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Conifers are keystone species in many ecosystems, dominating the vast boreal forests of the northern hemisphere as well as many temperate and mountainous forest ecosystems. These forests are critically important for terrestrial carbon fixation, as sources of energy and bio-based products, and for recreation. The conifers are an ancient group of gymnosperms with a phylogenetic history that goes back at least 200 million years. Throughout this period conifers have been challenged by insects and pathogens and have evolved complex defense mechanisms to combat these threats. These defenses, which may be either preformed or inducible in nature, are integrated into a coordinated, multi-purpose defense strategy that has stood the test of time (Franceschi *et al*. 2005). Conifer defenses are molecular, chemical or physical in nature, but all reside in different cell structures and thus have an anatomical basis. The ultimate purpose of defenses in conifer stems is to maintain the vital functions of the bark, vascular cambium, and sapwood. The first line of defense is made up of preformed (constitutive) defenses. These include components with a physical or mechanical mode of action, such as lignified stone cells in the bark, and components with a chemical mode of action, such as the terpenoid resin stored in specialized resin ducts found throughout the bark and sapwood (Figure 1). The second line of defense consists of inducible defense responses that are activated by an attack or infection. Inducible defenses range in organizational complexity from activation of existing defense structures, such as resin ducts or polyphenolic parenchyma cells, to the formation of entirely new tissues such as traumatic resin ducts and wound periderms (Figure 1). In addition to these short-term inducible defenses, conifers also have the ability to prime or induce additional defenses that increase tree resistance to future attacks (Krokene 2015). The conifers' ecological successes are the result of their unique and proven defenses, which have allowed them to develop into some of the largest and oldest organisms on earth.

Today, many conifer forests worldwide are facing new challenges due to anthropogenic disturbances such as climate change-induced drought, pest range expansions and invading nonindigenous species (Allen *et al*. 2010, Aukema *et al*. 2010). These challenges accentuate the need for more in-depth understanding of conifer defenses and their interactions with insect and pathogen pests. So far, our understanding of the basic structure/function aspects of conifer defense has progressed surprisingly slowly considering the conifers' great ecological and economic importance. For example, the role of the most important cell type in conifer defense, polyphenolic parenchyma (PP) cells, was only thoroughly characterized as late as 1998 (Franceschi *et al*. 1998). Stone cells, another conspicuous cell type in conifer bark, has long been implicated in defense against insects, but the paper by

Whitehill *et al*. in this issue of *Plant, Cell & Environment* is the first comprehensive report on this important resistance trait. This study is an excellent example of how our understanding of conifer defenses can be advanced by adopting a comprehensive, multipronged approach that combines sophisticated microscopy with chemical and molecular analyses (Figure 2).

The stone cells of conifer bark are massive, irregularly shaped cells with extremely thick lignified secondary cell walls (Figure 1). They occur as single cells or clusters in the bark of the 225 species making up the pine family, including the economically important Sitka spruce *Picea sitchensis* studied by Whitehill *et al*. (2015). Their physical toughness and distribution in bark suggest that stone cells function as a preformed physical defense against bark feeding insects. Stone cells are one of many different cell types involved in conifer stem defenses, and these defenses are generally organized in concentric layers around the stem circumference (Franceschi *et al*. 2005). The periderm forms the outer boundary of the bark and is a multi-purpose barrier with cell types that protect against desiccation and form a physical and chemical barrier to invading organisms (Figure 1). Just beneath the periderm is the cork cambium – a secondary meristem that produces cork tissues outwards and secondary cortex inwards. The cortex, consisting of large undifferentiated cells with phenolic inclusions and large cortical resin ducts, is an important defensive barrier in young stems, such as the apical shoots studied by Whitehill *et al*. (2015), but its functions are gradually taken over by the secondary phloem in older tissues. The secondary phloem makes up the bulk of the living bark in mature trees and is the major site of conifer stem defenses. The three most important defensive cell types in the secondary phloem are (1) the PP cells with their characteristic phenolic inclusions, (2) cells with calcium oxalate crystals, and (3) lignified sclerenchyma cells (stone cells in members of the pine family, fiber cells in most non-pine conifers) (Figure 1). In addition to these concentrically arranged cell types are the radial rays and associated resin ducts that extends radially from the secondary phloem into the sapwood (Figure 1). At the interface of the phloem and sapwood is the vascular cambium, producing undifferentiated wood cells inwards and phloem cells outwards. The meristematic cells of the vascular cambium have no defense capabilities, but in response to an attack the cambium can quickly be reprogrammed to produce cells that are differentiated into PP cells or traumatic resin ducts. Together with the radial rays and the occasional axial resin duct the traumatic resin ducts are the only living defense structures in the sapwood, which mostly is made up of dead, water conducting tracheid cells (Figure 1). The defensive capability of conifer stems clearly relies heavily on anatomical defenses, yet only a handful of studies have so far explored the morphological basis of conifer resistance traits.

