

# Kairomone traps: a tool for monitoring the invasive spruce bark beetle *Dendroctonus micans* (Coleoptera: Scolytinae) and its specific predator, *Rhizophagus grandis* (Coleoptera: Monotomidae)

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## Summary

1. The Eurasian spruce bark beetle *Dendroctonus micans* is a major pest of spruce which is expanding its range in France, Turkey, England and Wales. Its monospecific predator *Rhizophagus grandis* has followed its prey naturally into most areas and since the 1960s has also been mass-produced and released within newly infested locations. Assessing bark-beetle presence at previously uninfested sites, or predator establishment after release or natural spread, currently depends on direct observation of *D. micans* brood chambers. This is a slow and unreliable process because bark-beetle attacks are solitary, cryptic and occur on living trees. An effective trapping method would thus be most useful.
2. A synthetic lure for *R. grandis* was first field-tested in release–recapture experiments. Release points were surrounded by one or two concentric rings of traps baited with either *D. micans* larval frass or with a blend of synthetic chemicals. Kairomone-trapping using the synthetic lure was then tested in the field, monitoring *R. grandis* in 58 spruce stands in Belgium and France.
3. In the release–recapture experiments, up to 65% of the released predators were caught within 48 h. Catches with the synthetic lure and with prey frass were not significantly different.
4. Field-trapping assays showed *R. grandis* establishment in 30 stands, including areas where the predator had been introduced artificially. Trapping was unsuccessful at the southern and western limits of *D. micans*' range. Combined visual and trapping approaches identified 32 stands containing the predators, with two false negatives with the trapping method, compared to 19 for the visual surveys.
5. Unexpectedly, male and female *D. micans* adults were trapped in some cases. The combined approach identified 43 attacked stands, among which there were four false negatives with the trapping method and 11 with the visual surveys.
6. *Synthesis and applications.* To our knowledge, this is one of the first cases of using kairomone traps to monitor a predator after releases for biocontrol. Furthermore, because this predator is strictly prey-specific, this technique also identifies stands colonized by *D. micans* bark beetles. Following inoculative predator releases, kairomone trapping could be used to survey for *D. micans* in areas under colonization or in European Union 'Protected Zones'.

**Key-words:** beetles trapping, biological control, invasive pests monitoring, lures, oxygenated monoterpenes, pheromones, predator–prey relationship, semiochemicals

## Introduction

The European spruce beetle *Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae) is a widespread pest of spruce in Eurasia. The females are fertilized by their brothers prior to

emergence, and they attack solitarily apparently healthy trees. One single female is thus able to start a new colony. Unlike many aggressive bark beetles colonizing living trees, *D. micans* does not need to mass-attack and kill its host because of its uncommonly high resistance to the host tree's monoterpenes (Grégoire 1988). Supposedly native to Siberia (Schedl 1932), *D. micans* started invading Europe in the 19th century and is still expanding its range. Today, it is progressing and developing outbreaks in north-western and south-western

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France (Brittany, Normandy, Languedoc-Roussillon, Midi-Pyrénées: Pauly & Meurisse, unpublished data), in the United Kingdom (Cumbria, Devon, Kent, Lancashire, Shropshire and Wales: Fielding *et al.* 1991a; Fielding & Evans 1997; Burgess 2003) and in Turkey (Eastern Black Sea Region: Yüksel 1996). After a few years, however, the outbreaks subside, even though the beetles remain present at very low densities in all colonized sites (Grégoire 1988). These changes have been attributed to a specific natural enemy, *Rhizophagus grandis* Gyllenhal (Coleoptera: Monotomidae), which also originates from eastern Eurasia, follows *D. micans* in its spread and is considered a key factor in the population dynamics of *D. micans* (Bergmiller 1903; Kobakhidze, Tvaradze & Kraveishvili 1970; Grégoire 1988; Fielding, O'Keefe & King 1991b; Fielding & Evans 1997). *R. grandis*, which is strictly specific to *D. micans* (an extremely rare feature among predators), relies at least partly upon semiochemicals (specific long- and short-range attractants) to locate its prey (Grégoire *et al.* 1992a,b; Wyatt, Phillips & Grégoire 1993) and stimulate oviposition (Grégoire *et al.* 1991).

Biological control using mass-reared *R. grandis* is a common strategy used against *D. micans*. Since its development in Georgia in the early 1960s (Kobakhidze *et al.* 1970), the method has been used in France (Grégoire *et al.* 1985; Van Averbeke & Grégoire 1995), the United Kingdom (Fielding *et al.* 1991b; Fielding & Evans 1997) and Turkey (Yüksel 1996). *D. micans* populations almost always decline below the economic injury level 6–10 years after *R. grandis* releases (Tvaradze 1977; Van Averbeke & Grégoire 1995; Fielding & Evans 1997). A frequent approach is to inoculate newly colonized stands on invasion fronts, as early as possible, so that the demographic lag between predators and prey is minimal. In this respect, accurate monitoring of the spread of *D. micans* and of *R. grandis* is necessary.

Bark-beetle monitoring often relies upon pheromone trapping. The aggregation pheromones of most of the aggressive scolytine species have been identified and are commercially available. *D. micans*, however, does not aggregate on attacked trees, and there is no evidence that it uses pheromones in colonizing a new host (Vasechko 1976; Gilbert, Vouland & Grégoire 2001). Therefore, only visual surveys are currently possible. Foresters walk into stands and search for attacked trees. As the trees usually do not die unless colonized successively by several beetle generations, attack symptoms are only visible at close range: purple-brown resin tubes, entrance holes, boring dust and frass. Ground surveys for *D. micans* are thus highly time-consuming and inaccurate, especially in the initial stages of infestation (Grégoire 1988). Detection of *R. grandis* requires the additional step of exposing bark-beetle brood chambers to carefully examine their contents.

Here we report on the development of methods for kairomone-trapping *D. micans*' specific predator, *R. grandis*, to provide an easy way to monitor both species in stands of uncertain infestation status at the border of *D. micans*' range. First, long-range release–recapture experiments tested the attraction of kairomone-baited traps for *R. grandis*. Secondly, field-trapping surveys were conducted in stands where *D. micans*

was absent, scarce or abundant in Belgium and France to assess the efficiency of kairomone trapping for detecting wild *R. grandis* individuals and, unexpectedly, male and female *D. micans*.

