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Male-produced aggregation pheromone of *Carpophilus sayi*, a nitidulid vector of oak wilt disease, and pheromonal comparison with *Carpophilus lugubris*

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Abstract

1 *Carpophilus sayi*, a nitidulid beetle vector of the oak wilt fungus, *Ceratocystis fagacearum*, was shown to have a male-produced aggregation pheromone.

2 Six male-specific chemicals were identified from collections of volatiles. The two major compounds were (2*E*,4*E*,6*E*,8*E*)-3,5-dimethyl-7-ethyl-2,4,6,8-undecatetraene and (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-undecatetraene, in a ratio of 100 : 18. These compounds, in a similar ratio, were previously reported to be the pheromone of *Carpophilus lugubris*, a closely related species. The four minor *C. sayi* compounds (less than 4% as abundant as the first) were also alkyl-branched hydrocarbons and consisted of two additional tetraenes and two trienes.

3 The pheromone of *C. lugubris* was re-examined to refine the comparison with *C. sayi*, and *C. lugubris* was found to have the same additional, minor tetraenes as *C. sayi*, but not the trienes.

4 A synthetic mixture of the two major compounds was behaviourally active for both sexes of *C. sayi* in oak woodlands in Minnesota. The pheromone was tested in combination with fermenting whole wheat bread dough (a potent synergist of nitidulid pheromones). The combination of the 500-μg pheromone dose and dough attracted at least 30-fold more *C. sayi* than either pheromone or dough by itself. The synergized pheromone has potential as a tool for monitoring insect vector activity in an integrated management program for oak wilt.

5 Although *C. lugubris* was not present at the Minnesota test sites, two other *Carpophilus* species, *Carpophilus brachypterus* and *Carpophilus corticinus*, were clearly cross-attracted to the synergized pheromone of *C. sayi*.

Keywords *Carpophilus brachypterus*, *Carpophilus corticinus*, *Carpophilus lugubris*, *Carpophilus sayi*, *Ceratocystis fagacearum*, Coleoptera, Nitidulidae, oak wilt, pheromone.

Introduction

Carpophilus sayi Parsons is a small (4 mm) black sap beetle (Coleoptera: Nitidulidae), and is an implicated vector of the fungus that causes oak wilt disease, *Ceratocystis fagacearum* (Bretz) Hunt. The beetles are often found on the fungal mats that form on infected trees (Cease & Juzwik, 2001) and in fresh wounds on healthy red oaks during spring in Minnesota (Juzwik *et al.*, 1999). Connell (1956) provides a

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thorough description of *C. sayi*. He also notes that the species is frequently associated with tree wounds, and ranges from Quebec and Maine south to North Carolina, and west to Iowa and Minnesota; it was also reported in Texas.

The dispersal and flight behaviour of the beetles is central to their role in disease transmission, but monitoring beetle movements is difficult due to their inconspicuous appearance and cryptic habits. Aggregation pheromones have been isolated and identified from other *Carpophilus* species (Bartelt, 1999), and having one for *C. sayi* could provide the needed monitoring tool. A pheromone is already known for another nitidulid vector of oak wilt, *Colopterus truncatus* Randall (Cossé & Bartelt, 2000; Kyhl *et al*., 2002a).

The previously described *Carpophilus* pheromones are all male-produced and attract both sexes. Chemically, they are alkyl-branched triene and tetraene hydrocarbons. An important property is that the pheromones are strongly synergized by host-related fermentation volatiles, such as those from overripe fruits or certain microorganisms (Lin & Phelan, 1992). In practice, whole-wheat bread dough inoculated with baker's yeast (WWBD) is usually added to pheromone traps for *Carpophilus* species to enhance the effect of the pheromone (Bartelt, 1999).

It was previously reported that *C. sayi* was attracted to the pheromone of a closely related species, *Carpophilus lugubris* Murray (Williams *et al*., 1995). *Carpophilus lugubris* is a relatively abundant species that occurs in much of the U.S.A. and ranges south into Central and South America (Connell, 1956). It has become a significant pest of sweet corn in the U.S.A., with both the adults and larvae feeding on developing kernels. The species also develops on various fallen fruits and other decomposing plant materials (Connell, 1956).

