2001 Progress Report to the Oregon Processing Vegetable Commission

Subproject Title: Biology and Management of Fusarium Diseases on Sweet Corn in the PNW

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Background: Leaf-firing and an associated yield reduction has been observed on sweet corn plantings in the Willamette Valley. Sweet corn fields have been affected in the greater Pacific Northwest. Symptoms were originally observed on the widely-planted cultivar 'Golden Jubilee' but other sweet corn lines have been found to be susceptible. Root and crown rot are prevalent in symptomatic fields and we recovered a complex of *Fusarium* species from affected tissues. Darkening of nodes, atypical stalk rot, is also present and *Fusarium* species were recovered from darkened, internal tissues. *Fusarium* species associated with "firing" have also been found in 'Jubilee' seed- production fields, causing seedling death or stalk rot, and in kernels collected from processing fields in eastern Oregon and Washington state. It appears that a complex *Fusarium* syndrome is widespread in sweet corn production throughout the Willamette Valley and the greater Pacific Northwest. The predominate causal agents may include *F. oxysporum* var. *redolens, F. moniliforme, F. solani, F. proliferatum,* and other *Fusarium* spp. Other fungi (*Phoma* sp., *Pythium* spp., etc.) may be involved in root rot but the general strategies for managing root rot are the same regardless of fungal species. We need to decipher the importance of the stalk rot component, evaluate more firing fields to get a feel for the range of root pathogens, and continue to develop economically-feasible seed treatments for management crown and root disease for the Pacific Northwest.

During 1999 and 2000, we found that fields that were planted to sweet corn within a more limited rotation plan ("old" ground) had a greater level of root rot compared to plants grown in "new" ground. Site and other factors influenced the developmental stage at which these differences were observed. In some fields, the difference in root rot levels was observed as early as the 8-10 leaf stage. By tasseling, plants growing in "old" fields had more severe rot of subcrown internodes than plants in "new" fields. *Fusarium oxysporum* and *F. solani* were the prevalent species isolated from symptomatic plant parts.

Examination of plants in "firing" fields showed Fusarium root and crown rot and some external stalk rot. In one "firing" field examined during 2000, all 30 plants sampled had lesions on the primary root, subcrown internode, and adventitious roots. Fully half the plants had necrotic crowns or stalk rot. In addition, 7 ears appeared to have ear rot. Plants were examined from 6 "firing" fields in 2001; the crowns were rotted and varying levels of root rot were present. Nodal discoloration was also observed. Isolations were made from crowns and nodes above ground.

Five *Fusarium* taxa (*F. oxysporum, F. sambucinum, F. proliferatum, F. oxysporum* var. *redolens,* and *F. solani*) were screened on sweet corn germlings in laboratory evaluations. From this preliminary pathogenicity evaluation, 6 isolates of *F. oxysporum* var. *redolens*, 2 isolates of *F. oxysporum*, and a single strain of *F. sambucinum* were evaluated as a mixture in pasteurized field soil used in 5 gal pots studies in the greenhouse. At harvest, all plants growing in soil infested with the *Fusarium* mixture had lesions on the primary root, subcrown internode, and adventitious crown roots. Leaf "firing" was present as well as kernel dimpling and nodal discoloration.

Seed examinations have found a preponderance of *F. moniliforme* on kernels as well as other *Fusarium* species. Kernels produced in 'Golden Jubilee' plantings also contain *F. moniliforme* and *F. oxysporum* var. *redolens*.

Studies of fungicides, commercial biocontrol formulations, as well as some of my biocontrol acquisitions as seed treatments were evaluated in the greenhouse and small field plots during 2000 and 2001. Preliminary results in 2000 suggest that some materials are as good as, or better than, Captan/Thiram according to our evaluation (stand count, primary root rot, subcrown internode rot, adventitious root rot), however, our 2000 studies did not include yield evaluations. Larger plot studies were conducted in 2001 and yield measurements were made.