## Materials and methods

### RELEASE–RECAPTURE EXPERIMENT

#### Period and location

The experiment took place in July 1991 in the Forêt domaniale du Somail (Languedoc-Roussillon, France), an area in the Massif Central mountains that was far outside of the range of *D. micans* at the time of experiment (Fig. 1a). Sixteen *R. grandis* release–recapture trials were performed in 15 different spruce stands, located at least 500 m away from each other.

#### Beetle production and storage

*R. grandis* was mass-cultured in polystyrene boxes; larvae were fed frozen *Calliphora* sp. larvae. Adult beetles were stored upon emergence in a refrigerator at 4 °C, in groups of 150 individuals in moist bark powder.

#### Traps

Kreins<sup>(R)</sup> vane traps were used (Perfor SA, B-5060 Auvelais, Belgium). These consist of two rectangular panes of black polyvinylchloride (PVC) (width: 32 cm; length: 60 cm) crossing at right-angles and thus forming four 15 × 60 cm vanes erected vertically above a black PVC funnel (diameter: 33 cm; height: 18 cm) ending in a collecting bottle. The traps were baited either with larval *D. micans* frass or with synthetic attractants.

#### Frass

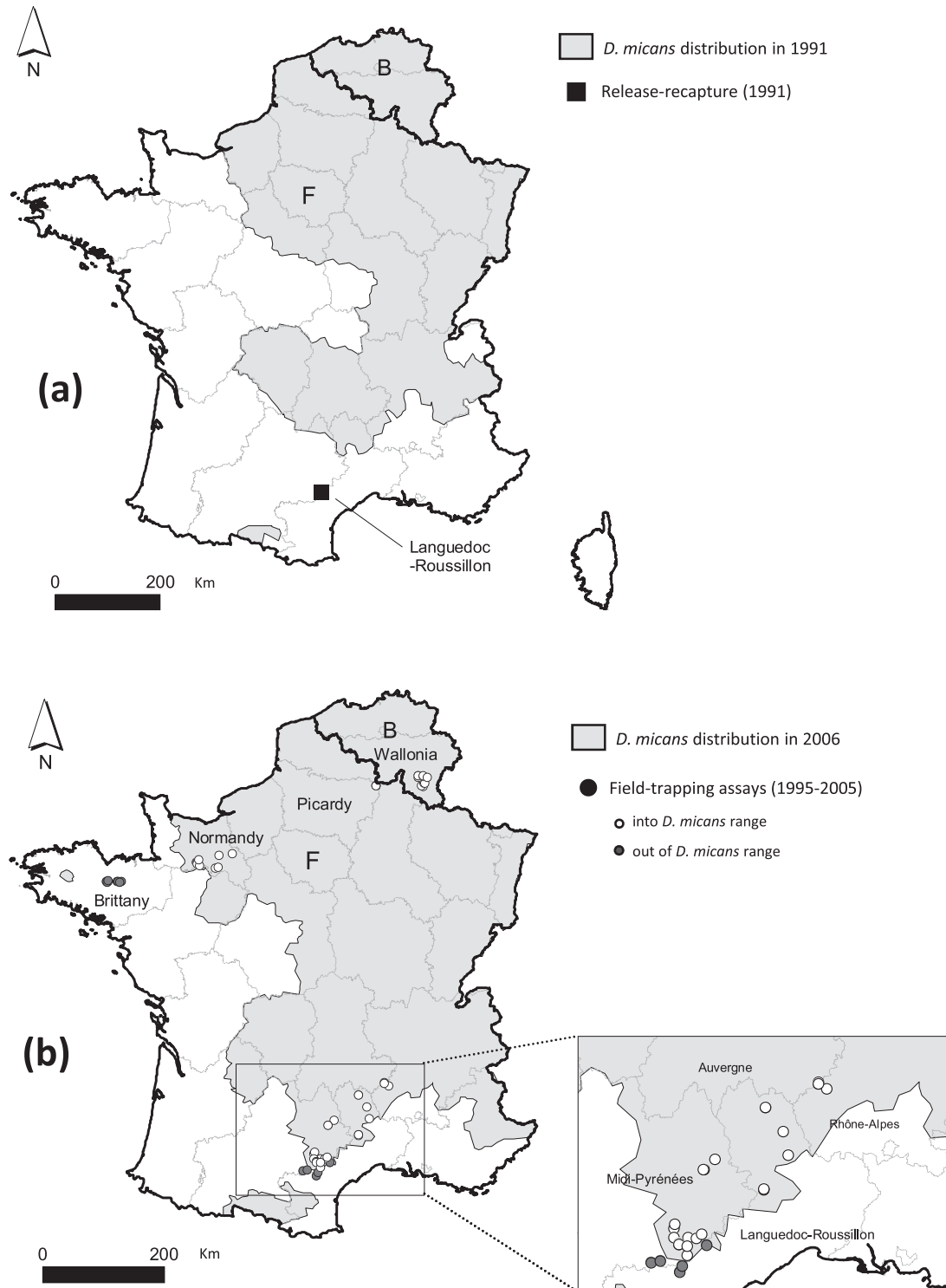
Larval frass of *D. micans*, collected in the same season in the Massif Central and stored at –18 °C, was used as a control. One dose of 130 mg was used per trap, packed into an Eppendorff disposable pipette tip (Wyatt *et al.* 1993). No attempt was made to measure the diffusion rate of volatiles released by the frass.

#### Synthetic attractants

A 190 mg mixture of equal amounts of all components identified by Grégoire *et al.* (1992b) was used: (1S)-(–)-verbenone [chemical purity: 94%; ( $\alpha$ )25: –130°]; (1R)-endo-(+)-fenchyl alcohol (97%); (1R)-(–)-fenchone [98%; ( $\alpha$ )24: –50.5°]; (1S)-endo-(–)-borneol [98%; ( $\alpha$ )20–35.6°]; (+)-terpinene-4-ol [96%, ( $\alpha$ )22 + 29°]; (–)- $\alpha$ -terpineol [90%; ( $\alpha$ )20–47°]; racemic camphor (96%). An equal amount of pentane (99%) was used as a solvent. All chemicals were purchased from Sigma-Aldrich NV (B-2880, Bornem, Belgium).

#### Dispensers: nature, size, release rates

Dispensers were made of 2 × 2 cm filter-paper wicks (Whatman no. 1) contained in sealed polyethylene bags (density: 4.13 mg cm<sup>–2</sup>). Previous measurements in the laboratory (still air; room temperature: 20–25 °C) showed a linear diffusion rate of 3.5 mg<sup>–1</sup> day during the first 16 days ( $R^2 = 0.94$ ; 18 d.f.;  $P < 0.001$ ) (Couillien & Grégoire 1994). No attempt was made to measure release rates in the field.