Cross-attraction has been frequently observed for *Carpophilus* pheromones because of shared, or very similar, pheromone components (Bartelt, 1999). However, kairomonal responses to pheromones of other species are also known. For example, *Carpophilus antiquus* Melsheimer and *C. lugubris* have entirely different pheromones, but *C. antiquus* still responds readily to the pheromone of *C. lugubris*, perhaps taking advantage of its host-finding ability (Bartelt, 1999). It was not known which of these explanations applied to the response of *C. sayi* to the pheromone of *C. lugubris*.

The aim of this study was to define the pheromone of *C. sayi* and, simultaneously, to test whether the *C. lugubris* pheromone could be used to monitor the flight behaviour of *C. sayi* in Minnesota oak woodlands where oak wilt was present. During this project, it was discovered that the pheromones of the two species are nearly identical; thus, the field experiment also became a test of the *C. sayi* pheromone. Finally, the pheromone of *C. lugubris* was reinvestigated, to clarify the differences and similarities between the two species.

Methods

Pheromone collections from *C. sayi*

The *C. sayi* were collected on 7 May 2001, by one of us (J.F.K.) from oak wilt mats on red or northern pin oaks in Anoka County, Minnesota. The beetles were sent to NCAUR in Peoria, Illinois, where the pheromone collections were made between 11 May and 6 June. In previous studies with other *Carpophilus* species, pheromone collections were made from beetles feeding on artificial diet, and production was consistently greater from small numbers of individuals than from large groups (Bartelt, 1999). Therefore, only two male or two female *C. sayi* were introduced into each collector, and approximately 5 g of brewer's yeast/wheat germ diet (Bartelt *et al*., 1993) was provided as a source of food and moisture. Seven collectors were set up with males, and one with females, for comparison. The collectors were kept in an incubator at 27 °C with a photoperiod of LD 16 : 8 h.

Each pheromone collector consisted of a 50-mL Erlenmeyer flask equipped with an inlet/outlet adapter (Part 5175, Ace Glass, Vineland, NJ); both pieces had 24/40 ground glass fittings, to allow assembly without corks or stoppers. A filter containing Super-Q porous polymer (Alltech Associates, Deerfield, IL) was attached to both the inlet and the outlet of the collector with a short length of Teflon tubing. Each filter consisted of a 4-mm inner diameter (I.D.) glass tube with a 300-mesh stainless steel screen fused into one end and filled to a depth of 6 mm with Super-Q; this was held in place with a plug of silanized glass wool. A gentle vacuum drew air through the assembled apparatus at about 100 mL/min. The upwind filter served to clean the incoming air, and the downwind filter captured the beetle emissions and food volatiles.

Volatiles were harvested every 2–4 days by rinsing the filters into a vial with 400 μL high-performance liquid chromatography grade hexane. Air was then drawn through the filter to evaporate residual hexane. Fresh diet was added if needed, and the apparatus was reassembled. A total of 54 collections were made from feeding males, and eight were made from feeding females.

Chemical analysis of collected volatiles

Each collection of volatiles was applied to a small (10 × 5 mm) column of silica gel in a Pasteur pipette, and the column was eluted first with 1 mL hexane and then with 1 mL diethyl ether. Primary focus was on the hydrocarbon (hexane) fractions because the pheromones of other *Carpophilus* species were known to be hydrocarbons. The recognition and identification of sex-specific hydrocarbons can be greatly simplified by prior chromatographic removal of polar constituents that are abundant in the diet. Nevertheless, the polar (ether) fractions were also retained for subsequent comparison of male- and female-derived samples, in case polar pheromone components existed. The chromatographic fractions were concentrated under a stream of nitrogen to approximately 10 μL just prior to analysis.

Gas chromatography/mass spectrometry (GC-MS) was the primary means of analysis. A Hewlett-Packard (Pato Alto, CA) 5973 Mass Selective Detector was coupled to a Hewlett-Packard 6890 Gas Chromatograph. A 30 m × 0.25 mm I.D. × 0.25 μm film thickness EC-20 capillary column (Alltech) was used for most analyses, but EC-5 and DB-1 columns of similar dimensions were also used (Alltech); these

less polar phases sometimes allowed successful separations of compounds that coeluted on EC-20. The oven temperature programme was 50 °C for 1 min, followed by an increase at 10 °C per min to 250 °C and a 5-min hold at 250 °C. The split/splitless inlet and the transfer line were kept at 250 °C. Injections were 1–2 μL in the splitless mode. Carrier gas was helium, and the inlet pressure was 42 kPa. The electron impact mass spectra were acquired at 70 eV ionization energy. Data system tools, such as background subtraction and extraction of single-ion chromatograms, made it possible to study spectra and measure relative amounts of collected compounds even when chromatographic separation between compounds was not complete.

