Ecology and Epidemiology

Association of Corn Stalk Rot *Fusarium* spp. and Western Corn Rootworm Beetles in Colorado

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We thank Patricia M. Anderson, Nancy D. Epsky, Deanne Casciano, and Jeremy Folger for technical assistance.

Research conducted by the first author in partial fulfillment of the requirements for the Ph.D. degree, Colorado State University.

Cooperative investigations of the USDA, Agricultural Research Service, and the Colorado Agricultural Experiment Station. Supported in part by USDA grant SRP-IPMS-WR-80-1 and Western Regional Pesticide Impact Fund.

Published with the approval of the director of the Colorado Agricultural Experiment Station as Scientific Journal Series Paper 3029.

Accepted for publication 29 May 1986 (submitted for electronic processing).

ABSTRACT

Gilbertson, R. L., Brown, W. M., Jr., Ruppel, E. G., and Capinera, J. L. 1986. Association of corn stalk rot *Fusarium* spp. and western corn rootworm beetles in Colorado. Phytopathology 76:1309-1314.

Western corn rootworm beetles (*Diabrotica virgifera*) were abundant on corn ears (*Zea mays*) in eastern Colorado in 1982, 1983, and 1984. Corn stalk rot fungi *Fusarium moniliforme* and *F. subglutinans* were consistently isolated from beetles collected from one site in 1982 and 1984, and two sites in 1983. Fungal contamination of beetles increased as the season progressed, and surface-disinfestation of beetles did not eliminate contamination, indicating internal contamination. In 1983, beetles collected from a corn field were more heavily contaminated by *F. moniliforme* and *F. subglutinans* (41%) than beetles collected from a nearby squash field (17%). Populations of beetles on corn ears and amount of kernel contamination by these fungi were correlated positively, suggesting that beetles were vectors of the fungi. Corn kernels from a field corn plot with Fusarium stalk rot and a heavy beetle infestation were more contaminated externally by *F. moniliforme* and *F. subglutinans* (73%), than kernels from a plot with no beetle infestation (27%); the difference in kernel contamination was attributed to rootworm beetles. Western corn rootworm beetles may be vectors for these fungi to corn ears and kernels.

Western corn rootworm beetles (*Diabrotica virgifera* Lec.) were abundant on field and sweet corn (*Zea mays* L.) ears in Colorado in 1982, 1983, and 1984. Twenty to 30 beetles were observed per ear at silking at two locations in Colorado (Fort Collins and Windsor), and, later in the season, *Fusarium moniliforme* Sheldon sporulated on beetle-damaged ear tips. The common occurrence of beetles, their high rate of activity on aboveground corn plant organs, and the incidence of Fusarium stalk and ear rot in Colorado led to a study of a potential *Fusarium*-beetle association. A preliminary report has been published (8).

F. moniliforme causes diseases in asparagus, corn, fig, and sugarcane. The fungus has been shown to be insect disseminated (3–6,10,12,16,20), and various insects can disseminate corn stalk and ear rot *Fusarium* spp. (1,4–8,16,20). *F. moniliforme* and *F. graminearum* Schwabe were isolated from picnic beetles (*Glischrochilus quadrisignatus* Say) collected from ears of standing corn in Minnesota, and *F. moniliforme* was consistently isolated externally and internally from picnic beetle larvae, pupae, and adults (20). Attwater and Busch (1) demonstrated that picnic beetles were vectors of conidia of *F. graminearum* and ascospores of *Gibberella zeae* (Schw.) Petch. to corn ears in Ontario. *Fusarium* spp., including *F. moniliforme* and *F. graminearum*, have been isolated from northern corn rootworm (*Diabrotica longicornis* Say) larvae and adults and rootworm-damaged corn roots (16). *F. moniliforme* and *F. graminearum* were pathogenic on corn, and Palmer and Kommedahl (16) concluded that larvae and adults of the northern corn rootworm were vectors of *Fusarium* spp. *F. subglutinans* (Wr. and Reink.) Nelson, Toussoun, and Marasas was associated with the pine weevil (*Pissodes nemorensis* Germar) in Georgia (2).

The ecology of corn stalk and ear rot *Fusarium* spp. differs throughout corn growing regions of the United States (6) and is not fully understood in Colorado. *F. moniliforme* is a common contaminant of corn seed, and a number of seed contamination mechanisms have been proposed, including airborne and rain-splashed inoculum, systemic infection, and insect-borne inoculum (1,3,6,8,17,19). The importance of insect-borne inoculum and the insects involved are not known. The objectives of this study were to: determine if corn stalk rot *Fusarium* spp. were associated with western corn rootworm beetles and, if so, the frequency of association; determine if beetle contamination was internal and/or external; ascertain if beetle contamination was related to beetle activity on corn plants and/or host plant species; attempt to correlate beetle populations on corn ears with subsequent kernel contamination by *Fusarium* spp.; and attempt to ascertain the potential importance of insect-borne inoculum to kernel contamination in the field.

