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Project Title: Management of Fusarium diseases of sweet corn in the PNW: Seed microflora influence on disease 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", where the leaves die prematurely starting at the base of the plant and then progress up the plant. 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 at least partly, perhaps greatly, the concomitant loss in yield.

Our pathogenicity experiments 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 are involved as well when yield declines involve Fusarium diseases; such as characteristics of specific corn genotypes, environmental conditions which stress plants or favor *Fusarium*, microbial population structure surrounding and within infection points of the corn plant. We've developed evidence that the Western Spotted Cucumber Beetle preferentially feeds on *Fusarium*-infected plants and possibly vectors *Fusarium* species to non-infected plants.

Commercial sweet corn seed lots have been found to contain high percentages of seed containing *Fusarium* (Miller and Ocamb, unpublished). Several species of seed-borne *Fusarium* are recovered from seed produced throughout the world; *F. verticillioides* and *F. proliferatum* are commonly reported. These two species are reported to cause seed rot, seedling blight, stalk rot, root rot, or ear rot. Thus 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*. Not all strains of specific *Fusarium* species are equal in virulence and some strains are apparently non-pathogenic, especially notable for *F. verticillioides* strains which some argue to be endophytic. Different species of *Fusarium* can cohabitate the same corn plant, and the interaction among strains on seed and with populations of *Fusarium* in the soil is not understood. Removal of all *Fusarium* from seed and planting of these seeds does not necessarily reduce losses from Fusarium diseases if pathogenic populations are present in the soil, but removal of seed-borne *Fusarium* and subsequent biological seed treatment can lead to reductions in root rot severity and crown rot incidence as well as improving yields. So perhaps a weaker pathogen that is present on seed primes the plant for subsequent infection by more aggressive soilborne strains of *Fusarium*. Removal of the *Fusarium* presence on sweet corn seed is also associated with a reduction in root worm injury.

Objectives for 2009 and Accomplishments:

Objective 1: Evaluate sweet corn inbred germplasm for colonization of silks by *Fusarium* species and subsequent seed infection/contamination.

Sweet corn genotypes used for Objectives 1 & 2
GH1861 inbred
GH1861 inbred
GH1861
Jubilee-C inbred
Jubilee-C inbred
Jubilee-C
GSS1477 inbred
GSS1477 inbred
GSS1477
GSS9372 inbred
GSS9372 inbred
GSS9372

Kernels of the lines listed in the table on the left were treated with Apron Maxx RTA and then sown with a hand-pushed belt planter into our Fusarium crown rot field on the OSU Botany Field Lab (Electric Rd., Corvallis). Each corn line was replicated in seven 20-foot long rows. A plot code was used so that treatments were not known while disease evaluations were made. Plants were irrigated weekly with 1.5" of water. Stand counts were taken several weeks after sowing. Plants were evaluated at silking, 66-67 days after planting, for rot of roots, crown, and stalk nodes as well as Western Spotted Cucumber beetle feeding on leaves or roots. Five plants were dug from each plot (35 plants total per treatment), soil was washed from the root balls of each plant, each internal crown was exposed by longitudinally splitting through the lower stalk, and crowns were digitally scanned for crown grayscale analysis (grayscale data not presented). Rot of each plant was also determined visually. Incidence of crown rot was also visually determined by C. Ocamb after splitting open each crown, noting whether crown rot was present, developing, or appeared healthy.

The rot of the primary root (radicle), adventitious root system, and subcrown-internode (mesocotyl) was visually estimated on a percentage basis while rot in the crown and stalk nodes as well as rootworm feeding was rated as follows:

Nodal rating
0 = no discoloration of stalk nodes above crown
1 = node 1 above crown is discolored (dark brown)
2 = node 2 above crown is discolored (dark brown)
3 = node 3 above crown is discolored (dark brown)

Crown rot rating
0 = no discoloration of crown area (creamy-colored) or tan-light brown crown area (normal)
1 = crown rot

Root worm feeding
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
3 = $\geq$ 75 % of adventitious roots at one whorl or $\geq$ 50 % of adventitious roots at 2 whorls have feeding

During October, ears were sampled from the 3 sets of inbred parents for 1861, 1477, and Jubilee. Silks were excised in the laboratory and plated onto *Fusarium*-selective medium. Late November, ears were harvested from five plants/plot of each the three seed parents, ear weights were recorded, and kernels were excised and then plated onto *Fusarium*-selective medium.