**Fig. 1.** Study areas in Belgium and France. Black square on the first map (a) indicates release–recapture study site, relative to the 1991 distribution of *Dendroctonus micans*. Black and white circles on the second map (b) indicate kairomone trapping sites, relative to the distribution of *D. micans* in 2006.

#### Experimental set-up and procedure

Different trap arrangements were used (Table 1). Simple linear arrangements, where a trap was established either at 50 m or at 100 m from the release point, were first deployed as preliminary feasibility

tests (trials 1–3). Later, in circular arrangements, each release point was surrounded by one or two concentric rings of traps, either 50 and/or 100 m distant from the release point (trials 4–16). There were four traps in the first ring and eight traps in the second ring. Circumference section between traps in any ring was 78.5 m; distance

**Table 1.** Conditions and results of the *Rhizophagus grandis* release–recapture experiments

Trial	No. of beetles released	No. of dead beetles	Attractant <sup>1</sup>	Trap arrangement <sup>2</sup>	Numbers caught (real)		% caught <sup>3</sup> (estimated)	Temperature at release time/remarks
					24 h	48 h		
1	150	33	A	L <sub>50</sub>	0	0	0%	14 °C (cloudy/rain)
2	140	30	A	L <sub>100</sub>	2	0	4.5%	25 °C (sun)
3	150	40	A	L <sub>50</sub>	1	0	2.3%	29 °C (sun)
4	420	94	A	S <sub>50</sub>	0	0	0%	19 °C (cloudy)
5	420	80	A	S <sub>100</sub>	0	0	0%	19 °C (cloudy)
6	420	70	A	D	0	0	0%	14 °C (cloudy/rain)
7	256	22	C	S <sub>50</sub>	0	0	0%	30 °C (sun/clouds)
8	407	75	A	S <sub>50</sub>	41	0	30.9%	29 °C (sun)
9	382	15	A	S <sub>50</sub>	18	1	12.9%	28 °C (sun/clouds)
10	431	18	A	S <sub>50</sub>	46	0	23.7%	31 °C (sun)
11	437	10	F	S <sub>50</sub>	51	6	28.4%	24 °C (sun)
12	422	98	A	S <sub>100</sub>	39	1	30.9%	29 °C (sun)
13	430	12	A	S <sub>100</sub>	49	0	24.9%	31 °C (sun)
14	420	60	A	D	74	0	51.4%	23 °C (sun)
15	415	81	F	D	85	3	65.9%	25 °C (sun)
16	446	20	A	D	113	1	56.9%	24 °C (sun)

<sup>1</sup> F = frass; A = synthetic attractants; C = control (no attractant). <sup>2</sup> S<sub>50</sub> = one single ring of four traps, 50 m from release point; S<sub>100</sub> = one single ring of eight traps, 100 m from release point; D = two rings, 50 and 100 m from release point (12 traps in total); L<sub>50</sub>; L<sub>100</sub> = linear set-up with one single trap 50 or 100 m from release point. <sup>3</sup>Proportion caught calculated as follows:  $100 \times \text{no. caught} / [(\text{no. released} - \text{no. dead}) \times \text{flight capacity}]$ .

between two neighbouring traps in a ring was *c.* 71 m at 50 m from release point, and 77 m at 100 m. Traps were affixed either to wooden posts or to trees, facing the release point, with their base 1 m from the ground. Beetles were released from an open polystyrene Petri dish 14 cm in diameter, with the bottom lined with moist blotting paper laying on a wooden post 25 cm above ground level. The insects, stored previously in a refrigerator at 5–6 °C, were placed in the Petri dish at 9 a.m. to start the experiment. Traps were inspected after 24 h, 48 h and, in some cases, *c.* 1 week after the releases. At the same time as the first inspection of the traps, dead insects remaining in the Petri dish were also counted. The specific conditions for each trial are given in Table 1. Previous field surveys had suggested that both *D. micans* and *R. grandis* were absent from these areas. However, the traps were baited 24 h before each release, so that wild *R. grandis* could be detected. No wild *R. grandis* were caught, and therefore the released beetles were not marked.

#### STATISTICAL ANALYSIS

To allow comparisons between trials, *R. grandis* catches were converted into proportions of flying insects trapped. Numbers of flying insects per trial were estimated by first excluding those beetles which died before take-off, then by using a take-off coefficient (proportion of beetles taking off) established in the laboratory during the same year with samples of insects coming from the same breeding batches (Couillien & Grégoire 1994), and which varied linearly with the age of the beetles. As the beetles used in these experiments were either 2 or 6 months old, a coefficient of either 0.40 (trials 2–3, 8–9, 12, 14–15) or 0.47 (trials 10–11, 13, 16) was applied. The spatial distribution of catches was analysed separately within each trial using  $\chi^2$  tests, except for trials 9 and 14 (ring at 100 m), where expected values were less than 5. In these cases the binomial law was used to compute the probability associated with the catches in each trap. According to

each experimental arrangement, either four directions (N, S, E, W: four traps at 50 m from release point) or eight directions (N, S, E, W, NE, NW, SE, SW: eight traps at 100 m from release point) were compared. With experiments involving two rings (trials 14–16), relative catches within each ring were analysed separately. Proportions of flying beetles caught per trial or per trap ring within a trial were transformed ( $y = 2\arcsin\sqrt{x}$ ) for all other statistical comparisons. Comparison between frass and synthetic attractant was performed using a Mann–Whitney *U*-test on releases from similar set-ups (trials 8–11, one ring of four traps 50 m distant from release point). Trap arrangements were compared using one-way analysis of variance (ANOVA) (trials 14–16, double rings with 12 traps; trials 8–11 and 12–13, single rings with four or eight traps). Effects of trapping distance (trials 8–11; 12–13) and the presence of another ring in the trial (trials 14–16) were compared using two-way ANOVA. Data processing was performed using spss (version 13 for Windows) statistical package.

#### FIELD-TRAPPING EXPERIMENTS

##### Periods and locations

*R. grandis* wild populations were surveyed from 1995 to 2005 in a total of 58 spruce stands. Study sites were chosen to represent a range of different *D. micans* epidemiological conditions (absent, limit of known range, endemic): in Belgium (Wallonia), northern France (Picardy), north-western France (Normandy, Brittany) and in central France (Massif Central: Auvergne, Languedoc-Roussillon, Midi-Pyrénées, Rhône-Alpes) (Table 2, Fig. 1b).

