Flight Periodicities, Phoresy Rates, and Levels of *Pseudopityophthorus minutissimus* Branch Colonization in Oak Wilt Centers

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Abstract: Oak bark beetles, *Pseudopityophthorus minutissimus* and *P. pruinosus,* are considered important vectors of the oak wilt fungus, *Ceratocystis fagacearum*, in Missouri and Ohio. However, the frequency of the species' association with diseased oaks in Minnesota and their relative importance in pathogen spread in the state are unclear. Window traps were placed in canopies of recently killed northern pin oaks to determine seasonal flight periodicities of dispersing oak bark beetles and their phoresy (pathogen presence) rates. Branch samples were collected from diseased northern pin and northern red oak canopies in May and Aug., life history data obtained, and oak bark beetles emerging from the branches assayed for the pathogen. Only *P. minutissimus* was found in the study areas. In 2003, peak flight of *P. minutissimus* in Minnesota occurred 12 May to 19 May, with 869 beetles being trapped. Dispersing beetles carried viable pathogen propagules at low frequencies (4 to 13 per 1,000) in May and June. Branches of oak wilt-killed trees were commonly colonized by the beetle. More beetles emerged from branch samples collected in May than in Aug., but none yielded *C. fagacearum*. These results support the hypothesis that the relative importance of *P. minutissimus* in the overland transmission of the pathogen in red oak species in Minnesota is minor. FOR. SCI. 52(3):243–250.

Key Words: Oak bark beetles, *Quercus ellipsoidalis*, *Quercus rubra*, *Ceratocystis fagacearum*.

OAK WILT, caused by the fungus *Ceratocystis fagacearum* (Bretz) (Hunt), is a vascular wilt disease that affects over 33 species of oak (*Quercus* sp.) (Tainter and Baker 1996) and kills thousands of trees annually in the north central United States. Oaks are a significant forest and shade tree resource in the Midwest, especially for urban landscapes. Thus, unexpected mortality of oaks results in serious consequences for the forest products industry, wildlife, and homeowner property values (Juzwik and Schmidt 2000).

Oak wilt spreads in two main ways: underground via functional root grafts, and overland via insect vectors (Gibbs and French 1980). Several groups of insects, such as sap beetles (Coleoptera: Nitidulidae) and bark and ambrosia beetles (Coleoptera: Scolytidae) have been implicated as vectors of *C. fagacearum* (Dorsey et al. 1953, Wood and Skelly 1968). Sap beetles have historically been considered the primary vectors of the pathogen in Minnesota (French 1995) and oak wilt control efforts for preventing overland spread are based on this premise. However, little direct evidence exists on the relative importance of oak bark beetles as vectors in the state. In contrast, oak bark beetles (*Pseudopityophthorus minutissimus* Zimm. and *P. pruinosus* Eichhoff) have historically been considered the primary vectors of *C. fagacearum* in Missouri and Ohio based on results of several studies (Buchanan 1958a, b, Rexrode and Jones 1970). Sap beetles were considered insignificant in Missouri because *C. fagacearum* sporulating mats were seldom observed in nature by early researchers (Rexrode and Jones 1970).

Basic information on *Pseudopityophthorus* species' association with *C. fagacearum* and oak wilt-killed trees in Minnesota is lacking. Data on timing and frequency of beetles colonizing diseased trees, dispersing from oak wilt-affected stands, and visiting suitable infection courts on healthy oaks as well as phoresy rates, i.e., frequencies of *C. fagacearum* contamination, rates for beetles in each of these situations is needed. Our objectives with this research were to (1) determine flight periodicities of *Pseudopityophthorus* species in stands affected by oak wilt during the growing season, (2) determine *C. fagacearum* contamination frequencies for dispersing oak bark beetles in such stands, and (3) quantify and characterize colonizing adult *Pseudopityophthorus* species in colonized branches of oaks recently killed by the pathogen. The studies were conducted with two red oak (Erythrobalanus) species, northern pin oak (*Quercus ellipsoidalis* E.J. Hill), and northern red oak (*Q. rubra* L.).

