Agricultural and Forest Entomology (2015), DOI: 10.1111/afe.12133

Monolithic dispensers for pheromones and their use in mating disruption of the ambrosia beetle *Megaplatypus mutatus* in poplar plantations

Hernán Funes, Eduardo Zerba and Paola Gonzalez-Audino

Centro de Investigaciones de Plagas e Insecticidas (CIPEIN-UNIDEF-CONICET), JB de La Salle 4397, B1603ALO Villa Martelli, Argentina

Abstract

1. *Megaplatypus mutatus* (formerly *Platypus mutatus*) (Chapuis) is an ambrosia beetle native to South America that attacks standing 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 sex pheromone. Volatiles emitted are composed of (+)-6-methyl-5-hepten-2-ol [(+)-sulcatol], 6-methyl-5-hepten-2-one (sulcatone) and 3-pentanol. Previously, we showed the potential of 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 and found that damage reduction was greater than 56% in all cases.
2. In the present study, the polymeric reservoir dispensers were replaced by matrix dispensers made by dispersion of the pheromone in natural waxes or polyethylene glycols that act as a matrix with the addition of a filler. After treatment, the number of mating galleries was significantly higher (70%) in control than in treated areas.
3. Using natural materials for dispensers, we confirmed that mating disruption is a viable tool for the management of *M. mutatus* in poplar plantations.

Keywords Paraffin, pheromone, sulcatol, sulcatone.

Introduction

Ambrosia beetles (Coleoptera: Platypodidae) are an important group of forest pests that colonize weakened or felled trees. *Megaplatypus mutatus* (= *Platypus mutatus*) (Chapuis) is an ambrosia beetle native to South America (Wood, 1993) that attacks standing 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 not only mainly in commercial plantations of poplar species such as *Populus deltoides* (Santoro, 1963; Achinelli *et al*., 2005; Alfaro *et al*., 2007), but also *Populus*, *Quercus*, *Ulmus*, *Casuarina* and fruit trees (Gimenez & Etiennot, 2003). Furthermore, the dark staining of the tunnels caused by associated fungi reduces the quality and commercial value of wood.

Megaplatypus mutatus was accidentally introduced in Italy in 1998 (Tremblay *et al*., 2000; Allegro & Della Beffa, 2001) and threatens poplar plantations that 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 it to the EPPO/OEPP Alert List in 2004 and, in 2007, recommended treating it 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. After copulation, *M. mutatus* pairs extend their galleries to produce offspring. In previous studies we showed that, to attract females, pioneer male *M. mutatus* emit (+)-6-methyl-5-hepten-2-ol [(+)-sulcatol], 6-methyl-5-hepten-2-one (sulcatone) (González-Audino *et al*., 2005) and 3-pentanol (Gatti-Liguori *et al*., 2008).

We developed controlled release dispensers for pheromones and various trap designs to be baited with a range of pheromones doses. In field studies in Argentina and Italy, we demonstrated the

Correspondence: Paola González Audino. Tel.: +54 11 4709 8224; fax: +54 11 4709 5334; e-mail: pgonzalezaudino@citedef.gob.ar

effectiveness of the pheromone blend for monitoring *M. mutatus* infestations (Funes *et al*., 2009, 2013; González-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. This disruption prevents reproduction. The strategy is frequently used for controlling lepidopteran pests, although it has seldom been exploited for coleopteran species. In the case of *M. mutatus*, there are several mitigating factors that favour the potential success of this strategy: the adults are relatively immobile with low dispersiveness (Santoro, 1962) and mated females lay their eggs in the host tree and die inside, making the risk of invasion from adjacent areas very low and and making the simultaneous treatment of thousands of hectares unnecessary. Also, *M. mutatus* has a cryptic lifestyle that protects it against treatments with conventional insecticides (Jutsum & Gordon, 1989) and systemic treatments are very expensive to apply in commercial poplar plantations. On the other hand, pheromones are relatively low cost, stable under field conditions during the flight period and can be formulated in controlled release devices.

