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## The Isolation of *Fusarium moniliforme* Sheld. from Corn Kernels<sup>1</sup>

RICHARD W. IKENBERRY

*Abstract.* Kernels representing six inbred corn lines were soaked in sterile distilled water for 24 and 48 hours, with and without aeration during soaking. When the kernels were plated on a nutritive medium following such treatments, frequency of appearance of *Fusarium moniliforme* on kernels was directly associated with longer periods of soaking, and with continuous aeration provided during soaking. Such variations in recovery of the organisms from the kernel, depending on isolation procedure used, strongly emphasize the fact that failure to isolate a fungus from host tissue does not necessarily mean the fungus is absent.

One of the organisms studied in connection with stalk rotting in corn (*Zea mays* L.) is *Fusarium moniliforme* Sheld. Although it is generally agreed that this fungus is frequently present in corn plant tissue, its pathogenicity in the rotting of stalks remains a subject of controversy among investigators. Some claim the organism to be an active parasite (Valleau, 1920; Peterson, 1961), whereas others attribute to it only a mild form of injury (Limber, 1927). It has been demonstrated that *F. moniliforme* is frequently present within corn kernels (Valleau, 1920; Edgerton and Kidder, 1925; Edwards, 1935), and recently the organism has been found to be systemic in the corn plant as early as July (Foley, 1959). It would seem likely that systemic infection could result in plants grown from infected kernels.

The value of plants free from infection with *F. moniliforme* for use as controls in experimentation with this organism is evident. To this end various methods of seed treatment have been examined without positive results. The fungus is probably well in the interior of the kernels, rendering surface treatments ineffective. In research on seed treatment methods it would be desirable to employ isolation techniques which would promote growth of the organism out of the kernel if the treatment is ineffective. The present study deals with problems involved in isolation procedures.

### MATERIALS AND METHODS

Five sets of test kernels were counted, using six inbred lines from 1959 and 1960 lots of seed. The kernels were wrapped in cheesecloth and suspended in sterile, distilled water in 12-oz. tall jars for periods of 24 and 48 hours. Continuous aeration

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was provided one set of kernels for each time period by passing filtered air through gas dispersion tubes immersed in the water. One set of kernels was left unsoaked, as a control.

Kernels were examined following the soaking procedures and found to show no growth of *F. moniliforme*. All kernels were then surface-sterilized, using 1.31% sodium hypochlorite solution, rinsed once in sterile distilled water and plated by pushing them into 1.7% nutritive agar medium in petri dishes. (Nutritive medium: 0.004M NH<sub>4</sub>NO<sub>3</sub>, 0.0038M CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.0038M KCl, 0.0021M KH<sub>2</sub>PO<sub>4</sub>, 0.0017M MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.055M glucose, plus trace amounts of MnCl<sub>2</sub>·4H<sub>2</sub>O, H<sub>3</sub>PO<sub>4</sub>, ZnSO<sub>4</sub>, CuSO<sub>4</sub> MoO<sub>3</sub> and Fe<sub>2</sub>O<sub>3</sub>.) The plates were incubated at room temperature.

RESULTS AND DISCUSSION

Successful isolation was recorded as the number of plated kernels showing *F. moniliforme* after an 8-day incubation period (Table 1).

Table 1. Isolation of *Fusarium moniliforme* from kernels of six inbred corn lines.<sup>1</sup>

Inbred line	Control	Soaked only		Soaked and aerated	
		24 hr.	48 hr.	24 hr.	48 hr.
1959 Seed Lot					
B14	x	xx	xxxx	xxx	xxxx
B37				xx	xxxx
Hy		x		x	xxxx
M14				x	xxxx
Oh51a	x	xx	xxxx	xxxx	xxxx
WF9	x	xxx	xxx	xxx	xxx
Totals	3	8	11	14	23
1960 Seed Lot					
B14	x		xxx	xx	x
B37	x		xxxx	xxxx	xxx
Hy	xxx	xxx	xxxx	xxxx	xx
M14	xxxx	x	x		x
Oh51a	x	xxx	xxx	xxxx	xxxx
WF9	x	x		x	x
Totals	11	8	15	15	12
Final Totals <sup>2</sup>	14	16	26	29	35

<sup>1</sup> Each x represents one kernel showing growth of *F. moniliforme*. Four kernels of each line plated for each of the five tests.

<sup>2</sup> 48 total possible for each column.

In some repetitions of the experiment it was found that the presence of bacteria on the seed may interfere with the results. Where improper surface-sterilization of the kernels allowed bacterial colonies to develop around the plated seeds very little,

if any, growth of *F. moniliforme* was observed. Some factor of antagonism was evidently active in such cases.

Valleau (1920), in recalling the findings of previous workers on seed infection by *F. moniliforme*, states, "The source of the remainder of field infection has not hitherto been ascertained, but it must be either from the soil or from more extensive seed infection than we have supposed to exist." It has been demonstrated in the present experiment that isolation of *F. moniliforme* from the kernel may vary according to isolation technique employed. Recovery of the organism was greater from soaked seed than from unsoaked, and aeration during soaking improved recovery even more. This would seem to bear out Valleau's supposition about extensive seed infection and also to emphasize the accepted concept that failure to isolate an organism from host tissue does not necessarily preclude its presence.

The method of aeration in water has also been found to bring about growth of *F. moniliforme* from kernel lots which showed no infection when germinated on moist blotter paper in petri dishes. In some cases 100% isolation of the organism has been obtained from samples of test kernels after using the aeration technique. Could a superior isolation technique, yet to be developed, demonstrate that no kernel is free from the organism?

It would seem that the growth of the organism is stimulated to an extent by increased moisture, and further by more available oxygen. Growth of the fungus from the seed does not usually occur when the kernel is germinated in soil under moisture conditions optimum for growth of the corn plant. Further work, involving the bubbling of oxygen-enriched air through the water, or much longer periods of soaking, may shed some light on the problem.

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