular ray parenchyma (rarely bi-cellular), where ‘typical’ axial parenchyma fractions are generally low and completely absent in some genera (e.g. *Taxus* spp., Morris *et al*., 2016b). The low parenchyma fraction and the subsequent arrangement means that all ray and in few cases axial parenchyma is in contact with water-conducting tracheids (Evert, 2006). Conifers use a different strategy that is both constitutive and

inducible, but preformed. Oleoresin, a terpenoid SM present in resin canals provides various conifers with both mechanical and chemical defence (Smith *et al*., 2016). The oleoresin terpenoids act as deterrents or are directly toxic to insects and pathogens, while the volatile components of oleoresin also act as airborne signaling molecules in recognition of the host by the bark beetles (Zulak and Bohlmann, 2010).

Constitutive resin canals (or intercellular canals; IAWA Committee, 2004) in *Picea* spp., for example, are arranged in defensive bands across xylem as seen in cross-section and are surrounded by epithelial cells that synthesise resin, once triggered (Evert, 2006). The banded arrangement of the canals, similar to marginal axial parenchyma in a range of angiosperm trees (Fig. 2C), likely form the constitutive element (wall 1 and 2 of CODIT), and are induced once living cells abutting the ducts are triggered into filling them with terpenoid SMs. However, marginal ducts in conifers appear to only serve in defence (Ferrenberg *et al*., 2014), while marginal axial parenchyma in angiosperms are likely to be multi-functional (Carlquist, 2018). For example, in angiosperm trees, prior to defence activation, marginal axial parenchyma serves to connect ray to ray, and the ray cells are connected to the phloem via the cambium for the transport of water and assimilate, which function in the osmoregulation of the water transport system (Morris *et al*., 2018a, b). Resin canals, when closed, can form a reaction zone (wall 1 and 2 of CODIT) that completely circumvents the sapwood in tangential and axial directions, which is similar in construct and alignment to the barrier zone (wall 4), the only key difference being that the former develops during wounding and the latter after wounding (Fig. 1, 2C, D, G, H). Resin canals are positioned radially among ray parenchyma (called fusiform rays) in some conifers (Evert, 2006), forming wall 3 of CODIT and helping prevent lateral fungal spread. The differences in anatomy and function in the context of three-dimensionality between conifers and

angiosperms are clear, where defence and hydraulic tradeoffs in each wood type allow for adaptations that suit their geographical distributions and site conditions.

REACTION ZONES AND SECONDARY METABOLITES

The formation of reaction zones

Reaction zones form through programmed cell death at the interface between sound and decayed wood. Macroscopically, reaction zones mostly appear as dark brown necrotic regions that should not be confused with zone lines (Fig. 2A). Zone lines are black on account of melanin accumulation and are produced by a number of fungal species in response to antagonism from another fungus, or strain from the same fungal species, or even in response to living cells to the outside of the reaction zone (Pearce, 1991). Reaction zones are rows of dead parenchyma filled with antimicrobial polyphenolic compounds (phytoalexins), and often structurally reinforced with lignin and suberin (phytoalexins) prior to programmed cell death (Fig. 2A, B, and D). Our knowledge of reaction zone formation in response to decay fungi remains poorly understood, as is our classification of the mode of nutrition of wood decay fungi along the gradient saprotrophic-(hemi)biotroph-necrotroph. Moreover, programmed cell death in trees (reaction zones 1-3) occurs against any desiccation-inducing threat, but with no specificity to a causative agent, as observed in a range of non-woody crop and model plants (Eyles *et al*., 2010; Germain and Séquin, 2011). In the latter studies, clear evidence of salicylic pathways activated in response to biotrophs was observed in crop plants, but not in trees. While there is plenty of evidence to support the role of jasmonic pathways for pattern triggered immunity in trees (Hudgins *et al*., 2004), evidence is scant for the role of the salicylic pathway in secondary xylem (Ollerstam and Larsson, 2003; Germain and Séquin, 2011). However, it is likely that salicylic acid does occur in secondary xylem and that there is molecular cross-talk between the two phytohormones.

Also, we cannot rule out involvement of salicylic pathways in reaction zone formation (CODIT 1-3). Germain and Séquin (2011) conclude that a comparable defence model to that found in short-lived herbaceous species (Durrant and Dong, 2004; Nasir *et al*., 2017) may be transferrable to the genetic model tree species *Populus trichocarpa*. However, reservations for this assumption are based on the longevity of trees versus the short generation time of herbaceous plants. Moreover, there is the question of plant height in relation to systemic acquired resistance and the role of salicylic acid in biotrophic and hemibiotrophic fungal interactions and whether or not sufficient doses of salicylic acid can be modulated in trees due to a plant size restriction. On this account, it is argued that salicylic pathways may not exist at all in trees regarding pattern triggered immunity. Although evidence of systemic induced resistance against necrotrophs (a form of pattern triggered immunity that is not to be confused with induced systemic resistance triggered by beneficial microbes; see table 1) is lacking in angiosperm trees, there are studies showing that it occurs in the xylem of some conifer species', *Pinus nigra* and *Picea abies* (Krekling *et al*., 2004; Luchi *et al*., 2005; Blodgett *et al*., 2007).

Several studies demonstrate how conifers respond specifically to decay fungi through SM production and reaction zone formation, and at distances from the initial site of infection (Krekling *et al*., 2004; Hudgins and Franceschi, 2004). Upon wounding and subsequent inoculation of Norway spruce by the pathogenic white rot fungus *Heterobasidion annosum* (necrotroph-saprotrophic lifestyle; Raffaello and Asiegbu, 2017), traumatic resin canals were triggered by a signal, propagating in an axial direction (wall 1 and 2 of CODIT) at about 2.5 cm per day and forming up to 30 cm above single inoculation points (Krekling *et al*., 2004). The size and number of traumatic ducts appeared to reduce with distance from the inoculation site, indicating a gradual decline in the signal that led to their formation (Krekling *et al*. 2004). Jasmonate-induced ethylene production was shown to be responsible for

reprogramming of the vascular cambial zone for the formation of traumatic resin ducts in conifer stems (Hudgins and Franceschi, 2004). Concerning the composition of constitutive and induced resin, the latter is more monoterpene-enriched (Martin *et al*., 2002) and thus less viscous, which facilitates resin flow in xylem. Reaction zone formation occurring at distances from the initial infection site is in line with microbe-associated molecular pattern (MAMP), microbe-triggered immunity (MTI), and effector triggered immunity described for herbaceous plants (Jones and Dangl, 2006; Dodds and Rathjen, 2010). There are conflicting reports on the toxicity of resin components towards wood decay fungi, with some reports for example indicating that volatile terpenes are more toxic than resin acids to white rot fungi (Cobb, 1968; Yamada, 1992). The inconsistencies in resin toxicity between different studies could partly be due to the fact that constitutive and induced resins may differ in composition and that certain tree species such as pines and spruce species produce two kinds of resin, supersaturated solution of resin acids produced by vertical ducts, and fatty acid enriched resin produced by ray-associated canals (Sjöström 1992).

So, although reaction zones are formed in response to wounding in general, there is a heightened response to fungal pathogens to some conifers, an important modification for our understanding of the CODIT model. Based on the premise that fungi follow wounding, a less robust response during the early stages of compartmentalisation is likely to be a form of priming and related to the protection of the hydraulic system, whereby maintaining NSC reserves in the event of a fungal attack.

Dynamic versus static reaction zones

Two kinds of reaction zone are produced in trees, dynamic and static reaction zones. Dynamic reaction zones are successive reaction zones formed at increasing distances from the wound into formerly healthy sapwood, which are likely related to a poor host response and characterised by a lack of polyphenolic compounds (e.g. in sapwood of a range of

conifers; Shain, 1995). Dynamic reaction zones may occur more frequently in species that lack defence specialisation and investment in axial parenchyma; however, some conifers invest heavily in a network of radial and axial resin canals (*P. abies*, Fig. 2, C-E). A static reaction zone, assumed in most angiosperm tree species, is formed with a higher quality and quantity of SMs and usually prevents any further progress of fungal spread in the region, as was shown in *F. sylvatica* when compared to *T. platyphyllos* (Baum and Schwarze, 2002). Furthermore, a second static reaction zone can be formed upon the failure of the first, but only if NSC reserves for conversion into SMs are in plentiful supply. Reaction zones, unlike the barrier zone (wall 4 of CODIT), are not continuous, where gaps between axial and ray parenchyma connections can provide entrance points for fungi to spread across xylem tissue. The level of symplastic connectivity between living cells varies greatly between angiosperm tree species and directly affects the ability of a host to compartmentalise. For instance, the effectiveness of *de novo* suberin synthesis against decay spread in parenchyma cell walls, vessel linings, and in tylosis of many tree species is limited by the degree of discontinuity between ray and axial parenchyma, and living fibres (Pearce, 1990; Yamada, 2011).

