Ceratocystis fagacearum and *C. piceae* on the Surfaces of Free-Flying and Fungus-Mat-Inhabiting Nitidulids

Jennifer Juzwik and D. W. French

Graduate student and professor, respectively, Department of Plant Pathology, University of Minnesota, St. Paul 55108.

A portion of the Ph.D. thesis of the senior author. We thank J. N. Gibbs, Forestry Commission, Great Britain, for valuable discussion and critical review of the manuscript; and we thank T. C. Skalbeck, former graduate student, Department of Entomology, University of Minnesota, for assistance in identifying the nitidulids.

This research was supported in part by the Minnesota Department of Agriculture through a special legislative research grant.

Scientific Journal Series Paper 13,002, Minnesota Agricultural Experiment Station, St. Paul.

Accepted for publication 6 December 1982.

ABSTRACT

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.

Free-flying and fungus-mat-inhabiting nitidulids (Coleoptera: Nitidulidae) were collected from two oak wilt areas in Minnesota during the spring of 1981. Washings from 1,043 free-flying beetles attracted to two types of bait traps from 30 March to 26 June in the Carlos Avery Wildlife Management Area, near Wyoming, MN, were plated on agar media. *Ceratocystis fagacearum* was isolated from seven nitidulids: one collected 1 April and all others collected from 18 May to 19 June, 2–3 wk after large numbers of sporulating fungus mats were detected. *C. piceae* was recovered from seven beetles trapped during 3 wk. *C. fagacearum* was recovered from 80 and *C. piceae* from 17 of 98 nitidulids collected on five dates from mats on northern pin oaks at Eagle Lake, near Big Lake, MN. From 30 to 760,000 viable spores of *C. fagacearum* were estimated to be present on the beetles. By scanning electron microscopy, conidia and ascospores of *C. fagacearum* were found on all external body parts of the insects. Conidia were the predominant spore type on beetles collected in April; ascospores prevailed in May. By carrying *C. piceae*, nitidulids may play a role in the natural biological control of *C. fagacearum*.

Additional key words: epidemiology, insect-vector relations, sap beetles.

New oak wilt infection centers are established by aboveground spread of the causal organism, *Ceratocystis fagacearum* (Bretz) Hunt, by insects. Transmission of the fungus by nitidulids, or sap beetles (Coleoptera: Nitidulidae) has received much attention (6). We hypothesize that, in Minnesota, nitidulids are the primary overland insect vector of *C. fagacearum*. Occurrence and distribution of nitidulids in oak forests and in oak wilt areas have been reported for several states (4,7,15).

The fungus has been isolated from free-flying nitidulids collected near an infection center on only one occasion (18). We suspected free-flying sap beetles would be carrying spores of *C. fagacearum* in oak wilt areas following peak periods of sporulation, or mycelial mat formation, in the spring and fall in Minnesota.

Isolation of *C. fagacearum* from nitidulids associated with mats has been reported (3,18). There are, however, no reports of the numbers of viable spores that may be carried by nitidulids as they leave the mats. Since the beetles crawl over, feed on, and burrow into the mats, spores would be expected to adhere to their body parts; however, location of spores on these insects has not been investigated. Speculation about the type of spores of *C. fagacearum* present on nitidulids has been largely based on the presence or absence of perithecia on the mats from which the beetles were collected.

To learn more about this vector relationship, we examined free-flying nitidulids attracted to bait traps in an oak wilt area and nitidulids collected from sporulating mats. Our objectives were to determine: the occurrence of different species of Nitidulidae during spring in oak wilt areas in Minnesota; the occurrence and frequency of *C. fagacearum* on free-flying and mat-inhabiting beetles; and amount, location, type, and viability of *C. fagacearum* spores on beetles associated with mats. Preliminary results from this study were reported in abstract form (9).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Nitidulid collection. Free-flying nitidulids were attracted to two different types of traps by odor baits. The study site was located in the Carlos Avery Wildlife Management Area, Anoka County, near Wyoming, MN (hereafter referred to as Carlos Avery). Ten flight traps, similar to those described by Castello et al (2), were baited with melon and fermenting flour dough and suspended at 10–15 m spacing from the most recently wilted oaks on the eastern periphery of an active oak wilt infection center.

