

Chapter 11

Genetic and Ecological Basis of Resistance to Herbivorous Insects in Mediterranean Pines



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11.1 Introduction

Pines represent a successful genus of trees that have occupied vast areas around the Mediterranean Basin for the last million years (Grivet et al. 2013). As massive, sessile, long-lived and prominent organisms potentially providing a huge amount of nutritional resources (Schulman 1984), Mediterranean pines are constantly exposed to a wide plethora of enemies that feed on their tissues. Insect herbivores stand out as one of the most important biotic threats for pine trees, causing important impacts on growth, reproduction, survival and ultimately on pine fitness (Paine and Lieutier 2016).

During a long co-evolutionary history, pines and herbivores have evolved multiple and complex mechanisms aimed at maintaining their fitness in the context of their interactions. Despite the potential evolutionary disadvantage imposed by the asymmetry between the generation times of pines and herbivores (Petit and Hampe 2006), pine trees have been able to persist and dominate extensive tracts of land around the Mediterranean Sea until current times (Carrión et al. 2000). Part of this success must rely on efficient physiological and ecological mechanisms that protect pine trees (i.e. resistance) or minimize the impact of insect damage on pine fitness (i.e. tolerance) (Mumm and Hilker 2006).

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A diverse community of enemies exerts complex and heterogeneous selection pressures to which pine defenses are expected to adaptively respond (Poelman and Kessler 2016). However, rather than by directional responses to specific pine-enemy interactions, pine defenses are expected to be the result of diffuse selective pressures exerted not only by the complex community of herbivore species (Wise and Rausher 2013), but also by the interaction with the abiotic environment (Vogan and Schoettle 2015). Pine defenses against insect herbivory are highly costly to produce (Sampedro et al. 2011a), and thus, resources invested in defense are no longer available for other plant functions such as growth or reproduction. Consequently, pine trees must thus optimize resource allocation among different functions according to the particular biotic and abiotic environment (Sampedro 2014).

Altogether, both the diffuse selection pressures exerted by the highly diverse enemy community and the integrated compromises for maximizing the different life functions have contributed to maintain the huge variation among and within pine species in defensive mechanisms and strategies against insect herbivory (Petit and Hampe 2006). Aiming to provide some insights into the huge variation of defensive traits and strategies within Mediterranean pines, in the present chapter we first introduce the main insect pests affecting Mediterranean pines, with special emphasis on their negative effects on the host tree. After that, we describe the defensive mechanisms implicated in biotic resistance to such pests in pine species, including direct (chemical and anatomical) and indirect defenses. We then discuss compromises between allocation to growth and defenses in Mediterranean pines. Finally, we review current knowledge on the sources of variation of pine resistance to insect herbivory, paying special attention to the genetic, plastic and ecological factors modulating investment in chemical and physical defenses and/or effective resistance against particular enemies. We focus on the four most abundant Mediterranean pine species (*Pinus pinaster* Ait., *P. halepensis* Mill., *P. pinea* L. and *P. brutia* Ten.) on which most of the available literature is focused.

11.2 Main Insect Herbivores of Mediterranean Pines

The number of phytophagous insect species that are associated with Mediterranean pines (*P. pinaster*, *P. halepensis*, *P. pinea* and *P. brutia*) is enormous. Based on a literature search and their own data, Mendel (2000) reported more than 110 insect species feeding on *P. halepensis* and *P. brutia* in southeast Europe, most of them (62%) belonging to five families: Scolytidae, Buprestidae, Cerambycidae, Lachnidae, and the superfamily Coccoidea. In Southwestern Europe, Muñoz-López et al. (2007) listed up to 66 species producing significant damage to native Pinaceae species. Insects feeding on Mediterranean pines include piercing-suckers, borers and chewers, producing damage in all pine tissues, including needles, buds, stems and cones. Despite the large diversity of insects feeding on Mediterranean pines, only a few cause damage of ecological and economic relevance with relatively high frequency (Fig. 11.1).

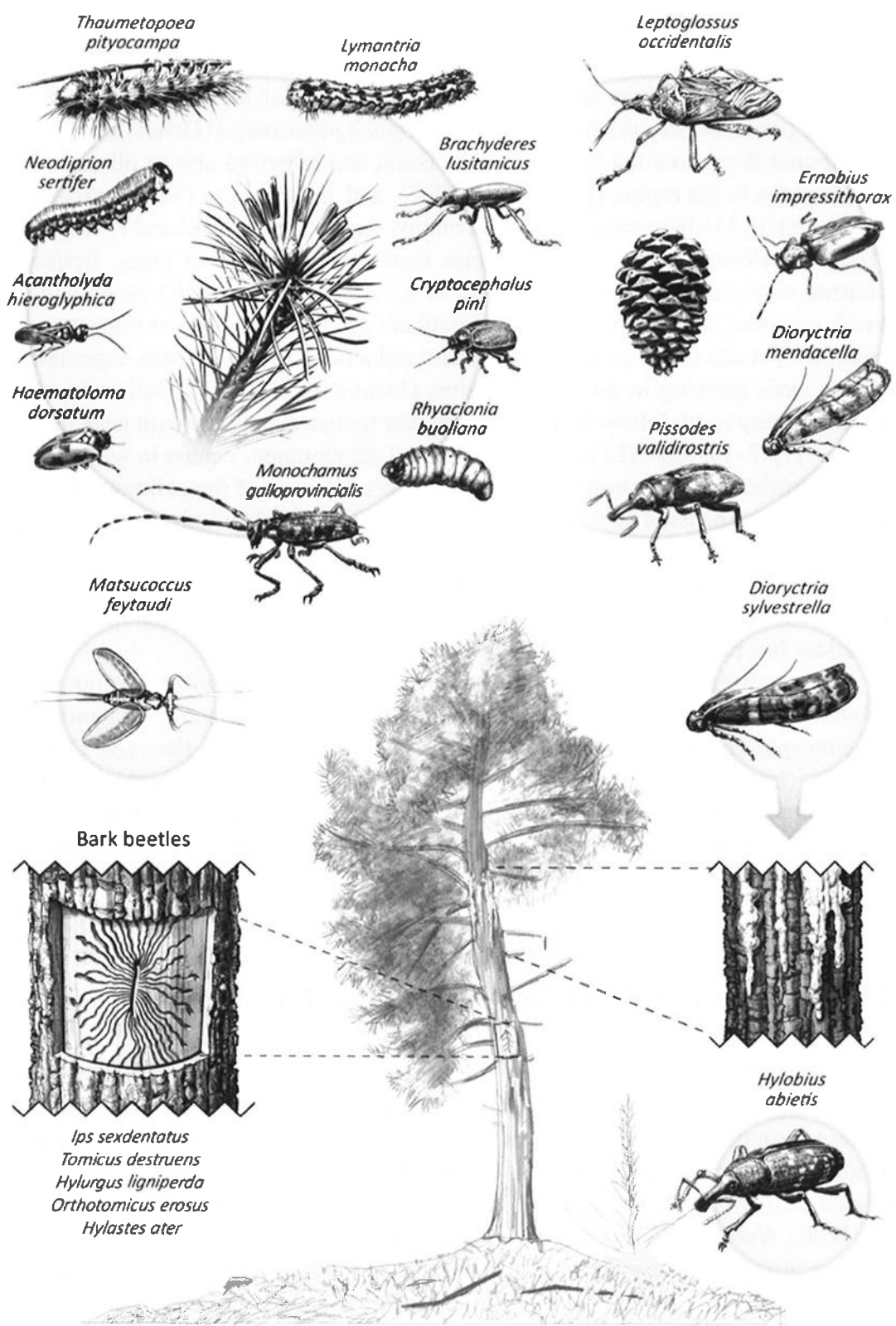


Fig. 11.1 Main insects feeding on living tissues of Mediterranean pines

11.2.1 Defoliators

Probably the most iconic and well-known insect pests of Mediterranean pines are the pine processionary moths, with *Thaumetopoea pityocampa* (Dennis and Schiff.), distributed throughout the Mediterranean Basin and affecting almost all native and exotic pines in the region (Hodar et al. 2002), and *T. wilkinsoni* (Tams), distributed in the eastern Mediterranean Basin and mainly damaging *P. brutia* and *P. halepensis* (Halperin 1990). Larvae of both species feed on the needles of pines, frequently causing severe defoliation rates (up to 100%). Although severe and prolonged outbreak episodes can eventually lead to significant mortality, the largest impact of processionary moth defoliations are important reductions in tree growth, especially in young trees growing in poor quality sites (Jacquet et al. 2012). Outbreaks of the processionary moth follow more or less regular temporal patterns with peak defoliations every 7–11 years (Li et al. 2015). Larval development occurs in winter and is mainly limited by minimum winter temperatures. Because of that, climate change is favoring the expansion of this insect to higher altitudes and latitudes (Pimentel et al. 2011; Battisti et al. 2016a). In the Mediterranean Basin, the processionary moth is probably the forest pest for which investment in control measures – mainly aerial insecticide applications – is the highest. However, the efficacy of such management practices has been questioned (Cayuela et al. 2011).