Previously, we 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 (Funes *et al*., 2011). For the present study, we manufactured matrix/monolithic dispensers in which the pheromone is dispersed in a matrix and filler materials are added. We selected the dispensers with a release rate in accordance with our previous results employing polyethylene dispensers, aiming to replace these in mating disruption field trials. We applied a mating disruption strategy of *M. mutatus* in an infested poplar plantation in Argentina using wax and polyethyleneglycol pheromone dispensers.

Materials and methods

Pheromone dispensers

Pheromones sulcatone (99%), (±) sulcatol (99%) and 3-pentanol (98%) were purchased from Sigma-Aldrich (Saint Louis, Missouri).

Dispensers used for monitoring purposes were of a reservoir type with zero-order kinetics and were deployed in the field during the female flying period. In reservoir systems, the pheromone is stored separately from the polymeric membrane controlling the release rate. In a previous study, we demonstrated that racemic sulcatol can be used to replace (+)-sulcatol because the (−) isomer does not interfere with its attracting capacity (Funes *et al*., 2013). For sulcatone and 3-pentanol, we used rectangular bags made with a nonpermeable side (high density polyethylene of 80 μm) and a semi-permeable side (low-density polyethylene of 40 μm). For sulcatol, we built bags with two semi-permeable sides (Table 1). The devices were homogeneously distributed by hand throughout the plot, representing 1.26, 1.82 and 1.58 g/ha for sulcatone, 3-pentanol and (±) sulcatol, respectively. The dispensers were attached to the trees with a pin 1.6 m above the ground. Pheromone dispensers were used in sets of three: one for each component.

For MD trials, the dispensers were monolithic/matrix type. In these systems, the pheromone is homogeneously dissolved and/or dispersed in a polymer matrix (Tojo, 1985). Dispensers were prepared by melting the matrix, thoroughly mixing with 20% of the filler component and 20% of each pheromone, pouring into a silicone mould containing half sphere shaped wells (diameter 3 cm) and cooling until solidification at 5–10 °C. Release rates were measured by weight loss per day in a wind tunnel (0.5 m/s at 27–28 °C) and monitored until pheromone depletion or until constant weight was achieved.

As polymeric matrices, we used polyethylene glycols of molecular weight of 1500 and 6000, paraffin wax m.p. 53–57, estearine and bees wax. As fillers, we used kaolin, talc and bentonite. Three replicates of each half sphere dispenser were prepared and their release rate measured. The curves were fitted and constants and regression coefficients (r) were calculated using OFFICE 2002 (Microsoft Corp., Redmond, Washington) and ORIGIN version 6.0 (Microcal Software, Northampton, Massachusetts) (Table 2). From the release rates curves, with the same software, we calculated the initial release rates V_i for sulcatone, sulcatol and 3-pentanol from the different matrices.

According to the previous results and the release rates required (Funes *et al*., 2011), we selected dispensers to be used in the field. Thus, for 3-pentanol and sulcatone, we used the dispenser made with paraffin and kaolin as filler and, for (±) sulcatol, we used polyethylene glycol 6000 with no filler added.

The pheromone dispensers were attached to the trees in sets of three, one for each component and adjacent to each other, by pinning them on a small nail on the tree's surface 1.6 m above the ground. The sets were distributed uniformly throughout each treatment plot at a density of 40 sets of three per hectare. Devices were checked weekly and replaced before total depletion.

Flight period detection

To determine the beginning of the female flying period with the aim of applying the pheromone dispensers, we monitored the

Table 1 Release rate (mg per day) (mean ± SE) of pheromone lures used for monitoring purposes containing sulcatone, 3-pentanol or sulcatol, at 27–28 °C and 0.5 m/s in a laboratory wind tunnel

Compound	Type of dispenser	Effective release area (cm^2)	Release rate (mg/day)	Initial pheromone load (mL)
Sulcatone (6-methyl-5-hepten-2-one)	Glass vials with polyethylene semipermeable cap	0.2	6.68 ± 0.63	0.5
3-Pentanol	Polyethylene bags	20	29.84 ± 1.8	1
(±) Sulcatol [(±)-6-methyl-5-hepten-2-ol]	Polyethylene bags	16	11.27 ± 0.51	0.5

