MANAGEMENT OF WHITE MOLD IN HYBRID SUNFLOWER SEED CROPS IN THE COLUMBIA BASIN OF CENTRAL WASHINGTON

By

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Abstract

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Hybrid sunflower seed production in the semi-arid Columbia Basin of central Washington increased from 20 ha in 2008 to >2,000 ha in 2014. White mold, caused by the soilborne fungus *Sclerotinia sclerotiorum*, is the main disease affecting sunflower seed crops in this region. Sclerotia, the survival structures of the fungus, are persistent in soil, and the fungus has a wide host range of >400 mostly dicotyledonous species, making *S. sclerotiorum* a difficult pathogen to manage. Sampling from Sclerotinia basal stalk rot foci in two sunflower seed crops in 2015 revealed genetically and genotypically diverse *S. sclerotiorum* isolates. The isolates were highly aggregated within foci, and the populations from each of the two fields were not differentiated genetically. White mold was observed in 92.5% of 40 sunflower seed crops surveyed in the Columbia Basin in 2016, but at a minimal incidence in most crops (average 3.8 ± 0.9% infected plants/field). In addition, very limited incidences of white mold in each of six grower-cooperator field trials completed in 2015 and 2016 precluded assessment of the efficacy of applications of Contans WG (*Coniothyrium minitans*, a mycoparasite of *S. sclerotiorum*) and

foliar fungicide applications for in-season management of white mold. Spore trapping revealed ascospore release over approximately a 4-week period, from one week prior to flowering to about 10 days after flowering. This stage of sunflower growth is highly susceptible to ascospore infection, indicating that timely, preventative applications of fungicides with efficacy against *S. sclerotiorum* should protect sunflower heads against ascospore infections. Microplot trials near Ephrata, WA showed that drench applications of Contans WG at 0.56 or 4.48 kg/ha only reduced survival of sclerotia on the soil surface, not sclerotia buried 15 cm deep, and the applications were never as effective as burial at reducing duration of sclerotium survival. A large-scale, postharvest Contans WG trial confirmed that burial of infested sunflower crop residues reduced sclerotium survival more rapidly than leaving residues on the soil surface, and postharvest application of Contans WG did not reduce the duration of sclerotium survival in crop residues.

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LITERATURE REVIEW

1.1. Sunflower.

1.1.1. Background. Sunflowers, *Helianthus* spp., are members of the Asteraceae, native to North America, and heliotropic from emergence to anthesis, hence the Spanish and French names, girasol and tournesol, respectively, which mean 'turn with the sun' (Putt 1997; Seiler 1997; Seiler and Rieseberg 1997). Although Linnaeus described only 9 species of sunflower, 67 species are now recognized, all of which originated from the Americas and most of which are found in the U.S. (Berglund 2007; Seiler and Rieseberg 1997). The base chromosome number of *Helianthus* is 17; and diploid, tetraploid, and hexaploid species exist (Berglund 2007; Seiler and Rieseberg 1997). Most species of *Helianthus* are perennial but there are annual species (Berglund 2007). *H. annuus* L., the cultivated sunflower, is an annual diploid (n = 17), and is used most commonly as an edible oil crop (Seiler and Rieseberg 1997). Because the focus of this review is on the cultivated sunflower, hereafter, when 'sunflower' or 'sunflowers' is used, it is in reference only to the cultivated sunflower, *H. annuus*.

1.1.2. Physical characteristics. The sunflower is differentiated from many other cultivated plants by a composite inflorescence, referred to as a head or capitulum, which is composed of ray and disk flowers borne by a fleshy tissue, the receptacle (Harveson et al. 2016; Seiler 1997). The head of the cultivated sunflower arises from a single stem, and the height of a sunflower plant varies depending on the cultivar and the environment, ranging from 50 to 500 cm with 160 to 180 cm generally the accepted ideotype (idealized appearance) for hybrid sunflowers (Harveson et al. 2016; Seiler 1997). The number of leaves on each stem varies and can be as few as 8 to as many as 70 (Harveson et al. 2016). Below ground, sunflowers are deep-

rooted, with rooting depths up to 2 m having been reported (Jones 1984; Sadras et al. 1989), and lateral roots can spread from 60 to 150 cm in the top 30 cm of the soil profile (Seiler 1997).

Ray flowers are typically golden yellow and located on the outer whorl of the head, giving sunflowers their characteristic appearance; each ray flower is formed from five elongated petals united together and is sterile (Seiler 1997). Inside the ray flowers are whorls of disk flowers; from 700 to 3,000 in oilseed type hybrids, and up to 8,000 in non-oilseed type hybrids (Seiler 1997). Each disk flower is perfect, i.e., each has a stamen and pistil, and can produce a seed (Seiler 1997). In hybrid seed production systems, the female lines are male sterile due to cytoplasmic male sterility, which renders the flowers unable to produce functional pollen and forces pollination to occur from an external pollen source (Seiler 1997). While cultivated hybrid sunflowers and female parental lines have non-branched stems which produce a single head, male parental lines may be branching and produce multiple heads (Harveson et al. 2016).

Most sunflowers are day-length neutral, i.e., flowering occurs independent of day-length (Seiler 1997). The stages of sunflower physiological development have been classified by Schneiter and Miller (1981). The beginning of anthesis, considered the R-5.1 stage (Schneiter and Miller 1981), occurs when the outermost whorl(s) of disk flowers open (Seiler 1997). One to four whorls of disk flowers open each successive day for 5 to 10 days thereafter (Seiler 1997). The flowering period is prolonged if the heads are large, or if the weather is cool and cloudy (Seiler 1997). Following the opening of the flower, the stigma is receptive for up to 4 to 5 days, the window for pollination (Harveson et al. 2016). Once the pollination period ends, achene development occurs. The achene is the fruit of sunflower and consists of a seed, referred to as a kernel, and the adhering pericarp, referred to as the hull (Seiler 1997). However, in common vernacular, sunflower seed refers to the achene (Anfinrud 1997), and hereafter in this document,

sunflower seed refers to the achene. Achenes mature from the periphery of the whorl to the center and generally are largest on the periphery of the head and smaller towards the center (Harveson et al. 2016). Regardless of fertilization status, achenes form hulls, but kernel development is dependent on fertilization (Seiler 1997). Seed yield is a product of the number and weight of fertile seeds per head (Robinson 1983).

1.1.3. Significance of sunflower crops and sunflower production. Sunflowers are grown for vegetable oil production (oilseed types), and for human food and bird-food markets (non-oilseed, confection types) (Berglund 2007). In 2016, the top six sunflower producing countries/regions were, in order, Ukraine, Russia, the European Union, Argentina, China, and Turkey at 13.50, 10.00, 8.30, 3.30, 2.51, and 1.20 million metric tons, respectively; the U.S. was seventh in sunflower production [United States Department of Agriculture (USDA) Foreign Agricultural Service (FAS) 2016]. Annual sunflower production in the U.S. was 1.12 million metric tons in 2016 (USDA FAS 2016) and ranged from 0.91 to 1.33 million metric tons between 2010 and 2015 [USDA National Agricultural Statistics Service (NASS) 2016a]. The top sunflower producing states in 2016, in terms of total area planted, were North Dakota and South Dakota, with >78.5% of the total sunflower hectares grown in the United States in 2016, and the rest planted in California (2.7%), Colorado (4.8%), Kansas (3.3%), Minnesota (4.9%), Nebraska (1.8%), and Texas (3.8%) for a total of 665,900 ha planted in the United States in 2016 (USDA NASS 2016b). Of the hectares of sunflower planted, 87% were oilseed type cultivars and 13% confection type cultivars (USDA NASS 2016b).

Sunflower production, in general, occurs in climates ranging from temperate to semi-arid (if irrigated) with ideal growing temperatures of 21 to 26^{o}C (Blamey et al. 1997; Putnam et al. 1990). While the adaptability of the sunflower plant allows for flexibility in some production

practices, other practices are less flexible. Sunflower production practices with some flexibility include stand density, row width, and tillage method for seedbed preparation. Similar yields have been reported over a range of stand densities (with lower stand densities correlated significantly with larger seed size) and row-widths (91 cm and narrower) (Ashley and Tanaka 2007; Putnam et al. 1990). Likewise, a range of tillage methods can be used to prepare a seedbed for sunflower production, from conventional tillage using moldboard, chisel, and/or disk plows followed by secondary tillage operations to prepare the seedbed, to minimum tillage or no-till to minimize erosion and increase the water storage capacity of the soil (Blamey et al. 1997; Putnam et al. 1990). The variety of possible row-widths and tillage methods for seedbed preparation are of benefit to growers who are able to use existing equipment and land management practices for sunflower production without adverse effects on crop yield.

However, there are many sunflower production practices which typically are less flexible, including planting date, crop sequencing, row orientation, weed control, and irrigation and nutrient management (Ashley and Tanaka 2007; Blamey et al. 1997; Endres 2007; Franzen 2007; Putnam et al. 1990; Robinson 1975; Scherer 2007; Unger 1990). Planting date selection is especially important in regions with cold winters as sunflowers are frost sensitive, and maturation of the crop depends on air and soil temperature as well as the length to maturity of a cultivar (Blamey et al. 1997). Sunflower seed will germinate at 3.9^{o}C but optimal germination requires temperatures of at least 7.8 to 10^{o}C; freezing temperatures can damage seedlings which become more sensitive to cold temperatures with age (Putnam et al. 1990). Planting date also is determined by the time it takes a cultivar to reach maturity relative to the length of the growing season in a region (Blamey et al. 1997). The average duration to crop maturity, i.e., the time from planting to physiological maturity, ranges from 75 to 150 days depending on cultivar and

environment; on average, a sunflower needs 1,372 growing degree day units, calculated as the summation of average degrees above 6.7^{o}C, to reach physiological maturity (Berglund 2007; Harveson et al. 2016).

Sunflower crops should be rotated with other crops because yields will decline without adequate crop rotation due to a combination of one or more of the following: i) increased sunflower disease and insect pest risk, ii) increased populations of certain weeds, iii) increased populations of volunteer sunflower plants, iv) soil moisture depletion, and v) allelopathy (Endres 2007). Row orientation, while not shown to have an effect on yield, may nevertheless need to run north-south to allow for equipment to move through the field in the growing season without causing lodging as a result of equipment hitting the heads as they move through a sunflower field due to heliotropy (Bill Wirth, Precision Seed Production, LLC, *personal communication*; Robinson 1975). Yield also is affected by competition from weed pressure, which is especially important early in the season because sunflowers do not grow fast enough to limit or prevent weed establishment (Putnam et al. 1990); depending on the method of seedbed preparation, pre-emergence herbicide application(s) along with post-emergence herbicide application and/or inter-row cultivation are used to control weeds (Ashley and Tanaka 2007; Blamey et al. 1997).

Much of the sunflower production in the United States is rain-fed, such as in North Dakota, so the water available for crop growth is determined by water stored in the soil and precipitation during the growing season (Scherer 2007). In semi-arid climates or in times of water shortage, however, irrigation may be necessary to obtain acceptable yields (Blamey et al. 1997). Adequate water supply is most critical during early crop development to promote germination, seedling emergence, and adequate leaf expansion; and from heading to achene filling to prevent adverse effects on seed yield (Blamey et al. 1997; Göksoy et al. 2004; Unger

1990). Heavier soils require fewer irrigations than sandier soil types (Unger 1990). Finally, proper nutrient management, particularly nitrogen (N), phosphorous (P), and potassium (K), is necessary to achieve high yields (Franzen 2007; Putnam et al. 1990).

1.1.4. Hybrid seed production. Hybrid sunflower seed production is currently accomplished using cytoplasmic male sterility and restorer genes possessed by the female and male parental lines, respectively (Anfinrud 1997). Cytoplasmic male sterility was discovered in sunflowers by Patrice Leclercq in 1968, and the fertility-restoring genes, which restore self-fertility to the female, were discovered by Murray Kinman in 1970 (Leclerq 1968; Kinman 1970). This system allows for the production of hybrid seed which is a genetically pure cross between the male and female parent lines, and is self-fertile when planted. Therefore, a hybrid seed production field is planted with both male and female parental lines, generally in a ratio ranging from 6:2 to 8:2 female:male rows (de Estrada et al. 2012). In comparison, a hybrid sunflower production field of oilseed or confection type, or an open-pollinated sunflower seed production field, is planted to a single cultivar (de Estrada et al. 2012).

Several challenges in hybrid sunflower seed production include management of floral synchrony or 'nicking' between the male and female lines, ensuring adequate pollen production by the male line, isolation of male lines to maintain genetic purity, management of pollinators (typically honeybees, *Apis mellifera* L.), and removal of male plants post-pollination (Anfinrud 1997; de Estrada et al. 2012; Bill Wirth, Precision Seed Production, LLC, *personal communication*). One means of ensuring good nicking, i.e., matching the duration of pollen-shedding in the male line with the duration of stigma receptivity in the female line, is the use of split-planting of the male line, a practice where some or all of the male rows are planted on a different date from the female rows; this practice has been improved in recent years by use of the

thermal time concept which relates the rate of crop development to growing degree days (Anfinrud 1997; de Estrada et al. 2012; Trudgill et al. 2005). Often the male line is branched, producing several inflorescences per plant, which allows for viable pollen to be produced for a month or longer to aid in nicking between the male and female lines (Anfinrud 1997). Assuming good nicking, adequate pollen production by the male line can be managed by the ratio of male to female rows in seed production fields (Anfinrud 1997).

Production of hybrid seed that is genetically pure requires that no pollen sources other than the relevant male line are present during anthesis (Anfinrud 1997). Possible sources of contaminating pollen include nearby sunflower fields, including sunflower production fields grown for oil or eating, sunflower seed production fields with different male parent lines, wild sunflowers, and volunteer sunflower plants (Anfinrud 1997). Rogueing of volunteer and wild sunflower plants from areas surrounding seed production fields can eliminate those sources of unwanted pollen. To avoid contamination from other male parent lines or from sunflower production fields, seed production fields with a particular male parent line must be isolated temporally and spatially from those sources (Anfinrud 1997; de Estrada et al. 2012). Globally, the distance required for spatial isolation of seed production fields varies (Anfinrud 1997). In California and Washington, seed production fields of different male parent lines must be isolated by a minimum distance of 2 km [Bill Wirth, Precision Seed Production, LLC, *personal communication*; California Crop Improvement Association (CCIA) 2015].

Even when appropriate nicking, adequate numbers of male plants, and isolation from undesirable pollen sources are accomplished, effective honeybee management also is important for maximizing seed set (Anfinrud 1997). The number of hives placed in a field should be a function of the percentage anthesis of the male sunflower plants (Anfinrud 1997). An

oversupply of honeybees can result in bees searching for alternative pollen sources, while an undersupply of bees can result in inadequate fertilization of the female flowers (Anfinrud 1997). Other factors affecting pollination include pollen desirability by honeybees, the level of dimorphism in morphology between male and female flowers, honeybee hive strength, and presence of wild bees (Anfinrud 1997; de Estrada et al. 2012; Greenleaf and Kremen 2006; Martin and Farina 2016). Finally, once pollination is finished the male rows must be removed prior to seed set to avoid harvesting undesired inbred male seed (Bill Wirth, Precision Seed Production, LLC, *personal communication*)

The major area of hybrid sunflower seed production in the U.S. is the Sacramento Valley of California, which produces 95% of the hybrid seed planted each year by U.S. sunflower growers and a significant amount of sunflower seed used by European and Asian sunflower producers (Lilliboe 2009). In 2009, sunflower seed comprised the largest acreage of seed crops in the state of California with 15,000 ha of sunflower seed crops certified by the CCIA (Lilliboe 2009). The number of hectares produced in California reflects the ideal sunflower seed production conditions in the Sacramento Valley and the high value of the crop, with an estimated gross return of $3,360/ha and an estimated net return of $1,410/ha in 2011 (Lilliboe 2009; Long et al. 2011).

The conditions in the Sacramento Valley of California which are ideal for the production of hybrid sunflower seed have been cited as: i) sunflower seed crops are irrigated, mitigating water stress; ii) daytime temperatures during the growing season range from warm to hot, and provide sufficient heat units for optimal crop development; iii) environmental conditions such as limited rain and low daytime relative humidity are unfavorable for many fungal, oomycete, and bacterial sunflower diseases that plague other production regions; iv) seed crops planted as early

as late March can be harvested by mid-August or early September, which provides adequate time to process and distribute seed to domestic and foreign customers; and v) temporal and spatial isolation can be used to prevent cross-pollination which maintains genetic purity because of the broad planting window and long growing season (Gulya et al. 2012; Lilliboe 2009). Still, the Sacramento Valley faces challenges in sunflower seed production. One challenge is finding enough isolated production areas needed to produce the desired quantity of sunflower seed crops as there are crops of many other plant species, both seed and commercial, produced in the area; and large numbers of sunflower seed crops of different cultivars or parent lines that must be isolated (Lilliboe 2009). Other challenges include control of volunteer sunflower plants to maintain genetic purity of the seed, honeybee hive strength, and availability of irrigation water, particularly during the recent protracted period of drought in California (Lilliboe 2009).

In 2008, production of hybrid sunflower seed was introduced to the Columbia Basin of central Washington, in part as a response to the above-mentioned challenges in the Sacramento Valley (Bill Wirth, Precision Seed Production, LLC, *personal communication*). The Columbia Basin of central Washington encompasses areas irrigated by the Columbia Basin Project which supplies 268,400 ha of farmland with irrigation water including the areas around Ephrata, Quincy, Moses Lake, and Othello, WA; many other areas in the Columbia Basin are irrigated with well water (Bill Wirth, Precision Seed Production, LLC, *personal communication*; Harrison 2008). The area in sunflower seed production in the Columbia Basin has increased from rapidly 50 acres in 2008 to >5,000 acres in 2014, with potential for additional growth. One reason for the rapid growth is that the gross return for a sunflower seed crop in the Columbia Basin ranges from $3,700 to $5,400/ha, which makes it a valuable rotational crop (Bill Wirth, Precision Seed Production, LLC, *personal communication*). The Columbia Basin has a similar hot and dry

summer climate to the Sacramento Valley; however, the growing season in Washington for sunflowers is shorter than in California, with the planting window extending from early to mid-May, and harvest occurring in September to October (Bill Wirth, Precision Seed Production LLC, *personal communication*). Parental male lines are isolated spatially to prevent cross-pollination, as the shorter growing season does not allow for the temporal isolation used in California. The result is that fields of different male parental lines are spread throughout the region. Also, because of the lack of precipitation during the summer months (average of 77 mm rainfall from May to October in Moses Lake, WA with an average of 10 mm in July, 6 mm in August, and 10 mm in September from 1981 to 2010), sunflower seed crops in the Columbia Basin are irrigated either by center-pivot or rill irrigation systems, with the frequency of irrigation dependent upon soil type, crop growth stage, and environmental conditions (Bill Wirth, Precision Seed Production LLC, *personal communication*; United States Climate Data 2017).

1.1.5. Diseases of sunflower. *Helianthus* spp. are native to North America and, thus, nearly all known sunflower pathogens can be found on the North American continent (Gulya and Rashid 1997). Sunflower pathogens, however, are not limited to North America as most have followed the introduction of sunflower around the world (Gulya and Rashid 1997). Therefore, regional climate and production practices typically are determinative of which pathogens are prevalent.

There are 7 known bacterial pathogens and 65 fungal and oomycete pathogens, as well as 18 species of parasitic nematodes, 8 viruses, and 2 phytoplasmas known to infect sunflower (Harveson et al. 2016). The most economically important foliar sunflower diseases and the corresponding pathogens are: Alternaria leaf blight and stem spot caused by *Alternaria* spp. and *Alternariaster helianthi*; downy mildew caused by *Plasmopara halstedii*; powdery mildew

caused by *Golovinomyces cichoracearum*, *Leveillula taurica*, and *Podosphaera xanthii*; and rust caused by *Puccinia helianthi* (Harveson et al. 2016). The most economically important root, stalk, and head sunflower diseases in the U.S., and the corresponding pathogens are: charcoal rot caused by *Macrophomina phaseolina*; Fusarium stalk rot caused by *Fusarium* spp.; Phoma black stem caused by *Phoma macdonaldii*; Phomopsis stem canker caused by the *Diaporthe/Phomopsis* spp. complex; Rhizopus head rot caused by *Rhizopus arrhizus, R. microsporus,* and *R. stolonifera*; Sclerotinia basal stalk rot and wilt, midstalk rot (stem rot), and head rot caused by *Sclerotinia sclerotiorum*; southern blight caused by *Sclerotium rolfsii*; sunflower necrosis caused by *Tobacco streak virus* (TSV); and Verticillium wilt or leaf mottle caused by *Verticillium dahliae* (Bradley et al. 2007; Harveson et al. 2016). For a thorough review of sunflower diseases, see Harveson et al. (2016).

1.1.5.1. Diseases in hybrid sunflower seed crops. One reason that California has been the major sunflower seed production region of the U.S. is that diseases in sunflower seed production fields in California have been present at very low incidences historically and currently (Gulya et al. 1991, 2012). Over the 15-year period from 1997 to 2011, diseases monitored by phytosanitary inspections were found in 6.9% of 7,231 sunflower seed production fields in California, with most of those fields located in the Sacramento River Valley. The diseases and respective pathogens which are part of the sunflower seed crop phytosanitary inspection program in California are as follows: Alternaria leaf blights caused by *Alternariaster helianthi* and *Alternaria zinnia*; Phoma black stem caused by *P. macdonaldii*; Phomopsis stem canker caused by *Diaporthe helianthi* (syn. *Phomopsis helianthi*); downy mildew caused by *P. halstedii*; bacterial leaf blights caused by *Pseudomonas syringae* pv. *helianthi* and *Pseudomonas syringae* pv. *tagetis*; rust caused by *P. helianthi*; white rust caused by *Albugo tragopogonis*;

Sclerotinia basal stalk, midstalk and head rot caused by *S. sclerotiorum*; Septoria leaf blight caused by *Septoria helianthi*; Verticillium wilt caused by *V. dahlia*; and sunflower mosaic caused by *Sunflower mosaic virus* (SuMV); and all other viruses known to infect sunflowers (Gulya et al. 2012).

In the inspected fields over the 15-year period, rust, Sclerotinia stalk rots, and downy mildew were confirmed in 4.3, 2.6, and 0.5% of the fields, respectively, with the majority of Sclerotinia stalk rots associated with basal stalk infections (i.e., Sclerotinia basal stalk rot) (Gulya et al. 2012). None of the other monitored pathogens was found in the sunflower seed production fields during this 15-year period (Gulya et al. 2012). Other pathogens reported in California sunflower seed production fields have included *M. phaseolina*, *Sclerotinia minor*, and *S. rolfsii* in 0.7, 0.4, and 0.2% of the fields inspected over the same years, respectively; powdery mildew caused by *G. cichoracearum* and Rhizopus head rot caused by *R. oryzae* were also reported (Gulya et al. 2012).

In the Columbia Basin of central Washington, the only sunflower seed crop disease that has occurred at levels of economic consequence to date is white mold caused by *S. sclerotiorum*, with reported losses ranging from <1% to 75%; more specifically, Sclerotinia basal stalk rot, midstalk rot, and head rot have all caused significant losses to sunflower seed crops in the area (Bill Wirth, Precision Seed Production, LLC, *personal communication*). There is a long history of *S. sclerotiorum* being present in the Columbia Basin due to white mold occurring in other susceptible crops grown in this region, such as potato, bean, pea, carrot, alfalfa, and various *Brassica* spp. (Bill Wirth, Precision Seed Production, LLC, *personal communication*; Pacific Northwest Plant Disease Management Handbook 2016).

1.2. *Sclerotinia.*

1.2.1. *Sclerotinia* species: Morphology and taxonomy. The genus *Sclerotinia* belongs to Sclerotiniaceae, a family of ascomycetes in the Kingdom Fungi, and includes three economically important plant pathogenic species: *S. sclerotiorum* (Lib.) de Bary (syn. *Whetzelinia sclerotiorum* (Lib.) Korf & Dumont, *S. libertania* Fuckel, *Peziza sclerotiorum* Lib.), *S. minor* Jagger, and *S. trifoliorum* Eriksson (Bolton et al. 2006; Kohn 1979b; Willets and Wong 1980). Some morphological features shared by, but not exclusive to, these *Sclerotinia* spp., are sclerotia formation, no known functional conidial state (anamorph), and production of a fruiting structure called an apothecium from the sclerotium in which inoperculate asci form, each with eight binucleate, hyaline ascospores (Kohn 1979b; Willets and Wong 1980). Ascospores of *S. sclerotiorum* are 4-6 x 9-14 µm (Kohn 1979a). While similar morphologically, *Sclerotinia* species were delimited by Kohn (1979b) based on morphological characteristics, and by Willets and Wong (1980) based on electrophoretic analysis of proteins and enzymes, sclerotial ontogenesis, mycelial interactions, and cytology. Kohn (1979b) separated *S. minor* from *S. sclerotiorum* and *S. trifoliorum* based on sclerotial size (0.5 to 2 mm for *S. minor* vs. 2 to 20 mm for *S. sclerotiorum* and *S. trifoliorum*), whereas *S. sclerotiorum* and *S. trifoliorum* were separated based on characteristics of the sclerotial rind (absence or presence of tomentum hyphae, respectively). Based on apothecial morphology, *S. minor*, *S. trifoliorum*, and *S. sclerotiorum* were separated based on characteristics of the ascospores and ectal excipulum. Notably, ascospores of *S. minor*, *S. trifoliorum*, and *S. sclerotiorum* are tetranucleate and monomorphic, tetranucleate and dimorphic, and binucleate and monomorphic, respectively. In addition, the ectal excipulum at the margin of the apothecium are composed of globose cells in *S. minor* vs. composed of prosenchyma 'turning out' perpendicularly to the apothecial surface in *S. sclerotiorum*.

1.2.2. *Sclerotinia sclerotiorum* as a plant pathogen. *S. sclerotiorum* is a necrotrophic, soilborne fungus that has a wide, phylogenetically diverse host range of >400, mostly dicotyledonous plant species (Boland and Hall 1994; Purdy 1979). Even though *S. sclerotiorum* is distributed throughout much of the world, the fungus is most common in temperate regions (Willets and Wong 1980). More than 60 names have been used to denote diseases caused by *S. sclerotiorum*, including cottony rot, watery soft rot, stem rot, drop, and, most commonly, white mold (Bolton et al. 2006; Purdy 1979). *S. sclerotiorum* can cause significant economic losses not only through reductions in crop yield and quality, as demonstrated by annual losses exceeding $200 million in the U.S. alone, but also by forcing growers to plant less profitable, non-susceptible crops to be planted instead of more profitable, susceptible crops (Bolton et al. 2006; Purdy 1979). In response to the economic impact of *S. sclerotiorum*, the National Sclerotinia Initiative was established by the USDA to conduct a coordinated research strategy to minimize the effects of *S. sclerotiorum* on selected crops, including soybean, canola, sunflower, and dry pea [USDA Agricultural Research Service (ARS) National Sclerotinia Initiative 2016]. Typical symptoms of plant tissue infected by *S. sclerotiorum* are light brown lesions that appear water-soaked and rotten followed by development of cotton-like mycelium growing over the infected tissue (Willets and Wong 1980). Other symptoms include wilting, stunting, premature ripening, and sudden collapse of the host plant (Willets and Wong 1980).

1.2.2.1. Biology of *S. sclerotiorum*. The primary survival structure of *S. sclerotiorum*, a homothallic fungus, is a sclerotium, a compact mycelial body surrounded by a melanized rind (Bolton et al. 2006). The center of the compact mycelial body, referred to as the medulla, is composed of carbohydrates, especially β-glucans, and proteins, and is protected from adverse environmental conditions by the melanized rind (Bolton et al. 2006; Le Tourneau 1979).

Formation of sclerotia occurs in three stages: i) initiation of hyphal branching and aggregation to form sclerotial initials; ii) development in the form of continued hyphal growth and aggregation causing enlargement; and iii) maturation during which the rind becomes clearly delimited, melanin is deposited into rind cells, and consolidation of medullary tissue occurs (Bolton et al. 2006; Le Tourneau 1979; Townsend and Willets 1954). The process, in general, is initiated by environmental stress such as nutrient scarcity (Christias and Lockwood 1973). In the form of sclerotia, *S. sclerotiorum* can survive in soil for up to 8 years and germinate when environmental conditions are favorable (Abawi and Grogan 1979; Bardin and Huang 2001).

Sclerotia of *S. sclerotiorum* germinate: i) carpogenically, or ii) myceliogenically (Bolton et al. 2006). Carpogenic germination occurs in several stages: i) formation of primordia (bulges in the rind layer); ii) development of initials erupting through the rind layer; iii) apothecial stipe formation and elongation, which is phototropic; and iv) apothecial disk formation, which is conditioned by the presence of light (Le Tourneau 1979). The disk of a mature apothecium is made up of an ectal and medullary excipulum, the hymenium, and subhymenium (Kosasih and Willets 1975). Inoperculate asci develop in the hymenial layer and each produce eight hyaline ascospores, which are the product of meiosis followed by a mitotic division (Willets and Wong 1980). Myceliogenic germination occurs when hyphae emerge or mycelia erupt from sclerotia, and is conditioned by the presence of exogenous nutrients (Bardin and Huang 2001). Unlike carpogenic germination, this process is asexual. Infection of a host plant can occur by ascospores or directly by mycelium that develops from sclerotia or from adjacent infected plants (Bardin and Huang 2001). Cool, moist conditions favor rapid invasion of host tissue (Willets and Wong 1980).

Production of oxalic acid by *S. sclerotiorum* in culture and infected plant tissue, and the relation of this acid to pathogenesis has been the subject of many studies (Bolton et al. 2006; Willets and Wong 1980; Xu et al. 2015). Several proposed mechanisms by which oxalic acid production functions in pathogenicity have been outlined (Bolton et al. 2006). For example, early in pathogenesis, oxalic acid accumulation lowers the extracellular pH to about 4 to 5, the optimal range for many cell-wall degrading enzymes, effectively enhancing enzyme activity (Bolton et al. 2006). While oxalic acid was once thought to be a pathogenicity determinant (Dickman 2007), research by Xu et al. (2015) showed that, in fact, a low pH defines the optimum condition for pathogenicity, whereas oxalate is not essential for pathogenicity of *S. sclerotiorum*.

1.2.2.2. Population biology of *S. sclerotiorum*. The genetic diversity, population structure, and genetic differentiation of *S. sclerotiorum* populations have been the subject of many studies worldwide. Recent studies, using robust molecular markers, e.g., microsatellite markers, have shown high genetic diversity, and both recombining and clonal population structures of the fungus in different regions of the world and in different host crops (Atallah et al. 2004; Attanayake et al. 2012, 2013; Gomes et al. 2011; Hemmati et al. 2009; Mert-Türk et al. 2007; Sexton et al. 2006). Recently, linkage disequilibrium decay analysis has provided further evidence of recombinance, i.e., outcrossing, in *S. sclerotiorum* populations (Attanayake et al. 2014). Genetic differentiation of *S. sclerotiorum* populations has been shown to occur in soil populations covering a 1 m^2 area (Attanayake et al. 2012), among populations from different crops in different regions of California and the Pacific Northwest (Malvárez et al. 2007), among populations from dry bean in the Brazilian Cerrado (Gomes et al. 2011), and among populations from the same crop species grown on different continents (Attanayake et al. 2013). However, other studies have not shown genetic differentiation among populations. For example, no

difference was found among populations of *S. sclerotiorum* from four potato fields in central Washington (Atallah et al. 2004), among populations of the fungus from sunflower crops in different regions of Australia (Ekins et al. 2011), among populations from canola in Northern Iran (Hemmati et al. 2009) and even among populations from the same crop species on different continents (Li et al. 2009). Generally, published research points to *S. sclerotiorum* populations having high genetic diversity, which has been shown to increase the ability of some fungal populations to adapt to their environment (Zhan et al. 2005).

When researchers have looked for a relationship between genotypes of *S. sclerotiorum*, characterized by microsatellite markers or mycelial compatibility groups, and putative phenotypic markers of fitness, e.g., virulence, many times no correlation has been found (Atallah et al. 2004; Attanayake et al. 2012; Kull et al. 2004; Sexton and Howlett 2004). For instance, even though fungicide resistance (Gossen et al. 2001; Zhou et al. 2014) and variability in virulence among isolates of *S. sclerotiorum* (Willbur et al. 2017) have been shown, several studies that have attempted to correlate genotypes with those characteristics have failed to detect significant associations (Atallah et al. 2004; Attanayake et al. 2012; Sexton and Howlett 2004). Other characteristics such as colony growth, oxalic acid production, host specificity, and preferential mode of infection (ascospore infection initiated by carpogenic germination or direct infection initiated by myceliogenic germination) have also not been found to be associated significantly with genotypes of the isolates (Atallah et al. 2004; Attanayake et al. 2012; Ekins et al. 2011; Kull et al. 2004). Regardless, high heritability (a measure of the phenotypic variation associated with genotypic variation) of phenotypic traits, including fungicide resistance, production of sclerotia, mycelial growth, and oxalic acid production, has been demonstrated for this pathogen and, thus, these traits are likely to respond to selection pressures (Attanayake et al.

2013). Low heritability in virulence was explained by strong environmental influence and strong selection pressure exerted on the trait, which erode genetic variation (Attanayake et al. 2013).

Other studies have shown significant associations between genotypic and phenotypic markers of fitness for *S. sclerotiorum* populations (Lehner et al. 2016; Otto-Hanson et al. 2011). For example, in a study looking at common bean resistance to white mold, Lehner et al. (2016) showed a significant interaction among isolates of *S. sclerotiorum* and genotypes of common bean in disease severity and area under the disease progress curve. This suggested some degree of bean cultivar specialization of the *S. sclerotiorum* genotypes screened. In contrast, Otto-Hansen et al. (2011) showed no significant interaction between isolates of *S. sclerotiorum* from different mycelial compatibility groups and bean genotypes. That study did show significant differences in *S. sclerotiorum* isolates for virulence between, but not within, mycelial compatibility groups (Otto-Hanson et al. 2011). This knowledge can be applied to breeding for resistance to white mold, as care should be taken in selecting isolates of *S. sclerotiorum* with which to assess host resistance (Otto-Hanson et al. 2011; Willbur et al. 2017).

1.2.2.3. Life cycle and epidemiology of *S. sclerotiorum*. The life cycle of *S. sclerotiorum* has two phases, an airborne, sexual phase and a soilborne, asexual phase based on the two types of sclerotium germination, which lead to aerial infections and basal infections of the susceptible host, respectively (Bolton et al. 2006). As stated above, soilborne sclerotia can germinate carpogenically or myceliogenically in response to different conducive environmental conditions (Bolton et al. 2006). Carpogenic germination results in the aerial release of ascospores during cool, moist conditions, which can infect the plant aboveground provided a nutrient source is present for the fungus, e.g., flower petals (Abawi and Grogan 1979; Willets and Wong 1980). This type of infection has been documented as the primary cause of white

mold outbreaks in crops such as bean (Cook et al. 1975), canola (Turkington and Morall 1993), carrot (Kora et al. 2005a), lettuce (Patterson and Grogan 1985), potato (Atallah and Johnson 2004), and sunflower (McCartney and Lacey 1991; Nelson and Lamey 2000). Myceliogenic germination is stimulated by exogenous nutrients, such as root exudates, resulting in the formation of hyphae which can infect susceptible host root and crown tissue of plants (Abawi and Grogan 1979; Gulya and Masirevic 1992; Huang and Dueck 1980). This mode of infection has been reported for many crops, including carrot, lettuce, and sunflower (Bardin and Huang 2001; Chitrampalam et al. 2010; Huang and Dueck 1980).

White mold lesion expansion is optimum at cool (15 to 20^{o}C) and moist conditions (Willets and Wong 1980). Sclerotia form from mycelia on diseased tissue when environmental stress is encountered by the pathogen (Adams and Ayers 1979; Willets and Wong 1980). In some crops, including bean, soybean, and sunflower, *S. sclerotiorum* also can be seedborne (Herd and Phillips 1988; Mueller et al. 1999; Tu 1988). Sclerotia that form on or in infected seed are then capable of myceliogenic and, limited (<8.0% of sclerotia), carpogenic germination, and can cause disease in the same growing season as planted (Mueller et al. 1999; Tu 1988). Storage rots are caused postharvest by continued development of mycelial infection in susceptible crops, such as carrots (Lumsden 1979). Return of sclerotia to the soil by tillage, on crop residues, or other means, completes the life cycle begun by either mode of sclerotial germination of *S. sclerotiorum*.

Diseases caused by *S. sclerotiorum*, whether initiated by carpogenic or myceliogenic germination, are considered monocyclic (Abawi and Grogan 1979). While populations of sclerotia of *S. sclerotiorum* have been shown to decline over time (Ben-Yephet et al. 1993; Duncan et al. 2006), 95% of sclerotia may remain viable for 2 years (Williams and Western

1965), and sclerotia have remained viable in some soils for up to 8 years (Ben-Yephet et al. 1993). The longevity of sclerotium survival can be affected by moistening and rewetting of soil (Adams 1975), high soil temperature and moisture (Cook et al. 1975; Wu et al. 2008), anoxic soil conditions (Wu et al. 2008), other soilborne microorganisms (Duncan et al. 2006; Merriman 1976), sclerotium size (Ben-Yephet et al. 1993; Harvey et al. 1995), and depth of sclerotium burial (Duncan et al. 2006; Merriman et al. 1979). Sclerotia can act as a fungal pathway into new areas when the sclerotia are moved with inadequately cleaned, infested seed lots, and in irrigation water, manure, and/or soil (Adams and Ayers 1979; Willets and Wong 1980).

In many crops, disease initiated by carpogenic germination of *S. sclerotiorum* is responsible for epidemics (Bolton et al. 2006). However, in sunflower (Nelson and Lamey 2000) and lettuce (Chitrampalam et al. 2010; Patterson and Grogan 1985) disease initiated by both carpogenic and myceliogenic germination can be significant. The development and severity of disease outbreaks initiated by carpogenic germination is affected by several factors: the prevalence and distribution of inoculum; microclimatic factors affecting carpogenic germination and the subsequent discharge, survival, and infectivity of ascospores; and the developmental stage of the host (Abawi and Grogan 1979).

Schwartz and Steadman (1978) showed that a low sclerotial density of 0.2 sclerotium/kg soil was sufficient to cause 46% incidence of white mold in bean crops in Nebraska, initiated by carpogenic germination. Even so, no consistent relationship between sclerotial density in the upper soil profile and disease incidence initiated by carpogenic germination was found in that study. One reason might be that apothecium production is related inversely to sclerotium depth and is maximized in the top 2 cm of the soil profile (Abawi and Grogan 1979; Mitchell and

Wheeler 1990). In the Schwartz and Steadman study (1978), sclerotial density was estimated over soil profile depth ranging from 7.5 to 15 cm.

Associations between apothecial density and white mold incidence have also been studied. Greater apothecial density has been correlated with greater disease incidence (Boland and Hall 1988), although intensive, large-scale sampling has been required in some cases to detect a significant relationship between apothecial density and disease incidence (Gugel and Morrall 1986). Furthermore, the spatial patterns of apothecia in soil and diseased plants have been reported to be highly clustered (Boland and Hall 1988). These results are in line with studies that have reported most ascospores are deposited near the apothecial source (Ben-Yephet and Bitton 1985; Hartill 1980; Wegulo et al. 2000), which would lead to sharp disease foci. For instance, although ascospores can be transported distances of several kilometers by wind (Abawi and Grogan 1979), Hartill (1980) reported the majority of ascospores are deposited within a few meters of the source apothecium.

Apothecial density may not always be correlated significantly with white mold incidence (Morall and Dueck 1982). One reason postulated for such a lack of correlation in a field is dispersal of ascospores from sources outside the field (Gugel and Morrall 1986; Hammond et al. 2008; Morall and Dueck 1982; Patterson and Grogan 1985). The hypothesis is supported by reports of high disease incidence in fields in which few apothecia were found (Morall and Dueck 1982), and was substantiated by a study by Wegulo et al. (2000) showing movement of ascospores from an infested corn field to an adjacent soybean field.

Microclimatic conditions in the field play an important role in the development and severity of white mold that develops from ascospore infection resulting from carpogenic germination, as ascospore survival, germination, and infection potential are all governed by

environmental conditions (Bolton et al. 2006). Carpogenic germination has been shown to be influenced by soil water potential, soil temperature, soil texture, light intensity, length and temperature of sclerotial vernalization, and temperature of sclerotial formation, with effects varying based on isolate of *S. sclerotiorum* (Clarkson et al. 2004, 2007; Huang and Kozub 1991; Mila and Yang 2008; Mitchell and Wheeler 1990; Twengstrom et al. 1998; Sun and Yang 2000; Wu and Subbarao 2008). Continuous high soil water potential for approximately 10 days was reported as a requirement for carpogenic germination by Abawi and Grogan (1979). Levels of soil water potential sufficient for carpogenic germination have been reported at ≥-300 kPa, and increasing carpogenic germination has been associated with increasing water potential (Clarkson et al. 2004; Wu and Subbarao 2008). Soil temperatures between 5 and 20 to 30^{o}C have been reported as sufficient for carpogenic germination with optimal temperatures between 15 and 20^{o}C (Clarkson et al. 2004; Sun and Yang 2000; Wu and Subbarao 2008). Fluctuations, either in soil water potential or soil temperature (latter exceeding 8^{o}C), has been shown to be detrimental to carpogenic germination, increasing the time to germination and reducing the number of apothecia produced (Mila and Yang 2008). Another factor affecting carpogenic germination is light intensity, with high light intensity increasing the number of sclerotial initials that develop into apothecia, and speeding up that process (Sun and Yang 2000). Time to carpogenic germination has also been shown to be inversely related to length of vernalization, with temperatures <10^{o}C optimal; at 5^{o}C, adequate vernalization can occur in 2 to 6 days compared to 30 to 80 days at 15^{o}C (Clarkson et al. 2007). However, a vernalization period is not always necessary as 1.2% of non-vernalized sclerotia formed on infected soybean seeds that were buried in the soil germinated carpogenically within 6 weeks, and 8.0% within 11 weeks in ideal conditions of saturated soil at 14^{o}C (Tu 1988). Finally, the origin of *S. sclerotiorum*

isolates is important, as isolates of *S. sclerotiorum* vary in vernalization requirement to germinate carpogenically depending on the temperature at which sclerotia are formed (Huang and Kozub 1991).

Ascospore release is not affected by light, and can occur continuously in saturated air (90 to 95% RH) and in moderately saturated air (65 to 75% RH) (Clarkson et al. 2003), although discharge is commonly triggered by a drop in RH that results in a "puff" of ascospores more than 1 cm above the surface of the apothecium (Abawi and Grogan 1979; Hartill and Underhill 1976; Ingold 1971). The duration for which an apothecium can produce ascospores is conditioned by temperature and soil moisture (Newton and Sequeira 1972; Twengstrom et al. 1998), with $\leq 3 \times 10^7$ ascospores discharged by a single apothecium (Abawi and Grogan 1979). Under optimal conditions, ascospores can be released continuously at a rate of 1,600 spores/h for >10 days (Clarkson et al. 2003). In field studies, apothecia have been shown to have lifespans ranging from 2 to 33 days (Twengstrom et al. 1998), with peak ascospore release generally occurring mid-day (Ben-Yephet and Bitton 1985; Gutierrez and Shew 1998; McCartney and Lacey 1991). In drier conditions, ascospore release has peaked between 2 and 7 am (Qandah and del Rio Mendoza 2011). Ascospore survival is affected by air temperature, RH, and ultraviolet (UV) radiation, with greater air temperature, RH, and UV exposure associated with shorter survival periods (Caesar and Pearson 1983; Clarkson et al. 2003). In the field, ascospores have survived up to 12 days on bean leaves in New York (Caesar and Pearson 1983).

