Chapter 15
Methyl Jasmonate as Chemical Elicitor of Induced Responses and Anti-Herbivory Resistance in Young Conifer Trees

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Abstract Exogenous application of phytohormones such as methyl jasmonate (MJ) can induce chemical and anatomical changes that lead to a reduction in insect herbivory and disease incidence in herbaceous and woody plants. However, exogenous application of MJ also produces notable disadvantages in terms of plant growth and physiology. In this chapter we review current published literature about the effects of exogenous application of MJ in defence responses and herbivory resistance of young conifer trees, as well as their implications for plant growth and physiology. Moreover, we proposed a series of recommendations for the use of MJ as chemical elicitor in young conifer trees.

15.1 Introduction

It is broadly recognized that the evolution of plants has been linked to the pressure exerted by their herbivores, and ever since plants appeared on land insects have been their most harmful herbivores. Forest trees are large, long-lived plants that are particularly exposed to herbivory [1], and insect pests are seen as a great threat for many types of temperate woodland owing to the large amounts of plant tissues they consume. Moreover, contemporary factors including global warming, the movement of genetic material and forest products, the decrease of genetic diversity in breeding

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programs and in planted forests and the difficulties of applying intensive control methods are combining to seriously increase the risk of forest pest damages [2]. As an illustrative example, about half of the area forested with ponderosa pine in British Columbia is now being destroyed by the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) and it has been predicted that around 80% of the province's pine volume will be killed by the time the infestation subsides [3]. This huge devastation potential of forest pests could lead to dangerous ecological and social consequences, since coniferous forests are of fundamental importance for both the biodiversity they support and for the environmental, social and ecological services they provide to humanity. Coniferous forests are, for example, currently the most significant net C sinks in the Earth, containing more than one-third of all carbon stored in terrestrial ecosystems [4]. Furthermore, coniferous forests are also highly valued from an economic point of view, as most of the conifer species are intensively used for timber, fuelwood, resins and other purposes [5].

During millions of years of co-existence with insect herbivores, conifer trees have evolved potent and effective mechanisms for defending themselves [6]. These mechanisms include structural, morphological or physical defences, such as resin canals, calcium oxalate structures, sclereid cells and lignin, and chemical defences based on the production of secondary chemical compounds [7]. Both physical and chemical defences are classified as either constitutive, preformed defences, which are always expressed in the plants, or induced defences that are synthesized or mobilized when the plant is injured.

A chemical defence of particular importance to conifers is the oleoresin, composed by a complex mixture of terpenoids. Monoterpenes (C_{10}) and sesquiterpenes (C_{15}) constitute the volatile fraction of oleoresin, and diterpene resin acids (C_{20}) are the main constituents of the non-volatile fraction [8]. Conifers produce and store copious amounts of resin in specialized anatomical structures such as resin ducts, resin blisters, or resin cells in stems, roots and needles [9]. The network of preformed resin ducts in the phloem is often the first defensive element encountered by organisms invading conifers. Resin fluxing from the storage sites out of injured resin ducts is a sticky physical barrier, and terpenes in the resin are highly toxic for insects and fungi [8].

Phenolic compounds are the other major constitutive defence in conifers. Phenolics are abundant in the phloem of all conifer species, especially in the polyphenolic parenchyma cells (PP cells) that are specialized in the synthesis and storage of phenolic compounds [7], but also appear in needle tissues [10]. Phenolic compounds are a complex group of diverse substances with diverse functions in plant physiology, but some types of phenolic are known to be active against a diverse array of herbivores and pathogens. Their effectiveness arises through a variety of mechanisms, such as the inhibition of insect digestive proteins or their toxicity to insects and fungi [11, 12].

A further group of chemicals involved in conifer defences are the alkaloids: a heterogeneous group of compounds with an organic base containing a nitrogen atom, most of them act as feeding deterrents and/or toxins to most insect herbivores