Analysis focused on compounds that were present from only one sex. Identification of these was by mass spectrum and GC retention time, and a compound was considered identified only when it matched an authentic standard by these criteria.

Re-examination of the *C. lugubris* aggregation pheromone

The results for *C. sayi* prompted re-examination of the *C. lugubris* pheromone. The *C. lugubris* were captured live on 7 June 2001, in Tazewell County, Illinois, in a wind-orientated pipe trap (Dowd *et al.*, 1992) baited with the synthetic pheromone for *C. lugubris* (Bartelt, 1999) plus WWBD. These beetles were set up as with *C. sayi* (two adults per collector; collections of 2–5 days duration); four collectors were set up with males and two with females. Overall, 24 collections were made from males and 12 from females between 8 June and 25 June 2001. Analysis was as for *C. sayi*.

Synthetic compounds

Various *Carpophilus* pheromone components had been synthesized at NCAUR during previous research. The synthetic compounds were used during the present project as analytical standards for pheromone identification and to prepare lures for the field tests. The specific compounds used in this project were: (2*E*,4*E*,6*E*)-3-methyl-5-ethyl-2,4,6-nonatriene (compound 1); (3*E*,5*E*,7*E*)-4-methyl-6-ethyl-3,5,7-decatriene (compound 2); (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-undecatetraene (compound 3); (2*E*,4*E*,6*E*,8*E*)-3,5-dimethyl-7-ethyl-2,4,6,8-undecatetraene (compound 4); (3*E*,5*E*,7*E*,9*E*)-4,6,8-trimethyl-3,5,7,9-dodecatetraene (compound 5); and (3*E*,5*E*,7*E*,9*E*)-4,6-dimethyl-8-ethyl-3,5,7,9-dodecatetraene (compound 6). References for their syntheses and spectral data are provided elsewhere (Bartelt, 1999).

Lures for field tests

The pheromone lures ('septa') were prepared at NCAUR for the 2001 field test. Synthetic compounds 3 and 4 had been purified by column chromatography on silica gel to remove polar byproducts but still contained small amounts of *Z*-isomers (Bartelt *et al.*, 1990c). For each lure, a hexane solution of compounds 3 and 4 (55 and 450 μg of all *E*-isomers, respectively, in 10 μL) was applied to the cup of a red rubber septum (10 mm O.D. × 18 mm, Aldrich Chemical Co., Milwaukee, WI), followed by 300 μL methylene chloride to aid penetration of the compounds into the rubber. After the solvent had soaked in, the septa were placed in a fume hood for several hours to cure and then into a tightly closed bottle. Septa were sent to Minnesota for the field trial, where they were stored at −70 °C until used.

Lures for the 2002 field test were prepared in the same way except that two doses were used. Septa made as in 2001 were designated as the 'high' dose, and a 10-fold dilution of the pheromone solution was used for the 'low' dose (6 μg of compound 3 + 45 μg of compound 4 per septum). Midway through the 2002 field season, pheromone septa became available from a commercial source (Great Lakes Integrated Pest Management, Vestaburg, MI). These had the same compositions as the septa prepared at NCAUR and were used to complete the study.

Analysis of volatiles from septa

Sample septa (high dose, from NCAUR) were placed in the pheromone collection apparatus as described above (but without diet) so that component ratios and emission rates could be determined over time. Collections were harvested every 2–3 days for 2 weeks. Analysis was by GC (Hewlett Packard 5890) with a flame ionization detector. The column was a 30 m × 0.25 mm I.D. × 0.25 μm film thickness DB-5 capillary (Alltech). Injections were in splitless mode. Nonadecane was used as the quantitative internal standard.

