IDENTIFICATION, ASSESSMENT AND HISTOPATHOLOGY OF PATHOGENIC *FUSARIUM OXYSPORUM* SPECIES COMPLEX ON SUGAR BEET

By

Kirsten D. Pollok

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Plant Pathology – Master of Science

2025

ABSTRACT

Fusarium yellows of sugar beet is caused by fungi in the *F. oxysporum* species complex (FOSC) and impacts the sugar beet industry from the field to post-harvest storage. The objectives of this work were to 1) identify pathogenic FOSC isolates collected in Michigan on sugar beet and assess their virulence levels and 2) to examine the infection process and to lay the groundwork for identification of potential histopathological differences between *F. commune,* a member of the FOSC and other FOSC members. The first set of objectives were addressed by isolating, identifying, and assessing virulence of Michigan FOSC isolates in a greenhouse assay using foliar and root severity ratings for Fusarium yellows. In comparison to the controls, of the 35 isolates screened in the greenhouse, 5.7% were classified as moderate virulence, and 60% were classified as low virulence, and 34.3% were non-pathogenic. The moderately virulent isolates will be of benefit to subsequent experiments and resistance screening trials targeted to manage Fusarium yellows in Michigan. For the second set of objectives, mature sugar beets were inoculated with an *F. commune* isolate and plants were collected every three days post inoculation through 18 days. The bottom half of the roots were fixed and stained for conventional and confocal microscopy. It was observed that *F. commune* initially colonized the root surface of the tap root and feeder roots. While penetration did occur in the feeder roots, the colonization of the vasculature of the feeder roots did not progress to the main tap root. On the tap root, penetration and colonization of the root interior occurred around the root tip. Hyphae subsequently grew into and traveled via the xylem in cambial rings and the stele from the root tip up to at least as far as the root groove. The knowledge acquired over the course of these experiments will help sugar beet breeding programs and growers make informed decisions on managing Fusarium yellows of sugar beet.

ACKNOWLEDGMENTS

I would like to thank my committee – Dr. Linda Hanson, Dr. Jaime Willbur, and Dr. Frances Trail for their counseling and support throughout my program. I am especially grateful to Dr. Hanson for her mentorship and the passion for knowledge and teaching she has imparted to me. I would also like to thank Tom Goodwill for his help, advice and humor in the lab and green house, as well as my family and friends for all their love and support throughout my program. Lastly, I'd like to acknowledge my dog, Hazel, for being a patient listener to all my practice presentations and proof-readings at home even though it puts her to sleep.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AUDPS	Area under disease progression steps
AV	Avirulent/Avirulence
GUS	Beta-glucuronidase gene
BT	Beta tubulin
BLAST	Basic local alignment search tool
CLA	Carnation leaf agar
V8B	Clarified half strength V8 broth
V8A	Clarified half strength V8 agar
CFU	Colony forming units
CMS	Cytoplasmic male sterility
DPI	Days post inoculation
EDTA	Ethylenediaminetetraacetic acid
dH2O	Distilled water
f. sp.	*formae specialis*
FOB	*Fusarium oxysporum formae specialis betae*
FOL	F. oxysporum f. sp. *lycopersici*
FORL	*F. oxysporum* f. sp. *radices-lycopersici*
FOS	*F. oxysporum* f. sp. *spinaciae*
FOSC	*F. oxysporum* species complex
FOV	*F. oxysporum* f. sp. *vasinfectum*
HV	High virulence/highly virulent
LSD	Least significant difference

LV	Low virulence
MI	Michigan
H1	Microscopy run 1
MIP	Maximum intensity projection
MV	Moderate virulence/moderately virulent
MLSA	Multilocus sequence analysis
NCBI	National Center for Biotechnology Information
Patho.	Pathogenicity
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PI	Propidium iodide
RAPD PCR	Random amplified polymorphic DNA-polymerase chain reaction
SVREC	Saginaw Valley Research and Extension Center
SNA	Spezieller Nährstoffarmer agar
TEF1a	Translation elongation factor 1α
TAE	Tris acetate EDTA
TES	N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid
USDA-ARS	United States Department of Agriculture's Agricultural Research Service
USA	United States of America
VCG	Vegetative compatibility group
WA	Water agar
WPE	Weeks post emergence
WPI	Weeks post inoculation

WGA	Wheat germ agglutinin
ZEN	Zearalenone

CHAPTER 1: LITERATURE REVIEW

Sugar Beet:

Globally, the United States (USA) was third for sugar beet production as of 2022, with the Russian Federation first and France second (Shahbandeh and FAO 2024). In the USA, the sugar beet industry is an agriculture cooperative, with grower-owner involvement from field to factory (Americansugarbeet.org, Accessed 2/21/2025). The majority of sugar beet production regions in the USA are west of the Mississippi river, with the exception of Michigan in the Great Lakes region (Abadam 2021). The production regions west of the Mississippi river include the Red River Valley (Minnesota, North Dakota, and Eastern Montana), the Great Plains (Colorado, Nebraska, Wyoming and Western Montana), and the Far West (California, Idaho, Oregon, and Washington). In regions west of the Red River Valley, sugar beet fields depend on irrigation, whereas in the Red River Valley and Michigan, the dependence is on rainfall (Abadam 2021). Historically, the Great Plains and Far West regions produced higher sugar beet yields, due to longer growing periods and length of daylight hours. These regions also benefitted from lower disease pressure from plant pathogens that benefit from high humidity or water retention in the soil, such as *Cercospora beticola* and *Aphanomyces cochlioides*, respectively (Abadam 2021, Jacobsen and Franc 2009, Windels and Harveson 2009). Through advancements in fungicide use, seed coat treatments, increasing plant spacing, and resistant sugar beet varieties, these differences in yield between the Eastern and Western growing regions have declined (Abadam 2021). The four states leading in sugar beet production by planted area, in order from first to fourth as of 2021 were: Minnesota, Idaho, North Dakota, and Michigan (Abadam 2021). In Michigan, the primary products produced from sugar beet in factories are granulated white sugar, brown sugar (with sugarcane molasses), confectioner's sugar, and liquid sugar. Secondary

products derived from sugar processing byproducts include dried and pressed sugar beet pulp, molasses, betaine and raffinate for animal feed and other industrial uses, and spent factory lime for agricultural topsoil remediation (DeSutter and Godsey 2010, Lewellen et al. 2009, Michigansugar.com, Accessed 11/14/2024). In 2022, the cash value for sugar beet commodities nationwide was $218 million, with Michigan accounting for 12% of nationwide sugar beet profits (USDA NASS 2024). As part of the cash value of this commodity, the sugar beet industry in MI that same year had an economic impact of $600 million directly and $1.5 billion indirectly (USDA NASS 2024).

Sugar beet is grown as a biennial herbaceous crop, with the first year devoted to vegetative growth and sugar accumulation in the root. In the second year, if plants are exposed to a period of vernalization at short day lengths and temperatures ranging from 1-12C, stalk proliferation, flowering and seed production can occur (Hoffman et al. 2021). The majority of sugar beet grown in the US are harvested after one growing season. In the second growing season, post-vernalization, the sugar stored in the roots is consumed by the plant to achieve stalk development and seed production (Hoffman et al. 2021). Sugar beet grown for seed production is grown through the second year primarily in the Willamette Valley of Oregon for certified seed in the USA (Abadam 2021). The primary growth phases of interest in the first year, with emphasis on maturation and development of plant defenses, were emergence, the two true leaf growth stage, and the six to eight leaf growth stage (Trebbi and McGrath 2009). Emergence was characterized by the visible emergence of the seedling from seed and soil, typically occurring as early as four days after planting depending on temperature (Trebbi and McGrath 2009). The two true leaf growth stage occurred at approximately 3 weeks post emergence (WPE) and corresponded to changes in expression of several genes, including the initiation of resistance

factors such as jasmonate, thaumatin and miraculin, leading to differences in susceptibility to diseases in comparison to after the six to eight leaf growth stage (Trebbi and McGrath 2009). Prior to the six to eight leaf growth stage, gene expression was focused on development and proliferation of root structures, through gene expression affiliated with protein metabolism and secretory system (Trebbi and McGrath 2009). After the six to eight leaf growth stage, gene expression shifted toward stress regulation, cell wall development, and more resistance functions – such as abscisic acid, peroxidase, and nematode resistance (Trebbi and McGrath 2009). The six to eight leaf growth stage occurred around 4-6 WPE and was the age at which a beet plant transitions from a seedling to a maturing sugar beet. This corresponded to transitions in a range of gene expression, maturation of the root, as well as shifting to sucrose and dry matter accumulation in the root (Trebbi and McGrath 2009). The development of the nine cambrial rings, a physiological sign of maturation in sugar beet, occurred during this period around 5 WPE. Root growth transitioned from primarily tap root elongation and feeder root proliferation to accumulation of dry matter and sucrose in the tap root around 5 WPE and was fully active after 6 WPE (Trebbi and McGrath 2009). After which the elongation and formation of feeder roots production continued throughout the life of the plant.

Fusarium:

Fusarium species, including isolates from the *Fusarium oxysporum* species complex, have been are associated with numerous sugar beet diseases. These included Fusarium yellows (also known as Fusarium wilt), Fusarium root rot (sometimes called Fusarium tip root rot), Fusarium stalk blight in seed production, and seedling damping off (Abada 1994, Gross and Leach 1973, Hanson 2009, Hanson and Jacobsen 2009, Harveson 2009, Martyn et al. 1989, Stewart 1931). Sugar beet diseases caused by *Fusarium* spp. have been observed across the

globe, including in Asia and Europe, as well as North America (Cao et al. 2018, Christ et al. 2011, Karadimos and Karaoglanidis 2006, Hanson and Jacobsen 2009). Fusarium yellows was first described as sugar beet yellows, caused by a *Fusarium* species provisionally called *Fusarium conglutinans* var. *betae* in 1931 (Stewart 1931). It was isolated from a commercial sugar beet field in the Arkansas Valley, Colorado; it caused disease in both mature sugar beets and seedlings. In mature infected sugar beets, leaves were described as brittle or wilting, developing interveinal yellowing, chlorosis, and necrosis (Harveson 2007, Stewart 1931). Symptoms began with exterior leaves, although with time the interior leaves could curl and twist and develop symptoms as well. The roots developed vascular discoloration ranging from gray to brown and could develop into a vascular dry rot when disease was severe. There were rarely external root symptoms and it was speculated that entry occurred through lateral roots (Stewart 1931). In seedlings, the disease was not shown to prevent emergence, but would cause the leaves of small seedlings to wilt, dry out, and fall off without displaying chlorotic foliar symptoms. Larger seedlings could develop foliar symptoms similar to those of mature sugar beet (Stewart 1931). Discoloration of the vascular system could be seen in seedlings, and was similar to that seen in mature sugar beet. Sugar beet infected with "*F. conglutinans* var. *betae"* had a 60% reduction in weight, and a 36% reduction in sugar content (Stewart 1931). Since this initial discovery, *F. conglutinans* var. *betae* has been reclassified as *F. oxysporum* f. sp. *betae* (FOB) and its role in sugar beet diseases has been expanded to include stalk blight during seed production (Gross and Leach 1973, Snyder and Hansen 1940). A similar organism, *F. oxysporum* f. sp. *radices-betae*, caused sugar beet root rot (Harveson 2007, Martyn et al. 1989). Since that initial report, the description of Fusarium yellows on sugar beet has remained largely unchanged, except for the addition of half-leaf chlorosis as an occasional symptom (Hanson and Jacobsen

2009, Harveson 2007, Windels et al 2007). In the USA, Fusarium yellows due to FOB has been reported in California, Michigan, Minnesota, Montana, Oregon, Nebraska, North Dakota, Texas and Wyoming (Fisher and Gerik 1994, Hanson 2006, Windels et al. 2007).

Fusarium Yellows-Like Diseases:

Other species of *Fusarium* could cause Fusarium yellows-like symptoms, including *F. avenaceum, F. acuminatum, F. solani, F. moniliforme, F. graminearum* and *F. secorum* (Hanson and Hill 2004, Hanson 2007, Ruppel 1991, Secor et al. 2014). In 1991, isolates of *F. avenaceum, F. acuminatum* and *F. solani* caused seedling damping off, while among these isolates, only *F. acuminatum* was able to cause yellows-like symptoms in mature sugar beets (Ruppel 1991). These findings were expanded upon in 2004 as similar species, in addition to *F. moniliforme,* were isolated from symptomatic sugar beets and determined to be pathogenic on mature sugar beet in greenhouse studies (Hanson and Hill 2004). While the 2004 study did not screen for pathogenicity and virulence on sugar beet seedlings, it did demonstrate that these *Fusarium* spp. can cause diseases on sugar beets at multiple growth stages. *F. graminearum* was first reported as a yellows-like pathogen of Minnesota and Wyoming sugar beet in 2007, causing similar symptoms to Fusarium yellows, including interveinal chlorosis, wilting, stunting and vascular discoloration along with rot of root tissue closer to the crown of the beet (Hanson 2007, Hanson and Hill 2004). More recently, a new disease called Fusarium yellowing decline, caused by *F. secorum,* in the *F. fujikuroi* species complex, has been identified (Secor et al. 2014). It was first reported in Minnesota in 2008 as a novel *Fusarium* species (Rivera et al. 2008). Since then, *F. secorum* has been reported throughout the Red River Valley including North Dakota (Webb et al. 2019). Fusarium yellowing decline displays similar foliar symptoms as those of Fusarium yellows, with the notable exception that infection by the fungus progresses beyond the root and

into the petioles to cause vascular discoloration and necrosis of the petioles, whereas with FOB of Fusarium yellows, infection and vascular discoloration was limited to the root in vegetative beets (Rivera et al. 2008, Secor et al. 2014). FOB has only been isolated from petioles of sugar beets post vernalization, in sugar beet exhibiting symptoms of Fusarium stalk blight (MacDonald and Leach 1976a, McFarlane 1981).

Fusarium Root Rot:

There are several *Fusarium* species that could cause root rot in sugar beets, including *F. graminearum, F. culmorum,* and *F. solani* (Harveson 2009), but for the purposes of this body of work, the emphasis will be on Fusarium root rot caused by *F. oxysporum*. Fusarium root rot caused by *F. oxysporum* (putatively f. sp. *radices-betae*) was first reported in Texas in 1989 (Martyn et al. 1989). The foliar and internal root symptoms associated with Fusarium root rot were consistent with those of Fusarium yellows, with the important exception that Fusarium root rot developed an exterior rot of the cortical tissue usually starting at the tip of the tap root (Harveson 2007). Since that initial report, Fusarium root rot caused by *F. oxysporum* has been reported in Colorado and Montana (Hanson and Jacobsen 2006). It was proposed in 1989 to classify *F. oxysporum* isolates that caused Fusarium root rot as a separate *formae specialis* from isolates that caused Fusarium yellows, as f. sp. *radices-betae* (Martyn et al. 1989). The reasons behind this consideration were preliminary evidence that only a handful of isolates caused Fusarium root rot and these isolates had different isozyme production than FOB isolates from the same production region (Texas, Martyn et al. 1989). In tomato, disease systems have been separated in a similar fashion, between *Fusarium oxysporum* f. sp. *lycopersici* for Fusarium wilt of tomato, and *F. oxysporum* f. sp. *radices-lycopersici* for tomato foot and root rot (Booth 1971). Th separation of *formae speciales* for sugar beet did not become prevalent as subsequent work

did not support such a distinction. *F. oxysporum* isolates that could cause Fusarium root rot did not fall into a single clade during phylogenetic analysis and had no evidence of a diverging lineage from that of FOB (Covey et al. 2014, Hill et al. 2011, Webb et al. 2012). It was also determined that susceptibility of the sugar beet variety could be a contributing factor to the development of Fusarium root rot, as some FOB isolates that caused Fusarium yellows in American sugar beet varieties caused Fusarium root rot on a set of susceptible Italian varieties (Hanson et al. 2018). However, there also were isolates in the FOSC that could cause root rot on the USA sugar beet varieties while most did not, so this is still an open question.

Fusarium Stalk Blight:

In 1973, *F. oxysporum* was isolated from sugar beet seed crops suffering from stalk blight and confirmed to be the causal agent (Gross and Leach 1973). Stalk blight was characterized by vascular discoloration of root tissue, foliar wilt, and seed stalk necrosis and death of bolting plants (MacDonald et al. 1976). Within the same variety, there was a difference in disease severity between root crops (first year) and seed crops (second year). Sugar beet varieties that developed severe stalk blight symptoms did not always have that same severity when grown solely as a root crop (MacDonald et al. 1976). It is worth noting that the assignment of disease severity was assessed using only a root rating scale that did not account for the other symptoms associated with Fusarium stalk blight and Fusarium yellows. Theories as to the cause of this difference included the time in the soil – root crops were grown on average for 5 months while seed crops were grown for 12 months on average. There were known to be physiological differences between vegetative and post-vernalization flowering plants, so it has been speculated that these differences also contributed to differences in disease severity between seed production beet and root crop beet (MacDonald et al. 1976). In 1981, it was confirmed that within infested

fields, FOB was not equally distributed, with different areas showing varied severity levels of Fusarium stalk blight as was consistent with the distribution of most soil borne diseases (McFarlane 1981, Nelson 1981). With this consideration in mind, subsequent studies made sure to grow sugar beet cultivars in various field locations across the years of research to best gauge the interaction of different cultivars with FOB in terms of resistance and susceptibility (McFarlane 1981, Nelson 1981). As part of the McFarlane (1981) study, trends were observed, although not statistically supported, regarding the susceptibility of various sugar beet breeding lines to stalk blight. It was observed that monogerm inbred lines tended to be more susceptible to FOB, while multigerm inbred lines tended toward greater resistance or moderate susceptibility. From this, it was hypothesized that resistance was controlled by multiple genes and that these genes may be dominant (De Lucchi et al. 2017, McFarlane 1981).

Seed-Borne:

Like many other pathogenic *F. oxysporum*, FOB could be seed-borne, although it was not believed to be a common source of transmission and infection across sugar beets whereas in other crops it was common (Macdonald and Leach 1976b, Nelson 1981). FOB could be carried on the exterior corky tissue of sugar beets fruits, and was only reported as present in the interior of seeds in cases of severe stalk blight disease. In MacDonald and Leach's work (1976b) heavily infected parent seed stalks led to 0.45% to 0.23% of the resulting seeds carrying FOB. In India, no *F. oxysporum* was detected, but *F. solani* was, on 3.7% of sugar beet screened from 1976 to 2005 (Agarwal et al. 2006). These levels could be further knocked down during the processing of seeds, specifically the milling away of corky tissue (MacDonald and Leach 1976b). *F. oxysporum* has been shown to have the potential to be seed-borne in a number of crops, so this was consistent with the known biology of the fungus (Nelson 1981).

Fusarium Yellows Disease Lifecycle:

Fusarium oxysporum lived in the soil and plant debris as a saprophyte, or survived as dormant macroconidia, chlamydospores or mycelia until a suitable host for endophytic or pathogenic colonization was available (Hanson and Jacobsen 2009, Nelson 1981). Suitable hosts included lambsquarter, black mustard, wild dill, spinach, onion, dry bean, and cultivars of garden beet and chard (Armstrong and Armstrong 1976, Hanson et al. 2001, Macdonald and Leach 1976a, Webb et al. 2012). Chlamydospores of pathogenic *F. oxysporum* have been shown to persist in the soil between 8 and 17 years at a temperature of 3-4C, although survival at varied temperatures can be different (McKeen and Wensley 1961). Macroconidia could persist in the soil from 3 to 4.5 months (El-Abyad and Afifi 1989). However, as demonstrated with *F. culmorum* and *F. graminearum*, the cells of macroconidia could develop into chlamydospores as early as days after introduction into the soil, which may extend viability (El-Abyad and Afifi 1989, Nelson 1981, Sitton and Cook 1981). In the presence of a suitable host, FOB entered the roots and grew into the vasculature, leading to vascular discoloration. It was hypothesized that the foliar symptoms were due to a combination phytotoxins and vascular obstruction via hyphal colonization of the xylem and plant defense responses, such as formation of tyloses in vasculature (Hanson and Jacobsen 2009, Nelson 1981). The development of foliar wilt symptoms due to *F. oxysporum* on various crops was often associated with the production of mycotoxins, such as the metallic chelation agents fusaric acid and phytonivein (Nelson 1981). These mycotoxins were reported to cause wilt by reducing foliar transpiration at least in part through altering the permeability of plant protoplasts (Desjardins 2006, Pegg 1981). In susceptible sugar beet cultivars, colonization of the vascular tissue may progress to necrosis of

the vascular and cortical tissue, increasing disease severity and may culminate in plant death (Hanson and Jacobsen 2009, Stewart 1931).

Etiology:

As is typical of *F. oxysporum*, no sexual stage has been observed for FOB (Leslie and Summerell 2006, Stewart 1931). In Stewart's first observations of "*F. conglutinans* var. *betae"* no macroconidia were observed, even after cultivating on ten different kinds of medium, but it did readily produce microconidia and chlamydospores (1931). The microconidia were reported as hyaline, 1-2 celled and straight to slightly curved ovals. The chlamydospores were globose and produced both as terminal single chlamydospores, typical of *F. oxysporum*, and intercalary chains – atypical for *F. oxysporum* (Stewart 1931). It had floccose white mycelium with aerial growth and no pigments were observed on glucose agar or steamed rice. The modern description of *F. oxysporum* was based on morphological attributes produced on carnation leaf agar (CLA) and potato dextrose agar for pigmentation (PDA, Leslie and Summerell 2006, Nelson et al. 1983). *Fusarium oxysporum* had variable macroconidia (singly or in sporodochia) production, and for FOB specifically, there was a widely observed correlation between low macroconidia production and virulence on sugar beet (Armstrong and Armstrong 1975, Cramer et al. 2003, Hill et al. 2011, Martyn et al. 1989, Ruppel 1991, Stewart 1931). Building on this observation, it was hypothesized that single-spore isolation and purification had led to a selection bias for non-pathogenic isolates due to this association of highly virulent isolates with low macroconidia production (Cramer et al. 2003, Hill et al. 2011). To the best of our knowledge, the relationship between low macroconidia production and virulence has not been investigated conclusively. When present, macroconidia may be produced in pale orange sporodochia or individually on hyphae. Macroconidia were usually 3 septate of medium length (in comparison to macroconidia

of other *Fusarium* species) with straight to slightly curved overall morphology, a blunt apical cell and a basal cell ranging from a distinct to an indistinct foot shape (Leslie and Summerell 2006). Microconidia morphology has remained similar to what was described in 1931, with additional information on production via short monophialides as false heads (Leslie and Summerell 2006, Stewart 1931). On PDA, mycelial morphology and pigmentation had wide variability – ranging from floccose to sparse, and white to pale purple, respectively. The pigment production in PDA ranged from no pigmentation to dark purple or reddish-purple, similar to the reports for the species complex as a whole (Leslie and Summerell 2006)

Post-Harvest Impact:

Campbell et al. (2011) reported that the greater the disease severity of Fusarium yellows in the field, the greater the rate of root rot, respiration and invert sugars, alongside increased loss of sugar content post-harvest. Based on these findings, it was recommended that roots from fields with a heavy presence of Fusarium yellows should be prioritized in sugar processing to minimize the accelerated loss of sugar content in comparison to healthy sugar beets. While it was shown that varied cultivars of sugar beet fared differently in terms of field foliar ratings, the authors were unable to group cultivars into distinct categories for increased or decreased sensitivity to post-harvest symptoms associated with Fusarium yellows (Campbell et al. 2011). When interpreting these results, it is necessary to do so with caution, as this experiment relied on natural *Fusarium* spp. populations in Minnesota, and *Fusarium* spp. acting upon the sugar beets were not identified. As such, it was not possible to know whether these findings could be attributed solely to Fusarium yellows and FOB isolates, to Fusarium yellows-like causal agents, to Fusarium yellowing decline due to *F. secorum*, to other *Fusarium* spp. that cause root rot, or some combination of these diseases and pathogens.

For the purposes of this review, the mycotoxin potential of FOSC isolates was our main focus. The production of mycotoxins is a concern associated with *Fusarium* spp., including those in the *F. oxysporum* species complex (FOSC). The process of extracting sugar filters out potential mycotoxin contaminants from the white sugar sucrose, but does not prevent its presence in byproducts, including sugar beet pulp and molasses (Bosch and Mirocha 1992). Both of these byproducts are commonly used in animal feed (Bosch and Mirocha 1992, Boudra et al. 2015, Michigansugar.com, Accessed 11/14/2024). In 1992 in a study focused on *Fusarium* spp., it was reported that zearlenone (ZEN) was the most frequently detected mycotoxin in harvested sugar beets, with a prevalence of 24% in root tissue samples and 41.3% in fiber samples (Bosch and Mirocha 1992). It also was reported that *F. oxysporum* and other *Fusarium* spp. (*F. sporotrichioides, F. equiseti, F. graminearum* and more) obtained from symptomatic sugar beet produced ZEN on rice and therefore were suspected to produce ZEN on sugar beet (Bosch and Mirocha 1992). It is worth noting that *F. oxysporum* was not typically reported as being capable of producing ZEN, and there was a possibility that the ZEN detected was not from *F. oxysporum*, but rather introduced into the rice substrate prior to the experiment by fungi that had colonized the grain; this has been reported as a common source of error in mycotoxin work (Desjardins 2006, Summerell 2001). ZEN is a fungal compound that can act as an estrogen mimic, causing uterine enlargement in rats and, in high concentrations, even death (Bosch et al. 1992, Mirocha et al. 1968). It has been identified as a contributing factor to infertility and hyperestrogenism in range of mammals, including swine, sheep, poultry and cattle (Desjardins 2006, Mirocha et al. 1968, Mirocha et al. 1976). In France, ZEN was detected in sugar beet pulp silage over one year at two of five regions, with two of the three samples exceeding the threshold set by European regulations for animal feed (Boudra et al. 2015, European Commission 2006). The ZEN detected

in sugar beet pulp silage could be due to the presence of an array of *Fusarium* spp. (Bosch and Mirocha 1992, Christ et al. 2011). Since isolation and identification of fungal species present in the silage was not undertaken as part of the Boudra et al. study, it was uncertain if the causal agent of Fusarium yellows was involved in production of ZEN in the silage (Boudra et al. 2015).

In 2011, the mycotoxin profile of FOSC isolated from sugar beet was expanded to include enniatins, moniliformin and beauvericin, which were produced on rice from FOSC isolates sampled from symptomatic sugar beets (Christ et al. 2011). The 2011 findings demonstrated that *F. oxysporum* isolates from symptomatic sugar beets were capable of producing mycotoxins, but could not be used as confirmation of their ability to do so in sugar beet. It has been determined that the substrate a species was grown on can impact expression of mycotoxin production (Bosch et al. 1992, Desjardins 2006). For instance, an isolate of *F. moniliforme* var. *subglutinans* grown on rice produced moniliformin, but moniliformin was not produced when the same isolate was grown on sugar beet (Bosch et al. 1992). Beauvericin and enniatins have implications in phytoxicity, can act as inhibitors of liver enzymes in rodent and other mammalian cell line cultures, and may have a role in insect and microbe toxicity (Desjardins 2006). Moniliformin has been demonstrated to cause cardiotoxicity, muscle weakness and respiratory distress in avian and rodent species, due to inhibition of liver mitochondria functions and pyruvate production (Desjdarins 2006). The presence and role of these mycotoxins in sugar beet need further investigation.

Taxonomy:

Isolates from the FOSC have distinct isolate populations in different regions; this has been observed across different geographic and production regions using tests such as random amplified polymorphic DNA-polymerase chain reaction (RAPD PCR) and vegetative

compatibility grouping (VCG, Fisher and Gerik 1994, Harveson and Rush 1997, Webb et al. 2012). However, FOSC clades from beet isolates assigned based on a multi-locus sequence analysis (MLSA) did not correspond to geographic location, indicating that additional factors contribute to the variability of isolates within FOSC on sugar beet (Hill et al. 2011). It has been speculated that contributing factors to clade separation within FOSC could be impacted by host specificity and cultivar susceptibility (Hanson et al. 2009, Hill et al. 2011). It also has been hypothesized that VCGs, mitochondrial DNA and virulence on sugar beets evolved independently of each other (Correll 1991). VCGs were not a reliable means of tracing the ancestral lineages and virulence within a *formae specialis* (Correl 1991). This finding was further validated and expanded upon to include an inability to use VCGs to classify FOSC to the level of *formae specialis* as it was determined there was no relationship between VCGs of *F. oxysporum* and ability to cause symptoms on sugar beets, onions and dry beans (Harveson and Rush 1997, Webb et al. 2012).

