

## Saprophytism and Survival of *Fusarium moniliforme* in Corn Stalks

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### ABSTRACT

Survival of *Fusarium moniliforme* within infected corn stalks was tested by placing corn fragments at different soil depths, soil moistures, and soil temp. *Fusarium moniliforme* survived best in corn fragments buried 30 cm deep, at 5-35% soil moisture content, and 5-10 C soil temp. It was recovered from corn tissues that decomposed the least, namely,

parenchyma and sclerenchyma adjacent to stalk epidermis, and to vascular tissues in nodes, internodes, and leaves. Survival was low in roots and small host fragments. *Phytopathology* 60:1233-1235.

*Additional key words:* soil microorganisms, soil inhabitant.

*Fusarium moniliforme* (Sheld.) Snyder & Hans. is frequently isolated from stalks and kernels of corn (*Zea mays* L.) in Minnesota. Its presence in kernels may (2) or may not (4) lead to stalk infection. Inoculum from infected stalks on the ground can also be blown onto shoots to infect nodes through leaf sheaths (4) or enter stalk tissues through injuries (5). Although this fungus has been found on roots, in stalks, and in rotted ears of corn (1), it is sometimes thought to be a wound parasite or a secondary organism. It behaves differently in combination with *F. oxysporum* than it does alone, and temp is also a factor in tests of pathogenicity to corn seedlings (7). Yet it is a common pathogen of corn in Minnesota (4, 5, 7) and other places in the Corn Belt (1). We showed previously that this fungus can survive in the field as thickened hyphae in infected stalks, and that roots of corn plants often grow into and through buried corn stalks (6). In this work, the factors of soil moisture, soil temp, and depth of burial are evaluated as they affect saprophytic development and survival in corn stalks.

**MATERIALS AND METHODS.**—Five varieties of corn were used: Minhybrids 313, 414, 417, 418, and 507. Isolates of *F. moniliforme* were obtained from Fairbault, Mountain Lake, and Rosemount, Minnesota, and all infected corn stalk fragments (1-2 × 2-4 cm) were inoculated with isolates from these locations, using the toothpick method (8). The tests were designed so that all isolates were used in a given study.

To evaluate survival of fungi, infested corn stalk fragments were (i) agitated in tap water plus detergent and rinsed in tap water to remove excess soil; (ii) surface treated in ethanol (95%) for 1 min, NaOCl (5%) for 3 min, and rinsed in running tap water; and (iii) placed on acid potato-dextrose agar (APDA) adjusted to pH 4.5-5.0 with lactic acid. After 3 days' incubation, fragments were examined for *F. moniliforme*.

Soil moisture was determined by the oven-dry method and recorded on a dry wt basis, and field-moisture capacity was based on a modified Hilgaard method. Soil was autoclaved at 121 C, 8 hr/day for 3 successive days.

**RESULTS.**—*Survival at four soil depths.*—Fifty fragments of infested corn stalks were placed in a plastic net (15 × 30 cm; 14 mesh/cm). One end was folded to make a bag (15 × 15 cm) and secured with tape. In October, a trench (60 cm deep) was dug in soil in which corn grew the previous summer. Bags containing fragments were placed at four depths (0, 10, 30, and 60 cm; initial soil moisture contents of 5, 17, 26, and 16%, respectively); care was taken to restore the original soil profile. At each sampling date, 50 fragments from each depth were examined for the presence of *F. moniliforme*.

There was little difference in survival of *F. moniliforme* among different depths during the first 3 months (Fig. 1). Since the snow was only 3 to 6 cm deep during this period, there was little protection of fungi in corn tissues, and the soil was frozen 10 cm deep. In June, 8 months after the test was started, *F. moniliforme* had survived best at 30 cm (94%). The moisture content of the soil was greatest at this depth, and fragments were moist when recovered. Also, there was little decomposition of tissues. Living mycelium was easily detected in sections of all fragments examined microscopically.

Fragments at the surface (0 cm) were dry, and *F. moniliforme* was recovered from fewer fragments than at any other depth (36%). Live mycelium was found only with difficulty in fragments on the surface. Pure, dense cultures of *F. moniliforme* grew from corn fragments stored at 30 cm but sparse cultures, overgrown with other fungi, grew from fragments stored at other depths and on the surface.

