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Development of natural waxes dispensers for pheromones and use in mating disruption of the ambrosia beetle *Megaplatypus mutatus* in poplar (*Populus* spp) plantations

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Abstract *Megaplatypus mutatus* (= *Platypus mutatus*) (Chapuis) is an ambrosia beetle native to South America that attacks live trees, mining deeply into the xylem through large tunnels. This activity weakens the structural integrity of the tree, causing severe stem-breakage and mortality. Attacks are initiated by pioneer males that select a host tree and build short nuptial galleries, to which they attract females using a sexual pheromone. Previously, we showed the potential for the strategy of pheromone-mediated mating disruption of *M. mutatus* in commercial poplar and hazelnut plantations in South America and Europe using polyethylene reservoir dispensers for pheromones. In the present work we replaced the polymeric reservoir dispensers by monolithic dispensers made by dispersion of the pheromone in natural waxes and the addition of kaolin and we found that: prior to pheromone deployment, the mean number of galleries per tree did not differ significantly between the control and treated plots and the same was observed after the mating disruption treatment for the control plot but not for treated plots, where the mean number of galleries were reduced. These findings confirm that mating disruption is a viable tool for management of *M. mutatus* in poplar plantations. Using natural wax dispensers has obvious advantages from an environmental point of view.

Keyword Ambrosia beetle · Pheromone · Wax dispenser · Controlled release · Sulcatol · Sulcatone

Introduction

Ambrosia beetles (Coleoptera: Platypodidae) are an important group of forest pests that colonise weakened or felled trees. *Megaplatypus mutatus* (= *Platypus mutatus*) (Chapuis) is an ambrosia beetle native to South America (Wood 1993) that attacks the xylem of many deciduous trees, forming large tunnels, known as galleries. This activity weakens the structural integrity of the tree, causing severe stem-breakage and mortality mainly in commercial plantations of poplar species (Populus spp) such as *Populus deltoides* (Santoro 1963; Achinelli et al. 2005; Alfaro et al. 2007) but also *Quercus, Ulmus, Casuarina* and fruit

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trees (Giménez and Etiennot 2003). Furthermore, the dark staining of the tunnels caused by the decaying ambrosia mycelium reduces the quality of wood for export.

M. mutatus was accidentally introduced in Italy in 1998 (Allegro and Della Beffa 2001; Tremblay et al. 2000) and threatens poplar plantations which are a highly important economic resource. In 2000, it was detected in *Populus canadensis* (Mönchh) in the Caserta province in the Campania region. Dispersal is facilitated by transportation of infested logs. The risk of spread of *M. mutatus* and its corresponding potential damage to other regions of Europe is of great concern to European regulatory authorities, who added this species to the EPPO/OEPP Alert List in 2004 and in 2007 recommended the inclusion as a quarantine pest (EPPO/OEPP 2004, 2007). North American forest resources are also at risk (Alfaro et al. 2007).

Attacks are initiated by pioneer males that select a host tree and build short nuptial galleries, to which they attract females using a pheromone. Following copulation, *M. mutatus* pairs extend their galleries to produce offspring. In previous studies we showed that in order to attract females, pioneer male *M. mutatus* emit (+)-6-methyl-5-hepten-2-ol ((+)-sulcatol), 6-methyl-5-hepten-2-one (sulcatone) (Gonzalez Audino et al. 2005) and 3-pentanol (Gatti Liguori et al. 2008).

In previous studies we developed controlled release dispensers for pheromones and tested various traps designs baited with a range of pheromone doses in the field and pheromone blend for monitoring *M. mutatus* infestations in Argentina and Italy (Funes et al. 2009, 2013; Gonzalez Audino et al. 2011, 2013; Griffo et al. 2012).

Mating disruption is a pest management technique based on the release in the field of large amounts of synthetic sex pheromones with the aim of disrupting the sexual communication between insects. It is frequently used for controlling lepidopteran pests, but it has seldom been exploited for coleopteran species. Several factors favour the potential success of pheromone-based management of *M. mutatus*: it is monogamous, it is of relatively low mobility because females do not leave the host tree after they mate (Santoro 1962), and and the pheromone is produced at relatively low-cost, stable under field conditions and can be formulated to be deployed in controlled release devices. Funes et al. (2011) performed three field trials testing mating disruption of *M. mutatus* in hazelnut and poplar plantations in South-America and Europe using polyethylene reservoir pheromone dispensers and reduced damage by more than 56 % in both countries. In this paper, monolithic devices made of wax mixtures were evaluated for controlled release of *M. mutatus* pheromone. Dispensers were deployed in field plots and evaluated for potential disruption of beetle mating.

