The aggregation pheromone of *Ips duplicatus* and its role in competitive interactions with *I. typographus* (Coleoptera: Scolytidae)

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Summary

Ips duplicatus with *I. typographus* co-inhabiting Norway spruce (*Picea abies* (L.) Karst.) would benefit from a pheromone blend distinct from that of the larger competitor *I. typographus*. GC-MS analysis showed that *I. duplicatus* males feeding in the host produced ipsdienol (Id), *cis*-verbenol (cV), *trans*-verbenol (tV), myrtenol (Mt), and *E*-myrcenol (EM) and traces of 2-methyl-3-buten-2-ol (MB). *I. duplicatus* produced Id in approximately racemic form (48.9–54.5% (+)-(*S*)-isomer). The amounts of Id and EM released over a 9 day period had a maximum of 250 and 5 ng/h/male, respectively, on day 2. Exposure of *I. duplicatus* males to myrcene and α-pinene resulted in the production of small amounts of Id, cV, tV, Mt, and *trans*-pinocarveol, but not of EM. In laboratory bioassays with walking beetles, the pheromone component Id alone was weakly attractive while EM was inactive, but in binary combination with Id strongly synergized attraction. A combination of EM and Id at a release rate equivalent to 100–200 males was more attractive in the field than 70 unmated males in a spruce log. The addition of myrcene (a suggested pheromone precursor of Id) to Id did not enhance trap caches, while addition of EM increased catches >10-fold. Subtracting EM from a blend of Id, EM, cV and MB drastically reduced trap catches while subtraction of cV or MB or both had no significant effect. Addition of EM over a wide concentration range to the synthetic pheromone of *I. typographus* did not reduce the attraction of females of this species in the laboratory. A two-species pheromone interaction field test releasing *I. typographus* pheromone components (MB + cV) at 10–1000 male equivalents (ME) and *I. duplicatus* pheromone (Id + EM) at 0, 10–1000 ME in all possible combinations showed both positive intraspecific dose-response effects and an interspecific inhibition. Higher release rates of EM appeared to inhibit *I. typographus*, especially males. In a tree colonization model, the response of the two competing species to their respective pheromones show a good separation during the mass-attack with a small initial cross-attraction. It remains to be shown whether either of the two pheromone systems have in fact evolved in the present sympatry, or if they are an incidental effect of ancestry of these phylogenetically distant *Ips*.

Key words

competition, inhibition, sex-ratio, mass-attack model, ipsdienol, *E*-myrcenol, *cis*-verbenol, 2-methyl-3-buten-2-ol, Coleoptera, Scolytidae

Introduction

The double-spined spruce engraver of Eurasia, *Ips duplicatus* (Sahlb.), occurs from central Scandinavia, across eastern Europe to Siberia, and in the mountains of central Europe (Postner 1974; Lekander *et al.* 1977). Its distribution overlaps extensively with its larger congener and competitor *I. typographus* L.. *Ips duplicatus* usually occurs on trees under attack by *I. typographus* and occupies the top position of the tree trunk (Ringsgård 1975; Schlyter & Anderbrant 1992). *I. duplicatus* is not considered a major pest, in contrast to *I. typographus* (Postner 1974). On trees baited with commercial pheromone dispensers for *I. typographus* (IPSLURE®), *I. duplicatus* appears to mix with *I. typographus*, even on the lower part of the trunk (Schlyter & Anderbrant 1992). Competition experiments in the laboratory with both species have shown that the smaller *I. duplicatus* is more strongly affected by interspecific larval competition than is *I. typographus* (Schlyter & Anderbrant 1993).

The sympatric distributions and the assymmetric competition for breeding substratum suggest that *I. duplicatus* would benefit from producing and responding to a pheromone blend distinct from that of *I. typographus*. However, it is well known that *I. duplicatus* is attracted in substantial numbers to the commercial IPSLURE®/PHEROPRAX® synthetic pheromone formulations for *I. typographus*, containing 2-methyl-3-buten-2-ol (MB), (*S*)-*cis*-verbenol (cV), and ipsdienol (Id) (Selander & Nourteva 1980; Bakke 1981a). This attraction of *I. duplicatus* is probably due to Id, a pheromone component of *I. duplicatus* (Bakke 1975; Byers *et al.* 1990). The racemate of Id elicited significant attraction as compared to a host log or the blank (Bakke 1975). Using racemic components, Schlyter *et al.* (1987a, b) showed Id to be essential for the attraction of *I. duplicatus*, both by substraction of Id from a 7-component blend of *I. typographus* (Schlyter *et al.*

1987a), and by addition of Id or both Id and ipsenol (Ie) to a combination of MB + cV (Schlyter *et al.* 1987b). Addition of Ie to a MB + cV + Id bait, however, decreases the attraction of *I. duplicatus* caused by Id (Schlyter *et al.* 1987c; A. Bakke unpubl.), while addition of ipsenol to MB + cV caused no attraction (Schlyter *et al.* 1987c).

The aggregation signal of *I. typographus* consists of MB + cV (Bakke *et al.* 1977; Krawielitzki *et al.* 1977; Schlyter *et al.* 1987a), produced when the male bores into the tree before he is joined by females (Birgersson *et al.* 1984). The male may produce small amounts of Id when females have joined the male and egg-galleries are being prepared (Francke & Vité 1983; Birgersson *et al.* 1984, 1988) but only if associated fungi are not established in the gallery wall phloem (Birgersson & Leufvén 1988). Thus, *I. duplicatus* could be attracted to an already occupied patch by a chemical blend produced in later attack phases by *I. typographus*, when this superior competitor has already started egg laying - potentially a reproductive disadvantage for *I. duplicatus*.

However, several mechanisms for species discernment between the aggregation pheromones seem possible, such as the chirality of Id or specific pheromone components produced by *I. duplicatus*. Recently, Byers *et al.* (1990) reported that *E*-myrcenol, a likely oxidation product of myrcene, was an essential synergistic pheromone component of *I. duplicatus*. Here we provide chemical analyses of the production and release of volatiles including pheromone components of *I. duplicatus* and the response of walking and flying beetles to these compounds and natural pheromone. The interspecific effects of synthetic pheromone blends on attraction of *I. typographus* and *I. duplicatus* were also investigated.