Other defoliators of Mediterranean pines include *Neodiprion sertifer* Geoff. (Hymenoptera, Diprionidae), *Lymantria monacha* L. (Lepidoptera, Lymantriidae), *Cryptocephalus pini* L. (Coleoptera, Curculionidae), *Panolis flammea* Denis & Schiff (Lepidoptera, Noctuidae) and *Acantholyda hieroglyphica* Christ. (Hymenoptera, Pamphiliidae) to mention a few, but in all cases both their incidence on Mediterranean pines and the information available about them are much more limited (Battisti et al. 2016b).

11.2.2 Stem Borers, Phloem Feeders and Bud Miners

An important group of insects that causes severe damage in Mediterranean pine forests are bark beetles (Coleoptera, Scolytidae). Bark beetles are an extremely diverse group of insects, with more than 40 species associated with typical Mediterranean conifers, many of them highly specialized to one or a few highly-related host species (Lieutier et al. 2016). *Ips sexdentatus* Börner, *Tomicus destruens* Woll., *Hylurgus ligniperda* Fab., *Orthotomicus erosus* Woll., and *Hylastes ater* Pay. are among the most frequent bark beetles infesting Mediterranean pines. Bark beetles oviposit in galleries beneath the bark of branches, trunks or roots, where developing larvae feed on the innermost part of the bark, causing tree mortality due to the breakdown of the vascular system. Bark beetles typically attack weakened trees, but epidemic populations during outbreaks can also kill healthy trees. As their counterparts in boreal pine forest, bark beetles of Mediterranean pines can act on

host trees in symbiotic association with an array of microorganisms, including pathogenic fungi such as *Ophistoma* sp. (Kirisits 2004). Pine host resistance to bark beetles has been reviewed elsewhere (Krokene 2015).

Another important stem borer of Mediterranean pines is the European stem borer *Dioryctria sylvestrella* (Ratz.) (Lepidoptera, Pyralidae), which causes extensive damage in pine forest plantations, through trunk malformations and resin pockets within the stem wood, and an increase in the risk of stem breakage (Budde et al. 2017).

One insect that is gaining increasing attention in the Mediterranean Basin is the pine sawyer *Monochamus galloprovincialis* Olivier (Coleoptera, Cerambycidae), which feeds in the inner stem of pine branches. Although the direct feeding impact of these longhorn beetles is not considered to be very significant (Evans et al. 2004), adults of this species vector the exotic nematode, *Bursaphelenchus xylophilus* (Steiner and Buhner) Nickle, responsible for pine wilt disease (Sousa et al. 2001). The devastating effects of this exotic invasive organism have motivated vast research efforts aimed at controlling its expansion throughout Europe; an important part of this effort has been to study the biology, ecology and control of the insect vector (Naves et al. 2016).

Insect damage on Mediterranean pines can occur at all tree ages; the early stages of development are assumed to be more critical, as insect damage can easily lead to seedling mortality (Barton and Koricheva 2010). One insect that can cause great damage at early ontogenetic stages of Mediterranean pines is the pine weevil, *Hylobius abietis* L. (Coleoptera, Curculionidae). Adults of this insect feed on the phloem and bark of young seedlings, easily leading to stem girdling and seedling death, with damage being especially problematic in natural or artificial regenerations after clear-cuts (Day et al. 2004). This insect has an enormous economic impact for the regeneration of conifer forests in northern and central Europe, but it can also attack Mediterranean pines such as *P. pinaster* (Sampedro et al. 2009), *P. halepensis* (Suarez-Vidal et al. 2019), or *P. brutia* (Semiz et al. 2017).

Bud miners of Mediterranean pines include lepidopteran species such as *Rhyacionia buoliana* (Dennis and Schiff.) and *R. duplana* Hübner (Lepidoptera, Tortricidae), two moths that can cause important economic damage on young plantations producing fancy stem deformations such as forked or crooked stems.

11.2.3 Sap Suckers

Several sap-sucker species of aphids, coccids and bugs are also known to affect Mediterranean pines, reducing tree vigor and favoring the entrance of secondary pests (Sallé and Battisti 2016). Among them, *Haematoloma dorsatum* Ahrens (Hemiptera: Cercopidae), *Leucaspis pusilla* Loew, *L. pini* Hartig, and *L. lowi* Colvée (Hemiptera: Diaspididae) are considered potential pests (Sallé and Battisti 2016). Two pine bast scales (*Matsucoccus* sp., Hemiptera, Matsucoccidae) are also potentially highly destructive for Mediterranean pines (Mendel et al. 2016). *Matsucoccus*

feytaudi Duc. is native to the western Mediterranean Basin and is specific to maritime pine, while *M. josephi* Bodenhiemer and Harpaz appears in the eastern Mediterranean and feeds mainly on *P. brutia* and *P. halepensis*. In their native range, both species cohabit with their host in equilibrium. However, the two species have recently spread out of their natural range, causing extensive damage and great mortality (Mendel et al. 2016; Roversi et al. 2013). The absence of efficient defensive mechanisms in the native pine populations of the invaded range due to absence of a co-evolutionary history with the insect (Schvester and Ughetto 1986) and lack of natural enemies in the new range (Jactel et al. 2006) seem to underlie these contrasting patterns of susceptibility.

11.2.4 Cone and Seed Feeders

Cone and seed feeders are another important group of phytophagous insects associated with Mediterranean pines (Boivin and Auger-Rozenberg 2016). Most of the knowledge about them comes from the study of the damage and control of insects affecting the valued cones of stone pine (*P. pinea*) (Bracalini et al. 2013). Two cone moths, *Dioryctria pineae* Staudinger and *D. mendacella* Staudinger (Lepidoptera: Pyralidae), one cone weevil, *Pissodes validirostris* Gyll (Coleoptera: Curculionidae), and the anobiid beetle *Ernobius impressithorax* Pic (Coleoptera: Anobiidae) are probably the most abundant cone-damaging agents (Bracalini et al. 2013; Innocenti and Tiberi 2002). The larvae of these insects cause severe visual and internal damage to the cones and ultimately lead to high seed loss rates, and a notable decrease in the weight and viability of the remaining unattacked seeds (Bracalini et al. 2013; Calama et al. 2017). Another exotic insect, *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae), native to North America and recently introduced into Europe (Bernardinelli and Zandigiacomo 2001), is also causing large seed yield losses in *P. pinea* orchards, but signs of its damage on the cones are not so evident (Oliveira Farinha et al. 2018).

11.3 Mechanisms and Strategies Providing Resistance to Insect Herbivores in Mediterranean Pine Trees

11.3.1 Direct Resistance Mechanisms

Pine trees have evolved a complex defensive system with multiple layers of chemical and anatomical defences (see Box 11.1). Pine defenses are always present at a baseline level, and are referred to as constitutive defenses, providing direct resistance against a vast array of herbivores and pathogens (Table 11.1). Direct resistance traits are toxic, repellent, anti-digestive chemical compounds or physical barriers that directly reduce the performance or efficiency of herbivores feeding on plant tissues, affecting their fitness at diverse stages of their life cycles.

Box 11.1: Mechanisms of Resistance to Insect Herbivores in Mediterranean Pines

Direct resistance mechanisms are those mechanisms that act directly killing or repelling the insect herbivores, and include:

- *Mechanical defenses* are displayed as concentric layers acting as defensive barriers aimed at avoiding the entrance of invader organisms. In Mediterranean pines, these include the outer bark (periderm), a highly suberized or lignified cell types, and a highly developed system of resin ducts.
- *Chemical defenses* are stored in different anatomical or cellular structures, in all pine tissues (needles, stem, roots and cones). In Mediterranean pines, chemical compounds, including at least phenolic compounds and terpenes, are released into the intercellular space upon insect damage, causing deterrence, digestive or feeding dysfunction, direct toxicity and might ultimately lead to insect mortality.

Indirect resistance mechanisms: Indirect defenses involve third partners, usually predators of insect herbivores, in mutualistic association with plant hosts that positively influence plant resistance throughout top-down regulation of herbivore populations. Indirect defenses may involve a ‘call for help’ carried out by plants often mediated by the emission of volatile organic compounds (VOCs) which the predator can recognize.

