Characterization of Fusarium Dry Rot Pathogens of Potato and Fusarium Dry Rot Disease Management in the Pacific Northwest of the United States

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Abstract

Fusarium dry rot is a worldwide fungal disease of potato causing seed decay at planting and postharvest tuber decay in storage with lesions appearing as sunken, wrinkled, darkened areas on the periderm and dry, crumbly decayed tissue internally. With average yield losses of 6 to 25%, Fusarium dry rot is one of the most important postharvest potato diseases worldwide. The disease is managed through cultural practices such as wound management and crop rotation, chemical fungicides and use of less susceptible potato varieties. Recently, an increase of Fusarium dry rot has been observed by growers in the Pacific Northwest (PNW) of the United States, a major potato production region of the world where the states of Idaho, Oregon and Washington collectively produce around 11.3 million metric tons of potatoes annually. The reported increase in the disease justified an investigation of potential contributing factors. This study characterized *Fusarium* diversity associated with Fusarium dry rot of potato in the PNW using molecular and phylogenetic techniques, confirmed pathogenicity of PNW *Fusarium* isolates to potato, assessed relative aggressiveness of prevalent PNW Fusarium dry rot pathogens to selected potato varieties, screened *Fusarium* species *in vitro* for sensitivity to fungicides typically used for dry rot management and investigated fungicide efficacy in both seed treatment application and a field trial.

Fusarium isolates were recovered from tuber samples from seed and commercial storages using standard isolation and culturing techniques and single-spored to obtain pure cultures. Isolates were identified to species or species complex by sequencing portions of the translation elongation factor 1-alpha (*TEF*) and/or phosphate permease (*PHO*) genes, and identification was confirmed by phylogenetic analysis of selected isolates. For *Fusarium* species not previously reported as dry rot pathogens in the PNW or worldwide, whole asymptomatic tubers were wounded, inoculated, and incubated, with lesion presence or absence used to determine pathogenicity. The relative aggressiveness of four prevalent PNW *Fusarium* species on seven potato varieties important to the PNW was determined by wounding, inoculating and incubating whole asymptomatic tubers and then comparing lesion size among both species and varieties. The sensitivity of PNW *Fusarium* isolates to difenoconazole, fludioxonil and thiabendazole was assessed via three different methods: laboratory sensitivity screening tests using on fungicide-amended agar plates, laboratory tests of fungicide effectiveness as a seed treatment and a field trial testing effectiveness of fludioxonil against Idaho *F. sambucinum* isolates.

Twenty *Fusarium* species were recovered in this survey of PNW tubers, with *F. sambucinum* the most prevalent species in Idaho and *F. oxysporum* the most prevalent in Washington. Both species

combined accounted for approximately 50% of isolates recovered from each state. Pathogenicity to potato tuber was confirmed for 14 *Fusarium* species (*F. acuminatum*, *F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. equiseti*, *F. flocciferum*, *F. graminearum*, *F. oxysporum*, *F. redolens*, *F. sambucinum*, *F. solani*, *F. sporotrichioides*, *F. stercicola* and *F. venenatum*) including eight species not previously reported as Fusarium dry rot pathogens in the PNW. More severe *Fusarium* dry rot was observed with certain combinations of *Fusarium* species and potato variety, e.g., Umatilla Russet infected with *F. sambucinum* and Dark Red Norland infected with *F. oxysporum*. For main effects, *F. sambucinum* was determined to be the most aggressive of all tested species, followed by *F. avenaceum* and *F. oxysporum*. *Fusarium redolens* and *F. culmorum* were least aggressive.

Results of the fungicide sensitivity experiments indicate that difenoconazole remains a viable dry rot management fungicide. For fludioxonil, *in vitro* resistance of *F. sambucinum* was found in 67% of isolates tested while 43% of *F. oxysporum* isolates tested were resistant to the fungicide. However, in an experiment testing efficacy of fludioxonil as a seed treatment, *in vitro* resistance of *F. oxysporum* to fludioxonil had much less impact on disease levels in fludioxonil-treated seed tubers than *F. sambucinum*. Data from the two-year field trial indicated fludioxonil-resistant *F. sambucinum* isolates may increase dry rot incidence and severity in the field and decrease marketable and overall yield when a fludioxonil seed treatment is used. Thiabendazole resistance was found to persist in PNW *F. sambucinum* isolates, with 71% of isolates tested considered resistant. These findings emphasize the importance of variety selection and fungicide resistance management plans when addressing problems associated with Fusarium dry rot.

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Dedication

WITH ALL MY HEART, I am grateful to my husband, my parents, my grandmothers, my brother, my parents-in-law, my friends and my dogs for all their love, support, encouragement, sacrifice and longsuffering as I traveled this journey. *Ich liebe dich, mein Schatz!*

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Problem Statement

Fusarium dry rot is a worldwide fungal disease of potato causing seed decay at planting and postharvest tuber decay in storage. Growers around the world strive to manage Fusarium dry rot during potato production. The Pacific Northwest (PNW) region of the United States is a major potato production region of the world, and the PNW states of Idaho, Oregon and Washington collectively produce around 11.3 million metric tons of potatoes annually. Potato growers in the PNW have recently observed an increase in Fusarium dry rot. Speculated contributing factors to the reported disease increase include use of varieties that are more susceptible to Fusarium dry rot and/or bruising and shifts in cropping practices, handling practices, fungicide use, species composition of the pathogens, or fungicide insensitivity within species. A change in any one of these potential contributing factors would require refined management strategies.

Literature Review

The potato is an annual, herbaceous, dicotyledonous crop that can be propagated from true seed for breeding purposes, from tissue culture plantlets for mini-tuber production to be used for seed tuber production and from tubers for commercial production (Loria 2001). Vegetative propagation from tubers is the predominant method in commercial production. In contrast, in nature, the potato is a perennial plant that survives from season to season using modified underground stems, i.e., tubers. There are five growth stages in the potato life cycle: Stage I – sprout development, Stage II – vegetative growth, Stage III: tuber initiation, Stage IV – tuber growth, and Stage V – tuber maturation (Western Regional IPM Project (U.S.) and University of California Integrated Pest Management Program 2006). In North America, vegetative propagation of potatoes can be accomplished using whole tubers or by cutting larger tubers into smaller seed pieces approximately 42.5 to 85.0 grams in size (Duellman et al. 2021).

Potato is among the top five major food crops grown globally, along with wheat, rice, maize, and soybean and accounts for 2.5% of human calorie intake worldwide, compared to 18.3% for wheat, 18.9 for rice, 5.4 for maize and 3.3 for soybean (Savary et al. 2019). In 2019, potatoes accounted for 4% of global crop production (sugarcane 21%, maize 12%, wheat 8%, rice 8%, oil palm fruit 4%; FAO 2021). Asia produced 51% of the global potato supply followed by Europe 29% (FAO 2021). The top potato producing country was China accounting for about 25% of global output, followed by India, Russia, Ukraine and United States (FAO 2021). Together, China, India and Russia produce about 40% of the world's potatoes (FAO 2021). Top producing European countries include Germany,

France, The Netherlands, Poland, Belarus, and the United Kingdom (FAO 2021). Three of the top four potato producing states in the United States are in the PNW region. Potato production for the PNW was approximately 11.8 million metric tons in 2020, with approximately 6.1 million from Idaho, 1.2 million from Oregon and 4.5 million from Washington, and 11.2 million metric tons in 2021, with approximately 5.9 million from Idaho, 1.2 million from Oregon and 4.2 million from Washington (USDA-NASS 2022).

Fusarium Dry Rot of Potato

Fusarium dry rot is a worldwide fungal disease of potato causing seed decay at planting and postharvest tuber decay in storage (Azil et al. 2021; Baturo-Ciesniewska et al. 2015; Corsini and Pavek 1986; Du et al. 2012; Gachango et al. 2012; Heltoft et al. 2016; Kawchuk et al. 1994, 2002; Peters et al. 2008a; Secor and Salas 2001; Stefańczyk et al. 2016). This disease is caused by several species in the genus *Fusarium*. Yield losses due to Fusarium dry rot make it one of the most important postharvest potato diseases worldwide. Average decreased yield has been cited as 6 to 25% with some situations resulting in up to 60% storage loss (Baturo-Ciesniewska et al. 2015; Corsini and Pavek 1986; Du et al. 2012; Gachango et al. 2012; Heltoft et al. 2016; Kawchuk et al. 1994, 2002; Peters et al. 2008a; Secor and Salas 2001; Stefańczyk et al. 2016). Most seed lots and commercial crops have some dry rot infection. This can exclude seed lots from sale, may affect crop emergence and health and may affect the amount of time potatoes can be stored without sustaining financial loss (Peters et al. 2008a). The phase of the disease that affects seed potatoes in-season, i.e., either before, at, or shortly after planting, is also known as Fusarium seed decay. Fusarium dry rot affects most potato varieties, but varietal susceptibility to the disease is variable while no commercial varieties have high resistance (Azil et al. 2021; Aprasad et al. 1997; Bojanowski et al. 2013; Corsini and Pavek 1986; Esfahani 2005; Peters et al. 2008a; Stefańczyk et al. 2016). Within a variety, susceptibility may differ by *Fusarium* species (Bojanowski et al. 2013; Choiseul et al. 2007; Du et al. 2012; Esfahani 2005; Gachango et al. 2012; Ocamb et al. 2007; Peters et al. 2008a).

Fusarium Dry Rot Disease Symptoms

Symptoms and signs of Fusarium dry rot disease begin as small, brown shallow lesions on the tuber surface under the periderm, which can be seen early on in the first month of storage (Gachango et al. 2012; Heltoft et al. 2016; Nolte 1994; Peters et al. 2008a; Secor and Salas 2001; Wharton et al. 2007a). In early infection, the tuber flesh is honey- or chocolate-colored and still fairly firm. In later infection, lesions appear as sunken, wrinkled, darkened areas on the periderm, possibly in concentric rings with necrotic, desiccated tissue beneath. Mycelia and spores may come to the surface of tuber lesions in clumps. Mummification may result during continued storage with total decay of the tuber

flesh and shriveling of the desiccated periderm. Dissection of internal lesions often reveals dry, crumbly, tan to brown to black necrotic tissue. Decay of internal tissue can leave behind cavities containing visible mycelium of various colors such as white, yellow, and pink. Lesions may be evenly distributed from the original wound site or irregular in shape with tunneling patterns. Margins of lesion advancement may be quite distinct. Lesions may be invaded by secondary pathogens such as soft rot bacteria after dry rot has begun, e.g. *Erwinia* and *Clostridium* spp., especially if storage humidity is too high or moisture is allowed to accumulate on tuber surfaces (Secor and Salas 2001).

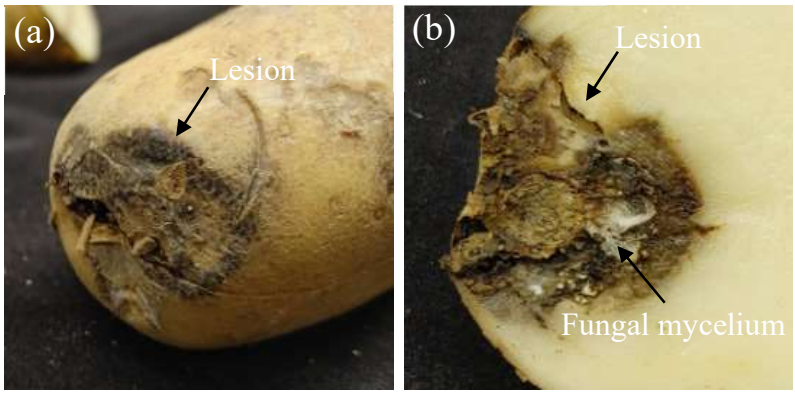

Figure 1-1. Fusarium dry rot disease symptoms. (a) External view of tuber lesion exhibiting darkened, sunken periderm. (b) Internal view of tuber lesion exhibiting dry, crumbly brown necrotic tissue and fungal mycelium. Photo credit: Kasia Duellman

Fusarium Dry Rot Disease Cycle

Wounds on the potato surface are required for initiation of disease as Fusarium dry rot pathogens cannot penetrate and infect healthy, intact tuber surfaces (Heltoft et al. 2016; Kawchuk et al. 1994; Peters et al. 2008a; Secor and Salas 2001). Periderm wounds occur during normal potato production activities and mechanized operations, i.e., seed cutting, planting, harvesting, handling, and grading) (Gachango et al. 2012; Peters et al. 2008a; Secor and Salas 2001). Insect, rodent and frost wounds can also occur (Gachango et al. 2012; Peters et al. 2008a; Secor and Salas 2001). Wound periderm is as resistant to *Fusarium* pathogens as native periderm (Lulai 2001b).

In the Fusarium dry rot disease cycle, *Fusarium* pathogens overwinter as spores on stored seed potatoes, i.e., seed-borne, or in the soil, i.e., soil-borne (Figure 1-1). These spores are the source of primary inoculum (Gachango et al. 2012; Peters et al. 2008a; Secor and Salas 2001). Most seed lots contain tubers with dry rot disease, i.e., infected, or inoculum, i.e., infested, or both. Infested soil may remain on tuber surfaces after harvest, bringing inoculum into storages. Planting contaminated seed infests the soil with the pathogen. Some *Fusarium* species produce chlamydospores, i.e., thick-walled asexual survival spores, that can survive in the soil without a host for long periods. As such, pathogen

inoculum can build up over time in soils where potatoes are routinely planted (Piepenbring 2015; Schumann and D'Arcy 2010; Secor and Salas 2001).

In the spring, whole tubers are cut into pieces for planting or planted whole. If seed tubers are already infected, spores can be moved during seed cutting onto cut seed surfaces or other wounds to start new infections. This is particularly a problem if cut seed is mishandled prior to planting, not given an opportunity to heal, or planted under conditions that promote the disease. Healthy seed is vulnerable to contamination or infection by Fusarium dry rot pathogens during cutting, handling and storage prior to planting. In the presence of infested or infected seed tubers, the risk of dry rot development increases (Gachango et al. 2012; Secor and Salas 2001). Infection begins when *Fusarium* spores germinate and hyphae enter through wounds on the seed tubers. Decay begins after infection and can develop even before seed is planted. Dry rot lesions develop most often on the cut surfaces of seed pieces. After one week, mycelium and brown and black flecks of decayed tissue are visible, and diseased depressions and pits can form by the second week after cutting. After planting, infected seed may continue to decay, and developing sprouts of the seed tubers can also become infected. Seed infection and decay can affect the rate of plant emergence, stand evenness, plant health, plant size, growth rate of plant, number of sprouts, sprout rot on seed tubers and number of marketable tubers (Gachango et al. 2012; Peters et al. 2008a; Secor and Salas 2001; Wharton et al. 2007a).

Spores that develop on decaying seed remain in the soil, in proximity to daughter tubers as they develop during the growing season. However, daughter tubers are not infected unless they incur wounds. At harvest, these spores either remain in the soil or adhere to tuber surfaces. Wounds incurred during harvest and handling provide entry points for spores on the tuber surface to start new infections. Tubers infected at harvest can decay while in storage. Non-infected tubers are contaminated with *Fusarium* spores and can serve as primary inoculum sources the next spring if the tubers are used for seed.

Critical time periods in the potato life cycle with regard to risk of Fusarium dry rot occur during the wound healing period after cutting seed, after planting, prior to skin-set, or periderm maturation, in the field, the wound healing period after harvest and the storage period for mature tubers. Environmental conditions affect the outcome of these critical time periods. For wound healing rate, important environmental factors are temperature, humidity and aeration. The growing season itself is not a critical time period for dry rot infection because tubers are not subjected to wounding.

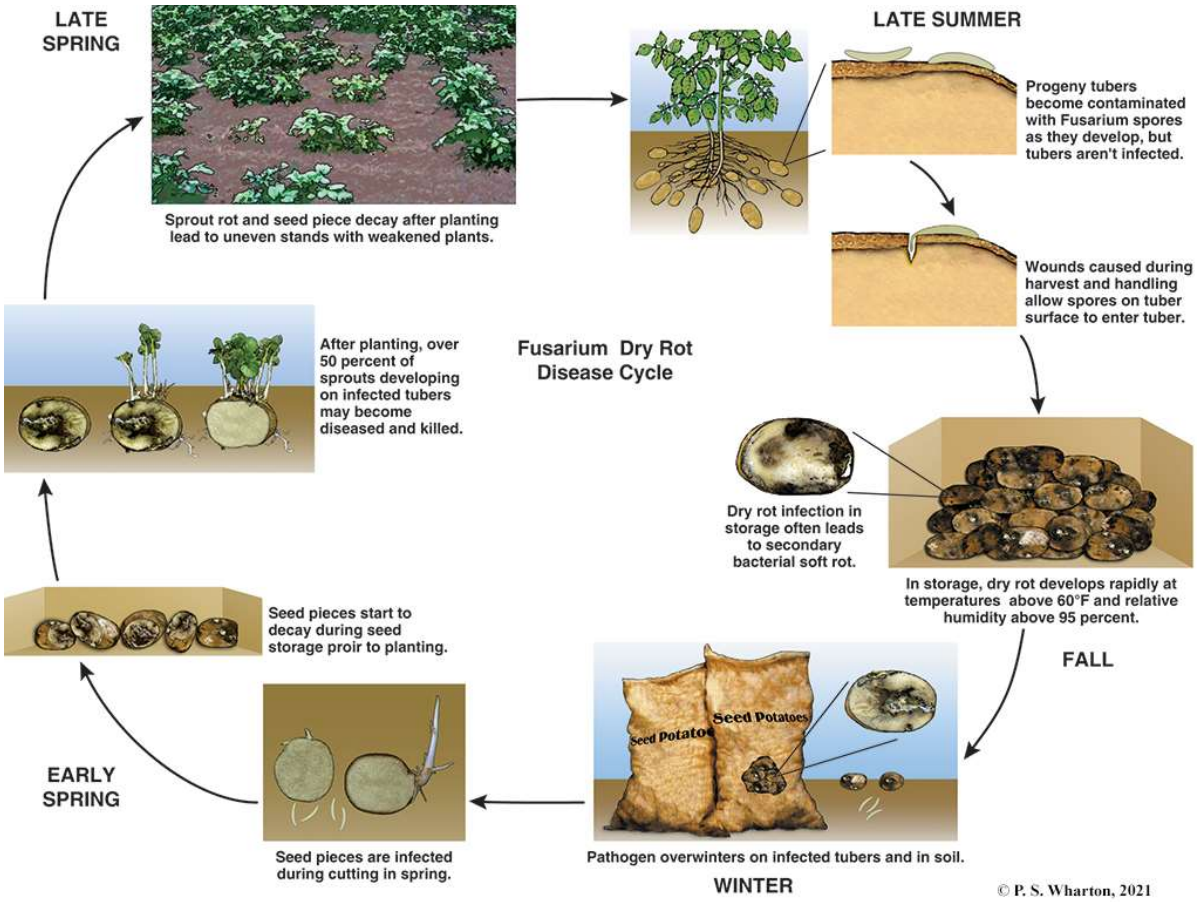

Figure 1-2. Fusarium dry rot disease cycle (Wharton et al. 2007).

Disease Management

Dry rot can be managed by specific sets of strategies at seed cutting, planting, harvest and post-harvest stages of potato production (Baturo-Ciesniewska et al. 2015; Bojanowski et al. 2013; Duellman et al. 2018; Gachango et al. 2012; Heltoft et al. 2016; Lulai 2001a, 2001b; Peters et al. 2008a; Secor and Salas 2001; Stefańczyk et al. 2016; Western Regional IPM Project (U.S.) and University of California Integrated Pest Management Program 2006; Wharton et al. 2007a). The goal is to reduce decay of seed in the field and harvested tubers in storage. Dry rot management is not actively pursued after planting as no post-planting, in-season dry rot management strategies are available. Cultural practices for dry rot management include using clean seed, implementing proper seed handling after cutting and treating and planting under conditions that favor plant growth and discourage disease. At harvest, tailoring harvest and handling practices to minimize wounds and promote wound healing is important. During storage, using appropriate storage temperature and humidity to discourage dry rot and maintain tuber quality is important. Chemical fungicide options are available to manage Fusarium dry rot as seed treatments, at planting in-furrow and post-harvest

prior to storage. Variety selection is an important factor in disease management as Fusarium dry rot susceptibility differs among potato varieties. Overall, fungicide seed treatments, wound minimization, and promotion of wound healing are the key strategies for Fusarium dry rot management to manage dry rot disease incidence and severity (Baturo-Ciesniewska et al. 2015; Peters et al. 2008a).

Cultural Practices

Pre-Plant and Planting

Most seed lots contain tubers with dry rot, and removal of infected seed before cutting and planting is recommended. Additionally, it is recommended that seed is provided conditions for wound healing after receiving it since transport of the seed can cause tuber wounds that allow disease development.

Planting seed into cold wet soil delays emergence, allowing more time for the pathogen to cause seed decay or sprout rot. After planting, seed decay and sprout rot results in non-emergence or delayed emergence of plants that are ultimately weaker than healthy plants (Wharton et al. 2007a). Dry rot causes delayed emergence because the seed tuber will produce secondary sprouts after the primary sprouts are killed. However, this often results in reduced plant vigor due to the extra energy needed to produce secondary sprouts (Wharton et al. 2007a). Sprout growth rate is temperature-dependent, and as such, planting in warm, well-drained soil will help prevent sprout rot because sprouts will grow faster and emerge sooner, decreasing the possibility of infection (Loria 2001). Ideally, planting should be done when soil temperature is 7°C or above and soil moisture is 60 to 80% field capacity.

Excessively wet soil leads to enlarged tuber lenticels (Lulai 2001a). As lenticels are perforations in the periderm, they are open entry points for pathogens. Normal-sized lenticels are protected from pathogen entry by layers of underlying suberin. Enlargement of lenticels raises the lenticel tissue above the tuber surface in white tufts, increases lenticel diameter and bypasses the protective suberin, thus, increasing the risk of pathogen entry. In wet soil, lenticels enlarge to help increase gas diffusion at the tuber surface for absorption of oxygen and evolution of carbon dioxide, increasing risk of pathogen entry. Additionally, tubers in waterlogged soil do not get enough oxygen and start to respire anaerobically. Since oxygen is required for host defense pathways, bacteria can cause infection when these pathways are blocked.

Prior to handling and cutting, stored seed tubers should be gradually warmed to 10°C over one to two weeks to decrease bruising, encourage wound healing after cutting, and quicken sprout growth. Cold tubers bruise more easily, and if cold tubers are planted in warm soils, excessive condensation may occur, which may encourage disease development. To decrease new infections on seed, especially cut

seed pieces, fungicide seed treatments should be used, especially when bruising and dry rot is known to be present.

Storage of contaminated cut seed should be avoided before planting because disease incidence will increase during storage. Miller et al. (2019) described a study of how dry rot incidence was affected by pre-cut seed versus direct planting of cut seed when seed was inoculated with either *F. sambucinum* or *F. coeruleum* and then left untreated or treated with one of three fungicide seed treatments. *Fusarium sambucinum* appeared to benefit from the lag time between cutting and planting, regardless of whether treated with fungicide, and much less dry rot occurred when seed inoculated with *F. sambucinum* was directly planted. After cutting, seed must be stored in piles no deeper than approximately 1.2 to 1.8 meters with good ventilation. The storage period should be no longer than ten days to minimize dry rot development before planting (Secor and Salas 2001).

At planting, seed and soil temperatures should vary no more than 5˚C of each other. Soil that is too cold or wet will hinder rapid sprout emergence and growth, and extremely wet soils after planting will increase the incidence of bacterial soft rot. Having the ideal range of temperature and moisture in soil for rapid sprout growth and emergence may result in minimal seed decay after planting (Secor and Salas 2001). Presence of soft rot bacteria as secondary invaders is also a factor in seed decay. Decay is slower if *Fusarium* is the only pathogen but much quicker if soft rot pathogens are also involved (Secor and Salas 2001).

Harvest and Post-Harvest

It is important for potatoes to achieve complete skin-set before they are harvested in order to decrease wounding of tubers (Wharton et al. 2007a). The phellem, i.e., suberized outermost layer of the periderm, matures completely by becoming tightly attached to the adjacent layer of periderm (Lulai 2001a). Killing mature vines two to three weeks before harvest using chemical methods will encourage good skin-set at a faster rate and, thereby, decrease tuber wound incidence and severity during harvest operations (Lulai 2001a). However, skin-set can be delayed by nitrogen applications that are too high (Lulai 2001a). In addition, tuber core temperature should be greater than 10°C before harvest to decrease bruise susceptibility (Lulai 2001a; Wharton et al. 2007a). Colder tubers will bruise more easily, and wounds of cold tubers will heal more slowly (Lulai 2001a). Green harvesting, i.e., harvesting without killing the vines beforehand, should be avoided when tubers are destined for long-term storage as green harvesting may create large stem-end wounds that may encourage greater disease development. Green harvest may be acceptable for tubers that will be moved quickly to market or processing.

During harvest and handling, physical impacts to tubers can be minimized by operating equipment at full capacity and with proper adjustments. However, tuber wounds are ultimately inevitable during harvesting and post-harvest handling. Postharvest dry rot management primarily focuses on avoidance of wounds and promotion of rapid wound healing with optimal conditions (Gachango et al. 2012; Heltoft et al. 2016; Secor and Salas 2001). Any wound on the tuber surface, including bruises, is a location where Fusarium dry rot pathogens can penetrate and start an infection, therefore yield loss due to dry rot can be reduced by wound prevention (Kawchuk et al. 1994; Wharton et al. 2007a).

Potato tubers can naturally heal surface wounds by developing wound periderm to replace the original periderm. This is an important defense response that will stave off invading pathogenic microbes who have yet to arrive (Lulai 2001a; Ray and Hammerschmidt 1998; Secor and Salas 2001). The healing process is called suberization, which, when complete, will prevent infection by microbes (Ray and Hammerschmidt 1998). However, non-suberized or incompletely suberized wounds are vulnerable (Ray and Hammerschmidt 1998). Infection sites can be walled off in the tuber tissue by developing a continuous layer of wound periderm cells and depositing suberin (Lulai 2001a; Secor and Salas 2001). Suberin deposition alone will not stop lesion spread. Wound periderm cells must be made. Periderm development requires 14 to 21 days, and suberin can be made in three days. A sufficiently long wound healing time period is crucial to minimization of dry rot severity by limiting lesion size.

Fully suberized tissue is very resistant to pathogen entry, but the suberin matrix is deposited in layers that have differential resistance to pathogens (Lulai 2001b). First, a bacterial resistant portion of suberin is deposited, a polyphenolic domain that attaches to cell walls in tuber tissue (Lulai 2001b). Second, a fungal resistant portion of suberin is deposited, a polyaliphatic hydrophobic domain attaches to the polyphenolic domain (Lulai 2001b). Therefore, the suberin component that confers resistance to fungal pathogens requires a longer time period to develop, underlining the importance of allowing a long enough time period for wound healing in storage (Lulai 2001b). Under favorable lab conditions, a bacterial barrier can develop after one to three days while a fungal barrier takes from five to eight days. This process takes longer in the cooler temperature environmental of a storage bin (Lulai 2001b).

Suberin deposition between resistant and susceptible potato varieties infected by *Fusarium* was found to differ in that a uniform suberin layer was deposited by resistant varieties, delaying *Fusarium* spread in the tuber until a wound periderm layer was formed. The susceptible variety only exhibited localized, non-uniform suberin deposition and remained vulnerable to pathogen spread (Ray and Hammerschmidt 1998).

A wound healing period of 14 to 21 days after harvesting at 10 to 16˚C with high relative humidity, e.g., 95%, and good air flow is optimal (Lulai 2001b; Secor and Salas 2001). Tuber respiration must be accounted for during the wound healing period. Tuber respiration in storage necessitates regulation of oxygen and carbon dioxide levels through ventilation of storage areas (Lulai 2001b). Respiration rates are higher just after harvest due to tuber injury incurred during the removal of tubers from soil, handling equipment and movement into storage. Without ventilation, the rapid respiration of the freshly harvested tubers will build up carbon dioxide levels and deplete oxygen levels in the storage environment. Sufficient oxygen is needed for proper wound healing and to prevent cell death in the tuber. Gas diffusion occurs through lenticels, and ventilation keeps water films from forming on the tuber surface which will block gas diffusion though lenticels, thereby depleting oxygen supply in the tuber. If ventilation is not used in storage areas during the wound healing period of up to two weeks, then high respiration rates will result in increased carbon dioxide and decreased oxygen that in turn inhibits proper suberization of wounds. Lack of suberization of wounds increases the risk for Fusarium dry rot, bacterial soft rot and physiological disorders like blackheart, internal browning, and internal heat necrosis. Storage temperature also influences respiration rate. At high temperatures, i.e., greater than 15°C, increased tuber respiration rate can lead to increases in these problems.

Storage

Potato storage should consider three factors: temperature, humidity and air circulation (Olsen et al. 2006). Generally, dry rot development in storage is slow. However, dry rot can still develop at the lowest temperature safe for potato storage (Secor and Salas 2001). Potatoes must be stored at cool temperatures because rapid dry rot will occur in high relative humidity conditions at higher temperatures (e.g., 15 to 20˚C; Wharton et al. 2007a). Ventilation using moving air should be continuous during storage (Olsen et al. 2006). Temperatures ranging from 10 to 12.8˚C and high relative humidity of 95% or above is recommended for the wound healing period (Olsen et al. 2006). After wound healing, the temperature selected for long term storage should be based on the intended end-use of the tubers (Olsen et al. 2006). For example, colder storage temperatures inhibit sprouting in seed potatoes since chemical sprout inhibition is not an option for seed potatoes. However, colder storage temperatures for processing potatoes may cause undesirable browning in the flesh if the tubers will be fried. Seed and fresh market tubers can be stored at 2.8 to 7.2˚C, and tubers meant for processing, e.g., French fries, potato chips, should be stored at 6.7 to 12.8˚C (Olsen et al. 2006).

Crop Rotation

In potato production, the aims of crop rotation include reducing populations of soilborne pathogens that cause adverse impacts on tuber quality and yield, improving soil fertility, improving soil

structure, reducing erosion and increasing soil organic matter (Hills et al. 2018; Western Regional IPM Project (U.S.) and University of California Integrated Pest Management Program 2006). Ideally, a crop rotation schedule should reduce inoculum of soilborne pathogens by depriving them of host plants that will sustain them. Therefore, to be most effective at decreasing pathogen populations, suitable rotation crops should be non-hosts (Hills et al. 2018; Western Regional IPM Project (U.S.) and University of California Integrated Pest Management Program 2006).

A minimum rotation schedule of three years is recommended for potato production. Potatoes are often grown on shorter rotations due to economic factors. However, longer time periods between potato crops can realize greater benefits to the future potato crop (Hills et al. 2018). Common rotation crops for potato vary from region to region and can include forage crops such as red clover and alfalfa, small grains such as wheat and barley, corn, canola, dry beans, peas, green manures such as mustard and oilseed radish, sugar beet and other vegetables (Delgado et al. 2010; Estrada Jr et al. 2010; Hills et al. 2018; Western Regional IPM Project (U.S.) and University of California Integrated Pest Management Program 2006). Different rotation crops can impart different benefits to soil health. For example, the fibrous roots of small grains help decrease soil compaction, perennial forage crops minimize tillage and alfalfa adds substantial amount of nitrogen to the soil (Hills et al. 2018).

Crop rotation schedules and crop choices vary within the PNW potato growing regions (Hills et al. 2018). There are four production areas in Idaho: the Treasure Valley, which extends from southwestern Idaho into northeastern Oregon, the Magic Valley in southern Idaho, southeastern Idaho and eastern Idaho. With a longer growing season, Treasure Valley potato growers can utilize longer four- to five-year rotations and a larger variety of rotation crops, including corn, onions, sugar beets, wheat, and alfalfa, than other potato cropping regions in Idaho, with wheat or corn preceding potato (Hills et al. 2018). Typical rotation schedules in the Magic Valley and southeastern Idaho are three- to four years. For example, potato/sugar beet/small grain for 3 years and potato/small grain/sugar beet/small grain for four years. Rotation crops may also include corn, alfalfa and beans (Hills et al. 2018). Due to a shorter growing season, shorter rotations of two to three years are predominant in eastern Idaho using small grains such as wheat or barley (Hills et al. 2018). Rotations of three to four years in seed production are common (Hills et al. 2018).

There are three production areas in Oregon: Columbia Basin, Klamath Valley, and Treasure Valley, which has a similar rotation to the Treasure Valley area in Idaho, with seed potatoes grown in Jefferson, Klamath, Gillian, Morrow, Union and Baker Counties. Two- to three-year rotations are typically used in seed production. In the Columbia Basin, three-year rotations are commonly used,

and rotation choices include alfalfa, beans, carrots, corn, onions, peas and wheat, with potato usually following corn. The Klamath Basin utilizes three- to seven-year rotations including alfalfa, green manure and small grains. Washington potato production mostly occurs in eastern Washington in proximity to the irrigation water available from the Snake River and Columbia Basin with most seed production occurring in Whatcom and Lincoln counties. In the primary production areas, a common rotation schedule is three years with alfalfa, bean, corn or onion often preceding potato while some acreage uses mustard green manure prior to potato (Hills et al. 2018).

The green manure approach is increasing in use in the Columbia Basin and eastern Idaho. A mustard or oilseed radish cover crop is incorporated into the soil prior to planting potatoes, but more research is needed into best practices to be used with this technique in the various potato growing areas (Hills et al. 2018). Green manure crops may be useful in eastern Idaho due to lack of choice in rotation crops and the need for more drought tolerant rotation species (Hills et al. 2018).

The role of previous crops in the risk of Fusarium dry rot in potato is somewhat unclear because the grain and forage crops commonly used in potato rotations harbor several of the same *Fusarium* species that can cause Fusarium dry rot in potato (Ali et al. 2005; Estrada Jr et al. 2010; Heltoft et al. 2016; Peters et al. 2008a). The most prominent of these is *F. graminearum*, which causes *Fusarium* head blight in wheat and barley and *Fusarium* corn diseases, e.g., ear rot, root rot, stalk rot, but *F. graminearum* is generally considered a minor potato dry rot pathogen (Peters et al. 2008b). However, a dry rot epidemic in North Dakota storages in 2003-2004 illustrated the ability of *F. graminearum* to act as a serious dry rot pathogen in some instances. In this case, it was speculated that harvesting potatoes while plant vines were still green led to large wounds as stems were torn from tubers during harvest, creating a situation that favored infection and more aggressive disease development by *F. graminearum* (Estrada Jr et al. 2010).

Regarding corn, researchers in South Dakota isolated eight *Fusarium* species from corn samples with root rot, and all eight species caused root rot in greenhouse aggressiveness testing (Okello et al. 2019). This group of corn pathogens includes known potato dry rot pathogens *F. oxysporum* and *F. graminearum.* Also, *Fusarium* can cause root and wilt diseases in forages like clover and alfalfa. In a study of Canadian *Fusarium* isolates obtained from cereal and forage crops, i.e., wheat, barley, clover and alfalfa, researchers observed dry rot symptoms in potato tubers inoculated with *F. graminearum* from cereals, *F. avenaceum* from alfalfa and red clover, and *F. oxysporum* from clover (Peters et al. 2008b). In this study, it was also found that *F. solani* from clover, *F. oxysporum f. sp. medicaginis*

from alfalfa, and *F. poae* and *F. sporotrichioides* from cereals were non-pathogenic to potato (Peters et al. 2008b).

In Norway, *F. avenaceum* was the most commonly observed *Fusarium* species in cereals and the second most commonly isolated *Fusarium* species from potato in areas where potato and cereal crops are rotated, another example of a cereal rotation maintaining the population of a known potato dry rot pathogen (Heltoft et al. 2016). Estrada, Jr. et al. (2010) found that all tested isolates from potato, wheat, and sugar beet were pathogenic to potato, although no significant difference in disease severity was observed between host origin of the isolates. Therefore, from these examples, it is apparent that rotation crops for potato can potentially harbor *Fusarium* pathogens that may cause potato dry rot, but whether use of these rotation crops has a substantial adverse impact on potato production in the field is unclear.

Chemical Options

Several effective fungicides are available as seed treatments for Fusarium dry rot, including difenoconazole, fludioxonil and mancozeb. Applying these to whole or cut seed prior to planting will help to decrease new infections on seed that can result in seed decay and sprout rot. Applying a postharvest fungicide application as tubers are put into storage is another dry rot management option but is not feasible in all cases (Baturo-Ciesniewska et al. 2015; Gachango et al. 2012). For example, Mertect, a benzimidazole fungicide product (Syngenta), is approved for postharvest use, but resistance to benzimidazoles by the prevalent dry rot pathogen *F. sambucinum* is well-documented in the United States. Thus, this fungicide is not recommended for seed treatment or postharvest use in the PNW (Nolte 1994; Ocamb et al. 2007; Gudmestad and Ivors 2019). Stadium (Syngenta) is another postharvest product containing active ingredients effective against dry rot (fludioxonil, difenoconazole, azoxystrobin), but United States growers cannot use Stadium in commercial storages if they intend to sell to those international markets that prohibit difenoconazole residues from this product (Duellman et al. 2018). A recently released formulation called Archive omits the difenoconazole component while retaining the fludioxonil and azoxystrobin components for Fusarium dry rot control postharvest.

Variety Selection

Dry rot affects most potato varieties, but varietal susceptibility to dry rot ranges from low to moderately high while no commercial varieties have high resistance (Baturo-Ciesniewska et al. 2015; Bojanowski et al. 2013; Gachango et al. 2012; Secor and Salas 2001; Stefańczyk et al. 2016). Potato variety susceptibility differs by *Fusarium* species and differs even among isolates of the same

Fusarium species (Baturo-Ciesniewska et al. 2015; Stefańczyk et al. 2016). Generally, tubers are "most tolerant" at harvest, become increasingly susceptible over time during storage and are most susceptible in early spring around planting time (Secor and Salas 2001). Furthermore, some researchers have documented an interaction between dry rot susceptibility of potato variety and aggressiveness of *Fusarium* species, as shown by *in vitro* rot volume data (Peters et al. 2008a). This indicates two intertwining contributors to dry rot incidence and severity: a) considering a single *Fusarium* species, susceptibility varies among different potato varieties and b) considering a single potato variety, pathogenicity, or ability to cause disease, and aggressiveness, or disease severity, varies among different *Fusarium* species. Thus, diversity of pathogenicity and aggressiveness among and within *Fusarium* species should be accounted for in potato breeding programs (Baturo-Ciesniewska et al. 2015).

Pathogen Characteristics

Fusarium as a Plant Pathogen

Fusarium is a fungal genus containing plant pathogenic species affecting numerous and diverse agricultural crops. These species can cause in substantial losses in both crop quality and yield (Laraba et al. 2021; Leslie and Summerell 2006; Summerell 2019). Just as importantly, *Fusarium* can act as an opportunistic pathogen in humans and animals and contributes to food and feed toxicity for humans and livestock due to production of mycotoxins (Laraba et al. 2021; Leslie and Summerell 2006; Summerell 2019). In general, *Fusarium* are soilborne fungi, mostly growing as saprotrophs (Leslie and Summerell 2006; Piepenbring 2015). However, *Fusarium* can play several other ecological roles including endophytes in living plants, rhizosphere symbionts, and pathogens of plants, fungi, humans and other animals (Leslie and Summerell 2006; Piepenbring 2015). A recovered *Fusarium* isolate may have been the primary dry rot pathogen, a secondary opportunistic pathogen causing dry rot or another disease, or an opportunistic saprophyte taking advantage of necrotic tissue resulting from the primary and/or secondary diseases (Stefańczyk et al. 2016).

Plant diseases caused by *Fusarium* are quite variable in severity and type, e.g. root rot, stem rot, canker, wilt, fruit rot, seed rot, leaf disease, and branch dieback (Leslie and Summerell 2006). *Fusarium* species considered to be major plant pathogens include *F. oxysporum*, *F. graminearum*, *F. culmorum*, *F. fujikuroi*, and *F. solani* (Leslie and Summerell 2006). Other *Fusarium* species are generally considered to be weak plant pathogens or frequent secondary colonizers where a different organism was the primary cause of disease, e.g., *F. chlamydosporum*, *F. incarnatum* and *F. scirpi* in tropical/subtropical areas and *F. sporotrichioides*, *F. tricinctum*, *F. acuminatum* and *F. equiseti* in more temperate climates (Leslie and Summerell 2006). *Fusarium equiseti* in particular is a

cosmopolitan, soil inhabitant that is primarily saprophytic or a secondary colonizer of dead, dying or damaged plant tissue (Leslie and Summerell 2006). *Fusarium equiseti* is commonly associated with disease symptoms caused by other species, but many reports of this association with plant disease do not include Koch's postulates providing evidence of *F. equiseti* pathogenicity (Leslie and Summerell 2006). Also, *F. equiseti* is known to be a root colonizer that may prevent some root association with mycorrhizal fungi (Leslie and Summerell 2006).

Fusarium is currently known to contain over 400 phylogenetically distinct species, i.e., phylospecies (O'Donnell et al. 2022). About one-third of those species lack formal Latin binomials, and many are morphologically indistinguishable, i.e., cryptic (O'Donnell et al. 2022). Many *Fusarium* species are members of species complexes, which are subgeneric groups of several species that share a monophyletic clade within the *Fusarium* genus and have similar characteristics (Summerell 2019). *Fusarium* species have either cosmopolitan distribution or are found on a more limited basis in certain climates, hosts or ecological niches (Leslie and Summerell 2006). If not cosmopolitan, *Fusarium* species are distributed climatically in tropical to subtropical areas, temperate regions or hot, arid locations (Leslie and Summerell 2006). Climate and host are informative in determining the *Fusarium* species acting as a plant pathogen (Leslie and Summerell 2006). The same disease can occur on the same host plant and be caused by a different *Fusarium* species if the climate differs (Leslie and Summerell 2006). For example, maize stalk rot is caused by *F. verticillioides* in a warm dry area such as Kansas, while *F. subglutinans* is the pathogen in a cool, moist area such as Minnesota (Leslie and Summerell 2006). *Fusarium culmorum* is the causal organism of wheat crown disease in cooler temperate areas, while *F. pseudograminearum* is the pathogen in warm subtropical and arid areas (Leslie and Summerell 2006).

Generally, the *Fusarium oxysporum* species complex (FOSC) and the *Fusarium solani* species complex (FSSC) are very common in soil and plant matter worldwide engaging in several ecological functions (Leslie and Summerell 2006). Some *Fusarium* species tend to be more common in temperate regions, e.g., *F. avenaceum*, *F. acuminatum*, *F. torulosum*, *F. cerealis*, *F. culmorum*, and *F. sambucinum*, while others are more common in tropical and subtropical areas, e.g., *F. fujikuroi*, *F. decemcellulare*, and *F. chlamydosporum* (Leslie and Summerell 2006).

The cosmopolitan and common soil saprophyte *F. oxysporum* is the most important plant pathogenic *Fusarium* species due to its wide host range and the magnitude of crop losses incurred from infection. The soilborne *F. oxysporum* is the most widely distributed and most commonly recovered *Fusarium* species and has been found in in Arctic soil (Leslie and Summerell 2006; Lombard et al. 2019).

Fusarium oxysporum is most accurately described as a species complex, the *F. oxysporum* species complex, or FOSC (Garcia Bayona et al. 2011; Leslie and Summerell 2006; Lombard et al. 2019). Detailed taxonomic work on FOSC is currently underway, but for the time being, it is recommended that FOSC isolates are reported as "*Fusarium oxysporum*" until further phylogenetic analyses using larger sample sizes can be conducted (Lombard et al. 2019; O'Donnell et al. 2022). Ranking fifth in a list of the top ten fungal pathogens, *F. oxysporum* is largely a soilborne vascular wilt pathogen, causing wilt disease in a wide variety of crops such as banana, tomato, cotton, melon, strawberry and lettuce (Dean et al. 2012; Garcia Bayona et al. 2011; Leslie and Summerell 2006). *Fusarium oxysporum* is also known to cause damping off and crown and root rots in plants as well as being a human and animal pathogen (Garcia Bayona et al. 2011; Leslie and Summerell 2006; Lombard et al. 2019). Non-pathogenic isolates are often recovered, and as a soil inhabitant, *F. oxysporum* is associated with plant roots in general (Garcia Bayona et al. 2011; Lombard et al. 2019). *Formae speciales* are an important informal classification for the FOSC both historically and currently (Lombard et al. 2019). More than 150 defined *formae speciales* of *F. oxysporum* reflect detected host specificity and are used commonly in literature (Garcia Bayona et al. 2011; Leslie and Summerell 2006; Lombard et al. 2019; Piepenbring 2015).

Generally, the informal *forma specialis* designation refers to differing host specificity within a pathogenic species (abbreviation f. sp.; Garcia Bayona et al. 2011; Leslie and Summerell 2006; Schumann and D'Arcy 2010. However, an organism with a *forma specialis* designation may have more than one host or simply have an endophytic role rather than pathogenic (Garcia Bayona et al. 2011; Lombard et al. 2019). As such, pathogenicity testing is required to establish whether an isolate should be designated *forma specialis*. Vegetative compatibility testing and molecular markers may also help establish whether a isolate is within a *forma specialis* (Garcia Bayona et al. 2011). However, *formae speciales* are not phylogenetically informative as many described *formae speciales* are polyphyletic or paraphyletic, meaning the *forma specialis* isolates are not from closely related lineages that have a common ancestor, i.e., not monophyletic. Examples include *F. oxysporum* f. sp. *batatas*, *F. oxysporum* f. sp. *cubense* and *F. oxysporum* f. sp. *vasinfectum* (Garcia Bayona et al. 2011; Lombard et al. 2019). Polyphyly in *formae speciales* may be due to lateral gene transfer or mutations (Dean et al. 2012; Garcia Bayona et al. 2011; Lombard et al. 2019).

The diverse members of the cosmopolitan *F. solani* species complex (FSSC; > 90 species) are found in a range of substrates but most commonly recovered from soil. FSSC members act in several ecological roles, commonly as saprophytes as well as endophytes, pathogens and insect symbionts. As a plant pathogen, FSSC has a wide host range, including trees, and most commonly causes rots in

both soft and woody plant tissues (Leslie and Summerell 2006; O'Donnell et al. 2020; Schroers et al. 2016). Economically important diseases are caused by FSSC in soybean, such as sudden death syndrome caused by *F. virguliforme* in North America and *F. tucumaniae* in South America, dry bean rot, root rot of pea and stem and fruit rot of greenhouse pepper (Leslie and Summerell 2006). As a symbiont with ambrosia beetles, FSSC contribute to trunk lesions on avocado, also known as sugar volcano (Leslie and Summerell 2006).

Fusarium species are very diverse in tropical regions as far as species number, host range and distribution (Leslie and Summerell 2006). The *Fusarium oxysporum* and *Fusarium solani* species complexes are the most common plant pathogens in tropical regions, having a wide diversity of forms that are typically polyphyletic (Leslie and Summerell 2006). Vascular wilts are very important diseases economically in the tropics (Leslie and Summerell 2006). *Fusarium* diseases affect coffee, cacao, cotton, tobacco, mango, pineapple, banana, passionfruit, dragonfruit, palm trees, sugarcane, rice, sorghum, maize, black pepper and vanilla. The *Fusarium fujikuroi* species complex (FFSC) members, which includes at least 32 species in 10 mating populations, infect corn, sugarcane, rice, sorghum, coffee, mango, and grasses (Leslie and Summerell 2006). *Fusarium fujikuroi* (FFSC) is the causal agent of bakane disease of rice, also known as "foolish seedling" disease, in which rice plants form elongated stems due to excess gibberellins produced in the plant (Leslie and Summerell 2006). *Fusarium sacchari* (FFSC) is a pathogen of sugarcane, causing Pokkah Boeng disease (Leslie and Summerell 2006). *Fusarium decemcellulare* is commonly found in the tropics and causes disease in various trees, including flower cushion gall on cacao, branch canker and dieback on avocado, cacao, mango and others (Leslie and Summerell 2006).

Fusarium species are diverse in temperate regions, and many species having a cosmopolitan distribution tend to be more common in the cooler, wetter temperate areas. *Fusarium graminearum*, having temperate to subtropical distribution, is a very important plant pathogen, causing Fusarium head blight, also known as scab, on cereals, especially wheat, barley and oat, and ear rot and stalk rot of maize (Leslie and Summerell 2006). In these same regions, *F. culmorum* is responsible for head blight, crown rot, or foot rot, and root rot of cereals, especially in northern Europe (Hellin et al. 2017; Leslie and Summerell 2006). *Fusarium redolens* is a common soilborne temperate species that causes root rot on a variety of plant hosts but especially asparagus, beans, carnation, peas, rose and spinach (Leslie and Summerell 2006).

Fusarium is an ecological member of both native and agricultural communities, but differences in these populations are present (Leslie and Summerell 2006). *Fusarium* communities in native settings

differ from agricultural settings in that they are more genetically diverse, found in fewer locations and have less opportunity for dispersal to new locations (Leslie and Summerell 2006). Compared to native communities, agricultural *Fusarium* populations have more biomass and availability of a large area of very uniform ecological niche (Leslie and Summerell 2006). However, agricultural *Fusarium* are exposed to infrequent but intense selection pressures associated with changes in crop plant and cropping practices, which may lead to population boom-or-bust (Leslie and Summerell 2006). The selection pressures in agricultural settings lead *Fusarium* populations to clonal reproduction of individuals having the most successful genetic combinations in the field environment (Leslie and Summerell 2006).

Fusarium as a Dry Rot Pathogen

Worldwide, several *Fusarium* species have been reported as causal organisms of Fusarium dry rot in potato storages and planted seed tubers in the field: *F. acuminatum* Ellis & Everh., *F. avenaceum* (Fr.: Fr.) Sacc., *F. ciliatum*, *F. crookwellense* L.W. Burgess, P.E. Nelson & T.A. Toussoun syn. *F. cerealis* (Cooke) Sacc., *F. coeruleum* (previously *F. solani* (Mart.) Sacc. var. *coeruleum* (Lib. ex Sacc.) C. Booth), *F. culmorum* (Wm. G. Sm.) Sacc., *F. equiseti* (Corda) Sacc., *F. flocciferum* Corda, *F. graminearum* (Schwabe), *F. lateritium*, *F. merismoides*, *F. oxysporum* Schlectend.: Fr.; *F. proliferatum*, *F. redolens* Wollenweber, *F. reticulatum*, *F. sambucinum* Fückel syn. *F. sulphureum* Schlectend.; *F. scirpi* Lambotte & Fautrey, *F. semitectum* Berk. & Ravenel, *F. solani*, *F. sporotrichioides* Sherb., *F. torulosum*, *F. tricinctum* (Corda) Sacc., *F. venenatum*, and *F. verticillioides* (Azil et al. 2021; Ali et al. 2005; Aprasad et al. 1997; Baturo-Ciesniewska et al. 2015; Bojanowski et al. 2013; Choiseul et al. 2007; Cullen et al. 2005; Du et al. 2012; Esfahani 2005; Estrada Jr et al. 2010; Gachango et al. 2011, 2012; Garcia Bayona et al. 2011; Hanson et al. 1996; Heltoft et al. 2016; Hide et al. 1992; Kawchuk et al. 1994, 2002; Merlington 2014; Nolte 1994; Ocamb et al. 2007; Peters et al. 2008a, 2008b; Sagar et al. 2011; Schroers et al. 2016; Secor and Salas 2001; Stefańczyk et al. 2016; Theron 1999; Tiwari et al. 2022).

Variability in aggressiveness among *Fusarium* species has been observed as measured by lesion size in potato tubers after inoculation in laboratory tests. *F. sambucinum* has been shown to be more aggressive *in vitro* on potato tubers as measured by lesion size as compared to *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. oxysporum*, *F. cerealis*, *F. solani* and others (Esfahani 2005; Gachango et al. 2012; Stefańczyk et al. 2016). As an example, among isolates of *F. sambucinum*, *F. solani* and *F. oxysporum* from Iran causing potato dry rot, *F. sambucinum* was found to be most aggressive while *F. oxysporum* was least aggressive (Esfahani 2005). In addition, potato varieties are known to vary in their susceptibility to Fusarium dry rot pathogens (Azil et al. 2021; Aprasad et al.

1997; Choiseul et al. 2007; Du et al. 2012; Esfahani 2005; Merlington 2014; Peters et al. 2008a, 2008b; Stefańczyk et al. 2016). As an example, Esfahani (2005) found variable susceptibility to *Fusarium* among 43 tested cultivars, with certain cultivars being more susceptible to one *Fusarium* species or the other, while one variety, Saturna, was most resistant to all three species among all cultivars.

Fusarium Taxonomy and Phylogenetics

H.F. Link delineated the genus *Fusarium* in 1809 using the canoe-shaped macroconidia as a key characteristic (Leslie and Summerell 2006). Historically, *Fusarium* taxonomy has alternated between "lumping" genus members into a few species and "splitting" the genus into numerous species (Leslie and Summerell 2006). Today, a key morphological characteristic of *Fusarium* is still the multi-septate, banana- or canoe-shaped macroconidia with a constriction in foot cell (Piepenbring 2015; Summerell 2019).

Before the advent of DNA sequencing and molecular techniques, delineation of species was based on morphology and, if a sexual stage was present, biological information gained from mating types and isolate crossing (Leslie and Summerell 2006; Summerell 2019). However, now that DNA sequences are available for isolate comparison and phylogenetic analyses, species that were previously defined are being broken into multiple new species because the available morphological characteristics were too few to be able to accurately separate species. In fact, molecular and phylogenetic techniques are necessary to identify cryptic species (Lombard et al. 2019; Summerell 2019).

In July 2011, the 18th International Botanical Congress in Melbourne, Australia abolished the use of dual nomenclature for pleomorphic fungi by revision of Article 59 (Braun 2012). In other words, a fungus with different morphs for asexual and sexual states, i.e., pleomorphic, would no longer be allowed to have two names, one name for the anamorph, i.e., asexual state and one name for the teleomorph, i.e., sexual state. Examples of dual nomenclature for *Fusarium* are anamorph *Fusarium sambucinum* / teleomorph *Gibberella pulicaris* and anamorph *Fusarium graminearum* / teleomorph *Gibberella zeae*. Under the revised Article 59, anamorph-typified and teleomorph-typified names became nomenclaturally equal, and one name must be chosen to represent the taxon to which both names refer, i.e. "One Fungus, One Name". The rules governing nomenclature are contained in the International Code of Nomenclature for algae, fungi and plants. Braun (2012) stressed that the International Code of Nomenclature only rules nomenclature but does not rule taxonomy itself. The methods used to make a taxonomic decision are under the control of the researcher, whether morphology, molecular analyses, pathogenicity tests, physiological data, biochemical data, and/or

reproductive data. Taxonomists decide if different morphs belong to the same taxon, but the different morphs are no longer allowed to have separate names. Concepts of genera, circumscriptions of genera and species, typification of a genus, i.e., definition of a single type species for the genus, and phylogenetic determinations are not under the control of International Code of Nomenclature.

The effects of "One Fungus, One Name" on *Fusarium* nomenclature have produced some competing theories on the best way forward, and the debate currently continues (Crous et al. 2021; Geiser et al. 2013, 2021; O'Donnell et al. 2013; Summerell 2019). After the 18th International Botanical Congress, more than sixty *Fusarium* researchers co-authored a letter to the editor of *Phytopathology* outlining a way forward for *Fusarium* taxonomy and nomenclature (Geiser et al. 2013). They proposed three criteria for the limits of the *Fusarium* genus: 1) monophyly, 2) tradition and 3) "*Fusarium* first". They proposed the *Fusarium* genus consist of the most inclusive monophyletic group containing the type species *Fusarium sambucinum*. The genus would also conserve the historical meaning of *Fusarium* genus concepts as understood by many basic and applied users of *Fusarium* taxonomy. Finally, they argue that the usefulness of the teleomorphs of the genus is low and proposed use of the anamorph name *Fusarium* over the teleomorph names ("*Fusarium* first"). Overall, Geiser et al. (2013) seek stability of *Fusarium* nomenclature such that it can be used for practical purposes by many kinds of researchers and reflects scientific data.

Fusarium has long been used as the anamorphic name for multiple teleomorphs, and the *Fusarium* anamorph has an easily recognized macroconidial shape (Geiser et al. 2013). The name "*Fusarium*" has gained recognition for over 100 years among a wide variety of professions including plant pathologists, mycologists, mycotoxicologists, plant breeders, food producers, farmers, physicians, geneticists (Geiser et al. 2013). The *Fusarium* anamorph is the most frequently observed form in culture by applied scientists, and teleomorphs are unknown for many species (Geiser et al. 2013). For these reasons, Geiser et al. (2013) recommended "*Fusarium* first".

Fusarium phylogenies published shortly after "One Fungus, One Name" indicated that a large group of *Fusarium* researchers supported the *Fusarium* generic limits as one of two hypotheses. Firstly, the F1 hypothesis included 23 informally named species complexes, the type species *F. sambucinum* and, provisionally, the *F. dimerum* and *F. ventricosum* species complexes. Alternatively, the F2 hypothesis omitted the *F. dimerum* and *F. ventricosum* species complexes due to lack of strong statistical support for these branches (Geiser et al. 2013; O'Donnell et al. 2013). Both the F1 and F2 hypotheses include the "terminal *Fusarium* clade" as described even prior to the abolition of dual nomenclature, which is the most inclusive monophyletic clade that contains the type species.

There have been several teleomorph generic names in use during the history of *Fusarium* nomenclature, including *Gibberella*, *Nectria*, *Neocosmospora*, *Cyanonectria*, *Geejayessia* and *Albonectria*, and these names have become synonyms for their *Fusarium* anamorph names with the *Fusarium* names now taking precedence due to new code. Some researchers have proposed separating out some of these teleomorph names as genera separate from the *Fusarium* genus, in effect, splitting the monophyletic *Fusarium* genus proposed by Geiser et al. (2013) and O'Donnell et al. (2013) into seven different genera (Crous et al. 2021; Geiser et al. 2021; Lombard et al. 2015; O'Donnell et al. 2020; Sandoval-Denis and Crous 2018; Sandoval-Denis et al. 2019; Summerell 2019). Lombard et al. (2015) retain the "*Gibberella*" clade, which includes type species *Fusarium sambucinum* and many species that have had *Gibberella* teleomorphs. Dissenters purport that the "*Gibberella* clade" is too limiting and cuts out the portion of a legitimate monophyletic group that includes several important *Fusarium* species such as *F. solani*, *F. dimerum* and *F. decemcellulare* that have the classical anamorph morphology and have long been part of the *Fusarium* genus (Geiser et al. 2013, 2021; O'Donnell et al. 2013, 2020). Furthermore, Geiser et al. (2013, 2021) do not consider the rarely observed sexual stages on which the 7-way split is based to be sufficient grounds for justifying the split. The "*Gibberella* clade" viewpoint mentioned above also emphasizes the adherence to the view of using the teleomorph name to define genera, but Geiser et al. (2013) point out three reasons that *Fusarium* is the better choice. Firstly, *Fusarium* is the oldest name, which was established in 1809. Secondly, *Fusarium* has been used most consistently as the anamorph name for multiple teleomorph names. Thirdly, in the *Fusarium* genus, teleomorphs contribute little to any evolutionary pattern based on morphology. The "*Gibberella* clade" also omits the inclusion of *Fusarium solani* species complex in the *Fusarium* genus and instead moves the *Fusarium solani* species complex into the genus *Neocosmospora* (Crous et al. 2021; Lombard et al. 2015; Sandoval-Denis and Crous 2018; Sandoval-Denis et al. 2019; Summerell 2019).

The omission of the *Fusarium solani* species complex from the *Fusarium* genus is not supported by a large group of *Fusarium* researchers because *F. solani* is an important pathogenic species in medicine and agriculture, being responsible for two-thirds of *Fusarium* infections in humans while also being widely resistant to the available suite of clinical antifungals (O'Donnell et al. 2020). Also, *F. solani* shares genetic, ecological, and morphological features with *F. oxysporum* as well as clinical connections, and although *F. solani* and *F. oxysporum* are in different species complexes, both are in the same monophyletic lineage defined as the *Fusarium* genus. O'Donnell et al. (2020) state that "monophyly is the principal criterion for taxon recognition in modern taxonomy". The *F. solani* species complex is species rich, but species names are not assigned currently in many cases because

of the limits of morphological characters. A group of *Fusarium* researchers continues efforts to move *F. solani* species to the genus *Neocosmospora* (Sandoval-Denis and Crous 2018; Sandoval-Denis et al. 2019), but this move is strongly opposed by a larger group of scientists based on phylogenetic and clinical, i.e., human medicine, grounds (O'Donnell et al. 2020; Geiser et al. 2021). The opposition to *Neocosmospora* is based on the confusion that the name changes will likely cause among medical mycologists and practitioners. It will affect clinicians trying to diagnose fusarioses in a timely manner and that will affect patient treatment in acute ways (O'Donnell et al. 2020). *Neocosmospora* opposition also offers the notion that the shift of species from *Fusarium* to *Neocosmospora* will adversely affect efficient access to the abundance of *Fusarium* knowledge scattered among several scientific disciplines, e.g., medical, clinical, phytopathological, as well as resulting in a "*de facto* dual nomenclature" that researchers and clinical practitioners will have to navigate when consulting information and literature pre-*Neocosmospora* and post-*Neocosmospora* (O'Donnell et al. 2020).

The currently proposed hypotheses F1 and F2 do not include the seven genera proposed by Lombard et al. (2015) but rather all species represented in the seven genera are kept in the single genus *Fusarium* with the subgeneric clades called "species complexes" used to represent major groups within *Fusarium* rather than splitting them out of *Fusarium* (Summerell 2019). In response to the call from Lombard et al. (2015), Sandoval-Denis and Crous (2018), and Sandoval-Denis et al. (2019) for splitting the genus *Fusarium* and moving the *Fusarium solani* species complex into the genus *Neocosmospora*, Geiser et al. (2021) presented a new *Fusarium* phylogeny based on 19 loci that reiterated the conclusion of Geiser et al. (2013) and O'Donnell et al. (2013) to place the *Fusarium solani* species complex in the *Fusarium* genus whether under the F1 or F2 hypothesis. The new Geiser et al. (2021) phylogeny metrics strongly support the F1 node that for which previous phylogenetic studies (Geiser et al. 2013; O'Donnell et al. 2013) did not provide strong evidence. However, the placement of the *F. ventricosum* and *F. dimerum* species complexes, while unambiguously a part of the F1 node, is still not strongly supported in their analysis (Geiser et al. 2021).

Ultimately, we are currently left with the question of where the generic boundary of *Fusarium* will be placed. Researchers cited in Geiser and O'Donnell publications advocate for a broader *Fusarium* genus that does not focus on the sexual morph and includes many more clades that share the fusioid shape macroconidia as the asexual spore and is more in line with traditional ideas of what *Fusarium* is. Researchers cited in publications led by Crous, Lombard and Sandoval-Denis advocate for a stricter definition of the *Fusarium* genus, splitting out many organisms traditionally considered *Fusarium* into new or existing genera of the Nectriaceae family due to their focus on limiting

Fusarium to a strict set of characteristics emphasizing sexual morph and mycotoxin production. The sexual morph is further emphasized in their movement of *Fusarium* species out of the F1 node into different genera. The current study of potato dry rot-associated *Fusarium* in the PNW adopts the broader concept of the genus *Fusarium* as described by Geiser et al. (2021) and O'Donnell et al. (2022), and taxonomy used for the current study is in agreement with these recent publications.

Species Complexes in *Fusarium*

Fusarium species complexes are subgeneric groups of several species that share a monophyletic clade within the larger *Fusarium* genus, that have similar morphological/phenotypic characteristics, that share phylogenetic markers, for which cryptic species are present and that may exhibit similar toxin production characters (O'Donnell et al. 2022; Summerell 2019). There are currently recognized 23 species complexes, but their designation is not official nomenclature recognized by the International Code of Nomenclature for algae, fungi and plants (O'Donnell et al. 2022; Summerell 2019). Although the "species complex" designation is unofficial, it is very helpful to applied users of *Fusarium* taxonomy for comprehension of the breadth and organization of the *Fusarium* genus and for communication with other researchers regarding *Fusarium* (Summerell 2019). These monophyletic lineages contain one to many species, and for the plant pathology and mycotoxin research areas, six of these 23 species complexes are especially important, *fujikuroi*, *incarnatum-equiseti*, *oxysporum*, *sambucinum*, *solani*, and *tricinctum* (O'Donnell et al. 2022). Of these six species complexes, all except *fujikuroi* are connected to reports of Fusarium dry rot of potato.

Fusarium incarnatum-equiseti species complex (FIESC)

The FIESC contains more than 30 phylogenetic species that were previously informally designated using Arabic numbers indicating haplotype by O'Donnell et al. (2009) and for which 16 phylospecies were since given Latin binomials (Xia et al. 2019). FIESC 14-a was identified to be *F. equiseti* sensu stricto, and two main clades are defined, the Equiseti clade and the Incarnatum clade (O'Donnell et al. 2009; Xia et al. 2019). The Incarnatum clade is generally common in the tropics and subtropics while Equiseti clade members are more cosmopolitan (O'Donnell et al. 2009). Recently, the FIESC phylogeny was updated by Xia et al. (2019), leaving only six phylospecies without Latin binomials. An 18 additional phylospecies were named, and a new species complex, *Fusarium camptoceras* species complex, and the *F. incarnatum* epitype were defined (Xia et al. 2019). The new FIESC phylogeny showed correspondence between recently assigned Latin binomials and the informal haplotype classification, which will facilitate the sorting out of this important species complex henceforth (O'Donnell et al. 2022; Xia et al. 2019).

Fusarium oxysporum species complex (FOSC)

The FOSC has long been in "taxonomic chaos" due to the large number of cryptic species arising from utilization of broad morphological characteristics, the size of the complex and the widespread use of the subspecific *forma specialis* concept to describe FOSC members, and the lack of living type material on which to base fundamental phylogenetic analyses of the FOSC (Lombard et al. 2019). Furthermore, additional subspecific systems are applied to *formae speciales* such as haplotype, race and vegetative compatibility groups (Lombard et al. 2019). Lombard et al. (2019) have defined an epitype for *F. oxysporum* sensu stricto using isolates collected from the original location and host, *Solanum tuberosum* from Berlin, Germany bearing dry rot symptoms. In addition, Lombard et al. (2019) described 15 new species in FOSC. However, other researchers have cautioned against use of the new FOSC taxonomic work until further analyses using larger sample sizes can be conducted and, for the time being have recommended reporting FOSC isolates as the single species *Fusarium oxysporum* (O'Donnell et al. 2022).

Fusarium oxysporum has long been a puzzle due to the contrast of host ranges for the species and for individual isolates (Dean et al. 2012). The species host range is very wide while it has been found that individual *F. oxysporum* isolates may only be able to infect one or a few plant hosts (Dean et al. 2012). Since only the asexual morph of *F. oxysporum* is known, the source of this host range contrast has been elusive (Dean et al. 2012; Lombard et al. 2019). However, recent research has shown that new lineages may arise from the horizontal transfer of small accessory chromosomes containing host-specific virulence genes that enable pathogenicity to develop absent sexual recombination (Dean et al. 2012; Lombard et al. 2019). Newer studies have also detailed the possibility of a still-cryptic sexual cycle (Lombard et al. 2019).