Materials and methods

Production and release of volatiles

GC-MS analysis of males feeding in the field and laboratory

Male *Ips duplicatus* beetles were collected at Torsby, Värmland, Sweden in May 1982 during their attack of Norway spruce trees (*Picea abies* (L.) Karst.) that had been baited with synthetic pheromone (IPSLURE®). The site and the sampling method, including storage of sampled beetles in liquid nitrogen, have been described (Birgersson *et al.* 1984, 1988). Beetles were pooled in different phases of gallery construction from males alone in nuptial chambers to males with 1-2 females with egg-galleries ≤ 2 cm long (corresponding to "attack phases" from 3 to 5 in *I. typographus*; Birgersson *et al.* 1984).

A laboratory culture of living beetles originally collected from Torsby has been maintained on host logs continuously at the Ecology Building since 1982 (Byers *et al.* 1993). Reared beetles (from generation 6, April 1983) were separated by sex according to the size and shape of the double spine (Postner 1974). After flight-exercise, males were allowed to colonize 5-7 cm diameter bolts with pre-drilled holes (25 °C, 70% r.h., 20:4 L:D). After 24 h the males were excised from their nuptial chambers ("attack phase 3") and stored as the field samples.

Hindguts were dissected from the beetles and extracted in pentane with heptyl acetate as an internal quantification standard (Birgersson *et al.* 1984). Extracts of laboratory and field beetles were analyzed by gas chromatography-mass spectrometry (GC-MS, Finnigan 4021). A fused silica capillary column (l = 25 m, ID = 0.15 mm), coated with Superox® FA (Alltech, terephthalic acid (TPA) treated polyethyleneglycol (PEG), df = 0.3 µm) was temperature programmed at 50 °C for 4 min, 8 °/min to 200 °C and isothermal for 10 min, with He as carrier gas at 25 cm/s.

GC-MS analysis of beetles exposed to monoterpene hydrocarbons

Laboratory-reared males (generation 6, April 1983) were exposed to either enantiomer of α-pinene and to β-myrcene in Petri dishes with moist filter paper as previously described (Birgersson *et al.* 1988). Hindguts were dissected and analysed by GC-MS as above, but using a fused silica capillary column (l = 23 m, ID = 0.20 mm), coated with OV®-351 (Ohio Valley, Nitro-TPA treated PEG, df = 0.4 m), temperature programmed at 50 °C for 4 min, 5 °/min to 200 °C and isothermal for 10 min, with He as carrier gas at 25 cm/s.

Quantification of male-released ipsdienol and *E*-myrcenol

Groups of naturally colonizing laboratory males (no pre-drilled holes) excavating nuptial chambers in a small (approx. Ø5 cm) spruce log were aerated in a glass chamber, 10 cm diam. × 32 cm high, in the laboratory. Volatiles were trapped on a Porapak® Q trap (Waters Assoc., Inc., 350 mg, mesh 80-100) which was replaced every 12 h and extracted once with 2 ml diethyl ether. The extracts were analyzed on a HP 5830 GC with a fused silica capillary column (l = 46 m, ID = 0.32 mm) coated with OV-351 (df = 0.59 µm) and temperature programmed at 50 °C for 4 min, 5 °/min to 200 °C and isothermal for 10 min, with N_2 carrier gas at 20 cm/s. All columns were prepared by A.-B. Wassgren at the department of Chemical Ecology, Göteborg.

Enantiomer analysis of ipsdienol

Males excavating nuptial chambers were excised from logs and extracted as described above. The extract was derivatized with isopropylisocyanate (König *et al.* 1982) and the resulting chiral diastereomers were analyzed on a HP 5880 GC equipped with a nitrogen/phosphorous detector using a fused silica capillary column, l = 50 m, ID = 0.23 mm, coated with chiral CP = XE-60-*S*-Valin-*S*-phenylethylamide (Chrompack, df = 0.14 µm). The temperature program was 120 °C for 3 min, 1 °/min to 150 °C and isothermal for 15 min, and N_2 carrier gas at 20 cm/s.

Response tests

Laboratory tests

Beetles from generations 59-60 (Byers *et al.* 1993) were bio-assayed in an open arena olfactometer with a laminar air flow. Substances are released in diethyl ether from a capillary at the air inlet side. A positive response is scored when a walking beetle approaches within 2 cm of the odour source (Byers *et al.* 1989). Id, MB, cV, and EM were tested in

Table 1 Release rates and dispensers (and baits) for field test of *I. Typographus* and *I. duplicatus* at Ås, Norway, May–June 1990

Compound	Purity (%)	Source	Release (mg/day) Nominal	Measured[a]	Dispensers
A Test of *E*-myrcenol activity					
Id †	90	Borregaard	1	1.2 ±0.1	2 open #730 PE-vials (6 mm ID, 3 cm high)
EM	96	Francke	0.2	0.15 ±0.07	open #730
M	95	Aldrich	30	30[b]	open #730
B Subtractive assay					
Id	90	Borregaard	1.2	1.2 ±0.1	2 × open #730
EM	96	Francke	0.06	0.1 ±0.05	screw cap glass vial ID 5 mm, 3 cm high
MB	97	Aldrich	0.06	0.065 ±0.033[c]	0.5 µl 'Microcaps' capillary (0.14 mm ID, one end closed)
cV	99	Borregaard	0.06	0.052 ±0.001	#730 with a short (2 mm) 150 µl 'Microcaps' capillary through the lid
Natural pheromone		males	69 ME	Id[d]: 0.24 EM: 0.06 MB: <0.001 cV: <0.001	*I. duplicatus* from lab. colony gen. 68, 69 males in 80 cm netted spruce log inside pipe trap.
C Pheromone interactions					
Id	90	Borregaard	6.	6.6 ±0.3[e]	3 × open 3 ml PE-vials
			0.6	0.58 ±0.05	open #730 PE-vial
			0.06	0.05 ±0.005	closed #730 PE-vial
EM	96	Francke	0.3	0.45 ±0.21	3 × open #730 PE-vials
			0.03	0.1 ±0.05	screw cap glass vial ID 5 mm, 3 cm high
			0.003	-	3 µl 'Microcaps' capillary (0.24 mm ID, one end closed)
MB	97	Aldrich	160.	140 ±8	2 × #733/4 8 ml PE-vials with 2.0 mm opening
			16.	17.4 ±0.9	3 × #730 with a short (2 mm) 150 µl 'Microcaps' capillary (0.9 mm ID) through the lid
			1.6	1.5 ±0.15	3 × closed #730
			0.3	0.5 ±0.05	closed #730
			0.03	0.065 ±0.033[c]	0.5 µl 'Microcaps' capillary (0.14 mm ID, one end closed)
			0.003	0.006 ±0.003	diluted 1:10 in heptane in 0.5 µl 'Microcaps' capillary (0.14 mm ID, one end closed)
cV	99	Borregaard	1	1.03 ±0.05	9 mm diam. hole, 2 ml hard PE-vial
			0.3	0.31 ±0.06	6 × #730 with a short 150 µl capillary through the lid
			0.1	0.104 ±0.03	2 × #730 with a short 150 µl capillary through the lid
			0.03	0.009 ±0.006	3 × closed #730 fit inside closed 3 ml PE-vial
			0.05	0.015 ±0.010	5 × #730 fit inside 3 ml vial
			0.003	0.0016±[c]	diluted 1:10 in heptane in 0.5 µl 'Microcaps' capillary (0.14 mm ID, one end closed)