*Survival affected by soil moisture.*—Waukegan-Dakota silt loam soil on which corn had been growing for 6 years was oven-dried at 35 C. Silt loam, peat, and sand were mixed (6:3:1, v/v) and autoclaved. Field and autoclaved soils were each added separately to sterile, 2-liter jars. Then sterile water was added to each jar and a field moisture capacity of 35% was obtained for the field soil and 26% for the autoclaved soil. Corn stalk fragments infested with *F. moniliforme* were buried in soil of each jar. Jars were weighed weekly, and sufficient water was added to maintain

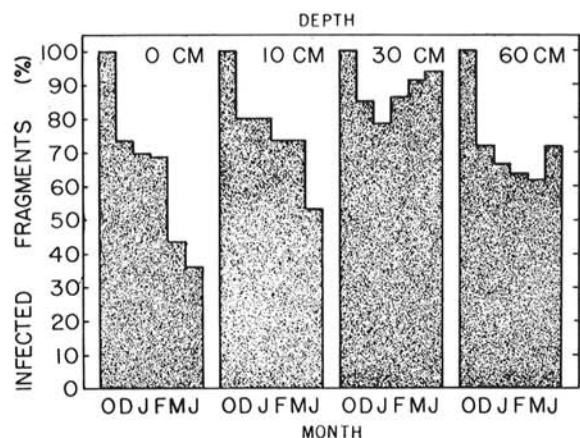


Fig. 1. Survival of *Fusarium moniliforme* as judged by recovery of fungus from infested corn stalk fragments buried in October at different soil depths. Isolations made in December (D), January (J), February (F), March (M), and June (J) in comparison to recovery at the start of the test in October (O). Each value is based on 50 corn fragments.

the initial moisture capacity. Controls consisted of noninfested corn stalk fragments in autoclaved soil.

As 0% moisture content, survival of *F. moniliforme* within corn fragments buried in silt loam decreased gradually in 8 months but remained high in autoclaved soil up to the last 3 months of the test (Table 1). At 5-35% moisture contents, there was no statistically significant difference (5% level) in survival among moisture contents of the nonautoclaved silt loam (48-68% recovery). Survival of *F. moniliforme* was greater in autoclaved soil than in the silt loam in moisture

contents from 5% to 15-35% range. Species of *Alternaria*, *Chaetomium*, *Fusarium*, *Helminthosporium*, and *Trichoderma* were isolated frequently from fragments buried in silt loam at 35% moisture content but infrequently at other moisture contents. Apparently these fungi had little effect on recovery of *F. moniliforme*. At 45% moisture content (soil saturation), *F. moniliforme* was isolated from 16% of infested fragments in autoclaved soil and 12% from fragments in silt loam. *F. moniliforme* was never isolated from fragments of controls at any moisture content.

*Survival affected by soil temp.*—Two hundred g of Waukegan-Dakota silt loam (12% moisture content) together with 25 infested corn stalk fragments were put into a polyethylene bag (2-liter capacity). A thermometer was inserted into each bag; the bags were rolled and secured with rubber bands. Bags were stored at different temp for 8 months, one bag at each temp: -15, 1, 5, 10, 20, and 30 C.

Recovery of *F. moniliforme* from infested tissues varied from 28% at -15 C to 100% at 5 and 10 C, followed by a decline in recovery at 20 and 30 C, after 8 months' burial in soil (Table 1). Temperatures of 6 and 10 C favored survival throughout the 8 months of test. Hyphae of *F. moniliforme* grew profusely on APDA only from fragments stored at 10 C, and grew sparsely and often were overrun with other fungi, such as *Trichoderma* sp., from fragments stored at other temp.

In another test, the effect of alternate freezing and thawing was studied. The test was similar to that shown in Table 1, except that the soil was adjusted to 15% moisture content. One bag of soil and infested fragments was stored at 25 C, one at -15 C, and one

TABLE 1. Effect of soil moisture and temp on survival of *Fusarium moniliforme* in previously inoculated corn fragments buried 1 to 8 months in soil

Soil factor		Autoclaved (+) soil or not (-) <sup>c</sup>	% Fragments yielding fungus <sup>a</sup>						
Temp	Moisture <sup>b</sup>		Nov <sup>d</sup>	Dec	Jan	Feb	Mar	May	June
C		%							
Temp constant, moisture variable									
25	0	+	100	92	96	100	92		12
		—	100	100	96	88	48		52
	5	+	100	100	80	84	80		92
		—	100	100	72	80	76		48
	15	+	100	80	76	72	76		96
		—	100	96	60	68	48		64
	25	+	100	100	80	84	88		72
		—	100	96	96	76	84		56
	35	+	100	76	44	44	48		64
		—	100	88	84	76	76		68
	45	+	100	20	8	0	0		16
		—	100	48	40	32	32		12
Moisture constant, temp variable									
-15	12	—	100	40	36		16	20	28
1	12	—	100	60	68		80	72	52
5	12	—	100	80	84		80	80	100
10	12	—	100	80	84		84	84	100
20	12	—	100	72	64		60	68	76
30	12	—	100	76	76		68	64	56

<sup>a</sup> Corn plants in the field were inoculated by the toothpick method. Each percentage is an average of 25 corn fragments.

