AN ABSTRACT OF THE DISSERTATION OF

Nathan L. Miller for the degree of Doctor of Philosophy in Botany and Plant Pathology, presented on May 16, 2007.
Title: Responses and Relationships among *Fusarium* Species, Sweet Corn, and Western Spotted Cucumber Beetles.

Abstract approved:

Cynthia M. Ocamb

Sweet corn (*Zea mays* L.) yields in the Willamette Valley of Oregon have been declining since the early 1990's. Studies were done to determine if there is a relationship between ear weight and several disease parameters including necrotic crowns or stalk nodes, nodal root rot, radicle root rot, and sub-crown internode rot. Regression analysis indicated that plants with more necrotic crown tissues have lower ear weights and crown necrosis was the best predictor of ear weight at harvest. Fungal isolations from crown tissues indicated that presence of *Fusarium oxysporum* was associated with more necrotic crowns and lower ear weights. *Fusarium verticillioides* was also associated with darker crowns and nodes. Fluid conductance tests indicated that plants with darker stalk nodes had a reduction in fluid passage through a 30-cm stalk section. Isolates of several species of *Fusarium* were examined for their ability to cause crown and stalk node rot in two field and two greenhouse trials. Seed kernel inoculation with *F. oxysporum* and *F. verticillioides* resulted in plants with darker crowns and lower ear weights,

although differences were not always significant. Both of these fungi were recovered from crown tissues of the majority of mature corn plants sampled in pathogenicity trials. It was also observed that seed kernel inoculation with these *Fusarium* species resulted in plants having greater root damage attributed to the western spotted cucumber beetle (WSCB), *Diabrotica undecimpunctata undecimpunctata*. Studies were conducted to determine if the beetles show feeding or oviposition preferences when sweet corn plants were grown from kernels inoculated with *Fusarium* species. Generally, plants grown from *Fusarium*-treated kernels had significantly more leaf-feeding injury of corn seedling from adult WSCB compared to the disinfested control. Plants grown from *Fusarium*-treated kernels were also found to have increased larval root feeding in field pathogenicity trials. It appears that sweet corn ear yield reductions in the Willamette Valley is at least partly attributed to crown and stalk node rot incited by *Fusarium* species, and WSCB injury could be compounded with *Fusarium* injury, causing further stress of diseased sweet corn plants when these beetles are present.

Responses and Relationships among *Fusarium* Species, Sweet Corn, and Western Spotted Cucumber Beetles

by

Nathan L. Miller

A DISSERTATION

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APPROVED:

Major Professor, representing Botany and Plant Pathology

Chair of the Department of Botany and Plant Pathology

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Nathan L. Miller, Author

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

Chapter 1

Introduction

Nathan L. Miller

Sweet corn (*Zea mays* L.) growers in the Willamette Valley of Oregon reported declining yields in the early 1990s, and by the mid 1990s, this decline could be seen valley-wide (Figure 1.1). Based on conversations with growers and the processing industry, sweet corn fields should yield a minimum of 23 to 24.5 metric tons/ha for production profitability. In 1995, more than 50 % of fields planted to the cultivar 'Jubilee', the most widely planted cultivar at the time, yielded less than 23 metric tons/ha, based on processor records. The percentage of fields below this tonnage threshold increased, and peaked at 90 % during 2000 and 2001. Growers and processors began planting other cultivars instead of 'Jubilee'; a change that appears to be associated with an increase in overall yields. There is still concern in the local sweet corn industry because some of replacement cultivars that looked higher yielding in small acreage exhibited a yield decline and associated disease symptoms similar to those seen in fields of 'Jubilee'. It is necessary to understand the factors leading to declining yields before long term management practices, including resistant cultivars, can be adopted.

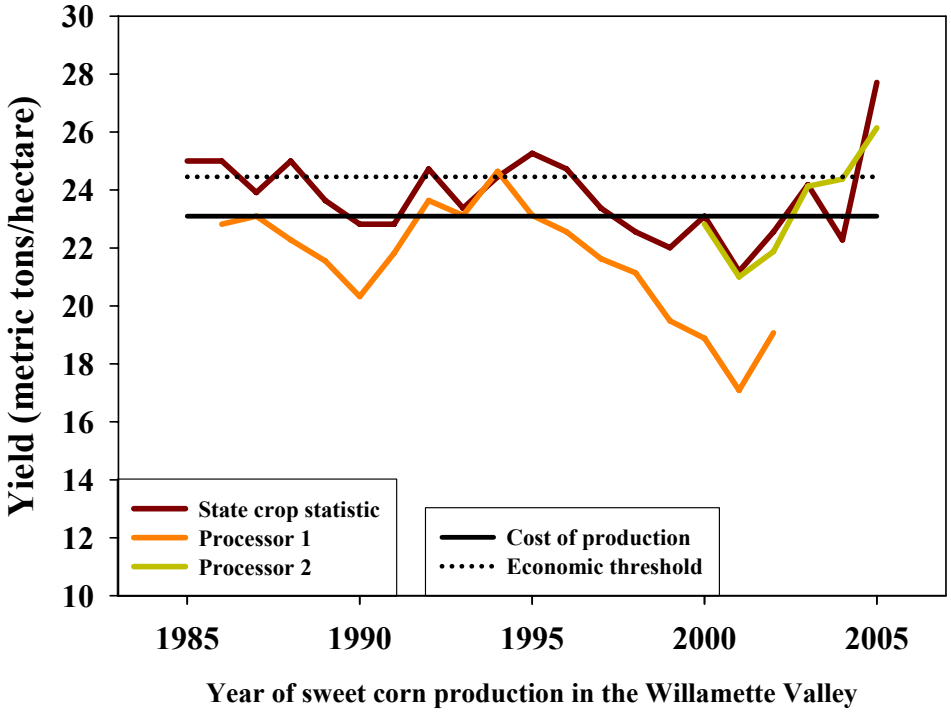

Figure 1.1 Average yield of sweet corn fields in the Willamette Valley based on data from three different sources. The cost of production represents the approximate yield necessary to recoup production costs and the economic threshold represents the level needed for a sustainable profit, based on conversations with growers and processor representatives, but may not be constant through entire timeline shown.

The decline in yields was initially associated with leaf "firing", where the leaves prematurely die starting at the base of the plant, and then leaf necrosis progresses up the plant. Root rot was implicated as the primary cause of the this yield decline in the Willamette Valley (1). However, sweet corn plants can show firing symptoms, and affected fields can have reduced yields, with little to no root rot present. Something other than root rot must also cause leaf firing and yield loss, and Ocamb (unpublished) observed necrosis of the tissues in the crown and stalk nodes. The crown is itself a series of nodes with shortened internodes (2). Nodes are where most of the connections between xylem vessels occur in a corn stalk, and at these connections fluid movement

is constricted by porous vessel end walls that filter particulates and gas bubbles (3). The node regions are points of increased resistance to movement of fluids within a stalk, so disease at nodes could further restrict the ability of plants to move fluid through the xylem. Root rots and vascular disease can both result in similar secondary symptoms on the external aboveground portions of plants. Thus, it is possible that leaf necrosis and low ear weight observed in sweet corn fields in the Willamette Valley are the result of a compromised crown and stalk node tissues as well as root rot.

This dissertation attempts to establish which disease symptoms are most closely associated with reduced ear yields in processing sweet corn fields in the Willamette Valley. A series of studies were also conducted to determine whether the plants with stalk node necrosis had alterations in the movement of fluid through the vascular system. Once the role of node and crown necrosis in resulting ear weight was established, the organisms that inhabited these regions were examined for their ability to cause necrosis. Previous work by Ocamb (unpublished) found that *Fusarium* species predominately inhabited afflicted sweet corn crowns and stalk nodes. Additional studies for this dissertation were conducted to determine which *Fusarium* species were associated with crown and stalk node necrosis, and yield decline in commercial fields. Pathogenicity studies were performed to determine if three *Fusarium* species, commonly found in corn roots, stalks and crowns, could cause crown and stalk node symptoms, and subsequent yield decline. The three species of *Fusarium* that were chosen for the pathogenicity experiments were *Fusarium oxysporum* var. *redolens*

(Wollenw.) Gordon, *F. verticillioides* Sacc. (Nirenberg) (synonym *F. moniliforme* Sheld.), and *F. proliferatum* (T. Matsushima) Nirenberg.

During pathogenicity experimentation, it was observed that plants grown from kernels inoculated with *Fusarium* species suffered higher levels of root damage from the larvae of *Diabrotica undecimpunctata undecimpunctata* Mannerheim, otherwise known as the western spotted cucumber beetle (WSCB). The larvae of this beetle have limited mobility, so studies were conducted to determine if WSCB adults showed leaf feeding or oviposition preferences for plants grown from *Fusarium* inoculated kernels. WSCBs can cause significant damage as adults if excessive silk feeding occurs, but they are usually more problematic as larvae feeding on corn roots. Although their reproduction appears to occur mostly on corn, WSCBs are also significant pests as adults on other important crops in the Willamette Valley. Interactions between WSCBs and *Fusarium* species would provide further justification for management of *Fusarium* diseases of corn since there may be the additional benefit of reducing WSCB damage.

1. Hoinacki, E. V. 2003. Sweet Corn Decline Syndrome in Oregon's Willamette Valley. Ph.D dissertation, Botany and Plant Pathology, Oregon State University, Corvallis, 104 pp.

2. Kiesselbach, T. A. 1999. The Structure and Reproduction of Corn, 50th Anniversary Edition. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. 101 pp.

3. Shane, M. W., McCully, M. E., and Canny, M. J. 2000. The vascular system of maize stems revisited: Implications for water transport and xylem safety. Annals of Botany 86:245-258.

Chapter 2

The Relationship between Yield, Xylem Conductance, and Disease in Crown and Node Tissues of Sweet Corn Grown in the Willamette Valley

Nathan L. Miller and C. M. Ocamb

Abstract

Sweet corn (*Zea mays* L.) yields in the Willamette Valley of Oregon declined during the 1990's. Severe root rot affected some plants, but was absent in other plants that showed secondary symptoms of low ear yield and leaf death; necrosis of stalk nodes and crowns was found instead. Studies were done to determine if there is a relationship among yield and necrosis of crowns, stalk nodes, nodal roots, radicles, or sub-crown internodes. Plants were sampled from each of ten grower fields. An image analysis program was used to quantify the grayscale value of the crown and node tissues. Bayesian information criteria model selection indicated that the model which best explained ear weight was a model containing field location and the grayscale value of crowns. Regression analysis indicates that plants with more necrotic crown tissues have lower ear weights. Rot of the nodal roots, radicle, or sub-crown internode were poor predictors of ear weight at harvest. Plants with darker stalk nodes had reduced fluid movement through a 30-cm stalk section. Reduced ear yields may be partly a result of decreased vascular conductance in symptomatic crown and stalk nodal tissues.

Introduction

Sweet corn (*Zea mays* L.) growers in the Willamette Valley of Oregon reported declining yields during the early 1990s. The decline in yields was initially associated with leaf "firing", where the leaves die prematurely starting at the base of the plant and then progressing up the plant. Root rot has been implicated as the primary cause of this yield decline in Oregon (12), however, sites afflicted with reduced yields may have little to no root rot. Something other than root rot must also cause leaf firing and yield loss in affected sweet corn fields in the Willamette Valley, and Ocamb (unpublished) has observed necrosis of the tissues in the crown and stalk nodes.

The crown region of maize plants is a stack of approximately eight nodes with shortened internodes (13). In sweet corn plants exhibiting this crown and node rot, both the crown and lower stalk nodes will be a dark brown to black in coloration (Figure 2.1). Most of the vascular bundle connections and branches occur at nodes, while within internodes, the individual vascular bundles can be easily traced and rarely branch (6,13,14,23,25). The end walls of vessels in the nodes act as filters and collect solid particles or gas bubbles that have gained entry into a vessel. Ninety-seven percent of all axial vessels are interrupted by an end wall in each node and 20-nm gold particles are too large to pass through these end walls (23). Even smaller particles (5-nm) are filtered at the vessel junctions between branch and main roots (24).

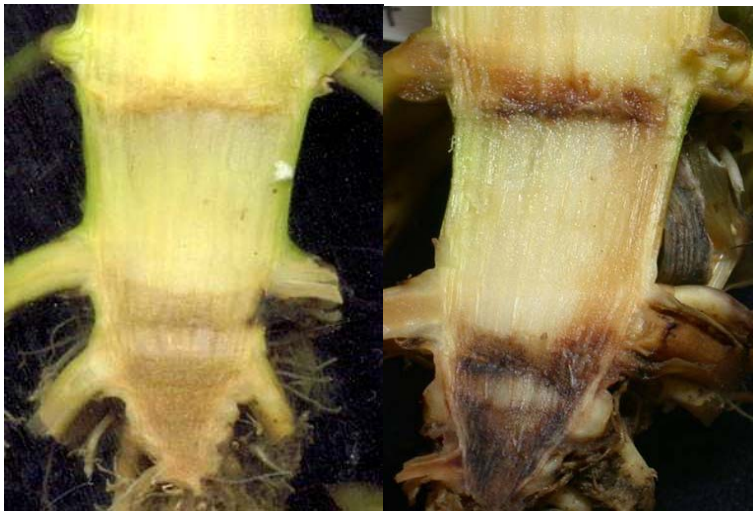

Figure 2.1. Longitudinal cross section of sweet corn plants showing a plant with healthy (left) and diseased (right) crown and stalk nodes.

Fluid columns in xylem are often maintained at pressures that are more negative than the vapor pressure of water, a state referred to as metastable (27). The cohesive properties of water allow vessels in this situation to function normally, so long as the transpiration stream is not interrupted (17). Once a break in the water column occurs, a gas embolism may form, known as a cavitation event. This can then fill with water vapor and dissolved gases coming out of solution. When the fluid in a vessel becomes interrupted by gas embolisms, that vessel becomes temporarily nonfunctional. Embolisms in one vessel may seed air into neighboring vessels through the pit membranes (26,30). Gas embolisms occur daily in maize plants, and up to half the vessels may be affected during a day with drought stress (15,28). These affected vessels can refill when transpiration drops at night, by dissolving gases and absorbing water from adjacent tracheids which are less likely to cavitate. Excessive cavitation and refilling can weaken pit membranes and make vessels more susceptible to further cavitation (8). End walls of the xylem vessels are concentrated in the nodes (23),

which results in the nodes acting as filters to stop movement of particulates or gas embolisms through the vessels. Since stalk node or crown necrosis occurs in regions where particulates in the vascular system would be accumulated, it was hypothesized that affected node tissues compromise the ability of the plant to move fluid up through the xylem.

Since the relationships between root rot, crown and stalk node rot, and yield loss are not clear, the objective of this study was to determine the association between root, crown, and stalk node rot to ear weight in sweet corn plants in the Willamette Valley. It was hypothesized that plants with rot within the crown and lower stalk node tissues, as indicated by darker coloration of tissues, will have lower ear weights. A second objective of this study was to determine if there is increased resistance to xylem conductance associated with affected stalk nodes.

Materials and Methods

Association of yield and symptoms

Plants in commercial sweet corn fields were examined to determine which disease symptoms are associated with reductions in ear weight. Ten commercial sweet corn sites in the Willamette Valley (Table 2.1) were sampled a few days before commercial harvest to control for developmental stage of the plants, and were chosen based on availability. A number of sweet corn varieties were sampled because of grower shifts from the formerly predominate cultivar, ‘Jubilee’.

Table 2.1. Varieties, field location, and sampling date of commercial sweet corn plants in the Willamette Valley used for symptom-yield association studies

Field	Variety	County	Date sampled
1	Jubilee	Marion	August 25, 2003
2	Jubilee	Marion	August 25, 2003
3	Prelude	Benton	September 8, 2003
4	GH2298	Benton	September 22, 2003
5	GH2298	Benton	October 6, 2003
6	Coho	Benton	August 18, 2004
7	SR1292	Linn	August 23, 2004
8	Coho	Benton	August 23, 2004
9	WSS3681	Lane	September 6, 2004
10	WSS3681	Benton	September 7, 2004

After surveying the general size and shape of each site, the site was divided by visual assessment into ten sections similar in size. One transect was sampled in each of the ten sections. To avoid edge effects, samples were not collected within 10 rows from any field edge. The number of foot steps taken to reach the first plant in each transect was determined by using random numbers between 20 and 100. In each transect, 10 consecutive plants within one row were sampled. Plants were cut above the ears, dug, and returned to the lab. Root balls were washed clean of soil and the percentage of nodal roots with rot was rated on the following scale: 1-25 % of the nodal root ball was rotted = 1, 26-50% = 2, 51-75% = 3, or 76-100% = 4. The rot of the primary root (radicle) and sub-crown internode (mesocotyl) were rated separately using a similar 1 to 4 scale based on 25 % increments. Weights of individual, developed ears were recorded after removing husks. Roots were also rated for rootworm larval feeding (*Diabrotica undecimpunctata undecimpunctata* Mannerheim) using the following 0 to 3 scale: 0 = no root tunneling observed on any roots, 1 = one to three roots had tunnels, 2

= more than three roots with tunnels but less than half the roots in the root ball had larval feeding damage, or 3 = more than half the roots in the root ball had larval feeding damage.

Stalks and crowns were split longitudinally, parallel to the leaf ranks, and the cut surface was digitally captured on a flatbed scanner (HP scanjet 2400, Hewlett Packard, Palo Alto, CA). Multiple layers of black fabric were used to cover the stalks while scanning to minimize extraneous light. The resulting images were then analyzed using the digital image analysis program, ImageJ version 1.34s (US Department of Health and Human Services, National Institutes of Health). A histogram for each plant was generated for the crown region and the first stalk node immediately aboveground that lacked rooted brace roots. The histogram calculated the coloration of each pixel within the region of the crown or stalk node on a 256-bit grayscale (Figure 2.2). Mean grayscale measurement was recorded and used for analysis of crown and lower stalk node necrosis.

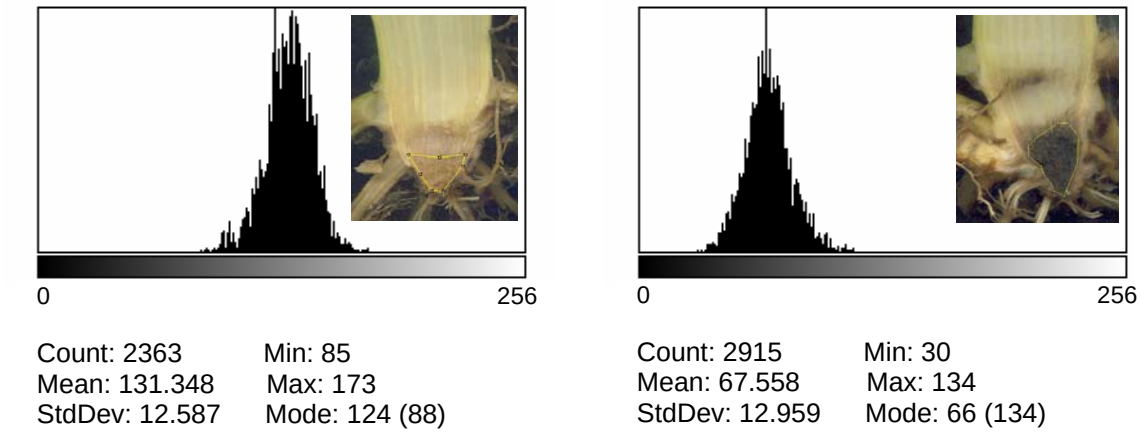

Figure 2.2. Examples of sweet corn crown images (inset) and ImageJ grayscale histogram output of the crown region. Stalks were cut longitudinally at the base and scanned on a flatbed scanner. Each pixel of the specified region is measured on a 256-bit grayscale and the histogram is generated using the number of pixels in each grayscale shade. Larger means indicate lighter grayscale values.

The symptom measurements were analyzed using mixed linear models (SAS 9.1, SAS institute, Cary, NC), and transect means were used for statistical analysis of all variables. Statistical models were tested for goodness-of-fit using Bayesian Information Criterion to find which models best explained the weight of the apical ear on the plants, as this was the only ear that matured on nearly all of the plants. Fields were sampled on different dates and spread across a wide geographic area of the Willamette Valley, in addition to representing more than one sweet corn cultivar, so models included site as a random effect. Models with each variable alone, without field, were also included. When models included field as an explanatory variable, all possible combinations of crown, lower stalk node, sub-crown internode, radicle, and nodal root rot as linear variables, without interactions, were tested. Also tested were models containing field and one of the rot variables as well as an interaction between site and the other variable. Field 3 appeared inconsistent with the other nine sites, so

the same analyses were conducted without field 3. A separate set of analyses were conducted removing single sites, to determine the relative contribution of each site on the outcome of the BIC analysis. Models are generally not considered to be significantly different if the difference in their BIC values is less than 2.0 (3). Pearson's correlation coefficients were generated between all explanatory variables to assist interpretation of slope estimates for variables' effects on ear weight among different models. Pearson's correlations were calculated because highly-correlated explanatory variables will influence estimates of their effects when they are included in the same model. Regression models were additionally run to determine if correlations of crown rot with other rot variables were consistent by field, using crown grayscale as the response variable.

Rootworm feeding was not included in the first Bayesian Information Criterion model selection because fields 1 through 3 did not include rootworm ratings and the difference in sample size would bias the results. Separate analyses were conducted using only sites where rootworm feeding was quantified.

Xylem conductance experiments

Fluid conductance through corn stalks was measured to determine whether nodal necrosis affected the ability of stalks to conduct sap. Five commercial sweet corn fields in the Willamette Valley were sampled (Table 2.2), based on availability. Six transects, and number of foot steps taken to reach the first plant in each transect, were chosen in the same manner as previously described. Five plants were sampled within each transect along a single row, and ten steps were taken between each plant to avoid

collecting root balls that overlapped spatially. Stalk sections were cut above the top ear and discarded. The rest of each plant, including soil associated with root balls, were dug up, bagged, and immediately brought to the lab. Plants were collected from each respective site before 10:00 AM on the day of the experiment. Plants with a bottom-most stalk internode diameter less than 1.5 cm were excluded from sampling because the stalks were too narrow for the sampling apparatus.

Table 2.2. Varieties, field locations by county, sampling dates, and the number of sweet corn plants evaluated for xylem conductance

Site	Variety	County	Date sampled	Number of Plants sampled
1	GH2298	Benton	09/12/2003	18
2	SS White	Polk	10/12/2003	21
3	ACX642A	Benton	09/14/2004	26
4	SS Jubilee	Benton	09/15/2004	25
5	Basin EX 84	Lane	09/16/2004	25

A 50-cm section of stalk containing two nodes was excised from each plant just above the top whorl of nodal roots. The stalk section was cleaned of any soil and debris, stripped of leaves, and then immediately placed under a solution of 5-mM KCl in reverse osmosis (RO) water. The excised stalk sections were cut again at each end while in this solution, leaving a 30-cm stalk section with one intact internode and two nodes. While under the solution, a piece of Tygon plastic tubing (2.45-cm inner diameter x 30-cm length) was placed over the lower node and then sealed with petroleum jelly and a screw-tightening, stainless steel hose clamp. The stalk section was then inverted on a ring stand with three-fingered flask clamps, and the Tygon tube

was filled to 20 cm above the cut-end of the stalk with 5-mM KCl. The outside of the stalk was towel-dried and any drips from the cut-end were dabbed off at time zero. A preweighed, zip-sealing sandwich bag (16.5-cm x 15-cm) containing one Kimwipe for wicking was used to collect the solution flowing out of the cut-end of the stalk. The solution was collected from each plant for 20 minutes and then the bag was reweighed. Data were excluded if hose seals visibly leaked. The stalk diameters in the center of the intact internode were recorded perpendicular to the leaf ranks. Individual flow rates were adjusted by the cross-sectional surface area inferred from the diameter. After xylem conductance tests, stalks were split, scanned, and the bottom stalk node was analyzed with ImageJ as previously described. Images of both stalk halves were analyzed and averaged for each stalk (Figure 2.3). Up to 30 plants per site were tested for fluid conductance, but no plants were tested later than 4 hours after returning to the lab. Ear weight was also recorded for each plant.

