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Project Title: Sweet corn diseases and their management in the PNW: Seed treatments and development of *Fusarium*-free seed

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Background: Sweet corn growers in the Willamette Valley of Oregon reported declining yields during the early 1990s. The decline in yields was associated with leaf-firing, in which the leaves die prematurely starting at the base of the plant and then progressing upwards. Initially, the firing and associated yield loss was thought to be caused by root rot. Recent investigations have shown that crown rot, accompanied by a stalk node rot, appears to explain much of the observed leaf firing and the concomitant loss in yield, rather than root rot. Pathogenicity experiments by Miller and Ocamb show that *Fusarium* spp. (*F. oxysporum*, *F. verticillioides*, and *F. proliferatum*) can have a negative impact on sweet corn health and yield. But other factors contribute as well when yield declines involve Fusarium diseases, including characteristics of specific corn genotypes, environmental conditions which stress plants or favor *Fusarium*, and the microbial population associated with seeds or soil.

Commercial sweet corn seed lots have been found to have high percentages of seed containing *Fusarium* (Miller and Ocamb, unpublished). Numerous species of seed-borne *Fusarium* are recovered from corn seed produced throughout the world; of these, *F. verticillioides* and *F. proliferatum* commonly predominate. These two species are known to cause seed rot, seedling blight, stalk rot, root rot, or ear rot of corn, and we have found them in consortium with *F. oxysporum* inside necrotic crown and stalk node tissues. Sweet corn seed is usually treated with fungicides which normally prevent seedling rot and damping-off. However, fungicidal seed treatments may not prevent seedlings from becoming infected by seed-borne *Fusarium*, even though these treatments can assist seedlings in reaching the 2-leaf stage. Reduction or eradication of *Fusarium* from sweet corn seed is associated with an improvement in crown and stalk health as well as potential improvements in ear yields (Miller and Ocamb, unpublished), but removal of *Fusarium* from corn seed does not necessarily reduce losses from Fusarium diseases if pathogenic populations are present in the soil where the seeds are planted. Reduction in seed-borne *Fusarium* accompanied by applications of biological control microbes can lead to reductions in root rot severity and crown rot incidence as well as increased ear yields (Ocamb, unpublished).

Fusarium species can associate with sweet corn seed through a number of different routes, including infection through corn silks or by *Fusarium* growing up through the stalk to the seed. We have examined silks of inbred females for susceptibility to *Fusarium* colonization for one season and have observed differences among the few lines examined. The use of biological control microbes to prevent kernel infection has shown some promising but preliminary results in our 2009 and 2010 studies. During 2011, we focused on the parents of a single corn hybrid (Jubilee) and found a significant reduction in *Fusarium* spp. on silks and kernels of the 'Jubilee' seed parent. We proposed to continue examinations into biocontrol treatment of seed parents and its subsequent effects on seed produced. During 2012, we hoped to be able to examine the first generation of seed from biocontrol-treated seed parent plants, but unfortunately found that the seeds were not viable.

By understanding how *Fusarium* associates with sweet corn seeds and investigating practices that may reduce the incidence of seed-borne *Fusarium*, it may be possible to reduce Fusarium diseases and their associated losses in sweet corn production. While we are becoming more confident that reducing *Fusarium* on seed will benefit the industry, more data are needed to support changes in vegetable seed production. The proposed investigations would aid in improving the sustainability of sweet corn production in Oregon because of the potential disease reduction and yield improvements that can be attained without additional chemical inputs.

Objectives for 2012 and Accomplishments:

1. Conduct an evaluation of materials currently registered on sweet corn for control of seed rot and seedling blight.

Seedling emergence was variable among treatments, but conventional fungicides had relatively greater stands than the biological materials (biocontrols or plant extracts). In contrast, two biocontrol formulations resulted in less rot of the seminal roots while another biological appeared to enhance rot of the seminal roots (Actinovate). There was severe rot of the radicle and mesocotyl tissues in general and no treatment differences. Overall, the adventitious root rot was low in severity and no treatment controlled severity on this date of sampling. Most treatments appear to delay the ingress or onset of symptoms in the nodes, which were beginning to become infected in the stalk. Incidence of crown rot was reduced in the Captec and MicroAFD treatments.

