EXAMINATION OF SOILBORNE PATHOGENS OF CELERY IN MICHIGAN

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ABSTRACT

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Michigan celery field and greenhouse yield and quality is negatively impacted by soilborne pathogens. Celery growers have observed a crown rot, wilt, and vascular discoloration called “meltdown”. Symptomatic celery plants were collected from seven grower commercial grower fields across four counties and two years. Isolations showed the most common organisms observed were *Fusarium solani* species complex (52%) and *Fusarium oxysporum* species complex (45%) as identified by sequencing of the translation elongation factor-1α. Isolates identified as *Fusarium oxysporum* f. sp. *apii* race 2 (*Foa* race 2), the cause of Fusarium yellows, using diagnostic primers caused disease in a growth chamber pathogenicity assay. *Foa* race 2 isolates are capable of causing disease symptoms but are infrequent so “meltdown” may have additional cause(s). Pythium root rot decreases quality of greenhouse grown celery transplants. *Pythium* spp. isolated from symptomatic celery seedlings were characterized by virulence and fungicides tested for control of Pythium root rot. The most frequent *Pythium* species collected from Michigan celery were *P. mastophorum* (55%), *P. intermedium* (16%), *P. sulcatum* (10%), *P. aff. diclinum* (6%), and *P. sylvaticum* (5%). *P. sulcatum* and *P. sylvaticum* caused disease symptoms on celery seedlings in a greenhouse virulence assay. Phosphorous acid salts, ethaboxam, and mefenoxam controlled Pythium root rot in a greenhouse setting. Results indicate which *Pythium* spp. are of most concern to celery growers and identify effective chemical controls. Understanding presence and virulence of soilborne plant pathogens in Michigan celery production will be used to develop strategies to limit disease

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INTRODUCTION

Celery production. Michigan produces diverse crops including celery. In 2018, 2000 acres of celery were planted in Michigan and the celery produced was valued at $19.5 million (USDA 2021). Michigan was second in the United States in celery acreage following California, making up 4.9% of production (USDA 2021). Most celery in Michigan is grown in the southwest region of the state along Lake Michigan (Newaygo, Oceana, Muskegon, Ottawa, Kent, Allegan, and Van Buren counties) and there is scattered production elsewhere in the state (Hausbeck 2011) .

Celery production has a long history in Michigan. Celery was first grown in the United States in Kalamazoo, Michigan as early as 1856 (Lucas and Wittwer 1952). Muck soil and comparatively cooler summers allowed growers to compete in the vegetable market (Halligan 1913). In early celery production, a blanched crop with white to yellow stalks was marketable (Halligan 1913; Lucas and Wittwer 1952; Zandstra et al. 1986). The process of blanching works by blocking light from the celery stalks using wooden boards or by hilling soil on the side of the plants, leaving the stalks with a light color (Halligan 1913). By the 1950s, the practice of blanching became less common because of a decreased market for blanched celery (Lucas and Wittwer 1952) and a labor shortage due to World War II (Hudgins 1994; Toth 1989). Green cultivars were also preferred because of their resistance to Fusarium yellows (Toth 1989). Disease pressure, including from Fusarium yellows caused by either *Fusarium oxysporum* f. sp. *apii* race 1 or race 2, has influenced cultivars produced in Michigan (Hudgins 1994; Zandstra et al. 1986).

Celery transplants are produced in the greenhouse using plug trays and peat-based media to ensure a uniform stand and allow for a faster maturing crop (Hausbeck 2011; Zandstra et al.

1986). Celery is grown in muck soils rich in organic matter and requires high inputs of fertility and moisture (Hausbeck 2011; Phillips et al. 2020). Overhead sprinkler or drip irrigation is used to apply water and fertilizer frequently as celery has a shallow root system (Hausbeck 2011). Transplanting begins in April and ends in late July with planting scheduled so that celery is harvested each week since there is a narrow window for harvesting (6-8 days) before the crop will lose marketability (Hausbeck 2011; Phillips et al. 2020). Fields are successively replanted and harvest continues until mid-October (Hausbeck 2011; Phillips et al. 2020). Celery in Michigan is mechanically harvested. Fresh market celery is trimmed, sized, washed, and packed into 50- pound cartons at on-farm packing sheds (Hausbeck 2011). The majority of Michigan celery is packed for fresh market (60%) and the remaining portion (40%) is processed in frozen foods, soups, juice blends, and other products (Hausbeck 2011; Zandstra et al. 1986). Celery is often rotated with onions and corn to avoid build-up of pests and pathogens in the soil (Hausbeck 2011; Phillips et al. 2020; Zandstra et al. 1986).

Pests of celery. The most important insect pests of celery in Michigan include aphids (*Aphis fabae, Myzus persicae, Aphis helianthil*; Hemiptera Aphididae), aster leafhopper (*Macrosteles quadrilineatus*; Hemiptera: Cicadellidae), the tarnished plant bug (*Lygus lineolaris*; Hemiptera: Miridae), and cutworms (Lepidoptera: Noctuidae). Minor pests include carrot weevil larvae (*Listronotus oregonensis*; Coleoptera: Curulinonidae), loopers (Lepidoptera: Noctuidae) and spider mites (Arachnida: Acari: Tetranychidae) (Colquhoun et al. 2022; Hausbeck 2011; Ingwell and Szendrei 2020).

Plant parasitic nematodes also negatively impact celery production. Stunting, wilting, yellowing, and poor stand development in patches are symptoms of nematode damage (Hausbeck 2011; Sherf and Macnab 1986). Nematodes of concern in Michigan production are

northern root-knot nematode (*Meloidogyne hapla*) and pin nematode (*Paratylenchus hamatus*) (Hausbeck 2011). Root-knot nematode causes severe yellowing, stunting and formation of root galls in ball-like masses. Affected roots are short with few lateral roots and secondary infections by pathogens such as *Pythium* spp. may occur (Roberts and Mullens 2002; Sherf and Macnab 1986). A unique symptom caused by pin nematode is the development of a witches broom (roots have brush like development and many roots arising at or close to the same point) (Hausbeck 2011; Sherf and Macnab 1986). Control of plant parasitic nematodes is achieved through crop rotation and soil fumigation (Egel 2020; Sherf and Macnab 1986).

Diseases of celery. Diseases caused by fungi, bacteria, and viruses decrease celery crop quality and yield. Foliar leaf blights include early blight (*Cercospora apii*) (Berger 1973), late blight (*Septoria apiicola*) (Sheridan 1968), and bacterial leaf blight (*Pseudomonas* spp.) (Lacy et al. 1996a). A diagnostic characteristic of *S. apiicola* infection is production of pycnidia which appear in lesions as black dots just visible to the naked eye (Hausbeck 2002). Bacterial blight lesions are water-soaked and greasy with a yellow halo (Sherf and Macnab 1986). Bacterial streaming in water can be observed on the margin of lesions under a microscope (Little et al. 2002).

One of the most impactful soilborne diseases of celery, Fusarium yellows (*Fusarium oxysporum* f. sp. *apii*), causes significant losses where resistant varieties are not available (Epstein et al. 2017; Hausbeck 2011; Lacy et al. 1996a). Symptoms include stunting, wilting, chlorotic foliage, and red-brown discoloration of roots and vascular tissue (Lacy et al. 1996a; Sherf and Macnab 1986; Subbarao and Elmer 2002) Pythium root rot (*Pythium* spp.) primarily impacts greenhouse transplant production causing root rot and stunting (Hausbeck 2011; Hershman et al. 1986; Koike et al. 2007). Soilborne diseases that are an occasional problem

depending on season and location in Michigan (Hausbeck 2011) are soft rot (*Pseudomonas marginalis* and *Pectobacterium carotovorum* subsp. *carotovorum*) (Robbs et al. 1996) and crater rot (*Rhizoctonia solani*) (Pieczarka 1981). Soft rot is identified by water-soaked lesions at the petiole base that expand and become soft, mushy, and sunken with a distinct edge (Farrar 2002; Sherf and Macnab 1986). Symptoms of crater rot are pale, brown, water-soaked lesions with a white or gray mat of mycelium on the lower parts of the petiole (Koike et al. 2007; Sherf and Macnab 1986). These diseases are controlled using a combination of cultural practices, chemical controls, and genetic resistance.

FUSARIUM YELLOWS

Causal agent and symptoms. Fusarium yellows, caused by *F. oxysporum* f. sp. *apii* (*Foa*), is one of the most important soilborne diseases of celery where resistant varieties are not available. The disease occurs in the United States across production regions (Awuah et al. 1986; Otto et al. 1976; Toth and Lacy 1991b), Canada (Cerkauskas and Chiba 1991; Cerkauskas and McDonald 1989) and Argentina (Lori et al. 2016; Lori et al. 2008).

The monocyclic disease cycle of Fusarium yellows begins with germination of chlamydospores, the survival structure of *F. oxysporum*, in response to root exudates (Subbarao and Elmer 2002). Germinating chlamydospores, microconidia or macroconidia germinate and infect celery plants through the root tips (Beckman 1987; Gordon 2017; Hart and Endo 1981). Fungal mycelium grows through the xylem tissue (Beckman 1987; Gordon 2017) leading to plant symptoms of stunting, chlorosis, and wilting, and red-brown discoloration of the vascular tissue (Lacy et al. 1996a; Sherf and Macnab 1986; Subbarao and Elmer 2002). As disease progresses, the crown breaks down, becoming watery and soft as it is colonized by secondary organisms (Koike et al. 2007). Symptom development takes 30-40 days after transplanting

seedlings into infested soil (Henry et al. 2020; Subbarao and Elmer 2002). *Foa* can persist almost indefinitely in the soil as chlamydospores, plant debris, and through living as a endophyte on weeds and other crops (Elmer et al. 1986; Subbarao and Elmer 2002; Toth 1989). In severely affected fields with susceptible cultivars, losses can be substantial, and the crop may not be harvestable (Lacy et al. 1996a; Sherf and Macnab 1986). *F. oxysporum* f. sp. *apii* can be isolated from symptomatic vascular tissue (Koike et al. 2007; Otto et al. 1976).

Foa is classified into four races based on pathogenicity by cultivar. Fusarium yellows caused by *Foa* was first described in 1914 in Kalamazoo, Michigan on the self-blanching cultivar Golden Self-Blanching as *Foa* race 1 (Coons 1915; Nelson et al. 1937). Fusarium yellows reemerged caused by *Foa* race 2, emerged in California, then in Michigan in 1982 (Elmer and Lacy 1982; Otto et al. 1976). *Foa* race 2 is virulent on both older self-blanching cultivars and modern green cultivars (Elmer et al. 1986; Hart and Endo 1978). A resistance gene, *Fu1*, was introduced to celery cultivars from celeriac, controlling Fusarium yellows caused by *Foa* race 2 (Orton et al. 1984; Quiros 1993). *Foa* race 2 resistant cultivars were developed in Michigan using somaclonal variation (Lacy et al. 1996b; Toth and Lacy 1991b) and derived from cultivar Tall Utah 52-70 HK, which is moderately resistant to *Foa* race 2 (Elmer et al. 1986). *Foa* race 3 was reported in California and differentiated from *Foa* race 2 as virulent on Tall Utah cultivars (green cultivars) but avirulent on Golden Self Blanching (Puhalla 1984). Disease caused by *Foa* remerged in California celery production caused by *Foa* race 4, which is highly virulent on cultivars resistant to *Foa* race 2 (Epstein et al. 2017).

Foa race 4, which has only been observed in California celery production, causes more severe disease symptoms called Fusarium wilt of celery (Kaur et al. 2022). *Foa* race 4 causes severe stunting, wilting, occasional leaf chlorosis, and young plant death (Henry et al. 2020;

Kaur et al. 2022). An additional symptom of Fusarium wilt of celery is water-soaked lesions around the vasculature in the crown and larger roots. This vascular discoloration may extend from the roots and crown to the petioles (Kaur et al. 2022). In infection with *Foa* race 2 vascular discoloration is often limited to the roots and crown and chlorosis is a common symptom (Kaur et al. 2022; Subbarao and Elmer 2002). Disease caused by *F. oxysporum* f. sp. *apii* decreases celery yield and quality where susceptible cultivars are grown.

Biology and epidemiology of *Fusarium oxysporum* species complex (FOSC). The genus *Fusarium* is as an ascomycete (Sordariomycetes: Hypocreales: Nectriaceae) (Ma et al. 2013; Michielse and Rep 2009) and recognized by its teleomorph names *Nectria* and *Gibberella* (Michielse and Rep 2009). *Fusarium* was first described in 1809 by H.F. Link as described in Snyder and Hanson (1940) and is easily recognized by its canoe or banana shaped macroconidia (Snyder and Hansen 1940). *Fusarium* spp. can act as plant pathogens causing wilts, blights, rots and cankers and producing mycotoxins that contaminate agricultural products (Ma et al. 2013).

The species *Fusarium oxysporum* was described by Snyder and Hanson (Snyder and Hansen 1940). *Fusarium oxysporum* is called a species complex because it is made up of diverse clonal lineages and there is a lack of boundaries in genetic exchange between strains (O'Donnell et al. 1998; Michielse and Rep 2009). *F. oxysporum* species complex (FOSC) can infect both monocots and dicots (Agrios 2005) and well as cause opportunistic infections in humans and animals (O'Donnell et al. 2004; Ortoneda et al. 2004). FOSC is found worldwide (Parkinson 1981) in both cultivated (Gordon and Martyn 1997) and non-agricultural soils (Burgess et al. 1989; Gordon et al. 1992; Windels and Kommedahl 1974).

FOSC does not have a known sexual cycle and is characterized by its asexual spores: macroconidia, microconidia, and chlamydospores (Gordon and Martyn 1997; Nelson et al.

1983). Characteristics that help in identification of *Fusarium* species, generally, and FOSC, in particular, are the shape of the macroconidia, structure of the conidiophore, and formation of chlamydospores (Nelson et al. 1983). Macro and microconidia are produced throughout the life stages of *F. oxysporum* and both have the ability to infect the host (Nelson et al. 1994).

FOSC is a soilborne pathogen containing more than 100 host specific strains called *formae speciales* which cause wilt diseases on crops including tomato, cotton, and banana (Gordon 2017; Michielse and Rep 2009). The symptoms of these diseases generally include vascular browning, stunting, and progressive wilting (Agrios 2005). Several *formae speciales* of FOSC are transmitted through seed including asparagus, watermelon and basil (Elmer et al. 1994; Martyn and McLaughlin 1983; Stephens and Elmer 1988). *Foa* is not known to be transmitted by seed. However, the simultaneous appearance of *Foa* race 2 in Michigan and New York in the 1980s and the emergence of the pathogen in Argentina (Lori et al. 2016; Lori et al. 2008) indicates that a very low incidence on the seed may have introduced the pathogen to new areas (Elmer 1985; Elmer 2001). *Foa* is primarily spread primarily through celery transplants grown in seedbeds infested with the pathogen and secondary spread is by contaminated field equipment (Awuah et al. 1986; Sherf and Macnab 1986). The pathogen persists between celery crops as chlamydospores, as a saprophyte or endophyte on crops debris, or on weed hosts (Elmer et al. 1986; Toth 1989).

Biology and epidemiology of *Fusarium solani* species complex (FSSC). The *Fusarium solani* species complex (FSSC) is a diverse group including at least 26 phylogenetically distinct species (Aoki et al. 2014; O'Donnell 2000; Schroeder et al. 2013; Šišić et al. 2018) and seven biological species or mating populations (Mauto and Snyder 1972; O'Donnell 2000; Šišić et al.

2018). Sexual reproduction is observed in FSSC and its teleomorph designation of *Nectria haematococca* (O'Donnell 2000; Zhang et al. 2006).

FSSC live in agricultural or non-agricultural soils (although more propagules are reported in cultivated soil) as well as saprophytically or parasitically in plant hosts (Coleman et al. 2009; Hall and Phillips 1992; Mandell 1996; Zhang et al. 2006). FSSC causes vascular wilts and root rots in over 100 crops (Coleman et al. 2009). Individual species or mating populations within FSCC are often associated with only one or a few plant hosts and are divided into *formae speciales* and races (Bueno et al. 2014; Chung et al. 2011; Mauto and Snyder 1972; O'Donnell 2000; Šišić et al. 2018). FSSC is reported to cause disease on Apiaceae crops including dry rot on carrots (Davis 2002; Zhang et al. 2014) and root rot on coriander/cilantro (Bhaliya 2014; Estévez De Jensen 2009; Satyanarayana et al. 2021). FSSC has not been reported to cause disease on celery (Davis and Raid 2002; Koike et al. 2007; Sherf and Macnab 1986).

FSSC is a soilborne pathogen transmitted through infested soil, plant material, or through seed for some *formae speciales* (Martyn 2017; Mehl and Epstein 2007; Nelson 2015). FSSC survives as chlamydospores in the soil for several years (Hall and Phillips 1992; Martyn 2017; Nash et al. 1961). Although FSSC has not been reported to cause disease on celery, this species complex causes root rot diseases and inhabits a similar ecological niche to FOSC.

Classifying FOSC: Species concept, *formae speciales* and race. FOSC is classified by *formae speciales* (f. sp.), race, and vegetative compatibility group (VCG). *Formae speciales* and race are based on pathogenicity on a plant species or cultivar, respectively (Correll 1991; Puhalla 1985). Classification by *formae speciales* and race is important because both pathogenic and non-pathogenic strain can be isolated from infected plant tissue (Gordon et al. 1989; Schneider 1984). *Formae speciales* cannot be distinguished morphologically (Baayen et al.

2000; Leslie and Summerell 2006). VCGs are identified based on the ability of the strains to exchange genetic materials through anastomoses (Gordon and Martyn 1997; Puhalla 1984).

Fusarium oxysporum f. sp. *apii* (*Foa*) causes disease on celery. *Foa* is a polyphyletic group made up of four clades (Epstein et al. 2017). Clade 3 contains *Foa* race 2 while clade 2 contains *Foa* races 1, 3, and 4 (Epstein et al. 2017). *Foa* race 2 is a clonal group that corresponds to one VCG while Foa race 1 is made up of multiple VCGs and genetically distinct from *Foa* race 2 (Correll et al. 1986; Epstein et al. 2017; Toth and Lacy 1991a). *Foa* races 3 and 4 are closely related (Henry et al. 2020). *Foa* race 2 has been most recently reported in Michigan celery production (Correll et al. 1986; Toth and Lacy 1991a) and race 4 recently emerged in California celery production (Epstein et al. 2017). Classification of FOSC and *Foa* allow for better understanding of the diseases they cause on celery.

Host-pathogen interactions in diseases caused by FOSC. Most *Fusaria* are classified as hemibiotrophs as their infection initially relies on live host tissue, but eventually transitions to killing and consuming host cells (Ma et al. 2013). FOSC initially penetrates roots asymptomatically, then triggers wilting, necrosis, and chlorosis of aerial plants parts (Hart and Endo 1981; Husaini et al. 2018; Ma et al. 2013). The pathogen invades, trying to evade recognition by the host, while the plant recognizes the pathogen and initiates defenses.

As the infection process begins, the host recognizes the pathogen and initiates defenses. PAMP (pathogen associated molecular pattern) recognition by the host triggers production of reactive oxygen species, chitinases, and glucanases (Husaini et al. 2018) and signaling pathways that trigger broad spectrum immunity (Muthamilarasan and Prasad 2013). In response to infection, the host plant starts the synthesis and accumulation of secondary metabolites (phytoalexins) within 24 hours of infection (Beckman 1987), in turn these act as a signal to the

fungus triggering synthesis of enzymes such as cutinases and pectinases by the fungus that are required for host penetration (Woloshuk and Kolattukundy 1986). The hypersensitive (HR) response in the plant host is characterized by the accumulation of reactive oxygen species (ROS) and allows the host to use localized cell death to contain spread of the pathogen. ROS are induced in response to pathogen elicitors (Davies et al. 2006; Husaini et al. 2018).

The plant responds to infection by slowing movement of the pathogen through the vascular tissue. The plant cell wall is the first line of defense. In resistant banana varieties a strengthened cell wall contributes to resistance (Husaini et al. 2018; Li et al. 2012). Once the pathogen enters the host and begins to colonize the vascular tissue, how the plant responds determines if the interaction will be susceptible or resistant (Beckman 1987). Localization of the pathogen in the vascular tissue contributes to a resistant interaction and this is visible using electron light microscopy (Beckman 1987; Beckman et al. 1991; Beckman et al. 1989). In infection of celery and celeriac with *Foa* race 2 indicated that occlusion of xylem vessel pores on the incompatible host prevents spread of the pathogen through the plant (Jordon et al. 1989). Callose is produced by the host plant and lipodial material forms deposits and globular occlusions that may block further infection by the pathogen in infection of tobacco and tomato (Beckman 1987; Gao et al. 1995; Mueller and Morgham 1996).