Materials and methods

Materials

Components of the pheromone were: Sulcatone (6-methyl-5-hepten-2-one) and Sulcatol (6-methyl-5-hepten-2-ol) from Vigon International, located in East Stroudsburg, PA, USA. 3-Pentanol from Sigma-Aldrich®, Germany.

Waxes formulated as matrix dispensers were: Paraffin wax of m.p. 53–57 °C and m.p. 70–80 °C from Sigma Aldrich®. Paraffin oil was obtained from Fluka, Germany, Lanolin wax and bee wax from Parafarm S.A, Argentina. Pentaerythritol ester of lanolin sent by Rolex Lanolin Limited, Mumbai, India as a free sample, Carnauba wax of Brazilian origin and Stearin from Serain Juarez S.A, Buenos Aires, Argentina.

Fillers added to the wax matrices to alter pheromone release profiles were: Glass spheres and kaolin from a local supplier; activated charcoal from Merck, Germany, and molecular sieve of 5 Å porosity and 4–8 mesh from Aldrich Chemical Company, Inc., Milwaukee, WI.

Monolithic dispensers and release rate determination

In monolithic dispensers the pheromone is homogeneously dissolved and/or dispersed in a polymer matrix (Tojo 1985). So, dispensers for release rate studies were prepared by melting the matrix, addition and mixing of the filler component in a proportion of 30 %, and final addition of 20 % of the pheromone component. The system was thoroughly mixed and poured into a mold with half sphere shaped wells of 3 cm diameter, and solidified in the fridge (Fig. 1).

The waxes used individually as matrix were carnauba wax, lanolin wax, bee wax and pentaerythritol ester of lanolin, stearin, paraffin wax m.p. 70–80 °C and

Fig. 1 Monolithic dispensers. *Left* Preparation. *Right* Display in the field

paraffin oil. The fillers added individually to each matrix were activated charcoal, kaolin, molecular sieve and glass spheres. Not all the combinations of fillers/waxes were tested. The combinations of fillers and waxes tested are described in Table 1, with three replicates for each combination.

The pheromone components release rate from the monolithic dispensers was measured in a wind tunnel at 29–30 °C with a wind speed of 0.5–0.6 m/s, weighing the mass loss during 30–70 days or until the release rate was less than 10 mg per day.

Graphics of residual mass in the dispensers versus time in days were plotted using SigmaPlot 11.0 software. From the release rate curves, and using the same software we calculated the release rates for day one, V_i, for sulcatone, sulcatol and 3-pentanol in the different matrices.

According to the release rates used in our previous work (Funes et al. 2011) we selected dispensers to be used in the field. So, for 3-pentanol and sulcatol we used the dispensers made of carnauba wax and kaolin as filler, and for sulcatone we used paraffin 70–80 °C.

The pheromone dispensers were attached to the trees in sets of three, one of each component and adjacent to each other, by pinning them on the tree surface at 1.8 m above the ground. The sets were distributed uniformly throughout each treatment plot by using Pheromone Dispenser Locator (Dolinko and Ceriani-Nakamurakare 2015) at a density of 14 sets of three per ½ ha. Devices were replaced every two weeks.

Field trial locations

The poplar plantation used in this experiment was located at Bragado, Buenos Aires Province, Argentina (34°50′ S, 60°30′ W, 55 m m.a.s.l.). Field trials were performed during the flight season of *M. mutatus,* starting on 12 February and ending on 15 April of 2015 in two different sites, both with *Populus deltoides* cv. Stoneville 66 of approximately 8 year-old, with a mean stand density of 453 trees per hectare and a mean diameter at breast height (DBH) of 67 ± 0.47 cm. Site A consisted in two replicates and one control, each of ½ ha and with a minimum separation of 200 m between the sites. Site B had one replica and one control with same characteristics. Both sites were located at the extremes of the plantation lot with a distance of 3000 m between them.