Ascospore germination occurs in a film of water (Lumsden 1979), but infection also depends on availability of a nutrient source (Abawi and Grogan 1979; Lumsden 1979). In beans,16 to 24 h vs. >72 h of leaf wetness were needed to initiate infection by moist, infested vs. dry, infested bean blossoms, respectively (Abawi and Grogan 1979). The dependency of

carpogenic germination on high soil water potential for relatively long periods (Abawi and Grogan 1979; Clarkson et al. 2004, 2007) and of ascospore infection on moisture and a nutrient source (Lumsden 1979) generally limits ascospore-initiated white mold to the flowering stage of the host plant (Bolton et al. 2006). Flower petals are generally the primary nutrient source (Abawi et al. 1975; Atallah and Johnson 2004; Turkington and Morall 1993), and flowering typically coincides with crop canopy closure which shades the ground and helps maintain high soil water potential and RH (Bolton et al. 2006). However, this is not always the case. For instance, in fresh market carrot crops, canopy cover >95% and senescent leaves are sufficient for disease development under conducive environmental conditions without the presence of flower petals (Kora et al 2005a).

Macroclimatic conditions, are also important as they affect the microclimate within a field and, thus, white mold incidence. For instance, above-normal precipitation was cited as the reason for an outbreak of Sclerotinia head rot in North Dakota in 1986 (Gulya et al. 1989). While a study by Workneh and Yang (2000) did not find a significant relationship between Sclerotinia stem rot of soybean and precipitation, they did find that prevalence of the disease was related to cumulative departures from normal maximum and minimum temperatures in July and August, with disease more prevalent when these monthly temperatures were below normal. In addition, they noted that the incidence of Sclerotinia stem rot of soybean was related exponentially to latitudinal position of the fields, which reflected the effects of north-south variations in temperature (Workneh and Yang 2000).

The development and severity of white mold initiated by myceliogenic germination also is affected by several factors. These include the prevalence and distribution of sclerotial inoculum, exposure to exogenous nutrient sources, such as root exudates, and environmental

conditions (Bardin and Huang 2001; Holley and Nelson 1986). Greater sclerotial density in the upper layer of the soil profile has been shown to be positively correlated with greater disease incidence when white mold is initiated by myceliogenic germination in sunflower (Holley and Nelson 1986). The sclerotial density reported to cause significant disease incidence was low (<1 sclerotium/800 cm^3 or ~850 g soil) compared to other sclerotia-producing soilborne pathogens (Holley and Nelson 1986). The result may be explained by the dense lateral root system of sunflower plants, as *S. sclerotiorum* mycelium seldom extends beyond 2 to 3 cm from a sclerotium to colonize a root, although root-to-root spread has been reported (Gulya and Masirevic 1992; Holley and Nelson 1986; Huang and Hoes 1980). A study by Chitrampalam et al. (2010) on lettuce drop initiated by myceliogenic germination of sclerotia of *S. sclerotiorum*, found a direct, significant relationship between sclerotial density and disease incidence.

Myceliogenic sclerotial germination is reported to be induced by the presence of exogenous nutrients (Abawi and Grogan 1979). In vitro research by Burgess and Hepworth (1996) showed that root exudates of sunflower enhanced myceliogenic germination of a related species, *S. minor*, suggesting a role of root exudates in stimulating germination. Studies on environmental conditions have shown incomplete melanization of the sclerotial rind layer or mechanical damage to the rind layer (Huang 1985), exposure to repeated wet and dry cycles (Smith 1972), and sub-freezing temperatures (Huang 1991) can all cause myceliogenic germination without stimulus from an exogenous nutrient source. In addition, Huang et al. (1998) reported that sclerotia formed at 20 to 25°C vs. cooler temperatures, and desiccant-dried vs. air-dried sclerotia germinated myceliogenically more readily when incubated at high RH.

1.2.2.4. *S. sclerotiorum* as a pathogen of sunflower crops. Sclerotinia basal stalk rot (Sclerotinia wilt) usually is the result of myceliogenic germination; hyphae of a germinated

sclerotium directly infect the sunflower plant in the root tissue and/or crown of the plant, and the disease can then spread from plant-to-plant by the roots of adjacent plants (Huang and Dueck 1980; Huang and Hoes 1980). In North Dakota and South Dakota, Sclerotinia basal stalk rot is, historically, the most prevalent form of the disease caused by *S. sclerotiorum* (Bradley et al. 2007). Typical symptoms of basal stalk rot include wilting of the sunflower plant and water-soaked lesions on the tap root and some fibrous roots; severely infected plants develop a characteristic lesion at the stem base (Huang and Dueck 1980). The lesion can extend from the taproot up the hypocotyl, as much as 50 cm up the stem. White mycelium develops with dark sclerotia interspersed over the plant surface and within the root and stem throughout the infected area, with an estimated 50 to 100 sclerotia potentially forming in each infected sunflower stem (Gulya and Masirevic 1992; Huang and Dueck 1980). In western Canada, Sclerotinia basal stalk rot has been shown to occur during two main periods in sunflower development: i) at seedling establishment, and ii) from budding through anthesis and seed development (Huang and Kozub 1990). Significant yield losses occur when sunflower plants wilt during any growth stage from flowering to near maturity, because of reductions in seed weight and yield (Dorrell and Huang 1978). Seed quality measured by test weight, oil content, and protein content are also reduced when wilting occurs within six weeks of flowering (Dorrell and Huang 1978).

Sclerotinia midstalk and head rot are typically the result of carpogenic germination by sclerotia of *S. sclerotiorum*; ascospores released from apothecia infect the sunflower plants when and where senescent tissue and adequate free water are present for a prolonged period (42 h for head rot) (Gulya et al. 1989; Nelson and Lamey 2000). Midstalk rots typically occur where the leaf petiole branches off the upper or middle-stem and a nutrient source, such as pollen grains or petals are present or deposited; head rots occur on the head of the sunflower with the flowers

providing the nutrient source (Gulya and Masirevic 1992; Nelson and Lamey 2000). While senescent tissue is a general requirement for infection, midstalk rot has also been shown to occur sans senescent tissue at sites of high sucrose secretion located where the leaf petiole and upper or middle-stem conjoin (Sedun and Brown 1987). Symptoms of midstalk rot appear during or after flowering and those of head rot generally after flowering (Nelson and Lamey 2000). Midstalk rot is expressed as a water-soaked lesion, which may become covered with dense white mycelium; as the lesion ages, the tissue dies, bleaches and dries out, and may take on a shredded appearance with the stalk lodging under the weight of the head (Nelson and Lamey 2000). Sclerotia are formed on the outside and within the stem as the lesion matures.

Typical symptoms of early head rot infection are white mycelium growing over flower parts and/or water-soaked spots on the receptacle (fleshy part) of the head (Nelson and Lamey 2000). Sunflowers are most susceptible to head rot infection from the beginning of floral initiation to 2 weeks after flowering, and plants on which the stem arches after flowering to place the head deeper within the crop canopy favor disease development (Gulya and Masirevic 1992; Seiler 1997). As infection progresses, *S. sclerotiorum* decays the receptacle and can produce many large, black sclerotia, in the tissue of the receptacle and/or over part or all of the seed layer of the head; generally sclerotia start forming in the receptacle 7 to 10 days post-infection (Nelson and Lamey 2000). The infected head tissue eventually dies, dries out, and appears bleached and/or shredded if the seed layer completely rots (Nelson and Lamey 2000).

Although aerial infections, including midstalk rots and head rots, are caused by ascospores of *S. sclerotiorum*, symptoms may only develop long after ascospores are released. In a study by McCartney and Lacey (1991) in the United Kingdom, symptoms did not develop until 30 to 40 days after ascospore release began. In that study, the rate of appearance of new

infections was positively correlated with airborne ascospore concentration 5 weeks earlier. From year-to-year, there can be great variability in aerial disease incidence (Gulya et al. 1989; McCartney and Lacey 1991). In North Dakota, aerial infections occur sporadically, and a particularly severe level of head rot in 1986 (average of 10.2% incidence over the 80 fields surveyed compared to 0.05% in 1984) was hypothesized to be the result of above-normal precipitation that year (Gulya et al. 1989). When head rot does occur, significant reductions in seed yield, seed weight, number of seed/head, and oil content can occur; sunflower crop losses worth $100 million have occurred as a result of head rot (Bolton et al. 2006; Gulya et al. 1989).

With head rots, many sclerotia may be harvested along with the sunflower seed, and become a seed cleaning issue; sclerotia can have similar size, shape and specific gravity as seed, making it hard to remove all the sclerotia from an infested seed lot (Gulya et al. 1989). While planting contaminated seed lots is known to introduce sclerotia to fields, *S. sclerotiorum* can also be seed-borne as dormant mycelia or sclerotia that form inside the hull of an achene (Herd and Phillips 1988; Harveson et al. 2016). However, treatment of seed with fungicides effective against the pathogen can limit the risk of *S. sclerotiorum* being introduced on infested seed (Herd and Phillips 1988).

1.2.3. Management of diseases caused by *S. sclerotiorum*. As white mold is a monocyclic disease (Abawi and Grogan 1979), reducing sclerotial populations in the soil prior to planting a susceptible crop is important for managing the disease (Steadman 1979). Several approaches have been used to accomplish this goal, including crop rotation, tillage practices, and application of biocontrol products (Bardin and Huang 2001; Kurle et al. 2001; Steadman 1979; Subbarao et al. 1996). Other approaches include modification of microclimatic conditions to prevent or reduce carpogenic germination, and foliar application of fungicides to prevent

infection by ascospores (Bardin and Huang 2001; Bradley et al. 2006; Steadman 1979). Planting cultivars partially resistant or tolerant to white mold is another management option, when available for the plant species and cultivar market characteristics required (Miller 2007). Complete resistance to white mold has not been found in sunflower or bean germplasm, but partial resistance has been identified (Schwartz and Singh 2013; Talukder et al. 2014). Complicating the breeding for resistance in sunflowers is the fact that resistance to Sclerotinia basal stalk rot and resistance to Sclerotinia head rot were not correlated significantly in an evaluation of the USDA sunflower germplasm collection (Talukder et al. 2014), and there can be variability in white mold resistance screening results depending on the isolate(s) of the pathogen and environmental conditions during the evaluation (Schwartz and Singh 2013).

1.2.3.1. Effect of crop rotation, crop residue, tillage, burial, and soil microflora on incidence of white mold and survival of sclerotia. Crop rotation, while a recommended practice for managing white mold (Nelson and Lamey 2000; Steadman 1979), is not always effective at reducing sclerotia populations within the practical consideration of some farming systems (Mueller et al. 2002; Schwartz and Steadman 1978). In relatively short-term study of white mold on beans, Schwartz and Steadman (1978) reported that populations of sclerotia did not decrease or increase appreciably under continuous cropping of a non-host crop or beans, respectively, over 3 years, and similar results were reported in a study on soybeans (Mueller et al. 2002). Likewise, no consistent reduction in apothecia production due to crop rotation was found in several other studies (Gracia-Garza et al. 2002; Kurle et al. 2001; Morall and Dueck 1982; Mueller et al. 2002). Another reason short-term crop rotation may not always be effective at controlling white mold, is that ascospore inoculum can move into susceptible crops from adjacent fields planted to non-host crops (Steadman 1979; Wegulo et al. 2000). Despite these

studies, crop rotation has been shown to be an important factor in white mold forecasting models for some crops (Koch et al. 2007; Twengstrom et al. 1998), and can be an effective disease management tool, e.g., white mold incidence in soybeans was reduced in oat-soybean and corn-soybean rotations (Kurle et al. 2001).

The use of tillage, to bury sclerotia deeper in the soil profile, is another cultural practice to control white mold (Nelson and Lamey 2000). Moldboard plowing can be used effectively to bury sclerotia >10 cm in the soil profile (Kurle et al. 2001; Mueller et al. 2002), which can prevent carpogenic germination (Abawi and Grogan 1979). However, when moldboard plowing is practiced on an annual basis, some sclerotia buried by previous plowing will be brought back to the upper layer of soil by the next round of plowing and will then be able to germinate carpogenically (Mueller et al. 2002). Hence, it is recommended growers only use shallow tillage for several subsequent growing seasons after a white mold outbreak in a field to prevent bringing sclerotia back up to the surface, if white mold control is the primary purpose of the tillage (Nelson and Lamey 2000). Indeed, this strategy led to lower levels of the disease in soybeans compared with no tillage and mulch tillage (Mueller et al. 2002). In contrast, no tillage, mulch tillage, or chisel plowing leave most sclerotia near the soil surface (Kurle et al. 2001; Mueller et al. 2002). Although sclerotia left near the surface can germinate carpogenically (Mitchell and Wheeler 1990), studies have found that fewer apothecia may form when no tillage is practiced in contrast to tillage by disking and chisel plowing (Gracia-Garza et al. 2002). No-till practices were also shown to reduce disease incidence compared to fields receiving minimum tillage, defined as maintaining 15 to 30% plant residue on the soil surface (Workneh and Yang 2000), or chisel or moldboard plowing yearly (Kurle et al. 2001; Mueller et al. 2002). Reduced tillage and

no tillage have the benefit of not bringing deeper buried sclerotia to the soil surface, and not redistributing sclerotia to areas of a field not previously infested (Subbarao et al. 1996).

Burial, including deep burial, have been shown to reduce sclerotial viability over time (Adams 1975; Duncan et al. 2006; Merriman 1976; Merriman et al. 1979; Wu et al. 2008). In a study by Duncan et al. (2006), sclerotia on the surface and sclerotia buried at 5 and 10 cm depths had declined from 80% viability to 57.5, 12.5, and 2.5%, respectively, over a period of 12 months. Merriman et al. (1979) reported that burial reduced sclerotial viability, but that sclerotial viability declined faster when sclerotia were exposed and buried compared to when sclerotia were protected by bean residues and buried.

Soil microflora, including fungi (Harvey et al. 1995; Merriman et al. 1979) and bacteria (Duncan et al. 2006) have been associated with sclerotia of *S. sclerotiorum* in the soil. Merriman et al. (1976) reported frequently isolating *Fusarium* spp., *Mucor* spp. and *Trichoderma* spp. associated with sclerotia and, less frequently, *Alternaria* spp., *Coniothyrium minitans*, *Epicoccum* spp., and *Penicillium* spp. That study showed that sclerotia naturally infested with fungi had viability reduced faster when placed in/on soil vs. sclerotia grown in culture on sterile potato cubes. In a study by Duncan et al. (2006), an inverse relationship between bacterial colonization levels and sclerotial viability was demonstrated; moreover, 268 morphologically different bacterial isolates were identified in association with sclerotia in the soil, 29 of which were inhibitory to *in vitro* mycelial growth of *S. sclerotiorum*. The 29 isolates comprised 15 species of bacteria, with over half being *Bacillus* spp.

In particular, the coelomycete *C. minitans* has been studied as a biocontrol agent for white mold as the fungus infects sclerotia of many ascomycetes, including *S. sclerotiorum* (Whipps and Gerlagh 1992; Whipps et al. 2008). In one study, *C. minitans* survived in the soil

for up to 30 days (Bennett et al. 2003), and, in another study, was recovered from sclerotia for up to 2 years after application (McQuilken et al. 1995). Maximum parasitism of sclerotia of *S. sclerotiorum* by *C. minitans* has been shown to occur between 15 and 20ºC (Budge et al. 1995), and low soil water potential adversely affects *C. minitans* in the soil since growth, conidial germination, and parasitism of sclerotia of *S. sclerotiorum* are reduced as water potential decreases (Jones et al. 2011). *C. minitans* can spread with movement of soil and water within soil (McQuilken et al. 1995), but does not have saprotrophic activity as the fungus is an obligate mycoparasite (Whipps et al. 2008).

The ability of *C. minitans* to reduce *S. sclerotiorum* populations in the soil is well documented (Budge and Whipps 1991; Chitrampalam et al. 2010; Gerlagh et al. 1999; McQuilken et al. 1995), and *C. minitans* has been shown to be effective at reducing lettuce drop incidence both in the glasshouse (Budge and Whipps 1991; Jones et al. 2004; Van Beneden et al. 2010) and in fields (Chitrampalam et al. 2010; McLaren et al. 1994). However, in crops such as canola and potato, *C. minitans* has not always been effective at reducing white mold incidence in-season (Hammond et al. 2008; McQuilken et al. 1995). Long-term, repeated use of *C. minitans* has been reported to reduce *S. sclerotiorum* populations and white mold incidence in a rotation of potato, bean, carrot, and chicory (Gerlagh et al. 1999). Application rate and timing also affect the efficacy of *C. minitans* (Gerlagh et al. 2003; Jones et al. 2003, 2004). Greater application rates led to reduced carpogenic germination, recovery, and viability of sclerotia (Jones et al. 2003), but research also has demonstrated that applications must be made far enough in advance of a crop for in-season control (Jones et al. 2004), or immediately after symptoms appear for optimum infection of sclerotia formed in a diseased crop (Gerlagh et al. 2003).

1.2.3.2. Effect of plant density, canopy density, and irrigation on carpogenic germination and white mold incidence. Canopy density and irrigation both affect the microclimate in a crop and, thus, contribute to the overall favorability of the microclimate to carpogenic germination of *S. sclerotiorum* and ascospore infection (Steadman 1979). Reducing the crop plant density can be a useful management tool (Jurke and Fernando 2008; Vieira et al. 2010) as greater white mold incidence has been observed in more densely planted crops due to increased canopy density and, therefore, RH (Jurke and Fernando 2008; Turkington et al. 1991; Vieira et al. 2010). In bean, another means used to lessen canopy density is the planting of upright cultivars (Saindon et al. 1993). In other crops, such as fresh market carrot, foliage is "clipped" in order to open the canopy and make the microclimate less favorable to disease development by increasing air and soil temperatures and lowering RH (Kora et al. 2005b). In addition, avoiding over-fertilizing with nitrogen is important in reducing canopy density/coverage for some crops, e.g., sunflower (Gulya and Masirevic 1992).

Reduction in irrigation frequency also can be very effective at decreasing carpogenic germination, and is especially important when a dense crop canopy favors high soil moisture (Schwartz and Steadman 1978). Furthermore, reduction in irrigation frequency can successfully reduce carpogenic germination even when the same amount of total water is applied over the same time span by using less frequent irrigation intervals (Twengstrom et al. 1998). The caveat is that flexibility in the frequency of irrigation is conditioned on the water storage capacity of a soil, with a lower water storage capacity necessitating more frequent irrigations, and irrigation must be managed to minimize crop stress that could impact yield (Unger 1990). Plant density also reflects the spacing of plant root systems, but this was not correlated with greater disease incidence caused by basal infections initiated by myceliogenic germination of *S. sclerotiorum* in

sunflower crops (Holley and Nelson 1986; Nelson et al. 1989). This was true even though the lowest plant density evaluated resulting in a within-row spacing >30 cm, which should have limited root-to-root spread of *S. sclerotiorum* (Holley and Nelson 1986; Huang and Hoes 1980).

1.2.3.3. Prevention of aerial infection using foliar fungicide applications. Fungicides applied to the canopy of a susceptible crop can be used to prevent infection by ascospores of *S. sclerotiorum* (Steadman 1979). Several fungicide chemistries belonging to different Fungicide Resistance Action Committee (FRAC) groups have been shown to be effective at suppressing *S. sclerotiorum* ascospore infection, including azoxystrobin (Group 11), boscalid (Group 7), fluazinam (Group 29), iprodione (Group 2), tebuconazole (Group 3), and thiophanate-methyl (Group 1) (Bradley et al. 2006; Johnson and Atallah 2006; Mahoney et al. 2014; FRAC 2016). For these chemistries to be effective in-season and to prevent the development of fungicide-resistant populations, which is imperative to the effectiveness long-term, proper fungicide management practices should be followed (Mueller et al. 2013). These include: i) use of appropriate rates of application, ii) use of effective sprayer equipment and calibration to get thorough coverage of the canopy, iii) proper timing of the application of fungicides and only when necessary, iv) rotating fungicides with different modes of action, v) tank mixing or applying pre-mixed fungicides with different modes of action effective against the pathogen, vi) monitoring baseline sensitivity levels of the pathogen to fungicides, and vii) assessing product effectiveness after application (Mueller et al. 2013).

For in-season efficacy, good crop coverage and proper timing of fungicide applications have been shown to be critical in controlling infections by ascospores of *S. sclerotiorum* (Bradley et al. 2006; Johnson and Atallah 2006; Morton and Hall 1989). The importance of good coverage was demonstrated in a bean study in which the level of control was related to the

percentage of blooms that received fungicide (Morton and Hall 1989). For several crops, including bean, canola, carrot, lettuce and potato, research has been done to ascertain when preventative fungicide applications should be made relative to crop development for optimum efficacy (Foster et al. 2011; Johnson and Atallah 2006; Kora et al. 2005a; Morton and Hall 1989; Turkington and Morall 1993). In bean, canola and potato, different times during bloom were identified as optimum for fungicide application to control white mold (Johnson and Atallah 2006; Morton and Hall 1989; Turkington and Morall 1993). In contrast, in fresh market carrot crops, >95% canopy cover with senescing leaves indicated fungicide applications were necessary, while in lettuce the vegetative growth stage for optimal fungicide application timing varied among years (Foster et al. 2011; Kora et al. 2005a; Patterson and Grogan 1985).

Even when the susceptible stages of crop development for *S. sclerotiorum* have been ascertained, assessment of disease risk against an action threshold, when possible, should be used to determine if a fungicide application should be made (Mueller et al. 2013). Risk of white mold has been assessed in several ways, including from estimates of inoculum potential as petal infestation (Turkington and Morall 1993) or ascospore concentration (Foster et al. 2011), and from a combination of inoculum potential, environmental conditions, and crop related factors (Koch et al. 2007; McDonald and Boland 2004; Twengstrom et al. 1998). In canola, levels of petal infestation during bloom were used to forecast white mold incidence and determine if fungicide application were necessary (Turkington and Morall 1993). More recently, real-time PCR assays (Almquist and Wallenhammar 2015; Yin et al. 2009; Ziesman et al. 2016) have been developed to assess petal infestation and obviate the need for a 3 to 5-day incubation period to assess petal infestation, so that fungicide applications can be made during a short window of susceptible host plant growth stage(s) (McLaren et al. 2004). In fresh market carrot crops, a

threshold of ascospores trapped on a *Sclerotinia* semi-selective agar medium has been used to guide successfully the number and timing of fungicide applications (Foster et al. 2011). Since a 3 to 5-day incubation period is necessary to detect ascospore inoculum of *S. sclerotiorum*, a real-time PCR assay was developed for use with a volumetric air sampler to speed the spore sampling and quantification process (Parker et al. 2014). However, as ascospore trapping may not always be practical, white mold risk in fresh market carrot crops has been modeled using canopy cover, soil temperature, and soil water potential, with fungicide applications recommended when the risk index exceeds a threshold value (Foster et al. 2011).

Other white mold risk models have incorporated a wider array of factors (Koch et al. 2007; McDonald and Boland 2004). Twengstrom et al. (1998) developed a risk-based model of disease incidence caused by *S. sclerotiorum* for use in canola based on macroclimatic variables, crop rotation (an indirect measure of inoculum potential), crop density, and regional risk of carpogenic germination assessed by the number of apothecia formed from bags of 100 sclerotia placed throughout provinces in Sweden. A study by Koch et al. (2007) in canola, which elucidated the SkleroPro forecasting system, took a similar approach of incorporating crop rotation, regional climate, and host development stage into the risk calculation. In addition, the model had the benefit of incorporating explicitly an economic threshold into the decision matrix of whether or not to spray (Koch et al. 2007). Even with the wide array of factors considered in the models developed by Twengstrom et al. (1998) and Koch et al. (2007), trapping and quantification of ascospores was investigated as a means of improving assessments of white mold risk in canola in the United Kingdom (Rogers et al. 2009).

1.2.3.4. Use of fungicides to prevent introduction of seed-borne inoculum. Seed infected with *S. sclerotiorum* can be treated with fungicides to reduce or prevent seed

transmission and sclerotia formation from infected seed (Harveson et al. 2016; Herd and Phillips 1988; Mueller et al. 1999). Seed treatments with the fungicide active ingredients benomyl, iprodione, procymidone, and vinclozolin were effective at preventing seed transmission from infected sunflower seeds gathered from heads that were partially rotted by *S. sclerotiorum* (Herd and Phillips 1988). The study showed that the fungicides applied with acetone as a solvent reduced the percentage of seeds infected with *S. sclerotiorum* from 65 to 0% for all products evaluated. When applied as a dust, benomyl and vinclozolin were just as effective as when applied with acetone, while iprodione and procymidone applied as a dust reduced the percentage of seeds infected with *S. sclerotiorum* to 4 and 1%, respectively. Similarly, infection of soybean seed by *S. sclerotiorum* was reduced from 4.0% (out of 200 seeds) to ≤0.7% by treating the seed with one or more of the active ingredients captan, fludioxonil, thiabendazole, or thiram (Mueller et al. 1999). In addition, fludioxonil, thiram, and thiabendazole significantly reduced the number of sclerotia that formed on seed from an 80:20 blend of healthy to infected soybean seed, i.e., from 100 seeds, an average of 2 sclerotia formed from seed treated with fludioxonil or thiram compared to 118.5 from the non-treated seed (Mueller et al. 1999).

1.2.3.5. Integrated management of white mold in sunflower crops. Management of white mold in sunflower crops is focused on limiting soilborne sclerotia populations and planting into relatively non-infested land away from infested areas, as is borne out by typical disease management recommendations for white mold (Bradley et al. 2007; Harveson et al. 2016; Nelson and Lamey 2000; Steadman 1979). For instance, it is recommended that growers use a 3 to 5-year rotation with non-susceptible host crops, such as corn or other cereals, when Sclerotinia basal stalk rot reaches 1 to 2% incidence, as well as appropriate tillage practices and applications of *C. minitans* to keep sclerotia populations limited (Bradley et al. 2007; Harveson et al. 2016;

Nelson and Lamey 2000). For tillage practices, it is suggested that growers either use no-till practices that leave sclerotia near the surface of the soil, exposed to fungi and weathering; or use deep tillage to place sclerotia deep in the soil profile and then, subsequently, only use shallow tillage to avoid bringing sclerotia back to the soil surface (Bradley et al. 2007; Harveson et al. 2016; Nelson and Lamey 2000). Other recommendations, such as control of white mold-susceptible broadleaf weeds or incorporating any sunflower crops with >20% basal stalk rot incidence 4 to 5 weeks after planting, also focus on preventing inoculum buildup (Nelson and Lamey 2000). In addition, cultivars that are more resistant to Sclerotinia basal stalk rot and/or head rot can be used to control the disease (Harveson et al. 2016). All of these recommendations are applicable to the control of both Sclerotinia basal stalk rot, and Sclerotinia midstalk rot and head rot.

In addition, there are recommended in-season practices targeted towards the control of Sclerotinia midstalk rot and head rot. One suggested practice for irrigated sunflower crops on soils with high water holding capacity is to avoid irrigation events during flowering (R-5.1 to R-5.9) to inhibit carpogenic germination of sclerotia (Scherer 2007; Schneiter and Miller 1981). Another suggested practice is to avoid over-fertilizing with nitrogen to prevent an overly dense crop canopy (Gulya and Masirevic 1992). Judicious use of foliar fungicide applications have also been recommended to control midstalk rot and head rot; however, inconsistent results tempered expectations for control using fungicides in sunflower crops (Harveson et al. 2016; Mueller et al. 2013).

Current management practices used in the Columbia Basin to mitigate Sclerotinia basal stalk rot, midstalk rot, and head rot in sunflower seed crops are based on the recommendations described above (Bill Wirth, Precision Seed Production, LLC, *personal communication*).

Sunflower seed crops in this region are typically grown on a 3 to 4-year rotation with at least one year of a non-susceptible, monocotyledonous crop (Bill Wirth, Precision Seed Production, LLC, *personal communication*). In addition, applications of *C. minitans* (Contans WG, Bayer CropScience, Research Triangle Park, NC) are made at planting and following cultivation in some crops in an effort to control Sclerotinia basal stalk infections. Also, applications of *C. minitans* have been made after harvest and in subsequent years, especially following a severely infected sunflower seed crop, to try and reduce survival of sclerotia. The types of tillage practices used are dictated mostly by the soil and irrigation type, with deep tillage practiced yearly in areas that are rill-irrigated, while tillage practices used in other areas of the Columbia Basin range from conventional tillage by ripping and disking to minimum tillage in areas where soil erosion is a concern (Bill Wirth, Precision Seed Production, LLC, *personal communication*).

Management practices targeted specifically to control Sclerotinia midstalk rot and head rot in sunflower seed crops in the Columbia Basin also follow many of the above recommendations. To inhibit carpogenic germination, sunflower seed growers use the least amount of irrigation possible based on the water holding capacity of the soil, without stressing the crop adversely (Bill Wirth, Precision Seed Production, LLC, *personal communication*). Growers have also adopted the use of reduced rates of nitrogen fertilizer application to limit crop canopy density. When consultants, field representatives, or growers determine a field to have a high risk of white mold, up to three foliar applications of fungicide are used to attempt to control ascospore infection, i.e., at pre-bloom, 10-50% bloom (R-5.1 to R-5.5), and 50% bloom to petal fall (R-5.5 to R-6) (Bill Wirth, Precision Seed Production, LLC, *personal communication*). Currently, sunflower seed growers in Washington State are limited to fungicides with active ingredients in one or more of FRAC Groups 3 (e.g., metconazole and tebuconazole), 7 (e.g.,

boscalid and fluopyram), and 11 (e.g., azoxystrobin and pyraclostrobin) that are registered for foliar application in sunflower crops in this state to control diseases caused by *S. sclerotiorum* [Washington State Pest Management Resource Service (WSPMRS) Pesticide Information Center Online (PICOL) 2017].

There is one exception to the general recommended practices listed above for white mold, the planting of resistant cultivars, that is difficult to use effectively in the Columbia Basin. This is not out of choice, but is dictated by the fact that, generally, the resistance of the parent lines to white mold used in seed crops may be unknown and, even when known, growers have little choice in the selection of the proprietary parent lines to plant because of the need for isolation of male parent lines, and the contractual, proprietary nature of the seed crops (Bill Wirth, Precision Seed Production LLC, *personal communication*).

1.3. Conclusion and research needs. *S. sclerotiorum* is a cosmopolitan pathogen with a wide-host range, and complex life cycle and epidemiology. Even though this plant pathogen has been the subject of many studies, the fungus continues to cause significant losses in crops like sunflower (Bolton et al. 2006). While the incidence and development of Sclerotinia basal stalk rot is reflective of soilborne sclerotial density, the incidence and development of Sclerotinia midstalk rot and head rot are strongly influenced by environmental conditions favorable to carpogenic germination and ascospore infection within the same field or adjacent fields, in addition to sclerotial density within the field (Gulya et al. 1989; Holley and Nelson 1986; McCartney and Lacey 1991; Nelson et al. 1989; Nelson and Lamey 2000).

The current practices used to manage Sclerotinia basal stalk rot, midstalk rot, and head rot in sunflower seed crops in the Columbia Basin fit with recommended practices overall. However, the literature in support of these practices is: i) inconclusive on the benefits of several

practices, e.g., applications of *C. minitans* for controlling these diseases in-season, ii) limited in research done in sunflower seed crops, or iii) the practices have not produced consistent results in sunflower crops, e.g., foliar fungicide applications for controlling aerial infections. Moreover, the Columbia Basin is a large and diverse cropping area with variable soils and production practices, including crop rotations, irrigation types, frequency of irrigation, and tillage practices (Bill Wirth, Precision Seed Production, LLC, *personal communication*), so the risk of these diseases is not homogeneous throughout the region even though the recommended practices are followed to some degree throughout the region. Therefore, there is a need for research to assess the efficacy of management practices currently used for *S. sclerotiorum* in sunflower seed crops in the Columbia Basin, and to optimize these management practices.

Even with improved control practices, diseases caused by *S. sclerotiorum* will continue to occur at some level in sunflower seed crops and other susceptible crops grown in the Columbia Basin, such as carrot, potato, and canola (Atallah and Johnson 2004; Bill Wirth, Precision Seed Production, LLC, *personal communication*). Therefore, furthering our understanding of the population biology of *S. sclerotiorum* in sunflower seed crops in this region is potentially important for the long-term management of white mold in the Columbia Basin. While populations of *S. sclerotiorum* from potato and alfalfa in the Columbia Basin have been studied, and showed high genetic diversity (Atallah et al. 2004; Attanayake et al. 2012), there has been no study to date of *S. sclerotiorum* populations in sunflower seed crops in the region. Sclerotinia diseases of sunflower have the potential to produce significant amounts of inoculum in the soil for subsequent susceptible crops. For instance, a severe Sclerotinia basal stalk rot infection can yield <100 sclerotia/plant, all of which will be returned to the soil (Gulya and Masirevic 1992). As basal stalk infection by *S. sclerotiorum* is an asexual process, such infections amplify the

genotype of the sclerotium that initiated the infection and, thus, increase the likelihood of persistence of that genotype in the field. Therefore, assessing the genetic diversity of populations of *S. sclerotiorum* in sunflower seed crops resulting from basal stalk infections is potentially valuable because fungal populations with greater genetic diversity may be able to adapt better to adverse environments and have an increased risk of fungicide resistance development (Bradley et al. 2016; Zhan et al. 2005). In addition, assessing the likelihood of root-to-root spread of the pathogen in sunflower seed crops in the Columbia Basin is important as root-to-root spread implies a potentially greater rate of multiplication of sclerotia in one growing season than if root-to-root spread does not occur.

Taking into consideration these research needs, the objectives of this thesis project are to:

1. Survey sunflower seed crops in the Columbia Basin of central Washington for incidence of Sclerotinia basal stalk rot, midstalk rot, and head rot in conjunction with current production practices and environmental conditions to assess the overall importance of the diseases in the area and the potential relative contributions of production practices and environmental conditions to disease incidence;
2. Assess the genetic diversity, population structure, and genetic differentiation of *S. sclerotiorum* populations from sunflower seed crops in the Columbia Basin arising from foci of basal stalk infections; and, assess the likelihood of root-to-root spread of *S. sclerotiorum* isolates from sunflower plants with sequential basal stalk rot infections;
3. Assess the effectiveness of current in-season management practices at reducing the incidence of Sclerotinia diseases in sunflower seed crops in the Columbia Basin, including:

a. Assess the degree to which *C. minitans* applications to the soil may contribute to control of Sclerotinia basal stalk rot in-season;
b. Correlate ascospore release with microclimatic field conditions during flowering and seed set in sunflower seed crops to assess disease risk and optimize foliar fungicide application timing;
c. Pursue registration of fungicides from FRAC Groups 1, 2, and 29 with the active ingredients thiophanate-methyl, iprodione, and fluazinam, respectively, in Washington State by testing the efficacy of these products at preventing Sclerotinia midstalk rot and head rot, and assessing potential phytotoxicity of foliar applications of these fungicides;
d. Assess the potential incidence of *S. sclerotiorum* on sunflower seed harvest from infected crops;

4. Determine the effect of *C. minitans* applications to the soil, and incorporation of sclerotia, on survival of sclerotia of *S. sclerotiorum* in the Columbia Basin of central Washington.

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CHAPTER 2

PREVALENCE OF WHITE MOLD AND GENETIC DIVERSITY OF *SCLEROTINIA SCLEROTIORUM* CAUSING BASAL STALK ROT IN SUNFLOWER SEED CROPS IN THE COLUMBIA BASIN OF CENTRAL WASHINGTON

2.1. Introduction

The cultivated sunflower, *Helianthus annuus* L., is grown for vegetable oil production (oilseed types), and for human food and bird-food markets (non-oilseed, confection types) (Berglund 2007). Sunflower production, in general, occurs in climates ranging from temperate to semi-arid (the latter with irrigation) (Blamey et al. 1997; Putnam et al. 1990). On average, the time from planting to physiological maturity for sunflower crops ranges from 75 to 150 days, depending on cultivar and environment, with production limited to warm, frost-free months as sunflowers are frost sensitive (Blamey et al. 1997; Harveson et al. 2016). In 2016, 40.36 million metric tons of sunflower seed were produced worldwide, which ranked third in global oilseed production behind soybean and rapeseed, and a total of 23.46 million ha were planted for sunflower production [United States Department of Agriculture (USDA) Foreign Agricultural Service (FAS) 2016].

In the U.S., production of hybrid sunflower seed has occurred predominantly in the Sacramento Valley of California, where ~95% of the hybrid seed planted by U.S. sunflower growers, and a significant amount of sunflower seed used by European and Asian sunflower growers, was produced in 2009 (Lilliboe 2009). In the Sacramento Valley, hybrid sunflower seed crops typically are furrow-irrigated, and the growing season extends from March through October (Lilliboe 2009). A principal reason hybrid sunflower seed production primarily occurs

in the Sacramento Valley has been the hot, dry summer climate, which facilitates high seed yields and low incidences of most foliar diseases of sunflower (Gulya et al. 1991, 2012). However, growers in the Sacramento Valley have faced challenges in hybrid sunflower seed production, including finding enough isolated fields to produce the number and diversity of hybrid sunflower seed crops in demand. This is the result of the many crops of other species, both seed and commercial, produced in the area (Lilliboe 2009), and the spatial separation needed for crops of numerous different sunflower male parent lines that must be isolated by at least 2 km if the blooming periods of nearby hybrid sunflower seed crops overlap to prevent undesired cross-pollination [Anfinrud 1997; California Crop Improvement Association (CCIA) 2015]. In part to address this issue, hybrid sunflower seed crops were introduced into the Columbia Basin of central Washington in 2008, when 20 ha were produced (Bill Wirth, Precision Seed Production, LLC, *personal communication*). Production has expanded rapidly, with >2,000 ha planted to hybrid sunflower seed crops in 2014. The expected gross return for a hybrid sunflower seed crop in the Columbia Basin ranges from $3,700 to $5,400/ha, which also makes these seed crops potentially valuable rotational crops.

Despite the short history of sunflower seed production in the Columbia Basin, some of these crops have been affected by white mold caused by the necrotrophic fungus *Sclerotinia sclerotiorum*, at incidences ranging from <1 to 75% (Bill Wirth, Precision Seed LLC, *personal communication*). *S. sclerotiorum* is a homothallic fungus that survives in the soil as sclerotia, and has a wide, phylogenetically diverse host range of >400, mostly dicotyledonous plant species, including sunflower (Boland and Hall 1994; Bolton et al. 2006). *S. sclerotiorum* caused an estimated $100 million in losses in sunflower crops in the U.S. in 1999 due to head rot, illustrating the potential destructiveness of this pathogen in sunflower crops (Bolton et al. 2006).

Infection of a sunflower plant by *S. sclerotiorum* can be initiated either by myceliogenic or carpogenic germination of sclerotia (Nelson and Lamey 2000). Myceliogenic germination results in basal stalk rot when mycelia originating directly from sclerotia in the soil infect the roots and/or crowns of sunflower plants (Huang and Dueck 1980). Carpogenic germination, favored by 7 to 14 days of high soil moisture, can lead to midstalk rot and head rot, as sclerotia germinate to form apothecia, from which ascospores are released (Nelson and Lamey 2000). If ascospores are deposited on susceptible host tissue, the spores germinate in the presence of a water film and an exogenous nutrient source, and infect the tissue. In hybrid sunflower seed crops in the Columbia Basin, both types of infection, which cause basal stalk rot and midstalk rot and head rot, can occur, providing impetus for assessing and optimizing management tactics for the two modes of infection that lead to white mold (Bill Wirth, Precision Seed Production LLC, *personal communication*).

S. sclerotiorum is not new to the Columbia Basin as the fungus causes white mold in many other susceptible crops grown in the region, including alfalfa, bean, carrot, pea, potato and various *Brassica* spp. (Bill Wirth, Precision Seed Production, LLC, *personal communication*; Pacific Northwest Plant Disease Management Handbook 2016). Therefore, tactics used to control white mold in hybrid sunflower seed crops in the Columbia Basin have been adopted from management practices in those crops. Crop rotation is used, with typically at least one year of a non-susceptible, monocotyledonous crop in the 3- to 4-year rotation between hybrid sunflower seed crops in order to reduce sclerotial populations in the soil. Irrigation frequency is reduced after canopy closure in hybrid sunflower seed crops in an attempt to minimize apothecium formation from sclerotia near the soil surface by reducing the length of periods of high surface soil moisture (Twengstrom, Kopmans, et al. 1998). In addition, the male sunflower

plants are removed just after the female sunflower plants finish blooming, to facilitate greater airflow in the crop canopy, which can reduce the length of periods of high soil moisture. Other management practices adopted by hybrid sunflower seed growers in areas of the Columbia Basin with a history of white mold include soil applications of the coelomycete *Coniothyrium minitans*, a mycoparasite of *S. sclerotiorum* (Campbell 1947; Whipps et al. 2008), as Contans WG (Bayer CropScience, Research Triangle Park, NC); and up to three protective foliar fungicide applications made during the period just prior to bloom and extending through petal fall, similar to recommendations for canola (Paulitz et al. 2015) and potato (Johnson and Atallah 2006).

Even with these management practices, infections by *S. sclerotiorum* continue to occur in hybrid sunflower seed crops and other susceptible crops grown in the Columbia Basin. Evidence that long range dispersal of ascospores is limited to several kilometers (Abawi and Grogan 1979), with most ascospores dispersed much closer to the apothecial source (Ben-Yephet and Bitton 1985; Hartill 1980; Wegulo et al. 2000), e.g., Ben-Yephet and Bitton (1985) reported that up to 90% of ascospores are deposited within 100 m of the apothecial source, and the homothallic nature of *S. sclerotiorum* suggests that populations of *S. sclerotiorum* may have limited genetic diversity and limited gene flow over long distances. However, sclerotia can act as a pathway for movement of *S. sclerotiorum* genotypes into new areas by movement with infested seed lots; and in irrigation water, manure, and/or soil moved between locations (Adams and Ayers 1979; Willets and Wong 1980). Research on the spatial structure of *S. sclerotiorum* populations has provided evidence for substantial population differentiation over large geographic areas (Attanayake et al. 2013; Kull et al. 2004; Malvárez et al. 2007; Sexton and Howlett 2004). For example, Sexton and Howlett (2004) detected substantial genetic differentiation among *S. sclerotiorum* populations from canola fields in Australia that were more

than 400 km apart; while Malvarez et al. (2007) found populations of the fungus from California, Washington, and Ontario, Canada were differentiated genetically. In addition, *S. sclerotiorum* isolates from different countries were shown to be differentiated genetically in several studies, e.g., Attanayake et al. (2013) demonstrated this for populations from North Dakota and China, and Kull et al. (2004) for populations from Illinois and Argentina. However, some studies of *S. sclerotiorum* populations have not found this type of genetic differentiation, even over wide geographic areas, e.g., Ekins et al. (2011) detected no genetic differentiation of *S. sclerotiorum* populations from sunflower crops distributed 350 km apart in Australia; Atallah et al. (2004) found similar results among populations of the fungus from four potato crops in the Columbia Basin; as did Hemmati et al. (2009) for populations from canola crops in Iran; and Dunn et al. (2017) from soybean, snap bean, dry bean, and lima bean crops in New York State. Minimal genetic differentiation among *S. sclerotiorum* populations could signal that gene flow is occurring among those populations (Milgroom 2015).

