Association of Fusarium Species With Picnic Beetles on Corn Ears

Carol E. Windels, Mark B. Windels, and Thor Kommedahl

Assistant Scientist, Department of Plant Pathology; Scientist, Department of Entomology, Fisheries and Wildlife; and Professor, Department of Plant Pathology, respectively, University of Minnesota, St. Paul 55108.

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ABSTRACT

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All corn ears buried by tillage operations in the fall of 1971 and 1973 were infested with picnic beetles, *Glischrochilus quadrisignatus,* and *Fusarium* spp. in July 1972 and 1974. More than 90% of the kernels from buried ears were colonized by *Fusarium* spp. From kernels of buried ears in the 2 years, *F. solani* grew from 66-84%, *F. moniliforme* from 25-39%, and *F. oxysporum* from 12-14%. These three *Fusarium* spp. also were isolated from larvae (85% internally in 1972; 96% externally and 82% internally in 1974), pupae (63% externally and 13% internally in 1974), and from newly emerged beetles (68% externally and 11% internally in 1974). In addition, *F. roseum* 'Equiseti' was isolated from pupae and newly emerged beetles. Beetles collected from ears on plants harbored more species and in greater frequency than beetles newly emerged from buried ears in 1972 and 1974 and included *F. oxysporum, F. tricinctum, F. moniliforme, F. solani,* and *F. roseum* 'Acuminatum', 'Avenaceum', 'Equiseti', 'Gibbosum', and 'Graminearum'. *Geotrichum candidum* was isolated from kernels and from all insect stages in both years and from 13 of 14 fields in 1974. Buried ears may, therefore, serve as inoculum sources for ear-infecting *Fusarium* spp. through the activities of the picnic beetle.

Fusarium spp. have been associated with corn roots, stalks, and ears as pathogens (2) and as secondary organisms whose role in the disease complex is not clear. *Fusarium roseum* 'Graminearum' and *F. moniliforme* are frequently cited as corn pathogens, but other *Fusarium* spp., and other fungi may function in the disease complex. Insects associated with corn may play an equally important role in the complex either as vectors or as predators and their activities may facilitate entry of fungi into plants.

One of the insects frequently observed on corn ears is the picnic beetle, *Glischrochilus quadrisignatus,* which is widely distributed in northern USA; its biology and seasonal history have been described by McCoy and Brindley (8) and Luckmann (7). Adult picnic beetles overwinter under bark of logs, in soil, and in or beneath decaying vegetation. In Minnesota, these beetles are active in April, and are attracted to decomposing fruits and vegetables, and to tree wounds. They oviposit in May and June on or near decomposing plant materials on or buried in soil.

Foott and Timmins (3) reported that ears of corn missed by harvesting machinery served as the principal reproductive sites for picnic beetles in southwest Ontario. Larvae feed on decaying plant material in June, pupate in soil, and the adults emerge from mid-July to August. Picnic beetles are attracted to ripe, damaged, or decomposing plants including corn ears damaged by the European corn borer [*Ostrinia nubilalis* (Hübner)] or other insects.

We have observed picnic beetles as apparent primary invaders of corn ears where husks incompletely cover the ear, or where silks are damaged by other insects; they can also invade corn ears buried in soil. Because we found *Fusarium* spp. frequently associated with picnic beetles (16), we attempted to ascertain the degree of association of *Fusarium* spp. with various stages of the insect. The number and species of *Fusarium* were identified for two growing seasons, 1972 and 1974, from buried corn ears, larvae, pupae, beetles emerging from buried ears, and beetles visiting ears of growing field corn.

MATERIALS AND METHODS

In early July 1972, 20 corn ears (*Zea mays* L.) buried by tillage operations the previous fall were randomly selected and removed from a field located at the Southern Experiment Station, Waseca, Minnesota. Buried ears of corn were located by unearthing clumps of volunteer corn. Ten kernels were chosen from various sites on each ear; five larvae of *Glischrochilus quadrisignatus* (Say) were also collected per ear. In mid-August, beetles were collected from ears of standing field (dent) corn.

In mid-June and early July 1974, two to five corn ears buried by tillage operations the previous fall were unearthed in each of 14 fields, and corn kernels, larvae, pupae, and newly emerged adults were collected. Newly emerged adults were identified by an incompletely tanned cuticle. Picnic beetle adults were again collected in mid-August from ears of standing field corn.

