

Pheromone Combination Lures for *Carpophilus* (Coleoptera: Nitidulidae) Species

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ABSTRACT Combinations of aggregation pheromones for 3 nitidulid beetle species, *Carpophilus hemipterus* (L.), *C. mutilatus* Erichson, and *C. freemani* Dobson, were evaluated as trap baits in a date garden in southern California. All possible 2- and 3-way combinations were compared with the single pheromones; fermenting whole wheat bread dough was present with all pheromone treatments as a synergist, and dough by itself was the experimental control. Each species was strongly attracted to its own pheromone, and species specificity of the pheromones was high. Generally, responses to conspecific pheromones were not inhibited by the presence of other pheromones. Only for *C. freemani* was the 3-pheromone combination significantly less attractive than species pheromone tested alone, but the combination was still more attractive to *C. freemani* than the control. A subsequent wind-tunnel study confirmed that the response of *C. freemani* to its pheromone could be reduced when the major component of the *C. mutilatus* pheromone was present. Two minor species at the test site, *C. obsoletus* Erichson and *C. (Urophorus) humeralis* (F.), exhibited significant cross attraction to the pheromones of the major species in the field. This cross attraction was not hindered by the presence of pheromone combinations. The demonstrated effectiveness of the pheromone combinations would simplify the use of the pheromones for mass trapping. One multispecies lure type would replace a series of specific lures for individual species with no or slight loss of efficacy for any target species.

KEY WORDS *Carpophilus*, pheromone combination, attraction, trapping

SAP BEETLES (COLEOPTERA: Nitidulidae) are abundant inhabitants of date, *Phoenix dactylifera* L., gardens of southern California. The beetles flourish in the waste dates that fall to the ground, and beetles can inflict serious damage to the developing crop (Lindgren and Vincent 1953, Warner et al. 1990a). Prophylactic application of pesticides to the dates ripening on the trees and fumigation of the harvested crop reduce economic loss (Warner et al. 1990b). However, the trend toward less insecticide use, the danger of insecticide resistance developing (Kehat et al. 1976), and the elimination of fumigants such as methyl bromide make it desirable to develop alternative control strategies. The pheromones of these beetles could become important tools in beetle management because pheromone-baited traps could be useful for monitoring beetle populations or for protecting the crop through mass trapping.

Male-produced aggregation pheromones, to which both sexes respond, are known for 3 major

species occurring in date gardens, *Carpophilus mutilatus* Erichson, *C. hemipterus* (L.), and *C. freemani* Dobson, and a minor species, *C. obsoletus* Erichson (Bartelt et al. 1994b). All of these pheromones are synergized by food volatiles such as those from fermenting bread dough or decomposing fruit (Bartelt et al. 1992, 1994a, b; Blumberg et al. 1993). *C. mutilatus* is generally the most abundant species, and as many as 114,000 of these beetles have been caught per trap per 3-d period when the bait was *C. mutilatus* pheromone plus dough (Bartelt et al. 1994a).

The complex of nitidulid species in date gardens could present a barrier to the practical use of pheromones because of the expense to deploy different lures for a series of species. This could be avoided if the pheromones can be combined successfully into a single, multispecies lure. In this study, the individual pheromones for *C. mutilatus*, *C. hemipterus*, and *C. freemani* and all possible combinations of these were compared for all 3 species.

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Materials and Methods

Trap Baits. The 7 synthetic compounds used in this study were (3E,5E,7E)-5-ethyl-7-methyl-3,5,7-undecatriene (1), (3E,5E,7E)-6-ethyl-4-methyl-3,5,7-decatriene (2), (2E,4E,6E,8E)-3,5,7-trimethyl-2,