In some host-pathogen interactions including tomato, a dominant resistance (R) gene enables host recognition of the pathogen (Huang and Lindhout 1997). Resistance genes are involved in biosynthesis of jasmonic acid, indole glucosinolate, camalexin, and callose (Bednarek et al. 2009; Kidd et al. 2009; Liu et al. 2010; Manzo et al. 2016; Pfalz et al. 2009; Zhu et al. 2012) In the host, R gene product recognizes the product of a corresponding avirulence

genes (Avr) in the pathogen, leading to defense expression and resistance in a host gene to pathogen gene relationship (Dean et al. 2012).

On the other side of the host-pathogen interaction, pathogen recognizes the host. Once the pathogens initiate infection, multiple signaling pathways are activated including mitogen activated protein (MAP), kinase signaling pathways, G-protein signaling pathways, and cAMP pathways (Husaini et al. 2018). The MAP kinase module processes signals in the cell and transmits the signal through the cell by phosphorylating various proteins including gene regulatory proteins (Widmann et al. 1999) and is required for full virulence (Husaini et al. 2018).

The FOSC genome is made up of the core genome and the adaptive or accessory genome that is responsible for pathogen virulence and located on pathogenicity chromosomes (Croll and McDonald 2012; Ma et al. 2013). The SIX (secreted in xylem) genes found in *F. oxysporum* f. sp. *lycopersici* are an example of pathogenicity genes (Michielse and Rep 2009; Pietro et al. 2003). The SIX genes encode at least 14 small proteins, also called effectors, secreted in the xylem system during infection (Houterman et al. 2009; Michielse and Rep 2009; Schmidt et al. 2013). Among the Six effectors, at least 3 are required for xylem colonization and are present in all pathogens causing tomato wilt but absent in avirulent strains (de Sain and Rep 2015; Houterman et al. 2009; Ma et al. 2014; Michielse et al. 2009; Rep et al. 2004). Six proteins are recognized by resistance proteins of tomato, making them are Avr (avirulence) proteins (Houterman et al. 2009; Ma et al. 2014; Rep et al. 2004). Henry et al. (2020) determined that FOSC clade 3 (*Foa* race 2) isolates have two SIX1 homologs. SIX1 serves as an avirulence gene eliciting a host response in tomatoes with the I-3 gene (Rep et al. 2004). Characteristics of the SIX gene regions suggest a distinct evolutionary origin from the rest of the genome, indicating horizontal gene transfer (Ma et al. 2010). SIX genes are also involved in defining variation in

host specificity among other *F. oxysporum formae speciales* including differentiating between cotton-specific *F. oxysporum* f. sp. *vasinfectum* isolates in Australia (Chakrabarti et al. 2011) and between races of *F. oxysporum* f. sp. *cubense* based on the presence and copy number of SIX1 (An et al. 2019; Fraser-Smith et al. 2014; Guo et al. 2014).

Foa race 4 putative effectors and pathogenicity factors were identified by comparing gene expression *in planta* versus *in vitro* (Henry et al. 2020). Host infection and virulence in *F. oxysporum* infection in a polygenic trait whose expression is controlled by regulation of many genes and transcriptional factors regulating their expression (Husaini et al. 2018). Both pattern triggered immunity (PTI), the recognition of PAMPS by the host, and effector triggered immunity (ETI) play a role in resistance. Additionally, effectors such as Six proteins produced by the pathogen play a role in pathogenicity (Henry et al. 2020; Michielse and Rep 2009).

The infection process is made up of the "arms race" between host and pathogen. For example, in celery *Fu1* gene gives resistance to *Foa* race 2 (Orton et al. 1984; Quiros 1993). But, with the recent emergence of *Foa* race 4 in California (Epstein et al. 2017), the pathogen has overcome this resistance.

Optimal conditions for growth of *Foa* and disease development of Fusarium yellows/Fusarium wilt. The optimal temperature for *Fusarium* spp. growth is between 25°C (Nelson et al. 1990) and 28°C (Fravel et al. 1995). As temperature increases, Fusarium wilt disease severity increases in celery (Kaur et al. 2022), cucurbits (Bosland et al. 1988), lettuce (Scott et al. 2010), and chickpeas (Navas-Cortés et al. 2007). Disease was greatest at 28° C in cucurbits and 26-28°C in chickpeas (Bosland et al. 1988; Navas-Cortés et al. 2007).

The optimal growth temperature for *Foa* (presumably *Foa* race 1) as is 28°C as observed by Ryker in 1935 as reported in Correll et al. (1986). *Foa* race 2 and race 4 grow most quickly at

25°C and 28°C respectively (Kaur et al. 2022). The most chlamydospores are produced by *Foa* race 2 at 24-27° C and pH 7.1 (Opgenorth and Endo 1985).

Fusarium yellows is generally favored by warmer temperatures (Koike et al. 2007). As shown in a growth chamber assay, a temperature increase from 16°C to 26°C caused an increase in vascular discoloration in plants inoculated with *Foa* race 2 or race 4 (Kaur et al. 2022).

Management of diseases causing by *Foa* and FOSC. The most important cultural control method for *Foa* is preventing introduction into a field. Eliminating *Foa* from a field is challenging since the chlamydospores survive for several years and *Foa* can survive on weed hosts or crops used in rotation with celery (Elmer 1987; Toth 1989). Using clean transplants, cleaning machinery between areas where *Foa* is present, and avoiding planting into fields with a history of Fusarium yellows prevent infestation of new fields (Koike et al. 2007; Sherf and Macnab 1986). Although adding mustard to soil in greenhouses combined with solarization reduced disease caused by FOSC (Gamliel and Katan 2009), adding mustard amendments as tested in a Michigan muck research plot did not decrease Fusarium yellows (Hudgins 1994; Toth 1989).

Effective use of fungicides has not been reported for either *Foa* race 1 or *Foa* race 2 (Awuah et al. 1986; Otto et al. 1976). Chemical controls for *Apiaceae* diseases caused by *Fusarium* spp. are limited. To control root rot and damping off of coriander/cilantro caused by *Fusarium* spp. fludioxonil or biocontrol products reduced disease (Koike and Gordon 2005; Pscheidt and Ocamb 2021). *Fusarium* spp. cause root rot of carrot (Stanković et al. 2015; Zhang et al. 2014) and application of metam-sodium through irrigation reduced fungal propagules (Roberts et al. 1988). Chemical controls are not effective for root rot of parsley caused by *Fusarium* spp. (Minchinton et al. 2006). In all of these crops, cultural control and genetic control

of diseases caused by *Fusarium* spp. is more effective than chemical control (Davis and Raid 2002; Sherf and Macnab 1986).

There is potential for development of biological controls for *Foa*. *Trichoderma harzianum* combined with other control methods reduced disease caused by *F. oxysporum* and enhanced plant defenses in tomato and cucumber (Chen et al. 2019; Sivan and Chet 1993). In areas of heavy celery production in California, FOSC suppressive soils were observed (Schneider 1984) and antagonistic *Fusarium* strains may form suppressive soils against pathogenic *Fusarium* strains by competing for nutrients in the soil and infection sites on the root (Larkin and Fravel 2002). Control of pathogenic *Fusarium* strains by non-pathogenic strains was observed in Fusarium tomato wilt and Fusarium wilt on basil (Larkin and Fravel 1998; Minuto et al. 1997). Biological controls play a minor role in controlling diseases caused by *Fusarium* spp.

Development of resistant cultivars is the most effective control of Fusarium yellows (Elmer et al. 1986; Epstein et al. 2017). Green cultivars including Tall Utah 52-70 and Florida 683 controlled *Foa* race 1 until the emergence of *Foa* race 2 (Elmer et al. 1986; Zandstra et al. 1986). *Foa* race 2 resistant cultivars used in Michigan include Tall Utah 52-70 HK and Deacon (Zandstra et al. 1986). Additionally, breeding efforts using somaclonal variation are a rapid method to produce cultivars that can be quickly screened for resistance (Toth and Lacy 1991). Cultivars resistant to *Foa* race 2 developed through somaclonal variation include MSU-SHK5 and UC-T3 (Lacy et al. 1996; Heath-Pagliuso and Rappaport 1990). Commercial varieties grown in Michigan are maintained by a small breeding effort supported directly by the largest celery growers in Michigan (Phillips et al. 2020). Cultivars currently grown in Michigan with resistance to *Foa* race 2 include Dutchess, Green Bay, CR-1, and WA-7 (Hausbeck 2011). Breeding efforts identified the major resistance gene *Fu1* that gives resistance to *Foa* race 2 and it was introduced

into commercial cultivars including Challenger, Stix, Sabroso, and Command (Epstein et al. 2017; Orton et al. 1984). Use of resistant cultivars is the best control for Fusarium yellows.

PYTHIUM ROOT ROT AND DAMPING OFF

Causal agent and symptoms. Pythium root rot and damping off (caused by *Pythium* spp.) primarily impacts celery production in the greenhouse but root damage caused by *Pythium* infection of seedlings can lead to stunting and poor growth in the field (Hershman et al. 1986; Koike et al. 2007; Sherf and Macnab 1986). Chlorotic foliage, wilting, and stunted growth are symptoms of Pythium root rot. Below ground, infected plants have reduced feeder roots with water-soaked red-brown discoloration (Koike et al. 2007; Nunez and Westphal 2002). *Pythium* spp. have a wide host range cause damping off and root rot in many greenhouse grown crops (Daughtrey and Benson 2005; Martin and Loper 1999). Pythium root rot and damping off are ubiquitous in Apiaceae species across production regions (Hershman et al. 1986; Koike et al. 2007; McCracken 1984; Nunez and Westphal 2002).

Biology and epidemiology of *Pythium* spp.. The genus *Pythium* contains about 120 species that are common soil inhabitants (Koike et al. 2007; Nunez and Westphal 2002). *Pythium* spp. include plant pathogens, saprophytes (Van der Plaats-Niterink 1981), parasites of insects (Saunders et al. 1988), mammals (Decock et al. 1987) algae and fish (Martin and Loper 1999; Van der Plaats-Niterink 1981). *Pythium* spp. grow vegetatively by aseptate, colorless hyphae, producing oospores and sporangia or hyphal swellings (Nunez and Westphal 2002).

The disease cycle of Pythium root rot or damping off starts with the germination of a *Pythium* oospore, which are sexually produced, thick, walled, and resistant to desiccation (Martin and Loper 1999). The oospore becomes thin walled and germinates to generate a germ tube which directly infects seeds or seedlings, or forms sporangia (Agrios 2005; Nunez and Westphal

2002). Zoospores then develop within the sporangia, and when released move through free-water using their flagella and encyst in host tissue (Agrios 2005; Nunez and Westphal 2002). If the conditions are not suitable for infection, the zoospores can also encyst in the soil and remain as long as moisture and temperature conditions in the soil are favorable (Martin and Loper 1999). After infection, intracellular mycelium develops in the roots and leading to disease symptoms of seed or root rot, wilting, stunting and chlorosis (Nunez and Westphal 2002). The pathogen persists primarily as oospores in soil, plant material, or on surfaces such as pots or benches in a greenhouse (Koike et al. 2007; Nunez and Westphal 2002).

Oospores are the primary survival structure for *Pythium* (Martin and Loper 1999). Air dried oospores of *P. ultimum* survived after 8 months of storage (Lumsden et al. 1975). Since *Pythium* spp. act as saprophytes by colonizing organic material in the soil, and can persist through oospores, they can persist as long as there is organic material to be colonized and conditions are favorable for oospore survival (Nunez and Westphal 2002). *Pythium* spp. compete with each other and other soil microbes to colonize substrates. For example, *P. ultimum* will not colonize organic matter when it has already been colonized by other saprophytes (Hancock 1977; Martin and Hancock 1986; Rush et al. 1986). However, *P. nuun* can colonize substrate that was previously colonized by *P. ultimum*, which appears to be an exception to this characteristic of *Pythium* spp.(Paulitz and Baker 1988).

Mechanism of infection by *Pythium* spp.. Infection by *Pythium* spp. generally occurs in seeds and seedlings although older plants can be affected (Martin and Loper 1999; Moorman et al. 2002). Germination of oospores and development of sporangia and zoospores is enhanced by germinating seed and developing root exudates, making these plant parts vulnerable to infection (Deacon and Donaldson 1993; Martin and Loper 1999). Germ tubes produced from oospores,

sporangia, or encysted zoospores have a chemotactic response to these exudates, attracting them to roots and germinating seeds (Lifshitz et al. 1986; Nelson 1987, 1990; Paulitz 1991). Free water helps zoospores move easily and rapidly and allows exudates to diffuse further from the root or seed in the soil, attracting zoospores (Martin and Loper 1999). Exudate compounds that regulate the attraction of zoospores to plants include L-glutamine, glucose, and certain amino acids (Deacon and Donaldson 1993). The amino acids and carbohydrates in root exudates from a specific plant may contribute to the host range and host specificity of *Pythium* spp. (Deacon and Donaldson 1993; Tripathi and Grover 1978).

Infection of a susceptible host by *Pythium* spp. occurs very rapidly. Sporangia of *P. ultimum* began to germinate within 1 hour after exposure to exudates from sugar beet seeds and infection occurred 14 hours after planting (Osburn and Schroth 1989). Rapid infection of germinating seeds has also been observed in other crops such as cotton (Nelson 1988, 1990), soybean (Schlub and Schmitthenner 1978), and pea (Lifshitz et al. 1986).

Soil pH influences growth of *Pythium* spp.. Soil pH mediates the availability of many soil minerals impacting the ability of *Pythium* to grow saprophytically. Several studies indicate that a lower pH (pH 5.0 compared to pH 7.3) increased saprophytic activity of *P. ultimum* and *P. nuun* (Lifshitz et al. 1984; Paulitz and Baker 1987). Plant age, soil moisture, and soil pH all impact the infection process of *Pythium* spp.

Pythium spp. vary in their ideal growth temperature (Martin and Loper 1999). *P. sulcatum* and *P. sylvaticum* have ideal growth temperatures of 28-30° and 25°C respectively (Pratt and Mitchell 1973; Serrano and Robertson 2018; Van der Plaats-Niterink 1981). Oospores are produced at the greatest rate by *P. intermedium* and by *P. sylvaticum* at 15°C (Hsu and Hendrix 1972). An ideal growth temperature for *P. mastophorum* has not been reported.

Identification and characterization of *Pythium* spp.. Morphological characteristics (oogonia, sporangia) and/or sequencing of the internal transcribed spacer (ITS) gene are used for identification of *Pythium* species (Del Castillo Munera et al. 2019; Robideau et al. 2011; Van der Plaats-Niterink 1981).

Multiple *Pythium* spp. can be isolated from the same environment, and they may vary in pathogenicity and virulence to a specific host (Del Castillo Munera and Hausbeck 2016; Stephens and Powell 1982). Virulence assays characterize *Pythium* spp. In-vitro assay are used to test virulence of *Pythium* isolates on seeds (Broders et al. 2007; Del Castillo Munera and Hausbeck 2016) and in-planta assays are used to test for virulence on seedlings (Broders et al. 2007; Kirkpatrick et al. 2006; Quesada-Ocampo et al. 2009).

Control of damping off and Pythium root rot. Sanitation and cultural controls prevent and limit Pythium root rot and damping off. Wet soil conditions favor pathogen growth and increases zoospore movement and contact with roots (Koike et al. 2007; Martin 2002). Sanitation practices include cleaning trays and bench tops, and using pathogen-free rooting media and irrigation water (Nunez and Westphal 2002; Egel 2020).

Preventive fungicide application is part of Pythium root rot control. Registered biorational products for use on celery in the greenhouse include those that act through fungi (*Trichoderma* spp.), bacteria (*Streptomyces* spp., *Bacillus* spp.), and phosphorus acid salts. Mefenoxam is registered for use on celery in the field, but not for use greenhouse. Resistance to mefenoxam and metalaxyl can be problematic in floriculture greenhouses and has been reported in celery production greenhouses (Del Castillo Munera and Hausbeck 2016; Hausbeck and Escobar-Ochoa 2015). Prevention of Pythium root rot and early treatment of seedlings before

they are transplanted into the field leads to a more even stand (Egel 2020). Cultural controls, fungicides, and biological controls are used to control Pythium root rot and damping off.

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CHAPTER 1: DETERMINING CAUSAL AGENTS ISOLATED FROM MICHIGAN CELERY WITH "MELTDOWN" SYMPTOMS

ABSTRACT

Michigan celery growers have observed a crown rot, wilt, and vascular discoloration called “meltdown”. *Fusarium solani* species complex (FSSC) (52% of isolates) and *Fusarium oxysporum* species complex (FOSC) (45%) were collected from symptomatic plants from seven commercial growers across four counties in 2018-19. *Fusarium oxysporum* f. sp. *apii* race 2 (*Foa* race 2) is the causal agent of Fusarium yellows, which caused severe economic losses to the Michigan celery industry in the 1980s and 90s until the development of resistant cultivars. A new race of *Foa* (race 4) has recently emerged in California celery production. Diagnostic primers for identification of the *Foa* race 2 and *Foa* race 4 haplotypes developed by Henry et al. (2020) were used to screen FOSC isolates collected from Michigan celery with “meltdown” symptoms. No *Foa* race 4 isolate was detected. *Foa* race 2 isolates as identified by diagnostic primers were tested for virulence in a growth chamber assay. Celery seedlings ‘Tall Utah 52-70R Improved’ (susceptible to *Foa* race 2), ‘Challenger’ (resistant to *Foa* race 2), and ‘CR-1’ (resistant to *Foa* race 2) were transplanted into soil artificially infested with a *Fusarium* isolate. *Foa* race 2 isolates caused the most severe symptoms of root rot and vascular discoloration on ‘Tall Utah 52-70R Improved’, intermediate disease on ‘CR-1’, and the lowest level of symptoms on ‘Challenger’. FSSC isolates were tested for virulence and no significant differences in symptoms was observed between inoculated and control plants. *Foa* race 2 isolates are capable of causing disease but are observed at a low frequency in “meltdown” plants so further investigation is needed to understand the cause(s) of celery “meltdown”.

INTRODUCTION

Michigan celery makes up 4.9% of U.S. production (USDA 2021). The majority of celery is produced in southwest Michigan (Newaygo, Oceana, Muskegon, Ottawa, Kent, Allegan, and Van Buren counties) with limited production elsewhere in the state (Hausbeck 2011; USDA 2021). Celery is grown on muck soils which have a high organic matter content (Hausbeck 2011; Phillips et al. 2020). Greenhouse-grown seedlings are planted in the field beginning in April; fields are successively planted and harvested until mid-October (Hausbeck 2011; Phillips et al. 2020).

Celery yield and quality is negatively impacted by soilborne diseases including Fusarium yellows (*Fusarium oxysporum* f. sp. *apii*) (*Foa*), Pythium root rot (*Pythium* spp.), crater rot (*Rhizoctonia solani*) and soft rot (*Pseudomonas* spp.) (Koike et al. 2007; Lacy et al. 1996a; Pieczarka 1981; Robbs et al. 1996). Fusarium yellows causes devastating economic losses where resistant cultivars are not available (Epstein et al. 2017; Hausbeck 2011; Lacy et al. 1996a).

Fusarium oxysporum causes disease on over 100 hosts (Michielse and Rep 2009) and is called *Fusarium oxysporum* species complex (FOSC) because of its diversity, lack of sexual reproduction, and its many clonal lineages (Michielse and Rep 2009; O'Donnell et al. 2015). FOSC strains are classified by *formae speciales* and race. *Formae speciales* are defined by the pathogenicity of strains on a host plant (i.e. *Fusarium oxysporum* f. sp. *apii* causes disease on celery) (Armstrong and Armstrong 1981; Snyder and Hansen 1940). A *formae speciales* can be further classified into races based on pathogenicity on specific cultivars (Correll 1991). *Foa* is classified into four races based on its virulence on specific celery cultivars (Table 1.1).

Fusarium yellows of celery was first described in 1914 in Kalamazoo, Michigan on the self-blanching cultivar Golden Self-Blanching (Correll et al. 1986b; Hart and Endo 1978). The

pathogen, designated *Foa* race 1, spread on infested seed or seedlings throughout the Midwest, eastern U.S. and California's Central Valley (Epstein et al. 2017). Fusarium yellows caused by *Foa* race 1 caused economic damage until resistant green cultivars such as Tall Utah 52-70 were developed (Elmer et al. 1986; Otto et al. 1976). Resistant cultivars are the primary disease control strategy for *Foa* (Elmer et al. 1986; Epstein et al. 2017; Orton et al. 1984a) as neither fungicides (Awuah et al. 1986; Otto et al. 1976) or rotation (Elmer and Lacy 1987; Toth 1989) are effective.