Five dough traps were placed in the surrounding healthy northern pin oak stand, ~20 m from the periphery of the same infection center and at a height of 1 m. The traps were spaced ~15 m apart and were NE, E, or SE of the center. Dough traps consisted of trays containing sticky, fermenting flour dough covered with a layer of flattened and crumpled moistened paper towel under a large mesh screen (12). Insects were removed from all traps three times each week; baits were changed weekly.

Mat production on wilted trees was recorded during bi-weekly surveys in the Carlos Avery infection center and in a second oak wilt area at Eagle Lake near Big Lake, MN. Only new mats that had ruptured the bark were recorded. Nitidulids were obtained from mats by removing the bark, exposing the fungus, and collecting the insects present.

Insects obtained by all three methods were placed individually in gelatin capsules and stored at 0 C. The beetles were tentatively identified, sorted into groups of similar species, and arranged by week of collection and collection method. Approximately one-fourth of all nitidulids collected were reserved for species determination. Confirmations and, in some cases, species determination were made by T. C. Skalbeck.

Fungus isolation. Presence of viable propagules of *C. fagacearum* and *C. piceae* (Munch) Bakshi on beetles was determined by a dilution technique. Mycelium and spore characteristics were used to identify *C. fagacearum*; synnemata and mycelial conidia were used for *C. piceae*. Each of the beetles tested was shaken from its storage capsule into a vial containing 1.5 ml of sterile distilled water. The vial was capped and held in a water-bath

Bransonic 220 sonicator for 1 min to dislodge fungus propagules from the insects. Three serial 10-fold dilutions of the resulting suspension were made with sterile distilled water and 0.5-ml amounts of each dilution were spread on potato-dextrose agar and Barnett's oak wilt agar (1) in petri dishes and incubated at room temperature. Colonies were counted after 7 and 14 days. All beetles tested were processed within 3 mo after collection. Storage of beetles infested with *C. fagacearum* for this period of time at 9 C did not appreciably affect fungus viability (18).

Fungus observation. Ascospores and conidia of *C. fagacearum* on the external surfaces of nitidulids collected from mats were observed by using scanning electron microscopy (SEM) (Fig. 1). A pair of similar nitidulid species collected on the same date were mounted on an aluminum stub with the ventral surface of one and the dorsal surface of the other uppermost. Specimens were coated with 40% gold–60% palladium in a Kinney KSE-2A-M vacuum evaporator and observed with a Philips scanning electron microscope at 12 kV.

RESULTS

Glischrochilus quadrisignatus Say was the beetle species most commonly trapped at the Carlos Avery site throughout the 13-wk period (Table 1). *Carpophilus* species included *C. brachypterus* (Say), *C. dimidiatus* (F.), and *C. sayi* Parsons. Two species of *Epuraea* identified were *E. labilis* Erich. and *E. peltoides* Horn; *Cryptarcha ampla* Erich. and *C. concinnea* Melsh. also were identified.

Of 1,043 free-flying nitidulids trapped between 30 March and 26 June at the Carlos Avery site and tested for *C. fagacearum*, seven yielded the fungus in culture. The collection dates of these beetles and the numbers yielding the fungus per total tested for those collection dates were: 30 March–3 April, 1/16; 18 May–22 May, 1/24; 25 May–29 May, 1/18; 1 June–5 June, 2/120; and 15 June–19 June, 2/9. Five of the seven nitidulids with *C. fagacearum* were collected from flight traps during four of the five weeks. The number of viable propagules of the fungus estimated to be present on the seven beetles ranged from 30 to 6,100. Because of the isolation technique employed, 30 was the lowest number of propagules that could be detected. Nitidulids carrying *C. fagacearum* were of at least five species: *G. sanguinolentus*, *G. fasciatus*, *Carpophilus* sp., *Epuraea* spp., and *G. siepmanni* or immature *G. quadrisignatus*.

The *Pesotum* state of *C. piceae* was also isolated from seven free-flying nitidulids. Four of these beetles had been trapped on 10 April, two on 27 April, and one on 5 June; the latter beetle was one that also had yielded *C. fagacearum*. Two of the seven nitidulids with *C. piceae* were collected from flight traps; the others were from dough traps. Species carrying this fungus were *G. fasciatus*, *G. sanguinolentus*, *Epuraea* sp., and *G. siepmanni* or immature *G. quadrisignatus*.

