

Seasonal Dispersal of the Oak Wilt Fungus by *Colopterus truncatus* and *Carpophilus sayi* in Minnesota

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ABSTRACT

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Sap beetles (Nitidulidae) are considered important overland vectors of the oak wilt pathogen, *Ceratocystis fagacearum*, in the north central United States. *Colopterus truncatus* and *Carpophilus sayi* are thought to be the principal sap beetle vectors in Minnesota. Field studies using wind-oriented funnel traps baited with aggregation pheromones of the insects were conducted during 2 years in east central Minnesota. The studies compared temporal flight dynamics of *Colopterus truncatus* and *Carpophilus sayi* from April through October, measured the proportion of dispersing adults of each species carrying viable pathogen propagules, and estimated the populations of contaminated dispersing beetles in oak (*Quercus* spp.) stands with and without oak wilt. Abundance of *Colopterus truncatus* peaked in either April or May, but the pathogen was most frequently isolated (20 to 45%) from beetles obtained from oak wilt sites from July through September. However, the highest contaminated insect population (CIP) generally occurred during April and May. Abundance of *Carpophilus sayi* peaked in October of both years, but the pathogen was most commonly isolated from beetles collected during May and June. The CIP was highest in June. The infective insect potential, as indexed by CIP, was greatest for both species in the spring and was greater for *Colopterus truncatus* than for *Carpophilus sayi*.

Additional keywords: fungal phoresy rates, vascular wilt disease

Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, is spread overland by insect vectors such as sap beetles (Coleoptera: Nitidulidae) (15). Insect transmission is significant because it is the means by which new oak wilt infection centers are established. Sap beetles transmit infectious propagules from the pathogen's sporulating mats formed on recently killed oaks (*Quercus* spp.) to fresh wounds on healthy oaks (8,16,21,23,26).

Many species of sap beetles have been associated with the oak wilt disease cycle. Based on his review of the literature, Skalbeck (27) reported 27 different species

associated with oak wilt fungal mats and 38 different species attracted to wounds on oaks. However, little distinction has been made as to the relative importance of species-specific vectors in pathogen transmission (25). Norris (26), however, suggested that some species might have a greater ecological specialization in their association with the pathogen than others. Sap beetle species dispersing in oak wilt centers, the species associated with fungal mats, and the frequency with which each carried viable propagules of the pathogen were studied in Minnesota in the early 1980s (18). *Glischrochilus quadrisignatus* Olivier, *G. fasciatus* Say, *G. sanguinolentus* Olivier, and *G. siepmanni* Brown were the most commonly captured free-flying nitidulid species (in descending order of frequency) trapped in dough- and melon-baited traps. *Colopterus truncatus* Randall was the most common species present in oak wilt mats, and *Glischrochilus*, *Epuraea*, *Colopterus*, and *Carpophilus* species were contaminated with *C. fagacearum* (17).

Further studies were conducted in Minnesota to test the hypothesis that all sap beetles that have been implicated as *C. fagacearum* vectors are important in the pathogen's transmission in nature (5). *Colopterus truncatus* and *Carpophilus sayi* Parsons were found to account for 54% of the 2,542 nitidulid beetles collected from

fungal mats. Fungal isolations revealed these two species also were carrying high numbers of viable propagules (>3,000 CFU per beetle) (5). *Colopterus truncatus* and *Carpophilus sayi* also were found to account for 95% of all sap beetles attracted to fresh wounds in a subsequent study (20,21). Furthermore, 78% of the beetles were contaminated with the pathogen. Thus, these two species are currently considered the principal sap beetle vectors of *C. fagacearum* during spring in Minnesota.

Studies were undertaken in 2002 to learn more about the temporal flight dynamics of *Colopterus truncatus* and *Carpophilus sayi* throughout the growing season in Minnesota. Such knowledge could lead to new control methods for overland transmission or lead to refinements of existing guidelines for disease prevention. New light also may be shed on the relative importance of each species in spreading the pathogen. New quantitative information could be useful in developing models to predict seasonal risks associated with the infective insect potential for *C. fagacearum* by specific insects. In order to accomplish this, the time of year when *C. fagacearum*-contaminated beetles are dispersing in oak wilt stands must be identified, as well as any differences in vectoring potential that may exist between the species.

Temporal flight dynamics of *Colopterus truncatus* have been studied in Minnesota to some extent. Peak abundance of this species has been shown to occur in early spring (22,27). There has been some characterization of *Carpophilus sayi* flight activity in Wisconsin (23); however, temporal flight dynamics for *Carpophilus sayi* in Minnesota have not been determined. Difference in occurrences of *Colopterus truncatus* and *Carpophilus sayi* in wounds and mats has been documented. In Iowa, Norris (26) showed *Colopterus truncatus* arrives in the mats earlier than *Carpophilus sayi* and colonizes younger mats. *Colopterus truncatus* also was more abundant than *Carpophilus sayi* in "infectable" wounds.

Field studies were initiated in east central Minnesota to compare and contrast *Colopterus truncatus* and *Carpophilus sayi* in relation to the oak wilt disease cycle. Specific objectives were to: (i) determine the abundance of dispersing *Colopterus truncatus* and *Carpophilus sayi* over the

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growing season in oak stands with and without oak wilt, (ii) determine the proportion of the dispersing beetles of each species contaminated with *C. fagacearum*, and (iii) estimate the contaminated beetle populations in sampled stands as a measure of the infective insect potential of each species during spring, summer, and fall.

MATERIALS AND METHODS

Study sites. Beetle populations were studied at six oak stands near the Minneapolis/St. Paul, MN, metropolitan area in 2002 and 2003. Selected stands included oak wilt sites with coalescing infection centers at Carlos Avery Wildlife Management Area, Washington County; and a privately owned woodlot in Columbus Township, Anoka County. Non-oak wilt sites included a privately owned woodlot in Lake Elmo, Washington County; and a privately owned woodlot in Pine Springs, Washington County. Two additional sites (one oak wilt and one non-oak wilt) were studied in 2003 in the southern part of the Minneapolis/St. Paul metropolitan area at Murphy-Hanrehan Park, Scott County.

Abundance of beetles. *Carpophilus sayi* and *Colopterus truncatus* were captured with wind-oriented funnel traps (10). Traps were placed approximately 1.5 m above the ground and 10 m apart. The common collection vial on each trap contained loosely packed, shredded paper

intended to reduce the possibility of cross-contamination (of pathogen propagules) among captured insects. Baits used for the *Colopterus truncatus* traps in 2002 and 2003 consisted of a high dose (3.5, 500, and 280 µg of components 1, 2, and 3, respectively) of the *Colopterus truncatus* pheromone (Great Lakes IPM, Vestaburg, MI) and 10 ml of viscous, fermenting whole wheat bread dough (22). Two *Colopterus truncatus* traps were placed at each site. Baits used for the *Carpophilus sayi* traps in 2002 were according to Bartelt et al. (4), where the species was captured in four different types of traps. These included high-dose (55 and 450 µg of components 3 and 4, respectively) *Carpophilus sayi* pheromone (Great Lakes IPM) and 10 ml of viscous, fermenting bread dough; low-dose (6 and 45 µg of components 3 and 4, respectively) *Carpophilus sayi* pheromone plus bread dough; high-dose *Carpophilus sayi* pheromone only; and low-dose *Carpophilus sayi* pheromone only. Four traps (one of each type) were placed at each site in 2002. Baits used for the *Carpophilus sayi* traps in 2003 consisted of a high dose (55 and 450 µg of components 3 and 4, respectively) of *Carpophilus sayi* pheromone (Great Lakes IPM) and 10 ml of viscous, fermenting bread dough. Two such traps were placed at each site in 2003. Traps were emptied and re-baited weekly from

