AN ABSTRACT OF THE THESIS OF

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Title: Presence and Pathogenicity of *Fusarium* and *Verticillium* Species in Commercial Red Radish (*Raphanus sativus*) Seed Production in the Willamette Valley of Oregon.

Abstract Approved:

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Commercial radish seed producers in the Willamette Valley of Oregon have observed a late season wilt in their seed fields. Twenty-two fields were surveyed for wilt in the Willamette Valley during June through August 2012 and 2013. Plants exhibiting wilt symptoms were collected from the fields and examined for vascular discoloration in the storage root; isolations were made when discolored vascular tissue was found. In 2012, *Fusarium* species were recovered from 11% of the 440 plants examined, while *V. dahliae* (=*V. longisporium*) was recovered from 0.2% of the 440 plants. In 2013, *Fusarium* and *Verticillium* species were recovered from 24% and 0.7% of plants, respectively. Greenhouse studies conducted in 2013 with 11 isolates of *F. oxysporum,* as well as one *V. dahliae,* and one *F. solani,* obtained from discolored storage roots in 2012, showed that all isolates evaluated have a degree of pathogenicity on red radish seedlings. This study confirms the presence of pathogenic *V. dahliae* and *F. oxysporum* in commercial radish seed fields in the Willamette Valley of Oregon. This is the first report of *F. solani* causing wilt symptoms on radish.

Some *Fusarium* and *Verticillium* species, including the species responsible for radish wilt in the Willamette Valley, have been found to seed-borne. *Fusarium* species were recovered from 0.352% of inbred stock seed treated with a 30 second dip in a 10% household bleach solution [0.006% NaClO] and 2.000% of nontreated stock seed while, no *V. dahliae* was recovered from stock seed surveyed when seeds were embedded in streptomycin amended Nash-Snyder and water agar mediums. *Fusarium proliferatum* and *F. oxysporum* accounted for 59% and 29% of the *Fusarium* species recovered from seed. Four strains of *F. proliferatum* and 3 *F. oxysporum* were evaluated in greenhouse studies during 2013 and all strains tested caused disease on red radish seedlings. To more closely examine the link

between wilting seed parent plants and the hybrid daughter seed they produce we surveyed the daughter seed of 35 randomly selected, commercially-grown radish seed parent plants with wilt in addition to the daughter seed of plants where *Fusarium or Verticillium* species were recovered from vascular discolorations in the storage root. *Fusarium oxysporum* was recovered from 12% of seed surveyed, while *F. culmorum* and *F. sambucinum* were recovered from 0.6% and 0.9%, respectively, of daughter seed surveyed. Forty-two percent of *F. oxysporum* strains recovered from daughter seed came from parent plants from which *F. oxysporum* was recovered from discolored vascular tissue. This study confirms that *F. oxysporum* can be associated with red radish seed and can be vertically transmitted. This is the first report of *F. proliferatum* as a pathogen on red radish and can be associated with seed.

Presence and Pathogenicity of *Fusarium* and *Verticillium* Species in Commercial Red Radish (*Raphanus sativus*) Seed Production in the Willamette Valley of Oregon.

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

Rachel A. Bomberger, Author

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CONTRIBUTION OF AUTHORS

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES Continued

AN INTRODUCTION TO RADISH AND THE PATHOGENS RESPONSIBLE FOR WILT IN RADISH, *FUSARIUM OXYSPORUM* AND *VERTICILLIUM DAHLIAE*

Radish belongs to the family, Brassicaceae, which contains 370 genera and over 3,000 species. In addition to the cultivated species, *Raphanus sativus*, there are multiple wild types, *R. raphanistrum* and *R. maritimus*, from both of which *R. sativus* was likely derived (69). Radish has been cultivated as far back as 4000 years ago. As a food crop, radish is renowned for its large storage root that is highly variable in regards to shape, size, color, and flavor; sprouted seeds and immature seedpods are also important food products. In addition to food crops, radish is grown for forage, fodder, oil, and as a cover crop (70). Despite, or maybe as a consequence of, its long history of cultivation, the geographic origin of commercial radish is unclear. From polymerase chain reaction (PCR) analysis, it is believed that there are multiple geographic origins; *R. maritimus* has a coastal European distribution while *R. raphanistrum* is found in across Europe, Asia, Africa, and North America (69, 70).

Radish as a cover crop can be used for managing soil compaction/hardpans through elongation and development of the storage root in no-till or reduced tillage systems (66, 69). Like other members of the Brassicaceae, radish produces high levels of secondary compounds like glucosinolates and the enzyme myrosinase, which can have biofumigant properties; as the plant tissue breaks down glucosinolates undergo a chemical reaction with myrosinase to release isothiocyanates, indoles, thiocyanates, nitriles, and oxozolidiinethions. These chemicals can reduce the populations of specific soilborne pathogens under certain conditions when sufficient concentrations are produced (44, 46, 60, 73).

The Pacific Northwest has an ideal climate for Brassica seed production, and currently produces up to 70% of the world's radish seed supply (16). For hybrid radish seed production in the Willamette Valley, inbred lines are either started in a greenhouse then transplanted into the field, or they are directly seeded into the field, commonly at three or four females for each male. Honeybees are brought in to pollinate plants, after which the males are cut down and the female plants mature and set seed. When 60-70% of the seedpods are brown and papery, stems are cut and seed heads are left to dry in the field as

windrows for around 10 days. The indehiscent seedpods are then threshed and the seed is separated from remaining pod debris (49, personal communication with growers).

A disease affecting seed production is wilt. More than one fungal pathogen could induce the wilt of red radish observed in the Willamette Valley, characterized by a darkening or necrosis of the vascular tissue in storage and fine roots as well as the stem. Foliar chlorosis may initially present on one side of the leaf, progressing until the affected leaves senesce, and eventually the plant may die (16). Radish yellows or Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *raphani,* has been reported in the United States as early as 1934, in Japan by 1952 (27), and in seed radishes in Washington State in 2003 (16). The disease caused by *F. oxysporum* is characterized by vascular browning and root rot, which likely causes the subsequent chlorosis of leaves and overall wilting and death of the plant (16, 27, 62). Fusarium wilt symptoms are often not seen until bolting and flowering, a time when demands are greater on the radish, even under ideal conditions, due to the resources needed to support the increased shoot growth, development of inflorescences, and seed set (17, 73).

A potential causal agent of the vascular wilt observed on radish seed crops in the Willamette Valley, *F. oxysporum* is known for its various *formae speciales* attributed to wilts and blights on many economically important crops (43). The *F. oxysporum* complex is characterized by sickle- or canoe-shaped septate macroconidia; hyaline, elliptic, single cell microconidia; and long-lived resting structures called chlamydospores that can persist longer than ten years (18, 23). *Fusarium oxysporum* is a soilborne species and both nonpathogenic and pathogenic strains are found across the globe (23). Some nonpathogenic *F. oxysporum* isolates can colonize roots of hosts without inciting disease due to either an inability to infect or because of the timely response of the host against the invading fungi before it can affect the health of the plant (23). The pathogenic wilt-forms typically cause vascular discoloration and wilting due to plugging of xylem vessels, which reduces the plant's ability to transport water (23, 58). *Fusarium oxysporum* is easily disseminated by soil movement, and possibly wind-borne microconidia and macroconidia (47). There is concern that the radish wilt pathogen may be seed-borne since many *Fusarium* wilt problems are known to be seed-borne; *F. oxysporum* f. sp. *raphani* is seed-borne on wild

and cultivated rocket (*Diplotaxis tenuifolia,* and *Eruca vesicaria*), *F. oxysporum* f. sp. *conglutinans,* which can be seed-borne on some *Brassica* species (16, 20).

Fusarium oxysporum has been described as cosmopolitan because of its ability to cause disease in over 200 species of monocotyledous and dicotyledous plants (43, 74). The cosmopolitan nature of this fungus is due in part to the 150 different *formae speciales* and numerous races reported (18, 23, 74). These *formae speciales* may appear morphologically identical and often share similar genetic makeup but there are small genetic differences as well as differing vegetative compatibility groups (VCG) which all may contribute to variations in virulence on closely related plant hosts (74). In as few as 30 *formae speciales* within *F. oxysporum,* 125 VCGs have been reported; isolates are said to be in the same VCG if when paired they are able to form a heterokaryon that results in normal aerial hyphae formation (74). The number of *formae speciales* and VCGs is extensive considering that there is no known sexual stage of *F. oxysporum*; the vast diversity of the fungus is currently attributed to mutation and selection factors (74). *Formae speciales* can be host-specific or infect numerous hosts, and when a broad range of hosts are affected, this presents an extra challenge when implementing a key cultural control method, crop rotation (74).

A known key to pathogenicity in strains of *F. oxysporum* f. sp. *lycopersici,* which cause wilt on tomato, are genes for effector proteins including necrosis- and ethylene-inducing peptides as well as enzymes believed to aid in the degradation and modification of plant cell walls, all of which make plant nutrients available to the fungus (43). The origin of these pathogenicity or effector genes is unknown but some hypothesize that a common ancestor acquired these genes as a result of genomic duplication or by horizontal gene transfer (43). Species of *Fusarium* that have less diverse host ranges, such as *F. graminearum* on cereals, may have lost some of these effector genes during vertical transmission of genes (43).

The different *formae speciales* of *F. oxysporum* are considered genetically and reproductively isolated, and are hypothesized to be evolving into so called ‘sibling’ species (4). The theory behind the evolution of host specific strains or sibling species is that there is a decreased amount of competition between strains and in turn, a reduction in the ability to outcross, limiting the risk of the fungal strain

losing fitness in sexual species (4). But, since *F. oxysporum* is asexual, this theory does not explain the evolution of these sibling species (4). Another hypothesis for the occurrence of host specific strains is that a novel pathogenic strain could arise from an existing strain through small mutations to the genome. Selection pressure helps pathogens overcome resistance genes in hosts, or enables the emergence of pathogens from a nonpathogenic population (23). *Fusarium oxysporum* f. sp. *raphani* has often been grouped as a race within *F. oxysporum* f. sp. *conglutinans*, a pathogen of many *Brassica* species. But unique VCGs, the resulting genetic isolation, an extremely narrow host range (limited to *Raphanus*, wild and cultivated rocket (*Diplotaxis tenuifolia,* and *Eruca vesicaria),* all suggest a unique *formae specialis* designation should be used at this time (4, 16, 20). Environmental conditions can play a role in the ability of *F. oxysporum* f. sp. *raphani* to infect a wide host range. *Fusarium oxysporum* f. sp. *raphani* did not cause disease on species in the cabbage tribe of the *Brassicaceae* at 24˚C, but when the temperature was increased to 28˚C, a few individuals that are not considered hosts were infected; resistance is overcome with increased temperature (4).

Another potential causal agent of vascular wilt in red radish is *Verticillium dahliae*. Both of these fungi, *Fusarium* and *Verticillium*, cause wilts with similar symptoms and rival each other in isolate diversity and economic importance. *Verticillium dahliae*, like *F. oxysporum*, is a cosmopolitan, soilborne fungus infecting over 200 different species of plants, and has six known VCGs (36, 63). Another parallel of *V. dahliae* to *F. oxysporum* as a wilt pathogen is that new wilt diseases are occurring on hosts that were once considered to not susceptible, especially when the plant is under stress (1). Diseased plants that were previously nonhosts, may exhibit symptoms that are less severe than the symptoms produced on the original plant host, possibly due to selection pressure for isolates to specialize and become hyper-aggressive on a specific plant species (1, 61). Lacking a sexual stage, *V. dahliae* relies heavily on recombination and genetic variation due to heterokaryon formation from VCGs to overcome host resistance genes and infect new hosts (1).

Verticillium dahliae is characterized by branched conidiophores with whorls capped with flask-shaped phialides that bear conidia (36). The symptoms common to *V. dahliae* include angular chlorotic areas on leaves that become necrotic, and a darkening of the vascular system due to accumulation of a

gum-like substance after hyphae have penetrated xylem vessels through pit pairs and perforation plates (63). *Verticillium dahliae* produces melanized microsclerotia that can remain viable in the soil for up to 13 years (68). Microsclerotia of *V. dahliae* can be seed-borne in lettuce, cotton, eggplant, tomato, safflower, sunflower and spinach (17, 36, 63,). Disease occurs after microsclerotia germinate and hyphae penetrate plant tissue, typically in the cortical tissue of roots. Conidia are produced in the xylem vessels, which can then infect neighboring vessels, eventually spreading disease throughout the host (36). After root colonization and prior to host death, conidia are produced on infected roots and contribute to secondary infections and spread of disease. However, the conidia produced by *V. dahliae* are not as adept as microsclerotia at long-term survival in the soil (24, 63).

