MANAGEMENT OF DAMPING-OFF CAUSED BY *PYTHIUM* SPP. IN ORGANIC VEGETABLE PRODUCTION IN THE PACIFIC NORTHWEST

By

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MANAGEMENT OF DAMPING-OFF CAUSED BY *PYTHIUM* SPP.

IN ORGANIC VEGETABLE PRODUCTION

IN THE PACIFIC NORTHWEST

Abstract

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Vegetable production is an important sector of the organic food industry in the Pacific Northwest USA. Significant acres of organic vegetable crops, particularly processing green pea and sweet corn, are grown in the semi-arid Columbia Basin of central Washington and north central Oregon, where Pythium damping-off can cause losses in early spring when cool and wet soil conditions are conducive to the disease. In this study, 37 certified organic fields were surveyed in the Columbia Basin for *Pythium* species, from which 305 isolates were baited and identified to 19 species. Pathogenicity tests of isolates of each species on pea in cool and wet soil conditions revealed isolates of 9 species were pathogenic, with differences in virulence among species and among isolates within species. *Pythium ultimum* (24.6% of the 305 isolates), *P. irregulare* group 1 (15.1%), and *P. abappressorium* (4.9%) were the most prevalent pathogenic species. Real-time PCR assays detected *P. ultimum* in 100% of the 37 fields compared to 78% for *P. abappressorium*, and 57% for *P. irregulare* group 1.

Given the lack of highly effective seed treatments for damping-off control in organic production, organic seed and drench treatments were evaluated in five pea field trials in the

Columbia Basin, and two pea trials plus one sweet corn trial in maritime western Washington in 2011-12. Nordox seed treatment and seed priming demonstrated the greatest potential for damping-off control. Pea seed priming with 16 h of seed soaking + 10 h of air drying optimized priming for rapid emergence. In two field trials in 2012, combining Nordox seed treatment with seed priming using biochar as an alternative to air-drying, was promising for damping-off control.

Seed exudates produced during germination are measured using electrical conductivity (EC). The EC levels of 17 pea seed lots (six cultivars) significantly affected emergence and susceptibility to Pythium damping-off. A negative linear relationship was demonstrated between EC level and emergence, with a stronger regression at higher inoculum levels of *P. ultimum*. Measuring the EC of pea seed lots, and quantifying *Pythium* inoculum in soil using real-time PCR assays may enable organic pea growers to assess accurately the risk of damping-off.

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Literature review

1.1. Organic vegetable production in the Pacific Northwest

The dramatic increase in growth of the organic food industry in the US started in 1990 and has continued to the present with significant dollar contributions to total food sales (Greene and Dimitri, 2007; Organic Monitor, 2011a). In 2011, organic food sales reached $31 billion, which represented 4.2% of total US food sales, with organic vegetable and fruit sales having the greatest contribution to the increase (Haumann, 2012). Acreage of land devoted to organic production has also increased immensely over the years, reaching 1.9 million ha in 2008 [United States Department of Agriculture National Agricultural Statistics Service (USDA, NASS), 2008; Organic Trade Association, 2011]. Similar trends in organic industries are occurring worldwide (Organic Monitor, 2011b; Organic World, 2011) with global organic sales reaching $59.4 billion in 2009 and a total of 15.1 million acres managed organically (Organic Trade Association, 2011). With the continued demand for expansion in the availability of organic foods, a bright future is projected for the organic industry (Greene and Dimitri, 2007; Organic Trade Association, 2011).

The Pacific Northwest (PNW) is the center of a large area of organic production. An eight-fold increase in organic acreage in Washington State was recorded from 1993 to 2002, prior to which Idaho was the leader in organic acreage in the US (Granatstein et al., 2005). Idaho is a leading state in producing wildcrafted plants such as St. Johnsworth, Oregon is recognized for the large certified organic acreage of ornamental nursery plants, and Washington's primary contribution is organic fruit production, particularly apples, pears, and cherries (Granatstein et

al., 2005). By 2008, Washington and Oregon ranked third and fifth among the top 10 states with the greatest number of certified organic farms in the US (USDA NASS, 2008).

The certified organic vegetable industry experienced the fastest growth of the organic sector over the last five years (Organic Trade Association, 2011; USDA NASS, 2010). Nationally, organic vegetables ranked first in production acreage and highest in sales in the organic industry (USDA NASS, 2010). In 1998, vegetables were grown on about 0.6 million ha in the US, 1% of which was devoted to organic vegetables (USDA, 1998). By 2001, approximately 28,975 ha were planted to certified organic vegetable crops, which accounted for 1.6% of the total US vegetable acreage (Rawson, 2007). Within the three PNW states, Washington had the greatest number of hectares devoted to organic vegetable production, followed by Oregon and Idaho, although similar trends in organic vegetable sales value were observed for the three states (Kirby and Granatstein, 2009). A USDA survey in 2005 showed that Washington was second to California in organic vegetable production in the US, contributing 10% to the national total acreage in organic vegetable production (Kirby and Granatstein, 2009). In Washington and Oregon, vegetables ranked second among the leading organic crops produced, contributing 21 and 5%, respectively, to the two states' total organic production (Kirby and Granatstein, 2009). Sweet corn, green pea, snap bean, onion, potato, and carrot were the major organic vegetables grown in the PNW (Kirby and Granatstein, 2009). The growth trends for acreage devoted to organic vegetable production doubled every year from 2004 to 2007. The trend was interrupted from 2008 and to 2010 during the US economic recession, but resumed in 2011 (Kirby and Granatstein, 2012).

Processing and fresh produce are the two main markets of organically produced vegetables. Although fresh produce comprises the bulk of organic production, an increase in

acreage devoted to processing vegetables is projected as the demand for ingredients for processed organic foods continues to rise [Washington State Department of Agriculture (WSDA), 2009]. The establishment of the USDA National Organic Program (NOP) in 2002 promoted expansion of certified organic crop acreage that led to security of supply for major processors, and encouraged the introduction of many new organic products (WSDA, 2009). In Washington State, the majority of the large-scale organic vegetable production is concentrated in the semi-arid, irrigated Columbia Basin east of the Cascade Mountains (Alcala and du Toit, 2009; Granatstein et al., 2005). A majority of the organic growers in the Columbia Basin produce one or two crops every season, with most of the production devoted to processing markets (Alcala and du Toit, 2009; Granatstein et al., 2005). In contrast, small-scale organic vegetable farms, typically characterized as diverse operations, that grow many types of crops in one cropping season, are common west of the Cascades (Alcala and du Toit, 2009; Granatstein et al., 2005). The produce from these smaller-scale farms typically satisfies local organic vegetable demands, e.g., farmer's markets, CSAs, and local stores (Alcala and du Toit, 2009; Granatstein et al., 2005).

1.2. Organic pea production in the Pacific Northwest

Organic, processed, green pea is an economically important vegetable crop in Washington (Kirby and Granatstein, 2009). It is a short-season crop that is typically double-cropped with sweet corn in the Columbia Basin (Kirby and Granatstein, 2009). Two-thirds of Washington's organic vegetable acreage is planted to green peas and sweet corn, making the state the largest producer of these organic vegetables in the US (Kirby and Granatstein, 2009). Green pea is an annual, cool-season, legume crop (Deshpande and Adsule, 1998). Pea is among the crops that can be planted early in the spring in temperate regions of the world (Kraft and

Pfleger, 2001). In the PNW, planting can start as early as the last week of February in the southern Columbia Basin and in the Willamete Valley in western Oregon, to as late as June in Grays Harbor Co. in Washington, although the ideal seeding date, particularly in central Washington, is March to mid-May (Mansour et al., 1984). Planting as early as possible in some areas is recommended for higher yields, since pods develop and mature during cooler months; however, yields from early spring planting are less predictable because of a greater risk of encountering poor stands (Alcala and du Toit, 2009; Mansour et al., 1984).

Pea production in the PNW is divided into two regions, west and east of the Cascades, dictated primarily by differences in climatic zones and crops grown in rotations (Granatstein, 2005). Peas grown west of the Cascades receive moisture from the abundant rainfall of the region, while crops grown east of the Cascades are mostly irrigated (Mansour et al., 1984). In Washington, most organic pea farms are situated in the semi-arid, irrigated area of the Columbia Basin, where peas are typically grown as a short rotation crop (Granatstein et al., 2005; Kirby and Granatstein, 2009).

The majority of organic pea crops grown in the PNW are produced largely for processing markets, either for freezing or canning (Kirby and Granatstein, 2009). The growing demand for organic processing pea is dictated by the market expansion for processed organic foods (Dimitri and Greene, 2007). Growers of organic peas for the processing market are classified as highly specialized, characterized by growing only two to five types of crops on large areas of certified organic land (Buck et al., 1997). According to Buck et al. (1997), large organic farms are more likely to operate like conventional farms than smaller-scale organic farms. Growers are often bound by contracts with processors before planting begins and, hence, must adhere to the timing and conditions required by the processors (Alec McErlich, *personal communication*).

1.3. Damping-off in organic crop production

Damping-off is a soilborne disease of worldwide importance that can be a significant problem in conventional or organic production (Laemmlen, 2001). In organic production, problems related to this disease are aggravated by the lack of highly effective seed treatments (Groot et al., 2004). Juvenile and succulent plant parts such as seeds, seedlings, and roots are particularly susceptible to infection (Agrios, 2005; Laemmlen, 2001). The greatest damage usually occurs when infection occurs early, prior to or immediately after emergence (Agrios, 2005). Infection of seeds can result in failure of germination, where seeds become rotten, soft and mushy, and disintegrate, causing pre-emergence damping-off (Agrios, 2005; Hendrix and Campbell, 1973). In post-emergence damping-off, seedlings are infected in the roots or the stems at the soil line, and the invaded tissues become water-soaked, discolored, and soon collapse (Agrios, 2005; Hendrix and Campbell, 1973). Damping-off often results in poor stands, which can be mistaken for poor seed quality or seed maggot damage rather than damping-off (Hendrix and Campbell, 1973; Laemmlen, 2001). *Pythium* spp., *Fusarium* spp., and *Rhizoctonia* spp. are the soilborne pathogens commonly associated with damping-off (Laemmlen, 2001). Occurrence of pre-emergence damping-off during cool and wet soil conditions is usually associated with *Pythium* species (Hendrix and Campbell, 1973; Laemmlen, 2001). If *Pythium* infection occurs after emergence, infection typically is restricted to the feeder roots and root tips (Hendrix and Campbell, 1973). *Pythium* infection usually begins as a root rot, while *Rhizoctonia* often infect plants at or near the soil surface (Laemmlen, 2001). Root pruning or necrosis, as a result of infection, reduces lateral root development in plants and may lead to lack of vigor and, later, poor yields (Koike et al., 2007; Kraft and Pfleger, 2001). *Fusarium* spp. typically cause wilt,

attack older seedlings, and are more virulent pathogens in warm soil conditions, e.g., 28°C (Bruehl, 1987).

Damping-off can be a concern in organic vegetable production. In the Columbia Basin of the PNW, damping-off is encountered more commonly when planting is done early in the spring in cool and wet soil conditions, which favor development of the disease (Alcala and du Toit, 2009). Under these conditions, damping-off is typically associated with *Pythium* spp. (Agrios, 2005), and is most significant in crops such as pea (Kraft and Pfleger, 2001). Damping-off can be more readily avoided when planting is delayed until after soil temperatures reach > 10°C (Cook, 2002). However, organic growers may not always have the flexibility to choose later planting dates, particularly when the crops are contracted with processors (Alec McErlich, *personal communication*). Often, in anticipation of encountering poor stands, growers increase the amount of seeds planted (Alcala and du Toit, 2009). This, however, adds to production costs, especially for expensive certified organic seeds (Alcala and du Toit, 2009). Damping-off infection in the worst situations can lead to the growers replanting entire fields (Alcala and du Toit, 2009). In conventional production, damping-off typically is controlled effectively by the use of seed treatments with fungicides such as metalaxyl or the isomer, mefenoxam (McGee, 1992). The lack of availability of such highly effective organic seed treatments exacerbates the problem in certified organic production. Therefore, growers of certified organic crops rely mostly on planting high quality seed lots with good vigor as a defense against damping-off. Although there are commercially available organic seed treatments with efficacy against damping-off stated on the labels, a majority of these products lack consistent efficacy or have limited efficacy under field conditions, especially in cold soils (McSpadden Gardener, 2002). Damping-off also can be particularly problematic in fields that have been transitioned from

conventional production to organic production practices, as no natural suppression from many soilborne diseases may have yet built-up (Baysal et al., 2008). During the transition period for organic certification, growers have to deal with maintaining yields while combating diseases (Zinati, 2002). Managing soilborne pathogens that cause damping-off is critical during this period, to avoid build-up of inoculum in the soil (Koike et al., 2000).

1.3.1. *Pythium* spp.

The genus *Pythium* consists of diverse and ubiquitous species, many of which are important soilborne plant pathogens that cause disease of plants in agriculture, forestry, and greenhouse environments (Hendrix and Campbell, 1973). There are about 140 recognized *Pythium* species (Bala et al., 2010; Lévesque, 2011; Vander Plaats-Niterink, 1981), although currently there are approximately 280 legitimate species listed in the MycoBank database (http://www.mycobank.org/). *Pythium* is a member of the Pythiaceae in the class Oomycota and order Peronosporales, which was recently placed in the kingdom Chromista of the phylum Stramenopiles (Kirk et al., 2008). Once classified as fungi, the oomycetes are now described as more closely related to the diatoms and golden-brown algae (Bauldaf et al., 2000). Some important characteristics that separate oomycetes from the true fungi include: (1) the cell wall is composed mostly of cellulose instead of chitin, (2) zoospores are bi-flagellated while zoospores of true fungi only have one flagellum, (3) a different metabolic pathway is utilized for synthesizing lysine than the true fungi, and (4) mitochondria are tubular instead of flattened cristae (Van der Auwera et al., 1995).

Many *Pythium* species are known to be important soilborne plant pathogens, while others are non-pathogenic, and some even have potential use for biological control (Hendrix and Campbell, 1973; Martin and Loper, 1999). Plant pathogenic *Pythium* species can affect a wide

variety of economically important agricultural crops (Agrios, 2005; Hendrix and Campbell, 1973). These species are most commonly identified as causal agents of pre- and post-emergence damping-off, leading to poor stands and low crop vigor (Agrios, 2005). Some *Pythium* species can infect mature plants, causing significant damage to yields, such as the species that cause carrot cavity spot (Martin and Loper, 1999; Suffert and Guibert, 2007).

Pythium species survive in the soil via oospores or sporangia (Hendrix and Campbell, 1973; Van der Plaats-Niterink, 1981). The oospore, a sexual spore, is the primary survival structure of many *Pythium* species because of the thick-wall enables the spore to survive in the absence of a host, even in the presence of unfavorable soil conditions (Dick, 1990; Vander Plaats-Niterink, 1981). The ability of oospores to exhibit constitutive dormancy, wherein spores fail to germinate even under favorable conditions, ensures a continuous supply of *Pythium* propagules in the soil (Martin and Loper, 1999). Many factors affect the germination of oospores in soil, including the age of spores, CO_2 concentration, alternate wetting and drying of the soil, and for some species, light (Adams, 1971; Ayers and Lumsden, 1975; Guo and Ko, 1994; Johnson and Doyle, 1986; Lifshitz and Hancock, 1984; Schmithenner, 1972).

The sporangia and hyphal swellings of *Pythium*, on the other hand, serve as the asexual reproductive structures for many of species (Hendrix and Campbell, 1973, Van der Plaats-Niterink, 1981). Dick (1990) and Van der Plaats-Niterink (1981) referred to sporangia as the asexual structures that produce zoospores, while hyphal swellings do not produce zoospores. Structures vary from spherical to inflated filamentous or lobate forms (Van der Plaats-Niterink, 1981). Spherical structures apparently survive longer in dry soil than inflated or lobate sporangia (Martin and Loper, 1999). Production of zoospores by sporangia occurs under certain conditions, such as the presence of certain nutrients or high moisture in the soil (Stanghellini and Hancock,

1971a). Zoospores are released under moist conditions, and are attracted to seeds or seedlings by exudates produced by germinating seeds, where they encyst and penetrate the host (Royle and Hickman, 1964). Temperature and moisture are important factors in survival of the zoospores in the soil, and unfavorable conditions zoospores encyst in the soil until conditions become favorable (Hardman et al., 1989; Stanghellini and Burr, 1973). In addition, saprophytic growth of *Pythium* is an important survival strategy in the soil and is favored by high soil moisture (Hendrix and Campbell, 1973). As saprophytes, *Pythium* species exist as mycelium in the soil, but are prone to lysis in the absence of susceptible hosts or a food base (Martin and Loper, 1999). Favorable conditions cause mycelium to divert into the production of resting structures (Martin and Loper, 1999).

To be an effective pathogen, *Pythium* propagules in the soil must germinate to colonize the host, either through germ tubes or zoospore production (Hendrix and Campbell, 1973). Many factors have been reported to stimulate or activate the germination of dormant spores in the soil. Root and seed exudates have been one of the most reported and studied factors stimulating germination of dormant spores (Milton et al., 1964; Nelson, 1990). Amino acids and sugars, the primary components of seed exudates, were reported to play important roles in the stimulation of *Pythium* propagules, but research has pointed to the volatile compounds in exudates in causing this response in *Pythium* propagules (Hendrix and Campbell, 1973; Nelson, 1987; Nelson, 1990; Nelson and Craft, 1989). Germination of propagules was demonstrated in one study to begin 1.5 h after initial contact with seed exudates (Stanghellini and Hancock, 1971a).

Soil moisture and temperature are two factors that have great influence on the growth of many *Pythium* species and on damping-off development (Broders et al., 2009; Lumsden et al., 1976; Martin and Loper, 1999). *Pythium* is active in fields with high soil moisture, i.e., moisture

at or greater than field capacity (Cook, 2002). Damping-off severity and incidence, ability of the pathogen to initiate infection, and inoculum density were all positively correlated with soil moisture for many *Pythium* species (Martin and Loper, 1999). Soil moisture can also affect the type of propagules prevalent in soil. Bainbridge (1970) found that formation of oospores predominates in wet soils, while sporangia are more common in soil with lower moisture. Liftshitz and Hancock (1984) reported that soil matric potential affects the saprophytic activity of *Pythium* spp. with a decrease in activity observed at saturated soil conditions or soils above field moisture capacity. Soil moisture also mediates the composition of microbial communities in organic matter, with *Pythium* spp. the primary saprophytes present at high soil moisture, while other fungi predominated as soil moisture decreased (Kouyeas, 1964). Stanghelleni and Hancock (1971b) explained that the size of the spermosphere, the area immediately surrounding the seed, is influenced by soil moisture, with germination of pathogen propagules stimulated at greater distances from germinating seeds as soil moisture increases. Carbon dioxide (CO_2) and oxygen ($O_{2)}$ concentration in the soil are also affected by soil moisture, e.g., an increase in soil moisture tends to increase CO_2 but decrease O_2 (Mitchell and Mitchell, 1973). It was reported that some *Pythium* species are favored by high CO_2 concentrations (Mitchell and Mitchell, 1973). For example, saprophytic growth of *P. irregulare* and *P. vexans* was favored by the presence of elevated CO_2 in the soil (Gardner and Hendrix, 1973).

Soil temperature can have varying effects on pathogenic *Pythium* spp. as well as on the host and damping-off. Some *Pythium* species are reported to be pathogenic in cooler temperatures, while others cause disease at higher temperatures (Hendrix and Campbell, 1973). Various authors have reported that *P. ultimum* and *P. irregulare* are more important species causing disease at low temperatures (Leach, 1947; Mundel et al., 1995; Pieckzarcka and Abawi,

1978; Sippell and Hall, 1982; Thomson et al., 1971). *P. ultimum* can still be active at temperatures < 10°C, while *P. irregulare* can be active at < 5°C, and these two species are common in soils of the PNW (Cook, 2002). *P. aphanidermatum* and *P. myriotylum* can cause more severe disease at higher temperatures (25 to 30°C) (Littrell and MacCarter, 1970). Furthermore, soil temperature influences the spore germination and germ tube growth of *Pythium* species, with more zoospore discharge observed at lower temperatures, while greater oospore germination and germ tube growth occurred at relatively higher temperatures (Adams, 1971; Lipps, 1980; McDonalds and Duniway, 1978).

Inoculum potential in the soil determines the incidence and severity of soilborne diseases. Cook (2002) sampled wheat fields in the inland PNW to quantify *Pythium* inoculum in the soil and found that most fields had at least 200 CFU/g soil, with an average of 350 to 400 CFU/g soil. Temporal and spatial variations in the fields influence the severity and incidence of diseases caused by many *Pythium* spp. (Martin and Loper, 1999). Seasonal and short term variations have been observed in the populations of *Pythium* spp., e.g., species of *Pythium* favored by low temperatures, such as *P. ultimum*, increased in population during fall and decreased in spring and summer (Lumsden et al., 1976). In addition, Hardman and Dick (1987) showed that short term fluctuations in inoculum density of *Pythium* spp. exist where temperature exerts a more pronounced effect on soils than rainfall. Furthermore, the distribution of *Pythium* soil inoculum in the soil strata corresponds with the distribution of roots and crop debris that are available for saprophytic colonization by *Pythium* spp. (Pankhurst et al., 1995). The primary concentration of inoculum in the upper soil profile (15 cm) is likely responsible for a greater number of infection sites on shallow roots than deeper roots (Hancock, 1985). With respect to the influence of inoculum density on the amount of damping-off, some authors have reported a linear relationship

between these two factors (e.g., Ferris, 1982; Stasz and Harman, 1980), although the amount of initial inoculum necessary to initiate disease development can vary among species (Mitchell, 1975).

1.3.2. Isolation, identification, and quantification of *Pythium* spp.

Identification of *Pythium* species traditionally has been done based on morphological characteristics (Dick, 1990; Van der Plaats-Niterink, 1981). Morphology of sporangia, oogonia, and antheridia, the type and size of oospores, homothallism vs. heterothallism, growth habit, and rate of growth in culture media are some of the common criteria used to differentiate among species (Dick, 1990; Van der Plaats-Niterink, 1981). However, because *Pythium* consists of biologically and ecologically diverse species, identification using these characteristics can be challenging. These characteristics also can vary under different culture conditions, and many species are very similar morphologically (e.g., Chen and Hoy, 1993). Some of these characteristics can also change or be acquired or lost readily (Lévesque and De Cock, 2004; Martin, 2000; Matsumoto et al., 1999). Also, the criteria traditionally used for species differentiation has not always correlated with the major clades in *Pythium* determined by molecular methods (e.g., Lévesque and De Cock, 2004).

The use of molecular methods for the identification of *Pythium* species began more than a decade ago with the work of Martin (1990) and Lévesque et al. (1994). Such methods use DNA based tools that differentiate genera, species, subspecies, races, and even strains of fungi, oomycetes, bacteria, and etc., as well as clones or individuals within a population (Glass and Donaldson, 1995). The methods are generally more rapid, accurate, sensitive, and specific than the traditional method of using morphological characteristics; therefore, these methods provide promising alternatives for identification, even without significant taxonomic expertise

(Schroeder et al., 2013). The application of polymerase chain reaction assays (PCR) has elevated the use of molecular tools further for the identification and detection of *Pythium* spp., and since many researchers have developed species-specific primers that delineate *Pythium* species (e.g., Kageyama, 1997; Tambong et al., 2006; Wang et al., 2003). For example, Kageyama (1997) developed a species-specific primer using the ribosomal DNA (rDNA) of internal transcribed spacer (ITS) region of *P. ultimum* to detect the pathogen from seedlings using a PCR assay. The ITS region of the nuclear rDNA has been established to be variable at the family, genus, and species level for *Pythium* (Chen, 1992; Chen and Hoy, 1993; Chen et al., 1992). Use of the ITS region seems to be the most popular choice of many researchers working with detection of this pathogen (Lévesque, 2011; Schroeder et al., 2013). Aside from high sequence variability in this region, the availability of primers that supply sequence data is also an important factor (Lévesque and de Cock, 2004). Paulitz and Adams (2003) developed a PCR assay followed by sequencing of the ITS region to identify *Pythium* species composition in wheat fields of eastern Washington. Other gene markers can be used for species identification, but are more commonly used for phylogenetic studies, such as the *cytochrome oxidase* (*cox*) II and *β-tubulin* genes (Villa et al., 2006). Martin (2000) first reported the use of *cox* II for assessing phylogenetic relationships among *Pythium* species. *Cox* II is a mitochondrially encoded gene and, therefore, is predicted to be more variable than nuclear DNA (Villa et al., 2006). The study by Villa et al. (2006) was the first to use *β-tubulin*, together with ITS rDNA and *cox* II, as genetic markers for a phylogenetic study of *Pythium* and *Phytophthora*. The *β-tubulin* gene in the nuclear DNA, encodes one of two conserved families of tubulin genes, and has been useful in reconstructing the phylogenetic relationships among fungi at all levels (Thon and Royse, 1999).

Detection and quantification methods for *Pythium* species have been done traditionally through baiting and soil dilution plating on a selective media (e.g., Broders et al., 2007; Conway, 1985; Paulitz and Adams, 2003). However, these methods are time-consuming and laborious, since detection of individual *Pythium* species requires different optimum growth conditions, and detection of the same species present in particular types of samples may be difficult especially when isolates of some species grow faster than isolates of other species. Therefore, most often, only an estimate and not the actual population profile can be detected by traditional methods using selective agar media (Schroeder et al., 2006). The real-time PCR assay is another molecular tool that can be valuable, not only for detection of *Pythium* species, but also for quantification of the population directly from soil or diseased plant material (e.g., Okubara et al., 2005; Schroeder et al., 2006; Schroder et al., 2013). This can be particularly useful for those pathogens that cannot be extracted or cultured readily from the host tissue or soil, or which are present at low populations in soil samples (Okubara et al., 2005). Numerous researchers have reported the applicability of this technique for identification and quantification of other plant pathogenic fungi such as *Pyrenophora* spp. (Bates et al., 2001), *Phytophthora infestans* and *P. citricola* (Bohm et al., 1999), *Helminthosporiumsolani* (Cullen et al., 2001), *Colletotrichum coccodes* (Cullen et al., 2002), *P. ramorum* (Hayden et al., 2004), and *R. solani* AG3 (Lees et al., 2002). Schroeder et al. (2006) used real-time PCR assays for the identification of pathogenic *Pythium* spp. in soils using species-specific primers designed for each of nine commonly isolated *Pythium* spp. in eastern Washington (Paulitz and Adams, 2003; Schroeder et al., 2006). The forward and reverse primers, based on the ITS 1 and ITS 2 sequence, respectively, were designed to be species-specific to reduce the likelihood of amplifying non-target species (Schroeder et al., 2006). The assays were sensitive enough to allow detection of *Pythium* DNA from naturally

infested and inoculated soil samples to as low as 10 fg/g soil (Schroeder et al., 2006). As techniques like this become more available, the detection and quantification of *Pythium* species in the soil has become less tedious and more accurate (Schroeder et al., 2013). Similar research using real-time PCR assays has been developed for detection and quantification of *Pythium* from soil. Kernaghan et al. (2008) quantified *Pythium* populations from ginseng soils by real-time PCR assays and compared the results to traditional dilution plating methods, while Li et al. (2010) developed real-time PCR assay to estimate population density of *P. intermedium* in forest soils.

Another application of molecular methods developed for the detection and identification of *Pythium* spp. was suggested by Asano et al. (2010) using multiplex PCR assays. In contrast to traditional PCR assays method, multiplex PCR assays can amplify multiple DNA regions simultaneously in a single PCR reaction. Thus, such assays can be less laborious and more rapid compared with traditional or individual real-time PCR assays, as it does not require separate PCR assays for each species being detected.

1.3.3. Damping-off management in organic crop production

As growth of the organic industry continues to increase, the need to maintain a steady supply of good quality produce is fundamental. However, as with conventional production of food, growers of organic vegetables face many challenges through the cropping season, one of which is the presence of pests and diseases (Koike et al., 2000; Letourneau and van Bruggen, 2006). For organic growers, the challenge lies in successfully growing good quality crops without using synthetic pesticides or fertilizers. For most growers, this means relying mostly on cultural practices and the use of resistant cultivars as management strategies to combat pests and diseases, since there are very few effective crop protection products that can be used in certified

organic production (Letourneau and van Bruggen, 2006). Koike et al. (2000) pointed out the importance of innovative disease control strategies in organic farming that have an ecological basis, in order to encourage microorganisms that are antagonistic to or otherwise inhibit pathogens. Title 7 Part 205.206 of the USDA National Organic Program (NOP) handbook provides a guideline on allowed practices for disease management in certified organic production, which focuses on cultural and biological control strategies (USDA NOP, http://www.ams.usda.gov).

As stated above, resistance and cultural management practices are the primary strategies organic growers use to address disease problems (Fernandez-Cornejo et al., 1998; Koike et al., 2000; Sullivan, 2004). Organic certifying agencies that follow USDA NOP guidelines and Organic Materials Review Institute (OMRI) guidelines permit growers to use materials for pest management that are categorized as naturally-occurring product that are not known to cause significant toxic effects to non-target organisms, and are considered environmentally sound (Fernandez-Cornejo et al., 1998; OMRI, 2010; USDA NOP National List, http://www.ecfr.gov). The use of resistant cultivars can be the most important weapon for disease control in organic systems. However desirable cultivars with resistance to important soilborne diseases are not always available (Koike et al., 2000). Site selection, such as choosing land without history of soilborne diseases, and planting in fields with well-drained soil, is important for preventing damping-off in organic production (Gaskell et al., 2000; Koike et al., 2000; Rao et al., 1978).

Exclusion by avoiding introduction of diseased material into a field is an effective preventive management strategy for some diseases, although avoidance may be difficult for the pathogens that tend to be widespread in agricultural areas (Agrios, 2005; Hendrix and Campbell, 1973). If a pathogen is already present in a field and the disease of concern is alarming, applying

materials approved for organic production that should be coupled with appropriate cultural practices (Koike et al., 2000*)*. Irrigation management, e.g., avoiding excessive soil moisture, is another logical strategy for managing damping-off because most *Pythium* species thrive in moist soil (Gaskell et al., 2000; Rao et al., 1978). It can also be important for growers to avoid planting when the soil temperature is < 5 to 10°C, because some *Pythium* species can be active even at temperatures that are suboptimal for growth of the host crop, and some species can initiate infection more readily when plants are stressed (Cook, 2002; Nederhoff, 2000). *Pythium* suppressive soils can sometimes be developed over time with the use of compost amendments, and can be an effective and long-term strategy to manage damping-off (Ben-Yephet and Nelson, 1999; Tamm et al., 2010). The build-up of microbial communities that metabolize fatty-acids can induce suppressiveness by inhibiting stimulation of pathogen propagules (McKellar and Nelson, 2003; Tamm et al., 2010). Many other studies have looked into the effects of using compost for managing soilborne diseases, some of which have reported effectiveness at suppressing *Pythium* species (Ben-Yephet and Nelson, 1999; Chen et al., 1988; McKellar and Nelson, 2003; Scheuerell et al., 2005).

There are other cultural practices that can improve soil health. In an ecologically balanced soil environment, antagonistic organisms can maintain the population of pathogenic ones to a level that will not cause a significant amount of disease, particularly those associated with soilborne pathogens. Abawi and Widmer (2000) reviewed the impact of some cultural practices on soil health management practices in controlling soilborne pathogens, nematodes and root diseases in vegetable crop production. They concluded that these cultural practices such as cover cropping and green manure incorporation, use of composts, crop rotation, and tillage practices improve soil compaction, increase drainage, and increase soil temperature, all of which

have an impact on the physical soil characteristics as well as increasing diversity of the soil biota. Baysal et al. (2008) examined the effects of organic transitional strategies, i.e., tilled, fallowing preceding rye or wheat cover crop, planting eight mixed species of hay, low-intensity vegetable production, and intensive vegetable production in high tunnels, on damping-off of soybean and tomato. They observed a significant reduction in damping-off of soybean and tomato with hay amendment, both in the field and in growth chamber trials. Njoroge and Kabir (2009) and Baldwin (2006) discussed the benefits of using crop rotation in organic production. Crop rotation is a key practice for growing organic and conventional crops because it not only serves to improve overall soil quality and structure, but also plays a role in controlling diseases, specifically those caused by soilborne pathogens that can build up due to continuous cropping of single crop. However, the study by Njoroge and Kabir (2009) using crop rotation of lettuce after broccoli with incorporation of broccoli residues to address soilborne pathogens common in organic and conventional strawberry production, demonstrated that while this crop rotation could effectively reduce the soilborne *Verticillumdahliae* population, the effect on *Pythium* was not consistent in the two-year cropping period evaluated.

Seed treatments can be an inexpensive and effective approach to manage damping-off in conventional or organic production (Taylor and Harman, 1990). Seed treatments are used on many crops to control diseases and pests, and ensure uniform stand establishment, by protecting against soilborne pathogens and insects (Paulsrud et al., 2001). In recent years, there has been an increase in the number of commercially available organic seed treatments for damping-off control (Gatch, 2011). Some advantages of using seed treatments include: (1) optimum timing since seeds and the seedling phase of growth are generally vulnerable to damping-off; (2) a relatively small dose of the product is used for seed treatments compared to broadcast sprays or

soil drenches; (3) ease of application; and (4) precise targeting of the application to the site of the host-pathogen interaction, as spray drift is avoided, and little product is wasted on non-target sites (Paulsrud et al., 2001). Many biological control agents are formulated for seed or slurry treatment to benefit from direct contact with the seeds (Taylor and Harman, 1990). Some organic seed treatments are microbial products with biological control activity (McSpadden Gardener, 2002; Raudales and McSpadden Gardener, 2008). The success, however, of microbial seed treatments depends on the ability of the microorganisms to grow well after application to the seeds and planting of the treated seeds. Therefore, efficacy depends on the seed treatment system used being strongly conducive for the beneficial microorganism to establish well in the environment in which the seed is planted (Taylor and Harman, 1990). Competitive microflora and unfavorable edaphic factors are two factors that can restrict growth of biological control agent seed treatments (Taylor and Harman, 1990). Other seed treatments are classified as non-biological or biochemical-based treatments that contain plant extracts, sulfur or copper-based products, or plant derived regulators (Cao et al., 2010). Many of these seed treatments are available commercially and are registered by OMRI, although many are registered as crop management and production aids rather than as disease control tools despite the evidence of the fungicidal or fungistatic activity (Abawi et al., 2012; McSpadden Gardener, 2002; OMRI, 2010).

Cummings et al. (2009) evaluated 14 seed and drench treatments in a greenhouse trial for efficacy against pre- and post-emergence damping-off of spinach caused by *P. ultimum*, *Rhizoctoniasolani*, and *Fusarium oxysporum*f.sp. *spinaceae*. Promising results were detected only for two proprietary seed treatments, and no product was effective against all three pathogens. Furthermore, none of the products was very effective when tested in field trials (Cummings et al., 2007a; Cummings et al., 2007b). Porter and Coffman (2007), and Porter

(2008) assessed a range of organic seed treatments in field trials, and found Nordox, a copper-based product from Monterey Chemical Co. (Fresno, CA), to be effective at controlling seed rot of pea. A study by Leisso et al. (2009) comparing commercially available biological seed treatments with fungicide seed treatments for *Pythium* damping-off control in chickpea demonstrated that the biological control treatments were ineffective compared to the fungicide treatments, both in greenhouse and field trials, and in some trials the biological seed treatments inhibited germination and growth of chickpea in the greenhouse trial. Although organic seed treatments provide a promising strategy to control soilborne diseases, particularly damping-off, organic growers typically do not use seed treatments because of problems with lack of efficacy, very limited efficacy, or inconsistency in performance in field conditions (Alcala and du Toit, 2009; McSpadden Gardener, 2002). Furthermore, efficacy data validating claims by the registrants of these products for particular disease is often lacking or not very robust. This emphasizes the need for independent efficacy studies for organic seed treatments for control of soilborne pathogens, particularly for efficacy trials to be carried out in the conditions that mimic field conditions where the diseases are important.

Priming is another form of seed treatment that is a potential strategy that organic and conventional growers can use to improve field emergence and control damping-off. Seed priming involves hydrating seeds to initiate the early stages of germination while preventing radicle emergence (Rajjou et al., 2012; Taylor et al., 1998). Water is added to seeds once to allow rapid imbibition, or slowly over time to facilitate slow imbibition (Taylor et al., 1998). Several benefits of seed priming include: (1) uniform and rapid emergence even in suboptimal conditions (Khan et al., 1992; Knypl and Khan, 1981); (2) improved vigor of low vigor seed lots (Bradford, 1986); and (3) reduced seed leakage during germination (Basra et al., 2003; Perry,

1973). Most seeds are dehydration tolerant until radicle emergence, i.e., after imbibing by priming, the seeds can be dried back to the initial moisture content for storage without losing the benefits of priming (Rajjou et al., 2012; Taylor et al., 1998). However, little attention has been directed on the post-priming effect, which could be an oversight because priming can be lost by improper post-treatment handling (Taylor et al., 1998). Improper drying of primed seeds can negate the beneficial effects of priming, such as observed by Weges and Karssen (1990) for lettuce seed lots that were dried to < 10% moisture following priming. Even storage conditions, e.g., relative humidity (RH) and temperature, can have a profound effect on primed seed efficacy and longevity. A study evaluating the effect of storage conditions at 42°C and 92% RH on primed seeds compared to non-primed seeds of tomato (Hacisalihoglu and Khan, 1997) demonstrated that, although more rapid germination was observed for the primed seeds initially, the differences in germination rate decreased with duration of storage. After 6 days, the primed seeds had lower germination than the non-primed seeds. Two ways by which priming can potentially control damping-off include: (1) more rapid and uniform emergence of the primed seeds, even at suboptimal conditions for seed germination, which decreases the window of susceptibility of seeds to infection; and (2) significant reduction in seed leakage may facilitate escape from infection by soilborne pathogens, particularly *Pythium* spp. which are highly responsive to the presence of exudates (Nelson, 1990). Decreased seed exudation causes a reduction in the size of the spermosphere, with less exudates that can activate pathogens and, thus, reduced seed colonization by pathogens (Osborn and Schroth, 1988; Schroth and Cook, 1964). For these reasons, several researchers have investigated priming for control of damping-off, e.g., Hardman et al. (1989) reported the benefit of combining priming and the biological control agent, *Trichodermaharzianum*, to protect pea against *Pythium.* This was contrary to what

Pill et al. (2001) observed when priming of cucumber seeds was combined with seed treatment with *Trichoderma* spp. for control of damping-off. Rush (1991), and Osborn and Schroth (1988) investigated different priming techniques for the effects on Pythium damping-off in sugarbeet seed, and found significant disease reduction when seeds were primed compared to non-primed seeds.