Fig. 15.1 Xylem cross sections from stems of 1 year-old *Pinus pinaster* seedlings treated with (**a**) 0 mM (control) and (**b**) 22 mM MJ, showing traumatic resin ducts in the xylem
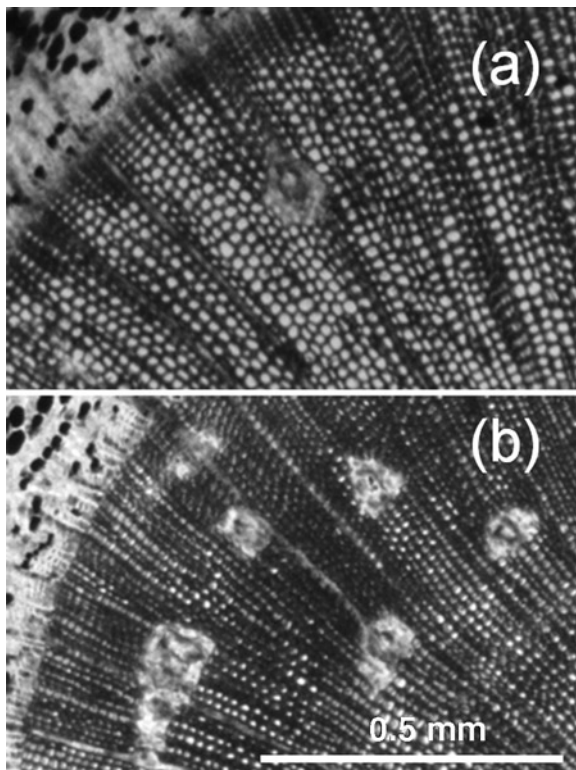
[13]. Alkaloids are restricted to some taxa within conifers, while terpenoids and defensive phenolic compounds are extensively found in gymnosperms [14].

Mechanical and chemical constitutive defences usually require large amounts of resources to be synthesized, and they are produced during the regular growth of conifers. On the other hand, the production of induced defences in response to herbivory is considered to be a cost-saving strategy since defences are only deployed when protection is needed and is thus less energy demanding [15]. Induced defences in conifers include several morphological and physiological changes, such as enhanced resin flow and traumatic resin duct formation [16, 17], swelling and proliferation of polyphenolic parenchyma cells in the bark [18, 19], and some qualitative changes and increased production of phenolic compounds [20] and terpenoids [21]. Differentiation and development of traumatic resin canals (TRC) in the secondary xylem of conifers begins soon after herbivory damage [22]. These resin canals are formed in the newly growing rings where they appear in one or two tangential lines within the annual-ring (Fig. 15.1). The formation of traumatic resin canals markedly increases the resin duct density in the xylem and thus the potential resin flow when the wood of conifer trees is injured [16]. Besides oleoresin,

chemical components that are more toxic to insect herbivores may also be present in TRC [16, 23].

Induced defences are usually produced in response to the damage or stress caused by biotic and abiotic factors, especially by insect herbivores and plant pathogens (see revision by Eyles et al. [24] on trees). During recent decades, several plant phytohormones such as jasmonic acid (JA), ethylene or salicylic acid have been discovered to be implied in the pathways of defence signalling and in the synthesis of chemical defences [25, 26]. These phytohormones also play regulatory roles in other aspects of plant physiology, but they have been found to be especially responsive to biotic aggressions. In particular, the methyl jasmonate pathway has been found to be intimately related to the wounding response to defoliating caterpillars, budworms and bark beetles [27–29]. Thus, exogenous application of some of these compounds can be used as chemical elicitors in order to study the nature of induced responses against herbivory and the environmental factors that determine them, in manipulative experiments in conifer trees.

Jasmonic acid is known to be involved in the expression of defence genes induced by wounding insects, and its production is regulated by the octadecanoid pathway (reviewed by Koo and Howe [29]). Jasmonic acid is synthesized from α-linolenic acid, which can be released from the cell membranes of injured tissues. Following several steps mediated by lipoxygenase and ciclase enzymes, α-linolenic acid is transformed into 12-oxo-phytodienoic acid. This last compound undergoes reduction and beta oxidation to form JA. The methyl ester of JA, methyl jasmonate (MJ), is the most commonly studied elicitor of induced defences against insect and pathogen attacks, in everything from annual to woody plants [30, 31]. In particular, over the last decade, several authors have investigated the role of MJ in wound-induced defence production and in the herbivory resistance of young conifer trees [21, 31–33]. Resistance to herbivory during the initial stages of a conifer's life is extremely important since insect herbivores are a major cause of early seedling mortality in coniferous stands. As an illustrative example, damage by the pine weevil *Hylobius abietis* L. often causes up to 60–80% of seedling mortality in young conifer stands in Europe during the first years after establishment [34].

In recent years, Holopainen and their collaborators have reviewed the potential use of several exogenous elicitors (including MJ) in the pest management of conifer seedlings, and their implications for plant growth and development [35]. The use of chemical elicitors for seedling protection against arthropod herbivores and pathogens appears attractive due to the low ecological risks associated [35]; it does, however, bring considerable disadvantages in terms of negative impacts on plant growth, reproduction and physiology [35].

