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Chapter 1

Chemical Plant Defense Against Herbivores

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Abstract

Herbivores can damage plant productivity and fitness because plants have improved defense mechanisms such as physical barriers, association with other organisms such as ants, and chemical defense. In that, separate plant species produce different chemical molecules. Chemical compounds involved in plant defense can act in several facts: decreased palatability, like a poison, such as a stunner, and increased gene defense expression, among others. In this chapter, we approach several examples of chemical molecules produced by plants to defend themselves, including biochemical metabolic pathways, as well as ecological and evolutive implications.

Keywords: plant chemical defense, alkaloids, resins, herbivores

1. Introduction

Interactions between plants and insect herbivores are important determinants of plant productivity in managed and natural vegetation. In response to attack, plants have evolved a range of defenses to reduce the threat of injury and loss of productivity [1]. Plants are exposed to threats of resource loss by herbivory in natural conditions experiencing damage; to mitigate losses many plant species develop defensive traits against herbivores, such as primary and secondary metabolites [2–4]. Among herbivores are many arthropods, mollusks, vertebrates, and nematodes, and these groups consume between 5 and 20% of plant biomass annually [5].

The cost on investing in defense can be quantified in reduced growth, lower photosynthetic production, and reduced plant fitness [6, 7]. Plant defenses reduce the ability of herbivores to obtain nutrients from plant tissue. Plants with diminished defense capability may suffer

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greater herbivore damage and exhibit lower overall fitness under conditions of herbivore stress than well-defended plants [7].

Plants respond to herbivory through various morphological, biochemical, and molecular mechanisms [8] and exhibit multifactorial traits against herbivory that are constitutively expressed or induced upon attack [9]. The plant defense activated upon herbivory is a complex network of different pathways composed of direct and indirect defenses. Direct defense compounds such as glucosinolates or protease inhibitors directly influence the insect performance and feeding behavior, while indirect defenses like emission of volatile organic compounds after herbivore attack function as attractant for parasitic wasp which in turn predate on the attacker. While plants develop new defense compounds or mechanisms to enhance the resistance against herbivores, their attackers find new ways to bypass or detoxify these [8, 10].

Insect herbivory induces several internal signals from wounded tissues, including calcium ion fluxes, phosphorylation cascades, and systemic and jasmonate signaling. These are perceived in undamaged tissues, which thereafter reinforce their defense producing low molecular weight defense compounds [11]. Some compounds produced by plants constitutively or induced by herbivore damage are toxic or impair gut function in arthropod; examples include alkaloids, benzoxazinoids, glucosinolates, and terpenoids [1].

Added to this, there are some other defense mechanisms, such as mechanical defenses, indirect defenses, interactions with other organisms, etc. In this review, we focus in different traits defensive in plants and its effect on population dynamics and evolution in both plants and invertebrates. Finally, we integrate all traits in a specific example in *Pinus* genera.

2. Induced defenses

Plants respond to herbivore attack through a dynamic defense system that includes structural barriers, toxic chemicals, and attraction of natural enemies of target pests. Both defense mechanisms may be present constitutively or induced after damage by the herbivores. Most of chemicals are produced in response to herbivore attack. Induced defenses make the plants phenotypically plastic, and high variability in defensive chemical exhibits a better defense [8].

The induced defenses occur when past or current herbivory is a reliable cue of future attack and defenses are costly; while in environments where herbivory is constantly high, constitutive defenses should be favored [4].

Herbivorous insects produce oral secretions which contain compounds that elicit plant responses [12] and plant elicitor peptides prevalence across wide-ranging plant families [13]. In response, plant produces diverse chemical active compounds such as benzyl cyanide, fatty acid-amino acid conjugates, and proteins such as β-glucosidase [14]. Plants can recognize herbivore elicitor and initiate a cascade of responses, including changes in plasma membrane potential and activation of networks of kinases and phytohormones [15]. Three major plant hormones, jasmonic acid (JA), salicylic acid (SA), and ethylene (ET), function in a complex regulatory network essential in herbivore-induced defense responses [16].

3. Chemical compounds in plant defense

Plants produce defensive metabolites, which do not affect the normal vegetative growth and development, but reduce the palatability of tissues in which are produced. Can be constitutive stored as inactive forms or induced in response to insect or microbe attack [8]. The defensive metabolites are bioactive specialized compounds used to protect plant against herbivores, and these compounds can use as target systems unique to herbivores, such as the nervous, digestive, and endocrine organs, may act as repellents for generalist herbivores, while specialists are forced to invest resources in detoxification mechanisms [11, 17].

Plant defense include changes in transmembrane potential immediately upon herbivory damage and are tightly followed by changes in the intracellular Ca^{2+} concentration and generation of H_2O_2. Kinases phytohormone jasmonic acid (JA), ethylene (ET), salicylic acid (SA), and nitric oxide (NO) are detectable within minutes. After roughly 1 h, gene activation is followed by metabolic changes [11, 13].