Fusarium sambucinum species complex (FSAMSC)

The FSAMSC is one of the most species-rich lineages in the genus *Fusarium* with 41 named species (Laraba et al. 2021). *Fusarium sambucinum* is the type species of the FSAMSC, which contains genetically closely related phylogenetically distinct species across six clades within the FSAMSC, *Brachygibbosum* with two named and 11 novel species, *Longipes* with four *F. longipes* and five novel species, *Sambucinum* with six named and 10 novel species, *Graminearum* with 22 named and one unnamed species, *Novel* with two phylogenetically distinct species previously unknown and *Sporotrichioides* with seven described and four novel species (Laraba et al. 2021). The *Graminearum* Clade (also known as the *Fusarium graminearum* species complex) contains *F. graminearum* and other closely related species that have been responsible for highly destructive Fusarium head blight and Fusarium crown rot outbreaks in cereals globally (Laraba et al. 2021). Members of FSAMSC are

well-known for trichothecene production, which are mycotoxins contributing to *Fusarium* pathogenicity on hosts but which can contaminate food and feed and cause health problems in humans and other animals that ingest contaminated food (Laraba et al. 2021; Munkvold et al. 2021). Laraba et al. (2021) advocate discontinuing use of the term "*Fusarium graminearum* species complex" and replacing it with "*Graminearum* Clade" to emphasize this group of species as a monophyletic group within the larger *F. sambucinum* species complex that has several other clades.

Fusarium solani species complex (FSSC)

The FSSC contains more than 90 species, most of which are morphologically cryptic, indicating that phylogenetic analyses are necessary to differentiate between species beyond morphological characters that are indistinguishable between species (O'Donnell et al. 2020; Schroers et al. 2016). Due to the abundance of cryptic morphology, the identity of *F. solani* senso stricto was unclear until 2016, when Schroers et al. (2016) epitypified *F. solani* senso stricto as the phylogenetic species FSSC 5 in FSSC clade 3. The selected *F. solani* senso stricto epitype was an isolate from a field in Slovenia causing dry rot of potato (Schroers et al. 2016). *Fusisporium solani* was described by von Martius in 1842, also being the first description of dry rot about 10 years after an epidemic in Germany within the current Hesse and Rhineland-Palatinate provinces (Schroers et al. 2016). As a part of the epitypification study, three fresh isolations from rotted potato in Slovenia were tested for pathogenicity to potato, and all three isolates caused variable dry rot incidence and severity of dry rot, none in some tubers, "small rot" in 50% of tubers and "extensive rot" in 20 to 50% of tubers (Schroers et al. 2016). The three isolates were identified using *TEF* as FSSC 5, verifying that FSSC 5, now known as *F. solani* senso stricto, is a potato dry rot pathogen (Schroers et al. 2016).

There are three phylogenetic clades defined in FSSC (O'Donnell et al. 2008, 2020; Schroers et al. 2016). Clades 1 (New Zealand) and 2 (South America) are geographically limited and only associated with plants (O'Donnell et al. 2008). Clade 2 includes the agronomically important pathogens causing sudden death of soybean and root rot of *Phaseolus* spp. (O'Donnell et al. 2008). Clade 3 contains a much larger number of species that are more common in populated areas, grow at a faster rate and have greater conidial production and contain all species that have been associated with human and animal infection (O'Donnell et al. 2008, 2020; Schroers et al. 2016). O'Donnell et al. (2008) noted that clinical isolates were identified across the breadth of clade 3, which indicates that any Clade 3 FSSC species may be pathogenic to humans and animals and be able to induce opportunistic infections that may be fatal. Interestingly, some FSSC species, such as *F. neocosmosporiellum* in FSSC 8, which was previously known as *Neocosmospora vasinfecta*, have been observed to be pathogenic to both plants and humans (O'Donnell et al. 2008, 2022).

Fusarium tricinctum species complex (FTSC)

Recent work on the FTSC taxonomy and mycotoxin production was initiated due to the emergence of FTSC species members as more prevalent pathogens of small-grain cereals, causing head blight and crown rot on wheat, barley, oat, rye and of pulses, soybean and canola, causing root rot, in North America, Brazil, Europe and China (Laraba et al. 2022). FTSC members were previously considered to be weak pathogens and secondary invaders, but a shift to a role as more prevalent pathogens is especially apparent for *F. avenaceum* and *F. acuminatum* in North America, Brazil and southern Europe (Laraba et al. 2022). *Fusarium avenaceum* has been commonly cited as a Fusarium dry rot pathogen, especially in Europe, Great Britain and Canada (Choiseul et al. 2007; Du et al. 2012; Heltoft et al. 2016; Peters et al. 2008b; Stefańczyk et al. 2016). In addition, mycotoxin production by FTSC is known to include "emerging" mycotoxins beauvericin, enniatins, fusarins and moniliformin (Laraba et al. 2022; Munkvold et al. 2021). Laraba et al. (2022) showed that FTSC phylogenetic diversity is greater than previously thought (at least 36 species) with nine novel species resolved in their study and established that the phosphate permease (*PHO*) gene is highly suitable for species-level resolution within FTSC. They demonstrated the utility of *PHO* to help correctly characterize previously misidentified FTSC isolates, which will facilitate future study of this species complex (Laraba et al. 2022).

Fungicide Sensitivity

Fungicide applications, especially those that are repeated, frequent, and non-rotated with other modes-of-action, can lead to selection for pathogen individuals that are naturally resistant and to subsequent shift to a pathogen population in which fungicide resistance poses a noticeable problem for growers. A small or negligible disease problem may become substantial as fungicide-resistant individuals survive fungicide applications, successfully reproduce and pass on their resistance traits to progeny (Nolte 1994; Pasche and Gudmestad 2019).

Each fungicide has a mode-of-action (MOA) by which it can affect the target pathogen adversely, and modes-of-action vary widely on the target metabolic process and biochemical site they affect (FRAC 2022). The Fungicide Resistance Action Committee (FRAC) sorts fungicides into "groups" based on their mode-of-action, and generally, fungicides have a single-site or multi-site activity (FRAC 2022). Single-site MOA chemicals act on a single specific target site while multi-site chemicals can affect multiple metabolic processes or biochemical sites. Risk of resistance development varies among fungicide MOA. Generally, single-site MOA risk varies from low to high while multi-site chemicals have very low risk (FRAC 2022). Many multi-site chemicals have been used for decades without showing any signs of resistance development and they are broad spectrum. However, many multi-site

MOA chemicals are contact fungicides and with the need for re-application, for some crops, systemic single-site MOA fungicides are more economical choices. However, chemicals with single-site activity generally are narrow spectrum and more prone to resistance development with repeated use.

The goals of fungicide resistance management programs are to delay resistance development to fungicides and to hold the level of fungicide resistance in a pathogen population to an economically acceptable level (Agrios 2005; Damicone 2017; FRAC 2022; Schumann and D'Arcy 2010). Resistant individuals are present in every pathogen population, and a small level of fungicide insensitivity is unavoidable. However, fungicide resistance can develop when resistant individuals are exposed to a fungicide to which they are insensitive. Under high fungicide pressure, resistant individuals are more reproductively successful than sensitive individuals. This selection pressure increases the proportion of resistant individuals in a pathogen population and renders disease management fungicides less effective, increasing disease levels in the host.

Fungicide resistance can develop abruptly or gradually (Damicone 2017). Abrupt, also called qualitative, resistance develops when a single mutation confers resistance in individuals, and a pathogen population that initially had only a few resistant individuals can quickly become dominated by resistant individuals. This results in abrupt loss of disease control with the fungicide. Gradual, also called quantitative, resistance develops when multiple genes are involved in conferring resistance, and a fungal population gradually gains reduced sensitivity to the fungicide as mutations accumulate within the population. However, with gradual resistance development, abrupt or total loss of disease control is unlikely. Development of resistance involving multiple genes makes shifts toward insensitivity within a pathogen population more difficult to detect. Thus, monitoring for gradual resistance requires more frequent testing of larger numbers of individuals to detect the shifts toward resistance.

Certain practices lead to more rapid development of fungicide resistance (Damicone 2017; Schumann and D'Arcy 2010). Repeated use of the same active ingredient or same MOA increases selection pressure on the resistant individuals of a pathogen population. Use of chemical groups that the pathogen can overcome by mutation in a single gene means the fungicide is more likely to be overcome in the short-term. Unnecessary applications, either too frequently or at times when risk of infection is low, give the pathogen additional opportunities to develop resistance. Improper timing of application, inadequate coverage or improper dosage also increases risk of resistance development. Cross-resistance must be considered in maintaining fungicide efficacy. Cross-resistance among a group of two or more fungicides means that if a fungus has resistance to one of the fungicides in the

group, then it has resistance to the other fungicides in the group (Brent and Hollomon 2007). Usually this phenomenon is due to a common mode-of action and/or chemical structure shared by the fungicides, but this is not always the case (Brent and Hollomon 2007).

There is a greater risk for resistance development among foliar diseases versus soilborne diseases (Damicone 2017). Only one or two fungicide applications per growing season are required to help prevent soilborne diseases whether applied to the soil or as a seed treatment. Generally, soilborne pathogens tend to be monocyclic and produce fewer progeny per season than foliar pathogens, which tend to be polycyclic. Since fewer progeny face selection pressure from fungicides, resistance in the pathogen population is slower to develop (Damicone 2017). Polycyclic pathogens having many and short reproductive cycles during a growing season have a greater risk for fungicide resistance development (Damicone 2017; FRAC 2019). Compared to soil pathogens, foliar pathogens have much greater spore dispersal over time and across physical space, further increasing resistance development (FRAC 2019). Also, seed treatments reduce primary inoculum, which really decreases the amount of disease that could potentially develop by preventing the pathogen from starting a new life cycle with successful spore germination.

Development of strategies to avoid fungicide resistance must consider three main factors: fungicide MOA, target pathogen and plant host. Fungicides are classed as either contact or systemic (Schumann and D'Arcy 2010). Contact fungicides are applied to and remain on the host surface until rain or other weathering processes remove them and generally must be re-applied thorough the growing season for foliar diseases. After infection, once the pathogen is inside the plant tissue, it cannot be affected by fungicide that is restricted to the plant surface. Systemic fungicides can penetrate the plant surface and are taken inside the host plant to various degrees after application. Systemic fungicides help manage disease by inhibition of fungal growth and sporulation and can be locally systemic, translaminar, xylem-mobile or truly systemic, i.e., xylem- and phloem-mobile. If infection has already occurred, systemic fungicides may help to slow the spread of the pathogen and minimize new infections. Broad spectrum fungicides are those that can affect a wider range of organisms and are advantageous for the crop grower who needs to manage multiple diseases with high effectiveness. Contact fungicides tend to be broad spectrum. Narrow spectrum fungicides affect specific pathogens and tend to be systemic chemicals taken up into the plant.

Efforts to detect fungicide resistance in a plant pathogenic population tend to include laboratory, i.e., *in vitro*, testing using either traditional poison agar method or the spiral gradient dilution method. The spiral gradient dilution method was developed for use with fungi and fungicides as a modification of

the spiral gradient endpoint method used for testing inhibitory concentrations of antibacterial agents (Förster et al. 2004). The spiral gradient dilution method requires far less time and resources to complete than the traditional poison agar method (Förster et al. 2004). The spiral gradient dilution method produces a value for 50% effective concentration (EC_{50}), i.e. the fungicide concentration at which the fungal response is reduced by 50% as compared to control plates containing non-amended agar. The fungal responses typically measured are mycelial growth or spore germination (Förster et al. 2004). As a measure of fungicide toxicity to a fungal pathogen, the EC_{50} value is useful in gauging how effective the fungicide will be at managing disease through pathogen inhibition. By testing a specific population for fungicide sensitivity, changes in sensitivity can be detected over time. If possible, it is important to obtain baseline fungicide sensitivity data for a pathogen population that has not yet been exposed to a fungicide in order to be able to detected reduction in sensitivity or resistance to a fungicide at a future time (Förster et al. 2004; Pasche and Gudmestad 2019; Russell 2004).

The ability of fungicides to inhibit fungal pathogens *in vitro* does not necessarily predict fungicide effectiveness in a disease situation in the field. *In vitro* assays should be accompanied by *in vivo* and/or field data correlating the *in vitro* fungicide resistance with loss of disease mitigation (Pasche and Gudmestad 2019; Russell 2004). It is not prudent to rely on laboratory testing alone to make fungicide use recommendations to growers given the possibility that laboratory and field tests may not correspond with each other. Risk involved in extrapolating pathogen-fungicide responses from the laboratory to the field stems from the following concepts from Russell (2004). 1) Resistance of a fungal isolate to a specific fungicide can be complicated. One or more mutations could be involved, raising the question of whether the mutations act alone or synergistically. The frequency of the mutation(s) in the cells of the individual may affect its level of sensitivity to a fungicide. 2) Disease management in a field situation will be dependent on the level of resistance in the field population, which raises the question of what frequency of resistant individuals is required to substantially impact or cause failure of disease management strategies. Therefore, the correlation between *in vitro* and *in vivo*/field data may not be straightforward.

Intuitively, observation of high *in vitro* sensitivity to a fungicide would lead to the expectation of high disease management in the field, and low sensitivity or insensitivity, i.e., resistance, to a fungicide would be expected to result in some measurable loss of disease reduction. Bauske et al. (2018) conducted *in vitro* and *in vivo* fungicide sensitivity assays on *Alternaria solani* isolates with multiple types of point mutations known to confer resistance to SDHI fungicides, and mutants with similar *in vitro* sensitivity displayed variable *in vivo* early blight disease reduction depending on which mutation

they possessed. Additionally, *in vitro* sensitivity to boscalid was correlated with reduced disease *in vivo*, and *A. solani* isolates with the D123E mutation had high level sensitivity to fluopyram *in vitro* but *in vivo* disease inhibition was significantly reduced (Bauske et al. 2018). Conversely, Thomas et al. (2012) consistently correlated laboratory-detected fungicide resistance with increased disease in the field for watermelon gummy stem blight. Fonseka and Gudmestad (2016) detected reduced pyrimethanil sensitivity *in vitro* for *A. solani* isolates, and this was correlated with increased early blight disease *in vivo* as compared to pyrimethanil-sensitive isolates. It is clear from these examples that field efficacy of fungicides cannot always be inferred from *in vitro* fungicide sensitivity testing.

Development of resistance in Fusarium dry rot pathogens to fungicides used for dry rot management is hypothesized to be one of the factors driving the observed increase in dry rot in the PNW. Fungicide resistance among Fusarium dry rot pathogens has long been known, e.g. thiabendazole resistance in multiple *Fusarium* species, including *F. sambucinum* (Gudmestad and Ivors 2019; Nolte 1994; Ocamb et al. 2007) and reports of fludioxonil resistance in *F. sambucinum*, *F. oxysporum* and *F. coeruleum* (Gachango et al. 2012; Peters et al. 2008b). The most recent work for the PNW on potato dry rot and disease management fungicides was published over a decade ago regarding methyl benzimidazole carbamates, including thiabendazole (Desjardins et al. 1993; Desjardins 1995; Nolte 1994; Ocamb et al. 2007). In a group of *F. sambucinum* isolates collected in 1990 and 1991 from Idaho, Michigan and North Dakota, 24 of 25 isolates were observed to have thiabendazole resistance. In contrast, 17 isolates collected from various North American locations between 1963 and 1991 were all highly sensitive to thiabendazole (Desjardins et al. 1993). Resistance to benzimidazole fungicides was found in approximately 85% of *F. sambucinum* isolates tested in Idaho from 1993 to 1995 (Gallian et al. 2006; Nolte 1994). Methyl benzimidazole carbamate fungicides, including the benzimidazole called thiabendazole and a thiophanate called thiophanate methyl, were used as both seed and post-harvest treatments for potatoes in the PNW but are no longer used because of insensitivity in the prevalent *F. sambucinum* dry rot pathogen (Nolte 1994; Miller et al. 2019). Several fungicides available in various commercial formulations have been shown to be effective for dry rot management in seed and commercial production by researchers in Idaho, including difenoconazole, fludioxonil, and mancozeb (Miller et al. 2019). These fungicides have different FRAC Codes, indicating they have different modes-of-action against *Fusarium* (Table 1-1).

Table 1-1. Characteristics of fungicides commonly used for management of Fusarium dry rot of potato.

Fungicide name	FRAC code [a]	Group name / chemical group	Effect on fungi	Resistance risk [b]	Resistance in Fusarium dry rot pathogens reported prior to this study	Mode-of-action type
Difenoconazole	3	DMI (demethylation inhibitor) / triazole	Inhibits ergosterol synthesis, a necessary component of fungal cell membrane, thereby, inhibits mycelia growth and causes leaky, less rigid and dysfunctional membranes	Medium	Fusarium sensitivity tested but resistance not detected [c]	Single-site
Fludioxonil	12	PP / phenylpyrrole	Indirectly triggers High Osmolarity Glycerol (HOG1) pathway, leading to cell-cycle arrest, glycerol accumulation, cell swelling and rupture	Low to medium	Reported in three species [d]: *F. sambucinum* - Canada and Michigan, *F. coeruleum* - Canada, *F. oxysporum* - Michigan	Single-site
Thiabendazole	1	MBC (methyl bendimidazole carbamate) / benzimidazole	Inhibits beta-tubulin polymerization, which is needed for building microtubules in the cell cytoskeleton, thereby inhibiting cell division	High	Detected in several species, especially *Fusarium sambucinum* [e]	Single-site
Mancozeb	M 03	Dithiocarbamates and relatives / Dithiocarbamates and relatives	After exposure to water and UV light, toxic products interfere with enzymes in at least six types of biochemical processes, leading to inhibition of spore germination	Low	None	Multi-site

[a] FRAC = Fungicide Resistance Action Committee; FRAC codes organize fungicides according to their modes-of action.

[b] Resistance risk as determined by FRAC.

[c] Burlakoti et al. 2010; Gachango et al. 2012; Hellin et al. 2017

[d] Gachango et al. 2011, 2012; Peters et al. 2008c

[e] Choiseul et al. 2007; Desjardins et al. 1993; Gudmestad and Ivors 2019; Hanson et al. 1996; Hide et al. 1992; Kawchuk et al. 1994; Nolte 1994; Ocamb et al. 2007

Additional references: Becher and Wirsel 2012; Brandhorst and Klein 2019; Brandhorst et al. 2019; Damicone 2017; FRAC 2018, 2022; Gullino et al. 2010; Kawchuk et al. 2002; Kilani and Fillinger 2016; Miller et al. 2019

Difenoconazole

Difenoconazole is a triazole in FRAC Code 3, which contains demethylation inhibitors that specifically inhibit sterol biosynthesis in membranes, causing general membrane dysfunction (Becher and Wirsel 2012; FRAC 2022; Hellin et al. 2017). Due to their effectiveness against a wide variety of fungi, triazoles are the most extensively used antifungals in both agricultural and clinical settings (Becher and Wirsel 2012). The DMI fungicides are unique amongst single-site mode-of-action fungicides in that they are still able to be widely used since their introduction in the 1970s due to lack of widespread resistance development (Cools et al. 2013).

Group 3 fungicides prevent cell membranes from forming or being repaired, thus membranes may become leaky and mycelial growth is inhibited. Ergosterol is a key component of fungal cell membranes and is exclusively found in fungi (Becher and Wirsel 2012; Piepenbring 2015). DMIs have medium risk for resistance development (FRAC 2022). Resistance to triazoles develops stepwise as mutations in multiple genes or alleles are involved (Damicone 2017; Hellin et al. 2017). Cross-resistance should be supposed between DMI fungicides, but cross resistance of DMIs with other sterol biosynthesis inhibitor classes is not known (FRAC 2022). Cross-resistance means that if a fungal pathogen is resistant to one Code 3 fungicide, then it can be supposed to be resistant to other Code 3 fungicides. FRAC recommends assumption of the presence of cross-resistance between Code 3 fungicides, which contributes to the "medium" risk of resistance development for Code 3 (FRAC 2022).

Based on the literature review of *Fusarium* sensitivity to triazoles, EC_{50} values less than a threshold of 5 mg/L appear to indicate sensitivity of *Fusarium* species to various triazoles in a laboratory setting (Burlakoti et al. 2010; Gachango et al. 2012; Hellin et al. 2017). Difenoconazole is also available as in single ingredient formulations, but as Group 3 fungicides are at medium risk for resistance development, it is most advisable to apply difenoconazole in mixtures or alternated with other modes of action during and between growing seasons (FRAC 2022).

Prior to the current study, no specific information on *Fusarium* difenoconazole sensitivity in the PNW was available. Difenoconazole is used as a partner fungicide in some commercial dry rot fungicide products to help protect against fludioxonil resistance (Miller et al. 2019). In addition, a commercial potato seed treatment containing prothioconazole is also labeled for use in the PNW. While prothioconazole, which is a triazolinthione, is in a different chemical group than difenoconazole, which is a triazole, it is still a Group 3 fungicide with a medium risk for resistance development and likely potential for cross-resistance (Miller et al. 2019; FRAC 2022). Prothioconazole is an active

ingredient in a commercial formulation called Emesto Silver (Bayer CropScience) that is labeled for the PNW states to help manage dry rot. It is currently unknown whether cross-resistance exists between difenoconazole and prothioconazole, two Group 3 fungicides used in Fusarium dry rot management in the PNW.

Fludioxonil

Fludioxonil is a FRAC Code 12 phenylpyrrole that is thought to affect osmotic signal transduction by interfering with MAP/Histidine kinase genes (os-2 and HOG1), leading to effects such as membrane hyperpolarization, the inability of exposed cells to control their internal pressure and possibility of swelling or bursting (FRAC 2022; Kilani and Fillinger 2016). It has been established through several studies that both a group III hybrid histidine kinase (HHK) and the HOG-1 (High Osmolarity Glycerol) pathway are required components for fludioxonil activity in fungi (Brandhorst et al. 2019). HOG-1 is an environmental stress response pathway activated constitutively by HHKs (Brandhorst et al. 2019).

The most recent theory presented is that fludioxonil creates a stress state in exposed fungal cells, with the key stressor proposed to be aldehydic stress caused by methylglyoxal, a very reactive molecule that is damaging to DNA and cellular proteins (Brandhorst and Klein 2019; Brandhorst et al. 2019). Methylglyoxal causes modification of sensitive cysteine entities in the HHK sensor kinases, converting HHKs from kinase to phosphatase activity (Brandhorst and Klein 2019; Brandhorst et al. 2019). Then, HHK acts as a phosphatase to dephosphorylate Ypd1, which directly triggers the HOG-1 pathway. The effects of HOG-1 are “cell-cycle arrest, glycerol accumulation, cell swelling, and [cell] rupture” (Brandhorst and Klein 2019; Brandhorst et al. 2019). Therefore, when fludioxonil indirectly triggers the HOG-1 cascade in fungi, cell death occurs, and organism death is inevitable. This new theory contradicts older theories that proposed direct binding of fludioxonil to the HHK, thereby triggering the HOG-1 pathway (Brandhorst and Klein 2019; Brandhorst et al. 2019).

Brandhorst et al. (2019) demonstrated that increased methylglyoxal in the cell is generated by the interference of fludioxonil with triosephosphate isomerase, a key enzyme in sugar metabolism. The presence of fludioxonil may introduce steric interactions at the interface of the triosephosphate isomerase dimer that cause one of the loops to become inflexible, allowing the release of methylglyoxal into the cytosol (Brandhorst et al. 2019). Normally, methylglyoxal is produced during sugar breakdown in cells but is catabolized by enzymes before it becomes toxic and damaging (Hamilton 2019).

Fludioxonil resistance may develop due to modification in the Group III HHKs in fungi. These HHKs are structurally different from other HHKs by presence of HAMP domain repeats at the N-terminus (Brandhorst et al. 2019). Research has shown that deletion or modification of these HAMP repeats causes the protein to become a constitutive kinase (Brandhorst et al. 2019). Thus, fludioxonil sensitivity disappears because the Group III HHK protein can no longer be induced to become a phosphatase that activates the HOG1 self-destruction pathway (Brandhorst et al. 2019).

Fludioxonil resistance has only been documented occasionally, and risk of resistance development is considered low to medium (FRAC 2022; Kilani and Fillinger 2016). As such, fludioxonil can be a prudent choice for disease management for many pathogen types, especially if used in combination or alternation with a multi-site fungicide with known efficacy. Though the mechanism of fludioxonil is not entirely solved, the fact that is has been used for several decades with few reports of resistance development indicates this chemistry can be relied upon similarly to a chemical with multi-site activity (Kilani and Fillinger 2016). In general, documentation of very little field resistance to fludioxonil may be due to severe fitness penalties incurred in fludioxonil resistant mutants (Kilani and Fillinger 2016). As compared to wild type in laboratory testing, resistant isolates may not be able to survive well in field conditions if they possess the mutation(s) to overcome fludioxonil (Kilani and Fillinger 2016).

Prior to this study, no specific information on *Fusarium* fludioxonil sensitivity in the PNW was available. However, for isolates from dry rot tubers, fludioxonil resistance was reported in *F. sambucinum* and *F. coeruleum* in Canada and in *F. sambucinum* and *F. oxysporum* in Michigan (Gachango et al. 2011, 2012; Peters et al. 2008c). Fludioxonil is available in formulations as a single ingredient and mixtures, but use of fludioxonil alone is strongly discouraged. The mixtures of fludioxonil with mancozeb or difenoconazole are commonly used for dry rot management in the PNW, with the addition of difenoconazole intended to help with resistance management for fludioxonil.

Thiabendazole

Benzimidazoles are in FRAC Code 1, indicating a mode-of-action that inhibits beta-tubulin polymerization, which is needed for building microtubules for the cell cytoskeleton, and thereby inhibits cell division (Damicone 2017; FRAC 2022; Kawchuk et al. 1994, 2002). Benzimidazoles bind directly to beta-tubulin, and fungal resistance to benzimidazoles is due to the ability of the fungus to block the benzimidazole molecules from binding (Kawchuk et al. 2002). Benzimidazoles have a high risk of resistance development because loss of efficacy was realized in only a few years

after introduction in one or more target pathogens, with resistance being widespread and severe (Brent and Hollomon 2007). Resistance is known to develop abruptly under selection pressure from fungicide application (Damicone 2017). Once resistance is established, it is likely to persist as researchers have found that growth rates and pathogenicity of benzimidazole resistant and sensitive isolates were similar, even in the absence of the fungicide, implying that there is no major fitness penalty incurred (Damicone 2017; Kawchuk et al. 1994, 2002). In addition, there is cross-resistance in benzimidazoles due to similar modes-of-action and structures (Kawchuk et al. 2002). For example, *F. sambucinum* exhibited cross-resistance to thiabendazole and thiophanate methyl in Canada (Kawchuk et al. 2002).

Thiabendazole resistance among *Fusarium* dry rot pathogens has been long known, in multiple *Fusarium* species, including *F. sambucinum* (Desjardins et al. 1993; Gudmestad and Ivors 2019; Hide et al. 1992; Nolte 1994; Ocamb et al. 2007). Thiabendazole was commonly used for dry rot management in the 1970s and 1980s, but *Fusarium* resistance to thiabendazole became widespread by the 1990s (Desjardins et al. 1993; Gudmestad and Ivors 2019; Hanson et al. 1996; Hide et al. 1992). The first reports of thiabendazole resistance in Europe were published in the 1980s, citing resistance in both *F. sambucinum* and *F. coeruleum* (Hide et al. 1992) with the first reports of *F. sambucinum* thiabendazole resistance in the United States following in the 1990s (Desjardins et al. 1993; Hanson et al. 1996). Kawchuk et al. (1994) observed that while Canadian isolates of *F. sambucinum* displayed resistance to thiabendazole, *F. avenaceum*, *F. equiseti*, *F. culmorum*, *F. solani* were sensitive.

Multisite Fungicides

To manage development of fungicide resistance, multisite fungicides, e.g., mancozeb, are specifically recommended as a component of fungicide programs that utilize single-site fungicides and/or medium to high risk fungicides, both as a partner fungicide in in a mixture and within an alternating rotation (FRAC 2018). Multisite fungicides acts to inhibit the fungus in more than one way biochemically Mancozeb has been shown to work very well for dry rot management in studies conducted in Idaho (Miller et al. 2019). Also, in Idaho studies, Polyram liquid fungicide seed treatment, which contained active ingredient metiram, was not sufficiently effective against either *F. sambucinum* or *F. coeruleum,* but MZ Dust fungicide seed treatment, which contained active ingredient mancozeb, was more effective against both *Fusarium* species (Miller et al. 2019). Interestingly, mancozeb and metiram are in the same chemical group, i.e., FRAC Code M 03, but these studies showed that one is highly effective against Fusarium dry rot while the other is not.

Dry Rot Pathogens in North America

Generally, *F. sambucinum* is considered the predominant dry rot pathogen in North America (Choiseul et al. 2007; Kawchuk et al. 1994; Ocamb et al. 2007; Peters et al. 2008b; Secor and Salas 2001). Storage surveys in the Atlantic region of Canada have revealed that 60-70% of tuber dry rot is caused by *F. sambucinum*, 20% by *F. coeruleum* and 10% by *F. avenaceum* (Peters et al. 2008b). Isolates recovered from dry rot tubers in Alberta storages in Canada represented the species *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. sambucinum* and *F. solani*, with *F. sambucinum* being the most commonly recovered species (Kawchuk et al. 1994). In the northeastern United States, *F. sambucinum*, *F. solani* and *F. oxysporum* were the most frequently isolated species from samples collected in 1992 and 1993 (Hanson et al. 1996). Other *Fusarium* species reported as less common or rare causal agents in North America are *F. acuminatum*, *F. culmorum*, *F. cerealis*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. sporotrichioides*, and *F. torulosum* (Ocamb et al. 2007; Secor and Salas 2001).

More recent reports in North America have indicated a shift in prevalence of dry rot pathogens. In 2004-2005, a limited survey of symptomatic dry rot tubers from North Dakota and Minnesota, an area where *F. sambucinum* was considered to be the main dry rot pathogen for the previous 25 years, recovered similar numbers of *F. sambucinum* and *F. graminearum* isolates, 58% and 42%, respectively (Estrada Jr et al. 2010). In Michigan, an area where *F. sambucinum* has historically been considered the predominant dry rot pathogen according to 1990s reports, *F. oxysporum* was found to be the most prevalent pathogen in more recent surveys (Gachango et al. 2012; Merlington 2014). In a 2009-2010 survey of seed tuber storages in Michigan, of 228 *Fusarium* isolates representing 11 species observed to pathogenic to Dark Red Norland tubers, 30% were *F. oxysporum*, 19% *F. equiseti*, 14% *F. sambucinum* and 14% *F. avenaceum* (Gachango et al. 2012). In a 2011-2012 survey of commercial production storages in Michigan, of 730 *Fusarium* isolates representing 11 species observed to be pathogenic to four tested potato varieties, 67% were *F. oxysporum*, 14% FIESC, 6% *F. solani* and 6% *F. sambucinum*. Comparison of the two Michigan storage surveys even revealed a difference in *Fusarium* species composition between seed and commercial storages (Gachango et al. 2012; Merlington 2014).

Dry Rot Investigations for the Pacific Northwest

Knowledge of *Fusarium* populations responsible for dry rot is limited for the PNW. In Idaho, only two species, *F. sambucinum* and *F. coeruleum*, have been considered important dry rot pathogens based on work that is more than 25 years old (Desjardins 1995; Nolte 1994). Idaho surveys of dry rot in storages in the 1990s indicated that *F. sambucinum* was more common than *F. coeruleum* (Miller

et al. 2019). *Fusarium coeruleum* has been observed to grow faster in soil and be a more destructive seed piece decay pathogen in the field than *F. sambucinum* (Miller et al. 2019). On the other hand, in storage, *F. sambucinum* grows faster than *F. coeruleum*, making *F. sambucinum* a more destructive pathogen in storage. Further, seed piece decay due to *F. sambucinum* can be an issue in precut seed without proper storage conditions, even if a seed piece treatment is applied (Miller et al. 2019).

A previous study of symptomatic tubers collected from commercial storages in the Columbia Basin of Washington and Oregon identified three predominant species associated with dry rot lesions, *F. sambucinum*, *F. oxysporum* and *F. solani*. Other isolated species were *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. proliferatum* and *F. sporotrichioides* (Ocamb et al. 2007). The two-year survey of *Fusarium* in storages in the Columbia Basin indicated that *F. sambucinum*, *F. oxysporum* and *F. solani* were the most widespread, being found each year in 7 and 23 storages, 20 and 14 storages and 10 and 23 storages, respectively (Ocamb et al. 2007). However, data from the Ocamb et al. (2007) study are also over 20 years old, do not specify from which state in the Columbia Basin each *Fusarium* species was recovered, i.e., Washington or Oregon, and do not include Idaho isolates. Additionally, in these previous dry rot surveys in the PNW including tubers from seed lots, storages and fields, the fungal pathogens were not molecularly characterized, indicating that identity is not entirely certain.

Project Justification and Expected Contribution

The potato production industry in the PNW serves local, national and international markets and is an economic driver for the region. As a disease with the potential to substantially decrease marketable yield, successful management of Fusarium dry rot is a crucial component in supporting abundant production of this food staple. As such, a recent reported increase in Fusarium dry rot in the PNW justified an investigation of potential contributing factors.

The expected contribution of this project is to improve Fusarium dry rot management in PNW by developing a cohesive set of information that identifies factors that may be contributing to the reported disease increase in the region. Factors to be studied included species composition, pathogenicity and phylogenetic relationships of *Fusarium* species associated with symptomatic dry rot tubers collected from the PNW, relative aggressiveness of *Fusarium* species as dry rot pathogens on potato varieties important to the region, and sensitivity of *Fusarium* species to fungicide modes-of-action frequently used for dry rot management.

Objectives

1. Collect *Fusarium* isolates from symptomatic dry rot tubers originating in the PNW and identify to species using molecular methods
2. Confirm pathogenicity of isolates, i.e., Koch's postulates, representing *Fusarium* species that are known dry rot pathogens but not previously reported in the PNW and not known to be dry rot pathogens
3. Evaluate fungicide sensitivity for a subset of isolates
4. Evaluate relative aggressiveness of different *Fusarium* species on selected potato varieties
5. Analyze phylogenetic relationships of a subset of isolates
6. Determine relationship between *in vitro* fungicide resistance of Fusarium dry rot pathogens and disease development *in vivo* and under field conditions in the presence of fungicide

Literature Cited

Agrios, G. 2005. Plant Pathology. 5th ed. Elsevier Academic Press.

Ali, S., Rivera, V. V., and Secor, G. A. 2005. First report of *Fusarium graminearum* causing dry rot of potato in North Dakota. Plant Dis. 89:105–105.

Aprasad, K. S., Bateman, G. L., and Read, P. J. 1997. Variation in pathogenicity on potato tubers and sensitivity to thiabendazole of the dry rot fungus *Fusarium avenaceum*. Potato Res. 40:357–365.

Azil, N., Stefańczyk, E., Sobkowiak, S., Chihat, S., Boureghda, H., and Śliwka, J. 2021. Identification and pathogenicity of *Fusarium* spp. associated with tuber dry rot and wilt of potato in Algeria. Eur. J. Plant Pathol. 159:495–509.

Baturo-Ciesniewska, A., Lenc, L., Grabowski, A., and Lukanowski, A. 2015. Characteristics of Polish isolates of *Fusarium sambucinum*: molecular identification, pathogenicity, diversity and reaction to control agents. Am. J. Potato Res. 92:49–61.

Bauske, M. J., Yellareddygari, S. K. R., and Gudmestad, N. C. 2018. Potential impact of fluopyram on the frequency of the D123E mutation in *Alternaria solani*. Plant Dis. 102:656–665.

Becher, R., and Wirsel, S. G. R. 2012. Fungal cytochrome P450 sterol 14α-demethylase (CYP51) and azole resistance in plant and human pathogens. Appl. Microbiol. Biotechnol. 95:825–840.

Bojanowski, A., Avis, T. J., Pelletier, S., and Tweddell, R. J. 2013. Management of potato dry rot. Postharvest Biol. Technol. 84:99–109.

Brandhorst, T. T., Kean, I. R. L., Lawry, S. M., Wiesner, D. L., and Klein, B. S. 2019. Phenylpyrrole fungicides act on triosephosphate isomerase to induce methylglyoxal stress and alter hybrid histidine kinase activity. Sci. Rep. 9:5047.

Brandhorst, T. T., and Klein, B. S. 2019. Uncertainty surrounding the mechanism and safety of the post-harvest fungicide fludioxonil. Food Chem. Toxicol. 123:561–565.

Braun, U. 2012. The impacts of the discontinuation of dual nomenclature of pleomorphic fungi: the trivial facts, problems, and strategies. IMA Fungus. 3:81–86.

Brent, K. J., and Hollomon, D. W. 2007. Fungicide resistance: The assessment of risk. Available at: https://www.frac.info/docs/default-source/publications/monographs/monograph-2.pdf?sfvrsn=749d419a_8 [Accessed May 5, 2022].

Burlakoti, P., Rivera, V. V., Burlakoti, R. R., Nelson, R., Adhikari, T. B., Secor, G. A., et al. 2010. Baseline sensitivity of *Fusarium* species associated with *Fusarium* diseases to metconazole, triticonazole, and thiabendazole fungicides. J. Sugar Beet Res. 47:23–34.

Choiseul, J., Allen, L., and Carnegie, S. F. 2007. Fungi causing dry tuber rots of seed potatoes in storage in Scotland. Potato Res. 49:241–253.

Cools, H. J., Hawkins, N. J., and Fraaije, B. A. 2013. Constraints on the evolution of azole resistance in plant pathogenic fungi. Plant Pathol. 62:36–42.

Corsini, D., and Pavek, J. J. 1986. Fusarium dry-rot resistant potato germplasm. Am. Potato J. 63:629–638.

Crous, P. W., Lombard, L., Sandoval-Denis, M., Seifert, K. A., Schroers, H.-J., et al. 2021. *Fusarium*: More than a node or a foot-shaped basal cell. Stud. Mycol. 98:100116.

Cullen, D. W., Toth, I. K., Pitkin, Y., Boonham, N., Walsh, K., Barker, I. and Lees, A. K. "Use of quantitative molecular diagnostic assays to investigate Fusarium dry rot in potato stocks and soil. Phytopathology 95:1462–71.

Damicone, J. 2017. Fungicide resistance management. EPP-7663. Oklahoma State University, Oklahoma Cooperative Extension Service. 8 pages.

Dean, R., van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., and Foster, G. D. 2012. The top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13:414–430.

Delgado, J. A., Schwarz, P. B., Gillespie, J., Rivera-Varas, V. V., and Secor, G. A. 2010. Trichothecene mycotoxins associated with potato dry rot caused by *Fusarium graminearum*. Phytopathology 100:290–296.

Desjardins, A. E. 1995. Population structure of *Gibberella pulicaris* (anamorph *Fusarium sambucinum*) from potato tuber dry rot in North America and Europe. Am. Potato J. 72:145–156.

Desjardins, A. E., Christ-Harned, E. A., McCormick, S. P., and Secor, G. A. 1993. Population structure and genetic analysis of field resistance to thiabendazole in *Gibberella pulicaris* from potato tubers. Phytopathology 83:164–170.

Du, M., Ren, X., Sun, Q., Wang, Y., and Zhang, R. 2012. Characterization of *Fusarium* spp. causing potato dry rot in China and susceptibility evaluation of Chinese potato germplasm to the pathogen. Potato Res. 55:175–184.

Duellman, K., McMoran, D., Inglis, D., and Frost, K. 2018. High and dry: Managing Fusarium dry rot in storage. Potato Grow. :48–49.

Duellman, K. M., Price, W. J., Lent, M. A., Christian, C. L., Bertram, M. C., and Nolte, P. 2021. Fungicide seed treatment improves performance of single-drop whole and cut seed potatoes. Am. J. Potato Res. 98:315–327.

Eranthodi, A., Schneiderman, D., Harris, L. J., Witte, T. E., Sproule, A., Hermans, A., et al. 2020. Enniatin production influences *Fusarium avenaceum* virulence on potato tubers, but not on durum wheat or peas. Pathogens. 9:75.

Esfahani, M. N. 2005. Susceptibility assessment of potato cultivars to Fusarium dry rot species. Potato Res. 48:215–226.

Estrada Jr, R., Gudmestad, N. C., Rivera, V. V., and Secor, G. A. 2010. *Fusarium graminearum* as a dry rot pathogen of potato in the USA: prevalence, comparison of host isolate aggressiveness and factors affecting aetiology. Plant Pathol. 59:1114–1120.

FAO (Food and Agriculture Organization of the United Nations). 2021. World Food and Agriculture - Statistical Yearbook 2021. Rome: Food and Agriculture Organization of the United Nations. Available at: https://doi.org/10.4060/cb4477en.

Fonseka, D. L., and Gudmestad, N. C. 2016. Spatial and temporal sensitivity of *Alternaria* species associated with potato foliar diseases to demethylation inhibiting and anilino-pyrimidine fungicides. Plant Dis. 100:1848–1857.

Förster, H., Kanetis, L., and Adaskaveg, J. E. 2004. Spiral gradient dilution, a rapid method for determining growth responses and 50% effective concentration values in fungus-fungicide interactions. Phytopathology 94:163–170.

FRAC (Fungicide Resistance Action Committee). 2018. Importance of multisite fungicides in managing pathogen resistance. Available at: https://www.frac.info/docs/default-source/publications/statement-on-multisite-fungicides/frac-statement-on-multisite-fungicides-2018.pdf?sfvrsn=3c25489a_2 [Accessed September 7, 2022].

FRAC (Fungicide Resistance Action Committee). 2019. Pathogen risk list. Available at: https://www.frac.info/docs/default-source/publications/pathogen-risk/frac-pathogen-list-2019.pdf [Accessed May 5, 2022].

FRAC (Fungicide Resistance Action Committee). 2022. FRAC Code List 2022: Fungal control agents sorted by cross-resistance pattern and mode of action. Available at: https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2022--final.pdf?sfvrsn=b6024e9a_2 [Accessed May 5, 2022].

Gachango, E., Hanson, L. E., Rojas, A., Hao, J. J., and Kirk, W. W. 2012. *Fusarium* spp. causing dry rot of seed potato tubers in Michigan and their sensitivity to fungicides. Plant Dis. 96:1767–1774.

Gachango, E., Kirk, W., Hanson, L., Rojas, A., Tumbalam, P., and Shetty, K. 2011. First report of in vitro fludioxonil-resistant isolates of *Fusarium* spp. causing potato dry rot in Michigan. Plant Dis. 95:228–228.

Gallian, J. J., Miller, J. S., and Nolte, P. 2006. Managing fungicide resistance. CIS1130. University of Idaho. 8 pages.

Garcia Bayona, L., Grajales, A., Cardenas, M.E., Sierra, R., Cepero de Garcia, M.C., Bernal, A., et al. 2011. First report of *Fusarium oxysporum* causing potato dry rot in *Solanum tuberosum* in Colombia. New Dis. Rep. 24:14–14.

Geiser, D. M., Al-Hatmi, A. M. S., Aoki, T., Arie, T., Balmas, V., Barnes, I., et al. 2021. Phylogenomic analysis of a 55.1-kb 19-gene dataset resolves a monophyletic *Fusarium* that includes the *Fusarium solani* species complex. Phytopathology 111:1064–1079.

Geiser, D. M., Aoki, T., Bacon, C. W., Baker, S. E., Bhattacharyya, M. K., et al. 2013. One Fungus, One Name: Defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. Phytopathology 103:400–408.

Gudmestad, N. C., and Ivors, K. 2019. Development of fungicide resistance in potato and tomato pathogens. In Fungicide Resistance in North America, St. Paul: American Phytopathological Society, p. 295–308.

Gullino, M. L., Tinivella, F., Garibaldi, A., Kemmitt, G. M., Bacci, L., and Sheppard, B. Mancozeb: Past, present, and future. *Plant Disease* 94: 1076–87.

Hamilton, E. 2019. New mechanism of action found for agricultural pesticide fludioxonil. Univ. Wis.-Madison News. Available at: https://news.wisc.edu/new-mechanism-of-action-found-for-agricultural-pesticide-fludioxonil/ [Accessed May 5, 2022].

Hanson, L., Schwager, S., and Loria, R. 1996. Sensitivity to thiabendazole in *Fusarium* species associated with dry rot of potato. Phytopathology 86:378–384.

Hellin, P., Scauflaire, J., Van Hese, V., Munaut, F., and Legrève, A. 2017. Sensitivity of *Fusarium culmorum* to triazoles: impact of trichothecene chemotypes, oxidative stress response and genetic diversity. Pest Manag. Sci. 73:1244–1252.

Heltoft, P., Brurberg, M. B., Skogen, M., Le, V. H., Razzaghian, J., and Hermansen, A. 2016. *Fusarium* spp. causing dry rot on potatoes in Norway and development of a real-time PCR method for detection of *Fusarium coeruleum*. Potato Res. 59:67–80.

Hide, G. A., Read, P. J., and Hall, S. M. 1992. Resistance to thiabendazole in *Fusarium* species isolated from potato tubers affected by dry rot. Plant Pathol. 41:745–748.

Hills, K., Collins, H., Yorgey, G., McGuire, C., and Kruger, C. 2018. Safeguarding potato cropping systems in the Pacific Northwest through improved soil health. Center for Sustaining Agriculture and Natural Resources, Washington State University. Available at: https://s3-us-west-2.amazonaws.com/wp2.cahnrs.wsu.edu/wp-content/uploads/sites/32/2018/12/Safeguarding-Potato-Cropping-Systems-in-the-PNW-Through-Improved-Soil-Health.pdf [Accessed July 23, 2022].

Kawchuk, L. M., Holley, J. D., Lynch, D. R., and Clear, R. M. 1994. Resistance to thiabendazole and thiophanate-methyl in Canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani*. Am. Potato J. 71:185–192.

Kawchuk, L. M., Hutchison, L. J., Verhaeghe, C. A., Lynch, D. R., Bains, P. S., and Holley, J. D. 2002. Isolation of the β-tubulin gene and characterization of thiabendazole resistance in *Gibberella pulicari*s. Can. J. Plant Pathol. 24:233–238.

Kilani, J., and Fillinger, S. 2016. Phenylpyrroles: 30 years, two molecules and (nearly) no resistance. Front. Microbiol. 7.

Laraba, I., Busman, M., Geiser, D. M., and O'Donnell, K. 2022. Phylogenetic diversity and mycotoxin potential of emergent phytopathogens within the *Fusarium tricinctum* species complex. Phytopathology 112:1284–1298.

Laraba, I., McCormick, S. P., Vaughan, M. M., Geiser, D. M., and O'Donnell, K. 2021. Phylogenetic diversity, trichothecene potential, and pathogenicity within *Fusarium sambucinum* species complex. PLOS ONE 16:e0245037.

Leslie, J. F., and Summerell, B. A. 2006. The *Fusarium* Laboratory Manual. Ames, IA: Blackwell Publishing.

Lombard, L., van der Merwe, N. A., Groenewald, J. Z., and Crous, P. W. 2015. Generic concepts in Nectriaceae. Stud. Mycol. 80:189–245.

Lombard, L., Sandoval-Denis, M., Lamprecht, S. C., and Crous, P. W. 2019. Epitypification of *Fusarium oxysporum* – clearing the taxonomic chaos. Persoonia - Mol. Phylogeny Evol. Fungi. 43:1–47.

Loria, R. 2001. Development and anatomy of the potato plant. In Compendium of Potato Diseases, St. Paul: The American Phytopathological Society, p. 2–3.

Lulai, E. 2001a. Tuber periderm and disease resistance. In Compendium of Potato Diseases, St. Paul: The American Phytopathological Society, p. 3–6.

Lulai, E. 2001b. Tuber respiration and the storage environment. In Compendium of Potato Diseases, St. Paul: The American Phytopathological Society, p. 6–7.

Merlington, A. A. 2014. Management options for control of Fusarium dry rot (*Fusarium* spp.) and potato common scab (*Streptomyces* spp.) of potato (*Solanum tuberosum* L.) in Michigan. M.S. thesis. Michigan State University.

Miller, J., Miller, T., Duellman, K., and Olsen, N. 2019. Managing Fusarium dry rot. Available at: https://millerresearch.com/wp-content/uploads/2019/04/Managing_Fusarium_Dry_Rot.pdf [Accessed May 5, 2022].

Munkvold, G. P., Proctor, R. H., and Moretti, A. 2021. Mycotoxin production in *Fusarium* according to contemporary species concepts. Annu. Rev. Phytopathol. 59:373–402.

Nolte, P. 1994. Managing benzimidazole resistance in the potato dry rot fungus. EXT 769. University of Idaho Extension. 8 pages.

Ocamb, C. M., Hamm, P. B., and Johnson, D. A. 2007. Benzimidazole resistance of *Fusarium* species recovered from potatoes with dry rot from storages located in the Columbia Basin of Oregon and Washington. Am. J. Potato Res. 84:169–177.

O'Donnell, K., Al-Hatmi, A. M. S., Aoki, T., Brankovics, B., Cano-Lira, J. F., Coleman, J. J., et al. 2020. No to *Neocosmospora*: Phylogenomic and practical reasons for continued inclusion of the *Fusarium solani* species complex in the genus *Fusarium*. mSphere. 5:e00810-20.

O'Donnell, K., Rooney, A. P., Proctor, R. H., Brown, D. W., McCormick, S. P., Ward, T. J., et al. 2013. Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. Fungal Genet. Biol. 52:20–31.

O'Donnell, K., Sutton, D. A., Fothergill, A., McCarthy, D., Rinaldi, M. G., Brandt, M. E., et al. 2008. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex. J. Clin. Microbiol. 46:2477–2490.

O'Donnell, K., Sutton, D. A., Rinaldi, M. G., Gueidan, C., Crous, P. W., and Geiser, D. M. 2009. Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-F. equiseti* and *F. chlamydosporum* species complexes within the United States. J. Clin. Microbiol. 47:3851–3861.

O'Donnell, K., Whitaker, B. K., Laraba, I., Proctor, R. H., Brown, D. W., Broders, K., et al. 2022. DNA sequence-based identification of *Fusarium*: A work in progress. Plant Dis. 106:1597–1609.

Okello, P. N., Petrović, K., Kontz, B., and Mathew, F. M. 2019. Eight species of *Fusarium* cause root rot of corn (*Zea mays*) in South Dakota. Plant Health Prog. 20:38–43.

Olsen, N., Miller, J., and Nolte, P. 2006. Diagnosis & management of potato storage diseases. Available at: http://www.extension.uidaho.edu/publishing/pdf/CIS/CIS1131.pdf [Accessed July 24, 2022].

Pasche, J., and Gudmestad, N. C. 2019. Laboratory methods for evaluating fungicide resistance in vitro. In Fungicide Resistance in North America, St. Paul, MN: American Phytopathological Society, p. 161–173.

Peters, J. C., Lees, A. K., Cullen, D. W., Sullivan, L., Stroud, G. P., and Cunnington, A. C. 2008a. Characterization of *Fusarium* spp. responsible for causing dry rot of potato in Great Britain. Plant Pathol. 57:262–271.

Peters, R. D., MacLeod, C., Seifert, K. A., Martin, R. A., Hale, L. R., et al. 2008b. Pathogenicity to potato tubers of *Fusarium* spp. isolated from potato, cereal and forage crops. Am. J. Potato Res. 85:367–374.

Peters, R. D., Platt, H. W., Drake, K. A., Coffin, R. H., Moorehead, S., et al. 2008c. First report of fludioxonil-resistant isolates of *Fusarium* spp. causing potato seed-piece decay. Plant Dis. 92:172–172.

Piepenbring, M. 2015. Introduction to Mycology in the Tropics. St. Paul, MN: American Phytopathological Society.

Ray, H., and Hammerschmidt, R. 1998. Responses of potato tuber to infection by *Fusarium sambucinum*. Physiol. Mol. Plant Pathol. 53:81–92.

Russell, P. E. 2004. Sensitivity baselines in fungicide resistance research and management. Available at: https://www.frac.info/docs/default-source/publications/monographs/monograph-3.pdf?sfvrsn=629d419a_8 [Accessed May 5, 2022].

Sagar, V., Sharma, S., Jeevalatha, A., Chakrabarti, S., and Singh, B. 2011. First report of *Fusarium sambucinum* causing dry rot of potato in India. New Dis. Rep. 24:5–5.

Sandoval-Denis, M., and Crous, P. W. 2018. Removing chaos from confusion: Assigning names to common human and animal pathogens in *Neocosmospora*. Persoonia - Mol. Phylogeny Evol. Fungi. 41:109–129.

Sandoval-Denis, M., Lombard, L., and Crous, P. W. 2019. Back to the roots: A reappraisal of *Neocosmospora*. Persoonia - Mol. Phylogeny Evol. Fungi. 43:90–185.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., and Nelson, A. 2019. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3:430–439.

Schroers, H.-J., Samuels, G. J., Zhang, N., Short, D. P. G., Juba, J., and Geiser, D. M. 2016. Epitypification of *Fusisporium* (*Fusarium*) *solani* and its assignment to a common phylogenetic species in the *Fusarium solani* species complex. Mycologia. 108:806–819.

Schumann, G. L., and D'Arcy, C. J. 2010. Essential Plant Pathology. 2nd ed. St. Paul, MN: American Phytopathological Society.

Secor, G. A., and Salas, B. 2001. Fusarium dry rot and Fusarium wilt. In Compendium of Potato Diseases, St. Paul: The American Phytopathological Society, p. 23–25.

Stefańczyk, E., Sobkowiak, S., Brylińska, M., and Śliwka, J. 2016. Diversity of *Fusarium* spp. associated with dry rot of potato tubers in Poland. Eur. J. Plant Pathol. 145:871–884.

Summerell, B. A. 2019. Resolving *Fusarium*: Current status of the genus. Annu. Rev. Phytopathol. 57:323–339.

Theron, D. J. 1999. Fusarium dry rot of potatoes: etiology, epidemiology, toxicity and control.

Thomas, A., Langston, D. B., Sanders, H. F., and Stevenson, K. L. 2012. Relationship between fungicide sensitivity and control of gummy stem blight of watermelon under field conditions. Plant Dis. 96:1780–1784.

Tiwari, R. K., Bashyal, B. M., Shanmugam, V., Lal, M. K., Kumar, R., Sharma, S., et al. 2022. First report of dry rot of potato caused by *Fusarium proliferatum* in India. J. Plant Dis. Prot. 129:173–179.

USDA-NASS (United States Department of Agriculture, National Agricultural Statistics Service). 2022. Potato stocks (quarterly report). Available at: https://downloads.usda.library.cornell.edu/usda-esmis/files/47429915b/8s45rg53p/5d86q505p/post0622.pdf [Accessed July 16, 2022].

Víglaš, J., and Olejníková, P. 2021. An update on ABC transporters of filamentous fungi – from physiological substrates to xenobiotics. Microbiol. Res. 246:126684.

Western Regional IPM Project (U.S.), and University of California Integrated Pest Management Program. 2006. Integrated pest management for potatoes in the western United States. 2nd ed. Oakland, Calif.: Oakland, Calif. : Division of Agriculture and Natural Resources, University of California.

Wharton, P., Hammerschmidt, R., and Kirk, W. 2007. Fusarium dry rot. E-2992. Michigan State University Extension Bulletin. 6 pages.

Xia, J. W., Sandoval-Denis, M., Crous, P. W., Zhang, X. G., and Lombard, L. 2019. Numbers to names - restyling the *Fusarium incarnatum-equiseti* species complex. Persoonia - Mol. Phylogeny Evol. Fungi. 43:186–221.

Chapter 2: Pathogenicity of *Fusarium* Species Associated with Potato Dry Rot in the Pacific Northwest

Abstract

Fusarium dry rot is a worldwide fungal disease of potato causing seed decay at planting and postharvest tuber decay in storage. A greater understanding of the current *Fusarium* species composition associated with Fusarium dry rot in the Pacific Northwest (PNW) is expected to aid in refinement of current management strategies. In this study, 325 *Fusarium* isolates from PNW tuber samples were identified using molecular and phylogenetic techniques, and selected isolates were tested for pathogenicity to potato tubers. Twenty *Fusarium* species were recovered, eight of which have not previously been reported as Fusarium dry rot pathogens in the PNW. *Fusarium sambucinum* was the most prevalent species (45.4%), followed by *Fusarium oxysporum* (14.1%). Pathogenicity testing on potato tubers showed *Fusarium* species have differing levels of aggressiveness and that aggressiveness can vary within a single species. On potato cv. 'Russet Burbank', *F. sambucinum* was determined to be the most aggressive of all tested species. *Fusarium avenaceum* and *F. oxysporum* were somewhat aggressive followed by *Fusarium redolens* and *Fusarium culmorum*. The diversity of *Fusarium* species pathogenic to potato in the PNW is greater than previously reported. *Fusarium sambucinum* and *F. oxysporum* are the most predominant in the PNW and thus pose the greatest risk. Other *Fusarium* species that were notably aggressive were not commonly encountered in this study, indicating low risk of dry rot generally but the possibility of high disease risk in specific situations. Other species were weak pathogens, and, even for those that were more prevalent, dry rot risk is considered low.

Introduction

Fusarium dry rot is a worldwide fungal disease of potato causing seed decay at planting and postharvest tuber decay in storage, known as Fusarium seed decay and Fusarium dry rot, respectively (Azil et al. 2021; Baturo-Ciesniewska et al. 2015; Corsini and Pavek 1986; Du et al. 2012; Gachango et al. 2012; Heltoft et al. 2016; Kawchuk et al. 1994, 2002; Peters et al. 2008a; Secor and Salas 2001; Stefańczyk et al. 2016). This disease is caused by several species in the genus *Fusarium*. Lesions appear as sunken, wrinkled, darkened areas on the periderm while internally there is dry, crumbly decayed tissue that is tan to brown to black in color (Gachango et al. 2012; Heltoft et al. 2016; Nolte 1994; Peters et al. 2008a; Secor and Salas 2001; Wharton et al. 2007a). Yield losses due to Fusarium dry rot make it one of the most important postharvest potato diseases worldwide. Reported average decreased yield ranges from 6 to 25% with up to 60% storage loss reported (Baturo-Ciesniewska et al.

2015; Corsini and Pavek 1986; Du et al. 2012; Gachango et al. 2012; Heltoft et al. 2016; Kawchuk et al. 1994, 2002; Peters et al. 2008a; Secor and Salas 2001; Stefańczyk et al. 2016). Most seed lots and commercial crops have some dry rot infection. High incidence of the disease in seed may affect crop emergence and health and the amount of time potatoes can be stored without sustaining financial loss (Peters et al. 2008a). Potato growers around the world strive to manage Fusarium dry rot through cultural practices such as wound management and crop rotation, application of chemical fungicides and use of less susceptible potato varieties (Bojanowski et al. 2013).

The Pacific Northwest (PNW) region of the United States is a major potato production region of the world, and the PNW states of Idaho, Oregon and Washington collectively produce around 11.3 million metric tons of potatoes annually (USDA-NASS 2022). Fusarium dry rot continues to be a disease of concern for the industry. Recently, isolated instances of severe outbreaks of the disease have been reported in the region. A greater understanding of the species of *Fusarium* associated with Fusarium dry rot in the PNW may allow refinement of management strategies to help reduce dry rot incidence and severity in this region. Such knowledge may also help guide efforts to breed for resistance. Consequently, the objectives of this study were to determine species composition of Fusarium dry rot pathogens in the PNW and characterize pathogenicity of isolates for species not previously reported in the PNW or not previously known to cause dry rot.

Materials and Methods

Isolate Collection

Sample Collection, Wound Inducement on Asymptomatic Tubers and Isolation from Dry Rot Lesions

Samples were collected from commercial and seed storage units from the PNW region during 2016 to 2019 (Table 2-1). For most samples, tubers with dry rot samples were arbitrarily selected from commercial and seed potato storages. Additional samples were obtained from growers and other individuals who submitted samples to our laboratory for diagnosis. Each sample consisted of one to twenty tubers from the same lot, i.e., same variety and same field or seed lot. Additionally, some samples were received as cultures from collaborators. A total of 399 symptomatic and 151 asymptomatic tubers were collected across 123 samples.

Isolations were made from lesions of symptomatic tubers shortly after receipt. For asymptomatic tubers, dry rot symptoms were induced by wounding tubers by cutting two circular wounds on two different sides of tubers using a sterilized four-millimeter cork borer, but no tissue was removed. Wounded tubers were incubated at 10°C in the dark for up to six weeks in plastic containers. To maintain air circulation, holes were placed in the container lids. To maintain sufficient humidity in the

plastic bins, layers of moistened paper towels were placed in the bottom of the bins with plastic canvas mesh overlying the towels to keep the tubers dry. After incubation, the wounds were cut through to evaluate whether dry rot lesions had formed, and isolations were made from these lesions.

Table 2-1. Number of samples and isolates by potato growing region obtained from samples of Pacific Northwest potato tubers received for a survey focused on Fusarium dry rot of potato, 2016 to 2019.

Growing region		Total number of samples received	Number of samples from commercial storage	Number of samples from seed storage	Number of samples from unknown facility	Number of asymptomatic samples [a]	Number of Fusarium isolates recovered	Number of Fusarium species recovered
Eastern Idaho		45	11	33	1	9	183	18
Southeastern Idaho		3	1	2	0	2	6	5
Magic Valley		10	5	3	2	5	34	6
Treasure Valley		5	0	5	0	5	7	4
Columbia Basin		3	0	3	0	3	4	3
Klamath Basin		2	0	2	0	2	2	2
Northwestern Washington		4	3	1	0	1	13	4
Unknown [c]	Total	51	6	16	29	3	76	14
	Idaho	11	4	6	1	3	31	11
	Wash-ington	40	2	10	28	0	45	10
Total [d]		123	26	65	32	30	325 [b]	n/a

[a] All other samples exhibited dry rot symptoms.
[b] Isolate information is detailed in Table A-1.
[c] Some isolates were obtained from samples with unknown county origin, therefore, the growing region is also unknown. For these samples, state location is known.
[d] Values for Idaho and Washington unknown samples are not included in the total.

Putative *Fusarium* isolates were obtained from symptomatic tissue using standard culturing techniques as follows. Tubers were washed with tap water and cut into sections containing both healthy and diseased tissue that were placed in new, clean Petri dishes. The tuber sections were surface sanitized in freshly mixed 0.5% sodium hypochlorite solution for two minutes and then triple-rinsed with sterilized distilled water. Using sterilized scalpels and sterilized paper plates, tuber sections were further dissected into small pieces containing both healthy and diseased tissue with excess rot removed. Four to six small tuber pieces were plated on acidified half-strength potato dextrose agar (PDA; HiMedia Potato Dextrose Agar with 3% agar; RICCA Lactic Acid, 1.00 Normal, added at 4.4 mL per 1 liter water), incubated for three to five days under ambient light or a 12-hour light/dark cycle at 20 to 25°C, and monitored for fungal growth. Resulting growth resembling *Fusarium* and free of contamination was transferred onto new, 3.9% PDA (Difco) via hyphal tipping. Hyphal tipping was performed by using a sterilized tool to remove a small piece of fungal hyphae

from the actively growing edge of the culture and placing it on a new agar plate (Gachango et al. 2012).

Single-Sporing to Obtain Pure Cultures

Pure fungal cultures were obtained by single sporing each putative *Fusarium* isolate. To obtain single macro- or micro-conidia, one four- to five-millimeter section of a putative *Fusarium* culture was placed in a microcentrifuge tube containing one milliliter of sterile distilled water and vortexed for 30 seconds to create a spore suspension. Spore suspensions were diluted 1:9 with sterile distilled water. An EddyJet 2 spiral plater (IUL) was used to apply the spore suspension to the surface of a 100-millimeter water agar plate (2% Difco BactoAgar) in a spiral pattern with progressively lower spore concentrations towards the plate edge using the EddyJet 2 spiral distribution setting of "Log Mode 100 µL" to dispense a total of 98.4 µL spore suspension per plate. After incubation for approximately 24 hours at 25 to 30°C, several single germinated macro- or micro-conidia were selected for transfer to 0.975% PDA plates. Growth was monitored until each individual colony was visually uniform. Pure colonies were subcultured onto several new 3.9% PDA plates to be prepared for long-term storage. Cultures were grown for seven to ten days, sectioned into four- to five-millimeter circles through both the mycelium and agar layers using a heat-sanitized cork borer, and air dried in a laminar flow hood for one to two days. The dried agar plugs were transferred to screw-cap cryovials for long-term storage at -20˚C.

Isolate Identification

Preliminary identification of isolates was conducted using the nuclear ribosomal internal transcribed spacer region (ITS). Some species were readily resolved to the species level using ITS, e.g., *F. sambucinum* and *F. oxysporum*, but many isolates had ambiguous identification at the species level, e.g., members of the *Fusarium tricinctum* species complex (FTSC). Further molecular analysis utilized a portion of the translation elongation factor 1-alpha (*TEF*) gene as it is well-documented to be informative for the *Fusarium* genus (O'Donnell et al. 2022). Additionally, a portion of the phosphate permease (*PHO*) gene was utilized specifically for the FTSC (Laraba et al. 2022).

Molecular Identification of Putative *Fusarium* Isolates

For DNA extraction, fungal tissue was collected from three- to five-day old cultures by gently scraping the surface of fungal colonies with clean glass cover slips. Collected tissue was transferred to microcentrifuge tubes and frozen for subsequent DNA extraction at a later time. For extraction, previously frozen mycelium was briefly thawed and transferred to screw cap vials containing an abrasive matrix (Fast Prep 245G Lysing Matrix vials) to facilitate tissue maceration via vortexing at

high speed. DNA was extracted using Qiagen DNeasy Plant Mini (Qiagen, Germany) extraction kits per manufacturer's instructions. For some heavily pigmented samples, the tissue disruption step was slightly altered from the published Qiagen protocol. Addition of one-half of the lysing buffer volume to the sample prior to vortexing resulted in more successful DNA extraction for these heavily pigmented samples (Spence et al. 2016).

Initial screening for species identification was conducted via conventional polymerase chain reaction (PCR) using universal primers ITS5 and ITS4 to amplify a portion of the ITS region (White et al. 1990). Primer sequences are shown in Table 2-2. PCR reaction mixtures of 25 microliter volume consisted of one microliter of ITS5 forward primer, one microliter of ITS4 reverse primer, 12.5 microliters of master mix (Promega PCR Master Mix, 2X), and 8.5 microliters of nuclease free water (Promega). Amplification was conducted using a Bio-Rad T100 thermal cycler with the following program: initial denaturation at 95°C for three minutes, followed by 30 cycles of 95°C for 30 seconds for denaturation, 55°C for 30 seconds for primer annealing and 72°C for one minute for extension, and a final extension at 72°C for five minutes. Crude PCR amplicons were visualized by electrophoresis in a 1% agarose gel containing 2-µL Biotium Gel Red Nucleic Acid Stain (Cat. 41002) using a Bio-Rad Sub-Cell GT Agarose Gel Electrophoresis System. Amplicons were purified using QIAquick PCR purification kit (Qiagen, Germany) and submitted to a commercial laboratory for ITS sequencing (Molecular Cloning Laboratories, San Francisco, California, USA).