MATERIALS AND METHODS

Beetle-*Fusarium* spp. association on sweet corn. An initial study was conducted on the western corn rootworm beetle-*Fusarium* association at a heavily beetle-infested sweet corn plot in Fort Collins, CO, in early August 1982. This site was considered to provide good potential beetle-*Fusarium* interaction. Fifteen damaged (lodged) plants, 10 damaged ears, and 155 beetles were randomly selected for isolation of *Fusarium* spp. Root systems, stalks, and damaged ears were placed individually in plastic bags. Beetles were individually collected and capped in sterile, 12-mm-diameter test tubes. All samples were brought into the laboratory and stored at 4 C until isolations were made. Corn roots were excised, washed under running tap water for 5 min, surface-disinfested in 0.6% sodium hypochlorite solution for 5 min, and placed on Komada's *Fusarium*-selective medium (KM) (11). Stalks were sectioned longitudinally, and pieces excised from inner nodal tissues were surface-disinfested as described for root sections and placed on KM. One or two damaged corn kernels were excised from each ear, damaged silks were removed, and kernels and silks placed directly on KM. Eighty beetles were washed five times in sterile distilled water, and 75 were surface-disinfested in 25 ml of 0.3% sodium hypochlorite solution containing 5 ml of surfactant (polyethylenesorbitan monolaurate) for 10 min and blotted; all beetles were placed on KM and crushed with sterile forceps. All plates were incubated at 23 C for 5–7 days, and colonies of *Fusarium* identified directly or subcultured on acidified potato carrot agar (PCAL) (9) for identification based on descriptions of Nelson et al (15).

Beetle-*Fusarium* spp. association on field corn. Two northeastern Colorado locations were selected to study the beetle-*Fusarium* spp. association on field corn ears in 1983, based on the common occurrence of beetles in these areas in 1982. Northrup King field corn cultivars PX9144 and PX9288 were planted in Fort Collins, CO, on 5 May 1983. Plant population and nitrogen fertilizer rates were increased over normal, and no larval rootworm control was implemented to ensure favorable stalk rot conditions (6). Field (dent) corn also was planted in Windsor, CO, in early May under normal agronomic practices. In 1982, the field was cropped in corn, and carbofuran was applied to control rootworm larvae in 1982 and 1983. Field corn at Fort Collins was harvested for grain, whereas the Windsor field was harvested for silage. Both sites were monitored for appearance of beetles.

Beetles appeared in late July at corn silking at Fort Collins and in mid-August at Windsor when corn was beginning to mature. One hundred beetles were collected from each location at three corn growth stages (based on beetle emergence and activity): Fort Collins late silk, early ear development (8 August), early maturity, ears fully developed (15 August), maturity, plants senescing (15 September); Windsor early maturity, ears fully developed (29 August), maturity (17 September), and postharvest (25 September). Collections were made from corn ears and leaves, except for the last date at Windsor when beetles were captured in corn stubble. Beetles were cooled (4 C), placed on KM, and crushed with sterile forceps.

Two hundred beetles were similarly collected in the Fort Collins field on 15 September 1983, and 200 beetles were collected at the Windsor field on 14 and 21 September to ascertain internal contamination. From each location, 100 beetles were surface-disinfested as described and 100 not treated; all beetles were placed on KM as described.

Another 70 beetles were collected from corn ears in the Fort Collins field, and an additional 70 were collected from a planting of zucchini (*Curcurbita pepo* L. var. *medullosa* Alef.) and summer squash (*C. pepo* L. var. *Melopepo* Alef.) located 0.4 km from the corn plot on 15 September 1983. Beetles were surface-disinfested and plated as described.

In 1984, beetles were collected at the Fort Collins location only. Field corn (Northrup King cultivar PX9288) was planted on 21 May 1984. Beetles appeared in early August, and 200 beetles were collected individually from corn ears, silks, or leaves at four growth stages: silk stage, early ear development (7 August); early maturity, ears fully developed (25 August); maturity, plants beginning to senesce (8 September); preharvest, plants senescent and dry (3 October). For each date, 100 beetles were surface-disinfested as described and 100 not treated; all were placed on KM as described.

Virulence of stalk rot *Fusarium* spp. from beetles. Two isolates each of *F. moniliforme* and *F. subglutinans*, and one isolate of *F. graminearum* from western corn rootworm beetles from Fort Collins were evaluated for ability to cause stalk rot. A modified version of the toothpick inoculation technique (6) was used. Mature (ears fully developed) field corn plants (Northrup King cultivar PX15) were inoculated on 20 August 1983, at the second node with toothpicks colonized by *Fusarium*. Four plants were inoculated with each isolate, whereas control plants were inoculated with sterile toothpicks. Stalks were harvested on 6 September. Virulence was evaluated by sectioning stalks longitudinally, measuring the amount of rot, and rating each stalk on a scale of 0–4 (0 = no rot, 1 = 0–25% of upper internode rotted, 2 = 26–50% rotted, 3 = 51–75% rotted, and 4 = 76–100% rotted). Rot constituted discolored and degraded tissue.