Cultural practices and environmental conditions can have significant effects on root, crown, and stalk rots. Stress from drought or prolonged periods of high soil moisture can increase disease severity and incidence. Soil compaction and hard pan formations can also enhance disease development, partially through limiting the rooting depth as well as hampering soil drainage. Slower draining soil types can be more prone to root rot. Typically, if root rot is more severe, the likelihood of crown root increases. When severe crown rot is present, stalk rot should be anticipated.

Cropping sequences, among other factors, can also influence Fusarium diseases by either perpetuating/increasing pathogen populations in soil as well as affecting microbial antagonists that could suppress *Fusarium* populations. Sweet corn production areas have generally seen an increase in grass seed fields. Wheat and oats are also very common in cover-cropping. Concentration of these monocots and a build-up of their residues in the production cycle may be increasing the pathogen population, survival, and spread. Other cropping practices (herbicide residues, fertility program, liming, covercrop kill-method, etc.) may also influence disease levels. But perhaps the pathogen populations could be rendered ineffective or could be sufficiently reduced so as to no longer cause economic losses.

2001 Objectives:

1. Continue greenhouse and laboratory screenings of seed treatments and evaluate the best seed treatments in large plot field studies for reduction of sweet corn root, crown and stalk rot.

2. Evaluate use of microbial seed treatment on wheat cover crop for reduction of sweet corn root, crown and stalk rot.

3. Evaluate rhizosphere populations of *F. oxysporum* var. *redolens* on rotational crops in laboratory and greenhouse studies.

4. Evaluate 'Golden Jubilee' and 'Supersweet Jubilee' seeds for presence of *Fusarium* species.

5. Cooperate with other sweet corn research projects.

Procedures

Objective 1: Continue greenhouse and laboratory screenings of seed treatments and evaluate best seed treatments in large plot field studies for reduction of sweet corn root, crown and stalk rot.

Fusarium isolates were obtained from symptomatic roots, purified by the single-spore method, and stored on silica gel at 5 C. For laboratory studies, 'Golden Jubilee' seeds were surface-disinfected in H_2O_2 for removal of seed-borne *Fusarium* species, air-dried, coated with seed treatments, and placed in moist chambers in the laboratory. As germlings developed, *F. oxysporum* var. *redolens* was inoculated onto the primary root and disease symptoms were noted.

Soil from firing fields was pasteurized and used in greenhouse studies. After pasteurization, a mixture of *Fusarium* species isolated from symptomatic roots and stalks, was added as a thin layer to the lower portion of pots or as a drench. Soil was placed in small pots. 'Golden Jubilee' seeds were surface-disinfected, air-dried, coated with seed treatments, and planted in the greenhouse pots. Below-ground plant parts were examined for disease at various times after sowing. Data from these studies won't be presented in this report

For field studies, a fallow field received a truckload of root balls in Sep 2000 from a symptomatic "firing" field and these root balls were disked in. 'Golden Jubilee' seeds from one lot were surface-disinfected, air-dried, and then coated with seed treatments. Treatments included various chemical and biological seed treatments listed in Table 4 and were tested in 40' experimental plots replicated on the Botany farm. Additional *Fusarium* inoculum was added to the field at sowing. Plants were examined at maturity for root, crown, and stalk rot.

Four larger plot (1-ac) studies were used to test a biocontrol mixture on different sites with a history of sweet corn "firing" syndrome. Field plots were sown by growers between June 7 and the 25th, 2001 at approximately the same time they sowed the rest of the field with their 'Golden Jubilee' seed. Two weeks after the planting date, stand counts and height measurements were taken. Thereafter, plants were sampled every 2 weeks and evaluated for rot/discoloration. A harvest weight comparison was taken by the grower at the end of the season.