Although FOB is largely specific to beets, there is evidence of host cross over between FOB and *F. oxysporum* f. sp. *spinaciae* (FOS, Armstrong and Armstrong 1976, Macdonald and Leach 1976a). In 1976, it was reported that FOS caused disease in both sugar beets and spinach, with a stronger preference for spinach. FOB, however, was only pathogenic on sugar beets in that study (Armstrong and Armstrong 1976). In a different study, FOB was able to cause disease in sugar beets, spinach and pigweed, while the tested FOS was only pathogenic on spinach (Macdonald and Leach 1976a). The results across these two studies provide contradictory results, and as such the suggestion that FOB should be reclassified as a race of FOS was rejected (Armstrong and Armstrong 1976). This pair of studies did support that while *formae speciales* in *F. oxysporum* may have some host specificity, this preference may not be to the point of

exclusion. This has been reported in other cropping system as well (Nelson 1981, Summerell et al. 2001) There was evidence that some *F. oxysporum* isolated from sugar beets were also capable of host crossover with dry bean, as an isolate of *F. oxysporum* from sugar beet was able to cause symptoms in beans and onions (Hanson et al. 2001, Webb et al. 2012).

Fusarium commune:

In 2003, a new species in the FOSC, *F. commune,* was identified from coniferous trees in Denmark (Skovgaard et al. 2003). This new species was a sister taxon to *F. oxysporum*, and described as closely related to, but distinct from the *F. oxysporum* and *F. fujikuroi* species complexes (Skovgaard et al. 2003). It was reported to be morphologically distinct from *F. oxysporum* when cultured in the dark, as it developed long mono and polyphialides whereas *F. oxysporum* had relatively short monophialides and did not produce polyphialides (Leslie and Summerell 2006, Skovgaard et al. 2003). However, this morphological marker has been found to be variable, and it was observed in 2006 that putative *F. commune* can only be "reliably distinguished" from *F. oxysporum* through molecular markers. This conclusion was reached after observing that all the "*F. commune"* isolates in a 2006 study were morphologically identical to *F. oxysporum* (Stewart et al. 2006). This variability in polyphialide production and reliance on molecular identification has since been reported across numerous studies of isolates genetically similar to *F. commune* across multiple countries, including the United States, China, Japan, Algeria and Australia (Carnegie et al. 2022, Deng et al. 2022, Edel-Hermann V. 2012, Osawa et al. 2020, Stewart et al. 2006). This has raised concerns about the separation of *F. commune* from *F. oxysporum* as the taxonomic code requires that species be distinguishable based on more than just molecular analysis (Turland et al. 2018).

In 2019, a number of isolates originally identified as *F. oxysporum* f. sp. *betae* were putatively re-classified as *F. commune* based on molecular markers (Webb et al. 2019). Amongst these isolates, of particular note was the isolate F19 that was initially isolated from Oregon and identified in Colorado as a highly virulent isolate of *F. oxysporum* f. sp. *betae*. F19 has been used across multiple studies as a reliable positive control and model organism for work on Fusarium yellows of sugar beet (Covey et al. 2014, De Lucchi et al. 2017, Hanson et al. 2009, Hill et al. 2011, Lai et al. 2020, Li and Smigocki 2019, Webb et al. 2015). There has been no published description of whether these reclassified isolates were morphologically consistent with *F. commune*, or could only be putatively separated from *F. oxysporum* based on molecular methods. The isolates that have since been tentatively reclassified as *F. commune* thus far all belonged to a common genetic clade in the FOSC, Clade A (Hill et al. 2001, Webb et al. 2012, Webb et al. 2019).

Impact of Temperature and pH:

Temperature and pH have both been demonstrated as impacting the growth and optimal development of sugar beet and FOB. The optimal temperature range for sugar beet germination and emergence ranged from 25 to 35C, with 27C as the optimal temperature, and a minimal germination temperature of 8 to 15C (O'Sullivan and Kavanagh 1991, Radke and Bauer 1969). Optimal sucrose yield occurred within a range of 18 to 32C, and dry matter accumulation at 23 to 26.3C (Radke and Bauer 1969). FOB grew at temperatures ranging from 6 to 33C, with the optimal growth range between 24 and 28C (Harveson and Rush 1998, Stewart 1931, Twomey 1952). The optimal conditions for development of Fusarium yellows and Fusarium root rot in sugar beets ranged from 25 to 30C (Harveson and Rush 1998). It was also reported that the majority of isolates tested were rendered non-pathogenic at green house temperature of 20C or

lower (Harveson and Rush 1998). In 2015, further work was done on assessing virulence at different day/night temperatures in the greenhouse. From this work, it was reported that Fusarium yellows severity was low at 16/6C, optimal at 26/11C, and had mixed results at higher temps of 32/16C, day/night temperatures (Webb et al. 2015). These findings were consistent with those of Harveson and Rush (1998) and general Fusarium wilt diseases, and point to temperature as a contributing factor in development of disease symptoms and severity (Nelson 1981). There was an attempt in 2017 to apply these findings to the field setting; however, the results were inconclusive as across the three growing seasons assessed, the environmental temperature was consistent (Webb et al. 2017). Overall, both sugar beet and FOB shared a common range of temperatures for favorable growth.

In addition to tolerance of a wide temperature spectrum, FOB also tolerated a wide pH range between 3.7 to 9.2, with optimal growth occurring between 4.8 and 5.9 (Stewart 1931**,** Twomey et al. 1952). This tolerance of a wide pH range was hypothesized to play a role in FOB's ability to survive in a wide range of environments, particularly in soil and plant tissue. The optimal soil pH for sugar beet growth and sugar yield was 7.0 to 9.5, while sugar beet growth and sugar development declined at less than 4.5 to 5.9 (Geng et al. 2021, McEnroe and Coulter 1964, Wang et al. 2022). Within plants, different tissues had varying pH levels, with the xylem having a pH range of 4.5 to 8, the phloem with a narrower pH range of 7.5 to 8.6, and between cortical cells the apoplast has a pH range of 5.1 to 5.6 (Dinant et al. 2010, Felle et al. 1998, Pramsohler 2022, Wilkinson et al. 1998). The optimal pH for germination of FOB macroconidia was around 6.0 (El-Abyad and Afifi 1989). To the best of our knowledge, there has been no work to assess the impact of environmental pH on pathogenicity and virulence of FOB. Although, it has been hypothesized that soil pH was not likely to be a control factor for

disease development, as FOB tolerated a wide pH range and for soil to be in a range that was inhospitable to FOB it would also be problematic to cultivate sugar beet (Geng et al. 2021).

Impact of Irrigation, Humidity, and Water Movement:

Consistent high humidity (96-100%) increased sporulation for both macroconidia and microconidia of *F. oxysporum* (Gracia-Garza and Fravel 1998). With fluctuating relative humidity, optimal sporulation occurred when it fell within a range of 75-100% humidity (Gracia-Garza and Fravel 1998). This humidity might lead to sporulation of conidia on the surface of the soil, such as when it was naturally harbored on plant debris, but was unlikely to impact germination in the soil (Hanson and Jacobsen 2009). The movement of water in soil can contribute to the movement of spores downwards in soil, although the amount of distance traveled varied from 2 to 10cm depending on spore size and how porous the soil itself was (Gracia-Garza and Fravel 1998). Reported in 1998, Harveson and Rush were unable to find an association between disease severity and irrigation methods. This may be due to the fact that both regimens in their tests the plants were initially inoculated via infested soil and were watered equally at the beginning of the experiment. After four to six weeks, plants were inoculated a second time via root dip, after which the plants transitioned to high or low watering regimens. It was probable that infection occurred prior to the shift in watering regimens (Harveson and Rush 1998). In other studies, water has been shown to be important to *F. oxysporum* for infection (Nelson 1981). In Harveson and Rush's later work focusing on total sugar beet root rot pathogens, including *F. oxysporum*, *Rhizoctonia solani* and *Aphanomyces cochlioides*, it was found that there was a reduction in total root rot between a "dry" irrigation strategy in comparison to a "wet" irrigation strategy, although "dry" irrigation did not prevent the development of root rot (Harveson and Rush 2002). In this experiment, "wet" irrigation referred

to a standard regimen of 5-6 bimonthly irrigations, while "dry" irrigation referred to a regimen of 2-3 irrigations at 5-6 week intervals (Harveson and Rush 2002). A similar positive correlation was observed between soil moisture and end of season disease severity for moderately susceptible and resistant cultivars for Fusarium yellows (Webb et al. 2017). In contrast, it was observed that the susceptible cultivars had the same disease severity regardless of moisture level in that study (Webb et al. 2017). These results across studies indicate that regulating irrigation alone was not sufficient to manage disease caused by *F. oxysporum* in sugar beet, although it could reduce disease severity as reported in other cropping systems (Nelson 1981).

Impact of Cultivar and Geographic Origin:

One of the most effective and common means of managing Fusarium yellows was through selecting for resistant sugar beet varieties (Schwartz et al. 2001, Wilson and Smith 2001). Mixed cultivar plots are not currently recommended for use in disease management, as in one study it did not impact the number of total sugar beet root diseases for better or worse (Harveson and Rush 2002). In 2009, it was reported that resistant varieties had variable results against different *Fusarium* isolates from four geographic regions (Hanson et al. 2009). Of the 14 varieties listed as having reported resistance to Fusarium yellows, only two varieties had below 40% plant deaths across all of the nine isolates used in screening. The other resistant varieties had variable responses depending on with which isolate they were inoculated. This highlighted a major issue with selecting resistant sugar beet varieties: broad-spectrum resistance was not easily achieved. This variability also indicated that geographic region could play a role in some of the observed variable resistance. However, definitive conclusions could not be drawn using all of this data, as the location of initial resistant varieties and their screenings were proprietary information of the production companies and as such, could not be matched against the known

geographic origin of the *Fusarium* isolates (Hanson et al. 2009). It was possible to see that a sugar beet variety, FC716 from Colorado that was initially reported as susceptible to Fusarium yellows, demonstrated resistance to an FOB isolate from Michigan (Hanson et al. 2009).

In 2017 in Colorado, it was reported that susceptible and resistant cultivars maintained consistent results across various years of field studies within that region, while the moderately susceptible cultivar demonstrated variability over the years (Webb et al. 2017). While this study demonstrated that selected resistant and highly susceptible cultivars might maintain their susceptibility level regardless of environmental conditions in the field in a given region, it was worth noting that there was one tested cultivar in each susceptibility category, and as such could not be taken as representative of all cultivar trends. In addition, the field trials were performed in only one geographic region, and the greenhouse work used only local isolates. It did not take into account that other sugar beet growing regions have different isolates than Colorado (Hanson et al. 2009, Harveson and Rush 1997, Ruppel 1991, Secor et al. 2012, Webb et al. 2017, Webb et al. 2019). The significance of accounting for different geographic regions was highlighted in a 2017 study that compared susceptible sugar beet cultivars from two completely different geographic regions, the USA and Italy. Severe root rot was observed in all four Italian varieties that were inoculated with three FOB isolates from the USA (Hanson et al. 2017). These FOB isolates were not associated with Fusarium root rot at their point of origin, and until this test had only been reported to cause Fusarium yellows in USA sugar beet varieties. The sugar beet breeding lines used in the USA, Western and Eastern Europe originated from the work of Ottavio Munerati (Llewellyn 1992). Wherein the USA and Western European countries prioritized total beet yield and disease resistance, also referred to as the Western breeding line, while Eastern European countries chose the breeding lines with high sucrose percentages but no disease

resistance – Eastern breeding lines (Llewellyn 1992). Since the acquisition of Munerati's Western sugar beet lineage, sugar beets in the USA have been bred divergently from that of Eastern Europe. European countries, including Italy, do not have as prevalent Fusarium yellows and root rot as in the United States (Christ et al. 2011). The Italian breeding lineages used by Hanson et al. (2017) were from Eastern breeding lines which did not prioritize breeding resistance to diseases caused by *Fusarium* spp. (De Lucchi et al. 2017, Llewellyn 1992, Hanson et al. 2017). This could be a factor in the varied responses that were reported.

Interaction with Cyst Nematodes:

In 1970, it was reported that some *F. oxysporum* strains could substantially reduce *Heterodera schachtii* (sugar beet cyst nematode) maturation and invasion of sugar beets (Jorgenson 1970). When sugar beets were inoculated with *F. oxysporum* alone, there was a reduction in plant mass, but not as severely as when treated with nematodes alone. The combination of both nematodes and *F. oxysporum* substantially reduced the damage from nematodes and the fresh weight of sugar beets increased, although not to the level of the negative control. It was not recommended to use *F. oxysporum* to treat sugar beet cyst nematode infestations, as some *F. oxysporum* could on their own cause a reduction in sugarbeet yield mass (Jorgenson 1970). This study did not specify the cultivar of sugar beet used or the strains of *F. oxysporum*, limiting our ability to draw comparisons to other varieties and strains in subsequent studies. The ability of *F. oxysporum* to antagonize other nematodes, such as *H. cruciferae* has been reported on varied crops as well, including cabbage and soybean (Kerry 1988, Mennan et al. 2005). The study on cabbage and cabbage cyst nematodes provided an alternative description of a potential mechanism behind this antagonistic relationship between *F. oxysporum* species and *Heterodera* species. *F. oxysporum* colonized nematode cysts and penetrated cyst cell walls to

infect the majority of the eggs, with the juveniles having a deformed "C" shape (Mennan et al. 2005). This study suggested using some *F. oxysporum* as biological control agents for cabbage cyst nematodes, but did not address the concerns around introducing *F. oxysporum formae speciales* that could cause Fusarium wilt of cabbage or diseases on rotation crops. In 1988, the ability of some *F. oxysporum* strains to colonize sugar beet and soy bean cyst nematodes (*H. glycines*) was reported (Kerry 1988). The *F. oxysporum* in this study rarely infected eggs and when it did, was reported as low virulence. It instead colonized deceased female nematodes – the cysts and their eggs (Kerry 1988). This has not been an exhaustive review, but the varying results from 2005 and 1988 demonstrated variation in the activity of some *F. oxysporum formae speciales* against nematodes for different hosts, and warrants further investigation before consideration for recommending the use of *F. oxysporum* as a biological control in varied crops (Kerry 1988, Mennan et al. 2005).

In 2009, the interaction of an array of FOB isolates and sugar beet cultivars was assessed, including the impact of co-infection with sugar beet cyst nematodes (Hanson et al. 2009). When FOB was co-inoculated with *H. schachtii*, the symptoms associated with Fusarium yellows varied widely in comparison to when FOB was applied alone. Amongst the isolates and varieties tested, the co-inoculation caused a decrease, no difference, or increase in Fusarium yellows disease severity (Hanson et al. 2009). There was no clear correlation or trend for the interaction of individual FOB isolates and sugar beet varieties, although it did provide grounds for several hypotheses worth further investigation. For instance, a reduction in both Fusarium yellows disease severity and sugar beet cyst nematode damage could relate to a preference for some strains of FOB for infecting cysts over sugar beets – leading to a reduction in both viable cysts and colonization of sugar beet by FOB as seen with one isolate (Hanson et al. 2009, Mennan et

al. 2005). In cases where disease severity increased synergistically, juvenile nematodes could serve as a mechanism for the introduction of FOB into sugar beets through nematode feeding wounds, as seen in several crops including cotton and beans (Nelson 1981).

Histopathology Work:

Prior histopathology work on plant-pathogenic *F. oxysporum* encompassed a wide array of diseases and hosts, including monocots such as banana and sweet corn (Lawrence et al. 1981, Rocha et al. 2022). For the purposes of this review, the focus will be on a small selection of dicot hosts and wilt diseases caused by *F. oxysporum*: tomato (*Solanum lycopersicum*), cotton (*Gossypium herbaceum*) and *Arabidopsis thaliana* (Lagopodi et al. 2002, Martinez-Soto et al. 2023, Olivain and Alabouvette 1999, Rodríguez-Gálvez and Mendgen 1995). In tomato, two different *formae speciales* have been studied, *F. oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *radices-lycopersici* (FORL). FOL is the causal agent of Fusarium wilt of tomato, while FORL is the causal agent of tomato foot and root rot (aka Fusarium crown and root rot). Both of these diseases are characterized by colonization and infection of the root in order to cause disease. In FORL, the hyphae predominantly showed initial colonization at root hairs and approached the lateral root surface (Lagopodi et al. 2002). Hyphae grew around the perimeter of plant cell walls and directly penetrated via thickened hyphae. There were no observed appressoria or structures to facilitate penetration of plant tissue, as seen in some other causal agents of wilt disease, including *Verticillium* spp. (Lagopodi et al. 2002, Schnathorst 1981). Brown discoloration in the root corresponded to densely growing mycelium, and the presence of both intracellular and intercellular growth of hyphae. Around the same time as discoloration developed on the main root, root hairs and lateral roots were also colonized at apparently random positions (Lagopodi et al. 2002). When wilting occurred, hyphae had

colonized the central cylinder and were growing in the xylem. Subsequently, FORL fully colonized and caused necrosis of the root system, then transitioned to sporulation to produce macroconidia (Lagopodi et al. 2002).

In 1999, the metabolic activity of a pathogenic FOL isolate and a non-pathogenic isolate of *F. oxysporum* were beta-glucuronidase gene (GUS) transformed and visualized for comparison of colonization and plant response. From this comparison of metabolic activity, the authors were able to describe a plant-pathogen interaction and a plant-non-pathogen interaction in tomato roots. The non-pathogenic isolate was demonstrated to penetrate and colonize the superficial cork of the plant without causing disease and thus was classified as endophytic. The pathogenic isolate grew past the cortex into the vascular tissue and led to symptoms associated with Fusarium wilt of tomato (Olivain and Alabouvette 1999). It was hypothesized that plant defenses prevented the non-pathogenic isolate from growing past the cortex and into the vascular tissue, as indicated by developing thickened plant cell walls and forming a barrier between the epidermis and the cortex (Olivain and Alabouvette 1999). Similar responses were seen in other studies as well (Beckman and Talboys 1981,). Olivain and Alabouvette (1999) also reported "buckling" of plant tissue around penetrating hyphae. This "buckling" was speculated to be a plant defense that might trap the fungus and limit further colonization (Olivain and Alabouvette 1999). While "buckling" and thickened plant cell walls were observed with the pathogenic isolate as well, it was not sufficient to prevent further colonization of the root tissue, leading to a hypothesis that pathogenicity was about quantity of inoculum rather than type (Olivain and Alabouvette 1999). That is, with a sufficiently large population of hyphae, FOL would be able to overcome plant defenses. However, other studies have not shown high inoculation load was

required for pathogenic strains to infect hosts, as an inoculum load of at least 10^2 to 10^4 cfu/g soil was sufficient for wilt symptoms to occur (Hill et al. 2011, Summerell et al. 2001).

In tomato the growth of both pathogenic and non-pathogenic *F. oxysporum* isolates followed a similar pattern, with hyphae moving between and alongside plant cells. It was hypothesized by Olivain and Alabouvette that this pattern of growth was facilitated by digestion of cell walls but this was not confirmed (1999). In response to both isolates, plant cell walls thickened and occlusions were formed that could impede penetration (Olivain and Alabouvette 1999). This study also described, but did not identify, electron-dense material and osmiophilic globular deposits that accumulated around infected vascular tissue and hyphae respectively. It was speculated that these accumulations were a part of the plant defenses, but the authors provided no explanation of what mechanism(s) by which they might benefit a plant. At the apex of developing roots, the sloughing off of exterior plant cells in inoculated roots was observed, whereas in the uninoculated roots, sloughing of exterior plant cells was not observed (Olivain and Alabouvette 1999). Olivain and Alabouvette hypothesized that this was a defense mechanism, though it could be argued that it was debris from necrotrophied plant cells. There were several shortcomings associated with this work that raised questions as to the dependability of these findings. This included the use of microconidia instead of macroconidia or hyphal fragments for inoculum, and a high concentration of them at that, 100 times the concentration seen in the field naturally (Nelson 1981). Samples were also fixed for a quarter of the recommended time, which could be a factor in the observed misshapen plant cells and inconsistency in observations (Klomparens et al. 1986). It's unclear if the "buckling" the authors referred to could be attributed as a plant defense or a byproduct of fixation, as regardless of if the plant was inoculated or not, buckling plant cells was observed in plant tissue.

In 2023, in order to further characterize the differences in colonization and plant responses between endophytic and pathogenic *F. oxysporum*, histopathology on tomato and *A. thaliana* was performed. The pathogens used were FOL on tomato and *F. oxysporum* f. sp. *conglutinans* on *A. thaliana* and an endophytic *F. oxysporum* that was known to colonize both hosts (FOC, Martinez-Soto et al. 2023). The endophyte in both tomatoes and *A. thaliana* were able to penetrate and colonize the epidermis, endodermis, and xylem of lateral roots and the root elongation zone. In comparison, the pathogens also colonized the xylem beyond the lateral roots and root elongation zone (Martinez-Soto et al. 2023). Additionally, the endophytes formed chlamydospores (resting structures) earlier than the pathogens within the plant vascular systems. In terms of plant response to colonization, lignification of xylem tissue and callose accumulation was stimulated by the endophytes, particularly at the transition points between the elongation zone and the lateral roots to the main xylem. This increase in biosynthesis of lignin and callose was not observed for the pathogenic strains. There were no observed differences in suberin deposition at plant cell walls regardless of if the *F. oxysporum* was pathogenic or endophytic (Martinez-Soto et al. 2023). The elevated presence of lignin and callose with endophytic *F. oxysporum* was hypothesized to be the cause of limited colonization observed for the endophytes (Martinez-Soto et al. 2023). It also demonstrated that FOL and FOC were either able to evade detection or suppressed plant responses during early infections, enabling further colonization into the vascular system of their respective hosts. Fusarium wilt pathogens have been shown in other studies to delay detection, and elicit plant responses such as tyloses and phytoalexins after they were well established in susceptible hosts (Nelson 1981). One shortcoming of the Martinez-Soto work was that the endophytic and pathogenic *F. oxysporum* were not compared to negative

control plants, limiting our ability to draw conclusive and compelling results from the plant response to various *F. oxysporum* isolates.

In cotton, the infection process at the root tip by *F. oxysporum* f. sp. *vasinfectum* (FOV, causal agent of Fusarium wilt in cotton) was investigated by quantifying penetration hyphae under a conventional light microscope and characterized under a transmission electron microscope (Rodríguez-Gálvez and Mendgen 1995). After spore germination, the root surface was first densely colonized to form a mycelial network and then penetrated by hyphae both intracellularly and intercellularly without appressoria (Rodríguez-Gálvez and Mendgen 1995). The meristematic zone at the root tip had the most penetration hyphae with a 40% difference from the elongation zone and root hairs. There were no penetration hyphae observed in the lateral roots (Rodríguez-Gálvez and Mendgen 1995). In response to colonization and prior to penetration by FOV in the meristematic zone, cell wall appositions and papilla were formed in order to minimize intercellular and intracellular penetration. At the same time, hyphae were surrounded by two separate materials, one that was electron dense and the other was electron translucent. Penetration by hyphae did not lead to plant cell organelle damage or plasmolysis, which led Rodríguez-Gálvez and Mendgen to hypothesize that in the early stages of infection in Fusarium wilt of cotton, FOV was in a biotrophic phase in which it colonized as an endophyte and, once well established in the plant tissue and under the right conditions, transitioned to a more necrotrophic lifestyle to cause disease (Rodríguez-Gálvez and Mendgen 1995).

LITERATURE CITED

Abada K. A. 1994. Fungi causing damping-off and root-rot on sugar-beet and their biological control with *Trichoderma harzianum*. Agriculture, Ecosystems and Environment. 51:333-337.

Abadam V. 2021. Sugars and Sweeteners Background. USDA Economic Research Service. https://www.ers.usda.gov/topics/crops/sugar-and-sweeteners/background/

Agarwal P. C., Singh B., Dev U., Rani I., Chand D. and Khetarpal R. K. 2006. Seed-borne fungi detected in sugar beet seeds imported into India during last three decades. Plant Health Progress. 7:1-5. DOI:10.1094/PHP-2006-1211-01-RS.

Armstrong G. M and Armstrong J. K. 1976. Common hosts for *Fusarium oxysporum formae speciales spinaciae* and *betae*. Phytopathology. 66:542-545.

Beckman C. H. and Talboys P. W. 1981. Anatomy of resistance. In: Mace M. E., Bell A. A. and Beckman C. H. eds. Fungal Wilt Diseases of Plants. Academic Press Inc., New York, NY USA. Pp. 487-518.

Booth C. 1971. The Genus *Fusarium*. Centre for Agriculture and Bioscience International, Wallingford, UK. 237 pages.

Bosch U. and Mirocha C. J. 1992. Toxin production by *Fusarium* species from sugar beets and natural occurrence of zearalenone in beets and beet fibers. Applied and Environmental Microbiology. 58:3233-3239.

Bosch U., Mirocha C. J. and Wen Y. 1992. Production of zearalenone, moniliformin and trichothecenes in intact sugar beets under laboratory conditions. Mycopathologia. 119:167-173.

Boudra H., Rouillé B., Lyan B., and Morgavi D. P. 2015. Presence of mycotoxins in sugar beet pulp silage collected in France. Animal Feed Science and Technology. 205:131-135.

Burlakoti P., Rivera V., Secor G. A., Qi A., Del Rio-Mendoza L. E. and Khan M. F. 2012. Comparative pathogenicity and virulence of *Fusarium* species on sugar beet. Plant Disease. 96:1291-1296.

Campbell L. G., Fugate K. K. and Niehaus W. S. 2011. Fusarium yellows affects postharvest respiration rate, sucrose concentration, and invert sugar in sugarbeet. Journal of Sugar Beet Research. 48:17-39.

Cao S., Yang N., Zhao C., Han C., and Wu X. 2018. Diversity of *Fusarium* species associated with root rot of sugar beet in China. Journal of General Plant Pathology. 84:321-329.

Carnegie A. J., Callaghan S. E., Laurence M. H., Plett K. L., Plett J. M., Green P. J., Wildman O., Daly A. and Summerell B. A. 2022. Response to the detection of an exotic fungal pathogen, *Fusarium commune*, in a *Pinus radiata* production nursery in Australia. Australian Forestry. 85:187-198.

Christ D. S., Märländer B. and Varrelmann M. 2011. Characterization and mycotoxigenic potential of *Fusarium* species in freshly harvested and stored sugar beet in Europe. Phytopathology. 101:1330-1337.

Correll J. C. 1991. The relationship between *formae speciales*, races, and vegetative compatibility groups in *Fusarium oxysporum*. Phytopathology. 81:1061-1064.

Covey P. A., Kuwitzky B., Hanson M. and Webb K. M. 2014. Multilocus analysis using putative fungal effectors to describe a population of *Fusarium oxysporum* from sugar beet. Phytopathology. 104:886-896.

Cramer R. A., Byrne P. F., Brick M. A., Panella L., Wickliffe E. and Schwartz H. F. 2003. Characterization of *Fusarium oxysporum* isolates from common bean and sugar beet using pathogenicity assays and random-amplified polymorphic DNA markers. Journal of Phytopathology. 151:352-360.

De Lucchi C., Stevanato P., Hanson L., McGrath M., Panella L., De Biaggi M., Broccanello C., Bertaggia M., Sella L. and Concheri G. 2017. Molecular markers for improving control of soil-borne pathogen *Fusarium oxysporum* in sugar beet. Euphytica Vol. 213. DOI: 10.1007/s10681-017-1859-7.

Deng S., Ma X., Chen Y., Feng H., Zhou D., Wang X., Zhang Y., Zhao M., Zhang J., Daly P., and Wei L. 2022. LAMP Assay for distinguishing *Fusarium oxysporum* and *Fusarium commune* in lotus (*Nelumbo nucifera*) rhizomes. Plant Disease. 106:231-246.

Desjardins A. E. 2006. *Fusarium* Mycotoxins, Chemistry, Genetics, and Biology. APS Press, St. Paul, MN. 260 pages.

DeSutter T. M. and Godsey, C. B. 2010. Sugar-beet-processing lime as an amendment for low pH soils. Communications in Soil Science and Plant Analysis. 4:1789–1796.

Dinant S., Bonnemain J. –L., Girousse C., and Kehr J. 2010. Phloem sap intricacy and interplay with aphid feeding. Comptes Rendus Biologies. 333:504-515.

Edel-Hermann V., Gautheron N. and Steinberg C. 2012. Genetic diversity of *Fusarium oxysporum* and related species pathogenic on tomato in Algeria and other Mediterranean countries. Plant Pathology. 61:787-800.

El-Abyad M. S. and Afifi M. A. 1989. Effect of some environmental factors on sugar beet *Fusarium* pathogen 1st communication. germination and survival of macroconidia. Zentralblatt für Mikrobiologie. 144:489-96.

European Commission. 2006. Commission recommendation No 2006/576 of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Official Journal of the European Union. 229:7-9.

Felle H. H. 1998. The apoplastic pH of the *Zea mays* root cortex as measured with pH-sensitive microelectrodes: aspects of regulation. Journal of Experimental Botany. 49: 987–995.

Fisher G. A. and Gerik J. S. 1994. Genetic diversity of *Fusarium oxysporum* isolates pathogenic to sugar beets (Abstr.). Phytopathology. 84:1098.