Beetle populations on corn ears and kernel contamination. The relationship between western corn rootworm beetles and contamination of corn kernels by *Fusarium* spp., was examined in 1982, 1983, and 1984. In 1982, a 0.2-ha plot of field corn (Northrup King cultivar PX15) was planted at Fort Collins in early May under standard agronomic practices. Beginning at the green silk stage (9 and 10 August), ears were selected randomly and beetles collected from the area were placed in specified numbers on ears. Fine, nylon-mesh bags were placed over ears to maintain beetle populations. Populations of 0, 3, 6, 9, 10, and 20 beetles were each established on 20 ears. Ears were harvested in mid-October and allowed to air dry to below 20% moisture. Fourteen months later, five kernels from the tip half and five from the basal end of each ear were randomly selected. Kernels were removed with sterile forceps, placed directly on KM, and incubated 5–7 days at 20 C under continuous (24 hr) light in the laboratory.

In similar 1983 and 1984 experiments, a 0.2-ha plot of Northrup King cultivar PX15 was planted at Fort Collins in early May as before. In 1983, at green silk stage (13 and 14 August) and brown silk stage (20 and 21 August) beetle populations of 0, 5, 10, and 20 per ear were each established on 20 randomly selected ears. Ears were harvested in mid-October, allowed to dry to below 20% moisture, and isolations made from kernels 2 mo later. In 1984, at the green silk stage (7 August), beetle populations of 0, 5, and 20 per ear were each established on 25 randomly selected ears. Ears were harvested in mid-October, allowed to dry to below 20% moisture, and isolations made from kernels 2.5 mo later.

Inoculum source for kernel contamination in 1983. To attempt to determine the potential importance of western corn rootworm beetles as an inoculum source of *Fusarium*, we investigated kernel contamination in two experimental field corn plots in Fort Collins. Plot one, planted with Northrup King field corn cultivar PX9288 on 5 May 1983, was described previously. The plot had been in field corn the previous two seasons, and had a history of Fusarium stalk rot and western corn rootworm infestation. Plot two was located 30 m across from plot one and was planted with Northrup King field corn cultivar PX15. Plot two had not been cropped in field corn the previous season and had no history of Fusarium stalk rot or western corn rootworm infestation. Two routes of kernel contamination were considered: systemic and insect-borne. We attempted to determine the level of kernel contamination and how kernels became contaminated in each plot. To determine if corn kernels were contaminated internally or externally and the extent of contamination at each plot, 10 ears were collected randomly from each plot on 19 October 1983. Twenty kernels were randomly selected from each ear, and 10 were surface-disinfested in 0.3% sodium hypochlorite for 5 min. Isolations from kernels were made as described.

RESULTS

Beetle-*Fusarium* spp. association on sweet corn in 1982. Rootworm-damaged sweet corn roots showed extensive larval tunneling, reddish-brown discoloration, and root rot. Tunneling and discoloration extended into crown tissue. Longitudinal stalk sections revealed discoloration of nodal tissues, with pronounced discoloration of lower nodes. There was a heavy western corn rootworm beetle infestation of corn ears, and ears, kernels, and silks were damaged from beetle feeding.

F. moniliforme and *F. subglutinans* were isolated from rootworm-damaged sweet corn plant organs and western corn rootworm beetles (Table 1). *Fusarium* spp., including *F. moniliforme* and *F. subglutinans*, were consistently isolated from surface-disinfested root sections; incidence of *F. subglutinans* was slightly greater than *F. moniliforme*, *F. equiseti* (Corda) Sacc. sensu Gordon, *F. solani* (Mart.) Appel and Wollenw. emend. Snyd. and Hans., and *F. oxysporum* Schlect. emend. Snyd. and Hans. also were isolated. *F. moniliforme* and *F. subglutinans* were isolated from surface-disinfested stalk sections, whereas only *F. moniliforme* was isolated from damaged kernels and silks. Other *Fusarium* spp. were infrequently isolated from aboveground plant parts.

TABLE 1. Isolation of *Fusarium* spp. from western corn rootworm beetles (*Diabrotica virgifera*) and rootworm-damaged sweet corn roots, stalks, silks, and kernels in early August 1982

		Colonies of *Fusarium* isolated (%)			
Source[a]	Treatment[b]	*F. moniliforme*	*F. subglutinans*	*Fm* & *Fs*[c]	Other *Fusarium* spp.
Roots	Surface-disinfested	16	23	4	100
Stalks	Surface-disinfested	35	8	2	0
Kernels	None	96	0	0	4
Silks	None	70	0	0	0
Beetles	Washed	66	14	4	16
Beetles	Surface-disinfested	25	12	4	4

[a]75 root sections, 60 stalk sections, 25 kernels, 10 silks, and 155 beetles assayed.