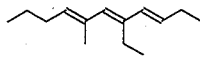
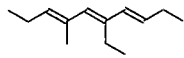
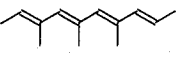
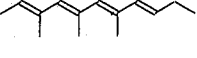
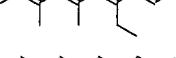
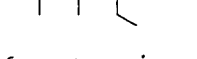
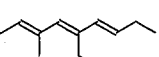
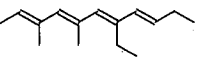
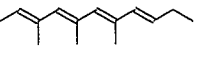
SPECIES AND STRUCTURES	PHEROMONE BAITS
<i>C. mutilatus</i>	BLEND "M"
1 	468 µg
2 	32 µg
<i>C. hemipterus</i>	BLEND "H"
3 	334 µg
4 	103 µg
5 	37 µg
6 	26 µg
<i>C. freemani</i>	BLEND "F"
7 	481 µg
6 	19 µg
<i>C. obsoletus</i>	BLEND "O"
4 	500 µg

Fig. 1. Structures of pheromone components used in the study and assigned structure numbers. Compositions of the pheromone blends are defined. Weights indicate all-*E* isomers. Blends for *C. mutilatus*, *C. hemipterus*, *C. freemani*, and *C. obsoletus* indicated by M, H, F, and O, respectively.

4,6,8-decatetraene (3), (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-undecatetraene (4), (2*E*,4*E*,6*E*,8*E*)-7-ethyl-3,5-dimethyl-2,4,6,8-decatetraene (5), (2*E*,4*E*,6*E*,8*E*)-7-ethyl-3,5-dimethyl-2,4,6,8-undecatetraene (6), and (2*E*,4*E*,6*E*)-5-ethyl-3-methyl-2,4,6-nonatriene (7). Synthetic pheromones for *C. hemipterus*, *C. mutilatus*, *C. freemani*, and *C. obsoletus* (denoted H, M, F, and O, respectively) were blends of these compounds (Fig. 1). For each blend, the dose per septum was 500 µg of the active, all-*E* isomers. Septa were prepared for 2- and 3-blend combinations involving H, M, and F, as well as for the 4 single blends. For each combination, all chemicals were added to a single septum. Synthesis of the compounds and the preparation method for the septa were described previously (Bartelt et al. 1994b).

Experiment. The test was conducted in a garden of 'Deglet Noor' dates at Rancho Eileen, near Oasis, CA. The date palms were in rows, with ≈10

m between trees in a row and 10 m between rows. Each of the 2 experimental blocks was a line of trees. Wind-oriented funnel traps (Dowd et al. 1992) were attached by redwood stakes to alternate trees along each line (trap spacing was 20 m). Trap height was ≈1.5 m.

This study is an extension of Bartelt et al. (1994b), who compared responses to the pheromone blends, H, M, F, and O. All of these were used with fermenting whole wheat bread dough (≈15 ml per trap), and the dough alone was the control. The current study began on 26 May 1992, when 4 additional treatments were added to the 5 noted above: H + M + dough, H + F + dough, M + F + dough, and H + M + F + dough. The 9 traps in each block were then checked and rerandomized weekly until 29 September. The dough co attractant was replaced weekly. The pheromone septa were replaced every 2 wk, but replacement was staggered so that only 1 block received new septa each week, as described by Bartelt et al. (1994b). Initiation of the pheromone-combination study was delayed until the massive spring flight of *C. mutilatus* had abated (Bartelt et al. 1994b). Accurate counts of *C. freemani* could not have been made amidst tens of thousands of *C. mutilatus*.

Data Collection and Analysis of Trap Catches. Trapped beetles were frozen and shipped to the National Center for Agricultural Utilization Research (NCAUR) at Peoria, Illinois, for analysis. Beetles were sorted by species and sex.

Results for the single-pheromone treatments and dough control for 14 April to 29 September are presented by Bartelt et al. (1994b); here we include a subset of these data (for 26 May to 29 September) to serve as a basis of comparison for the combination treatments. All trap counts were transformed to the log($x + 1$) scale to stabilize variance and subjected to analysis of covariance. Calculations were done using the General AOV/AOCV procedure of Statistix software (Analytical Software 1992). The model included treatments, weeks, and blocks as main effects and also the treatment-by-week interaction. Septum ages for the 4 types of pheromones were the covariates. Estimates of aging parameters for H, M, and F represented averages over the 3 combination treatments and the 1 individual treatment involving each of the pheromones.