Table 2 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

Pheromone (20%)	Matrix	Inert (20%)	First-order release parameters			V_i (mg/day)[a]
			a	*b*	r^2	
Sulcatone (6-methyl-5-hepten-2-one)	Paraffin wax	Talc	4.14	0.028	0.984	63.6
		Kaolin	5.76	0.062	0.977	140.6
		Bentonite	3.51	0.097	0.979	77.3
		–	1.19	0.113	0.989	60.3
	Estearine	Talc	4.41	0.025	0.981	43.1
		Kaolin	4.64	0.032	0.984	33.7
		Bentonite	3.84	0.035	0.989	37.9
		–	1.05	0.1154	0.984	27.3
	Bees wax	Talc	6.65	0.059	0.966	240.9
		Kaolin	3.85	0.078	0.966	226.8
		Bentonite	3.27	0.045	0.973	25.4
		–	2.76	0.119	0.989	95
	PEG 1500		0.46	0.245	0.938	14
	PEG 6000		1.71	0.438	0.980	59.3
3-Pentanol	Paraffin wax	Talc	2.30	0.070	0.983	39.4
		Kaolin	4.19	0.080	0.98	85.7
		Bentonite	4.70	0.044	0.988	84.9
		–	0.64	0.093	0.994	31.9
	Estearine	Talc	5.41	0.027	0.996	59.2
		Kaolin	3.94	0.020	0.992	31.6
		Bentonite	4.71	0.025	0.995	37.7
		–	1.39	0.048	0.991	21.9
	Bees wax	Talc	5.23	0.038	0.976	134.2
		Kaolin	6.15	0.050	0.984	187.7
		Bentonite	4.12	0.022	0.996	44.4
		–	1.62	0.096	0.991	50.4
	PEG 1500		2.48	0.110	0.997	46
	PEG 6000		3.60	0.216	0.993	140
(±) Sulcatol [(±)-6-methyl-5-hepten-2-ol]	Paraffin wax	Talc	1.28	0.050	0.98	26.5
		Kaolin	1.38	0.050	0.98	26.9
		Bentonite	1.36	0.040	0.99	28.8
		–	0.63	0.070	1	16.7
	Estearine	Talc	0.58	0.040	0.99	12.1
		Kaolin	1.82	0.040	1	22.1
		Bentonite	1.19	0.170	1	28.2
		–	0.77	0.040	0.99	9.7
	Bees wax	Talc	1.73	0.050	0.99	36.9
		Kaolin	0.58	0.1200	0.93	35.6
		Bentonite	4.11	0.0400	0.99	63.2
		–	1.5	0.0900	0.99	34.7
	PEG 1500		1.82	0.130	0.98	100
	PEG 6000		3.75	0.090	1	127.8

[a] Release rate at day 1. Calculated from the differentiation of the pheromone release curve Mass versus time.
Parameters *a*,*b*, from the equation $M = ae^{-bt}$. r^2, correlation coefficient.
PEG, poly(ethylene glycol).

population using pheromone-baited traps (Funes *et al.*, 2011). The temporal monitoring was performed in an adjacent plantation (35°10′00.9″S and 60°16′N, 68 m.a.s.l.), with the same agro meteorological characteristics and the same clones. The baits used were rectangular bags with lower release rates than those used for mating disruption. Thirty CIPEIN-F-type cross-vane traps made of two acrylic panels in a cross-arrangement above a funnel were deployed (Funes *et al.*, 2009, 2011, 2013). All traps were checked at least weekly in accordance with weather conditions.

Field trial locations

Field trials were performed during the flight season of *M. mutatus* between 2 December 2011 and 7 January 2012, in a 12-ha stand of 11 year-old *Populus* × *canadensis* 'Conti 12' located at Alberti, Buenos Aires Province, Argentina (35°10′00.1″S, 60°17′W, 68 m a.s.l.). Mean stand density was 1111.1 trees per hectare ($3 \times 3\ \mathrm{m}^2$) with a mean diameter at breast height of 28.55 ± 0.47 cm.

