REPORT TO THE AGRICULTURAL RESEARCH FOUNDATION
FOR THE OREGON PROCESSED VEGETABLE COMMISSION
December 2008

Project Title: Fusarium Crown and Root Rot of Sweet Corn in the PNW: Host Resistance and Association of Cucumber Beetles with Infected Hosts

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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 a 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*) have a negative impact on plant health and yield, but other factors are probably involved as well. We've developed evidence that the Western Spotted Cucumber Beetle preferentially feeds on *Fusarium*-infected plants and possibly vectors *Fusarium* spp. to non-infected plants. The majority of our studies has been done in an experimental corn field located on Oregon State University's Botany and Plant Pathology farm (OSU-BPP); a site we've developed for corn crown and stalk node rot screenings. This experimental corn field was originally infested with pathogens via incorporation of sweet corn crowns, roots, and lower stalk portions of severely affected plants collected from a grower's field during 2000. *Fusarium*-colonized cornmeal-sand and/or oat kernel cultures were incorporated into the soil during 2002-2006, resulting in a uniform, high-disease pressure screening site for sweet corn germplasm.

Host tolerance or resistance is a major management tactic for sweet corn root and crown rot, and associated yield decline, and efforts continue on the development of increasingly resistant sweet corn lines. Screenings of Syngenta sweet corn inbreds showed that some inbred lines have greater tolerance to crown rot and root rot. Historically, crown rot ensues and then spreads through the internodes of the lower stalk, giving rise to "classic" stalk rot, which can result in lodging. However, we're finding crown rot and necrosis only at the stalk nodal plates in affected sweet corn plants grown throughout the Oregon (as well as other places). In our disease screening in our experimental corn field, we have shown that one parent of 'Jubilee' exhibits both stalk node rot and stalk internode rot while the other inbred shows susceptibility to only stalk node rot, which quite pointedly suggests that "crown and stalk node rot" appears to be a disease syndrome distinctly different from the "classic" stalk rot.

A body of work shows that rootworm feeding by *Diabrotica* spp. (cucumber beetles, rootworms) can result in more severe root rot and that the injury makes a plant more susceptible to certain soilborne pathogens, such as some *Fusarium* strains. Recent work by Miller and Ocamb indicates that Western Spotted Cucumber beetles (*Diabrotica undecimpunctata undecimpunctata*) can preferentially feed or lay eggs on plants that are grown from seed inoculated with certain *Fusarium* species. Plants grown from *Fusarium*-infested seed had significantly more adult leaf feeding damage and egg masses present at the seedling stage in laboratory studies. Larval root feeding at harvest maturity was also increased on *Fusarium*-infested plants in field studies conducted during 2004 and 2005. Why beetles would preferentially choose infected plants is not clear, but there may be a fitness benefit for the offspring that develop on plants infected with pathogenic fungi. It is unknown whether adult beetles vector pathogenic *Fusarium* after feeding on infected plants or plant parts.

Most seed lots of sweet corn sampled have also been found to contain a high percentage of seed contaminated with *Fusarium* species (Miller and Ocamb, unpublished). Corn seed grown throughout most of the world is often infected by several species of seed-borne *Fusarium*. *Fusarium verticillioides*, *F. subglutinans*, and *F. proliferatum* are commonly recovered. These three species are all capable, under certain environmental conditions, of causing seed rot, seedling blight, stalk rot, root rot, or ear rot. Seed is often treated with fungicides and normally does prevent seedling rot and damping-off by these *Fusarium* species and other fungi. However fungicidal seed treatments may not prevent seedlings from becoming infected by seed-borne *Fusarium*. Both *F. verticillioides* and *F. proliferatum* have been shown to have negative effects on resulting plant health (Miller & Ocamb) or field corn (Ocamb).

A number of methods for seed disinfestation have been reported but generally involve techniques damaging to seed viability or are not met with commercial acceptance. A technique that we have begun investigating involves germicidal lights, which are readily available and currently used to sterilize air and solid surfaces. One advantage of germicidal light is that it can used with dry seed, unlike many other seed disinfestation techniques which require seeds to be wetted and then re-dried. If corn plants are grown from seed protected from *Fusarium* spp. by beneficial organisms (biocontrol), may be the plants would be less attractive to the beetles, perhaps even affecting the reproductive potential of the beetles. Most biocontrol formulations that suppress Fusarium diseases of seedlings are less efficacious when *Fusarium* species are already present on seed, so removal of *Fusarium* from seed may enhance biocontrol. A better understanding of beetle-seed-microbe interactions could aid in making seed treatment choices that reduce the overall beetle populations as well as Fusarium diseases and their associated losses in corn yield.

Objectives for 2008 and Accomplishments:

Objective 1: Evaluate commercial sweet corn varieties and inbred germplasm in small plots for susceptibility to root rot, stalk node rot, and crown rot.

