



# Volatile Organic Compounds Influence Pine Processionary Moth Behavior<sup>†</sup>

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**Abstract:** The pine processionary moth (PPM), namely *Thaumetopoea pityocampa* or *T. wilkinsoni*, feeds on pine needles, causing defoliation and promoting tree decline. The production and emission of volatile organic compounds is one of the most effective plant defense mechanisms against herbivory. The present work reviewed available reports on the influence of plant volatiles on PPM behavior. Eighteen volatiles were reported to be detected by the PPM, to inhibit feeding and oviposition, or to reduce attraction to pheromones. High biological activities were reported for limonene or  $\beta$ -pinene enantiomers. Infested pines showed an increased production of bornyl acetate,  $\beta$ -pinene and caryophyllene oxide. Uncovering the volatile cues that influence parasitic PPM behavior provides an important contribution to the establishment of safer pest-management practices to control this pine pest.

**Keywords:** enantiomers; limonene; pest management; *Pinus*; terpenes; *Thaumetopoea pityocampa*; volatiles;  $\beta$ -pinene



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## 1. Introduction

The pine processionary moth (PPM), namely *Thaumetopoea pityocampa* or *T. wilkinsoni* (Lepidoptera: Thaumetopoeidae), is a tree phytophagous insect that mainly affects *Pinus* and *Cedrus* spp. The PPM is considered one of the most destructive pine defoliators and can be found in South Europe, North Africa, Asia and the Middle East. As a probable result of global warming, its geographical distribution has been expanding northwards, endangering the vast European pine forests [1].

The life cycle of the PPM shows two phases, an aerial phase comprising the egg, larvae and adult stages, and a below-ground phase, during pupa development. The PPM emerges from underground in the summer, reproduces and oviposits in the branch tips of the upper crown of pine trees. As eggs hatch, the larvae begin to feed on pine needles and moult. By the fourth instar, the recognizable silken nests that characterize this pest have enlarged to the definitive winter nest. Fully grown larvae (fifth instar) emerge in the beginning of spring and seek out pupation sites in the soil, in long head-to-tail processions [2].

In addition to the severe impact on biodiversity and forest ecosystem health, the PPM has a dangerous societal impact due to its urticating hairs, produced from the third instar onwards, which cause strong allergic reactions in mammals. Its increasing dispersion in

urban areas and recreational pine parks has led to several occurrences of public-health problems and high economic losses, resulting in increasing public awareness [1].

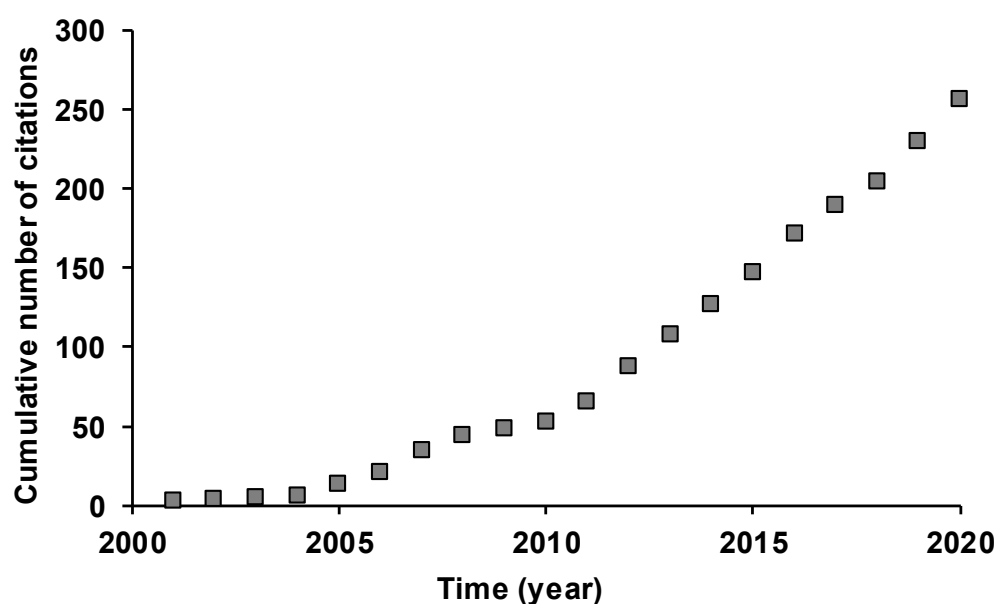
## 2. Emission of Tree Volatiles due to Herbivory

Over the past decade, tree defoliation was estimated to be over 20% in European forest ecosystems. Insect herbivore pests are key stress factors responsible for vegetation damage and have greatly contributed to the increase in defoliation estimates in several forest ecosystems worldwide. Herbivory greatly influences the amounts and classes of volatiles emitted by trees. Forest ecosystems are the largest source of atmospheric volatile organic compounds that play an important role in atmospheric chemistry and physics. The emitted volatiles are major players in plant communication, particularly plant-plant interactions, which promote defense responses, or plant-insect interactions (with either parasites or pollinators) as a direct defense mechanism [3]. These volatile emissions are comprised mainly of terpenoid compounds, well known modulators of insect development and behavior [1,4,5].