Rot of subcrown internodes (SCIN) and root balls were evaluated as follows:

SCIN rot rating
0 = no decay
1 < 25 % of SCIN decayed/necrotic
2 = 26-50 % of SCIN decayed/necrotic
3 = 51-75 % of SCIN decayed/necrotic
4 >75 % of SCIN decayed/necrotic

Root rot rating
0 = no necrosis (decay) on primary roots/seminal roots/brace roots
1 = < 50 % primary root necrotic & no other root rot
2 = > 50 % primary root necrotic or seminal root necrosis < 25 %
3 = primary root necrotic > 50% plus seminal root necrosis = 26 - 50 %
4 = seminal root necrosis = 50 -75 %

5 = seminal root necrosis >75 % and brace root necrosis < 25 %
6 = seminal root necrosis >75 % and brace root necrosis = 26 - 50 %
7 = seminal root necrosis >75 % and brace root necrosis = 51 - 75 %
8 = >75 % of all roots are decayed

Objective 2: Evaluate use of microbial seed treatment on wheat cover crop for reduction of sweet corn root, crown and stalk rot.

Wheat seeds were disinfested and treated with a mixture of Ocamb's biocontrol agents. One-acre plots were sown in the fall (one in 2000 and two in 2001). Spring stand counts are collected and plants are assayed for *Fusarium* species in their rhizosphere. Thirty plants from each treatment were randomly selected, each placed in individual 2-cm glass tubes (40-ml volume) containing 15 ml of 0.01% water agar. Each plant sampled was agitated in the tube for 15 s to remove the rhizosphere soil. Shoot weight was recorded for each wheat plant. Sweet corn was planted and plants were sampled as described above.

Objective 3: Evaluate rhizosphere populations of *F. oxysporum* var. *redolens* on rotational crops in laboratory and greenhouse studies.

We are evaluating conventional rotation and covercrops (wheat, grass seed species, bean, crucifers) for ability to perpetuate *F. oxysporum* var. *redolens* in laboratory and greenhouse studies. Soil artificially infested with a mixture of *Fusarium* species will be placed in small pots. Disinfested seeds of the specific rotational crops will be sown and allowed to grow up to 4 weeks. Rhizosphere soil samples will be assayed by a dilution method for population of *Fusarium* species.

Objective 4: Evaluate 'Golden Jubilee' and 'Supersweet Jubilee' seeds for presence of *Fusarium* species.

Fusarium presence is examined by plating 50 seeds/lot on supplemented Nash medium then incubating at 24 C for 18 days. Five seeds are embedded in each culture plate. Putative *Fusarium* colonies are transferred to two other media, potato dextrose agar and carnation leaf agar. These plates are placed under fluorescent lamps supplemented with black light in a 12-hr photoperiod at 25 C. Isolates were identified to species through microscopic examinations according to the taxonomic system described by Nelson, et al. (1983). Seeds from some infected fields and commercial seed lots were be examined for *Fusarium* presence. Identifications will be completed during 2002.

Results:

Overall conclusions from accomplishments made in 2001

Various fields exhibiting stalk rot were examined in 2001 and all fields had various degrees of root rot and severe crown rot. Nodes exhibited discoloration and *Fusarium* species were recovered from these nodes; out of 209 nodes sampled during 2001, approximately 130 nodes contain a putative *Fusarium* species (or mix). *Fusarium* species were also recovered from kernel samples; 44 kernels with putative *Fusarium* species, out of 81 kernels sampled. It appears

that a pathogenic *Fusarium* complex, associated frequently with *F. oxysporum* var. *redolens*, *F. proliferatum*, *F. moniliforme*, *F. solani*, and other *Fusarium* species, is wide-spread in sweet corn production throughout the Willamette Valley.

Biological seed treatments can decrease root rot severity but didn't decrease infections of the subcrown internode. A yield increase was found with the biological seed treatment at one site. Firing and root rot were present in the experimental sweet corn field on the Botany Farm and some seed treatments appear to suppress some rots.