Damage assessment

Damage was assesed by means of two parameters: number of active galleries (AG) and number of mating galleries (MG) (Funes et al. 2011). AG are developed galleries with larval activity, and precede future adult emergence. MG are new galleries, with the mated couple inside extending the gallery inwards (Santoro 1962). After a couple of months, an MG becomes an AG. Then, AG are the entrance holes where a male initiated attack, lured a female, mating took place, females laid their eggs, offspring were produced, and feeding larvae expelled the sawdust outside.

Megaplatypus mutatus damage in control and pheromone-treated plots was assessed before placing the pheromone devices and 7 days after treatment. The prior assessment is necessary so that relative differences in damage between pre- and post-treatment may be compared between control and treated plots. Damage assessment was conducted before the trial by examining tree trunks, identifying active galleries

Table 1 Release rate of monolithic type pheromone lures containing sulcatone, 3-pentanol, or sulcatol, at 27–28 °C and a wind speed of 0.5 m/s in a laboratory wind tunnel

Matrices	R^2	a	b	V_i (mg/day)[d]
Release rate parameters of the curve $m = ae^{-b\sqrt{t}}$ for sulcatone (20 %)				
Paraffin m.p. 70–80 °C+10 % kaolin	0.9853	5.204	0.02404	79.5
Paraffin m.p. 70–80 °C+20 % kaolin	0.9871	4.970	0.02476	71.7
Paraffin m.p. 70–80 °C+30 % kaolin	0.9553	4.670	0.03556	123.3
Paraffin m.p. 70–80 °C+20 % glass spheres	0.9823	4.646	0.01756	56.2
Paraffin m.p. 70–80 °C+20 % molecular sieve	0.9878	5.765	0.02176	84.3
Paraffin m.p. 70–80 °C+20 % activated charcoal	0.9707	5.709	0.02460	137.6
Paraffin m.p. 70–80 °C	0.9978	7.336	0.01582	70.8
Pentaerythritol ester of lanolin+20 % kaolín	0.7793	5.570	0.04167	449.2
Pentaerythritol ester of lanolin	0.9528	7.202	0.03362	241.3
Lanolin wax+20 % kaolin	0.7604	4.199	0.03062	325.0
Lanolin wax	0.8501	5.112	0.04299	333.3
Carnauba wax+10 % kaolin	0.9733	4.073	0.01990	61.9
Carnauba wax+20 % kaolin	0.9957	5.208	0.01876	61.7
Carnauba wax+30 % kaolin	0.9934	6.448	0.01809	77.1
Carnauba wax+20 % glass spheres	0.6152	4.307	0.03025	410.1
Carnauba wax+20 % molecular sieve	0.8436	5.720	0.02035	138.9
Carnauba wax+20 % activated charcoal	0.9523	5.152	0.02171	92.3
Carnauba wax	0.8869	5.838	0.03766	269.4
Release rate parameters of the curve $m = ae^{-b\sqrt{t}}$ for 3-pentanol (20 %)				
Paraffin m.p. 70–80 °C+10 % kaolin	0.9790	4.220	0.01284	47.3
Paraffin m.p. 70–80 °C+20 % kaolin	0.9450	4.787	0.01480	86.8
Paraffin m.p. 70–80 °C+30 % kaolin	0.9408	5.145	0.02255	124.8
Paraffin m.p. 70–80 °C+20 % glass spheres	0.9559	5.857	0.01012	74.2
Paraffin m.p. 70–80 °C+20 % molecular sieve	0.9674	6.346	0.01453	83.2
Paraffin m.p. 70–80 °C+20 % activated charcoal	0.9869	6.764	0.01105	47.0
Paraffin m.p. 70–80 °C	0.9978	6.807	0.00754	37.6
Pentaerythritol ester of lanolin+20 % kaolín	0.9268	6.669	0.04386	328.9
Pentaerythritol ester of lanolin	0.9528	6.942	0.03196	196.2
Lanolin wax+20 % kaolin	0.9304	5.775	0.02416	203.2
Lanolin wax	0.9498	5.734	0.02717	190.8
Carnauba wax+10 % kaolin	0.7024	3.713	0.03432	230.8
Carnauba wax+20 % kaolin	0.7334	5.465	0.03440	378.7
Carnauba wax+30 % kaolin	0.8743	5.847	0.03335	263.6
Carnauba wax+20 % glass spheres	0.3973	4.374	0.01380	401.5
Carnauba wax+20 % molecular sieve	0.5009	7.066	0.01556	538.7
Carnauba wax+20 % activated charcoal	0.6494	4.984	0.02965	403.7
Carnauba wax	0.7727	5.692	0.04911	402.9
Release rate parameters of the curve $m = ae^{-b\sqrt{t}}$ for sulcatol (20 %)				
Paraffin m.p. 70–80 °C	0.9916	5.531	0.00784	15.8
Lanolin wax	0.9715	3.000	0.04083	106.3
Carnauba wax	0.9924	5.278	0.01983	32.0
Pentaerythritol ester of lanolin	0.988	6.735	0.03040	108.4