Induced resistance: The production of direct and indirect defenses is not constant throughout time. Conversely, their production is increased when the biotic damage is perceived, producing the so called ‘induced defenses’, which are specially relevant to deter the negative impacts of an insect attack. These include:

- *Induced direct defenses* in Mediterranean pines involve processes leading to increased production of physical and chemical defenses, including cell differentiation, gene expression, protein biosynthesis and hormonal regulation.
- *Induced emission of volatile organic compounds (VOCs)* lead to changes in the tree chemical airborne signature that might lead to faster within-plant and between-plant communication and to a strong ‘call for help’.
- *Induced tolerance responses* refer to phenotypic changes in the host aimed at minimizing the impact caused by the herbivore on plant fitness, with no effects on the herbivore survival, growth or reproduction.

Priming of resistance: Priming of defensive mechanisms act as a ‘vaccination effect’ by which plants are able to perceive the risk of an attack and activate the cellular and molecular machinery to carry out a faster induced response once the damage finally occurs. Up to date priming has been described in *Picea abies*, but not yet in Mediterranean pines.

Table 11.1 Defensive traits providing resistance to specific insect herbivores in Mediterranean pines

Host	Insect herbivore	Trait	Type ^a	Pattern ^b	Observations	References
<i>Pinus</i> spp.	<i>Thaumetopoea pityocampa</i>	Terpenes	C	R	Oviposition deterrence	Paiva et al. (2011)
		Total phenols	C	R	Larval mortality	Schopf and Avtzis (1987)
		Silica	P	R	Larval mortality	Schopf and Avtzis (1987)
	<i>Matsucoccus josephi</i>	Wound periderm	P	R	Faster response in non-host species	Liphschitz and Mendel (1989)
		Tissue hypertrophy	P	S	Abnormal tissue formation in host species	Liphschitz and Mendel (1989)
	<i>Leptoglossus occidentalis</i>	Cone and seed size	O	S	<i>P. pinea</i> > <i>P. pinaster</i> = <i>P. halepensis</i>	Farinha et al. (2018)
<i>P. pinea</i>	<i>Tomicus destruens</i>	Terpenes	C	R	Limonene, β -caryophyllene	Faccoli et al. (2011)
		Terpenes	C	S	α -pinene, β -myrcene, α -terpinolene	Faccoli et al. (2008, 2011)
	<i>Thaumetopoea pityocampa</i>	Terpenes	C	R	(1S)-(-)- β -pinene and (R)-(+)-limonene reduced egg laying. Limonene reduce oviposition preference	Panzavolta et al. (2015) and Tiberi et al. (1999)
	<i>Neodiprion sertifer</i>	Terpenes	C	R	Low limonene concentration is attractive, whereas high concentration is repellent	Martini et al. (2010)
	<i>Marchalina hellenica</i>	Terpenes	C	R	Limonene increases resistance	Mita et al. (2002)
<i>P. pinaster</i>	<i>Dioryctria sylvestrella</i>	Terpenes	C	R	Terpinolene as a candidate compound providing resistance for this insect	Jactel et al. (1999)
		Terpenes	C	S	Limonene, copaene, linalool and longipinene contribute to susceptibility	Jactel et al. (1996) and Kleinhenz et al. (1999)

(continued)

Table 11.1 (continued)

Host	Insect herbivore	Trait	Type ^a	Pattern ^b	Observations	References
		Bark thickness	P	R	Bark thickness reduces infestation	Carisey et al. (1994)
	<i>Hylobius abietis</i>	Terpenes	C	R	Limonene provides constitutive resistance	López-Goldar et al. (2018)
			C		Induced resin acids increase resistance	
		Phenolics	C	R	Induced lignans increase resistance	López-Goldar et al. (2018)
		Non-volatile resin	C	R	MJ induction of non-volatile resin increases resistance	Sampedro et al. (2011b)
	<i>Diprion pini</i>	Terpenes	C	R	Resin acids reduce oviposition	Auger et al. (1994)
	<i>Thaumetopoea pityocampa</i>	Terpenes	C	R	Sprayed limonene reduces female oviposition on needles	Tiberi et al. (1999)
<i>P. halepensis</i>	<i>Thaumetopoea pityocampa</i>	Needle length	P	R	Shorter needles reduce survival of pine processionary eggs	Hezil et al. (2018)
		Needle asymmetry	P	S	Pine processionary females select asymmetric needles for oviposition	Pérez-Contreras et al. (2008)
	<i>Tomicus piniperda</i>	Terpenes	C	R	High levels of (+)- α -pinene, +-3-carene; low levels of (-)- α -pinene are deterrent	Almquist et al. (2006)
	<i>Tomicus destruens</i>	Ethanol	C	S	Increased ethanol in drought-stressed pines, which is attractive for the insect	Kelsey et al. (2014)
	<i>Marchalina hellenica</i>	Terpenes	C	S	High levels of α -pinene and low levels of limonene and α -terpinyl acetate	Mita et al. (2002)
<i>P. brutia</i>	<i>Tomicus destruens</i>	Bark thickness	P	R	Attacked trees are damaged only in thin-bark parts	Ciesla et al. (2011)

(continued)

Table 11.1 (continued)

Host	Insect herbivore	Trait	Type ^a	Pattern ^b	Observations	References
	<i>Tomicus minor</i>	Bark thickness	P	R	Attacked trees are damaged only in thin-bark parts of the mid and upper bole	Ciesla et al. (2011)
	<i>Hylobius abietis</i>	Terpenes	C	R	<p>γ-terpinene, sabinene are negatively correlated to pine weevil damage.</p> <p>Limonene and α-terpinolene showed negative trends with weevil damage.</p>	Semiz et al. (2017)

^aType of defenses: *C* Chemical, *P* Physical, *O* Other

^bResistance pattern analyzed: *R* Resistance, *S* Susceptibility

11.3.1.1 Mechanical Defenses

Pine trees display a model mechanical defensive system in their stem and branches, comprising several concentric layers (Franceschi et al. 2005). The outer bark (periderm), among other functions, provides a first barrier of suberized, lignified, dead cells that hinder the entrance of insects into the stem (Krokene 2016). In the bark, the main defensive mechanisms are the polyphenolic parenchyma, with living cells containing large vacuoles filled with phenolic compounds (Nagy et al. 2004), and stored starch and lipids that could be readily released in case of attack (Franceschi et al. 2000). Vacuoles with proteinase inhibitors, phenol oxidases and other enzymes reducing the digestibility of ingested host tissues may also be present, as found in other plant taxa (Huffaker et al. 2013). Cell walls may be reinforced with compounds such as suberin, lignin and callose. Other layers of defensive cells with calcium oxalate crystals or stone cells with a defensive function have been described in other conifer species (Moreira et al. 2012a; Whitehill et al. 2016) but not yet in Mediterranean pines.

A major defensive mechanism in pine trees are resin-based defenses (Franceschi et al. 2005). Particularly, pine trees have developed a complex three-dimensional network of interconnected axial and radial resin ducts within the xylem and phloem. Resin ducts are the structures where oleoresin (a complex mixture of diterpenes and resin acids solubilized by a volatile fraction of monoterpenes and sesquiterpenes) is synthesized and stored (Celedon and Bohlmann 2019). Pine oleoresin is a sticky, toxic fluid that flows out when the resin ducts are injured, thus preventing the entrance of invading organisms.

Resin synthesis also takes place in needles (Turner et al. 2018), where axial resin ducts are also present and connected to the tree resin duct network. Cuticular waxes

may prevent oviposition or reduce the mobility of defoliating insects (Hilker et al. 2002). Trichomes and spines have also been described as mechanical barriers against small insects in other species. Small spines are present in primary needles of pine seedlings but they have been not yet been associated with insect resistance.

11.3.1.2 Chemical Defenses

Among direct chemical defenses, mono-, sesqui- and diterpene acids are highly toxic compounds for many invading organisms (Whitehill et al. 2019). Pine phenolic compounds are very diverse in nature and contribute in different forms to resistance, such as the tannins binding digestive proteins or the stilbenes with direct toxicity (Nagy et al. 2004). Both terpenes and phenolics are carbon-based compounds and have been traditionally considered to be the most relevant chemical defenses for pine resistance. Up to 93 different terpenoid chemical species and 35 different phenolic compounds have been found in young *P. pinaster* seedlings, reaching a concentration up to 27 mg g⁻¹ and 15 mg g⁻¹, respectively, in some tissues (López-Goldar et al. 2019). Piperidine alkaloids have been described in other conifers and could provide direct toxicity to insect herbivores or their microbial symbionts (Gerson and Kelsey 2002). Similarly, acetophenones have been reported as chemicals providing resistance against spruce budworm (Parent et al. 2020). However, alkaloids and acetophenones remain understudied in Mediterranean pines.