Results for Objective 1: Very little rootworm injury was found on plants when sampled at silking, probably due to the cool conditions that occurred during the 2009 growing season (Table. 1). Crown rot was prominent in certain lines, such as one parent of 1861, and both parents of Jubilee as well as 9372. Primary roots and mesocotyl were rotten by early silking but

adventitious root rot levels were low, as we typically find for sweet corn plants grown in our crown rot field.

Table 1. 2009 rot ratings of sweet corn inbreds and hybrids

Sweet corn genotype	Mean stalk node # discolored[1]		Incidence of crown rot[1]		Mean % primary root with rot[1]		Mean % adventitious roots with rot[1]		Mean % mesocotyl with rot[1]		Mean rootworm injury[1]	
GH1861 inbred	3.1	d	94	ab	100	a	5.8	g	99.7	a	0.03	ef
GH1861 inbred	1.2	e	20	f	100	a	13.0	ab	100	a	0.20	cd
GH1861	1.8	e	41	e	100	a	8.6	def	95	abc	0.34	bc
Jubilee-C inbred	4.8	a	97	ab	100	a	6.8	fg	96.5	abc	0.15	def
Jubilee-C inbred	4.3	abc	97	ab	100	a	14.6	a	99.0	ab	0.54	a
Jubilee-C	3.9	bcd	76	c	99.3	a	9.4	cde	94.7	abc	0.11	def
GSS1477 inbred	1.7	e	54	d	99.8	a	10.4	cd	99.4	a	0.14	def
GSS1477 inbred	1.2	e	62	d	100	a	11.2	bc	96.9	ab	0.18	de
GSS1477	1.2	e	44	e	99.7	a	11.2	bc	90.5	c	0.45	ab
GSS9372 inbred	4.0	bc	100	a	100	a	8.5	ef	100	a	0.04	ef
GSS9372 inbred	3.6	cd	100	a	100	a	13.9	a	100	a	0.00	f
GSS9372	4.5	ab	90	b	97.7	a	6.8	fg	92.9	bc	0.11	def
LSD	0.8		10		3.3		1.9		6.3		0.1	0.66

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

When silks of the seed parents were examined, Jubilee inbreds had little *Fusarium* on the silks sampled (Table 2). One parent of both 1861 and 1477 averaged at least one *Fusarium* colony per silk.

Table 2. Recovery of *Fusarium* spp. from silks of inbreds

Sweet corn genotype	Mean # *Fusarium* colonies/silk[1]	
GH1861 inbred	1.4	a
GH1861 inbred	0.4	cd
Jubilee-C inbred	0.4	cd
Jubilee-C inbred	0.2	cd
GSS1477 inbred	1.2	ab
GSS1477 inbred	0.6	bcd

[1]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 silks and subsequent *Fusarium* presence in ears.

Within the field study described above, treatments were also included where seed parents received applications of MicroAF (an experimental biocontrol formulation used at 12.8 fl oz/A) at planting as a soil drench and later as a spray on silks during ear development.

Results for Objective 2: *Fusarium* spp. were absent from silks while the silks were still green. Generally, the levels of Fusarium recovered from silks, once the silks started turning brown, were lower in plants that received biocontrol applications but there were significant differences only for 1477. Weight of the primary ear was generally unaffected but the weight of the secondary ear was significantly increased for 1477 when the biocontrol treatment was applied (Table 4). The percentages of kernels contaminated with *Fusarium* spp. were also decreased in 1477 as well as 1861 with biocontrol applications.