Verticillium dahliae strains have a host preference in the sense that some strains cause more severe symptoms in certain plant species relative to others; VCG's are reported to explain this apparent host specificity of *V. dahliae* (61). Disagreements exists over the classification of the *Brassica*-infecting *V. dahliae* strain because of morphological differences; conidia are more elongate, microsclerotia are more diffuse than *V. dahliae*, the strain infecting *Brassica* species are amphihaploid, containing a complete haploid chromosome from each parent, rather than haploid as is typical of *V. dahliae*; these differences suggest a new species, *V. longisporum* (36). Similar to how *formae specialis* of *F. oxysporum* are thought to arise from the hybridizing of two different *F. oxysporum* strains, it is speculated that *V. longisporum* arose from the hybridization between *V. dahliae* and *V. ablo-atrum* (36). Other evidence that *V. longisporum* is a unique species includes the colonization of oilseed rape (*Brassica napus)*; after infection through the roots, *V. longisporum* colonizes the entire plant whereas *V. dahliae* was limited to the lower portion of the plant (73). In addition, *V. longisporum* invades neighboring xylem cells via plant cells plasmodesmata, which has not been observed in *V. dahliae* infections (73). However, the usage of *V. longisporum* is not universal; many prefer to group the pathogenic isolates of *Brassicas* into three separate molecular types and on their ability to produce different microsclerotia (9, 61). The three molecular types of the *Brassica* isolates are molecularly distinct from *V. albo-atrum*, a similar species that differs from *V. dahliae* by having melanized hyphae, rather than microsclerotia and a darkening at the base of the conidiophore at maturity (9, 17).

In addition to the presence of wilt, another contributing and complicating factor affecting radish seed yields in the Willamette Valley is the cabbage maggot (*Delia radicum*). Cabbage maggot larvae were found in the soil around the roots and in the roots themselves (16; Ocamb and Bomberger, data not shown, 2012-2013). Cabbage maggot is a member of order Diptera and family *Anthomyiidae* and is a pest on many *Brassica* crops and weeds (12, 44). The pest was introduced to North America from Europe as early as the 1880s (44). The larval stage impacts many crops due to the tunneling caused by the larva feeding. This tunneling results in cosmetic damage for many food crops but can also serve as an entryway for pathogens and other microbes (12). There are three to five generations of cabbage maggot a year, with higher population densities in the spring and fall compared to the summer with infested spring-planted fields serving as the maggot sources for fall-seeded fields (12). Cabbage maggots can overwinter in the soil as pupae to a depth of at least six inches; the pupae emerge as adults in the spring (12, 21). Since the pupae overwinter in soil, many growers will rotate a field out of *Brassica* crops to avoid the pest and limit the increase in cabbage maggot population (12). The female maggot flies that emerge from the overwintering pupae, mate within seven days and then begin to locate hosts for oviposition by the isothiocyanates present in the plants, chemicals produced by the breakdown of glucosinolates; there is a preference of radish for oviposition because of the plants' specific glucosinolate composition (12, 22, 30, 44). The white, oval eggs are laid near the host plant, hatch within three to seven days, and then the maggots begin to tunnel and feed on the host plant (12, 44). Maggots attack plants throughout bolting, flowering, and during seed set by feeding on the outer tissue of the roots and other tissues in contact with the soil (22, 30). Cabbage maggot-infested plants can have an appearance resembling wilt: reduced foliar growth and chlorosis, flagging, and wilting due to damage of the vascular tissues, which is especially noticeable during the hottest months and when plants are flowering. This vascular damage in cabbage can result in a seed yield reduction (21, 22). Cabbage maggot infestations are typically more severe during cool, wet springs (21). Chemical control of maggot is difficult since the maggot can have three to five generations per year, and few insecticides are available for use. Insecticide seed treatments have had mixed results; the use of some insecticides applied to seed as a film coating has been shown to protect transplants grown from seed for up to ten weeks (31). Cultural practices for cabbage maggot include crop

rotation, field sanitation, removal of other *Brassica* hosts, timing to avoid peak population densities, and trap cropping (31). There are also options such as using tar paper and rubber collars around the base of plants to prevent the deposition of eggs by female flies, but these methods would be extremely labor intensive and costly for the many thousands of individual plants in radish seed fields (31). Few insecticides are available and so there is the risk of developing insecticide resistance when only a few chemical options are available; recent work is focused more on the development of maggot-resistant cultivars (30).

The wilt observed in Willamette Valley red radish seed fields has gone unstudied despite likely being present for many years. The presence of fungal wilt pathogens or pests such as cabbage maggot often go unnoticed until the plants are under stress, even though the pathogens or pests may affect the vascular tissue earlier in the plant development. Symptoms of vascular distress look similar among the many causes, including; pathogens, insects, environment, and physiological problems. The objective of this thesis was to determine the cause of the red radish wilt in Willamette Valley seed fields. Commercial seed fields were surveyed during May through August 2012 and 2013, and plants were collected for laboratory evaluations. Seed was examined for the presence of pathogens known to cause wilt. Strains of potential pathogens isolated from seeds and storage roots were tested for pathogenicity on radish in greenhouse tests to confirm Koch's postulates.

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PRESENCE OF *FUSARIUM* AND *VERTICILLIUM* WILT IN RED RADISH (*RAPHANUS SATIVUS)* GROWN FOR SEED IN THE WILLAMETTE VALLEY OF OREGON

Abstract. Commercial radish seed producers in the Willamette Valley of Oregon have observed a late season wilt in their seed fields. Twenty-two fields were surveyed for wilt in the Willamette Valley during June through August 2012 and 2013. Plants exhibiting wilt symptoms were collected from the fields and examined for vascular discoloration in the storage root; isolations were made when discolored vascular tissue was found. In 2012, *Fusarium* species were recovered from 11% of the 440 plants examined, while *V. dahliae* (=*V. longisporum*) was recovered from 0.2% of the 440 plants. In 2013, *Fusarium* and *Verticillium* species were recovered from 24% and 0.7% of plants, respectively. Greenhouse studies conducted in 2013 with 11 isolates of *F. oxysporum*, as well as one *V. dahliae*, and one *F. solani*, obtained from discolored storage roots in 2012, showed that all evaluated have a degree of pathogenicity on red radish seedlings. This study confirms the presence of pathogenic *V. dahliae* and *F. oxysporum* in commercial radish seed fields in the Willamette Valley of Oregon. This is the first report of *F. solani* causing wilt symptoms on radish.

Introduction. Radish (*Raphanus sativus* L.) is a member of the Brassicaceae known for its fleshy root and unique taste; as much as 50% of red radish seed grown in the U.S. is produced in the Pacific Northwest (4). During the later part of the growing season, many commercial hybrid red radish seed growers in western Oregon observe a wilt of radish that upon closer examination is characterized by vascular discoloration, especially in the xylem vessel elements of the storage root and basal stem (4, Bomberger and Ocamb). Fusarium wilt, also known as radish yellows, caused by *Fusarium oxysporum* f. sp. *raphani*, was reported in seed radishes in Washington State (4). *Fusarium oxysporum* is a common soilborne organism that has both non-pathogenic strains and pathogenic strains (6). Pathogenic strains, which can consist of *formae specialis* considered to be host specific, may cause wilt on many economically important crops, and threat of Fusarium wilt in radish seed field requires 10 to 20 year rotations out of radish (3, 6, 8). *Verticillium dahliae* (Kleb.) (synonym=*V. longisporum)*, another economically important soilborne pathogen also causes a wilt of seed radishes (5, 12). In the Willamette Valley of Oregon, commercial radish seed fields have been experiencing late season wilting and death

from possibly either or both of the aforementioned pathogens. These wilt pathogens can be difficult to distinguish in the field and require a laboratory examination to determine what is causing the wilt symptoms. The objectives of this research were to determine: (i) what potential wilt pathogens were present in symptomatic plants in commercial seed fields located in the Willamette Valley and (ii) the pathogenicity of the *Fusarium* and *Verticillium* strains recovered on red radish.

Materials and Methods

Field Sampling. During May and June of 2012, early in host plant development before bolting and flowering, four sets of rows were randomly chosen and 10 plants were sampled at a random distance from the end of the row; each set consisted of 6 rows of seed parent and 2 rows of pollen parent (industry standard row arrangement for F1 hybrid seed). Plants that were showing stunting, chlorosis, wilting, or otherwise appeared unthrifty, were collected within each set of rows; plants healthy in appearance were also sampled. In May and June of 2013, 20 symptomatic plants per field were arbitrarily sampled prior to bolting, along with two plants that appeared healthy. Wilt symptoms were apparent during the later stages of plant development, bolting through seed set, during July and August of both years. Twenty symptomatic plants were arbitrarily collected while surveying the entire field, along with four plants that appeared healthy. Storage roots and basal stems of all plants sampled were placed into one-gallon plastic bags, transported to the laboratory in a cooler with ice packs, and then stored at 5˚C.

Storage root and basal stem sampling. Basal stems and storage roots were rinsed with cool tap water to remove soil and then air-dried on paper towels. Once dry, the stems and roots were cut longitudinally with a knife that was cleaned with 95% ethanol between each cut. The storage roots were examined for presence of vascular discoloration. When found, areas of discolored vascular tissue were scraped with a sterilized scalpel to expose underlying layers of cells, and then using another sterile scalpel, tissue was excised as two halves at each point of sampling. One half of the tissue sample was embedded in streptomycin-amended Nash-Snyder medium (1 g streptomycin sulfate liter^{-1}) (6, 9) and the other half was embedded in water agar (2% Difco® agar). After 21 days of incubating at room temperature (~24˚C), suspect *Fusarium* colonies on amended Nash-Snyder and *Verticillium* species on water agar were transferred onto streptomycin-amended half strength potato dextrose agar (sPDA)

(Difco® potato dextrose agar, 1 g streptomycin sulfate liter^{-1}); *Fusarium* species were also transferred to carnation leaf agar (CLA) (7). *Fusarium* cultures were incubated for 2 weeks under 3 fluorescent lights (General Electric 40-W tubes) and a black light (Sylvania 40-W tubes, BLB series) for a 12-hr photoperiod at room temperature (8) while *Verticillium* cultures were incubated in the dark at room temperature (11). Cultures were macroscopically examined; *Fusarium* (8, 10) and *Verticillium* (3) were identified to species based on morphology. *Fusarium* isolates were then single-spored while the *Verticillium* isolate was hyphal-tipped. When *Fusarium* was single-spored, sporodochia or colonized carnation leaves were placed into sterile 50-ml test tubes containing 3 mL of sterile RO water with a drop of 25% lactic acid and vortexed for 60 s. Approximately 1 ml of each suspension was poured onto each of three 3% water agar plates (Difco® agar). Plates were tilted to spread suspension, and left to grow at a slant for 12-18 hr. Single germinating spores were transferred to CLA and incubated for 4 weeks under the lighting described above. For *Verticillium*, a small agar plug of each strain was put onto potato dextrose agar (PDA) (Difco® agar) and allowed to grow in the dark for 24 hr. Individual hyphal tips were transferred to fresh PDA plates and incubated in the dark at room temperature.

Inoculum production. Fungal strains shown in Table 1 were used in pathogenicity tests and were representative of the potential pathogen species recovered from plant samples with vascular discoloration in addition to foliar symptoms of wilt. Agar plugs of each *Fusarium* isolate were placed in potato dextrose broth (PDB) (Difco® potato dextrose broth) and incubated for 14 to 18 days at room temperature on an Innova 2300® Platform Shaker (New Brunswick Scientific, Enfield, CT, USA) set at 90 rpm. The shaker was incidentally located 95 cm from the lighting previously described. Each culture flask was vacuum filtered using Whatman® #2 filter paper (GE Healthcare Bio-Sciences Corp, Piscataway, NJ, USA) inside a Buchner funnel attached to a sink faucet via plastic hosing. Contents on filter paper were washed three times with 100 ml aliquots of sterile RO water, resuspended in 100-ml of sterile RO water, blended with Tissue-Tearor® (Biospec Products INC, Bartlesville, OK, USA) at 5000 rpm for 1 minute and then conidia were collected by filtration through 100 µm Nitex® cloth. The spore suspension was adjusted to a concentration of $1x10^4$ conidia ml^{-1} as determined with hemacytometer counts (7). *Verticillium dahliae* was prepared by placing an agar plug onto PDA for 10 days at room

temperature in the dark; the agar surface was then washed with sterile RO water to dislodge the conidia and the conidia concentration was adjusted to $1x10^6$ (11). *Fusarium* inoculum was plated onto both sPDA and amended Nash-Snyder medium while the *V. dahliae* isolate was plated onto PDA as a dilution series to determine the colony forming units for each fungal strain.

Pathogenicity testing. Seeds of a susceptible hybrid red radish cultivar, Cherry Belle, and of a resistant cultivar, Alta Globe, were surface disinfested with a 3% hydrogen peroxide (VWR International, Radnor, PA, USA) solution for 60 min and then dried on sterile germination paper (Anchor Paper, Minneapolis, MN, USA) overnight in a Type II Biological Safety Cabinet (Baker Co., Sanford, ME, USA). Seeds were sown into green plastic, 6-inch pots (McConkey Co., Sumner, WA, USA) that had been steam pasteurized at 71˚C for 60 minutes followed by soaking in a 10% household bleach solution [0.6% NaClO] for 60 min. Pots were filled with a sandy loam soil that had been pasteurized at 115˚C for 45 min (per 1800 cc soil, arranged in a layer no thicker than 7.6 cm) on each of two consecutive days. For each fungal strain, there were three pots of the susceptible hybrid and one pot from the resistant hybrid, and each pot contained 15 seeds. A single pot of each hybrid variety served as a negative control for each experimental run. Ten days after sowing, 5 ml of inoculum was pipetted around the base of each plant while 5 ml of sterile RO water was applied to the control plants (7). After 21 to 28 days, the plants were examined using a rating system modeled after the P.H. Williams Interaction Phenotype (14). We used a 0-5 rating class to evaluate the aboveground portions of the plants for chlorosis and necrosis: 0=no symptomatic leaves, 1= one symptomatic leaf, 2= two symptomatic leaves, 3= three symptomatic leaves, 4= four symptomatic leaves or that all leaves were stunted or chlorotic, and 5= all of the leaves and the entire plant was dead. A 0 to 3 rating class was used to evaluate storage roots: 0= root appears healthy, 1= slight discoloration, 2= vascular discoloration in $\leq$ 50% of the storage root parenchyma tissue or discoloration in the vascular cambium, and 3= > 50% vascular discoloration. Plants inoculated with *V. dahliae* were rated using the system described above and also evaluated for the presence of microsclerotia. Symptomatic vascular tissue was then excised onto amended Nash-Snyder medium and WA for *Fusarium* and *Verticillium*, respectively; colonies were transferred to sPDA and CLA for identification to fungal species.