In this plantation, we marked off three 1.5-ha plots separated by at least 300 m around the centre of the stand. Each plot was split into two equal areas for control and treatment.

Damage assessment

Two parameters were used to evaluate damage by *M. mutatus*: mated galleries (MG) and active galleries (AG). MG are galleries where a male initiated attack, lured a female and mating took place, and where both male and female are extending the gallery length inwards. These galleries are in straight line with no twists, 5–6 cm in length and the female is in the lead position. The frass particles surrounding the gallery are light brown, 2–3 mm long, 0.13–0.15 mm wide and needle-shaped. After 2 months, MG become AG. Thus, AG are the entrance holes where a male initiated attack, lured a female, mating took place, females laid their eggs, eggs hatched and feeding larvae produced sawdust expelled outside by adults. This gallery expelled sawdust are disaggregated particles of irregular shape and flour-like quality (0.13–0.15 mm) (Santoro, 1963) and the length of the gallery is more than 6–7 cm. In each case, the length of the gallery was measured with a small calibrated wire, which is sufficiently accurate to measure the first 10 cm.

Both AG and MGs represent galleries that achieved successful mating. We use the parameters AGs or MGs in a comparative way between control and treated plots. Before treatment, we use AGs in control versus treated plots as damage indicator because they are representative of succesful attacks from the previous flying season. After treatment, we chose MG in control versus treated plots because they represent successful new attacks. The comparison of MGs in control versus treated plots after treatment represents the success of the pheromone treatment.

Megaplatypus mutatus damage in control and pheromone-treated plots was assessed before placing the pheromone devices and from 10 to 35 days after the end of the experiment. 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 carefully carried out before the trial by examining tree trunks, identifying active galleries (AG) and numbering them individually. In each experimental area, and in both treated and control plots, we randomly sampled 30% of the trees (Sower *et al.*, 1982). We assigned every tree a number and, afterwards, we picked random numbers until 30% of individuals were reached. The surveillance of galleries was performed up to 2 m high for each tree. Old galleries (dry) from previous seasons were also marked to avoid confusion when the galleries were quantified later on.

Damage was expressed as mean number of AG or MG per tree, and the means of treated and control areas were compared using a *t*-test after the trial using STATISTICA, version 5.0 (StatSoft, Tulsa, Oklahoma).

Results

Release rates of monolithic dispensers

Release rates of sulcatol, sulcatone and 3-pentanol from matrices aged in the wind tunnel varied in a nonlinear manner and were characterized by the exponential equation $M = ae^{-bt}$, where M is mass (g) time t, a is the amplitude constant, e is the base of natural logarithm, b is the decaying constant and t is time (days). For all determinations, P was < 0.0001.

From the same curves, we calculated V_i (i.e. release rate at day 1) for sulcatol, sulcatone and 3-pentanol, as well as for each matrix and its mixture with the fillers, respectively (Table 2). Values ranged from 10 to 250 mg of pheromone released per day.

Based on these results, the optimal dispensers for mating disruption trials were selected taking into account the release rates used in our previous study (Funes *et al.*, 2011) and deployed them in a commercial poplar plantation in Buenos Aires, Argentina, during the flight season of *M. mutatus*. Thus, for sulcatol, we used polyethylene glycol 6000 (V_i 128 mg/day), and for sulcatone (V_i 141 mg/day) and 3-pentanol (V_i 86 mg/day), paraffin wax/kaolin 20%, with 20% pheromone in all cases.

Flight period detection

Catches of female *M. mutatus* were monitored in a plantation adjacent to the treatment area (Fig. 1). *Megaplatypus mutatus* peaked initially around November 18, confirming the arrival of the flight period. When a second raise in the population was detected, dispensers for mating disruption were placed (12 December). After two consecutive falls of insect catches, dispensers were withdrawn (7 January).

Mating disruption of M. mutatus in the field

Prior to pheromone deployment (30 November to 1 December), the damage level measured as AG was similar both in treated and control plots because the mean number of AG per tree did not differ significantly between the treated and control plots in the poplar plantations. AGs are larval galleries that originated after the attacks during the previous flying season.