The order in which plants from each field were evaluated for xylem conductance was randomized. The data were analyzed using mixed linear models (SAS 9.1, SAS institute, Cary, NC). All models containing mean stalk node grayscale and site as explanatory variables for individual flow rate were tested for goodness-of-fit using Bayesian Information Criterion.

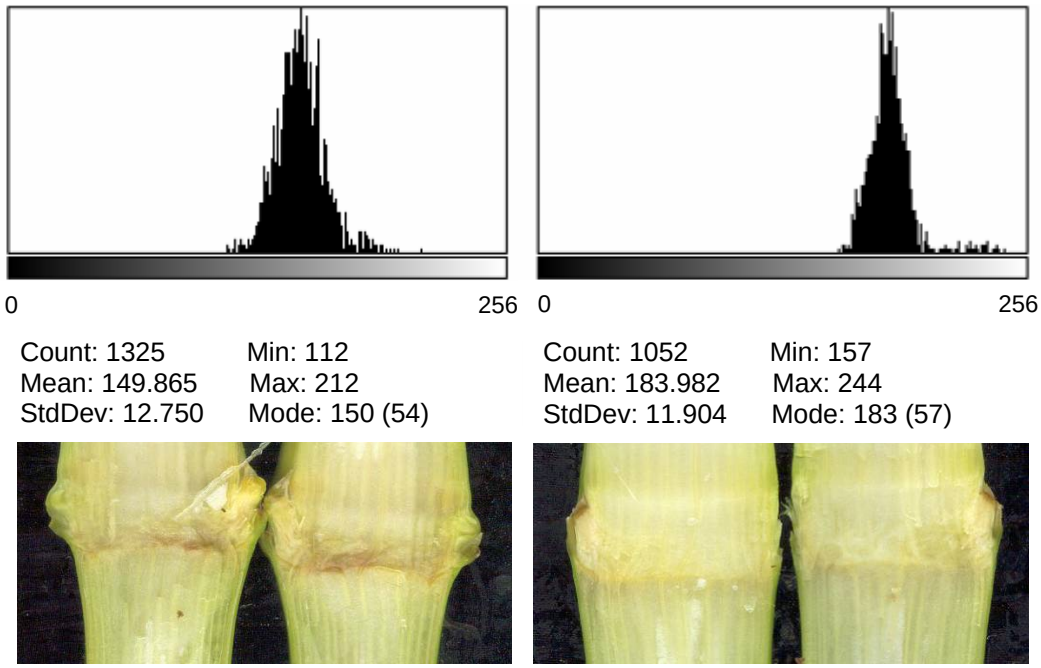

Figure 2.3. Examples of sweet corn stalk node images and ImageJ grayscale histogram output of the node region. Stalks were cut longitudinally and scanned on a flatbed scanner. Each pixel of the specified region is measured on a 256-bit grayscale and the histogram is generated using the number of pixels in each grayscale shade. Larger means indicate lighter grayscale values.

Results

Association of yield and symptoms

Plants from commercial sweet corn fields were examined to determine whether disease symptoms are significantly associated with reductions in ear weight. Disease symptoms measured included rot of the nodal roots, radicle, sub-crown internode, crown, and first stalk node without rooted brace roots. Rootworm tunneling was also assessed. Regression models were developed for an array of disease symptoms/pest levels and tested with the Bayesian Information Criterion (BIC) to determine which disease variables measured best explain ear weight. BIC values differing by less than 2.0 indicate that there is little difference in the quality of fit between two models (3).

All models that include crown grayscale (models 1A through 17A) as an explanatory variable had a better fit, as indicated by their smaller BIC values (Table 2.3), than models which did not include crown grayscale, except for the model that includes only crown (not site, model 34A). The best-fitting model (Table 2.3), based on Bayesian Information Criterion selection, is the model that estimates a different slope and intercept for the crown effect at each site (model 1A, Figure 2.4). In this model, darker crown grayscales (or more necrotic crowns) are associated with lower ear weights in 9 of the 10 sites sampled. The slopes of ear weight vs. crown grayscale were 3.19*, 0.83, -0.55, 2.21*, 3.18, 3.60*, 4.18*, 0.89, 3.64*, and 1.32* for sites 1 through 10, respectively, and six of the estimated slopes were significant at the $P = 0.05$ level (indicated by *). When field 3 is excluded, this model (1A) drops to the 16th best model and model 3A becomes the best-fitting model (slope = 2.1 g decrease in ear weight per darker crown grayscale unit, $P < 0.0001$).

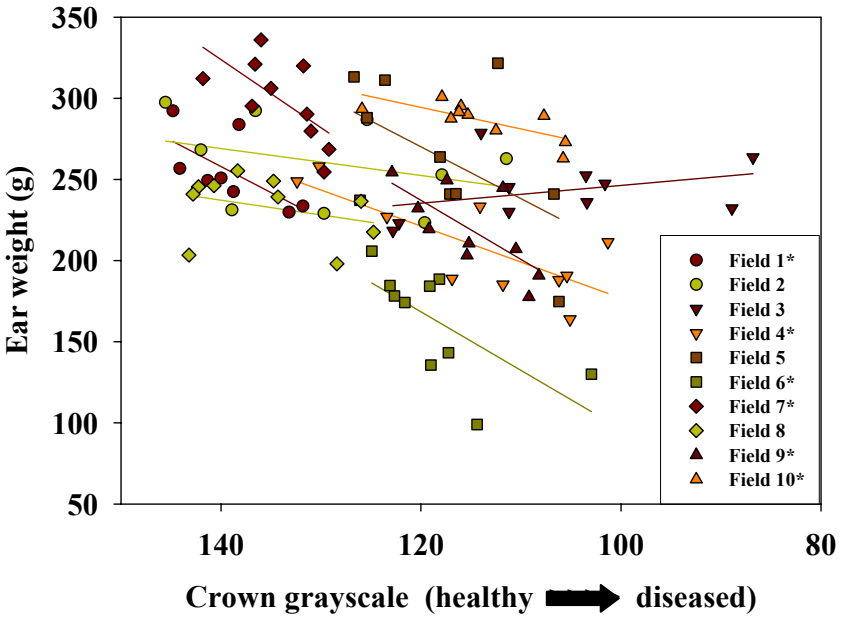

Figure 2.4. The best-fitting mixed regression model based on Bayesian Information Criteria model selection. Grayscale means of sweet corn crowns vs, mean plant ear weight presented by field. The regression model includes field and mean grayscale of the crown as explanatory variables, with an interaction between field and crown grayscale. Each point represents the transect mean based on 10 plants/transect in 10 transects for each field. Fields 1-5 were sampled in 2003 and 6-10 were sampled in 2004. * indicates statistically significant at $P \leq 0.05$ relative to a slope of zero (null).

Table 2.3. Bayesian Information Criterion selection of best-fitting models to explain variation in mean ear weight per transect (10 plants/transect, 10 transects/field) in 10 commercial sweet corn fields in Oregon's Willamette Valley

M# [α]	BIC [β]	Variables in model [γ]	Crown	NRR	PR	SCI	N1
1A	914.1	Site Site●Crown	-0.55 to 4.19*[6]	-	-	-	-
2A	917.2	Site Crown NRR	1.57**	14.4	-	-	-
3A	918.2	Site Crown	1.44**	-	-	-	-
4A	918.5	Site Crown PR	1.53**	-	7.2	-	-
5A	919.1	Site Crown SCI	1.56**	-	-	5.4	-
6A	919.3	Site Crown NRR N1	1.69**	14.5	-	-	-0.2
7A	919.3	Site Crown NRR PR	1.64**	11.9	2.8	-	-
8A	919.4	Site Crown NRR SCI	1.58**	12.8	-	1.8	-
9A	920.0	Site Crown PR N1	1.59**	-	8.6	-	-0.37
10A	920.3	Site Crown N1	1.51**	-	-	-	-0.2
11A	920.7	Site Crown PR SCI	1.66**	-	5.5	2.4	-
12A	920.9	Site Crown SCI N1	1.67**	-	-	6	-0.28
13A	921.1	Site Crown NRR N1 PR	1.56**	10.7	4.3	-	-0.3
14A	921.3	Site Crown NRR SCI N1	1.69**	12.4	-	2.4	-0.24
15A	921.6	Site Crown NRR SCI PR	1.68**	11.6	2.3	0.83	-
16A	922.2	Site Crown SCI N1 PR	1.59**	-	6.8	2.3	-0.38
17A	923.4	Site Crown NRR SCI N1 PR	1.70**	10.4	3.7	1	-0.3
18A	935.1	Site	-	-	-	-	-
19A	935.9	Site N1	-	-	-	-	0.5
20A	936.8	Site NRR	-	7.2	-	-	-
21A	937.2	Site PR	-	-	1.2	-	-

[α]Number of each mixed regression model with mean ear weight as the response variable.
[β]Lower Bayesian Information Criterion (BIC) statistics indicate better fitting models.
[γ]Estimated slopes for the effect of each variable in the model as explanatory variables; Crown = crown mean grayscale, NRR = rating of nodal root rot, PR = primary root (radicle) rot, SCI = rating of sub-crown internode rot, N1 = mean grayscale of the first stalk node aboveground, and RW = rootworm damage rating.
* indicates statistically significant at $P \leq 0.05$ and ** at $P \leq 0.01$ for each estimated slope. Numbers in superscript represent the number of fields which had significant effects when interactions (indicated by ●) were included.

Table 2.3 (Continued). Bayesian Information Criterion selection of best-fitting models to explain variation in mean ear weight per transect in 10 commercial sweet corn fields in Oregon's Willamette Valley (10 plants/transect, 10 transects/field)

M# [α]	BIC [β]	Variables in model [γ]	Crown	NRR	PR	SCI	N1
22A	937.3	Site SCI	-	-	-	-3.02	-
23A	937.5	Site NRR N1	-	6.9	-	-	0.52
24A	938.1	Site PR N1	-	-	1.3	-	0.48
25A	938.1	Site SCI N1	-	-	-	-0.84	0.5
26A	938.3	Site Site●NRR	-	-58.2 to 97.0*[2]	-	-	-
27A	938.7	Site NRR SCI	-	14.2	-	-7.1	-
28A	939.1	Site NRR PR	-	9.3	-2.3	-	-
29A	939.2	Site PR SCI	-	-	6.3	-6.5	-
30A	939.3	Site NRR SCI N1	-	11	-	-4.1	0.54
31A	939.6	Site Site●SCI	-	-	-	-58.9 to 39.5*[2]	-
32A	939.7	Site NRR PR N1	-	9.1	-2.37	-	0.55
33A	940.2	Site PR SCI N1	-	-	3.6	-2.9	0.46
34A	940.3	Crown	1.19**	-	-	-	-
35A	940.9	Site NRR SCI PR	-	12.7	2.8	-8.4	-
36A	941.6	Site NRR SCI N1 PR	-	10.6	0.38	-4.3	0.54
37A	942.1	Site Site●N1	-	-	-	-	-1.59 to 5.79
38A	949.1	Site Site●PR	-	-	-66.1 to 77.4*[2]	-	-
39A	955.9	Null	-	-	-	-	-
40A	956.4	N1	-	-	-	-	0.9*
41A	957.9	PR	-	-	-21.2**	-	-
42A	957.9	SCI	-	-	-	-20.6**	-
43A	958.2	NRR	-	-15.2*	-	-	-

[α]Number of each mixed regression model with mean ear weight as the response variable.
[β]Lower Bayesian Information Criterion (BIC) statistics indicate better fitting models.
[γ]Estimated slopes for the effect of each variable in the model as explanatory variables; Crown = crown mean grayscale, NRR = rating of nodal root rot, PR = primary root (radicle) rot, SCI = rating of sub-crown internode rot, N1 = mean grayscale of the first stalk node aboveground, and RW = rootworm damage rating.
* indicates statistically significant at $P \leq 0.05$ and ** at $P \leq 0.01$ for each estimated slope. Numbers in superscript represent the number of fields which had significant effects when interactions (indicated by ●) were included.

The second best model (model 2A) based on Bayesian Information Criterion selection is a model that includes nodal root rot and crown rot with no interactions, so the crown and nodal root rot effects are held constant across all fields (Table 2.3). In this model, increased nodal root rot has a positive, nearly statistically significant effect on yield (slope = 14.4 g increase in ear weight as nodal root rot increases, $P = 0.07$), while darker, more necrotic crowns, indicated by lower grayscale values, had a negative and highly significant effect on yield (slope = 1.57 g decrease in ear weight per darker crown grayscale unit, $P < 0.0001$). Models 2A and 3A differed by only 1.0 BIC value, suggesting that model 2A is not a significantly better fitting model than 3A. Models 2A through 17A range over 6.2 BIC values, and increase in increments less than 2.0, indicating only minor reductions in the fit of these consecutive models. Model 18A is the first model where crown grayscale is not included and there is an associated 11.7 point increase in BIC values between model 17A and 18A. This would suggest that the addition of disease variables other than crown grayscale add little, if any, explanatory value to the models predicting ear weight. The coefficients (slopes) of disease variables other than crown grayscale are also nonsignificant ($P = 0.05$) in all models that include crown grayscale. The nonsignificant increases in BIC values in models 2A through 17A and the lack of significant slope values ($P = 0.05$) for variables other than crown, indicate that crown grayscale is likely the best predictor of ear weight at harvest. Since crown rot appears to be the most predictive variable, and only one field (field 3) is affecting the consistency of the slope of crown grayscale, model 3A will be used as the for predictive purposes for the rest of this manuscript.

Model 3A, the third best-fitting model (Table 2.3), is the parallel lines model for crown grayscale (Figure 2.5), which estimates a single slope for the crown effect (1.44 g decrease in ear weight per darker crown grayscale unit, $P < 0.0001$) and a different intercept for each field. In this model, a slope of 1.44 results in a reduction in predicted ear weight values by 6 to 20 % per site (Table 2.4). In addition, the parallel lines model for crown rot is the best-fitting model if field 3 is eliminated

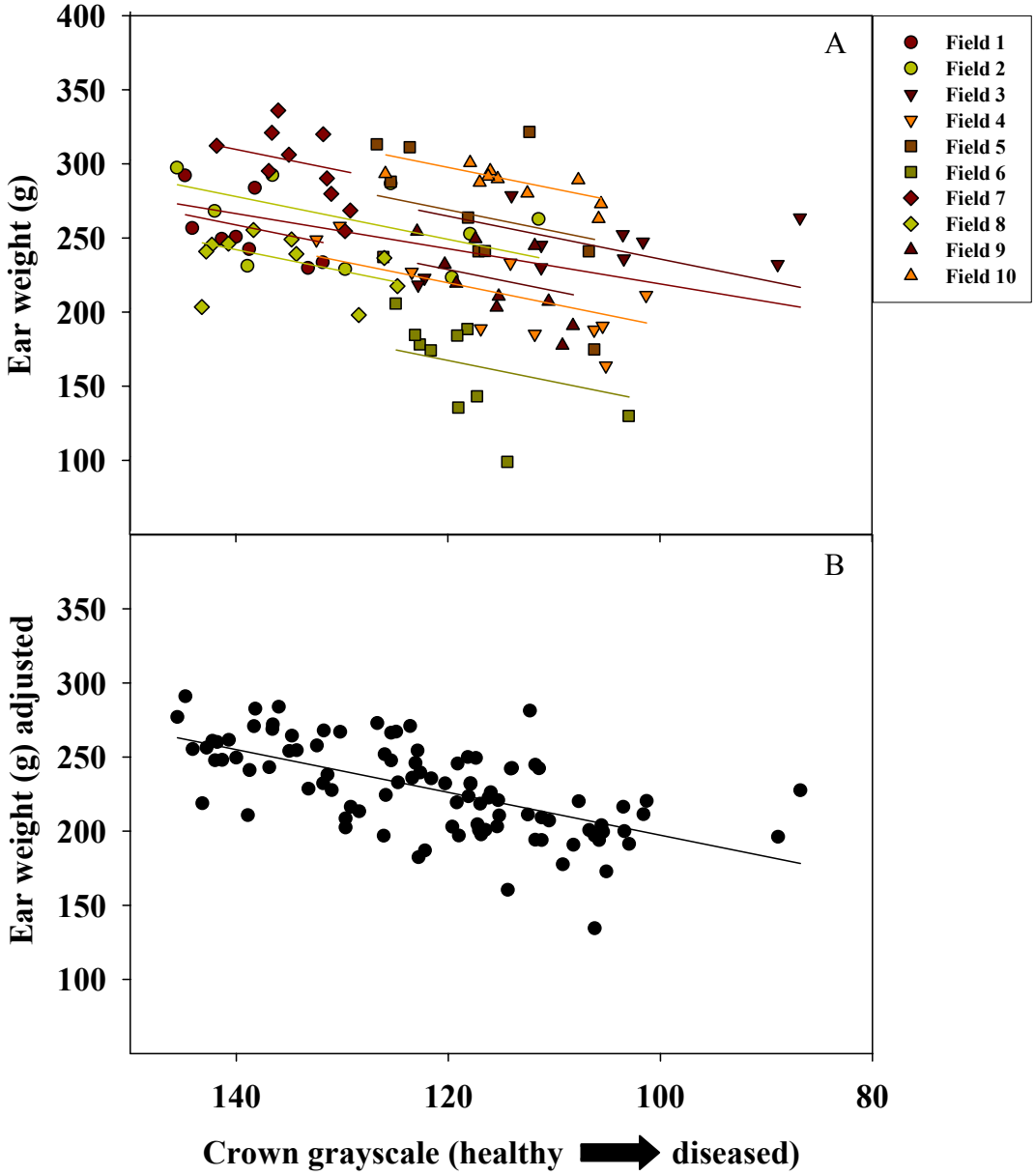

Figure 2.5. Grayscale means of sweet corn crowns versus mean ear weight by field sites. The regression model includes mean grayscale of the crown and field site as explanatory variables without interactions (A) (crown grayscale parallel lines model). The same model is adjusted so all fields fit a common intercept (B) to better show the relationship between crown grayscale and ear weight. Each point represents the transect mean based on 10 plants/transect in 10 transects in each field. Fields 1-5 were sampled in 2003 and 6-10 were sampled in 2004.

Table 2.4. Percent reduction of ear weight of sweet corn plants based on predicted values generated with parallel lines regression model with site and crown grayscale as explanatory variables

Site	Variety	Intercept	Percent reduction [ά]
10	WSS3681	121.70	9.7
7	SR1292	104.88	5.9
5	GH2298	93.01	10.7
3	Prelude	88.72	19.5
2	Jubilee	73.10	17.4
1	Jubilee	53.98	7.1
9	WSS3681	52.85	9.2
4	GH2298	43.80	19.1
8	WSS3681	37.32	10.9
6	Coho	-8.60	18.5

[ά]Percent reduction in predicted ear weights based on a slope of 1.44 g reduction per grayscale unit. Ear weights were derived from transect means based on 10 plants/transect in 10 transects in each field. Fields 1-5 were sampled in 2003 and 6-10 were sampled in 2004.

When field site is not included in the regression models, all disease variables measured appear to affect ear weight (Tables 2.3 and 2.7, Figure 2.6, models 34A, 40 through 43A, and 73B). However, for variables other than crown grayscale, there is a lack of consistency in slopes between models where field site is included compared to when field is not included. This inconsistency creates a residual pattern of non-constant variance for each field, violating statistical assumptions. The constant variance assumption is met by including field site in each model.

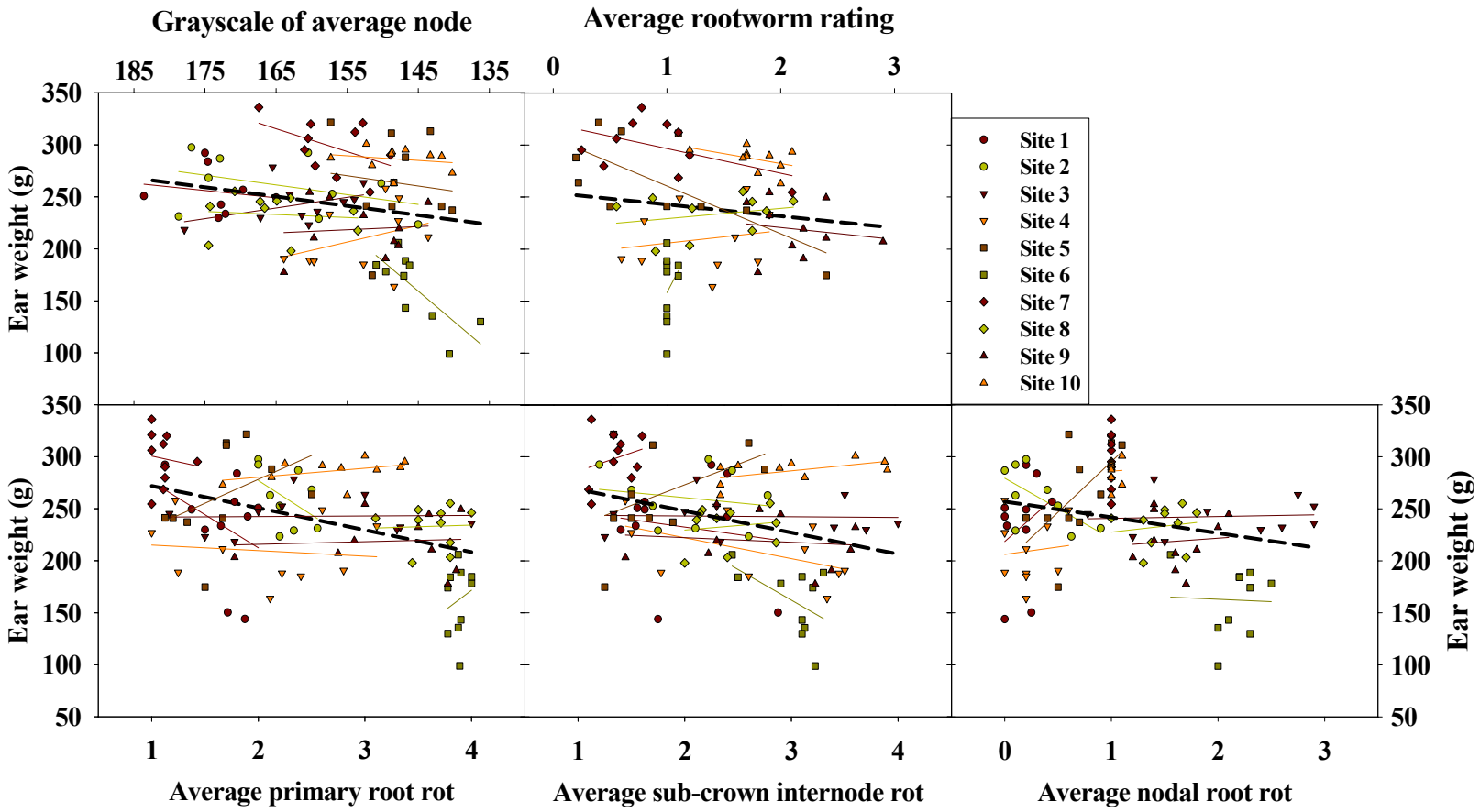

Figure 2.6. Relationships between mean sweet corn ear weight and disease parameters measured other than crown grayscale. Dashed lines represent regression models when site is not included as an explanatory variable and colored lines show the relationship for each site independently. Each point represents the transect mean based on 10 plants/transect in 10 transects for each field. Fields 1-5 were sampled in 2003 and 6-10 were sampled in 2004.

