Comparison of Three Types of Traps Baited With Sexual Pheromones for Ambrosia Beetle *Megaplatypus mutatus* (Coleoptera: Platypodinae) in Poplar Plantations

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ABSTRACT *Megaplatypus mutatus* (Coleoptera: Platypodinae) is an ambrosia beetle native to South America that only attacks standing live trees and is a serious problem for commercial poplar (*Populus* L.; Salicaceae) plantations in Argentina. The development of traps baited with synthetic pheromones that can be used for monitoring *M. mutatus* in infested poplar plantations is an important goal in preventive programs. Pioneer male *M. mutatus* emit a pheromone composed mainly by (+)-sulcatol and sulcatone. In the current study, we tested their release rates from several polymeric reservoir systems, to develop and manufacture a pheromone-releasing device. The efficacy of three different types of traps was evaluated in the field. Single funnel traps equipped with cross-vanes (CIPEIN-CV) captured significantly more insects than multiple funnel traps (LINDGREN) and simple funnel traps (CIPEIN-F).

KEY WORDS *Megaplatypus mutatus*, pheromone, (+)-sulcatol, sulcatone, trap design

Platypodid ambrosia beetles (Coleoptera: Platypodidae: Platypodinae: Platypodini) are an important group of forest pests that attack mainly felled or weakened trees. The common name "ambrosia beetle" derives from the fungus carried and inoculated by the adult beetles and on which the larval stages feed. The dark-stained tunnels caused by the decaying ambrosia mycelium of *Raffaelea santoroi* (Guerrero) (Bascialli et al. 1996) reduce the quality of the wood required for export.

Megaplatypus mutatus (Chapuis) is an ambrosia beetle native to South America that attacks only living trees, penetrating into the xylem of its host by boring large tunnels. This weakens the stem, causing it to break during wind storms, and represents a serious problem in poplar, *Populus deltoides* Bartram ex Marshall, commercial plantations (Achinelli et al. 2005, Alfaro et al. 2007). The attack is initiated when the male excavates a tunnel through the bark. With the boring dust it builds a crown-shaped rim around the gallery entrance from which it emits volatile emissions with to attract individuals of the opposite sex (Santoro 1962). A similar behavior has been reported for *Platypus apicalis* White and *Platypus gracilis* Broun in New Zealand (Milligan and Ytsma 1988).

In a previous study, we showed that volatile emissions of *M. mutatus* are composed mainly of (+)-6-methyl-5-hepten-2-ol [(+)-sulcatol, or retusol] and 6-methyl-5-hepten-2-one (sulcatone) (González Audino et al. 2005). Behavioral bioassays in olfactometer and electroantennogram recordings confirmed an attractive response, with (+)-sulcatol and sulcatone specifically attracting females. These results suggested that *M. mutatus* emits a sex pheromone composed mainly by (+)-sulcatol and sulcatone. Other platypodids (Renwick et al. 1977, Shore and McLean 1983) and scolytids (Byrne et al. 1974, Borden and McLean, 1979, Fletchmann and Berisford 2003) also respond to sulcatol but not to sulcatone.

Although *M. mutatus* is native to South America, it has recently been introduced to Italy (Allegro and Della Beffa 2001), causing great concern because poplar plantations are a highly important economic resource. Therefore, the development of traps baited with synthetic pheromones that can be used for the management of *M. mutatus* in infested poplar plantations as well as for detection of low level populations is an important goal for control programs.

The aim of the current study was to develop a plastic pheromone dispenser using a range of doses of (+)-sulcatol and sulcatone to test the efficacy of various traps designs in the field.

Materials and Methods

Synthetic Chemicals. Analytical grade sulcatone (6-methyl-5-hepten-2-one) was obtained from Sigma (St. Louis, MO) and (+)-sulcatol, (+)-6-methyl-5-hepten-2-ol, also known as retusol, 99% was obtained from Con-Tech Systems (Delta, BC, Canada).

