Insect Transmission of Ceratocystis Species Associated with Aspen Cankers

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ABSTRACT

Numerous insects which frequent fresh aspen bark injuries can transmit the following species of *Ceratocystis: C. alba, C. crassivaginata, C. fimbriata, C. moniliformis, C. minor, C. pilifera, C. populina,* and *C. tremulo-aurea.* Certain insects belonging to the Nitidulidae, Rhizophagidae, Staphylinidae, and Drosophilidae families spend parts of their life cycles in fungus mats formed in the wounds. Stem inoculations proved that nitidulid beetles, *Epuraea* spp. and *Colopterus truncatus*; rove beetles, *Nudobius corticalis* and *Quedius raevigatus*; and a root-eating beetle, *Rhizophagus brunneus* were all vectors of *C. fimbriata* and other *Ceratocystis* species. Three of the insects carried at least three species of *Ceratocystis* upon emergence in the spring from overwintering pupae. Five species of *Ceratocystis* were recovered from surface-sterilized *Glischrochilus vittatus.* Nitidulids are considered the principal vectors of Ceratocystis canker of aspen in Colorado.

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Additional key words: perithecial succession, soil isolation, fungus inoculum.

Ceratocystis canker of aspen (*Populus tremuloides* Michx.) caused by the fungus *Ceratocystis fimbriata* Ell. & Halst., was first confirmed in Minnesota by Wood & French (13) in 1962, although the cankers, whose cause was unknown at the time, had been reported earlier from the western United States (2, 4, 9, 11).

Insect transmission of *Ceratocystis* species is common; two specific studies have demonstrated ability of insects to transmit *C. fimbriata*. Crone & Bachelder (6) obtained infection on London plane tree in 6 of 11 trials by placing beetles on cultures of the fungus prior to using them as inoculating agents. Insects collected from diseased plane trees, however, only transmitted *C. fimbriata* directly in two of seven trials. Moller & DeVay (12) provided circumstantial evidence for transmission of *C. fimbriata* by insects to almond. They isolated the fungus from field-collected insects, and transferred insects reared on cultures of the fungus to healthy trees. They demonstrated direct transmission and subsequent infection with two of the many insect species tested. The exclusion of insects and mites by a screen covering fresh bark wounds prevented infection and canker development on the trunks of control trees.

The fact that *C. fimbriata* is insect-transmitted in other hosts suggested that insects may be involved in the transmission of *C. fimbriata* in aspen, particularly as insects are commonly present on or about Ceratocystis cankers on aspen, and infection normally occurs at trunk wounds (7). This paper reports on studies conducted to investigate this possibility.

MATERIALS AND METHODS.—To attract insects, aspen trees in two areas of the Roosevelt National Forest, Larimer County, Colo., were wounded biweekly throughout the summers of 1967 and 1968. An area of bark about 10 X 10 cm was cut loose from the trunk with a hatchet on each of two trees. Insects attracted to each wound were collected with an aspirator biweekly and put in separate vials. Those to be used for disease transmission studies were collected individually in sterile gelatin capsules. Specimens of each apparent species of insect were preserved in alcohol, and most were identified by the Insect Identification and Parasite Introduction Research Branch, Entomology Research Division, USDA, Beltsville, Md. The staphylinids were identified by Ian Moore, El Cajon, Calif.

RESULTS.—*Association of insects with trunk wounds.*—Time of insect emergence in the spring was unknown; trunk wounds made the first week in June did not attract insects until 3 weeks later. Wounds made in July, August, and the first week in September, however, attracted adult insects within a few days. The wounds became infected with different species of *Ceratocystis* soon after insect visitation, and a succession of perithecial formations was apparent. Mature perithecia of *C. alba* DeVay, Davidson, & Moller formed in 4-5 days; *C. moniliformis* (Hedgc.) C. Moreau, in 5-7 days; *C. fimbriata* and *C. populina* Hinds & Davidson, in 7-10 days; *C. tremulo-aurea* Davidson & Hinds, in 10-14 days; and *C. crassivaginata* Griffin, within 21 days. Perithecia of the various species were frequently intermixed on wounds, but those of *C. alba* soon disintegrated after maturing. Sapwood invaded with *C. moniliformis* appeared pink for the first few days, then turned the characteristic blue-black discoloration similar to that caused by other species of blue-stain fungi. Infected wounds had the unique ester odors typical of *C. fimbriata* and *C. moniliformis* in culture.