Geng G., Wang G., Stevanato P., Lv C., Wang Q., Yu L., and Wang Y. 2021. Physiological and proteomic analysis of different molecular mechanisms of sugar beet response to ascidic and alkaline pH environment. Frontiers in Plant Science Vol. 12. DOI: 10.3389/fpls.2021.682799

Gracia-Garza J. A. and Fravel D. R. 1998. Effect of relative humidity on sporulation of *Fusarium oxysporum* in various formulations and effect of water on spore movement through soil. Phytopathology. 88:544-549.

Gross D. C. and Leach L. D. 1973. Stalk blight of sugarbeet seed crop caused by *Fusarium oxysporum* f. sp. *betae* (Abstr.). Phytopathology. 63:1216.

Hanson L. E. Hill A., Wickliffe E., Schwartz H., and Panella E. 2001. *Fusarium* in sugar beet and bean (Abstr.). Journal of Sugar Beet Research. 38:70.

Hanson L. E. and Hill A. L. 2004. *Fusarium* species causing Fusarium yellows of sugar beet. Journal of Sugar Beet Research. 41:163-178.

Hanson L. E. 2006. First report of Fusarium yellows of sugar beet by *Fusarium oxysporum* in Michigan. Plant Disease. 90:1554.

Hanson L. E. and Jacobsen B. J. 2006. Beet root-rot inducing isolates of *Fusarium oxysporum* from Colorado and Montana. Plant Disease. 90:247.

Hanson L. E. 2007. Fusarium yellowing of sugar beet caused by *Fusarium graminearum* from Minnesota and Wyoming. Plant Disease. 91:686.

Hanson L. E. 2009. Other *Fusarium* associated problems. In: Harveson R. M., Hanson L. E., Hein G. L., eds. Compendium of Beet Diseases and Pests, 2nd ed. St. Paul, MN, USA: APS Press. Pp. 28–30.

Hanson L. E. and Jacobsen B. J. 2009. Fusarium yellows. In: Harveson R. M., Hanson L. E., Hein G. L., eds. Compendium of Beet Diseases and Pests, 2nd ed. St. Paul, MN, USA: APS Press. Pp. 28–30.

Hanson L. E., Hill A. L., Jacobsen B. J. and Panella L. 2009. Response of sugarbeet lines to isolates of *Fusarium oxysporum* f. sp. *betae* from the United States. Journal of Sugar Beet Research. 46:11-26.

Hanson L. E., De Lucchi C., Stevanato P., McGrath M., Panella L., Sella L., De Biaggi M. and Concheri G. 2018. Root rot symptoms in sugar beet lines caused by *Fusarium oxysporum* f. sp. *betae*. European Journal of Plant Pathology. 150:589-593.

Harveson R. M. and Rush C. M. 1997. Genetic variation among *Fusarium oxysporum* isolates from sugar beet as determined by vegetative compatibility. Plant Disease. 81:85-88.

Harveson R. M. and Rush C. M. 1998. Characterization of Fusarium root rot isolates from sugar beet by growth and virulence at difference temperatures and irrigation regimes. Plant Disease. 82:1039-1042.

Harveson R. M. and Rush C. M. 2002. The influence of irrigation frequency and cultivar blends on the severity of multiple root diseases in sugar beets. Plant Disease. 86:901-908.

Harveson R. M. 2007. Identifying and distinguishing seedling and root rot diseases of sugar beets. Plant Health Progress Vol. 7. DOI:10.1094/PHP-2006-0915-01-DG.

Harveson, R. M. 2009. Fusarium root rot. In: Harveson R. M., Hanson L. E., Hein G. L., eds. Compendium of Beet Diseases and Pests, 2nd ed. APS Press, St. Paul, MN, USA. Pp. 30-31.

Hill A. L., Reeves P. A., Larson R. L., Fenwick A. L., Hanson L. E. and Panella L. 2011. Genetic variability among isolates of *Fusarium oxysporum* from sugar beet. Plant Pathology. 60:496-505.

Hoffmann C. M., Koch H-J., and Märländer B. 2021. Sugar beet. In: Sadras V. O. and Calderini D. F. eds. Crop Physiology Case Histories for Major Crops. Elsevier. Amsterdam, the Netherlands, 384-406.

Jacobsen B. J. and Franc G. D. 2009. Cercospora leaf spot. In: Harveson R. M., Hanson L. E., Hein G. L., eds. Compendium of Beet Diseases and Pests, 2nd ed. APS Press, St. Paul, MN, USA. Pp. 7-9.

Jorgenson E. C. 1970. Antagonistic interaction of *Heterodera schachtii* (Schmidt) and *Fusarium oxysporum* (Woll.) on sugarbeets. Journal of Nematology. 2:393-398.

Karadimos D. A. and Karaoglanidis G. S. 2006. Survey of root rot diseases of sugar beet in Central Greece. Proceedings of the National Academy of Sciences. 110:129-131.

Kerry B. 1988. Fungal parasites of cyst nematodes. Agriculture, Ecosystems and Environment. 24:293-305.

Klomparens K. L., Flegler S. L., and Hooper G. R. 1986. Procedures for Transmission and Scanning Electron Microscopy for Biological and Medical Science: A Laboratory Manual 2nd Ed. Ladd Research Industries, Burlington, VT USA. Pp. 13-20.

Lai X., Qi A., Liu Y., Del Río Mendoza L. E., Liu Z., Lin Z. and Khan M. F. 2020. Evaluating inoculation methods to infect sugar beet with *Fusarium oxysporum* f. *betae* and *F. secorum*. Plant Disease. 104:1312-1317.

Lagopodi A. L., Ram A. F. J., Lamers G. E. M., Punt P. J., Van den Hondel C. A. M. J. J., Lugtenberg B. J. J., and Bloemberg G. V. 2002. Novel aspects of tomato root colonization and infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. Molecular Plant Microbe Interactions. 15: 172-9.

Lawrence E. B., Nelson P. E., and Ayers J. E. 1981. Histopathology of sweet corn seed and plants infected with *Fusarium moniliforme* and *F. oxysporum*. Phytopathology. 71:379-386.

Leslie J. F. and Summerell B. A. 2006. *F. oxysporum* Schlechtendahl emend. Snyder and Hansen. In:The *Fusarium* Laboratory Manual. First ed. Blackwell Publishing, Ames, Iowa. Pp. 212-218

Lewellyn R. T. 1992. Use of plant introductions to improve populations and hybrids of sugarbeet. In: Use of Plant Introductions in Cultivar Development, Part 2. Crop Science Society of America Special Publication. 20:117-136. DOI:10.2135/cssaspecpub20.c5.

Lewellen R. T., Panella L., and Harveson R. M. Introduction. 2009. In: Harveson R.M., Hanson L.E., Hein G.L., eds. Compendium of Beet Diseases and Pests, 2nd ed. APS Press, St. Paul, MN, USA. Pp. 1-3.

Li H. and Smigocki A. C. 2019. Suppression of *Fusarium oxysporum* with recombinant polygalacturonase inhibiting proteins (BvPGIPs) extracted from sugar beet roots. Plant Cell, Tissue and Organ Culture. 136:197-203.

MacDonald J. D. and Leach L. D. 1976a. Evidence for an expanded host range of *Fusarium oxysporum* f. sp. *betae*. Phytopathology. 66:542-545.

MacDonald J. D. and Leach L. D. 1976b. The association of *Fusarium oxysporum* f. sp. *betae* with nonprocessed and processed sugarbeet seed. Phytopathology. 66: 868-872.

MacDonald J. D., Leach L. D. and McFarlane J. S. 1976. Susceptibility of sugarbeet lines to the stalk blight pathogen *Fusarium oxysporum* f. sp. *betae*. Plant Disease Reporter. 60:192-196.

Martinez-Soto D., Yu H., Allen K. S. and Ma L. J. 2023. Differential colonization of the plant vasculature between endophytic versus pathogenic *Fusarium oxysporum* strains. Molecular Plant Microbe Interactions. 36:4-13.

Martyn R. D., Rush C. M., Biles C. L. and Baker E. H. 1989. Etiology of a root rot disease of sugar beet in Texas. Plant Disease. 73:879-884.

McEnroe P. and Coulter B. 1964. Effect of soil pH on sugar content and yield of sugar beet. Irish Journal of Agricultural Research. 3:63-69.

McFarlane J. S. 1981. Fusarium stalk blight resistance in sugar beet. Journal of the American Society of Sugar Beet Technologists. 21:175-183.

McKeen C. D. and Wensley R. N. 1961. Longevity of *Fusarium oxysporum* in soil tube cultures. Science. 134:1528-1529.

Mennan S., Akson H. M., and Ecevit O. 2005. Antagonistic effect of *Fusarium oxysporum* on *Heterodera cruciferae*. Journal of Phytopathology. 153:221-225.

Mirocha C. J., Harrison J., Nichols A. A. and McClintock M. 1968. Detection of a fungal estrogen (F-2) in hay associated with infertility in dairy cattle. Applied and Environmental Microbiology. 16:797-798.

Mirocha C. J., Schauerhamer B. and Christensen C. M. 1976. Natural occurrence of *Fusarium* toxins in feedstuff. Applied and Environmental Microbiology. 31:553-556.

Moshari S., Mahmoudi S. B., Hemmati R., Naderpour M., Uemura M., Pedram A. and Azizi H. 2021. Evaluation of yield and resistance of sugar beet cultivars to fungal root rots under drought stress. Australasian Plant Pathology. 51:91-100.

Nelson P. E. 1981. Life cycle and epidemiology of *Fusarium oxysporum*. In: Mace M. E., Bell A. A. and Beckman C. H. eds. Fungal Wilt Diseases of Plants. Academic Press Inc., New York, NY USA. Pp. 51-78.

Nelson P. E., Toussoun T. A. and Marasas W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University, University Park. 193 pages.

Olivain C. and Alabouvette C. 1999. Process of tomato root colonization by a pathogenic strain of *Fusarium oxysporum* f. sp. *lycopersici* in comparison with a non-pathogenic strain. New Phytologist. 141:497-510.

Osawa H., Sakamoto Y., Akino S. and Kondo N. 2020. Autumn potato seedling failure due to potato dry rot in Nagasaki Prefecture, Japan, caused by *Fusarium acuminatum* and *Fusarium commune*. Journal of General Plant Pathology. 87:46-50.

O'Sullivan E. and Kavanagh J. A. 1991. Characteristics and pathogenicity of isolates of *Rhizoctonia* spp. associated with damping-off of sugar beet. Plant Pathology. 40:128-135.

Pegg G. F. 1981. Biochemistry and Physiology of Pathogenesis. In: Mace M. E., Bell A. A. and Beckman C. H. eds. Fungal Wilt Diseases of Plants. Academic Press Inc., New York, NY USA. Pp. 193-253.

Pramsohler M., Lichtenberger E., and Neuner G. 2022. Seasonal xylem sap acidification is governed by tree phenology, temperature and elevation of growing site. Plants Vol. 11. DOI: 10.3390/plants11152058.

Radke J. K. and Bauer R. E. 1969. Growth of sugar beets as affected by root temperatures part I: greenhouse studies. Agronomy Journal. 61:829-972.

Rivera V., Rengifo J., Khan M., Geiser D. M., Mansfield M. and Secor G. 2008. First report of a novel *Fusarium* species causing yellowing decline of sugar beet in Minnesota. Plant Disease. 92:1589.

Rocha A. J., Soares J. M. D. S., Nascimento F. D. S., Rocha A. D. S., Amorim V. B. O., Ramos A. P. S., Ferreira C. F., Haddad F., and Amorim E.P. 2022. Molecular, histological and histochemical responses of banana cultivars challenged with *Fusarium oxysporum* f. sp. *cubense* with different levels of virulence. Plants (MDPI). 11:2339. DOI: 10.3390/plants11182339.

Rodríguez-Gálvez E. and Mendgen K. 1995. The infection process of *Fusarium oxysporum* in cotton root tips. Protoplasma. 189:61-72.

Ruppel E. G. 1991. Pathogenicity of *Fusarium* spp. from diseased sugar beets and variation among sugar beet isolates of *F. oxysporum*. Plant Disease. 75:486-489.

Secor G. A., Rivera-Varas V., Christ D. S., Mathew F. M., Khan M. F. R., Varrelmann M., and Bolton M. D. 2014. Characterization of *Fusarium secorum*, a new species causing Fusarium yellowing decline of sugar beet in north central USA. Fungal Biology. 118:764-775.

Shahbandeh M. and Food and Agriculture Organization of the United Nations (FAO). 2024. Worldwide leading sugar beet producers worldwide in 2022, based on production volume. FAOSTAT Statistical Database. www.statista.com/statistics/264670/top-sugar-beet-producers-worldwide-by-volume/ and www.fao.org/faostat.

Schnathorst W. C. 1981. Life cycle and epidemiology of *Verticillium*. In: Mace M. E., Bell A. A. and Beckman C. H. eds. Fungal Wilt Diseases of Plants. Academic Press Inc., New York, NY USA. Pp. 81-108.

Schneider C. L. and Whitney E. D. 1986. Fusarium yellows. In: Whitney E. D. and Duffus J. E., eds. Compendium of Beet Diseases and Insects 1st ed. APS Press, St. Paul, MN, USA. Pp. 18-19.

Schwartz, H. F., Panella, L. W., Brick, M. A. and Byrne, P. F. 2001. Fusarium wilt and yellows of sugar beet and dry bean. Colorado State University Extension Fact Sheet 2.950. Fort Collins, CO. 3pgs.

Sitton J. W. and Cook R. J. 1981. Comparative morphology and survival of chlamydospores of *Fusarium roseum* '*culmorum*' and '*graminearum*.' Phytopathology. 71:85-90.

Skovgaard K., Rosendahl S., O'Donnell K., and Nirenberg H. I. 2004. *Fusarium commune* is a new species identified by morphological and molecular phylogenetic data. Mycologia. 95:630-636.

Snyder W. C. and Hansen H. N. 1940. The species concept in *Fusarium*. American Journal of Botany. 27:64-67.

Stewart D. 1931. Sugar-beet yellows caused by *Fusarium conglutinans* var. *betae*. Phytopathology. 21:59-70.

Stewart J. E., Kim M. S., James R. L., Dumroese R. K., Klopfenstein N. B. 2006. Molecular characterization of *Fusarium oxysporum* and *Fusarium commune* from a conifer nursery. Phytopathology. 96:1124-1133.

Summerell B. A., Leslie J. F., Backhouse D., Bryden W. L. and Burgess L. W. 2001. *Fusarium*: Paul E. Nelson Memorial Symposium. APS Press, St. Paul, MN, USA. 392 pgs.

Trebbi D. and McGrath M. J. 2009. Functional differentiation of the sugar beet root system as indicator of developmental phase change. Physiologia Plantarum. 135:84–97.

Turland N. J., Wiersema J. H., Barrie F. R., Greuter W., Hawksworth D. L., Herendeen P. S., Knapp S., Kusber W.-H., Li D.-Z., Marhold K., May T. W., McNeill, J., Monro A. M., Prado J., Price M. J. and Smith G. F. (eds.). 2018. International code of nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress

Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. DOI: 10.12705/Code.2018

Twomey J. A. 1952. Factors affecting the growth of *Fusarium oxysporum* f. *betae* (Abstr.). Journal of Colorado-Wyoming Academy of Science. 4:73.

Wang G., Dong Y., Stevanato P., Lv C., Liu Y., Cheng S., Geng G., Yu L., and Wang Y. 2022. Growth status and physiological changes of sugar beet seedlings in response to acidic pH environments. Journal of Plant Physiology Vol. 277. DOI: 10.1016/j.jplph.2022.153771

Webb K. M., Covey P. A. and Hanson L. E. 2012. Pathogenic and phylogenetic analysis of *Fusarium oxysporum* from sugarbeet in Michigan and Minnesota. Journal of Sugar Beet Research. 49:38-57.

Webb K. M., Case A. J., Brick M. A., Otto K. and Schwartz H. F. 2013. Cross pathogenicity and vegetative compatibility of *Fusarium oxysporum* isolated from sugar beet. Plant Disease. 97:1200-1206.

Webb K. M., Brenner T. and Jacobsen B. J. 2015. Temperature effects on the interactions of sugar beet with Fusarium yellows caused by *Fusarium oxysporum* f. sp. *betae*. Canadian Journal of Plant Pathology. 37:353-362.

Webb K. M., Delgrosso S. J., West M. S., Freeman C. and Brenner T. 2017. Influence of environment, crop age and cultivar on the development and severity of Fusarium yellows in field-grown sugar beet. Canadian Journal of Plant Pathology. 39:37-47.

Webb K. M., Shresthab S., Trippe III R., Rivera-Varas V., Covey P. A., Freeman C., de Jonge R., Secor G. A. and Bolton M. 2019. Phylogenetic relationships and virulence assays of *Fusarium secorum* from sugar beet suggest a new look at species designations. Plant Pathology. 68:1654-1662.

Wilkinson S., Corlett J. E., Oger L., and Davies W. J. 1998. Effects of xylem pH on transpiration from wild-type and *flacca* tomato leaves. Plant Physiology. 117:703–709.

Wilson R. and Smith J. 2001. Sugarbeet Production Guide. University of Nebraska-Lincoln Extension, Scottsbluff NE, USA. Pp. 173-174.

Windels C. E., Brantner J. R., Bradley C. A. and Khan M. F. R. 2007. First report of *Fusarium oxysporum* causing yellows on sugar beet in the red river valley of Minnesota and North Dakota. Plant Disease. 89:341

Windels C. E. and Harveson R. M. 2009. Aphanomyces root rot. In: Harveson R.M., Hanson L.E., Hein G.L., eds. Compendium of Beet Diseases and Pests, 2nd ed. APS Press, St. Paul, MN, USA. Pp. 24-27.

USDA NASS. 2024. Michigan Agricultural Statistics 2022-2023. https://www.nass.usda.gov/Statistics_by_State/Michigan/index.php

CHAPTER 2: VIRULENCE SCREENING OF *FUSARIUM OXYSPORUM* SPECIES COMPLEX ISOLATES FROM MICHIGAN SUGAR BEET FIELDS

Abstract:

The sugar beet industry in Michigan (MI) is an important source of revenue for the state; in 2022, this culminated in $500 million in direct economic benefit to Michigan, and $1.5 billion indirectly. Species within the *F. oxysporum* species complex (FOSC) can cause diseases in sugar beet, including Fusarium yellows (aka Fusarium wilt) and Fusarium root rot. These diseases can lead to yield losses due to reduced crop size, reduction in sugar production, and may be a contributing factor in post-harvest storage losses. The primary strategy to reduce *Fusarium*-caused diseases in sugar beet has been by selecting for resistant sugar beet varieties. However, it has been demonstrated that resistance to isolates in one region did not always carry over to other regions, such as Minnesota to Michigan. The objectives of this study were to identify local MI FOSC isolates that were pathogenic on sugar beet and assess their virulence levels. Isolates belonging to the FOSC were screened for virulence in the greenhouse on a pair of USDA sugar beet germplasm that were moderately resistant and moderately susceptible to Fusarium yellows based on prior testing from several production regions. In comparison to the virulent controls, of the 35 isolates screened, 23 were pathogenic, with 21 isolates that were classified as low virulence and 2 classified as moderate virulence. No highly virulent isolates from Michigan were identified. The moderately virulent isolates have downstream applications in screening for resistance to *Fusarium* spp. These isolates could also be useful in developing a *Fusarium* resistance screening system for selecting cultivars suitable to grow in Michigan.

Introduction:

Sugar beet diseases caused by *Fusarium oxysporum* and related species are a growing problem in Michigan (MI) as the optimal disease conditions are fulfilled at an increased frequency. For diseases such as Fusarium yellows and Fusarium root rot to occur, a minimum temperature of 25C is needed, and disease is enhanced by water stress such as drought, flooding, or a mixture of both conditions (Gracia-Garza and Fravel 1998). There is ample evidence that if the temperature remains below 25C, *F. oxysporum* may infect a plant but remain in an endophytic lifestyle and will not cause symptoms in sugar beet (Hanson et al. 2009, Harveson and Rush 1998, Webb et al. 2015). The past decade has seen increased water extremes throughout the field season in addition to higher temperatures, resulting in ideal conditions for *Fusarium*-induced disease progression (Frankson et al. 2022, MiDHHS 2024). In the main sugar beet production region of Michigan, the number of days at 25C or higher in a year has increased from 71 days in 1979 to 98 days in 2022 (NOAA 2024). The average precipitation per year has been above the extreme (2 inches or more) since 1999 and the wettest consecutive 5 year span occurred from 2016 to 2020, with 2021 to 2025 on track to exceed that 5 year span (Frankson et al. 2022). Therefore, *Fusarium*-induced diseases are likely to remain an issue for MI sugar beet growers and if current weather trends persist, increase in prevalence.

Fusarium yellows (also known as Fusarium wilt) was named for its foliar symptoms of interveinal and half-leaf chlorosis, although it also produces wilt and stunting progressing to half-leaf and full-leaf necrosis and eventually plant death (Hanson and Jacobsen 2009, Stewart 1931). The historical causal agent, *F. oxysporum* f. sp. *betae* (FOB) infects and colonizes the vascular tissue of the root to cause these symptoms (Hanson and Jacobsen 2009, Stewart 1931). The symptoms within the root are characterized by interveinal discoloration and vascular

necrosis, typically originating at the tip of the root (Harveson 2007, Stewart 1931). Fusarium root rot from *F. oxysporum* (also known as Fusarium tip rot) develops similar foliar and internal root symptoms as Fusarium yellows, with the addition of necrosis on the surface of the root usually starting at or near the root tip (Harveson 2007, Harveson 2009, Martyn et al. 1989). It has been proposed to use a different *formae specialis* (*radices-betae*) for Fusarium root rot (Martyn et al. 1989), but there was uncertainty over if there was evidence to support the designation of a separate *formae specialis* as no grouping based on phylogenetic analysis or vegetative compatibility has been reported (Hill et al. 2011, Webb et al. 2012).

In recent years, over a dozen FOB isolates across various states (OR, CO, MN) have been re-classified as *F. commune* based solely on molecular identification using translation elongation factor 1α (TEF1a, Webb et al. 2019). The majority of these re-classified isolates belong to what was defined as "Clade A" of the FOB phylogeny as originated by Hill et al., with one isolate each in Clades B and C (Hill et al. 2011, Webb et al. 2019, Pollok and Hanson unpublished data). *Fusarium commune* belongs to the *F. nisikadoi* species complex, a sub complex within the *F. oxysporum* species complex (FOSC, Crous et al. 2021, Skovgaard et al. 2004). The single morphological difference between these two species is the development of polyphialides when grown in the dark on conidiation specific agar, such as carnation leaf agar (CLA) or Spezieller Nährstoffarmer agar (SNA). *Fusarium commune* forms both monophialides and polyphialides whereas *F. oxysporum* forms only monophialides (Leslie and Summerell 2006, Skovgaard et al. 2004). However, this morphological difference has been widely reported as a flexible trait (Carnegie et al. 2022, Deng et al. 2022, Edel-Hermann et al. 2012, Osawa et al. 2020, Stewart 2006). As such, *F. commune* could only be putatively identified using molecular methods, which is not supported by the current taxonomic code for fungi (Carnegie et al. 2022,

Deng et al. 2022, Stewart 2006, Turland et al. 2018). To the best of our knowledge, there have been no reports of phenotypic differences between *F. commune* from beet and FOB isolates in regards to morphology, geographic origin, and pathogenicity. Without a reliable morphological difference, identification as a separate species is questionable, and as such isolates that are genetically similar to *F. commune* in this study are described as "putative" classifications.

There are several strategies to reduce *Fusarium*-caused diseases and symptom severity in sugar beet including the use of cultural practices and irrigation, but by far the most effective is selecting for resistant sugar beet varieties (Schwartz et al. 2001). While there are variety screenings in place to assess resistance in sugar beet to *F. oxysporum*, varieties used in Michigan are screened in Minnesota fields, so only isolates local to the Red River Valley are included (Lai et al. 2020, Panella et al. 2018). This presents a dilemma, as it has been observed that *F. oxysporum* has distinct pathogenic isolates in various production regions (Hanson et al. 2009, Harveson and Rush 1997, Hill et al. 2011, Webb et al. 2012). As such, a sugar beet variety that is resistant to strains in one region may not be resistant to isolates in another, as seen by Ruppel with a variety originally from Oregon that was planted in Colorado and similar results have been observed by others (Ruppel 1991, Hanson et al. 2009). It has been demonstrated that having representative isolates for a production region provides a more accurate prediction of what sugar beet varieties will show a reduced disease severity from *F. oxysporum* in that region (Godby, personal communication, Hanson et al. 2009, Hill et al. 2011). In addition, up to 92% of isolated pathogenic *Fusarium* spp. in the Red River Valley are *Fusarium secorum*, the causal agent of Fusarium yellowing decline in sugar beet (Rivera et al. 2008, Secor et al. 2014). In Michigan, *F. secorum* has been observed rarely, and to our knowledge, Fusarium yellowing decline has not been reported in Michigan. As such, screening for resistance to *F. secorum* is not a priority for

Michigan growers at this time. With this in mind, it would be beneficial to identify pathogenic Michigan isolates and incorporate them into local resistance screening programs in Michigan. This also would provide us with pathogen strains to use in future research to better understand the mechanisms by which FOSC strains cause disease, examine potential resistance mechanisms, and test other management tools. The objectives of this study were to identify pathogenic Michigan isolates from the FOSC, and to assign a virulence level to each identified pathogenic isolate to obtain promising moderate to high virulence isolates for use in MI sugar beet variety screening and scientific applications.

Methods and Materials:

Symptomatic sugar beets were provided by Sugar Beet Advancement and Michigan Sugar field staff, or collected directly from grower fields or research plots at the Saginaw Valley Research and Extension Center (SVREC) by lab personnel. Sugar beet roots were cross-sectioned and 5mm segments of symptomatic root tissue, especially showing vascular discoloration, were surface disinfected with 0.5% sodium hypochlorite for 60 seconds and placed on potato dextrose agar (PDA, Sigma-Aldrich St. Louis, MO, Nelson et al. 1983). Plates were incubated on a bench top at room temperature with ambient light. Through successive hyphal tip transfers to half strength clarified V8 agar (V8A, Miller 1955), pure fungal cultures were isolated, taking note of other fungal species observed and selected for likely *Fusarium* spp. based on pigmentation and mycelial morphology (Leslie and Summerell 2006). When bacteria were confluent on and around fungal cultures, hyphal tips were transferred to 2% water agar (WA) instead of V8A. Resulting single hyphal tips that appeared to be bacteria-free were transferred to V8A plates. Each cleaned *Fusarium* isolate was transferred via a size 3 core borer (7mm diameter) to a replicate PDA and a carnation leaf agar plate (CLA, Fisher et al. 1982, Snyder and

Hansen 1947). The CLA plates were incubated under continuous cool fluorescent lights at room temperature for at least two weeks. During that same time, the PDA plates were incubated on the bench top at room temperature with ambient light. The PDA plates were examined for pigmentation, mycelium color and texture, sclerotia presence, and abundance and color of sporodochia. The CLA plates were examined under a light microscope (Olympus, Tokyo, Japan) for phialide type(s), and the presence and morphology of chlamydospores, microconidia and macroconidia (Appendix Table 16). From these observations, the *Fusarium* species of each isolate was determined based on the identification key of Leslie and Summerell (2006).

For isolates that could not be reliably identified based solely on morphology, mycelium was grown in half strength clarified V8 broth (V8B) for 1 week on a shaking incubator at 150 rpm, 27C in ambient light. The resulting mycelium was harvested using a funnel filter and collected in sterile 15ml capped plastic tubes. The tubes were frozen at -80C for at least 1 hour, lyophilized (Freezone 6L -84C, Labconco, Kansas City, MO), and ground to a fine powder via a modified paint mixer (Miracle Paint Rejuvenator Co., Inver Grove Heights, MN) for DNA extraction. DNA was extracted using a Nucleospin® Plant II DNA extraction kit (Macherey-Nagel, Düren, Germany) with a modified protocol. Modifications to the DNA extraction kit protocol included increasing the cell lysis time at 65C from 10 minutes to 1 hour, and omitting RNase A from the lysis solution. The approximate concentration and purity of extracted DNA was determined via spectrophotometer (ND-8000, NanoDrop, Wilmington, DE) and diluted to approximately 50ng/ul in sterile distilled water. The diluted DNA was amplified via polymerase chain reaction (PCR), using TEF1a primers developed by O'Donnell et al. (1998, Table 1) with a negative water control in 25µl reactions. Each reaction contained a final concentration of 1x Phusion™ HF Buffer (Thermoscientific, Waltham, MA), 200 µM of each dNTP (Promega,

Madison, WI), 0.5 μM of each primer (Integrated DNA Technologies, Coralville, IA), 1 unit of Phusion™ HF DNA polymerase (Thermoscientific), and 50ng of genomic DNA. The PCR was performed in a C1000 Touch Thermocycler (BioRad, Hercules, CA). Based on the manufacturer's protocol for Phusion™ HF DNA polymerase, the amplification conditions were an initial denaturation of 3 min at 98C, 35 cycles of denaturation for 10 sec at 98C, annealing for 15 sec at 54C, elongation for 15 sec at 72C, a final elongation time of 7 min at 72C, and products were held at 4C until analysis. PCR products were separated on 1.5% agarose gel in 1x Tris acetate EDTA (TAE, Maniatis et al. 1982) buffer and stained with RedSafe™ (iNtRON Biotechnology, Seongnam, South Korea). The resulting bands were visualized with a gel imaging system (UVP ChemStudio; Analytikjena, Jena, Germany), and the sizes of products were estimated via comparison to Quick-Load 100bp ladder (New England Biolabs, Ipswich, MA).