New mats were found on three dates in the infection center at the Carlos Avery site during the insect collection period (Fig. 2). The date for maximum number of nitidulids trapped coincided with the time of maximum mat production.

Species and numbers of nitidulids collected from individual mats occurring on four trees on 11 May and 27 May in the Carlos Avery infection center include *C. truncatus*, 19; *Epuraea* spp., 11; *G. sanguinolentus*, 6; *G. fasciatus*, 3; and *Carpophilus* spp., 2. *C. fagacearum* was isolated from 7 of 11 mycelial-mat-inhabiting nitidulids tested, while *C. piceae* was obtained from all 11. Conidia of *C. fagacearum* were observed on one, and ascospores on 12 of 15, beetles examined with SEM. The spores were distinguishable on the basis of morphology and size (Fig. 1). Other fungus spores were present but not identified. Conidia of *C. piceae* were distinguishable from spores of *C. fagacearum*, being tapered on one end and noticeably smaller. It was not possible, however, to differentiate between conidia of *C. piceae* and those of other unidentified spores also observed on mat-inhabiting nitidulids.

Nitidulids were collected from mats at the Eagle Lake site on 1 April, 10 April, 29 April, 11 May, and 27 May (Table 1). *C. fagacearum* was recovered from 80 of 98 nitidulids collected from mats at Eagle Lake (Table 2). Peak mat production occurred between 25 April and 8 May with 173 new mats detected on 8 May. Number of mats recorded for the other six survey dates were: 30 March, 37; 10 April, 23; 24 April, 52; 27 May, 95; 5 June, 16; and 19 June, 7.

