Predominant nitidulid species (Coleoptera: Nitidulidae) associated with spring oak wilt mats in Minnesota[1]

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Abstract: Nitidulids are primary vectors of the oak wilt pathogen, *Ceratocystis fagacearum* (Bretz) Hunt, in the north-central United States. Species of adult nitidulids associated with different ages of oak wilt fungus mats on red oaks (*Quercus rubra* L. and *Quercus ellipsoidalis* E.J. Hill) during spring in east-central Minnesota were determined. The exoskeletal surfaces of representative specimens were assayed for the presence and abundance of the pathogen. Six species comprised 94% of 2542 adults, representing at least 12 species, collected between 1994 and 1996. Of these six species, *Colopterus truncatus* Randall and *Epuraea corticina* Erichson were the most abundant ones on immature mats (94% of 154 nitidulids). They were also more abundant than the other species on mature mats (77% of 868 nitidulids). *Carpophilus sayi* Parsons was the most common species (28% of 1134 nitidulids) on aging mats, while *Carpophilus sayi* and three *Glischrochilus* species (*Glischrochilus sanguinolentus* (Oliv.), *Glischrochilus fasciatus* (Oliv.), and *Glischrochilus quadrisignatus* Say) were predominant on declining mats (80% of 214 nitidulids). Multiple numbers of individuals of each species were commonly found on the mats. The smaller bodied species, *C. truncatus* and *E. corticina*, had the lowest numbers of fungal propagules on their bodies, while the highest incidence and numbers of viable propagules were found for the three largest bodied species (*Glischrochilus* spp.). These results are important to elucidating the principal nitidulid species involved in successful transmission of the pathogen in Minnesota.

Résumé : Les nitidules sont les principaux vecteurs du pathogène responsable de la flétrissure du chêne, *Ceratocystis fagacearum* (Bretz) Hunt, dans le centre-nord des États-Unis. Les espèces de nitidules adultes associées au feutre mycélien du champignon de différents âges ont été identifiées sur des chênes rouges (*Quercus rubra* L. et *Quercus ellipsoidalis* E.J. Hill) au printemps dans le centre-est du Minnesota. La surface de l'exosquelette de spécimens représentatifs a été testée pour la présence et l'abondance du pathogène. Six espèces regroupent 94% des 2542 adultes qui comptent au moins 12 espèces, collectionnés entre 1994 et 1996. De ces six espèces, *Colopterus truncatus* Randall et *Epuraea corticina* Erichson étaient les plus abondantes sur les feutres mycéliens immatures (94% des 154 nitidules). Elles étaient aussi plus abondantes que les autres espèces sur les feutres mycéliens matures (77% des 868 nitidules). *Carpophilus sayi* Parsons était l'espèce la plus commune (28% des 1134 nitidules) sur les vieux feutres mycéliens tandis que *C. sayi* et trois espèces de *Glischrochilus* (*Glischrochilus sanguinolentus* (Oliv.), *Glischrochilus fasciatus* (Oliv.) et *Glischrochilus quadrisignatus* Say) étaient prédominantes (80% des 214 nitidules) sur les feutres mycéliens dépérissants. Plusieurs individus de chaque espèce étaient communément retrouvés sur les feutres mycéliens. Les plus faibles quantités de propagules fongiques ont été retrouvées sur le corps des espèces les plus petites, *C. truncatus* et *E. corticina*, tandis que la plus forte incidence et les quantités les plus élevées de propagules ont été observées sur le corps des trois espèces les plus volumineuses (*Glischrochilus* spp.). Ces résultats sont importants pour identifier les principales espèces de nitidules responsables de la transmission du pathogène au Minnesota.

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Introduction

Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, is an important disease of oaks (*Quercus* spp.) in the eastern United States. The disease occurs in 22 states (USDA Forest Service 2000) and is considered the most important forest disease problem in Illinois, Iowa, Minnesota, Texas, and Wisconsin (Billings 2000). Although species of both the red (section *Lobatae*) and white oak (section *Quercus*) groups are affected, the disease is most serious in the more highly susceptible red and black oaks and in live oak (Tainter and Baker 1996). The pathogen causes mortality of thousands of native oaks annually across the midwestern United States in urban and natural forests.

The pathogen is transmitted underground from diseased to healthy trees through root grafts or overland by insect vectors (Gibbs and French 1980). While a large proportion of oaks contract the disease via root grafts each year, insect transmission is significant as the means for establishment of new infection centers in adjacent as well as distant forest stands. Nitidulids (Coleoptera: Nitidulidae), or sap beetles, are considered primary vectors of the fungus in the north-

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central states of the United States (Gibbs 1984; French 1995). These beetles are attracted to *Ceratocystis fagacearum* sporulation mats that form on recently killed oaks, primarily red and black oak species. After crawling over, burrowing into, and feeding on the mats, the insects leave with pathogen spores on and in their bodies (Juzwik and French 1983) and may transmit the pathogen to fresh wounds on healthy oaks. Oak wilt control programs in Minnesota often include measures to prevent acquisition of inoculum from oak wilt fungus mats by nitidulids and reduce or eliminate the occurrence of fresh wounds on healthy oaks during the critical spring period (French 1995).