An experimental field on the OSU-Botany Farm was inoculated during several growing seasons since 2000 with a complex of *Fusarium* species, and has been repeatedly shown to have severe disease pressure for Fusarium crown and stalk node rot of sweet corn. Treatments included conventional fungicides as well as biocontrol formulations and are shown in Table 1. Treated and nontreated seeds were sown in the "Fusarium" field in 20-ft long, 1-row plots with seven replicates in a randomized block design on 22 June 2012 using hand-planters. Plants were irrigated weekly with about 1.5 inches of water, depending on the weather, commencing the day of sowing. Stand counts were made 27 days after planting. On days 54 and 55 after sowing, plants were at the 4- to 5-leaf stage and were assessed for root, crown, and stalk disease. Five plants were dug from each plot, soil was washed from the root balls so the percentage of the underground portion of the plant with rot could be estimated, and then each plant's crown as well as the lower stalk portion was split open longitudinally for an evaluation of crown and stalk node rot. Crown grayscale was measured with a grayscale analysis (ImageJ, NIH) after each crown was digitally-captured on a flatbed scanner.

Table 1. Treatments examined in sweet corn 'Jubilee' for stand and plant health effects

Trt #	Treatment [x]
1	Captec 4L at 4 fl oz/100 lb seed (*FRAC M4, ai=captan*)
2	42-S Thiram at 5 fl oz/100 lb seed (*FRAC M3, ai=thiram*)
3	Allegiance-FL at 0.75 fl oz/100 lb seed (*FRAC 4, ai=metalaxyl*)
4	Apron XL LS at 0.64 fl oz/100 lb seed (*FRAC 4, ai=metalaxyl*)
5	Maxim 4FS at 0.08 fl oz/100 lb seed (*FRAC 12, ai=fludioxonil*)
6	Dynasty at 0.153 fl oz/100 lb seed (*FRAC 11, ai=azoxystrobin*)
7	Dynasty + Maxim 4FS + Apron XL
8	Raxil 2.6F at 0.1 fl oz/100 lb seed (*FRAC 3, ai=tebuconazole*)
9	MicroAFD at 2% seed wt (*biocontrol mixture*)
10	Actinovate AG at 6 fl oz/100 lb seed (*biocontrol: Streptomyces lydicus)*
11	Sonata at 5.0 fl oz/100 lb seed (*biocontrol: Bacillus pumilis)*
12	Serenade ASO/Max at 5.0 fl oz/100 lb seed (*biocontrol: Bacillus subtilis)*
13	Regalia at 2 quarts/A (*extract of Reynoutria sachalienensis)*
14	Prestop at 3.5 oz/100 sq. ft (*biocontrol: Gliocladium catenulatum)*
15	Taegro at 2 quarts/A (*biocontrol: Bacillus subtilis)*
16	nontreated

[x] Stamina (FRAC 11, ai=pyraclostrobin) was requested from BASF but was not provided in time for planting.

Lorsban Advanced at 2 pints/A was applied preplant but rootworm damage was visible on the root systems by 27 days after sowing and was rated as follows:

0 = no root worm feeding is evident;
1 = slight root worm feeding is evident;
2 = < 75 % of adventitious roots at a single whorl have feeding; and
3 = ≥ 75 % of adventitious roots at one whorl or ≥ 50 % of adventitious roots at 2 whorls have feeding.

Stand number (averaged across 7 replicate blocks) was significantly different among a few treatments examined on Jubilee (Fig. 1A). Generally, biocontrols had lower establishment numbers than the traditional fungicides, Captec or Raxil, except for the biocontrol, Serenade. While most of the treatments had relatively similar rootworm feeding on the roots, Captec was associated with less while Actinovate had greater rootworm injury (Fig. 1B). The primary root (radicle) was almost completely rotted in all treatments at the time of sampling (≥99 % of the root length was rotten), and the mesocotyl findings were nearly identical (≥98.9 % of the mesocotyl was rotten), so these data are not shown. There were significant differences among treatments in rot of the % of seminal and there is a trend towards less severe root rot in the biocontrol treatments compared to the more traditional fungicides, except for Actinovate, which had an increase in severity of seminal roots rotted (Fig. 1C). The adventitious root rot severity was low overall, and no treatment reduced severity relative to the nontreated control plants (Fig. 1D). Most of the treatments examined resulted in a reduction in the number of stalk nodes with necrosis compared to plants that grew from nontreated seed, except for Actinovate as well as Raxil and Maxim (Fig. 1E). The symptom with the greatest affect on yield in this experimental *Fusarium* field, crown rot, was present at higher percentages in most treatments, including the nontreated control, and though there was little statistical separation among the treatments, MicroAFD and Captec appeared to reduce, or more likely, delay crown rot (Fig. 1F).