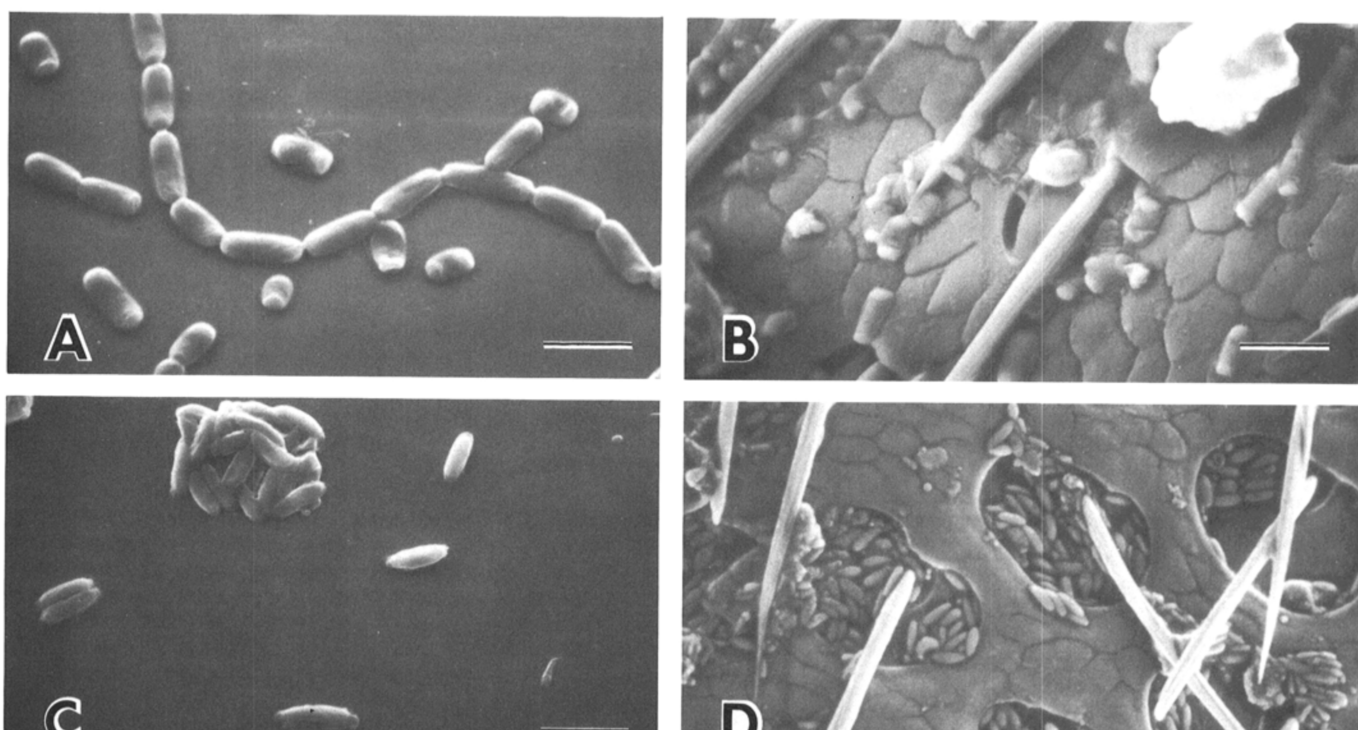

Fig. 1. Scanning electron micrographs of *Ceratocystis fagacearum* spores. **A,** Conidia from culture of fungus (bar = 10 μm). **B,** Conidia observed on dorsal surface of a nitidulid collected from a sporulating mycelial mat (bar = 10 μm). **C,** Ascospores from perithecia found on a mat in nature (bar = 10 μm). **D,** Ascospores on dorsal surface on a nitidulid collected from a mat (bar = 10 μm).

C. piceae was isolated from 17 of the same group of 98 mat-inhabiting nitidulids. The occurrence and frequency of the fungus on the beetles according to species were: *Epuraea* spp., 4; *G. sanguinolentus*, 5; *G. obtusus*, 3; *C. truncatus*, 4; and *G. quadrisignatus*, 1. Number of nitidulids with *C. piceae* per total number cultured for each date include: 10 April, 5/16; 29 April, 9/62; 11 May, 3/12; and 27 May, 0/5. Seven beetles yielded both *C. fagacearum* and *C. piceae*.

Conidia and ascospores were observed on all areas of the dorsal and ventral surfaces of nitidulids collected from mats at Eagle Lake (Table 3). Spores were sometimes present singly; other times in clusters of few to many. Grooves between body segments, troughs of the thorax and elytra edges, pitlike depressions in the elytra, and body hairs were common collecting points for spores on the dorsal surface. Spores were especially common on the legs were clusters were observed in grooves of the tibia and femur, around hairs and bristles of the tarsi, and between tarsal segments. Grooves in the thoracic surface and abdominal segments were also areas of spore concentration. Large numbers of spores were smeared across abdominal segments of several beetles.

TABLE 1. Species of nitidulids and their percentage of the total population collected from oak wilt areas during spring 1981 in two Minnesota locations

Species	Percent of total (%) Carlos Avery[a]	Eagle Lake[b]
Glischrochilus quadrisignatus	38.9	5.0
G. fasciatus	18.7	5.0
G. sanguinolentus	13.6	15.9
G. siepmanni	10.7	1.0
Epuraea spp.	6.9	9.0
Cryptarcha spp.	6.2	...[c]
Colopterus truncatus	3.3	62.3
Carpophilus spp.	0.8	2.5
Lobiopa undulata	0.5	1.0
G. obtusus	0.4	...

[a]Of 1,898 free-flying nitidulids trapped near an oak wilt infection center, Carlos Avery Wildlife Management Area, near Wyoming, MN.
[b]Of 201 nitidulids collected from oak wilt fungus mats on northern pin oaks at Eagle Lake, near Big Lake, MN.
[c]Species not collected.

TABLE 2. Occurrence and estimated number of viable *Ceratocystis fagacearum* spores on surfaces of nitidulid species collected from mats during spring 1981 at Eagle Lake