Numerous nitidulid species have been appropriately implicated as vectors of *Ceratocystis fagacearum* based on the four requirements established by Leach (1940). These implicated species and the mat-associated species vary between regions as well as within regions of the eastern United States (Merrill and French 1995). This variability plus the relatively inefficient manner in which *Ceratocystis fagacearum* is transmitted by these insects have led to broad generalizations about the Nitidulidae as oak wilt vectors (J. Juzwik, unpublished data). Although there is ample evidence supporting the adaptability and opportunistic nature of the numerous nitidulids associated with the oak wilt disease cycle, additional evidence suggests that some species have higher degrees of ecological specialization in this association than the others (Norris 1956).

We hypothesize that not all nitidulid species that have been implicated as vectors of *Ceratocystis fagacearum* are important in the transmission of the pathogen in nature. Specifically, we suspect that only a few species are responsible for the majority of overland spread of oak wilt that occurs during spring in Minnesota. We initiated a series of studies to test this hypothesis. Our first step has been to investigate the species of nitidulids associated with oak mats during spring.

Skalbeck (1976) summarized published reports on nitidulid species visiting oak wilt mats in Illinois (Curl 1955), Iowa (Norris 1956), West Virginia (Dorsey and Leach 1956; Jewell 1956), and Wisconsin (McMullen and Shenefelt 1961). Overall, 27 species were found on oak wilt mats, with eight of the species producing larvae on the mats. Additional nitidulid species have since been reported on oak wilt mats in Texas (Appel et al. 1986) and during spring and fall in Minnesota (Juzwik and French 1983; Juzwik and French 1986).

Few studies have documented the occurrence of nitidulid species by biological age of oak wilt mats while considering the sequence of species presence. Norris (1956) summarized monthly occurrence and abundance of nitidulid species found on mats from May through November over 1.5 years in northern Iowa. He also documented the succession of insect populations (nitidulids and numerous other groups of insects) in relation to mat age defined in terms of ascospore development. Curl (1955) reported on occurrence of different species of nitidulids present on different condition classes of oak wilt mats collected over a 10-month period in Illinois. Quantitative analyses, however, were not conducted with the data in either study.

Likewise, few studies have involved quantitative assessment of pathogen presence on nitidulids collected from oak wilt mats. The presence, abundance and viability of *Ceratocystis fagacearum* propagules vary by mat age (Curl 1955; Juzwik and Meyer 1997). Numbers of viable *Ceratocystis fagacearum* propagules on external surfaces were determined for spring-collected, mat-inhabiting nitidulids (Juzwik and French 1983), but ages of these mats were not determined. In a later study investigating the role of *Ophiostoma quercus* (Georgev.) Nannf. in biocontrol of *Ceratocystis fagacearum*, numbers of pathogen propagules were determined for nitidulids from control mats (Juzwik et al. 1998). However, results were not reported by individual species.

Results of our investigation of nitidulid species associated with spring oak wilt mats over a 3-year period in east-central Minnesota are reported here. The specific objectives of this study were to determine (*i*) the frequencies of incidence and abundance of adult nitidulid species in relation to age of spring oak wilt mats and (*ii*) the frequencies of incidence and numbers of viable *Ceratocystis fagacearum* propagules on the insects' bodies. A preliminary abstract has been published (Cease et al. 1997).

Materials and methods

Study sites and tree selection

Two study areas, including northern and southern sites in the Minneapolis – St. Paul metropolitan region of east-central Minnesota, were selected based on presence of *C. fagacearum*-infected red oak species (*Quercus rubra* L. and *Quercus ellipsoidalis* E.J. Hill). Wilted oaks were selected in July and August of the year preceding sample collection within identified oak wilt infection centers in these areas. The trees were monitored for oak wilt mat production beginning in mid-April of the spring following the year of wilting. Mat production generally increased significantly in mid-May. Nitidulids were then collected from oak wilt mats during the peak sporulation period (i.e., middle to late May) in the two areas. In the northern area, insect collections were made during May 1994 and 1995 from scattered properties in the city of Blaine, and during May 1995 and 1996 from various properties within Ham Lake and Columbus Township. In the southern area, collections were made from scattered sites within a park reserve near Burnsville and on a private property in Savage during May 1994 and 1995.

