

# Relationship Between Nitidulids and *Ceratocystis fagacearum* During Late Summer and Autumn in Minnesota

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## ABSTRACT

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Free-flying nitidulids (Coleoptera: Nitidulidae) were collected from an oak wilt area in Minnesota from 30 August to 22 October 1982. *Ceratocystis fagacearum* was isolated from two of 1,145 (0.17%) beetles. None of 11 oaks with nitidulids (collected from sporulating mats) caged over wounds wilted; however, two of five oaks receiving 1 ml of a water wash of similarly collected beetles in a fresh wound wilted. About 3,300 nitidulids marked with fluorescent powder were released near wounded trees in September and October. Marks indicating insect visitation as well as marked beetles were found in wounds on eight trees during the 11 days after release. Unmarked nitidulids were collected from wounds on three trees. From these observations, nitidulids might transmit *C. fagacearum* to susceptible oaks during late summer and autumn in Minnesota, but this is probably rare.

Nitidulids (Coleoptera: Nitidulidae) are vectors of *Ceratocystis fagacearum* (Bretz) Hunt, the cause of oak wilt (6). Infection may occur when fungus-infested beetles visit fresh wounds on susceptible oaks. Natural overland spread almost always occurs during spring. For all but one report, infection occurred only in trees wounded between 24 April and 22 June (5). In the single exception, 21 of 101 red oaks pruned during winter in Pennsylvania wilted the following summer (2). No evidence of overland transmission during autumn has been observed in Minnesota (D. W. French, unpublished).

The question of whether overland spread of oak wilt can occur in Minnesota other than April through June is important. Currently, owners of oak trees in southeastern Minnesota are advised to avoid pruning or wounding oaks during the critical spring period; paint or wound dressing may be used to cover the surface if wounds occur at this time. If transmission can occur more than on rare occasions during autumn, this recommendation should be revised.

Inoculum (conidia and/or ascospores) produced on mats on oaks recently killed by *C. fagacearum* is most abundant in Minnesota during spring and autumn (1).

Three species of nitidulids known to carry the fungus during spring (7) are also most abundant during autumn in oak forests in Minnesota (12). In addition, nitidulids can be found on mats in Minnesota during these periods. Thus, it seems plausible that inoculation via insects might occur during autumn as well as spring. Jewell (6) reported that wounds made in autumn were not attractive to nitidulids. When fungus-infested insects were caged over or placed on fresh wounds, infection resulted only during spring (3,6,8,11,13); however, *C. fagacearum* has been isolated from nitidulids collected from fresh mats during autumn (14). Isolation from free-flying nitidulids during autumn has not been attempted previously. Studies reported here were designed to determine 1) occurrence of species of Nitidulidae in areas with oak wilt in Minnesota during late summer and autumn, 2) occurrence and frequency of *C. fagacearum* on free-flying nitidulids in the same areas, 3) whether caging infested nitidulids over fresh wounds on oak during autumn would result in infection, and 4) frequency of nitidulids in fresh wounds during autumn.

## MATERIALS AND METHODS

**Nitidulidae collection.** The study site was located in the Carlos Avery Wildlife Management Area, Anoka County, near Wyoming, MN. Free-flying nitidulids in or near an oak wilt infection center were attracted to two types of traps by odor baits. Ten flight traps (7), baited with honeydew melon and fermenting flour dough, were suspended from trees around the periphery of a group of 14 oaks that wilted from mid-July to mid-August 1982. Traps were 5–10 m from the nearest wilted tree. Oak blocks

soaked in vinegar, similar to those used by Neel et al (10), were placed on platforms 1.2 m above the ground. These were interspersed with the flight traps. Insects were removed from all traps three times each week; baits were changed weekly. The oak blocks were replaced every 2 wk. Mat production on the wilted trees was recorded biweekly. Only new mats that ruptured the bark were recorded. Trapped insects were placed individually in gelatin capsules and stored at 0 C. The beetles were identified and sorted according to species, week of collection, and method of collection.

Fungus-infested nitidulids for the caging study were obtained from fresh, sporulating mats on northern pin oak (*Quercus ellipsoidalis* E. J. Hill) at a second oak wilt area (Sherburne County) 64 km west of the Carlos Avery site. In the laboratory, the nitidulids were divided by species, and 10 of each species were placed in sterile plastic petri dishes with a paper disk, then stored at 8 C for 1–2 days before the caging experiment.