Direct evidence for outcrossing in the Columbia Basin was provided in a study by Atallah et al. (2004), who showed that ascospores from a single apothecium in a potato crop belonged to different mycelial compatibility groups. Further evidence of outcrossing of *S. sclerotiorum* populations from the U.S. and China was provided by Attanayake et al. (2014) using linkage disequilibrium decay analysis. Evidence for outcrossing in *S. sclerotiorum* populations has also been found in other studies (Attanayake et al. 2013; Ekins et al. 2011; Hemmati et al. 2009; Malvárez et al. 2007; Sexton and Howlett 2004).

The introduction of hybrid sunflower seed crops to the Columbia Basin presents new challenges in managing white mold in the region, particularly because of the copious numbers of sclerotia that can be produced in infected sunflower plants, e.g., ~100 sclerotia were recovered

from individual infected stalks following a severe white mold outbreak in a hybrid sunflower seed crop in 2015 (John Weber, *personal observation*). Therefore, research on the prevalence of white mold in hybrid sunflower seed crops in the Columbia Basin, and the association of production practices and environmental conditions with the incidence of white mold, could potentially lead to improved control of white mold in hybrid sunflower seed crops and other crops in this region. Additionally, long-term management of white mold may be enhanced by further understanding of the genetic diversity and spatial structure of *S. sclerotiorum* populations in the Columbia Basin. In hybrid sunflower seed crops, *S. sclerotiorum* isolates obtained from basal stalk infections, an asexual process of infection, represent a subset of the isolates present in the soil, or isolates potentially introduced into the field on infested stock seed lots (Herd and Phillips 1988). Therefore, the objectives of this study were to: i) assess the prevalence of white mold in hybrid sunflower seed crops in relation to production practices and environmental conditions; and ii) assess the genetic diversity and population structure of *S. sclerotiorum* populations in hybrid sunflower seed crops in the Columbia Basin arising from foci of basal stalk infections.

2.2. Materials and Methods

2016 White mold survey of sunflower seed crops in the Columbia Basin. In 2016, 40 hybrid sunflower seed crops were surveyed for white mold incidence in August and September. The fields were located in regions specified by the nearest towns to the crops, i.e., Coulee City (3 fields), George (7), Kittitas (6), Moses Lake (4), Odessa (4), Quincy (7), Ritzville (6), Royal City (1), and Warden (2) (Table 2.1), and, thus, were distributed over an area approximately 170 km

east to west and 100 km north to south. These crops represented 16 proprietary female parent lines, 10 of which were for oil type and 6 for confection type sunflower cultivars (Table 2.1).

To estimate white mold incidence in each sunflower seed crop, the field was divided into eight strata, and 100 plants from each of two adjacent female rows were rated along a transect in each stratum. Each plant was rated for the presence or absence of Sclerotinia basal stalk rot, midstalk rot, and head rot to calculate the incidence (%) of plants with each type (location on the plant) of infection. White mold ratings occurred at or near physiological maturity of the female line, after the male line had been swathed (all 40 fields had been planted to hybrid sunflower seed crops). The total incidence of white mold (Sclerotinia basal stalk rot, midstalk rot, and/or head rot) was estimated by adding the incidences of the three types of infection as infection of one plant at more than one site was <0.1% in all fields.

Data on the field sites, including production and cultural practices associated with each field, were collected: i) field location and size; ii) grower; iii) female parent; iv) sunflower type (confection or oilseed); v) planting dates; vi) plant spacing (between and within rows) and plant population; vii) cropping history for the previous three years; viii) soil type [USDA National Resources Conservation Service (NRCS) 2016]; ix) type, frequency, and amount of irrigation in July and August, as these two months correspond to the timing of canopy closure, flowering, and seed set in sunflower seed crops in the Columbia Basin; and x) for selected fields, Contans WG application(s) at planting and fungicide application(s) during bloom of hybrid sunflower seed crops. In addition, data on the monthly average for daily maximum, minimum, and mean temperature; monthly average relative humidity (RH), soil temperature (latter at a depth of 20 cm); and total precipitation in July and August were collected from the nearest AgWeatherNet station [Washington State University (WSU) AgWeatherNet 2016] within 15 km of each of 33 of

the 40 fields surveyed; soil temperature at a depth of 20 cm was used because this measurement was available from all the AgWeatherNet stations. Pearson's correlation coefficients were estimated between each of white mold incidence, basal stalk rot incidence, midstalk rot incidence, head rot incidence, and aerial white mold (midstalk rot + head rot) incidence and production practices that could be quantified as (relatively) continuous variables, including number of irrigation days, total irrigation (mm), plant population (plants/ha), and the regional weather data described above. Correlation coefficients were calculated in R using *rcorr*. Descriptive statistics, such as minimum, maximum, mean, and standard errors of each variable were calculated using Excel.

Genetic diversity and population structure of *S. sclerotiorum* in sunflower seed crops. ***Collection of* S. sclerotiorum *isolates.*** Sclerotia or sections of basal stalk tissue with symptoms of basal stalk rot were collected from sequentially infected sunflower plants (disease foci) in each of two ~53-ha, center pivot-irrigated, hybrid sunflower seed crops in central Washington on 28 August 2015. One field was located near Ephrata (N 47°07' 343" W 119°36' 217") and the other near Odessa (N 47° 31' 611" W 118° 46' 147"). Odessa is approximately 66 km east of Ephrata and the fields were located approximately 77 km apart. In total, 270 isolates of *S. sclerotiorum* were obtained from the two fields. Sclerotia or infected plant tissue were collected from 137 sunflower plants that comprised 39 disease foci in the field near Odessa, with a minimum of 2 and maximum of 5 sequentially infected plants in each focus. The disease foci sampled were located along two female rows in the northwest quadrant of the seed crop. Similarly, sclerotia or infected plant tissue were collected from 133 infected plants that comprised 35 disease foci in the field near Ephrata, with a minimum of 2 and maximum of 8

sequentially infected plants in each focus. The disease foci were sampled along two female rows in the southeast quadrant of the seed crop.

Isolates of *S. sclerotiorum* were obtained directly by plating sclerotia collected off the taproot or symptomatic basal stalk, or by plating a segment of infected basal stalk tissue of each of the sunflower plants sampled in each disease focus. Each sclerotium was dried for 48 h in a laminar flow hood, and then stored at 20 ± 2°C. Infected basal stalk tissue was dried at 20 ± 2°C for up to two weeks, and any sclerotia that developed on the stem pieces were then sampled and treated as described above, or a part of the infected stem tissue was stored at 20 ± 2°C. Each dried sclerotium was surface-sterilized for 1 min each in 70% ethanol and 0.06% sodium hypochlorite, rinsed three times with sterilized distilled water, bisected aseptically with a scalpel, and plated onto water agar (WA) in a petri plate. Each piece of infected stem tissue was cut into 1 cm^2 squares, and the sections surface-sterilized in 0.06% sodium hypochlorite for 1 min, rinsed three times in sterilized distilled water, dried, and plated onto WA. *S. sclerotiorum* isolates that developed from the sclerotia or stem pieces were then purified by hyphal tip transfers onto half-strength potato dextrose agar (1/2 PDA).

Microsatellite genotyping. Microsatellite markers are considered selectively neutral and are highly polymorphic due to higher mutation rates compared to other neutral loci, making microsatellite markers ideally suited for population genetic studies (Milgroom 2015). Eight microsatellite loci developed by Sirjusingh and Kohn (2001) were used to investigate genetic variation of the isolates of *S. sclerotiorum* from a subset of the disease foci for each of the Ephrata and Odessa hybrid sunflower seed crops sampled. A sample of 96 isolates encompassing 30 disease foci were used from the field located near Odessa. A subset of 94 isolates encompassing 26 foci were used from the field located near Ephrata.

The fluorescent labeling method of Schuelke (2000) was used in a multiplex polymerase chain reaction (PCR) assay with the modifications described by Attanayake et al. (2012). For each isolate, total genomic DNA was extracted from mycelium using a Qiagen DNeasy Plant Mini Kit (Qiagen, Germantown, MD) following the manufacturer's instructions with minor modifications: 50 to 100 mg of mycelium, 420 µl AP1 buffer, and 350 mg of 0.7 mm zirconia beads (BioSpec, Bartlesville, OK) were placed into a 1.7 ml microcentrifuge tube, and the mycelial cells lysed by bead beating (Minibeadbeater; Biospec Products, Inc., Bartlesville, OK) for 5 min. The lysed product was centrifuged for 15 min at 14,000 rpm, the resulting supernatant pipetted to a 1.7 ml microcentrifuge tube, and DNA extracted using the manufacturer's protocol. The quality of the DNA was verified on a 1.5% agarose gel, and DNA concentration quantified using a Qubit fluorometer (Thermo Fisher Scientific, Bothell, WA). Each PCR reaction included: 500 pg template genomic DNA, 1.5 units of Taq DNA polymerase (Bioline, Taunton, MA), 0.2 mM dNTPs, and PCR buffer containing 1.5 mM $MgCl_2$, 50 nM forward primer, 200 nM fluorescent dye (Vic, Fam, Pet, or Ned), and 250 nM reverse primer in a total reaction volume of 12 µl. PCR parameters were similar to those of Sirjusingh and Kohn (2001) with minor modifications — each locus was amplified using the following PCR reaction parameters: i) 94°C denaturation step for 4 min; ii) 35 cycles of 94°C for 20 s, 58°C for 25 s, and 67°C for 23 s; and iii) a final extension step of 72°C for 5 min. PCR products labeled with each fluorophore were multiplexed and diluted with molecular grade water: 0.12-, 0.16-, and 0.24-fold (volume labeled of PCR product/volume of multiplex solution) for the Vic or Fam, Ned, and 6-Pet labels, respectively. A 3 µl aliquot was then mixed with 5.0 µl Hi-Di formamide and 5.0 µl of Cassul-445 size standard in a 96-well plate, and denatured for 5 min at 95°C, before assembly in an ABI plate sandwich and running the sample on an ABI 3730xl DNA Analyzer (Applied Biosystems,

Foster City, CA). Fragment analysis was completed using GeneMarker (SoftGenetics, State College, PA). The microsatellite genotyping was carried out in the USDA — Agricultural Research Service (ARS) Western Regional Small Grain Genotyping Laboratory of Dr. Deven See in Pullman, WA.

Assessment of homoplasy among microsatellite loci. Representative DNA fragments of each base-pair size were amplified for each locus, and sequenced to check for homoplasy. All putative alleles present in ≥10% of the observed loci were sequenced from at least three isolates, with at least one isolate from each of the Ephrata and Odessa populations. If a particular allele was present in <10% of the observed loci, then the allele was sequenced from a single isolate if the allele was present in only one population, and from two isolates if the allele was present in both populations. Sequencing was performed in 12 µl reactions: 500 pg template genomic DNA, 1.5 units of Taq DNA polymerase (Bioline, Taunton, MA), 0.2 mM dNTPs, and PCR buffer containing 1.5 mM MgCl2, 250 nM forward primer, and 250 nM reverse primer. PCR parameters were as described above. The quantity and quality of PCR product was determined using a Qubit fluorometer and by gel electrophoresis. One 5-µl aliquot of each PCR product was treated with ExoSAP-IT (USB Corp., Cleveland, OH), and prepared for sequencing by mixing 2 to 8 ng of treated DNA (depending on fragment size) with 8 pmol of the appropriate forward or reverse primer in water, for a total volume of 15 µl. The premixed samples were sequenced in both directions by Elim Biopharmaceuticals, Inc. (Hayward, CA).

Statistical analysis of microsatellite data. Arlequin Version 3.5 (Excoffier et al. 2005) was used to assemble multilocus haplotypes and calculate frequencies of the haplotypes, to estimate the number of polymorphic loci, mean number of alleles/locus (allelic richness), expected heterozygosity (Nei 1978), and private alleles (those restricted to either the Ephrata or

Odessa population). Genotypic diversity was estimated by Stoddart and Taylor's *G* (Stoddart and Taylor 1988), and genotypic evenness by the index E_5 (Grünwald et al. 2003) in POPPR (Kamvar et al. 2014, 2015). A Chi-square test was used to assess spatial aggregation of haplotypes within and among disease foci in each population. Analysis of molecular variance (AMOVA) was used to test the null hypothesis that the Ephrata and Odessa populations were not differentiated genetically after clone-correction, with 1,023 permutations implemented using Arlequin by the nonparametric approach. AMOVA was completed using variances computed with a matrix of genetic distances between all pairs of haplotypes, estimated assuming the stepwise mutation model (SMM) (Michalakis and Excoffier 1996; Slatkin 1995).

To illustrate visually the evolutionary relationship among haplotypes of *S. sclerotiorum* in the sunflower seed crops sampled, a minimum spanning network (MSN) of the Ephrata and Odessa populations was produced in POPPR using a matrix of Bruvo's distance (Bruvo et al. 2004) between all pairs of haplotypes. Bayesian clustering analysis in STRUCTURE Version 2.3.4 (Pritchard et al. 2000) combined with the Evanno method (Evanno et al. 2005) implemented in Structure Harvester Version 0.6.94 (Earl and vonHoldt 2012), were used to infer the most probable number of genetic populations among the clone-corrected Ephrata and Odessa populations. For the Bayesian clustering analysis completed using STRUCTURE, the admixture model, which allows individuals to have ancestry from multiple populations, was assumed to assess the probable number of genetic clusters without considering geographic origin. Five independent runs were used for each k value (the assumed number of genetic clusters) of 1 to 15, with 100,000 Markov Chain Monte-Carlo iterations and a burn-in of 50,000 iterations. Multilocus and pairwise locus estimates of the index of association (I_A) and modified index of association (r_d) were made using Multilocus Version 1.3b (Agapow and Burt 2001) for each

population, and also for the clone-corrected Ephrata and Odessa populations, both with and without taking into account inferred subpopulations from Bayesian analysis in STRUCTURE, to test the random mating model. The statistical significance of the null hypothesis of random mating within a population was tested by comparing the observed dataset to 1,000 randomizations of the dataset created by imposing unlimited recombination, i.e., randomly shuffling the alleles among individuals, independently for each locus.

MAT genotyping. For each isolate of *S. sclerotiorum* that was genotyped using microsatellite markers, two PCR assays were performed separately for both the Inv– and Inv+ mating type (*MAT*) alleles using allelic specific primers (Chitrampalam et al. 2013). The novel *MAT* allele, Inv+, identified by Chitrampalam et al. (2013), differed from the previously identified *MAT* allele, Inv-, by a 3.6-kb inversion. PCR assay parameters used were as described by Chitrampalam et al. (2013) except that the reaction mixture included 15 µl (0.25 ng/µl) of genomic DNA and 2 µl of PCR grade water. A Chi-square test was used to determine if the proportions of Inv- and Inv+ *MAT* alleles in each population differed from a 1:1 ratio.

2.3. Results

2016 White mold survey of sunflower seed crops in the Columbia Basin. White mold was observed in 37 of the 40 hybrid sunflower seed fields surveyed, with total white mold incidence (basal stalk rot + midstalk rot + head rot) ranging from 0 to 32.4%/field, a median incidence of 1.5% white mold, and a mean of 3.8 ± 0.9% white mold/field (Fig. 2.1A). White mold incidence was >1% in 24 of the 40 fields (60%), including at least one field of each of 12 of the 16 female parent lines, although only 3 of the fields had >10% incidence of white mold, 2 fields with female F and one field with female J. The greatest contribution to total incidence of

white mold was basal stalk rot, which was observed in 36 of the 40 fields (90%), at incidences ranging from 0 to 8.9%/field, with a median incidence of 0.8%, and a mean incidence of 1.8 ± 0.4%/field (Fig. 2.1B). Head rot was the second greatest contributor to total white mold incidence, and was observed in 27 of the 40 fields (67.5%), at incidences ranging from 0 to 28.8%/field, a median incidence of 0.2%, and a mean incidence of 1.6 ± 0.7%/field (Fig. 2.1C). However, the incidence of plants with head rot was <1% in 31 of the 40 fields, with the other 9 fields planted to female F (of a total 10 fields planted with that female parent). In contrast, basal stalk rot incidence was >1% in 18 fields (45.0%), including at least one field of each of 12 of the 16 female parent lines. Midstalk rot was observed in 18 of the 40 fields (45.0%) at incidences ranging from 0 to 7.2%/field, a median incidence of 0%, and a mean incidence of 0.4 ± 0.2%/field (Fig. 2.1D). Midstalk rot was observed on <1% of the plants in all 40 fields except 2 fields planted to female J, which also had some of the greatest incidences of basal stalk rot. Although basal stalk rot was the most prevalent type of white mold symptom observed in these sunflower seed crops in the Columbia Basin in 2016, the greatest incidence of white mold was head rot in one field (field 19) planted to female F (28.8%) (Table 2.2). The potential for head rot to occur at very high incidences was also illustrated by the maximum incidence of head rot in any one stratum rated, 50% in one stratum in field 19, compared to relatively lower maximum incidences of basal stalk rot and midstalk rot in any one stratum, 18 and 19% in fields 17 and 27, respectively (Table 2.2).

White mold incidence varied by location as the mean incidence was 2.9 ± 0.3% for fields near Coulee City (3 hybrid sunflower seed crops), 3.4 ± 1.4% for fields near George (7), 0.2 ± 0.1% for fields near Kittitas (6), 13.1 ± 6.7% for fields near Moses Lake (4), 1.3 ± 0.4% for fields near Odessa (4), 4.9 ± 2.0% for fields near Quincy (7), 3.2 ± 1.4% for fields near Ritzville

(6), 4.1% for the field near Royal City (1), and 0.7 ± 0.4% for fields near Warden (2). Information on the use of Contans WG and foliar fungicide applications was not provided for all crops surveyed, but an application of 0.56 kg Contans WG/ha at or soon after planting in May, and at least one foliar fungicide application during bloom were made for each field near George (7) and Moses Lake (4) (Table 2.1). Even with both a Contans WG application soon after planting in May and a foliar fungicide application made at ~50% bloom (R5.5; Schneiter and Miller 1981), white mold incidence was greatest in field 19 near Moses Lake with a white mold incidence of 32.4% and aerial white mold incidence of 28.9%.

Prior to these sunflower seed crops being planted in 2016, 29 of the 40 fields (72.5%) had been planted with at least one white mold-susceptible crop (alfalfa, bean, canola, carrot seed, coriander, garbanzo bean, pea, or potato) from 2013 to 2015. In those fields, mean white mold incidence was 4.6%/field, and ranged from 0.1 to 32.4%/field; compared to a mean white mold incidence of 1.6%/field and a range of 0 to 7.8%/field for the 11 hybrid sunflower seed crops in which no white mold-susceptible crop was grown from 2013 to 2015 (Tables 2.1 and 2.2). Slight differences in white mold incidence were found based on irrigation type. White mold incidence and aerial white mold (midstalk rot + head rot) incidence averaged 3.8 ± 1.1% and 2.2 ± 1.0% for the 30 pivot-irrigated fields vs. 3.5 ± 1.5% and 1.4 ± 0.8% for the 10 rill-irrigated fields, respectively (Table 2.1). Mean white mold incidence for the 33 fields surveyed that were planted to oil type sunflower seed crops (4.3 ± 1.1%) was greater than the mean white mold incidence in the 7 fields planted to confection type sunflower seed crops (1.3 ± 0.4%). Soil textures of the 40 fields were categorized as sandy (15.0% of the fields), sandy loam (5.0%), loamy sand (12.5%), loamy (12.5%), and silty loam (55.0%) with the latter the most common

(Table 2.1). Mean white mold incidence for each of those soil textures was 7.6 ± 5.2, 5.6 ± 2.2, 3.2 ± 1.0, 1.1 ± 0.7, and 3.3 ± 0.9%, respectively (Table 2.1).

Planting dates for female parent lines in the fields ranged from 28 April 2016 to 30 May 2016. Because planting date and female parent line significantly affect crop development, including when the crop blooms, the relationship between planting date and white mold incidence was investigated only for female parent line F, for which there were enough fields (10) to justify the analysis. The correlations between planting date of crops with female F and mean white mold incidence were not significant (r = -0.545 to 0.200 at P = 0.103 to 0.579). Inter-row spacing in the fields surveyed was either 56 or 76 cm, with in-row spacing of plants ranging from 19 to 30 cm for the former, and 26 to 41 cm for the latter. Sunflower plant populations in the 40 fields ranged from 43,100 to 69,100 plants/ha, with a median of 54,400 plants/ha. The correlations was found between plant population and mean total white mold incidence, mean basal stalk rot incidence, mean midstalk rot incidence, mean head rot incidence, or mean aerial white mold incidence were not significant (r = -0.138 to -0.039 at P = 0.394 to 0.813). Pearson's correlation coefficients and the associated significance levels were similar for maximum incidences of white mold as mean incidences.

The number of days of irrigation (a measure of irrigation frequency) in July ranged from 1 to 21, with a median of 12 days; while the number of days of irrigation in August ranged from 0 to 12, with a median of 6.5 days. The total number of days of irrigation in July + August ranged from 1 to 32, with a median of 19 days for the 40 crops surveyed. There were significant positive correlations between the number of days of irrigation in July and each of mean head rot incidence (r = 0.414 at P = 0.008), mean aerial white mold incidence (r = 0.406 at P = 0.009; Fig. 2.2A) and mean total white mold incidence (r = 0.433 at P = 0.005), but no significant

correlation was found between the number of days of irrigation in July and either mean basal stalk rot incidence or mean midstalk rot incidence. Similar correlation results were found between total number of days of irrigation in July + August with mean head rot incidence, mean aerial white mold incidence, and mean total white mold incidence; however, the correlations between number of days of irrigation in August were not significant for incidence of white mold.

The amount of irrigation water applied in July ranged from 91 to 323 mm, with a mean of 240 ± 10 mm; while the amount of irrigation water used in August ranged from 0 to 191 mm, with a mean of 103 ± 7 mm; and the amount of irrigation water used over July + August ranged from 101 to 508 mm, with a mean of 344 ± 16 mm. The only significant positive correlation between amount of irrigation water and any measure of white mold incidence was a positive correlation between the amount of water irrigated in July and mean total white mold incidence ($r = 0.317$ at $P = 0.046$); although the incidence of mean aerial white mold and amount of irrigation in July was almost significant ($r = 0.260$ at $P = 0.077$; Fig 2.2B). However, since Pearson's correlation coefficient is strongly influenced by outliers, data for field 19, which had 28.9% aerial white mold incidence, was removed from the correlation calculation, resulting in no significant correlations of any measure of white mold incidence with the number of days of irrigation or total amount of irrigation (Fig. 2.2A and 2.2B). Pearson's correlation coefficients and the associated significance levels were similar for maximum incidences of white mold as for mean incidences (*data not shown*).

There were few significant correlations between any measure of white mold incidence and environmental conditions in July and August for the 33 fields located within 15 km of a WSU AgWeatherNet station. Total precipitation in July + August and mean basal stalk rot incidence were significantly negatively correlated ($r = -0.352$ at $P = 0.045$); average minimum

daily temperature in July, August, and July + August were significantly positively correlated with mean midstalk rot incidence (r = 0.418 at *P* = 0.016, r = 0.376 at *P* = 0.031, and r = 0.407 at *P* = 0.019, respectively); and total precipitation in July + August was negatively correlated with mean head rot incidence (r = -0.409 at *P* = 0.018). When field 19 was removed from the analyses because the white mold ratings for that field were outliers, head rot incidence was almost significantly correlated with precipitation in July + August (*P* = 0.077). Also, the incidence of plants with basal stalk rot was almost significantly correlated with total precipitation in July + August when field 19 was excluded (*P* = 0.060). The significance of the correlations between mean midstalk rot incidence and average minimum daily temperature in July, August, and July + August remained significant when field 19 was excluded as an outlier (r = 0.419 at *P* = 0.017, r = 0.376 at *P* = 0.034, and r = 0.408 at *P* = 0.020, respectively). Correlation results were similar when maximum white mold incidence in each field was used to calculate Pearson's correlation coefficient (*data not shown*).

Genetic diversity and population structure of *S. sclerotiorum* in sunflower seed crops. ***Assessment of homoplasy among microsatellite loci.*** Homoplasy, the presence of which leads to underestimating genetic diversity and genetic differences among fungal isolates, was not detected among any representative DNA fragments in each size class amplified by PCR assay for each of the eight microsatellite loci of *S. sclerotiorum* isolates obtained from foci off basal stalk rot infections in two hybrid sunflower seed crops sampled in the Columbia Basin in 2016. Differences in fragment sizes for all but one locus, were due to expansions and contractions in the microsatellite repeat motifs. For locus 110-4, one of the differences in size was due to a 7-bp insertion in the flanking region rather than expansion or contraction of the repeat motif.

Therefore, the stepwise mutation model was assumed as the evolutionary model of mutations at microsatellite loci used in this study (Slatkin 1995; Bruvo et al. 2004).

Genetic diversity and population structure. The Ephrata and Odessa populations of *S. sclerotiorum* isolates originating from basal stalk infections were diverse both genetically and genotypically (Table 2.3). In each population, all eight microsatellite markers were polymorphic, with 27 alleles across all eight loci (Tables 2.3 and 2.4). Of the 27 alleles, 24 were shared between the two populations, with the Ephrata population having 1 private allele and the Odessa population 2 private alleles (Table 2.3). Nei's expected heterozygosity (H_e) increased when the populations were clone-corrected, but neither population differed in the mean number of alleles/locus nor H_e (Table 2.3). Comparison of the number of alleles/locus and H_e after clone-correction between the populations indicated similar levels of genetic diversity in the Ephrata and Odessa populations. However, the Ephrata population had greater levels of genotypic diversity than the Odessa population, indicated by a Stoddart and Taylor's *G* value of 12.14 vs. 8.33, respectively; and the haplotypes were more evenly distributed in the Ephrata population, indicated by an E_5 value of 0.66 compared to 0.58 for the Odessa population (Table 2.3). In total, there were 28 unique multilocus haplotypes in the Ephrata population compared to 22 in the Odessa population, with 9 multilocus haplotypes shared between the two populations (Table 2.3 and Fig. 2.3).

Multilocus linkage disequilibrium estimates, I_A and r_d, for each population before and after clone-correction, indicated that neither population was under linkage equilibrium (Table 2.3), i.e., there was significant non-random association of alleles among loci. Within each clone-corrected population, significant linkage disequilibrium was found between eight pairs of loci for the Ephrata population, and nine pairs of loci for the Odessa population, with five pairs of loci

[(7-2,17-3), (7-2, 114-4), (17-3, 55-4), (17-3, 114-4), and (55-4, 110-4)] under significant linkage disequilibrium in both populations (Table 2.4). However, when subpopulations defined by Bayesian clustering analysis in STRUCTURE were taken into account, multilocus linkage equilibrium could not be rejected in either population (Table 2.3), and significant pairwise linkage disequilibrium between loci was found only for two pairs of loci in the Ephrata population (7-2, 13-2 at $P = 0.026$; and 55-4, 110-4 at $P = 0.006$), and one pair in the Odessa population (13-2, 114-4 at $P = 0.010$).

The optimal number of subpopulations in the clone-corrected Ephrata and Odessa populations was 2, as $\Delta k = 97.9$ was maximized for $k = 2$ subpopulations (the criterion of Evanno et al. (2005) for choosing the optimal number of subpopulations). The membership probabilities for each *S. sclerotiorum* isolate in each of the clone-corrected Ephrata and Odessa populations are depicted in Fig 2.4. Visual inspection of the minimum spanning network (Fig. 2.3) showed that the isolates from the clone corrected populations did not cluster based on location of the population, Ephrata or Odessa. Similarly, the AMOVA results showed that the Ephrata and Odessa populations were not genetically distinct ($P = 0.32$; Table 2.5), further supporting the conclusion that the isolates were not clustered genetically based on population origin.

The Chi-square test revealed significant spatial aggregation of isolates of *S. sclerotiorum* with the same multilocus haplotype within disease foci (Table 2.6). When the observed frequencies of isolate-pairs from adjacent plants in the same focus with the same microsatellite haplotype were tested against expected frequencies of the same haplotype being recovered from adjacent plants based on a random distribution, the Chi-square test was extremely significant for both populations ($P < 10^{-37}$ and $< 10^{-48}$ for the Ephrata and Odessa populations, respectively)

(Table 2.6). However, when the observed frequencies of isolate-pairs with the same multilocus haplotypes from plants closest to each other between foci were tested against expected frequencies of the same haplotype being recovered from plants closest to each other between foci based on a random distribution, the Chi-square test was not significant for either population, suggesting a random distribution of isolates among foci (Table 2.6).

MAT *genotyping.* Of the 94 *S. sclerotiorum* isolates evaluated from the Ephrata population, there were 42 Inv- (44.7%) and 51 Inv+ (54.3%) with 1 *MAT* heterokaryon; of the 96 *S. sclerotiorum* isolates from the Odessa population, 49 were Inv- (51.0%) and 44 were Inv+ (45.8%) with 3 *MAT* heterokaryons. In both populations, the ratio of Inv- to Inv+ genotypes did not differ significantly from 1:1 ($X^2 = 0.87$, df = 1, $P = 0.351$ and $X^2 = 0.27$, df = 1, $P = 0.604$ for the Ephrata and Odessa populations, respectively).

2.4. Discussion

Although white mold was observed in a majority (92.5%) of 40 hybrid sunflower seed crops surveyed in 2016 in the Columbia Basin of central Washington, the incidence of infection was relatively low, averaging 3.8 ± 0.9%/field, and ranging 0 to 32.4%/field. Basal stalk rot was the most prevalent symptom of infection caused by *S. sclerotiorum* (90.0% of 40 fields), although midstalk rot and head rot were also observed in a significant percentage of the crops (45.0 and 67.5%, respectively). The survey results indicated that production practices such as reduced irrigation frequency and rotation with crops that are not susceptible to white mold for at least three years prior to hybrid sunflower seed crops could reduce white mold risk, although the potential confounding effects of multiple factors influencing white mold in these crops limited the ability to assign relative risk to any particular factor. The *S. sclerotiorum* isolates collected

from basal stalk rot foci in each of two hybrid sunflower seed crops in 2015 were clonal within foci, but the population from each field was genetically and genotypically diverse. The survey was limited to 40 fields in on year, and the population assessment to two hybrid sunflower seed crops. Nevertheless, the 40 fields surveyed represented 100% of the acreage of sunflower seed crops planted in the Columbia Basin in 2016, and the population assessment showed a lack of genetic differentiation between the two *S. sclerotiorum* populations based on field location.

In the Columbia Basin, akin to reports from the Sacramento Valley of California and North Dakota, basal stalk rot was the most prevalent type of white mold infection observed in the 40 hybrid sunflower seed crops surveyed (Gulya et al. 1989, 1991, 2012; Nelson and Lamey 2000). In a 1989 survey of diseases in hybrid sunflower seed crops in the Sacramento Valley (Gulya et al. 1991), basal stalk rot and head rot were detected in 23.0 and 3.4% of 87 crops surveyed, respectively. While basal stalk rot was more prevalent than head rot in the Columbia Basin in 2016, the proportion of crops in which basal stalk rot (90.0%) and head rot (67.5%) were detected was much greater. In North Dakota, white mold was detected in 53% of 49 hybrid sunflower seed crops surveyed from 1995 to 2011 by the North Dakota Department of Agriculture and in 54% of 1,214 commercial sunflower crops surveyed from 2001 to 2011 by the National Sunflower Association (Gulya et al. 2012), which was less than the 92.5% of the 40 hyrbid sunflower seed crops surveyed in the Columbia Basin in 2016. In contrast, the proportion of hybrid sunflower seed crops in the Sacramento Valley in which white mold was detected by phytosanitary certification inspections was much less than the proportion of crops for which white mold was detected in North Dakota and in the Columbia Basin, i.e., only 2.6% of 7,231 crops inspected in the Sacramento Valley from 1997 to 2011 had white mold (Gulya et al. 2012).

Although both the Columbia Basin and Sacramento Valley are hot and dry during the summer, the Sacramento Valley is significantly warmer than the Columbia Basin. The average daily high temperature and total precipitation for July + August from 1981 to 2010 was 30.2°C and 11 mm, respectively, in Quincy, WA compared to 34.5°C and 3 mm for Woodland, CA (United States Climate Data 2017). Therefore, while precipitation in both regions is limited and, thus, irrigation is the primary means of supplying hybrid sunflower seed crops with water in both regions, the much greater temperatures of the Sacramento Valley during bloom of hybrid sunflower crops likely aid in the suppression of lengthy periods of high soil moisture, leaf wetness, and high RH, making this region even less favorable than the Columbia Basin to development of white mold initiated by apothecium formation and ascospore release (Weiss et al. 1980; Twengstrom et al. 1998; Wu and Subbarao 2008). Similarly, in a survey in the Midwestern U.S., Workneh and Yang (2000) found a significantly greater percentage of soybean crops were infected with Sclerotinia stem rot at cooler, northern latitudes compared to warmer, southern latitudes. Therefore, it should be expected that more severe outbreaks of white mold and a greater prevalence of the disease can be expected in the Columbia Basin vs. the Sacramento Valley.

In this study, only a few significant correlations were found between regional weather data measured across the Columbia Basin and any measure of the incidence of white mold (total, aerial, head, midstalk, or basal stalk rot) in hybrid sunflower seed crops. Even when only the 29 of 40 fields with a history of at least one white mold-susceptible crop planted in the previous three years were included in the correlation analysis, in an attempt to control for inoculum potential of *S. sclerotiorum* in each field surveyed, correlation results were similar to when all 40 fields were included. However, cropping history is an imperfect indicator of *S. sclerotiorum*

inoculum potential (as susceptible crops may not necessarily be affected by white mold), and the strength and/or significance of correlations between regional weather data and white mold incidence might have been strengthened if the history of white mold in the 40 fields was known so that those fields without a record of white mold could be removed from the correlation calculations. Significant negative correlations were detected between head rot incidence, aerial white mold incidence, and total white mold incidence with total precipitation in July and July + August, and between midstalk rot incidence and average minimum daily temperature in July, August, and July + August which retained significance when the outlier, field 19, with % aerial white mold incidence was removed from the calculation. The results suggest that development of midstalk rot is favored by moderate temperatures, and precipitation after canopy closure in the Columbia Basin in 2016 was not a major factor in promoting development of white mold. Abawi and Grogan (1975) showed that *S. sclerotiorum* ascospore germ tube length and lesion expansion rate were less at temperatures $\leq 15^{o}C$ compared to optimal temperatures of 20 to $25^{o}C$. That precipitation did not have a significant positive correlation with aerial white mold incidence in the Columbia Basin survey suggests, as expected for a semi-arid area, that irrigation of these crops is much more important for promoting development of white mold in the Columbia Basin than precipitation.

Effects of regional weather conditions on white mold incidence in hybrid sunflower seed crops in the Columbia Basin could be more feasibly assessed, if weather data and white mold ratings were collected over a period of several years. Workneh and Yang (2000) found that cumulative departures below normal minimum and maximum temperatures in July and August were strongly correlated with the percentage of soybean crops in the Midwestern U.S. in which Sclerotinia stem rot was detected. Based on conversations with growers and observations in

hybrid sunflower seed crops, including those surveyed in the Columbia Basin in this study and field trials conducted in growers' crops in 2015 and 2016 (see Chapter 3), the percentage of hybrid sunflower seed crops with aerial white mold was greater in 2016 than in 2015, although an extensive survey of most hybrid sunflower seed crops was not carried out in 2015. This probably reflected differences in regional weather conditions in 2016 vs. 2015, as the average daily maximum temperature was 32.9 vs. 29.7°C, and total precipitation was 0.1 vs. 7.5 mm in July of 2015 vs. 2016, respectively (compared to the 8-year average from 2009 to 2016 of 31.4°C and 5.0 mm, respectively, measured at the Ephrata and Moses Lake WSU AgWeatherNet stations). However, local factors influencing development of white mold in each field can override generally unfavorable weather conditions, as demonstrated in a rill-irrigated sunflower seed crop near Quincy in 2015 that developed >50% incidence of white mold (basal stalk rot, midstalk rot, and head rot incidences all >10%; *personal observation*). Interestingly, the grower of that crop had no recollection of white mold having occurred in previous white mold susceptible crops in that field for the prior 4 years, suggesting that *S. sclerotiorum* could have been introduced on the stock seed (Herd and Phillips 1988) and/or ascospores might have been blown into the hybrid sunflower seed crop from adjacent infested fields (Hammond et al. 2008; Wegulo et al. 2000).

This investigation of the relationship between production practices and white mold incidence in hybrid sunflower seed crops was limited by the lack of complete information on white mold history and production practices in each field. For instance, information was not provided on whether or not white mold had been observed in a previous crop in any of the fields surveyed, even though this is a very important indicator of white mold risk (Nelson and Lamey 2000). In addition, information on foliar fungicide applications by the growers was only

provided for the 11 crops located near George and Moses Lake, with at least one foliar fungicide application made during bloom in all those fields, although information on the timing of the application(s) relative to percent bloom was not always recorded. Even for those fields for which timing of fungicide application(s) was known in relation to crop maturity, the timing of the application(s) were not always optimal. For example, only one fungicide application of boscalid (Endura; BASF Corp., Research Triangle Park, NC) was applied at 50% bloom, R5.5 (Schneiter and Miller 1981), in field 19, in which the mean head rot incidence was 28.8%. This might illustrate why inconsistent results have been reported on the efficacy of foliar applications of fungicides to control aerial white mold in sunflower crops (Mueller et al. 2013), and also the need to optimize the timing of fungicide applications relative to the initiation and duration of ascospore release (see Chapter 3). As a result, the survey data could not be used to assess the efficacy of foliar fungicide application(s) made by growers in these fields for white mold control. Information on Contans WG applications was provided for the 11 crops near George and Moses Lake, each of which was treated with Contans WG at planting in May. Therefore, the data were not suitable for assessing whether the Contans WG applications were effective at minimizing basal stalk infections from soilborne sclerotia, or aerial infections resulting from carpogenic germination of sclerotia. Even if data were provided on the use of Contans WG for all 40 crops, the data likely would not have been suitable for assessing if Contans WG applications provided control of white mold because the use of Contans WG is targeted to regions of the Columbia Basin with a known history of white mold (Bill Wirth, Precision Seed Production, LLC, *personal communication*). Fields with similar inoculum potential that were and were not treated with Contans WG would have been difficult to identify even if a suitable measure of inoculum potential, such as sclerotial density, could have been assessed for all crops surveyed. It is of note

that both field 19, which had the most head rot, and field 17, which had the most basal stalk rot (8.9%) of all 40 fields surveyed, were treated with Contans WG at or soon after planting in May.

Crop rotation is an important practice for controlling white mold in sunflower crops (Harveson et al. 2016). In North Dakota, rotations of three years with non-susceptible, monocotyledonous crops are generally recommended for minimizing the risk of white mold in sunflower crops (Nelson and Lamey 2000). Only 11 of the hybrid sunflower seed crops surveyed in the Columbia Basin in 2016 met this criterion with a mean white mold incidence of 1.6% in those 11 crops vs. 4.6% for the 29 crops that did not meet the criterion. While the same recommendation is made for canola crops in the Columbia Basin (Paulitz et al. 2015), canola was grown in one of the three years preceding 2016 for 6 of the 40 hybrid sunflower seed crops surveyed. This may reflect the fact that, in general, it is not always feasible economically to rotate hybrid sunflower seed crops for as many as three years with white mold non-susceptible crops such as wheat or corn (Bill Wirth, Precision Seed Production LLC, *personal communication*).

While strongly influenced by the outlier field that had 28.9% incidence of aerial white mold caused by *S. sclerotiorum*, the significant positive correlation between number of days of irrigation in July and incidence of aerial white mold indicates that reducing irrigation frequency may be efficacious for reducing aerial white mold incidence in hybrid sunflower seed crops in the Columbia Basin. Twengstrom et al. (1998) showed that less frequent irrigation reduced carpogenic germination of *S. sclerotiorum* even when the same total amount of water was applied over the same duration by using less frequent irrigation intervals combined with applying more water at each irrigation. A constraint of this control tactic is that adequate water is needed from flower development through achene fill in sunflower seed crops to prevent adverse effects

on seed yield, i.e., from the initiation of flowering until two weeks after flowering, which is also the period during which sunflower crops are particularly susceptible to infections of the heads by ascospores of *S. sclerotiorum* (Blamey et al. 1997; Göksoy et al. 2004; Gulya and Masirevic 1992; McCartney and Lacey 1991; Unger 1990).

Plant populations in the hybrid sunflower seed crops surveyed in this study ranged from 43,000 to 69,000 plants/ha, which is similar to those reported by Holley and Nelson (1986), i.e., 37,000 to 74,000 plants/ha. As in that study, there was no significant correlation between basal stalk rot incidence and plant population. In addition, the correlations between plant population and midstalk rot incidence, head rot incidence, or white mold incidence were not significant.

The *S. sclerotiorum* isolates collected from Sclerotinia basal stalk rot foci in two hybrid sunflower seed crops in the Columbia Basin in 2015 were diverse genetically and genotypically. The isolates were all confirmed to be *S. sclerotiorum* based on sclerotial size (Kohn 1979b) and ITS rDNA sequences. The foci of basal stalk infections were identified and selected based on symptoms on the taproot and lower stalk of multiple sequential plants. Apothecia were not observed during weekly visits to both fields through the summer of 2015, and <0.05% of the sunflower plants in each field had symptoms of aerial infection out of 8,000 plants rated for white mold in trials completed in these fields in 2015 (see Chapter 3). Therefore, the isolates of *S. sclerotiorum* obtained from the base of sunflower stalks were presumed to be a subsample of isolates present in the soil or, potentially, introduced with the sunflower stock seed at planting (Herd and Phillips 1988), with limited possibility of isolates originating from infections caused by ascospores, which are the products of sexual recombination.

Isolates of *S. sclerotiorum* in the Ephrata and Odessa fields with identical haplotypes, based on microsatellite markers, were highly aggregated within the foci sampled. This suggests

that adjacent plants within a basal stalk rot focus were infected from a sclerotium or multiple sclerotia that originated from the same isolate. Root-to-root spread of *S. sclerotiorum* has been demonstrated (Huang and Hoes 1980) and might explain, in part, why relatively low inoculum densities can cause significant basal stalk rot outbreaks in sunflower crops (Nelson and Lamey 2000). Sunflower plants also can develop extensive root systems (Seiler 1997), which facilitates root-to-root spread of *S. sclerotiorum* (Holley and Nelson 1986). However, caution should be exercised in this assertion because sclerotia of a single *S. sclerotiorum* isolate, arising from a previously infected crop, could readily be distributed the 19 to 23 cm distance between adjacent sunflower plants in the fields from which the *S. sclerotiorum* isolates were collected in this study. Regardless, the results suggest that basal stalk rots in sunflower seed crops can amplify few genotypes of *S. sclerotiorum* within limited areas in fields, whereas genotypic diversity throughout each field reflects a greater number of genotypes present and amplified during the growing season.