A particular feature of secondary metabolism in pine trees is that the large concentration of mono- and sesquiterpenes in pine tissues is actively stored in the internal network of resin ducts, rather than in cell vacuoles, external glandular trichomes or directly solved in the cell content as found for other plant groups such as oaks and most aromatic herbs. As mono- and sesquiterpenes are volatile at atmospheric temperatures, they can diffuse or be more actively released to the atmosphere from the stomata and exposed resin ducts. The emission rate and profile of such terpene mixtures have been found to vary across populations and families of maritime pine (Blanch et al. 2012; Sampedro et al. 2010). Emission of volatile organic compounds (VOCs) also depends on light conditions, temperature, and nutrient availability and thus informs about plant performance and nutritional status (Blanch et al. 2012). The emission of such an amount of VOCs confers an airborne signature variable among individuals or lineages that may have great ecological and evolutionary implications for herbivore deterrence, host selection by herbivores, host selection by herbivore predators and plant–plant interactions.

11.3.2 Indirect Resistance Mechanisms

Besides mechanisms providing direct resistance, plants have also evolved mechanisms of indirect resistance (i.e. indirect defenses) (see Box 11.1). They are based on boosting mutualistic relationships with other organisms that are potentially

antagonistic to plant enemies (Heil 2008). Most of those third partners are predators and parasitoids of herbivore insects, and in most cases indirect resistance involves an active, inducible, and fairly specific ‘call for help’ from the plant counterpart in response to herbivore attack (Heil 2014). Many particular cases of indirect resistance have been discovered in annual plants and some have currently been exploited in agricultural crops. In pines and other trees such mechanisms remain understudied. However, it has been reported that some insectivorous birds may play such a role in scots pine (Mäntylä et al. 2018). Furthermore, there is strong evidence that wasp parasitoids provide indirect defenses against sawflies in some pine species (Hilker et al. 2002; Mumm and Hilker 2006). A particular case is the protective mutualism potentially provided by aphid-tending ant species on pine trees. Aphids feed on the pine phloem while ants feed on the honeydew produced by tended aphids. Ants clean their aphid colonies and surrounding pine tissues of any other insects and debris. Some studies have showed effective protection of ants against other herbivores (Maňák et al. 2013) and a lack of costs in terms of growth in young pines of several species (Moreira et al. 2012b). More research is needed to obtain a clear understanding of the costs and benefits of these particular aphid-mediated potential plant mutualisms along the ontogeny of the tree host and across environments.

11.3.3 Induced Resistance

Provided that plant species have the ability to perceive, identify and signal changes in the environment, they are also able to adjust their defensive phenotype to maximize survival. Plant defence theory predicts that this kind of plastic response must be favored in long-lived slow-growing species such as pine trees. For instance, when plants perceive an herbivore attack, the allocation priorities change and plants can express an “induced response” to protect their tissues (Eyles et al. 2010) (Fig. 11.2). The constitutive levels of defenses may be raised to obtain an extra-defended phenotype. Expressing induced responses is supposed to be a cost-saving strategy, as associated costs are only materialized when needed, after the attack (Cipollini and Heil 2010). Induction may be systemic, when phenotypic change affects the whole organism, or local, when changes are detectable just in localized plant parts or specific tissues (Moreira et al. 2009). In pine trees, induced responses can be classified into the following types of responses, described below.

11.3.3.1 Induced Direct Resistance

This includes those phenotypic changes in response to damage that aim to repel the attack and weaken insect performance. Producing an induced defensive phenotype implies that plants are able to identify the challenge, signal the alarm message to target tissues, and activate gene expression, protein biosynthesis and gene and

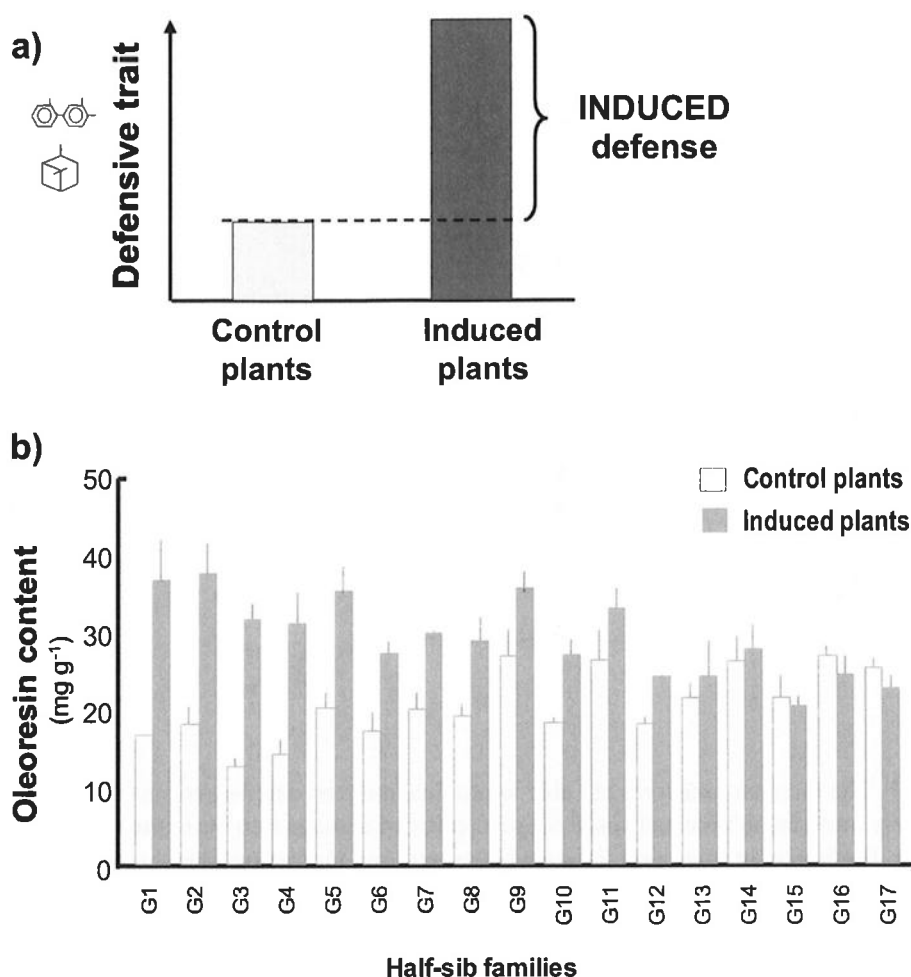


Fig. 11.2 Panel a) presents three common concepts regarding induced responses in trees: (i) the concentration of a given defensive trait providing resistance in control plants (light grey bar), which is the baseline level, already present before any attack (constitutive defenses); (ii) the concentration of the same defensive trait in plants exposed to herbivory, induced in response to a biotic challenge (dark grey bar, induced level); and (iii) inducibility – the difference between the two, i.e. the ability to express a plastic, induced, response in a given defensive trait, sometimes reversible after a given time. Panel b) illustrates how constitutive and induced defenses in pine trees, as well as the inducibility of a given defense, may be genetically variable across genetic entries, as is the case of oleoresin in the stem of *P. pinaster* half-sib families. (Modified from Sampedro et al. 2011b)

enzyme regulation to produce the proper phenotypic changes leading to improved resistance (Fig. 11.3). Upregulation of biosynthesis of plant hormones associated with herbivore signaling (such as jasmonic acid, ethylene and salicylic acid) and chemical defenses (terpenoids and phenolics) have been reported in several conifer species in response to herbivore attack (Verne et al. 2011). Reconfiguration of the entire primary metabolism may be required, with a complex integration of potential cross-talks with other plant hormonal signaling pathways (auxins, abscisic acid, brassinosteroids and gibberellins) to balance plant growth, abiotic stress tolerance and defense. Changes in physical and chemical defenses may contribute to improve effective resistance and plant fitness in time and space, thus reducing insect