Results. Vascular discoloration was observed in all of the 22 fields examined during 2012 and 2013. During the 2012 growing season, 89% of symptomatic seed parents had vascular discoloration, 78% of symptomatic pollen parents had vascular discoloration, and 69% of healthy appearing seed parent plants were found to have vascular discoloration in storage root tissue (Table 2). In 2013, #% of symptomatic seed parents, #% of symptomatic pollen parents, and % of healthy appearing parent plants were found to have vascular discoloration (Table 2). *Fusarium* species were recovered more frequently from symptomatic plant tissue than *Verticillium* species; during 2012 *Fusarium* and *Verticillium* was recovered from 11% and 0.2% of plants sampled with vascular discoloration, respectively. While in 2013, 31% *Fusarium* and 1% *Verticillium* were recovered from plants sampled with vascular discoloration. *Fusarium oxysporum* and *F. solani* were both recovered from plant samples with vascular discoloration. Whereas *V. dahliae* was the only species of *Verticillium* recovered from the radish plants sampled. Cabbage maggot, *Delia radicum* L., was found in all the 22 fields examined during the two years (Table 2).

When representative fugal strains were examined in greenhouse pathogenicity studies (Table 1) on both *Fusarium* resistant and susceptible cultivars, the inoculated plants had generally a greater incidence of plants with foliar chlorosis and/or necrosis as well as vascular discoloration of the storage root compared to the non-inoculated control plants (Table 3). All strains of *Fusarium* used in greenhouse pathogenicity tests caused symptoms of wilt on the susceptible red radish cultivar in terms of leaf chlorosis and necrosis (Table 4, Figure 1) and necrosis of vascular tissue in storage roots (Table 5, Figure 2). Although the non-inoculated plants that serve as negative controls sometimes had foliar symptoms, usually only one or two leaves showed chlorosis or necrosis and rarely was vascular discoloration found in the storage root tissue (Tables 4 and 5). The control plants are most frequently have class 0 foliar ratings; the inoculated plants were most frequently rated as class 2, and the inoculated plants exhibited severity ratings greater than 2 more frequently for *F. oxysporum* strains Fo1, Fo4, and Fo6. Inoculated plants had 35% of storage root ratings of 2 or above; while non-inoculated controls had 93% of storage roots sampled in rating class 0 (Table 5). One strain of *F. oxysporum*, Fo5, did not cause observable symptoms on the *Fusarium* resistant cultivar, Alta Globe, but appeared aggressive on 'Cherry Belle.' All

other strains evaluated did incite vascular discoloration on both the susceptible cultivar, Cherry Belle, and the *Fusarium* resistant cultivar, Alta Globe, but overall less frequently on 'Alta Globe.' One 'Cherry Belle' negative control replicate set out of 9 runs had plants from which *Fusarium oxysporum* was recovered from discolored vascular tissue samples.

Discussion. This study confirms the suspected presence of Fusarium wilt in commercial radish seed fields located in the Willamette Valley of Oregon and the pathogen population may include *F. oxysporum* f. sp. *raphani.* However, since pathogenicity tests were not conducted on non-host species for *F. oxysporum* f. sp. *raphani*, it is possible that *formae specialis* of other *F. oxysporum*, such as *F. oxysporum* f. sp. *conglutinans*, are inciting wilt in the radish seed fields studied (2). *Verticillium dahliae* has been previously shown to be a pathogen of radish and through pathogenicity studies it was confirmed as a pathogen but this is the first report of *V. dahliae* on radish in Oregon (12, 13).

This is the first report of *Fusarium solani* being pathogenic on radish. *Fusarium solani* has been previously reported to cause vascular discoloration on horseradish, *Armoracia rusticana*, another member of the Brassicaceae (1). Our research found *Fusarium solani* in tissue samples from regions with vascular discoloration within the storage root of plants with wilt symptoms from certain commercial radish seed fields. In our pathogenicity greenhouse testing, *F. solani* caused disease on 36% of the susceptible 'Cherry Belle' plants and 14% of the resistant 'Alta Globe' plants while recovery of *F. solani* from samples with vascular discolorations was 100%, illustrating the potential for *F. solani* to invade vascular tissues of red radish.

Wilt of radish has been observed in the Willamette Valley prior to our research, and the threat of these diseases prompted rotations out of radish for 10+ years to minimize disease pressure because soilborne species with long-lived resting structures, like some *Fusarium* and *Verticillium* species, are difficult to control. While crop rotation may limit the build-up of disease inoculum, preventing the introduction of the pathogen through the use of clean seed and minimizing movement of soil should also be a focus of management practices to protect virgin soil.

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Table 1. Fungal species isolated in 2012 from storage roots of commercial red radish plants grown for seed and used in greenhouse pathogenicity studies on red radish during 2013

Strain ID	Oregon County	Species	CFU[a] counts[b]
Fo1	Lane	*Fusarium oxysporum*	--
Fo2	Lane	*Fusarium oxysporum*	4
Fo3	Lane	*Fusarium oxysporum*	200
Fo4	Lane	*Fusarium oxysporum*	12
Fo5	Lane	*Fusarium oxysporum*	--
Fo6	Linn	*Fusarium oxysporum*	7
Fo7	Linn	*Fusarium oxysporum*	72
Fo8	Linn	*Fusarium oxysporum*	200
Fo9	Yamhill	*Fusarium oxysporum*	130
Fo10	Yamhill	*Fusarium oxysporum*	229
Fo11	Linn	*Fusarium oxysporum*	361
Fs1	Yamhill	*Fusarium solani*	250
Vd1	Lane	*Verticillium dahliae*	150

[a] Colony forming units.
[b] As determined from a $1x10^3$ serial dilution; some isolates do not have this data.

Table 2. Recovery of *Fusarium* and *Verticillium* species from storage roots and incidence of storage root vascular discoloration in red radish inbred plants grown for seed in commercial plantings in the in the Willamette Valley of Oregon during 2012 and 2013

Year[a]	Oregon County	Field[b]	Parent[c]	Percent of plants sampled with vascular discoloration[d] (Total)[e]	Percent of plants with *Fusarium, Verticillium* species	Percent of plants sampled with maggot injury[f]
2012	Lane	1[d]	F	85 (20)	10,0	50
			F-A	75 (4)	25,0	25
2012	Lane	2	F	95 (19)	74,0	30
			F-A	75 (4)	0,0	50
2012	Lane	3	F	100 (20)	20,0	65
			F-A	67 (6)	0,0	67
2012	Lane	6	F	85 (52)	17,2	50
			F-A	33 (6)	17,0	33
			M	78 (9)	11,0	33
2012	Lane	7	F	82 (55)	18,0	62
			F-A	100 (6)	17,0	67
			M	75 (8)	38,0	50
2012	Lane	8	F	87 (52)	8,0	50
			F-A	33 (6)	17,0	50
			M	50 (8)	13,0	13
2012	Yamhill	12	F	88 (32)	6,0	100
			F-A	50 (4)	0,0	100
			M	88 (8)	13,0	88
2012	Yamhill	13	F	90 (30)	0,0	77
			F-A	100 (4)	0,0	75
			M	75 (8)	13,0	63
2012	Yamhill	14	F	93 (32)	6,0	97
			F-A	75 (4)	0,0	100
			M	100 (8)	0,0	100
2012	Linn	19	F	97 (31)	26,0	94
			F-A	100 (4)	0,0	75
2013	Lane	4	F	78 (58)	9,0	33
			F-A	50 (6)	0,0	0
			M	17 (6)	0,0	0

[a] 440 and 529 plants were sampled in 2012 and 2013 respectively from commercial red radish seed fields in Willamette Valley, OR.
[b] Plants were sampled at multiple phases over plant development for each respective year.
[c] F denotes symptomatic seed parent, F-A is asymptomatic seed parent, and M is symptomatic pollen parent. [d] Vascular discoloration in parenchyma tissue distal to cabbage maggot feeding injury.
[e] Number in parenthesis is the number of plants sampled.
[f] Evidence of cabbage maggot (*Delia radicum)* was determined by feeding tunnels or physical presence.

Table 2 Continued. Recovery of *Fusarium* and *Verticillium* species from storage roots and incidence of storage root vascular discoloration in red radish inbred plants grown for seed in commercial plantings in the in the Willamette Valley of Oregon during 2012 and 2013

Year[a]	Oregon County	Field[b]	Parent[c]	% of plants sampled with vascular discoloration[d] (Total)[e]	% of plants with *Fusarium, Verticillium* species	% of plants sampled with maggot injury[f]
2013	Lane	5	F	75 (57)	14, 4	35
			F-A	67 (6)	17	33
			M	43 (7)	0	29
2013	Lane	9	F	44 (32)	9	22
			F-A	0 (4)	25	50
			M	1 (4)	0	0
2013	Linn	10	F	85 (58)	45	83
			F-A	89 (9)	33	89
			M	63 (8)	0	50
2013	Linn	11	F	93 (59)	34	86
			F-A	88 (8)	13	38
			M	67 (6)	33	17
2013	Yamhill	15	F	90 (20)	60	40
			F-A	100 (2)	50	50
2013	Yamhill	16	F	90 (20)	55	75
			F-A	100 (2)	50	100
2013	Linn	17	F	100 (20)	20	36
			F-A	50 (2)	0	0
2013	Linn	18	F	100 (20)	60	63
			F-A	100 (2)	0	50
			M	100 (2)	0	0
2013	Linn	20	F	78 (60)	32	68
			F-A	75 (8)	50	63
			F	57 (7)	0	29
2013	Linn	21	F	100 (20)	60	100
			F-A	100 (2)	0	100
			M	100 (2)	0	100
2013	Clackamas	22	F	100 (10)	30	90
			F-A	100 (3)	0	0

[a] 440 and 529 plants were sampled in 2012 and 2013 respectively from commercial red radish seed fields in Willamette Valley, OR.
[b] Plants were sampled at multiple phases over plant development for each respective year.
[c] F denotes symptomatic seed parent, F-A is asymptomatic seed parent, and M is symptomatic pollen parent.
[d] Vascular discoloration in parenchyma tissue distal to cabbage maggot feeding injury.
[e] Number in parenthesis is the number of plants sampled.
[f] Evidence of cabbage maggot (*Delia radicum)* was determined by feeding tunnels or physical presence.

Table 3. Percentage of a susceptible red radish cultivar (S), Cherry Belle, and a *Fusarium*-resistant red radish cultivar (R), Alta Globe, with wilt symptoms in greenhouse studies

Strain ID	Red radish cultivar[a] (Total)	Foliar symptom disease severity index[b]	% of storage roots with vascular discoloration	Recovery of *Fusarium* or *Verticillium* species from plants (%)
Control	S	36	17	5.35
	R	34	17	2.2
Fo1	S	62	90	82
	R	53	100	100
Fo2	S	56	36	33
	R	41	13	13
Fo3	S	43	64	53
	R	51	18	18
Fo4	S	56	49	49
	R	76	33	22
Fo5	S	45	59	51
	R	29	9	0
Fo6	S	58	14	16
	R	58	38	13
Fo7	S	33	50	45
	R	25	33	33
Fo8	S	41	41	38
	R	30	20	20
Fo9	S	50	54	40
	R	40	25	13
Fo10	S	48	56	54
	R	50	50	36
Fo11	S	38	46	46
	R	23	14	14
Fs1	S	33	38	36
	R	37	14	14
Vd1	S	46	75	44
	R	51	36	27
Mix[c]	S	40	44	41
	R	37	27	7

[a] Strains were collected from storage roots of commercial red radish plants grown for seed.
[b] Foliar symptom disease severity index was calculated using the formula ∑[(class no.)*(plants in class)]/[(total number of plants)*(number of classes-1)].
[c] Inoculum composed of strains Fo9, Fo9, Fo11 and Fs1.
[d] Cultures were not developed by submission deadline.