When variables are strongly correlated, regression analysis can give misleading results, so correlations between explanatory variables were examined to help interpret regression results. There are strong correlations among many explanatory variables and regression analyses using one variable as a response and another as an explanatory variable with field site, and an interaction term, helps determine if these correlations are maintained within a field site. Crown grayscale values positively correlate ($R = 0.54$, $P < 0.0001$) with node grayscale values (Table 2.5, Figure 2.7A), suggesting that stalk node discoloration is positively correlated with crown rot in sweet corn. There were significant correlations among most of the explanatory variables which suggests that root rot and crown rot are not always independent. However, when data are analyzed by site using crown grayscale as the response variable, there is no relationship between nodal root rot and crown symptoms for seven of nine field sites (Figure 2.7E, Table 2.6). This also appears to be true for primary root (radicle) rot and crown grayscale relationships (Figure 2.7C, Table 2.6), where there is no correlation for nine of 10 sites. Sub-crown internode rot is the most closely-correlated rot variable with crown mean grayscale ($R = -0.47$, $P < 0.0001$) (Figure 2.7B). The correlations were strong between sub-crown internode and primary root (radicle) rot ($R = 0.71$, $P < 0.0001$), sub-crown internode rot with nodal root rot ($R = 0.48$, $P < 0.0001$), and primary root (radicle) rot with nodal root rot ($R = 0.66$, $P < 0.0001$) (Table 2.5). The correlation between rootworm damage and crown mean grayscale ($P = 0.014$) is more difficult to interpret because there was little variation in rootworm damage within sites (Figure 2.7D, Table 2.6).

Table 2.6. Relationships between sweet corn crown grayscale and other explanatory variables when a separate slope and intercept is estimated for each field site

		PR[α]		SCI[α]		NRR[α]		RW[α]		N1[α]	
Site	Variety	Slope	P	Slope	P	Slope	P	Slope	P	Slope	P
1	Jubilee	-0.01	0.72	0.03	0.42	0.02	0.46	.	.	-0.06	0.89
2	Jubilee	<0.01	0.83	-0.02	0.10	<0.01	0.72	.	.	0.83	<0.01
3	Prelude	-0.04	<0.01	-0.05	<0.01	-0.04	<0.01	.	.	0.36	0.01
4	GH2298	-0.02	0.18	-0.06	<0.01	<0.01	0.90	-0.01	0.66	-0.15	0.32
5	GH2298	0.01	0.76	0.05	0.01	0.02	0.04	-0.02	0.26	-0.40	0.07
6	Coho	0.01	0.79	-0.02	0.50	-0.01	0.47	<0.01	0.96	0.64	0.02
7	SR1292	<0.01	0.91	<0.01	0.93	.[β]	.[β]	-0.05	0.16	0.23	0.59
8	Coho	<0.01	0.95	-0.02	0.47	<0.01	0.75	-0.01	0.58	0.92	<0.01
9	WSS3681	-0.03	0.29	-0.03	0.35	-0.01	0.42	-0.01	0.67	0.24	0.47
10	WSS3681	0.02	0.41	0.05	0.06	<0.01	0.93	<0.01	0.98	0.40	0.14

[α]PR = primary (radicle) root rot, SCI = sub-crown internode rot, NRR = nodal root rot, RW = rootworm damage, and N1 = mean grayscale of the internal lower stalk node tissue.

[β]There was insufficient variability in nodal root rot at site 7 to estimate slope and the P-value. There are 98 transects means for each variable except RW, which has 70, and each transect mean consists of 10 consecutive sweet corn plants in one row. Ten transects were sampled at each of 10 sites.

Table 2.5. Pearson correlation coefficients and associated P-values of explanatory variables used for model testing to determine the association between specific disease symptoms and ear yield of sweet corn plants in the Willamette Valley

	SCI[α]	NRR[α]	RW[α]	Crown[α]	N1[α]
PR[α]	0.71 <0.0001	0.66 <0.0001	0.24 0.04	0.21 0.37	0.10 0.32
SCI[α]		0.48 <0.0001	0.29 0.016	0.47 <0.0001	0.20 0.045
NRR[α]			0.12 0.34	0.34 0.0007	0.22 0.0338
RW[α]				0.29 0.01	0.089 0.46
Crown[α]					0.54 <0.0001

[α]PR = primary root (radicle) rot, SCI = sub-crown internode rot, NRR = nodal root rot, RW = rootworm damage, Crown = mean grayscale of the internal crown tissue, N1 = mean grayscale of the internal lower stalk node tissue. Crown and node grayscale values are inversed to make increased disease always positive.
There are 98 transects means for each variable except RW, which has 70, and each transect mean consists of 10 consecutive sweet corn plants in one row. 10 transects were sampled at each of 10 sites.

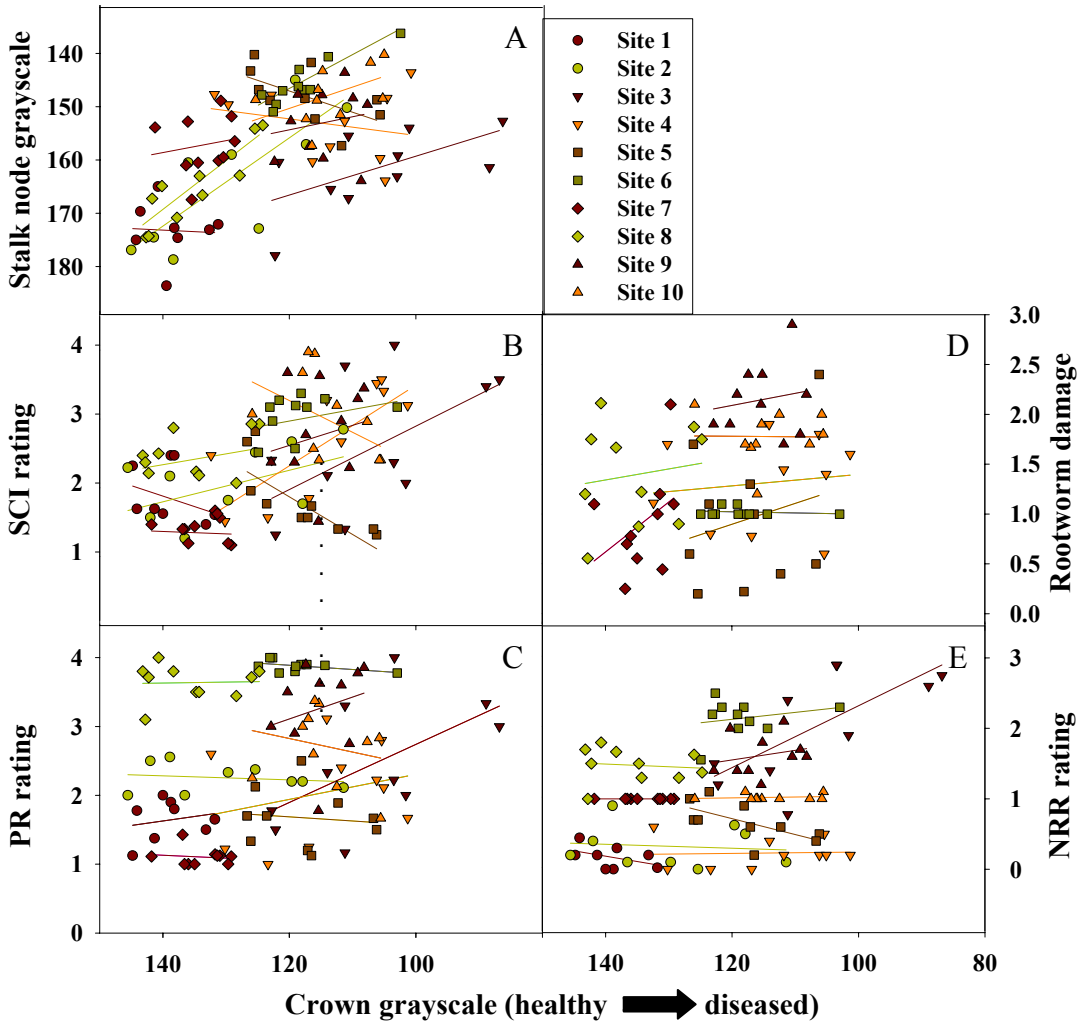

Figure 2.7. Relationships between crown mean grayscale and other explanatory variables by sweet corn fields. Grayscale means of the crown (x-axis) versus the node mean grayscale (A), sub-crown internode rot (SCI) (B), primary root (radicle) rot (PR) (C), rootworm damage (D), and nodal root rot (NRR) (E). Each point represents the mean of ten plants from the same transect. Sites 1-5 were sampled in 2003 and 6-10 were sampled in 2004.

When the Bayesian Information Criterion model selection analysis was repeated with only the fields where rootworm feeding was recorded, the best model (model 1B) to explain ear weight included field, crown mean grayscale (Slope = 2.3, $P < 0.0001$), nodal root rot (Slope = 23.5, $P < 0.06$), and rootworm damage (Slope = -13.2, $P < 0.03$) (Table 2.7). The second best model (model 2B) included only crown mean grayscale (Slope = 2.3, $P < 0.0001$) and rootworm damage (Slope = -12.9, $P < 0.04$) as explanatory variables. Crown grayscale is present in the top 34 models and always has significant slope values ($P \leq 0.05$ for slope coefficients), again suggesting that crown grayscale has the most predictive value of ear weight at harvest. Rootworm damage appears to be the next most predictive variable, and is present in 13 of the top 14 models with significant slope values. The addition of disease variables other than crown grayscale add little, if any explanatory value to the models predicting ear weight and do not have significant slope values in the best-fitting models (top 34 models).

Table 2.7. Bayesian Information Criterion selection of best-fitting models to explain variation in mean ear weight per transect in 7 commercial sweet corn fields in Oregon's Willamette Valley (10 plants/transect, 10 transects/field)

M# [α]	BIC [β]	Variables in model [γ]	Crown	NRR	PR	SCI	N1	RW
1B	648.3	Site Crown NRR RW	2.3**	23.5	-	-	-	-13.2*
2B	649.8	Site Crown RW	2.3**	-	-	-	-	-12.9*
3B	649.9	Site Crown NRR SCI RW	2.2**	27	-	-3.0	-	-13.5*
4B	650.1	Site Crown NRR PR RW	2.3**	20.7	2.6	-	-	-12.8*
5B	650.2	Site Crown NRR N1 RW	2.3**	23.3	-	-	-0.1	-13.5*
6B	650.2	Site Crown PR RW	2.4**	-	7.4	-	-	-12.0*
7B	651.0	Site Crown NRR	2.4**	22.8	-	-	-	-
8B	651.4	Site Crown NRR SCI PR RW	2.3**	23.7	5.5	-5.3	-	-13.1*
9B	651.6	Site Crown SCI RW	2.3**	-	-	2.1	-	-12.7*
10B	651.6	Site Crown N1 RW	2.3**	-	-	-	-0.2	-13.4*
11B	651.6	Site Crown PR N1 RW	2.4**	-	8.4	-	-0.3	-12.9*
12B	651.9	Site Crown NRR PR N1 RW	2.3**	19.3	3.5	-	-0.2	-13.3*
13B	651.9	Site Crown NRR SCI N1 RW	2.2**	26.7	-	-2.9	0.1	-13.8*
14B	651.9	Site Crown PR SCI RW	2.3**	-	9.2	-2.8	-	-12.0*
15B	652.0	Site Crown PR	2.5**	-	8.7	-	-	-
16B	652.1	Site Crown	2.4**	-	-	-	-	-
17B	652.5	Site Crown NRR PR	2.4**	17.8	4.6	-	-	-
18B	652.9	Site Crown NRR SCI	2.4**	24.6	-	1.6	-	-
19B	652.9	Site Crown NRR N1	2.4**	23	-	-	0.1	-
20B	653.2	Site Crown NRR SCI PR N1 RW	2.3**	22.4	6.3	5.7	-0.2	-13.5*

[α]Number of each mixed regression model with mean ear weight as the response variable.
[β]Lower Bayesian Information Criterion (BIC) statistics indicate better fitting models.
[γ]Estimated slopes for the effect of each variable in the model as explanatory variables; Crown = crown mean grayscale, NRR = rating of nodal root rot, PR = primary root (radicle) rot, SCI = rating of sub-crown internode rot, N1 = mean grayscale of the first stalk node aboveground, and RW = rootworm damage rating.
* indicates statistically significant at $P \leq 0.05$ and ** at $P \leq 0.01$ for each estimated slope.

Table 2.7 (continued). Bayesian Information Criterion selection of best-fitting models to explain variation in mean ear weight per transect in 7 commercial sweet corn fields in Oregon's Willamette Valley (10 plants/transect, 10 transects/field)

M# [α]	BIC [β]	Variables in model [γ]	Crown	NRR	PR	SCI	N1	RW
21B	653.3	Site Crown SCI N1 RW	2.4**	-	-	2.4	-0.2	-13.3*
22B	653.4	Site Crown SCI PR N1 RW	2.4**	-	10.3	-2.8	-0.4	-13.0
23B	653.8	Site Crown SCI	2.5**	-	-	3.1	-	-
24B	653.8	Site Crown PR SCI	2.5**	-	10.4	-2.5	-	-
25B	653.9	Site Crown PR N1	2.5**	-	9.1	-	-0.1	-
26B	654.0	Site Crown NRR SCI PR	2.4**	20.3	7.2	-4.6	-	-
27B	654.1	Site Crown N1	2.4**	17.8	-	-	0.1	-
28B	654.4	Site Crown NRR PR N1	2.4**	25.4	4.6	-	0.01	-
29B	654.7	Site Crown NRR SCI N1	2.3**	-	-	-2.0	0.2	-
30B	655.7	Site Crown SCI PR N1	2.5**	-	10.9	-2.5	-0.1	-
31B	655.7	Site Crown SCI N1	2.5**	-	-	3	0.01	-
32B	655.9	Site Crown NRR SCI PR N1	2.4**	20.5	7.1	-4.7	0.03	-
33B	657.4	Site Site●Crown	0.9 to 4.2*[5]	-	-	-	-	-
34B	672.5	Crown RW	2.3**	-	-	-	-	-12.3*
35B	674.1	Site NRR SCI RW	-	39.4*	-	-10	-	-19.5**
36B	674.1	Crown	2.4**	-	-	-	-	-
37B	674.3	Site NRR RW	-	28.1	-	-	-	-18.7*
38B	675.0	Site Site●RW	-	-	-	-	-	-45.9 to 210.8*[1]
39B	675.4	Site NRR SCI N1 RW	-	41*	-	-10.7	0.4	-18.1*
40B	675.7	Site RW	-	-	-	-	-	-18.5*
41B	675.8	Site NRR SCI PR RW	-	37*	4.0	-11.7	-	-19.2**

[α]Number of each mixed regression model with mean ear weight as the response variable.
[β]Lower Bayesian Information Criterion (BIC) statistics indicate better fitting models.
[γ]Estimated slopes for the effect of each variable in the model as explanatory variables; Crown = crown mean grayscale, NRR = rating of nodal root rot, PR = primary root (radicle) rot, SCI = rating of sub-crown internode rot, N1 = mean grayscale of the first stalk node aboveground, and RW = rootworm damage rating.
* indicates statistically significant at $P \leq 0.05$ and ** at $P \leq 0.01$ for each estimated slope. Numbers in superscript represent the number of fields which had significant effects when interactions (indicated by ●) were included.

Table 2.7 (continued). Bayesian Information Criterion selection of best-fitting models to explain variation in mean ear weight per transect in 7 commercial sweet corn fields in Oregon's Willamette Valley (10 plants/transect, 10 transects/field)

M# [α]	BIC [β]	Variables in model [γ]	Crown	NRR	PR	SCI	N1	RW
42B	676.0	Site NRR N1 RW	-	28.6	-	-	0.3	-17.7*
43B	676.1	Site NRR PR RW	-	30.9	-2.7	-	-	-19.0*
44B	677.3	Site PR RW	-	-	4.7	-	-	-18.0*
45B	677.3	Site NRR SCI PR N1 RW	-	39.4	2.4	-11.7	0.4	-18.0*
46B	677.5	Site SCI RW	-	-	-	-2.8	-	-18.7*
47B	677.5	Site N1 RW	-	-	-	-	0.2	-17.6*
48B	677.7	Site NRR PR N1 RW	-	33	-4.3	-	0.4	-17.9*
49B	678.2	Site PR SCI RW	-	-	10	-8	-	-18.0*
50B	678.8	Site NRR	-	27.4	-	-	-	-
51B	679.2	Site PR N1 RW	-	-	3.9	-	0.2	-17.5*
52B	679.2	Site SCI N1 RW	-	-	-	-3.0	0.3	-17.8*
53B	679.2	Site Site●SCI	-	-	-	-58.9 to 39.5*3	-	-
54B	679.3	Site NRR SCI	-	37.1*	-	-8.6	-	-
55B	679.3	Site NRR SCI N1	-	40.3*	-	-10.2	0.8	-
56B	679.4	Site NRR N1	-	28.5	-	-	0.7	-
57B	679.7	Site	-	-	-	-	-	-
58B	680.1	Site PR SCI N1 RW	-	-	9.5	-7.9	0.2	-17.5*
59B	680.4	Site Site●NRR	-	-4.6 to 97*1	-	-	-	-
60B	680.7	Site N1	-	-	-	-	0.6	-
61B	680.7	Site NRR PR	-	27.4	-0.03	-	-	-
62B	680.7	Site NRR SCI PR	-	33.2	6.6	-11.3	-	-

[α]Number of each mixed regression model with mean ear weight as the response variable.
[β]Lower Bayesian Information Criterion (BIC) statistics indicate better fitting models.
[γ]Estimated slopes for the effect of each variable in the model as explanatory variables; Crown = crown mean grayscale, NRR = rating of nodal root rot, PR = primary root (radicle) rot, SCI = rating of sub-crown internode rot, N1 = mean grayscale of the first stalk node aboveground, and RW = rootworm damage rating.
* indicates statistically significant at $P \leq 0.05$ and ** at $P \leq 0.01$ for each estimated slope. Numbers in superscript represent the number of fields which had significant effects when interactions (indicated by ●) were included.

Table 2.7 (Continued). Bayesian Information Criterion selection of best-fitting models to explain variation in mean ear weight per transect in 7 commercial sweet corn fields in Oregon's Willamette Valley (10 plants/transect, 10 transects/field)

M# [α]	BIC [β]	Variables in model [γ]	Crown	NRR	PR	SCI	N1	RW
63B	681.0	Site PR	-	-	6.2	-	-	-
64B	681.2	Site NRR SCI PR N1	-	38.1	3.4	-11.5	0.7	-
65B	681.2	Site NRR PR N1	-	32.1	-3.3	-	0.7	-
66B	681.3	Site Site●N1	-	-	-	-	-1.6 to 5.8*[1]	-
67B	681.6	Site SCI	-	-	-	-1.8	-	-
68B	682.0	Site PR SCI	-	-	11.8	-8.1	-	-
69B	682.3	Site PR N1	-	-	4.6	-	0.5	-
70B	682.5	Site SCI N1	-	-	-	-2.6	0.6	-
71B	683.3	Site PR SCI N1	-	-	10.2	-7.8	0.5	-
72B	687.7	Site Site●PR	-	-	-22.4 to 77.4*[1]	-	-	-
73B	695.9	RW	-	-	-	-	-	-18.0*
74B	699.2	Null	-	-	-	-	-	-
75B	700.1	N1	-	-	-	-	0.6	-
76B	700.5	NRR	-	12.9	-	-	-	-
77B	700.7	SCI	-	-	-	-4.4	-	-
78B	701.1	PR	-	-	.48	-	-	-

[α]Number of each mixed regression model with mean ear weight as the response variable.
[β]Lower Bayesian Information Criterion (BIC) statistics indicate better fitting models.
[γ]Estimated slopes for the effect of each variable in the model as explanatory variables; Crown = crown mean grayscale, NRR = rating of nodal root rot, PR = primary root (radicle) rot, SCI = rating of sub-crown internode rot, N1 = mean grayscale of the first stalk node aboveground, and RW = rootworm damage rating.
* indicates statistically significant at $P \leq 0.05$ and ** at $P \leq 0.01$ for each estimated slope. Numbers in superscript represent the number of fields which had significant effects when interactions (indicated by ●) were included.

Xylem conductance experiments

Since diseased stalk nodes may affect fluid conductance through xylem, conductance tests were done to measure fluid movement through stalks and then stalk nodes were examined for necrosis. Bayesian Information Criterion was used to determine if there is a relationship between node grayscale and fluid movement through the stalk and if so, whether the relationship is constant across field sites. The best-fitting model based on Bayesian Information Criterion selection (Table 2.8) is the parallel lines model (Figure 2.8), a model that estimates the same node grayscale slope effect for all field sites. The parallel lines model slope for node mean grayscale vs. flow is a constant value of 0.00093 ml/cm^2/min/grayscale unit, and allows each field site to have its own intercept. This suggests that the relationship between node mean grayscale and flow rate is similar across sites, even though the overall flow rate varies among sites. The flow rate ranged from 0.001 to 0.1882 ml/cm^2/min. The mean node grayscale ranged from 135 to 204 on a 0 to 255 scale. The effect of the mean grayscale (0.00093 ml/cm^2/min) reduced the predicted values from the model of the flow rate by 26 to 65% (Table 2.9).

Table 2.8. Bayesian Information Criterion (BIC) model selection for flow rates through a 30-cm lower stalk section of 18 to 26 sweet corn plants from each of five commercial field sites

BIC[α]	Variables in model	Slope for node grayscale effect
-472.6	Node[β] Site	0.00093**
-469.2	Node*Site	0.0012 to 0.00023**[6]
-466.0	Node Site Mean*Site	0.0022 to 0.00023**[2]
-464.9	Site	-
-394.3	Node	0.0013**
-383.7		Null

[α]More negative Bayesian Information Criterion statistics indicate better fitting models.
[β]Mean grayscale of both halves of the lowest stalk node without brace roots.
** indicates statistically significant at $P \leq 0.05$ or 0.01 for each estimated slope.
Numbers in superscript represent the number of sites which had significant node effects when interactions were included.