1.4. Electrolyte leakage of seeds

Germinating seeds normally exude a large amount of leachates during the water imbibition phase (Rajjou et al., 2012). The components of leachates are largely cell constituents, including sugars, amino acids, organic acids, and electrolytes (Pandey, 1992). In pea, potassium was reported to be the main constituent in seeds soaked in water, carbohydrates as well as simple sugars, and amino acids (Larson, 1968; Matthews and Rogerson, 1976). The amounts of these constituents when measured were correlated with conductivity in the steep water (Matthews and Carver, 1971).

Soilborne pathogens such as *Pythium* are influenced greatly by seed exudates, as the exudates stimulate germination of oospores and sporangia, with *Pythium* regarded as the most responsive of the soilborne pathogens to the seed exudates (Martin and Loper, 1999; Nelson, 1990; Schroth and Hilderbrand, 1964). Conditions that typically enhance seed exudation also influence susceptibility of the host to disease. The direct correlation between seed rot and exudates in pea has been documented (Kraft, 1986; Matthews and Whitebread, 1968). Short and Lacy (1976) demonstrated that pea seeds that were soaked for 48 h at 22°C prior to planting reduced the incidence of seed and seedling rot due to the removal of bulk exudates stimulatory to the pathogen. Nelson (1987) suggested that the volatiles, rather than the carbohydrate components of seed exudates, moderate the response of *Pythium* to seed exudates.

The amount of seed exudation is directly correlated with field emergence, suggesting that the amount of exudation can be used as an indicator of the vigor of a seed lot, therefore, to predict field emergence (Pandey, 1992). In organic production, the use of good quality, vigorous seed lots is key to providing a better chance of producing healthy crop that will perform well, particularly, in the presence of cold soil, soilborne pathogens, and/or other unfavorable conditions (Elias et al., 2011). According to Duke and Harvey (1983), one strongly correlated indicator of seed vigor is the leakage of substances from imbibing seeds. Poor quality or weak seeds possess poor membrane structure, and produce greater electrolyte leakage and, hence, greater electrical conductivity measurements (EC values) (Matthews and Bradnock, 1968; Pandey, 1992). Low EC values are associated with good seed qualities such as high germination rate, and vigorous and uniform seedling growth (Steere et al., 1981). An increase in electrolyte leakage can also be an indicator of loss of cell integrity as a consequence of seed aging, as suggested by Bewley and Black (1985) and Parish et al. (1982). Short and Lacy (1975) reported that the amount of seed exudation, specifically carbohydrates, in pea seeds depends on cultivar, seed age, seed color and temperature.

A high EC measurement correlates with low vigor of a seed lot and, consequently, poor field emergence and field stands (Pandey, 1992). In a study on common bean seed during early planting in a cool climate, it was found that of the standard germination tests, only EC could accurately predict seedling emergence in the field (Kolasinska et al., 2000). For garden pea, soybean, French bean, mungbean, and field bean, the EC is a routine vigor test to predict field emergence (Matthews and Powell, 2006). In corn, the measurement of EC as a tool to predict seed lot shelf life or storability was explored by Marks and Stroshine (1998) and Fessel et al. (2006). deCarvalho-Miguel and Filho (2002) suggested that the use of a potassium leachate test,

in contrast to the overall EC test, is a more consistent method to predict seed quality in maize. The EC test is acknowledged as one of the best tests for the evaluation of the loss of cell membrane integrity due to seed deterioration (Matthews and Powell, 2006). Lower vigor seed lots exhibit higher intensity of cellular constituent losses, such as inorganic ions (Delouche, 1976; Powell, 1986).

1.5. Conclusion and research needs

The significance of damping-off in organic vegetable production and the need for effective management strategies warrant investigation of the *Pythium* species that cause the disease during early spring planting conditions in certified organic production systems. Specifically, there is a need understand and identify the major species that are important in causing damping-off in organic fields during early spring planting in the Columbia Basin, a primary region of organic vegetable production in the PNW, USA. A greater understanding of the inoculum potential of the major pathogenic species associated with damping-off in organic soils is a key step towards effective management of damping-off. There is also a need to evaluate organic seed or drench treatments as management options that growers in organic production can use, particularly when planting in conditions favorable to damping-off. The EC test as a way to measure vigor of seed lots is a potential tool to determine the susceptibility of specific cultivar and seed lots to damping-off prior to planting. This could provide a valuable tool for growers to assess the risk of damping-off. In consideration of these research needs, the objectives of this dissertation were to:

(1) Survey and identify *Pythium* species present in certified organic fields of the semi-arid, irrigated Columbia Basin of central Washington, specifically fields used for vegetable production with pea included in the rotations;

(2) Identify which of these *Pythium* species are pathogenic to pea under the cool and moist soil conditions typical of early spring planting conditions in the Columbia Basin;

(3) Quantify the three most prevalent and pathogenic *Pythium* species in soil sampled from the organic fields in the Columbia Basin using real-time PCR assays, and develop a standard curve for each of these *Pythium* species to relate DNA concentration detected with the number of propagules of each species and use this to assess the inoculum potential of the three pathogenic *Pythium* species in organic fields in the Columbia Basin;

(4) Evaluate seed and drench treatments that can be used in certified organic pea production in Washington for control of damping-off caused by *Pythium* spp. during early spring field conditions;

(5) Optimize pea seed priming conditions for rapid emergence and damping-off suppression;

(6) Evaluate combinations of seed priming and other organic seed treatments for control of damping-off in pea during spring planting conditions;

(7) Assess the susceptibility of seed lots of various pea cultivars to Pythium damping-off at different inoculum levels;

(8) Examine the relationship between EC and seedling emergence in soil inoculated with *P. ultimum*, and develop a model to predict the risk of Pythium damping-off.

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CHAPTER 2

Survey of *Pythium* species associated with damping-off of pea in certified organic fields in the Columbia Basin of central Washington

2.1. Introduction

The genus *Pythium* consists of many species that are important plant pathogens (Hendrix and Campbell, 1973). At present, there are approximately 280 legitimate *Pythium* species listed in MycoBank (http://www.mycobank.org/), a majority of which are recognized soilborne pathogens. Many of these pathogenic species are ubiquitous and can infect a diversity of agricultural crops, causing problems both in the field and in greenhouses (Agrios, 2005; Martin and Loper, 1999). *Pythium* spp. are described as necrotrophic, early colonizers and opportunistic plant pathogens that attack vulnerable and stressed plants, particularly the immature, succulent tissues of germinating seeds, feeder roots, and seedling stems, leading to pre- and post-emergence damping-off (Agrios, 2005; Hendrix and Campbell, 1973; Martin and Loper, 1999). In the field, such damping-off causes poor stands that can lead to significant yield losses (Cook, 1992; Cook et al., 1987; Oyarzun, 1993).

Surveys of the diversity of *Pythium* species in agricultural soils have demonstrated that it can be common to isolate more than one species in a field, including both pathogenic and non-pathogenic species (Abad et al., 1994; Broders et al., 2007; Paulitz and Adams, 2003; Weiland, 2011). In fact, in a survey by Paulitz and Adams (2003) of 80 wheat fields in eastern Washington, approximately 30% of the isolates belonged to non-pathogenic *Pythium* species. Several pathogenic *Pythium* species also can be associated with damping-off in a particular field,

making management of the disease more complex (Broders et al., 2007; Broders et al., 2009; Campion et al., 1997).

Accurate identification of *Pythium* spp. is imperative in understanding the etiology of outbreaks of damping-off, and the cornerstone to development of effective management strategies (Lévesque, 2001). Identification typically has required successful isolation of the causal agent, and differentiation from other organisms present. Many *Pythium* species can be isolated successfully from symptomatic roots or from soil samples by baiting methods, e.g., using grass leaves (Paulitz and Adams, 2003). Seeds of a susceptible plant species and cultivar can also be planted in soil, and root sections of the seedlings that develop plated onto selective media to bait for *Pythium*, as described by Broders et al. (2009). Soil dilution onto selective media has been used traditionally to isolate *Pythium* species from soil (Ali-Shtayeh et al., 1986). However, these methods do not necessarily guarantee isolation of all possible species and/or may be selective for some species (Weiland, 2011). Examination of the cultural and morphological features can be used to identify *Pythium* isolates to species level (van der Plaats-Niterink, 1981). However, this can be time-consuming and requires expertise for accurate identification. Furthermore, heterothallic or asexual species may be more difficult to identify than homothallic species (Hendrix and Campbell, 1973; Paulitz et al., 2003). The application of molecular tools such as polymerase chain reaction (PCR) assays using universal eukaryotic primers (Bakkeren et al., 2000), followed by sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA), has been used widely for identification of *Pythium* species (e.g., Paulitz and Adams, 2003; Wang et al., 2003). Other candidate genes that are used for identification include *cytochrome oxidase* (*COX*) II, the large subunit (LSU) of the ribosomal DNA, and β-

tubulin, although these genes are used more commonly for phylogenetic studies than for identification (Bala et al., 2010; Martin, 2000).

Pythium species are very diverse plant pathogens. Some species, such as *Pythium ultimum*, have a wide host range while others can infect only a limited number of plant species (Augspurger, 2007; Hendrix and Campbell, 1973; Martin, 1992). The ability of each species to infect plants is dictated by recognition of the host as well as the presence of the environmental factors that favor disease development (Park, 1963). Soil moisture and temperature are two examples of environmental factors that have great influence on the growth and ability of *Pythium* species to incite disease (Hendrix and Campbell, 1973; Martin and Loper, 1999). In general, *Pythium* species are more prevalent in fields with higher soil moisture content (at or above field capacity) than drier soils (Cook, 2002; Rao et al., 1978). The amount of soil moisture can affect the type of *Pythium* propagules produced in the soil, with oospores formation favored in wet soils and sporangia in drier soils (Bainbridge, 1970). The spermosphere, the immediate vicinity surrounding a seed, is affected by the amount of exudates released during seed germination, which is influenced by soil moisture (Nelson, 2004). Exudates, in turn, stimulate germination of *Pythium* oospores (Stanghellini and Burr, 1973). As the soil moisture level increases, the germination of *Pythium* propagules is stimulated at a greater distance from the germinating seed (Short and Lacy, 1976).

Soil temperature affects both *Pythium* species and the host plants. Some *Pythium* species are favored by lower soil temperatures (5 to 10°C), such as *P. ultimum* and *P. irregulare*, while others thrive in warmer temperatures (as warm as 25 to 30°C), such as *P. aphanidermatum* (Hendrix and Campbell, 1973; Hershman, 1986; van der Plaats-Niterink, 1981). Spore germination and germ tube growth are influenced by soil temperature, with greater zoospore

discharge from sporangia at lower soil temperatures, while oospore germination and germ tube growth occur at relatively higher temperatures (Agrios, 2005). Temperature can also affect host susceptibility and seed exudation, e.g., planting seed at soil temperatures <10°C slowed seed germination, whereas the rate of seed exudation was greater at 22 to 30°C but lasted only 18 h compared to the seed exudation rate at 10°C, which lasted for 48 h (Martin and Loper, 1999; Nederhoff, 2000; Short and Lacy, 1976). Conditions that favored greater seed exudation also favored greater levels of pathogen germination and seedling decay (Short and Lacy, 1976). Therefore, because environmental factors are important to disease development, testing *Pythium* isolates for pathogenicity on particular plant species and cultivars requires providing the conditions that mimic disease development in field conditions.

The inoculum potential of *Pythium* in the soil dictates disease development and severity (Martin and Loper, 1999). Understanding the role of inoculum density has been of interest to many researchers for quantifying disease on a particular host species (Baker, 1971; Xi et al., 1995). For example, several studies have demonstrated that a linear relationship exists between inoculum density and disease severity caused by *P. ultimum* (Ferris, 1982; Stasz and Harman, 1980). Soil dilution plating techniques onto selective agar media have been the traditional methods to assess inoculum density of *Pythium* spp. in soil (Ali-Shtayeh et al., 1986; Mircetich and Kraft, 1973). However, these methods may not always account accurately for the *Pythium* population present in soil. For example, dilution plating alone does not differentiate between pathogenic and non-pathogenic species, is more selective for fast-growing than slow-growing species, and does not detect inoculum present in the form of dormant oospores (Kernagahan et al., 2008; Schroeder et al., 2006). Recent studies have explored the use of real-time PCR assays for the amplification and quantification of *Pythium* species directly from soil samples using

species-specific primers, hence bypassing the need to obtain pure cultures of *Pythium* spp. from the soil (Kernagahan et al., 2008; Li et al., 2010; Schroeder et al., 2006; Schroeder et al., 2013). However, in order to make the application of this technique more valuable for assessing *Pythium* populations in soil, DNA concentration of the *Pythium* spp. detected in the soil must be correlated significantly with the number of propagules present in the soil, which is typically determined by dilution plating (e.g., Kernagahan et al., 2008; Li et al., 2010; Schroeder et al., 2006).

Organic crop production has expanded greatly over the past decade in Washington State (Granatstein et al., 2005). In 2011, there were 729 certified organic farms in Washington, with approximately 90,000 acres devoted to certified organic production (Kirby and Granatstein, 2012). Most of these organic farms are situated in the semi-arid, irrigated area of central Washington, referred to as the Columbia Basin, which is characterized by large-scale agricultural production (Kirby and Granatstein, 2012). Sweet corn and pea are two of the major organic vegetable crops grown on significant acreage in Washington with 2,122 and 3,112 ha devoted to organic pea and sweet corn production in 2009, respectively, out of a 17,550 ha of organic vegetable production in Washington State that year. Pea and sweet corn also make up two-thirds of the organic vegetables harvested in the state (Kirby and Granatstein, 2012). Organic processing pea crops accounted for total sales of $4.2 million and ranked second among the organic vegetables grown in Washington in 2009 (Kirby and Granatstein, 2009; Kirby and Granatstein, 2012), while total sweet corn sales $4.6 million placed this vegetable as the top ranking in organic vegetable crop produced in the Pacific Northwest USA (Kirby and Granatstein, 2009; Kirby and Granatstein, 2012). *Pythium* induced damping-off can be a concern in these organic crops, especially for those species planted early in the season, such as pea

(Alcala and du Toit, 2009). Early spring planting in the Columbia Basin of Washington not only exposes the seeds to cool and moist soil conditions, but the water used to irrigate the crop is cold (< 10°C), contributing to conditions that slow seed germination and provide an environment conducive to damping-off caused by *Pythium* spp., therefore, increasing the risk of poor stands (Agrios, 2005; Cook, 2002; Hendrix and Campbell, 1973). However, early planting of processing pea crops in the Columbia Basin may be difficult to avoid for some growers because of the widespread use of early planting to: (1) reduce weed competition, particularly from nightshade species common in the area (e.g., *Solanum nigrum, S. physalifolium, S. ptycanthum,* and *S. triflorium*) which produce berries toxic to human and animals that cannot be separated readily from peas by the processing equipment; (2) harvest and process organic pea crops ahead of conventional pea crops to avoid having to clean the processing plant between conventional and organic crops; and (3) double-crop pea with sweet corn in the same season (Alcala and du Toit, 2009; Lyndon Porter, USDA ARS, *personal communication*; Mansour et al., 1984; Miller and Parker, 2006).

The use of seed treatments with metalaxyl or the isomer mefenoxam proved to be an effective strategy to control damping-off from *Pythium* spp. (McGee, 1992). However, these fungicides are not approved for certified organic production (Organic Materials Review Institute, 2010; United States Department of Agriculture National Organic Program). Currently, the lack of highly effective seed treatments available for use in certified organic production limits the ability to manage damping-off, but there is great interest from growers in the use of organic seed treatments that work consistently under conditions conducive to damping-off. This has been the focus of recent research in Washington State (Alcala et al., 2012a, 2012b, 2013a, 2013b;

Cummings et al., 2007a, 2007b; Cummings et al., 2009; Porter and Coffman, 2007; Porter, 2008).

The significance of damping-off in organic vegetable production and the need for effective management strategies warrant investigation of the primary *Pythium* species that cause the damping-off during early spring planting conditions in certified organic fields in the Columbia Basin of Washington, and the inoculum potential of the major pathogenic species detected in these organic soils. Previous studies that have investigated *Pythium* species diversity in Washington soils have focused on the wheat production areas of eastern Washington (Charmswarng and Cook, 1985; Paulitz and Adams, 2003). Dryland cereal production in eastern Washington is very different from agriculture in the semi-arid, irrigated Columbia Basin of central Washington. Therefore, *Pythium* species composition and diversity in organic fields in the Columbia Basin may be very different from the former. For this reason, this study was carried out to: (1) survey and identify *Pythium* species present in 37 certified organic fields located in the semi-arid, irrigated Columbia Basin of Washington, specifically fields used for vegetable production with pea crops in the rotations; (2) identify which of these *Pythium* species are pathogenic to pea under the cool and moist soil conditions typical of early spring planting conditions in the Columbia Basin; and (3) detect and quantify the three most prevalent and pathogenic *Pythium* species in soil sampled from the organic fields in the Columbia Basin using real-time PCR assays. For the latter, a standard curve was developed for each of the *Pythium* species to relate DNA concentration detected of the target *Pythium* sp. with the amount of propagules in inoculated soils, and the regression equation was then used to quantify inoculum density of the three pathogenic *Pythium* species in soil from each of 37 organic fields surveyed in

the Columbia Basin in 2009. Preliminary results of the study have been presented (Alcala et al., 2011).

2.2. Materials and methods

2.2.1. Fields surveyed. In the fall of 2009, 37 fields used for certified organic vegetable production in the Columbia Basin were surveyed for *Pythium* species. The fields were chosen based on vegetable cropping history, i.e., planted to pea and/or sweet corn in the previous five years; and to represent the north (17 fields), central (5 fields), and south (15 fields) regions of the Columbia Basin (Fig. 2.1). Soil samples were collected from each field in October 2009 when most of the fields were either fallow or planted to a cover crop. Pertinent information about the fields (e.g., crop production history, location, etc.) was obtained from the growers or managers of the farms (Table 2.1). All of the fields surveyed were irrigated using center pivots.

2.2.1.1. Soil sampling. Soil samples were collected from each of the 37 fields in October 2009. For full center pivot fields, sampling was done along each of four transects in a grid pattern (Fig. 2.2A). For half-circle fields, samples were collected along four parallel transects (Fig. 2.2B). Soil sampling was done by four people in each field, with each person assigned to collect a total of 20 soil cores/transect to a depth of 15 cm using a soil probe with a diameter of 2.5 cm. The soil cores for each transect were then pooled into a 7.6 liter polyethylene bag, mixed thoroughly, and a sub-sample placed into a 50 ml Falcon tube. The 50 ml sub-samples were stored at -20°C and later used to quantify selected *Pythium* species by real- time PCR assays (described below). All of the remaining soil was pooled for the four transects sampled in a field, placed in a 7.6 liter Ziploc bag, mixed thoroughly, and a 500 g sub-sample sent to Soiltest Farm Consultants, Inc. (Moses Lake, WA) for nutrient analysis. The remaining soil from each field was used for baiting *Pythium* spp., as described below. At the time of sampling, the soil samples

were placed in a cooler with ice, and transferred to a cold storage facility (4 ± 2°C) at the Washington State University (WSU) Pullman campus.

2.2.1.2. Isolation and baiting of *Pythium* species. Two methods were used for baiting *Pythium* species from the soil samples. Grass leaf baiting was done following the method described by Paulitz and Adams (2003). A 20 g soil sample from each field was placed in a 15 cm diameter petri plate, and moistened to field capacity with 3 to 5 ml tap water. Plates were incubated at room temperature (22 ± 2˚C) on a laboratory bench for 24 to 48 h. Distilled water (10 ml) was added to each plate, and five grass clippings of *Poa pratensis* L., cut to about 2.5 cm long and sterilized by autoclaving at 1.1 kg/cm^2 and 121˚C for 20 min, were floated on the water in each plate. After 24 to 36 h, the grass clippings were removed, blotted dry on sterilized paper towel, and plated on a *Pythium* selective agar medium (PSM) (Mircetich and Kraft, 1973). After another 24 to 36 h, emerging hyphal tips from the grass leaves were transferred onto plates of water agar (WA). Hyphal growth patterns on WA were observed microscopically to select and verify isolation of *Pythium* species. A pure culture of each isolate of *Pythium* was maintained on WA and stored at 4°C. For long term storage, a WA block (1 cm^3) colonized by the *Pythium* isolate was placed in a glass vial containing sterilized water and four sterilized hemp seeds (HBD International Inc., Brentwood, TN), and stored at room temperature.

Pythium species were also baited from each soil sample following the method described by Broders et al. (2009), with some modifications. A sub-sample of soil from each field was placed in each of two 10 cm diameter pots, saturated with tap water for 24 h, and then drained. The saturated soil was then incubated for 14 days at 14°C in a growth chamber. *Pythium* was baited from the soil by planting pre-germinated seeds of the sweet corn cv. Chase (Monsanto Vegetable Seeds, St. Louis, MO) in one pot, and of the pea cv. Tonic (Brotherton Seed Co., Inc.,

Moses Lake, WA) in the other pot. Germination of the pea and sweet corn seeds was initiated by wrapping the seeds in a moist paper towel at room temperature for 3 to 5 days until the radicle was about 2.5 cm long. Five germinated seeds of each plant species were planted in a separate pot, and maintained in a growth chamber at 10°C by night and 15°C by day with a 12 h photoperiod/day to mimic the cold soil conditions typical of early spring planting in the Columbia Basin. The plants were irrigated with cold water that was stored in the growth chamber, mimicking the cold water used to irrigate fields in the Columbia Basin. Five days after planting, the pots were flooded with water for 24 h to promote damping-off from *Pythium* spp. that might be present in the soil. Four days after flooding, seeds and seedlings in each of the pots were removed, washed, and the roots cut off and blotted dry on sterilized paper towel. Root sections were plated onto PSM and incubated at 22 ± 2°C for 24 to 36 h, prior to making hyphal tip transfers of the emerging mycelium onto WA plates. Pure cultures of the isolates were maintained on WA and kept at 4°C for species identification. Long-term storage was done with sterilized water and hemp seeds following the procedure described above.

2.2.1.3. Identification of *Pythium* species. The *Pythium* isolates obtained from the soil using grass leaves or by pea and sweet corn root baiting were identified initially through microscopic examination of typical morphological features of *Pythium* (Van der Plaats-Niterink, 1981). This was done by first growing a culture of each isolate in a petri plate containing pond water (from the pond in Reaney Park, Pullman, WA) that had been sterilized at 1.1 kg/cm^2 and 121˚C for 20 min. Five sterilized grass leaf clippings were floated on the water in each of the plates, and the plates kept on a laboratory bench for 24 to 36 h at room temperature. When mycelial growth became visible, the grass clippings were removed from each plate and a slide

mount prepared for microscope examination of sporangia, oospores, antheridia, and oogonia characteristic of *Pythium* spp.

2.2.1.4. DNA extraction and sequencing. To verify the species of the *Pythium* isolates obtained by baiting, the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) of each of the isolates was sequenced using the method described by Paulitz and Adams (2003). Each isolate was grown in potato dextrose broth (PDB) in petri plates for 5 to 14 days (depending on whether the isolate was slow- or fast-growing) or until at least three quarters of the plate was covered with mycelial growth. The mycelium was then removed from the PDB, and excess liquid drained by squeezing the mycelium against the edge of the plate with a sterilized pipette tip. The harvested mycelium was washed twice with sterilized distilled water, then drained and placed in a 1.7 ml Eppendorf tube. Harvested mycelia were kept at -20°C until used for DNA extraction.

DNA extraction from the mycelium of each isolate was done using the FastDNA Kit (MP Biomedicals, Santa Ana, CA) along with the Fast Prep FP120 cell disruptor (American Instrument Exchange, Inc., Havervill, MA) following the manufacturer's protocol. PCR amplification of the ITS region was done as described by Schroeder et al. (2006), using the primers UNUP18S42 (5'-CGTAACAAGGTTTCCGTAGGTGAAC-3') and UNLO28S576B (UN-LO28S22) (5'GTTTCTTTTCCTCCGCTTATTAATATG-3') (Bakkeren et al., 2000). The reaction mixture contained: 2 µl DNA template, 6 µl 5x buffer, 1.5 mM $MgCl_2$, 0.2 mM of each of the dNTPs, 10 pmol of each of the primers, and 1.25 U Taq polymerase (Invitrogen Life Technologies, Grand Island, NY) in a total volume of 30 µl. Amplification of DNA was carried out using a PTC-200 Peltier thermal cycler (MJ Research, Reno, NV) with the following program: 3 min initial denaturation at 94°C for 3 min; followed by 30 cycles of 92°C for 45 s, 45

s at the annealing temperature of 60°C, and 60 s at 72°C; and a final extension step for 10 min at 72°C. The PCR products were separated on a 1.0 to 1.5% agarose gel along with a 100 bp DNA ladder (Invitrogen Life Technologies) to verify the sample templates before proceeding with sequencing. The amount of DNA was quantified using a NanoDrop 1000 (Thermo Scientific Co., Wilmington, DE) or by Qubit fluorometric quantification using the Quant-iT dsDNA assay kit (Invitrogen Life Technologies). DNA products were treated with Exo SAP-IT (USB Corp., Cleveland, OH) using 2 µl for every 5 µl of DNA product to remove remaining dNTPs and primers. Premixed sample templates consisted of 10 to 15 ng template, 8 pmole forward primer, and PCR grade water added to achieve a total volume of 15 µl; and were sent to Elim Biopharmaceuticals, Inc. (Hayward, CA) for sequencing. Sequences were edited manually using Chromas Lite (Technelysium Pty. Ltd., South Brisbane, Australia) and compared against the GenBank database using the NCBI online BLAST tool. Isolates with 97 to 100% homologous ITS rDNA sequences to ITS sequences in GenBank were considered the same species.

2.2.2. Pathogenicity tests

2.2.2.1. Isolates of *Pythium* species. Isolates of each of the 19 *Pythium* species identified in the survey were tested for pathogenicity on the pea cv. Tonic using cool and moist soil conditions typical of early spring planting in the Columbia Basin: *P. abappressorium, P. adhaerens, P. aristosporum, P. camurandrum, P. catenulatum, P. diclinum, P. dissotocum, P. echinulatum, P. inflatum, P. intermedium, P. irregulare, P. middletonii, P. oligandrum, P. splendens, P. sylvaticum, P. radiosum, P. torulosum, P. ultimum,* and *P. violae* (Table 2.2). Up to nine representative isolates of each species (three from fields sampled in each of the north, central, and south regions of the Columbia Basin) were used in the pathogenicity tests. For the species for which less than nine isolates were obtained, all isolates were tested for pathogenicity

(Table 2.2). A known pathogenic isolate of *P. ultimum*, isolate 030141 (obtained from T. Paulitz, Wheat Pathology Lab, USDA-ARS, Pullman, WA), was included in every pathogenicity test to serve as a positive control treatment. Another pathogenic isolate, *P. irregulare* 110305 (also from T. Paulitz), was used as a positive control treatment in the first pathogenicity test of the *P. irregulare* isolates.

2.2.2.2. Soil-oatmeal inoculum production. Inoculum production for each of the isolates tested for pathogenicity was done using a mixture of soil and ground oatmeal. A sandy loam soil from a grower's field in Skagit Co., WA was used. The soil was spread on kraft butcher paper on a greenhouse bench for 3 to 5 days to dry, then passed through a sieve (1-mm diameter pore size) to remove plant debris, and crushed with a marble rolling pin. Ground oatmeal (Quaker Oats Brand, Chicago, IL) was added to the soil (1% by weight) and mixed using a PK Blendmaster soil blender (Patterson-Kelley Co. division of Harsco Corp., East Stroudsberg, PA). The soil and oatmeal were mixed for 10 min with deionized water (15% w/w) the latter was added slowly through a funneled hose attached to the blender during the last 5 min of mixing. Approximately 400 g or 1 kg of soil was then placed in a liter size Kerr mason jar or a 3.8 liter high-density polyethylene (HDPE) milk jug, respectively, depending on the container used during preparation. The mason jars were each covered with an autoclavable plastic lid typically used for mushroom spawning (Fungi Imperfecti, Olympia, WA), with a 1.3-cm diameter hole drilled into the lid and a 70-mm diameter synthetic filter disk (Fungi Imperfecti) placed beneath the lid. For the milk jugs, autoclaved, 3.8-cm diameter foam plugs (VWR, Baltimore, MD) were used to seal the jugs, and each foam plug was covered with 2 layers of aluminum foil. The jars or jugs of soil-oatmeal mix were autoclaved at 1.1 kg/cm^2 and 121˚C for 50 min, cooled for 24 h, and autoclaved a second time using the same conditions. A 10 or 20 ml

volume of sterilized, distilled water was added to each jar or jug, respectively, and left overnight prior to inoculation with the appropriate *Pythium* isolate. A mycelial mat of a 5 to 7-day old culture of the appropriate *Pythium* isolate grown in PD broth, was used to inoculate the soil-oatmeal jars or jugs. The inoculated jars or jugs were then kept in the dark at ambient temperature (22 ± 2°C) for at least 3 weeks, and shaken manually every three days to facilitate thorough colonization of the soil-oatmeal mix.

Inoculum of each isolate of *Pythium* was then quantified on WA using soil-dilution plating. Each jar or jug was shaken vigorously to mix the inoculum, and a 10 g sample was added to a French square bottle (250 ml capacity) containing 100 ml sloppy agar (0.1% WA). The bottle with the inoculum was then placed on a platform rotary shaker (New Brunswick Scientific Innova 2100, Enfield, CT) for 10 min at 250 rpm. Five-fold dilutions were carried out using 10 ml aliquots transferred serially to French square bottles (100 ml capacity), each containing 40 ml sloppy agar. Three 0.5 ml aliquots of each dilution were then spread on the surface of a WA plate using a bent glass rod. The plates were incubated on a laboratory bench at room temperature. Colony counts were done after 24, 30, and 36 h. The number of colony forming units (CFU)/g soil was determined using the average number of colonies counted from the three replicate plates/dilution.

2.2.2.3. Soil pasteurization. Field soil obtained from a certified organic farm in the Columbia Basin (Lenwood Farms, Inc., Connell, WA) was used in the pathogenicity tests. Prior to pasteurization, approximately 19 liters of soil was moistened by adding tap water slowly while mixing the soil in an 84 liter cement mixer. Once completely moist, the soil was pasteurized for 2 h at 70°C using a custom-built steam pasteurizer (Patzek, 2013). The soil was cooled, placed on kraft butcher paper, and dried on a greenhouse bench for 2 to 3 days. The dry soil was then

crushed using a marble rolling pin. The pasteurized soil was stored at 22 to 25°C in plastic bins, each covered with a lid to minimize the risk of contamination.

2.2.2.4. Seed surface-disinfestation. Pea seeds of the cv. Tonic were surface-disinfested using a protocol provided by L. Porter (Legume Pathology Lab, USDA-ARS, Prosser, WA). Seeds (50 g) were placed in a 400 ml plastic beaker with 10 holes (each 0.1 cm diameter) in the bottom, and rinsed with tap water. The beaker containing the seeds was then submerged into a 500 ml beaker with 10 g Alconox (Alconox, Inc., White Plains, NY) dissolved in a liter of water, and stirred for 30 s. The seeds were then rinsed with deionized water to remove the soap, and submerged in 95% ethanol for 60 s with constant stirring, followed by submersion in 15% hydrogen peroxide for another 60 s. The seeds were then rinsed thoroughly with deionized water, drained, and spread to dry on a plastic tray lined with two layers of sterilized paper towels. The dried, disinfested seeds were stored at 6°C and 45% RH, and planted the next day.

2.2.2.5. Preparation of inoculated soil and planting of pea seeds. For each of the *Pythium* isolates tested for pathogenicity on pea, 4 kg of pasteurized soil was mixed with soil-oatmeal inoculum of the isolate in a ratio required to achieve a final inoculum density of 500 CFU/g soil. The soil was mixed using a Gustafson lab batch seed treater (Gustafson LLC, Shakopee, MN) for 5 min, and 500 ml tap water was added while mixing. The inoculated soil was added to five 10-cm diameter plastic pots (Anderson Die Manufacturing Co., Portland, OR). Five negative control pots were prepared similarly using non-inoculated, pasteurized soil that was moistened and mixed using the same method. Five replicate positive control pots were prepared similarly using the isolate 030141 of *P. ultimum*. Each pot was planted with 10 surface-disinfested pea seeds placed approximately 2 cm deep. Pots were kept in a PGC Controlled Environment Chamber (Percival Scientific Inc., Perry, IA) at 13°C by day and 10°C by night

with a 12 h photoperiod/day to mimic cold, spring planting conditions. Watering was done approximately every other day to ensure saturated soil, with the water stored in a container inside the same growth chamber. The pots were arranged in a randomized complete block design (RCBD) with five replicate pots/isolate of the appropriate *Pythium* species. Because of limited growth chamber space, a maximum of nine *Pythium* test isolates plus a non-inoculated control treatment and the *P. ultimum* 030141 control treatment were included in each pathogenicity test.

2.2.2.6. Data collection. The number of seedlings that emerged in each pot was counted weekly for four weeks, starting 7 days after planting (dap). The seedlings were removed carefully from each pot 28 dap, and the roots washed to remove adhering soil. Total plant fresh weight (g), shoot height (cm), and shoot dry biomass (g) were measured for all seedlings in each pot. Shoot height was measured from the point of seed attachment to the tip of the most distal leaf. For shoot dry weight, all shoots collected in a pot were placed in a paper bag in an oven set at 90°C. After 2 to 3 days, total dry weight of the shoots/pot was measured. Representative root samples from each pot were plated onto PSM. After 36 h, mycelia growing on the root sections were transferred to WA and examined for *Pythium* species identification after 5 days.

2.2.2.7. Repeat pathogenicity tests. To validate results of the first set of pathogenicity tests of isolates of the 19 *Pythium* species, repeat tests were done. All isolates that proved pathogenic on the pea cv. Tonic in the first set of tests were included in the repeat tests. For the species for which no isolate was pathogenic in the first test, a single representative isolate was re-tested (Table 2.2). The methods described above for the first pathogenicity tests were followed in the repeat tests, with addition of a root rot severity rating measured using a 0 to 5 scale, where: 0 = no visible symptoms on the roots; 1 = a few, small, discolored lesions on the roots; 2 = minor discoloration covering most of the roots and epicotyl; 3 = brown discoloration

of the epicotyl and roots, with most of the lateral roots missing; 4 = complete root discoloration and lateral roots missing; 5 = rotten seeds with no roots developed.

2.2.2.8. Statistical analyses. For each pathogenicity test, data were analyzed as a RCBD using analysis of variance (ANOVA), and Fisher's least significant difference (LSD at $P < 0.05$) was used for treatment means comparisons using SAS Proc GLM (Version 9.2, SAS Institute, Cary, NC) for each of the dependent variables: pea emergence measured 14 and 28 dap, and shoot length, total fresh weight, shoot dry weight, and root rot severity measured 28 dap. Isolates were considered fixed effects and replications random effects in the model. Dependent variables that did not meet the parametric assumptions of normality and equal variances were transformed by logarithmic, square root, arcsine, or rank transformation (latter using Friedmans' non-parametric rank test). If none satisfied the assumptions completely, the transformation was used that best met the assumptions for ANOVA.

Virulence of the isolates causing damping-off symptoms on pea in the growth chamber conditions was determined relative to isolate *P. ultimum* 030141 and to the non-inoculated control plots. Isolates for which a significant reduction ($P < 0.05$) was observed for at least two of the dependent variables measured, compared to that of reference isolate *P. ultimum* 030141, were considered more virulent than the control isolate (+++); isolates not significantly different than the reference isolate for at least two of the variables measured, were considered similar in virulence to the control isolate of *P. ultimum* (++); and isolates with a significant reduction in at least two of the dependent variables measured compared to the non-inoculated control plants, but not as great as that of *P. ultimum* 030141, were considered less virulent (+) than the control isolate of *P. ultimum* (Table 2.5).

2.2.3. Quantification of *Pythium* spp. by real-time PCR assays

Real-time PCR assays using species-specific primers were used to detect and quantify the amount of inoculum of the three most prevalent and pathogenic *Pythium* species detected in soil from the 37 organic fields surveyed: *P. abappressorium*, *P. irregulare* group I, and *P. ultimum*. Each field was represented by a soil sample from each of the four transects sampled. Each of the four soil samples was considered a replicate sample for that field in each of the real-time PCR assays.

