# AGGREGATION PHEROMONE OF AUSTRALIAN SAP BEETLE, *Carpophilus davidsoni* (COLEOPTERA:  $NITIDULIDAE$ <sup>1</sup>

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Abstract--A male-produced aggregation pheromone was identified for the Australian sap beetle, *Carpophilus davidsoni* Dobson (Coleoptera: Nitidulidae), by bioassay-guided fractionation of volatiles collected from feeding beetles. The most abundant components were: *(2E,4E,6E)-5-ethyl-3-meth*yl-2,4,6-nonatriene, *(3E,5E,7E)-6-ethyl-4-methyl-3,5,7-decatriene, (2E,4E,6E,8E)-3,5,7-trimethyl-2,4,6,8-undecatetraene,* and *(2E,4E,6E,8E)-*  7-ethyl-3,5-dimethyt-2,4,6,8-undecatetraene. The relative abundance of these components in collections from individual males feeding on artificial diet was 100 : 7 : 9 : 31, respectively. Pheromone production began within several days after males were placed onto diet medium and continued for at least 20 weeks. Peak production was  $>3 \mu$ g total pheromone per male per day. Males in groups of 50-60 emitted less pheromone (the peak level was 0.09  $\mu$ g per beetle per day), and the emissions from groups contained relatively little tetraene (proportions of the components listed above were 100 : 7 : 2 : 7, respectively). Three additional trienes and one additional tetraene were identified in minor amounts; the entire eight-component male-specific blend is qualitatively identical and quantitatively similar to that of the North American sibling species, *C. freemani* Dobson. A synthetic blend of the four major components on rubber septa, prepared to emit in the same proportions as from individual males, was highly attractive in the field when synergized with fermenting whole-wheat bread dough. Cross-attraction was observed in the field involving

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<sup>&</sup>lt;sup>1</sup> Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standards of the products, and the use of the names by USDA implies no approval of the products to the exclusion of others that may also be suitable.

the pheromones of *C, davidsoni, C. hemipterus* (L.), and *C. mutilatus* Erichson. Potential uses of the pheromones in pest management arc discussed.

Key Words--Aggregation pheromone, triene, tetraene, hydrocarbon, Coleoptera, Nitidulidae, *Carpophilus davidsoni*, Australian sap beetle, trapping.

### INTRODUCTION

*Carpophilus davidsoni* Dobson is a small (2.5 to 3.5-mm-long), reddish brown sap beetle that occurs in Australia, New Zealand, and Micronesia (Gillogly, 1962; Williams et al., 1983). In his original description, Dobson (1952) noted that the species was found infesting Australian sweet corn, figs, and peaches, and he predicted that it would grow in economic importance, given the pest status of related species. Apparently that prediction has been fulfilled; *C. davidsoni* was the most abundant nitidulid collected from major stone-fruit growing regions of southeastern Australia during 1992-1994 (James et al., in preparation). Nitidulid damage to ripening stone fruit during this period was severe. Major Australian fruit pests such as the Oriental fruit moth, *Grapholita molesta*  Busck, are increasingly being managed by pheromonal disruption of mating, and the nitidulid problem has worsened as insecticides are used less (James et al., 1994). While reduced use of insecticide is desirable environmentally, new tools must be developed to manage those species inadvertently elevated to pest status by avoidance of insecticides.

Nitidulid pheromones have potential as pest management tools. Male-produced aggregation pheromones, to which both sexes respond, are known for a number of *Carpophilus* species, including two in the Australian nitidulid complex, *C. hemipterus* (L.) and *C. mutilatus* Erichson (Bartelt et al., 1990a, 1992, 1993). These pheromones were identified from North American strains of the beetles, but synthetic pheromones for these species have worked well in Australia also (James et al., 1993, 1994). In all cases, a food-related synergist is required for optimal pheromone activity; fermenting bread dough has generally been used for this purpose.

