

SEMIOCHEMICAL-MEDIATED FLIGHT RESPONSES OF SAP BEETLE VECTORS OF OAK WILT, *Ceratocystis fagacearum*

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Abstract—The sap beetle, *Colopterus truncatus* (Coleoptera: Nitidulidae), is one of the primary vectors of the oak wilt pathogen, *Ceratocystis fagacearum*, in the north-central United States. Field behavioral assays utilizing various release rates and blends of three methyl-branched hydrocarbon aggregation pheromone components showed that flight responses of this beetle were similar in Illinois and Minnesota populations. In both locations, both sexes of the beetle responded synergistically to a combination of the three-component pheromone and fermenting whole-wheat bread dough. Further, *Colopterus truncatus* preferred a high release rate over a low release rate of the three-component blend. In both locations, the response of *C. truncatus* to a simplified version of the pheromone consisting of (2*E*,4*E*,6*E*)-3,5-dimethyl-2,4,6-octatriene (**1**) and (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene (**3**) was not significantly different from the response to the three-component blend. An experiment in Illinois with all possible combinations of the components demonstrated that the decatetraene (**3**) was the crucial component in the blend; of all treatments, the maximal response was elicited by **3** + dough. Chipped bark, phloem, and xylem from northern pin oak, *Quercus ellipsoidal*, was not attractive to *C. truncatus* in Minnesota. During a weekly survey over two seasons in Minnesota, *C. truncatus* flew in response

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to the three-component pheromone between early April and early July, with the maximum responses coming on May 4, 2000 and April 20, 2001. During both years, more than 98% of the beetles were trapped between April 14 and June 1. During the same survey, *Glischrochilus* spp. (Nitidulidae) flew during longer periods of the summer, particularly in 2001. The sex ratio of *C. truncatus* responding during all experiments was female-biased (1.8:1, female-male), which is characteristic of other male-produced coleopteran aggregation pheromones. Other sap beetles that play a minor role in the pathobiology of *C. fagacearum* also responded in experiments conducted in Minnesota. *Carpophilus brachypterus* Say was cross-attracted to the two- and three-component blends of the *C. truncatus* pheromone and dough, whereas two *Glischrochilus* spp. were attracted to all treatments that contained dough.

Key Words—*Colopterus truncatus*, oak wilt, *Ceratocystis fagacearum*, aggregation pheromone, Coleoptera, Nitidulidae, sap beetle, *Glischrochilus* spp., *Carpophilus brachypterus*, methy-branched hydrocarbons, monitoring, phenology.

INTRODUCTION

Oak wilt, *Ceratocystis fagacearum*, Bretz (Hunt), is a fungal disease that annually kills many oak trees (Fagaceae: *Quercus* spp.) across a large area in the eastern and north-central United States (Tainter and Baker, 1996). For example, by the mid-1990s, aerial photographs recorded 3000 oak wilt infection centers (areas) in Minnesota that contained 100,000 recently killed trees (French, 1995). This disease has had a significant effect on the vegetative composition of natural, periurban, and urban forests and is the disease of most concern to forest health specialists in 5 of the 22 states in which it occurs (Billings, 2000).

One way in which new oak wilt infection centers are established in the landscape is through the dispersal of oak wilt spores during the feeding and reproductive activities of sap beetles (Coleoptera: Nitidulidae). These insects are attracted to and aggregate on oak wilt fungal fruiting tissues (mats) that occur on oaks recently killed by the pathogen (Lin and Phelan, 1992; Cease and Juzwik, 2001). The beetles feed, mate, and oviposit on the mats from which oak wilt fungal propagules are ingested and accumulate on the cuticular surfaces of the adults and larvae (Curl, 1955; Jewell, 1956; Juzwik, 1983). Spore-laden sap beetles also aggregate, feed, mate, and oviposit in sap fluxes associated with fresh wounds on uninfected oak trees, thereby propagating the disease (Jewell, 1956). The response of sap beetles to wounded oak tissue can be remarkably rapid; some species may be attracted to experimentally created wounds within 15 min (J.J., personal observation). In one study, up to 78% of sap beetles that responded to wounds carried viable oak wilt spores in or on their bodies (Juzwik et al., 1999). Behaviors associated with exposure to inoculum and the visitation of fresh wounds have led to the identification of two sap beetles, *Colopterus truncatus* (Randall) and *Carpophilus sayi* (Parsons),

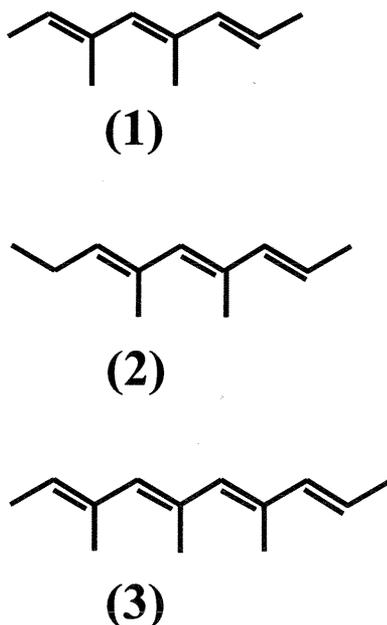


FIG. 1. Hydrocarbon components of the male-produced *Colopterus truncatus* aggregation pheromone: (1) $(2E,4E,6E)$ -3,5-dimethyl-2,4,6-octatriene, (2) $(2E,4E,6E)$ -4,6-dimethyl-2,4,6-nonatriene, and (3) $(2E,4E,6E,8E)$ -3,5,7-trimethyl-2,4,6,8-decatetraene.

as the primary vectors of oak wilt in the north-central United States (Juzwik et al., 1999; Cease and Juzwik, 2001).

In order to improve our understanding of the life history of *C. truncatus*, we investigated the response of this insect and associated sap beetles to semiochemicals in oak forests of Illinois and Minnesota. Little is known about the chemical ecology of the response of *C. truncatus* to oak wilt mats or to wounded oaks in the field. However, a three-component, male-produced aggregation pheromone was recently isolated and identified from *C. truncatus* (Cossé and Bartelt, 2000). Three methyl-branched hydrocarbons, $(2E,4E,6E)$ -3,5-dimethyl-2,4,6-octatriene (1), $(2E,4E,6E)$ -4,6-dimethyl-2,4,6-nonatriene (2), and $(2E,4E,6E,8E)$ -3,5,7-trimethyl-2,4,6,8-decatetraene (3) (Figure 1), elicited electroantennographic activity from male and female antennae, and when combined with fermenting whole-wheat bread dough as a trap bait, synthetic hydrocarbon 2, the combination of 1 and 3, and the combination of 1, 2, and 3, all elicited flight responses from both sexes that significantly exceeded the response to dough alone (Cossé and Bartelt, 2000). The nonatriene (2) was the most abundant of the three components; volatiles collected from males indicated emission rates of 1.8:100:3.3 for 1, 2, and

3, respectively. However, Cossé and Bartelt (2000) did not unequivocally establish the relative significance of the components for the flight response of *C. truncatus*, particularly in light of the differences in volatility of components 2 and 3 after they had been loaded on rubber septa for slow release in the field.