Date	Species	No. with *C. fagacearum*/ no. tested	Range of maximum no. of spores per beetle
1 April	*Epuraea* spp.	3/3	2.4 -3.6×10^4
10 April	*Epuraea* spp.	4/6	$2.1 \times 10^3-7.3 \times 10^5$
	Colopterus truncatus	3/4	$1.5 \times 10^2-4.8 \times 10^4$
	Glischrochilus sanguinolentus	4/4	$4.5 \times 10^3-6.0 \times 10^4$
	Lobiopa undulata	0/2	0
29 April	*C. truncatus*	42/48	$3.0 \times 10^1-1.2 \times 10^5$
	G. sanguinolentus	10/10	$6.1 \times 10^1-7.6 \times 10^5$
	G. siepmanni	2/2	$3.0 \times 10^3-2.7 \times 10^4$
	G. quadrisignatus	2/2	$2.4 \times 10^3-1.0 \times 10^5$
11 May	*Epuraea* spp.	2/3	$6.1 \times 10^1-3.0 \times 10^3$
	G. truncatus	4/5	$3.0 \times 10^1-3.0 \times 10^3$
	G. sanguinolentus	2/3	1.2 -3.0×10^3
	Carpophilus spp.	1/1	$3.0 \times 10^2-2.4 \times 10^3$
27 May	*Epuraea* spp.	0/1	0
	G. sanguinolentus	1/1	1.6 -2.4×10^4
	G. quadrisignatus	0/1	0
	Carpophilus spp.	0/2	0

DISCUSSION

Skalbeck (15) collected 34 species of nitidulids from healthy northern pin oaks stands in Minnesota during April and May. Fewer species were obtained from our collection in oak wilt areas. The limited number of beetles collected in this study compared to the thousands of nitidulids collected by Skalbeck could readily explain the difference in number of species.

The small proportion of nitidulids carrying viable *C. fagacearum* suggested that only a low percentage of the total population of

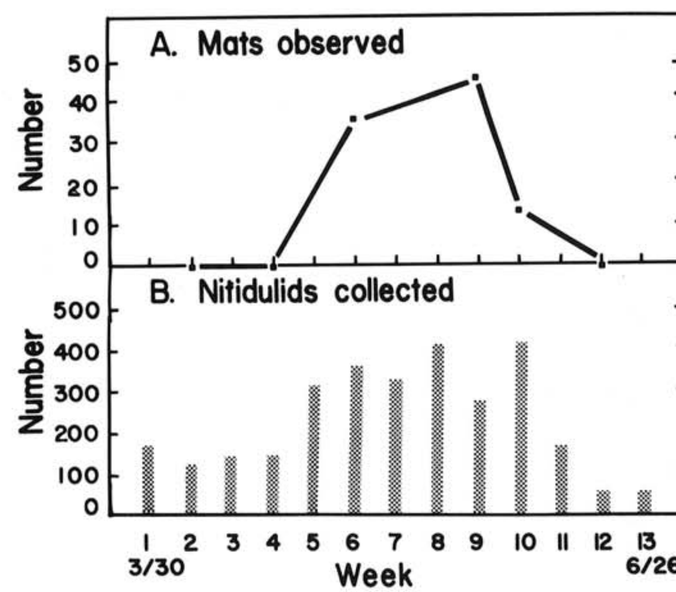

Fig. 2. Temporal distribution of new oak wilt fungus mats and number of nitidulids collected from 30 March to 26 June 1981 at Carlos Avery Wildlife Management Area near Wyoming, MN.

TABLE 3. Numbers of nitidulids with spores of *Ceratocystis fagacearum* observed on their surface using scanning electron microscopy. Beetles were collected from sporulating mats on northern pin oaks at Eagle Lake, near Big Lake, MN, in 1981

Date collected	Species	Dorsal surface: Total observed	Dorsal: Number with: Conidia	Dorsal: Ascospores	Dorsal: Both	Ventral surface: Total observed	Ventral: Number with: Conidia	Ventral: Ascospores	Ventral: Both
1 April	*Epuraea* spp.	2	2	1	1	2	2	0	0
29 April	*Epuraea* spp.	...[a]	...	...	...	1	1	0	0
	Colopterus truncatus	13	8	1	1	13	11	0	0
	Glischrochilus sanguinolentus	3	2	1	1	3	3	2	2
	G. quadrisignatus	2	2	0	0	1	1	0	0
	G. fasciatus	...	...	...	...	1	0	1	0
11 May	*Epuraea* spp.	1	0	0	0	2	0	2	0
	C. truncatus	3	0	3	0	3	1	3	1
	G. sanguinolentus	3	0	3	0	2	1	1	0

[a]Species not examined.

nitidulids in an area with recently wilted oaks carried viable propagules of this fungus. Yount et al (18) reported a similar frequency of fungus recovery from nitidulids collected at biweekly intervals from freshly cut stumps and bait traps located near an oak wilt infection center from 10 February to 11 June in Pennsylvania where *C. fagacearum* was isolated from 2 of 120 beetles in one of seven lots of free-flying nitidulids tested.