The treatment sum of squares was partitioned into contrasts for the main effects caused by H, M, and F. Eight treatment means were used for each contrast; for example, H versus no H was the sum of treatment means for H + dough, H + M + dough, H + F + dough, and H + M + F + dough, minus the sum for dough, M + dough, F + dough, and M + F + dough. (The net effects of M and F disappear in this contrast because each appears twice in positive terms and twice in negative terms.) Analogous contrasts, M versus no M and F versus no F, were also constructed, as were the 4 interaction contrasts. (The 9th treatment in the

study, O + dough, was not included in this part of the analysis; its effect was removed from the overall treatment sum of squares by partitioning out the contrast, O + dough versus the mean for all other treatments.)

Finally, individual treatment means were also compared by *t*-tests (least significant difference [LSD], $P < 0.05$, for when the covariates were at their overall means) All statistical computations were done with Statistix software (Analytical Software, 1992).

Wind Tunnel Studies with *C. freemani*. After completion of the field experiment, responses of *C. freemani* to selected combinations of pheromone components were compared in a wind tunnel. Preference tests were conducted using methodology and equipment described by Bartelt et al. (1990). The wind tunnel contained ≈ 500 beetles, with ≈ 50 – 100 being in flight at any instant. Test materials were applied to 2 filter paper baits that were hung side-by-side, 30 cm apart, in the up-wind end of the wind tunnel. Beetles were counted as they alighted on the baits; test duration was 3 min. For all tests, both baits contained 1.3 ng of the major pheromone component of *C. freemani* (compound 7), an amount that consistently elicits a strong response. In addition, one of the baits contained either the major component of *C. mutilatus* (1) or the major component of *C. hemipterus* (3). Compounds 1 and 3 were used at doses of either 1.3 or 13 ng. Purity of samples used in wind-tunnel tests exceeded 95%. Finally, the entire experiment was repeated with a food-related co-tractant added to each bait (20 μ l of a 10% solution of propyl acetate in mineral oil). This solution has negligible activity by itself, but it increases the response to the *C. freemani* pheromone by ≈ 3 -fold (Bartelt et al. 1990). Data were analyzed in the $\log(x + 1)$ scale by paired *t*-tests.

Results and Discussion

Responses of *C. hemipterus*, *C. mutilatus*, and *C. freemani* to Their Pheromones. Each species responded to every treatment containing its own pheromone (with dough) better than to the dough control (Fig. 2). Responses to treatments not containing the conspecific pheromone were generally poor. The main-effect contrasts (Table 1) reflected these trends; the 1-df contrasts for attraction to conspecific pheromones accounted for between 68 and 95% of the total variation (sums of squares) among the 8 treatments. The overall effect of each pheromone is summarized in Table 1 as the ratio of total catches for traps containing the pheromone to the total for those that did not. (The effects were not calculated directly from the contrasts in the $\log[x + 1]$ scale because the transformation distorts ratios when means are near zero.)

One main effect of a heterospecific pheromone was that the presence of blend F always resulted in an increased catch of *C. mutilatus*, relative to