In this study, we report new information on the chemical ecology of *C. truncatus* and other sap beetles associated with oak wilt. The specific goals of this study were to: (1) determine and compare the responses of *C. truncatus* to its pheromone at two different locations in the north-central United States; (2) optimize the blend and release rate of the *C. truncatus* synthetic aggregation pheromone components and explore the potential of specific synergy of the pheromone with oak tissue; and (3) determine the flight period of *C. truncatus* in oak forests in Minnesota to more clearly define the high risk seasonal period for overland transmission of oak wilt.

METHODS AND MATERIALS

Semiochemicals and Baits. As attractants, we used the synthetic aggregation pheromone components for *C. truncatus* [1, 2, and 3 (Cossé and Bartelt, 2000) (Figure 1)] combined with fermenting whole-wheat bread dough or oak chips. Our standard attractant for *C. truncatus* consisted of dough in a plastic cup (see below) and 3.5, 500, and 280 μg each of the all *E* isomers of 1, 2, and 3, respectively, applied in hexane to a rubber septum. Solution concentrations were quantified by capillary GC using an internal standard, and all pheromone components were formulated at the USDA-ARS laboratory in Peoria (Cossé and Bartelt, 2000). With these initial amounts of 1, 2, and 3 loaded on a septum, laboratory analyses have indicated that the proportions released during the first day from a trap are very close to those emitted by male beetles (Cossé and Bartelt, 2000). In two experiments, the treated septa were placed within 1.5 ml polyethylene Eppendorf microcentrifuge tubes each with a single pinhole in the lid (low release rate). Otherwise, the treated septa were pinned directly to the trap (high release rate).

The synthetic compounds had ca. 80% chemical purity and after passage through silica gel with hexane to remove polar contaminants, the remaining impurities were small amounts of *Z* isomers. The *Z* isomers result both as artifacts of synthesis and from slow isomerization of the *E* isomers, and there is presently no simple, large-scale method available for their removal. However, with other nitidulids, small amounts of *Z* isomers have not significantly reduced trap catch (Bartelt, 1999).

When compound 3 is synthesized (Bartelt et al., 1990), about 1–2% of the product consistently is compound 1 (which arises because a Wittig-Horner condensation does not quite go to completion). When a 100:1 mixture of 3 and 1 is applied to a rubber septum, 3 and 1 are released with an initial ratio of about 5:1 instead of 100:1 because of the impurity and because of their different boiling

points (Cossé and Bartelt, 2000). Thus, synthetic **3** on a rubber septum will deliver both **3** and **1** into the air at approximately the same ratio as from the male beetles (3.3:1.8). No complication of this sort arises with **2** because neither **3** nor **1** are produced in its synthesis, nor is **2** produced in the synthesis of **1** or **3**.

For one experiment (IV), samples of **1**, **2**, and **3** were purified further so that they could be tested individually and in all possible combinations. Synthetic **3** was purified by HPLC on a AgNO₃-coated silica column (Heath and Sonnet, 1980), using 0.5% 1-hexene in hexane as the solvent. The procedure removed all traces of **1** and a substantial fraction of the Z isomers [final chemical purity 90%, by capillary GC, instrumentation as in Cossé and Bartelt (2000)]. The major triene **2** was similarly purified to >90% by HPLC; the purity of minor triene **1** was 81%. After HPLC purification, this sample was free of compounds **2** and **3**.

Fermenting whole-wheat bread dough (dough) was used in these experiments because it is an attractive food bait to a variety of nitidulid species and because it has also been shown to act as a synergist for all nitidulid pheromones tested (Bartelt, 1997). Dough was prepared in the laboratory between 2 and 6 hr before use and consisted of whole-wheat flour, table sugar, water, and baker's yeast (3:1:3–4:0.1). Approximately 10–15 ml of dough was placed in a 35-ml plastic cup, and the cup was placed in the trap.

To test for the possibility of attraction of *C. truncatus* to volatiles produced by oaks, tissue was collected from a healthy northern pin oak, *Quercus ellipsoidalis* (E. J. Hill), in mid-April 2001. Live branches (<6 cm diam.) were cut and chipped using a chipper/shredder. All branch chips were mixed together and stored in heavy plastic bags in a –55°C freezer until they were placed in the field.

Traps and Field Sites. All studies used wind-oriented funnel traps (Dowd et al., 1992), which were hung from tree branches or posts ca. 1–1.5 m above the ground. Traps were placed at least 10 m apart. In one experiment, the traps were slightly modified to hold approximately 300 ml of oak chips. In Illinois, all studies were conducted in a single mixed hardwood forest near East Peoria (40.7°N latitude, 89.8°W longitude), while in Minnesota, four separate northern red/white oak (*Quercus rubra* L./*Quercus alba* L.) forests near Minneapolis-St. Paul were used as field sites. All four Minnesota field sites were separated by at least 5 km: Carlos Avery Wildlife Management Area (45.4°N latitude, 93.0°W longitude), a private residence in Columbus township (45.2°N latitude, 93.1°W longitude), Katherine Abbott Park in Mahtomedi (45.1°N latitude, 93.0°W longitude), and a private residence in Lake Elmo (45.0°N latitude, 92.9°W longitude). If more than one block was located at a site, blocks were separated by at least 100 m.

Field Bioassays. Six experiments were conducted in this study. They took place in Illinois in 2000 and in Minnesota in 2000 and 2001.

Experiment I. A study was conducted from late March to mid-April, 2000 in Illinois to establish the importance of the dough coattractant and to determine which of two pheromone release rates was most attractive to *C. truncatus*. The

experiment consisted of: (1) a high release rate of **1 + 2 + 3**, (2) a low release rate of **1 + 2 + 3**, (3) dough, (4) a high release rate of **1 + 2 + 3 + dough**, (5) a low release rate of **1 + 2 + 3 + dough**, and (6) an unbaited control. The experiment was initiated on March 24 with two completely randomized blocks (six traps per block), and the traps were emptied semiweekly until April 18. Treatment locations were rerandomized within blocks and dough baits were replaced after each trap was emptied. Pheromone baits were replaced weekly, but the replacement was staggered so that it was done in just one of the blocks after each trap check. Thus, after March 28 pheromone baits of two ages were always present, and in principle, changes in bait attractiveness over time would not be completely confounded with day effects.