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Table 1. Geometrical shape, material, effective area for pheromone release, commercial source and daily release rate in laboratory conditions of plastic pheromone lures containing 100 mg (+)-sulcatol or sulcatone except for the devices tested with high release rates (≥40 mg/d) where the amount used was 200 mg

Device	Semipermeable material (thickness of the wall)	Effective area (cm^2)	Source	Release rate (+)-sulcatol ± SE (mg/d)	Release rate sulcatone ± SE (mg/d)
Vials with flip-top cap PN Z136166	Polyethylene	0.5	Sigma	<1	<1
Bottles, 5 ml	Polyethylene (1 mm)	2.2	Envitap S.A.	<1	11.3 ± 1.1, R^2 = 0.99
Commercial bubble caps, PN L2 2022/000	Polyethylene	7.5	Con-Tech Systems	11.2 ± 0.4, R^2 = 0.99	—[a]
Plastic 1.5-ml tubes, PN 022364111	Polyethylene	7.5	Eppendorf	<1	—
Rectangular bags with a nonpermeable side and a semipermeable side	Polyethylene (40 μm)	4	Polisur	5.50 ± 0.30, R^2 = 0.99	41.2 ± 4.5, R^2 = 0.92
	Polyethylene (40 μm)	8	Polisur	9.02 ± 0.08, R^2 = 0.99	58.7 ± 2.9, R^2 = 0.97
	Polyethylene (40 μm)	24	Polisur	19.7 ± 0.02, R^2 = 0.99	—
	Polyethylene (40 μm)	4	Alfatech	11.5 ± 0.01, R^2 = 0.99	—
	Polyethylene (80 μm)	4	Alfatech	11.5 ± 0.01, R^2 = 0.99	—
	Polyethylene (80 μm)	8	Alfatech	—	60.1 ± 0.7, R^2 = 0.99
Glass vial with a plastic semipermeable cap	Rubber cap (70 mm) PN[b] 27226	0.8	Supelco, Bellefonte, PA	—	<1
	Terbutilic rubber (30 mm) PN Z166065	0.8	Supelco	—	<1
	Polyethylene (40 μm)	0.07	Polisur	—	<1
	Polyethylene (40 μm)	0.2	Polisur	—	7.5 ± 0.01, R^2 = 0.93
	Polyethylene (80 μm)	0.2	Polisur	—	8.4 ± 0.02, R^2 = 0.99
	Polyethylene (40 μm)	0.2	Alfatech	—	9.5 ± 0.3, R^2 = 0.99
	Polyethylene (80 μm)	0.2	Alfatech	—	9.8 ± 0.4, R^2 = 0.99
	Clear silicone rubber (20 mm) PN Z166081	0.8	Supelco	—	11.9 ± 0.01, R^2 = 0.97

Linear correlation coefficients, R^2, are means of three replicates.
[a] —-, not tested.
[b] PN, part number.

Pheromone Release Devices. The release rates of (+)-sulcatol and sulcatone from different polymeric reservoir systems were tested. In these systems, the pheromone is stored separately from the release rate controlling polymeric membrane.

Table 1 and Fig. 1 show the characteristics of the plastic reservoir systems (devices) tested for the delivery of (+)-sulcatol and sulcatone. All the devices initially contained 100 mg (+)-sulcatol or sulcatone (Table 1). To test their release rate in the laboratory, we monitored the daily weight loss at 30°C until they reached constant weight, indicating that all the pheromone was gone. To test devices with high release rates, we used 200 mg of pheromone.