PCR products were cleaned using spin filtration columns by loading equal volumes of Sephadex^{TM} G-50 in the wells of a 96 well filter plate loaded using a Sephadex loading block (Molitor, unpublished, GE Healthcare Bio-Sciences, Piscataway, NJ). Each well of Sephadex was suspended using 300μl of sterile distilled water. The filter plate was placed on top of a 96 well collection plate and centrifuged for 2 minutes at 377xg. The flow through in the collection plate was discarded, and resulting gel filtration columns were loaded with the PCR products and placed on a new collection plate. The loaded wells were centrifuged for 2 minutes at 671xg to purify PCR products. The filter plate was discarded and the collection plate with the purified PCR products was sealed with adhesive aluminum foil. In preparation for Sanger sequencing, 2μl (5-20ng total DNA) of purified DNA were loaded into 2 wells on a 96 well plate, with 30 picomoles of the forward and reverse primers added to separate wells for a total volume of 12μl

in each well. The purified PCR products were subjected to forward and reverse Sanger sequencing by Michigan State University's Research Technology Support Facility Genomics Core. Sequence cleanup and consensus sequence assembly were performed using Geneious Prime (2024.0.5, https://www.geneious.com). The resulting consensus sequences were run through a Geneious Prime BLAST search in the databases NCBI, FUSARIUM-ID v3.0, and FUSARIOID-ID (Crous et al. 2022, Torres-Cruz et al. 2022) to confirm the identity of each isolate. Once identified, the *Fusarium* isolates were stored on sterile glass fiber filter paper in a -20C freezer, using Peever and Milgroom's protocol (1979) as modified by Hanson and Hill (2004). For isolates that most closely matched *F. commune* through TEF1a sequence analysis, an additional set of sequence analysis was performed using Beta-tubulin primers C and D (Koenraadt et al. 1992). The same thermocycler, gel, and sequence analysis protocol was used as with TEF1a, with the exception of the annealing temperature, which was changed from 54C to 55C.

Table 1: Primer information for PCR amplification of *Fusarium* isolates from sugar beet.

Primer	**Direction**	**Sequence (5'→3')**	**Citation**
EF-1	Forward	ATGGGTAAGGARGACAAGAC	O'Donnell et al. 1998
EF-2	Reverse	GGARGTACCAGTSATCATG	O'Donnell et al. 1998
Btub C	Forward	GAGGAATTCCCAGACCGTATGATG	Koenraadt et al. 1992
Btub D	Reverse	GCTGGATCCTATTCTTTGGGTCGAACAT	Koenraadt et al. 1992

Sugar beet germplasm used for this experiment were from the USDA-ARS breeding programs, described in the germplasm resources information network (USDA-ARS GRIN, Beltsville, MD). Initially, the two germplasm used were C869 cytoplasmic male sterile (C869cms) for a moderately susceptible form (Lewellen 2004) and FC716 for a moderately resistant form (Panella et al. 1995); however, FC716 was replaced with F1042 (Campbell 2015) after the first two runs due to low germination rates. Seeds were surface disinfected in 0.5% sodium hypochlorite for 20 minutes and rinsed twice with sterile distilled water. Prior to

planting, seeds were imbibed with a 0.3% hydrogen peroxide solution overnight on a shaking incubator at 150 rpm and 27C to enhance germination (Hanson 2017, McGrath et al. 2000). After imbibition, most of the 0.3% hydrogen peroxide solution was poured off, keeping just enough to coat the bottom of the flask. 2µl of Allegiance FL fungicide (active ingredient Metalaxyl, Bayer Crop Science, Whippany, New Jersey) was applied to the seeds to manage Pythium damping-off (Harveson et al. 2007). Approximately 50 seeds were planted per plastic pot (6in tall, 6.5in diameter,) with SureMix^{TM} potting mix (Michigan Grower Products Inc. Galesburg, MI). Plants were grown in a greenhouse in a completely randomized arrangement, with conditions set to a minimum temperature of 22C, maximum temperature of 31C, and a 16:8 hour light-dark cycle. Plants were watered every day to every other day as needed for maintaining soil moisture for plant growth. At the first two true leaf growth stage (roughly 3 weeks after planting), the sugar beet seedlings were transplanted to 2.25gal plastic pots (9.25in tall, 10in diameter) with SureMix^{TM} potting mix (Michigan Grower Products Inc.). There were three seedlings per pot for observational units, and three replicate pots per experimental run of each variety for each treatment. Pots were arranged at random on a greenhouse bench. Watering conditions remained the same. Greenhouse temperature was tracked and monitored throughout the duration of experiments using a Watchdog A-series Data Logger (Spectrum® Technologies, Inc. Aurora, Illinois) and analyzed using Specware 9 basic software (Spectrum® Technologies) to monitor the temperature in the greenhouse and adjust the greenhouse settings accordingly to achieve optimal conditions for disease development.

The selection criteria for *Fusarium* isolates from the Hanson lab fungal collection to use in this experiment were isolates collected in Michigan from 2007 onwards, presence of Fusarium yellows symptoms observed on the original host plant, low macroconidia production, and

belonging to the FOSC based on morphology (Leslie and Summerell 2006, Ruppel 1991). Low macroconidia production has been demonstrated to correlate to virulence, with FOSC isolates in both sugar beets and dry beans (Cramer et al. 2003, Hanson et al. 2001, Hill et al. 2011, Ruppel 1991). FOSC isolates (Table 2) were removed from long-term paper copy storage and plated onto replicate V8A and PDA plates. The PDA plates were used to confirm morphology was consistent with prior descriptions, and the V8A plates were used for subsequent maintenance. After a week of growth at room temperature on a bench top with ambient lighting, a size 3 core borer (7mm diameter) was used to transfer colonized agar plugs to flasks with 50ml of sterile V8B, with three flasks per FOSC isolate. Broth cultures were incubated on a shaking incubator for one week at 150 rpm and 27C with ambient light. The resulting broth cultures were strained through 3 layers of sterile cheesecloth to separate spores and mycelia. The mycelium was collected from the cheesecloth, combining the contents of the three flasks for each isolate into a sterile plastic bottle (200ml) and 50ml of sterile distilled water (dH2O) was added to re-suspend the harvested mycelia and spores. Using sterile industrial grinders (Waring Commercial, Stamford, CT), each suspension of mycelium and spores was ground at 10 second intervals on the lowest setting, checking for fine hyphal fragments between each interval. When the slurry was fairly even and no large chunks of mycelium were visible, the contents were poured into a plastic bottle and set aside. Grinders were treated with 0.5% sodium hypochlorite for at least 5 minutes and rinsed once in sterile dH2O between isolates. A haemocytometer was used to count macroconidia and hyphal fragments. The relative abundances of chlamydospores and microconidia were noted as well. The combined concentration of macroconidia and hyphal fragments (aka colony forming units, cfu) was calculated using the equation:
(Average cfu)x10,000] based on instructions with the haemocytometer (Thermo Fisher

Scientific, Waltham, MA). The volume of cfu/ml suspension and sterile DH2O needed to produce two sets of 250ml stocks at 4x10^4 cfu/ml for each isolate was calculated using the dilution formula: $c_1v_1 = c_2v_2$.

Sugar beets were inoculated at the 6 to 8 leaf growth stage (roughly 5 weeks after planting) via a root dip (Hanson and Hill 2004). Sugar beets were removed from potting mix and the majority of the potting mix was rinsed off roots with potable water before submerging the roots in an inoculum stock for 8 minutes with gentle agitation every 60 seconds (Hanson and Hill 2004). The control plants were soaked in sterile dH2O serving as the negative control. After the root dip, sugar beets were replanted in the 2.25gal pots and gently watered. This was repeated with each variety, and each inoculum source (Hanson and Hill 2004). The positive controls were FOB220a (moderately to highly virulent, Webb et al. 2012, Wickliffe 2001) and F19 (highly virulent, Hill et al. 2011, Webb et al. 2012). Pots were arranged at random on a greenhouse bench, with the same greenhouse conditions as described earlier and watering regime for 1 week to allow recovery from transplant stress. One week after inoculation, damaged leaves were pruned, each pot was fertilized with 1 tablespoon of Osmocote Plus (15-9-12 and micronutrients, Miracle-Gro, Marysville, OH). At this time, the greenhouse thermostat was adjusted to a minimum temperature of 25C, and the watering regime transitioned to biweekly watering to produce mild drought conditions. Initial observations were also taken, noting if any plants had died due to suspected transplant stress. Foliar ratings were taken once a week for 6 weeks after inoculation using the USDA Fusarium yellows disease rating scale (Table 3). After the final foliar rating, plants were removed from the potting mix and sugar beet roots were examined and cross-sectioned transversely starting at the root tip and progressing up the root, in order to rate root symptom severity caused by each isolate (Table 4). At least one root from each inoculum,

including the water control, were collected for re-isolation. The water control was included to ensure neither the potting mix nor seeds were not naturally harboring *Fusarium* spp. Fungal species were isolated from root samples using the same techniques as described for initial isolation. The morphology of re-isolated fungi was noted, and *Fusarium*-like fungi were identified using the same DNA and TEF1a sequencing protocol as described for initial identification. Experimental runs were repeated for moderately virulent isolates, and a representative from each of the low virulence and avirulence categories. The repeated experimental runs were performed at a different time of year than the first run to ensure that the time of year did not impact symptom severity.

The foliar and root ratings of the observational units within each experimental unit were averaged. The series of Fusarium yellows foliar symptom ratings were interpreted using the area under the disease progression steps (AUDPS, Simko and Piepho 2012) for each experimental unit. The Fusarium yellows root ratings were assessed as the mean root rating of each experimental unit. Data was analyzed using R 2023.12.1.402 to run multifactor analyses of each experimental run (Posit team 2024, see Appendix Table 6 for R package information). Each experimental run was analyzed as a completely randomized design, with the sugar beet varieties and isolates as independent factors, and the foliar AUDPS and mean root rating as dependent factors in their respective statistical analyses. Significant interactions were determined using a preliminary analysis of variance (ANOVA, p-value <0.05). When data from an experimental run did not meet the equality of variances assumption required for ANOVA, data was transformed using $\log10(x+1)$ where x was the original data. Depending on the findings from ANOVA (Appendix Tables 9 and 10), experimental runs were sliced by significant factors for Fisher's

Table 2: *Fusarium* isolates used in virulence screening on sugar beet.

Geographic Origin	Isolate	Taxonomy	Year Isolated	Provided by
Colorado	Fob220a	*F. commune**	1998	H. F. Schwartz
Oregon	F19	*F. commune**	2001	A. Hill
Michigan	F05-284	*F. oxysporum*	2005	L. E. Hanson
	F07-43	*F. oxysporum*	2007	L. E. Hanson
	F07-48	*F. oxysporum*	2007	L. E. Hanson
	F07-49	*F. oxysporum*	2007	L. E. Hanson
	F08-193	*F. oxysporum*	2008	L. E. Hanson
	F08-207	*F. oxysporum*	2008	L. E. Hanson
	F09-16	*F. oxysporum*	2009	L. E. Hanson
	F09-53	*F. oxysporum*	2009	L. E. Hanson
	F09-87	*F. oxysporum*	2009	L. E. Hanson
	F10-58	*F. oxysporum*	2010	L. E. Hanson
	F11-2	*F. oxysporum*	2011	L. E. Hanson
	F11-3	*F. oxysporum*	2011	L. E. Hanson
	F11-63	*F. oxysporum*	2011	L. E. Hanson
	F11-67	*F. oxysporum*	2011	L. E. Hanson
	F12-12	*F. oxysporum*	2012	L. E. Hanson
	F12-24	*F. oxysporum*	2012	L. E. Hanson
	F12-25	*F. oxysporum*	2012	L. E. Hanson
	F12-36	*F. oxysporum*	2012	L. E. Hanson
	F12-44	*F. oxysporum*	2012	L. E. Hanson
	F13-10	*F. oxysporum*	2013	L. E. Hanson
	F14-22	*F. oxysporum*	2014	L. E. Hanson
	F21-2	*F. oxysporum*	2021	L. E. Hanson
	F21-8	*F. commune**	2021	L. E. Hanson
	F21-22	*F. oxysporum*	2021	L. E. Hanson
	F21-42	*F. oxysporum*	2021	L. E. Hanson
	F21-76	*F. oxysporum*	2021	L. E. Hanson
	F22-4	*F. oxysporum*	2022	L. E. Hanson
	F22-7	*F. oxysporum*	2022	L. E. Hanson
	F22-12	*F. oxysporum*	2022	L. E. Hanson
	F22-24	*F. oxysporum*	2022	L. E. Hanson
	F22-28	*F. oxysporum*	2022	L. E. Hanson
	F22-34	*F. oxysporum*	2022	L. E. Hanson
	F22-35	*F. oxysporum*	2022	L. E. Hanson
	F23-7	*F. oxysporum*	2023	L. E. Hanson
	F23-11	*F. oxysporum*	2023	L. E. Hanson
	F23-27	*F. oxysporum*	2023	L. E. Hanson

* While the closest sequence match using TEF1a was to *F. commune*, these isolates lacked its distinctive morphological characters for this species, and instead had the morphology of *F. oxysporum*

protected least significant difference (LSD) to assign statistically significant differences across factors with a p-value less than 0.05, and assigned confidence interval of 10%. Tables and figures were generated to interpret these findings used the least square means and confidence intervals of the LSD table (Appendix Tables 11, 12, 13, and 14). In the case of the log transformed experimental runs, the resulting least square means and confidence intervals were reverse transformed using (1x10^y)-1 where y was the transformed data. The virulence levels of each isolate were assigned in comparison to the positive and negative controls. When an isolate was not statistically different from the water control, it was classified as avirulent. When an isolate was different from the water control, it was classified as pathogenic. Low virulence isolates were statistically different from both the water control and the moderately virulent control, Fob220a. Moderately virulent isolates were either not significantly different from Fob220a, or statistically different from low virulence isolates, in the event that Fob220a had a higher rating than F19 (Figure 3C). Among the pathogenic isolates, to be considered highly virulent, it would need to have no significant difference from F19, the highly virulent positive control.

Table 3: USDA Fusarium yellows foliar severity rating scale for sugar beet (Hanson et al. 2009).

Rating	**Symptoms**
0	No visible disease
1	Leaves wilted, small chlorotic areas on older leaves, majority of leaves were still green
2	Leaves might have half leaf chlorosis, or interveinal chlorosis
3	Leaves had the beginning of necrosis, but less than half of leaves affected
4	Half or more of the leaves were dead or symptomatic
5	Death of entire plant

Table 4: Fusarium yellows root severity rating scale for sugar beet.

Rating	Symptoms
0	No visible disease
1	Less than 25 percent of xylem discolored
2	25 to 50 percent of xylem discolored
3	50 to 75 percent of xylem discolored, and/or small pockets of interior necrosis
4	Over 75 percent of xylem discolored, and/or large pockets of interior necrosis.
5	The majority of root was necrotic or dead.

Figure 1: Foliar (top) and root (bottom) rating examples, from left to right rated from 0 to 5.

Results:

The sugar beet germplasm performed as expected, as the moderately susceptible sugar beet variety, C869cms, consistently displayed more severe symptoms for both foliar and root symptoms than the moderately resistant controls (FC716 and F1042) in the majority of the trials. This difference was observed visually and validated with statistical analysis through post hoc analysis using Fisher's protected LSD (Appendix Tables 11 and 12). In one instance, F1042 had a statistically significantly more severe foliar symptoms than C869cms, during experimental run 2, corresponding to the introduction of virus yellows into the sugar beet via aphids (Appendix Table 11).

Of the 35 MI isolates screened, 65.7% were pathogenic, with 60% low virulence, 5.7% moderate virulence, and no high virulence isolates. There were occasional complications

throughout the screening process due to external factors. For instance, due to an aphid infestation, one or more unknown members of the virus yellows complex were introduced into sugar beets during experimental run 2. The virus yellows complex had similar foliar chlorotic symptoms to Fusarium yellows, limiting our ability to draw conclusions from the foliar ratings, but did not impact the root symptoms (Figure 2B, Wintermantel 2009). Isolates F09-16 and F08-207 were redone in subsequent experimental runs to confirm potential pathogenicity and virulence independent of confounding factors, based on the root symptom severity ratings (Table 3). F11-63 was not repeated, given that the root symptom severity was not statistically significantly different from the water control.

Also worth noting was that in March 2023, the greenhouse thermostat was reset on March 1 and was not corrected until March 31 (Appendix Table 7). During this time interval, temperatures were sub-optimal for disease development, although not prohibitive (Harveson and Rush 1998, Webb et al. 2015). Experimental runs at this time included the end of the third experimental run and the beginning of the fourth experimental run. The third run's foliar and root ratings did not show an adverse impact (Figure 2). Isolates in the fourth run presented typical foliar symptoms, but had a sharp reduction in severity of root symptoms (Figure 2). During the fifth and sixth experimental runs, an infestation of spider mites and thrips occurred. Between the insect damage from the thrips and the regime of foliar spray treatments, foliar symptoms from the *Fusarium* isolates were inconsistent and in the case of the sixth run statistically significant differences could not be drawn even between the water control and the positive controls (Figure 2). For experimental runs 1, and 7 through 13, they were completed without complications.

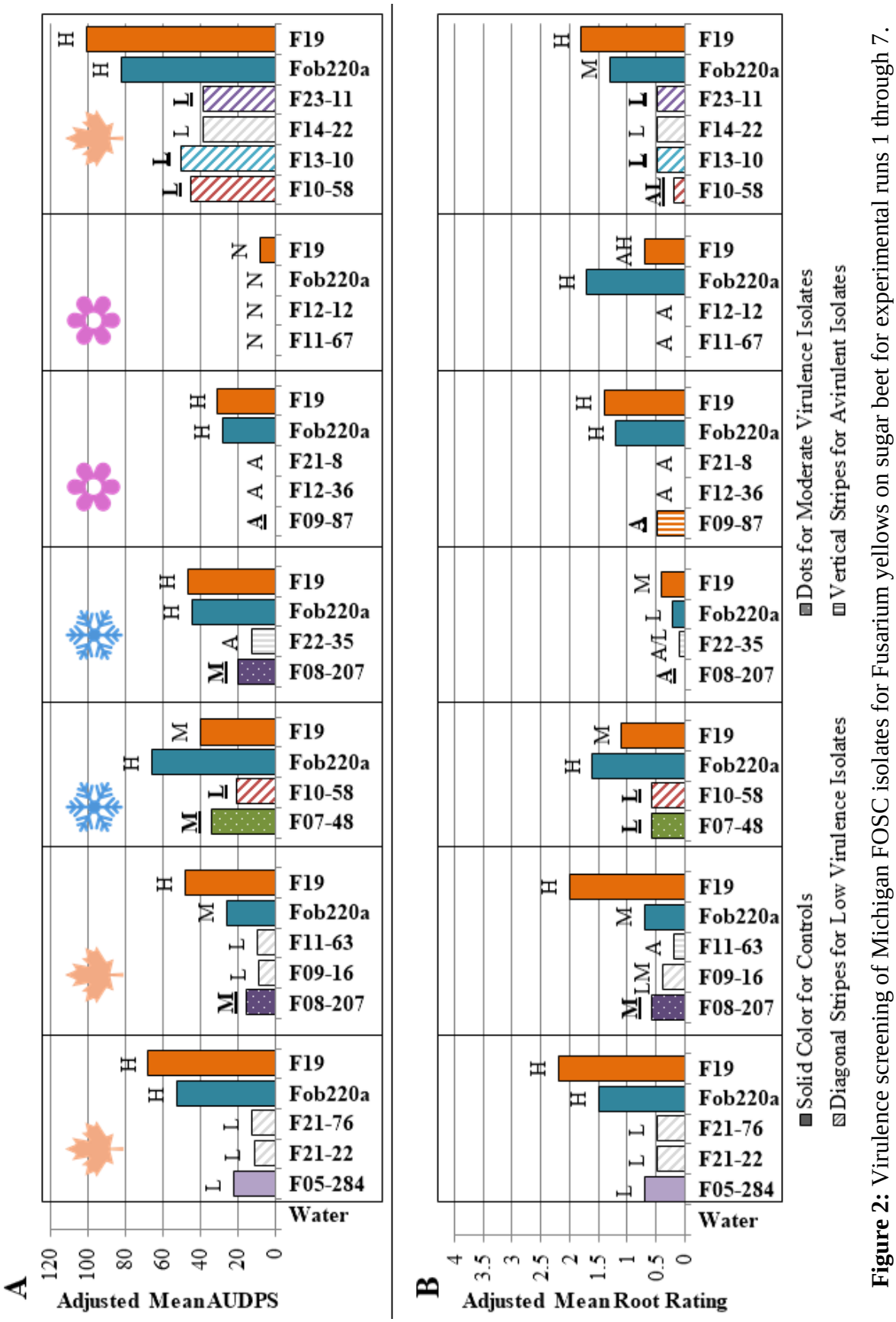

Figure 2: Virulence screening of Michigan FOSC isolates for Fusarium yellows on sugar beet for experimental runs 1 through 7.

Figure 2 (cont'd)
Runs 1-4 were in 2022 and runs 5-7 were in 2023. Data presented in each figure was minus the mean water rating for each experimental run. **A,** results from foliar ratings; **B,** results from root ratings. Virulence levels were assigned based on statistically significant differences between the negative control (water) and the positive controls (F19 for high virulence and Fob220a for moderate virulence) using Fisher's Protected LSD (α=0.05) on the raw data for each experimental run. Data presented in each figure is minus the mean water rating for each experimental run. The labels indicate statistically significant virulence levels assigned as avirulent (A), low virulence (L) moderate virulence (M), and high virulence (H), and no statistically significant differences (N). The leaf, snowflake and flower symbols represent the time of year the experimental run was screened: autumn (between September and November), winter (between December and February) and spring (between March and early June) respectively. The bolded and underlined letters emphasize isolates were run in multiple experimental runs.

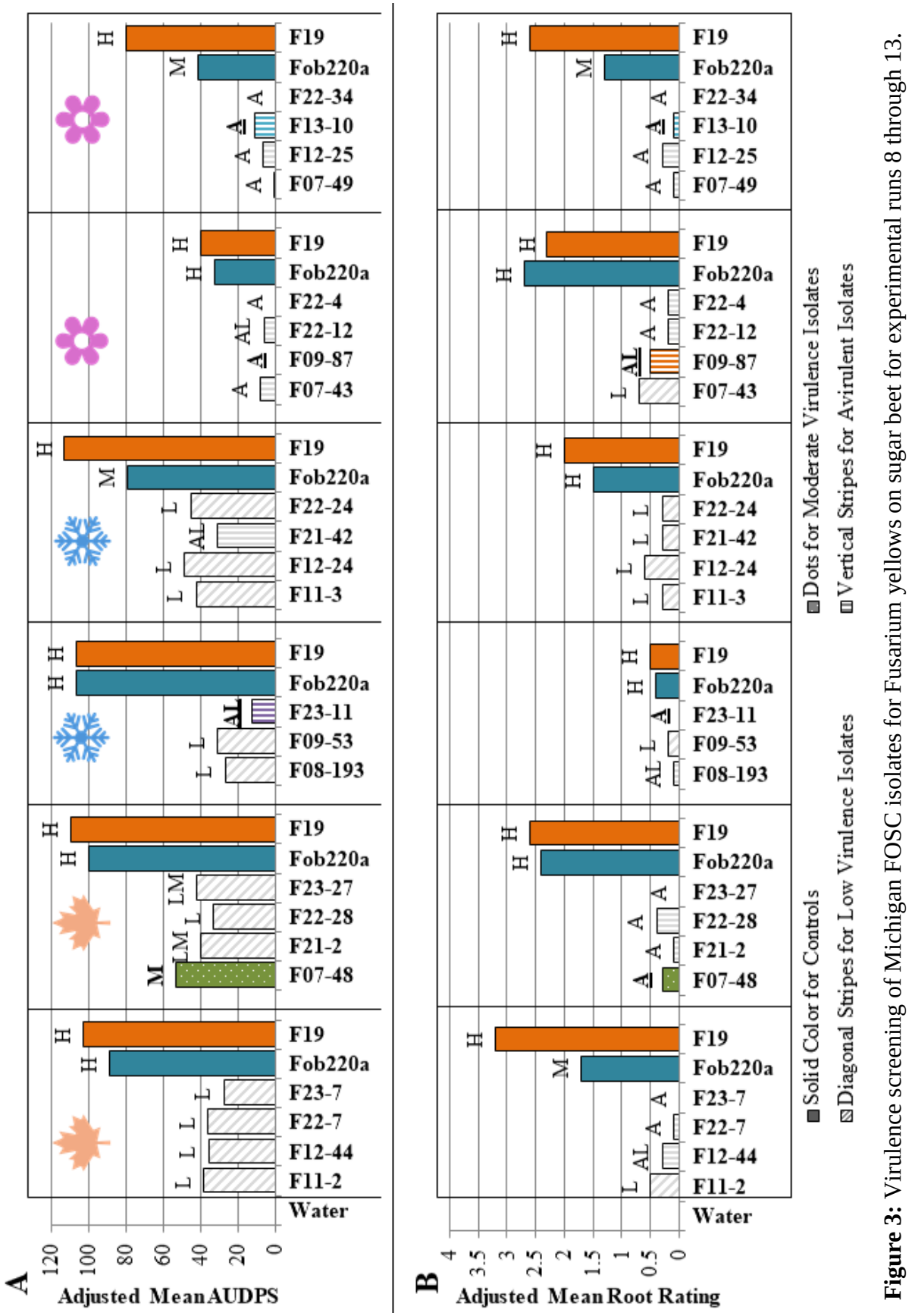

Figure 3: Virulence screening of Michigan FOSC isolates for Fusarium yellows on sugar beet for experimental runs 8 through 13.

Figure 3 (cont'd)
Runs 8-11 were in 2023 and runs 12-13 were in 2024. Data presented in this figure was minus the mean water rating for each experimental run. **A,** results from foliar ratings; **B,** results from root ratings. Virulence levels were assigned based on statistically significant differences between the negative control (water) and the positive controls (F19 for high virulence and Fob220a for moderate virulence) using Fisher's Protected LSD (α=0.05) on the raw data for each experimental run. Data presented in each figure is minus the mean water rating for each experimental run. The labels indicate statistically significant virulence levels assigned as avirulent (A), low virulence (L) moderate virulence (M), and high virulence (H), and no statistically significant differences (N). The leaf, snowflake and flower symbols represent the time of year the experimental run was screened: autumn (between September and November), winter (between December and February) and spring (between March and early June) respectively. The bolded and underlined letters emphasize isolates were run in multiple experimental runs.

Table 5: Summary of Fusarium yellows on sugar beet pathogenicity and virulence results (2022-2024).

Isolate	Identification	Foliar Symptoms		Root Symptoms		Repeats
		Patho.[a]	Virulence[b]	Patho.[a]	Virulence[b]	
F19	*F. commune**	Yes	MV/HV	Yes	HV	All
Fob220a	*F. commune**	Yes	MV/HV	Yes	MV/HV	All
F05-284	*F. oxysporum*	Yes	LV	Yes	LV	1
Water	--	No	AV	No	AV	All
F07-49	*F. oxysporum*	No	AV	No	AV	1
F09-87	*F. oxysporum*	No/No	AV/AV	Yes/No	LV/AV	2
F11-67	*F. oxysporum*	No	AV	No	AV	1
F12-12	*F. oxysporum*	No	AV	No	AV	1
F12-25	*F. oxysporum*	No	AV	No	AV	1
F12-36	*F. oxysporum*	No	AV	No	AV	1
F21-8	*F. commune**	No	AV	No	AV	1
F21-42	*F. oxysporum*	No	AV	Yes	LV	1
F22-04	*F. oxysporum*	No	AV	No	AV	1
F22-12	*F. oxysporum*	No	AV	No	AV	1
F22-34	*F. oxysporum*	No	AV	No	AV	1
F22-35	*F. oxysporum*	No	AV	No	AV	1
F07-43	*F. oxysporum*	Yes	LV	Yes	LV	1
F08-193	*F. oxysporum*	Yes	LV	No	AV	1
F09-16	*F. oxysporum*	Yes	LV	Yes	LV	1
F09-53	*F. oxysporum*	Yes	LV	Yes	LV	1
F10-58	*F. oxysporum*	Yes/Yes	LV/LV	Yes/No	LV/AV	2
F11-2	*F. oxysporum*	Yes	LV	Yes	LV	1
F11-3	*F. oxysporum*	Yes	LV	Yes	LV	1
F11-63	*F. oxysporum*	Yes	LV	No	AV	1
F12-24	*F. oxysporum*	Yes	LV	Yes	LV	1
F12-44	*F. oxysporum*	Yes	LV	No	AV	1
F13-10	*F. oxysporum*	Yes/No	LV/AV	Yes/No	LV/AV	2
F14-22	*F. oxysporum*	Yes	LV	Yes	LV	1
F21-2	*F. oxysporum*	Yes	LV	No	AV	1
F21-22	*F. oxysporum*	Yes	LV	Yes	LV	1
F21-76	*F. oxysporum*	Yes	LV	Yes	LV	1
F22-7	*F. oxysporum*	Yes	LV	No	AV	1
F22-24	*F. oxysporum*	Yes	LV	Yes	LV	1
F22-28	*F. oxysporum*	Yes	LV	No	AV	1
F23-7	*F. oxysporum*	Yes	LV	No	AV	1
F23-11	*F. oxysporum*	Yes/No	LV/AV	Yes/No	LV/AV	2
F23-27	*F. oxysporum*	Yes	LV	No	AV	1
F07-48	*F. oxysporum*	Yes/Yes	MV/MV	Yes/No	LV/AV	2
F08-207	*F. oxysporum*	Yes/Yes	MV/LV	Yes/No	MV/AV	2

Table 5 (cont'd)

* While the closest sequence match using TEF1α was to *F. commune*, these isolates lacked its distinctive morphological characters for this species, and instead had the morphology of *F. oxysporum*

[a] Pathogenicity abbreviated to Patho. Isolates different from the water control were classified as pathogenic based on statistically significant differences by Fisher's Protected LSD (α=0.05).