Table 3. Recovery of *Fusarium* spp. from inbred silks with and without biocontrol applications to soil and silks

Sweet corn genotype	Biocontrol treatment	Mean # *Fusarium* colonies/silk[1]	
GH1861 inbred	none	1.4	a
GH1861 inbred	none	0.4	cd
Jubilee-C inbred	none	0.4	cd
Jubilee-C inbred	none	0.2	cd
GSS1477 inbred	none	1.2	ab
GSS1477 inbred	none	0.6	bcd
GH1861 inbred	MicroAF	0.8	abc
GH1861 inbred	MicroAF	0.2	cd
Jubilee-C inbred	MicroAF	0.4	cd
Jubilee-C inbred	MicroAF	0.2	cd
GSS1477 inbred	MicroAF	0	d
GSS1477 inbred	MicroAF	0	d

[1]Means labeled with the same letters are not significantly different (P=0.05) as determined by Fisher's LSD test.

Table 4. Ear weights and recovery of *Fusarium* spp. from kernels of plants with and without biocontrol applications to soil and silks

Seed parent of	Biocontrol treatment	Incidence of rot at top ear node[1]		Incidence of rot at 2nd ear node[1]		Weight (g) of top ear[1]		Weight (g) of 2nd ear[1]		% kernels tested with *Fusarium* spp.[1]	
GH1861	none	100	a	100	a	159	a	96	ab	45	a
Jubilee	none	97	b	100	a	127	d	91	ab	14	c
GSS1477	none	97	b	94	b	143	bc	88	b	50	a
GH1861	MicroAF	100	a	100	a	155	ab	101	ab	25	bc
Jubilee	MicroAF	100	a	100	a	129	cd	84	b	11	c
GSS1477	MicroAF	100	a	100	a	149	ab	114	a	30	b

[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 seed disinfestation and location of remaining *Fusarium* on or in seed.

Seeds of four commercial sweet corn cultivars were treated with UV light, 3% Hydrogen peroxide, or 10% bleach. A new UV treatment chamber was constructed for 2009 studies and it is estimated that 1 hour in the new chamber is approximately equivalent to 5 hours in the old chamber used for 2008 studies. Seeds were exposed to UV light while being agitated on a shaker

for 1 hour. Seeds treated with bleach and hydrogen peroxide were soaked for 3 hours with agitation and then rinsed with reverse osmosis water. Dry, non-treated seeds were included for comparison. Seeds (50 per cultivar-treatment combination) were embedded in a *Fusarium*-selective medium and evaluated 10 days later.

Results for Objective 3: All three seed disinfestation treatments reduced the overall level of *Fusarium* incidence on seed. Bleach and hydrogen peroxide appear to be more effective in removing *Fusarium* from seed compared to UV light. This is probably because the wet treatments penetrate further into the seed. However, these wet treatments require a re-drying step, and may reduce seed quality; making wet seed treatment a less favorable option commercially. Utilizing UV light avoids the problems associated with wetting of seed and still results in a considerable reduction in *Fusarium* incidence on sweet corn seed. With incidence data, we could not differentiate heavily-infested seed from lightly-infested. There may also still be a significant reduction in *Fusarium* propagules on a seed, even if the seed yields *Fusarium* in our assay.

Table 5. Recovery of *Fusarium* spp. from seeds with and without seed-disinfestation treatments

	% Seeds with *Fusarium* after Seed Treatment			
Sweet corn cultivar	**non-treated**	**UV for 1 hr**	**10% Bleach for 3hr**	**3% H_2O_2 for 3hr**
2007 Jubilee	96	18	0	6
GH1861	100	26	4	2
Jubilee C	18	0	0	0
XP08705808	100	20	0	2

Objective 4: Examine the yield and disease levels of sweet corn plants grown from seeds treated with germicidal light.