11 April to 18 October 2002, and 10 April to 16 October 2003.

Beetle processing. Adult beetles were removed from traps, identified, their sex determined, and stored individually in sterilized, micro-centrifuge tubes at -2°C until further processed. Representative individuals of each species were deposited in the Insect Museum, Department of Entomology, University of Minnesota, St. Paul, by A. Ambourn. Within 6 months of capture, 0.5 ml of water was added to each dead specimen and homogenized for 5 s using an ultrasonic tip sonicator to dislodge fungal propagules on the exoskeleton and in the gut. The resulting solution was then serially diluted (10-, 100-, and 1,000-fold), and 0.5-ml aliquots of each dilution were spread onto triplicate lactic acid-amended potato dextrose agar (PDA) plates, for a total of nine plates per beetle. The number of *C. fagacearum* colonies was recorded 10 days after the plates had been incubated at 24°C in the dark (5). Colony appearance and asexual spore characteristics were used to identify *C. fagacearum* (3).

Statistical analyses. Counts of *Colopterus truncatus* and *Carpophilus sayi* obtained in the traps were analyzed using Poisson regression in ARC (6). Results from each year were analyzed separately because variances were different between years. For each year, counts of beetles caught in wind-oriented funnel traps were the response variables, and site type (oak wilt versus non-oak wilt), month, sex, and all two-way interactions were the predictor variables. The most parsimonious model was obtained through backward elimination, which sequentially removed inconsequential terms based on the Mallows C₁ statistic (6). The significance of each remaining term was interpreted using the Wald χ² test.

Fitted mean beetle counts per site for each species and sex by month and site type were obtained from the Poisson regression analysis table data function. These values were used for graphing beetle temporal flight dynamics over the course of the study. Standard errors for the fit means were calculated as

$$SE = \sqrt{\frac{\bar{x}}{n}}$$

because in a Poisson distribution the mean is assumed to be equal to the variance (6).

Presence of *C. fagacearum* propagules on, or in, *Colopterus truncatus* and *Carpophilus sayi* was analyzed using binomial regression (6). Each specimen was scored as 0 if propagules were absent, or 1 if present. Results for each species and year were analyzed separately, with initial model terms for site, month, sex, and all two-way interactions. Again, backward elimination was used to obtain the most parsimonious model (6).

Table 1. Best-fitting models resulting from Poisson regression analyses of significant explanatory variables that affect mean numbers of *Colopterus truncatus* adults caught in wind-oriented funnel traps in oak wilt and non-oak wilt sites in east central Minnesota from April to October 2002 and 2003^a

Year	Variable	Level	Estimate	SE	Wald χ ²	P value
2002	Constant		1.98	0.05	43.04	<0.0001
	Month	May	-1.19	0.07	-17.83	<0.0001
		June	-2.47	0.12	-20.31	<0.0001
		July	-4.39	0.34	-13.07	<0.0001
		August	-4.82	0.34	-14.35	<0.0001
		September	-4.39	0.30	-14.45	<0.0001
		October	-5.12	0.50	-10.20	<0.0001
	Site type	Non-oak wilt	-2.36	0.13	-17.90	<0.0001
	Sex	Female	-0.09	0.05	-1.75	>0.07
	Interaction	May*site	1.68	0.16	10.42	<0.0001
		June*site	2.27	0.20	13.63	<0.0001
		July*site	1.77	0.57	3.09	<0.002
		August*site	0.16	1.06	0.15	>0.80
		September*site	-8.88	50.38	-0.18	>0.80
October*site		0.97	1.13	0.87	>0.30	
2003	Constant		0.53	0.10	5.26	<0.0001
	Month	May	1.39	0.11	13.14	<0.0001
		June	-0.78	0.16	-4.87	<0.0001
		July	-0.94	0.16	-6.00	<0.0001
		August	-0.20	0.14	-1.49	>0.10
		September	-0.76	0.16	-4.80	<0.0001
		October	-0.49	0.16	-3.07	<0.002
	Site type	Non-oak wilt	-0.32	0.15	-2.09	<0.03
	Sex	Female	-0.37	0.05	-7.85	<0.0001
	Interaction	May*site	-0.34	0.17	-2.08	<0.03
		June*site	0.30	0.23	1.28	>0.10
		July*site	-0.45	0.27	-1.71	>0.08
		August*site	-1.11	0.26	-4.21	<0.0001
		September*site	-0.01	0.25	-0.05	>0.90
October*site		0.35	0.23	1.50	>0.10	

^a Fitted model for: Monthly mean no. beetles caught = constant + month + site + sex + month*site. Results of contrasts between levels of the coefficients and the first level (implied to be 0) are presented.

The proportion of *C. fagacearum*-contaminated beetles by species and sex for each sampling month and site type was determined by dividing the number of beetles testing positive for *C. fagacearum* by the total number of beetles tested. The standard error for the proportion contaminated was calculated using

$$SE = \sqrt{\frac{p(1-p)}{n}}$$

as per Esker and Nutter (12), where p is the proportion of samples positive, $1-p$ is the proportion of samples negative, and n is the total number of *Carpophilus sayi* or *Colopterus truncatus* tested.

To combine trap catches with levels of contamination, the contaminated insect population (CIP) was calculated for male and female *Colopterus truncatus* and *Carpophilus sayi* for each month and year. CIP is an estimation of the population of presumably infective insects (i.e., those having the ability to effect the transmission of a pathogen), and was indexed by the product of the fitted monthly beetle catches per site and the corresponding proportions that were contaminated (12). The standard error for CIP was calculated according to Freese (13).

RESULTS

Seasonal abundance of *Colopterus truncatus*. In 2002, results of Poisson regression indicated that numbers of captured beetles varied across months ($P < 0.0001$) and by site type ($P < 0.0001$) (Table 1). Beetle catches were highest in April (Fig. 1). Catches in oak wilt sites were greater than in non-oak wilt sites (Table 1). Overall, abundance trends in both site types decreased as the season progressed (Fig. 2A and B), but they differed slightly, as indicated by the difference in site \times month interaction for April through July ($P < 0.01$, Table 1). There was no difference in mean number of males and females trapped; terms for sex and all other two-way interactions were not significant ($P > 0.1$). Abundance of *Colopterus truncatus* in 2002 exhibited a unimodal pattern (Fig. 2A and B). Peak flight occurred in the week of 18 April, when 599 insects were trapped. Over the entire year, 97% ($N = 1,461$) of the specimens were trapped between 18 April and 30 June. The sex ratio was 1.0:1.1 females to males ($N = 1,461$).

In 2003, results of Poisson regression indicated that catches varied by month ($P < 0.0001$, except August, $P > 0.1$), across sites ($P < 0.03$), and by sex ($P < 0.0001$) (Table 1). Catches in oak wilt sites were greater than in non-oak wilt sites (Fig. 1).

