Conifer Cell Response to

Invasion of

Dendroctonus brevicomis LeConte

(Western Bark Beetle)

Biology 196 Lab
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Conifers and bark beetles are both organisms that have existed for millions of years. They have both evolved mechanisms of survival, the beetle relying on shear numbers, while the pine tree primarily on chemical defense systems. *Dendroctonus brevicomis* is well adapted at utilizing conifer terpenes in mounting attacks on *Pinus ponderosa.* In defense, conifers have multiple chemical and mechanical abilities in their cache of weaponry, of which we are only now beginning to understand. The cellular response of the pine remains an area for much needed research if we are to fully appreciate chemical mechanisms for the long term survival of this long-lived organism.

Conifers are life forms possessing many adaptive capabilities for enduring great hardship. The trees have been around for millions of years and some species may exist for up to 4,000 years. They have evolved into exquisitely developed living organisms with varying traits and unique features (Trapp and Croteau, 2001). The western pine beetle (*Dendroctonus brevicomis* LeConte) has evolved to attack the *Pinus* spp., specifically the ponderosa pine and Coulter pine, and play a useful role in ecosystem cycling of dead and dying woody material. Recently however, beetle attack has been extremely widespread and devastating with depletion of timber supplies, disruption to forest management and operations, and increased forest fire danger with increased fuels, not to mention large ecosystem devastation and destruction (DeMars and Roettgering, 1982). The complex mechanism of conifer response to attack is currently being intently studied and continues to elude researchers from concrete answers. It appears that Ponderosa Pine has multiple systems in place to counter attacks by the offensive beetle. *D. brevicomis* continues to redesign its own strategies of species survival, leading to a complex interaction of the victorious and the defeated.

Western bark beetles may target trees 15cm in diameter or larger. These beetles reside in mid-elevation regions between 600-1800m, and are capable of finding and invading healthy hosts (DeMars and Roettgering, 1982). The insects typically attack conifers that may be slow growing, diseased or damaged by lightening, fire, or mechanical injury. Some pines may be drought stressed, or be large in size and easier for the beetle to locate (Valdez, 2002). Evidence of attack is seen with ‘pitch tubes’ of 6-13mm in length forming around tiny entry holes in the cracks between bark plates (Pielou, 1988).

Typically, it is the female beetle that selects the host tree and mating location. She bores through the bark. The eggs are laid in 'galleries' within the inner surface of the bark, also known as the cambial zone or secondary phloem (Franceschi et al., 2005). C-shaped legless larvae develop, and then rice-grain sized pupae form. Characteristic tunneling is seen on the outer and inner surfaces of the trees, appearing as 'squiggly' burrows radiating out from initial hatch site with widening diameter as the grubs increase in size (Pielou, 1988). Adult beetles continue to live under the host bark, perpetuating the life cycle multiple times, depending on the season and conditions (Valdez, 2002). The feeding of beetles in the inner phloem layer of the bark eventually kills the tree, usually within a year's time (Hagle et al., 2003). In addition, fungal spores from *Cera minor* are vectored by the western pine beetle into the vulnerable phloem and xylem tissue, where xylem water flow may be interrupted by pathogenically formed emboli (Franceschi et al., 2005). It only takes a few weeks during summer months for the fungus to invade and block the flow of nutrients within the inner bark (DeMars and Roettgering, 1982). Foliage color changes from green to light brown, then to red on the tree crown, evidencing extreme stress and eventual loss of life. Tissue necrosis, cellular desiccation, localized autolysis, and costly secondary resin formation all contribute to host demise. Death at this point is usually inevitable from a series of progressive events, but sometimes the tree is able to remain viable through a variety of mechanisms (Nebeker, et al., 1993).