In this chapter we review the role of the exogenous application of MJ in defence responses and herbivory resistance of young conifer trees, as well as the implications for plant growth and physiology. We also focus on the methodological approaches for the exogenous application of MJ in studies of induced resistance in young conifer trees (<5 years), as an easy, practical and operative means to elicit responses similar to those caused by real herbivory.

15.2   Activation of Young Conifer Defences After Exogenous Application of MJ

15.2.1   Chemical Defences

It is broadly accepted that exogenous application of MJ elicits the pathways of biosynthesis of chemical defences in young conifer trees, involving both resin- and phenolic-based defences. Exogenous application of MJ has direct consequences in the synthesis of resin terpenoids. Since early works by Phillips and Croteau [8] and Trapp and Croteau [9], some of the huge advances made in recent years in the knowledge of the pathways of resin biosynthesis in conifers were aided by the use MJ as chemical elicitor of induced responses. Nowadays, it is known that MJ increases the transcript levels, the enzyme concentration and the enzymatic activity of several terpene synthases and monoxygenases involved in the biosynthesis of mono-, sesqui- and diterpenes in conifer trees [36–39]. Although not identical, these MJ-induced responses were equivalent to those elicited after mechanical wounding and real insect attack, at the transcript level [37]. Quantitative accumulation of terpenoids in young conifer trees induced by MJ treatment has also been reported [32, 40–42]. Interestingly, a 12-fold increase in total monoterpene concentration and a 38-fold increase in total diterpene concentration were observed in spruce saplings treated with 10 mM MJ, when compared with control plants [40]. The effects of MJ application on the accumulation of terpenoids in needles are, however, not as clear as they are in stem tissues. Whereas the concentration of diterpene acids in the needles of young conifers has been reported to increase after MJ application [32, 41], no changes or only minor alterations in mono and sesquiterpenoids were reported in maritime pines [43], Douglas-fir [44], Scots pine [32] and spruce [42], when compared with other tissues of those plants such as stem wood and roots. These results suggest that the response to MJ application in terms of terpenoid chemistry in leaves could differ from that observed in stems or roots, and depends on the chemical fraction under consideration.

Exogenous application of MJ also has a relevant effect on the phenylpropanoid pathway leading to the synthesis of phenolic compounds in conifer plants, although at present this area is little understood compared with our knowledge of terpenoids. Wounding and MJ application are known to induce the transcript accumulation of the enzyme chalcone synthase in needles, which is involved in the first steps of flavonoid biosynthesis in pine trees [45]. Foliar MJ application also notably increased the concentration of phenolic compounds in the needles of maritime [46] and Monterrey (Moreira et al. unpublished data) pine seedlings.

Finally, exogenous application of MJ also stimulates the synthesis of other defensive compounds such us pathogenesis-related (PR) proteins [47–49]. PR proteins include chitinases, peroxidases, proteinase inhibitors and several other proteins. Local wounding and MJ application similarly induced the expression of PR proteins in *P. monticola* [47, 49] and *Picea glauca* seedlings [48].

15.2.2   Anatomical Defences

That exogenous application of MJ provokes anatomical changes in young trees of several conifer species has been known since the early work carried out with MJ and conifers by Franceschi and collaborators at the beginning of the last decade [50–52]. Reported anatomical changes included the formation of additional traumatic resin ducts in the xylem and additional phenolic deposition in PP cells in the phloem. In a convincing study with seedlings belonging to twelve conifer species, Hudgins et al. [51] reported that the application of 100 mM MJ induced PP cell activation in all the studied species and xylem traumatic resin duct formation and cell wall lignification in most of them. Similarly, exogenous application of MJ induced the formation of traumatic resin ducts in the xylem of young Douglas-fir [44, 53], giant redwood [53], Scots pine [32] and Norway spruce [54] trees. Wounding and MJ application were reported to induce early lignification of phloem fibres and the accumulation of transcripts of cynnamyl alcohol dehydrogenase, an enzyme involved in the lignification of the cell wall in the xylem of *P. taeda* trees [55].