[b]Root systems washed in tap water; sections excised, surface-disinfested in 0.6% sodium hypochlorite solution for 5 min, and placed on Komada's *Fusarium*-selective medium (KM). Stalks sectioned longitudinally; pieces excised, surface-disinfested in 0.3% sodium hypochlorite solution for 5 min, and placed on KM. Eighty beetles washed in sterile distilled water, and 75 beetles surface-disinfested in 25 ml of 0.3% sodium hypochlorite solution containing 5 ml polyethylenesorbitan monolaurate for 10 min and blotted; all beetles were placed on KM and crushed with sterile forceps.

[c]Both *F. moniliforme* (*Fm*) and *F. subglutinans* (*Fs*) isolated from individual beetles or plant pieces (mixed isolations). Percent contamination by *Fm* and/or *Fs* was calculated by substracting percent mixed isolations from combined percent isolation of *Fm* and *Fs*.

F. moniliforme and/or *F. subglutinans* were isolated from 76% of washed beetles and 33% of surface-disinfested beetles. Three washed (4%) and three (4%) surface-disinfested beetles yielded both *F. moniliforme* and *F. subglutinans* (mixed isolations); unless otherwise stated, mixed isolations were considered as one contaminated beetle for calculation of percent contamination by *F. moniliforme* and/or *F. subglutinans*. *F. moniliforme* was more frequently isolated from beetles than *F. subglutinans* (Table 1). Other *Fusarium* spp. were infrequently isolated from beetles.

Beetle-*Fusarium* spp. association on field corn. In 1983, beetles were observed earlier in the Fort Collins field (late July) than in the Windsor field (mid-August). *F. moniliforme* and *F. subglutinans* contamination of beetles collected at different time intervals at Fort Collins is shown in Figure 1A, and at Windsor in Figure 1B. Beetle contamination at Fort Collins was initially low, increased by midseason (early maturity), and reached 77% by September 15 (maturity) (Fig. 1A). There were no mixed isolations on 8 August, one on 25 August, and one on 15 September. At Windsor, beetle contamination was initially greater than at Fort Collins, and reached 100% by 7 September (maturity), but contamination decreased after the corn (dent) was harvested (Fig. 1B). There was one mixed isolation on 29 August, seven on 7 September, and eight on 25 September. Slightly more *F. subglutinans* than *F.*