For isolates identified to be *Fusarium*, a portion of the *TEF* gene was amplified via PCR using primers EF1 and EF2 (Geiser et al. 2004). Primer sequences are shown in Table 2-2. PCR reaction mixtures were prepared as above except using forward primer EF1 and reverse primer EF2. The amplification program was as follows: initial denaturation at 95°C for three minutes, followed by 40 cycles of 95°C for 30 seconds for denaturation, 53°C for one minute for primer annealing and 72°C for one minute for extension, and a final extension at 72°C for five minutes. Amplicons were submitted to a commercial laboratory for *TEF* sequencing (Eurofins, Louisville, Kentucky, USA).

For isolates identified to be within FTSC, the *PHO* gene was sequenced to aid in species resolution using primers PT-F1 and PT-R6 (Laraba et al. 2022). PCR reaction mixtures were prepared as above except using forward primer PT-F1 and reverse primer PT-R6. Primer sequences are shown in Table 2-2. The amplification program was as follows: initial denaturation at 94°C for 1 minute 30 seconds, followed by 40 cycles of 94°C for 30 seconds for denaturation, 57°C for 30 seconds for primer annealing, and 68°C for three minutes for extension, and a final extension at 68°C for five minutes. For *PHO* sequencing, crude PCR amplicons were purified using PureLink PCR Purification Kit

(Invitrogen). Amplicons were submitted to a commercial laboratory for *PHO* sequencing (Eurofins, Louisville, Kentucky, USA).

The three loci were analyzed separately. The BLAST tool was used to compare sequences of PNW isolates for similarity with type sequences deposited in the databases National Center for Biotechnology Information GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi), Fusarium-ID, version 1.0, 2.0, 3.0 (Geiser et al. 2004; Torres-Cruz et al. 2022), Fusarioid-ID (www.fusarium.org), and Fusarium MLST (https://fusarium.mycobank.org; O'Donnell et al. 2022).

Table 2-2. Primer sequences used to identify *Fusarium* isolates to species or species complex.

DNA locus	**Primer name**	**Primer sequence (5′ to 3′)**	**Direction**	**Reference**
ITS	ITS5	GGA AGT AAA AGT CGT AAC AAG G	Forward	White et al. 1990
	ITS4	TCC TCC GCT TAT TGA TAT GC	Reverse	
TEF	EF1	ATG GGT AAG GA(A/G) GAC AAG AC	Forward	Geiser et al. 2004
	EF2	GGA (A/G)GT ACC AGT (G/C)AT CAT GTT	Reverse	
PHO	PT-F1	CAA GAC AGC AGG AGG CAA YTC	Forward	Laraba et al. 2022
	PT-R6	GGA TTG TAA GAC GAT CTG CG	Reverse	

Phylogenetic Analyses

Phylogenetic analyses were used to confirm species level identification for selected isolates as determined by *TEF* and/or *PHO* DNA sequencing. These analyses adopted the broader concept of the genus *Fusarium* as described by Geiser et al. (2021) and O'Donnell et al. (2022), and taxonomy used here is in agreement with these recent publications.

Sequence chromatograms were checked for accuracy and edited using MEGA X, Version 10.0.5 (Kumar et al. 2018). Forward and reverse sequences were aligned using ClustalW in MEGA X to confirm similarity between the forward and reverse sequences and retain information from the 5' and 3' ends. These aligned sequences were saved for further analysis.

TEF sequence datasets were compiled for the *Fusarium sambucinum* species complex (FSAMSC), the *Fusarium incarnatum-equiseti* species complex (FIESC), the *Fusarium solani* species complex (FSSC), the clade within *Fusarium* containing *F. oxysporum*, the *F. nisikadoi* species complex, the *F. newnesense* species complex and *F. redolens*, and the entire *Fusarium* genus. A *PHO* sequence dataset was compiled for the *Fusarium tricinctum* species complex (FTSC). Sequence datasets consisted of a) selected PNW *Fusarium* isolates from the current study and b) selected reference and

outgroup sequences based on supporting literature for the *Fusarium* groups (Crous et al. 2021; Geiser et al. 2021; Laraba et al. 2021, 2022; O'Donnell et al. 2008, 2009, 2013, 2022; Schroers et al. 2016; Xia et al. 2019). Sequence datasets were aligned using MUSCLE (Edgar 2004) in MEGA X (Kumar et al. 2018) to produce multiple sequence alignments. The multiple sequence alignments were used to infer individual trees using maximum likelihood as implemented in the IQ-TREE web server (Nguyen et al. 2015; Trifinopoulos et al. 2016). ModelFinder in IQ-TREE was utilized to find the best-fit substitution model, and branch supports were obtained with ultrafast bootstrap (UFBoot) for 1,000 replications (Hoang et al. 2018; Kalyaanamoorthy et al. 2017) for all trees except for the full *Fusarium* genus tree, in which 5,000 replications were used. The best-fit models according to Bayesian Information Criterion (BIC) were as follows: FSAMSC, model TIM2e+I+G4, BIC=15785.858; FSSC, model TPM2+F+G4, BIC = 8872.425; FTSC, model TIM2e+I+G4, BIC = 13667.726; FIESC, model TNe+I+G4, BIC = 9561.862; *Fusarium* genus, model TIM2e+I+G4, BIC = 39671.766. Phylogenies were visualized and annotated using Interactive Tree of Life, version 6.5.7 (Letunic and Bork 2021). The *TEF* and *PHO* sequences of the PNW isolates used in the phylogenetic analyses were deposited in the NCBI GenBank database.

Pathogenicity Testing

Fifteen *Fusarium* species selected for pathogenicity tests (Table 2-3) were determined to be either dry rot species not previously reported in one or more of the PNW states or not previously reported to be a dry rot pathogen. Two sets of pathogenicity tests were conducted ("A" and "B"). Pathogenicity Test A (PTA) was completed by testing 34 *Fusarium* isolates from 13 *Fusarium* species and 13 non-*Fusarium* isolates from up to seven fungal genera on a single potato variety, Russet Burbank, which is commonly grown and dry rot susceptible (Table 2-3). Pathogenicity Test B (PTB) was designed to focus on weak pathogens as determined by PTA and to test their pathogenicity on potato varieties from which they were originally isolated, as well as Russet Burbank, to determine if Russet Burbank was less susceptible (Table 2-3). PTB was completed for 25 *Fusarium* isolates spanning 12 *Fusarium* species, including two species that were not tested in PTA. Representative isolates were chosen from each state for each species that was tested.

Both PTA and PTB were conducted twice. The experiments were established in a randomized complete block design with six replications for PTA and five replications for PTB, with one tuber per replication. A mock-inoculated control treatment was included in all experiments, serving as a negative control in which the tuber was inoculated with a sterile agar plug. An inoculated control treatment was included in all experiments, serving as a positive control in which the tuber was inoculated with a known, pathogenic *F. sambucinum* isolate (FID 71-6). All control treatments used

Russet Burbank tubers. Test isolate cultures were prepared by placing a disk of previously air-dried and frozen mycelium in the center of a culture plate containing 3.9% PDA, followed by 7 to 14 days incubation at ambient temperature, i.e., 20 to 25°C to produce a pure culture.

Visually healthy whole tubers without dry rot symptoms weighing 85 to 142 grams were rinsed by hand with tap water to remove soil, surface sanitized by immersion in freshly mixed 0.5% sodium hypochlorite solution for 10 minutes and air dried on sterile absorbent cloth towels in a laminar flow hood. After drying, tubers were marked with a permanent marker to indicate the stem end. Both the stem and bud ends of the tuber were inoculated by wounding the tuber end with a cork borer to remove a five-millimeter diameter plug of tuber tissue, placing a four-millimeter diameter agar plug of a *Fusarium* culture into the wound with the mycelium side facing inward, and replacing the excised tuber plug. Each tuber was individually placed inside a small paper bag with the top loosely folded over. All paper bags representing all treatments in an individual replication were randomly placed inside a large plastic bin for incubation. Tubers were incubated for four weeks in conditions conducive to dry rot development, i.e., approximately 19˚C and 90-100% relative humidity.

After incubation, tubers were visually assessed by cutting in half lengthwise through the wounds, i.e., longitudinally across tuber, with a freshly surface-sterilized knife on a clean paper plate. For each end of the tuber, if a lesion had developed, the length and width of the lesion was recorded by first measuring the longest point in the lesion from the center of the tuber end toward the tuber center, and then measuring the widest point in the lesion from side to side, approximately perpendicular to where the lesion length was measured. If the tissue around the plug was suberized with no apparent development of dry rot, then the tuber was recorded to have no lesion development. Dry rot severity was represented by total lesion area, which was the sum of the areas of the stem and bud end lesions.

Tubers with lesions were stored for two to three days at 4˚C in small plastic bins until pathogens could be re-isolated. Isolations were made as described previously from tuber lesions from at least two tubers per replication Candidate cultures were subcultured onto new full-strength potato dextrose agar plates and monitored for visual similarity to the original isolate until uniform cultures were obtained. To confirm identity, isolates were identified molecularly using the *TEF* gene as described previously. Sequences were compared to the original isolate to complete Koch's postulates and confirm the pathogenicity of the isolate.

Data were analyzed using the generalized linear mixed model (GLIMMIX) procedure in SAS® version 9.4 software (Cary, North Carolina) with species as the fixed effect and random effects being run and replication within experimental run. Least square means were separated using pairwise

comparisons (alpha = 0.05). For isolates tested on two varieties in PTB, pairwise comparisons were also made between the varieties (alpha = 0.05).

Table 2-3. *Fusarium* species used to challenge Russet Burbank potato tubers to test pathogenicity using isolates originating from tuber samples collected from seed and commercial storages in the Pacific Northwest states of Idaho, Oregon, and Washington.

***Fusarium* species isolated from Pacific Northwest potato tubers**		**Pathogenicity test name [z] and isolate code**			**For Pathogenicity Test B, potato variety of original isolate tested in addition to Russet Burbank [y]**
		Idaho	Oregon	Washington	
Known dry rot pathogen not previously reported in the PNW by state [x]	*acuminatum*	AB: B050	n/r	AB: E099	E099, proprietary variety
	avenaceum [x]	A: A020, H038	n/r	A: C013	n/a
	cerealis	A: F044	n/r	A: E011, F007	n/a
	culmorum [x]	AB: C050	AB: I023	AB: B010	Original varieties not available for testing
	equiseti [x]	AB: C012	n/r	n/r	C012, Dark Red Norland
	flocciferum	AB: C007, C053, C056; B: C058	n/r	A: F059; B: F068	F068, Dark Red Norland; C007, Pacific Russet; C056, All Blue; C053, original variety not available for testing
	graminearum	B: G098	n/r	n/r	G098, Challenger
	oxysporum [x]	A: B083, I053	A: I005, I021	A: I008, I009	n/a
	redolens	AB: I019, I030	AB: I007, I028	n/r	I030, Dark Red Norland; I007, original variety not available for testing
	sambucinum [x]	--	n/r	AB: E089; A: I010	E089, proprietary variety
	solani [x]	AB: H025, H045	n/r	A: F085	H045, Challenger
	sporotrichioides [x]	AB (C059)	n/r	n/r	n/a
	venenatum	n/r	n/r	B: F009, F022, G002	F009, F022, G002, Dark Red Norland
Species not previously reported as a dry rot pathogen worldwide	*stercicola*	--	n/r	AB: E087	E087, proprietary variety
	toxicum	AB: B088	--	n/r	n/a

[z] Pathogenicity test name is "A" (34 *Fusarium* isolates from 13 *Fusarium* species) or "B" (25 *Fusarium* isolates from 12 *Fusarium* species).

[y] Original variety was Russet Burbank unless otherwise noted. Russet Burbank was used for all Pathogenicity Test A isolates, while both Russet Burbank and the variety from which an isolate was originally recovered was used in Pathogenicity Test B.

[x] The following species were previously reported in the Columbia Basin by Ocamb et al. (2007), but the state of origin (Washington or Oregon) was not specified: *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. sambucinum*, *F. solani*, and *F. sporotrichioides*.

Abbreviations: n/a = not applicable; n/r = species not recovered from this location in this survey; -- = species recovered but not tested.

Results

Isolate Identification

From 2016 to 2019, 123 samples containing 550 tubers were received with 93 samples symptomatic for dry rot (Table 2-1). Sample origin included seven potato growing regions of the PNW (Table 2-1). The eastern Idaho region included samples from Bingham, Butte, Custer, Fremont, Jefferson, Madison and Teton Counties. The southeastern Idaho region included samples from Bannock and Caribou Counties. The Magic Valley region of south-central Idaho included samples from Cassia, Minidoka and Power Counties. The Treasure Valley region spans from southwestern Idaho to northeastern Oregon and included samples from Union County in Oregon. The Columbia Basin spans eastern Washington and northern Oregon and included samples from Lincoln and Spokane Counties in Washington. The Klamath Basin in Oregon included samples from Klamath County. The northwestern Washington growing region included samples from Skagit and Whatcom Counties.

From these samples, 802 single-spored isolates were obtained. The identity of 534 isolates was resolved using ITS, *TEF* and/or *PHO* loci. Sixty-four isolates were identified as non-*Fusarium*, and 470 isolates were determined to be species of *Fusarium*. For many tubers, multiple isolates were recovered from the same tuber, and some of the isolates from the same tuber were identified to be the same species (i.e. duplicate isolates). Therefore, to reduce inflation of the true importance of a species within the PNW dry rot survey, a representative isolate was selected in these situations. For example, five isolates were recovered from Sample 87, Tuber 6, and three of those isolates (E070, F044, F077) were identified as *F. cerealis*. Therefore, one isolate, F044, was selected to be the representative isolate for all three *F. cerealis* isolates. Of the 470 *Fusarium* isolates, 249 isolates represented seven potato growing regions in the PNW, 76 isolates were from an unspecified growing region while 145 were duplicate isolates (Table 2-1). Generally, the greater the number of samples collected from a growing region, the greater the total number of isolates and *Fusarium* species recovered (Table 2-1).

Twenty different *Fusarium* species were recovered from this survey of PNW tubers (Figure 2-1). Based on the total number of isolates collected, the predominant species associated with dry rot in the PNW was *F. sambucinum,* followed by *F. oxysporum,* with these two species accounting for nearly two-thirds of isolates (Table 2-4).

Eight counties in the eastern Idaho and the Magic Valley growing regions produce approximately 77% of Idaho potatoes: Bingham 20.4%, Power 10.3%, Madison 8.6%, Fremont 8.5%, Jefferson 8.5%, Cassia 8.0%, Minidoka 7.7%, and Bonneville 6.8% (Idaho Potato Commission, date unknown). Forty-five percent of samples were from these two growing regions (Table 2-1). Many samples were

from Fremont (23) and Bingham (10) counties in eastern Idaho, and these two counties had the highest number of species recovered within a single county, with 19 species recovered from samples originating from Bingham County, an important commercial production area, and 12 species recovered from samples originating from Fremont County, an important seed production area (Table A-3). Although fewer samples were obtained from Butte County, an area with seed potato production, and Minidoka County, an area with commercial potato production, *Fusarium* diversity was greater in these areas than in the remaining Idaho counties, i.e., Bannock, Caribou, Cassia, Custer, Jefferson, Madison, Power (Table A-3).

Recovery of some species was limited geographically (Table A-3). Ninety-five percent of *F. sambucinum* isolates were recovered from Idaho samples with *F. sambucinum* rarely occurring in Washington samples and absent from the limited Oregon samples, indicating that *F. sambucinum* was prevalent in the eastern Idaho, southeastern Idaho and Magic Valley growing regions. Isolates of *F. clavum*, *F. coeruleum*, *F. gamsii*, *F. graminearum*, *F. iranicum*, and *F. mori* were found only in Idaho. *Fusarium gamsii* accounted for 7% of eastern Idaho isolates, and most of those isolates were from Butte, Bingham and Fremont counties. The three isolates of *F. iranicum* were found in the seed producing counties Fremont and Butte. *Fusarium acuminatum* in Idaho samples was only observed from the eastern Idaho growing region. *Fusarium venenatum* was only recovered from Washington samples in the northwestern Washington growing region definitively and possibly the Columbia Basin. Of the five isolates of *F. stercicola* from Idaho and Washington, four of them were from Washington. *Fusarium redolens* and *F. toxicum* were not recovered from the Columbia Basin and northwestern Washington growing regions. Only *F. oxysporum* was recovered from tuber samples from all seven growing regions (Table A-2).

Some variation in the *Fusarium* species isolated from potato varieties was apparent from the PNW survey data (Table A-4). *Fusarium sambucinum*, *F. oxysporum*, *F. culmorum* and *F. gamsii* were recovered from the most potato varieties, 16, 13, 14 and 11 varieties, respectively. A greater diversity of *Fusarium* species was observed for some potato varieties. Nine to eleven different *Fusarium* species per variety were recovered from samples of Russet Burbank, Ranger Russet and Russet Norkotah. In particular, *F. sambucinum* isolates were especially prevalent in Ranger Russet, Teton Russet, Umatilla Russet, Payette and Dark Red Norland samples.

Table 2-4. Number of *Fusarium* isolates recovered from symptomatic dry rot tubers from commercial and seed storages in the Pacific Northwest by species and potato growing region.

Fusarium species	No. [a]	% [a]	E ID [b]	SE ID [b]	MV [b]	TV [b]	CB [b]	KB [b]	NW WA [b]
acuminatum	11	3.4	9	0	0	0	0	0	0
avenaceum	6	1.8	4	0	0	0	0	0	0
cerealis	9	2.8	4	0	0	0	0	0	0
clavum	2	0.6	1	0	0	0	0	0	0
coeruleum	2	0.6	2	0	0	0	0	0	0
culmorum	21	6.4	9	0	4	3	0	0	0
equiseti	20	6.1	10	1	6	1	1	0	0
flocciferum	9	2.8	6	0	0	0	0	0	0
gamsii	16	4.9	13	0	2	0	0	0	0
graminearum	1	0.3	1	0	0	0	0	0	0
iranicum	3	0.9	3	0	0	0	0	0	0
mori	1	0.3	1	0	0	0	0	0	0
oxysporum	45	14.1	14	1	1	1	1	1	7
redolens	5	1.8	0	1	1	2	0	0	0
sambucinum	148	45.4	98	2	20	0	2	0	0
solani	12	3.7	4	1	0	0	0	0	2
sporotrichioides	1	0.3	1	0	0	0	0	0	0
stercicola	5	1.5	0	0	0	0	0	0	2
toxicum	3	0.9	2	0	0	0	0	1	0
venenatum	4	1.2	0	0	0	0	0	0	2

[a] Number and percent of isolates out of 325 isolates resolved to *Fusarium* species.
[b] Isolates from an unknown growing region are not included in this table.
Abbreviations: No. = number; % = percent; Potato growing region: E ID = eastern Idaho; SE ID = southeastern Idaho; MV = Magic Valley; TV = Treasure Valley; CB = Columbia Basin; KB = Klamath Basin; NW WA = northwestern Washington

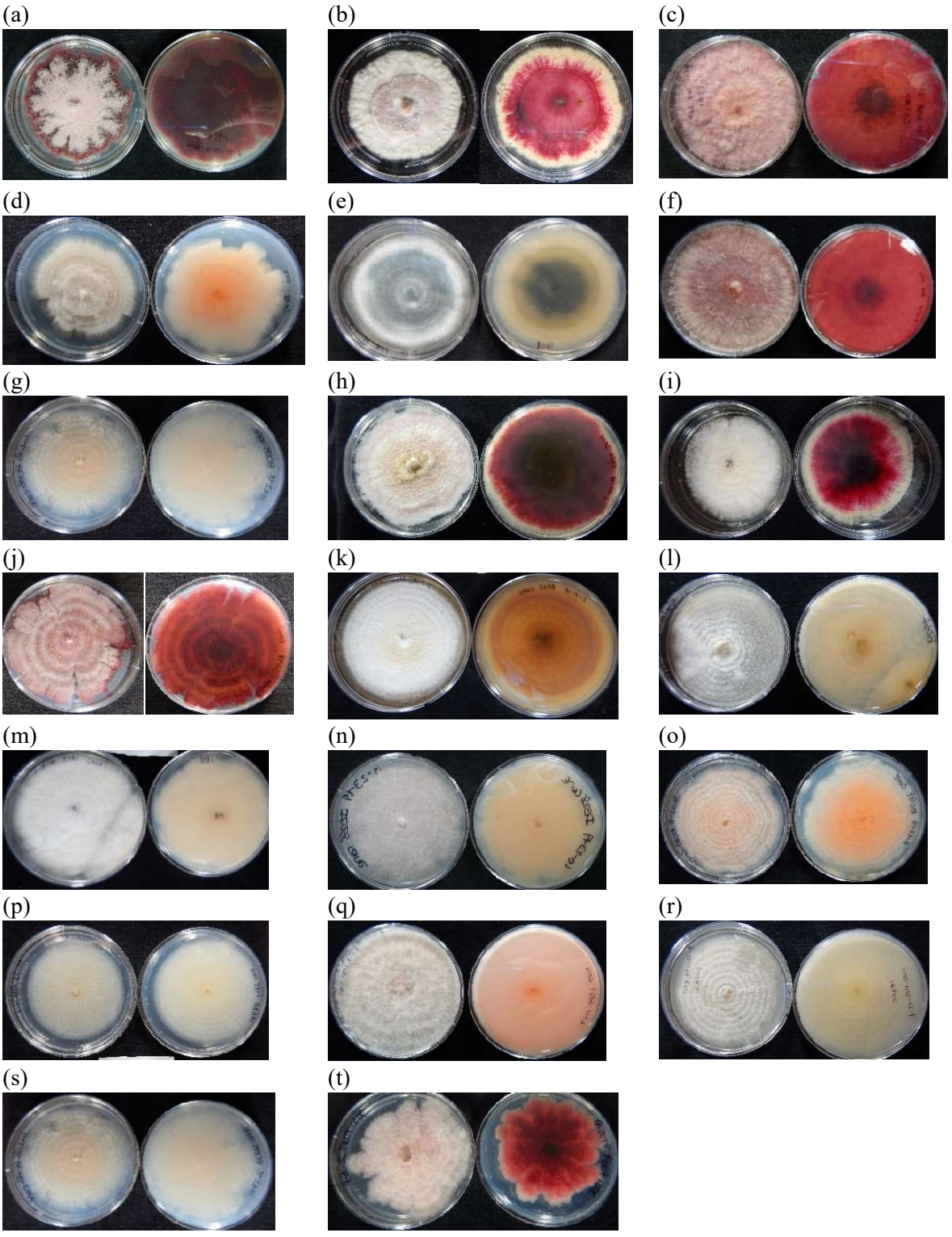

Figure 2-1. Colony morphology of *Fusarium* species associated with Fusarium dry rot of potato in the Pacific Northwest. (a) *F. acuminatum*; (b) *F. avenaceum*; (c) *F. cerealis*; (d) *F. clavum*; (e) *F. coeruleum*; (f) *F. culmorum*; (g) *F. equiseti*; (h) *F. flocciferum*; (i) *F. gamsii*; (j) *F. graminearum*; (k) *F. iranicum*; (l) *F. mori*; (m) *F. oxysporum*; (n) *F. redolens*; (o) *F. sambucinum*; (p) *F. solani*; (q) *F. sporotrichioides*; (r) *F. stercicola*; (s) *F. toxicum*; (t) *F. venenatum*

Phylogenetic Analyses

Fusarium sambucinum species complex (FSAMSC)

The FSAMSC dataset contained 114 *TEF* sequences with 261 parsimony-informative sites in the 789 bp alignment, and the inferred FSAMSC tree had a log likelihood of -7114.091 and was rooted on NRRL 13338 *F. nelsonii* from the *Fusarium chlamydosporum* species complex, which is a sister complex to FSAMSC (Figure 2-2; Table 2-6). Six clades were resolved in the tree, as was expected based on a recently published phylogeny of FSAMSC (Laraba et al. 2021; Figure 2-2). Thirty-one PNW *Fusarium* isolates representing six species were resolved within species clades as expected based on previous BLAST analyses of sequence databases (Figure 2-2). Phylogenetic distinction of the *Brachygibbosum*, *Longipes*, *Novel* and *Sambucinum* Clades was well supported to highly supported with node bootstrap values of 76.8 to 98.1. Separation of the *Sporotrichioides* and *Graminearum* Clades from the other four clades was well supported with bootstrap value of 76.8, but somewhat less support was present for distinction between *Sporotrichioides* and *Graminearum* with a bootstrap value of 61.7. The node separating the closely related *F. culmorum* and *F. cerealis* was well supported with a bootstrap value of 77.5. Three PNW isolates were previously unresolved to species (E090, E073, E092), and this analysis identified them as *F. cerealis* based on *TEF*.

Fusarium tricinctum species complex (FTSC)

For the FTSC, the *TEF* locus was insufficient to resolve many isolates to species. Using *TEF*, PNW isolates from the FTSC were identified as *F. acuminatum*, *F. avenaceum*, *F. flocciferum* or as FTSC. More specifically, 31 Idaho isolates were identified as FTSC, which was 9% of unique isolates in Idaho. Therefore, the *PHO* locus was utilized based on recent work by Laraba et al. (2022). Using *PHO*, the 31 FTSC isolates from Idaho were identified to species level as 24 isolates of *F. gamsii*, six isolates of *F. acuminatum* and one isolate of *F. avenaceum*. Further, *PHO* sequences helped differentiate the closely related species *F. flocciferum* and *F. iranicum*, and five *F. flocciferum* isolates identified by *TEF* were subsequently identified as *F. iranicum* using *PHO*.

The FTSC *PHO* dataset contained 54 sequences with 258 parsimony-informative sites in the 1,154 bp alignment, and the inferred FTSC tree had a log likelihood of -6445.572 and was rooted on NRRL 36452 *F. nurragi* based on Laraba et al. (2022) (Figure 2-3). Twenty-three PNW *Fusarium* isolates representing five species were resolved within species clades as expected based on previous BLAST analyses of sequence databases (Figure 2-3). Although blasting of *PHO* sequences sometimes led to inconclusive species identification for *F. flocciferum* and *F. iranicum*, the node separating these two closely related species received strong support with a bootstrap value of 99.7. The PNW isolate C015 was previously unresolved by BLAST analyses and was identified as *F. iranicum* in the phylogeny.

The tree structure was generally in agreement with Laraba et al. (2022) except for the placement of FTSC 18. All nodes were well to highly supported as indicated by bootstrap values with the exception of the node separating the youngest lineages, i.e., FTSC 12, *F. reticulatum, F. tricinctum, F. acuminatum*, *F. negundinis*, from the remainder, which had a bootstrap value of 42.7.

Fusarium incarnatum-equiseti species complex (FIESC)

The FIESC dataset contained 62 *TEF* sequences with 148 parsimony-informative sites in the 776 bp alignment, and the inferred FIESC tree had a log likelihood of -4364.308 and was rooted on NRRL 13459 *F. concolor* as demonstrated in Xia et al. (2019) and O'Donnell et al. (2009) (Figure 2-4). Separation of the two main clades of the FIESC was accomplished with phylogenetic analysis of *TEF* sequences. Resolution of the Incarnatum Clade was well supported by bootstrap value of 78.5 (Figure 2-4). Apart from the *F. mucidum* and *F. croceum* isolates, all members of the Equiseti Clade were placed together separate from both the outgroup and Incarnatum Clade. PNW *Fusarium* isolates resolved to three species within FIESC: one isolate of *F. clavum*, four isolates of *F. equiseti* and three isolates of *F. toxicum* (Figure 2-4). Separation of the closely related *F. equiseti* and *F. toxicum* species, which are both considered to be within haplotype FIESC 14, was highly supported with a bootstrap value of 99.7.

Fusarium solani species complex (FSSC)

The FSSC dataset contained 56 *TEF* sequences with 135 parsimony-informative sites in the 1049 bp alignment, and the inferred FSSC tree had a log likelihood of -4026.274 and was rooted on NRRL 22316 *F. staphyleae* based on O'Donnell et al. (2009) and Schroers et al. (2016) (Figure 2-5). The three FSSC clades were resolved in the tree, as was expected based on previously published phylogenies of FSSC (Figure 2-5; O'Donnell et al. 2008; Schroers et al. 2016). PNW *Fusarium* isolates were resolved to three species within Clade 3 of FIESC, *F. solani*, *F. mori* and *F. stercicola* (Figure 2-5).

Fusarium genus

The *Fusarium* genus dataset contained 252 sequences with 468 parsimony-informative sites in the 903 bp alignment, and the tree inferred from *TEF* sequences had a log likelihood of -18,063.580 and was rooted on outgroup *Trichoderma brevicompactum* (GenBank accession AY937453.1) based on Geiser et al. (2021) (Figure 2-6; Table A-6). Species complexes containing PNW *Fusarium* isolates were placed in agreement with Geiser et al. (2021), including FSAMSC, FIESC, FTSC, FRSC (*Fusarium redolens* species complex), and FSSC. Additionally, apart from the *F. dimerum* and *F. buxicola* species complexes, all species complexes were placed in general agreement with Geiser et

al. (2021) with good bootstrap support in most cases. In the *Fusarium* phylogeny, three species of PNW isolates were included that were not addressed in the species complex level trees: *F. oxysporum*, *F. redolens* and *F. coeruleum*. All isolates of these three species resolved together in separate clades.

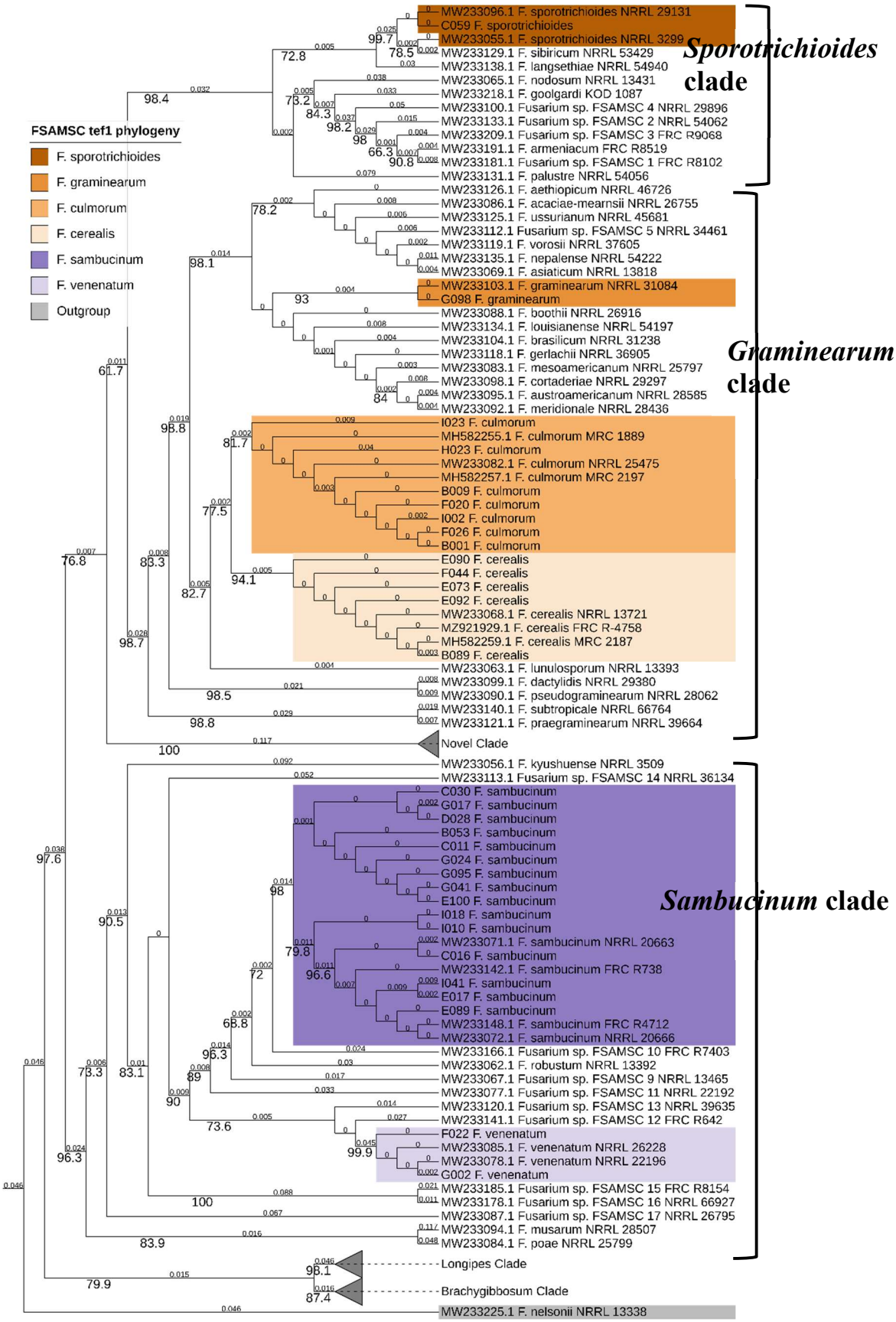
FSAMSC tef1 phylogeny
F. sporotrichioides
F. graminearum
F. culmorum
F. cerealis
F. sambucinum
F. venenatum
Outgroup
MW233096.1 F. sporotrichioides NRRL 29131
C059 F. sporotrichioides
MW233055.1 F. sporotrichioides NRRL 3299
MW233129.1 F. sibiricum NRRL 53429
MW233138.1 F. langsethiae NRRL 54940
MW233065.1 F. nodosum NRRL 13431
MW233218.1 F. goolgardi KOD 1087
MW233100.1 Fusarium sp. FSAMSC 4 NRRL 29896
MW233133.1 Fusarium sp. FSAMSC 2 NRRL 54062
MW233209.1 Fusarium sp. FSAMSC 3 FRC R9068
MW233191.1 F. armeniacum FRC R8519
MW233181.1 Fusarium sp. FSAMSC 1 FRC R8102
MW233131.1 F. palustre NRRL 54056
Sporotrichioides clade
MW233126.1 F. aethiopicum NRRL 46726
MW233086.1 F. acaciae-mearnsii NRRL 26755
MW233125.1 F. ussurianum NRRL 45681
MW233112.1 Fusarium sp. FSAMSC 5 NRRL 34461
MW233119.1 F. vorosii NRRL 37605
MW233135.1 F. nepalense NRRL 54222
MW233069.1 F. asiaticum NRRL 13818
MW233103.1 F. graminearum NRRL 31084
G098 F. graminearum
MW233088.1 F. boothii NRRL 26916
MW233134.1 F. louisianense NRRL 54197
MW233104.1 F. brasilicum NRRL 31238
MW233118.1 F. gerlachii NRRL 36905
MW233083.1 F. mesoamericanum NRRL 25797
MW233098.1 F. cortaderiae NRRL 29297
MW233095.1 F. austroamericanum NRRL 28585
MW233092.1 F. meridionale NRRL 28436
Graminearum clade
I023 F. culmorum
MH582255.1 F. culmorum MRC 1889
H023 F. culmorum
MW233082.1 F. culmorum NRRL 25475
MH582257.1 F. culmorum MRC 2197
B009 F. culmorum
F020 F. culmorum
I002 F. culmorum
F026 F. culmorum
B001 F. culmorum
E090 F. cerealis
F044 F. cerealis
E073 F. cerealis
E092 F. cerealis
MW233068.1 F. cerealis NRRL 13721
MZ921929.1 F. cerealis FRC R-4758
MH582259.1 F. cerealis MRC 2187
B089 F. cerealis
MW233063.1 F. lunulosporum NRRL 13393
MW233099.1 F. dactylidis NRRL 29380
MW233090.1 F. pseudograminearum NRRL 28062
MW233140.1 F. subtropicale NRRL 66764
MW233121.1 F. praegraminearum NRRL 39664
Novel Clade
MW233056.1 F. kyushuense NRRL 3509
MW233113.1 Fusarium sp. FSAMSC 14 NRRL 36134
C030 F. sambucinum
G017 F. sambucinum
D028 F. sambucinum
B053 F. sambucinum
C011 F. sambucinum
G024 F. sambucinum
G095 F. sambucinum
G041 F. sambucinum
E100 F. sambucinum
I018 F. sambucinum
I010 F. sambucinum
MW233071.1 F. sambucinum NRRL 20663
C016 F. sambucinum
MW233142.1 F. sambucinum FRC R738
I041 F. sambucinum
E017 F. sambucinum
E089 F. sambucinum
MW233148.1 F. sambucinum FRC R4712
MW233072.1 F. sambucinum NRRL 20666
Sambucinum clade
MW233166.1 Fusarium sp. FSAMSC 10 FRC R7403
MW233062.1 F. robustum NRRL 13392
MW233067.1 Fusarium sp. FSAMSC 9 NRRL 13465
MW233077.1 Fusarium sp. FSAMSC 11 NRRL 22192
MW233120.1 Fusarium sp. FSAMSC 13 NRRL 39635
MW233141.1 Fusarium sp. FSAMSC 12 FRC R642
F022 F. venenatum
MW233085.1 F. venenatum NRRL 26228
MW233078.1 F. venenatum NRRL 22196
G002 F. venenatum
MW233185.1 Fusarium sp. FSAMSC 15 FRC R8154
MW233178.1 Fusarium sp. FSAMSC 16 NRRL 66927
MW233087.1 Fusarium sp. FSAMSC 17 NRRL 26795
MW233094.1 F. musarum NRRL 28507
MW233084.1 F. poae NRRL 25799
Longipes Clade
Brachygibbosum Clade
MW233225.1 F. nelsonii NRRL 13338

Figure 2-2. Maximum likelihood phylogeny of the *Fusarium sambucinum* species complex (FSAMSC) inferred from translation elongation factor 1-alpha (*TEF*) sequences and including *Fusarium* isolates recovered from symptomatic dry rot potato tubers from Pacific Northwest (PNW) seed and commercial potato storages in Idaho, Oregon and Washington. The phylogeny was rooted on outgroup *Fusarium nelsonii* from the sister complex to FSAMSC, *Fusarium chlamydosporum* species complex. The phylogeny was generated in the IQ-TREE Web Server, and support values shown at nodes are maximum likelihood bootstrap values indicating the percentage of replicate trees out of 1,000 replicates resulting in isolate placement as shown. GenBank accession numbers, the originating location and plant of the isolate, if known, and culture collection identification numbers are shown for all reference sequences either in the figure or in Appendix C, Table A-5 (FRC = *Fusarium* Research Center, The Pennsylvania State University, University Park, PA; KOD = Kerry O'Donnell; MRC = National Research Institute for Nutritional Diseases, South Africa; NRRL = ARS Culture Collection, Peoria, IL). All PNW isolates are indicated by 4-character alpha-numeric names (e.g., C059) with species identity noted. Collapsed clades are represented by gray triangles and do not contain any PNW isolates, only reference isolates, which are listed in Table A-5. Reference and outgroup sequences were selected based on Laraba et al. 2021. In this phylogeny, six clades were identified within FSAMSC as in Laraba et al. (2021), and all PNW isolates resolved with their expected species within FSAMSC.

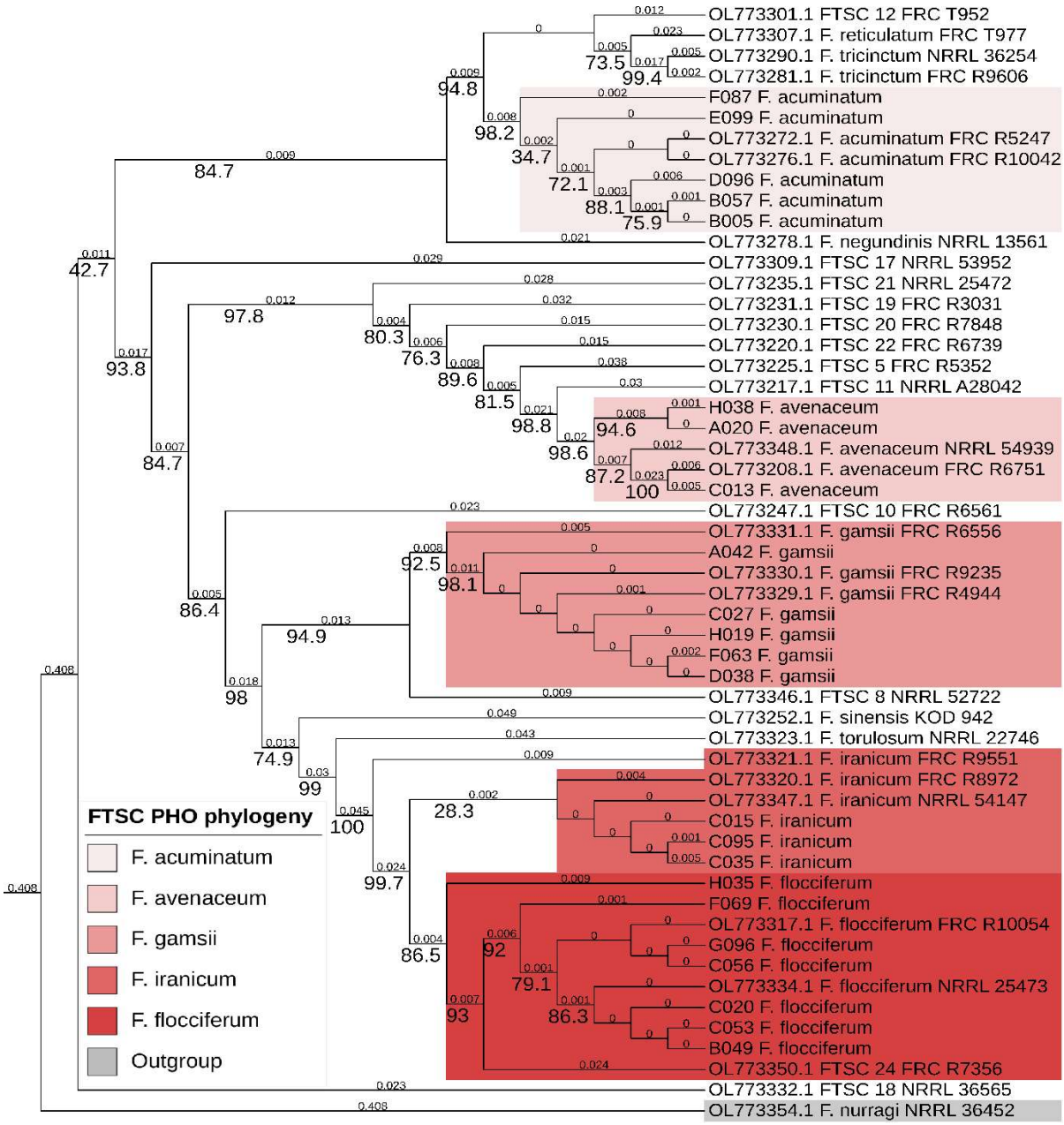

Figure 2-3. Maximum likelihood phylogeny of the *Fusarium tricinctum* species complex (FTSC) inferred from phosphate permease (*PHO*) sequences and including *Fusarium* isolates recovered from symptomatic dry rot potato tubers from Pacific Northwest (PNW) seed and commercial potato storages in Idaho and Washington. The phylogeny was rooted on outgroup *Fusarium nurragi* and generated in the IQ-TREE Web Server. Support values shown at nodes are maximum likelihood bootstrap values indicating the percentage of replicate trees out of 1,000 replicates resulting in isolate placement as shown. Branch lengths are shown along branches and represent the number of nucleotide substitutions per nucleotide site. GenBank accession numbers and culture collection identification numbers are shown for all reference sequences (FRC = *Fusarium* Research Center, The Pennsylvania State University, University Park, PA; KOD = Kerry O'Donnell; NRRL = ARS Culture Collection, Peoria, IL). All PNW isolates are indicated by 4-character alpha-numeric names (e.g.,

F087). Species-level clades containing PNW isolates are indicated by color as shown in the legend. Reference and outgroup sequences were selected based on Laraba et al. (2022). In this phylogeny, all PNW isolates resolved with their expected species within FTSC.

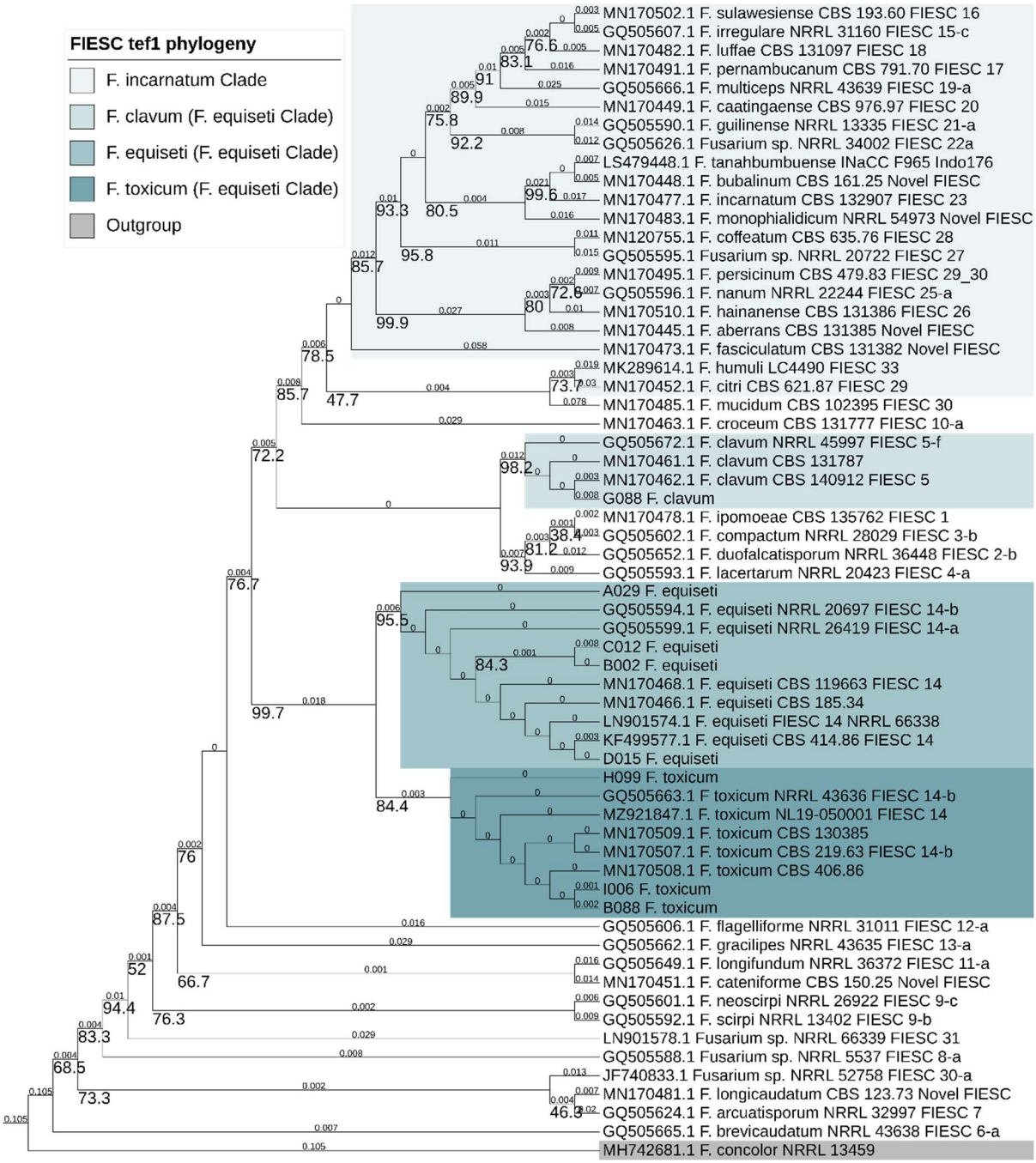

Figure 2-4. Maximum likelihood phylogeny of the *Fusarium incarnatum-equiseti* species complex (FIESC) inferred from translation elongation factor 1-alpha (*TEF*) sequences and including *Fusarium* isolates recovered from symptomatic dry rot potato tubers from Pacific Northwest (PNW) seed and commercial potato storages in Idaho and Oregon. The phylogeny was rooted on outgroup *Fusarium concolor* and generated in the IQ-TREE Web Server. Support values shown at nodes are maximum likelihood bootstrap values indicating the percentage of replicate trees out of 1,000 replicates resulting in isolate placement as shown. Branch lengths are shown along branches and represent the number of nucleotide substitutions per nucleotide site. GenBank accession numbers and culture collection identification numbers are shown for reference sequences (CBS = Westerdijk Fungal

Biodiversity Institute, Utrecht, The Netherlands; FRC = *Fusarium* Research Center, The Pennsylvania State University, University Park, Pennsylvania, USA; NRRL = ARS Culture Collection, Peoria, Illinois, USA). All PNW isolates are indicated by 4-character alpha-numeric names (e.g., G088). Species-level clades containing PNW isolates are indicated by the legend (*F. clavum*, *F. equiseti*, *F. toxicum*). Reference and outgroup sequences were selected based on O'Donnell et al. (2009), Xia et al. (2019) and Geiser et al. (2021). The *F. incarnatum* species complex and Outgroup are indicated in the legend while the remainder of the tree collectively represents the *F. equiseti* Clade.

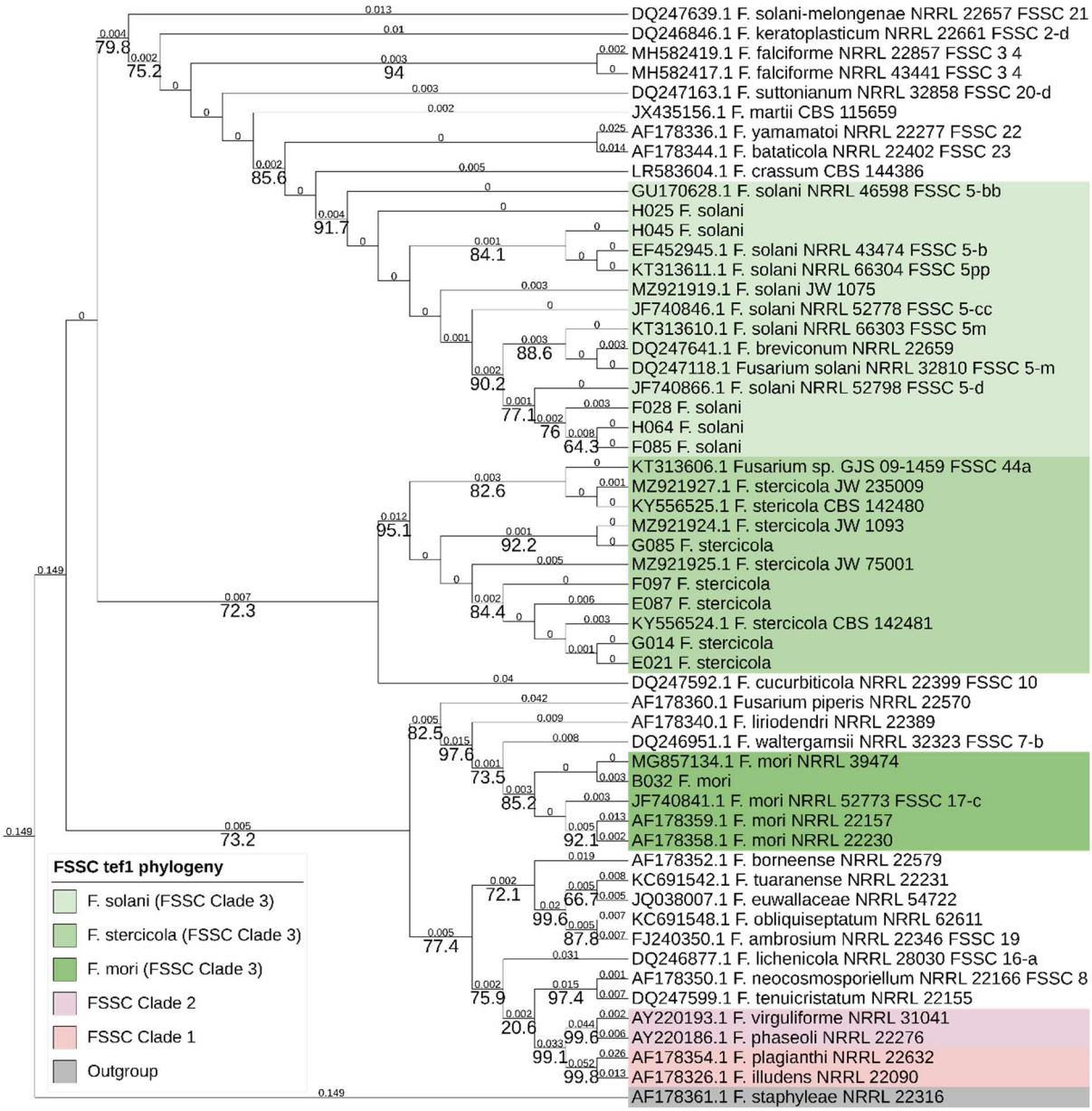

Figure 2-5. Maximum likelihood phylogeny of the *Fusarium solani* species complex (FSSC) inferred from translation elongation factor 1-alpha (*TEF*) sequences and including *Fusarium* isolates recovered from symptomatic dry rot potato tubers from Pacific Northwest (PNW) seed and commercial potato storages in Idaho and Washington. The phylogeny was rooted on outgroup *Fusarium staphyleae* based on O'Donnell et al. (2008) and Schroers et al. (2016). The phylogeny was generated in IQ-TREE Web Server, and support values shown at nodes are maximum likelihood bootstrap values indicating the percentage of replicate trees out of 5,000 replicates resulting in isolate placement as shown. Branch lengths are shown along branches and represent the number of nucleotide substitutions per nucleotide site. GenBank accession numbers and culture collection identification numbers are shown for all reference sequences (NRRL = ARS Culture Collection, Peoria, Illinois, USA; CBS = Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; GJS = collection of G.J. Samuels, Systematic Mycology and Microbiology Laboratory, USDA, Beltsville, Maryland, USA). All PNW isolates are indicated by 4-character alpha-numeric names (e.g., H025)

with their species identity noted. Species-level clades containing PNW isolates are indicated by the legend (*F. mori*, *F. solani*, *F. stercicola*). Reference and outgroup sequences were selected based on O'Donnell et al. (2008), Schroers et al. (2016) and Geiser et al. (2021).

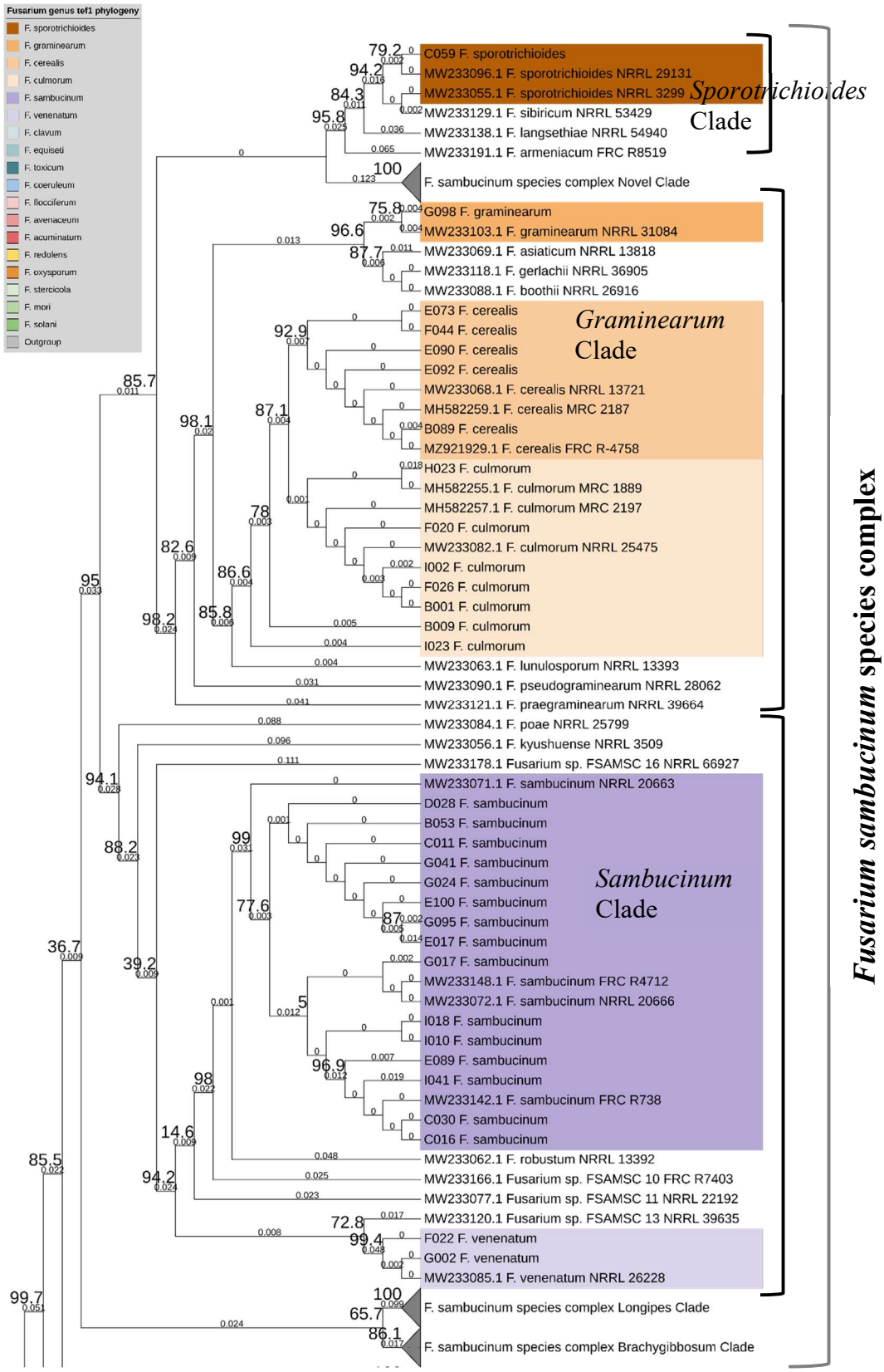

Figure 2-6 (top section of four-section figure– caption at bottom of figure)

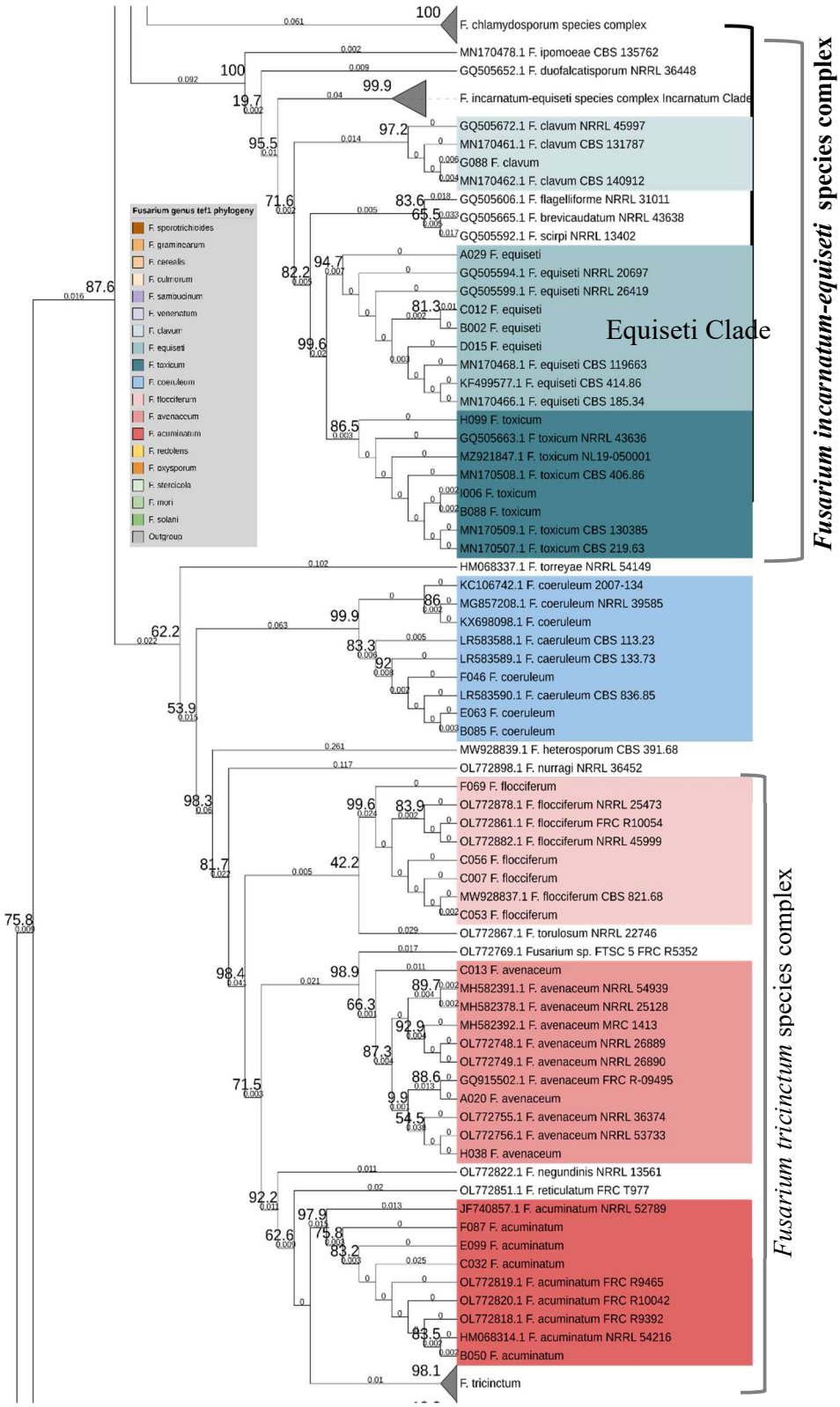

Figure 2-6 (second section of four-section figure– caption at bottom of figure)

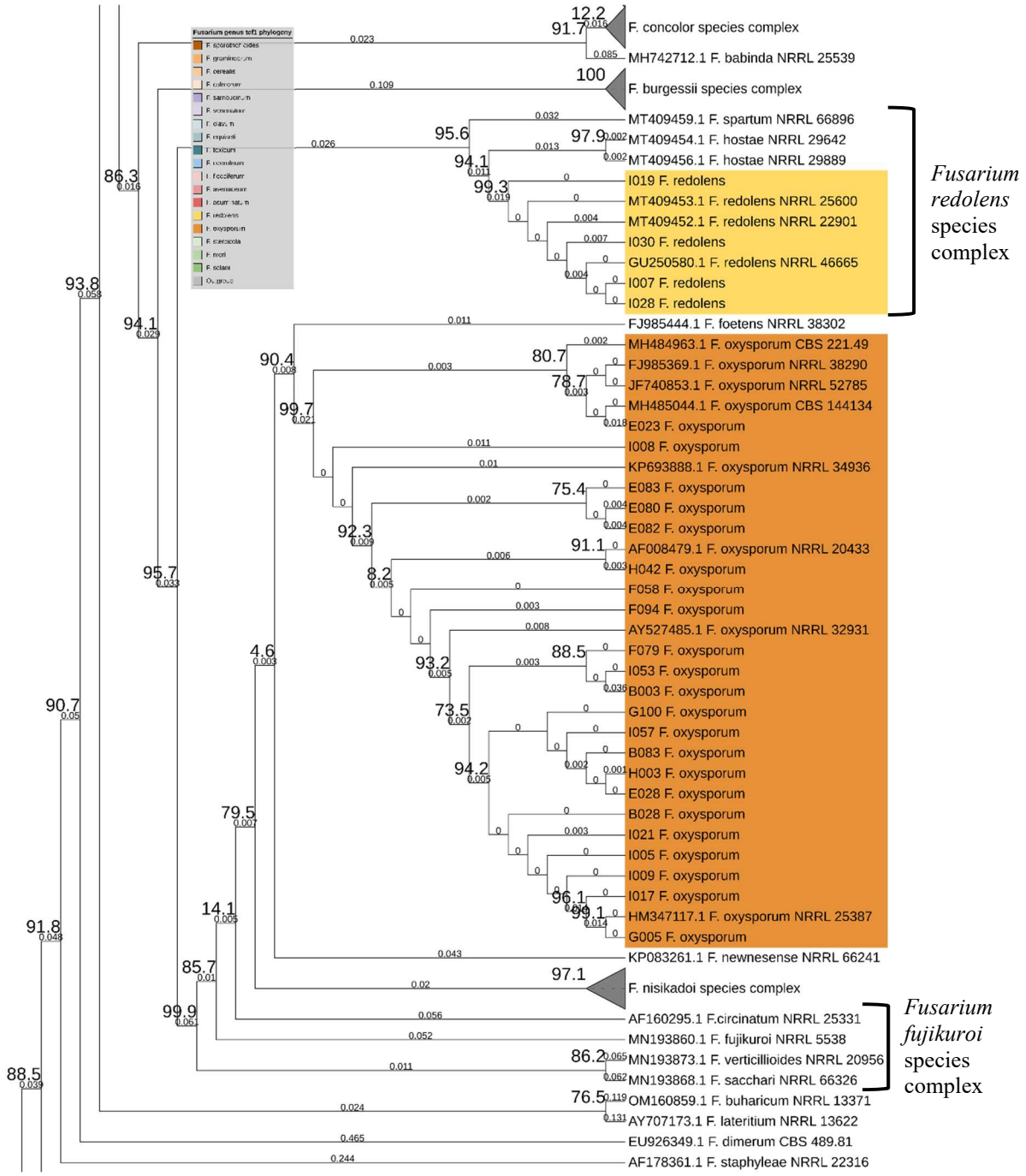

Figure 2-6 (third section of four-section figure– caption at bottom of figure)

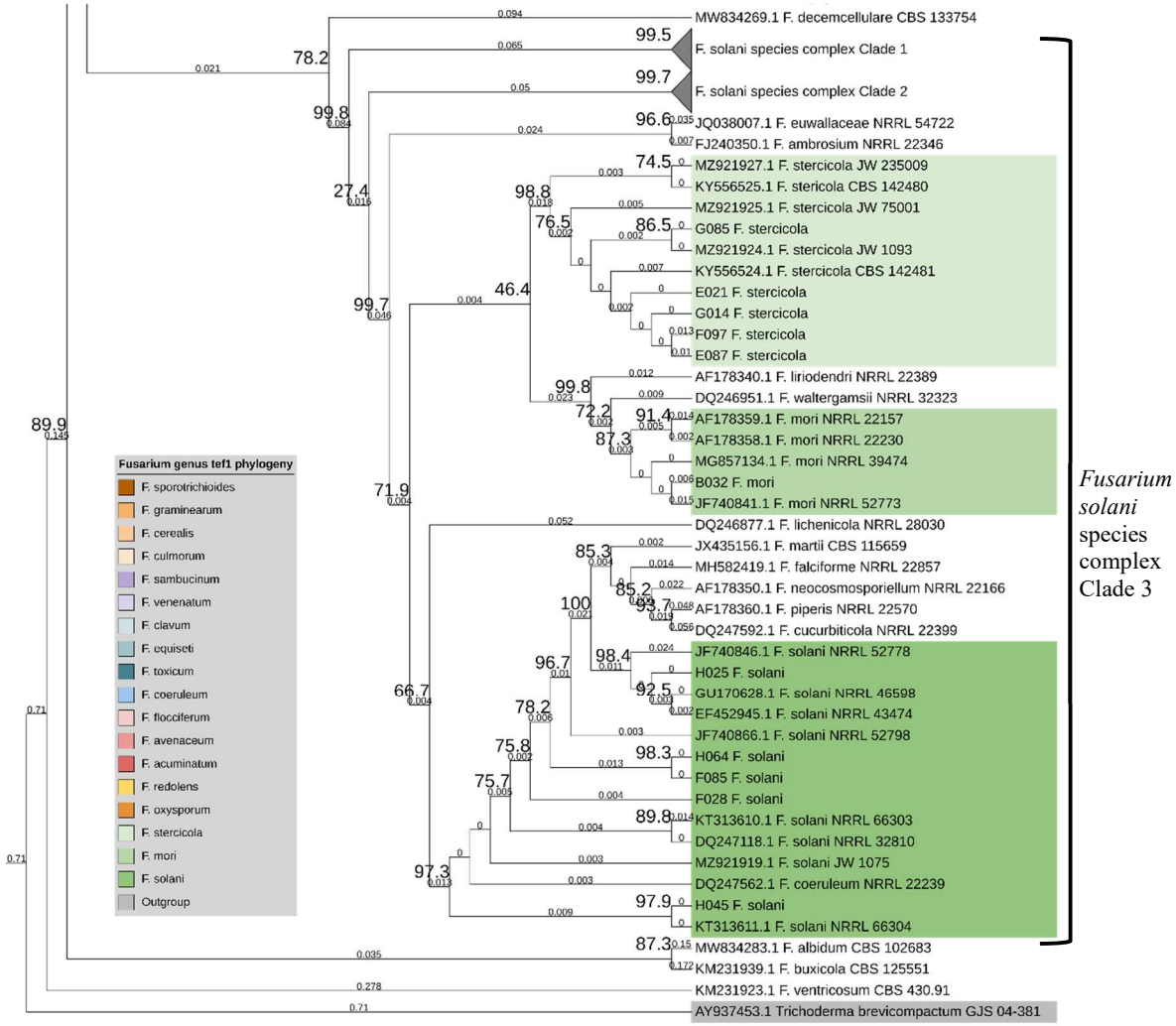

Figure 2-6. Maximum likelihood phylogeny of the *Fusarium* genus inferred from translation elongation factor 1-alpha (*TEF*) sequences and including *Fusarium* isolates recovered from symptomatic dry rot potato tubers from Pacific Northwest (PNW) seed and commercial storages in Idaho, Oregon, Washington. The phylogeny was rooted on outgroup *Trichoderma brevicompactum* and generated in IQ-TREE Web Server. Support values shown at nodes are maximum likelihood bootstrap values indicating the percentage of replicate trees out of 5,000 replicates resulting in isolate placement as shown. Branch lengths are shown along branches and represent the number of nucleotide substitutions per nucleotide site. GenBank accession numbers and culture collection identification numbers are shown for all reference sequences either in the figure or in Appendix C, Table A-6 (NRRL = ARS Culture Collection, Peoria, IL; FRC = *Fusarium* Research Center, The Pennsylvania State University, University Park, PA; CBS = Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands). All PNW isolates are indicated by 4-character alpha-numeric names (e.g., E089). Reference and outgroup sequences were selected based on Geiser et al. (2021), Crous et al. (2021), Laraba et al. (2021) and Laraba et al. (2022). Collapsed clades are represented by gray triangles and do not contain any PNW isolates, only reference isolates, which are listed in Table A-6.