Catches of males were greater than of females, except in oak wilt sites in August. Overall abundance increased sharply in May (Fig. 2C and D), decreased, and then leveled off, except in August and October, as indicated by the site \times month interaction. All other two-way interactions were not significant ($P > 0.1$). Patterns of abundance in 2003 were different from that observed in 2002 (Fig. 2C and D). Peak flight occurred in the week of 1 May, when 356 insects were caught. Over the entire year, 74% ($N = 1,814$) of all specimens were caught between 17 April and 26 June. In 2003, there was a second peak in the week of 21 August ($N = 86$) and a third peak in the week of 9 October ($N = 76$). The sex ratio was 1.0:1.5 females to males ($N = 1,814$).

Seasonal abundance of *Carpophilus sayi*. In 2002, results of Poisson regression indicated that number of beetles captured varied across months ($P < 0.002$) and by site type ($P < 0.0001$) (Table 2). Catches of the beetles were lowest in April (Fig. 1). Catches were greater in non-oak wilt sites than in oak wilt sites (Table 2). Overall, abundance increased from April to July, decreased in August and September, and increased sharply in October (Fig. 3A and B). Sex and all two-way interactions were not significant ($P > 0.07$). Peak flight oc-

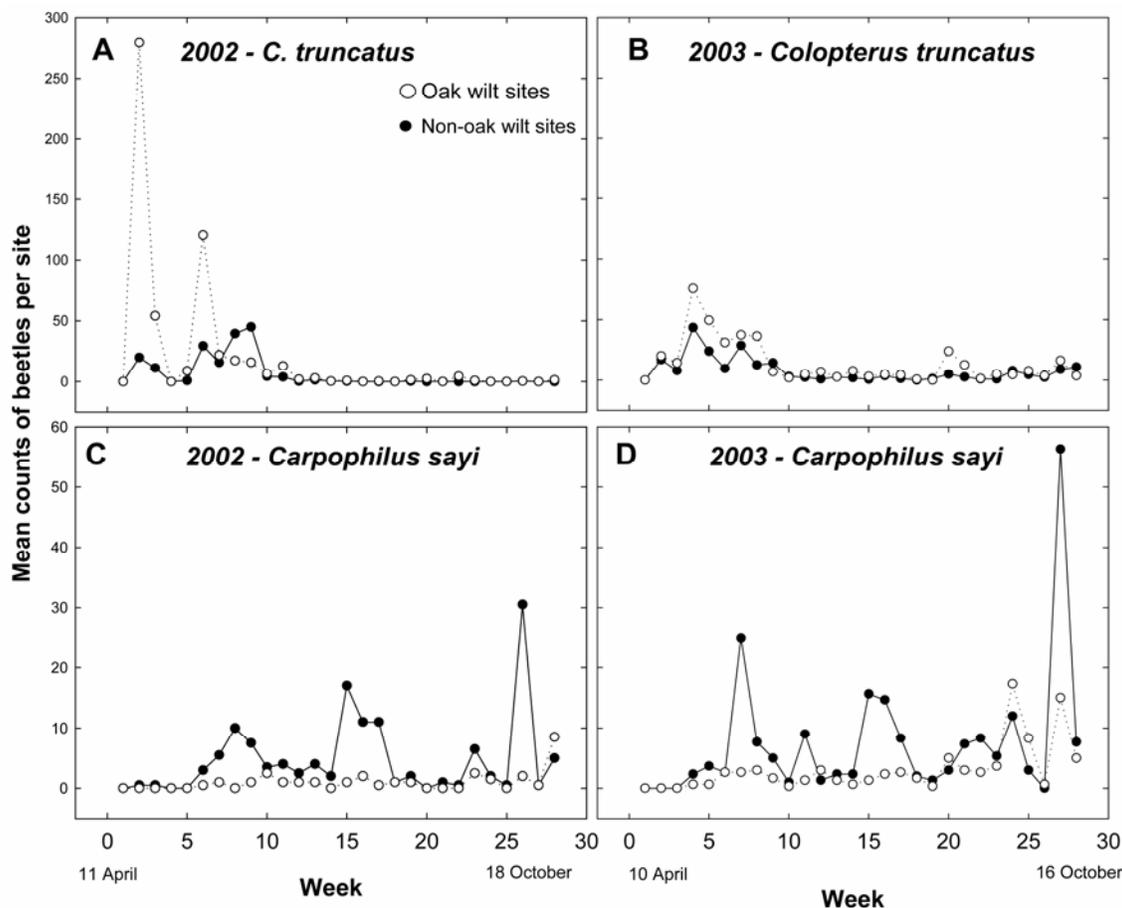


Fig. 1. Mean numbers of *Colopterus truncatus* (A and B) and *Carpophilus sayi* (C and D) captured each week in four sites, by year and site type. Insects were caught in wind-oriented funnel traps using species-specific pheromones from April to October in east central Minnesota.

curred in the week of 4 October, when 68 insects were captured (Fig. 1). Two other very small peaks occurred in the week of 20 May ($N = 20$) and in the week of 18 July ($N = 36$). Over the entire season, 58% ($N = 320$) of the specimens were captured between 16 May and 1 August. The sex ratio was 1.0:1.0 females to males ($N = 319$).

In 2003, results of Poisson regression indicated that catches were greater in non-oak wilt sites than in oak wilt sites ($P < 0.0001$) (Table 2). Overall abundance increased as the season progressed (Fig. 1, Fig. 3C and D). Terms for month, sex, and all two-way interactions were not significant ($P > 0.1$). Patterns of abundance in 2003 were similar to those observed in 2002, but more insects were caught overall. The highest number of beetles ($N = 214$) was captured during the week of 9 October (Fig. 1). Interestingly, 163 of the

specimens were caught in one trap. Three other very small peaks were found the weeks beginning 22 May ($N = 83$), 24 July ($N = 51$), and 18 September ($N = 88$). Over the entire season, 44% ($N = 887$) of all specimens were caught between 1 May and 7 August. The sex ratio was 1.1:1 ($N = 887$).

Contamination by *C. fagacearum*. In 2002, results of binomial regressions indicated the presence of *C. fagacearum* on *Colopterus truncatus* varied across months ($P < 0.03$, except July and October, $P > 0.8$), by site type ($P < 0.002$), and by sex ($P < 0.006$) (Table 3). May, June, August, and September had higher proportions of individuals with *C. fagacearum* propagules than were present in April. The proportion of individuals contaminated with *C. fagacearum* in oak wilt sites was greater than in non-oak wilt sites. Finally, a higher proportion of males were carrying *C. fagacearum* propagules than females. No

two-way interactions were significant ($P > 0.8$). The proportion of *Colopterus truncatus* carrying viable propagules of *C. fagacearum* in 2002 ranged from 0.00 to 0.63 (Table 4). For beetles yielding *C. fagacearum*, the average CFU per beetle ranged from 6 to 1,800 (Table 4).

The proportions of *Colopterus truncatus* carrying *C. fagacearum* in 2003 varied across sites ($P < 0.0001$) (Table 3). Greater proportions of individuals were carrying *C. fagacearum* propagules in the oak wilt sites than in non-oak wilt sites ($P < 0.0001$). Terms for month, sex, and two-way interactions were not significant ($P > 0.1$). The proportion of *Colopterus truncatus* carrying *C. fagacearum* in 2003 ranged from 0.07 to 0.46 (Table 4). For beetles yielding *C. fagacearum*, the average CFU per beetle ranged from 55 to 28,000.