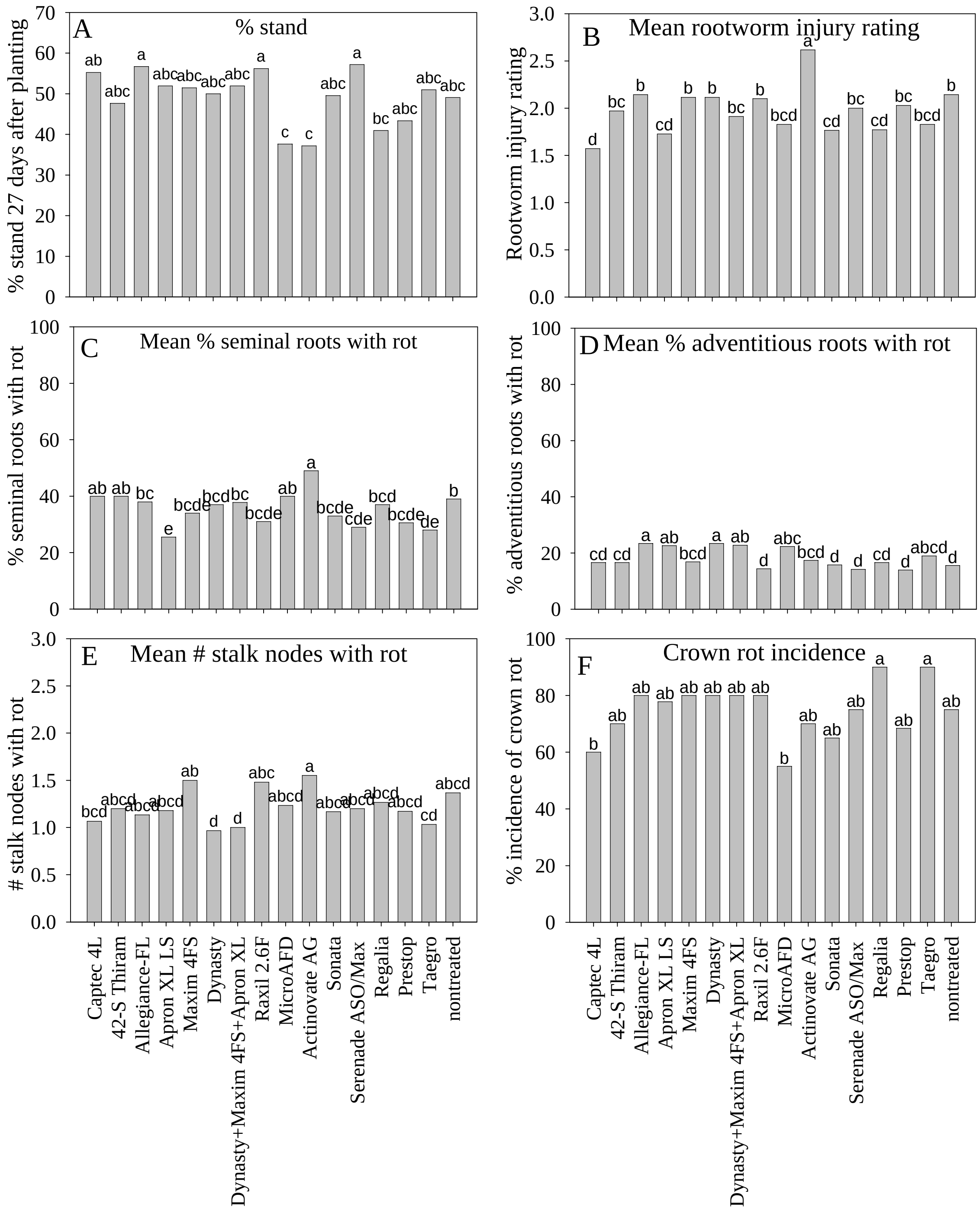

Figure 1. Stand, rootworm injury on roots, and disease assessments of sweet corn 'Jubilee' during 2012. Within each figure, means labeled with the same letters are not significantly different (P=0.05) as determined by Fisher's LSD test.