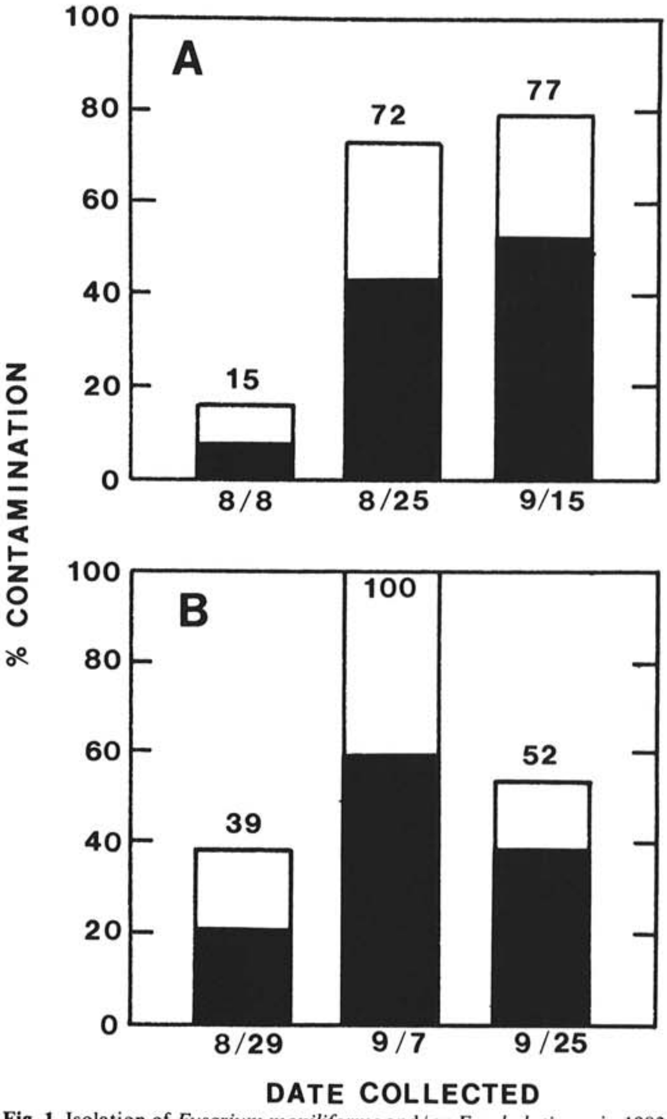

Fig. 1. Isolation of *Fusarium moniliforme* and/or *F. subglutinans* in 1983 from 100 western corn rootworm beetles on corn at three field corn growth stages at **A,** Fort Collins; and **B,** Windsor, CO. Solid bars are percent isolation of *F. subglutinans*, open bars are percent isolation of *F. moniliforme*. Successive growth stages were: early ear development, early maturity, and maturity at Fort Collins; and early maturity, maturity, and postharvest at Windsor.

moniliforme was isolated from beetles at both locations on all dates, except for 8 August at Fort Collins (Fig. 1A and B).

Surface-disinfestation of beetles reduced but did not eliminate contamination by *Fusarium* spp. as compared with non-disinfested beetles (Table 2). Contamination of surface-disinfested beetles from Fort Collins and Windsor by *F. moniliforme* and/or *F. subglutinans* was 46 and 43%, respectively, whereas contamination of non-disinfested beetles was 76 and 95%, respectively. *F. subglutinans* was isolated more frequently than *F. moniliforme* from beetles from both locations regardless of treatment. Other *Fusarium* spp. were isolated less frequently (5–9%); *F. equiseti* was the most common other species isolated.

Beetles from squash that were surface-disinfested were less contaminated by *F. moniliforme* and *F. subglutinans* (17%) than those from corn (41%); there were no mixed isolations. Percentage of contamination by total *Fusarium* spp. was similar for beetles from both hosts (Table 3).

Contamination of beetles from Fort Collins by *F. moniliforme* and *F. subglutinans* in 1984 is shown in Figure 2. Few mixed isolations were detected the first sampling date; on the second sampling date, three mixed isolations were detected from non-disinfested beetles and one from surface-disinfested beetles. On the third sampling date, there were 19 and 24 mixed isolations from non-disinfested and disinfested beetles, respectively; on the fourth sampling date, there were 21 mixed isolations from each treatment group. The isolation frequency of *F. subglutinans* was slightly higher, regardless of sampling date or treatment. Contamination increased as the season progressed and, by September, over 80% of all beetles were contaminated by *F. moniliforme* and/or *F. subglutinans*, regardless of treatment. Unlike 1983, surface-disinfestation did not consistently reduce beetle contamination, and surface-disinfested beetles were more contaminated than non-disinfested beetles on two sampling dates.

Virulence of stalk rot fusaria from beetles. The five isolates of *Fusarium* from western corn rootworm beetles caused stalk rot in toothpick inoculation tests. *F. graminearum* was most virulent, with a stalk rot rating of 3.5. *F. moniliforme* and *F. subglutinans* were less virulent (ratings ranged from 2.0–2.5); control toothpicks resulted in no stalk rot (rating of 0.1).

Beetle populations and kernel contamination. Combined *F. moniliforme* and/or *F. subglutinans* contamination of corn kernels from corn ears with different western corn rootworm populations is shown in Figure 3. Mixed isolations were not quantified. *F. subglutinans* (473 isolations) was more frequently isolated than *F. moniliforme* (352 isolations). In 1982 and 1983, beetle population was positively and significantly correlated with kernel contamination: $r = 0.94$ in 1982, and $r = 0.96$ for both green and brown silk ears in 1983 (Fig.3A–C). In 1982, 5% of the kernels from ears with zero and three beetles were contaminated, whereas 18 and 25% were contaminated from ears with 10 and 20 beetles, respectively. In 1983, 11.5% of kernels from green silk ears with zero beetles were contaminated, whereas 94% were contaminated from ears with 20 beetles. With brown silk ears, approximately 20% of the kernels from ears with zero and five beetles were contaminated, whereas 70% were contaminated from ears with 20 beetles. In 1984, beetle population was positively but not significantly correlated with kernel contamination. Kernels from ears with 0, 5, and 20 beetles were 38, 75, and 100% contaminated, respectively.

Inoculum source for kernel contamination. By late August 1983, corn plants in plot one had Fusarium stalk rot, and corn ears were infested with western corn rootworm beetles. Plants in plot two had little stalk rot, and beetle infestation of ears was limited to outer rows of the plot. Corn stalks in plot one were infected by *F. moniliforme* and *F. subglutinans* after silking (19 August) (7), and in plot two after kernel development (6 September) (Gilbertson, *unpublished*). However, 5 and 0% of surface-disinfested kernels were contaminated by *F. moniliforme* and/or *F. subglutinans* in plots one and two, respectively, whereas 73 and 27% of non-disinfested kernels were contaminated from plots one and two, respectively.