Table 4. Percentage of red radish plants in each foliar chlorosis and necrosis severity rating class after inoculation with *Fusarium* or *Verticillium* species in greenhouse pathogenicity studies

		Severity class rating of foliar chlorosis and necrosis symptoms [c]					
Strain ID	**Red radish cultivar[a,b]**	**0**	**1**	**2**	**3**	**4**	**5**
Control	S	50	26	23	1	1	0
	R	60	16	22	0	2	0
Fo1	S	13	28	38	0	0	21
	R	13	50	25	0	0	13
Fo2	S	10	22	49	20	0	0
	R	27	40	33	0	0	0
Fo3	S	33	19	47	0	0	0
	R	18	9	73	0	0	0
Fo4	S	7	27	51	11	4	0
	R	0	0	44	33	22	0
Fo5	S	28	21	49	3	0	0
	R	73	9	18	0	0	0
Fo6	S	7	29	45	7	12	0
	R	0	33	44	22	0	0
Fo7	S	48	39	14	0	0	0
	R	73	27	0	0	0	0
Fo8	S	26	46	28	0	0	0
	R	50	50	0	0	0	0
Fo9	S	22	27	51	0	0	0
	R	50	0	50	0	0	0
Fo10	S	23	14	63	0	0	0
	R	0	50	50	0	0	0
Fo11	S	34	44	22	0	0	0
	R	86	14	0	0	0	0
Fs1	S	46	41	13	0	0	0
	R	43	29	29	0	0	0
Vd1	S	22	26	52	0	0	0
	R	9	27	64	0	0	0
Mix[d]	S	22	57	20	2	0	0
	R	53	33	0	0	13	0

[a] *Fusarium*-susceptible red radish cultivar, Cherry Belle (S).
[b] *Fusarium*-resistant cultivar, Alta Globe (R).
[c] Class 0= no chlorotic or necrotic leaves, class 1= one symptomatic leaf, class 2= two symptomatic leaves, class 3= three symptomatic leaves, class 4= four symptomatic leaves or all leaves are stunted or chlorotic, and class 5= all of the leaves and/or the entire plant are dead
[d] Inoculum composed of strains Fo9, Fo9, Fo11 and Fs1.

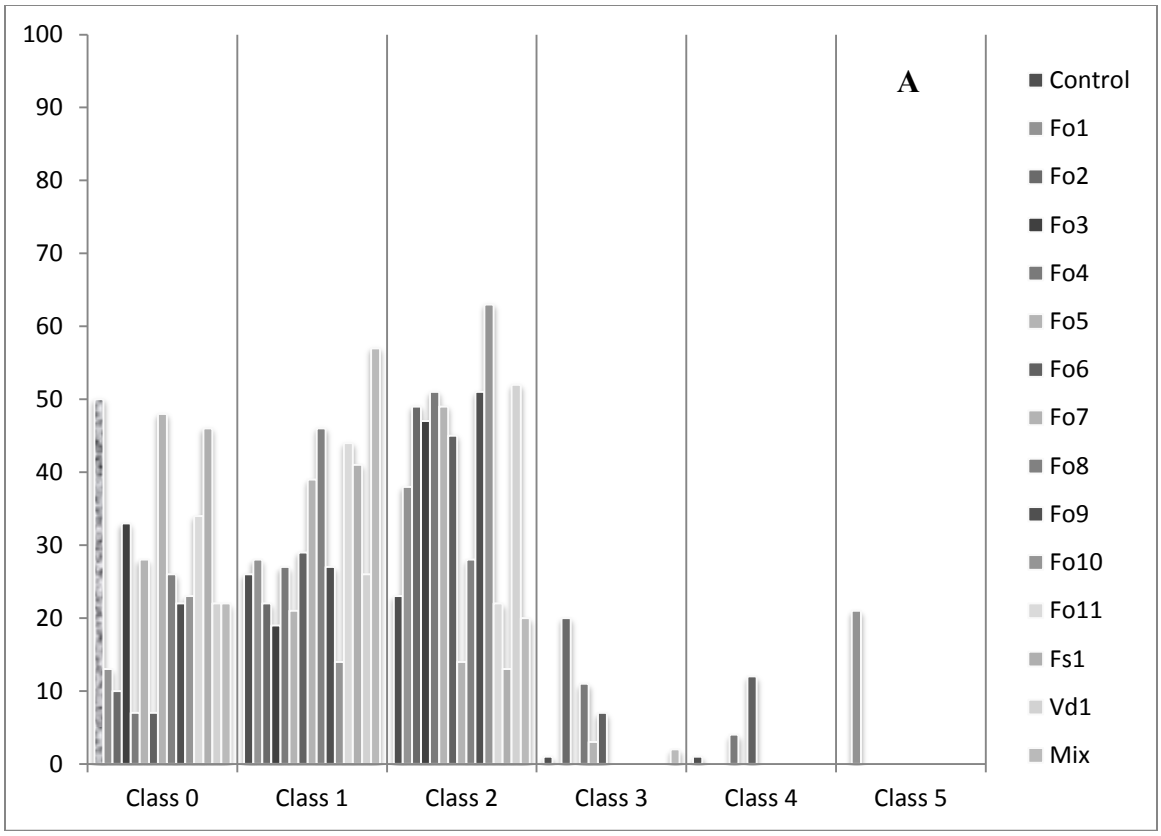

Figure 1. Percentage of (A) *Fusarium* susceptible (S) red radish cultivar, Cherry Belle, and (B) *Fusarium*-resistant (R) red radish cultivar, Alta Globe, plants inoculated with *Fusarium* or *Verticillium* species during greenhouse pathogenicity studies in each foliar chlorosis and necrosis severity rating class. Class 0= no chlorotic or necrotic leaves, class 1= one symptomatic leaf, class 2= two symptomatic leaves, class 3= three symptomatic leaves, class 4= four symptomatic leaves or all leaves are stunted or chlorotic, and class 5= all of the leaves and the entire plant are dead. Plants inoculated with "Mix" means plants were inoculated with inoculum composed of strains Fo9, Fo9, Fo11 and Fs1.

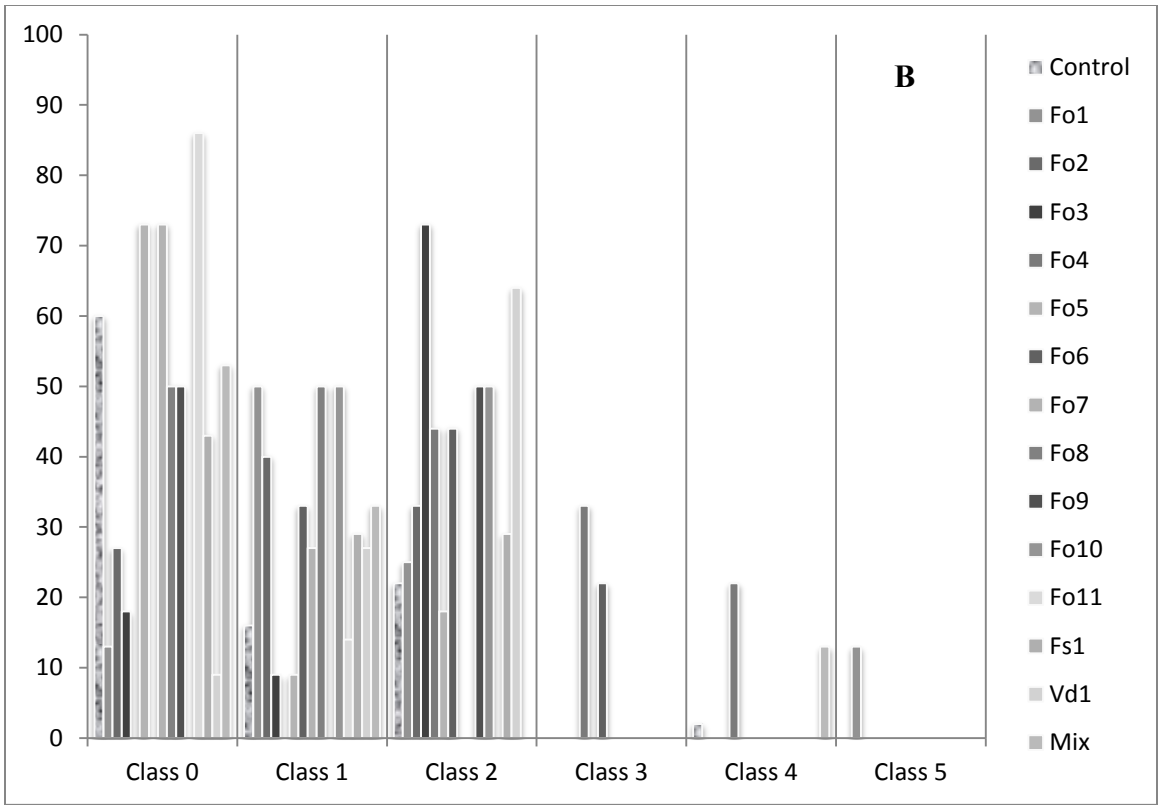

Figure 1 Continued. Percentage of (A) *Fusarium* susceptible (S) red radish cultivar, Cherry Belle, and (B) *Fusarium*-resistant (R) red radish cultivar, Alta Globe, plants inoculated with *Fusarium* or *Verticillium* species during greenhouse pathogenicity studies in each foliar chlorosis and necrosis severity rating class. Class 0= no chlorotic or necrotic leaves, class 1= one symptomatic leaf, class 2= two symptomatic leaves, class 3= three symptomatic leaves, class 4= four symptomatic leaves or all leaves are stunted or chlorotic, and class 5= all of the leaves and the entire plant are dead. Plants inoculated with "Mix" means plants were inoculated with inoculum composed of strains Fo9, Fo9, Fo11 and Fs1.

Table 5. Percentage of red radish plants in each storage root severity rating class after inoculations with *Fusarium* or *Verticillium* species in greenhouse pathogenicity studies .

		Severity class rating of storage root discoloration[c]			
Strain ID	**Cultivar[a,b]**	**0**	**1**	**2**	**3**
Control	S	81	12	7	0
	R	84	8	8	0
Fo1	S	10	10	62	18
	R	0	13	75	13
Fo2	S	64	5	31	0
	R	88	0	13	0
Fo3	S	36	39	25	0
	R	45	18	36	0
Fo4	S	51	4	44	0
	R	67	22	11	0
Fo5	S	36	31	33	0
	R	91	9	0	0
Fo6	S	86	7	7	0
	R	67	22	11	0
Fo7	S	50	5	45	0
	R	67	13	20	0
Fo8	S	59	0	38	3
	R	80	0	20	0
Fo9	S	44	17	37	2
	R	50	38	0	13
Fo10	S	46	11	4	0
	R	75	0	25	0
Fo11	S	54	0	46	0
	R	86	0	14	0
Fs1	S	64	3	31	3
	R	86	0	14	0
Vd1	S	25	0	75	0
	R	64	9	27	0
Mix[d]	S	57	2	37	4
	R	73	7	20	0

[a] *Fusarium*-susceptible red radish cultivar, Cherry Belle (S).
[b] *Fusarium*-resistant cultivar, Alta Globe (R).
[c] Class 0= root appears healthy, class 1= slight discoloration in storage root, class 2= vascular discoloration in ≤ 50% of the storage root parenchyma tissue or discoloration in the vascular cambium, and class 3= > 50% vascular discoloration in storage root parenchyma tissue.
[d] Inoculum composed of strains Fo9, Fo9, Fo11 and

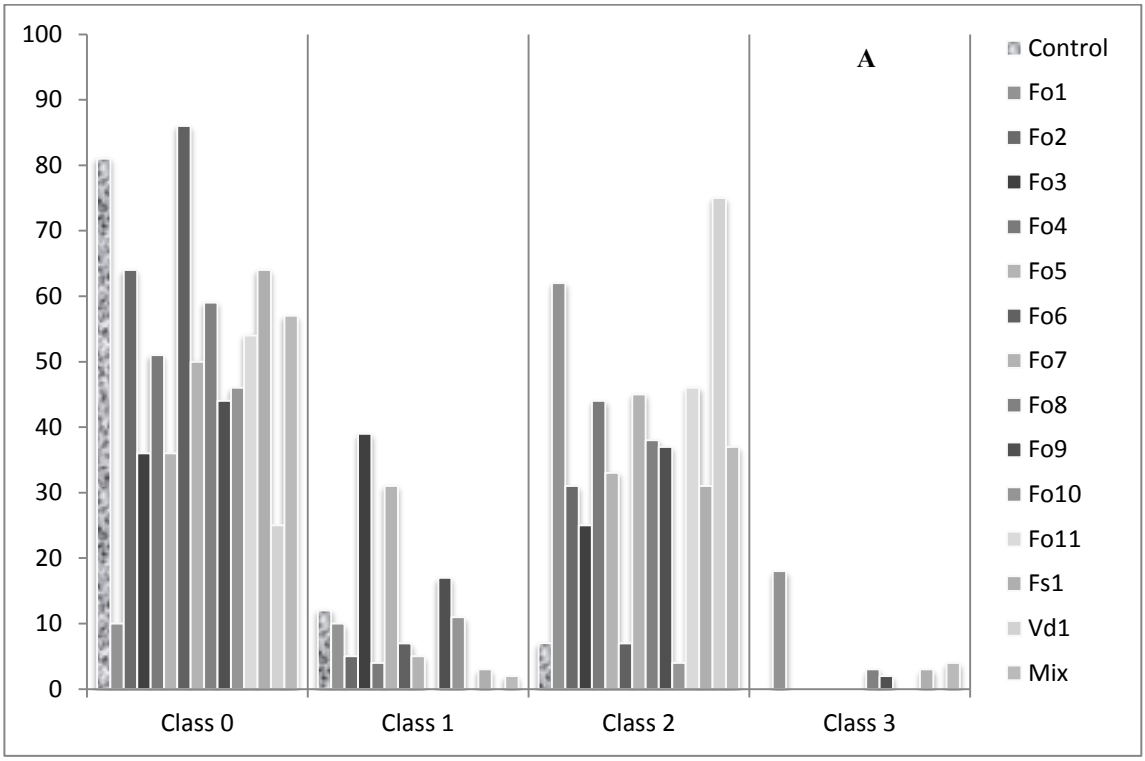

Figure 2. Percentage of (A) *Fusarium*-susceptible (S) red radish cultivar, Cherry Belle, and (B) *Fusarium*-resistant (R) red radish cultivar, Alta Globe, plants inoculated with *Fusarium* or *Verticillium* species during greenhouse pathogenicity studies in each storage root vascular discoloration severity rating class. Class 0= root appears healthy, class 1= slight discoloration in storage root, class 2= vascular discoloration in ≤ 50% of the storage root parenchyma tissue or discoloration in the vascular cambium, class 3= > 50% vascular discoloration in storage root parenchyma tissue. Plants inoculated with "Mix" means plants were inoculated with inoculum composed of strains Fo9, Fo9, Fo11 and Fs1.