Materials and Methods

Trapping of Dispersing Beetles

Dispersing *Pseudopityophthorus* species were sampled using nonbaited window traps. Each trap consisted of a

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20 × 28 cm piece of Plexiglas attached to the top of an inverted plastic milk carton (3.8 l). The opening of the carton led to a collection cup (250 ml, plastic). The bottom of the cup was replaced with fine mesh screen to allow for water drainage. A crumpled paper towel was placed in each cup. Two traps were placed in the canopy of each of three northern pin oaks that had wilted mid- to late-summer of the previous year in four different oak wilt disease centers. The centers were 1 to 4 km apart, located in the Carlos Avery Wildlife Management Area east and west of Stacy, Washington County, MN. A professional tree climber installed traps on pulley systems for easy trap access. Collection cups were emptied weekly between 17 Apr. and 22 Sept., 2003. Collections from a tree's two traps were combined to constitute one sample per tree per week. Cups were cleaned with 70% EtOH, and new paper towel inserted each week. The species and sex of each trapped oak bark beetle were determined and the beetles were stored in groups of five in microcentrifuge tubes at −2°C.

Fungal Isolations from Dispersing Beetles

Serial dilution plating procedures were used to detect viable *C. fagacearum* propagules on the beetles (Juzwik et al. 2004). Sterilized water (0.5 ml) was added to each microcentrifuge tube containing five adult beetles, and the suspension subjected to sonication (5 seconds) using a tip sonicator (Ultrasonic Homogenizer, Cole-Parmer, Vernon Hills, IL) to dislodge fungal propagules present on the exoskeletons and in the gut of each insect. The resulting solution was then serially diluted (3×) and 0.5 ml aliquots of each dilution spread onto lactic acid-amended potato dextrose agar plates. Three replicate plates were used per dilution. The plates were placed in an incubator (dark conditions, 24°C) for 10 days (Cease and Juzwik 2001). The number of *C. fagacearum* colonies were then determined and recorded. Identification of the fungi was based on morphology and presence of characteristic endoconidia (Barnett 1953). All beetles were processed within 4 months of capture.

Observation of Beetles in Colonized Slash

Observational data were gathered on the life stages of oak bark beetles present in branches from *C. fagacearum*-killed *Q. ellipsoidalis* and *Q. rubra* piled on the ground (=slash) in various oak wilt centers near Stacy, Washington County, MN, in Carlos Avery Wildlife Management Area, and in Blaine, Anoka County, MN, on the Metropolitan Airport Commission property. Observations of the slash piles were made on 10 Apr., 8 May, 16 May, 21 May, 18 June, 8 July, 15 July, 21 July, 4 Aug., 11 Aug., and 25 Aug., 2003. Branches within each pile were closely examined to detect insect presence. The bark of branches exhibiting holes characteristic of *Pseudopityophthorus* species was carefully peeled back using a knife. Data on the presence of larvae, pupae, and adults (including tenerals) were recorded.

Sampling of Colonized Branches in Tree Crowns

Branches were inspected and samples taken from crowns of oak wilt-killed northern pin and northern red oaks to determine presence and abundance of colonization by oak bark beetles and to obtain emerging adults. The trees were located in several oak wilt centers from 1 to 4 km apart near Stacy, Washington County, MN, in the Carlos Avery Wildlife Management Area and in Blaine, Anoka County, MN, on the Metropolitan Airport Commission property. These activities occurred before anticipated beetle emergence in the spring (May) and mid-summer (Aug.), 2003.

Branches in the crowns of eight trees exhibiting evidence of oak bark beetle colonization were sampled on 8 and 12 May. The trees had died of oak wilt during the previous summer. The sampled trees were not evenly distributed among the sites; thus, the number of branches collected varied by site. Trees used for the Aug. sampling had exhibited wilting symptoms in late summer 2002, but did not completely wilt until early summer 2003. These trees were felled in late June to facilitate sampling. Branches from six such trees were sampled between 1 and 13 Aug. 2003. Numbers of branches collected varied by site for the reason previously stated.

