Sap Beetle Species (Coleoptera: Nitidulidae) Visiting Fresh Wounds on Healthy Oaks During Spring in Minnesota

Jennifer Juzwik, Thomas C. Skalbeck, and Marc F. Neuman

ABSTRACT. Many species of sap beetles have been implicated as vectors of the oak wilt pathogen, (*Ceratocystis fagacearum*), but the species responsible for most aboveground transmission of the fungus is unknown. The abundance of adult sap beetle species inhabiting <7-day-old, artificially made wounds on healthy oaks during Apr.–June of 2 years in Minnesota, and the frequencies with which viable propagules of the fungi were present on each, were determined. Sap beetles were most abundant on wounds created in May, and 1–3-day-old wounds yielded higher numbers of insects than 4–6-day-old wounds. Of the beetles collected, >95% were *Colopterus truncatus* and *Carpophilus sayi*. *C. fagacearum* was isolated from 75% of 594 adults assayed for the fungus. The average number of viable propagules for pooled data of individuals of each species ranged from <100 to 18,000. Frequencies of fungus isolation differed by days after wounding, study location, and sap beetle species. These results, when coupled with previous findings on predominant species associated with oak wilt fungi mats, support the hypothesis that *Co. truncatus* and *Ca. sayi* are the principal sap beetle species transmitting *C. fagacearum* from diseased to healthy oaks in Minnesota. FOR. SCI. 50(6):757–764.

Key Words: Oak wilt, nitidulids, phoresy rates, *Quercus ellipsoidalis*, *Quercus rubra*.

OAK WILT IS AN IMPORTANT DISEASE of oaks in the eastern United States. In the north-central states, thousands of trees in urban forests, woodlots, and natural stands die annually from infection by the oak wilt pathogen, *Ceratocystis fagacearum* (Bretz) Hunt. The disease is most serious on the more highly susceptible red and black oaks (section *Lobatae*) and in live oaks (section *Protobalanus*), although species of the white oak group (section *Quercus*) are also affected (Tainter and Baker 1996).

The fungal pathogen is transmitted from diseased to healthy oaks either below ground, through common root systems or grafted roots between adjacent trees, or aboveground by insect vectors (Gibbs and French 1980). Although the large majority of individual trees that die annually in states such as Minnesota and Wisconsin are infected as a result of below-ground spread, insect spread is important because it is the means by which infection centers are initiated. Sap beetles (Coleoptera: Nitidulidae), or nitidulids, are considered the primary insect vector group in

Jennifer Juzwik, Research Plant Pathologist, North Central Research Station, USDA Forest Service, 1561 Lindig Street, Street Paul, MN 55108: Phone: (651) 649-5114; Fax: (651) 649-5055; jjuzwik@fs.fed.us. Thomas C. Skalbeck, Entomologist, 8879 Jane Road N., Lake Elmo, MN 55042. Phone: (651) 770-8295; beacon85@hotmail.com. Marc F. Neuman, Former Biological Technician, North Central Research Station, USDA Forest Service, 1561 Lindig Street, Street Paul, MN 55108: Phone: (507) 663-7642; marc.neuman@syngenta.com.

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most of the region (Gibbs 1984, French 1995). Species of this group are attracted to the sporulating structures (oak wilt mats) of the oak wilt fungus and to wounds on healthy trees.

Mats commonly form on species of the red oak group (Gibbs and French 1980); less commonly, to rarely, on members of the white oak group (Engelhard 1955, Cones 1967); and never on live oaks (Appel 1994). They most commonly form during spring and fall months in the northern portion of the oak wilt range (Juzwik 1983). Mats appear from ~2 to 10 months after tree death caused by *C. fagacearum*, depending on tree diameter and timing of crown wilt, among other factors (Juzwik 1983).

At least 12 species of sap beetles have been associated with oak wilt mats in Minnesota (Cease and Juzwik 2001). Sap beetles are attracted by the aromatic volatiles produced by the growing fungus (Lin and Phalen 1992) and by oak wilt mats on disease-killed trees. The beetles crawl over, and may tunnel into, the mats as they feed on the fungal tissues, acquiring viable fungal propagules on their external surfaces, and internally, as they ingest fungal material. Mating, oviposition, and larval development also may occur on oak wilt mats (Norris 1956, Kyhl 2004).