In 1976, outbreaks of Fusarium yellows were reported in California on green and self-blanching cultivars (Otto et al. 1976). This pathogen strain was designated *Foa* race 2 (Elmer et al. 1986; Hart and Endo 1978; Otto et al. 1976). Fusarium yellows caused by *Foa* race 2 spread across Michigan (Elmer and Lacy 1982), New York (Awuah et al. 1986), Texas (Martyn et al. 1987 as reported in Lori et al. 2008), and Ontario, Canada (Cerkauskas and McDonald 1989) and to Argentina (Lori et al 2008). Symptoms of Fusarium yellows caused by *Foa* race 2 include leaf chlorosis in lower leaves, stunting, red-brown discoloration of vascular tissue and a decrease in quality and yield (Lacy et al. 1996a; Sherf and Macnab 1986; Subbarao and Elmer 2002). Commercial cultivars with resistance to *Foa* race 2 including 'Challenger', 'Stix', 'Sabroso', and 'Command' (Epstein et al. 2017) were developed using the resistance gene *Fu1* identified from celeriac (Orton et al. 1984a; Quiros 1993). In Michigan, development of celery cultivars resistant to *Foa* race 2 was pursued using somaclonal variation (Lacy et al. 1996b; Toth 1989; Toth and Lacy 1991). Currently, 'CR-1', developed by a Michigan grower, is the dominant cultivar grown in the state and is considered resistant to *Foa* race 2

Table 1.1: Races of *F. oxysporum* f. sp. *apii* and their virulence on celery cultivars

Race	Date of identification and presence in U.S. celery production	Observed in Michigan?	Virulence on 'Golden Self Blanching'	Virulence on 'Tall Utah 52-70R Improved'	Virulence on 'CR-1'	Virulence on 'Challenger'
Race 1	Identified in Michigan in 1914 (Nelson et al. 1937)	Yes	Highly virulent	None	None	None
Race 2	Identified in California in 1976 (Hart and Endo 1978; Otto et al. 1976) , then in Michigan in 1982 (Elmer and Lacy 1982)	Yes	Highly virulent	Highly virulent	None	None
Race 3	Identified in California in 1984 (Puhalla 1984), not currently observed in California (Epstein et al. 2017).	No	None	Highly virulent	Unknown	Unknown
Race 4	Identified in California in 2013, has not been observed in Michigan (Epstein et al. 2017)	No	Highly virulent	Highly virulent	Unknown	Highly virulent

Puhalla (1984) reported an additional *Foa* race, *Foa* race 3, from California that is virulent on 'Tall Utah' but nonpathogenic on 'Golden Self Blanching'. Among 174 isolates collected in California between 1993 and 2013, none were *Foa* race 3 (Epstein et al. 2017), indicating that this race is not important in celery production.

In 2013, a new highly virulent race designated as *Foa* race 4 was identified in California celery. This race causes disease on *Foa* race 2 resistant 'Challenger', several commercial cultivars grown in California, and *Foa* race 2 susceptible 'Tall Utah 52-70R Improved' (Epstein et al. 2017). The disease caused by *Foa* race 4 is called Fusarium wilt as it is characterized by slightly different symptoms than those associated with Fusarium yellows. *Foa* race 4 causes severe stunting, death in young plants, and a water-soaked lesion around the crown vasculature (Henry et al. 2020; Kaur et al. 2022). Severe disease outbreaks of Fusarium wilt, caused by *Foa* race 4, currently occur in California celery (Epstein et al. 2017; Henry et al. 2020; Kaur et al. 2022).

Identifying pathogens using *formae speciales* and race classification is needed to understand *Fusarium* isolates causing Fusarium yellows or Fusarium wilt disease. Both pathogenic and nonpathogenic strains can be isolated from infected plant tissue (Correll 1991; Epstein et al. 2017; Puhalla 1985). *Foa* isolates are classified using diagnostic primers and their virulence. Pathogenicity testing identifies *formae speciales* and race based on their ability to cause disease on a host (O'Donnell et al. 2009; Recorbet et al. 2003). Diagnostic primers aid in rapid identification of races or *formae speciales* that are monophyletic (Baayen et al. 2000; Chiocchetti et al. 2001; Fernandez et al. 1998; Okubara et al. 2013). Diagnostic primers have been developed to identify *Foa* races 2 and 4 (Henry et al. 2020). This is possible because *Foa* is a polyphyletic group where *Foa* race 2 is part of FOSC clade 3 and *Foa* races 1, 3, and 4 are part

of FOSC clade 2. Both *Foa* races 2 and 4 are clonal groups (Epstein et al. 2017; Henry et al. 2020).

Since approximately 2015, celery producers in southwest and western Michigan have annually observed a crown rot, wilt, and vascular discoloration called "meltdown" (Fig. 1). Symptoms are often accompanied by a soft bacterial rot of crown and petioles. Discoloration of crown tissue is red-brown to dark brown in color. Significant losses have occurred due to plant death in the field and quality losses in the packing shed. The wilt symptoms and discolored vascular tissue have led to a hypothesis that *Foa* is the causal agent of "meltdown". Thus, the objective was to determine the causal agent of "meltdown" in Michigan celery. Identifying the *Fusarium* spp. associated with "meltdown" symptoms and determining whether they are *Foa* race 2, and pathogenic on selected celery cultivars was also of interest.

MATERIALS AND METHODS

Sampling and fungal identification. 'CR-1', 'WA-7', and 'Dutchess' celery plants exhibiting "meltdown" symptoms including crown rot, wilting, chlorosis, and/or stunting were sampled from seven commercial farms across four Michigan counties (Van Buren, Ottawa, Allegan, Kent) from August to October, 2018 and July to October, 2019. An opportunistic sampling strategy was employed. Severely rotted plants were avoided due to likely colonization by secondary organisms. A shovel or harvesting fork was used to remove the plants while keeping the crown and petioles intact. Plants were collected and immediately transported to the Hausbeck Laboratory where they were kept in a walk-in cooler maintained at 5°C until processing (Michigan State University, East Lansing, MI).

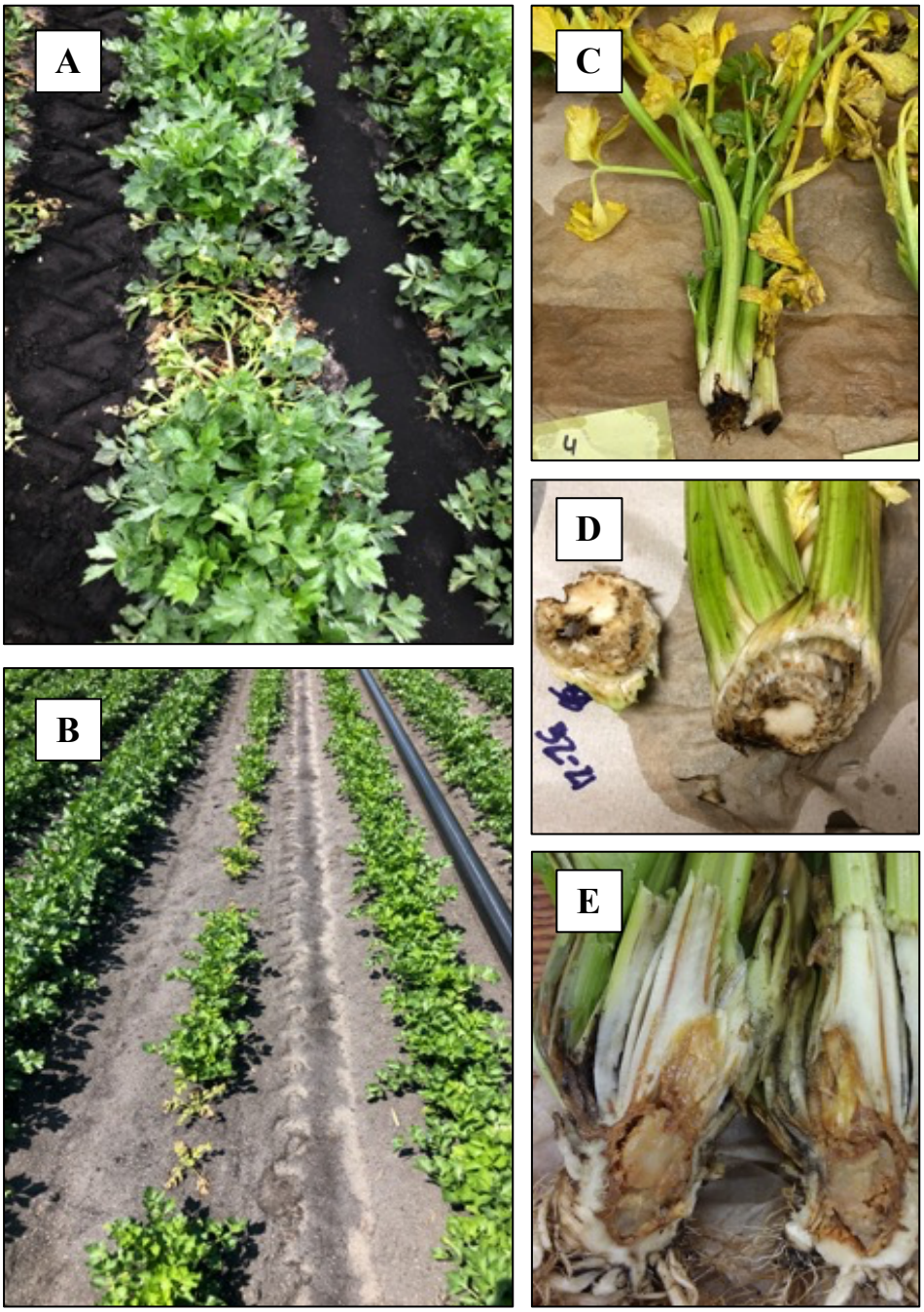

Figure 1.1: "Meltdown" symptoms. (**A**) (**B**) (**C**) celery "meltdown" symptoms including chlorosis, wilting, stunting, and plant death. (**D**) (**E**)"Meltdown" symptoms of crown rot and vascular discoloration.

Samples were processed following the methods presented in Epstein et al. (2017) with modifications. Plants were washed with tap water until soil was no longer visible. The crown was cut diagonally to observe the crown and vascular tissue using a knife whose blade (17 cm x

by 4.5 cm) was cleaned with 70% ethanol between each plant. When discolored vascular tissue was observed, it was traced up the petiole to find the interface of healthy and discolored tissue where 0.5 to 1 cm^2 petiole samples were taken. All tissue was taken from this interface if possible otherwise additional samples were taken from discolored tissue. When multiple petioles from one plant exhibited vascular discoloration, at least one tissue sample was taken from each petiole. If neither crown or vascular discoloration was visible, 0.5 to 1 cm^2 samples were taken from the vascular tissue of the crown. Based on the severity of symptoms, 3 to 9 samples were taken per plant; more samples were taken from plants with severe symptoms.

Each tissue piece was surface sterilized in a fume hood by rinsing first with a bleach solution (121 ml 8.25% sodium hypochlorite; 879 ml water) for 30 seconds, then rinsing in sterile water twice, and drying on a paper towel. Surface sterilized tissue pieces were plated on 1.6% water agar (16 g technical agar, 1000 ml distilled water) (Leslie and Summerell 2006; Nelson et al. 1983) (BD Biosciences, San Jose, CA, USA) amended with rifampicin and ampicillin in 2018 and pentachloronitrobenzene peptone agar (PPA) for selective isolation of *Fusarium* species (Leslie and Summerell 2006; Nash and Snyder 1962) in 2019. Plates were sealed with Parafilm® (Amcor Limited and Bemis Company, Inc., Neenah, WI) and allowed to grow for 3 to 4 days. All culture incubation steps during the isolation process took place at room temperature (approximately 20°C).

Mycelial growth from tissue samples was transferred to potato dextrose agar (PDA) (39 g PDA and 1000 ml distilled water) (Neogen, Lansing, MI, USA). Petri plates were sealed with Parafilm® and allowed to grow for 5 to 7 days. Growth from one tissue sample was considered one isolate unless mycelia with multiple morphologies were observed in which case each unique morphology was transferred to a separate plate and considered a distinct isolate. Cultures

identified as *Fusarium* spp. based on presence of microconidia, macroconidia, and chlamydospores (Leslie and Summerell 2006; Nelson et al. 1983) were transferred to water agar and a single hyphal tip was transferred to PDA after 3 to 4 days of growth on a plate sealed with Parafilm® to obtain a pure culture. This protocol was adapted from Epstein et al. (2017) by adding a rinse in sterile water after surface sterilization and using PPA for isolation in place of amended PDA. Additionally, isolates were transferred to water agar to perform the hyphal tip transfer.

Isolate storage. Isolates were stored on silica gel crystals following a method presented by Windels et al. (1988) modified from that described by Perkins (1962). Sterile skim milk prepared using 10 g Difco skim milk powder (BD Biosciences, Franklin Lakes, NJ) per 100 ml distilled water was autoclaved for 20 min at 115°C, cooled, and used to make a spore suspension from 7-10 day old PDA plates colonized with the *Fusarium* isolate to be stored. The screw cap glass culture tubes (22mm foil lined 20 ml low background glass scintillation vials DWK Life Sciences, Millville, NJ) were half filled with silica gel crystals (Sigma-Aldrich, St. Louis, MO) and sterilized using dry heat (121°C, 1.5 h) and cooled. The milk spore suspension (200 µl) was pipetted onto the gel and the opening of the vial was flame sterilized, capped, and wrapped with Parafilm®. Vials were shaken by hand for approximately 10 seconds to evenly distribute the spore suspension and stored at 4-6° C. Two replicates of each vial were prepared.

DNA extraction. Isolates were removed from storage by shaking 20-30 silica gel crystals onto the surface of a water agar plate and pressing them into the media with a scalpel (Perkins 1962; Windels et al. 1988). After the growth was observed from the crystals (3 to 4 days after crystals were plated) the culture was transferred to a PDA plate and allowed to grow for another 7 to 10 days under continuous light at room temperature. Mycelia scraped from the surface of the

agar plate weighing 50-70 mg wet and a single 0.4 cm stainless steel ball bearing (Grainger, Lake Forest, IA) were placed into 1.1 ml polypyrene 8-strip collection tubes (Thomas Scientific, Swedsboro, NJ). Samples were covered with a layer of cheesecloth and freeze-dried using a Genesis Pilot Lyophilizer (SP Scientific, Warminster, PA) overnight until completely desiccated. The dried tissue was then lysed using the TissueLyser II (Qiagen, Hilden, Germany) for 3 min at 30Hz. DNA was extracted from the tissue using the Omega 1130 Mag-Bind DNA Extraction kit following the kit manufacturer's instructions (Omega Bio-Tek, Norcross, GA) using the KingFisher Flex extraction instrument (ThermoFisher, Waltham, MA). Extracted DNA was stored at -80°C and working stocks of DNA were stored at -20°C.

PCR and sequencing. *Fusarium* isolates were identified to species by sequencing the translation elongation factor 1α (TEF1) coding region, commonly used for identification of *Fusarium* isolates to species (O'Donnell et al. 2015). The TEF1 region was PCR-amplified using the EF-1 and EF-2 primers described by O'Donnell et al. (1998). The 700-bp portion of TEF1 was amplified in reaction with a total volume of 20 µl containing 3.6 µl buffer 10X High Fidelity PCR buffer (Invitrogen, Carlsbard, CA), 0.5 µl 2.5 mM dNTPS (Promega, Madison, WI), 3 µl 25 mM magnesium chloride (Promega, Madison, WI), 0.5 µl 10 µg/µl BSA (New England BioLabs, Ipswich, MA), 0.15 µl Platinum Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbard, CA) and 0.5 µl each of the 5 µM EF-1 and EF-2 primers (Integrated DNA Technologies, Coralville, IA), sample DNA equal to 8-10 ng of template DNA with water added to reach total reaction volume. Reactions were run in a 96-well plate with a well volume of 0.2 ml (Alkali Scientific Inc., Pompano Beach, FL) or 0.2 ml 8-strip tubes (VWR, Randor, PA). For isolates collected in 2018, this protocol was modified by using 0.15 µl GoTaq® polymerase (Promega, Madison, WI) and 3.6 µl 5X Green GoTaq® Reaction Buffer in the 20 µl reaction (Promega, Madison, WI).

Samples were amplified in a VapoProtect MasterCycler Pro thermocycler (Eppendorf, Hauppauge, NY) using the following program modified from O'Donnell et al. (1998): 1 min at 94°C, 24 cycles of 30 sec at 94°C, 45 sec at 59°C, 1 min at 72°C, followed by 1 min at 72°C. Sample quality was confirmed by gel electrophoresis using PowerPac (BioRad, Hercules, CA) and observation of the 700 base pair product. The PCR product was purified using QIAquick PCR Purification kit (Qiagen, Germantown, MD) following the manufacturer's instructions and DNA was quantified using the Qubit 4 Fluorometer (Thermo Scientific, Waltham, MA). Forward and reverse sequences of the TEF1 regions were sequenced using EF-1 and EF-2 primers (Epstein et al. 2017; O'Donnell et al. 1998) using Sanger sequencing by Macrogen (Rockville, MD) in 2018 and the Michigan State University Research Technology Support Facility-Genomics Core (East Lansing, MI) in 2019. Sequence assembly was performed using the DNA sequence analysis program Geneious© (Biomatters, New Zealand) for 2018 samples and MEGA (Molecular Evolutionary Genetics Analysis Version 10.1.8) (Stecher et al. 2020) for 2019 samples. Low quality bases at the beginning and end of sequences were trimmed and forward and reverse sequences were aligned. After sequence assembly, consensus sequences were run through National Center for Biotechnology Information Basic Local Alignment Search Tool Nucleotide (NCBI BLAST-N) and cross-referenced with the *Fusarium*-ID database (Geiser et al. 2004; Torres-Cruz et al. 2021). If identification of the isolate differed between the two databases, the result from *Fusarium*-ID was followed.

Screening isolates using race-specific diagnostic primers. Purified DNA of isolates identified as *Fusarium oxysporum* species complex (FOSC) using sequencing of TEF1 region were screened using diagnostic primers developed for identification of *F. oxysporum* f. sp. *apii* (*Foa*) races 2 and 4 (Henry et al. 2020). Primers for identification of *Foa* races 2 (primer FOA

R2-76k) and 4 (primers FOAR4-447 and N3875-2) (Henry et al. 2020) were ordered from Integrated DNA Technologies (Coralville, IA). These primers were used in a PCR reaction to screen FOSC isolate DNA collected from symptomatic celery plants following the reaction conditions outlined in Epstein et al. (2017) for amplification of *Foa* races 2 and 4 DNA and primers published in Henry et al. (2020). Positive control DNA for *Foa* races 2 and 4 were provided by the Epstein lab at University of California (UC)-Davis.

A positive for *Foa* races 2 or 4 was confirmed by gel electrophoresis using PowerPac (BioRad, Hercules, CA) and observation of 226 base pair product for the *Foa* race 2 primers or the 196 and 189 base pair products for the *Foa* race 4 primers. A positive band indicated a positive for *Foa* race 2 while a positive band with both *Foa* race 4 primer sets is necessary to indicate a positive for *Foa* race 4 (Henry et al. 2020).

Pathogenicity testing. Preliminary studies. Tests were conducted in the greenhouse to determine the best methods and included soaking celery seedling roots in a conidial suspension for 1 hour (Burkhardt et al. 2019) and transplanting them into sandy field soil (Elmer et al. 1986). However, the dense field soil did not drain well, leading to seedling death (>10%) within 2 weeks of inoculation (data not shown). In a second greenhouse study, roots were soaked in a conidial suspension (Burkhardt et al. 2019; Epstein et al. 2017; Henry et al. 2017), or planted into a mixture of millet or straw colonized with the isolate and sandy field soil (Elmer et al. 1986; Hart and Endo 1981) (data not shown). However, the celery plants became infected with Tomato Spotted Wilt Virus (TSWV) leading to foliar chlorosis which was indistinguishable from *Fusarium* symptoms.

To avoid TSWV, pathogenicity tests were conducted in growth chambers. As described in Henry et al. (2020), millet colonized with *Fusarium* was used to inoculate celery seedlings.

Soil infestation has been reported to be a better method for reproducible plant symptoms than conidial suspension root dips (Elmer et al. 1986; Hart and Endo 1981; Henry et al. 2020). Temperature influences symptom development for *Foa* races 2 and 4; *Foa* race 4 is more affected by temperature that *Foa* race 2 (Henry et al. 2020; Kaur et al. 2022). When *Foa* was tested for pathogenicity on a susceptible celery cultivar in a 27-29° C greenhouse (Henry et al. 2020), the concentration of *Foa* race 2 in plant tissue increased from 16°-26°C; plant stunting of infected plants was not temperature dependent (Kaur et al. 2022).