Objective 2. Evaluate biological applications to sweet corn seed parents and subsequent *Fusarium* presence on silks, in stalk nodes, and subsequent seed infection/contamination.

> *Fusarium crown and stalk node rot as well as root rots were present but little difference was found between the biocontrol and nontreated Jubilee seed parent plants in terms of disease severity, though stalk node rot was significantly more severe in the nontreated plants. Biocontrol applications resulted in a significant increase in ear weights, both for the primary ear and total ear yield. Evaluation of presence of Fusarium species associated with silk and seeds are currently under way at the time of writing of this report.*

Kernels of the inbreds for ‘Jubilee’ were seed-treated with Apron Maxx RTA and then sown with a 4-row planter into a field not commonly rotated to corn on the OSU Botany Field Lab (Electric Rd., Corvallis) on 6 July 2012. The seed parent of ‘Jubilee’ was replicated twice as 55-ft long, 4-row plots for each treatment, with a 1-row border on each side for the ‘Jubilee’ pollen parent. There were two treatments for the seed parent: nontreated or MicroAF (an experimental biocontrol formulation) at 12.8 fl oz/A as a soil drench at planting and later as applications to silks each week until the outer silks turned brown (Sep 27 , Oct 4, Oct 11, and Oct 18). Plants were irrigated every week with approximately 1.5” of water, depending on the weather conditions. The seed parent plants were subsampled for disease evaluations later in the season, 123 days after planting (Nov. 6). On 15 Nov 2012, the primary ear was harvested from each of 30 plants in each row of the seed parent of Jubilee. Individual, non-husked ear weights were recorded. Then most husks were stripped off (leaving two layers), external silk portions were removed , individual ears were placed in paper bags, and then stored in a room maintained at <85ºF for drying before sampling for *Fusarium*. Silks pieces were excised in the laboratory and plated onto a *Fusarium*-selective medium. Five kernels were excised from each ear from the same area that the silks were sampled from and then plated onto *Fusarium*-selective medium. The rest of the kernels were removed and weighed, and then the kernels were placed in paper bags (kept as individual ears). On 27 Nov 2012, ears were harvested from 10 additional plants and husked ear weight was recorded for all ears produced while ears were saved for drying to obtain additional, perhaps more mature seeds.

Not unexpectedly, the level of root rot, rootworm injury, and crown rot was not significantly between the two seed parent treatments at this later sampling time (immature ears) during the growing season. Rot of the radicle and mesocotyl were ~ 100% and crown rot incidence was 100%, while adventitious root rot averaged ~ 30%. The number of stalk nodes with rot varied significantly ($P \leq 0.05$), 3.1 and 2.2 nodes, respectively, for the nontreated plants compared to the plants receiving biocontrol (MicroAF) applications. There was a significant increase in husked ear yield in the plants that were treated with MicroAF (Table 2), which yielded about 74% more in average yield per plant and a 27% greater primary ear weight compared to the nontreated plants. However, there was no difference between the two treatments in non-husked ear weights.

Table 3. Effect of biocontrol treatment on ear yield

Sweet corn inbred	Biocontrol treatment	Average ear yield per plant (g)[1]	Primary ear wt (g)[1]	Non-husked primary ear wt (g)[1]
Female parent of Jubilee	nontreated	138 b	132 b	172 a
Female parent of Jubilee	MicroAF	240 a	168 a	174 a

[1]Within each column, means labeled with the same letters are not significantly different (P=0.05) as determined by Fisher’s LSD test.

Objective 3. Evaluate 1st generation ‘Jubilee’ seed produced from seed parent plants treated with biocontrol applications.

Kernels produced by the inbreds for ‘Jubilee’ during 2011 were treated with Apron Maxx RTA and then sown with a 4-row planter into the experimental Fusarium field on the OSU Botany Field Lab (Electric Rd., Corvallis) on 21 June 2012. Seed had an extremely low emergence rate (<10%), and upon further investigation, it was recognized that probably embryos did not survive the temperature (100°F) used for drying ears in 2011.

Objective 4. Cooperate with other sweet corn projects within and outside of OSU.

Ocamb compared two sweet corn varieties (‘Jubilee’ and ‘GSS1477’) for development of Fusarium crown and stalk node rot. Plants were sampled on seven different dates during the growing season, beginning at the 2-leaf stage and through immature ear development. Data will be presented at the oral reports.

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