2.2.3.1. DNA Extraction. DNA was extracted from 500 to 510 mg of the soil sampled/ transect/field for each of the 37 certified organic fields surveyed, using the Ultraclean Soil DNA isolation kit (Mo-Bio Laboratories, Carlsbad, CA), following the manufacturer's procedure with some modifications. The samples were processed with a FP-24 cell disruptor (MP Biomedicals) for 45 s at speed 5. Washed, 10% insoluble polyvinyl-polypyrrolidone (PVP) was added upon eluting the DNA from each spin column to help remove potential PCR inhibitors (Zhou et al., 1996).

2.2.3.2. Real-time PCR assays. The DNA extract from each soil sample was used to quantify the three primary *Pythium* species using species-specific primer pairs and SYBR Green real-time PCR assays as described by Schroeder et al. (2006). For the PCR assay for each species, a 20 µl reaction was prepared in a LightCycler 96 plate (Roche Applied Sciences, Indianapolis, IN), that included 12 µl PCR grade water, 4 mM $MgCl_2$, 8 pmole of each of the forward and reverse primers, 2 µl LightCycler Fast Start DNA Master SYBR Green 1 (Roche Applied Science), and 2 µl DNA template. Each real-time PCR assay was completed in a LightCycler 480 (Roche Applied Science) using the following program: 10 min initial denaturation at 95°C; followed by 50 cycles of 10 s at 95°C, 15 s at 70°C, and 30 s at 72°C.

Melting curves were then generated by increasing the temperature to 95°C for 5 s, then decreasing to 65°C for 1 min, and ramping up the temperature at the rate of 0.1°C/s to 95°C with continuous measurement of fluorescence. Data were analyzed with LightCycler 480 software using the second derivative maximum analysis, and compared with the standard curve developed by K.L. Schroeder (Wheat Pathology Lab, USDA ARS Pullman) using a 1 ng standardized DNA concentration for each of the species from DNA extracted from mycelium of *P. ultimum* isolate 030141 and *P. irregulare* group 1 isolate 0900101. The real-time PCR assay was carried out as described above with a 10-fold dilution series prepared from the standardized DNA concentration for each species, from 1 ng to 5 fg DNA.

For *P. abappressorium*, a standard curve was generated using total DNA extracted from the mycelium of a pure culture of *P. abappressorium* isolate 020162 (obtained from T. Paulitz, Wheat Pathology Lab, USDA ARS Pullman). Extraction was performed with the FastPrep kit (MP Biomedicals) and Fast prep FP 120 cell disruptor following the manufacturer's instructions, as described above. Quantification of total DNA extracted was performed using Hoechst 33258 dye for a 200 µl volume in a 96-well plate and a Safire fluorescence plate reader (TECAN Research, Triangle Park, NC), standardized to a 5 ng DNA final concentration. A 10-fold dilution from 1 ng to 5 fg was prepared, and the real-time PCR assay was performed in a 96-well plate using a Roche Light Cycler (Roche Applied Science). The assay was performed using FastStart DNA Master SYBR Green I (Roche) in 20 µl reactions, as described above (Schroeder et al., 2006). Each dilution sample was tested in triplicate wells in the real-time PCR assay.

A soil standard curve was developed for each of the three prevalent pathogenic *Pythium* species to examine the relationship of DNA concentration with number of CFU/g soil. The objective of the assay was to develop six soil dilutions with target concentrations of 5, 10, 50,

100, 500, and 1,000 CFU/g soil, to represent the *Pythium* population densities reported in soils surveyed previously in eastern Washington (Cook, 2002; Schroeder et al., 2006). Three soil samples, each obtained from different fields (Baillie 2N, S-44, and WB-14: see Table 2.1) representing the central, north, and south regions of the Columbia Basin, respectively, were inoculated with an isolate of each of the three primary pathogenic *Pythium* species. A steam-pasteurized sample of each soil was inoculated with *P. ultimum* isolate 030141, *P. irregulare* group 1 isolate B1-22, or *P. abappressorium* isolate B11-111. The three inoculated soils for each of the *Pythium* species were incubated at room temperature for three weeks, and then quantified by dilution plating onto PSM, as described above. A soil dilution series was prepared with a target population range of 5, 10, 100, 500, and 1,000 CFU/g soil. Three 500 mg soil samples were used for each soil dilution for DNA extraction using the Ultraclean Soil DNA isolation kit (Mo-Bio Laboratories), following the manufacturer's protocol. A subsample of 1 g soil was also used from each soil dilution to quantify by dilution plating to verify the actual viable *Pythium* population (CFU/g soil) after the dilution series preparation. The total DNA extracted/soil dilution was used for the real-time PCR assay with the species-specific primers for each of the three *Pythium* species, following the protocol described above. Regression analyses were calculated using Sigma Plot Version 11 (Systat, San Jose, CA) to examine the relationship between target DNA concentration detected and pathogen population in the inoculated soil (CFU/g soil) for each of the three target *Pythium* species.

The number of CFU/g quantified in the soil after preparing the dilution series resulted in a much higher population calculated than the targeted 5 to 1,000 CFU/g soil (see results below). Therefore, a repeat experiment was carried out to attempt to obtain a population dilution series closer to the targeted range of ≤ 1,000 CFU/g soil. The repeat inoculation was carried out using

soil from one field (WB-14), since there was no evidence of significant differences among soils from the three fields tested previously (*data not shown*). A set of soil dilution series was prepared similarly with a target range of 5 to 1, 000 CFU/g soil. Total DNA was extracted following the method described above, followed by the real-time PCR assays. A 1 g subsample from each soil dilution was used to carry out soil dilution plating on PSM to determine the number of CFU/g soil for each dilution. Regression analyses were calculated to examine the relationship between DNA concentration and the number of CFU/g soil for each of the three *Pythium* species.

2.3. Results

2.3.1. Survey of *Pythium* spp.

A total of 305 *Pythium* isolates was obtained from the soils sampled from 37 certified organic fields in the Columbia Basin of Washington in October 2009 by baiting using grass leaves of *P. pratensis* or isolated from the roots of pea or sweet corn seedlings planted in pots filled with soil collected from each of the fields. Of the 305 isolates, 41% were from fields in the north region, 17% from fields in the central region, and 42% from fields in the south region of the Columbia Basin (Table 2.3), which may reflect the number of fields surveyed from each region (17, 5, and 15, respectively). Identification of 269 out of 305 isolates to *Pythium* species by sequencing the ITS rDNA, revealed that the presence of 19 species (Tables 2.2 and 2.3). Fourteen *Pythium* species were detected in the 17 fields from the north region, 9 species were from the 5 fields of the central region, and 15 species were from the 15 fields in the south region of the Columbia Basin. *P. ultimum* isolates made up 24.6% of all the isolates, followed by *P. torulosum* at 18.4%, and *P. irregulare* group 1 at 15.1%. A single isolate of each of *P. camurandrum* (0.3%), *P. diclinum* (0.3%), *P. radiosum* (0.3%), and *P. splendens* (0.3%) was

obtained out of the 305 isolates baited from all 37 fields (Table 2.3). The incidence of other species ranged from 0.7% (*P. intermedium*) to 4.9% (*P. abappressorium*) (Table 2.3). *P. ultimum, P. torulosum, P. irregulare* group I, *P. abappressorium,* and *P. middletonii* were present in 86, 65, 38, 24, and 24% of the 37 fields, respectively (Fig. 2.3). Four of the species, *P. camurandrum, P. diclinum, P. radiosum,* and *P. splendens* were each baited from a single field (Table 2.3 and Fig. 2.3). The other 10 species were detected in 5 to 22% of the fields (Fig. 2.3). The isolates of *P. echinulatum, P. oligandrum,* and *P. radiosum* were obtained only from fields in the north region; *P. camurandrum, P. diclinum,* and *P. splendens* were present only in fields in the south region; and the two *P. intermedium* isolates were present only in fields in central region (Tables 2.2 and 2.3).

The soil analyses for the 37 organic fields revealed an average of 13.9 mg NO_3-nitrogen (N)/kg soil, with low levels of NO_3-nitrogen (< 10 mg/kg) in 54% of the fields, medium levels (10 to 20 mg/kg) in 30% of the fields, and excessive NO_3-nitrogen levels (> 30 mg/kg) in 16% of the fields (Table 2.4) (Marx et al., 1999). The typical range of NH_4^+-N in soils, according to Marx et al. (1999), is 2 to 10 mg/kg soil. Of the 37 fields surveyed, 76% had NH_4^+-N levels below this range, while 24% fell within this range (Table 2.4). The average amount of phosphorus (P) and potassium (K) in the 37 fields was high (33 and 491 mg/kg, respectively). In fact, 32% of the fields had excessive P (> 40 mg/kg), 41% had high P levels (20 to 40 mg/kg), 22% had intermediate levels (10 to 20 mg/kg), and only 5% had low levels (< 10 mg/kg) (Table 2.4). For K, 11% of the fields had excessive levels (> 800 mg/kg), a majority (81%) had high levels (250 to 800 mg/kg), and 5 and 2% had medium and low levels, respectively (160 to 250 and < 50 mg/kg, respectively) (Table 2.4) (Marx et al., 1999).

Calcium (Ca) was present at high levels (> 10 meq/100 g soil) in 43% of the fields, at a medium range (5 to 10 meq/100 g) in 54% of the fields, and at a low level (< 5 meq/100 g) in only 3% of the fields (Table 2.4). Magnesium (Mg) was present at high levels (> 1.5 meq/100 g of soil) in a majority (86%) of the fields, and at medium levels (0.5 to 1.5 meq/100 g soil) for the remaining 14% of the fields. Most (95%) of the fields had high sulfur (S) levels (> 10 ppm), and the rest (5%) had medium levels (5 to 10 ppm) (Table 2.4). Boron (B) was mostly at low levels, with 62% of the fields at < 0.5 ppm, and 38% at medium levels (0.5 to 2 ppm). Zinc (Zn), manganese (Mn), and copper (Cu) were present at > 1.0, > 1.5, and > 0.6 ppm in 84, 35, and 92% of the fields, respectively, which are classified as sufficient levels by Marx et al. (1999); while 16, 65, and 8% of the fields, respectively, were deficient in these nutrients (Marx et al., 1999) (Table 2.4). The iron (Fe) levels in 16% of the fields were similar to the average of the 37 fields (9 mg/kg), while 27% of the fields had greater Fe levels and 57% of the fields were below the average (Table 2.4).

The average organic matter (OM) content in the 37 fields was 1.2%, with 43% of the fields below this average (0.7 to 1.1%), 41% above 1.2% OM (1.3 to 2.0%), and 13% at 1.2% OM (Table 2.4). A highly alkaline soil pH (> 8.5) characterized 19% of the 37 fields, while the majority of the fields (73%) were moderately to strongly alkaline (pH 7.4 to 8.4), and 8% were neutral (soil pH 6.6 to 7.3) (Marx et al., 1999) (Table 2.4). EC, an indicator of the amount of soluble salts in the soil, averaged 0.45 m.mhos/cm across the 37 fields, with 13% of the fields similar in EC level to the average, 49% below the average, and 38% above the average EC level (Table. 2.4).

2.3.2. Pathogenicity tests

Isolates of nine *Pythium* species, *P. abappressorium, P. dissotocum, P. echinulatum, P. intermedium, P. irregulare* group I, *P. splendens, P. sylvaticum, P. ultimum,* and *P. violae,* showed pathogenicity to the pea cv. Tonic under cool and moist soil conditions in at least one of the two growth chamber trials (Appendix Table 2.1 and Fig. 2.4 to 2.13). Pea seedlings in pots inoculated with isolates of these species had significant reductions ($P < 0.05$) in emergence, shoot length, and/or shoot dry weight 28 dap compared to the seedlings in non-inoculated control pots (Fig. 2.4 and Appendix Table 2.1). Isolates of 10 species, *P. adhaerens, P. aristosporum, P. camurandrum, P. catenulatum, P. diclinum, P. inflatum, P. middletonii, P. oligandrum, P. radiosum,* and *P. torulosum,* were non-pathogenic to pea under these cool and moist soil conditions, as there were no significant differences in emergence, shoot length, and/or shoot dry weight of the pea plants grown in soil inoculated with isolates of these species compared to plants in the non-inoculated control pots (Fig. 2.4D and 2.4F). The control isolate of *P. ultimum* 030141 was pathogenic on pea in every trial.

Some of the *P. ultimum* isolates were more virulent than the reference isolate, *P. ultimum* 030141, in causing damping-off symptoms on pea under cool and moist soil conditions in at least one of the two pathogenicity trials (Fig. 2.4A vs. Fig. 2.4E). Some isolates of five other *Pythium* species were similar in virulence to the *P. ultimum* 03014 in trial 1, including isolates of *P. abappressorium, P. intermedium, P. irregulare* group 1, *P. splendens,* and *P. violae* (Fig. 2.4B vs. Fig. 2.4E). Some isolates of three other pathogenic species, *P. dissotocum, P. echinulatum,* and *P. sylvaticum,* were less virulent than the reference isolate, *P. ultimum* 030141 (Fig. 2.4C vs. Fig. 2.4E).

In *P. abappressorium*- inoculated soil in the first pathogenicity trial, mean pea emergence for the seven pathogenic isolates of the eight isolates tested was 26 to 60%, shoot length was 4.1 to 5.5 cm, and shoot dry weight was 0.07 to 0.22 g compared to 44% emergence, 5.8 cm shoot length, and 0.15 g shoot dry weight of pea plants in soil inoculated with the reference isolate *P. ultimum* 030141(Fig. 2.5A, 2.5C, and 2.5E and Appendix Table 2.1). Isolate B11-97 was not pathogenic (Fig. 2.5A, 2.5C, and 2.5E and Appendix Table 2.1). In the repeat pathogenicity trial, all the isolates had reduced virulence on pea (mean emergence of 46 to 70%, shoot length of 4.0 to 5.7 cm, and shoot dry weight of 0.12 to 0.37 g) compared to the reference isolate (mean emergence of 20%, shoot length of 3.3 cm, and shoot dry weight of 0.04 g), but seven of the eight isolates were still rated as pathogenic, and three isolates were as virulent as the control isolate of *P. ultimum* (Fig. 2.5B, 2.5D, and 2.5F and Appendix Table 2.1). Interestingly, isolate B3-94, which was pathogenic in trial 1 was not pathogenic in trial 2, and vice versa for B11-97 (Fig. 2.5 and Appendix Table 2.1).

Only four of the eight isolates of *P. dissotocum* tested in the first pathogenicity trial were pathogenic on pea based on criterion of at least two dependent variables being reduced significantly compared to the non-inoculated control plants. Plants inoculated with these four isolates had a mean emergence of 44 to 66% (Fig. 2.6A), mean shoot length of 5.3 to 6.6 cm (Fig. 2.6C), and mean shoot dry weight of 0.14 to 0.26 g (Fig. 2.6E), greater compared to the 4% emergence, 1.1 cm shoot length, and 0.01 g shoot dry weight of pea plants in pots inoculated with the reference isolate (Fig. 2.5A, 2.5C, and 2.5E, respectively; and Appendix Table 2.1). However, in the repeat pathogenicity trial, only two isolates were pathogenic on pea (B1-32 and B2-49), one of which (B1-32) was not pathogenic in the first trial (Fig. 2.6B, 2.6D, and 2.6F, and Appendix Table 2.1).

Two of the seven isolates of *P. echinulatum* were pathogenic on pea in the first pathogenicity trial, but less so than the reference isolate of *P. ultimum* (Fig.2.7 and Appendix Table 2.1). Mean emergence of pea plants in pots inoculated with these two isolates ranged from 60 to 68% (Fig. 2.7A), shoot length from 6.0 to 6.1 cm (Fig. 2.7C), and shoot dry weight from 0.25 to 0.27 g (Fig. 2.7E), which was greater than 34% emergence, 3.9 cm shoot length, and 0.08 g shoot dry weight of plants in soil inoculated with the reference isolate but less than that of the non-inoculated plants (Fig. 2.7A, 2.7C, and 2.7E, respectively). When the trial was repeated, both of these isolates were not rated as pathogenic, but isolate B3-69 was pathogenic, unlike in the first trial (Fig. 2.7B, 2.7D, and 2.7F, and Appendix Table 2.1).

Only one of the two *P. intermedium* isolates was pathogenic on pea (B3-76), and was as virulent as the reference isolate of *P. ultimum* in both trials (Fig. 2.8 and Appendix Table 2.1), with mean pea emergence of 8%, shoot length of 4.5 cm, and shoot dry weight of 0.04 g compared to 6% emergence, 1.9 cm shoot length, and 0.01 g shoot dry weight of seedlings in soil inoculated with the reference isolate (Fig. 2.8 and Appendix Table 2.1). Isolate B2-55 of *P. intermedium* was not pathogenic in trial 1 and was not evaluated in trial 2 (Fig. 2.8A, 2.8C, and 2.8E; and Appendix Table 2.1).

The seven *P. irregulare* group I isolates included in the first trial were all pathogenic and as virulent as the reference isolate *P. ultimum* on pea (Fig. 2.9 and Appendix Table 2.1). Mean emergence of the peas in soil inoculated with isolates of this species ranged from 32 to 74%, with a range in shoot length of 4.0 to 5.2 cm, and shoot dry weight of 0.10 to 0.50 g compared to 66% emergence, 3.6 cm shoot length, and 0.13 g shoot dry weight of pea plants in the soil inoculated with the reference isolate of *P. ultimum* (Fig. 2.9A, 2.9C, and 2.9E, respectively). When the trial was repeated, six of the seven isolates were pathogenic on pea, but less virulent

than the reference isolate. Mean pea emergence in pots inoculated with the six pathogenic isolates in trial 2 ranged from 38 to 66%, with shoot length of 4.3 to 6.3 cm, and shoot dry weight of 0.16 to 0.33 g, while there was no emergence in pots inoculated with the reference isolate (Fig. 2.9B, 2.9D, and 2.9F).

The single isolate of *P. splendens* obtained from organic soils in the Columbia Basin was pathogenic on pea and resulted in no emergence of pea plants (Fig. 2.10A, 2.10C, and 2.10E; and Appendix Table 2.1). In the repeat pathogenicity test, the isolate of this species was similarly virulent on pea (Fig. 2.10B, 2.10D, and 2.10F; and Appendix Table 2.1).

Only one of the four isolates of *P. sylvaticum* was pathogenic on pea in the first trial (Fig. 2.11A, 2.11C, and 2.11E); however, when the trial was repeated, all four isolates were pathogenic on pea, although less virulent than the *P. ultimum* reference isolate (Fig. 2.11B, 2.11D, and 2.11F). Mean emergence of pea plants ranged from 64 to 78% (Fig. 2.11B), shoot length from 4.5 to 5.5 cm (Fig. 2.11D), and shoot dry weight from 0.18 to 0.27 g (Fig. 2.11F), while there was no emergence in soil inoculated with the reference isolate (Fig. 2.11B, 2.11D, and 2.11F; and Appendix Table 2.1).

All nine of the *P. ultimum* isolates tested were pathogenic on pea, six of which were more virulent than the reference isolate, *P. ultimum* 030141 (Fig. 2.12A and 2.12C, and 2.12E; and Appendix Table 2.1). In soil inoculated with the six most virulent *P. ultimum* isolates, the mean emergence of pea plants ranged from 6 to 20% (Fig. 2.12A), mean shoot length ranged from 1.7 to 5.1 cm (Fig. 2.12C), and shoot dry weight ranged from 0.01 to 0.05 g (Fig. 2.12 E), all of which were significantly reduced compared to 50% mean emergence, 5.3 cm mean shoot length, and 0.19 g mean shoot dry weight of peas in soil inoculated with the reference isolate (Fig. 2.12A, 2.12C, and 2.12E, respectively). The three other *P. ultimum* isolates were similar in

virulence as the reference isolate, and inoculation of these isolates resulted in mean pea emergence of 42 to 48%, shoot length of 3.3 to 5.9 cm, and shoot dry weight of 0.12 to 0.18 g (Fig. 2.12A, 2.12C, and 2.12E, respectively). In the repeat trial, all of the isolates were still pathogenic on pea but showed reduced virulence compared to the first trial (Appendix Table 2.1). Four of the isolates were similar in virulence to the reference isolate, resulting in mean pea emergence of 0 to 28%, shoot length of 0 to 5.3 cm, and shoot dry weight of 0 to 0.08 g (Fig. 2.12B, 2.12D, and 2.12F, respectively); while four isolates were less virulent, resulting in pea emergence of 48 to 78%, shoot length of 5.7 to 7.5 cm, and shoot dry weight of 0.14 to 0.39 g, which were significantly greater than those of plants inoculated with reference isolate (mean pea emergence of 3%, shoot length of 3 cm, and shoot dry weight of 0.03 g) (Fig. 2.12B, 2.12D, and 2.12F, respectively). One isolate (B3-73) was not pathogenic on pea in the repeat trial.

The nine *P. violae* isolates tested were all pathogenic on pea of which eight were as virulent and one isolate (B13-197) was less virulent than the reference isolate of *P. ultimum* in trial 1 (Fig. 2.13A, 2.13C, and 2.13E; and Appendix Table 1). Inoculation of soil with these isolates resulted in pea plants with a mean emergence of 14 to 32% (Fig. 2.13A), plants as tall as 2.6 to 7.0 cm (Fig. 2.13C), and shoot dry weight of 0.03 to 0.12 g (Fig. 2.13E); while soil inoculated with the reference isolate had pea plants with a mean emergence of 14%, shoot length of 5.80 cm, and shoot dry weight of 0.07 g (Fig. 2.13A, 2.13C, and 2.13E, respectively) (Fig. 2.13A, 2.13C, and 2.13E). In the repeat trial, only three of the nine *P. violae* isolates were as virulent as the reference isolate of *P. ultimum*, and the other six were less virulent but still pathogenic on pea (Fig. 2.13B, 2.13D, and 2.13F; and Appendix Table 2.1). Interestingly, isolate B13-197 was as virulent as the reference isolate in the repeat trial (Fig. 2.13B, 2.13D, and 2.13F; and Appendix Table 2.1).

2.3.3. Quantification of *Pythium* spp. by real-time PCR assays

Of the three predominant pathogenic *Pythium* species detected in the 37 organic fields surveyed, *P. ultimum* was detected in 100% of the 37 fields using the real-time PCR assays, with DNA concentrations ranging from 42 ± 26 (mean ± standard error) to 18,698 ± 1,880 fg DNA/g soil (Table 2.5). Overall, 30% of the fields had ≥ 2,000 fg DNA of *P. ultimum*/g soil, 62% of the fields had 200 to 2,000 fg DNA of *P. ultimum*/g soil, while the remaining 8% of the fields had < 200 fg DNA of *P. ultimum*/g soil (Table 2.5). In contrast, with the grass leaf baiting method, *P. ultimum* was recovered from only 86% of the fields (Fig.2.3). Interestingly, in the fields where this species was not recovered by baiting (ANW 644, Baillie 2N, S8, S-44, and WB-12), a considerable amount of DNA (1,112 ± 628, 1,678 ± 994, 338 ± 196, 238 ± 168, and 2,108 ± 1,234 fg/g soil) was detected in the soil samples by the real-time PCR assay, at even greater levels than in the fields from which this species was baited successfully (Tables 2.2 and 2.5).

For *P. abappressorium*, 78% of the 37 fields tested positive for this species based on the real-time PCR assay (Table 2.5), whereas this species was recovered from only 24% of the fields by the baiting method (Table 2.3). There were five fields in which DNA of *P. abappressorium* was detected with the real-time PCR assay, but the DNA concentration was below the quantifiable limit. The amount of DNA detected for this species in the 29 fields was much less compared to that of *P. ultimum*, with a range of 4 ± 2 to 226 ± 134 fg DNA of *P. abappressorium*/g soil. For this species, 16% of the 37 fields had > 100 fg DNA/g soil detected, 62% had < 100 fg DNA/g soil, and the remaining 22% of the fields had DNA levels either below the quantifiable limit or not detected (Table 2.5).

The DNA of *P. irregulare* group I was detected in 57% of the 37 fields using the real-time PCR assay (Table 2.5), in contrast to only 38% of the fields testing positive for this species

by the baiting method (Table 2.3). The amount of DNA detected ranged from 14 ± 0 fg DNA/g soil to as high as 760 ± 296 fg DNA/g soil; however, for most fields, *P. irregulare* was detected from only one or two of the four replicate soil samples tested, and rarely from all four replicate samples/field (Table 2.5). For this species, 32.4% of the fields had < 200 fg DNA/g soil, 24.3% had > 200 fg DNA/g soil, and the remaining 43.2% of the fields did not test positive (Table 2.5).

For the standard curve developed for each of the three *Pythium* species, dilution plating of triplicate soil samples inoculated with each of *P. ultimum*, *P. irregulare* group 1, and *P. abappressorium* at a targeted rate of 1,000 CFU/g soil resulted in recovery of a greater number of CFU than expected (Table 2.6). In trial 1 of the real-time PCR assays, the ANOVA main effect of field soils did not differ significantly ($P = 0.7854$, 0.4059, and 0.1776 for *P. abappressorium*, *P. irregulare* group 1, and *P. ultimum*, respectively). In contrast, the ANOVA main effect of soil dilutions was significant ($P < 0.0001$ for all three *Pythium* species), i.e., there were significant differences in the amount of DNA detected among the soil dilutions with a significant decrease in DNA concentration detected with an increase in soil dilution (Table 2.6, Trial 1).

In addition, the interaction term between fields and soil dilutions was significant for *P. irregulare* group 1 ($P = 0.0061$), but not for the other two species ($P = 0.2664$ and 0.1135 for *P. ultimum* and *P. abappressorium*, respectively), demonstrating that the DNA extraction and real-time PCR assay for *P. irregulare* group 1 were affected significantly by soil from the three fields tested. Therefore, results are presented individually for each soil inoculated with *P. irregulare* group 1 but pooled across the three field soils for each of *P. abappressorium* and *P. ultimum* (Table 2.6). The amount of *P. irregulare* group 1 DNA quantified from WB-14 soil differed significantly among the six soil dilutions, demonstrating that there was a significant decrease in

the amount of DNA detected with a decrease in CFU/g soil, except for the DNA concentrations detected in soil infested at the two intermediate rates, 480 and 240 CFU/g soil (Table 2.6). When the Baillie 2N soil was used to generate the inoculated soil dilutions, significant differences were observed in the amount of *P. irregulare* DNA detected/g soil at all dilutions except the two highest dilutions (soil samples with 55 and 28 CFU/g soil) (Table 2.6). For the S-44 soil, samples with the least CFU/g recovered by dilution plating, i.e., 17 and 34 CFU/g soil did not differ significantly in terms of fg DNA detected/g soil by real-time PCR assay; as well as soil samples from the two lowest dilutions (3,400 and 1,700 CFU/g soil) (Table 2.6).

A significant linear regression relationship ($P < 0.0001$) of DNA concentration vs. the number of CFU/g soil detected by soil dilution plating was observed for each of the three *Pythium* species in all three field soils tested (Fig. 2.14), with the coefficient of determination (R^2) ranging from 0.8866 to 0.9716, and a comparable rate of increase calculated for the three *Pythium* species (Fig. 2.14A to 2.14C for *P. abappressorium*, Fig. 2.14D to 2.14F for *P. irregulare* group I, and Fig. 2.14G to 2.14I for *P. ultimum*). There was greater variability in the DNA concentration detected at the higher soil dilutions (lower CFU/g soil) for each *Pythium* species in each soil. For this reason, results for the highest dilution were removed from the regression analyses to satisfy the assumption of equal variances among the soil dilutions.

When the soil assays were repeated with soil from a single field (WB-14) inoculated with the same three *Pythium* species, but starting with a lower inoculum concentration (see Table 2.6, trial2), significant differences were observed ($P < 0.0001$) in the amount of DNA detected (fg/g soil) by real-time PCR assay among the soil dilutions. A similar significant linear relationship was detected between the CFU/g recovered soil and DNA concentration detected by real-time PCR assay for each *Pythium* species ($P < 0.0001$ for all three species, with $R^2 = 0.9214$, 0.7989,

and 0.9541 for *P. abappressorium*, *P. irregulare* group 1, and *P. ultimum*, respectively) (Fig. 2.14C, 2.14F, and 2.14I, respectively). The results confirmed the linear increase in DNA concentration with increasing CFU/g soil, and that the real-time PCR assays were repeatable, although DNA detection was again highly variable at the lowest inoculum concentration (5 CFU/g soil), and results differed most for *P. irregulare* group 1 real-time PCR assay between repeated soil assays (Fig. 2.14F).

Regression analyses of the pooled standard curve data from the three field soils for each *Pythium* species showed similar positive linear relationships ($P < 0.0001$) of DNA concentration detected vs. *Pythium* populations (CFU/g soil), with $R^2 > 0.9700$ for each species. The regression equations from the standard curves pooled across results for the three soils for each of three *Pythium* species were used to estimate the number of propagules (CFU/g soil) in the soil of each of 37 organic fields surveyed, by calculating CFU using the DNA concentration from the real-time PCR assays (Table 2.5). The levels of DNA detected for *P. abappressorium* were below the quantifiable limit (< 1,000 CFU/g soil) that could be estimated accurately by the regression equation for this species (Fig. 2.14A, 2.14C, 2.15A, and Table 2.5). The number of propagules of *P. irregulare* group 1 estimated by the regression equation for the 37 fields ranged from 25 ± 0 to 228 ± 4 CFU/g soil (Fig. 2.15B and Table 2.5). The *P. ultimum* DNA concentrations detected in soil from the 37 fields led to an estimated range of 14 ± 3 to 322 ± 32 CFU *P. ultimum*/g soil (Fig. 2.15C and Table 2.5). Five and 14 of the 37 fields had DNA levels of *P. irregulare* group 1 and *P. ultimum* below the quantifiable limit that could be estimated accurately using the regression equations for these two species (Fig. 2.15B and 2.15C, respectively).

2.4. Discussion

A total of 37 certified organic fields was surveyed in 2009 for *Pythium* species associated with damping-off in the semi-arid Columbia Basin of Washington State, an important region for large-scale organic vegetable crops like pea and sweet corn (Kirby and Granatstein, 2009). The prevalence of damping-off in this area is affected, in particular, by the cool and wet soil conditions typical during early spring planting. Organic vegetables planted early in the spring such as processing pea, are prone to damping-off, which can result in poor stands and may even necessitate having to replant entire fields (Alcala and du Toit, 2009). Therefore, this study surveyed the diversity of *Pythium* species in organic fields in the Columbia Basin to identify the primary species potentially associated with damping-off during cool spring conditions. From a total of 305 isolates baited from the 37 organic fields, 19 *Pythium* species were identified by sequencing the ITS region of rDNA. *P. ultimum*, *P. torulosum*, and *P. irregulare* group 1 were the three most frequently isolated species, followed by *P. abappressorium*, *P. adhaerens*, *P. middletonii*, *P. dissotocum*, *P. violae*, *P. inflatum*, *and P. echinulatum*; while *P. sylvaticum*, *P. aristosporum*, *P. catenulatum*, *P. oligandrum*, *P. intermedium*, *P. camurandrum*, *P. diclinum*, *P. radiosum* and *P. splendens* were each isolated from only one or two fields. Interestingly, *P. camurandrum*, a species isolated from only one of the 37 fields, is a new species reported recently in Canada by Bala et al. (2010). This, potentially, is the first report of this species occurring in Washington State.

In comparison to the surveys by Paulitz and Adams (2003) and Schroeder et al. (2006), a very different composition of *Pythium* species was found to be dominant in the organic vegetable production fields surveyed in the semi-arid, irrigated Columbia Basin of central Washington in this study compared to the wheat fields of eastern Washington. Differences in soil moisture,

temperature, and cropping systems may have profound effects on the composition of *Pythium* species in these two distinct regions of Washington State. In this study, at least three *Pythium* species were isolated from the soil sampled from a majority of the fields (43%), while one of the fields had as many as seven species isolated, and another field had only one species isolated. This is not unexpected, however, based on surveys for *Pythium* species in agricultural or forestry soils (e.g., Broders et al., 2007; Paulitz and Adams, 2003; Weiland, 2011). This potential association of different combinations of *Pythium* species across fields makes the development of effective disease management strategies more complicated because of the potential variation in sensitivity of different species to various production practices, fungicides, or environmental conditions (Broders et al., 2007).

Results of the pathogenicity tests in growth chamber conditions showed that, of the 19 *Pythium* species baited and identified from the certified organic soils of the Columbia Basin, isolates of only nine species were pathogenic on pea in cool and moist soil conditions. There were also differences in virulence among species and among isolates within species, as determined by the severity of damping-off symptoms. *P. ultimum* isolates consistently were the most virulent of the nine species, while isolates of *P. abappressorium, P. intermedium, P. irregulare* group 1, *P. splendens*, and *P. violae* caused symptoms as severe as those caused by the reference isolate of *P. ultimum* used in the study, an isolate that originated from a wheat field in eastern Washington. In contrast, isolates of *P. dissotocum*, *P. echinulatum*, and *P. sylvaticum* caused mild symptoms on pea, i.e., the isolates were less virulent than the reference isolate of *P. ultimum* under cool and moist soil conditions. *P. ultimum* and *P. irregulare* group 1 have been described extensively in association with damping-off of a variety of crops in cool and moist soil conditions, e.g., as reported by Ingram and Cook (1990) for isolates of these species on wheat,

pea, lentil, and barley. *P. abappressorium* is a recently described species that is an important soilborne pathogen causing root rot of wheat in eastern Washington (Paulitz and Adams, 2003; Paulitz et al., 2003). Wheat is commonly used as a cover crop in rotation with organic vegetable crops in the Columbia Basin (Kok et al., 2008), which may explain a similar prevalence of *P. abappressorium* in these organic fields as in the wheat fields in eastern Washington. *P. intermedium* is an important soil inhabitant of cool, temperate forest soils (Li et al., 2010), while *P. splendens* is a common root rot pathogen of many plants in warmer areas (Kidney, 1979; Van der Plaats-Niterink, 1981). *P. violae* is a pathogen widely associated with carrot cavity spot (Campion et al., 1997), and isolates of this species were baited from a field that had been planted to certified organic carrot crops in 2009, i.e., WB-5 field in the south region of Columbia Basin. Weiland (2011) recently reported *P. dissotocum* as the second most commonly isolated *Pythium* species from forest nursery soils in Oregon and Washington. This species also was isolated frequently from corn and soybean fields in Ohio, along with *P. sylvaticum* (Broders et al., 2007). In the latter study, *P. dissotocum* was reported to be more pathogenic on soybean than on corn, and vice versa for *P. sylvaticum*. *P. echinulatum* is also commonly isolated from soils and was reported to be very virulent on strawberry (van der Plaats-Niterink, 1981). Isolates of all these species were pathogenic on pea in cool spring planting conditions evaluated in this study.

In this study, *P. torulosum* was the second most commonly isolated species from organic fields in the Columbia Basin, although none of the isolates of this species was pathogenic on pea in cool and moist soil conditions. Other studies have reported this species to be an important pathogen causing damping-off of corn, soybean, and turf grasses (Broders et al., 2007; Hsiang et al., 1995; Zhang et al., 1996). Similarly, *P. adhaerens* and *P. middletonii* were isolated frequently in this study, but were not pathogenic on pea. Sparrow (1932) reported *P. adhaerens*

as a pathogen of pea and other crops, while *P. middletonii* has been associated with root rot of crucifers (Singh and Pavgi, 1978). The cool and moist soil conditions used during the pathogenicity tests in this study may have affected the ability of isolates of some of the *Pythium* species to infect pea seeds or seedlings, and some of these species may have proven pathogenic on pea if the trials were carried out at warmer temperatures, because environmental conditions can have profound effects on the ability of some *Pythium* species to infect plants and cause symptoms (Martin and Loper, 1997).

Variability in results of the repeated pathogenicity trials with isolates of the nine *Pythium* species with pathogenic isolates was observed in this study. There was an observed reduction in the virulence of isolates compared to the reference isolate of *P. ultimum* when the trial was repeated. Seven isolates were not pathogenic in the repeated trial, while five isolates displayed pathogenicity even though these isolates were not pathogenic in the first trial. This variation could have been caused, in part, by age of the inoculum used in each of the trials. In the first trial for each set of isolates, freshly prepared inoculum (3 to 4-weeks-old), was used for each of the isolates tested. The inoculum remaining after the first trial was kept in cold storage (4 ± 1°C) for 3 to 6 months, and used in the repeat trial. In comparison, a new batch of inoculum was used for the reference isolate *P. ultimum* 030141, in every trial because this isolate was included in each test. The inoculum of each isolate was quantified by dilution plating onto WA in every trial to ensure an inoculation rate of 500 CFU/g soil. However, the virulence of the isolates may have been affected by the duration of storage. Johnson et al. (1980) demonstrated that the level of exogenous nutrients, e.g., nitrogen and carbohydrates, amended in the inocula of *P. ultimum*, influenced virulence of the pathogen, i.e., high concentrations of these nutrients were required for maximum disease development following infection of cotton hypocotyls. Higginbotham et al.

(2004) also observed variability between repeats of pathogenicity trials of *Pythium* isolates on wheat and associated this variation with a reduction in inoculum potential between the two trials. The authors pointed out the importance of accurate determination of inoculum potential when conducting virulence assays for *Pythium*, particularly in preparation of inoculum and determination of spore density, since a relatively small amount of inoculum typically is mixed in a much greater volume of soil. The method used to determine virulence of the isolates in this study was all relative to the reference isolate of *P. ultimum*, for which a new batch of inoculum was prepared for every trial, which may have confounded the variation observed between repeat trials for the test isolates from the organic fields. Differences in the vigor of the two seed lots of the pea cv. Tonic used in each trial may also have contributed to variation between the two trials. The seed lot used in the repeat trial generally had better vigor than the seed lot used in the first trial, based on the early emergence count (14 dap) of pea in the non-inoculated soil control treatments between the two pathogenicity trials (*data not shown*). A higher vigor seed lot will be less susceptible to infection by less virulent *Pythium* isolates, such as in the repeat pathogenicity trial. However, this does not explain results for those isolates that were pathogenic in the repeat trial but not in the first trial.