Table 1 continued

Matrices	R^2	*a*	*b*	V_i (mg/day)[d]
Carnauba wax+10 % kaolin	0.9762	5.592	0.02370	18.6
Carnauba wax+20 % kaolin	0.9838	5.092	0.03275	39.8
Carnauba wax+30 % kaolin	0.9928	6.772	0.02190	39.4

Parameters *a,b*, from the eq $M = ae^{-b\sqrt{t}}$. R^2 = correlation coefficient

[a] *a, b,* R^2, V_i and *t* parameters of pheromone components curves from wax monolithic dispensers for sulcatone

[b] *a, b,* R2, *Vi* and *t* parameters of pheromone components curves from wax monolithic dispensers for 3-pentanol

[c] *a, b,* R^2, V_i and *t* parameters of pheromone components curves from wax monolithic dispensers for sulcatol

[d] Calculated from the differentiation of the pheromone component release curve Mass vs. time

(AG), and numbering them individually. In each experimental area, in both treated and control plots, we randomly sampled 30 % of the trees (Sower et al. 1982). We assigned every tree a unique number, and random selection was made using Pheromone Dispenser Locator until 30 % of the individuals were sampled. The search for galleries was performed up to 2.2 m high for each tree. Old galleries (dry) from previous seasons were also marked to avoid confusion with later assessments.

Damage was expressed as the mean number of MG or AG per tree, and the means of treated and control areas were compared by a *t* test after the trial (Statistica 5.0; StatSoft, Tulsa, OK, USA).

Results

Release rates of monolithic dispensers

We found that depletion of pheromone components from formulations was characterized by an exponential equation $M = ae^{-b\sqrt{t}}$, where M is the remaining daily mass of the formulation e is the base natural of logarithm, *a* is an amplitude constant, *b* is a decay constant and *t* is the time in days. In all determinations P was < 0.0001 (Table 1).

From the same curves, we calculated Vi, that is, release rate at day 1 for sulcatol, sulcatone and 3-pentanol, for each matrix and its mixture with the fillers respectively (Table 1). Values ranged from 10–250 mg of pheromone released per day.

Pentaerythritol ester of lanolin+20 % kaolín had the highest Vi (449.2 mg/day) and paraffin m.p. 70–80 °C+20 % glass speheres produced the lowest Vi for sulcatone (56.2 mg/day). For 3-pentanol, carnauba wax+20 % molecular sieve had the highest Vi (538.7 mg/day) and paraffin m.p. 70–80 °C without filler produced the lowest Vi (37.6 mg/day). For sulcatol, pentaerythritol ester of lanolin had the higher Vi (108.4 mg/day) and paraffin m.p. 70–80 °C without filler produced the lowest Vi (15.8 mg/day).

Based on the release rates used in our previous work (Funes et al. 2011) and the hardness and melting points of the matrices, the optimal dispensers for mating disruption trials were selected. In the case of sulcatol we used carnauba wax/kaolin 20 % for 3-pentanol and sulcatol and for sulcatone, paraffin wax 70–80/kaolin 20 %, with 20 % pheromone in all cases.

Mating disruption of *Megaplatypus mutatus* in the field

Prior to pheromone deployment, the mean number of AG and MG per tree did not differ significantly between the treated and control plots (Table 2). However, after the mating disruption treatment the mean number of MG and AG were reduced for treated versus control plot.