Results of binomial regressions for 2002 data indicated contamination rates for

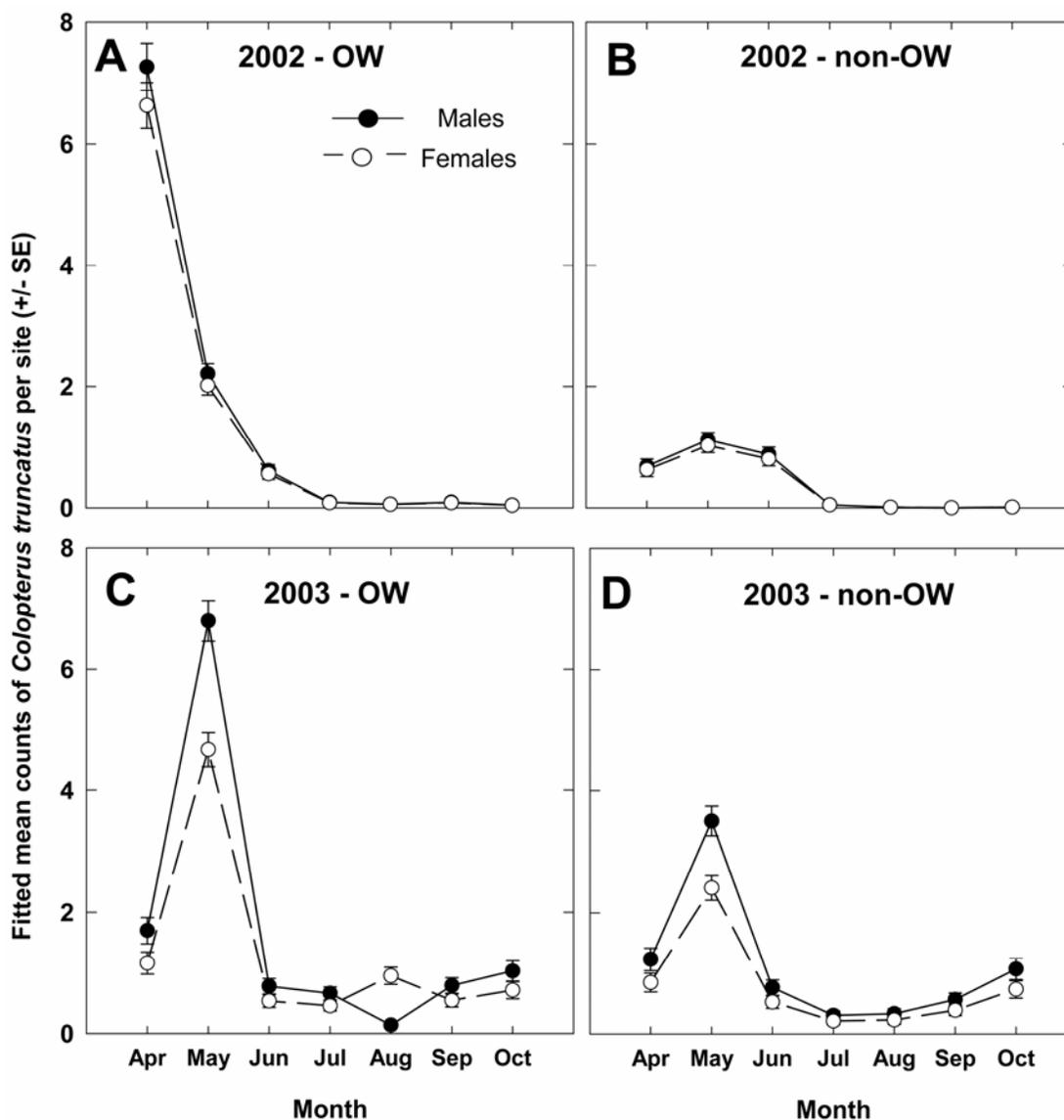


Fig. 2. Fitted mean counts per site \pm SE for *Colopterus truncatus* males (closed circle/solid line) and females (open circle/dashed line) captured per month in four sites, by year and site type (oak wilt, **A**, 2002 and **C**, 2003, or non-oak wilt, **B**, 2002 and **D**, 2003). Insects were caught in wind-oriented funnel traps using species-specific pheromones from April to October in east central Minnesota.

Table 2. Best-fitting models resulting from Poisson regression analyses of significant explanatory variables that affect mean numbers of *Carpophilus sayi* adults caught in wind-oriented funnel traps in oak wilt and non-oak wilt sites in east central Minnesota from April to October 2002 and 2003^a

Year	Variable	Level	Estimate	SE	Wald χ^2	P value
2002	Constant		-5.60	0.72	-7.82	<0.0001
	Month	May	2.48	0.73	3.44	<0.0006
		June	2.85	0.72	3.95	<0.0001
		July	3.56	0.71	4.98	<0.0001
		August	2.35	0.73	3.24	<0.0012
		September	2.31	0.73	3.17	<0.0015
		October	3.85	0.71	5.40	<0.0001
Site type	Non-oak wilt	1.53	0.15	10.47	<0.0001	
2003	Constant		-14.04	43.38	-0.32	>0.70
	Month	May	13.06	43.38	0.30	>0.70
		June	12.47	43.38	0.29	>0.70
		July	13.07	43.38	0.30	>0.70
		August	12.52	43.38	0.29	>0.70
		September	13.46	43.38	0.31	>0.70
		October	14.08	43.38	0.03	>0.70
Site type	Non-oak wilt	0.87	0.72	11.78	<0.0001	

^aFitted model for: Monthly mean no. beetles caught = constant + month + site + sex + month*site. Results of contrasts between levels of the coefficients and the first level (implied to be 0) are presented.

Carpophilus sayi did not differ among months ($P > 0.9$), by site type ($P > 0.9$), or by sex ($P > 0.6$) (Table 5). The proportion of *Carpophilus sayi* carrying viable propagules of *C. fagacearum* in 2002 ranged from 0.06 to 0.25 (Table 6). For beetles yielding *C. fagacearum*, the average CFU per beetle ranged from 21 to 17,000.

In 2003, results of binomial regression indicated contamination levels on *Carpophilus sayi* varied across months ($P < 0.02$, except August, $P > 0.8$), among sites ($P < 0.0001$), and between sexes ($P < 0.03$) (Table 5). The proportion of individuals carrying *C. fagacearum* in all months except June was less than in May. The proportion contaminated was greater in oak wilt sites than in non-oak wilt sites, and more females were carrying fungal propagules than males. No two-way interactions were significant ($P > 0.1$). The proportion of beetles contaminated with *C. fagacearum* in 2003 ranged from 0 to