Twelve non-treated, commercial seed lots were obtained from four seed companies (Table 6). Cultivars were chosen based on what processing companies reported as the most commonly-grown in Willamette Valley as well as cultivars that we had used in earlier studies. Seeds were exposed to UV light while being agitated on a shaker for 0 or 1 hour. A subset of treated seeds was then evaluated for *Fusarium* contamination by embedding 20 seeds of each variety-treatment combination in a *Fusarium*-selective medium. Seeds for planting in our experimental *Fusarium* field were treated with Apron/Max (5 fl oz/cwt) and then planted in 20' plots in 7 blocks on the OSU Botany and Plant Pathology Field Lab. At midseason (40 days post planting), 5 plants from each plot were dug, cleaned of soil, and rated for percentage rot of the primary root, sub-crown internode, and nodal roots as well as damage associated with rootworm feeding on a 0 to 3 scale (0 = no tunneling observed, 1 = up to 3 roots with tunneling, 2 = more than 3 roots but less than half with tunneling, and 3 = more than half the roots with tunneling). Crowns were split, scanned, and analyzed for degree of necrosis based on grayscale. Isolations for *Fusarium* were made from tissues of the primary root (PR), sub-crown internode (SCI), crown, and 1st node above the crown (N1). At processing maturity, ears from10 plants from each plot were weighed. Plot means were calculated and compared using MIXED procedure in SAS 9.1 with seed, UV treatment and block as main effects to evaluate differences in ear weights, *Fusarium* infection, crown grayscale and root rot/feeding ratings. Difference in *Fusarium* contamination on seed was analyzed using a paired t-test. Regression analysis was used to evaluate relationships between variables.

Table 6. Seed varieties used in 2009 UV seed disinfestation studies

Variety	Lot	Company	Type
Jubilee-C	OC6005XILF	Rogers	su
GH2298	CC4092MF	Rogers	su
GH1861	NC7309LF	Rogers	su
GSS1477	NW8137LF	Rogers	sh2
Evita	78210-MF	Crookham	su1
XP08705808	964352LR-27	Seminis	SH2+SH2
Basin-R	962412LR-27	Seminis	SH2+SH2
Coho	Q71054	Harris Moran	su
Kokanee	Q73679	Harris Moran	su
HM2390	Q60868	Harris Moran	su
Trustart	CW6035LF	Rogers	sh2
07Jub	NC4276MR	Rogers	su

Results for Objective 4: Treating seed with UV light significantly reduced (P = 0 .0007) the level of *Fusarium verticillioides* in all seed lots tested (Fig. 1H). Plants grown from seed treated with UV light tended to have better yields than non-treated plants but the overall differences were not highly significant (P = 0.056 for total ear weight and 0.07 for weight of ear 1), and results vary among varieties (Figs. 1A, 1B). Trustart, a 'Supersweet Jubilee' variety, had significantly better yields when grown from UV-treated seeds. This trend was also observed during 2008 in a similar trial. Crown rot, based on grayscale values, was also significantly reduced (P = 0.014) when seed was treated with UV light (Fig. 1C) which was also seen in two of the three cultivars tested during 2008. Levels of *Fusarium* infection were significantly reduced (P < 0.001) in all plant parts sampled at mid-season when plants were grown from seed treated with UV light (Figs. 1D-1G). Root worm damage was also reduced in GH2298 and Kokanee when seed was treated with UV light (Fig. 1I). *Fusarium verticillioides* infection levels correlated strongly with seed contamination levels (Fig. 2). As in previous studies, crown grayscale was significantly correlated with ear weights (Fig. 3) (P = 0.0002 for total ear weight; 0.041 for weight of ear 1).

While there is some variability between seed lots, UV seed treatment overall appears to benefit plant health, and in these studies plant health was not negatively affected as a result of our UV treatment.