Studies have shown variability in virulence among isolates of *S. sclerotiorum* (e.g., Willbur et al. 2017), possible specialization of *S. sclerotiorum* genotypes to cultivars of particular crops (e.g., Lehner et al. 2016), and differences in virulence based on mycelial compatibility groups of *S. sclerotiorum* isolates (Otto-Hanson et al. 2011). Therefore, it is important to use a representative panel of isolates of *S. sclerotiorum* in breeding for resistance to white mold. With partial resistance to *S. sclerotiorum* having been identified in sunflower germplasm (Talukder et al. 2014), the isolates collected in this study could potentially be used to select for resistance to basal stalk rot and aerial infection, particularly if the isolates prove to differ in virulence. Further research is needed to assess whether the isolates collected in this study differ in virulence for causing basal infections and/or aerial infections.

The spatial structure of the *S. sclerotiorum* populations detected in two sunflower seed crops in the Columbia Basin in 2015 were consistent with results of a previous study of potato crops in the Columbia Basin by Atallah et al. (2004). Both the AMOVA and STRUCTURE analyses provided evidence that the two populations were not differentiated genetically, similar to the results reported by Atallah et al. (2004) for populations from potato crops. The STRUCTURE analysis demonstrated that the isolates, regardless of population of origin, grouped into two genetic clusters. A lack of genetic differentiation between/among populations of this fungus in fields located over distances <400 km apart is in not unique to the Columbia Basin, and has been reported for *S. sclerotiorum* populations in Australia (Ekins et al. 2011; Sexton and Howlett 2004) and New York State (Dunn et al. 2017). The two hybrid sunflower seed crops from which the *S. sclerotiorum* populations from hybrid sunflower seed crops were isolated 77 km apart, including a 3.5 km-wide buffer of high desert habitat. Therefore, gene flow of *S. sclerotiorum* isolates between the two fields via ascospore dispersal or movement of inoculum in soil or water was very unlikely. However, gene flow could have occurred as a result of introducing inoculum on infected stock seed or stock seed lots contaminated with sclerotia in these hybrid sunflower seed crops or previous susceptible crops. Future studies looking at *S. sclerotiorum* populations from locations where sunflower stock seed lots are grown for seed crops to be planted in the Columbia Basin may provide further evidence for this being a potential factor contributing to the lack of genetic differentiation of *S. sclerotiorum* populations among fields.

Evidence of outcrossing, similar to the results of Attanayake et al. (2012), was found in each of the Ephrata and Odessa populations of *S. sclerotiorum* in this study when the hypothesis of linkage equilibrium was tested, taking into account the two subpopulations defined by the

STRUCTURE analysis. The significant multilocus linkage disequilibrium detected in both populations prior to accounting for the subpopulations defined by STRUCTURE, illustrated the importance of correcting for underlying population structure for these analyses as stated in the Multilocus manual (Agapow and Burt 2001). Given the results from Attanayake et al. (2012) and Atallah et al. (2004) in the Columbia Basin, these results were expected.

Chitrampalam et al. (2013) discovered a 3.6 kb inversion in the mating type locus of *S. sclerotiorum* that inverts *MAT1-2-1* and *MAT1-2-4*, and truncates the *MAT1-1-1* allele. The inversion occurred each time the *S. sclerotiorum* isolates studied underwent meiosis, resulting in a 1:1 distribution of Inv-:Inv+ isolates. Both Inv- and Inv+ isolates are self-fertile so the potential significance to outcrossing is not clear (Chitrampalam et al. 2013). In a subsequent study, Chitrampalam et al. (2015) more thoroughly assessed the prevalence of Inv- and Inv+ in isolates of *S. sclerotiorum* across the U.S., and showed a preponderance of the isolates (55.5%) were *MAT* heterokaryons possessing both Inv- and Inv+ mating type loci. The Ephrata and Odessa *S. sclerotiorum* populations collected from hybrid sunflower seed crops in the Columbia Basin had *MAT* heterokaryon frequencies of 1.1 and 3.1%, respectively, well below those reported by Chitrampalam et al. (2015).

Overall, this hybrid sunflower seed crop survey suggests that good management practices for control of white mold are needed as >90% of the sunflower seed crops surveyed in 2016 had white mold. In particular, crop rotation of 3 years with a white mold non-susceptible crop preceding a hybrid sunflower seed crop, and reduced frequency of irrigation appear to be important production practices for reducing the risk or severity of losses to white mold. While effective assessment of the efficacy of applications of Contans WG at planting and foliar fungicide applications for controlling white mold could not be made with the limited data

provided for each field, the evidence of limited control of white mold by those practices suggests that research is necessary to optimize these practices for hybrid sunflower seed growers in the Columbia Basin. The high level of genetic diversity in the two *S. sclerotiorum* populations evaluated in this study indicates a highly adaptable pathogen population, which suggests a high risk of the pathogen adapting to production practices, e.g., developing resistance to widely used fungicides in the Columbia Basin (Bradley et al. 2016); and, in fact, isolates of *S. sclerotiorum* from the Columbia Basin have shown putative insensitivity to benomyl and fluazinam (Attanayake et al. 2012). The lack of genetic differentiation between the populations from each of Ephrata and Odessa, despite the spatial isolation of these fields, suggests that populations of *S. sclerotiorum* are not differentiated genetically within the Columbia Basin, based on limited sampling in two sunflower seed fields in one year, but supported by the results of Atallah et al. (2004). This also indicates that management practices for white mold that are effective in one area of the Columbia Basin are likely to be effective throughout the region, and a relatively limited number of isolates could be used effectively to screen for fungicide resistance in populations of the pathogen in the Columbia Basin.

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Table 2.1. Field, nearest town, grower, female parent line, sunflower type, field size (ha), soil texture, and cropping history (for 2013, 2014, and 2015) of hybrid sunflower seed crops rated for white mold incidence in 2016 in the Columbia Basin of central Washington

Field[a]	Nearest town	Grower[a]	Female parent[b]	Type	Irrigation type[c]	Size (ha)[b]	Soil texture	Cropping history[d]		
								2013	2014	2015
1	Coulee City	1	D	Oil	Pivot	20.2	Sandy loam	Wheat	Pea**	Wheat
2	Coulee City	2	F	Oil	Pivot	36.4	Loam	Alfalfa**	Alfalfa**	Alfalfa**
3	Coulee City	2	F	Oil	Pivot	10.1	Loam	Alfalfa**	Alfalfa**	Alfalfa**
4*[e]	George	3	E	Oil	Pivot	89.0	Loamy sand	Timothy grass	Timothy grass	Timothy grass
5*	George	3	F	Oil	Pivot	74.9	Sand	Canola**	Corn	Potato**
6*	George	3	F	Oil	Pivot	42.5	Sand	Pea**	Wheat	Carrot seed**
7*	George	4	F	Oil	Pivot	33.2	Sandy loam	Timothy grass	Timothy grass	Timothy grass
8*	George	3	G	Oil	Pivot	50.6	Sand	Canola**	Corn	Potato**
9*	George	3	G	Oil	Pivot	52.6	Sand	Canola**	Wheat	Potato**
10*	George	3	G	Oil	Pivot	52.6	Sand	Canola**	Corn	Potato**
11	Kittitas	5	B	Oil	Pivot	30.4	Loam	Timothy grass	Timothy grass	Oat
12	Kittitas	6	D	Oil	Pivot	24.3	Silt loam	Timothy grass	Timothy grass	Timothy grass
13	Kittitas	6	D	Oil	Pivot	48.6	Loam	Timothy grass	Timothy grass	Timothy grass
14	Kittitas	6	D	Oil	Rill	24.3	Silt loam	Timothy grass	Timothy grass	Timothy grass
15	Kittitas	7	O	Confection	Rill	14.2	Loam	Timothy grass	Timothy grass	Timothy grass
16	Kittitas	5	P	Oil	Rill	16.2	Silt loam	Timothy grass	Timothy grass	Timothy grass
17*	Moses Lake	8	D	Oil	Pivot	26.3	Silt loam	Sunflower**	Wheat	Blue grass
18*	Moses Lake	9	F	Oil	Pivot	52.6	Silt loam	Corn	Wheat	Potato**
19*	Moses Lake	10	F	Oil	Pivot	52.6	Sand	Wheat	Potato**	Wheat
20*	Moses Lake	3	G	Oil	Pivot	56.7	Loamy sand	Canola**	Corn	Potato**
21	Odessa	11	A	Confection	Pivot	21.0	Silt loam	Wheat	Wheat	Corn
22	Odessa	11	A	Confection	Pivot	24.3	Silt loam	Wheat	Canola**	Wheat
23	Odessa	11	A	Confection	Pivot	6.1	Silt loam	Wheat	Wheat	Wheat
24	Odessa	11	F	Oil	Pivot	48.6	Silt loam	Blue grass	Garbanzo bean**	Wheat
25	Quincy	12	D	Oil	Rill	19.4	Silt loam	Bean**	Corn	Wheat

Table 2.1. (Continued)

Field[a]	Nearest town	Grower[a]	Female parent[b]	Type	Irrigation type[c]	Size (ha)[b]	Soil texture	Cropping history[d] 2013	2014	2015
26	Quincy	13	I	Oil	Rill	13.8	Loamy sand	Bean**	Wheat	Pea** /Wheat
27	Quincy	14	J	Oil	Rill	10.1	Silt loam	Corn	Corn	Corian-der**
28	Quincy	15	J	Oil	Rill	17.0	Silt loam	Corn	Corian-der**	Corn
29	Quincy	16	J	Oil	Rill	12.1	Silt loam	Wheat	Bean**	Wheat
30	Quincy	17	K	Oil	Rill	12.1	Loamy sand	Bean**	Bean**	Corn
31	Quincy	12	M+L+N	Confection	Rill	8.9+1.2+0.4	Silt loam	Corn	Bean**	Fruit tree root stock
32	Ritzville	18	C	Oil	Pivot	24.3	Silt loam	Fallow	Wheat	Grass
33	Ritzville	19	C	Oil	Pivot	35.2	Silt loam	Pea**	Wheat	Wheat
34	Ritzville	20	F	Oil	Pivot	52.6	Silt loam	Wheat	Potato**	Wheat
35	Ritzville	20	H	Confection	Pivot	23.5	Silt loam	Alfalfa**	Alfalfa**	Wheat
36	Ritzville	20	H	Confection	Pivot	51.4	Silt loam	Wheat	Canola**	Wheat
37	Ritzville	19	I	Oil	Pivot	25.9	Silt loam	Wheat	Bean**	Wheat
38	Royal City	21	F	Oil	Pivot	40.5	Loamy sand	Alfalfa**	Alfalfa**	Potato**
39	Warden	22	D	Oil	Pivot	27.5	Silt loam	Alfalfa**	Alfalfa**	Potato**
40	Warden	23	E	Oil	Pivot	14.2	Silt loam	Alfalfa**	Potato**	Triticale

[a] Field, grower, and female parent line are coded for anonymity.

[b] The letters correspond to different female parent lines, and the numbers to the hectares of the field planted to each female parent. Field 31 was planted to three female parents and one male parent. All fields were planted to hybrid sunflower seed crops, but only female parent lines were rated for white mold incidence.

[c] Rill irrigation = furrow irrigation. All other fields were irrigated by center pivots.

[d] Alfalfa (*Medicago sativa*), bean (*Phaseolus vulgaris*), blue grass (*Poa* sp.) canola (*Brassica napus*), carrot (*Daucus carota*), corn (*Zea mays*), coriander (*Coriandrum sativum*), garbanzo bean (*Cicer arietinum*), oat (*Avena sativa*), pea (*Pisum sativum*), potato (*Solanum tuberosum*), sunflower (*Helianthus annuus*), timothy grass (*Phleum pratense*), tree fruit root stock (Rosaceae), triticale (x *Triticosecale*), wheat (*Triticum aestivum*). ** = crop susceptible to white mold.

[e] Fields with an * = Contans WG application was made at planting in May, and at least one foliar fungicide application was made during bloom to control white mold. Information on these applications was not provided for the other 29 sunflower seed crops surveyed.

Table 2.2. Weather data from the nearest Washington State University (WSU) AgWeatherNet station, and mean and maximum incidence of white mold caused by *Sclerotinia sclerotiorum* rated in each of eight strata in each of 40 sunflower seed crops surveyed in 2016 in the Columbia Basin of central Washington

	Average daily air temp. (ºC) in July and August 2016[a]			Mean RH[a]	Mean soil temp.[a]	Total precip.[a]	Mean white mold incidence (%)					Maximum white mold incidence (%)				
Field	Min	Mean	Max	(%)	(ºC)	(mm)	Basal	Mid-stalk	Head	Aerial	Total	Basal	Mid-stalk	Head	Aerial	Total
1	-	-	-	-	-	-	2.5	0.0	0.9	0.9	3.4	12.5	0.0	3.0	3.0	15.5
2	-	-	-	-	-	-	0.2	0.0	2.1	2.1	2.3	0.5	0.0	11.5	11.5	11.5
3	-	-	-	-	-	-	0.3	0.3	2.6	2.9	3.1	1.5	2.0	14.5	16.5	18.0
4*	13.0	21.4	29.6	52	23.6	10.7	2.4	0.0	0.8	0.8	3.2	4.5	0.0	1.5	1.5	4.5
5*	13.3	22.1	30.4	49	28.2	10.7	0.1	0.0	0.2	0.2	0.3	0.5	0.0	0.5	0.5	0.5
6*	13.3	22.1	30.4	49	28.2	10.7	5.0	0.8	3.7	4.5	9.5	12.0	3.5	16.8	19.3	30.5
7*	13.0	21.4	29.6	52	23.6	10.7	0.4	0.0	7.4	7.4	7.8	1.0	0.0	14.0	14.0	15.0
8*	12.7	21.9	30.4	51	28.2	8.6	0.4	0.0	0.4	0.4	0.9	3.0	0.0	3.0	3.0	3.0
9*	13.0	21.4	29.6	52	23.6	10.7	0.9	0.0	0.4	0.4	1.4	2.0	0.0	1.0	1.0	3.0
10*	13.0	21.4	29.6	52	23.6	10.7	0.8	0.0	0.2	0.2	0.9	1.0	0.0	0.5	0.5	1.0
11	11.0	19.6	27.7	57	25.1	15.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	11.0	19.6	27.7	57	25.1	15.8	0.8	0.0	0.0	0.0	0.8	2.5	0.0	0.0	0.0	2.5
13	11.0	19.6	27.7	57	25.1	15.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	11.0	19.6	27.7	57	25.1	15.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	11.0	19.6	27.7	57	25.1	15.8	0.3	0.0	0.0	0.0	0.3	1.0	0.0	0.0	0.0	1.0
16	11.0	19.6	27.7	57	25.1	15.8	0.3	0.0	0.1	0.1	0.4	2.5	0.0	0.5	0.5	2.5
17*	12.9	21.5	29.0	47	25.3	6.9	8.9	0.1	0.1	0.2	9.1	18.0	1.0	0.5	1.0	18.0
18*	12.9	21.5	29.0	47	25.3	6.9	3.5	0.1	6.6	6.7	10.2	6.0	0.5	11.0	11.0	15.0
19*	12.9	22.1	30.5	49	25.8	5.8	3.4	0.2	28.8	28.9	32.4	6.5	0.5	49.5	50.0	53.0
20*	12.7	21.9	30.4	51	28.2	8.6	0.9	0.1	0.0	0.1	0.9	3.0	0.5	0.0	0.5	3.0
21	-	-	-	-	-	-	1.3	0.4	0.3	0.8	2.0	4.0	1.5	2.5	4.0	8.0
22	-	-	-	-	-	-	0.1	0.0	0.1	0.1	0.2	1.0	0.0	0.5	0.5	1.0
23	-	-	-	-	-	-	1.5	0.1	0.2	0.3	1.8	7.0	1.0	2.0	2.0	7.0
24	-	-	-	-	-	-	1.2	0.0	0.0	0.0	1.2	4.0	0.0	0.0	0.0	4.0
25	13.3	22.1	30.4	49	28.2	10.7	0.7	0.0	0.3	0.3	1.0	2.5	0.0	1.0	1.0	3.0
26	15.3	21.6	28.5	52	21.2	11.7	5.9	0.0	0.4	0.4	6.3	11.0	0.0	2.5	2.5	11.0
27	15.3	21.6	28.5	52	21.2	11.7	7.5	7.2	0.3	7.4	14.9	10.5	19.0	0.5	19.0	29.0
28	15.3	21.6	28.5	52	21.2	11.7	2.9	3.8	1.2	5.0	7.9	8.5	9.0	2.5	10.0	18.5
29	15.3	21.6	28.5	52	21.2	11.7	0.4	0.0	0.6	0.6	1.0	1.0	0.0	1.5	1.5	2.0
30	15.3	21.6	28.5	52	21.2	11.7	1.3	0.1	0.0	0.1	1.4	2.5	0.5	0.0	0.5	2.5
31	13.3	22.1	30.4	49	28.2	10.7	1.3	0.1	0.1	0.3	1.6	4.0	1.0	0.5	1.0	4.0
32	11.0	20.9	29.6	42	22.8	9.4	1.8	0.0	0.1	0.1	1.9	4.5	0.0	0.5	0.5	4.5

Table 2.2. (Continued)

Field	Average daily air temp. (ºC) in July and August 2016[a]			Mean RH[a]	Mean soil temp.[a]	Total precip.[a]	Mean white mold incidence (%)					Maximum white mold incidence (%)				
	Min	Mean	Max	(%)	(ºC)	(mm)	Basal	Mid-stalk	Head	Aerial	Total	Basal	Mid-stalk	Head	Aerial	Total
33	11.0	20.9	29.6	42	22.8	9.4	0.0	0.1	0.0	0.1	0.1	0.0	0.5	0.0	0.5	0.5
34	11.0	20.9	29.6	42	22.8	9.4	6.1	0.1	3.3	3.4	9.5	9.5	0.5	7.5	8.0	15.0
35	11.0	20.9	29.6	42	22.8	9.4	0.2	0.1	0.0	0.1	0.3	1.0	0.5	0.0	0.5	1.0
36	11.0	20.9	29.6	42	22.8	9.4	2.6	0.0	0.0	0.0	2.6	5.5	0.0	0.0	0.0	5.5
37	11.0	20.9	29.6	42	22.8	9.4	4.6	0.0	0.0	0.0	4.6	11.0	0.0	0.0	0.0	11.0
38	16.4	22.7	28.8	43	27.4	7.1	0.8	0.2	3.2	3.4	4.1	2.0	0.5	7.0	7.0	8.5
39	13.1	21.5	29.3	51	24.9	9.4	0.4	0.4	0.3	0.7	1.1	2.0	1.5	1.5	2.0	2.5
40	13.1	21.5	29.3	51	24.9	9.4	0.2	0.1	0.0	0.1	0.3	0.5	1.0	0.0	1.0	1.5

[a] Weather data collected from the WSU AgWeatherNet stations with corresponding fields numbers in parentheses: Broadview (11, 12, 13, 14, 15, 16), Ephrata (5, 6, 25, 31), Frenchman Hills (4, 7, 9, 10), Mae (8, 20), Moses Lake (19), Quincy (26, 27, 28, 29, 30), Ritzville (32, 33, 34, 35, 36, 37), Royal East Slope (38), Warden Golf (39, 40), Wheeler (17, 18). Weather data for fields 1 to 3 (near Coulee City) and 21 to 24 (near Odessa) were not collected due to the lack of a nearby AgWeaetherNet stations in those regions. Mean data were collected over July and August for relative humidity (RH), and soil temperature (soil temp.), and total precipitation (total precip.).

[b] Fields with an * = Contans WG application was made at planting in May, and at least one foliar fungicide application was made during bloom to control white mold. Information on these applications was not provided for the other 29 sunflower seed crops surveyed.

Table 2.3. Comparison of gene diversity, genotypic diversity, and random association of alleles of *Sclerotinia sclerotiorum* populations sampled from basal stalk infections in sunflower seed crops near each of Ephrata and Odessa, WA[a]

Population characteristic	Ephrata (N = 94)[a]	Odessa (N = 96)[a]
Gene diversity		
Total number of alleles	25	26
Number of private alleles	1	2
Mean number of alleles/locus	3.13 ± 1.25	3.25 ± 1.28
H_e[b]	0.53 ± 0.16	0.48 ± 0.16
H_e clone-corrected[b]	0.57 ± 0.13	0.56 ± 0.14
Genotypic diversity		
Unique multilocus haplotypes	28	22
Stoddart and Taylor's *G*	12.14	8.33
Genotypic richness (g/N)	0.30	0.23
Genotypic evenness, E_5[c]	0.66	0.58
Test of recombination[d]		
Whole population		
I_A (*p*)	0.98 (*P* = 0.001)	1.65 (*P* = 0.001)
r_d (*p*)	0.14 (*P* = 0.001)	0.24 (*P* = 0.001)
Clone-corrected population		
I_A (*p*)	0.39 (*P* = 0.001)	0.76 (*P* = 0.001)
r_d (*p*)	0.06 (*P* = 0.001)	0.11 (*P* = 0.001)
Clone-corrected/subpopulations defined by STRUCTURE[f]		
I_A (*p*)	0.39 (*P* = 0.499)	0.76 (*P* = 0.558)
r_d (*p*)	0.06 (*P* = 0.499)	0.11 (*P* = 0.558)

[a] *S. sclerotiorum* isolates collected from sequential basal stalk infections in each of two hybrid sunflower seed fields to determine population structure and genetic diversity of the populations; N = 94 isolates of *S. sclerotiorum* collected from 26 disease foci in a 2015 hybrid sunflower seed crop near Ephrata, WA; N = 96 isolates of *S. sclerotiorum* from 30 disease foci in a 2015 hybrid sunflower seed crop near Odessa, WA.

[b] Nei's expected heterozygosity (Nei 1978) for eight microsatellite loci calculated using Arlequin (Excoffier et al. 2005).

[c] Genotypic evenness as defined by Grünwald et al. (2003).

[d] Estimates of multilocus linkage disequilibrium and significance of the null hypothesis tested using Multilocus (Agapow and Burt 2001).

[e] I_A = index of association; r_d = modified multilocus I_A independent of the number of loci (*P* value in parenthesis).

[f] Subpopulations defined by Bayesian clustering analysis using STRUCTURE (Pritchard et al. 2000).

Table 2.4. Number of alleles in each locus, and pair-wise linkage disequilibrium (*P* value) for eight microsatellite loci estimated using Multilocus[c] software for the clone-corrected *Sclerotinia sclerotiorum* population from a sunflower seed crop near each of Ephrata and Odessa, WA[a]

Field and locus[b]	Number of alleles	Microsatellite locus						
		5-2	7-2	12-2	13-2	17-3	55-4	110-4
Ephrata								
7-2	2	-0.024						
		(0.661)						
12-2	3	-0.032	-0.089					
		(0.023)	(0.883)					
13-2	5	-0.005	0.318	-0.107				
		(0.531)	(0.010)	(0.924)				
17-3	2	0.101	0.165	-0.0118	0.028			
		(0.105)	(0.023)	(0.432)	(0.101)			
55-4	3	-0.032	0.024	0.077	-0.044	0.198		
		(0.605)	(0.271)	(0.065)	(0.947)	(0.008)		
110-4	3	-0.027	0.005	-0.008	0.088	-0.001	0.242	
		(0.878)	(0.199)	(0.558)	(0.058)	(0.503)	(0.001)	
114-4	5	0.094	0.119	-0.022	0.100	0.396	0.038	0.048
		(0.038)	(0.047)	(0.680)	(0.086)	(<0.001)	(0.220)	(0.122)
Odessa								
7-2	2	0.231						
		(0.099)						
12-2	3	0.016	-0.092					
		(0.415)	(0.748)					
13-2	4	-0.061	0.186	-0.145				
		(0.614)	(0.085)	(0.857)				
17-3	2	0.394	0.516	-0.014	0.101			
		(0.004)	(0.003)	(0.577)	(0.121)			
55-4	5	-0.072	-0.013	0.016	-0.153	0.135		
		(0.701)	(0.545)	(0.332)	(1.000)	(0.049)		
110-4	3	0.216	0.105	-0.05	0.073	0.281	0.176	
		(0.018)	(0.129)	(0.769)	(0.105)	(0.008)	(0.014)	
114-4	5	0.145	0.222	0.011	0.280	0.411	-0.031	0.119
		(0.077)	(0.024)	(0.466)	(0.006)	(<0.001)	(0.599)	(0.064)

[a] *S. sclerotiorum* isolates collected from sequential basal stalk infections in each of two hybrid sunflower seed fields to determine population structure and genetic diversity of the populations; 94 isolates of *S. sclerotiorum* collected from 26 disease foci in a 2015 hybrid sunflower seed crop near Ephrata, WA; 96 isolates of *S. sclerotiorum* from 30 disease foci in a 2015 hybrid sunflower seed crop near Odessa, WA.

[b] Locus definition from Sirjusingh and Kohn (2001).

[c] Pair-wise linkage disequilibrium estimated in Multilocus (Agapow and Burt 2001).

Table 2.5. Analysis of molecular variance for clone-corrected *Sclerotinia sclerotiorum* populations isolated from infected sunflower seed crops near each of Ephrata and Odessa, WA[a]

Source of variation[b]	df	Sum of squares	Variance components	Percentage of variation	*P* value
Among populations	1	24.90	0.17	0.82	0.32
Within populations	48	993.58	20.70	99.18	
Total	49	1018.48	20.87		

[a] *S. sclerotiorum* isolates collected from sequential basal stalk infections in each of two hybrid sunflower seed fields in the Columbia Basin of Washington to determine population structure and genetic diversity of the fungal populations.

[b] Genetic distance was based on the stepwise mutation model (SMM) implemented in Arlequin (Excoffier et al. 2005). Multilocus haplotypes determined from eight microsatellite loci defined by Sirjusingh and Kohn (2001). Estimated based on the clone-corrected (one representative isolate for each unique multilocus haplotype) for the Ephrata (n = 28) and Odessa (n = 22) populations of *S. sclerotiorum* to reduce bias due to overrepresentation of clones.

Table. 2.6. Chi-square test of the spatial aggregation of *Sclerotinia sclerotiorum* isolates within and between foci of adjacent plants with basal stalk rot in a sunflower seed crop near each of Ephrata and Odessa, WA[a]

		Observed[b]		Expected[b]			
Population	**Pairs from**	**Identical pairs**	**Non-identical pairs**	**Identical pairs**	**Non-identical pairs**	***X*² value**	***P* value**
Ephrata	Within foci	35	33	5.60	62.40	168.10	$<10^{-37}$
	Between foci	3	23	2.14	23.86	0.37	0.54
Odessa	Within foci	47	19	7.92	58.08	219.11	$<10^{-48}$
	Between foci	1	29	3.60	26.40	2.13	0.14

[a] *S. sclerotiorum* isolates collected from sequential basal stalk infections in each of two hybrid sunflower seed fields in the Columbia Basin of Washington to determine population structure and genetic diversity of the fungal populations.

[b] The observed frequencies of isolate-pairs from adjacent plants with the same multilocus haplotype were tested against expected frequencies of the same haplotype being recovered from adjacent plants based on a random distribution of haplotypes. Multilocus haplotypes determined from eight microsatellite loci defined by Sirjusingh and Kohn (2001). Estimates were made using 94 isolates from 26 basal stalk rot foci in a hybrid sunflower seed field near Ephrata, WA; and 96 isolates from 30 basal stalk rot foci in a hybrid sunflower seed field near Odessa, WA.

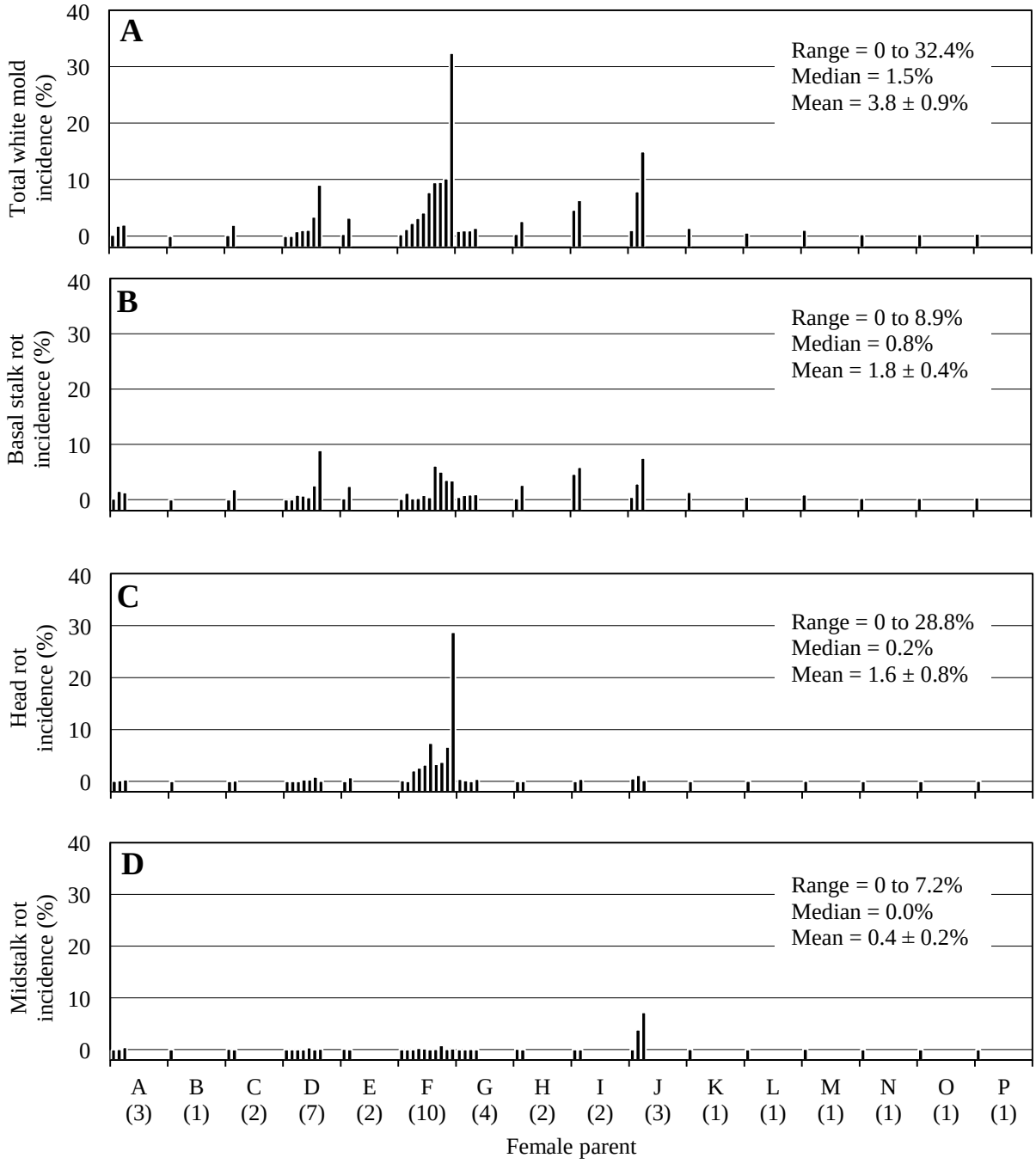

Fig. 2.1. Mean incidence of total white mold (**A**), basal stalk rot (**B**), midstalk rot (**C**), and head rot (**D**) for eight strata rated in each of 40 sunflower seed crops surveyed for white mold in the Columbia Basin of central Washington in 2016. Female parent lines are identified by capital letters, with the number of fields planted to each female parent noted in parentheses. Details on the sunflower seed crops surveyed can be found in Tables 2.1 and 2.2.

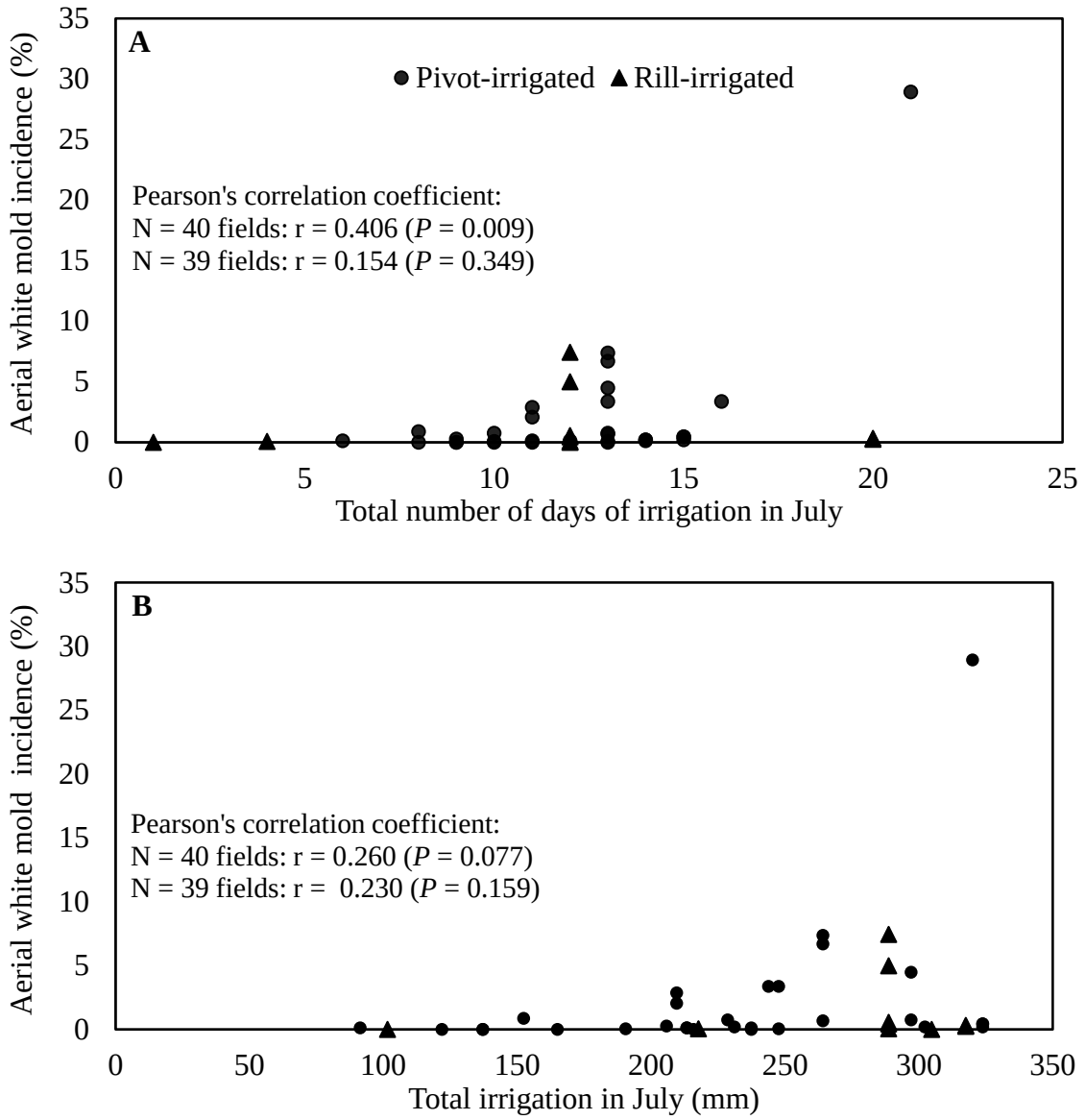

Fig. 2.2. Mean incidence (%) of aerial white mold (midstalk rot + head rot) for eight strata rated in each hybrid sunflower seed crop in relation to the total number of days of irrigation (a measure of irrigation frequency) (**A**), and total amount of irrigation (mm) in July (**B**) for 40 sunflower seed crops surveyed in the Columbia Basin of central Washington in 2016. Pearson's correlation coefficients were calculated for all 40 fields and for all fields except one field with 28.9% aerial white mold incidence (outlier). Details on the sunflower seed crops surveyed can be found in Tables 2.1 and 2.2.

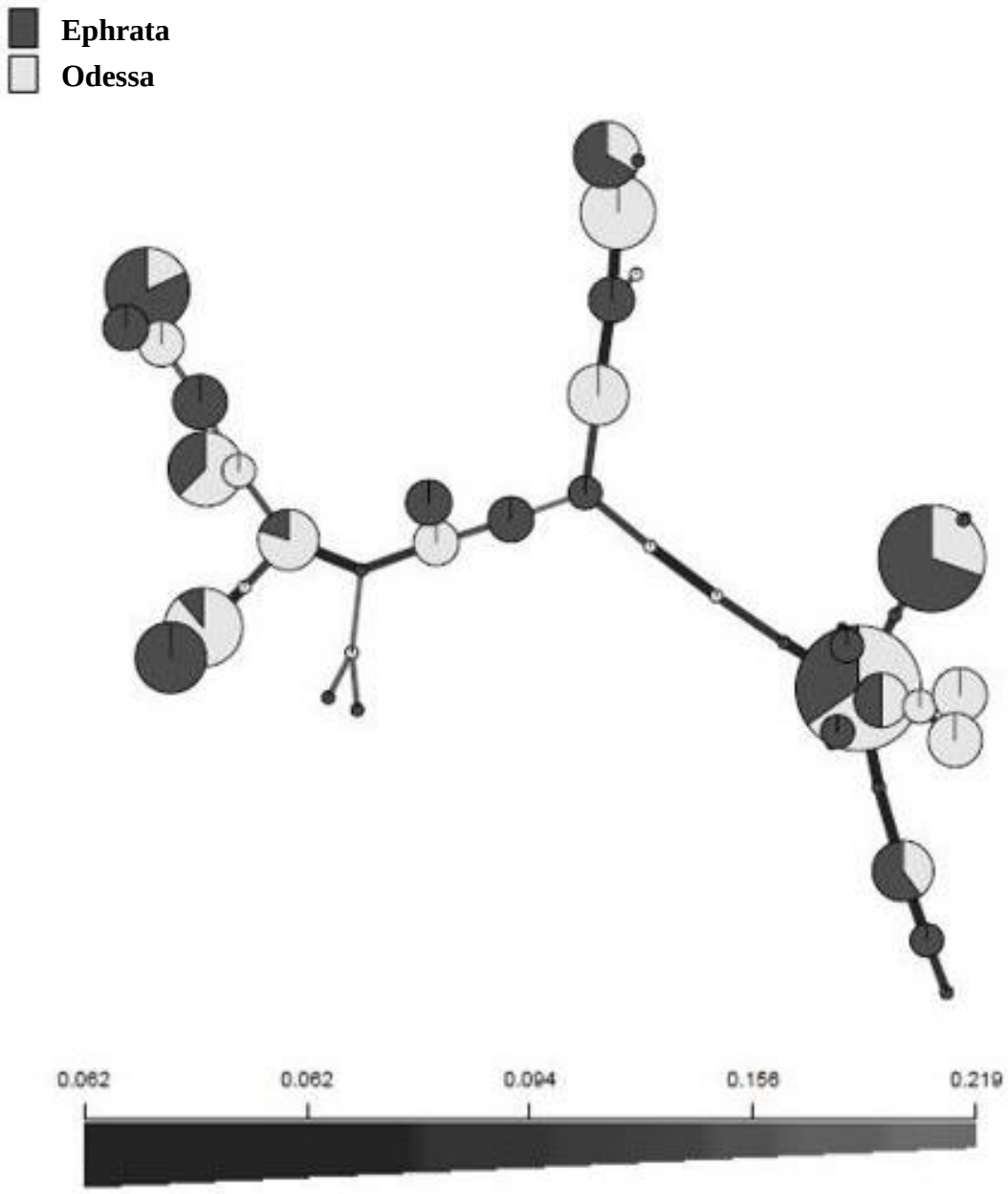

Fig. 2.3. Minimum spanning network of multilocus haplotypes for isolates of *Sclerotinia sclerotiorum* obtained from plants with basal stalk rot in a sunflower seed crop near each of Ephrata and Odessa, WA. Each circle (node) represents a unique multilocus haplotype. The radius of each node is proportional to the number of isolates in both populations sharing that multilocus haplotype. The proportion of each node shaded dark gray or light gray indicates the number of isolates from each of the Ephrata and Odessa populations, respectively, that share the same multilocus haplotype. The line (edge) width between nodes is proportional to the genetic distance between multilocus haplotypes, with the lines becoming thicker for more related multilocus haplotypes. Genetic distance was calculated as described by Bruvo et al. (2004) using POPPR.

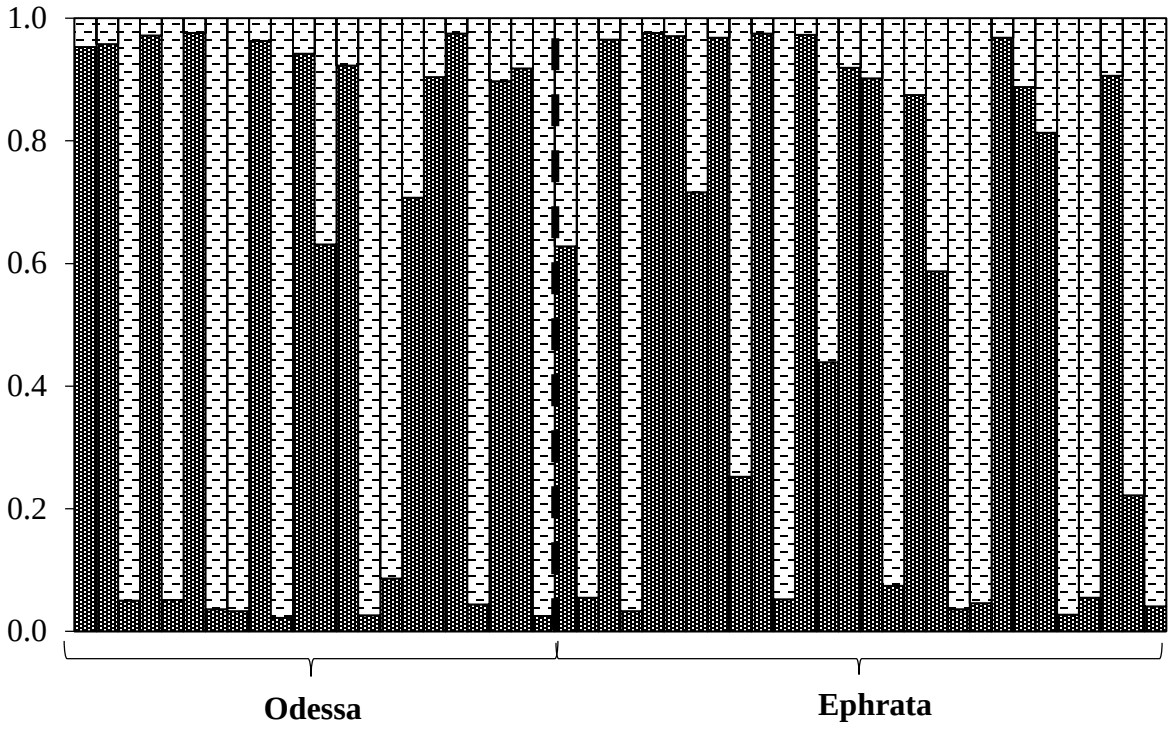

Fig. 2.4. Posterior probabilities of population assignment from Bayesian clustering analysis using the software STRUCTURE (Pritchard et al. 2000) for clone-corrected *Sclerotinia sclerotiorum* populations isolated from foci of plants with basal stalk infections in a sunflower seed crop near each of Ephrata and Odessa, WA. Shaded and hatched bars correspond to the posterior probability of each isolate belonging to one of two genetic clusters defined from Bayesian clustering analysis using STRUCTURE. Analysis was based on the clone-corrected (one representative isolate for each unique multilocus haplotype) Ephrata (n = 28) and Odessa (n = 22) populations.

