Urban Forestry & Urban Greening 14 (2015) 538–543

Contents lists available at ScienceDirect

Urban Forestry & Urban Greening

journal homepage: www.elsevier.com/locate/ufug

Original article

Pine monoterpene deterrence of pine processionary moth oviposition

T. Panzavolta*, G. Tellini Florenzano, R. Tiberi

Department of Agrifood Production and Environmental Sciences, University of Florence, via Maragliano 77, 50144 Florence, Italy

ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 29 April 2015
Accepted 9 May 2015

Keywords:
Egg-laying
Limonene
Pest control management
β-Pinene
Pinus pinea
Thaumetopoea pityocampa

ABSTRACT

The pine processionary moth (PPM) is a major pest of the Mediterranean area. Considering the severe damage the PPM may cause to pine forests, humans, and other warm-blooded animals control management is particularly needed to protect urban areas. Many plant volatiles have been studied as pest control tools. In this paper we look at (1S)-(−)-β-pinene, (±)-limonene, (R)-(+)-limonene, and (S)-(−)-limonene, which were tested in 2003 and 2004 against the PPM. The chemical cues were explored for both years: (1S)-(−)-β-pinene and (R)-(+)-limonene both clearly affected PPM egg-laying, reducing the number of egg masses per tree. Pine tree height and distance from the plantation's edge also proved to be influential, with highest trees, and trees closest to the plantation edge, having greater numbers of egg masses. Climatic factors, affecting monoterpene evaporation rates, may need to be taken into consideration by pest control managers.

Introduction

The pine processionary moth (PPM), *Thaumetopoea pityocampa* (Denis & Schiffermüller), (Lepidoptera, Notodontidae) is a pest of the Mediterranean area. It is present in the Mediterranean regions of Europe, Africa, and Asia (EPPO, 2004; Kerdelhué et al., 2009), from sea level to upper elevations (variable from north to south). However, its distribution range is expanding both in altitude and in latitude due to climate changes (Battisti et al., 2005; Roques et al., 2015). The PPM has one generation per year; nevertheless, it can adapt its life cycle to local climatic conditions through changes in life-cycle timing. In addition, the occurrence of a prolonged diapause in the pupal stage may affect life-cycle duration, causing changes to insect abundance and infestation levels.

The PPM may cause severe damage to host trees, the environment, as well as to humans and other warm-blooded animals. PPM larvae feed on *Pinus* spp. needles; however, high infestation occurs frequently on *Cedrus* spp. and occasionally on other conifers (Battisti et al., 1991). Furthermore, some pine species are preferred for PPM egg-laying (Tiberi et al., 2002). Young trees suffer higher damages, with consequent delay in radial and longitudinal growth and subsequent high economic losses. In addition, PPM larvae may cause indirect damage in urban and human-frequented areas, due to its urticating hairs (Vega et al., 2000; Battisti et al., 2011). These hairs are extremely dangerous to humans and other warm-blooded animals, as the toxins they contain spill out when they penetrate mammalian skin. Mammalian immune systems then recognize these substances as foreign, causing severe reactions (Battisti et al., 2011).

Consequently, in urban and other areas of human activity which are particularly affected by PPM indirect damage, pest control is necessary. The attack level in a forest setting is considered high when five tents per tree occur on more than 75% of the pines, whereas in urban areas two tents per tree are enough (Tiberi, 1989). Given that the PPM is a serious public hazard, particularly to humans but also to other warm-blooded animals, pest control is mandatory in Italy (Ministerial Decree, October 30th, 2007). Among pesticides, environmentally-safe control products, like *Bacillus thuringiensis* (Berliner) var. *kurstaki*, are preferred (Battisti et al., 1998). However, few other products, which are both environmentally friendly and effective against the PPM, are available (FAO, 2009).

On the other hand, plant volatiles, involved in plant–pest–natural enemy interactions, offer significant potential as tools for pest control. Several studies show that many plant-eating insects use plant volatiles to locate host plants (Schoonhoven et al., 2005; Charalampos et al., 2012), particularly during egg-laying activity (Städler, 1974; Leather, 1987; Jactel et al., 1996). As a consequence, many of these substances have been studied as pest control tools, being used: to confuse insects during the location of the host plant (*deterrent effect*), to capture them (*attractant effect*) or to negatively affect their development (*toxic effect*) (Koul et al., 2008).