TABLE 2. Isolation of *Fusarium moniliforme* and *F. subglutinans* from surface-disinfested and non-disinfested western corn rootworm beetles collected from field corn in 1983

		Colonies of *Fusarium* isolated (%)		
Location[a]	Treatment[b]	*F. moniliforme*	*F. subglutinans*	*Fm* & *Fs*[c]
Fort Collins	Non-disinfested	28	49	1
	Surface-disinfested	11	35	0
Windsor	Non-disinfested	44	58	7
	Surface-disinfested	19	29	5

[a] 200 beetles collected from Fort Collins on 15 September, and 200 from Windsor on 14 and 21 September.

[b] 100 beetles from each location surface-disinfested (footnote b, Table 1) and 100 from each location not treated; all beetles placed on Komada's *Fusarium*-selective medium and crushed with sterile forceps.

[c] Both *F. moniliforme* (*Fm*) and *F. subglutinans* (*Fs*) isolated from individual beetles or plant pieces (mixed isolations). Percent contamination by *Fm* and/or *Fs* was calculated by subtracting percent mixed isolations from combined percent isolation of *Fm* and *Fs*.

TABLE 3. Isolation of *Fusarium* spp. from surface-disinfested western corn rootworm beetles collected from corn and squash plants at Fort Collins, CO, on 15 September 1983

	Colonies of *Fusarium* isolated (%)[a]			
Source[b]	*F. moniliforme*	*F. subglutinans*	Other *Fusarium* spp.	Total
Squash	10	7	29	46
Corn	17	24	13	54

[a] 70 beetles collected from each host; all beetles were surface-disinfested (footnote b, Table 1), placed on Komada's *Fusarium*-selective medium, and crushed with sterile forceps.

[b] Squash planting located 0.4 km from corn plot.

DISCUSSION

Western corn rootworm beetles physically damage corn plants and cause poor kernel set by feeding on silks, ears, and leaves (14). In Colorado, beetles are not considered a major pest; however, our research indicated that these beetles may disseminate the stalk and ear rot fungi *F. moniliforme* and *F. subglutinans* to corn kernels.

In an initial study, western corn rootworm-damaged sweet corn roots, stalks, silks, and kernels were heavily infested with *F.*

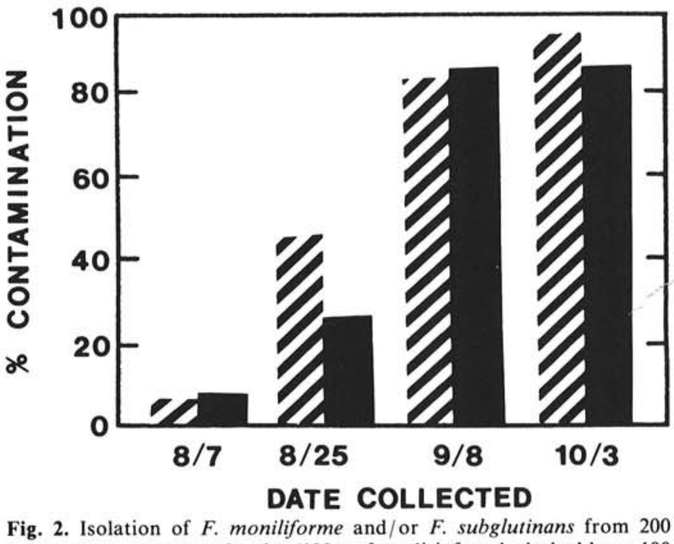

Fig. 2. Isolation of *F. moniliforme* and/or *F. subglutinans* from 200 western corn rootworm beetles (100 surface-disinfested, slashed bars; 100 directly plated, solid bars) on corn at four field corn growth stages at Fort Collins, CO, 1984. Successive growth stages were silk, early maturity, maturity, and preharvest, respectively.

moniliforme and/or *F. subglutinans*, as were western corn rootworm beetles from these plants. Contamination of silks, kernels, and beetles suggested an association between the insects and these fungi on aboveground corn plant organs. Palmer and Kommedahl (16) isolated *F. moniliforme* from northern corn rootworm beetles and larvae in Minnesota, and reported that the incidence of infections by *Fusarium* in corn roots increased with an increase in rootworm larva-infested roots. It appeared that western corn rootworm larvae similarly favored *Fusarium* infection of sweet corn roots, crowns, and stalks by *Fusarium* spp. in our experimental plot.