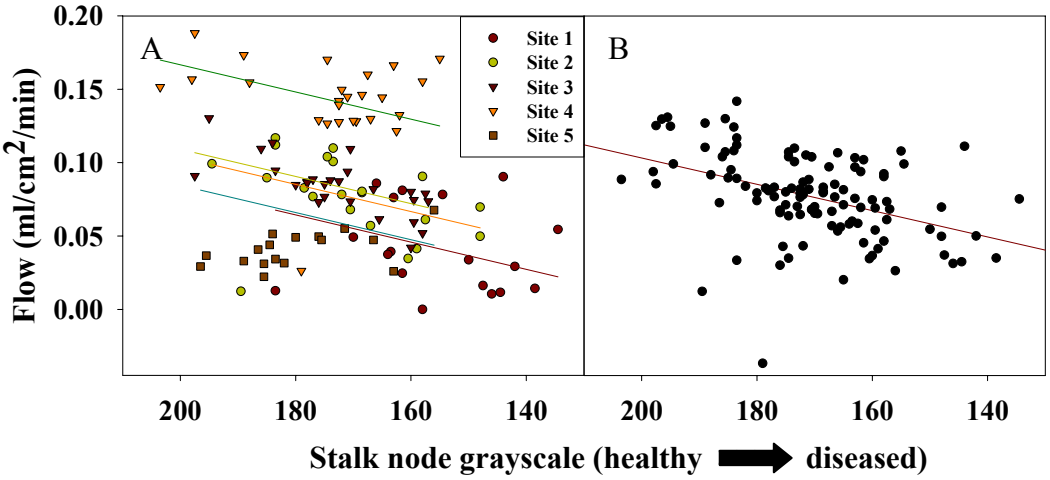

Figure 2.8. Best-fitting model based on Bayesian Information Criterion model selection. Grayscale means of the lowest stalk node used in conductance tests versus the flow rate of solution through the 30-cm lower stalk section of sweet corn plants (18 to 26) from each of five commercial field sites, by field sites (A) (slope = 0.00093, $P < 0.0001$). Regression model includes field site and mean grayscale of the lowest stalk node as explanatory variables (without interactions) for rate of solution flow through the stalk. The same model was adjusted so all fields fit a common intercept (B) to better show the relationship between stalk node grayscale and flow rates.

Table 2.9. Percent reduction of fluid movement through xylem of a 30-cm lower stalk section of 18 to 26 sweet corn plants from each of five commercial field sites based on predicted values generated with a parallel lines regression model with site and node mean grayscale as explanatory variables

Site	Variety	Intercept	Percent reduction[α]
Site 4	SS Jubilee	-0.021	26.8
Site 3	ACX642A	-0.079	35.6
Site 2	SS White	-0.084	44.3
Site 1	GH2298	-0.102	65.3
Site 5	Basin EX 84	-0.104	46.8

[α]Percent reduction in predicted flow rates based on a slope of 0.00093 ml reduction per grayscale unit.

Discussion

Decline of sweet corn yields in the Willamette Valley are probably a result of disease symptoms in addition to, or other than, root rot. The results of the studies reported here indicate that there is a decrease in ear weight associated with increasing crown rot, and that crown rot levels explain more ear weight variation than any other disease variable measured, based on the best-fitting mixed regression models. Other variables measured, such as rot of the radicle, sub-crown internode, or nodal root system, were poor predictors of ear weight at harvest. Nodal root rot, which had the most predictive value of those variables, was positively associated with ear weight in most models; higher ear weights associate with increasingly severe root disease. A relationship was also found between stalk node rot and increased resistance to fluid movement through stalks. This is probably happening in the crown as well, since the crown is essentially a stack of nodes with shortened internodes. Sap flow rates could be reduced by a variety of factors that may affect xylem vessels. The experimental

technique used in this paper may introduce particulates or embolisms into the xylem, or excessive physiological stress which may cause plant responses to block vessels. For these reasons, flow rates for some plants may be artificially low, and there are perhaps two plants with data points that are suspect, but the relationship between sap flow impediment and crown/node rot still appears strong.

Increased resistance to fluid movement through a vessel will require an increase in the pressure gradient difference moving the fluid if the flow rate is to remain constant. This means that when resistance to flow is increased in a column of fluid under tension, one of two things must happen; either the flow rate will decrease or the pressure above the resistance point must become more negative. Either scenario will ultimately decrease the rate of transpiration because as the pressure in the xylem becomes more negative, cavitation events become more likely, which limit the amount of functional xylem. To prevent excessive xylem cavitation, plants may close stomata, which can reduce their overall photosynthetic ability (4), in addition to reducing transpiration rates.

There may be a number of causes of apparent rot of crown and node tissues. Chapter 3 of this dissertation investigates several *Fusarium* species as possible causal agents. Since nodes are acting as filters for xylem sap and darker nodes are associated with increased resistance to xylem conductance, then it is appropriate to investigate organisms that may inhabit the xylem. Since the filters do not allow the passage of particles that are larger than 20 nm, most xylem-inhabiting or invading organisms, being orders of magnitude larger, would be trapped at the nodes. Excessive

accumulation of microorganisms could be partially responsible for the resistance to xylem conductance, which can then cause water stress. Conversely, there are many examples where water stress can predispose plants to infection and disease (2,21,22) and the negative effects of both water stress and xylem infection can be additive (16).

Root-rotting pathogens and root-feeding insects may provide entry into the xylem for microorganisms. Rootworm feeding and root rot variables were positively correlated with darker crowns. Rootworm feeding is also known to reduce transpiration in corn (19). Excessive growth of xylem-inhabiting/invading organisms would likely cause wilting to occur, which was not usually seen. However, low levels of growth of these organisms could block sap flow enough to cause drought stress. Sucrose concentration in the xylem increases when corn is drought stressed (7), which could further enhance microbial growth in xylem sap, which is normally a dilute growth medium.

Similar node symptoms have been associated with accumulation of iron and aluminum compounds (1,9-11). Hoffer (9) reported that nodal discoloration was seen before the development of any root lesions, and root rot developed more severely in places where nodal discoloration was more prevalent. Hoffer concluded that the cause of the symptoms was an iron and aluminum toxicity, but reported finding a number of microorganisms present in the symptomatic tissues. Similar nodal symptoms were produced with the injection of ferrous salts but not aluminum (1). This same study reported that, "stalk and root-rot organisms were usually associated with the accumulation of iron and aluminum." A survey of node tissue microorganisms (18)

found a similar relationship between iron accumulation and the presence of microbes. Both were more likely to be found in lower nodes. However, a study by Salter and Ames reported that little or no correlation between iron compounds and node color could be seen (20). None of these aforementioned studies on node discoloration included pathogenicity tests with any microorganisms found in the nodes. The presence of some siderophore-producing microbes inhibit the germination of chlamydospores and root colonization of *Fusarium* spp. (5,29). The loss of those competing microbes could allow more available iron to *Fusarium* spp. which colonize and enter plants.

The studies in this chapter indicate that plants with darker, more necrotic crown tissues have lower ear weights while plants with darker stalk nodes have increased resistance to fluid movement through the stalk. The crown region is a series of nodes with shortened internodes so it is not unexpected that both symptoms are related. The grayscale values of the crown were positively correlated with the stalk node grayscale values. Although most explanatory variables were correlated, crown and node grayscale values had stronger correlations with each other than with any root or sub-crown internode rot variables, and the relationship between crown and node grayscale values appeared more consistent across field sites. There was also a strong positive correlation between darker crown grayscale and sub-crown internode rot. It is possible that plant pathogens which gain entry into the sub-crown internode will be able to easily move into the crown since both structures are so in close proximity. Crown grayscale appears to be the best predictor variable for ear weight. Root rot variables

may be good predictors of yield on a coarse landscape level, but they appear to do little to explain variation within a site. There were significant differences among sites for both the crown effects on ear weight and the node effects on xylem conductance. Differences among sites could be due to many factors, such as sweet corn variety, soil properties, grower practices, or environmental parameters.

The nodes in corn stalks are the point where vascular bundles branch out to leaves. Our observations indicate that the lowest nodes are typically darker than nodes further up the stalk; however, Ocamb (unpublished) has observed dark nodes at the point of ear attachment as well. Firing symptoms are reported to occur in the lower leaves first and can be associated with nodal discoloration (10). Even if sufficient water is moving up the stalk there may be significant blockage at the connections where the vessels branch out to the leaves. Further investigations are needed to measure transport from the stalk region to the leaves through these darker nodes.

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Chapter 3

The Relationship between *Fusarium* Species, Crown and Node Necrosis, and Yield Decline in Sweet Corn in the Willamette Valley of Oregon

Nathan L. Miller and C. M. Ocamb

Abstract

Fusarium oxysporum, *F. verticillioides*, and *F. proliferatum* were examined for their association with and ability to incite crown and node necrosis of sweet corn. Necrosis was quantified using digitally-scanned images of cut stalks; grayscale values of the affected regions were calculated. When either *F. oxysporum* or *F. verticillioides* were isolated from crowns of commercial sweet corn plants, these crowns had significantly darker grayscale values than those from which neither species was isolated; ear weights were also lower when *F. oxysporum* was isolated from the crown or first stalk node. Kernel inoculations with each of the three *Fusarium* species, as well as a mixture of all three species, resulted in plants with darker crowns in two field trials and one greenhouse trial. Ear weights were reduced but less consistently so; each *Fusarium* treatment reduced ear weight in at least one of the four trials. In greenhouse pathogenicity studies, all three species were recovered at frequencies greater than 70% from the crowns of plants that developed from inoculated kernels. In field pathogenicity studies, *Fusarium oxysporum* and *F. verticillioides* were recovered from crowns at 67-70% and 52-56%, respectively. These studies indicate that *Fusarium* species are capable of causing crown disease and reducing yields in the Willamette Valley.

Introduction

Commercial sweet corn (*Zea mays* L.) yields in the Willamette Valley of Oregon exhibited a steady decline during the 1990s. Initially, this decline in yield was associated with leaf "firing", where leaves prematurely die, starting at the base of the plant, and then progressing up the plant. Root rot has been implicated as the primary cause of the yield decline (14). However, necrosis of the crown and stalk nodes is also found in many fields (Figure 3.1), including those where plants lacked even moderate root rot, and the crown grayscale was a better predictor of ear weight than root rot in the studies reported in chapter 2 of this dissertation.

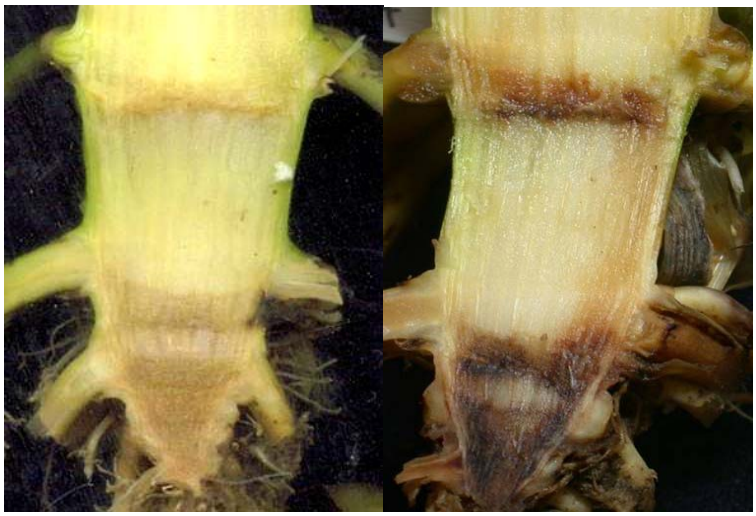

Figure 3.1. Longitudinal cross section of sweet corn plants showing a plant with healthy (left) and diseased (right) crown and stalk nodes.

The crown region of maize plants is a stack of approximately eight nodes with shortened internodes (16). End walls of the xylem vessels are concentrated in the nodes (39), which results in nodes acting as barriers to movement of particulates or gas embolisms through the vessels. These node regions do not allow the passage of

particles that are 20 nm or greater in size. Most xylem-inhabiting or invading organisms, being orders of magnitude larger, would be stopped at the nodes.

Plants were evaluated from commercial fields to determine if crown and stalk node darkening, and the accompanying yield decline, were associated with any of the three *Fusarium* species commonly found in corn roots, stalks, and crowns. Pathogenicity studies were also conducted to determine if these *Fusarium* species could incite crown rot or stalk node rot, and a subsequent ear yield decline. Three species of *Fusarium* were chosen for the pathogenicity experiments because of their prevalence on diseased corn roots, crown, and stalk nodes in the Willamette Valley (Ocamb, unpublished): *Fusarium oxysporum* var. *redolens* (Wollenw.) Gordon, *F. verticillioides* Sacc. (Nirenberg) (synonym *F. moniliforme* Sheld.), and *F. proliferatum* (T. Matsushima) Nirenberg. *Fusarium oxysporum* was among the most prevalent *Fusarium* species found on diseased corn roots, crown, and stalk nodes in the Willamette Valley (Ocamb, unpublished) and it is known to cause root rot and internode stalk rot (17,20,23,43). Strains of *F. oxysporum* have been reported to be prevalent in the rhizosphere of maize (15,34,42). This fungus also survives well in soil or in plant debris or other organic matter, and produces durable, long-living chlamydospores (42). *Fusarium proliferatum* was prevalent on maize seed kernels used in these studies and can also incite ear rot (36) and root rot (43). *Fusarium verticillioides* is commonly found in many corn tissues and on kernels, and it is known to cause disease on roots, seedlings, stalks, and ears (6,18,43). These three *Fusarium* species can survive long periods of time in maize residue in the soil (8,32), for up to

two years during fallow (8). They can also colonize the rhizosphere of many plant species; so once introduced they can become a perpetual component of the soil microflora.

Fusarium verticillioides and *F. proliferatum* were formerly considered the same species (*F. moniliforme* Sheldon), and as many as six different biological species (38) and possibly many more (33) have been referred to as *F. moniliforme*. Nirenberg (31) proposed *F. verticillioides* as a distinct species in 1976, and Nelson et. al. in 1983 (30) distinguished *F. proliferatum* and *F. subglutinans* from *F. moniliforme* (*F. verticillioides*). Reports after 1983 that make reference to *F. moniliforme* are probably dealing with *F. verticillioides*, and research prior to 1983 could be referring to any of these now separate species, including *F. proliferatum*. Studies have been conducted on the effects of kernel contamination by *F. moniliforme* that are relevant to this study but the exact species identification is unclear.

Under the pre-1983 classification system, hyphae of *F. moniliforme* have been shown to spread from infected seed kernels into mesocotyls, crowns, and roots (12,40). Incidence of kernel association is commonly 100% for *F. moniliforme*, but reports vary on the significance this fungus has on plants grown from contaminated kernels (18,28). *Fusarium moniliforme* has been shown to result in reduced yields (41) as well as reduced germination and seedling vigor (9,13,22) compared to plants that developed from kernels free of *F. moniliforme*. Other studies reported however, that there is little effect from kernel contamination on germination, seedling vigor, or yield (10,24,46). This fungus is sometimes said to be capable of asymptomatic infections (1,2), but it is

not clear if symptoms would develop in these infections given more time or conducted under different conditions.

It is important to understand the causes of yield losses when planning disease management strategies. Information is needed about the role that these three *Fusarium* species have in disease and ear yield of sweet corn in the Willamette Valley. Environmental factors and genetic diversity play a role in the development of disease, which may account for the differing results observed by various researchers among corn production zones across the US. The objectives of these studies were to: 1) evaluate plants from commercial fields for crown and stalk node rot, ear yield, and associated infections by *F. oxysporum*, *F. verticillioides*, and *F. proliferatum* in symptomatic crown and stalk nodes; and 2) determine if these *Fusarium* species can incite crown rot or stalk node rot, and a subsequent decline in ear weights.

Materials and Methods

Fungal isolations from plants in commercial fields

Assays for the presence of *Fusarium* species in crown and stalk nodes of sweet corn plants were conducted with plant samples from eight commercial sweet corn fields in the Willamette Valley (Table 3.1) during 2003 and 2004. These fields were sampled within a few days of commercial harvest to control for developmental stage of the plants, and were chosen based on availability. A number of sweet corn varieties were sampled because of grower shifts away from the formerly predominant cv., 'Jubilee'.

Table 3.1. Varieties, field location, and sampling date of commercial sweet corn plants in the Willamette Valley examined for disease and *Fusarium* populations

Field	Variety	County	Date sampled
1	Prelude	Benton	September 8, 2003
2	GH2298	Benton	September 22, 2003
3	GH2298	Benton	October 6, 2003
4	Coho	Benton	August 18, 2004
5	SR1292	Linn	August 23, 2004
6	Coho	Benton	August 23, 2004
7	WSS3681	Lane	September 6, 2004
8	WSS3681	Benton	September 7, 2004

After surveying the general size and shape of each field, the field was divided by visual assessment into ten sections similar in size. One transect was sampled in each of the ten sections. To avoid edge effects, samples were not collected within 10 rows from any field edge. The number of foot steps taken to reach the first plant in each transect was determined by the use of random numbers between 20 and 100. In each transect, 10 consecutive plants within one row were sampled. Plants were cut above the ears, dug, and returned to the lab. Stalks and crowns were split longitudinally, parallel to the leaf ranks, and the cut surface was digitally scanned on a flatbed scanner (HP scanjet 2400, Hewlett Packard, Palo Alto, CA). Multiple layers of black fabric were used to cover the stalks while scanning to minimize extraneous light. The resulting images were then analyzed using the digital image analysis program, ImageJ version 1.34s (US Department of Health and Human Services, National Institutes of Health). A histogram for each plant was generated for the crown region and the first aboveground stalk node which lacked rooted brace roots. The histogram

calculated the coloration of each pixel within the region of the crown or stalk node on a 256-bit grayscale.

Plants (30 to 40) from the set of 100 plants collected from each field were selected randomly by a random-number table for determining the presence of *Fusarium* species in crown and stalk node tissues. A portion (< 5 mm x 2 mm x 2 mm) of crown or node tissue was dissected under sterile conditions, dipped in 0.5 % NaClO solution, and then rinsed with sterile reverse osmosis (RO) water. The tissue piece was then embedded in Nash-Snyder medium (27) amended with 120 µg/mL chlortetracycline HCl (19). Putative *Fusarium* colonies were transferred to carnation leaf agar (CLA) (11) and potato dextrose agar amended with streptomycin sulfate (1g/L) (SPDA) for microscopic classification.

Pathogenicity experiments

Assays with specific strains of *Fusarium* species were done to evaluate the ability of these *Fusarium* species to cause crown and stalk node necrosis in sweet corn plants. Three *F. oxysporum* var. *redolens* isolates from diseased sweet corn, and two cultures each of *F. verticillioides* and *F. proliferatum* isolated from sweet corn seed kernels, were evaluated for their ability to cause crown and stalk node rot of sweet corn (Table 3.2). *Fusarium oxysporum* cultures were grown from single spores (30) on CLA and stored on silica gel at 5 C (44,45). *Fusarium verticillioides* and *F. proliferatum* cultures were grown from single spores, transferred to dilute water agar (3g/liter), increased at 24 C on a platform shaker (New Brunswick Scientific, Edison, NJ) at 200 RPM for one month, and then stored at 5 C. Inoculum was increased in 60-ml aliquots

of potato dextrose broth (Difco, Sparks, MD) on a platform shaker at 175 RPM for seven days at 24 C. Broth cultures were rinsed by twice centrifuging, pouring off the supernatant and resuspending the pellet in 50 ml of sterile RO water. Cultures were then homogenized for 20 seconds with a tissue tearor (985-370 Biospec, Bartlesville, OK). Isolates of the same species were then bulked together. Equal amounts of these suspensions were then combined to make the mixed species inoculum. A hemocytometer was used to estimate the number of spores and hyphal fragments (Table 3.3).

Table 3.2. *Fusarium* isolates used for sweet corn pathogenicity studies

Isolate name	*Fusarium* species	Year collected	Part of sweet corn from which obtained
F 47	*F. oxysporum* var. *redolens*	1999	Root
F 44	*F. oxysporum* var. *redolens*	1999	Root
F 33	*F. oxysporum* var. *redolens*	1999	Root
F 230	*F. proliferatum*	2003	Kernel
F 231	*F. proliferatum*	2003	Kernel
F 232	*F. verticillioides*	2002	Kernel
F 233	*F. verticillioides*	2003	Kernel

Table 3.3. Concentrations of *Fusarium* inoculum for each inoculum treatment used for infesting sweet corn kernels in pathogenicity studies

	No. of conidia and hyphal fragments	
Fusarium species	2004 studies	2005 studies
F. oxysporum var. *redolens*	2×10^7	1×10^7
F. proliferatum	5×10^7	1×10^7
F. verticillioides	5×10^7	3×10^7
Mix of the *Fusarium* species listed above	4×10^7	2×10^7

Sweet corn kernels (cv. Jubilee) were disinfested for 3 hours in 3 % H_2O_2 and rinsed with running RO water for five minutes. Inoculum was added (30 ml of spore suspension) to 230 g of kernels and thoroughly mixed by shaking in a plastic bag for five minutes by hand. Other treatments included disinfested kernels with no additional microbial infestation, and nontreated kernels which are naturally infested with a variety of fungi including *F. verticillioides* and *F. proliferatum*.

Pathogenicity evaluations of *Fusarium* species in the field

Field assays with specific strains of *Fusarium* species were done to evaluate the ability of these *Fusarium* species to cause crown and stalk node necrosis in sweet corn plants. All field experiments were conducted at a site located at the Oregon State University Botany and Plant Pathology field lab (Corvallis, Oregon) which has been planted with sweet corn since 2001. The soil is a Chehalis series silty loam with 10.8% sand, 63.2 % silt, and 26% clay. In the fall of 2000, this field was infested with the lower portions of plants collected from a commercial symptomatic sweet corn field. Kernels were planted using a hand push-type belt planter with the foot set for a 2.4 cm planting depth and were sown on June 21, 2004 in 12-m rows with 60 kernels per row using a randomized complete block design. Six blocks were established in a portion of the field that had been inoculated with *Fusarium* species via infested oats, and nine blocks were set up in an oat-free part of the field. Oats were added as part of variety trials being conducted in the same field.

When plants were approaching processing maturity on September 21, 2004 (92 days post planting), 10 plants from each plot were sampled in each of four blocks from

both the oat-inoculum portion of the field and the oat-free area. Plants were cut above the ears, dug, and returned to the lab. Root balls were washed clean of soil and the percentage of nodal roots with rot was rated on the following scale: 1 to 25 % of the nodal root ball necrotic = 1, 26 to 50 % = 2, 51 to 75 % = 3 and 76 to 100 % = 4. The rot of the primary root (radicle) and sub-crown internode (mesocotyl) were rated separately and used the following scale: 0 to 25 % of the primary root (radicle) or sub-crown internode was rotted = 1, 26 to 50 % = 2, 51 to 75 % = 3, and 76 to 100 % = 4. Weights of individual, developed ears were also recorded after removing husks. Stalks and crowns were split longitudinally, parallel to the leaf ranks, and the cut surface was scanned as previously described.

In 2005, the field was treated with *Fusarium*-infested oats prior to sowing. Six blocks were planted on June 21, with 50 kernels per row in 9-m rows in the same part of the field that was inoculated with oats. Ears from ten plants from each plot in all six blocks were harvested at processing maturity (September 16, 87 days post planting), and plants were dug four days later. *Fusarium* isolations were done as previously described from crowns and stalk nodes of the first five plants sampled in each plot during both years of study.