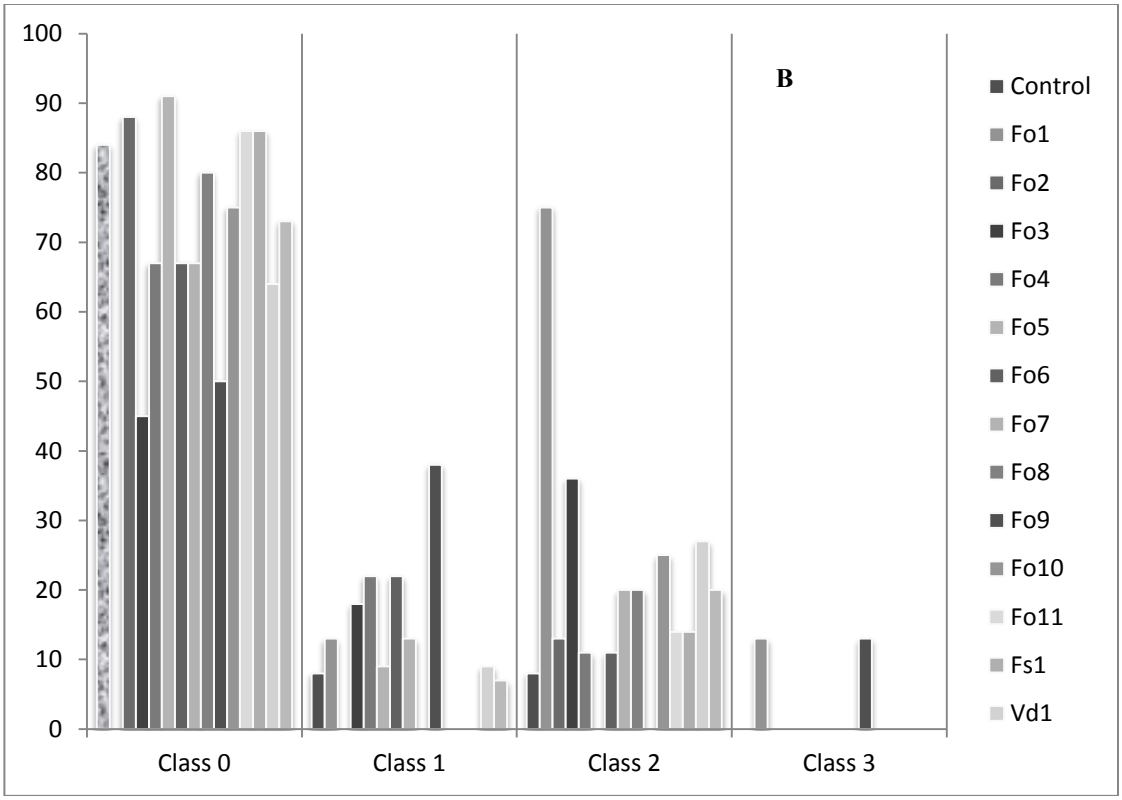

Figure 2 Continued. Percentage of **(A)** *Fusarium*-susceptible (S) red radish cultivar, Cherry Belle, and (B) *Fusarium*-resistant (R) red radish cultivar, Alta Globe, plants inoculated with *Fusarium* or *Verticillium* species during greenhouse pathogenicity studies in each storage root vascular discoloration severity rating class. Class 0= root appears healthy, class 1= slight discoloration in storage root, class 2= vascular discoloration in ≤ 50% of the storage root parenchyma tissue or discoloration in the vascular cambium, class 3= > 50% vascular discoloration in storage root parenchyma tissue. Plants inoculated with "Mix" means plants were inoculated with inoculum composed of strains Fo9, Fo9, Fo11 and Fs1.

SEED-BORNE FUSARIUM ON INBRED RED RADISH (*RAPHANUS SATIVUS*) STOCK SEED AND TRANSMISSION TO DAUGHTER SEEDS THROUGH INFECTED SEED PARENT PLANTS

Abstract. Certain *Fusarium* and *Verticillium* species, including the species responsible for red radish wilt in the Willamette Valley, have been found to seed-borne. *Fusarium* species were recovered from 0.352% of inbred stock seed treated with a 30 second dip in a 10% household bleach solution [0.6% NaClO] and 2.000% of nontreated stock seed while no *V. dahliae* was recovered from stock seed surveyed. *Fusarium proliferatum* and *F. oxysporum* accounted for 59% and 29% of the *Fusarium* species recovered from radish seed. Four strains of *F. proliferatum* and three of *F. oxysporum* were evaluated in greenhouse studies during 2013, and all strains tested caused wilt disease on red radish seedlings. To more closely examine the link between diseased seed parent plants and the hybrid daughter seed produced, we surveyed seed of 35 randomly-selected, commercially-grown radish seed parent plants with wilt. *Fusarium oxysporum* was recovered from 12% of seed surveyed while *F. culmorum* and *F. sambucinum* were recovered from 0.6% and 0.9%, respectively, of daughter seed surveyed. Forty-three percent of *F. oxysporum* strains recovered from daughter seed came from parent plants from which *F. oxysporum* was recovered from discolored vascular tissue. This study confirms that *F. oxysporum* can be associated with red radish seed and appears to be vertically transmitted. This is the first report of *F. proliferatum* as a being seed-borne and a pathogen of red radish.

Introduction. Radish (*Raphanus sativus* L.) is a member of the Brassicaceae known for its fleshy root and unique taste; as much as 50% of the United States red radish seed is produced in the Pacific Northwest (7). Fusarium wilt of red radish, also know as radish yellows, is caused by *Fusarium oxysporum* f. sp. *raphani* (Schlect.) and was found in Washington State during 2003 (7). Fusarium wilt of radish is responsible for 10-20 year rotations for radish seed fields (7, 16, 19). *Verticillium dahliae (*Kleb.) (synonym *V. longisporum*) is another pathogen that can cause a wilt on radish and can be seed-borne on many crops including spinach, as found in Washington state (8, 22). *Fusarium oxysporum* f. sp. *raphani* can also be seed-borne, as reported for wild and cultivated rocket, *Diplotaxis tenuifolia,* and *Eruca vesicaria,* respectively (9). *Fusarium oxysporum* f. sp. *conglutinans,* closely related to *F. oxysporum* f. sp. *raphani,* is seed-borne on many *Brassicaceae* crops (7, 9). Given the long-lived

tendencies of the chlamydospores produced by *F. oxysporum*, introducing a small amount of inoculum via seed can ultimately result in yield loss due to disease unless long rotations are practiced. The objectives of this research were (i) examination of inbred red radish stock seed used for commercial production of hybrid seed in Oregon for the presence and pathogenicity of *Fusarium* and *Verticillium* species and (ii) evaluate the vertical transmission of *Fusarium* and *Verticillium* species from symptomatic seed parent plant to hybrid seed of seed fields in western Oregon.

Materials and Methods

Isolation of fungi from stock seed. Seed of seven hybrid red radish lines, as seventeen different lots obtained from one company, were examined for *Fusarium* and *Verticillium* spp. A subset of seeds were disinfested by soaking seeds for 30 sec in a household bleach [0.6% NaClO] solution and sterile reverse osmosis (RO) water and then rinsing with sterile RO water. Fifty disinfested seeds per lot were embedded into both streptomycin amended Nash-Snyder medium (1 g streptomycin sulfate liter^{-1}) (18, 13) and water agar (2% Difco® agar) for isolation of *Fusarium* and *Verticillium* species, respectively. Fifty nontreated seeds per lot were embedded into both media as well and incubated for 21 days at room temperature (~24˚C) with ambient lighting. Suspect *Fusarium* colonies were transferred from the amended Nash-Snyder medium onto carnation leaf agar (CLA) (19) and streptomycin amended half-strength potato dextrose agar (sPDA) (1g streptomycin sulfate liter^{-1}; Difco® potato dextrose agar) and were incubated for 2 weeks under 3 fluorescent lights (General Electric 40-W tubes) and a black light (Sylvania 40-W tubes, BLB series) for a12-hr photoperiod at room temperature (19).

Putative *Fusarium* cultures were macroscopically examined and were identified based on morphology (16, 19). A subset of *Fusarium* isolates was then single-spored by placing a conidia-bearing sporodochium or colonized carnation leaf into a sterile 50-ml test tube filled with 3 of ml sterile RO water and one drop of 25% lactic acid and then vortexing for 60 s. Approximately 1 ml of each suspension was poured onto each of three water agar plates (3% Difco® agar) and left to grow for 12-18 hr. Single germinating spores were transferred to fresh CLA media and *Fusarium* cultures were incubated for two weeks under the lighting described above.

Vertical transmission study. From seven fields during 2012) with at least two leaves showing one-sided chlorosis were flagged two weeks prior to harvest. Within one week of commercial harvest, each plant was removed from the field; the storage root and basal stem were cut off and were placed into new gallon plastic bags and transported in a cooler with ice packs to the laboratory where samples were stored at 5˚C until processing.

Basal stems and storage roots were rinsed with cold tap water to remove soil and air-dried on paper towels. Once dry, the stems and roots were cut longitudinally on a plastic cutting board using a large knife that was cleaned with 95% ethanol between each cut. The storage roots were examined for presence of vascular discoloration. When discolored vascular tissue was found in the storage root or basal stem, the areas were scraped with a flame-sterilized scalpel to expose underlying layers of cells, then using another sterile scalpel, the discolored tissue was excised as two halves from each discolored area. Using flame-sterilized forceps, one half of the tissue sample was embedded in streptomycin amended Nash-Snyder medium and the other half was embedded in water agar (2% Difco® agar) for isolation of *Fusarium* and *Verticillium* species, respectively. After 21 days, the suspect colonies were subcultured and single spored as described above.

The seedpods from each plant were placed in paper bags and dried for four days at 37.8˚C. After drying, seedpods were crushed open on aluminum sheets with wooden rolling pins. Seedpod debris and chaff was then separated from the seed by nesting two strainers and gently shifting out the lighter chaff. The oven liner, rolling pin, and strainers were sprayed with 95% ethanol between each plant. Seeds were placed in paper envelopes and stored at 5˚C. Twenty-five seeds from each of five randomly-chosen plants from each of the seven fields were embedded into amended Nash-Snyder medium. In addition, 25 seed from 44 plants found to contain *Fusarium* or *Verticillium* species in symptomatic vascular tissue within the storage root or basal stem were embedded into amended Nash-Snyder medium and WA for *Fusarium* and *Verticillium* recovery, respectively.

Production of *Fusarium* inoculum. Fungal strains used in pathogenicity tests (Table 1) are representative of the range of potential pathogens recovered from radish stock seed. Agar plugs of each *Fusarium* isolate were placed in potato dextrose broth (PDB) (Difco® potato dextrose broth) and placed

on Innova 2300® Platform Shaker (New Brunswick Scientific, Enfield, CT, USA) at 90 rpm for 14 to 18 days at room temperature. The shaker was incidentally located 95 cm from the lighting detailed previously. Each culture flask was vacuum filtered using Whatman® #2 filter paper (GE Healthcare Bio-Sciences Corp, Piscataway, NJ, USA) inside a Buchner funnel attached to a sink faucet via plastic hosing. Contents on filter paper were washed three times with 100 ml aliquots of sterile RO water, resuspended in 100 ml sterile RO water, blended with Tissue-Tearor® (Biospec Products INC, Bartlesville, OK, USA) at 5000 rpm for one minute; and then conidia were collected by filtration through 100 µm Nitex® cloth. The spore suspension was then diluted with sterile RO water to a concentration of $1x10^4$ conidia ml^{-1} as determined with hemacytometer counts (15). Inoculum was plated onto sPDA and amended Nash-Snyder medium as a dilution series to determine the colony forming units for each fungal isolate.