Experiment II. Experiment I (Illinois) was duplicated in 2000 in Minnesota with the additional goal of monitoring the seasonal flight periodicity of *C. truncatus*. The same six treatments and the same trap type from experiment I were used. Experiment II was initiated on April 14 in a completely randomized block design in each of four field sites. Traps were emptied, rerandomized, and rebaited weekly until November 3. Traps were not baited in a staggered fashion as described in experiment I.

Experiment III. In 2001, the response to different treatments and the seasonal flight periodicity of *C. truncatus* were again monitored in Minnesota. This experiment consisted of: (1) a high release rate of **1 + 2 + 3 + dough**, (2) dough, and (3) an unbaited control. The experiment was initiated on April 5 and continued until November 9. Sites, rebaiting, and rerandomization schedules were the same as in 2000.

Experiment IV. In 2000, in a second experiment in Illinois, the effects of **1**, **2**, and **3** on the response of *C. truncatus* were studied. In this case, the compounds were released individually and in all possible combinations. The amounts of each component loaded on the septa were 3.5, 500, and 280 μg , respectively, no matter what combination was tested. Based on the results of experiment I, high release rates of **1**, **2**, and **3** were always combined with dough. The experiment consisted of: (1) **1 + dough**, (2) **2 + dough**, (3) **3 + dough**, (4) **1 + 2 + dough**, (5) **1 + 3 + dough**, (6) **2 + 3 + dough**, (7) **1 + 2 + 3 + dough**, and (8) dough alone control. The experiment was established as two completely randomized blocks, each with eight traps, in the same woodlot used for experiment I. Traps were set on posts at a height of 1 m and were baited fresh at ca. 07:00 AM on mornings with a favorable weather forecast (i.e., temperature $> 15^{\circ}\text{C}$, with minimal wind and cloud cover). As peak flight activity of *C. truncatus* occurs in mid- to late afternoon (R.J.B., personal observation), traps were emptied either near dusk or early the following morning. Treatment locations were rerandomized within blocks each day. Seven replications of the experiment were run between April 21 and May 5, 2000, and each replication was of short duration to ensure that the component ratios and emission rates of the pheromone components would be essentially stable throughout the study.

Experiment V. Based on the results of experiment IV in Illinois, an experiment was conducted in 2001 in Minnesota (same four sites) to compare responses of *C. truncatus* to the high release rate of 1 + 2 + 3 and to the high release rate of 1 + 3. As in experiment IV, the amounts of 1, 2, and 3 loaded on the septa were always 3.5, 500, and 280 μg , respectively. However, in this instance, component 3 also consisted of 1–2% of component 1. The experiment consisted of: (1) 1 + 2 + 3 + dough, (2) 1 + 3 + dough, (3) dough, and (4) an unbaited control. Traps were hung from trees at a height of 1.5 m, baited fresh between 07:00 and 10:00 AM on days with a favorable weather forecast (as above), and emptied 24 hr later. This experiment was conducted for eight days between April 28 and May 31.

Experiment VI. An experiment was conducted in 2001 in Minnesota to compare responses of *C. truncatus* to oak chips (i.e., their volatiles) with responses to oak chips plus the high release rate of 1 + 3. The experiment consisted of: (1) 1 + 3 + 300 ml oak chips, (2) 300 ml oak chips, and (3) an unbaited control. The experiment was set up as completely randomized blocks in each of the four previously described sites. Traps were placed, emptied, rebaited, and rerandomized on the same schedule and dates as in experiment V. The oak chips were replaced daily, and the previous day's chips were always moist when they were removed from the traps, increasing the likelihood that volatiles were still being released.

Statistical Analyses. Data were tabulated by species and sex, with the exception of experiment II, in which the sexes of responding *C. truncatus* were not determined. Nitidulid beetles other than *C. truncatus* were also tallied in the Minnesota experiments. Data from all experiments were analyzed by Friedman's nonparametric two-way ANOVA. Data from experiment IV also were analyzed by standard two-way ANOVA, after transformation of the trap-catch counts by $\log(X + 1)$, because several linear contrasts were of particular interest. When *F* statistics were significant, means were compared using LSD (Bonferroni's method) with an experiment-wise $\alpha = 0.05$. Since trap catches of *C. truncatus* dropped off dramatically after mid-June in Minnesota, statistical analyses of responses of this species in 2000 and 2001 were only carried out on data collected during the mid-April to the mid-June periods. In some cases, analyses of trap catches of other nitidulid species spanned the entire trapping season. Voucher specimens from Minnesota trap catches were deposited in the University of Minnesota Insect Collection.

RESULTS

Experiment I. The high release rate of 1 + 2 + 3 + dough was the most attractive treatment to *C. truncatus* (Friedman's $\chi^2 = 367.1$, $df = 5$, $P < 0.001$; Figure 2A). The high release rate of 1 + 2 + 3 and dough were clearly synergistic; without the dough, *C. truncatus* did not respond to the high release rate of 1 + 2 + 3, and dough alone attracted about one sixth the mean number of *C. truncatus* that responded to the combined treatments. There was no difference