† **Id** ipsdienol, **EM** *E*-myrcenol, **MB** 2-methyl-3-buten-2-ol, **cV** 4*S*-(−)-*cis*-verbenol

[a] Measured in a mini-windtunnel at 20 °C and 0.7 m/s. Rates calculated as the slope ± its 95 % confidence limits of the regression of weight loss vs time (Schlyter *et al.* 1987c)

[b] At 18 °C Byers *et al.* 1985

[c] Calculated from the release of MB from a 0.25 mm ID capillary

[d] Calculated from the release by *I. duplicatus* males in the laboratory (Fig. 1)

[e] Calculated from the release of verbenone of 7.7±0.4 mg/day compared to 0.5±0.055 mg/day of Vn from an open #730 where Id has 0.58

additive and in dose-response assays. Dose-response tests of *I. duplicatus* females to varying release rates of three blends of monoterpene alcohols present in the hindgut of males: ipsdienol (Id) and Id + (−)-(4*S*)-*cis*-verbenol (cV) blends were tested 12 October 1988 while the *E*-myrcenol (EM) + Id + 2-methyl-3-buten-2-ol (MB) + cV blend was tested 15 December 1988. Females of *I. typographus* were tested with a range of EM (10^{-10} to 10^{-6} × 2 g/min in 5 decadic steps) added to a blend of MB + cV + Id at MB 10^{-7}, cV 10^{-8}, and Id 10^{-8} × 2 g/min. Statistical analysis was done by χ^2-tests adjusted for number of comparisons at $\alpha = 0.05$.

Field tests

Field test of synthetic I. typographus *blends* – Beetles attracted to baits with Id, MB, and cV were trapped in Värmland, Sweden, 1982, in pipe traps with a barrier trap on the top and a sticky trap at the lower end (Schlyter *et al.* 1987c).

Test of E-*myrcenol activity and subtractive assay* – Ipsdienol alone or in combination with EM was tested with non-sticky, standard Norwegian drainpipe traps (N79) to replicate the test in Värmland 1989 (Byers *et al.* 1990). The test sites in Norway spruce were clear-cuts from recent winter logging near Ås, southernmost Norway (150 km SE of the 1982 test site) in May-June 1990. In addition, myrcene (a likely host monoterpene precursor of both Id and EM), was tested by adding it to Id as a test for possible host compound synergism. A subtractive assay of EM, cV and MB was performed with a natural pheromone source as a control. The *I. typographus* pheromone components (MB and cV) were included since *I. duplicatus* produces trace amounts of them and there was an indication of activity of cV in the 1982 test. The subtraction of Id was judged to be unnecessary since Id was known from several earlier tests of various designs to be attractive to *I. duplicatus* (Bakke 1975; Schlyter 1987a, b, 1987c; Byers *et al.* 1990). Testing was done at Ås during two

Table 2 Male content of hindgut volatiles identified by GC-MS in *I. duplicatus* in ng/male

Compound (in approximate eluation order)	Tree		Laboratory log	Exposure[a]	
# Name	(31)[b]	(30)	(13)	1 µl (17)[c]	10 µl (16)
1 2-methyl-3-buten-2-ol (MB)	18	21	+[d]	-[e]	-
2 Ipsdienol (Id)	55	17	540	2.7	21
3 Ipsenol (Ie)	0.3	1.5	< 1	< 0.05	< 0.05
4 *E*-Myrcenol (EM)	12	0.8	91	-	-
5 *cis*-Verbenol (cV)	16	10	ca. 3	*	*
6 *trans*-Verbenol (tV)	18	9.0	ca. 3	23	25
7 Myrtenol	3.9	1.5	2.1	3.4	5.6
8 *trans*-Myrtanol	3.1	1.5	3.3	-	-
9 Myrtenal	-	-	-	0.4	< 0.4
10 *trans*-Pinocarveol	-	-	-	8.3*	5.8*
11 α-Terpineol	0.3	0.1	-	0.4	-
12 Borneol	0.6	0.4	2.8	-	-
13 Verbenone	0.8	1.5	-	-	-
14 Linalool	-	-	1.2	-	-
15 Isopiperitenol	-	-	-	0.5	0.5
16 Geraniol-isomer	-	-	2.4	-	-
17 Geraniol	-	-	11.4	-	-
18 *trans*-Carveol	-	-	-	3.2	2.9
19 Piperitenone	0.3	0.3	-	-	-
20 Perilla alcohol	-	-	-	-	2.9
21 2-Phenylethanol	0.8	0.9	≤ 1	0.5	1.7
22 Unidentified I	5.5	-	-	-	-
23 Unidentified II	-	-	-	1.0	1.4

[a] Exposure to volatiles from 1 or 10 µl capillaries of myrcene, plus 1 µl capillary each of (+)-α-pinene and (−)-α-pinene, in paper lined Petri-dishes
[b] Sample size (number of males in extract)
[c] Mean of two analyses of this extract
[d] Compound identified by MS but below limit of quantification (LOQ)
[e] Denotes value below a limit of detection (LOD) of ≤0.25 ng/male
* cV and *trans*-pinocarveol co-elutes on two of the columns used; cV is the minor compound

periods, the first (8–11 May 1990) included a 80 cm long log of Norway spruce (*Picea abies* (L.) Karst.) infested with 80 males (69 were active at end of test) from our laboratory colony (originating from Lardal, Norway 1983). The second period (31 May–7 June) did not have the natural pheromone control but instead a treatment with the subtraction of both MB and cV. Compounds were released in approximately the ratio they were found in hindgut extracts of *I. duplicatus*, at about 1:5 EM:Id (Tables 1 and 2).

Pheromone interactions – The response to different doses of pheromone of the two species, corresponding to the first stages of mass-attack on a tree, was tested to evaluate cross-attraction, inhibition, and species separation.