If the pheromone for *C. davidsoni* could be identified, then pheromonal approaches to nitidulid management could be developed/applied simultaneously for all of the major Australian species. In previous field tests, *C. davidsoni*  responded significantly to the synergized synthetic pheromones for five other *Carpophilus* species; these were *C. hemipterus, C. mutilatus,* and three non-Australian species, *C. freemani* Dobson, *C. lugubris* Murray, and *C. obsoletus*  Erichson (James et al., 1993, 1994). Cross-attraction suggested that the pheromone of *C. davidsoni* was similar to those of the other species, but the modest responses ( $2 \times$  to  $10 \times$  more beetles to the pheromone-plus-bread-dough treatments than to the dough control) indicated that none of the other pheromones

were correct for *C. davidsoni; C. hemipterus,* for example, typically responded over  $100 \times$  better to its pheromone plus dough than to dough alone.

The pheromone of *C. davidsoni* has now been analyzed, and we present here the chemical details and evidence for activity of the synthetic pheromone under field conditions.

#### METHODS AND MATERIALS

*Beetle Culture.* A laboratory culture of *C. davidsoni* was established at NCAUR, Peoria, Illinois, from beetles collected at Leeton in southern New South Wales by one of us (D.G.J.). The beetles were easily reared by the method of Dowd and Weber (1991) using a modified diet (Bartelt et al., 1993).

*Volatile Collections.* Volatiles from beetles feeding on artificial diet were collected on Super Q porous polymer (Alltech Associates, Deerfield, Illinois) as described previously (Bartelt et al., 1990a). The temperature during collections was 27°C, and the photoperiod was 14L: 10D. Initially, collections were from groups of 50-60 male or female beetles, but most later collections were from single beetles, after it was determined that analysis of the single-beetle samples was possible.

The volatiles were flushed from the Super Q with 500  $\mu$ l hexane. Nonadecane (2.5  $\mu$ g) was added to each sample as an internal standard for GC. Collections from individual beetles were usually made daily, but the interval was three to seven days for two individuals monitored for 20 weeks. Collection intervals were two to four days for the beetle groups.

*Bioassay-Guided Isolation of Active Compounds.* Activity of volatile collections and chromatographic fractions was assessed by wind-tunnel bioassay (Bartelt et al., 1990a). Briefly, ca. 300 beetles (both sexes) were released into a wind tunnel and began to fly about after several hours without food. Two filter paper test baits to be compared (usually a chemical sample versus a control) were hung side by side in the upwind end of the wind tunnel. Beetles approached attractive baits with a casting, hovering flight and alighted readily on them. Counts of beetles landing on the baits during 3-min test periods were recorded. Tests could be initiated every 5 min for up to 4 hr with one group of beetles. Paired t tests were conducted on transformed data  $\lceil \log(x + 1) \rceil$  to detect treatment differences; residual error was pooled over all tests dealing with a given set of chromatographic fractions.

Active collections were partitioned by polarity on open columns of silica gel. The most active silica-gel fraction (eluted with hexane) was then subjected to HPLC on a silver-nitrate-coated silica column; the solvent was 10% toluene in hexane. Details of procedures and equipment were as in Bartelt et al. (1990a).

*Chemical Analysis.* All volatile collections and derived fractions were ana-

lyzed by capillary gas chromatography (GC). The Hewlett Packard 5890 gas chromatograph was equipped with a splitless injector, flame ionization detector, autosampler, and Hewlett Packard 3396A integrator. The DB-1 capillary column (J & W Scientific, Folsom, California) had a length of 15 m, internal diameter of 0.25 mm, and film thickness of 1.0  $\mu$ m. Carrier gas was helium, and head pressure was 7.7 psig. Oven temperature program was  $50^{\circ}$ C to  $250^{\circ}$ C at  $10^{\circ}$ C/ min. All injections were made in splitless mode. Retention indices of selected GC peaks were calculated relative to  $n$ -alkanes by linear interpolation (Poole and Schuette, 1984, p. 24).

Electron impact mass spectra were obtained for the sex-specific compounds in the active fractions and corresponding synthetic standards. The spectra were taken on a Hewlett Packard 5970 mass selective detector or on a Finnigan TSQ700 tandem mass spectrometer. Sample introduction was always by capillary GC.