CHAPTER 3

EVALUATION OF CONTANS WG AND FOLIAR FUNGICIDE APPLICATIONS FOR MANAGEMENT OF WHITE MOLD IN SUNFLOWER SEED CROPS IN THE COLUMBIA BASIN OF CENTRAL WASHINGTON

3.1. Introduction

The cultivated sunflower, *Helianthus annuus* L., is grown for vegetable oil production (oilseed types), and for human food and bird-food markets (non-oilseed, confection types) (Berglund 2007). Sunflower production, in general, occurs in climates ranging from temperate to semi-arid, the latter with irrigation (Blamey et al. 1997). On average, the duration from planting to physiological maturity for sunflower crops ranges from 75 to 150 days, depending on cultivar and environment, with production limited to warm, frost-free months as sunflowers are frost sensitive (Blamey et al. 1997; Harveson et al. 2016). In 2016, 40.36 million metric tons of sunflower seed were produced worldwide, which ranked third in global oilseed production behind soybean (*Glycine max*) and canola (*Brassica napus*), and a total of 23.46 million ha were planted for sunflower production [United States Department of Agriculture (USDA) Foreign Agricultural Service (FAS) 2016].

In the U.S., production of hybrid sunflower seed that is sold for planting by Asian, European, and U.S. growers has occurred predominantly in the Sacramento Valley of California (Lilliboe 2009). However, challenges in finding enough isolated fields to produce the number and diversity of hybrid sunflower seed crops in demand has, in part, caused expansion of hybrid sunflower seed production into the Columbia Basin of central Washington (Bill Wirth, Precision Seed Production, LLC, *personal communication*; Lilliboe 2009). Production of hybrid sunflower

seed has expanded rapidly in this region, from 20 ha in 2008 to >2,000 ha in 2014, with potential for additional growth. Hybrid sunflower seed crops are valuable rotational crops as the expected gross return in the Columbia Basin ranges from $3,700 to $5,400/ha.

Although sunflower seed crops have been produced in the Columbia Basin for less than 10 years, some of these crops have been affected by white mold caused by the necrotrophic fungus *Sclerotinia sclerotiorum*, at incidences ranging from <1 to 75% (Bill Wirth, Precision Seed LLC, *personal communication*). *S. sclerotiorum* survives in the soil as sclerotia, and has a wide, phylogenetically diverse host range of >400, mostly dicotyledonous plant species, including sunflower (Boland and Hall 1994; Bolton et al. 2006). *S. sclerotiorum* caused an estimated $100 million in losses in sunflower crops in the U.S. in 1999, primarily due to head rot, illustrating the potential destructiveness of this pathogen in sunflower crops (Bolton et al. 2006).

Infection of a sunflower plant by *S. sclerotiorum* can be initiated either by myceliogenic or carpogenic germination of sclerotia, depending on environmental conditions (Nelson and Lamey 2000). Sclerotia that germinate myceliogenically give rise to mycelia, which can infect the root and crown tissues of a host plant (Bolton et al. 2006). Sclerotia that germinate carpogenically bear apothecia, with each apothecium capable of releasing up to 3×10^7 ascospores over the duration of viability of the apothecium, which can range from 2 to 33 days (Abawi and Grogan 1979; Twengstrom et al. 1998a). The ascospores can infect the stalks and heads of sunflower plants in the presence of an exogenous nutrient source, most typically florets, and >42 h of continuous water film on the plant tissue surface (Gulya and Masirevic 1992). Carpogenic germination is favored by 7 to 14 days of high soil moisture (≥-400 kPa), which is typically not achieved in crops until after canopy closure (Kora et al. 2005; Nelson and Lamey

2000; Teo and Morrall 1985), i.e., sunflower crops usually are susceptible to ascospore infection from canopy closure, through bloom, until about two weeks following flowering (Gulya and Masirevic 1992).

Several strategies are recommended to control white mold in sunflower crops, including tillage to bury sclerotia >6 cm deep in order to inhibit sclerotial germination (Mitchell and Wheeler 1990), 3- to 4-year rotations with non-susceptible crops, applications of *Coniothyrium minitans* as a biocontrol agent for reduction of sclerotial viability and density in the soil, and timely foliar applications of fungicides (Harveson et al. 2016). Planting cultivars with partial resistance to white mold, while an important means of control of white mold in sunflower crops (Harveson et al. 2016), is seldom an option in hybrid sunflower seed production because the parental lines planted are dictated to the growers by the proprietary contracts with seed companies, and the resistance levels of the parental lines often are not known (Bill Wirth, Precision Seed Production, LLC, *personal communication*). In addition, growers can use reduced irrigation frequency after canopy closure to limit the duration of high levels of soil moisture near the soil surface that are favorable for apothecial formation (Twengstrom et al. 1998a).

The coelomycete *Coniothyrium minitans*, a mycoparasite of *S. sclerotiorum*, can be used to reduce sclerotial populations of this pathogen in the soil (Budge and Whipps 1991; Budge et al. 1995; Campbell 1947; Whipps et al. 2008). Applications of *C. minitans* reduced the incidence of lettuce (*Lactuca sativa*) drop caused by *S. sclerotiorum* in greenhouses (Budge and Whipps 1991; Jones et al. 2004; Van Beneden et al. 2010) and in fields the same season the applications were made (Chitrampalam et al. 2010; McLaren et al. 1994). However, when applied at planting to fields of canola and potato (*Solanum tuberosum*), *C. minitans* has not

always been effective at reducing white mold incidence in the season of application (Hammond et al. 2008; McQuilken et al. 1995). Contans WG Biological Fungicide (Contans WG), a commercial formulation of *C. minitans*, is marketed in the U.S. by Bayer CropScience (Research Triangle Park, NC), and is registered for use in sunflower seed crops in the Columbia Basin. Hybrid sunflower seed growers in the Columbia Basin have been applying Contans WG to their fields, typically at about the time of planting using a ground rig and spray boom or by chemigation through center-pivots, to try and control white mold in the season of the Contans WG application(s) (Bill Wirth, Precision Seed Production, LLC, *personal communication*). The applications are aimed particularly at controlling basal stalk infections resulting from myceliogenic germination of sclerotia in close proximity to sunflower plants.

Protective applications of fungicides to the heads and foliage of sunflower plants is another tactic that can be used by sunflower seed growers to try and control midstalk rot and head rot caused by *S. sclerotiorum* ascospore infections (Bill Wirth, Precision Seed Production, LLC, *personal communication*). However, there have been reports of inconsistent efficacy of such fungicide applications in sunflower crops (Harveson et al. 2016). In other white mold-susceptible crops such as bean (*Phaseolus vulgaris*) (Mahoney et al. 2014; Morton and Hall 1989), canola (Bradley et al. 2006; Turkington and Morall 1993), and potato (Johnson and Atallah 2006), foliar fungicide applications have controlled white mold effectively when timed during bloom with thorough coverage of the crop canopy. Similarly, sunflowers are most susceptible to ascospore infection, especially on the face of the heads, from the beginning of flowering until two weeks after flowering, when fungicide coverage of the face of the heads is important for effective control of head rot, since infection most commonly begins on florets on the face of sunflower heads (Gulya and Masirevic 1992).

The timing of foliar fungicide sprays for control of white mold has been optimized in other crops by identifying when peak ascospore release occurs in relation to susceptible stage(s) of crop development, as well as key microclimatic factors that contribute to white mold development (Atallah and Johnson 2004; Johnson and Atallah 2006; Kora et al. 2005; Turkington and Morall 1993). A study by McCartney and Lacey (1991) in the UK showed that ascospore release in sunflower crops can occur for up to 6 weeks, demonstrating the potential for a sustained period of carpogenic germination and ascospore release in sunflower crops. In North Dakota, growing seasons with abnormally high amounts of precipitation have been cited as promoting sunflower head rot epidemics (Gulya et al. 1989); however, to our knowledge, there have been no studies to date in sunflower crops that have examined the microclimate in relation to white mold development.

In the Columbia Basin, inconsistent results with control of white mold have been observed by some sunflower seed growers when applications of Contans WG have been made at planting, and/or protective applications of fungicides have been made to the foliage and head of sunflower plants (Bill Wirth, Precision Seed Production, LLC, *personal communication*). These management tactics are perceived as important in areas of the Columbia Basin with a history of white mold in crops of the many other susceptible species grown in this region, and for growers concerned about reducing irrigation sufficiently for white mold management without compromising seed yields (Göksoy et al. 2004). Currently, many sunflower seed growers make a Contans WG application in May at the time hybrid sunflower seed crops are planted, sometimes followed by a second application in June following cultivation for weed control and/or dammer-diking to prevent water run-off [Washington State University (WSU) Irrigated Agriculture 2016]. Growers typically make Contans WG applications by chemigation or with a

ground rig and spray boom; however, some growers have been evaluating the use of, a banded application of Contans WG at the time of planting, which facilitates a greater rate of Contans WG in 15 cm-band over the row compared to a broadcast or chemigated application to the whole field. By concentrating the product near the crown of the plant, growers seek to enhance the efficacy of Contans WG for controlling basal stalk rot. Growers may also apply Contans WG after harvest of the sunflower seed crop in the fall, if the crop had a severe white mold outbreak. Applications of fungicides to the foliage and head of the sunflower plants typically are initiated pre-bloom (approximately the R2 growth stage; Schneiter and Miller 1981), with a strobilurin product [Fungicide Resistance Action Committee (FRAC) group 11] to enhance yield (Nelson and Meinhardt 2011; Vincelli 2002), and followed by applications of fungicides in FRAC group 7 and/or 3 targeted for 10% bloom and 80 to 90% bloom (the latter two occur about 10 days apart) (FRAC Committee 2016; Bill Wirth, Precision Seed Production, LLC, *personal communication*).

The objectives of this research were to evaluate the relative efficacy of Contans WG applications and protective foliar fungicide applications for control of white mold in hybrid sunflower seed crops in the Columbia Basin, as currently implemented by growers in this region. An additional objective was to assess potential strategies to improve control of white mold using foliar fungicide and Contans WG applications, including: i) optimizing the application of foliar fungicides based on the timing and duration of ascospore release; ii) monitoring microclimatic conditions to identify conditions conducive to apothecial formation and ascospore infection (given the significant difference in sunflower architecture from other white mold-susceptible crops such as bean and potato); and iii) determining if the efficacy of Contans WG for control of

basal stalk rot can be improved using a banded application over the sunflower rows vs. a broadcast application.

3.2. Materials and Methods

Foliar fungicide application trials. ***2015 trial.*** A field trial was carried out to determine the efficacy of three fungicides applied to the foliage for control of Sclerotinia head rot and midstalk rot in sunflower seed crops: 1) Rovral Brand 4 Flowable Fungicide (41.6% iprodione; Bayer CropScience); 2) Topsin 4.5FL (45.0% thiophanate-methyl; United Phosphorous, Inc., King of Prussia, PA); and 3) Omega 500F (40.0% fluazinam; Syngenta Crop Production, LLC., Greensboro, NC). The fungicides are not currently registered for use in sunflower seed crops in Washington State (Washington State Pest Management Resource Service Pesticide Information Center Online 2017), but have proved effective for control of white mold in other crops (Bradley et al. 2006; Johnson and Atallah 2006; Mahoney et al. 2014). The trial was located in the northeast quadrant of a 52-ha, center pivot-irrigated, hybrid sunflower seed crop near Ephrata, WA, in Block 89, Unit 146 of the Columbia Basin Irrigation Project (47° 7'20.33"N 119°36'12.61"W) (U.S. Department of the Interior Bureau of Reclamation 2016). The soil type was a Quincy loamy fine sand [United States Department of Agriculture (USDA) National Resources Conservation Service (NRCS) 2016]. The cropping history of the field was canola in 2012, potato in 2013, and wheat (*Triticum aestivum*) in 2014.

The fungicides were evaluated using a randomized complete block (RCB) design with five replications of four treatments: i) no foliar fungicide application (control treatment), ii) Rovral Brand 4 Flowable Fungicide applied at 1.75 liters/ha, iii) Topsin 4.5FL at 1.83 liters/ha, and iv) Omega 500F at 0.58 liters/ha. Each fungicide was applied pre-bloom on 18 June, at 10%

bloom on 15 July, and at 80% bloom on 29 July, with each application done in 374 liters water/ha with 89 ml/ha of the surfactant Syl-Tac (Wilbur-Ellis Co., Fresno, CA), using a 30.5-m wide, tractor-mounted, spray boom. Each plot was 30.5 m x 30.5 m, with a 30.5 m wide border on all four sides of the plot (borders included rows of sunflower plants treated in the same manner as the rest of the crop that was maintained by the grower-cooperator). Following strip-tillage, the whole field (including plots, alleys, and borders) was planted with proprietary male and female parent lines of an oilseed-type cultivar in an 8:2 female:male ratio of rows. On 8 May, the female rows and the first male row in each pair of male rows were planted; and the second male row for each pair of male rows was planted on 15 May. The seeding depth was 2.5 cm, inter-row spacing was 55 cm, and intra-row spacing was 23 cm. The corners of each plot were marked with flags on 8 May.

Management of the sunflower seed crop included typical grower production practices for the Columbia Basin area. The field was fertilized on 5 May, prior to planting, with 100-0-100-10 (sulfur)-2 (manganese) at 112 kg/ha; and again on 21 May and 1 June with 32-0-0 applied at 39 kg/ha by chemigation. The herbicides Brawl (83.7% s-metolachlor; Tenkoz, Inc., Alpharetta, GA) and Satellite (37.9% pendimethalin; United Phosphorus, Inc.) were applied at 1.46 liters/ha and 2.34 liters/ha, respectively, on 9 May, prior to seedling emergence; and Express (50% tribenuron methyl; DuPont, Wilmington, DE) was applied post-emergence on 28 May at 0.037 liters/ha by chemigation. Contans WG was applied to the remainder of the field outside of plot areas on 11 May, followed by irrigation, between the split-plantings of the male line, with the exception of specific plots in the 2015 foliar fungicide application + Contans WG trial located in the southeast quadrant of this field, as described below. Contans WG was applied at 0.84 kg/ha in 374 liters water/ha using the same spray boom as for the foliar fungicide applications. The

insecticides Delta Gold (16.6% deltamethrin; Winfield Solutions, LLC, St. Paul, MN) and Belt (39% flubendiamide; Bayer CropScience) were applied to the whole field including all plots by chemigation at 0.11 liters/ha on 8 July and 0.29 liters/ha on 17 July, respectively, to control the sunflower moth (*Homoeosoma electellum*). In addition, the fungicide Priaxor Xemium Brand Fungicide (14.33% fluxapyroxad + 28.58% pyraclostrobin; BASF Corp., Research Triangle Park, NC) was applied at 0.58 liters/ha on 18 June, the fungicide Endura (70% boscalid; BASF Corp.) was applied at 0.56 kg/ha on 15 July, and the fungicide Topsin 4.5FL was applied at 1.83 liters/ha on 29 July 2015, each in 374 liters water/ha to the heads and foliage of sunflower plants over the whole field using the same tractor-mounted spray boom, with the exception of the plots in this trial as well as specific plots in the 2015 foliar fungicide application + Contans WG trial described below.

Sunflower plants of the female line in each of the 20 plots for this trial were rated for white mold incidence on 27 August, by which time the male line had been removed and the female line was senescing. In each plot, 200 contiguous plants in the center-most two female rows were rated for white mold incidence, with rating completed for plants located at least 3 m from the ends of the plot, for a total of 400 plants/plot. Each plant was rated for the presence or absence of Sclerotinia basal stalk rot, midstalk rot, and head rot, and the incidence (%) of plants with each type of symptom was calculated for each plot. The total incidence of white mold was estimated by adding the incidences of Sclerotinia basal stalk rot, midstalk rot, and head rot since infection of any one plant at more than one of the three locations was minimal to non-existent.

Statistical analysis of the data was performed in R using the *aov* procedure for analysis of variance (ANOVA), and descriptive statistics (means, ranges, and standard errors) were calculated in Excel. Replications (blocks) were treated as random effects and fungicide

treatments as fixed effects in the ANOVA model. For variables with a significant treatment effect, means were compared using Tukey's honestly significant difference (HSD) at $P < 0.05$.

2016 trial. The 2015 trial was repeated in 2016 in the northwest quadrant of a 42.5-ha, center pivot-irrigated, hybrid sunflower seed crop near Ephrata, WA (Township 19 N, Range 26 E, Section 20, southwest quadrant = 47° 7'19.81"N 119°35'35.70"W). The soil type was a Quincy fine sand (USDA NRCS 2016). The cropping history of the field was pea (*Pisum sativum*) in 2013, wheat in 2014, and carrot (*Daucus carota*) seed in 2015. The trial design, treatments, and dimensions were the same as the 2015 trial, except that each fungicide treatment was applied pre-bloom on 12 July, at 50% bloom on 26 July, and at petal fall on 6 August. The whole field, including plots, alleys, and borders, was planted after strip tillage of the field, with proprietary male and female parent lines of an oilseed-type cultivar planted in an 8:2 female:male ratio of rows on 11 May, when one of the two male rows in each pair of male rows was planted. The second male row of each pair of male rows was planted on 18 May. The seeding depth was 2.5 cm, inter-row spacing was 55 cm, and intra-row spacing was 36 cm. The corners of the plots were marked with flags on 17 May.

Management of the sunflower seed crop included typical grower production practices. The field was fertilized, beginning on 6 June, with five successive chemigated applications of 32-0-0 for a total of 112 kg N/ha. The herbicide Eptam (87.8% s-ethyl dipropylthiocarbamate; Gowan, Yuma, AZ) was applied at 3.51 liters/ha post-planting on 19 May by chemigation, and Beyond (12.1% ammonium salt of imazamox; BASF Corp.) was applied at 0.29 liters/ha on 8 June using the 30.5 m-wide, tractor-mounted spray boom. Contans WG was applied to the whole field on 26 May, after seedling emergence, followed by irrigation; and again on 5 July after dammer-diking, with the exception of the treatment plots in this trial as well as specific

plots in the 2016 foliar fungicide applications + Contans WG trial in the southwest quadrant of the field, described below. The rates of Contans WG applications were 0.84 kg/ha on 26 May and 0.56 kg/ha on 5 July, with each application made in 374 liters water/ha using the 30.5-m wide, tractor-mounted, spray boom. The field was dammer-diked on 21 June to reduce run-off of water. The insecticide Warrior (11.4% lambda-cyhalothrin; Syngenta Crop Protection, Inc.) was applied by aeroplane on 16 July at 0.14 liters/ha, and Belt was applied on 21 July by chemigation at 0.29 liters/ha to control the sunflower moth. In addition, the fungicides Priaxor Xemium Brand Fungicide, Endura, and Topsin 4.5FL were applied at 0.58 liters/ha on 12 July 2016, 0.56 kg/ha on 26 July 2016, and 1.83 liters/ha on 6 August 2016, respectively, in 374 liters water/ha to the heads and foliage of sunflower plants in the whole field using the same tractor-mounted spray boom, with the exception of plots in this trial as well as specific plots in the 2016 foliar fungicide application + Contans WG trial described below. The desiccant Gramoxone SL (30.1% paraquat dichloride; Syngenta Crop Protection, LLC) was applied by aeroplane on 6 September 2016, preceding harvest.

Since greater soil moisture can be expected in areas of a field with lower altitude ('valleys'), and taller plants potentially result in a more humid crop canopy and greater soil moisture, the relationship between counts of apothecia with plot altitude as well as plant height were determined. Apothecia were counted on 10 August between two female rows in each of six sections, each 3 m long, between the centermost female rows for a total area of 10.2 m^2 in each plot. Each section was located at least 6 m from the edge of the plot to minimize the risk of interplot interference, with 3 m between adjacent sections measured in the same plot. The altitude of the soil in each plot was calculated using Google Earth Pro Version 7.1.7.2600 from a plot map created using GPS coordinates of the trial. Four altitude measurements were taken in

4.6 m-increments in the center rows of each plot, at least 6 m from the edges of the plot. Normalized altitude was then calculated by subtracting the minimum altitude measured from the mean altitude measured for all 20 plots. The height of sunflower plants was estimated to the nearest 0.15 m over a 10.2 m^2 area in each of the 20 plots on 10 August 2016.

Sunflower plants of the female line in each of the 20 plots were rated for white mold incidence on 13 September 2016, as described for the 2015 trial. By this date the male line had been swathed, the female line had reached physiological maturity and a desiccant had been applied. On 13 September 2016, 10 sunflower heads of the female line were harvested from each plot by cutting the head off every fifth plant from one of the centermost female rows that had been rated for white mold incidence. The sunflower heads were stored at 30 ± 2°C until the seed moisture level was <9% as measured using the sunflower-oilseed setting on an agraTronix MT-16 Grain Moisture Tester (AgraTronix, Streetsboro, Ohio). The seed was then harvested manually, and sized using seed cleaning screens with circular perforations of 7.9 mm and 4.8 mm diameter, corresponding to the maximum and minimum seed size retained, respectively. On 14 October, a seed germination assay was set up for the seed harvested from each plot to assess potential phytotoxicity of the foliar fungicide treatments. For each plot, 100 seed were placed on moist blotter paper (heavy weight germination paper, 76 #, Anchor Paper Co., St. Paul, MN) and incubated at 20^{o}C for 7 days in the dark in accordance with the blotter protocol of the Association of Official Seed Analysts (2008). Germination counts were made after 4 and 7 days, and the incidence of non-germinated, decayed, and abnormally germinated seed counted at 7 days. Following observation of white mycelium and sclerotia on a few seed during the germination assay, the germination assay was repeated on 4 November to quantify the number of seeds infected with *S. sclerotiorum* after 7 days. Statistical analyses of the data were performed

as described for the 2015 trial, with the addition of calculating Pearson's correlation coefficients for apothecial counts, normalized plot altitude, and average plant height.

Foliar fungicide application + broadcast Contans WG trials. ***2015 trial.*** A second field trial was conducted in the southeast quadrant of the same field near Ephrata as the 2015 foliar fungicide application trial, to determine the relative contribution of applications of Contans WG for control of basal stalk rot caused by *S. sclerotiorum*, and a foliar fungicide application program for control of Sclerotinia head rot and midstalk rot. The foliar fungicide program included sequential applications of: 1) Priaxor Xemium Brand Fungicide applied pre-bloom, 2) Endura applied at 10% bloom, and 3) Topsin 4.5FL applied at 80% bloom as described above for the general grower practices to the whole field. The trial was set up as a RCB design with five replications of a 2 x 2 factorial treatment design: i) no application of Contans WG and no foliar fungicide applications (control treatment); ii) a broadcast application of Contans WG at 0.84 kg/ha in 374 liters water/ha on 11 May, three days after planting, followed by irrigation; iii) the foliar fungicide application program described above of Priaxor Xemium Brand Fungicide at 0.58 liters/ha on 18 June, Endura at 0.56 kg/ha on 15 July, and Topsin 4.5FL at 1.83 liters/ha on 29 July; and iv) the combination of Contans WG application and the foliar fungicide application program. Foliar fungicide applications were each done in 374 liters water/ha with 89 ml Syl-Tac/ha with the same tractor-mounted spray boom used in the 2015 foliar fungicide application trial. Plot and border dimensions, and grower production practices were as described for the 2015 foliar fungicide application trial. White mold incidence ratings were completed on 27 August 2015, and statistical analysis of the data were completed as described for the 2015 foliar fungicide application trial.

2016 trial. The trial was repeated in 2016 in the southwest quadrant of the same field as the 2016 foliar fungicide application trial. The trial design, treatments, and dimensions were the same as for the 2015 trial, except that Priaxor Xemium Brand Fungicide was applied pre-bloom on 12 July 2016, Endura was applied at 50% bloom on 26 July 2016, and Topsin 4.5FL was applied at petal fall on 6 August 2016; and plots were treated with Contans WG at 0.84 kg/ha on 26 May after seedling emergence, and again at 0.56 kg/ha on 5 July 2016, after dammer-diking, followed by irrigation. Both Contans WG applications were made in 374 liters water/ha with the same tractor-mounted spray-boom. Foliar fungicide applications were each done using 374 liters water/ha with 89 ml Syl-Tac/ha applied with the tractor-mounted spray-boom. Plot and border dimensions, and grower production practices were as described for the 2016 foliar fungicide application trial. Apothecia counts, plant height, normalized altitude measurements, sunflower white mold incidence ratings, and statistical analysis were completed as described for the 2016 foliar fungicide application trial.

Combined analysis of 2016 Ephrata field trials. Analysis of the relationship between aerial white mold incidence (head rot plus midstalk rot), basal stalk rot incidence, apothecia counts, plant height, and normalized plot altitude was completed for data combined from both of the 2016 trials in order to increase the statistical power with 40 plots (vs. 20 plots for each trial) for calculating Pearson's correlation coefficients. Descriptive statistics were calculated in Excel.

Banded vs. broadcast Contans WG application trials. ***2015 trial.*** A field trial was carried out in 2015 to determine the efficacy of banded vs. broadcast applications of Contans WG for control of basal stalk rot caused by *S. sclerotiorum* in sunflower seed crops. The trial was established at planting on 7 May in the northwest quadrant of a 54-ha, center pivot-irrigated, hybrid sunflower seed crop located near Odessa, WA in Township 24, Range 32, Section 34

southeast quadrant (47º 31’611” N and 118º 46’147”W). The soil type was a Ritzville silt loam (USDA NRCS 2016). The cropping history of the field was wheat in 2012, garbanzo bean (*Cicer arietinum*) in 2013, and wheat in 2014.

The trial was set up as a RCB design with five replications. Four Contans WG treatments were applied to the plots in each replication: i) no application of Contans WG (control treatment); ii) a 15-cm-wide banded application of Contans WG over each sunflower row at 3.36 kg/ha in 61 liters water/ha at planting on 7 May; iii) two post-emergence broadcast applications of Contans WG, one at 0.84 kg/ha on 2 June, and the other at 0.56 kg/ha on 20 June after dammer-diking (each applied in 187 liters water/ha with a 30.5 m-wide tractor-mounted, spray boom); and iv) a combination of the banded and broadcast treatments (all three Contans WG applications). The dimensions of plots and borders were as described above for the other field trials. The whole field, including plots, alleys, and borders, was planted with proprietary male and female parent lines of a confection-type cultivar in a 6:2 female:male ratio of rows on 7 May, when the female rows and the first male row in each pair of male rows were planted; the second male row of each pair was planted on 12 May. The seeding depth was 2.5 cm, inter-row spacing was 76 cm, and intra-row spacing was 19 cm.

Management of the sunflower seed crop by the grower included typical production practices for this region. The field was prepared twice for planting with a disk and packer. The field was then fertilized on 1 May 2015, prior to planting, with 112 kg N/ha applied as 32-0-0 by chemigation; included in the application were the herbicides Dual Magnum (83.7% s-metolachlor; Syngenta Crop Protection, Inc.) at 1.56 liters/ha and Prowl (38.7% pendimethalin; BASF Corp.) at 2.34 liters/ha for pre-emergent weed control. The field was fertilized again with 36.5 kg N/ha applied as 32-0-0 by chemigation on 8 June, and dammer-diked on 20 June to limit

run-off of water. Two fungicide applications were made by the grower to the three quadrants of the field in which the trial was not located: Priaxor Xemium Brand Fungicide was applied at 0.58 liters/ha on 26 June, and Endura at 0.58 liters/ha on 20 July, both by chemigation in 2.58 ha-mm of water. Warrior was applied aerially at 0.14 liters/ha on 10 July, and Belt was applied by chemigation at 0.29 liters/ha on 20 July for control of sunflower moth.

Sunflower plants of the female line in each of the 20 plots were rated for white mold incidence on 28 August 2015, as described for the 2015 field trials, by which time the male line was removed and the female line was senescing. Statistical analyses of the data were done as described above for the other two 2015 field trials.

2016 trial. The repeat trial was carried out in a wedge-shaped, 6.1-ha sunflower seed crop located in the northwest quadrant of a 235-ha, center-pivot irrigated field near Odessa, WA (Township 24, Range 32, Section 35 northwest quadrant = 47°31'49.92"N and 118°45'10.90"W). The soil type was a Ritzville silt loam (USDA NRCS 2016). The cropping history of the field was wheat in each of 2013, 2014, and 2015. The trial was very similar to the 2015 trial, except plots were located only 6.1 m apart in the north-to-south direction, and only three treatments were included because of the limited size of the field: i) no application of Contans WG (control treatment); ii) a 15-cm-wide banded application of Contans WG over each row at 3.36 kg/ha in 61 liters water/ha at planting on 9 May when the second set of male rows was planted; and a second banded application at the same rate on 18 May when the female rows were planted; iii) two broadcast applications of Contans WG, a post-emergence application at 0.84 kg/ha in 187 liters water/ha on 15 June, and an application following dammer-diking of the field at 0.56 kg/ha in 187 liters water/ha on 1 July. The whole field, including plots and borders, was planted with proprietary male and female parent lines of a confection-type cultivar in a 6:2 female:male ratio

of rows, the first set of male rows was planted on 4 May, the second set on 9 May, and the female rows on 18 May. The seeding depth was 1.9 cm, inter-row spacing was 76 cm, and intra-row spacing was 19 cm. At the time of planting the second male row, the corners of each plot were marked with flags. The Contans WG banded applications were made to the whole field as described above, except for the control plots and the plots that received the broadcast Contans WG treatments. The whole field was dammer-diked by the grower-cooperator on 30 June 2016.

Management of the sunflower seed crop by the grower included similar practices as described for the 2015 trial. The field was prepared twice for planting with a disk and packer, and a fertilizer application of 112 kg N/ha was made and incorporated with the tillage operation on 27 April. In addition, the field was fertilized, beginning on 22 June, with three weekly chemigated applications of 32-0-0, for a total of 34 kg N/ha. On 2 May, Dual Magnum was applied at 1.56 liters/ha and Prowl at 2.34 liters/ha by chemigation for pre-emergent weed control. Beyond was applied at 0.29 liters/ha on 13 June for post-emergent weed control using a 30.5 m-wide, tractor-mounted, spray boom. Warrior was applied aerially at 0.14 liters/ha on 30 July, and Belt was applied by chemigation at 0.29 liters/ha on 4 August to control the sunflower moth. Gramoxone SL was applied aerially at 2.34 liters/ha on 30 September to desiccate the crop for harvest.

Sunflower plants of the male line were rated in all 15 plots on 9 August, based on evidence from prior years that the male line in this hybrid crop was more susceptible to white mold than the female line (Bill Wirth, Precision Seed Production, LLC, *personal communication*). In addition, sunflower plants in the female line in each of the 15 plots were rated for white mold incidence on 19 September, as described above, by which time the male line had been removed and the female line was senescing. In each plot at each rating date, 200

contiguous plants of the center-most male or female rows were rated for white mold, at least 3 m from the ends of the plot to avoid interplot interference, for a total of 400 plants rated for each parent line/plot. Statistical analyses were completed as described above.

Ascospore trapping. ***2015 trials.*** A Burkard 7-day volumetric spore trap (Burkard Scientific Ltd., Uxbridge, UK) was placed in a border region within each of the 2015 field trials: i) the foliar fungicide application trial near Ephrata, ii) the foliar fungicide application + broadcast Contans WG trial near Ephrata, and iii) the Contans WG banded vs. broadcast application trial near Odessa. The spore traps were used to monitor the timing of ascospore release of *S. sclerotiorum* in these sunflower seed crops. A 100:18 (w:w) mixture of petroleum jelly and paraffin wax was used to coat the Melinex spore trap tape after placing two spore traps in the Ephrata field on 17 June. The Melinex tape was replaced on 23 June, and every seven days thereafter until 11 August, when the spore traps were removed from the field as the female rows were senescing and the male rows had been removed. Similarly, a spore trap was placed in the field trial near Odessa on 24 June, and the Melinex tape was replaced on 30 June and every seven days thereafter until 11 August, when the spore trap was removed from the field. The Melinex tape from each weekly sampling for each of the three spore traps was cut into daily sections within 24 to 48 h of collection, and the sections stored at -20^{o}C. However, almost no aerial white mold infections were detected in the three 2015 field trials (<1% incidence of midstalk rot + head rot), so the tape sections were not tested.

2016 trials. A Burkard 7-day volumetric spore trap was placed into a border region on 1 July within each of three sunflower seed crops in the Columbia Basin: i) the 2016 foliar fungicide trial near Ephrata; ii) the 2016 Contans WG banded vs. broadcast trial near Odessa; and iii) a 2016 rill-irrigated, hybrid sunflower seed crop near Quincy (47°15'4.49"N and

119°41'54.30"W) that had been cropped to corn (*Zea mays*) in 2013, beans in 2014, and nursery tree fruit rootstock (Rosaceae species) in 2015. The Melinex tape was replaced on 5 July and again at 7-day intervals, except for a 6-day interval between 23 and 29 August. The spore traps were removed from the Ephrata, Odessa, and Quincy field trials on 23 August, 5 September, and 16 August 2016, respectively. The Melinex tape from each weekly sampling was cut into 12-h sections within 24 to 48 h of collection, and the tape sections stored at -20°C. Because aerial infections only occurred at significant levels in one of the three sunflower seed crops, the foliar fungicide application trial near Ephrata (see Results below), only the Melinex tape sections from the spore trap at that location were used to assess the timing of ascospore release by *S. sclerotiorum*. In addition, near each spore trap, three random sections between two rows of sunflowers (each section 3 m long for an area of 1.7 m^2/section), were surveyed weekly for the presence of apothecia throughout the period the spore traps were in each of the three fields.

Detection of spores from Melinex tape. Detection of the timing of ascospore release was estimated in each of the three fields in 2016 in which spore traps were placed, based on extraction of DNA from ascospores of *S. sclerotiorum* adhering to the tape. DNA was extracted from each 12-h section of Melinex tape as described by Freeman et al. (2002), and eluted into 20 µl of TE buffer. The DNA extract from each tape section was then tested with the real-time PCR assay developed by Ziesman et al. (2016), which targets a 70 bp fragment of the SSIG_00263 gene using the primer set SSBZF (forward primer) and SSBZR (reverse primer), and a hydrolysis probe, SSBZP. The PCR setup and the conditions of each reaction were as described by Ziesman et al. (2016), except that the reactions were carried out in 0.1 ml polypropylene PCR strip tubes (Axygen, Union City, CA) and run on a Corbett Rotor-Gene 6000 thermal cycler (Qiagen, Germantown, MD), with results analyzed using the software Rotor-Gene Q Pure

Detection Version 2.3.1 (Qiagen). A quantification cycle (C_q) value, as defined by Bustin et al. (2009), of <2 above the mean C_q value for 4.0 x 10^{-4} ng of genomic DNA in the DNA standard curve (described below), was considered a positive detection of *S. sclerotiorum* DNA from each 12-h tape section.

Specificity of the real-time PCR assay. The specificity of the real-time PCR assay had been tested against several species of fungi closely related to *S. sclerotiorum*, including *S. minor*, *S. trifoliorum*, and *B. cinerea*, as well as seven isolates of *S. sclerotiorum*, in the study by Ziesman et al. (2016). Therefore, the assay was only tested for specificity against 12 *S. sclerotiorum* isolates collected from the Columbia Basin in the fall of 2015 from two hybrid sunflower seed crops: isolates OD 31-1, OD 39-3, and OD 40-2 were from a seed crop near Odessa, WA; and isolates EP 2-3, EP 4-1, EP 17-1, EP 31-2, EP 33-2, EP 33-3, EP 35-1, EP 35-2, and EP 35-3 were obtained from a seed crop near Ephrata, WA.

Sensitivity of the real-time PCR assay. The limit of detection (LOD) of the real-time PCR assay was determined using 10-fold serial dilutions of genomic DNA of *S. sclerotiorum* isolate OD 40-2. Each DNA dilution series was run in duplicate, the dilution series was tested two times, and the LOD was calculated as the lowest quantity of DNA that could be detected with a standard deviation of the mean C_q of <0.5 for duplicate samples within each run. With each run of the real-time PCR assay, a standard curve was calculated from a duplicate, standard, 10-fold dilution series of DNA from 4.0 to 4.0 x 10^{-4} ng; and the efficiency and coefficient of determination (R^2) were calculated for each run (Bustin et al. 2009). The reproducibility of the standard curve was assessed by calculating the standard deviation at each dilution point for four replications of separate runs of the assay. An average standard deviation <0.5 for the average C_q values over the four replications indicated the assay was reproducible.

To quantify the minimum number of ascospores of *S. sclerotiorum* that could be detected on the Melinex tape using the real-time PCR assay, two 10-fold dilution series of spore suspensions were made, each from 10^5 to 1 spores/µl, and 10 µl of each dilution from each series were pipetted onto Melinex tape coated the same mounting medium used on the tape in the spore traps in the fields. The tape and spores for each dilution were then placed into a 1.7 ml microcentrifuge tube. In addition, a separate 10 µl aliquot of each spore suspension dilution was pipetted into a separate 1.7 ml microcentrifuge tube. DNA was then extracted from the contents of each tube, as described above, and 4 µl of extracted DNA subjected to the real-time PCR assay along with a duplicate, 10-fold dilution series of DNA from 4.0 ng to 4.0 x 10^{-4} ng. The LOD for spores using the real-time PCR assay was based on the minimum number of spores for which extracted *S. sclerotiorum* DNA was detected with a $C_q <$ (C_q for the 4.0 x 10^{-4} ng of genomic DNA of the standard curve) + 2.

Field trial microclimate. ***2015 trials.*** A WatchDog air temperature and relative humidity sensor, as well as a WatchDog leaf wetness sensor (Spectrum Technologies, Aurora, IL), and with two 10HS large soil moisture sensors (Decagon Devices, Inc., Pullman, WA) were used to track hourly air temperature, relative humidity, and leaf wetness in the sunflower canopy as well as soil volumetric water content (latter at a depth of 7.6 to 12.7 cm), respectively. This sensor suite was placed into a border region of the trial near each of the spore traps described above, i.e., within the 2015 foliar fungicide application + Contans WG trial on 17 June, and in the Contans WG banded vs. broadcast trial near Odessa on 24 June 2015, prior to canopy closure (when leaves of the sunflower plants from adjacent rows overlapped). The sensor suites were removed from each of the fields on 11 August, and the microclimate data analyzed using Excel (means, range, standard errors, etc.).

2016 trials. In 2016, the sensor suites were augmented with a soil temperature sensor placed at a depth of 10 cm. A sensor suite was placed near the spore trap at the 2016 field sites near each of Ephrata, Odessa, and Quincy, as described above for the spore trapping sites, just prior to canopy closure. The sensors were removed from the fields near Ephrata, Odessa, and Quincy on 23 August, 5 September, and 16 August 2016, respectively. The air temperature, relative humidity, leaf wetness, and soil temperature data from the sensors at the 2016 trial near Ephrata could not be downloaded as a result of problems with the datalogger. Therefore, to supplement the soil volumetric water content data collected at that site, air temperature, relative humidity, leaf wetness, and soil temperature measurements (latter at a depth of 5 cm) were obtained from the Ephrata weather station of the Washington State University AgWeatherNet system (WSU AgWeatherNet 2016). Data were analyzed using Excel as described above for the 2015 sunflower seed crop microclimate data recorded.

3.3. Results

Foliar fungicide application trials. ***2015 trial.*** Total white mold incidence averaged 1.9 ± 0.5% (mean ± standard error) over all 20 plots in the 2015 foliar fungicide trial, with a range from 0 to 7.0%/plot. This was equivalent to the basal stalk rot incidence because midstalk rot or head rot were not detected on any of the 8,000 plants rated for white mold in the trial. Although, the effects of foliar fungicide applications on basal stalk rot incidence was not significant (P = 0.4290) (Table 3.1), the very limited incidence of basal stalk rot and complete lack of both midstalk rot and head rot precluded differentiation of the effects of the fungicide treatments on white mold in this sunflower seed crop trial.

2016 trial. Total white mold incidence averaged 7.5 ± 1.2% over all 20 plots in the 2016 foliar fungicide trial, and ranged from 1.5 to 21.8%/plot, with an average of 4.9 ± 0.5% basal stalk rot incidence (1.5 to 11.8%/plot), 0.6 ± 0.3% midstalk rot incidence (0 to 4.5%/plot), and 2.1 ± 0.7% head rot incidence (0 to 9.5%/plot) for the 8,000 plants rated for white mold in this trial. As in 2015, the ANOVA revealed no significant foliar fungicide application effects on basal stalk rot incidence ($P = 0.1174$), midstalk rot incidence ($P = 0.1478$), head rot incidence ($P = 0.2278$), aerial (midstalk rot + head rot) disease incidence ($P = 0.1999$), or total white mold incidence ($P = 0.1072$) (Table 3.1). However, as in the 2015 trial, the very limited amount of white mold in this 2016 field trial prevented effective differentiation of fungicide treatment effects on the various measurements of white mold.

Seed germination assays completed to assess potential phytotoxic effects of the fungicides Topsin 4.5FL, Rovral Brand 4 Flowable Fungicide, and Omega 500F on sunflower seeds harvested from plots in the 2016 trial revealed no adverse effects of any of the treatments on seed quality (Table 3.2). The incidence of seeds with normal germination, abnormal germination, decay, and no germination averaged 87.1 ± 1.3, 5.9 ± 0.6, 1.5 ± 0.4, and 5.6 ± 1.2% across all 20 plots for the first germination assay; and 87.0 ± 1.3, 5.6 ± 0.7, 6.6 ± 1.1, and 1.0 ± 0.3% for the second assay. In addition, the incidence of seed on which *S. sclerotiorum* developed averaged 0.3 ± 0.3% in the second germination assay (this was not recorded in the first assay). Although there was a significant main effect of foliar fungicide treatments on normal seed germination ($P = 0.0012$) in the first assay, with significantly more normally germinated seed harvested from the Rovral Brand 4 Flowable Fungicide plots (92.2 ± 1.9%) than normally germinated seed harvested from the Topsin 4.5FL plots (82.2 ± 2.4%), none of the fungicide treatments reduced normal seed germination significantly compared to that of seed

harvested from the control plots (86.6 ± 1.6%) (Table 3.2). The main effect of foliar fungicide applications also was significant for the incidence of non-germinated seed (P = 0.0497) in the first assay, with significantly fewer seed not germinated for the Rovral Brand 4 Flowable Fungicide plots (2.2 ± 0.7%) compared to the Topsin 4.5FL plots (9.0 ± 3.8%); however, none of the fungicide treatments affected the percentage of non-germinated seed significantly compared to non-germinated seed harvested from the control plots (5.0 ± 2.2%) (Table 3.2). Similarly, foliar fungicide treatments had no significant effects on the incidence of seed with normal germination, abnormal germination, decay, no germination, or infection with *S. sclerotiorum* in the second germination assay (Table 3.2).