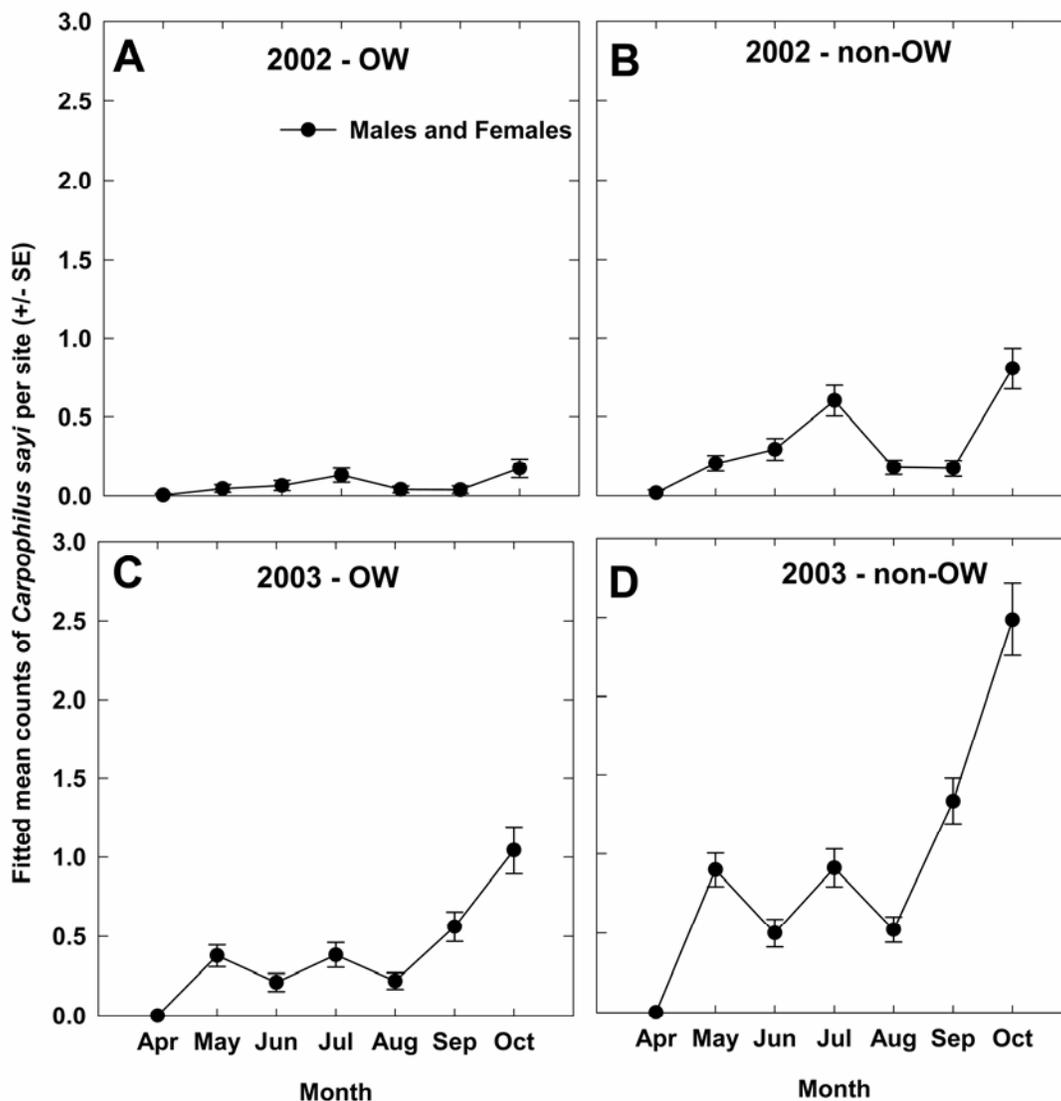


Fig. 3. Fitted mean counts per site \pm SE for *Carpophilus sayi*, sexes combined, captured per month in four sites, by year and site (oak wilt, A, 2002 and C, 2003, or non-oak wilt, B, 2002 and D, 2003). Insects were caught in wind-oriented funnel traps using species-specific pheromones from April to October in east central Minnesota.

0.833 (Table 6). For beetles yielding *C. fagacearum*, the average CFU per beetle ranged from 9 to 43,000.

Contaminated insect populations. Contaminated insect populations (CIP) for *Colopterus truncatus* in 2002 were greatest in April, May, and/or June in both site types (Fig. 4A and B). Peak CIP occurred in oak wilt sites in April (approximately 0.6 infective beetles per trap). *Colopterus truncatus* males represented a higher CIP than females in the oak wilt sites in April and May (Fig. 4A). In the non-oak wilt sites, there was little difference between males and females (Fig. 4B).

In 2003, male and female *Colopterus truncatus* CIP was similar to levels observed in 2002 (Fig. 4C and D). Peak CIP occurred in the oak wilt sites in May (ap-

proximately 1.3 infective beetles per trap), and the overall CIP decreased as the season progressed. Contaminated insect populations were highest in May and June, then decreased and leveled off through September and October when a slight peak occurred (Fig. 4D). In both site types, the CIPs for males were slightly greater than for females.

Contaminated insect populations for *Carpophilus sayi* in 2002 were very low (Fig. 5A and B). Peak CIP occurred in the non-oak wilt sites in June (approximately 0.08 infective beetles per trap) (Fig. 5B). In general, CIP decreased after June and then increased again in October. Overall, the CIP of females was slightly higher than for males in both oak wilt and non-oak wilt sites.

In 2003, the CIPs for *Carpophilus sayi* were different than in 2002 (Fig. 5C and D). Peak CIP in oak wilt and non-oak wilt sites occurred in June (approximately 0.16 and 0.07 infective beetles per trap, respectively). Overall, CIP increased until June, declined through July and August, and increased again in September and October in oak wilt sites. In general, CIP of males was higher in the spring than for females, but dropped off sharply as the season progressed. Contaminated insect population of females was higher than of males in July, September, and October.

For both *Colopterus truncatus* and *Carpophilus sayi*, CIP estimations of zero were either due to no beetles being trapped in a given month (as in September 2002 for *Colopterus truncatus*, Fig. 3A and B) or to the fact that no beetles yielded *C. fagacearum* upon isolation (as in all other cases).

Table 3. Best-fitting models resulting from binomial regression analyses of significant explanatory variables that affect presence of *Ceratocystis fagacearum* propagules on *Colopterus truncatus* adults caught in wind-oriented funnel traps in oak wilt and non-oak wilt sites in east central Minnesota from April to October 2002 and 2003^a

Year	Variable	Level	Estimate	SE	Wald χ^2	P value	
2002	Constant		-3.60	0.30	-12.10	<0.0001	
		Month					
		May	1.19	0.31	3.86	<0.0001	
		June	2.33	0.33	7.03	<0.0001	
		July	-7.65	42.82	-0.18	>0.80	
		August	1.85	0.84	2.20	<0.03	
		September	2.95	0.66	4.44	<0.0001	
		October	-7.82	80.42	-0.10	>0.90	
		Site type	Non-oak wilt	-0.91	0.28	-3.24	<0.002
		Sex	Males	0.64	0.23	2.77	<0.006
2003	Constant		-13.70	54.10	-0.25	>0.80	
		Month					
		May	12.65	54.10	0.23	>0.80	
		June	12.96	54.10	0.24	>0.80	
		July	12.80	54.10	0.24	>0.80	
		August	-0.12	365.60	0.00	>0.90	
		September	0.14	125.40	0.001	>0.90	
		October	10.93	54.10	0.20	>0.80	
		Site type	Non-oak wilt	-1.29	0.20	-6.59	<0.0001
		Sex	Males	-0.02	0.15	-1.40	>0.10

^a Fitted model for: Probability of isolating fungus = constant + month + site + sex. Results of contrasts between levels of the coefficients and the first level (implied to be 0) are presented.

DISCUSSION

Highest numbers of *Colopterus truncatus* dispersing in oak stands occurred in early to mid-spring of both years, with numbers decreasing as the growing season progressed. This time of peak abundance coincided with the historical "critical spring period" for overland transmission of *C. fagacearum* to healthy oaks in Minnesota (14). The peak also was consistent with results of Kyhl et al. (22) in which 98% of similarly trapped *Colopterus truncatus* were found between mid-April and mid-June. A major spring flight peak followed by a smaller fall peak as was seen in this study was reported for *Colopterus truncatus* in several previous studies in Illinois, Minnesota, and Wisconsin (7,11,19,23,27).