Pathogenicity Testing

Differences among treatments were observed in both pathogenicity tests for both dry rot incidence and severity (Tables 2-5 and 2-6). Generally, a higher percentage of tubers with observed lesions was associated with higher mean lesion number per tuber. Treatments exhibiting dry rot in 90 to 100% of tubers had 1.6 to 2.0 lesions per tuber, and treatments with 60% or fewer tubers with dry rot had fewer than 1.2 lesions per tuber (Tables 2-5 and 2-6). In both PTA and PTB, no lesions were recorded for the mock-inoculated negative control tubers and lesions were observed on all inoculated positive control tubers (Tables 2-5 and 2-6; Figure 2-7). Of 15 tested *Fusarium* species, 14 species produced dry rot lesions and were determined to be pathogenic (Tables 2-5 and 2-6; Figure 2-8). The same fungal species used for the inoculation of healthy tubers was re-isolated from lesion tissue of the inoculated tubers, which completed Koch's postulates. No lesions were observed from inoculation with *F. toxicum*, demonstrating non-pathogenicity of the tested PNW isolate (Table 2-5, Figure 2-9).

Incidence was greatest in tubers inoculated with *F. sambucinum* and *F. oxysporum*. All tested isolates of *F. sambucinum* produced two dry rot lesions in all inoculated tubers (Tables 2-5 and 2-6). *Fusarium sambucinum* lesions were the most severe of all tested species with lesion size ranging from 19.5 to 27.7 cm^2 while lesions for all other species were approximately half the size or smaller than *F. sambucinum* lesions (Tables 2-5 and 2-6; Figure 2-10). Most *F. oxysporum* isolates produced dry rot in most tubers with 1.8 to 2.0 lesions per tuber with lesion size ranging from 4.3 to 6.9 cm^2 except for one isolate, I008 at 2.0 cm^2 (Tables 2-5 and 2-6).

Dry rot incidence was also high for *F. avenaceum* and *F. cerealis* but less than *F. sambucinum* and *F. oxysporum*. Both species caused lesions in 83 to 100% of inoculated tubers with 1.3 to 1.9 lesions per tuber (Table 2-5). Although *F. avenaceum* was less frequently observed, *F. avenaceum* lesions were more severe than with a lesion size of 5.3 to 8.5 cm^2 compared to 4.3 to 6.9 cm^2 (Table 2-5, Figure 2-10). With lesion size of 2.2 to 2.7 cm^2, severity of *F. cerealis* lesions was less than *F. sambucinum*, *F. oxysporum*, and *F. avenaceum* although incidence was similar (Table 2-6, Figure 2-10).

For tubers inoculated with *F. graminearum* and *F. culmorum*, dry rot incidence was very high in PTB, but severity was intermediate with lesion size of 4.2 cm^2 for *F. graminearum* and 3.0 to 4.2 cm^2 for *F. culmorum* (Table 2-6; Figures 2-9 and 2-10). Incidence and severity were much lower in PTA for *F. culmorum*. Overall, *F. redolens* caused greater incidence of dry rot in some cases, 80 to 100% in PTB and 9 to 58% in PTA, but severity was lower in some cases, 0.2 to 1.6 cm^2 in PTA and 2.6 to 5.3 cm^2 in PTB with one isolate having lesion size of 13.1 cm^2 (Tables 2-5 and 2-6; Figures 2-9 and 2-10).

Dry rot incidence and severity was variable for five isolates of *F. flocciferum* with two isolates (C056 and F069) producing zero lesions (Tables 2-5 and 2-6). Three isolates (C007, C056, C053) were tested in both PTA and PTB with similar results between the two tests, indicating low dry rot incidence and severity (Tables 2-5 and 2-6). Two *F. flocciferum* isolates (C058, F068) were only tested in PTB and exhibited much greater mean incidence and severity, 60 to 100% incidence and lesion sizes from 2.2 to 10.1 cm^2, than the rest of the tested *F. flocciferum* isolates, 0 to 20% incidence and 0 to 0.3 cm^2 lesion size (Tables 2-5 and 2-6). For analysis of dry rot incidence and severity by species, both incidence and severity are greater for *F. flocciferum* in PTB due to the greater aggressiveness of isolates C058 and F068, which were not included in PTA (Figures 2-9 and 2-10).

Three isolates of *F. venenatum* were tested for pathogenicity on two varieties in PTB with 60 to 90% of tubers developing lesions with 1.1 to 1.6 lesions per tuber and mean lesion size of 1.4 to 3.0 cm^2 (Table 2-6). Incidence and severity of *F. venenatum* infection was similar for the two varieties, Dark Red Norland and Russet Burbank (Table 2-6). Dry rot incidence and severity was intermediate for *F. sporotrichioides* infection in both pathogenicity tests with 30 to 80% incidence, 0.4 to 1.5 lesions per tuber and 0.8 to 2.8 cm^2 lesion size (Tables 2-5 and 2-6). Incidence and severity of *F. venenatum* and *F. sporotrichioides* were similar and at intermediate levels relative to the rest of the tested *Fusarium* species for PTB (Figures 2-9 and 2-10).

Overall, dry rot incidence and severity was lowest among tested *Fusarium* species for *F. acuminatum*, *F. equiseti*, *F. solani* and *F. stercicola* (Figures 2-9 and 2-10). One isolate of *F. acuminatum* was non-pathogenic while the other isolate (B050) caused lesions on three tubers in PTB out of a total of 22 tubers inoculated in both PTA and PTB (Tables 2-5 and 2-6). The *F. acuminatum* lesions ranged in size from 1.2 to 2.4 cm^2. *Fusarium equiseti* produced lesions on one Russet Burbank tuber in PTA (1.3 cm^2 lesion area) and one Dark Red Norland tuber in PTB (2.0 cm^2 lesion area) (Tables 2-5 and 2-6). *Fusarium stercicola* caused dry rot in one Russet Burbank tuber in PTB (lesion area 1.42 cm^2). Lesions were recorded for all three tested isolates of *F. solani*, but only on one tuber per treatment with lesion sizes ranging from 1.6 to 2.2 cm^2. Although incidence was low for these species, individual lesion size in infected tubers was comparable to other tested *Fusarium* species that can be considered more aggressive overall (Tables 2-5 and 2-6).

Isolates of non-*Fusarium* genera that were tested (*Clonostachys*, *Trichocladium*/*Chaetomium*/*Humicola*, *Plectosphaerella*, and *Galactomyces*/*Geotrichum*) were determined to be non-pathogenic to potato tuber. No lesions were recorded for the isolates

representing *Clonostachys* and *Trichocladium*/*Chaetomium*/*Humicola* genera (Table 2-5). Only one lesion in one tuber was recorded for one isolate of *Plectosphaerella* (I025 – lesion area 2.13 cm^2) while the other three isolates produced no lesions, and only one lesion in one tuber was recorded for one isolate of *Galactomyces*/*Geotrichum* (H028 – lesion area 2.27 cm^2) while the other two isolates produced no lesions (Table 2-5). These lesions were uncharacteristic for *Plectosphaerella* and *Galactomyces*/*Geotrichum* based on their isolated occurrence and relatively large size as compared to the rest of PTA data. Further, *Fusarium* species were re-isolated from these lesions, therefore, the lesions were concluded to be the result of inadvertent contamination of the wounding tool or tuber surface during inoculation.

Evidence for variation in aggressiveness toward potato tuber within a pathogenic species was observed in statistical differences in dry rot incidence and severity between isolates of the same species: *F. oxysporum*, *F. avenaceum* and *F. cerealis* in PTA, *F. flocciferum* in PTB and *F. redolens* in both PTA and PTB (Tables 2-5 and 2-6). For example, out of six *F. oxysporum* isolates, isolate I008 caused significantly less dry rot incidence and severity than all other isolates, and isolate I005 produced less severe lesions than four of the isolates despite having the same incidence (Table 2-5).

Differences in dry rot incidence and severity between Russet Burbank and the variety an isolate originally came from were observed for three isolates representing species *F. flocciferum*, *F. redolens* and *F. sambucinum* (Table 2-7). Dry rot incidence was higher in Dark Red Norland than Russet Burbank after inoculation with *F. flocciferum* isolate F068 ($p = 0.01$), but severity was similar (Table 2-7). Inoculation with *F. redolens* isolate I030 resulted in significantly higher dry rot incidence ($p = 0.004$) and much higher severity ($p < 0.0001$) in Dark Red Norland than Russet Burbank (Table 2-7). Incidence of dry rot was similar between two varieties inoculated with *F. sambucinum* isolate E089, but, based on significantly greater lesion severity, Russet Burbank was much more susceptible than the proprietary variety from which E089 was originally isolated ($p < 0.0001$).

Notably, four species tested in both PTA and PTB had greater mean incidence (percent of inoculated tubers with lesions) and severity (mean lesion size) in PTB: *F. culmorum* (PTA – 22%, 0.48 cm^2; PTB – 100%, 3.47 cm^2); *F. flocciferum* (PTA – 4%, 07 cm^2; PTB – 33%, 2.68 cm^2); *F. redolens* (PTA – 33%, 0.80 cm^2; PTB – 90%, 4.27 cm^2); *F. sporotrichioides* (PTA – 33%, 0.83 cm^2; PTB – 80%, 2.83 cm^2) (Tables 2-5 and 2-6; Figures 2-9 and 2-10). In addition, the pathogenic *F. acuminatum* isolate B050 only caused lesions in PTB, the only *F. stercicola* lesion was observed in PTB and two *F. solani* isolates only produced lesions in PTB (Table 2-6).

These differences in incidence and severity from PTA to PTB for these isolates is attributed to maintenance of high humidity in PTB. In the first experimental run of PTA, all six replications had less than 90% RH for the first eight to eleven days of incubation, and only four replications were maintained at 90 to 100% RH for only one to three days. In the second experimental run of PTA, two replications had only 40 to 50% RH for the entire incubation period, and only two replications maintained 90 to 100% RH for two to three days. For PTB, all replications in both experimental runs were successfully maintained at 90 to 100% RH for most of the incubation period.

Table 2-5. Dry rot incidence and severity for Pathogenicity Test A (PTA), where potato variety Russet Burbank was challenged with selected *Fusarium* species associated with dry rot symptoms in Pacific Northwest potato tubers.

***Fusarium* species or fungal genus name(s)**	**Isolate name**	**Number of tubers with a lesion**	**Mean lesion number per tuber (incidence) [a b]**		**Mean lesion size (cm^2) (severity) [a b]**	
Clonostachys	C066	0	0	h	0	k
Clonostachys	D079	0	0	h	0	k
Clonostachys	F081	0	0	h	0	k
Clonostachys	I039	0	0	h	0	k
F. acuminatum	B050	0	0	h	0	k
F. acuminatum	E099	0	0	h	0	k
F. avenaceum	A020	10	1.3	c	6.0	de
F. avenaceum	H038	12	1.8	a	8.5	c
F. avenaceum	C013	10	1.4	bc	5.3	ef
F. cerealis	F044	10	1.3	c	2.2	gh
F. cerealis	E011	11	1.8	a	2.7	g
F. cerealis	F007	12	1.9	a	2.6	g
F. culmorum	C050	3	0.3	fgh	0.4	jk
F. culmorum	I023	3	0.4	fg	0.5	ijk
F. culmorum	B010	2	0.3	fgh	0.5	ijk
F. equiseti	C012	1	0.1	gh	0.1	jk
F. flocciferum	C007	2	0.3	fgh	0.3	jk
F. flocciferum	C053	0	0	h	0	k
F. flocciferum	C056	0	0	h	0	k
F. flocciferum	F069	0	0	h	0	k
F. oxysporum	B083	12	2.0	a	6.9	d
F. oxysporum	I053	12	1.9	a	6.8	de
F. oxysporum	I005	12	1.8	ab	4.3	f
F. oxysporum	I021	11	1.8	ab	5.8	def
F. oxysporum	I008	8	1.1	cd	2.0	ghi
F. oxysporum	I009	12	1.9	a	5.9	de
F. redolens	I019	1	0.2	gh	0.2	jk
F. redolens	I030	3	0.3	fgh	0.4	jk
F. redolens	I007	7	0.9	de	1.6	ghij
F. redolens	I028	5	0.6	ef	1.0	hijk
F. sambucinum	FID 71-6	12	2.0	a	20.7	ab
F. sambucinum	E089	12	2.0	a	19.5	b
F. sambucinum	I010	12	2.0	a	21.3	a
F. solani	H025	0	0	h	0	k
F. solani	H045	0	0	h	0	k
F. solani	F085	1	0.1	gh	0.1	jk
F. sporotrichioides	C059	4	0.4	fg	0.8	hijk
F. stercicola	E087	0	0	h	0	k
F. toxicum	B088	0	0	h	0	k
Galactomyces or *Geotrichum*	E059	0	0	h	0	k
Galactomyces or *Geotrichum*	H028	1	0.2	gh	0.2	jk
Galactomyces or *Geotrichum*	D086	0	0	h	0	k
Plectosphaerella	H083	0	0	h	0	k
Plectosphaerella	G070	0	0	h	0	k
Plectosphaerella	I025	1	0.2	gh	0.2	jk
Trichocladium/*Chaetomium*/*Humicola*	E066	0	0	h	0	k
Trichocladium/*Chaetomium*/*Humicola*	H027	0	0	h	0	k
Mock-inoculated	Mock	0	0	h	0	k

[a] Values followed by the same letter within columns do not differ based on pairwise comparisons (alpha = 0.05).

[b] The tests were conducted twice. The data shown are for both tests combined.

Table 2-6. Dry rot incidence and severity for Pathogenicity Test B (PTB), where potato variety Russet Burbank and the variety from which the isolate originated were challenged with selected *Fusarium* species associated with dry rot symptoms in Pacific Northwest potato tubers.

***Fusarium* species**	**Potato variety**	**Isolate name**	**Number of tubers with lesion**	**Mean lesion number per tuber (incidence) [a b]**		**Mean lesion size (cm^2) (severity) [a b]**	
acuminatum	Russet Burbank	B050	3	0.4	g	0.6	ij
acuminatum	Proprietary	E099	0	0	g	0	j
acuminatum	Russet Burbank	E099	0	0	g	0	j
culmorum	Russet Burbank	C050	10	1.9	ab	4.2	ef
culmorum	Russet Burbank	I023	10	1.8	abc	3.0	fgh
culmorum	Russet Burbank	B010	10	1.7	abcd	3.2	efgh
equiseti	Dark Red Norland	C012	1	0.2	g	0.2	j
equiseti	Russet Burbank	C012	0	0	g	0	j
flocciferum	Pacific Russet	C007	0	0	g	0	j
flocciferum	Russet Burbank	C007	1	0.2	g	0.2	j
flocciferum	Russet Burbank	C053	1	0.1	g	0.1	j
flocciferum	All Blue	C056	0	0	g	0	j
flocciferum	Russet Burbank	C056	0	0	g	0	j
flocciferum	Russet Burbank	C058	10	2.0	a	10.1	d
flocciferum	Dark Red Norland	F068	9	1.8	abc	2.2	fghij
flocciferum	Russet Burbank	F068	6	1.2	ef	2.9	fgh
graminearum	Challenger	G098	10	2.0	a	5.3	e
graminearum	Russet Burbank	G098	10	2.0	a	4.2	ef
redolens	Russet Burbank	I019	9	1.7	abcd	5.3	e
redolens	Dark Red Norland	I030	10	2.0	a	13.1	c
redolens	Russet Burbank	I030	8	1.3	def	2.6	fghi
redolens	Russet Burbank	I007	9	1.5	bcdef	4.0	efg
redolens	Russet Burbank	I028	10	1.8	abc	5.3	e
sambucinum	Russet Burbank	FID 71-6	10	2.0	a	26.7	a
sambucinum	Proprietary	E089	10	2.0	a	21.6	b
sambucinum	Russet Burbank	E089	10	2.0	a	27.7	a
solani	Russet Burbank	H025	1	0.2	g	0.2	j
solani	Challenger	H045	1	0.2	g	0.2	j
solani	Russet Burbank	H045	1	0.2	g	0.2	j
sporotrichioides	Russet Burbank	C059	8	1.5	bcdef	2.8	fgh
stercicola	Proprietary	E087	0	0	g	0	j
stercicola	Russet Burbank	E087	1	0.2	g	0.1	j
toxicum	Russet Burbank	B088	0	0	g	0	j
venenatum	Dark Red Norland	F009	6	1.1	f	1.2	hij
venenatum	Russet Burbank	F009	6	1.2	ef	1.4	hij
venenatum	Dark Red Norland	F022	7	1.4	cdef	1.9	ghij
venenatum	Russet Burbank	F022	7	1.4	cdef	3.0	fgh
venenatum	Dark Red Norland	G002	9	1.6	abcde	2.5	fghi
venenatum	Russet Burbank	G002	8	1.6	abcde	2.0	ghij
Mock-inoculated	Russet Burbank	Mock	0	0	g	0	j

[a] Values followed by the same letter within columns do not differ based on pairwise comparisons (alpha = 0.05).

[b] The tests were conducted twice. The data shown are for both tests combined.

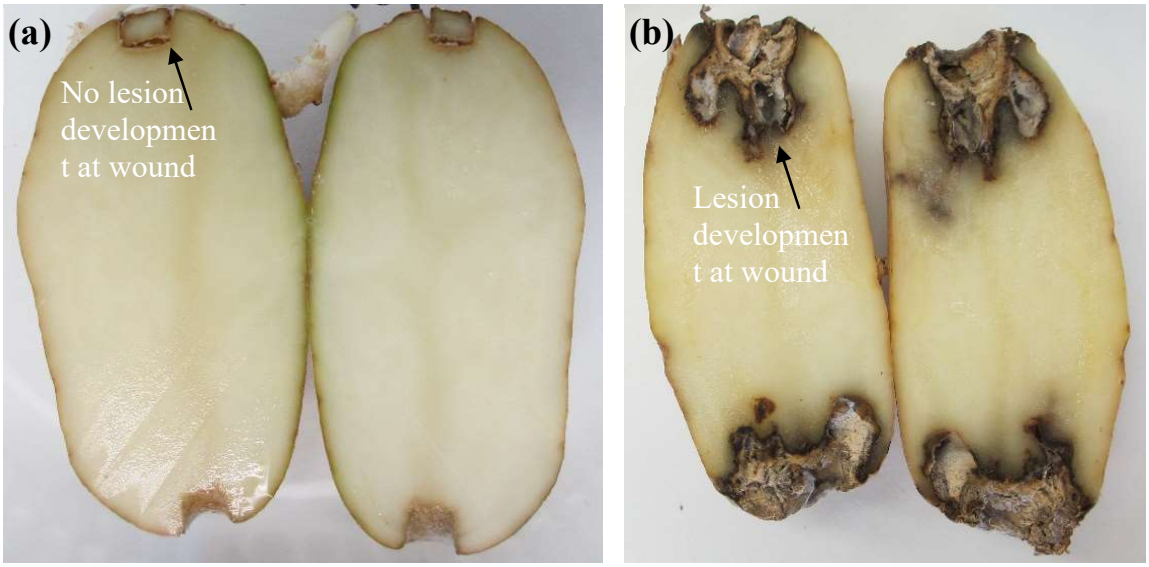

Figure 2-7. Examples of a mock-inoculated, negative control tuber (a) and an inoculated, positive control tuber (b) inoculated with *Fusarium sambucinum,* a known dry rot pathogen, for pathogenicity testing.

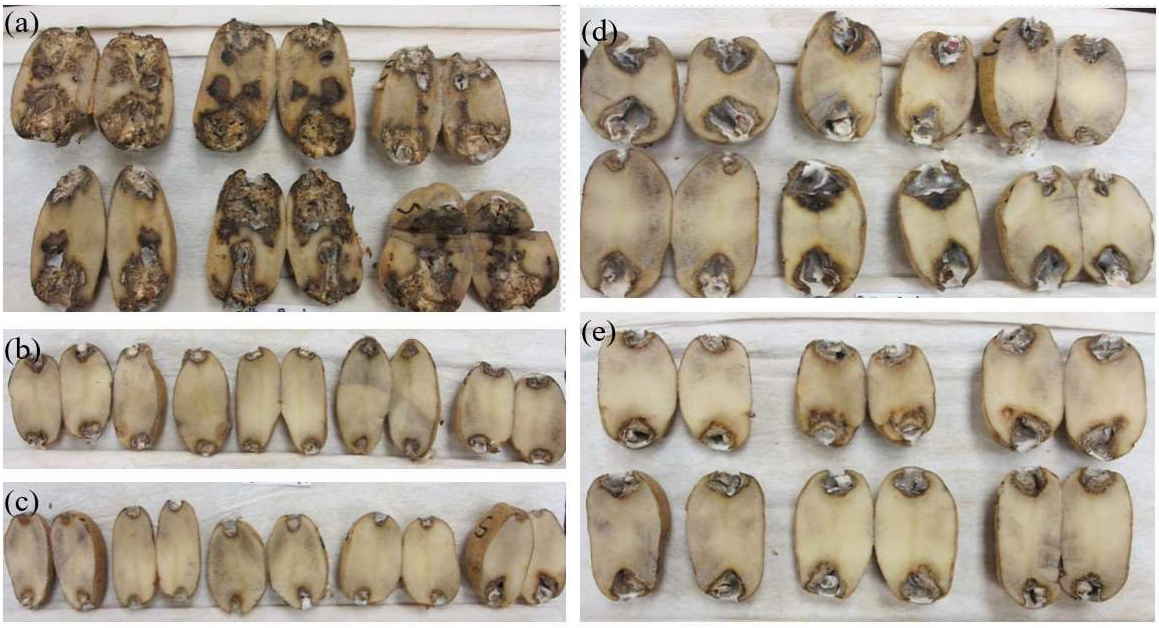

Figure 2-8. Dry rot lesions in Russet Burbank potato tubers that developed after inoculation with *Fusarium* dry rot pathogens obtained from Pacific Northwest tubers. (a) *Fusarium sambucinum*; (b) *Fusarium cerealis*; (c) *Fusarium redolens*; (d) *Fusarium avenaceum*; (e) *Fusarium oxysporum*

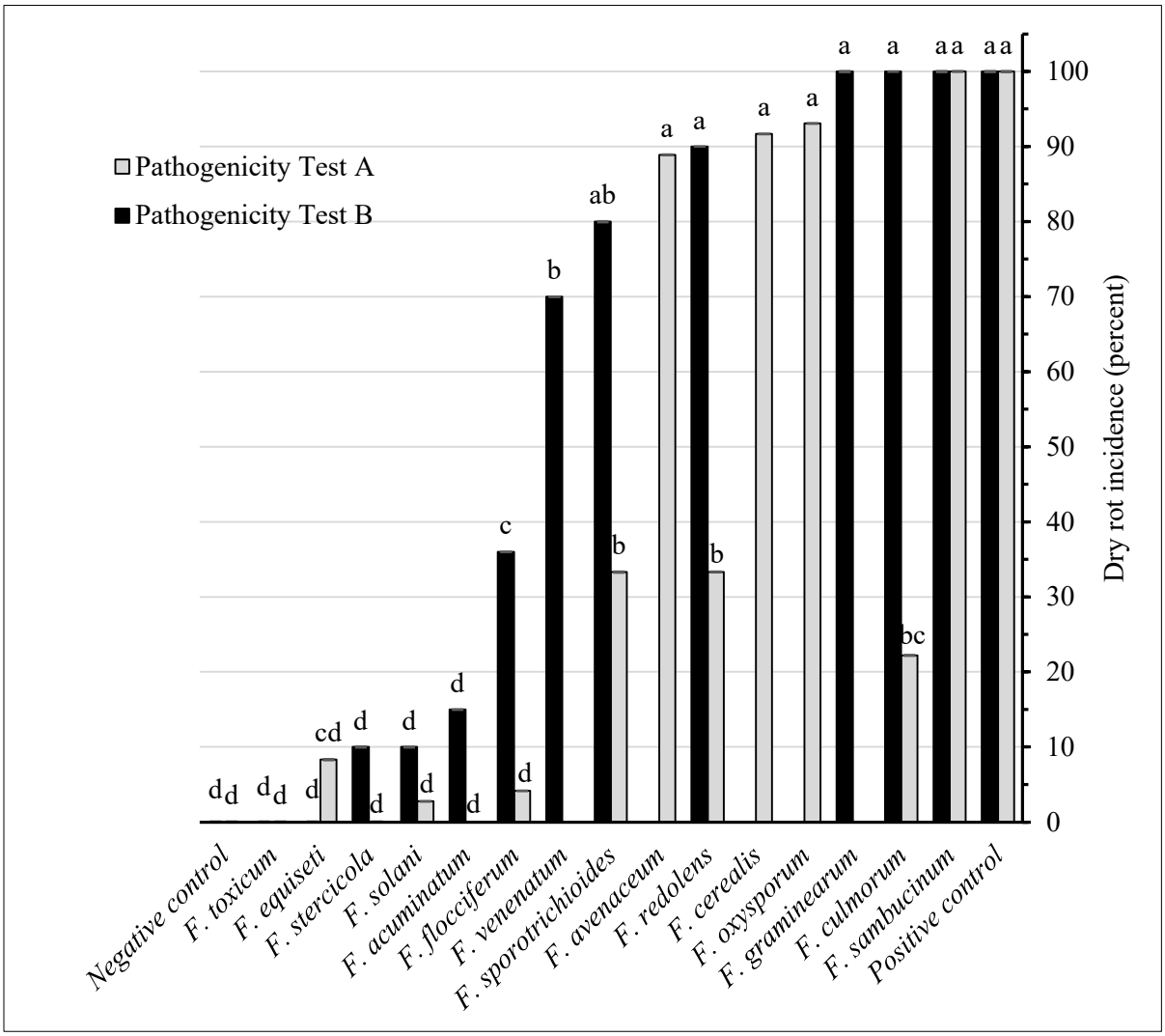

Figure 2-9. Dry rot incidence as determined by the presence of at least one dry rot lesion on either the stem or bud end of inoculated tubers in one or two pathogenicity tests (A and/or B) challenging potato tubers with selected isolates of *Fusarium* species obtained from Pacific Northwest tubers. Only Russet Burbank was challenged in Pathogenicity Test A while both Russet Burbank and the potato variety from which an isolate was obtained were challenged in Pathogenicity Test B. The positive control was inoculated with a known pathogenic isolate of *Fusarium sambucinum* (FID 71-6), and the negative control was mock-inoculated with a sterile agar plug. Bars followed by the same letter do not differ based on pairwise comparisons (alpha = 0.05). Standard error values were 0.1 or less.

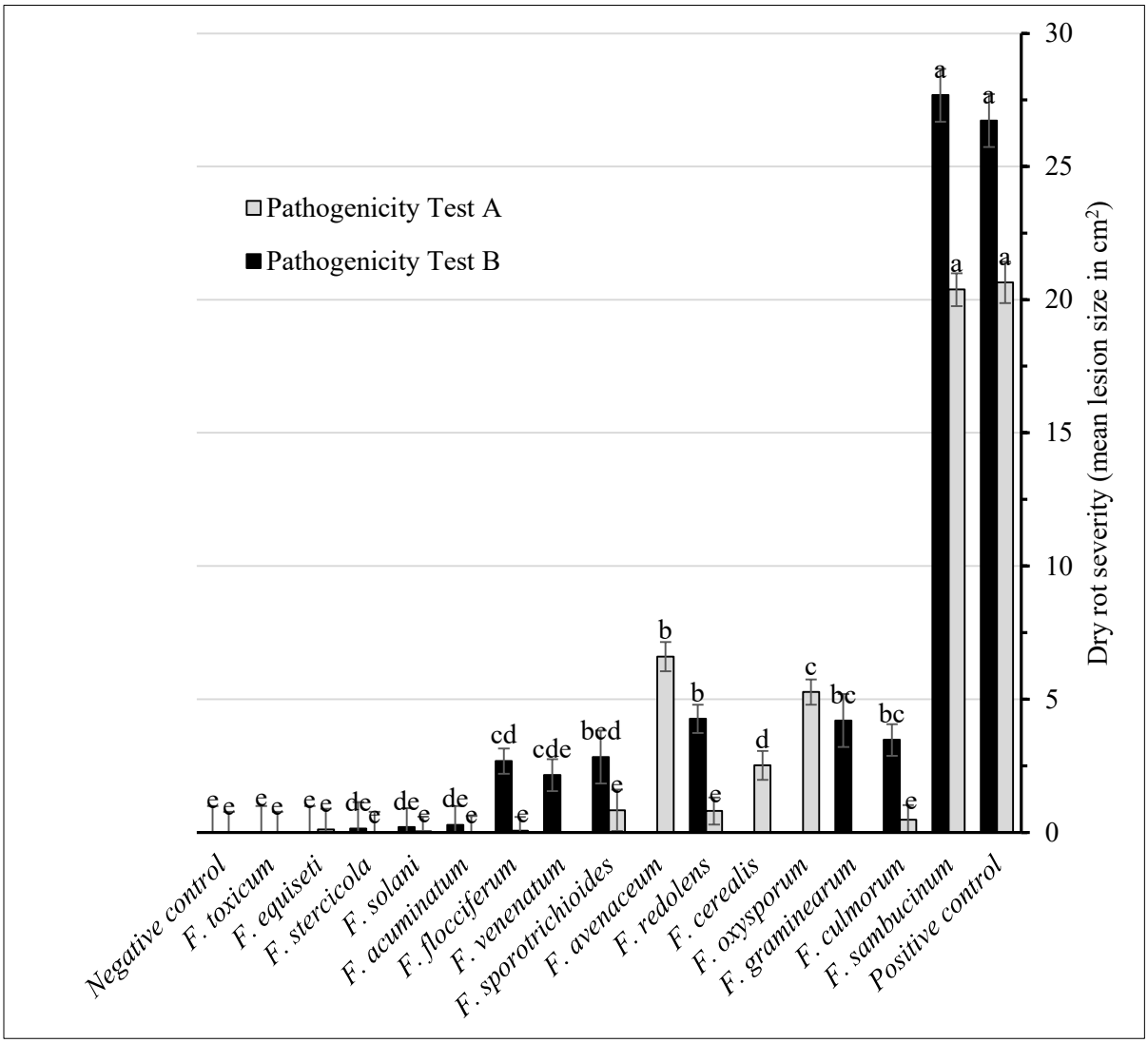

Figure 2-10. Dry rot severity as determined by mean lesion size (cm^2) in one or two pathogenicity tests (A and/or B) challenging potato tubers with selected isolates of *Fusarium* species obtained from Pacific Northwest tubers. Only Russet Burbank was challenged in Pathogenicity Test A while both Russet Burbank and the potato variety from which an isolate was obtained were challenged in Pathogenicity Test B. Lesion size is represented by the sum of the lesion areas that developed at the bud and stem end inoculation sites. The positive control was inoculated with a known pathogenic isolate of *Fusarium sambucinum* (FID 71-6), and the negative control was mock-inoculated with a sterile agar plug. Bars followed by the same letter do not differ based on pairwise comparisons (alpha = 0.05). Error bars indicate standard error.

Table 2-7. Difference in dry rot incidence and severity between the potato variety of original isolation and the Russet Burbank variety, as measured in Pathogenicity Test B.

Isolate code/ ***Fusarium*** species	**Dry rot incidence (mean number of lesions per tuber)**				**Dry rot severity [a] (mean lesion size in cm^2)**			
	Original variety	Russet Burbank	Difference in means	p-value [b]	Original variety	Russet Burbank	Difference in means	p-value [b]
E089 / *sambucinum*	Proprietary[c] 2.0	2	0	1	Proprietary 21.6	27.7	6.1	**<0.0001**
F009 / *venenatum*	DRN 1.1	1.2	0.1	0.68	DRN 1.2	1.4	0.2	0.84
F022 / *venenatum*	DRN 1.4	1.4	0	1	DRN 1.9	3.0	1.1	0.29
F068 / *flocciferum*	DRN 1.8	1.2	0.6	**0.01**	DRN 2.2	2.9	0.7	0.50
G002 / *venenatum*	DRN 1.6	1.6	0	1	DRN 2.5	2.0	0.5	0.62
E099 / *acuminatum*	Proprietary 0	0	0	1	Proprietary 0	0	0	1
C012 / *equiseti*	DRN 0.2	0	0.2	0.41	DRN 0.2	0	0.2	0.86
C007 / *flocciferum*	Pacific R. 0	0.2	0.2	0.41	Pacific R. 0	0.2	0.2	0.83
C056 / *flocciferum*	All Blue 0	0	0	1	All Blue 0	0	0	1
G098 / *graminearum*	Challenger 2.0	2	0	1	Challenger 5.3	4.2	1.1	0.31
I030 / *redolens*	DRN 2.0	1.3	0.7	**0.004**	DRN 13.1	2.6	10.6	**<0.0001**
E087 / *stercicola*	Proprietary 0	0.2	0.2	0.41	Proprietary 0	0.1	0.1	0.90
H045 / *solani*	Challenger 0.2	0.2	0	1	Challenger 0.20	0.2	0.02	0.99

Abbreviations: DRN and Pacific R. refer to potato varieties Dark Red Norland and Pacific Russet, respectively.

[a] Dry rot severity was represented by mean lesion size, which was the sum of the lesion areas on both the stem and bud end inoculated sites.

[b] alpha = 0.05

[c] Original variety listed as Proprietary refers to a single, unnamed variety.

Discussion

A greater understanding of the current *Fusarium* species composition associated with Fusarium dry rot in PNW potatoes is expected to aid in refinement of current management strategies for the grower. In this study, the diversity of *Fusarium* species associated with PNW potato tubers is greater than previously reported with recovery of 20 species in this survey. Pathogenicity to potato tuber was confirmed for 14 PNW *Fusarium* species, and eight of these species have not previously been reported as Fusarium dry rot pathogens in the PNW. The prevalence and aggressiveness levels of *F. sambucinum* and *F. oxysporum* confirm that they continue to impart the greatest dry rot risk overall in the PNW.

Of the 20 species recovered in this survey, 14 have been previously reported as causal organisms of Fusarium dry rot of potato in locations worldwide while six species (*F. clavum*, *F. gamsii*, *F. iranicum*, *F. mori*, *F. stercicola*, *F. toxicum*) have not been documented as dry rot pathogens (Azil et al. 2021; Ali et al. 2005; Aprasad et al. 1997; Baturo-Ciesniewska et al. 2015; Bojanowski et al. 2013; Choiseul et al. 2007; Cullen et al. 2005; Du et al. 2012; Esfahani 2005; Estrada Jr et al. 2010; Gachango et al. 2011, 2012; Garcia Bayona et al. 2011; Hanson et al. 1996; Heltoft et al. 2016; Hide et al. 1992; Kawchuk et al. 1994, 2002; Merlington 2014; Nolte 1994; Ocamb et al. 2007; Peters et al. 2008a, 2008b; Sagar et al. 2011; Schroers et al. 2016; Secor and Salas 2001; Stefańczyk et al. 2016; Theron 1999; Tiwari et al. 2022). Only eight of the 20 recovered species have been reported previously as Fusarium dry rot pathogens in the PNW (*F. avenaceum*, *F. coeruleum*, *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. sambucinum*, *F. solani*, and *F. sporotrichioides* (Desjardins 1995; Nolte 1994; Ocamb et al. 2007). Regarding *Fusarium* diversity, the prevalent species observed in this survey agreed in part with previous work by Ocamb et al. (2007), with *F. sambucinum* being the most prevalent species associated with dry rot tubers, followed by *F. oxysporum*. However, the Ocamb et al. (2007) study was limited to the Columbia Basin of Washington and Oregon and recorded only eight *Fusarium* species while the current survey recorded 20 different *Fusarium* species from potato tubers over a wider geographic area, including Idaho, the top potato producing state in the United States. The proportion of *F. sambucinum* (45.4% of 325 isolates) recovered in the current survey was similar to Ocamb et al. (2007) (35 to 57%) while *F. oxysporum* appeared less prevalent now (14.1%) than in the Ocamb study (18 to 36%). Both surveys recovered *F. avenaceum, F. equiseti, F. oxysporum, F. sambucinum, F. solani* and *F. sporotrichioides*. *Fusarium proliferatum* was found in the 1999-2001 survey but only in a single storage facility in a single year, and this species was not recovered in the current survey. *Fusarium solani* accounted for a much smaller percentage of the total number of isolates recovered in the current survey (1%) as compared to the Ocamb et al. (2007)

survey (14.8 to 16.5%), indicating that *F. solani* is now less prevalent in PNW tubers storages. Although *F. sporotrichioides* was recovered in both the Ocamb et al. and the current surveys, it accounted for a very small portion of all isolates recovered.

Fusarium sambucinum and *F. coeruleum* have both been previously reported in Idaho as dry rot pathogens (Miller et al. 2019; Nolte 1994). Interestingly, these results also indicated *F. coeruleum* is not an important contributor to storage dry rot problems. However, the remarkably low incidence of *F. coeruleum* recovered in our survey may be a consequence of the predominant type of sample obtained in the survey. The samples were primarily from commercial and seed storages, rather than from in-field plants showing symptoms of Fusarium seed piece decay, where *F. coeruleum* may play a more important role as previously described by Miller et al. (2019). The classification of the *Fusarium* species referred to as *F. coeruleum* is currently unsettled and additional study of pathogenicity, morphology and phylogeny of this species is recommended by some *Fusarium* researchers (Sandoval-Denis et al. 2019). The information obtained about *F. coeruleum* from this study is scant due to the paucity of isolates recovered from PNW tubers with dry rot from commercial and seed storages and should be viewed cautiously. Since all species complexes currently defined in the *Fusarium* genus were included in the genus phylogeny in this study, then *F. coeruleum* appeared to be most closely related to the *F. torreyae* species complex. However, since the *F. torreyae* species complex was not placed as expected basal to FFSC, then the apparent close relationship between *F. coeruleum* and *F. torreyae* may be falsely represented. From this phylogenetic analysis, it can be cautiously concluded that *F. coeruleum* likely belongs somewhere between FIESC and FIESC and may be most closely related to *F. torreyae* species complex. Additionally, from the *TEF* phylogeny, it is clear that the *F. coeruleum* isolates represented are from a younger lineage than FSSC, which was the species complex in which *F. coeruleum* was previously classified.

Most recently, O'Donnell et al. (2022) stated that the most informative loci for identifying unknown *Fusarium* isolates to species or species complex are translation elongation factor 1-alpha (*TEF*) and two subunits of a DNA-directed RNA polymerase (*RPB1* and *RPB2*). In contrast, two frequently used loci in fungal identification are now known to be too conserved to be useful in resolving *Fusarium* to the species level, ITS rDNA, or the nuclear ribosomal internal transcribed spacer region, and 28S rDNA, the 5' end of the nuclear ribosomal large subunit (O'Donnell et al. 2022; Leslie and Summerell 2006). In this study, the phylogenetic analyses of PNW *Fusarium* isolates confirmed species identity of PNW *Fusarium* isolates as initially determined by analyses of ITS, *TEF* and *PHO* sequences. Concerning the full Fusarium genus phylogeny (Figure 2-5), the evolutionary placement of most species complexes and species within Fusarium aligned well with the most recently published

phylogeny by Geiser et al. (2021). For the purposes of this study, the goal of the phylogenetic analyses was accomplished in that species identity was better defined and/or confirmed by the addition of phylogenetic information to BLAST analyses of DNA sequences.

However, the misplacement of the *F. dimerum* and *F. buharicum* species complexes was notable. According to O'Donnell et al. (2022), *TEF* amplicons align less accurately among more distantly related species complexes within *Fusarium* because of greater instance of insertions, deletions and substitutions within the introns. This is the most likely reason for the misplacement of the *F. dimerum* and *F. buharicum* species complexes in the full *Fusarium* genus phylogeny generated in this study (Figure 2-5). In comparison, the single locus *TEF* tree by Geiser et al. (2021) shows bootstrap values of 58 and 61 for the nodes leading to *F. dimerum* species complex placement while the single locus trees for both *RPB1* (86 to 100) and *RPB2* (100) have much higher bootstrap support for placement of *F. dimerum* species complex, which was accurately placed at the basal position of the *Fusarium* genus (Geiser et al. (2021); see Supplementary Figure 2). This limitation of the *TEF* locus also likely contributed to the lack of high bootstrap support shown in a few nodes in the genus phylogeny for this study. For example, a lower bootstrap value of 36.7 was obtained for the node separating the Brachygibbosum and Longipes Clades from the rest of the FSAMSC Clades.

The phylogenies also revealed some unanticipated species among the PNW isolates, i.e., *F. iranicum*, *F. gamsii*, *F. toxicum*, *F. clavum*, *F. mori* and *F. stercicola.* Most of these species have only been formally described and named in the past four years in the midst of a substantial amount of work on *Fusarium* taxonomy since the 2012 implementation of "One Fungus, One Name" and with vast improvement of molecular technologies enabling separation and classification of morphologically cryptic species and the establishment and expansion of DNA sequence databases, some of which are focused on *Fusarium*. It is currently unknown if *F. iranicum* and *F. gamsii* are pathogenic to potato and can cause Fusarium dry rot. *Fusarium gamsii* has been previously recovered from potato tuber from Idaho and soil in Washington and North Dakota (all potato-producing states) but was reported as *F. reticulatum* and the Idaho sample did not indicate if dry rot symptoms were present (Laraba et al. 2022). *Fusarium toxicum* has only recently been described formally as a species by Xia et al (2019). *Fusarium equiseti* and *F. toxicum* are within the same haplotype (*F. equiseti* = 14-a; *F. toxicum* = 14-b), i.e., closely related sister species (O'Donnell et al. 2009). Isolates of both *F. equiseti* (C012) and *F. toxicum* (B088) were tested for pathogenicity to potato in this study, but *F. equiseti* was determined to be a weak pathogen while *F. toxicum* was non-pathogenic. Two Idaho isolates were resolved as *F. clavum* (FIESC haplotype 5 described by O'Donnell et al. (2009)) using *TEF* sequences, however, it is currently unknown if PNW isolates of *F. clavum* are pathogenic to potato

and can cause Fusarium dry rot. However, *F. clavum* has been recently reported in Italy to cause leaf spot and fruit rot of tomato, a solanaceous relative of potato (Gilardi et al. 2021). Additionally, a Mexican isolate of *F. clavum* was reported to cause Fusarium head blight in wheat (Leyva-Mir et al. 2022), and an isolate of *F. clavum* has also been recovered from potato leaf in Russia (Xia et al. 2019). PNW isolates of *F. stercicola* was shown to be weak pathogens, but the pathogenicity of PNW *F. mori* to potato tuber is currently unknown. *F. mori* (Iran) has been previously isolated from potato tubers in Iran, but those from Iran were determined to be non-pathogenic (Chehri et al. 2014).

Utilization of the *PHO* gene for species resolution within FTSC was successful. Both the misplacement of FTSC 18 in the tree structure and the lower bootstrap value (42.7) for the node separating the youngest lineages were attributed to the parameters of the dataset used to construct the FTSC phylogeny. Based on the guidance in Laraba et al. (2022), the most phylogenetically informative 5′ portion of the *PHO* gene was sequenced for this study, using only two primers to gain sufficient information for confirmation of species identity of PNW isolates instead of sequencing the entire *PHO* gene. The tree constructed in this study used a dataset with a shorter alignment (1154 bp versus 2112 bp in Laraba et al.) and fewer phylogenetically informative sites (258 versus 569 in Laraba et al.) as compared to the dataset used by Laraba et al. (2022) to construct the single-locus *PHO* tree for their study (see Laraba et al. 2022, Supplementary Fig. S1). The bootstrap value for the node separating the youngest lineages in the Laraba et al. (2022) FTSC *PHO* tree was 99 in comparison to the 42.7 value obtained in this study. Overall, the use of the 5′ portion of the *PHO* gene as described was satisfactory for species resolution of PNW isolates in FTSC.

Overall, bootstrap values would very likely be improved by using a multigene dataset, especially for the full Fusarium genus phylogeny, as O'Donnell et al. (2022) states that *TEF* is not the most accurate locus for analysis across distantly related species complexes. However, for the purposes of confirming species identity for this study, *TEF* was satisfactory in all cases except FTSC, for which *PHO* gave satisfactory results.

Pathogenicity testing of *F. clavum*, *F. gamsii*, *F. iranicum*, and *F. mori* on potato is warranted as these are *Fusarium* species newly reported in the PNW that were not tested for pathogenicity in the current study. The identification of these isolates was recently made based on newly published phylogenetic information that was not available at the time these pathogenicity tests were conducted (2020 to early 2021) and newly acquired *PHO* sequences for FTSC species *F. gamsii* and *F. iranicum* in 2022 (Geiser et al. 2021; Laraba et al. 2022; Xia et al. 2019). The pathogenicity of all four of these species to potato tuber is unknown based on the literature review conducted for this study, and their

ability to cause Fusarium dry rot needs to be ascertained since these species are being recovered PNW potato tubers. In particular, *F. gamsii* and *F. iranicum* were only observed in samples from Idaho growing regions, with *F. gamsii* accounting for 7.5% of isolates in eastern Idaho and 5.9 isolates in Magic Valley. *Fusarium gamsii* has been recovered from head-blighted wheat and barley as well as root-rotted canola in Canada, and *F. iranicum* has also recently been associated with blighted wheat and barley (Laraba et al. 2022). The presence of these species in potato also raises the question of whether these *Fusarium* species may also be acting as cereal pathogens in Idaho and, if so, whether potato-cereal crop rotation schedules may be increasing disease risk to both crop types.

Determination of pathogenicity was based on the presence of dry rot lesions and re-isolation of the same *Fusarium* species initially used for inoculation. Some *Fusarium* species that caused dry rot lesions were statistically similar to the negative mock-inoculated control, but if a species was able to cause a lesion of any size, it was considered to be pathogenic. As such, tested PNW isolates of four species, *F. acuminatum*, *F. equiseti*, *F. solani*, and *F. stercicola*, were determined to be weak pathogens based on infrequent lesion occurrence, small lesion size and statistical similarity to the negative control. It is possible that for species exhibiting only one lesion out of all inoculated tubers, contamination may have played a role in these occurrences. However, given that many *Fusarium* species are known dry rot pathogens, it is reasonable to conclude that these lesions were due to a weak pathogen encountering ideal environmental conditions for successful disease initiation in the tuber. Du et al. (2012) also found that tested isolates of *F. acuminatum* and *F. equiseti* were least aggressive based on lesion size measured in pathogenicity tests that included more aggressive species like *F. sambucinum* and *F. avenaceum*. Not all species and not all isolates within a species which were associated with tubers collected in this dry rot survey of PNW were found to be pathogenic. These non-pathogenic isolates may have been secondary colonizers of the tuber wounds rather than pathogenic primary pathogens or may have been saprophytes feeding on necrotic tissue resulting from the original pathogenic infection.

F. sambucinum was determined to be the most aggressive of all tested species based on high disease incidence and severity. Although similarly high incidence to *F. sambucinum* was observed for some species (*F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. oxysporum*, *F. redolens*, *F. sporotrichioides*), lesion severity from *F. sambucinum* infection was much higher (19.5 to 27.7 cm^2) than those other species (2.2 to 8.5 cm^2). *Fusarium sambucinum* has also been reported as the most aggressive species by other researchers conducting pathogenicity and aggressiveness tests (Azil et al. 2021; Du et al. 2012; Esfahani 2005; Gachango et al. 2012; Stefańczyk et al. 2016).

Among other species with high incidence (*F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. graminearum, F. oxysporum*, *F. redolens*, *F. sporotrichioides*), *F. avenaceum* and *F. oxysporum* were more aggressive followed by *F. redolens* and *F. culmorum*, based on dry rot severity. *Fusarium culmorum*, *F. redolens* and *F. sporotrichioides* were observed to have intermediate aggressiveness to potato overall, especially based on PTB data. The difference in relative humidity levels between the two pathogenicity tests, i.e., PTA and PTB, appeared to affect both incidence and severity of dry rot lesions caused by *F. culmorum*, *F. redolens* and *F. sporotrichioides*, with greater dry rot observed in PTB for all three species. Also accounting for dry rot differences observed for *F. flocciferum*, *F. acuminatum*, *F. stercicola* and *F. solani* between the PTA and PTB datasets and for greater dry rot severity for *F. sambucinum* in PTB, it can be concluded that higher humidity levels in PTB increased the pathogenic ability of some *Fusarium* species, resulting in greater dry rot incidence and severity.

F. sambucinum and *F. oxysporum* were the most prevalent species recovered from symptomatic dry rot tubers in the PNW with *F. sambucinum* being highly aggressive to potato and *F. oxysporum* demonstrating intermediate aggressiveness. Both *F. sambucinum* and *F. oxysporum* pose a threat to PNW potato production based on their prevalence in the PNW and ability to produce high incidence and intermediate to high severity of dry rot infections. However, not all tested species pose such a high risk to potato. Although *F. avenaceum* produced larger lesions than all species except *F. sambucinum* (5.3 to 8.5 cm^2) and has been cited by many as a prevalent dry rot pathogen in Europe, Great Britain and Canada (Choiseul et al. 2007; Du et al. 2012; Heltoft et al. 2016; Peters et al. 2008b; Stefańczyk et al. 2016), there were relatively few *F. avenaceum* isolates recovered in the PNW dry rot tuber samples (six isolates accounting for 2% of all isolates). Therefore, *F. avenaceum* is not likely to cause a substantial or widespread problem in PNW potato production. However, Aprasad et al. (1997) observed that *F. avenaceum* isolates from various sources (lupin, barley, wheat, field bean, potato, pine, oilseed rape), were aggressive on potato tuber but pathogenicity of the isolates was not related to their original host, indicating that dry rot risk can be higher if *F. avenaceum* inoculum is present from any source. Leslie and Summerell (2006) note that climate and host are informative in determining the *Fusarium* species acting as a plant pathogen, meaning that the same disease can occur on the same host plant and be caused by a different *Fusarium* species if the climate differs. Since *F. avenaceum* is often reported as a prevalent dry rot pathogen in climates that can be much cooler and wetter during the growing season than the PNW potato growing regions, then it may be that climatic conditions play a role in *F. avenaceum* being a minor dry rot pathogen in the PNW, despite its demonstration as a very aggressive dry rot pathogen.

The same conclusion can be made for *F. cerealis* and *F. sporotrichioides* posing a low risk to PNW potato production. *Fusarium cerealis* readily causes dry rot lesions based on high incidence data, but with relatively smaller lesion sizes (less than 3.0 cm^2) and relatively few isolates recovered (nine isolates accounting for 3% of all isolates), and only a single isolate of *F. sporotrichioides* was recovered in the PNW dry rot survey.

A single isolate of *F. graminearum* was recovered in the PNW dry rot survey, and it had very high incidence on both varieties it was tested on and similar intermediate lesion severity to *F. culmorum*, *F. redolens* and others (4.2 to 5.3 cm^2). *Fusarium graminearum* has been cited as the causal organism of a dry rot epidemic in North Dakota storages in 2003-2004 (Estrada Jr et al. 2010), and this pathogenicity test showed that *F. graminearum* from PNW can act as a dry rot pathogen. However, given the lack of prevalence of *F. graminearum* in PNW samples of dry rot tubers, *F. graminearum* appears to pose low risk to PNW potato production at this time.

In these pathogenicity tests, *F. flocciferum* isolates demonstrated a wide range of aggressiveness toward potato, with two isolates causing zero lesions, two isolates displaying weak pathogenicity and low aggressiveness, and two isolates causing high incidence of dry rot with intermediate to high aggressiveness. *Fusarium flocciferum* has been previously implicated as a Fusarium dry rot pathogen of potato, but none of the available reports are very recent. Theron (1999) cited four reports dating from 1971 to 1981. More recently, Summerell et al. (2010) noted that *F. flocciferum* was isolated from potato in Victoria, Australia in which there were “symptoms of a black rot”, but the report did not specify from which part of the potato plant the isolation was made. *F. flocciferum* (a species within the FTSC) may be an emerging Fusarium dry rot pathogen of potato that has not been yet widely documented and/or may be an emerging potato pathogen specifically in the PNW. Species in the FTSC have previously been considered to be weak pathogens and secondary invaders, but a shift to a greater pathogenic role is being documented due to the emergence of FTSC species members as more prevalent pathogens of small-grain cereals, pulses, soybean and canola in North America, Brazil, Europe and China (Laraba et al. 2022). This may be the case also for *F. flocciferum* as a potato dry rot pathogen in the PNW based on the data presented here. It is also possible that *F. flocciferum* has been a potato dry rot pathogen for many decades, but isolates were either mis-identified or not identified to species. For example, Laraba et al. (2022) included five *F. flocciferum* isolates in their recent study, three of which were previously identified as other *Fusarium* species (*F. reticulatum*, *F. lateritium*, and *F. acuminatum*). Use of the *PHO* gene using the primers developed by Laraba et al. (2022) may help correctly identify past and future isolates of *F. flocciferum* more readily, perhaps

eventually providing a more accurate picture of the prevalence of *F. flocciferum* as a Fusarium dry rot pathogen of potato in the PNW.

In PTB, a few differences in dry rot incidence and severity were observed between Russet Burbank and the variety from which an isolate came. These results highlight how susceptibility to the same *Fusarium* species can differ between potato varieties. For example, *F. sambucinum* isolate E089 had 100% incidence on both the original proprietary variety and Russet Burbank, but severity was significantly greater on Russet Burbank, indicating higher susceptibility of Russet Burbank to this isolate of *F. sambucinum*. *Fusarium flocciferum* isolate F068 had greater incidence on the original variety Dark Red Norland, but lesion severity was similar to Russet Burbank. Despite similar severity, presence of dry rot in a storage situation may incur more yield loss based on this data if the variety was Dark Red Norland rather than Russet Burbank as incidence may be higher. For the *F. redolens* isolate I030, the susceptibility of Dark Red Norland was clearly much greater than Russet Burbank for both incidence and severity, demonstrating the relatively high susceptibility of the Dark Red Norland variety to this particular dry rot pathogen.

In conclusion, the diversity of *Fusarium* species pathogenic to potato in the PNW is greater than previously reported. The prevalence and aggressiveness levels of *F. sambucinum* and *F. oxysporum* confirm that they continue to impart the greatest dry rot risk overall in the PNW. Given the number of different species of Fusarium dry pathogens, their varying prevalence in the PNW and their varying aggressiveness to potato, knowledge which species is acting as the pathogen in a given situation may help guide adjustments to current management strategies to reduce dry rot.

Literature Cited

Ali, S., Rivera, V. V., and Secor, G. A. 2005. First report of *Fusarium graminearum* causing dry rot of potato in North Dakota. Plant Dis. 89:105–105.

Aprasad, K. S., Bateman, G. L., and Read, P. J. 1997. Variation in pathogenicity on potato tubers and sensitivity to thiabendazole of the dry rot fungus *Fusarium avenaceum*. Potato Res. 40:357–365.

Azil, N., Stefańczyk, E., Sobkowiak, S., Chihat, S., Boureghda, H., and Śliwka, J. 2021. Identification and pathogenicity of *Fusarium* spp. associated with tuber dry rot and wilt of potato in Algeria. Eur. J. Plant Pathol. 159:495–509.

Baturo-Ciesniewska, A., Lenc, L., Grabowski, A., and Lukanowski, A. 2015. Characteristics of Polish isolates of *Fusarium sambucinum*: molecular identification, pathogenicity, diversity and reaction to control agents. Am. J. Potato Res. 92:49–61.

Bojanowski, A., Avis, T. J., Pelletier, S., and Tweddell, R. J. 2013. Management of potato dry rot. Postharvest Biol. Technol. 84:99–109.

Burkhart, C. R., Christ, B. J., and Haynes, K. G. 2007. Non-additive genetic variance governs resistance to Fusarium dry rot in a diploid hybrid potato population. Am. J. Potato Res. 84:199.

Chehri, K., Ghasempour, H. R., and Karimi, N. 2014. Molecular phylogenetic and pathogenetic characterization of *Fusarium solani* species complex (FSSC), the cause of dry rot on potato in Iran. Microb. Pathog. 67–68:14–19.

Choiseul, J., Allen, L., and Carnegie, S. F. 2007. Fungi causing dry tuber rots of seed potatoes in storage in Scotland. Potato Res. 49:241–253.

Corsini, D., and Pavek, J. J. 1986. Fusarium dry-rot resistant potato germplasm. Am. Potato J. 63:629–638.

Crous, P. W., Lombard, L., Sandoval-Denis, M., Seifert, K. A., Schroers, H.-J., et al. 2021. *Fusarium*: More than a node or a foot-shaped basal cell. Stud. Mycol. 98:100116.

Cullen, D. W., Toth, I. K., Pitkin, Y., Boonham, N., Walsh, K., et al. 2005. Use of quantitative molecular diagnostic assays to investigate Fusarium dry rot in potato stocks and soil. Phytopathology 95:1462–1471.

Desjardins, A. E. 1995. Population structure of *Gibberella pulicaris* (anamorph *Fusarium sambucinum*) from potato tuber dry rot in North America and Europe. Am. Potato J. 72:145–156.

Du, M., Ren, X., Sun, Q., Wang, Y., and Zhang, R. 2012. Characterization of *Fusarium* spp. causing potato dry rot in China and susceptibility evaluation of Chinese potato germplasm to the pathogen. Potato Res. 55:175–184.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.

Esfahani, M. N. 2005. Susceptibility assessment of potato cultivars to Fusarium dry rot species. Potato Res. 48:215–226.

Estrada Jr, R., Gudmestad, N. C., Rivera, V. V., and Secor, G. A. 2010. *Fusarium graminearum* as a dry rot pathogen of potato in the USA: prevalence, comparison of host isolate aggressiveness and factors affecting aetiology. Plant Pathol. 59:1114–1120.

Gachango, E., Hanson, L. E., Rojas, A., Hao, J. J., and Kirk, W. W. 2012. *Fusarium* spp. causing dry rot of seed potato tubers in Michigan and their sensitivity to fungicides. Plant Dis. 96:1767–1774.

Gachango, E., Kirk, W., Hanson, L., Rojas, A., Tumbalam, P., and Shetty, K. 2011. First report of in vitro fludioxonil-resistant isolates of *Fusarium* spp. causing potato dry rot in Michigan. Plant Dis. 95:228–228.

Garcia Bayona, L., Grajales, A., Cardenas, M. E., Sierra, R., Cepero de Garcia, M. C., et al. 2011. First report of *Fusarium oxysporum* causing potato dry rot in *Solanum tuberosum* in Colombia. New Dis. Rep. 24:14–14.

Geiser, D. M., Al-Hatmi, A. M. S., Aoki, T., Arie, T., Balmas, V., et al. 2021. Phylogenomic analysis of a 55.1-kb 19-gene dataset resolves a monophyletic *Fusarium* that includes the *Fusarium solani* species complex. Phytopathology 111:1064–1079.

Geiser, D. M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., et al. 2004. FUSARIUM-ID v.1.0: A DNA sequence database for identifying *Fusarium*. Eur. J. Plant Pathol. 110:473–479.

Gilardi, G., Matic, S., Guarnaccia, V., Garibaldi, A., and Gullino, M. L. 2021. First report of *Fusarium clavum* causing leaf spot and fruit rot on tomato in Italy. Plant Dis. 105:2250.

Hanson, L., Schwager, S., and Loria, R. 1996. Sensitivity to thiabendazole in *Fusarium* species associated with dry rot of potato. Phytopathology 86:378–384.

Heltoft, P., Brurberg, M. B., Skogen, M., Le, V. H., Razzaghian, J., and Hermansen, A. 2016. *Fusarium* spp. causing dry rot on potatoes in Norway and development of a real-time PCR method for detection of *Fusarium coeruleum*. Potato Res. 59:67–80.

Hide, G. A., Read, P. J., and Hall, S. M. 1992. Resistance to thiabendazole in *Fusarium* species isolated from potato tubers affected by dry rot. Plant Pathol. 41:745–748.

Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., and Vinh, L. S. 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol. Biol. Evol. 35:518–522.

Idaho Potato Commission. Date unknown. Major Idaho potato growing areas by county. Infographic. https://idahopotato.com/uploads/media/17-1004GrowingAreaMap-8.5x11.pdf

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., and Jermiin, L. S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods. 14:587–589.

Kawchuk, L. M., Holley, J. D., Lynch, D. R., and Clear, R. M. 1994. Resistance to thiabendazole and thiophanate-methyl in Canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani*. Am. Potato J. 71:185–192.

Kawchuk, L. M., Hutchison, L. J., Verhaeghe, C. A., Lynch, D. R., Bains, P. S., and Holley, J. D. 2002. Isolation of the β-tubulin gene and characterization of thiabendazole resistance in *Gibberella pulicaris*. Can. J. Plant Pathol. 24:233–238.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35:1547–1549.

Laraba, I., Busman, M., Geiser, D. M., and O'Donnell, K. 2022. Phylogenetic diversity and mycotoxin potential of emergent phytopathogens within the *Fusarium tricinctum* species complex. Phytopathology 112:1284–1298.

Laraba, I., McCormick, S. P., Vaughan, M. M., Geiser, D. M., and O'Donnell, K. 2021. Phylogenetic diversity, trichothecene potential, and pathogenicity within *Fusarium sambucinum* species complex. PLOS ONE 16:e0245037.

Leslie, J. F., and Summerell, B. A. 2006. The *Fusarium* Laboratory Manual. Ames, IA: Blackwell Publishing.

Letunic, I., and Bork, P. 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 49:W293–W296.

Leyva-Mir, S. G., García-León, E., Camacho-Tapia, M., Villasenor-Mir, H.-E., Leyva-Madrigal, K. Y., et al. 2022. Occurrence of the *Fusarium incarnatum-equiseti* species complex causing Fusarium head blight of wheat in Mexico. Plant Dis. Available at: http://apsjournals.apsnet.org/doi/10.1094/PDIS-11-21-2467-PDN [Accessed August 7, 2022].

Merlington, A. A. 2014. Management options for control of Fusarium dry rot (*Fusarium* spp.) and potato common scab (*Streptomyces* spp.) of potato (*Solanum tuberosum* L.) in Michigan. M.S. thesis. Michigan State University.

Miller, J., Miller, T., Duellman, K., and Olsen, N. 2019. Managing Fusarium dry rot. Available at: https://millerresearch.com/wp-content/uploads/2019/04/Managing_Fusarium_Dry_Rot.pdf [Accessed May 5, 2022].

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., and Minh, B. Q. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32:268–274.

Nolte, P. 1994. Managing benzimidazole resistance in the potato dry rot fungus. EXT 769. University of Idaho Extension. 8 pages.

Ocamb, C. M., Hamm, P. B., and Johnson, D. A. 2007. Benzimidazole resistance of *Fusarium* species recovered from potatoes with dry rot from storages located in the Columbia Basin of Oregon and Washington. Am. J. Potato Res. 84:169–177.

O'Donnell, K., Rooney, A. P., Proctor, R. H., Brown, D. W., McCormick, S. P., et al. 2013. Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. Fungal Genet. Biol. 52:20–31.

O'Donnell, K., Sutton, D. A., Fothergill, A., McCarthy, D., Rinaldi, M. G., et al. 2008. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex. J. Clin. Microbiol. 46:2477–2490.

O'Donnell, K., Sutton, D. A., Rinaldi, M. G., Gueidan, C., Crous, P. W., and Geiser, D. M. 2009. Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-F. equiseti* and *F. chlamydosporum* species complexes within the United States. J. Clin. Microbiol. 47:3851–3861.

O'Donnell, K., Whitaker, B. K., Laraba, I., Proctor, R. H., Brown, D. W., et al. 2022. DNA sequence-based identification of *Fusarium*: A work in progress. Plant Dis. 106:1597–1609.

Peters, J. C., Lees, A. K., Cullen, D. W., Sullivan, L., Stroud, G. P., and Cunnington, A. C. 2008a. Characterization of *Fusarium* spp. responsible for causing dry rot of potato in Great Britain. Plant Pathol. 57:262–271.

Peters, R. D., MacLeod, C., Seifert, K. A., Martin, R. A., Hale, L. R., et al. 2008b. Pathogenicity to potato tubers of *Fusarium* spp. isolated from potato, cereal and forage crops. Am. J. Potato Res. 85:367–374.

Sagar, V., Sharma, S., Jeevalatha, A., Chakrabarti, S., and Singh, B. 2011. First report of *Fusarium sambucinum* causing dry rot of potato in India. New Dis. Rep. 24:5–5.

Sandoval-Denis, M., Lombard, L., and Crous, P. W. 2019. Back to the roots: A reappraisal of *Neocosmospora*. Persoonia - Mol. Phylogeny Evol. Fungi. 43:90–185.