Sweet corn genotypes planted on the OSU-BPP farm during 2008
GH1861 inbred
GH1861 inbred
GH1861
GH8267 inbred
GH8267 inbred
GH8267
Jubilee-C inbred
Jubilee-C inbred
Jubilee-C
GSS1477 inbred
GSS1477 inbred
GSS1477
Jubilee 2007
Jubilee 2007 (2^{nd} seed lot)
Jubilee 2006
Jubilee 2004
Trustart

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. 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 the 4-leaf, silking, and post-silking stages for rot of roots, crown, and stalk nodes as well as Western Spotted Cucumber beetle feeding on leaves or roots. At the 4-leaf stage (36 days after sowing), three plants were dug from each plot (21 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. At early silking (~67 days after seeding) and postsilking (~87 days after seeding), five plants were sampled from each plot. Rot of each plant was recorded in addition to the crown grayscale analyses. Incidence of crown rot was also visually determined at silking and post-silking after splitting open each crown, noting whether crown rot was present, developing, or that the crown 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 = ≥ 75 % of adventitious roots at one whorl or ≥ 50 % of adventitious roots at 2 whorls have feeding

Results for Objective 1: Very little rootworm injury was found on plants when sampled at silking and postsilking stages, probably due to the cool conditions that occurred during the 2008 growing season (Fig. 1). Crown grayscale values indicate increasingly darker crowns (smaller values) as the growing season progressed (Fig. 2) from the 4-leaf stage through postsilking.

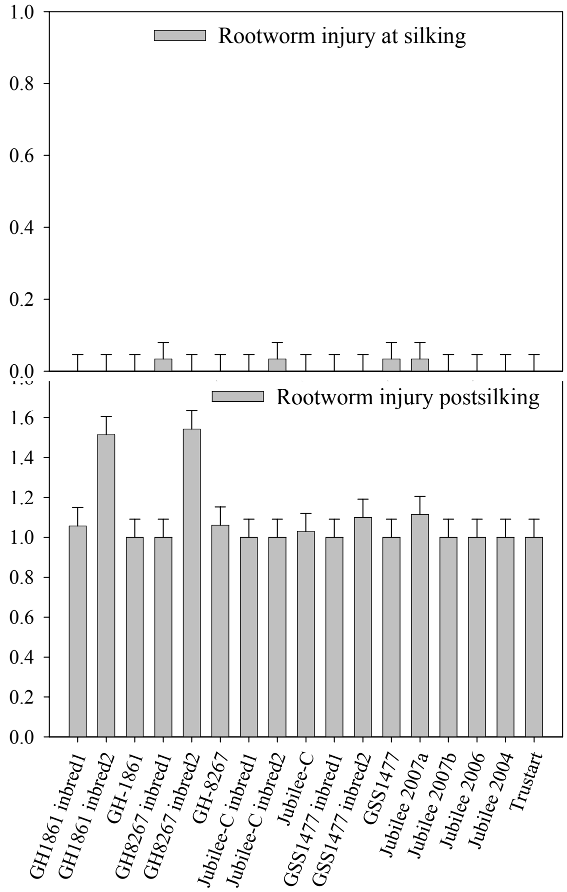


Fig. 1. Rootworm injury on hybrids and Inbreds at silking and postsilking (~67 & 87 days). Bars show the least significant difference at $P \leq 0.05$ as determined by Fisher's protected LSD test.

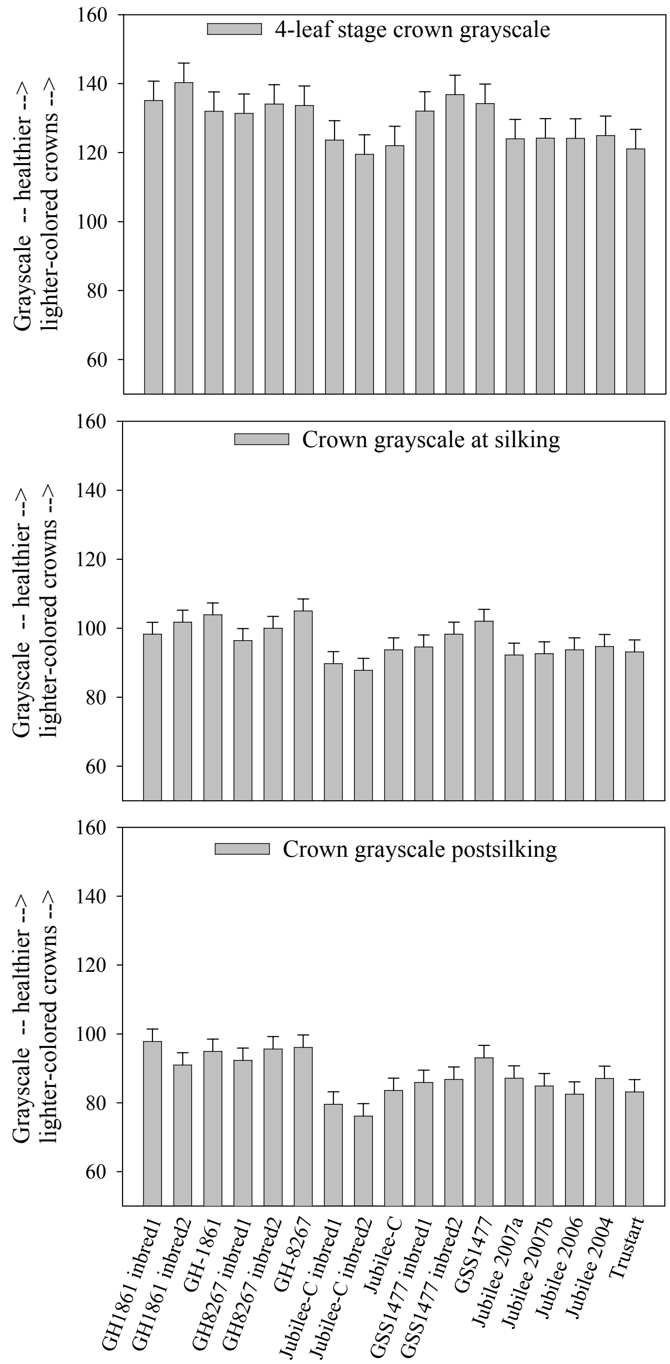


Fig. 2. Crown grayscale values of hybrids and inbreds at the 4-leaf stage silking and postsilking (36, ~67 & 87 days). Bars show the least significant difference at $P \leq 0.05$ as determined by Fisher's protected LSD test.

