AN ABSTRACT OF THE THESIS OF

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Impact of natural infection of common corn smut (*Ustilago maydis*) on processing characteristics of three F_1 hybrid sweet corn (*Zea mays L.*) cultivars was evaluated in a two-year study with early and late spring planting dates. At harvest maturity, size and location of galls were recorded and quality characteristics measured. Galls on the lower stalk, upper stalk or tassel reduced fresh weight and diameter of husked ears while galls on the base reduced fresh weight only. Ear length was reduced by galls on the upper stalk. As gall size increased from 0 to larger than 10.2 cm. diameter, ear fresh weight and diameter decreased. The presence of galls larger than 10.2 cm diameter reduced ear length. Kernel depth was not affected by size or location of a gall. Additional ears of the same three cultivars were sampled from commercial fields planted in mid-season near Walla Walla and Patterson, Wa. Galls located on the upper and lower stalk reduced fresh weight, length, diameter and kernel depth, while galls on the tassel or base had little or no effect on these parameters. As gall size increased, fresh weight, length, diameter and kernel depth decreased.

A white yeast-like fungus was observed associated with kernels of sweet corn in mature fields, and was hypothesized to be *U. maydis*. Frequently kernels associated with

this symptom leaked their contents to surrounding tissue. These kernels would become dark during processing and resulted in reduced ear yield and quality. Several tests were conducted to confirm the identity of the fungus. Using a Polymerase Chain Reaction (PCR) technique referred to as a CAPS (Cleaved Amplified Polymorphic Sequence) procedure, isolates of this unknown fungus, when compared to known *U. maydis* isolates, were identical. To support the data from the PCR test a traditional mating test was done which paired known and unknown isolates. In addition, greenhouse inoculation tests using both known and unknown isolates resulted in symptom development consistent with *U. maydis* infection.

The Effect of Common Corn Smut (*Ustilago maydis*) on Sweet Corn (*Zea mays L.*) in the Columbia Basin

by

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

Sarah Blatchford, Author

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (Continued)

Effect of Common Corn Smut (*Ustilago maydis*) on Sweet Corn (*Zea mays L.*) in the Columbia Basin

Chapter 1

INTRODUCTION

The Midwest and the Pacific Northwest are the leading producers of processed sweet corn (Washington Agricultural Statistical Service, 1997). The majority of processed sweet corn acreage in the Pacific Northwest is produced in the Columbia Basin (central Washington and northeastern Oregon). Approximately 98,000 acres of sweet corn for processing are produced annually. In 2002, the farm market value of sweet corn produced in Washington was $66,000,000 (Washington Ag Stats, 2002).

Common smut (*Ustilago maydis*), a fungus, occurs world wide and can cause losses in dent corn that range from a trace to 10% (Shurtleff, 1980). Losses occur when galls replace the kernels of the ear, or when a gall on the plant causes the quality measurements of the ear to decrease. Losses due to *U. maydis* on sweet corn crops can be much greater due to the high susceptibility of flint corn, an ancestor of sweet corn (Bojanowski, 1969).

Common smut of corn is caused by a gall producing basidiomycete, characterized by chlorosis, stunting and the production of galls that can grow on any above-ground location of the plant. (Christensen, 1963; Banuettt and Herskowitz, 1988). Galls of common smut are filled with dark soot-like spores called teliospores. Teliospores are the over-wintering structures of the pathogen. In the spring, when the temperature rises to 26-38 C, the teliospore germinates and forms a promycelium (Shurtleff, 1980). From the promycelium, haploid sporidia are dispersed into the wind and onto young corn plants.

Two compatible sporidia fuse to form a dikaryon which infects the plant (Christensen, 1963). Development of teliospores from current season infections can also contribute to infection in corn seeded later in the season.

In 1996, *U. maydis* was identified on 'Supersweet Jubilee' in the southern Columbia Basin near Hermiston, OR. In the next few years *U. maydis* became widespread throughout the Columbia Basin, causing serious economic losses. In the late 1990's, a variety trial was begun at the Hermiston Agricultural Research and Extension Center (HAREC) to assess the susceptibility of different corn varieties based on natural infection. The varieties were planted in an area that had high levels of disease for several seasons. The results of that trial showed that some of the most common varieties grown in the Columbia Basin were consistently susceptible to *U. maydis* (Clough et al., 2003)

Throughout the 1990's, sweet corn growers in the Columbia Basin produced a limited number of varieties for processing. 'Jubilee', 'Supersweet Jubilee', 'Krispy King' and 'Sheba' were among the varieties that were grown in the highest acreages. Apparently due to the susceptibility of these varieties, in addition to a favorable environment, *U. maydis* spread quickly from the southern Columbia Basin to the northern Columbia Basin (Philip B. Hamm., pers. comm.).

Processors in the Columbia Basin have experienced yield reductions due to *U. maydis* in several ways. When *U. maydis* infects the silks of a corn plant, it can replace the kernel of corn with a gall (Christensen, 1963). As the plant matures, a bouquet of galls can emerge from the sheath of corn where a healthy ear would grow. When this occurs, the ear is a complete loss.

Additional losses occurred in the processing plant when ears either were made into kernel corn, to be either canned or frozen, or cut into 10.2 cm sections for "cobbettes." In processing plants that only produce kernel corn, any ear of corn with a gall on it is discarded (Bill Ficket, Symon Frozen Foods, pers. comm.). Likewise, when producing cobbettes, sold to restaurants, grocery stores, and fast food franchises such as Kentucky Fried Chicken, cobbettes cannot be made when galls develop, making the ear a cull.

Additional losses also occurred due to the dark, soot-like teliospores produced within the galls of *U. maydis*. When these spores enter the processing complex, they contaminate the wash water and the spores become embedded between the kernels of the corn on the cob. Once lodged between the kernels, the spores cause a discoloration. In this case processors can only use the ear for kernel corn, whereas cob corn is a more valuable product.

Lastly, before cob or kernel corn is frozen, it is blanched. Once blanched, cob or kernel corn was found to have kernels darkened by the process. Although the cause of the dark kernels was unknown, the incidence of smut in the field correlated with the incidence of dark kernel after processing (John Louma, AgriFrozen Foods, pers. comm.).

In fields where the dark kernel syndrome was, a white fungus was observed on the kernels under the leaf sheaths, often times associated with moisture or "leaks". Initially, this appeared to be Fusarium stalk rot of corn (*Fusarium moniliforme*), or Gibberella ear rot of corn (*Fusarium graminearum*), but microscope observation of spores associated with this problem suggested another cause. *F. moniliforme* and *F. graminearum* generally produce conidia that are sickle-shaped. This unknown fungus

produced cigar-shaped spores, through budding, similar to a yeast. Interestingly, the haploid stage of *U. maydis* is documented as being a nonpathogenic, saprophytic yeast (Christensen, 1963).

Yield losses due to *U. maydis* have in the past been related primarily to infection of the ear itself. One objective of the work reported here was to determine whether a relationship existed between ear quality and galls located elsewhere on the corn plant. Previous research from the early 1900's suggests that there is quality loss to the ear when the plant is infected with a smut gall (Immer and Christensen; 1928, Johnson and Christensen, 1935), but an accurate assessment of the extent of loss on infected modern sweet corn varieties has not been explored. The second objective of this project was to determine whether the yeast-like fungus associated with kernels was *U. maydis*.

Chapter 2

LITERATURE REVIEW

Life Cycle of *Ustilago maydis*

Smut pathogens infect many other cereals as well as corn. Diseases such as loose smut of wheat (*Ustilago tritici*) and loose smut of barley (*Ustilago nuda*) are seed borne and attack the plant systemically, and therefore can be controlled through the use of chemical seed treatments (Agrios,1997). In contrast, common smut of corn *(Ustilago maydis)* is a local infection, and can not be controlled through seed treatment (Christensen, 1963).

Common smut of corn is caused by a gall producing basidiomycete. Symptoms produced by the pathogen are chlorosis, stunting and the formation of galls on a corn plant (Christensen, 1963; Banuettt and Herskowitz, 1988). Common smut has been documented wherever corn is grown (Alexopoulos, 1996). Galls of common smut are filled with dark soot-like spores called teliospores. Teliospores can be the over-wintering structures of the pathogen. In the spring, when the air temperature rises to 26-38 C, the teliospores germinate and forms a promycelium (Shurtleff, 1980). From the promycelium haploid sporidia are dispersed in the air or water and onto corn plants. Additional spread by teliospores can occur in- season from plants infected early, spreading spores to later plantings.

Two compatible haploid sporidia must fuse to form a dikaryon. The dikaryon of *U. maydis* is an obligate parasite (Christensen, 1963). Development in the plant as well as teliospore formation is dependent on the formation of the dikaryon (Christensen,

1963). *U. maydis* is frequently polycyclic. The sequence from teliospore to teliospore takes approximately three weeks (Shurtleff, 1980). There can be several cycles of infection each growing season.

Resistance to *U. maydis* has been reported (Clough et al. 2001), although little is known about how the plant defends itself from this pathogen (Thakur et al., 1989). Griffiths (1928), Platz (1929), and Kyle (1929) suggested that the mechanism of defense may be due to morphological features of the sweet corn variety such as how tight the husk is wrapped around the silks or thickness of husk. This may form a physical barrier that prevents the fungus from entering the ear of the plant. This explanation is unlikely, since the fungus is believed to enter the ear through the silks of the ear which extend beyond the husk. In some cases resistance may be a polygenic trait that involves several genes that may condition morphological, functional, and physiological characteristics (Smith and White, 1988).

It has been reported that the dikaryon is the only part of the pathogen which is capable of infecting the plant (Christensen, 1963). The dikaryon grows filamentously with septate hyphae and may enter a plant through direct penetration (Walter, 1934). Snetselaar and Mims (1992, 1993) observed sporidia on leaves and silks to mate, fuse together, become dikaryotic hypha, and then form a terminal appressorium that directly penetrates the host. In the silks, hyphae were observed to grow through many cells in the direction of the ovary, but observance of the fungus growing to the ovary has not been seen, even though this is how kernels are hypothesized to become infected (Shurtleff, 1980). *U. maydis* can also enter a host passively through wounds or through stomata (Mills and Kotze, 1981). The dikaryon first grows intracellularly and then later

intercellularly. It causes the cells of the corn plant to undergo hypertrophy (enlargement of the cells), as well as hyperplasia (uncontrollable division of the cell). The tumors are composed of abnormally growing host cells and filamentous hyphae (Snetselaar and Mims, 1994; Banuett and Herskowitz, 1996). When the tumor replaces a kernel of corn it forms in such a way that the gall is actually hollow. When Snetselaar et al. (2001) examined a hollow gall they reported

> "...it appeared that the ovary wall formed the tumor, and not the gametophyte or embryo. Examination of sections through very young ovaries from ears inoculated 4 days before they were fixed for microscopy confirmed that while the ovary wall contained both plant and fungal cells, the entire ovule inside the ovary was atrophied, and the integuments were collapsed. By contrast, ovaries that were from ears of similar age but had not been inoculated contained well-developed ovules surrounded by integuments."