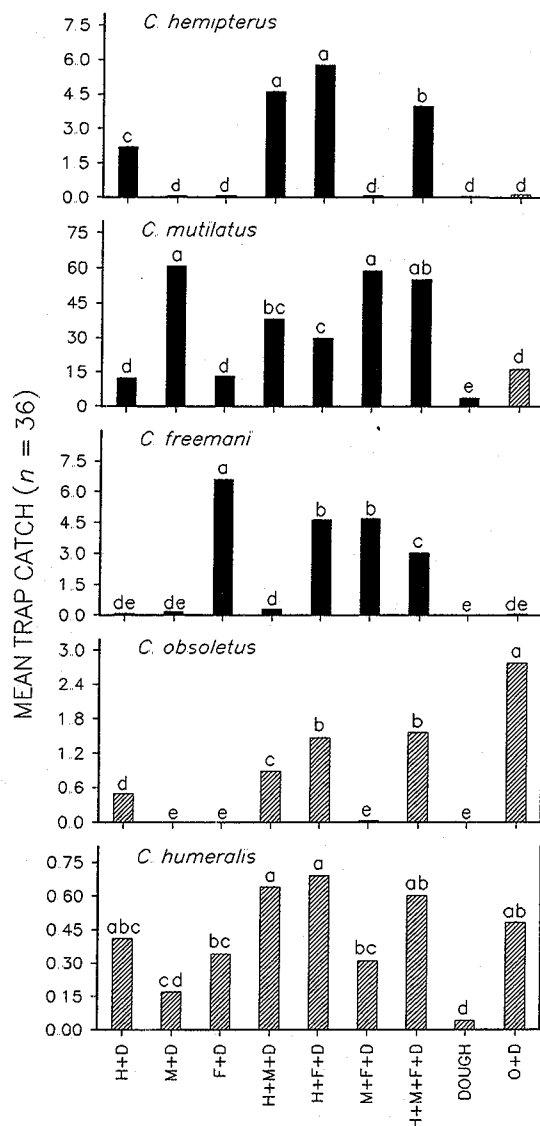


Fig. 2. Responses of 5 *Carpophilus* species to synthetic pheromones and 2- and 3-way combinations of these. Black graph bars represent data for the study as originally planned. Shaded graph bars represent supplementary information involving 2 minor species present at the test site. In each panel, treatments not accompanied by the same letter are significantly different (LSD, 0.05 level). Means were calculated in the analysis scale ($\log[x + 1]$) but were transformed back to the numerical scale for presentation. Pheromones of *C. hemipterus*, *C. mutilatus*, *C. freemani*, and *C. obsoletus*, are indicated by H, M, F, and O, respectively. For the pheromone treatments, D indicates fermenting whole wheat bread dough.

the corresponding bait without blend F (Table 1; Fig. 2). However, other responses to heterospecific pheromones were more complicated and involved highly significant interactions (that is, the effect of one pheromone depended on whether another pheromone was present or absent; Table 1). Blends

Table 1. Responsiveness of 5 *Carpophilus* species to all possible combinations of the pheromones of *C. hemipterus*, *C. mutilatus*, and *C. freemani*: contrasts for main effects of the pheromones and interactions

Responding species	Source of variation ^a	df	F ratio ^b	Overall effect of pheromone ^c
<i>C. hemipterus</i>	H vs no H	1	886.25***	91×
	M vs no M	1	0.08	0.83×
	F vs no F	1	5.52*	1.3×
	Interactions	4	15.53***	—
<i>C. mutilatus</i>	H vs no H	1	14.52***	0.74×
	M vs no M	1	254.40***	13×
	F vs no F	1	36.48***	1.3×
	Interactions	4	16.91***	—
<i>C. freemani</i>	H vs no H	1	3.28	0.76×
	M vs no M	1	1.04	0.51×
	F vs no F	1	641.61***	50×
	Interactions	4	6.70*	—
<i>C. obsoletus</i>	H vs no H	1	214.04***	227×
	M vs no M	1	2.04	1.4×
	F vs no F	1	17.98***	1.7×
	Interactions	4	4.99*	—
<i>C. humeralis</i>	H vs no H	1	26.83***	2.9×
	M vs no M	1	0.88	1.0×
	F vs no F	1	6.45*	1.6×
	Interactions	4	0.95	—

Each contrast is a linear combination of 8 treatment means, including those for H, M, and F plus dough, the 2- and 3-way combinations of these plus dough, and the dough control.

^a Pheromones of *C. hemipterus*, *C. mutilatus*, and *C. freemani* denoted by H, M, and F, respectively.

^b Denominators of F ratios had 156 df. Significance of statistics at the 0.05, 0.01, and 0.001 levels denoted by *, **, and ***, respectively.

^c Each factor was calculated as the total catch for the 4 treatments containing the pheromone divided by the total catch for the 4 treatments that did not.