Through the chemical and physical nature of a reaction zone that involves metabolising of NSC into antimicrobial materials (e.g. polyphenolic compounds, suberin, etc), the boundary is particularly important against ingress of air and decay fungi. However, seasonality plays an important factor regarding how effective reaction zones are against a fungal threat, which directly concerns the tree's ability to synthesise SMs (Schwarze and Baum, 2000; Dujesiefken *et al*., 2005). During the dormant season, Schwarze and Fink (1997) observed no phenolic SMs in front and behind the reaction zone after colonisation by the white rot fungus *Inonotus hispidus* in *Platanus* x *acerifolia*, allowing the fungus to successfully breach wall 3 (ray parenchyma) of the tree's defence system. The inability to produce phenolic SMs during certain times of the year highlights the shortcomings of the

defence system where preformed boundaries (i.e. the parenchyma cell interconnectivity of wall 1-3) are presumably only partially effective against fungal pathogens when phytoalexins are not induced. While heartwood and the dead outer bark can solely have a constitutive role in defence, the design of sapwood (and secondary phloem) is based around having an integrated system of preformed and induced defences. The primary role of the three-dimensional interconnected symplastic network is in providing a transport link between phloem and xylem and ultimately in preserving the integrity of the hydraulic system and protecting the cambium (Tyree and Zimmermann, 2002). However, the bidirectional channel for transport is sacrificed for defence purposes, culminating in the death of parenchyma and the blocking of tracheary elements. This changeover presents a critical tradeoff between the conservation of xylem (and phloem) hydraulic and storage space, and successful compartmentalisation of the decayed/diseased region.

SECONDARY METABOLITES IN SECONDARY XYLEM

Taxonomy and definition of secondary metabolites in microbe-plant interactions

Phytoalexins are a heterogeneous group of antibiotics, which fall into a number of classes including terpenoids, alkaloids, lignans, and glycosteroids (Smith, 1996). Phytoanticipins are constitutive by nature or a preformed component of defence, such as those found in heartwood (e.g. polyphenols, quinones, saponins, terpenoids, stilbenes, tannins; refer to Fig. 3, 4 and Supplementary data Table S1 for a breakdown of phytoalexins and phytoanticipins and the SM classes they belong.

Secondary metabolites of heartwood and sapwood

In the absence of infection, heartwood has a range of characteristics that define it from sapwood. Heartwood is a dead substance, except for rare circumstances where parenchyma activity has been detected (i.e. sandalwood; Celedon and Bohlmann, 2017).

Also, heartwood is low in free oxygen, has comparatively low moisture and high CO_2 content (Hietala *et al*., 2015), and generally has an increased durability with some species having a much higher resistance to rot than others (Metsä-Kortelainen and Viitanen, 2009), making the environment hostile to fungi. At the transition zone during heightened metabolic activity just prior to programmed cell death, ray parenchyma releases antimicrobial SMs, phytoanticipins that accumulate in heartwood (Chattaway, 1949; Magel *et al*., 2001). In *Cryptomeria japonica* (Japanese cedar) ferruginol, a diterpenoid SM, was found to accumulate in both ray and axial parenchyma at the transition zone (Imai *et al*., 2005). Similarly, axial parenchyma is thought to play a role in heartwood formation of *Juglans nigra* (black walnut), with contrasting luminance values between inclusions in ray and axial parenchyma suggesting qualitative differences between synthesised phenolic SMs (Phelps and McGinnes, 1983). However, the key functional differences may lie between vessel-associated ray and axial parenchyma and their vessel-distant counterparts, where the former develop gels and tyloses to fill vessel or tracheid lumina at the transition zone, while the latter release mostly SMs synthesised via phenylpropanoid and isoprenoid pathways (Fig. 3 and 4). Raman spectroscopy used together with image analysis software might provide us with a way to detect different tones of colouration in heartwood related to the parenchymal source from where SMs are derived, along with the area (fraction %) the shaded regions occupy (see Felhofer *et al*., 2018).

Three heartwood models have been described, which follow an order based on SM production at the sapwood-heartwood interface and its subsequent accumulation within heartwood: the *Robinia* type I, the *Juglans* type II (Magel *et al*., 1994; Kampe and Magel, 2013), and the newly proposed *Santalum* type III, with the latter based on the retention of living parenchyma in heartwood (Celedon and Bohlmann, 2018). There is no appreciable production of SMs at the transition zone in the *Robinia* type I model from ray parenchyma,

with significant increases of SMs from ray parenchyma at the transition zone in the *Juglans* type II model. The darker the colouration of heartwood, the greater the resistance to microbial rot in general, as characterised by a higher quantity and quality of phenolic and terpenoid based SMs (Shigo and Hillis, 1973; Hillis, 1987). When heartwood forms, the active defence associated with living parenchyma is replaced with a constitutive or passive defence (Shain, 1995). Aside from phenolic SMs filling the lumina of fibres and vessels (tracheids in conifers), aspirated and encrusted pits together with suberin and lignin deposit in cell walls form physical barriers. Some species invest heavily in heartwood defence (phytoanticipins), predominantly owing to their genetic makeup (Hillis, 1987; Hazenburg and Yang, 1991), but also from environmental influences such as growth rate and photosynthate production (Wilkins and Stamp, 1990), nutrient availability (Brix and Mitchell, 1983), and site conditions (Climent *et al*., 2002). Also, the parenchyma fraction of the sapwood was found to be related to heartwood extractive richness in *Pseudotsuga menziesii* (Hemingway and Hillis, 1970) and heartwood diameter in *Pinus canariensis*, which influences the ratio between sapwood and heartwood (Climent *et al*., 1998). Secondary metabolites released from ray and axial parenchyma at the transition zone include the following broad classifications, which all play a role in heartwood durabilty: (1) flavonoids (Harborne and Williams, 2000), (2) quinones (Lukmandaru and Takahashi, 2009), (3) stilbenes (Felhofer *et al*., 2018), and (4) tannins (Haslam, 1989).

In a few studies, SMs extracted from sapwood were shown to be analogous to phytoanticipins extracted from heartwood (Shain, 1995; Burden and Kemp, 1984). One of these SMs is mansonone, a sesquiterpenoid found in *Ulmus* spp. Mansonone is constitutively synthesised in heartwood and actively produced in sapwood in response to Dutch elm disease, a vascular wilt disease. In *Sorbus aucuparia*, the antifungal compound aucuparin, a byphenyl derivative, is normally associated with heartwood, but has been found in the

sapwood as a phytoalexin (Kokubun and Harborne, 1995). Compounds occurring in both heartwood and sapwood (along with bark) are probably quite common across species, but research is still limited in the quantification of SMs in trees, particularly regarding phytoalexin accumulation. Carbon investment into heartwood likely trades off against inducible defences in sapwood (and bark), where a balance between constitutive defences and induced defences may well depend on the nature of the threat and the frequency of attacks as well as the ecological strategy (life history) of the tree species.

Two other tree defence-focused models that could help explain investment in heartwood (constitutive defence) and possible tradeoffs with sapwood (constitutive and induced defence) in relation to heartwood-sapwood defence allocation, include the extended growth/differentiaton model (GDBe; Herms and Mattson, 1992) and the protein competition model (PCM; Jones and Hartley 1999). Both conceptual models address partitioning to SMs by the phenylpropanoid biosynthetic pathway but predict opposite trends in response to environmental constraints, e.g. increased levels of CO_2. The protein competition model predicts an inverse relationship between protein and phenolic synthesis at the cellular, tissue, and whole plant level. In the latter model, both proteins and phenolics use the same phenylalanine (PHE) precursor. However, a biochemical tradeoff occurs in response to phenylalanine ammonia (PAL) activity, where PAL activation leads to the synthesis of phenolics, while its inhibition results in proteins; this is on account of environmental pressure dictating demands for growth and development (proteins), or structure, protection, and defence (phenolics; Fig. 3 and 4). Stressful environmental conditions and subsequent slow growth should cause the activation of PAL and phenolic synthesis, while conditions that promote growth should result in protein synthesis at the expense of defence investment. However, tests using the GDBe model showed a rise in constitutive SMs (i.e. tannins and lignins) in *Betula papyrifera* and *Populus tremuloides* wood in response to CO_2 enrichment

and increased biomass (Mattson *et al*., 2005), but did not discriminate between heartwood and sapwood. More research is required here with a heartwood-sapwood focus on a range of conifers and angiosperm trees to see if CO_2 and growth affects the proportion of SMs being allocated into constitutive versus induced defences. Although the environment certainly plays a role in partitioning of SMs, heartwood SM accumulation for many species of conifer, and diffuse and ring porous angiosperm trees is under strong genetic control (Zobel and Jett, 1995).