Commercial bubble caps (Con-Tech Systems) where used as models of a probable release rate of pheromones for ambrosia beetles, but unfortunately they were only available only for sulcatol but not for sulcatone. Polyethylene vials and tubes were tested only with sulcatol (see Results). Semipermeable rectangular bags with different effective areas were also tested. To obtain higher release rates with rectangular bags we increased the surface area as necessary by increasing the size of the bag. For low release rates for sulcatone, we used a glass vial equipped with a small evaporation surface in the cap made with semi permeable low density polyethylene. The devices contained 100 mg (+)-sulcatol or sulcatone except for the devices tested with high release rates (≥40 mg/d) where the amount used was 200 mg. We performed three replicates of this experiment for each device and for each compound. Linear regressions of the weight loss values were calculated to determine the release rates and to establish their profile of linearity. For this, Microsoft Office Package (2002) (Microsoft, Redmond, WA) was used.

Comparison of Three Types of Traps for Capture of *M. mutatus* in Poplar Plantations. Field trials were performed during the flight season of *M. mutatus* between 17 November 2006 and 21 February 2007, in a commercial poplar (*P. deltoides*) plantation. The experimentation area consisted of 9 ha within an 11-yr-old plantation, with a tree density of 1,111 trees per ha (square of plantation 3 by 3 m) and a mean diameter at breast height of 24.5 ± 1.02 cm.

The goal of this trial was to compare the efficacy of three different types of traps, all baited with the same lures, (+)-sulcatol and sulcatone in rate 10:10 mg/d (nominal). Emission rates selected to test in the field were based on the release rate of commercial sulcatol bubble cups as determined in our laboratory (see Results) and previous information available for other ambrosia beetles (Borden et al. 1980, Deglow and Borden 1988, Young-Biao and McLean 1989), because pheromone rates emitted by male *M. mutatus* are highly variable between insects according to the number of days from gallery initiation (L. Gatti et al., unpublished data). For example, Deglow and Borden (1998) use with good results retusol bubble cups from Con-Tech Systems (release rate under laboratory conditions, 10 mg/d; see Results).

The devices used for sulcatol were rectangular bags of 8 cm^2 of semipermeable surface (low-density polyethylene, 40 μm) and for sulcatone we used glass vials with a plastic semipermeable cap of polyethylene (40 μm; Alfatech, RS, Brasil) with an effective area of 0.2

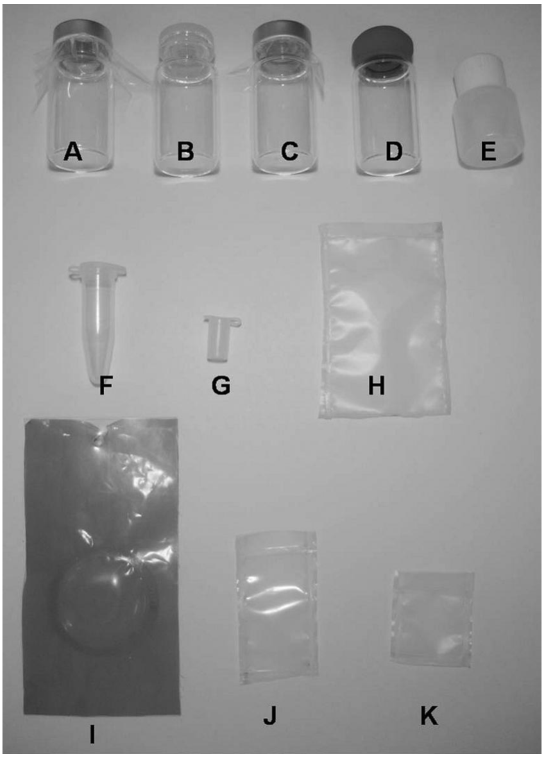

Fig. 1. Pheromone release devices tested for sulcatol and sulcatone. (A and C) Glass vial with a polyethylene semipermeable cap. (B) Glass vial with a clear silicone cap. (D) Glass vial with a terbutilic rubber cap. (E) Bottles, 5-ml polyethylene (Envitap S.A., Buenos Aires, Argentina). (F) Polyethylene, 1.5-ml tubes (Eppendorf, Hamburg, Germany). (G) Vials with flip-top caps (Sigma). (H, J, and K) Rectangular polyethylene bags with a nonpermeable side and a semipermeable side (Alfatech and Polisur [Buenos Aires, Argentina]). (I) Commercial bubble caps (Con-Tech Systems).