Isolate selection. Initially, a group of FOSC isolates with a variety of genotypes was tested for pathogenicity (trial 1). A second experiment was conducted using isolates initially identified as *Foa* race 2 (trial 2). Finally, *F. solani* isolates were also tested for virulence (trial 3). Each experiment was conducted twice FOSC isolates were tested for pathogenicity (trial 1). FOSC isolates collected in 2018 were identified to clade by genotyping performed by the Epstein Lab at UC-Davis. This information was used to select isolates that represented a variety of genotypes and isolates collected across several farms. Eight isolates tested included one isolate matching the *Foa* race 2 haplotype and two isolates within the FOSC clade 3 (containing *Foa* race 2) but not matching the *Foa* race 2 haplotype. Additionally, three of the isolates included were in FOSC clade 2 (containing *Foa* races 1, 3, and 4). An additional FOSC isolate that was not identified to clade was also included (Table 1.2). The 2018 isolates were collected from four celery farms.

Pathogenicity of isolates initially identified as *Foa* race 2 using diagnostic primers was tested (trial 2). Isolate 26-27.1a was initially mis-identified as *Foa* race 2 (Table 1.3). PCR of DNA extracted from the culture of isolate 26-27.1a used to inoculate seedlings showed no positive band using race 2 primers. The DNA of this isolate in the original DNA extraction plate

had a positive band when tested again; mis-identification was due to contamination in the DNA extraction plate. Isolates 04-2, 32-20.1a, and 72-7.1a consistently amplify with *Foa* race 2 primers (Table 1.3). The final trial (trial 3) Included a group of isolates identified as *Fusarium solani* species complex (FSSC) that represent both years and several sampling locations (Table 1.4).

Table 1.2: Isolates used in *Fusarium* pathogenicity testing trial 1. Celery seedlings inoculated with *Fusarium oxysporum* species complex isolates (FOSC). FOSC clade 3 contains *Foa* race 2 and FOSC clade 3 contains *Foa* races 1, 3, and 4 (Epstein et al. 2017).

Isolate	***Fusarium* spp.**	**Isolate details**
04-2	FOSC	FOSC clade 3, positive band with *Foa* race 2 primers
04-3	FOSC	FOSC clade 3, no positive band with *Foa* race 2 primers
04-9	FOSC	FOSC clade 2
04-7	FOSC	FOSC clade 2
26-2	FOSC	FOSC clade 2
26-3	FOSC	FOSC, clade not identified
84-1	FOSC	FOSC clade 3, no positive band with *Foa* race 2 primers
72-1	FOSC	FOSC, clade not identified

Table 1.3: Isolates used in *Fusarium* pathogenicity testing trial 2. Celery seedlings inoculated with *Fusarium oxysporum* species complex (FOSC) isolates that amplify with *Fusarium oxysporum* f. sp. *apii* race 2 (*Foa* race 2) primers (Henry et al. 2020).

Isolate	***Fusarium* spp.**	**Isolate details**
04-2	FOSC	Positive band with *Foa* race 2 primers
32-20.1a	FOSC	Positive band with *Foa* race 2 primers
72-7.1a	FOSC	Positive band with *Foa* race 2 primers
26-27.1a	FOSC	No band with *Foa* race 2 primers

Table 1.4: Isolates used in *Fusarium* pathogenicity testing trial 3. Celery seedlings inoculated with *Fusarium solani* species complex (FSSC) isolates.

Isolate	*Fusarium* spp.
26-5.8a	FSSC
4-31.1c	FSSC
54-4.4a	FSSC
7-20.1b	FSSC

Inoculation, experimental methods, and data collection. Methods for all trials conducted were the same except where noted. Isolates were taken out of storage as previously described for DNA extraction. The methods for pathogenicity testing were modified from those described in (Henry et al. 2020). Inoculum was prepared by adding 150 (trials 1 and 3) or 300 cc (trial 2) of millet that had been covered with water and allowed to soak overnight to a mushroom growing bag (RJC Sales/Shroom Supply, Brooksville, FL). The millet in a sealed mushroom bag was autoclaved for 45 min on two consecutive days to sterilize and then inoculated with six PDA plugs that were 1 cm in diameter from a 1-week old culture that had grown at room temperature in continuous light. For the non-infested control, non-colonized PDA plugs were added to the millet bag. The inoculum was allowed to grow for 7 to 10 days under continuous light at room temperature and the bag was shaken every 1 to 2 days to ensure uniform colonization.

Celery seed was planted into 288-cell flats filled with soilless potting mix (SunGro, Agawam, MA) that had been autoclaved for 45 min to steam sterilize. Seedlings were watered with 200 ppm Jack's 20-20-20 fertilizer (JR Peters Inc., Allentown, PA) as needed. Seedlings were treated with SubdueMAXX (mefenoxam) (Syngenta, Woodland, CA) at a rate of 0.5 oz/100 gal 10 days before inoculation in the pathogenicity test to prevent rot root caused by *Pythium* spp.. Seedlings were allowed to grow for 6 to 7 weeks after seeding. Celery cultivars included CR-1 (provided by grower cooperator), Dutchess (provided by grower cooperator), Tall Utah 52-70R Improved (Burpee Seed Co. Warminster, PA), Golden Self Blanching (Seeds n'

Such, Graniteville, SC), and Challenger (Syngenta, Woodland, CA). Cultivars included in each trial as listed in table 1.5.

Table 1.5: Cultivars used in each *Fusarium* spp. pathogenicity trial. Celery seedlings inoculated with *Fusarium* spp. isolates.

	Cultivar used in each trial				
Trial	**Golden Self Blanching**	**Tall Utah 52-70R Improved**	**CR-1**	**Dutchess**	**Challenger**
Trial 1	yes	yes	yes	yes	no
Trial 2	no	yes	yes	no	yes
Trial 3	yes	yes	yes	no	yes

The planting medium was prepared by mixing steam sterilized MSU greenhouse soil (GHS) with perlite (Therm-O-Rock East Inc., New Eagle PA in trial 1; FoxFarm Soil and Fertilizer Co., St. Samoa, CA in trials 2 and 3) in a 3:1 perlite to GHS mixture by volume. This mixture was used to fill planting tubes (4 cm diameter, 20.5 cm deep). Inoculum and soil perlite mixture were added as described in Henry et al. (2020). Seedlings were transplanted into planting tubes with infested soil or soil mixed with a non-inoculated millet control and maintained in a growth chamber at 28°C. Seedlings were overhead irrigated as needed with 200 ppm Jack's water soluble 20-20-20 general purpose fertilizer (JR Peters Inc., Allentown, PA). Modifications from Henry et al. (2020) include growing millet inoculum in a mushroom grow bag instead of a flask, adding non-inoculated millet to the negative control, inoculating 6-week-old celery seedlings, and growing inoculated plants in a growth chamber instead of a greenhouse.

Plant height was measured weekly. Eight (trial 1) or six (trials 2 and 3) weeks after inoculation, plants were harvested, washed, and scored for vascular discoloration. The following scale was used: 0 = asymptomatic; 1= some discoloration in the lateral root vasculature; 2 = some discoloration in the main root vasculature; 3 = some discoloration in the crown vasculature;

4 = extensive discoloration of the crown vasculature; and 5 = plant dead (Epstein et al. 2017; Henry et al. 2020).

In trials 1 and 3, four single plant replicates were included for each cultivar x isolate combination in a completely randomized design (CRD); the experiment was conducted twice. Trial 2 included 20 single plant replicates per isolate x cultivar combination across two growth chambers. The plants were randomly assigned to a growth chamber and cultivar x isolate treatment and the experiment was blocked by growth chamber in a randomized complete block design (RCBD). The experiment was conducted twice.

Pathogen confirmation. Symptomatic root and crown tissue were sampled from 20% of the plants in each trial. One root (1-cm in length) and crown tissue sample (0.5-1 cm^2) per plant were surface sterilized as described previously and plated on water agar plates. Fungal growth was transferred to PDA and identified based on morphological characteristics.

Statistical analysis. Statistical analysis was conducted using SAS Studio SAS OnDemand for Academics (SAS Institute Inc. Cary, NC).

Trial 1**.** A model was established with cultivar and isolate as fixed factors and experiment as a random factor. Disease severity rating was treated as an ordinal variable. To allow for simplified interpretation of the data, the disease score (0-5) was reclassified as a binomial variable based on the procedure described in Kaur et al. (2022). If the original disease severity score was 0 or 1, this data point was designated as 0, indicating no disease. If the original disease severity score was 2, 3, 4, or 5, this data point was designated as 1, indicating disease was observed. A ordinal logistic regression model could not be established in PROC GLIMMIX as the model did not converge, presumably as there was too much variability in the dataset.

Disease incidence (%) was calculated based on the binomial rating (0=asymptomatic, no disease or 1=vascular discoloration with a score ≥2) and was treated as a continuous variable and modeled in PROC GLIMMIX. The data met the assumption of normality based on residual plots. Data were tested for equal variance using Levene's test and the Kenward-Roger degrees of freedom approximation (ddfm=kr) was included to account for unequal variance. The ANOVA (analysis of variance) for the main effects was significant, so the isolates and treatments were compared. PROC GLIMMIX was used to determine statistical separation between isolate and cultivar groups based on Fisher's least square means difference at $P < 0.05$.

Trial 2. The model included fixed factors of cultivar and isolate and experiment and block as random factors, with block nested in experiment. Plant height was analyzed as a continuous variable. Data were tested for equal variance using Levene's test. Normal distribution was observed using residual plots. Plant height was analyzed using repeated measures analysis with a model in PROC MIXED analysis to determine if there were differences in the rates of growth of plants in each treatment group. The height was compared across time to show the trends in plant growth for each treatment. Statistical differences were determined based on Fisher's least square means significant difference at $P<0.05$.

Next, disease severity rating was analyzed in a similar manner to trial 1. PROC GLIMMIX was used to model the data using a multinomial (binomial) distribution and ordinal logistic regression. The 0-5 rating scale was simplified to a binomial rating in the same manner (0=asymptomatic, no disease or 1=vascular discoloration with a score ≥2). The data were analyzed by logistic regression in PROC GLIMMIX. The model for this dataset converged and contrasts were used to estimate the odds ratios and determine significant differences between comparisons. Additionally, a predicted probability of disease based on the logistic regression and

binary rating scale (0=asymptomatic, no disease or 1=vascular discoloration with a score ≥2) was calculated for each isolate within each cultivar using the LSMEANS statement in PROC GLIMMIX. Incidence (%) was determined for each cultivar x isolate treatment in the same manner as experiment 1 and Kenward-Roger degrees of freedom approximation was included for unequal variance.

Trial 3. The data from trial 3 were analyzed using the same methods as for trial 1 with a model treating isolate and cultivar as fixed variables and experiment as a random variable. Binary disease severity rating data (0=asymptomatic, no disease or 1=vascular discoloration with a score ≥2) was analyzed using binary logistic regression. The model for this dataset converged and contrasts were used to estimate the odds ratios and determine significant differences between comparisons.

RESULTS

Isolate collection and identification. *Fusarium* spp. were the most frequently recovered organisms from celery plants with "meltdown" symptoms collected from commercial farms in Van Buren, Ottawa, Allegan, and Kent counties in 2018-2019 (Table 1.6). *Fusarium* spp. isolates were collected from 66 plants in 2018 (209) and from 209 plants in 2019 (219) (Table 1.6).

Table 1.6: *Fusarium* spp. isolates collected in 2018-2019 from Michigan celery fields with "meltdown" symptoms. Plant were collected across four Michigan counties and seven commercial celery growers. 66 and 209 total plants were sampled and 209 and 2019 *Fusarium* spp. isolates were collected in 2018 and 2019, respectively.

	County					
Year	**Van Buren**	**Ottawa**	**Allegan**	**Kent**	**Other**	**Total**
2018	20	76	15	0	98	209
2019	140	29	27	23	0	219
Total	160	103	42	23	100	**428**

The most common species recovered were *Fusarium oxysporum* species complex (FOSC) (45%) and *Fusarium solani* species complex (FSSC) (52%). *F. commune* and *F. sporotrichiodes* were identified from the remaining 3% of the isolates. The proportion of FOSC and FSSC collected differed between 2018 and 2019. In 2018, FOSC and FSSC made up 67% and 25% of isolates, respectively. In 2019, FOSC and FSSC made up 33% and 67% of isolates, respectively (Table 1.7).

Table 1.7: *Fusarium* spp. collected from celery from fields with "meltdown" symptoms identified to species level by sequencing. Isolates were identified using sequencing of translation elongation factor 1-α (TEF1). Of *Fusarium* spp. isolates collected in 89 collected in 2018 and 162 collected in 2019 were identified by sequencing.

			2018	**2019**	**2018-19**
***Fusarium* spp.**	***F. oxysporum* species complex**	Number of isolates	60	53	113
		Percentage of isolates	67%	33%	45%
	***F. solani* species complex**	Number of isolates	22	108	130
		Percentage of isolates	25%	67%	52%
	F. commune	Number of isolates	6	1	7
		Percentage of isolates	7%	<1%	3%
	F. sporotrichiodes	Number of isolates	1	0	1
		Percentage of isolates	1%	0	<1%

FOSC isolates collected from plants with "meltdown" symptoms were screened with primers developed for identification of *Foa* races 2 and 4 (Henry et al. 2020). Isolates identified as *Foa* race 2 using primers were observed in three of four farms sampled in each of 2018 and 2019 although different farms were sampled each year (Table 1.8). *Foa* race 2 isolates made up 25% and 17% of FOSC isolates collected in 2018 and 2019, respectively; no *Foa* race 4 isolates were observed (Table 1.9). *Foa* race 2 isolates were collected from plants that had "meltdown" symptoms of crown rot and vascular discoloration and asymptomatic plants (Fig. 1.2).

Table 1.8: Preliminary identification of isolates to *Fusarium oxysporum* f. sp. *apii* race 2 by grower. Results of screening *Fusarium oxysporum* species complex isolates collected from celery from fields with "meltdown" symptoms using diagnostic primers for *Fusarium oxysporum* f. sp. *apii* race 2 (*Foa* race 2) and race 4 (Henry et al. 2020) by grower.

	Positive band using *Foa* race 2 primers	
Grower	**2018**	**2019**
04	Yes	Yes
26	Yes	Yes
84	No	N/A
05	N/A	Yes
07	N/A	No
72	Yes	N/A

Table 1.9: Preliminary identification of isolates to *Fusarium oxysporum* f. sp. *apii* race 2 by year. Results of screening *Fusarium oxysporum* species complex DNA collected from celery from fields with "meltdown" symptoms using diagnostic primers for *Fusarium oxysporum* f. sp. *apii* race 2 (*Foa* race 2) and race 4 published in Henry et al. (2020) by year.

	2018		**2019**	
	Band using *Foa* 2 primers	**Band using *Foa* 4 primers**	**Band using *Foa* 2 primers**	**Band using *Foa* 4 primers**
Number of isolates	12	0	9	0
Percentage of isolates	25%	0%	17%	0%

Figure 1.2: Celery plants where an isolate with preliminary identification as *Fusarium oxysporum* f. sp. *apii* race 2 using diagnostic primers was detected. DNA was screened using diagnostic primers (Henry et al. 2020).

Pathogenicity testing results. Trial 1**.** FOSC isolates differed in their ability to incite symptoms of root rot and vascular discoloration when compared to a non-inoculated control (Fig. 3). A logistic regression model was established based on disease severity rating scores that were converted to a binary rating where 0=asymptomatic, no disease and corresponded to a score <2 on the scale from 0 to 5 and 1=vascular discoloration with a score ≥2 on scale from 0 to 5. The binary logistic regression model did not converge so results are not presented.

Incidence (%) of disease was calculated based on the binomial rating and the ANOVA of the main effects of isolate ($P = 0.0003$) and cultivar ($P = 0.0008$) was statistically significant while the interaction between cultivar and isolate ($P = 0.5358$) was not. The interaction of cultivar x isolate interaction was of interest as pathogenicity by cultivar is used for identification to *formae speciales* and race (Armstrong and Armstrong 1981; Correll 1991; Snyder and Hansen 1940). The results were sliced by cultivar and the differences among isolates were observed (Fig. 1.3).

Disease incidence was based on the mean disease incidence of eight single plant replicates across two replicated experiments. Isolates 04-2 (*Foa* race 2), 04-9 and 04-7 incited significantly greater disease incidence compared to the control in 'Tall Utah 52-70R Improved' (referred to as 'Tall Utah') which is susceptible to *Foa* race 2 (Fig. 3). Five FOSC isolates tested incited greater disease incidence compared to the control in 'Golden Self Blanching' including isolate 04-2; 'Golden Self Blanching' is susceptible to *Foa* race 2. FOSC isolates tested did not differ from the control when tested on 'CR-1'. When 04-2 (*Foa* race 2) and 04-9 were tested on 'Dutchess', the amount of disease differed from the control. When isolate 04-2 was compared to the control, disease incidence differed from the control in 'Tall Utah', 'Golden Self Blanching', and 'Dutchess'. In 'CR-1' isolate 04-2 incited disease incidence of 62.5%, which did not significantly differ from the control.

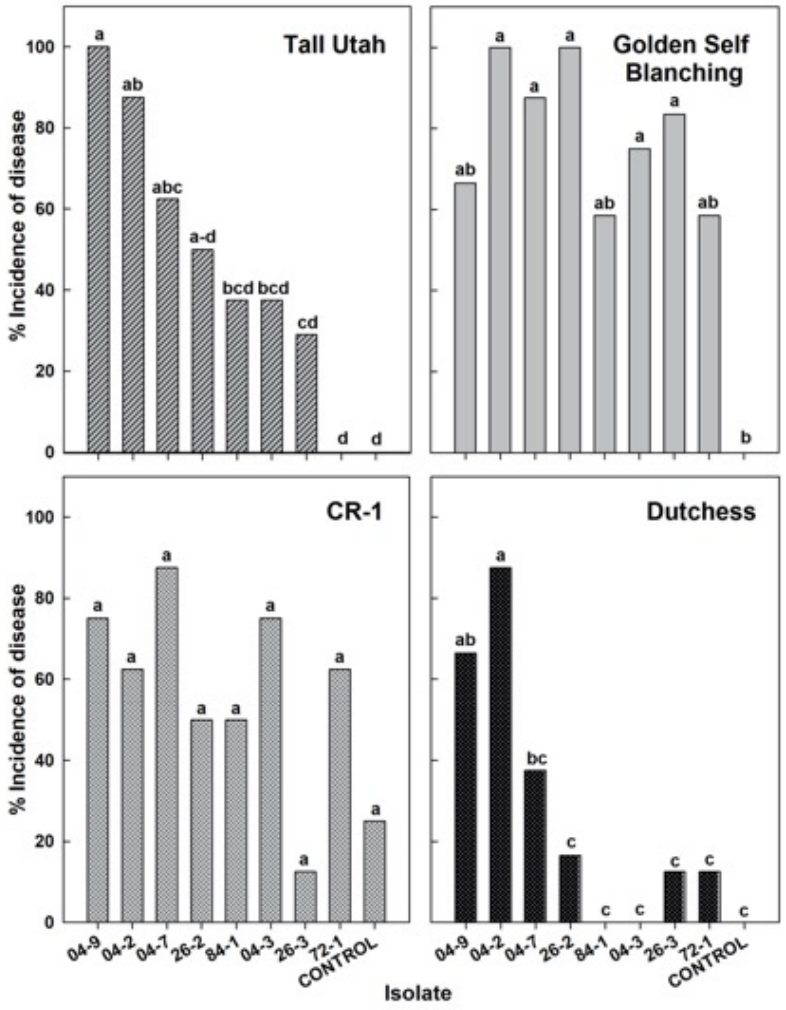

Figure 1.3: Incidence of disease in celery seedlings inoculated *Fusarium oxysporum* species complex (FOSC) isolates. 6-week old seedlings of cultivars Tall Utah 52-70R Improved (Burpee Seed Co. Warminster, PA, USA), Golden Self Blanching (Seeds n' Such, Graniteville, SC, USA), CR-1 (provided by grower cooperator), Dutchess (provided by grower cooperator) were transplanted into un-infested (control) soil or soil infected with an FOSC isolate in a growth chamber maintained at 28°C. Disease symptoms were evaluated based on a scale of 0= asymptomatic, 1= lateral roots discolored, 2=main root discolored, 3=crown discolored, 4=crown extensively discolored, 5=plant dead at 6 weeks after inoculation (Epstein et al. 2017). This scale was converted to a binary scale where a rating of 0 or 1 on 0-5 scale indicated no disease and a rating of 2, 3, 4, or 5 indicated disease (Kaur et al. 2022) to calculate percent incidence. Each bar is based on the mean disease incidence of eight single plant replicates across an experiment conducted twice. Bars with a letter in common are not significantly different based on Fisher's least square means significant difference at $P < 0.05$. Statistical comparisons are within each cultivar.