At the time of tumor induction the dikaryon undergoes karyogamy and becomes diploid. The diploid cells become teliospores and they emerge from the gall to begin the process again (Banuett and Herskowitz, 1996).

Mating types of *U. maydis*

The mating and pathogenic capabilities of *U. maydis* are regulated by two loci in an unusual tetrapolar mating system. The two loci are denoted "a" and "b". Sporidia are completely compatible when they have different alleles at the "a" locus as well as the "b" locus. For example, a haploid sporidia that carries the alleles a1b1 is completely compatible with a haploid sporidia that carries the alleles a2b2 (Rowell and DeVay, 1954), but not with a2b1.

The "a" loci controls mating by attracting two compatible (one a1 the other a2) haploid sporidia (Rowell and Devay, 1954; Rowell, 1955; Puhalla, 1969). The "a" locus encodes pheromones on gene "mfa" and pheromone receptors on gene "pra" (Bolker et. al., 1992). Pathenogenicity is dependent on the compatibility of the "b" loci (Holliday, 1961). The "b" locus has at least 25 different alleles (Puhulla, 1968). The "b" locus encodes transcriptional regulators. These regulators control filamentous growth and pathenogenicity (Romeis et al., 2000; Kahman et al, 1995).

The alleles of *U. maydis*

The research conducted in this trial focuses on the "a" locus because it is easier to obtain two known loci than have to deal with 25 possible alleles at the "b" locus. The "a" locus has been sequenced by Bolker et al. (1992). Their work revealed that the "a1" and "a2" alleles are flanked by sequences of homology. The DNA sequence unique for the "a1" is about 4.5 kb long and the DNA sequence unique for the "a2" allele is about 8 kb long. These data are consistent with Froelinger and Leong (1991) who found through molecular analysis that the "a" alleles have large regions of DNA that have no similarity to each other. However, on either side of the unique DNA sequences are regions of nearly identical DNA sequences. In these sequences of DNA there are single base pair exchanges and small gaps.

The enzyme FNU 4HI can be used to cut the "a1" allele and "a2" allele into fragments that differ by a couple hundred base pairs. This allows easy differentiation between the two alleles (and hence mating types) when used in a modified PCR technique called a CAPS (Cleaved Amplified Polymorphic Sequence) procedure.

Infection of plants with compatible isolates of *U. maydis*

Thakur et al. (1989) developed a consistent technique to inoculate compatible isolates of *U. maydis* and produce galls on the ears. In 1992, D. D. Pope evaluated different techniques of inoculating *U. maydis* into the ear of the corn plant to produce galls. He found that injecting a few milliliters of compatible isolates at the concentration $1x10^6$ sporidia per milliliter through the husk of the cob at the time of silking would give very consistent gall formation. du Toit and Pataky (1999) found that compatible isolates at $1x10^6$ sporidia per milliliter would consistently produce galls when 1-3 ml was injected with a syringe into the silk channel of the ear.

Infection of haploid sporidia in seedlings

Hanna (1929) and Rowell and DeVay (1954) reported that inoculation of sweet corn seedlings with haploid strains of *U. maydis* reduced elongation of leaves and shoots, increased basal shoot diameter, and reduced plant fresh weight. Munnecke (1949) found that certain haploid strains would cause distorted morphologies; curl reactions in the leaves of seedlings and distortions of corn tissue similar to the hyperplasic effect of normal infection. Andrews et al. (1981) also reported haploid isolates of *U. maydis* inoculated into sweet corn seedlings do not form a gall, but affect the health of the plant by causing distortions in plant morphology such as curling of the leaves. In addition, Snetselaar and Mims (1992) concurred that haploid strains of *U. maydis* did cause abnormal morphologies. Strains remained in their yeast like phase and did not promote gall formation.

Yield loss due to *U. maydis*

Yield loss in field corn occurs when the ear of the corn plant is replaced with a smut gall. An entire ear can be completely replaced by galls, resulting in complete loss in that ear. Johnson and Christensen (1935) showed that plants infected with a single gall would have a yield reduction of about 25%. Yield was reduced approximately 50% on plants that had multiple galls. When a single gall was found between the ear and the tassel, yield was reduced about twice as much as a gall between the base of the plant and the ear. Johnson and Christensen also reported large galls reduced yield more than small galls. Gall size is thought to be dependent on the condition of the infected plant, and the environment (Thakur et al., 1989).

This 1930's study was conducted on dent corn, also known as field or grain corn. Because of the highly susceptible nature of the flint corn in sweet corns' ancestry, sweet corn is far more susceptible to *U. maydis* than field corn (Bowjanowski, 1969). Therefore the work reported by Johnson and Christensen (1935) may not accurately describe effects of *U. maydis* infection on sweet corn ear quality characteristics.

Chapter 3

MATERIALS AND METHODS

Ear evaluation

Field trials trials were conducted at the Hermiston Agricultural Research and Experiment Center (HAREC), Hermiston, OR during the summers of 2002 and 2003 to evaluate the impact of *U. maydis* on important sweet corn ear quality characteristics. The objectives of the trial were:

1. To determine the effect of gall location of *U. maydis* on ear quality,
2. To determine the effect of gall size of *U. maydis* on ear quality.

Two plantings, approximately one month apart were established each year. The three hybrids evaluated, 'Sheba' (Hollis Kiel, Harris Moran, pers.comm.), 'FMX 516' (Asgrow) and 'Supersweet Jubilee' (Rogers) represented early, mid, and late maturing varieties, respectively. 'Sheba' requires 1510 heat units (Steve Marshall, Seminis Seed Co., pers. comm), 'FMX 516' 1687 heat units, and 'Supersweet Jubilee' 1750 heat units (Steve Marshall, Syngenta, pers. comm) to reach harvest maturity.

'FMX 516' belongs to the sugary (su) class, while 'Sheba' and 'Supersweet Jubilee' belong to the shrunken 2 (sh_2) class of sweet corn endosperm types. The major difference between these two types of mutants is their endosperm composition. Sugary (su) mutants have endosperms that have very high levels of water soluble polysaccharides. A shrunken 2 (sh_2) mutant variety will accumulate sugar at the expense of starch and lacks the enzyme which converts sugar to starch, thus retaining its

"sweetness." This class of endosperm mutant generally has two to three times as much sugar as a sugary (su) endosperm mutant.

The experimental design was a randomized complete block with four replications. Data were analyzed with SAS Proc GLM (SAS Institute, Cary, N.C.). Means were separated using Duncan's Multiple Range Test.

Plots-2002

In 2002 the plots were established in Adkins Series fine sandy loam (coarse-loamy, mixed mesic Xerollic Camborthid, pH 6.7, 0.9% organic matter) by broadcasting fertilizer (84N-22P-28K-22S-1B) $kg \cdot ha^{-1}$ and disking on 1 Apr. Plots were disked and cultipacked on 29 Apr. and seed was planted on 4 May. Plots were four rows wide, 9.1 m long, and 76.2 cm apart. The in-row spacing was 22.9 cm. Atrazine (1.12 kg-ai/ha) and Dual (metolachor) (1.68 kg-ai/ha) were applied preemergence and incorporated with 0.64 cm of irrigation water on 13 May. In-season irrigation was applied according to the Agrimet crop water requirement calculated for HAREC (IRZ, Hermiston OR, www.irz.com) with an overhead center pivot irrigation system. Solution 32 (NH_4NO_3 • $CO(NH_2)_2$) was applied at N rates of 28, 39 and 28 $kg \cdot ha^{-1}$ on 27 June, 5 July, and 12 July respectively. Insecticides were always applied through the irrigation system for earworm control. Asana XL (esfenvalerate) was applied on 11 July and 31 July at 0.50 $kg \cdot ha^{-1}$ ai, Ambush (permethrin) at 0.67 $kg \cdot ha^{-1}$ ai. on 17 July, and Warrior T (lambda-cyholthrin) at 0.15 $kg \cdot ha^{-1}$ ai. on 25 July.

Fertilizer was broadcast on 1 Apr. for the second planting, but discing and cultipacking did not take place until 10 June. Seed was planted on 13 June (su /se) and

14 June (sh_2). The pre-emergence herbicide was applied on 19 June as previously described. Additional fertilizer (Solution 32) was applied at 33, 39 and 28 kg·ha^{-1} on 24 July, 31 July, and 9 Aug. respectively. Insecticides were applied on 7 Aug., (Ambush at 0.67 kg·ha^{-1} ai.), 13 Aug. (Warrior T at 0.15 kg·ha^{-1} ai.), and on 21 Aug. (Asana XL was applied at 0.49 kg·ha^{-1}).

Plots-2003

In 2003 plots were established in late March using the same methods as 2002. On 28 Apr. Gly Star Plus (glyphosate) was applied to emerged weeds at 0.90 l·ha^{-1}. Atrazine (1.12 kg·ha^{-1}) and Dual (1.46 kg·ha^{-1}) were applied preemergence and incorporated with 0.64 cm irrigation. Seed was planted on 2 May. Fertilizer (84N-22P-28K-22S-4Cu-3Zn-1B kg·ha^{-1}) was broadcast on 22 May. Solution 32 was applied at 56, 34 and 34 kg·ha^{-1} N on 10 June, 1 July, and 12 July with 0.64 cm of irrigation water respectively. Earworm control was applied through the irrigation system. Asana XL was applied on 7 July and 28 July at 0.49 kg·ha^{-1} ai., Ambush on 14 July and 12 Aug. at 0.67 kg·ha^{-1} ai., Warrior T on 21 July at 0.15 kg·ha^{-1} ai., and *Bacillus thuringiensis* on 5 Aug. at 1.68 kg·ha^{-1} ai., and Ambush on 12 Aug. at 0.67 kg·ha^{-1} ai.