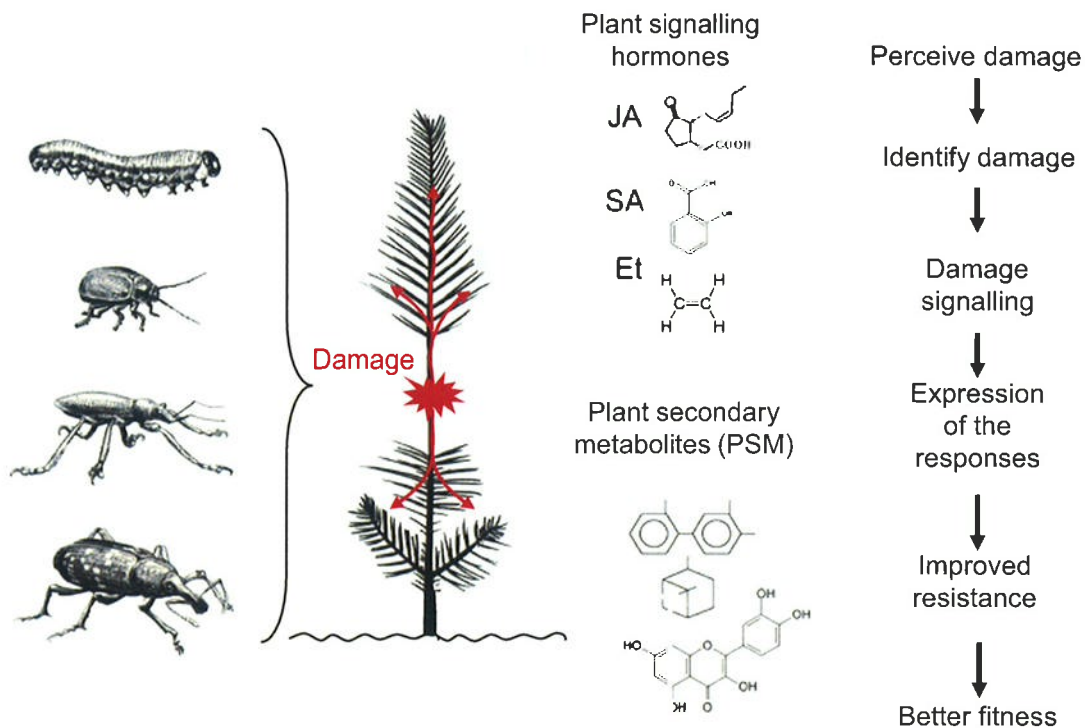


Fig. 11.3 The fact that pine trees are able to produce induced defenses means that they are able to adequately integrate in time and space the perception of damage into the expression of an adequate response, leading to an improved resistant phenotype and increasing fitness under herbivore damage. Plant hormones such as jasmonic acid, salicylic acid, ethylene and others are essential in this process, leading to the induction of defensive proteins, chemicals and anatomical defenses

performance. Most of the knowledge in this field comes from model plants and a strong translational effort to pine trees would be required.

11.3.3.2 Induced Emission of Organic Volatile Compounds (VOCs)

Changes in the emission of VOCs may contribute to faster within-plant damage-signaling among plant parts, separated anatomically but physically close (Heil and Bueno 2007; Heil 2014). Changes in the airborne message from infested pine trees may also modulate other biotic interactions leading to induced indirect resistance, with a strong “call for help” from attacked plants attracting insect predators and parasitoids (Heil and Bueno 2007). Furthermore, changes in airborne scents in the tree’s neighborhood could be perceived by relatives of the focal host plant, leading to early perception of the risk of herbivore attack (Heil and Karban 2010). Again, knowledge about this kind of induced indirect resistance mechanism in pine trees is still very limited.

11.3.3.3 Induced Tolerance Responses

These include those phenotypic changes aimed at minimizing the impact of the attack on plant fitness, irrespective of the performance of the attacker. Tolerance is a plant defense strategy that seeks to maintain plant fitness with no effect on herbivore growth, survival and/or reproduction. This last point is essential for understanding this strategy. No effects on herbivore fitness will result in no selective pressure and thus no room to further co-evolutionary arms race. Induced direct tolerance responses in pine trees may include changes in growth priorities among tissues, for instance favoring apical leader recovery and diminishing branching differentiation, or stimulating fine root differentiation for water uptake. Such changes in growth patterns could be favored by active mobilization of carbon and nutrients to sink tissues, or, alternatively, by moving resources away from tissues targeted by the herbivores (roots, stems, needles). A particular case of responses aiming to achieve induced indirect tolerance, could be those boosting carbon exudation through the roots to favor decomposition and nutrient mineralization by soil microbes and/or more intense ectomycorrhizal (ECM) colonization in fine roots, which may favour water and nutrient uptake and thus quicker recovery after attack. Although pine trees are obligate mutualists with ECM, this kind of response has been understudied in pines. However, there is evidence of drastic changes in carbon allocation to fine roots and nitrogen and phosphorus remobilization in young pines in response to biotic stimuli (Moreira et al. 2012c).

11.3.4 Priming of Resistance

Although expressing induced defenses is an energy-saving strategy, the benefits of induced defenses rely on effective damage recognition and timely expression of the improved defensive phenotype. A good match between damage recognition and activation time – the time required for expressing an effective defensive phenotype – is essential for a efficient, rapid response to improve resistance. Similarly, when herbivore risk diminishes, the lag time and decay time – the time for returning to basal defensive investment – is essential for balancing the costs. Thus, time is an essential concept for understanding the costs and benefits of expressing induced defenses in each environmental circumstance.

Research on model species has shown that plants, besides recognizing herbivore damage, can perceive the risk of damage. Perception of the risk of damage prior to herbivore arrival may be crucial for activating induced defenses in time, for instance, recognizing the herbivore loading in the neighborhood, probably via changes in the airborne messages of VOC from the surroundings. Upon perception of such risk, pine trees may express an intermediate state of immune activation before herbivore attack. This state is known as the ‘priming state’ (see reviews by Hilker et al. 2016; Martinez-Medina et al. 2016; Hilker and Schmülling 2019). The ability to be primed after recognition of the risk of damage is an evolutionary step of adaptive induced

resistance. Knowledge about priming in long-lived plants such as trees is only in its early stages; future work will lead to great advances in basic and applied research (Mumm et al. 2003; Hilker and Meiners 2006; Hilker and Fatouros 2016; Bittner et al. 2019; Hilker and Schmölling 2019; Mageroy et al. 2019).

Discovering that plants may react with a plastic response to pests and pathogens has signaled a brand new era in our understanding of plant science and led to a new discipline of plant behavior and plant immunity. The first reports of plant-induced defenses were published in the early 1950s, whereas induced defenses in conifers were reported some 30 years later (Lewinsohn et al. 1991). A literature search to date in the ISI-Web engine showed that published articles in the last 5 years with the words ‘tree + induced + responses’ and ‘pine + induced + responses’ accounted just for 15% and 2%, respectively, of those published with the words ‘plant + induced + responses’, the latter with a yearly average of 3050 items. Thanks to the last 20 years of research on model plants, we now know that plant resistance does not rely only on the presence and abundance of secondary chemicals. Plant resistance depends on a complex and integrated immune system that is able to recognize specific molecular patterns of plant self-damage and herbivore associated molecular patterns (HAMPs), among others (Heil and Land 2014). Moreover, herbivore offense commonly involves effector proteins and RNAs that confound and disturb the plant immune system, molecular pattern recognition or plant hormonal damage signaling. In turn, plants have evolved resistance proteins that block pathogen and herbivore effectors triggering plant triggered immunity. Effector-triggered immunity, where plants detect phytopathogenic factors that trigger a response, has been identified in other genera of trees such as *Malus*, *Salix*, *Vitis*, *Prunus*, *Castanea* and *Eucalyptus* in response to insect gillers, but not yet in conifers (Yamaguchi et al. 2012; Tooker and Helms 2014; Oates et al. 2016).

Herbivore-plant interactions are more complex than we could imagine a few years ago. The induced defensive phenotype is the outcome of many molecular steps, pathways and interactions, including recognition of insect-associated molecular patterns, insect effectors, modulators, plant defensive proteins, etc. Furthermore, induced responses against one organism can lead to susceptibility to others, as specific responses and defensive mechanisms to each organism might be affected by hormonal crosstalks. The effects of the combined exposure to multiple organisms are, however, mostly still unknown.