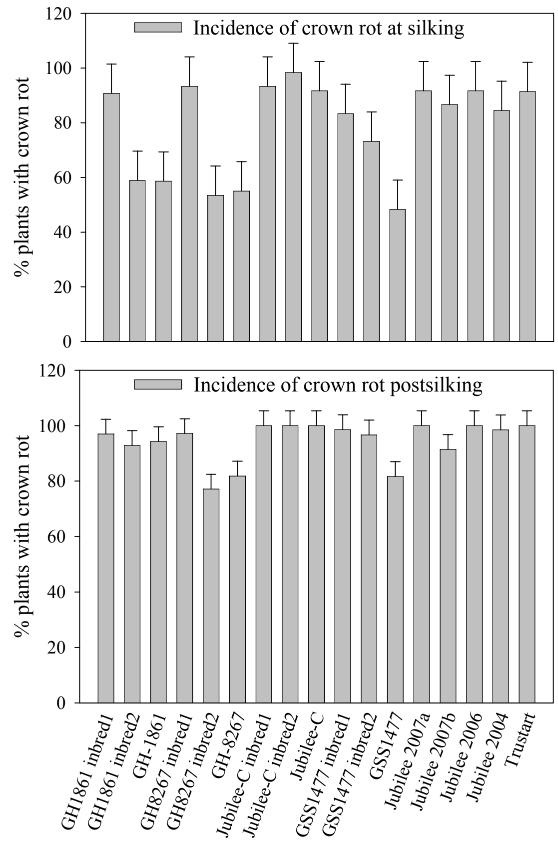


Fig. 3. Percentage of plants with crown rot at silking and postsilking (~67 & 87 days). Bars show the least significant difference at $P \leq 0.05$ as determined by Fisher's protected LSD test.

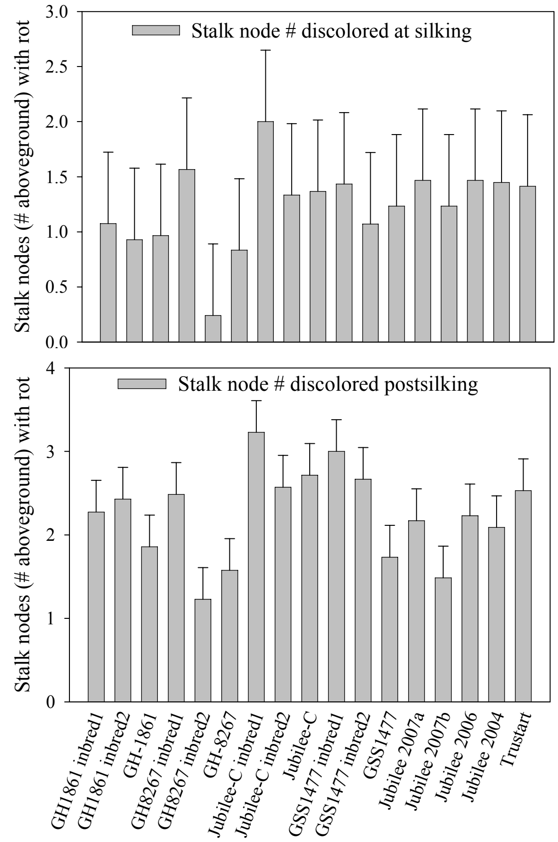


Fig. 4. Number of stalk nodes with rot at silking and postsilking (~67 & 87 days). Bars show the least significant difference at $P \leq 0.05$ as determined by Fisher's protected LSD test.

The incidence of crown rot increased over the season and 'Jubilee' lines had very high incidence of crown rot by silking (Fig. 3). The number of stalk nodes with rot was also greater at postsilking than silking and 'Jubilee' generally had affected nodes further up the stalk (Fig. 4). Root rot develops on sweet corn plants growing in our experimental corn field but root rot of adventitious roots did not occur at especially high levels by silking (Fig. 5) while the primary root and the sub-crown internode were rotted along nearly their entire length by silking (Fig. 6). Sweet corn hybrid 'GSS1477' had a significantly higher ear yield than the hybrids, in spite of a rather dark crown, similar to that of '1861' but 'GSS1477' has a much lower incidence of crown compared to the other hybrids. However, as discussed below, crown grayscale is strongly correlated with ear yield, so 'GSS1477' is most likely suffering a reduction in ear yield that would be apparent in a comparison to a healthy field.

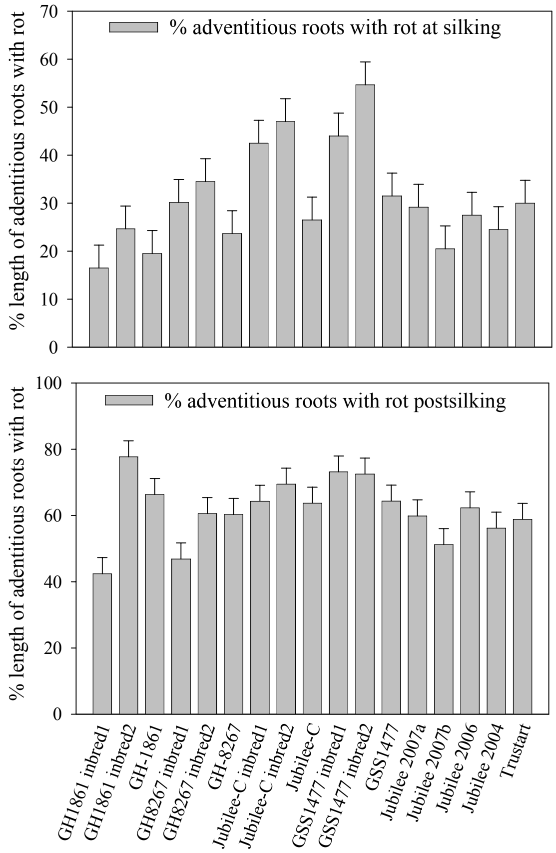


Fig. 5. Percentage of adventitious roots with rot at silking, and postsilking. Bars show the least significant difference at $P \leq 0.05$ as determined by Fisher's protected LSD test.