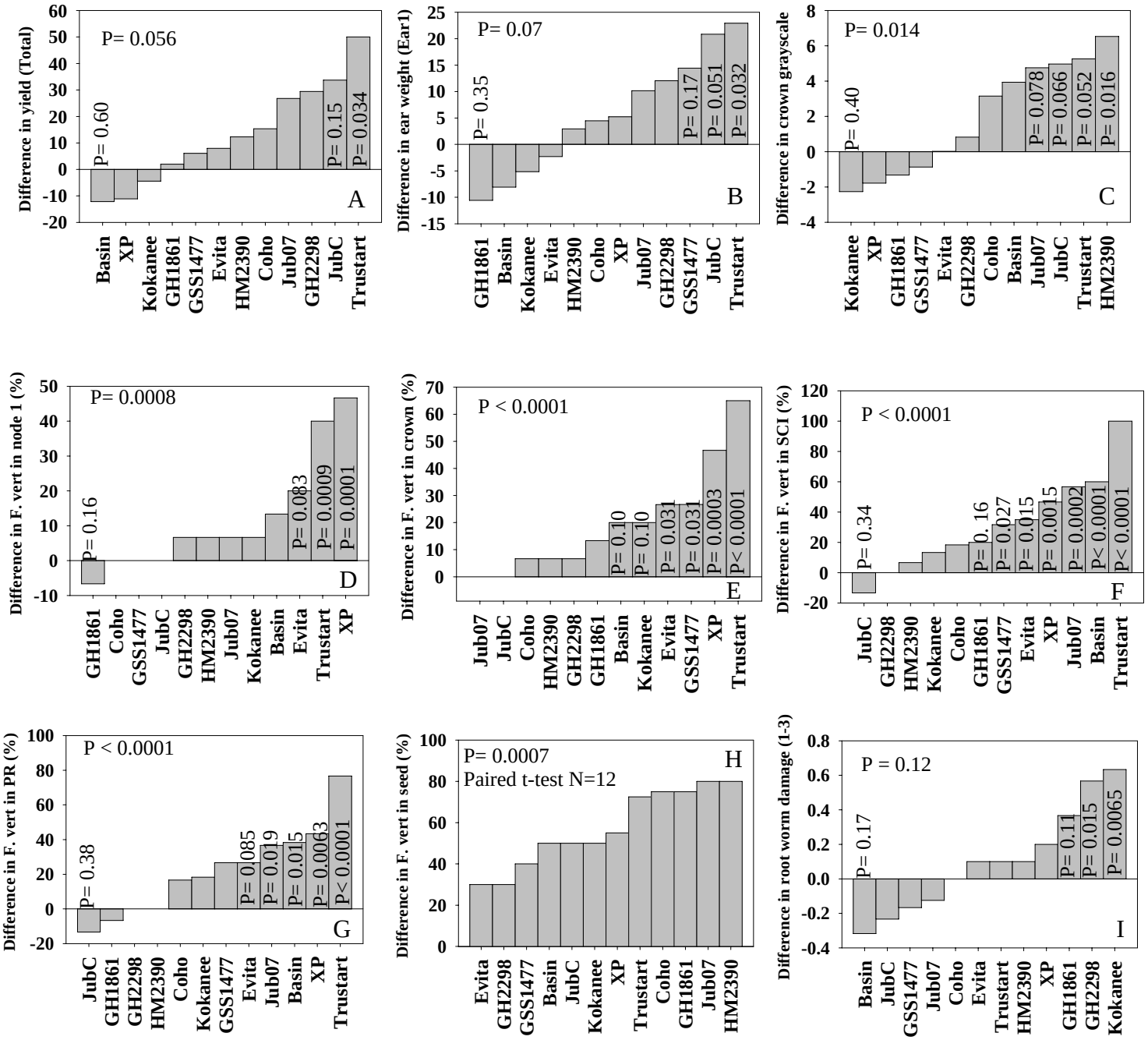

Figure 1. Differences between UV-treated and non-treated sweet corn seed in *Fusarium* presence on seed, disease ratings, crown grayscale, rootworm damage, and ear yield. Each bar represents the difference between plants grown from UV-treated and non-treated seed. Bars are arranged in order of the least affected cultivar on the left, to the most affected on the right. Upper-left P-values indicate the overall significance of the UV effects across all sweet corn cultivars. Bars labeled with a P-value indicate the significance of the pair-wise comparison between treated and non-treated seed of respective cultivars. Differences in percentages = (Percent contaminated UV treated) – (Percent contaminated non-treated treated).