Nitidulids for the release experiment were collected from five dough traps (7) on platforms in a different area of the oak stand at Carlos Avery. These insects were separated into lots of 20–30, placed in sterile petri dishes with paper disks, and stored at 8 C for no longer than 2–3 days before their release.

**Fungus isolation.** Presence of viable propagules of *C. fagacearum* on free-flying nitidulids was determined by a previously described dilution technique (7). Aliquots (0.5 ml) of the insect wash were spread on potato-dextrose agar in petri dishes and incubated at room temperature. All beetles tested were processed within 3 mo of collection.

**Insect caging.** A total of 24 healthy northern pin oaks 9.6–18.3 cm dbh (1.4 m) at North Oaks, MN (Ramsey County), were wounded in October 1982, exposing a cambial area of 30–40 cm<sup>2</sup>. Each tree received one of the following treatments: A) 10 *Glischrochilus quadrisignatus* Say, obtained from mats, placed on a small cardboard tray stapled 3–4 cm above the wound; B) five *G. quadrisignatus* from mats, placed in 5 ml of sterilized distilled water, subjected to sonication for 1 min, and 1 ml of the wash pipetted onto the fresh wounds, C) wounded only, and D) 1 ml of sterile distilled water added to wounds. Immediately after treatment, fine-mesh nylon screening about 1 m wide was placed around the wounded section

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of each stem. Wood blocks 10 cm above and below the edge of the wound kept the screen from touching the wound surface. The top and bottom portions of the screen were secured against the bark with twine and sealed to prevent escape of nitidulids and entry by other insects.

**Nitidulid release.** Two circular plots (30-m radius) were each divided into eight equal sections. Immediately before insect release, 15 trees in each section received two axe wounds into the xylem on the main stem 1.4 m above the ground. One wound faced the plot center (inner), the other faced away from the plot center (outer). The wounded trees were distributed uniformly in each section.

A marking technique described by Foott (4) was modified for use in this study. Green and orange fluorescent powders (Day-Glo Color Corp., Cleveland, OH) were used to mark beetles. A small amount of powder was placed in a cardboard box and shaken to distribute the powder. Nitidulids were then placed in the box in lots of 300–400 and the box was agitated slightly. The marked beetles were gently shaken onto a 0.25-m<sup>2</sup> platform 1.4 m above the ground in the plot center. Beetles were released only on sunny afternoons when the temperature was higher than 20 C, because flight is inhibited in cool weather (4).

On 9 September 1982, 1,800 nitidulids were released at one plot center at noon. *G. quadrisignatus* and *G. siepmanni* made up 90% of those released. About 200 beetles died immediately after treatment. Weather conditions were recorded off-site (3 km distant). The temperature was 29 C, relative humidity was 65%, and the wind was from the southwest at 1.34 m/sec, gusting at times to 2.2 m/sec.

On 4 October 1982 at 2:30 P.M., 1,500 beetles were released; about 100 of them did not survive marking. Weather conditions were recorded on-site. The temperature was 22 C and relative humidity was 50%. The wind direction alternated from south-southwest to northeast; velocity varied from 0.5 to 0.9 m/sec in a 1.5-hr period.

Observations on initial flight were made possible by the visibility of the fluorescent powder. Observations on takeoff and initial flight of the beetles were recorded.

Wounds on trees were checked for nitidulids or evidence of their visitation (i.e., powder mark) 2–3 hr after release and for 5 days after release.

## RESULTS AND DISCUSSION

*G. quadrisignatus* and *G. fasciatus* Oliv. were the nitidulid species most commonly trapped at Carlos Avery from 30 August to 22 October 1985, accounting for 37.5 and 36.5%, respectively, of the total 2,180. Other species included *G. siepmanni* (18.8%), *Colopterus truncatus* Rand (2.9%), *G. sanguinolentus* Oliv.

(1.5%), *Cryptarcha ampla* Erich. (1.1%), *C. concinna* Melsh (0.6%), *Epuraea* spp. (0.5%), and *Carpophilus* spp. (0.2%). The greatest number of nitidulids was collected between 27 September and 1 October (Fig. 1).