Field tests

The first field experiment had the initial goal of assessing the synthetic *C. lugubris* pheromone (consisting of compounds 3 + 4) for attracting *C. sayi*, based on the report of Williams *et al.* (1995). The pheromone treatment consisted of a pheromone lure ('septum') plus fermenting WWBD (approximately 20 mL, not in contact with the septum but in the same trap), and the experimental control was WWBD by itself. Wind orientated pipe traps (Dowd *et al.*, 1992) were used. One treatment and one control trap were deployed at each of two oak woodland sites known to have populations of *C. sayi*. The first was in the Carlos Avery Wildlife Management Area, Washington County, Minnesota, and the second was at a privately owned woodland in Columbus Township, Anoka County, Minnesota. Trap height was approximately 1.5 m, and trap spacing was at least 10 m. Trapped beetles were collected approximately weekly between 8 June and 18 October 2001. Treatments were re-randomized over the two trap stations at each site and baits were replaced when the traps were checked.

The second field experiment further explored the synergistic effect of compounds 3 + 4 and WWBD and tested the effect of two doses of compounds 3 + 4 on the flight response of *C. sayi*. The methods for this experiment were generally as before but, in this case, six treatments were

used: (i) no bait; (ii) WWBD; (iii) low dose of compounds 3 + 4; (iv) high dose of compounds 3 + 4; (v) low dose of compounds 3 + 4 with WWBD, and (vi) high dose of compounds 3 + 4 with WWBD.

All six treatments were placed at four different oak woodland sites in Minnesota (for a total of 24 traps). Two of these sites were identical to the sites from the 2001 experiment (i.e. Carlos Avery Wildlife Management Area, Washington County and Columbus Township, Anoka County). The other two sites were privately owned properties in Pine Spring (Washington County) and in Lake Elmo (Washington County).

The study ran from 11 April to 18 October 2002. Traps were emptied, baits were replaced, and treatments were re-randomized over the six trap stations approximately each week. However, treatments (iii), (iv) and (v) were not present in the field during the trapping weeks of 3–11 July and 11–18 July, when the pheromone source was switched. After 18 July, all treatments were again present and all replacement baits came from Great Lakes Integrated Pest Management.

Statistical analysis

The trap catch data were subjected to two-way analysis of variance (ANOVA). The *log* $(x+1)$ transformation was applied to stabilize variance. Each trapping period at each site was considered as a 'block.' Only those blocks with non-zero totals were used in the analysis because those in which no beetles were caught provided no information about treatment differences. For 2002, the period during which three of the treatments were not present in the field (3–18 July) was also disregarded to simplify analysis. Separation of means was by the least significant difference (LSD) method ($P < 0.05$). Chi-square analysis was used to test for consistency of sex ratios among the various treatments. The analysis was performed on the total number of males and females caught by the six treatments in the 'complete' blocks in 2002 (a 2×6 contingency table).

Results

Analysis of volatiles from *C. sayi*

Male-specific compounds were readily detected in some collections from *C. sayi* (Fig. 1). The most abundant of these compounds was identified as tetraene compound 4 (Fig. 2), which is also the major pheromone component of *C. lugubris* (Bartelt *et al.*, 1991). This compound was detectable in 33 of the 54 collections, based on the occurrence of three characteristic ions (*m*/*z* 204, 175 and 133, in the appropriate ratios and at the proper GC retention time). Compound 4 was never detected in the initial collections after males were brought in from the field, but it eventually appeared in all collectors with males. Mean time to first appearance of compound 4 was 10 days after setup (range 4–22 days) but, once emission began, the compound was always found in subsequent collections. It was never detected from females; (in Fig. 1, the small GC peak in the female sample at a retention similar to compound 4 had a distinctly different mass spectrum). All beetles were alive and apparently healthy when the collections were terminated after 27 days. The typical amount of compound 4 emitted per male was calculated to be approximately 2 ng per day, and the maximum was approximately 50 ng per day.

The collections with the greatest amounts of compound 4 were studied for additional components. Careful comparison of the male- and female-derived hydrocarbons revealed five additional male-specific compounds that had carbon skeletons different from compound 4 (Figs 1 and 2).