15.3   Changes in Volatile Organic Compounds Induced by MJ

Exogenous application of MJ was found to have a significant effect on the amount of volatile organic compounds (VOCs, mono- and sesquiterpenes) emitted from needles of young conifer trees, increasing their emission between 1 and 3 days after MJ treatment [41, 42]. These results have a great relevance from an ecological point of view since VOCs emitted from injured tissues are airborne molecular messengers involved in plant-animal, animal-animal and plant-plant signalling [8, 56, 57]. Thus, VOCs could simultaneously act as herbivore repellent and as olfactory clues for herbivore host selection [58], as well as attracting herbivore predators and parasitoids [10, 59].

15.4   Effects of MJ on Resistance to Insect Herbivores and Plant Pathogens

The defensive responses to MJ application have been commonly associated with enhanced resistance to biotic damage in young conifer trees. In one of the first studies relating to this, [60] observed that fumigation with a volatile MJ treatment (25 µl 100 l^{-1} air) protected Norway spruce seedlings against the plant pathogen *Pythium ultimum* Trow. (Pythiales: Pythiaceae). These authors concluded that MJ increased seedling resistance by stimulating the accumulation of salicylic acid in all the parts of the seedlings. Similarly, application of different MJ concentrations significantly decreased the disease incidence of the pathogen *Diplodia pinea* (Desm.) Kickx. (also named *Sphaeropsis sapinea*, Sphaeropsidales: Sphaeropsidaceae) in *P. radiata* seedlings

[33, 61]. In contrast with these results, soil application of MJ in field-grown *P. radiata* seedlings did not reduce root rot incidence by *Phytophthora cactorum* (Lebert & Conh) Schröeter (Pythiales: Pythiaceae) compared with the untreated controls [62].

MJ application can also protect conifer seedlings against pest arthropods. For example, the exogenous application of MJ over the foliage significantly increased the resistance of pine seedlings against the phloem-feeder *Hylobius abietis* L. (Coleoptera: Curculionidae), both in a cafeteria experiment (*in vitro* feeding test) [31] and in living plants (*in vivo* feeding test) [21, 32]. Some of these authors observed significant increases in the concentration of stem resin after MJ application, and suggested a direct link between the increase in resin and the observed enhanced resistance [21, 31]. The ingestion of needles by the pine processionary moth *Thaumetopoea pityocampa* Schiff. (Lepidoptera: Thaumetopoeidae) was also significantly reduced by exogenous application of MJ (Moreira et al. unpublished data). Specifically, larvae consumed 10% more needles in the control plants than in MJ-treated plants. The increase in the concentration of total phenolics in the needles after MJ application was suggested as the main factor responsible for the increased resistance against the pine processionary moth.

15.5 Effects of MJ Application on Growth, Primary Metabolism and Physiology

In recent years, exogenous application of MJ to conifer seedlings in forest nurseries has been increasingly proposed as a potential means of protecting seedlings against forest pests [44]. Enhancing plant resistance through the activation of induced responses appears attractive as an environmental friendly tool in the fight against pests and diseases in both agricultural crops and forests. However, this proposal has an important weak point in that the induction of plant defences is beneficial and increases plant fitness when the plant enemies are present, but induced defences are costly for plants to produce and maintain. In the absence of damage, the activation of induced defences could negatively affect plant fitness, as they divert resources that could be used instead for growth, development or reproduction [15]. Indeed, the exogenous application of MJ to conifer seedlings has been found to lead to a decline in growth in many conifer species in numerous studies [21, 32, 33, 61–63]. Remarkable reductions in height and diameter growth [21, 32, 61], as well as shoot [32, 61] and root [32] biomass were observed a few weeks following MJ treatment in pine seedlings. As small seedlings may suffer serious problems of competition with herbaceous forbs and grasses, so MJ application can in fact drastically reduce seedling survival. Exogenous application of MJ can also negatively influence other plant physiological functions. For example, large decreases in photosynthetic rate of pine seedlings have been observed after foliar application of MJ [32, 33, 61]. Many of these authors also observed decreases in needle water potential and transpiration rate in seedlings treated with MJ [33, 61]. Foliar application of MJ was also reported to depress starch reserves in the stem of pine seedlings [46].
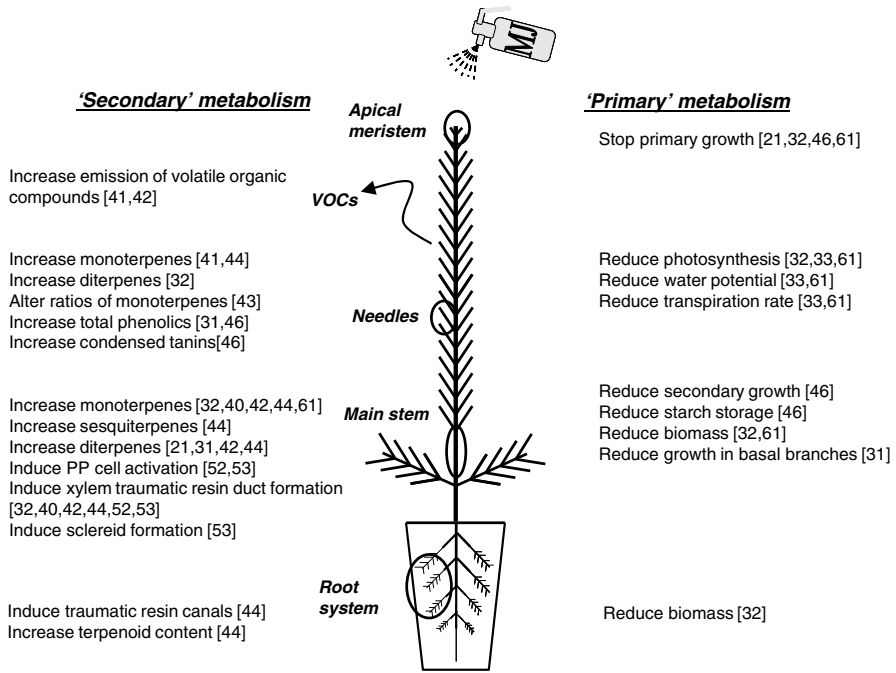
Fig. 15.2 Summary of the reported effects of MJ application on primary and secondary metabolism of young conifer trees. Reference numbers within brackets