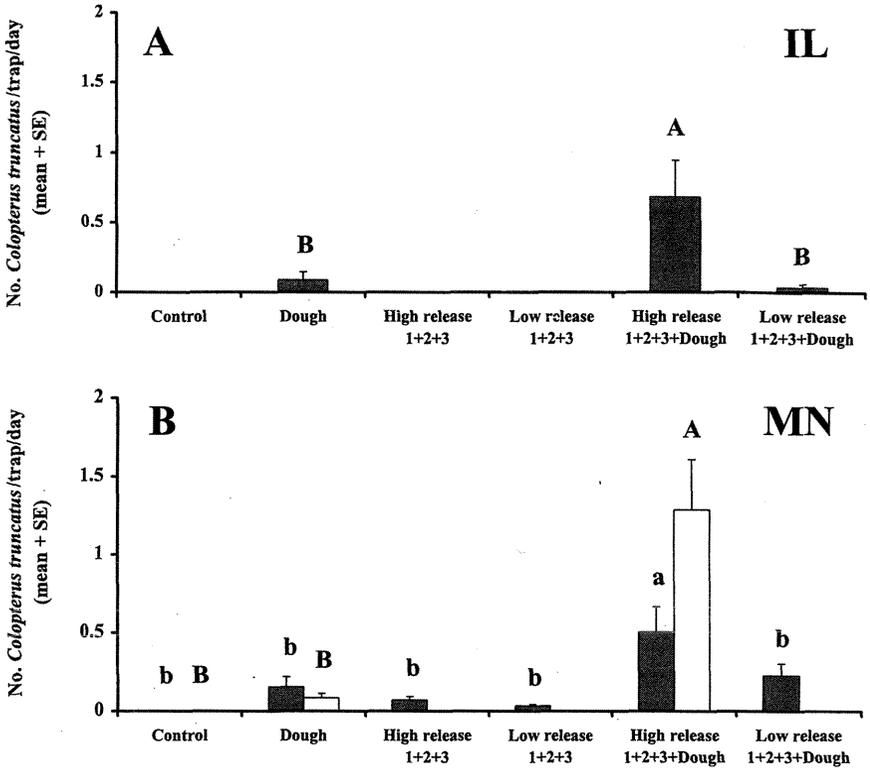


FIG. 2. Flight response of *Colopterus truncatus* to high and low release rates of synthetic aggregation pheromone components 1 + 2 + 3, with and without dough, from (A) March 24 to April 18, 2000 in Illinois (experiment I) and from (B) April 4 to June 15, 2000 (dark bars) and April 5 to June 15, 2001 (open bars) in Minnesota (experiments II and III). Responses to treatments are significantly different if histogram bars within a year are marked with different letters.

between the responses of *C. truncatus* to dough alone and to dough combined with 1 + 2 + 3 released at the low rate. Overall, trap catches were too low to take advantage of the information from staggered pheromone replacement. Both sexes of *C. truncatus* (31 females and 21 males) responded in this experiment, and the sex ratio was not significantly different among treatments ($\chi^2 = 0.07$, $df = 5$, $P = 0.97$). No *C. truncatus* were collected in control traps.

Experiments II and III. *Colopterus truncatus* responded to the two release rates of its aggregation pheromone in Minnesota as it did in Illinois (Figure 2B). In 2000, the high release rate of 1 + 2 + 3 combined with dough was clearly the most attractive treatment to *C. truncatus* (Friedman's $\chi^2 = 3,414.9$, $df = 5$, $P < 0.001$;

Figure 2B). In experiments II, III, V, and VI, there were no significant effects for site or the site \times treatment interaction for any of the taxa that responded. In experiments I and IV, only one site was used. As in Illinois, the responses of *C. truncatus* to dough alone and to the high release rate of 1 + 2 + 3 alone were low and did not differ significantly from one another or from the response to the unbaited trap. However, when the dough and high release rate of aggregation pheromone were combined, the response increased more than threefold above the response to the high release rate of 1 + 2 + 3 alone, indicating that the treatments were synergistic. Both treatments involving the low release rate of 1 + 2 + 3 elicited responses that were not significantly different from the response to the unbaited trap. However, when the low release rate of 1 + 2 + 3 was combined with the dough, the response was only exceeded by the comparable high release rate treatment. In 2001, the high release rate of 1 + 2 + 3 + dough was the most attractive treatment to *C. truncatus* (Friedman's $\chi^2 = 363.7$, $df = 2$, $P < 0.001$; Figure 2B). Although the numbers of trapping days were nearly identical, overall *C. truncatus* trap catch was nearly twice as great in 2001 (385) when compared to 2000 (206). Both sexes of *C. truncatus* responded in 2001 (the only year sex ratio data were collected); 247 females and 138 males were captured, and the sex ratio did not vary across treatments ($\chi^2 = 0.28$, $df = 2$, $P = 0.59$). No *C. truncatus* were collected in control traps during either year.

Two other nitidulid beetles, *Glischrochilus quadrisignatus* (Say) and *G. fasciatus* (Olivier), responded to the treatments in experiments II and III in a different way than did *C. truncatus* (Figure 3). In both years, these species responded to all traps containing dough at a higher level than those without dough (2000: Friedman's $\chi^2 = 3,722$, $df = 5$, $P < 0.001$; 2001: Friedman's $\chi^2 = 588.1$, $df = 2$, $P < 0.001$). No synergism was found, and there was no discernible response to either release rate of 1 + 2 + 3 alone. Hence, there was no evidence for cross-attraction of *G. quadrisignatus* and *G. fasciatus* to the pheromone of *C. truncatus*. The total pooled trap catch for *G. quadrisignatus* and *G. fasciatus* was nearly twice as great in 2000 (542) when compared to 2001 (337). Two other sap beetles, *Carpophilus sayi*, and *Epuraea corticina* Erichson were trapped in experiments II and III, but not in sufficiently high enough numbers to analyze.

Seasonality in Experiments II and III. There was a definite seasonality to the flight activity of *Colopterus truncatus* in Minnesota. In both years, adult activity was initiated in mid- to late April, peaked between late April and early May, and largely disappeared by early and mid-June (Figure 4A). Continued monitoring until November caught only three additional *C. truncatus*, one on September 15, 2000, and one each on August 24 and 31, 2001. The maximum flight responses came on May 4 (2000) and April 20 (2001), and during both years, more than 98% of the beetles were trapped between April 14 and June 1.

The seasonal pattern of flight activity of *G. quadrisignatus* and *G. fasciatus* (both species pooled) was different than *C. truncatus* (Figure 4B). In 2000, these

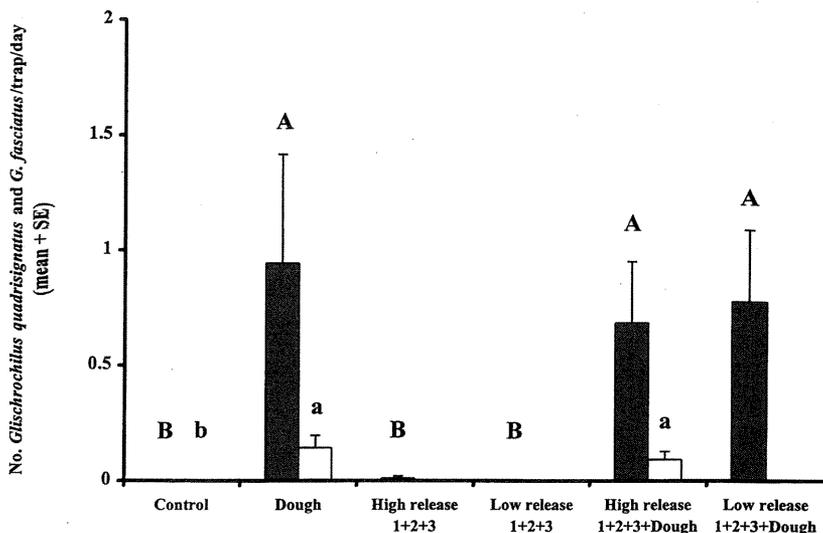


FIG. 3. Pooled flight responses of *Glischrochilus quadrisignatus* and *G. fasciatus* to high and low release rates of synthetic *Colopterus truncatus* aggregation pheromone components 1 + 2 + 3, with and without dough, from April 4 to June 15, 2000 (dark bars) and April 5 to June 15, 2001 (open bars) in Minnesota (experiments II and III). Responses to treatments are significantly different if histogram bars within a year are marked with different letters.