SECONDARY METABOLITES AND THE REACTION ZONES OF CODIT

Reaction zones and phytoalexins

Although walls 1-3 of the CODIT concept are in place at the time of wounding, it is important to note that they are only constitutive in terms of their spatial positioning and close alignment (Morris *et al*., 2016a, 2018b), and the degree of lignification of fibres at the growth-ring boundary of temperate trees (wall 2; Fig. 1). Parenchyma becomes activated into phytoalexin synthesis on recognition of a threat, which is triggered in the cell wall before sending signals to the plasma membrane (Hammerschmidt, 1999). Phytoalexin SM compounds in reaction zones are most commonly derived via the phenylpropanoid pathway (Deng and Lu, 2017). However, compounds derived through other pathways have been extracted from reaction zones in response to fungi, including alkaloids (Chen *et al*., 1976) and terpene-based phytoalexins in both angiosperms (Burden and Kemp, 1984; Melcher *et al*., 2003), and conifers (Flodin, 1979; Schuck, 1982; Keeling and Bohlmann, 2006).

Other than histochemical confirmation by microscopy, there are still relatively few accounts characterising polyphenolic SMs (phytoalexins) in reaction zones (and the barrier zone). Of polyphenolic compounds, flavonoids have been successfully extracted from reaction zones in a range of temperate tree species (Feucht and Treutter, 1999; Baum and

Schwarze, 2002). Through HPLC-CRD and spectrophotometric analysis, flavanols (a class of flavonoid) were found to differ significantly in quantity and quality between the reaction zones of *Fagus sylvatica* (common beech) and *T. platyphyllos* (large-leaved lime). Catechin, a monomeric flavanol with antifungal properties against decay fungi (Ardi *et al*., 1998) is found in high amounts in *F. sylvatica* compared to *T. platyphyllos* (Baum and Schwarze, 2002), whereas the fungitoxic epicatechin is found only in *T. platyphyllos*. Of interest is that the highest amounts of phenolic SMs were found on the healthy side of the reaction zone, while the reaction zone margin facing the decay column showed a build-up of cell wall bound proanthocyanidins (Baum and Schwarze, 2002). In another study, *Prunus cerasus* (sour cherry) and *Prunus avium* (gean) also showed great differences in flavanol content (Feucht and Treutter, 1999), demonstrating that even congeneric trees (*Prunus* in this case) can show great variation in SM concentration and chemical composition, which likely plays a role in their ability to compartmentalise successfully after wounding.

During the formation of reaction zones in sapwood, phytoalexin SMs are generally thought to be synthesised in large doses only in response to fungi and bacteria, while being found to accumulate to a lesser degree against abiotic stresses (Yang *et al*., 1989); however, abiotic wounding versus wounding associated with fungi and the level of response in host cells has long been debated in tree defence studies (Shigo and Tippett, 1981; Boddy and Rayner, 1983). Although there is evidence of a heightened response to the Dutch elm wilt fungus *Ophiostoma nova-ulmi* in *Ulmus americana*, where mansonone accumulation is correlated with resistance to the pathogen (Jeng *et al*., 1983; Duchesne *et al*., 1985), there is no work to our knowledge demonstrating a similar result for decay fungi. However, a study by Shortle and Cowling (1978) demonstrated how early non-decay colonisers failed to induce discolouration (reaction zone formation) from living parenchyma, while decay fungi prompted strong discolouration (defence response) in excised live sapwood tissue. The latter

work shows that trees' can differentiate harmful from non-harmful fungi and respond accordingly, demonstrating a level of recognition at the molecular level. One must bear in mind that the success of fungitoxic SMs during reaction zone formation is largely dependent on the inoculum potential and pathogenicity of the fungus; if pathogenicity exceeds SM toxicity, reaction zones can be breached (Boddy and Rayner, 1983).

Reaction zones, wood anatomy and its modification

Reaction zones comprise of numerous cell types, including vessel-distant ray and axial parenchyma, libriform fibres, tracheids (conifers and angiosperms) and vessels. Vessel-associated cells often form an airtight sleeve around the conduits (Braun, 1984; Morris *et al*., 2018a, b). In the reaction zone, *de novo* synthesised polyphenolic SMs from ray and axial parenchyma leak into neighbouring libriform fibres, vascular tracheids, and vessels, thus strengthening the region (Schwarze and Fink, 1997). Through exocytosis along the plasma membrane of parenchyma cells, polyphenols can be secreted from parenchyma cells through pit membranes into adjacent vessels or fibres as precursor molecules with subsequent polymerisation within vessel and fibre lumina. However, we do not know precisely how polyphenols cross the plasmalemma, amorphous layer, and pit membrane of cell walls at the parenchyma-vessel or parenchyma-tracheid pit membranes. Moreover, SM compounds were often involved in the modification of fibre and vessel cell wall structures by forming a narrow black-stained dense layer along the inner wall surface, perhaps through cross-linking with cell wall molecules (Melcher *et al*., 2003; Schmitt and Koch, 2009). The *de novo* enhancement of preexisting lignin along with the synthesis of suberin in parenchyma in the reaction zones are also reported for a range of tree species (Biggs, 1987; Geiger *et al*., 1989). Localised lignification of xylem cell walls was observed in *Hevea brasiliensis* (rubber tree) in response to the decay fungus *Rigidoporus lignosus*, resulting in a 30% increase in lignin content (Geiger *et al*., 1989). Also, in a large range of temperate angiosperm and coniferous

tree species, lamellar suberin was frequently found to form along the inner cell wall structure of ray parenchyma (wall 3 of CODIT; Fig. 1), but less often in marginal axial parenchyma (wall 2 of CODIT; Fig.1) (Biggs, 1987). The latter finding reveals differences in the defence functions between ray and axial parenchyma, and is among the key evidence for wall 3 being the strongest of the walls in place at the time of wounding. Suberin initiation in parenchyma cell walls of *Ulmus minor* and subsequent increased resistance to the wilt pathogen *O. nova-ulmi* was found to be induced by the application of exogenous phenolic compounds (Martín *et al*., 2008). Although the latter study concerns tree phenols originating from external application, it could be suggested that phenol accumulation in parenchyma triggers suberin and lignin deposition in response to pathogenic decay fungi; the amount of suberin and lignin synthesised *de novo* may be related to the composition of the simple phenols produced in the cytoplasm. Simple phenols could form phenolic moieties that are used in liginin and suberin biosynthesis.

Vessel-associated cells in reaction zones are an anatomically and chemically distinct cell type compared to vessel-distant parenchyma and react differently to wounding due to their association with conduits (Morris *et al*., 2018b). Regarding defence in angiosperm trees, the core function of the vessel-associated cells is their involvement in clogging the vessels to prevent longitudinal spread of fungal pathogens (wall 1 of CODIT). Along with preventing fungal spread, the spread of air is restricted following desiccation of wound associated tissue. However, vessel-associated cells are also defensively involved along ray margins in contact with vessels and laterally where axial parenchyma contact vessels (wall 2 and 3 of CODIT). Contrary to vessel distant parenchyma, vessel-associated cells are smaller with few amyloplasts (starch storing organelles), few plastids, and small vacuoles, but have dense cytoplasm, more plentiful mitochondria, large nuclei and many ribosomes (Morris *et al*., 2018a). The combination of smaller vacuoles that house polyphenolic SMs such as tannins

and fewer amyloplasts for storage of NSC suggest that vessel-associated cells are limited in their capacity to manufacture polyphenolic SMs compared to vessel-distant parenchyma, where water transport related functions (and tylosis) take precedence in the former (Holbrook and Zwieniecki, 1999, Goldstein *et al*., 1998; Morris et al., 2018a).

Reaction zones, tyloses, and gels

When tylosis/gels (formed of cell wall content, lignin, suberin, phenolic compounds, and pectins; De Micco *et al*., 2016) are triggered into clogging the vessels either indirectly following embolism formation or directly in response to vascular pathogens from the vessel side, vessel-associated cells die (Fig. 2A). Tylosis development results in the death of vessel-associated cells together with the cessation of translocation in the affected vessels. There are exceptions, however, where vessel-associated cells remain alive and have functions post-vessel translocation cessation (Spicer, 2014), and where localised lignification results in thickening of the secondary walls of parenchyma-vessel pit membrane of rice and eggplant preventing bacterial and fungal spores from entering parenchyma (Benhamou, 1995; Hilaire *et al*., 2001). For the latter, vessel-associated cells remain alive and continue a normal but perhaps reduced role in hydraulic upkeep. Further studies are required here with a tree-focus to provide evidence for the occurrence of localised lignification in response to decay fungi.