Schroers, H.-J., Samuels, G. J., Zhang, N., Short, D. P. G., Juba, J., and Geiser, D. M. 2016. Epitypification of *Fusisporium* (*Fusarium*) *solani* and its assignment to a common phylogenetic species in the *Fusarium solani* species complex. Mycologia 108:806–819.

Secor, G. A., and Salas, B. 2001. Fusarium dry rot and Fusarium wilt. In Compendium of Potato Diseases, St. Paul: The American Phytopathological Society, p. 23–25.

Spence, A., Haj-Ahmad, Y., and Kim, W.-S. 2016. Application Note 88, Plant/Fungi Sample Preparation: Optimizing bead homogenization of plant tissues for DNA and RNA isolation. Norgen Biotek Corp. https://norgenbiotek.com/sites/default/files/resources/App%20Note%2088%20-%20Bead%20Beating%20Optimization.pdf.

Stefańczyk, E., Sobkowiak, S., Brylińska, M., and Śliwka, J. 2016. Diversity of *Fusarium* spp. associated with dry rot of potato tubers in Poland. Eur. J. Plant Pathol. 145:871–884.

Summerell, B. A., Leslie, J. F., Liew, E. C. Y., Laurence, M. H., Bullock, S., et al. 2011. *Fusarium* species associated with plants in Australia. Fungal Divers. 46:1–27.

Theron, D. J. 1999. Fusarium dry rot of potatoes: etiology, epidemiology, toxicity and control.

Tiwari, R. K., Bashyal, B. M., Shanmugam, V., Lal, M. K., Kumar, R., Sharma, S., et al. 2022. First report of dry rot of potato caused by *Fusarium proliferatum* in India. J. Plant Dis. Prot. 129:173–179.

Torres-Cruz, T. J., Whitaker, B. K., Proctor, R. H., Broders, K., Laraba, I., et al. 2022. FUSARIUM-ID v.3.0: An updated, downloadable resource for *Fusarium* species identification. Plant Dis. 106:1610–1616.

Trifinopoulos, J., Nguyen, L.-T., von Haeseler, A., and Minh, B. Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44:W232–W235.

Wharton, P., Hammerschmidt, R., and Kirk, W. 2007. Fusarium dry rot. E-2992. Michigan State University Extension Bulletin. 6 pages.

White, T. J., Bruns, S. L., and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A guide to methods and applications, New York: Academic Press, Inc.

Xia, J. W., Sandoval-Denis, M., Crous, P. W., Zhang, X. G., and Lombard, L. 2019. Numbers to names - restyling the *Fusarium incarnatum-equiseti* species complex. Persoonia - Mol. Phylogeny Evol. Fungi. 43:186–221.

Chapter 3: Relative Aggressiveness of Pacific Northwest Fusarium Dry Rot Pathogens on Seven Potato Varieties

Abstract

Fusarium dry rot is a worldwide fungal disease of potato causing seed decay at planting and postharvest tuber decay in storage. This study investigated the relative aggressiveness of *Fusarium* species as dry rot pathogens on potato varieties important to the Pacific Northwest region of the United States. Using Pacific Northwest *Fusarium* isolates and potato varieties grown in the region, the objective was to challenge seven varieties with four pathogenic *Fusarium* species to determine aggressiveness to potato as measured by lesion size, or disease severity. *Fusarium sambucinum* and *F. oxysporum* were the most aggressive species with mean lesion sizes ranging from 7.2 to 12.8 cm^2 and 5.2 to 13.8 cm^2, respectively. *Fusarium culmorum* was the least aggressive of the four species with the smallest lesion sizes ranging from 1.8 to 4.0 cm^2. The importance of considering the combination of *Fusarium* species and potato variety in management decisions is highlighted by these data as the interaction between *Fusarium* species and potato variety was found to be significant. More severe Fusarium dry rot was observed with certain combinations of *Fusarium* species and potato variety, e.g., Umatilla Russet infected with *F. sambucinum* and Dark Red Norland infected with *F. oxysporum*. Knowledge of *Fusarium* species identity in specific situations may assist in dry rot management. Variety selection may play a role in alleviation of dry rot issues if a different variety can be utilized by a grower where a specific *Fusarium* species is causing disease.

Introduction

Fusarium dry rot is a worldwide fungal disease of potato causing seed decay at planting and postharvest tuber decay in storage, known as Fusarium seed decay and Fusarium dry rot, respectively. This disease is caused by several species in the genus *Fusarium*. Lesions appear as sunken, wrinkled, darkened areas on the periderm while internally there is dry, crumbly decayed tissue that is tan to brown to black in color (Gachango et al. 2012; Heltoft et al. 2016; Nolte 1994; Peters et al. 2008a; Secor and Salas 2001; Wharton et al. 2007). Yield losses due to Fusarium dry rot make it one of the most important postharvest potato diseases worldwide. Average decreased yield has been cited as 6 to 25% with some situations resulting in up to 60% storage loss (Baturo-Ciesniewska et al. 2015; Corsini and Pavek 1986; Du et al. 2012; Gachango et al. 2012; Heltoft et al. 2016; Kawchuk et al. 1994, 2002; Peters et al. 2008a; Secor and Salas 2001; Stefańczyk et al. 2016). Most seed lots and commercial crops have some dry rot infection. This can exclude seed lots from sale, may affect crop emergence and health and may affect the amount of time potatoes can be stored without sustaining

financial loss (Peters et al. 2008a). Potato growers around the world strive to manage Fusarium dry rot through cultural practices such as wound management and crop rotation, application of chemical fungicides and use of less susceptible potato varieties (Bojanowski et al. 2013).

The Pacific Northwest (PNW) region of the United States is a major potato production region of the world, and the PNW states of Idaho, Oregon and Washington collectively produce around 11.3 million metric tons of potatoes annually. Potato growers in the PNW have recently observed an increase in Fusarium dry rot. One possible contributing factor to the reported disease increase is increased production of varieties that are more susceptible to Fusarium dry rot. Variability in aggressiveness among *Fusarium* species has been observed as measured by lesion size in potato tubers after inoculation in laboratory tests. *Fusarium sambucinum* has been shown to be more aggressive *in vitro* on potato tubers as compared to *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. oxysporum*, *F. cerealis*, *F. solani* and others (Esfahani 2005; Gachango et al. 2012; Stefańczyk et al. 2016). As an example, among isolates of *F. sambucinum*, *F. solani* and *F. oxysporum* from Iran causing potato dry rot, *F. sambucinum* was found to be most aggressive while *F. oxysporum* was least aggressive among these three species (Esfahani 2005). In addition, potato varieties are known to vary in their susceptibility to Fusarium dry rot pathogens (Azil et al. 2021; Aprasad et al. 1997; Choiseul et al. 2007; Du et al. 2012; Esfahani 2005; Merlington 2014; Peters et al. 2008a, 2008b; Stefańczyk et al. 2016). As an example, Esfahani (2005) found variable susceptibility to three *Fusarium* species among 43 tested cultivars, with certain cultivars being more susceptible to one *Fusarium* species or another, while one variety, Saturna, was most resistant to all three species among all cultivars.

This study investigated the relative aggressiveness of *Fusarium* species as dry rot pathogens on potato varieties grown in the PNW region. The objective was to challenge seven potato varieties with four pathogenic *Fusarium* species to determine aggressiveness as measured by lesion size, or disease severity. The seven varieties were Russet Burbank, Clearwater Russet, Umatilla Russet, Dark Red Norland, Ciklamen, Atlantic and Snowden and represented three potato types, i.e., russet, red, and chipping. These seven varieties accounted for approximately 23 to 53% of seed and/or commercial acreage for 2018-2019 in the PNW states of Idaho, Oregon and Washington (Idaho Crop Improvement Association 2019a, 2019b; Oregon Potato Commission and Oregon State University Extension Service 2019; Washington Seed Potato Commission and Washington Certified Seed Potatoes 2019). Based on species prevalence data from a recent survey of dry rot in the Pacific Northwest and subsequent results from pathogenicity assays based on lesion presence or absence, four *Fusarium* species were tested: *F. sambucinum*, *F. oxysporum*, *F. redolens* and *F. culmorum*.

Materials and Methods

A total of 120 treatments was tested using a randomized complete block design with four replications and one tuber per replication, and the experiment was conducted twice. Treatments were a combination of *Fusarium* isolate and potato variety. A mock-inoculated control treatment was included in which a Russet Burbank tuber was inoculated with a sterile potato dextrose agar plug instead of a *Fusarium* isolate. *Fusarium* isolates used for the experiment are listed in Table 3-1. Cultures of each test isolate were prepared by aseptically placing a piece of previously air-dried and frozen mycelium in the center of a potato dextrose agar plate mycelium side down and then incubating for 7 to 14 days at room temperature under a 12-hour light/dark cycle.

Visually healthy whole tubers without dry rot symptoms weighing 113 to 227 grams were rinsed with tap water to remove soil, surface sanitized by immersion in freshly mixed 0.5% sodium hypochlorite solution for 10 minutes and air dried on sterile absorbent cloth towels in a laminar flow hood. After drying, tubers were inoculated by wounding the stem end with a flame-sterilized cork borer to remove a plug of tuber tissue five millimeters in diameter and five millimeters long, placing a four millimeter diameter agar plug of a *Fusarium* culture into the wound and replacing the tuber plug. Each tuber was individually placed inside a small paper bag with the top loosely folded over. For each replication, the 120 bags representing all treatments were randomly placed inside a large plastic bin for incubation for approximately four weeks at 19˚C and 90 to 100% relative humidity.

After incubation, tubers were cut in half lengthwise through the wound, placed onto a one millimeter thick glass plate cut side down and digitally imaged alongside a ruler using a flatbed scanner. Lesion area was measured using Adobe Photoshop software (version 22.4.2 or later). The Measurement Scale tool was calibrated with the ruler in the scanned image, and lesion area in square centimeters was measured using the Polygonal Lasso Tool. Lesion area was analyzed using the generalized linear mixed model (GLIMMIX) procedure in SAS® version 9.4 software (Cary, North Carolina) with *Fusarium* species and potato variety as the main factors. Data are presented as the estimated least square mean of lesion area, and differences were identified using pairwise comparisons (alpha = 0.05).

Results

Both main effects, *Fusarium* species and variety, and their interaction were significant (Table 3-2). Relative aggressiveness of the four *Fusarium* species was variable as determined in two separate statistical analyses, one analysis within *Fusarium* species (Table 3-3) and one analysis within potato variety (Table 3-4). Generally, *F. sambucinum* and *F. oxysporum* were most aggressive (Tables 3-3

and 3-4). *F. sambucinum* was particularly aggressive on Umatilla Russet (Tables 3-3 and 3-4; Figure 3-1). Differences in susceptibility to *F. sambucinum* were observed for all three potato types. The three russet varieties were differentially susceptible with Umatilla being most and Clearwater Russet being least susceptible (Tables 3-3 and 3-4; Figure 3-1). Dark Red Norland was more susceptible than Ciklamen, and Atlantic was more susceptible than Snowden (Tables 3-3 and 3-4; Figure 3-1). *F. oxysporum* was particularly aggressive on Dark Red Norland while the other red variety, Ciklamen, was much less susceptible (Tables 3-3 and 3-4; Figure 3-2). *Fusarium oxysporum* was more aggressive on Russet Burbank than the other two russets, Clearwater Russet and Umatilla Russet (Tables 3-3 and 3-4; Figure 3-2). The two chipping varieties were similarly susceptible to *F. oxysporum* (Tables 3-3 and 3-4; Figure 3-2). Generally, *F. redolens* and *F. culmorum* were less aggressive on the seven tested varieties than *F. sambucinum* and *F. oxysporum* (Tables 3-3 and 3-4). *Fusarium culmorum* was the least aggressive of the four species with the smallest lesion sizes and was similarly aggressive on all seven varieties (Tables 3-3 and 3-4; Figure 3-3). *Fusarium redolens* was most aggressive on Dark Red Norland and less aggressive on the other six varieties (Tables 3-3 and 3-4; Figure 3-4). Variation in mean lesion size among isolates of the same *Fusarium* species was observed, with the largest range for *F. sambucinum* (Table 3-5).

Table 3-1. *Fusarium* species and isolates used for inoculation of seven potato varieties to measure relative aggressiveness of Fusarium dry rot pathogens recovered from the Pacific Northwest states of Idaho, Oregon and Washington.

***Fusarium* species** [a]	**Isolate code**	**State of origin**
F. culmorum	C050	Idaho
F. culmorum	I023	Oregon
F. culmorum	B010	Washington
F. oxysporum	B083	Idaho
F. oxysporum	I053	Idaho
F. oxysporum	I005	Oregon
F. oxysporum	I021	Oregon
F. oxysporum	I008	Washington
F. oxysporum	I009	Washington
F. redolens	I030	Idaho
F. redolens	I007	Oregon
F. sambucinum	C030	Idaho
F. sambucinum	D083	Idaho
F. sambucinum	F060	Idaho
F. sambucinum	FID 71-6	Idaho
F. sambucinum	E089	Washington
F. sambucinum	E100	Washington

[a] For statistical analysis, data for all isolates of the same species were combined. Therefore, all analyses were by species, not by isolate.

Table 3-2. Analysis of variance for the main effects of *Fusarium* species and potato variety and the resulting interaction on mean lesion size resulting from inoculation of seven potato varieties with four *Fusarium* species to measure relative aggressiveness of potato dry rot pathogens recovered from the Pacific Northwest states of Idaho, Oregon and Washington.

Source	**df**	**F**	**P > F**
Species	3	9.51	0.0272
Variety	6	8.25	0.0107
Species x Variety	18	7.66	<0.0001

Table 3-3. Mean dry rot lesion area (square centimeters) compared within *Fusarium* species for the interaction of *Fusarium* species and potato variety in an experiment to measure relative aggressiveness of different *Fusarium* species on potato tubers inoculated with *Fusarium* dry rot pathogens recovered from the Pacific Northwest states of Idaho, Oregon and Washington.

Potato variety	***F. culmorum*** [a]	***F. oxysporum*** [a]	***F. redolens*** [a]	***F. sambucinum*** [a]
Russet Burbank	2.1 [b] a	8.6 [b] b	4.5 [b] ab	9.6 [b] b
Clearwater Russet	2.8 a	5.8 c	2.9 b	7.4 cd
Umatilla Russet	1.8 a	5.4 c	3.3 b	12.8 a
Dark Red Norland	2.5 a	13.8 a	7.3 a	9.2 bc
Ciklamen	2.0 a	5.2 c	2.4 b	7.2 d
Atlantic	4.0 a	8.0 b	4.1 b	9.7 b
Snowden	2.1 a	8.2 b	3.9 b	7.7 cd

[a] Multiple isolates of each species were included in the experiment. For statistical analysis, data for all isolates of the same species were combined. Therefore, all analyses were by species, not by isolate.
[b] Means within columns followed by the same letter are not significantly different based on pair-wise comparisons (alpha = 0.05.)

Table 3-4. Mean dry rot lesion area (square centimeters) compared within potato variety for the interaction of *Fusarium* species and potato variety in an experiment to measure relative aggressiveness of different *Fusarium* species on potato tubers inoculated with *Fusarium* dry rot pathogens recovered from the Pacific Northwest states of Idaho, Oregon and Washington.

***Fusarium* species**	**Russet Burbank**	**Clearwater Russet**	**Umatilla Russet**	**Dark Red Norland**	**Ciklamen**	**Atlantic**	**Snowden**
F. culmorum [a]	2.1 [b] b	2.8 [b] b	1.8 [b] c	2.5 [b] c	2.0 [b] b	4.0 [b] b	2.1 [b] b
F. oxysporum [a]	8.6 a	5.8 ab	5.4 b	13.8 a	5.2 ab	8.0 a	8.2 a
F. redolens [a]	4.5 b	2.9 b	3.3 bc	7.3 b	2.4 b	4.1 b	3.9 b
F. sambucinum [a]	9.6 a	7.4 a	12.8 a	9.2 b	7.2 a	9.7 a	7.7 a

[a] Multiple isolates of each species were included in the experiment. For statistical analysis, data for all isolates of the same species were combined. Therefore, all analyses were by species, not by isolate.
[b] Means within columns followed by the same letter are not significantly different based on pair-wise comparisons (alpha = 0.05.)

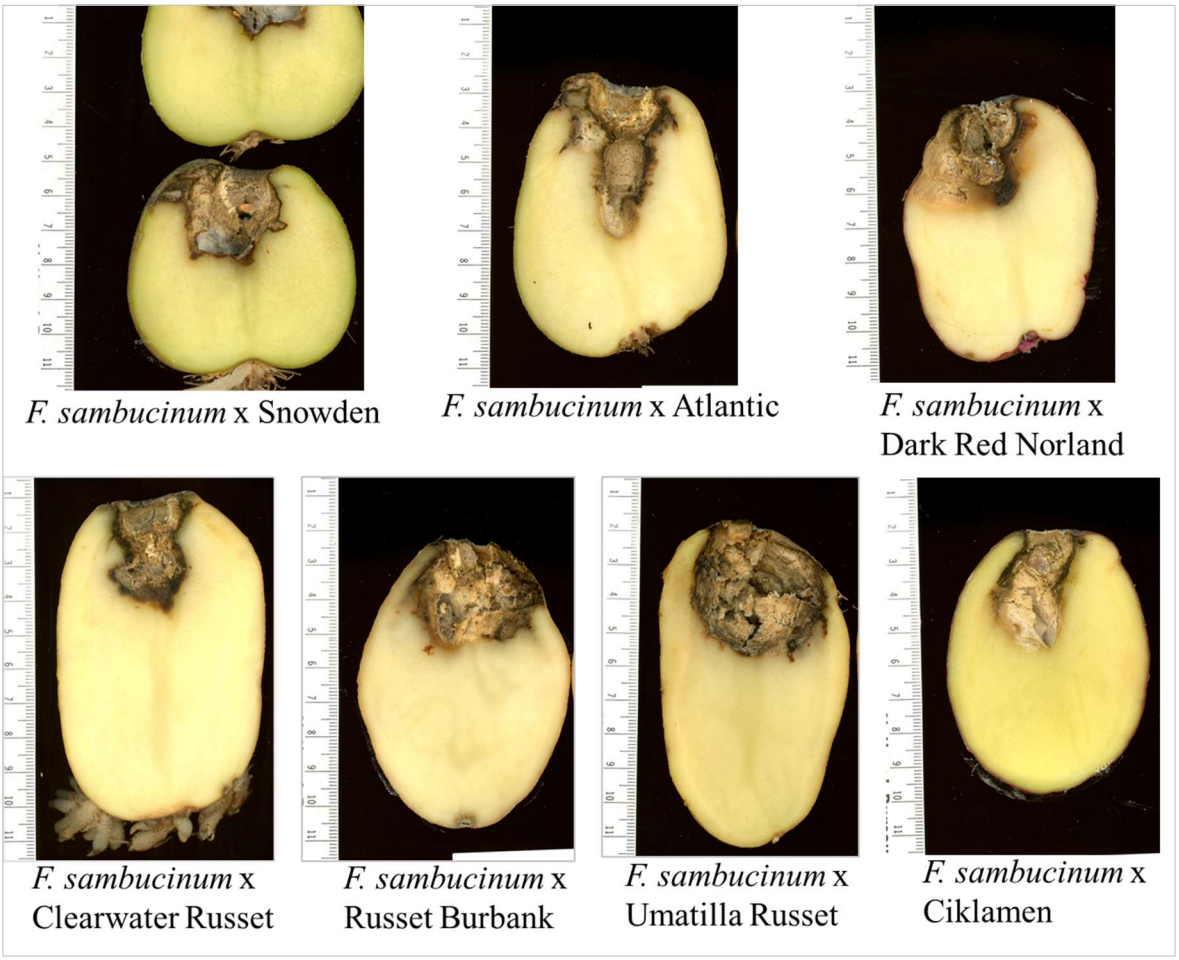

Figure 3-1. Visual comparison of lesion severity caused by *Fusarium sambucinum* infection on seven potato varieties in an experiment to measure relative aggressiveness of different *Fusarium* species on potato tubers inoculated with Fusarium dry rot pathogens recovered from the Pacific Northwest states of Idaho, Oregon and Washington. The ruler scale is shown in centimeters.

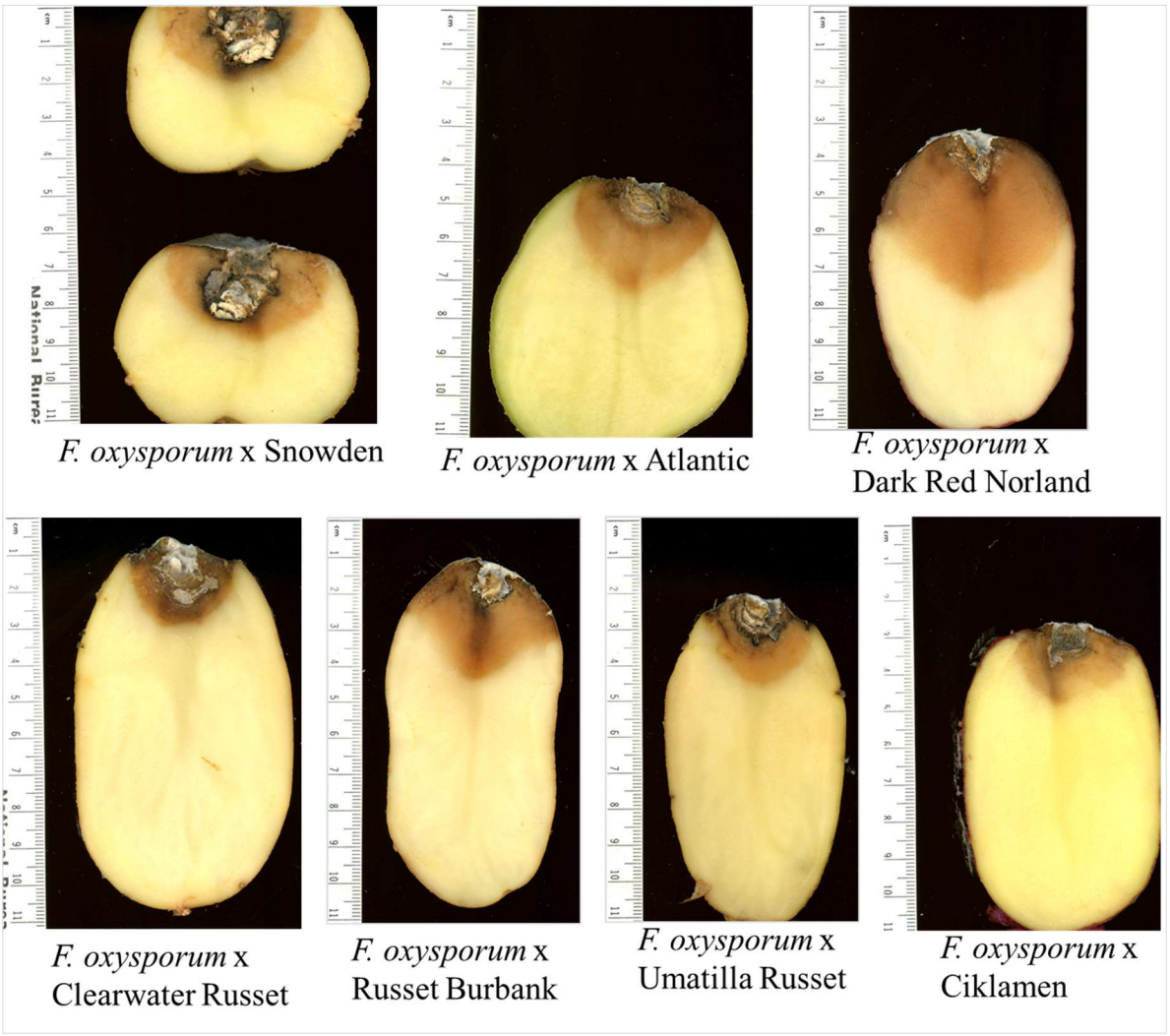

Figure 3-2. Visual comparison of lesion severity caused by *Fusarium oxysporum* infection on seven potato varieties in an experiment to measure relative aggressiveness of different *Fusarium* species on potato tubers inoculated with Fusarium dry rot pathogens recovered from the Pacific Northwest states of Idaho, Oregon and Washington. The ruler scale is shown in centimeters.

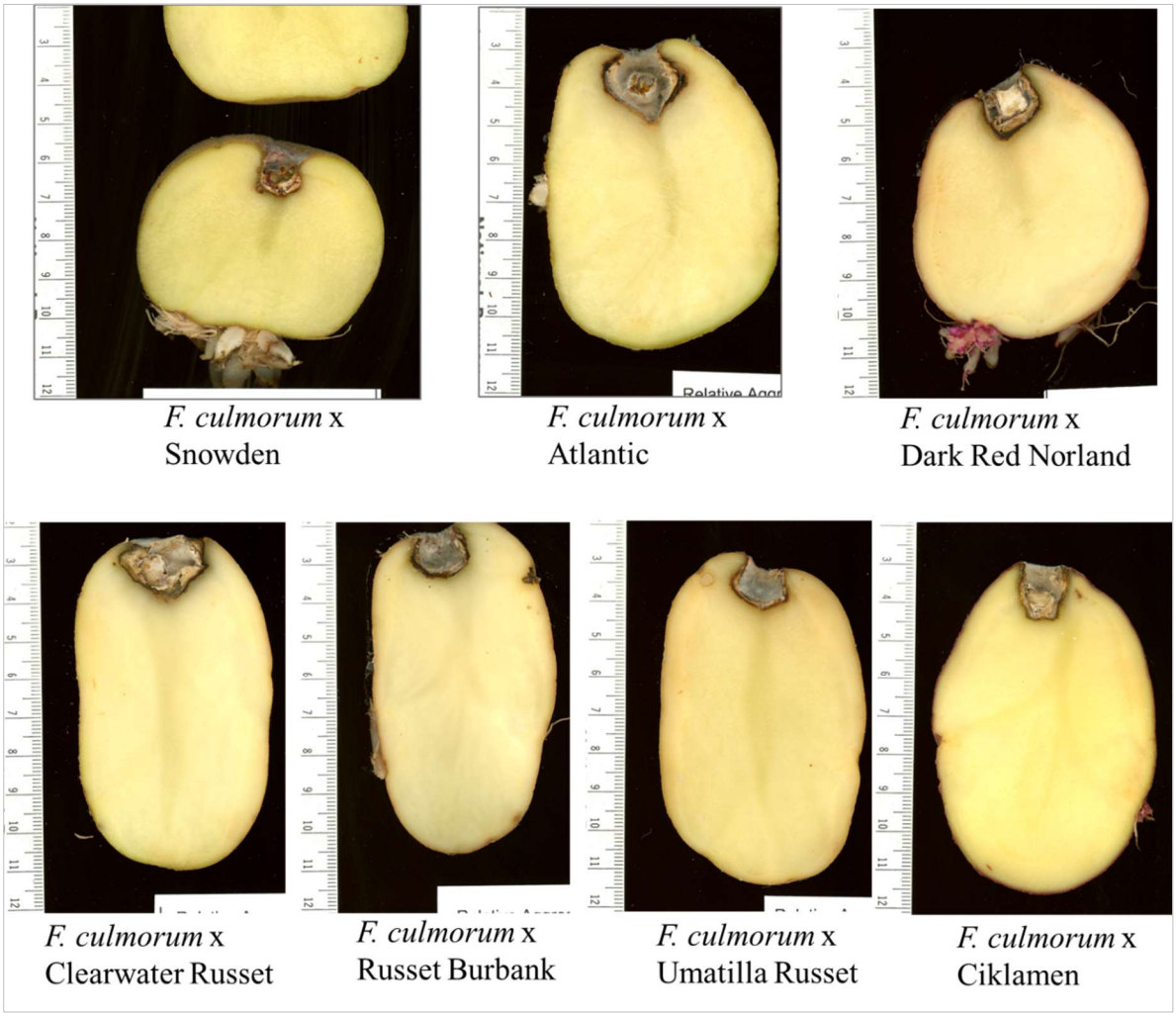

Figure 3-3. Visual comparison of lesion severity caused by *Fusarium culmorum* infection on seven potato varieties in an experiment to measure relative aggressiveness of different *Fusarium* species on potato tubers inoculated with Fusarium dry rot pathogens recovered from the Pacific Northwest states of Idaho, Oregon and Washington. The ruler scale is shown in centimeters.

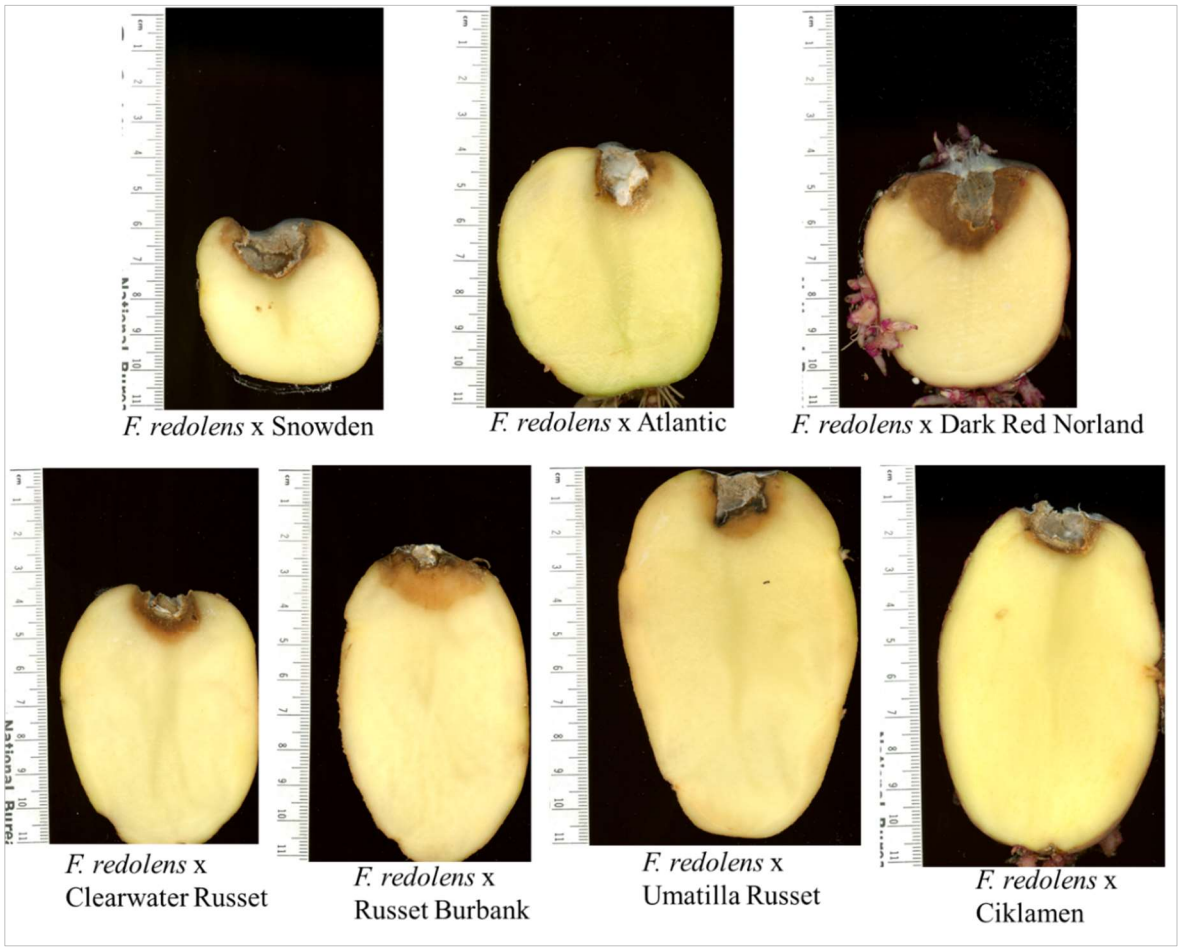

Figure 3-4. Visual comparison of lesion severity caused by *Fusarium redolens* infection on seven potato varieties in an experiment to measure relative aggressiveness of *Fusarium* on potato tubers inoculated with Fusarium dry rot pathogens recovered from the Pacific Northwest states of Idaho, Oregon and Washington. The ruler scale is shown in centimeters.

Table 3-5. Variation in aggressiveness among isolates of the same *Fusarium* species in an experiment to measure relative aggressiveness of different *Fusarium* species on potato tubers inoculated with *Fusarium* dry rot pathogens recovered from the Pacific Northwest states of Idaho, Oregon and Washington.

F. culmorum		*F. oxysporum*		*F. redolens*		*F. sambucinum*	
Isolate code	Mean lesion area (cm^2)	Isolate code	Mean lesion area (cm^2)	Isolate code	Mean lesion area (cm^2)	Isolate code	Mean lesion area (cm^2)
C050	3.5 [a] a	I021	8.9 a	I007	4.5 a	E100	11.8 a
I023	2.0 b	B083	8.8 a	I030	3.6 b	FID 71-6	11.3 ab
B010	2.0 b	I009	8.2 a			E089	10.1 bc
		I053	7.8 a			C030	8.8 cd
		I005	7.4 ab			F060	8.5 d
		I008	6.0 b			D083	4.0 e

[a] Means within columns followed by the same letter are not significantly different based on pair-wise comparisons (alpha = 0.05.)

Discussion

This study investigated the relative aggressiveness of PNW *Fusarium* species as dry rot pathogens on potato varieties important to the PNW. Seven potato varieties were challenged with four pathogenic *Fusarium* species. The results provided new, specific information about *Fusarium* dry rot in the PNW, specifically the severity of infection in potato varieties grown in the PNW when challenged with pathogenic *Fusarium* isolates originating from the region. For example, dry rot was more severe when Umatilla Russet was infected with *F. sambucinum* and when Dark Red Norland was infected with *F. oxysporum,* as compared to other tested potato varieties. Knowledge of the identity of the *Fusarium* species in specific situations may assist in dry rot management. Variety selection may play a role in alleviation of dry rot issues if a different variety can be utilized by a grower where a specific *Fusarium* species is causing disease.

The importance of considering the combination of *Fusarium* species and potato variety in management decisions is highlighted by these data as the interaction between *Fusarium* species and potato variety was found to be significant. In similar laboratory studies of tuber inoculation with various *Fusarium* species resulting in dry rot lesions, the interaction of species and variety was also found to be significant (Azil et al. 2021; Peters et al. 2008a; Stefańczyk et al. 2016). Peters et al. (2008a) also specifically emphasized the value of knowing the species identity of the *Fusarium* pathogen in specific situations to help assess the risk level for dry rot development and guide decision-making.

Fusarium sambucinum and *F. oxysporum* were the most aggressive species with mean lesion size ranging from 7.2 to 12.8 cm^2 and 5.2 to 13.8 cm^2, respectively. *Fusarium sambucinum* has also been

reported as the most aggressive species by other researchers conducting pathogenicity and aggressiveness tests. *Fusarium sambucinum* has been shown to be more aggressive *in vitro* on potato tubers as measured by lesion size as compared to *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. oxysporum*, *F. cerealis*, *F. solani* and others (Azil et al. 2021; Du et al. 2012; Esfahani 2005; Gachango et al. 2012; Ocamb et al. 2007; Peters et al. 2008a; Stefańczyk et al. 2016). For example, Du et al. (2012) observed that *F. sambucinum* lesions were nearly twice as large as *F. acuminatum* and *F. equiseti* lesions for Chinese isolates, and Gachango et al. (2012) reported that *F. sambucinum* lesions were more than 200 mm^2 larger on Dark Red Norland than lesions caused by ten other *Fusarium* species.

Fusarium sambucinum and *F. oxysporum* were not similarly aggressive to all seven tested potato varieties. *F. sambucinum* was much more aggressive on Umatilla Russet with lesion sizes from three to over five centimeters larger than the other six varieties. *F. oxysporum* was much more aggressive on Dark Red Norland with lesion sizes from five to more than eight centimeters larger than the other six varieties. Many other studies have also reported variation in susceptibility of potato varieties to Fusarium dry rot pathogens (Azil et al. 2021; Aprasad et al. 1997; Choiseul et al. 2007; Du et al. 2012; Esfahani 2005; Merlington 2014; Peters et al. 2008a, 2008b; Stefańczyk et al. 2016). Within each potato type, less susceptible varieties were recorded for both *F. sambucinum* and *F. oxysporum* infection, highlighting the importance of variety selection in dry rot management. For example, Clearwater Russet was the least susceptible of the three russet varieties to *F. sambucinum* and Snowden was less susceptible than Atlantic. For infection by *F. oxysporum*, *F. sambucinum* and *F. redolens*, Dark Red Norland was more susceptible than Ciklamen in all cases. Merlington (2014) reported that the chipping varieties Snowden and Atlantic were more susceptible to Fusarium dry rot than Russet Norkotah. This distinction between potato types was observed also in this study with *F. oxysporum* infections in that both chipping varieties were more susceptible to *F. oxysporum* than all three russet varieties tested.

Evaluation of data gathered in recent pathogenicity testing of PNW *Fusarium* isolates (see Chapter 2) provided additional information regarding relative aggressiveness of PNW *Fusarium* isolates to potato. These tests provided additional evidence for the relatively high aggressiveness of *F. sambucinum*, followed by *F. oxysporum*. *Fusarium avenaceum* was observed to cause more severe lesions than all tested species except for *F. sambucinum*, indicating the ability of *F. avenaceum* to cause substantial dry rot problems. Merlington (2014) also reported *F. avenaceum* as one of the three most aggressive to potato out of 11 species tested while Du et al. (2012) reported that *F. avenaceum* was the most aggressive species tested out of five species. *Fusarium avenaceum* has been reported as

a prevalent dry rot pathogen in some regions, comprising 10 to 49% of isolates recovered in dry rot surveys (Choiseul et al. 2007; Du et al. 2012; Gachango et al. 2012; Heltoft et al. 2016; Peters et al. 2008a, 2008b; Stefańczyk et al. 2016). However, *F. avenaceum* was recovered rarely in the PNW samples collected for this study, accounting for only 2% of isolates, and can be expected to generally pose low risk of dry rot in PNW for the time being.

The differing aggressiveness among isolates of the same species was noted for all four species in this dataset as well as in the above-referenced pathogenicity tests for *F. oxysporum*, *F. avenaceum*, *F. cerealis*, *F. flocciferum* and *F. redolens*, with some isolates of each species causing higher incidence and/or severity of dry rot than other isolates (see Chapter 2). This variability within species has been noted by other researchers. Du et al. (2012) observed differential aggressiveness in two *F. oxysporum* isolates, with one isolate as aggressive as *F. sambucinum* and *F. avenaceum* while the other was about half as aggressive, similar to *F. acuminatum* and *F. equiseti*. Peters et al. (2008b) reported differences in aggressiveness among *F. oxysporum* isolates that were obtained from different host plants, i.e., clover, potato, cereals. This phenomenon indicates that dry rot problems in certain locations could be due to the presence of a more aggressive, local pathogen population. Screening field soil for aggressive Fusarium isolates may be helpful if a grower is experiencing frequent issues with storage dry rot.

The pathogenicity testing for this study (see Chapter 2) also provided evidence for differences in aggressiveness of a *Fusarium* isolate on two different potato varieties. For example, one *F. flocciferum* isolate caused greater incidence on Dark Red Norland than Russet Burbank but similar severity. Dark Red Norland was more susceptible to *F. redolens* isolate I030 than Russet Burbank. Russet Burbank was shown to be more susceptible than a proprietary variety to *F. sambucinum* isolate E089. Like the relative aggressiveness test results, these examples indicated that the combination of *Fusarium* species and potato variety can affect dry rot incidence and severity.

Notably, Clearwater Russet was less susceptible to *Fusarium* than expected in this experiment despite the reputation that Clearwater Russet has as being susceptible to dry rot. For storage trials comparing dry rot incidence and severity in Russet Burbank and Clearwater Russet after bruising and inoculation with *F. sambucinum*, Novy et al. (2010) rated the disease response of both Clearwater Russet and Russet Burbank as susceptible but reported higher incidence and severity for Clearwater Russet, 63% and 31%, respectively, than Russet Burbank, 39% and 12%, respectively. In this study, when infected with *F. sambucinum*, Clearwater Russet was less susceptible than both Umatilla Russet and Russet Burbank. Also, *F. oxysporum* infection was less severe in Clearwater Russet than Russet Burbank.

Though this study and the Novy et al. (2010) methods differed, the data reported here indicate that Clearwater Russet may not be as susceptible to Fusarium dry rot as previously reported. Additional focused investigation on the disease response of Clearwater Russet to PNW Fusarium dry rot pathogens is warranted, especially in light of increasing acreage of Clearwater Russet in the PNW, e.g., certified Idaho seed potato acres increased from 4.8% in 2019 to 6.2% in 2022 (Idaho Crop Improvement Association 2019a, 2022).

In conclusion, this experiment showed the interaction between *Fusarium* species and potato variety to be significant. More severe *Fusarium* dry rot was observed with certain combinations of *Fusarium* species and potato variety, e.g., Umatilla Russet infected with *F. sambucinum* and Dark Red Norland infected with *F. oxysporum*. For dry rot management, growers can consider the potato variety being produced in terms of which *Fusarium* species are present. Determining the species composition of *Fusarium* in field soil may help manage for both seed decay and storage rot, keeping in mind that soil-borne *Fusarium* could infect newly planted cut seed pieces and can also adhere to harvested tubers and infect new tuber wounds post-harvest. For example, if field soil contains an abundance of *F. sambucinum*, varieties with a relatively high susceptibility to *Fusarium* should be avoided in the field, especially since *F. sambucinum* is known to be a highly aggressive species by many accounts. Knowledge of *Fusarium* species could also help in post-harvest. A post-harvest tuber sample could be tested for presence of *Fusarium* species using a wound induced inoculation of symptomless tubers. If an aggressive *Fusarium* species is found to be present on tuber surface, especially if the variety may be known to be more susceptible to *Fusarium*, then proactively selling those tubers first may help mitigate possible yield loss due to potential future disease during the storage season.

Literature Cited

Aprasad, K. S., Bateman, G. L., and Read, P. J. 1997. Variation in pathogenicity on potato tubers and sensitivity to thiabendazole of the dry rot fungus *Fusarium avenaceum*. Potato Res. 40:357–365.

Azil, N., Stefańczyk, E., Sobkowiak, S., Chihat, S., Boureghda, H., and Śliwka, J. 2021. Identification and pathogenicity of *Fusarium* spp. associated with tuber dry rot and wilt of potato in Algeria. Eur. J. Plant Pathol. 159:495–509.

Baturo-Ciesniewska, A., Lenc, L., Grabowski, A., and Lukanowski, A. 2015. Characteristics of Polish isolates of *Fusarium sambucinum*: molecular identification, pathogenicity, diversity and reaction to control agents. Am. J. Potato Res. 92:49–61.

Bojanowski, A., Avis, T. J., Pelletier, S., and Tweddell, R. J. 2013. Management of potato dry rot. Postharvest Biol. Technol. 84:99–109.

Choiseul, J., Allen, L., and Carnegie, S. F. 2007. Fungi causing dry tuber rots of seed potatoes in storage in Scotland. Potato Res. 49:241–253.

Corsini, D., and Pavek, J. J. 1986. Fusarium dry-rot resistant potato germplasm. Am. Potato J. 63:629–638.

Du, M., Ren, X., Sun, Q., Wang, Y., and Zhang, R. 2012. Characterization of *Fusarium* spp. causing potato dry rot in China and susceptibility evaluation of Chinese potato germplasm to the pathogen. Potato Res. 55:175–184.

Esfahani, M. N. 2005. Susceptibility assessment of potato cultivars to Fusarium dry rot species. Potato Res. 48:215–226.

Gachango, E., Hanson, L. E., Rojas, A., Hao, J. J., and Kirk, W. W. 2012. *Fusarium* spp. causing dry rot of seed potato tubers in Michigan and their sensitivity to fungicides. Plant Dis. 96:1767–1774.

Heltoft, P., Brurberg, M. B., Skogen, M., Le, V. H., Razzaghian, J., and Hermansen, A. 2016. *Fusarium* spp. causing dry rot on potatoes in Norway and development of a real-time PCR method for detection of *Fusarium coeruleum*. Potato Res. 59:67–80.

Idaho Crop Improvement Association. 2019a. 2019 Certified Seed Potato Acreage Report.

Idaho Crop Improvement Association. 2019b. 2019 Certified Seed Potato Grower Directory.

Idaho Crop Improvement Association. 2022. 2022 Certified Seed Potato Acreage Report.

Kawchuk, L. M., Holley, J. D., Lynch, D. R., and Clear, R. M. 1994. Resistance to thiabendazole and thiophanate-methyl in Canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani*. Am. Potato J. 71:185–192.

Kawchuk, L. M., Hutchison, L. J., Verhaeghe, C. A., Lynch, D. R., Bains, P. S., and Holley, J. D. 2002. Isolation of the β-tubulin gene and characterization of thiabendazole resistance in *Gibberella pulicaris*. Can. J. Plant Pathol. 24:233–238.

Merlington, A. A. 2014. Management options for control of Fusarium dry rot (*Fusarium* spp.) and potato common scab (*Streptomyces* spp.) of potato (*Solanum tuberosum* L.) in Michigan. M.S. thesis. Michigan State University.

Nolte, P. 1994. Managing benzimidazole resistance in the potato dry rot fungus. EXT 769. University of Idaho Extension. 8 pages.

Novy, R. G., Whitworth, J. L., Stark, J. C., Love, S. L., Corsini, D. L., Pavek, J. J., et al. 2010. Clearwater Russet: A dual-purpose potato cultivar with cold sweetening resistance, high protein content, and low incidence of external defects and sugar ends. Am. J. Potato Res. 87:458–471.

Ocamb, C. M., Hamm, P. B., and Johnson, D. A. 2007. Benzimidazole resistance of *Fusarium* species recovered from potatoes with dry rot from storages located in the Columbia Basin of Oregon and Washington. Am. J. Potato Res. 84:169–177.

Oregon Potato Commission, and Oregon State University Extension Service. 2019. 2019 Certified Seed Potato Report and Directory of Growers.

Peters, J. C., Lees, A. K., Cullen, D. W., Sullivan, L., Stroud, G. P., and Cunnington, A. C. 2008a. Characterization of *Fusarium* spp. responsible for causing dry rot of potato in Great Britain. Plant Pathol. 57:262–271.

Peters, R. D., MacLeod, C., Seifert, K. A., Martin, R. A., Hale, L. R., et al. 2008b. Pathogenicity to potato tubers of *Fusarium* spp. isolated from potato, cereal and forage crops. Am. J. Potato Res. 85:367–374.

Secor, G. A., and Salas, B. 2001. Fusarium dry rot and Fusarium wilt. In Compendium of Potato Diseases, St. Paul: The American Phytopathological Society, p. 23–25.

Stefańczyk, E., Sobkowiak, S., Brylińska, M., and Śliwka, J. 2016. Diversity of *Fusarium* spp. associated with dry rot of potato tubers in Poland. Eur. J. Plant Pathol. 145:871–884.

Washington Seed Potato Commission, and Washington Certified Seed Potatoes. 2019. Crop Directory - 2019 Certified Seed Potato Acreage.

Wharton, P., Hammerschmidt, R., and Kirk, W. 2007. Fusarium dry rot. E-2992. Michigan State University Extension Bulletin. 6 pages.

Chapter 4: Fungicide Sensitivity of *Fusarium* Species Associated With Fusarium Dry Rot of Potato in the Pacific Northwest

Abstract

Fusarium dry rot is a worldwide fungal disease of potato causing seed decay at planting and postharvest tuber decay in storage. Development of resistance in Fusarium dry rot pathogens to dry rot management fungicides is hypothesized to be one of the factors driving a reported increase in dry rot in the PNW in recent years. In this study, three experimental methods were utilized to gain information about the fungicide sensitivity of Fusarium dry rot pathogens in the PNW: laboratory sensitivity screening tests using fungicide-amended agar plates, laboratory tests of fungicide effectiveness as a seed treatment and a field trial testing fludioxonil effectiveness against *F. sambucinum*. Fungicide-amended agar tests revealed sensitivity to difenoconazole among all tested *Fusarium* species, resistance to fludioxonil in five tested species, and resistance to thiabendazole in one tested species, *F. sambucinum*. When applied as a seed treatment on cut seed pieces, difenoconazole reduced dry rot in all five tested species, fludioxonil reduced dry rot for sensitive isolates of *F. sambucinum* and *F. oxysporum*, resistant isolates of *F. oxysporum*, and some resistant isolates of *F. sambucinum*, indicating that laboratory testing is not entirely predictive of fungicide effectiveness in presence of the potato host, and thiabendazole reduced dry rot for sensitive *F. sambucinum* isolates but not for most resistant isolates. In a field trial testing the effectiveness of a fludioxonil seed treatment against sensitive and resistant isolates of *F. sambucinum*, sensitive isolates were observed to have less frequent and less severe seed piece decay than resistant isolates, and yields were somewhat greater for sensitive isolates than resistant isolates. To our knowledge, this is the first study on *Fusarium* isolates from the PNW region connecting laboratory fungicide sensitivity data to dry rot incidence and severity when fungicides were used as seed treatments *in vivo* and in the field.

Introduction

Fusarium dry rot is a worldwide fungal disease of potato causing seed decay at planting and postharvest tuber decay in storage, known as Fusarium seed decay and Fusarium dry rot, respectively (Azil et al. 2021; Baturo-Ciesniewska et al. 2015; Corsini and Pavek 1986; Du et al. 2012; Gachango et al. 2012; Heltoft et al. 2016; Kawchuk et al. 1994, 2002; Peters et al. 2008a; Secor and Salas 2001; Stefańczyk et al. 2016). This disease is caused by several species in the genus *Fusarium*. Lesions appear as sunken, wrinkled, darkened areas on the periderm while internally there is dry, crumbly decayed tissue that is tan to brown to black in color (Gachango et al. 2012; Heltoft et al. 2016; Nolte 1994; Peters et al. 2008a; Secor and Salas 2001; Wharton et al. 2007a). Yield losses due to Fusarium

dry rot make it one of the most important postharvest potato diseases worldwide. Average decreased yield has been cited as 6 to 25% with some situations resulting in up to 60% storage loss (Baturo-Ciesniewska et al. 2015; Corsini and Pavek 1986; Du et al. 2012; Gachango et al. 2012; Heltoft et al. 2016; Kawchuk et al. 1994, 2002; Peters et al. 2008a; Secor and Salas 2001; Stefańczyk et al. 2016). Most seed lots and commercial crops have some dry rot infection. This can exclude seed lots from sale, may affect crop emergence and health and may affect the amount of time potatoes can be stored without sustaining financial loss (Peters et al. 2008a). Potato growers around the world strive to manage Fusarium dry rot through cultural practices such as wound management and crop rotation, application of chemical fungicides and use of less susceptible potato varieties (Bojanowski et al. 2013).

The Pacific Northwest (PNW) region of the United States is a major potato production region of the world, and the PNW states of Idaho, Oregon and Washington collectively produce around 11.3 million metric tons of potatoes annually. Development of resistance in Fusarium dry rot pathogens to fungicides used for dry rot management is hypothesized to be one of the factors driving a reported increase in dry rot in the PNW in recent years. Resistance to a dry rot management fungicide called thiabendazole has been documented in the PNW and elsewhere in multiple *Fusarium* species, including *F. sambucinum* (Carnegie and Cameron 1992; Desjardins et al. 1993; Gudmestad and Ivors 2019; Hanson et al. 1996; Hide et al. 1992; Kawchuk et al. 1994; Langerfeld 1990; Nolte 1994; Ocamb et al. 2007). The most recent work related to thiabendazole resistance in Fusarium dry rot pathogens in the PNW was published more than 15 years ago (Desjardins 1995; Nolte 1994; Ocamb et al. 2007). Additionally, resistance to the dry rot management fungicide fludioxonil has not been previously reported in the PNW but has been observed in *F. sambucinum*, *F. oxysporum* and *F. coeruleum* in other potato-growing regions (Gachango et al. 2012; Peters et al. 2008c).

In a group of *F. sambucinum* isolates collected in 1990 and 1991 from Idaho, Michigan and North Dakota, 24 of 25 isolates were observed to have thiabendazole resistance (Desjardins et al. 1993). In contrast, 17 isolates collected from various North American locations between 1963 and 1991 were all highly sensitive to thiabendazole (Desjardins et al. 1993). Resistance to thiabendazole was found in approximately 85% of *F. sambucinum* isolates tested in Idaho from 1993 to 1995 (Gallian et al. 2006; Nolte 1994). Thiabendazole was regularly used as both seed and post-harvest treatments for potatoes in the PNW to manage Fusarium dry rot, but use of thiabendazole is no longer recommended due to its ineffectiveness against widespread resistance in the PNW population of *F. sambucinum* (Nolte 1994; Miller et al. 2019). For symptomatic dry rot tubers collected from commercial potato storages in the Columbia Basin of Washington and Oregon, Ocamb et al. (2007) reported

thiabendazole resistance in *F. sambucinum* (71% of tested isolates), *F. oxysporum* (17%), *F. solani* (21%) and at least one isolate each of *F. avenaceum*, *F. culmorum*, *F. equiseti* and *F. sporotrichioides*. In a subset of isolates screened for thiabendazole sensitivity, thiabendazole-resistant *F. sambucinum* was recovered from 20 of 25 sampled storages, *F. oxysporum* from 8 of 23 storages, and *F. solani* from 3 of 15 storages, indicating that *F. sambucinum* resistance was affecting dry rot risk on a larger scale than either *F. oxysporum* or *F. solani* (Ocamb et al. 2007).

Petri-dish based laboratory screening methods are commonly conducted to determine fungicide sensitivity of plant pathogens to gain information that will assist in disease management (Aprasad et al. 1997; Carnegie and Cameron 1992; Choiseul et al. 2007; Desjardins et al. 1993; Fairchild et al. 2013; Förster et al. 2004; Gachango et al. 2012; Gudmestad and Ivors 2019; Hanson et al. 1996; Hide et al. 1992; Kawchuk et al. 1994; Ocamb et al. 2007; Peters et al. 2001, 2008a, 2008c). However, the ability of fungicides to inhibit fungal pathogens *in vitro* does not necessarily predict fungicide effectiveness in a disease situation in the field (Pasche and Gudmestad 2019; Russell 2004). *In vitro* assays should be accompanied by *in vivo* and/or field data correlating the *in vitro* fungicide resistance with loss of disease mitigation (Pasche and Gudmestad 2019; Russell 2004). It is not prudent to rely on laboratory testing alone to make fungicide use recommendations to growers given the possibility that laboratory and field tests may not correspond with each other.

As such, this study of fungicide sensitivity of Fusarium dry rot pathogens in the PNW was conducted to gain information in both laboratory and field settings. First, in a laboratory setting, *Fusarium* isolates were grown on fungicide-amended agar in a Petri dish to screen for their level of fungicide sensitivity based on measured growth as compared to a control plate without fungicide. Second, *Fusarium* isolates were tested for fungicide sensitivity in the presence of living potato tuber tissue, i.e., an *in vivo* setting, based on the results of the sensitivity screening in the lab. A modification of a method known as the “bag test” as described by Nolte (1994) and Bohl, Nolte and Thornton (1992) was used, where Fusarium seed decay was evaluated after freshly cut seed pieces were inoculated with selected isolates immediately prior to applying a fungicide seed treatment and placed in a paper bags for incubation for approximately three weeks. For the bag test, *Fusarium* isolates with different fungicide sensitivity levels were used, with the general expectation that dry rot incidence and severity would be lower for treatments inoculated with fungicide sensitive isolates and greater for resistant isolates. Third, a focused field experiment was conducted to determine fludioxonil effectiveness as a seed treatment against *F. sambucinum*.

Three fungicides were selected for this study. Fludioxonil is widely used in PNW potato production as a seed treatment, but sensitivity data for PNW *Fusarium* isolates was not available prior to this study. Since fludioxonil resistance has been reported in *F. sambucinum* and *F. oxysporum* from other North American locations (Gachango et al. 2012; Peters et al. 2008c), fludioxonil was included to determine sensitivity of the prevalent PNW dry rot pathogen *F. sambucinum* in particular and other PNW *Fusarium* species in general. Difenoconazole was tested because it is used commonly in mixtures with fludioxonil for dry rot management in the PNW and sensitivity data for PNW *Fusarium* isolates was not available prior to this study. Although thiabendazole use for dry rot management declined after widespread resistance developed in the PNW, it was included in these experiments to determine whether the PNW population of *F. sambucinum* still exhibits a high proportion of resistance and if individuals from other *Fusarium* species in the PNW possess resistance to thiabendazole.

For *Fusarium* species associated with dry rot in the PNW, the objectives of this study were to a) conduct *in vitro* screening of fungicide sensitivity in the laboratory using fungicide-amended agar, b) test fungicide effectiveness as a seed treatment in the laboratory using a bag test method to mimic the seed cutting and treating portion of potato production, and c) determine the effectiveness of fludioxonil as a seed treatment in a field experiment using seed inoculated with Idaho *F. sambucinum* isolates displaying either sensitivity or resistance to fludioxonil *in vitro*. To our knowledge, this is the first study on *Fusarium* isolates from the PNW region connecting laboratory fungicide sensitivity data to dry rot incidence and severity when fungicides were used as seed treatments *in vivo* and in the field.

Materials and Methods

In Vitro Fungicide Sensitivity Screening

In vitro fungicide sensitivity testing was conducted using the spiral gradient dilution method according to the protocol described in Fairchild et al. (2013). Selected isolates were tested at least twice for sensitivity to three fungicides used in potato dry rot management, difenoconazole, fludioxonil and thiabendazole. The experiment was repeated two additional times in cases where results from the first two rounds of testing did not agree. The tested isolates were included to reflect the species proportions of sequenced isolates obtained in a recent survey of *Fusarium* species associated with potato dry rot in the PNW. Additionally, three isolates maintained in the laboratory for inoculum sources in other projects were tested for fungicide sensitivity, two *F. sambucinum* isolates (FID 212 and FID 71-6) and one *F. coeruleum* isolate (MR-6), all originating from Idaho.

For all tests, three replicate plates were made for each combination of test isolate and fungicide and for the control. The control plates received no fungicide amendment, and spore suspensions were applied as for the fungicide-amended plates. For each test isolate, a fresh culture was grown by aseptically placing a frozen disk of dried mycelium in the center of a 10-centimeter Petri dish of potato dextrose agar and incubating at room temperature under a 12-hour light/dark cycle for 10 to 14 days. When test isolate cultures were ready, 50 milliliters of antibiotic-amended potato dextrose agar were dispensed into 15-centimeter Petri dishes. The antibiotic solution for amendment contained 0.2 grams ampicillin, 0.075 grams rifamycin and 1 milliliter of DMSO per one liter of potato dextrose agar. After cooling, plates were amended with fungicide solutions. Commercial formulations of fungicides with a single active ingredient (ai) were mixed with sterile distilled water to obtain a stock solution with a final concentration of 10,000 parts per million of active ingredient. The three fungicides were Inspire with 23.2% difenoconazole ai, Maxim 4FS with 40.3% fludioxonil ai, and Mertect 340F with 42.3% thiabendazole ai, all from Syngenta. Fungicide stock solutions were applied to the 15 cm Petri plates using an EddyJet 2 spiral plater (IUL) set for the exponential spiral distribution setting M3K Exp Slow 54.30 µL, which provided a fungicide concentration range of 0.9 mg/L to 506.9 mg/L on each plate. Plates were allowed to dry for at least four hours in a sterile biosafety cabinet before inoculating with the test isolate. Prior to plate inoculation, a 15-millimeter core of agar was removed from the center of the plate to prevent fungal growth into the plate center and into nearby areas that were separately inoculated.

Spore suspensions were prepared by flooding the 10- to 14-day old fungal culture plates with sterile distilled water, gently dislodging spores by scraping across the top of the culture with a sterile stainless-steel instrument and straining the resulting spore suspension through four layers of cheesecloth. Plates were inoculated by applying 10 microliters of the spore suspension in straight line along the agar surface from plate edge to center. On each plate, three separate transects of spore suspension were applied radially from the center of the plate to the edge using a micropipettor with the plate placed over a colony counter grid (IUL) to aid transect placement. Two transects together spanned the plate diameter, while the third transect was perpendicular to the first two and spanned only the radius, to form a pattern of growth that resembled the capital letter “T”. After drying for about an hour, plate edges were sealed with parafilm and incubated for four days on the bench in ambient light. After incubation, images of the plates were scanned using a flatbed scanner (Epson Perfection V600 Photo) alongside a physical ruler with centimeter markings.

Using Adobe Photoshop software (version CC 20.4 or later), fungal growth was measured using the Ruler Tool. The Measurement scale was calibrated to the physical ruler in the scanned image. The

fungal growth measurements were used to calculate the fungicide concentration that inhibited fungal growth by 50% as compared to the control. This is known as the 50% Effective Concentration (EC_{50}). On each plate, two measurements of the width of fungal growth were recorded, one from the widest point along the two combined transects that spanned the plate diameter, and the second from the widest point along the transect that spanned only the radius. These two measurements were averaged and recorded as the fungal growth for the plate. For a set of three replicate plates for a test isolate grown on fungicide-amended agar, the mean fungal growth was calculated and compared with the mean fungal growth for the control plates of that test isolate. This comparison was called "relative growth" and was calculated as follows: relative growth = (mean fungicide-amended growth / mean control growth) * 100. The relative growth measurement accounted for growth of the isolate within the entire concentration range of the fungicide applied to the plate.

If the isolate had less than 50% relative growth, then the EC_{50} was below the range of fungicide concentrations on the plate, i.e., less than 0.9 mg/L (Figure 4-1). If the isolate had greater than 50% relative growth, the EC_{50} was determined. First, the 50% value of the mean fungal growth for the control plates was established (e.g., 50% of a mean fungal growth of 2.0 centimeters was 1.0 centimeter). Second, the 50% mean control fungal growth width was located on the image of the fungicide-amended plate, and the IUL colony counter grid was placed over the plate image such that the circumference of the grid physically overlaid the circumference of the plate edge. Finally, the name of the grid line matching the location of the 50% mean control fungal growth width was noted, and the corresponding fungicide concentration based on the EddyJet spiral distribution mode was the EC_{50} value in mg/L (Figure 4-1). If the fungal growth width was greater than 50% across all grid lines on the IUL colony counter grid, then the EC_{50} was above the range of fungicide concentrations on the plate, i.e., greater than 506.9 mg/L (Figure 4-1).

Based on other published fungicide screening tests, threshold sensitivity values were adopted for the EC_{50} values obtained in this study. For difenoconazole tests, isolates were considered difenoconazole-sensitive with EC_{50} values of less than 5 mg/L, following the threshold set by Gachango et al. (2012). Those isolates with EC_{50} values of 5 mg/L or higher were considered to have "reduced sensitivity" to difenoconazole in this study. For fludioxonil tests, isolates were considered sensitive using a threshold EC_{50} value of less than 5 mg/L to indicate sensitivity, following the threshold used by Gachango et al. (2012). Isolates with EC_{50} values greater than 100 mg/L were considered fludioxonil-resistant, following the threshold used by Gachango et al. (2012) and Peters et al. (2008b). For thiabendazole tests, isolates with an EC_{50} value of less than 5 mg/L were considered thiabendazole-sensitive, following the threshold used by Gachango et al. (2012). Isolates with EC_{50} values of 5 mg/L

to 100 mg/L were considered to have "reduced sensitivity" to thiabendazole in this study. Isolates with EC_{50} values greater than 100 mg/L were considered thiabendazole-resistant, following the threshold used by Gachango et al. (2012). In addition, a previous screening of *F. avenaceum* for thiabendazole sensitivity by Aprasad et al. (1997) defined sensitivity as lack of fungal growth on agar amended with 5 mg/L thiabendazole and resistance as presence of fungal growth on agar amended with 100 mg/L thiabendazole.

Figure 4-1. Examples of fungal growth on fungicide-amended agar as obtained from fungicide sensitivity screening tests. (a) Control plate using agar not amended with fungicide; (b) EC_{50} determined to be less than 0.9 mg/L, i.e. isolate is "sensitive" to the fungicide; (c) EC_{50} determined to be greater than 506.9 mg/L, i.e. isolate is "resistant" to the fungicide; (d) EC_{50} determined to be within the range of fungicide concentrations of the test, i.e. $0.9 > EC_{50} < 506.9$ mg/L. Note: EC_{50} = 50% Effective Concentration, or the fungicide concentration inhibiting fungal growth by 50% as compared to the fungal growth on the control plate.

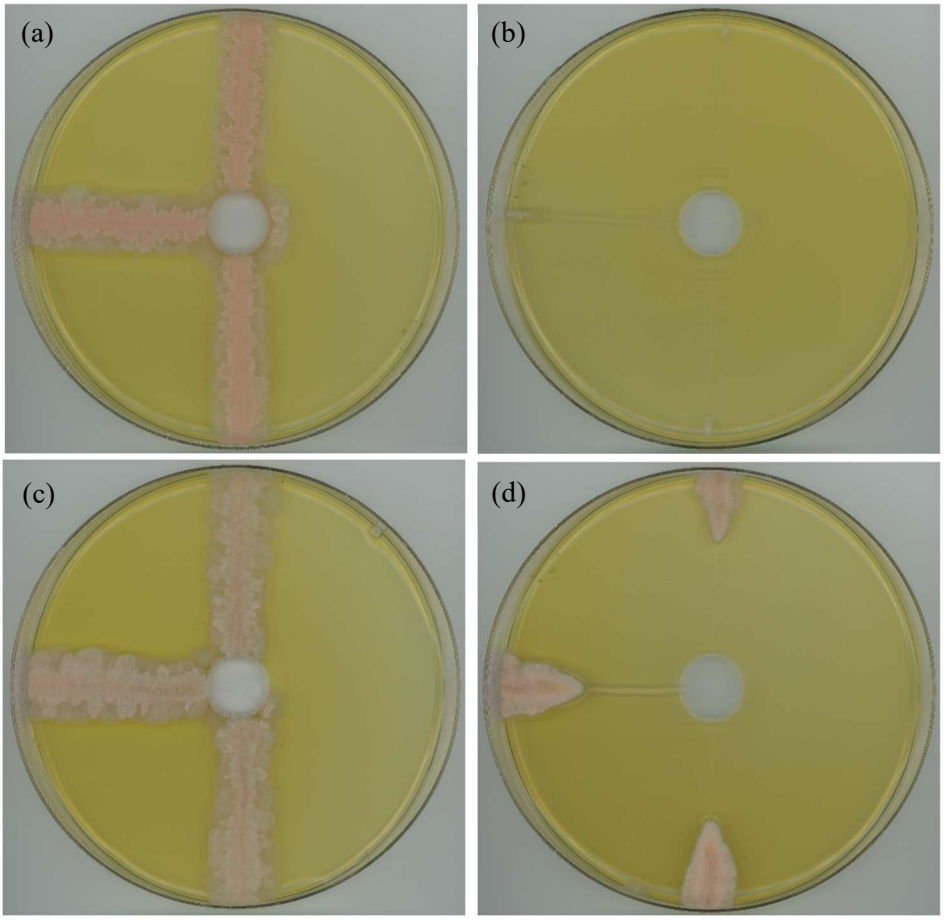

Figure 4-1. Examples of fungal growth on fungicide-amended agar as obtained from fungicide sensitivity screening tests. (a) Control plate using agar not amended with fungicide; (b) EC50 determined to be less than 0.9 mg/L, i.e. isolate is "sensitive" to the fungicide; (c) EC50 determined to be greater than 506.9 mg/L, i.e. isolate is "resistant" to the fungicide; (d) EC50 determined to be within the range of fungicide concentrations of the test, i.e. 0.9 > EC50 < 506.9 mg/L.