[b] Virulence levels were abbreviated for brevity, and assigned based on statistically significant differences by Fisher's Protected LSD (α=0.05). AV was avirulent for isolates that were not significantly different from the water control; LV was low virulence for isolates that were significantly different from both the water control and Fob220a; MV was moderate virulence for isolates that were either not significantly different from Fob220a or were significantly different from the low virulence isolates; HV was high virulence for isolates that were not significantly different from F19.

Discussion:

Prior work identifying pathogenic FOB isolates had a success rate of 28 to 40.7 percent of screened isolates collected from symptomatic plants as pathogenic on sugar beet, in comparison to the 65.7 percent achieved in this work (Hill et al. 2011, Ruppel 1991, Webb et al. 2012). Part of the low success rate was hypothesized to be due to the ability of FOSC species to live as endophytes and hemi-biotrophs on plant hosts (Thaler et al. 2004). As such, part of identifying pathogenic FOB strains required distinguishing the endophytic FOSC strains from hemi-biotrophic pathogens. The increase in the ability to select for pathogenic isolates could be attributed to several causes; including selecting for low macroconidia production which was reported as a characteristic of virulence and adapting the inoculation protocol to include hyphal fragments to accommodate low macroconidia production (Ruppel 1991). To the best of our knowledge, this was the first experimental protocol that used low macroconidia production as a criterion in selecting for potentially pathogenic *F. oxysporum* isolates. Prior to this, it had been observed that low or no macroconidia production correlated with virulence (Stewart 1931, Martyn et al. 1989). In the early 2000's, Hill et al. (2011) and Cramer et al. (2003) speculated that single-spore isolation and culture purification might selectively favor non-pathogenic FOSC

species, and recommended transitioning to hyphal tip transfers instead; that increased recovery of pathogenic isolates as noted by Hill et al. (2011). Although the idea to take it a step further and select isolates with low macroconidia production for subsequent virulence screenings was not applied (Cramer et al. 2003, Hill et al. 2011).

To reduce the likelihood that hemi-biotrophic FOB would colonize sugar beet as an endophyte rather than a pathogen, efforts were taken to keep the greenhouse at temperatures conducive to disease development. The optimal temperature for mycelial growth for FOB occurred at 24-28C (Stewart 1931, Twomey 1952) and optimal conditions for Fusarium yellows disease development were at 25-30C (Harveson and Rush 1998, Stewart 1931, Twomey 1952, Webb et al. 2015). As temperatures decreased toward 20C the disease severity from Fusarium yellows (and Fusarium root rot) declined, and temperatures below 20C could result in no disease development (Harveson and Rush 1998, Webb et al. 2015). The importance of this was highlighted in the current study when the greenhouse temperature averaged 22C during experimental run 4 (Appendix Table 7). This temperature was not prohibitive to the development of Fusarium yellows, as seen with the consistency of foliar symptoms, but it was observed to be detrimental to the development of root symptoms (Figure 2).

The root rating scale used in this experiment was developed by drawing from the literature and from observations gathered in a pilot experiment (unpublished data). The objective was to determine the possible extent and severity of root symptoms to provide a more complete picture of Fusarium yellows disease severity, as well as monitor for potential production of symptoms described in Fusarium root rot. The disease Fusarium root rot was characterized by necrosis starting on the exterior of the root, typically originating at the tip of the root (Harveson 2007, Harveson 2009, Martyn et al. 1989). While an external root rot was observed in some

cases in the current study, the necrosis began in the interior of the root, as vascular discoloration escalated to vascular degradation and subsequent necrosis of surrounding parenchymal cells until it reached the exterior of the root (Figure 1, Harveson 2009, Martyn et al. 1989). In the majority of works assessing Fusarium yellows severity, the emphasis has been on foliar symptoms and briefly noting the presence of vascular discoloration with no assigned scale (Hanson and Hill 2004, Hanson et al. 2009, Ruppel 1991). In addition, in many of the works that used root ratings focused on Fusarium root rot rather than Fusarium yellows. In 1994 and 1998, a scale of 0 to 4 was used to assess both Aphanomyces root rot and Fusarium root rot, and focused on only vascular necrosis and total tap root rot (Harveson and Rush 1994, Harveson and Rush 1998). This scale did not consider vascular discoloration, and given the scale was used synonymously with *Aphanomyces cochlioides* severity, the specificity of this rating scale to Fusarium root rot was questionable. In 2018, a scale of 0 to 5 was used to assess Fusarium root rot with emphasis on percentage of discoloration and surface rot (Hanson et al. 2018). Both of these Fusarium root rot scales placed emphasis on the severity of surface rot, a diagnostic trait for distinguishing Fusarium root rot from Fusarium yellows.

The rating scale used for the current experiment aimed to take the best components of each root rating scale to develop a more complete look at Fusarium yellows, with consideration of vascular discoloration, necrosis, and interior necrosis. In the case of the isolates and germplasm screened throughout this experiment, rot originating on the root surface did not occur (Figure 1). When surface rot was visible, it occurred after advanced interior necrosis (Figure 1). The incorporation of the root ratings alongside foliar ratings provided a more complete view of overall plant health. In instances where foliar symptoms were sub-optimal due to external factors, the inclusion of root ratings enabled results to still be gathered as seen in experimental

runs 5 and 6 (Figure 2A, 2B). Both of these experimental runs' foliar ratings were impacted by external factors – insect and insecticide treatments, but root ratings showed consistency with other experimental runs (Appendix Tables 7 and 8).

Of the putative *F. commune* isolates examined, none of them were morphologically distinct from *F. oxysporum*. The key morphological difference between these two species was the presence of polyphialides forming false heads in *F. commune* isolates grown in the dark on SNA or CLA (Skovgaard et al. 2004). In comparison, *F. oxysporum* would only form monophialides. The isolates of putative *F. commune* used in this study, Fob220a, F19 and F21-8 did not develop polyphialides under any conditions tested, including incubation on SNA and CLA under either light or dark conditions.

It has been hypothesized that *F. commune* has increased virulence in comparison to FOB, especially given that two of the commonly used pathogenic FOB isolates – Fob220a and F19, as well as isolates belonging to clade A of Hill et al. 2011, have since been putatively re-identified as *F. commune*, based on a single gene: TEF1a (Webb et al. 2019). Fob220a and F19 were reliably pathogenic, with F19 being one of the most virulent isolates collected in the United States (Hill et al. 2011, Webb et al. 2019). The findings of the virulence screening in the current study show that putative *F. commune* isolates are not always pathogenic on sugar beet, as seen with F21-8. Prior phylogenetic screening demonstrated variability in virulence. Two separate studies comparing phylogenetics and pathogenicity observed varying results across the same panel of FOSC isolates, some of which have been re-classified as *F. commune* (Covey et al. 2014, Hill et al. 2011, Webb et al. 2012, Webb et al. 2019, Appendix Table 18). This indicated that like *F. oxysporum*, the ability of putative *F. commune* to cause disease on sugar beet was

influenced by additional factors. To the best of our knowledge, these factors have not been investigated or differentiated from those for *F. oxysporum*.

The identity of Fob220a is a potentially contentious classification. In 2019, a number of FOB isolates were re-identified as *F. secorum* based on phylogenetic work (Webb et al. 2019). However, morphological and phylogenetic work prior to and after the 2019 study still upheld their designation as members of the FOSC; this included Fob257c, Fob220a, Fob216b, and H7 (Covey et al. 2014, Hill et al. 2011, Webb et al. 2012). Pulling from the original collection maintained by the Hanson Lab, Fob220a and H7 were identified as *F. commune* and Fob257c and Fob216b as *F. oxysporum* based on TEF1a sequence analysis and morphology (Leslie and Summerell 2006, Appendix Table 18). One hypothesis was that the difference in identification might be due to human error. This hypothesis was supported by the inconsistency between the results of Webb's work in 2019 and their prior work in 2012 and 2014 (Covey et al. 2014, Webb et al. 2012, Webb et al. 2019). In their 2012 and 2014 work, the contended FOB isolates grouped with FOSC clade B, whereas in their 2019 work, the isolates grouped with the *F. fujikuroi* species complex.

The identified moderately virulent isolates, F08-207 and F07-43 may be incorporated into Michigan-based resistance screening trials to better serve local growers. They are also available for use in local research, including assessment of cover crops as potential hosts and reservoirs for *Fusarium* spp. pathogenic on sugar beets and beans. In addition, these isolates can also be used to address a wide array of questions and concerns. This includes identification of resistant sugar beet varieties in MI breeding programs, and testing potential management strategies such as biological controls and fungicides.

LITERATURE CITED

Campbell L. G. 2015. Details for: PI 674103, *Beta vulgaris* L. subsp. *vulgaris*, F1042. U.S. National Germplasm System. https://npgsweb.ars-grin.gov/gringlobal/accessiondetail?id=1923721

Carnegie A. J., Callaghan S. E., Laurence M. H., Plett K. L., Plett J. M., Green P. J., Wildman O., Daly A. and Summerell B. A. 2022. Response to the detection of an exotic fungal pathogen, *Fusarium commune*, in a *Pinus radiata* production nursery in Australia. Australian Forestry. 85:187-198.

Covey P. A., Kuwitzky B., Hanson M. and Webb K. M. 2014. Multilocus analysis using putative fungal effectors to describe a population of *Fusarium oxysporum* from sugar beet. Phytopathology. 104:886-896.

Cramer R. A., Byrne P. F., Brick M. A., Panella L., Wickliffe E. and Schwartz H. F. 2003. Characterization of *Fusarium oxysporum* isolates from common bean and sugar beet using pathogenicity assays and random-amplified polymorphic DNA markers. Journal of Phytopathology. 151:352-360.

Crous P. W., Lombard L., Sandoval-Denis M., Seifert K. A., Schroers H. -J., P. Chaverri P., Gene J., Guarro J., Hirooka Y., Bensch K., Kema G. H. J., Lamprecht S. C., Cai L., Rossman A. Y., Stadler M., Summerbell R. C., Taylor J. W., Ploch S., Visagie C. M., Yilmaz N., Frisvad J. C., Abdel-Azeem A. M., Abdollahzadeh J., Abdolrasouli A., Akulov A., Alberts J. F., Araújo J. P. M., H.A. Ariyawansa H. A., Bakhshi M., Bendiksby M., Ben Hadj Amor A., Bezerra J. D. P., Boekhout T., Camara M. P. S., Carbia M., Cardinali G., Castaneda-Ruiz R. F., Celis A., Chaturvedi V., Collemare J., Croll D., Damm U., Decock C. A., de Vries R. P, Ezekiel C. N., Fan X. L., Fernandez N. B., Gaya E., Gonzalez C. D., Gramaje D., Groenewald J. Z., Grube M., Guevara-Suarez M., Gupta V. K., Guarnaccia V., Haddaji A., Hagen F., Haelewaters D., Hansen K., Hashimoto A., Hernandez Restrepo M., Houbraken J., Hubka V., Hyde K. D., Iturriaga T., Jeewon R., Johnston P. R., Jurjevic Z., Karalti I., Korsten L., Kuramae E. E., Kusan I., Labuda R., Lawrence D. P., Lee H. B.,Lechat C., Li H. Y., Litovka Y. A., Maharachchikumbura S. S. N., Marin-Felix Y., Matio Kemkuignou B., Matocec N., McTaggart A. R., Micoch P., Mugnai L., Nakashima C., Nilsson R. H., Noumeur S. R., Pavlov I. N., Peralta M. P., Phillips A. J. L., Pitt J. I., Polizzi G., Quaedvlieg W., Rajeshkumar K. C., Restrepo S., Rhaiem A., Robert J., Robert V., Rodrigues A. M., Salgado-Salazar C., Samson R. A., Santos A. C. S., Shivas R. G., Souza-Motta C. M., Sun G. Y., Swart W. J., Szoke S., Tan Y. P., Taylor J. E., Taylor P. W. J., Tiago P. V., Vaczy K. Z., van de Wiele N., van der Merwe N. A., Verkley G. J. M., Vieira W. A. S., Vizzini A., Weir B. S., Wijayawardene N. N., Xia J. W., Yanez-Morales M. J., Yurkov A., Zamora J. C., Zare R., Zhang C. L., and Thines M. 2021. *Fusarium*: more than a node or a foot-shaped basal cell. Online. Studies in Mycology. 98:1-184. DOI: 10.1016/j.simyco.2021.100116.

Crous P. W., Sandoval-Denis M., Costa M. M., Groenewald J. Z., van Iperen A. L., Starink-Willemse M., Hernández-Restrepo M., Kandemir H., Ulaszewski B., de Boer W., Abdel-Azeem A. M., Abdollahzadeh J., Akulov A., Bakhshi M., Bezerra J. D. P., Bhunjun C. S., Câmara M. P. S., Chaverri P., Vieira W. A. S., Decock C. A., Gaya E., Gené J., Guarro J., Gramaje D., Grube M., Gupta V. K., Guarnaccia V., Hill R., Hirooka Y., Hyde K. D., Jayawardena R. S., Jeewon R., Jurjević Ž., Korsten L., Lamprecht S. C., Lombard L.,

Maharachchikumbura S. S. N., Polizzi G., Rajeshkumar K. C., Salgado-Salazar C., Shang Q.-J., Shivas R. G., Summerbell R. C., Sun G. Y., Swart W. J., Tan Y. P., Vizzini A., Xia J. W., Zare R., González C. D., Iturriaga T., Savary O., Coton M., Coton E., Jany J.-L., Liu C., Zeng Z.-Q., Zhuang W.-Y., Yu Z.-H., and Thines M. 2022. *Fusarium* and allied fusarioid taxa (FUSA). 1. Fungal Systematic and Evolution. 9:161–200.

Deng S., Ma X., Chen Y., Feng H., Zhou D., Wang X., Zhang Y., Zhao M., Zhang J., Daly P., and Wei L. 2022. LAMP assay for distinguishing *Fusarium oxysporum* and *Fusarium commune* in lotus (*Nelumbo nucifera*) rhizomes. Plant Disease. 106:231-246.

Edel-Hermann V., Gautheron N. and Steinberg C. 2012. Genetic diversity of *Fusarium oxysporum* and related species pathogenic on tomato in Algeria and other Mediterranean countries. Plant Pathology. 61:787-800.

Fisher N. L., Burgess L. W., Toussoun T. A., and Nelson P. E. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology. 72:151-153.

Frankson R., Kunkel K. E., Champion S. M., and Runkle J. 2022. Michigan State Climate Summary 2022. NOAA Technical Report NESDIS 150-MI. NOAA/NESDIS, Silver Spring, MD. 4 pages. statesummaries.ncics.org/chapter/mi/

Gracia-Garza J. A. and Fravel D. R. 1998. Effect of relative humidity on sporulation of *Fusarium oxysporum* in various formulations and effect of water on spore movement through soil. Phytopathology. 88:544-549.

Hanson L. E. Hill A., Wickliffe E., Schwartz H., and Panella E. 2001. Fusarium in sugar beet and bean (Abstr.). Journal of Sugar Beet Research. 38:70.

Hanson L. E. and Hill A. L. 2004. *Fusarium* species causing Fusarium yellows of sugar beet. Journal of Sugar Beet Research. 41:163-178.

Hanson L. E. and Jacobsen B. J. 2006. Beet root-rot inducing solates of *Fusarium oxysporum* from Colorado and Montana. Plant Disease. 90:247.

Hanson L. E., Hill A. L., Jacobsen B. J. and Panella L. 2009. Response of sugarbeet lines to isolates of *Fusarium oxysporum* f. sp. *betae* from the United States. Journal of Sugar Beet Research. 46:11-26.

Hanson L. E. and Jacobsen B. J. 2009. Fusarium yellows. In: Harveson R.M., Hanson L.E., Hein G.L., eds. Compendium of Beet Diseases and Pests, 2nd ed. St. Paul, MN, USA: APS Press, 28–30.

Hanson L. E., De Lucchi C., Stevanato P., McGrath M., Panella L., Sella L., De Biaggi M. and Concheri G. 2018. Root rot symptoms in sugar beet lines caused by *Fusarium oxysporum* f. sp. *betae*. European Journal of Plant Pathology. 150:589-593.

Harveson R. M. and Rush C. M. 1994. Evaluation of fumigation and rhizomania-tolerant cultivars for control of a root disease complex of sugar beet. Plant Disease. 78:1197-1202.

Harveson R. M. and Rush C. M. 1997. Genetic variation among *Fusarium oxysporum* isolates from sugar beet as determined by vegetative compatibility. Plant Disease. 81:85-88.

Harveson R. M. and Rush C. M. 1998. Characterization of Fusarium root rot isolates from sugar beet by growth and virulence at difference temperatures and irrigation regimes. Plant Disease. 82:1039-1042.

Harveson R. M. 2007. Identifying and distinguishing seedling and root rot diseases of sugar beet. Plant Health Progress. Vol. 7. DOI:10.1094/PHP-2006-0915-01-DG

Harveson R. M., Windels C. E., Smith J. A., Brantner J. R., Cattanach A. W., Giles J. F., Hubbell L., and Cattanach N. R. 2007. Fungicide registration and a small niche market: a case history of hymexazol seed treatment and the U.S. sugar beet industry. Plant Disease. 91:780-790.

Harveson, R. M. 2009. Fusarium root rot. In: Harveson R.M., Hanson L.E., Hein G.L., eds. Compendium of Beet Diseases and Pests, 2nd ed.. St. Paul, MN, USA: APS Press, 30-31.

Hill A. L., Reeves P. A., Larson R. L., Fenwick A.L., Hanson L.E. and Panella L. 2011. Genetic variability among isolates of *Fusarium oxysporum* from sugar beet. Plant Pathology. 60:496-505.

Koenraadt H., Somerville S. C., and Jones A. L. 1992. Characterization of mutations in the beta-tubulin gene of benomyl-resistant field strains of *Venturia inaequalis* and other plant pathogenic fungi. Phytopathology. 82:1348-1354.

Leslie J. F. and Summerell B. A. 2006. The *Fusarium* Laboratory Manual. First ed. Blackwell Publishing, Ames, Iowa. 388 pages.

Lewellen R. T. 2004. Registration of Rhizomania resistant, monogerm populations C869 and C869CMS sugarbeet. Crop Science. 44:357–358.

Maniatis T., Fritsch E. F., Sambrook J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Pg. 156.

McGrath J. M., Derrico C. A., Morales M., Copeland L. O., and Christenson D. R. 2000. Germination of sugar beet *Beta vulgaris* L. seed submerged in hydrogen peroxide and water as a means to discriminate cultivar and seed lot vigor. Seed Science and Technology. 28:607-620.

MiDHHS. 2024. Number of Extreme Precipitation Days (Greater than 1 Inch) and Number of Extreme Heat Days (Daily Heat Index above 90°F). MI Environmental Public Health Tracking. mitracking.state.mi.us/

Miller, P. M. 1955. V-8 juice agar as a general purpose medium for fungi and bacteria. Phytopathology. 45:461-462.

NOAA. 2024. Climate Records and Information for Southeast Michigan: Average Monthly Temperatures and Monthly Precipitation. National Oceanic and Atmospheric Administration. www.weather.gov/dtx/decadetable

Nelson P. E., Toussoun T. A. and Marasas W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University, University Park. 193 pages.

O'Donnell K., Kistler H. C., Cigelnik E., and Ploetz R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences. 95:2044-2049.

Osawa H., Sakamoto Y., Akino S. and Kondo N. 2020. Autumn potato seedling failure due to potato dry rot in Nagasaki Prefecture, Japan, caused by *Fusarium acuminatum* and *Fusarium commune*. Journal of General Plant Pathology. 87:46-50.

Panella L., Ruppel E. G. and Hecker R. J. 1995. Registration of four multigerm sugarbeet germplasms resistant to Rhizoctonia root rot: FC716, FC717, FC718, and FC719. Crop Science. 35:291-292.

Panella L., Fenwick A. L., Stevanato P., Eujayl I., Strausbaugh C. A., Richardson K.L., Wintermantel W.M. and Lewellen R.T. 2018. Registration of FC1740 and FC1741 multigerm, Rhizomania-resistant sugar beet germplasm with resistance to multiple diseases. Journal of Plant Registrations. 12:257–263.

Peever T. L. and Milgroom M. G. 1992. Inheritane of triadimenol resistance in *Pyrenophora teres*. Phytopathology. 82:821-828.

Posit team. 2024. RStudio: Integrated Development Environment for R. Posit Software PBC, Boston, MA.

Rivera V., Rengifo J., Khan M., Geiser D. M., Mansfield M. and Secor G. 2008. First report of a novel *Fusarium* species causing yellowing decline of sugar beet in Minnesota. Plant Disease. 92:1589.

Ruppel E. G.1991. Pathogenicity of *Fusarium* spp. from diseased sugar beet and variation among sugar beet isolates of *F. oxysporum*. Plant Disease. 75:486-489.

Schwartz, H. F., Panella, L. W., Brick, M. A. and Byrne, P. F. 2001. Fusarium wilt and yellows of sugar beet and dry bean. Colorado State University Extension Fact Sheet 2.950. Fort Collins, CO. 3pgs.

Secor G. A., Rivera-Varas V., Christ D. S., Mathew F. M., Khan M. F. R., Varrelmann M., and Bolton M. D. 2014. Characterization of *Fusarium secorum*, a new species causing Fusarium yellowing decline of sugar beet in north central USA. Fungal Biology. 118:764-775.

Simko I. and Piepho H.-P. 2012. The area under the disease progress stairs: Calculation, advantage, and application. Phytopathology. 102:381-389.

Skovgaard K., Rosendahl S., O'Donnell K., and Nirenberg H. I. 2004. *Fusarium commune* is a new species identified by morphological and molecular phylogenetic data. Mycologia. 95:630-636.

Snyder W. C. and Hansen H. N. 1947. Advantages of natural media and environments in the culture of fungi. Phytopathology. 37:420-421.

Stewart D. 1931. Sugar-beet yellows caused by *Fusarium conglutinans* var. *betae*. Phytopathology. 21:59-70.

Stewart J. E., Kim M. S., James R. L., Dumroese R. K., Klopfenstein N. B. 2006. Molecular characterization of *Fusarium oxysporum* and *Fusarium commune* from a conifer nursery. Phytopathology. 96:1124-1133.

Thaler J. S., Owen B. and Higgins V. J. 2004. The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. Plant Physiology. 135:530–538.

Torres-Cruz T. J., Whitaker B. K., Proctor R. H., Broders K., Laraba I., Kim H.-S., Brown D. W., O'Donnell K., Estrada-Rodríguez T. L., Lee Y.-H., Cheong K., Wallace E. C., McGee C. T., Kang S., and Geiser D. M. 2022. FUSARIUM-ID v.3.0: an updated, downloadable resource for *Fusarium* species identification. Plant Disease. 106:1610-1616.

Turland N. J., Wiersema J. H., Barrie F. R., Greuter W., Hawksworth D. L., Herendeen P. S., Knapp S., Kusber W.-H., Li D.-Z., Marhold K., May T. W., McNeill, J., Monro A. M., Prado J., Price M. J. and Smith G. F. (eds.). 2018. International code of nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. DOI: 10.12705/Code.2018

Webb K. M., Case A. J., Brick M. A., Otto K. and Schwartz H. F. 2013. Cross pathogenicity and vegetative compatibility of *Fusarium oxysporum* isolated from sugar beet. Plant Disease. 97:1200-1206.

Webb K. M., Brenner T. and Jacobsen B. J. 2015. Temperature effects on the interactions of sugar beet with Fusarium yellows caused by *Fusarium oxysporum* f. sp. *betae*. Canadian Journal of Plant Pathology. 37:353-362.

Webb K. M., Shresthab S., Trippe III R., Rivera-Varas V., Covey P. A., Freeman C., de Jonge R., Secor G. A. and Bolton M. 2019. Phylogenetic relationships and virulence assays of *Fusarium secorum* from sugar beet suggest a new look at species designations. Plant Pathology. 68:1654-1662.

Wickliffe E. R. 2001. Characterization of *Fusarium oxysporum* f. sp. *phaseoli* and f. sp. *betae* in dry bean and sugar beet by pathogenicity and vegetative compatibility grouping. Fort Collins, CO, USA: Colorado State University, MS thesis.

Wintermantel W. M. 2009. Virus yellows complex. In: Harveson R.M., Hanson L.E., Hein G.L., eds. Compendium of Beet Diseases and Pests, 2nd ed. St. Paul, MN, USA: APS Press, 46-47.

CHAPTER 3: HISTOPATHOLOGY FOR EARLY STAGES OF FUSARIUM YELLOWS IN SUGAR BEET ROOTS

Abstract:

Prior histopathological work on *Fusarium oxysporum* has been performed on a variety of hosts, but no histopathological work has focused on Fusarium yellows of sugar beet to the best of our knowledge. The most prevalent hypothesis was that *F. oxysporum* f. sp. *betae* (FOB) and putative *F. oxysporum* f. sp. *radices-betae* entered through the tip of the tap root. This was in keeping with the observation that early symptoms of Fusarium yellows and Fusarium root rot such as vascular discoloration and necrosis were first observed in the root tip. In recent years, over a dozen FOB isolates have been putatively reclassified based on single gene identification as *F. commune*. At present, nothing is known about the infection process of pathogenic *F. commune*. The primary objective of this study was to identify the initial site of entry and examine progress of infection in sugar beet roots inoculated with *F. commune* that cause Fusarium yellows. A secondary objective was to lay the groundwork for identification of potential histopathological differences between *F. commune* and *F. oxysporum*. Plants were inoculated via a soil drench with hyphal fragments and macroconidia, and destructive sampling was performed every three days post inoculation. Root samples were stained with AlexaFluor 488 to visualize fungal structures (chitin) and propidium iodide for plant tissue (lignin). While *F. commune* initially colonized the surface of sugar beet roots, particularly the feeder roots, progress from the feeder root into the tap root was not observed even when the xylem of feeder roots was colonized. Initial entry into the plant interior occurred at the tip of the tap root and progressed up the root through the xylem vessels of the plant vascular system, as hypothesized based on observed symptom progression. When necrosis of the root occurred, it originated from the stele, progressed toward the exterior of the root, and halted at the cork. These findings were

in keeping with histopathology performed on other *formae speciales* of *F. oxysporum*. As of yet, no differences in penetration and colonization distinctive to *F. commune* or sugar beet have been observed.

Introduction:

When Fusarium yellows of sugar beet was first described in 1931, it was hypothesized that initial entry occurred through the feeder roots (Stewart 1931). Fusarium yellows was characterized as having minimal exterior necrosis, indicating that *F. oxysporum* f. sp. *betae* (FOB) had preferential sites of entry and was unlikely to penetrate through the epidermis of the tap root indiscriminately (Stewart 1931). Since Stewart's work in the 1930's, the hypothesized site of entry for FOB has shifted from feeder roots to the tip of the tap root (Hanson and Jacobsen 2009). This was due to the common observation that the initial development of vascular discoloration developed at the root tip (Hanson and Jacobsen 2009). In addition, another disease on sugar beets caused by *F. oxysporum* supported this hypothesis: Fusarium root rot (Fusarium tip rot) disease progression reported earliest symptoms developing at the root tip as well (Martyn et al. 1989, Harveson 2009). The key difference between Fusarium yellows and Fusarium root rot was necrosis of the root surface, often beginning at the tip of the root (Marten et al. 1989). It was proposed that Fusarium yellows and Fusarium root rot were caused by separate *formae speciales* – FOB and *F. oxysporum* f. sp. *radices-betae* respectively (FORB, Martyn et al. 1989, Harveson 2009). However, multiple studies devoted to phylogenetic analysis of FOB and FORB isolates have found no difference in phylogeny between FOB and FORB (Covey et al. 2014, Hill et al. 2011, Webb et al. 2012). In addition, the ability of *F. oxysporum* isolates to cause Fusarium root rot has been demonstrated to be partly dependent on the sugar beet host (Hanson et al. 2018). Once inside the root, it had been reported that FOB preferentially

colonized and moved through the vascular tissue over the parenchymal tissue of sugar beet, based on observations at the localization of hyphal, isolations, and discoloration to the stele and cambial rings of sugar beet (Hanson and Jacobsen 2009, Stewart 1931). This also was reported as standard behavior of *F. oxysporum* wilt pathogens on other hosts (Nelson 1981). This preference for colonization of the xylem was also demonstrated in isolates that cause Fusarium root rot on sugar beet as seen through scanning electron microscopy (Martyn et al. 1989). In the 1989 study, Martyn et al. reported that the hyphae colonized and obstructed the xylem and adjoining tissue which was common for *F. oxysporum* (Martyn et al. 1989).