Antinutritive proteinase inhibitors (PINs) are locally and systemically induced upon insect attack, but many other proteins contribute to antiherbivory responses. Enzymes such as polyphenol oxidase a threonine deaminase limit protein availability in the midgut, whereas others destabilize insect peritrophic membranes [13, 18]. Plants also draw upon a complex arsenal of small-molecule chemical defenses including terpenoids, alkaloids, phenylpropanoids, glucosinolates, lipids, and nonprotein amino acids [19].

Volatiles which can alert neighbor plants or tissues to potential attacks are promoted by herbivory and are a complex blend. Volatiles induces indirect defenses inhibits oviposition and attracts natural enemies such as parasitoids and predators [13].

3.1. Alkaloids

Efficient feeding deterrents against herbivore group of compounds are the alkaloids, particularly such derived from quinolizidine, like cytisine and sparteine. These molecules are alkaline and contain nitrogen in a heterocyclic ring [20]. Alkaloids are biosynthesized in roots from amino acids [21] and probably are involved in defense against insect herbivory. Twenty percent of vascular plants synthesized alkaloids, particularly in plant families Leguminosae, Liliaceae, Solanaceae, and Amaryllidaceae [11, 19].

3.2. Phenolics

Phenolics are produced by plants as compounds able to repel herbivores, inhibit enzymes, attract pollinators and fruit dispersers, absorb UV radiation, and decrease competition between plant neighbors [11, 22]. There are approximately 10,000 plant phenolics derived from shikimic y/o malonic acids [23]. Phenolics can bind covalently to herbivore's digestive enzymes and inactivate them [24] or halt the growth and development of larvae [25]. Phenolics can be regulated for external conditions like light and nutrients; when a plant is stressed, it produces less phenolics than nonstressed plants [22].

3.3. Terpenoids

The most diverse class of bioactive natural products in plants is terpenoids, with approximately 40,000 structures. Terpenoids are synthesized from acetyl-CoA and play a role in plant defense, can act like active compounds in resin or as volatiles, repellents, and toxins, or can modify development in herbivores [26]. Another characteristic in monoterpenes and sesquiterpenes is its ability to form essential oils, like limonene in citrus plants; these essential oils have repellent and toxic effects on insects [27]. Many terpenoids can have synergistic effects upon release [28].

3.4. Nonprotein amino acids

Amino acid g-aminobutyric acid (GABA), a four carbon nonproteinogenic widespread in animals, plants, and microorganisms, can be implicated in defense responses. Wounding plant tissue and cell disruption caused by feeding insects is sufficient to induce rapid jasmonate-independent GABA synthesis and accumulation. When ingested the elevated GABA levels become toxic for the insects. GABA is synthesized by decarboxylation of L-glutamate bay glutamate decarboxylases (GAD) in shoots and roots and is a component in a plant's first line of general, rapid defense against invertebrate pests [29].

One metabolite induced in plants is tyrosine, which can be redirected into other primary and secondary metabolites, and its accumulation in excess in young leaves may not be adaptive as they would persist once the leaf is full in size and protected by toughness [30]. In contrast to tyrosine, physiological constraints on catabolism may be selected against induction of phenolics and saponins [4]. When plants exceed the capacity to store constitutive secondary metabolites could avoid autotoxicity [31].

3.5. Sulfur

Sulfur is a crucial element for plants, determining plant development, maintenance, and resistance to environmental stress. Sulfur is taken up by plants as inorganic sulfate and incorporated in different sulfated metabolites including glucosinolates, selected flavonoids, phytosulfokines, and hormones by distinct pathways. Some sulfated metabolites function in plant defense against pathogens and herbivores such as defensin and thionin peptide, antimicrobial defenses with widespread distribution, whereas antifeedant glucosinolates are limited to the Brassicales order. *Bacillus subtilis* activates plant growth by producing IAA y/o gibberellins and emits volatile metabolites (VOCs), which can activate transcripts related to cell wall modifications, primary and secondary metabolism, stress responses, hormone regulation, iron homeostasis, and sulfur-rich aliphatic and indolic glucosinolates. Plants exposed to *Bacillus subtilis* with elevated glucosinolates exhibit greater protection against generalist herbivores. Then, plant-growth-promoting rhizobacteria can enhance plant sulfur assimilation and integrate in plant defense [32].

3.6. Lipids

Fatty acids (FAs) are essential macromolecules present in all living organisms, are the major source of reserve energy, are essential components of cellular membranes, and are implicates as signaling molecules, modulating normal and disease-related physiologies in microbes,

insects, animals, and plants. In plants, fatty acids regulate salt, drought, heavy metal tolerance, and herbivore feeding, especially by JA is a FA derivate molecule [33]. In *Nicotiana attenuata* fatty acid-amino acid conjugates (FACs) in the herbivore *Manduca sexta* oral secretions are the major elicitors that induce herbivory-specific signaling [34]. FAs increased plant defense against pathogens and insects by stimulation of key short- and long-term regulatory process [35, 136].