Nitidulid collection and processing

A hatchet and mallet were used to expose recently formed mats associated with visibly open cracks in the bark. Immediately after a mat was exposed, any adult nitidulids present on the xylem or the phloem portions of the mat were captured and placed in individual gelatin capsules. All the collected insects from a mat were placed in a polyethylene bag and stored at –2°C until processed in the laboratory. The biological age of each mat was determined according to Curl's (1955) definition of immature, mature, aging, and declining mats. Collection site, tree number, mat number, and mat age were recorded for each collected beetle. All nitidulids collected were identified to species (confirmed by T.C. Skalbeck, Department of Entomology, University of Minnesota).

The presence and number of viable *Ceratocystis fagacearum* propagules on the nitidulids were determined by serial dilution plating techniques. All beetles were processed within 9 months of collection. Each beetle was placed in 2.5 mL of sterile distilled water and agitated with a vortex mixer. The fungal propagules were then dislodged from the insect using a water bath sonicator for 15 min. The resulting suspension was then serially diluted (10× dilutions) and 0.5-mL aliquots of each dilution were spread onto lactic acid amended potato dextrose agar (PDA) plates. Each dilution

Table 1. Frequencies of incidence for six predominant species on different age-classes of oak wilt mats collected between 1994 and 1996 from two east-central Minnesota locations.

Location* and nitidulid species	Percent mats with >1 adult of the species present[†]				Statistical results[‡]
	Immature	Mature	Aging	Declining	
Northern					
Colopterus truncatus	90*a*[§]	59*a*	61*a*	43*a*	*y*
Epuraea corticina	48*b*	64*a*	34*b*	9*b*	*y*
Carpophilus sayi	10*c*	25*b*	37*b*	37*a*	*z*
Glischrochilus sanguinolentus	10*c*	29*b*	34*b*	34*a*	*z*
Glischrochilus fasciatus	—[¶]	17*b*	28*b*	34*a*	*z*
Glischrochilus quadrisignatus	—	4*c*	2*c*	6*b*	*z*
Southern					
Colopterus truncatus	83*a*	59*a*	32*ab*	20*b*	*y*
Epuraea corticina	3*b*	50*a*	20*bc*	6*b*	*y*
Carpophilus sayi	8*b*	26*b*	30*ab*	48*a*	*z*
Glischrochilus sanguinolentus	—	34*b*	35*a*	68*a*	*z*
Glischrochilus fasciatus	8*b*	1*c*	5*d*	12*b*	*z*
Glischrochilus quadrisignatus	8*b*	7*c*	14*c*	20*b*	*z*

*Frequencies of nitidulid incidence on mats differed by location for each species ($P < 0.01$); thus, data were not combined.

[†]Percentages are based on the following number of mats yielding any nitidulid. Northern locations: immature, 21; mature, 75; aging, 118; declining, 32. Southern locations: immature, 12; mature, 76; aging, 111; declining, 25. Mat age-classes are based on Curl (1955).

[‡]Distributions followed by different letters are significantly different ($P < 0.05$). Letters show the results of singly ordered Kruskal–Wallis analyses (Mehta and Patel 1995) comparing distributions for each species across mat age-classes.

[§]Values followed by different letters within each column for a location are significantly different ($P < 0.05$). Letters show the results of the unordered likelihood ratio test (Agresti 1996) comparing the number of mats yielding a particular species versus the number of mats yielding other nitidulid species within the mat age-class.

[¶]No adults of this species collected from the mat age-class.

was applied to three plates and the number of colonies of *Ceratocystis fagacearum* was counted after approximately 14 days. Colony appearance and asexual spore characteristics were used to identify *Ceratocystis fagacearum* colonies.

Data summarization and analyses

Numbers of nitidulids present on mats were summarized by species, mat age, and study area (northern vs. southern sites).

The analysis for nitidulid incidence on mats within mat age-classes was conducted with the unordered likelihood ratio test with calculation of exact Monte Carlo *P* values (Agresti 1996; Mehta and Patel 1995) comparing the number of mats yielding a particular species versus the number of mats yielding other nitidulid species within the mat age-class. Distributions for each species' incidence on mats were compared across mat age-classes using the singly ordered Kruskal–Wallis analyses (Mehta and Patel 1995). Nitidulid species abundance on mats was analyzed using the unordered likelihood ratio test with calculation of exact Monte Carlo *P* values. Comparisons were made among species using the number of individuals of any one species present on a particular mat age and the number of individuals of the same species absent on that particular mat age (i.e., present on mats of the other three age-classes). Distributions for each nitidulid species' occurrence on mats were compared across mat age-classes using the singly ordered Kruskal–Wallis analyses. Analysis of the frequency of *Ceratocystis fagacearum* isolation from nitidulid species within a mat age-class was conducted using the unordered likelihood ratio test with calculation of exact Monte Carlo *P* values based on the ratio numbers of beetles yielding the fungus compared with the number that did not. Data on aggregation of individuals of the same species on a mat were analyzed using the unordered likelihood ratio test with calculation of exact *P* values via Monte Carlo simulation. Quantitative data involving colony-forming units (CFU) of *Ceratocystis fagacearum* isolated from nitidulids were averaged for all beetles of each species obtained from a mat. Only values from *Ceratocystis fagacearum* positive nitidulids were used in calculating mean CFU. The means were transformed to the natural log and grouped by mat age and location. Analyses of variance (ANOVA) were performed on transformed means and mat age comparisons were made using Fisher's least significant difference test (Wilkinson 1992).