Each sampled branch was cut into subsamples, placed in polyethylene bags, and stored at 3°C in the lab until processed. A subset of this collection was used to determine diameter (cm) and length (cm) for each sampled branch. The number of emergence or exit holes in a selected 15 cm^2 area of bark was also determined. The bark in that area was then removed and the number of galleries counted. The presence of larvae, pupae, and/or adults was noted.

The remaining branch subsamples were placed in emergence boxes. Four boxes, constructed per Browne (1972), were used. Collection vials associated with each box were emptied weekly until no further adult emergence occurred. The collected beetles were counted, identified to sex, and stored in groups of five in microcentrifuge tubes at −2°C. Serial dilution plating techniques (previously described) were used to assay emerged beetles for presence of *C. fagacearum*. All beetles were stored for no longer than 3 months before processing.

Statistical Analyses

Numbers of adult oak bark beetles obtained from canopy window traps were analyzed using Poisson regression in the program ARC (Cook and Weisberg 1999). We assumed the counts conformed to a Poisson distribution. The full model tested counts of beetles as the response, with site, tree, week, and all two-way interactions as the terms or predictors. The most parsimonious model to fit the data was obtained through backward elimination. The significance of each factor was interpreted with the Wald χ^2 test, where a high *P*-value suggests the term is not needed in the model.

The minimum infestation rate (*f*) of *C. fagacearum* per group of dispersing beetles was calculated for each month

according to the equation, $f = x/(km)$, where x is the total number of positive groups, k is the number of beetles per pool (group), and m is the number of pools tested. The standard error is calculated as $\sqrt{(1-f)/(km)}$. This statistic estimated the frequency of individuals carrying the fungus per 1,000 (Venette et al. 2002).

Presence or absence of larvae, pupae, and adults in the branch samples taken from the canopies of oak wilt-infected trees were analyzed using binomial regression in ARC (Cook and Weisberg 1999). Samples were grouped by season (May = spring, Aug. = summer) and were analyzed separately by site (oak wilt center). The full model tested presence and absence of larvae, pupae, adults, or any combination of the three (=infestation) as the response and diameter, site, and the two-way interaction as the predictors. Backward elimination was used to obtain the most parsimonious model (Cook and Weisberg 1999).

Differences in branch sample characteristics (diameters of subsamples) and colonization characteristics (number of entrance and exit holes per unit area, number of galleries per unit area) between the oak wilt sites were analyzed using a SAS completely randomized design PROC GLM (SAS Institute 1996). Seasonal samples were analyzed separately by site. Means were separated using the Ryan-Eniout-Gabriel-Welsch Multiple Range for the response variable at $P < 0.05$ (SAS Institute 1996).

Results

Dispersing Beetles

Window flight traps yielded 1,852 adult *P. minutissimus* during the trapping period, 17 Apr. to 22 Sept., 2003 (Figure 1A); no other *Pseudopityophthorus* species were captured. The overall sex ratio was 1.5:1.0 (1,100 females:752 males). The highest numbers of beetles were trapped during two weeks in May: 869 (534 females:335 males) between 12 May and 19 May, and 468 (280 females:188 males) between 19 May and 26 May (Figure 1A). Of all beetles caught in 2003, 72% were obtained during these two consecutive weeks; furthermore, 86% were trapped between 1 May and 5 June. Week of collection was the significant factor affecting bark beetle abundance in the flight traps during the trapping period, based on results of Poisson regression ($P < 0.0004$) (Table 1). Site and interactions between site and week were not significant ($P > 0.7$).

Ceratocystis fagacearum was isolated from groups of oak bark beetles caught in May and June (Table 2). For beetles collected in May, 2.1% of the 280 assayed groups yielded the fungus (Figure 1B). These beetles were obtained on either 19 May (4 positive groups of 175) or 26 May (2 positive groups of 95) from traps in the same tree on one site. The number of colony-forming units (CFU) for the positive groups ranged from 100 to 3,300 per group. The minimum *C. fagacearum* infestation rate for May was 0.004, i.e., 4 in 1,000 beetles were carrying the fungus in May was 4 in 1,000 beetles. For beetles collected in June, 6.6% of the 120 assayed groups yielded *C. fagacearum*. These beetles were collected on either 2 June (6 positive groups of 38) or 16 June (2 positive groups of 10) from single trees in site 3 and site 1, respectively. The number of viable pathogen propagules for the positive groups ranged from 33 to 530 CFU/group. The minimum infestation rate for June was 0.013, i.e., 13 beetles in 1,000 were carrying the fungus. *C. fagacearum* was not isolated from 15 groups of beetles collected in July, or one group collected in Sept.