Finally, exogenous application of MJ in *P. pinaster* seedlings can also have relevant effects on C and nutrient reallocation. Increased nutrient concentrations in the shoots along with unchanged or reduced concentrations in the roots have been observed after spraying MJ over the foliage of maritime pine seedlings (Moreira et al. unpublished data). We also observed that MJ application promoted a marked reduction in aboveground biomass but a strong enhancement of fine roots. Both induced responses were likely short-term induced tolerance responses to simulated herbivory, that would likely help to reduce the impact of herbivore damage on plant fitness. Effect of exogenous MJ is summarised in Fig. 15.2.

15.6 Methodological Issues When Using MJ as Experimental Elicitor of Induced Defences

15.6.1 Effective Concentration and Method of Application

The effects of MJ application strongly depend on the concentration of MJ in the applied solution, but seedling age and physiological status are also relevant. For example, exogenous application of 100 mM MJ notably increased terpenoid concen-

tration in the stem of pine seedlings, but no significant differences were observed between the effects of the application of lower concentrations of MJ and untreated controls in 2 year old seedlings [31, 32]. In 1 year old seedlings, however, the application of 22 mM MJ produced significant effects on quantitative defences, growth [46] and carbon and nutrient allocation (Moreira et al. unpublished data). The induction of anatomical defences has also been reported to be MJ dose-dependent. For example, traumatic resin duct size of young giant redwood and Douglas fir trees notably increased with increasing MJ concentration [53]. In contrast with these results, Martin et al. [40] observed lower monoterpene and diterpene content in the wood of young spruces treated with 100 mM MJ as compared with those treated with 10 mM MJ.

Enhancement of the effective resistance against pest and pathogens is also MJ dose-dependent, although the relation is not always linear. For example, one application of either 1.0 or 4.5 mM MJ promoted resistance to *D. pinea* in *P. radiata* seedlings, whereas a concentration of 18 mM MJ did not reduce the incidence of the plant pathogen [61]. Adults of *H. abietis* consumed 80% less phloem in 100 mM MJ treated seedlings than in control *P. pinaster* seedlings, and 5 mM and 50 mM MJ treatments produced intermediate values [31]. More surprisingly, young spruce seedlings were reported to be more susceptible to *P. ultimum* infection at higher concentrations of gaseous MJ [60].

Exogenous application of MJ at high concentrations could result in local phytotoxicity for plants. For example, Moreira et al. [31] observed that although total phenolic concentration in the needles of 5 mM MJ-treated *P. pinaster* seedlings was slightly greater than in control plants, phenolic concentration significantly dropped in seedlings treated with 100 mM MJ concentration, which showed visual signs of stress or toxicity in the form of chlorotic or dead needles. Similarly, phytotoxicity symptoms or plant mortality were observed in pine seedlings treated with high MJ concentrations [32, 61].