The second planting of 2003 was maintained weed free with applications of glyphosate on 28 Apr. at 2.20 l·ha^{-1} and on 21 May at 3.19 l·ha^{-1}. Fertilizer (84N-22P-28K-22S-4Cu-3Zn-1.5B kg·ha^{-1}) was broadcast on 22 May. The plot area was roller-harrowed and cultipacked on 4 June, and planted on 6 June. Atrazine (1.12 kg·ha^{-1} ai.) + Dual (1.45 kg·ha^{-1} ai.) was applied on 11 June with 0.64 cm of irrigation water. Solution 32 was added at 34, 56, and 35 kg·ha^{-1} on 1 July, 10 July, 19 July, respectively with 0.64

cm of water througth the irrigation system. Asana XL was applied at 0.49 $kg \cdot ha^{-1}$ ai. on 28 July and 25 Aug., *bacillus thuringiensis* at 1.68 $kg \cdot ha^{-1}$ on 5 Aug., and Ambush at 0.67 $kg \cdot ha^{-1}$ ai. on 12 Aug, and Warrior T was applied on 18 Aug at 0.15 $kg \cdot ha^{-1}$ ai.

Data collection

Corn ears were harvested and evaluated at the same stage of maturity that is required by processors. The processing window of harvest depends on the endosperm mutant type of corn. Sugary (su) endosperm mutants are harvested when kernel moisture ranges from 74%-71%. Shrunken 2 (sh_2) endosperm mutants are harvested in the 77%-75% moisture range (Steve Boyd, Smith Frozen Foods, pers.comm).

Three ears were taken from each plot to determine moisture levels and ear maturity. Moisture readings were determined by using a microwave to dry down the sample in a procedure developed by Becwar (1977). In this method, kernels are removed from three ears and blended. A sample of 10 grams is weighed in a glass Petri dish. Samples are then heated in a commercial microwave oven (Litton FS-10EVP) for 2 minutes at 50% power and weighed again. Moisture content was calculated as *{(original weight – final weight) / original weight} x 100.*

At the appropriate maturity, plants were selected that had one gall or no galls. The location of the gall was recorded.

0 = no gall
1 = gall at the base of the corn plant (brace roots to soil line)
2 = gall between the base of the plant and the ear of the plant
4 = gall between the ear of the plant and the tassel of the plant
5 = gall on the tassel of the plant.

Then the size of the gall was recorded as:

None = 0 cm
Small = less than 5.08 cm diameter
Medium = 5.08 cm -10.16 cm diameter
Large = over 10.16 cm diameter.

These categories were the same as those used by Immer and Christensen (1928). Ten plants that had no gall on them were harvested from each plot. The number of plants harvested with galls in other location and sizes was dependent on how many plants were found in that plot that had galls in the desired locations. The ear was harvested from the plant and brought into the laboratory.

Corn ears were husked, weighed, and measured. Quality characteristics evaluated were fresh weight, diameter of ear, length of ear, and kernel depth (Stall et al., 1989) Measurements for diameter and kernel depth were made with a ninety-two cm caliper (General no 142).

In 2002, the first planting of Sheba (sh_2) was harvested at 72% moisture on 30 July. FMX 516 (su) was harvested on 2 Aug. at 74% moisture, and Supersweet Jubilee (sh_2) on 8 Aug., at 77% moisture. In the second planting Sheba was harvested on 27 Aug. at 76% moisture, FMX 516 on 30 Aug. at 74% moisture, and Supersweet Jubilee on 9 Sept. at 78% moisture.

In 2003, the first planting of Sheba was not sampled due to lack of galls on the plants. FMX 516 was harvested on 11 Aug. at 74% moisture, and Supersweet Jubilee was harvested on 15 Aug at 71% moisture. For the second planting, Sheba was harvested on 22 Aug. at 76% moisture, FMX 516 on 28 Aug. at 74% moisture, and Supersweet Jubilee on 2 Sept. at 75% moisture.

In 2003, additional samples of each of these varieties were taken from commercial production fields planted in late June. Ears were chosen and evaluated as previously described from four areas in each field. Supersweet Jubilee ears were harvested at 78% moisture from a field planted near Walla Walla, Washington on 4 Sept. FMX 516 was harvested at 76% moisture, and Sheba was harvested at 80% moisture on 2 Oct. from commercial fields near Patterson, Washington.

Collection of unknown fungal isolates and experiments

Ears that were infected with the unknown yeast like fungus were collected on 27 Sept. 2002, from commercial fields located near Mesa and Patterson, Washington, and Hermiston, Oregon (Table 1). Supersweet Jubilee (sh_2) and Krispy King (sh_2) varieties were represented. An inoculation loop and sterile technique was used to put the yeast-like fungus on Potato Dextrose Agar (PDA Difco #8) amended with 1 ml streptomycin per liter agar. Single spore colonies were collected from streaked plates and grown and maintained on new PDA plates. Twenty four isolates were randomly selected for additional study. The known a1 and a2 mating types were acquired. These isolates were used throughout laboratory and field experiments.

Table 1. Isolate number, location found and corn variety.

Isolate number	Location	Variety
1	Patterson[1]	Krispy King (sh_2)
2	Patterson	Krispy King (sh_2)
3	Patterson	Krispy King (sh_2)
4	Mesa[2]	Supersweet Jubilee (sh_2)
5	Patterson	Krispy King (sh_2)
6	Patterson	Krispy King (sh_2)
7	Patterson	Krispy King (sh_2)
8	Mesa	Supersweet Jubilee (sh_2)
9	Patterson	Krispy King (sh_2)
10	*U. maydis* a2 isolate (known)	
11	*U. maydis* a1 isolate (known)	
12	Mesa	Supersweet Jubilee (sh_2)
13	Patterson	Krispy King (sh_2)
14	Patterson	Krispy King (sh_2)
15	Mesa	Supersweet Jubilee (sh_2)
16	Patterson	Krispy King (sh_2)
17	Hermiston[3]	Supersweet Jubilee(sh_2)
18	Mesa	Supersweet Jubilee (sh_2)
19	Hermiston	Supersweet Jubilee (sh_2)
20	Mesa	Supersweet Jubilee (sh_2)
21	Mesa	Supersweet Jubilee (sh_2)
22	Mesa	Supersweet Jubilee(sh_2)
23	Patterson	Krispy King (sh_2)
24	Mesa	Supersweet Jubilee (sh_2)
25	Mesa	Supersweet Jubilee(sh_2)
26	Patterson	Krispy King (sh_2)

[1] Patterson, WA
[2] Mesa, WA
[3] Hermiston, OR

Pairing known *U. maydis* with unknown isolates

Day and Anagnostakis (1971) report that when two compatible haploid strains of *U. maydis* are put on a complete medium they will become filamentous, indicating that the yeast like phase has transformed into a filamentous dikaryon. These pairings were done to determine if the unknown yeast-like cultures from the ears would fuse with known *U. maydis* isolates, and if a dikaryon formed, identify the mating type (Table 1). Each isolate was freshly streaked on PDA and allowed to grow for 3 days. Streaks were then placed on one side of each plate of new PDA of either the known mating type a1 (Isolate 11) or the known mating type a2 (Isolate 10). An additional streak was made with the known isolate in the middle of the plate. On the opposite side of the plate was an individual streak of the remaining isolates 1-9 and 12-26. The unknown isolate was also combined in the middle of the plate with the known isolate. Each plate therefore had on either side a haploid isolate, the known on one side and the unknown on the other, with both isolates combined in the middle. The same technique was used with the known mating types a1 (11) and a2 (10). Compatible isolates would form a colony in the center containing filamentous hyphae, and each single streak on each side would maintain a yeast-like growth. The single streak on each side served as a control to confirm filamentous hyphae did not form in the haploid isolate. Plates were observed seven days later for the development of filamentous hyphae.

Determining the identity of the unknown yeast with molecular techniques

Untyped fungal cultures were maintained on PDA at 21C. Fungal DNA was purified from pure *in vitro* cultures by the procedure of Elder et al. (1983) and using a

DNeasy Plant Mini Kit (Qiagen, Valencia, CA). Cultures were typed using two primer sets. The general fungal primers TW81 (5'-GTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (Curran et al., 1994) were used to amplify the ITS1-5.8S tRNA-ITS2 spanning region between the 16S rDNA and 28S rDNA and classify cultures as putative *Ustilago* or non-*Ustilago* based on the amplified product size. A second primer set, *a1a2*-F1 (5'-TATTCTCGTTGCTCTCTATCGTCC-3') and *a1a2*-R1 (5'-TCGATTTCG-GCGTTGCTAGCG-3'), were designed based on alignments of the *U. maydis a1* and *a2* mating type alleles (Urban et al., 1996). This primer set would specifically amplify an approximately 500bp conserved region of the *U. maydis* mating type alleles that contained specific diagnostic CAPS (cleaved amplified polymorphic sequence) sites to be used for assigning mating type. Each unknown isolate, as well as control cultures of the two known mating types (*a1* and *a2*) were used as a template for the diagnostic typing reactions. For both primer sets, 30 μl PCR reactions were performed which contained 1 μl of DNA, 3 μl of 10X Qiagen Taq Polymerase reaction buffer, 0.6 μl 10 mM dNTP, 0.6 μl each primer, 0.12 μl Qiagen Taq polymerase and 24.08 μl of ddH_2O. Reactions were heated to 94C for 2 minutes, then subjected to 30 cycles at 94C for 20 seconds, 50C for 30 seconds, and 72C for 40 seconds in a Gene Amp PCR System 9600. A final extension at 72C for 10 minutes was carried out to complete reactions. Aliquots of each reaction were separated by electrophoresis in a 1% agarose gel and results evaluated following ethidium bromide staining.

For the CAPS-based diagnostic mating type evaluation procedure, the restriction enzyme *Fnu*4HI (New England Biolabs, Beverly, MA) was used. Aliquots from each PCR reaction were digested in a 15 μl reaction which contained 5 μl PCR product, 1X

NEB4 Buffer, 0.1% BSA, and 2.5 units *Fnu*4HI. Half of the digestion reaction was then separated by electrophoresis in a 6% polyacrylamide gel and results evaluated following ethidium bromide staining.