Life Stages in Colonized Branches

Larvae were found in branches of oak wilt-killed trees in slash piles during inspections in early to mid-spring (10 Apr. and 8 May) and in mid-summer (15 July through 25 Aug.) (Figure 1C). Pupae were present from early summer (30 June) through mid-summer (25 Aug.). Adults *P. minutissimus* (including tenerals) were observed in the same material in mid-spring (28 Apr. to 2 June) and in mid-summer (4 Aug. to 25 Aug.). No other *Pseudopityophthorus* species were found.

In general, only adult *P. minutissimus* were observed in branches sampled in mid-May from standing oak wilt-killed trees (Table 3) and no *P. pruinosus* were found. The average numbers of entrance or exit holes found on these branches ranged from 2.0 to 6.4 holes per 15 cm^2 bark area. The average number of parent galleries in the same bark area ranged from 1.3 to 3.8. Both measures of beetle colonization differed by site of branch collection (Table 3). All life stages were observed in branches sampled in Aug. from standing oak wilt-killed trees in three of six sites (Table 3). The average number of entrance or exit holes found on these branches ranged from 4.2 to 7.5 per 15 cm^2 bark area, and no differences were found by study site. The average number of oak bark beetle parent galleries in the same bark area ranged from 4.2 to 12 (Table 3). Based on binomial regressions of the May and Aug. data, neither site nor diameter, nor the interaction, were significant factors in explaining the presence of larvae, pupae, adults, or a combination of any of the three in oak wilt-killed branches ($P > 0.8$).

Number of adult *P. minutissimus* that emerged from sampled branches ranged from 12 to 2,283 for the May collection, and from 0 to 3,453 for the Aug. (Table 4); no other *Pseudopityophthorus* species were found. The sex ratio of emerging adults was 1.4:1.0 (4,540 females:3,336 males). The length of time over which beetles emerged from the branch samples differed by sampling period. For the May sample, adults emerged from branches collected from all sites within a 2 week period (9 May to 23 May). Branches from all but one of the sample sites in Aug. yielded adult beetles, but emergence occurred over a much longer period (11 Aug. to 10 Oct.) than in the spring. *C. fagacearum* was not isolated from any of the 365 groups (191 female-only; 172 male-only; 1 mixed sex) of emerged beetles assayed.

Discussion

The only oak bark beetle species found in our study was *P. minutissimus*. This is consistent with a previous investigation in Wisconsin where *P. minutissimus* was the only