The mechanism triggering tyloses or gels (also known as gums) together with the composition of the substance likely depends on whether it is trauma or embolism induced. Trauma induced gels have a high abundance of antimicrobial SMs from different chemical pathways including catechol, flavonoids and coumarins (Rioux *et al*., 1998; Del Rio *et al*., 2001; Baum and Schwarze, 2002), indicating that a different secondary metabolic pathway is possibly triggered in vessel-associated cells in response to pathogenesis (Wallis and Truter, 1978). A recent study by Lesniewska *et al*. (2017) revealed that down regulation of *Pectin Methlesterase1* (PtxtPME1) via jasmonates (i.e. jasmonic acid and metyl jasmonate)

mediated ethylene signaling, when working in synergy with 1- aminocyclopropane-1-carboxylic acid (ACC), trigger gel and tylosis development in aspen plantlets. Jasmonates and ACC were found to depend on ethylene signaling, while treatments with salicylic acid had no effect. The latter finding demonstrates the key role SMs play in tylosis and gel production in vessel-associated cells, but it is not clear if greater jasmonic acid concentrations occur in response to pathogenesis when compared to normal wounding. Jasmonic acid concentrations may vary depending on the extent of the wounding and on the size of the interface between responsive host cells and the fungus.

Trauma-induced gels (i.e. polyphenols, pectin and hemi-celluloses), compared to tyloses, can be secreted through smaller pit apertures than parenchyma-vessel pit membranes, and may be complementary to tylosis in the wider development of reaction zones, where the gels can fill both fibres and vascular tracheids together with vessels (Fig. 2A, B). Studies of two temperate genera, *Betula* and *Tilia*, show that in response to wounds, vessel-associated cells produce and secrete gels into neighbouring imperforate tracheary elements and vessels through interconnecting pit membranes, which involves modification of both the protective layer located on the parenchyma side of the pit membrane and the pit membrane itself, which allow antimicrobial substances through (Schmitt and Liese, 1990; 1992). Deposits of polyphenols in fibre-tracheids originating from axial parenchyma in response to *I. hispidus* (a white rot fungus) were also found in *Platanus* x *acerifolia* (London plane tree) (Schwarze and Fink, 1997). The effectiveness of the constitutive and induced barrier (wall 1 of CODIT; Fig. 1) is seasonal with the most intense defensive responses occurring between May and November (Schmitt and Liese, 1992), which is linked to carbohydrate mobilisation (Tixier *et al*., 2019). However, the exact period during the growing season in which the response is most effective against fungal pathogens would likely depend on NSC availability (Essiamah and Eschrich, 1985; Sauter and Wisniewski, 1996; Hoch *et al*., 2003).

In extant conifers, ray contact cells are not known to produce tyloses and clog tracheids. However, the epithelial cells that surround resin canals appear to completely clog intercellular resin canals in response to fungi by a similar process to tylosis, but intrusions in the case of conifers are referred as tylosoids (Evert, 2006). Tylosoids have been defined by the IAWA Committee (1964) as differing to tylosis in that they do not pass through a pit cavity as found in parenchyma-vessel pit membranes in angiosperms (Fig. 2E). However, from a functional perspective, the latter difference should be regarded as superficial; similar to the function of vessel-associated cells in angiosperms, the blocking of resin canals in reaction zone formation prevents axial and radial spread of decay (wall 1 and wall 2 of CODIT; Fig. 2D and E).

Secondary metabolites and the barrier zone

The parenchyma of the barrier zone is filled with NSC in the form of starch, which becomes mobilised and converted into polyphenolic SMs while in many species lignin and suberin are induced in the cell walls, making the barrier zone both a chemical and anatomical construct (Tippett and Shigo, 1981; Shigo, 1984; Rioux *et al*., 1995). The strength of the barrier zone varies between species and is largely genetically controlled, with successful compartmentalisation of the fungus dependent on the effectiveness of wall 4 of CODIT and the tree's ability to synthesise phenolic based SMs (Fig. 1, 2 G-I). For example, Schwarze *et al*. (2007) speculated that the higher resistance of the barrier zone in *Platanus* x *acerifolia* compared to *Fraxinus excelsior* when colonised by *I. hispidus* was due to the impregnation of the cell walls with suberin in the former and the complete absence of suberin in the latter. Suberin is especially difficult to degrade by fungi, which may explain why the barrier zone is regarded as the strongest wall of the CODIT model (Pearce and Rutherford, 1981; Pearce, 1996; Schwarze, 2000a). White rot fungi in genus *Armillaria* represent some of the few fungi that are capable of degrading suberin (Swift, 1965), but suberin degradation has only been

reported in periderm. The timing of SM synthesis is also a critical factor determining the effectiveness of the barrier zone, which depends on the genetic propensity of the species, the fitness of the individual at the time of infection, the type of SM being produced, and the time of the year. For instance, polyphenolic SMs can be produced more rapidly than lignin and suberin, which is related to the molecular complexity of the compounds, the pathways involved, and the degree of carbon investment (Fig. 2G, I). In general, the accumulation of polyphenolic SMs in the large vacuoles of temperate tree species was followed by lignin synthesis and deposition of suberin (Rioux and Ouellett, 1991a). The deposition of lignin was found to be higher in barrier zones than in cells of uncolonised xylem (Fig. 2H), demonstrating its crucial importance in structural defence (Pearce, 1996; Rioux and Ouellette, 1991a). Interestingly, determining when parenchyma dies after suberin deposition is difficult, but the process towards death is thought to be irreversible once suberisation is initiated (Rioux and Ouellette, 1991b).

SECONDARY METABOLITES AND TREE-FUNGI INTERACTIONS

Secondary metabolites and wood-decay fungi

There are three main fungal wood-decay, brown rot, soft rot, and white rot, with the latter divided into sub-types, simultaneous white rots and selective delignification (Table 1). The decay mode and the genetic potential of the fungus to interact with tree defences can influence whether constitutive and/or induced plant defences are used. For instance, brown rot fungi are generally confined to heartwood (phytoanticipins) and are weakly pathogenic, whereas white rot and soft rot fungi are more likely to be associated with sapwood (phytoalexins and phytoanticipins) and have a higher level of pathogenicity (Supplementary data Table S2). Brown rot fungi have high stress tolerance and use a heart rot strategy to colonise heartwood, a hostile substance to most fungi, being low in O_2, high in CO_2, and high in antifungal SMs (Rayner and Boddy, 1988). The release of small molecules of hydrogen

peroxide and hydroxyl radicals by brown rot fungi allow them to degrade cellulose and hemi-cellulose in cell walls, but leave lignin largely unmodified due to an inability to produce lignin peroxidase, manganese peroxidase and laccase (Hatakka, 1994; Goodell, 2003). Heart rot specialists have generally low ability to detoxify *de novo* synthesised fungitoxic or antifeedant SMs associated with reaction zones and rarely establish in sapwood. Some white rot fungi such as *Phellinus igniarus* are also heart rot specialists and are usually delimited to the heartwood (Seehann, 1979), while *Ganoderma lipsiense*, a weakly pathogenic white rot involved in selective delignification of cell walls, can slowly migrate into sapwood; however, mycelia is usually upheld by strong reaction and barrier zones (Terho *et al*., 2007). In contrast, *Ganoderma adspersum* is moderately to highly pathogenic, with an ability to degrade polyphenols and suberin along reaction zones (Schwarze and Ferner, 2003).

The brown rot fungal basidiomycetes *Fistulina hepatica* and *Laetiporus sulphureus* use different strategies when decaying the durable heartwood of *Quercus robur, Q. petraea* and *Castanea sativa* (all Fagaceae family). Unable to degrade cell walls until a late stage, *F. hepatica* readily breaks down tannins (polyphenolic SMs) giving the heartwood a red-brown colouration, while *L. sulphureus* degrades the cellulose and hemicellulose in the cell wall, but cannot breakdown tannins (Schwarze *et al*., 2000c, Schwarze *et al*., 2003). *F. hepatica* deploys the phenoloxidases, laccase, and the hydrolytic enzyme tanninase to degrade tannins into simple sugars for consumption, whereas *L. sulphureus* lacks tanninases and the ability to metabolise parenchyma cell walls (Schwarze *et al*., 2000a).

Fungal pathogenicity should not be viewed as being in conflict with their ecological significance, especially regarding nutrient recycling. The degree of pathogenicity between different decay fungi just highlights the huge diversity in strategies used by them in the context of CODIT. The strategies vary depending on the key fungal lifestyles that range in a gradient from necrotrophy to saprotrophy. No decay fungi are completely necrotrophic or

saprotrophic, but fall somewhere along a gradient between them. Pathogenic decay fungi have what could be considered stage-specific necrotrophy upon sapwood colonisation, suggesting tradeoffs between wood decay and parasitism (Olson *et al*., 2012). For instance, in a range of deciduous broadleaved trees, the decay fungi *Chondrostereum purpureum* (silver-leaf disease fungus) and *Amylostereum laevigatum* combine necrotrophic and saprotrophic strategies to kill trees rapidly through hydraulic dysfunction from reaction zone formation and direct toxicity. Moreover, the infected trees are relatively small in diameter, so formation of reaction zones likely comes with significant sacrifice. Biphenyl phytoalexin aucuparin has been isolated in the reaction zones of *Malus pumila* presumably in response to *C. purpureum* (Kemp and Burden, 1986), which may form successful walls 2 and 3 of CODIT; Fig. 1). However, CODIT wall 1 is easily bypassed through the excretion of fungal sesquiterpene SM toxins directly into the transpiration stream that cause foliar silvering followed by tree death (Strunz *et al*., 1997). Such fungi that use multiple strategies take advantage of wall 1, the weakest defence construct of the CODIT system (Fig. 1). *A. laevigatum* and *L. sulphureus* both cause decay in the European yew, *Taxus baccata*. However, the differences in decay strategies the latter conifer has with deciduous broadleaf trees against these fungi is undocumented.