H and M attracted slightly more *C. freemani* than the dough control, but these blends significantly decreased the response of *C. freemani* to its own pheromone (Fig. 2). Also, attraction of *C. hemipterus* to blend H was enhanced when either M or F was added to the lure, but less enhancement occurred when all 3 pheromones were present together (Fig. 2). *C. mutilatus* responded significantly to H by itself, but H lessened the effectiveness of M when H and M were together in 2- and 3-way combinations.

The interactions have little practical effect, however. For *C. hemipterus*, the 3-way combination exceeded the individual pheromone in activity, and for *C. mutilatus*, the combination was not significantly ($P = 0.52$, $df = 156$, $t = 0.64$) different from the conspecific pheromone (Fig. 2). Only for *C. freemani* was the 3-pheromone combination significantly ($P < 0.001$, $df = 156$, $t = 5.05$) less attractive than the pheromone of this species used alone.

Wind Tunnel Studies with *C. freemani*. The reduced attraction of *C. freemani* in the field to pheromone combinations was examined in the wind tunnel with the major components for *C. mutilatus* and *C. hemipterus*. The *C. mutilatus* com-

Table 2. Wind tunnel responses of *C. freemani* to its major pheromone component (7), with or without additional test chemicals

Dose of 1 or 3 (ng)	Co-attractant present ^a	Effect of 1		Effect of 3	
		1 + 7 ^b	7 alone	3 + 7	7 alone
1.3	No	33.9	26.8	33.9	29.8
13	No	3.4**	16.3	38.4	29.8
1.3	Yes	50.9	52.0	45.9	39.5
13	Yes	4.6*** ^c	18.8 ^c	51.9**	34.7

Data values are mean numbers of landings per 3-min test. Within a row and test compound, significant differences between the test combination and compound 7 alone at $P < 0.01$ and $P < 0.001$ are indicated by ** and ***, respectively (t -tests on data in $\log[x + 1]$ scale); for the other comparisons, $P > 0.05$.

^a Coattractant is propyl acetate in mineral oil.

^b Dose of 7 was 1.3 ng in all cases.

^c Number of replications was 16 for this comparison; $n = 8$ for all others.

ound (1) at the 13-ng dose substantially reduced the number of *C. freemani* responding to its own major component (7); this occurred when a food-related co-attractant was either present or absent (Table 2). The reduction was not significant at the 1.3 ng dose of compound 1. Compound 3 did not cause reductions in response to 7, but it caused a slight yet significant increase in response when the co-attractant was present.

The presence of triene 1 might account for reduced responses exhibited by *C. freemani* in the field to mixtures containing *C. mutilatus* pheromone. This aversion may suggest a semiochemical mechanism for *C. freemani* to avoid competition with *C. mutilatus* under natural conditions. A site co-colonized by both species would be less attractive to *C. freemani* than one with only *C. freemani*. However, *C. mutilatus* demonstrated no comparable avoidance of the *C. freemani* pheromone and might even be somewhat attracted to sites colonized by only *C. freemani* (Fig. 2). Interpretation of semiochemical interactions between these sibling species is complicated by their sharing of minor pheromone components (Bartelt et al. 1990).

No comparable biological basis was suggested for the avoidance of the *C. hemipterus* pheromone by *C. freemani*, and the response reduction in the field remains unexplained. Impurities in the technical grade compounds used in field tests or effects from unusual component doses or ratios may have influenced trap catches. Nevertheless, neither the *C. mutilatus* nor *C. hemipterus* pheromones completely suppressed the response of *C. freemani* to its own pheromone.

Results with *C. obsoletus* and *C. humeralis*. The *C. obsoletus* pheromone was not part of the combination lures, but the species was present in small numbers during the study, and its responses are reported (Fig. 2; Table 1). *C. obsoletus* was attracted most readily to its own pheromone (the 9th experimental treatment), but the species also responded well to all treatments containing the

pheromone of *C. hemipterus*. (A minor component of the *C. hemipterus* pheromone, compound 4, is the only known component for *C. obsoletus* [Fig. 1].) The pheromones of the other species were not active by themselves but enhanced the pheromone of *C. hemipterus*. Of the 3 major species in this study, only *C. mutilatus* was significantly ($P < 0.001$, $df = 156$, $t = 7.24$) cross-attracted to the *C. obsoletus* pheromone.