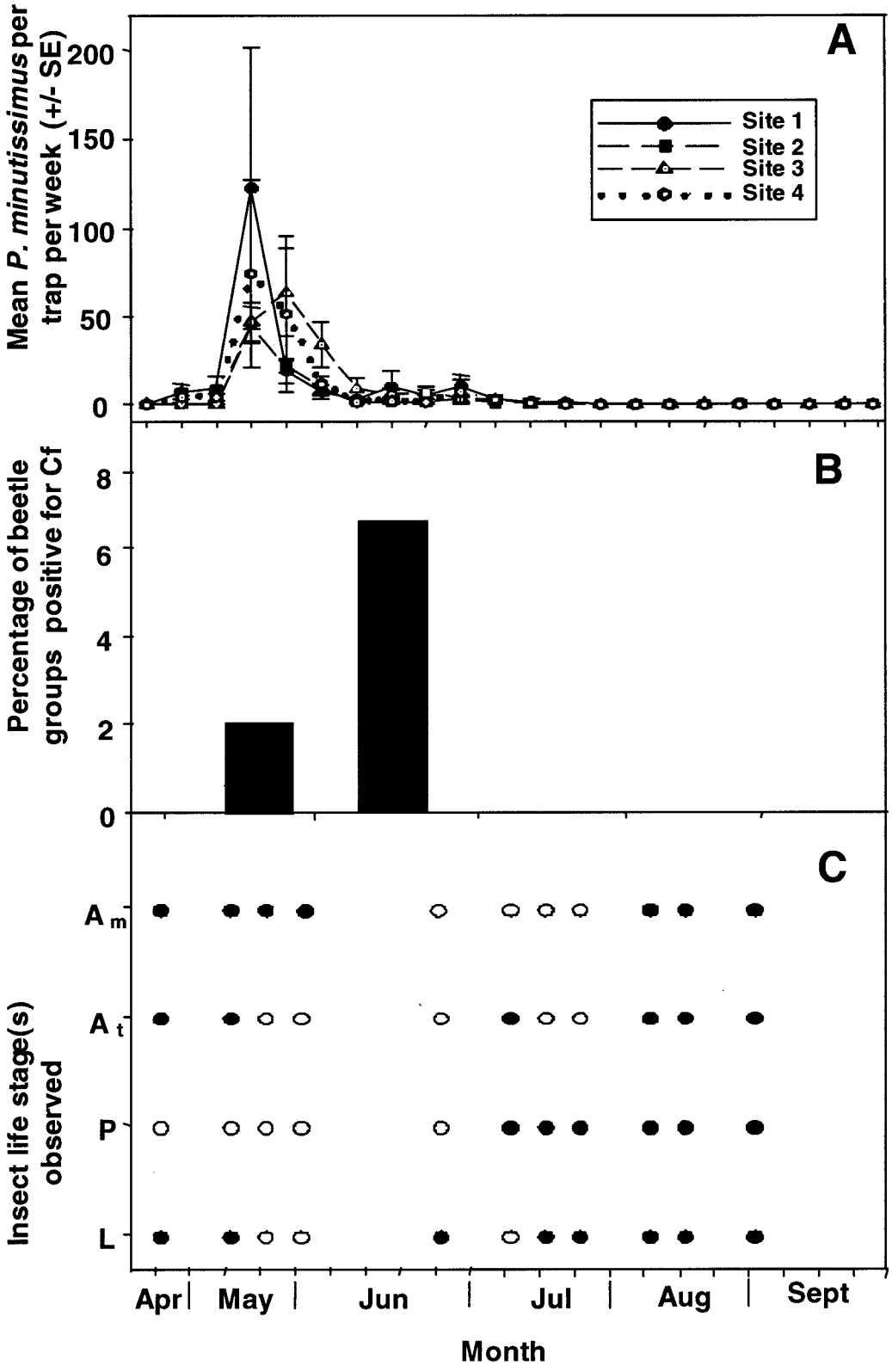

Figure 1. (A) Mean *Pseudopityophthorus minutissimus* per pooled trap (two traps per tree, combined in the field) per week by site +/ − SE. Insects were collected from canopy window traps in *Ceratocystis fagacearum*-killed northern pin oaks from 28 Apr. to 22 Sept. 2003 in east central Minnesota. (B) Percentages of groups of *P. minutissimus* (five/group) carrying *C. fagacearum* (Cf) based on serial dilution plating assays. Adult insects tested were those collected in the canopy window traps from Figure 1A. (C) Life stages of *P. minutissimus* observed in branches of *C. fagacearum*-killed northern pin and northern red oaks in slash piles. ●, presence of life stage; ○, absence of life stage. A_m = mature adult, A_t = teneral adult, P = pupae, and L = larvae.

Table 1. Poisson regression model for numbers of adult *Pseudopityophthorus minutissimus* caught in canopy window flight traps placed in crowns of *Ceratocystis fagacearum*-killed northern pin oaks

Variable	Estimate	SE	Wald χ^2	*P*-value*
Constant	−1.39	0.58	−2.40	<0.01
Week				
2 (125)†	2.46	0.60	4.08	<0.0001
3 (132)	2.59	0.60	4.33	<0.0001
4 (139)	5.67	0.58	9.80	<0.0001
5 (147)	5.05	0.58	8.72	<0.0001
6 (153)	4.12	0.58	7.07	<0.0001
7 (160)	2.61	0.60	4.33	<0.0001
8 (167)	2.94	0.59	4.97	<0.0001
9 (174)	2.61	0.60	4.33	<0.0001
10 (181)	3.16	0.60	5.37	<0.0001
11 (188)	2.16	0.61	3.54	<0.0004

* *P*-value reflects statistical difference between each stated level (of week) and week 1.
† Julian (date) corresponds to the last day or collection day of the week.