In the pheromone interaction test, May–June 1990 at Ås, all 12 possible combinations of 10, 100, and 1000 male equivalents (ME) of *I. duplicatus* and 0, 10, 100, and 1000 ME of *I. typographus* were tested simultaneously. Compounds were released at natural rates estimated from both species [*I. typographus* cV:MB = 1:100–150 (Birgersson & Bergström 1989) and *I. duplicatus* EM:Id = 1:40–80 (Fig. 1)] except that the EM release was too high (Table 1). The 0 ME *I. duplicatus* column in the test matrix was omitted (Fig. 4), as this series of treatments would be equivalent to the dose-response of *I. typographus* to its pheromone. This has been tested before, and is known to give a linear dose-response for *I. typographus* (Bakke *et al.* 1983; Sauerwein 1981; Schlyter *et al.* 1987c), but essentially no catch of *I. duplicatus* (Schlyter *et al.* 1987c). Two test periods were run, 8–11 May and 30 May–7 June, in all 9 replicates. In the first period the dispenser of EM for 100 ME *I. duplicatus* was faulty, and the captures for these treatments were coded as missing values for this period (3 replicates) in the ANOVA. Statistical analysis of field data, ANOVA followed by Duncans multiple-range test or factorial ANOVA (Perry 1986) with SPSS/PC+™ V3.0 and V4.0, was done on log(catch + 1) or *arcsin* $\sqrt{p}$ transformed data to approach normality and homoscedasticity as judged by Barletts and Cochrans C tests. For the 1982 test there remained a significant heteroscedasticity for *I. duplicatus* catches due to the many zero values for baits without Id.

Results

Production and release of volatiles

GC-MS analysis of field and laboratory males

In addition to the major compound ipsdienol (Id), another 22 compounds were quantified from hindguts of male beetles boring in phloem (Table 2). In the extracts from beetles in attacked trees a higher number of compounds was found (14), than in extracts of beetles from logs in the laboratory (12). However, some of the compounds (Table 2; #10–13 and #19) are very common in spruce phloem (Heeman & Francke 1977) and thus may not have been produced by the beetles. The compounds most frequently found in *Ips* beetles, Id, Ie, cV, and tV (Byers 1989), were all present in detectable amounts in males from the field. Beetles from laboratory logs

contained very little Ie, but substantial amounts of geraniol, as well as small amounts of a geraniol-isomer and linalool, which were absent in other samples.

Three unidentified compounds occurred, of which two were only found sporadically in small amounts in samples from the host tree and monoterpene exposure (Table 2; #22, 23) and were not studied further. The other unknown compound (Table 2; #4), which was consistently present in samples from naturally excavating males, eluted in the region of the sesquiterpenes and had the MS characteristics of a monoterpene alcohol. It was recently identified as *E*-myrcenol [(*E*)-2-methyl-6-methylene-2,7-octadien-1-ol] by comparison with the synthetic reference (Byers *et al.* 1990). The ratio of EM:Id was between 1:4.6 and 1:21 (Table 2).

GC-MS analysis of monoterpene hydrocarbon exposed beetles

Exposure to α-pinene and myrcene vapours at two doses induced males to produce Id, tV, and cV (Table 2). Male hindguts contained fragments of earlier eaten bark (brown) at their rectum, as well as clumps of paper fibre (pure white) throughout the hindgut, which had been ingested from the moistened filter paper. However, Id production was low compared to that of naturally attacking males, even at the high dose (10 µl) of myrcene, the presumed monoterpene hydrocarbon precursor. Traces of Ie could be detected. However, no trace of *E*-myrcenol, which is a likely product of myrcene oxygenation, could be detected at a limit of detection (LOD) of ≤0.25 ng/male.

Release of ipsdienol and *E*-myrcenol over time

Odour collections from aerations of unmated males in the laboratory showed a high amount of Id released, up to about 250 ng/h·male after about 2 days, but then a decline over time (Fig. 1). No ipsenol was detected, in contrast to the extract of "mixed attack phases" from the field. *E*-myrcenol was released in lower amounts up to about 5 ng/h·male), at a ratio of about 1:40–80 EM:Id, and lagged behind in production about 0.5 days relative to Id (Fig. 1).

Enantiomeric composition of ipsdienol

Repeated analyses of Id from *Ips duplicatus* males feeding in logs in the laboratory showed an enantiomeric composition close to a racemic mixture (50/50). A value of 48.9% (+)-(*S*)-isomer was found for a pooled sample of 20 males. Eight separate analyses from a larger extract of 40 males gave a mean value of 52.8% (+)-(*S*) (S.D.:0.87, min.-max.: 51.7–54.5).

Response tests

Synthetic *E*-myrcenol in the laboratory

In a series of dose-response assays, Id by itself was not significantly more attractive to *Ips duplicatus* than the solvent control (7% response, 2–21%, 95% B.C.L., N = 40) over a range of 5 orders of magnitude (from 10^{-11} to $10^{-6} \times 2$ g/min (0–13% response)). Id with cV seemed to give some response between 10^{-11} and 10^{-9} g/min, but was not significantly different from the control at any concentration

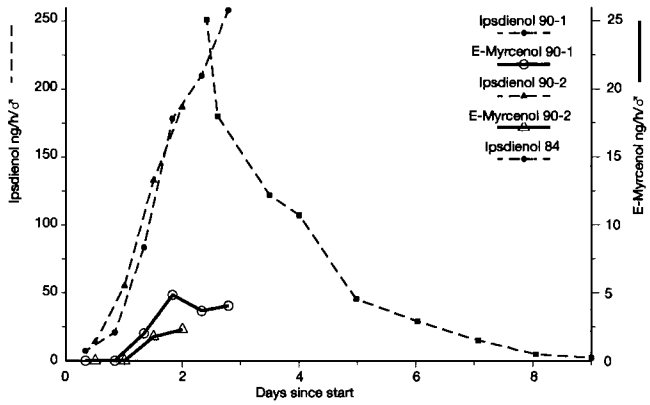

Fig. 1 Release of ipsdienol and *E*-myrcenol over time after start of gallery excavation from logs with unmated male *I. duplicatus* in the laboratory (runs '90–1' and '90–2' 50 males each, run '84' 37 males)