Fungicide Seed Treatment Experiment

After the laboratory screening of PNW *Fusarium* isolates for fungicide sensitivity in the absence of the potato host, isolates were exposed to fungicides in the presence of living potato tissue using the bag test as described by Nolte (1994) and Bohl, Nolte and Thornton (1992). Three fungicides, difenoconazole, fludioxonil and thiabendazole, were evaluated separately. For all bag tests, the following treatment design applied per fungicide, and each test was conducted twice. Selected *Fusarium* isolates previously screened for fungicide sensitivity *in vitro* were exposed to the fungicide applied as a seed treatment to Russet Burbank seed pieces. Isolates with differing sensitivity to the fungicide were included. Four control treatments were included for each fungicide, a) non-treated non-inoculated control, a negative control, to account for Fusarium seed decay as a result of inoculum naturally present on seed pieces (NTC-NI), b) non-treated mock-inoculated control using distilled water as the inoculum, a negative control, to show that the inoculation method did not cause elevated Fusarium seed decay beyond that resulting from naturally present inoculum (NTC-MI), c) non-treated inoculated control, a positive control, to confirm success of the inoculation method with a known pathogenic isolate of *Fusarium sambucinum* (NTC-I FID 71-6 and/or NTC-I A054), and d) a treated inoculated control, a positive control, that was treated with the fungicide and inoculated with a pathogenic *Fusarium* isolate that is known to be sensitive to the fungicide being tested to confirm that the seed treatment was effective in reducing Fusarium seed decay (TC-I).

The experiment was established in a randomized complete block design with four replications. Each bag (i.e., experimental unit was a brown paper grocery bag) contained one treatment applied to 25 seed pieces. Each *Fusarium* isolate was a separate treatment. One replication consisted of all treatments arranged randomly in a large plastic bin (i.e., the "block"). The response variables for statistical analysis were percent dry rot incidence and percent severity.

For inoculum, cultures of *Fusarium* isolates were prepared by aseptically placing a piece of frozen mycelium onto the center of a potato dextrose agar plate and incubating at room temperature for approximately two weeks under a 12-hour light/dark cycle. After cultures were ready, spore suspensions were prepared by flooding the culture plate with sterile distilled water, gently dislodging

fungal spores and tissue by scraping across the top of the culture with a heat-sterilized stainless-steel instrument and straining the resulting spore suspension through four layers of cheesecloth. Spore concentrations were adjusted to approximately 2.0 x 10^5 spores per milliliter using a hemacytometer.

Freshly cut potato seed pieces (Russet Burbank variety) were inoculated with the spore suspension using a hand-held spray bottle at a rate of 12 milliliters per 9.1 kilograms of 70.9-gram seed pieces. Inoculation was conducted in a plastic-coated cement mixer that was rotated as the spore suspension was sprayed onto the seed pieces. Immediately following inoculation, the fungicide seed treatment was applied in water suspension using a hand-held CO_2 powered sprayer at 10-12 psi and a TXVS 1 ConeJet nozzle at the appropriate rate. Fungicide application was also made in the plastic-coated cement mixer by rotating for 70 seconds, with fungicide being applied at 10-second intervals. For each treatment of a bag test, 25 randomly selected seed pieces were placed in a brown paper grocery bag and allowed to dry overnight. The following day, bag tops were folded over, and bags were randomly arranged in a large plastic bin for incubation. Bins were incubated at room temperature (approximately 23°C) for three weeks at high relative humidity (80-100%).

After incubation, the seed pieces were visually assessed for dry rot. Seed pieces were cut into quarters, and dry rot severity was recorded as the visually estimated percent of each seed piece that exhibited dry rot symptoms, which essentially was an estimate of the volume the seed piece that was decayed. The response variables for statistical analyses were calculated for each bag of 25 seed pieces (bag = experimental unit) as follows:

- Percent dry rot incidence = [(number of seed pieces exhibiting dry rot symptoms / total number of seed pieces) * 100]
- Percent dry rot severity (mean for 25 seed pieces of percent dry rot per seed piece *100)

Field Experiment for Fludioxonil Efficacy on Fusarium sambucinum in Idaho

Field experiments were conducted in 2020 and 2021. A total of 28 treatments was included in the experiment, twenty fludioxonil-resistant *F. sambucinum* isolates, three fludioxonil-sensitive *F. sambucinum* isolates and five control treatments, a non-treated, non-inoculated control and two fludioxonil-sensitive control isolates, each tested as both treated and non-treated with fludioxonil. Test isolates were exposed to a commercial formulation of fludioxonil potato seed treatment, i.e., Maxim 4FS, 40.3% active ingredient, Syngenta, applied to Russet Burbank seed pieces grown under field conditions using standard field management practices.

Treatments were arranged in a randomized complete block design with five replications. Each replication consisted of a plot that was a 9.1 meter-long row with seed pieces planted at intervals of 30.5 centimeters and a total of 30 seed pieces per plot. At the end of each plot, 1.5 meters of a red potato variety (LaDonna in 2020 and Dark Red Norland in 2021) was planted to enable accurate plot separation at harvest. Row spacing was 0.91 meters, and there were no additional buffer rows between blocks.

Seed pieces were cut, inoculated with *F. sambucinum* spore suspensions, and treated with Maxim 4FS seed treatment on May 7-8, 2020 and May 5-6, 2020. For inoculum production, all Idaho *F. sambucinum* isolates selected for the field trial were grown on 3.9% potato dextrose agar (PDA) for two weeks at 24˚C. Culture plates were flooded with sterile distilled water, the agar surface was gently scraped to release fungal spores and tissue, and the liquid suspension was filtered through double-layered cheesecloth to collect the spores. Suspensions were adjusted with sterile distilled water to approximately 2.0×10^5 to 2.6×10^5 spores per milliliter. Freshly cut Russet Burbank seed pieces were inoculated with the spore suspension at a rate of 15 milliliters per 11.3 kilograms of seed (approximately 150 seed pieces weighing 71 grams each). Immediately following inoculation, the fludioxonil seed piece treatment was applied in water suspension at the labeled rate of Maxim 4FS using a hand-held CO_2 powered sprayer at 30 psi and a TXVS 4 ConeJet nozzle in a total volume of 2.37 milliliters of active ingredient per 45.4 kilograms of seed. Fludioxonil application was made in a plastic-coated cement mixer that was rotated for 60 seconds, with fungicide being applied at 10 second intervals. Seed pieces were stored in labeled brown paper bags at ambient temperatures until planting on May 14, 2020 and May 11, 2021.

Seed pieces were planted in plots as described above. Approximately three weeks after planting, row hilling and herbicide and insecticide applications were carried out uniformly for all plots. Emergence counts were recorded three times after planting until most or all plants emerged in each plot at 22, 26

and 36 days after planting (DAP) in 2020 and 28, 35 and 42 DAP in 2021. For both years, a relative area under the emergence progress curve (RAUEPC) was calculated from planting date to the final emergence assessment (Wharton et al. 2007b). Generally, a greater RAUEPC value for a treatment indicated that the plants emerged faster and in greater numbers as compared to other treatments. In-season destructive sampling occurred 54-55 DAP in 2020 and 59 DAP in 2021. During destructive sampling, ten plants per plot were gently removed from the ground and assessed for vigor, tuber count, stem count, stolon count, and seed piece decay incidence and severity. The remaining 20 plants were harvested at end of season at 145 and 149 DAP in 2020 and 2021, respectively, and marketable and total yield data were collected the following week.

Statistical Analysis

Fungicide seed treatment data were analyzed using the generalized linear mixed model (GLIMMIX) procedure in SAS 9.4 (SAS Institute, Cary, North Carolina), and least square means were separated using pairwise comparisons (alpha = 0.05). For the field experiment, seed piece decay incidence and severity, RAUEPC and marketable (US No. 1) and total yield were analyzed using the generalized linear mixed model (GLIMMIX) procedure in SAS 9.4 (SAS Institute, Cary, North Carolina), and least square means were separated using pairwise comparisons with the Tukey-Kramer multiple comparison adjustment method (alpha = 0.05). Prior to combined analyses of data for both years of the field experiment, data were subjected to the Levene test to ensure homogeneity of variance.

Results

In Vitro Fungicide Sensitivity Screening

Fungicide screening assays using the spiral gradient dilution endpoint method were conducted on 81 isolates representing 17 *Fusarium* species (Table 4-1). Species from the *Fusarium tricinctum* species complex (*F. acuminatum, F. avenaceum, F. flocciferum*, *F. gamsii* and *F. iranicum*) were all sensitive to all three fungicides, except for one *F. acuminatum* isolate in one thiabendazole test (Table 4-1).

Difenoconazole

For all tests, no resistance as defined by this method was detected for difenoconazole. Difenoconazole resistance was not observed in any of the 33 *F. sambucinum* isolates tested (Table 4-1). Most *F. sambucinum* isolates were sensitive to difenoconazole in both experiments (Table 4-1). Interestingly, both lab-maintained *F. sambucinum* isolates (FID 212 and FID 71-6) exhibited reduced sensitivity to difenoconazole and had the greatest EC_{50} values for tested *F. sambucinum* isolates across both experiments (7.5 to 17.8 mg/L), in contrast to all other *F. sambucinum* isolates tested, which were recently isolated from tubers (Table 4-1).

All thirteen *F. oxysporum* isolates tested were sensitive to difenoconazole or had slightly reduced sensitivity with EC_{50} values less than 8 mg/L (Table 4-1). Two species from the *F. solani* species complex, *F. mori* and *F. stercicola*, exhibited reduced sensitivity to difenoconazole with EC_{50} values ranging from 10.3 to 99.4 mg/L (Table 4-1). Three isolates belonging to the *F. incarnatum-equiseti* species complex (one *F. toxicum* and two *F. equiseti*) showed reduced sensitivity to difenoconazole with EC_{50} values ranging from 5.0 to 20.5 mg/L (Table 4-1). Of four isolates of *F. redolens*, one was sensitive to difenoconazole while the other three exhibited reduced sensitivity with EC_{50} values ranging from 5.3 to 14.9 mg/L. Four of five *F. cerealis* isolates were sensitive to difenoconazole but only one of five *F. culmorum* isolates was difenoconazole sensitive (Table 4-1). Two *F. coeruleum* isolates exhibited reduced sensitivity to difenoconazole with EC_{50} values ranging from 7.5 to 12.5 mg/L (Table 4-1).

Fludioxonil

Of 32 *F. sambucinum* isolates tested, 20 isolates from Idaho exhibited *in vitro* resistance to fludioxonil (Table 4-1). Therefore, 77% of Idaho *F. sambucinum* isolates (20 of 26 isolates) obtained from a recent PNW dry rot survey were fludioxonil-resistant in the lab (Table 4-1). All five Washington isolates (100%) were fludioxonil-sensitive while only six of 26 Idaho isolates (23%) were sensitive (Table 4-1). Notably, no *F. sambucinum* isolates had EC_{50} values within the range of fungicide concentrations on the fungicide-amended plates, i.e., all isolates had EC_{50} values < 0.9 mg/L or > 506.9 mg/L (Table 4-1).

Thirteen *F. oxysporum* isolates were tested for fludioxonil sensitivity with seven isolates exhibiting resistance (Table 4-1). From the *F. solani* species complex, the *F. mori* isolate was fludioxonil-resistant, and the *F. stercicola* isolate was fludioxonil-sensitive (Table 4-1). From the *F. incarnatum-equiseti* species complex, two *F. equiseti* isolates were fludioxonil-sensitive while the *F. toxicum* isolate had slightly reduced sensitivity in one test (Table 4-1). All four tested isolates of *F. redolens* were fludioxonil-resistant (Table 4-1). Isolates of other species from the *F. sambucinum* species complex (*F. cerealis, F. culmorum, F. graminearum*, *F. venenatum*) were all sensitive to fludioxonil (Table 4-1). Two *F. coeruleum* isolates were tested for fungicide sensitivity, one from Idaho and one lab-maintained isolate (MR-6). The lab isolate was sensitive to fludioxonil while the Idaho isolate was resistant (Table 4-1).

Thiabendazole

Of 32 *F. sambucinum* isolates tested, 23 Idaho isolates were found to be resistant to thiabendazole. Thiabendazole sensitivity was observed in only four Idaho isolates and all five Washington isolates

(Table 4-1). In most cases, *F. sambucinum* isolates resistant to fludioxonil were also resistant to thiabendazole, except for C011 (Table 4-1). All thirteen *F. oxysporum* isolates tested were sensitive to thiabendazole or had slightly reduced sensitivity with EC_{50} values ranging from 5.7 to 16.9 mg/L (Table 4-1). From the *F. solani* species complex, both *F. mori* and *F. stercicola* were thiabendazole-sensitive (Table 4-1). From the *F. incarnatum-equiseti* species complex, two *F. equiseti* isolates and one *F. toxicum* were thiabendazole-sensitive (Table 4-1). All *F. redolens* isolates showed reduced sensitivity to thiabendazole with EC_{50} values ranging from 7.5 to 18.4 mg/L (Table 4-1). Greater thiabendazole sensitivity was observed for *F. cerealis* and *F. venenatum* than for *F. culmorum* and *F. graminearum* (Table 4-1). Both tested *F. coeruleum* isolates exhibited reduced sensitivity to thiabendazole with EC_{50} values ranging from 5.9 to 21.0 mg/L (Table 4-1).

Table 4-1. EC_{50} values of *in vitro* fungicide sensitivity screening experiments on *Fusarium* species isolated from potato tubers collected from seed and commercial storages in the Pacific Northwest states of Idaho, Oregon and Washington using the spiral gradient dilution method [a].

			EC_{50} [b] (mg/L)							
Isolate	***Fusarium***	**State of**	Difenoconazole [e, f]		Fludioxonil [e, g]				Thiabendazole [e, h]	
code	**species [c]**	**origin [d]**	*Exp [i] 1*	*Exp 2*	*Exp 1*	*Exp 2*	*Exp 3*	*Exp 4*	*Exp 1*	*Exp 2*
B057	*acuminatum*	ID	<0.9 (S)	1.64	<0.9 (S)	<0.9 (S)	-	-	4.8	5.4
D096	*acuminatum*	ID	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	2.7	3.1
E099	*acuminatum*	WA	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
A020	*avenaceum*	ID	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	2.8	4.3
C013	*avenaceum*	WA	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
E073	*cerealis*	ID	4.7	2.3	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
F044	*cerealis*	ID	6.8	11.4	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
B089	*cerealis*	WA	<0.9 (S)	4.6	>506.9 (R)	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	2.2
E011	*cerealis*	WA	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
E074	*cerealis*	ID	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
E090	*cerealis*	WA	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
B085	*coeruleum*	ID	7.5	12.5	>506.9 (R)	>506.9 (R)	-	-	17.0	21.0
MR-6	*coeruleum*	ID; Lab	4.7	7.7	<0.9 (S)	<0.9 (S)	-	-	5.9	16.3
C021	*culmorum*	ID	<0.9 (S)	3.8	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	2.4
C050	*culmorum*	ID	6.4	5.9	<0.9 (S)	<0.9 (S)	-	-	1.7	3.3
I002	*culmorum*	OR	14.8	7.5	<0.9 (S)	<0.9 (S)	-	-	9.8	3.4
I023	*culmorum*	OR	9.9	6.5	<0.9 (S)	<0.9 (S)	-	-	10.2	2.9
B010	*culmorum*	WA	7.6	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	9.4	3.8
A029	*equiseti*	ID	12.5	19.9	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
B059	*equiseti*	ID	13.9	20.5	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	4.6
C056	*flocciferum*	ID	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	1.7	1.7
C027	*gamsii*	ID	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	2.1	1.2
C028	*gamsii*	ID	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	2.5
G098	*graminearum*	ID	5.1	4.9	<0.9 (S)	<0.9 (S)	-	-	9.1	4.3
C035	*iranicum*	ID	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	1.4	<0.9 (S)
B032	*mori*	ID	10.7	99.4	>506.9 (R)	>506.9 (R)	-	-	<0.9 (S)	<0.9 (S)
B028	*oxysporum*	ID	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
B083	*oxysporum*	ID	<0.9 (S)	<0.9 (S)	291	204	-	-	2.0	2.8
D098	*oxysporum*	ID	<0.9 (S)	<0.9 (S)	>506.9 (R)	<0.9 (S)	<0.9 (S)	<0.9 (S)	1.6	<0.9 (S)
E028	*oxysporum*	ID	<0.9 (S)	1.8	>506.9 (R)	159	<0.9 (S)	>506.9 (R)	3.4	3.4
I053	*oxysporum*	ID	6.2	3.0	<0.9 (S)	<0.9 (S)	-	-	6.7	6.4
I005	*oxysporum*	OR	7.9	1.9	>506.9 (R)	467	-	-	16.9	7.1
I021	*oxysporum*	OR	<0.9 (S)	5.4	<0.9 (S)	<0.9 (S)	-	-	15.8	9.4
E023	*oxysporum*	WA	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
E080	*oxysporum*	WA	1.3	<0.9 (S)	>506.9 (R)	>506.9 (R)	-	-	<0.9 (S)	<0.9 (S)
E082	*oxysporum*	WA	<0.9 (S)	1.4	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
E083	*oxysporum*	WA	<0.9 (S)	1.7	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
I008	*oxysporum*	WA	5.6	5.2	>506.9 (R)	>506.9 (R)	-	-	7.2	4.3
I009	*oxysporum*	WA	<0.9 (S)	<0.9 (S)	>506.9 (R)	>506.9 (R)	-	-	5.7	6.2
I019	*redolens*	ID	5.3	5.7	>506.9 (R)	>506.9 (R)	-	-	15.4	7.8
I030	*redolens*	ID	4.8	11.0	>506.9 (R)	>506.9 (R)	-	-	18.4	8.0
I007	*redolens*	OR	14.9	11.0	>506.9 (R)	inconclusive	-	-	18.4	9.8
I028	*redolens*	OR	4.1	3.2	>506.9 (R)	>506.9 (R)	-	-	17.9	7.5
A031	*sambucinum*	ID	<0.9 (S)	4.4	<0.9 (S)	<0.9 (S)	-	-	>506.9 (R)	>506.9 (R)
A035	*sambucinum*	ID	<0.9 (S)	2.9	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	>506.9 (R)
A038	*sambucinum*	ID	2.1	7.9	>506.9 (R)	>506.9 (R)	-	-	404	>506.9 (R)
A054	*sambucinum*	ID	<0.9 (S)	<0.9 (S)	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
B097	*sambucinum*	ID	<0.9 (S)	4.1	<0.9 (S)	<0.9 (S)	-	-	>506.9 (R)	>506.9 (R)
C009	*sambucinum*	ID	<0.9 (S)	2.1	>506.9 (R)	>506.9 (R)	-	-	170	>506.9 (R)
C011	*sambucinum*	ID	<0.9 (S)	2.0	>506.9 (R)	>506.9 (R)	-	-	<0.9 (S)	<0.9 (S)
C030	*sambucinum*	ID	<0.9 (S)	2.0	>506.9 (R)	456	>506.9 (R)	>506.9 (R)	>506.9 (R)	408
C049	*sambucinum*	ID	<0.9 (S)	2.3	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	>506.9 (R)
C081	*sambucinum*	ID	2.3	1.6	404	>506.9 (R)	>506.9 (R)	>506.9 (R)	179	>506.9 (R)
C084	*sambucinum*	ID	<0.9 (S)	2.2	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	>506.9 (R)
C086	*sambucinum*	ID	<0.9 (S)	4.6	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	>506.9 (R)
C090	*sambucinum*	ID	12.8	2.8	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	>506.9 (R)
C100	*sambucinum*	ID	<0.9 (S)	<0.9 (S)	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	335
D018	*sambucinum*	ID	<0.9 (S)	<0.9 (S)	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	>506.9 (R)
D024	*sambucinum*	ID	<0.9 (S)	2.2	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	>506.9 (R)
D028	*sambucinum*	ID	<0.9 (S)	4.6	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	2.2

Table 4-1 continued on next page

Table 4-1, continued

Isolate code	*Fusarium* species [c]	State of origin [d]	EC_{50} [b] (mg/L)							
			Difenoconazole [e, f]		Fludioxonil [e, g]				Thiabendazole [e, h]	
			Exp [i] 1	*Exp 2*	*Exp 1*	*Exp 2*	*Exp 3*	*Exp 4*	*Exp 1*	*Exp 2*
D050	*sambucinum*	ID	1.4	2.5	>506.9 (R)	97.4	> 506.9 (R)	-	43.9	162
D062	*sambucinum*	ID	12.5	2.6	>506.9 (R)	>506.9 (R)	-	-	249	>506.9 (R)
D064	*sambucinum*	ID	<0.9 (S)	1.5	>506.9 (R)	>506.9 (R)	-	-	112	>506.9 (R)
D077	*sambucinum*	ID	3.8	1.7	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	>506.9 (R)
D083	*sambucinum*	ID	3.0	<0.9 (S)	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	216
E041	*sambucinum*	ID	<0.9 (S)	<0.9 (S)	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	>506.9 (R)
E051	*sambucinum*	ID	<0.9 (S)	3.9	>506.9 (R)	>506.9 (R)	-	-	>506.9 (R)	>506.9 (R)
F060	*sambucinum*	ID	<0.9 (S)	4.4	<0.9 (S)	<0.9 (S)	-	-	>506.9 (R)	>506.9 (R)
F071	*sambucinum*	ID	<0.9 (S)	6.1	<0.9 (S)	<0.9 (S)	-	-	2.2	2.4
FID 212	*sambucinum*	ID; Lab	8.7	7.5	<0.9 (S)	<0.9 (S)	-	-	2.7	4.9
FID 71-6	*sambucinum*	ID; Lab	13.0	17.8	<0.9 (S)	<0.9 (S)	-	-	>506.9 (R)	>506.9 (R)
E089	*sambucinum*	WA	<0.9 (S)	3.8	<0.9 (S)	<0.9 (S)	-	-	1.6	<0.9 (S)
E100	*sambucinum*	WA	3.0	1.9	<0.9 (S)	<0.9 (S)	-	-	1.4	<0.9 (S)
F001	*sambucinum*	WA	2.0	2.6	<0.9 (S)	<0.9 (S)	-	-	1.7	<0.9 (S)
F004	*sambucinum*	WA	<0.9 (S)	1.7	<0.9 (S)	<0.9 (S)	-	-	2.3	<0.9 (S)
I010	*sambucinum*	WA	5.7	5.2	<0.9 (S)	<0.9 (S)	-	-	2.3	<0.9 (S)
E021	*stercicola*	WA	10.7	10.3	<0.9 (S)	<0.9 (S)	-	-	<0.9 (S)	<0.9 (S)
B088	*toxicum*	ID	<0.9 (S)	5.0	<0.9 (S)	9.8	-	-	<0.9 (S)	1.8
G002	*venenatum*	WA	5.2	3.8	<0.9 (S)	<0.9 (S)	-	-	1.9	<0.9 (S)

[a] Spiral gradient dilution method (Fairchild et al. 2013); the fungicide concentration range for testing was 0.9 to 506.9 mg/L.

[b] EC_{50} = 50% Effective Concentration; the fungicide concentration at which mycelial growth is inhibited by 50% as compared to control growth. EC_{50} values are derived from mycelial growth measurements and reported in milligrams per liter (mg/L). For results outside of the fungicide concentration range of testing, i.e., 0.9 to 506.9 mg/L, an "S" designates sensitivity for EC_{50} values less than 0.9 mg/L, and an "R" designates resistance for EC_{50} values greater than 506.9 mg/L.

[c] Species identity resolved with translation elongation factor 1-alpha (*TEF*) and/or phosphate permease (*PHO*) DNA sequences.

[d] State of origin: ID = Idaho; OR = Oregon; WA = Washington; Lab = lab-maintained isolate

[e] Fungicide percent active ingredient (ai): fludioxonil 40.3% ai, difenoconazole 23.2% ai, thiabendazole 42.3% ai.

[f] For difenoconazole tests, isolates were considered sensitive to difenoconazole using a threshold EC_{50} value of less than 5 mg/L to indicate sensitivity, following the threshold used by Gachango et al. (2012). Those isolates with EC_{50} values of 5 mg/L or higher were considered to have "reduced sensitivity" to difenoconazole in this study.

[g] For fludioxonil tests, isolates were considered sensitive to fludioxonil using a threshold EC_{50} value of less than 5 mg/L to indicate sensitivity, following the threshold used by Gachango et al. (2012). Isolates with EC_{50} values greater than 100 mg/L were considered to be resistant to fludioxonil, following the threshold used by Gachango et al. (2012) and Peters et al. (2008b).

[h] For thiabendazole tests, isolates were considered sensitive to thiabendazole using a threshold EC_{50} value of less than 5 mg/L to indicate sensitivity, following the threshold used by Gachango et al. (2012). Those isolates with EC_{50} values of 5 to 100 mg/L were considered to have "reduced sensitivity" to thiabendazole in this study. Isolates with EC_{50} values greater than 100 mg/L were considered to be resistant to thiabendazole, following the threshold used by Gachango et al. (2012).

[i] Exp = experiment. Data for Exp 1 were generated by the Wharton Lab and Exp 2, 3 and 4 by the Duellman Lab, respectively.

Fungicide Seed Treatment Experiment

Fusarium isolates were chosen as test treatments for all three fungicides based on the laboratory fungicide sensitivity results and are listed in Tables 4-2, 4-3 and 4-4.

Table 4-2. *Fusarium* isolates selected for *in vivo* **difenoconazole** sensitivity testing using the bag test method[a] designed to test efficacy of fungicide seed treatments on potato tubers.

***Fusarium* species**	**Treatment / isolate code**	**State of origin**	**EC_{50} (mg/L) [b]**
Non-treated, non-inoculated	NTC-NI	n/a	n/a
Non-treated, mock-inoculated with water	NTC-MI-Water	n/a	n/a
Non-treated, inoculated with *F. sambucinum*	NTC-I FID 71-6	ID; Lab	15.4
Treated, inoculated with *F. sambucinum*	TC-I FID 71-6	ID; Lab	15.4
Non-treated, inoculated with *F. sambucinum*	NTC-I A054	ID	< 0.9 (S)
Treated, inoculated with *F. sambucinum*	TC-I A054	ID	< 0.9 (S)
F. cerealis	E011	WA	< 0.9 (S)
F. cerealis	F044	ID	9.1
F. cerealis	E074	ID	< 0.9 (S)
F. culmorum	C050	ID	6.2
F. culmorum	I002	OR	11.2
F. oxysporum	B028	ID	< 0.9 (S)
F. oxysporum	E023	WA	< 0.9 (S)
F. oxysporum	I053	ID	4.6 (S)
F. oxysporum	I005	OR	4.9 (S)
F. oxysporum	I021	OR	3.1 (S)
F. oxysporum	I008	WA	5.4
F. redolens	I007	OR	12.9
F. redolens	I028	OR	3.6 (S)
F. sambucinum	C100	ID	< 0.9 (S)
F. sambucinum	I010	WA	5.5
F. sambucinum	A038	ID	5.0
F. sambucinum	C090	ID	7.8
F. sambucinum	D062	ID	7.5

Abbreviations: *F.* = *Fusarium*; NTC = non-treated control; NI = non-inoculated; MI = mock-inoculated; I = inoculated; TC = treated control; ID = Idaho; OR = Oregon; WA = Washington; Lab = lab-maintained isolate; S = sensitive.

[a] The bag test method is referenced in Nolte (1994) and Bohl, Nolte and Thornton (1992).
[b] EC_{50} = 50% Effective Concentration, i.e., the fungicide concentration at which mycelial growth is inhibited by 50% as compared to control growth. EC_{50} values are derived from mycelial growth measurements in a laboratory screening of isolate sensitivity using fungicide-amended agar and reported in milligrams per liter (mg/L). Isolates were considered sensitive to difenoconazole using a threshold EC_{50} value of less than 5 mg/L to indicate sensitivity (S), following the threshold used by Gachango et al. (2012). Those isolates with EC_{50} values of 5 mg/L or higher were considered to have "reduced sensitivity" to difenoconazole.

Table 4-3. *Fusarium* isolates selected for *in vivo* **fludioxonil** sensitivity testing using the bag test method[a] designed to test efficacy of fungicide seed treatments on potato tubers.

***Fusarium* species**	**Treatment / isolate code**	**State of origin**	**EC_{50} (mg/L) [b]**
Non-treated, non-inoculated	NTC-NI	n/a	n/a
Non-treated, mock-inoculated with water	NTC-MI-Water	n/a	n/a
Non-treated, inoculated with *F. sambucinum*	NTC-I FID 71-6	Lab	< 0.9 (S)
Treated, inoculated with *F. sambucinum*	TC-I FID 71-6	Lab	< 0.9 (S)
F. oxysporum	B028	ID	< 0.9 (S)
F. oxysporum	B083	ID	248 (R)
F. oxysporum	I021	OR	< 0.9 (S)
F. oxysporum	I005	OR	487 (R)
F. oxysporum	E023	WA	< 0.9 (S)
F. oxysporum	I009	WA	> 506.9 (R)
F. sambucinum	F060	ID	< 0.9 (S)
F. sambucinum	E089	WA	< 0.9 (S)
F. sambucinum	C011	ID	> 506.9 (R)
F. sambucinum	C009	ID	> 506.9 (R)
F. sambucinum	D077	ID	> 506.9 (R)
F. sambucinum	D083	ID	> 506.9 (R)
F. sambucinum	C081	ID	456 (R)
F. sambucinum	C030	ID	481 (R)

Abbreviations: *F.* = *Fusarium*; NTC = non-treated control; NI = non-inoculated; MI = mock-inoculated; I = inoculated; TC = treated control; ID = Idaho; OR = Oregon; WA = Washington; Lab = lab-maintained isolate; S = sensitive; R = resistant.

[a] The bag test method is referenced in Nolte (1994) and Bohl, Nolte and Thornton (1992).
[b] EC_{50} = 50% Effective Concentration, i.e., the fungicide concentration at which mycelial growth is inhibited by 50% as compared to control growth. EC_{50} values are derived from mycelial growth measurements in a laboratory screening of isolate sensitivity using fungicide-amended agar and reported in milligrams per liter (mg/L). Isolates with EC_{50} values greater than 100 mg/L were considered to be resistant (R) to fludioxonil, following the threshold used by Gachango et al. (2012) and Peters et al. (2008b). For this study, isolates with EC_{50} values less than 0.9 mg/L were considered to be sensitive (S) to fludioxonil.

Table 4-4. *Fusarium* isolates selected for *in vivo* **thiabendazole** sensitivity testing using the bag test method[a] designed to test efficacy of fungicide seed treatments on potato tubers.

***Fusarium* species**	**Treatment / isolate code**	**State of origin**	**EC_{50} (mg/L) [b]**
Non-treated, non-inoculated	NTC-NI	n/a	n/a
Non-treated, mock-inoculated with water	NTC-MI-Water	n/a	n/a
Non-treated, inoculated with *F. sambucinum*	NTC-I FID 71-6	Lab	> 506.9 (R)
Treated, inoculated with *F. sambucinum*	TC-I FID 71-6	Lab	> 506.9 (R)
Non-treated, inoculated with *F. sambucinum*	NTC-I A054	ID	< 0.9 (S)
Treated, inoculated with *F. sambucinum*	TC-I A054	ID	< 0.9 (S)
F. sambucinum	C011	ID	< 0.9 (S)
F. sambucinum	E089	WA	1.3 (S)
F. sambucinum	D050	ID	103 (R)
F. sambucinum	D062	ID	378 (R)
F. sambucinum	F060	ID	> 506.9 (R)
F. sambucinum	C084	ID	> 506.9 (R)
F. redolens	I007	OR	14.1
F. oxysporum	B028	ID	< 0.9 (S)
F. oxysporum	I053	ID	6.5
F. oxysporum	I021	OR	12.6
F. oxysporum	E023	WA	< 0.9 (S)
F. oxysporum	I009	WA	6.0
F. cerealis	E074	ID	< 0.9 (S)
F. culmorum	I023	OR	6.5

Abbreviations: *F.* = *Fusarium*; NTC = non-treated control; NI = non-inoculated; MI = mock-inoculated; I = inoculated; TC = treated control; ID = Idaho; OR = Oregon; WA = Washington; Lab = lab-maintained isolate; S = sensitive; R = resistant.

[a] The bag test method is referenced in Nolte (1994) and Bohl, Nolte and Thornton (1992).
[b] EC_{50} = 50% Effective Concentration, i.e., the fungicide concentration at which mycelial growth is inhibited by 50% as compared to control growth. EC_{50} values are derived from mycelial growth measurements in a laboratory screening of isolate sensitivity using fungicide-amended agar and reported in milligrams per liter (mg/L). Isolates were considered sensitive (S) to thiabendazole using a threshold EC_{50} value of less than 5 mg/L, following the threshold used by Gachango et al. (2012). Isolates with EC_{50} values of 5 mg/L or higher were considered to have "reduced sensitivity" to thiabendazole. Isolates with EC_{50} values greater than 100 mg/L were considered to be resistant (R) to thiabendazole, following the threshold used by Gachango et al. (2012).

Difenoconazole

There were no differences in dry rot severity among all treatments except for higher severity for the non-treated control inoculated with *F. sambucinum* isolate FID 71-6 (Table 4-5). Statistically, for all treatments but one (NTC-I FID 71-6), dry rot incidence was the same as the background level treatment, i.e., NTC-NI, when difenoconazole was used (Table 4-5). Difenoconazole appeared to be similarly effective at keeping dry rot incidence and severity near background levels for most isolates (Table 4-5).

The background level, i.e., NTC-NI, of Fusarium dry rot incidence and severity for the difenoconazole bag test was 19.5% and 0.7%, respectively (Table 4-5). The mock-inoculated control, i.e., NTC-MI, was similar to background levels, demonstrating that the inoculation method did not cause dry rot beyond the background level (Table 4-5). Control treatments included an isolate of *F. sambucinum* with reduced sensitivity to difenoconazole (FID 71-6) and one with difenoconazole sensitivity (A054). Both isolates were non-treated (no fungicide application) and treated (difenoconazole applied). For the isolate with reduced sensitivity (FID 71-6), the non-treated, i.e., NTC-I FID 71-6, confirmed that the inoculation method was successful, resulting in dry rot infection well above background levels (Table 4-5). The treated, i.e., TC-I FID 71-6 in which difenoconazole was applied had incidence and severity lower than background levels, demonstrating difenoconazole sensitivity of this isolate, successful fungicide application and fungicide efficacy (Table 4-5). For the difenoconazole-sensitive isolate (A054), when the fungicide treatment was not applied, dry rot infection was statistically similar to background levels (Table 4-5). Isolate A054, when treated with difenoconazole, incidence and severity as compared to the non-treated isolate did not differ (Table 4-5). Overall, the difenoconazole-sensitive control isolate A054 was shown to be a less aggressive *F. sambucinum* isolate than FID 71-6 in the absence of the fungicide, but when difenoconazole was used, similar dry rot incidence and severity was observed for both isolates.

Table 4-5. Effect of **difenoconazole** seed piece treatment on dry rot incidence and severity on potato seed pieces inoculated with different species of *Fusarium* isolates that exhibited sensitivity or reduced sensitivity to difenoconazole [a] *in vitro*. [bc]

Treatment or isolate code	*Fusarium* species	*In vitro* EC_{50} (mg/L) [d]	Mean dry rot incidence (%) [e]		Mean dry rot severity (%) [e]	
NTC-NI	Non-treated control, non-inoculated	n/a	19.5	bcde	0.7	b
NTC-MI	Non-treated control, mock-inoculated	n/a	22.5	bcde	0.8	b
NTC-I FID 71-6	*Fusarium sambucinum*	15.4	65.2	a	10.7	a
TC-I FID 71-6	*Fusarium sambucinum*	15.4	13.4	e	0.4	b
NTC-I A054	*Fusarium sambucinum*	< 0.9 (S)	27.3	bcd	1.8	b
TC-I A054	*Fusarium sambucinum*	< 0.9 (S)	19.9	bcde	0.4	b
E011	*Fusarium cerealis*	< 0.9 (S)	20.7	bcde	0.4	b
F044	*Fusarium cerealis*	9.1	19.5	bcde	0.4	b
E074	*Fusarium cerealis*	< 0.9 (S)	14.6	de	0.4	b
C050	*Fusarium culmorum*	6.2	29.1	b	1.3	b
I002	*Fusarium culmorum*	11.2	28.2	bc	0.8	b
B028	*Fusarium oxysporum*	< 0.9 (S)	23.0	bcde	0.7	b
E023	*Fusarium oxysporum*	< 0.9	17.5	bcde	0.4	b
I053	*Fusarium oxysporum*	4.6	18.7	bcde	0.4	b
I005	*Fusarium oxysporum*	4.9	22.8	bcde	0.6	b
I021	*Fusarium oxysporum*	3.1	17.5	bcde	0.4	b
I008	*Fusarium oxysporum*	5.4	16.1	cde	0.4	b
I007	*Fusarium redolens*	12.9	16.6	bcde	0.3	b
I028	*Fusarium redolens*	3.6	12.7	e	0.4	b
C100	*Fusarium sambucinum*	< 0.9 (S)	19.3	bcde	0.6	b
I010	*Fusarium sambucinum*	5.5	15.7	cde	0.6	b
A038	*Fusarium sambucinum*	5.0	18.1	bcde	0.6	b
C090	*Fusarium sambucinum*	7.8	27.1	bcd	1.2	b
D062	*Fusarium sambucinum*	7.5	19.3	bcde	0.4	b

Abbreviations: NTC = non-treated control in which no fungicide seed treatment was applied; NI = non-inoculated control that was not inoculated with *Fusarium*; MI = mock-inoculated with distilled water); I = inoculated with *Fusarium* isolate; TC = treated control in which fungicide seed treatment was applied; FID 71-6 = inoculated with *F. sambucinum* isolate FID 71-6; FID 212 = inoculated with *F. sambucinum* isolate FID 212

[a] Difenoconazole was applied as the commercial formulation Inspire, Syngenta.
[b] *In vitro* fungicide sensitivity screening was conducted using the spiral gradient dilution method according to the protocol described in Fairchild et al. (2013).
[c] The seed piece treatment experimental method was based on the "bag test" protocol described by Nolte (1994).
[d] Isolates were considered sensitive to difenoconazole using a threshold EC_{50} value of less than 5 mg/L to indicate sensitivity, following the threshold used by Gachango et al. (2012). Those isolates with EC_{50} values of 5 mg/L or higher were considered to have "reduced sensitivity" to

difenoconazole. EC_{50} = 50% Effective Concentration, i.e., the fungicide concentration at which mycelial growth is inhibited by 50% as compared to control growth. EC_{50} values are derived from mycelial growth measurements in a laboratory screening of isolate sensitivity using fungicide-amended agar and reported in milligrams per liter (mg/L). For results outside of the fungicide concentration range of testing, i.e., 0.9 to 506.9 mg/L, an "S" designates sensitivity for EC_{50} values less than 0.9 mg/L, and an "R" designates resistance for EC_{50} values greater than 506.9 mg/L.

[e] Means within columns followed by the same letter do not significantly differ based on pair-wise comparisons (alpha = 0.05.)

Fludioxonil

In the presence of fludioxonil, both fludioxonil-sensitive *F. sambucinum* isolates (E089 and F060) had similar dry rot incidence and severity to background levels, indicating that the seed piece treatment was effective in reducing dry rot (Table 4-6). Four of six fludioxonil-resistant *F. sambucinum* isolates had greater dry rot incidence and severity than background levels, indicating that the seed piece treatment was not effective in reducing dry rot in the presence of resistant isolates (Table 4-6). Two *F. sambucinum* isolates (D083 and C081) exhibiting fludioxonil resistance *in vitro* appeared to be inhibited by the fludioxonil treatment as shown by lower incidence and severity, similar to background levels and contrary to the expected result (Table 4-6).

Dry rot incidence observed among five of six *F. oxysporum* isolates did not differ from background levels (Table 4-6). However, one *F. oxysporum* isolate (I021) had lower dry rot incidence and was inhibited by fludioxonil below background level incidence (Table 4-6). No difference in dry rot severity was observed among five of six *F. oxysporum* isolates as compared to background levels. However, one fludioxonil-resistant isolate (I009) had significantly greater severity than three of the other isolates (Table 4-6).

The background level, i.e., NTC-NI, of dry rot incidence and severity for the fludioxonil bag test was 51% and 3.8%, respectively (Table 4-6). The mock-inoculated control, i.e., NTC-MI, was similar to background levels, demonstrating that the inoculation method did not cause dry rot (Table 4-6). For the fludioxonil-sensitive control isolate FID 71-6, non-treated, i.e., NTC-I FID 71-6, confirmed that the inoculation method was successful, resulting in dry rot infection well above background levels (Table 4-3). Treated, i.e., TC-I FID 71-6, confirmed that fungicide application was successful and that the fungicide solution effectively lowered incidence and severity as compared to the non-treated (Table 4-6).

Table 4-6. Effect of **fludioxonil** seed piece treatment on dry rot incidence and severity on potato seed pieces inoculated with isolates of *Fusarium sambucinum* and *Fusarium oxysporum* that exhibited sensitivity or resistance to fludioxonil [a] *in vitro*. [bc]

Treatment or isolate code	***Fusarium* species**	***In vitro* EC_{50} (mg/L) [d]**	**Mean dry rot incidence (%) [e]**	**Mean dry rot severity (%) [e]**
NTC-NI	Non-treated control, non-inoculated	n/a	50.6 b	3.8 cde
NTC-MI	Non-treated control, mock-inoculated	n/a	53.9 b	3.3 de
NTC-I FID 71-6	*Fusarium sambucinum*	< 0.9 (S)	80.2 a	8.7 a
TC-I FID 71-6	*Fusarium sambucinum*	< 0.9 (S)	48.0 bc	1.9 de
B028	*Fusarium oxysporum*	< 0.9 (S)	46.6 bc	1.3 e
B083	*Fusarium oxysporum*	248	42.8 bc	1.3 e
I021	*Fusarium oxysporum*	< 0.9 (S)	32.6 c	1.3 e
I005	*Fusarium oxysporum*	487	54.5 b	2.8 de
E023	*Fusarium oxysporum*	< 0.9 (S)	48.0 bc	2.0 de
I009	*Fusarium oxysporum*	> 506.9 (R)	49.5 b	4.6 bcd
F060	*Fusarium sambucinum*	< 0.9 (S)	39.3 bc	1.4 e
E089	*Fusarium sambucinum*	< 0.9 (S)	44.4 bc	1.5 e
C011	*Fusarium sambucinum*	> 506.9 (R)	73.5 a	8.9 a
C009	*Fusarium sambucinum*	> 506.9 (R)	74.7 a	7.6 ab
D077	*Fusarium sambucinum*	> 506.9 (R)	82.4 a	8.7 a
D083	*Fusarium sambucinum*	> 506.9 (R)	50.6 b	1.3 e
C081	*Fusarium sambucinum*	456	43.0 bc	1.4 e
C030	*Fusarium sambucinum*	481	74.6 a	6.7 abc

Abbreviations: NTC = non-treated control in which no fungicide seed treatment was applied; NI = non-inoculated control that was not inoculated with *Fusarium*; MI = mock-inoculated with distilled water); I = inoculated with *Fusarium* isolate; TC = treated control in which fungicide seed treatment was applied; FID 71-6 = inoculated with *F. sambucinum* isolate FID 71-6

[a] Fludioxonil was applied as the commercial formulation Maxim4FS, Syngenta.

[b] *In vitro* fungicide sensitivity screening was conducted using the spiral gradient dilution method according to the protocol described in Fairchild et al. (2013).

[c] The seed piece treatment experimental method was based on the "bag test" protocol described by Nolte (1994).

[d] Isolates were considered sensitive to fludioxonil using a threshold EC_{50} value of less than 5 mg/L to indicate sensitivity, following the threshold used by Gachango et al. (2012). Isolates with EC_{50} values greater than 100 mg/L were considered resistant to fludioxonil, following the threshold used by Gachango et al. (2012) and Peters et al. (2008b). EC_{50} = 50% Effective Concentration, i.e., the fungicide concentration at which mycelial growth is inhibited by 50% as compared to control growth. EC_{50} values are derived from mycelial growth measurements in a laboratory screening of isolate sensitivity using fungicide-amended agar and reported in milligrams per liter (mg/L). For results outside of the fungicide concentration range of testing, i.e., 0.9 to 506.9 mg/L, an "S" designates sensitivity for EC_{50} values less than 0.9 mg/L, and an "R" designates resistance for EC_{50} values greater than 506.9 mg/L.

[e] Means within columns followed by the same letter do not significantly differ based on pair-wise comparisons (alpha = 0.05.)

Thiabendazole

Two thiabendazole-sensitive *F. sambucinum* isolates (C011 and E089) were inhibited by thiabendazole applied as seed piece treatment as shown by dry rot incidence and severity levels at or below background (Table 4-7). Thiabendazole application did not reduce dry rot incidence and severity for three thiabendazole-resistant *F. sambucinum* isolates (D050, F060, C084) (Table 4-7). A fourth thiabendazole-resistant *F. sambucinum* isolate (D062), contrary to the expected result, was actually inhibited by the thiabendazole treatment as shown by incidence and severity similar to background levels (Table 4-7).

The background level, i.e., NTC-NI, of dry rot incidence and severity for the thiabendazole bag test was 58.9% and 2.8%, respectively (Table 4-7). The mock-inoculated control, i.e., NTC-MI, was similar to background levels, demonstrating that the inoculation method did not cause dry rot beyond background levels (Table 4-7). Control treatments were included for both a thiabendazole-resistant (FID 71-6) and a thiabendazole-sensitive (A054) isolate of a known pathogenic species *Fusarium sambucinum*, including both non-treated without fungicide application and treated with thiabendazole applied for each isolate. For the thiabendazole-resistant isolate FID 71-6, non-treated, i.e., NTC-I FID 71-6, confirmed that the inoculation method was successful, resulting in dry rot infection above background levels (Table 4-7). Treated, i.e., TC-I FID 71-6, had similar incidence to non-treated but lower severity (Table 4-7). Therefore, the FID 71-6 isolate demonstrated resistance to thiabendazole but the fungicide was able to decrease disease severity to some extent (Table 4-7). For the thiabendazole-sensitive isolate (A054), non-treated, i.e., NTC-I A054, confirmed that the inoculation method was successful, resulting in dry rot infection above background levels (Table 4-7). Treated, i.e., TC-I A054, confirmed that fungicide application was successful and the fungicide solution was effective with significantly lower incidence and severity as compared to the non-treated. Also, compared to background, TC-I A054 dry rot incidence was 25% less and severity was 50% less (Table 4-7).

Table 4-7. Effect of **thiabendazole** seed piece treatment on dry rot incidence and severity on potato seed pieces inoculated with different species of *Fusarium* isolates that exhibited differing sensitivity to thiabendazole [a] *in vitro*. [bc]

Treatment or isolate code	***Fusarium* species**	***In vitro* EC_{50} (mg/L) [d]**	**Mean dry rot incidence (%) [e]**		**Mean dry rot severity (%) [e]**	
NTC-NI	Non-treated control, non-inoculated	n/a	58.9	bcd	2.8	ed
NTC-MI	Non-treated control, mock-inoculated	n/a	49.7	def	2.4	ed
NTC-I FID 71-6	*Fusarium sambucinum*	> 506.9 (R)	79.3	a	13.2	a
TC-I FID 71-6	*Fusarium sambucinum*	> 506.9 (R)	71.3	abc	5.1	cd
NTC-I A054	*Fusarium sambucinum*	< 0.9 (S)	75.2	ab	6.1	c
TC-I A054	*Fusarium sambucinum*	< 0.9 (S)	34.3	f	1.3	e
C011	*Fusarium sambucinum*	< 0.9 (S)	53.5	cde	2.4	ed
E089	*Fusarium sambucinum*	1.3	38.5	ef	1.6	e
D050	*Fusarium sambucinum*	103	85.8	a	6.4	bc
D062	*Fusarium sambucinum*	378	45.0	def	1.1	e
F060	*Fusarium sambucinum*	> 506.9 (R)	87.7	a	9.5	b
C084	*Fusarium sambucinum*	> 506.9 (R)	81.5	a	8.1	bc
I007	*Fusarium redolens*	14.1	45.1	def	1.4	e
B028	*Fusarium oxysporum*	< 0.9 (S)	41.4	def	0.9	e
E023	*Fusarium oxysporum*	< 0.9 (S)	37.1	ef	0.9	e
I053	*Fusarium oxysporum*	6.5	45.3	def	1.5	e
I009	*Fusarium oxysporum*	6.0	46.8	def	1.4	e
I021	*Fusarium oxysporum*	12.6	48.5	def	1.3	e
E074	*Fusarium cerealis*	< 0.9 (S)	35.8	ef	1.0	e
I023	*Fusarium culmorum*	6.5	39.0	ef	1.1	e

Abbreviations: NTC = non-treated control in which no fungicide seed treatment was applied; NI = non-inoculated control that was not inoculated with *Fusarium*; MI = mock-inoculated with distilled water); I = inoculated with *Fusarium* isolate; TC = treated control in which fungicide seed treatment was applied; FID 71-6 = inoculated with *F. sambucinum* isolate FID 71-6

[a] Thiabendazole was applied as the commercial formulation Mertect 340F, Syngenta.

[b] *In vitro* fungicide sensitivity screening was conducted using the spiral gradient dilution method according to the protocol described in Fairchild et al. (2013).

[c] The seed piece treatment experimental method was based on the "bag test" protocol described by Nolte (1994).

[d] Isolates were considered sensitive to thiabendazole using a threshold EC_{50} value of less than 5 mg/L, following the threshold used by Gachango et al. (2012). Isolates with EC_{50} values of 5 to 100 mg/L were considered to have "reduced sensitivity" to thiabendazole. Isolates with EC_{50} values greater than 100 mg/L were considered resistant to thiabendazole, following the threshold used by Gachango et al. (2012). EC_{50} = 50% Effective Concentration, i.e., the fungicide concentration at which mycelial growth is inhibited by 50% as compared to control growth. EC_{50} values are derived from mycelial growth measurements in a laboratory screening of isolate sensitivity using fungicide-amended agar and reported in milligrams per liter (mg/L). For results outside of the fungicide concentration range of testing, i.e., 0.9 to 506.9 mg/L, an "S" designates sensitivity for EC_{50} values less than 0.9 mg/L, and an "R" designates resistance for EC_{50} values greater than 506.9 mg/L.

[e] Means within columns followed by the same letter do not significantly differ based on pair-wise comparisons (alpha = 0.05.)

Field Experiment for Fludioxonil Efficacy on Fusarium sambucinum in Idaho

There were no statistically significant differences in emergence rates (RAUEPC) among treatments, but a few fludioxonil-resistant isolates had slower emergence rates, e.g., C100, C011, C009 (Table 4-8; Figure 4-2). When a seed treatment containing only fludioxonil was applied, fludioxonil-sensitive isolates caused lower incidence and severity of seed decay as compared to fludioxonil-resistant isolates (Table 4-8; Figures 4-3 and 4-4). Yield for fludioxonil-sensitive isolates was greater than for fludioxonil-resistant isolates (Table 4-8; Figures 4-5 and 4-6). Although yield differences were not statistically different between most isolates, numerical differences in yield indicate a downward trend showing potentially greater yield loss for fludioxonil-resistant isolates compared to sensitive isolates when a fludioxonil seed treatment is used (Table 4-8; Figures 4-5 and 4-6).

Table 4-8. Combined data for a field experiment conducted in 2020 and 2021 using a fludioxonil fungicide seed treatment for Fusarium dry rot against *Fusarium sambucinum* isolates recovered from Idaho potato tuber samples.

Treatment or isolate code	RAUEPC [z q]	Seed piece decay incidence (%) [z]	Seed piece decay severity (%) [z]	US No. 1 yield, ≥ 133 grams, (metric tons/hectare) [z]	Total yield (metric tons/hectare) [z]	*In vitro* EC_{50} (mg/L) [p]
NTC-NI [y x]	16.4 a	46 fg	20 efgh	16.0 a	35.1 a	n/a [s]
NTC-I-716 [w v]	16.2 a	51 ef	33.1 bcde	14.5 ab	31.4 ab	< 0.9 (S)
NTC-I-212 [u]	15.6 a	97 a	49.7 ab	12.7 ab	29.8 ab	< 0.9 (S)
TC-I-716 [t]	16.0 a	18 h	4.4 h	15.3 a	32.6 ab	< 0.9 (S)
TC-I-212	16.3 a	32 fgh	8.3 fgh	14.7 ab	32.7 ab	< 0.9 (S)
F060	15.7 a	14 h	4.9 gh	14.5 ab	32.3 ab	< 0.9 (S)
D028	15.8 a	30 gh	8.4 fgh	14.0 ab	35.3 a	< 0.9 (S)
F071	15.4 a	28 gh	7.9 fgh	14.3 ab	33.4 ab	< 0.9 (S)
D077	15.2 a	67 de	25.5 defgh	13.2 ab	31.3 ab	>506.9 (R)
D050	15.1 a	79 abcd	19.8 efgh	15.4 a	32.4 ab	302.1
A038	15.7 a	75 cd	26.9 cdefg	14.2 ab	30.3 ab	>506.9 (R)
D083	15.7 a	27 gh	7.4 fgh	12.6 ab	32.2 ab	>506.9 (R)
D064	15.2 a	92 abc	38.0 bcde	13.1 ab	30.1 ab	>506.9 (R)
D062	15.9 a	80 abcd	26.6 cdefgh	12.9 ab	31.9 ab	>506.9 (R)
E041	15.9 a	86 abcd	52.2 ab	12.1 ab	31.7 ab	>506.9 (R)
C081	16.5 a	70 de	27.3 cdef	13.5 ab	31.5 ab	455.6
C086	15.1 a	96 ab	40.5 bcde	14.9 ab	31.6 ab	>506.9 (R)
D018	14.5 a	92 abc	45.9 abcd	12.5 ab	29.3 ab	>506.9 (R)
C030	16.4 a	95 abc	64.6 a	12.0 ab	29.2 ab	481.3
D024	14.6 a	94 abc	51.0 ab	11.2 ab	26.8 b	>506.9 (R)
C090	15.8 a	76 bcd	33.3 bcde	12.8 ab	31.5 ab	>506.9 (R)
E051	15.3 a	94 abc	52.7 ab	13.7 ab	30.8 ab	>506.9 (R)
C049	15.7 a	93 abc	46.0 abcd	9.7 b	28.8 ab	>506.9 (R)
A035	15.4 a	95 abc	45.9 abcd	12.9 ab	30 ab	>506.9 (R)
C100	13.9 a	94 abc	45.2 abcd	11.3 ab	29.0 ab	>506.9 (R)
C084	14.4 a	92 abc	54.8 ab	11.6 ab	28.0 b	>506.9 (R)
C011	14.0 a	91 abc	48.3 abc	12.2 ab	29.7 ab	>506.9 (R)
C009	14.0 a	92 abc	45.3 abcd	10.9 ab	27.4 b	>506.9 (R)
P > F [r]	0.1016	<0001	<0001	0010	0002	n/a
F value	1.39	55.42	18.24	2.20	2.46	n/a

[z] Values are estimated least square means for five replications per treatment. Least square means within the same column and followed by the same letter are not significantly different as determined by pairwise comparisons with the Tukey-Kramer multiple comparison adjustment (α = 0.05).

[y] NTC = non-treated control (no fungicide seed treatment applied)

[x] NI = non-inoculated control (not inoculated with *Fusarium*)

[w] I = inoculated with *Fusarium* isolate

[v] 716 = inoculated with *F. sambucinum* isolate FID 71-6, which has known fludioxonil sensitivity

[u] 212 = inoculated with *F. sambucinum* isolate FID 212, which has known fludioxonil sensitivity

[t] TC = treated control (fungicide seed treatment applied)

[s] n/a = not applicable

[r] P > F is the probability associated with the F value.

[q] Relative area under the emergence progress curve

[p] EC_{50} = 50% Effective Concentration, i.e., the fungicide concentration at which mycelial growth is inhibited by 50% as compared to control growth. EC_{50} values are derived from mycelial growth measurements in a laboratory screening of Fusarium isolate fungicide sensitivity using fungicide-amended agar and reported in milligrams per liter (mg/L). Laboratory screening was conducted using the spiral gradient dilution method according to the protocol described in Fairchild et al. (2013). For results outside of the fungicide concentration range of testing, i.e., 0.9 to 506.9 mg/L, an "S" designates sensitivity for EC_{50} values less than 0.9 mg/L, and an "R" designates resistance for EC_{50} values greater than 506.9 mg/L.

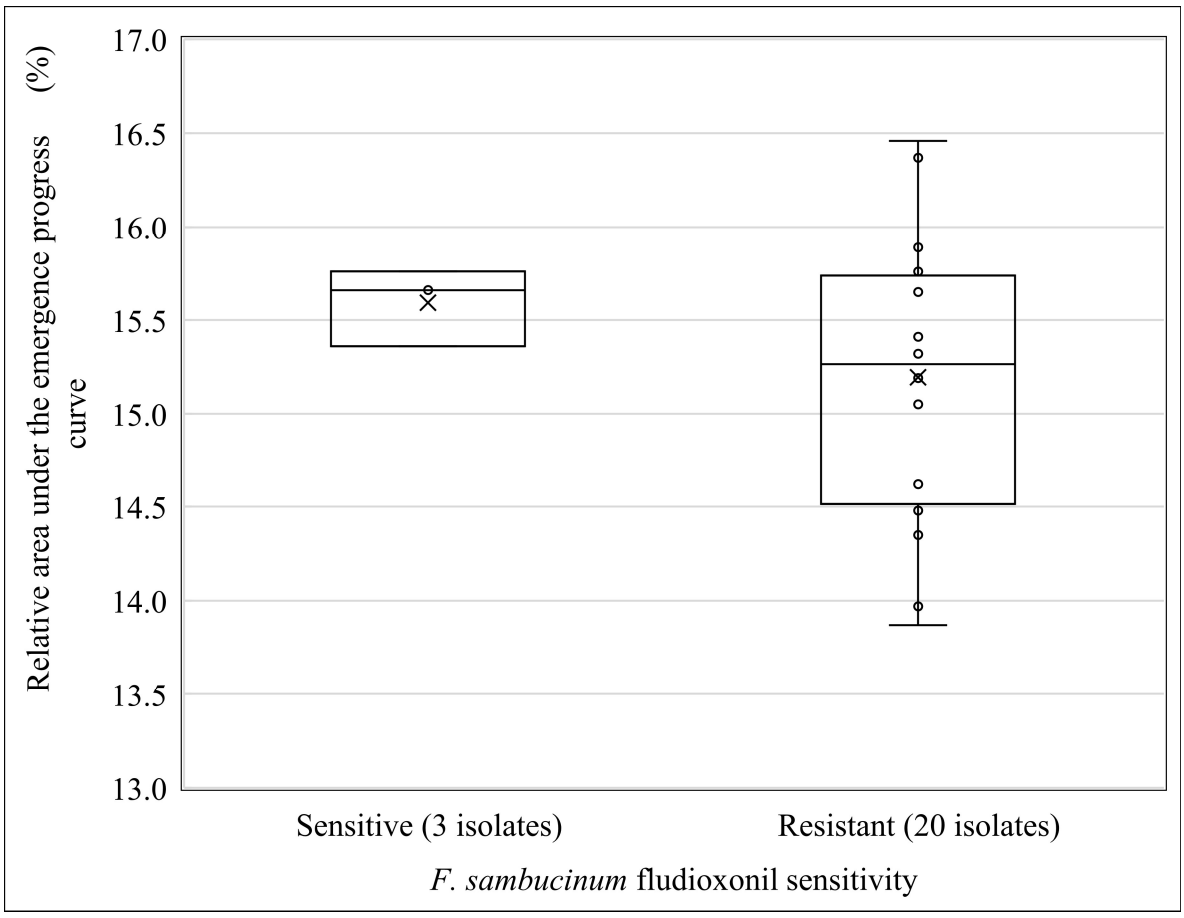

Figure 4-2**.** Box plots of mean plant emergence rates for dry rot field trials conducted in 2020 and 2021 in Idaho. Plant emergence was calculated as the Relative Area Under the Emergence Progress Curve (RAUEPC). Efficacy of the seed piece treatment fludioxonil (Maxim 4FS, Syngenta) was evaluated against 23 *Fusarium sambucinum* isolates from Idaho that were either sensitive or resistant to fludioxonil. A lower RAUEPC percentage indicates a slower emergence rate.

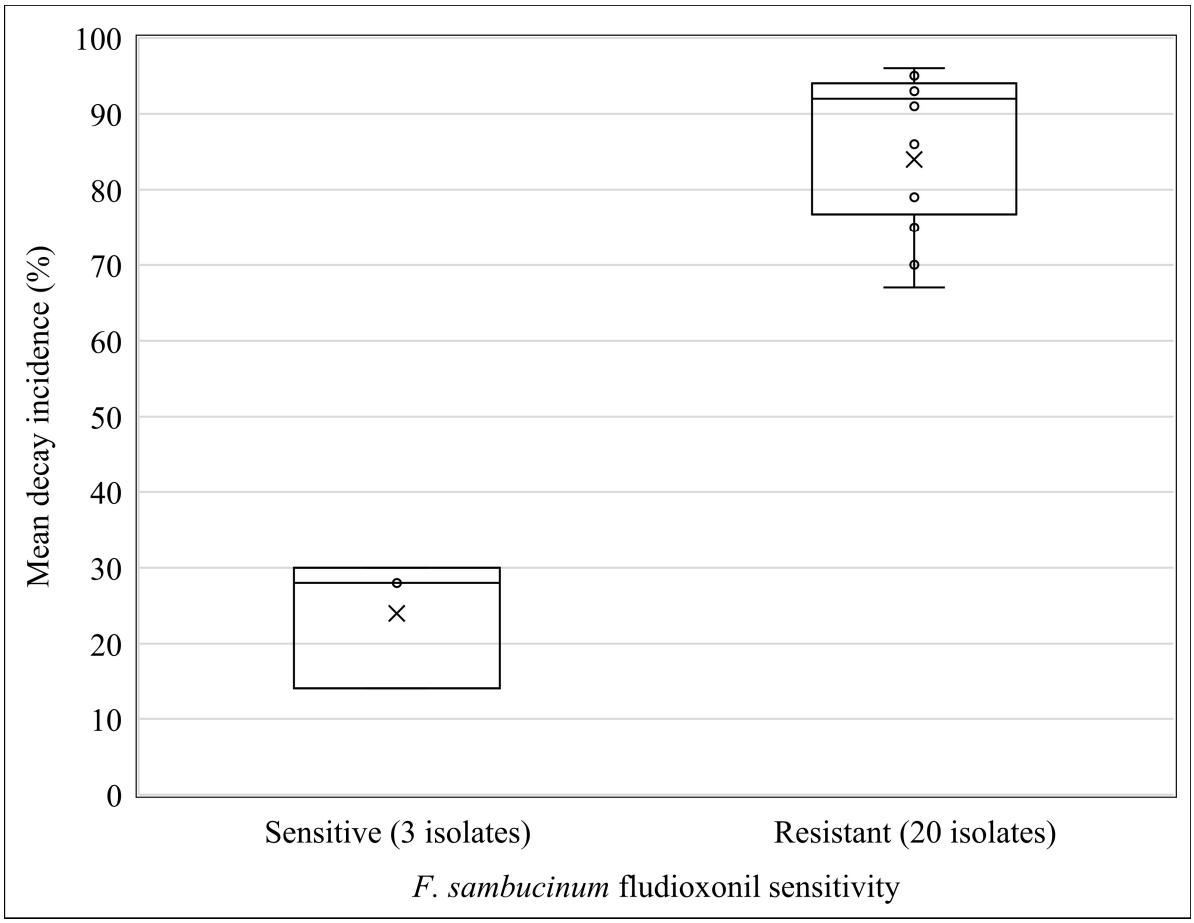

Figure 4-3**.** Box plots of mean seed piece decay incidence of Fusarium dry rot from field trials conducted in 2020 and 2021 in Idaho. Efficacy of the seed piece treatment fludioxonil (Maxim 4FS, Syngenta) was evaluated against 23 *Fusarium sambucinum* isolates from Idaho that were either sensitive or resistant to fludioxonil.

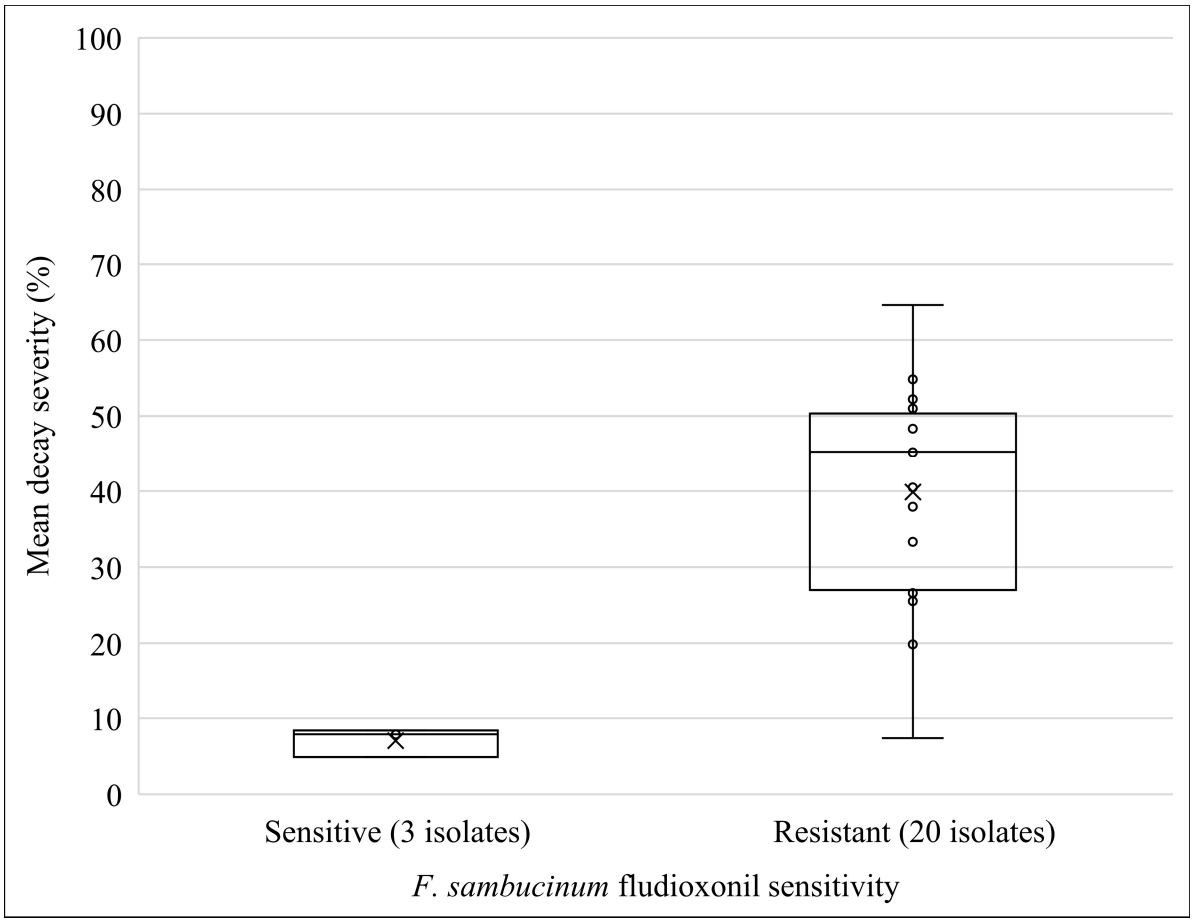

Figure 4-4. Box plots of seed piece decay severity in Fusarium dry rot infections for field trials conducted in 2020 and 2021 in Idaho. Efficacy of the seed piece treatment fludioxonil (Maxim 4FS, Syngenta) was evaluated against 23 *Fusarium sambucinum* isolates from Idaho that were either sensitive or resistant to fludioxonil.