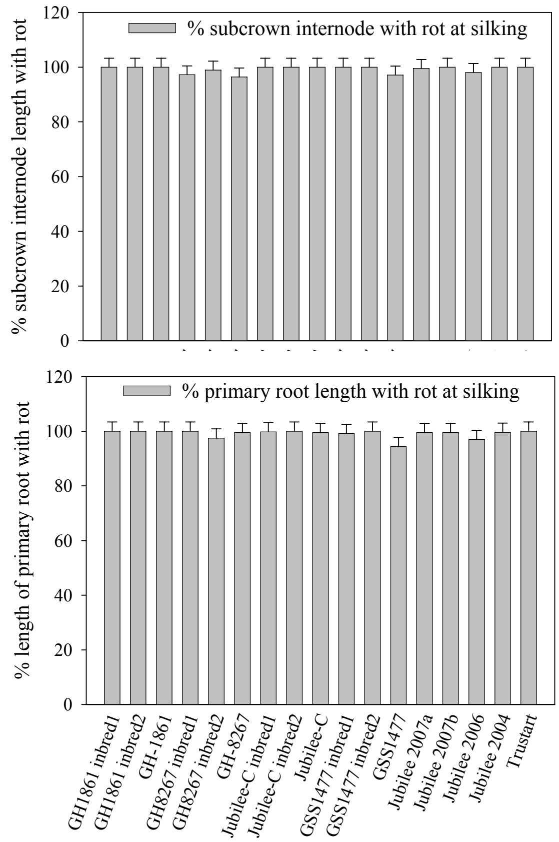
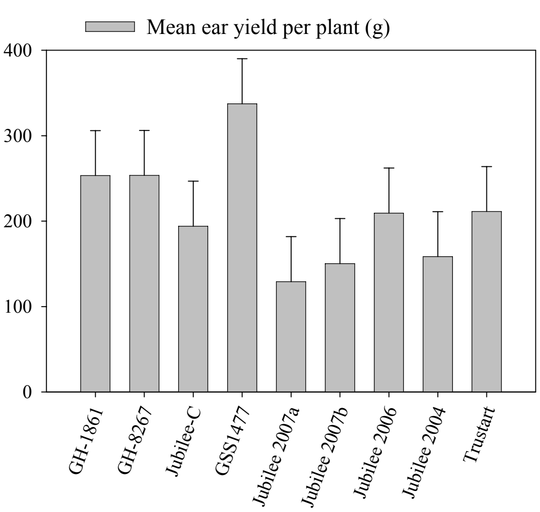


Fig. 6. Percentage of sub-crown internode and primary root with rot at silking. Bars show the least significant difference at $P \leq 0.05$ as determined by Fisher's LSD test.



Examination of data shows sweet corn hybrids ear weight (Fig. 7) to be correlated with crown disease and it appears that crown disease has the most explanatory power for ear yield similar to results seen in previous studies. Ear yield was strongly correlated with crown rot at both silking and postsilking as well as with crown grayscale at 4-leaf and silking (Table 1). Regression of ear weight with crown grayscale values are shown in Figure 8. No other disease parameters measured had nearly as strong of correlation with yield compared to the crown disease measurements. Rot of the adventitious (nodal) roots at both sampling dates showed no negative effect on ear yield, suggesting that crown rot, not root rot, is responsible for declining sweet corn yields.

Fig. 7. Ear yield of sweet corn hybrids are presented as average per plant. Bars show the least significant difference at $P \leq 0.05$ as determined by Fisher's

Table 1. Correlations among disease symptoms in a 2008 sweet corn hybrid trial on the OSU-BPP farm

	Yield (g)	Crown grayscale postsilking	Stalk node rot postsilking	Crown rot incidence postsilking	Ear node rot postsilking	Adventitious root rot postsilking	Adventitious root rot at silking	Stalk node rot at silking	Crown rot incidence at silking	Crown grayscale at silking	Crown grayscale at 4-leaf
Yield (g)		0.215	0.000	-0.389	0.132	0.158	0.006	-0.091	-0.507	0.555	0.315
		0.074	0.999	0.001	0.274	0.192	0.962	0.492	<.0001	<.0001	0.008
Crown grayscale postsilking			-0.416	-0.380	-0.255	-0.284	-0.288	-0.310	-0.485	0.572	0.421
			<.0001	<.0001	0.004	0.001	0.003	0.001	<.0001	<.0001	<.0001
Stalk node rot postsilking				0.451	0.246	0.254	0.361	0.458	0.404	-0.422	-0.158
				<.0001	0.006	0.004	0.000	<.0001	<.0001	<.0001	0.078
Crown rot incidence postsilking					0.156	0.189	0.065	0.213	0.453	-0.412	-0.301
					0.082	0.034	0.503	0.027	<.0001	<.0001	0.001
Ear node rot postsilking						0.061	0.045	0.236	0.196	-0.252	-0.233
						0.496	0.644	0.014	0.042	0.009	0.009
Adventitious root rot postsilking							0.326	0.024	-0.197	0.020	-0.049
							0.001	0.805	0.041	0.836	0.588
Adventitious root rot at silking								0.186	0.227	-0.352	-0.113
								0.053	0.018	0.000	0.246
Stalk node rot at silking									0.482	-0.419	-0.297
									<.0001	<.0001	0.002
Crown rot incidence at silking										-0.768	-0.472
										<.0001	<.0001
Crown grayscale at silking											0.433
											<.0001
Crown grayscale at 4-leaf											