A pathogenic isolate of *F. oxysporum* var. *redolens* was successfully transformed with the green fluorescent protein and laboratory studies are underway to determine pathogenic and asymptomatic host range as well as elucidate fungal spread on sweet corn roots. Preliminary runs on various rotational crops have been conducted as well as biocontrol selections.

Objective 1: Continue greenhouse screenings of seed treatments and evaluate best seed treatments in large plot field studies for reduction of sweet corn root, crown and stalk rot.

and Objective 2: Evaluate use of microbial seed treatment on wheat cover crop for reduction of sweet corn root, crown and stalk rot.

Germlings were used in laboratory studies to evaluate inoculation effects by *F. oxysporum* var. *redolens* on root development and whether biological seed treatments would improve root growth. As can be seen in Table 1, disinfested seed inoculated with *F. oxysporum* var. *redolens* had the shortest root length on day 13.

Table 1. 'Golden Jubilee' germling evaluations of biological seed treatments

Seed treatment	Mean root length on day 13
BC19	27.5
BC20	26.1
BCB175	27.5
BCB176	26.5
BCB270	30.4
BCB272	30.1
BCB333	26.8
BCT144	30.6
BCT156	19.0
BCT157	22.4
BCT196	29.7
BCT19b	27.5
BCT41b	28.4
BCT53	28.9
BCT5A	24.9
Biocontrol mix 1	33.0
Biocontrol mix 2	24.7
Biocontrol mix 3	20.6
Disinfested	21.0
Disinfested + Fusarium	2.2
Mycostop	24.7

Field studies in grower plots were used to evaluate a microbial mixture seed treatment for reduction in rot and yield improvement. Seeds of 'Golden Jubilee' were treated and tested at three different sites. One site (G1) was previously cover-cropped with biocontrol-treated wheat seed and was planted with conventional 'Golden Jubilee' seeds. Stand counts within biocontrol plots were similar to conventional plantings alongside. Harvest amounts were compared at three sites, the forth site was not used because the conventional plot was planted to a different sweet corn variety ('Cinch'). There was a yield increase with biocontrol treatments in Field H (both harvested plots were equal in area). Field B did not show a yield improvement, however; treated seed at this site was held 14 days before planting. We have not tested longevity of treated seed when stored above 5 C and it is possible that temperatures in mid-late June were detrimental to the microbial populations augmented on corn kernels. However, it is also possible that the biocontrol mixture just wasn't effective against pathogens encountered in Field B.

Table 2. Weight of 'Golden Jubilee' harvest from on-farm plots

Field	Biocontrol Treatment	Yield of BCA-trt	Yield of Conventional
B	'Golden Jubilee' seed	5.7 tons/ac	6.07 tons/ac
H	'Golden Jubilee' seed	19100 lbs	17400 lbs
G1	Wheat covercrop	4.92 tons/ac	6.07 tons/ac

In addition to obtaining harvest data, we also collected disease data every two weeks. When overall means by field location are examined, we can see that there is significant variation due to location (By field in Table 3). When mean disease ratings are compared among dates, the severity of rot in both the subcrown internode and root system progressed with time. Also, nodal tissues at the first node aboveground got increasingly darker with time. When means are calculated by seed treatment; the biocontrol seed treatment was associated with shorter plants and slightly higher rot ratings of the subcrown internode. The overall root rot ratings were less in the biocontrol treatments compared to the conventional seed treatment.