After the mating disruption treatment, the damage level was instead surveyed as MG (10–15 January). MGs represent males successful to mate a female, and we found that the mean number of MG per tree was significantly lower in the pheromone-treated plot than in the control plots ($t = -2.258$; d.f. = 58; $P = 0.027$) (Table 3).

Discussion

Efficient controlled-release systems are essential for delivering behaviourally relevant aerial concentrations of sex pheromones for both monitoring and mating disruption purposes in the field. In previous work, we obtained very good results with respect to monitoring and mating disruption of *M. mutatus* using polymeric controlled release dispensers for pheromones of a reservoir type. In the present study, we replaced polyethylene reservoir dispensers by matrix dispensers made with natural waxes and polyethylene glycols and obtained similar results. Release rates for the matrix dispensers followed first-order kinetics, as expected for monolithic systems. The use of natural materials for dispensers has obvious advantages from an environmental point of view and, furthermore, these dispensers deployed in the field do not require additional work for their ultimate disposal after the treatment.

After the experiment, we found that the number of mating and/or active galleries was significantly higher (70%) in control than in treated areas, as in our previous findings with polymeric dispensers (Funes *et al.*, 2011), thus confirming that the strategy

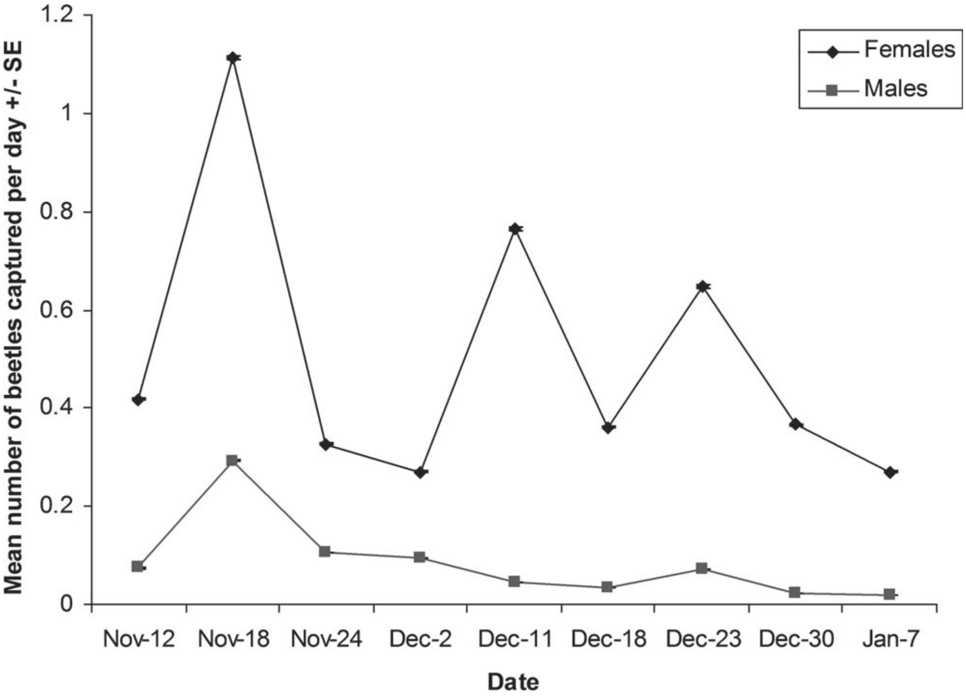

Figure 1 Number of male and female *Megaplatypus mutatus* captured per trap per day during the 2009–2010 season in Alberti, Buenos Aires, using pheromone-baited traps in a plantation adjacent to experiment site. Arrows indicate the duration of the mating disruption trial.

Table 3 Mean number of *Megaplatypus mutatus* active galleries (AG) before the trial and mating galleries (MG) after the trial per tree in infested control and pheromone-treated plots of poplar plantations

Plot	AG/tree Before trial	MG/tree After trial
Control	0.92 ± 0.19^{a}	0.76 ± 0.14^{a}
Treated	0.97 ± 0.17^{a}	0.23 ± 0.08^{b}

Means within a column followed by the same superscript letter are not significantly different ($P < 0.05$).