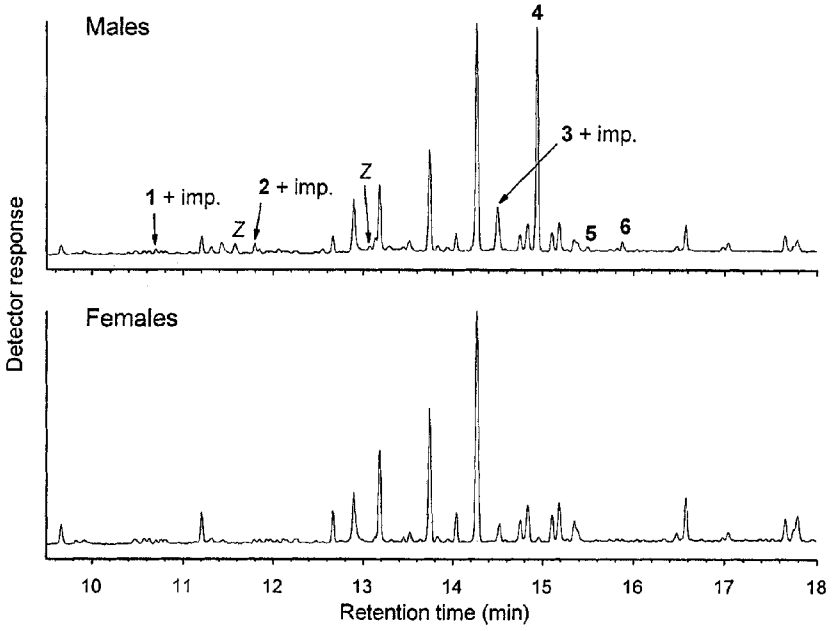


Figure 1 Gas chromatograms (TIC) of volatiles collected from two male and two female *Carpophilus sayi* feeding on artificial diet. Peaks 1–6 represent male-specific compounds described in the text. *Z*-isomers of compound 4 are denoted by *Z*; the presence of coeluting impurities in some GC peaks is denoted by 'imp'. Based on study of mass spectra, compounds 1–6 were never detected from females.

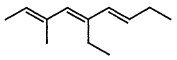
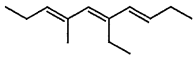
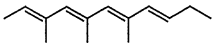
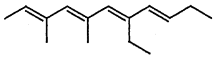
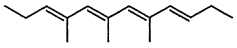
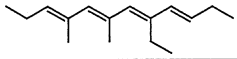
Compound		Relative abundance	
		C. sayi	*C. lugubris*
1		1.6 ± 0.3	-
2		3.7 ± 0.6	-
3		17.9 ± 2.9	14.0 ± 1.8
4		100	100
5		1.5 ± 0.5	0.7 ± 0.3
6		3.7 ± 0.5	1.5 ± 0.5

Figure 2 Structures of compounds 1–6 and relative abundances in volatiles collected from male *Carpophilus sayi* and *Carpophilus lugubris* feeding on artificial diet. The abundance of compound 4 is defined as 100; the other compounds are scaled accordingly (mean ± SD; based on six observations for *C. sayi* and 15 for *C. lugubris*).

Compounds 1, 2 and 6 were first encountered from *Carpophilus freemani* Dobson (Bartelt *et al.*, 1990b), and compounds 3 and 5 were first identified from *Carpophilus hemipterus* (L.) (Bartelt *et al.*, 1990a, 1992). Synthetic standards for compounds 1–6 matched the respective compounds from male *C. sayi*, both with respect to mass spectrum and GC retention on all columns tested. The mass spectra for synthetic and natural tetraene (compound 5) are given as examples (Fig. 3). This demonstrates that acceptable quality spectra could be obtained for even the least abundant male-specific *C. sayi* compounds and without sample purification beyond column chromatography on silica gel. Complete mass spectra for the other five compounds have been reported previously (Bartelt *et al.*, 1990a,b, 1991), but that for compound 5 had not been. As with compound 4, none of these additional compounds was ever detected from females, although coeluting compounds with different mass spectra were often seen in female samples (Fig. 1).

The proportions of the *C. sayi* compounds shown in Fig. 2 were based on the six collections with the greatest amounts of compound 4 (in the range of 5–50 ng per male per day). The relative abundance of compound 4 was defined as 100, and the other compounds were scaled accordingly. The proportions in the other *C. sayi* collections appeared consistent with Fig. 2, to the extent that the minor components could be detected.