Wounds attracted insects (Table 1) throughout the summer. Nitidulids usually appeared first, followed in a few days by staphylinids and flies. Of the nitidulids, three species of *Epuraea* and *Colopterus truncatus* were most abundant and most frequently collected. Morphological differences between the three *Epuraea* species are minor, and they are combined here for discussion. Insects mated and oviposited under the

TABLE 1. Frequency of *Ceratocystis* spp. associated with insects collected from fresh aspen trunk wounds in Colorado

Insect family and species	No. insects cultured	*Ceratocystis* spp. recovered				
		C. fimbriata	*C. populina*	*C. moniliformis*	*C. crassivaginata*	*C. pilifera*[b]
		%	%	%	%	%
Nitidulidae (sap-feeding beetles)						
Epuraea spp.[a]	51	16	90	13	2	
Colopterus truncatus Randall	50	44	41	11	2	3
Glischrochilus moratus Brown	10	40	100		10	20
Glischrochilus vittatus Say	35	49	94	9	14	17
Rhizophagidae (root-eating beetles)						
Rhizophagus brunneus Horn	41	32	83	29	15	17
Staphylinidae (rove beetles)						
Nudobius corticalis Casey	50	38	88	18	12	4
Quedius raevigatus Gyllenhal	31	26	100	26		
Quedius sp.	28	11	71	7		2
Drosophilidae (Pomace flies)						
Chymomyza aldrichi Sturtevant	27	22	70	22		15
Tachinidae (Tachina flies)						
Nowickia sp. [probably *N. latifrons* (Tothill)]	10		30			
Aphididae (aphids, plant lice)						
Plerocomma pseudopopulea Palmer	3	66		33		
Gelechiidae (Gelechid moths)						
Anacampis niveopulvella Chamberlin	8	50	12			
Oecophoridae (moths)						
Ethmia coloradella Chamberlin	2		50			50
Scolytidae (bark beetles)						
Trypodendron retusum LeConte	5	40	80			
Cicadellidae (leafhoppers)						
Idiocerus lachrymalis Fitch	27	37	52	19		
Anthocoridae (minute pirate bugs)						
Anthocoris musculus Say	3	33	100			33

[a] Three species of *Epuraea*: *E. avara* Randall; *E. erichsoni* Reitter; and *E. terminalis* Mann.
[b] 1968 collections only.

loose bark, and larvae of the nitidulids, staphylinids, and flies were common. Adult nitidulids died after oviposition. Nitidulid eggs hatched in ca. 1 week, and larvae matured in about 3 weeks, and dropped to the ground to pupate. At least two generations were produced during the summer, and larvae of the last generation overwintered as pupae in the soil at the base of the trees. *Glischrochilus moratus* and *G. vittatus*, while not so numerous, were present throughout the summer, and were more common in September and October. *Chymomyza aldrichi* adults, larvae, and pupae were common on wounds throughout the summer, whereas adult Gelechiidae, Oecophoridae, and Tachinidae occupied wounds only during cold weather. *Trypodendron retusum* and *Idiocerus lachrymalis* were collected on healthy bark adjacent to wounds. Numerous other insects visited the wounds, particularly during cold periods, but were not common enough to collect for culturing and identification.

Insect-Ceratocystis associations.—For isolation of fungi, individual insects were placed in petri dishes containing a 2% Fleischmann's diamalt and 2% Difco-Bacto agar medium within 2 hr after collection. The insects were allowed free movement in the plates for 24 hr to several days, after which they were pushed down into the agar.

The carrot technique recommended by Moller & DeVay (12) was used for the initial screening of insects for *C. fimbriata*. The method was effective, but too selective. Placing live insects directly in plates containing diamalt agar medium allowed growth and identification of the various species of *Ceratocystis* involved.

The nitidulids frequently laid eggs which hatched in the plates. Larvae and adults would consume