species were abundant in the spring and their presence continued at a low, but discernible level throughout the rest of the year, showing a pattern somewhat similar to *C. truncatus*. In 2001, there was no large spring peak, and the abundance varied throughout the remainder of the year, peaking in mid- to late July. While there was a large amount of variation in trap catches during both years of the study, *G. quadrisignatus* and *G. fasciatus* were generally more abundant than *C. truncatus*, and they did not show the same distinct limited seasonality in Minnesota.

Experiment IV. The effect of all possible combinations of 1 + 2 + 3 on *C. truncatus* in Illinois, showed a strong treatment effect, using either the Friedman's nonparametric two-way ANOVA (Friedman's $\chi^2 = 2,104.2$, $df = 7$, $P < 0.001$) or the standard two-way ANOVA with data transformed using $\log(X + 1)$ ($F = 21.7$, $df = 7,49$, $P < 0.001$) (Figure 5). The day \times treatment interaction was not significant ($F = 1.3$, $df = 42,49$, $P < 0.185$), indicating that relationships among treatments were fairly consistent from day to day. Individual block effects were allowed to vary from day to day in the analysis (i.e., the day \times block interaction was fitted); this precaution allowed for the possibility that the *C. truncatus* population density changed in different ways over

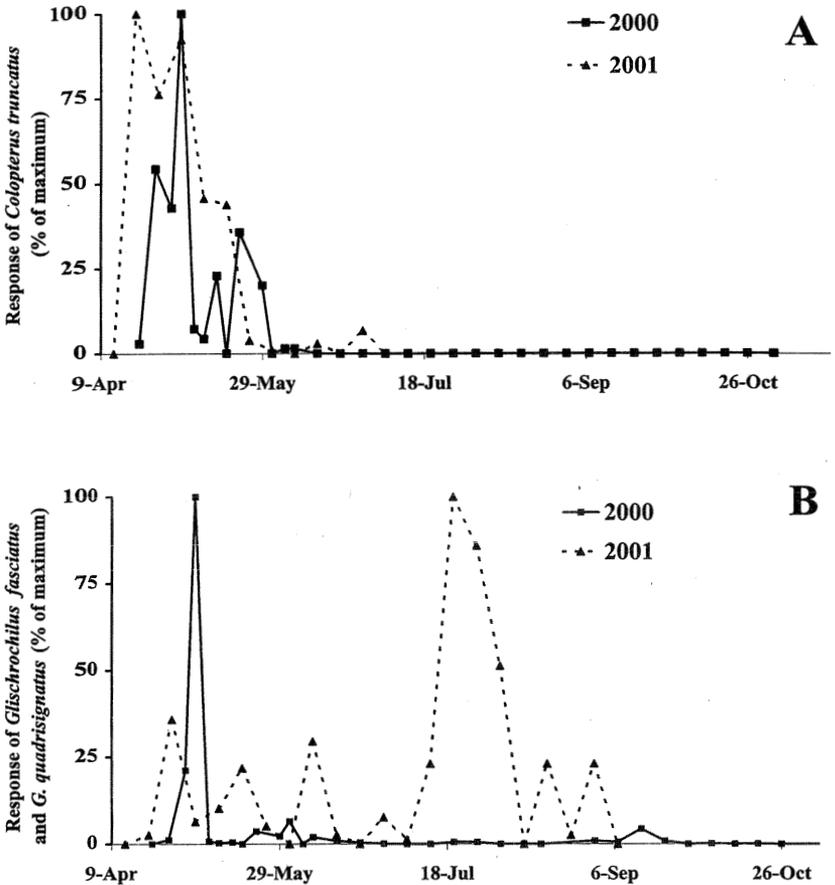


FIG. 4. Relative seasonal flight responses of (A) *Colopterus truncatus* to all treatments in experiments II and III from April 4 to November 3, 2000, and April 5 to November 9, 2001, in Minnesota and (B) *Glischrochilus quadrisignatus* and *G. fasciatus* to all treatments in experiments II and III from April 4 to November 3, 2000, and April 5 to November 9, 2001, in Minnesota. Of the 591 *C. truncatus* trapped in 2000 and 2001, only three beetles were trapped after early July (September 15, 2000; and August 24 and 31, 2001—both females). Maximum trap catch per week in 2000 was 40 beetles (May 4), whereas maximum trap catch per week in 2001 was 97 beetles (April 20). For *Glischrochilus* spp., maximum trap catch per week in 2000 was 136 beetles (May 11), whereas the maximum trap catch per week in 2001 was 76 beetles (July 18).

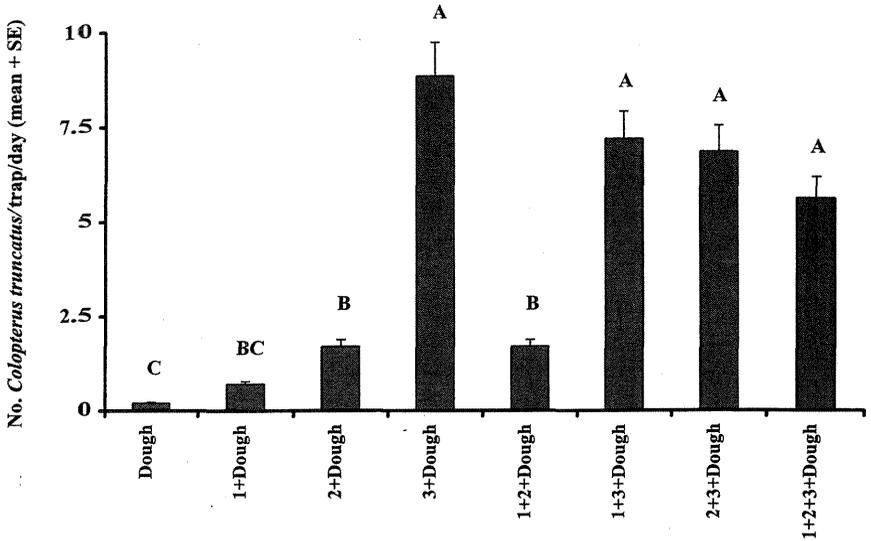


FIG. 5. Flight response of *Colopterus truncatus* to high release rate of all possible combinations of synthetic aggregation pheromone components 1, 2, and 3 with dough (experiment IV) in Illinois. Responses to treatments are significantly different if histogram bars are marked with different letters.