A number of sap beetle species also visit fresh wounds and fermented sap flow in older wounds on healthy oak trees (Dorsey et al. 1953, Norris 1956). Occurrences of mating adults, female egg-laying, and developing larvae on the wounds have been reported (Jewell 1955). Successful disease transmission occurs when *C. fagacearum*-infested sap beetles visit susceptible wounds on healthy oaks. Susceptibility of wounds to infection by the pathogen diminishes quickly over time. For example, Zuckerman (1954) reported that fresh wounds are susceptible for only 48 hours after their creation. In Wisconsin studies, Kuntz and Drake (1957) found wounds to be susceptible for up to 4 days after wounding when surfaces were inoculated artificially with pathogen spores. However, no natural infection occurred in trees with >24-hour-old wounds in the same study. Wound type also is important; xylem-penetrating wounds are a prerequisite for successful infection (Zuckerman 1954).

Many species of sap beetles have been implicated as vectors of the oak wilt fungus. Lists of such species vary by US region within the known range of oak wilt (Merrill and French 1995). Following a review of historical reports, we hypothesized that only a subset of the numerous implicated species is actually responsible for the majority of aboveground transmission occurrences in Minnesota (Juzwik et al. 1996). We recently reported that six sap beetle species accounted for 94% of the total number of sap beetles found on 429 mats during spring of three years in the east-central region of the state (Cease and Juzwik 2001). Additional studies were planned to determine whether any sap beetle species predominate in fresh wounds.

Several previous studies investigated the sap beetle species associated with wounds on oaks. Based on multiyear studies in northern Iowa, Norris (1956) reported the species of sap beetles occurring in ≤2-week-old wounds (considered "infectable wounds") and recorded the seasonal changes in occurrences of insects on such wounds. Eighteen sap beetle species were found on wounds, with the wounds sometimes yielding larvae and adults of the same species. However, analyses of the quantitative data were lacking. Sap beetles were the most numerous species associated with fresh wounds on oaks in West Virginia (Dorsey et al. 1953). The species most commonly visiting artificial wounds within a few days of their creation in early May were *Glischrochilus fasciatus* (Oliv.), *G. sanguinolentus* (Oliv.), *Colopterus semitectus* Say, and *Carpophilus lugubris* Murray. As many as 50 adult sap beetles were collected at one time from a single injured tree.

Information on how frequently wound-visiting sap beetles carry viable propagules of *C. fagacearum* is scant. Reports are limited about how frequently freshly wounded trees that were visited by sap beetles subsequently wilted. In a Missouri study (Buchanan 1960), sap beetles were caught in traps stapled in auger holes on trees that subsequently developed oak wilt. The beetles were not identified to species.

Results of our investigation of sap beetle species associated with fresh wounds during 2 years in east-central Minnesota are reported here. The specific objectives were to determine: (1) the abundance of different adult sap beetle species visiting 1–6-day-old wounds on healthy oaks during spring months, and (2) the frequencies of beetles with viable *C. fagacearum* and numbers of fungus propagules on the insects' bodies. A preliminary abstract has been published (Juzwik et al. 1999).

Materials and Methods

Study Sites and Tree Selection

Healthy northern red oak (*Quercus rubra* L.) and northern pin oak (*Q. ellipsoidalis* E.J. Hill) located adjacent to, or between, primary and secondary root-graft barrier lines of treated oak wilt infection centers were selected for studies in 1998 and 1999. Potential oak wilt mat-producing trees were not removed. Oak wilt mats were observed at various times on several such trees during the course of this study. The trees were on public land associated with the Anoka County Airport, in Blaine, MN, and on several scattered sites within a large park reserve near Burnsville, MN.

Wounding Treatment

Using a hole saw (5-cm dia) and battery-powered portable drill, wounds were created just into the outer xylem of the tree's main stem. The trees were wounded at ~1.4 m in height on each wounding date—one on the east and the other on the west aspect of each tree. To retard desiccation and create a darkened niche, the bark plug was loosely placed back over and nailed to the wound immediately after it was made. A flap of waterproof cardboard was nailed above each wound and silicone caulking used to seal the bark edge of the flap to the ridges and fissures of the bark. The flaps prevented rainwater from washing out any insects visiting the wounds.