Trial 2: The virulence of three FOSC isolates identified as a *Foa* race 2 using diagnostic primers (Henry et al. 2020) was evaluated on three celery cultivars (Tall Utah, CR-1,

Challenger). Isolates 32-20.1a, 72-7.1a, and 04-02 were identified as *Foa* race 2 using diagnostic primers; 26-27.1a is an FOSC isolate that does not match the *Foa* race 2 haplotype (Table 1.3). The frequency of each rating by isolate and cultivar indicate that the inoculated plants had a higher occurrence of root and vascular discoloration (Fig. 1.4) and frequency of disease compared to the control (Fig. 1.5). Disease severity ratings were converted to a binary rating as previously described to analyze the results. Factors of cultivar (P=<0.0001) and isolate (P=<0.0001) were significant in the binary logistic regression model, but their interaction was not (P=0.8550). The data were both sliced to analyze the effect of cultivar or isolate. Additionally, since each cultivar x isolate was of interest, the interaction was examined.

Across isolates tested, 'Tall Utah' was significantly more likely to develop symptoms than 'CR-1' or 'Challenger'. Isolates 04-02, 32-20.1a, and 72-7.1a had a significantly greater likelihood of disease than the control while 26-27.1a did not differ from the control when compared across cultivars.

Predicted probability of disease indicated that for 'Tall Utah', isolates 32-20.1a and 72-7.1a had the highest likelihood of inciting disease (Fig. 1.6). Isolates 04-2 and 26-27.1a differed from both the most virulent isolates and the control in their predicted probability of disease (Fig. 1.6). Within 'CR-1' plants, isolates 32-20.1a, 72-7.1a, and 04-2 had the highest predicted probability of disease. Plant inoculated with 26-27.1a differed in disease from the control but had a significantly lower predicted probability of disease than isolates 32-20.1a and 72-7.1a. Within 'Challenger', isolates 32.20.1a and 72.7.1a had the highest predicted probability of disease and isolate 04-2 did not differ from the two most virulent isolates or from the control. Plants inoculated with 26-27.1a did not differ from the control (Fig. 2.6). Isolates 32-20.1a and 72-7.1a caused the highest predicted levels of disease across cultivars. However, the differences in

predicted probability of disease between the inoculated plants and the control is greater in ‘CR-1’ and ‘Tall Utah’ than in ‘Challenger’. For example, 72.7.1a was 3.3 times more likely to cause disease symptoms in the inoculated than control plants in ‘Challenger’. In ‘CR-1’ and ‘Tall Utah’, isolate 72.7.1a was 7.9X and 7.8X more likely to incite disease compared to the control, respectively.

Plant height was recorded weekly for six weeks and the interaction of isolate and cultivar was compared over time by repeated measures analysis. In ‘Tall Utah’, all isolates were stunted compared to the control when height was analyzed over the course of the experiment. In ‘CR-1’, isolates 04-2, 26-27.1a, and 32-20.1a caused stunting compared to the control. Finally, in ‘Challenger’, isolates 04-2, 26-27.1a, and 32-20.1a caused stunting compared to the control (Fig. 1.7).

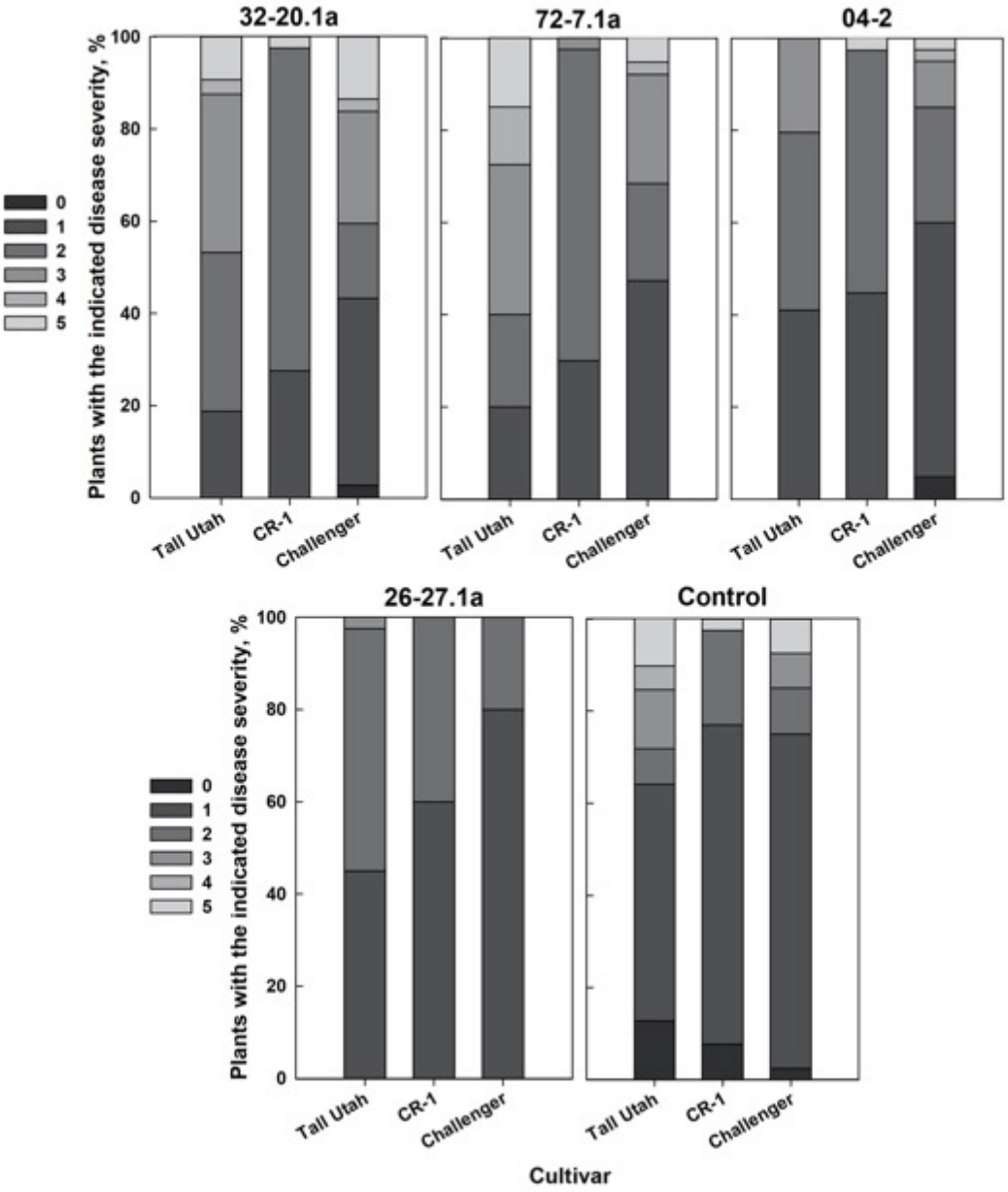

Figure 1.4: The effect of inoculation with *Fusarium oxysporum* f. sp. *apii* race 2 (*Foa* race 2) on root and vascular discoloration symptoms on a scale from 0 to 5. 6-week old seedlings of cultivars Tall Utah 52-70R Improved (Burpee Seed Co. Warminster, PA, USA), CR-1 (provided by grower cooperator), and Challenger (Syngenta, Woodland, CA, USA) were transplanted into un-infested (control) soil or soil infected with an FOSC isolate in a growth chamber maintained at 28°C. Disease symptoms were evaluated based on a scale of 0= asymptomatic, 1= lateral roots discolored, 2=main root discolored, 3=crown discolored, 4=crown extensively discolored, 5=plant dead at 6 weeks after inoculation (Epstein et al. 2017). Percentage of plants (n=40 across experiment conducted twice) with the disease severity scores shown on the right.

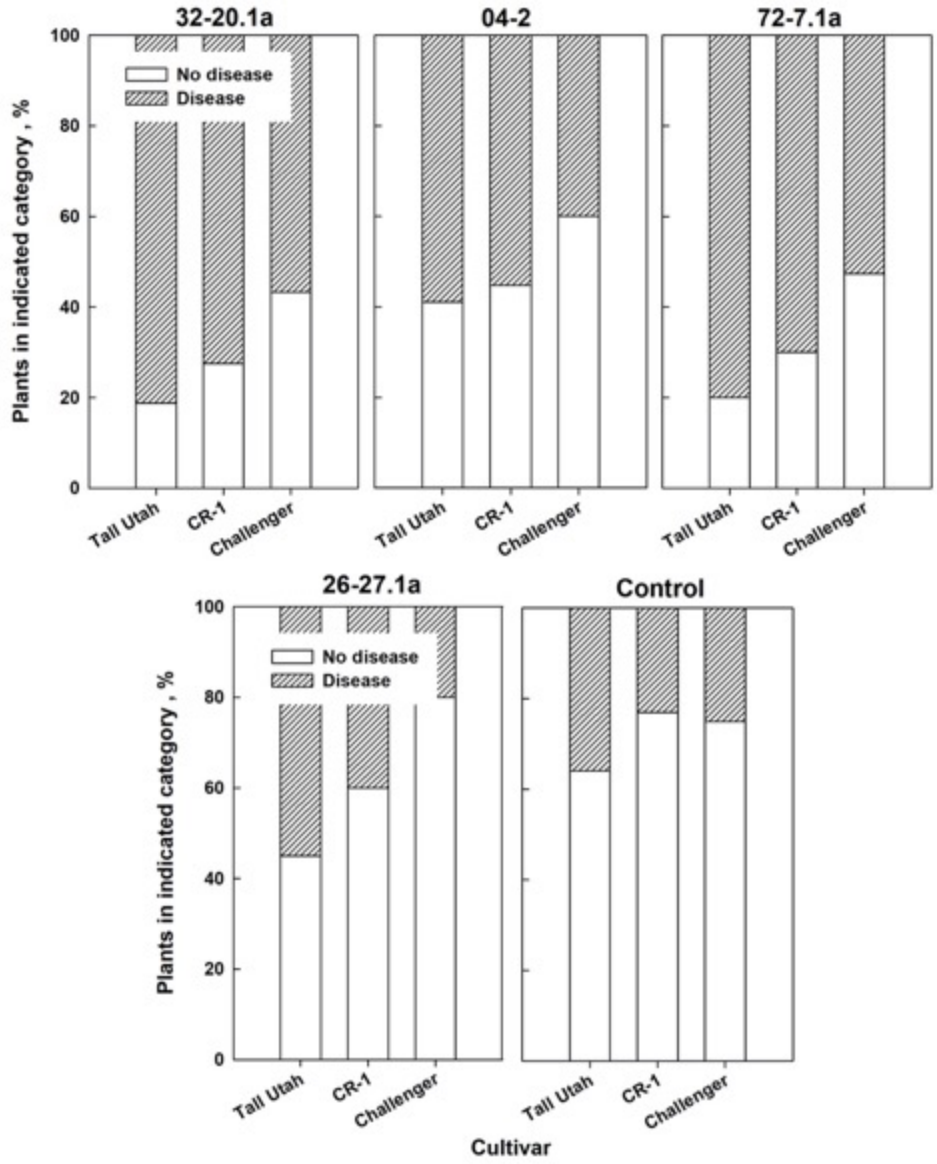

Figure 1.5: The effect of inoculation with *Fusarium oxysporum* f. sp. *apii* race 2 (*Foa* race 2) on root and vascular discoloration symptoms. 6-week old seedlings of cultivars Tall Utah 52-70R Improved (Burpee Seed Co. Warminster, PA, USA), CR-1 (provided by grower cooperator), and Challenger (Syngenta, Woodland, CA, USA) were transplanted into un-infested (control) soil or soil infected with an FOSC isolate in a growth chamber maintained at 28°C. Disease symptoms were evaluated based on a scale of 0= asymptomatic, 1= lateral roots discolored, 2=main root discolored, 3=crown discolored, 4=crown extensively discolored, 5=plant dead at 6 weeks after inoculation (Epstein et al. 2017). This scale was converted to a binary scale where a rating of 0 or 1 on 0-5 scale indicated no disease and a rating of 2, 3, 4, or 5 indicated disease (Kaur et al. 2022). Percentage of plants (n=40 across an experiment conducted twice) with or without disease symptoms is shown.

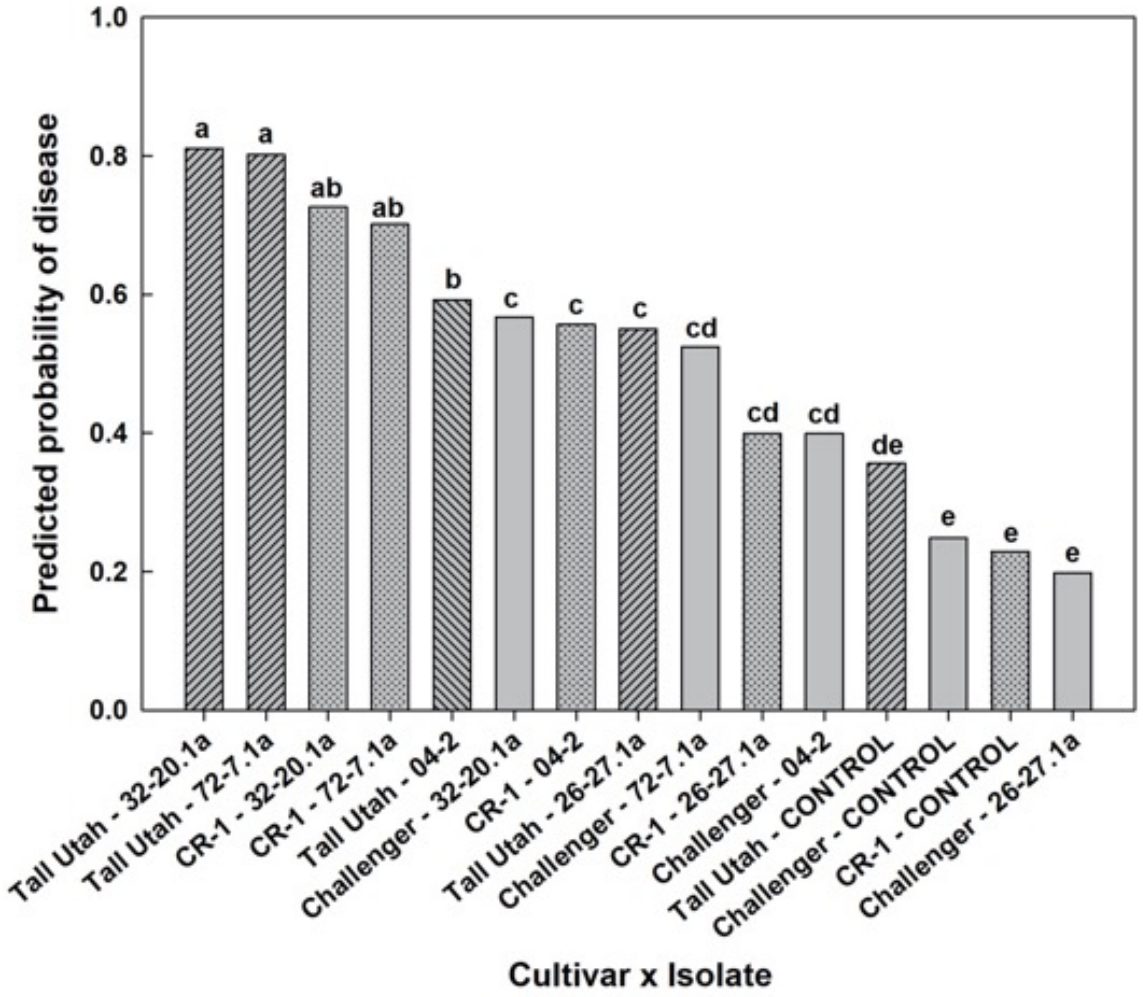

Figure 1.6: Predicted probability of disease in celery seedlings inoculated with isolates identified as *Fusarium oxysporum* f. sp. *apii* race 2 (*Foa* race 2). Isolates 32-20.1a, 72-7.1a and 04-2 were identified as *Foa* race 2 with diagnostic primers (Henry et al. 2020). 26-27.1a is an FOSC isolate that does not match the *Foa* race 2 haplotype. 6-week old seedlings of cultivars Tall Utah 52-70R Improved (Burpee Seed Co. Warminster, PA, USA), CR-1 (provided by grower cooperator), and Challenger (Syngenta, Woodland, CA, USA) were transplanted into un-infested (control) soil or soil infected with an FOSC isolate in a growth chamber maintained at 28°C. Disease symptoms were evaluated based on a scale of 0= asymptomatic, 1= lateral roots discolored, 2=main root discolored, 3=crown discolored, 4=crown extensively discolored, 5=plant dead at 6 weeks after inoculation (Epstein et al. 2017). This scale was converted to a binary scale where a rating of 0 or 1 on 0-5 scale indicated no disease and a rating of 2, 3, 4, or 5 indicated disease for percent predicted probability of disease data. The responses were then analyzed by logistic regression (Kaur et al. 2022). Each bar represents 40 single plant replicates across an experiment conducted twice. Bars with a letter in common are not significantly different based on 95% confidence intervals of predicted probabilities calculated in binary logistic regression analysis.

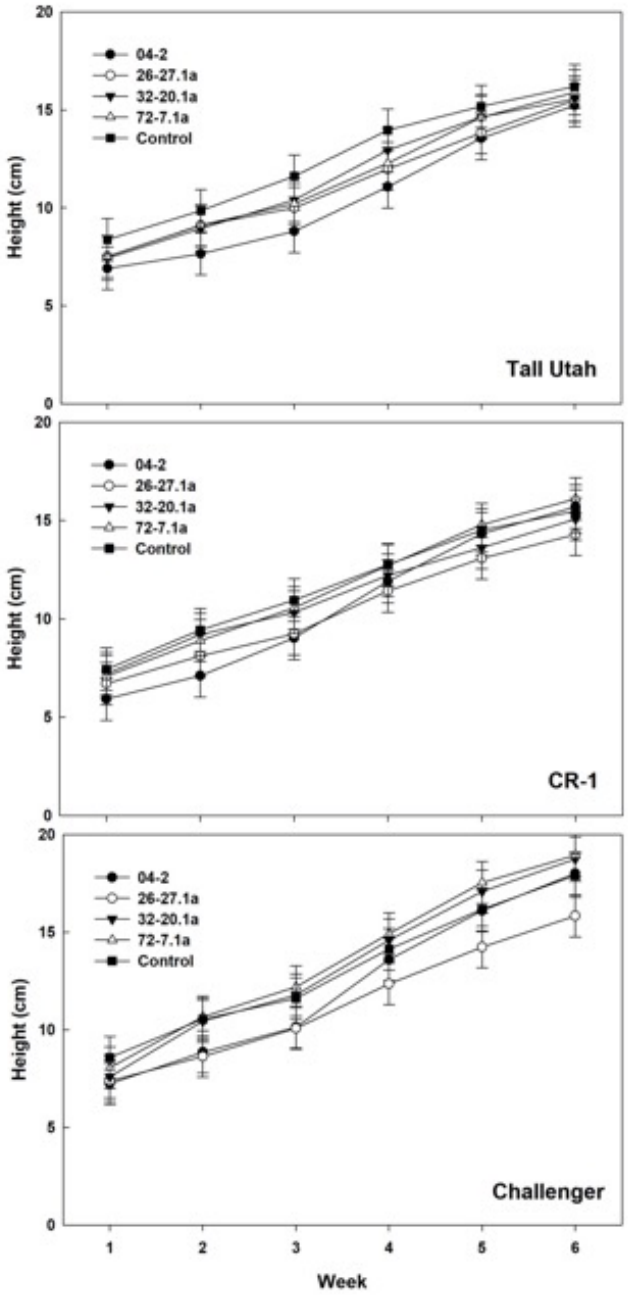

Figure 1.7: Plant height over time of celery seedlings inoculated with *Fusarium oxysporum* f. sp. *apii* race 2 (*Foa* race 2). 6-week old seedlings of cultivars Tall Utah 52-70R Improved (Burpee Seed Co. Warminster, PA, USA), CR-1 (provided by grower cooperator), and Challenger (Syngenta, Woodland, CA, USA) were transplanted into un-infested (control) soil or soil infected with an *Foa* race 2 isolate in a growth chamber maintained at 28°C. Plant height was recorded weekly for 6 weeks after inoculation. Error bars are based on least squared means difference. Each data point is based on 40 single plant replicates across an experiment conducted twice.

