Acquisition of *Ophiostoma quercus* and *Ceratocystis fagacearum* by Nitidulids from *O. quercus*–Colonized Oak Wilt Mats

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ABSTRACT

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Field experiments were conducted to determine whether the frequency of *Ceratocystis fagacearum* and *Ophiostoma quercus* propagule acquisition by nitidulids visiting oak wilt fungal mats is affected by the presence of *O. quercus* on the mats. Augmentation sprays with *O. quercus* were used to achieve different levels of mat colonization by that fungus. The extent of colonization by *O. quercus* 7 to 14 days after spraying was greatest for postmature mats with open cracks (>0.5 cm) observed on the spray date ($P < 0.02$). Specifically, all six subsamples assayed per mat for 16 of 18 open, *O. quercus*-sprayed, postmature mats yielded *O. quercus*. The frequency of nitidulids with *O. quercus* was also highest (83%) for those collected from *O. quercus*-treated, postmature mats that were open on the spray date. The frequency of nitidulids with viable *C. fagacearum* (88%), however, was also greatest for beetles from the postmature mats. Thus, *O. quercus* colonization did not affect vector acquisition of *C. fagacearum* from the mats. In addition, *O. quercus* vectored by nitidulids probably does not result in natural biological control of overland transmission of *C. fagacearum* at fresh wound surfaces on healthy oaks, as previously suggested.

Additional keywords: *Graphium pirinum, Ophiostoma picea*

Oak wilt infection centers are initiated by overland spread of the causal fungus, *Ceratocystis fagacearum* (T. W. Bretz) J. Hunt, by insects (10). Sap-feeding beetles (Coleoptera: Nitidulidae) are considered a primary vector of the pathogen in the north central states (7,9). Successful transmission by sap-feeding beetles depends on several coexisting conditions, including (i) the availability of viable inoculum on *C. fagacearum* mycelial mats; (ii) the presence of active nitidulids to transmit the pathogen; (iii) a fresh, xylem-exposing wound receptive to infection by the pathogenic propagules present on the beetles; (iv) the coexistence of these events during the spring when *Quercus* spp. are most susceptible to infection; and (v) the absence of microbial deterrents to infection at or introduced to the wound surface (21). *Ophiostoma quercus* (Georgev.) Nannf. (previously the hardwood type of *O. piceae* (Münch.) Syd. & P. Syd.) (19), a common sapwood colonizer of hardwood species, has been implicated as a biological control organism in the oak wilt disease cycle (8, 20). In an inoculation experiment on healthy northern pin oak, *Q. ellipsoidalis* E. J. Hill, Gibbs (8) found that when *O. quercus* was introduced into a fresh wound 24 h before *C. fagacearum* was introduced, infection by the pathogen was prevented. He speculated that *O. quercus* colonization of fresh wounds on healthy oaks may be partly responsible for the wounds being receptive to *C. fagacearum* for only a few days (3,14,17).

Propagules of *O. quercus* have been isolated from both free-flying (2,12) and mat-inhabiting nitidulids (12). In addition, nitidulids are common in fresh wounds on healthy oaks (4,18). Thus, it is very likely that nitidulids initiate *O. quercus* colonization when they visit fresh wounds on oaks. Oak wilt mats overgrown by the *Graphium* anamorph of *O. quercus* are the most likely places for nitidulids to acquire *O. quercus* (8). On the basis of microscopic and isolation studies, Reutze and Parameswaran (20) suggested that *O. quercus* colonization of mats would reduce the acquisition of *C. fagacearum* by insect vectors. They speculated that nitidulids leaving such mats might well be carrying spores of *O. quercus* more frequently than *C. fagacearum*, thus increasing the probability that nitidulids would successfully vector *O. quercus* to fresh wounds more often than they would vector the oak wilt pathogen. The *O. quercus*-colonized wound would then no longer be receptive to *C. fagacearum* infection (8); and in this sense, nitidulids and *O. quercus* would appear to play a role in biological control of overland transmission of *C. fagacearum* in nature (11,12).