Simulated herbivory dramatically increased salicylic acid-induced protein kinase (SIPK) activity and jasmonic acid (JA) levels in damaged leaves and undamaged systemic leaves, whereas wounding alone had no detectable systemic effects. The activation of SIPK and elevation of JA in specific systemic leaves increase in the activity of an important antiherbivore defense, trypsin proteinase inhibitor (TPI). Then, *N. attenuata* can identify FACs produced by herbivory in damaged leaves and activate MAPK and JA signaling for activated defenses [34].

Another lipids produced by plants are alkamides. Natural alkamides are often insecticidal [35, 36]. Chrysanthemum cultivars show a wide variation in degree of host-plant resistance to the western flower thrips *Frankliniella occidentalis*. Extracts of chrysanthemum leaves revealed the presence of an unsaturated isobutylamide, N-isobutyl-(E,E,E,Z)-2,4,10,12-tetradecatetraen-8-ynamide. Alkamides account for natural host resistance to thrips. The participation of alkamides in host resistance to insects can be due to their role as elicitors of plant defense responses. For instance, it has been reported that linolenoyl-L-glutamine, an amide produced in oral secretions of caterpillars, is able to induce the production of volatile chemicals from plants that attract predators and parasites of the caterpillar while it feeds [36, 37].

3.7. Jasmonic acid and ethylene

Jasmonic acid (JA) is an important regulator of defense responses against chewing insects, necrotrophic pathogens, and cell-content feeders such as spider mites and thrips [16]. Herbivores stimulate JA production by octadecanoid pathway. In *Arabidopsis*, JA is conjugated with isoleucine [135] through the enzyme jasmonoyl isoleucine conjugate synthase1 (JAR1) that conjugates binding to the F-box protein coronatine insensitive1 (COI1) and degrades jasmonate ZIM domain (JAZ) repressor proteins [38, 39]. Then, JA-responsive genes, including JAZ, which involves a negative feedback loop are activated [16]. There are two possible pathways: MYC2 regulates positively vegetative storage protein 2 (VSP2) and lipoxygenase 2 (LOX2), which are JA-responsive inducible by wound. The another pathway implicates the ethylene response factor (ERF) (JA and ET are synergic) and induces ERF1 and ORA59; both are JA/ET-responsive transcription factors which regulate responsive genes like plant defensin 1.2 (PDR1.2) [40]. MYC2 regulates defense against herbivores, and ERF is involved in induced defense especially against necrotrophic pathogens [16].

3.8. Salicylic acid

Salicylic acid (SA) is an essential signaling molecule that mediates pathogen-triggered signals perceived by different immune receptors to induce downstream defense responses. SA is

a small phenolic phytohormone, which plays a major role in mediating defense; its accumulation is essential for induction of defense responses [40, 137].

Induced plant responses are regulated by SA when herbivores bite phloem [16]. Plant responses synthesizing SA from chorismate by isochorismate and phenylalanine ammonium lyase pathways [41]. Increases in SA concentrations lead to nuclear translocation of pathogenesis-related genes 1 (NPR1), which results in the expression of defense proteins, the pathogenesis-related (PR) proteins [42].

When a plant faces multiple herbivore attack, induced defense is regulated through interconnection of the JA, SA, and ET signal transduction pathways. Cross talk between JA and SA signaling is mutually antagonistic, resulting in the prioritization of SA-dependent defense responses over JA-dependent responses or vice versa [42].

4. Mechanical defenses

The first layer of defense in plant is mechanical, and the major components contributing to mechanical defenses are trichomes. These structures negatively influence on herbivore feeding behavior and insect mobility [43]. Another trait in plant defense is the palatability, and one form to modify this character is to produce dense trichomes; for example, in *Phaedon* species, the host preference of adult beetles was less for *Brassica* cultivars that produced dense trichomes, while adult beetles were inclined to attack glabrous leaves [3]. That is particularly important on young leaves of hairy plants, which produce denser trichomes than those of mature leaves. Therefore, trichomes might play an important role in the defense of younger leaves and contribute to future development of leaves [3, 44]. Trichomes tend to be more effective against insects that are small relative to trichome size; additionally, trichomes tend to deter sap-feeding or leaf-chewing insects to a greater extent than those feeding within plant tissues [45]. Spinescence, including spines, thorns, and prickles, also defends the plants against many insects [8].

Epicuticular waxes form a slippery film or crystals that prevent from attaching to the plant surface, oviposition, or feeding [1]. The biosynthesis and composition of waxes vary during plant development, and the physical-chemical properties of the cuticle respond on changes in season and temperature [46].

Another mechanical defense is to deposit granular minerals in tissues that deter insect attack and feeding. For example, Si accumulation, especially in Poaceae family, which is abrasive, damages herbivore feeding structures and reduces digestibility. Si accumulation can be induced by herbivory. Si in leaf surface can be abrasive in grasses with silicified spines, while others deposited Si in short cells. Si allocation to spines impacts palatability, while allocation to short cells may impact digestibility [1, 45].

The cell walls of leaves are also reinforced during the feeding through the use of different macromolecules, such as lignin, cellulose, suberin, and callose, together with small organic molecules, such as phenolics and Si [47].