Trees were wounded during spring, when oaks are most susceptible to oak wilt infection via overland transmission in Minnesota (Juzwik et al. 1985). Once a month, in Apr., May, and June, the fresh wounds were made on the same set of trees within the study year. Wounds that were created in a subsequent month were sealed with the bark plug and plumber's putty around the edges, and around the center nail hole. A new set of trees was selected for the study in the second year. In 1998, wounding dates were 22 Apr., 19 May, and 16 June; in 1999, wounding dates were 26 Apr., 17 May, 25 May, and 14 June. Because of inclement weather (rain, moderate winds, and cool temperatures) at the Blaine site from 19–21 May 1999, wounding was repeated 1 week later. At the Blaine site, 15 trees were wounded in 1998, and 11 trees were wounded in 1999. In the Burnsville study, 18 trees were wounded in 1998, and 20 were wounded in 1999.

Sample Collection and Processing

Wounds were checked daily for six days after wound creation at both sites for both years. The bark plug was carefully removed and any sap beetle(s) observed were quickly moved from the wound with fine forceps, a small curved spatula, or a fine paint brush into a sterile petri dish with moistened filter paper covering the bottom. The petri dish with beetles was immediately placed on an ice block to slow down beetle activity so that adults could then be placed individually into gelatin capsules (00 size). The encapsulated beetles then were grouped by wound aspect and tree in polyethylene bags, transported in ice coolers, and stored at −2° C in the laboratory. All adult sap beetles were identified to species before further processing.

The numbers of viable propagules of *C. fagacearum* on the sap beetles were determined using a serial dilution plating technique. Each beetle was placed into a sterile micro-centrifuge tube containing 0.6 ml of sterile distilled water. Using a tip homogenizer (Ultrasonic Homogenizer, Cole-Palmer, Vernon Hills, IL), the beetle suspension was sonicated for 10 seconds. With the smaller beetles, this treatment resulted in total maceration. The resulting suspension was serially diluted (10-fold dilutions; 3 dilutions), and 0.5-ml aliquots of each dilution spread on lactic acid-amended potato dextrose agar petri dishes. Three dishes were used per dilution. Resulting colonies of *C. fagacearum* were counted after 10–14 days of growth (dark conditions, 24° C). Colony appearance and asexual spore characteristics were used to identify *C. fagacearum* colonies.

Statistical Analyses

Poisson regression was used to determine whether the abundances of total sap beetles, or of three different species of sap beetles, varied by selected factors. The numbers of sap beetles collected from the wounds were the response variables. Year, location, and number of days after wounding were the predictor variables. The Poisson loglinear model has the form,

$$\text{Log}\ \mu = \alpha + \beta x,$$

where the mean represents the exponential relationship

$$\mu = \exp(\alpha + \beta x).$$

If $\beta = 0$, then the mean of the response variable does not change as the predictor variable changes. If $\beta > 0$, the mean of y increases as x increases; but if $\beta < 0$, the mean decreases as x increases (Agresti 1996). Thus, interpretation of the coefficient estimates and general trends from the resulting Poisson regression models can be offered.

Logistic regression was used to determine the probabilities of isolating *C. fagacearum* from individuals of two sap beetle species and one combined group collected in a location and within either 1–3 or 4–6 days after wounding in May only (Agresti 1996). Data from Apr. and June were too sparse to analyze. Data from May for two years were collapsed because of sparseness in some combinations of wound age and species. The logistic regression model is

$$\theta(x) = \eta_0 + \eta_1 x_1 + \eta_2 x_2 + \cdots + \eta_i x_i,$$

where θ is the probability of isolation of the fungus, η_i are coefficients, and x_i are variables $i = 1, 2, \ldots, n$ (Cook and Weisberg 1999). Estimation of the model coefficients is based on maximum likelihood. The significance of a single variable was investigated with the Wald χ^2 test, in which a high *P*-value suggests that the variable is not needed in the model. The hypothesis that θ is equal for the levels within each significant variable was tested using the Z statistic (Cook and Weisberg 1999). The odds of success of isolating the fungus for each level were also calculated. All statistics were performed using the computer program ARC, as described in Cook and Weisberg (1999).

Results

Sap Beetles Visiting Wounds

The greatest numbers of sap beetles were collected in May for each location and year (Figure 1). The ranges of total numbers collected each month for locations and years combined were 2–7 in Apr., 50–228 in May, and 0–40 in June. Within each month, the highest numbers of sap beetles generally were collected during the first three days after wounding. One major exception to this trend occurred during May 1999 in Burnsville, where no beetles were collected during the first three days compared to a large number (102) on day 4. The significant variables affecting sap beetle abundance in fresh wounds were number of days after wounding, location, and a year * number-of-days-after-wounding interaction based on results of Poisson regression analyses ($P < 0.0001$) (Table 1).