Our research was undertaken to determine whether the frequency of *O. quercus* and *C. fagacearum* propagule acquisition by nitidulids visiting oak wilt mats is impacted by the presence of *O. quercus* on the mats. Because *O. quercus* is a common natural colonizer of the mats (13), we wished to artificially establish a different level of colonization for comparison, i.e., either no colonization (via exclusion) or complete colonization (via augmentation). The latter strategy was chosen for this study. Our objectives were to determine (i) whether augmentation sprays with *O. quercus* would result in significantly greater colonization of oak wilt mats compared with the untreated mats and (ii) the frequencies with which nitidulids associated with differentially colonized mats acquire *C. fagacearum* and/or *O. quercus.*

MATERIALS AND METHODS

Study sites and tree selection. *C. fagacearum*-infected northern red (*Q. rubra* L.) and northern pin oaks that had wilted and died during July or August, 1994, were selected for this study. The trees were located in a number of oak wilt infection centers on scattered properties in Minnesota in Blaine and in a park reserve in Hennepin County near Burnsville. We began monitoring these trees for oak wilt mat production in mid-April, 1995. A sharp increase in the number of new mats was observed in midspring, and *O. quercus* spray treatments were conducted during this peak sporulation period at each location.

***O. quercus* spray treatment.** An *O. quercus* isolate obtained from an oak wilt mat on a recently killed northern pin oak in North Oaks, Minnesota, was used for the study. The isolate species was confirmed by E. B. Smalley (Department of Plant Pathology, University of Wisconsin, Madison) through pairing with known mating types of the fungus. The single-spored iso-

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late had been stored on silica gel at 4°C for less than 2 years prior to the study. Two agar plugs (3 mm in diameter) were removed from 10-day-old *O. quercus* colonies grown on potato-dextrose agar (PDA) in Petri dishes at 22°C in the dark. The plugs were placed in 125 ml of potato-dextrose extract medium in 250-ml Erlenmeyer flasks and incubated (24 h of light, 20°C) on an orbital shaker with moderate agitation for 7 days. On the treatment date, the conidial concentration in each flask (averaged for three flasks) was determined with a hemacytometer. Appropriate amounts of the inoculum concentrate were removed from the flasks and placed in 3.8 liters of nonsterilized 0.6% malt extract broth (Difco, Detroit, MI) on the morning of the spray treatment to achieve a spray suspension of 10^6 conidia per milliliter. In the field, all spray trees were examined for oak wilt mats whose pressure pads had cracked open the bark approximately 0.5 cm or more. Those mats were marked and considered to be open on the spray date. Other mats that had cracked open the bark only slightly (<0.5 cm) or were not yet apparent because the bark was not cracked were considered to be closed on the spray date. The *O. quercus* suspension was applied to the trees designated for *O. quercus* spray treatment with a 15-liter tank-type sprayer (Hudson, St. Paul, MN) by using the unit's hand pump. Pressure was maintained at 0.27 MPa. The sprayer was equipped with an extended, bent-angle (45°) wand with a flat fan nozzle (TJ 005, Tee-Jet, Spraying Systems, Wheaton, IL). Trees were sprayed from two opposite cardinal directions for 60 to 80 s in order to cover the lower 3.5 m of the main stem with 1.0 liter of inoculum suspension. In the Burnsville study, 25 trees located in scattered infection centers in the northern half of the park reserve were sprayed on 11 May; 20 additional trees located in scattered infection centers in the southern half of the park were not treated. In the Blaine study, 18 trees on four properties received the treatment on 10 May, while 19 trees on three different properties were not treated.

Sample collection and processing. Oak wilt mats and the associated mat-inhabiting nitidulids were collected 7 and 14 days after the *O. quercus* spray treatment. Mats were exposed by removing the bark with a hatchet and mallet, and the biological age of each mat was determined according to Curl's (5) description of immature, mature, aging, and declining mats. Adult nitidulids on the mats were collected immediately after each mat was exposed and then placed individually into gelatin capsules, grouped by source mat number in polyethylene bags, and stored at −2°C until processed in the laboratory. The phloem side of each fungal mat was wrapped in aluminum foil, placed in a polyethylene bag, and stored at −2°C until processed. Tree number, mat number, and mat age were recorded for each nitidulid and mat collection.