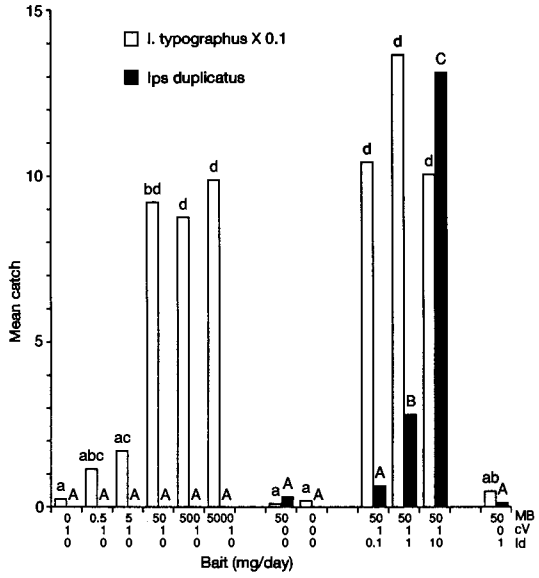

Fig. 2 Response of *I. duplicatus* and *I. typographus* to volatiles produced by *I. typographus*. Pooled data from combined pipe, sticky, and barrier trap (pheromone inside pipe trap), Värmland, Sweden, May 1982. Bait designation based on release rate of compounds from separate dispensers with their rates estimated in the laboratory (Schlyter *et al.* 1987c). Bars with the same letter within a species are not significantly different by ANOVA followed by Duncans multiple range test

(from 10^{-11} to 10^{-6} g/min). Only the combination of *E*-myrcenol (EM) with Id + MB + cV was significantly active (at 10^{-8} 47%, 30–64% B.C.L. and 57%, 39–73% B.C.L. at $10^{-7} \times 2$ g/min) (the four compound blend tested only between 10^{-9} and $10^{-7} \times 2$ g/min).

The possible inhibitory property of EM on attraction of *I. typographus*, which could enhance species separation, was also tested. An increase of EM, from 2×10^{-10} to 10^{-6} g/min in five decadic steps, released with an attractant mix of MB + cV + Id showed, however, neither positive nor negative effects on the attraction of *I. typographus* females (35–42% response; N = 40 tested per dose).

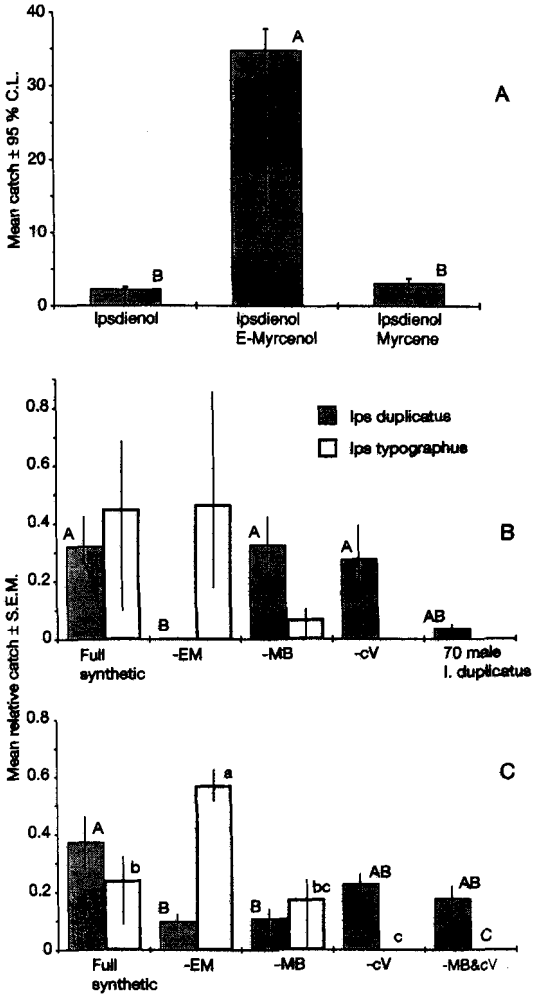

Fig. 3 **A** Response of *I. duplicatus* to the two major compounds produced by *I. duplicatus* and the host monoterpene hydrocarbon myrcene. $N = 20$, Norway May 2–5, 1990. Bars with the same letter are not significantly different by Duncans multiple range test following ANOVA on log $(x+1)$ transformed catches. Error bars are ±95% confidence limits (retransformed from log $(x+1)$). **B** Subtractive assay and comparison with natural pheromone. $N = 3$, Norway, May 7–11, 1990. **C** Subtractive assay – subtracting from the quaternary blend (Id + EM + MB + cV) either EM, MB, cV or MB & cV combined, $N = 4$, Norway, May 29–June 7, 1990. Bars with the same letter within a species are not significantly different by Duncans multiple range test following ANOVA on arcsin $\sqrt{p}$ transformed catches. In B and C error bars are ±S.E.M. (retransformed from arcsin $\sqrt{p}$). **Id** ipsdienol; **EM** *E*-myrcenol; **MB** 2-methyl-3-buten-2-ol; **cV** 4*S*-(−)-*cis*-verbenol

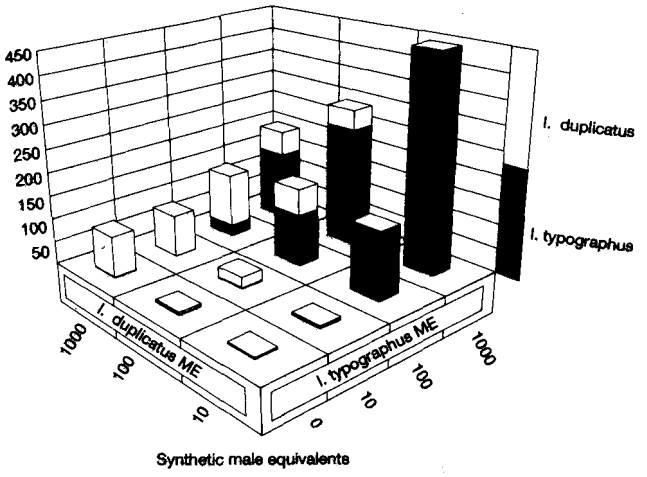

Fig. 4 The catches of the synthetic pheromones from the two species in the pheromone interaction dose-response test (untransformed mean catch per replicate). Pipe traps, 3 + 6 replicates, Norway, May 7–11 and May 29–June 7, 1990. Apart from the dose-response effects for each species to its own pheromone (front row and right column), strong interactions were present. The negative effect by high doses of *I. duplicatus* pheromone on the catch of *I. typographus* at 1000 *I. typographus* **ME** (male equivalents) is shown by the back row. For statistical analysis (ANOVA) see Table 3

Field tests

Synthetic I. typographus *blends – Ips duplicatus* was significantly attracted only to baits including Id (racemic), and showed a clear positive dose-response to increased Id release (in baits with MB + cV) over a range of 2 orders of magnitude (0.1 to 10 mg/day) (Fig. 2). A high release of Id with MB + cV was the most active bait. The removal of cV seemed to reduce catch, but exact significance levels for range tests for all the baits for *I. duplicatus* could not be assigned as heteroscedasticity was significant due to zero catches for several baits.