Overall, *P. ultimum*, *P. irregulare* group 1, and *P. abappressorium* were the dominant pathogenic *Pythium* species detected in certified organic fields surveyed in the semi-arid Columbia Basin of central Washington. Isolates of all three species caused damping-off in cool and moist soil conditions, and are likely associated with poor stands in early spring planted pea crops in this area, although direct isolations from pea seedlings with damping-off symptoms in growers' fields would be necessary to confirm this. Knowledge of the major *Pythium* species associated with damping-off in organic vegetable cropping systems is important for effective

management of this disease, especially for evaluating potential organic seed treatments that might be used by growers when planting in conditions conducive to damping-off (see Chapter 3).

The three dominant pathogenic *Pythium* species detected by baiting were also quantified in soil samples from the 37 fields by real-time PCR assays using species-specific primers. *P. ultimum* was detected in all 37 fields with the real time PCR assay, with DNA concentrations of this species generally detected at greater concentrations than the DNA levels detected for the two other species. *P. abappressorium* was detected by real time PCR assay in 78% of the fields, but at lower DNA concentrations than *P. ultimum*, while *P. irregulare* group 1 was detected in only 57% of the fields and seldom detected in all four of the soil transects assayed per field, unlike the other two species. Nevertheless, the frequency of detection of the three species in soils from the 37 organic fields using the real-time PCR assays was generally greater than the frequency of recovery of these species by baiting with grass leaves or pea and corn seeds planted in the soil samples. Schroeder et al. (2006) used the same species-specific primer pairs for detecting these three *Pythium* species from fields in eastern Washington, and detected greater amounts of DNA of *P. abappressorium* and *P. irregulare* group 1 from dry soils (e.g., 580 to 2,775 fg/g soil for *P. abappressorium* detected in three fields, and 5,360 fg/g soil for *P. irregulare* group 1 detected in only one out of six fields), and wet soil samples (680 to 6,065 fg/g soil for *P. abappressorium*, and 7,980 fg/g soil for *P. irregulare* group 1) compared to what was detected in this study from soil samples from certified organic fields in the Columbia Basin (see Table 2.5). In contrast, *P. ultimum* was detected frequently with greater amount of DNA quantified in the soils of the 37 certified organic fields in the Columbia Basin compared to what was found in the soils of wheat fields in eastern Washington, in which the amount of DNA quantified for this species was very low (< 10 fg/reaction) (Schroeder et al., 2006).

Schroeder et al. (2006) reported that variation in the amount of *Pythium* DNA detected in eastern Washington soils increased greatly as the population of *Pythium* in the soil approached the detection limit of 10 to 100 fg/g soil (~1 *Pythium* propagule/g soil) for the real-time PCR assay used, although one of the species, *P. abappressorium*, was detected to as low as 4 fg/g soil in this study. The detection of very low *Pythium* populations in the soil dilution series resulted in highly variable results across samples from the four transects/field. Of the three target *Pythium* species, *P. abappressorium* was detected at relatively low DNA concentrations compared to the two other species. Overall, the results of the real-time PCR assays in this study were consistent with those of other studies in the application of real-time PCR assay to detect and quantify specific pathogens in soil (Li et al., 2010; Schroeder et al., 2006). The potential ability to use real-time PCR assays to detect and quantify *Pythium* species in soil at a greater level of sensitivity (lower concentrations) than baiting methods has been documented by others (Li et al., 2010; Schroeder et al., 2006). However, real-time PCR assays detect total DNA of the target pathogen(s), regardless of the viability of the propagules, which could also account for part of the greater amount of detection using this method vs. baiting. In contrast, dilution plating detects only viable propagules (Li et al., 2010). There was variability in the amount of DNA detected from the four soil transects assayed per field for each of the three *Pythium* species. This may reflect the typical aggregated distribution of *Pythium* propagules within soil, an important consideration when sampling soils to detect target organisms (Okubara et al., 2005), although the collection of 20 soil cores/transect followed by mixing the cores thoroughly, should have addressed this issue to some degree.

A standard curve for each of the three primary *Pythium* species detected was developed by inoculating each of three soils from different regions of the Columbia Basin with each of the

Pythium species, to relate *Pythium* population detected in the soil by dilution plating with DNA concentration detected using the real-time PCR assays. Regression analyses showed a positive linear relationship between *Pythium* population and DNA concentration, regardless of the soil used, i.e., the DNA extraction method and real-time PCR assay results were consistent among soils for two of the three *Pythium* species. This demonstrated that the real-time PCR assays for *P. abappressorium* and *P. ultimum* could be used to quantify for DNA concentration of these two *Pythium* species in different soils, similar to results of other studies (Kernagahan et al., 2008; Li et al., 2010; Schroeder et al., 2006), but not for the *P. irregulare* group 1 real-time PCR assay. When the real-time PCR assays were repeated for the three *Pythium* species using one soil with a lower range of soil inoculation rates, results were consistent with the first trial, i.e., DNA concentration was strongly positively correlated with the *Pythium* species inoculum level, further demonstrating reproducibility of the real-time PCR assay results. However, as the *Pythium* population in the soil approached the detection limit, i.e., 10 fg (Schroeder et al., 2006), the amount of DNA detected across replicate samples from the soil became highly variable such that regression analyses failed the test for equal variances. This revealed the lower detection limit for the reliable quantification results with these real-time PCR assays (~10^2 to 10^3 fg/g soil).

When the regression equations, for the three *Pythium* species were used to estimate the inoculum concentration (CFU/g soil) from the 37 organic fields surveyed in 2009, based on the DNA quantified/species with the real-time PCR assays, very few of the fields had similar inoculum levels (200 CFU/g soil) to those reported by Cook et al. (1990) for the wheat fields of inland PNW. This suggests a need to investigate further whether the amount of CFU detected in the certified organic fields of Columbia Basin for the three *Pythium* species would lead to a significant damping-off in organic pea crop during early spring planting conditions. The

regression equation developed in this study for *P. ultimum* estimated inoculum concentrations of 1.6 CFU per 100 fg DNA/g soil, which is similar to what was detected by K.L. Schroeder and T.C. Paulitz for eastern Washington wheat fields (*unpublished data*), i.e., 0 to 100 *Pythium* DNA fg/1 propagule/g soil sampled from inoculated soil samples. However, it must be noted that the equation used to estimate inoculum concentration was developed using pasteurized soil inoculated with each of the three *Pythium* species, and has not been validated for field soil samples with natural *Pythium* populations as well as in the presence of other soil microflora that readily affect the survival and inoculum potential of *Pythium* propagules in soils (Martin and Loper, 1999).

In summary, this survey revealed the presence of 19 *Pythium* species in organic vegetable fields in the semi-arid Columbia Basin of Washington, of which three were most prevalent and pathogenic on pea in cool, moist soil conditions: *P. abappressorium*, *P. irregulare* group 1, and *P. ultimum*. Variability in virulence on pea was detected among species and among isolates within each species. The isolates of *P. ultimum* were the most virulent on pea under cool, moist soil conditions and may be the most important species associated with damping-off in organic fields in the Columbia Basin. Species-specific primer pairs were used in real-time PCR assays for each of these three species for detection and quantification of the pathogens in soils from 37 fields in this region. Detection of these three *Pythium* species in field soil by real-time PCR assays was more sensitive than the baiting methods evaluated in this study, as a greater percentage of the soil samples tested positive with the real-time PCR assays than the baiting assays. Greater DNA concentrations were detected for *P. ultimum* in these fields compared to the two other target *Pythium* species with the real-time PCR assays, which further supports the prevalence of these species in these fields in the Columbia Basin. Furthermore, the DNA

concentration of the target species was positively associated with the number of propagules detected in the soil by dilution plating.

The regression equations developed in this study were used to estimate the inoculum concentration of the three *Pythium* species in each of the 37 fields. However, there is a need to validate these equations further using other naturally-infested field soils since the equations were developed using *Pythium* populations added to pasteurized soil. Accurate estimation of inoculum concentration in field soil was limited to DNA concentrations of 10^3 fg/g soil for *P. abappressorium* and *P. ultimum*, and ~ 10^2 for *P. irregulare* group 1 based on the regression equations. Further investigations are needed to determine the inoculum concentrations at which growers typically experience significant losses from these *Pythium* species in pea and other crops, and to validate the regression equations developed in this study.

If a consistent relationship between DNA concentration and CFU/g soil detected on selective agar media can be established with naturally infested soil samples, the DNA-based inoculum concentration prediction using real-time PCR assays might be a rapid and accurate way to predict the risk of damping-off. This will help with developing and implementing appropriate management tactics ahead of planting, which could assist the growers of certified organic crops who have limited control options for Pythium damping-off. Ultimately, a multiplex PCR assay can be developed to detect and quantify multiple target *Pythium* species associated with damping-off in a single assay, which will not only increase the efficiency of DNA-based assessment of inoculum concentration in a field, but will also make it possible to assay a greater number of samples thereby improving the accuracy of results. Such an assay would provide a tool that growers can use for more timely risk assessment, and timely application of control strategies to manage damping-off in organic production. Such an assay could be of similar value

to conventional growers of crops that are highly susceptible to damping-off by *Pythium* spp. However, even if such a diagnostic tool is made available, the inoculum potential of a field for damping-off is a function not only of inoculum concentrations at the time of planting and seedling establishment, but also cultural practices implemented by the growers.

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Table 2.1. Field, survey date, location, farm (and grower), and cropping history of 37 certified organic fields in the Columbia Basin of Washington surveyed for *Pythium* spp. in 2009

Field code[a]	Survey date	Location[b]	Farm (grower)	Crop(s) grown during 2009
1	7 Oct.	North Basin	Hyers' Farm	Wheat and mustard cover crop
2	15 Oct.	North Basin	Hyers' Farm	Wheat
3	15 Oct.	North Basin	Hyers' Farm	Wheat
4	15 Oct.	North Basin	Hintz Farm	Sweet corn
5	15 Oct.	North Basin	Hintz Farm	Sweet corn
6	9 Oct.	North Basin	Hyers' Farm	Pea
7	9 Oct.	North Basin	Hyers' Farm	Wheat cover crop
8	9 Oct.	North Basin	Hyers' Farm	Pea
9	7 Oct.	North Basin	Hyers' Farm	Mustard cover crop and pea
10	9 Oct.	North Basin	Hyers' Farm	Onion
11	9 Oct.	North Basin	Hyers' Farm	Pea
12	9 Oct.	North Basin	Hyers' Farm	Pea
13	9 Oct.	North Basin	Hyers' Farm	Mustard and pea
14	15 Oct.	North Basin	Hyers' Farm	Wheat
15	7 Oct.	North Basin	Hyers' Farm	Winter wheat and pea
16	7 Oct.	North Basin	Hyers' Farm	Sweet corn and wheat
17	7 Oct.	North Basin	Hyers' Farm	Wheat
18	9 Oct.	Central Basin	Lennwood Farms (Brad Baillie)	Onion
19	9 Oct.	Central Basin	Lennwood Farms (Brad Baillie)	Spelt, camelina, and emmer
20	9 Oct.	Central Basin	Lennwood Farms (Brad Baillie)	Not known
21	9 Oct.	Central Basin	Lennwood Farms (Brad Baillie)	Pea
22	9 Oct.	Central Basin	Lennwood Farms (Brad Baillie)	Potato and spelt
23	21 Oct.	South Basin	Baybee Farms, Plymouth WA	Pea, sweet corn, and green beans
24	21 Oct.	South Basin	Baybee Farms, Plymouth WA	Pea, sweet corn, and green beans
25	21 Oct.	South Basin	Baybee Farms, Plymouth WA	Green beans
26	21 Oct.	South Basin	Baybee Farms, Plymouth WA	Onion
27	21 Oct.	South Basin	Baybee Farms, Plymouth WA	Green beans
28	10 Oct.	South Basin	100 Circles Farm (Watts Brother)	Sweet corn
29	10 Oct.	South Basin	100 Circles Farm (Watts Brother)	Silage
30	10 Oct.	South Basin	100 Circles Farm (Watts Brother)	Carrots
31	10 Oct.	South Basin	100 Circles Farm (Watts Brother)	Pea and sweet corn
32	10 Oct.	South Basin	100 Circles Farm (Watts Brother)	Silage

Table 2.1. *continued*….

Field code[a]	Survey date	Location[b]	Farm (grower)	Crop(s) grown during 2009
33	10 Oct.	South Basin	100 Circles Farm (Watts Brother)	Pea
34	10 Oct.	South Basin	100 Circles Farm (Watts Brother)	Pea and mustard cover crop
35	10 Oct.	South Basin	100 Circles Farm (Watts Brother)	Sweet corn
36	10 Oct.	South Basin	100 Circles Farm (Watts Brother)	Pea and sweet corn
37	10 Oct.	South Basin	100 Circles Farm (Watts Brother)	Pea and sweet corn

[a] Soil samples were collected from four transects in each field, and used to bait for *Pythium* species as well as for quantification of the three primary pathogenic *Pythium* species on pea, the latter using real-time PCR assays (as described in the main text). Up to 20 soil cores were collected from each transect using a 2.5 cm diameter soil probe, to a depth of 15 cm, and cores pooled within each transect.

[b] Region of the Columbia Basin where the field was located. The north region represented fields located north of Warden, WA; the central region represented fields between Warden and Eltopia, WA; and the south region represented fields between Eltopia and Paterson, WA (see Fig. 2.1).

Table 2.2. Isolates of 19 *Pythium* species baited from soil sampled from each of 37 certified organic fields in the Columbia Basin of Washington in October 2009, and tested for pathogenicity on the pea cv. Tonic

Species[a]	Isolate	Field code[b]	Baiting method[c]	Pathogenicity test[d]	
				Trial 1	Trial 2
P. abappressorium	B3-94	22	Grass leaves	+	+
	B13-168	34	Pea	+	+
	B12-160	20	Corn	+	+
	B11-97	20	Corn	+	+
	B11-111	3	Pea	+	+
	B10-16	27	Corn	+	+
	B10-2	27	Corn	+	+
	B11-86	11	Corn	+	+
P. adhaerens	B3-81	1	Grass leaves	+	
	B3-86	37	Grass leaves	+	
	B2-57	10	Grass leaves	+	
	B3-87	10	Grass leaves	+	
	B1-35	37	Grass leaves	+	
	B2-52	23	Grass leaves	+	
	B3-66	37	Grass leaves	+	
	B1-10	25	Grass leaves	+	+
	B3-67	10	Grass leaves	+	
P. aristosporum	B4-29	12	Grass leaves	+	+
	B10-38	34	Corn	+	
P. camurandrum	B8-104	26	Grass leaves	+	+
P. catenulatum	B9-157	7	Grass leaves	+	+
	B3-88	37	Grass leaves	+	
P. diclinum	B1-9	34	Grass leaves	+	+
P. dissotocum	B4-4	9	Grass leaves	+	+
	B4-5	9	Grass leaves	+	+
	B3-84	20	Grass leaves	+	+
	B5-59	20	Grass leaves	+	+
	B1-32	30	Grass leaves	+	+
	B4-2	6	Grass leaves	+	+
	B2-49	34	Grass leaves	+	+
	B3-79	34	Grass leaves	+	+
P. echinulatum	B3-69	9	Grass leaves	+	+
	B12-118	17	Corn	+	+
	B5-62	20	Grass leaves	+	+
	B5-47	2	Grass leaves	+	+
	B5-45	2	Grass leaves	+	+
	B1-16	3	Grass leaves	+	+
	B2-51	14	Grass leaves	+	+
P. inflatum	B3-89	36	Grass leaves	+	+
	B1-13	33	Grass leaves	+	+
	B3-93	33	Grass leaves	+	+
	B10-27	33	Corn	+	+
	B1-30	5	Grass leaves	+	+

Table 2.2. *continued*….

Species[a]	Isolate	Field code[b]	Baiting method[c]	Pathogenicity test[d]	
				Trial 1	Trial 2
P. intermedium	B2-55	22	Grass leaves	+	
	B3-76	19	Grass leaves	+	+
P. irregulare	B2-44	24	Grass leaves	+	+
	B1-19	9	Grass leaves	+	+
	B1-14	26	Grass leaves	+	+
	B1-20	19	Grass leaves	+	+
	B3-80	1	Grass leaves	+	+
	B1-22	21	Grass leaves	+	+
	B2-43	31	Grass leaves	+	+
P. middletonii	B3-60	15	Grass leaves	+	
	B5-63	17	Grass leaves	+	
	B5-61	16	Grass leaves	+	
	B1-15	37	Grass leaves	+	+
	B3-68	30	Grass leaves	+	
	B4-35	30	Grass leaves	+	
	B2-50	5	Grass leaves	+	
P. oligandrum	B3-77	8	Grass leaves	+	
	B3-64	3	Grass leaves	+	
	B4-16	8	Grass leaves	+	+
P. radiosum	B3-93	33	Grass leaves	+	+
P. splendens	B5-45	2	Grass leaves	+	+
P. sylvaticum	B10-37	33	Pea	+	+
	B12-148	4	Corn	+	+
	B12-151	4	Corn	+	+
	B12-147	4	Corn	+	
P. torulosum	B4-1	21	Grass leaves	+	+
	B1-37	27	Grass leaves	+	
	B1-11	11	Grass leaves	+	
	B1-5	36	Grass leaves	+	
	B3-72	4	Grass leaves	+	
	B4-20	15	Grass leaves	+	
	B1-24	6	Grass leaves	+	
	B3-70	35	Grass leaves	+	
	B4-25	4	Grass leaves	+	
P. ultimum	B8-113	32	Grass leaves	+	+
	B2-42	22	Grass leaves	+	+
	B4-9	15	Grass leaves	+	+
	B1-1	30	Grass leaves	+	+
	B8-96	19	Grass leaves	+	+
	B9-141	19	Grass leaves	+	+
	B3-82	12	Grass leaves	+	+
	B1-23	37	Grass leaves	+	+
	B3-73	2	Grass leaves	+	+

Table 2.2. *continued*…

Species[a]	Isolate	Field code[b]	Baiting method[c]	Pathogenicity test[d]	
				Trial 1	Trial 2
P. violae	B11-102	6	Corn	+	+
	B11-77	37	Pea	+	+
	B13-198	35	Pea	+	+
	B12-139	35	Corn	+	+
	B10-53	35	Pea	+	+
	B13-194	30	Pea	+	+
	B12-149	31	Pea	+	+
	B12-145	31	Pea	+	+
	B13-197	36	Pea	+	+

[a] *Pythium* species identification was done by sequencing the internal transcribed spacer (ITS) region of the ribosomal DNA using universal eukaryotic primers following the protocol described by Bakkeren et al. (2000), and Paulitz and Adams (2003).

[b] Details of the 37 certified organic fields sampled are described in Table 2.1 and Fig. 2.1.

[c] Baiting for *Pythium* was done following the procedures described in the main text and based on those of Paulitz and Adams (2003), and Broders et al. (2007).

[d] Pathogenicity trials were done in a growth chamber at 13°C by day and 8°C by night with 12 h photoperiod/day, to mimic early spring planting conditions in the Columbia Basin of Washington. '+' indicates the isolate was evaluated in that pathogenicity trial.

Table 2.3. Prevalence of *Pythium* species isolated from soil sampled from 37 certified organic fields in the Columbia Basin of central Washington in 2009[a]

	No. of isolates collected/species for each region in the Columbia Basin				
***Pythium* species**[b]	**North**	**Central**	**South**	**Total**	**%**[c]
P. ultimum	36	18	21	75	24.6
P. torulosum	20	8	28	56	18.4
P. irregulare group I	7	9	30	46	15.1
P. abappressorium	9	4	2	15	4.9
P. adhaerens	5	0	8	13	4.3
P. middletonii	8	2	3	13	4.3
P. dissotocum	4	3	3	10	3.3
P. violae	1	1	8	10	3.3
P. echinulatum	7	0	0	7	2.3
P. inflatum	0	1	4	5	1.6
P. sylvaticum	0	3	1	4	1.3
P. aristosporum	1	0	2	3	1.0
P. oligandrum	3	0	0	3	1.0
P. catenulatum	1	0	2	3	1.0
P. intermedium	2	0	0	2	0.7
P. diclinum	0	0	1	1	0.3
P. splendens	0	0	1	1	0.3
P. camurandrum	0	0	1	1	0.3
P. radiosum	1	0	0	1	0.3
Not identified[d]	19	3	14	36	11.8
Total (% 305 isolates)	124 (41%)	52 (17%)	129 (42%)	305	

[a] Details of the 37 fields, regions of the Columbia Basin, and sampling protocol are described in Tables 2.1 and 2.2, and in Figs. 2.1 and 2.2.

[b] *Pythium* species isolated from soils sampled from the 37 certified organic fields, and identified to species by sequencing the internal transcribed spacers (ITS) of the ribosomal DNA (rDNA) using universal eukaryotic primers as described by Bakkeren et al. (2000), and Paulitz and Adams (2003).

[c] Percentage isolates of the 19 *Pythium* species out of a total of 305 isolates baited.

[d] Of 305 isolates obtained from the 37 fields, 36 were not identified to species because of failure to amplify and sequence the ITS rDNA.

Table 2.4. Soil nutrient analysis of the soils sampled from the 37 certified organic fields in the Columbia Basin of Washington surveyed in 2009[a]

Field code[b]	Columbia Basin region[c]	Macronutrients				Micronutrients								OM (%)[d]	pH	EC (m.mhos/cm)[e]
		NO_3-N (mg/kg)	NH_4-N (mg/kg)	P (mg/kg)	K (mg/kg)	Ca (meq/100 g)	Mg (meq/100 g)	S (mg/kg)	B (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)			
1	N	2.9	1.3	18	265	8.6	2.4	12	0.30	1.0	1.0	1.0	7	1.2	8.4	0.31
2	N	9.4	3.4	31	599	7.5	2.6	9	0.39	1.8	4.0	0.8	6	1.6	7.9	0.42
3	N	9.7	4.3	19	471	11.3	2.7	17	0.51	1.2	6.5	1.0	9	1.6	8.2	0.48
4	N	5.9	2.6	37	457	4.7	2.2	18	0.18	0.9	0.9	0.9	9	0.7	8.1	0.26
5	N	36.1	3.4	39	143	6.9	2.2	15	0.19	3.4	2.5	1.3	41	1.6	6.6	0.40
6	N	50.4	1.0	54	748	7.2	2.1	29	0.40	1.8	1.6	1.6	10	1.4	8.0	0.97
7	N	19.7	1.0	56	810	7.6	2.2	17	0.43	2.5	1.2	1.6	9	1.2	8.2	0.58
8	N	17.8	4.6	37	521	11.3	2.1	18	0.28	0.7	3.7	1.6	18	1.1	7.0	0.40
9	N	1.4	2.1	19	286	6.1	2.1	12	0.31	1.0	3.3	1.4	17	1.0	7.7	0.24
10	N	47.9	1.4	51	567	9.7	1.8	28	0.55	2.6	2.1	1.2	7	1.3	8.4	0.85
11	N	3.5	0.9	27	352	11.5	2.2	15	0.60	1.1	1.3	1.3	5	1.2	8.7	0.29
12	N	35.4	1.1	24	497	14.1	2.2	26	0.54	1.2	1.4	1.2	5	1.0	8.6	0.45
13	N	4.0	0.9	41	658	13.3	1.9	10	0.42	1.2	1.1	0.9	7	1.0	8.7	0.39
14	N	2.2	3.3	66	910	7.5	2.5	15	0.42	2.8	5.7	1.3	13	1.4	8.7	0.45
15	N	36.5	1.1	36	269	9.9	2.6	32	0.56	1.7	1.5	1.0	12	1.7	7.9	0.46
16	N	12.3	2.2	47	273	14.4	2.4	41	0.77	2.3	5.6	2.1	12	1.3	8.3	0.46
17	N	6.8	1.7	22	267	11.7	2.7	17	0.69	1.4	5.2	1.2	7	1.4	8.4	0.45
18	C	17.2	0.9	35	822	6.9	2.0	42	0.44	1.8	1.7	1.6	9	1.1	8.6	0.48
19	C	6.1	0.4	46	1,044	7.4	2.2	27	0.61	3.0	1.6	3.6	10	1.4	8.6	0.40
20	C	20.2	1.0	32	792	8.2	2.1	42	0.42	2.3	1.4	2.3	7	2.0	8.0	0.59
21	C	6.4	1.0	33	484	7.1	2.4	39	0.51	2.7	1.6	3.6	11	1.2	8.2	0.45
22	C	12.4	1.4	17	487	6.2	2.5	42	0.38	1.3	1.5	2.3	11	1.1	7.3	0.44
23	S	4.9	0.5	5	204	8.4	1.4	13	0.2	0.4	0.6	0.6	5	0.7	8.1	0.22
24	S	4.5	0.5	6	235	8.3	1.5	14	0.16	0.4	0.4	0.5	3	0.8	8.0	0.25
25	S	13.9	0.5	12	312	5.9	1.4	16	0.23	0.7	0.5	0.5	5	1.0	7.8	0.29
26	S	6.5	1.5	15	362	6.6	1.8	25	0.3	1.2	0.9	0.7	5	1.2	7.8	0.31
27	S	12.2	0.8	12	323	6.6	1.6	12	0.25	0.8	0.8	0.9	5	1.0	7.6	0.19
28	S	19.0	1.0	45	551	9.5	1.4	25	0.42	2.8	1.1	0.6	7	1.2	7.9	0.45
29	S	10.9	0.8	53	698	13.9	2.1	38	0.69	2.7	1.2	0.8	9	1.4	8.2	0.51
30	S	2.2	0.7	31	401	15.9	2.3	17	0.49	1.9	1.0	0.9	6	1.4	8.3	0.28
31	S	13.9	0.8	42	511	14.5	2.0	54	0.50	1.9	1.0	0.7	6	1.3	8.0	0.60
32	S	9.3	0.8	79	776	12.0	2.2	50	0.84	4.0	1.4	2.0	9	1.4	8.0	0.54
33	S	6.0	2.1	27	468	13.0	1.5	53	0.54	2.0	0.9	0.6	5	1.0	8.2	0.48
34	S	4.1	1.6	22	332	14.7	2.2	76	0.51	1.9	0.9	0.5	7	0.9	8.2	0.55
35	S	31.7	1.0	49	555	13.9	2.4	67	0.49	3.0	1.3	0.7	7	1.1	7.9	0.74
36	S	4.0	0.8	20	316	14.5	2.8	31	0.41	1.0	0.7	0.7	6	0.6	8.5	0.40
37	S	6.8	0.4	22	392	15.2	3.2	35	0.57	1.2	0.7	0.8	5	0.8	8.4	0.44
Average		13.9	1.5	33	491	10.1	2.2	28	0.45	1.8	1.9	1.2	9	1.2	8.1	0.45

[a] Soil samples were collected from four transects/field using a 2.5- cm diameter soil probe to a depth of 15 cm. All the soil cores/field were pooled in a Ziploc bag, mixed thoroughly, and a 500 g subsample sent to Soiltest Farm Consultant, Inc. (Moses Lake, WA) for nutrient analysis.

[b] Field code for each of 37 certified organic fields surveyed in 2009.

[c] North (N), Central (C), and South (S) regions where the 37 Columbia Basin of central Washington fields were surveyed in 2009 as defined in Table 2.1.

[d] OM = organic matter.

[e] EC = electrical conductivity, the method used to measure soluble salts in the soil samples.

Table 2.5. Quantification by real-time PCR assays of three *Pythium* species pathogenic on pea, from the soil sampled in each of 37 certified organic fields in the Columbia Basin of Washington in 2009

Columbia Basin region[a]	Field code[b]	*P. abappressorium* DNA (fg/g soil)[c]	N[d]	*P. abappressorium* Mean estimated CFU/g soil[e]	*P. irregulare* group 1 DNA (fg/g soil)	N	*P. irregulare* group 1 Mean estimated CFU/g soil	*P. ultimum* DNA (fg/g soil)	N	*P. ultimum* Mean estimated CFU/g soil
North	1	62 ± 30	3	BQL	760 ± 296	4	264 ± 115	436 ± 286	4	7 ± 5
North	2	102 ± 48	4	BQL	-	-	-	918 ± 558	4	15 ± 9
North	3	198 ± 92	4	BQL	262 ± 10	3	103 ± 6	1,662 ± 1,148	4	28 ± 19
North	4	6 ± 2	4	BQL	148 ± 20	2	62 ± 11	2,114 ± 1,178	4	36 ± 20
North	5	BQL[f]	-	BQL	644 ± 6	2	228 ± 4	128 ± 8	4	BQL
North	6	168 ± 22	4	BQL	206 ± 4	2	83 ± 3	832 ± 192	4	14 ± 3
North	7	20 ± 6	4	BQL	-	-	-	2,864 ± 822	4	48 ± 14
North	8	88 ± 28	4	BQL	-	-	-	430 ± 244	4	BQL
North	9	52 ± 52	4	BQL	-	-	-	338 ± 196	4	BQL
North	10	28 ± 8	4	BQL	-	-	-	1,322 ± 208	4	22 ± 3
North	11	30 ± 10	3	BQL	-	-	-	362 ± 152	4	BQL
North	12	58 ± 42	4	BQL	250 ± 0	1	99 ± 0	4,462 ± 2,466	4	76 ± 42
North	13	44 ± 22	4	BQL	-	-	-	2,140 ± 1,704	4	36 ± 29
North	14	12 ± 6	3	BQL	-	-	-	238 ± 168	4	BQL
North	15	184 ± 74	4	BQL	54 ± 0	1	25 ± 0	1,986 ± 796	4	33 ± 13
North	16	154 ± 46	4	BQL	-	-	-	18,698 ± 1,880	4	322 ± 32
North	17	16 ± 6	3	BQL	-	-	-	5,956 ± 904	4	101 ± 15
Central	18	66 ± 20	4	BQL	368 ± 108	4	139 ± 47	5,394 ± 1,918	4	92 ± 32
Central	19	28 ± 6	4	BQL	330 ± 104	2	126 ± 45	3,020 ± 814	4	51 ± 14
Central	20	24 ± 12	4	BQL	14 ± 4	2	BQL	1,678 ± 994	4	28 ± 17
Central	21	34 ± 20	4	BQL	34 ± 0	1	BQL	5,546 ± 2,102	4	94 ± 35
Central	22	226 ± 134	4	BQL	-	-	-	42 ± 26	4	BQL
South	23	BQL	1	BQL	302 ± 0	1	117 ± 0	656 ± 210	4	11 ± 3
South	24	BQL	3	BQL	38 ± 0	1	BQL	6,808 ± 6,182	4	116 ± 105
South	25	BQL	1	BQL	20 ± 0	-	BQL	498 ± 238	4	BQL
South	26	18 ± 14	3	BQL	-	-	-	1,112 ± 628	4	19 ± 10
South	27	10 ± 6	3	BQL	14 ± 1	2	BQL	232 ± 52	4	BQL
South	28	BQL	-	BQL	74 ± 34	2	34 ± 17	138 ± 28	4	BQL
South	29	4 ± 2	2	BQL	132 ± 88	2	56 ± 39	416 ± 332	4	BQL

Table 2.5. *continued….*

Columbia Basin region[a]	Field code[b]	*P. abappressorium* DNA (fg/g soil)[c]	N[d]	*P. abappressorium* Mean estimated CFU/g soil[e]	*P. irregulare* group 1 DNA (fg/g soil)	N	*P. irregulare* group 1 Mean estimated CFU/g soil	*P. ultimum* DNA (fg/g soil)	N	*P. ultimum* Mean estimated CFU/g soil
South	30	-[g]	-	BQL	154 ± 0	1	64 ± 0	338 ± 232	4	BQL
South	31	58 ± 50	4	BQL	-	-	-	1,542 ± 886	4	26 ± 15
South	32	-	-	BQL	116 ± 0	1	50 ± 0	858 ± 504	4	14 ± 8
South	33	-	-	BQL	136 ± 0	1	57 ± 0	1,990 ± 920	4	34 ±15
South	34	4 ± 1	2	BQL	-	-	-	1,314 ± 792	4	22 ± 13
South	35	28 ± 16	4	BQL	222 ± 0	1	89 ± 0	2,108 ± 1,234	4	36 ± 21
South	36	8 ± 6	2	BQL	-	-	-	1,058 ± 826	4	18 ± 14
South	37	22 ± 20	2	BQL	-	-	-	352 ± 162	4	BQL

[a] North, central, and south regions of the Columbia Basin are defined in Table 2.1.

[b] Field codes and details are described in Table 2.1.

[c] Mean ± standard error of fg DNA/g soil extracted and quantified from each of N samples assayed/field. Real-time PCR assays were completed using the protocols described in the main text for each species, based on the methods of Schroeder et al. (2006).

[d] N = number of soil samples assayed/field. Twenty soil cores were sampled in each of four transects/field, and the samples pooled/transect. Subsamples of 500 mg were used/DNA extraction in the real-time PCR assays.

[e] Mean ± standard error of propagules (CFU)/g soil of each target *Pythium* species estimated based on a regression equation developed by quantifying DNA in a soil dilution series using real-time PCR assays developed by Schroeder et al. (2006). Soil dilutions were prepared from pasteurized field soil inoculated with each of the three *Pythium* species, and quantified by dilution plating onto a *Pythium* selective medium (Mircetich and Kraft, 1973). Regression analyses of DNA concentrations and number of propagules (CFU)/g soil in the soil dilutions were analyzed using SigmaPlot Version 11 (Systat, San Jose, CA). Refer to Fig. 2.15 for details of the regression analyses.

[f] BQL = the species was detected by real-time PCR assay but the DNA concentration was below the quantifiable limit.

[g] ‘ – ‘ = soil was tested but no DNA was detected for that *Pythium* species.

Table 2.6. DNA concentration detected by real-time PCR assays of soil inoculated with each of *Pythium abappressorium*, *P. irregulare* group 1, and *P. ultimum*, and used to develop a standard curve for each of the three *Pythium* species

Species/Field[a]	Target soil inoculation (CFU/g soil)[b]	Trial 1[c] Recovered CFU/g soil[e]	Trial 1[c] DNA detected (fg/g soil)[f]	Trial 2[d] Recovered CFU/g soil	Trial 2[d] DNA detected (fg/g soil)
P. abappressorium	1,000	4,706	37,366 ± 4,533	2,660	31,400 ± 1,900
	500	2,353	16,665 ± 759	1,330	17,000 ± 1,230
	100	470	3,937 ± 154	266	3,953 ± 856
	50	235	2,124 ± 396	133	1,362 ± 263
	10	47	224 ± 120	27	331 ± 152
	5	23	129 ± 73	13	335 ± 66
P. ultimum	1,000	3,636	187,977 ± 13,287	1,660	39,540 ± 3,631
	500	1,818	111,890 ± 17,646	830	17,853 ± 958
	100	364	25,657 ± 7,685	166	4,626 ± 330
	50	182	9,802 ± 1,463	83	3,084 ± 636
	10	36	2,790 ± 582	17	192 ± 21
	5	18	938 ± 207	8	100 ± 45
P. irregulare group 1					
WB-14	1,000	4,800	14,936 ± 1,936	860	45,073 ± 11,170
	500	2,400	9,666 ± 933	430	18,046 ± 2,031
	100	480	873 ± 136	86	3,746 ± 256
	50	240	692 ± 195	43	2,296 ± 835
	10	48	121 ± 14	9	1,495 ± 1,372
	5	24	32 ± 23	4	59 ± 39
Baillie 2N	1,000	5,540	21,810 ± 1,678		
	500	2,770	7,370 ± 283		
	100	554	1,682 ± 390		
	50	277	635 ± 185		
	10	55	89 ± 25		
	5	28	99 ± 49		
S-44	1,000	3,400	15,556 ± 1,902		
	500	1,700	11,160 ± 3,535		
	100	340	2,408 ± 707		
	50	170	714 ± 205		
	10	34	61 ± 24		
	5	17	48 ± 19		

Error! Not a valid link.[a] Soil from each of three fields in the Columbia Basin (WB-14, Baillie 2N, and S-44, as described in Table 2.1) was inoculated with each of three *Pythium* species: *P. abappressorium*, *P. irregulare* group 1, and *P. ultimum*, at a target population of 1,000 CFU/g soil. Details of the inoculation procedure are described in the main text. Mean DNA concentration (fg/g soil) detected for *P. abappressorium* and *P. ultimum* did not differ significantly among the field soils based on the analysis of variance (ANOVA). Therefore, data were pooled for the three fields for each of these two *Pythium* species. Mean DNA concentration

(fg/g soil) detected for *P. irregulare* group 1 differed significantly among soil from the three fields, so results for the three fields are presented separately.

[b] Targeted *Pythium* population (CFU/g soil) for each of six soil dilutions prepared from the inoculated soil for developing a standard curve for the real-time PCR assay for each *Pythium* species (Schroeder et al., 2006).

[c] First trial to quantify DNA concentration by real-time PCR assay from soil dilutions prepared for soil from each of the three fields inoculated with different levels of *Pythium* (CFU/g soil), and used to develop the standard curves shown in Fig. 2.15 for each of the three *Pythium* species.

[d] In the repeat trial, only one soil (WB-14) was inoculated with each of the three *Pythium* species, and used to quantify DNA concentrations in the soil dilution series detected by real-time PCR assay to develop a standard curve for each *Pythium* species (Fig. 2.16). A lower range of CFU/g soil was recovered from the soil dilutions in trial 2 compared to trial 1, that was closer to the targeted inoculation rates.