Within 1 week of collection, six subsamples were taken from each mat to obtain an estimate of the area of each mat colonized by *O. quercus* or that had viable *C. fagacearum* propagules. Subsamples were aseptically taken with a 3-mm cork borer at a distance of 0.5 cm from the edge of the mat in a systematic oval pattern. The subsamples were stored in individual vials at −2°C until further processed. Nitidulids obtained from the mats were tentatively identified to species and grouped by species according to study location, mat age, and treatment. All nitidulids collected were identified to species. Determinations were confirmed by T. C. Skalbeck (Department of Entomology, University of Minnesota).

The presence and number of viable propagules of *O. quercus* and *C. fagacearum* on mat subsamples and of nitidulids were determined by serial dilution plating techniques. Defrosted mat subsamples were processed according to previously described methods (11). Aliquots (0.5 ml) of suspensions from mat subsamples were spread on acidified (10% lactic acid) PDA in Petri dishes and incubated in the dark at 22°C. Each nitidulid tested was placed in a sterile test tube containing 2.5 ml of sterile distilled water. The tube was capped, agitated on a vortex mixer, and held in a water bath sonicator for 15 min to dislodge the fungal propagules from the insect. The resulting suspension was then serially diluted (10-fold dilutions), and 0.5-ml aliquots of each dilution were spread on lactic acid-amended PDA plates. Three plates were used per dilution for both mat subsample and beetle washing platings. Resulting colonies of *O. quercus* and *C. fagacearum* were counted after 7 and 14 days. Colonies of *O. quercus* were generally well developed in 1 week, but *C. fagacearum* colonies often required 12 to 14 days to develop. All mat subsamples were processed within 4 months of the collection date, while nitidulids were processed within 9 months of collection. Colony appearance and the presence of the *Chalara* anamorph were used to identify *C. fagacearum.* The presence of synnemata and colony appearance were used to identify *O. quercus.*

Data summarization and analyses. Mat subsample and insect data were summarized by mat age, mat exposure on *O. quercus* spray date, and treatment for each location. For frequency analyses, a mat subsample or an insect was considered positive for *O. quercus* or *C. fagacearum* if at least one colony was detected on any of three Petri dishes used per mat subsample or insect for the dilution series counted.

Subsample frequency data were analyzed by both the stratified Wilcoxon-Mann-Whitney test and the singly ordered Kruskal-Wallis test for categorical data (1,16) to investigate whether the extent of *O. quercus* colonization of oak wilt mats

Table 1. Oak wilt fungus mat colonization by *Ophiostoma quercus* during *O. quercus* spray trials in Burnsville and Blaine, MN[t]

Mat age[u]	Mat exposure on spray date	Treatment[v]	No. trees producing processed mats	Number of mats[x]							Statistical results[y]
				0%[w]	17%	33%	50%	67%	83%	100%	
Immature	Closed	*O. quercus*	18	20	8	3	1	0	1	2	a
	Closed	None	15	21	4	3	2	1	1	2	a
Mature	Open[z]	*O. quercus*	6	0	0	1	0	1	1	5	bc
	Open	None	5	0	0	0	1	0	1	6	c
	Closed	*O. quercus*	16	4	4	2	2	3	2	9	b
	Closed	None	13	2	5	2	3	1	7	7	b
Postmature	Open	*O. quercus*	9	0	0	0	0	0	2	16	e
	Open	None	12	0	0	2	1	4	3	4	d
	Closed	*O. quercus*	9	1	1	0	1	2	3	9	d
	Closed	None	12	2	2	1	1	2	6	7	d

[t] Number of mats with different frequency of isolation is reported by mat age.
[u] Ages based on descriptions by Curl (5). Postmature category includes aging mats from Burnsville and aging plus declining mats from Blaine study trees.
[v] Either *O. quercus* spray or no spray (none).
[w] Percentage of subsamples per mat yielding *O. quercus.*
[x] Data were derived from isolation of *O. quercus* from six 3-mm-diameter mat subsamples by serial dilution plating techniques.
[y] Results of singly ordered Kruskal-Wallis analyses (1,16) comparing distributions across mat age. Distributions followed by different letters were not similar ($P < 0.05$).
[z] No mature open mats were collected from Blaine study trees.