The earliest isolation of *C. fagacearum* was from a nitidulid trapped on 1 April. The nitidulids from which Yount et al (18) recovered the fungus had been collected on 6 April. The presence of viable *C. fagacearum* spores on a nitidulid collected in April suggests infection could occur in early spring. Possible explanations for the presence of *C. fagacearum* on a nitidulid in early spring are that the fungus may have overwintered on the beetle; or the beetle, just prior to being trapped, could have acquired the propagules from newly formed mats which were not detected in the first mat survey; or the beetle could have acquired the fungus just prior to being trapped from mats which had formed late in fall 1980 and remained viable through the winter.

The time period during which six of the beetles carrying *C. fagacearum* were trapped coincides with the rate of decline of mats during May and June, and with the behavior of nitidulids associated with mats. Curl (3) found that mats newly formed in May, June, and July in Illinois deteriorated rapidly, sometimes within 14 days. When present, nitidulids contributed greatly to the deterioration. It is hypothesized that nitidulids are attracted to trees with fresh mats by the fruit-like odor of the fungus; they gain access to the sporulating structures through narrow cracks in the bark created by the pressure pads; the insects then acquire spores as they crawl over and feed on the fungus, contributing to the deterioration of the mats; and after several weeks, when the structures are in poor condition, the nitidulids leave the mats (3,6,13). In this study, the recovery of *C. fagacearum* from six free-flying beetles 2–3 wk after maximum numbers of new mats were reported is consistent with this sequence of events.

C. truncatus was the most abundant species found on mats at both the Carlos Avery and Eagle Lake sites. A large percentage of the *C. truncatus* collected from mats at both locations carried *C. fagacearum*. Because of these observations one might suspect *C. truncatus* to be one of the more important nitidulid species involved in overland transmission of the fungus; however, none of the 41 free-flying *C. truncatus* collected at the Carlos Avery site yielded *C. fagacearum*. Norris (13) considered *C. truncatus* the most important of the potential vectors in his study.

The occurrence and frequency of conidia and ascospores of *C. fagacearum* over time is consistent with the progress of fungus sporulation on trees where sexually compatible strains are present and perithecia as well as conidia occur. Conidia are the predominant spores in early spring and ascospores are more common on beetles later in the spring.

Yount et al (18) suggested that spores of *C. fagacearum* may not adhere well to nitidulids. We observed large clusters of spores of the fungus on all parts of the surfaces of nitidulids collected from mats. The location of these clusters indicates that nitidulids can leave mats with large numbers of spores adhering to their bodies.

Nitidulids are commonly attracted to sap exudates of oaks. Spores of *C. fagacearum* present on a free-flying nitidulid which visits a wound could presumably be easily dislodged by sap flow or by contact with the wound surface. In view of the large number of viable spores present on nitidulids collected from mats and traps, we postulate that the probability of infection would be quite high if a *C. fagacearum*-infested beetle reached a fresh wound with exposed xylem vessels.

The *Pesotum* state of *C. piceae* was also isolated from free-flying and mat-inhabiting nitidulids. This fungus commonly colonizes oak wilt mats in Minnesota (5) and has been frequently isolated from wounds on healthy oaks in West Virginia (14). Jewell (8) found the fungus to be constantly associated with nitidulids in wounds on healthy trees, and suggested that *C. piceae* is probably carried to tree wounds by insects. We have shown that nitidulids may acquire the fungus from mycelial mats of *C. fagacearum* and that free-flying nitidulids in nature carry viable spores of *C. piceae*.

Experimental inoculation of fresh wounds on northern pin oaks with *C. piceae* 24 hr before challenge inoculation with *C. fagacearum* prevented infection by the latter (5). Attraction of a beetle with *C. piceae* to a wound on a healthy oak could result in the introduction and establishment of the fungus on the wound surface prior to the introduction of *C. fagacearum* by the same vector group. Wounds on oaks are only susceptible to infection by *C. fagacearum* for a short period of time (10,11). Natural colonization of wounds by *C. piceae* may be partly responsible for the altered susceptibility (5). As carriers of *C. piceae*, nitidulids may well play a role in this example of natural biological control.