[e] *Pythium* population (CFU/g soil) recovered after preparation of a soil dilution for each of the soils inoculated with each of the three *Pythium* species, determined by plating an aliquot of the lowest soil dilution (targeted 1,000 CFU/g soil) onto plates of a *Pythium* selective agar medium (PSM) (Mircetich and Kraft, 1973).

[f] Mean ± standard error of DNA concentration (fg/g soil) detected by real-time PCR assay for each of the three target *Pythium* species (Schroeder et al., 2006) for each soil dilution, which was then used to develop a standard curve for each *Pythium* species (Fig. 2.15).

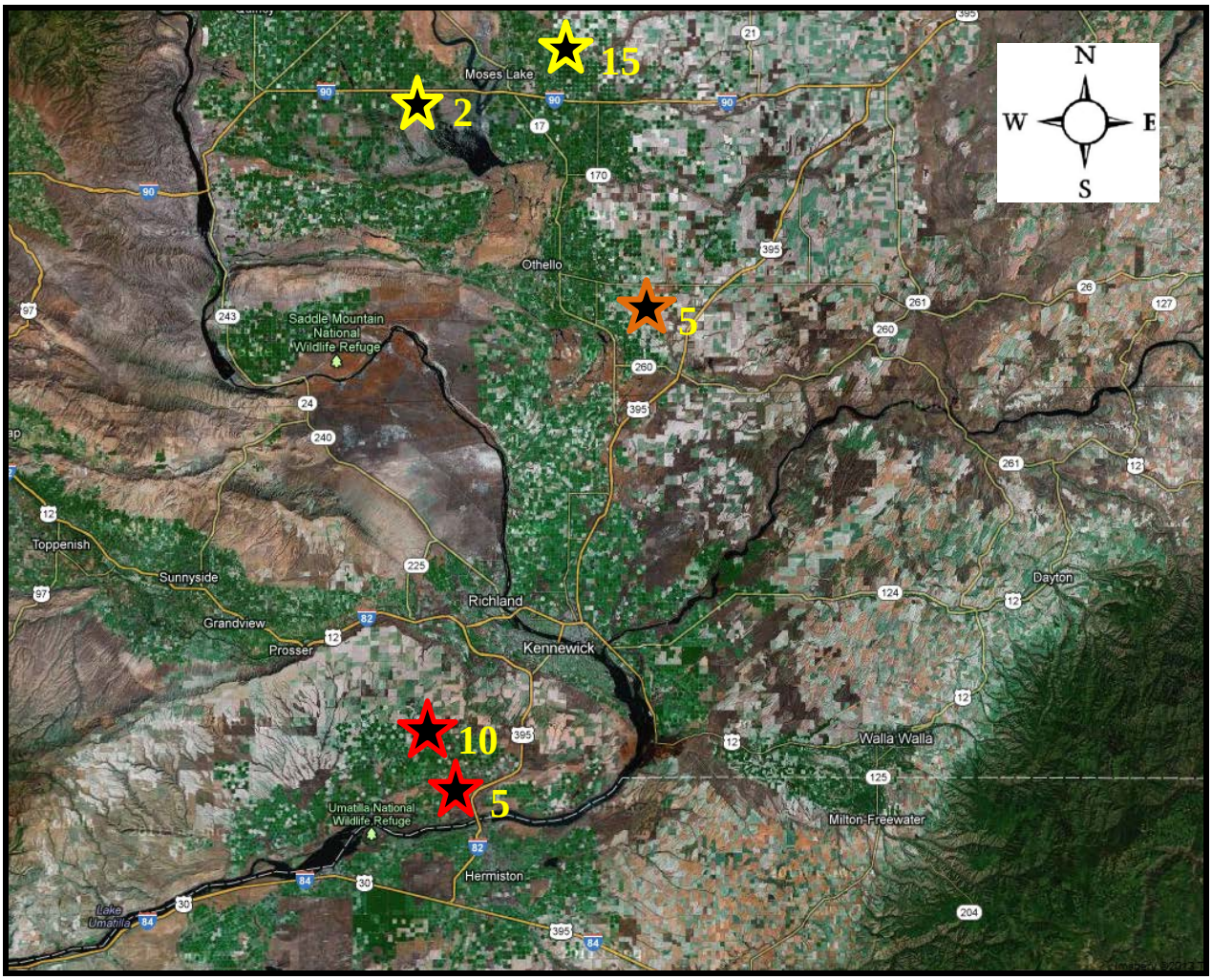

Fig. 2.1. Approximate locations of certified organic fields in the Columbia Basin of central Washington surveyed in October 2009 for *Pythium* spp. ★ = fields (n = 17) located in the north region, ★ = fields (n = 5) located in the central region, and ★ = fields (n = 15) located in the south region of the Columbia Basin. The number indicates the number of fields surveyed in that particular location. Map source: http://maps.google.com/ (accessed February 2013).

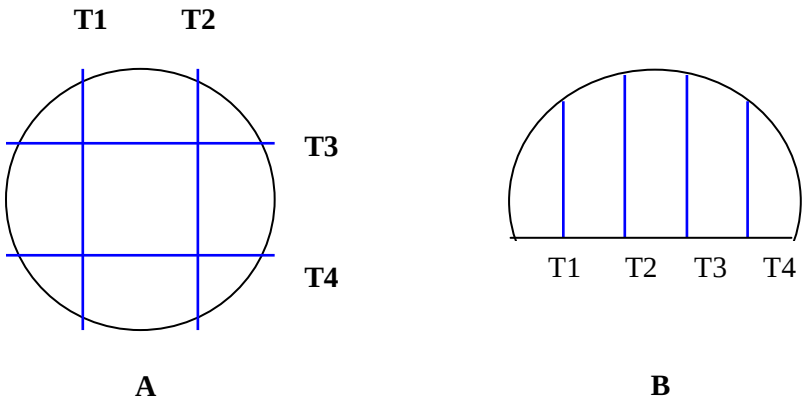

Fig. 2.2. Sampling pattern used for soil collection in 37 certified organic fields surveyed in the Columbia Basin of central Washington in 2009 for *Pythium* spp. A = full circle fields, and B = half-circle fields; T1 = transect 1, T2 = transect 2, T3 = transect 3, and T4 = transect 4. Twenty soil cores were collected within each transect of each field, then pooled and used to bait for *Pythium* spp.

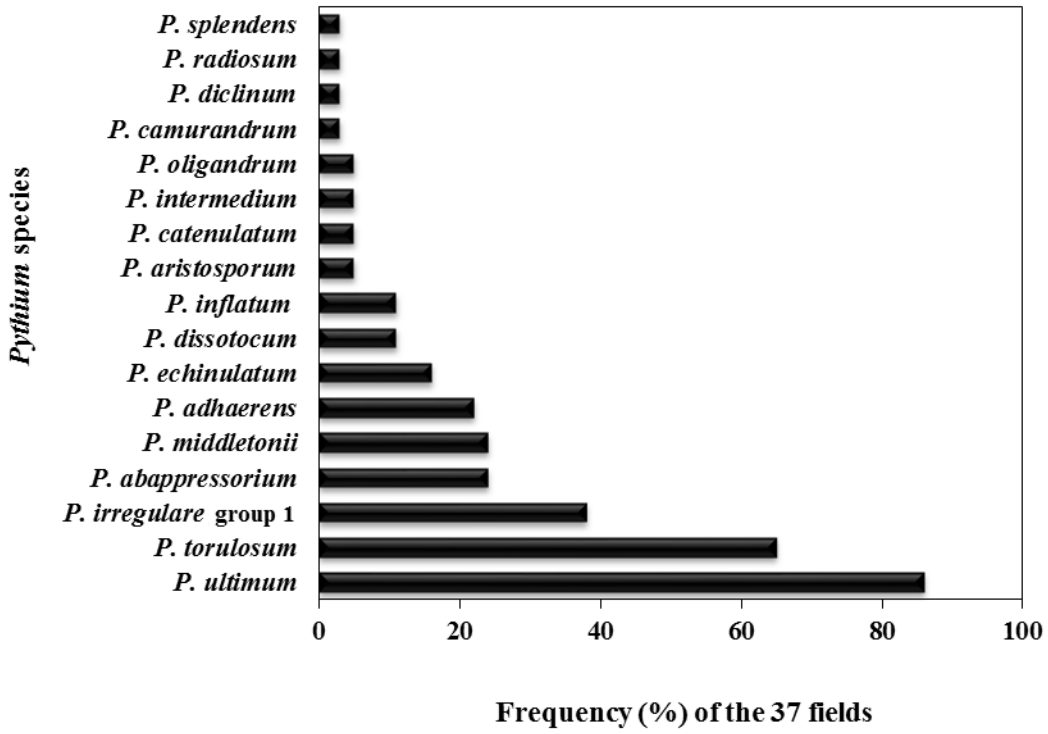

Fig. 2.3. Frequency of occurrence of each 19 *Pythium* species isolated from soil sampled from each of 37 certified organic fields in the Columbia Basin of Washington in 2009.

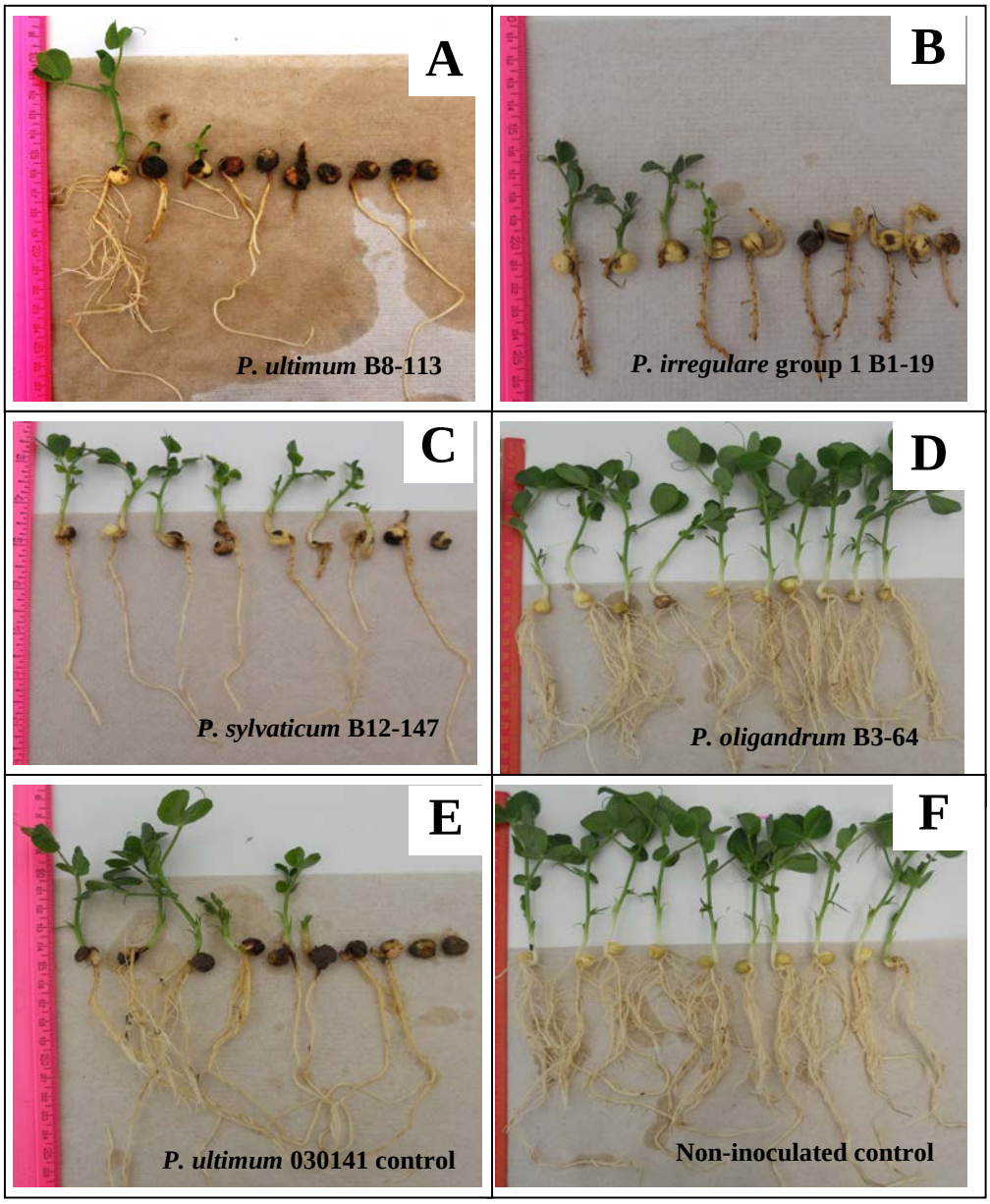

Fig. 2.4. Symptoms caused by representative isolates of each of four *Pythium* species on the pea cv. Tonic in pathogenicity trials in a growth chamber set at 13°C for day and 8°C for night with a 12 h photoperiod/day. Symptom severity caused by *P. ultimum* B8-113 (A) represents typical symptoms from isolates of *Pythium* more virulent than the control isolate, *P. ultimum* 030141 (E); *P. irregulare* B1-19 (B) caused symptoms similar in severity to the control isolate; and *P.*

sylvaticum B12-147 (C) represents isolates that caused less severe symptoms than the control isolate (E). Non-pathogenic isolates, e.g., *P. oligandrum* B3-64 (D), did not cause any symptoms, as observed on the non-inoculated control seedlings (F).

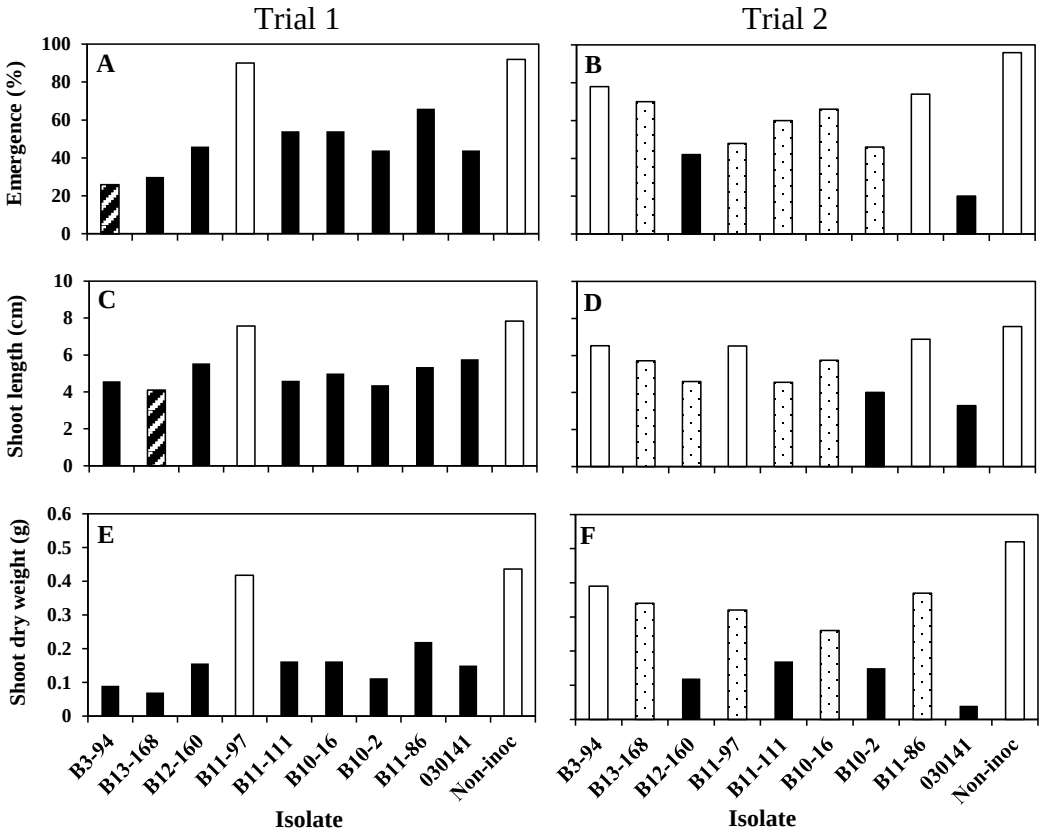

Fig. 2.5. Effect of eight isolates of *Pythium abappressorium* on emergence 28 days after planting (dap) (A and B), shoot length (C and D), and shoot dry weight (E and F) of pea plants in two pathogenicity trials carried out in a growth chamber set at 8°C by night and 13°C by day with 12 h photoperiod/day. Each data point is the mean of five replicate pots/isolate. "Non-inoc" represents the non-inoculated control treatment, and "030141" represents the pathogenic control isolate, *P. ultimum* 030141. White bars represent isolates that did not differ significantly from the non-inoculated control treatment for that dependent variable, black bars represent isolates that did not differ significantly from *P. ultimum* 030141, dotted bars represent isolates with intermediate virulence between the two control treatments, and hashed bars represent isolates that were significantly more virulent than *P. ultimum* 030141 for the dependent variable shown.

Bars with the same pattern are not significantly different according to Fisher's protected least significant difference (LSD, $P < 0.05$). Refer to Appendix Table 2.1 for details of the means separations.

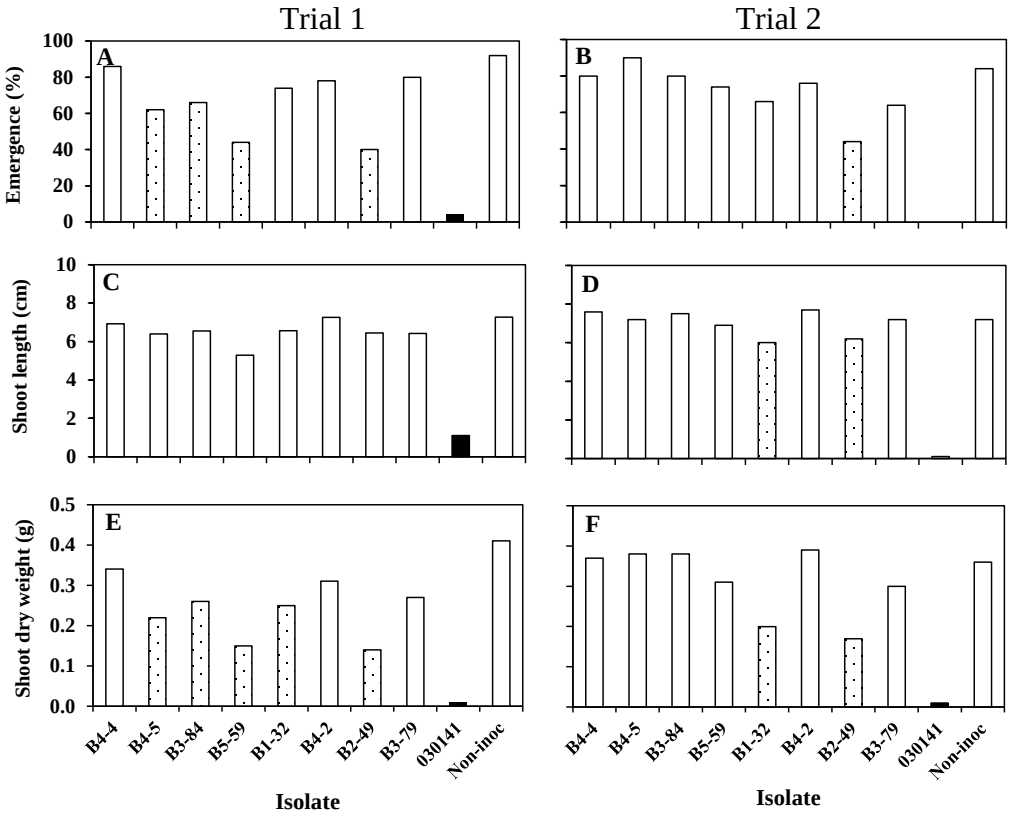

Fig. 2.6. Effect of eight isolates of *Pythium dissotocum* on emergence 28 days after planting (dap) (A and B), shoot length (C and D), and shoot dry weight (E and F) of pea plants in two pathogenicity trials carried out in a growth chamber set at 8°C by night and 13°C by day with 12 h photoperiod/day. Each data point is the mean of five replicate pots/isolate. "Non-inoc" represents the non-inoculated control treatment, and "030141" represents the pathogenic control isolate, *P. ultimum* 030141. White bars represent isolates that did not differ significantly from the non-inoculated control treatment for that dependent variable, black bars represent isolates that did not differ significantly from *P. ultimum* 030141, and dotted bars represent isolates with intermediate virulence between the two control treatments for the dependent variable shown. Bars with the same pattern are not significantly different according to Fisher's protected least significant difference (LSD, $P < 0.05$). Refer to Appendix Table 2.1 for details of the means separations.

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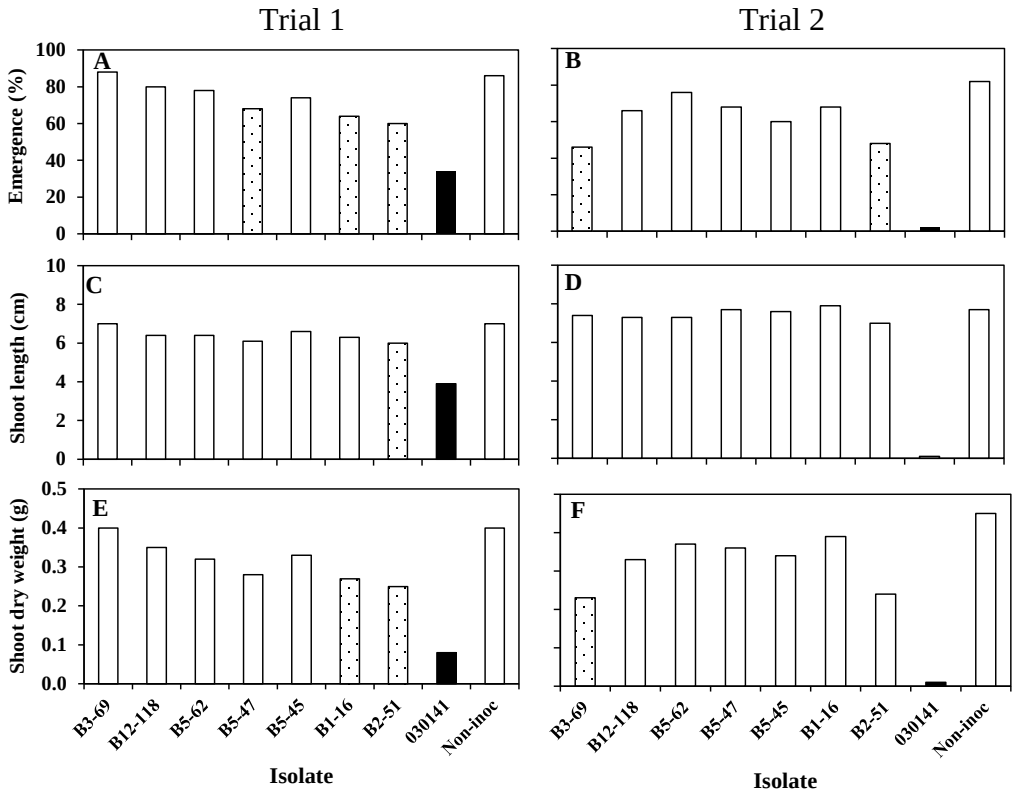

Fig. 2.7. Effect of seven isolates of *Pythium echinulatum* on emergence 28 days after planting (dap) (A and B), shoot length (C and D), and shoot dry weight (E and F) of pea plants in two pathogenicity trials carried out in a growth chamber set at 8°C by night and 13°C by day with 12 h photoperiod/day. Each data point is the mean of five replicate pots/isolate. "Non-inoc" represents the non-inoculated control treatment, and "030141" represents the pathogenic control isolate, *P. ultimum* 030141. White bars represent isolates that did not differ significantly from the non-inoculated control treatment for that dependent variable, black bars represent isolates that did not differ significantly from *P. ultimum* 030141, and dotted bars represent isolates with intermediate virulence between the two control treatments for the dependent variable shown. Bars with the same pattern are not significantly different according to Fisher's protected least significant difference (LSD, $P < 0.05$). Refer to Appendix Table 2.1 for details of the means separations.

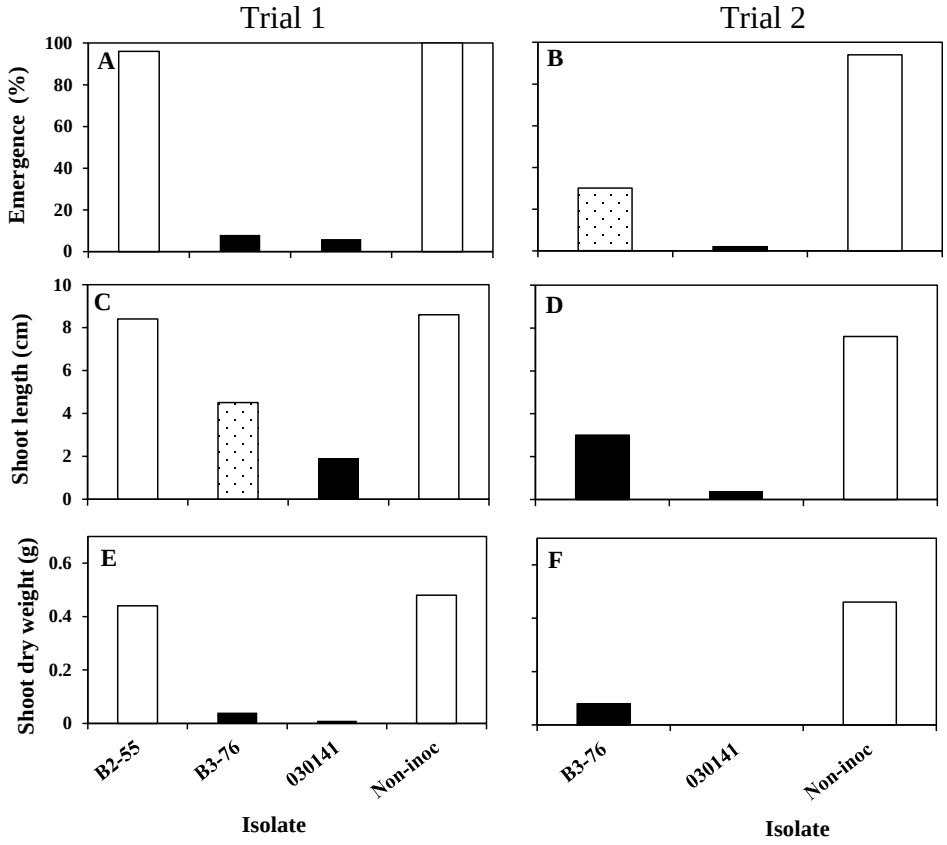

Fig. 2.8. Effect of the isolates of *Pythium intermedium* on emergence 28 days after planting (dap) (A and B), shoot length (C and D), and shoot dry weight (E and F) of pea plants in two pathogenicity trials carried out in a growth chamber set at 8°C by night and 13°C by day with 12 h photoperiod/day. Each data point is the mean of five replicate pots/isolate. "Non-inoc" represents the non-inoculated control treatment, and "030141" represents the pathogenic control isolate, *P. ultimum* 030141. White bars represent isolates that did not differ significantly from the non-inoculated control treatment for that dependent variable, black bars represent isolates that did not differ significantly from *P. ultimum* 030141, and dotted bars represent isolates with intermediate virulence between the two control treatments for the dependent variable shown. Bars with the same pattern are not significantly different according to Fisher's protected least significant difference (LSD, $P < 0.05$). Refer to Appendix Table 2.1 for details of the means separations.

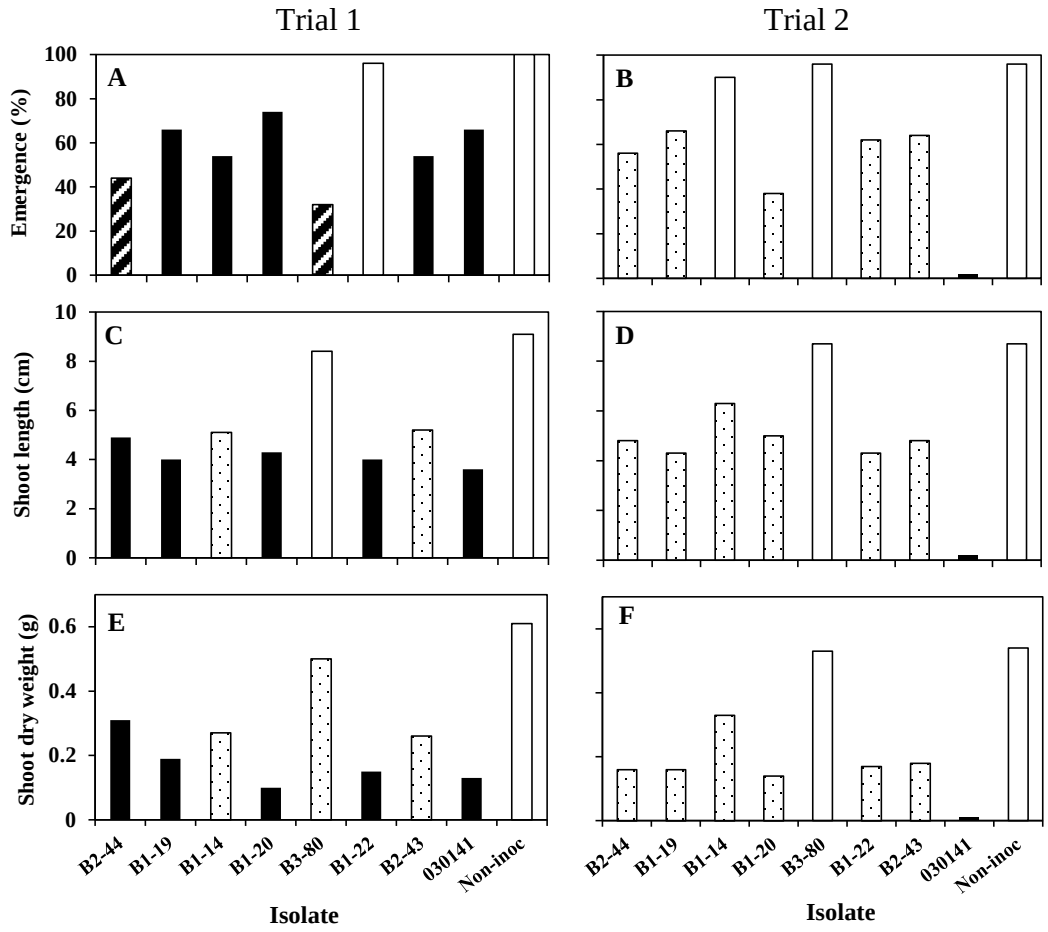

Fig. 2.9. Effect of seven isolates of *Pythium irregulare* group 1 on emergence 28 days after planting (dap) (A and B), shoot length (C and D), and shoot dry weight (E and F) of pea plants in two pathogenicity trials carried out in a growth chamber set at 8°C by night and 13°C by day with 12 h photoperiod/day. Each data point is the mean of five replicate pots/isolate. "Non-inoc" represents the non-inoculated control treatment, and "030141" represents the pathogenic control isolate, *P. ultimum* 030141. White bars represent isolates that did not differ significantly from the non-inoculated control treatment for that dependent variable, black bars represent isolates that did not differ significantly from *P. ultimum* 030141, dotted bars represent isolates with intermediate virulence between the two control treatments, and hashed bars represent isolates that were significantly more virulent than *P. ultimum* 030141 for the dependent variable shown.

Bars with the same pattern are not significantly different according to Fisher's protected least significant difference (LSD, $P < 0.05$). Refer to Appendix Table 2.1 for details of the means separations.

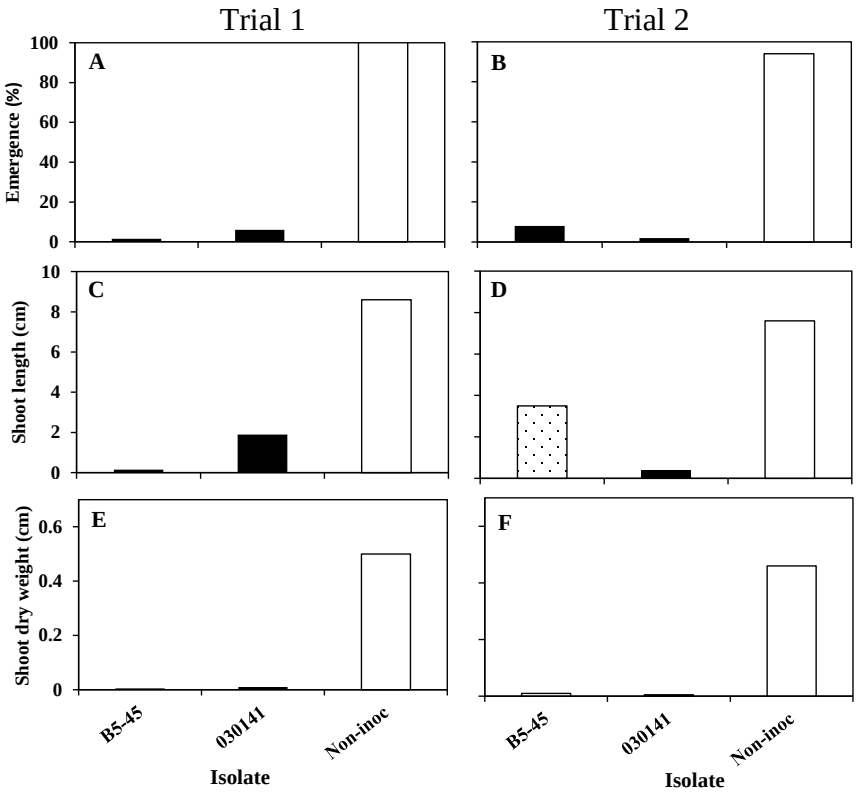

Fig. 2.10. Effect of the isolates of *Pythium splendens* on emergence 28 days after planting (dap) (A and B), shoot length (C and D), and shoot dry weight (E and F) of pea plants in two pathogenicity trials carried out in a growth chamber set at 8°C by night and 13°C by day with 12 h photoperiod/day. Each data point is the mean of five replicate pots/isolate. "Non-inoc" represents the non-inoculated control treatment, and "030141" represents the pathogenic control isolate, *P. ultimum* 030141. White bars represent isolates that did not differ significantly from the non-inoculated control treatment for that dependent variable, black bars represent isolates that did not differ significantly from *P. ultimum* 030141, and dotted bars represent isolates with intermediate virulence between the two control treatments for the dependent variable shown. Bars with the same pattern are not significantly different according to Fisher's protected least significant difference (LSD, $P < 0.05$). Refer to Appendix Table 2.1 for details of the means separations.

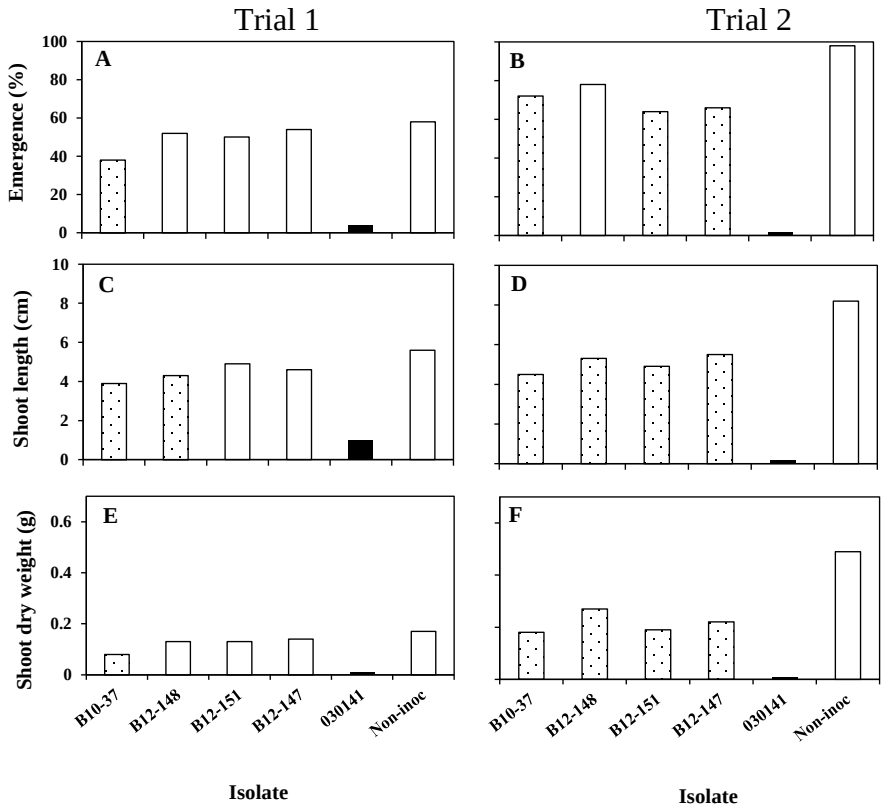

Fig. 2.11. Effect of four isolates of *Pythium sylvaticum* on emergence 28 days after planting (dap) (A and B), shoot length (C and D), and shoot dry weight (E and F) of pea plants in two pathogenicity trials carried out in a growth chamber set at 8°C by night and 13°C by day with 12 h photoperiod/day. Each data point is the mean of five replicate pots/isolate. "Non-inoc" represents the non-inoculated control treatment, and "030141" represents the pathogenic control isolate, *P. ultimum* 030141. White bars represent isolates that did not differ significantly from the non-inoculated control treatment for that dependent variable, black bars represent isolates that did not differ significantly from *P. ultimum* 030141, and dotted bars represent isolates with intermediate virulence between the two control treatments for the dependent variable shown. Bars with the same pattern are not significantly different according to Fisher's protected least significant difference (LSD, $P < 0.05$). Refer to Appendix Table 2.1 for details of the means separations.