time in different areas of the site. The dominant effect in the experiment was that trap catches were dramatically higher whenever compound 3 was present in the bait (the linear contrast for the presence versus absence of 3 was significant, $F = 135.7$, $df = 1,49$, $P < 0.001$). On the other hand, the corresponding contrasts for 1 and 2 were not significant ($F = 0.0009$, $df = 1,49$, $P = 0.973$; $F = 1.932$, $df = 1,49$, $P = 0.170$, respectively), although the response to 2 plus dough was nominally more attractive than and significantly different from the response to the dough control (Figure 5). The higher-order effects for interactions among treatments were significant ($F = 3.595$, $df = 4,49$, $P = 0.012$), but these were minor when compared with the contrast for 3. That the interaction was significant at all was due to catches for 3 being somewhat lower when 2 was also present versus when 2 was absent, for unknown reasons. This trend was not significant when the means were considered just two at a time with LSD tests. Overall, 291 female and 171 male *C. truncatus* were captured in experiment IV. The sex ratio did not differ significantly among treatments ($\chi^2 = 6.2$, $df = 7$, $P = 0.52$).

Experiment V. In Minnesota, the responses of *C. truncatus* to the high release rate of 1 + 2 + 3 + dough and to the high release rate of 1 + 3 + dough were not significantly different (Friedman's $\chi^2 = 1,453.2$, $df = 3$, $P < 0.001$; Figure 6A). Both pheromone-containing treatments were more attractive than the

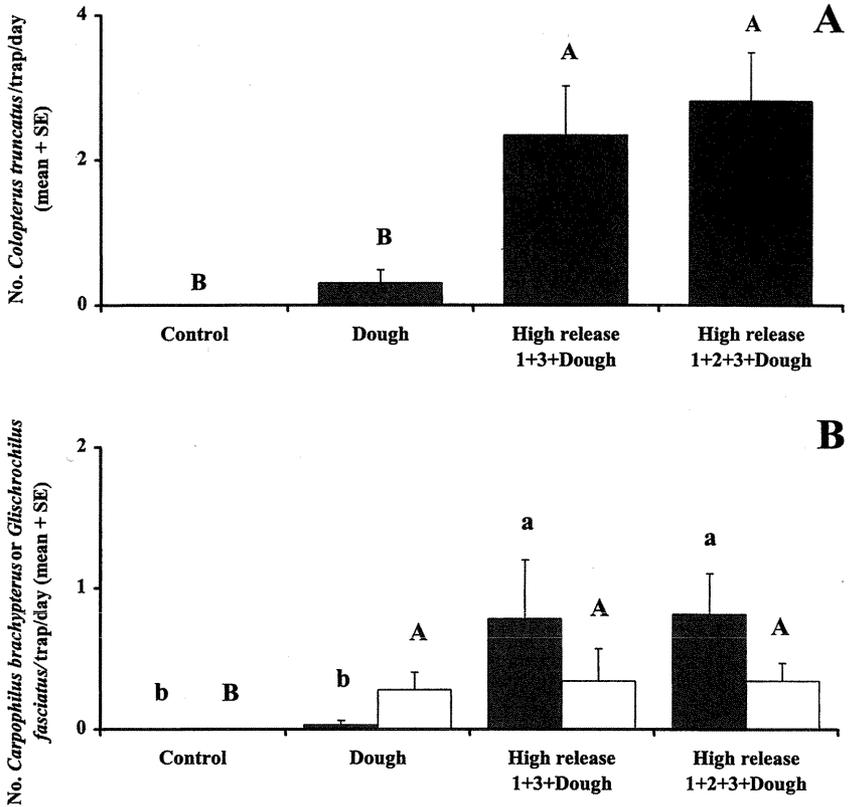


FIG. 6. Flight responses of (A) *Colopterus truncatus* and (B) *Carpophilus brachypterus* (shaded bars) and *Glischrochilus fasciatus* (open bars) to high release rate of synthetic *Colopterus truncatus* aggregation pheromone components 1 + 2 + 3 + dough, high release rate of 1 + 3 + dough, dough alone, and unbaited control in Minnesota (experiment V). Responses to treatments are significantly different if histogram bars within a species are marked with different letters.

dough alone or unbaited control treatments. Of the 182 beetles in the traps, there were 117 females and 65 males, and the sex ratio was not significantly different among treatments ($\chi^2 = 0.48$, $df = 3$, $P = 0.79$).

Two other nitidulids, *Carpophilus brachypterus* (Say) (52 beetles caught) and *Glischrochilus fasciatus* (31 beetles caught), also responded to treatments in experiment V (Figure 6B). *Carpophilus brachypterus* responded to the treatments similarly to *Colopterus truncatus* (albeit at lower levels), with a significant and similar response to both pheromone-dough combinations (Friedman's $\chi^2 = 813.8$, $df = 3$, $P < 0.001$; Figure 6B). *Glischrochilus fasciatus* responded

to the treatments differently than *C. truncatus* and *C. brachypterus*. With *G. fasciatus*, there were no significant differences among the responses to traps containing dough (Friedman's $\chi^2 = 269.1$, $df = 3$, $P < 0.001$; Figure 6B). Although some *G. quadrisignatus* were collected in this study, the catches (18 beetles) were too low to discern any pattern in the response.

Experiment VI. The high release rate of **1 + 3** + oak chips was the most attractive treatment to *C. truncatus* (Friedman's $\chi^2 = 147.0$, $df = 2$, $P < 0.001$). Overall trap catches in this experiment were quite low: no beetles were caught in either the control or oak chips-alone treatments, and more than 75% of the traps baited with **1 + 3** + oak chips were empty. Of the 16 *C. truncatus* caught in the traps baited with **1 + 3** + oak chips, there were 12 females and 4 males. No other sap beetles were caught in this experiment.