* Pearson's correlations and associated P-values of disease variables measured, P values are < 0.05 or 0.01 when significant correlations occur.

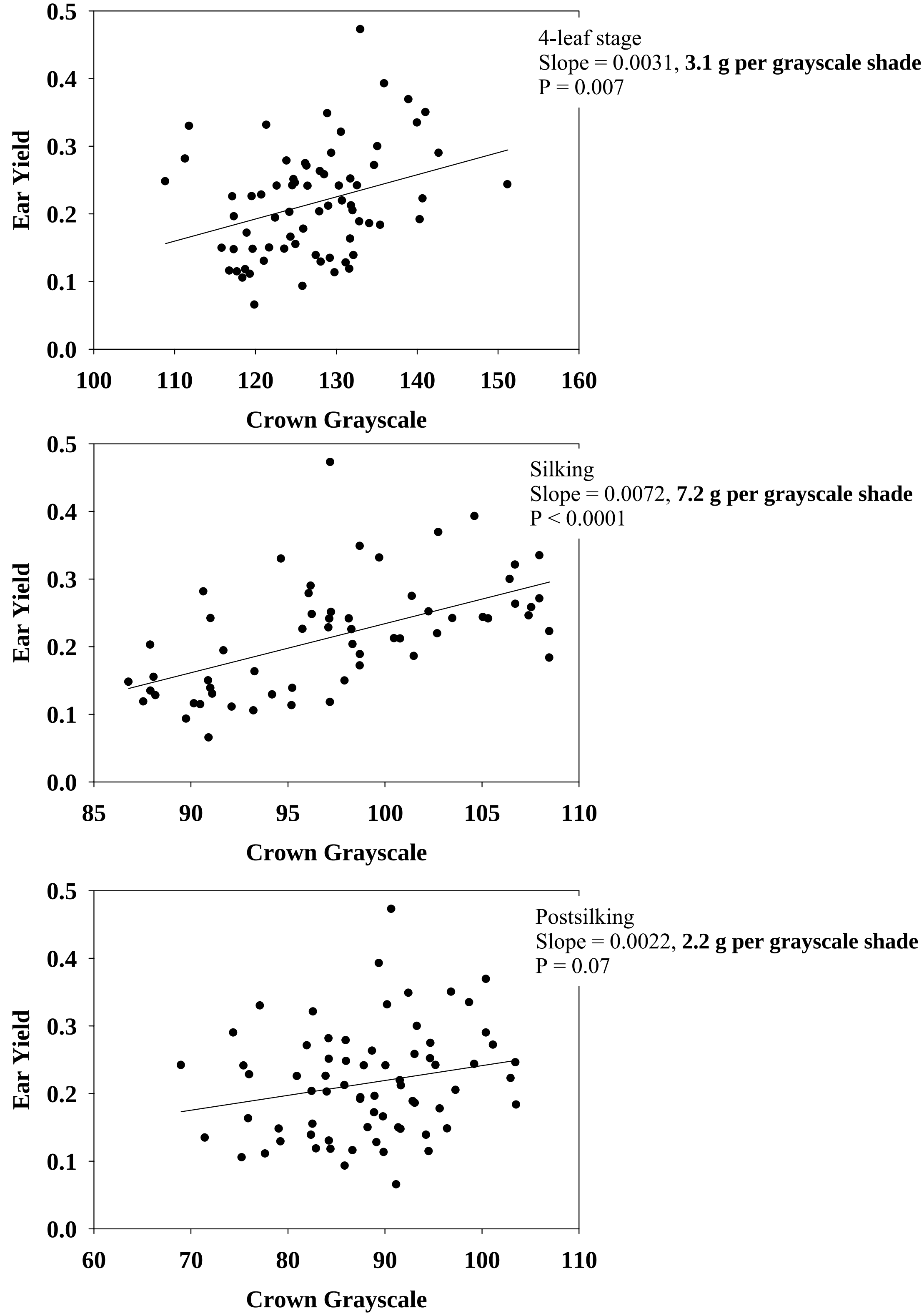


Fig. 8. Regression of sweet corn hybrids ear yield (weight per plant in grams) on crown grayscale values at the 4-leaf stage, silking, and postsilking stages (36, ~67, and ~87 days after seeding, respectively).

Objective 2: Examine the relationship between Western Spotted Cucumber Beetle reproduction and seed microbial communities.

The purpose of these studies was to look for reproductive advantage for beetles that develop on plants grown from contaminated sweet corn seed, test the effects of germicidal light for reduction in *Fusarium* levels on or in seed, and to compare seed treatments on disinfested seeds in terms of plant growth, disease development, and *Diabrotica* damage.