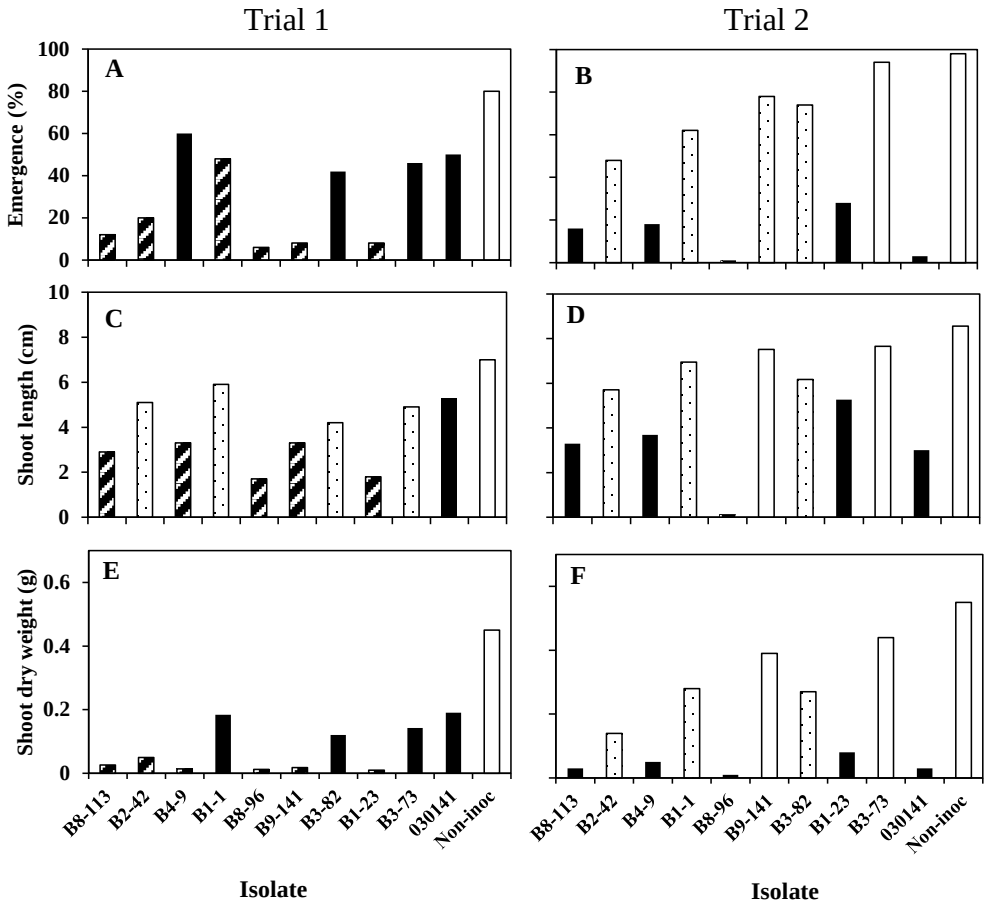

Fig. 2.12. Effect of nine isolates of *Pythium ultimum* on emergence 28 days after planting (dap) (A and B), shoot length (C and D), and shoot dry weight (E and F) of pea plants in two pathogenicity trials carried out in a growth chamber set at 8°C by night and 13°C by day with 12 h photoperiod/day. Each data point is the mean of five replicate pots/isolate. "Non-inoc" represents the non-inoculated control treatment, and "030141" represents the pathogenic control isolate, *P. ultimum* 030141. White bars represent isolates that did not differ significantly from the non-inoculated control treatment for that dependent variable, black bars represent isolates that did not differ significantly from *P. ultimum* 030141, dotted bars represent isolates with intermediate virulence between the two control treatments, and hashed bars represent isolates that were significantly more virulent than *P. ultimum* 030141 for the dependent variable shown.

Bars with the same pattern are not significantly different according to Fisher's protected least significant difference (LSD, $P < 0.05$). Refer to Appendix Table 2.1 for details of the means separations.

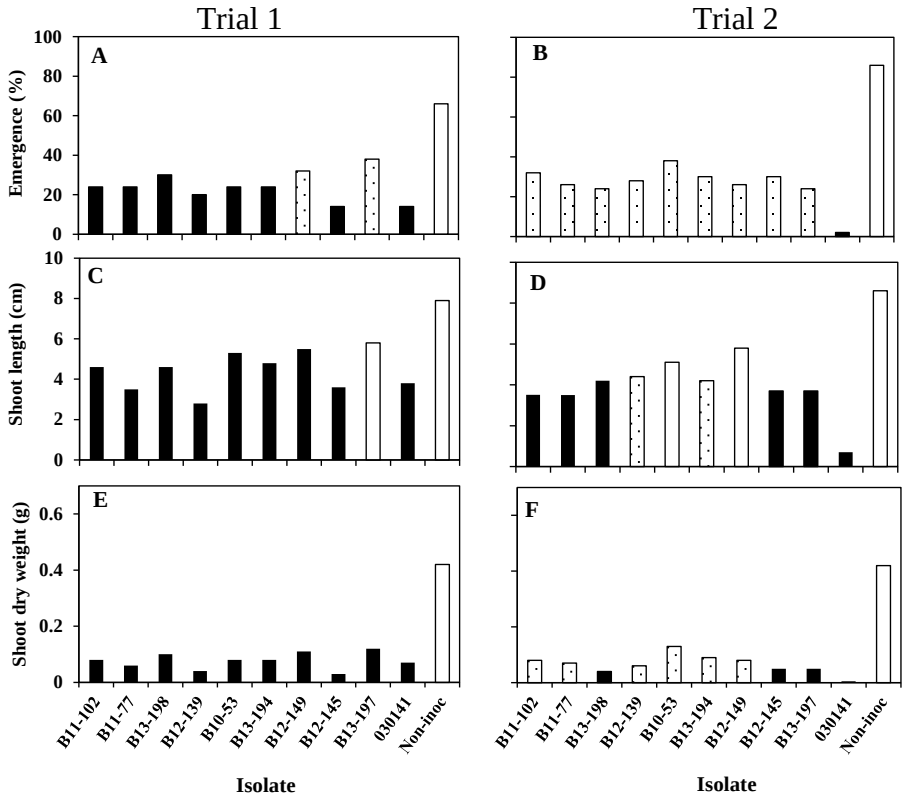

Fig. 2.13. Effect of nine isolates of *Pythium violae* on emergence 28 days after planting (dap) (A and B), shoot length (C and D), and shoot dry weight (E and F) of pea plants in two pathogenicity trials carried out in a growth chamber set at 8°C by night and 13°C by day with 12 h photoperiod/day. Each data point is the mean of five replicate pots/isolate. "Non-inoc" represents the non-inoculated control treatment, and "030141" represents the pathogenic control isolate, *P. ultimum* 030141. White bars represent isolates that did not differ significantly from the non-inoculated control treatment for that dependent variable, black bars represent isolates that did not differ significantly from *P. ultimum* 030141, and dotted bars represent isolates with intermediate virulence between the two control treatments for the dependent variable shown. Bars with the same pattern are not significantly different according to Fisher's protected least significant difference (LSD, $P < 0.05$). Refer to Appendix Table 2.1 for details of the means separations.

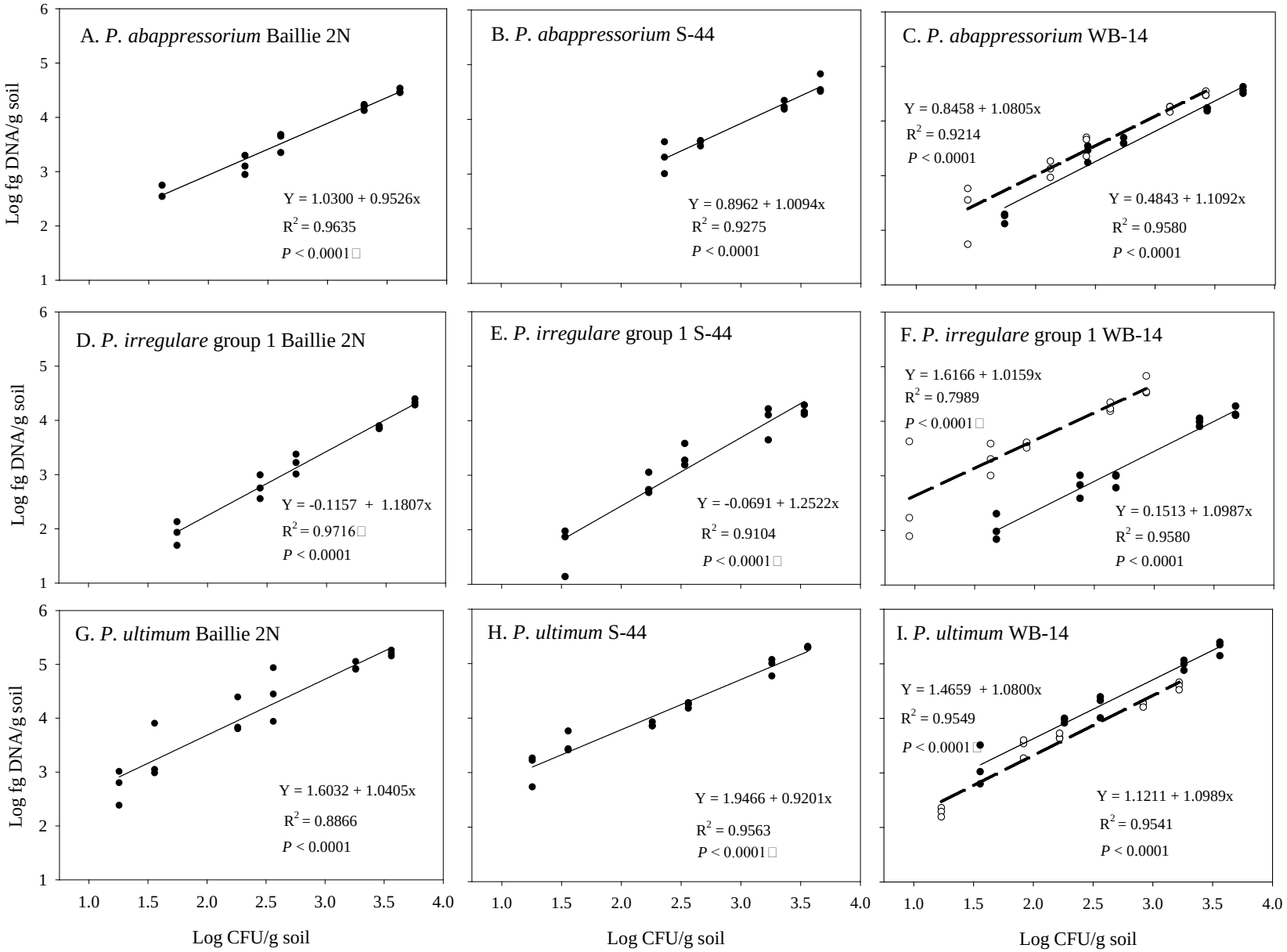
A. P. abappressorium Baillie 2N
Y = 1.0300 + 0.9526x
R2 = 0.9635
P < 0.0001
B. P. abappressorium S-44
Y = 0.8962 + 1.0094x
R2 = 0.9275
P < 0.0001
C. P. abappressorium WB-14
Y = 0.8458 + 1.0805x
R2 = 0.9214
P < 0.0001
Y = 0.4843 + 1.1092x
R2 = 0.9580
P < 0.0001
D. P. irregulare group 1 Baillie 2N
Y = -0.1157 + 1.1807x
R2 = 0.9716
P < 0.0001
E. P. irregulare group 1 S-44
Y = -0.0691 + 1.2522x
R2 = 0.9104
P < 0.0001
F. P. irregulare group 1 WB-14
Y = 1.6166 + 1.0159x
R2 = 0.7989
P < 0.0001
Y = 0.1513 + 1.0987x
R2 = 0.9580
P < 0.0001
G. P. ultimum Baillie 2N
Y = 1.6032 + 1.0405x
R2 = 0.8866
P < 0.0001
H. P. ultimum S-44
Y = 1.9466 + 0.9201x
R2 = 0.9563
P < 0.0001
I. P. ultimum WB-14
Y = 1.4659 + 1.0800x
R2 = 0.9549
P < 0.0001
Y = 1.1211 + 1.0989x
R2 = 0.9541
P < 0.0001
Log fg DNA/g soil
Log CFU/g soil
1
2
3
4
5
6
1.0
1.5
2.0
2.5
3.0
3.5
4.0

Fig. 2.14. Regression analyses of the soil population density (CFU/g soil) vs. soil DNA concentration (fg/g soil) detected by real-time PCR assays for each of *Pythium abappressorium* (A to C), *P. irregulare* group 1 (D to F), and *P. ultimum* (G to I). Soils from each of three fields, Baillie 2N (A, D, and G), S-44 (B, E, and H), and WB-14 (C, F, and I), was inoculated with each of the three *Pythium* species, and then used in a soil dilution series to achieve populations ranging from 5 to 1,000 CFU/g soil. The actual CFU detected on a *Pythium* selective medium agar (PSM) (Mircetich and Kraft, 1973), however, was always greater than the target range when quantified by dilution plating. The dashed line in C, F, and I represents results of the regression analyses when the assays were repeated using a lower target range in population of the three *Pythium* species in soil from field WB-14. Due to excessive variability in the amount of DNA detected across replicate samples at the highest dilution (lowest CFU/g soil), the data points for the highest dilutions were not included in the regression analyses to satisfy the assumption of equal variances. Each data point is the mean of three replicate soil DNA extractions tested with the real-time PCR assay, using the species-specific primers for each of the three *Pythium* species, as described by Schroeder et al. (2006). R^2 = coefficient of determination, *P* = probability of no significant regression relationship between the two variables, Y = predicted log (fg DNA/g soil), and x = log CFU/g soil. Details of the three fields from which soil samples were tested are described in Table 2.1.

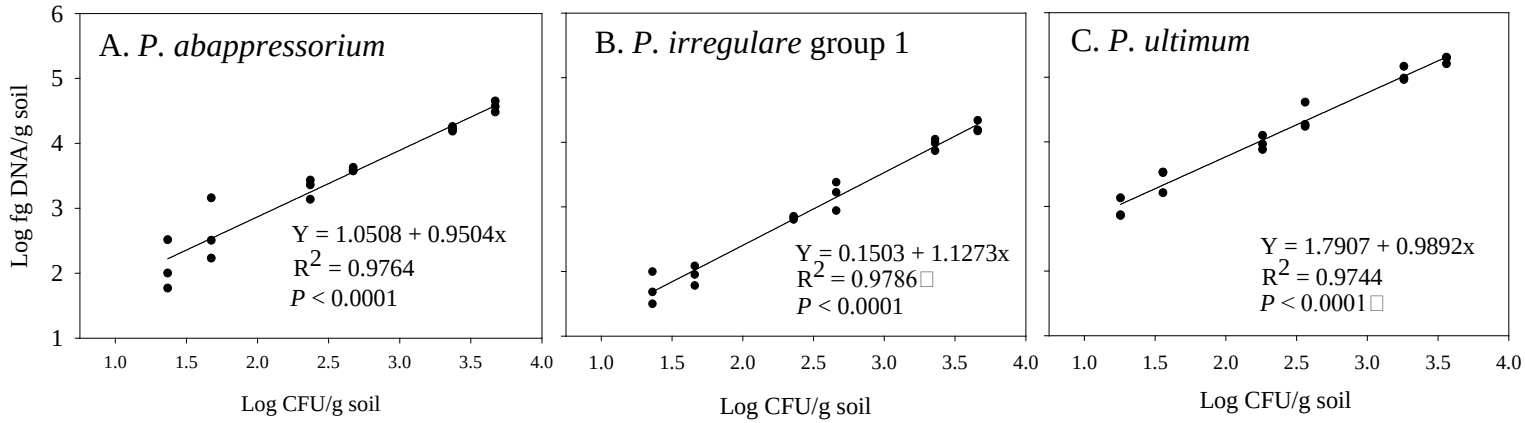

Fig. 2.15. Regression analyses of soil population density (CFU/g soil) and soil DNA concentration (fg DNA/g soil) detected by real-time PCR assays for *Pythium abappressorium* (A), *P. irregulare* group 1 (B), and *P. ultimum* (C). Each data point represents the mean DNA concentration for soil sampled from each of three fields (Baillie 2N, S-44, and WB-14; see Table 2.1), inoculated with each *Pythium* species and used to generate a soil dilution series to achieve populations ranging from 5 to 1,000 CFU/g soil. To satisfy the assumption of equal variances in the regression analysis, the two highest dilutions of soil inoculated with *P. abappressorium* were removed from the analysis. Each regression equation was used to estimate the mean number of propagules (CFU)/g soil for each *Pythium* species from soil sampled from each of 37 organic fields in the Columbia Basin of central Washington, for which the DNA concentration was quantified using real-time PCR assays as described by Schroeder et al. (2006) (refer to Table 2.5). R^2 = coefficient of determination, *P* = probability of no significant regression relationship between the two variables, Y = predicted log (fg DNA/g soil), and x = log CFU/g soil. Details of the three fields from which soil samples were tested are described in Table 2.1.

CHAPTER 3

Evaluation of seed and drench treatments for the control of damping-off in organic pea production in the Pacific Northwest

3.1. Introduction

Organic crop production is a viable industry in the USA, European Union, and many other countries worldwide (Organic Monitor, 2011a; Organic Monitor, 2011b). About 37 million ha of agricultural land were devoted to certified organic production worldwide in 2010, with approximately 1.6 million organic producers (Willer and Kilcher, 2012). Organic production continues to expand due to increasing demand for organic foods (Offner, 2013; Organic Trade Association, 2011; Organic World, 2011). Reliable and efficient organic crop production systems are paramount to keep up with the increasing demand for certified organic produce. However, producers of certified organic crops face many of challenges. Among the challenges are biotic threats, such as plant pathogens that can cause economically important diseases (Granatstein et al., 2005; Letourneau and van Bruggen, 2006). Growers have to be vigilant with these threats to minimize crop losses. The challenge of disease management in an organic production system is there are not as many disease management options available as in conventional production (Letourneau and van Bruggen, 2006; van Bruggen and Termorshuizen, 2003). Disease management using cultural practices (e.g., crop rotation, compost amendments, sanitation, etc.) and resistant cultivars are typically utilized by for growers of certified organic crops (Fernandez-Cornejo et al., 1998; Koike et al., 2000; Sullivan, 2004). However, cultural practices alone may not provide adequate disease control (Felsot and Racke, 2007), and resistant cultivars may not always be available (Koike et al., 2000).

Soilborne diseases causing seed rot or seedling damping-off contribute to poor stands (Agrios, 2005; Martin and Loper, 1999), and are at the top of the list among the plant diseases with which growers have to contend. The soilborne pathogens *Pythium*, *Fusarium*, and *Rhizoctonia*, are common causal agents of pre- and post-emergence damping-off in many crops, including vegetables (Koike et al., 2000; Laemmlen, 2001). Each damping-off pathogen is unique (Campbell and Neher, 1996) and, therefore, a generic control strategy for damping-off in all crops is not always effective. Different disease management strategies may be needed based on the disease etiology and epidemiology (Campbell and Neher, 1996).

An informal survey conducted by Alcala and du Toit (2009) of a limited number (9) of certified organic vegetable growers in Washington State revealed that poor stands can be a significant problem in organic vegetable production. Some organic growers practice over-seeding routinely to compensate for potential poor stands. Under severe damping-off conditions, some growers have had to replant entire fields, which incurs extra expenses, particularly given the high cost of organic seeds (Alcala and du Toit, 2009). Also, delayed planting as a result of the need to replant means delayed harvest. This can be a significant issue if growers have contracts with processors that require adhering to the processor's harvest schedule (Alec McErlich, Earthbound Farms, *personal communication*). In the Pacific Northwest (PNW) region of the USA, growers in the semi-arid Columbia Basin of central Washington and north central Oregon typically practice early spring planting of pea crops for three reasons: (1) to avoid weed competition, particularly nightshade species common in the PNW (e.g., *Solanum nigrum*, *S. physalifolium*, *S. ptycanthum*, and *S. triflorium*) since these weeds produce berries toxic to humans that cannot be separated from peas by the processing equipment; (2) to harvest and process organic pea crops ahead of conventional pea crops to avoid having to clean the

processing equipment between crops; and (3) to double-crop pea with sweet corn, beans and other short season crops in the same season (Lyndon Porter, USDA ARS, *personal communication*; Mansour et al., 1984; Miller and Parker, 2006). However, there is a greater risk of poor stands when planting is done early in the season. In early spring, when the soil and irrigation water in the Columbia Basin are still cold (< 5 to 10°C), conditions are highly favorable for infection by *Pythium* species (Alcala and du Toit, 2009; Cook, 2002; Martin and Loper, 1999).

There are preventive strategies against damping-off that organic growers can include in their production systems. Site selection, e.g., choosing a field with no history of emergence problems, can reduce the risk of the disease (Koike at al., 2000). However, this strategy may not always work for *Pythium* spp. because pathogenic strains tend to be ubiquitous in agricultural soils (Agrios, 2005; Hendrix and Campbell, 1973). Also, planting into fields with well-drained soil is important for preventing damping-off, as infection of seedlings by *Pythium* spp. is favored by saturated soil conditions (Gaskell et al., 2000; Rao et al., 1978). Timing the planting of seed when the soil temperature is >10°C also can minimize the risk of poor emergence (Cook, 2002), as many *Pythium* spp. are favored by cool soil conditions (Ayers and Lumsden, 1975). Planting seed into cold and/or water-logged soil subjects the seeds to stressful conditions, rendering the seeds highly susceptible to *Pythium* infection (Nederhoff, 2000). Slower germination leads to a longer window of opportunity for *Pythium* infection as many crops are only susceptible to infection by *Pythium* spp. during the seedling phase of growth (Martin and Loper, 1999). Another approach to manage damping-off is the use of compost, as repeated compost soil amendments can eventually result in a *Pythium*-suppressive soil (Ben-Yephet and Nelson, 1999;

Tamm et al., 2010). Suppressive soils have been associated with the build-up of fatty-acid-metabolizing microbial communities (McKellar and Nelson, 2003; Tamm et al., 2010).

The use of resistant cultivars can be an effective approach to manage some soilborne diseases in organic production (Fernandez-Cornejo et al., 1998; Koike et al., 2000). Quantitative resistance to *Pythium* spp. has been reported (Navarro et al., 2008, York et al., 1977). For example, in common beans, the resistance to *Pythium ultimum* was associated with colored seeds, and was conferred, partly, by the same genes that control emergence rate and seed vigor (Campa et al., 2010; Lucas and Griffiths, 2004). Qualitative resistance also can be effective against some pathogens but can easily be overcome through selection pressure on the pathogen (Letourneau and van Bruggen, 2006; Riggs and Winstead, 1959). Recently, the soybean cultivar Archer was reported to have a high level of resistance to *Pythium aphanidermatum* and possibly other *Pythium* spp. (Rupe et al., 2011). The resistance was associated with the gene, *Rpa1*, which is thought to have a role in regulating compounds present in seed exudates and in induction of active defense pathways in plants (Rupe et al., 2011). There have been other reports on resistance to *Pythium* spp. conferred by single genes (e.g., Mahuku et al., 2005; Otsyula et al., 2003). While these developments are promising, the lack of availability of commercially-acceptable cultivars with resistance to many important soilborne pathogens is still a shortcoming of this strategy for control of soilborne diseases (Koike et al., 2000).

A potentially effective and relatively inexpensive strategy to manage damping-off is the use of seed treatments (Taylor and Harman, 1990). In conventional agricultural production, seed treatment with fungicides is a common practice, especially when planting during conditions conducive to soilborne diseases (Cook, 2002). Metalaxyl, or the active isomer mefenoxam, are chemical seed treatments that can work effectively at controlling *Pythium* spp. (McGee, 1992)

unless dealing with *Pythium* spp. or isolates resistant to these active ingredients, which can develop readily (Cook and Zhang, 1985; Porter et al., 2009). However, these fungicides are not approved for certified organic production (Organic Materials Review Institute, 2010; United States Department of Agriculture National Organic Program (205.206 (d)). There are commercially available biological and non-biological seed treatments for certified organic production (Gatch, 2011); however, the efficacy of such products against damping-off typically is less than that of conventional fungicide seed treatments (El-Mohamedy and Abd El-Baky, 2008; Guetsky et al., 2001). In a greenhouse study evaluating 14 organic seed and drench treatments against the soilborne pathogens *P. ultimum*, *Rhizoctonia solani*, and *Fusarium oxysporum* f. sp. *spinaceae* on spinach, few products showed efficacy against damping-off and seedling blight; and no product worked effectively against all three pathogens (Cummings et al., 2009). Furthermore, none of the products proved effective in related field studies (Cummings et al., 2007a; Cummings et al., 2007b). Although many organic seed treatments are developed specifically for controlling soilborne diseases (Taylor and Harman, 1990), most organic growers do not use seed treatments because of problems with inconsistency in performance in field conditions (Alcala and du Toit, 2009; McSpadden Gardener, 2002). There remains a need for independent efficacy studies for organic seed treatments for which the labels may claim efficacy against soilborne pathogens. However, such efficacy trials should be carried out in similar conditions to where the diseases are important.

Seed priming is a form of seed treatment that represents a potential strategy organic growers can use for reducing losses to damping-off (Taylor and Harman, 1990). Seed priming involves hydrating seed to initiate the early stages of germination while preventing radicle emergence (Rajjou et al., 2012). Most seeds are dehydration tolerant until radicle emergence, i.e.,

up to this stage, the seeds can be dried back to the initial moisture content for storage without losing the benefits of priming (Rajjou et al., 2012; Taylor et al., 1998). Benefits of seed priming include: (1) uniform and rapid emergence even in suboptimal conditions (Khan et al., 1992; Knypl and Khan, 1981), (2) improved vigor of low vigor seed lots (Bradford, 1986), and (3) reduced seed exudation during germination (Basra et al., 2003; Perry 1973). More rapid and uniform emergence can occur with primed seeds, which decreases the window of susceptibility to infection by some soilborne pathogens. Several researchers have looked at the potential of priming for damping-off control. Harman et al. (1989) reported that priming enhanced the ability of the biological control agent, *Trichoderma harzianum*, to protect peas against *Pythium*. Rush (1991) investigated three priming techniques for the effects on seedling emergence and Pythium damping-off in sugar beet and found that solid matrix priming of sugar beet seeds resulted in better emergence, damping-off suppression, and final stand than the two osmopriming treatments evaluated in the study.

Many soilborne pathogens, especially *Pythium* spp., are favored by the leachates exuded from seeds during germination (Martin and Loper, 1999; Nelson, 1990). The exudates can serve as a nutrient source and stimulate the growth of dormant propagules (e.g., oospores) of *Pythium* spp., thereby facilitating infection. Several studies showed a positive relationship between the amount of seed leakage and poor emergence associated with soilborne pathogens (Matthews, 1971; Matthews and Bradnock, 1967; Matthews and Whitebread, 1968; Nelson, 1990; Nelson and Craft, 1989; Perry, 1973). Reducing exudates upon planting of seeds in soil is a potential approach to reducing the risk of damping-off (Nanayakara, 2001). In some studies, primed seeds produced less seed exudates than non-primed seeds (Basra et al., 2003; Jett et al., 2006; Khan et al.,1992; Perrera and Cantliffe, 1991).

The overall purpose of this study was to evaluate the efficacy of various organic seed and drench treatments that are approved for use in certified organic vegetable crops in Washington State, commercially available, and registered for control of damping-off and other related soilborne pathogens. Pea was used as a model vegetable crop for the seed treatment evaluations since pea crops are commonly grown early in the spring in the Columbia Basin of Oregon and Washington (Mansour et al., 1984). Also, organic processing pea crops are economically important in the Columbia Basin, with total sales of $ 4.2 million and produced on 2,122 ha in 2009, ranking pea second among the organic vegetables grown in Washington (Kirby and Granatstein, 2009; Kirby and Granatstein, 2012). Sweet corn is another major organic vegetable crop in the Columbia Basin of Oregon and Washington. In 2009 in the PNW, sweet corn was grown in 3,112 ha with total sales of $ 4.6 million, ranking corn first in organic vegetables production in this region (Kirby and Granatstein, 2009; Kirby and Granatstein, 2012). Sweet corn and pea make up two-thirds of the organic vegetables grown in Washington (Kirby and Granatstein, 2012). Therefore, sweet corn was included in the initial seed treatment evaluations in this study, but was dropped from subsequent trials because of limited time and resources. The specific study objectives were to: (1) evaluate various seed and drench treatments that can be used in certified organic pea production in Washington for control of damping-off caused by *Pythium* spp. during early spring planting under field conditions; (2) optimize pea seed priming conditions for rapid emergence and damping-off suppression (completed in growth chamber trials); and (3) evaluate combinations of seed priming with other organic seed treatments for control of damping-off in pea crops during spring planting. Preliminary results of the study have been published (Alcala et al., 2012a; 2012b; 2013a; 2013b).

3.2. Materials and methods

Fields trials were carried out in 2011 and 2012 to evaluate organic seed and drench treatments for control of damping-off (Table 3.1 and 3.2). In 2011, pea trials were located at a grower-cooperator field near each of Boardman, OR and Soap Lake, WA in the Columbia Basin. A third site was at the Washington State University Mount Vernon Northwestern Washington Research and Extension Center (WSU Mount Vernon NWREC), where both a pea trial and a sweet corn trial were completed. In 2012, four pea trials were carried out at grower-cooperator fields near Ephrata, WA (one trial) and Royal City, WA (two trials) in central Washington, and WSU Mount Vernon NWREC (one trial). Tables 3.1 and 3.2 summarize the treatments evaluated at each of the sites.

3.2.1. 2011 Field trials

3.2.1.1. Field sites. The trial near Boardman was at Threemile Canyon Farm, LLC in a field that had been planted to an organic carrot crop in 2010 followed by a triticale (*x Triticosecale*) winter cover crop in 2010-11. The Soap Lake trial was in a field of a grower-cooperator, Gilbert Hintz, and had only been in agricultural production for two years, prior to which the field was in native sagebrush. At the WSU Mount Vernon NWREC site, where a pea trial and a sweet corn trial were carried out in a field that was previously in small fruit production, and was managed organically except for plots where conventional seed treatment was applied (Tables 3.1 and 3.2).

The trials located in the semi-arid Columbia Basin of Oregon and Washington were all irrigated by center pivot which is standard practice in the warm and dry conditions of this region (30 year monthly total precipitation averages were 1.7, 1.6, and 1.0 cm for May, June, and July,

respectively) (Mansour, 1984; Prism Climate Group, http://www.prism.oregonstate.edu/). The trial at Mount Vernon was not irrigated as the mild, maritime climate of Skagit Co., WA in spring and summer is typically cool and wet (30 year total monthly precipitation average of 6.5, 5.3, and 2.3 cm, for May, June, and July, respectively) (Mansour, 1984; Prism Climate Group, http://www.prism.oregonstate.edu/). Weeds were a significant problem at the Boardman site due to excessive regrowth of the overwintered triticale cover crop and the presence of nightshade, and at the Mount Vernon site because of the abundant presence of tall oat grass (*Arrhenatherum elatus* var. *bulbosum*).

3.2.1.2. Field trial lay-out and experimental design. The plots for each of the field trials were set up in a randomized complete block design (RCBD) with five replications, except for the trial near Boardman where only four replications were used. Fifteen and fourteen treatments were evaluated in each of the Boardman and Soap Lake trials, respectively, including two control treatments: (1) non-treated seed (referred to hereafter as NTS) and (2) a water drench control treatment applied to plots planted with NTS (referred to hereafter as WD) (Tables 3.1 and 3.2). A similar set of organic seed and drench treatments was evaluated in both the pea and sweet corn trials in Mount Vernon (Tables 3.1 and 3.2). However, inoculation of the plots with a pathogenic isolate of *P. ultimum* (isolate 030141) was done at the Mount Vernon site to try to increase disease pressure, for a total of 20 treatments including the following five control treatments: (1) non-inoculated soil (referred to hereafter as NI), (2) inoculated soil, (3) inoculated soil drenched with water after planting (referred to hereafter as drench control), (4) soil amended with the soil-oatmeal carrier used to produce the *P. ultimum* inoculum but without the pathogen (referred to hereafter as carrier), and (5) a conventional seed treatment of Apron XL (mefenoxam; Syngenta Crop Protection, Inc., Greensboro, NC) to compare with the organic treatments (Tables 3.1and

3.2). Plots of the last four control treatments listed in Table 3.1 were planted with NTS in the Mount Vernon pea and sweet corn trials.

Each pea plot in the Boardman and Soap Lake trials measured 9.9 m^2, consisted of six 6 m-long rows each spaced 28 cm apart, and was surrounded by a 60 cm-wide alley. For the pea trial in Mount Vernon, each plot measured 6.3 m^2 with six 6 m-long rows each spaced 18 cm apart, and was surrounded by a 20 cm-wide alley. Each plot in the sweet corn trial measured 18 m^2, with four 6 m-long rows each spaced 76 cm apart, and was surrounded by 105 cm-wide alley. The Boardman and Soap Lake trials were surrounded by border plots of the same dimensions to separate the experimental plots from the surrounding commercial pea crop.

3.2.1.3. Inoculum preparation for the Mount Vernon field trials. Inoculum for the Mount Vernon trials was prepared using a mixture of ground oatmeal and a sandy loam soil collected from a grower's field in Skagit Co., WA. The soil was spread on kraft butcher paper on a greenhouse bench for 3 to 5 days to dry, then passed through a sieve (1 mm diameter pore size) to remove plant debris, and crushed with a marble rolling pin. Ground oatmeal (Quaker Oats Brand, Chicago, IL) was added to the soil (1% by weight) and mixed using a PK Blendmaster (Patterson-Kelley Co. division of Harsco Corp., East Stroudsberg, PA). The soil and oatmeal were mixed for 10 min with deionized water (15% v/w), which was added slowly through a funneled hose attached to the blender during the last 5 min of mixing. Approximately 1 kg of soil was then placed in a clean, 3.8 liter high density polyethylene (HDPE) milk jug; sealed with an autoclavable, 3.8 cm diameter foam plug (VWR, Baltimore, MD); and the foam plug covered with two layers of aluminum foil. The jugs of soil-oatmeal mix were autoclaved at 1.1 kg/cm^2 at 121°C for 50 min, cooled for 24 h at room temperature (23 ± 1°C), and autoclaved a second time using the same conditions. A 20 ml volume of sterilized distilled water was added to each jug,

and the jug was left overnight at room temperature prior to inoculation with *P. ultimum* isolate 030141 (T. Paulitz, USDA ARS, Pullman, WA).

Inoculum of *P. ultimum* was quantified using soil-dilution plating on water agar (WA). Each jug was shaken vigorously to mix the inoculum, and a 10 g sample was added to a French square bottle (250 ml capacity) containing 100 ml sloppy agar (0.1% WA). The bottle was then placed on a platform rotary shaker (New Brunswick Scientific Innova 2100, Einfield, CT) for 10 min at 250 rpm. Five-fold dilutions were carried out using 10 ml aliquots transferred serially to French square bottles (100 ml capacity), each containing 40 ml sloppy agar. Three 0.5 ml aliquots of each of the dilutions were then each spread on the surface of a WA plate using a bent glass rod. The plates were incubated on a laboratory bench at room temperature with natural light from a north-facing window. Colony counts were done after 24, 30, and 36 h. The number of colony forming units (CFU)/g soil was determined using the average number of colonies counted from the triplicate plates/dilution.

3.2.1.4. Seed and drench treatments. Microbial and non-microbial organic seed and drench treatments that were available commercially in the USA and/or labeled for damping-off control were included in the field trials (Tables 3.1 and 3.2). The seed and drench treatments evaluated in the two growers' certified organic fields in 2011 were all Organic Materials Review Institute (OMRI) and/or Washington State Department of Agriculture (WSDA) listed, while products that were not yet registered for organic production but could potentially become registered were included only in the Mount Vernon trial. All of the seed treatments were done at the Vegetable Seed Pathology lab of L.J. du Toit at the WSU Mount Vernon NWREC, except for Natural II, a proprietary seed treatment that was applied by Agricoat, LLC at their facility in Soledad, CA. Actinovate STP (referred to hereafter as Actinovate), T-22 HC, Nordox 75 WG

(referred to hereafter as Nordox), Heads Up Plant Protectant (referred to hereafter as Heads Up), and Acadian Marine Plant Extract (referred to hereafter as Acadian Marine), were each applied as a slurry using the highest recommended rate on the product label (Tables 3.2). Seed treatments with non-microbial products were done a week prior to the scheduled planting date, while the microbial products were applied 3 to 4 days or at the closest time possible prior to planting. Both Mycostop Mix and Myco Seed Treat were applied as a dust powder on the day of planting (Table 3.2). Serenade Soil, Soilgard 12G, and Stimplex Crop Biostimulant (referred to hereafter as Soilgard and Stimplex for the last two products, respectively) were each applied as a drench treatment after planting. A repeat application of each of Soilgard and Stimplex was done 14 days after planting. Drench treatments were applied immediately after planting, at the highest recommended rate on the label (Table 3.2). Prestop WP was applied using a CO_2 backpack sprayer at 2.1 kg/cm^2 in 3,279 liters of water/ha (R&D sprayers, Bellspray, Inc., Opelousas, LA) fitted with a single nozzle boom (8003 flat spray tip; Teejet, Wheaton, IL). In the Soap Lake trial, strong wind at the time of planting prevented the application of this product.