Table 3. Average height, rot ratings, and darkening of nodal tissues by field site, sample date, and seed treatment

		Average rot rating		Mean of darkening of nodal tissues	
By Field	**Average plant height (cm)** [1]	**Subcrown internode**[2]	**Root system**[3]	**Node +1**[4]	**Ear node**[5]
B	28.5 a	1.02 c	2.25 d	0.26 d	.
H	24.1 b	2.09 b	3.02 b	0.35 c	0.50 a
G2	19.6 c	2.64 a	3.69 a	0.52 a	0.55 a
G1	18.5 d	2.04 b	2.86 c	0.43 b	0.67 a
By Date					
1	3.3 c	.	.	.	.
2	17.4 b	0.31 g	0.44 g	0 d	.
3	42.4 a	0.65 f	1.23 f	0 d	.
4	.	1.54 e	2.56 e	0.07 d	.
5	.	2.42 d	3.60 d	0.59 c	.
6	.	2.98 c	4.34 c	0.75 b	.
7	.	3.66 b	4.88 b	0.81 b	0.43 b
8	.	3.97 a	5.35 a	0.92 a	0.70 a
By Trt					
Standard	24.9 a	1.92 b	3.14 a	0.42 a	0.54 a
BCA-trt'd	20.5 b	2.10 a	2.83 c	0.39 a	0.62 a
CC-BCA-trt'd	.	2.13 a	3.03 b	0.41 a	0.59 a

[1] Means based on 30 plants from each treatment sampled on each date from each field. Numbers labeled with the same letters are not significantly different (P=0.05) to others in its subset column, according to Tukey's W statistic.
[2] Subcrown internode rot rating was based on 0 = no infection; 1 < 25 %; 2 = 26-50 %; 3 = 51-75 %; and 4 >75 % of subcrown internode was decayed/necrotic.
[3] Root system rating is based on 0 = no necrosis on roots; 1 = < 50 % primary root necrotic & no other root rot; 2 = > 50 % primary root necrotic or seminal root necrosis < 25 %; 3 = primary root necrotic > 50% plus seminal root necrosis = 26 - 50 %; 4 = seminal root necrosis = 50 -75 %; 5 = seminal root necrosis >75 % and brace root necrosis < 25 %; 6 = seminal root necrosis >75 % and brace root necrosis = 26 - 50 %; 7 = seminal root necrosis >75 % and brace root necrosis = 51 - 75 %; and 8 = >75 % of all roots are decayed
[4] Node +1 signifies the first node above the soil line. A zero was given for healthy appearance. A "1" was assigned to a node with necrosis. Highest possible disease value is 1.
[5] Ear node signifies a node from which an ear also developed. Highest possible disease value is 1.

The wheat cover crop was also examined. Thirty plants were dug up in the spring from each treatment . Shoot weight, rhizosphere soil weight, and the colony-forming units of *Fusarium* species per gram of rhizosphere soil was also determined. As seen in Table 4, the cover crop seeds treated with the biocontrol mixture resulted in slightly larger shoots and rhizosphere soil amounts.

Table 4. Preliminary field experiment using biocontrol-treated covercrop seeds

Treatment	Mean wheat shoot weight (g)	Mean rhizosphere soil weight (g)	CFUs of *Fusarium* per g of rhizosphere soil
Wheat covercrop seed + biocontrol mixture	3.2	0.2	54947.2
Conventional wheat covercrop	1.1	0.1	83640.5

The seed treatments tested in the Botany Farm plot are listed in Table 5. Each plot was a single corn row, 40 ft row in length, and replicated 6 times. Due to the late planting date and sampling weather, only 3 replicates could be sampled in a timely manner.

Table 5. 'Golden Jubilee' seed treatments tested in small plots on Botany Farm

BAS 570 (High rate)
BAS 570 (Low rate)
BAS 500 (High rate)
BAS 500 (Low rate)
Ocamb biocontrol mixture 1
Ocamb biocontrol mixture 2
Ocamb biocontrol mixture 3
'Bonus' + Captan/Thiram standard
Captan/Thiram standard
Disinfested in 3 % hydrogen peroxide
Gustafson L1028-C4
Gustafson L1028-C4 + Captan/Thiram standard
Mycostop (*Streptomyces* sp.)
Nontreated

The Ocamb mixes utilized the following microorganisms:

Acquisition code	Microbial identification via fatty acid analyses
BC 19	*Methylobacterium mesophilicum*
BC 20	*Rhodococcus erythropolis/Kocuria varians/Pseudomonas diminuta*
BCB 175	*Bacillus megaterium*
BCB 176	*Streptomyces lavendulae*
BCT 5a	*Streptomyces violaceusniger* subsp. *violaceusniger*
BCT 19b	*Streptomyces rochei* subsp. *rochei*
BCT 156	Unknown

Mix 1 contained BC19, BC20, BCB175, BCB176, BCT5a, and BCT19b.
Mix 2 contained the above, minus BCB175. Mix 3 contained BC20 and BCT156.