In contrast, the highest numbers of *Carpophilus sayi* were caught in October of both years. In general, abundance of this

Table 4. *Ceratocystis fagacearum* (Cf) contamination proportions and CFU from male and female adult *Colopterus truncatus* resulting from serial dilution plating assays in 2002 and 2003

Year	Site type	Month	Males				Females			
			No. assayed	No. positive	Proportion with Cf	Mean CFU/ beetle \pm SE ^a	No. assayed	No. positive	Proportion with Cf	Mean CFU/ beetle \pm SE
2002	Oak wilt ^b	April	204	16	0.08	390 \pm 250	192	0	0.00	... ^c
		May	147	20	0.14	280 \pm 150	129	11	0.09	710 \pm 510
		June	41	11	0.27	200 \pm 150	48	14	0.29	510 \pm 230
		August	6	1	0.17	55 \pm 55	3	1	0.33	250 \pm 250
		September	8	5	0.63	1,800 \pm 950	3	0	0.00	...
	Non-oak wilt ^d	May	90	7	0.08	89 \pm 71	63	2	0.03	6 \pm 5
		June	31	3	0.10	43 \pm 29	46	7	0.15	60 \pm 32
2003	Oak wilt ^e	May	399	80	0.20	2,400 \pm 1,200	260	75	0.29	5,700 \pm 2,500
		June	24	11	0.46	28,000 \pm 19,000	41	10	0.24	89 \pm 97
		July	12	4	0.33	2,300 \pm 1,600	9	3	0.33	770 \pm 730
	Non-oak wilt ^f	May	195	15	0.08	1,800 \pm 1,700	155	12	0.0	2,300 \pm 2,100
		June	34	4	0.12	55 \pm 29	16	1	0.06	160 \pm 160
		October	42	3	0.07	233 \pm 230	15	0	0.00	...

^a CFU of *C. fagacearum* counted on potato dextrose agar following the 10-day incubation period after serial dilution plating.

^b In July, 5 males and 4 females were assayed; none were positive. In October, 2 males and 1 female were assayed; none were positive.

^c Calculation not applicable.

^d In April, 28 males and 25 females were assayed; none were positive. July to October, only 7 males were assayed; none were positive.

^e In April, 71 males and 39 females were assayed; none were positive. August to October, 35 males and 28 females were assayed; none were positive.

^f In April, 47 males and 20 females were assayed; none were positive. July to September, 25 males and 13 females were assayed; none were positive.

species increased as the growing season progressed, with several distinct peaks occurring during the summer months. This is the first published data on *Carpophilus sayi* based on adults caught in pheromone-baited flight traps. Flight periods were previously reported for *Carpophilus sayi* caught in banana-baited traps in Wisconsin (23). In that study, the highest numbers of beetles were caught in May, late July, and mid-September.

Differences in biology, life history, and temporal flight dynamics of these two species may be responsible for varying numbers of beetles in the baited flight traps. Skalbeck (27) showed a progressive arrival of nitidulid species in fungus-baited traps over the growing season in Minnesota. *Colopterus truncatus* was one of the first species present in the spring, whereas *Carpophilus* species did not appear until a week or more later. Skalbeck (27) sug-

gested that differences in capture rates of the two species of beetles at a given site may be affected by differences in type of overwintering site, developmental stage that overwinters, and the amount of heat that is absorbed by developmental substrate of overwintering sites in the spring.

The abundance of *Colopterus truncatus* and *Carpophilus sayi* in oak wilt-affected stands can be measured in at least three different situations: those dispersing through the stand, those in fresh wounds on healthy trees, and those in oak wilt fungal mats. In this study, dispersing beetles were measured using pheromone-food synergist bait traps not located on live or dead oak trees. These captured beetles may have come from outside the stand, or may have departed recently from aging oak wilt mats or wounds on trees in the stand. The arrival and departure of beetles from fresh wounds and mats have been documented

(5,21,26). Cease and Juzwik (5) reported *Colopterus truncatus* as the most abundant species on immature and mature mats in mid- to late May, but the species' presence was much reduced on aging mats during the same time period. *Carpophilus sayi* was present only in low numbers on the immature and mature mats, but accounted for approximately one-third of the total nitidulid beetles on aging and declining mats. J. F. Kyhl (*unpublished*) also found differences in adult nitidulid beetles species and their abundances on mats between spring and fall.

Temporal differences in sap beetle species occurrence also have been reported for wounds. In West Virginia, Dorsey and Leach (9) found the highest numbers of sap beetle species in wounds in May and early June; none were found in wounds in July. Norris (26) found *Colopterus truncatus* was most abundant in <2-week-old wounds in Iowa during April, May, and June; *Carpophilus sayi* also was present during this same time period. In a Minnesota spring wounding study, >95% of sap beetles collected from <6-day-old wounds were *Colopterus truncatus* and *Carpophilus sayi* (21). Very few beetles were collected in April and June; the majority were collected in May. McMullen et al. (24) found both *Colopterus truncatus* and *Carpophilus sayi* common in 1-week-old axe blaze wounds on oaks from the end of May through June in Wisconsin.

For beetles collected during spring in this study, the proportions of beetles contaminated with *C. fagacearum* were the highest in June. However, the highest contamination rates for *Colopterus truncatus* were found for the September (45%) and July (33%) collections. The pathogen also was isolated from *Carpophilus sayi* trapped in July, September, and October, but at a low rate (<5%). The high isolation frequency for *Colopterus truncatus* trapped during summer months could be

Table 5. Best-fitting models resulting from binomial regression analyses of significant explanatory variables that affect presence of *Ceratocystis fagacearum* propagules on *Carpophilus sayi* adults caught in wind-oriented funnel traps in oak wilt and non-oak wilt sites in east central Minnesota from April to October 2002 and 2003^a

Year	Variable	Level	Estimate	SE	Wald χ^2	P value	
2002	Constant		-14.23	520.55	-0.03	>0.90	
		Month					
		May	0.11	535.17	0.00	>0.90	
		June	13.23	520.54	0.03	>0.90	
		July	0.18	527.61	0.00	>0.90	
		August	0.21	534.77	0.00	>0.90	
		September	0.18	539.43	0.00	>0.90	
		October	9.80	520.55	0.02	>0.90	
		Site type	Non-oak wilt	0.03	0.89	0.04	>0.90
		Sex	Males	-0.33	0.68	-0.49	>0.90
2003	Constant		-1.40	0.48	-2.95	<0.003	
		Month					
		June	-1.75	0.54	3.23	<0.001	
		July	-2.5	1.08	-2.33	<0.02	
		August	-11.44	53.12	-0.02	>0.80	
		September	-2.64	0.70	-3.79	<0.0002	
		October	-2.86	0.81	-3.54	<0.0004	
		Site type	Non-oak wilt	-3.09	0.52	-5.98	<0.0001
		Sex	Males	0.98	0.47	2.10	<0.03

^a Fitted model for: Probability of isolating fungus = constant + month + site + sex. Results of contrasts between levels of the coefficients and the first level (implied to be 0) are presented.