Good few plants contain laticifers and resin ducts that canals produce and store latex and resins under internal pressure; when the channels are broken, they are secreted and might entrap or intoxicate the herbivore [11, 48]. However, several specialist herbivores can block the flow of latex cutting the leaf veins, for example, the milkweed beetles *Labidomera clivicolis*, *Tetraopes melanurus*, and *T. tetrophthalmus* for feeding *Asclepias* cut veins and wait stop flow [49].

Oleoresins produced by conifers are a blend of terpenoids and phenolics accumulated in intercellular channels. When bark beetles bite that channels resin flow and get out the insect until outside, when oleoresins solidifying [11, 50].

5. Indirect defenses

Indirect defense can be used when plants attract, nourish, or house other organisms to reduce enemy pressure [51]. For example, ant association in *Mallotus japonicus* (Euphorbiaceae) the damage leaf areas of ant excluded plants were much larger than those of control plants in middle-age leaves [44]. This is done by producing volatiles, extrafloral nectar, food bodies, and nesting or refuge sites [11].

Extrafloral nectar is secreted on leaves and shoots to attract predators and parasitoids and consists mainly of sugars, amino acids, lipids, proteins, antioxidants, and mineral nutrients; its production increases by herbivory and decreases in the absence of herbivory [52]. Extrafloral nectar has been associated to protective ants, which have the ability to defend their food sources. Increases in extrafloral nectar production augment the numbers of protective ants. In *Catalpa bignonioides* and Fabaceae family, extrafloral nectar attracts mites, ladybird beetles, wasp, lacewing larvae, and spiders [53].

6. Another influent factors in plant defense

The composition and dynamics of the insect community that interacts with plants are influenced by plant traits such as chemistry, physiology, and morphology, which have a genetic basis. Plant traits may affect the sizes of herbivores and therefore the sizes of parasitoids that develop in the herbivores and even the sizes of hyperparasitoids.

Induction of defense timing was examined by Bixenmann and collaborators [4] in *Inga* genus using lepidopteran larvae on young leaves. While young leaves are expanding, they are tender and high in protein, the two traits that make them a target for herbivores, receiving 70% of the leaf's lifetime herbivore damage despite being vulnerable for only few weeks. Once leaves reach their full size, they rapidly toughen, and rates of herbivore drop to almost zero. The amount of damage, the timing, and the identity of damage agent impact directly induced responses. When increasing leaf area removed in *Phaseolus lunatus*, extrafloral nectar production, and ant recruitment decreases significantly, then extrafloral nectar production is inversely correlated with leaf area and therefore with the amount of intact photosynthetic surface [54].

Herbivory risk depends not only on the traits of an individual plant but also on those of neighboring plants [3]. In that sense, the "associational effects" may mediate the local frequency of the density dependence of herbivory [55].

Volatile organic compounds (VOCs), such as aldehydes, alcohols, esters, and terpenoids, are released from plant flowers, vegetative parts or roots to attract pollinators and predators, repel herbivores, and communicate between or within plants [56, 57]. When a plant is attacked, it is able to communicate with other plants and alert them of a possible future attack [58]; thereby, the alerted plants will respond stronger once attacked [59]. For example, when molasses grass, *Melinis minutiflora*, was planted in a maize field, the herbivore damage decreased. The grass emits a compound in response to caterpillar damage to attract parasitoids, and the amount of caterpillar in a maize decreased by parasitoids, after induction of JA to release more VOCs [60, 61].

The perception of herbivory by plants involved not only mechanical injury to plant and the presence of herbivore-derived elicitors released during feeding but also the presence of microbes associated with the herbivore [62]. Microbial symbionts can influence their hosts including providing nutrition, digestion, and detoxifying toxins; insect symbionts have a role in mediating plant defenses [60]. Different microbes in insects may have species-specific effects on different host plants, specifically herbivores' microbiota are perceived by plants during herbivory and thus may alter the outcome plant responses [62].

7. Plant defenses against herbivores and fitness

Insects find and select their host plants and deal with plant defenses, as well as herbivores modify plant phenotypes. However, plants interact with multiple attackers and interact at different levels of biological organization [39].

Herbivory affects the expression of floral traits, plant-pollinator interactions, and costs-benefits to controlling reproductive systems and defense strategies. Plant-herbivore interaction promotes myriad defenses that protect plants from damage. In recent years, it has been considered whether reproductive traits and antiherbivore defenses are interdependent as a result of pollinator- and herbivore-mediated selection [63]. Floral traits are most likely to affect susceptibility to herbivores. There are pollinating herbivores, which when adult insects pollinate the plants their larvae use as host, for example, figs and fig wasps [64], the larvae feed directly on ovules and developing seeds. A diversity of floral traits influences the susceptibility of plants to herbivores; for example, taller inflorescences often result in greater herbivory, phenology also affects herbivory risk, and plants that flower early or late typically receive less damage than plants that flower during peak flowering [63].