AG, galleries with larval activity that precede future adult emergency; MG, galleries with a mated couple extending the gallery inwards. Comparisons are valid within the same column and the same location.

of mating disruption using the pheromone delivery doses is a viable tool for the management of infested poplar plantations.

Although the discussion about the behavioural mechanisms involved in the mating disruption is beyond the scope of the present study, the high-release dispensers (compared with the monitoring lures) also showed an attractant effect on beetles, and females were frequently observed walking over the bark around the dispensers attached to the tree trunk.

Several aspects of the biology of *M. mutatus* contribute to the promising success of mating disruption for controlling this species. First, the adults are relatively immobile with low dispersiveness (Santoro, 1962) and mated females lay their eggs in the host tree and die inside, making the risk of invasion from adjacent areas very low and also making the simultaneous treatment of thousands of hectares unnecessary. Second, *M. mutatus* has a cryptic lifestyle that protects it against treatments with conventional insecticides (Jutsum & Gordon, 1989) and systemic treatments are very expensive to apply in commercial poplar plantations. Third, *M. mutatus* does not feed on host phloem or xylem before or after emergency (Santoro, 1962; P. Gatti, unpublished data) and so it is free-living for only a limited amount of time, with that time being spent fully on the search of a mate. This may enhance the influence of pheromones more than in species that spend time on foraging (Hasewaga *et al.*, 1993). Fourth, the sex ratio is 1 : 1 (Santoro, 1963), making the location of males by females less likely than in cases where there is a bias towards females. In that case, the timing of emergency is expected to mediate female mating success through its effect on the operational sex ratio (Bessa-Gomes *et al.*, 2004). Fifth, poplar plantations have extensive foliage during summer, reducing the diffusion of pheromones outside the plantation (Cardé, 1990). Last, because of its relatively low fecundity (50–300 eggs per female), mating must be reduced to a lower degree than with other more fecund insects to affect population change (Kolodny-Hirsch & Schwalbe, 1990).

Inhibiting female orientation may produce delays in the process of finding a male that could prove to be fatal because females may soon begin to suffer from dehydration under the hot summer conditions (P. Gatti, unpublished data). Even if the pheromone does not prevent mating and only delays finding males, a definitive effect is produced on the population because males very frequently die when waiting for a female to arrive (Santoro, 1963). Also, protandry minimizes the pre-reproductive death of females and restricts the mating success of late emergent females at low population density (Calabrese & Fagan, 2004; Rhainds, 2010); thus, if females fly in the search of males because of the confusion generated by high releases of male pheromones, this effect could be increased.

The level of attack or the density of the beetle affects the succes of mating disruption. Indeed, insect density is a major limiting factor that can affect the mating disruption strategy (Howell *et al.*, 1992). In the present study, the initial density was one attack per tree, and the difference in attack density between control and treated plots after treatment was 70%. In our previous work conducted in Italy and Argentina, we obtained differences of 77%, 65% and 56% in attack density between control and treated plots, with inital densities of 0.5, 2 and 4 attacks per tree, respectively (Funes *et al.*, 2011), which indicates that there is probably a relationship between attack density and the success of mating disruption. Also, in the treatment for *Cydia pomonella*, the efficacy of different types of formulations with pheromones is highly dependent on codling moth density (Vickers & Rothschild, 1991; Trimble, 1995; Stelinski *et al.*, 2008). Mating disruption of *Lobesia botrana* was less effective with aggregated populations because of the increased chance of a male entering into the active space of a calling female (Schmitz *et al.*, 1995).

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

The direct and indirect costs of mating disruption applications depend on the country where it is applied because labour costs are very country-dependent. In the case of Italy, and probably in the rest of Southern Europe, the cost of labour and materials per hectare for mating disurption are in the same order of costs as for insecticide treatments and insecticide application is less effective than mating disruption.

Acknowledgements

We are very grateful to Carlos Urionaguena and Daniel Sama from Establecimientos San José, Aserraderos Euskadi, SA, Alberti, Buenos Aires, Argentina. PGA and EZ are members of the CONICET. HF received a grant from ANPCyT. We thank Dr Edward P. Horty for the careful revision of the manuscript. This study received financial support from the ANPCyT of Argentina (PICT 2010-305).