Table 6. *Ceratocystis fagacearum* (Cf) contamination proportions and CFU from male and female adult *Carpophilus sayi* resulting from serial dilution plating assays in 2002 and 2003

Year	Site type	Month	Males				Females			
			No. assayed	No. positive	Proportion with Cf	Mean CFU/ beetle \pm SE ^a	No. assayed	No. positive	Proportion with Cf	Mean CFU/ beetle \pm SE
2002	Oak wilt ^b	June	2	0	0.00	... ^c	5	1	0.20	66 \pm 66
		October	6	0	0.00	...	16	1	0.06	21 \pm 21
	Non-oak wilt ^d	June	20	5	0.25	17,000 \pm 10,000	18	5	0.27	5,300 \pm 5,200
2003	Oak wilt ^e	May	14	3	0.21	290 \pm 240	15	2	0.13	250 \pm 250
		June	6	5	0.83	5,700 \pm 2,600	8	6	0.75	43,000 \pm 4,000
		July	14	0	0.00	...	10	1	0.1	4,000 \pm 4,000
		September	52	0	0.00	...	44	3	0.07	30 \pm 18
		October	23	0	0.00	...	41	1	0.02	9 \pm 8
	Non-oak wilt ^f	May	51	2	0.39	66 \pm 65	60	2	0.03	48 \pm 33
		June	25	0	0.00	...	25	4	0.16	1,300 \pm 1,100

^a CFU of *C. fagacearum* counted on potato dextrose agar following the 10-day incubation period after serial dilution plating.

^b No beetles were assayed in April or May. July to September, 7 males and 13 females were assayed; none were positive.

^c Calculation not applicable.

^d In April and May, 13 males and 24 females were assayed; none were positive. July to October, 101 males and 86 females were assayed; none were positive.

^e In April, no beetles were assayed. In August, 12 males and 13 females were assayed; none were positive.

^f In April, no beetles were assayed. July to October, 210 males and 210 females were assayed; none were positive.

misleading, however, because of the relatively low numbers of adults collected ($N = 41$, July to September) compared with the spring months ($N = 2,115$, April to June). If one assumes that the pheromone baits for *Carpophilus sayi* and *Colopterus truncatus* are equally efficient in attracting beetles of the same species, the threat or infective insect potential posed by these *C. fagacearum*-contaminated beetles to healthy oaks over the growing season is perhaps better expressed by the CIP value.

Proportions of contaminated beetles differed between sites with oak wilt and those without, and is likely attributable to the presence or absence of oak wilt mats (i.e., sites of propagule acquisition by beetles) in the sampled stands. It is interesting that even when sap beetle adults were collected directly from oak wilt mats, the frequency of isolation of the pathogen from the beetles was not necessarily 100%. For example, of 57 *Colopterus truncatus* collected from mats between 1 April and 27 May,

94% were contaminated, while only 33% of mat-inhabiting *Carpophilus sayi* were contaminated (18). Cease and Juzwik (5) recovered *C. fagacearum* from *Colopterus truncatus* less frequently than from five other species (including *Carpophilus sayi*) that accounted for 94% of all beetles on oak wilt mats in May. Thus, not all *Colopterus truncatus* and *Carpophilus sayi* exiting oak wilt mats are necessarily contaminated. Proportions of *C. fagacearum*-contaminated beetles in wounds on healthy oaks in a stand with active oak wilt in May also were high (84% for *Colopterus truncatus* and 71% for *Carpophilus sayi*) (21).

The proportions of dispersing sap beetles contaminated with *C. fagacearum* in this study were much higher than reported previously. Dispersing beetles trapped and assayed in Texas in the mid-1980s (1,2) and earlier in Minnesota in the late 1970s and 1980s (18,19) were collected from fruit or fungus culture, and/or fermenting flour dough-baited traps. For example,

only 0.65% of the 4,126 sap beetles in Texas (trapped October to May) and 0.67% of the 1,043 sap beetles in Minnesota (trapped March to June) yielded *C. fagacearum*. This difference may be attributable to the use of aggregation pheromones specific to the beetle species and closely related species.

The temporal flight dynamics of dispersing *Colopterus truncatus* and *Carpophilus sayi* during the spring in stands with active oak wilt and the proportions found to be contaminated with *C. fagacearum* further support the current thought that these two species are the principal sap beetle vectors of the pathogen in Minnesota. Differences in flight dynamics also suggest that *Colopterus truncatus* may be relatively more important than *Carpophilus sayi* in this role. Furthermore, higher proportions of contaminated beetles and higher CIP values were found for *Colopterus truncatus* than for *Carpophilus sayi* in this study. These results, in turn, suggest