Foliar fungicide application + broadcast Contans WG trials. ***2015 trial.*** Total white mold incidence averaged 4.4 ± 1.0% for the 20 plots in the 2015 foliar fungicide + Contans WG trial (ranging from 0 to 14.3%/plot), which was equivalent to the basal stalk rot incidence because midstalk rot and head rot were not detected on any of the 8,000 plants rated for white mold. The very limited incidence of basal stalk rot precluded assessment of whether the Contans WG applications, foliar fungicide applications, or the interaction of these treatments had significant effects on basal stalk rot incidence (P = 0.5942, 0.9305, and 0.1265, respectively) (Table 3.3).

2016 trial. Total white mold incidence averaged 11.5 ± 2.1% for all 20 plots in the 2016 trial evaluating both Contans WG applications and foliar fungicide applications for control of white mold, with a range from 1.8 to 30.5%/plot, and an average of 5.2 ± 0.8% basal stalk rot incidence (1.3 to 12.0%/plot), 1.0 ± 0.2% midstalk rot incidence (0 to 3.5%/plot), and 5.3 ± 1.3% head rot incidence (0 to 16.8%/plot) for the 8,000 plants rated for white mold. Neither the Contans WG treatments or the foliar fungicide program had a significant effect on basal stalk rot

incidence (P = 0.3153 and 0.7329, respectively), midstalk rot incidence (P = 0.6510 and 0.8797, respectively), head rot incidence (P = 0.5731 and 0.6547, respectively), aerial (midstalk rot + head rot) disease incidence (P = 0.6905 and 0.6756, respectively), or white mold incidence (P = 0.9118 and 0.6644, respectively), although there was a significant interaction between Contans WG treatments and fungicide treatments on basal stalk rot incidence (P = 0.0439) (Table 3.3). However, there was no significant difference in basal stalk rot incidence in the nontreated control plots compared to plots treated with Contans WG only, the foliar fungicide program only, or the combination of both sets of treatments (Table 3.3).

Combined analysis of 2016 Ephrata field trials. Normalized altitude of the plots in the 2016 field trials were significantly negatively correlated with the incidences of aerial white mold (midstalk rot + head rot) (r = -0.620 at P <0.0001) and basal stalk rot (r = -0.618 at P <0.0001), apothecia counts (r = -0.452 at P = 0.0034), and plant height (r = -0.396 at P = 0.0115), reflecting the fact that incidence of aerial white mold, basal stalk rot, apothecia counts, and plant height were less in plots with higher elevation in the two trials ('peaks') vs. plots with lower elevations ('valleys') (Table 3.4). In contrast, significant positive correlations were calculated between plant height and aerial white mold incidence (r = 0.408 at P = 0.0090), basal stalk rot incidence (r = 0.409 at P = 0.0088), and apothecia counts (r = 0.367 at P = 0.0199) (Table 3.4). Apothecia counts and aerial white mold incidence were also significantly positively correlated (r = 0.476 at P = 0.0019), demonstrating a strong relationship between apothecia as the source of ascospores and midstalk rot + head rot. In addition, apothecia counts were significantly positively correlated with basal stalk rot incidence (r = 0.386 at P = 0.0138) (Table 3.4), indicating that apothecia counts reflected the sclerotial density in close enough proximity to sunflower plants to initiate basal stalk infections. This was further demonstrated by the

significant positive correlation between aerial white mold incidence and basal stalk rot incidence (r = 0.559 at $P = 0.0002$) (Table 3.4).

Banded vs. broadcast Contans WG application trials. ***2015 trial.*** Total white mold incidence averaged 4.2 ± 1.0% over all 20 plots in the 2015 trial examining banded vs. broadcast application of Contans WG for control of basal stalk rot , with a range from 0 to 14.3%/plot. This was similar to the basal stalk rot incidence as only two aerial infections were detected (both plants with midstalk rot) out of the 8,000 plants rated for white mold in the trial. Neither the banded nor the broadcast Contans WG treatments had a significant effect on basal stalk rot incidence ($P = 0.8065$ and $P = 0.3137$), and the interaction term between the two types of applications of Contans WG was not significant ($P = 1.0000$ for Friedman's rank analysis). However, the very limited amount of white mold in this trial basically precluded the ability to differentiate the effects of the two types of Contans WG applications on white mold.

2016 trial. Only 13 of 6,000 male sunflower plants (0.22%) rated in the banded vs. broadcast Contans WG trial near Odessa in 2016 displayed symptoms of white mold, with all 13 having basal stalk rot. Similarly, only 27 of 6,000 female sunflower plants (0.34%) rated in the 2016 trial had symptoms of white mold, 23 of which had basal stalk rot. Since the incidence of basal stalk rot was extremely minimal, an ANOVA was not calculated to assess the effectiveness of the banded vs. broadcast applications of Contans WG at preventing basal stalk rot.

Ascospore trapping. The real-time PCR assay enabled the detection of DNA from 11 of the 12 *S. sclerotiorum* isolates (91.7%) tested that were obtained from sunflower seed crops in the Columbia Basin in 2015. Surprisingly, DNA of isolate OD 39-3 was not detected by the real-time PCR assay, suggesting the assay is not robust enough to detect all isolates of this pathogen. The limit of detection (LOD) of extracted DNA of *S. sclerotiorum* for the real-time

PCR assay was 4.0 x 10^{-4} ng, as the standard deviation of the mean C_q was <0.5 for the 4.0 x 10^{-4} dilution of the standard curves calculated from duplicate runs of the assay. The efficiency was >90 and the R^2 was >0.99 for each real-time PCR assay run (Fig. 3.1A). None of the points on the standard curves had a standard deviation >0.5, indicating the assay was reproducible (Bustin et al. 2009). The LOD of spores of *S. sclerotiorum*, based on DNA extracted from spores placed onto Melinex tape coated with adhesive, was 10^4 spores/12-h section of tape; and the LOD of DNA extracted from a spore suspension of *S. sclerotiorum* was 10^4 spores/200 µl molecular grade water. However, the real-time PCR reactions using DNA extractions from 10^5 spores/12-h section of tape and 10^5 spores/200 µl molecular grade water did not result in consistently lower C_q values than when using 10^4 spores/12-h section of tape and 10^4 spores/200 µl of water, respectively (*data not shown*). Therefore, the concentration of spores of the pathogen could not be quantified effectively using C_q values with this real-time PCR assay. As a result, the real-time PCR assay was only used to determine the presence or absence of ascospores of *S. sclerotiorum* on each 12-h section of tape.

Based on very limited detection of midstalk rot and head rot in the three 2015 field trials in which the Burkard spore traps were located, the timing of ascospore release could not be determined in any of those trials. In 2016, aerial white mold incidence was >1.0% in only one of the three field sites in which spore traps were located, i.e., the field located near Ephrata in which the foliar fungicide application trial was carried out. In addition, apothecia only were observed in that field out of the three fields monitored for apothecia and ascospore release in 2016. Apothecia were observed on the soil surface near the location of the spore trap in that field. Therefore, only the tape from the Burkard spore trap in that trial was used to assess the timing of ascospore release. Real-time PCR assays of DNA extracted from each 12-h section of

tape revealed the presence of ascospores on the tape sections from 14 July to 10 August 2016, and was corroborated by the observation of apothecia in this field from 26 July to 9 August (Fig. 3.3B). This period of detection of DNA of the white mold pathogen on the spore traps spanned the crop developmental stages from pre-bloom (late R3), flowering (R4 and R5 from approximately 19 to 31 July), petal fall, and the beginning of seed set (R6) (Schneiter and Miller 1981).

Field trial microclimate. In the field trial sites in both 2015 and 2016, the crop canopies were relatively warm with limited hours over which the air in the canopy was near saturation (RH >90%) and very limited periods of leaf wetness >0 (Fig. 3.2 and Fig. 3.3). For the months of July and August in the 2015 field locations, the average daily air temperature, average number of hours/day with RH >90%, and average number of hours/day of leaf wetness >0 were: 20.9 and 21.1°C, 6.7 and 4.8 h/day, and 3.2 and 3.9 h/day, respectively, in the 2015 foliar fungicide + Contans WG trial near Ephrata; and 20.1 and 19.8°C, 0.2 and 0.2 h/day, and 3.2 and 2.4 h/day, respectively, in the 2015 Contans WG banded vs. broadcast trial near Odessa. For the months of July and August in the 2016 field locations in which microclimate was monitored, the average air temperature, average number of hours/day with RH >90%, and average number of hours/day of leaf wetness >0 were: i) 18.9 and 18.1°C, 5.1 and 5.8 h/day, and 3.7 and 11.0 h/day, respectively, for the 2016 Contans WG banded vs. broadcast trial near Odessa; ii) 21.8 and 21.3°C, 0.4 and 0.2 h/day, and 2.0 and 3.5 h/day, respectively, for the rill-irrigated sunflower seed crop near Quincy; and iii) 21.8 and 22.5°C, 0.5 and 0 h/day, and 1.8 and 0.6 h/day, respectively, for the 2016 foliar fungicide trial. For the 2016 foliar fungicide trial, air temperature, RH, and leaf wetness measurements were collected from the WSU AgWeatherNet Station near Ephrata due to

datalogger failure for the sensor suite in that field, i.e., these data do not reflect the microclimate of that sunflower seed crop canopy.

Soil temperature in the 2016 banded vs. broadcast Contans WG trial near Odessa averaged 18.2 and 17.1^{o}C in July and August, respectively; and in the 2016 sunflower seed crop near Quincy, soil temperature averaged 20.5 and 20.6^{o}C in July and August, respectively. The average soil temperatures were ~1^{o} less than air temperatures in each site, but average soil temperatures fluctuated less than average air temperatures, as expected (Fig. 3.3B and 3.3C). In all but one of the five field sites monitored for microclimate conditions in 2015 and 2016, average soil volumetric water content declined from July to August, reflecting the fact that growers reduce the amount of irrigation after the male rows have been cut (Fig. 3.2 and Fig. 3.3). The lack of decline in average soil volumetric water content detected from July to August in the 2016 sunflower seed crop near Quincy reflected both the amount of irrigation water applied by the grower and the fact that the sensors had to be removed before the male rows were destroyed as the grower requested the sensor suite be placed in rows of the male line (Fig. 3.3C).

3.4. Discussion

In the six white mold field trials completed in sunflower seed crops in the Columbia Basin over two years in this study, white mold incidence was very limited, ranging from <1.0 to 11.5 ± 2.1% averaged over all plots in each trial. The limited incidences of white mold, in conjunction with interplot variation in each trial precluded effective assessment of the efficacy of the Contans WG treatments and the foliar fungicide treatments in all six trials. Successful assessment of the efficacy of those practices in the Columbia Basin for control of white mold was precluded despite using a relatively large plot size and five replications of each treatment

combination in each trial over the two seasons. Apothecia were not observed and aerial white mold incidence (midstalk rot + head rot) was <0.1% in all three trials completed in 2015. In 2016, apothecia were observed in two of three trials that season, the foliar fungicide application and foliar fungicide application + Contans WG trials near Ephrata. Aerial white mold incidence in the 2016 foliar fungicide trial was 2.1 ± 0.7%, and based on the real-time PCR assays of DNA extracted from a Burkard spore trap installed in one location in the field, ascospore release extended from 14 July, just prior to the onset of flowering in that crop, to 10 August, approximately 10 days after bloom. Fungicide applications in the trial were made on 12 July (approximately a week prior to bloom), 26 July (approximately 50% bloom), and 6 August (petal fall). Therefore, the face of the heads of the sunflowers, which were not exposed until bloom occurred, would not have been protected by the initial fungicide application, only the second application that occurred after 50% bloom. Coverage of the face of sunflower heads has been demonstrated to be important for optimizing white mold control as head rot most commonly starts as a result of ascospore infection of florets on the face of the head (Gulya and Masirevic 1992).

Plots in the two trials in the 2016 hybrid sunflower seed crop near Ephrata that had lower normalized altitude also had more apothecia of *S. sclerotiorum*, taller plants, and greater incidences of basal stalk rot and aerial white mold. This illustrated that lower areas of fields in which sunflower seed crops are grown are suitable sites for growers to monitor fields for apothecia to detect early risk of white mold compared to higher areas of the fields, and scouting such areas of the crops could be used to optimize the timing of foliar fungicide applications to protect sunflower plants when ascospores are being released. This observation is similar to recommendations for canola crops in the inland Pacific Northwest (Paulitz et al. 2015).

However, the real-time PCR assay detected ascospores on the spore trap almost 2 weeks before apothecia were observed near the spore trap in the 2016 foliar fungicide trial, where the trap was located in a low spot of the field. The spore trap was located >300 m within the hybrid sunflower seed crop, with the orifice of the spore trap below the top of the canopy. Therefore, the ascospores trapped probably originated from within the sunflower crop. The delay in observation of apothecia in the field relative to detection of ascospores by spore trapping and real-time PCR assay suggests a more extensive area should have been surveyed in the field to detect apothecia during earlier periods of conducive microclimatic conditions for carpogenic germination. In addition, more frequent surveys for apothecia than just weekly would have been ideal to provide a more accurate determination of when apothecia formation started.

The field trials in this study were all carried out in grower-cooperator fields using large plots and similarly wide borders between plots, to accommodate the width of the growers' spray booms (30.5 m) and to minimize the risk of interplot interference from ascospore dispersal, since a majority of the ascospores released by apothecia are dispersed in relatively close proximity to the apothecial source (Ben-Yephet and Bitton 1985; Hartill 1980). However, utilizing grower-cooperator field trials negated the ability to take adequate measures to enhance development of white mold, rendering the results inconclusive as the incidence of white mold was too limited to assess the effects of the Contans WG treatments or the foliar fungicide treatments on white mold. A few hybrid sunflower seed crops with >25% incidence of aerial white mold and >10% incidence of basal stalk rot were observed in the Columbia Basin in 2015 and 2016 (see Chapter 2). Despite efforts by the grower-cooperators involved in this study to identify fields with greater risk of white mold for these trials, the trials were completed in fields that had relatively

high value to the growers and contracting seed companies, with reluctance on the part of the growers to attempt to create conditions more conducive to white mold.

Although the very limited amount of white mold precluded assessment of the efficacy of foliar applications of the fungicides Topsin 4.5FL, Rovral Brand 4 Flowable Fungicide, and Omega 500F at controlling white mold in sunflower seed crops in the Columbia Basin, the germination assays of seed harvested from plots treated with these products demonstrated that the fungicide treatments did not have phytotoxic effects on the seed. However, the germination assays also revealed that sunflower seeds harvested from asymptomatic heads can be infected with *S. sclerotiorum*. While only 5 of 2,000 seeds tested in the germination assay for the 2016 foliar fungicide trial were infected with the pathogen, this is not an insignificant incidence of infection considering the plant populations in sunflower crops, ranging from 37,000 to 74,000 plants/ha (Holley and Nelson 1986). Herd and Phillips (1988) showed that seed treatments with the fungicides benomyl, iprodione, procymidone, and vinclozolin were effective at preventing seed transmission of *S. sclerotiorum* from infected sunflower seeds collected from heads that were partially rotted by white mold. Similarly, infection of soybean seed by *S. sclerotiorum* was reduced from 4.0% (of 200 seeds tested) to ≤0.7% by treating the seed with one or more of the fungicides captan, fludioxonil, thiabendazole, or thiram (Mueller et al. 1999). In addition, fludioxonil, thiabendazole, and thiram significantly reduced the number of sclerotia that formed on the soybean seed, i.e., an average of 2 sclerotia formed on 100 seed treated with fludioxonil or thiram compared to 119 sclerotia observed on 100 non-treated seed (Mueller et al. 1999). Therefore, fungicide seed treatments could be effective at preventing seed transmission of *S. sclerotiorum* in sunflower crops. This management tool for white mold has already been incorporated into sunflower seed production in the Columbia Basin, as all of the sunflower stock

seed lots planted in the Columbia Basin in 2016 for hybrid sunflower seed crops were treated with fludioxonil (Bill Wirth, Precision Seed Production, LLC, *personal communication*).

Ascospores were detected from 14 July to 10 August 2016, a 4-week period, in the 2016 foliar fungicide applications trial, while apothecia were observed from 26 July to 9 August, a 2-week period. The former spanned crop developmental stages from approximately a week before bloom, through flowering, petal fall, and the beginning of seed set. This fell within the durations of ascospore dispersal reported by McCartney and Lacey (1991) in a study in sunflower crops in the UK, which ranged from 2 to 6 weeks. Thus, ascospores were present in the 2016 sunflower seed crop in the Columbia Basin for most of the duration over which sunflower plants are most susceptible to head infections, from the beginning of flowering until two weeks after flowering, i.e., 19 July to 14 August in this particular crop (Gulya and Masirevic 1992). The last two of the three foliar fungicide applications during flowering were on the 26 July and 6 August. As a result, the faces of the sunflower heads were not protected by a white mold-efficacious fungicide for the first week of flowering, as the faces of the heads would not have been exposed to the fungicide during the first foliar application made on 12 July, about a ~ week before the onset of flowering. The heads of some sunflower female parent lines, including the female line grown in the 2016 foliar fungicide trial in this study, lean forward into the crop canopy after flowering. This makes conditions more conducive to head rot infection (Gulya and Masirevic 1992) as the crop canopy is more humid and the face of the sunflower head points downwards at an angle, potentially reducing the ability to get thorough coverage of the faces of the heads during fungicide applications after flowering, such as during the third fungicide application made on 6 August at petal fall (growth stage R6) in the 2016 foliar fungicide trial (Schneiter and Miller 1981).

Based on conversations with some sunflower seed growers in the Columbia Basin, Priaxor Xemium Brand Fungicide, with the active ingredients fluxapyroxad and pyraclostrobin, is sometimes applied pre-bloom to try and benefit from the growth enhancing effect that has been reported for strobilurins fungicides such as pyraclostrobin (Nelson and Meinhardt 2011; Vincelli 2002), rather than timing this initial fungicide application for white mold control (Bill Wirth, Precision Seed Production, LLC, *personal communication*). This is not a recommended practice, however, as Vincelli (2002) noted that the use of strobilurin fungicides for growth enhancing effects can increase the selection pressure for fungicide resistance to develop in pathogen populations. Currently, only fungicides belonging to FRAC Groups 3 (e.g., metconazole and tebuconazole), 7 (e.g., boscalid and fluopyram), and 11 (e.g., azoxystrobin and pyraclostrobin) are registered for use in sunflower seed crops in the Columbia Basin. Therefore, this practice of using fungicides for a growth regulator effect could be highly disadvantageous for control of white mold in the multiple crops grown in the Columbia Basin that are susceptible to this disease [Washington State Pest Management Resource Service (WSPMRS) Pesticide Information Center Online (PICOL) 2017].

Assessment of the relationship between microclimatic conditions after sunflower seed crop canopy closure with apothecia formation and ascospore release was hindered in this study by the lack of field sites in which apothecia were observed and ascospores were detected over the two years of field trials. In only one field location out of six over the duration of the study, the 2016 foliar fungicide trial, were apothecia observed and ascospores detected. This trial also was the only location of the six with >1% incidence of aerial white mold. Unfortunately, datalogger failure at that location meant that the only microclimatic data recorded within the crop canopy was soil moisture. The air temperature, relative humidity, soil temperature, and leaf wetness data

from the Ephrata AgWeatherNet station were used as substitute data, but did not approximate microclimatic conditions in the field adequately because that AgWeatherNet site was not exposed to the irrigation events that occurred in the sunflower crop and the station was not located within the crop canopy.

The soil moisture sensors indicated that the field in which the 2016 foliar fungicide trial was completed was maintained at a relatively high soil moisture throughout July, including the 7 to 14 days of high soil moisture required for apothecia formation as reported by Kora et al. (2005) and Nelson and Lamey (2000), starting ~2 weeks prior to ascospores being detected in the field and ~4 weeks prior to apothecia being observed. However, in order to confirm that conclusion and compare the soil moisture levels for carpogenic germination with those reported in previous studies (e.g., Clarkson et al. 2004; Kora et al. 2005; Phillips 1986; Teo and Morrall 1985; Wu and Subbarao 2008) or to the soil moisture status in other field locations and years, soil moisture characteristic curves would need to be developed to convert soil volumetric water content into soil water potential (Brady and Weil 2008). Soil moisture characteristic curves were not developed for the fields sites in this study because of the lack of detection of apothecia, ascospore release, and <1% aerial white mold incidence in all but one field location across both years. In future research, assessment of the relationship between apothecia formation and ascospore release with microclimatic conditions could be enhanced by placing vernalized sclerotia at field sites along with microclimate sensors within lower areas in hybrid sunflower seed crops to quantify microclimatic conditions that are conducive to apothecium development (Clarkson et al. 2007; Phillips 1986; Twengstrom et al. 1998b). This might also improve detection of the initiation of apothecium formation in fields, as ascospores were detected using spore traps and the real-time PCR assay in the 2016 foliar fungicide trial about two weeks before

apothecia were observed in the field. It would also be of benefit to start the monitoring of microclimatic conditions at least several weeks prior to canopy closure; this was not done in this study because of cultivation/dammer-diking operation in the fields that occurred within 2 weeks of canopy closure, preventing the placement of microclimate sensor suites in the fields until after these soil-disturbing activities had been completed.

The microclimatic data recorded in the sunflower seed crop canopies in 2015 and 2016 revealed that the air did not reach saturation (>90% RH) for the majority of each day in July and August. This probably explains why a continuous leaf wetness period of >42 h, which has been reported as necessary for sunflower head infection by *S. sclerotiorum* ascospores, was never recorded in these trials in the Columbia Basin (Gulya and Masirevic 1992), and why the Columbia basin overall has a suitable climate for sunflower seed production with relatively low risk of white mold. However, leaf wetness sensors do not simulate actual periods of leaf wetness adequately (Rowlandson et al. 2015). In addition, the florets on the face of a sunflower head, and the junction of leaf petioles with the stalk (Gulya and Masirevic 1992), which are areas of sunflower plants most susceptible to infection by ascospores, tend to support longer periods of wetness than would be detected on a sensor placed at a 45^{o} angle in the crop canopy. How long ascospores can survive in the sunflower crop canopy is a matter of conjecture, but each field site monitored in 2015 and 2016 averaged >8 h/day of air temperatures >21^{o}C and >35% RH in July (except for the 2016 foliar fungicide trial site for which canopy environmental conditions were not recorded because of datalogger failure). Based on the results of Caesar and Pearson (1983), ascospore survival is not favored for the duration of those conditions.

In summary, the sunflower seed crops in which white mold field trials were completed in this study developed very limited incidences of white mold, which prevented effective

differentiation of treatment effects on white mold. Ideally this research should be done in sunflower trials in which conditions can be made much more conducive for white mold development to assess effectively the efficacy of Contans WG and foliar fungicide applications for in-season control of white mold. However, the study did illustrate several ways growers can potentially increase the effectiveness of foliar fungicide sprays, e.g., by surveying low spots in sunflower seed crops for early detection of the onset of apothecia formation in order to optimize the timing of initiation of foliar fungicide programs; and applying fungicides to the canopy in a manner that improves the coverage of sunflower heads, particularly for parent lines that tend to mature with the heads facing downwards.

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Table 3.1. Analysis of variance (ANOVA) for incidence (%) of basal stalk rot, midstalk rot, head rot, total aerial rot (midstalk rot + head rot), and total white mold (basal stalk rot + midstalk rot + head rot) for 2015 and 2016 foliar fungicide application trials in the Columbia Basin of Washington

	2015 trial[b]	2016 trial				
ANOVA and treatment[a]	**Basal[c]**	**Basal[c]**	**Midstalk[c]**	**Head[c]**	**Aerial[c]**	**Total[c]**
ANOVA						
Block	0.0960[d]	0.3586	0.7276	0.9434	0.9618	0.8708
Fungicide	0.4290	0.1174	0.1478	0.2278	0.1999	0.1072
Transformation[e]	-	-	-	Sq root	Sq root	-
Treatment						
Control	1.85 a[f]	4.90 a	0.40 a	2.05 a	2.45 a	7.35 a
Topsin 4.5FL	2.30 a	5.10 a	0.10 a	2.05 a	2.15 a	7.25 a
Rovral Brand 4 Flowable Fungicide	2.60 a	3.05 a	0.00 a	0.10 a	0.10 a	3.15 a
Omega 500F	0.70 a	6.50 a	1.70 a	4.20 a	5.90 a	12.40 a

[a] Data were analyzed as a randomized complete block design with five replications. Fungicide treatments included nontreated control plots and plots treated with Topsin 4.5FL (thiophanate methyl), Rovral Brand 4 Flowable Fungicide (iprodione), and Omega 500F (fluazinam), each applied pre-bloom, at 10 to 50% bloom, and at 75% bloom to petal fall.

[b] Sclerotinia midstalk rot and head rot were not detected in any plots in the 2015 trial; therefore, basal disease incidence = total white mold incidence for that trial.

[c] Incidence of basal stalk rot, midstalk rot, head rot, aerial white mold (midstalk rot + head rot), total white mold (basal stalk rot + midstalk rot + head rot) out of 400 sunflower plants rated/plot and averaged over five replicate plots/treatment.

[d] Probability of means square > F in the ANOVA for that main effect.

[e] "Sq root" indicates the data were subjected to square root transformation to meet assumptions of parametric analysis. "-" indicates the data were not transformed for that variable.

[f] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$).

Table 3.2. Results of a seed germination assay of hybrid sunflower seed harvested from plots in a 2016 foliar fungicide trial to assess potential phytotoxicity of foliar fungicide applications of Topsin 4.5FL (thiophanate-methyl), Omega 500F (fluazinam), and Rovral Brand 4 Flowable Fungicide (iprodione) in a hybrid sunflower seed crop in the Columbia Basin of central Washington

Assay, ANOVA factor, and treatment[a]	Normal seed germination (%)	Abnormal seed germination (%)	Decayed seed (%)	Non-germinated seed (%)	White mold incidence (%)
First assay					
ANOVA factor					
Block	0.0035**[b]	0.8492	0.0237*	0.0039**	-
Fungicide	0.0012**	0.2335	0.7224	0.0497*	-
Transformation[c]	-	-	-	-	-
Treatment					-
Control	86.6 ab[d]	7.4 a	1.0 a	5.0 ab	-
Topsin 4.5FL	82.2 b	6.8 a	2.0 a	9.0 b	-
Rovral Brand 4 Fl. Fungicide	92.2 a	4.2 a	1.4 a	2.2 a	-
Omega 500F	87.2 ab	5.2 a	1.4 a	6.2 ab	-
Second assay					
ANOVA factor					
Block	0.4034	0.576	0.0258*	0.7257	1.0000
Fungicide	0.1983	0.3355	0.0562	0.1435	0.0951
Transformation	-	-	Sq root	-	Rank
Treatment					
Control	84.6 a	6.0 a	8.6 a	0.8 a	0.0 a
Topsin 4.5FL	83.6 a	7.0 a	8.2 a	1.2 a	0.0 a
Rovral Brand 4 Fl. Fungicide	90.6 a	3.0 a	6.2 a	0.0 a	0.0 a
Omega 500F	89.0 a	6.0 a	3.2 a	1.8 a	1.0 a

[a] Data were analyzed as a randomized complete block design with five replications. Fungicide treatments included nontreated control plots and plots treated with foliar sprays of Topsin 4.5FL (thiophanate methyl), Rovral Brand 4 Flowable Fungicide (iprodione), and Omega 500F (fluazinam), each applied pre-bloom, at 10 to 50% bloom, and at 75% bloom to petal fall.

[b] Probability of means square > F in the analysis of variance (ANOVA) for that main effect. * and ** = significant at $P = 0.05$ and 0.01, respectively.

[c] "Rank" and "Sq root" indicate the data were subjected to non-parametric rank transformation and square root transformation, respectively, to meet assumptions of parametric analysis. "-" indicates the data were not transformed for that variable.

[d] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$).

Table. 3.3. Analysis of variance (ANOVA) for white mold incidence measured as basal, midstalk, head, aerial (midstalk + head), and total disease incidence for the 2015 and 2016 foliar fungicide application + broadcast Contans WG trials in the Columbia Basin of central Washington

ANOVA and treatment[a]	2015[b]	2016				
	Basal[c]	Basal[c]	Midstalk[c]	Head[c]	Aerial[c]	Total[c]
ANOVA						
Block	0.3073[d]	0.0739	0.4541	0.0475*	0.0551	0.0493*
Contans WG	0.5942	0.3153	0.6510	0.5731	0.6905	0.9118
Foliar fungicides	0.9305	0.7329	0.8797	0.6547	0.6756	0.6644
Contans WG x Fungicides	0.1265	0.0439*	0.1147	0.1542	0.1221	0.0603
Treatment						
Control	5.40 a[e]	3.25 a	0.55 a	4.80 a	5.35 a	8.60 a
Contans WG only	3.25 a	7.50 a	1.60 a	6.75 a	8.35 a	15.85 a
Foliar fungicides only	2.35 a	5.70 a	1.30 a	7.00 a	8.30 a	14.00 a
Contans WG + Foliar fungicides	6.65 a	4.15 a	0.70 a	2.65 a	3.35 a	7.50 a

[a] Data were analyzed as a randomized complete block design with five replications. Contans WG treatments were applied soon after planting and following dammer-diking (latter in the 2016 trial). Fungicide treatments included Priaxor Xemium Brand Fungicide (fluxapyroxad and pyraclostrobin), Endura (boscalid), and Topsin 4.5FL (thiophanate methyl), applied pre-bloom, at 10 to 50% bloom, and at 75% bloom to petal fall, respectively.

[b] Sclerotinia midstalk rot and head rot were not detected in plots in the 2015 trial; therefore, basal disease incidence = total white mold incidence for that trial.

[c] Incidences of basal stalk rot, midstalk rot, head rot, aerial white mold (midstalk rot + head rot), total white mold (basal stalk rot + midstalk rot + head rot) out of 400 sunflower plants rated/plot and averaged over five replicate plots/treatment.

[d] Probability of means square > F in the ANOVA for that main effect or interaction term. * = significant at $P = 0.05$.

[e] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$).

Table 3.4. Pearson's correlation coefficients (and associated *P* values) for incidences of aerial white mold and basal stalk rot caused by *Sclerotinia sclerotiorum*, apothecia counts, plant height, and normalized altitude of plots in both the 2016 foliar fungicide application trial and the 2016 foliar fungicide application + Contans WG trial in a hybrid sunflower seed crop near Ephrata in the Columbia Basin of central Washington

Variable measured[a]	Aerial white mold incidence	Basal stalk rot incidence	Apothecia counts	Plant height	Normalized plot altitude
Aerial white mold incidence	-	0.559 (<0.0002)**[b]	0.476 (0.0019)**	0.408 (0.0090)**	-0.620 (<0.0001)**
Basal stalk rot incidence		-	0.386 (0.0138)*	0.409 (0.0088)**	-0.618 (<0.0001)**
Apothecia counts			-	0.367 (0.0199)*	-0.452 (0.0034)**
Plant height				-	-0.396 (0.0115)*

[a] Aerial disease incidence = midstalk rot + head rot incidence (400 plants rated/plot). Basal disease incidence = basal stalk rot incidence (400 plants rated/plot). Apothecia counts = average number of apothecia of *S. sclerotiorum* count in each of six 1.7 m^2 sections in each of 20 plots/trial. Plant height = average plant height in six 1.7 m^2 sections in each plot. Normalized plot altitude = difference between the average altitude measured at four locations per plot vs. the lowest altitude measured over all 40 plots.

[b] * and ** significant at *P* = 0.05 and 0.01, respectively.

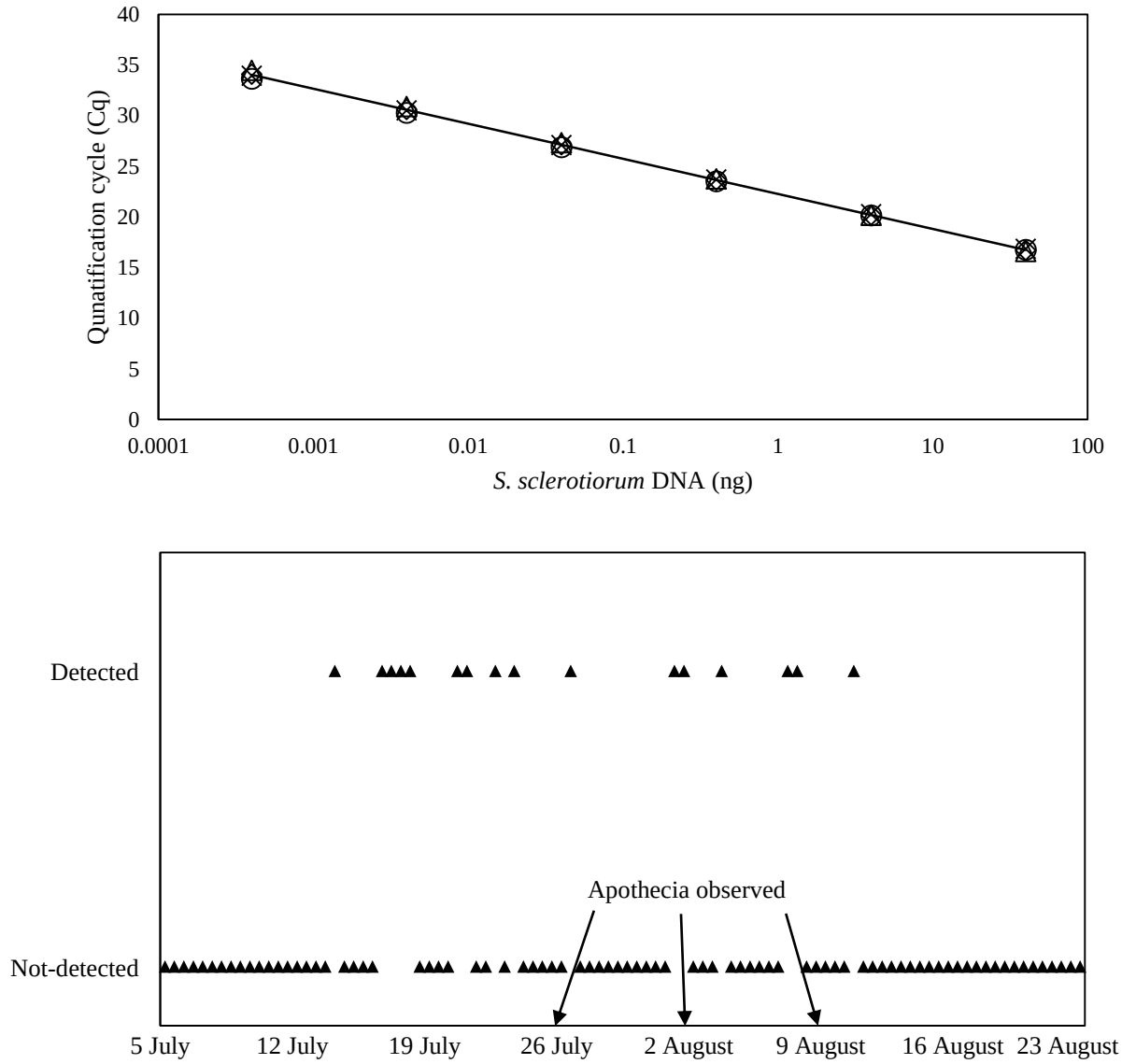

Fig. 3.1. (**A**) Four replicate standard curves, each denoted by a different symbol obtained using genomic DNA of *Sclerotinia sclerotiorum* that was subjected to a real-time, quantitative polymerase chain reaction (PCR) assay using the primer set and hydrolysis probe developed by Ziesman et al. (2016). The standard curves were prepared from a serial dilution of 4.0 to 4.0 x 10^{-4} ng of genomic DNA of *S. sclerotiorum*. PCR efficiency ranged from 90.54 to 97.77 and the coefficient of determination (R^2) ranged from 0.9973 to 0.9996 for individual standard curves. (**B**) Detection of DNA of ascospores of *S. sclerotiorum* from DNA extracted from 12-h sections of Melinex tape collected from a Burkard 7-day volumetric spore trap placed in a border of the 2016 foliar fungicide trial located in a hybrid sunflower seed crop near Ephrata, WA. Arrows indicate the dates when apothecia of *S. sclerotiorum* were observed on the soil surface near the spore trap.

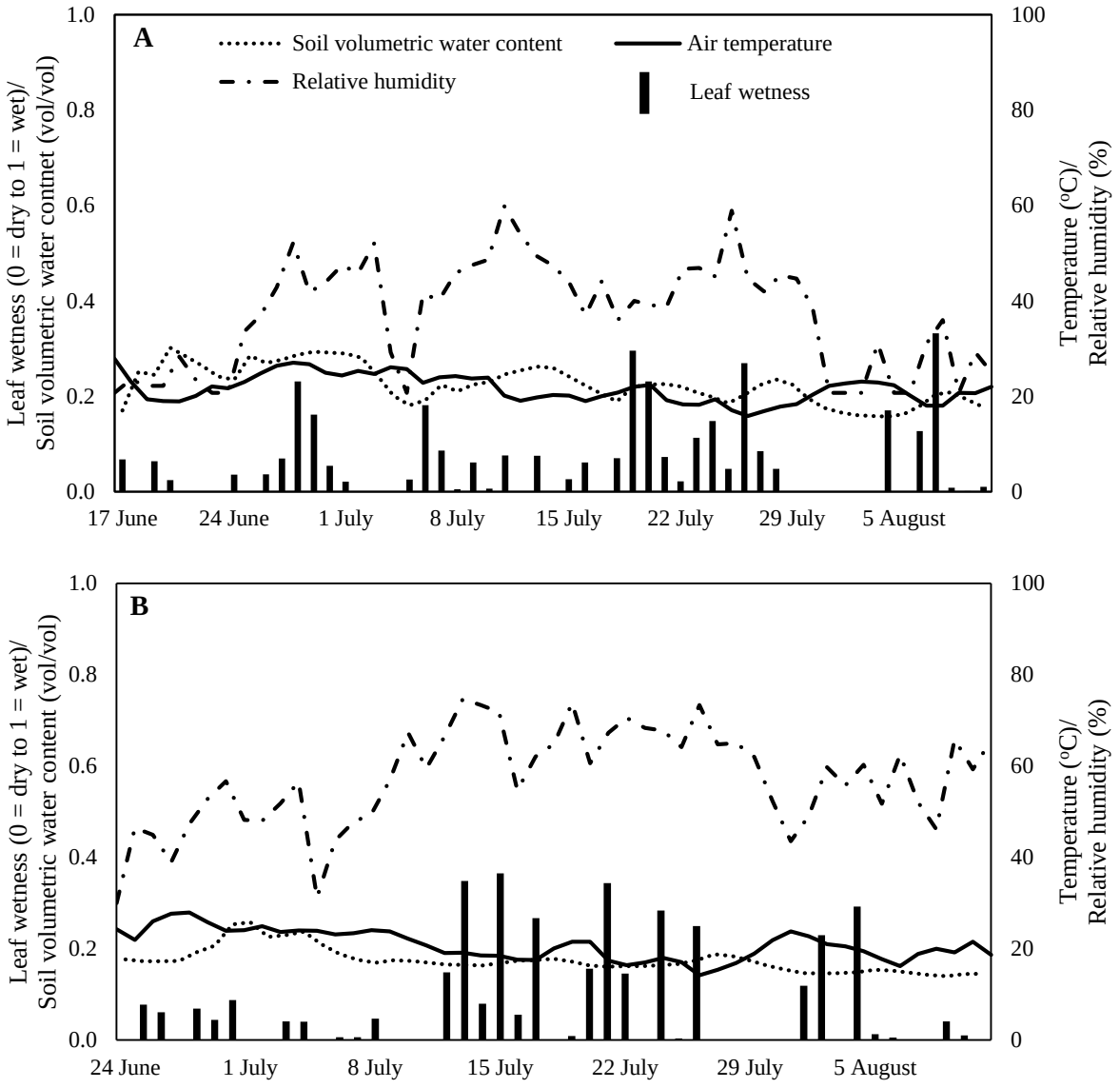

Fig. 3.2. Temperature (ºC), relative humidity (%), leaf wetness (0 = dry to 1 = wet), and soil volumetric water content (vol/vol) data for 2015 field trials in hybrid sunflower seed crops in the Columbia Basin of central Washington. (**A**) Data from a field trial near Ephrata, WA that was completed to evaluate the efficacy of selected fungicides for control of white mold caused by *Sclerotinia sclerotiorum*. (**B**) Data from a field trial near Odessa, WA that was completed to evaluate banded vs. broadcast applications of Contans WG (*Coniothyrium minitans*) for control of white mold.