*Synthetic Compounds.* Eight synthetic hydrocarbons were used in this research (Figure 1). The compounds were: *(2E,4E,6E)-5-ethyl-3-methyl-2,4,6*  octatriene (1), *(2E,4E,6E)-3,5-dimethyl-2,4,6-nonatriene* (2), *(2E,4E,6E)-5*  ethyl-3-methyl-2,4,6-nonatriene (3}, *(3E,5E,7E)-6-ethyl-4-methyl-3,5,7-deca*triene (4), *(3E,5E,7E)-5-ethyl-7-methyl-3,5,7-undecatriene* (5), *(2E,4E,6E,8E)-*  3,5,7-trimethyl-2,4,6,8-undecatetraene (6), *(2E,4E,6E,8E)-7-ethyl-3,5-dimeth*yl-2,4,6,8-undecatetraene (7), and *(3E,5E,7E,9E)-8-ethyl-4,6-dimethyl-*3,5,7,9-dodecatetraene (8). These were all available from a previous project (Bartelt et al., 1990b, and references therein).



FIG. 1. Structures, assigned structure numbers, and GC retention indices (relative to  $n$ -alkanes) for the compounds used in this research.

Septa were prepared with compounds 3, 4, 6, and 7 for use as baits in field traps; the general procedure was described by James et al. (1993). Small quantities of various  $Z$  isomers were also present unavoidably in the baits, but with other *Carpophilus* species, Z isomers appear not to decrease attraction to the all-E pheromones (see Bartelt et al., 1992). The proportions of components in the baits and the rationale for choosing these are given in the Results and Discussion section.

*Field Studies.* Two experiments were conducted in two unsprayed peach orchards near Leeton in southern New South Wales. Both orchards were floodirrigated, with fruit ripening occurring during February-March. The pheromones of *C. hemipterus* and *C. mutilatus* were used in the field studies in addition to that of *C. davidsoni* [details as in James et al. (1993)]. All pheromones were loaded at a rate of 500  $\mu$ g/septum. Fermenting whole-wheat bread dough was used as the pheromone synergist ( $\approx$  15 ml per trap) (Bartelt et al., 1992). Windoriented funnel traps [Figure 3 in Dowd et al. (1992), the "modified design"] were used in both experiments. Traps were examined, beetles collected, and dough replaced weekly. Septa were replaced fortnightly. Trapping data were transformed to the  $log(x + 1)$  scale and subjected to analysis of variance and least significant difference procedures.

Experiment I was run at the end of the 1993 field season (April 27 to May 19), shortly after pheromone identification was completed, and this test allowed initial evaluation of the *C. davidsoni* pheromone. Five traps containing the pheromone of C *davidsoni* plus dough were added to an ongoing experiment dealing with the pheromone of *C. hernipterus.* The treatments already present were *C. hemipterus* pheromone plus dough (four traps) and dough by itself (four traps). The traps were assigned randomly in a block of approximately 30 trees. Traps were separated by at least 6-12 m and hung from trees at a height of 1.5 m.

Experiment 2 was of longer duration and included most of the 1993/1994 growing season (September 22, 1993, to March 16, 1994), It further evaluated the *C. davidsoni* pheromone and gathered evidence for cross-attraction among *C. davidsoni, C. hemipterus,* and *C. mutilatus.* Three treatments were the pheromones of *C. davidsoni, C. hemipterus,* and *C. mutilatus,* all plus dough, and the fourth was dough alone (three replications per treatment). Traps were hung from trees as in experiment 1 and were 5-6 m apart; treatments were randomized.

#### RESULTS AND DISCUSSION

*Initial Observations.* In the wind tunnel, volatiles from groups of male beetles were clearly more attractive than those from groups of females or blank controls (mean landings were 9.5, 3.0, and 0.5, respectively,  $n = 4$ ). Com-

parison of gas chromatograms from these volatile collections revealed a malespecific peak at retention index 1224; this suggested the presence of triene 3 (Figure 1), a compound encountered previously (Bartelt et al., 1990b). The identity was confirmed by mass spectrometry. Pheromone collections were continued both from groups of males and from individual males to confirm the initial result and to search for additional components.