11.4 Trade-Offs Associated with Mediterranean Pine Defenses

Since defense production is costly and resources are limited, optimal resource allocation to defenses is expected to come at the expense of growth and reproduction, leading to the expression of trade-offs among life functions (Herms and Mattson 1991, 1992) (Fig. 11.4a). Particularly, growth–defense trade-offs have been of

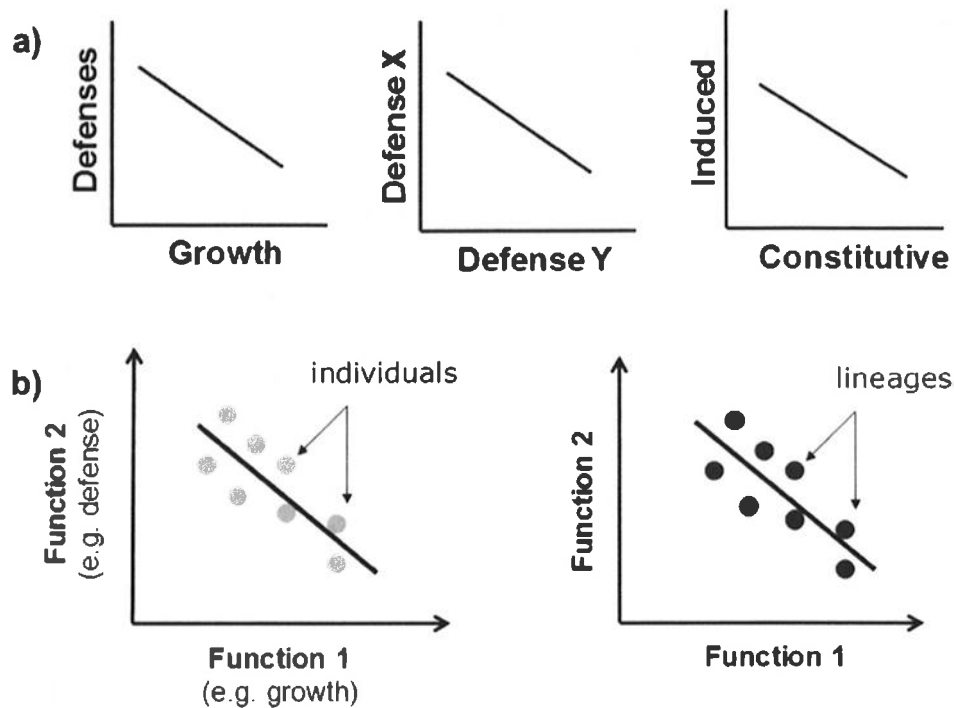


Fig. 11.4 Some of the trade-offs potentially associated with pine defenses. **(a)** As defensive investment is energetically costly and the required carbon resources are shared with growth, constraints between growth and defenses have sometimes been reported. As defenses of different nature (x and y in **(a)**) may share the resources required for their synthesis, a trade-off in the expression of both types of defenses could be expected. The same pattern could be observed if both types of defenses are to some extent functionally redundant. Moreover, a trade-off between constitutive defenses and inducibility of defenses could be also expected. The balance of costs and benefits of both strategies depends on environmental factors such as resource availability, herbivore loading and risk and predictability of herbivore attacks. Furthermore, evolutionary conflicts between both strategies may arise because the benefits of induced defenses in terms of fitness could differ for plant lineages with high and low constitutive defenses. **(b)** Conflicts in the allocation to two traits of functions sharing a common resource usually emerge as negative phenotypic correlations at the individual level (grey points in **(b)**-left), where points in the negative correlation are subjects or individuals, indicating a physiological trade-off among source-related traits. Negative genetic associations between pairs of traits may emerge as negative correlations, where points in the plot are entities with a genetic basis (black points in **(b)**-right; families, lineages, populations, species) delimiting a surface of all the possible combinations of values for both traits leading to similar fitness. Such negative genetic correlations have heritable and evolutionary relevance, and denote potential constraints to the simultaneous selection on both functions

special consideration in plant defense research since their expression is thought to maintain patterns of variation in defensive allocation among and within species (Stamp 2003; Agrawal 2011, 2020). Several ecological and evolutionary hypotheses have been postulated to provide a mechanistic basis that explains constraints between growth and defenses (Stamp 2003). For instance, the growth–differentiation balance hypothesis (GDBH – Herms and Mattson 1992) states that when resource shortage (mainly in water or key nutrients such as nitrogen and phosphorus) limits growth but not photosynthesis, a physiological trade-off between growth and defenses might be expressed, as the exceed of photoassimilates can be

reallocated to defense production. Likewise, the resource availability hypothesis (RAH – Coley et al. 1985; Endara and Coley 2011) addresses the existence of an evolutionary trade-off among species. According to the RAH, habitats with high resource availability select for species with enhanced growth rates and low defensive capability. Conversely, habitats characterized by poor resource conditions select for slow-growing but highly defended plants, given the costs associated with the replacement of tissue lost by herbivore damage.

The defensive system of pine species is costly to produce, particularly in relation to resin-based defenses, as it involves the development of a system of interconnected resin ducts and the production of abundant oleoresin comprising carbon-enriched terpenoid molecules (Gershenzon 1994; Moreira et al. 2015). Furthermore, environmental conditions in the Mediterranean Basin are often strongly limiting (e.g. pronounced, long-lasting drought periods), and may therefore exert strong constraints on resource allocation among life-functions. Growth–defense trade-offs (i.e., negative correlations between both functions) are therefore expected to be expressed in Mediterranean pines, although empirical evidence is scarce and suggests that their expression is strongly context-dependent. For instance, glasshouse studies with *P. pinaster* saplings have revealed that trade-offs between chemical or anatomical defenses and growth emerged only under nutrient-limited conditions, particularly in phosphorous availability (Sampedro et al. 2011a; Moreira et al. 2015). Likewise, an experimental study with *P. halepensis* saplings also showed that growth–defense constraints in this species only emerged in response to biotic competition with other tree species (Fernandez et al. 2016). More importantly, finding evidence of negative genetic correlation (i.e. across families or populations within species; Fig. 11.4b) between growth and defenses might indicate the heritable and therefore evolutionary basis of such trade-offs (Agrawal 2020) (Fig. 11.4b). For instance, Moreira et al. (2015) reported negative intraspecific genetic correlations between growth and anatomical defenses among *P. pinaster* families, indicating that fast growing genotypes were less defended and vice versa. Likewise, Di Matteo and Voltas (2016) found that *P. pinaster* populations adapted to more favorable conditions and with higher growth potential were more susceptible to the insect *M. feytaudi*. Conversely, slow-growing populations adapted to harsher environmental conditions showed reduced insect damage (i.e., higher resistance). This latter study provides evidence of a trade-off between growth and resistance at the population level and suggests that *P. pinaster* populations evolved divergently towards differential defensive strategies.

Mediterranean pine species occupy wide distribution ranges and are therefore subject to heterogeneous environmental conditions leading to genetic adaptation and different life-history strategies (Tapias et al. 2004; Grivet et al. 2010, 2013). If growth–defense constraints are genetically determined, as seems to be the case (Moreira et al. 2015; Di Matteo and Voltas 2016), populations with higher intrinsic growth rates (i.e. growth potential) might have compromised their defensive capability. This would have immediate implications for the future management of forest genetic resources, since characterization of optimal population performance is often based on growth potential or abiotic resistance (Alía et al. 1995; Taïbi et al. 2014).

However, biotic resistance markers are less frequently considered, despite the evidence of increased biotic stresses associated with global change (Peñuelas and Filella 2001; Bale et al. 2002; Benito-Garzón and Fernández-Manjarrés 2015). Given the current evidence of genetic growth–defense trade-offs, there is an urgent need to understand to what extent and under what conditions (water or nutrient availability) such constraints are expressed between and within Mediterranean pine populations. This knowledge will help us anticipate population responses to predicted environmental shifts in the Mediterranean Basin and facilitate decision making in adaptive forest management under current scenarios of global change.

11.5 Variation in Defensive Investment

Anatomical and chemical defenses in Mediterranean pine species vary widely both among and within species (Table 11.2). Such variation is likely the result of genetic adaptation to environmental variation, both biotic (i.e. differential selective pressures imposed by phytophagous organisms) and abiotic (i.e., environmental gradients in abiotic factors along the species distribution range). This variation is translated into notable inter- and intraspecific differences in the susceptibility to many particular insect herbivores (Table 11.3). Investment in defensive traits and resistance to specific herbivores is also largely determined by plastic responses to the abiotic and biotic environmental conditions (e.g. Lombardero et al. 2000), the ecological context in which the host trees are immersed (e.g. Castagneyrol et al. 2014) and complex interactions with other symbiotic, mutualistic and higher-trophic level organisms (e.g. Giffard et al. 2012; Kanekar et al. 2018). Understanding how genetic and plastic responses modulate defensive investment in long-lived species such as pine trees is required to forecast their future performance, particularly in the current context of environmental change. In the following sections we review current knowledge on the sources of variation of defensive traits and effective resistance against particular enemies in Mediterranean pines.