References

Achinelli, F.G., Liljersthröm, G., Aparicio, A., Delgado, M., Jouanny, M. & Mastrandrea, C. (2005) Daños por taladrillo (*Megaplatypus mutatus* (=*Platypus sulcatus*)) en plantaciones de álamo (*Populus* spp.) de Alberti, Buenos Aires: análisis preliminar de la magnitud y distribución de fustes quebrados. *Revista Asociación Forestal Argentina*, **59**, 8–11.

Alfaro, R., Humble, L.M., Gonzalez Audino, P., Villaverde, R. & Allegro, G. (2007) The threat of the ambrosia beetle *Megaplatypus mutatus* (Chapuis) [= *Platypus mutatus* Chapuis] to world poplar resources. *Forestry*, **80**, 471–479.

Allegro, G. & Della Beffa, G. (2001) Un nuovo problema entomologico per la pioppicoltura Italiana: *Platypus mutatus* Chapuis (Coleoptera, Platypodidae). *Sherwood Foreste ed Alberi Oggi*, **66**, 31–34.

Bessa-Gomes, C., Legendre, S. & Clobert, J. (2004) Allee effects, mating systems and the extinction risk in population with two sexes. *Ecology Letters*, **7**, 802–812.

Calabrese, J.M. & Fagan, W.F. (2004) Lost in time, lonely, and single: reproductive synchrony and the Allee effect. *American Naturalist*, **164**, 25–37.

Cardé, R.T. (1990) Principles of mating disruption. *Behavior-Modifying Chemical for Insect Management* (ed. by R. Ridgway, R. Silverstein and M. Inscoe), pp. 47–71. Marcel Dekker, Inc., New York, New York.

EPPO/OEPP (2004) *First Report of* Platypus mutatus *in Italy: Addition to the EPPO Alert List*. EPPO Reporting Service No. 04 2004/061. EPPO/OEPP Pest Risk Analysis Reporting Service, France.

EPPO/OEPP (2007) *Report of the 39th Meeting of the Panel on Phytosanitary Measures (Paris, 2007-03-06/09) 07-13694.* [WWW document]. URL http://archives.eppo.org/EPPOStandards/PM1_GENERAL/pm1-02 (16)_A1A2_2007.pdf [accessed on September 2007].

Funes, H., Zerba, E. & González-Audino, P. (2009) Comparison of three types of traps baited with sexual pheromones for Ambrosia beetle *Megaplaytpus mutatus* in poplar plantations. *Journal of Economic Entomology*, **102**, 1546–1550.

Funes, H., Zerba, E. & González-Audino, P. (2011) Mating disruption of the ambrosia beetle *Megaplatypus mutatus* in poplar and hazelnut plantations using reservoir systems for pheromones. *Entomologia Experimentalis et Applicata*, **139**, 226–234.

Funes, H., Zerba, E. & González-Audino, P. (2013) Effect of release rate and enantiomeric composition on response to pheromones of *Megaplatypus mutatus* (Chapuis) in poplar plantations of Argentina and Italy. *Bulletin of Entomological Research*, **103**, 564–569.

Gatti-Liguori, P., Zerba, E., Alzogaray, R. & González-Audino, P. (2008) 3-pentanol: a new attractant present in volatile emissions from the ambrosia beetle, *Megaplatypus mutatus*. *Journal of Chemical Ecology*, **34**, 1446–1451.

Gimenez, R.A. & Etiennot, A.E. (2003) Host range of *Platypus mutatus* (Chapuis, 1865) (Coleoptera: Platypodidae). *Entomotropica*, **18**, 89–94.

González-Audino, P., Villaverde, R., Alfaro, R. & Zerba, E. (2005) Identification of volatile emissions from *Platypus mutatus* (= *sulcatus*) (Coleoptera: Platypodidae) and their behavioral activity. *Journal of Economic Entomology*, **98**, 1506–1509.