Pathogenicity evaluations of *Fusarium* species in a greenhouse

Greenhouse assays with specific strains of *Fusarium* species were done to evaluate the ability of these *Fusarium* species to cause crown and stalk node necrosis in sweet corn plants under more controlled conditions. Two pathogenicity studies were conducted in a greenhouse using 5-gallon pots under two high pressure sodium, and

one metal halide, 1000 W lights (Ruud lighting, Racine, WI) on a 16-hour day length. A sandy loam soil with 63.0 % sand, 29.5.2 % silt, and 7.5% clay (Green & White Rock products, Corvallis, OR) was steam-pasteurized for one hour on each of two consecutive days. In each experiment, 10 'Jubilee' kernels of each kernel treatment used in field pathogenicity studies, excluding the nontreated kernels, were used. The first study (study A) was planted on June 22, 2004, and used seed saved from the field study planted on the previous day. The second study (study B) was planted June, 29 2004 with seed disinfested and treated that day with the same inoculum used for the field studies, which had been stored at 5 C for one week. In each study, pots were arranged in a complete random design in rows of 10 pots with a 30-cm alley between every other row. Six weeks after sowing, 6 g of fertilizer (12 % ammoniacal N, 29 % phosphate, 10 % soluble potash, 8% sulfur; Wilco, Mt. Angel, OR) was added to the surface of the soil. Plants were watered as needed. Study A was sampled on October, 14, 2004 (114 days post planting) and Study B was sampled November 1, 2004 (125 days post planting). Temperatures in the greenhouse averaged 20 to 25 C during the summer months, with an average difference between day and night temperatures of 10C.

Statistical analysis

Statistical analyses were done using SAS (SAS institute, version 9.1, Cary, NC). Plants sampled from commercial fields and found to have *Fusarium oxysporum* in the crown tissues (+) were compared to plants in which *F. oxysporum* was not isolated (-) from the crown. T-tests were used to compare ear weights and crown grayscale of the

two groups. The same comparisons were made for *F. verticillioides* (+/-), *F. proliferatum* (+/-), and any *Fusarium* (+/-) recovered from the crown tissues. Similar tests were conducted for recovery of the three *Fusarium* species from stalk nodes of plants from commercial fields and the associated node grayscale values.

Data from pathogenicity experiments were analyzed using a mixed procedure analysis of variance, and means were compared to the control with the Dunnett adjusted statistic. The means of each plot were used as the experimental unit when comparing treatment effects for field pathogenicity studies. Data from greenhouse pathogenicity studies were analyzed in a similar fashion except treatment effects were analyzed with individual plants as the experimental unit. Data were also analyzed using the same technique as the commercial field isolation data, where plants were grouped according to *Fusarium* species isolated. An additional analysis was done for the pathogenicity studies; plants grown from disinfested kernels and from which no *Fusarium* was isolated were compared to plants from which the inoculated fungal species was reisolated. For plants grown from mixed-*Fusarium* treated kernels, plants were included if any of the three *Fusarium* species were isolated.

Results

Fusarium isolations from plants in commercial fields

There were 159 *Fusarium* cultures isolated from 136 of the 305 randomly selected crowns sampled from commercial fields and crown grayscale values ranged from 69 to 168. Isolations from the lowest node without rooted brace roots yielded 137 *Fusarium* cultures from 120 of the 305 plants sampled and node grayscale values

ranged from 87 to 193. The most prevalent *Fusarium* species isolated from crowns and nodes were *F. oxysporum* and *F. verticillioides* (Table 3.4). *Fusarium oxysporum* was isolated from both the crown and first stalk node of the same plant 22 times and this occurred 13 times with *F. verticillioides*.

Table 3.4. Percentage of plants from which *Fusarium* cultures were recovered from 305 plants sampled in commercial sweet corn fields

	Percentage of plants from which each species were recovered			
Tissue sampled	*F. oxysporum*	*F. verticillioides*	Other species	Any *Fusarium* spp.
Crown	26	13	13	45
Stalk node	19	15	10	39

Crowns from which *F. oxysporum* was isolated were significantly darker (lower grayscale values, $P \leq 0.05$) (Figure 3.2A), indicative of more severe crown rot, and had significantly lower ear weights associated with these plants (Figure 3.2C) compared with plants from which no *F. oxysporum* nor any other *Fusarium* species was recovered from crown tissue. When *F. verticillioides* was isolated from crowns, these tissues were significantly darker compared to crowns from which the fungus was not isolated or compared to plants from which no *Fusarium* was isolated. Plants with crowns that yielded any species of *Fusarium* also had significantly darker grayscale values compared to plants from which no *Fusarium* was isolated from the crown. *Fusarium proliferatum* was recovered infrequently from crowns or stalk nodes and no differences in yield or grayscale were detected (data not shown).

When *F. oxysporum* was recovered from the lowest stalk node without rooted brace roots, ear weights were significantly lower ($P \leq 0.05$) but there was no difference in node grayscale values (Figure 3.2B, 3.2D) compared to plants from which this fungus was not recovered from stalk nodes. When *F. verticillioides* was recovered from the lowest node without rooted brace roots, nodes were significantly darker but ear weights were not reduced. Plants from which any *Fusarium* species was isolated from the stalk node had significantly darker nodes than plants from which no *Fusarium* was isolated.

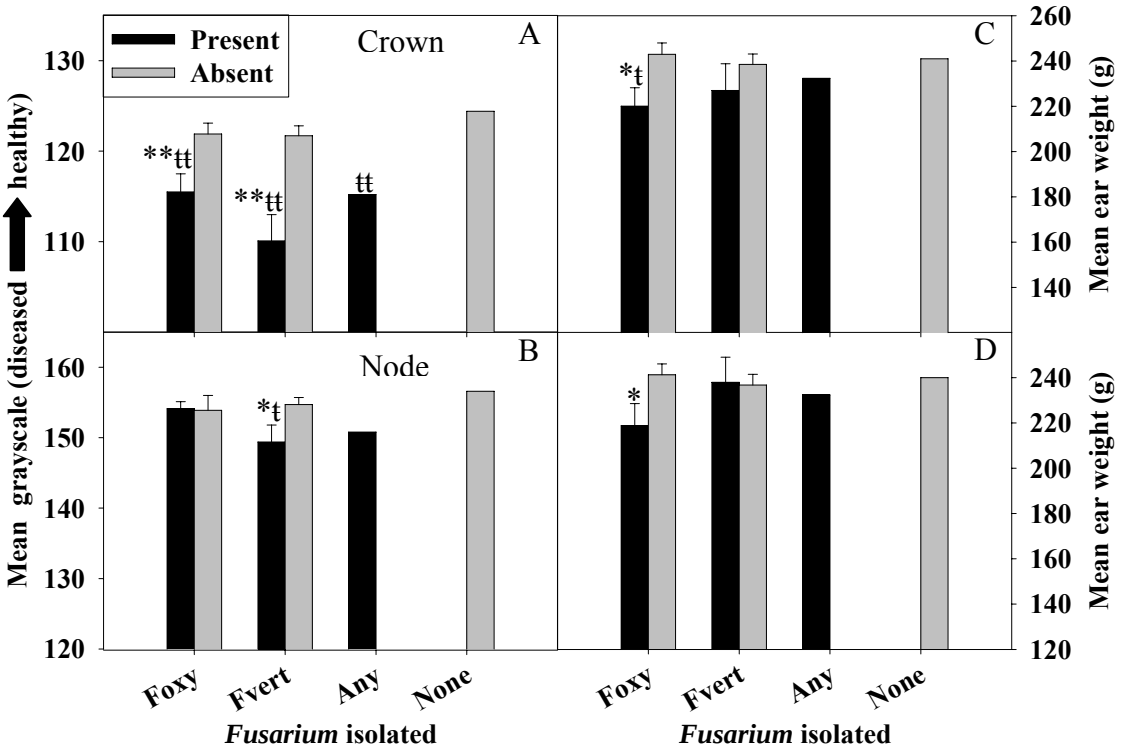

Figure 3.2. Mean crown grayscale (A), mean stalk node grayscale (B), and ear weights (C, D) of sweet corn plants sampled from commercial fields in the Willamette Valley when *Fusarium* species were detected or absent. Foxy = *Fusarium oxysporum*, present in 80 crowns and 58 nodes of 305 plants sampled, Fvert = *F. verticillioides*, present in 39 crowns and 46 nodes, Any = any *Fusarium* species, present in 136 crowns and 120 nodes, and none = no *Fusarium* species recovered. Within each bar graph, * indicates significant differences at P = 0.05 and ** at P = 0.01 between adjacent black and gray bars, and ŧ indicates significant differences at P = 0.05 and ŧŧ at P = 0.01 compared to the "no *Fusarium* recovered" group ("None", lone gray bar) based on two-sample t-tests. Error bars represent pooled error from a two-sample test comparing adjacent black and gray bars.

Pathogenicity evaluations of *Fusarium* species in the field

Field assays with specific strains of *Fusarium* species were done to evaluate the ability of these *Fusarium* species to cause crown and stalk node necrosis in sweet corn plants. In 2004, half of the plants were grown in a field portion inoculated with *Fusarium*-colonized oats while the other half was grown in an "oat-free" area. The oat-treatment did not have a significant effect on ear weight or severity of disease

symptoms when compared to plants grown in the oat-free portion of the field, so the data were combined for analyses of treatment effects. Plants grown from kernels treated with *F. oxysporum*, *F. verticillioides*, or the mixed-*Fusarium* treatments had significantly darker ($P < 0.05$) crown grayscale values, indicative of more severe crown rot, than plants grown from disinfested kernels (Figure 3.3A). In 2005, plants grown from kernels treated with *F. oxysporum* or the mixed-*Fusarium* treatments had significantly darker crown grayscale means than the plants grown from disinfested kernels. In 2005, plants were less healthy overall compared to 2004 indicated by lower ear weights, so data are presented separately for 2004 and 2005 field pathogenicity studies.

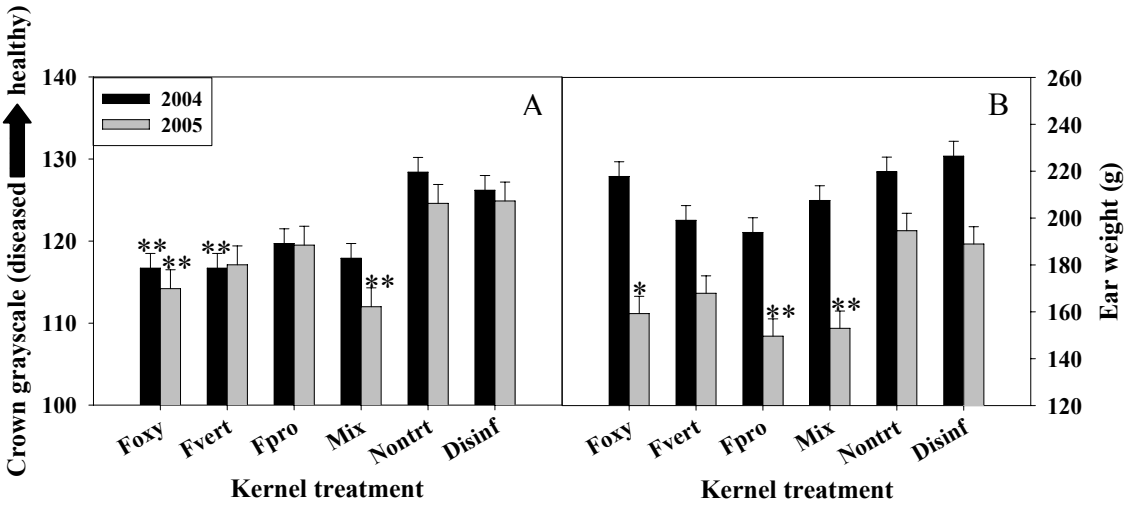

Figure 3.3. Mean crown grayscale (A), and ear weight (B) of sweet corn plants grown from various kernel treatments in 2004 and 2005 field pathogenicity studies. Kernels were infested with *F. oxysporum* var. *redolens* (Foxy), *F. verticillioides* (Fvert), *F. proliferatum* (Fpro), all three *Fusarium* species combined (Mix), nontreated (Nontrt), or surface disinfested with 3% H_2O_2 (Disinf). Means represent 80 and 60 plants/treatment in 2004 and 2005, respectively. Within each bar graph, * indicates significantly different (Dunnett adjusted) at $P = 0.05$ and ** at $P = 0.01$, compared to the disinfested control of the same year. Error bars represent pooled error from mixed model, analyzed separately for each year.

In 2004, plants grown from kernels treated with *F. proliferatum* resulted in reduced ear weights compared to plants grown from disinfested kernels (Figure 3.3B), and plants grown in 2005 from kernels treated with *F. oxysporum*, *F. proliferatum*, or the mixed-*Fusarium* treatments had significantly lower ($P \leq 0.05$) ear weights than the plants grown from disinfested kernels. There was significantly more nodal root rot in plants grown from all *Fusarium*-treated kernels in 2005, and the rot levels increased in 2005 relative to 2004 in all treatments (Table 3.5). Germination was reduced in 2004 in the *F. oxysporum*-treated kernels. There were no treatment differences seen in node grayscale, primary root (radicle) rot, or sub-crown internode rot in either year, but disease symptoms worsened in 2005 relative to 2004.

Table 5. Mean germination and disease severity ratings of sweet corn plants grown from various kernel treatments in 2004 and 2005 field pathogenicity studies

Trt[α]	RRR[β]		PR[β]		SCI[β]		N1[β]		Germ[β]	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Foxy	1.3	2.1*	2.0	3.6	2.2	3.7	167.2	146.4	0.78*	0.83
Fvert	1.2	2.0*	1.9	3.9	2.0	3.7	169.7	149.3	0.81	0.83
Fpro	1.4	2.1*	1.9	3.5	2.1	3.5	168.0	148.8	0.81	0.80
Mix	1.2	2.1*	1.8	3.7	1.7	3.8	168.3	148.8	0.80	0.81
Nontrt	1.3	1.9	2.0	3.8	2.4	3.8	169.3	152.4	0.81	0.84
Dis	1.4	1.7	1.8	3.4	2.3	3.6	173.8	149.1	0.83	0.79
SE[γ]	0.09	0.09	0.17	0.14	0.2	0.12	2.1	3.1	0.014	0.02

[α]Kernel treatments include *F. oxysporum* var. *redolens* (Foxy), *F. verticillioides* (Fvert), *F. proliferatum* (Fpro), all three *Fusarium* species combined (Mix), nontreated (Nontrt), and surface disinfested with 3 % H_2O_2 (Disinf).

[β]Mean disease severity ratings: RRR = nodal root rot ratings on a 0-4 scale. 0 = no disease, 1 = 1-25 % necrotic, 2 = 26-50 %, 3 = 51-75 %, and 4 = 76-100 %. PR = primary root (radicle) and SCI = sub-crown internode rot ratings on a 1-4 scale: 1 = 0-25% necrotic, 2 = 26-50%, 3 = 51-75 %, and 4 = 76-100 %. N1 = Node grayscale of the lowest stalk node without rooted brace roots. Means represent 80 and 60 plants/treatment in 2004 and 2005, respectively. Germ = arcsin transformed proportion of germination per plot.

[γ]Standard error from mixed model.

* indicates significantly different at $P \leq 0.05$ (Dunnett adjusted) and ** at $P \leq 0.01$, compared to the disinfested control of the same year, within each column.

Fusarium oxysporum, *F. verticillioides* and *F. proliferatum* species were recovered from crowns and nodes of plants grown from all kernel treatments. When a solo *Fusarium* species was used to inoculate kernels, the same species was the most prevalent species recovered from crowns (Figure 3.4). *Fusarium oxysporum* and *F. verticillioides* were the most abundant species of *Fusarium* in the stalk nodes of their respective treatments. *Fusarium proliferatum* was found less frequently in the lowest stalk nodes of plants grown from *F. proliferatum*-treated kernels than *F. verticillioides* in both years of study. *Fusarium proliferatum* was also less frequently found in plants from the mixed-*Fusarium* treatment.

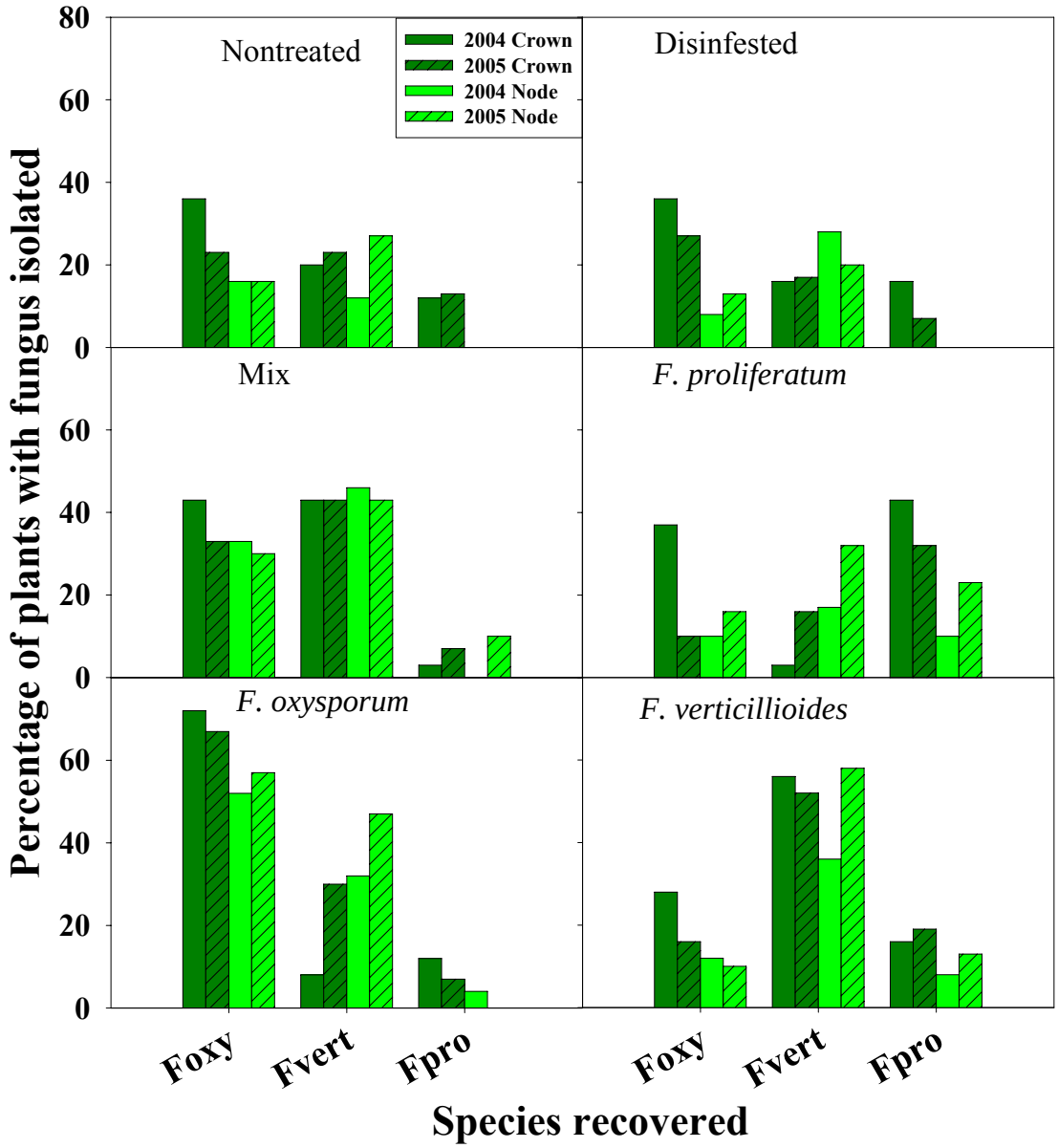

Figure 3.4. Recovery (percentage) of *Fusarium oxysporum* (Foxy), *F. verticillioides* (Fvert), and *F. proliferatum* (Fpro) from crowns and nodes of sweet corn plants grown from variously treated kernels in field pathogenicity experiments by year. Plants were grown from kernels infested with one of the three *Fusarium* species alone (individual graphs labeled for kernel treatment), all three species combined, nontreated kernels, or surface disinfested with 3% H_2O_2. Means represent 80 and 60 plants/treatment in 2004 and 2005, respectively.

When comparing crowns from which each *Fusarium* species was isolated to those from which that species was not isolated, regardless of kernel treatment, crown grayscale was significantly darker ($P < 0.05$) when *F. oxysporum* was recovered compared to plants in which it was not detected during both 2004 and 2005 (Figure 3.5A). Node grayscale was also significantly darker when *F. oxysporum* or *F. verticillioides* was isolated from the lowest stalk nodes without rooted brace roots in 2005 (Figure 3.5B). Ear weights were significantly lower when *F. oxysporum* was isolated from the node as well (when recovered = 190.1, SE = 8.0, when absent = 216.6, SE = 4.3, $P = 0.0053$).

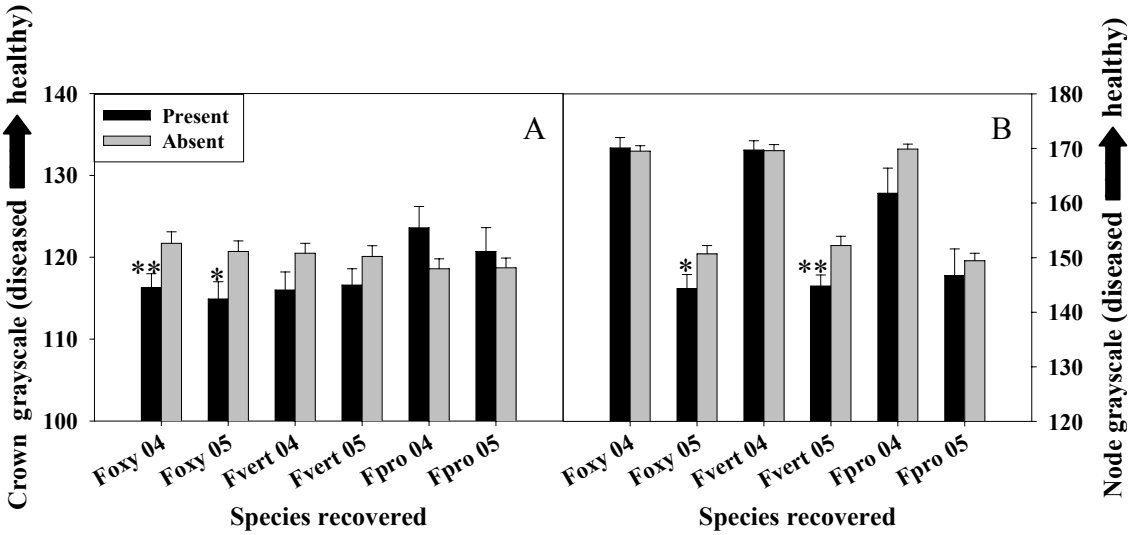

Figure 3.5. Mean crown grayscale (A) and node grayscale (B) of sweet corn plants from 2004 (04) and 2005 (05) field pathogenicity studies from which *Fusarium oxysporum* (Foxy), *F. verticillioides* (Fvert), or *F. proliferatum* (Fpro) was isolated (black bars) or not recovered (gray bars) regardless of kernel treatment. Graph A contains crown isolation data while (B) contains isolation data for the first stalk node lacking rooted brace roots. Means represent 80 and 60 plants/treatment in 2004 and 2005, respectively. Within each bar graph, * indicates significant differences at $P \leq 0.05$ and ** at $P \leq 0.01$ between adjacent bars based on two sample t-tests. Error bars represent pooled error from two-sample tests.

Reanalysis using only plants from which the respective inoculated species was recovered, showed mean crown grayscale was significantly darker ($P < 0.05$) in both years of study when kernels were treated with *F. oxysporum* or the mixed-*Fusarium* treatments, and the same inoculated species was recovered (Figure 3.6A). Crown grayscale values in 2004 were also significantly darker when *F. verticillioides* inoculated on kernels and recovered from crowns. No significant differences were seen in node grayscale either year of study by this analysis (Figure 3.6B). When any of the three *Fusarium* species were recovered from the stalk nodes of plants from the mixed-*Fusarium* kernel treatment in 2004, the ear weight was lower relative to plants of the disinfested kernel treatment from which no *Fusarium* species were recovered from node (Mix = 197.4 g, SE = 9.2, Disinf = 236.9, SE = 10.1, P = 0.04, Dunnett adjusted).