Table 2. Frequency of *Ceratocystis fagacearum* (Cf) isolation from groups of *Pseudopityophthorus minutissimus* caught in canopy window flight traps placed in crowns of *Ceratocystis fagacearum*-killed northern pin oaks

Month of collection	No. groups assayed*			No. groups yielding Cf	Minimum infestation rate ± SE†	Mean no. CFU per positive group ($\times 10^2$) ± SE‡
	Male	Female	Mixed			
May	95	168	17	6	0.004 ± 0.001	16.0 ± 6.5
June	52	56	12	8	0.013 ± 0.04	3.7 ± 1.0
July	4	9	2	0	—	—§
August	0	0	0	0	—	—
September	1	0	0	0	—	—

* Beetles were assayed in groups of five according to sex. If numbers of one sex from a site were insufficient to make a group, the sexes were combined (mixed).
† Minimum infestation rate based on group testing (see Methods).
‡ CFU = colony-forming unit of *C. fagacearum*.
§ Calculation not applicable.

Table 3. Characteristics of *Pseudopityophthorus minutissimus* branch colonization for *Ceratocystis fagacearum*-killed northern pin and northern red oaks

Sample collection			Branch sample diameter (cm)		Colonization measure: No. holes/15 cm^2 bark		Colonization measure: No. galleries/15 cm^2 bark		Frequency by life stage†		
Time	Site	No. branches	Mean	SE	Mean	SE	Mean	SE	Larvae	Pupae	Adults
Spring	1	8	2.0 B‡	0.18	6.4 A	1.1	2.9 A	0.7	0	0	0.9
(May)	2	11	1.9 B	0.17	2.0 B	0.4	1.3 B	0.5	0	0	0.9
	3	16	2.9 A	0.19	5.8 A	0.7	3.8 A	0.3	0	0	1.0
	5	10	1.8 B	0.14	5.9 A	0.96	2.6 AB	0.3	0.1	0	0.9
Summer	1	11	2.3 b	0.28	4.9 a	0.65	5.7 b	1.2	0.2	0.2	9.7
(August)	6	4	2.1 b	0.56	4.3 a	0.75	4.3 b	1.3	0.3	0.5	0.5
	7	8	2.8 b	0.51	4.4 a	0.46	6.0 b	1.2	0.1	0.9	0.9
	8	3	3.0 b	0.16	7.5 a	1.0	5.5 b	0.9	0.7	0	0.7
	9	2	19.4 a	17.9	6.5 a	2.5	12.0 a	6.5	0	0.5	1.0

Branches sampled from standing and felled trees killed by the pathogen.
† Proportion of the samples that contained larvae, pupae, and adults.
‡ Within columns values with different letters are significantly different ($P < 0.05$). Capital letters are used to designate significance among the spring sampling and lower case letters are used for the summer sampling.

Pseudopityophthorus species collected from wilting *Q. rubra*, *Q. velutina* Lamarck, and *Q. ellipsoidalis* between Apr. and Nov. (McMullen et al. 1955).

The highest numbers of dispersing *P. minutissimus* were found the third and fourth week of May in 2003. To our knowledge, this is the first report on dispersal of the small oak bark beetle based on window flight trap collections. The time and duration of this peak were consistent with emergence data for the species in other states (McMullen et al. 1955, Rexrode 1969). In Wisconsin, McMullen et al. (1955) reported maturing larvae on May 1, onset of emergence approximately 1 week later, and duration of emergence as 2 weeks. In Ohio, emergence also occurred over 2 weeks, but began the end of Apr. (Rexrode 1969).