Pathogenicity testing. Seeds of a susceptible hybrid red radish cultivar, Cherry Belle, and a resistant cultivar, Alta Globe, were surface disinfested with a 3% hydrogen peroxide (VWR International, Radnor, PA, USA)- sterile RO water solution for 60 min and then dried overnight on sterile germination paper (Anchor Paper, Minneapolis, MN, USA) in a Type II Biological Safety Cabinet (Baker Co, Sanford, ME, USA). Seeds were sown into green plastic 6-inch, thin-walled pots (McConkey Co., Sumner, WA, USA) that had been steam pasteurized at 71˚C for 60 minutes followed by a 60 minute soak in a 10% household bleach [0.6% NaClO] solution for 60 min. Pots were filled with a sandy loam soil that had been pasteurized at 115˚C for 45 min (per 1800 cc soil, arranged in a layer no thicker than 7.6 cm) on each of two consecutive days. For each *Fusarium* isolate evaluated, there were three pots of the susceptible hybrid and one pot of the resistant hybrid with 15 seeds per pot. A single pot of each hybrid variety served as a negative control for each run. Ten days after sowing, 5 ml of inoculum was pipetted around each the base of each plant while 5 ml aliquots of sterile RO water was applied to the base of the control plants (15). After 21 to 28 days, the plants were examined using a rating system modeled after the P.H. Williams Interaction Phenotype (23). We used a 0-5 rating class to evaluate the aboveground portions of the plants for chlorosis and necrosis: 0= no symptomatic leaves, 1= one symptomatic leaf, 2 =two symptomatic leaves, 3= three symptomatic leaves, 4= four symptomatic leaves or that all leaves were stunted or chlorotic, and 5= all of the leaves and/or the entire plant was dead. A 0

to 3 rating class was used to evaluate storage roots: 0= root appears healthy, 1= slight discoloration, 2= vascular discoloration in ≤ 50% of the storage root parenchyma tissue or discoloration in the vascular cambium, and 3= > 50% vascular discoloration. Discolored vascular tissue was transferred onto amended Nash-Snyder medium and colonies were transferred to sPDA and CLA for confirmation of *Fusarium* species.

Results. *Fusarium* species were recovered from 2% of the non-treated red radish inbred stock seeds examined while no *Verticillium* species were detected (Table 2). When inbred stock seed was surface-disinfested, only 0.4% of the seeds examined were found to have *Fusarium* species and still no *Verticillium* detected. *Fusarium oxysporum* and *F. proliferatum* strains were the predominant *Fusarium* species recovered (Figure 1).

When representative fungal strains (Table 1) were tested in greenhouse pathogenicity studies, both the resistant and susceptible hybrid cultivars, Alta Globe and Cherry Belle, had generally greater incidence of plants with leaf necrosis and/or chlorosis and storage root vascular discoloration when inoculated compared to the non-inoculated control plants (Table 3). The severity class rating of foliar symptoms of all plants were generally 2 or below; although the *F. proliferatum* strains, Fp1, Fp2, and Fp4 had plants with foliar class ratings greater than 2 (Table 4, Figure 2). Generally all strains of *Fusarium* obtained from red radish inbred stock seed that were examined caused more severe vascular discoloration compared to the non-inoculated control plants (Table 5, Figure 2) and were more severe in the inoculated susceptible cultivar, Cherry Belle, relative to 'Alta Globe.' Only one strain of *F. oxysporum*, Fo3, and one strain of *F. proliferatum*, Fp1, incited vascular discoloration in the storage roots of the resistant cultivar.

There is evidence of vertical transmission of *F. oxysporum* from symptomatic seed parent field plants to daughter seed. Of the seed surveyed from symptomatic parents, *F. oxysporum* was recovered from 12% of all seed while *F. culmorum* and *F. sambucinum* were each present on 0.6% and 0.9%, respectively, of the seeds surveyed (Figure 4). Forty-three percent of the *Fusarium oxysporum* strains recovered from hybrid seeds came from a commercially-grown, symptomatic seed parent plants where *F. oxysporum* was recovered from discolored vascular tissue in the storage roots (Table 6).

Discussion. Our research confirms that *Fusarium oxysporum* can be detected in radish inbred stock seed and that isolates examined were found to have some degree of pathogenicity when inoculated on red radish seedlings, as illustrated by our greenhouse studies. Also, we demonstrated that vertical transmission of *F. oxysporum* appears to possibly be occurring in seed parent plants to daughter seed. Previous studies have shown *F. oxysporum* to be seed-borne on other hosts, wild and cultivated rocket (9). Despite the small sample size of hybrid seed surveyed, we were able to recover *F. oxysporum* from 12% of the seeds sampled. Since pathogenicity tests were not conducted on non-host species for *F. oxysporum* f. sp. *raphani*, it is possible that other *formae specialis,* such as *F. oxysporum* f.sp. *conglutinans*, were recovered from inbred red radish stock seed (2). The exact *formae specialis* of the *F. oxysporum* strains we tested are unknown.

This is the first report of *F. proliferatum* causing disease on radish. *Fusarium proliferatum* is a pathogen reported on crops such as maize (12), onions (6), garlic (5), and pine seedlings (20). In our studies with red radish, *F. proliferatum* caused vascular discoloration in the storage root parenchyma tissue and in the vascular cambium. *Fusarium proliferatum* has previously been recovered from alfalfa (14) and onion seed (17); our findings support the ability of this species to be seed transmitted. *Fusarium proliferatum* was recovered from seeds after surface disinfestation with 0.6% sodium hypochlorite, which indicates that this pathogen could be more than superficially associated with red radish seeds.

While the percentage of seed from which *Fusarium* was recovered was low in lots examined, the number of individual plants necessary to produce commercial radish seed gives this finding a greater implication. For one acre of land that goes into radish seed production, there are roughly 12,000 radishes planted; so if 2% of seeds have an associated *Fusarium*, then in one acre, 240 of those plants could be developing from a seed with an associated, potentially pathogenic *Fusarium* strain. *Fusarium oxysporum* is capable of surviving as chlamydospores for at least 10 years in soil (11) and *F. proliferatum* can survive in crop debris, such as maize stalk residue, for a at least 21 months (3). So to avoid long-lived pathogens being introduced, it is important for radish seed growers to use clean seed; our study demonstrated how a quick surface disinfestation with household bleach brought the number of *Fusarium* species down seven-fold. The use of clean seed could help reduce pathogen build up and spread.

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Table 1. *Fusarium* strain isolated from inbred red radish stock seed during 2012 and used in greenhouse pathogencity studies on red radish during 2013

Strain I.D.	Stock Seed Lot	Seed Treatment[a]	Species	CFU counts[b]
Fp1	5	None	*Fusarium proliferatum*	90
Fp2	2	0.6% NaClO	*Fusarium proliferatum*	--
Fp3	5	None	*Fusarium proliferatum*	--
Fp4	3	None	*Fusarium proliferatum*	237
Fo1	6	None	*Fusarium oxysporum*	425
Fo2	5	None	*Fusarium oxysporum*	--
Fo3	3	None	*Fusarium oxysporum*	--

[a] Seeds either received no treatment indicated as, none, or were surface disinfested with a 30 sec soak in a 10% bleach solution [0.6% NaClO].
[b] Colony forming units as determined from a $1x10^3$ serial dilution; some isolates do not have this data.

Table 2. Percentage recovery of *Fusarium* and *Verticillium* from 3400 inbred red radish stock seeds after either soaking in 10% household bleach or receiving no treatment

Treatment	% of seed from which *Fusarium* was recovered.	% of seed from which *Verticillium* was recovered
No treatment[a]	2.00	0.00
[0.6%] NaClO dip[b]	0.35	0.00

[a] One-hundred seed per each of 17 samples of seed lots received no treatment; 50 seeds per sample were embedded into each streptomycin amended Nash Snyder medium and water agar.
[b] One-hundred seed per each of 17 sample of seed lots were surface disinfested with a 30 sec soak in a 10% [0.6% NaClO] bleach solution followed by a rinse with sterile water; 50 seeds per sample were embedded into each streptomycin amended Nash Snyder medium and water agar.

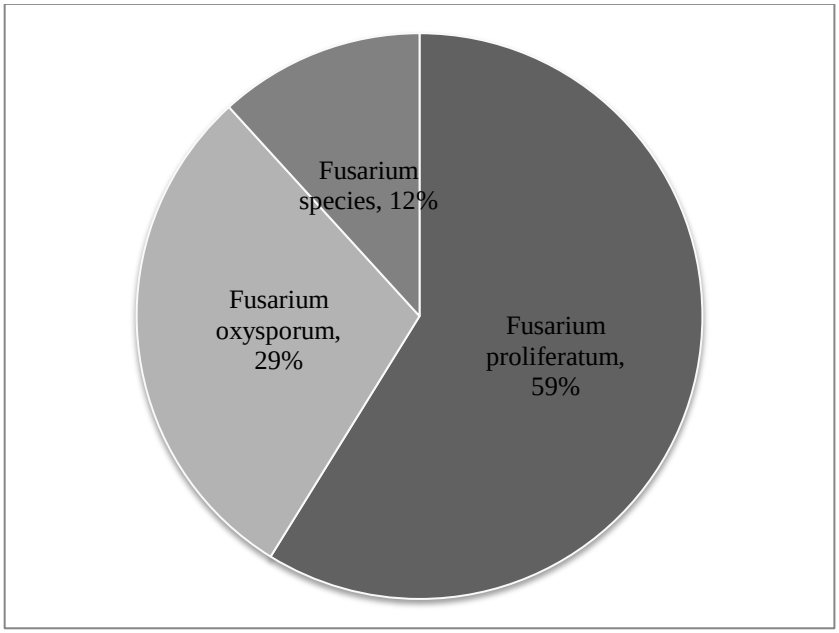

Figure 1. Proportion of fungal species recovered from all inbred red radish stock seed surveyed.

Table 3. Percentage of a susceptible red radish cultivar (S), Cherry Belle, and a *Fusarium*-resistant red radish cultivar (R), Alta Globe, with wilt symptoms in greenhouse studies

Strain ID	Red radish cultivar[a]	Foliar symptoms disease severity index[b]	% of storage roots with vascular discoloration	Recovery of *Fusarium* species from plants (%)
Control	S	36	17	5.35
	R	34	5	2.2
Fp1	S	47	53	n/d[d]
	R	30	50	n/d[d]
Fp2	S	42	44	29
	R	35	0	0
Fp3	S	40	58	58
	R	33	27	27
Fp4	S	45	56	n/d[d]
	R	42	10	n/d[d]
Fo1	S	43	69	71
	R	54	23	15
Fo2	S	46	74	28
	R	n/d[c]	n/d[c]	n/d[c]
Fo3	S	34	56	58
	R	23	0	0

[a] Strains were isolated from inbred red radish stock seed during 2012.
[b] Foliar symptom disease severity index was calculated using the formula ∑[(class no.)*(plants in class)]/[(total number of plants)*(number of classes-1)].
[c] Replicate was knocked over immediately prior to inoculation.
[d] Cultures were not developed by submission deadline.

Table 4. Percentage of red radish plants in each foliar rating class after inoculated with *Fusarium* species in greenhouse studies

		Foliar chlorosis and necrosis severity rating class[c]					
Strain ID	**Red radish cultivar[a,b]**	**0**	**1**	**2**	**3**	**4**	**5**
Control	S	50	26	23	1	1	0
	R	60	16	22	0	2	0
Fp1	S	25	15	60	0	0	0
	R	50	50	0	0	0	0
Fp2	S	37	22	37	2	2	0
	R	60	7	33	0	0	0
Fp3	S	33	36	31	0	0	0
	R	60	13	27	0	0	0
Fp4	S	28	19	53	0	0	0
	R	30	30	40	0	0	0
Fo1	S	23	40	37	0	0	0
	R	0	46	46	0	8	0
Fo2	S	23	23	54	0	0	0
	R	n/d	n/d	n/d	n/d	n/d	n/d
Fo3	S	49	33	16	2	0	0
	R	83	17	0	0	0	0

[a] *Fusarium* susceptible red radish cultivar, Cherry Belle (S).
[b] *Fusarium* resistant cultivar, Alta Globe (R).
[c] Class 0= no chlorotic or necrotic leaves, class 1= one symptomatic leaf, class 2= two symptomatic leaves, class 3= three symptomatic leaves, class 4= four symptomatic leaves or all leaves are stunted or chlorotic, class 5= all of the leaves and/or the entire plant are dead.