differed by mat age, mat exposure on spray date, and/or treatment within location and whether data could be combined across locations. Exact *P* values were calculated because of the occurrence of low cell frequencies. The same statistical methods were used to determine whether the extent of *C. fagacearum* recovery from the mats differed by the same factors. Quantitative data involving colony-forming units of *O. quercus* or *C. fagacearum* isolated from mat subsamples were averaged for each mat and grouped according to mat age, mat exposure, and treatment within location. Only values obtained from positive subsamples were included in the calculation. Analyses of variance were performed on transformed numbers (ln [CFU + 1]), and mat age comparisons were made (Fisher's least significant difference) when appropriate (24).

Data involving frequency of *O. quercus* and *C. fagacearum* occurrence on nitidulids were analyzed by using the likelihood ratio test for unordered categorical data with calculation of exact *P* values (1,16) (i) to investigate differences in occurrence by mat age, exposure, and treatment of mats from which insects were obtained and (ii) to determine whether data could be combined across locations. Quantitative data involving colony-forming units of either fungus isolated from nitidulids were averaged for all beetles obtained from a mat, and those averages were grouped according to location and mat-related factors (age, exposure, and treatment). Only values obtained from *O. quercus*- or *C. fagacearum*-positive nitidulids were used in calculating the average number of colony-forming units. Subsequent data analyses of beetle-derived colony-forming units were performed as described.

RESULTS

Isolation of fungi from oak wilt mats. More than 200 oak wilt mats were collected from 69 study trees in the two study locations. Frequencies of *O. quercus* and *C. fagacearum* isolation from mat subsamples were similar for mats collected from the two locations. Colonization of mats by *O. quercus* increased with mat age ($P < 0.001$). Differences ($P < 0.02$) in frequencies of fungal isolation were found for exposure-treatment combinations within mature and postmature mat categories (Table 1). For the postmature mats, the frequency of fungal isolation was greatest for open, *O. quercus*-sprayed mats (16 of 18 mats with 100% positive isolation) ($P < 0.02$). Frequency of *O. quercus* isolation for the other three postmature treatments were similar ($P > 0.26$); >50% of the subsamples from 43 of 52 mats were colonized.

Recovery of *C. fagacearum* from mat subsamples within each mat age was not affected by exposure or treatment differences (Table 2). Isolation frequencies for *C. fagacearum* from subsamples were similar for immature and mature mats, but frequencies were lower for postmature mats ($P < 0.001$). Specifically, 107 of 138 (78%) immature and mature mats yielded *C. fagacearum* on 100% of the subsamples compared with 37 of 70 (53%) postmature mats with positive isolation from all subsamples.

The numbers of *O. quercus* and *C. fagacearum* colony-forming units on mat subsamples were similar for Burnsville and Blaine mats, and data were therefore combined (Table 3). The average number of propagules of *O. quercus* isolated per square centimeter increased with mat age ($P < 0.001$), with each mat age group differing from the others ($P < 0.001$). The average numbers of colony-forming units recovered per mat age were 3.5×10^3, immature; 82×10^3, mature; and 600×10^3, postmature. Differences among exposure and treatment factors were found only for postmature mats. Specifically, the numbers of colony-forming units varied by treatment ($P < 0.001$) and exposure-treatment interaction ($P < 0.01$) factors. The highest number ($1{,}300 \times 10^3$ CFU/cm^2) was noted for the open, *O. quercus* treatment combination.

Recovery of *C. fagacearum* propagules per square centimeter ranged from 160 to 400×10^3. Levels were higher for postmature mats than for immature and mature ones ($P < 0.01$) on the basis of analysis of closed mat data (Table 3). No differences were found within the mat ages for mat exposure or treatment ($P > 0.05$).