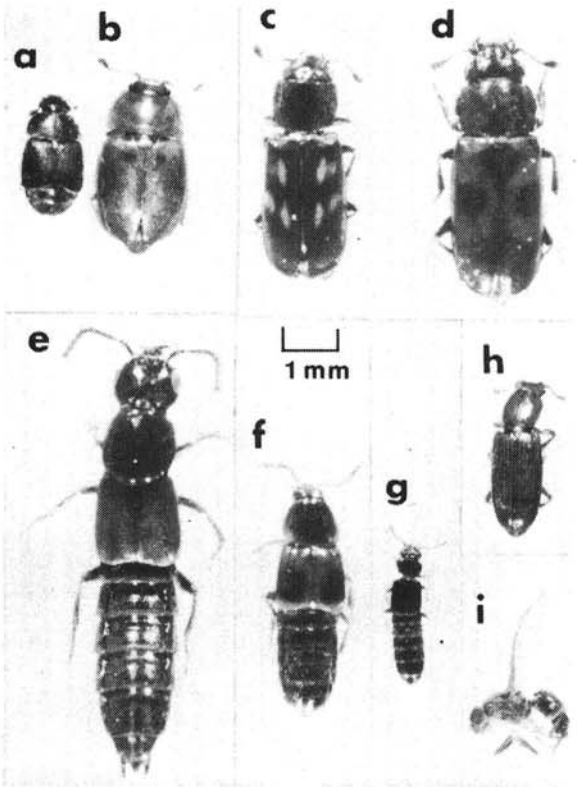

Fig. 1. Insects commonly found on fresh aspen trunk wounds in Colorado. Nitidulids: **a)** *Colopterus truncatus;* **b)** *Epuraea erichsoni;* **c)** *Glischrochilus vittatus;* and **d)** *Glischrochilus moratus;* staphylinids: **e)** *Quedius raevigatus;* **f)** *Quedius* sp.; and **g)** *Nudobius corticalis;* the root-eating beetle: **h)** *Rhizophagus brunneus;* and Pomace fly: **i)** *Chymomyza aldrichi*. Scale same for all insects.

perithecia, aerial mycelium, and yeast growing in the plates, but larvae seldom pupated; they died and became overgrown with fungi. Nematodes introduced by the insects were also common in some plates.

The insects (Fig. 1) often carried one or more species of *Ceratocystis*; it was not unusual to obtain at least three from a single insect. In addition to *C. fimbriata, C. crassivaginata, C. moniliformis, C. pilifera* (Fries) C. Moreau, and *C. populina* were frequently isolated. The common insects collected and the frequency of the various species of *Ceratocystis*, as obtained from plate cultures, associated with them are listed in Table 1.

It was not possible to identify all fungi which grew in the plates. It was not unusual to have 50 or more colonies of *Ceratocystis* in a plate (Fig. 2). Also, fast-growing species, such as *C. moniliformis*, would cover a plate within 10 days and obscure colonies of other species. *Ceratocystis crassivaginata*, which usually requires about 3 weeks for production of perithecia, was usually obtained only by subculturing. *Ceratocystis pilifera* was first detected in plates near the end of the 1st year of screening; consequently, identifications of this species in Table 1 pertain only to the 1968 collections. Although this fungus has a

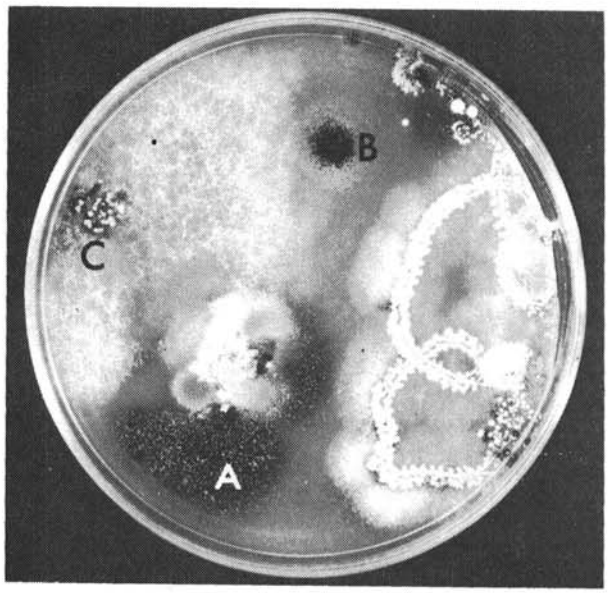

Fig. 2. Petri dish culture with numerous colonies of *Ceratocystis,* bacteria, yeasts, and other fungi 1 week after the introduction of an insect; **a)** *C. fimbriata;* **b)** *C. populina;* and **c)** *C. moniliformis.*

wide range of hosts including *P. tremuloides*, this is apparently the first report of its association with an insect. *Ceratocystis alba* grows slowly, and seldom produces perithecia on diamalt agar; consequently it was not detected in the plates.