Most of the compounds in the male- and female-derived samples (Fig. 1) were actually from the artificial diet (data for analyses of diet alone not shown). The more abundant diet-derived hydrocarbons were mostly sesquiterpenes, presumably from the wheat germ in the diet. *Z*-isomers of compound 4 were also recognized in the male collections with a large amount of compound 4. These isomers had similar mass spectra but earlier GC retention times than the all-E isomer of compound 4. The same isomers also appear in highly purified synthetic compound 4 over time and apparently result from isomerization/degradation (Bartelt *et al.*, 1992). It is unknown whether the *Z*-isomers from *C. sayi* are actually emitted by the males or are artifacts of deterioration. The male- and female-derived ether fractions were compared by GC-MS for those days when the male collections showed the greatest production of compound 4, but no additional sex-specific polar compounds were detected.

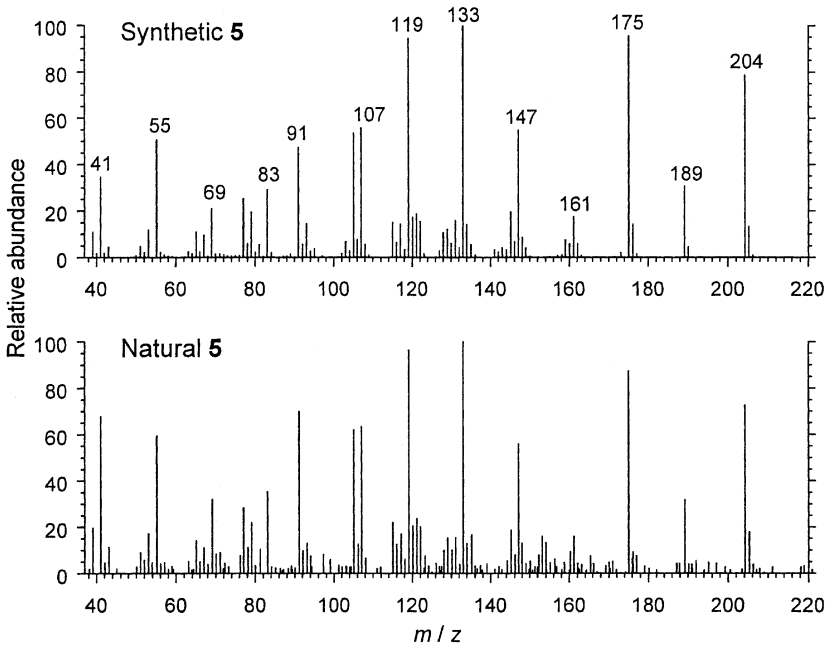


Figure 3 Mass spectra of tetraene compound 5: synthetic standard (above) and the natural compound as a minor constituent in the emission from male *Carpophilus sayi* (below). The upper spectrum represents approximately 100-fold more material than the lower one, which includes some baseline 'noise'.

Analysis of volatiles from *C. lugubris*

As with *C. sayi*, pheromone was nearly absent in the first collections after bringing male *C. lugubris* in from the field (just a trace of compound 4 was detected in one of the four initial 2-day collections), but levels eventually reached at least 100 ng per male per day in all four collectors with males. Other components were also detected, and the results are summarized in Fig. 2 for the 15 collections with the greatest amounts of compound 4 (in the range of 10–100 ng per male per day). Tetraene compound 3 was 14% as abundant as compound 4, similar to the level previously reported (Bartelt, 1999). In addition, tetraene compounds 5 and 6 were found from *C. lugubris* for the first time, but triene compounds 1 and 2 were not detected. None of the compounds were detected from females.

Analysis of volatiles from pheromone septa

The septa with synthetic compounds 3 + 4 initially emitted these compounds in a ratio of 17 : 100. This was higher than the ratio at which they were applied (12 : 100) because of the relatively greater volatility of compound 3. The ratio for the septa stayed quite stable over the 2-week laboratory test period. Mean rates of emission from the septa were 23 and 138 ng per h for compounds 3 and 4, respectively.

Field tests

The 2001 trapping study confirmed that the blend of compounds 3 + 4 was pheromonally active for *C. sayi* (Table 1). Overall, 64 *C. sayi* were captured in traps baited with compound 3 + 4 plus WWBD, whereas only two were caught traps baited with just WWBD. *Carpophilus sayi* were found in at least one trap at a site for 16 of the 36 weekly checks; thus, the ANOVA had 16 blocks. The *F*-statistic for treatment differences was 57.1 (1 and 15 d.f., $P < 0.00001$). The sexes responded to both treatments approximately equally (Table 1). *Carpophilus sayi* were captured at both sites in approximately equal numbers.