Nine non-treated commercial sweet corn seed lots were shaken on a flatbed shaker while being exposed to germicidal (UV) light for following time points: 0, 30, 60, 120, 180, 240, and 300 minutes. Sixteen garden varieties were also treated for 0 and 120 minutes. Seeds (20) from each seed lot by time point were embedded in *Fusarium*-selective media and monitored for two weeks for presence of *Fusarium* or other fungi. Seedling germination and growth was measured by growing seeds (approximately 20) in rolled moist germination paper (rag dolls). For commercial seed lots, there were five rag dolls made for each time point and for garden varieties there were three or four rag dolls made for each seed lot depending on seed availability. After ten days, average root and shoot weight were measured for each rag doll. All seedling experiments were conducted twice at 23°C (73°F) and 28°C (82°F). Data were analyzed the using MIXED procedure in SAS 9.1 with seed-lot and rep as random variables.

In commercial seed lots, *Fusarium* contamination was reduced as time under UV increased (Figure 9). In garden varieties, *Fusarium* contamination was reduced by about 47% with two hours of UV light (Figure 10). Seedling vigor in rag dolls also was improved based on increased root and shoot weight of 10-day old seedlings. Shoot and root weight was higher in all UV-treated seeds relative to nontreated controls at 23°C (Figure 11). A similar but less significant pattern was seen at 28°C (Figure 11). In the garden varieties, the average shoot and root weight increased 16% and 10%, respectively, at 23C and 18% and 8% at 28C (data not shown).

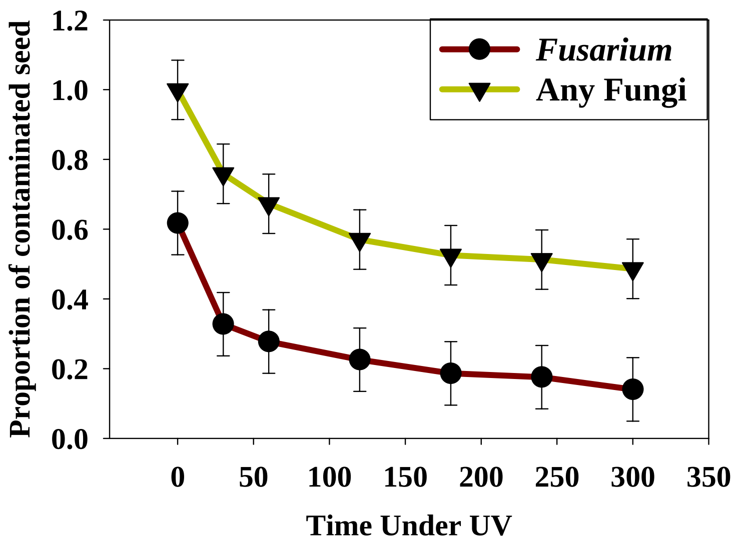


Figure 9. Proportion of seed (1.0 = 100 %) from which *Fusarium* (●) and any fungi (▼) was isolated after being treated with UV light for 0 to 300 minutes. Each point represents the mean of 20 seeds of each of nine cultivars, replicated twice. Error bars represent the standard error from a mixed model ANOVA with cultivar and replicate as random variables.

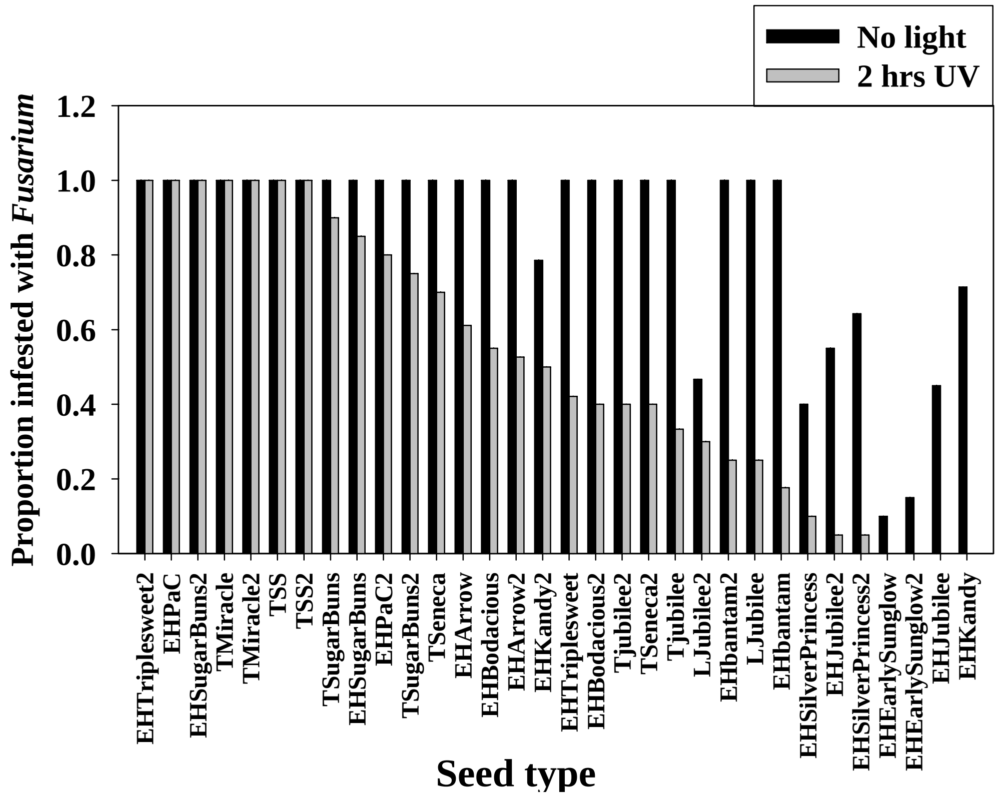


Figure 10. Proportion of seeds (1.0 = 100 %) from which *Fusarium* was isolated.