Inoculating *U. maydis* into seedlings of sweet corn - Greenhouse

This trial was conducted in 2003 at the Hermiston Agricultural Research and Experiment Center (HAREC). Supersweet Jubilee (Rogers) was chosen as the host variety due to its susceptibility to *U. maydis*. Sunshine Plug Mix No. 5 was used as a potting mix (peat moss, vermiculite, dolomitic lime added for pH adjustment, Gypsum, wetting agent) and Osmocote (14-14-14) was added at 0.5 cm^3 per cell. Trays with 7.62 x 7.62 x 10.2 cm cells were prepared and seeds were planted approximately 3.8 cm deep. Two seeds were planted in each cell on 15 Sept. Trays were put on benches in the greenhouse at 28C. Overhead irrigation was applied 3 times per day for 4 minutes, and by hand as needed. Seedlings were thinned to one seedling per cell on 22 Sept.

On 8 Oct. (Table 2) isolates were re-streaked on new PDA plates and allowed to grow until 11 Oct. Samples of each were removed from the plates and put into test tubes of distilled water. Tubes were agitated on the vortex to disperse spores. A hemocytometer based estimate of the concentration of sporidia was about 2 x 10^7 cfu/ml.

Haploid sporidia or combinations of compatible sporidia (Table 2) were injected at the base of the seedlings. A 20 gauge (B-D) hypodermic syringe was used and approximately 0.5 ml of solution was injected per seedling. Generally a small hole was poked through the stem and then the solution was injected in the middle of the stem. It was possible to see the liquid rise on the inside of the outer leaf. Control plants were

injected only with water and evaluations were made approximately 10 days later to determine which treatments developed symptoms or galls. Eight seedlings of each treatment were inoculated.

Inoculating *U. maydis* into large corn plants

Greenhouse Trial

On 1 Apr. 2003, Supersweet Jubilee was planted in 3.5 l pots. The soil mixture was composed of one-third Sunshine Plug mix no. 5 and two-thirds sandy soil (an Adkins series fine sandy loam). Three cm^3 of Osmocote (14-14-14) was added to each pot and mixed into the soil. Thirteen pots were planted with 20 to 30 seeds in each pot and then thinned to four plants/pot one week after planting.

The pots were placed in the greenhouse at 28C and received 150 ml of water five times per day. Fertilizer was applied at 100 ppm nitrogen/water once a week, beginning seven weeks after planting.

Tasseling began on 16 May. The silks were covered with paper bags (size 8) on 23 May. Four or five ears were covered for each treatment. The check was not covered.

On 2 June, sporidia were scraped from PDA plates using sterile technique and put into 10 ml of water. Isolates 1 (a1), 2 (a2), 10 (a2 known), and 11 (a1 known) (Table 1) were selected and used singly or in combination (Table 26). Sporidia were counted with a hemocytometer and estimated to be at a concentration of 2×10^7 cfu/ml. A 20 gauge (B-D) syringe was used to inject 1-2 ml of sporidia solution down the silk channel of each ear. Nothing was injected down the silk channel of the check treatment. Silks were also "painted" with sporidia. By this method silks were first cut to a uniform length, then

a paint brush was used to spread the sporidia solution over the tops of the silks. For one of the treatments, isolate 10 (a2 known) was painted on one half of the silks and isolate 11 (a1 known) was painted on the other half to see if the isolates would form galls or remain yeast-like. Four or five plants of most treatments were inoculated.

On 30 June 2003 ears were harvested at maturity. Husks were pulled back from the ears and analyzed for the presence of the yeast-like fungus. Four ears that had not been exposed to the environment were selected for sampling. The husk of the ear was peeled back and the yeast-like fungus was isolated on PDA + 1 ml streptomycin per liter agar using an inoculation loop. One isolation was made from each of four ears. Single spore isolates were then obtained. Two phenotypically different isolates appeared on one plate, so isolates were separated, resulting in five isolates total. Isolates D and E came from the same plate.

These isolates displayed two different phenotypes. Cultures A, B, and D were classified "normal" phenotype, indicating that they were the same consistency as the known mating types of *U. maydis*. Cultures C and E were "slimy" indicating that they were not of the typical *U. maydis* phenotype. Each isolate was paired with all other isolates as well as the known a1 and a2 mating types on agar plates as described previously. After one week plates were analyzed for presence of filamentous hyphae, indicating a compatible reaction between two haploid isolates of *U. maydis* (Day and Anagnostakis, 1971).

Table 2. Identification of treatments used when seedlings were inoculated in the greenhouse.

Treatment	Isolate(s)
1	1
2	2
3	1 x 2
4	10 (a2 known)
5	11 (a1 known)
6	10 x 1
7	11 x 2
8	10 x 11
9	control

[1] see table 1

Field trial

Plant preparation

The protocol used in this experiment was adapted from the one used by Snetselaar et al. (2001) to inoculate ears of corn with isolates of *U. maydis*.

On 10 July 2003, seventy ears of 'Supersweet Jubilee' of the same maturity phase, nearly silking, were selected from a four row plot 36.6 m long in the variety trial (for complete detail see Plots 2003). Only the middle rows were chosen to decrease border effects. White Weyerhaeuser (no. 12) bags were put on top of the tassel with a large clear produce bag on top to prevent overhead irrigation from spoiling the bags. Staples were used along the bottom edge of the bags, and one staple anchored the bag to the leaves of the plant.

The developing primary ear was similarly covered and topped with a clear plastic bag to prevent the silks from being exposed. Bags were paper clipped to the adjacent leaf to allow easy removal without damaging the leaves. On 15 July, silks were cut so that they formed a uniform length to allow easy inoculation the next day.

Inoculum preparation

Inoculum was prepared by inoculating 100ml of Potato Dextrose Broth (PDB) with one loopful of the isolates; 1 (a1), 2 (a2), 10 (a2), and 11 (a1), that had been growing on PDA for three days (Table 1). Flasks were put on a stir plate at low speed with a magnetic stir rod for 18 hours. A piece of Styrofoam 2.54 cm thick was put on top of the stir plate to prevent heat from reaching the flasks. This procedure mimicked the motion

of a rotary shaker. The flasks were left overnight (18 hrs) and the sporidia were harvested and the concentration was determined to be approximately 1.6 X 10^7 cfu/ml.

On 16 July 2003 one ml of inoculum was injected down the silk channel of each corn plant that had been covered. Each isolate was injected separately, then isolates 1 and 2 were combined and isolates 10 and 11 were combined and injected as well. The control was injected with 1 ml sterile PDB. Tassels were shaken after injection in an attempt to pollinate the silks, but high wind prevented good pollination. Bags were put back on top of the ears, but were removed from the tassels. On 17 July, tassels were shaken on exposed corn silks to promote pollination.

On 24 July 2003 two bags were removed from each treatment to determine the effect of taking off the bag, compared to leaving the bag on, for the rest of the growing season.

This experiment was replicated in the second planting on 'Supersweet Jubilee.' On 4 Aug. corn plants were bagged and on 7 Aug. the ears were inoculated the same as before. The concentration of the sporidia was 6.5 X 10^7 cfu/ml. Evaluations were made for the presence of the yeast-like fungus on 15 Sept. 2003. Corn ears were husked and observations were made.

Chapter 4

RESULTS

Ear quality evaluation – Research station

Sweet corn ear fresh weight was greater in 2003 than in 2002 (Table 3). Ear fresh weight decreased from the early to the late planting. The ear fresh weight of Supersweet Jubilee was greater than Sheba which was greater than FMX 516. Ear fresh weight was reduced by a gall on the base or tassel of the plant, and was further reduced by a gall on the lower or upper stalk. Ear fresh weight decreased as gall size increased. Fresh weight, however, was influenced by interactions between year, planting, variety, gall location and gall size.

In 2002 the fresh weight of FMX 516 and Sheba tended to increase from the early to the late planting, but the difference was not significant (Table 4). Supersweet Jubilee ear fresh weight, however, increased from the early to the late planting. In 2003 the early planting of Sheba was not sampled due to lack of galls, but the ear fresh weight of FMX 516 and Supersweet Jubilee decreased from the early to the late planting.

Gall location did not affect ear fresh weight for the early planting in 2002 (Table 5). At the late planting however, fresh weight was reduced by a gall on the upper stalk, and reduced further by a gall on the lower stalk. In 2003 for the early planting, ear fresh weight was reduced by a gall on either the tassel or the upper stalk (there were no galls found on the lower stalk); for the late planting, ear fresh weight was reduced by a gall on the base, lower stalk or tassel and was further reduced by a gall on the upper stalk.

Ear fresh weight of FMX 516 was reduced by a gall on the base, lower or upper stalk of the plant (Table 6). Ear fresh weight of Sheba was not reduced regardless of gall

location. However, ear fresh weight of Supersweet Jubilee was reduced by a gall on any location of the plant.

Ear fresh weight of FMX 516 in the early planting was not affected regardless of gall size (Table 7). Ear fresh weight at the late planting was reduced by a gall less than 5.1 cm in diameter, reduced further by a gall 5.1 – 10.2 cm, and reduced further by a gall larger than 10.2 cm in diameter. Ear fresh weight of Sheba in the early planting was not affected by gall size, however there were no galls larger than 10.2 cm. In the late planting, ear fresh weight was not affected by a gall up to 10.2 cm but was reduced by a gall larger than 10.2 cm. In the early planting of Supersweet Jubilee ear fresh weight was reduced by a gall up to 10.2 cm in diameter, and further reduced by a gall larger than 10.2 cm in diameter. In the late planting, ear fresh weight of Supersweet Jubilee was not reduced by a gall less than 5.1 cm in diameter, but was reduced by a gall 5.1 – 10.2 cm in diameter and was reduced further by a gall over 10.2 cm in diameter.

Fresh weight was reduced by a gall at the base of the plant when the gall was 5.1-10.2 cm and further reduced by a gall larger than 10.2 cm in diameter (Table 8). Fresh weight was reduced by a gall of any size on the lower stalk. Fresh weight was reduced by a gall on the upper stalk when the gall was 5.1-10.2 cm and further reduced when the gall was larger than 10.2 cm in diameter. Fresh weight was reduced by a gall on the tassel when the gall was less than 5.1 cm and was further reduced by a gall larger than 10.2 cm in diameter.

Table 3. Sweet corn ear quality as affected by year, planting, variety, gall location, and gall size, HAREC.