On the other hand, inbreeding can produce individuals with reduced fitness, but inbred plants are more susceptible to herbivores than outbreds [7]. In horsenettle (*Solanum carolinense* L.), the tobacco hornworm caterpillars (*Manduca sexta* L.) preferred to feed on inbred plants, and the females oviposited more frequently on inbred plants compared to outbreds [65, 66].

Inbreeding in horsenettle causes significant reduction in the plant's induced defense responses and resistance to herbivory [67–69]. The predilection for inbred plants exhibited by insects suggests that they are gaining fitness benefits by choosing inbred host plants, regulated by insect herbivore growth, oviposition, and flight capacity. Inbred plants, serve as better host for developing insects could be that inbred plants suffer from a limited ability to unregulate genes in defense biochemical pathways. In the system plant-insect horsenettle-tobacco hornworm suggests that biochemical changes in plant inbreeding can influence in the health of animals at a higher trophic level, particularly in insect herbivores which increases survival, growth, and flight metabolism when nurtured on inbred plants [7].

8. Tolerance traits

There is another plant defense strategy: tolerance. In resistance plant synthesizes structural or chemical traits to minimize herbivore damage, while in tolerance traits reduce the negative effects or herbivore damage [1].

The traits that maintain or promote plant fitness following damage before or after infestation can confer herbivore tolerance, and they are grouped in those that alter physiological process like photosynthesis and growth, phenology, and nutrient storage [1]. In many plant species, partial defoliation leads to increased photosynthetic rate in the remaining plant tissues, but is not universal [70]. Delayed growth, flower, and fruit production following herbivore damage could promote herbivore tolerance by postponing plant development until the threat of attack has passed [71].

Roots eaten by insect herbivores exhibit extensive regrowth, in density and quantity [72]. The former might be caused by additional lignification that could increase the toughness of the roots [73].

Mechanisms involved in increased tolerance are [i] increased net photosynthetic rate after damage, [ii] high relative growth rates, [iii] increased branching or tillering after release of apical dominance, [iv] preexisting high levels of carbon storage in roots for allocation to aboveground reproduction, and [v] ability to shunt carbon stores from roots to shoots after damage. The evolution of tolerance can promote an apparently mutualistic relationship between plant and herbivore populations [70].

9. Example conifer plant defense against bark beetle

Now, we examined how different responses can be used by *Pinus* genera to limit damage causes by attack of bark beetle, one of the principal plagues that affect *Pinus* populations.

Most herbivores are insects that feed on plants in various forms, for example, they adopt different feeding strategies throughout their life cycle and can feed both external [leaf buds or flowers] and internal structures of the plant [miners, stem borers, gillnet] [74].

Unlike other herbivores such as mammals, insects commonly feed on the leaves and other parts of the mature plant typically do not cause the death of the plant; as for insects to kill the plant, they will require much time [75]. Thus, the relationship between herbivorous insects and plants is more like the host-parasite than predator-prey relationship. Plants for their part have not become passive victims of herbivorous insects as they have been able to produce special metabolites and toxic proteins, which serve as repellents or have antinutritional effects for their attackers [76]. However, herbivorous insects successfully consume plant material, overcoming the complex set of defenses of plant [74, 77, 78]. Moreover, unlike other herbivores, insects are much more specialized, because they can feed exclusively from a plant species or a limited number of them [75, 79, 80]. Therefore, it is necessary to understand the relationship between herbivorous insects and their host plants from biochemical, ecological, behavioral, physiological, and genetic aspects, including the ways in which insects can affect the abundance and distribution of plant species [75].

9.1. Herbivory and regulation of plant populations

Herbivorous insects usually cause reduced growth, fertility, and even the survival of plants; some plants can counter or overcompensate significant amounts of damage in general [75, 81]; however, the insect damage as a group causes a multiple effect and simultaneously in succession with additive effects and multiplicative on the plant fitness, which results in a significant impact on the abundance of plants, distribution, or population dynamics [82].

The role of herbivorous insects in the regulation of plant populations and dynamics of communities has been poorly documented; most studies have focused mainly on explaining the role of herbivorous insects' native as agents that limit the distribution of its plant host [75]. However, it has been possible to distinguish that the effects of herbivorous insects on plants may differ depending of the different scenarios under which the interaction takes place as in the case of herbivorous insects (bark beetles) and pines.

On the one hand, if the evolutionary success involves adaptive radiation and overtime, the species survive and expand their geographical distribution, and then pines (*Pinus* sp.) can be considered successful, because they form the largest genus of conifers in the Pinaceae family. The pine group consists of more than 100 species, many subspecies, and varieties. Although mainly distributed in temperate regions of the northern hemisphere, pines also occupy other habitats and climates [83, 84].

Moreover, the great success of the pines can be attributed to their defense strategies against herbivorous insects or parasites [85]. For its wide distribution and its prolonged generational cycles, ranging from decades to more than 4000 years such as *Pinus longaeva* [85], pines are subject to deal with a wide range of attackers at which they have developed along its evolution complex defense mechanisms [84].