The isolate used in the current study, Fob220a (isolated in Colorado by H. F. Schwartz, 1998), has been used in the USA as a reliable moderately to highly virulent causal agent of Fusarium yellows (De Lucchi et al. 2017, Hanson et al. 2009, Hanson et al. 2018, Hill et al. 2011, Larson et al. 2007, Webb et al. 2012). There is some dispute over the taxonomy of Fob220a that needs clarification before proceeding through the current study. Fob220a was initially identified based on morphology and multilocus sequence analysis as *F. oxysporum* f. sp. *betae* (Hill et al. 2011). However, in 2019, a phylogenetic study using just translation elongation factor 1α (TEF1a) reclassified a number of FOB isolates, including Fob220a, as *F. secorum*, a member of the *F. fujikuroi* species complex (FFSC, Webb et al. 2019). It has since been hypothesized that the reclassification of Fob220a as *F. secorum* was due to human error, as morphological and phylogenetic work prior to 2019 placed Fob220a and the other isolates in the FOSC rather than in the FFSC (Hill et al. 2011), including work performed by Webb (Covey et al. 2014, Webb et al. 2012). Most recently, TEF1a sequence analysis of Fob220a had putatively identified it as *F. commune* (Chapter 1, Appendix Table 18), which was consistent with its morphology and the prior phylogenetic work by Hill et al. (2011). The 2019 study also putatively

reclassified a number of FOB isolates as *F. commune* based on sequence and phylogenetic analysis using just translation elongation factor 1α (TEF1a, Skovgaard et al. 2004, Webb et al. 2019). This included notable isolates such as F19, Fob216c and Fob13 which have been used in an array of studies (De Lucchi et al. 2017, Hanson et al. 2009, Hanson et al. 2018, Hill et al. 2011, Lai et al. 2020, Larson et al. 2007, Webb et al. 2017). *F. commune* is a member of the *F. nisikadoi* species complex, which falls within the FOSC (Crous et al. 2021). Phenotypic differences between *F. oxysporum* and *F. commune* have not been thoroughly explored. At present, the only published differences between *F. oxysporum* and *F. commune* were single gene differences based on TEF1a and the potential for *F. commune* to form false heads on polyphialides in the dark as part of the initial species description to fulfill the requirements for naming a new species under the taxonomic code (Skovgaard et al. 2004). This morphological attribute has yet to be observed for the putative *F. commune* isolates from sugar beet that were formerly classified as FOB. In other crops systems, this feature had an unreliable presence (Stewart et al. 2006). The unreliable presence of polyphialide production in *F. commune* does raise concerns as to the validity of classifying it as a separate species from *F. oxysporum*, as the taxonomic code requires that new species be phenotypically or morphologically distinct from other species (Turland at al. 2018). Nothing is known about potential variability in pathogenicity and virulence factors of *F. commune*, including differences in symptoms, disease severity, mycotoxin production, and mechanisms of infection. At present, the only "reliable" method to distinguish *F. oxysporum* and *F. commune* is through sequence analysis (Deng et al. 2022, Stewart et al. 2006).

Prior histopathological work on *F. oxysporum* performed on dicots has focused heavily on pathogens of tomato, specifically *F. oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f.

sp. *radices-lycopersici* (FORL). The causal agent of Fusarium wilt of tomato, FOL, has been demonstrated to utilize multiple sites of entry across different studies (Martinez-Soto et al. 2023, Olivain and Alabouvette 1999). In 1999, a comparison of pathogenic FOL and endophytic *F. oxysporum* demonstrated that both were able to penetrate and colonize the superficial cork of the plant, while the pathogenic isolate grew past the cork into vascular tissue to cause disease, with no site preference observed (Olivain and Alabouvette 1999). In contrast, a study of pathogenic FOL and endophytic *F. oxysporum* from tomato in 2023 reported preferential colonization and penetration from the feeder roots and the elongation zone (root tip) of the tap root (Martinez-Soto et al. 2023). In comparison, FORL, the causal agent of tomato foot and root rot (synonym Fusarium crown and root rot) demonstrated primarily initial colonization of feeder roots and progressed to colonize the surface of the tap root. Penetration occurred via cork of the tap root with no evidence of differentiation between sites of initial infection (Lagopodi et al. 2002).

There was also histopathological work on *F. oxysporum* f. sp. *vasinfectum* (FOV), the causal agent of Fusarium wilt in cotton (Rodríguez-Gálvez and Mendgen 1995). The primary site of infection and penetration occurred at the root tip, in the meristematic zone followed by the elongation zone and to a lesser extent the root hairs; there was no penetration in the lateral roots or differentiation zone of the tap root (Rodríguez-Gálvez and Mendgen 1995). Rodríguez-Gálvez and Mendgen (1995) hypothesized that FOV initially penetrated and colonized the interior of the root in a biotrophic lifestyle, as the penetration of plant tissue intracellularly and intercellularly during early infection did not cause organelle or cell damage. Under the right conditions and once well-established in the plant tissue, FOV transitioned to a necrotrophic lifestyle that caused symptoms in cotton (Rodríguez-Gálvez and Mendgen 1995).

The aims of the current study were to identify sugar beet root regions that were susceptible to infection progression and the host response to putative *F. commune* infection for Fusarium yellows. This could provide sugar beet breeders with knowledge of which regions of the sugar beet could be bolstered by known resistance mechanisms and provide insights into type and method of application for administering disease controls in the field.

Materials and Methods:

For these objectives, C869 cytoplasmic male sterile (C869cms) germplasm (Lewellen 2004), which is moderately susceptible to Fusarium yellows, was used. Seeds were disinfected and imbibed using the same protocol as described in Chapter 1 and planted in three equidistant rows in twice-autoclaved play sand (KolorScape All Purpose Sand, Conard-Pyle Company, West Grove, Pennsylvania) in plastic totes (25.5cm tall x 40.5cm x 25.25cm). The plastic totes were disinfested in a 0.5% sodium hypochlorite soak for 5 minutes and UV sterilized in a biosafety cabinet for 1 hour (1300 Series A2 Class II, Type A2 Biological Safety Cabinet, Thermo Fisher Scientific, Waltham, MA). Plants were maintained in a growth chamber at 25C with a 16/8 hour light/dark cycle. To maintain reduced contamination conditions, plants were watered with sterile distilled water and fertilized with sterile Hoagland solution #2 (Sigma-Aldrich, St. Louis, MO, Hoagland and Arnon 1950) as needed for optimal plant growth and no beneficial insects were applied to the plants (Appendix Table 8). At the two true leaf growth stage, plants were thinned for optimal spacing, with 18 to 24 plants per row for a total of 54 to 72 plants per container. At the six to eight leaf growth stage, plants were inoculated via root drench inoculation. The inoculum was prepared and concentrated using the protocol described in Chapter 1. 10ml of inoculum (4x10^4 hyphal fragments and macroconidia per ml) was poured at the base of each plant using a pipette controller (Drummond portable pipet aid, Drummond Scientific, Broomall,

PA). All the plants within a container were drenched with the same inoculum – with one container for Fob220a and one container for sterile distilled water. Samples were taken prior to inoculation on day 0 to establish a baseline of root morphology, after which samples were taken every three days post inoculation (DPI) for 18 days for a total of 7 sampling times.

Each sugar beet was rated using the USDA Fusarium yellows foliar severity rating scale and the Fusarium yellows root severity scale (Chapter 1; Tables 2 and 3). Samples were sliced with a razor blade in 5mm increments, at four regions of the root between the tip of the root and the bottom of the root groove, with two increments falling equidistant between these two distinct regions (Figure 4) and samples were placed in micromesh cassettes (Cancer Diagnostic Inc. Durham, NC). While encased in the cassettes, samples were fixed in 10% neutral buffered formalin (Thermo Fisher Scientific) for 4 days, and transferred to 50% ethanol (EtOH) for 1 day, before storing in a plastic Tupperware container with 50% EtOH until ready to submit for embedding (Investigative Histopathology Lab, Unpublished). The cassette samples were embedded in paraffin wax blocks by Michigan State University's Investigative Histopathology Laboratory (East Lansing, MI).

The paraffin-embedded samples were cross-sectioned using a Reichert-Jung 820 microtome (Reichert Technologies, Buffalo, NY) set to cut 10-12μm thick segments at a 9 degree angle. The resulting cross-sections were mounted on glass slides and dried overnight at 40C in an incubator. The paraffin wax was cleared from the samples in a chemical fume hood on a shaker at 75 rpm at room temperature; all solutions were applied for 10 minutes each. Slides with cross-sections were soaked in Neo-Clear twice (Sigma-Aldrich, St. Louis, MO), xylenes (Sigma-Aldrich), air dried in a fume hood for ten minutes, then soaked in an ethanol (EtOH) gradient of twice in 100% and 95%, and once in 70% and 50%, and lastly in distilled water

(Minier 2023). The wax-cleared slides were dried overnight at 40C and kept at room temperature in a glass slides container until ready for use (Minier 2023).

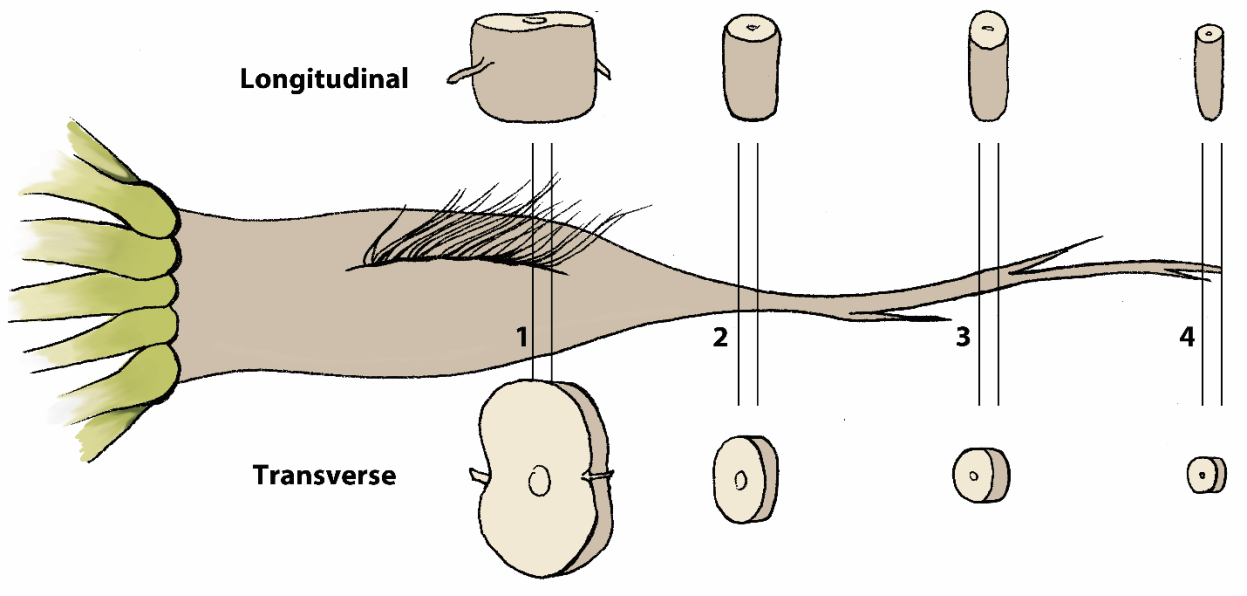

Figure 4: Diagram of location and type of sampled cross sections of sugar beet root tissue. Root tissue location 1 was at the lower region of the root groove, location 4 was the root tip, and locations 2 and 3 fell equidistant between locations 1 and 4.

Samples were rehydrated, cleared and stained based on the protocols of Carotenuto and Genre (2020) and Minier (2023). Specifically, samples were rehydrated by soaking in sterile dH2O for 15 minutes, cleared by soaking in 0.5% sodium hypochlorite for 10 minutes, and rinsed in dH2O for 2-3 minutes. Samples were stained in 50 µg/ml of Alexaflour 488 (wheat germ agglutinin, WGA, Thermo Fisher Scientific Waltham, MA, Appendix Table 15) for 45 minutes and rinsed for 5 minutes in dH2O. The secondary stain was applied by soaking samples in 50 µg/ml propidium iodide (PI, Appendix Table 15) for 5 minutes and rinsing for 1 minute in dH2O. Then a drop of N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES) buffered Fluoro-Gel (Electron Microscopy Sciences, Hatfield, PA) was applied to each slide and a coverslip was mounted over the sample. Mounting medium was allowed to set overnight in the dark before viewing slides. Initial microscopy screening was performed on Olympus (Olympus

Corporation, Tokyo, Japan) and EVOS (Thermo Fisher Scientific) conventional fluorescence microscopes (Appendix Table 16) to generate preliminary information, observations and determine optimum samples and methods for further testing. From this, a selection of informative slides was observed under confocal microscopes as described below and in Appendix Table 16, at MSU's Center for Advanced Microscopy (East Lansing, MI). Preliminary visualization of samples utilized an upright Nikon eclipse Ni conventional fluorescent microscope (Nikon Instruments, Tokyo, Japan). Image acquisition was performed on a Nikon C2 confocal laser microscope (Nikon Instruments). WGA fluorochromes were excited with a 488nm diode laser, and green fluorescence was recorded through a 525/50 band pass filter. Propidium iodide fluorochromes were excited with a 561nm diode laser, and red fluorescence was recorded through a 600/50 band pass filter. All samples were exposed to both excitation wavelengths simultaneously. The objective specifications and objectives used for each image are listed in the Appendix under Tables 16 and 17. On a Leica Stellaris 5 inverted microscope (Leica Microsystems, Wetzlar, Germany), WGA and PI were excited at 488nm and 561nm respectively using a white light laser. Emissions from the fluorochromes were filtered and directed to photomultiplier tubes via prism-based beam splitters, set to detect ranges of 499-584nm and 585-730nm for WGA and PI respectively.

Results

Colonization of sugar beet roots with *F. commune* was first observed at 9 DPI, on the exterior surface of the main root tip (Figure 6B), and both internally and externally on feeder roots (Figure 5B). The internal colonization of the feeder roots was not observed to progress into the main root at any of the sampled time points. There were no foliar or root symptoms among the inoculated or water control plants from 0 to 15 DPI (Figure 9). At 15 DPI, interior

colonization was observed at the tip of the main root in the central xylem (Figure 6C), while on the surface of the main root near the root groove (location 1) hyphal masses were observed but did not penetrate the periderm (Figure 5C).

Among the inoculated plants, two beet plants had died at 18 DPI, and the beginning of vascular discoloration and foliar chlorosis were observed, whereas in the water control, no Fusarium yellows symptoms or death had occurred (Figure 9). At 18 DPI it was observed that interior colonization had progressed up the root, to location 2, below the root groove (Figure 7C and 7D). Interior colonization progression included growth in the xylem (Figure 7D) and both intracellularly and intercellularly across the parenchyma, in a step-like fashion up the root and toward the vasculature (Figure 7C). On the surface, colonization of the periderm also favored hyphal growth between and to a lesser extent across plant cells (Figure 7A and 7B). The colonization of root tissue in a dead sugar beet included heavy colonization of the xylem of both the stele and the cambrium rings (Figure 8B and 8C). The parenchyma was also heavily colonized, favoring intercellular growth (Figure 8A and 8C), following a pattern of growth from the central stele, between the cambrial rings and toward the periderm (Figure 8A). This corresponded to the severe interior necrosis observed on the deceased plant, and the lack of exterior necrosis corresponded to no observed colonization and penetration of the periderm (Figure 8A).

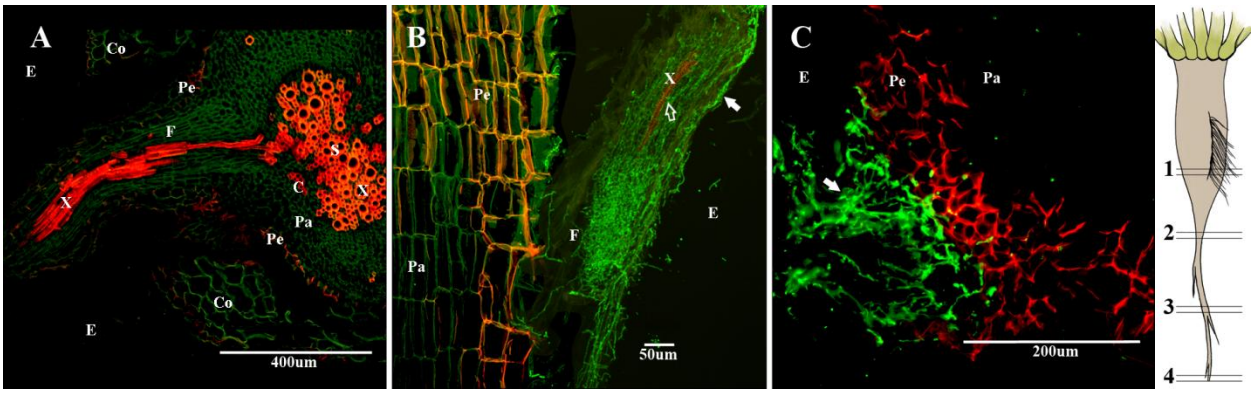

Figure 5: Example of microscopy of sugar beet root sampled near the root groove (location 1) at three time points, one prior to, and two post inoculation with *Fusarium commune*. A, Taken with Evos - transverse view of un-colonized tap root and feeder root prior to inoculation at 0 days post inoculation (DPI), prior to inoculation; B, Taken with Nikon C2 - longitudinal view of heavily colonized feeder root's xylem (arrow outline) and surface (solid arrow) and superficial colonization of tap root's periderm at 9 DPI; C, Taken with Nikon C2 – transverse view of hyphal mass (solid arrow) on periderm of tap root at 15 DPI;

Letter abbreviations used for Figures 5-8: C for cambium, Co for cork, E for exterior space, F for feeder root, Pa for parenchyma tissue, Pe for periderm, S for stele, and X for xylem.

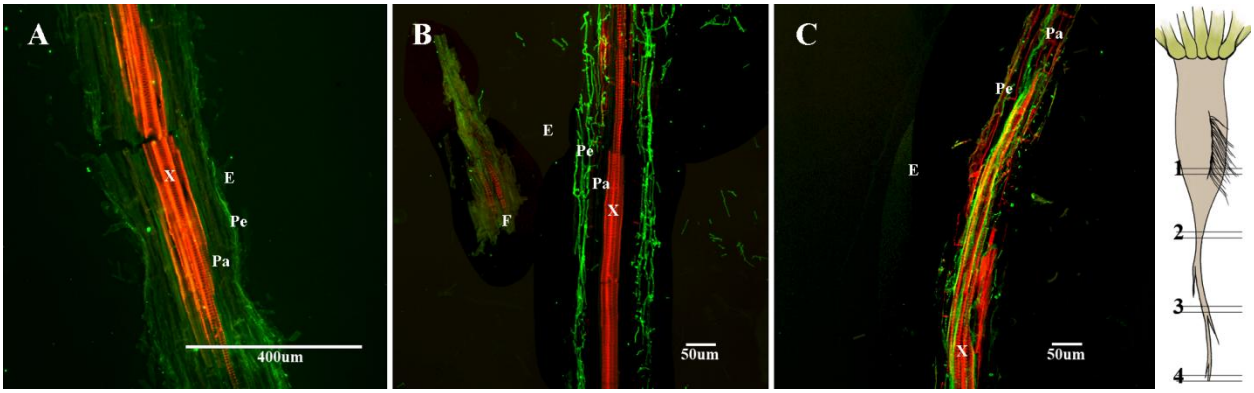

Figure 6: Example of longitudinal view of sugar beet root tips (location 4) sampled at time points post inoculation with sterile distilled water (A) or *Fusarium commune* (B and C). A, Taken with Evos - un-colonized root interior and exterior at location 3, 0 days post inoculation (DPI); B, Taken with Nikon C2 - colonized periderm of root tip with no interior colonization at 9 DPI; C, Taken on Nikon C2 - root tip colonized both internally (parenchyma and xylem) and externally (periderm) by hyphae at 15 DPI.

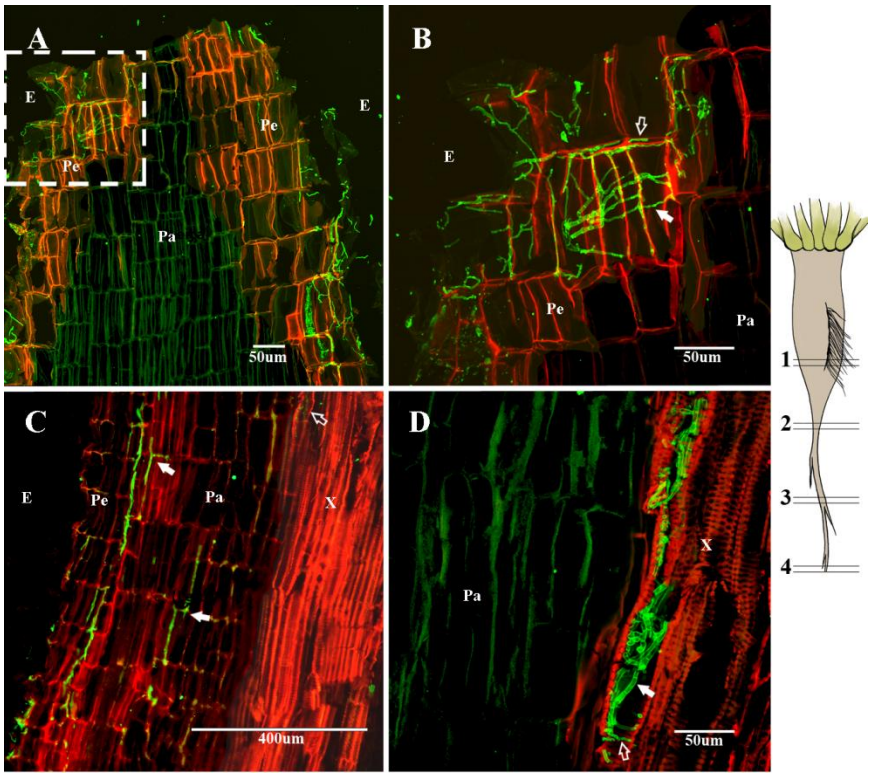

Figure 7: Representative images with a longitudinal view of sugar beet roots sampled at location 1, below the root groove (C and D) and location 2, one-third of the way below the root groove, and two-thirds above the root tip (A and B) at 18 days post inoculation with *Fusarium commune*. A, taken on Leica - surface of epidermis in periderm tissue colonized by hyphae, with no visible colonization of parenchyma; B, taken on Nikon C2 - magnified view of image A, to highlight the ability of the hyphae to grow both intercellularly (arrow outline) and intracellularly (solid arrow) across the periderm; C, taken on Evos –parenchyma colonized by hyphae moving toward the center of the root in a staircase pattern (solid arrows), and a portion of the xylem in the stele colonized with hyphae (arrow outline); D, taken on Nikon C2 – xylem in the stele colonized by hyphae growing both across (arrow outline) and along (solid arrow) the xylem.

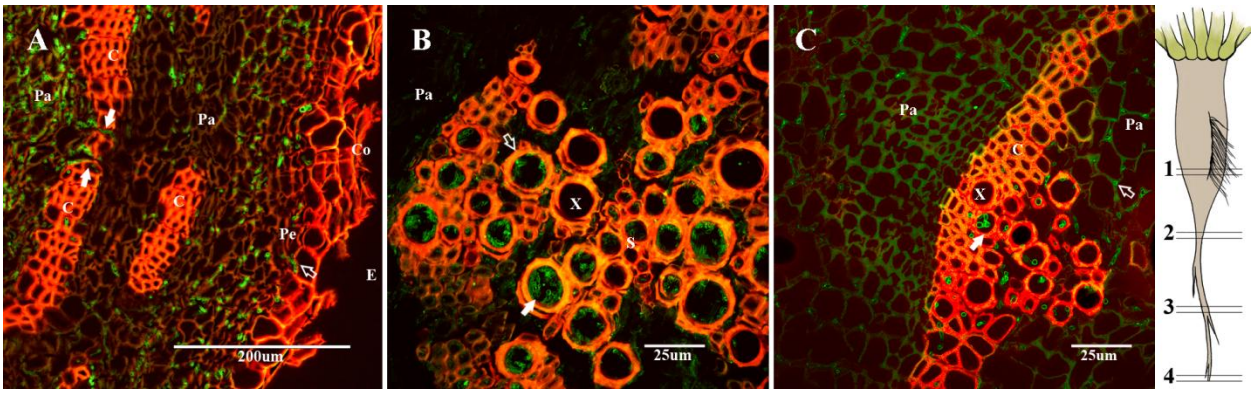

Figure 8: Example of transverse cross-sections of a dead sugar beet root sampled 18 days post inoculation with *Fusarium commune* at location 1, below the root groove (A) and location 2, one-third below the root groove and two thirds above the root tip (B and C). A, taken on Evos – colonization of parenchyma by *F. commune* originated from the central vascular bundle and spread toward the periderm, traveling between cambrium rings (solid arrows) and was not spread into the cork (arrow outline) No colonization of the root surface or penetration of hyphae through the root surface was observed; B, taken on Nikon C2 – hyphae in the xylem vessels in the stele, ranging from some hyphal growth (arrow outline) to complete obstructed of the xylem by hyphae (solid arrow); C, taken on Nikon C2 – hyphae in the xylem in the cambium ring (solid arrow) and the intercellular space of parenchyma (arrow outline).

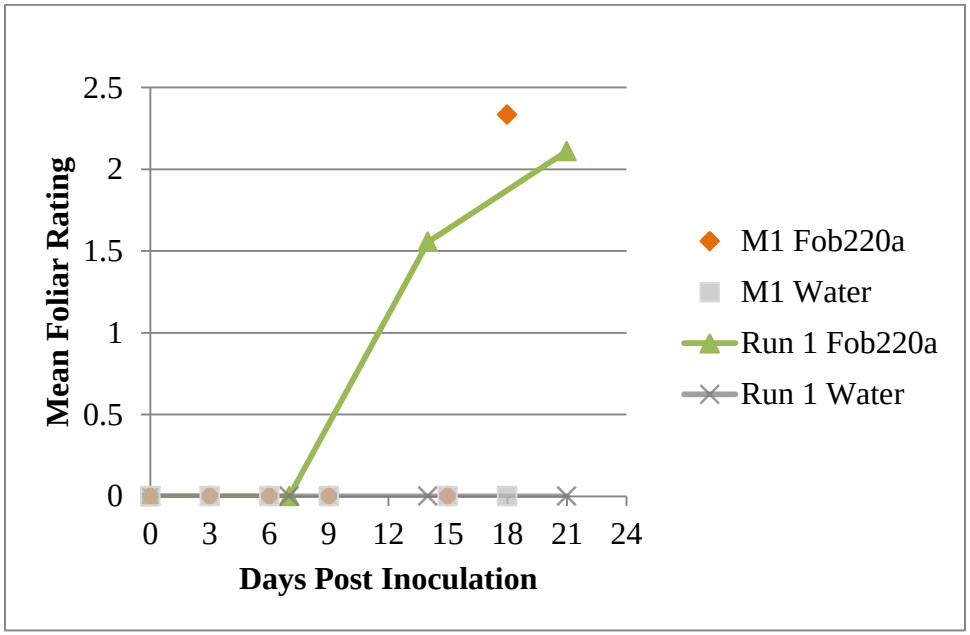

Figure 9: Area under disease progression curves (AUDPCs) comparing foliar symptom development for Fusarium yellows between the microscopy trial, H1 in orange (soil drench inoculation method, 0 to 18 DPI), in comparison to Run 1 from Chapter 1 in green (root dip inoculation method, 0 to 21 DPI), and the water controls in gray.

True aseptic conditions were not achieved, as coenocytic hyphae were observed colonizing the exterior of sugar beet roots (Appendix Figure 10). However, as *F. commune* had septate hyphae, and the contaminant did not penetrate into the sugar beet tissue, it could be reliably distinguished from the intended fungal pathogen. Efforts to re-isolate the contaminant and identify it were unsuccessful, as it did not grow on provided media. In the majority of sugar beet sampled, the contaminant was not observed, and to the best of our knowledge had a minimal impact on assessing *F. commune*'s progression of infection in sugar beet. The means by which the contaminant was introduced into the experimental design was unknown.