	Fresh weight (g)	Diameter (cm)	Length (cm)	Kernel depth (cm)
Year (Y)				
2002	275	4.68	21.0	0.83
2003	287	4.92	20.8	1.02
	****	****	NS	****
Planting (P)				
Early	285	4.83	20.5	0.97
Late	277	4.74	21.2	0.89
	*	****	****	*
Variety (V)				
FMX 516	262 c	4.67 b	20.7 b	0.86 b
Sheba	285 b	4.85 a	21.2 a	0.91 ab
SS. Jubilee	297 a	4.83 a	21.1 a	0.99 a
	****	****	****	*
Gall location (L)				
None	297 a	4.90 a	21.2 a	0.97
Base	282 b	4.83 a	20.8 a	0.89
Lower stalk	252 c	4.57 c	20.7 a	0.86
Upper stalk	242 c	4.45 c	19.9 b	0.84
Tassel	282 b	4.78 b	21.1 a	0.89
	****	****	*	NS
Gall size (cm) (S)				
0	297 a	4.90 a	21.2 a	0.97
< 5.1	277 b	4.75 b	20.9 a	0.89
5.1 – 10.2	267 c	4.65 c	20.8 a	0.86
> 10.2	242 d	4.50 d	20.3 b	0.84
	****	****	*	NS
Interactions				
P x V	**	**	NS	NS
Y x V	*	*	NS	NS
Y x P x V	*	*	**	*
Y x L	**	NS	NS	NS
P x L	*	NS	NS	NS
Y x P x L	*	****	NS	NS
V x L	*	*	NS	NS
P x S	*	NS	NS	NS
P x V x S	*	NS	NS	NS
L x S	*	*	NS	NS

****,**,*,NS Significant at $P \leq 0.0001$, 0.01, 0.05 or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 4. Sweet corn ear fresh weight as affected by year, planting, and variety interaction.

	Variety					
	FMX 516		Sheba		Supersweet Jubilee	
	2002	2003	2002	2003	2002	2003
Planting	Fresh weight (g)					
Early	245	312	277	----	260	370
Late	260	245	295	277	307	287
	NS	****	NS	----	****	****

****, NS Significant at $P \leq 0.0001$, or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 5. Sweet corn ear fresh weight as affected by year, planting, and gall location interaction.

	Year			
	2002		2003	
	Early	Late	Early	Late
Gall location	Fresh weight (g)			
None	267	292 a	347 ab	297 a
Base	267	295 a	365 a	265 b
Lower stalk	240	262 c	----	247 b
Upper stalk	212	270 bc	337 b	192 c
Tassel	265	287 ab	312 c	265 b
	NS	*	**	***

***, **, *, NS Significant at $P \leq 0.001$, 0.01, 0.05 or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 6. Sweet corn ear fresh weight as affected by variety and gall location interaction.

	Variety		
	FMX 516	Sheba	Supersweet Jubilee
Gall location	Fresh weight (g)		
None	277 a	290	322 a
Base	240 b	272	295 b
Lower stalk	230 b	240	277 c
Upper stalk	235 b	257	242 d
Tassel	270 a	290	302 b
	***	NS	****

****, ***, NS Significant at $P \leq 0.0001$, 0.001, or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 7. Sweet corn ear fresh weight as affected by planting, variety and gall size interaction.

	Variety					
	FMX 516		Sheba		Supersweet Jubilee	
	Early	Late	Early	Late	Early	Late
Gall size (cm)	Fresh weight (g)					
0	280	275 a	277	295 a	327 a	317 a
< 5.1	280	252 b	270	290 a	285 b	307 ab
5.1 – 10.2	202	230 c	305	285 a	265 b	295 b
> 10.2	237	210 d	----	237 b	207 c	262 c
	NS	*	NS	***	****	****

****,***,*, NS Significant at $P \leq 0.0001$, 0.001, 0.05 or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 8. Sweet corn ear fresh weight as affected by gall location and gall size interaction.

	Gall location			
	Base	Lower stalk	Upper stalk	Tassel
Gall size (cm)	Fresh weight (g)			
0	297 a	297 a	297 a	297 a
< 5.1	295 a	257 b	295 a	280 b
5.1 – 10.2	282 b	255 b	255 b	297 a
> 10.2	270 c	237 b	152 c	262 c
	****	****	****	****
Contrast				
Gall vs. none	NS	----	****	----

****, NS Significant at $P \leq 0.0001$, or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Ear diameter was greater in 2003 than 2002, and greater in the early planting than the late planting (Table 3). The diameters of Supersweet Jubilee and Sheba were similar, and greater than the diameter of FMX 516. Ear diameter was reduced by a gall on the tassel of the plant and further reduced by a gall on the lower or upper stalk. Ear diameter was reduced by a gall less than 5.1 cm in diameter, reduced further by a gall 5.1-10.2 cm in diameter and reduced further by a gall larger than 10.2 cm in diameter. However, ear diameter was influenced by interactions between year, planting, variety, gall location and gall size.

The diameter of FMX 516 and Sheba did not change from the early to the late planting in 2002, but the diameter of Supersweet Jubilee increased significantly (Table 9). In 2003 the ear diameter of FMX 516 and Supersweet Jubilee decreased from the early to the late planting. The diameter of Sheba could not be compared.

In the early planting of 2002 and 2003 ear diameter was not affected by gall location (Table 10). In the late planting of 2002 and 2003 however, ear diameter was reduced by a gall on the upper or lower stalk of the plant.

Ear diameter of FMX 516 was reduced by a gall on the lower or upper stalk of the plant (Table 11). Ear diameter of Sheba was not affected by gall location. Ear diameter of Supersweet Jubilee was reduced by a gall on the base, lower stalk, or tassel of the plant and reduced further by a gall on the upper stalk of the plant.

Ear diameter was not affected by a gall up to 10.2 cm in diameter at any gall location (Table 12). Ear diameter was reduced by a gall larger than 10.2 cm in diameter on the upper stalk or tassel of the plant.

Table 9. Sweet corn ear diameter as affected by year, planting and variety interaction.

	Variety					
	FMX 516		Sheba		Supersweet Jubilee	
	2002	2003	2002	2003	2002	2003
Planting	Diameter (cm)					
Early	4.47	5.23	4.88	----	4.60	5.31
Late	4.52	4.62	4.80	4.90	4.83	4.85
	NS	****	NS	----	****	****

****, NS Significant at $P \leq 0.0001$, or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 10. Sweet corn ear diameter as affected by year, planting and gall location interaction.

	Year			
	2002		2003	
	Early	Late	Early	Late
Gall location	Diameter (cm)			
None	4.70	4.80 a	5.28	4.93 a
Base	4.65	4.75 ab	5.44	4.85 a
Lower stalk	4.47	4.55 c	----	4.67 b
Upper stalk	4.24	4.62 bc	5.26	4.04 c
Tassel	4.65	4.67 abc	5.23	4.80 ab
	NS	****	NS	***

****,***, NS Significant at $P \leq 0.0001$, 0.001, or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 11. Sweet corn ear diameter as affected by variety and gall location interaction.

	Variety		
	FMX 516	Sheba	Supersweet Jubilee
Gall location		Diameter (cm)	
None	4.78 a	4.90	5.02 a
Base	4.60 ab	4.88	4.83 b
Lower stalk	4.39 b	4.70	4.72 b
Upper stalk	4.47 b	4.80	4.45 c
Tassel	4.75 a	4.83	4.72 b
	****	NS	***

****,***,NS Significant at P ≤ 0.0001, 0.001, or not significant, respectively. Means followed by different letters significantly different at P ≤ 0.05 (Duncan's Multiple Range Test).

Table 12. Sweet corn ear diameter as affected by gall location and gall size.

	Gall location			
	Base	Lower stalk	Upper stalk	Tassel
Gall size (cm)		Diameter (cm)		
0	4.90	4.90	4.90 a	4.90 a
< 5.1	4.83	4.57	4.93 a	4.78 a
5.1 – 10.2	4.88	4.60	4.52 a	4.75 a
> 10.2	4.72	4.52	3.76 b	4.47 b
	NS	NS	*	**

**,*,NS Significant at P ≤ 0.01, 0.05 or not significant, respectively. Means followed by different letters significantly different at P ≤ 0.05 (Duncan's Multiple Range Test).

Ear length was similar in 2002 and 2003 (Table 3). Ear length increased from the early to the late planting. Ears of Supersweet Jubilee and Sheba were similar in length, and larger than the ears of FMX 516. Ear length was reduced by a gall on the upper stalk. Ear length was also reduced by a gall larger than 10.2 cm in diameter. These values were influenced by interactions between variety, year, and planting.

In 2002, ear length of Sheba and Supersweet Jubilee increased from the early to the late planting, and while length tended to increase for FMX 516, the difference was not significant (Table 13). In 2003 ear length increased from the early to the late planting for FMX 516, but not for Supersweet Jubilee. (There were no data for the first planting of Sheba.)

Kernel depth was greater in 2003 than in 2002 (Table 3). Ear kernel depth decreased from the early to the late planting. Ear kernel depth of Supersweet Jubilee was greater than that of FMX 516, while kernel depth of Sheba was intermediate. Kernel depth was not affected by gall location or gall size. Kernel depth was influenced by an interaction between year, planting and variety.

Ear kernel depths for the 2002 did not differ from the early to the late planting (Table 14). In 2003, however, kernel depth of FMX 516 decreased from the early to the late planting. Ear kernel depth for Supersweet Jubilee in the late planting tended to be reduced, but was not significantly different.

Table 13. Sweet corn ear length as affected by year, planting and variety interaction.

	Variety					
	FMX 516		Sheba		Supersweet Jubilee	
	2002	2003	2002	2003	2002	2003
Planting	Length (cm)					
Early	20.5	19.6	20.7	----	20.4	22.2
Late	21.1	22.6	21.6	20.9	21.4	21.1
	NS	****	***	----	****	NS

****,***, NS Significant at $P \leq 0.0001$, 0.001, or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 14. Sweet corn ear kernel depth as affected by year, planting and variety interaction.

	Variety					
	FMX 516		Sheba		Supersweet Jubilee	
	2002	2003	2002	2003	2002	2003
Planting	Depth (cm)					
Early	0.79	1.02	0.89	----	0.86	1.02
Late	0.79	0.91	0.86	0.99	0.86	0.94
	NS	****	NS	----	NS	NS

****, NS Significant at $P \leq 0.0001$, or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Ear quality evaluation- Commercial production fields

Although differences in ear fresh weight of the three varieties were significant in the analysis of variance test, Duncan's Multiple Range Test failed to separate them because of outliers in the data set caused by exceptionally small ears (Table 15). Ear fresh weight was reduced by a gall on the base of the plant, further reduced by a gall on the lower stalk and reduced most by a gall on the upper stalk. Ear fresh weight was reduced by a gall 5.1 – 10.2 cm and reduced further by a gall larger than 10.2 cm in diameter. Ear fresh weight was influenced by interactions between variety, gall location, and gall size.