Fusarium spp. were isolated from corn kernels and from the outside and inside of various developmental

stages of the insect. Kernels were rinsed with water several times, surface treated in 1% NaOCl for 30 seconds to 2 minutes, and placed on pentachloronitrobenzene agar (PCNB-peptone), a selective medium for isolation of *Fusarium* spp. (9) but supplemented with Aureomycin (250 mg/liter).

Larvae collected in 1972 were washed in 1 ml sterile water; the wash water was then dispensed onto PCNB agar to determine externally carried *Fusarium* spp. These larvae were then surface treated in 1% NaOCl for 30 seconds, cut in half and placed on PCNB agar to determine internally contained *Fusarium* spp. Adults collected from standing-corn ears in 1972 and 1974 and larvae, pupae, and newly emerged adults collected from buried corn ears in 1974 were frozen prior to isolation of *Fusarium* spp. to kill the insects but not the *Fusarium* propagules (11). Insects were then placed directly on PCNB agar to determine externally carried *Fusarium* spp. or were surface treated in 1% NaOCl for 30 seconds, cut in half, and placed on PCNB agar to determine internally carried *Fusarium* spp.

Petri plates were incubated at room temperature (approximately 24 C) under fluorescent lights (12-hour/12-hour cycle) for 7 or more days. *Fusarium* colonies were then transferred to homemade (not preformulated) potato-dextrose agar (PDA), incubated 7-14 days and identified according to the system of Snyder and Hansen (12, 13, 14).

RESULTS

Buried corn ears.—At each collection time, kernels of buried ears were in various stages of decay and strongly aromatic when removed from the soil. Kernels ranged from relatively sound to decayed and only the pericarp remained. The later the ears were collected, the more advanced the degree of kernel decomposition.

All of the buried ears collected in July of both 1972 and 1974 were infested with *G. quadrisignatus,* and several stages of development (larvae, pupae, and newly emerged adults) were often associated with one ear. In June of 1974, 92% of the ears were infested with picnic beetle larvae; the remaining 8% were not examined closely for small larvae or eggs. Pupae and newly emerged adults were not found in June, but overwintered adults were present in 30-40% of the buried ears.

In 1972 and 1974, all buried ears were colonized by *Fusarium* spp. The percentage of kernels colonized was equally high, and the same three *Fusarium* spp. colonized kernels in the same frequency for both years. Of the 200 kernels collected in 1972, 94% were colonized by *Fusarium* spp. and 84, 25, and 12% of the kernels were colonized by *F. solani* (Mart.) App. et Wr. emend. Snyd. et Hans., *F. moniliforme* (Sheld.) emend. Snyd. et Hans., and *F. oxysporum* Schl. emend. Snyd. et Hans., respectively. In 1974, of the 14 fields sampled, *F. solani, F. moniliforme,* and *F. oxysporum* were isolated from ears of 100, 92, and 70% of the fields sampled, respectively. Of 252 kernels collected in 1974, 90% were colonized by *Fusarium* spp. and 66, 39, and 14% of the kernels were colonized by *F. solani, F. moniliforme* and *F. oxysporum,* respectively. Kernels of most ears were frequently colonized by more than one species of *Fusarium*.

Larvae.—*Fusarium solani* occurred externally on larvae from all ears, and *F. moniliforme* was isolated from larvae of 25% of the ears collected in 1972. By dispensing water used to wash these larvae onto agar media, only the presence or absence of *Fusarium* spp. could be detected. The colonies were too numerous to count for all water washes and 25 colonies were randomly