Discussion:

While bearing in mind that the current study was being compared to *F. oxysporum* on different hosts, it was worth noting that there were no notable difference in penetration and colonization between putative *F. commune* and *F. oxysporum* as expected with similar disease expression (Lagopodi et al. 2002, Martinez-Soto et al. 2023, Olivain and Alabouvette 1999). Both species grew intercellularly and intracellularly in the plant tissue (Figures 7B and 8C, Olivain and Alabouvette 1999). The initial entry of *F. commune* into the tap root occurred at the root tip and progressed up the root through xylem vessels. This was consistent with what was reported for Fusarium wilt of tomatoes and for Fusarium wilt of cotton (Martinez-Soto et al. 2023, Rodríguez-Gálvez and Mendgen 1995). While it was possible to get close to the root tip in the current study, none of the samples had an intact root cap. There was sufficient data to ascertain that preliminary entry into the tap root occurred around the root tip, but it was unclear if that point of entry was specific to the root cap, the elongation zone or that general region. While there was colonization of feeder roots both on the surface and internally, this colonization did not correspond to the timing of development of foliar symptoms and did not show evidence of

progression from the feeder root into the tap root (Figures 5B and 9). Based on these findings, *F. commune* as the causal agent of Fusarium yellows showed evidence of a specific site of entry rather than multiple sites, but replication of this experiment is necessary before drawing conclusive findings. It also would be beneficial to perform histopathology with emphasis on the root tip to identify the specific point(s) of entry through which *F. commune* entered the tip of the root. This could be done with growth in soil extract agar, hydroponics, or a visualization system that allow in situ characterization. To draw definitive conclusions, performing histopathology on multiple FOSC isolates that cause Fusarium yellows is needed in the future.

Interior necrosis caused by *F. commune* was characterized by degradation of cellular material and heavy colonization of vascular and parenchyma tissue, generally originating from the central stele (Figure 8, Hanson and Jacobsen 2009). This differed from the description of Fusarium root rot, which was characterized by an exterior rot that progressed inward, typically originating at the root tip (Martyn et al. 1989, Harveson 2009). There has been no comprehensive histopathological work on Fusarium root rot in beet, and as such the precise nature of penetration and development of root necrosis in this crop system was unknown. In 2018, Fob220a was among the isolates that caused Fusarium yellows on USA sugar beet cultivars and Fusarium root rot on cultivars from Italy (Hanson et al. 2018). A comparison of colonization and penetration of Fob220a on a susceptible Italian cultivar would be beneficial to characterize differences in disease progression between Fusarium root rot and Fusarium yellows. The literature on tomato histopathology with FOL and FORL indicated that a potential difference between Fusarium wilt versus root rot could be related to site preference for penetration and infection. It was observed that Fusarium wilt of tomato had some site preference for the root tip, whereas Fusarium foot and root rot of tomato did not show a preference (Lagopodi et al. 2002, Martinez-Soto et al.

2023, Olivain and Alabouvette 1999). Adding additional isolates could support the broad spectrum applicability of such observations. It is also needed to confirm that FOB acts similarly in sugar beet.

While fungicide and biological control treatments are not currently used to manage Fusarium yellows of sugar beet, the results of the current study are informative as to application methods that might minimize disease severity. For instance, treatments that reach the tip of the sugar beet root, such as in-furrow and soil drench treatments, would be recommended over topical foliar and soil sprays unless they include systemic activity. In addition, it would be beneficial to compare sugar beet varieties that are highly susceptible and highly resistant to Fusarium yellows to identify plant defense mechanisms that contribute to disease resistance. This would benefit both sugar beet breeders and growers in making informed decisions on cultivar selection to promote resistance to Fusarium yellows.

LITERATURE CITED

Carotenuto G. and Genre A. 2020. Fluorescent staining of arbuscular mycorrhizal structures using wheat germ agglutinin (WGA) and propidium iodide. In: N. Ferrol and L. Lanfranco, eds. Arbuscular Mycorrhizal Fungi. Humana, New York, NY. Pp. 53-59.

Covey P. A., Kuwitzky B., Hanson M. and Webb K. M. 2014. Multilocus analysis using putative fungal effectors to describe a population of *Fusarium oxysporum* from sugar beet. Phytopathology. 104:886-896.

Crous P. W., Lombard L., Sandoval-Denis M., Seifert K. A., Schroers H. -J., P. Chaverri P., Gene J., Guarro J., Hirooka Y., Bensch K., Kema G. H. J., Lamprecht S. C., Cai L., Rossman A. Y., Stadler M., Summerbell R. C., Taylor J. W., Ploch S., Visagie C. M., Yilmaz N., Frisvad J. C., Abdel-Azeem A. M., Abdollahzadeh J., Abdolrasouli A., Akulov A., Alberts J. F., Araújo J. P. M., H.A. Ariyawansa H. A., Bakhshi M., Bendiksby M., Ben Hadj Amor A., Bezerra J. D. P., Boekhout T., Camara M. P. S., Carbia M., Cardinali G., Castaneda-Ruiz R. F., Celis A., Chaturvedi V., Collemare J., Croll D., Damm U., Decock C. A., de Vries R. P, Ezekiel C. N., Fan X. L., Fernandez N. B., Gaya E., Gonzalez C. D., Gramaje D., Groenewald J. Z., Grube M., Guevara-Suarez M., Gupta V. K., Guarnaccia V., Haddaji A., Hagen F., Haelewaters D., Hansen K., Hashimoto A., Hernandez Restrepo M., Houbraken J., Hubka V., Hyde K. D., Iturriaga T., Jeewon R., Johnston P. R., Jurjevic Z., Karalti I., Korsten L., Kuramae E. E., Kusan I., Labuda R., Lawrence D. P., Lee H. B.,Lechat C., Li H. Y., Litovka Y. A., Maharachchikumbura S. S. N., Marin-Felix Y., Matio Kemkuignou B., Matocec N., McTaggart A. R., Micoch P., Mugnai L., Nakashima C., Nilsson R. H., Noumeur S. R., Pavlov I. N., Peralta M. P., Phillips A. J. L., Pitt J. I., Polizzi G., Quaedvlieg W., Rajeshkumar K. C., Restrepo S., Rhaiem A., Robert J., Robert V., Rodrigues A. M., Salgado-Salazar C., Samson R. A., Santos A. C. S., Shivas R. G., Souza-Motta C. M., Sun G. Y., Swart W. J., Szoke S., Tan Y. P., Taylor J. E., Taylor P. W. J., Tiago P. V., Vaczy K. Z., van de Wiele N., van der Merwe N. A., Verkley G. J. M., Vieira W. A. S., Vizzini A., Weir B. S., Wijayawardene N. N., Xia J. W., Yanez-Morales M. J., Yurkov A., Zamora J. C., Zare R., Zhang C. L., and Thines M. 2021. *Fusarium*: more than a node or a foot-shaped basal cell. Studies in Mycology Vol. 98:100116. 1-184 pages. DOI: 10.1016/j.simyco.2021.100116.

De Lucchi C., Stevanato P., Hanson L., McGrath M., Panella L., De Biaggi M., Broccanello C., Bertaggia M., Sella L. and Concheri G. 2017. Molecular markers for improving control of soil-borne pathogen *Fusarium oxysporum* in sugar beet. Euphytica. 213:71. 1-14 pages. DOI: 10.1007/s10681-017-1859-7.

Deng S., Ma X., Chen Y., Feng H., Zhou D., Wang X., Zhang Y., Zhao M., Zhang J., Daly P., and Wei L. 2022. LAMP Assay for distinguishing *Fusarium oxysporum* and *Fusarium commune* in lotus (*Nelumbo nucifera*) rhizomes. Plant Disease. 106:231-246.

Hanson L. E. and Jacobsen B. J. 2009. Fusarium yellows. In: Harveson R.M., Hanson L.E., Hein G.L., eds. Compendium of Beet Diseases and Pests, 2nd ed. St. Paul, MN, USA: APS Press. Pp. 28–30.

Hanson L. E., Hill A. L., Jacobsen B. J. and Panella L. 2009. Response of sugarbeet lines to isolates of *Fusarium oxysporum* f. sp. *betae* from the United States. Journal of Sugar Beet Research. 46:11-26.

Hanson L. E., De Lucchi C., Stevanato P., McGrath M., Panella L., Sella L., De Biaggi M. and Concheri G. 2018. Root rot symptoms in sugar beet lines caused by *Fusarium oxysporum* f. sp. *betae*. European Journal of Plant Pathology. 150:589-593.

Harveson, R. M. 2009. Fusarium root rot. In: Harveson R.M., Hanson L.E., Hein G.L., eds. Compendium of Beet Diseases and Pests, 2nd ed. APS Press, St. Paul, MN, USA. Pp. 30-31.

Hill A. L., Reeves P. A., Larson R. L., Fenwick A. L., Hanson L. E. and Panella L. 2011. Genetic variability among isolates of *Fusarium oxysporum* from sugar beet. Plant Pathology. 60:496-505.

Hoagland D. R. and Arnon D. I. 1950. The water-culture method for growing plants without soil. Page 31 in: California Agricultural Experiment Station, 347 ed. University of California, Berkeley, CA.

Lai X., Qi A., Liu Y., Del Río Mendoza L. E., Liu Z., Lin Z. and Khan M. F. Evaluating inoculation methods to infect sugar beet with *Fusarium oxysporum* f. *betae* and *F. secorum*. Plant Disease. 104:1312-1317.

Lagopodi A. L., Ram A. F. J., Lamers G. E. M., Punt P. J., Van den Hondel C. A. M. J. J., Lugtenberg B. J. J., and Bloemberg G. V. 2002. Novel aspects of tomato root colonization and infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. Molecular Plant Microbe Interactions. 15: 172-9.

Larson R. L., Hill A. L., Fenwick A., Kniss A. R., Hanson L. E. and Miller S. D. 2007. Influence of glyphosate on Rhizoctonia and Fusarium root rot in sugar beet. Pest Management Science. 62:1182-1192.

Lewellen R. T. 2004. Registration of Rhizomania resistant, monogerm populations C869 and C869CMS sugarbeet. Crop Science. 44:357–358.

Martinez-Soto D., Yu H., Allen K. S. and Ma L. J. 2023. Differential colonization of the plant vasculature between endophytic versus pathogenic *Fusarium oxysporum* strains. Molecular Plant Microbe Interactions. 36:4-13.

Martyn R. D., Rush C. M., Biles C. L. and Baker E. H. 1989. Etiology of a root rot disease of sugar beet in Texas. Plant Disease. 73:879-884.

Minier D. H. 2023. Population genetics, intraspecific groups, and histopathology of *Rhizoctonia solani* AG 2-2 on sugar beet. Michigan State University. Pp.181-186. DOI: 10.25335/5xke-4114.

Nelson P. E. 1981. Life cycle and epidemiology of *Fusarium oxysporum*. In: Mace M. E., Bell A. A. and Beckman C. H. eds. Fungal Wilt Diseases of Plants. Academic Press Inc., New York, NY USA. Pp. 51-78.

Olivain C. and Alabouvette C. 1999. Process of tomato root colonization by a pathogenic strain of *Fusarium oxysporum* f. sp. *lycopersici* in comparison with a non-pathogenic strain. New Phytologist. 141:497-510.

Rodríguez-Gálvez E. and Mendgen K. 1995. The infection process of *Fusarium oxysporum* in cotton root tips. Protoplasma. 189:61-72.

Skovgaard K., Rosendahl S., O'Donnell K., and Nirenberg H. I. 2004. *Fusarium commune* is a new species identified by morphological and molecular phylogenetic data. Mycologia. 95:630-636.

Stewart D. 1931. Sugar-beet yellows caused by *Fusarium conglutinans* var. *betae*. Phytopathology. 21:59-70.

Stewart J. E., Kim M. S., James R. L., Dumroese R. K., Klopfenstein N. B. 2006. Molecular characterization of *Fusarium oxysporum* and *Fusarium commune* from a conifer nursery. Phytopathology. 96:1124-1133.

Turland N. J., Wiersema J. H., Barrie F. R., Greuter W., Hawksworth D. L., Herendeen P. S., Knapp S., Kusber W.-H., Li D.-Z., Marhold K., May T. W., McNeill, J., Monro A. M., Prado J., Price M. J. and Smith G. F. (eds.). 2018. International code of nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. DOI: 10.12705/Code.2018

Webb K. M., Covey P. A. and Hanson L. E. 2012. Pathogenic and phylogenetic analysis of *Fusarium oxysporum* from sugarbeet in Michigan and Minnesota. Journal of Sugar Beet Research. 49:38-57.

Webb K. M., Delgrosso S. J., West M. S., Freeman C. and Brenner T. 2017. Influence of environment, crop age and cultivar on the development and severity of Fusarium yellows in field-grown sugar beet. Canadian Journal of Plant Pathology. 39:37-47.

Webb K. M., Shresthab S., Trippe III R., Rivera-Varas V., Covey P. A., Freeman C., de Jonge R., Secor G. A. and Bolton M. 2019. Phylogenetic relationships and virulence assays of *Fusarium secorum* from sugar beet suggest a new look at species designations. Plant Pathology. 68:1654-1662.

CHAPTER 4: GENERAL SUMMARY

To address grower concerns in Michigan regarding the increased observance of pathogenic *Fusarium oxysporum* species complex isolates on sugar beet, research was undertaken to identify local pathogenic *F. oxysporum* isolates and assess mechanisms of penetration and colonization of sugar beet root tissue.

The major limitations of the virulence screening experiment were largely due to environmental factors. Under ideal conditions there would be precise control of the greenhouse temperature and complete management of undesired pests in the greenhouse unit. Since the completion of this study, an updated heating and cooling system program has been installed, placing both heating and cooling under one system. This will hopefully improve the regulation of temperature for future studies in the greenhouse and has the added benefit that it can be remotely monitored. This should reduce the chance for an issue like the undetected temperature change that affected experimental run four to be undetected and more rapidly corrected in future experiments. For management of pests, the use of preventative biological controls had been demonstrated to have promising results, in particular the control of green peach aphids (*Myzus persicae*) with parasitoid wasps (Flint and Dreistadt 1998) The use of parasitoid wasps has been incorporated into greenhouse units, with the aim of managing aphids and the virions they could vector for sugar beet viral diseases.

In the future, a similar greenhouse virulence screening protocol can be applied to identifying pathogenic *Fusarium* spp. on sugar beet seedlings. One of the objectives of the seedling screening project would be to compare findings to some existing reports on the wider range of *Fusarium* spp. causing diseases on sugar beet seedlings (Hanson and Hill 2004, Ruppell 1991). Another objective of the seedling screening would be to identify *Fusarium* spp. to be used

in seedling resistance screening in the field and greenhouse for the benefit of Michigan sugar beet growers. There is a need for this work, as sugar beet seedlings and more mature sugar beets display differences in plant defenses and susceptibility to pathogens, as reported with *Rhizoctonia solani* and observed in the field with *Fusarium* spp. (Engelkes and Windels 1994, Liu et al. 2019, Trebbi and McGrath 2009, unpublished data). In Michigan in particular, seedling damping off due to *Fusarium* spp. has contributed to early season losses (Hanson and McGrath 2011, Hanson et al. 2012, Ruppel 1991).

There were several limitations of the microscopy experiment, including the partial loss of the outermost layer of root, the cork. This was due to the abrasive interaction of the sand against plant tissue when removed from the potting container and during gentle rinsing prior to sample fixation. Based on the presence of hyphae on the remaining tissue, it was cautiously hypothesized that *F. commune* was able to colonize and penetrate the cork. Another limitation was the method of inoculation, as a soil drench, while more representative than a root dip of natural inoculum conditions in the field, is prone to plants that escape inoculation (Lai et al. 2020, Resende et al. 1995). One reported hypothesis for the cause of escapes was that the movement of spores in soil is variable, leading to uneven distribution of spores at different depths of soil (Gracia-Garza and Fravel 1998, Lai et al. 2020). Gracia-Garza and Fravel (1998) reported that the proportion of spores at a depth of 0-2 cm was ten times that of spores at 8-10cm deep. There were sugar beets that potentially escaped inoculation during the current experiment, with about two-thirds of plants successfully inoculated, and one-third of plants were not colonized by hyphae. In hindsight, it would have been beneficial to explore alternative growth substrates for the beet plants, such as an agar based medium (Bornman and Barnard 1993). It would hypothetically provide more control of inoculation as the inoculum could be precisely

injected into the agar along the length of the root, instead of the variable water movement of inoculum through sand. It also could allow for extraction of fully intact sugar beet roots, as the agar could be dissolved via chemical buffer degradation as used in gel extraction kits such as dimethyl sulfoxide or guanidinium thiocyanate (Roux et al. 2023) or gentle heating to remove the agar without damaging the root tissue. This could preserve the cork and periderm and reduce the loss of plant tissue due to the abrasive removal of sand. However, this would be artificial environment, and potentially less representative of what would occur in field soil, so additional testing would be needed to ensure similar behavior in soil.

Another limitation of the microscopy experiment was the limited range of symptom severities achieved in sampled sugar beets. Samples were taken from pre-infected, asymptomatic, mildly symptomatic, and deceased hosts during the course of the experiment. Ideally, there also would have been moderate to severe symptomatic sugar beet to sample from, to fully correlate the advancement of foliar symptom severity to what was occurring in the root. Options to increase the odds of sampling a wider range of symptom severity would have been to increase the number of replicates at each time point, and to increase the range of time sampled. However, this would have incurred additional costs by prolonged use of the growth chamber and increased the number of samples to prepare and visualize under the microscope.

Lastly, our ability to draw conclusions on differences, or lack thereof, between *F. commune* and *F. oxysporum* on sugar beet was limited. At the time of performing the microscopy experiment, a number of the prior moderately virulent isolates have since been putatively reclassified as *F. commune* based on sequence analysis, including Fob220a itself, as well as Fob216c and F17 (Hanson et al. 2009, Hill et al. 2011, Webb et al. 2012, Webb et al. 2019, Appendix Table 18). The moderately virulent isolates identified during the greenhouse virulence

screening experiment, F08-207 and F07-43, could be promising candidates for an additional microscopy screening to validate the hypotheses formed in response to preliminary data acquired in Chapter 3. In the future, it could be beneficial to perform this microscopy protocol with various sugar beet varieties to potentially provide breeders with an indication of some plant defenses they might emphasize in selective breeding. In particular, the comparison of two different sugar beet germplasm with a range of susceptibility to Fusarium yellows could be used to inform *Fusarium* resistance breeding programs. This could be informative as to some of the plant defenses that could be stimulated in response to infection by *Fusarium* oxysporum species complex strains and would be complimentary to research comparing the presence and expression of resistance genes in the respective germplasm.

Across both experiments, isolates of putative *F. commune* and *F. oxysporum* that were utilized showed no differences in phenotype, including morphology. This raised concerns as to the validity of taxonomic separation between these two species since a morphological difference currently is required to separate a species. If they are indeed separate species, the similarity between infection process and diseases symptoms is an encouraging find, as this might indicate that management strategies currently in use for *F. oxysporum*-induced diseases of sugar beet would not need to be modified to manage pathogenic *F. commune*.

LITERATURE CITED

Bornman J. J. and Barnard R. O. 1993. The possible use of agar gel in plant nutritional studies. South African Journal of Plant and Soil. 10:146-149.

Engelkes C. A. and Windels C. E. 1994. Relationship of plant age, cultivar, and isolate of *Rhizoctonia solani* AG-2-2 to sugar beet root and crown rot. Plant Disease. 78:685-689.

Flint M. L and Dreistadt S. H. 1998. Natural Enemies Handbook: The Illustrated Guide to Biological Pest Control. UC Division of Agriculture and Natural Sciences, Oakland CA, USA. 154 pages.

Hanson L. E. and Hill A. L. 2004. *Fusarium* species causing Fusarium yellows of sugar beet. Journal of Sugar Beet Research. 41:163-178.

Hanson L. E. and McGrath M. J. 2011. Seedling damping-off in sugar beet in Michigan. International Institute for Beet Research Workshop Proceedings. Abstract.

Hanson L. E., Goodwill T. R., McGrath M. J. 2012. Seedling disease survey in Michigan. In: Research Results 2011. Michigan Sugarbeet REACh. Pp. 39-40.

Gracia-Garza J. A. and Fravel D. R. 1998. Effect of relative humidity on sporulation of *Fusarium oxysporum* in various formulations and effect of water on spore movement through soil. Phytopathology. 88:544-549.

Hanson L. E., Hill A. L., Jacobsen B. J. and Panella L. 2009. Response of sugarbeet lines to isolates of *Fusarium oxysporum* f. sp. *betae* from the United States. Journal of Sugar Beet Research. 46:11-26.

Hill A. L., Reeves P. A., Larson R. L., Fenwick A. L., Hanson L. E. and Panella L. 2011. Genetic variability among isolates of *Fusarium oxysporum* from sugar beet. Plant Pathology. 60:496-505.

Lai X., Qi A., Liu Y., Del Río Mendoza L. E., Liu Z., Lin Z. and Khan M. F. 2020. Evaluating inoculation methods to infect sugar beet with *Fusarium oxysporum* f. *betae* and *F. secorum*. Plant Disease. 104:1312-1317.

Liu Y., Qi A., and Khan M. F. R. 2019. Age-dependent resistance to *Rhizoctonia solani* in sugar beet. Plant Disease. 103:2322-2329.

Resende M. L. V., Flood J., and Cooper R. M. 1995. Effect of method of inoculation, inoculum density and seedling age at inoculation on the expression of resistance of cocoa (*Theobroma cacao* L.) to *Verticillium dahliae* Kleb. Plant Pathology. 44:374-383.

Roux D. C. D., Jeacomine I., Maîtrejean G., Caton F., and Rinaudo M. 2023. Characterization of agarose gels in solvent and non-solvent media. Polymers. 15:2162. Pages 1-16. DOI: 10.3390/polym15092162

Ruppel E. G. 1991. Pathogenicity of *Fusarium* spp. from diseased sugar beets and variation among sugar beet isolates of *F. oxysporum*. Plant Disease. 75:486-489.

Trebbi D. and McGrath M. J. 2009. Functional differentiation of the sugar beet root system as indicator of developmental phase change. Physiologia Plantarum. 135:84–97.

Webb K. M., Covey P. A. and Hanson L. E. 2012. Pathogenic and phylogenetic analysis of *Fusarium oxysporum* from sugarbeet in Michigan and Minnesota. Journal of Sugar Beet Research. 49:38-57.

Webb K. M., Shresthab S., Trippe III R., Rivera-Varas V., Covey P. A., Freeman C., de Jonge R., Secor G. A. and Bolton M. 2019. Phylogenetic relationships and virulence assays of *Fusarium secorum* from sugar beet suggest a new look at species designations. Plant Pathology. 68:1654-1662.

APPENDIX

Supplementary Tables:

Table 6: R packages and their corresponding references for statistical analysis of pathogenicity of *F. oxysporum* species complex isolates and Fusarium yellows virulence on sugar beet.

Packages	**References**
lme4	Bates D., Mächler M., Bolker B., and Walker S. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 67:1-48. Bates D., Maechler M., and Jagan M 2024. Matrix: Sparse and Dense Matrix Classes and Methods. R package version 1.6-5.
car	Fox J. and Weisberg S. 2019. An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. Fox J., Weisberg S., and Price B. 2022. carData: Companion to Applied Regression Data Sets. R package version 3.0-5.
emmeans	Lenth R. 2023. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.9.0.
lattice	Sarkar D. 2008. Lattice: Multivariate Data Visualization with R. Springer, New York.
multcomp	Genz A. and Bretz F. 2009. Computation of Multivariate Normal and t Probabilities, series Lecture Notes in Statistics. Springer-Verlag, Heidelberg. Hothorn T. 2023. TH.data: TH's Data Archive. R package version 1.1-2. Hothorn T., Bretz F., and Westfall P. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal. 50:346-363. Therneau T. 2023. A Package for Survival Analysis in R. R package version 3.5-7. Therneau T. and Grambsch P. 2000. Modeling Survival Data: Extending the Cox Model. Springer, New York. Venables W. N., and Ripley B. D. 2002. Modern Applied Statistics with S, Fourth edition. Springer, New York.
multcompview	Graves S., Piepho H., Dorai-Raj L. 2023. multcompView: Visualizations of Paired Comparisons. R package version 0.1-9.
phia	De Rosario-Martinez H. 2024. phia: Post-Hoc Interaction Analysis. R package version 0.3-1. Fox J. and Weisberg S. 2019. An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. Fox J., Weisberg S., and Price B. 2022. carData: Companion to Applied Regression Data Sets. R package version 3.0-5.
nlme	Pinheiro J., Bates D., R Core Team. 2023. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-163. Pinheiro J. and Bates D. 2000. Mixed-Effects Models in S and S-PLUS. Springer, New York.
rstatix	Kassambara A. 2023. rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.7.2.

Table 7: Supplementary data on pest and temperature (Watchdog, Spectrum® Technologies, Inc.) data for virulence screening for Fusarium yellows of sugar beet in the greenhouse.

Month	Year	Experimental Run	Day Mean Temperature (C)	Night Mean Temperature (C)	Pest(s) Present[a]
September	2022	Pilot, Run 1	24.12	22.03	PM
October	2022	Run 1, 2	24.01	22.84	
November	2022	Run 1, 2	23.75	23.08	
December	2022	Run 1, 2, 3	27.00	25.79	A, FG, VYC,
January	2023	Run 3, 4	27.22	25.93	
February	2023	Run 3, 4, 5	26.61	25.78	
March	2023	Run 3, 4, 5, 6	22.04	22.26	T, SM
April	2023	Run 5, 6	28.05	25.20	T, SM
May	2023	Run 6	27.38	21.84	T, SM
June	2023	Run 6	29.80	23.38	
September	2023	Run 7, 8	25.98	24.52	
October	2023	Run 7, 8, 9	27.99	26.89	A
November	2023	Run 8, 9	28.24	27.76	A
December	2023	Run 8, 9, 10, 11	28.16	27.34	
January	2024	Run 9, 10, 11, 12	27.55	27.71	
February	2024	Run 10, 11, 12, 13	28.54	28.96	
March	2024	Run 11, 12, 13	28.16	28.10	
April	2024	Run 12, 13	27.48	28.58	
May	2024	Run 13	28.20	27.93	
June	2024	Run 13	28.84	27.52	

[a] Abbreviated pest names, PM for powdery mildew (*Erysiphe betae*), A for aphids (*Myzus persicae*), FG for fungus gnats (*Mycetophilidae* family), VYC for sugar beet virus yellows complex, T for thrips (*Thrips* sp.), and SM for spider mites (*Tetranychus urticae*).

Table 8: Treatments for pest management in research greenhouse and growth chamber units.

Location	Date (Month, Year)	Treatment	Target
Greenhouse	Sept 2022 – June 2024	Yellow sticky paper traps, suspended between plants	General insect pests, monitoring pest levels
Greenhouse	Sept 2022 – June 2024	AMBLYforce™ biocontrol (*Amblyseius cucumeris*)	Preventative thrips
Greenhouse	Oct 2023 – June 2024	(pouch, biotreatment)	Preventative aphids
Greenhouse	Sept 2022	2% Sodium bicarbonate + 0.1% Tween20	Spray, powdery mildew
Greenhouse	Dec 2022	Marathon ii	Drench, aphids
Greenhouse	Dec 2022	Enstar Aq	Drench, fungus gnats
Greenhouse	March-May 2023	Enstar Aq + Mainspring GNL + Floramite SC Suffoil-X, Hexygon, Xxpire Tristar 8.5 SL + Shuttle O Mainspring GNL + Akari	Spider mites and thrips
Greenhouse	Sept 2023	Altus + Captiva and Capsil	Aphids
Greenhouse	Oct 2023	Endeavor + Captiva and Capsil	Aphids
Greenhouse	Oct 2023	Suffoil-X and Ventigra	Aphids
Greenhouse	Jan 2024	Suspend Polyzone	Cockroaches, (not applied directly on or for plants)
Greenhouse	Feb 2024	NEMAforce™ SF biocontrol (*Steinernema feltiae* nematodes)	Fungus gnats
Growth chamber	2024	AMBLYforce™ biocontrol (*Amblyseius cucumeris*)	Preventative thrips, used on other plants in unit

Table 9: ANOVA Table (α=0.05) for Fusarium yellows foliar severity ratings in sugar beet, based on foliar ratings on a scale of 0 (no symptoms) to 5 (plant death).