The basic defense strategy of conifers including pines is both morphological structures [physical barriers] and chemical mechanisms [85]. Physical barriers are formed by static structures such as lignified cells, calcium oxalate crystals, or hard foliage; they act primarily against herbivores, ovipositors, and defoliating insects [84, 85]. The bark of the trunk on his part is of particular interest because it forms the first barrier against herbivorous insects such as bark

beetles, whose evolution has specialized to kill the tree [85]. Then, conifers produce a plethora of chemical defenses where the most important are phenolic compounds and oleoresins which contain numerous terpenoids. Chemical defense mechanisms may be directed against herbivorous insects to prevent oviposition and food or affect their physiology to reduce survival or fecundity [86].

9.2. Defense and resistance strategies of conifers against bark beetles and fungal pathogens

Conifers throughout their life cycle face the challenges of a variety of organisms cycle, conifers face the challenges of a variety of organisms, the more severe are the bark beetle and fungal pathogens associated [85]. Conifer defenses against insects and pathogens that infect the trunk are classified as constitutive and induced [84, 85].

9.2.1. Constitutive defense systems

Mechanisms that produce a stable set of structural defenses (cells and resin canals), toxic chemicals such as phenolics and terpenes, and mechanical properties of the cortex (suberized layers of cells and lignified oxalate crystal calcium) are permanent. The constitutive systems are defenses with great resilience against a number of organisms trying to penetrate the cortex during the history of the tree and against common secondary invasions of opportunistic organisms. The constitutive defenses are of two basic types:

A. *Mechanical defenses*: Structural elements that provide hardness or thickness to tissues and inhibit mastication or piercing in the bark. Impregnating plant tissues with polymers such as suberin and lignins can add resistance to the mechanical properties against penetration, degradation, and ingestion/mastication by insects.

B. *Chemical defenses*: Formed by chemical compounds stored, like phenolics, terpenoids, and alkaloids, and released under attack. Antinutritive defenses include chemical, toxins, defensive proteins, enzymes, and resin deposits that can flow to repel or physically trap small organisms. These defenses are scattered in the tissues of the bark [periderm, cortex, and secondary phloem]. The constitutive strategies vary depending on the physical or chemical nature of defense and its distribution within the bark and trunk [85].

9.2.1.1. Periderm defenses

Periderm forms a permeable barrier for controlling the gas exchange in the trunk and is the first line of defense against biotic and abiotic factors. It is characterized by the presence of multiple layers of cells, most of which are dead, are also structurally and chemically different, and have lignified or suberized its walls. Cells may contain high amounts of phenolic compounds, and one or more layers have encrusted calcium oxalate crystals. These mechanical defenses (hard walls lignified, crystallization, and suberization) provide a hydrophobic barrier, combined with the chemical properties of the phenolic compounds and form

a multifunctional barrier against the external environment. However, the periderm is not a continuous barrier, due to the presence of lenticels to allow gas exchange at the surface, although it is not an open system that may allow entry of invading organisms as in the case of small bark beetles (*Pityogenes chalcographus*) in *Picea abies* [87, 88].

9.2.1.2. Cortex defenses

The cortex is formed during the early development of the stem, so it is an important general barrier, especially during the early development of the stem. It remains alive for several years during the secondary growth and contains high amounts of phenolic compounds within vacuoles of cortical parenchyma; in many Pinaceae, the cortex has axial duct resins, which participate in defense, although its function is replaced by the secondary phloem [87].

9.2.1.3. Secondary phloem defenses

The secondary phloem is the most important site of constitutive defense mechanisms of conifers and is made up of phenolic bodies, sclerenchyma, and calcium oxalate crystals; the relative amount of these components varies considerably between species [89]. A fourth constitutive strategy of defense in certain taxa as Pinaceae is the production of resin structures comprising radial ducts extended from xylem, axial ducts, blisters, and resin cells. The amount and combination of each of these components define defense strategies. In the secondary phloem, there are specialized structures, such as phenolic bodies, sclerenchyma, calcium oxalate crystals, and resins.

The phenolic bodies are parenchymal cells of the axial phloem, also called polyphenolic parenchymal cells [PP cells], specializing in the synthesis and storage of phenolic compounds [89, 90], making nonedible tissues or antifungal capacity [85, 91]. Different species produce different phenolic compounds depending on the type of organisms that commonly attack, so that the relative resistance to pathogens may be due in part to the type of phenolic compounds they produce [92, 93].

Moreover, the PP cells are responsible for responses of induced defense and, even when they have thickened walls, allow the exchange of axial and tangential information and signaling for defense because they contain lots of plasmodesmata. The PP cells represent a very dynamic component in defense strategies in conifers and are most abundant in the secondary phloem [94]. Another important feature of the PP cells located along the radial ducts parenchyma is that they are an important site that stores starch and/or lipids [94], which are considered the target for bark beetles and fungi; however, the presence of phenolic compound constituent allows cells to protect themselves and prevent the penetration of fungi into the area of the cambium. In any case, the layers of PP cells form a sieve maintaining the physical and chemical resistance to prevent penetration into the cortex [95].