E-myrcenol activity and subtractive assay – The addition of EM to Id gave a dramatic increase of *I. duplicatus* catch with >10 times more caught on the combination Id + EM than on Id alone (Fig. 3A). No *I. typographus* were caught. The difference for *I. duplicatus* was even larger than in earlier tests with a rotating sticky trap pair (Byers *et al.* 1990). The addition of myrcene to Id seemed to give an increase in mean catch but was far from significant (Fig. 3A).

The subtractive test of Id, EM, cV, and MB showed again that EM was an essential synergist for *I. duplicatus*: its removal caused a 50-fold drop in catch. Subtraction of cV or MB or both had no effect (Fig. 3B, C). The log with 70 unmated males had a lower catch than the baits releasing EM + Id at 200 male equivalents. For *I. typographus* the effects were the opposite: no catch on heterospecific males (Fig. 3B), an increase of catch when EM was subtracted (Fig. 3C), and a decreased (or zero) catch when MB, cV, or both were subtracted (Fig. 3B, C).

Pheromone interactions – The multiple dose-response test of the two species synthetic pheromones gave the expected positive increase in catch with dose for both species (species specificity) (Fig. 4; front left row for *I. duplicatus*, front right column for *I. typographus*). Species interactions were observed both in a lowered catch at higher levels of pheromone from the other species (inhibition, Fig. 4; back row for *I. typographus*) and in an increase at lower levels (cross-attraction, Fig. 5B). A distinct specificity with almost no catch of *I. typographus* with an increase of *I. duplicatus* pheromone release was evident (Fig. 4, front row). The response of *I. duplicatus* was proportional to the dose of its pheromone, as indicated by homogeneous subsets of Duncan ranges for mean catches within doses of its pheromone (Fig. 5A). However, although there was a dose-effect from its own pheromone, *I. typographus* was also affected by the presence of *I. duplicatus* pheromone, as indicated by the more heteroge-

Table 3 ANOVA of log 10 Catch of *I. duplicatus* and log 10 catch of *I. typographus* as affected by the dose of *I. duplicatus* pheromone (male equivalents 10–1000) and the dose of *I. typographus* pheromone (male equivalents 10–1000) in pheromone interaction test Ås, Noway, May–June 1990

Source of Variation	df	Mean Square	F	Significance of F
log 10 Catch of *I. duplicatus*				
Main Effects				
IDPHER[a]	2	7.6	26.033	.000
ITPHER[b]	2	.04	.695	.88
2-way Interactions				
IDPHER x ITPHER	4	.17	.141	.69
Explained	8	2.1	.752	.000
Residual	66	.30		
Total	74	.50		
log 10 Catch of *I. typographus*				
Main Effects				
IDPHER[a]	2	.25	.332	.321
ITPHER[b]	2	19.9	96.858	.000
2-way Interactions				
IDPHER x ITPHER	4	3.9	3.379	.03
Explained	8	5.9	25.987	.000
Residual	66	.2		
Total	74	.8		

[a] The dose of *I. duplicatus* pheromone (male equivalents 10–1000: 10 = 0.06/0.003/0.003/0.003 [Id/EM/cV/MB, mg/day], 100 = 0.6/0.03/0.03/0.03, 1000 = 6/0.3/0.3/0.3) in all 9 possible combinations with the other species pheromone

[b] The dose of *I. typographus* pheromone (male equivalents 10–1000: 10 = 0/0/0.01/1.6 [Id/EM/cV/MB, mg/day], 100 = 0/0/0.1/16, 1000 = 0/0/1/160) combined as in [a]

neous Duncan ranges for catches within doses and different slopes between doses (Fig. 5B).

Variances of the mean catches with all baits included was heteroscedastic by Barletts but not by Cochrans test, while for treatments including both species (baits 4–12) it was homogeneous by both tests. A formal ANOVA of baits 4–12 (excluding the combinations with 0 ME *I. typographus*) showed as expected a very significant effect of conspecific pheromone doses ($p < 0.1\%$) and a non significant effect of interspecific dose ($P > 50\%$) (Table 3). However, for *I. typographus* there was a significant interaction term ($P < 2\%$), corresponding to the differences in slope in Figure 5B.

Sex-ratios for the baits with *I. typographus* inhibition was of special interest as *I. typographus* females were not inhibited by EM in the laboratory. The baits with a high *I. duplicatus* ME and low *I. typographus* ME dose, like 1000+0 (ME *I. duplicatus*+ME *I. typographus*) and 1000+10, had 20% (15.1–26.5) and 19% (13.5–25.3) (95% binomial C.I.) males, respectively. Of the baits with the highest *I. typographus* dose (1000 ME) and an increase in *I. duplicatus* dose the male percentage was 14 (11.1–17.7), 16 (12.4–19.2) and 7% (5.4–9.97), for 10, 100, and 1000 ME *I. duplicatus*, respectively. These three baits are those in the back row of Figure 4 and the top row in Figure 5B, which show the clear inhibition of *I. typographus* with an increase of *I. duplicatus* pheromone. Thus, at the highest *I. duplicatus* dose the *I. typographus* males were relatively more inhibited than the females.

Discussion

Our findings show that *Ips duplicatus* and *I. typographus* can discriminate well between their respective pheromone blends, although they have many compounds in