Ear fresh weight of FMX 516 was reduced by a gall on the base and further reduced by a gall on the lower stalk (no galls were found on the upper stalk) (Table 16). Ear fresh weight of Sheba was reduced by a gall on the base or lower stalk, and further reduced by a gall on the upper stalk. Fresh weight of Supersweet Jubilee was reduced by a gall on the upper stalk.

Ear fresh weight of FMX 516 and Supersweet Jubilee were reduced by a gall larger than 10.2 cm in diameter (Table 17) while ear weight of Sheba was reduced by a gall 5.1 cm diameter or larger.

Table 15. Sweet corn ear quality as affected by variety, gall location and gall size, commercial fields, 2003.

	Fresh weight (g)	Diameter (cm)	Length (cm)	Kernel depth (cm)
Variety (V)				
FMX 516	250	4.85 b	19.6 b	0.89 b
Sheba	257	5.03 a	18.3 c	0.94 a
S. Jubilee	255	4.55 c	20.6 a	0.89 b
	****	****	****	*
Gall location (L)				
None	277 a	4.88 b	20.3 a	0.91
Base	250 b	4.78 bc	19.8 a	0.89
Lower stalk	232 c	4.63 c	18.9 b	0.89
Upper stalk	215 d	4.37 d	18.9 b	0.84
Tassel	287 a	5.16 a	20.0 a	0.97
	****	**	****	NS
Gall Size (cm) (S)				
0	277 a	4.88 a	20.3 a	0.91 a
< 5.1	262 ab	4.88 a	19.6 b	0.91 a
5.1 – 10.2	247 b	4.75 b	19.4 bc	0.89 ab
> 10.2	220 c	4.55 c	19.0 c	0.86 b
	***	*	**	**
Interactions				
V x L	****	*	****	NS
V x S	*	NS	NS	NS
L x S	****	****	*	****

****,***,**,*, NS Significant at $P \leq 0.0001$, 0.001, 0.01, 0.05 or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 16. Sweet corn ear fresh weight as affected by variety and gall location interaction.

	Variety		
	FMX 516	Sheba	Supersweet Jubilee
Gall location		Fresh weight (g)	
None	264 a	291 a	275 a
Base	238 b	249 b	261 a
Lower stalk	216 c	213 b	259 a
Upper stalk	----	126 c	226 b
Tassel	282 a	295 a	----
	****	****	*

****,* Significant at $P \leq 0.0001$, or 0.05, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 17. Sweet corn ear fresh weight as affected by variety and gall size interaction.

	Variety		
	FMX 516	Sheba	Supersweet Jubilee
Gall size (cm)		Fresh weight (g)	
None	264 a	291 a	275 a
< 5.1	250 ab	272 a	269 a
5.1 – 10.2	244 ab	199 b	273 a
> 10.2	218 b	209 b	224 b
	*	****	****

****,* Significant at $P \leq 0.0001$, or 0.05, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Ear fresh weight decreased similarly regardless of gall size when the gall was located on the base of the plant (Table 18). Fresh weight was reduced by a gall on the lower stalk when the gall was 5.1-10.2 cm, and reduced further when the gall was larger than 10.2 cm in diameter. Fresh weight was reduced by a gall on the upper stalk when the gall was 5.1-10.2 cm and reduced more when the gall was larger than 10.2 cm in diameter. Ear fresh weight increased slightly in response to a gall less than 5.1 cm on the tassel.

Ear diameter was greatest for Sheba, intermediate for FMX 516, and smallest for Supersweet Jubilee (Table 15). Ear diameter was reduced by a gall on the base, further reduced by a gall on the lower stalk, and reduced further by a gall on the upper stalk. Ear diameter slightly increased when a gall was on the tassel. Ear diameter was reduced by a gall 5.1-10.2 cm, and further reduced by a gall larger than 10.2 cm in diameter. Diameter was influenced by interactions between variety and gall location, and gall location and gall size.

Ear diameter of FMX 516 was reduced by a gall on the base or lower stalk (Table 19). Ear diameter of Sheba was reduced by a gall on the lower stalk and further reduced by a gall on the upper stalk. Ear diameter of Supersweet Jubilee was not affected by gall location.

Ear diameter was not affected by galls on the base regardless of gall size (Table 20). Diameter was reduced by a gall on the lower stalk when the gall was less then 5.1 cm and reduced further when the gall was larger than 10.2 cm in diameter. Diameter was reduced by a gall on the upper stalk when the gall was larger than 5.1 cm in diameter.

Diameter showed a slight increase when a gall was on the tassel and was less than 5.1 cm in diameter.

Supersweet Jubilee produced the longest ears, followed by FMX 516; ears were shortest for Sheba (Table 15). Ear length was reduced by a gall on the lower or upper stalk. Ear length was shortened by a gall up to 10.2 cm, and further reduced by a gall larger than 10.2 cm in diameter. Results, however, were influenced by interactions between variety and gall location, and gall location and gall size.

Ear length of FMX 516 was reduced by a gall on the lower stalk (Table 21). Galls were not found on the upper stalk of FMX 516 or on the tassel of Supersweet Jubilee (Supersweet Jubilee was detasseled by the grower). Ear length of Sheba was reduced by a gall on the lower stalk, and reduced further by a gall on the upper stalk. Ear length of Supersweet Jubilee was reduced by a gall on the upper stalk.

Ear length was not reduced regardless of gall size when the gall was located on the base of the plant (Table 22). Ear length was reduced by a gall on the lower stalk when the gall was less than 5.1 cm and reduced further when the gall was larger than 10.2 cm. Ear length was reduced by a gall on the upper stalk when the gall was larger than 5.1 cm in diameter.

Kernel depth was greater for Sheba than for FMX 516 and Supersweet Jubilee, which were similar (Table 15). Kernel depth was not affected by gall location. Kernel depth was reduced by a gall larger than 10.2 cm in diameter. Results, however, were influenced by an interaction between gall location and gall size.

Kernel depth was not affected by a gall at the base or lower stalk regardless of gall size (Table 23). Kernel depth was reduced by a gall on the upper stalk when the gall was 5.1-10.2 cm and further reduced when the gall was larger than 10.2 cm in diameter.

Table 18. Sweet corn ear fresh weight as affected by gall location and gall size interaction.

	Gall location			
	Base	Lower stalk	Upper stalk	Tassel
Gall size (cm)	Fresh weight (g)			
0	276 a	276 a	276 a	276 b
< 5.1	248 b	236 c	275 a	288 a
5.1 – 10.2	249 b	258 b	229 b	----
> 10.2	255 b	214 d	186 c	----
	***	****	****	***
Contrast				
Gall vs None	****	****	****	----

****,***, Significant at P ≤ 0.0001, or 0.001, respectively. Means followed by different letters significantly different at P ≤ 0.05 (Duncan's Multiple Range Test).

Table 19. Sweet corn ear diameter as affected by variety and gall location interaction.

	Variety		
	FMX 516	Sheba	Supersweet Jubilee
Gall location	Diameter (cm)		
None	4.95 b	5.16 ab	4.65
Base	4.75 c	5.03 ab	4.60
Lower stalk	4.65 c	4.88 b	4.57
Upper stalk	----	4.24 c	4.39
Tassel	5.11 a	5.21 a	----
	****	*	NS

****, *, NS Significant at P ≤ 0.0001, 0.05 or not significant, respectively. Means followed by different letters significantly different at P ≤ 0.05 (Duncan's Multiple Range Test).

Table 20. Sweet corn ear diameter as affected by gall location and gall size interactions.

	Gall location			
	Base	Lower stalk	Upper stalk	Tassel
Gall size (cm)	Diameter (cm)			
0	4.87	4.87 a	4.87 a	5.04 b
< 5.1	4.70	4.70 bc	4.90 a	5.16 a
5.1 – 10.2	4.83	4.85 ab	4.47 b	----
> 10.2	4.88	4.57 c	4.15 b	----
	NS	***	****	*

****,***,*, NS Significant at P ≤ 0.0001, 0.001, 0.05 or not significant, respectively. Means followed by different letters significantly different at P ≤ 0.05 (Duncan's Multiple Range Test).

Table 21. Sweet corn ear length as affected by variety and gall location interaction.

	Variety		
	FMX 516	Sheba	Supersweet Jubilee
Gall location		Length (cm)	
None	19.8 a	19.3 a	21.2 a
Base	19.3 ab	18.6 a	20.7 a
Lower stalk	18.4 b	16.8 b	20.4 ab
Upper stalk	----	12.1 c	19.8 b
Tassel	20.1 a	19.7 a	----
	***	****	**

****,***,** Significant at $P \leq 0.0001$, 0.001, or 0.01 respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 22. Sweet corn ear length as affected by gall size and gall location interaction.

	Gall location			
	Base	Lower stalk	Upper stalk	Tassel
Gall size (cm)		Length (cm)		
0	20.3	20.3 a	20.3 a	19.6
< 5.1	19.8	18.8 bc	19.5 ab	19.9
5.1-10.2	19.6	19.6 ab	18.8 b	----
> 10.2	20.1	18.1 c	18.8 b	----
	NS	****	****	NS
Contrast				
Gall vs. None	*	****	----	----

****,*,NS Significant at $P \leq 0.0001$, 0.05, or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Table 23. Sweet corn ear kernel depth as affected by gall location and gall size interaction.

	Gall location			
	Base	Lower stalk	Upper stalk	Tassel
Gall size (cm)		Depth (cm)		
0	0.90	0.90	0.90 a	0.92
< 5.1	0.88	0.87	0.97 a	0.95
5.1 – 10.2	0.91	0.90	0.86 b	----
> 10.2	0.93	0.88	0.76 c	----
	NS	NS	****	NS

****,NS Significant at $P \leq 0.01$, or not significant, respectively. Means followed by different letters significantly different at $P \leq 0.05$ (Duncan's Multiple Range Test).

Pairing known *U. maydis* isolates and unknown isolates

Isolates 1, 3, 5, 12, 20, and 25 developed filamentous hyphae when they were paired with the known a2 *U. maydis* mating type (isolate 10) and did not develop hyphae when they were paired with the known a1 *U. maydis* mating type ((isolate 11) [Table 24]). No filamentous growth developed in any of the streaks with the individual haploid isolate. This would indicate that these isolates were a1 *U. maydis* isolates. Isolates 2, 4, 6, 9, 18, 21, and 22 developed filamentous hyphae when they were paired with the known a1 *U. maydis* isolate, and no hyphae when they were paired with the known a2 isolate. This indicates that these isolates were a2 *U. maydis*. Isolates 7, 8, 13, 14, 15, 16, 17, 19, 23, 24, and 26 failed to develop filamentous hyphae when paired with either mating type. This may be because of identical alleles at the 'b' allele, or because of experimental error.