Whitehill *et al*. (2015) present the most comprehensive characterization of conifer stone cells to date and thus fill a significant gap in our basic understanding of conifer defenses. The few previous studies on stone cells have focused on microchemical analyses of laser-microdissected cells in Norway spruce *Picea abies* (Li *et al*. 2007) and stone cell abundance/lignin content in Sitka spruce and their effect on insect performance (Wainhouse *et al*. 1990). Whitehill *et al*. have a much wider scope, and by combining careful anatomical characterization using confocal and electron microscopy with metabolite analysis, immuno-histochemical staining, transcript analysis and fluorescence-tagged monolignol incorporation assays they provide a comprehensive overview of the defensive roles of stone cells in Sitka spruce. Indeed, their study is perhaps the most complete single-publication characterization of any defense structure in conifer stems. Their broad methodological scope, combining an array of different methods, is an excellent example of the multipronged approach needed to unravel the morphology, development and function of conifer defenses (Figure 2).

The study's experimental design is centered around a comparison of two clonally propagated Sitka spruce genotypes that are either resistant or susceptible to attack by the white pine weevil *Pissodes strobi*. The morphology, development and chemical composition of stone cells in these two genotypes are compared using microscopy, antibody/immuno-histochemical labeling, and transcript analysis of a set of genes involved in the monolignol biosynthetic pathway. Through skillful combination of different microscopy techniques and carefully crafted illustrations Whitehill *et al*. show how the distribution and structure of stone cells differ between the resistant and susceptible genotype as well as between stem parts. Stone cells were found to be more abundant in the upper parts of apical shoots, which is consistent with a role in defense against the white pine weevil that preferentially oviposits and feeds in these plant parts. More than 30% of the cross-sectional cortex area in the upper shoot parts consisted of stone cells in the resistant spruce genotype, which had many more stone cells in these tissues than the susceptible genotype. Through detailed biochemical characterization, Whitehill *et al*. also demonstrate differences in the biochemical composition of lignin in stone cells from resistant and susceptible genotypes.

The thorough anatomical, chemical, and molecular characterization of stone cells presented by Whitehill *et al*. (2015) is a valuable first step to describe the roles of stone cells in conifer defense. However, to fully understand the defensive functions of stone cells it is necessary to also study how these cells affect insect herbivores. Plant resistance traits are those that increase plant fitness by decreasing the performance of an attacker (Karban & Baldwin 1997), implying that plant resistance can only be precisely characterized by studying both the plant traits and their effects on the aggressor. The next step to fully characterize the role of stone cells in defense of Sitka spruce against the white pine weevil is thus to explore how stone cells affect weevil performance, thereby completing the full circle of complementary approaches illustrated in Figure 2. Stone cells are probably less likely to interact with fungal pathogens since mature stone cells are dead and chemically inert, but some evidence suggests that stone cells also may interfere with fungal colonization (Wainhouse & Ashburner 1996). Possible modes of action of stone cells against white pine weevil larvae could be that the physically tough stone cells disrupt larval feeding and performance by wearing down the mouth parts or by interfering with digestion. Functional studies of how stone cells interfere with an insect herbivore would fill an important gap in our understanding of conifer defense mechanisms. More studies on stone cells and other defenses in conifers are needed to effectively manage these important tree species in an increasingly changeable future. The multipronged approach used by Whitehill *et al*. is an excellent model for such studies.