DISCUSSION

Both Illinois and Minnesota populations of *C. truncatus* responded similarly to the low- and high-release rate formulations of **1 + 2 + 3** with dough and to dough alone. Both populations preferred the high release rate formulation. Since the low release rate pheromone formulation keeps the ratios of emitted volatiles constant for a longer period of time than the high release rate (i.e., the unenclosed septum) formulation (R.J.B., unpublished data), our trapping results suggest that the sustained emission of the constant ratio of pheromone components is not the most important factor in the attraction of *C. truncatus*. Rather, they suggest that the total amount of components emitted was the most important factor in trap catch.

In both Illinois and Minnesota populations, there was no difference in the response to the combination of **1 + 2 + 3** with dough or the combination of **1 + 3** with dough. In contrast to earlier work in Illinois on *C. truncatus* (Cossé and Bartelt, 2000), this study showed directly in Illinois, and by inference in Minnesota, that component **2** was not necessary for, and in fact perhaps reduced, the attraction of *C. truncatus*. Even though component **2** was the most abundantly produced of the three male-specific hydrocarbons investigated in this study, it was component **3** that dominated the response. Component **3** had not been tested alone in previous behavioral studies, but it did show considerable electrophysiological activity when presented alone to male and female antennal preparations (Cossé and Bartelt, 2000). Although there were no apparent differences in the responses of Illinois and Minnesota populations of *C. truncatus* to various release rates and blends of the aggregation pheromone, there may be differences in the seasonal pattern of response to aggregation pheromone (see below).

Since *C. truncatus* and other nitidulids have a relatively rapid and strong response to fresh wounds in oak trees (Juzwik et al., 1999; J.J., personal observation), we hypothesized that the volatiles produced by oak tissue would synergize the *C. truncatus* aggregation pheromone. Based on the evidence shown here

(experiment VI), volatiles produced from chipped oak tissue are not attractive, and these volatiles likely do not synergize the pheromone. Trap catches in experiment VI were quite low, and are probably explained by a low-level response to the pheromone without the dough synergist as observed in experiments I and II. The oak tissue in our experiments might not have been attractive to *C. truncatus* because the process of chipping might produce different volatiles (or different amounts of volatiles) than those normally encountered by nitidulids when searching for fresh sap-laden wounds on living trees. Further work is needed to identify and characterize the volatiles of wounded oaks, how they change over time, and when they are most attractive to nitidulids searching for feeding, mating, and ovipositional sites. Preliminary chemical analyses, however, show that volatiles from individuals within an oak species are quite variable and suggest that this work will be difficult (R.J.B., unpublished data).

Since sap beetles aggregate on oak wilt mats (Cease and Juzwik, 2001), it is possible that oak wilt mat volatiles synergize the aggregation pheromone emitted by *C. truncatus* and other species while on mat tissue. Volatiles from laboratory cultures of oak wilt have been described and are attractive to sap beetles (*C. truncatus* was not tested) in wind-tunnel bioassays (Lin and Phelan, 1992). Further study on the relationship between oak wilt mat volatiles and nitidulid pheromones will be conducted when a stable *Ceratocystis fagacearum* volatile formulation is available for field tests.

The distinct seasonality shown by *C. truncatus* in our study is very important in understanding and managing the overland spread of oak wilt. Peak production of oak wilt mats (Campbell and French, 1955) and the window of peak susceptibility to infection (Prey and Kuntz, 1995) both occur during the spring in Minnesota (Juzwik et al., 1985). Our results show that in Minnesota, *C. truncatus* is attracted to pheromone + dough-baited traps in April, May, and June, but very few *C. truncatus* are caught in the same traps with the same baits later in the summer and in the fall. Skalbeck (1976), when trapping with fermenting dough and *Ceratocystis fimbriata* baits over two years in three Minnesota forest types, also found *C. truncatus* only in the spring and early summer, with the peak abundance in April and May. In another instance in Minnesota, of 40 *C. truncatus* trapped in response to combinations of dough and (2E,4E,6E,8E)-7-ethyl-3,5-dimethyl-2,4,6,8-decatetraene, 95% of the trap catch flew before July 6 (one male and one female were trapped in September) (R.J.B., unpublished data). However, McMullen and Shenefelt (1961), Levesque and Levesque (1992), Dowd and Nelsen (1994), and Cossé and Bartelt (2000) have all reported trapping *C. truncatus* in flight during the fall to various stimuli in Wisconsin, Quebec (Canada), Illinois, and Illinois, respectively. Climatic differences between Minnesota and these other north-central areas may influence the life history, and, specifically, the flight behavior of *C. truncatus*.

Sampling *C. truncatus* from oak wilt mats in Minnesota has revealed that it is univoltine. Adults disperse in flight for mating in the spring and early summer,

larvae only occur in the mats in spring and early summer, and adult densities are approximately eight times higher in the mats in the fall than in the spring (J.F.K., unpublished data). As the fall cohort of oak wilt mats is newly formed each fall, it is likely that *C. truncatus* has flown to the mats at this time. These adults are also likely to feed on these mats and are less likely to disperse from them again through flight. Rather, they are more likely to drop to the litter layer on the forest floor to overwinter. Further work in Minnesota needs to be done to determine whether *C. truncatus* flies selectively in the spring and early summer in response to synergized synthetic pheromone (as a mating and feeding response) and selectively in the early fall to mat odors (as a feeding and preoverwintering response). Alternatively, *C. truncatus* may fly in response to pheromone in the fall, but at much lower densities or it may reach the mats in the fall by crawling to them. It should be noted that the seasonally restricted flight behavior of *C. truncatus* in Minnesota is not unique among sap beetles. Some species in the genus *Carpophilus* also show strong seasonal flight activity periods in California (Bartelt et al., 1994), South Carolina (Bartelt et al., 1995), and Australia (James et al., 1994), while species of *Colopterus* and *Cryptarcha* show a similar seasonal pattern in Texas (Appel et al., 1986).