During the study, eight sap beetle species were collected from fresh wounds. These species included *Colopterus truncatus* Randall, *Co. semitectus*, *Carpophilus sayi* Parsons, *Ca. dimidiatus* L., *Ca. hemipterus* L., *Epuraea corticina* Erich., *Cryptarcha ampla* Erich., and *Cychramus adustus* Erich.

Overall, *Ca. sayi* and *Co. truncatus* accounted for 57 and 39%, respectively, of all the sap beetles collected (Table 2). *Co. truncatus* was the predominant species (63% of 321) obtained for all days after wounding in 1998, and the first three days after wounding in 1999 in Blaine. However, *Ca.*

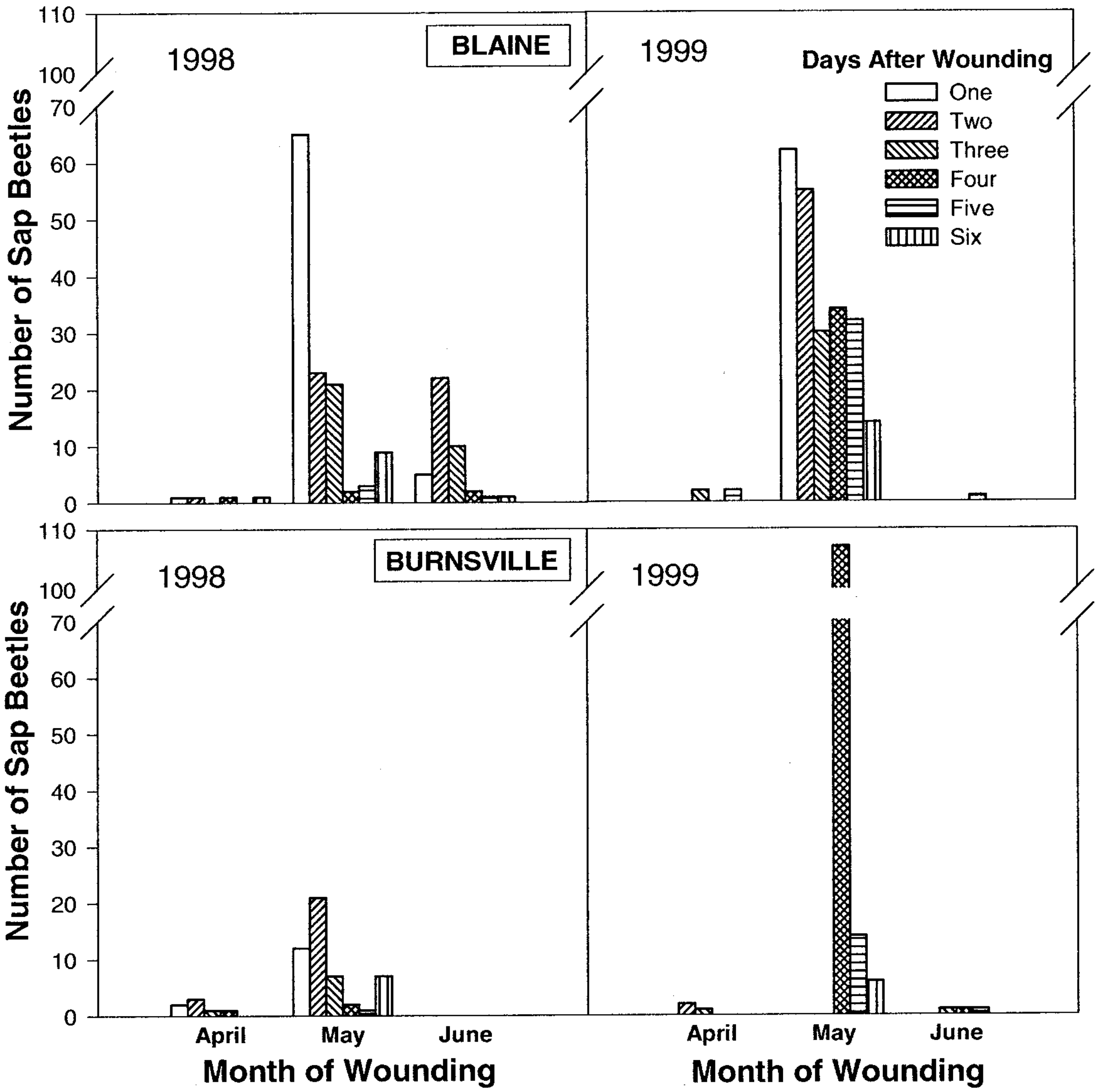

Figure 1. Numbers of sap beetles collected from wounds on healthy red oaks for six consecutive days after wounding during spring 1998 and spring 1999 in two east-central Minnesota locations.