Another important tissue with mechanical function is the sclerenchyma, which is common in the bark of conifers; quantity and type vary among taxa. It consists in cells with thickened lignified secondary wall, which are known as “stone cells” because they are high hardness cells or sclereids, so they can serve as structural element and mechanical defense. This organization is massive and irregular in many Pinaceae or organized form rows as in the case of Taxaceae [96]. Their physical strength can detain predation or perforation of the

bark by insects forming a screen of dead cells that progressively collapse under pressure of new layers of inner cells [90, 97–99].

The crystals of calcium oxalate formed are stored intracellularly in the secondary phloem of conifers, particularly in Pinaceae, and represents a defense mechanism because the physical nature of the crystals and their relative abundance could imply a role in deterring penetration bark or chewing by herbivores. However, being chemically inert, it is unlikely to have any effect on fungal attack [85].

One of the common deposits in plants is crystals of calcium oxalate [85, 100–103], and its role in most of them is the regulation of calcium [104–106]; however, also they have secondary functions of defense [85, 107]. In Pinaceae the calcium oxalate crystals embedded within the phenolic bodies in PP cells vacuoles present typically form scattered axial lines crystallized cells. The combination of several layers of fibers and dense encrustation with crystals can provide a powerful defense against bark beetles [85].

One of the main constitutive defenses is resins, particularly for Pinaceae. The resin production and storage structures for this include radial resin ducts, axial ducts or channels, blisters, and resin cells. Ducts and blisters have a coating epithelial cell enriched by plastids that synthesize terpenoid resins and secreted into the extracellular lumen, which is accumulated under pressure. After injuries are caused by damage from invading organism, the pressed resin is released and may expel the invading organism from the bark and catch it thanks to its sticky consistency or kill it because of its toxic nature. Volatile resin components evaporate and nonvolatile crystallize to sterilize and seal the damaged region effectively. It has been shown that the resin is an effective defense against insect bark borers [108].

9.2.1.4. Secondary xylem defenses

Secondary xylem is a general system of defense in trunk, which is involved in the synthesis and storage of resin and phenolic compounds and other secondary products such as lignins [109], and provides a defense against wood-rotting fungi and other organisms. The constituent axial ducts of resin found in the xylem of some conifers can contribute to resin flow when connected to the radial ducts that traverse the xylem and phloem [110].

9.2.2. Induced defense systems: second level of defense

Induced defense system or responses due to herbivore attack involves the synthesis "de novo" or activation of a wide range of chemical defenses, including terpenoids, phenolic compounds, PR proteins, reactive oxygen species, and enzymes. The induced defense system can act against a current infection presenting a hypersensitive response and local resistance or against future infections or attacks by bark beetles generating responses with acquired resistance [85, 111].

A. *Induced structural defenses*: Structural defenses in bark are important, because they improve the overall defense capability of the plant; these are diverse and include structural changes and synthesis of chemical and biochemical agents. They are a combination of responses apparently targeting specific organisms, including the general increase in hypersensitivity re-

sponses, aimed at limiting the spread of detected damage and isolating the invading organism, repairing damaged tissues, and limiting the attack or later invasion of opportunistic organisms. In addition, long term results in acquired resistance [85, 111, 112]. Among these structural defenses are hypersensitive response, callus tissue formation, and scarring in the periderm.

9.2.2.1. Hypersensitive response

Damage produces a hypersensitive response in the plant, which quickly stops invading organisms sacrificing a small piece of tissue [112]. The hypersensitive response occurs locally at the site of infection or attack, producing reactive oxygen species causing rapid cell death, which tries to stop organisms such as pathogenic fungi, bacteria, and virus killing only the damaged plant tissue that has been attacked [87, 111].

9.2.2.2. Callus tissue formation

A more generalized response in the case of wounds in plants is the formation of callus tissue that can subsequently lignify, suberize, or impregnate phenolic compounds to provide a barrier, part of the wound periderm. This reaction provides protection against new intrusions and blocking an organism such as a fungal pathogen. The callus can also repair damaged tissues so that its functions can be restored [87].

9.2.2.3. Scarring in the periderm

Periderm scars are produced around damaged regions of the cortex, which cause activation of the PP cells of the secondary phloem, which begin to divide to form new tissue. Periderm scar acts as a wall that essentially isolates the damaged area preventing the supply of nutrients to the wound area, which eventually dies if not already dead by the attack of an invading organism. These scars also have permanent effects of tissue repair and generally are formed within the limits of induced injuries by bark beetles or fungal attacks in the trunks of conifers or well around any damaged tissue [112].

B. *Induced chemical defenses*: While the constituent chemical defenses are generally nonselective for pest species, induced chemical defenses can be broad-spectrum and specific components. Chemical defenses are extremely diverse and therefore cover a wide range of pests. Nonprotein chemicals, such as products of the phenylpropanoid routes (phenolic) and isoprenoids (terpenoids resin) products, as well as alkaloids can have potent effects on invading organisms.

These compounds are produced more rapidly than protein-based defense because the path usually exists in tissues and only requires activation. However, some of the biochemical pathways are created "de novo" in the tissues [90, 96].