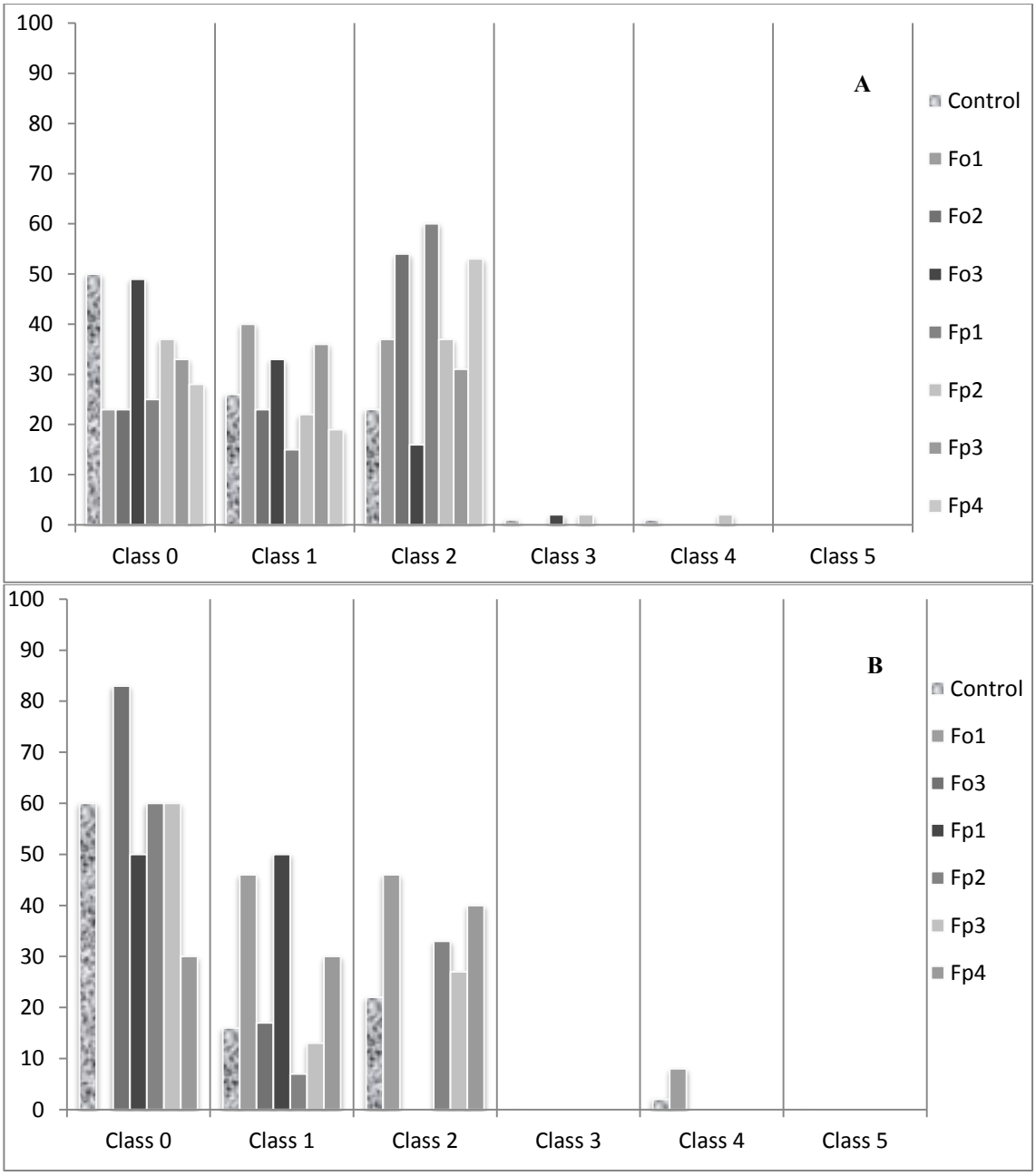

Figure 2. Percentage of (A) *Fusarium* susceptible (S) red radish cultivar, Cherry Belle, and (B) *Fusarium*-resistant (R) red radish cultivar, Alta Globe, plants in each foliar rating class after inoculation with *Fusarium* species in greenhouse studies . Class 0= no chlorotic or necrotic leaves, class 1= one symptomatic leaf, class 2= two symptomatic leaves, class 3= three symptomatic leaves, class 4= four symptomatic leaves or all leaves are stunted or chlorotic, and class 5= all of the leaves and/or the entire plant are dead.

Table 5. Percentage of red radish plants in each storage root rating class after inoculation with *Fusarium* species in greenhouse studies

		Storage root vascular discoloration severity rating class[c]			
Strain ID	**Red radish cultivar[a,b]**	**0**	**1**	**2**	**3**
Control	S	81	12	7	0
	R	84	8	8	0
Fp1	S	47.5	2.5	50	0
	R	50	25	25	0
Fp2	S	52	21	26	0
	R	100	0	0	0
Fp3	S	42	22	36	0
	R	67	33	0	0
Fp4	S	48	2	50	0
	R	90	10	0	0
Fo1	S	29	17	54	0
	R	77	8	15	0
Fo2	S	26	33	38	3
	R	n/d	n/d	n/d	n/d
Fo3	S	42	14	42	2
	R	100	0	0	0

[a] *Fusarium* susceptible red radish cultivar, Cherry Belle (S).
[b] *Fusarium* resistant cultivar, Alta Globe (R).
[c] Class 0= root appears healthy, class 1= slight discoloration in storage root, class 2= vascular discoloration in ≤ 50% of the storage root parenchyma tissue or discoloration in the vascular cambium, and class 3= > 50% vascular discoloration in storage root parenchyma tissue.
[d] Replicate was knocked over immediately prior to inoculation.

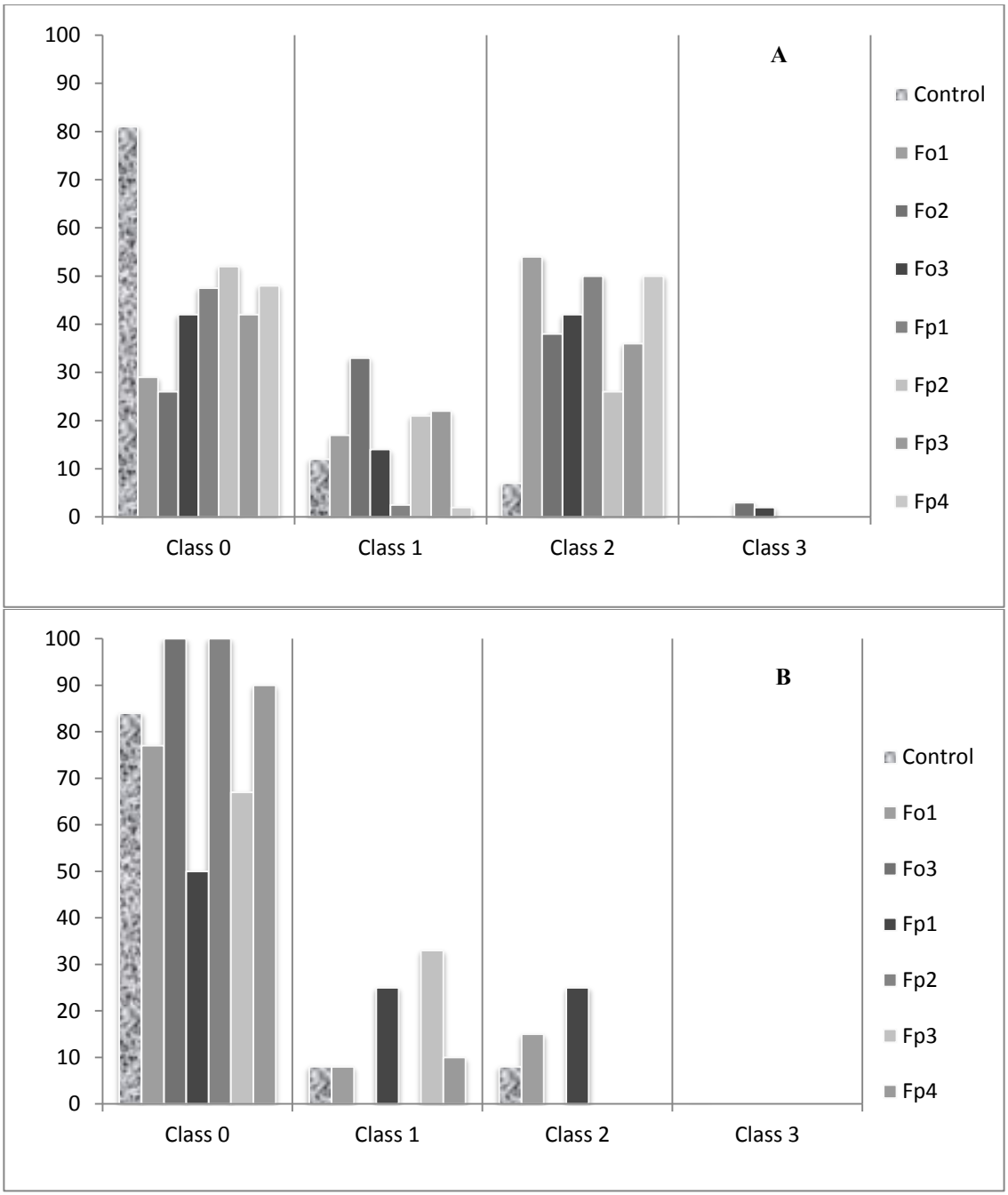

Figure 3. Percentage of (A) *Fusarium*-susceptible (S) red radish cultivar, Cherry Belle, and (B) *Fusarium*-resistant (R) red radish cultivar, Alta Globe, plants in each storage root rating class after inoculation with *Fusarium* species in greenhouse studies. Class 0= root appears healthy, class 1= slight discoloration in storage root, class 2= vascular discoloration in ≤ 50% of the storage root parenchyma tissue or discoloration in the vascular cambium, and class 3= >50% vascular discoloration in storage root parenchyma tissue.

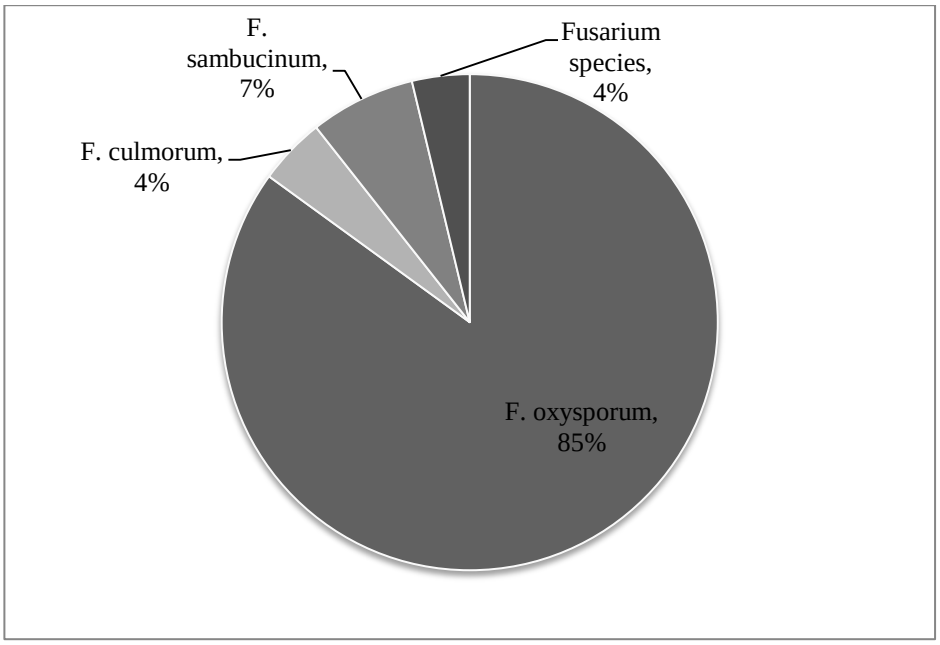

Figure 4. Fungal species recovered from the daughter seed of symptomatic field plants.

Table 6. Recovery of *Fusarium* and *Verticillium* species from daughter seed from randomly selected respective parent plant with wilt symptoms and discolored vascular tissues in storage roots

Field-Plant	Species Recovered from vascular discoloration[b]	Percent of *Fusarium* or *Verticillium* species recovered from seed[c]	Fungal species recovered
1-1[a]	none	0	-
1-4[a]	none	0	-
1-8[a]	none	0	-
1-9[a]	none	8	*F. oxysporum*
1-10[a]	none	4	*F. oxysporum*
2-3[a]	*F. oxysporum*	12	*F. oxysporum*
2-4[a]	none	28	*F. oxysporum*
2-5[a]	*F. oxysporum*	12	*F. oxysporum*
2-9[a]	*F. oxysporum*	0	-
2-10[a]	none	0	-
3-1[a]	*F. oxysporum*	0	-
3-3[a]	*F. oxysporum*	8	*F. oxysporum*
3-4[a]	none	0	-
3-6[a]	none	0	-
3-8[a]	none	0	-
4-1[a]	none	48	*F. oxysporum*
4-3[a]	none	0	-
4-4[a]	none	36	*F. oxysporum*
4-5[a]	none	4	*Fusarium* species
4-8[a]	none	20	*F. oxysporum*
4-10[a]	*F. oxysporum*	4	*F. oxysporum*
5-1[a]	none	16,4	*F. oxysporum, Fusarium* species
5-4[a]	*F. oxysporum*	0	-
5-8[a]	*F. oxysporum*	16	*F. sambucinum*
5-9[a]	*F. oxysporum*	0	-

[a] Daughter seed were randomly selected for surveying.
[b] Plants were sampled at four different locations within the basal stem and storage root during 2012.
[c] Twenty-five seed were embedded into amended Nash Snyder media for plants that had *Fusarium* recovered from vascular discolorations. Twenty-five seed of the single plant where *Verticillium* was recovered were embedded into water agar.

Table 6 Continued. Recovery of *Fusarium* and *Verticillium* species from daughter seed from randomly selected respective parent plant with wilt symptoms and discolored vascular tissues in storage roots

Field-Plant	Species Recovered from vascular discoloration[b]	Percent of *Fusarium* or *Verticillium* species recovered from seed[c]	Fungal species recovered
6-1[a]	none	28	*F. culmorum*
6-3[a]	none	12	*F. sambucinum*
6-6[a]	*F. oxysporum*	8	*F. oxysporum*
6-7[a]	none	72	*F. oxysporum*
6-8[a]	none	8	*F. oxysporum*
7-2[a]	*F. oxysporum*	32	*F. oxysporum*
7-3[a]	*F. oxysporum*	32	*F. oxysporum*
7-4[a]	*F. oxysporum*	56	*F. oxysporum*
7-8[a]	*F. oxysporum*	12	*F. oxysporum*
7-10[a]	none	0	-

[a] Daughter seed were randomly selected for surveying.
[b] Plants were sampled at four different locations within the basal stem and storage root during 2012.
[c] Twenty-five seed were embedded into amended Nash Snyder media for plants that had *Fusarium* recovered from vascular discolorations. Twenty-five seed of the single plant where *Verticillium* was recovered were embedded into water agar.