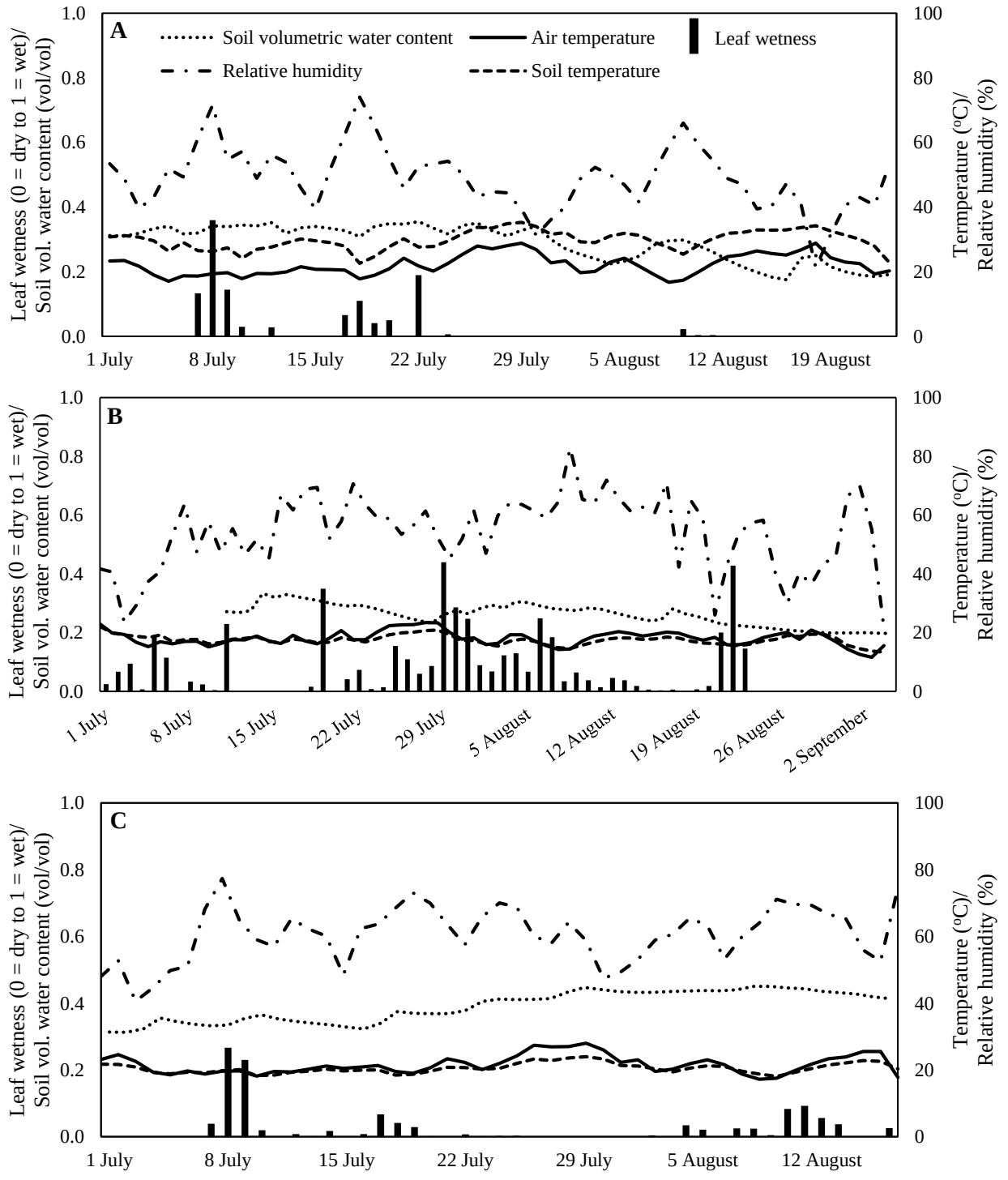

Fig. 3.3. Temperature (ºC), relative humidity (%) leaf wetness (0 = dry to 1 = wet), soil volumetric water content (vol/vol), and soil temperature (ºC) measured in 2016 hybrid sunflower seed crops in the Columbia Basin of central Washington in 2016. (**A**) Data from a field trial near Ephrata, WA that was completed to evaluate the efficacy of selected fungicides for control of white mold caused by *Sclerotinia sclerotiorum*. (**B**) Data from a field trial near Odessa, WA that

was completed to evaluate banded vs. broadcast applications of Contans WG (*Coniothyrium minitans*) for control of white mold. (**C**) Data from a rill-irrigated hybrid sunflower seed crop near Quincy, WA.

CHAPTER 4

EFFECT OF SCLEROTIUM BURIAL AND CONTANS WG APPLICATION ON SURVIVAL OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* IN THE COLUMBIA BASIN OF CENTRAL WASHINGTON

4.1. Introduction

Sclerotinia sclerotiorum is a necrotrophic fungus that survives in the soil as sclerotia, and has a wide, phylogenetically diverse host range of >400, mostly dicotyledonous plant species (Boland and Hall 1994; Bolton et al. 2006). In the U.S., annual losses to *S. sclerotiorum* have been documented to exceed $200 million, demonstrating the destructiveness of the pathogen (Bolton et al. 2006). The fungus is not a new pathogen to the Columbia Basin of central Washington, where *S. sclerotiorum* historically has caused white mold on many susceptible crops grown in the region, including alfalfa (*Medicago sativa*), bean (*Phaseolus vulgaris*), carrot (*Daucus carota*), pea (*Pisum sativum*), potato (*Solanum tuberosum*), and various *Brassica* spp. (Bill Wirth, Precision Seed Production, LLC, *personal communication*; Pacific Northwest Plant Disease Management Handbook 2017).

Hybrid sunflower (*Helianthus annuus*) seed crops were introduced into the Columbia Basin of central Washington fairly recently, starting with 20 ha planted in 2008 (Bill Wirth, Precision Seed Production, LLC, *personal communication*). Production has expanded rapidly, with >2,000 ha in 2014. However, sunflower crops are susceptible to white mold, and hybrid sunflower seed production in the Columbia Basin has been affected by this disease, with incidences of white mold ranging from <1 to 75% in individual crops (Bill Wirth, Precision Seed LLC, *personal communication*).

Sclerotia of *S. sclerotiorum* play a significant role in the disease cycle in susceptible crops, including sunflower. Sclerotia serve as the primary inoculum source and can be persistent in the soil, with sclerotia documented to survive up to 8 years in some soils (Abawi and Grogan 1979; Adams and Ayers 1979; Bolton et al. 2006). Depending on environmental conditions, sclerotia can germinate myceliogenically or carpogenically. Sclerotia that germinate myceliogenically give rise to mycelia, which can infect the root and crown tissues of a host plant (Bolton et al. 2006). Sclerotia that germinate carpogenically bear apothecia, with each apothecium capable of releasing up to 3×10^7 ascospores over the duration of viability of the apothecium, which can range from 2 to 33 days (Abawi and Grogan 1979; Twengstrom et al. 1998), and, once released, the ascospores can then infect a susceptible host plant such as bean (Cook et al. 1975), canola (*Brassica napus*) (Turkington and Morall 1993), carrot (Kora et al. 2005), lettuce (*Lactuca sativa*) (Patterson and Grogan 1985), potato (Atallah and Johnson 2004), and sunflower (McCartney and Lacey 1991; Nelson and Lamey 2000). While white mold typically is initiated by carpogenic germination in many crops, initiation of white mold in sunflower crops can occur by both myceliogenic and carpogenic germination (Abawi and Grogan 1979; Nelson and Lamey 2000; Steadman 1979).

Severe infections of sunflower crops by *S. sclerotiorum* can produce a great amount of sclerotial inoculum, e.g., up to 100 sclerotia can be recovered from a severely infected sunflower stalk (Gulya and Masirevic 1992). Therefore, the introduction of hybrid sunflower seed crops to the Columbia Basin has the potential to increase sclerotial inoculum of *S. sclerotiorum* in fields, and has heightened the importance of controlling sclerotial populations of the pathogen in the soil. One possible means of reducing sclerotial populations in the soil is by the use of the coelomycete *Coniothyrium minitans*, a mycoparasite of *S. sclerotiorum* (Campbell 1947; Whipps

et al. 2008). The capacity of *C. minitans* to reduce *S. sclerotiorum* populations in the soil has been documented (Budge and Whipps 1991; Budge et al. 1995), and applications of *C. minitans* have been shown to be effective at reducing the incidence of lettuce drop in greenhouses (Budge and Whipps 1991; Jones et al. 2004; Van Beneden et al. 2010) and in fields (Chitrampalam et al. 2010; McLaren et al. 1994). However, when applied to crops such as canola and potato at planting, *C. minitans* has not always been effective at reducing white mold incidence the season of application (Hammond et al. 2008; McQuilken et al. 1995). Long-term, repeated applications of *C. minitans* have been reported to reduce *S. sclerotiorum* populations and white mold incidence in a rotation of potato, bean, carrot, and chicory (*Cichorium intybus*) (Gerlagh et al. 1999). Contans WG Biological Fungicide (Contans WG), a commercial formulation of *C. minitans*, is marketed in the U.S. by Bayer CropScience (Research Triangle Park, NC), and is registered for use in sunflower seed crops in the Columbia Basin. Hybrid sunflower seed growers in the region are already applying Contans WG to their fields generally with a ground rig and spray boom or by chemigation through center-pivots.

Burial of sclerotia has also been shown to reduce the duration of survival of sclerotia compared to leaving sclerotia on the soil surface (Adams 1975; Duncan et al. 2006; Merriman 1976; Merriman et al. 1979). Merriman (1976) and Merriman et al. (1979) showed that a 4 cm-depth of burial was sufficient to reduce sclerotial survival in comparison to sclerotial survival on the soil surface. In addition, Merriman et al. (1979) reported that sclerotial viability declined faster when buried sclerotia were not protected within crop residues compared to sclerotia protected within crop residues. Duncan et al. (2006) showed that sclerotial viability was less at a burial depth of 10 cm compared to 5 cm, and sclerotial viability at both depths was less than that of sclerotia on the soil surface after a year in a field study in Manitoba, Canada. However,

Adams (1975) found that only burial to the depth of 61 cm reduced sclerotial viability significantly compared to depths of 3, 15, and 30 cm. Burial of sclerotia present on the soil surface or within infested residues can be achieved by sunflower growers through tillage practices as a means of managing soilborne inoculum of *S. sclerotiorum* (Kurle et al. 2001).

Based on white mold management practices in other crops grown in the region such as canola (Paulitz et al. 2015), hybrid sunflower seed growers in the Columbia Basin currently use crop rotation, application of Contans WG, and break up of and incorporation of crop residues to reduce sclerotial populations in the soil. Additional tactics used by sunflower seed growers in the Columbia Basin to control white mold are reduced irrigation frequency after canopy closure and use of foliar fungicide applications during bloom and seed set to try and prevent carpogenic germination and ascospore infection, respectively (Johnson and Atallah 2006; Twengstrom, Kopmans, et al. 1998). While it is recommended that growers rotate white mold-susceptible crops, such as sunflower and canola, with at least 3 years of non-susceptible host crops (Nelson and Lamey 2000; Paulitz et al. 2015), this is not always economically feasible for some growers in the Columbia Basin (Bill Wirth, Precision Seed Production, LLC, *personal communication*). Therefore, hybrid sunflower seed growers try typically use a rotation that incorporates at least one year of a non-susceptible host crop in the 3- to 4-year rotation between sunflower seed crops. A Contans WG application is typically made in May when hybrid sunflower seed crops are planted, and a second application may be applied in June following cultivation of the field for weed control. Growers may also apply Contans WG after harvest of the sunflower seed crop in the fall if those crops had severe white mold outbreaks. Following harvest growers generally break up and partially incorporate sunflower crop residues into the soil including using a flail shredder to break up the residues into smaller pieces, followed by disking and ripping the soil to

incorporate the residues partially. However, based on continued observation of losses to white mold in hybrid sunflower seed crops despite adoption of these practices, sunflower seed growers in the region have requested research on the degree of efficacy of practices such as Contans WG applications and burial of sclerotia for management of white mold (Bill Wirth, Precision Seed Production LLC, *personal communication*).

The objectives of this research were to determine the relative efficacy of drench applications of *C. minitans* in the form of Contans WG, as well as burial of sclerotia and/or white mold-infected sunflower seed crop residues at reducing the duration of survival of sclerotia of *S. sclerotiorum* in the Columbia Basin. This was accomplished using microplot trials in a growers' field in which Contans was drenched over sclerotia placed on the soil surface or buried 15 cm deep. A complementary, large-scale field trial was initiated to assess the degree to which a postharvest application of Contans WG to infested sunflower seed crop residues in a grower's field might reduce the duration of survival of *S. sclerotiorum* sclerotia, thereby decreasing the risk of white mold developing in susceptible crops that might be planted after the hybrid sunflower seed crops.

4.2. Materials and Methods

Microplot sclerotium survival trials. *Spring 2015 trial.* A microplot field trial was set up on 17 April 2015 to determine the effect of the application of Contans WG on survival of sclerotia of *S. sclerotiorum*. Sclerotia were either placed on the soil surface or buried in the soil in a field near Ephrata, WA (47º 09'748"N, 19º 32'810"W), and then treated or not treated with Contans WG. The soil was a Timmerman coarse sandy loam [United States Department of Agriculture (USDA) National Resources Conservation Service (NRCS) 2016]. The field was

ripped to a depth of 30 cm and disked 15 cm deep prior to placing sclerotia in/on the soil. The sclerotia used in this trial were collected from a mixture of sclerotia, sunflower seeds, and senesced sunflower debris from the screens in the sunflower seed cleaning facility of Precision Seed Production, LLC, after harvest of sunflower seed crops in the Columbia Basin in fall 2013. The sclerotia had been held in cold storage (4 ± 1^{o}C) from 2013 to 2015. The sclerotia and debris were placed on a soil sieve with 7.14 mm-diameter perforations to separate and collect the sclerotia, with a total of 5,400 sclerotia used for this trial.

The trial was set up as a split-plot, randomized complete block (RCB) experimental design with four replications of a 3 x 2 factorial treatment design, and nine sampling periods (repeated measures design) for assessing sclerotia viability and colonization by *C. minitans* at approximately 2-month intervals over 18 months. Each main plot was 2.7 m x 0.6 m, and each subplot was 2.7 m x 0.3 m. Three Contans WG treatments were applied to the main plots: i) no Contans WG (soil and sclerotia drenched with water as a control treatment), ii) soil and sclerotia drenched with the equivalent of 0.56 kg Contans WG/ha, and iii) soil and sclerotia drenched with the equivalent of 4.48 kg Contans WG/ha. Each Contans WG treatment was applied in the equivalent of 127,216 liters water/ha to simulate chemigation through a center pivot, as Contans WG is applied by chemigation in most hybrid sunflower seed crops grown under center pivot irrigation in the Columbia Basin (Bill Wirth, Precision Seed Production, LLC, *personal communication*). The split plot treatments consisted of two placements of sclerotia: i) on the soil surface, and ii) buried 15 cm deep. The entire trial was 7.9 m x 7.9 m, including a 0.6 m border and 0.6 m wide alleys between adjacent replicate blocks, except for a 1.2-m wide alley between the eastern and western halves of the trial to accommodate a lay-flat sprinkler irrigation line. Plots immediately adjacent to the sprinkler flat line were 0.6 m from the lay-flat line as a

precaution against a leak or water pooling which might affect the uniformity of wetting the soil during sprinkler irrigation. Within each split plot, nine sets of 25 sclerotia, each enclosed in a 15 cm x 15 cm mesh bag were placed either on the soil surface or buried at a depth of 15 cm, with 1 bag of 25 sclerotia/0.093 m^2. The mesh bags were made from window insect screening material (Phifer Inc., Tuscaloosa, AL) and sealed with a 20 cm impulse sealer (Uline, Pleasant Prairie, WI).

Each pair of bags of sclerotia, surface and buried, in each replicate block represented one of the nine sampling periods. To facilitate retrieval of the buried bags of sclerotia, a 0.6 m long section of orange baling twine was attached to each mesh bag with a zip tie, fastened through a hole punctured along one edge of the bag outside the sealed area. The pairs of buried and surface bags of sclerotia were sampled randomly within each main plot (Contans WG treatment) to minimize the confounding effect of variation associated with placement of the mesh bags at each sampling period. The pairs of buried and surface bags of sclerotia collected from the trial at each sampling interval were stored at 4 ± 1°C until the sclerotia could be processed, within one or two days after sampling. The sclerotia were then washed under running tap water in a strainer to remove soil and plant debris, and transferred to sterilized paper toweling in a laminar flow hood for at least 2 h to dry. The number of sclerotia recovered from each bag was recorded with the number of disintegrated sclerotia calculated as: 25 – (the number of sclerotia recovered from each mesh bag). Once dry, each sclerotium was surface-sterilized for 2 s in 70% ethanol followed by brief flaming, using sterilized forceps to handle each sclerotium. The sclerotium was then aseptically bisected, and both halves plated onto ½-strength potato dextrose agar (PDA) medium amended with 50 mg/liter of bromophenol blue (BPDA) (Steadman et al. 1994). The

sclerotia were then incubated on the plates for 14 days in the dark at 20°C, and assessed for viability and *C. minitans* colonization after 3, 7, and 14 days.

Sclerotium viability was determined based on: i) whether or not the sclerotium germinated and the resulting mycelial growth produced oxalic acid, which turned the BPDA medium yellow indicating the pH of the medium was lowered (Steadman et al. 1994), and ii) the production of new sclerotia from mycelial growth on the agar medium. Assessment of colonization of sclerotia by *C. minitans* was based on the observation of characteristic black pycnidia, dark-colored cirrhi, and typical dematiaceous, ellipsoid, 4.0-6.0 x 3.5-4.0 µm conidia of the fungus (Campbell 1947), determined using dissecting and compound microscopes. Since colonization of sclerotia by *C. minitans* could only be assessed for the sclerotia, out of 25 in each mesh bag, which had not disintegrated, colonization was expressed as number rather than a percentage. Four replicate sets of 25 sclerotia that had not been treated with Contans WG were plated onto BPDA at the time the trial was set up (0-month sampling interval) to determine the initial percentage of viable sclerotia and number of sclerotia colonized by *C. minitans*. Subsequent pairs of surface and buried bags of sclerotia were collected on 17 June and 11 August 2015, and processed as described above. Just prior to collecting sclerotia in October 2015, the grower-cooperator accidentally disked the entire field along with the trial site, destroying the trial.

Data were analyzed by restricted maximum likelihood (REML) estimation of variance components using the lmer procedure in the R package *lme4* (Bates et al. 2015) with descriptive statistics (e.g., means, standard errors and minimum and maximum values) calculated in Excel. Data that did not meet assumptions of homogeneity of variance and/or normality were subjected to log, square root, or arcsine square root transformations. If the data from all the sampling

periods, not including the initial sampling, met assumptions before or after transformation, then that data was analyzed as a split plot RCB design with sampling time as a repeated measure. Contans WG treatments, sclerotium location, and sampling time were treated as fixed effects in the model, and blocks as random effects. If assumptions were not met, then the data were analyzed separately for each sampling period as a split-plot RCB design, with Contans WG treatments and sclerotium location treated as fixed effects, and blocks as random effects. If analysis of the sampling periods separately and transformation of the data did not resolve the violation of assumptions, then Friedman's nonparametric rank test was used for analysis. Mean comparisons were made using Tukey's honestly significant difference (HSD) ($P < 0.05$).

2015 Fall trial. A repeat of the micro-plot field trial was set up on 17 October 2015 in a different location in the same grower-cooperator's field near Ephrata, WA, with the field prepared as for the 2015 spring trial. The plot dimensions and split-plot RCB experimental design of the 3 x 2 factorial treatment design were the same as in the 2015 spring trial, with enough pairs of mesh bags, each with 25 sclerotia, buried or placed on the soil surface for nine sampling periods. Sclerotia used in the trial were collected on 19 September 2015 from a rill-irrigated, hybrid sunflower seed crop on Rd K, Grant Co., WA (47º 09'748"N, 19º 32'810"W), with most of the sclerotia collected from inside infected sunflower stalks prior to harvest of the crop in October. The sclerotia were stored at 4 ± 1ºC until used to set up this microplot trial.

Four replicate sets of 25 sclerotia that had not been treated with Contans WG were plated onto BPDA at the time the trial was set up in October 2015 (0-month sampling interval) to determine the initial percentage of viable sclerotia and percentage of sclerotia colonized by *C. minitans*. Subsequent pairs of surface and buried bags of 25 sclerotia were collected on 19 March, 6 May, 24 June, 24 August, and 28 October 2016, and processed as described above.

Sampling will resume in March of 2017 and occur at ~2 month intervals for four more sampling periods, but will not be processed until after this thesis was written. Data were analyzed as described above.

2016 Spring trial. As a result of the accidental destruction of the spring 2015 trial after only two sampling periods, a third repeat of the microplot trial was set up on 20 March 2016 in an adjacent location to the 2015 fall trial in the same grower-cooperator's field near Ephrata, WA. The field was ripped to a depth of 30 cm and disked 15 cm deep prior to placing sclerotia in/on the soil. The plot dimensions and split-plot RCB experimental design of the 3 x 2 factorial treatment design were the same as for the 2015 trials, with enough pairs of mesh bags, each with 25 sclerotia, buried or placed on the soil surface for nine sampling periods. The sclerotia used in the trial were from the same source used in the 2015 spring trial.

Four replicate sets of 25 sclerotia that had not been treated with Contans WG were plated onto BPDA at the time the trial was set up in March (0-month sampling interval) to determine the initial incidence of viable sclerotia and percentage of sclerotia colonized by *C. minitans*. Subsequent pairs of surface and buried bags of 25 sclerotia were collected on 6 May, 24 June, 31 August, and 28 October 2016, and processed as described above. Sampling will resume in March of 2017 and occur at ~2 month intervals for five more sampling periods, but will not be processed until after this thesis was written. Data were analyzed as described above.

Postharvest Contans WG application trial. To complement the microplot sclerotium survival trials, a large-scale field trial was set up to determine the effect of postharvest application of Contans WG to sunflower seed crop residues on survival of sclerotia of *S. sclerotiorum* present in/on the residues. The trial was set up on 13 October 2015 in a rill-irrigated, hybrid sunflower seed crop on Rd K, Grant Co., WA (47º 09'748"N, 19º 32'810"W)

that had developed a very severe outbreak of white mold (>50% of plants infected). The soil was an Esquatzel silt loam (USDA NRCS 2016) and the seed crop had been harvested the week prior, with crop residues then partially broken up using a flail shredder. The field had been planted to bean in 2012, sugar beet (*Beta vulgaris* subsp. *vulgaris*) in 2013, and wheat (*Triticum aestivum*) in 2014.

A RCB design was used with four replications of two treatments: i) no application of Contans WG (control treatment), and ii) application of 4.48 kg Contans WG/ha in 748 liters water/ha on 13 October 2015 using a 30.5 m-wide, tractor-mounted spray boom. The field was then disked and ripped by the grower-cooperator to break up the residues further and incorporate a greater amount of the residues into the soil. Each plot was 30.5 m-wide (the width of the spray boom) and extended the length of the field from north to south (~276 m), parallel to the irrigation rills. Each replicate pair of Contans WG-treated and control plots was separated by a 30-m wide alley. In April 2016, the field was moldboard plowed by the grower to a depth of 30 cm, disked, and a corn crop planted with rill (furrow) irrigation. In late September 2016, the corn crop was harvested and the field ripped to a depth of 30 cm, then disked to a depth of 15 cm, after a flail shredder was used to break up the corn residues.

The method used to collect sclerotia from the plots varied over time based on the condition of the sunflower residues and the soil at the time of sampling, with five sampling periods from fall 2015 to fall 2016: i) on 17 October 2015, 50 sclerotia/plot were collected from the soil and sunflower residues to establish the initial percentage of viable sclerotia and percentage of sclerotia colonized by *C. minitans*; ii) on 1 March 2016, 50 sclerotia/plot were collected from the soil and sunflower residues aboveground, and another 50 sclerotia/plot were sampled from buried sunflower residues; iii) on 26 May 2016, 25 sclerotia/plot were collected by

sampling soil to a depth of 30 cm and then sifting the soil through a sieve with 1.19 mm-diameter perforations because very few sunflower stalk residues could be found by this sampling period; iv) on 2 to 4 August 2016, 40 sclerotia/plot were collected using the same protocol as in May; and v) on 8 October 2016, 25 sclerotia/plot were collected using the same protocol as in May. Sclerotia sampling always occurred at least 5 m from the border of each plot to avoid collecting sclerotia that potentially had been moved from an adjacent plot during disking and other cultivation practices. For the first two sampling periods, sclerotia were collected along a zig-zag pattern through each plot. For the next three sampling periods, soil was sampled from at least three holes at random sites in each plot to a depth of 15 cm. The mean sclerotial density per plot was estimated for those sampling time that necessitated sieving soil to recover sclerotia, based on the amount of soil that had to be sieved to recover the requisite number of sclerotia. The number of sclerotia collected from the soil in the manner varied among sampling times based on the difficulty of finding adequate sclerotia.

The collected sclerotia were stored at 4 ± 1°C and processed within a day or two of sampling, as described for sclerotia collected from the microplot trials. The sclerotia were incubated on BPDA plates for 14 days in the dark at 20°C, and assessed for viability and *C. minitans* colonization 3, 7, and 14 days after plating. Sclerotium viability and colonization of sclerotia by *C. minitans* were evaluated as described above for the microplot trials. Homogeneity of variance was satisfied between sampling periods for sclerotia collected on the 26 May, 2 to 4 August, and 8 October 2016; therefore, the data were analyzed as repeated measures for those sampling times using a split-plot framework with time as split plots. Contans WG treatments and time were treated as fixed effects, and blocks as a random effect in the model. All data analyses were done using restricted REML estimation of variance components

using the lmer procedure in the R package *lme4*. If the assumptions of homogeneity of variance or normality of residuals were violated, the data were subjected to logarithmic or square root transformations. Mean comparisons were made using Tukey's HSD ($P < 0.05$).

4.3. Results

Microplot sclerotium survival trials. ***Viability of sclerotia.*** The mean percentage of viable sclerotia was 98.0 ± 1.2 (mean ± standard error), 99.0 ± 1.0, and 95.0 ± 3.0% at the start of the spring 2015, fall 2015, and spring 2016 microplot field trials, respectively; and then declined over time in all three trials (Fig. 4.1A, 4.2A, and 4.3A, respectively). Averaged over all plots within each trial, the incidence of viable sclerotia had declined to 40.7 ± 7.7% by 11 August 2015 in the spring 2015 trial (4 months after trial initiation), 9.5 ± 3.3% by 28 October 2016 in the fall 2015 trial (12 months after trial initiation), and 18.2 ± 4.0% on 28 October 2016 in the spring 2016 trial (7 months after trial initiation).

Because of heterogeneity of variances among sampling periods for the fall 2015 and spring 2016 trials, sampling time could only be analyzed as a factor affecting viability of sclerotia for the spring 2015 trial (Table 4.1). The effect of sampling time on the viability of sclerotia was significant in that trial ($P = 0.0032$), as the mean percentage of viable sclerotia on 17 June 2015 (54.2 ± 8.3) was significantly greater than on 11 August 2015 (40.7 ± 7.7%) (Table 4.1 and Fig. 4.1A).

In each of the three microplot trials, burial of sclerotia caused a highly significant decrease in the survival of sclerotia of *S. sclerotiorum*, as the percentage of viable sclerotia recovered from buried mesh bags was significantly less than the percentage of viable sclerotia recovered from the surface mesh bags at each sampling time (Tables 4.1, 4.2, and 4.5). In the spring 2015 trial, an average 10.3 ± 2.6% of the buried sclerotia were viable compared to 84.5 ±

2.5% of the surface sclerotia (averaged over both the 17 June and 11 August sampling times) (Table 4.1, Fig. 4.1A). For the fall 2015 trial, the highly significant effect of burial at reducing sclerotium survival (P <0.0001 to 0.0138 for individual sampling dates from 19 March to 28 October 2016) was reflected in survival of 17.0 ± 4.4 vs. 71.0 ± 4.2% of the buried vs. surface sclerotia, respectively, on 19 March; 6.7 ± 2.2 vs. 61.7 ± 4.9%, respectively, on 6 May; 2.0 ± 0.9 vs. 32.3 ± 4.6%, respectively, on 24 June; and 4.7 ± 1.7 vs. 19.0 ± 6.8%, respectively, on 24 August 2016 (Table 4.2, Fig. 4.2A). Similarly, in the spring 2016 trial, burial had a highly significant effect on sclerotium viability at each of the sampling dates from 6 May to 28 October 2016 (P ranged from <0.0001 to 0.0016). The percentage of viable vs. surface sclerotia was 33.7 ± 4.3 vs. 90.7 ± 4.2% , respectively, on 6 May; 25.0 ± 5.2 vs. 86.0 ± 3.1%, respectively, on 24 June; and 9.0 ± 3.5 vs. 31.7 ± 6.1%, respectively, on 31 August 2016 (Table 4.5, Fig. 4.3A).

In the fall 2015 microplot trial, the Contans WG soil drench treatments had no significant effect on the percentage of viable sclerotia except by the 28 October 2016 (P = 0.0140), 12 months after initiation of the trial (Table 4.2), when there was also a significant interaction between sclerotium location and Contans WG treatments (P = 0.0392) (Table 4.2). The mean percentage of viable sclerotia recovered from mesh bags on the soil surface in plots drenched with 0.56 kg Contans WG/ha (6.0 ± 2.6%) and 4.48 Contans WG kg/ha (1.0 ± 1.0%) was significantly less than in control plots not treated with Contans WG (42.0 ± 7.0%) (Table 4.2). In contrast, there was no significant difference in the percentage of viable sclerotia recovered from buried bags among any of the Contans treatments (1.0 ± 1.0, 6.0 ± 2.6, and 1.0 ± 1.0% for plots treated with 0, 0.56, and 4.48 kg Contans WG/ha, respectively) (Table 4.2).

As described above, the effect of sclerotium location on or in the soil was significant on 28 October 2016 in the fall 2015 microplot trial (P = 0.0043), but the effect of sclerotium

location on/in the soil on survival of sclerotia varied depending on the Contans WG treatment (P = 0.0392). On this date, the mean percentage of viable sclerotia recovered from buried mesh bags (1.0 ± 1.0%) was significantly less than for surface sclerotia (42.0 ± 7.0%) in plots not treated with Contans WG, whereas the mean viability of buried and surface sclerotia was similarly low in plots drenched with Contans WG at 0.56 and 4.48 kg/ha (Table 4.2, Fig. 4.2A). In the spring 2016 trial, the survival of sclerotia on the soil surface vs. buried did not differ among Contans WG treatments until the 28 October 2016, seven months after initiating that trial, when the interaction term between sclerotium location and Contans WG treatment was significant (P = 0.0190) (Table 4.5). The percentage of viable buried sclerotia (3.0 ± 1.9%) was significantly less than that of the surface sclerotia (49.0 ± 3.4%) in plots not treated with Contans WG, but there was no significant difference in viability of buried vs. surface sclerotia in plots drenched with Contans WG at 0.56 or 4.48 kg/ha (Table 4.5, Fig. 4.3A).

***Colonization of sclerotia by* C. minitans.** The mean number of *S. sclerotiorum* sclerotia colonized by *C. minitans* (out of 25/mesh bag) peaked within five months of initiating each microplot trial (Fig. 4.1B, 4.2B, and 4.3B). The number of sclerotia colonized by the mycoparasite peaked at 3.2 ± 1.2 on 17 June 2015, 15.3 ± 0.9 on 19 March 2016, and 5.1 ± 1.2 on 24 June 2016 (averaged across all plots) in the spring 2015, fall 2015, and spring 2016 trials, respectively. Because of heterogeneous variances between sampling periods for the spring 2015 and fall 2015 trials, sampling time could only be included as a repeated measures factor affecting colonization of sclerotia by *C. minitans* for the spring 2016 trial (Table 4.5). In that trial, there was no significant effect of sampling time on the number of sclerotia colonized by *C. minitans* (P = 0.6847; Table 4.5), i.e., the number of sclerotia colonized by the mycoparasite did not

increase or decrease over time when averaged over both sclerotium locations (on or in the soil) and the three Contans WG treatments.

In each of the microplot trials, burial of sclerotia had a significant effect on colonization of the sclerotia by *C. minitans* as the number of sclerotia colonized was significantly less for buried sclerotia than for sclerotia on the soil surface (Tables 4.1, 4.3, and 4.5). In the spring 2015 trial, for each of the 17 June and 11 August 2015 sampling times, this effect of sclerotium location on colonization of sclerotia by the mycoparasite varied depending on the Contans WG treatment (P = 0.0190 and 0.0140, respectively, for the interaction term). For sclerotia sampled on 17 June 2015 in that trial, the mean number of surface sclerotia colonized by *C. minitans* in plots drenched with 0.56 kg and 4.48 kg Contans WG/ha was 8.0 ± 4.6 and 10.5 ± 2.1, whereas none of the buried sclerotia were colonized by *C. minitans* in any of the Contans WG plots (Table 4.1). By 11 August 2015, the mean number of surface sclerotia colonized by *C. minitans* in plots drenched with 0.56 kg and 4.48 kg Contans WG/ha had decreased to 2.5 ± 1.5 and 4.0 ± 0.7 out of 25/bag, respectively, whereas there was still no evidence of colonization of any of the buried sclerotia in plots drenched with Contans WG at either rate (Table 4.1). In the fall 2015 trial, the location of sclerotia on the soil surface or buried did not affect the number of sclerotia colonized by *C. minitans* at the first sampling date of 19 March 2016, but the effect of sclerotium location in/on the soil was highly significant for all subsequent sampling dates (P <0.0001 to 0.0071 from 6 May to 28 October 2016). The number of buried sclerotia colonized by *C. minitans* was significantly less than for surface sclerotia for each of 6 May (1.3 ± 0.4 vs. 15.8 ± 1.7 out of 25 sclerotia/bag, respectively), 24 June (0.3 ± 0.1 vs. 14.6 ± 1.2, respectively), 24 August (0.6 ± 0.4 vs. 5.7 ± 1.5, respectively), and 28 October 2016 (0.1 ± 0.1 vs. 6.5 ± 1.4, respectively) (Table 4.3). Similarly, in the spring 2016 trial, the effect of sclerotium placement

was highly significant (P = 0.0003, Table 4.5). The mean number of sclerotia colonized by *C. minitans* was far less for buried sclerotia (0.9 ± 0.2 averaged over all sampling times) than surface sclerotia (6.2 ± 0.7) (Table 4.5 and Fig. 4.2B).

The Contans WG treatments did not have a significant effect on the mean number of sclerotia colonized by *C. minitans*, except in the spring 2015 microplot trial (P = 0.0267 and P = 0.0210 for sclerotia sampled on 17 June and 11 August, respectively) (Table 4.1). In that trial, there also was a significant interaction between sclerotium location and Contans WG drench treatments (P = 0.0190 on 17 June and P = 0.0140 on 11 August 2016) (Table 4.1 and Fig. 4.1B). On 17 June 2015, the mean number of surface sclerotia colonized by *C. minitans* that had been drenched with 0.56 kg and 4.48 kg Contans WG/ha was 8.0 ± 4.6 and 10.5 ± 2.1, respectively, vs. 0.5 ± 0.5 for surface sclerotia in control plots not treated with Contans WG (Table 4.1 and Fig. 4.1B). Likewise, the mean number of surface sclerotia colonized by *C. minitans* on 11 August 2015 was greater in plots drenched with 0.56 kg and 4.48 kg Contans WG/ha (2.5 ± 1.5 and 4.0 ± 0.7, respectively) than in control plots drenched with water (0.0) (Table 4.1 and Fig. 4.1B). In contrast, the mean number of buried sclerotia colonized by *C. minitans* was 0 on 17 June and 0 on 11 August 2015 for all three Contans WG drench treatments (Table 4.1 and Fig. 4.1B).

Disintegration of sclerotia. The percentage of sclerotia of *S. sclerotiorum* that disintegrated increased over the duration of sampling in all three microplot trials (Fig. 4.1C, 4.2C, and 4.3C). In the 2015 spring trial, the incidence of disintegrated sclerotia increased from 0.0% on 16 April 2015 when the trial was set up, to 13.2 ± 3.4% on 17 June and 26.3 ± 5.6% on 11 August 2015, averaged over all replicate plots of all treatment combinations at each sampling date. In the fall 2015 trial, the incidence of disintegrated sclerotia increased from 0% on 17

October 2015 at the time of trial initiation to 63.0 ± 7.7% on 28 October 2016, when averaged over all plots. Similarly, in the spring 2016 trial, the incidence of disintegrated sclerotia increased from 0% on 19 March 2016 at the onset of the trial to 30.5 ± 6.0% on 28 October 2016. Because of heterogeneous variances among sampling periods in all three trials, sampling time could not be analyzed as a repeated measures factor for the sclerotium disintegration data.

In all three microplot trials, sclerotium location in/on soil had a significant effect on the incidence of disintegrated sclerotia for all sampling times except the first sampling date after setup of the fall 2015 trial, 19 March 2016 (Table 4.4). In the spring 2015 trial, the effect of sclerotium location on disintegration of sclerotia was affected by Contans WG treatments on 17 June ($P = 0.0061$) but not on 11 August 2015 ($P = 0.3327$) (Table 4.1). However, at both sampling times the percentage of disintegrated buried sclerotia was significantly greater than that of surface sclerotia (Table 4.1 and Fig. 4.1C). Likewise, in the fall 2015 trial, the effect of sclerotium placement was highly significant on each of 6 May, 24 June, 24 August, and 28 October 2016 ($P < 0.0001$), with a significantly greater percentage of buried sclerotia disintegrated than surface sclerotia (77.3 ± 4.7 vs. 0%, respectively, on 6 May; 89.7 ± 2.6 vs. 1.0 ± 1.0%, respectively, on 24 June; 87.7 ± 4.9 vs. 9.7 ± 5.5%, respectively, on 24 August; and 95.3 ± 1.5 vs. 30.7 ± 7.6%, respectively, on 28 October 2016) (Table 4.4, Fig. 4.2C). Similarly, the effect of sclerotium location was significant on each of 24 June ($P = 0.0037$), 31 August ($P = 0.0002$), and 28 October 2016 ($P = 0.0164$) for the spring 2016 microplot trial, with far more buried sclerotia disintegrated than surface sclerotia (8.0 ± 2.8 vs. 0%, respectively, on 24 June; 27.3 ± 6.0 vs. 2.7 ± 2.3%, respectively, on 31 August; and 45.7 ± 8.3 to 15.3 ± 6.3%, respectively, on 28 October).

The Contans WG treatments did not have a significant effect on the percentage of disintegrated sclerotia, except on 17 June 2015 (P = 0.0105) in the spring 2015 trial when there was a significant interaction between Contans WG treatments and sclerotium location (P = 0.0061) (Table 4.1). However, on this sampling date the percentage of disintegrated buried sclerotia in plots drenched with 0.56 kg Contans WG/ha (35.0 ± 5.7%) or 4.48 kg Contans WG/ha (15.0 ± 9.0%) was not significantly greater than that of buried sclerotia in control plots drenched with water (29.0 ± 3.4%) (Table 4.1, Fig. 4.1C).

2015 Postharvest Contans application trial. ***Viability of sclerotia.*** In the postharvest Contans WG trial, the percentage of viable sclerotia collected from sunflower seed crop residues declined from 99.5 ± 0.5% at the time of trial initiation on 17 October 2015 to 87.5 ± 2.1% for surface residues and 73.0 ± 3.8% for buried residues by the first sampling date the following spring, 1 March 2016 (Table 4.7 and Fig 4.4A). The location of the residues on or in the soil had a significant effect on the percentage of viable sclerotia recovered (P = 0.0054), but the Contans WG treatment did not affect sclerotium survival (Table 4.7). Although the percentage of viable sclerotia collected from the soil at subsequent sampling dates did not vary significantly (78.5 ± 2.1% on 26 May, 71.9 ± 4.5% on 4 August, and 82.5 ± 4.3% on 8 October 2016), the estimated mean sclerotial density/plot decreased from 0.28 to 0.14 sclerotia/liter of soil over this duration.

***Colonization of sclerotia with* C. minitans.** The percentage of sclerotia collected from sunflower seed crop residues that were colonized by *C. minitans* increased from the date the trial was established on 17 October 2015 (4.8 ± 1.2%) to the first sampling date the next spring, 1 March 2016, for both surface residues (31.3 ± 3.6% colonized sclerotia) and buried residues (38.3 ± 3.9%) (Fig. 4.4B and Table 4.7). However, the percentage of sclerotia colonized by *C. minitans* subsequently declined (P = 0.0001 for the main effect of sampling time) from 9.5 ±

2.3% on 26 May, to 1.3 ± 0.8% on 4 August and 1.5 ± 0.7% on 8 October (Fig. 4.4B and Table 4.8). Sclerotium colonization on 26 May was significantly greater than on 4 August and 8 October with no significant difference in colonization of sclerotia between the latter two sampling dates (Table 4.8). However, neither crop residue location on the soil surface or buried nor Contans WG treatment had a significant effect on colonization of sclerotia by *C. minitans* on 1 March 2016 (Table 4.7). In addition, the application of Contans WG to sunflower seed crop residues immediately after harvest in fall 2015 did not have a significant effect on colonization of sclerotia by *C. minitans* for sclerotia sampled from the soil from May through October 2016 (Fig. 4.4B and Table 4.8).

4.4. Discussion

Burial of sclerotia of *S. sclerotiorum* and burial of infested sunflower crop residues were far more effective at reducing survival of sclerotia of this pathogen than application of *C. minitans* in the form of Contans WG. Drenching sclerotia of *S. sclerotiorum* with Contans WG was not effective at reducing survival of the sclerotia of *S. sclerotiorum* if the sclerotia were buried 15 cm deep, but did reduce the duration of survival of sclerotia left on the soil surface in the microplot trials in this study in the Columbia Basin of central Washington. This significant effect of Contans WG drench treatments on sclerotium survival was only detected ≥7 months after the Contans WG application. The reduction in survival of sclerotia on the soil surface as a result of Contans WG drench application was detected regardless of whether 0.56 vs. 4.48 kg Contans WG/ha was applied, i.e., there was no benefit to applying Contans WG at the higher rate. However, the results of the field trial evaluating postharvest application of Contans WG to infested sunflower seed crop residues revealed no effect of the postharvest Contans WG

application on survival of sclerotia present in the infested sunflower residues or sampled from the soil the following year. Likewise, the application did not increase the percentage of sclerotia colonized by *C. minitans* the season after initiating the postharvest trial. Burial of sclerotia, which exposes sclerotia to the soilborne microflora, was very effective at reducing the duration of survival of sclerotia in this study, as demonstrated in other studies (e.g., Duncan et al. 2006; Harvey et al. 1995; Merriman et al. 1979).

The limited reduction in survival of sclerotia on the soil surface following a Contans WG drench application was surprising as other studies have shown *C. minitans* can reduce sclerotium survival within the top 10 cm of soil (Budge and Whipps 1991; McQuilken et al. 1995; Zeng et al. 2012b), reduce the density of apothecia produced by sclerotia (Gerlagh et al. 1999; Zeng et al. 2012b), and/or reduce white mold incidence or severity (Budge and Whipps 1991; Chitrampalam et al. 2010; Gerlagh et al. 1999; McLaren et al. 1994; Zeng et al. 2012a). McQuilken et al. (1995) demonstrated a significant reduction in survival of sclerotia buried 1 to 2 cm deep in plots of oilseed rape treated with *C. minitans*, even 1 month after application compared with survival of sclerotia in non-treated control plots. In comparison, the microplot trials in this study revealed a significant effect of the *C. minitans* drench application only 7 months after the application, and then the effect was only detected for surface sclerotia, not sclerotia buried 15 cm deep. This may have been due, in part, to differences in conditions on the soil surface, 1 to 2 cm deep, and 15 cm deep, e.g. greater soil moisture and more moderate (14 to 22^{o}C) soil temperatures below the surface, which favor parasitism of sclerotia by *C. minitans* (Jones et al. 2011; Partridge et al. 2006); and the Contans WG drench application not moving the *C. minitans* spores as deep as 15 cm to the sclerotia buried in the microplot trials in this study. In addition, McQuilken et al. (1995) applied *C. minitans* to the soil formulated in colonized maizemeal perlite, which may be a

more effective medium for application of the mycoparasite in field conditions vs. a conidial suspension of the Contans WG formulation used in this study. Solid state fermentation of the fungus is used by the manufacturer to produce the inoculum, which is then incorporated into the wettable granular formulation of Contans WG (De Vrije et al. 2001; Jones et al. 2004; Whipps et al. 2008). However, Contans WG has been effective at reducing the incidence or severity of white mold in other studies (e.g., Chitrampalam et al. 2010; Zeng et al. 2012a).