Ten plants in each plot were rated for "firing" of side tillers and the number of nodes aboveground that had "firing" symptoms was recorded. Ten plants were dug up in each plot (3 replicate plots per treatment equals 30 plants sampled per treatment). Plants were cleaned with a power washer and rated for rot. 'Golden Jubilee' seed, disinfested or treated with

Captan/Thiram; resulted in firing of all side tillers (Fig. 1). There was variation in some of the treatments but several were significantly better; BCA mix 2 and 3, as well as the nontreated seed. The number of nodes that were "firing" was generally low, however, firing was present and some hybrids in Myer's varietal screening trial in the field had severe firing. Again seed disinfested or treated with Captan/Thiram resulted in the highest number of "firing" nodes (Fig. 2). One biological treatment, Mycostop, and nontreated seeds resulted in the lowest number of nodes "firing". Mean root rot ratings were similar, though some significant differences were apparent. These data analyses are preliminary and will continue in 2002.

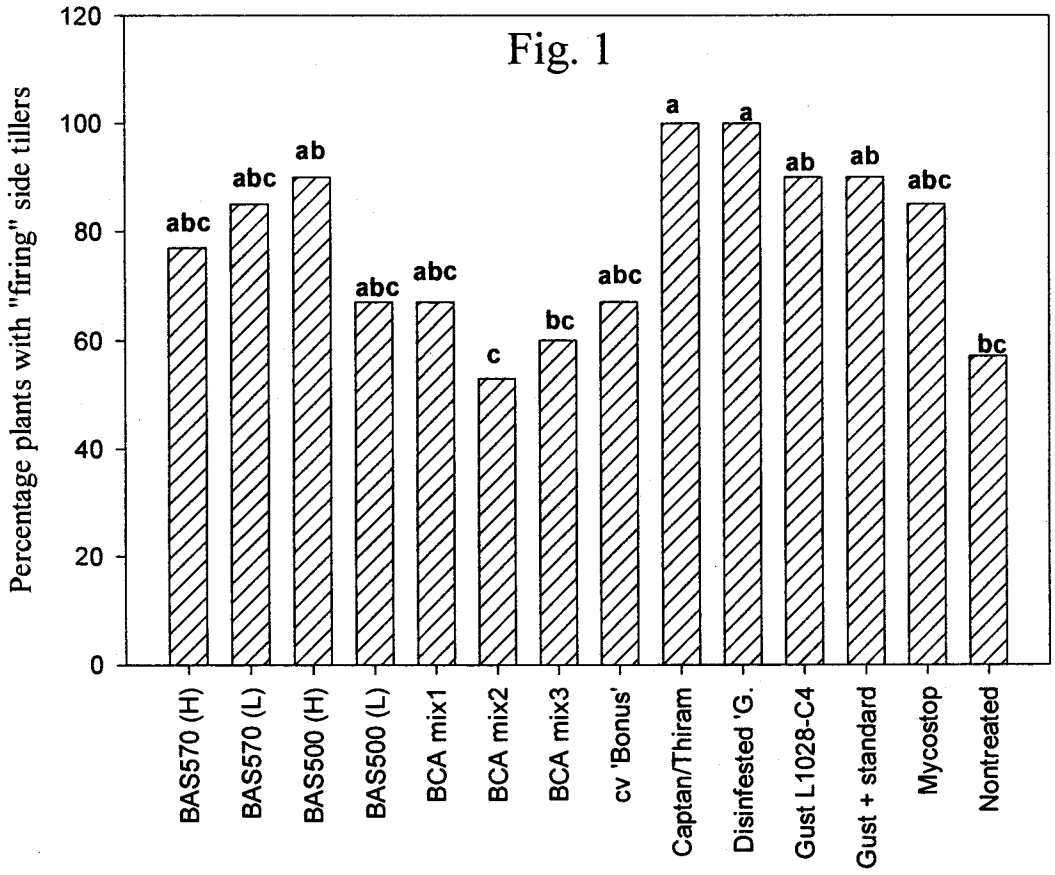

Figure 1. Mean % plants with "firing" side-tillers. Bars labeled with the same letters are not significantly different (P=0.05) according to Tukey's W statistic.

Objective 3: Evaluate rhizosphere populations of *F. oxysporum* var. *redolens* on rotational crops in laboratory and greenhouse studies.

An isolate of *F. oxysporum* var. *redolens* was transformed with a jellyfish gene, the green fluorescent protein (GFP). This transformed fungus glows green under a blue light; enabling us to recognize the pathogen's physical presence on or in the plant. Because we can measure populations without laborious fungal isolations; we can use this for rapid evaluations of pathogen growth, especially useful in the absence of disease symptoms. Since pathogenic *Fusarium* populations can increase asymptomatically in the rhizosphere of some plant species, knowing which plants support greater *Fusarium* populations requires measuring *Fusarium* growth. We have begun preliminary evaluations on a range of plant species