##### Traps

'Bottle traps' were used, made of longitudinally cut PET commercial 1.5-L transparent water bottles, turned upside-down in order to provide a 21 × 13 cm interception pane and connected by the bottleneck

**Table 2.** Field-trapping experiments and visual assessments

Year	Area	Coordinate selevation	Visual assessments		Trapping experiments									
			Attacked trees (%)	Species observed <sup>1</sup>	Trapping period	<i>R. grandis</i> catches			<i>D. micans</i> catches			Total no. traps	<i>R. grandis</i> release	
						(M : F)	period	No. active traps	(M : F)	period	No. active traps			
1995	Languedoc-Roussillon (F)	44-057°N, 3-522°E; 1250 m	5	<i>Dm</i>	4/5–6/11	2 : 3	18/9–6/11	4	–	–	–	20	1984	
		44-055°N, 3-521°E; 1255 m	5	<i>Dm</i>	4/5–6/11	3 : 2	18/9–6/11	3	–	–	–	20	1984	
1996	Auvergne (F)	44-914°N, 4-168°E; 1510 m	15	<i>Dm</i> <i>Rg</i>	5/4–7/10	3 : 2	5/4–22/5	1	–	–	–	20	1987	
							22/5–14/7	2						
							14/7–18/8	1						
							18/8–8/10	1						
		44-924°N, 4-163°E; 1580 m	7	<i>Dm</i> <i>Rg</i>	5/4–7/10	1 : 4	22/5–14/7	2	–	–	–	20	1987	
							14/7–8/8	2						
							8/8–8/10	1						
1996	Languedoc-Roussillon (F)	44-726°N, 3-553°E; 1410 m	75	<i>Dm</i>	21/5–8/10	4 : 6	22/5–14/7	3	–	–	–	20	1978	
				<i>Rg</i>			14/7–17/8	2						
		44-526°N, 3-747°E; 1300 m	13	<i>Dm</i>	22/5–8/10	1 : 0	14/7–18/8	1	–	–	–	20	1978	
				<i>Rg</i>										
		44-330°N, 3-800°E; 1070 m	6	<i>Dm</i>	5/4–8/10	2 : 0	14/7–18/8	1	–	–	–	20	1990	
				<i>Rg</i>			18/8–8/10	1						
		44-057°N, 3-522°E; 1250 m	7	<i>Dm</i>	22/5–8/10	0	–	0	–	–	–	20	1984	
		43-603°N, 2-862°E; 1000 m	0	–	23/5–9/10	0	–	0	–	–	–	20	1990 <sup>3</sup>	
		43-384°N, 2-558°E; 720 m	0	–	23/5–9/10	0	–	0	–	–	–	19	–	
1996	Rhône-Alpes (F)	44-872°N, 4-259°E; 900 m	18	<i>Dm</i> <i>Rg</i>	5/4–7/10	1 : 1	22/5–14/7	2	–	–	–	20	1987	
1999	Auvergne (F)	44-914°N, 4-168°E; 1510 m	5	<i>Dm</i> <i>Rg</i>	5/6–9/12	0 : 1	5/6–13/8	1	–	–	–	20	1987	
1999	Languedoc-Roussillon (F)	44-726°N, 3-553°E; 1410 m	90	<i>Dm</i> <i>Rg</i>	6/6–10/12	5 : 2	6/6–14/8	4	–	–	–	20	1978	
							14/8–7/10	3						
1999	Midi-Pyrénées (F)	44-304°N, 2-968°E; 935 m	20	<i>Dm</i>	8/6–13/12	0 : 2	8/6–15/8	2	–	–	–	20	1996	
		44-222°N, 2-835°E; 880 m	40	<i>Dm</i>	8/6–13/12	2 : 1	8/6–15/8	3	–	–	–	20	1996	
		44-221°N, 2-827°E; 895 m	40	<i>Dm</i>	8/6–13/12	2 : 3	8/6–15/8	3	–	–	–	20	1996	
							15/8–8/10	2						
2003	Wallonia (B)	50-037°N, 5-221°E; 340 m	0	–	20/10–7/10	1 : 0	29/5–6/7	1	0 : 0	–	0	20	–	
		50-057°N, 5-215°E; 400 m	1	<i>Dm</i>	20/10–7/10	2 : 2	19/3–13/4	1	0 : 1	8/9–7/10	1	20	–	
								5/8–8/9	3					
		50-060°N, 5-228°E; 350 m	3	<i>Dm</i>	20/10–7/10	0 : 0	–	0	0 : 0	–	0	20	–	
		49-874°N, 5-344°E; 430 m	2	<i>Dm</i>	20/10–7/10	0 : 2	29/5–6/7	1	0 : 2	29/5–6/7	2	20	–	
		49-883°N, 5-302°E; 460 m	0	–	20/10–7/10	1 : 2	7/11–26/11	1	0 : 2	10/5–29/5	1	20	–	
						29/5–6/7	1		29/5–6/7	1				

Table 2. (Continued)