Treatments of MJ in young conifer trees are usually applied by spraying over the foliage with a handheld sprayer [32, 33, 40–43, 61]. This method of MJ application showed positive results in the context of induction of chemical and anatomical defences [31, 32, 40, 41] and herbivory resistance [21, 31–33, 61]. However, alternative methods for applying MJ treatments have been also tested [44, 51, 53, 60]. In one study, soil irrigation of MJ increased the production of terpenoids in the needles, stem and roots of Douglas fir seedlings [44], while in other experiments the application of MJ to the stem with a small brush induced xylem traumatic resin duct formation and PP cell activation in several conifer species [51, 53]. Finally, application of MJ to young spruces using cotton wool to allow evaporation increased the resistance against the pathogen *P. ultimum* [60].

Polyoxyethylene-20-sorbitan monolaurate (commercially also known as Tween-20®) is commonly used as surfactant which favours the suspension of MJ in water, and control treatment in experiments evaluating MJ-induced responses are therefore frequently sprayed with a Tween-20 solution [31, 32, 44, 51, 53, 61]. Recent studies in conifer seedlings have reported, however, significant induced responses in control plants treated with Tween-20 treatment [31, 42]. For example, application of 0.1% Tween-20 to *Picea sitchensis* Bong

seedlings increased the concentration of diterpenes and sesquiterpenes in the outer stem tissue with respect to the untreated control [42]. Similarly, resin content in *P. pinaster* branches treated with Tween-20 was higher than that in untreated control branches [31]. Alternatives to the use of Tween-20 for dissolving MJ without interfering in plant defences do exist [40, 41, 43]. For example, Sampedro et al. [43] used a carrier solution based on deionized water with 2.5% ethanol (v:v), while other authors did not use any surfactant to dissolve MJ [40, 41].

15.6.2 Activation Time, Decay Time and Systemic Effect

Like other induced responses, plant defensive responses to MJ application require a threshold time to be expressed (activation time), and continue for a period of time until defensive levels relax back to their initial state (decay time) [64]. Specifically, maximum accumulation of monoterpenes and sesquiterpenes in the stem and needles of young spruces treated with MJ occurs around 10–15 days after MJ application and then progressively declines back to control levels in around 25–35 days [40, 41]. Similarly, the concentration of α-pinene and β-pinene in the stems of *P. radiata* seedlings increased 7 and 14 days after MJ application [61]. Contrastingly, the effect of MJ on the emission of volatile terpenes from the needles is much faster. In spruces, the emission peak occurs just a few hours after MJ application (around 45–57 h after MJ treatment in Sitka spruce and between 24 and 30 h in Norway spruce) and remained high up to 4–7 days after treatment [41, 42]. The activation time and decay time appears thus to depend on the defensive trait considered. The relatively short decay time of the responses to MJ application may explain why the protection of *P. radiata* seedlings to *D. pinea* infection through MJ application is only effective during the first 2 weeks after treatment [33]. All these results highlight the great relevance of the time course of MJ responses, which should be taken into account in any use of MJ as a simulator of real herbivory.

Many studies on mature conifer trees have demonstrated that MJ can elicit a response in untreated tissues above or below the treated area [50, 52]. For example, the exogenous application of 100 mM MJ to the bark of 30-year-old Norway spruce induced the formation of traumatic resin ducts some distance away from its application site [50]. These authors also found that the greater the distance from the application area, the smaller the traumatic resin ducts were, indicating the existence of a dose-response phenomenon. However, little is known about the distal effects of proximal MJ application in young conifer trees. Moreira et al. [31] observed that the local application of a solution of 100 mM MJ on a basal branch distally elicited defensive responses on the upper main stem, but not on the opposite branch. Soil application of MJ increased resin terpenoids in the stem of young Douglas fir seedlings [44].