Trial 3: Celery seedlings ('Tall Utah 52-70', 'Golden Self Blanching', 'CR-1', and 'Challenger') were inoculated with four FSSC isolates. Disease severity scores were converted to binary ratings as previously described. A frequency table showing the occurrence of disease in plants inoculated with FSSC revealed no differences in disease by isolate or cultivars or between inoculated and control plants (Fig. 1.8). When the data were modeled using binary logistic regression, the main effects of isolate (P=0.76), cultivar (P=0.08) and their interaction (P=0.51) were not significant at the level of $P < 0.05$. Additionally, there were no differences in incidence (%) by isolate (P=0.85) or cultivar (P=0.36) and their interaction was not significant (P=0.83). There were no differences between cultivar x isolate treatment groups.

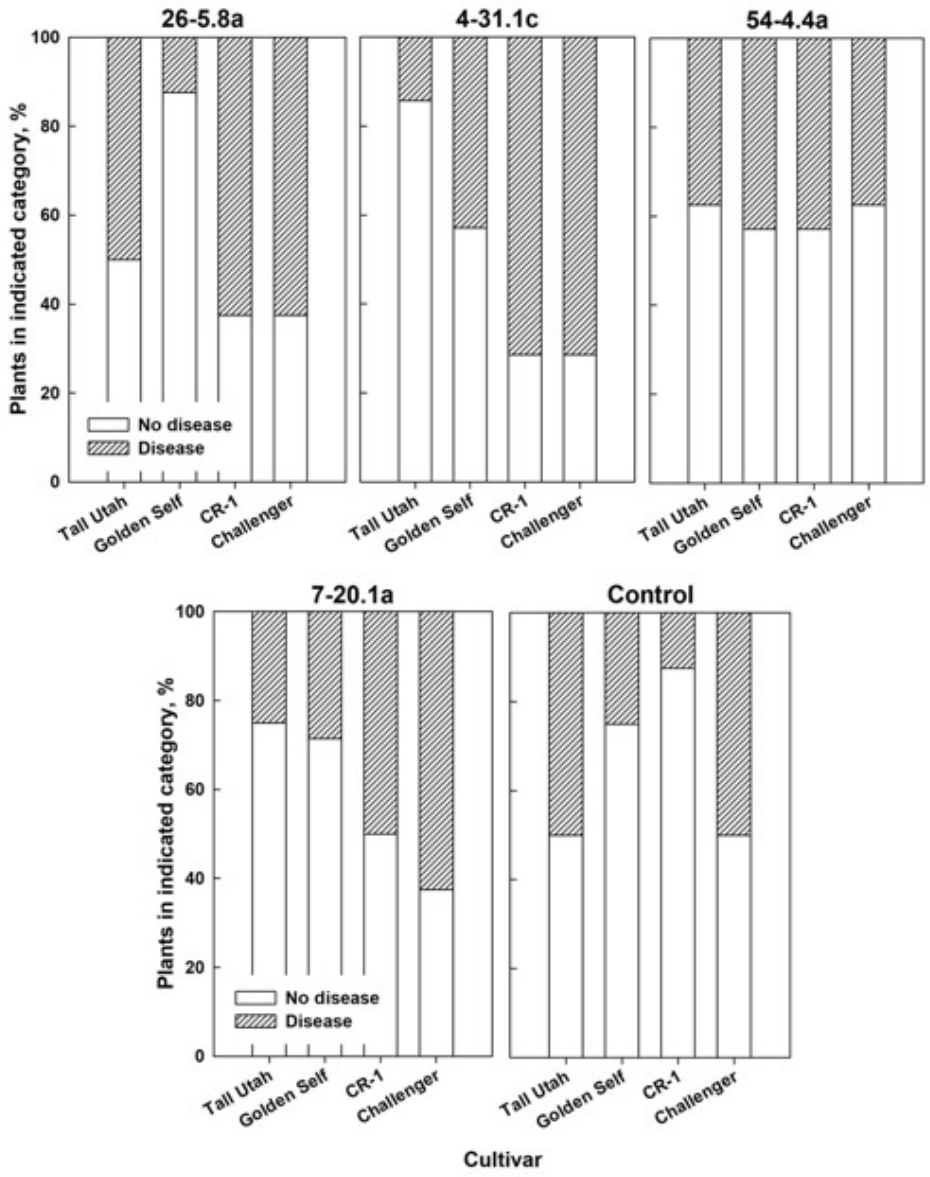

Figure 1.8: The effect of inoculation with *Fusarium solani* species (FSSC) complex on root and vascular discoloration symptoms. 6-week old seedlings of cultivars Tall Utah (Burpee Seed Co. Warminster, PA, USA), Golden Self Blanching (Seeds n' Such, Graniteville, SC, USA), CR-1 (provided by grower cooperator), Dutchess (provided by grower cooperator) were transplanted into un-infested (control) soil or soil infected with an FSSC isolate in a growth chamber maintained at 28°C. Disease symptoms were evaluated based on a scale of 0= asymptomatic, 1= lateral roots discolored, 2=main root discolored, 3=crown discolored, 4=crown extensively discolored, 5=plant dead at 6 weeks after inoculation (Epstein et al. 2017). This scale was converted to a binary scale where a rating of 0 or 1 on 0-5 scale indicated no disease and a rating of 2, 3, 4, or 5 indicated disease (Kaur et al. 2022). Percentage of plants (n=8 across an experiment conducted twice) with or without disease symptoms is shown.

DISCUSSION

The first objective of this research was to understand the causal agents of "meltdown" symptoms on celery. Michigan celery growers have noted plant death in the field and reduced quality on the packing lines due to crown rot, wilting, stunting, chlorosis, and plant death; these symptoms are collectively referred to as "meltdown". FOSC and FSSC were the most frequently recovered organisms from plants with "meltdown" symptoms in 2018 and 2019. However, FOSC was more frequent in 2018 while FSSC was more frequent in 2019. A greater proportion of plants with minor symptoms were sampled in 2019 to reduce contamination by secondary organisms (Epstein et al. 2017). In 2019, samples (209) ranged from seedlings to mature plants while most samples (66) in 2018 were mature plants.

The second research objective was to identify FOSC isolates to *formae speciales* and race based on diagnostic primers and virulence testing. This research focused on Fusarium yellows, caused by *Fusarium oxysporum* f. sp. *apii* race 2, as this disease resulted in severe losses in the Michigan celery industry during the 1980s and 1990s until resistant cultivars were developed (Elmer et al. 1986; Lacy et al. 1996a; Orton et al. 1984b; Quiros 1993). Cultivar CR-1 was developed by a Michigan grower and is considered resistant to *Foa* race 2, although its genetic background is unknown. Germplasm used to develop 'CR-1' originated from celery lines developed through somaclonal variation. Michigan celery varieties are maintained by a small breeding effort supported directly by the largest growers (Phillips et al. 2020). Although Fusarium yellows has not caused significant losses for the Michigan celery industry since the development of resistant cultivars, it is probable that *Foa* race 2 is still present in Michigan celery production fields. *Foa* survives in soil but its population decreases overtime (Elmer and Lacy 1987; Toth 1989). *Foa* race 2 survives through chlamydospores and by asymptomatically

colonizing roots of plants also present in celery fields including onion, weed species, and moderately susceptible celery cultivars (Elmer and Lacy 1987; Toth 1989).

The observation of diseased plants originating from a field foci and slowly moving outward is common with soilborne pathogens including those that cause Fusarium yellows (Agrios 2005; Otto et al. 1976). Michigan growers have observed field patterns of "meltdown" that appear to differ from Fusarium yellows. However, many symptoms between "meltdown" and Fusarium yellows may be similar making it necessary to determine if FOSC isolates were *Foa* race 2.

Diagnostic primers can aid in identification of *formae speciales* and race (Baayen et al. 2000; Chiocchetti et al. 2001; Fernandez et al. 1998; Okubara et al. 2013) and primers for identification of *Foa* race 2 and 4 haplotypes were developed by Henry et al. (2020). Screening FOSC isolates from symptomatic plants during 2018 and 2019 with diagnostic primers for *Foa* race 2 and 4 markers (Epstein et al. 2017; Henry et al. 2020) revealed that about 20% of FOSC isolates collected (21 of 101) had a positive band when tested with the *Foa* race 2 primers and none had a positive band using race 4 primers.

The proportion of FOSC identified as *Foa* race 2 isolated from symptomatic plants in this study differed from that reported in Epstein et al. (2017). Isolates collected from celery with Fusarium yellows symptoms in California fields included 77 *Foa* race 2 isolates (44%) and 83 nonpathogenic isolates (47%) of the 174 isolates collected (Henry et al. 2020). Epstein et al. (2017) reports that isolates collected from the margin of symptomatic tissue will include at least one *F. oxysporum* f. sp. *apii* isolate and up to two nonpathogenic strains. Isolation of nonpathogenic strains from celery with Fusarium yellows has been previously reported in other studies (Correll et al. 1986a; Schneider 1984).

Foa race 2 is monomorphic with no evidence of a change over time or location based on next generation sequencing of a selection of *Foa* race 2 isolates by Epstein et al. (2017). Additionally, ATCC isolate 52626 (American Type Culture Collection, Manassas, VA) which is a highly virulent historic *Foa* race 2 isolate collected from celery in Michigan (Elmer et al. 1986) was screened using *Foa* race 2 primers (Henry et al. 2020) and a positive band was observed. This Michigan *Foa* race 2 which was deposited prior to 1986, did not appear to differ from the California *Foa* race 2 population for which the diagnostic primers where developed (Epstein et al. 2017; Henry et al. 2020).

FOSC can live saprophytically in soil or cause disease (Gordon 2017); identification of *formae speciales* and race within FOSC is based on virulence on a host or cultivar (Correll et al. 1986b; Hart and Endo 1981). After optimizing pathogenicity methods, trial 1 included a diverse group of FOSC isolates for pathogenicity indicating FOSC isolates cause varying levels of root rot and vascular discoloration. The *Foa* race 2 isolates tested consistently caused disease symptoms; further experiments focused on FOSC isolates identified as *Foa* race 2 as Fusarium yellows is a historically important disease in Michigan celery production.

Three *Foa* race 2 isolates collected from plants with "meltdown" symptoms were pathogenic. Isolate 26-27.1a was initially misidentified as *Foa* race 2. Thus, it could serve as a unintentional negative control. It consistently caused a lower level of symptoms than the *Foa* race 2 isolates tested. *Foa* race 2 susceptible 'Tall Utah' caused a higher level of disease than resistant 'Challenger' (Epstein et al. 2017); 'CR-1' showed an intermediate level of symptoms. 'Challenger's' *Fu1* gene denotes resistance to *Foa* race 2 (Epstein et al. 2017; Orton et al. 1984a; Quiros 1993). 'CR-1' is considered to be resistant to Fusarium yellows by Michigan growers although replicated, controlled tests have not been conducted.

Foa race 2 isolates made up a low percentage of the organisms recovered from plants with "meltdown" symptoms and FSSC made up approximately 50% of the isolates. A small group of FSSC isolates were tested for virulence. When plants were inoculated with FSSC isolates the plants were similar to the control plants. Increasing the number replicates could decrease variability between treatments. FSSC occupies a similar ecological niche to FOSC. It can inhabit agricultural and nonagricultural soils, asymptomatically colonize plant tissues (Coleman 2016; Šišić et al. 2018) and cause root rot on a number of crops (Coleman 2016). FSSC is not reported to cause disease on celery (Davis and Raid 2002; Koike et al. 2007; Sherf and Macnab 1986), but can cause disease on other Apiaciae crops including carrot (Davis 2002; Zhang et al. 2014) and coriander/cilantro (Bhaliya 2014; Estévez De Jensen 2009; Satyanarayana et al. 2021).

FOSC isolates are classified by virulence, but pathogenicity testing is time consuming and influenced by the environment, host age, disease rating scale, and inoculation method (Correll 1991; Hart and Endo 1981; Hopkins et al. 1992; Kraft and Haglund 1977; Leslie and Summerell 2006; Priest and Letham 1996). Uniform disease across *Foa* virulence experiments is difficult to achieve (Correll et al. 1986b; Hart and Endo 1981). Our data indicate that *Foa* race 2 isolates from plants with "meltdown" symptoms cause disease, but variability was observed among and within trials.

Foa race 2 is associated with a relatively small proportion of plants with "meltdown" symptoms. In comparison, the highly virulent *Foa* race 4 appeared in one field in California (Epstein et al. 2017) and has not been reported outside of a restricted area of the state (Epstein et al. 2017; Henry et al. 2020). FSSC and FOSC isolates, including some identified as *Foa* race 2

using diagnostic primers and pathogenicity testing are associated with “meltdown” symptoms but further research is needed to understand celery “meltdown”.

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CHAPTER 2: CHARACTERIZING VIRULENCE OF *PYTHIUM* SPP. AND EVALUATION OF PLANT PROTECTANTS FOR ROOT ROT CONTROL ON CELERY GREENHOUSE SEEDLINGS

ABSTRACT

Michigan commercial celery production starts with greenhouse grown transplants ensuring a uniform and fast maturing crop. Root rot caused by *Pythium* spp. affects celery transplants. The objectives of this research were to compare the virulence of *Pythium* spp. isolates collected from celery and determine efficacy of fungicides to control Pythium root rot on celery transplants. *Pythium* spp. were isolated from celery plants ('CR-1', 'WA-7', and 'Dutchess') in greenhouses and fields during 2014 to 2015. The most common *Pythium* spp. observed when identified by sequencing of the internal transcribed spacer (ITS) region were *P. mastophorum* (55%), *P. intermedium* (16%), *P. sulcatum* (10%), *P. aff. diclinum* (6%), and *P. sylvaticum* (5%). Virulence by *Pythium* spp. was evaluated by transplanting seedlings into soil mixed with millet colonized with the *Pythium* spp.. *P. sulcatum* and *P. sylvaticum* caused symptoms of root rot and stunting. Biorational products registered for use on celery in the greenhouse with the active ingredients *Streptomyces lydicus* (Actinovate) and phosphorus acid salts (Kphite) were evaluated. Additionally, unregistered ingredients were tested including mefenoxam (SubdueMaxx), ethaboxam (Elumin), and cyazofamid (Segway). Phosphorous acid salts, ethaboxam, and mefenoxam were effective at controlling Pythium root rot, having a similar level of root rot symptoms compared to the uninoculated control. An understanding of the different *Pythium* spp. in celery production greenhouse settings, their virulence, and the identification of effective options for chemical control can direct management of Pythium root rot.

INTRODUCTION

Celery production has a long history in Michigan and the crop was first grown in Kalamazoo as early as 1856 (Lucas and Wittwer 1952). Since celery seed is small and may not germinate readily, all commercial field production begins with greenhouse transplants (Hausbeck 2011; Phillips et al. 2020) ensuring a uniform and fast-maturing crop (Hausbeck 2011; Zandstra et al. 1986). Seed is sown into plug trays filled with a peat-based media (Hausbeck 2011; Zandstra et al. 1986). Historically, transplants were produced in seed beds inside of greenhouses made of wood with a removable sash or in a glass greenhouse structure heated by a stove (Halligan 1913). Celery transplants in seedbeds or in the greenhouse may be affected by damping-off and root rot caused by *Pythium* spp. or *Rhizoctonia solani* (Egel 2020; Sherf and Macnab 1986); healthy transplants are important for an even plant stand in the field (Phillips et al. 2020). An extension publication from 1952 recommended a steam-sterilized or disease-free muck soil for producing greenhouse transplants as celery seedlings are susceptible to damping off (Lucas and Wittwer 1952).

Cultural strategies to limit Pythium root rot include water management and sanitation. Recommended greenhouse sanitation practices include cleaning trays, bench tops and equipment, and using pathogen-free rooting media and irrigation water (Nunez and Westphal 2002; Egel 2020). Avoiding overwatering is important as wet soil conditions promote zoospore movement and contact with roots (Koike et al. 2007; Martin 2002). Cultural practices may be integrated with biorational agents and/or chemical treatments to limit Pythium root rot.

Biorational products may play an important role in controlling Pythium root rot in greenhouse celery transplants as few fungicides are registered for this use. *Pythium* spp. are a good candidate to be managed using biological controls as they are susceptible to competition

and antagonism during their saprophytic growth phase (Martin and Loper 1999). The activity of the biorational agent must coincide with pathogen infection (Martin and Loper 1999; Whipps and Lumsden 1991). A differential response to biological controls within a pathogen population has been noted resulting in inconsistent control of soilborne pathogens (Martin and Loper 1999; Weiland 2014). Since biorational agents work in different ways, one proposed strategy is to combine complementary biorational agents to optimize control (Guetsky et al. 2002; Otto-Hanson et al. 2013; Weiland et al. 2013; Xu et al. 2011).

The biorational agent, *Streptomyces lydicus* WYEC108 available as Actinovate (Valent, Walnut Creek, CA) has demonstrated efficacy against *Rhizoctonia solani, Pythium aphanidermatum, Pythium irregulare,* and *Pythim ultimum* (Del Castillo Munera and Hausbeck 2015; Yuan and Crawford 1995). When phosphorus acid salts (Fungicide Resistance Action Committee (FRAC) 33) were evaluated on greenhouse celery seedlings inoculated with *Pythium* spp., the treated and inoculated seedlings were similar in health to the uninoculated untreated control (Hausbeck and Glaspie a,b).

Pythium spp. can live as saprophytes on organic matter in soil or cause disease (Martin and Loper 1999; Nunez and Westphal 2002). It is common for several *Pythium* spp. to be isolated from a single symptomatic root, making it difficult to attribute disease to a single species (Broders et al. 2007). *Pythium* spp. vary in their host range and virulence (Abad et al. 1994; Davison and McKay 2003; Larkin et al. 1995; Moulin et al. 1994). In greenhouse grown floriculture and vegetable transplants, several *Pythium* spp. may cause disease but may vary in virulence (Del Castillo Munera and Hausbeck 2016; Moorman et al. 2002). *Pythium* spp. observed in Michigan floriculture greenhouses varied by crop (Del Castillo Munera and Hausbeck 2016). Fungicides tested for control of Pythium root rot on greenhouse floriculture

crops varied in their efficacy based on the host and *Pythium* spp. that were evaluated (Del Castillo Munera and Hausbeck 2015). Understanding the *Pythium* spp. populations associated with specific crops and/or environments is important to develop effective root rot management strategies. The objectives of this study were to identify effective products for Pythium root rot of greenhouse celery seedlings and determine the virulence of *Pythium* spp. isolated from celery in Michigan greenhouses and fields.

MATERIALS AND METHODS

Isolate collection, selection and storage. *Pythium* spp. were isolated from 'CR-1', 'WA-7', and 'Dutchess' celery plants with symptoms of root rot obtained from Michigan greenhouses and fields during 2014 and 2015 (Hausbeck and Escobar-Ochoa 2014, 2015). Plants with symptoms of stunting, wilting, and/or chlorosis were collected from five farms and/or greenhouses and transported to the Hausbeck Lab at Michigan State University (MSU). Discolored roots were rinsed with tap water and sections were placed onto 1.6% water agar (BD Biosciences, San Jose, CA, USA). After incubation at room temperature under artificial lights for 4-5 days, the mycelial growth was transferred to potato dextrose agar (39 g PDA and 1000 ml distilled water) (Neogen, Lansing, MI, USA) (Hausbeck and Escobar-Ochoa 2014, 2015). Hyphal tip transfer was used to obtain pure cultures and identification to species was accomplished by sequencing the internal transcribed spacer (ITS) region. The sequence was run through to GenBank- NCBI BLAST to identify the isolate to species (Hausbeck and Escobar-Ochoa 2014, 2015).

Pure *Pythium* cultures were stored by adding 1 ml sterile water, and one sterile hemp seed, and two 0.5 cm^2 plugs of a culture growing on field isolation (FI) media to a sterile 1.7 ml microcentrifuge tube stored in an incubator at 10°C (Del Castillo Munera and Hausbeck 2016;

Hausbeck and Escobar-Ochoa 2015; Quesada-Ocampo et al. 2009). FI media consists of an amended V8 media modified from the BARP media described in Lamour and Hausbeck (2000) prepared using 12 g technical agar (BD Biosciences, San Jose, CA, USA), 40 ml V8 juice (Campbell Soup Company, Camden, NJ), 0.6 g calcium carbonate, 25 ppm (0.05 g) benomyl, 100 ppm (0.1 g PCNB (pentachloronitrobenzene)), 100 ppm (2 ml ampicillin stock), 30 ppm rifampicin (2 ml rifampicin stock) and 960 ml distilled water. Isolates were taken out of storage by placing the hemp seed from the microcentrifuge tube on a FI plate. Isolates used in experiments described below were selected from this culture collection maintained in the Hausbeck Lab at MSU.