A western corn rootworm beetle-*Fusarium* association was detected on field corn, which makes up most of Colorado's corn acreage. Beetles from field corn were consistently contaminated by *F. moniliforme* and *F. subglutinans*, and contamination appeared to be external and internal. Beetle contamination increased as the season progressed, suggesting that beetle activity in the field was necessary for higher rates of contamination. At Windsor,

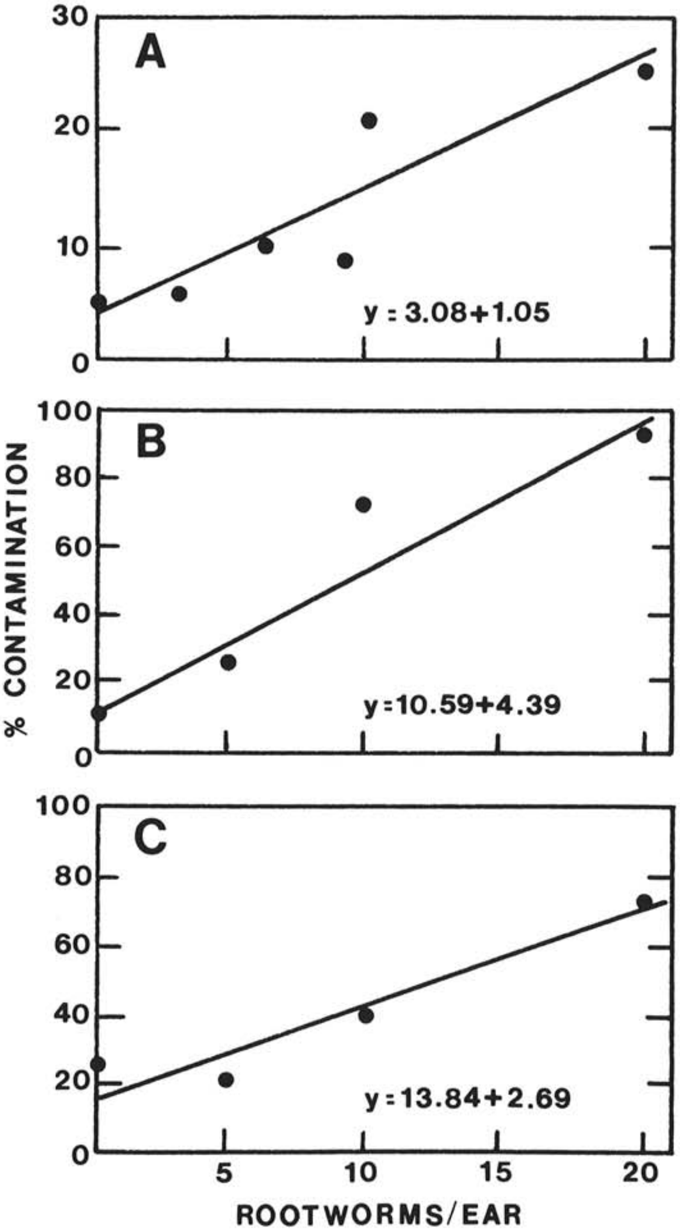

Fig. 3. Contamination of field corn kernels by *Fusarium moniliforme* and/or *F. subglutinans* from corn ears with different populations of field-captured western corn rootworm beetles established at green silk stage in **A**, 1982, and **B**, 1983; and **C**, at brown silk stage in 1983 at Fort Collins, CO. Each point on the graph is mean percent contamination of 10 kernels from each of 20 ears. Correlation coefficients (r) were significant at $P < 0.05$.

contamination rate was lowest initially, peaked in early September at plant maturity, and decreased by late September after the field was harvested for silage corn. Corn plants may be necessary to provide sites for beetles to contact propagules of *F. moniliforme* and *F. subglutinans*; both species have been isolated from corn plants by other workers (6–9,13). Windels et al (20) isolated more *Fusarium* spp. from picnic beetles collected from ears of standing corn than from newly emerged beetles, and it was concluded that the beetles acquired *Fusarium* spp. from a variety of habitats after leaving the soil.

Initial external contamination of beetles may occur as beetles move through the corn rhizosphere after emerging from pupae in soil, and in 1984, *F. moniliforme* and *F. subglutinans* were detected in corn rhizosphere soil in the Fort Collins field during beetle emergence (18). Newly emerged non-disinfested beetles were 7% contaminated at this time, versus 42, 84, and 94% contamination of beetles on later sampling dates. Thus, most contamination probably came from beetles contacting inoculum on corn plants. Beetles could contact fungal inoculum on plant debris or weeds. However, most beetle activity was on aboveground corn plant organs.

Internal contamination of beetles was suggested when surface-disinfestation did not eliminate contamination by *Fusarium* spp. Although some internal contamination may persist from larvae, internal contamination increased with time in 1984. Internal contamination increased from 9% at the first sampling date to 25, 85, and 85% as the season progressed. Internal contamination probably occurred as beetles fed on aboveground corn organs and ingested fungal propagules. The high rates of internal contamination seen in 1984 may have been caused by the high incidence of Fusarium stalk rot in the field and a large amount of inoculum for beetles to ingest.