Table 1. Model resulting from Poisson regression analyses of significant explanatory variables that affect the abundance of total adult sap beetles in wounds <7 days old on oak trees in two locations in 1998 and 1999.

Variable	Estimate	SE	*P*-value
Constant	4.5947	0.0656	<0.0001
Days	−1.7851	0.2082	<0.0001
Location	−0.6818	0.0920	<0.0001
Years * days	2.1138	0.2117	<0.0001

sayi was most commonly found (71% of 83) 4–6 days after wounding in 1999 in the same location (Table 2). *Ca. sayi* was the predominant sap beetle species (94% of 193) collected from wounds on oaks in Burnsville during both years (Table 2).

Because the total number of sap beetles in wounds varied by age of wound (days) and location (Table 1), the Poisson analysis for this data set was conducted for each wound-age category in each location (Table 3). The significant variables affecting abundance of selected species groups (*Co. truncatus*, *Ca. sayi*, and others combined) varied within the number of days after wounding categories for each location (Table 3). For 1–3 days after wounding in Blaine, two sap beetle species (*Co. truncatus* and *Ca. sayi*) were the only significant factors ($P < 0.0001$). The same two beetle species were also significant in explaining total beetle abundance in 4–6-day-old wounds in Blaine, but an interaction between *Ca. sayi* and year was also significant. In Burnsville, abundance of beetles varied by year, and by one species (*Co. truncatus*) for collections made from 1–3-day-old wounds. However, for collections made from 4–6-day-old wounds, abundance was affected by two species (*Co. truncatus* and *Ca. sayi*), but not by year.

Fungi Isolation from Beetles

Frequencies and numbers of viable *C. fagacearum* propagules on external surfaces of sap beetles were determined for

Table 2. Numbers of various sap beetle species collected from <7-day-old wounds during spring 1998 and 1999 in two east-central Minnesota locations.

Location	Year	No. days after wounding	No. of adults by sap beetle species		
			Co. truncatus	*Ca. sayi*	Other*
Blaine	1998	1–3	96	45	10
		4–6	16	3	4
	1999	1–3	91	55	1
		4–6	22	59	5
Burnsville	1998	1–3	4	39	4
		4–6	0	11	0
	1999	1–3	0	4	1
		4–6	5	124	1

* Other species include *Colopterus semitectus*, *Carpophilus dimidiatus*, *Carpophilus hemipterus*, *Epuraea corticina*, *Cryptarcha ampla*, and *Cychramus adustus*.

Table 3. Models resulting from Poisson regression analyses of explanatory variables that affect the abundance of *Colopterus truncatus* and *Carpophilus sayi* adults in wounds <7 days old on red oak trees in two east-central Minnesota locations, spring 1998 and 1999.

Location	No. days after wounding	Variable*	Estimate	SE	*P*-value
Blaine	1–3	Constant	1.8718	0.2773	<0.0001
		Cot	2.6662	0.2868	<0.0001
		Cas	2.0402	0.2948	<0.0001
Blaine	4–6	Constant	1.5041	0.3333	<0.0001
		Cot	1.2685	0.4167	0.0023
		Cas	−0.4055	0.6667	0.5431
		Cot * Year	0.3185	0.3286	0.3324
		Cas * Year	2.9789	0.5918	<0.0001
Burnsville	1–3	Constant	1.1760	0.5039	0.0196
		Year	−1.4522	0.3349	<0.0001
		Cot	0.0000	0.7069	1.0000
Burnsville	4–6	Constant	−2.6027	0.8320	0.0018
		Year	0.9163	0.8364	0.2733
		Cot	4.1589	0.7123	<0.0001
		Cas	3.2581	0.4557	<0.0001

* *Cot* = sap beetle species *Colopterus truncatus*; *Cas* = sap beetle species *Carpophilus sayi*.