Discussion

Efficient controlled-release systems are essential to deliver behaviorally relevant aerial concentrations of sex pheromones, for both monitoring and mating disruption purposes in the field. In previous works we showed very good results of monitoring and mating disruption of *M. mutatus* using polymeric controlled

Table 2 Mean number of *Megaplatypus mutatus* mating galleries (MG) and active galleries (AG) (±SE) in poplar plantations, before and after treatment with mating disruption pheromones

Location	AG per tree		MG per tree	
	Before[a]	After	Before	After
Site A				
Plot 1	1.01 ± 0.128a	0.66 ± 0.080a	1.21 ± 0.141a	0.85 ± 0.111a
Plot 2	1.04 ± 0.123a	0.68 ± 0.097a	1.28 ± 0.165a	0.82 ± 0.100a
Control plot	0.99 ± 0.132a	1.18 ± 0.124b	1.07 ± 0.153a	1.18 ± 0.120b
Site B				
Plot	0.66 ± 0.083a	0.38 ± 0.070a	0.82 ± 0.137a	0.49 ± 0.082a
Control plot	0.63 ± 0.098a	0.93 ± 0.110b	0.87 ± 0.134a	0.96 ± 0.097b

[a] Means within a column followed by the same letter are not significantly different (Student t-test: $P > 0.05$)

Prior to pheromone deployment: (Site A. Replicate 1. AG: $t = 0.16$; $df = 67$; $p = 0.87$. MG: $t = 0.66$; $df = 67$; $p = 0.57$. Site A. Replicate 2. AG: $t = 0.34$; $df = 67$; $p = 0.73$. MG: $t = 0.84$; $df = 67$; $p = 0.4$. Site B. AG: $t = 0.21$; $df = 67$; $p = 0.83$. MG: $t = 0.23$; $df = 67$; $p = 0.81$)

After mating disruption treatment: (Site A. Replicate 1. AG: $p = 0.00095$. MG: $p = 0.045$. Site A. Replicate 2. AG: $p = 0.0029$. MG: $p = 0.022$. Site B. AG: $p = 0.00012$. MG: $p = 0.00075$)

release dispensers for pheromones of reservoir type. In this work we replaced polyethylene reservoir dispensers by monolithic dispensers made with natural waxes and obtained similar results. treatment.

The depletion of pheromone components from formulations was characterized by an exponential equation proportional to $\sqrt{t}$. Pentaerythritol ester of lanolin produced very high release rates and paraffin showed the lowest release rates. The addition of fillers increased the release rates. Filler materials are incorporated in polymers to modify physical properties and improve handling characteristics (Kydonieus and Beroza 1982).

For the selection of monolithic dispensers for field trials, not only should V_i be relatively high and constant, but also the melting point and hardness of the mixtures of matrices+fillers+pheromones should be considered, as dispensers could easily melt in field conditions during warm summer temperatures or be easily eroded by heavy rains.

We selected the optimal dispensers deployed them in a commercial poplar plantation during the flight season of *M. mutatus*. After treatment, we found that the number of mating and active galleries had increased in control plots and decreased in treated plots, confirming our previous findings with polymeric dispensers with zero order kinetics (Funes et al. 2011) that the strategy of mating disruption using the pheromone delivery doses is a viable tool for management of infested poplar plantations. In our previous work we found damage reductions in Argentina and Italy greater than 56 % using reservoir systems for pheromones.

The use of a mating disruption strategy is favoured by several aspects of the biology of *M. mutatus*.That is, mated females do not leave the host tree avoiding the threaten of invasion by gravid females. *M. mutatus* does not feed on host phloem or xylem before or after emergency (Santoro 1962; Gatti unpubl.) enhancing the influence of pheromones compared to species that spend time on foraging, as time spent in the search of a new host becomes critical (Hasewaga et al. 1993). Also, *M. mutatus* is monogamous (Santoro 1962) so mated beetles that survived treatment are not a threat for repeat mating or host infestation. Finally,the sex ratio 1:1 (Santoro 1963), makes the location of males by females less likely than in when this proportion is in favour to females. In that case, timing of emergence is expected to mediate female mating success through its effect on the operational sex ratio (Bessa-Gomes et al. 2004).

Although synthetic pheromones of *M. mutatus* are not expensive to produce, the application process is labor intensive. Also, it is critical to have an effective monitoring schedule to detect the beginning of the flying period with pheromone-baited traps in order to maximize the benefit-cost ratio of the control treatment.

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