Year	Area	Coordinate selevation	Visual assessments		Trapping experiments									
			Attacked trees (%)	Species observed <sup>1</sup>	Trapping period	<i>R. grandis</i> catches			<i>D. micans</i> catches			Total no. traps	<i>R. grandis</i> release	
						(M : F)	period	No. active traps	(M : F)	period	No. active traps			
2003	Picardy (F)	49-935°N, 5-380°E; 510 m	2	<i>Dm</i> <i>Rg</i>	20/10-7/10	0 : 0	–	0	0 : 3	29/5-6/7	2	20	–	
		49-930°N, 5-391°E; 540 m	1	<i>Dm</i>	20/10-7/10	0 : 1	29/5-6/7	1	0 : 0	–	0	20	–	
		50-043°N, 5-357°E; 480 m	0	–	20/10-7/10	2 : 1	29/5-6/7 5/8-8/9	2 1	0 : 0	–	0	20	–	
		50-050°N, 5-351°E; 520 m	0	–	20/10-7/10	4 : 5	29/5-6/7 5/8-8/9 8/9-7/10	3 3 1	0 : 0	–	0	20	–	
		50-019°N, 5-454°E; 490 m	0	–	20/10-7/10	3 : 2	29/5-6/7	5	0 : 0	–	0	20	–	
		49-917°N, 4-133°E; 325 m	0	–	31/3-11/8	0 : 1	11/6-30/6	1	0 : 0	–	0	20	–	
		49-917°N, 4-133°E; 260 m	2	<i>Dm</i> <i>Rg</i>	31/3-11/8	3 : 1	11/6-30/6	1	0 : 4	21/4-15/5	1	20	–	
									2 NI <sup>2</sup>	15/5-11/6	2			
										30/6-21/7	1			
										–	0	20	–	
2004	Normandy (F)	49-917°N, 4-133°E; 285 m	0	–	31/3-11/8	5 : 1	21/4-15/5 15/5-11/6	3 2	0 : 0	–	0	20	–	
		49-917°N, 4-133°E; 260 m	0	–	31/3-11/8	1 : 1	11/6-30/6	1	0 : 1	11/6-30/6 30/6-21/7	1 1	20	–	
		48-765°N, 0-434°E; 291 m	17	<i>Dm</i>	19/5-31/10	2 : 3	19/5-30/6	4	0 : 0	–	0	20	–	
		48-728°N, 0-106°E; 232 m	23	<i>Dm</i>	19/5-31/10	1 : 0	19/5-30/6	1	0 : 3	31/7-31/8 31/8-30/9	1 2	20	–	
		48-612°N, 0-464°E; 265 m	3	<i>Dm</i>	19/5-31/10	0 : 0	–	0	0 : 0	–	0	20	–	
		48-602°N, 0-445°E; 238 m	0	–	19/5-31/10	1 : 0	19/5-30/6	1	0 : 0	–	0	20	–	
		48-582°N, 0-444°E; 227 m	0	–	19/5-31/10	1 : 3	19/5-30/6	4	0 : 0	–	0	20	–	
		48-559°N, 0-382°E; 190 m	0	–	19/5-31/10	0 : 3	19/5-30/6	2	0 : 0	–	0	20	–	
		48-649°N, 0-408°E; 252 m	37	<i>Dm</i>	19/5-31/10	1 : 0	19/5-30/6	1	0 : 4	30/6-31/7 31/8-30/9	3 1	20	–	
		48-514°N, 0-024°E; 387 m	0	–	19/5-31/10	0 : 0	–	0	0 : 0	–	0	20	–	
48-539°N, 0-085°E; 273 m	3	<i>Dm</i>	19/5-31/10	0 : 1	31/8-30/9	1	1 : 0	31/7-31/8	1	20	–			
2005	Brittany (F)	48-208°N, 2-673°E; 170 m	0	–	20/5-26/12	0 : 0	–	0	0 : 0	–	0	3	2004 <sup>3</sup>	
		48-204°N, 2-716°E; 180 m	0	–	20/5-26/12	0 : 0	–	0	0 : 0	–	0	6	2004 <sup>3</sup>	
		48-194°N, 2-685°E; 210 m	0	–	20/5-26/12	0 : 0	–	0	0 : 0	–	0	12	2004 <sup>3</sup>	
		48-224°N, 2-663°E; 185 m	0	–	20/5-26/12	0 : 0	–	0	0 : 0	–	0	24	2004 <sup>3</sup>	
		48-220°N, 2-427°E; 175 m	0	–	20/5-26/12	0 : 0	–	0	0 : 0	–	0	3	2004 <sup>3</sup>	
		48-216°N, 2-414°E; 155 m	0	–	20/5-26/12	0 : 0	–	0	0 : 0	–	0	3	2004 <sup>3</sup>	
		48-228°N, 2-332°E; 135 m	0	–	20/5-26/12	0 : 0	–	0	0 : 0	–	0	3	2004 <sup>3</sup>	
		48-204°N, 2-368°E; 135 m	0	–	20/5-26/12	0 : 0	–	0	0 : 0	–	0	3	2004 <sup>3</sup>	

**Table 2.** (Continued)

Year	Area	Coordinate selevation	Visual assessments		Trapping experiments								
			Attacked trees (%)	Species observed <sup>1</sup>	Trapping period	<i>R. grandis</i> catches			<i>D. micans</i> catches			Total no. traps	<i>R. grandis</i> release
						(M : F)	period	No. active traps	(M : F)	period	No. active traps		
2005	Languedoc-Roussillon (F)	43·597°N, 2·646°E; 677 m	95	<i>Dm</i>	4/5–16/12	0 : 0	–	0	17 : 64	4/5–31/5 31/5–30/6 30/6–31/7 31/7–31/8 31/8–30/9	3 3 8 13 3	20	1990 <sup>3</sup>
		43·457°N, 2·241°E; 863 m	0	–	02/5–13/12	0 : 0	–	0	0 : 0	–	0	20	–
		43·472°N, 2·342°E; 698 m	0	–	02/5–13/12	0 : 0	–	0	0 : 0	–	0	20	–
		43·431°N, 2·588°E; 846 m	0	–	4/5–15/12	0 : 0	–	0	0 : 0	–	0	20	–
		43·664°N, 2·657°E; 896 m	29	<i>Dm</i>	3/5–14/12	0 : 0	–	0	1 : 9	30/6–31/7 31/7–31/8 31/8–30/9 30/9–14/12	4 1 1 1	20	–
2005	Midi-Pyrénées (F)	43·655°N, 2·751°E; 967 m	0	–	3/5–14/12	0 : 0	–	0	0 : 0	–	0	20	–
		43·692°N, 2·805°E; 939 m	0	–	3/5–14/12	0 : 0	–	0	0 : 1	30/6–31/7	1	20	–
		43·740°N, 2·497°E; 653 m	48	<i>Dm</i>	3/5–14/12	0 : 0	–	0	0 : 8	30/6–31/7 31/8–30/9	6 1	20	–
		43·775°N, 2·504°E; 687 m	44	<i>Dm</i>	3/5–14/12	0 : 0	–	0	22 : 48 2 NI <sup>2</sup>	30/6–31/7 31/7–31/8 31/8–30/9 30/9–14/12	8 4 1 1	20	–
		43·665°N, 2·491°E; 608 m	6	<i>Dm</i>	3/5–15/12	0 : 0	–	0	0 : 1	30/9–14/12	1	20	–
		43·618°N, 2·552°E; 767 m	4	<i>Dm</i>	3/5–13/12	0 : 0	–	0	0 : 0	–	0	20	2004
		43·612°N, 2·550°E; 750 m	21	<i>Dm</i> <i>Rg</i>	3/5–13/12	0 : 0	–	0	0 : 5 2 NI <sup>2</sup>	30/6–31/7 31/7–31/8 30/9–13/12	2 3 1	20	2004
		43·607°N, 2·551°E; 679 m	24	<i>Dm</i>	3/5–13/12	0 : 0	–	0	0 : 0	–	0	20	2004
		43·517°N, 2·645°E; 900 m	10	<i>Dm</i>	4/5–15/12	1 : 0	–	1	0 : 1	31/5–30/6 30/6–31/7	1 1	20	2004

<sup>1</sup>*Dm* = *Dendroctonus micans*; *Rg* = *Rhizophagus grandis*. <sup>2</sup>NI = sex not identified. <sup>3</sup>Preventive *R. grandis* releases in areas where *D. micans* was still supposed absent.

to a 50-mL clear polystyrene collecting tube filled with ethylene glycol as a preservative.