*Analysis of Pheromone from Individual Males.* A combined sample from individual males (53 volatile collections, 167 total beetle-days) was active in the wind tunnel (Table 1), and most of the activity resided in the hexane fraction derived from this sample. After  $AgNO<sub>3</sub> HPLC$  of the hexane fraction, significant pheromonal activity was found in five consecutive fractions (Table 1). Analysis of these fractions by GC and GC-mass spectrometry revealed the presence of compounds 1-8 (Figure 1). The GC retentions, HPLC retentions, and mass spectra matched those of synthetic standards (see Bartelt et al., 1990b). Traces of Z isomers were detected, primarily related to components 3 and 7, but no other analogous hydrocarbons were found. It is believed that degradation of the all- $E$  compounds produced these isomers (Bartelt et al., 1992). The blend of male-specific hydrocarbons emitted by *C. davidsoni* was qualitatively identical to that reported previously for the North American species, *C. freemani* (Bartelt et al., 1990b).

The existence of these hydrocarbons in *C, davidsoni* made the field responses of *C. davidsoni* to other *Carpophilus* pheromones reported earlier (James et al., 1993) more understandable. The synthetic pheromone for C. *hemipterus* contained 6 and 7 (plus other compounds); that for *C. mutilatus*  contained only 4 and 5; that for *C. freemani,* 3 and 7; that for *C. lugubris, 6*  and 7; and that for *C. obsoletus,* only 6. The relative weakness of responses to these may have been due to missing components and/or improper ratios. Field experiments were used to verify the pheromonal activity of the most abundant components and are presented below; preparation of field samples was guided by the following laboratory results.

*GC of Crude Volatile Collections from Individual Males.* The four most abundant components, 3, 4, 6, and 7, were readily observed in volatile collections from individual males feeding on artificial diet, without any prior chromatographic purification (Figure 2). In fact, the mate-produced pheromone components were the only prominent beetle-related compounds in the volatile collections. Collections from virgin female beetles (e.g., Figure 2, lower panel) were virtually identical to those from diet only. (The diet volatiles probably account for the slight activity of volatile collections from feeding females, noted in initial wind-tunnel tests). The prominence of the major male components allowed analysis of single collections from individual males and from groups of males without combining samples to gain sensitivity.



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collection, The dose for the whole collection and for all fractions was 0.1 beetle-day per test, Test duration was 3 rain. Differences between the test material and control that were significant at the 0,001 level (t tests) are indicated by \*\*\*. No other comparisons

between the test material and control that were significant at the 0.001 level ( $t$  tests) are indicated by  $***$ . No other comparisons

were significant at even the 0.05 level.

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FIG. 2. Gas chromatograms of whole volatile collections from a single male and a single virgin female *C. davidsoni* feeding on artificial diet. Collection period was one day; beetles were 7 days old. Male-specific hydrocarbons are highlighted. The internal standard (I.S.) was nonadecane (2.5  $\mu$ g/sample). The GC temperature program was 50- $250^{\circ}$ C at  $10^{\circ}$ C/min; run duration was 20 min. Retentions of *n*-alkane standards are noted along bottom of chromatogram. Each injection was 2  $\mu$ l from a 500- $\mu$ l crude volatile collection; there was no chromatographic purification or concentration prior to injection

*Amounts of Pheromone Produced.* Pheromone production was dramatically higher for individual males than for beetles relatively crowded in groups (Table 2). For both the individuals and the groups, production was very low for the first two days during which the beetles were in the collectors, but even the oldest beetles tested (up to 20 weeks) still produced pheromone. Peak production usually occurred between one and two weeks after the beetles were introduced into the collectors. Most individuals tested were newly emerged, but the onset and peaking of pheromone production followed the pattern in Table 2 even when beetles were transferred from the culture two weeks after emergence.