Run	Factor	Degrees of freedom	p-value
Run 1*	Isolate	5	<0.0001
	Variety	1	0.0277
	Isolate:Variety	5	0.3781
Run 2*	Isolate	5	<0.0001
	Variety	1	0.0029
	Isolate:Variety	5	<0.0001
Run 3*	Isolate	4	<0.0001
	Variety	1	<0.0001
	Isolate:Variety	4	<0.0001
Run 4	Isolate	5	<0.0001
	Variety	1	<0.0001
	Isolate:Variety	5	<0.0001
Run 5	Isolate	5	0.0012
	Variety	1	0.2471
	Isolate:Variety	5	0.0174
Run 6	Isolate	4	0.0903
	Variety	1	0.0087
	Isolate:Variety	4	0.1961
Run 7	Isolate	6	<0.0001
	Variety	1	0.0420
	Isolate:Variety	6	0.0015
Run 8	Isolate	6	<0.0001
	Variety	1	<0.0001
	Isolate:Variety	6	<0.0001
Run 9	Isolate	6	<0.0001
	Variety	1	<0.0001
	Isolate:Variety	6	<0.0001
Run 10	Isolate	5	<0.0001
	Variety	1	0.1043
	Isolate:Variety	5	<0.0001
Run 11	Isolate	6	<0.0001
	Variety	1	0.7318
	Isolate:Variety	6	<0.0001
Run 12	Isolate	6	<0.0001
	Variety	1	0.01
	Isolate:Variety	6	<0.0001
Run 13	Isolate	6	<0.0001
	Variety	1	<0.0001
	Isolate:Variety	6	<0.0001

* Denotes log10 transformed data, when original data didoes not meet the equality of variances assumption.

Table 10: ANOVA table (α=0.05) for Fusarium yellows root symptom severity rating in sugar beet, based on a scale of 0 (no symptoms) to 5 (plant death).

Run	Factor	Degrees of Freedom	p-value
Run 1*	Isolate	5	<0.0001
	Variety	1	0.0024
	Isolate:Variety	5	0.0738
Run 2*	Isolate	5	<0.0001
	Variety	1	0.0009
	Isolate:Variety	5	0.0618
Run 3*	Isolate	4	<0.0001
	Variety	1	0.6304
	Isolate:Variety	4	0.1878
Run 4	Isolate	5	<0.0001
	Variety	1	0.0770
	Isolate:Variety	5	<0.0001
Run 5*	Isolate	5	<0.0001
	Variety	1	0.5116
	Isolate:Variety	5	0.1324
Run 6	Isolate	4	0.0080
	Variety	1	0.0824
	Isolate:Variety	4	0.2829
Run 7*	Isolate	6	<0.0001
	Variety	1	<0.0001
	Isolate:Variety	6	<0.0001
Run 8	Isolate	6	<0.0001
	Variety	1	0.0002
	Isolate:Variety	6	<0.0001
Run 9	Isolate	6	<0.0001
	Variety	1	<0.0001
	Isolate:Variety	6	<0.0001
Run 10*	Isolate	5	<0.0001
	Variety	1	0.0106
	Isolate:Variety	5	0.0115
Run 11*	Isolate	6	<0.0001
	Variety	1	<0.0001
	Isolate:Variety	6	<0.0001
Run 12	Isolate	6	<0.0001
	Variety	1	<0.0001
	Isolate:Variety	6	<0.0001
Run 13*	Isolate	6	0.0003
	Variety	1	<0.0001
	Isolate:Variety	6	<0.0001

* Denotes log10 transformed data, when original data did not meet the equality of variances assumption.

Table 11: Statistical analysis of the impact of sugar beet variety on Fusarium yellows foliar symptom severity, by Fisher's Protected LSD (α=0.05).

Run	Variety	Mean AUDPS	10% Confidence Interval
Run 1*	FC716	15.6a	(11.9, 20.9)
	C869cms	24.1b	(20.9, 32.1)
Run 2*	F1042	23.5a	(20.4, 27.2)
	C869cms	32.1b	(32.1, 37)
Run 3*	F1042	37.9a	(32.88, 42.65)
	C869cms	53.9b	(46.86, 62.09)
Run 4	F1042	37.7a	(31.5, 43.81)
	C869cms	55.8b	(49.6, 62)
Run 5	--[a]	--	--
Run 6	F1042	41.1a	(31, 51.3)
	C869cms	61.2b	(51, 71.4)
Run 7	F1042	62a	(52.5, 71.5)
	C869cms	75.9b	(66.5, 85.4)
Run 8	F1042	46.3a	(39, 53.6)
	C869cms	76.2b	(98.9, 83.5)
Run 9	F1042	56.9a	(49.5, 64.3)
	C869cms	79.1b	(71.7, 86.5)
Runs 10 and 11	--	--	--
Run 12	C869cms	89.9a	(84.5, 95.3)
	F1042	99.6b	(94.2, 105)
Run 13	F1042	67.4a	(60.1, 74.7)
	C869cms	89.7b	(82.3, 97)

* denotes log10 transformed data, when original data did not meet the equality of variances assumption. [a] the use of -- represents experimental runs where Fisher's protected LSD could not be applied due to an insufficient p-value in the preliminary ANOVA.

Table 12: Statistical analysis of the impact of sugar beet variety on Fusarium yellows root symptom severity, by Fisher's Protected LSD (α=0.05).

Run	Variety	Mean Disease Rating	10% Confidence Interval
Run 1*	FC716	0.9a	(0.7, 1.1)
	C869cms	1.3b	(1.1, 1.5)
Run 2*	F1042	0.5a	(0.3, 0.6)
	C869cms	0.9b	(0.7, 1.1)
Runs 3*, 4, 5*, 6	--[a]	--	--
Run 7*	F1042	0.7a	(0.5, 0.8)
	C869cms	1.4b	(1.2, 1.6)
Run 8	F1042	0.9a	(0.8, 1.1)
	C869cms	1.3b	(1.2, 1.5)
Run 9	F1042	0.8a	(0.6, 1)
	C869cms	1.5b	(1.7, 1.7)
Run 10*	F1042	0.5a	(0.2, 0.3)
	C869cms	0.3b	(0.3, 0.4)
Run 11*	F1042	0.6a	(0.4, 0.7)
	C869cms	1.1b	(0.9, 1.2)
Run 12	F1042	0.7a	(0.5, 0.9)
	C869cms	1.4b	(1.3, 1.6)
Run 13*	F1042	0.5a	(0.3, 0.6)
	C869cms	0.8b	(0.7, 1)

* denotes log10 transformed data, when original data did not meet the equality of variances assumption. [a] the use of -- represents experimental runs where Fisher's protected LSD could not be applied due to an insufficient p-value in the preliminary ANOVA.

Table 13: Statistical analysis of the impact of *Fusarium* spp. isolates on Fusarium yellows foliar symptom severity in sugar beet, by Fisher's Protected LSD (α=0.05).

Run	Isolate	Mean AUDPS	10% Confidence Interval
Run 1*	Water	2.2a	(1, 4.1)
	F21-22	13.7b	(8.3, 22.1)
	F21-76	14.7b	(8.3, 22.1)
	F05-284	24.5b	(15.2, 39.4)
	Fob220a	55c	(34.4, 87.3)
	F19	70c	(44, 110.9)
Run 2*	Water	12.8a	(10, 16.4)
	F09-16	21.9b	(17.2, 27.8)
	F11-63	22.4b	(17.6, 28.5)
	F08-207	28.5bc	(22.4, 36.2)
	Fob220a	38.8c	(30.6 50.3)
	F19	60.7d	(48, 76.6)
Run 3*	Water	18.1a	(14.14, 22.99)
	F10-58	38.8b	(31.36, 47.98)
	F07-48	52.7c	(42.65, 65.07)
	F19	57.9c	(46.86, 71.44)
	Fob220a	84.1d	(68.18, 103.71)
Run 4	Water	23.9a	(13.2, 34.6)
	Cy22-1	36.2ab	(25.5, 46.9)
	F22-35	36.9ab	(26.3, 47.6)
	F08-207	44.3b	(33.6, 55)
	Fob220a	68.4c	(57.8, 79.1)
	F19	70.6c	(59.9, 81.3)
Run 5	F12-36	42.6a	(26, 59.2)
	F21-8	55.9a	(37.3, 74.4)
	F09-87	56a	(39.4, 72.6)
	Water	60.7a	(44, 77.3)
	Fob220a	88.7b	(72, 105.3)
	F19	91.3b	(74.7, 107.9)
Run 6	--[a]	--	--
Run 7	Water	17.9a	(0.2, 35.6)
	F14-22	57b	(39.2, 74.7)
	F23-11	57b	(39.2, 74.7)
	F10-58	63.2b	(45.5, 80.9)
	F13-10	68.6b	(50.9, 86.4)
	Fob220a	100.3c	(82.6, 118.1)
	F19	118.8c	(101.1, 136.5)

Table 13 (cont'd)

Run 8	Water	14a	(0.3, 27.7)
	F23-7	41.8b	(28.2, 55.5)
	F12-44	50b	(36.3, 63.6)
	F22-7	50.4b	(36.7, 64)
	F11-2	52.7b	(39, 66.3)
	Fob220a	103.1c	(89.4, 116.7)
	F19	116.9c	(103.2, 130.5)
Run 9	Water	13.8a	(-0.1, 27.7)
	F22-28	47.4b	(33.6, 61.3)
	F21-2	53.7bc	(39.8, 67.5)
	F23-27	56.2bc	(42.3, 70)
	F07-48	67.3c	(53.4, 81.1)
	Fob220a	113.9d	(100.1, 127.8)
	F19	123.7d	(109.8, 137.5)
Run 10	Water	19.1a	(4.4, 33.7)
	F23-11	31.9ab	(17.2, 46.5)
	F08-193	46.1b	(31.4, 60.7)
	F09-53	50.3b	(35.6, 64.9)
	Fob220a	125.7c	(111.1, 140.3)
	F19	125.9c	(111.2, 140.5)
Run 11	Water	37.3a	(27.3, 47.3)
	F21-42	50.2ab	(60.2, 60.2)
	F11-3	61.8bc	(71.8, 71.8)
	F22-24	64.8c	(74.8, 74.8)
	F12-24	67.9c	(77.9, 77.9)
	Fob220a	98.2d	(108.2, 108.2)
	F19	132.2e	(142.2, 142.2)
Run 12	F22-4	77.6a	(67.5, 87.7)
	F09-87	78a	(67.9, 88.1)
	Water	84.2ab	(74.1, 94.3)
	F22-12	90.6ab	(80.5, 100.7)
	F07-43	92.4b	(82.3, 102.5)
	Fob220a	116.7c	(106.6, 126.8)
	F19	124.1c	(114, 134.2)
Run 13	F22-34	58.3a	(44.6, 72.1)
	Water	58.7a	(45, 72.4)
	F07-49	59.3a	(45.6, 73)
	F12-25	65.5a	(51.8, 79.2)
	F13-10	69.6a	(55.9, 83.3)
	Fob220a	99.8b	(86, 113.5)
	F19	138.4c	(124.7, 152.2)

* denotes log10 transformed data, when original data did not meet the equality of variances assumption. [a] the use of -- represents experimental runs where Fisher's protected LSD could not be applied due to an insufficient p-value in the preliminary ANOVA.

Table 14: Statistical analysis of the impact of *Fusarium* spp. isolates on Fusarium yellows root symptom severity in sugar beet, by Fisher's Protected LSD (α=0.05).

Run	Isolate	Mean Disease Rating	10% Confidence Interval
Run 1*	Water	0.3a	(0.1, 0.5)
	F21-76	0.8b	(0.5, 1.1)
	F21-22	0.8b	(0.5, 1.1)
	F05-284	1b	(0.7, 1.4)
	Fob220a	1.8c	(1.4, 2.4)
	F19	2.5c	(2, 3.2)
Run 2*	Water	0.1a	(-0.1, 0.3)
	F11-63	0.3ab	(0.1, 0.5)
	F09-16	0.5bc	(0.3, 0.8)
	F08-207	0.7c	(0.4, 1)
	Fob220a	0.8c	(0.5, 1.2)
	F19	2.1d	(1.6, 2.7)
Run 3*	Water	0a	(-0.2, 0.4)
	F10-58	0.6b	(0.3, 0.9)
	F07-48	0.6b	(0.4, 1)
	F19	1.1bc	(0.6, 1.7)
	Fob220a	1.6c	(1, 2.4)
Run 4	Water	0.1a	(-0.01, 0.18)
	F08-207	0.1a	(0.05, 0.24)
	Cy22-1	0.2a	(0.08, 0.28)
	F22-35	0.2ab	(0.11, 0.3)
	Fob220a	0.3b	(0.24, 0.43)
	F19	0.5c	(0.39, 0.59)
Run 5*	F12-36	0.3.a	(0, 0.6)
	Water	0.3ab	(0.1, 0.6)
	F21-8	0.3ab	(0.1, 0.6)
	F09-87	0.8b	(0.4, 1.2)
	Fob220a	1.5c	(1, 2)
	F19	1.7c	(1.2, 2.4)
Run 6	F11-67	0a	(-0.8, 0.8)
	Water	0.2a	(-0.6, 0.9)
	F12-12	0.2a	(-0.6, 0.9)
	F19	0.9ab	(0.2, 1.7)
	Fob220a	1.9b	(2.7, 2.7)
Run 7*	Water	0.4a	(0.2, 0.7)
	F10-58	0.6ab	(0.3, 0.8)
	F23-11	0.9b	(0.6, 1.2)
	F13-10	0.9b	(0.6, 1.2)
	F14-22	0.9b	(0.7, 1.3)
	Fob220a	1.7c	(1.3, 2.2)
	F19	2.2c	(1.7, 2.7)

Table 14 (cont'd)

Run 8	Water	0.3a	(0, 0.5)
	F23-7	0.3a	(0, 0.5)
	F22-7	0.4a	(0.2, 0.7)
	F12-44	0.6ab	(0.4, 0.9)
	F11-2	0.8b	(0.6, 1.1)
	Fob220a	2c	(1.7, 2.3)
	F19	3.5d	(3.2, 3.8)
Run 9	Water	0.3a	(-0.1, 0.7)
	F23-27	0.3a	(0, 0.7)
	F21-2	0.4a	(0.1, 0.8)
	F07-48	0.6a	(0.2, 1)
	F22-28	0.7a	(0.3, 1)
	Fob220a	2.7b	(2.3, 3)
	F19	2.9b	(3.3, 3.3)
Run 10*	Water	0.1a	(0, 0.2)
	F23-11	0.1a	(0, 0.2)
	F08-193	0.2ab	(0.1, 0.3)
	F09-53	0.3b	(0.2, 0.4)
	Fob220a	0.5c	(0.5, 0.6)
	F19	0.6c	(0.5, 0.7)
Run 11*	Water	0.2a	(0, 0.3)
	F11-3	0.5b	(0.3, 0.7)
	F21-42	0.5bc	(0.3, 0.7)
	F22-24	0.5bc	(0.3, 0.8)
	F12-24	0.8c	(0.6, 1.1)
	Fob220a	1.7d	(1.3, 2.1)
	F19	2.2d	(1.7, 2.7)
Run 12	Water	0.1a	(-0.2, 0.5)
	F22-12	0.3a	(-0.1, 0.6)
	F22-4	0.3a	(-0.1, 0.6)
	F09-87	0.6ab	(0.2, 1)
	F07-43	0.8b	(0.5, 1.2)
	F19	2.4c	(2.1, 2.8)
	Fob220a	2.8c	(2.5, 3.2)
Run 13*	F22-34	0.1a	(-0.1, 0.3)
	Water	0.2ab	(0, 0.4)
	F07-49	0.3ab	(0.1, 0.5)
	F13-10	0.3ab	(0.1, 0.5)
	F12-25	0.5b	(0.3, 0.7)
	Fob220a	1.5c	(1.1, 1.9)
	F19	2.8d	(2.2, 3.4)

* denotes log10 transformed data, when original data did not meet the equality of variances assumption. [a] the use of -- represents experimental runs where Fisher's protected LSD could not be applied due to an insufficient p-value in the preliminary ANOVA.

Table 15: Fluorochrome information for histopathological studies of *Fusarium commune* infection in sugar beet.

Stain	Brand and Product Description	Binding	Excitation (nm)	Emission (nm)
Propidium iodide	Sigma-Aldrich, #P4864	Pectin[1]	300, 535*	622*
Wheat germ agglutinin	Thermofisher, #W11261: AlexaFluor 488 Conjugate	Chitin[2, 3]	498*	519*

[1] Rounds C. M., Lubeck E., Hepler P. K., and Winship L. J. 2011. Propidium Iodide Competes with Ca^{2+} to Label Pectin in Pollen Tubes and *Arabidopsis* Root Hairs. Plant Physiology. Volume 157: 175–187.

[2] Allen A. K., Neuberger A., and Sharon N. 1973. The purification, composition and specificity of wheat-germ agglutinin. Biochemical Journal. 131:155–162.

[3] Meyberg, M. 1988. Selective staining of fungal hyphae in parasitic and symbiotic plant-fungus associations. Histochemistry. 88: 197–199.

*Excitation and emission wavelength determined using Thermofisher's Fluorescence Spectraviewer_www.thermofisher.com/order/fluorescence-spectraviewer

Table 16: Conventional and confocal fluorescence microscopes' objective information for histopathological studies of *Fusarium commune* infection in sugar beet.

Olympus BX60 (Conventional; Olympus Corporation, Tokyo, Japan)				
Objectives	**Immersion**	**Numerical Aperture**	**Lens**	**Additional Features**
4x	Dry	0.10	Plan	
10x	Dry	0.25	Plan	Ph1
20x	Dry	0.40	Plan	Ph1
40x	Dry	0.75	Plan	Ph2
100x	Oil	1.3	U Plan FL	
EVOS FL Auto (Conventional; Thermo Fisher Scientific, Waltham, MA)				
2x	Dry	0.06	L Plan	
4x	Dry	0.13	L Plan	Ph2
10x	Dry	0.30	L Plan	Ph2
20x	Dry	0.40	L Plan FL	Ph2
40x	Dry	0.65	L Plan FL	Ph2
Nikon Eclipse Ni and Nikon C2 (Conventional and Confocal Respectively; Nikon Instruments, Tokyo, Japan)				
10x	Dry	0.30	Plan Fluor	WD .13
20x	Dry	0.75	Plan APO	OFN25, Ph2 DLL
40x	Dry	0.75	Plan Fluor	WD, OFN25, DIC N2
60x	Oil	1.40	Plan APO	OFN25, DIC L/N1
Leica Stellaris 5 (Conventional and Confocal; Leica Microsystems, Wetzlar, Germany)				
10x	Dry	0.40	HC PL APO	--
20x	Dry	0.75	HC PL APO	--
40x	Oil	1.30	HC PL APO	--
63x	Oil	1.40	HC PL APO	--
100x	Oil	1.40	HC PL APO	--

Table 17: List of slides and relevant information used for histopathological studies of *Fusarium commune* infection in sugar beet sections.

Figure	Slide	File Name	Merged Channel Acquisitions	Taken On
Figure 10	D0H2A	M1D0H2Ar3a	20x, single image	Evos
Figure 5A	D0F1E	M1D0F1Er1a	20x, single image	Evos
Figure 6A	D0F2E	M1D0F2Er4a	20x, single image	Evos
Figure 5B	D9F2A	M1D9F2Ar1a	20x, average 2, MIP*, z-step 0.9µm, 18µm width	Nikon C2
Figure 6B	D9F2B	M1D9F2Br3a	20x, average 4	Nikon C2
Figure 5C	D15F1A	M1D15F1Ar1a	40x, single image	Evos
Figure 6C	D15F2O	M1D15F2Or4a	20x, average 2	Nikon C2
Figure 7A	D18F2A	M1D18F2Ar1	40x, average 2, zoom 1.5x, MIP xyz step series, z-step 0.34µm, 9.35µm width, xy area 722.3x718.8µm	Leica
Figure 7B	D18F2A	M1D18F2Art1z_40x	40x, average 1, MIP, z-step 0.8µm, 14.4µm width,	Nikon C2
Figure 7C	D18F2F	M1D18F2Fr2	40x, average 2, zoom 1.5x, MIP, z-step 0.34µm, 10.39µm width, xy total area 3309x4213µm	Leica
Figure 7D	D18F2F	M1D18F2F	40x, average 1, MIP, z-step 0.7µm, 15.4µm width	Nikon C2
Figure 8A	D18FdD	M1D18FdDr1a2	40x, single image	Evos
Figure 8B	D18FdE	M1D18FdE1	40x, average 1	Nikon C2
Figure 8C	D18FdE	M1D18FdE2	40x, average 1	Nikon C2

*MIP stands for maximum intensity projection of z-series

Table 18: Translation elongation factor 1α (TEF1a) and beta tubulin (BT) sequence data for isolates used in pathogenicity screening and histopathology.

	Isolate	Identification	Gene	Accession ID in NCBI
Chapter 1: Virulence Screening Original Isolates	F07-43	*F. oxysporum*	TEF1a	PV523650
	F07-48	*F. oxysporum*	TEF1a	PV523651
	F07-49	*F. oxysporum*	TEF1a	PV523652
	F08-193	*F. oxysporum*	TEF1a	PV523653
	F09-16	*F. oxysporum*	TEF1a	PV523654
	F09-53	*F. oxysporum*	TEF1a	PV523655
	F09-87	*F. oxysporum*	TEF1a	PV523656
	F10-58	*F. oxysporum*	TEF1a	PV523657
	F11-2	*F. oxysporum*	TEF1a	PV523658
	F11-3	*F. oxysporum*	TEF1a	PV523659
	F11-63	*F. oxysporum*	TEF1a	PV523660
	F11-67	*F. oxysporum*	TEF1a	PV523661
	F12-12	*F. oxysporum*	TEF1a	PV523662
	F12-24	*F. oxysporum*	TEF1a	PV523663
	F12-25	*F. oxysporum*	TEF1a	PV523664
	F12-36	*F. oxysporum*	TEF1a	PV523665
	F12-44	*F. oxysporum*	TEF1a	PV523666
	F13-10	*F. oxysporum*	TEF1a	PV523667
	F14-22	*F. oxysporum*	TEF1a	PV523668
	F21-2	*F. oxysporum*	TEF1a	PV523669
	F21-8	*F. commune*	TEF1a	PV523670
	F21-22	*F. oxysporum*	TEF1a	PV523671
	F21-42	*F. oxysporum*	TEF1a	PV523672
	F21-76	*F. oxysporum*	TEF1a	PV523673
	F22-4	*F. oxysporum*	TEF1a	PV523674
	F22-7	*F. oxysporum*	TEF1a	PV523675
	F22-12	*F. oxysporum*	TEF1a	PV523676
	F22-24	*F. oxysporum*	TEF1a	PV523677
	F22-28	*F. oxysporum*	TEF1a	PV523678
	F22-34	*F. oxysporum*	TEF1a	PV523679
	F22-35	*F. oxysporum*	TEF1a	PV523680
	F23-11	*F. oxysporum*	TEF1a	PV523681
	F23-7	*F. oxysporum*	TEF1a	PV523682
	F23-27	*F. oxysporum*	TEF1a	PV523683
Chapter 1: Virulence Screening Re-isolations from symptomatic and asymptomatic plant tissue	F19r	*F. commune*	TEF1a	PV523684
	Fob220a-r	*F. commune*	TEF1a	PV523685
	F07-43r	*F. oxysporum*	TEF1a	PV523686
	F07-48r	*F. oxysporum*	TEF1a	PV523687
	F07-48r2	*F. oxysporum*	TEF1a	PV523688
	F08-193r	*F. oxysporum*	TEF1a	PV523689

Table 18 (cont'd)

Chapter 1: Virulence Screening Re-isolations from symptomatic and asymptomatic plant tissue	F08-207r	*F. oxysporum*	TEF1a	PV523691
	F09-53r	*F. oxysporum*	TEF1a	PV523692
	F09-87r	*F. oxysporum*	TEF1a	PV523693
	F09-87r2	*F. oxysporum*	TEF1a	PV523694
	F10-58r	*F. oxysporum*	TEF1a	PV523695
	F10-58r2	*F. oxysporum*	TEF1a	PV523696
	F11-2r	*F. oxysporum*	TEF1a	PV523697
	F11-3r	*F. oxysporum*	TEF1a	PV523698
	F12-12r	*F. oxysporum*	TEF1a	PV523699
	F12-24r	*F. oxysporum*	TEF1a	PV523700
	F12-25r	*F. oxysporum*	TEF1a	PV523701
	F12-44r	*F. oxysporum*	TEF1a	PV523702
	F13-10r	*F. oxysporum*	TEF1a	PV523703
	F13-10r2	*F. oxysporum*	TEF1a	PV523704
	F14-22r	*F. oxysporum*	TEF1a	PV523705
	F21-42r	*F. oxysporum*	TEF1a	PV523706
	F21-8r	*F. commune*	TEF1a	PV523707
	F22-4r	*F. oxysporum*	TEF1a	PV523708
	F22-12r	*F. oxysporum*	TEF1a	PV523709
	F22-24r	*F. oxysporum*	TEF1a	PV523710
	F22-28r	*F. oxysporum*	TEF1a	PV523711
	F22-34r	*F. oxysporum*	TEF1a	PV523712
	F22-35r	*F. oxysporum*	TEF1a	PV523713
	F23-7r	*F. oxysporum*	TEF1a	PV523714
	F23-11r	*F. oxysporum*	TEF1a	PV523715
	F23-11r2	*F. oxysporum*	TEF1a	PV523716
	F23-27r	*F. oxysporum*	TEF1a	PV523717
Addressing Sequence Inconsistencies	F19	*F. commune*	TEF1a	PV523718
	F19	*F. commune*	BT	PV449160
	F42	*F. secorum*	TEF	PV523719
	F46	*F. commune*	TEF1a	PV523720
	F49	*F. commune*	TEF1a	PV523721
	Fob216b	*F. oxysporum*	TEF1a	PV523722
	Fob220a	*F. commune*	TEF1a	PV523723
	Fob220a	*F. commune*	BT	PV449161
	Fob257c	*F. oxysporum*	TEF1a	PV523724
	Fob266a	*F. secorum*	TEF1a	PV523725
	H7	*F. commune*	TEF1a	PV523726

Supplementary Figure:

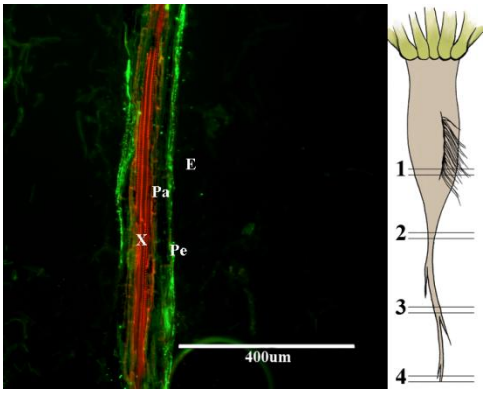

Figure 10: Longitudinal view of sugar beet root tip (location 4) sampled prior to inoculation with sterile distilled water, 0 days post inoculation. Taken with Evos - un-colonized root interior parenchyma and xylem and the periderm colonized with coenocytic hyphae. Abbreviations used: E for exterior space, Pa for parenchyma tissue, Pe for periderm, and X for xylem.

Media Recipes:

Unless otherwise stated, all media was autoclaved for 45 minutes on a liquid cycle.

Carnation Leaf Agar (CLA)

To make in 500ml distilled water: combine water with 10g bacteriological agar, autoclave and pour agar in 35x15mm petri dishes; once solidified, place 3 to 4 chopped and irradiated carnation leaves on the surface of agar.

Fisher N. L., Burgess L. W., Toussoun T. A., and Nelson P. E. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology. 72:151-153.

Leslie J. F. and Summerell B. A. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Ames, Iowa. Pg. 6.

Snyder W. C. and Hansen H. N. 1947. Advantages of natural media and environments in the culture of fungi. Phytopathology. 37:420-421.

Clarified Half-Strength V8 Agar (V8A)

To make in 500ml distilled water: combine 450ml water, 50ml of clarified V8, and 10g bacteriological agar, autoclave, and pour agar in 100x15mm petri dishes.

Clarification Protocol: per 100ml of V8, incorporate 1g of calcium carbonate and centrifuge at 6750g for 10 minutes. Filter the clarified V8 and calcium carbonate through cheese cloth and store in -20C freezer until used. Discard the pellet of V8 pulp left over from centrifugation.

Miller, P. M. 1955. V-8 juice agar as a general purpose medium for fungi and bacteria. Phytopathology. 45:461-462.

Spezieller Nährstoffarmer Agar (SNA)

To make in 500ml distilled water: Combine water with listed ingredients, autoclave, and pour agar in 35x15mm petri dishes; once solidified, place 3 to 4 pieces of sterile filter paper on the surface.

KH_2PO_4	0.5g
KNO_3	0.5g
$MgSO_4 \cdot 7H_2O$	0.25g
KCl	0.25g
Glucose	0.1g
Sucrose	0.1g
Agar	10g

Nirenberg H. I. 1976. Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion *Liseola*. Mitteilungen aus der Biologischen Bundesanstalt Für Land- und Fortstwirtschaft (Berlin-Dahlem). 169:1-117.

Leslie J. F. and Summerell B. A. 2006. The *Fusarium* Laboratory Manual. First ed. Blackwell Publishing, Ames, Iowa. Pp. 6-7.