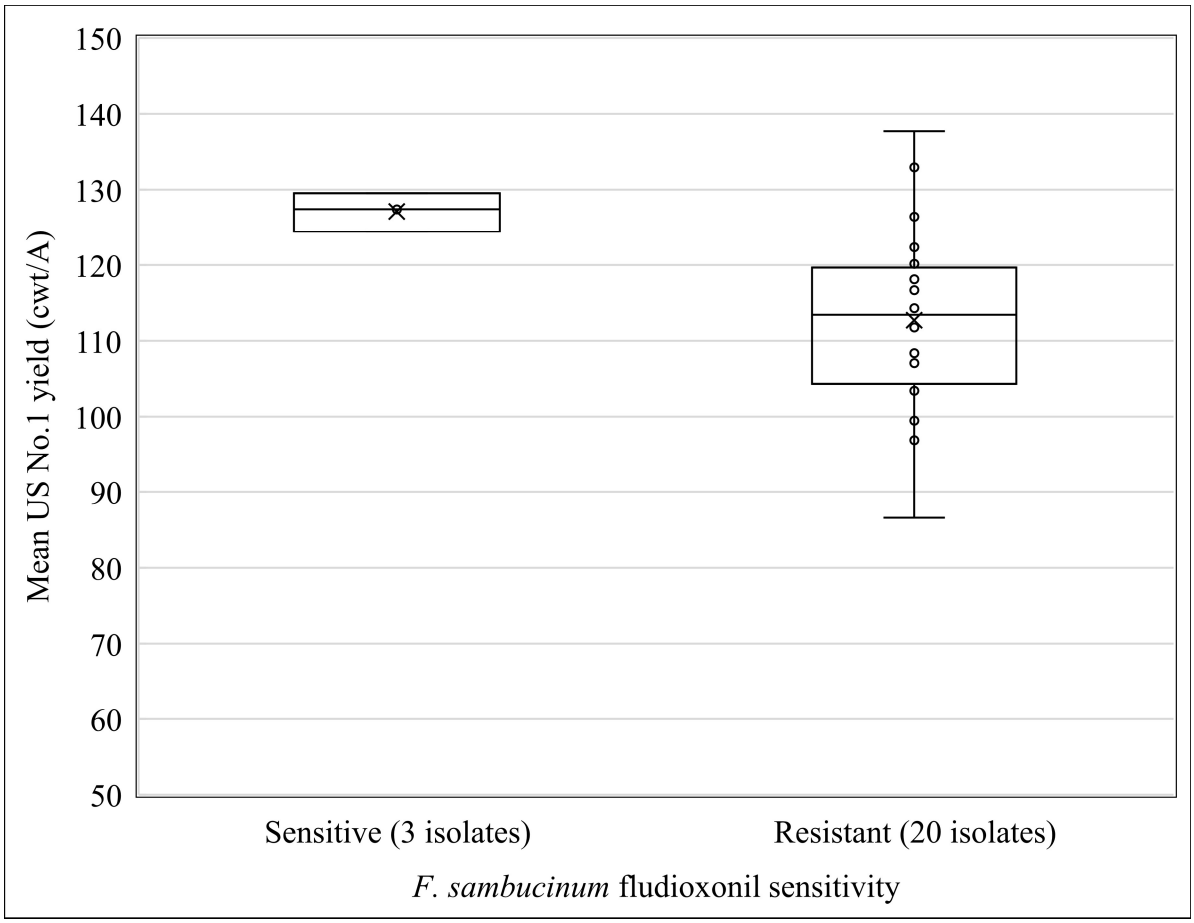

Figure 4-5. Box plots of mean US No. 1 yields for potatoes harvested from Fusarium dry rot field trials conducted in 2020 and 2021 in Idaho. Efficacy of the seed piece treatment fludioxonil (Maxim 4FS, Syngenta) was evaluated against 23 *Fusarium sambucinum* isolates from Idaho that were either sensitive or resistant to fludioxonil.

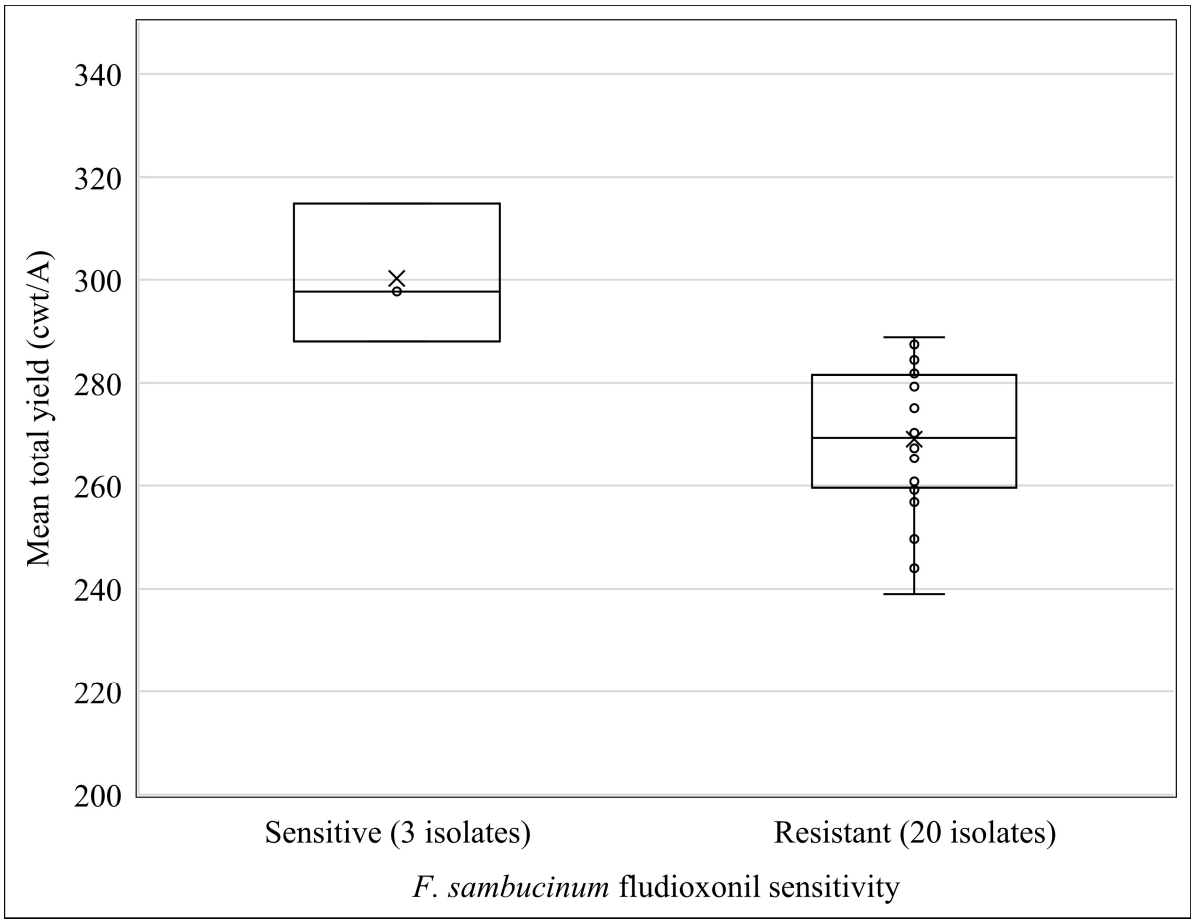

Figure 4-6. Box plots of mean total yields for potatoes harvested from Fusarium dry rot field trials conducted in 2020 and 2021 in Idaho. Efficacy of the seed piece treatment fludioxonil (Maxim 4FS, Syngenta) was evaluated against 23 *Fusarium sambucinum* isolates from Idaho that were either sensitive or resistant to fludioxonil.

Discussion

Development of resistance in Fusarium dry rot pathogens to fungicides used for dry rot management is hypothesized to be one of the factors driving the reported increase in Fusarium dry rot in the PNW in recent years. In this study, three experimental methods were utilized to gain information about the fungicide sensitivity of Fusarium dry rot pathogens in the PNW: laboratory sensitivity screening tests using fungicide-amended agar plates, laboratory tests of fungicide effectiveness as a seed treatment and a field trial testing fludioxonil effectiveness against *F. sambucinum*. Fungicide-amended agar tests revealed sensitivity to difenoconazole among all tested *Fusarium* species, resistance to fludioxonil in five tested species, and resistance to thiabendazole in one tested species, *F. sambucinum*. When applied as a seed treatment on cut seed pieces, difenoconazole reduced dry rot in all five tested species. When a fludioxonil seed treatment was used against *F. sambucinum* and *F. oxysporum* isolates with lab-detected sensitivity or resistance, fludioxonil reduced dry rot for sensitive isolates of both species, resistant isolates of *F. oxysporum*, and some resistant isolates of *F. sambucinum*, indicating that laboratory testing is not entirely predictive of fungicide effectiveness in presence of the potato host. For *F. sambucinum* isolates with lab-detected thiabendazole sensitivity and resistance, a thiabendazole seed treatment reduced dry rot for sensitive isolates but not for most resistant isolates. In a field trial testing the effectiveness of a fludioxonil seed treatment against sensitive and resistant isolates of *F. sambucinum*, which is the most prevalent and aggressive dry rot pathogen in the PNW, sensitive isolates were observed to have less frequent and less severe seed piece decay than resistant isolates, and yields were somewhat greater for sensitive isolates than resistant isolates.

Based on the data, difenoconazole remains a viable dry rot management fungicide. The presence of fludioxonil resistance among PNW *F. sambucinum* is possibly a contributing factor in the reported increase in dry rot in the PNW in recent years, and *F. oxysporum* fludioxonil resistance may play a role as well. As these two species are the most prevalent dry rot pathogens in the PNW based a recent survey (see Chapter 2) and fludioxonil is a commonly used seed treatment in PNW potato production, identification of fludioxonil resistance among isolates of these two species is very concerning. However, increased disease levels due to fludioxonil-resistant *F. oxysporum* were not apparent in the seed treatment bag tests. Therefore, currently, fludioxonil resistance in *F. sambucinum* appears more likely to negatively impact dry rot management than *F. oxysporum*. Many fludioxonil resistant *F. sambucinum* isolates were observed to cause increased seed piece decay and decreased yield in the field trial. The persistence of thiabendazole-resistant *F. sambucinum* isolates in the PNW was

demonstrated here, indicating that continued omission of thiabendazole use in dry rot management is wise in the PNW region.

In this study, all *F. sambucinum* isolates were sensitive to difenoconazole or had low EC_{50} values, indicating that this fungicide is likely still effective in PNW dry rot management. Similarly, Gachango et al. (2012) concluded that multiple *Fusarium* species isolated from potatoes with dry rot in Michigan were sensitive to difenoconazole using a threshold EC_{50} value of less than 5 mg/L to indicate sensitivity. In this study, difenoconazole sensitivity was the same for both fludioxonil-sensitive and fludioxonil-resistant isolates. Therefore, if used in conjunction with fludioxonil, difenoconazole can help control dry rot even if the *F. sambucinum* isolates present are fludioxonil-resistant. Also, the sensitivity of *Fusarium tricinctum* species complex isolates, i.e., *F. avenaceum, F. acuminatum*, *F. flocciferum*, *F. gamsii*, and *F. iranicum*, to difenoconazole bodes well for dry rot management, in particular for the aggressive dry rot pathogen *F. avenaceum* and any aggressive isolates of *F. flocciferum*.

Burlakoti et al. (2010) determined triazole (triticonazole and metconazole) and thiabendazole sensitivity of *F. oxysporum* and *F. graminearum* isolates from sugar beet in Minnesota. Using a EC_{50} threshold of 10 mg/L to indicate resistance, they observed both *Fusarium* species to be sensitive to both triazoles and thiabendazole with mean EC_{50} values less than 2.2 mg/L. In addition, Burlakoti et al. (2010) used a thiabendazole-resistant isolate of *F. sambucinum* from potato as a check and observed resistance to thiabendazole but sensitivity to both triazoles. The results from the *in vitro* fungicide sensitivity assay in this study show similar results for another triazole, difenoconazole. All tested *F. oxysporum* and *F. graminearum* isolates had EC_{50} values less than 8 mg/L and, in most cases, thiabendazole-resistant *F. sambucinum* isolates had EC_{50} values less than 8 mg/L. Similar to triazole sensitivity reported by Hellin et al. (2017), PNW isolates of *F. culmorum*, *F. graminearum* and *F. cerealis* were sensitive to difenoconazole or have slightly reduced sensitivity with EC_{50} values ranging from 5.1 to 14.8 mg/L. Further, triazole sensitivity in thiabendazole-resistant *F. sambucinum* isolates is very helpful for dry rot management.

Overall, the PNW isolates of species for which triazole sensitivity data is reported by other researchers are largely sensitive to the triazole difenoconazole. These studies together are evidence for the continued efficacy of triazoles on *Fusarium* pathogens. Since both difenoconazole and prothioconazole are used in commercial formulations of potato seed treatment products, monitoring of PNW Fusarium isolates for sensitivity would be prudent for early detection of potential cross resistance in these two triazoles used for dry rot management.

In vitro fludioxonil resistance was detected in PNW isolates of five *Fusarium* species, *F. coeruleum*, *F. mori*, *F. oxysporum*, *F. redolens* and *F. sambucinum*. Fludioxonil resistance in Idaho *F. sambucinum* isolates is of particular concern since *F. sambucinum* is a prevalent dry rot pathogen in Idaho. Fludioxonil is an important seed treatment fungicide chemistry for PNW potato production, however, 77% of Idaho *F. sambucinum* isolates tested *in vitro* were fludioxonil-resistant. Loss of fludioxonil effectiveness on PNW *F. sambucinum* would substantially increase difficulty in dry rot management in the PNW. As such, these laboratory screening results were further investigated using fungicide seed treatment experiments and a field trial. Fludioxonil resistance has also been reported previously for *F. sambucinum* isolates from tubers collected in Michigan and Canada, as measured by lack of fungal growth inhibition at 100 mg/L fludioxonil (Gachango et al. 2011, 2012; Peters et al. 2008c). Gachango et al. (2012) observed a high frequency of fludioxonil resistance among tested *F. sambucinum* isolates (81%) from Michigan tuber samples.

Interestingly, no fludioxonil resistance was found in Washington isolates while 77% of Idaho isolates were resistant. None of the major PNW potato growing regions span locations in both Washington and Idaho, therefore, the *F. sambucinum* isolates used in this study are reasonably supposed to be from separate populations. The lack of resistance in the *F. sambucinum* isolates from Washington and large proportion of resistant isolates from Idaho suggested a notable difference in these two *F. sambucinum* populations. The difference in fludioxonil sensitivity may be due to less exposure of the Washington population to fludioxonil and/or due a difference in disease pressure between the two ptoat production areas.

Fludioxonil-sensitive *F. sambucinum* isolates have also been detected in Michigan. However, there was a notable difference between fludioxonil-sensitive *F. sambucinum* isolates recovered from a seed storage survey (Gachango et al. 2012) and a commercial storage survey (Merlington 2014) in Michigan. All four (100%) tested *F. sambucinum* isolates from commercial storage samples were observed to be fludioxonil-sensitive (Merlington 2014) while only 19% of *F. sambucinum* isolates from seed storage samples were fludioxonil-sensitive (Gachango et al. 2012). The differences in proportions of fludioxonil-sensitive *F. sambucinum* isolates from the two different Michigan storage types and between the Idaho and Washington isolates is evidence for the spread of fludioxonil resistance within some populations but not others.

As with *F. sambucinum*, the observation of fludioxonil resistance in some *F. oxysporum* isolates was of particular concern as *F. oxysporum* displays intermediate to high aggressiveness as a dry rot pathogen and is the second most prevalent dry rot pathogen in the PNW. Gachango et al. (2012) and

Merlington (2014) also observed fludioxonil resistance among tested *F. oxysporum* isolates (79% and 17% of tested isolates, respectively) from Michigan tuber samples with no growth inhibition at 100 mg/L fludioxonil. However, compared to the Michigan studies, an intermediate frequency of *F. oxysporum* fludioxonil resistance was observed in PNW with 43% of tested isolates exhibiting resistance.

In contrast to *F. sambucinum*, all other tested species in the *Fusarium sambucinum* species complex, i.e., *F. cerealis*, *F. culmorum*, *F. graminearum* and *F. venenatum*, were sensitive to fludioxonil. *F. venenatum* was only recovered from Washington potato samples. *Fusarium venenatum* has previously been reported as a dry rot pathogen but not in the PNW. Although this species displayed an intermediate level of aggressiveness to potato tuber, fludioxonil and difenoconazole sensitivity was detected, indicating that *F. venenatum* should be inhibited by seed treatments containing these fungicides as active ingredients. Both *F. culmorum* and *F. cerealis* isolates from the PNW were shown to have intermediate aggressiveness levels to potato but should be inhibited by fludioxonil and difenoconazole according to the sensitivity screening data of this study. *Fusarium culmorum* has also decreased in importance as a *Fusarium* head blight pathogen in small grains (Bissonnette et al. 2018), further mitigating disease risk from *F. culmorum* due to crop rotation of potato and small grains.

F. graminearum is also both a Fusarium dry rot pathogen of potato and a Fusarium head blight pathogen of small grains. Although *F. graminearum* has become the prevalent *Fusarium* head blight pathogen attacking wheat in southeastern Idaho over *F. culmorum* (Bissonnette et al. 2018), only one *F. graminearum* isolate was recovered from Idaho in the recent dry rot survey (less than 1% of Idaho isolates), indicating that *F. graminearum* is a very minor potato dry rot pathogen in the PNW. However, *F. graminearum* was observed to have intermediate aggressiveness as a dry rot pathogen in the laboratory for this study and was observed to be a catastrophic dry rot pathogen in North Dakota in 2011. In the North Dakota event, it was speculated that harvesting potatoes while plant vines were still green led to large wounds as stems were torn from tubers during harvest, creating a situation that favored infection and more aggressive disease development by *F. graminearum* (Estrada Jr et al. 2010). Therefore, though the current dry rot risk to PNW from *F. graminearum* is concluded to be very low, the potential risk indicates that the presence of *F. graminearum* in potato production should be considered as needed. Fludioxonil and difenoconazole sensitivity was observed during screening of the Idaho *F. graminearum* isolate, indicating that *F. graminearum* should be inhibited by seed treatments containing these fungicides as active ingredients.

The sensitivity of *Fusarium tricinctum* species complex isolates, i.e., *F. avenaceum, F. acuminatum*, *F. flocciferum*, *F. gamsii* and *F. iranicum*, to fludioxonil bodes well for dry rot management. Although pathogenicity testing indicated that *F. avenaceum* is a relatively aggressive dry rot pathogen, the sensitivity of *F. avenaceum* to fludioxonil and difenoconazole coupled with its scarcity in the PNW region indicated that *F. avenaceum* does not pose a high risk to PNW potato production at this time. Based on pathogenicity testing, *F. acuminatum* is a weak dry rot pathogen and also is not prevalent in the PNW. All three tested *F. acuminatum* isolates were sensitive to both fludioxonil and difenoconazole, therefore, the efficacy of these two fungicides on *F. acuminatum* is beneficial to dry rot management in the PNW. Pathogenicity testing indicated intraspecific variability in aggressiveness for *F. flocciferum* isolates, with some isolates acting as weak dry rot pathogens while others were as more aggressive. However, only one *F. flocciferum* isolate was screened for fungicide sensitivity (C056) and though sensitive to fludioxonil and difenoconazole, it also displayed non-pathogenicity to potato. Therefore, currently, the fungicide sensitivity of pathogenic *F. flocciferum* isolates is unknown. However, since *F. flocciferum* was found in samples from both Idaho and Washington and can act as a relatively aggressive dry rot pathogen, more information regarding its fungicide sensitivity would be useful for dry rot management in general. In addition, presuming that *in vitro* data indicate that fludioxonil and difenoconazole will also be effective in field situations, reliable reduction of the *Fusarium tricinctum* species complex populations in potato fields may be helpful for common rotation crops with potato in the PNW, wheat and barley. In North America, increases in *F. avenaceum* and *F. acuminatum*, as well as the recently described novel *Fusarium* species *F. gamsii* and *F. iranicum*, as wheat and barley pathogens have been documented (Laraba et al. 2022). The ability of *F. gamsii* and *F. iranicum* to cause Fusarium dry rot of potato is currently unknown.

Although *F. redolens* was not an overly prevalent species in the PNW, its intermediate level of aggressiveness as a dry rot pathogen and its fludioxonil resistance makes *F. redolens* a species worth monitoring in the PNW. Dry rot risk could be high in specific situations where *F. redolens* is present in a seed lot or in field soil. Fludioxonil resistance was detected in the Idaho *F. coeruleum* isolate B085 recently collected from a storage sample of symptomatic seed tubers. Peters et al. (2008c) also observed fludioxonil resistance in *F. coeruleum* in Canada, and this was the first report of fludioxonil resistance in *Fusarium* causing potato seed piece decay.

Fludioxonil resistance was detected *in vitro* in isolates of five PNW *Fusarium* species originating from tuber samples, *F. coeruleum*, *F. mori*, *F. oxysporum*, *F. redolens*, and *F. sambucinum*. Fludioxonil sensitivity for these five species was distinct, with the tested isolates being either

decidedly resistant or sensitive, as indicated by EC_{50} values well-above the threshold EC_{50} value of > 100 mg/L, which indicated resistance, or well-below the threshold EC_{50} value of 5 mg/L, which indicated sensitivity. Distinct fludioxonil resistance or sensitivity was also observed in *F. sambucinum* and *F. oxysporum* isolates from Michigan (Gachango et al. 2012) and *F. sambucinum* and *F. coeruleum* isolates from Canada (Peters et al. 2008c) with no tested isolates exhibiting EC_{50} values between the 5 mg/L sensitivity threshold and the 100 mg/L resistance threshold. This lack of a continuum of sensitivity may indicate that the mutation needed to overcome the fludioxonil mode-of-action is a change in a single gene, i.e., the abrupt, qualitative resistance described by Damicone (2017). In light of a revised theory for the fludioxonil target site and mechanism, this all-or-nothing resistance observed *in vitro* may be due to the catastrophic effect(s) fludioxonil has on sensitive isolates of fungus. Based on the Brandhorst et al. (2019) theory, the fungus may suffer from either an inability to meet basic energy needs due to a mutation in triosephosphate isomerase, thereby conferring resistance along with a severe fitness penalty, or an inability to catabolize a toxic by-product of sugar metabolism that increases to such high concentrations as to constitutively turn on the cell self-destruction mode.

The exact mechanism and target site of fludioxonil is not completely solved. The most recent mechanism theory states that fludioxonil indirectly triggers the HOG-1 pathway by creating a substantial stress state in the fungal cell, which ultimately leads to fungal cell death (Brandhorst and Klein 2019; Brandhorst et al. 2019). Brandhorst et al. (2019) report that fludioxonil sensitivity has been shown to disappear when HAMP repeats of the Group III HHK, which are unique from other HHKs, are modified or deleted, causing the HHK protein to become a constitutive kinase and no longer be able to be induced to become a phosphatase that ultimately activates the HOG1 self-destruction pathway (Brandhorst et al. 2019). The fludioxonil target site proposed by Brandhorst et al. (2019) is triosephosphate isomerase, a key enzyme in sugar metabolism. The interaction of the fludioxonil molecule may be the interface of the triosephosphate isomerase dimer, where fludioxonil may introduce steric interactions that cause one of the loops in triosephosphate isomerase to become inflexible, allowing the release of methylgloxal into the cytosol (Brandhorst et al. 2019). Methylglyoxal, an aldehydic stress molecule that is very reactive and damaging to DNA and cellular proteins, is produced on a normal basis during sugar breakdown in cells but is catabolized by enzymes before it becomes toxic and damaging (Hamilton 2019). The excess methylglyoxal induces Group III HHK to convert from kinase to phosphatase, which then acts on Ypd1 to trigger HOG-1 pathway activation and fungal cell death (Brandhorst et al. 2019; Hamilton 2019).

The source of fludioxonil resistance in the PNW *Fusarium* isolates can be contemplated in light of this new theory proposed by Brandhorst et al. (2019). The isolates may be obtaining fludioxonil resistance due to some modification of the HAMP repeats in the Group III HHK protein or a mutation in triosephosphate isomerase. However, since triosephosphate isomerase is a key enzyme in sugar metabolism, a triosephosphate isomerase mutation that excludes interaction with fludioxonil may also induce a substantial fitness penalty to the mutated fungus due to reduction or other alteration in sugar metabolism that reduces the ability of the mutant to meet basic energy needs.

Thiabendazole resistance was expected in *F. sambucinum* based on past data (Choiseul et al. 2007; Desjardins et al. 1993; Gallian et al. 2006; Hide et al. 1992; Kawchuk et al. 1994; Miller et al. 2019; Nolte 1994; Ocamb et al. 2007). Both sensitive and resistant isolates were recovered from Idaho, but all tested Washington isolates were thiabendazole-sensitive. The sensitivity observed in Washington isolates was interesting, again highlighting a difference in the Idaho and Washington *F. sambucinum* populations. Hanson et al. (1996) also observed a variation in thiabendazole sensitivity in a collection of *F. sambucinum* isolates from tuber samples in the northeastern United States. Thiabendazole is no longer recommended for dry rot management in Idaho due to the high occurrence of resistance observed in *F. sambucinum* (Miller et al. 2019). Additionally, thiabendazole sensitivity for *F. sambucinum* was distinct with the tested isolates being either decidedly resistant or sensitive, with few EC_{50} values between the threshold values of 5 mg/L and 100 mg/L, indicating sensitivity or resistance, respectively. This lack of a continuum of sensitivity may indicate that the mutation needed to overcome the thiabendazole mode-of-action is a change in a single gene.

Multi-class fungicide resistance in *F. sambucinum* isolates was observed in this study for the chemical groups phenylpyrroles (fludioxonil) and benzimidazoles (thiabendazole), based on *in vitro* fungicide sensitivity screening data. Multi-class resistance to fludioxonil and thiabendazole has been observed *in vitro* for *F. sambucinum* isolates from potato seed lots with substantial dry rot in Prince Edward Island and Saskatchewan, Canada (Peters et al. 2008c). It could be suggested that the mechanism conferring resistance in *F. sambucinum* could be the same for both fungicides, but this seems very unlikely given the proposed mechanisms in the literature (Brandhorst et al. 2019; Kawchuk et al. 2002). Also, in this study, multi-class resistance was not detected in the other four PNW *Fusarium* species exhibiting fludioxonil resistance *in vitro*, i.e., thiabendazole resistance as defined by $EC_{50} > 100$ mg/L was not detected. Therefore, again, it appears that the mechanisms conferring resistance could not be similar. However, *F. sambucinum* could be unique among Fusarium dry rot pathogens in its ability to develop resistance to both fludioxonil and thiabendazole. Given that thiabendazole was used to combat Fusarium dry rot well before fludioxonil, it could be

suggested that development of thiabendazole resistance prior to fludioxonil exposure in *F. sambucinum* populations may pre-dispose *F. sambucinum* to fludioxonil resistance development in some way.

Estrada, Jr. et al. (2010) stated that 127 *F. graminearum* isolates from tubers with dry rot that were screened for thiabendazole sensitivity were found to be sensitive, which is in contrast to *F. sambucinum*, and that thiabendazole may be a feasible option for postharvest application when *F. graminearum* is the pathogen. The *F. graminearum* isolate recovered from Idaho in the current study had EC_{50} values of 4.3 and 9.1 mg/L for two separate tests, which does not indicate complete sensitivity, but the isolate is clearly not resistant to thiabendazole.

Generally, *F. coeruleum* is considered to be a soil-borne species that has contributed to dry rot problems in Idaho via seed piece decay in the field after planting (Miller et al. 2019). *F. coeruleum* has been observed to grow faster in soil and can be a more destructive seed piece decay pathogen in the field than *F. sambucinum* (Miller et al. 2019). Considering the fungicide screening data for *F. coeruleum* isolate B085, if other *F. coeruleum* isolates in Idaho fields are also fludioxonil-resistant and have reduced sensitivity to thiabendazole, this may help explain why this species has been implicated over the years in Idaho as a predominant dry rot pathogen. In the past when thiabendazole was used frequently for dry rot management, if the generally seed borne pathogen *F. sambucinum* and the generally soil-borne pathogen *F. coeruleum* were both resistant or had reduced sensitivity to thiabendazole, then this could explain why both seed piece decay in the field and storage decay were common. Currently, fludioxonil is commonly used as a seed piece treatment, and if there are resistant *F. coeruleum* isolates in the field, then seed piece decay could occur even if the fludioxonil inhibits dry rot pathogens associated with the seed lot. This could especially be an issue if fludioxonil is applied as a single active ingredient, a practice which is not recommended in any case as a guard against fungicide resistance development. Considering the reduced sensitivity to difenoconazole observed in the lab for *F. coeruleum*, application of a seed piece treatment containing both fludioxonil and difenoconazole as active ingredients could also be less effective against seed piece decay than intended if *F. coeruleum* isolates like B085 tested in this study are present and have both fludioxonil resistance and reduced sensitivity to difenoconazole.

Laboratory-based evidence of fludioxonil resistance development in *F. sambucinum* and *F. oxysporum* to fungicides used to manage dry rot in the PNW is concerning. Fludioxonil resistance in both species has also been detected in Michigan and Canada. Targeted application of management strategies to slow development of fludioxonil resistance is highly recommended. However, a

connection between the laboratory data indicating resistance and negative impacts on dry rot management and yield is needed to help demonstrate to the potato production industry that specific action regarding fludioxonil resistance development is justified. Based on this *in vitro* fungicide sensitivity screening dataset, it was prudent and logical to develop an experiment to provide information about the relationship between *in vitro* testing of fungicide efficacy in a Petri plate and *in vivo* testing of fungicide efficacy on potato seed. The objective was to determine if *in vitro* tests indicate what fungicide performance would be in a real-world situation.

Comparison of the results for the *F. sambucinum* control treatments (isolates FID 71-6 and A054) with and without difenoconazole application indicated that difenoconazole can reduce dry rot incidence and severity. Reduced disease levels similar to background dry rot levels were observed for all five tested *Fusarium* species, indicating that difenoconazole is effective on these PNW *Fusarium* species. However, the ability of laboratory fungicide screening results in a Petri dish to predict difenoconazole performance as a seed treatment on potato tubers was not clear. For example, there was lack of statistical evidence for the F. redolens isolates, but numerically the sensitive *F. redolens* isolate I028 had lower incidence than the *F. redolens* isolate with reduced sensitivity. Also, for example, the *F. sambucinum* isolate C090 with reduced sensitivity had numerically higher incidence and severity than the rest of the *F. sambucinum* isolates but this was not statistically significant. The ambiguity from this dataset may be related to the arbitrary definition of what constitutes "reduced sensitivity" in this study, which was following the work of Gachango et al. (2012). Considering both the *in vitro* sensitivity screening data and the seed treatment bag test data together, it appeared the threshold EC_{50} value of less than 5 mg/L may be too stringent for indicating difenoconazole sensitivity. However, a higher EC_{50} value may be appropriate to indicate a threshold at which reduced difenoconazole sensitivity results in increased dry rot. Damicone (2017) and Hellin et al. (2017) noted that resistance to triazoles, e.g. difenoconazole, develops stepwise as mutations in multiple genes or alleles are involved. Additional study would be required to determined what this higher threshold would be.

In conclusion, difenoconazole continues to be an effective dry rot management fungicide in the PNW based on this data. As such, difenoconazole use is unlikely to be a factor in the reported increase in dry rot in the PNW. For a few isolates, greater sensitivity to difenoconazole did appear to result in less dry rot compared to isolates with larger EC_{50} values when difenoconazole was used as a seed treatment, but evidence for this relationship was weak within this dataset. A threshold EC_{50} value for which difenoconazole would be rendered ineffective on Fusarium dry rot pathogens was not established by this dataset. Fortunately, the PNW *Fusarium* population does not appear to have

evolved this level of insensitivity to difenoconazole. A suggested strategy to help curb development of insensitivity to difenoconazole in the PNW is annual alternation of Group 3 fungicide use in seed treatment application. For example, in one year, a fludioxonil-difenoconazole mixture could be used, then the next year a fludioxonil-mancozeb mixture could be used, then in the third year, a prothioconazole product could be used.

It is important to keep in mind that reduction in fungal sensitivity to Group 3 fungicides like difenoconazole is known to develop gradually since multiple genes are involved in conferring resistance (Damicone 2017; Hellin et al. 2017). For the time being, continuing to screen PNW *Fusarium* isolates using the *in vitro* spiral gradient dilution method demonstrated in this study would be a sensible way to monitor difenoconazole sensitivity of PNW dry rot pathogens, keeping in mind that EC_{50} values up to approximately 20 mg/L were shown to not result in increased dry rot when potato tubers were inoculated with those isolates in the bag test experiment conducted in this study. If observation of higher EC_{50} values begins to occur regularly in tested isolates, this may be an indication of resistance development in PNW *Fusarium*. At that time, additional bag test experiments may be helpful in determining if the apparent decrease in fungicide sensitivity, i.e., EC_{50} values greater than 20 mg/L, is affecting efficacy as a potato seed treatment.

In addition, further study of PNW *Fusarium* sensitivity to prothioconazole, another Group 3 fungicide that is an active ingredient in a commonly applied potato seed treatment formulation Emesto Silver (Bayer CropScience), is warranted. Cross-resistance is known to be prevalent in Group 3 fungicides, meaning that if a *Fusarium* isolate is resistant to one Group 3 fungicide, then it can be supposed that the same isolate is also resistant to other Group 3 fungicides. Monitoring sensitivity to both Group 3 active ingredients used in potato seed treatments (difenoconazole and prothioconazole) would be prudent, and the spiral gradient dilution method used in this study was shown to be useful as a fungicide sensitivity screening tool.

Laboratory fungicide screening generally predicted performance of fludioxonil as a seed treatment on potato tubers inoculated with *F. sambucinum* isolates. Two fludioxonil-sensitive isolates caused similar dry rot incidence and severity to background levels, indicating that the fludioxonil treatment effectively reduced disease levels associated with these isolates. Out of six fludioxonil-resistant *F. sambucinum* isolates, four caused significantly greater dry rot incidence and severity than background levels, demonstrating that the fludioxonil treatment was not effective. In fact, incidence and severity for these four isolates were the same as disease levels for the treatment that was inoculated with *F. sambucinum* but not treated with fludioxonil, i.e., NTC-I FID 71-6. However, two *F. sambucinum*

isolates, C081 and D083, exhibited fludioxonil resistance *in vitro* but were apparently controlled by fludioxonil in the seed treatment test using tubers, which is contradictory to the behavior of the other four *F. sambucinum* isolates. Alternatively, for isolates C081 and D083, the lack of a strict relationship between fungicide resistance measured *in vitro* and decreased disease control on potato tuber may instead indicate differences in aggressiveness among these isolates. This is not surprising as variability in aggressiveness on potato within and among *Fusarium* species has been observed in this study and by other researchers (e.g., Gachango et al., 2012; Stefańczyk et al., 2016). Whether these isolates differ in aggressiveness, are susceptible to fludioxonil under field conditions but not lab conditions or respond unexpectedly due to other factors warrants further study.

Looking at all six fludioxonil-resistant *F. sambucinum* isolates that were included here, the data indicated that *in vitro* sensitivity data do not necessarily indicate *in vivo* infection incidence and severity but that the laboratory screening can generally help predict whether use of fludioxonil seed treatment will result in reduced Fusarium seed decay in potato seed. This dataset shows that two of eight (25%) *F. sambucinum* isolates tested did not cause disease levels as expected based on lab screening, but 75% were controlled or not controlled by the fludioxonil as expected. Overall, this indicated that the laboratory screening is an effective tool to predict fludioxonil effectiveness as a potato seed treatment in the presence of *F. sambucinum*. From a disease management perspective, the knowledge of the presence of fludioxonil resistant isolates as demonstrated by laboratory screening is cause enough to indicate that an adjustment to fungicide application programs would be prudent, even if one or a few isolates in a pathogen population being considered may actually still be controlled by fludioxonil contrary to laboratory results.

For *F. oxysporum*, laboratory fungicide screening assays did not strongly predict fludioxonil performance as a seed treatment on potato tubers. Significant differences related to *in vitro* sensitivity results were inconsistent within the groups of resistant and sensitive isolates, but the significant results did follow a pattern indicating that more resistant isolates cause greater dry rot. This lack of consistency between laboratory fungicide screening and performance of fludioxonil may be related to variability in aggressiveness among isolates.

Fludioxonil resistance was detected *in vitro* in isolates of five PNW *Fusarium* species originating from tuber samples, *F. coeruleum*, *F. mori*, *F. oxysporum*, *F. redolens*, and *F. sambucinum*. Two of these species, *F. sambucinum* and *F. oxysporum* were the most prevalent dry rot pathogens in the PNW based a recent survey. As fludioxonil is a commonly used seed treatment in PNW potato production, identification of fludioxonil resistance among isolates of these two prevalent species is

very concerning. *In vitro* resistance of *F. sambucinum* was found in 67% of isolates tested while 43% of *F. oxysporum* isolates tested were resistant to the fungicide. However, within this dataset, *in vitro* resistance of *F. oxysporum* to fludioxonil had much less impact on disease levels in fludioxonil-treated seed tubers than *F. sambucinum*. In the seed treatment bag tests, disease incidence for *F. sambucinum* treatments was 25 to 30% higher and disease severity was 45 to 60% higher than background levels, in contrast to fludioxonil-resistant *F. oxysporum* isolates, where disease levels did not differ from background disease. Therefore, currently, fludioxonil resistance in *F. sambucinum* appears much more likely to negatively impact dry rot management than *F. oxysporum*.

Presence of fludioxonil resistance among PNW *F. sambucinum* is possibly a contributing factor in the reported increase in dry rot in the PNW in recent years, and *F. oxysporum* fludioxonil resistance may play a role as well. If growers have used fungicide applications of fludioxonil as the single active ingredient and/or have repeatedly used fludioxonil year after year without rotating to other fungicide modes-of-action, and unknowingly used these application patterns in the presence of resistant *F. sambucinum* and/or *F. oxysporum* isolates, this has likely led to greater dry rot problems. To effectively manage dry rot in the face of fludioxonil resistance among isolates of the most prevalent and aggressive *Fusarium* dry rot pathogens in the PNW, growers can take additional steps to minimize the risk associated with this newfound pathogen resistance in the PNW. These actions should be in addition to the most important dry rot management strategy, which is wound management.

When dry rot is identified by growers in their operations, submission of samples for pathogen identification and fungicide screening would be helpful in determining if fungicide resistance played a role in disease occurrence. Before planting, performing bag tests on every seed lot to ascertain dry rot potential and test the effectiveness of fungicide(s) planned to be used may help avoid use of an ineffective fungicide product, especially if planning to use a fludioxonil product under the distinct possibility that the seed lot may contain one or both prevalent PNW dry rot pathogens with known fludioxonil resistance. Practicing recommended strategies for curbing resistance development in pathogen population such as rotating fungicide modes-of-actions and using fungicide mixtures should help reduce the rate of resistance development among Fusarium dry rot pathogens.

Reduced sensitivity to thiabendazole (EC_{50} values 5.7 to 18.4 mg/L) as measured in the lab did not result in dry rot incidence and severity above background levels in seed treatment bag tests for *F. culmorum*, *F. oxysporum,* and *F. redolens*, indicating that low levels of insensitivity to this fungicide do not decrease efficacy on these dry rot pathogens. In contrast, reduced sensitivity, as defined by

EC_{50} values from 5 to 100 mg/L, was not observed for *F. sambucinum* isolates. Gachango et al. (2012) also only detected thiabendazole resistance in *F. sambucinum*, but not in other *Fusarium* species they tested, *F. avenaceum*, *F. acuminatum*, *F. cerealis*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. sporotrichioides*, *F. solani*, *F. tricinctum*, and *F. torulosum*. In contrast to this study and Gachango et al. (2012), Ocamb et al. (2007) reported thiabendazole resistance in *F. oxysporum*, *F. solani*, *F. avenaceum*, *F. culmorum*, *F. equiseti* and *F. sporotrichioides*, based on the ability of isolates to grow on agar containing 5 mg/L thiabendazole. However, the Ocamb et al. (2007) study uses a different criterium to define "resistance", so the data among all three studies actually appear comparable. Thiabendazole resistance is only abundantly clear for the dry rot pathogen *F. sambucinum* in United States locations while other *Fusarium* species appear sensitive.

Laboratory fungicide screening results in a Petri dish predicted thiabendazole performance as a seed treatment when potato tubers were inoculated with *F. sambucinum* in all but one instance. Two thiabendazole-sensitive isolates caused similar dry rot incidence and severity to background levels, indicating that the thiabendazole treatment effectively reduced disease levels associated with these isolates. Out of four thiabendazole-resistant *F. sambucinum* isolates, three of them caused significantly greater dry rot incidence and severity than background levels. In fact, incidence and severity for these three isolates were the same as disease levels for the control treatment that was inoculated with *F. sambucinum* but not treated with thiabendazole, i.e., NTC-I FID 71-6, which demonstrated that the thiabendazole application for these three isolates was ineffective for disease reduction. However, one *F. sambucinum* isolate (D062) exhibited thiabendazole resistance *in vitro* but was controlled by thiabendazole in the seed treatment test using tubers, which is contradictory to the behavior of the other three *F. sambucinum* isolates. The lack of a strict relationship between *in vitro* fungicide resistance and decreased disease control may also indicate differences in aggressiveness among these isolates. This is not surprising as variability in aggressiveness on potato within and among *Fusarium* species has been observed in this study and by other researchers (e.g., Gachango et al., 2012; Stefańczyk et al., 2016). Whether these isolates differ in aggressiveness, are susceptible to thiabendazole under field conditions but not lab conditions or respond unexpectedly due to other factors warrants further study.

Looking at all four tested thiabendazole-resistant *F. sambucinum* isolates, the data indicated that *in vitro* sensitivity data do not necessarily indicate *in vivo* infection incidence and severity but that the laboratory screening can generally help predict whether the thiabendazole seed treatment will result in dry rot reduction on potato tuber seed pieces. This dataset shows that out of eight *F. sambucinum*

isolates tested (including the controls and consisting of three thiabendazole-sensitive and five thiabendazole-resistant isolates), only one isolate did not cause disease levels as expected based on the laboratory screening. Overall, this indicated that the laboratory fungicide screening is an effective tool to predict thiabendazole effectiveness as a potato seed treatment in the presence of *F. sambucinum*.

Thiabendazole is no longer recommended in the PNW as a Fusarium dry rot management fungicide due to the widespread resistance of the prevalent dry rot pathogen *F. sambucinum*, resistance which has been demonstrated by this study and many past studies. From a disease management perspective, the persistence of thiabendazole-resistant isolates as demonstrated here is cause enough to recommend continued omission of thiabendazole as a dry rot management fungicide, even if one or a few isolates in the pathogen population being considered may actually still be inhibited by the fungicide. This is undoubtedly the recommendation for Idaho potato production. However, it is notable that none of the tested *F. sambucinum* isolates from Washington exhibited thiabendazole resistance in the lab, and the Washington isolate included in the bag test (E089) was inhibited by the thiabendazole seed treatment as predicted by the lab screening result indicating thiabendazole sensitivity. Although Washington *F. sambucinum* isolates tested in this study were determined to be thiabendazole-sensitive, use of thiabendazole on this dry rot pathogen is still not recommended as development of resistance is likely inevitable. Concerning continued use of thiabendazole in dry rot control, Gachango et al. (2012) stated that specific knowledge of the *Fusarium* species present in a potato lot would be needed to support use of thiabendazole, and this recommendation is applicable to the PNW, as use of thiabendazole in the presence of *F. sambucinum*, whether the specific isolates were sensitive or resistant, would be counterproductive for dry rot management in the region.

Combined analyses of both years of field data indicated an apparent relationship between *in vitro* fungicide resistance and decreased control of dry rot in the field for many *F. sambucinum* isolates identified as fludioxonil-resistant in a laboratory sensitivity screening. Overall, field data indicated that sensitive isolates had less frequent and less severe seed piece decay than resistant isolates when a fludioxonil seed piece treatment was used, and that yields were somewhat greater for sensitive isolates than resistant isolates.

Interestingly, some isolates that were identified as fludioxonil-resistant *in vitro* displayed unexpected behavior in the field by causing less seed piece decay than other resistant isolates. Isolate D083, deemed resistant to fludioxonil in the lab, did not significantly differ in seed piece decay incidence and severity from isolates that were sensitive to fludioxonil, i.e., F060, D028, and F071 (Table 4-8).

Isolates A038, D050 and D077 showed fludioxonil resistance *in vitro* but caused less severe decay than expected with the amount of decay being statistically similar to decay severity of sensitive isolates. However, decay incidence was similar to most other resistant isolates. The unexpected behavior of these lab resistant isolates indicates that the *in vitro* fungicide sensitivity assay does not necessarily predict decreased fungicide efficacy in the field. The lack of a strict relationship between *in vitro* fungicide resistance and decreased disease control may also indicate differences in aggressiveness among these isolates. This is not surprising as variability in aggressiveness on potato within and among *Fusarium* species has been observed in this study and by other researchers (e.g., Gachango et al. (2012), Stefańczyk et al. (2016)). It has also been suggested that mutations in fungi conferring fludioxonil resistance induces a fitness penalty severe enough to result in effects such as reduced sporulation or decreased pathogenicity (Kilani and Fillinger 2016). Whether these isolates differ in aggressiveness, are susceptible to fludioxonil under field conditions but not lab conditions or respond unexpectedly due to other factors warrants further study.

Development of fludioxonil resistance among PNW *F. sambucinum* is possibly a contributing factor in the reported increase in dry rot in the PNW in recent years. Data from this field study indicated fludioxonil-resistant *F. sambucinum* isolates may increase dry rot incidence and severity in the field and decrease marketable and overall yield. *Fusarium sambucinum* is a prevalent and aggressive *Fusarium* dry rot pathogen in the PNW. To effectively manage dry rot in the face of fludioxonil-resistant *F. sambucinum* isolates, growers can take additional steps to minimize the risk associated with this newfound pathogen resistance in the PNW. When dry rot is identified by growers in their operations, submission of samples for pathogen identification and fungicide screening would be helpful in determining if fungicide resistance played a role in disease occurrence. Before planting, performing bag tests on every seed lot to assess dry rot potential and test the effectiveness of fungicide(s) planned for seed treatment may help avoid use of an ineffective fungicide product, especially if planning to use a fludioxonil product and in light of the distinct possibility that the seed lot may contain fludioxonil-resistant *F. sambucinum*.

Additionally, even though a storage component was not included in this study, it should be noted that this data may be applicable for storage situations as well. Fludioxonil-resistant *F. sambucinum* isolates present in the soil may adhere to tuber surfaces after harvest. The same fludioxonil inefficacy could be encountered in post-harvest fungicide application, and cultural practices promoting wound minimization and wound healing are especially important along with the choice of a post-harvest fungicide.

It should be reiterated that fludioxonil was the only active ingredient in the seed treatment used for this field experiment. In practice, use of fludioxonil in conjunction with other fungicide modes of action is strongly recommended, such as the multisite fungicide mancozeb or the Group 3 fungicide difenoconazole. Even if fludioxonil-resistant *F. sambucinum* isolates are present in a specific seed lot, field, or storage, the other fungicide in the mixture will most likely inhibit these isolates. For example, in recent tests, this study did not find difenoconazole resistance in PNW *F. sambucinum* isolates, and resistance to mancozeb has never been reported, including in various University of Idaho studies. Therefore, both of these fungicides are sound partner fungicides to fludioxonil for dry rot management.

Since the mechanism is still unsolved, the possibility of multiple paths to fludioxonil resistance by *Fusarium* can be considered, in that some isolates may obtain resistance from HAMP repeat modifications while others may have triosephosphate isomerase mutations. Considering a possible fitness penalty in basic sugar metabolism due to triosephosphate isomerase mutations, this idea of multiple paths conferring resistance is illustrated by the isolates in this study. Four Idaho *F. sambucinum* isolates (D083, A038, D050 and D077) that were identified as fludioxonil-resistant *in vitro* displayed unexpected behavior in the field, in that they caused less seed piece decay than other isolates with lab-detected resistance. Smaller lesions produce less mycelia, and this could be considered a fitness penalty. In theory, these four isolates may have possessed a triosephosphate isomerase mutation that may have reduced fitness of the isolates in the field while the other isolates with lab-detected resistance that caused greater incidence and severity of dry rot in the field may have possessed a HAMP repeat modification that conferred fludioxonil resistance without decreasing fitness. Investigation of the genetic and biochemical source of *Fusarium* resistance to fludioxonil could potentially provide information that would help avoid development of fludioxonil resistance in important potato dry rot pathogens and maintain greater efficacy of fludioxonil as a dry rot management fungicide.

Advantageously, very little field resistance has been documented within agriculture in the three decades since the release of fludioxonil as a fungicide (Kilani and Fillinger 2016). However, fludioxonil resistance may be developing within Fusarium dry rot pathogen populations in North America (this study, Gachango et al. 2012, Peters et al. 2008b). Based on data from the field trial described here, the lab-detected fludioxonil resistance appeared to reliably predict increased disease and reduced yield when the fungicide is used in the presence of resistant pathogens. Although the extent of *Fusarium* fludioxonil resistance in the PNW and the extent of effects on potato yield for individual growers and the industry as a whole is currently unknown, this dataset can be viewed as a

canary in the coal mine, an early indicator to drive a more intense effort to promote a) increased vigilance with wound management, b) appropriate fungicide application program design to reduce risk of fungicide resistance development, and c) identification of *Fusarium* species and fungicide resistance or sensitivity status when Fusarium dry rot does occur to facilitate future dry rot management efforts.

Literature Cited

Aprasad, K. S., Bateman, G. L., and Read, P. J. 1997. Variation in pathogenicity on potato tubers and sensitivity to thiabendazole of the dry rot fungus *Fusarium avenaceum*. Potato Res. 40:357–365.

Azil, N., Stefańczyk, E., Sobkowiak, S., Chihat, S., Boureghda, H., and Śliwka, J. 2021. Identification and pathogenicity of *Fusarium* spp. associated with tuber dry rot and wilt of potato in Algeria. Eur. J. Plant Pathol. 159:495–509.

Baturo-Ciesniewska, A., Lenc, L., Grabowski, A., and Lukanowski, A. 2015. Characteristics of Polish isolates of *Fusarium sambucinum*: molecular identification, pathogenicity, diversity and reaction to control agents. Am. J. Potato Res. 92:49–61.

Bissonnette, K. M., Wharton, P., Chen, J., and Marshall, J. M. 2018. Survey of *Fusarium* species associated with Fusarium head blight of spring wheat (*Triticum aestivum*) in southeastern Idaho. Plant Health Prog. 19:125–127.

Bohl, W. H., Nolte, P., and Thornton, M. K. 1992. Potato seed management: Seed certification and selection (CIS 974).

Bojanowski, A., Avis, T. J., Pelletier, S., and Tweddell, R. J. 2013. Management of potato dry rot. Postharvest Biol. Technol. 84:99–109.

Brandhorst, T. T., Kean, I. R. L., Lawry, S. M., Wiesner, D. L., and Klein, B. S. 2019. Phenylpyrrole fungicides act on triosephosphate isomerase to induce methylglyoxal stress and alter hybrid histidine kinase activity. Sci. Rep. 9:5047.

Brandhorst, T. T., and Klein, B. S. 2019. Uncertainty surrounding the mechanism and safety of the post-harvest fungicide fludioxonil. Food Chem. Toxicol. 123:561–565.

Burlakoti, P., Rivera, V. V., Burlakoti, R. R., Nelson, R., Adhikari, T. B., Secor, G. A., et al. 2010. Baseline sensitivity of *Fusarium* species associated with *Fusarium* diseases to metconazole, triticonazole, and thiabendazole fungicides. J. Sugar Beet Res. 47:23–34.

Carnegie, S. F., and Cameron, A. M. 1992. Resistance to thiabendazole in isolates of *Polyscytalum pustulans* (skin spot) and *Fusarium solani* var. *coeruleum* (dry rot) in Scotland. Plant Pathol. 41:606–610.

Choiseul, J., Allen, L., and Carnegie, S. F. 2007. Fungi causing dry tuber rots of seed potatoes in storage in Scotland. Potato Res. 49:241–253.

Corsini, D., and Pavek, J. J. 1986. Fusarium dry-rot resistant potato germplasm. Am. Potato J. 63:629–638.

Damicone, J. 2017. Fungicide resistance management. EPP-7663. Oklahoma State University, Oklahoma Cooperative Extension Service. 8 pages.

Desjardins, A. E. 1995. Population structure of *Gibberella pulicaris* (anamorph *Fusarium sambucinum*) from potato tuber dry rot in North America and Europe. Am. Potato J. 72:145–156.

Desjardins, A. E., Christ-Harned, E. A., McCormick, S. P., and Secor, G. A. 1993. Population structure and genetic analysis of field resistance to thiabendazole in *Gibberella pulicaris* from potato tubers. Phytopathology 83:164–170.

Du, M., Ren, X., Sun, Q., Wang, Y., and Zhang, R. 2012. Characterization of *Fusarium* spp. causing potato dry rot in China and susceptibility evaluation of Chinese potato germplasm to the pathogen. Potato Res. 55:175–184.

Estrada Jr, R., Gudmestad, N. C., Rivera, V. V., and Secor, G. A. 2010. *Fusarium graminearum* as a dry rot pathogen of potato in the USA: prevalence, comparison of host isolate aggressiveness and factors affecting aetiology. Plant Pathol. 59:1114–1120.

Fairchild, K. L., Miles, T. D., and Wharton, P. S. 2013. Assessing fungicide resistance in populations of *Alternaria* in Idaho potato fields. Crop Prot. 49:31–39.

Förster, H., Kanetis, L., and Adaskaveg, J. E. 2004. Spiral gradient dilution, a rapid method for determining growth responses and 50% effective concentration values in fungus-fungicide interactions. Phytopathology 94:163–170.

Gachango, E., Hanson, L. E., Rojas, A., Hao, J. J., and Kirk, W. W. 2012. *Fusarium* spp. causing dry rot of seed potato tubers in Michigan and their sensitivity to fungicides. Plant Dis. 96:1767–1774.

Gachango, E., Kirk, W., Hanson, L., Rojas, A., Tumbalam, P., and Shetty, K. 2011. First report of in vitro fludioxonil-resistant isolates of *Fusarium* spp. causing potato dry rot in Michigan. Plant Dis. 95:228–228.

Gallian, J. J., Miller, J. S., and Nolte, P. 2006. Managing fungicide resistance. CIS1130. University of Idaho. 8 pages.

Gudmestad, N. C., and Ivors, K. 2019. Development of fungicide resistance in potato and tomato pathogens. In Fungicide Resistance in North America, St. Paul: American Phytopathological Society, p. 295–308.

Hamilton, E. 2019. New mechanism of action found for agricultural pesticide fludioxonil. Univ. Wis.-Madison News. Available at: https://news.wisc.edu/new-mechanism-of-action-found-for-agricultural-pesticide-fludioxonil/ [Accessed May 5, 2022].

Hanson, L., Schwager, S., and Loria, R. 1996. Sensitivity to thiabendazole in *Fusarium* species associated with dry rot of potato. Phytopathology 86:378–384.

Hellin, P., Scauflaire, J., Van Hese, V., Munaut, F., and Legrève, A. 2017. Sensitivity of *Fusarium culmorum* to triazoles: impact of trichothecene chemotypes, oxidative stress response and genetic diversity. Pest Manag. Sci. 73:1244–1252.

Heltoft, P., Brurberg, M. B., Skogen, M., Le, V. H., Razzaghian, J., and Hermansen, A. 2016. *Fusarium* spp. causing dry rot on potatoes in Norway and development of a real-time PCR method for detection of *Fusarium coeruleum*. Potato Res. 59:67–80.

Hide, G. A., Read, P. J., and Hall, S. M. 1992. Resistance to thiabendazole in *Fusarium* species isolated from potato tubers affected by dry rot. Plant Pathol. 41:745–748.

Kawchuk, L. M., Holley, J. D., Lynch, D. R., and Clear, R. M. 1994. Resistance to thiabendazole and thiophanate-methyl in Canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani*. Am. Potato J. 71:185–192.

Kawchuk, L. M., Hutchison, L. J., Verhaeghe, C. A., Lynch, D. R., Bains, P. S., and Holley, J. D. 2002. Isolation of the β-tubulin gene and characterization of thiabendazole resistance in Gibberella pulicaris. Can. J. Plant Pathol. 24:233–238.

Kilani, J., and Fillinger, S. 2016. Phenylpyrroles: 30 years, two molecules and (nearly) no resistance. Front. Microbiol. 7 Available at: http://journal.frontiersin.org/article/10.3389/fmicb.2016.02014/full [Accessed February 14, 2022].

Langerfeld, E. 1990. Thiabendazole resistance in *Fusarium coeruleum*. Nachrichtenblatt Dtsch. Pflanzenschutzdiestes. 42:79.

Laraba, I., Busman, M., Geiser, D. M., and O'Donnell, K. 2022. Phylogenetic diversity and mycotoxin potential of emergent phytopathogens within the *Fusarium tricinctum* species complex. Phytopathology 112:1284–1298.

Merlington, A. A. 2014. Management options for control of Fusarium dry rot (*Fusarium* spp.) and potato common scab (*Streptomyces* spp.) of potato (*Solanum tuberosum* L.) in Michigan. M.S. thesis. Michigan State University.

Miller, J., Miller, T., Duellman, K., and Olsen, N. 2019. Managing Fusarium dry rot. Available at: https://millerresearch.com/wp-content/uploads/2019/04/Managing_Fusarium_Dry_Rot.pdf [Accessed May 5, 2022].

Nolte, P. 1994. Managing benzimidazole resistance in the potato dry rot fungus. EXT 769. University of Idaho Extension. 8 pages.

Ocamb, C. M., Hamm, P. B., and Johnson, D. A. 2007. Benzimidazole resistance of *Fusarium* species recovered from potatoes with dry rot from storages located in the Columbia Basin of Oregon and Washington. Am. J. Potato Res. 84:169–177.

Pasche, J., and Gudmestad, N. C. 2019. Laboratory methods for evaluating fungicide resistance in vitro. In Fungicide Resistance in North America, St. Paul, MN: American Phytopathological Society, p. 161–173.

Peters, J. C., Lees, A. K., Cullen, D. W., Sullivan, L., Stroud, G. P., and Cunnington, A. C. 2008a. Characterization of *Fusarium* spp. responsible for causing dry rot of potato in Great Britain. Plant Pathol. 57:262–271.

Peters, R. D., Macdonald, I. K., MacIsaac, K. A., and Woodworth, S. 2001. First report of thiabendazole-resistant isolates of *Fusarium sambucinum* infecting stored potatoes in Nova Scotia, Canada. Plant Dis. 85:1030–1030.

Peters, R. D., MacLeod, C., Seifert, K. A., Martin, R. A., Hale, L. R., et al. 2008b. Pathogenicity to potato tubers of *Fusarium* spp. isolated from potato, cereal and forage crops. Am. J. Potato Res. 85:367–374.

Peters, R. D., Platt, H. W., Drake, K. A., Coffin, R. H., Moorehead, S., et al. 2008c. First report of fludioxonil-resistant isolates of *Fusarium* spp. causing potato seed-piece decay. Plant Dis. 92:172–172.

Russell, P. E. 2004. Sensitivity baselines in fungicide resistance research and management. Available at: https://www.frac.info/docs/default-source/publications/monographs/monograph-3.pdf?sfvrsn=629d419a_8 [Accessed May 5, 2022].

Secor, G. A., and Salas, B. 2001. Fusarium dry rot and Fusarium wilt. In Compendium of Potato Diseases, St. Paul: The American Phytopathological Society, p. 23–25.

Stefańczyk, E., Sobkowiak, S., Brylińska, M., and Śliwka, J. 2016. Diversity of *Fusarium* spp. associated with dry rot of potato tubers in Poland. Eur. J. Plant Pathol. 145:871–884.

Wharton, P., Hammerschmidt, R., and Kirk, W. 2007a. Fusarium dry rot. E-2992. Michigan State University Extension Bulletin. 6 pages.

Wharton, P. S., Kirk, W. W., Berry, D., and Tumbalam, P. 2007b. Seed treatment application-timing options for control of Fusarium decay and sprout rot of cut seedpieces. Am. J. Potato Res. 84:237–244.

Chapter 5: Fusarium Dry Rot Management Recommendations for Pacific Northwest Potato Production

Summary of Findings

A recent reported increase in Fusarium dry rot in the Pacific Northwest (PNW) justified an investigation of potential contributing factors with the goal of improving Fusarium dry rot management in PNW such that disease levels are meaningfully reduced. This research study has characterized *Fusarium* diversity associated with Fusarium dry rot of potato in the PNW using molecular and phylogenetic techniques, confirmed pathogenicity of PNW *Fusarium* isolates to potato, assessed relative aggressiveness of prevalent PNW Fusarium dry rot pathogens to selected potato varieties, screened *Fusarium* species for sensitivity to fungicides typically used for dry rot management and investigated fungicide efficacy in both seed treatment application and a field trial.

Twenty *Fusarium* species were recovered in this survey of PNW tubers, 13 of which have not been previously reported in association with Fusarium dry rot of potato in the PNW: *F. acuminatum, F. cerealis*, *F. clavum*, *F. culmorum*, *F. flocciferum*, *F. gamsii*, *F. graminearum*, *F. iranicum*, *F. mori*, *F. redolens*, *F. stercicola*, *F. toxicum* and *F. venenatum*. *Fusarium sambucinum* was the most prevalent species, followed by *F. oxysporum*, confirming that these two species are still prevalent in the PNW. However, this survey indicated that several additional species may also be playing an important role in the development of potato dry rot in the region.

Pathogenicity to potato tuber was confirmed for 14 *Fusarium* species, *F. acuminatum*, *F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. equiseti*, *F. flocciferum*, *F. graminearum*, *F. oxysporum*, *F. redolens*, *F. sambucinum*, *F. solani*, *F. sporotrichioides*, *F. stercicola* and *F. venenatum*, with inoculation resulting in Fusarium dry rot disease symptoms. Eight species have not been previously reported as Fusarium dry rot pathogens in the PNW, *F. acuminatum, F. cerealis*, *F. culmorum*, *F. flocciferum*, *F. graminearum*, *F. redolens*, *F. stercicola*, and *F. venenatum*. Four species, *F. acuminatum*, *F. equiseti*, *F. solani*, *F. stercicola*, were determined to be weak pathogens based on infrequent lesion occurrence, small lesion size and statistical similarity to the negative control.

Pathogenicity tests showed that *Fusarium* species have differing levels of aggressiveness toward potato tuber, that aggressiveness can vary within a single species, and that aggressiveness can vary with potato variety. *Fusarium sambucinum* was determined to be the most aggressive of all tested species based on high disease incidence and severity. Although similarly high incidence to *F.*

sambucinum was observed for some species, i.e., *F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. oxysporum*, *F. redolens*, and *F. sporotrichioides*, lesion severity from *F. sambucinum* infection was much higher than all other species. Among other species with high incidence, *F. avenaceum* and *F. oxysporum* were more aggressive followed by *F. redolens* and *F. culmorum*, based on dry rot severity. Both *F. sambucinum* and *F. oxysporum* pose a threat to PNW potato production based on their prevalence in the PNW and ability to produce high incidence and intermediate to high severity of dry rot infections. However, the other tested species pose lower dry rot risk due to lack of aggressiveness or lower presence within the PNW *Fusarium* population.

Additional study of *Fusarium* aggressiveness was conducted based on species prevalence in the region and level of aggressiveness shown in pathogenicity testing. Seven potato varieties, i.e., Russet Burbank, Clearwater Russet, Umatilla Russet, Dark Red Norland, Ciklamen, Atlantic and Snowden, representing three potato types, i.e., russet, red, chipping, were challenged with four species of *Fusarium*, i.e., *F. sambucinum*, *F. oxysporum*, *F. culmorum* and *F. redolens*, to determine relative species aggressiveness based on disease severity.

The relative aggressiveness experiment showed the interaction between *Fusarium* species and potato variety to be significant. More severe *Fusarium* dry rot was observed with certain combinations of *Fusarium* species and potato variety, e.g., Umatilla Russet infected with *F. sambucinum* and Dark Red Norland infected with *F. oxysporum*. Notably, Clearwater Russet was less susceptible to *Fusarium* than expected in this experiment despite the reputation Clearwater has as being very susceptible to dry rot. When infected with *F. sambucinum*, Clearwater Russet was less susceptible than both Umatilla Russet and Russet Burbank while infection by *F. oxysporum* was less severe in Clearwater Russet than Russet Burbank. Knowledge of the identity of the *Fusarium* species in specific situations may assist in management of dry rot. Variety selection may play a role in alleviation of dry rot issues if a different variety can be utilized by a grower where a specific *Fusarium* species is causing disease.

Based on the dataset in this study, difenoconazole remains a viable dry rot management fungicide for the time being. Although *in vitro* screening tests detected some reduced sensitivity to difenoconazole based on the fungicide concentration threshold adopted, efficacy of difenoconazole as a seed treatment applied to seed pieces appeared to be mostly unaffected. A threshold EC_{50} value for which difenoconazole would be rendered ineffective on Fusarium dry rot pathogens was not established by this dataset. Fortunately, the PNW *Fusarium* population does not appear to have evolved a level of

insensitivity to difenoconazole that results in greater dry rot when difenoconazole was used as a seed treatment.

Presence of fludioxonil resistance among PNW *F. sambucinum* is possibly a contributing factor in the reported increase in dry rot in the PNW in recent years, and *F. oxysporum* fludioxonil resistance may play a role as well. As these two species are the most prevalent dry rot pathogens in the PNW based a recent survey and fludioxonil is a commonly used seed treatment in PNW potato production, identification of fludioxonil resistance among isolates of these two species is very concerning. *In vitro* resistance of *F. sambucinum* was found in 67% of isolates tested while 43% of *F. oxysporum* isolates tested were resistant to the fungicide. However, in an experiment testing efficacy of fludioxonil as a seed treatment, *in vitro* resistance of *F. oxysporum* to fludioxonil had much less impact on disease levels in fludioxonil-treated seed tubers than *F. sambucinum*. In the seed treatment bag tests, disease incidence in *F. sambucinum* treatments was 25 to 30% higher and disease severity was 45 to 60% higher than background levels, in contrast to fludioxonil-resistant *F. oxysporum* isolates, where disease levels did not differ from background levels. Therefore, currently, fludioxonil resistance in *F. sambucinum* appears more likely to negatively impact dry rot management than *F. oxysporum*. Data from the two-year field trial indicated fludioxonil-resistant *F. sambucinum* isolates may increase dry rot incidence and severity in the field and decrease marketable and overall yield when a fludioxonil seed treatment is used.