### Lures

In 1995–2003, lures were identical to those used in the release–recapture experiments. In 2004–05, the kairomone doses were increased to 285 mg, absorbed on  $50 \times 50 \times 2$  mm pieces of viscose, folded into aluminium foil to prevent photo-oxidation, and enclosed in  $60 \times 80$  mm griplock LDPE minibags (density:  $5 \text{ mg}^{-1} \text{ cm}^2$ ) (Joakim S.A., B-1120 Brussels, Belgium).

Under laboratory conditions at 20–24 °C, release rate was shown to decrease slowly for the first 40 days ( $y = 6.47 - 0.06X$ ;  $R^2 = 0.47$ ; 10 d.f.;  $P = 0.14$ ). At the end of this period, the release rate [ $\pm$  standard deviation (SD)] was still  $4.2 \pm 1.1 \text{ mg}^{-1} \text{ day}^{-1}$  in the laboratory, but fell to  $1.6 \pm 0.2 \text{ mg}^{-1} \text{ day}^{-1}$  under summertime field conditions.

### Procedure

In most cases, 20 traps were set up along a 300–400 m transect, with 15–20 m between each trap. Each trap was stapled onto a tree, c. 1.5 m above ground, and orientated so that they could be seen from the previous trap when collecting the catches. The release–recapture experiment had shown that trap orientation does not influence the catches (see results below). The traps were established in March–early June, and recovered in October–December except in the Belgian experiments (which were conducted over a whole year), and experiments conducted in Picardy (which were stopped in August; details in Table 2). Kairomone dispensers and collecting tubes were replaced monthly.

In each experimental site, 100 spruce trees were examined along the transect at the end of the trapping period in order to evaluate the *D. micans* attack rate in the stand. As some attractive effect of the traps on the bark beetle cannot be excluded, a possible overestimation of global attack rate in the stands is conceivable. In 2005, the 20 trees carrying the traps were searched for attack symptoms as well as their 80 nearest neighbours, in order to evaluate trap influence on infestations.

## Results

### RELEASE–RECAPTURE EXPERIMENT

Catches in each trial are given in Table 1. In some releases insects did not take off, presumably because of a relatively

strong wind (observed but not measured; trials 4–5), or because the temperature at release time was too low (trials 1 and 6: see Table 1). These trials were excluded from the analyses. No insects were caught in control traps (trial 7, no bait: see Table 1).

Most of the recaptured beetles were recovered within 24 h (516 of 528), with only a few insects recaptured between 24 h and 48 h after release (12 of 528; Table 1), and no further catches after a week. There were no significant differences between directions of captures in any trial (Table 3).

There was no significant difference ( $U = 2.000$ ,  $P = 0.770$ ) between catches in the frass-baited traps (trial 11) and in the lure-baited traps (trials 8–10; Table 4). Higher catches ( $F_{2,6} = 16.931$ ;  $P = 0.003$ ) were observed in the double rings with 12 traps (trials 14–16) compared to the single rings with four or eight traps (trials 8–11; 12–13; Table 4).

There was no significant difference in catches due to trapping distance ( $F_{1,8} = 0.238$ ;  $P = 0.638$ ) or to the presence of another ring in the trial ( $F_{1,8} = 0.746$ ;  $P = 0.413$ ), and there was no significant interaction ( $F_{1,8} = 2.778$ ;  $P = 0.134$ ) between trapping distance and number of rings in a trial (trials 8–11; 12–13; 14–16; Table 4).

### FIELD-TRAPPING EXPERIMENTS

Catches and field observations are given in Table 2.

*R. grandis* was caught in almost all sites where *D. micans* was considered endemic (northern study sites in Wallonia and Picardy: 41 captures in 12 of 14 sites; 0–3% attacked trees per site). Similar captures were obtained at the western limits of *D. micans*' range (Normandy: 16 captures in seven of nine sites; 0–37% attacked trees per site), with one stand with no captures showing symptoms of previous *D. micans* establishment but no *R. grandis*, whereas both species seemed to be lacking from the other. In the southern study sites, devoid of predators, *R. grandis* releases were performed between 1984 and 2004, and followed by trapping campaigns between 1995 and 2005 (Massif Central; 0–95% attacked trees per site). Successful establishment of *R. grandis* was observed when traps were deployed at least 3 years after releases (1995–99 experiments:

**Table 3.** Spatial distribution of captures in the *Rhizophagus grandis* release–recapture experiments (italic type: percentages of total catches in trial; bold type: absolute totals)

Trial	Numbers caught								Total	Statistics
	North	South	East	West	NE	SE	SW	NW		
<b>8</b>	24.4	22	21.1	26.8	26.8	–	–	–	<b>41</b>	$\chi^2_3 = 0.27$ ; $P = 0.966$
<b>9</b>	26.3	36.8	23.9	15.8	–	–	–	–	<b>19</b>	Binomial tests ( $P \gg 0.05$ )
<b>10</b>	21.7	28.3	15.8	26.1	–	–	–	–	<b>46</b>	$\chi^2_3 = 0.44$ ; $P = 0.933$
<b>11</b>	33.3	19.3	12.5	31.6	–	–	–	–	<b>57</b>	$\chi^2_3 = 5.25$ ; $P = 0.155$
<b>12</b>	12.5	10	12.2	12.5	12.5	12.5	12.5	15	<b>40</b>	$\chi^2_7 = 0.40$ ; $P = 1$
<b>13</b>	8.2	14.3	24.4	8.2	10.2	16.3	16.3	14.3	<b>49</b>	$\chi^2_2 = 3.08$ ; $P = 0.877$
<b>14–50 m</b>	26.7	22.2	26.7	–	–	–	–	–	<b>45</b>	$\chi^2_3 = 0.24$ ; $P = 0.970$
<b>14–100 m</b>	10.3	13.8	10.3	13.8	17.2	10.3	10.3	13.8	<b>29</b>	Binomial tests ( $P \gg 0.05$ )
<b>15–50 m</b>	22.9	22.9	18.8	35.4	–	–	–	–	<b>48</b>	$\chi^2_3 = 3.00$ ; $P = 0.392$
<b>15–100 m</b>	12.5	12.5	12.5	15	10	10	15	12.5	<b>40</b>	$\chi^2_7 = 0.80$ ; $P = 0.997$
<b>16–50 m</b>	32.8	20.3	15.6	31.3	12	12	12	14	<b>64</b>	$\chi^2_3 = 5.38$ ; $P = 0.146$
<b>16–100 m</b>	16	10	10	14	–	–	–	–	<b>50</b>	$\chi^2_7 = 1.20$ ; $P = 0.991$