98% of 602 collected beetles (Tables 4 and 5). The fungus was isolated from four of the eight sap beetle species obtained from the wounds. Specifically, *Co. truncatus*, *Co. semitectus*, *E. corticina*, and *Ca. sayi* yielded *C. fagacearum*, whereas the fungus was not obtained from the few individuals (total of eight) of *Ca. dimidiatus*, *Ca. hemipterus*, *Cr. ampla*, and *Cy. adustus* tested. Frequency of *C. fagacearum* isolation from all collected adults was 75%. Frequency of *C. fagacearum* isolation was greatest for *Co. truncatus* (84%) and *Ca. sayi* (71%) compared to other species assayed. For beetles collected during the first three days after wounding, 82% yielded *C. fagacearum* compared to 66% of those collected 4–6 days after wounding. The fungi were obtained more frequently from sap beetles collected from wounds on trees in Blaine (82%) (Table 4) than from those obtained in Burnsville (60%) (Table 5). When present, the average number of *C. fagacearum* propagules isolated from pooled data for individuals of a species ranged from <100 to 18,000 colony-forming units (Tables 4 and 5).

Because data from Apr. and June were too sparse to analyze, only May data were used in the logistic regression analysis of fungal isolation frequencies. The May data for 1998 and 1999 also were combined because of sparseness in some wound-age–species categories. Of 536 assayed beetles collected in May of both years, 79% yielded *C. fagacearum*. Frequencies of positive isolation differed by wound age ($P = 0.0001$), location ($P < 0.0001$), and sap beetle species ($P = 0.0007$) (Table 6). When all other variables were kept constant in each comparison, the odds of success (shown in parentheses) of isolating *C. fagacearum* from beetles visiting wounds were significantly higher ($P < 0.02$) for: (a) 1-3-day-old (10–1) than 4–6-day-old wounds (2–1), (b) beetles from Blaine (9–1) compared to Burnsville (2–1), and (c) *Co. truncatus* individuals (6–1) than *Ca. sayi* individuals (3–1).

Discussion

The greatest numbers of total sap beetles associated with wounds in this study were collected in May for both years and sites. The period of greatest wound attractiveness to insects in West Virginia studies also was in May (Dorsey and Leach 1956), and sap beetles were the most numerous of all insects found on wounds in an earlier study (Dorsey et al. 1953). In the 1956 study, maximum populations of sap beetle species on wounds occurred during the early spring and fall when the mean weekly temperature was approximately 10°C;

Table 4. Frequency of *Ceratocystis fagacearum* isolated from sap beetles collected from fresh wounds on red oaks in Blaine, MN.*

Collection month	Sap beetle species[†]	No. days after wounding	No. beetles tested	Percent with fungus	CFU fungus per beetle[‡]	
					Ave. ($\times 10^3$)	SE ($\times 10^3$)
April	*Cot*	1–3	1	100	0.4	–[§]
	Other	1–3	5	0	–	–
	Cot	4–6	1	0	–	–
	Cas	4–6	1	100	0.1	–
	Other	4–6	1	0	–	–
May	*Cot*	1–3	165	94	1.3	0.2
	Cas	1–3	86	91	8.8	2.0
	Other	1–3	6	57	1.1	0.9
	Cot	4–6	35	71	2.0	0.6
	Cas	4–6	58	90	18	3.7
	Other	4–6	3	0	–	–
June	*Cot*	1–3	22	59	0.1	<0.1
	Cas	1–3	14	21	0.9	0.6
	Other	1–3	1	0	–	–
	Cot	4–6	2	0	–	–
	Cas	4–6	3	33	1.4	–

* Sap beetles assayed were taken from collections of adults obtained from fresh wounds made on healthy oaks during spring of two years.
[†] *Cot* = sap beetle species *Colopterus truncatus*; *Cas* = sap beetle species *Carpophilus sayi*. Other = other sap beetle species assayed included *Colopterus semitectus*, *Epuraea corticina*, *Cychramus adustus*, *Carpophilus dimidiatus*, *Carpophilus hemipterus*, and *Cryptarcha ampla*.
[‡] CFU = colony-forming units of *Ceratocystis fagacearum* on petri dishes of growth medium after serial dilution plating.
[§] "–" = calculation not applicable.