González-Audino, P., Gatti-Liguori, P. & Zerba, E. (2011) Traslucent pheromone traps increase trapping efficiency of ambrosia beetle *Megaplatypus mutatus*. *Crop Protection*, **30**, 745–747.

Gonzalez-Audino, P., Griffo, R., Gatti, P., Allegro, G. & Zerba, E. (2013) Pheromone detection of the introduced forest pest *Megaplatypus mutatus (=Platypus mutatus)* (Chapuis) (Platypodinae, Curculionidae) in Italy. *Agroforestry Systems*, **87**, 109–115.

Griffo, R., Pesapane, G., Funes, H., Gonzalez-Audino, P. & Germinara, G.S. (2012) Diffusione e controllo di platipo in Campania. *L'Informatore Agrario*, **31**, 66–68.

Hasewaga, M., Leal, W.S. & Sawada, M. (1993) Field evaluation of *Anomala schondeldti* Ohaus (Coleoptera: Scarabaeidae) synthetic sex pheromone. *Journal of Chemical Ecology*, **19**, 1453–1459.

Howell, J.F., Knight, A.L., Unruh, T.R., Brown, D.F., Krysan, J.L., Sell, C.R. & Kirsch, P.A. (1992) Control of the codling moth in apple and pear with sex pheromone-mediated mating disruption. *Journal of Economic Entomology*, **58**, 918–925.

Jutsum, A.R. & Gordon, R.F.S. (1989) Pheromones: importance to insects and role in pest management. *Insect Pheromones in Plant Protection*, pp. 89–92. John Wiley and Sons, U.K.

Kolodny-Hirsch, D.M. & Schwalbe, C.P. (1990) Use of dispalure in the management of the gypsy moth. *Behavior-Modifying Chemical for Insect management* (ed. by R. Ridgway, R. Silverstein and M. Inscoe), pp. 363–379. Marcel Dekker, Inc., New York, New York.

Rhainds, M. (2010) Female mating failures in insects. *Entomologia Experimentalis et Applicata*, **136**, 211–226.

Santoro, F.H. (1962) La cópula en *Platypus sulcatus*. *Revista de Investigaciones Forestales*, **3**, 25–27.

Santoro, F.H. (1963) Bioecología de *Platypus sulcatus* Chapuis (Coleoptera – Platypodidae). *Revista de Investigaciones Forestales*, **4**, 47–78.

Schmitz, V., Roehrich, R. & Stockel, J. (1995) Disruption mechanisms of pheromone communication in the European grape moth *Lobesia botrana*. *Journal of Applied Entomology*, **199**, 303–308.

Sower, L.L., Overhulser, D.L., Daterman, G.E., Saterman, C., Laws, D.E. & Koerber, T.W. (1982) Control of *Eucosma sonomana* by mating disruption with synthetic sex attractant. *Journal of Economic Entomology*, **75**, 315–318.

Stelinski, L.L., McGhee, P., Grieshop, M., Brunner, J. & Gut, L.J. (2008) Efficacy and mode of action of female – equivalent dispensers of pheromone for mating disruption of codling moth. *Agricultural and Forest Entomology*, **10**, 389–397.

Tojo, K. (1985) Intrinsic release rate from matrix-type drug delivery systems. *Journal of Pharmaceutical Sciences*, **74**, 685–687.

Tremblay, E., Espinosa, B., Mancini, D. & Caprio, G. (2000) Un coleottero proveniente dal Sudamerica minaccia i pioppi. *L'Informatore Agrario*, **56**, 89–90.

Trimble, R.M. (1995) Mating disruption of controlling the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Torticidae) in organic apple production in southwestern Ontario. *Canadian Entomologist*, **127**, 493–505.

Vickers, R.A. & Rothschild, G.H.L. (1991) Used of sex pheromone for control of codling moth. *World Crop Pest: Tortricid Pest: Their Biology, Natural Enemies and Control* (ed. by L. P. S. Van der Geest and H. H. Evenhuis), pp. 339–354. Elsevier, New York, New York.

Wood, S.L. (1993) Revision of the genera Platypodidae (Coleoptera). *Great Basin Naturalist*, **53**, 259–281.

Accepted 9 August 2015