**Table 4.** Distances of captures in the *Rhizophagus grandis* release–recapture experiments

Trial	Traps at 50 m		Traps at 100 m		Total
	No. caught	Proportion of flying beetles caught	No. caught	Proportion of flying beetles caught	
8	41	30.9%	–	–	41
9	19	12.9%	–	–	19
10	46	23.8%	–	–	46
11	57	28.4%	–	–	57
12	–	–	40	31.10%	40
13	–	–	49	24.8%	49
14	45 (60.8%) <sup>1</sup>	31.26%	29 (39.2%) <sup>1</sup>	20.14%	74
15	48 (54.5%) <sup>1</sup>	35.84%	40 (45.5%) <sup>1</sup>	29.86%	88
16	64 (56.1%) <sup>1</sup>	32%	50 (43.9%) <sup>1</sup>	25%	114

<sup>1</sup>Percentage of total catches in trial.

32 captures, 11 of 11 sites). When performed after 1 year (the minimal delay after which any beetle from the original release would have died), *R. grandis* was caught in only one case (2005 experiments: one capture, one of four sites). When *R. grandis* had been released preventively in pest-free areas no insects were trapped subsequently, confirming, as expected, *R. grandis*' incapacity to establish in areas devoid of prey (Massif Central: one of one site; Brittany: eight of eight sites). Finally, in control areas where no predator had been released, no captures were observed, irrespective of the presence (Massif Central: six of six sites) or absence of the bark beetle (Massif Central: five of five sites).

Unexpectedly, *D. micans* adults were also captured in all areas located inside the pest's range. Detailed figures are available from the 2003–05 experiments (Massif Central, 181 beetles caught; Normandy, eight; Picardy, seven; Wallonia, eight). Higher catches were observed at the limit of *D. micans*' range, especially in sites with higher percentages of attacked trees (Massif Central). This observation is supported by a significant linear relation between bark beetle catches per site and the proportions of attacked trees counted at the end of the trapping period ( $R^2 = 0.72$ ; 45 d.f.;  $P < 0.001$ ).

*R. grandis* was caught in 30 stands. The combined visual and trapping approach identified 32 stands containing the predators and, hence, *D. micans*, with two false negatives with the trapping method, against 19 with the visual surveys. *D. micans* was also caught in nine stands without its predator. Prey and predator catches thus identified 39 stands colonized by the bark beetle (32 identifications by visual surveys). Combined visual and trapping approach identified 43 attacked stands, among which there were four false negatives with the trapping method and 11 with the visual surveys.

*R. grandis* was caught from April to November with slightly higher catches in June (Table 2). *D. micans* was caught from May to October, with a major peak occurring in July and August (Table 2). The overall sex-ratio of *R. grandis* in the traps was balanced [1 : 1.02 (55 : 56);  $\chi_1^2 = 0.009$ ;  $P = 0.924$ ; Massif Central: 1 : 1 (27 : 27); Normandy: 1 : 1.67 (6 : 10); Picardy: 1 : 0.44 (9 : 4); Wallonia: 1 : 1.15 (13 : 15)]. In *D.*

*micans*, the overall sex-ratio was biased largely towards females [1 : 3.83 (41 : 157);  $\chi_1^2 = 67.960$ ;  $P < 0.001$ ]; some males were captured in one Norman (1 : 7) and three Massif Central stands [1 : 9; 1 : 2.18 (22 : 48); 1 : 3.76 (17 : 64)]. Visual inventories in 2005 indicated higher *D. micans* captures on trees showing attack symptoms, with 147 catches (33 ♂, 114 ♀) obtained from 30 of the 67 traps located on attacked trees and 30 catches (7 ♂, 23 ♀) from 15 of the 213 traps located on unattacked trees ( $\chi_1^2 = 53.80$ ,  $P < 0.001$ ), with no difference according to sex ( $\chi_1^2 = 0.11$ ,  $P = 0.916$ ). The presence of frass deposits in a few collecting bottles (26 of 1459) suggests that beetles emerging from the trees sometimes fall into the traps (maximum catches in a single trap: 41). Conversely, some captures occurred in traps (2%; six traps) located more than 20 m away from any attacked trees; and one bark beetle was captured in an apparently unattacked stand. As inventories were performed at the end of the trapping period, it cannot be excluded that the lures attracted *D. micans* to the trees. This last hypothesis is supported by the fact that trees carrying traps show attack symptoms more often than the other trees. A total of 51 of 160 trees carrying traps were attacked, but only 148 trees without traps of the 640 neighbouring individuals were attacked ( $\chi_1^2 = 5.24$ ,  $P = 0.022$ ).

## Discussion

### RELEASE–RECAPTURE EXPERIMENT

Trapping was distributed evenly, irrespective of trap orientation; this was due possibly to the near-absence of wind. Absence of wind, sunny conditions and a temperature threshold above 19 °C seems necessary for *R. grandis* optimal flight and response. Wyatt *et al.* (1993) observed little flight activity in wind-tunnel experiments with windspeeds of *c.* 0.5 m<sup>-1</sup> s<sup>-1</sup>, but many more take-offs in speeds around 0.05 m<sup>-1</sup> s<sup>-1</sup> at 19–25 °C. In similar release–recapture experiments, the absence of any perceptible wind (< 1 m<sup>-1</sup> s<sup>-1</sup>) was a prerequisite for *Scolytus multistriatus* (Marshall) to take flight (Lanier, Silverstein & Peacock 1976).