Results

Incidence of nitidulid species on mats

At least 12 species of nitidulids were found on 429 mats in the two study areas between 1994 and 1996. Six species comprised 94% of the total 2542 adult nitidulids collected from these mats. The predominant species were *Colopterus truncatus* Randall, *Epuraea corticina* Erichson, *Carpophilus sayi* Parsons, *Glischrochilus sanguinolentus* (Oliv.), *Glischrochilus fasciatus* (Oliv.), and *Glischrochilus quadrisignatus* (Say). The species accounting for the remaining 6% of the total collection included *Colopterus semitectus* Say, *Cryptarcha ampla* Erichson, *Glischrochilus siepmanni* Brown, *Phenolia grossa* (Fabricus), *Prometopia sexmaculata* Say, and other unidentified *Epuraea* spp.

Incidences based on presence–absence of each species on the mats differed by study area ($P < 0.01$) and the data were not combined. Different trends in frequencies of incidence for the six species across the four mat age-classes were found ($P < 0.05$, Table 1). The frequency of *Colopterus truncatus* incidence on mats decreased as mat age increased, with the highest frequency (≥83%) found for immature mats in both study areas. A unimodal trend was observed with *E. corticina*, with the highest frequencies found for mature mats in both locations and lower frequencies for all other mat age-classes. However, statistically the trend for *Colopterus*

Table 2. Frequencies of abundance for six predominant adult nitidulid species collected between 1994 and 1996 from different oak wilt mat age-classes in two east-central Minnesota locations.

Nitidulid species*	Total no. of individuals	Percent adult species abundance by mat age†				Statistical results‡
		Immature	Mature	Aging	Declining	
Colopterus truncatus N	508	36*a*§	19*c*	24*b*	9*b*	*v*
Colopterus truncatus S	355	31*a*	22*b*	9*c*	6*b*	*w*
Epuraea corticina	501	27*a*	36*a*	12*c*	5*b*	*x*
Carpophilus sayi	478	3*b*	9*e*	28*a*	35*a*	*y*
Glischrochilus sanguinolentus	346	2*b*	10*d*	17*b*	28*a*	*y*
Glischrochilus fasciatus	98	<1*b*	2*e*	5*b*	11*a*	*z*
Glischrochilus quadrisignatus	84	<1*b*	2*e*	5*b*	6*a*	*z*

*Frequencies of occurrence for nitidulid species on mats differed by location for only *Colopterus truncatus* ($P < 0.05$); thus, data were combined for all but this species. The letter following *Colopterus truncatus* indicates the northern (N) and the southern (S) collection locations.

†Based on the following number of mats within each mat age-class: immature, 34; mature, 151; aging, 188; declining, 57. Mat age-classes are based on Curl (1955).

‡Distributions followed by different letters are significantly different ($P < 0.05$). Letters show the results of singly ordered Kruskal–Wallis analyses (Mehta and Patel 1995) comparing distributions for each species across mat age-classes.

§Values followed by different letters within each column were significantly different ($P < 0.05$) using the unordered likelihood ratio test (Agresti 1996). Comparisons were made among species using the number of individuals of any one species present on a particular age mat and the number of individuals of the same species absent from that particular mat age (i.e., present on mats of the other three ages).

truncatus and *E. corticina* did not differ ($P > 0.05$). The frequencies of occurrence for *Carpophilus sayi* and the three *Glischrochilus* species did not differ ($P > 0.66$), initially increasing in an exponential manner with increasing mat age. In northern locations, the frequencies reached a plateau on aging and declining mats; while in southern locations they continued to increase with aging and declining mats (Table 1).