The seed priming treatment was done by soaking seed for 16 h in deionized water (1:5 v/v seed:water ratio) on a rotary shaker (150 rpm), followed by drying the seeds in a fume hood by spreading the seeds on wire mesh rack lined with two layers of paper towels. Primed seeds (referred to hereafter as PS) used in the Boardman trial were dried for 8 h, while for the Soap Lake and Mount Vernon trials, a 12 h drying duration was used so that seeds were more fully dry in order to facilitate planting or storage in case planting was delayed.

Two of the microbial seed treatments, Germains' II and Prestop Mix, and a conventional seed treatment, Apron XL, were evaluated at the Mount Vernon site in both the pea and sweet corn trials. These products were not registered by the WSDA for use in certified organic ground

and, hence, could be included only at the Mount Vernon site. Prestop Mix and Apron XL were applied to the seed as a slurry 3 to 4 days prior to planting, at the highest rate on the product labels; while Germains' II, a proprietary seed treatment, was applied by the company Germains Technology Group at their facility in Gilroy, CA (Table 3.2).

3.2.1.5. Inoculum application at the Mount Vernon site. The plots in the pea and sweet corn trials in Mount Vernon were inoculated with *P. ultimum* isolate 030141 at 5 x 10^4 CFU/m linear row. For the pea trial, inoculum was applied at the same time as planting the pea seeds, by dispensing the inoculum with the seeds on a belt planter (custom-built by W.H. Haglund, Professor Emeritus, WSU Mount Vernon NWREC). Manual inoculum application was done in the sweet corn trial, in which the rows for each plot were made using a John Deere 71-Flexi planter (Deere and Company, Moline, IL) with the press wheels lifted to leave the rows open. A set amount of inoculum was then spread manually in the furrow of each of the open rows to achieve the desired inoculation rate as uniformly as possible, prior to planting the sweet corn seeds, and closing the rows manually.

3.2.1.6. Planting. The Boardman trial was planted on 28 March, and the Soap Lake trial on 5 April 2011. The pea cvs. Bistro and Boogie (Brotherthon Seeds Co., Inc., Moses Lake, WA) were planted in the Boardman and Soap Lake trials, respectively, to match the cultivar in the surrounding pea crop at each site. A six-row cone planter (Almaco 8000 6-row grain drill, Nevada, IA), was used for planting a total of 70 seeds/m^2 at a seeding depth of 3.8 to 4.5 cm.

Soil samples were collected from each site using a 2.5 cm-diameter soil probe to a sampling depth of 15 cm. Two cores were collected per plot; soil cores were pooled among plots within each replication in a 3.8 liter Ziploc bag, mixed thoroughly, then a 500 g subsample was submitted to Soiltest Farm Consultants, Inc. (Moses Lake, WA) for soil nutrient analysis. A

Spectrum data logger (model # 450, Spectrum Technologies, Inc., Plainfield, IL) was installed at each of the trial sites immediately after planting to monitor soil temperature.

The pea trial at Mount Vernon was planted with the pea cv. Ice pack (Monsanto Vegetable Seeds, Warden, WA) on 21 April 2011. A six-row belt planter (custom-built by W.H. Haglund) was used for planting a total of 110 seeds/m^2 at a seeding depth of 2.5 to 3.8 cm, with rows spaced 18 cm apart. Proganics, an organic fertilizer (Wilbur-Ellis, Burlington, WA), was applied at 444 kg/ha at the time of planting by mixing the fertilizer with the seed and inoculum on the belt planter. The non-inoculated control plots were planted first to avoid potential contamination of these plots with the *P. ultimum* inoculum. Soil samples were also collected following the procedure described above for the trials in the growers' field.

The sweet corn trial in Mount Vernon was planted on 19 May 2011. Proganics fertilizer was applied at 778 kg/ha a week prior to planting. Plots were staked a day before planting and the rows prepared as described above. The *P. ultimum* inoculum was applied as described above and the sweet corn seeds were then placed manually in each row at a 2.5-cm planting depth and 15-cm spacing within the row for a total of 9 seeds/m^2. Each of the rows was closed manually by covering the furrow with soil immediately after planting to avoid drying out the inoculum.

3.2.1.7. Plot maintenance. Manual weeding was done as needed at all four sites, particularly at the Boardman and Mount Vernon trials where weed pressure was severe as a result of regrowth of triticale cover crop at the Boardman site and tall oat-grass at the Mount Vernon pea trial, respectively. Rototilling, followed by spot hand-weeding, were employed as needed for the sweet corn trial at Mount Vernon. Irrigation and fertilization of the pea crops at the growers' site were done by the grower-cooperators using the acceptable practices for the

commercial organic pea crops surrounding each trial. The Mount Vernon pea and sweet corn trials were not irrigated or fertilized again after planting.

3.2.1.8. Data collection. Pea and sweet corn stand counts were done 14 days after planting (dap) and weekly thereafter until 35 dap. For the Boardman and Soap Lake trials, seedling emergence data were obtained from the entire length of two middle rows/plot in which a total of 230 seeds were planted. At the Mount Vernon site, emergence counts were done from 1 m^2 in the center of each plot, because the belt planter used in this trial did not distribute the seeds equally along the six rows unlike the planter used in the two other sites in the Columbia Basin, which distributed 230 seeds/two rows. Seedling emergence data for the sweet corn trial were collected by counting seedlings that emerged from the entire length of four rows/plot.

Isolations for root rot pathogens were done 35 dap by sampling five seedlings randomly from each of three plots/treatment. Seedling roots were washed with tap water to remove adhering soil, and blotted dry on paper towels. A root section from each of the sampled seedlings was placed on *Pythium* selective agar medium (PSM) (Mircetich and Kraft, 1973) and incubated in the dark at room temperature (23 ± 1°C) for 24 h. Emerging mycelial growth was transferred by hyphal tip onto WA plates. Isolates were examined with a microscope to confirm the presence of *Pythium*, *Rhizoctonia*, or other soilborne pathogens. *Rhizoctonia* and *Pythium* isolates were subcultured to obtain clean cultures, and stored at 4 ± 2°C before further identification. Isolations were not done 35 dap for the sweet corn trial in Mount Vernon as there were insufficient plants/plot to sample without affecting data collection for other variables measured.

At pea full bloom (Muehlbauer and McPhee, 1997) in each of the trials, root rot severity, plant height, and shoot dry biomass were measured by sampling 16 plants taken from two feet from both ends of the two center rows/plot (8 plants/row). Rating for root rot severity was done

using a modified Vandermark and Grünwald (2005) scale of 0 to 5, where: 0 = no visible symptoms; 1 = a few, small, discolored lesions on the epicotyl or root system; 2 = minor discoloration covering most of the root system, including the epicotyl; 3 = brown discoloration of the entire root system and epicotyl; 4 = brown discoloration of the entire root system with shriveled and brown epicotyl and hypocotyl; 5 = dead plant. Plant height was measured from the point of seed attachment to the tip of the last leaf. All of the shoots of the 16 plants sampled/plot were dried in an oven at 65°C, and the dry weight measured after 5 days.

For the sweet corn trial, sampling was done 8 weeks after planting. Plant height and shoot dry weight were measured from all plants sampled from the center 3 m of the two middle rows/plot. Root rot rating was not done for the sweet corn trial as there were no visible rot symptoms on most of the roots.

3.2.1.9. Harvest. The Boardman trial was harvested on 28 June (89 dap), and the Soap Lake trial on 5 July 2011 (92 dap). In each plot, plants from the center 1 m of the four middle rows were harvested manually by pulling plants from the ground, and placing the plants in a burlap sack labeled with the appropriate plot number. The plants were taken immediately after harvest to the USDA ARS Legume Pathology facility in Prosser, WA. The plants were passed through a customized thresher (model # 520, Taylor Manufacturing Co., Moultrie, GA) to remove the peas from the pods. The peas were cleaned further by removing any plant debris, prior to measuring total pea weight/plot. Peas were then placed in a strainer washed lightly with running water before two successive tenderometer readings were taken using a TU-12 tenderometer (Food Technology Corporation, Sterling, VA).

The Mount Vernon pea trial was harvested on 11 July 2011 (81 dap) by removing the plants manually from the center 1 m of the four middle rows/plot. Plants were taken to Prosser,

WA immediately after harvest and stored in a cold room overnight. The plants were then processed using the method described above. The sweet corn trial was not taken to harvest as there were insufficient plants to obtain accurate harvest data as a result of very cool, wet spring conditions for more than a month after planting.

3.2.2. Seed priming

Based on the promising results observed for the pea and sweet corn seed priming treatments in some of the 2011 field trials, soaking and drying durations for optimizing the priming of pea seeds were evaluated further in winter 2011-12, prior to the 2012 pea field trials. A RCBD was used with four replications of a factorial combination of three seed soaking durations (8, 16, and 24 h), three drying durations (8, 10, and 12 h), and two soil treatments (inoculated with *P. ultimum* isolate 030141 and non-inoculated). Pea seeds of the cv. Boogie were soaked in deionized water (1:5 v/v ratio) on a rotary shaker (150 rpm) for 8, 16, or 24 h. The water was then drained, and the imbibed seeds washed once with deionized water. The seeds then were dried in a fume hood by spreading the seeds on a wire mesh screen lined with a single layer of paper towel. The imbibed seeds soaked for the different durations were then air-dried for 8, 10, or 12 h. The airflow in the fume hood was maintained at 1.6 to 1.8 m/s at room temperature. Primed seeds were stored at 6°C and 45 RH until planted.

The various combinations of priming conditions for pea seeds were evaluated in a growth chamber trial by planting seeds of the various treatment combinations in non-inoculated soil or in soil inoculated with *P. ultimum* at 500 CFU/g soil. The *P. ultimum* inoculum was prepared and quantified as described above. The soil used for the growth chamber trial was obtained from a certified organic field (Lenwood Farms, Inc., Connell, WA), pasteurized at 70°C for 2 h, dried on a greenhouse bench for 2 to 3 days, and then crushed prior to use. A 3.5 kg soil sample was

then mixed with *P. ultimum* inoculum using a Gustafson batch seed treater (Gustafson LLC, Shakopee, MN) for 5 min, while slowly adding 500 ml water to the soil. A similar soil:water mixing procedure was used for the non-inoculated soil, except that no inoculum was added. The soil was then placed in five 10 cm-diameter plastic pots (replicates) (Anderson Die Manufacturing Co., Portland, OR) for each treatment combination. Each pot was planted with 10 seeds of the appropriate treatment combination. Non-primed seed served as a control treatment in both inoculated and non-inoculated soil. The planted pots were kept in a PGC Controlled Environment Chamber (Percival Scientific Inc., Perry, IA) at 13°C by day and 8°C by night with a 12 h photoperiod. The pots were watered every other day. Seedling emergence counts were done weekly from14 to 28 dap. Shoot length, root rot ratings, total fresh weight, and shoot dry weight were measured 28 dap.

An electrical conductivity test was completed for four replicate subsamples of 50 seeds of each of the nine priming treatment combinations and the non-primed control seed, following the protocol of the Processors and Growers Research Organization (1981) as described below. A seed germination assay, following the method of the Association of Official Seed Analysts (2008) for pea seeds, as described below, was also carried out for four replicate samples of 100 seeds for each of the priming treatment combinations.

3.2.3. 2012 Field trials

3.2.3.1. Field sites and experimental design. Based on results of the 2011 field trials, the 2012 field trials were focused on evaluating the potential benefits of combining seed priming with select organic seed treatments that showed some potential benefit in at least one of the 2011 field trials. Four field trials were carried out in 2012 at three sites in Washington (Table 3.1): (1) one trial was in a grower's certified organic pea field near Ephrata; (2) one trial was in a field

that was managed organically, but not certified, at the WSU Mount Vernon NWREC (adjacent to the 2011 Mount Vernon pea trial site); and (3) two trials were in a separate fields approximately 8 km apart near Royal City. The growers' organic field near Ephrata had been planted to sweet corn in 2011, the Mount Vernon field was fallow in 2011, and the two Royal City trials had been in corn production in 2011. Treatments were laid out in a RCBD with five replications/treatment in each trial. Fourteen seed treatments were evaluated in the Ephrata trial including seven seed treatments applied to both PS and non-primed seeds (referred to hereafter as dry seed or DS), and the two control treatments (PS and DS) (Table 3.1). In the Mount Vernon trial, where plots were inoculated with *P. ultimum* isolate 030141, twenty treatments were evaluated, including 10 organic seed treatments each applied to PS and DS, as well as the control treatments of inoculated soil and NI soil each planted with NTS (Table 3.1).

The two field trials established near Royal City, WA in 2012 focused on evaluating seed priming with the use of biochar as an alternative to air drying for removing excess moisture from the imbibed seeds (Table 3.1). Biochar is highly porous, activated charcoal that is a by-product of the lumber industry and has good moisture absorbing capacity (Biochar, http://www.biochar-international.org/; Chun et al., 2004; Liang et al., 2006). In addition, PS and DS, with and without Nordox seed treatment, were included in these trials because this product was the most effective seed treatment in the 2011 Boardman trial in which damping-off was severe. A total of seven treatments, including the two control treatments of non-treated PS and DS, was arranged in RCDB with four replications with identical treatments at each site (Table 3.1).

3.2.3.2. Seed treatments. Six of the organic seed treatments evaluated in the 2011 pea and sweet corn field trials showed potential for control of damping-off and, therefore, were included in the 2012 Ephrata and Mount Vernon field trials: Acadian Marine, Heads Up, Myco

Seed Treat, Mycostop Mix, Natural II, and Nordox (Table 3.2). These products were each applied to PS and DS in the Ephrata and Mount Vernon trials. Non-treated PS and DS served as the control treatments in both trials. At the Mount Vernon trial, Acadian Powder, a seaweed extract that is not OMRI-listed, was also evaluated, as well as the conventional fungicide, Apron XL. All the plots at Mount Vernon were inoculated with *P. ultimum* using a soil-oatmeal carrier as described above except for NI plots planted with PS and DS which served as two additional control treatments in that trial.

The pea cv. Boogie, the same cultivar planted in the surrounding grower's field in Ephrata, was used in the 2012 Ephrata and Mount Vernon trials. The priming protocol entailed 16 h of seed soaking in distilled water (1:5 v/v ratio) on a rotary shaker set at 150 rpm, similar to the protocol used in the 2011 field trials; however, the seeds were then dried in a fume hood for 10 h instead of 8 or 12 h. After imbibing, the seeds were drained and rinsed twice with tap water to remove dirt and exudates adhering to the seed surface. The seeds were then spread on mesh wire racks lined with two layers of paper towels, and dried for 10 h in a fume hood. Airflow in the fume hood was maintained at 1.6 to 1.8 m/s. The seeds were then packed in a wax-lined seed bag and stored at 6°C and 45% RH.

Application of the seed treatments was done at the Vegetable Seed Pathology laboratory in Mount Vernon. The amount of product applied to the seeds was the highest rate recommended on the label (Table 3.2). Seed treatments were applied at least one week prior to the anticipated date of planting, except for Mycostop Mix and Myco Seed Treat, which were each applied as a dust treatment the day before planting. Heads Up and Nordox were applied as a slurry by suspending the product in water to obtain a 30% final seed moisture content for treating DS, and 60% final seed moisture content for treating PS. Acadian Marine was applied as a liquid seed

treatment at the rate recommended by the manufacturer. Natural II was applied by Agricoat LLC at their facility in Soledad, CA, a week prior to planting.

The pea cv. Gallant (Pure Line Seeds, Inc., Warden, WA), the same cultivar planted in the surrounding grower's fields, was used in the two trials near Royal City. Priming and seed treatment with Nordox were done at the USDA ARS Legume Pathology facility in Prosser, WA. The seeds were primed 48 and 24 h prior to the anticipated planting dates for Trial 1 and Trial 2, respectively, by soaking the seeds in tap water for 16 h. Seeds were then coated immediately in the biochar (CharBiologicals, Red Boiling Springs, TN) at 0.82 g in 2.5 ml water/100 g seeds by placing the seeds in a Ziploc bag to cover the seed surface thoroughly after shaking for 2 to 3 min. Biochar with particle size that can fit through a 710 µm opening sieve (no. 25, U.S.A. Standard Testing Sieve, Tyler equivalents 24 mesh, W.S. Tyler Industrial Group, Mentor, OH) was used to coat the seeds. Nordox was applied as a slurry after biochar application, using either the full label rate of 0.14 g/kg seeds (referred to hereafter as the Nordox FR) or a half rate of 0.07 g/kg seeds (referred to hereafter as the Nordox HR) on both PS and DS.

3.2.3.4. Electrical conductivity test and germination assay for the 2012 trials. An electrical conductivity (EC) test was carried out for the PS and DS used in the 2012 Ephrata and Mount Vernon trials to determine any differences in seed exudation between PS and DS. The EC test was carried out following the Processors and Growers Research Organization (1981) protocol. Fifty seeds for each of five replications of both PS and DS seeds were weighed and soaked in a 400 ml glass beaker containing 250 ml deionized water that had been stored at 20°C for 24 h. The beakers with the seeds and water were then kept for another 24 h at 20°C, after which, the EC of the steeped water was measured using an EC meter (model HI98312 Hannah Instruments Inc., Woonsocket, RI).

A pea seed germination assay was completed for the PS and DS for each seed treatment combination in the four 2012 trials to determine if the organic seed treatments applied to PS and DS had any effects on germination. Following the protocol of the Association of Official Seed Analysts (2003), a total of 100 treated seeds was sampled/replication/treatment. The 25 seeds were arranged in two rows on Anchor seed germination blotters (two 25.4 cm x 38.1 cm blotters placed to overlap by 2 cm, creating a 25.4 cm x 61.0 cm blotter; Anchor Paper Co., St. Paul, MN) that had been soaked in distilled water for 5 to 10 min and then passed through a ringer (Lake City Industries, Inc., Lake City, PA) to remove excess water. The blotter was then folded lengthwise, rolled, and placed in a 1 liter Ziploc bag. The bags with blotters were placed in a germinator at 20°C without light. Seed germination readings were done after 5 and 8 days.

3.2.3.5. Plot inoculation at the 2012 Mount Vernon trial. Inoculum of *P. ultimum* isolate 030141was produced using soil and ground oatmeal as a carrier, and quantified following the procedure described for the 2011 Mount Vernon trial (see 3.2.1.3). Inoculum was applied to each plot in the 2012 Mount Vernon trial five days prior to the scheduled planting date. A six-row belt planter was used to deliver the inoculum at a target rate of 5×10^4/m linear row. The non-inoculated plots, in which the soil-oatmeal carrier was applied without the pathogen, served as an additional control treatment. However, strong winds at the time of inoculum application blew some inoculum from the belt planter, resulting in a significantly reduced rate of inoculation than intended. A second application of inoculum was done on 27 April 2012, after planting, by broadcasting 1 kg inoculum over the top of each plot manually, as uniformly as possible.

3.2.3.5 Planting. Planting at the Ephrata trial was done on 5 April, and for the Mount Vernon trial on 19 April 2012 while the two field trials near Royal City were both planted on 6 April 2012. Each plot at the Ephrata and Royal City trials measured 9.9 m^2 with rows spaced 28

cm apart, while plots at the Mount Vernon trial each measured 6.3 m^2 with rows spaced 18 cm apart. Each of the plots at all locations was surrounded by a 60-cm-wide alley. A six-row cone planter (Almaco 8000 series 6-row grain drill) was used at the Ephrata and Royal City trials, and a drill/planter (1000 series, HEGE Equip., Inc., Colwich, KS) at the Mount Vernon trial. A total of 70 seeds/m^2 was planted in the trials at Ephrata and Royal City and 110 seeds/m^2 in the trial at Mount Vernon at a seeding depth of 2.5 to 3.8 cm for all the trials. Soil samples were collected from each plot to a depth of 15 cm using a soil probe with a diameter of 2.5 cm. The soils cores from all the replications in a trial were pooled in a 19-liter bucket and mixed thoroughly. A 500 g sub-sample of the soil from each trial was sent to Soiltest Farm Consultants, Inc. for nutrient analysis. A Spectrum data logger (model # 450, Spectrum Technologies, Inc) data logger was installed at the site immediately after planting to monitor soil temperature.

3.2.3.6 Data collection. Seedling emergence counts were done 14 dap and weekly thereafter until 35 dap, from the entire length of the two middle rows/plot at the 2012 Ephrata and Royal City trials. At the 2012 Mount Vernon trial, the planter did not distribute the seeds uniformly along the six rows, so emergence counts were done from the full length of all six rows/plot initially. Excessive rain, however, washed out the two outer rows of some of the plot; so the two outer rows were not included in the later emergence counts for this trial.

Plant height was measured from 10 seedlings randomly sampled from each of the two center rows/plot 35 dap in each of the four trials in 2012. A 3-m PVC pole marked with colored tape at 30 cm increments was used to determine which plants to measure in each row. Plant height was measured from the soil line to the tip of the growing point.

At full bloom for each of the four trials, plant height, root rot severity, and shoot dry biomass were measured from 20 plants sampled 60 cm from the ends of each of the two middle

rows/plot. Roots were washed to remove adhering soil, and blotted dry. The root system of each plant was rated for severity of root rot using the 0 to 5 scale described above for the 2011 trials. The root system of each plant was then cut, and plant height measured from the point of seed attachment to the tip of the apical leaf. Shoots of the plants sampled from each plot were dried in an oven at 65°C and dry weight measured after 5 to 7 days of drying.

3.2.3.7. Harvest. The trial in Ephrata was harvested on 2 July (88 dap), the Mount Vernon trial on 19 July (91 dap), and the two Royal City trials were harvested on 27 June 2012 (82 dap). Plants from each plot were harvested manually by pulling plants from the center 1.5 m of each of the four middle rows/plot. The plants were placed in a burlap sack, closed with a wire string, and transported to the USDA ARS facility in Prosser, WA for processing. Peas were separated from the pods using a commercial pea thresher/de-viner, cleaned, total weight measured, and the tenderometer readings taken as described for the 2011 trials.

3.2.4. Data analyses

Analyses of variance (ANOVA) and means comparisons were done using Fisher's least significant difference (LSD, $P < 0.05$) with Proc GLM of SAS Version 9.2 (SAS Institute, Cary, NC) for each of the dependent variables measured in the 2011 and 2012 field trials, and the pea seed priming optimization trials. Replications were considered as random effects, and seed treatments (as well as priming conditions and inoculation treatments for the priming optimization trials) were fixed effects in the models. Linear regression analysis, using SigmaPlot Version 11 (Systat Software, Inc., San Jose, CA) was carried out for seedling emergence, total fresh weight, shoot length, and shoot dry weight variables measured in relation to seed soaking and drying durations in the seed priming optimization trials. Arcsine, log, or square root transformations were used when needed to satisfy the assumptions for parametric analyses. Rank transformation

using Friedman's non-parametric rank test was used when none of the transformations satisfied these assumptions.

3.3. Results

3.3.1. 2011 Field trials

3.3.1.1. Environmental data. Boardman and Soap Lake were warmer and drier than Mount Vernon from March through July 2011 (Fig. 3.1A to 3.1C). The Mount Vernon site received the greatest precipitation with total monthly rainfall of 9.9, 10.6, 2.3, and 3.5 cm in April, May, June, and July, respectively; compared to 3.5, 0.6, 3.9, and 1.4 cm for Boardman; and 0.4, 3.0, 0.4, and 0.4 cm for Soap Lake, respectively (Fig. 3.1A). The most rainfall was recorded in April (9.9 cm) and May (10.6 cm) at the Mount Vernon site, and the least rainfall (0.4 cm) in each of April, June, and July at the Soap Lake site (Fig. 3.1A). Mean monthly air and soil temperatures in 2011 were consistently higher at the Boardman and Soap Lake sites compared to the Mount Vernon site, which is typical for spring and summer weather in the semi-arid Columbia Basin vs. the maritime western Washington, respectively (Fig. 3.1B and 3.1C). The lowest mean temperatures were recorded in March at the Boardman site (mean air temperature of 7.2°C and soil temperature of 8.3°C), and in April in Soap Lake (7.4 and 11.2°C, respectively) and Mount Vernon (7.3 and 9.6°C, respectively), when planting occurred at these sites. The warmest mean temperatures were in July at the Soap Lake site (mean air temperature of 19.8°C and soil temperature of 27.2°C) compared to June in Boardman (18.3 and 22.9°C, respectively) and in July in Mount Vernon (15.7 and 17.7°C, respectively), when the pea crops were harvested at these sites.

Soil properties based on the soil nutrient analyses of the three trial sites at the time of planting in 2011 are presented in Table 3.3. The Boardman field had higher nitrate-N levels (35

ppm) compared to Mount Vernon (10 ppm) and Soap Lake (8 ppm), while Boardman and Mount Vernon had higher phosphorus levels (70 and 66 ppm, respectively) compared to Soap Lake (16 ppm). Potassium was highest at Boardman and Soap Lake (482 and 484 ppm, respectively) compared to Mount Vernon (204 ppm). The B, Mn, and Fe levels were all higher at the Mount Vernon site (0.4, 3.0, and 77.0 ppm, respectively) compared to Boardman (0.3, 1.5, and 15.0 ppm, respectively) and Soap Lake (0.2, 1.3, and 14.0 ppm, respectively). Zn and Cu were greatest in the soil at the Boardman site (3.5 and 3.9 ppm, respectively) compared to Mount Vernon (2.4 and 3.6 ppm, respectively) and Soap Lake (0.6 and 1.4 ppm, respectively). All three sites had similar Ca levels (6.1 to 6.5 meq/100 g soil). Soap Lake had the highest level of Mg (2.5 meq/100 g soil) followed by Boardman and Mount Vernon (1.4 and 0.8 meq/100 g soil, respectively). The Soap Lake and Boardman soils had similar Na levels (0.2 meq/100 g soil) which were greater than at the Mount Vernon site (0.1 meq/100 g soil) (Table 3.3).

The soil pH at the Boardman and Soap Lake sites was neutral (7.1) while the Mount Vernon soil was slightly acidic pH (6.5) (Table 3.3). Organic matter was 3.7% in the Mount Vernon site which was greater compared to 1.4% in Boardman and 1.0% in Soap Lake. The cation exchange capacity (CEC) of the soil from Soap Lake (12.7 meq/100 g soil) was greater than the soils in Boardman (9.7 meq/100 g soil) and Mount Vernon (8.5 meq/100 g soil).

3.3.1.2. Pea trials

3.3.1.2.1. Seedling emergence. In general, the spring of 2011 was cold in both the Columbia Basin and western Washington, with average March and April monthly temperatures of 5.3 and 7.4°C in the Columbia Basin, and 7.5 and 7.3°C in western Washington, respectively. This was lower than the 30-year average March and April temperatures of 6.6 and 10.8°C for the Columbia Basin, and 9.7°C for April in western Washington (Prism Climate Group,

http://www.prism.oregonstate.edu/). This resulted in delayed emergence of pea plants across the three trial sites, so the earliest stand counts were measured 21 dap, when emergence across all plots averaged 26 and 59% in the Boardman and Soap Lake trials, respectively (Fig. 3.2). In the Mount Vernon trial, where stand counts were done by counting all plants that emerged in the center m^2, there was an average of 62 plants/m^2 emergence 21 dap across all plots (Fig. 3.2). At the final stand count, 35 dap, emergence at the Boardman trial still averaged only 32% across all plots in contrast to 81% at the Soap Lake trial and 68 plants/m^2 at the Mount Vernon trial (Fig. 3.2). The poor emergence at the Boardman site was primarily attributed to two factors: first, high disease pressure from *Pythium* and *Rhizoctonia* based on isolations completed from the symptomatic pea roots sampled in this trial (*data not shown*), and second, competition from weeds such as nightshade, and the regrowth of the triticale cover crop. In the presence of such severe soilborne disease pressure, the main effect of the organic seed treatments on emergence was significant at 21 dap ($P < 0.0001$) and 35 dap ($P < 0.0001$) in the ANOVAs. The Nordox and priming seed treatments significantly increased emergence compared to the NTS control plots and WD control plots at 21 dap (from 25 ± 2% for NTS plots and 18 ± 1% for WD control plots to 40 ± 4% for Nordox plots and 51 ± 2% for PS plots, respectively) and similarly at 35 dap (Fig. 3.2A). No significant improvement in emergence was observed for any of the other organic seed and drench treatments evaluated in Boardman compared to the NTS and WD control plots; and none of the seed, drench, or spray treatments showed a negative effect on emergence of pea seeds (Fig. 3.2A and Appendix Table 3.1).

At the Soap Lake trial, where very few damping-off and root rot symptoms were observed and the site was relatively weed-free, pea stands were good overall. Although the main effect of seed treatments on pea emergence 21 and 35 dap was significant ($P < 0.0001$ and $P =$

0.0163, respectively), none of the treatments improved emergence compared to that of the NTS control plots (Fig. 3.2B). However, all of the drench treatments, including the WD control plots, resulted in significantly reduced stand counts 21 dap compared to stand counts in plots with the various organic seed treatments and the NTS control plots, demonstrating the potential negative effect of increased soil moisture on pea emergence following the drench applications (Fig. 3.2B and Appendix Table 3.1). Seeds treated with Acadian Marine, Heads Up, Myco Seed Treat, Natural II, and Nordox produced significantly greater emergence than the NTS in the WD control plots at 35 dap (Fig. 3.2B and Appendix Table 3.1).

At the Mount Vernon trial, there was a significant seed treatment effect on emergence at 21 dap ($P < 0.0001$) and 35 dap ($P < 0.0001$). Although the cold and wet spring conditions contributed to poor emergence, inoculation of the plots with *P. ultimum* 030141 did not result in a significant increase in damping-off compared to the NI control plots planted with NTS (Fig. 3.2C). Emergence 21 and 35 dap were reduced only by 14 and 13% in inoculated control plots compared to the NI control plots. At this low disease pressure site, plots planted with Acadian Marine treated seeds had significantly greater emergence 21 dap compared to the NTS in inoculated control plots but not in NI control plots (Fig. 3.2C and Appendix Table 3.1). Similarly, Acadian Marine, Heads Up, Nordox, and Prestop WP treated seeds resulted in greater emergence 35 dap than NTS in inoculated control plots (Fig. 3.2C and Appendix Table 3.1). Plots planted with PS had fewer pea plants than NI plots planted with NTS at 21 dap, and NTS planted in both inoculated and NI control plots at 35 dap (Fig. 3.2C and Appendix Table 3.1). In addition, plots sprayed with Prestop WP had lower stand counts 35 dap than inoculated plots with NTS (Fig. 3.2C and Appendix Table 3.1). However, the conventional seed treatment, Apron XL, increased emergence 21 and 35 dap by 53 and 59%, respectively, compared to emergence of

NTS planted in inoculated control plots, and increased emergence by 46 and 42% compared to emergence in NI control plots planted with NTS, respectively (Fig. 3.2C and Appendix Table 3.1).

3.3.1.2.2. Plant height. Measurement of plant height at full bloom of the pea crop in Boardman revealed no significant differences among seed treatments ($P = 0.7453$), with a mean height of 49 ± 5 cm across all plots (Fig. 3.3A). Although a significant effect of seed treatments ($P = 0.0153$) on pea plant height was observed at the Soap Lake trial, none of the organic seed and drench treatments improved pea height compared to plants in the control plots planted with NTS; but plots planted with seeds treated with Mycostop Mix or Nordox had significantly taller plants (9%) compared to plants in the WD control plots (Fig. 3.3B). In the Mount Vernon trial, plant height was affected significantly by the seed and drench treatments ($P < 0.0001$), where plots planted with seeds treated with Apron XL had 24 and 25% taller plants compared to NTS planted in inoculated control plots and NI control plots, respectively. However, none of the organic treatments improved plant height in the Mount Vernon trial (Fig. 3.3C and Appendix Table 3.1).

3.3.1.2.3. Root rot rating. There was no significant effect of organic seed and drench treatments on root rot in the Boardman ($P = 0.2615$), Soap Lake ($P = 0.7613$), and Mount Vernon ($P = 0.1030$) trials. In the Boardman trial, root rot was observed on most plants sampled (mean rating of 1.57 ± 0.04 out of a maximum of 5.00), and both *Rhizoctonia* spp. and *Pythium* spp. were isolated from the symptomatic roots. In contrast, plants sampled from the Soap Lake trial showed almost no symptoms of root rot (mean rating of 0.30 ± 0.03). Inoculation with *P. ultimum* 030141 at the Mount Vernon trial resulted in minimal pea root rot (mean rating of 0.91

± 0.30) across all plots, although isolations from the symptomatic roots of sampled plants confirmed the presence of the pathogen in inoculated and non-inoculated plots (*data not shown*).

3.3.1.2.4. Shoot dry biomass. There were no significant differences among organic seed/drench treatments for pea shoot dry weight in the Boardman (P = 0.4338), Soap Lake (P = 0.2741), and Mount Vernon (P = 0.4333) trials. The mean shoot dry weights across all plots at full bloom were 147 ± 3, 105 ± 2, and 42 ± 1 g for the Boardman, Soap Lake, and Mount Vernon trials, respectively.

3.3.1.2.5. Tenderometer readings (TR) and yield. The mean TR of peas harvested from plots did not differ significantly among treatments at the Boardman and Soap Lake trials (P = 0.3596 and 0.1914, respectively) in the ANOVA. In the Mount Vernon trial, the mean TR was affected significantly by treatments (P = 0.0415), wherein the peas harvested from the NI control plots and plots with Nordox seed treatments had the greatest TR (both with a mean of 91 ± 3) and were significantly greater compared to the TR of the inoculated control treatment (85 ± 5), while peas harvested from plots with Apron XL had the lowest TR (82 ± 1). The mean TR of peas harvested at the three trials was 85 ± 1 for Boardman, 92 ± 2 for Soap Lake, and 86 ± 1 for Mount Vernon.

At the Boardman trial, there was a significant organic seed and drench treatment main effect on pea yield (P = 0.0044). At this site, plots planted with PS had significantly greater yields (71 and 111%) compared to control plots with NTS and NTS + WD , respectively, while plots planted with Nordox treated seeds had 71% more yield compared to the yields of the WD (Fig. 3.4A and Appendix Table 3.1). Likewise, there was a significant seed treatment effect (P = 0.0135) on the yields of pea plants at the Soap Lake trial. Nordox seed treatment resulted in significantly greater yields compared to the yields of control plots with NTS and NTS + WD

control plots (26 and 44% greater, respectively). Plants in plots with the Heads Up, Myco Seed Treat, PS, and Nordox treatments produced significantly greater yields (40, 32, 31, and 44%, respectively) than the yields of plants in the WD control plots in the Soap Lake trial (Fig. 3.4B and Appendix Table 3.1). At the Mount Vernon trial, seed treatments affected pea yields significantly ($P = 0.0005$). However, none of the organic seed and drench treatments improved yields. Only plots with Apron XL seed treatment had significantly greater yields compared to any other treatments, and had 111 and 61% more pea yield than the yield in inoculated and NI control plots with NTS (Fig. 3.4C and Appendix Table 3.1).

3.3.1.3 Sweet corn trial

In the 2011 sweet corn trial, there was a significant main effect of seed and drench treatments for both 21 and 35 dap emergence counts ($P < 0.0001$). A significant increase in emergence was detected 21 dap for plots with Natural II, PS and Apron XL treated seed, and at 35 dap for plots with Natural II, PS, and Apron XL treated seeds compared to the NTS in inoculated plots and in NI control plots + WD (Fig. 3.5A). Shoot dry weight was also significantly greater ($P < 0.0001$) in plots with PS and Apron XL treated seed compared to any other treatments, while Natural II and Nordox, treated seed were different compared only to the NTS in inoculated control plots (Fig. 3.5B). Plant height (mean of 22.0 ± 0.4 cm) was not affected significantly ($P = 0.3250$) by treatments. Examination of the plants sampled from each plot did not show the presence of root rot symptoms (*data not shown*). Because of very poor emergence, the sweet corn trial was not harvested as there were not enough plants in some plots to obtain sufficient data for statistical analyses.

3.3.2. Optimization of the priming protocol for pea seeds.

The ANOVAs revealed a significant main effect of the soaking duration of pea seeds in the priming protocol on the emergence measured 14 dap ($P = 0.0020$) and 28 dap ($P < 0.0001$), total fresh weight ($P = 0.0066$), and shoot dry weight ($P = 0.0094$), but not for shoot length ($P = 0.6395$) and root rot ratings ($P = 0.1675$). However, there was no significant main effect of the pea seed drying duration after soaking in any of the dependent variables measured ($P > 0.05$). The main effect of soil treatment (i.e., planting PS into soil inoculated and not inoculated with *P. ultimum*) was significant ($P < 0.0001$) for all the variables measured, except emergence 14 dap ($P = 0.1154$).

There was a significant interaction between soaking duration and soil treatment for emergence 14 and 28 dap ($P = 0.0210$ and $P < 0.0001$, respectively), a significant interaction between drying duration and soil treatment for emergence 28 dap ($P = 0.0201$) and shoot length ($P = 0.0297$), and a significant interaction between soaking and drying durations for emergence 28 dap ($P = 0.0202$). There were no significant three-way interactions for any of the variables measured ($P > 0.05$).