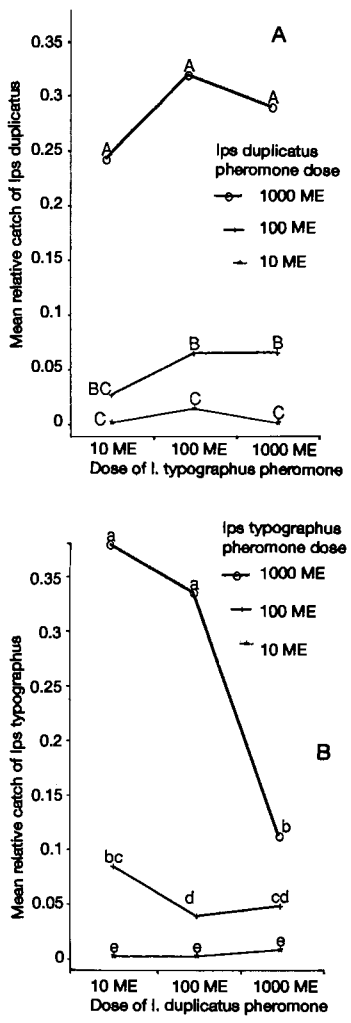

Fig. 5 Mean relative catch per replicate in the pheromone interaction dose-response test (retransformed from arcsin $\sqrt{p}$). **A** *I. duplicatus* catch with doses of *I. typographus* pheromone. **B** *I. typographus* catch with doses of *I. duplicatus* pheromone. Points with the same letter are not significantly different by Duncans multiple range test following ANOVA on arcsin $\sqrt{p}$ transformed catches. ME male equivalents in release rate. For statistical analysis of the simultaneous effects and interactions of the pheromone doses of the two species (ANOVA) see Table 3

Table 4 Production of and response to pheromone components from *I. duplicatus* and *I. typographus* as known from the literature and this study[a]

Compound[b]	Production				Response to synthetics		Reference[c]	
	in host material		host monoterpene exposure[d]					
	I. duplicatus	*I. typographus*	*I. duplicatus*	*I. typographus*	*I. duplicatus*	*I. typographus*	*I. duplicatus*	*I. typographus*
MB	+	+ + +	**0**	+	**0?**	+ + +	1, 8, 17	3, 6, 12, 17
(*S/R*)-Id	+ + +	+[L]	+ +	**0**	+ + +	**−/+/0**	1, 8, 17	2, 6, 7, 9, 13–16
(*R*)-(−)-Id	**50%**	**95%**	**50%**	**?**	**?**	**0**	17	9, 11
(*S/R*)-Ie	+[L]?	+[L]	**0**	**0**	−	−	5, 14, 16	4, 7, 13–16
(*R*)-(+)-Ie	**?**	**95%**	**0**	**60%**	**?**	**0**	−	11
cV	+	+ +	+	+ + +	**0**	+ + +	17	2, 6, 10, 12, 13, 15
EM	+ +	**0**	**0**	**0**	+ +.	**0/−**	8, 17	6, 17

[a] +, + +, + + + Relative magnitude of production (approx. orders of magnitude) or positive response (attraction), − negative response (inhibition of attraction), **0** not detectable in beetle or no effect on behaviour

[b] **MB** 2-methyl-3-buten-2-ol, **cV** (−)-(4*S*)-*cis*-verbenol, **Ie** (*S/R*)-ipsenol, **Id** (*S/R*)-ipsdienol, and **EM** (*E*)-myrcenol

[c] 1 Bakke 1975, 2 Bakke 1976, 3 Bakke *et al.* 1977, 4 Bakke 1981b, 5 A. Bakke unpubl., 6 Birgersson *et al.* 1984, 7 Birgersson *et al.* unpubl., 8 Byers *et al.* 1990, 9 Dickens 1981, 10 Hackstein & Vité 1978, 11 Kohnle *et al.* 1991, 12 Krawielitzky *et al.* 1977, 13 Schlyter *et al.* 1987a, 14 Schlyter *et al.* 1987b, 15 Schlyter *et al.* 1987c, 16 Schlyter *et al.* 1989, 17 this paper

[d] Myrcene and α-pinene

[L] Product only in later attack phases (after mating)

common (Table 4). The essential pheromone components are ipsdienol (Id) + *E*-myrcenol (EM) for *I. duplicatus*, and 2-methyl-3-buten-3-ol (MB) + *cis*-verbenol (cV) for *I. typographus* (Table 4). The combination of Id + EM may represent the complete pheromone blend of *I. duplicatus*, as there was no significant difference between attraction to synthetics or to naturally infested material in the field at comparable ME rates. It could be calculated that our release of Id in the field tests (0.1–0.2 mg/day), is comparable to a natural pheromone source of moderate size, as the laboratory estimate of an average release, from only 37 males, over the first 2 days corresponded to ≥0.2 mg/day for the log. The release rate found here for Id by *I. duplicatus* in the laboratory, 200–250 ng/h·male is slightly lower than reported for *I. pini*, 830 ng/h·male collected from aerations of males in phloem sandwiches (Gries *et al.* 1988b) and 660 ng/h·male in pine logs (Gries *et al.* 1990). The possibility that other compounds, such as cV or MB, could be active as cross-attractant mediators to the *I. typographus* blend in some combination, remains to be tested with other ratios and amounts of released compounds in the field.

In bark beetles, *E*-myrcenol of *E*/Z-isomer mixes, have been reported from *Dendroctonus ponderosae* and *D. brevicomis* (Renwick *et al.* 1976; Conn 1981; Hunt *et al.* 1986; Pierce *et al.* 1987), *I. schmutzenhoferi* (Francke *et al.* 1988), and *I. pini* (Gries *et al.* 1988a), *I. sexdentatus* (W. Francke unpubl.), and *I. grandicollis* (G. Birgersson unpubl.). *E*-Myrcenol was first identified from the essential oil of thyme (Granger *et al.* 1972) and was also found in the boll weevil *Anthonomus grandis* (Coleoptera: Curculionidae; Hedin 1977) and in *Pseudomonas* spp. (aerobic bacterium; Maclyarta 1984).

Our results support Byers *et al.* (1990) that *I. duplicatus* produces EM and aggregate in response to EM with Id. Earlier bioassays with EM and other bark beetles have given inconsistent results, being attractive in the laboratory but slightly inhibitory in the field (*D. ponderosae;* Conn 1981; Conn *et al.* 1983), or inhibitory in traps but attractive on logs (*I. pini*; Gries *et al.* 1988a; Miller *et al.* 1990).

Exposure of *I. duplicatus* males to myrcene (M) vapours indicate that this host monoterpene hydrocarbon can serve as a precursor to its alcohol pheromone component, ipsdienol (Id), as earlier data has suggested for other *Ips* species (*cf.* Byers 1989). However, the amount of Id produced was small compared to the amount in males from natural material, although the males had been feeding from the filter paper. *I. typographus* under similar exposure conditions do not appear to produce any Id (Table 4). These data, and the poor correspondence between Id production in the Nearctic *I. paraconfusus* and the myrcene content in serveral of its pine hos. species (Byers & Birgersson 1990), indicate that the biosynthesis of Id from myrcene may not be the major natural pathway. Thus, in quantitative terms the reaction M→Id may not be important in bark beetle chemical ecology. Moreover, *E*-myrcenol, another very plausible hydroxylation product of myrcene, was surprisingly not produced at all upon myrcene exposure. The biosynthesis under natural conditions of the two open chain monoterpene alcohols (Id & EM) remains unknown, although in the laboratory it is has been shown with isotope labelling that M is converted to Id *in vivo* in *I. paraconfusus* (Hendry *et al.* 1980).