Isolation of fungi from nitidulids. More than 1,500 nitidulids were obtained from oak wilt mats collected at the two locations. The species represented in the insect collection included *Epuraea peltoides* Horn., *Colopterus truncatus* Randall, *C. semitectus* (Say), *Carpophilus sayi* Parsons., *Glischrochilus sanguinolentus* (Oliv.), and *G. quadrisignatus* Say. Occurrences of viable *O. quercus* and *C. fagacearum* propagules on external beetle surfaces were determined for a subset (383) of the collected nitidulids. No location effects were found for the different factors where frequencies were high enough to test. A higher proportion (57%) of nitidulids from postmature mats were carrying *O. quercus* than those from immature (10%) or mature (22%) mats (Table 4). Treatment and treatment-exposure interaction effects were found only for beetles collected from postmature mats. Within both open and closed mat categories, higher frequencies of *O. quercus*-positive nitidulids were obtained from mats collected from *O. quercus*-sprayed trees compared with those from untreated trees. The highest frequency (83%) of *O. quercus*-positive nitidulids was found among those collected from open, *O. quercus*-sprayed mats ($P < 0.04$). Frequencies were similar ($P > 0.05$) for nitidulids obtained from closed, *O. quercus*-sprayed (64%)

Table 2. Extent of *Ceratocystis fagacearum* recovery from oak wilt mats collected during *Ophiostoma quercus* spray trials in Burnsville and Blaine, MN[t]

Mat age[u]	Mat exposure on spray date	Treatment[v]	No. trees producing processed mats	Number of mats[x]							Statistical results[y]
				0%[w]	17%	33%	50%	67%	83%	100%	
Immature	Closed	*O. quercus*	18	0	0	0	0	3	3	29	a
	Closed	None	15	0	0	1	0	3	5	25	a
Mature	Open[z]	*O. quercus*	6	0	0	0	0	0	2	6	a
	Open	None	5	0	0	0	0	0	3	5	a
	Closed	*O. quercus*	16	0	0	0	0	1	6	19	a
	Closed	None	13	1	0	0	0	1	2	23	a
Postmature	Open	*O. quercus*	9	0	1	0	1	8	2	6	b
	Open	None	13	0	0	0	2	2	1	9	b
	Closed	*O. quercus*	9	0	1	0	1	2	3	10	b
	Closed	None	12	0	0	2	1	1	5	12	b

[t] Number of mats with different frequency of isolation is reported by mat age.
[u] Ages based on descriptions by Curl (5). Postmature category includes aging mats from Burnsville and aging plus declining mats from Blaine study trees.
[v] Either *O. quercus* spray or no spray (none).
[w] Percentage of subsamples per mat yielding *C. fagacearum.*
[x] Data were derived from isolation of *C. fagacearum* from six 3-mm-diameter mat subsamples by serial dilution plating techniques.
[y] Results of singly ordered Kruskal-Wallis analyses (1,16) comparing distributions across mat age. Distributions followed by different letters were not similar ($P < 0.05$).
[z] No mature open mats were collected from Blaine study trees.

and open control mats (51%) and lowest for those collected from closed control mats (31%).

A higher proportion (88%) of *C. fagacearum*-positive nitidulids was collected from postmature mats than from immature (64%) or mature (83%) mats ($P < 0.01$) (Table 4). Neither mat exposure nor treatment consistently affected the *C. fagacearum* frequencies from nitidulids obtained from immature, mature, and postmature mats. Isolation frequencies from mat-inhabiting nitidulids for both *O. quercus* and *C. fagacearum* increased with mat age from 8% on immature mats to 21 and 54% on mature and postmature mats, respectively.

The average number of *O. quercus* colony-forming units isolated from each beetle increased with the age of the mats from which they were collected. The average numbers of colony-forming units found for each mat age were less than 1.1×10^3, immature; 7.2×10^3, mature; and 60×10^3, postmature ($P < 0.01$) (Table 4). Exposure of mats on treatment date and treatment did not affect isolation of *O. quercus* from nitidulids collected from each mat age group. The average number of *C. fagacearum* propagules isolated from the same insects was lower for those collected from immature mats (1×10^3 CFU per nitidulid) compared with that for nitidulids from mature and postmature mats (108×10^3 CFU per nitidulid) ($P < 0.01$) (Table 4). Exposure of mats on treatment date and treatment did not affect isolation of *C. fagacearum* from nitidulids collected from each mat age group.