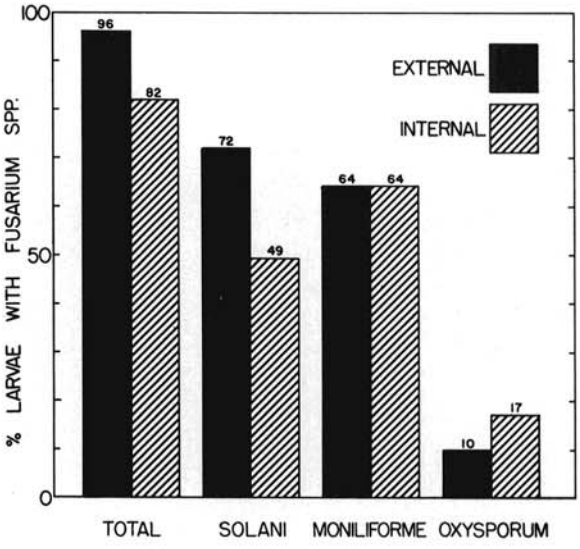

Fig. 1. Percentage of picnic beetle larvae carrying *Fusarium solani, F. moniliforme,* or *F. oxysporum* externally and internally in 1974. One hundred and sixty-eight larvae were used to determine external *Fusarium* spp. and 162 were used to determine internal *Fusarium* spp.

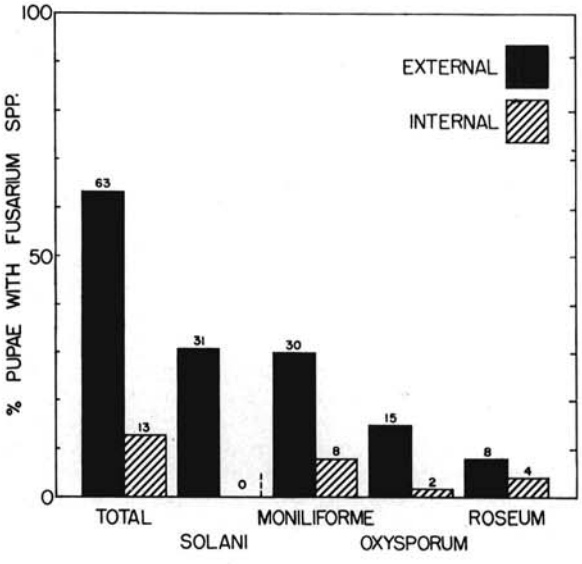

Fig. 2. Percentage of picnic beetle pupae carrying *Fusarium solani, F. moniliforme, F. oxysporum,* or *F. roseum* externally and internally. Eighty pupae were used to determine external *Fusarium* spp. and 84 pupae were used to determine internal *Fusarium* spp.

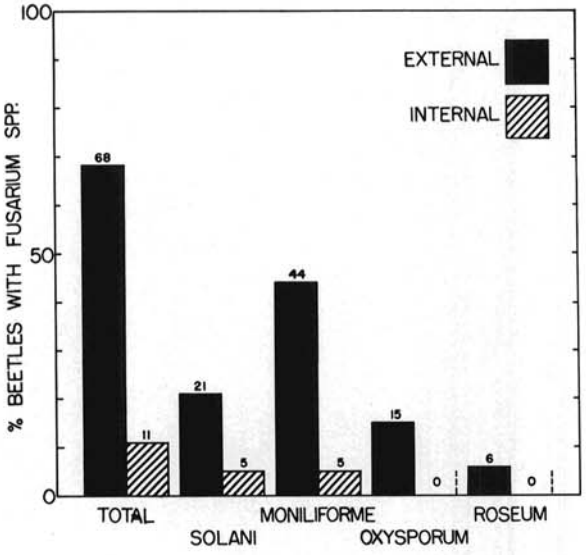

Fig. 3. Percentage newly emerged picnic beetles carrying *Fusarium solani, F. moniliforme, F. oxysporum,* or *F. roseum* externally and internally. Thirty-four beetles were used to determine external *Fusarium* spp. and 37 beetles were used to determine internal *Fusarium* spp.