Some *R. grandis* were caught at a relatively short distance (50 m) from the release point, but all insects did not respond at this distance because, in an arrangement comprising two rings, a second ring of traps at 100 m caught the same numbers of beetles as the ring at 50 m. Moreover, a single ring at 100 m caught as many insects as did a ring at 100 m associated with a ring at 50 m, suggesting either that the insects that would respond at 50 m are not the same as those responding at 100 m, or that the traps' attraction radius was smaller than half the distance between them, therefore allowing some beetles to pass a first ring of traps. Salom & McLean (1989) recorded similar trends with *Trypodendron lineatum* (Olivier): while a total of 24.9% of released beetles were recaptured with three rings of traps at 5, 25 and 100 m from the release point and, respectively, comprising four, eight and 16 traps, only 12.4% were caught with a single ring of 16 traps at 100 m. Lanier *et al.* (1976) compared *S. multi-striatus* catches in a ring of three traps at 20 m from release point (traps 20 m apart) with four surrounding rings at 100, 200, 400 and 600 m, respectively, comprising three, three, six, 12 and 18 traps (all traps 200 m apart); the largest proportions of beetles were captured in the innermost (7% of catches, 781 beetles per trap) and the two outer circles (21% and 62% of catches, 561 and 1109 beetles per trap). Furthermore, other bark beetle species need to fly prior to responding to semiochemicals: *D. frontalis* Zimmermann (Turchin & Odendaal 1996), *D. pseudotsugae* Hopkins (Bennett & Borden 1971; Dodds & Ross 2002), *Ips sexdentatus* Herbst (Jactel 1991), *I. typographus* (Linné) (Duelli *et al.* 1997) and *Tomicus piniperda* (Linné) (Perttunen, Oksanen & Kangas 1970).

Numerous captures in the ring at 100 m also suggest that a significant proportion of the predators are able to disperse longer distances. Grégoire *et al.* (1985) and Fielding *et al.* (1991b) observed that released predators can colonize *D. micans* brood galleries within *c.* 200 m.

#### FIELD-TRAPPING EXPERIMENTS

*R. grandis* trapping was successful in all areas where *D. micans* is endemic (Belgium and north of France). There is general agreement today that the endemic situation of *D. micans* at very low population levels is related to high predation by *R. grandis* (Grégoire 1988). Numerous predator catches were also made in Normandy, on the western limits of *D. micans*' range. Although its arrival is recent and various outbreaks still occur, most Norman study sites shelter both *D. micans* and *R. grandis*, the latter probably stemming from northern releases performed in 1989. In western Brittany, *R. grandis* appears not to have established 1 year after releases in 2004. This is consistent with the fact that *D. micans* has not yet been found there in our experimental stands and is still limited to a 320-ha Sitka spruce stand occurring 70 km away.

In the Massif Central, *D. micans* was not followed by *R. grandis*, justifying releases of laboratory-bred predators. In our study, predator trapping was always successful at least 3 years after releases. However, although a new generation of predators was observed in bark beetle brood chambers in the

year following a release, kairomone traps sometimes failed to document predator establishment after such a short time. Possible reasons for this include: (i) low predator population levels; (ii) competition between lures and numerous bark beetle galleries; and (iii) inadequate developmental stages at the time of trapping (immature stages). *R. grandis* was never captured in areas devoid of its prey, even where preventive releases had been performed (parts of Brittany and Massif Central). This result confirms the impossibility for the predator to establish elsewhere than in *D. micans* brood systems. To date, *R. grandis* has never been found in the field on an alternative prey (Francke-Grosman 1954; Grégoire 1988).

*R. grandis* seems to fly most of the year (April–November) while its prey has a shorter flying period (May–October). Francke-Grosman (1954) observed predator activity above a 11–12 °C threshold; Fielding & Evans (1997) estimate the threshold for *D. micans* to be 16–17 °C.

Bark beetle catches in some of our experimental stands remain difficult to explain. *D. micans* is a solitary colonizer of live spruce. This species does not use aggregation pheromones (Vasechko 1976; Grégoire 1988), and no significant response towards attacked trees could be detected in previous experiments (Grégoire, unpublished data). As we caught mainly *D. micans* in heavily attacked stands, most of the beetles could have come from the trees carrying the traps or from attacked trees nearby. However, the traps are small and their area represents less than 1% of that of the trunk, hence excluding random interception of landing beetles. Male *D. micans* were caught in four experimental sites during our 2003–05 experiments, in particular in two heavily attacked stands. The overall male to female sex-ratio among our catches (1 : 4) matches that observed in brood chambers in the field by Francke-Grosman (1954) (1 : 5–1 : 48), and by Chararas (1962) (1 : 5).

#### PROSPECTS

Further colonization of European spruce stands by *D. micans* is considered inevitable. However, accurate predictions about its future expansion are not possible. *D. micans* has a well-developed dispersal capacity, probably over 10 km (Forsse 1989; Gilbert & Grégoire 2003). Moreover, this species can also disperse over much longer distances with commercial timber movements. For example, in France, extensive timber transportation after the 1990 and the 1999 windstorms probably caused the sudden appearance of the pest in the French Pyrénées and in Brittany (Douzon, unpublished data), located 120 and 230 km, respectively, from the nearest known bark beetle populations.

As-yet uncolonized European spruce stands located outside *D. micans*' present range are thus all threatened in the long term. In areas isolated by geographical barriers from the main range of spruce, *R. grandis* may not naturally follow *D. micans* (as happened in the Caucasus mountains and the United Kingdom), thus increasing the risk of damaging outbreaks.

There is a need both for pest monitoring and for early biological control in areas susceptible to colonization. The success of predator establishment can be monitored easily

using traps baited with synthetic kairomones. Another very exciting possibility offered by this technique is indirect monitoring of the pest itself. *D. micans* does not respond to aggregation pheromones and does not kill the host trees when at low population levels, therefore making early detection in newly colonized areas very difficult. The predators, which are extremely efficient at locating their prey, even at very low densities, could thus be used as indicator of *D. micans* presence. Small inoculative releases in supposedly yet uncolonized areas would be followed by successful establishment only if the prey were present. Although the grounds already exist for developing such a technique, careful calibration of the method (sensitivity and range of the traps, phenological constraints) is still needed.

#### SYNTHESIS AND APPLICATION

To our knowledge, there is only one other case to date of kairomone traps used for monitoring predator establishment after releases for biocontrol (Borgemeister *et al.* 1997). Because *R. grandis* is strictly prey-specific, this technique also allows for identification of stands colonized by its target pest after natural or artificial introduction of the predator. A final promising prospect is to use *R. grandis* as an indicator of *D. micans* presence in areas of uncertain infestation status. If successful kairomone-trapping is observed in the years following preventive predator releases, its establishment means that this area is colonized by the bark beetle. In this context, kairomone trapping could be used for surveying the pest in areas under colonization or in European Union 'Protected Zones'.

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