Inoculum preparation and inoculation. Millet was colonized with *Pythium* spp. using isolates from the culture collection described above. Celery seedlings were inoculated by transplanting them into soil mixed with the colonized millet. *Pythium* species were tested for virulence. Fungicides and a biorational were tested for control of Pythium root rot with millet seed inoculum prepared as described by Quesada-Ocampo et al. (2009) and inoculation accomplished as outlined by Del Castillo Munera and Hausbeck (2015). To prepare inoculum, eight plugs (1.5 cm in diameter) were removed from actively growing mycelia from three-week old cultures growing on dilute V8 media (16 g technical agar, 40 ml V8 juice, 1.5 g calcium carbonate, and 960 ml distilled water (Hausbeck and Glaspie 2008b; Quesada-Ocampo et al. 2009)) and added to mushroom bags (RJC Sales/Shroom Supply, Brooksville, FL) containing 200 g of millet seeds mixed with L-asparagine (0.16 g) and water (144 ml) (Quesada-Ocampo et al. 2009). One-half of the amount of millet was prepared per *Pythium* sp. for the virulence trial. A control millet bag was prepared for each trial and received dilute V8 plugs. The mushroom bag containing millet was autoclaved for 45 minutes on two consecutive days prior to adding the

plugs of colonized media (Quesada-Ocampo et al. 2009). The inoculated millet was kept at room temperature (approximately 20°C) for three weeks and bags were shaken every two to three days for even colonization of the inoculum (Del Castillo Munera and Hausbeck 2015; Hausbeck and Glaspie 2008b).

Celery 'CR-1' seed provided by a grower cooperator was sown in 128-cell plug trays (Ball Horticultural Company, West Chicago, IL) filled with Suremix soilless potting media (Michigan Grower Products, Inc., Galesburg, MI) that had been autoclaved for 45 minutes to steam sterilize. Six to seven-week old seedings were inoculated by adding colonized millet (4 g) and a celery seedling to a well in the center of a square pot with dimensions 9.0 cm tall with the top of the pot measuring 9.0 x 9.0 cm and the base of pot measuring 6.5 x 6.5 cm were used for all trials. Each experiment was arranged in a completely randomized experimental design with eight single plant replicates; each plant replicate was grown in an individual pot with a saucer. The celery seedlings were inoculated and maintained in the greenhouse on a raised plant bench. Seedlings were overhead irrigated as needed to maintain moist soil with 200 ppm Jack's water soluble 20-20-20 general purpose fertilizer (JR Peters Inc., Allentown, PA). The trials were conducted in the Plant Sciences Research Greenhouses located on the campus of MSU.

Disease assessment. A visual assessment scale was modified from the disease severity scale presented in Del Castillo Munera and Hausbeck (2015) where 1= healthy plant, 2-9= progressive symptoms of wilting, chlorosis and stunting, and 10= dead plant. Root rot disease severity was assessed at the completion of each trial. Plant roots were carefully washed to observe root rot symptoms and rated according to the following scale: 1=normal, healthy appearance of the root system; 1.5=occasional lesions or slight discoloration; 2=≤25% of the primary and lateral roots with lesions or discoloration; 2.5=26-50% of the primary or lateral

roots with lesions or discoloration plus lateral roots pruned; 3=51-75% of the roots with lesions or discoloration plus many lateral roots pruned; 3.5=76-90% of the lateral roots missing or completely rotted and primary roots extensively discolored; 4=>90% lateral roots missing and primary roots completely discolored; 5=root system completely disintegrated and detached from the stem (Hausbeck and Escobar-Ochoa 2015). Foliar plant weight was recorded at the completion of the trial.

Pythium spp. virulence trial. The inoculum used contained one to four isolates of each of the following: *P. sylvaticum*, *P. sulcatum*, *P. mastophorum*, *P. aff. diclinum*, and *P. intermedium*. A separate bag of inoculum for each species and for the control was prepared. When multiple isolates were available for a species, an equal number of plugs from each isolate was added to the inoculum for a total of eight plugs (1.5 cm in diameter). The isolates used to prepare inoculum originated from celery plants and included: *P. aff. diclinum* (1 isolate from a field plant), *P. sylvaticum* (2 isolates from field plants), *P. sulcatum* (1 isolate each from the field and greenhouse, *P. intermedium*, (2 isolates each from the field and greenhouse), and *P. mastophorum* (2 isolates from the greenhouse). An uninoculated control was also included in each experiment. Trials were conducted from 27 July to 24 August (trial 1), 2 to 30 September (trial 2), and 1 to 29 October (trial 3), 2021.

The mean air temperatures were 26.9°C (trial 1), 25.1°C (trial 2), and 24.6°C (trial 3). Plant height and health rating were recorded weekly from one to four weeks after inoculation.

Plant protection trial. Inoculum contained one isolate each of *P. sylvaticum*, *P. oopapilum*, *P. sulcatum*, *P. aff. diclinum*, *P. coloratum,* and three isolates of *P. intermedium*. Isolates of *P. coloratum*, *P. oopapilum*, *P. mastophorum*, and two isolates of *P. intermedium* originally obtained from celery transplants. Isolates of *P. sylvaticum*, *P. aff diclinum*, *P.*

coloratum and one isolate of *P. intermedium* originated from field plants. Multiple isolates of *P. intermedium* were used to represent the prevalence of these isolates within the culture collection. Not all *P. mastophorum* isolates could be recovered from storage so one was included in the plant protection trial and two were included in the *Pythium* spp. virulence trial.

Treatments included three fungicides, two biorational products, an untreated uninoculated control, and an untreated inoculated control (Table 2.1). Actinovate containing *Streptomyces lydicus* (Valent USA Corp., Walnut Creek, CA) and Kphite containing phosphorus acid salts (Plant Food Systems Inc., Zellwood, FL) are registered for use on celery seedlings in the greenhouse. Mefenoxam may be used on celery to control Pythium root rot in the field but not in the greenhouse (Egel 2020). Elumin containing ethaboxam (Valent USA Corp., Walnut Creek, CA) and Segway containing cyazofamid (OHP Inc., Mainland, PA) are not registered for use on celery in the greenhouse or the field . The experiment was conducted from 2 April to 3 May (trial 1), 6 May to 7 June (trial 2) and 15 November to 7 December (trial 3) of 2021 at the Plant Sciences Research Greenhouses located on the campus of MSU. Each of the 42 plants was considered an experimental unit with six single 'CR-1' plant replicates per treatment. A single fungicide treatment was assigned to each plant.

Six- to seven-week old seedings were inoculated as described in the *Pythium* spp. virulence trial by transplanting seedings into soil infested with millet inoculum. Fungicide and biorational treatments were applied as a drench with a volume sufficient to overflow the pot (approximately 80 ml) about four hours after inoculation and again two weeks after inoculation according to labeled rates (Table 2.1). Plants were maintained in the greenhouse in the same manner as previously described in *Pythium* spp. virulence trial. Controls consisted of untreated uninoculated plants (millet not colonized with *Pythium* spp.) and untreated inoculated plants

(inoculated with millet colonized with *Pythium* spp. but no fungicide or biological control product applied).

The mean air temperature was not recorded during trial 1. The average greenhouse air temperature was 26.7°C during trial 2 and 21.9 during trial 3. Plant height and plant health rating (1-10) were recorded weekly from one to four weeks after inoculation. At the end of trials 2 and 3, root rot severity was also visually assessed using a scale from 1-5 in the same manner as described previously.

Table 2.1: Fungicides used in plant protection trial. All products were applied as a drench at a 14-day interval at labeled rate.

Treatment	**Active Ingredient**	**Manufacturer[y]**	**FRAC Code[z]**	**Rate/L**
Untreated uninoculated	-	-	-	-
Untreated inoculated	-	-	-	-
SubdueMaxx	Mefenoxam	Syngenta	4	0.08 mL
Actinovate[x]	*Streptomyces lydicus*	Valent	-	0.45 g
Kphite[x]	Phosphorous acid salts	Plant Food Systems	33	3.74 mL
Elumin	Ethaboxam	Valent	22	0.62 mL
Segway	Cyazofamid	OHP	21	0.22 mL

[x]Registered for use on greenhouse grown celery transplants
[y]Syngenta = Syngenta Crop Protection Inc., Greensboro, NC; Valent = Valent USA Corp., Walnut Creek, CA; Plant Food Systems = Plant Food Systems Inc., Zellwood, FL; OHP = OHP Inc., Mainland, PA
[z]FRAC = Fungicide Resistance Action Committee

Pathogen confirmation. On completion of each experiment, approximately 20% of the symptomatic inoculated plants and at least one uninoculated control plant, were randomly selected for pathogen confirmation. Two pieces of root (1 cm length) with brown discoloration were rinsed in bleach solution (121 ml 8.25% sodium hypochlorite; 879 ml water) for 30 seconds, and then rinsed twice in sterile distilled water. The root pieces were allowed to air dry on a paper towel before being placed onto 1.6% water agar (BD Biosciences, San Jose, CA, USA). The resulting mycelial growth was then transferred to dilute V8 media for identification.

Pythium spp. was re-isolated from symptomatic plants and confirmed via microscopic observation of sporangia. In all experiments, *Pythium* spp. was isolated from symptomatic root pieces but not from control plant roots.

Statistical analysis. *Pythium* spp. virulence trial**.** Statistical analysis was performed using SAS Studio SAS OnDemand for Academics (SAS Institute Inc. Cary, NC) using the PROC MIXED and PROC GLIMMIX procedures. A model was established with species considered a fixed factor and trial considered a random factor. Trial was used as a blocking factor to analyze the data from the trial conducted three times.

Height and weight data were modeled as continuous variables using PROC MIXED to determine that the data met assumptions of normality using plots of residuals and data was tested for equal variance using Levene's test. PROC GLIMMIX was used to test for statistical differences in plant height and weight using Fisher's least significant difference (LSD) test. Kenward-Roger degrees of freedom approximation (ddfm=kr) was included to account for the unequal variance where needed. A repeated measures analysis was performed for weekly plant height data so that the height of plants inoculated with each species was compared each week. A autoregressive with heterogeneous variances variance-covariance structure was used.

Although root rating data were taken as an ordinal score (1-5), it was treated as a continuous variable in this analysis as it was based on the roots (%) that were discolored, and the data set met the assumption of normality after log transformation. The Kenward-Roger degrees of freedom approximation (ddfm=kr) was included to account for unequal variance. Statistical differences were determined using Fisher's LSD. Back-transformed means are presented in the results.

Plant protection trial. Plant protection trial data were modeled with treatment as a fixed factor and trial as a random factor to analyze the data from the trial repeated three times. Root rating data collected in trials 2 and 3 were analyzed by modeling the data in PROC MIXED. Log transformation allowed the data to meet the assumption of a normality. The Kenward-Roger degrees of freedom approximation (ddfm=kr) was included to account for unequal variance. The data were then analyzed as a continuous variable and back transformed means are presented in the results. Statistical differences were determined using Fisher's LSD. Plant height taken at the end of the trial was analyzed in the same manner as in the *Pythium* spp. virulence trial; repeated measures analysis was not performed.

RESULTS

Isolate collection. *Pythium* spp. isolates were obtained from plants collected from the field (19) and greenhouse (48) and included *P. mastophorum* (55%), *P. intermedium* (16%), *P. sulcatum* (10%), *P. aff. diclinum* (6%), *P. sylvaticum* (5%), P. *oopapillum* (5%), and *P. coloratum* (3%) (Table 2). *Pythium* spp. differed by farm and plant origin (greenhouse versus field). *P. mastophorum* was isolated from seedlings in one greenhouse but was not observed in the field. *P. intermedium* was found in three greenhouses and two fields. *P. sulcatum* was isolated from plants collected from one greenhouse and two fields. Finally, *P. sylvaticum* was isolated from plants sampled from one field.

Table 2.2: *Pythium* spp. isolates collected from celery with root rot symptoms in Michigan commercial greenhouses and fields in 2014-2015.

Species	Number of isolates collected	Percentage of collection
P. mastophorum	37	55%
P. intermedium	11	16%
P. sulcatum	7	10%
P. aff. diclinum	4	6%
P. sylvaticum	3	5%
P. oopapillum	3	5%
P. coloratum	2	3%

***Pythium* spp. virulence trial.** *Pythium* spp. differed in virulence according to a greenhouse assay. *P. sulcatum* and *P. sylvaticum* caused more severe root rot symptoms and plant stunting compared to other species tested and the uninoculated control (Table 2.3; Fig. 2.1). *P. sulcatum* and *P. sylvaticum* caused the most severe root discoloration (Fig. 2.1). Plants inoculated with *P. intermedium* had more root rot symptoms than the control but less severe symptoms than plants inoculated with *P. sulcatum* and *P. sylvaticum*. Plants inoculated with *P. mastophorum* and *P. aff. diclinum* were similar to the uninoculated control for root discoloration symptoms.

Isolates of *P. sulcatum* and *P. sylvaticum* caused stunting as indicated by plant height and weight (Table 2.3). *P. sulcatum* caused the most stunting. Plants inoculated with *P. sylvaticum* were less stunted than those inoculated with *P. sulcatum*, but were significantly shorter than the control. The remaining species tested (*P. intermedium*, *P. mastophorum*, and *P. aff. diclinum*) did not cause reduced plant height or weight compared to the control (Table 2.3). Interestingly, *P. mastophorum* and *P. aff. diclinum* had a significantly greater height than the uninoculated control (Table 2.3). Stunting caused by *P. sulcatum* was evident within one week following inoculation and stunting caused by *P. sylvaticum* was observed within two weeks after inoculation (Fig. 2.2).

Plant health rating (1-10) data are not presented as there were no significant differences among treatments.

Table 2.3: Virulence by five *Pythium* spp. on celery 'CR-1' seedlings. 6 to 7-week old celery seedlings were inoculated with *Pythium* spp. in a greenhouse. Root rating, height and above ground weight were recorded four weeks after inoculation.

***Pythium* spp.**	**Root rating (1-5)[y]**	**Height (cm)**	**Above ground weight (g)**
P. sulcatum	2.56 a[z]	11.42 d	7.26 d
P. sylvaticum	2.13 a	13.71 c	10.33 c
P. intermedium	1.41 b	15.98 a	16.02 a
P. aff. diclinum	1.16 c	16.44 a	15.66 ab
P. mastophorum	1.18 c	14.75 b	13.35 b
Control	1.13 c	14.92 b	14.15 ab

[y]A rating scale for disease observed on roots was established where 1=normal, healthy appearance of the root system; 1.5=occasional lesions or slight discoloration of the roots; 2=≤25% of the primary and lateral roots with lesions or discolored; 2.5=26-50% of the primary or lateral roots with lesions or discolored plus lateral roots pruned; 3=51-75% of the roots with lesions or discolored plus many lateral roots pruned; 3.5=76-90% of the lateral roots missing or completely rotted and primary roots extensively discolored; 4=>90% lateral roots missing and primary roots completely discolored; 5=root system completely disintegrated and detached from the stem (Hausbeck and Escobar-Ochoa 2015).

[z]Values in a column followed by the same letter are not significantly different according to Fisher's least square means significant difference (LSD) at $P < 0.05$. Root rating, height, and above ground weight were analyzed using a PROC GLIMMIX procedure. Values represent the means of 24 single plant replicates across a trial replicated three times.

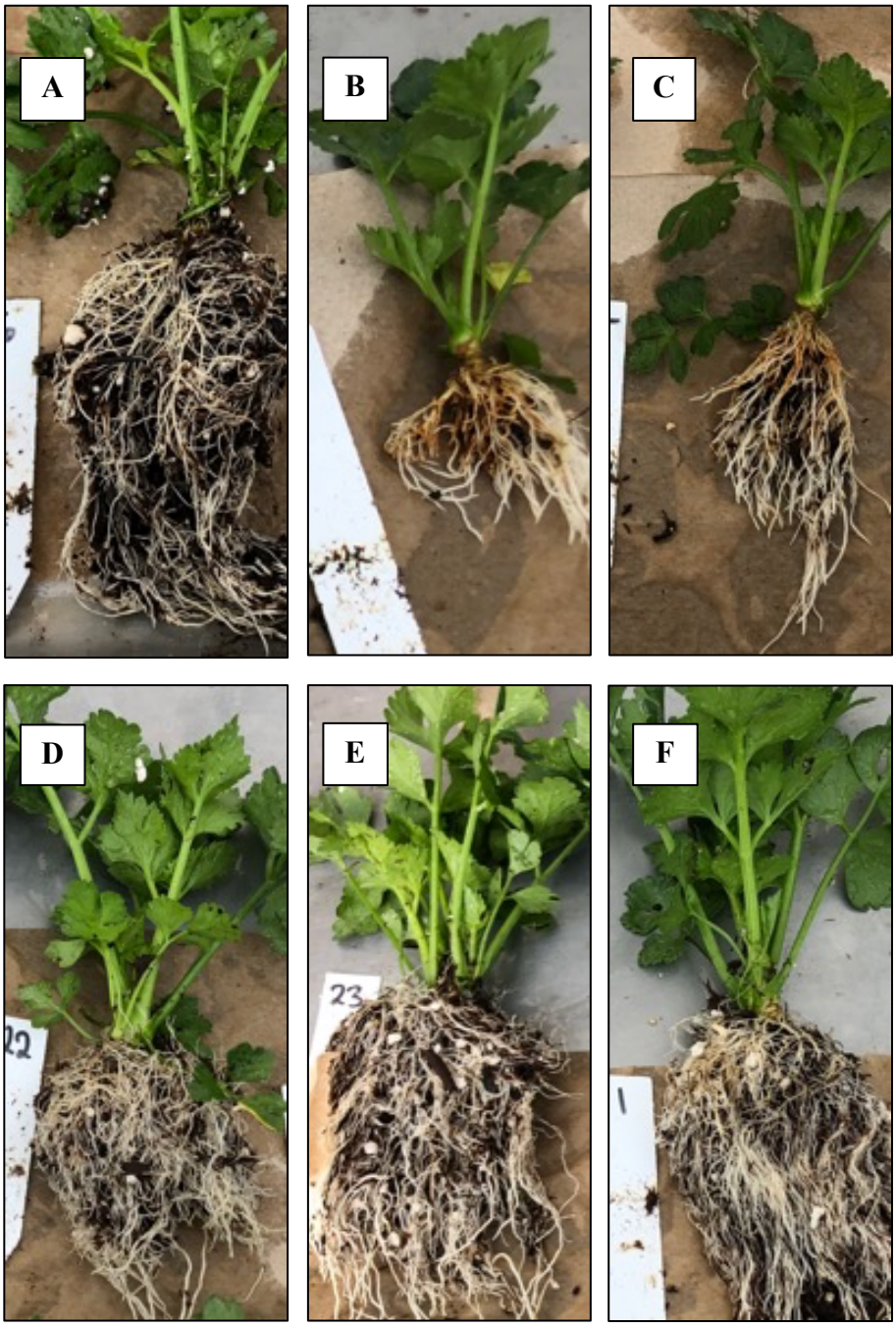

Figure 2.1: Root rot symptoms four weeks post-inoculation for celery 'CR-1' seedlings growing in the greenhouse in inoculated with the following *Pythium* spp.. (**A**) uninoculated control (**B**) *Pythium sulcatum* (**C**) *P. sylvaticum* (**D**) *P. intermedium* (**E**) *P. mastophorum* (**F**) *P. aff. diclinum.*

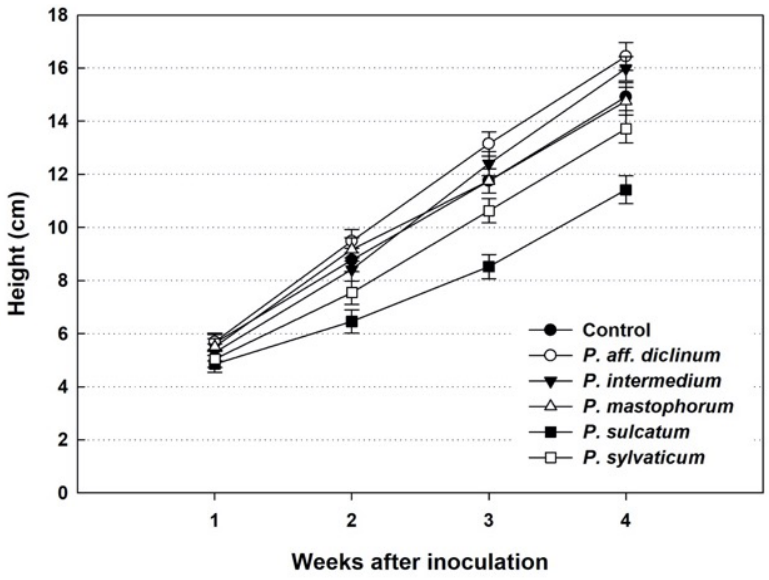

Figure 2.2: Plant height of celery 'CR-1' seedlings grown in a greenhouse inoculated with *Pythium* spp. over time. Values represent the mean height of 24 single plant replicates across an experiment conducted three times. Bars with a letter in common are not significantly different based on Fisher's LSD at $P < 0.05$.