with this transformant *Fusarium* isolate and will continue more intensively in 2002. We can evaluate *Fusarium* spread in the rhizosphere, on and in the roots, up the plant, and associated with debris. We can evaluate one population alone or in raw soil or mixed with other pathogens. More importantly, we can use it to rapidly evaluate control strategies like seed treatments.

Objective 4: Evaluate 'Golden Jubilee' and 'Supersweet Jubilee' seeds for presence of *Fusarium* species.

Fresh kernels from 'Golden Jubilee' plantings with "firing" syndrome were evaluated for the presence of *Fusarium* species. The survey was quite small because we've been focusing on Objectives 1 & 2. However, in our limited examination of darker-orange kernels, we recovered putative *Fusarium* species from 44 kernels out of 81 kernels sampled. Species identification will be finalized during 2002.

Sweet corn seeds have been in an on-going examination of *Fusarium* presence and a larger cultivar evaluation will be undertaken during 2002. The two cultivars examined so far, 'Golden Jubilee' and 'Bonus', were found to have *F. moniliforme* propagules present on virtually all seeds tested. Other *Fusarium* species have been recovered but are difficult to obtain and require much more time to define their presence.

Objective 5: Cooperate with other sweet corn research projects.

We participated in variety evaluations with Jim Myers, planting one replicate of his varietal screening set on the Botany Farm. Disease was lower in our study (see Table 6) but some rankings were similar, including the top variety (GH9595).

Table 6. Nodal "firing" ratings of various sweet corn varieties plant on the Botany Farm

Sweet corn variety	Node # with firing (up stem)
GH9595	1.00
GH5702	1.90
HMX7384	1.90
FMX516	2.00
GH2042	2.00
Bonus	2.10
Cinch	2.10
EX8452067	2.10
GH1829	2.10
GH2385	2.10
GH2386	2.10
GH5703	2.10
HMX0395	2.10
Legacy	2.10
Ocamb Jubilee standard	2.10
GH2298	2.20
Climax	2.40
Esquire	2.40
GH5704	2.40
Untreated Jubilee	2.40
EX8441107	2.50
Disinfested Jubilee	2.50
GH2041	2.60
GH4809	2.60
Jubilee	2.70
Chase	3.20
GH9590	3.60
Reward	4.60
EX8473488	overmature