Table 5. Frequency of *Ceratocystis fagacearum* isolated from sap beetles collected from fresh wounds on red oaks in Burnsville, MN.*

Collection month	Sap beetle species[†]	No. days after wounding	No. beetles tested	Percent with fungus	CFU fungus per beetle[‡]	
					Ave. ($\times 10^3$)	SE ($\times 10^3$)
April	*Cot*	1–3	1	0	–[§]	–
	Cas	1–3	7	14	0.007	–
	Other	1–3	1	0	–	–
	Cas	4–6	1	100	1.1	–
May	*Cot*	1–3	3	100	1.7	0.9
	Cas	1–3	35	74	1.3	0.8
	Other	1–3	2	100	1.2	1.1
	Cot	4–6	5	20	0.75	–
	Cas	4–6	132	61	4.7	1.3
June	*Cas*	1–3	1	0	–	–
	Cas	4–6	2	0	–	–

* Sap beetles assayed were taken from collections of adults obtained from fresh wounds made on healthy oaks during spring of two years.
[†] *Cot* = sap beetle species *Colopterus truncatus*; *Cas* = sap beetle species *Carpophilus sayi*. Other = other sap beetle species assayed included *Colopterus semitectus*, *Epuraea corticina*, *Cychramus adustus*, *Carpophilus dimidiatus*, *Carpophilus hemipterus*, and *Cryptarcha ampla*.
[‡] CFU = colony-forming-units of *Ceratocystis fagacearum* on petri dishes of growth medium after serial dilution plating.
[§] "–" = calculation not applicable.

Table 6. Coefficients of the logistic regression model fitted to the *Ceratocystis fagacearum* isolation data for *Colopterus truncatus* and *Carpophilus sayi* collected during May 1998 and 1999.

Variable*	Coefficient	SE	Wald	*P*-value
Constant	4.0832	0.4795	8.516	<0.0001
Days	−0.0371	0.0092	−4.021	0.0001
Location	−2.0126	0.3788	−5.313	<0.0001
Sapbspp	−1.3686	0.4021	−3.404	0.0007

* Sapbspp = Sap beetle species.

very few adult beetles were found in June (Dorsey and Leach 1956). In Wisconsin studies, however, McMullen et al. (1960) found the largest number of insects on wounds made in mid-June, coinciding with cessation of mat production and deterioration of existing mats in the area.

We found eight sap beetle species in the spring wounds for both sites and years. Norris (1956) found 18 species of sap beetles in 2-week-old or younger wounds in studies conducted from Apr. through Oct. over a four-year period in Iowa. The sap beetles' occurrences varied by site and year in his studies. In our study, *Co. truncatus* and *Ca. sayi* were the predominant species, accounting for >95% of the total sap beetles found on the wounds. Six other species made up the remaining 2%. The most abundant species (in decreasing order) found on wounds on oaks in Iowa over a 2-year period were *Co. truncatus*, *Ca. sayi*, *G. quadrisignatus*, and *Epuraea* sp. (Norris 1956). In general, *Co. truncatus* and *Ca. sayi* were the most abundant species on the wounds. However, the predominant species present differed by time and location. *Ca. lugubris* and *Co. semitectus* were the predominant species found on wounds in an earlier West Virginia study (Dorsey et al. 1953). In subsequent studies, the sap beetle species found on oak wounds included *G. fasciatus*, *G. sanguinolentus*, *Ca. lugubris*, *Carpophilus* sp., *Co. truncatus*, *Co. morio* En., *Cryptarcha ampla*, *Stelidota germinata* Say, *Lobiopa undulata* Say, and *Epuraea* sp. (Dorsey and Leach 1956).

The sap-beetle catches in our study generally were greatest for the youngest wounds (i.e., 1–3-day-old). Either *Co. truncatus* or *Ca. sayi* was the most abundant species within each wounding month, depending on year and site. These findings generally agree with the sequence and timing of sap beetle species arrival in two-week-old or less wounds in Iowa (Norris 1956). In the latter studies, *Co. truncatus* was commonly present on wounds within 3–4 h of their creation and then was common throughout the two-week period monitored. Four species (*Co. semitectus*, *G. quadrisignatus*, *Ca. sayi*, *Epuraea* sp.) were first picked up in 3-day-old wounds whereas *G. fasciatus* was first found in 11-day-old wounds. The two *Glischrochilus* species usually were found on very sappy and fermented wounds.

On healthy oaks, the incidence and abundance of *Co. truncatus* and *Ca. sayi* on spring wounds can be related to flight activities. Based on 3 years of monitoring with pheromone bait traps, *Co. truncatus* has been trapped frequently in Apr. and May and trapped infrequently in June in oak stands with active oak wilt in several Minnesota locations (Kyhl et al. 2002). Using similar methods at the same locations, adults of *Ca. sayi* were not commonly trapped until late May or early June based on subsequent monitoring in 2002 and 2003 (A. Ambourn, University of Minnesota, Mar. 10, 2004).