Table 4. Number of *Pseudopityophthorus minutissimus* emerging from branches of *Ceratocystis fagacearum*-killed northern pin and northern red oaks

Sample collection			No. emerged adults	
Time	Site	No. branches	Males	Females
Spring (May)	1	8	357	478
	2	11	3	9
	3	16	944	1339
	5	10	160	163
Summer (August)	1	11	1464	1989
	6	4	29	24
	7	8	222	336
	8	3	0	0
	9	2	97	129

Branches sampled from standing and felled trees killed by the pathogen.

A second peak in flight activity was expected for late July through early Sept. based on published emergence studies (McMullen et al. 1955, Rexrode 1969) and the large numbers of beetles emerging from branches collected from tree crowns in Aug.; however, no beetles were trapped during this time period in 2003. Interestingly, Ambourn and Seybold (2002, Naturally produced attractants of the small oak bark beetle, a potentially important vector of the oak wilt fungus in Minnesota, *in* Forest insect and disease newsletter, 18 November, 2002. Minn. Dept. of Nat. Res., St. Paul, MN. Available online at http://www.dnr.state.us/fid/index.html; accessed 25 October 2005) also failed to detect a mid- to late-summer peak in a pheromone-baited, sticky-trap study in Roseville, MN in an urban oak stand with no oak wilt. We conducted additional window trapping in 2004 in four oak wilt-affected stands near our 2003 sites. In this case, traps were placed in crowns of northern pin oaks that had wilted in June 2004. Only 47 beetles were obtained from 12 traps between 20 July and 7 Sept. We are unable to explain why summer-emerging beetles were not caught (2003) or infrequently caught (2004) in our window flight traps.

Abundant *P. minutissimus* were found to colonize red oaks dying from *C. fagacearum* infection and large numbers of beetles emerge from such trees in Minnesota. In contrast, Gibbs (1980) reported that no adults emerged during May from colonized branches of *C. fagacearum*-infected trees that had wilted in July the previous year in Minnesota, although dead larvae were found in the galleries when branches were dissected. The author concluded that very few oak wilt branches were being used for *P. minutissimus* breeding. Results of this study, however, agree with reports from Missouri, where colonization and emergence were found to be common (Buchanan 1958b). Over 100,000 beetles emerged from colonized, naturally infected oaks in the Missouri study and emerged beetles were used in successful transmission studies.

The frequency of standing oak wilt-killed trees successfully attacked by *P. minutissimus* in Missouri and Ohio ranges from 11 to 50%, with intensity of attack either light or medium, i.e., from 1 to 35 galleries per linear foot of branches (Rexrode 1967, Rexrode and Jones 1972). Our observations in Minnesota are similar. Based on our branch sampling efforts, 40% ($n = 20$) and 33% ($n = 18$) of recently killed red oaks examined in May and Aug., respectively, showed evidence of oak bark beetle activity. Interestingly, the frequency of standing oak wilt-killed red oaks successfully attacked by *P. minutissimus* is less than that on girdled oaks (either *C. fagacearum*-infected or not) (Rexrode 1967, Rexrode and Jones 1972). Adults were the predominant life stages present in branch samples in May, and this coincided with high emergence numbers, flight of adults, and observations from slash. In Aug., however, the frequency of life stages present was much more variable, with all stages being present in slash and in sampled branches. This is probably due to the overlap of generations caused by female re-emergence after the first egg laying (Faccoli and Battisti 1997).

The oak wilt fungus was detected at low levels on dispersing beetles in tree canopies within oak wilt centers, but not on beetles emerging from branches collected in May or Aug. from crowns of *C. fagacearum*-killed trees. The latter results are contrary to previous reports from other states. In Missouri, *P. minutissimus* were found to carry the pathogen after over-wintering and emerging in spring from oaks that had wilted the previous June and from adults emerging from diseased branches collected in Aug. and Sept. (Berry and Bretz 1966, Rexrode and Jones 1970). Rexrode and Jones (1971) reported that up to 33% of the beetles emerging from oak wilt-killed trees in West Virginia were carrying viable pathogen propagules.