Differences were also found when frequencies of incidence were compared across species within each mat age-class (Table 1). *Colopterus truncatus* was present on the highest percentage of immature mats ($P < 0.05$) compared with the other species, while *Carpophilus sayi* and the three *Glischrochilus* species were not found on mats of this age-class or were less frequent. For mature mats, *Colopterus truncatus* and *E. corticina* were the species present on the highest percentage of mats in both locations (≥50% of mats) ($P < 0.05$). The lowest incidence for a species varied somewhat by location, but *G. quadrisignatus* occurred at low frequencies in both locations (≤7%).

Abundance of nitidulid species on mats

The frequency of occurrence of the six predominant nitidulid species on all mats were as follows: *Colopterus truncatus*, 35%; *E. corticina*, 20%; *Carpophilus sayi*, 19%; *G. sanguinolentus*, 14%; *G. fasciatus*, 5%; and *G. quadrisignatus*, 4%. The other six or more nitidulid species observed occurred at much lower levels (<1%). Because of their low frequency of occurrence, these other species were not included in data summarization and analyses.

The distributions of numbers of adult nitidulid species among the four mat age categories did not differ between the two study areas for all species except *Colopterus truncatus* ($P < 0.01$). Data for all species except *Colopterus truncatus* were thus combined across locations (Table 2). Frequencies of adult abundance across mat age-classes did not differ for *Carpophilus sayi* and *G. sanguinolentus* ($P > 0.05$) and for *G. fasciatus* and *G. quadrisignatus* ($P > 0.38$). However, frequencies of abundance differed for *E. corticina*, for *Colopterus truncatus* in northern sites (N), and for *Colopterus truncatus* in southern sites (S) ($P < 0.05$).

Abundance of different species varied within a mat age category ($P < 0.001$). *Colopterus truncatus* (N and S) and *E. corticina* were the predominant species on immature mats ($P < 0.001$), accounting for 94% of 154 nitidulids collected from the 34 immature mats. These two taxa were also more abundant on mature mats than the other four taxa ($P < 0.004$), with *E. corticina* most prominent on mature mats ($P < 0.025$), followed by *Colopterus truncatus* (S) and *Colopterus truncatus* (N). Together these three species comprised 77% of 868 adult nitidulids from 151 mats of that age-class. Of 1134 sap beetles collected from 188 aging mats, however, *Carpophilus sayi* was the most abundant taxa ($P < 0.001$), accounting for 28% of the adults. Abundance of *Carpophilus sayi* and the three *Glischrochilus* species did not differ on declining mats ($P > 0.231$). They were the predominant species collected from these mats, comprising 80% of the 214 nitidulids collected from 57 declining mats.

Nitidulid species aggregation on mats

In addition to the common occurrence of multiple nitidulid species on a given mat, multiple numbers of individuals of each species were also found. Data on occurrence of aggregation within each species were summarized into three abundance categories, i.e., 2–10, 11–20, and >20 individuals of one species present on a mat. The distribution of aggregation frequencies for the six predominant species did not vary by mat age ($P > 0.05$), and so the data were combined. The relationship between frequencies of increasing numbers of individual species present on a given mat and the number of mats yielding those individuals was found to be an exponentially decreasing one, regardless of species. *Colopterus truncatus* and *E. corticina* aggregated more than 50% of the time, with two or more individuals found per mat when collected from immature, mature, and aging mats. Similar frequencies of aggregation were found for *Carpophilus sayi*

Table 3. Frequency of *Ceratocystis fagacearum* isolation from nitidulid species collected between 1994 and 1996 from different age-classes of oak wilt mats from two east-central Minnesota locations.

Location* and nitidulid species	Immature		Mature		Aging		Declining	
	No. assayed	% positive	No. assayed	% positive	No. assayed	% positive	No. assayed	% positive
Northern								
Colopterus truncatus	52	69*a*†	107	65*b*	227	71*b*	19	58*a*
Epuraea corticina	26	73*a*	100	59*b*	76	73*b*	0	—
Carpophilus sayi	0	—‡	23	91*a*	103	86*a*	16	81*a*
Glischrochilus sanguinolentus	0	—	19	89*a*	56	93*a*	7	43*a*
Southern								
Colopterus truncatus	49	87*a*	52	60*b*	30	63*b*	5	100*a*
Epuraea corticina	15	93*a*	22	68*ab*	29	86*ab*	5	100*a*
Carpophilus sayi	0	—	35	74*ab*	38	97*a*	8	88*a*
Glischrochilus sanguinolentus	0	—	20	90*a*	26	96*a*	11	82*a*

Note: Values are the total number of beetles assayed on the mats and the percentage from which the fungus was isolated. Mat age-classes are based on Curl (1955).

*Isolation frequencies differed by location ($P < 0.05$); thus, data were not combined. Northern location included sites in Blaine, Columbus Township, and Ham Lake, Minnesota. Southern location included sites in Burnsville and Savage, Minnesota.