11.5.1 Genetic Variation

Mediterranean pines show several evolutionary particularities (Grivet et al. 2013; see also Grivet and Olsson [this volume](#), Chap. 1). For instance, since pine species, as other trees, display lower mutation rates per unit of time than other plants, low intraspecific genetic variation within populations might be expected (Petit and Hampe 2006). However, within-population variation is high for many quantitative traits (Zas et al. 2005; Hernandez-Serrano et al. 2014) probably favored by significant among-population gene flow in these outcrossing species (De-Lucas et al. 2008). Such intra-population variation ultimately allows for rapid local adaptation processes to environmental change (Grivet et al. 2017). Indeed, Mediterranean pine

Table 11.2 Studies reporting inter and intraspecific genetic variation in physical and chemical defenses of Mediterranean pine species

Species	Genetic variation	Trait	Reported pattern	References
<i>Pinus</i> spp.	Among species	Terpenes	Wide genetic variation in chemical composition and concentration	Rodrigues et al. (2017)
		Phenolics	Wide genetic variation in chemical composition and concentration	Kaundun et al. (1997) and Pimentel et al. (2017)
<i>P. pinaster</i>	Among populations	Terpenes	Constitutive monoterpenes and diterpenes: $Q_{ST} = F_{ST}$	López-Goldar et al. (2019)
			Constitutive sesquiterpenes: $Q_{ST} > F_{ST}$	
		Phenolics	Constitutive phenolic groups: $Q_{ST} = F_{ST}$	López-Goldar et al. (2019)
			Constitutive hydroxycinnamic acids: $Q_{ST} < F_{ST}$	
	Inducibility of eugenols			
	Resin canals	Wide genetic variation among populations for constitutive resin duct features, but not for inducibility	Zas et al. (2015) and Vázquez-González et al. (2019)	
	Among families	Terpenes	Constitutive diterpenes	López-Goldar et al. (2019)
		Phenolics	Constitutive hydroxycinnamic acids and lignans	López-Goldar et al. (2019)
		Non-volatile resin	Genetic variation in constitutive and MJ-induced defenses	Sampedro et al. (2011a)
		Total phenolics	Genetic variation in constitutive and MJ-induced defenses	Sampedro et al. (2011a)
		Lignin	Additive genetic variation ($h^2 = 0.34$)	Gaspar et al. (2011)
		Resin canals	Wide genetic variation among families in inducibility, but not in constitutive resin duct features.	Moreira et al. (2015)
		Growth (tolerance)	Strong compensatory growth and large genetic variation in tolerance after herbivory by a stem-chewer insect.	Sampedro et al. (2009) and Zas et al. (2011)

(continued)

Table 11.2 (continued)

Species	Genetic variation	Trait	Reported pattern	References
<i>P. halepensis</i>	Among populations	Terpenes	Three differentiated provenance clusters based on chemotypic variation	Djerrad et al. (2015)
		Flavonoids	Wide genetic variation, grouped into three distinct clusters.	Kaundun et al. (1998b)
	Among and within families	Resin flow	Large inter- and intrafamilial variation Among families: $h^2 = 0.65$ Within families $h^2 = 0.84$	Moulalis (1991)
<i>P. brutia</i>	Among populations	Terpenes	Wide genetic variation showing geographic clustering	Schiller and Grunwald (1987) and Schiller and Genezi (1993)
		Flavonoids	Wide genetic variation showing geographic clustering	Kaundun et al. (1998a)
<i>P. pinea</i>	Among populations	Terpenes	Wide genetic variation in volatile terpenes. Limonene and α -pinene as dominant metabolites	Nasri et al. (2011)

Q_{ST} = Genetic differentiation among populations displayed in quantitative traits. F_{ST} = Differentiation among populations measured on neutral loci. h^2 = narrow sense heritability, or additive variance contributing to total phenotypic variance. MJ = Methyl Jasmonate, hormone implicated in damage signaling and the production of induced defenses

species are strongly genetically differentiated among populations (Gómez et al. 2005) including large quantitative differences in almost every life-history trait (e.g. Santos-Del-Blanco et al. 2012; Voltas et al. 2015), resulting from both neutral and adaptive evolutionary processes.

Defensive traits are not an exception and are also highly variable among and within Mediterranean pine populations (Table 11.2). Intraspecific variation in defensive traits is reflected in significant differences among populations and families within populations in effective resistance to specific herbivores, although the available information about this is scarce (Table 11.3). Population differentiation in plant secondary metabolites, for example, is particularly acute in species such as maritime pine, with highly fragmented and isolated populations (Meijón et al. 2016; López-Goldar et al. 2019). The singular demographic history of Mediterranean pine species with several independent postglacial refugia suffering relevant bottle-necks, and posterior migration routes from such refugia (Petit et al. 2003) has surely contributed to this marked population differentiation (López-Goldar et al. 2019; Vázquez-González et al. 2019). Furthermore, genetic adaptation to differential selection pressures imposed by the great environmental heterogeneity within the natural distribution range of Mediterranean pines is also assumed to explain the

Table 11.3 Studies reporting among or within species variation in susceptibility of Mediterranean pines to diverse insect herbivores

Insect herbivore/ pine species	Genetic level	Outcome	References
<i>Hylobius abietis</i>			
<i>P. pinaster</i>	Families	Additive variation among 40 half-sibs in susceptibility	Zas et al. (2005)
<i>P. pinaster</i>	Populations	Significant variation in susceptibility among 10 pine populations	López-Goldar et al. (2018)
<i>P. pinaster</i>	Populations	Significant variation in susceptibility among 3 pine populations	Suarez-Vidal et al. (2017)
<i>P. halepensis</i>	Populations	No significant variation in susceptibility among 3 pine populations	Suarez-Vidal et al. (2019)
<i>Matsucoccus feytaudi</i>			
<i>P. pinaster</i>	Populations	Significant variation in density of nymphs among 8 pine populations	Di Matteo and Voltas (2016)
<i>P. pinaster</i>	Populations	Significant variation in insect density and symptoms among 8 pine populations	Schvester and Ughetto (1986)
<i>P. pinaster</i>	Populations	Significant variation in symptoms among 25 pine populations	Harfouche et al. (1995)
<i>Thaumatopoea pytiocampa</i>			
<i>P. halepensis</i>	Populations	Variation in susceptibility among 57 pine populations	Sbay and Zas (2018)
	Species	<i>P. halepensis</i> more attacked than <i>P. brutia</i>	Sbay and Zas (2018)
<i>P. halepensis</i>	Populations	Variation in susceptibility among 57 pine populations	Bariteau and Pommery (1992)
	Species	Differences in oviposition and larvae development on different hosts	Hodar et al. (2002)
	Species	Oviposition deterrence: <i>P. pinea</i> > <i>P. halepensis</i> > <i>P. pinaster</i> > <i>P. brutia</i>	Paiva et al. (2011)
	Species	Larval mortality: <i>P. pinea</i> > <i>P. brutia</i> > <i>P. halepensis</i> > <i>P. pinaster</i>	Schopf and Avtzis (1987)
<i>Thaumatopoea wilkinsoni</i>			
	Species	Susceptibility variation: <i>P. eldarica</i> > <i>P. brutia</i> > <i>P. halepensis</i>	Mendel (1988)
<i>Monochamus galloprovincialis</i>			
	Species	Differences among species in insect feeding and oviposition	Sanchez-Husillos et al. (2013)
	Species	Differences among species in insect feeding and oviposition	Naves et al. (2006)
<i>Dyorictria sylvestrella</i>			
<i>P. pinaster</i>	Families	Significant differences in resistance among full-sibs	Jactel et al. (1999)
<i>P. pinaster</i>	Families	Significant differences in resistance among full-sibs	Kleinhentz et al. (1998)

observed genetic differentiation among populations in defensive traits (Elvira-Recuenco et al. 2014). Both the biotic environment (e. g., variation in the herbivorous insect communities and the frequency of insect outbreaks) and the pronounced heterogeneity in the abiotic environment across the range of pine species are expected to have shaped defensive strategies and traits across populations (Serravarela et al. 2015; Di Matteo and Voltas 2016).