Table 7. Recovery of *Fusarium* and *Verticillium* species from daughter seed and from respective parent plant that *Fusarium* or *Verticillium* species were recovered from discolored storage root or basal stem vascular tissue

Field-Plant	Species Recovered from vascular discoloration[a]	Percent of *Fusarium* or *Verticillium* species recovered from seed[b]	Fungal species recovered
1-6	*F. oxysporum*	4	*Fusarium* species
2-6	*F. oxysporum*	8,4	*F. oxysporum, Fusarium* species
2-8	*F. oxysporum*	0	-
3-2	*F. oxysporum*	32	*F. oxysporum*
4-2	*V. dahliae*	0	-
4-6	*F. oxysporum*	80,4	*F. oxysporum, V. dahliae*
4-9	*F. oxysporum*	4,4,4	*F. oxysporum, F. sambucinum, F. proliferatum*
5-7	*F. oxysporum*	12,4	*F. sambucinum, F. oxysporum*
7-9	*F. oxysporum*	0	-

[a] Plants were sampled at four different locations within the basal stem and storage root during 2012.
[b] Twenty-five seed were embedded into amended Nash Snyder media for plants that had *Fusarium* recovered from vascular discolorations. Twenty-five seed of the single plant where *Verticillium* was recovered were embedded into water agar.

CONCLUSION OF FINDINGS AND THEIR IMPLICATIONS FOR COMMERCIAL RADISH SEED PRODUCTION

After surveying commercial red radish seed fields in the Willamette Valley, it was apparent that there were a number of plant with obvious wilt symptoms, especially later in the growing season which often coincides with the hotter and drier months of the summer. Many of these plants exhibiting wilt were found to have vascular discoloration in the storage roots and *Fusarium oxysporum* was often recovered from storage root parenchyma with vascular discoloration. A subset of strains that were representative of the species recovered from discolored vascular tissues within storage roots were used in greenhouse pathogenicity studies to confirm Koch's postulates. Koch's postulates is a set of criteria that helps to distinguish a pathogenic organism from other microorganisms present on the plant and confirms the disease-causing ability of the suspected pathogen. The criteria are i) the suspected organism must consistently be associated with the disease, ii) the suspected organism must be isolated from the infected plant, iii) when a healthy host plant is inoculated with a pure culture of the suspected organism, symptoms of the original disease must develop and, iv) the same organism used to inoculate must be recovered from the plants experimentally infected. We confirmed Koch's postulates with *F. oxysporum* isolates obtained from affected commercial radish plants; indicating that pathogenic *F. oxysporum* strains are present in commercial radish seed fields. Though *F. oxysporum* strains tested were pathogenic on radishes, indicating that the recovered strains are possibly *F. oxysporum* f. sp. *raphani,* another *formae specialis* that is pathogenic on other members of the Brassicaceae, *F. oxysproum* f. sp. *conglutinans*, is capable of causing wilt symptoms on radish when the soil temperature increases (3). So the exact *formae specialis* will remain unknown until the *Fusarium* strains are tested for pathogenicity on nonhost Brassicaceae members for each *formae specialis*.

Fusarium oxysporum was recovered from samples of commercial inbred stock seed used for the production of hybrid red radish seed as well as from hybrid red radish daughter seed collected from wilted seed parent plants. The recovery of *F. oxysporum* from both kinds of seed demonstrates the potential for this pathogen to be transmitted via seed and build up in seed production. Previous

work has shown *F. oxysporum* to be seed-borne on wild and cultivated rocket *(Diplotaxis tenuifolia,* and *Eruca vesicaria)*, also hosts for *F. oxysporum* f. sp. *raphani* (7, 8). When first examining seed for *Fusarium* presence, 3400 total inbred stock seeds, representing different seed lots and varieties, were examined; we were able to show that there was *Fusarium* present on seeds at low levels in multiple lots. To further confirm our suspicion that *F. oxysporum* can be seed-transmitted, we examined the link between seed parents showing wilt symptoms in commercial fields and the subsequent daughter seed produced. Of the 70 parent plants collected in a survey, we randomly chose 35 parent plants and examined the seed produced from these plants; some of these plants had vascular discolorations from which *F. oxysporum* was recovered from while others did not yield *Fusarium* species. In addition to these randomly chosen plants, we focused on the seed of plants where *Fusarium* was recovered from vascular discoloration. We found that when *F. oxysporum* occurred in parent plant vascular tissues, then often *F. oxysporum* was also recovered from the daughter seed although sometimes *F. oxysporum* was not recovered from plant's daughter seed despite having recovered *F. oxysporum* from the parent plant's vascular tissue. Also, *F. oxysporum* was recovered from daughter seed of parent plants where we did not recover *F. oxysporum* from the parents; these parent plants may have had *F. oxysporum* or other wilt pathogens present but they were not recovered from the discolored vascular tissue, and the *Fusarium* may have been overrun with secondary microbes when isolations were done, or perhaps the seed or seed pods were incidentally contaminated with *Fusarium* in the field or during processing. In our greenhouse pathogenicity studies, we did not always recover the inoculated pathogen from symptomatic tissue. Pathogens such as *Fusarium* can be masked by organisms like *Penicilium, Trichoderma,* and other fungi that commonly occur, and cohabitate with or colonize subsequent to, *Fusarium* species, and thusly will occur on the semi-selctive *Fusarium* medium. It is also possible that the portions of vascular tissue that were sampled were discolored due to the host's response to the pathogen; a vessel outside of the *Fusarium*-colonized area was sampled .

Verticillium dahliae, was rarely recovered from vascular tissue sampled from commercial red radish seed fields. *Verticillium dahliae* has been previously shown to be pathogenic on radish (11).

The single finding of *V. dahliae* during 2012, was from a plant collected for the vertical transmission study, so the daughter seed produced by this parent plant was included in the study. *Verticillium dahliae* was not recovered the plant's daughter seeds that were examined.

The interaction between cabbage maggot, *Fusarium*, and *Verticillium* species isolated in this research is unknown. Further research should be done to see if cabbage maggot potentially vectored wilt pathogens into radish plants, similar to how corn earworm larvae or corn rootworm beetles can vector *Fusarium* species to corn (9, 17). Feeding by western corn rootworm has also been shown to increase root colonization of maize by *F. verticillioides* at a high inoculum density (12), so it is possible that cabbage maggot feeding could increase radish root colonization by *F. oxysporum.* In muskmelon seedlings, severity of *F. oxysporum* wilt and incidence of disease at low inoculum levels was increased in the presence of striped cucumber beetles (13). The feeding of cabbage maggot larvae may result in increased severity and incidence of wilt in radish seed fields in the Willamette Valley. The injury caused by the cabbage maggot feeding may also make the severity of the fungal wilts appear worse as the cabbage maggot damage is also disrupting the vascular system and the storage root in general. In light of the potential vectoring of microbes, pathogenic or saprophytic, when sampling examined storage roots we sampled discoloraed vascular tissues that were distal to caggabe maggot feeding injuryto limit the potential contamination by secondary organisms.

This is the first report to confirm that *F. oxysporum* is present in commercial radish seed fields in the Willamette Valley of Oregon, though this pathogen has obviously been present for some time. It is not uncommon for a low incidence of *F. oxysporum* wilt to go without notice because initially the disease is usually found as a small patch of affected plants, but if it is seed-borne at high rates or if it spreads rapidly within a planting, the patch of diseased plants can grow larger, affecting more plants, until the disease presence becomes obvious (15). Despite this being the first study reporting the presence of this disease, many of the commercial fields practice long rotations out of radish (20 years) in attempt to reduce the build-up of *F. oxysporum* inoculum in the soil. Our examinations of seeds for the presence of potential wilt pathogens showed the recovery of two

Fusarium species, albeit in low numbers. While this low level does not seem initially alarming, one acre of radish grown for seed contains 12,000 plants, and if there is a 2% seed association rate for *Fusarium* species then there are 240 plants per acre that are introducing *Fusarium* strains. The use of clean seed would greatly limit the introduction of these potentially long-lived pathogens into the fields. In our stock seed survey, we demonstrated that a quick treatment with 10% household bleach [0.6% NaClO] reduced the pathogen load seven-fold.

Verticillium dahliae has also been assumed to be present and causing disease in radish seed fields, but again has not been confirmed in Oregon radish seed fields until this research showed that vascular discoloration in greenhouse inoculated plants. *Verticillium dahliae* has previously been shown to be seed-borne on other crops like spinach (6), so the results of this study confirm the ability of this pathogen to be seed-transmitted in addition to showing that it maybe vertically transmitted in radish. To confirm vertical transmission, we would need more parent plant and daughter seed samples with *V. dahliae*. While *V. dahliae* was present in commercial radish seed fields at a much lower incidence, there are other potential hosts commonly grown in the Willamette Valley, such as mint and members of the Brassicaceae; isolates of *V. dahliae* may have host range specificity, such as cabbage isolates causing disease on cauliflower and vice versa but neither being pathogenic on mint (2). Thus, it is important that growers take into account what potential host crops susceptible to *Verticillium dahliae* are planted prior to radish and what crops can be planted after radish to limit the buildup of inoculum and prevent the loss of future crops due to wilt disease

This is the first report of *Fusarium proliferatum* and *Fusarium solani* being pathogenic on red radish. The isolates of *F. proliferatum* were isolated from inbred stock seed and through greenhouse pathogenicity studies we showed that *F. proliferatum* causes vascular discoloration in storage root parenchyma and vascular cambium of radish seedlings; ambiguous leaf chlorosis and necrosis was also observed. *Fusarium proliferatum* has been previously recovered from both alfalfa seed and onion seed (10; 14); our study shows that this species has the ability to be associated with radish seed. *Fusarium proliferatum* was recovered from seed that had been surface-disinfested, which

might indicate that this species could be more than just a superficial seed pathogen. *Fusarium proliferatum* does not produce robust chlamydospores, but its macroconidia have been shown to survive in crop debris, such as maize stalk residue, for at least 21 months (4). This again illustrates the importance of using clean seed to limit the introduction of these potentially long-lived *Fusarium* pathogens into fields. However, *F. proliferatum* was not recovered from symptomatic plants in commercial seed fields, so it may not be pathogenic when radish plants are grown in non-pasteurized soils, or the affected seeds may result in disease seedlings that are rogued from the greenhouse before transplanting into the field.

We isolated *F. solani* from a vascular discoloration in the storage root parenchyma of a plant that showed symptoms of wilt. This strain was inoculated onto healthy radish seedlings and subsquently recovered from discolored vascular tissues in the storage root of the test plants, thus fulfilling Koch's postulates. The most notable symptoms of this pathogen were the discolorations in the storage root parenchyma and vascular cambium. Foliar symptoms of chlorosis and necrosis, though ambiguous in appearance, were also observed. *Fusarium solani* has been previously reported to be pathogenic on horseradish, *Armoracia rusticana*, another member of the Brassicaceae (1). The symptoms observed in horseradish were discoloration of the cortex and vascular system but there was no mention of above-ground symptoms associated with the pathogen (1). Chlamydospores formed by *F. solani* can survive in plant debris or in the soil for many years (15), which will influence the disease management decisions for radish seed crops.

Due to resource and time constraints, we did not screen all of the fungal isolates at one time. Efforts were taken to lessen the impact of microsite variation by randomizing the pots arranged on the bench tops and what isolate strain they received. *Fusarium oxysporum* was recovered from 8 out of 95 non-inoculated control plants despite efforts to minimize such issue; the number of plants that had symptomatic tissue that *F. oxysporum* was recovered from was low but still warrants mention. Other *Fusarium* strains were recovered from a subset of control and inoculated plants: *F. polyphialidicum* was recovered from three 'Cherry Belle' negative control plants and *F. poae* was recovered from one

inoculated ‘Cherry Belle’ plant. The pathogenicity studies were conducted in a greenhouse that was not a closed system, so fungal strains could have immigrated from the outside airflow or from neighboring greenhouses. A neighboring greenhouse section housed wheat (*Triticum* spp.), a known host of *F. poae.* Also, the sandy loam soil used in greenhouse studies was pasteurized, so the subsequent soil environment was vulnerable to invasion by early colonists such as *Fusarium* species (15). Although the seeds used in the greenhouse pathogenicity studies were disinfested with a 3% hydrogen peroxide solution prior to sowing, the treatment may not have removed all *Fusarium* strains associated with the seed.

This study confirmed the suspicions that soilborne wilt pathogens, *F. oxysporum* and *V. dahliae*, are present in commercial red radish seed fields in the Willamette Valley. Our study also shows that both of these fungi can also be found in association with red radish seed. In addition to confirming the presence of these pathogens, we have identified additional *Fusarium* species that can be pathogenic on radish, *F. proliferatum* and *F. solani.* We have also found that *F. proliferatum* can be associated with radish seed. Our findings demonstrate the complexity of the wilt observed in commercial red radish seed fields and the diversity of *Fusarium* species associated with red radish seed.

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