†Values followed by different letters within each mat age for each location were significantly different ($P < 0.05$) based on analyses of beetle numbers using the unordered likelihood ratio test (Agresti 1996).

‡No beetles were assayed.

and *G. sanguinolentus* on aging mats. The greatest frequency of aggregation occurred with *Colopterus truncatus* on mature mats (72%). Large numbers of individuals of one species were found on mats in several cases. For example, eight mature mats were found with ≥15 *Colopterus truncatus* on each. Three aging mats contained >20 *Carpophilus sayi*, with one mat having 62 individuals. A fourth aging mat yielded 26 *G. quadrisignatus*.

Frequency of nitidulids yielding *Ceratocystis fagacearum*

Incidence of viable *Ceratocystis fagacearum* propagules on external surfaces of nitidulids was determined for the four most common species (*Colopterus truncatus*, *E. corticina*, *Carpophilus sayi*, and *G. sanguinolentus*) (Table 3). Distributions of *Ceratocystis fagacearum* isolation frequencies for each of the nitidulid species across mat ages were significantly different ($P < 0.01$) between the northern and southern areas and, thus, the data were not combined. Although variation in frequency occurred across mat age for each species, overall the fungus was commonly isolated (43–100%) from assayed beetles. When compared across species within the different mat ages, no differences were found among species in the number of beetles yielding the fungus from immature or for declining mat collections ($P > 0.05$) (Table 3). Variations in frequencies of isolation were found among species from mature and aging mats ($P < 0.05$). Frequencies of fungal isolation were lower from *Colopterus truncatus* and *E. corticina* (59–73%) than from *Carpophilus sayi* and *G. sanguinolentus* (86–93%) from mature and aging mats in the northern location. Incidence of fungal isolation was higher for beetles from aging mats compared with mature mats in the southern location.

Number of viable *Ceratocystis fagacearum* propagules on beetles

The mean number of *Ceratocystis fagacearum* propagules isolated from nitidulids collected from oak wilt mats varied by location and mat age ($P < 0.05$) and the data were not combined (Tables 4 and 5). Differences in mean CFU varied by species when collections were from mature, aging, and declining mats. For mature and postmature mats in both study areas, the smallest mean CFUs ($31 \times 10^3 - 170 \times 10^3$) were consistently associated with *Colopterus truncatus*. In general, the highest CFU levels ($170 \times 10^3 - 5100 \times 10^3$) were found for the *G. fasciatus*, *G. quadrisignatus*, and *G. sanguinolentus* collected from mature and aging mats in both study locations.

Discussion

Six nitidulid species accounted for 94% of 2542 adult nitidulids, representing 12 species, collected from spring oak wilt mats in Minnesota. Among these six species, *Colopterus truncatus* was the most common species found, with *E. corticina* and *Carpophilus sayi* being the next most abundant species. These findings agree with previous studies in Iowa (Norris 1956), Minnesota (Juzwik and French 1983), and Wisconsin (McMullen et al. 1960), where *Colopterus truncatus* was found to be the most abundant species (65, 62, and 86%, respectively) associated with spring oak wilt mats. At the other end of the abundance spectrum, *G. fasciatus* and *G. quadrisignatus* each accounted for only 4% and *Cryptarcha ampla* Erch. for <1% of the 2542 individuals collected in our study. Although there may be little correlation between the composition of nitidulid species collected from oak wilt mats in this study compared with those caught using artificial baits in other studies (which may be more attractive to some species than others), our results provide an interesting contrast. Specifically, *G. fasciatus*, and *G. quadrisignatus* were found to be the most abundant nitidulids, while *Colopterus truncatus* was one of the least abundant during spring in red oak stands in three separate bait studies conducted in Minnesota and Wisconsin. These results were the same regardless of the different types of baits used, i.e., liquid fun-

Table 4. Numbers of colony-forming units (CFU) of *Ceratocystis fagacearum* (Cf) isolated from nitidulid species collected between 1994 and 1996 from oak wilt mats in northern study location.

Mat age-class and nitidulid species	No. of mats yielding Cf-positive beetles	No. of Cf-positive beetles	CFU/beetle ($\times10^3$)* Mean	SE
Immature				
Colopterus truncatus	19	36	17*a*[†]	5
Epuraea corticina	8	19	18*a*	15
Carpophilus sayi	3	4	130*a*	96
Glischrochilus sanguinolentus	2	2	10*a*	3
Mature				
Colopterus truncatus	32	70	31*c*	14
Epuraea corticina	27	59	150*bc*	120
Carpophilus sayi	12	21	1300*a*	970
Glischrochilus sanguinolentus	12	17	440*a*	190
Glischrochilus fasciatus	5	8	170*ab*	130
Aging				
Colopterus truncatus	53	161	170*c*	55
Epuraea corticina	25	56	510*c*	290
Carpophilus sayi	45	89	1800*b*	380
Glischrochilus sanguinolentus	31	52	1900*ab*	410
Glischrochilus fasciatus	33	49	5100*a*	1100
Declining				
Colopterus truncatus	6	11	34*c*	30
Carpophilus sayi	10	13	1200*a*	500
Glischrochilus sanguinolentus	3	3	7900*a*	6500
Glischrochilus fasciatus	6	8	840*ab*	730

Note: The locations included study sites in Blaine, Columbus Township, and Ham Lake, Minnesota. Mat age-classes are based on Curl (1955).