Overall, the laboratory fungicide screening was an effective tool to predict thiabendazole effectiveness as a potato seed treatment in the presence of *F. sambucinum* based on the seed treatment bag test data. Thiabendazole is no longer recommended in the PNW as a Fusarium dry rot management fungicide due to the widespread resistance of the prevalent dry rot pathogen *F. sambucinum*, resistance which has been demonstrated by this study and many past studies. From a disease management perspective, the persistence of thiabendazole-resistant isolates as demonstrated here is cause enough to recommend continued omission of thiabendazole as a dry rot management fungicide, even if one or a few isolates in the pathogen population being considered may actually still be inhibited by the fungicide.

Fusarium Dry Rot Management Recommendations

Overall, understanding which species are prevalent in the PNW region, occurrence of fungicide resistance, and relative aggressiveness of the most important *Fusarium* species and corresponding susceptibility of selected varieties have implications on management decisions, such as which seed

piece treatment to use, crop rotation decisions, and variety selection. These are the main findings from this study that can impact dry rot management in the PNW.

The diversity of Fusarium species pathogenic to potato in the PNW is greater than previously reported.

The prevalence and aggressiveness levels of *F. sambucinum* and *F. oxysporum* confirm that they continue to impart the greatest dry rot risk overall in the PNW. Some of the other *Fusarium* species are quite aggressive to potato but were not commonly encountered, indicating low risk of dry rot from these species generally but the possibility of high disease risk in specific situations where the pathogen is present. Other species were weak pathogens, and, even for those weak pathogens that are more prevalent, dry rot risk is low.

The observation of fludioxonil resistance in F. sambucinum and F. oxysporum, two prevalent dry rot pathogens in the PNW, is of particular concern as fludioxonil is a commonly used seed treatment fungicide for PNW dry rot management. Difenoconazole remains a viable dry rot management fungicide. Thiabendazole resistance in F. sambucinum persists.

Fludioxonil resistance has developed in a large proportion of *F. sambucinum* isolates, the most prevalent and aggressive dry rot pathogen in the PNW, and may be developing in another prevalent, somewhat aggressive species, *F. oxysporum*. Although inhibition of *F. oxysporum* with lab-detected fludioxonil resistance was observed using a fludioxonil seed treatment *in vivo*, this was not the case with *F. sambucinum*. Further, a field trial demonstrated that fludioxonil-resistant *F. sambucinum* isolates may increase dry rot incidence and severity in the field and decrease marketable and overall yield when a fludioxonil seed treatment is used.

To effectively manage dry rot in the face of fludioxonil-resistant *F. sambucinum* isolates, growers can take additional steps to minimize the risk associated with this newfound pathogen resistance in the PNW. When dry rot is identified by growers in their operations, submission of samples for pathogen identification and fungicide screening would be helpful in determining if fungicide resistance played a role in disease occurrence. Continual sampling of the pathogen population over time will help detect shifts in fungicide sensitivity more readily, especially for those fungicides to which resistance can develop gradually (e.g., difenoconazole) (Damicone 2017; Pasche and Gudmestad 2019; Russell 2004). Additionally, monitoring PNW *F. sambucinum* and *F. oxysporum* populations for fludioxonil and difenoconazole sensitivity will be helpful in characterizing the extent of fungicide resistance over time. Before planting, performing bag tests on every seed lot to assess dry rot potential and test the effectiveness of fungicide(s) planned for seed treatment may help avoid use of an ineffective

fungicide product, especially if planning to use a fludioxonil product and considering the distinct possibility that the seed lot may contain fludioxonil-resistant *F. sambucinum*. The bag test has been recommended for many years to help assess seed lots for dry rot potential prior to planting (Bohl et al. 1992; Nolte 1994). Also with seed lots, tuber samples could also be submitted, both with and without dry rot symptoms, to have any pathogens identified and fungicide sensitivity tested if warranted based on species identification. Although a grower may be unable to wait for results of laboratory testing before planting, the results of the grower-conducted bag tests will serve well to assist in selecting appropriate seed treatment for the seed lot. The laboratory tests will serve the role of continued monitoring of PNW *Fusarium* population for species prevalence and fungicide sensitivity.

Additionally, even though a storage component was not included in this study, it should be noted that this data may be applicable for storage situations as well. Fludioxonil-resistant *F. sambucinum* isolates present in the soil may adhere to tuber surfaces after harvest. The same fludioxonil inefficacy could be encountered in post-harvest fungicide application and cultural practices promoting wound minimization and wound healing are especially important along with the choice of post-harvest fungicide.

It should be reiterated that fludioxonil was the only active ingredient in the seed treatment used for the experiment. In practice, use of fludioxonil in conjunction with other fungicide modes-of-action is strongly recommended, such as the multisite fungicide mancozeb or the Group 3 fungicide difenoconazole (FRAC 2018, 2022). Even if fludioxonil-resistant *F. sambucinum* isolates are present in a specific seed lot, field, or storage, the other fungicides will most likely inhibit these isolates. For example, in recent tests, we did not find difenoconazole resistance in PNW *F. sambucinum* isolates, and resistance to mancozeb has never been reported, including in various University of Idaho studies. Therefore, both fungicides would be sound partner fungicides to fludioxonil for dry rot management.

It is important to keep in mind that reduction in fungal sensitivity to Group 3 fungicides like difenoconazole is known to develop gradually since multiple genes are involved in conferring resistance (Damicone 2017; Hellin et al. 2017). For the time being, continuing to screen PNW *Fusarium* isolates using the *in vitro* laboratory method demonstrated in this study would be a prudent way to monitor difenoconazole sensitivity of PNW dry rot pathogens, keeping in mind that EC_{50} values up to approximately 20 mg/L were shown to not result in increased dry rot when potato tubers were inoculated with those isolates in the bag test experiment conducted in this study. If observation of higher EC_{50} values begins to occur regularly in tested isolates, this may be an indication of resistance development in PNW *Fusarium*. At that time, additional bag test experiments may be

helpful in determining if the apparent decrease in fungicide sensitivity, i.e., EC_{50} values greater than 20 mg/L, is affecting efficacy as a potato seed treatment.

In conclusion, fungicide programs for dry rot management should aim to prevent further fludioxonil resistance development by using mixtures and alternating modes-of-action, placing high priority on wound management, never using fludioxonil alone, continuing to omit use of thiabendazole as a seed potato treatment, testing seed lots for dry rot potential and fungicide efficacy, and identifying the *Fusarium* species present in seed potatoes, field soil, and tubers at harvest along with their fungicide sensitivity. Gachango et al. (2012) have also suggested that fungicide choice can be guided by knowledge of the pathogen species in a given a situation.

The importance of some combinations of Fusarium species and potato variety with regard to dry rot severity was revealed by relative aggressiveness tests.

In similar laboratory studies of tuber inoculation with various *Fusarium* species resulting in dry rot lesions, the interaction of species and variety was also found to be significant (Azil et al. 2021; Peters et al. 2008a; Stefańczyk et al. 2016). Peters et al. (2008a) also specifically emphasized the value of knowing the identity of species present in specific situations to help assess the risk level for dry rot development and guide decision-making. For dry rot management, growers can consider the potato variety being produced in terms of which *Fusarium* species are present. Determining the species composition of *Fusarium* in field soil may help manage for both seed decay and storage rot, keeping in mind that soil-borne *Fusarium* could infect newly planted cut seed pieces and can also adhere to harvested tubers and infect new tuber wounds post-harvest.

For example, if field soil contains an abundance of *F. sambucinum*, varieties with a relatively high susceptibility to *Fusarium* should be avoided in the field, especially since *F. sambucinum* is known to be a highly aggressive species by many accounts. However, if fungicide sensitivity of those soil *F. sambucinum* isolates is determined, namely to fludioxonil and difenoconazole, then planting a more susceptible variety in that field may be less risky if a seed piece treatment with efficacy against those isolates is applied. For example, if the *F. sambucinum* isolates are susceptible to fludioxonil, then a fludioxonil-containing seed treatment mixture would be reasonable to apply. However, if the *F. sambucinum* isolates are fludioxonil-resistant, then application of an effective fungicide not containing fludioxonil would be advisable. This example scenario illustrates how the knowledge of *Fusarium* species in a particular situation can be used to better manage dry rot risk. Additionally, if the presence of a dry rot pathogen in field is known, a post-harvest fungicide application can be considered to reduce dry rot risk in storage, especially in situations where the storage time may be

longer. As another example, if PNW field soil contains weak dry rot pathogens such as *F. acuminatum* or *F. equiseti*, then planting a variety that is generally more susceptible to dry rot present lower dry rot risk.

Knowledge of *Fusarium* species could help in post-harvest. A post-harvest tuber sample could be tested for presence of *Fusarium* species using a wound induced inoculation of symptomless tubers. If an aggressive *Fusarium* species is found to be present on tuber surfaces, especially if the variety may be known to be more susceptible to *Fusarium*, then proactively selling those tubers first may help mitigate possible yield loss due to potential future disease in the storage. The same test of post-harvest tubers may mitigate possible yield loss even if a post-harvest fungicide was used. Any detected species in the stored tubers could also be tested for fungicide resistance to the post-harvest fungicide used. If resistance is detected, then moving that group of tubers to market sooner may be advantageous.

Other considerations

When growers observe dry rot in their fields, storages, or seed lots, submission of a representative sample to extension personnel would be helpful to monitoring the status of PNW dry rot. Samples could be processed to identify the pathogen species, screen for fungicide sensitivity and determine aggressiveness to different potato varieties. These samples may help identify if new issues are developing or if current issues are improving or worsening. For example, development of increased insensitivity to difenoconazole in *F. sambucinum* would necessitate a change in fungicide use to maintain better control over this prevalent and aggressive pathogen that already has shown resistance to fludioxonil and thiabendazole as well as a little reduced sensitivity to difenoconazole. Grower submitted samples may help identify other combinations of *Fusarium* species and potato variety that would be prudent to avoid, such as the the Dark Red Norland x *F. oxysporum* combination tested in this study. For Washington growers, development of fludioxonil resistance in *F. sambucinum* would be a key piece of information needed to continue effective dry rot management, and tuber samples providing evidence for continued *F. sambucinum* fludioxonil sensitivity would help alleviate concerns. Additionally, lack of recovery of *F. avenaceum* from dry rot samples would be helpful toward knowing that this aggressive dry rot pathogen is not gaining a foothold in the PNW potato growing regions of the world.

Future Research

Pathogenicity and Fungicide Sensitivity of Newly Observed Fusarium Species

During this study, six *Fusarium* species, *F. clavum*, *F. gamsii*, *F. iranicum*, *F. mori*, *F. stercicola*, and *F. toxicum*, were observed for the first time in association with potato dry rot in the PNW. However, their identification was confirmed late in the project due to recent updates in *Fusarium* taxonomy, and some questions remain regarding pathogenicity and fungicide sensitivity. Pathogenicity was tested for *F. stercicola* and *F. toxicum*, determining *F. stercicola* to be a very weak pathogen and *F. toxicum* to be non-pathogenic. However, the pathogenicity of *F. clavum*, *F. gamsii*, *F. iranicum* and *F. mori* to potato tuber is currently unknown and should be tested. Determining pathogenicity seems especially important for *F. gamsii* as, a) it accounted for 5% of PNW isolates and was only recovered from Idaho samples, and b) it is in the *Fusarium tricinctum* species complex, which contains species that are both strong and weak dry rot pathogens in the PNW. If any of these four species are dry rot pathogens, information on fungicide sensitivity may be useful for dry rot management. Data from this study indicated resistance to fludioxonil and reduced sensitivity to difenoconazole for *F. mori*. Although only one *F. mori* isolate was recovered, the potential pathogenicity of this species would be important to know given the fungicide insensitivity exhibited by the isolate.

Monitoring of Group 3 Triazole Seed Treatment Fungicides for Insensitivity and Cross-Resistance

Since both difenoconazole and prothioconazole are used in commercial formulations of potato seed treatment products, monitoring of PNW *Fusarium* isolates for sensitivity to these two fungicides would be prudent for early detection of potential cross-resistance. Based on generally known cross-resistance between fungicides in this chemical group, it is possible that insensitivity could develop in one fungicide or the other, which would possibly result in rendering both fungicides ineffective on *Fusarium* dry rot pathogens. Screening of the PNW *Fusarium* species for prothioconazole sensitivity is warranted along with representative seed treatment bag tests in cases of reduced sensitivity.

Characterization of Fludioxonil Resistance Mechanism in Five Pacific Northwest Fusarium Species

Fludioxonil resistance was detected *in vitro* in isolates of five PNW *Fusarium* species originating from tuber samples collected from all three states in the region (*F. coeruleum*, *F. mori*, *F. oxysporum*, *F. redolens*, *F. sambucinum*). Fludioxonil sensitivity for these five species was distinct, with the tested isolates being either decidedly resistant or sensitive as indicated by EC_{50} values well-above the threshold EC_{50} value of > 100 mg/L, which indicated resistance, or well-below the threshold EC_{50}

value of 5 mg/L, which indicated sensitivity. Distinct fludioxonil resistance or sensitivity was also observed in *F. sambucinum* and *F. oxysporum* isolates from Michigan (Gachango et al. 2012) and *F. sambucinum* and *F. coeruleum* isolates from Canada (Peters et al. 2008c) with no tested isolates exhibiting EC_{50} values between the 5 mg/L sensitivity threshold and the 100 mg/L resistance threshold. This lack of a continuum of sensitivity may indicate that the mutation needed to overcome the fludioxonil mode of action is a change in a single gene, i.e., the abrupt, qualitative resistance described by Damicone (2017). In light of a revised theory for fludioxonil target site and mechanism, this all-or-nothing resistance observed *in vitro* may be due to the catastrophic effect(s) fludioxonil has on sensitive isolates of *Fusarium*. Based on the Brandhorst et al. (2019) theory, the fungus may suffer from either an inability to meet basic energy needs due to a mutation in triosephosphate isomerase, thereby conferring resistance along with a severe fitness penalty, or an inability to catabolize a toxic by-product of sugar metabolism that increases to such high concentrations as to constitutively turn on the cell self-destruction mode.

The exact mechanism and target site of fludioxonil is not completely solved. The most recent mechanism theory states that fludioxonil indirectly triggers the HOG-1 pathway by creating a substantial stress state in the fungal cell, which ultimately leads to fungal cell death (Brandhorst and Klein 2019; Brandhorst et al. 2019). Brandhorst et al. (2019) report that fludioxonil sensitivity has been shown to disappear when HAMP repeats of the Group III HHK, which are unique from other HHKs, are modified or deleted, causing the HHK protein to become a constitutive kinase and no longer be able to be induced to become a phosphatase that ultimately activates the HOG1 self-destruction pathway (Brandhorst et al. 2019). The fludioxonil target site proposed by Brandhorst et al. (2019) is triosephosphate isomerase, a key enzyme in sugar metabolism. The interaction of the fludioxonil molecule may be the interface of the triosephosphate isomerase dimer, where fludioxonil may introduce steric interactions that cause one of the loops in triosephosphate isomerase to become inflexible, allowing the release of methylgloxal into the cytosol (Brandhorst et al. 2019). Methylglyoxal, an aldehydic stress molecule that is very reactive and damaging to DNA and cellular proteins, is produced on a normal basis during sugar breakdown in cells but is catabolized by enzymes before it becomes toxic and damaging (Hamilton 2019). The excess methylglyoxal induces group III HHK to convert from kinase to phosphatase, which then acts on Ypd1 to trigger HOG-1 pathway activation and fungal cell death (Brandhorst et al. 2019; Hamilton 2019).

The source of fludioxonil resistance in the PNW *Fusarium* isolates can be contemplated in light of this new theory proposed by Brandhorst et al. (2019). The isolates may be obtaining fludioxonil

resistance due to some modification of the HAMP repeats in the Group III HHK protein or a mutation in triosephosphate isomerase. However, since triosephosphate isomerase is a key sugar metabolism enzyme, a triosephosphate isomerase mutation that excludes interaction with fludioxonil may also induce a substantial fitness penalty to the mutated fungus due to reduction or other alteration in sugar metabolism that reduces the ability of the mutant to meet basic energy needs.

Since the mechanism is still unsolved, the possibility of multiple paths to fludioxonil resistance by *Fusarium* can be considered, in that some isolates may obtain resistance from HAMP repeat modifications while others may have triosephosphate isomerase mutations. Considering a possible fitness penalty in basic sugar metabolism due to triosephosphate isomerase mutations, circumstantial evidence from the data presented here suggest the possibility that some of the fludioxonil-resistant isolates identified in this work may have acquired fludioxonil resistance via a triosephosphate isomerase mutation. Four of twenty Idaho *F. sambucinum* isolates (D083, A038, D050 and D077) that were identified as fludioxonil-resistant *in vitro* displayed unexpected behavior in the field, in that they caused less seed piece decay than the other isolates with lab-detected resistance when fludioxonil was used. Smaller lesions produce less mycelia, and this could be considered a fitness penalty. In theory, these four isolates may have possessed a triosephosphate isomerase mutation that may have reduced fitness of the isolates in the field while the remaining isolates with lab-detected resistance that caused greater incidence and severity of dry rot in the field may have possessed a HAMP repeat modification that conferred fludioxonil resistance without decreasing fitness. Investigation of the genetic and biochemical source of *Fusarium* resistance to fludioxonil could potentially provide information that would help avoid development of fludioxonil resistance in important potato dry rot pathogens and maintain greater efficacy of fludioxonil as a dry rot management fungicide.

Literature Cited

Azil, N., Stefańczyk, E., Sobkowiak, S., Chihat, S., Boureghda, H., and Śliwka, J. 2021. Identification and pathogenicity of *Fusarium* spp. associated with tuber dry rot and wilt of potato in Algeria. Eur. J. Plant Pathol. 159:495–509.

Bohl, W. H., Nolte, P., and Thornton, M. K. 1992. Potato seed management: Seed certification and selection (CIS 974).

Brandhorst, T. T., Kean, I. R. L., Lawry, S. M., Wiesner, D. L., and Klein, B. S. 2019. Phenylpyrrole fungicides act on triosephosphate isomerase to induce methylglyoxal stress and alter hybrid histidine kinase activity. Sci. Rep. 9:5047.

Brandhorst, T. T., and Klein, B. S. 2019. Uncertainty surrounding the mechanism and safety of the post-harvest fungicide fludioxonil. Food Chem. Toxicol. 123:561–565.

Damicone, J. 2017. Fungicide resistance management. EPP-7663. Oklahoma State University, Oklahoma Cooperative Extension Service. 8 pages.

FRAC. 2018. Importance of multisite fungicides in managing pathogen resistance. Available at: https://www.frac.info/docs/default-source/publications/statement-on-multisite-fungicides/frac-statement-on-multisite-fungicides-2018.pdf?sfvrsn=3c25489a_2 [Accessed September 7, 2022].

FRAC. 2022. FRAC Code List 2022: Fungal control agents sorted by cross-resistance pattern and mode of action. Available at: https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2022--final.pdf?sfvrsn=b6024e9a_2 [Accessed May 5, 2022].

Gachango, E., Hanson, L. E., Rojas, A., Hao, J. J., and Kirk, W. W. 2012. *Fusarium* spp. causing dry rot of seed potato tubers in Michigan and their sensitivity to fungicides. Plant Dis. 96:1767–1774.

Hamilton, E. 2019. New mechanism of action found for agricultural pesticide fludioxonil. Univ. Wis.-Madison News. Available at: https://news.wisc.edu/new-mechanism-of-action-found-for-agricultural-pesticide-fludioxonil/ [Accessed May 5, 2022].

Hellin, P., Scauflaire, J., Van Hese, V., Munaut, F., and Legrève, A. 2017. Sensitivity of *Fusarium* culmorum to triazoles: impact of trichothecene chemotypes, oxidative stress response and genetic diversity. Pest Manag. Sci. 73:1244–1252.

Nolte, P. 1994. Managing benzimidazole resistance in the potato dry rot fungus. EXT 769. University of Idaho Extension. 8 pages.

Pasche, J., and Gudmestad, N. C. 2019. Laboratory methods for evaluating fungicide resistance in vitro. In Fungicide Resistance in North America, St. Paul, MN: American Phytopathological Society, p. 161–173.

Peters, J. C., Lees, A. K., Cullen, D. W., Sullivan, L., Stroud, G. P., and Cunnington, A. C. 2008a. Characterization of *Fusarium* spp. responsible for causing dry rot of potato in Great Britain. Plant Pathol. 57:262–271.

Peters, R. D., Platt, H. W., Drake, K. A., Coffin, R. H., Moorehead, S., et al. 2008b. First report of fludioxonil-resistant isolates of *Fusarium* spp. causing potato seed-piece decay. Plant Dis. 92:172–172.

Russell, P. E. 2004. Sensitivity baselines in fungicide resistance research and management. Available at: https://www.frac.info/docs/default-source/publications/monographs/monograph-3.pdf?sfvrsn=629d419a_8 [Accessed May 5, 2022].

Stefańczyk, E., Sobkowiak, S., Brylińska, M., and Śliwka, J. 2016. Diversity of *Fusarium* spp. associated with dry rot of potato tubers in Poland. Eur. J. Plant Pathol. 145:871–884.

Appendix A: Supplementary Information

Table A-1. *Fusarium* isolates collected from potato tuber samples from the Pacific Northwest.

Fusarium species	Isolate code	Growing region [a]	State [b]	County	Potato variety	Sample source [c]	Dry rot symptoms [d]	DNA locus [e]	Utilized in experiments [f]
acuminatum	E053	-	I	Unknown	Payette	S	Yes	*PHO*	-
acuminatum	E099	-	W	Unknown	Proprietary	S	Yes	*PHO*	PSY
acuminatum	B005	E ID	I	Bingham	Russet Burbank	C	Yes	*PHO*	Y
acuminatum	B050	E ID	I	Butte	Russet Burbank	S	Yes	*PHO*	PY
acuminatum	B057	E ID	I	Butte	Russet Burbank	S	Yes	*PHO*	SY
acuminatum	B060	E ID	I	Butte	Umatilla	S	Yes	*PHO*	-
acuminatum	C032	E ID	I	Fremont	Umatilla Russet	S	Yes	*PHO*	Y
acuminatum	D074	E ID	I	Custer	Ranger Russet	S	Yes	*PHO*	-
acuminatum	D096	E ID	I	Jefferson	Russet Burbank	C	Yes	*PHO*	SY
acuminatum	F087	E ID	I	Butte	Umatilla	S	Yes	*PHO*	Y
acuminatum	H074	E ID	I	Fremont	Unknown	S	No	*PHO*	-
avenaceum	A056	-	I	Unknown	Ranger Russet	C	Yes	*PHO*	-
avenaceum	C013	-	W	Unknown	Elfe	U	Yes	*PHO*	PSY
avenaceum	A020	E ID	I	Fremont	Princess	S	Yes	*PHO*	PSY
avenaceum	E072	E ID	I	Bingham	Purple Majesty	S	Yes	*PHO*	-
avenaceum	H038	E ID	I	Fremont	Unknown	S	No	*PHO*	PY
avenaceum	H075	E ID	I	Fremont	Unknown	S	No	*PHO*	-
cerealis	B089	-	W	Unknown	Chieftain	U	Yes		SY
cerealis	E011	-	W	Unknown	Elfe	U	Yes		BPS
cerealis	E090	-	W	Unknown	Proprietary	S	Yes		SY
cerealis	E092	-	W	Unknown	Proprietary	S	Yes		Y
cerealis	F007	-	W	Unknown	Unknown	C	Yes		P
cerealis	E068	E ID	I	Bingham	Purple Majesty	S	Yes		-
cerealis	E073	E ID	I	Bingham	Purple Majesty	S	Yes		SY
cerealis	E074	E ID	I	Bingham	Purple Majesty	S	Yes		BS
cerealis	E079	E ID	I	Bingham	Purple Majesty	S	Yes		-
cerealis	F044	E ID	I	Bingham	Purple Majesty	S	Yes		BPSY
clavum	G088	-	I	Unknown	Russet Norkotah	C	Yes		Y
clavum	H090	E ID	I	Bingham	Russet Norkotah	C	No		-
coeruleum	MR-6	-	I	-	Lab culture	-	-		SY
coeruleum	B085	E ID	I	Fremont	Russet Norkotah	S	Yes		SY
coeruleum	F046	E ID	I	Bingham	Dark Red Norland	S	Yes		Y
culmorum	B009	-	W	Unknown	Chieftain	U	Yes		Y
culmorum	B010	-	W	Unknown	Elfe	U	Yes		APS
culmorum	C050	-	I	Unknown	Almera	C	Yes		ABPS
culmorum	F020	-	W	Unknown	Unknown	C	Yes		Y

Fusarium species	Isolate code	Growing region [a]	State [b]	County	Potato variety	Sample source [c]	Dry rot symptoms [d]	DNA locus [e]	Utilized in experiments [f]
culmorum	G086	-	I	Unknown	Russet Norkotah	C	Yes		-
culmorum	A084	E ID	I	Fremont	Princess	S	Yes		-
culmorum	B001	E ID	I	Fremont	Princess	S	Yes		Y
culmorum	B030	E ID	I	Fremont	Dark Red Norland	C	Yes		-
culmorum	B087	E ID	I	Bingham	Russet Burbank	C	Yes		-
culmorum	B095	E ID	I	Bingham	Russet Burbank	C	Yes		-
culmorum	C021	E ID	I	Bingham	Russet Burbank	C	Yes		S
culmorum	C023	E ID	I	Fremont	Dark Red Norland	C	Yes	ITS	-
culmorum	F026	E ID	I	Bingham	Purple Majesty	S	Yes		Y
culmorum	F027	E ID	I	Bingham	Purple Majesty	S	Yes		-
culmorum	G094	MV	I	Minidoka	Mountain Gem	C	No		-
culmorum	H017	MV	I	Minidoka	Clearwater Russet	C	No		-
culmorum	H020	MV	I	Minidoka	Clearwater Russet	C	No		-
culmorum	H023	MV	I	Minidoka	Clearwater Russet	C	No		Y
culmorum	I002	TV	O	Union	Ranger Russet	S	No		BSY
culmorum	I022	TV	O	Union	Shepody	S	No		-
culmorum	I023	TV	O	Union	Alturas	S	No		ABPSY
equiseti	I016	-	I	Unknown	Russet Burbank	S	No	ITS	-
equiseti	I029	CB	W	Spokane	Russet Burbank	S	No	ITS	-
equiseti	A004	E ID	I	Butte	Ranger Russet	S	Yes	ITS	-
equiseti	A029	E ID	I	Fremont	Pacific Russet	S	Yes		SY
equiseti	B002	E ID	I	Butte	Russet Burbank	S	Yes		Y
equiseti	B059	E ID	I	Butte	Ranger Russet	S	Yes	ITS	S
equiseti	B092	E ID	I	Bingham	Russet Burbank	C	Yes	ITS	-
equiseti	C012	E ID	I	Madison	Dark Red Norland	S	Yes		PY
equiseti	C014	E ID	I	Butte	Russet Burbank	S	Yes	ITS	-
equiseti	D015	E ID	I	Fremont	Payette	S	Yes		Y
equiseti	H036	E ID	I	Fremont	Unknown	S	No		-
equiseti	I056	E ID	I	Fremont	Russet Burbank	S	No	ITS	-
equiseti	G028	MV	I	Power	Ranger Russet	C	Yes	ITS	-
equiseti	G097	MV	I	Minidoka	Clearwater Russet	C	No	ITS	-
equiseti	H021	MV	I	Minidoka	Clearwater Russet	C	No	ITS	-
equiseti	H022	MV	I	Minidoka	Clearwater Russet	C	No	ITS	-
equiseti	I012	MV	I	Power	Dark Red Norland	S	No	ITS	-
equiseti	I038	MV	I	Power	Umatilla Russet	S	No	ITS	-
equiseti	H033	SE ID	I	Bannock	Russet Burbank	C	Yes	ITS	-
equiseti	I003	TV	O	Union	Alturas	S	No	ITS	-
flocciferum	F068	-	W	Unknown	Unknown	C	Yes	*PHO*	P
flocciferum	F069	-	W	Unknown	Unknown	C	Yes	*PHO*	PY

Fusarium species	Isolate code	Growing region [a]	State [b]	County	Potato variety	Sample source [c]	Dry rot symptoms [d]	DNA locus [e]	Utilized in experiments [f]
flocciferum	G096	-	I	Unknown	Russet Norkotah	C	Yes	*PHO*	Y
flocciferum	B049	E ID	I	Fremont	Pacific Russet	S	Yes	*PHO*	Y
flocciferum	C007	E ID	I	Fremont	Pacific Russet	S	Yes	*PHO*	PY
flocciferum	C020	E ID	I	Fremont	Princess	S	Yes	*PHO*	Y
flocciferum	C053	E ID	I	Fremont	Princess	S	Yes	*PHO*	PY
flocciferum	C056	E ID	I	Fremont	All Blue	S	Yes	*PHO*	PSY
flocciferum	C058	E ID	I	Bingham	Russet Burbank	C	Yes	*PHO*	PS
flocciferum	F042	E ID	I	Bingham	Purple Majesty	S	Yes	*PHO*	-
flocciferum	H035	E ID	I	Fremont	Unknown	S	No	*PHO*	Y
gamsii	C028	-	I	Unknown	Almera	C	Yes	*PHO*	S
gamsii	A023	E ID	I	Butte	Umatilla	S	Yes	*PHO*	-
gamsii	A042	E ID	I	Fremont	Pacific Russet	S	Yes	*PHO*	Y
gamsii	A043	E ID	I	Butte	Ranger Russet	S	Yes	*PHO*	-
gamsii	B014	E ID	I	Fremont	All Blue	S	Yes	*PHO*	-
gamsii	B016	E ID	I	Fremont	All Blue	S	Yes	*PHO*	-
gamsii	B026	E ID	I	Butte	Russet Burbank	S	Yes	*PHO*	-
gamsii	B051	E ID	I	Fremont	All Blue	S	Yes	*PHO*	-
gamsii	C027	E ID	I	Fremont	Russet Burbank	S	Yes	*PHO*	SY
gamsii	C031	E ID	I	Fremont	Umatilla Russet	S	Yes	*PHO*	-
gamsii	D038	E ID	I	Fremont	Payette	S	Yes	*PHO*	Y
gamsii	F055	E ID	I	Bingham	Purple Majesty	S	Yes	*PHO*	-
gamsii	F063	E ID	I	Bingham	Dark Red Norland	S	Yes	*PHO*	Y
gamsii	H082	E ID	I	Fremont	Unknown	S	No	*PHO*	-
gamsii	H006	MV	I	Minidoka	Clearwater Russet	C	No	*PHO*	-
gamsii	H019	MV	I	Minidoka	Clearwater Russet	C	No	*PHO*	Y
graminearum	G098	E ID	I	Bingham	Challenger	S	No		PSY
graminearum	H048	E ID	I	Bingham	Challenger	S	No		-
iranicum	B065	E ID	I	Butte	Ranger Russet	S	Yes	*PHO*	-
iranicum	C015	E ID	I	Butte	Ranger Russet	S	Yes	*PHO*	Y
iranicum	C035	E ID	I	Butte	Ranger Russet	S	Yes	*PHO*	SY
iranicum	C095	E ID	I	Fremont	Alturas	S	Yes	*PHO*	Y
iranicum	D010	E ID	I	Fremont	Alturas	S	Yes	*PHO*	-
mori	B032	E ID	I	Butte	Ranger Russet	S	Yes		SY
oxysporum	D090	-	W	Unknown	Elfe	U	culture	ITS	-
oxysporum	D091	-	W	Unknown	Unknown	U	culture	ITS	-
oxysporum	E023	-	W	Unknown	Nadine	U	Yes		BSY
oxysporum	E080	-	W	Unknown	Proprietary	S	Yes		SY
oxysporum	E082	-	W	Unknown	Proprietary	S	Yes		SY
oxysporum	E083	-	W	Unknown	Proprietary	S	Yes		SY
oxysporum	E085	-	W	Unknown	Proprietary	S	Yes		-

Fusarium species	Isolate code	Growing region [a]	State [b]	County	Potato variety	Sample source [c]	Dry rot symptoms [d]	DNA locus [e]	Utilized in experiments [f]
oxysporum	F014	-	W	Unknown	Unknown	C	Yes	ITS	-
oxysporum	F032	-	W	Unknown	Unknown	C	Yes	ITS	-
oxysporum	F035	-	W	Unknown	Unknown	C	Yes		-
oxysporum	F058	-	W	Unknown	Unknown	C	Yes		Y
oxysporum	F079	-	W	Unknown	Unknown	C	Yes		Y
oxysporum	H042	-	W	Unknown	Unknown	S	Yes		Y
oxysporum	H043	-	W	Unknown	Unknown	S	Yes		-
oxysporum	H052	-	W	Unknown	Unknown	S	Yes		-
oxysporum	H056	-	W	Unknown	Unknown	S	Yes		-
oxysporum	H059	-	W	Unknown	Unknown	S	Yes		-
oxysporum	H061	-	W	Unknown	Unknown	S	Yes		-
oxysporum	I017	-	W	Unknown	Russet Burbank	S	No		Y
oxysporum	I009	CB	W	Spokane	Russet Burbank	S	No		ABPSY
oxysporum	B003	E ID	I	Fremont	All Blue	S	Yes		Y
oxysporum	B012	E ID	I	Fremont	Pacific Russet	S	Yes		-
oxysporum	B028	E ID	I	Fremont	Dark Red Norland	C	Yes		BSY
oxysporum	B083	E ID	I	Bingham	Russet Burbank	C	Yes		ABPSY
oxysporum	D098	E ID	I	Jefferson	Ciklamen	C	Yes		S
oxysporum	E003	E ID	I	Jefferson	Ciklamen	C	Yes	ITS	-
oxysporum	E004	E ID	I	Jefferson	Ciklamen	C	Yes	ITS	-
oxysporum	E028	E ID	I	Jefferson	Ciklamen	C	Yes		SY
oxysporum	G015	E ID	I	Bingham	Unknown	C	No	ITS	-
oxysporum	H003	E ID	I	Bingham	Challenger	S	No		Y
oxysporum	H086	E ID	I	Bingham	Russet Norkotah	C	No		-
oxysporum	H092	E ID	I	Bingham	Russet Norkotah	C	No		-
oxysporum	H095	E ID	I	Bingham	Russet Norkotah	C	No		-
oxysporum	I057	E ID	I	Bingham	Russet Norkotah	C	No		Y
oxysporum	I005	KB	O	Klamath	Russet Norkotah	S	No		ABPSY
oxysporum	G100	MV	I	Minidoka	Clearwater Russet	C	No		Y
oxysporum	F094	NW WA	W	Skagit	Unknown	C	Yes		Y
oxysporum	G004	NW WA	W	Skagit	Unknown	C	Yes		-
oxysporum	G005	NW WA	W	Skagit	Unknown	C	Yes		Y
oxysporum	G006	NW WA	W	Skagit	Unknown	C	Yes		-
oxysporum	G007	NW WA	W	Skagit	Unknown	C	Yes		-
oxysporum	G009	NW WA	W	Skagit	Unknown	C	Yes		-
oxysporum	I008	NW WA	W	Whatcom	Clearwater Russet	S	No		ABPSY

Fusarium species	Isolate code	Growing region [a]	State [b]	County	Potato variety	Sample source [c]	Dry rot symptoms [d]	DNA locus [e]	Utilized in experiments [f]
oxysporum	I053	SE ID	I	Caribou	Russet Burbank	S	No		ABPSY
oxysporum	I021	TV	O	Union	Shepody	S	No		ABPSY
redolens	I019	-	I	Unknown	Russet Burbank	S	No		PSY
redolens	I030	MV	I	Power	Dark Red Norland	S	No		APSY
redolens	I052	SE ID	I	Caribou	Russet Burbank	S	No		-
redolens	I007	TV	O	Union	Shepody	S	No		ABPSY
redolens	I028	TV	O	Union	Russet Burbank	S	No		BPSY
sambucinum	A054	-	I	Unknown	Ranger Russet	C	Yes		BS
sambucinum	A058	-	I	Unknown	Ranger Russet	C	Yes		-
sambucinum	A059	-	I	Unknown	Ranger Russet	C	Yes		-
sambucinum	A062	-	I	Unknown	Ranger Russet	C	Yes		-
sambucinum	A064	-	I	Unknown	Ranger Russet	C	Yes	ITS	-
sambucinum	B019	-	I	Unknown	Unknown	S	Yes		-
sambucinum	B020	-	I	Unknown	Unknown	S	Yes	ITS	-
sambucinum	B042	-	I	Unknown	Unknown	S	Yes		-
sambucinum	B044	-	I	Unknown	Unknown	S	Yes	ITS	-
sambucinum	C064	-	I	Unknown	Unknown	U	Yes		-
sambucinum	D048	-	I	Unknown	Payette	S	Yes		-
sambucinum	D050	-	I	Unknown	Payette	S	Yes		BFS
sambucinum	D052	-	I	Unknown	Payette	S	Yes		-
sambucinum	D059	-	I	Unknown	Payette	S	Yes	ITS	-
sambucinum	E017	-	W	Unknown	Elfe	U	Yes		Y
sambucinum	E089	-	W	Unknown	Proprietary	S	Yes		ABPSY
sambucinum	E100	-	W	Unknown	Proprietary	S	Yes		ASY
sambucinum	F001	-	W	Unknown	Proprietary	S	Yes		S
sambucinum	F003	-	W	Unknown	Proprietary	S	Yes		-
sambucinum	F004	-	W	Unknown	Proprietary	S	Yes		S
sambucinum	FID 212	-	I	Unknown	Lab culture	-	-		FS
sambucinum	FID 71-6	-	I	Unknown	Lab culture	-	-		ABFPS
sambucinum	G063	-	I	Unknown	Russet Norkotah	C	Yes		-
sambucinum	G084	-	I	Unknown	Russet Norkotah	C	Yes		-
sambucinum	G090	-	I	Unknown	Russet Norkotah	C	Yes		-
sambucinum	I051	-	I	Unknown	Russet Burbank	S	No		-
sambucinum	I010	CB	W	Lincoln	Russet Norkotah 296	S	No		BPSY
sambucinum	I041	CB	W	Spokane	Snowden	S	No		Y
sambucinum	A013	E ID	I	Butte	Umatilla	S	Yes		-
sambucinum	A014	E ID	I	Fremont	Russet Norkotah	S	Yes		-
sambucinum	A026	E ID	I	Fremont	Russet Norkotah	S	Yes		-

Fusarium species	Isolate code	Growing region [a]	State [b]	County	Potato variety	Sample source [c]	Dry rot symptoms [d]	DNA locus [e]	Utilized in experiments [f]
sambucinum	A031	E ID	I	Fremont	All Blue	S	Yes		S
sambucinum	A032	E ID	I	Fremont	Dark Red Norland	C	Yes		-
sambucinum	A035	E ID	I	Madison	Viviana	S	Yes		FS
sambucinum	A038	E ID	I	Butte	Umatilla	S	Yes		BFS
sambucinum	A046	E ID	I	Bingham	Russet Burbank	C	Yes	ITS	-
sambucinum	A068	E ID	I	Butte	Umatilla	S	Yes		-
sambucinum	A081	E ID	I	Butte	Umatilla	S	Yes		-
sambucinum	A086	E ID	I	Butte	Umatilla	S	Yes		-
sambucinum	A090	E ID	I	Fremont	All Blue	S	Yes		-
sambucinum	B011	E ID	I	Fremont	All Blue	S	Yes		-
sambucinum	B031	E ID	I	Fremont	Dark Red Norland	C	Yes		-
sambucinum	B034	E ID	I	Fremont	Umatilla Russet	S	Yes		-
sambucinum	B035	E ID	I	Fremont	Umatilla Russet	S	Yes		-
sambucinum	B037	E ID	I	Fremont	Umatilla Russet	S	Yes		-
sambucinum	B066	E ID	I	Butte	Umatilla	S	Yes	ITS	-
sambucinum	B077	E ID	I	Madison	Viviana	S	Yes		-
sambucinum	B086	E ID	I	Fremont	Umatilla Russet	S	Yes		-
sambucinum	B093	E ID	I	Butte	Umatilla	S	Yes		-
sambucinum	B097	E ID	I	Fremont	Russet Norkotah	S	Yes		S
sambucinum	C002	E ID	I	Fremont	Umatilla Russet	S	Yes		-
sambucinum	C003	E ID	I	Fremont	Umatilla Russet	S	Yes		-
sambucinum	C009	E ID	I	Fremont	Umatilla Russet	S	Yes		BFS
sambucinum	C011	E ID	I	Madison	Dark Red Norland	S	Yes		BFSY
sambucinum	C016	E ID	I	Butte	Umatilla	S	Yes		Y
sambucinum	C025	E ID	I	Fremont	Umatilla Russet	S	Yes		-
sambucinum	C029	E ID	I	Fremont	Umatilla Russet	S	Yes		-
sambucinum	C030	E ID	I	Fremont	Ranger Russet	S	Yes		ABFSY
sambucinum	C047	E ID	I	Fremont	Ranger Russet	S	Yes	ITS	-
sambucinum	C049	E ID	I	Fremont	Umatilla Russet	S	Yes		FS
sambucinum	C079	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	C080	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	C081	E ID	I	Fremont	Teton Russet	S	Yes		BFS
sambucinum	C082	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	C083	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	C084	E ID	I	Fremont	Teton Russet	S	Yes		BFS
sambucinum	C085	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	C086	E ID	I	Fremont	Teton Russet	S	Yes		FS
sambucinum	C087	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	C088	E ID	I	Fremont	Teton Russet	S	Yes	ITS	-
sambucinum	C089	E ID	I	Fremont	Teton Russet	S	Yes		-

Fusarium species	Isolate code	Growing region [a]	State [b]	County	Potato variety	Sample source [c]	Dry rot symptoms [d]	DNA locus [e]	Utilized in experiments [f]
sambucinum	C090	E ID	I	Fremont	Teton Russet	S	Yes		BFS
sambucinum	C092	E ID	I	Fremont	Teton Russet	S	Yes	ITS	-
sambucinum	C094	E ID	I	Fremont	Alturas	S	Yes		-
sambucinum	C097	E ID	I	Fremont	Unknown	S	Yes		-
sambucinum	C100	E ID	I	Fremont	Ranger Russet	U	Yes		BFS
sambucinum	D003	E ID	I	Fremont	Ranger Russet	U	Yes		-
sambucinum	D004	E ID	I	Fremont	Ranger Russet	U	Yes		-
sambucinum	D006	E ID	I	Fremont	Ranger Russet	U	Yes	ITS	-
sambucinum	D017	E ID	I	Fremont	Ranger Russet	U	Yes		-
sambucinum	D018	E ID	I	Fremont	Ranger Russet	U	Yes		FS
sambucinum	D019	E ID	I	Custer	Ranger Russet	S	Yes	ITS	-
sambucinum	D024	E ID	I	Fremont	Payette	S	Yes		FS
sambucinum	D025	E ID	I	Fremont	Payette	S	Yes		-
sambucinum	D026	E ID	I	Fremont	Teton Russet	S	Yes	ITS	-
sambucinum	D028	E ID	I	Custer	Ranger Russet	S	Yes		FSY
sambucinum	D034	E ID	I	Custer	Ranger Russet	S	Yes		-
sambucinum	D039	E ID	I	Fremont	Ranger Russet	U	Yes		-
sambucinum	D043	E ID	I	Custer	Ranger Russet	S	Yes		-
sambucinum	D062	E ID	I	Fremont	Teton Russet	S	No		BFS
sambucinum	D064	E ID	I	Fremont	Teton Russet	S	No		FS
sambucinum	D077	E ID	I	Fremont	Teton Russet	S	No		BFS
sambucinum	D080	E ID	I	Fremont	Teton Russet	S	No		-
sambucinum	D081	E ID	I	Fremont	Teton Russet	S	No		-
sambucinum	D083	E ID	I	Fremont	Payette	S	No		ABFS
sambucinum	D084	E ID	I	Fremont	Payette	S	No		-
sambucinum	E031	E ID	I	Fremont	Payette	S	Yes		-
sambucinum	E033	E ID	I	Fremont	Payette	S	Yes		-
sambucinum	E034	E ID	I	Fremont	Payette	S	Yes		-
sambucinum	E036	E ID	I	Fremont	Payette	S	Yes	ITS	-
sambucinum	E039	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	E041	E ID	I	Fremont	Teton Russet	S	Yes		FS
sambucinum	E042	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	E043	E ID	I	Fremont	Teton Russet	S	Yes	ITS	-
sambucinum	E044	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	E049	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	E051	E ID	I	Fremont	Payette	S	Yes		FS
sambucinum	E052	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	E058	E ID	I	Fremont	Dark Red Norland	S	Yes		-
sambucinum	E067	E ID	I	Bingham	Purple Majesty	S	Yes	ITS	-
sambucinum	F034	E ID	I	Bingham	Dark Red Norland	S	Yes		-

Fusarium species	Isolate code	Growing region [a]	State [b]	County	Potato variety	Sample source [c]	Dry rot symptoms [d]	DNA locus [e]	Utilized in experiments [f]
sambucinum	F040	E ID	I	Bingham	Dark Red Norland	S	Yes		-
sambucinum	F043	E ID	I	Bingham	Dark Red Norland	S	Yes		-
sambucinum	F045	E ID	I	Bingham	Dark Red Norland	S	Yes		-
sambucinum	F060	E ID	I	Bingham	Purple Majesty	S	Yes		ABFS
sambucinum	F061	E ID	I	Bingham	Dark Red Norland	S	Yes		-
sambucinum	F062	E ID	I	Bingham	Dark Red Norland	S	Yes	ITS	-
sambucinum	F066	E ID	I	Bingham	Dark Red Norland	S	Yes		-
sambucinum	F071	E ID	I	Bingham	Dark Red Norland	S	Yes		FS
sambucinum	G017	E ID	I	Bingham	Russet Burbank	C	Yes		Y
sambucinum	G019	E ID	I	Bingham	Russet Burbank	C	Yes		-
sambucinum	G021	E ID	I	Fremont	Teton Russet	S	Yes		-
sambucinum	G024	E ID	I	Teton	Clearwater Russet	S	No		Y
sambucinum	H037	E ID	I	Fremont	Unknown	S	No		-
sambucinum	I015	E ID	I	Teton	Russet Norkotah (Jorde line)	S	No		-
sambucinum	I020	E ID	I	Fremont	Russet Burbank	S	No		-
sambucinum	A041	MV	I	Cassia	Clearwater Russet	C	Yes		-
sambucinum	B053	MV	I	Cassia	Clearwater Russet	C	Yes		Y
sambucinum	D014	MV	I	Cassia	Russet Burbank	U	Yes		-
sambucinum	G030	MV	I	Power	Ranger Russet	C	Yes		-
sambucinum	G033	MV	I	Power	Ranger Russet	C	Yes		-
sambucinum	G034	MV	I	Power	Ranger Russet	C	Yes		-
sambucinum	G035	MV	I	Power	Ranger Russet	C	Yes		-
sambucinum	G038	MV	I	Power	Ranger Russet	C	Yes		-
sambucinum	G040	MV	I	Power	Ranger Russet	C	Yes		-
sambucinum	G041	MV	I	Power	Ranger Russet	C	Yes		Y
sambucinum	G045	MV	I	Power	Ranger Russet	C	Yes		-
sambucinum	G059	MV	I	Power	Ranger Russet	C	Yes		-
sambucinum	G065	MV	I	Power	Ranger Russet	C	Yes	ITS	-
sambucinum	G074	MV	I	Power	Ranger Russet	C	No	ITS	-
sambucinum	G075	MV	I	Power	Ranger Russet	C	No	ITS	-
sambucinum	G076	MV	I	Power	Ranger Russet	C	Yes		-
sambucinum	G093	MV	I	Minidoka	Mountain Gem	C	No		-
sambucinum	G095	MV	I	Minidoka	Mountain Gem	C	No		Y
sambucinum	H008	MV	I	Power	Ranger Russet	C	Yes	ITS	-
sambucinum	H013	MV	I	Minidoka	Mountain Gem	C	No		-
sambucinum	I013	SE ID	I	Caribou	Alturas	S	No		-
sambucinum	I018	SE ID	I	Caribou	Russet Burbank	S	No		Y
solani	E019	-	W	Unknown	Harmony	U	Yes		-

Fusarium species	Isolate code	Growing region [a]	State [b]	County	Potato variety	Sample source [c]	Dry rot symptoms [d]	DNA locus [e]	Utilized in experiments [f]
solani	E086	-	W	Unknown	Proprietary	S	Yes		-
solani	F028	-	W	Unknown	Unknown	C	Yes		Y
solani	F085	-	W	Unknown	Unknown	C	Yes		PY
solani	H064	-	W	Unknown	Unknown	S	Yes		Y
solani	A016	E ID	I	Butte	Ranger Russet	S	Yes		-
solani	H045	E ID	I	Bingham	Challenger	S	No		PY
solani	H050	E ID	I	Bingham	Challenger	S	No		-
solani	H096	E ID	I	Bingham	Russet Norkotah	C	No		-
solani	F091	NW WA	W	Skagit	Unknown	C	Yes		-
solani	F098	NW WA	W	Skagit	Unknown	C	Yes		-
solani	H025	SE ID	I	Bannock	Russet Burbank	C	Yes		PY
sporotrichioides	C059	E ID	I	Bingham	Russet Burbank	C	Yes		PSY
stercicola	E021	-	W	Unknown	Unknown	U	Yes		SY
stercicola	E087	-	W	Unknown	Proprietary	S	Yes		PY
stercicola	G085	-	I	Unknown	Russet Norkotah	C	Yes		Y
stercicola	F097	NW WA	W	Skagit	Unknown	C	Yes		Y
stercicola	G014	NW WA	W	Skagit	Unknown	C	Yes		Y
toxicum	B088	E ID	I	Bingham	Russet Burbank	C	Yes		PSY
toxicum	H099	E ID	I	Bingham	Russet Norkotah	C	No		Y
toxicum	I006	KB	O	Klamath	Russet Burbank	S	No		Y
venenatum	F009	-	W	Unknown	Unknown	C	Yes		PS
venenatum	F022	-	W	Unknown	Unknown	C	Yes		PSY
venenatum	G002	NW WA	W	Skagit	Unknown	C	Yes		PSY
venenatum	G003	NW WA	W	Skagit	Unknown	C	Yes		-

[a] Potato growing regions: CB = Columbia Basin, E ID = eastern Idaho, KB = Klamath Basin, MV = Magic Valley, NW WA = northwestern Washington, SE ID = southeastern Idaho, TV = Treasure Valley

[b] States: ID = Idaho, OR = Oregon, WA = Washington

[c] C = commercial storage, S = seed storage, U = unknown

[d] For samples with dry rot symptoms, isolations were made from lesion margins. For asymptomatic samples, isolations were made from lesion induced by artifical wounding.

[e] DNA sequences for the listed locus were deposited in NCBI GenBank.

[f] Selected isolates were utilized in various experiments. A = relative aggressiveness, B = seed treatment bag test, F = seed treatment field test, P = pathogenicity, S = screening for fungicide sensitivity, Y = phylogenetic analysis

Table A-2. Number of *Fusarium* isolates recovered from symptomatic dry rot tubers from commercial and seed storages in the Pacific Northwest by species and by potato growing region.

***Fusarium* species or species complex**	**E ID**		**SE ID**		**MV**		**TV**		**CB**		**KB**		**NW WA**		**Unknown**	
	No.	% [a]	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
acuminatum	9	4.9	0	0	0	0	0	0	0	0	0	0	0	0	2	2.6
avenaceum	4	2.2	0	0	0	0	0	0	0	0	0	0	0	0	2	2.6
cerealis	4	2.2	0	0	0	0	0	0	0	0	0	0	0	0	5	6.6
clavum	1	0.5	0	0	0	0	0	0	0	0	0	0	0	0	1	1.3
coeruleum	2	1.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
culmorum	9	4.9	0	0	4	11.8	3	42.9	0	0	0	0	0	0	5	6.6
equiseti	10	5.5	1	16.7	6	17.6	1	14.3	1	25	0	0	0	0	1	1.3
flocciferum	6	3.3	0	0	0	0	0	0	0	0	0	0	0	0	3	3.9
gamsii	13	7.1	0	0	2	5.9	0	0	0	0	0	0	0	0	1	1.3
graminearum	1	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
iranicum	3	1.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mori	1	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
oxysporum	14	7.1	1	16.7	1	2.9	1	14.3	1	25	1	50	7	53.8	19	25.0
redolens	0	0.0	1	16.7	1	2.9	2	28.6	0	0	0	0	0	0	1	1.3
sambucinum	98	53.8	2	33.3	20	58.8	0	0	2	50	0	0	0	0	26	34.2
solani	4	2.2	1	16.7	0	0	0	0	0	0	0	0	2	15.4	5	6.6
sporotrichioides	1	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
stercicola	0	0.0	0	0	0	0	0	0	0	0	0	0	2	15.4	3	3.9
toxicum	2	1.1	0	0	0	0	0	0	0	0	1	50	0	0	0	0
venenatum	0	0.0	0	0	0	0	0	0	0	0	0	0	2	15.4	2	2.6

Abbreviations: No. = number; % = percent; E ID = eastern Idaho; SE ID = southeastern Idaho; MV = Magic Valley; TV = Treasure Valley; CB = Columbia Basin; KB = Klamath Basin; NW WA = northwestern Washington

[a] The percent of the total number of isolates from the growing region resolved to *Fusarium* species.

Table A-3. Number of isolates recovered from symptomatic dry rot potato tubers in the Pacific Northwest by sample location and *Fusarium* species.

Sample location (state, county / potato growing region)	**Number of samples**	**Total number of *Fusarium* species**	**Isolates identified to *Fusarium* species by DNA sequencing of ITS, *TEF* and/or *PHO* loci**																					
			Total number for all samples by species																					
			Total number for all samples	*acuminatum*	*avenaceum*	*cerealis*	*clavum*	*coeruleum*	*culmorum*	*equiseti*	*floccifferum*	*gamsii*	*graminearum*	*iranicum*	*mori*	*oxysporum*	*redolens*	*sambucinum*	*solani*	*sporotrichioides*	*stercicola*	*toxicum*	*venenatum*
Idaho	69	19	252	10	5	4	2	2	14	19	7	16	1	3	1	17	3	137	5	1	1	2	0
Custer/ E ID	2	2	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
Bingham/ E ID	10	18	46	1	1	4	1	1	5	1	2	2	1	0	0	7	0	13	3	1	0	2	0
Butte/ E ID	3	7	22	4	0	0	0	0	0	4	0	3	0	1	1	0	0	8	1	0	0	0	0
Fremont/ E ID	23	10	100	2	3	0	0	1	4	4	4	8	0	2	0	3	0	68	0	0	0	0	0
Jefferson/ E ID	3	2	5	1	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
Madison/ E ID	2	2	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	0	0	0	0	0
Teton/ E ID	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Cassia/ MV	3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Minidoka/ MV	3	5	13	0	0	0	0	0	4	3	0	2	0	0	0	1	0	3	0	0	0	0	0
Power/ MV	4	3	18	0	0	0	0	0	0	3	0	0	0	0	0	0	1	14	0	0	0	0	0
Bannock/ SE ID	1	2	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Caribou/ SE ID	2	3	4	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0
Unknown	11	11	31	1	1	0	1	0	2	1	1	1	0	0	0	1	1	20	0	0	1	0	0
Oregon	7	5	9	0	0	0	0	0	3	1	0	0	0	0	0	2	2	0	0	0	0	1	0
Klamath/ KB	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Union/ TV	5	4	7	0	0	0	0	0	3	1	0	0	0	0	0	1	2	0	0	0	0	0	0
Washington	47	11	62	1	1	5	0	0	3	1	2	0	0	0	0	27	0	7	7	0	4	0	4
Lincoln/ CB	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Spokane/ CB	2	3	3	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0
Skagit/ NW WA	3	4	12	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	2	0	2	0	2
Whatcom/ NW WA	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Unknown	40	10	45	1	1	5	0	0	3	0	2	0	0	0	0	18	0	6	5	0	2	0	2

Abbreviations: ITS = internal transcribed spacer; TEF = translation elongation factor 1-alpha; PHO = phosphate permease; E ID = eastern Idaho; MV = Magic Valley; SE ID = southeastern Idaho; KB = Klamath Basin; TV = Treasure Valley; CB = Columbia Basin; NW WA = northwestern Washington

Table A-4. Number of *Fusarium* isolates recovered by potato variety from tubers collected from seed and commercial storages in the Pacific Northwest including tubers both symptomatic and asymptomatic for Fusarium dry rot.

		Number of *Fusarium* isolates																					
Potato variety (no. samples / no. tubers)	**No. tubers with *Fusarium* isolates**	*acuminatum*	*avenaceum*	*cerealis*	*clavum*	*coeruleum*	*culmorum*	*equiseti*	*flocciferum*	*gamsii*	*graminearum*	*iranicum*	*mori*	*oxysporum*	*redolens*	*sambucinum*	*solani*	*sporotrichioides*	*stercicola*	*toxicum*	*venenatum*	**Total no. *Fusarium* species**
All Blue (1/10)	6	-	-	-	-	-	-	-	1	3	-	-	-	1	-	3	-	-	-	-	-	4
Almera (1/1)	1	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	2
Alturas (4/6)	6	-	-	-	-	-	1	1	-	-	-	2	-	-	-	2	-	-	-	-	-	4
Challenger (1/2)	2	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	2	-	-	-	-	3
Chieftain (1/4)	2	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Ciklamen (1/4)	4	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	1
Clearwater R. (4/8)	7	-	-	-	-	-	3	3	-	2	-	-	-	1	-	3	-	-	-	-	-	5
Dark Red Norland (6/18)	15	-	-	-	-	1	1	2	-	2	-	-	-	1	1	13	-	-	-	-	-	7
Elfe (16/18)	5	-	1	1	-	-	1	-	-	-	-	-	-	1	-	1	-	-	-	-	-	5
Harmony (2/2)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
Mountain Gem (1/6)	3	-	-	-	-	-	1	-	-	-	-	-	-	-	-	3	-	-	-	-	-	2
Nadine (1/1)	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Pacific Russet (1/8)	4	-	-	-	-	-	-	1	1	1	-	-	-	1	-	-	-	-	-	-	-	4
Payette (4/23)	14	1	-	-	-	-	-	1	-	1	-	-	-	-	-	12	-	-	-	-	-	4
Princess (1/4)	3	-	1	-	-	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	3
Proprietary (8/17)	14	1	-	2	-	-	-	-	-	-	-	-	-	4	-	5	1	-	1	-	-	6
Purple Majesty (1/8)	8	-	2	3	-	-	2	-	1	1	-	-	-	-	-	2	-	-	-	-	-	6
Ranger Russet (8/55)	39	1	1	-	-	-	1	2	-	1	1	-	1	-	-	33	1	-	-	-	-	9
Russet Burbank (20/42)	23	4	-	-	-	-	3	6	1	2	-	-	-	2	2	4	1	1	-	2	-	11
Russet Norkotah (7/20)	15	-	-	-	2	1	1	1	1	-	-	-	-	5	-	8	-	-	1	1	-	9
Shepody (2/2)	2	-	-	-	-	-	1	-	-	-	-	-	-	1	1	-	-	-	-	-	-	3
Snowden (1/1)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
Teton Russet (4/35)	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28	-	-	-	-	-	1
Umatilla Russet (4/22)	19	3	-	-	-	-	-	1	-	2	-	-	-	-	-	18	-	-	-	-	-	3
Viviana (1/2)	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	1
Unknown (17/55)	36	1	2	1	-	-	1	-	3	1	-	-	-	16	-	7	2	-	3	-	4	11
Total no. potato varieties	**na**	6	5	5	1	2	14	9	7	11	2	1	1	13	5	16	6	1	3	2	1	na

Abbreviations: no. = number; "-" = 0 (count of zero); na = not applicable

Table A-5. Translation elongation factor 1-alpha (*TEF*) reference sequences utilized for the *Novel*, *Longipes* and *Brachygibbosum* Clades of the *Fusarium sambucinum* species complex (FSAMSC) within a phylogenetic analysis of *Fusarium* isolates associated with Fusarium dry rot of potato in the Pacific Northwest.

Clade name	Species name	NCBI GenBank accession number	Isolate number	Geographic origin	Host/substrate
Brachygibbosum	*F. brachygibbosum*	MW233198.1	FRC R-8851	Niger	pearl millet
Brachygibbosum	*F. transvaalense*	MW233161.1	FRC R-7052	Pennsylvania, USA	oat horse feed
Brachygibbosum	*F.* sp. nov.-23	MW233169.1	FRC R-7513	Texas, USA	sorghum soil debris
Brachygibbosum	*F.* sp. nov.-24	MW233212.1	NRRL 66936	China	soil
Brachygibbosum	*F.* sp. nov.-25	MW233186.1	NRRL 66928	Tunisia	olive grove soil
Brachygibbosum	*F.* sp. nov.-26	MW233154.1	NRRL 66923	North Dakota, USA	pasture soil debris
Brachygibbosum	*F.* sp. nov.-27	MW233193.1	NRRL 66930	Illinois, USA	corn soil
Brachygibbosum	*F.* sp. nov.-28	MW233217.1	NRRL 66939	China	soybean root
Brachygibbosum	*F.* sp. nov.-29	MW233194.1	FRC R-8779	Georgia, USA	sorghum soil debris
Brachygibbosum	*F.* sp. nov.-30	MW233215.1	NRRL 66937	Botswana	pearl millet
Brachygibbosum	*F.* sp. nov.-31	MW233221.1	NRRL 66919	Ghana	soybean roots
Brachygibbosum	*F.* sp. nov.-32	MW233216.1	NRRL 66938	Thailand	corn
Brachygibbosum	*F.* sp. nov.-33	MW233214.1	FRC R-9125	Australia	soil
Longipes	*F. longipes* 1	MW233060.1	NRRL 13368	Australia	soil
Longipes	*F. longipes* 2	MW233061.1	NRRL 13374	New Guinea	soil debris
Longipes	*F. longipes* 3	MW233074.1	NRRL 20723	United Kingdom	unknown
Longipes	*F. longipes* 4	MW233073.1	NRRL 20695	Florida, USA	soil debris
Longipes	*F.* sp. nov.-18	MW233160.1	NRRL 66924	Australia	soil debris
Longipes	*F.* sp. nov.-19	MW233203.1	NRRL 66932	Australia	grassland soil
Longipes	*F.* sp. nov.-20	MW233177.1	NRRL 66926	Thailand	rice paddy soil
Longipes	*F.* sp. nov.-21	MW233210.1	NRRL 66935	Australia	soil debris
Longipes	*F.* sp. nov.-22	MW233208.1	NRRL 66934	Nigeria	sorghum soil debris
Novel	*F.* sp. nov.-6	MW233091.1	NRRL 28066	Japan	corn
Novel	*F.* sp. nov.-7	MW233127.2	NRRL 46743	Ethiopia	corn

Table A-6. Translation elongation factor 1-alpha (*TEF*) reference sequences utilized for selected *Fusarium* species and species complexes within a phylogenetic analysis of the *Fusarium* genus using of *Fusarium* isolates associated with Fusarium dry rot of potato in the Pacific Northwest.

Species complex name	Clade name	Species name	NCBI GenBank accession number	Isolate number	Geographic origin	Host / substrate
F. babinda	n/a	*F. babinda*	MH742712.1	NRRL 25539	Australia	rainforest soil
F. burgessii	n/a	*F. beomiforme*	MF120507.1	NRRL 13606	Australia	pasture soil
F. burgessii	n/a	*F. beomiforme*	MF120508.1	NRRL 53548	Australia	soil
F. chlamydosporum	n/a	*F. chlamydosporum*	GQ505403	NRRL 13444	unknown	unknown
F. chlamydosporum	n/a	*F. nelsonii*	MW233255.1	NRRL 13338	unknown	unknown
F. concolor	n/a	*F. concolor*	MH742681.1	NRRL 13459	South Africa	plant debris
F. concolor	n/a	*F. verrucosum*	KM231940.1	CBS 102163	Venezuela	bamboo culm
F. heterosporum	n/a	*F. heterosporum*	MW928839.1	CBS 391.68	Germany	*Triticum* sp.
F. incarnatum-equiseti	Incarnatum	*F. caatingaense*	MN170449.1	CBS 976.97	USA	*Juniper chinensis*
F. incarnatum-equiseti	Incarnatum	*F. coffeatum*	MN120755.1	CBS 635.76	South Africa	*Cynodon lemfuensis*
F. incarnatum-equiseti	Incarnatum	*F. incarnatum*	MN170477.1	CBS 132907	Iran	*Triticum* sp.
F. incarnatum-equiseti	Incarnatum	*F. luffae*	MN170482.1	CBS 131097	Iran	*Setaria verticilata*
F. incarnatum-equiseti	Incarnatum	*F. nanum*	GQ505596.1	NRRL 22244	China	*Oryza* sp.
F. niskadoi	n/a	*F. gaditjirri*	MN193881.1	NRRL 45417	Australia	unknown
F. niskadoi	n/a	*F. miscanthi*	MN193878.1	NRRL 26231	Denmark	*Mischanthus*
F. niskadoi	n/a	*F. nisikadoi*	MN193879.1	NRRL 25179	Japan	*Phyllostachys nigra* var. *henonis*
F. redolens	n/a	*F. hostae*	MT409454.1	NRRL 29642	The Netherlands	hyacinth
F. redolens	n/a	*F. hostae*	MT409456.1	NRRL 29889	South Carolina, USA	hosta
F. sambucinum	*Brachygibb-osum*	*F. brachygibbosum*	MW233198.1	FRC R-8851	Niger	pearl millet
F. sambucinum	*Brachygibb-osum*	*F. transvaalense*	MW233161.1	FRC R-7052	Pennsylvania, USA	oat horse feed
F. sambucinum	*Longipes*	*F. longipes* 1	MW233060.1	NRRL 13368	Australia	soil
F. sambucinum	*Longipes*	*F. longipes* 2	MW233061.1	NRRL 13374	New Guinea	soil debris
F. sambucinum	*Novel*	*F.* sp. nov.-6	MW233091.1	NRRL 28066	Japan	corn
F. sambucinum	*Novel*	*F.* sp. nov.-7	MW233127.2	NRRL 46743	Ethiopia	corn
F. solani	Clade 1	*F. illudens*	AF178326.1	NRRL 22090	New Zealand	*Beilschmiedia tawa*
F. solani	Clade 1	*F. plagianthi*	AF178354.1	NRRL 22632	New Zealand	*Hoheria glabrata*
F. solani	Clade 2	*F. phaseoli*	AY220186.1	NRRL 22091	unknown	unknown
F. solani	Clade 2	*F. virguliforme*	AY220193.1	NRRL 31041	Illinois, USA	soybean
F. torreyae	n/a	*F. torreyae*	HM068337.1	NRRL 54149	Florida, USA	*Torreya* sp.
F. tricinctum	n/a	*F. tricinctum*	OL772834.1	NRRL 36254	Germany	wheat
F. tricinctum	n/a	*F. tricinctum*	OL772833.1	NRRL 25481	unknown	unknown