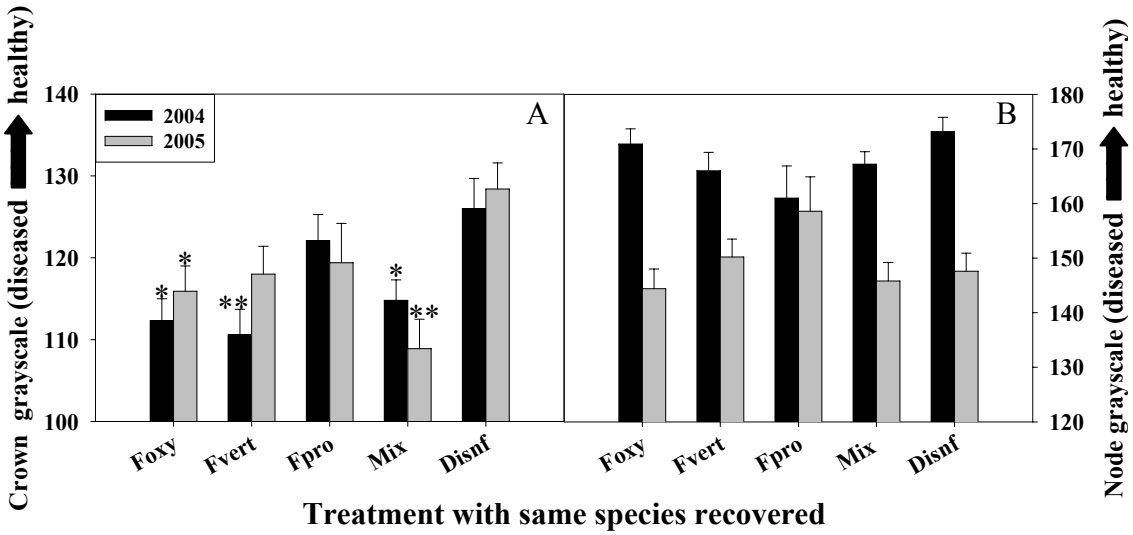

Figure 3.6. 2004 and 2005 mean crown grayscale (A) and node grayscale (B) of sweet corn plants grown from kernels infested with *F. oxysporum* var. *redolens* (Foxy), *F. verticillioides* (Fvert), *F. proliferatum* (Fpro), all three *Fusarium* species combined (Mix), or surface disinfested with 3% H_2O_2 (Disinf) and the same *Fusarium* species used in inoculations was/were recovered from crowns or the first stalk node lacking rooted brace roots in field pathogenicity studies. Means represent 80 and 60 plants/treatment in 2004 and 2005, respectively. Within each bar graph for each respective year, * indicates significantly different at $P \leq 0.05$ (Dunnett adjusted) and ** at $P \leq 0.01$, compared to plants in the disinfested group where no *Fusarium* species was found. Error bars represent pooled error from mixed model.

Pathogenicity evaluations of *Fusarium* species in a greenhouse

Pathogenicity studies with specific strains of *Fusarium* species were also conducted in a greenhouse to determine the ability of these fungi to incite crown and node necrosis under more controlled conditions. Plants in the second greenhouse experiment (study B) had more diseased crowns ($P = 0.0021$, two-sample t-test) than plants in study A. Generally, *Fusarium*-treated kernels resulted in darker crowns, though differences in crown means were significant ($P \leq 0.05$) only for study B. In study B, plants grown from *Fusarium*-inoculated kernels had significantly darker

crowns than plants grown from disinfested kernels (Figure 3.7A). There was a wide range of ear development in the greenhouse studies and the variance increased as ear weight increased so the data were transformed using ln(ear weight +1). Plants grown from kernels inoculated with either *F. verticillioides* or the mixed-*Fusarium* treatment had significantly lower ear weights compared to plants grown from disinfested kernels in study A (Figure 3.7B). In study B, *F. oxysporum* and mixed-*Fusarium* treated kernels resulted in significantly lower ear weights. There were no significant increases in disease rating of the primary root (radicle), sub-crown internode, or stalk node with any *Fusarium* treatments (Table 3.6).

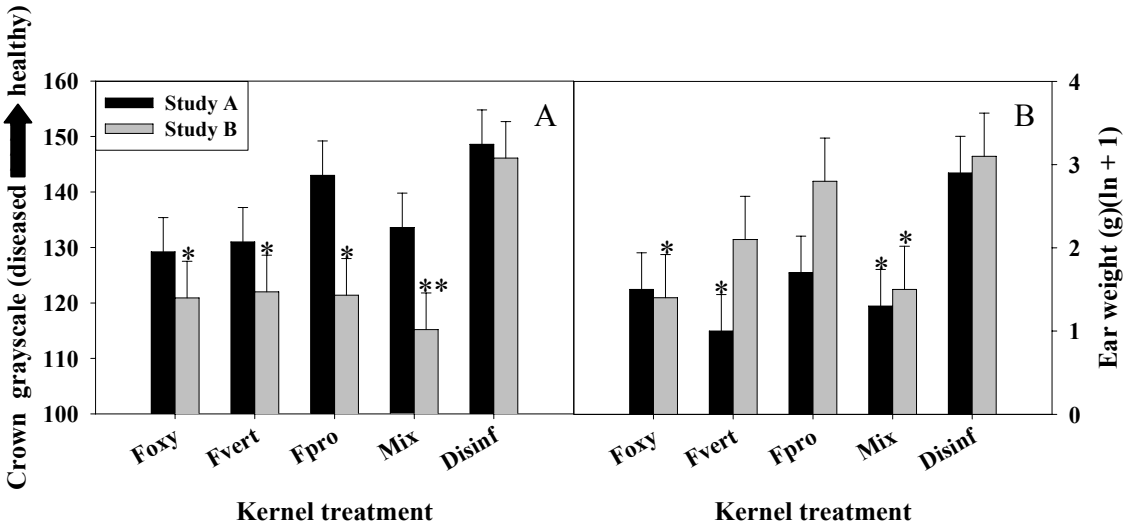

Figure 3.7. Mean crown grayscale (A) and ear weight (B) of sweet corn plants grown from various kernel treatments in two greenhouse pathogenicity tests. Plants were grown from kernels infested with *F. oxysporum* var. *redolens* (Foxy), *F. verticillioides* (Fvert), *F. proliferatum* (Fpro), all three *Fusarium* species combined (Mix), or surface disinfested with 3% H_2O_2 (Disinf). Means represent 10 plants/treatment. Within each bar graph, * indicates significantly different at $P \leq 0.05$ (Dunnett adjusted) and ** at $P \leq 0.01$, compared to disinfested control of the same study. Error bars represent pooled error from mixed model, analyzed separately for each study.

Table 3.6. Mean disease severity ratings of plants by kernel treatment in greenhouse pathogenicity studies

Treatment[α]	**PR**[β]		**SCI**[β]		**N1**[γ]	
	A[ε]	B[ε]	A	B	A	B
Foxy	3.0	3.3	3.0	2.7	154.9	144.5
Fvert	1.9	1.9	2.7	2.0*	145.3	152.1
Fpro	2.8	2.7	2.9	2.7	153.2	153.4
Mix	2.5	2.3	3.1	1.7**	155.3	139.4
Dis	2.6	3.3	2.9	3.3	151.8	156.1
SE[δ]	0.43	0.43	0.43	0.38	7.6	6.8

[α]Treatments included *F. oxysporum* var. *redolens* (Foxy), *F. verticillioides* (Fvert), *F. proliferatum* (Fpro), all three *Fusarium* species combined (Mix), and surface disinfested with 3% H_2O_2 (Disinf).
[β]Primary root (radicle) (PR) and sub-crown internode (SCI) rot ratings on a 1-4 scale. 1 = 0-25% necrotic, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100%. Means represent 10 plants/treatment.
[γ]Node grayscale of the lowest node without rooted brace roots. Means represent 10 plants/treatment.
[δ]Standard error from mixed model.
[ε]Studies A and B.
* indicates significantly different at $P \leq 0.05$ (Dunnett adjusted) and ** at $P \leq 0.01$, compared to disinfested kernels within the same experimental study.

Recovery of each inoculated species from nodes and crowns was higher relative to the pathogenicity studies in the field. In the greenhouse, the inoculated species was the most prevalent species recovered (Figure 3.8). The same *Fusarium* species used as inoculum was recovered from 70 to 90 % of crowns when kernels were inoculated with *F. oxysporum*, *F. verticillioides*, or *F. proliferatum*. Similar to field pathogenicity studies, *F. proliferatum* was less frequently isolated in plants from the mixed-*Fusarium* treatment.

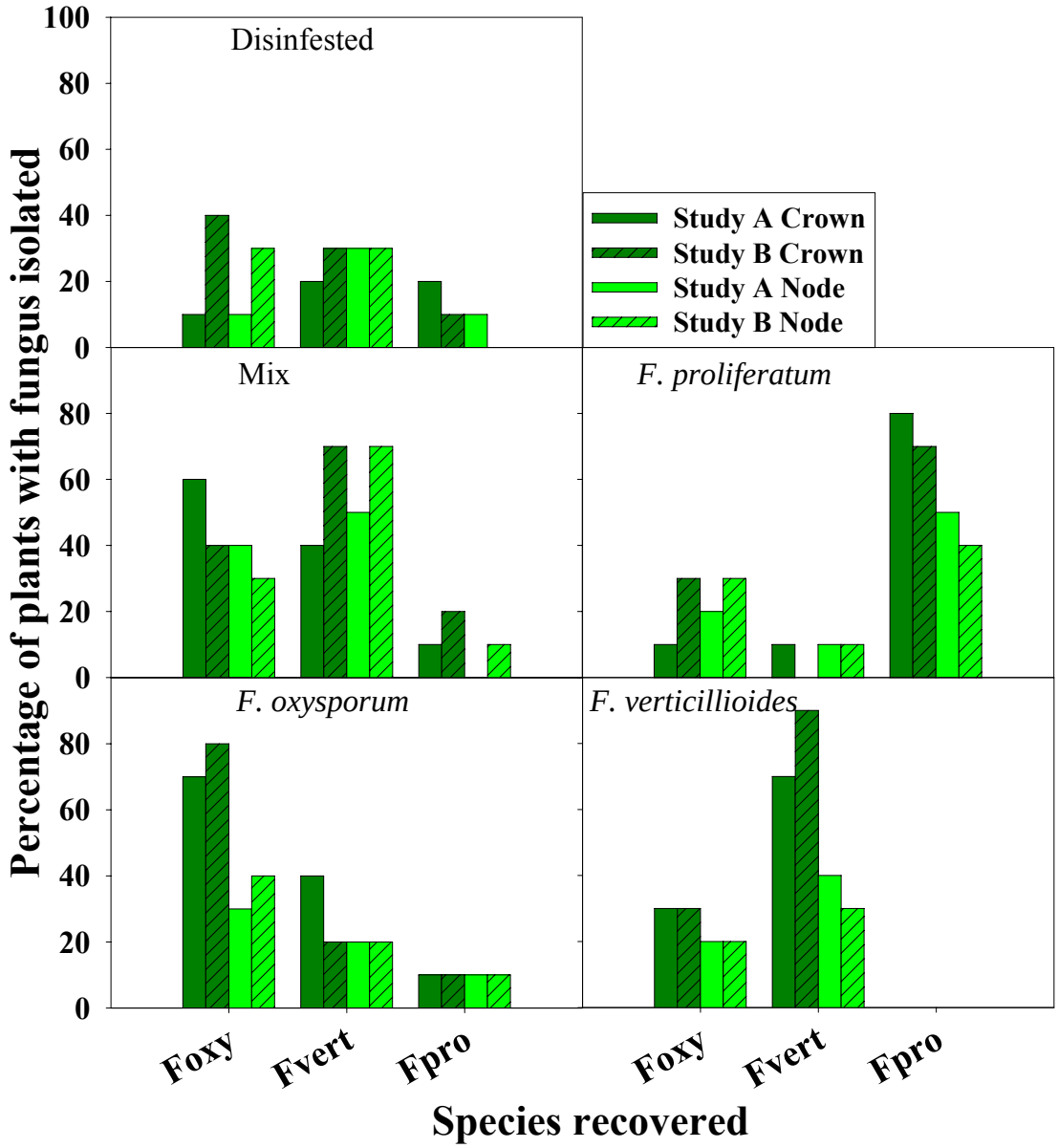

Figure 3.8. Recovery (percentage) in two greenhouse pathogenicity experiments of *Fusarium oxysporum* (Foxy), *F. verticillioides* (Fvert), and *F. proliferatum* (Fpro) from crowns and nodes of sweet corn plants grown from treated seed kernels. Plants were grown from kernels infested with one of the three *Fusarium* species alone (individual graphs are labeled for kernel treatment), all three species combined, nontreated kernels, or surface disinfested with 3% H_2O_2. Percentages are based on 10 plants/treatment.

When comparing crowns from which each species was isolated to those from which that species was not isolated regardless of kernel treatment, crown grayscale was generally lower (darker), although not significant at the P = 0.05 level, when *F. oxysporum* or *F. verticillioides* was isolated compared to when it was absent in both studies (Figure 3.9A). Node grayscale was significantly lower when *F. oxysporum* or *F. verticillioides* was isolated in the lowest stalk nodes without rooted brace roots in one study (Figure 3.9B). Ear weights were lower when *F. verticillioides* was isolated from the node in study B (when recovered = 1.5 ln transformed, SE = 0.35, when absent = 2.5, SE = 0.25, P = 0.03).

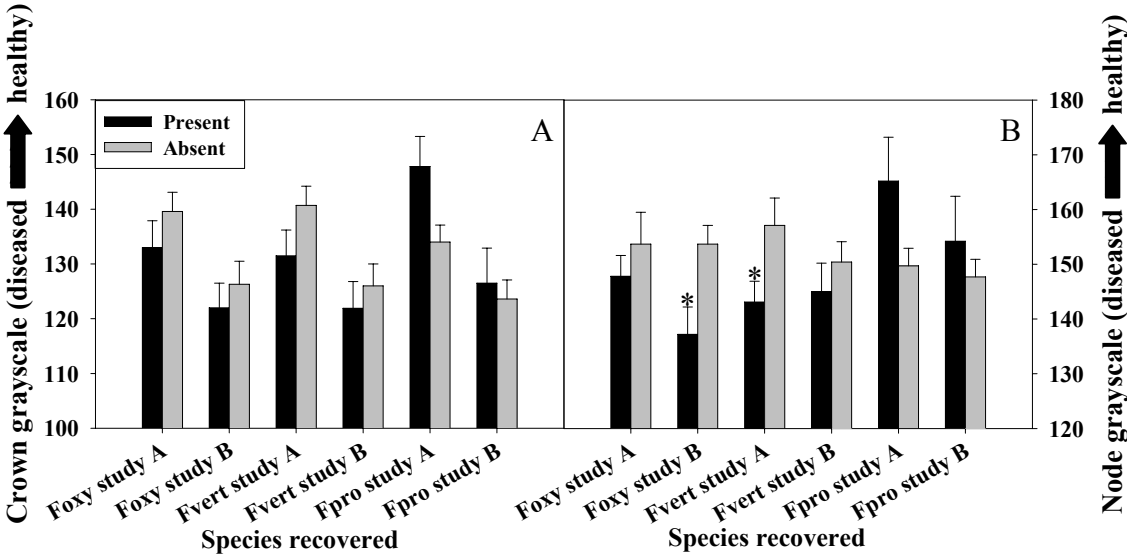

Figure 3.9. Mean crown (A) and node (B) grayscales of sweet corn plants in greenhouse pathogenicity studies from which *Fusarium oxysporum* (Foxy), *F. verticillioides* (Fvert), or *F. proliferatum* (Fpro) was recovered (black bars), or absent (gray bars), regardless of kernel treatment. Graph A contains crown isolation data while B contains isolation data from the first stalk node lacking rooted brace roots. Means represent 10 plants/treatment. Within each bar graph, * indicates significant ($P \leq 0.05$) differences between adjacent bars based on two sample t-tests. Error bars represent pooled error from two sample tests.

Discussion

The results of these studies indicate that strains of *F. oxysporum*, *F. verticillioides*, and *F. proliferatum* used in the investigations reported here can have negative effects on corn ear weight and crown health. *Fusarium oxysporum* and *F. verticillioides* were recovered most frequently from commercial sweet corn plants in the Willamette Valley and both were recovered at higher frequency from mature crowns in field and greenhouse pathogenicity studies. Crowns of commercial sweet corn plants from which *F. verticillioides* and *F. oxysporum* were recovered were darker, more necrotic, than crowns in which neither species was detected. The presence of *F. oxysporum* within crown and stalk node tissues was associated with lower ear weights. In both field and greenhouse pathogenicity studies, kernel inoculation with all three species separately, and a mix of the three species, resulted in plants which had darker, more diseased crowns. These differences were statistically significant for *F. oxysporum* and the mixed *Fusarium* treatment in most studies while *F. verticillioides* effects were significant for half of the pathogenicity studies. Mean ear weights were typically reduced when plants were grown from *Fusarium*-inoculated kernels, but the differences were not always significant. *Fusarium oxysporum* was recovered with the highest frequency from crowns of plants grown in field studies from inoculated kernels, followed by *F. verticillioides* and *F. proliferatum*. This could be because *F. oxysporum* may be more efficient infecting crowns from seed kernels, but also probably reflects infections by propagules in field soil. *Fusarium oxysporum* was the most frequently isolated species from crowns of plants from disinfested and nontreated treatments,

which indicates its presence in soil. In the greenhouse studies, all three species could be recovered from crowns in high frequencies when a single species was used to inoculate kernels. In plants that were grown from kernels treated with a mix of all three *Fusarium* species, *F. proliferatum* was less frequently recovered in both the field and greenhouse pathogenicity tests. These findings, and the low isolation frequency seen in commercial fields, suggest that *F. proliferatum* is less competitive than *F. oxysporum* or *F. verticillioides* under local conditions. Each *Fusarium* species studied could be found in plants outside its treatment group. In field studies, this is likely due to natural populations present in the soil. In greenhouse studies, there may be movement attributed to the strong air currents made by the ventilation system. It could also be that *Fusarium* species can quickly colonize soil once it has been pasteurized and had the populations of competing fungi reduced. Sweet corn plants grew more vigorously in the field studies than in our greenhouse setting, so more normal plant development may occur in the field but the greenhouse studies provided a more controlled setting for the studies.

Though root rot was not the focus of this paper, field testing showed a slight but significant increase in nodal root rot when seed kernels were inoculated with *F. oxysporum, F. verticillioides,* or *F. proliferatum*, compared to plants that developed from disinfested or nontreated kernels. Numerous *Fusarium* species are reported to cause root rot of corn, but most commonly reported are *F. oxysporum* and *F. solani* (43). These were also the two most commonly found *Fusarium* species isolated from symptomatic roots in sweet corn in the Willamette Valley, but they weren't considered

primary pathogens in a previous report (14). *Fusarium oxysporum* has been shown to be more pathogenic at higher temperatures (35,42), which may account for the inconsistent results and low pathogenicity seen in greenhouse studies in the previous report (14) as well as in some studies in this dissertation. Studies of diseased corn roots have found that *F. oxysporum* accounts for 95% (35), 70% (14), and 62% (42) of *Fusarium* species isolated but it is often considered a wound pathogen (35,42), inferring that its role in root disease may become more significant when plants are injured by root-feeding insects or nematodes. Because of the widespread nature of *F. oxysporum*, it has been suggested that more attention be given to this fungus when evaluating diseased roots (35). *Pythium arrhenomanes*, *Phoma terrestris*, *Drechslera* species were shown to be capable of causing root rot on sweet corn in the Willamette Valley and were implicated as the primary cause of root rot (14), but recovery of the latter two species have not been reported in symptomatic plants of commercial sweet corn plantings located in the Willamette Valley.

Fusarium oxysporum typically causes either root rots or vascular wilts on a wide variety of hosts (5,29). The frequency at which it has been found in crown and node tissues in studies reported in this dissertation, where xylem-inhabiting/invading microbes would accumulate, suggest that it is invading vascular tissues. This is supported by the ability of *F. oxysporum* to cause darkening in the crown because darker nodes have also been associated with lower xylem conductance (Chapter 2). The fungus is however, also frequently isolated from diseased roots, so it may be acting as both a root rot and vascular pathogen. In many vascular diseases, infection starts in

roots, which may or may not require wounding, and the pathogen must cross the endodermis, which is usually a more effective barrier to fungi than the epidermis. This can be done by directly penetrating between cells, or by initiating infection at root tips where vascular tissues are less developed (4). Hyphae of *F. oxysporum* are not usually seen penetrating the endodermis within the roots or sub-crown internode of corn but occlusion of xylem vessels is one of the earliest symptoms seen when corn plants are infected (23). When roots are damaged by rootworms, *F. oxysporum* made up 95% of *Fusarium* species isolated from roots in one report (35). This damage may help overcome the fungus' limited ability to penetrate the endodermis, and move it into the xylem. In typical wilt diseases, spores of the fungus are found in the xylem where they can move up the plant with the transpiration stream until they hit a vessel end-wall. At the end-wall, spores get trapped and must germinate into the next cell. Wilting is typically explained either by disruption of cell membrane permeability by a toxin or by increased resistance to flow from physical blockage of xylem (7). Higher temperatures increase water demand of plants, so increased temperature can increase the movement of pathogens up the stem, and can trigger symptom development if there is too much resistance to flow in the xylem. Corn nodes can act as filters for particulates due to the high number of end-walls present. Shane (39) reported that only 3% of axial vessels passed through a node without an end wall, and latex particles, 1/20 the size of microconidia of a *Fusarium* species, were rarely able to make it past the first node when put into the transpiration stream. If a fungus inhabits the xylem, it could then be expected to be found in the first node upstream from the point of inoculation. If initial

infection occurs in the roots, then the introduced spores or particulates would accumulate in the crown and then the lower nodes if they passed the crown nodes. This may explain why *F. oxysporum* was more frequently found in the crown than in the stalk node above the crown without rooted brace roots, and why it could be recovered with a higher frequency from crowns after kernel inoculation.

Fusarium verticillioides is known to infect plants systemically from seed kernel-borne inoculum, (25) but many infections also result from air-borne conidia infecting through leaf axils (21). Leaf axil infections could explain why this fungus was found more frequently from the first stalk node without functional brace roots, and it was recovered from that node about as frequently as it was recovered from the crown after seed kernel inoculation in field studies. This was not the case in greenhouse pathogenicity studies where there are less opportunity and less favorable conditions for airborne conidia to infect stalks through the leaf axils. Reports vary on the abilitiy of *F. verticillioides* to enter the vascular tissue and it is not considered a vascular wilt pathogen, (3,26,37) so it unclear what mechanism is responsible for ear yield reduction. One possible mechanism is the interaction with the western spotted cucumber beetle (*Diabrotica undecimpunctata undecimpunctata*). This beetle appears to preferentially feed and oviposit on corn plants grown from seed kernels inoculated with *Fusarium* (Chapter 4). The subsequent larval damage to the root systems may account for yield loss, and allow *Fusarium* entry into the vascular system of the roots. This may also help account for the mixed results seen on the importance of seed kernel-borne inoculum by researchers in different parts of the United States.