*The number of colony forming units (CFU) was based on mean number of propagules obtained from all nitidulids of each particular species that yielded the fungus and were collected from the same mat. ANOVA was conducted on CFU averaged on a per-mat basis.

[†]Values followed by different letters within a mat age are significantly different ($P < 0.05$) using Fisher's least-squares difference test (Wilkinson 1992).

gus culture baits and fermenting bread dough (Skalbeck 1976), fermenting bread dough and melon baits (Juzwik and French 1983), and banana baits (McMullen and Shenefelt 1961).

Based on species' presence and abundance data, there were general sequences of arrival of the dominant species that were related to the biological ages of mats. *Colopterus truncatus* and *E. corticina* were the predominant nitidulid occupants of immature and mature mats. *Carpophilus sayi* and *G. sanguinolentus* were detected in increasing numbers on aging and declining mats. Finally, *G. fasciatus* and *G. quadrisignatus* were more common on aging and declining compared with the younger aged mats. *Colopterus truncatus* and *E. corticina* appear to vacate occupied mats between the aging and declining stages. Our results agree with earlier findings for nitidulids on oak wilt mats in Iowa (Norris 1956) and Illinois (Curl 1955). Norris (1956) reported that larvae of *Colopterus truncatus*, *Colopterus semitectus* Say, and *E. corticina* fed on mats, and the adults left the mats before they deteriorated. Further, he noted that *G. fasciatus* and *G. quadrisignatus* remained on the mats until they had desiccated and deteriorated.

At least two possible explanations for this temporal partitioning of mats by these nitidulid species exist. Relative body size of the nitidulid species may explain why *Colopterus truncatus* and *E. corticina*, and not *Glischrochilus* spp., occupy immature and mature mats. The small vertical cracks often associated with immature mats (J. Juzwik, personal observation) may physically limit entry by the larger bodied *G. fasciatus*, *G. sanguinolentus*, and *G. quadrisignatus*. These cracks usually continue to expand until, upon maturation, the pressure pads are fully formed on the mat. However, this explanation is not consistent with a report that *Glischrochilus obtusus* (Say), one of the largest nitidulid species in the north-central states, was frequently collected on immature mats in Illinois (Curl 1955). A more plausible explanation for the temporal partitioning of nitidulids on oak wilt mats may be the differential olfactory-based attraction of the species to substrate volatiles, insect produced semiochemicals, or a synergism of the two. Four species (*Carpophilus sayi*, *G. sanguinolentus*, *G. fasciatus*, and *G. quadrisignatus*) were generally absent from immature mats, but were the predominant species present on declining mats in our study. Norris (1956) and Curl (1955) reported similar results for *G. fasciatus* and *G. quadrisignatus*. The four species may prefer the more "ripe" odor of older oak wilt mats colonized by secondary microorganisms (Shigo 1958; Gibbs 1980; Juzwik and Meyer 1997) and invertebrates. In comparison with older mats, the volatiles associated with younger oak wilt mats that are not yet overrun by secondary colonists may not seem attractive to these four species.

Table 5. Numbers of colony-forming units (CFU) of *Ceratocystis fagacearum* (Cf) isolated from nitidulid species collected between 1994 and 1996 from oak wilt mats in southern study location.

Mat age-class and nitidulid species	No. mats yielding Cf-positive beetles	No. of Cf-positive beetles	CFU/beetle (×10³)*	
			Mean	SE
Immature				
Colopterus truncatus	9	43	6*a*†	2
Epuraea corticina	3	14	14*a*	10
Mature				
Colopterus truncatus	19	31	13*c*	5
Epuraea corticina	12	15	160*bc*	130
Carpophilus sayi	14	26	310*ab*	98
Glischrochilus sanguinolentus	10	18	460*a*	150
Glischrochilus quadrisignatus	3	6	380*ab*	220
Aging				
Colopterus truncatus	12	19	40*c*	20
Epuraea corticina	12	25	81*bc*	27
Carpophilus sayi	17	37	230*ab*	85
Glischrochilus sanguinolentus	16	25	460*a*	170
Declining				
Colopterus truncatus	3	5	22*b*	15
Epuraea corticina	2	5	84*a*	83
Carpophilus sayi	3	7	19*b*	8
Glischrochilus sanguinolentus	5	9	110*a*	60

Note: Locations included study sites in Burnsville and Savage, Minnesota. Mat age-classes are based on Curl (1955).