Secondary metabolites, beneficial endophytes, and wood-decay fungi

Microorganisms are known to critically affect host physiology and performance where evolution and ecology of plants and animals might be better understood in the context of a holobiont, a concept explaining the relationship between host and all associated microbes (Agler *et al*., 2016). Many endophytes that have developed a co-evolutionary relationship with trees were found to be beneficial to tree health by suppressing decay causing fungi, while other endophytes that may lack a close evolutionary coupling with the host become pathogenic under the right conditions. The endophytic fungus *Taxomyces andreanae,*

considered specific to Pacific yew (*Taxus brevifolia*), produces the SM paclitaxel (taxol), an antifungal diterpenoid that can be produced *de novo* in response to decay-causing fungi (Zhou *et al*., 2010; Jia *et al*., 2016). Paclitaxel is present in all *Taxus* species as well as in *Ginkgo biloba* and *Wollemia nobilis*, two extremely long-lived gymnosperm trees. Several host-specific endophytes can produce paclitaxel, including *Paraconiothyrium* sp., associated with the European yew *T. baccata* (Talbot, 2015). Through *in vitro* experiments with *Taxus* plantlets and fungal culture tests, the pathogenic wood-decay fungi *Heterobasidion annosum*, *Phaeolus schweinitzii*, and *Perenniporia subacida* were found to be suppressed by paclitaxel produced from the endophyte *Paraconiothyrium* (Soliman *et al*., 2015). The authors showed how the endophyte, on recognition of the threat, migrates to pathogen entry points (cracks in the bark) where paclitazel is synthesised in extracellular hydrophobic bodies, only to be released by exocytosis upon contact with fungi. Here, the released antifungal compound from the extracellular bodies coalesce to form a continuous barrier blocking the entrance way to the decay fungi. The latter demonstrates remarkably how endophytes can form a crucial part of tree defence systems that may hold the key to their host's longevity.

Another important genus of ‘mostly’ symptomless (absence of disease expression) endophytes are *Trichoderma* spp., root symbionts that behave as mycoparasites while inducing systemic plant defences to protect the plant through the secretion of various SMs and proteins (Harman *et al*., 2004; Mukherjee *et al*., 2013). *Trichoderma* are even known to suppress and kill a range of pathogenic decay fungi through several mechanisms (Grosclaude *et al*., 1973; Harman *et al*., 2004; Schubert *et al*., 2008; Ribera *et al*., 2017). *Trichoderma harzianum* was successful against *Phellinus noxius* (brown root-rot disease) in a wood block test (Schwarze *et al*., 2012; Ribera *et al*., 2017), while *T. atroviride* showed a high antagonistic potential against the moderately pathogenic *Ganoderma adspersum* and *I. hispidus* and the ascomycete *Kretzschmaria deusta* after fresh pruning wounds were

inoculated with the decay fungi but only had a weak effect against the basidiomycete *Polyporus squamosus* (Schubert *et al*., 2008). *Trichoderma* uses SM compounds, volatile organic compounds, fungal toxic cell wall degrading enzymes (chitinases and glucanases), and appressoria (clamp-like structures) to parasitise other fungi. For instance, *Trichoderma* spp. are regarded as the richest source of peptaibiotics (Zeilinger *et al*., 2016), where this class of SMs act in concert with hydrolytic enzymes to antagonise pathogenic fungi (Schirmböck *et al*., 1994). Other SMs associated with the antagonistic behaviour in *Trichoderma* include: terpenes, peptides, gliovirn, gliotoxin, pyrenes, siderophores and isocyane metabolites (Zeilinger *et al*., 2016).

Plant systemic resistance works through the manipulation of the SM hormones, jasmonic acid, salicylic acid, and ethylene by asymptomless endophytes (e.g. *Trichoderma* spp. and mycorrhizae). The latter fungi can induce host phytoalexin production (priming) against a specific target, a process referred to as induced systemic resistance (Harman *et al*., 2004; Pieterse *et al*., 2014). The induction of resistance in the whole body of herbaceous plants is clear (Vinale *et al*., 2008; Eyles *et al*., 2010; Tytgat *et al*., 2013; Martínez-Medina *et al*., 2017), but how *Trichoderma* (or similar endophytes) activates the defence system in trees remains poorly understood. There is certainly no known evidence to support salicylic signalling pathways in secondary xylem (Eyles *et al*., 2010; Germain and Séquin, 2011). Only a few studies on conifers provide evidence for induced systemic resistance in trees, but only in response to necrotrophs (Bonello *et al*., 2001). The production of phenolic phytoalexins is considered to be terminal for the wood parenchyma involved (reaction zones, walls 1-3), so perhaps signalling is only conducted between roots and leaves to alleviate symptoms from foliar pathogens, with secondary xylem only used to transport the defence molecules; however, overcoming the long distance signalling pathway in trees challenges this hypothesis (but see Kollist *et al*., 2019).

Tree defence systems and non-beneficial endophytes

Relatively few endophytes are host-specific, and have undergone co-evolutionary adaptation with their host plant as in the case of *Taxus* spp. (Sieber, 2007). Symbiosis of most tree endophytes appear to sway more towards commensalism, where it is not clear what health benefits are gained by the host other than a fast decomposition rate at the ecological level. Such endophytes rest as chlamydospores in the xylem of healthy trees until conditions become suitable, such as the onset of drought, changes in nutrient availability, or oxygen levels (Rayner, 1986). In a range of Chilean tree species, the chlamydospores of important decay-causing fungi were found in living xylem ray parenchyma, including the basidiomycetes *Bjerkandera adusta* and *Inonotus* sp. as well as the ascomycete *Xylaria* sp. (Oses *et al*., 2008), while the defence system remained apparently inactivated. *Fomes fomentarius*, a white-rot decay causing basidiomycete not previously considered an endophyte, was also found in the healthy wood of *F. sylvatica* (Baum *et al*., 2003). For the latter fungus, the chlamydospore stage was found in the rays of *F. sylvatica* and more rarely in the vessels of *F. sylvatica* and *Quercus robur* (Schwarze *et al*., 2000a). The spores of decay causing endophytes are thought to enter wood passively through wounds and possibly lenticels, allowing the fungus to eventually spread throughout xylem before switching from latent to active colonisation (Schwarze *et al*., 2000a; Baum *et al*., 2003). It remains unknown how spores enter xylem parenchyma, as there is a size restriction to spore entry. The pore size of intervessel pit membranes in fresh (i.e. never-dried) samples for a number of trees species were found to range between 5 and 20 nm after using colloidal gold particles (Choat *et al*., 2003), and vessel-parenchyma pits are presumably of a similar size. Aside from sapwood endophytes, the brown rot fungus *Laetiporus sulphureus* provides an example of a heartwood endophyte whose chlamydospores have been observed in the ray cells and fibres of *Robinia pseudoacacia* (false locust tree) heartwood (Fig. 2F). We suspect they enter the ray system of

heartwood from the vascular system in functional sapwood and likely remain there in a spore state until xylem dysfunction allows for hyphal development. Once germinated, the hyphae spread centripetally (from sapwood to heartwood) by degrading cell wall structures using chitinase and hydrogen peroxide to access fibres and ray parenchyma, or they await the transition from sapwood to heartwood. Remarkably, there was no visual evidence in cross-section of the presence of hyphae in the ray cells where the chlamydospores rest (Fig. 2F). We speculate that an individual hypha of *L. sulphureus* burrows through the vessel-parenchyma pit membrane, releasing a single chlamydospore in each ray cell (Fig. 2F), before undergoing self-parasitism. However, although self-parasitism is an important part of nitrogen recycling in many fungi at a higher ecological level (Gruber and Seidl-Seiboth, 2012), there is no direct evidence for it in this species.