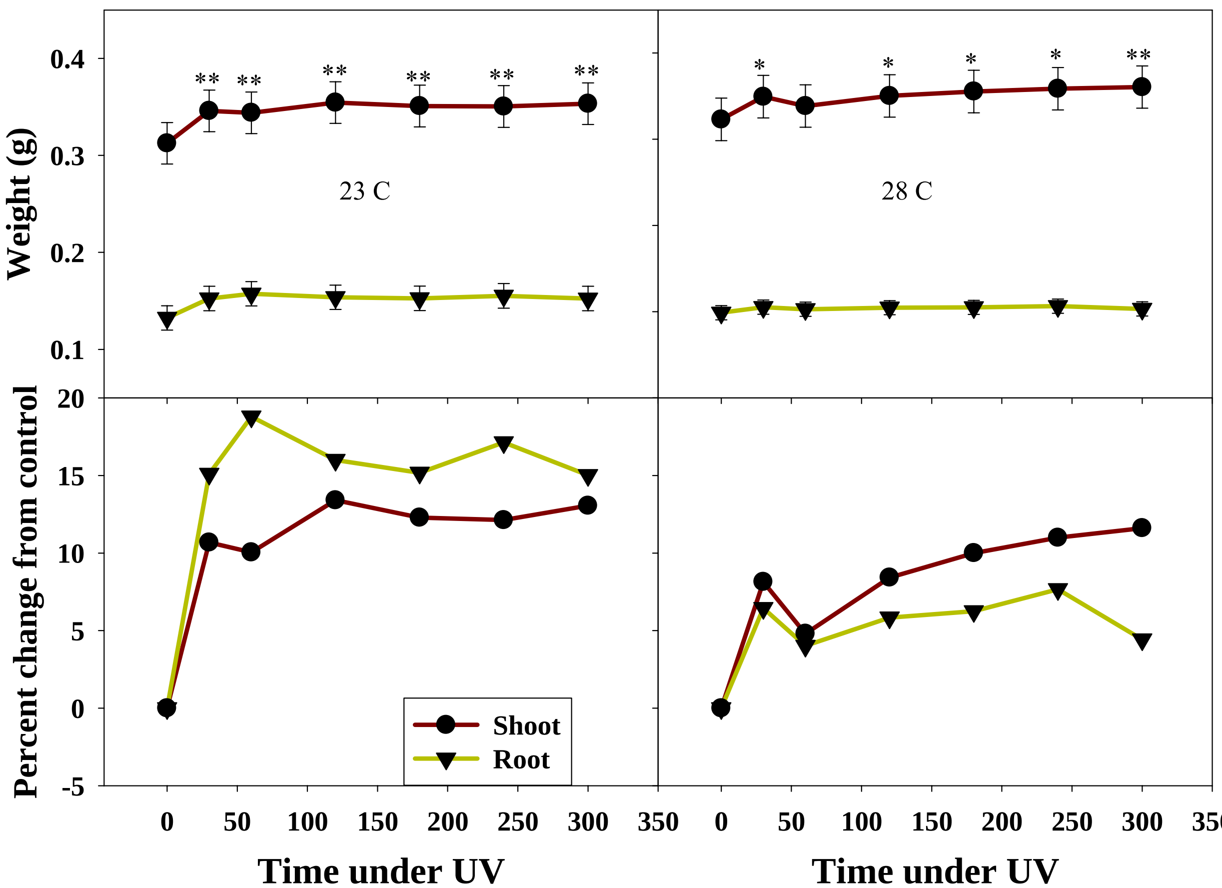


Figure 11. Average weight and percentage increase compared to control of seedlings grown for 10 days at 23°C (73°F) or 28°C (82°F). Error bars represent the standard error from a mixed model ANOVA with cultivar and replicate as random variables. * Represents significant differences at $P < 0.05$ and ** significant differences at $P < 0.001$ compared to 0 hr of UV.

Seeds of 'Jubilee' were disinfested with 3% hydrogen peroxide, treated with *F. oxysporum* or MicroAFD (biocontrol seed treatment), and planted in small plots (10 seeds/ft^2). Eight pairs of plots were planted in each of three fields on June 25th. A second set was planted in two of the fields on August 12th. Damage to seedlings from adults was monitored three weeks after sowing and then exclusion cages were placed over the a subset of the plots with the bottom of the cage being buried 3 inches under the soil (40 total cages split between treatments and planting dates). Seedling damage by adult feeding was only seen in one field. Sticky traps were placed inside the cages to capture beetles that emerge from soil. Beetle presence was monitored weekly. Germination was reduced in seed treated with *Fusarium* (33% stand) relative to MicroAFD (55% stand, $P < 0.0001$), but no other noticeable differences were seen. Beetles were not found on the sticky traps. Rootworm damage was observed on plants grown in the same field though at very low levels by silking. But it is unclear why beetles were not detected in the caged studies. It may be that more time is needed to allow beetles to lay eggs or perhaps the time of planting and cage placement.