*The number of colony-forming units (CFU) was based on mean number of propagules obtained from all nitidulids of each particular species that yielded the fungus and were collected from the same mat. ANOVA was conducted on CFU averaged on a per-mat basis.

†Values followed by different letters within a mat age are significantly different ($P < 0.05$) using Fisher's least-squares difference test (Wilkinson 1992).

Ceratocystis fagacearum and many other oak wilt mat associated fungi produce an array of volatile metabolites (Hanssen 1993; Hanssen and Abraham 1988; Hanssen et al. 1986; Collins and Kalnins 1965). A number of volatiles associated with *Ceratocystis* species are even reported as insect kairomones (Metcalf 1987). Significant attraction of three nitidulid species (*Carpophilus hemipterus* (Linne), *Carpophilus lugubris* Murray, and *Stelidota geminata* (Say) was elicited by *Ceratocystis fagacearum* in wind tunnel bioassays (Lin and Phelan 1992). Similar studies using predominant nitidulid species reported here are needed to assess the role of substrate volatiles in attraction of nitidulids to oak wilt mats.

Insect-produced semiochemicals may also contribute to temporal partitioning of nitidulid species on oak wilt mats. A male-produced aggregation pheromone has been identified for *Colopterus truncatus* (Cosse and Bartelt 2000). Furthermore, different blends of this pheromone were highly attractive to *Colopterus truncatus* when synergized with fermenting bread dough. This semiochemical, and possible synergism in a mat habitat, may explain the high frequency of aggregation observed for *Colopterus truncatus* in this study. The use of synthetic pheromones and a coattractant has shown potential for nitidulid population suppression in stone fruit orchards (James et al. 1994, 1996). Before a similar strategy could be considered for managing the overland spread of oak wilt, more information is needed on the ecology and pheromone chemistry of oak wilt associated nitidulids.

Ceratocystis fagacearum was commonly found on the external surfaces of assayed nitidulid species, and the numbers of viable propagules were generally high, i.e., greater than 3×10^3 CFU. Numbers of *Ceratocystis fagacearum* propagules were sufficient to cause host infection regardless of nitidulid species on which they occurred. Viability of different types of *Ceratocystis fagacearum* propagules varies by mat ages (Curl 1955), and logically, incidence and numbers of viable propagules acquired by nitidulids visiting mats should also vary by mat age (Juzwik et al. 1998). However, incidences for all beetles combined within a mat age in this study did not show this trend (Table 3). In general, *Ceratocystis fagacearum* CFUs did increase as age of mats yielding that species increased in the northern location, but this was not true in the southern location (Tables 4 and 5). Lastly, the number of *Ceratocystis fagacearum* CFUs isolated from nitidulids collected from the southern location were generally lower than numbers obtained from beetles from the northern location. We cannot explain this difference.

Results presented in this paper show that frequency of *Ceratocystis fagacearum* incidence and CFUs on nitidulids also varies by relative size of beetle species. Based on measured lengths of the species in our collections, the predominant species we found associated with spring mats can be ranked as follows (mean lengths for 20 individuals/species): *Colopterus truncatus*, 2.6 mm; *E. corticina*, 3.3 mm; *Carpophilus sayi*, 4.2 mm, and the three *Glischrochilus* species, ranging from 5.4 to 5.9 mm (V. Cervenka, unpublished data). In general, frequencies of *Ceratocystis fagacearum* presence (based on incidence of isolation and CFU levels) on beetles from mature and aging mats were higher for the larger bodied species (*Carpophilus sayi* and the three *Glischrochilus* species) compared with the smaller bodied *Colopterus truncatus* and *E. corticina*. Although data are limited, a similar trend can be found in numbers reported by Juzwik and French (1983). Incidence of *Ceratocystis fagacearum* isolation from nitidulids collected from non-age-classified mats during April and May was the lowest for a smaller bodied species (*Epuraea* sp.) and highest for a larger bodied one (*G. sanguinolentus*).

In conclusion, our results are important in the larger context of identifying the relative importance of each of the numerous nitidulid species implicated as vectors of *Ceratocystis fagacearum*. Studies documenting nitidulid species associated with fresh wounds on healthy oaks during spring are underway (Juzwik et al. 1999), and those results will complement these in achieving our larger objective. If the number of nitidulid species of high importance is small, the potential will be greater for developing new oak wilt control tools based on insect vector management strategies.

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