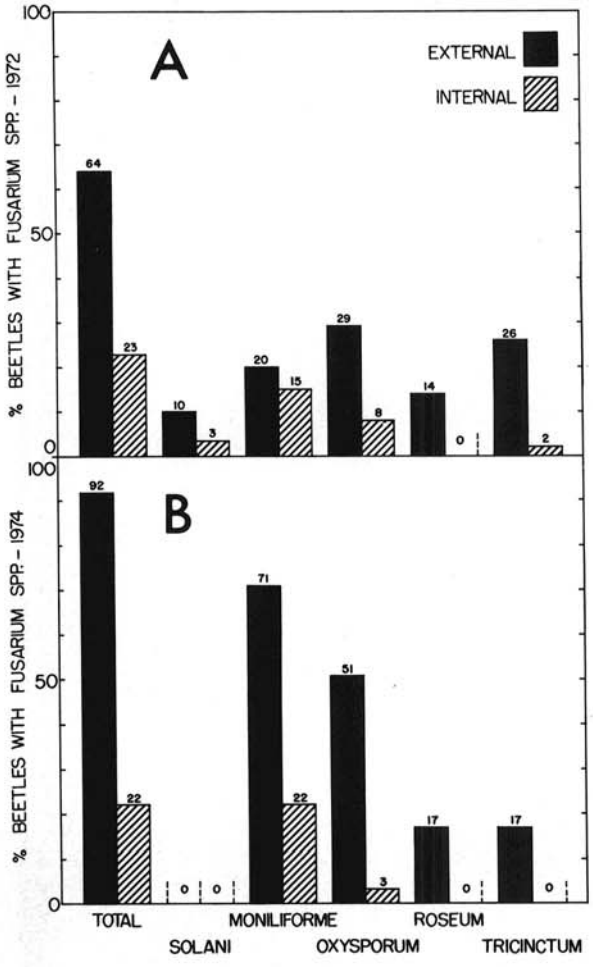

Fig. 4-(A, B). Percentage picnic beetle adults from standing-corn ears carrying *Fusarium solani, F. moniliforme, F. oxysporum, F. roseum,* or *F. tricinctum* externally and internally. **A)** Fifty beetles were used to determine external *Fusarium* spp. and 61 beetles were used to determine internal *Fusarium* spp. in 1972; **B)** 83 beetles were used to determine external *Fusarium* spp. and 81 beetles were used to determine internal *Fusarium* spp. in 1974.

selected per plate for identification to species. Of 100 larvae collected in 1972, 85% carried *Fusarium* spp. internally. *Fusarium solani, F. moniliforme,* and *F. oxysporum* were isolated from the inside of 72, 28, and 7% of the larvae, respectively. About 96% of the larvae collected in 1974 carried *Fusarium* spp. externally and 82% carried *Fusarium* spp. internally (Fig. 1). *Fusarium solani, F. moniliforme,* and (to a lesser extent) *F. oxysporum,* were isolated from the outside and inside of larvae and from larvae collected from all fields.

Pupae.—About 63% of the pupae carried *Fusarium* spp. externally and 13% carried *Fusarium* spp. internally (Fig. 2). In addition to the same three species isolated from larvae, *F. roseum* (Lk.) emend. Snyd. et Hans., 'Equiseti' was isolated. The isolation of *F. roseum* 'Equiseti' is not surprising, because it is a common soil fungus and pupae were found in soil adjacent to the buried ears. Each species of *Fusarium* was isolated more frequently from outside than inside the pupae. *Fusarium* spp. were less frequently isolated both externally and internally from pupae than from larvae. *Fusarium solani* was not isolated from the inside of any of the pupae.

Newly emerged adults from buried ears.—*Fusarium* spp. were carried externally by 68% and internally by 11% of the newly emerged adults (Fig. 3). The same four species isolated from pupae were isolated from the newly emerged adults; however, only *F. solani* and *F. moniliforme* were isolated from the inside of the beetles. The percentage of newly emerged adults carrying *Fusarium,* the frequency of isolation of the different species, and their location outside the beetles were similar to results found for pupae.

Adults from standing-corn ears.—A greater percentage of adults from standing-corn ears carried more *Fusarium* spp. both externally and internally than did newly emerged adults (Fig. 4 vs. Fig. 3). In 1972, *Fusarium* spp. were isolated from the outside of beetles in the following order of frequency: *F. oxysporum, F. tricinctum* (Cda.) emend. Snyd. et Hans., *F. moniliforme, F. roseum,* and *F. solani* (Fig. 4-A). Cultivars of *F. roseum* included 'Acuminatum', 'Avenaceum', 'Equiseti', 'Gibbosum', and 'Graminearum'. With the exception of *F. roseum,* the same species were isolated from inside the beetles although less frequently.