C. piceae was more frequently recovered from free-flying nitidulids collected in early spring than later on. This suggests that wounds made in early spring may be more frequently colonized by this fungus than those occurring later. More information is required on this point.

Gibbs (5) also reported that simultaneous inoculation of wounds with *C. fagacearum* and *C. piceae* did not stop infection and disease development. In this study, one free-flying and several mat-inhabiting nitidulids yielded both *C. piceae* and *C. fagacearum* in culture. Based on Gibbs' (5) results, it seems very likely that such a beetle would be able to initiate infection if it arrived at a susceptible wound.

In summary, few of the free-flying nitidulids trapped in oak woods surrounding an oak wilt infection center carried viable propagules of *C. fagacearum* and *C. piceae*. However, the numbers of spores of *C. fagacearum* on the surface of these beetles seem to be quite adequate for infection if the insects were to reach a fresh wound on a healthy oak. In addition, the location of the spores on the surface of the nitidulids seem to favor ready transfer of the fungus to the tree. At the same time, by carrying *C. piceae* to fresh wounds, nitidulids may play a role in the natural biological control of *C. fagacearum*.

LITERATURE CITED

1. Barnett, H. L. 1953. A unisexual male culture of *Chalara quercina*. Mycologia 45:450-457.
2. Castello, J. D., Shaw, C. G., and Furniss, M. M. 1976. Isolation of *Cryptoporus volvatus* and *Fomes pinicola* from *Dendroctonus pseudotsugae*. Phytopathology 66:1431-1434.
3. Curl, E. A. 1955. Natural availability of oak wilt inocula. Ill. Nat. Hist. Surv. Bull. 26:277-323.
4. 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.
5. Gibbs, J. N. 1980. Role of *Ceratocystis piceae* in preventing infection by *Ceratocystis fagacearum* in Minnesota. Trans. Br. Mycol. Soc. 74:171-174.
6. Gibbs, J. N., and French, D. W. 1980. The transmission of oak wilt. USDA For. Serv. Res. Pap. NC-185. 17 pp.
7. Himelick, E. B., and Curl, E. A. 1958. Transmission of *Ceratocystis fagacearum* by insects and mites. Plant Dis. Rep. 42:538-545.
8. Jewell, F. F. 1956. Insect transmission of oak wilt. Phytopathology 46:244-257.
9. Juzwik, J., and French, D. W. 1982. Occurrence and frequency of *Ceratocystis fagacearum* on free-flying nitidulids in Minnesota. (Abstr.) Phytopathology 72:958.
10. Kuntz, J. E., and Drake, C. R. 1957. Tree wounds and long distance spread of oak wilt. Phytopathology 47:22.
11. Morris, C. L., Thompson, H. E., Hadley, B. L., and Davis, J. M. 1955. Use of radioactive tracer for investigation of the activity pattern of suspected insect vectors of the oak wilt fungus. Plant Dis. Rep. 39:61-63.
12. Mussen, E. D. 1969. Some laboratory observations on the development and behavior of *Glischrochilus quadrisignatus*. M. S. thesis, Univ. of Minn., St. Paul. 76 pp.
13. Norris, D. M. 1956. Association of insects with the oak tree and *Endoconidiphora fagacearum* Bretz. Ph.D. thesis, Iowa State College, Ames. 284 pp.
14. Shigo, A. L. 1958. Fungi isolated from oak-wilt trees and their effects on *Ceratocystis fagacearum*. Mycologia 50:757-769.
15. Skalbeck, T. C. 1976. The distribution of Nitidulidae in deciduous

forests of Minnesota. Ph.D. thesis, Univ. of Minn., St. Paul. 260 pp.
16. Stambaugh, W. J., and Fergus, C. L. 1956. Longevity of spores of the oak wilt fungus on overwintered nitidulid beetles. Plant Dis. Rep. 40:919-922.
17. Stambaugh, W. J., Fergus, C. L., Craighead, F. C., and Thompson, H. E. 1955. Viable spores of *Endoconidiophora fagacearum* from bark and wood-boring beetles. Plant Dis. Rep. 39:867-871.
18. Yount, W. L., Jeffery, A. R., and Thompson, H. E. 1955. Spores of *Endoconidiophora fagacearum* on the external surface of the body of nitidulids. Plant Dis. Rep. 39:54-57.