Consistent with prior studies on the effect of burial on sclerotial viability (Duncan et al. 2006; Merriman 1976; Merriman et al. 1979) this study showed that burial of sclerotia can be very effective at reducing survival of *S. sclerotiorum*. For instance, Duncan et al. (2006), reported that sclerotia on the soil surface and sclerotia buried at 5 and 10 cm depths declined from 80% viability to 57.5, 12.5, and 2.5%, respectively, over a period of 12 months. In the microplot trials in this study, survival was measured for sclerotia buried 15 cm deep. This was deeper than the soil depth at which previous studies have looked for effects of *C. minitans* applications on sclerotium survival (Budge and Whipps 1991; McQuilken et al. 1995; Zeng et al. 2012a; Zeng et al. 2012b). A 15 cm depth of burial was used in the microplot trials to reflect the depth of burial that is achieved when fields are disked and plowed (Kurle et al. 2001). Furthermore, sclerotia at this depth can cause basal stalk rot of sunflower plants, according to Huang and Hoes (1980). However, the Contans WG application had no effect on survival of sclerotia that were buried 15 cm deep. In fact, the Contans WG treatments had no effect on the percentage of viable sclerotia, the percentage of disintegrated sclerotia, and the number of sclerotia colonized by *C. minitans* for sclerotia buried 15 cm deep, i.e., the drench applications were ineffective at getting the mycoparasitic fungus to that depth, despite using a volume of water for the drench that was equivalent to what sunflower seed growers use by chemigation.

Results of the sunflower seed crop postharvest Contans WG trial suggested that Contans WG applied by spray boom to sunflower crop residues infested with *S. sclerotiorum* is not likely to be effective at reducing survival of sclerotia of the pathogen. There were no significant differences in the percentage of viable sclerotia or the number of sclerotia colonized by *C. minitans* in the large-scale plots treated with Contans WG vs. the non-treated control plots. Approximately 35% of the sclerotia sampled from infested residues were colonized by *C. minitans* in spring 2016, the first sampling after the fall application of Contans WG, confirming that sclerotia within residues can be colonized by *C. minitans* as reported by Merriman et al. (1979). However, the incidences of sclerotium colonization by *C. minitans* in plots treated with Contans WG vs. the control plots did not differ, suggesting that the fact that a majority of the sclerotia are enclosed within the sunflower residues limits the capacity for application of Contans WG by spray boom to lead to a significant degree of colonization of sclerotia. A drench or chemigated application of Contans WG may be more effective than a spray boom application because of the much greater volume of water that can be applied with a drench or chemigated application. However, the microplot trials revealed no colonization by *C. minitans* of 'naked' sclerotia (sclerotia not enclosed in crop residues) that were buried 15 cm deep when Contans WG was drenched over the plots. Further research is needed to determine if drench or chemigated applications of Contans WG could improve the efficacy of the product for control of white mold when applied to infested sunflower crop residues.

Sampling of infested sunflower crop residues past March 2016 was planned for the postharvest Contans WG trial, but the almost complete lack of intact residues by the May 2016 sampling necessitated switching to sampling sclerotia from the soil, and sampling to a depth of 30 cm by sieving the soil in order to recover adequate numbers of sclerotia in a reasonable

amount of time. The moldboard plowing of the field in spring 2016 by the grower, prior to planting a corn crop, greatly enhanced the breakup of residues and buried most of the sclerotia to a depth of ~30 cm. There were no differences in the percentage of viable sclerotia or percentage of sclerotia colonized by *C. minitans* between plots treated with Contans WG vs. non-treated control plots when sclerotia were sampled from the soil in May, August, or October 2016. *C. minitans* survived in soil for up to 2 years when applied in the form of a maizemeal perlite inoculum in a study by McQuilken et al. (1995), and 5 months after application when applied as Contans WG in a study by Zeng et al. (2012a). However, the lack of increased colonization of sclerotia by this mycoparasite in the Contans WG-treated plots vs. the control plots in the fall 2015 and spring 2016 microplot trials, suggests that the level of soilborne *C. minitans* was not enhanced by the Contans WG treatment. This also contradicts the findings by Zeng et al. (2012a) that *C. minitans* colony forming units (cfus) were significantly greater in plots treated with Contans WG than in control plots in a soybean study, even 5 months post application. Also, the persistence of *C. minitans* in the soil for the postharvest Contans WG trial in this study, the average percentage of sclerotia colonized by *C. minitans* had declined to 1.5% 12 months after application, indicating very limited persistence of *C. minitans* in that soil, even at the estimated sclerotial density of 0.14 sclerotia/liter of soil.

Holley and Nelson (1986) found sclerotial densities in the top 16.5 cm of soil ranged from 0.14 to 2.09 sclerotia/liter of soil in sunflower fields in North Dakota, which were associated with Sclerotinia basal stalk rot incidences ranging from 16 to 79%. By comparison, sclerotial density in the Contans WG postharvest trial in this study decreased from 0.28 sclerotia/liter of soil to 0.14 sclerotia/liter of soil in October 2016, which was slightly greater than the range of sclerotial densities reported by Holley and Nelson (1986), although the

sclerotium densities in this study were estimated for the top 30 cm of soil, almost twice as deep as in the North Dakota study. The results illustrate the significant density of sclerotia that can be present in the soil following a hybrid sunflower seed crop with >50% incidence of white mold, and the reason growers have wanted to try a product like Contans WG to reduce sclerotial density following an infected sunflower seed crop.

While it would have been ideal to estimate sclerotial density for each plot at every sampling period once sclerotia had to be sampled from the soil (after crop residues decomposed extensively), it took a minimum of 12 h to collect 25 sclerotia/plot from all eight plots of the trial on 26 May 2016, and 24 h to collect 25 sclerotia/plot by the last sampling on 6 October 2016. Therefore, the estimates of sclerotial density were pooled across the whole trial. More soil could have been sieved and sampled from each plot to mitigate these problems, but significantly more labor would have been needed to sample each plot effectively.

Even if this study had indicated that drench or spray boom applications of Contans WG can be highly efficacious at reducing survival of sclerotia of *S. sclerotiorum*, this might not lead to significant reduction in white mold incidence within a field if the outbreak is initiated primarily by carpogenic germination of sclerotia (McQuilken et al. 1995). Furthermore, ascospores released from apothecia in adjacent fields could cause a white mold outbreak in a sunflower crop, as suggested by Hammond et al. (2008) who did not find a significant effect of *C. minitans* application at reducing the incidence of white mold in potato crops in-season or the season after the applications in the Columbia Basin. In fact, significant movement of ascospores between adjacent fields has been documented by Wegulo et al. (2000). Therefore, applications would have to be made not just in the targeted fields, but to adjacent fields with a history of white mold to avoid the potential of white mold outbreaks being caused by windborne

ascospores from adjacent fields. Thus, applications of Contans WG might not be a viable tool for managing white mold because applications would have to be made to many more fields than those targeted for production of high-value, susceptible crops such as potato or hybrid sunflower seed (Hammond et al. 2008).

Jones et al. (2004) hypothesized that *C. minitans* colonization can be displaced and/or the detection of this mycoparasite can be masked by secondary fungal colonizers of sclerotia. In support of that hypothesis, Jones and Stewart (2000) noted a decline in recovery of *C. minitans* from parasitized sclerotia of *S. sclerotiorum* over time and a concurrent increase in the isolation of other soil fungi, such as *Fusarium* spp. Evidence that *C. minitans* colonization of sclerotia of *S. sclerotiorum* can be masked by other soil fungi was provided by Jones et al. (2003) who reported increased detection of sclerotia parasitized by *C. minitans*, transformed with the hygromycin B resistance gene, when plated onto hygromycin B amended medium vs. detection of other soil fungi when compared to plating parasitized sclerotia on non-amended medium. Plating the sclerotia sampled from the microplot trials and postharvest Contans WG trial in this study regularly revealed the presence of soil fungi other than *C. minitans* on the surface-sterilized sclerotia, including Mucoraceous fungi, *Fusarium* spp., *Trichoderma* spp., *Alternaria* spp., *Epicoccum* spp., and *Penicillium* spp. based on morphological identification using compound and dissecting microscopes, similar to what was reported by Merriman et al. (1979). Therefore, it is possible that detection of sclerotia colonized by *C. minitans* in this study may have been reduced by the presence of other secondary fungal colonizers at the time of assessment. In addition, while *Trichoderma* spp. have been reported to parasitize sclerotia of *S. sclerotiorum* (Budge and Whipps 1991; Jones and Stewart 2000), the contribution of the soil fungi, other than *C. minitans*, to the reduction in viability of sclerotia of *S. sclerotiorum* in this

study is a matter of conjecture. Also, colonization of a sclerotium by *C. minitans* did not necessarily indicate that the sclerotium of *S. sclerotiorum* was non-viable as some sclerotia colonized by *C. minitans* in this study were able to grow on the agar medium and form daughter sclerotia. Whether or not these colonized sclerotia would be able to form apothecia was not assessed, but the observation indicates that sclerotia colonized by this mycoparasite may still have the potential to cause infections of host plants.

In summary, the results of this study suggest that applications of *C. minitans* to sunflower seed crops in the form of Contans WG have limited capacity to reduce sclerotial populations of *S. sclerotiorum* in soils in the Columbia Basin of central Washington. Applications of Contans WG did not affect survival of sclerotia except for sclerotia not enclosed in crop residues on the soil surface. In contrast, burying sclerotia and infested crop residues into the soil was a highly effective means to reduce sclerotium survival, demonstrating the importance of timely incorporation of infested crop residues for management of white mold regionally following outbreaks of the disease in susceptible crops.

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Table 4.1. Effect of Contans WG application rate, sclerotium location (on the soil surface or buried 15 cm deep), and sampling time on the viability, colonization by *Coniothyrium minitans*, and disintegration of sclerotia for a 2015 spring microplot sclerotium survival trial in the Columbia Basin of central Washington

Factor and treatment[a]	Mean incidence of viable sclerotia (%)[b]	Mean number of sclerotia colonized by *C. minitans*[b]		Mean incidence of disintegrated sclerotia (%)[b]	
	17 June + 11 August	17 June	11 August	17 June	11 August
Factor					
Contans	0.3360[c]	0.0267*	0.0210*	0.0105*	0.1594
Sclerotium location	<0.0001**	0.0201*	0.0268*	<0.0001**	<0.0001**
Sampling time	0.0032**	-	-	-	-
Contans x Sclerotium location	0.3677	0.0190*	0.0140*	0.0061**	0.3327
Time x Contans	0.5246	-	-	-	-
Time x Sclerotium location	0.5573	-	-	-	-
Time x Contans x Sclerotium location	0.8432	-	-	-	-
Transformation[d]	Arcsine	Rank	Rank	Rank	Square root
Sclerotium location					
Buried	10.3 a[e]	-	-	-	50.0 a
Surface	84.5 b	-	-	-	2.7 b
Sampling time					
June	54.2 a	-	-	-	-
August	40.7 b	-	-	-	-
Treatment combination					
Buried: 0.00 kg Contans WG/ha	-	0.0 a	0.0 a	29.0 a	-
Buried: 0.56 kg Contans WG/ha	-	0.0 a	0.0 a	35.0 a	-
Buried: 4.48 kg Contans WG /ha	-	0.0 a	0.0 a	15.0 b	-
Surface: 0.00 kg Contans WG /ha	-	0.5 a	0.0 a	0.0 c	-
Surface: 0.56 kg Contans WG /ha	-	8.0 b	2.5 b	0.0 c	-
Surface: 4.48 kg Contans WG /ha	-	10.5 b	4.0 b	0.0 c	-

[a] The data were analyzed as a repeated measures, split plot, randomized complete block (RCB) design with four replications. Contans WG treatments were the main plot factor, sclerotium location the split plot factor, and sampling time the repeated measure, if homogeneity of variance over time was satisfied. Otherwise, each sampling time was analyzed individually as a split plot, RCB design with four replications, with Contans WG treatments as the main plot factor and sclerotium location as the split plot factor.

[b] Percentage of sclerotia that were viable, mean number of sclerotia colonized by *C. minitans*, or percent of sclerotia disintegrated out of 25 sclerotia in each mesh bag/split plot at each sampling time. Percentage of sclerotia disintegrated was calculated as: 100 x [25 – (number of sclerotia recovered from the mesh bag)]/25.

[c] Probability of means square > F in the restricted maximum likelihood (REML) estimation of variance components for that main effect or interaction term. * and ** = significant at $P = 0.05$ and 0.01, respectively.

[d] "Arcsine", "Rank", and "Square root" indicate the data were subjected to arcsine square root transformation, non-parametric rank transformation, and square root transformation, respectively, to meet assumptions of parametric analysis.

[e] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$). For variables subjected to arcsine, square root, and rank transformations, original means are presented although means separations were calculated based on transformed data.

Table 4.2. Effect of Contans WG application rate and sclerotium location (on the soil surface or buried 15 cm deep) on viability of sclerotia in a 2015 fall microplot sclerotium survival trial in the Columbia Basin of central Washington

Factor and treatment[a]	Mean percentage of viable sclerotia (%)[b]				
	19 March	6 May	24 June	24 August	28 October
Factor					
Contans	0.4018[c]	0.7483	0.7007	0.2512	0.0140*
Sclerotium location	<0.0001**	<0.0001**	<0.0001**	0.0138*	0.0043**
Contans x Sclerotium location	0.7573	0.9740	0.5711	0.1169	0.0392*
Transformation[d]	None	None	Log	Log	Log
Sclerotium location					
Buried	17.0 a[e]	6.7 a	2.0 a	4.7 a	-
Surface	71.0 b	61.7 b	32.3 b	19.0 b	-
Treatment combination					
Buried: 0.00 kg Contans WG/ha	-	-	-	-	1.0 a
Buried: 0.56 kg Contans WG/ha	-	-	-	-	6.0 a
Buried: 4.48 kg Contans WG/ha	-	-	-	-	1.0 a
Surface: 0.00 kg Contans WG/ha	-	-	-	-	42.0 b
Surface: 0.56 kg Contans WG/ha	-	-	-	-	6.0 a
Surface: 4.48 kg Contans WG/ha	-	-	-	-	1.0 a

[a] Variances over sampling times were heterogeneous. Therefore, data for each sampling time were analyzed individually as a split plot, randomized complete design with four replications. Contans WG treatments were the main plot factor, and sclerotium location the split plot factor.

[b] Percentage of sclerotia that were viable out of 25 sclerotia per mesh bag/split plot at each sampling time.

[c] Probability of the means square > F in the restricted maximum likelihood (REML) estimation of variance components for that main effect or interaction term. * and ** = significant at $P = 0.05$ and 0.01, respectively.

[d] "Log" indicates the data were subjected log transformation to meet assumptions of parametric analysis.

[e] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$). For variables subjected to transformation, original means are presented although means separations were calculated based on transformed data.

Table 4.3. Effect of Contans WG application rate and sclerotium location (on the soil surface or buried 15 cm deep) on colonization of sclerotia by *Coniothyrium minitans* for a 2015 fall microplot sclerotium survival trial in the Columbia Basin of central Washington

Factor and treatment[a]	Mean number of sclerotia colonized by *C. minitans*[b]				
	19 March	6 May	24 June	24 August	28 October
Factor					
Contans	0.3528[c]	0.1992	0.2336	0.4450	0.0756
Sclerotium location	0.5704	<0.0001**	<0.0001**	0.0071**	<0.0001**
Contans x Sclerotium location	0.0990	0.2257	0.6598	0.4958	0.1907
Transformation[d]	None	Square root	Square root	Log	Rank
Sclerotium placement					
Buried	-	1.3 a[e]	0.3 a	0.6 a	0.1 a
Surface	-	15.8 b	14.6 b	5.7 b	6.5 b

[a] Variances over sampling time were heterogeneous. Therefore, data for each sampling time were analyzed individually as a split plot, randomized complete block design with four replications. Contans WG treatments were the main plot factor, and sclerotium location the split plot factor.

[b] Number of sclerotia colonized by *C. minitans* out of 25 sclerotia in one mesh bag/split plot at each sampling time.

[c] Probability of the means square > F in the restricted maximum likelihood (REML) estimation of variance components for that main effect or interaction term. * and ** = significant at $P = 0.05$ and 0.01, respectively.

[d] "Square root", "Log", and "Rank" indicate the data were subjected to square root transformation, log transformation, and non-parametric rank transformation, respectively, to meet assumptions of parametric analysis.

[e] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$). For variables subjected to transformation, original means are presented although means separations were calculated based on transformed data.

Table 4.4. Effect of Contans WG application rate and sclerotium location (on the soil surface or buried 15 cm deep) on disintegration of sclerotia for a 2015 fall microplot sclerotium survival trial in the Columbia Basin of central Washington

Factor and treatment[a]	Mean percentage of disintegrated sclerotia[b]				
	19 March	6 May	24 June	24 August	28 October
Factor					
Contans	0.9516[c]	0.8211	0.6132	0.3125	0.0521
Sclerotium location	0.1644	<0.0001**	<0.0001**	<0.0001**	<0.0001**
Contans x Sclerotium location	0.9511	0.8191	0.7314	0.6010	0.0649
Transformation[d]	Rank	Rank	Arcsine	Arcsine	Arcsine
Sclerotium placement					
Buried	-	77.3 a[e]	89.7 a	87.7 a	95.3 a
Surface	-	0.0 b	1.0 b	9.7 b	30.7 b

[a] Variances over sampling times were heterogeneous. Therefore, data for each sampling time were analyzed individually as a split plot, randomized complete block design with four replications. Contans WG treatments were the main plot factor, and sclerotium location the split plot factor.

[b] Percentage of sclerotia disintegrated out of 25 sclerotia in one mesh bag/split plot at each sampling time, calculated as: 100 x [25 – (number of sclerotia recovered from the mesh bag)]/25.

[c] Probability of the means square > F in the restricted maximum likelihood (REML) estimation of variance components for that main effect or interaction term. * and ** = significant at $P = 0.05$ and 0.01, respectively.

[d] "Rank" and "Arcsine" indicate the data were subjected to non-parametric rank transformation and arcsine square root transformation, respectively, to meet assumptions of parametric analysis.

[e] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$). For variables subjected to transformation, original means are presented although means separations were calculated based on transformed data.

Table 4.5. Effect of Contans WG application rate, sclerotium location (on the soil surface or buried 15 cm deep), and sampling time on the viability of sclerotia and *Coniothyrium minitans* colonization of sclerotia for a 2016 spring microplot sclerotium survival trial in the Columbia Basin of central Washington

Factor and treatment[a]	Mean percentage of sclerotia viable[b]				Mean number of sclerotia colonized by *C. minitans*[ab]
	6 May	24 June	31 August	28 October	
Factor					
Contans	0.5309[c]	0.0744	0.1768	0.4738	0.0923
Sclerotium location	<0.0001**	<0.0001**	0.0016**	0.0007**	0.0003**
Time	-	-	-	-	0.6847
Contans x Sclerotium location	0.4849	0.4681	0.0797	0.0190*	0.2138
Time x Contans	-	-	-	-	0.1535
Time x Sclerotium location	-	-	-	-	0.2746
Time x Contans x Sclerotium location	-	-	-	-	0.0649
Transformation[d]	None	None	Arcsine	Arcsine	Square root
Sclerotium placement					
Buried	33.7 a[e]	25.0 a	9.0 a	-	0.9 a
Surface	90.7 b	86.0 b	31.7 b	-	6.2 b
Treatment combination					
Buried: 0.00 kg Contans WG/ha	-	-	-	3.0 a	-
Buried: 0.56 kg Contans WG/ha	-	-	-	7.0 a	-
Buried: 4.48 kg Contans WG/ha	-	-	-	12.0 a b	-
Surface: 0.00 kg Contans WG/ha	-	-	-	49.0 b	-
Surface: 0.56 kg Contans WG/ha	-	-	-	20.0 a b	-
Surface: 4.48 kg Contans WG/ha	-	-	-	18.0 a b	-

[a] The data were analyzed as a repeated measures, split plot, randomized complete block (RCB) design with four replications. Contans WG treatments were the main plot factor, sclerotium location the split plot factor, and sampling time the repeated measure, if homogeneity of variance over time was satisfied. Otherwise, each sampling time was analyzed individually as a split plot, RCB design with four replications, Contans WG treatments as the main plot factor, and sclerotium location as the split plot factor. Variances were homogeneous for *C. minitans* colonization data from the 6 May, 24 June, 24 August, and 28 October sampling times and therefore the sampling times were analyzed together as described above.

[b] Percentage of sclerotia that were viable or mean number of sclerotia colonized by *C. minitans*, out of 25 sclerotia in each mesh bag/split plot at each sampling time.

[c] Probability of the means square > F in the restricted maximum likelihood (REML) estimation of variance components for that main effect or interaction term. * and ** = significant at $P = 0.05$ and 0.01, respectively.

[d] "Arcsine" and "Square root" indicate the data were subjected to arcsine square root transformation and square root transformation, respectively, to meet assumptions of parametric analysis.

[e] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$). For variables subjected to transformation, original means are presented although means separations were calculated based on transformed data.

Table 4.6. Effect of Contans WG application rate and sclerotium location (on the soil surface or buried 15 cm deep) on disintegration of sclerotia for a 2016 spring microplot sclerotium survival trial in the Columbia Basin of central Washington

Factor[a] and treatment	Mean percentage of disintegrated sclerotia[b]		
	24 June	31 August	28 October
Contans	0.2230[c]	0.3380	0.6845
Sclerotium location	0.0037**	0.0002**	0.0164*
Contans x Sclerotium location	0.2113	0.3474	0.3067
Transformation[d]	Rank	Square root	None
Sclerotium location			
Buried	8.0 a[e]	27.3 a	45.7 a
Surface	0.0 b	2.7 b	15.3 b

[a] Variances over sampling times were heterogeneous. Therefore, each sampling time was analyzed individually as a split plot, randomized complete block design with four replications. Contans WG treatments were the main plot factor, and sclerotium location was the split plot factor.

[b] Percentage of sclerotia disintegrated out of 25 sclerotia in one mesh bag/split plot at each sampling time, calculated as: 100 x [25 – (number of sclerotia recovered from the mesh bag)]/25.

[c] Probability of the means square > F in the restricted maximum likelihood (REML) estimation of variance components for that main effect or interaction term. * and ** = significant at $P = 0.05$ and 0.01, respectively.

[d] "Rank" and "Square root" indicate the data were subjected to non-parametric rank transformation and square root transformation, respectively, to meet assumptions of parametric analysis.

[e] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$). For variables subjected to transformation, original means are presented although means separations were calculated based on transformed data.

Table 4.7. Effect of Contans WG application and sclerotium location (on the soil surface or buried 15 cm deep) on viability and *Coniothyrium minitans* colonization of sclerotia collected on 1 March 2016 from sunflower residues in a 2015 postharvest Contans WG application trial

Factor and treatment[a]	Mean incidence of viable sclerotia (%)[b]	Mean incidence of sclerotia colonized by *C. minitans* (%)[b]
Factor		
Contans	1.0000[c]	0.3256
Sclerotium location	0.0054**	0.2165
Contans x Sclerotium location	0.3414	0.3698
Sclerotium location		
Buried residue	73.0 a[d]	-
Surface residue	87.5 b	-
Treatment combination		
Buried: 0.00 kg Contans WG/ha	-	38.0 a
Buried: 4.48 kg Contans WG/ha	-	38.5 a
Surface: 0.00 kg Contans WG/ha	-	26.0 a
Surface: 4.48 kg Contans WG/ha	-	36.5 a

[a] Data were analyzed as a split plot, randomized complete block design with four replications. Contans WG treatments were the main plot factor, and sclerotium location the split plot factor.

[b] Percentage of sclerotia that were viable or colonized with *C. minitans*, calculated based on 50 sclerotia collected from each of surface sunflower crop residues and buried sunflower crop residues.

[c] Probability of the means square > F in the restricted maximum likelihood (REML) estimation of variance components for that main effect or interaction term. ** = significant at $P = 0.01$.

[d] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$).

Table 4.8. Effect of Contans WG application and sampling time on the viability and *Coniothyrium minitans* colonization of sclerotia sampled on 26 May, 4 August, and 8 October 2016 from the soil to a depth of 30 cm in a 2015 postharvest Contans WG trial

Factor and treatment[a]	Mean incidence of viable sclerotia (%)[b]	Mean incidence of sclerotia colonized by *C. minitans* (%)[b]
Factor		
Contans	0.3854[c]	0.3969
Sampling time	0.1698	0.0001**
Sampling time x Contans	0.4464	0.2409
Transformation[d]	None	Square root
Sampling time		
6 May	-	9.5 a[e]
2 and 4 August	-	1.3 b
8 October	-	1.5 b

[a] Data were analyzed as a repeated measures, randomized complete block design with four replications. Contans WG treatments were the main plot factor and sampling time was the repeated measure.

[b] Percentage of sclerotia that were viable or colonized with *C. minitans* calculated from: i) 25 sclerotia/plot collected on 26 May 2016, ii) 40 sclerotia/plot collected on 2 to 4 August using the same protocol, and v) 25 sclerotia/plot collected on 8 October 2016 (by sampling soil to a depth of 30 cm and then sifting the soil to find sclerotia in four replicate plots/treatment).

[c] Probability of the means square > F in the restricted maximum likelihood (REML) estimation of variance components for that main effect or interaction term. ** = significant at $P = 0.01$.

[d] "Square root" indicates the data were subjected to a square root transformation to meet assumptions of parametric analysis.

[e] Within each column, means followed by same letter are not significantly different based on Tukey's honestly significant difference ($P < 0.05$).

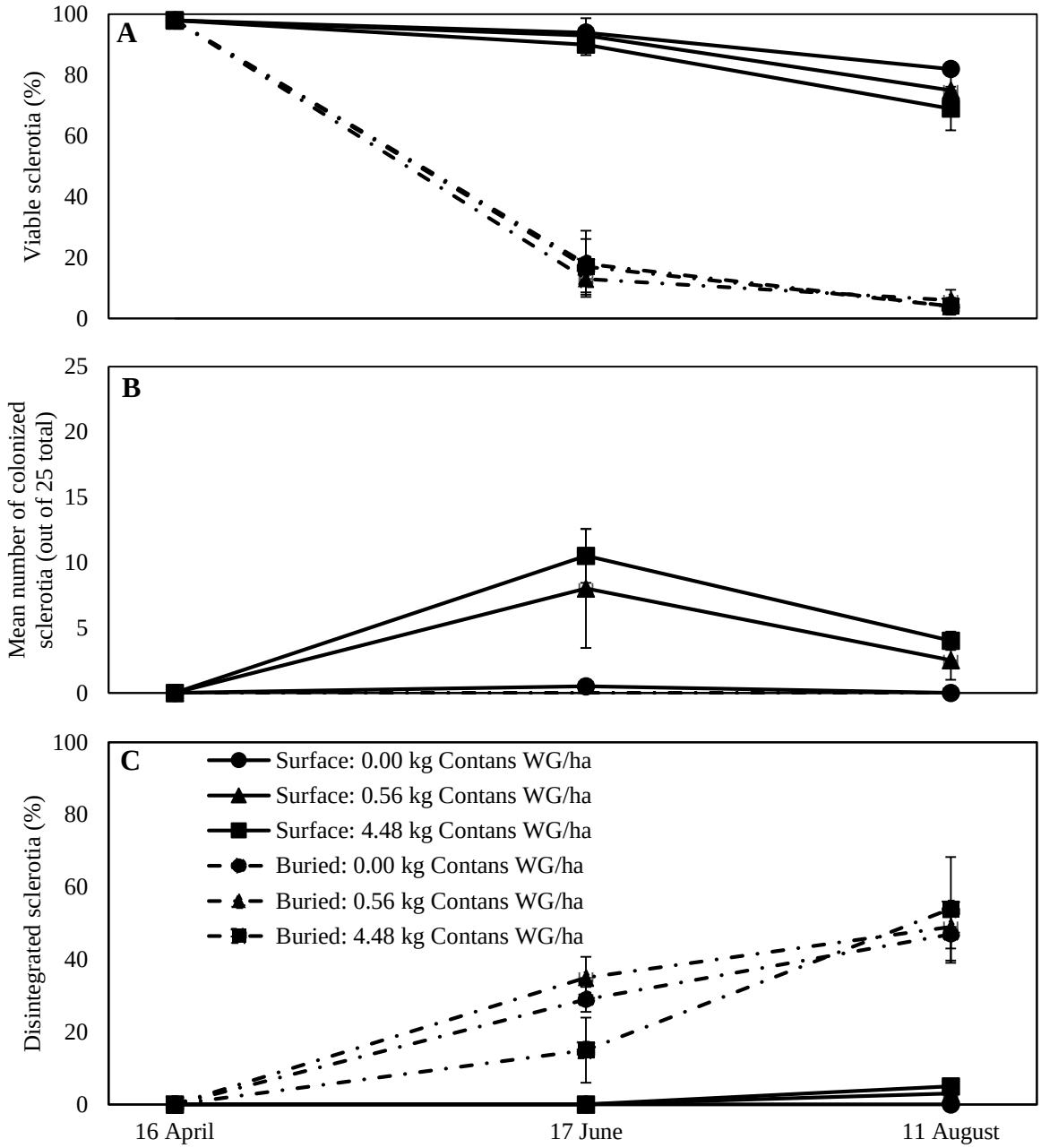

Fig. 4.1. (**A**) Mean percentage of sclerotia that were viable, (**B**) mean number of sclerotia colonized by the mycoparasitic fungus *Coniothyrium minitans*, or (**C**) mean percentage of disintegrated sclerotia (not recovered) from the 2015 spring micro-plot sclerotium survival trial. Contans WG drench treatments were applied at 0, 0.56, and 4.48 kg/ha. Surface = sclerotia in mesh bags (25/bag) were placed on the soil surface. Buried = sclerotia in mesh bags (25/bag) buried 15 cm deep. Colonized sclerotia data are expressed out of 25 because disintegrated sclerotia could not be assessed for *C. minitans* colonization. Each data point represents the mean ± standard error of four replicate plots. Refer to details in the main text.

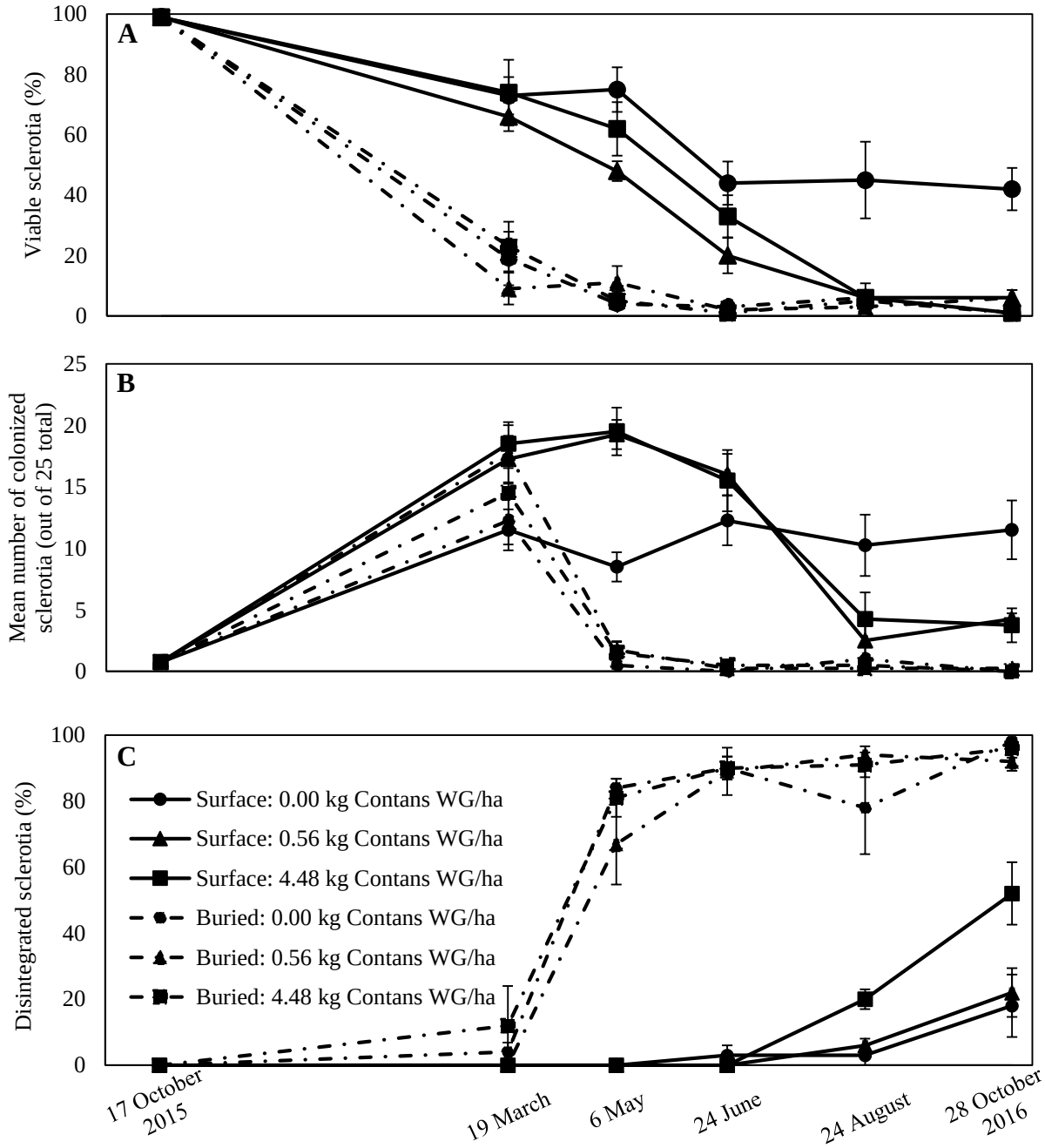

Fig. 4.2. (**A**) Mean percentage of sclerotia that were viable, (**B**) mean number of sclerotia colonized by the mycoparasitic fungus *Coniothyrium minitans*, or (**C**) mean percentage of sclerotia disintegrated (not recovered) from the 2015 fall micro-plot sclerotium survival trial. Contans WG drench treatments were applied at 0, 0.56, and 4.48 kg/ha. Surface = sclerotia in mesh bags (25/bag) were placed on the soil surface. Buried = sclerotia in mesh bags (25/bag) buried 15 cm deep. Colonized sclerotia data are expressed out of 25 because disintegrated sclerotia could not be assessed for *C. minitans* colonization. Each data point represents the mean ± standard error of four replicate plots. Refer to details in the main text.

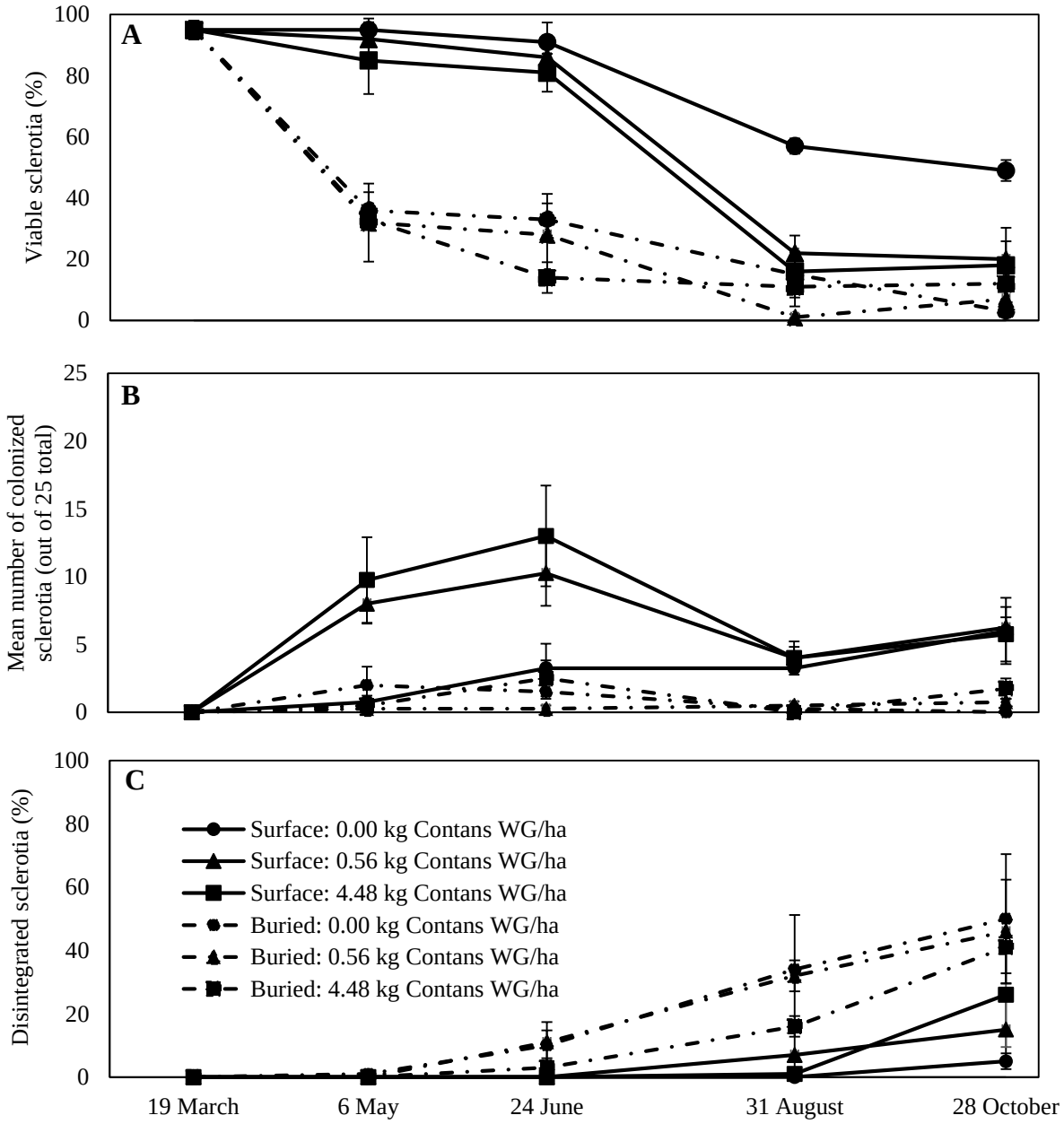

Fig. 4.3. (**A**) Mean percentage of sclerotia that were viable, (**B**) mean number of sclerotia colonized by the mycoparasitic fungus *Coniothyrium minitans*, and (**C**) mean percentage of sclerotia disintegrated (not recovered) from the 2016 spring micro-plot sclerotium survival trial. Contans WG drench treatments were applied at 0, 0.56, and 4.48 kg/ha. Surface = sclerotia in mesh bags (25/bag) were placed on the soil surface. Buried = sclerotia in mesh bags (25/bag) buried 15 cm deep. Colonized sclerotia data are expressed out of 25 because disintegrated sclerotia could not be assessed for *C. minitans* colonization. Each data point represents the mean ± standard error of four replicate plots. Refer to details in the main text.

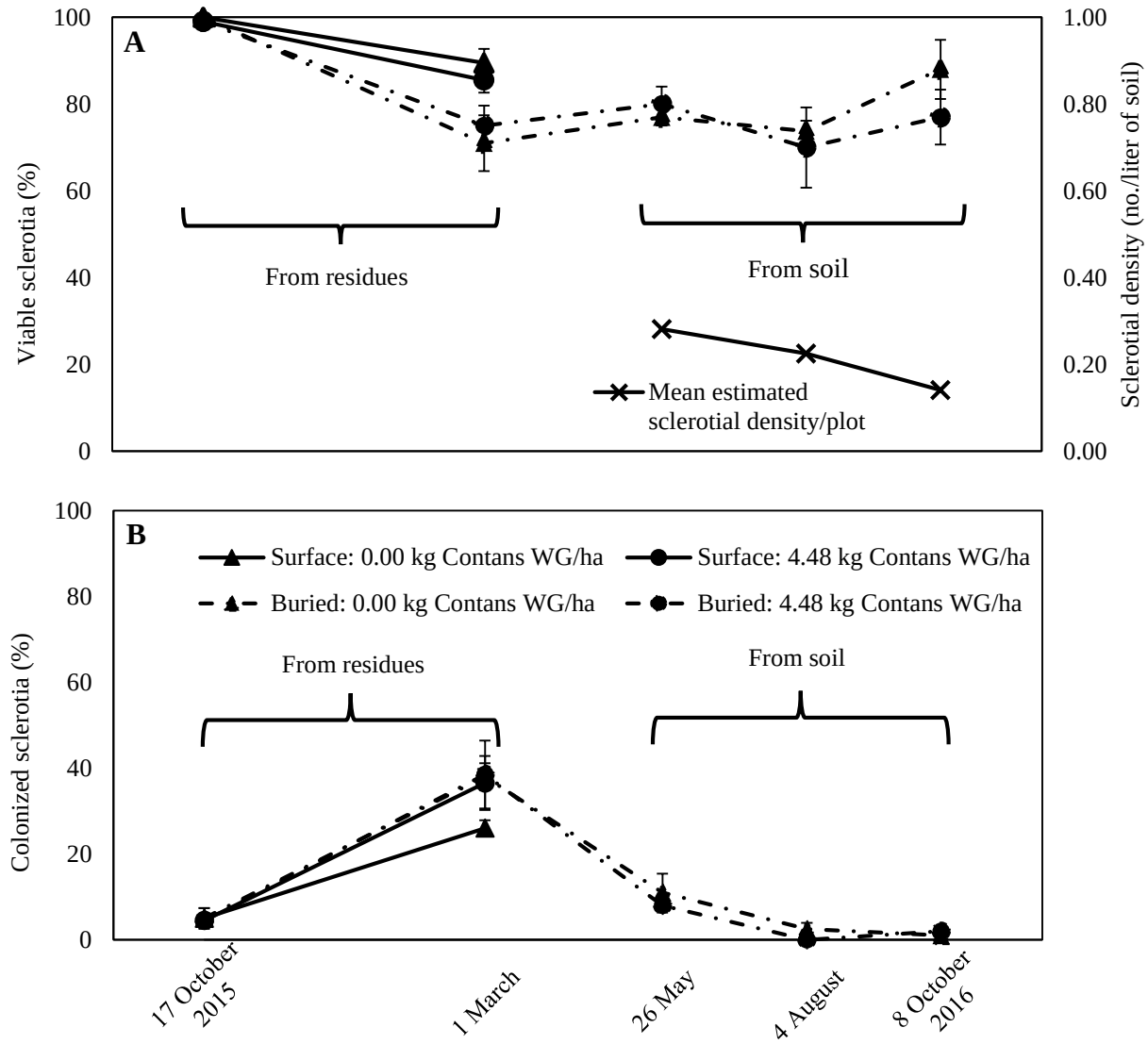

Fig. 4.4. (**A**) Mean percentage of sclerotia that were viable and mean estimated sclerotial density/plot, and (**B**) mean percentage of sclerotia colonized by the mycoparasitic fungus *Coniothyrium minitans* from a 2015 postharvest Contans trial near Quincy, WA. Sclerotia sampled in October 2015 and March 2016 were collected from sunflower stalk residues. Collections of 25, 40, and 25 sclerotia/plot were made in May, August, and October 2016, respectively, from soil sampled to a depth of 30 cm. Mean sclerotial density/plot for May, August, and October was estimated based on the amount of soil that had to be sieved to collect the particular number of sclerotia at each sampling time. Contans WG drench treatments were applied at 0 and 4.48 kg/ha. Surface = sclerotia sampled from surface sunflower crop residues. Buried = sclerotia sampled from buried residues or sampled by sieving soil. Each data point represents the mean ± standard error of four replicate plots. Refer to details in the main text.