Ceratocystis populina was first to produce perithecia in culture and the easiest to identify, which may account for its being the most frequently isolated species from all insects. *Ceratocystis tremulo-aurea* was isolated once from *Rhizophagus brunneus*, whereas *C. minor* (Hedgc.) Hunt was isolated once each from *Anthocoris musculus* and *Glischrochilus vittatus*. Of the 381 insects plated, 36 (9%) were so completely overgrown with other fungi that the presence of *Ceratocystis* could not be verified.

Inoculation via insect vectors.—*Ceratocystis* infection of fresh trunk wounds in the field, and the frequency of *Ceratocystis* spp. associated with insects attracted to these wounds, suggested that some insects were vectors of the fungi. To demonstrate insect transmission, 3-year-old aspen sprouts growing in the greenhouse were inoculated in July 1968 by placing two live, field-collected insects of the same species in a small aseptic bark wound (slit to the cambium). The slit was then covered with an adhesive tape bandage. The covering and insects were removed after 10 days. Controls consisted of bark slits and covering only.

Cankers were produced at all bark slits into which insects were inserted. None developed in the controls. Necrotic bark tissue surrounding the inoculation wound and *Ceratocystis* perithecia were evident when the insects and bandages were removed. Many insects were still alive at the end of 10 days. Canker formation and stem girdling, depending upon stem

TABLE 2. Transmission of *Ceratocystis* spp. by field-collected insects to aspen in the greenhouse

Insect	No. inoculations	No. times *Ceratocystis* isolated from canker tissue		No. times *Ceratocystis* perithecia observed at inoculation site				
		C. fimbriata	*C. populina*	*C. fimbriata*	*C. populina*	*C. alba*	*C. crassivaginata*	*C. moniliformis*
Epuraea spp.	18	7	4	14	16	18	3	3
Colopterus truncatus	10	1	1	1	6	6	0	0
Nudobius corticalis	11	3	2	7	10	10	0	1
Quedius sp.	5	0	0	3	6	4	0	1
Q. raevigatus	4	1	2	1	2	2	0	0
Rhizophagus brunneus	9	5	3	4	8	8	1	2
Control	10	0	0	0	0	0	0	0

size, were evident 7 weeks later, and continued until the end of the growing season. By then, all insect-inoculated stems, with the exception of two inoculated with *Colopterus truncatus*, were girdled. The stems were cut for microscopic examination of perithecia at the inoculation site, and for isolation of fungi from canker tissue. Table 2 lists the insects used, number of inoculations made, and fungi associated with the cankers.

Presence of fungi in insect.—A modification of Batra's technique (3) of "fractional sterilization" of insects was used to determine whether *Ceratocystis* species were present within the nitidulid *Glischrochilus vittatus*. This modification consisted of placing the insects alternately in moist and dry sterile chambers for three 24-hr periods each, prior to their placement on diamalt culture media.

Of nine surface-sterilized nitidulids, *C. fimbriata* was isolated from five, *C. populina* from nine, *C. pilifera* from three, *C. moniliformis* from one, and *C. crassivaginata* from one.

Persistence of fungi in insect.—Because *Ceratocystis* spp. were able to survive within *Glischrochilus vittatus*, the following study was made to determine their ability to overwinter in pupae.

Newly emerging insects were collected in the spring by placing muslin cloth and plastic cages (140 X 140 cm) on the ground in May 1969 around infected trees at two areas. Cage edges were embedded 15-30 cm into the ground and secured around the tree base with plastic tape. A zipper in the cloth and a covered jar lid ring in the plastic provided access to the cage's interior. Insects began to emerge the 1st week in June, and were trapped within the cages on a freshly cut piece of aspen. Insects were individually collected twice weekly during the month, and placed on culture media in petri dishes within 2 hr. Culture media used were diamalt agar and diamalt agar with the addition of 0.5% Difco yeast extract.

The various insects collected from eight ground cages and the species of *Ceratocystis* they carried as determined from cultures are given in Table 3. Identity of *Ceratocystis* fungi was often obscured by fast-growing soil fungi.

Acquisition of fungi from soil.—To determine whether *C. fimbriata* was present in the soil, samples were collected in mid-August from the soil at the base of two infected trees in each of two areas. Each sample was thoroughly mixed, and a small portion placed between carrot discs. Soil also was placed on fresh sterile aspen blocks (3 X 3 cm) on moist filter paper in petri dishes. All inoculations were incubated at 100% humidity at room temperature, and checked weekly for 3 weeks.