The results of these studies suggest that *Fusarium* species, especially *F. oxysporum* and *F. verticillioides*, can at least partially account for ear yield losses of sweet corn in the Willamette Valley. *Fusarium proliferatum* appears to have only a minor involvement in disease and yield loss under the conditions of these studies. Given the prevalence of *F. oxysporum* and *F. verticillioides*, more attention should be given to them when considering disease management options.

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Chapter 4

Sweet Corn seedlings Grown from *Fusarium*-infested Kernels and Feeding Damage by Adult Western Spotted Cucumber Beetles

Nathan L. Miller and C. M. Ocamb

Abstract

The western spotted cucumber beetle, *Diabrotica undecimpunctata undecimpunctata*, is an important pest of sweet corn (*Zea mays*) in the Willamette Valley of Western Oregon. Sweet corn injury by this beetle includes larval feeding on roots, and adult feeding on silks and leaves. Studies were conducted to determine if the western spotted cucumber beetle shows feeding or oviposition preferences when sweet corn is grown from kernels inoculated with *Fusarium oxysporum, F. verticillioides*, or *F. proliferatum*. In field trials, a higher proportion plants grown from *Fusarium*-treated kernels had leaf-feeding injury by the western spotted cucumber beetle compared to plants grown from disinfested kernels. Laboratory trials showed a similar injury trend. Plants grown from *Fusarium*-infested kernels were also found to have increased larval damage to roots in field trials. Affected plants may suffer root damage from larval feeding in addition to disease caused by pathogenic *Fusarium* species. This is the first report to suggest that the presence of *Fusarium* species on sweet corn kernels is correlated with increased damage by the western spotted cucumber beetle.

Introduction

Sweet corn (*Zea mays* L.) root rot became increasingly severe in the Willamette Valley of Western Oregon by the early 1990's. During the latter part of the 1990's, Ocamb (personal communication) found rot of crowns and stalk nodes to be prevalent in many of the problematic sweet corn fields. Several species of *Fusarium* are under investigation as causal agents for stalk node and crown disease. During preliminary pathogenicity studies for this dissertation, it was observed that increased insect feeding damage occurred on the roots of *Fusarium*-inoculated plants compared to non-inoculated control plants in field studies. This observation led to investigations of interactions between corn rootworm (*Diabrotica*) and *Fusarium* species. These insects may vector pathogenic *Fusarium* species as well as cause declines in sweet corn ear yields due to larval feeding on plant roots. Understanding interactions between rootworm beetles and pathogenic *Fusarium* species is important for developing management strategies for both the insect pest as well as Fusarium diseases of sweet corn.

Corn rootworms belonging to the genus *Diabrotica* (Coleoptera: Chrysomelidae) are known to be significant pests of corn throughout the United States. Larvae can cause considerable damage while feeding on corn roots and may reduce root integrity to the point that plant lodging occurs (24). *Diabrotica undecimpunctata undecimpunctata* Mannerheim, otherwise known as the western spotted cucumber beetle (WSCB), is the predominate *Diabrotica* species to attack sweet corn in the Willamette Valley of Oregon (16). In other parts of the US where corn is a planted in

larger acreage, considerable attention has been paid to other corn pests within *Diabrotica* species, relative to the WSCB. However, the biology of the WSCB has significant differences that may warrant different management strategies.

There are two major groups of *Diabrotica* species that are pests on corn, the *fucata*-type and *virgifera*-type (33). The western corn rootworm (*D. virgifera virgifera* Leconte) and the northern corn rootworm (*D. longicornis* Say) are closely related, well-studied members of the *virgifera* group and both can be significant pests of corn in the Midwestern US. The second group, the *fucata*-type, includes important pests such as the southern corn rootworm (*D. undecimpunctata howardi*) as well as the WSCB, which is a subspecies of southern corn rootworm. Important differences exist between the *fucata*-type and *virgifera*-type of *Diabrotica* species. The *fucata*-types live in regions where the climate is milder during the off-season, enabling gravid adult females to overwinter. The *virgifera*-type lives in regions such as Iowa, Minnesota, North Dakota, Colorado, Eastern Oregon and Mexico, where the winters or summers are too harsh for adult survival; the beetle population survives as eggs in the soil. Beetles in the *fucata* group have multiple generations a year (multivoltine) and the larvae are less host specific, while *virgifera*-type beetles are univoltine with larvae that are more host specialized (8). The dynamics of disease epidemics caused by pathogens vectored by *Diabrotica* species should vary between the *virgifera*- and *fucata*-types. Multivoltine, *fucata*-type beetles would have more opportunities to vector pathogens, as well as a greater window of time to potentially feed on host plant shoots and roots.

Larvae of the WSCB can reduce root volume, cause corn lodging, or increase root rot (5,29), while adults feed on aboveground corn tissues (leaves and silks). The adult beetles can reduce ear yield in corn when excessive silk feeding occurs and pollination is subsequently inhibited (4). Adult beetles feed on corn plants from plant emergence through harvest. Although adult feeding may not cause considerable damage to the plants, wounding occurs, potentially increasing diseases incited by weakly pathogenic or secondary organisms. Species of *Fusarium* pathogenic on corn have been shown to be associated with feeding by a number of insect species, including the *virgifera*-type of *Diabrotica* species (10,12,14,30,32,39). The western and northern corn rootworms can disseminate the ear-rotting fungi, *Fusarium verticillioides* Sacc. (Nirenberg) (synonym *F. moniliforme* Sheld.) and *F. subglutinans* (Wr. and Reink.) Nelson Toussoun and Marasas (14,30). *Fusarium verticillioides*, *F. oxysporum* (Schlecht.) Snyd. and Hans., *F. subglutinans*, and other *Fusarium* species have been isolated from surface-disinfested *Diabrotica* adults in the *virgifera* group (14,30). *Fusarium oxysporum* and *F. verticillioides* have also been isolated from larvae of northern corn rootworms and root infections by these fungi were higher when rootworms were present (30). Given that *virgifera*-type of *Diabrotica* species, as well as other insects, have been shown to disseminate *F. verticillioides* and other *Fusarium* species, it seems likely that the WSCB can also vector pathogenic *Fusarium* species.

Eggs of the WSCB hatch in seven to ten days, depending on weather conditions, while *virgifera*-type beetles diapause for a year or longer (25). Western spotted cucumber beetle eggs will hatch during the same growing season in which they were

laid, making the beetle's choice in oviposition site potentially significant since the plant used for oviposition will likely still be present when the eggs hatch. In contrast, *virgifera*-type beetle larva will be limited to development on whatever plant species are near the site of overwintering eggs. Because of this difference in life cycle, gravid females of all WSCB generations may select the individual plants for larval development and a variety of plant or other environmental cues may influence those choices.

The differences seen in rootworm damage during the preliminary pathogenicity studies may be due to preferential oviposition next to infected plants, infected plants having increased vulnerability to rootworm damage, or a combination of both factors. This raised the question of whether adult beetles show preference for plants grown from kernels infested with a *Fusarium* species. There are no reports to demonstrate that plant pathogens influence feeding or oviposition preference in any *Diabrotica* species. If adult WSCB can recognize and lay eggs near corn plants grown from *Fusarium*-infested kernels, then these plants would have potential injury from *Fusarium* infection compounded with rootworm larvae pressure. In order for adult females to preferentially lay eggs on *Fusarium*-infected hosts, beetles must somehow distinguish infected plants from non-infected plants and this ability could be exhibited if adult beetles show feeding or oviposition preferences when presented with plants developing from *Fusarium*-infected and *Fusarium*-free kernels.

Increased feeding by the WSCB could compound the stress on *Fusarium* infected plants and perhaps increase rot severity as well as potentially facilitate

dissemination of pathogenic *Fusarium* species. *Fusarium* contamination of adult beetles becomes increasingly important once corn plants initiate silking. When silks emerge, adult WSCB will feed on them, sometimes to the point where insufficient pollination occurs (4). Silks have been reported to be the most efficient inoculation point for kernel infection by *F. verticillioides* (27). This suggests that contaminated WSCBs may play a significant role in *Fusarium* infection of corn kernels. Ear rot can increase the levels of *Fusarium* contamination within the subsequent seed lot. *Fusarium verticillioides* (27) and *F. subglutinans* (19) have been shown to be transmitted from contaminated kernels to seedlings with high frequency. Currently, seed corn is treated with a variety of fungicides, and although fungicides help prevent seed and seedling rot, they may not stop kernel to seedling transmission. Treating corn kernels with Captan, a common seed treatment, after infestation with *F. verticillioides*, did not reduce transmission of the fungus to seedlings (26).

Studies were conducted to see if the adult WSCB shows preference for corn plants grown from kernels infested with *Fusarium* species. Three species of *Fusarium* have been chosen for these experiments: *Fusarium oxysporum* var. *redolens* (Wollenw.) Gordon, *F. verticillioides*, and *F. proliferatum* (T. Matsushima) Nirenberg. *Fusarium oxysporum* was chosen because of its prevalence on diseased corn roots and stalk nodes in the Willamette Valley, reputation to cause root rot, and association with stalk rot (20-22,36). *Fusarium verticillioides* is also commonly found in corn plants and on kernels, and it is known to cause disease of roots, stalks and ears (10,36).

Fusarium proliferatum was found on symptomatic sweet corn plants from commercial fields by Ocamb (unpublished) and is also a known ear rot (31) and root pathogen (36).

Materials and Methods

***Fusarium* inoculum production and kernel treatment**

Three *F. oxysporum* var. *redolens* isolates isolated from diseased sweet corn, and two cultures each of *F. verticillioides* and *F. proliferatum* isolated from sweet corn kernels, were used in these studies (Table 4.1). *Fusarium oxysporum* var. *redolens* cultures were grown from single spores (28) on carnation leaf agar (CLA) (13) and stored on silica gel at 5 C (37,38). *Fusarium verticillioides* and *F. proliferatum* cultures were single-spored, transferred to dilute water agar (3g/L), increased at 200 RPM on a platform shaker (New Brunswick Scientific, Edison, NJ) for one month, and then stored at 5 C. Inoculum was increased in 60-ml aliquots of potato dextrose broth (Difco, Sparks, MD) on a platform shaker at 175 RPM for seven days at 24 C. Broth cultures were rinsed twice by centrifuging, pouring off the supernatant, and resuspending in 50 ml of sterile reverse osmosis (RO) water. Cultures were homogenized for 20 seconds with a tissue tearor (985-370 Biospec, Bartlesville, OK). Isolates of the same species were bulked together. Equal amounts of these bulked cultures were then combined to make the mixed species inoculum. A hemocytometer was used to count conidia and hyphal fragments and estimate colony forming units (CFUs) of inoculum (Table 4.2).

Table 4.1. *Fusarium* isolates used for WSCB feeding and oviposition preference studies

Isolate name	*Fusarium* species	Year collected	Part of sweet corn from which obtained
F 47	*F. oxysporum* var. *redolens*	1999	Root
F 44	*F. oxysporum* var. *redolens*	1999	Root
F 33	*F. oxysporum* var. *redolens*	1999	Root
F 230	*F. proliferatum*	2003	Kernel
F 231	*F. proliferatum*	2003	Kernel
F 232	*F. verticillioides*	2002	Kernel
F 233	*F. verticillioides*	2003	Kernel

Table 4.2. Concentrations of *Fusarium* inoculum for each fungal treatment used for infesting sweet corn kernels

	No. of conidia and hyphal fragments	
Fusarium species	2004 studies	2005 studies
F. oxysporum var. *redolens*	2×10^7	1×10^7
F. proliferatum	5×10^7	1×10^7
F. verticillioides	5×10^7	3×10^7
mix of the *Fusarium* species listed above	4×10^7	2×10^7

Sweet corn kernels (cv. Jubilee, lot NC9114LF) were disinfested for 3 hours in 3% H_2O_2 and rinsed with running RO water for five minutes. Thirty ml of inoculum was added to 230 g of kernels and thoroughly mixed via shaking in a plastic bag for five minutes by hand. Disinfested kernels with no additional microbial infestation and nontreated kernels, which are naturally infested with a variety of fungi including *F. verticillioides* and *F. proliferatum,* were also included in the treatments.

Field studies

All field experiments were conducted at a site located at the Oregon State University Botany and Plant Pathology field lab (Corvallis, Oregon), which had been planted to sweet corn since 2001. The soil is a Chehalis series silty loam with 10.8 % sand, 63.2 % silt, and 26 % clay. In the fall of 2000, this field was infested with the lower portions of plants collected from a commercial sweet corn field exhibiting rot of crown, stalk nodes and roots. Kernels were planted using a hand push-type belt planter with the foot set for a 2.4 cm planting depth and were sown on June 21, 2004 in 12-m rows with 60 kernels per row using a randomized complete block design. Six blocks were established in a portion of the field that had been inoculated with *Fusarium* species via infested oats and nine blocks were set up in an oat-free part of the field.

The numbers of corn seedlings with leaf feeding damage were recorded in each row 11 days after sowing. A second trial (study B) was planted with three repetitions of each treatment in each of four blocks in unplanted portions of the same field on July 16. Ten kernels were planted in an approximately 20-cm diameter circle. At 10 and 14 days post sowing, the numbers of plants with leaf feeding damage were counted.

On September 21, 2004, (92 days post planting), 10 plants from each row in study A were sampled in each of four blocks from the oat-inoculum portion of the field as well as in the oat-free area. Plants were cut above the ears, dug, and returned to the lab. Root balls were washed clean of soil and rated on the following scale for rootworm feeding: 0 = no root tunneling observed on any roots, 1 = one to three roots had rootworm tunneling, 2 = more than three roots with tunneling but less than half the

roots in the root ball were affected, and 3 = more than half the roots in the root ball had tunneling.

The field was treated with *Fusarium*-infested oats prior to sowing again in 2005. Six blocks were planted on June 21, 2005 with 50 kernels per row in 9-m rows in the same part of the field that was inoculated with oats. Leaf feeding damage of seedlings was rated at 15 and 22 days after sowing, later than the previous year because unusually cool weather slowed both corn growth and beetle activity. Ten plants from each row in all six blocks were sampled on September 20 (91 days post planting) for rootworm damage. A second leaf feeding study (study B) was conducted by planting kernels in small circles as previously described, between rows on July 15; 10 blocks were planted and leaf feeding ratings were done on July 28.

Laboratory evaluations of leaf feeding

Feeding and oviposition by the WSCB on sweet corn seedlings was evaluated in laboratory studies using rolled germination papers ("rag dolls"). The germination paper (Anchor Paper, St. Paul, MN) was pre-moistened with RO water and autoclaved for 20 minutes at 121 C and 103 kPa. Rag dolls were made by lining up approximately 20 sweet corn kernels (cv. Jubilee, lot NC9114LF) along the long edge of a 25 cm x 38 cm sterile piece of germination paper with the pedicel of each kernel orientated inwards. A second sheet of germination paper was placed on top of the first so that kernels were sandwiched between the two. The pair of germination papers, with kernels, was then placed in between two pieces of wax paper. All four sheets were then rolled up so that the kernels were at one end (the top end) of the roll. A rubber band was used at the

bottom end to keep the papers rolled together. The rag dolls were then placed vertically in a container with a 5-cm depth of RO water so that the germination papers would remain moist through capillary action.

Corn kernels and *Fusarium* inoculum were prepared and treated in the same manner as previously outlined above in the field trials, and the same six treatments (*F. oxysporum* var. *redolens*, *F. verticillioides*, *F. proliferatum*, mix of these three *Fusarium* species, disinfested kernels, and nontreated kernels) were used in all laboratory and field experiments and were randomized within each block, cage or aquarium. So insects always had all six treatment choices available in each experiment. Four rag dolls were used in each treatment, were set up on July 19, 2004, and placed in a 50 x 50 x 50-cm square insect cage in the greenhouse under ambient light. Five days later (after kernels had germinated), 50 WSCB adults were caught at the BPP corn field and added to the cage. Beetles were collected in sweep nets from *Amaranthus retroflexus* L., *Hypochaeris glabra* L., and *H. radicata* L., and then transported to the lab in 3.8-L Ziploc bags. Beetles were cooled at 5 C for 30 minutes, sorted into groups of 50, and then introduced into the cage containing the rag dolls. Beetles were released at the bottom center of the cage and had to move upwards to reach seedlings. Nine days after placement in the insect cages, the number of feeding regions per plant were determined and the size class of the largest feeding region per plant were recorded as follows: 1 = small bite or scrape, 2 = visibly missing tissue up to 2 mm in length, and 3 = any feeding region is larger than 2 mm in length.

A second trial (study B) was set up on August 6, 2004 using twelve rag dolls for each kernel treatment split evenly between two cages (6 rag dolls/kernel treatment/cage). Fifty adults of the WSCB were collected on August 9, and were added to each cage. Due to insect mortality, 50 additional beetles were collected on August 16 and added to each cage. The experiment was rated 15 days after placing rag dolls in the insect cages.

Rag doll studies with *Fusarium*-treated kernels and adult WSCB were conducted during 2005 using four rag dolls for each kernel treatment in each of two cages for each of two experiments, (studies A and B). Fifty WSCB adults were added twice to both cages in both experiments because of insect mortality. The first set of beetles was added when the kernels had germinated (3 days after initial set-up) and the second set was added one week later. When the second set of beetles was added to study A, this experiment was moved into the laboratory because of concerns that mortality may have been caused by residues from greenhouse insecticide applications. For the 2005 study A, rag dolls were set up on July 1 and evaluations were made 13 days later. The 2005 study B was set up on July 15 in the laboratory and evaluated 13 days later. Temperatures in both the lab and greenhouse averaged 20 to 25 C during the summer months with an average difference between day and nighttime temperature of 10 C in the greenhouse and 7 C in the laboratory.

Laboratory evaluations of oviposition

Evaluation of oviposition on sweet corn plants from *Fusarium*-treated seed kernels experiments were conducted using rag dolls, which enabled eggs to be detected

on kernels and germination paper during evaluations. Two preliminary experiments were conducted in 50 x 27 x 25-cm aquariums to establish a protocol for the leaf feeding and oviposition studies. The first experiment consisted of two rag dolls for each kernel treatment, made on July 15, 2004. Thirty adults of the WSCB were added on July 20, and then plants were evaluated 7 days later. The second experiment consisted of four rag dolls for each kernel treatment made on July 15, 2005. Fifty adults of the WSCB were added on July 18, and evaluations were made 7 days later. Larger studies were conducted in 2005 using eight aquariums in each of two experiments. Two rag dolls for each kernel treatment were put in each aquarium on August 2, 2005 and thirty beetles were added to each aquarium on August 5. Oviposition was recorded on August 17, 15 days after set-up. The experiment was repeated with rag dolls set up on August 19. Thirty beetles were captured and added 6 days later, and the experiment was evaluated 13 days after setup.

Statistical analyses

Rows of corn plants in the field or within a rag doll were considered a single experimental unit, while plants were a sub-sample. The average of all plants sampled within a row was used for analyses for root injury, bites per plant, and severity of leaf feeding. For leaf injury incidence data, an arcsine$\sqrt{y}$ transformation of the proportion of plants with chewed leaves was used for statistical analyses. Data from all experiments were analyzed using a mixed procedure analysis of variance and means were compared to the disinfested kernel control with the Dunnett adjusted statistic (SAS 9.1, SAS institute, Cary, NC). To determine if oviposition was influenced by

treatment, egg mass data were analyzed via a randomization test using 5000 randomly generated data sets on combined oviposition data with the assumption that each egg mass was an independent choice made by a gravid female (Excel 2003, Microsoft Corporation, Redmond, WA). Random data sets were counted when the difference in egg masses between a *Fusarium* treatment and the control were as large as or larger than the observed differences. This was treated as a one-sided test so data sets were only counted if the *Fusarium* treatment had the higher number of egg masses. The frequencies were used as an estimate of one-sided P values.

Results

Adult damage (leaf feeding) of seedlings in 2004 and 2005 trials

Rows of sweet corn plants grown from *Fusarium*-inoculated and the nontreated kernels had a significantly ($P \leq 0.05$, Dunnett adjusted) higher proportion of plants exhibiting leaf feeding by adult WSCB compared to the disinfested control in the 2004 study A (Figure 4.1.2). There were no significant differences between plants grown in the oat-infested or noninfested portions of the field so the data were combined. On day 14 in 2004 study B, all plots grown from *Fusarium*-inoculated kernels had significantly greater proportion of plants with leaf feeding compared to the disinfested control plants (Figure 4.1.2).

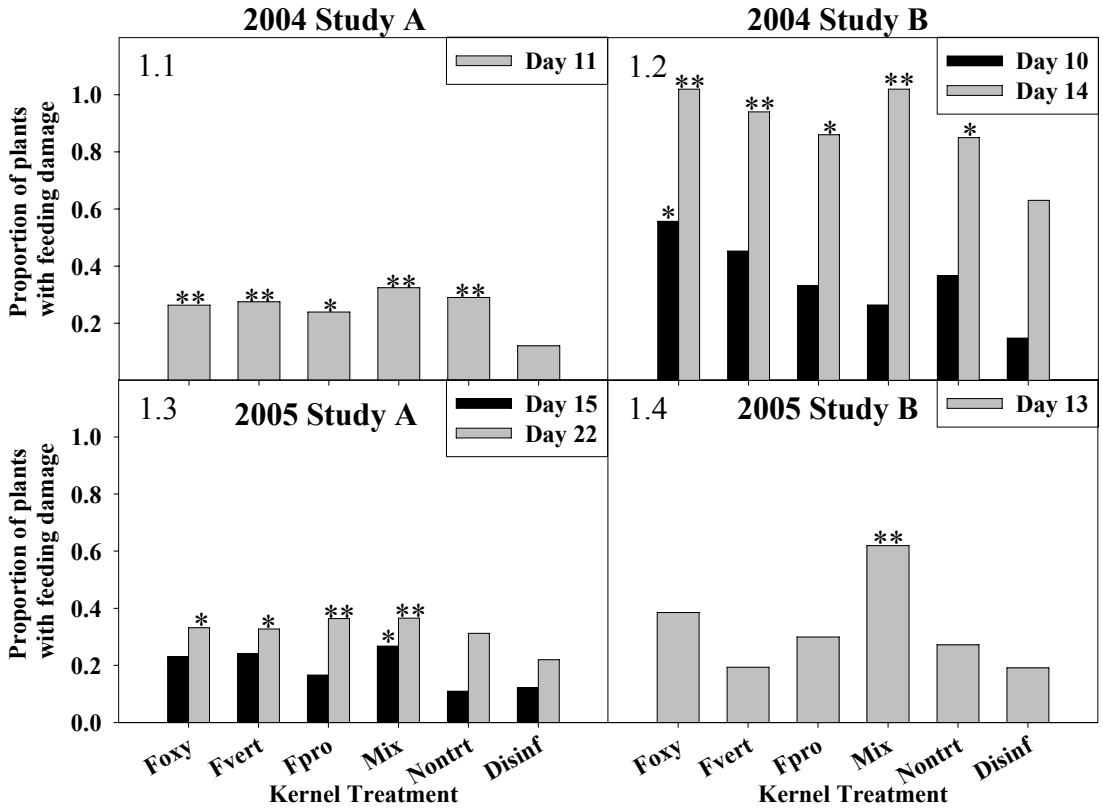

Figure 4.1. Mean proportion (transformed arcsine√y) of sweet corn plants with leaf feeding injury from WSCB adults. Plants were grown from kernels infested with *F. oxysporum* var. *redolens* (Foxy), *F. verticillioides* (Fvert), *F. proliferatum* (Fpro), all three *Fusarium* species combined (Mix), nontreated (Nontrt), or surface disinfested with 3% H_2O_2 (Disinf). In 2004 Study A and B, each bar represents the mean based on 15 and 12 plots respectively. In 2005 Study A and B, each bar represents the mean based on 6 and 10 plots respectively. For each graph, * and ** indicates significantly different at P = 0.05 (Dunnett adjusted) and P = 0.01, respectively, relative to plants grown from disinfested kernels.