*Ratios of Components.* The observed proportions of components are given in Table 3. The amount of 7 relative to 3 was fairly consistent (30.6%  $\pm$  9.0%) standard deviation) among the 237 samples from single beetles for which acceptable quantitation could be obtained (total pheromone in sample  $>0.5~\mu$ g). By one-way ANOVA, about half of the variance that did exist in the relative amount of 7 was due to systematic beetle-to-beetle differences among the 21 individual males from which collections were taken. The amount of 7 relative to 3 was consistently lower for collections from groups of 50-60 beetles than for single

Time after beetles placed in collectors"	Individual males		Groups of 50–60 males	
	$\mu$ g/male/day + SD	n	$\mu$ g/male/day $\pm$ SD	n
$0-2$ days	$0.22 \pm 0.35$	35	$0.0096 + 0.0083$	3
$2-4$ days	$1.6 + 1.1$	35	0.037 $+0.048$	2
$4-7$ days	$2.4 + 1.3$	45	0.026 $+0.014$	5
$7-10$ days	$3.3 \pm 1.1$	44	0.057 $+0.048$	5
$10 - 14$ days	$2.8 + 1.2$	42	$0.085 \pm 0.12$	8
$2-3$ weeks	$2.5 + 1.1$	48	0.081 $+0.056$	3
$3-7$ weeks	$2.2 + 0.4$	16		
$7-11$ weeks	2.0 $+0.4$	16		
$11 - 15$ weeks	$1.4 + 0.4$	16		
$15-20$ weeks	0.7 $+0.5$	14		

TABLE 2. PHEROMONE PRODUCTION BY INDIVIDUAL MALES AND GROUPS OF 50-60 MALES (TOTALS OVER COMPONENTS 3, 4, 6, AND 7)

"Each age category includes times greater than the lower limit and less than or equal to the upper limit.





"Amount of triene 3 was normalized to 100, and the proportional values for other components were calculated. Values for 4, 6, and 7 are means  $(\pm SD)$  for all single collections for which acceptable quantitation was obtained (i.e., total pheromone in sample  $> 0.5 \mu g$ ) (n = 237 for individual males and  $n = 21$  for groups).

<sup>h</sup>Values determined from a combination of 53 volatile collections from individual males, after AgNO<sub>3</sub> HPLC; collections from groups of males were not analyzed for 1, 2, 5, or 8.

beetles (6.6% versus 30.6%, Table 3). As with the individuals, significant systematic group-to-group differences existed; two of the five groups did not produce 7 at a detectable level. The abundance of 7 relative to 3 was strongly correlated with the total amount of pheromone produced per beetle per unit time. Considering collections from both individuals and groups, the amount of 7 relative to 3 increased by 5.4% for each increase of 1  $\mu$ g in daily pheromone production per beetle (simple linear regression,  $t = 13.6$ , 279 *df, P << 0.0001*,  $R^2 = 0.40$ . The relationship was still highly significant when either the individuals or the groups were considered separately.

The ratio of triene 4 to triene 3 and the ratio of tetraene 6 to tetraene 7 both remained quite stable, regardless of whether collections were from individuals or from groups. For individuals, 4 was 6.5% as abundant as 3, and 6 was 29% as abundant as 7. For groups, the respective values were 6.7% and 25%. The greatest distinction between the individual and group collections was in the overall ratio of trienes to tetraenes.

Under natural conditions, we suggest that beetles react to suboptimal (e.g., crowded) feeding/breeding sites by producing smaller amounts of pheromone, and furthermore, this pheromone has relatively little tetraene. Large numbers of beetles at such a site could compensate for low pheromone production per beetle. (In Table 2, for example, pheromone production per male in a group of 50-60 was roughly 1/50th of that by isolated individuals; thus total pheromone production per collector in the two situations was essentially the same.) However, the skewed ratio of components would still be detectable to responding beetles. Intuitively, a blend rich in 7 would be preferred by responding beetles because it would indicate a site with relatively little competition for food or mates. The earlier, mediocre response of *C. davidsoni* to the pheromone of C. *freemani*, which contained 7 and 3 in a 4:100 ratio (James et al., 1993), may have occurred because the blend signaled a suboptimal landing site in the chemical language of *C. davidsoni.* Consequently, we attempted to emulate the blend from individual male beetles when preparing baits for the field tests (Table 3).