Determining the identity of the unknown yeast with molecular techniques

Isolates were first typed using the fungal primers to amplify the region between the 16S and 28S rDNAs. In *U. maydis*, this primer set should yield a product of 601 bp. The control isolates 10 and 11 yielded products of the expected size (Figure 1). Isolates 1-6, 8-12, 14, 15, 18, 20, and 22-26 yielded a single band of the expected size for a *U. maydis* culture and were categorized as putative *U. maydis* isolates. Isolates 13, 17 and 19 yielded products of a size distinct from the *U. maydis* band and are therefore categorized as of different fungal origin (non-*U. maydis*). These fell into three distinct sizes, suggesting at least three different fungal species are represented among the non-*U. maydis* isolates. Isolate 7 yielded two bands, one of the expected size for *U. maydis*, and one matching the size of the product from isolates 19 suggesting 7 contains a *U. maydis*

culture contaminated with another fungal species. Isolate 16 failed to yield a band after multiple attempts and may not be a fungal isolate.

The isolates classified as putative *U. maydis*, based on the spacer amplifications were amplified with the a1a2 primer set and the products digested with *Fnu*4HI (Figure 2). This enzyme yields diagnostic bands of 119 and 174 base pairs for the a1 mating type and 293 base pairs for the a2 mating type. Following digestion of the products, isolates 1, 3, 5, 8, 12, 15, 20, 23, and 25, yielded a1 mating type bands, while isolates 2, 4, 6, 9, 18, 21, 22, and 14 yielded an a2 mating type band. Isolates 24 and 26 yielded both diagnostic band sets, implying they are a1a2 diploid cultures of *U. maydis*. Based on these results, it can be concluded that among the isolates evaluated, isolates 1, 3, 5, 8, 12, 15, 20, 23, and 25 are mating type a1, isolates 2, 4, 6, 9, 14, 18, 21, and 22 are mating type a2. Isolates 24 and 26 are *U. maydis* diploid cultures, and that the remaining isolates were not *U. maydis* (see Table 24).

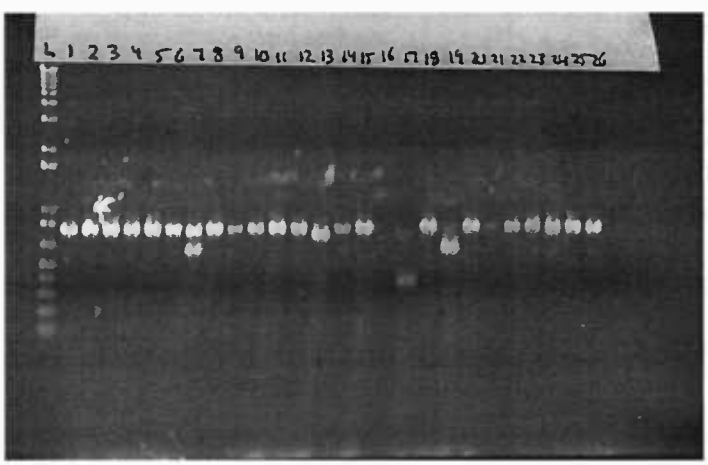

Fig. 1. Amplification of 16S and 28S rDNAs. Isolates 10 and 11 are the known U. maydis mating types

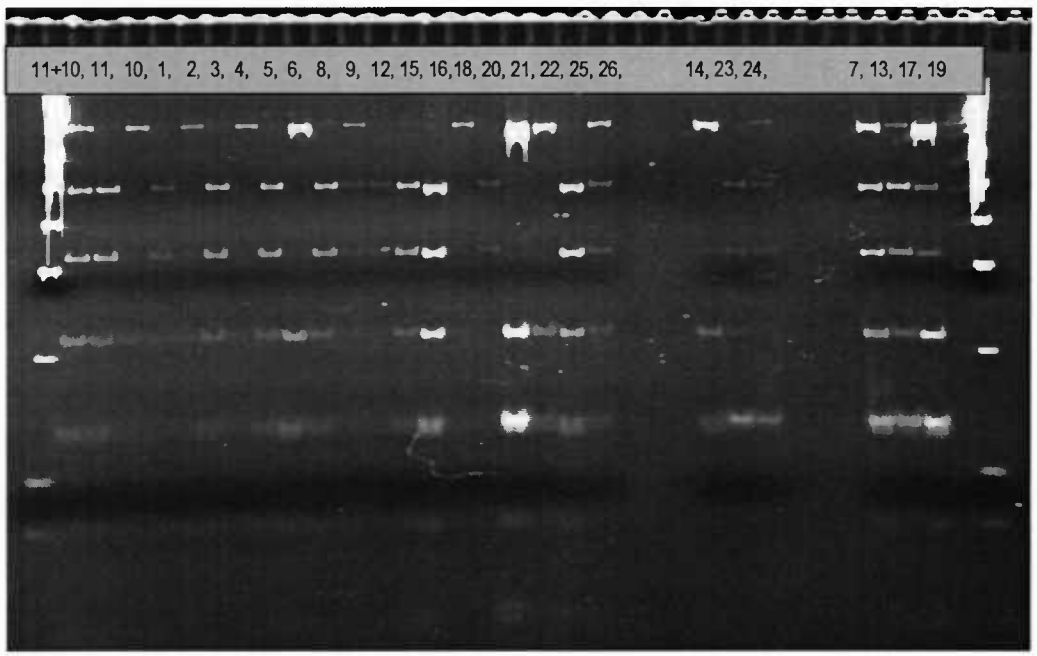

Fig. 2. Amplification of spacer amplifications amplified with a1a2 primer sets and digested with enzyme Fnu4HI. Isolates are in following order: a1a2, 11 (a1), 10 (a2), 1, 2, 3, 4, 5, 6, 8, 9, 12, 15, 16, 18, 20, 21, 22, 25, 26, 14, 23, 24, 7, 13, 17, 19.

Table 24. Mating type identity based on plate pairing and PCR testing.

	Mating test			
	Filamentous hyphae formation		Pairing	Results of
Isolate	11 (known a1)	10 (known a2)	results of unknown	2nd PCR test
1	no	yes	a1	a1
2	yes	no	a2	a2
3	no	yes	a1	a1
4	yes	no	a2	a2
5	no	yes	a1	a1
6	yes	no	a2	a2
7	no	no	--	not *U. maydis*
8	no	no	--	a1
9	yes	no	a2	a2
10	yes	no	a2	a2
11	no	yes	a1	a1
12	no	yes	a1	a1
13	no	no	--	not *U. maydis*
14	no	no	--	a2
15	no	no	--	a1
16	no	no	--	not *U. maydis*
17	no	no	--	not *U. maydis*
18	yes	no	a2	a2
19	no	no	--	not *U. maydis*
20	no	yes	a1	a1
21	yes	no	a2	a2
22	yes	no	a2	a2
23	no	no	--	a1
24	no	no	--	a1, a2
25	no	yes	a1	a1
26	no	no	--	a1, a2

Inoculating *U. maydis* into corn seedlings - Greenhouse

When isolate 1 (a1) was injected into corn plants, two of the plants had distorted morphologies after 7 days (Table 25). Distorted morphologies were characterized by curl reactions in the leaves of seedlings and distortions of corn tissue similar to the hyperplasic effect of normal infection (Munnecke, 1949). Isolate 2 (a2) produced three distorted plants out of eight, and the combined inoculation of two unknown isolates, 1 (a1) and 2 (a2), resulted in seven out of the eight plants producing galls characteristic of *U. maydis*. Inoculating isolate 11 (a1) resulted in three of the plants becoming distorted, similar to the symptoms seen in treatments 1 (a1) and 2 (a2). Isolate 10 (a2) produced four plants with distorted morphologies and when the two known isolates 10 (a2) + 11 (a1) were combined, galls were produced on all eight plants. In a similar manner, when isolate 10 (a2) was combined with isolate 1 (a1), galls were formed on six plants. In the opposite situation when isolate 11 (a1) was combined with isolate 2 (a2), galls developed on six plants. No symptoms resulted when plants were injected only with water.

Table 25. Plants that developed symptoms after being inoculated with *U. maydis*.

Treatment	Isolate(s)	Mating type	Results
1	1	a1	- - 0 0 0 0 0 0
2	2	a2	- - - 0 0 0 0 0
3	1 x 2	a1 x a2	+ + + + + + + 0
4	10	a2	- - - - 0 0 0 0
5	11	a1	- - - 0 0 0 0 0
6	1 x 10	a1 x a2	+ + + + + + 0 0
7	11 x 2	a1 x a2	+ + + + + 0 0 0
8	10 x 11	a2 x a1	+ + + + + + + + +
9	control		0 0 0 0 0 0 0 0

0 = healthy

- = distorted morphologies

+ = galls

Inoculating isolates of *U. maydis* into large corn plants.

Greenhouse trial

The yeast-like fungus was observed on several ears, but only re-isolated from four ears that had not been exposed to the environment outside of the laboratory (Table 26). The four ears were from the 1 (a1) + 2 (a2) painted treatment, isolate 10 (a2) injected treatment, and two ears were from the untreated check. One isolation plate yielded two phenotypically different cultures, and the cultures were separated. Isolate A originated from the 1 (a1) + 2 (a2) painted treatment group, isolate C from the 10 (a2) injected treatment group, and isolates B, D, and E were from the Check treatment group. Isolates D and E were separated from the mixed isolate plate.

When paired, the known U. maydis isolates 10 (a2) and 11 (a1), produced filamentous hyphae (Table 27). Isolates A and B produced filamentous hyphae when paired with the known a1 isolate and unknown isolate D. When A and B were combined, no filamentous hyphae was produced, demonstrating that both are the a2 mating type of *U. maydis*. Isolate D produced filamentous hyphae when paired with the a2 mating type of *U. maydis,* also demonstrating that Isolate D is the a1 mating type of *U. maydis.* Isolates C and E did not produce filamentous hyphae. Isolates C and E differed from the other isolates phenotypically.

Table 26. Results from inoculating *U. maydis* isolates into sweet corn ears in greenhouse.