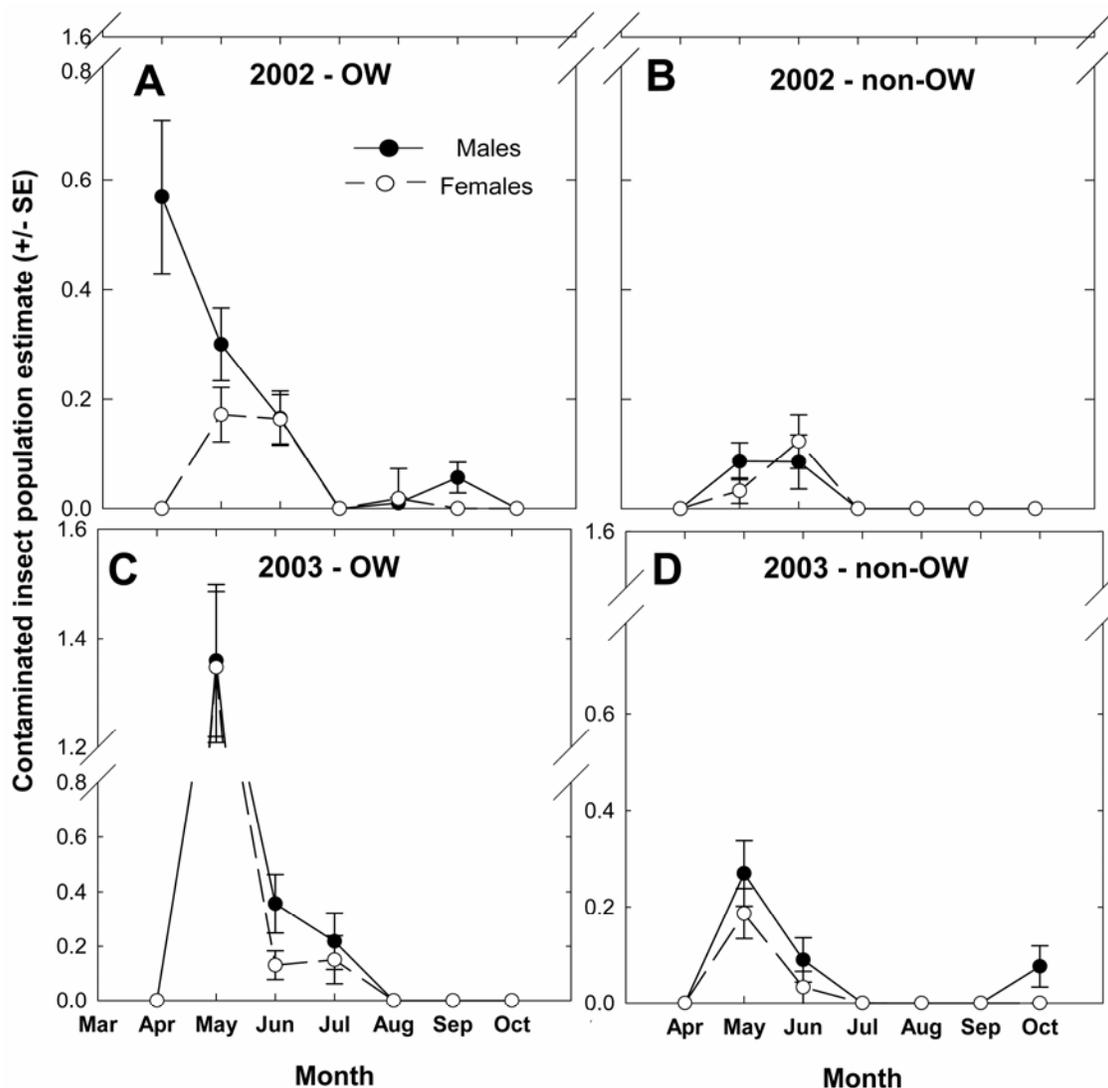


Fig. 4. Contaminated insect population estimates for male and female *Colopterus truncatus* (determined by multiplying the monthly fit mean per site × the proportion of individual insects that tested positive for *Ceratocystis fagacearum*) for four sites by year and site type (oak wilt, **A**, 2002 and **C**, 2003, or non-oak wilt, **B**, 2002 and **D**, 2003). Insects were trapped in wind-oriented funnel traps using species-specific pheromones from April to October in east central Minnesota.

that the infective insect potential is greater for *Colopterus truncatus* than for *Carpophilus sayi*, particularly during spring months. Based on his studies of numerous insect species associated with oak wilt mats and “infectable wounds” on oaks in Iowa, Norris (26) also concluded *Colopterus truncatus* is likely the most important sap beetle vector in that state, although quantitative analysis was not used to reach his conclusion.

As previously indicated, CIP proved useful in our study to compare relative importance of two implicated sap beetle vector species. It also may prove useful in comparing two different vector groups, such as oak bark beetles (Coleoptera: Scolytidae) to sap beetles. Additional factors, (e.g., environmental variables and amount of inoculum present) could be added to a risk model and tested for making such comparisons.

Current oak wilt management guidelines in Minnesota include rating the risk of oak

wilt transmission potential by month during the year (14). The guidelines specify that pruning, wounding, or felling of oaks during the high-risk period (April to June) should be avoided, while the summer months are considered to be a low-risk period, and late fall and winter are considered “safe” for such actions. Although dispersing *Colopterus truncatus* and *Carpophilus sayi* were contaminated with *C. fagacearum* during summer and fall months, the CIP values were relatively low. However, CIP values were higher during the spring months. Thus, relative risk in terms of infective insect potential agrees with previously published guidelines. The attractiveness of fresh summer and fall wounds to *Colopterus truncatus* and *Carpophilus sayi* is not clear. Future studies will address the relative attractiveness of fresh summer and fall wounds to sap beetles, and to *Colopterus truncatus* and *Carpophilus sayi* in particular. If *C. fagacea-*

rum-contaminated beetles are found in fresh wounds during these seasons, the risk designation will need to be reassessed.

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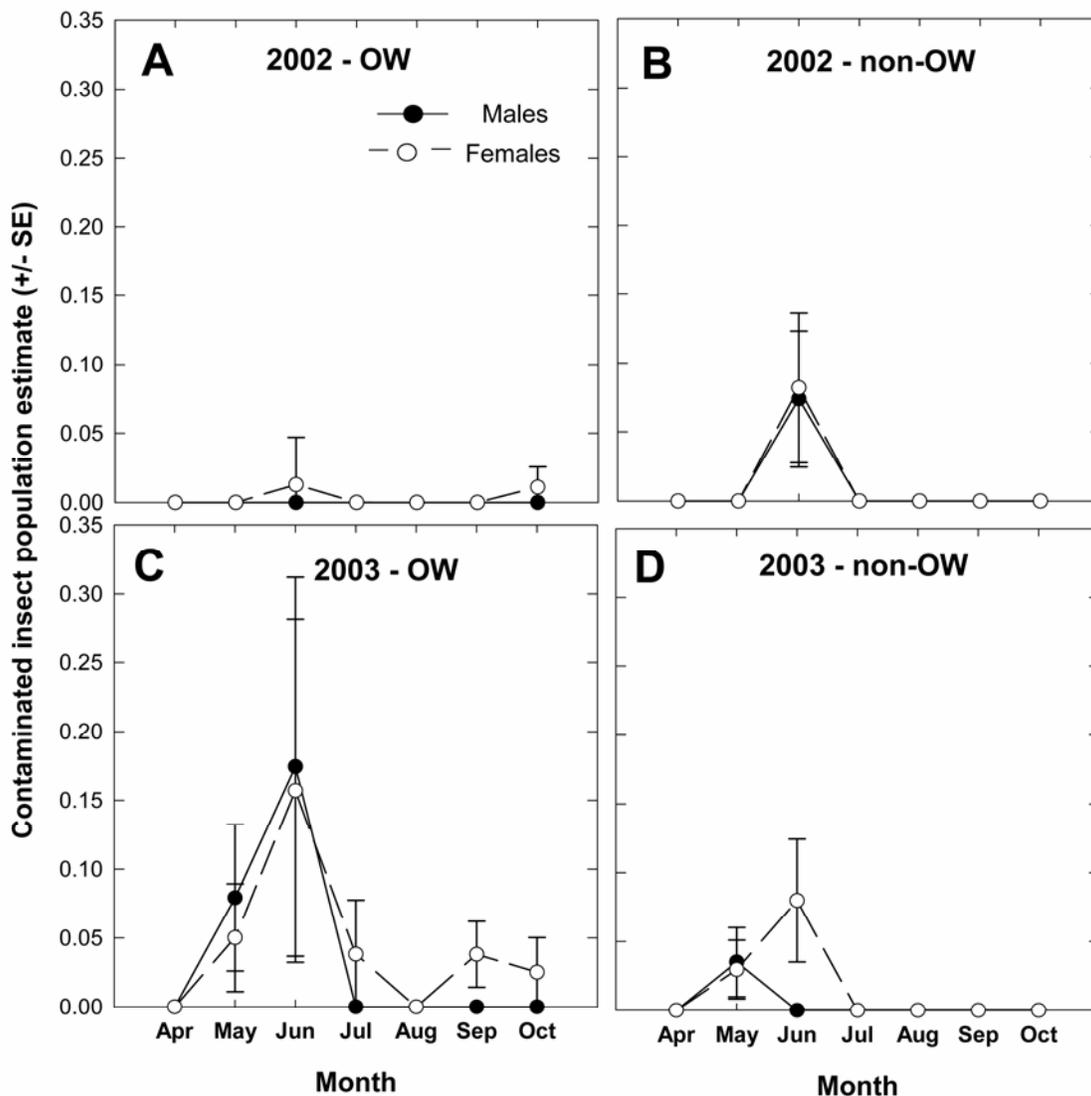


Fig. 5. Contaminated insect population estimates for male and female *Carpophilus sayi* (determined by multiplying the monthly fit mean per site \times the proportion of individual insects that tested positive for *Ceratocystis fagacearum*) for four sites by year and site type (oak wilt, **A**, 2002 and **C**, 2003, or non-oak wilt, **B**, 2002 and **D**, 2003). Insects were caught in wind-oriented funnel traps using species-specific pheromones from April to October in east central Minnesota.

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