An outstanding example of how variation in defensive traits may be associated with adaptive responses to differences in the biotic environment is the variation among maritime pine populations in their susceptibility to the bark scale *M. feytaudi*. Although factors related to the invasion process such as founder effects or lack of natural enemies in the new range (Jactel et al. 2006) can also explain variation patterns, common garden tests have demonstrated that pine populations genetically differ in their susceptibility to the insect (Schvester and Ughetto 1986; Harfouche et al. 1995; Di Matteo and Voltas 2016). Western populations that have coexisted with the insect are much more resistant than eastern populations that lack efficient resistance mechanisms due to the absence of a co-evolutionary history with the insect.

Intraspecific variation in defensive traits may also result from adaptations to abiotic environmental conditions and trade-offs with other life history traits. For example, some studies have reported that population variation in both chemical and anatomical pine defenses follows climatic clines, suggesting adaptive processes related to the abiotic environment (López-Goldar et al. 2019; Vázquez-González et al. 2019). However, rather than acting through direct evolutionary responses to climate in defensive-related traits, these adaptive processes likely act indirectly through links with other life functions such as growth, reproduction or tolerance to abiotic factors (see Sect. 11.4).

Population differentiation processes are typically associated with a reduction of variation within populations. However, intra-population genetic variation in defensive traits in Mediterranean pines is commonly very high, even higher than that among populations (Tables 11.2 and 11.3). High rates of gene flow among populations, high environmental heterogeneity within population ranges, and genetic correlations with other characters are likely contributing to maintaining such wide variation. Intra-population variation constitutes the fuel of evolutionary change where natural selection operates (Petit and Hampe 2006) and the basis for breeding programs aimed at improving resistance to biotic threats (Woodcock et al. 2018). Although heritability of some defensive traits may be notably high (Rosner and Hannrup 2004), resistance to insect herbivores typically shows low heritability estimates (Kleinhentz et al. 1998; Zas et al. 2005). However, the large phenotypic variation in defensive traits results in high additive coefficients of variation, allowing for rapid and strong evolutionary responses to natural or artificial selection (Snieszko and Koch 2017).

Evidence suggests that variation in defense production may be, at least in part, genetically determined in Mediterranean pine species. Future research should, however, deepen into the genetic and evolutionary basis of effective resistance and tolerance against key pests and associated defensive mechanisms. Such knowledge

would allow us to forecast the evolutionary responses of pine tree populations and create adaptive forest management programs for tackling the negative impacts of increasing biotic stresses associated with global change in the Mediterranean region.

11.5.2 Environmental Plasticity

Pine trees, like other plants, are exposed to constant environmental fluctuations in both abiotic and biotic factors. Particularly, abiotic factors such as soil nutrients, quality and amount of light, water availability and pollutants drive primary production but may also directly and indirectly affect defensive allocation. At the same time, pine trees host a large and diverse community of antagonistic and mutualistic organisms. Importantly, both biotic and abiotic factors vary in space and time, and thus pine trees must finely integrate all the environmental signals to enhance their fitness. Energy allocation must be therefore readjusted with respect to the balance between the costs and benefits of defensive allocation under a specific environmental context (Herms and Mattson 1992). Accordingly, plastic responses to both biotic and abiotic conditions are expected to play a key role in the optimal performance of pine populations. Plastic responses to biotic factors are covered in Sect. 11.3.3.

Studies exploring plastic responses to abiotic factors in Mediterranean pines often assess the effects of nutrient availability and drought under experimental conditions. For instance, higher nutrient availability has been shown to negatively affect the production of both chemical and anatomical defenses in *P. pinaster*, probably due to changes in resource allocation priorities for growth (Moreira et al. 2008, 2015; Sampedro et al. 2011a). Furthermore, reduced water availability treatments have been reported to constraint the inducibility of chemical defenses in *P. halepensis* (Suarez-Vidal et al. 2019). Similarly, light deprivation has also been shown to reduce the inducibility of chemical defenses in saplings of *P. pinaster* (Suárez-Vidal et al. 2017). It is also known that evapotranspiration rates and other climate-related variables affect resin yield and production of resin ducts (i.e. anatomical defenses) in *P. pinaster* (Rodríguez-García et al. 2015). There is a considerable knowledge gap, however, regarding the effect of environmental conditions, particularly climate, on defense production in mature trees growing under natural conditions.

Climate change scenarios anticipate considerably harsh conditions for Mediterranean regions in the near future, including increased atmospheric temperature and more extreme drought periods (IPCC 2014). Plasticity in functional traits may facilitate the persistence of locally adapted forest tree populations by buffering the negative impacts of shifting climate conditions and allowing range expansions (Valladares et al. 2014). Knowledge on how climate modulates defensive allocation in Mediterranean pine species is therefore needed in order to anticipate population responses to both increased biotic and abiotic stresses. As referenced by the literature cited above, there is some experimental evidence to expect that drought may negatively affect defensive investment in Mediterranean pine species (Suarez-Vidal et al. 2019). Further studies should therefore focus on disentangling the effect of

different climate factors on anatomical and chemical defensive traits in mature trees growing under natural environmental conditions. For instance, climatic response functions widely applied in dendrochronological studies have facilitated the accumulation of an extensive body of knowledge on how climatic constraints affect growth patterns in conifer species (Andreu et al. 2007; Tardif et al. 2003). In a similar way, tree-ring analysis could be applied to resin-based defenses in pine trees, particularly to axial resin ducts recorded in annual growth rings, to clarify the role of particular climate variables in defensive investment. This approach has already been applied to different Northern-American pine species demonstrating the sensitivity of axial resin ducts to temperature and precipitation (Saracino et al. 2017; Slack et al. 2017). Improving our understanding of which climate variables have the most significant effect on defensive investment will improve our ability to forecast the future responses of pine populations and the potential impacts of global warming on Mediterranean forests.

11.5.3 Other Sources of Variation

It is known that the environmental conditions experienced by parent plants may determine, at least in part, the phenotype of their progeny (Holeski et al. 2012). Such transgenerational plastic responses to the maternal environment are transmitted to the offspring phenotype without any change in the DNA sequence, and thus constitute a layer of phenotypic variation over the genomic variation. This kind of maternal environmental effect can influence the evolutionary processes and population dynamics of plant species. It has been showed that the abiotic environment experienced by mother maritime pine trees may modify germination rate, early growth patterns and resistance to root rot fungi in their progeny (Vivas et al. 2013; Zas et al. 2013; Zas and Sampedro 2015). Whether the biotic environment experienced by mother trees may modify the resistance of the progeny against insect herbivores remains unexplored in pine trees. This kind of transgenerational priming could generate powerful and relevant applications in forest management.

As discussed above (Sect. 11.5.2), differences in resource availability (light, nutrients, water) can modulate allocation to defenses, and interact with the expression of induced defenses and their costs. Heterogeneity in resource availability usually follows non-random spatial patterns in nature. Moreover, biotic interactions are usually spatially aggregated, and individuals in close proximity are more likely to share the same herbivore loadings, mycorrhizal partners, pathogen risks and exposure to VOCs. In summary, it is more likely that neighboring trees share the same biotic interactions and resource availability. Even when genetic relatedness could be expected to also show some degree of spatial aggregation, neighboring trees may express a more similar defensive phenotype than expected from their genetic relatedness. In general, we can say that biotic interactions have been overlooked as a source of spatial autocorrelation, and thus biotic interactions and their effects on the plant phenotype should be considered in a spatially explicit context.

Similarly, the composition of a given plant community may alter the relative likelihood of a focal plant species to be attacked by a given herbivore (Barbosa et al. 2009). Factors determining the associational benefits include plant community diversity, the presence or abundance of a given species, patch size, structural composition of the plant community determining habitat type, and the nature of plant antagonists such as specialist/generalist, insect/mammal, feeding guild, diet breadth, etc. Associational resistance and associational susceptibility effects could spatially alter the pressure exerted by herbivores and thus the spatial distribution of plant phenotypes.

11.6 Concluding Remarks

The ecological and genetic bases of resistance to herbivore insects in Mediterranean pines generally match those of more studied model plants. However, there are some particularities. Mediterranean pines are natural or semi-natural populations of long-lived plants; they show large genetic diversity in functional and molecular traits, even when their particular demographic history of regression, recolonization and isolation has been greatly influenced by evolution; they live in a hotspot of diversity, but also in a hotspot of global change, on one hand subjected to range expansion of alien and native pests, pathogens and other plants, and on the other hand challenged by rapid climate change in the Mediterranean region. Combining the advances in model plants in the last decade in a translational research effort with the particular life history characteristics of Mediterranean pines will allow us to unravel the patterns of resistance and tolerance to multiple pests and pathogens in these species. This knowledge will ultimately help to efficiently manage forest genetic diversity in resistance traits under a changing environment and to forecast how species and populations will face the current challenges.

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