In 2002, *C. sayi* were found in at least one trap at a site for 61 of the 112 weekly checks, but five of these occurred during 3–18 July, when three of the treatments were absent (Table 1). Thus, the ANOVA was based on the 56 'complete' blocks. The *F*-statistic for treatment differences was 52.4 (5 and 275 d.f., $P < 0.00001$). The high dose of compound 3 + 4 with WWBD was significantly more attractive than all other treatments, followed by the low dose of compound 3 + 4 with WWBD. No differences among the other four treatments were significant at the 0.05 level. The differences in sex ratios among treatments (Table 1) were not significant (chi-square statistic 2.61, 4 d.f., $P = 0.63$, based on totals for the 56 analysed blocks). The variability in sex ratios evident in Table 1, especially in treatments with the lower trap totals, was not meaningful. *Carpophilus sayi* was captured at all four field sites; totals for the Carlos Avery, Columbus Township, Pine Spring, and Lake Elmo sites were 26, 26, 138 and 102, respectively.

In 2002, captures were recorded for two other *Carpophilus* species, *Carpophilus brachypterus* Say and *Carpophilus corticinus* Erichson (Table 1). *Carpophilus brachypterus* were found in at least one trap at a site for 42 of the 112 weekly checks, and *C. corticinus* were found for 49. For the two species, respectively, the *F*-statistics for tests of treatment differences were 37.6 (5 and 205 d.f., $P < 0.00001$) and 59.0 (5 and 240 d.f., $P < 0.00001$). For both species, combinations of compounds 3 + 4 with WWBD were the most attractive treatments, and like *C. sayi*, these species discriminated between the two doses of compounds 3 + 4 with WWBD. However, treatments with the pheromone or WWBD alone tended to have more activity, relative to the control, for *C. brachypterus* than for *C. corticinus* (Table 1). For *C. brachypterus*, the respective site totals for Carlos Avery, Columbus Township, Pine Spring, and Lake Elmo were 191, 19, 94 and 40; for *C. corticinus*, the respective totals were 35, 40, 20 and 140. No *C. lugubris* were captured during the experiment, but the species was not known to occur at any of the test sites. The seasonal patterns in trap catch for all three *Carpophilus* species and *C. fagacearum* isolation data from the captured *C. sayi* individuals will be reported at a later time by one of us (A.K.A.).

Discussion

Existence of a male-produced aggregation pheromone was demonstrated in *C. sayi*, and six male-specific compounds

Table 1 Summary of trapping results for three *Carpophilus* species*

Treatment	*Carpophilus sayi* (2001)	*Carpophilus sayi* (2002)	*Carpophilus brachypterus* (2002)	*Carpophilus corticinus* (2002)
Control (unbaited trap)		0^{c}	0^{d}	0^{c}
Whole wheat bread dough (WWBD)	2^{b} (50%)	5^{c} (60%)	$18^{c,d}$	2^{c}
Compounds 3 + 4, low dose		1^{c} (100%)	$3^{c,d}$	3^{c}
Compounds 3 + 4, high dose		3^{c} (67%)	24^{c}	1^{c}
Compounds 3 + 4, low dose + WWBD		70^{b} (59%)	54^{b}	46^{b}
Compounds 3 + 4, high dose + WWBD	64^{a} (45%)	213^{a} (50%)	244^{a}	183^{a}

*Overall treatment totals are given for each year and species. In each column, treatments having the same superscript letter were not significantly different (see text for analysis details). Composition of trap catch by sex is indicated for *C. sayi* (females as percentage of total catch). *Carpophilus brachypterus* and *C. corticinus* were not sexed.