In the present work, publications reporting on the influence of tree volatiles in PPM behavior and the induction/inhibition of pine volatile production as a result of PPM activity were reviewed.

## 3. Reports on Volatiles Involving the Pine Processionary Moth

Published works reporting on the activity of tree volatiles on PPM behavior and the influence of PPM herbivory on pine volatile composition were compiled using the Web of Science search engine, in all available databases, using the keywords “volatile” and “*Thaumetopoea*” or “processionary moth”. The eight reports retrieved dated from 1999 to 2020 and were published in journals specialized in the scientific fields of Plant sciences (63%), Entomology (25%) and Environmental Sciences (25%). These works generated 267 citations by a total of 240 works, with an average of 33 citations per work. The reports that cited these works were published in journals specialized in the scientific fields of Environmental Sciences (88%), Plant sciences (83%) and Forestry (71%). Since 2011, the cumulative number of citations has been steadily increasing, revealing a stable research interest in this field (Figure 1).



**Figure 1.** Cumulative number of citations on reports of volatiles involving the pine processionary moth. Data obtained from Web of Science (<https://www.webofknowledge.com>) using the keywords “volatile” and “*Thaumetopoea*” or “processionary moth”.

#### 4. Effect of Tree Volatiles on the Pine Processionary Moth

The influence of tree volatiles on PPM behavior was analyzed in five reports. Zhang et al. [6] analyzed the potential role of pine-emitted volatiles on a host selection of PPM females, namely the response of *T. pityocampa* female antennae to volatiles from freshly cut *Pinus sylvestris* branches, through coupled gas chromatographic-electroantennographic detection (GC-EAD). Surprisingly, a higher response was reported for pine volatiles commonly present in lower proportions, the monoterpenes  $\beta$ -myrcene,  $\beta$ -phellandrene, *trans*- $\beta$ -ocimene and terpinolene, while the more abundant pine volatiles induced no antennae response, namely the monoterpenes  $\alpha$ -pinene,  $\beta$ -pinene and  $\delta$ -3-carene (Table 1). Additionally, a differential response was reported for limonene enantiomers, with the (–)-enantiomer eliciting a response but not the (+)-enantiomer. The effect of separate enantiomers was also analyzed by Tiberi et al. [7] and Panzavolta et al. [8] on PPM oviposition in host trees. Strong egg-laying deterrent properties were reported for (–)- $\beta$ -pinene and (+)-limonene (Table 1). This limonene enantiomer was reported to provide a good degree of protection when applied in *P. sylvestris*, *P. nigra*, *P. pinaster* and *P. radiata* stands during the *T. pityocampa* oviposition period [7].

**Table 1.** Tree volatile compounds reported to influence the behavior of the pine processionary moth.

Compound	Activity <sup>1</sup>
<b>Monoterpene hydrocarbon</b>	
<i>cis</i> - $\beta$ -ocimene	Weak antennae response
<i>trans</i> - $\beta$ -ocimene	Strong antennae response
(–)-limonene	Weak antennae response, inhibited oviposition, may influence feeding
(+)-limonene	No antennae response, inhibited oviposition, may influence feeding
terpinolene	Strong antennae response, may influence feeding
$\alpha$ -pinene	No antennae response, may influence feeding
$\beta$ -myrcene	Strong antennae response, may influence feeding
$\beta$ -phellandrene	Strong antennae response
(–)- $\beta$ -pinene	No antennae response, inhibited oviposition, may influence feeding
(+)- $\beta$ -pinene	No antennae response, may influence feeding
$\gamma$ -terpinene	Weak antennae response
$\delta$ -3-carene	<no antennae response
<b>Sesquiterpene hydrocarbon</b>	
germacrene D	May influence feeding
$\beta$ -caryophyllene	May influence feeding
<b>Oxygenated sesquiterpene</b>	
caryophyllene oxide	May influence feeding
eudesmol	May influence feeding
<b>Branched chiral alcohol</b>	
2-ethyl-1-hexanol	Weak antennae response
<b>Phenylpropanoid</b>	
methyl salicylate	Strong antennae response, inhibited male attraction to pheromones

<sup>1</sup> Compound activities reported in [6–10].