<sup>b</sup> Dry wt basis.

<sup>c</sup> Autoclaved soil was a 6:3:1 (v/v) mixture of silt loam:peat:sand; whereas nonautoclaved soil was Waukegan-Dakota silt loam soil from the field.

<sup>d</sup> Indicates the month when the experiment was started.

alternately between these two temp every 3 days. Survival of *F. moniliforme* was 80, 84, 80, and 40% after 1, 3, 5, and 7 months, respectively, after burial in soil at 25 C. Survival was 36, 52, 36, and 0% after the same months of storage at -15 C. With the alternate freezing and thawing, survival was 80, 98, 84, and 12% after 1 to 7 months of storage. Thus exposure to -15 C reduced survival more than the alternating temp did.

*Survival in host tissues of different sizes.*—Survival of *F. moniliforme* might be related to size of fragment and to degree of decomposition of tissues. After corn stalks were inoculated in the field, they were cut into sections: 10 whole internodes (including 2 nodes); 25 half internodes (one node); and 50 quarter internodes. These fragments were buried in silt loam (nonautoclaved) at 21% moisture (wet wt basis) and stored outdoors for 1-8 months (November-June).

The incidence of infested stalk fragments, which was 100% in November, varied from 70 to 84% for all three sizes when tested in December and February. In April, however, 70% of whole, 56% of halved, and 34% of quartered internodes still contained live mycelium. By June, these percentages dropped to 64 for whole, 34 for halved, and 26 for quartered internodes. Thus, as fragments became smaller, survival was reduced.

Initially *F. moniliforme* was present in all tissues of the corn stalk. However, as parenchyma of the pith decayed, the fungus disappeared. Only in the parenchyma and sclerenchyma near the epidermis or near the vascular tissues did the fungus survive, either in the node or internode, as we reported previously (6). The longer the tissues remained intact, the greater the chance the fungus would survive.

When 50 inoculated fragments of roots, stalks, and leaves were buried 10 cm deep in silt loam soil (nonautoclaved, 15% moisture content) outdoors for 8 months, the incidence of infested organs decreased from 100% in October to 60% of stalks, 18% leaves, and 8% of roots, in June. The relatively rapid deterioration of roots accounted for the low survival there. Even in leaves, the fungus was found only in tissues near the vascular elements. The more decay-resistant tissues of stalks favored survival of *F. moniliforme* there.

**DISCUSSION.**—The presence of *F. moniliforme* in corn fragments buried in the field by tillage operations is explained by its parasitism or growth in live plants and its continued saprophytic growth in stalks after ears are harvested but before soil microorganisms have had a chance to invade corn tissues. This fungus is not a soil inhabitant, as it did not colonize sterile fragments of corn buried in field soil (6).

The conditions under which this fungus grows saprophytically on host tissues and the conditions for survival are not the same. Thus, conditions that favored saprophytic development of *F. moniliforme* in stalk tissues also favored microbial activity in general, and these conditions occurred at or near the soil surface, where aeration, moisture, and temp were optimum. As host tissues decayed, *F. moniliforme* gradually

disappeared, and eventually the fungus could only be found in tissues most resistant to decay, such as sclerenchyma and parenchyma, near the epidermal and vascular tissues. This accounts for its survival in stalks and not roots. As the saprophytic phase ends, the fungus is threatened with extinction unless conditions change. *Fusarium moniliforme* has not been found in soil apart from host tissue in several years of sampling soil at Minnesota.

The poor competitive saprophytic ability of *F. moniliforme* is shown also by its poor survival in soil of high moisture content, where antagonists, especially bacteria, would be numerous and would flourish, and by its lower survival at high (> 20 C) than at lower soil temp. If the soil temp is too low (< 1 C), survival is also low, probably because of desiccation. Survival is lower in small than in large fragments, again probably because of desiccation as well as by the greater chances of encounter with antagonists.

Garrett (3) noted "that those conditions which at one time encourage the activity of the fungus on the roots of the growing crop, viz., adequate soil moisture, good aeration and high temperature may at another time hasten its disappearance in the fallow soil" (p. 155). This generalization seems consistent with our results, where the thickened hyphae previously reported (6) seem to survive best when (i) host tissue is buried deeply in soil, where aeration is poor; (ii) soil is moist but not wet; (iii) soil temp is low (1-20 C); and (iv) there is little or no competition with other organisms.

Conversely, reduction of inoculum might be achieved by chopping crop debris into small fragments and either leaving them at the surface of soil or lightly disking them into soil, where the moisture and temp would favor microbial activity of soil saprophytes as well as hasten saprophytic development of the pathogen (*F. moniliforme*) and eventual deterioration of stalks. Growing mycelium is more subject to lysis, and if host material is decayed sufficiently, survival structures may not form to provide inoculum for the next season.

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