The strategy used by the decay-causing 'endophytes' appears to be a 'chink in the armour' of the tree's CODIT system, where fungal spores gain access to xylem tissue without activating parenchyma into reaction zone formation until at a late stage when the *in situ* latent fungi become infectious. Regarding the deposition of chlamydospores in living parenchyma from a range of white rot fungi, the question of how fungal spores avoid activating the synthesis of phytoalexins is unknown; they most likely degrade the vessel-parenchyma pit membrane structure (or tracheid-parenchyma pits in non-vessel bearing plants) from the vessel side, which normally activate either lignification of the cell wall to decrease the aperture size to prevent spore entry (common in the case of vascular wilts), or trigger tylosis (Wallis and Truter, 1978). We suggest that there is perhaps a specialised gelatinous layer surrounding the spore that makes it undetectable to parenchyma or that enzymes manipulate the defence signaling pathways (CODIT) rendering the defence system ineffective. Moreover, spores during transit in the water conducting system fail to germinate, which

might be related to low oxygen levels, or the interaction with various compounds in xylem sap. This is an important avenue of research that requires exploration.

DROUGHT STRESS, NON-STRUCTURAL CARBOHYDRATE RESERVES, AND SECONDARY METABOLITES

Considering current concerns about climate change and shifts in rainfall patterns at many places on this planet, the tree defence system may become more vulnerable to various pathogens. Serious pathogens during times of drought include opportunistic decay fungi such as the white rot complex *Armillaria mellea*, which in turn pave the way for secondary decay fungi (more saprotrophic). When the tree's immune system is low, the CODIT system can be overwhelmed and easily breached, which can result in complete failure and tree death. A key to successful tree defence upon compromise of constitutive tiers lies in the availability of NSC (Wargo, 1972; Kemp and Burden, 1986; Hartmann and Trumore, 2016). Starch reserves within the parenchyma of the secondary xylem are critical to offset the effects of short term drought, as shown by O'Brien *et al*. (2015) in temperate tree species. Moreover, total parenchyma fractions (ray and axial parenchyma combined) provide the uppermost limit for the amount of NSC that can amass and in turn be immobilised in sapwood of temperate trees (Plavcová *et al*., 2016). During drought, NSC act as a buffer through osmotic adjustments (Sala *et al*., 2012), but are also available for conversion to SMs for defence, as drought brings with it an increased risk of attack from pathogens. As SMs are converted from NSC, the amount of starch available for conversion may have serious implications in relation to tree defence capabilities during drought stress (Wargo, 1977b; Shigo and Tippett, 1981; Rolshausen *et al*., 2010). Therefore, temperate tree species with more parenchyma and a greater capacity for NSC storage, particularly in storage cells of ray parenchyma (Chattaway, 1951), should have greater defence potential owing to subsequent abundant chemical availability. Research into grapevines by Rolshausen *et al*. (2010) shows that concentrations

of polyphenolic compounds vary between cultivars, with higher concentrations proving more resilient against the fungal canker-and-decay- causing *Eutypa lata*, a disease, often coupled with *Phomopsis viticola*, and a serious concern for wine growers.

Much recent research has focused on secondary metabolite synthesis during drought stress; however, the focus appears to concentrate on water deficits in leaf xylem tissue rather than in wood (McKeirnan *et al*., 2014, 2016; Niinemets, 2016). The latter works describe how NSC is converted into SMs in response to drought stress irrespective of the presence of pathogens. With the exception of SMs involved in tylosis, which are mostly suberised, the production of SMs solely in response to drought is, as far as we know, undocumented for woody xylem tissue. Moreover, it is unclear and perhaps unlikely that leaf defence strategies in trees can be extrapolated to woody parts. Different plant organs likely have different strategies in response to drought. Here, we postulate that there is a tradeoff between leaves and secondary xylem in approach to drought stress. Leaves, like bark, are on the front line of defence in trees, and being disposable organs and the site of photosynthesis, can afford to invest in a more costly strategy.

In leaves, the onset of drought signals stress related phytohormones and enzymes from the phenolic pathway to activate secondary metabolism from primary pathways, where fresh reserves of NSC are available to be converted to SMs (McDowell, 2011; McKiernan *et al*., 2016). In addition, the accumulation of organic alkaloids such as quercitol, presumably arising from vascular parenchyma, has been found to function as osmotica in the leaves of *Eucalyptus* (Merchant *et al*., 2006; Arndt *et al*., 2008), but there is no evidence of this for stems. For leaves, this is outlined as a tradeoff between growth, leaf hydraulic function, and defence (Herms and Mattson, 1992; Caretto *et al*., 2015), and falls under the relatively recent discipline termed metabolomics (Trethewey, 2004). Also, in chlorenchyma of leaves (and bark of many species), the chloroplasts are specialist organelles involved in active defence

against pathogens: the first organelles to receive stress signals before shutting down photosynthesis, chloroplasts liaise with the cell membrane, endoplastic reticulum and nuclei to form an interorganellular immune response that is highly effective against pathogens (Serrano *et al*., 2016). While generally absent in wood parenchyma, chloroplasts may be a key distinction between leaves and stem defence capabilities, providing an explanation for greater defence plasticity in front-line defence along with more rapid plant immune responses.

In sapwood, post drought hydraulic recovery is largely dependent on the upkeep of vessel-associated cells and vessel or tracheid translocation. However, in temperate angiosperms, a reason for the cessation of translocation in vessels in compartmented regions of sapwood on account of embolism, might be speculated to preserve the integrity of the stem hydraulic system as a whole by preventing rapid spread of emboli and, indirectly, fungal spread. The latter scenario is explained by various models similar in principle to CODIT, including: hydraulic segmentation (Tyree and Zimmermann, 2002), hydraulic sectoriality (Orians *et al*., 2002; Zanne *et al*., 2006), and hydraulic redundancy (Schenk *et al*., 2008). Tree hydraulics is often viewed in separation to tree defence, but the two functions are entwined; when the hydraulic system falters, the defence system is activated. The integrity of the water conductive system against the ingress of air (embolism formation) is foremost to safeguard the tree, and all tree responses to fungal pathogens revolve around maintenance of the hydraulic system (Rayner and Boddy, 1988; Schenk *et al*., 2008). Safeguarding the tree requires careful readjustment of carbon imbalances (Sala *et al*., 2012; Oliva *et al*., 2014). Overcompensation in response to fungi (an overactive CODIT) can result in depletion of resources and can also disrupt hydraulic activity through excessive tylosis, a tipping point often resulting in premature death. Overcompensation is a known occurrence for *Ulmus* spp. (elm) in response to Dutch elm disease and for *Pinus* spp. in response to *Heterobasidion*

(Rishbet, 1951; Kirisits, 2007). An overactive CODIT is especially critical when fungal activity is coupled with abiotic stresses such as drought. Also, excessive compartmentalisation reduces xylem space otherwise used for NSC storage and transport, placing greater stress on the tree at the whole plant level (Shigo, 1982; Oliva *et al*., 2012; Smith, 2015; Hartmann and Trumbore, 2016). In light of this, the CODIT system, which is confined to xylem (largely sapwood), needs modification to integrate more distal organs for a whole tree approach.

CONCLUSION AND OUTLOOK

In this review we have extended the applicability of the CODIT model to help us further our understanding of tree defence processes at micro-scales in relation to decay fungi. Research at the micro-scale now clarifies that trees recognise differences between threats, and can even identify levels of threat within fungi, new findings which should be factored into the CODIT model. Moreover, the CODIT model and the 4 walls is mostly limited to the xylem (particularly sapwood), which fails to recognise that the tree is an interconnected modular-based system, where tradeoffs exist between the hydraulic system and defence in a continuum across leaves, bark, roots, and stem. To better understand bark for instance, detailed histology and experiments on a range of species with different bark thicknesses is required to interpret tradeoffs within bark (between the phloem and periderm) and between bark and xylem. The coordination between modules should form an all-encompassing system based on a new CODIT concept design that should make the model applicable to all tree defects, insects, diseases, and necrotrophic-saprotrophic decay fungi. Also, the CODIT model should not only belong under the remit of forestry, but become available to our understanding of compartmentalisation in plants from genetic to micro-macro scales across all plant-based disciplines. The concept's focus on wood decay has prevented its usefulness beyond forestry.

Increased parenchyma and vessel interconnectivity in angiosperm trees should allow for more rapid signalling, synthesis, and transport of SMs. However, greater vessel connectivity, although hydraulically more efficient, can result in an increase in embolism and fungal spread, the idea behind hydraulic-focused models, such as hydraulic sectoriality (Orians *et al*., 2002; Zanne *et al*., 2006), and hydraulic redundancy (Schenk *et al*., 2008). CODIT should envelop the latter models to embrace the important tradeoffs between defence and hydraulics, such as how reaction zones form at the expense of water transport resulting in often regionalised hydraulic cessation upon successful compartmentisation of the decayed or damaged region. Conifers lack a highly integrated three-dimensional system and are anatomically and functionally distinct from angiosperms, deserving a different approach when applying CODIT. Another important aspect of three-dimensional connectivity is related to signaling and the long distance transport of jasmonic and salicylic acid from root-based symbionts such as *Trichoderma* spp. and mycorrhizae. Large scale field studies are required to investigate if induced systemic resistance from the latter endophytic fungi can occur in trees as has been shown in model crop plants. Specifically, we need to understand if a heightened immunity against pathogenic fungi can be triggered across the whole plant body without activating cell death and reaction zone formation in sapwood, when considering the complexity, size, and longevity of trees.