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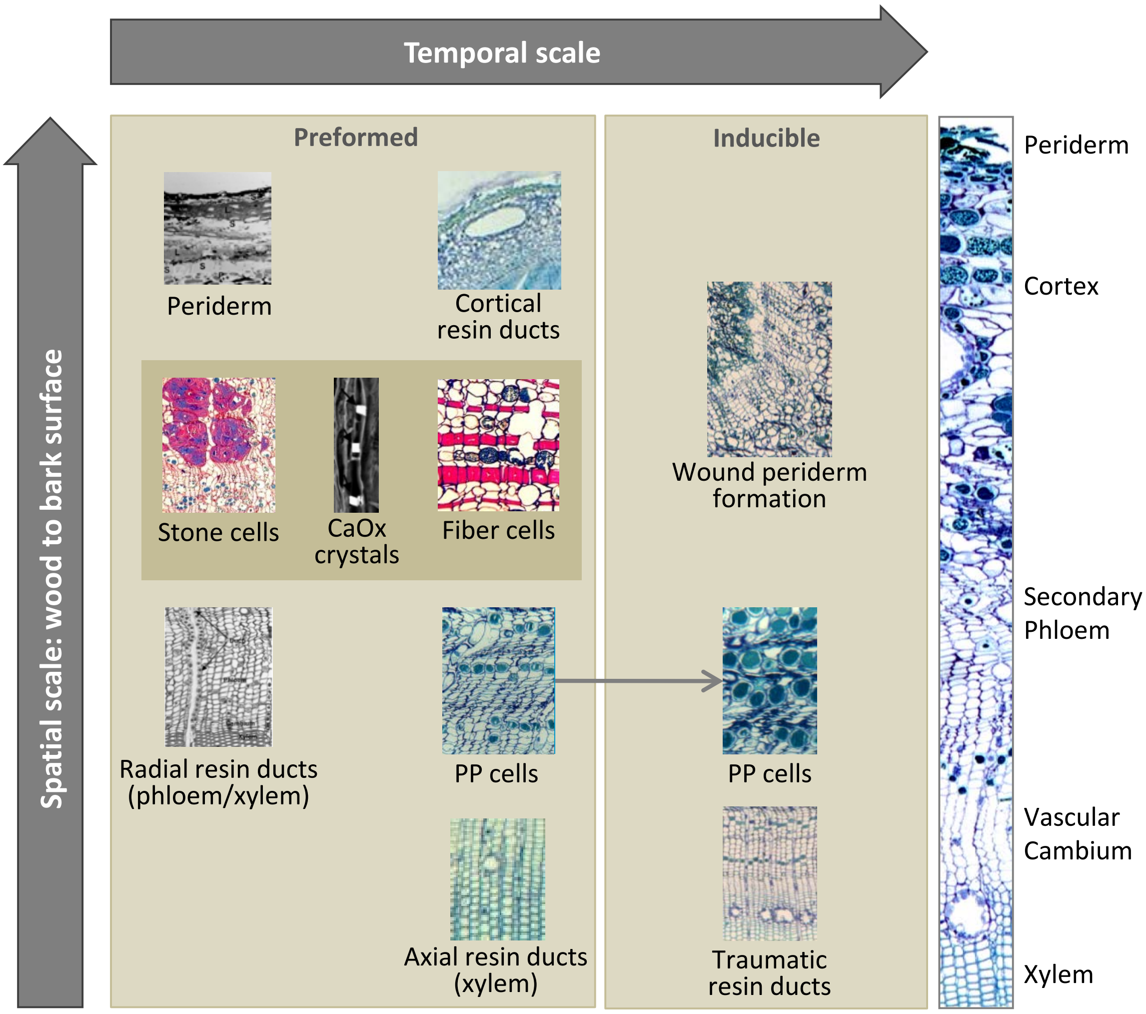

Figure 1. The multiple, overlapping defenses of conifer stems. Conifers have an array of preformed and inducible defense structures in their stems that work together to deter and stop attacking insects and pathogens. These defense structures occur along spatial and temporal scales and may have predominantly chemical or physical modes of action. Stone cells and other preformed physical defenses are highlighted.

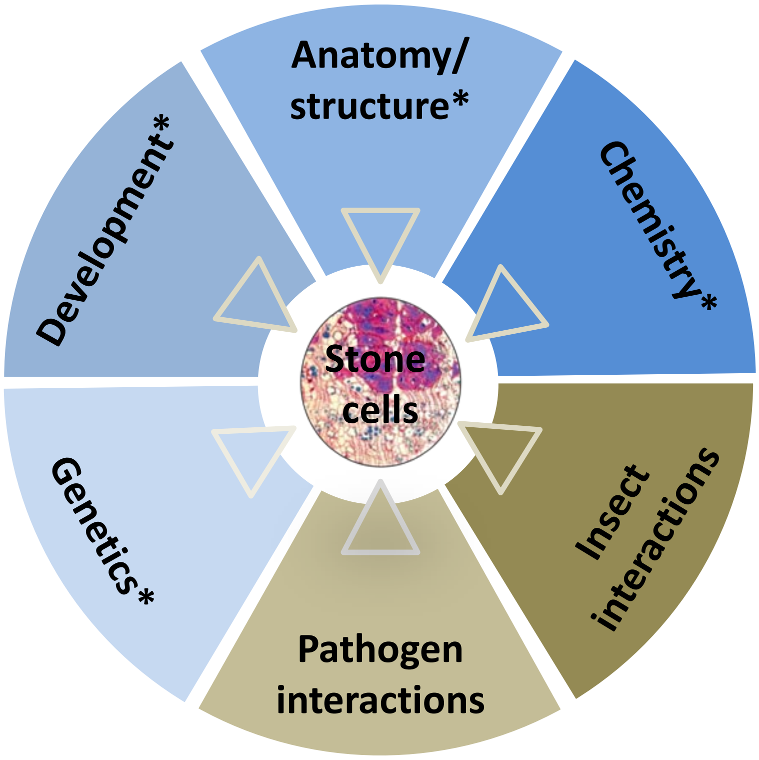

Figure 2. The multipronged approach to studying conifer defense. Conifers and other plants have multiple defenses that overlap in space and time, and these defenses, exemplified here by stone cells, can be thoroughly characterized only by combining an array of different approaches. The approaches used in the study by Whitehill *et al*. (2015) are highlighted (*).