cm^2 according to Table 1. The traps were (Fig. 2) as follows:

1. CIPEIN-CV is a cross-vane trap of two black acrylic panels in a cross arrangement above a funnel. The diameter of the funnel is 20 cm. The total height of the trap is 70 cm. The color of the trap is black.
2. LINDGREN is a multiple funnel trap made of eight black funnels trap (Con-Tech Systems). Diameter of the funnels is 18.5 cm and the color is black. Total height of the trap is 1.5 m.
3. CIPEIN-F consists of one funnel that acts as trapping surface and another that acts as collector. The diameter of the funnel is 26.5 cm and the color is dark red. Total height is 50 cm.

The effective trapping areas of the traps were as follows: CIPEIN-CV, 1,050 cm^2; LINDGREN trap, 5,796.2 cm^2; and CIPEIN-F trap, 1,844.6 cm^2. In the CIPEIN-CV traps, the dispensers were hung in the top of the vanes; in the in the LINDGREN traps, dispensers were hung inside the bottom funnel; and in the CIPEIN-F traps, the dispensers were placed inside the collector funnel (Fig. 2).

We used 10 baited and five unbaited traps of each type. The traps were hung from the trees with ropes 1.8 m above ground level and were set 30–40 m apart in five parallel lines of nine traps each in a randomized design. They were rotated in random way weekly or every 2 wk as access to the field site allowed. Lures were replaced before complete pheromone consumption.

Beetle captures in the different traps were analyzed statistically using the nonparametric Kruskal–Wallis test (STATISTICA, StatSoft, Tulsa, OK), followed by Dunn's method (Infostat 2004).

Results

Pheromone Release Rates. All the materials tested showed a linear release rate until complete consumption as was expected for diffusion controlled membrane-moderated reservoir systems (Tojo 1985). Table 1 shows the release rates of (+)-sulcatol and sulcatone from each device calculated from the slope of the line obtained and their respective linear correlation coefficients. Commercial bubble caps where used as models of a probable release rate of pheromones for ambrosia beetles, but unfortunately they were only available only for sulcatol but not for sul-

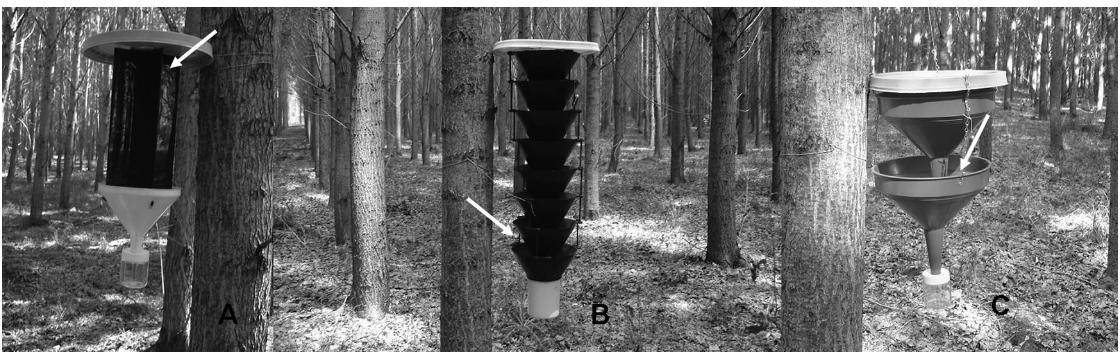

Fig. 2. Different traps types used for trapping *M. mutatus*. (A) CIPEIN-F cross-vane trap. (B) LINDGREN multiple funnel trap (Con-Tech Systems). (C) CIPEIN-F funnel trap. Arrows point the position of the dispensers.