Skalbeck (12) also had commonly collected these species during both seasons in oak forests in Minnesota. Fewer nitidulid species were collected in autumn than in spring as Skalbeck (12) had reported. Two of 1,146 (0.17%) free-flying beetles carried viable propagules of *C. fagacearum*. The *G. fasciatus* and the *G. siepmanni* with the fungus were collected from flight traps on 4 October. A similar low frequency had been observed in spring (7). Mat production, however, was less abundant in the area in autumn of 1982 than during spring of that year. The fungus-infested beetles had been collected during the mat production period. It has been suggested that infection through wounds is not likely unless mats at the right stage of decline are available at the time of wounding or shortly thereafter (9). Insects have been observed on wounds in mid-June coinciding with cessation or dramatic decrease in mat production and deterioration of existing mats (8). The situation appears to be different in autumn. The sequence of mat formation and decline during autumn is not the same as that during spring. In spring, fresh mats deteriorate more rapidly because of increasing average daily temperature, whereas diminishing average temperatures tend to prolong mat freshness in autumn (J. Juzwik, *personal observation*). The decrease in temperature in autumn below that associated with nitidulid activity (20 C) (4) would, however, lessen the likelihood of mat visitation by nitidulids and of subsequent wound visitation.

Trees receiving treatment on 9 September 1982 as part of the caging experiment were observed for wilt in September 1983. Two of the five wounded oaks that received 1 ml of wash water from beetles wilted, whereas none of the 11 wounded trees with caged nitidulids from mats wilted. All eight control trees (five wounded only and three treated with sterilized distilled water) remained healthy.

The failure of infection to occur after the infested nitidulids were caged over fresh wounds on susceptible oaks does not necessarily refute the possibility that transmission by nitidulids could occur in autumn in Minnesota. The percentage of successful transmission obtained in caging experiments with nitidulids has varied greatly (5).

During the first release of nitidulids, the beetles generally first rose 1–2 m above the platform, then flew east, northeast, or north. No fluorescent powder marks were observed in the wounds on surrounding trees 2 hr after release or during any of the subsequent

daily checks. One unmarked *Cryptarcha ampla* was collected from a wound in the south-southeast sector 8 days after the release. Because no trace of the powder was observed when the insect was dissected, the beetle evidently was not one of those released.

Within 2 hr of insect release on the second date, marked nitidulids had visited wounds. Three *G. fasciatus* were found in wounds on three trees at this time, two in wounds facing the plot center and one in a wound facing away from the plot center. In addition, on 4 October, orange marks were found in wounds of two trees facing the plot center and two unmarked beetles, *G. fasciatus* and *G. sanguinolentus*, were collected from wounds on two trees. Marks also were observed in wounds on three trees during subsequent daily checks on 6, 11, and 15 October.

Activity and behavior of nitidulids leaving fungus mats during autumn are probably different than during spring. In fact, they may prefer to remain in place in autumn. Jewell (6) stated that nitidulids do not visit fresh wounds in autumn. We have recovered both marked nitidulids after their release and naturally occurring nitidulids in fresh wounds in mid-September and early October. The insects apparently were attracted to the wounds rather than encountering them by chance because marks from released beetles were found on outward-facing wounds in the second experiment. If activity of nitidulids after experimental release can be compared to the situation in nature when nitidulids leave a mat, *C. fagacearum*-infested beetles could theoretically be similarly attracted to an available fresh wound; however, the attraction may not be as strong as during spring. Sap flow is not as abundant in autumn, and sap composition may be different from that in spring. It also is possible that behavior and instinct of the insects during autumn override this attraction. For example, an infested beetle leaving a mat may be inclined to seek an overwintering site rather than sap in a wound.

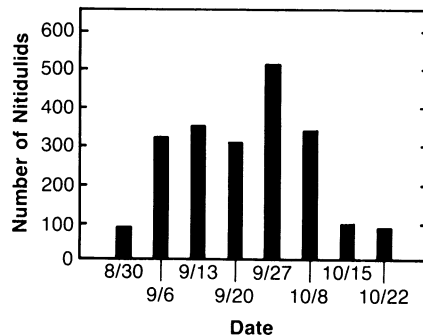


Fig. 1. Numbers of nitidulids collected from 30 August to 22 October 1982 at Carlos Avery Wildlife Management Area. New mats of *Ceratocystis fagacearum* were forming during the fifth and seventh weeks.

In summary, free-flying nitidulids were rarely found infested with *C. fagacearum* during late summer and autumn. Released nitidulids were also rarely attracted to fresh wounds on oak. Therefore, it appears that overland spread of *C. fagacearum* by nitidulids may occur during this time period but would be infrequent.

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