Ponderosa pine's ability to survive attack is possible through evolved responses which are currently being studied by scientists with all eagerness. Classically, resin production has been the favored defensive mechanism in the limited understanding of host defensive response. Regulation remains unknown. What is known as the process of 'pitching out' occurs initially when a violation to the organism occurs. Resin cells, a form of secretory epithelial cells, produce resin, which in turn release their products into ducts. Large amounts of resin are excreted through the wound site to slow the invasion, flush the wound and repel any intruders. Turpentine components of resin are toxic to beetles. When resin sap reaches the bark surface, terpenes evaporate leaving a semi-crystalline plug that hardens with atmospheric exposure.

Resin biosynthesis is a complicated chemical process. Resin terpenes are the main component in pine sap, and are made from the main building block, isopentenyl diphosphate. In plants, this compound may be found in the cytosol compartment of resin cells or in plastids within the cell. Plastids are organelles within a plant cell that are responsible for storage of starch, protein or lipids. Plastids also store plant pigments. These organelles house the monoterpenes, diterterpenes and tetraterpenes where the sesquiterpenes and triterpenes are formed (Trapp and Croteau, 2001). After isopentenyl diphosphate is created and isomerized to dimethylallyl diphosphate, the molecule is transformed by up to 3 units of isopentenyl diphosphate by another specific enzyme, prenyltransferase. All of this is expensive for the tree to produce (Valdez, 2002). Prenyltransferase works within the cell to generate the forerunners of the monterpenes, sesquiterpenes and diterpenes (Trapp and Croteau, 2001).

The next player in resin biosynthesis is terpenoid synthase which acts to convert the acyclic precursors to parent terpene families. This enzyme is known as a cyclase. The monoterpene synthases have been well studied. Conifer synthases for an individual of the same species are genetically more closely related than to the same type of synthase in other species of conifers. This suggests a species-specific synthase through inheritance and evolution. These are soluable proteins, and work in a pH range between 6.8 and 7.8. This enzyme requires metal ions such as Mg^{2+}, Mn^{2+}, and Fe^{2+} for catalysis, and use cations like K^{+} to become useful. Research on chemically optimal resin composition for conifer survival still needs to be conducted.

Remarkably, *Dendroctonus brevicomis* utilizes monoterpenes as starting material to produce pheromones for ‘mass attacks’. When terpenes are present outside of the trunk, pheromone signaling is used by the beetle for aggregation and colonization. Many of the attackers will actually produce the pheromones necessary for high levels of colonization from terpentine components, trans-verbenol, obtained from the host tree (Trapp and Croteau, 2001).

Conifers have several mechanisms to stem the tide of an invasion by *Dendroctonus brevicomis* once the outer tree barrier has been breached. Intracellular deposits of calcium oxalate crystals within the periderm or outside layer may work to deter boring and chewing. Little is known of this mechanism. Radial resin ducts and canals may secrete into the extracellular lumen when under pressure. Interestingly, polyphenolic parenchyma cells found throughout the bark and secondary phloem have been understudied, yet seem capable of multiple offensive and defensive roles (Franceschi et al., 2005). They hold in their large vacuoles components that appear to exhibit anti-feedant and anti-fungal properties. Polyphenolic parenchyma cells are also capable of the formation of callous tissue at the wound site to wall off the damaged area, in addition to deactivating hydrolytic enzymes secreted by the beetle (Franceschi et al., 2005). In addition, radial ray cells within the structure of the tree may possibly be involved with signal transduction and the phenolic pathway for elicited responses (Franceschi et al., 2005). Rays are small bands of cells that act as the connecting links between tracheids. Tracheids are new layers of cambrium added each new growing season (Pielou, 1988).

The *Pinus* genus has a large, complex genome which has not been sequenced. Much remains to be understood in just how these specially adapted life forms have evolved and compete with voracious enemies, and still remain victorious. We continue to search for knowledge on signaling cues between host and pest, the crystal structure for conifer terpene synthase for determining substrate specificity, for phenolic pathways, and control of resin formation and secretion. Ultimately, I believe conifers will win survival because of their remarkable chemical arsenal and proven ability to evolve.

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