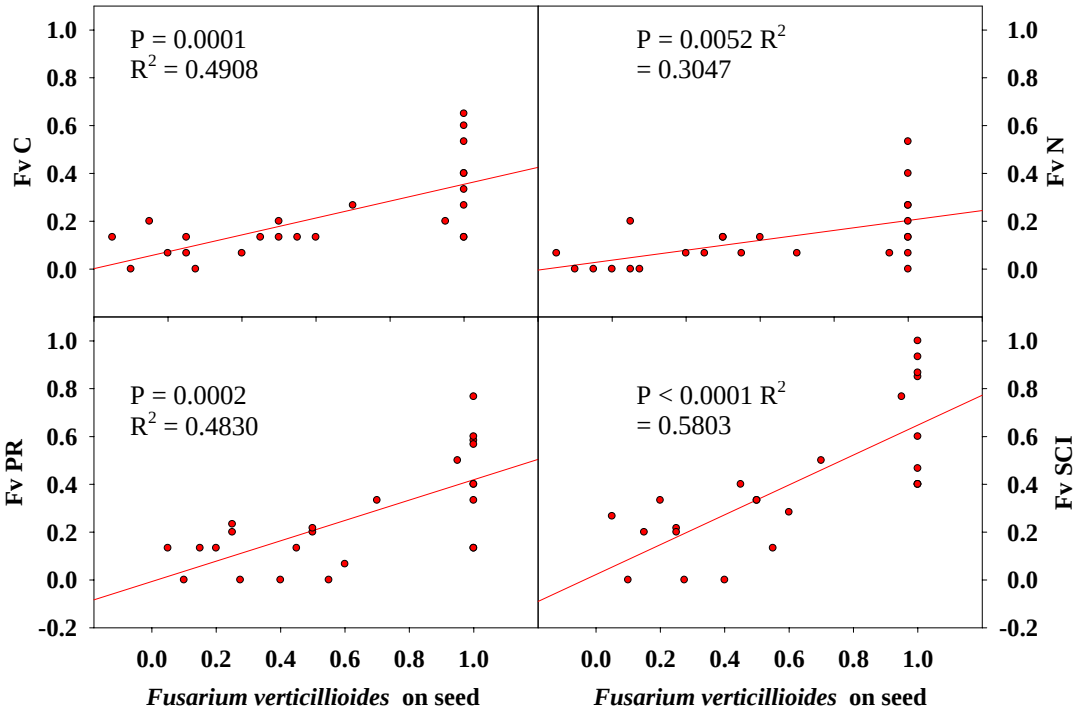

Figure 2. Regression analysis comparing seed contamination of *Fusarium verticillioides* with recovery of *F. verticillioides* (Fv) from the following plant parts: PR = primary root, SCI = sub-crown internode, C = crown and N = fist stalk node above crown.

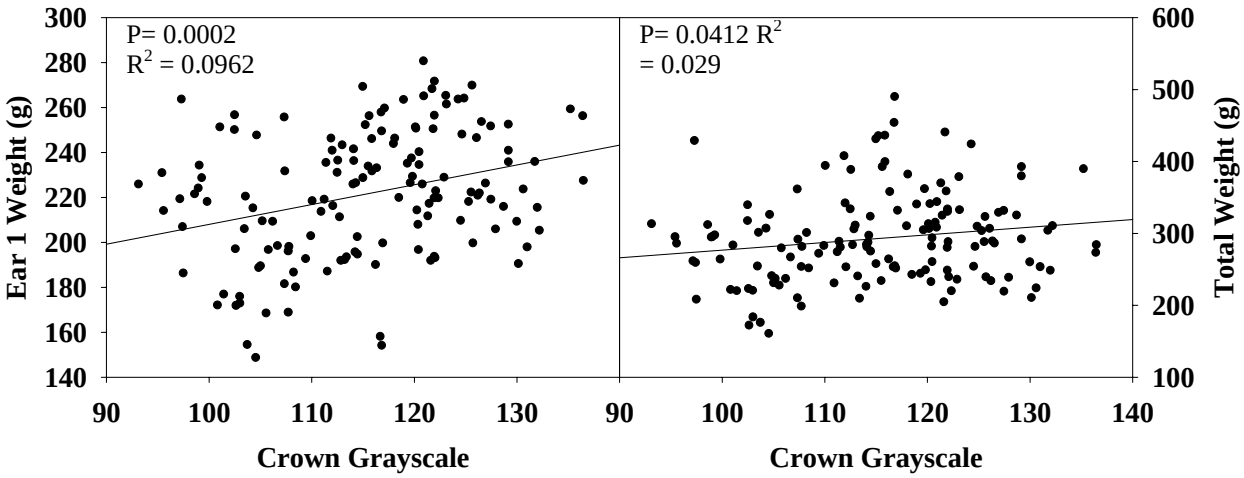

Figure 3. Regression analysis comparing crown grayscale with yield measurements. Smaller crown grayscale values indicate darker crowns.