Another advantage of these chemical defenses is often effective against a wide range of organisms and thus may delay an attack, while recognition mechanisms come into

play to identify the organism and then activate specific defenses against herbivore [77]. Among chemical compound induced by herbivores in conifers are phenols, resin terpenoids, and proteins.

9.2.2.4. Phenolic compounds

Phenolic compounds are abundant in the bark of conifers [113–115], mainly in the PP cells. Both phenolic compounds and tannins act as antifungal agents and block hydrolytic enzymes secreted by invading organisms, thereby inhibiting its progress in tissues [116–118]. By joining amino acids and proteins disturbed by plant tissues, phenolics and tannins reduce the nutritional value for attackers while coupling to digestive enzymes in the intestine decreases the ability to digest plant tissues. The wounds of the plant or invading organisms in the cortex activate PP cells, including cell expansion and accumulation of a higher amount of phenolic compounds [95, 119, 120]. Generally, the induced phenolic compounds are more toxic or more specific to an invading organism than the constituent phenols, whereby the conversion of polyphenolic compounds to soluble phenolic compounds during an attack adds to the defense capacity; evidence of this is the reduction of polyphenols in vacuoles of intact cells PP near the region of attack [121].

9.2.2.5. Resin terpenoids

Resin terpenoid production is induced by the attack of organisms. During and after attack, the resin flow in the wound can be quite extensive, especially in the Pinaceae. Part of this resin is stored in the structures that produce, while the constituent ducts can be activated to produce resin [89, 122, 123].

Within the first 2–3 weeks of the attack, the new resin ducts are induced to produce, being considered as traumatic resin ducts [124–127], and the resin forming these ducts can be different from the constitutive resin [103, 128, 129]. In Pinaceae and some other groups of conifers, traumatic ducts are formed in the xylem [130] and interconnected with the radial ducts phloem [131]. However, some species of conifers are induced to form more traumatic ducts in the phloem and the xylem [89]. Regardless of their origin, the end result of the development of traumatic resin ducts is to increase the formation and accumulation of resin and increase its flow [128, 129]. The increased flow helps to kill or expel the invaders and to seal the wound and resin-soaked regions of the bark and wood making them more resistant to microbial activity. Furthermore, it has been found that traumatic ducts can confer acquired resistance to subsequent attacks [131, 132] and the resin in traumatic ducts may be more toxic through changes of terpenoids or addition of phenolic compounds [133].

9.2.2.6. Proteins

Chemical defenses of the trees based on proteins include enzymes such as chitinases and glucanases that may degrade components of invading organisms and toxic proteins such as

porins, lectins, and enzyme inhibitors such as proteinases and amylase. Inhibiting enzymes interfere with the ability of the invading organism to use resources from invaded tissue. Other induced enzymes such as peroxidases and laccases can do more resistant cell walls through crisscrossed reactions or promotion of lignification or well included affecting invader organism. The protein-based defenses can be highly specific for certain organisms. For example, in Norway spruce, there are chitinases as a large family of proteins, but only a small subset of them can be regulated during the attack by a specific pathogenic fungus [133, 134], and it is presumed that these are effective against the wall cell of this organism. In general, chemical defenses induced mechanical follow a pattern similar to the induced structural defense, such as overlapping of multiple strategies. The production of a toxic cocktail with various chemical components maximizes the potential to stop or destroy an aggressive or virulent invading organism, in contrast to a more conservative production of one or few directed defenses.

9.2.3. Remark defense system importance in conifers

Multiple overlaying of structures and defense systems provides an efficient barrier against a wide range of possible attacks of organisms. However, conifers remain susceptible to certain organisms that have evolved strategies to overcome the defenses or avoid them. Nevertheless, the remarkable longevity of various species of conifers is a testament to the success of their defense strategies [87].

The first line of defense of the plant is given by a mechanical resistance to attack, due to the hardened cells either by thickening the walls or storing different compounds like calcium oxalate crystals that are joined to form a screen of high hardness. This first defense system is effective against most of the organisms that can attack the tree; however, bark beetles usually manage to overcome this barrier, bringing with them pathogenic fungi.

After that penetrate the bark beetles, thanks to its powerful masticatory apparatus, tree active chemical defense mechanisms, in which the phenolic bodies, resin and some proteins may be directed mainly beetles as organisms that are directly attacking the tree; however, these compounds also have an effect on fungi. Another unspecific compounds may function to attack bark beetles as in the case of some proteins and calcium oxalate crystals during the attack the hypersensitive response is activated, the formation of callous bodies and interaction with proteins and enzymes which are directed primarily by fungal attack. Also, answers that could be used for both bark beetles and fungi, as in the case of periderm scars, phenolic compounds, and terpenoids, can be triggered. But nevertheless, together, the beetle and the fungus can gradually block the tree's defenses, weakening to lead to death.

10. Conclusion

Plants have been developing a plethora of defense traits: chemical direct and indirect, mechanic, and uses interactions. All defense mechanisms aim survival with high photosynthetically rates, population maintenance, and fitness for plant.

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