In 2005 seedling evaluations, leaf feeding damage and plant growth was initially low overall, possibly due to cooler weather that occurred in late June and early July of that year, so the evaluations were done at later dates compared to 2004 studies. The proportion of plants with foliar feeding damage was greater ($P \leq 0.05$) when kernels were infested with a mix of *Fusarium* species compared to the plants grown

from disinfested kernels at day 15 in 2005 study A (Figure 4.1.3). By 22 days after sowing, the proportion of plants with foliar feeding damage was significantly greater in plants grown from *Fusarium*-treated kernels compared to plants grown from disinfested kernels. In 2005 study B, plants grown from only the mixed *Fusarium* species-treated kernels resulted in significantly greater proportion with leaf damage relative to plants grown from disinfested kernels (Figure 4.1.4).

Rootworm damage

In 2004, mean severity of root damage by larval WSCB in corn plants sampled at maturity was significantly greater ($P \leq 0.05$) for plants grown from any *Fusarium*-treated kernels compared to plants grown from disinfested kernels (Figure 4.2.1). In 2005, root injury levels were slightly higher than levels in 2004. Disinfested kernels resulted in plants with less root damage than most *Fusarium* treatments and the difference was significant for all except plants grown from *F. verticillioides*-treated kernels (Figure 4.2.2).

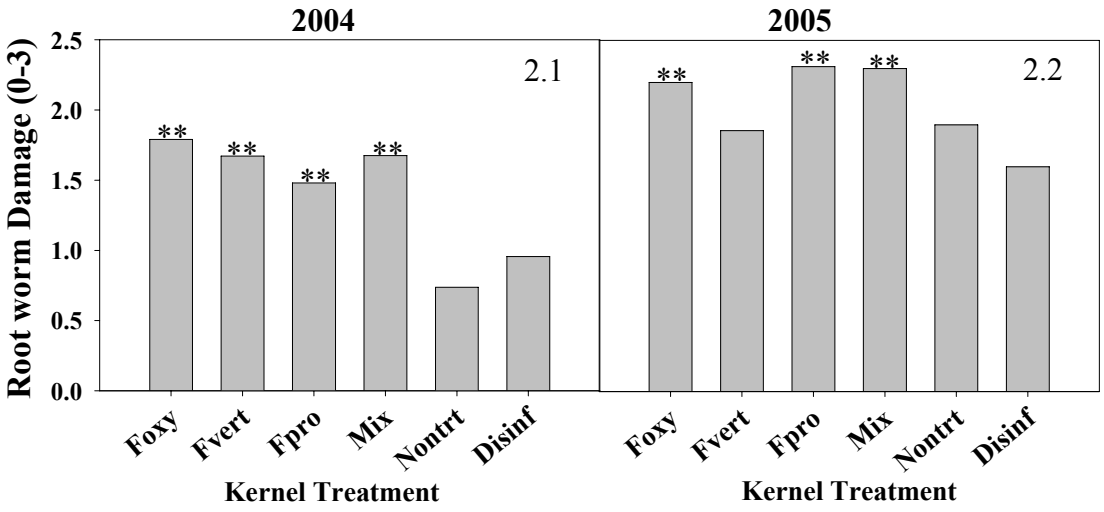

Figure 4.2. Mean severity rating of rootworm damage on sweet corn plants sampled at maturity. Severity ratings were based on: 0 = no root tunneling observed on any roots; 1 = one to three roots had rootworm tunneling; 2 = more than three roots with tunneling but less than half the roots in the root ball were affected; and 3 = more than half the roots in the root ball had tunneling. Plants were grown from kernels infested with *F. oxysporum* var. *redolens* (Foxy), *F. verticillioides* (Fvert), *F. proliferatum* (Fpro), all three *Fusarium* species combined (Mix), nontreated (Nontrt), or surface disinfested with 3% H_2O_2 (Disinf). For each graph, ** indicates significantly different ($P \leq 0.01$, Dunnett adjusted) from disinfested control. Each bar represents the mean based on 8 plots in 2004 and 6 plots in 2005.

Laboratory evaluations of leaf feeding

In both of the 2004 laboratory leaf feeding studies, seedlings grown from *Fusarium*-inoculated kernels tended towards greater numbers of feeding regions per plant than plants grown from disinfested kernels, but only means for plants grown from *F. verticillioides* and the *Fusarium* mixture-treated kernels were significantly different ($P \leq 0.05$) from the mean of plants grown from disinfested kernels (Figure 4.3.1). All plants from *Fusarium*-treated kernels had slightly greater, though generally nonsignificant, severity of the largest leaf feeding region compared to the plants from disinfested kernels. In 2004 plants grown from *F. verticillioides*-treated kernels had

significantly greater severity of leaf damage compared the disinfested control study A, and the mixed *Fusarium*-treated kernels had significantly greater severity of damage compared the disinfested control in study B (Figure 4.3.3). In both 2005 leaf feeding studies, all plants grown from *Fusarium*-inoculated kernels had more feeding regions per plant than the plants grown from disinfested kernels. The mean number of feeding regions on plants grown from disinfested kernels was significantly lower than plants grown from *F. oxysporum*-treated kernels (Figure 4.3.2). There were no significant differences in the severity data in 2005 (Figure 4.3.4).

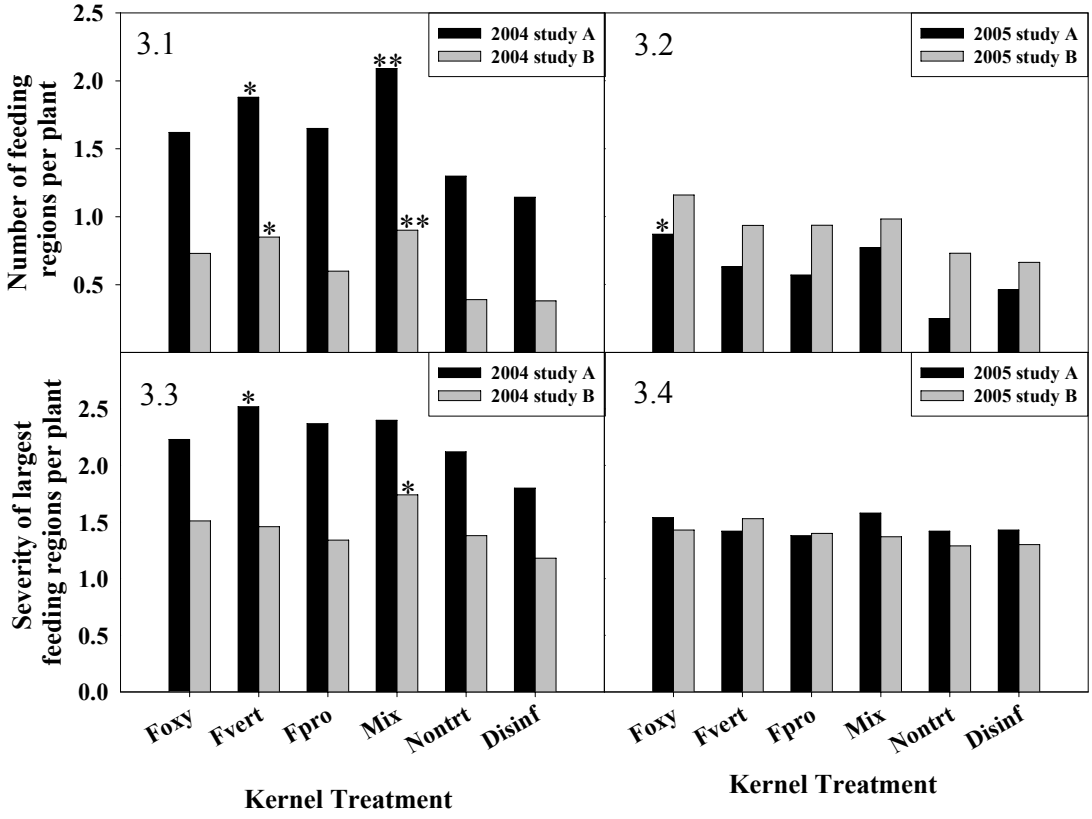

Figure 4.3. Mean number of WSCB leaf feeding regions and mean severity rating of leaf injury to laboratory grown sweet corn seedlings. Severity was rated on the following scale: 1 = small bite or scrape, 2 = visibly missing tissue up to 2 mm in length, and 3 = any feeding region is larger than 2 mm in length. Plants were grown from kernels infested with *F. oxysporum* var. *redolens* (Foxy), *F. verticillioides* (Fvert), *F. proliferatum* (Fpro), all three Fusarium species combined (Mix), nontreated (Nontrt), or surface disinfested with 3% H_2O_2 (Disinf). Each bar represents the mean based on 4 and 12 rag dolls in 2004 study A and B respectively, and 8 rag dolls in both 2005 studies. For each graph, * indicates significantly different at P = 0.05, (Dunnett adjusted) and ** at P = 0.01, relative to disinfested control.

Laboratory evaluation of oviposition

Eggs of *Diabrotica* could be found on kernels or adjacent germination paper in the rag doll studies. In all rag doll studies, all six treatments were available to the beetles in equal numbers in each cage or aquarium. Egg masses were recorded in all rag dolls experiments. A total of 72 egg masses were found in all experiments and 65 of the egg masses were found on plants grown from *Fusarium*-inoculated kernels (Table 4.3). Thirty egg masses were found in total in the 2005 aquarium oviposition study A while no egg masses were found in the 2005 aquarium oviposition study B. Oviposition occurred in both preliminary experiments and in the 2005 leaf feeding experiments, but not the 2004 leaf feeding experiments. the probability of any one treatment having only three egg masses, as in the disinfested kernels, or four, as in the nontreated kernels, of the 72 total egg masses occurring randomly was 0.007 and 0.023 respectively. The results of the randomization test on the combined data indicate that plants grown from *Fusarium* infested kernels were more likely to be chosen for oviposition than plants from disinfested or nontreated kernels. Plants grown from *F. verticillioides, F. oxysporum* var. *redolens, F. proliferatum,* and the *Fusarium* mixture-treated kernels had 15, 12, 12, and 14 more egg masses, respectively, compared to plants grown from disinfested kernels. Kernel treatments with *Fusarium* species had a greater probability for presence of egg masses than that would be expected by random chance as indicated by P-values.

Table 4.3. Number of egg masses observed on sweet corn seedlings grown in rag dolls of each *Fusarium* treatment in all rag doll experiments where eggs were found

	Number of egg masses in each study						
Kernel treatment[α]	Preliminary study 2004	2005 Leaf feeding study A	2005 Leaf feeding study B	Preliminary oviposition study 2005	2005 aquarium oviposition study A	Total[β]	P[γ]
Foxy	4	3	3	1	4	**15**	0.041
Fvert	3	0	1	4	10	**18**	0.004
Fpro	3	2	1	3	6	**15**	0.041
Mix	5	1	0	6	5	**17**	0.015
Nontrt	0	0	0	0	4	**4**	0.99
Disinf	0	0	1	1	1	**3**	.
Total	15	6	6	15	30	**72**	

[α] Plants were grown from kernels infested with *F. oxysporum* var. *redolens* (Foxy), *F. verticillioides* (Fvert), *F. proliferatum* (Fpro), all three *Fusarium* species combined (Mix), nontreated (Nontrt), or surface disinfested with 3% H_2O_2 (Disinf).
[β] the sum of egg masses found on seedlings of each treatment in all five experiments.
[γ] P values indicate the frequency in which any treatment had a difference as large as or larger than the observed difference seen in 5000 randomly generated data sets.

Discussion

Plants grown from *Fusarium*-infested kernels generally suffered more damage from adults and larvae of the WSCB relative to damage that occur on plants grown from disinfested kernels (generally *Fusarium*-free); it appears that adults of the WSCB will preferentially feed and oviposit on plants grown from *Fusarium*-infested kernels. In field studies using *Fusarium*-infested kernels, the mean number of leaf feeding regions attributed to adult WSCB was generally significantly greater for plants grown from kernels inoculated with *F. oxysporum*, *F. proliferatum*, *F. verticillioides*, or a mixture of these three species compared to plants grown from disinfested kernels. Evaluations of leaf-feeding by WSCB in laboratory studies showed greater injury when plants were grown from kernels infested with *F. verticillioides* or a mixture of the three

Fusarium species compared to plants grown from disinfested kernels. In laboratory leaf feeding and oviposition studies, oviposition occurred on disinfested kernels, presumably *Fusarium*-free, only three times out of 72 total events, while the other eggs were laid on seedlings grown from kernels inoculated with *Fusarium*. Plants grown from kernels infested with *F. oxysporum*, *F. proliferatum*, or a mixture of these three *Fusarium* species had greater levels of rootworm damage compared to plants grown from disinfested kernels in both 2004 and 2005 field trials. Plants grown from *F. verticillioides*-treated kernels had greater rootworm damage than plants grown from disinfested kernels in the 2004 field trial but not the 2005 field trial. Insects in other genera have been shown to be more prevalent on corn inoculated with *F. verticillioides* (9,32), and oviposition preference of stem and ear boring lepidopteran pests (Pyralidae) correlated with this *Fusarium* species has been reported in West Africa (2) but there are no reports to demonstrate that plant pathogens influence feeding or oviposition preference in any *Diabrotica* species.

Insect larvae often do not have options as to which plants they will develop on due to limited mobility compared to flying adults, so plant choice for oviposition is critical for larval survival. Rootworm damage was greater on plants grown from *Fusarium*-inoculated kernels compared to the disinfested controls in our studies and could be due to oviposition choices, survival rates, larval vigor, or a combination of these factors. Higher numbers of egg masses found on plants from *Fusarium*-treated kernels gives evidence that differences in larval damage to roots may be partly due to oviposition preferences.

The ability of adults to distinguish *Fusarium*-infected from non-infected seedlings could be due to gustatory, olfactory or visual cues, or some combination of these factors. When damage severity was recorded, the *Fusarium*-treated plants typically had greater severity but these differences, when significant, were relatively small. The severity ratings indicate how much feeding a beetle did once it had already chosen a particular plant. It could be that gustation plays only a small role in the beetles' decision to feed since severity differences were weak relative to incidence. It is unclear whether the fungi themselves or the plants' physiological responses to the fungi are attracting the beetles. It was occasionally observed in the laboratory leaf feeding studies that beetles would feed directly on the hyphae of the *Fusarium* isolates which had grown through the germination and wax papers. Beetles on two occasions chewed through papers and reached the inoculated kernels of the mixed-*Fusarium* species treatment. This suggests that the beetles can be directly attracted to *Fusarium* species. However, kernel-applied fungal inoculum was sown below the soil surface in field studies so an olfactory signal directly from the fungi would be minimized. Nonetheless, *Fusarium* could be present in aboveground plant tissues, as transmission from kernels to seedlings has been shown to be highly efficient with *F. verticillioides* (27) and another *Fusarium* species, *F. subglutinans* (19).

Pathogens which can be dispersed by insects would benefit from evolving mechanisms to attract insect vectors. Volatile compounds such as acetaldehyde, ethyl acetate, and some alcohols produced by *F. verticillioides* have been shown to be attractive to sap beetles (Coleoptera: Nitidulidae) (3). Fruity aromas associated with

many esters, including ethyl acetate, are considered to be important insect attractants (11,15). However, clover root borers have been shown to be attracted to diseased clover roots but not to isolates of *Fusarium* species that cause root rot on clover (23), suggesting that volatiles made by plants under stress may play a larger role in some circumstances.

This is the first report that shows that the presence of plant pathogens increases plant damage by *Diabrotica* species. *Fusarium*-infected seedlings may be attracting beetles through olfactory or visual cues, or the beetles may be retained at higher levels around *Fusarium*-infected once the beetles find the infected seedlings. It has been shown in cucumber that populations of southern corn rootworms are lower in cucumber plants inoculated with plant growth promoting rhizobacteria (40), indicating that plant-associated microbial populations may influence host preference in *fucata*-type of *Diabrotica* species. Information concerning the nature of the signal acting as an attractant would be useful for trying to develop control strategies. If the signal is a volatile chemical that attracts gravid females to a host then this could be used to bait traps and catch females before they oviposit on corn.

The question arises as to why adults of the WSCB would preferentially feed on plants grown from kernels inoculated with *Fusarium* species. The presence of a pathogen or endophytic fungus can have a range of effects on insect herbivory. Pathogens will often induce defense responses which may or may not have an effect against herbivory. There are examples where inducing one type of defense pathway comes at a cost to the plant's ability to induce defenses against a different form of

attack (6,7,17,34). Although pathogens and insects can induce many similar biochemical responses in plants, some responses are more specific to either insect or pathogen attack. Insect herbivory is associated with induced resistance through the jasmonic acid signal pathway and pathogen attack is more often associated with systemic acquired resistance (SAR) through the salicylic acid signal pathway (6). In some cases, growth rates of insect herbivores are increased when grown on plants where SAR is artificially induced (7,34). Insects would have an evolutionary advantage if they could recognize plants under stresses caused by pathogens or other non-insect stressors. Insects may also obtain beneficial compounds directly from fungal pathogens of plants. *Fusarium verticillioides* has been shown to produce a pigment that has growth promoting effects on coleopteran pests of stored grain (18). In contrast, fumonisin, zearalenone, fusaric acid and other secondary metabolites from *F. verticillioides*, *F. oxysporum*, and *F. solani* have been shown to reduce larval weight and inhibit pupation in *Heliothis virescens* F. (1). Further studies are needed to determine if there are any differences in growth and reproduction of WSCB reared on *Fusarium*-infected corn compared to non-infected corn.

Understanding the relationships among corn, pathogenic *Fusarium* species, and the WSCB could lead to control strategies that reduce pressure from both pathogenic *Fusarium* and the WSCB larval feeding. Although WSCB can use multiple larval hosts, much of its reproduction may be occurring on corn since corn is the host that typically suffers damaging larval feeding levels. Another *Diabrotica* species, *D. speciosa*, a *fucata*-type that can use several larval hosts, is reported to have the greater

emergence rates on corn, which is also the host plant on which it oviposits most, compared to other host crop plants (35). The WSCB is known to migrate between several important crop species grown in close proximity in the Willamette Valley. Most notably are migrations between snap beans (*Phaseolus vulgaris* L.) that provide a high protein diet for egg development in adult females, and sweet corn, the preferred larval host (J. Luna, personal communication). Control strategies that reduce reproduction of the WSCB on corn could benefit snap bean production, as well as other crops affected by adult WSCBs. Current fungicide seed treatments provide protection during germination and seedling stages but probably do not prevent *Fusarium* species from moving from the kernel to the seedling. If *Fusarium* infection is affecting oviposition, larval survival, or subsequent rootworm damage, then root damage may be reduced by controlling seed-borne *Fusarium* species. The results of these studies suggest that *Fusarium* infection is at least affecting oviposition, making efforts to reduce *Fusarium* in seed worth pursuing.

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Chapter 5

General Conclusions

Nathan L. Miller

Based on the studies reported here of plants from commercial sweet corn fields, crown rot, measured as a grayscale, appears to be the disease symptom most closely associated with reductions in ear weight. In regression models that included crown grayscale and site as explanatory variables, crown grayscale was highly significant, while no other rot variables, including root rots, were significant at the $P = 0.05$ level. Increased nodal root rot was not significantly associated with ear weight, and in most cases there was a positive slope, suggesting that there is more root rot when there are larger ears. It may be that when plants are further along in ear development before root rot incites or symptoms express and subsequently have more developed (larger) ears, these plants are susceptible to severe root rot during later ear maturation.

Nodal grayscale was strongly correlated with decreased fluid movement through the stalk, suggesting that darker nodes restrict sap passage through the xylem end-walls. Since the crown region is anatomically a stack of nodes, it is likely that the same type of relationship exists between fluid movement and crown grayscale. If transpiration is reduced or blocked at the lower portion of the stalk, affected plants may undergo moisture stress, resulting in a cascade of physiological events that are detrimental to foliage and developing ears.

The results of the pathogenicity studies indicate that all three *Fusarium* species investigated can have negative effects on corn plants, but the effects vary according to

species. *Fusarium oxysporum* and *F. verticillioides* showed significant reductions in ear weight and greater necrosis of crowns across most studies and should be considered potential agents of crown and node disease in the Willamette Valley. Seed kernel inoculations enable close contact between a pathogen during early stages of root or shoot development, and it appears that this position on corn kernels is conducive for crown infection by these fungal species. Kernel inoculations resulted in consistently darker crowns and lower ear weights when *F. oxysporum* and *F. verticillioides* were applied, although somewhat less consistently so for the latter species. *Fusarium oxysporum* is not typically considered an abundant seed-borne pathogen on sweet corn, but early infections can occur from soil-borne inoculum in close proximity to kernels at planting. *Fusarium oxysporum* was the most commonly isolated *Fusarium* species from crowns of sweet corn plants in growers' fields, and it was more frequently isolated from crowns than stalk nodes, suggesting that initial infections may take place lower on the plant. The presence of *F. verticillioides* was not lesser in the lower stalk node relative to the crown region, as was found for *F. oxysporum* treatments, but rather increased slightly, which may be due to infections starting also at leaf axils as well as possible seed-borne or soil-borne infections.

Having the ability to produce chlamydospores, *F. oxysporum* may to be better adapted to survive in soil, while *F. verticillioides* doesn't produce chlamydospores but does make long chains of microconidia that are adapted for aerial dispersal (1). These differences may explain some of the patterns seen in isolation data. But there may also be a successional population shift or colonization pattern that was beyond the scope of this dissertation.

Plants grown from *Fusarium*-infested kernels generally suffered more damage from adults and larvae of the Western spotted cucumber beetle (WSCB) relative to damage that occur on plants grown from disinfested kernels (generally *Fusarium*-free); it appears that adults of the WSCB will preferentially feed and oviposit on plants grown from *Fusarium*-infested kernels. Oviposition occurred more frequently on plants grown from *Fusarium*-inoculated kernels than on plants from disinfested or nontreated kernels, and it is likely that differences seen in larval damage is due to oviposition choices made by adult females rather than increased larval vigor.

These studies indicate that crown and stalk node rot can cause reductions in ear weight and should be considered when evaluating germplasm or disease management techniques. Root rot had little correlation with ear weight in these studies. However, when plants have severe root rot, a negative impact on yield would not be unexpected, so root rot should not be discounted when making management decisions. There is justification to investigate production or disease management practices that limit the presence or activity of potentially pathogenic *Fusarium* species on seed corn. For sweet corn, kernels are usually chemically treated to protect seedlings but this doesn't likely prevent systemic infection resulting from seed-borne inoculum. Disinfesting seed with hydrogen peroxide or other disinfectant may be useful for research purposes but not in commercial production of corn products. Understanding the relationship between *Fusarium* and the WSCB, if it is proven that *Fusarium* acts as some attractant, could provide additional incentive for managing *Fusarium* infection if it would also reduce root worm damage.

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