We also need to investigate SM production speed and SM composition in reaction zones and the barrier zone in both conifers and angiosperm trees using control tests with just mechanical damage versus mechanical damage together with a range of pathogenic decay fungi. The latter tests might universally demonstrate that heightened response occurs in response to pathogenic fungi with noticeable differences between pathogens depending on toxicity levels compared to only mechanical damage and air ingress. An understanding surrounding the allocation of constitutive SMs during ontogeny (genetic control) and

inducible SMs during growth and development also highlights shortcomings in our knowledge of xylem and beyond xylem tree defence, which need to be addressed. The CODIT system is a pragmatic approach to explaining and understanding defence processes in trees. With more research alongside the expansion of the current model we can make it a more widely used framework to better understand genetic influence, microbial-plant interactions, and cost-benefit tradeoffs in trees.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. **Table S1**: Low molecular weight and complex secondary metabolites found in the reaction zones and barrier zone of CODIT in a range of tree species. **Table S2:** Overview of the major fungi associated with xylem tissue in trees.

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Table 1. Overview of key terms used in tree defence systems

Term	Meaning and references
Biotroph	A specialised pathogenic fungus that feeds off living parenchyma without killing them and usually causes disease. Possesses appressoria or haustoria
Brown rot fungi	Decay fungi specialised in breaking down cellulose in plant secondary cells walls using specialised digestive enzymes, such as cellulase (Schwarze *et al*., 2000c)

Dynamic reaction zone	A migrating reaction zone that keeps pace with the advancing decay by developing into previously functional sapwood and preceded by drying wood. Typically found in conifers and some angiosperms (Shain, 1995), Barry *et al*., 2000; Baum and Schwarze, 2002)
Induced systemic resistance	A resistance mechanism in plants triggered by beneficial microbes, e.g. *Trichoderma harzianum* or *Mycorrhiza* spp. Priming of whole plant body is through activation of jasmonate and ethylene and by salicylic signalling pathways (Pieterse *et al*., 2014)
Jasmonic pathway	Leads to localised synthesis of jasmonic acid, a SM plant hormone essential for the immune response against necrotrophic pathogens (Pieterse *et al*., 2014)
Necrotroph	Pathogenic fungi that actively kill host cells as a source of nourishment
Pathogen	A pathogen is a disease-causing organism. A pathogen is interpreted here as the ability to kill or cause parenchyma death or pass reaction zone defences in sapwood. Pathogenicity ranges from low to high along a saprotroph-necrotroph gradient for decay fungi
Pattern triggered immunity	An innate immune plant recognition system triggered by activating the plasma-membrane in response to pathogenic fungi. Part of a two-tiered defence system alongside effector-triggered

	immunity (Jones and Dangl, 2006)
Phytoalexin	Secondary metabolites produced by parenchyma in response to a plant pathogen or saprotroph
Phytoanticipin	Pre-existing or constitutively produced SMs that interact and inhibit fungal colonisation, e.g. in heartwood
Salicylic pathway	Leads to an accumulation of the phenolic SM salicylic acid around the fungal infection or wound site in response to effector-triggered immunity (Betsuyaku *et al*., 2018)
Saprotroph	A decay fungus that solely lives on dead tissue, especially regarding heart rot decay. Has low pathogenicity (e.g. *Fistulina hepatica;* Schwarze, 2000c)
Soft rot fungi	Similar to a brown rot, these fungi selectively degrade lignin, but the fungal hyphae grow in the cell wall and follow the direction of the microfibrils (Schwarze *et al*., 2000a)
Static reaction zone	A non-migrating reaction zone formed between sound and discoloured wood in response to fungi or any desiccation-inducing threat. Typically found in angiosperms (Shain, 1995)
Systemic induced resistance	Infection by a pathogen which results in an induced or greater resistance to further challenges by the same pathogen (Bonello *et al*., 2001)
Vessel-associated cells	Parenchyma in direct association with the vessels that have specialised functions compared to vessel-distant cells (Morris *et al*., 2018b)

White rot fungi	Decay fungi specialised in breaking down lignin using specialised digestive enzymes, e.g. laccase. A simultaneous white rot can degrade cellulose and lignin at equal rates, whereas a selective delignification white rot primarily involves lignin (Schwarze *et al*., 2000a)

Figure captions

Figure 1. Conceptual drawing illustrating the three-dimensionality of xylem and the four walls of the CODIT mode in a typical temperate angiosperm tree (A). Numbers 1-4 with arrows represent the CODIT walls from weakest to strongest: wall 1, prevents decay spread in axial directions by plugging vessels with tyloses and gels from vessel-associated cells (VAC); wall 2, the annual rings, restrict decay in radial directions through lignified fibres and polyphenolic SM production from ray and marginal axial parenchyma (MAP); wall 3, ray parenchyma (RP) plugs vessels from VAC and is critical in reaction zone development; and wall 4 or the barrier zone, is a lignified and suberised parenchymatous zone of phenolic compounds initiated by the cambium after wounding that prevents decay spreading outwardly (and inwardly) into new sapwood. VDP: vessel-distant parenchyma connecting vessel-to-vessel (V) and ray-to-ray forming a continuous three-dimensional lattice in axial, radial, and lateral directions. Darkened regions represent decay columns.

Figure 2. Micrographs of a range of angiosperm and conifer tree species showing reaction zone (A-F) and barrier zone (G-I) development as part of the CODIT tree defence system. (A) *Fagus sylvatica* (Fagaceae), pseudosclerotial plates or dark strands of melanised mycelia

can be seen in the vessels and comprise zone lines formed by the ascomycete *Kretzschmaria deusta*; polyphenolic SMs occlude fibre-tracheids (white arrow), which are synthesised in vessel-associated cells (black arrows); scale bar, 50 µm. (B) *F. sylvatica*, reaction zone stained with acridine orange under fluorescence microscope showing polyphenols in fibre-tracheid lumina and in adjoining pits (white arrows); scale bar, 50 µm . (c) *Picea abies* (Pinaceae), a ring of uninduced constitutive resin canals tangentially oriented along the early wood (black arrows); scale bar, 200 µm. (D) *P. abies*, resin-induced traumatic resin canals forming a distinct tangentially oriented impervious barrier that operates to prevent axial and radial spread of fungi in wall 1 and wall 2 of CODIT, respectively. Epithelial cells produce tylosoids that protrude into the canals, completely closing them (black arrows); scale bar, 50 µm. (E) *P. abies*, close-up of tylosoid development from epithelial cells lining the resin canals; scale bar, 30 µm. (F) *Robinia pseudoacacia* (Fabaceae) a single chlamydospore deposit (arrows) in each ray cell from fungal hyphae belonging to the basidiomycete *Laetiporus sulphureus*; scale bar, 50 µm. (G) *Platanus* x *acerifolia* (Platanaceae), deposits of low molecular weight polyphenolic SMs accumulate in axial parenchyma of the barrier zone prior to suberisation and lignification of cell walls (Bz), an anatomically distinct region that differs to normal wood (Nw); scale bar, (H) *P.* x *acerifolia*, fluorescence microscopy image (Fig. 2G) shows suberin and lignification of axial parenchyma cells walls, two high molecular weight SMs that are part of inducible defences in barrier zone formation, (I) *Tilia platyphyllos* (Malvaceae), heavily suberised axial parenchyma with polyphenolic SM deposits in the barrier zone in response to treatments from a range of decay fungi (black arrows); scale bar, 50 µm. Images A, B, G, H, and I are the copyright of Francis W.M.R. Schwarze.

Figure 3. Diagram of major chemical groups and the main chemical pathways found in the secondary xylem of trees. Phytoanticipins (in red) are preformed secondary metabolites (SMs), while phytoalexins (in blue) are induced as part of a host response to decay fungi.

Figure 4. The biosynthesis of the major chemical defence groups in secondary xylem tissue, terpenoids, fatty acids, alkaloids, and simple to complex phenols. We include all compounds outside of primary metabolism under the framework of secondary metabolites, including complex molecular structures such as lignin. The latter is produced through the phenylpropanoid pathway.

Figure 1.