DISCUSSION

We were able to consistently achieve greater colonization of postmature oak wilt mats compared with naturally colonized mats by using spray applications of *O. quercus* on trees with exposed mats. This increased coverage was also accompanied by an increased number of colony-forming units of *O. quercus* per square centimeter on the exposed, postmature mats (Table 3). The frequencies of nitidulids collected from oak wilt mats carrying *O. quercus* did vary by mat age, exposure of the host mat on treatment date, and treatment (Table 4). Significantly higher frequencies of *O. quercus*-contaminated beetles were collected from *O. quercus*-sprayed mats compared with untreated mats within the open and closed exposure categories for postma-

Table 3. Numbers of colony-forming units of *Ophiostoma quercus* and *Ceratocystis fagacearum* isolated from oak wilt mats collected during *O. quercus* spray trials in Burnsville and Blaine, MN

			Mats yielding fungi[y]			
			O. quercus		*C. fagacearum*	
Mat age[w]	Mat exposure on spray date	Treatment[x]	Total no.	Average CFU/cm^2 ($\times 10^3$)	Total no.	Average CFU/cm^2 ($\times 10^3$)
Immature	Closed	*O. quercus*	15	5	33	200
	Closed	None	13	2	34	270
Mature	Open[z]	*O. quercus*	8	190	8	230
	Open	None	7	23	8	230
	Closed	*O. quercus*	22	76	26	160
	Closed	None	25	37	26	250
Postmature	Open	*O. quercus*	18	1,300	18	350
	Open	None	14	180	14	340
	Closed	*O. quercus*	16	580	17	310
	Closed	None	19	360	21	400

[w] Ages based on descriptions by Curl (5). Postmature category includes aging mats from Burnsville and aging plus declining mats from Blaine study trees.
[x] Either *O. quercus* spray or no spray (none).
[y] Data were derived from isolation of *O. quercus* and *C. fagacearum* from six 3-mm-diameter mat subsamples by using serial dilution plating techniques. Averages include values for mats where one or more subsamples yielded the fungus.
[z] No mature open mats were collected from Blaine study trees.

Table 4. Frequency and numbers of colony-forming units of *Ophiostoma quercus* and *Ceratocystis fagacearum* isolated from mat-inhabiting nitidulids collected during *O. quercus* spray trials in Burnsville and Blaine, MN

							Average CFU/beetle[x]	
				Percentage of beetles yielding:[x]				
Mat age[v]	Mat exposure on spray date	Treatment[w]	No. of nitidulids tested	*O. quercus*	*C. fagacearum*	Both fungi	*O. quercus* ($\times 10^3$)	*C. fagacearum* ($\times 10^3$)
Immature	Closed	*O. quercus*	33	12 a[y]	58 a	9 a	<0.1 a	0.2 a
	Closed	None	54	9 a	69 a	7 a	2.2 a	1.8 a
Mature	Open[z]	*O. quercus*	14	14 b	100 b	14 b	5.4 b	54 b
	Open	None	29	24 b	76 b	24 b	5.9 b	220 b
	Closed	*O. quercus*	36	25 b	81 b	22 b	16 b	77 b
	Closed	None	34	21 b	76 b	21 b	1.3 b	56 b
Postmature	Open	*O. quercus*	48	83 e	88 b	75 e	89 c	56 b
	Open	None	43	51 cd	88 b	44 cd	69 c	230 b
	Closed	*O. quercus*	50	64 d	96 b	64 de	16 c	110 b
	Closed	None	42	31 c	79 b	26 c	66 c	60 b

[v] Mat ages based on descriptions by Curl (5). Postmature category includes aging mats from Burnsville and aging plus declining mats from Blaine study trees.
[w] Either *O. quercus* spray or no spray (none).
[x] Data were derived from isolation of *O. quercus* and *C. fagacearum* from washings of individual beetles by using serial dilution plating techniques. Colony-forming unit data were averaged on a mat basis. Nitidulid species tested included *Epuraea peltoides*, *Colopterus truncatus*, *C. semitectus*, *Carpophilus sayi*, *Glischrochilus sanguinolentus*, and *G. quadrisignatus*.
[y] Values followed by different letters within each column are not similar ($P < 0.05$) based on analyses with the likelihood ratio test for unordered categorical data (1,16).
[z] No mature open mats were collected from Blaine study trees.

ture mats. This increased frequency, however, was not accompanied by an increase in propagule number for *O. quercus*-contaminated beetles.