I. duplicatus males produce relatively large amounts of both enantiomers of Id and respond to the racemate alone or in combination with EM, while it is not known which of the Id enantiomers is active or if both are active. *I. typographus*, on the other hand, produces small and variable amounts of Id (Table 4). The chirality of Id produced by *I. typographus* in later attack phases is *R*-(−) (Francke *et al.* 1980; Kohnle *et al.* 1991). The enantiomer ratio of *I. typographus* (≈90% e.e.) could be different enough from that of *I.*

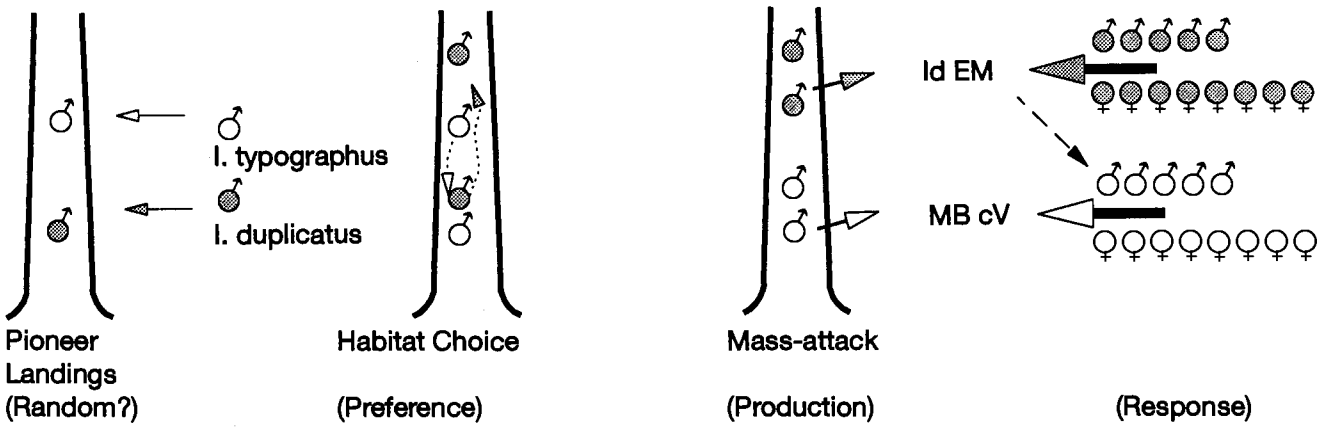

Fig. 6 Conceptual model of early stages of a hypothetical mass-attack with similar numbers of both species attacking at the same time. Solid symbols for sexes and arrows are used for *I. duplicatus* and outlined symbols for sexes and arrows for *I. typographus*. See Table 4 and Discussion for details. **Id** ipsdienol; **EM** *E*-myrcenol; **MB** 2-methyl-3-buten-2-ol; **cV** 4*S*-(−)-*cis*-verbenol. Dashed arrow from EM to *I. typographus* males indicates inhibition of response

duplicatus (≈0% e.e.) to lessen the response of *I. duplicatus*. The chirality of Id from *I. duplicatus* could not be shown to be different from racemic, since the range of values from several analyses of a single extract was 2.8%-units, which equals the difference between the mean and 50%.

While several *Ips* (and allied genera) breed in *Pinus* spp., the only *Ips* breeding in *Picea abies* in the Western Paleartic are *I. typographus*, *I. duplicatus*, and *I. amitinus* (Postner 1974). The signal from *I. amitinus* appears distinct from the two species studied here, as it has no MB but an additional, specific compound, the acyclic monoterpene alcohol amitinol (Francke *et al.* 1980). A slight cross attraction to *I. typographus* could be of benefit to *I. duplicatus*, giving a cue that the tree has been successfully attacked or weakened by *I. typographus*, but this could not be shown here.

The pheromone specificity was clearly shown in the interaction experiment, where the ANOVA only showed a significant effect of conspecific pheromone doses, but no significant effect of interspecific doses (Table 3). In addition, for *I. typographus* there was an interaction term which means that at different levels of the other species pheromone there was a difference in the dose-response to its own pheromone (Fig. 5B). The interaction term, then, indicates that, on one hand, a slight cross-attraction of *I. typographus* to *I. duplicatus* pheromone may exist, as when *I. duplicatus* has started to attack a tree but not *I. typographus*. On the other hand, an inhibition of *I. typographus* clearly existed when large doses of both pheromones were mixed (at 1000 *I. duplicatus* ME), corresponding later stages in a mass-attack when large numbers of both species are established.

The sex-ratio is of special interest for interpreting mechanisms affecting the behaviour of the pioneering sex, the male. Of the three baits in the back row of Figure 4 and the top row in Figure 5B, which show an inhibition of *I. typographus* by increase of *I. duplicatus* pheromone, the one with the highest *I. duplicatus* dose had the lowest percentage of males (7% or a male: female ratio of 1:12.5). *I. typographus* females were not inhibited by EM. These high doses of synthetic *I. duplicatus* pheromone had also high doses of racemic Id, and this compound inhibits the attraction of *I. typographus* to its pheromone (Schlyter *et al.* 1987b, 1989). However, Id did not change the proportion of males in these studies (Schlyter *et al.* 1987b, their tables 3 and 5; Schlyter *et al.* 1989, their tables 2A and 2B). Thus, it appears that EM at high doses inhibits mainly male *I. typographus*.

A mass-attack model can now be devised where the initial step is random landing on a tree or possibly as a result of attraction or cross-attraction from adjacent trees (Fig. 6). Host selection by diameter, probably based on bark structure preferences (Schlyter & Anderbrant 1993), would then assort most males, and mass-attack would follow where most individuals respond specifically to their pheromone.

The response of both species to their respective pheromone systems show good separation, which is adaptive. Recent research on North American *Ips* have shown the conservative character of pheromone systems within subgeneric groups (Cane *et al.* 1990a; Fox *et al.* 1990). Whether the specificity of the pheromone systems in the two European species examined here have evolved in response to their present sympatry, or is a result of an incidental effect of the ancestral systems in these two more distantly related *Ips* (belonging to different subgeneric groups; Hopping 1963, 1965; Wood 1982) remains to be shown.

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