Table 2. Means ± SE number per trap of *M. mutatus* caught from 16 November to 27 February [release rates of (+)-sulcatol and sulcatone were 10:10]

Trap	Total mean no. female caught per trap[a]
CIPEIN-CV	5.6 ± 1.4a
LINDGREN	1.0 ± 0.4b
CIPEIN-F	1.0 ± 0.4b

Means followed by the same letter are not significantly different ($P < 0.05$, Kruskal Wallis). Kruskal–Wallis test, $\chi^2 = 9.60$ df = 2, $P < 0.01$.

[a] There were no insects caught in any of the control unbaited traps.

catone. Polyethylene flip-top vials showed to be not permeable with non finite release rates to both sulcatol and sulcatone. Polyethylene bottles were not permeable to sulcatol, but they were to sulcatone. Rectangular bags were tested for both components, and release rates for sulcatol were similar to the commercial bubble caps. However, release of sulcatone was too high, so we incorporated another type of device made with a nonpermeable glass vial equipped with a small evaporation surface in the cap made with semipermeable low-density polyethylene. In this case, the release rates for sulcatone were similar to the rectangular bags for sulcatol and similar to commercial bubble caps.

Comparison of Efficacy of Different Types of Traps. Single cross-vane funnel traps (CIPEIN-CV) captured significantly more insects (5.6 ± 1.4) than LINDGREN and CIPEIN-F traps (Kruskal–Wallis test, $\chi^2 = 9.6$, df = 2, $P < 0.01$) (Table 2). There were no insects caught in any of the control unbaited traps, indicating that pheromone baits exhibit a significant attractive response compared with empty traps.

Discussion

Several experimental devices for the slow release of (+)-sulcatol and sulcatone were prepared in our laboratory. All of them showed a linear weight loss, and from the slopes we were able to calculate the respective release rates. We obtained release rates from <1 to 60 mg/d at 30°C. The rates obtained match well with those of commercial devices (Deglow and Borden 1988). Also, they match well with bibliography data, taking into account that these rates were determined at 22–24°C (Yong-Biao and McLean 1989).

Our results showed that pheromone lures exhibit a significant attractive response compared with control traps. The difference in effectiveness of the various traps tested is important in improving the efficacy of pheromone-baited traps. CIPEIN-CV traps captured significantly more insects than LINDGREN traps and CIPEIN-F traps.

Trap efficacy is also directly influenced by the lure release rate (Lindgren 1983). Although the dispensers were all the same, the lures were more or less exposed to the open environment depending on the trap type. In the CIPEIN-F trap and LINDGREN trap, the dispensers were set into the funnels, whereas in CIPEIN-CV traps they were hung over the vanes. So, it is probable that the pheromone plume is more obstructed in the funnel traps without vanes, and the lure was more exposed in the most efficient trap. This could mean in the field a slight difference in the release rates owing to factors such as wind and temperature. Similar results were obtained by Flechtmann et al. (2000) who compared the efficiency of different traps used in Brazil for survey of ambrosia beetles and other Scolytidae and found that the highest catches per unit surface in one of the traps tested was likely associated with a higher bait release rate.

Ethanol and α-pinene baits are frequently used as general attractants for trapping ambrosia beetles (Yong-Biao and McLean 1989, Flechtmann et al. 2000). Ethanol is a major product of anaerobic fermentation in dead or dying wood tissue (Moeck 1970), and α-pinene is a major naturally occurring terpene component in live and dead tree tissue (Fengel and Wegener 1984). However, that *M. mutatus,* unlike most ambrosia beetles, attacks only standing live trees and does not attack cut wood or weakened trees, and that it does not attack conifer species, negates the possibility of the use of these effective and low-cost baits. This is another reason why the development of traps baited with synthetic pheromones for surveillance programs and for managing *M. mutatus* in infested poplar plantations is an important goal for both European and South American control efforts. However, our present efforts are oriented to the determination of the optimal release rate of the pheromone components as well as their optimal blend.

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