were identified. All of these were encountered previously in other *Carpophilus* species. The emissions from *C. sayi* were remarkably like those of *C. lugubris*. The previous report of attraction of *C. sayi* to the two-component (compounds 3 + 4) synthetic pheromone for *C. lugubris* (Williams *et al*., 1995) was therefore due to shared pheromone components rather than a kairomonal response by *C. sayi* to the pheromone of *C. lugubris*. The 2001 field test, which was originally intended to assess the activity of the *C. lugubris* pheromone on *C. sayi* in Minnesota, effectively became a test of the *C. sayi* pheromone. The synthetic blend was clearly active. In both 2001 and 2002, synthetic compounds 3 + 4 greatly increased the attractiveness of WWBD. In 2002, this result held even when the dose of compounds 3 + 4 was reduced to one-tenth. Most striking was that neither the pheromone baits (compounds 3 + 4) nor WWBD alone was significantly more attractive than an empty trap; synergism of the two bait types was very dramatic. This pattern was consistent with responses of other *Carpophilus* species (Bartelt, 1999). In nature, the oak-wilt fungus (Lin & Phelan, 1992) or tree wound volatiles likely serve as the functional equivalent of WWBD.

The ratio of compounds 3 + 4 in emissions from the septa (17 : 100) was very similar to that from both *C. sayi* (17.9 : 100) and *C. lugubris* (14.0 : 100). It remains to be determined whether slight adjustments in the ratio would affect the attractiveness of the blend to *C. sayi*. *Carpophilus sayi* also has four minor male-specific compounds, two of which are now known to occur in *C. lugubris* as well. Further field tests will be required to determine whether these could enhance trap catch in either species.

The emission rate of the high-dose septa was measured to be approximately 70-fold higher than the maximum observed from *C. sayi*, on a per day basis, and the rate for the low-dose septa was therefore approximately seven-fold higher than the *C. sayi* maximum. It is not known whether pheromone emission from males is constant over time or has a daily cycle, but if it occurs primarily during a period of several hours per day, then the emission rate for a group of several males could be similar to a low-dose septum. Furthermore, it is possible that pheromone emission on a natural substrate (oak wilt mat or tree wound) would exceed that observed in the laboratory on artificial diet and that groups of males may release pheromone from such sites under natural conditions. Thus, it is likely that the septum emissions were in the same general range as natural emissions from beetles with respect to both the blend of compounds 3 + 4 and emission rate.

Geographically, the ranges of *C. sayi* and *C. lugubris* overlap broadly, but *C. sayi* appears to be less common and is more restricted in its host range than *C. lugubris*; *C. sayi* is primarily associated with wounds on hardwood trees (Connell, 1956) and regularly colonizes spring oak wilt mats in Minnesota (Cease & Juzwik, 2001). It is unclear whether pheromone differences between *C. sayi* and *C. lugubris* could provide a mechanism for species discrimination in nature. *Carpophilus sayi* emitted slightly more compound 3 than *C. lugubris*, relative to compound 4, but there was overlap in the ratios when sample-to-sample variability was taken into account, and *C. lugubris* and *C. sayi* have both responded clearly to synthetic blends that included compounds 3 + 4 in vastly different ratios (e.g. 100 : 0 and 0 : 100) than was tested here (Bartelt, 1999). A qualitative difference between the two species was that the minor trienes (compounds 1 and 2) were detected only from *C. sayi*. However, in some *Carpophilus* species, such minor male-specific compounds are believed to be merely biosynthetic artifacts rather than true, behaviourally relevant pheromone components (Bartelt, 1999). It appears likely that differences in ecological niche (such as host preference, habitat, and seasonal and diel timing of pheromone-related activity) are more important for species separation than the slight differences in pheromone composition.

Two other *Carpophilus* species responded to the *C. sayi* pheromone, *C. brachypterus* and *C. corticinus*. Neither of the latter species is known to be involved in oak wilt transmission, but both occur in woodland habitats. The attraction of *C. brachypterus* is probably due to shared pheromone components; the pheromone blend of this species includes both compounds 3 and 4 (Bartelt, 1999). It is likely that still higher catches of *C. brachypterus* could have been obtained if the entire *C. brachypterus* pheromone had been used (Williams *et al*., 1995). Nothing is known about the pheromone of *C. corticinus*, but Williams *et al*. (1995) noted that it was attracted to the synergized pheromone of *C. lugubris*.

Regardless of unresolved details about the pheromones of *C. sayi*, *C. lugubris* and other *Carpophilus* species, the pheromone for *C. sayi* promises to be a useful practical tool in the study and management of the oak wilt disease (Kyhl *et al*., 2002b). The pheromone will allow for more sensitive monitoring of beetle dispersal than has ever previously been possible.

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