Plant protection trial. Fungicide and biorational products (Table 2.1) tested varied in their efficacy against Pythium root rot (Figs. 2.3, 2.4; Table 2.4). Plant height did not differ among treatments or between treatments and the inoculated control (Table 2.4). Root rot data demonstrated differences in treatment efficacy (Table 2.4; Figs. 2.4). The inoculated untreated plants had significantly greater disease than the uninoculated control. Treatments of Elumin (ethaboxam), SubdueMaxx (mefenoxam), and Kphite (phosphorus acid salts) provided control and plants treated with these products did not differ in root rot compared to the uninoculated seedlings. Plants treated with Actinovate (*Streptomyces lydicus*) or Segway (cyazofamid) displayed root rot symptoms that were similar to the inoculated untreated plants. There were no significant differences in disease pressure based on root rating between trials (P=0.38). Plant health rating (1-10) data are not presented as there were no statistically significant differences among treatments.

Pathogen confirmation. Re-isolations from 20% of symptomatic plants were identified as *Pythium* spp. based on presence of sporangia and coenocytic hyphae. Control plants did not exhibit disease symptoms and *Pythium* spp. were not recovered from untreated uninoculated plants.

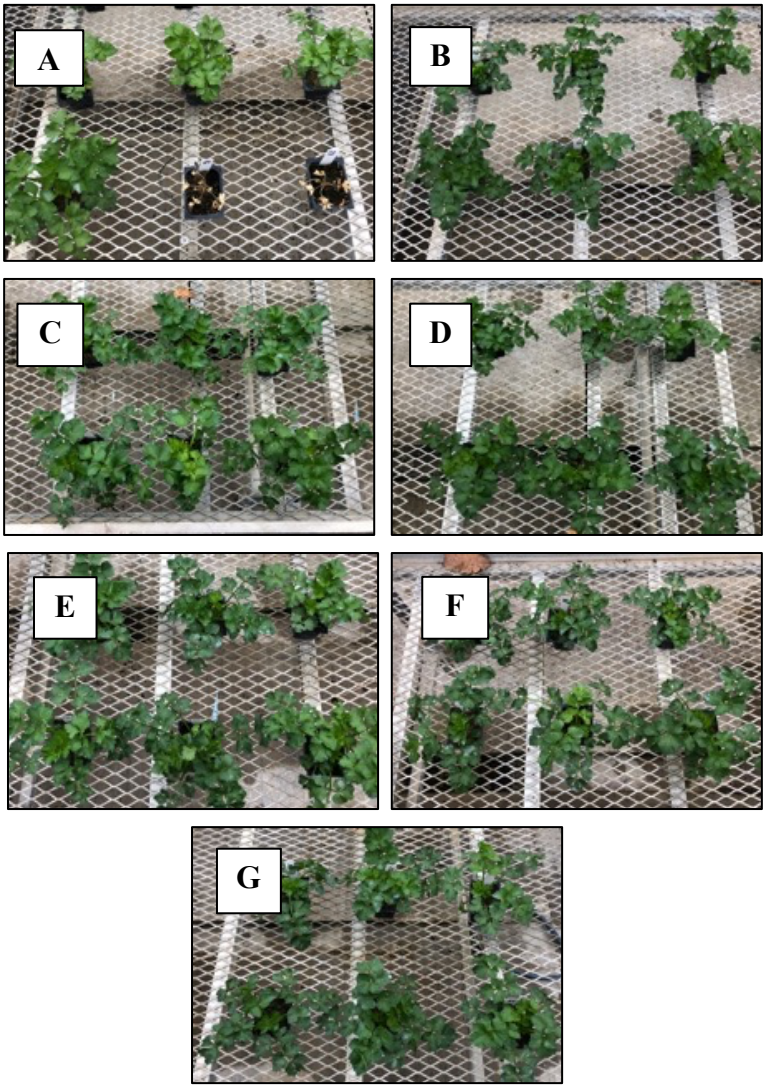

Figure 2.3: Symptom development of celery 'CR-1' seedlings inoculated with *Pythium* spp. and treated with fungicides in a greenhouse four weeks after inoculation in treatment groups (trial 1). (**A**) inoculated untreated control (**B**) uninoculated untreated control (**C**) SubdueMaxx (**D**) Actinovate (**E**) Elumin (**F**) Segway (**G**) Kphite.

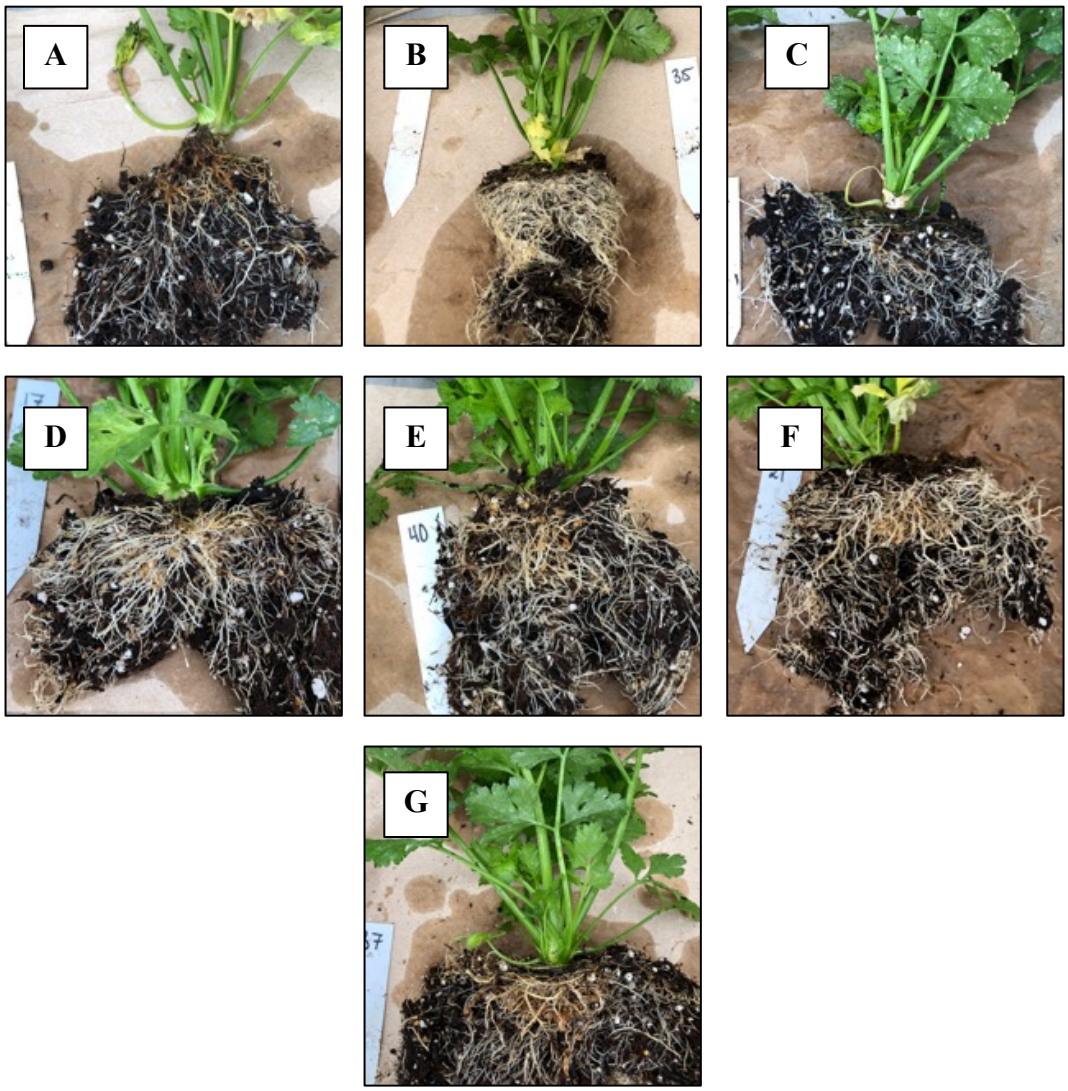

Figure 2.4: Symptom development of celery 'CR-1' seedlings inoculated with *Pythium* spp. and treated with fungicides in a greenhouse four weeks after inoculation in treatment groups (trial 2). (A) inoculated untreated control (B) uninoculated untreated control (C) SubdueMaxx (D) Actinovate (E) Elumin (F) Segway (G) Kphite.

Table 2.4: Efficacy of fungicide treatments in controlling Pythium root rot on greenhouse grown celery seedlings. 6 to 7-week old celery seedlings 'CR-1' were inoculated with *Pythium* spp.. Root rating and plant height were recorded four weeks after inoculation.

Treatment	**Root rating (1-5)** [x]	**Plant height (cm)**
Inoculated untreated	2.39 a [y]	13.56 [z]
Actinovate	2.34 a	16.31
Ranman	2.03 ab	14.78
Kphite	1.50 bc	17.53
SubdueMaxx	1.47 bc	17.03
Elumin	1.33 bc	16.36
Uninoculated untreated	1.16 c	17.31

[x]Symptoms of root rot were evaluated following the rating scale established in Hausbeck and Escobar-Ochoa (2015).
[y]Values in a column followed by the same letter are not significantly different according to Fisher's LSD at $P < 0.05$. Root rating, height, and above ground weight were analyzed using a PROC GLIMMIX procedure. Values represent the means of 18 single plant replicates across an experiment conducted three times.
[z]Height did not significantly differ between treatments.

DISCUSSION

Pythium root rot reduces transplant quality in Michigan celery production greenhouses; fungicide and biorational products can provide control (Hausbeck 2011; Hausbeck and Escobar-Ochoa 2015). Determining the *Pythium* spp. prevalent among celery plants in greenhouses and the field and their virulence is important to develop effective management strategies. Isolates in this study were identified by sequencing of the ITS region.

The most common *Pythium* spp. associated with root rot in Michigan celery from greenhouses and fields included *P. mastophorum*, *P. intermedium*, and *P. sulcatum*. This contrasts with the most common *Pythium* species observed in Michigan, Pennsylvania, and Ohio floriculture greenhouses that include *P. irregulare*, *P. ultimum*, and *P. aphanidermatum* (Del Castillo Munera and Hausbeck 2016; Moorman et al. 2002; Stephens and Powell 1982). *Pythium* spp. were found to vary by floriculture crop (Del Castillo Munera and Hausbeck 2016) suggesting they may differ in their ability to infect by host. The profile of *Pythium* spp. collected

from celery in this study differs from that observed in floriculture crops and those species reported to be associated with celery. Pythium root rot of celery is reported to be caused by *P. artotrogus*, *P. debaryanum*, *P. irregulare*, *P. mastophorum*, *P. paroecandrum*, and *P. ultimum* (Koike et al. 2007; Nunez and Westphal 2002; Starr and Aist 1976). *P. mastophorum*, obtained from one greenhouse in our study causes disease on greenhouse grown California celery (Vazquez et al. 1996). Additionally, *P. intermedium*, recovered from two greenhouses in this study, is associated with Pythium root rot of Apiaceae crops in Australia (Minchinton 2013). *P. sulcatum* and *P. sylvaticum* were observed to be most virulent on celery seedlings in our study, are not reported to cause disease on celery. *P. sulcatum* causes cavity spot on carrot in Washington (U.S), Australia and Egypt (Davison and McKay 2003; du Toit et al. 2014; El-Tarabily et al. 2004). *P. sulcatum* and *P. sylvaticum* are the most common species associated with cavity spot of carrot in Quebec (Allain-Boulé et al. 2004). *P. sulcatum* and *P. sylvaticum* isolates were collected in Michigan from celery greenhouse seedlings and field plants (*P. sulcatum)* or from field plants only (*P. sylvaticum*). Carrots are not currently grown in the celery production areas of the state and have not been grown in these regions in recent history.

Pythium root rot can become a chronic problem for individual growers or greenhouses depending on implantation of management strategies including sanitizing flats and fungicide use (Hausbeck et al. 2016). A resident population of *Pythium* spp. may become established in a greenhouse; this population can vary across greenhouses (Del Castillo Munera and Hausbeck 2016; Hausbeck et al. 2016). For isolates collected from Michigan celery, *Pythium* species varied by greenhouse. *P. mastophorum* isolates were collected from one of five greenhouses but was not found in the fields sampled. *P. intermedium* was observed in two greenhouses and two fields. *P. sulcatum* was isolated from plants from one greenhouse and three fields. *P. sylvaticum* was

not isolated from greenhouse samples but was found in field plants at one location. *P. sulcatum* and *P. sylvaticum* caused the most severe root rot symptoms compared to other *Pythium* spp. collected from Michigan celery production greenhouses. A range of virulence in *Pythium* spp. was observed in floriculture greenhouses (Del Castillo Munera and Hausbeck 2016; Moorman et al. 2002; Stephens and Powell 1982).

Soil temperature impacts growth of *Pythium* differently based on species (Martin and Loper 1999). *P. sulcatum* and *P. sylvaticum* have ideal growth temperatures of 28-30° and 25°C, respectively (Pratt and Mitchell 1973; Serrano and Robertson 2018; Van der Plaats-Niterink 1981). An ideal growth temperature has not been determined for the other *Pythium* spp. isolated in this study. However, *P. brassicum* which is closely related to *P. mastophorum*, has an ideal growth temperature of 25°C (Stanghellini et al. 2014) and *P. aff. diclinum* was reported to cause disease on dry bean at 20°C (Rossman et al. 2017). Greenhouse temperature averaged 25.5°C in the *Pythium* spp. virulence trial.

Virulence of *Pythium* spp. based on root rating did not differ significantly based on experiment (P=0.06). However, earlier trials had greater disease pressure leading to variability in ratings across trials. For example, *P. sulcatum* had an average root disease severity rating of 3.2 (trial 1) and 2.0 (trial 3). The average greenhouse temperature was 26.9°C and 24.6°C in trials 1 and 3, respectively; a higher greenhouse temperature might have favored disease caused by *P. sulcatum*. However, *P. sulcatum* and *P. sylvaticum* caused the most severe disease symptoms compared to other species tested across all trials. Understanding the *Pythium* spp. population in a greenhouse and their optimum temperature could lead to an integrated management approach to reduce the risk of root rot.

The isolates tested for virulence in this study were collected in 2014-2015 and were stored until testing in 2021. Only two *P. mastophorum* isolates were recovered from the 37 isolates originally collected. Since the isolates of other Pythium species were readily recovered from storage, the hemp seed storage method may not be optimal for *P. mastophorum*. Although our results indicated that *P. mastophorum* isolates were not virulent, they may not be representative of the samples originally collected.

There are limited products registered for control of Pythium root rot of celery seedlings in the greenhouse. Although Actinovate (*Streptomyces lydicus*) did not control Pythium root rot in our study, it was shown to control *P. aphanidermatum*, *P. irregulare*, *P. ultimum* from floriculture greenhouses (Del Castillo Munera and Hausbeck 2015), and *Pythium* spp., including *P. sylvaticum*, from tree nurseries (Weiland 2014; Weiland et al. 2013). However, in a diverse population sample of *Pythium* isolates from a forest nursery, *S. lydicus* inhibited all *Pythium* isolates but there were differences in growth inhibition, inhibition zone distance, and mortality among *Pythium* spp., isolates, and sampling location (Weiland et al. 2013). *Streptomyces* spp. act as antagonists of fungi and oomycetes by colonizing the rhizosphere and producing antibiotics and cell wall degrading enzymes including cellulases, hemicellulases, chitinases, and amylases (Yuan and Crawford 1995). The efficacy of biological control agents, such as Actinovate, vary in their ability to limit soilborne diseases (Handelsman and Stabb 1996; Linderman et al. 2008; Weiland 2014). Variability in the plant host, pathogen or biological control agent influence the success of the product (Handelsman and Stabb 1996; Weiland 2014). Mefenoxam (FRAC 4), was tested in this study, and is registered for control of Pythium root rot on celery in the field (Egel 2020). Mefenoxam has been widely used on greenhouse ornamentals and resistance in *Pythium* spp. is reported in Michigan and elsewhere (Del Castillo Munera and Hausbeck 2016;

Garzon et al. 2011; Lookabaugh et al. 2015; Moorman et al. 2002). Although mefenoxam effectively controlled Pythium root rot in this trial, it is unlikely that this fungicide would become registered for use on celery seedlings in the greenhouse due to the risk of pathogen resistance developing.

Fungicides containing ethaboxam (FRAC 22) (Scott et al. 2020; Wang et al. 2021) and cyazofamid (FRAC 21) (Kerns et al. 2009; Lookabaugh et al. 2021; Porter et al. 2006) are registered for control of other oomycete pathogens on vegetable crops including eggplant and pepper (Phillips et al. 2020). In this experiment, ethaboxam provided good control of Pythium root rot.

Products containing phosphorus acid salts (FRAC 33) provided control of Pythium root rot in this and previous experiments (Hausbeck and Glaspie 2008b, a). These products work through direct fungitoxic activity by stimulating host defenses (Guest and Grant 1991). Phosphorous acid salts are translocated to the xylem and phloem and have a relatively low risk of resistance development (Guest and Grant 1991; Landschoot 2016). Phosphorous acid salt products, including Kphite (Plant Food Systems Inc., Zellwood, FL), show promise for control of Pythium root rot in celery greenhouse transplant production.

Results indicate that control of Pythium root rot can be achieved with biorationals or fungicides. However, disease pressure was low in the experiments with an average root rating in the inoculated untreated control of 2.39 on a scale from 1 to 5. Experiments with higher disease pressure could provide an additional performance indicator of these products. A current survey of *Pythium* spp. in Michigan celery production greenhouses could be useful for comparison to earlier results. Fungicides and biorationals could also be tested for their efficacy in limiting specific *Pythium* spp.

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FUTURE WORK

Future investigation of celery "meltdown" could include additional sampling, pathogenicity testing and phylogenetic analysis of *Fusarium* spp. isolates. Sampling will include discussion of possible multiple symptom types previously classified as "meltdown" and isolation from plants by symptom type to determine if the microorganisms differ by farm, cultivar, plant age etc.. A small proportion of FOSC isolates match the *Foa* race 2 haplotype based on screening with *Foa* race 2 diagnostic primers (Henry et al. 2020) and are capable of causing disease. Questions remain about *Fusarium* spp. isolates collected that are not *Foa* race 2. Non-pathogenic FOSC isolates are diverse (Correll et al. 1986; Epstein et al. 2017) and *Foa* race 2 and race 4 are clonal haplotypes (Epstein et al. 2017; Henry et al. 2020; Puhalla 1984; Toth and Lacy 1991). Phylogenetic analysis to understand the diversity of FOSC isolates associated with "meltdown" would identify groups of genetically similar FOSC and/or FSSC isolates to be tested for pathogenicity. The pathogenicity tests presented here followed the methods described in Henry et al. (2020) with modifications including using a sterile millet in the control plants and conducting the trial in a growth chamber instead of a greenhouse. Some root and vascular discoloration was observed in the control plants, although the control still differed from those plants where a virulent *Fusarium* isolate was added. A control with no millet should be compared. Further research may identify the cause(s) of meltdown in Michigan which can direct management strategies.

Future study of Pythium root rot of Michigan celery could include a new survey of *Pythium* spp. in transplant production greenhouses and testing additional fungicides for efficacy. In the survey of celery production greenhouses and fields conducted in 2014-2015, *P. mastophorum* was the most frequently observed species but only two of 37 isolates stored using a hemp seed storage method (Quesada-Ocampo et al. 2009) could be recovered. The majority of

other species isolates were recovered from storage. Future research could compare survival of *Pythium* spp. by storage method. Another survey of celery transplant production should be conducted and new isolates for tested virulence to observe changes in the *Pythium* spp. population over time. Future studies evaluating virulence of *Pythium* spp. might include an in-vitro test of virulence of *Pythium* on germinating seed as described in Del Castillo Munera and Hausbeck (2016) and Broders et al. (2007). In the future, additional biorational products with active ingredients including *Trichoderma harizanum* could be tested for control of Pythium root rot. A factorial experiment testing efficacy of various fungicides by *Pythium* spp. would indicate if certain fungicides perform better in control of Pythium root rot based on which *Pythium* spp. are present informing fungicides used based on *Pythium* spp. present. Mefenoxam sensitivity is reported in floriculture production greenhouses (Del Castillo Munera and Hausbeck 2016; Garzon et al. 2011; Lookabaugh et al. 2015; Moorman et al. 2002). *Pythium* isolates from celery should be evaluated for mefenoxam sensitivity. Additional research can further understanding of *Pythium* spp. in Michigan celery production and fungicides to control Pythium root rot.

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