In contrast, the extent of mat area yielding *C. fagacearum* was not affected by exposure of the mat on spray date or by treatment within different mat ages. *C. fagacearum* recovery was less for the oldest mats, a phenomenon that has been previously observed (13,20). Conversely, the number of colony-forming units of *C. fagacearum* per square centimeter did increase with mat age for positive mat portions. This is perhaps partly the result of the maturation of perithecia and associated ascospores that occurs on aging mats. Insect vectors are largely responsible for introducing the opposite mating type needed for spermatization and teleomorph development (10,15). We speculate that these events occur on immature, mature, and possibly aging mats as different nitidulid vector species arrive. Frequency of *C. fagacearum*-positive nitidulids also was highest for beetles collected from mature and postmature mats, but this was not related to any of the other trial factors. The number of colony-forming units of *C. fagacearum* was highest for beetles from mature and postmature mats, but no correlation was observed with the other variables.

In summary, increased frequency of nitidulids carrying *O. quercus* was associated with increased colonization of mats by *O. quercus*. However, frequency of nitidulids with viable *C. fagacearum* also was greatest from these same mats. These results do not support the hypothesis (20) that *O. quercus* colonization of mats reduces or prevents the chance that insect vectors will acquire *C. fagacearum*. This hypothesis was based on electron microscopic observation of *O. quercus* synnemata with conidial masses extending above the more embedded *C. fagacearum* perithecia with exuded ascospores on the mats as well as hyphal overgrowth of ascospore masses on perithecial tips. Behavior of nitidulids on the mats, however, involves more than the insects traversing the mat surface and acquiring pathogen spores. These insects are known to feed on, tunnel into, oviposit, and even rear broods in the mats (6,11,18; *personal observation*), and these activities alone suggest that mat-inhabiting nitidulids would easily pick up *C. fagacearum* propagules, regardless of mat surface overgrowth.

References to natural biological control occurring in different pathosystems are common in the literature. In the oak wilt disease cycle, several reports have referred to fungi involved in natural biological control in the overland-transmission segment of this system (8,20,22,23). The hypothesis that *O. quercus* limits *C. fagacearum* infection of healthy oaks was based on the common occurrence of the fungus on mats, its occurrence on both mat-inhabiting and free-flying nitidulids, and its ability to protect against *C. fagacearum* infection when it arrives on a fresh wound surface first. Our results demonstrate that *O. quercus* colonization of mats does not affect availability of viable *C. fagacearum* propagules on oak wilt mats or acquisition of *C. fagacearum* propagules by nitidulids visiting the mats. Even though increased mat colonization by *O. quercus* leads to increased frequency of nitidulids carrying *O. quercus* and a greater number of spores carried per beetle, the frequency of *C. fagacearum* on nitidulids visiting mats and its occurrence in significant numbers on such beetles would preclude protection at the wound surface being mediated by contaminated nitidulids. This is based on the assumption that no protection is afforded when both fungi arrive simultaneously at the wound surface (8).

O. quercus vectored by nitidulids is probably not resulting in natural biological control of overland transmission of *C. fagacearum* at the wound surface. Other fungi (e.g., *Trichothecium roseum* (Pers.: Fr.) Link and *Gliocladium roseum* Bainier) that are known to be antagonistic to *C. fagacearum* growth and/or sporulation and that occur on oak wilt mats would be more likely candidates for directly interfering with the overland-transmission cycle (22, 23). In addition, other nonantagonistic microorganisms may well be responsible for rendering fresh wounds nonreceptive to *C. fagacearum*, but it seems that they would originate from a source other than oak wilt mats.