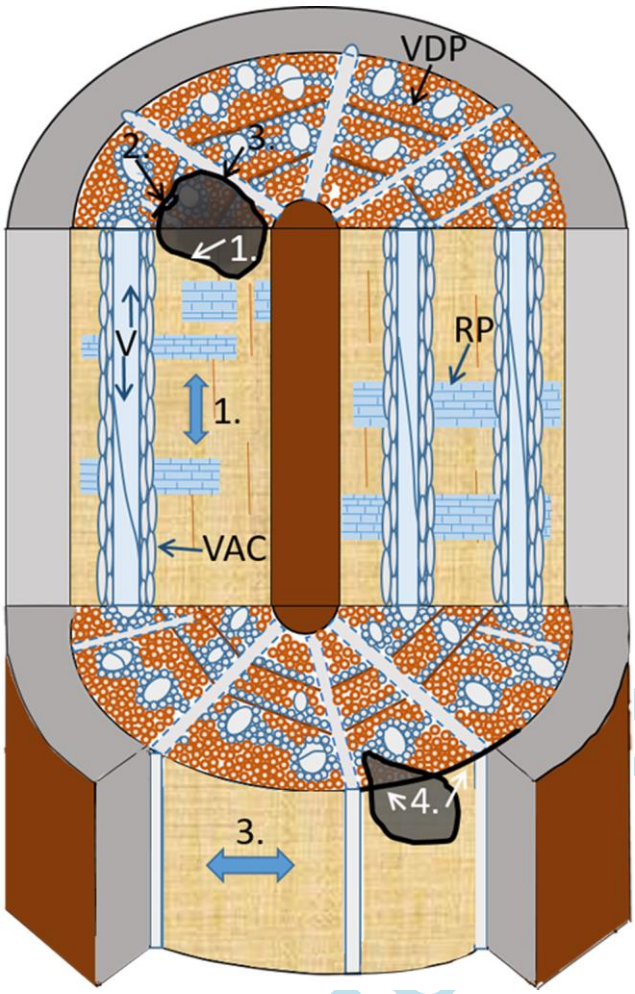

Figure 2.

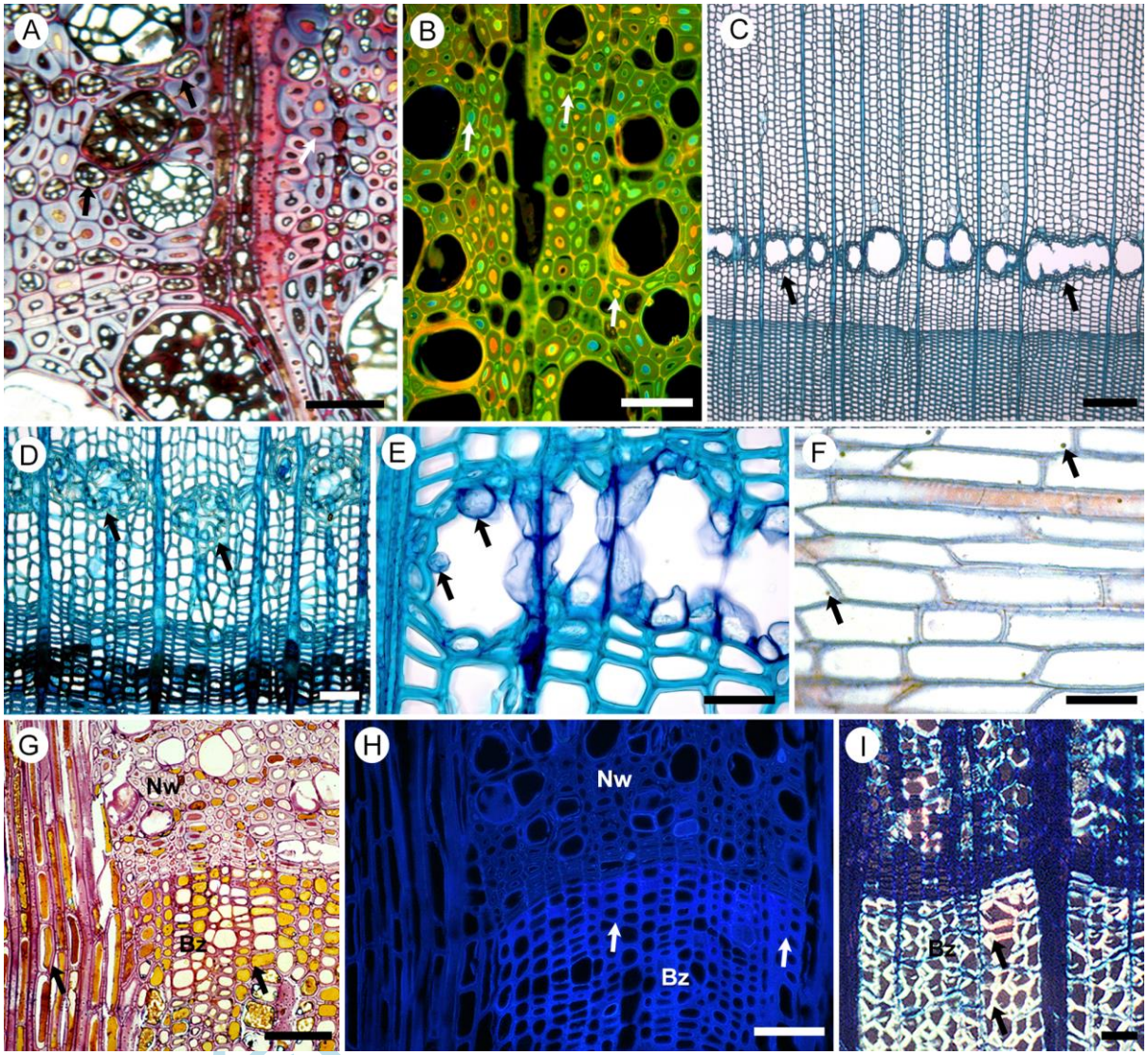

Figure 3.

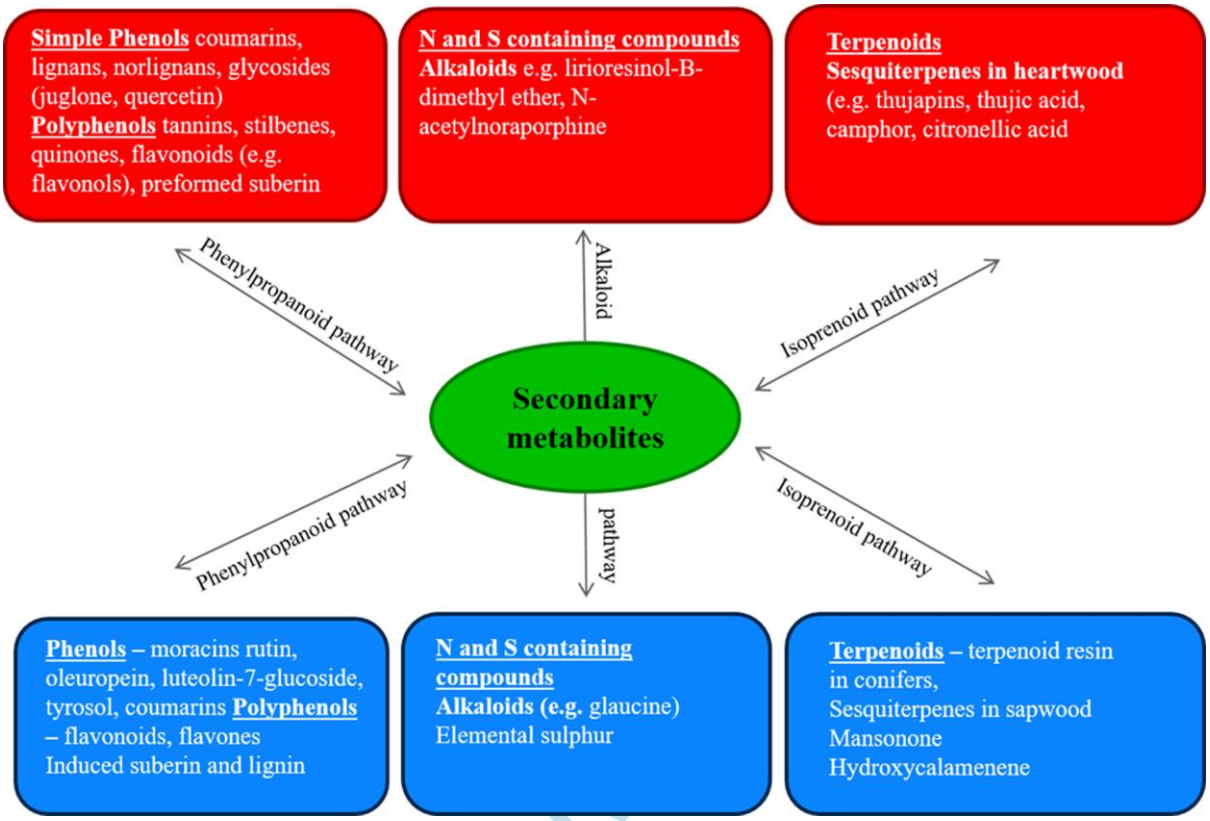

Figure 4.