Petrakis et al. [10] analyzed the direct activity of terpenoid extracts from 15 pine species on the feeding behavior of *T. pityocampa* caterpillars, and developed an analytical model to predict the influence of each terpene compound. Although no constant promoter or inhibitor role could be attributed to any volatile throughout PPM feeding stages, the terpenes caryophyllene oxide, terpinolene,  $\beta$ -myrcene, germacrene D, eudesmol, limonene,  $\beta$ -pinene,  $\beta$ -caryophyllene and  $\alpha$ -pinene allowed for the construction of the most robust model, which suggests that these volatiles may influence the PPM feeding behavior.

Jactel et al. [9] analyzed the influence of volatiles from non-host species on the PPM, based on the premise that richer plant communities release diverse organic volatiles capable of disturbing the host choice and mating behavior of predatory pests. Methyl salicylate, a birch-emitted volatile, elicited antennae response and interfered with male PPM attraction to pheromone traps. This suggests that volatile chemical diversity plays an important role in natural-forest-pest management and can be used in sustainable strategies for PPM control.

## 5. Effect of the Pine Processionary Moth on Tree Volatiles

Insect herbivory is known to induce strong chemical defenses in trees. The impact of PPM feeding on the pine-tissue volatile composition was profiled for five pine species, namely *P. nigra*, *P. pinaster*, *P. radiata*, *P. radiata* and *P. sylvestris*. Variation in volatile compounds in pine after PPM feeding seems to be dependent on the pine species and respective volatile composition. For example, limonene was reported to increase in *P. pinaster* in response to PPM activity, whereas for *P. radiata*, a reduction was reported (Table 2) [11]. Similarly, *cis*- $\beta$ -ocimene was reported to increase in *P. nigra*, while in *P. sylvestris*, *cis*- $\beta$ -ocimene was reported to decrease [12,13]. Variability in volatile production after PPM herbivory seems to be dependent on the chemical defense strategy of each species, which suggests that there may not be a single defense mechanism in these conifers.

**Table 2.** Changes reported for pine volatile compounds as a response to herbivory by the pine processionary moth.

Compound	Pine Species <sup>1</sup>
<b>Increase</b>	
limonene	<i>P. pinaster</i>
$\beta$ -pinene	<i>P. pinaster</i> and <i>P. radiata</i>
<i>cis</i> - $\beta$ -ocimene	<i>P. nigra</i>
bornyl acetate	<i>P. pinaster</i> and <i>P. nigra</i>
<b>Decrease</b>	
limonene	<i>P. radiata</i>
$\beta$ -caryophyllene	<i>P. sylvestris</i>
germacrene D	<i>P. sylvestris</i>
bicyclogermacrene	<i>P. sylvestris</i>
$\alpha$ -pinene	<i>P. sylvestris</i>
<i>cis</i> - $\beta$ -ocimene	<i>P. sylvestris</i>
borneol	<i>P. sylvestris</i>
Epi-bicyclosesquiphelandrene	<i>P. sylvestris</i>
$\gamma$ -muurolene	<i>P. sylvestris</i>
$\delta$ -cadinene	<i>P. sylvestris</i>
$\tau$ -cadinol	<i>P. sylvestris</i>

<sup>1</sup> Variation in pine volatiles reported in [11–13].

While  $\beta$ -pinene increased in *P. pinaster* and *P. radiata* after PPM feeding,  $\alpha$ -pinene decreased in *P. sylvestris*. Bornyl acetate was reported to increase in *P. pinaster* and *P. nigra*; however, borneol decreased in *P. sylvestris*.

Surprisingly, many of the pine-emitted volatiles that significantly changed in response to PPM herbivory (Table 2) were also reported to directly influence PPM behavior with respect to feeding, oviposition and antennae response (Table 1), namely limonene,  $\beta$ -pinene,  $\beta$ -ocimene, germacrene D and  $\beta$ -caryophyllene, suggesting an undiscovered volatile interplay between the PPM and its host.

## 6. Conclusions

The analysis of volatiles with activity on the PPM and involved in the response to PPM-induced defoliation is still preliminary. Nevertheless, the compilation of available research allowed us to conclude that the volatile chemical responses of forest species, in particular those of pines, can greatly influence PPM behavior. A higher number of studies will be required to propose effective anti-PPM mechanisms that can contribute to understanding PPM behavior and establishing sustainable pest-management strategies capable of efficiently protecting forest ecosystems.

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