We suggest three possible reasons for the lack of isolation of *C. fagacearum* from emerging beetles. First, the adults may have simply failed to acquire viable fungi propagules from the infested branches before emergence. Mycelium, or spores, have not been observed in oak bark beetle galleries, so the mechanism for pathogen propagule acquisition by beetles is not clear (Gibbs and French 1980). Second, the pathogen may have been present at one point in the colonized branches, but was no longer viable. Gibbs (1980) found the longest survival of *C. fagacearum* in small branches, where the small oak bark beetle breeds in trees that wilt in late summer. If the trees wilted earlier than late summer 2002, we would not expect to isolate the fungus. Third, the beetles may have acquired pathogen propagules, but their viability was lost before the laboratory assay. Loss of propagule viability could be related to length of time the infested beetles were in the emergence apparatus or in cool storage before processing. Environmental conditions in the emergence boxes (occasionally 28–30°C) or in the walk-in storage cooler (4°C with 60–80% RH) may have caused propagule death or promoted growth of competing fungi. Samples were maintained no longer than 4 weeks in cold storage before being placed in emergence boxes. Although branch samples may have remained in the emergence boxes for up to 3 weeks, the beetles that emerged were trapped in a collection jar in a refrigerated unit (5°C). Gibbs (1980)

Table 5. Comparison of estimated *Ceratocystis fagacearum*-contaminated insect populations in oak wilt-affected stands of northern pin or northern red oaks

	Oak bark beetle *Pseudopityophthorus minutissimus*		Sap beetles			
			Colopterus truncatus		*Carpophilus sayi*	
Month	N[†]	MIR[‡]	N	CIP[‡]	N	CIP
May	280	0.004	659	0.235	29	0.172
June	120	0.013	65	0.323	14	0.786
July	15	0	21	0.333	24	0.042

Bark beetles captured using nonbaited window flight traps; sap beetles captured in pheromone-baited funnel traps (per Ambourn et al. 2005).

[†] Bark beetles assayed in groups of five; sap beetles assayed singly.

[‡] MIR = minimum infestation rate (see Methods); CIP = contaminated insect population (see Methods in Ambourn et al. 2005).

suggested *Coryneum kunzei* Corda and *Dothiorella quercina* Cke. & Ell. were important in preventing *C. fagacearum* from invading the inner bark of small branches of infected trees. These fungi were found to be the most common colonizers of wood and bark of branches on oaks that had succumbed to the pathogen in the previous summer.

Historically, sap beetles have been considered the primary vectors of *C. fagacearum* in Minnesota (French 1995). Comparison of fungal contamination rates for this group to rates for *P. minutissimus* is useful when considering the relative importance between sap and oak bark beetles as oak wilt vectors. In a separate study (Ambourn et al. 2005), data were collected on dispersal and pathogen contamination rates of two sap beetle species during the same year and months from two similar stands with oak wilt. The stands were 4 and 10 km from the nearest *P. minutissimus* dispersal site of our study. The sap beetles, *Colopterus truncatus* Randall and *Carpophilus sayi* Parsons, were captured using pheromone-baited, wind-oriented funnel traps (Ambourn et al. 2005). The contamination rates were much higher for the two sap beetle species than for dispersing *P. minutissimus* captured using nonbaited window traps (Table 5).

In summary, comparison of *P. minutissimus* associated with oak wilt-killed trees in Missouri and Ohio to those in Minnesota found similarities in frequencies of trees colonized and in intensity of colonization. Ranges of numbers of *P. minutissimus* emerging from oak wilt-killed trees are also comparable; however, isolation of *C. fagacearum* from adults emerging from colonized branches differs, i.e., 0% in Minnesota versus 22 to 30% in Missouri (Bretz and Berry 1966). However, *P. minutissimus* dispersing in oak wilt centers in Minnesota do carry *C. fagacearum* on their bodies, albeit at a low level. In conclusion, we believe these study results support the hypothesis that *P. minutissimus* is of minor importance in the overland spread of oak wilt in Minnesota. A future study could compare frequencies of pathogen-contaminated beetles feeding in twig crotches, leaf petioles, and on small branches of healthy oaks (i.e., the inoculum court) in Minnesota, Missouri, and Ohio. Feeding beetles have been collected from these parts of healthy oaks in past studies (Rexrode and Jones 1972), but assays for the fungi were apparently not conducted. Findings of such studies would be a good measure of whether the vector's importance in oak wilt spread varies by region.

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