F. moniliforme and *F. subglutinans* probably survive in the beetle gut. Beetles were crushed on KM to extrude the gut onto the agar, and fungal colonies frequently grew from gut tissues. Fungal survival in the insect would enhance dissemination of the fungi, particularly via frass deposited on plants. Internal contamination also may lead to transovarial transmission. Eggs and frass of northern corn rootworms were contaminated by *Fusarium* spp. in Minnesota (16). More work is needed to determine if an internal association exists between the western corn rootworm and *Fusarium* spp. in Colorado, and the longevity of the association.

The importance of the host plant for western corn rootworm beetle contamination was evident with beetles collected from corn and squash hosts. Beetles from corn were more contaminated by *F. moniliforme* and *F. subglutinans*, reflecting the common occurrence of these fungi on aboveground corn in Colorado (7–9). Squash is not a host for these fungi, and beetles from squash were less contaminated by *F. moniliforme* and *F. subglutinans*, but were more contaminated by other *Fusarium* spp. The infrequent association of the more virulent *F. graminearum* with western corn rootworm beetles may have been due to the scarcity of this fungus at the two locations; *F. graminearum* was isolated from picnic beetles in Ontario, Canada, where it causes corn ear rot (1). Therefore, the association between western corn rootworm beetles and stalk rot *Fusarium* spp. may be coincidental rather than "specific." The association provides little apparent benefit for the beetles, whereas the beetles deliver fungal propagules to an infection court and provide entry sites for the fungi by feeding on silks, ears, and kernels.

Isolates of *Fusarium* spp. from beetles were pathogenic in corn. *F. graminearum* was most virulent, and disease ratings of stalk rot *Fusarium* spp. from beetles were similar to ratings of stalk rot *Fusarium* spp. from other sources (9). It is evident that beetles can serve as vectors for pathogenic isolates of stalk rot *Fusarium* spp.

The abundance and high activity rate of western corn rootworm beetles on corn plants enhance the chances of beetles to contact and disseminate these stalk rot *Fusarium* spp. Western corn rootworm beetles were the most abundant beetles on corn plants at both locations in this study, and 10–20 beetles were commonly observed on individual plants and ears. Silks were initially damaged, but beetles eventually fed on ear tips, husks, and developing kernels;

many ear tips were tattered and exposed by beetle feeding. Beetles were very active on corn plants during the day, frequently flying from plant to plant. Evidence of beetles as fungal vectors was provided by the beetle population experiments where kernel contamination by *F. moniliforme* and *F. subglutinans* was positively correlated to beetle populations established on corn ears. The greatest level of kernel contamination was from ears with 10 and 20 beetles at green silk stage, which may have been because of the early establishment of the fungi on ears via beetles.

Lower levels of kernel contamination detected in beetle population experiments in 1982 versus 1983 and 1984 may be due to the 14-mo storage period before conducting *Fusarium* spp. assays. In 1983 and 1984, ears were stored only 2–2.5 months. Storage may have reduced fungal viability on the 1982 kernels, or beetles established on ears in 1982 may not have been heavily contaminated by *Fusarium*. Grain corn may be fed to livestock 2–3 mo after harvest in Colorado, and a 14-mo storage period may not approximate actual storage time of feed. Heavy kernel contamination is a concern because of potential toxin production by fungi in feed (13,17,18). We assayed several grain corn samples sent by corn growers to the Colorado State University Plant Disease Clinic in 1982 and 1983, and found that contamination of non-disinfested kernels by *F. moniliforme* and *F. subglutinans* ranged from 26–100% (9). This indicates that samples of non-disinfested corn kernels may have a range of contamination.

Further evidence of western corn rootworm beetles as vectors of *F. moniliforme* and *F. subglutinans* to corn kernels came from the inoculum source study. In both plots, surface-disinfestation reduced kernel contamination greatly, indicating mostly external contamination of kernels, probably via the air or insects. In the plot with Fusarium stalk rot and heavy western corn rootworm beetle infestation, kernel contamination was 73%, whereas in the plot without stalk rot and beetle infestation kernel contamination was only 27%. We attribute the difference in kernel contamination between the two plots to the presence of western corn rootworm beetles.

Based on the high rate of western corn rootworm contamination by *F. moniliforme* and *F. subglutinans* and the correlation of beetle populations with kernel contamination by these fungi, we feel the association between the fungi and insects may play a role in the ecology of these fungi, particularly in kernel contamination. The high rate of beetle contamination, particularly internal contamination, increases the potential importance of these insects as fungal vectors. Beetle control in the field may reduce kernel contamination; however, further research is necessary to determine the significance of beetle-disseminated inoculum in respect to other means of kernel contamination, particularly contamination occurring during normal corn harvesting and shelling operations.

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