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LITERATURE CITED

1. Agresti, A. 1996. An Introduction to Categorical Data Analysis. John Wiley & Sons, New York.
2. Appel, D. N., Kurdyla, T., and Lewis, R. 1990. Nitidulids as vectors of the oak wilt fungus and other *Ceratocystis* spp. in Texas. Eur. J. For. Pathol. 20:412-417.
3. Cobb, F. W., Fergus, C. L., and Stambaugh, W. S. 1962. The effect of temperature on ascogonial and perithecial development in *Ceratocystis fagacearum*. Mycologia 53:91-97.
4. Craighead, F. C., Morris, C. L., and Nelson, J. C. 1953. A preliminary note on the susceptibility of wounded oaks to natural infection by the oak wilt fungus. Plant Dis. Rep. 37: 483-484.
5. Curl, E. A. 1955. Natural availability of oak wilt inocula. Ill. Nat. Hist. Surv. Bull. 26:277-323.
6. Dorsey, C. K., and Leach, J. G. 1956. The bionomics of certain insects associated with oak wilt with particular reference to the Nitidulidae. J. Econ. Entomol. 49:219-230.
7. French, D. W. 1995. Oak wilt management in Minnesota. Pages 117-120 in: Oak Wilt Perspectives. Proc. Natl. Oak Wilt Symp. D. N. Appel and R. F. Billings, eds. Texas Agricultural Experiment Station, Texas Forestry Service, and Texas Agricultural Extension Service, Austin.
8. Gibbs, J. N. 1980. Role of *Ceratocystis piceae* in preventing infection by *Ceratocystis fagacearum* in Minnesota. Trans. Br. Mycol. Soc. 74:171-174.
9. Gibbs, J. N. 1984. Oak wilt. For. Comm. For. Rec. 126. Her Majesty's Stationery Office, London.
10. Gibbs, J. N., and French, D. W. 1980. The transmission of oak wilt. U.S. Dep. Agric. For. Serv. Res. Pap. NC-185.
11. Jewell, F. F. 1956. Insect transmission of oak wilt. Phytopathology 46:244-257.
12. Juzwik, J., and French, D. W. 1983. *Ceratocystis fagacearum* and *C. piceae* on the surfaces of free-flying and fungus-mat-inhabiting nitidulids. Phytopathology 73:1164-1168.
13. Juzwik, J., and Meyer, J. M. 1997. Colonization of oak wilt fungal mats by *Ophiostoma piceae* during spring in Minnesota. Plant Dis. 81:410-414.
14. Kuntz, J. E., and Drake, C. R. 1957. Tree wounds and long distance spread of oak wilt. Phytopathology 47:22.
15. Leach, J. G., True, R. P., and Dorsey, C. K. 1952. A mechanism for liberation of spores from beneath the bark and for diploidization in *Chalara quercina*. Phytopathology 42:537-539.
16. Mehta, C., and Patel, N. 1995. StatXact 3 for Windows: Statistical Software for Exact Nonparametric Inference. User's Manual. CYTEL Software, Cambridge, MA.
17. Morris, C. L., Thompson, H. E., Hadley, B. L., and Davis, J. M. 1955. Use of radioactive tracers for investigation of the activity pattern of suspected insect vectors of the oak wilt fungus. Plant Dis. Rep. 39:61-63.
18. Norris, D. M. 1956. Association of insects with the oak tree and *Endoconidiophora fagacearum* Bretz. Ph.D. thesis. Iowa State College, Ames.
19. Pipe, N. D., Buck, K. W., and Braiser, C. M. 1995. Genomic fingerprinting supports the separation of *Ophiostoma piceae* into two species. Mycol. Res. 99:1182-1186.
20. Reutze, M., and Parameswaran, N. 1984. Observations on the colonization of oak wilt mats (*Ceratocystis fagacearum*) by *Pesotum piceae*. Eur. J. For. Pathol. 14:326-333.
21. Shelstad, D., Queen, L., French, D. W., and Fitzpatrick, D. 1991. Describing the spread of oak wilt using a geographical information system. J. Arboric. 17:192-199.
22. Shigo, S. L. 1958. Fungi isolated from oak-wilt trees and their effects on *Ceratocystis fagacearum*. Mycologia 50:757-769.
23. Turk, F. M. 1955. The biological relationship between the oak wilt pathogen and the fungi found in wilted oak trees. M.S. thesis. University of Minnesota, St. Paul.
24. Wilkinson, L. 1992. Systat for Windows: Statistics. Version 5.0. SYSTAT, Evanston, IL.