15.6.3   Comparison of Chemical, Biological and Mechanical Induction

It is likely that responses to real herbivory also involve other signalling pathways, and there is even evidence for annual plants that plant defensive responses elicited by closely related insect herbivores or by herbivores belonging to the same guild could differ [28]. As summarized in this review, it is well documented (Table 15.1) that MJ treatments are able to induce defensive responses that provide effective resistance in young conifer trees against pest and diseases, but it remains poorly understood whether defensive responses induced by real and MJ-simulated herbivory are comparable. Indeed, this could be an important drawback to supporting methodological approaches that use MJ to simulate herbivory treatments for the induction of defences. For example, the exogenous application of MJ to young spruces caused significant increases of total monoterpenes, sesquiterpenes and diterpenes in the inner stem tissue compared with untreated controls, but these increases were notably lower than those observed after real feeding by the weevil *Pissodes strobi* Peck. (Coleoptera: Curculionidae) [42]. In contrast, Moreira et al. (unpublished data) observed that chemical induction with 40 mM MJ, mechanical wounding in the stem, and real phloem herbivory by *H. abietis* produced similar quantitative defensive responses in *P. pinaster* seedlings, increasing the concentration of resin diterpenes in the stem and total phenolics in the needles by equivalent magnitudes (Fig. 15.3). Further research is needed to confirm at what extent qualitative defensive responses produced by real herbivory are equivalent to those observed after simulated herbivory with MJ in young conifer trees.

15.7   Conclusions and Recommendations

Exogenous application of MJ increases the concentration of secondary chemicals in conifer tissues and promotes the formation of anatomical defence structures in young conifer trees [31, 40, 51]. Defensive responses elicited by MJ also increase the resistance of young conifer trees to insect herbivores and to a lesser extent to some pathogenic fungi [31–33]. MJ could therefore be used as an experimental simulator of real herbivory for scientific purposes; however the extent to which MJ-induced responses reflect those elicited by insect herbivores needs to be further examined. Although there is evidence that MJ-induced responses could mimic those elicited by wounding caused by coleoptera feeding in phloem, bark and stem, little is known about the responses elicited by caterpillars, nematodes, and defoliators, which could involve responses based in different signalling pathways. The use of exogenous application of MJ as pre-treatment in nursery production instead of traditional and prohibited pesticides should be subjected to further evaluation, as MJ application has also been shown to reduce plant growth, photosynthesis rate and needle transpiration [21, 32, 33].

Table 15.1 Summary of the published works about the effect of exogenous application of MJ in induced defences and resistance of young conifer trees

Reference number[a]	Conifer species	MJ treatments[b]	Surfactant	Application	Plant age[c]	Plant type[d]	Effect of MJ
[61]	Pinus radiata	0.0, 1.0, 4.5, 18.0	Tween-20	Foliar spraying	5 m	Seedling	Increased α-pinene and β-pinene in the stem; Increased resistance to Diplodia pinea
[33]	Pinus radiata	0.0, 1.1, 4.5, 18.0	Tween-20	Foliar spraying	3 m	Seedling	Increased resistance to Diplodia pinea
[32]	Pinus sylvestris	0, 10, 100	Not described	Foliar spraying	2 y	Seedling	Induced xylem traumatic resin duct formation; Increased terpenoid concentration in needles and xylem; Increased resistance to Hylobius abietis
[44]	Pseudotsuga menziesii	0.00, 0.01% (v/v)	Tween-20	Soil irrigation	14–15 m	Seedling	Induced traumatic resin duct formation in the stem and roots; Increased the concentration of terpenoids in the stem, needles and roots
[52]	12 species	0, 100	Tween-20	Stem brushing	3 y	Sapling	Induced polyphenolic parenchyma cell activation; Induced xylem traumatic resin duct formation
[53]	Pseudotsuga menziesii, Sequoiadendron giganteum	0, 10, 25, 50, 100	Tween-20	Stem brushing	4 y	Sapling	Induced polyphenolic parenchyma cell activation and early sclereid formation; Induced xylem traumatic resin duct formation
[60]	Picea abies	0, 25 (μM/100 l^{-1})	None	Evaporation by cotton wool	7 d	Seedling	Increased salicylic acid concentration in roots, hypocotyls and cotyledons; Increased resistance to Pythium ultimum