Carpophilus humeralis was also present during the study and responded to all pheromone treatments except M + dough above the control level (Fig. 2; Table 1). The pheromones of *C. hemipterus* and *C. obsoletus* were most attractive to *C. humeralis*, and combinations generally had higher catches than the individual pheromones. The pheromone of *C. humeralis* is unknown.

Sex of Captured Beetles. Both beetle sexes were captured in similar numbers regardless of species or treatment. For *C. hemipterus*, 46% of the trapped beetles were females, and the range for the individual treatments was 41–55% (ignoring the treatments with 10 or fewer total captures). For *C. mutilatus*, 56% of the beetles were females, and the treatment range was 48–59%. For *C. freemani*, *C. obsoletus*, and *C. humeralis* the overall percentages (and ranges among treatments) were 65% (59–68%), 52% (50–53%), and 46% (35–61%), respectively. (The percentages for *C. humeralis* were least stable, probably because these involved the smallest numbers of individuals.)

Loss of Pheromone Activity Over Time. There was a highly significant ($P < 0.001$) decrease in the activity of blends H, M, and F over time ($df = 156$, $t = -5.44$, -3.98 , and -19.6 , respectively). For *C. hemipterus*, baits containing blend H were, on the average, 62% as attractive after 1 wk in the field as they were when first set out. For *C. mutilatus* and *C. freemani*, the corresponding percentages were 53 and 11%, respectively, for baits containing blends M and F. For *C. obsoletus*, the activity during the 2nd wk was 99% of that during the 1st wk (not significantly different from 100%). These results are similar to those of Bartelt et al. (1994a, b). Loss of activity was greatest for *C. freemani*, which uses the most volatile of the compounds (7) as its major component. Pheromone dispensers with greater field longevity than rubber septa are needed for this pheromone especially.

Ecological Implications. The generally good response of *Carpophilus* species to pheromone combinations was contrary to results with other systems. For example, the sympatric bark beetle species *Ips paraconfusus* and *I. pini* both strongly avoid the pheromone of the other species. When both *Ips* pheromones are present, neither species responds well. In nature, this is a mechanism for avoiding interspecific competition (Borden 1985).

Our data did not suggest a similar reliance on semiochemicals among the nitidulids for avoiding interspecific competition except, to a minor degree, for *C. freemani*. Bartelt et al. (1994b) showed

that dates sampled from the ground often contained 2 or more species. It is possible that a greater degree of avoidance of heterospecific pheromones would occur in the traps if all minor pheromone components were carefully included in the synthetic blends (Dunkelblum and Mazor 1993), but such discrimination among the pheromones is undesirable for many pest management purposes.

Practical Implications. This study demonstrates that pheromones for 3 nitidulid species can be combined into 1 lure without destroying the activity for any of them. This may simplify the use of these pheromones in applied pest management. The 3-pheromone combination was at least as effective as the single pheromones for *C. hemipterus* and *C. mutilatus*. Only for the least abundant of the species, *C. freemani*, was there an adverse effect from combining the pheromones, but this combination was still an effective bait.

Carpophilus obsoletus and *C. humeralis*, 2 minor species, were cross attracted to the pheromones of the major species, and the 3-pheromone combination was as active or more active than any of the single pheromones. Any practical benefit gained by luring these species to heterospecific pheromones would not be lost when the pheromones are combined into one bait.

Further improvements to the combination lure are still desirable. It may be possible to overcome the reduced effectiveness of the combination lure for *C. freemani* by adding a greater amount of its pheromone to the blend. Specific attractants for *C. humeralis* and another nitidulid, *Haptoncus luteolus* (Erichson), should be added as these become known; these species can have major importance in other date growing regions (Kehat et al. 1983). Also, field longevity of pheromone formulations could be improved and synthetic versions of the synergistic host volatiles (bread dough odor) could be evaluated in the date gardens.

Acknowledgments

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