Inoculation method	Treatments	Mating type	No. of ears inoculated	No. pollinated ears	Yeast or galls
Painted	11	a1	5	1	no
	10	a2	5	1	yeast
	1	a1	5	3	yeast
	2	a2	5	0	----
	.5 11 + .5 10	a1 x a2	4	0	----
	11 + 10	a1 x a2	5	0	----
	1 + 2	a1 x a2	5	1	galls
Injected	11	a1	4	1	no
	10	a2	4	3	yeast
	1	a1	5	1	yeast
	2	a2	4	2	yeast
	11 + 10	a1 x a2	5	0	no
	1 + 2	a1 x a2	4	1	yeast/galls
	check				yeast/galls

Table 27. Compatibility of isolates found in *U. maydis* inoculation trial in greenhouse.

Isolates	Filamentous Hyphae	Isolates	Filamentous Hyphae
A + B	no	C + D	no
A + C	no	C + E	no
A + D	yes	C + 11 (a1)	no
A + E	no	C + 10 (a2)	no
A + 11 (a1)	yes	D + E	no
A + 10 (a2)	no	D + 11 (a1)	no
B + C	no	D + 10 (a2)	yes
B + D	yes	E + 11 (a1)	no
B + E	no	E + 10 (a2)	no
B + 11 (a1)	yes	10 (a2) + 11 (a1)	yes
B + 10 (a2)	no		

[1] isolates originated from the re-isolation from inoculated ears

Field trial

In the first planting, poor pollination prevented most ears from developing. No yeast-like fungus was found on ears that were uncovered a few days after pollination or in the checks (Table 28). Galls did develop when isolates 11 (a1) and 10 (a2) were combined and when isolates 1 (a1) and 2 (a2) were combined. There was also no yeast found in the other treatments.

In the second planting, poor pollination also occurred. No yeast-like fungus was found. On some of the haploid treatments galls were found indicating cross contamination. Galls were also found on the check treatments.

Table 28. Results from inoculating *U. maydis* isolates into sweet corn ears in the field.

		Presence of yeast or galls	
Treatment	Mating type	First planting	Second planting
1	a1	no	galls
2	a2	no	no
10	a2	no	no
11	a1	no	galls
1 x 2	a1 x a2	galls	no
11 x 10	a1 x a2	galls	galls
check		no	galls

Chapter 5

DISCUSSION

Ear quality evaluation

Data from the field trials and the commercial fields were similar. Quality characteristics were reduced by a gall on the base of the plant, but the greatest impact occurred when the gall was on the lower or upper stalk of the plant (Tables 3, 15). The larger the gall size the greater the impact on ear quality.

Quality measurement loss, which depends on the location of the gall, can be explained by understanding the physiology of the plant. The xylem moves water and nutrients from the lower areas to the upper areas of the plant. The phloem transports photosynthate from the source of photosythate, the leaves, to the sink, the part of the plant that is growing (Ritchie et.al, 1986). Most of the photosynthate is produced in the upper leaves, but some is also produced in the lower leaves. When a gall infects the upper stalk of the plant it intercepts nutrients in the phloem and prevents them from reaching the ear of the plant. With less nutrients available to it, the ear will be smaller and less developed. Quite often in the field if there is a gall on the upper stalk of the plant, there will be little to no ear development at all. Galls on the lower stalk do not intercept as many photosynthates so less yield reduction occurs. Likewise, a gall on the base of the plant impedes photosynthate transport far less than a gall on the upper or lower stalk.

A gall on the tassel of the plant did not block photosynthates from getting to the ear, and did not cause a large yield reduction in the ear. The tassel is similar to the ear, it

is a reproductive structure of the plant that does not produce photosynthates, and is designed to receive large amounts of energy from the plant.

The small size of the tassel resulted in mostly small galls developed on the tassel leading to a bias in the data concerning gall size and location. Because this trial relied on natural infection, the number of plants infected in a plot were beyond control and the number of plants in the plot were limited. In the commercial fields it was possible to find as many plants with galls in desired gall locations as were needed.

Many of the interactions in this data set occurred when ears of the first planting in 2003 were larger than the second planting. Typically, ears of sweet corn planted in June will be larger than from corn planted in May, but in 2003 extreme heat prevented the June planting from developing larger ears.

The average temperature for June, July and August was 28.6C in 2002 and 31.0C in 2003 (Appendix A1). Not only was 2003 a hotter summer than 2002, but from 20 July 2003 to 1 Aug. 2003 the maximum temperature in Hermiston averaged 36.7C. This extreme heat in Hermiston occurred at the time the second planting was in the vegetative stage of development between planting and silking. According to Hortik and Arnold (1965) when the air temperature exceeds 29C there is a reduction of vegetative growth. This could cause a reduction in ear growth and quality measurements at the time of harvest maturity. This extreme heat could be responsible for the decreased yields in the second planting of 2003.

Although many of the interactions that occurred can be explained by the extreme heat that reduced the quality measurements in the second planting of corn in 2003, other

interactions occurred when quality reductions followed the same trend as the main effects, but to varying degrees and levels of significance.

In the 1930's Immer and Christensen conducted a study using dent corn that quantified the losses due to galls in more than 1800 plants that were smutted or smut free (Johnson et al, 1935). These lines consisted of hybrids, open pollinated varieties, and inbred lines. Johnson et al. analyzed yield reduction that occurred when galls were on different gall locations of the stalk and were of different sizes. They found the loss in yield was dependent on the size and location of the gall. On average, they concluded that a single smut gall would reduce yield of that ear about 25 percent. This study was conducted on dent corn that was dried and weighed without shelling to calculate yield loss. From the data in this report, little reduction takes place in the depth of the kernel regardless of the location or size of the gall, leaving the loss in yield due to reductions in the cob diameter and length of the ear. A loss in cob diameter or length of an ear of dent corn may have been inconsequential to the value of the crop because there would be little reduction in kernel yield from the ear.

In the current study approximately 1600 smutted and smut free ears of three F1 hybrids of sweet corn were measured. These data provide a more accurate estimate of yield loss for modern sweet corn varieties, particularly for those currently being grown in the Columbia Basin. These data also show that yield reduction is dependent on gall location and size. Each variety reacted similarly; the fresh weight data were contrasted to compare plants with no galls and plants with galls in the field trial and in the commercial fields.

In this study ear fresh weight was reduced 5.0-10.2% by a gall on the base depending on the size of the gall. (Tables 8, 18). Ear fresh weight was reduced 6.5-22.5% by a gall on the lower stalk of the plant depending on the size of the gall. Fresh weight was reduced 13.8-48.5% by a gall on the upper stalk depending on the gall size and fresh weight was reduced 5.7-11.4% by a gall on the tassel.

Another contrast was done to compare the length with and without a gall on the plant (Table 22). It was shown that a gall of any size would reduce the diameter of the ear when the gall was on the lower stalk.

Because kernel depth is not as significantly affected by gall location and gall size as the other quality measurements, fresh weight reduction translates into a reduction of other quality measurements of the ear, the diameter and the length. Because of the specific uses of sweet corn, a reduction in diameter or length may reduce the value of the ear significantly.

A reduction in cob diameter could cause the knives in a processing plant to cut into the kernel of the corn, reducing the percent cut off the corn product. Processors rely on the length of ears to get two "cobbettes" out of each ear. When the length is reduced only one "cobbette" can be cut, resulting in a loss of half of the product.

In trials conducted at the Hermiston Agricultural Research and Extension Center in 1999-2003, up to 67 sweet corn varieties were evaluated for presence and gall location of common smut (Clough et al., 2004). The three varieties in this trial, FMX 516, Sheba, and Supersweet Jubilee had 0.3%, 7.7%, and a 10.9% infection, respectively, of *U. maydis* in the ear. An infection of the ear results in a total yield loss for that ear and is subtracted directly off the tonnage of the crop.

Not only is there a direct yield loss when galls infect the ear, but there are also many implications when a gall infects other locations of the plant. While larger quality reductions take place when a gall is on the lower or upper stalk and when the gall is large, profit loss may occur when any reduction in ear quality characteristics occur, making even a small gall on any part of the plant a threat to processors. Profit margins to the grower are very thin and a small percent reduction in yield may negate any profit.

Determining the identity of the unknown yeast-like fungus

The work reported here confirms the identity of the yeast-like fungus associated with kernels without galls as haploid isolates of *U. maydis*. Mycological and molecular techniques were used to confirm the identity of the unknown isolates. Seventeen of 24 unknown isolates collected from ears were confirmed to be *U. maydis*. Further confirmation of their identity was achieved by pairing these haploid isolates individually with known *U. maydis* isolates of both mating types. Thirteen isolates formed filamentous hyphae when paired with the opposite mating type (Day and Anagnostakis, 1971). When unknown isolates of both mating types were inoculated into corn singly and in combination, only distorted morphologies occurred in the former trial, (Munnecke, 1949), but typical galls of *U. maydis* formed by the combination treatment (Thaker et al., 1989).

The results of this work suggest that neither the a1 or a2 mating type dominate the infection of ears. Of the 17 isolates confirmed to be haploid isolates of *U. maydis* from ears, 9 isolates were confirmed to be a1 and 8 isolates were a2.

While the identity of the yeast-like fungus was confirmed, no direct evidence was produced that proved the haploid isolates of *U. maydis* are responsible for the leaky kernel symptom. However, there is circumstantial evidence that suggests the two are correlated. The leaky kernel symptom has never been seen without the presence of the yeast-like growth on the kernels (Philip Hamm, pers. comm.). Similarly, there has been reported a high correlation of gall incidence in the field and the incidence of dark kernel, the result of heating kernels that have been leaking (John Louma, AgriFrozen Foods, pers. comm.)

In this research, the method developed by Curran et al. (1996) was used for determining whether an isolate was *U. maydis* or of other fungal origin. While the method did not specifically distinguish *U. maydis*, the addition of known *U. maydis* isolates allowed for that determination. A second method was developed to confirm isolate identity as *U. maydis* using a primer set unique to *U. maydis*. A similar method was used by Xu et al. (1996) to confirm *U. maydis*. This method was effective in determining that the unknown isolates were *U. maydis*. However, the unique set of primers and enzyme (Fnu 4HI, CAPS marker) used here not only confirmed the identity of the unknown isolates as *U. maydis* but also proved to be a reliable way to confirm mating type. Seventeen haploid isolates were identified using this technique, only 13 could be identified by pairing using traditional isolates on agar plates.

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APPENDIX

Table A1. Maximum temperature for 2002, 2003 in Hermiston, OR.