C. fagacearum was isolated from 75% of the wound-visiting sap beetles in this study. The highest frequencies of isolation were from the most abundant species found in the wounds, i.e., *Co. truncatus* and *Ca. sayi*. One or more individuals yielded the pathogen in four of the eight total species found present in the wounds. The incidence of pathogen presence on wound-visiting beetles in our study is much higher than the incidences reported for dispersing (i.e., free-flying or airborne) sap beetles caught in oak wilt center food-bait traps in Minnesota ($<1\%$) (Juzwik and French 1983) and Texas ($<0.7\%$) (Appel et al. 1990). Several explanations are possible for this difference. Numerous individuals (95% of total) of over six species of sap beetles were trapped in the earlier Minnesota dispersing insect study, whereas only two species accounted for almost all adults assayed in this wound study. The frequencies of *C. fagacearum* isolation from the dispersing beetle studies may have been greater for a particular species if calculated on a per-species basis. We hypothesize that there are species differences in the frequencies with which dispersing populations carry *C. fagacearum*. In studies of sap beetle species associated with oak wilt mats, Cease and Juzwik (2001) found differences among species harboring *C. fagacearum* when assayed adults were collected from mature and aging oak wilt mats. However, no differences were evident when species were from immature or declining mats. Finally, the food baits used in the earlier Minnesota dispersing insect study were more attractive to certain species than others. Four *Glischrochilus* species accounted for 82% of the dispersing beetles attracted to the food baits (Juzwik and French 1983). The behavioral response of sap beetles to volatiles released by freshly wounded, healthy oaks apparently differs from their response to food baits. Thus, we suggest that it is not valid to compare our level of *C. fagacearum* contamination rate directly with those obtained in the two earlier studies cited.

When present on beetles captured from wounds, the viable propagule load of *C. fagacearum* varied greatly, ranging from 10 to 18,000 per individual. The potential therefore existed (had we not intervened) for any of the fungi-infested beetles we assayed to transmit the fungus successfully based on these inoculum loads and age of the wounds used. This statement is supported by previously published reports on inoculum spore loads needed for successful wound infection of healthy oaks. Jones (1964) reported that main stem inoculations were more effective in causing tree infection than were inoculations made in the tree crown when 10, 100, 10,000 and 1,000,000 conidia per ml were used. Regardless of where inoculation was done, success increased with the inoculum spore load. Cobb et al. (1965) found no difference in the incidences of infection in trees inoculated with spore doses of between 1,000 and 1,000,000 spores per ml.

Two general conclusions are based on these results in conjunction with those reported by Cease and Juzwik (2001) for the same region of Minnesota. First, *Co. truncatus* and *Ca. sayi* appear to be more highly specialized in their relationship to the oak wilt disease cycle than a number of the other implicated sap beetle vectors that are viewed more as "generalists" (Norris 1956). The strong association of these two species to the oak wilt disease cycle in east-central Minnesota is found at the inoculum source (i.e., oak wilt mats) and the infection court (i.e., fresh wounds). Both species have been observed to feed on, oviposit in, and rear their broods on oak wilt mats (Kyhl 2004). In addition, these species are specifically and strongly attracted to the tree volatiles associated with fresh wounds on healthy oaks. In one instance, we observed *Ca. sayi* on wounds 15 minutes after we had created them, and Norris (1956) reported *Co. truncatus* arrival on a fresh wound within 10 minutes of its infliction.

Second, we deduce that *Co. truncatus* and *Ca. sayi* are the principal sap beetle species transmitting *C. fagacearum* from oak wilt-killed trees to healthy oaks during the spring months in Minnesota. With some extrapolation and interpretation of data presented by Norris (1956), this conclusion also may be applicable to northern Iowa. Given this knowledge of principal vector species, and with the recent commercialization (Great Lakes IPM, Vestaburg, MI) of the aggregation pheromones discovered for each of these species (Cosse and Bartelt 2000, Bartelt et al. 2004), seasonal monitoring of *Co. truncatus* and *Ca. sayi* occurrence in oak wilt stands each spring and summer may be a useful tool in annually defining the period of wound prevention and wound treatment guidelines as part of oak wilt management efforts.

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