As with other nitidulid pheromones (Bartelt, 1997; Zilkowski and Bartelt, 1999), the *C. truncatus* aggregation pheromone is cross-attractive to other species. In our experiments in Minnesota, as was the case in previous work in Illinois (Cossé and Bartelt, 2000), *Carpophilus brachypterus* responded to the synergized *Colopterus truncatus* pheromone. Since the decatetraene (**3**) is the main component of the aggregation pheromone of *Carpophilus brachypterus* (Williams et al., 1995), the response of *Carpophilus brachypterus* is most likely due to pheromone component similarity, reflecting the close phylogenetic relationship between the genera *Colopterus* and *Carpophilus* within the subfamily Carpophilinae (Downie and Arnett, 1996). The effect could also be interpreted as a kairomonal response. *Carpophilus brachypterus* may benefit by locating wounds in oaks or mats originally colonized by *Colopterus truncatus* and signaled by aggregation pheromone production by *C. truncatus*. Since *Carpophilus brachypterus* has been collected from oak wounds (Vogt, 1950; Norris, 1956), this chemically guided ecological relationship seems plausible. However, our review of the literature and a reexamination of summary data in Skalbeck (1976) suggests that *C. brachypterus* has not been collected to date from oak wilt mats (T.C. Skalbeck, personal communication). *Glischrochilus quadrisignatus* and *G. fasciatus* (subfamily Cryptarchinae) responded equally to all treatments containing dough. Thus, these species respond to food volatiles (Lin and Phelan, 1991), but do not show a kairomonal response to the *C. truncatus* aggregation pheromone.

One interesting outcome from these experiments is that female *C. truncatus* consistently responded to the *C. truncatus* aggregation pheromone in both Illinois and Minnesota at nearly twice the rate that males did. The sex ratio of *C.*

truncatus trapped in all experiments from both locations was approximately 1.8:1 (female-male) (Minnesota 1.8:1; Illinois 1.7:1). In the previous study with aggregation pheromone components of *C. truncatus* in Illinois, Cossé and Bartelt (2000) also reported female-biased sex ratios between 1.1 and 1.7:1. In other studies with sap beetles, Petroski et al. (1994) recorded a sex ratio of 1.2:1 in *Carpophilus obsoletus* Erichson responding to its aggregation pheromone in the field, whereas Bartelt et al. (1994) reported sex ratios "close to" 1:1 in five *Carpophilus* spp. and in *Haptoncus luteolus* (Erichson) responding to aggregation pheromones in the field. In the latter study, the sex ratios for four of the *Carpophilus* spp. and for *H. luteolus* were nearly 1:1 in date fruits, which are the hosts colonized by the beetles. The sex ratio of a sample of *C. truncatus* collected in oak wilt mats in Minnesota was also nearly 1:1 (1.1:1, $N = 128$; J.F.K., unpublished data).

In other coleopteran aggregation pheromone systems with a male-produced attractant, the sex ratio of beetles responding behaviorally to pheromone or the magnitude of the electrophysiological response to pheromones also tends to be female biased. Indeed, aggregation pheromones may, in part, serve a sex pheromone function (Borden, 1985). Female-biased responses have been reported in bark beetles (Scolytidae), e.g., the California five-spined ips, *Ips paraconfusus* Lanier (Wood et al., 1967, 1968; Byers, 1983), and the pine engraver, *Ips pini* (Say) (Seybold et al., 1992; Miller et al., 1990, 1995, 1996; Miller and Borden, 1990, 2000; Miller, 2000) and in grain beetles (Bostrichidae, Cucujidae, and Silvanidae), e.g., the larger grain borer, *Prostephanus truncatus* (Horn) (Birkenshaw and Smith, 2000), the rusty and flat grain beetles, *Cryptolestes ferrugineus* (Stephens) and *C. pusillus* (Schönherr) (Chambers et al., 1990), and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (White and Chambers, 1989). See Millar et al. (1985 a,b) and Pierce et al. (1988) for counter-examples with grain beetles. In the initial case of the isolation and identification of an aggregation pheromone from an insect (*I. paraconfusus*, Wood et al., 1967, 1968), the sex-specific production and the female-biased responses caused the investigators to view the signals as sex attractants. Since female *C. truncatus* are attracted at a greater rate than males in our study, this nitidulid example lends additional support to the hypothesis that male-produced aggregation pheromones evolved secondarily from their initial roles as sex pheromones to attract females (Raffa et al., 1993; Phillips, 1997; Plarre and Vanderwel, 1999).

A parallel can be made between sap beetles that colonize, develop on, and emerge from oak wilt mats (or wounds) and bark beetles that undergo similar activities in phloem in tree stems and branches. In the cases of both *C. truncatus* and *I. paraconfusus*, the sex ratios of the arriving insects (simulated by response to baited traps) do not appear to match the sex ratios of those that emerge from the developmental substrate. For example, the sex ratio of *I. paraconfusus* responding to the immediate source of naturally produced or synthetic pheromones in

various field studies ranges from 1.8 to 5.6:1 (Struble and Hall, 1955; Gara, 1963; Wood et al., 1967, 1968; Byers, 1983), whereas the sex ratio of insects emerging from ponderosa pine logs has been reported as approximately 1.2:1 (Gara, 1963; Cameron and Borden, 1967) and 1.3:1 (Struble and Hall, 1955). Similarly, with *C. truncatus* we have an arriving sex ratio of 1.8:1 and a sex ratio of 1.1:1 in the colonized oak wilt mats. Further investigation is needed to determine the sex ratio of *C. truncatus* that develop and emerge from oak wilt mats and wounds in oaks. However, higher differential mortality of males during dispersal has been offered as a potential explanation for this sex ratio difference in *I. paraconfusus* (Cameron and Borden, 1967), and this hypothesis may apply to the sex ratio difference for *C. truncatus* as well.

Finally, a number of conclusions with practical implications for monitoring the flight of *C. truncatus* and for managing overland spread of oak wilt can be drawn from this work. Experiments I and II demonstrated that the most attractive bait for *C. truncatus* is the high release rate of pheromone compounds with the bread dough co attractant. Further, experiment V showed that it is unnecessary to add synthetic **2** to the pheromone baits for monitoring *C. truncatus*. Thus, the simplest effective approach to preparation of pheromone baits would be to use synthetic **3**, without bothering to remove the **1** that is invariably present as a synthetic artifact. Since the two- and three-component blends of the *C. truncatus* aggregation pheromone are equally attractive, the less expensive two-component blend can be used effectively as a monitoring tool. As we recorded no response of *C. truncatus* or other sap beetles to 300-ml aliquots of oak chips (experiment VI), operational studies in the future might explore whether landowners, resource managers, or arborists could chip woody debris from uninfected oak trees and leave the chips on site without attracting nitidulid oak wilt vectors to the general area and accelerating overland transmission of the disease. Finally, land managers should be aware that the critical period of flight activity for *C. truncatus* in Minnesota occurs conservatively between early April and early July, and in order to limit the overland spread of oak wilt, pruning and other management activities that wound oaks should be avoided during this time. In a future study, a similar critical period needs to be defined for the other primary oak wilt vector, *Carpophilus sayi*.

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