| Ref	Species	Concentration	Surfactant	Application	Time	Stage	Response
[40]	*Picea abies*	0, 1, 10, 100	None	Foliar spraying	2 y	Sapling	Induced traumatic resin duct formation in the xylem. Increased the concentration of terpenoids in the wood
[41]	*Picea abies*	10	None	Foliar spraying	2 y	Sapling	Increased mono- and sesquiterpene concentration in needles. Increased terpene emission in needles
[42]	*Picea sitchensis*	0.00, 0.01% (v/v)	Tween-20	Foliar spraying	1 or 2 y	Seedling	Induced traumatic resin duct formation in the xylem. Increased terpenoid concentration in inner stem tissue. Increased volatile terpene concentration in needles
[31]	*Pinus pinaster*	0, 5, 50, 100	Tween-20	Foliar spraying	11 m	Seedling	Increased stem resin and needle total phenolics. Increased resistance to *Hylobius abietis*
[43]	*Pinus pinaster*	0, 22	Ethanol	Foliar spraying	6 m	Seedling	Decreased sesquiterpene and α-pinene concentration in needles. Increased β-pinene concentration in needles
[21]	*Pinus pinaster*	0, 100	Tween-20	Foliar spraying	16 m	Seedling	Increased resin in the stem. Increased resistance to *Hylobius abietis*
[46]	*Pinus pinaster*	0, 22	Ethanol	Foliar spraying	6 m	Seedling	Increased stem diterpenes and needle phenolic compounds
[62]	*Pinus radiata*	Unknown	Unknown	Foliar spraying	2 m	Seedling	No effect on phytophtora root rot disease

aReference number in the table coincides with the reference number in the text
bMJ concentration always in mM, unless other units are specified
cDays (d), months (m) or years (y)
dSeedlings: young plant sporophyte developing out of a plant embryo from a seed. Sapling: a young tree with a slender trunk

Fig. 15.3 Exogenous application of 40 mM MJ, mechanical wounding in the stem (*MW*) and real herbivory by *H. abietis* (*HYL*) increased the concentration of chemical defences by similar magnitudes. (**a**) Concentration of diterpenes in the stem, and (**b**) total phenolics in the needles in 1 year-old *P. pinaster* seedlings after application of MJ (*MJ*, *black bars*), mechanical wounding of the phloem (*MW*, *white bars*) and phloem feeding by the large pine weevil *Hylobius abietis* (*HYL*, *grey bars*). Data are the mean values ± s.e.m. (N = 8). All the treatments were significantly different to the untreated control (*P* < 0.05)
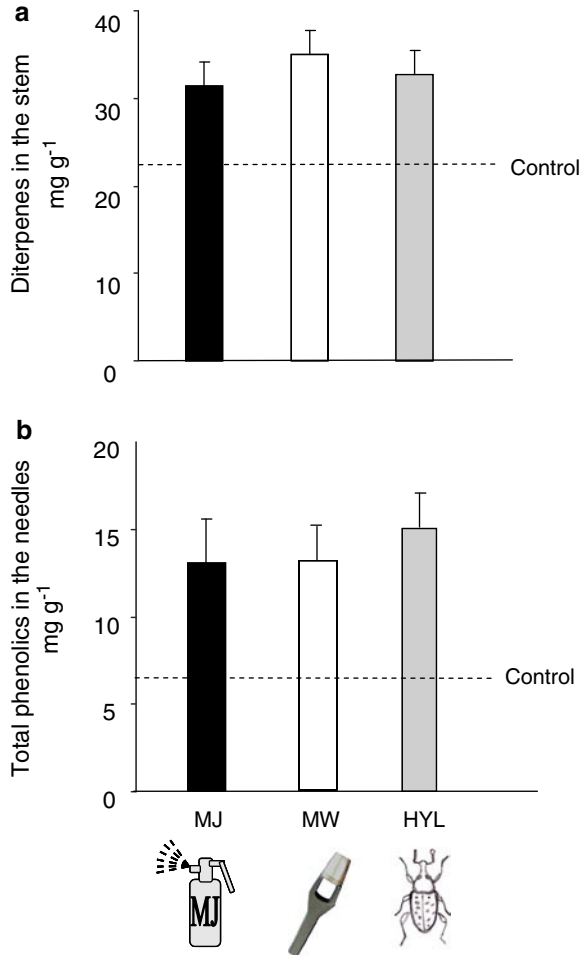
As a result of this review we can propose several methodological recommendations for the use of MJ as chemical elicitor in scientific studies. Firstly, a suspension of MJ in aqueous ethanol should be preferred to the use of the surfactant Tween-20, because the latter detergent increased defensive secondary compounds with respect to the untreated controls [31, 42]. Secondly, the use of the lowest concentrations of MJ as possible should be preferred, since high concentrations may provoke phytotoxicity or even the death of young trees, with unrealistic manipulation of the induction. Thirdly, as far as possible, plant responses to MJ application should be calibrated with respect to those elicited by real herbivory. Although it is known that MJ application can promote defensive responses quantitatively similar to those caused by insect herbivores [42], this is an area which has been relatively little studied and in which there is a relative lack of concrete knowledge.

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