A higher percentage of adults from standing-corn ears carried *Fusarium* spp. in 1974 (Fig. 4-B) than in 1972 (Fig. 4-A). Species carried externally in 1974 in order of decreasing frequency were: *F. moniliforme, F. oxysporum, F. roseum* 'Equiseti,' and *F. tricinctum*. Only *F. moniliforme* and *F. oxysporum* were isolated from inside the beetles (Fig. 4-B).

Geotrichum candidum in corn ears and insects.—*Geotrichum candidum* Lk. ex Pers. emend.

Carmichael (1) was also isolated on PCNB agar from corn kernels and various stages of the insect. The "fruity" odor associated with this fungus was similar to that of the decaying ears of buried corn. The fungus was isolated during both summers, but data from 1974 are presented because they are more complete. *Geotrichum candidum* was isolated from insects in 93% of the fields. Of the larvae, 29% carried the fungus externally and 21% carried it internally. The fungus was isolated externally from 9% of the pupae, internally from 2% of the pupae, and externally and internally from 3% of the newly emerged adults. Of the adults collected from standing-corn ears, 4% carried *G. candidum* externally, and 6% carried it internally.

DISCUSSION

Because of the consistent association of *Fusarium* spp. with (i) buried ears of corn and larvae, pupae, newly emerged adults, and (ii) adults on standing-corn ears, it could be inferred that *G. quadrisignatus* serves as a vector for *Fusarium* spp. on corn. Moreover the same *Fusarium* spp. are frequently isolated from kernels of harvested corn. To establish the vector relationship for *Fusarium* spp. it would have to be shown that larvae or beetles feeding on *Fusarium*-infected ears could infect ears of standing corn with *Fusarium* spp. This has not been done, but picnic beetles are vectors of the oak wilt fungus, *Ceratocystis fagacearum* (10), so their potential to serve as fungal vectors has been established.

The consistent isolation of *F. solani, F. moniliforme* and *F. oxysporum* in buried corn ears is different from *Fusarium* populations usually found in the soil. Six species of *Fusarium* are commonly found in cornfield soils of southern Minnesota. *Fusarium oxysporum* and *F. solani* predominate, with the remaining 30% of the *Fusarium* population consisting of *F. roseum, F. episphaeria, F. tricinctum,* and *F. moniliforme* (5, 15). *Fusarium oxysporum* and *F. solani* are soil inhabitants and therefore good competitors, so their colonization of buried corn ears is not surprising. *Fusarium moniliforme* colonized a high percentage of buried corn ears, but in soil it usually represents about 2% of the *Fusarium* population (15). It is known to infect ears of wounded or insect-infested field corn (4), and in some cases to cause kernel and ear rot of corn (4, 6). If these infected ears drop early or are missed during harvesting, they could provide not only a suitable substrate for *F. moniliforme* but a means of entry into soil. The buried ears and volunteer corn as well as the picnic beetles could provide a source of *F. moniliforme* in the spring.

Frequently, the *Fusarium* spp. on cobs were the same ones as on the outside and inside of picnic beetle larvae collected from those cobs. The *Fusarium* spp. within larvae were also on larvae collected from the same ear, but the converse did not always occur. The *Fusarium* spp. isolated inside the larvae could have been picked up during feeding. Luckmann (7) found that half-grown larvae on agar plates covered with mycelium, consumed that mycelium.

A lower percentage of pupae carried *Fusarium* externally and internally than did larvae. The larvae, when fully grown, leave their food source, and then pupate in the soil. As larvae enter and move through soil, spores and mycelium could become dislodged and new fungi acquired upon and within the larvae. Some of the internal *Fusarium* propagules could be lost through the feces or destroyed in the gut of the insect. However, there was little variation in the *Fusarium* spp. and their frequency of occurrence either upon or within pupae and newly emerged adults.

Occurrence of *Fusarium* spp. was more frequent and additional species were isolated from beetles collected from standing-corn ears than from newly emerged beetles on buried corn ears. This is probably because the beetles are attracted to a wide variety of habitats upon leaving the soil and could acquire various *Fusarium* spp. Beetles were frequently observed visiting corn ears and wounds produced by other insects. Exposure to *Fusarium* spp. is likely to occur in this way, particularly to *F. moniliforme* and *F. tricinctum*. Both species are rarely found in soil but are frequently isolated from corn plants; both species were frequently isolated from beetles.

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