As limonene and β-pinene are common volatile monoterpenes in pine species, they are expected to have a role in plant–phytophagous insect interactions (Langenhaim, 1994).

---

* Corresponding author. Tel.: +39 0552755552.
E-mail address: tpanzavolta@unifi.it (T. Panzavolta).

http://dx.doi.org/10.1016/j.ufug.2015.05.004
1618-8667/

However, enantiomers of the same drug may have different effects on insects, depending also on insect species: for example, limonene and β-pinene are oviposition deterrents for some defoliating insects (Ntiamoah et al., 1996; Zhang et al., 2004), while for others limonene and (−)-β-pinene proved to have a stimulant effect on egg-laying activity (Städler, 1974; Shu et al., 1997; Charalampos et al., 2012). In any case, as are other plant monoterpenes, these compounds are involved both in plant defense strategies (Langenhaim, 1994) and in host-finding behavior (Charalampos et al., 2012).

Finding and selecting an appropriate host plant for egg-laying is a challenging task, even for the PPM. During the laying period PPM females can fly for many kilometers to find their preferred hosts. In fact, although some authors hypothesized it has an unselective oviposition (Hódar et al., 2002), some pine species are less infested than others; in particular, the least infested is *Pinus pinea* L. (Demolin, 1969). Visual cues, such as needle size, tree size, and tree position in the forest, are significant in PPM egg-laying (Tiberi, 1983; Peréz-Contreras et al., 2008); however, olfactory cues may play the main role (Demolin, 1969; Paiva et al., 2011; Achotegui-Castellas et al., 2013). In general, limonene, β-pinene, and α-pinene are among the most abundant monoterpenes in pine needles (Mateus et al., 1998; Tiberi et al., 1999; Paiva et al., 2011). For *P. pinea*, limonene content proved from 24 to 70 times higher than in other pines, mainly in summer during the PPM flight period (Tiberi et al., 1999). However, less abundant pine monoterpenes are not to be underestimated, because they may also be important olfactory cues (Zhang et al., 2003). In conclusion, studying the effect of monoterpenes on PPM activity seemed promising for pest control.

Trials on the effects of monoterpenes on PPM larval feeding and egg-laying were carried out in Italy during the past decades. From these prior studies emerged a negative impact of (±)-limonene and (1S)-(−)-β pinene on PPM larvae (Niccoli et al., 2004). As regards PPM laying activity, instead, (R)-(+)-limonene, (S)-(−)-limonene, and (1S)-(−)-β pinene in a water solution sprayed onto pine crowns showed a deterrent effect (Tiberi et al., 1999, 2004). To overcome the difficulties of spraying pine crowns, such as tree height and an uneven distribution of substances, in another study monoterpenes were applied by dispensers placed on the pine crown; this showed an even higher effect against PPM females than the spraying treatment (Niccoli et al., 2008). In light of those findings, for this paper (1S)-(−)-β-pinene, (±)-limonene, (R)-(+)-limonene, and (S)-(−)-limonene were tested in 2003 and 2004 in dispensers of different sizes to verify their efficacy as PPM control tools, as well as their stability inside dispensers. Ten years after the above mentioned trials, the resulting data are still useful today, because PPM's range of distribution is constantly expanding due to increasing winter temperatures (Robinet et al., 2013). As a consequence, finding an effective and ecologically sustainable control method, particularly in urban forestry, is increasingly important.

Materials and methods

Surveys were carried out in a PPM-infested black pine stand planted within the Monte San Michele forest area (Province of Florence, Italy) (43°33′N, 11°22′E, 750–800 m a.s.l.). The pine stand was bordered by a lane road; pines (1.2–3.5 m high) were distributed along eight rows, spaced 4 m apart, with pines every 3 m, for a total of about 200 pines.