None of the 150 soil samples incubated between carrot discs yielded *Ceratocystis*. At the end of 3 weeks, only six *C. populina* colonies appeared on four of the 138 aspen blocks tested. No other species of *Ceratocystis* were detected.

DISCUSSION.—The habits of the various insects found on fresh aspen trunk wounds are variable and incompletely known. Nitidulids are generally considered saprophagus and mycetophagus. Adults and larvae of the *Epuraea* and *Colopterus* species observed in this study fed on mycelium and perithecia, as did the larvae of *Chymomyza aldrichi*. Larvae and adults of certain *Glischrochilus* and *Rhizophagus* species are predaceous on xylophagus

TABLE 3. *Ceratocystis* spp. isolated from insects emerging in the spring

Insect	No. insects	No. times *Ceratocystis* spp. isolated			
		C. fimbriata	*C. populina*	*C. pilifera*	*C. alba*
Epuraea spp.	20	6	15	4	1
Colopterus truncatus	1	1	1	0	1
Nudobius corticalis	32	9	4	0	7
Quedius raevigatus	10	0	0	0	0
Rhizophagus brunneus	2	0	0	0	0

insects (1). Predation was not observed. These insects were active in the fungus mats formed on the sapwood. Tachinidae are parasitic on many lepidopterous insects, which may account for their presence in wounds. The staphylinids are known predators, and were observed preying on the nitidulids collected in this study. Numerous aphids, nematodes, and mites were found in fresh wounds, but their role is unknown in the etiology of canker formation.

Several species of *Ceratocystis* soon form perithecia on fresh wounds, and the insects which frequent these wounds become contaminated with spores of these fungi. The fact that at least five species of *Ceratocystis* were transmitted by insects used in greenhouse studies confirm their potential as transmitting agents. The relative importance of each insect species is still uncertain, however. It has been shown that *C. fimbriata* causes cankers (7, 13, 14), but observations on 3-year-old field inoculations with *C. populina* and *C. crassivaginata* indicate that they too may cause cankers.

Others have determined the acquisition and persistence of *C. fimbriata* in or on vectors (6, 12). Hussain (8), who worked with aspen canker insects in Colorado, used Bretz's technique (5) to surface-sterilize 12 larvae of *Epuraea*, 22 adult *Epuraea*, and 15 adult *Colopterus truncatus* before placing them on culture media. *Ceratocystis fimbriata* was isolated from eight larvae, seven adult *Epuraea*, and six adult *Colopterus truncatus*. *Ceratocystis populina* was isolated from 12 larvae, 16 adult *Epuraea*, and two adult *Colopterus truncatus*. Hussain concluded that the adult nitidulids carried the two fungi internally. Hussain also cultured eight *Epuraea* and two *Colopterus truncatus* adults which had just emerged from pupae in the soil. *Ceratocystis fimbriata* was isolated from all of the newly emerged insects. His evidence that the fungus could pass through one generation suggested that it could overwinter in pupae. Hussain (8) might have recovered additional species of *Ceratocystis* had his insect isolates been subcultured. During the latter part of this study, *C. alba* commonly formed perithecia, and its identification was confirmed when the diamalt yeast extract medium was used. Had this medium also been used throughout the study, *C. alba* would probably have been recovered in the initial surveys (Table 1).

Soil fungi carried by the insects collected from the ground cages quickly covered some of the culture plates. Over half the plates were so overrun that species of *Ceratocystis* could not be isolated. Soil fungi were also common in the other plates, but the *Ceratocystis* spp. were identified before plates were completely overrun.

Moller & DeVay (12) investigated the possibility that *C. fimbriata* might be present in orchard soil. Using a carrot disc technique, they recovered the fungus from soil only occasionally, and then only under specific conditions. They concluded that "the soil apparently serves as a limited inoculum source, if at all". P. D. Manion (*personal communication*) occasionally isolated the organism from soil in greenhouse pots using sterile aspen blocks as a culture medium (10). No definite conclusions concerning soil as a source of inoculum can be drawn from this limited study in Colorado. The fact that *Ceratocystis* has been isolated from soil indicates the fungus may be able to survive there.

Nitidulid beetles, which are sap-and fungus-feeding insects, are ideally adapted for transmission of *Ceratocystis* spp. Although nitidulids were not commonly found on Ceratocystis cankers in early spring when *C. fimbriata* produces perithecia and ascospores, they readily infected fresh trunk wounds. Active cankers may thus be of secondary importance as an inoculum source, and the insects themselves the reservoirs of primary fungus inoculum.

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