'Jubilee' (2003 and 2007 seed lots) and 'Trustart' were disinfested with UV for 2 and 8.3 hours or left nontreated and were then divided into three seed treatments: none, ApronMaxx (5 fl oz/cwt), and Micro-AFD (2 % w/w). Plots consisted of 30 seeds sown in 25' rows with six blocks. At presilking (~ 56 days), 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, sub-crown internode, crown, and 1st node above the crown. Fungi were identified as either *Fusarium*, *F. verticillioides*, *F. proliferatum*, or not *Fusarium*. At processing maturity, 5 plants from each plot were dug and rated in the same fashion as presilking samples, but no isolations made. One week later, ear weights from ten plants per plot were determined. First plant means were calculated and tested for main effects; cultivar, UV-disinfestation, and seed treatments. Then plot means were calculated and analyzed using MIXED procedure in SAS 9.1 with block treated as a random variable.

There are differences between 'Trustart and' 'Jubilee' when data are combined across UV and seed treatments (Table 2). 'Trustart' averaged a slightly darker crown, less adventitious root rot, less rootworm damage, and greater ear yield compared to 'Jubilee'. When seed treatments were examined for effects across UV-treatments and cultivars, MicroAFD averaged less adventitious root rot and rootworm damage compared to the ApronMaxx treatment (Table 3). When UV treatments were examined for effects across seed-treatments and cultivars, UV-treated seeds resulted in plants with lighter crowns, less rootworm damage, and greater ear yield compared to no UV treatment (Table 4).

Table 2. Results for sweet corn cultivars across UV and seed treatments

Cultivar seed lot year	Crown [w, y]		% rot of adventitious roots [y]		Rootworm damage at harvest [x, y]		Mean 1st ear wt (g) [y]		Mean plant yield (g) [y]	
'Trustart'	96.8	b	40	b	2.26	b	205	a	228	a
'Jubilee' 2007	98.8	a	41	ab	2.30	ab	200	ab	223	a
'Jubilee' 2003	99.4	a	44	a	2.35	a	192	b	206	b

[w] Mean crown grayscale at 8 weeks. Smaller values indicate darker crowns.
[x] 0 = no root worm feeding is evident; 1 = up to 3 roots with root worm feeding is evident; 2 = more than 3 roots but less than half with root worm feeding; and 3 = more than half the roots with root worm feeding.
[y] Numbers within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined by Fisher's protected LSD test.

Table 3. Results of seed treatments for combined sweet corn cultivars and UV-treatments

Seed treatment	Crown [w]	% rot of adventitious roots [y]		Rootworm damage at harvest [x, y]		Mean 1st ear wt (g)	Mean plant yield (g)	
ApronMaxx (5 fl oz/cwt)	97.7	44	a	2.3	a	195	210	a
MicroAFD (2 % w/w)	99.3	41	b	2.2	b	197	223	ab
none	98.3	39	b	2.3	a	204	226	b

Table 4. Results of UV-disinfestation for combined sweet corn cultivars and seed treatments

UV (Hours)	Crown [w, y]	% rot of adventitious roots	Rootworm damage at harvest [x, y]	Mean 1st ear wt (g) [y]	Mean plant yield (g) [y]
0	96.6 b	40	2.5 a	191 b	208
2	98.7 a	42	2.3 b	201 ab	222
5	99.0 a	42	2.2 b	203 a	224

[w] Mean crown grayscale at 8 weeks. Smaller values indicate darker crowns.
[x] 0 = no root worm feeding is evident; 1 = up to 3 roots with root worm feeding is evident; 2 = more than 3 roots but less than half with root worm feeding; and 3 = more than half the roots with root worm feeding.
[y] Numbers within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined by Fisher's protected LSD test.

When UV effects are examined by cultivar/seed lot, UV treatment reduced the levels of *Fusarium verticillioides* recovered from the sub-crown internode and crown tissues of 'Trustart' or 'Jubilee' 2007 (Table 5). The recovery of *F. verticillioides* from the node above the crown was also reduced in 'Trustart' with UV treatment. In addition, UV treatment of 'Trustart' resulted in improved ear yield as well as slightly lighter-color crown tissue. Rootworm damage at harvest was reduced by UV treatment of all three seed lots, though not to a large degree. After a 5-hr UV exposure, *Fusarium* was still recovered from sweet corn seed except from the 2003 lot of 'Jubilee', and for this corn line, no improvement in ear yield was evident. 'Trustart' is characterized as *Fusarium*-susceptible and this line seemed to clearly benefit from the UV-treatment.

Table 5. Results of 2008 Sweet corn UV treatments planted in the field

	UV (hr)	Crown[1]	FusSCI[2]	FusC[2]	FusN[2]	% seed with *Fusarium*	Mean 1st ear wt (g)	Mean plant yield (g)	Rootworm damage at harvest[3]
'Trustart'	0	93	67	80	49	100	191	212	2.4
	2	97 *	35*	29*	20*	38	203	222	2.3*
	5	99 *	23*	29*	19*	28	216*	244*	2.1*
'Jubilee' 2007	0	97	51	58	18	100	191	210	2.5
	2	101*	16*	42	20	70	202	229	2.3*
	5	98	21*	29*	22	35	205	228	2.2*
'Jubilee' 2003	0	100	14	13	9	84	192	205	2.5
	2	99	8	9	7	4	196	215	2.3*
	5	99	10	11	13	0	187	197	2.3*

[1] Mean crown grayscale at 8 weeks. Smaller values indicate darker crowns.
[2] FusSCI, FusC, and FusN denote the recovery of *Fusarium verticilllioides* from the sub-crown internode, crown, and 1st stalk node above crown at 8 weeks, respectively.
[3] 0 = no root worm feeding is evident; 1 = up to 3 roots with root worm feeding is evident; 2 = more than 3 roots but less than half with root worm feeding; and 3 = more than half the roots with root worm feeding.
* Represents significant differences at $P < 0.05$ compared to 0 hr UV.