In the 2003 trial a randomized block design was used, considering pine height (PH) and distance of pines from the plantation edge (DPE) as blocking factors. A total of 100 pines, distributed in seven rows (the nearest to the plantation edge was excluded), were selected. Pines were sorted into 25 blocks, each block being of four pines. (1S)-(−)-β-pinene (99%), (±)-limonene (97%), (R)-(+)-limonene (97%), and (S)-(−)-limonene (96%) were tested separately. All chemicals were purchased from Sigma–Aldrich S.r.l. (Milan, Italy). Each treatment was applied to five blocks (20 pines in total), while five blocks were maintained as controls.

Monoterpenes evaporated from dispensers placed on the pine crowns. Each dispenser (a black cylindrical polyethylene vial having a 4 mm hole on its lower surface) was filled with 10 g of a monoterpene absorbed in a cotton swab. Four dispensers, containing the same monoterpene, were placed on each experimental pine immediately after the first PPM captures in pheromone traps, and were maintained in the field during the whole PPM flight period: two pheromone traps were used to monitor PPM flight. Dispensers were refilled two times during the study period. Each week, all the selected pines were checked to detect PPM egg masses, which were marked and left on the pine trees. At the end of the egg hatching period, egg masses were collected, counted and stored separately according to treatment, block and tree on which they had been laid.

In the 2004 trial a randomized block design was again used, again considering PH and DPE as blocking factors. A total of 105 pines, distributed in eight rows (now including that nearest to the plantation edge), were selected. Pines were sorted into 21 blocks of five pines each. This time only two treatments were tested; (1S)-(−)-β-pinene (99%) and (±)-limonene (97%). Per each monoterpene seven blocks (for a total of 35 pines per terpene) were treated, while seven blocks were maintained as controls. The methodology was largely the same as the previous year, except that in 2004 the dispensers were larger, being filled with 20 g of substance; furthermore, they were not refilled during the study period.

In both study years meteorological data, from a nearby meteorological station, were obtained from ARSIA, the Regional Agrometeorological Services of Tuscany, and from U.C.E.A, the Central Office of Agricultural Ecology. Half of the dispensers were weighed each week to estimate monoterpene evaporation. In addition, GC/MS and NMR analyses were carried out weekly by the Department of Organic Chemistry (University of Florence), on a sample of 10 dispensers from different points of the plantation, to verify monoterpene stability. Dispensers were analyzed individually, recovering monoterpenes by dichloromethane. GC/MS analyses were performed with a Shimadzu GC-17A coupled with a Shimadzu GCMS-Q5050A (Shimadzu Corp., Kyoto, Japan) using a factor four capillary column FU5MS (length 30 m, internal diameter 0.25 mm). Helium was the carrier gas employed and the ionization potential was 70 eV (column temperature: 50 °C; injector temperature: 250 °C; detector temperature: 250 °C; program: 2 min at 50 °C; rate: 15 °C/min; max temperature: 280 °C to 15 min). NMR analyses were obtained with Varian Gemini 200 and Varian Mercury 400 spectrometers (Varian, Inc., Palo Alto, CA, USA) ($CDCl_3$ solvent). NMR chemical shifts were referenced to non deuterated residual solvent signals (7.26 ppm for ^1H).

The same statistical analysis procedures were used for both the 2003 and the 2004 data. The Generalized Linear Model (GLM), with a Poisson distribution of error and a log link, was used to analyze the number of egg masses per tree, being a discrete variable with a non-normal distribution. This analysis is recommended for data with non-normal distribution and non-homogeneous variance (Draper and Smith, 1998). Models with a Poisson distribution of error and a log link are frequently used in entomology to study egg mass distribution (Elkinton et al., 1996; Hilbeck et al., 1998): furthermore, Rushton et al. (2004) proved their applicability in zoology. The Akaike Information Criterion (AIC) (Trexler and Travis, 1993), which selects the most informative model (Mac Nally, 2002), was used to select the optimal number of parameters (including interactions). The marginal effect of each variable was controlled and only significant ones ($P < 0.05$) were retained. The unpaired t-test