*Pheromone Baits for Field Tests.* Compounds 3, 4, 6, and 7 differ markedly in volatility; for example, 3 evaporates about five times faster from septa than 7 (R.J.B., unpublished). To achieve the desired release ratio of 3.3 : 1 for 3 and 7 (Table 3) required loading 3 and 7 in a ratio of about  $0.6:1$ ; load amounts for minor components 4 and 6 were chosen in a similar way. The first-order release property of septa presented a further complication; the release rate is proportional to the amount of pheromone remaining in the septum (McDonough, 1991). For 3, it takes only 1.5 day at 27°C for the amount of pheromone remaining in the septum to decrease by one half, but for 7, the half-life is 8.1 days (R.J.B., unpublished). Thus, proportions of emitted compounds would change considerably over the two weeks during which each septum was to be in the field. However, the emitted blend would become progressively richer in 7, moving away from, rather than approaching, the undesirable proportions observed from groups of males (Table 3).

*Field Studies.* The synthetic blend of *C. davidsoni* pheromone in combination with fermenting bread dough was highly attractive to this species in both field experiments (Figure 3). The effect of this pheromone, relative to the dough treatment and to the *C. hemipterus* pheromone, was somewhat weaker in experiment 2 than in experiment 1. (For example, the *C. davidsoni* pheromone plus dough treatment attracted 48 × more *C. davidsoni* than the dough in experiment 2, but  $377 \times$  more in experiment 1). The reason for this difference is unknown, but experiment 2 included the warmest part of the year, when depletion of the *C. davidsoni* pheromone septa would be especially rapid.

In all cases, *C. davidsoni, C. hemipterus,* and *C. mutilatus* responded in greatest numbers to the conspecific pheromones when these were present. This



FIG. 3. Responses of three *Carpophilus* species to synthetic aggregation pheromones in Australian field tests. Experiment 1 ran from April 27 to May 19, 1993, and experiment 2 from September 22, 1993, to March 16, 1994. The abbreviations, DAV, HEM, MUT, and D, refer to the pheromones of *C, davidsoni, C. hemipterus, C. mutilatus,* and to fermenting bread dough, respectively. For each graph, the responding species is indicated above the bars. In each graph, means accompanied by the same letter are not significantly different [0.05 level, analysis of data in the  $log(x + 1)$  scale]. For experiment 1, there were 16-20 observations per mean; for experiment 2, there were 78.

preference was significant in all but one case (the response of *C. hemipterus* to the pheromones of *C. hemipterus* and *C. davidsoni* in experiment 1). Each of the three species was significantly attracted to the pheromones of the other two, except that *C. hemipterus* did not respond significantly to the pheromone of C. *mutilatus* (experiment 2). The existence of cross-attraction is not surprising because the species share pheromone components in some cases: both the C. *davidsoni* and *C. mutilatus* blends contain compound 4 (Figure 1), and both the *C. davidsoni* and *C. hemipterus* blends contain 6 and 7. However, the attraction of *C. mutilatus* to the *C. hemipterus* pheromone can not be rationalized in this way, and slight kairomonal effects may be operating.

*Use of Nitidulid Pheromones in Future Pest Management.* This demonstration of an effective synthetic aggregation pheromone for *C. davidsoni* means pheromones are now available for the three nitidulid species that cause greatest concern to Australian stone-fruit growers. The potential of *C. hemipterus* and *C. mutilatus* pheromones as attractants for these species in Australia was reported previously (James et al., 1993, 1994). Synthetic pheromones have the potential to become important management tools for nitidulids in stone-fruit orchards. They could be used as the basis of a sensitive monitoring system to facilitate better timing of insecticide sprays, particularly early in the season when growers are often unaware of beetle presence. Improved spray timing would inevitably reduce insecticide inputs and the risk of residues on harvested fruit. Pheromones also provide an opportunity to develop novel control options such as masstrapping or disruption of fruit colonization by the beetles. However, more information on nitidulid biology and ecology in stone-fruit orchards is required before these options can be explored properly. We also need to know the effective trapping radius of nitidulid pheromones together with information on optimal doses. The prospects for developing pheromones as a management tool for nitidulids in stone-fruit orchards appear to be excellent.

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