Pea emergence 14 and 28 dap was significantly greater for seeds that had been soaked for 16 h (mean ± standard error of 79 ± 4 and 93 ± 2%, respectively) or 24 h (75 ± 4 and 90 ± 3%, respectively), than for seeds soaked for 8 h (60 ± 4 and 78 ± 4%, respectively) and for non-primed seeds (36 ± 7 and 71 ± 9%, respectively). Total fresh weight of pea seedlings measured 28 dap was greater for plants that grew from the seeds soaked for 24 h (18.2 ± 0.1 g) compared to seeds soaked for 8 h (15.2 ± 1.2 g) or 0 h soaking (13.0 ± 1.8 g), but not different than that of seeds soaked for 16 h (17.2 ± 1.2 g). Similarly, the dry weight of shoots produced by seeds

soaked for 24 or 16 h was significantly greater (0.49 ± 0.03 and 0.49 ± 0.04 g, respectively) than that of seeds soaked for 8 or 0 h (0.39 ± 0.04 and 0.31 ± 0.06 g, respectively).

Planting in NI soil resulted in greater seedling emergence 28 dap, shoot length , total fresh weight, and shoot dry weight (98 ± 1%, 5.6 ± 0.1 cm, 21 ± 1 g, and 0.56 ± 0.02 g, respectively) compared to seeds planted in the inoculated soil (73 ± 3%, 4.4 ± 0.1 cm, 12 ± 1 g, and 0.32 ± 0.02 g, respectively). Root rot rating was less severe for seedlings in NI soil (0.15 ± 0.10) than in inoculated soil (3.20 ± 0.10).

Pea emergence 14 dap of seeds planted in NI soil for seeds soaked for 8, 16, or 24 h (66 ± 7, 75 ± 6, and 85 ± 3%, respectively) was significantly greater compared to non-primed seeds (35 ± 13%), while emergence 14 dap in the inoculated soil was significantly greater for seeds soaked for 16 h (82 ± 4%) than seeds soaked for 24, 8, or 0 h (64 ± 5, 53 ± 5, and 36 ± 7%, respectively) (Fig. 3.6A). The emergence 14 dap from seeds that had been soaked for 24 h in the priming protocol was significantly greater than the emergence from non-primed seeds, but not different from that of the 8 h soaking duration (Fig. 3.6A). A similar incidence of emergence 28 dap was observed for all seed soaking durations planted in the NI soil (> 90%) (Fig. 3.6A). However, when PS were planted in the inoculated soil, emergence 28 dap was greater for seeds that had been soaked for 16 h (89 ± 6%) than 24 h (80 ± 4%), which was, in turn, greater than the emergence from seeds soaked for 8 or 0 h (58 ± 3 and 50 ± 7%, respectively) (Fig. 3.6A).

Regardless of the drying duration after soaking, seeds planted in NI soil had greater emergence than in the inoculated soil and similar emergence as non- primed seeds planted in NI soil (Fig. 3.6A). Emergence was greater for seeds that were soaked for 16 or 24 h followed by drying for 8, 10, or 12 h (i.e., for all three drying durations) compared to seeds soaked for 8 h and then dried for 8 or 10 h, or from non-primed seeds (Fig. 3.6B). Emergence from the seeds

soaked for 24 h did not differ significantly for any of the drying durations (95 ± 2 , 88 ± 7, and 86 ± 6%, for seeds dried for 8, 10, or 12, respectively) (Fig. 3.6B). Soaking seeds for 16 h followed by 8 h drying resulted in greater emergence (99 ± 1%), than drying the soaked seeds for 10 or 12 h (92 ± 3 and 89 ± 4%, respectively) (Fig. 3.6B). Drying seeds for 8, 10, or 12 h after seeds had been soaked for 8 h did not result in any significant differences in emergence 28 dap (75 ± 8, 78 ± 9, and 81 ± 7, respectively) (Fig. 3.6B).

There was also a significant interaction between seed drying and soil treatment for shoot length was also affected significantly by the drying and soil treatment interaction. Regardless of the drying durations after soaking, plants that grew from PS planted in NI soil were taller than the plants in inoculated soil. Non-primed seeds planted in NI soil produced plants as tall as those from PS dried for 8 h and planted in inoculated soil (*data not shown*).

Regression analyses revealed a significant relationship between pea seed soaking durations and seedling emergence 14 and 28 dap as well as shoot dry weight, for PS planted in NI soil regardless of drying duration; and for PS dried for 8, 10, or 12 h planted in inoculated soil (Fig. 3.7). Emergence 14 dap showed significant linear relationships with the soaking durations when the seeds were dried for 8 or 10 h and planted in inoculated soil ($P = 0.0004$ and $R^2 = 0.7772$, and $P = 0.0396$ and $R^2 = 0.2689$, respectively) (Fig. 3.7A), i.e., emergence in inoculated soil was greater the longer the seeds were soaked if the drying duration was 8 or 10 h. The shortest drying period (8 h) resulted in greater emergence than the 10 h drying duration (Fig. 3.7A). A quadratic relationship was detected between the soaking duration and emergence 14 dap in inoculated soil if the seeds were dried for 12 h, and similarly for seeds planted in the NI soil ($P = 0.0120$ and $R^2 = 0.4924$, and $P < 0.0001$ and $R^2 = 0.4636$, respectively) (Fig. 3.7A). Emergence increased for seeds with increasing soaking duration up to 16 h, but then declined

when soaking was extended to 24 h if the long drying period of 12 h was used and the seed planted in inoculated soil. The same was true for PS planted in NI soil, except that the decrease in emergence observed from 16 to 24 h was not as great (Fig. 3.7A).

Emergence of PS in inoculated soil measured 28 dap showed a quadratic relationship with the soaking duration regardless of the drying duration ($P = 0.0004$ and $R^2 = 0.7039$ for 8 h drying; $P = 0.0161$ and $R^2 = 0.4073$ for 10 h drying; and $P = 0.0042$ and $R^2 = 0.5693$ for 12 h drying, respectively) (Fig. 3.7B). The increase in emergence was greater the longer the seeds were soaked, up to 16 h, and then declined if seeds were soaked longer for 24 h, although the rate of decline was greater when seeds were dried for 10 or 12 h than for 8 h (Fig. 3.7B). Seeds planted in NI soil showed a linear increase in emergence 28 dap the longer the seed soaking duration ($P = 0.0240$ and $R^2 = 0.1050$) (Fig. 3.7B).

The shoot dry weights of seedlings growing in NI were greater than that of the seedlings in the inoculated soil (Fig. 3.7C). Pea shoot dry weights showed a quadratic relationships with seed soaking duration for each of drying durations tested when the seeds were planted in inoculated soil ($P = 0.0008$ and $R^2 = 0.6688$ for 8 h, $P = 0.0060$ and $R^2 = 0.5450$ for 10 h, and $P = 0.0019$ and $R^2 = 0.6184$ for 12 h, respectively); and also for seeds planted in NI soil ($P = 0.0213$, $R^2 = 0.1572$) (Fig. 3.7C). Pea dry weights were greater for seeds soaked for up to 16 h than 24 h (Fig. 3.7C).

Exudation of the PS was affected significantly by the main effects of soaking duration ($P < 0.0001$) and drying duration ($P < 0.0001$), but there was no significant interaction term. Exudation was significantly reduced for PS (7.8 ± 0.6, 3.3 ± 0.2, and 3.1 ± 0.3 µS/cm/g for seeds soaked 8, 16, and 24 h, respectively) compared to seeds that were not primed (23.8 ± 1.6 µS/cm/g) (Fig. 3.8A). PS dried for 8 or 10 h had significantly less exudation (3.7 ± 0.5 and 4.6 ±

0.8 µS/cm/g, respectively) than seeds dried for 12 h and seeds not primed (5.9 ± 0.7 and 23.8 ± 1.2 µS/cm/g) when averaged across all three soaking durations (Fig. 3.8A).

In the germination assay for the seed priming treatment combinations tested, seed germination at 5 days was affected significantly by the main effects of soaking duration ($P < 0.0001$) and drying duration ($P = 0.0337$), and there was a significant interaction between soaking and drying ($P = 0.0300$). Germination was greater for seeds soaked for 8 and 16 h (85 ± 1 and 87 ± 1%) vs. 24 h (76 ± 2%), and the latter did not differ from germination of non-primed seeds (76 ± 3%) . Drying the soaked seeds for 8, 10, or 12 h resulted in better germination (86 ± 1, 82 ± 2, and 81 ± 3%) than the non-primed seeds (76 ± 3%). When seeds were soaked for 8 or 16 h, the drying duration did not affect germination; however, when seeds were soaked for 24 h, germination decreased more the longer the drying duration (Fig. 3.8B).

3.3.3. 2012 Field trials

3.3.3.1. Environmental data. The weather data for the duration of the field trials at each of the four sites selected in 2012 showed that the Ephrata and the two Royal City sites had similar total monthly precipitation and mean monthly air temperatures in April through July (Fig. 3.1D and 3.1E). The monthly total precipitation in Mount Vernon (10.9, 6.1, 8.0, and 3.3 cm for April, May, June, and July, respectively), was much greater than the two sites in Columbia Basin (average of 1.7, 0.15, 2.7, and 1.7 cm for the two sites, respectively) (Fig 3.1D). Mean monthly air temperatures at the Ephrata and Royal City trials increased more rapidly from April to July (mean of 11, 15, 17, and 23°C in Ephrata; and 11, 15, 17, and 24°C in Royal City for April, May, June, and July, respectively) compared to air temperatures in Mount Vernon (10, 12, 14, and 16°C for April, May, June and July, respectively) (Fig. 3.1E). The mean monthly soil temperature was similar for the three sites for the month in April (13, 11, and 11°C for Ephrata,

Royal City, and Mount Vernon, respectively). However, as the season progressed, the soil temperature at the Ephrata trial increased more rapidly than at the other two sites reaching 29°C by July vs. 25°C at the Royal City trials and 19°C in Mount Vernon (Fig. 3.1C). Overall, spring was warmer and drier in Ephrata and Royal City compared to Mount Vernon in 2012, which is typical for spring conditions in these regions.

The soil nutrient analyses for each site showed that the three Columbia Basin sites had greater levels of nitrate-N (69, 54, and 39 ppm for Royal City Trial 2, Ephrata, and Royal City Trial 1, respectively) than the field in Mount Vernon (2 ppm) at the time of planting (Table 3.3). However, the Mount Vernon site had the most phosphorus (53 ppm), followed by the Ephrata site (39 ppm), Royal City Trial 2 (26 ppm), and Royal City Trial 1 (22 ppm) sites (Table 3.3). Potassium levels were similar in Royal City Trial 1 (275 ppm), Royal City Trial 2 (266 ppm), and Mount Vernon (247 ppm), while the Ephrata site had the least (153 ppm) (Table 3.3). Soil nutrient analyses for each of the sites showed that the nitrogen level was excessive (> 30 ppm) for the three Columbia Basin sites and low (< 10 ppm) for the Mount Vernon site at the time of planting (Marx et al., 1999). The phosphorus level in the soil of all sites was high, as well as the potassium level in the soil of the two Royal City sites, but potassium was intermediate level for Ephrata and Mount Vernon sites at the time of planting according to Marx et al., (1999).

The micronutrient levels were considered sufficient for all four sites. However, the Mount Vernon soil had the most B, Cu, and Fe of the four trial sites in 2012, whereas the Ephrata site had the most Zn and Na and the least B and Mn (Table 3.3). The two Royal City sites had highest level of Mg but similar Na level as the Ephrata site (Table 3.3).The Royal City Trial 1 and 2 sites had alkaline soil with 7.3 and 7.8 pH, respectively, while Ephrata soil pH was 6.6 and Mount Vernon soil pH was 5.8. However, the Mount Vernon soil had the highest organic matter

(4.0%) compared to the three other sites (1.4, 1.0, and 0.9% for Royal City Trial 2, Ephrata, and Royal City Trial 1, respectively) (Table 3.3).

3.3.3.2. Seed electrical conductivity (EC) test and germination assays for 2012 trials. Priming pea seeds by soaking the seeds for 16 h, followed by air drying for 10 h for the Ephrata and Mount Vernon trials or using biochar for the Royal City trials significantly reduced seed exudation ($P < 0.0001$) by 85, 86, and 88% in the 2012 field trials (EC of 3.2 ± 0.1 for Ephrata, 2.4 ± 0.3 for Mount Vernon, and 2.0 ± 0.1 µS/cm/g for Royal City, respectively) compared to the DS (21.7 ± 0.6, 18.4 ± 0.2, and 16.9 ± 0.8 µS/cm/g, respectively) (Fig. 3.9). In the blotter germination assay of PS and DS after the seed treatment applications, significant main effects of priming and seed treatments ($P = 0.0004$ and $P = 0.0001$, respectively) occurred at the 5 day count; there was a significant main effect of priming ($P = 0.0015$) at the 8 day count for the Ephrata trial. There was a significant interaction between priming and seed treatments for both the 5 and 8 day germination counts ($P < 0.0001$ and $P = 0.0349$, respectively) in the Ephrata trial. Except for the Natural II treatment applied to PS, the PS had greater emergence (74.6 ± 4.8%) compared to DS (70.9 ± 2.2%) at the 5 day count for the Ephrata seed lot (Fig. 3.10A). Within the PS and DS treatments, germination did not differ significantly for most of the seed treatments, except for Natural II applied to PS which resulted in the poorest germination at both readings (17.8 ± 2.7% at 5 day and 53.2 ± 2.7% at the 8 day count) compared to NTS (82.5 ± 3.1% and 95.0 ± 0.9% at the 5 and 8 day counts, respectively). At the 8 day count, mean germination of all the DS treatments (96.9 ± 0.3%) was significantly greater compared to that of PS (89.5 ± 2.9%) because of the low germination of PS treated with Natural II; there were no significant differences in germination among the other seed treatments (Fig. 3.10A).

The germination assay of the seeds used for planting the Mount Vernon trial showed a significant main effect of seed treatment ($P = 0.0422$) at the 5 day count only, and a significant main effect of priming ($P = 0.007$) at the 8 day count only. At the 5 day count, germination was not significantly different when averaged across all the PS or DS treatments. In contrast to the Ephrata seed lot, seeds treated with Natural II for the Mount Vernon trial had the greatest germination at 5 days, when averaged across PS and DS, although this was not significantly different from germination of the seed treated with Myscostop Mix, Acadian Powder, and Myco Seed Treat as well as NTS. At the 8 day count, DS had significantly greater overall emergence (96.7 ± 0.3%) than the PS (95.1 ± 0.4%). There was a significant interaction between seed priming and organic seed treatments in the ANOVA for both the 5 and 8 day germination counts ($P = 0.0138$ and $P = 0.0148$, respectively) for seeds used at the Mount Vernon site. PS treated with Natural II and non-treated PS control had the best germination at 5 day count but compared only to PS treated with Acadian Marine, Apron XL, Heads Up, and Nordox, and DS treated with Acadian Powder, and not with the other seed treatment and priming combinations (Fig. 3.10B). At the 8 day count, PS treated with Acadian Powder had better germination compared to PS treated with Acadian Marine, Apron XL, Heads Up, Natural II, and Nordox but not with the other treatments (Fig. 3.10B).

For the seed lots used in the two Royal City trials, there was no significant main effect of organic seed treatments at the 5 day germination count ($P = 0.4448$), but a significant treatment effect at the 8 day count ($P = 0.0005$). Germination was greater for non-treated DS (99.5 ± 0.3%) compared to non-treated PS, and PS + biochar + Nordox at the FR or HR (98.5 ± 0.3, 98.0 ± 0.4, and 97.0 ± 0.4%, respectively) (Fig. 3.10C).

3.3.3.3. Ephrata and Mount Vernon trials.

3.3.3.3.1. Seedling emergence. Overall, the mean emergence of pea seedlings 14 dap was greater at the Mount Vernon trial (62 ± 1%) compared to the Ephrata trial (31 ± 1%), probably as a result of rainfall that occurred at the former site soon after planting. Inoculation of plots at the Mount Vernon trial with *P. ultimum* 030141 did not increase the incidence of damping-off (*data not shown*). There was a significant main effects of priming and organic seed treatment for emergence at 14 and 28 dap in Ephrata ($P = 0.0001$ and $P = 0.0271$ for 14 dap, respectively; and $P < 0.0001$ and $P < 0.0001$ for 28 dap, respectively) and in Mount Vernon ($P < 0.0001$ at 14 and 28 dap, respectively). There was also a significant priming- by-seed treatment interaction for the Ephrata and Mount Vernon trials for emergence at 14 dap ($P = 0.0003$ and $P = 0.0002$, respectively) and 28 dap ($P < 0.0001$ and $P < 0.0001$, respectively).

The use of PS did not improve pea emergence for any of the organic seed treatments compared to DS in either trial. In fact, the PS treatments resulted in significantly lower emergence than the DS treatments at both locations. In Ephrata at 14 dap, there was 26 ± 2% emergence for PS treatments and 35 ± 2% for DS treatments (Fig. 3.11A), and in Mount Vernon trial there was 56 ± 1% emergence for PS treatments and 66 ± 1% for DS treatments (Fig. 3.11B). By 28 dap, similar differences were observed between plots with PS and DS treatments, with a mean of 60 ± 3% for PS and 80 ± 2% for DS in the Ephrata trial (Fig. 3.11A), and 68 ± 2% for PS and 80 ± 1% for DS in the Mount Vernon trial (Fig. 3.11B).

In the Ephrata trial at 14 dap, there was no significant difference in emergence between the non-treated PS and non-treated DS control plots; and plots planted with PS + Acadian Marine or DS + Natural II had significantly greater emergence compared to plots with non-treated DS, but not compared to plots with non-treated PS (Fig. 3.11A and Appendix Table 3.2). In contrast,

plots with PS + Natural II or PS + Heads Up had significantly fewer plants compared to the PS and DS control plots. By 28 dap in this trial, emergence was greater in plots with the DS + Acadian Marine, Heads Up, Myco Seed Treat, or Nordox compared to plots with non-treated PS or DS; and plots with DS + Mycostop Mix had more plants than the PS control plots (Appendix Table 3.2). In contrast, plots with PS + Acadian Marine, Heads Up, or Natural II had significantly fewer plants than both the DS and PS control plots, and plots with PS + Myco Seed Treat or PS + Nordox had fewer plants than the non-treated DS control plots (Fig. 3.11A and Appendix Table 3.2).

In the Mount Vernon trial, emergence 14 and 28 dap were greatest in three of the four control plots: DS + Apron XL (76 ± 5 and 89 ± 3%, respectively), PS + Apron XL (70 ± 4 and 84 ± 2, respectively), and DS alone (69 ± 2 and 81 ± 2%, respectively); which all had greater stand counts than the PS control plots (57 ± 4 and 71 ± 3%) (Fig. 3.11B and Appendix Table 3.2). Furthermore, plots with DS + Acadian Marine or DS + Nordox had statistically similar emergence to the DS + Apron XL control plots, while the five other DS combination treatments resulted in similar emergence to the DS control plots (Fig. 3.11B and Appendix Table 3.2). In contrast, plots with the seven organic seed treatments combined with PS had significantly less emergence than the DS control plots, and the PS + Nordox plots also had fewer plants (40 ± 2%) than the PS control plots (57 ± 3%). By 28 dap in the Mount Vernon trial, plots with DS + Acadian Marine and DS + Natural II had similar stand counts to plots with DS + Apron XL, while all five of the other DS organic treatment combinations did not result in different stands than in the DS control plots (Fig. 3.11B and Appendix Table 3.2). As in the Ephrata trial, PS combined with organic seed treatments had poorer emergence 28 dap in general than the DS control plots (Fig. 3.11B).

3.3.3.3.2. Plant height. There was a significant difference in plant height 35 dap in the Ephrata trial for the priming main effect ($P = 0.0015$), as plots planted with PS had significantly smaller plants (6.96 ± 0.2 cm) compared to plots planted with DS (7.6 ± 1.0 cm). At full bloom, priming and seed treatment main effects on plant height were both significant ($P = 0.0001$ and $P = 0.0232$, respectively). Plants in plots with DS remained taller (52.8 ± 0.5 cm) than in plots planted with the PS (50.1 ± 0.6 cm). Plants in plots with Nordox (48.9 cm tall) or Natural II (49.1 cm) were significantly shorter compared to the plants in plots with NTS (52.7 cm), while plant height in plots with the other four seed treatments did not differ from that of the NTS. The interaction effect was not significant for plant height at either 35 dap or at full bloom in the Ephrata trial (*data not shown*).

In the Mount Vernon trial, plant height 35 dap was not significantly affected by any of the main factors ($P > 0.05$). However, the main effects of both priming ($P < 0.0001$) and organic seed treatments ($P = 0.0004$) were significant for plant height measured at full bloom stage of the plant height measurement. At full bloom, plots planted with DS (54.5 ± 0.4 cm) had significantly taller plants than plots with PS (51.1 ± 0.5 cm). In addition, plots with Natural II (53.8 cm) and NTS had plants as tall (53.9 cm) as plots with the Apron XL seed treatment (55.9 cm). Seed treatment with Nordox resulted in smaller plants (50.6 cm) compared to the NTS. There was no significant interaction between priming and organic seed treatments for plant height measured 35 dap and at full bloom in the Mount Vernon trial (*data not shown*).

3.3.3.3.3. Shoot dry biomass. In the Ephrata trial, only the priming main effect was significant for shoot dry weight ($P = 0.0001$), and there was no seed treatment main effect or interaction detected in the ANOVA. Shoot dry weight of plants in plots with PS (151.8 ± 4.4 g) was greater than for plants from plots with DS (131.8 ± 2.7 g). In the Mount Vernon trial, there

were no significant effects of any of the factors were detected for shoot dry weight ($P = 0.1262$, $P = 0.5218$, and $P = 0.3983$ for the main effects of priming and seed treatment, and the interaction effect, respectively).

3.3.3.3.4. Root rot severity rating. There were no significant main effects of priming or organic seed treatments ($P = 0.3466$ and $P = 0.7429$, respectively) and no significant interaction term ($P = 0.9460$) for the root rot rating at pea full bloom in the Ephrata trial. Similarly, in the Mount Vernon trial there was no significant main effect of priming or seed treatments ($P = 0.1160$ and $P = 0.5329$, respectively) and no significant interaction term ($P = 0.4736$) at pea full bloom (*data not shown*).

3.3.3.3.5. Tenderometer reading and yield. Priming and seed treatment main effects ($P = 0.3019$ and $P = 0.5760$, respectively) and the interaction term ($P = 0.4111$) were not significant for TR at pea harvest in the Ephrata trial. Similarly, in the Mount Vernon trial, no significant priming and seed treatment main effects ($P = 0.9862$ and $P = 0.7118$, respectively) and no significant interaction term ($P = 0.7008$) was detected for the TR of harvested peas. However, there was a significant main effect of priming ($P < 0.0001$), but no seed treatment main effect, for yield in the Ephrata trial. The mean yield across all the DS treatments and the PS treatments was 13,926 ± 375 and 11,129 ± 505 kg/ha, respectively. A significant interaction term ($P = 0.0440$) showed that the plots with PS + Heads Up or PS + Natural II had significantly reduced yield compared to plots with any other treatments, NTS produced yields equivalent to all other treatments for both DS and PS control plots. Although DS + Myco Seed Treat plots had the greatest yield numerically in the Ephrata trial, this yield was not significantly different than the yield of the PS and DS control plots (Fig. 3.12A and Appendix Table 3.2). In the Mount Vernon trial, yield was not affected significantly by priming or seed treatment main effects ($P = 0.5486$

and $P = 0.5185$, respectively) or by the interaction term ($P = 0.2042$) (Fig. 3.12B and Appendix Table 3.2).

3.3.3.4. Royal City trials

3.3.3.4.1. Emergence. Overall, there was good emergence of pea plants in the field trials located at the two certified organic fields near Royal City in 2012. In the first field (Trial 1), pea emergence was affected significantly by the treatments at 14 dap ($P < 0.0001$) and 28 dap ($P < 0.0001$). Plots planted with PS had significantly greater pea emergence 14 and 28 dap (47 ± 4 and 88 ± 3%, respectively), compared to plots planted with DS (9 ± 2 and 66 ± 1%, respectively). In Trial 1, emergence 14 and 28 dap was greater in plots planted with PS + biochar + Nordox FR (59 ± 4 and 93 ± 3%, respectively) compared to plots with non-treated PS (47 ± 4 and 88 ± 3%, respectively) or PS + biochar (45 ± 6 and 86 ± 2%, respectively), while plots planted with PS + biochar + Nordox HR or PS + biochar had statistically similar emergence to the non-treated PS plots at 14 and 28 dap. Emergence in plots planted with any of the three DS treatments did not differ significantly at 14 and 28 dap (Fig. 3.13A and Appendix Table 3.3).

In the second Royal City field (Trial 2), there was a significant treatment main effect for emergence at 14 and 28 dap ($P < 0.0001$). All treatments with PS did not differ significantly in emergence at 14 dap; and similarly emergence of all three DS treatments did not differ. However, all PS treatments resulted in significantly greater emergence compared to the DS treatments (Fig. 3.13D and Appendix Table 3.3). At 28 dap, plots with PS + biochar + Nordox FR or HR had significantly greater emergence (83 ± 4 and 83 ± 2%) than plots with PS + biochar (73 ± 4%) but not compared to the plots with non-treated PS (81 ± 2%). PS + biochar plots did not differ significantly in emergence compared to plots with DS + Nordox FR. In plots planted

with DS, application of Nordox FR alone or with biochar did not affect emergence compared to non-treated DS (Fig. 3.13D and Appendix Table 3.3).

3.3.3.4.2. Plant height. A significant treatment main effect was detected for both Trial 1 (P = 0.0028) and Trial 2 (P = 0.0410) for plant height measured 35 dap. The plots in Trial 1 planted with PS + biochar + Nordox FR or HR, or PS + biochar had plants that did not differ significantly in height from the plants in the non-treated PS control plots. Similar results were observed for plots planted with DS + Nordox FR, with or without biochar, in which plant height was not improved compared to DS alone. However, the plots planted with non-treated PS had significantly taller plants (9.7 ± 0.4 cm) compared with plots planted with non-treated DS (8.5 ± 0.5 cm), while plants in the plots with PS + biochar did not differ significantly in height compared to plants in control plots with non-treated DS in Trial 1 (Fig. 3.13B and Appendix Table 3.3). Plant height at pea full bloom was not significantly affected by any of the treatments (P = 0.1136).

In Trial 2, the seed treatment main effect was significant (P = 0.0410) for plant height measured 35 dap. Although non-treated PS control plots produced significantly taller plants than the non-treated DS control plots, pea plant height in the former was not significantly different compared to plants in plots with PS + biochar + Nordox FR or HR, PS + biochar, and DS + Nordox FR (Fig. 3.13E and Appendix Table 3.3). Plant height at full bloom did not differ significantly among any of the treatments in Trial 2 (P = 0.1453).

3.3.3.4.3. Root rot severity rating. There were no significant differences in root rot severity rating among treatments in Royal City Trial 1 (P = 0.9931) or Trial 2 (P = 0.2456); however, there was more severe root rot across treatments in Trial 2 (3.0 ± 0.3) compared to Trial 1 (1.5 ± 0.1). Although isolations from symptomatic plants sampled at each site confirmed

the presence of *P. ultimum*, pea plants in Trial 2 also showed severe black roots and stunting that were not typical of *Pythium* infection. Examination of the symptomatic roots revealed the presence of *Thielaviopsis basicola* and the root lesion nematode, *Pratylenchus* sp.

3.3.3.4.4. Shoot dry biomass. Measurement of shoot dry weight at pea full bloom in Royal City Trial 1 showed significant differences among treatments ($P = 0.0008$). In general, pea shoot dry weights in plants planted with DS + biochar + Nordox FR (101 ± 2 g) or non-treated DS control plots (100 ± 3 g) were significantly greater compared to treatments with non-treated PS (85 ± 3 g). Treatment of DS + Nordox FR resulted in shoot dry weights that were not significantly different from that of PS + biochar + Nordox FR or HR, and non-treated PS; while PS + biochar resulted in the least shoot dry weight (81 ± 4 g). In Trial 2, shoot dry weight was not affected by any of the treatments ($P = 0.3499$).

3.3.3.4.4. Tenderometer reading. There was a significant seed treatment main effect on the TR for Trial 1 ($P = 0.0025$) but not for Trial 2 ($P = 0.4547$). In Trial 1, TR of peas harvested from DS and PS control plots did not differ significantly. Combining Nordox (FR or HR) with PS and biochar, or PS with biochar alone resulted in greater TR (92 ± 2, 94 ± 1, and 91 ± 1, respectively) than DS treated with Nordox, with or without biochar (83 ± 1 and 81 ± 4, respectively), but not with DS or PS alone (83 ± 3 and 85 ± 2, respectively). TR in Trial 2 revealed no significant differences among treatments ($P = 0.4547$) (Appendix Table 3.3).

3.3.3.4.5. Yield. The seed treatment main effect was significant in Trial 1 ($P = 0.0076$) and in Trial 2 ($P = 0.0474$) for pea yield. In Trial 1, plots with PS + biochar + Nordox FR or HR, and PS control plots had significantly greater yields (7,034 ± 224, 7,004 ± 1,154, and 6,819 ± 156 kg/ha, respectively) compared to plots planted with DS, and DS + Nordox FR (4,983 ± 208 and 4,583 ± 208 kg/ha, respectively). Yield of plots with DS + biochar + Nordox FR was not

significantly different compared to plots with PS + biochar + Nordox FR or PS + biochar (Fig. 3.13C and Appendix Table 3.3). In Trial 2, yields measured from plots with PS + biochar + Nordox FR and PS control plots were significantly greater (6, 191 ± 444 and 5,697 ± 174 kg/ha, respectively) compared to the yields in plots with DS + biochar + Nordox FR and DS treatments (3,341 ± 622 and 3,611 ± 507 kg/ha, respectively) (Fig. 3.13F and Appendix Table 3.3).

3.4. Discussion

Organic seed and drench treatments were evaluated over two growing seasons in a total of seven organic pea trials and one organic sweet corn trial, in search of effective treatments that growers of organic vegetables can use for managing damping-off in early spring planting conditions. The trials were located at five sites in certified organic grower-cooperator fields in the semi-arid, irrigated, Columbia Basin of central Washington and Oregon, and one research site managed organically in Skagit Co. in the maritime northwestern Washington. The differences in weather, soil properties, cropping systems, and production practices at these sites provided a range of environments for evaluating the treatments against damping-off.

In the 2011 field trials, Nordox seed treatment significantly improved pea stand and yield compared to the NTS control treatments at the Boardman, OR site, where damping-off pressure from *Pythium* spp. and *Rhizoctonia* spp. was severe. In contrast, at the Soap Lake, WA trial, where very little damping-off occurred, Nordox seed treatment did not improve pea stand compared to NTS control plots but did improve pea emergence compared to NTS + WD control plots; furthermore, pea yield was improved significantly in the Nordox plots compared to the yields of NTS control plots and also NTS + WD control plots at this site. Nordox seed treatment did not improve pea stand or yield at the 2011 Mount Vernon, WA trial; even though the plots at this site were inoculated with *P. ultimum* prior to planting and spring conditions were colder and

wetter at this site than the two sites in the Columbia Basin. The incidence of damping-off at this site was negligible.

Nordox, a formulation of cuprous oxide, is included in the OMRI list as a disease management tool, and approved by the USDA NOP (205.601 (2)) (http://www.ecfr.gov/) for use in organic production; however, currently, this product is not registered as an organic seed treatment (http://www.omri.org/). The copper is absorbed by germinating spores of fungi and oomycetes, like *Pythium*, and must be applied to the environment in which the pathogen is present prior to onset of infection in order to be effective (prophylactic) (Stone, 2007). Therefore, if Nordox is to be used for the control of soilborne pathogens causing damping-off, the product is more likely to be effective as a seed treatment than as a foliar treatment. The mode of action of copper involves nonspecific denaturation of cellular proteins of the pathogen (Brown Rosen et al., 2006). Porter (2008), and Porter and Coffman (2007) reported benefits of Nordox seed treatment for managing seed and root rot of processed peas by improving emergence and plant height. Nordox was also effective for late blight control in potato and tomato crops as a foliar treatment (Stone, 2007). However, copper should be used with caution since application at high rates can be phytotoxic, causing stunting in peas (L.D. Porter, *personal communication*). Also, copper is a regulated synthetic material and must be used in a manner that minimizes accumulation in the soil (section 205.601 (2) of the USDA NOP National List) (http://www.ecfr.gov/). Nevertheless, because of the non-biological nature of Nordox, its use shows promise over a wide range of environmental conditions as efficacy is not strongly influenced by conditions such as cool and wet soils that are conducive to damping-off. In contrast, the efficacy of many biological type products can be affected strongly by environmental conditions such as soil temperature (Harman et al., 1981; Lifshitz et al., 1986; Nelson, 1988). For

example, Harman et al. (1981) reported that seed treatment of pea and radish with *Trichoderma hamatum* was effective in controlling seed rot caused by *Pythium* spp. when evaluated in soil temperatures between 17 and 34°C but not in 12 or 35°C. Similarly, the efficacies of some strains of *Enterobacter cloacae* and *Erwinia herbicola*, in controlling Pythium seed rot in cotton, were as effective as metalaxyl when evaluated at soil temperature of 25°C, but were less effective at 15°C (Nelson, 1988).

In the 2011 pea field trials, increased emergence was observed in plots planted with seeds treated with Acadian Marine, Heads Up, Myco Seed Treat, or Natural II in the Soap Lake trial compared to emergence in control plots drenched with water, in which plants performed poorly due to the negative effects of the additional water on pea seed germination. In fact, the pea plants grew poorly in all the plots with drench treatments at this site. The very low incidence of damping-off and lack of root rot symptoms in this trial indicates the decreased emergence was the result of effects of excess moisture on the plants, not soilborne pathogens. At the Mount Vernon site, Acadian Marine, Heads Up, and Prestop WP seed treatments in the pea trial, and Natural II seed treatment in the sweet corn trial improved emergence compared to the inoculated control plots. However, in both the pea and the sweet corn trial, none of the organic seed treatments improved stand as much as Apron XL, the conventional fungicide seed treatment.

Myco Seed Treat is an organic treatment composed of a dry blend of beneficial bacteria and fungi (including mycorrhizae), Natural II is a proprietary formulation of *Streptomyces* sp. that has anti-fungal properties, and Acadian Marine is an extract of brown seaweed (*Aschophyllum nodosum*). These products are classified in the OMRI list as crop management tools and production aids based on the mode of action directed towards plant growth and root health promotion, but not for disease control, even though Myco Seed Treat and Natural II

contain active ingredients with fungicidal properties. Heads Up is derived from the plant *Chenopodium quinoa*, while Prestop WP contains *Gliocladium catenulatum* strain J1446 as the active ingredient. Both are classified as pest and disease control tools in the OMRI list, with labels of each stating efficacy against soilborne pathogens such as *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp.

Cummings et al. (2009) reported efficacy of Natural II for suppression of damping-off in spinach caused by *P. ultimum* and *R. solani*, but only in one of two greenhouse trials for each pathogen. In contrast, no significant improvement was observed in emergence of sweet corn following seed treatment with Natural II compared to NTS in a field trial evaluating the efficacy of various conventional and organic seed treatments against soilborne and seedborne pathogens of sweet corn in the Columbia Basin of Washington (Wohleb, 2010). In a greenhouse study, Prestop WP applied as a drench to spinach resulted in a greater incidence of damping-off in one of the two trials (Cummings et al., 2009). Acadian Marine has been demonstrated to improve seedling emergence and vigor of crops like sugar beets, tomato, and barley (Blunden et al., 1979; Crouch and Van Standen, 1992; Demir et al., 2006; Featonby-Smith and van Staden, 1987). In a more recent study, Rayorath et al. (2008) found that the application of seaweed extract on barley seeds led to the gibberellic acid independent induction of amylase activity, and the authors suggested that amylase could be acting in concert with the other gibberrellic acid-dependent amylase produced in barley, resulting in enhanced germination and seedling vigor. Many of the trials evaluating the above-mentioned products were done under greenhouse conditions. However, when such products were tested under field conditions, inconsistency in efficacy was often observed (Bennett et al., 1991; Cummings et al., 2007a and 2007b; McSpadden Gardener, 2002).

In this study, pea seed priming resulted in improved emergence and yield in the high disease pressure trial in Boardman, and improved emergence in the Mount Vernon sweet corn trial in 2011, demonstrating the potential benefits of this seed treatment for organic production. In contrast, the benefit of seed priming was not evident in the Soap Lake and Mount Vernon pea trials in 2011, both of which had a very low incidence of damping-off. In fact, plots with primed seeds performed poorly at the Mount Vernon pea trial in 2011. There are several potential reasons for the different results observed for the seed priming treatments evaluated in the four field trials in 2011. The different pea cultivars planted in each trial (Bistro, Boogie, and Ice pack in Boardman, Soap Lake, and Mount Vernon pea trials, respectively, based on the grower-cooperators' commercial crops) may have responded differently to the priming protocols used. In addition, the duration of drying following the seed soaking step of the priming protocol, may have affected the primed seeds. A longer air-drying period of 12 h in a fume hood was used in the seed priming protocol in the Soap Lake and Mount Vernon pea trials compared to the 8 h drying period in the Boardman trial, which may have negated the benefits of priming (Schwember and Bradford, 2005).

The priming of pea seeds for reducing germination time and improving seedling emergence has been reported (Farooq et al., 2007, Khan et al., 1978; Sivretepe and Dourado, 1995). However, osmotic priming with polyethylene glycol and/or other priming agents were used in these studies for more controlled imbibition of seeds than soaking the seeds in water. Tyron (1994) pointed out that liquid priming, i.e., hydropriming, is not recommended for all crop species because of the rapid and uncontrolled imbibition of this process, and should be done in small batches and only for high vigor seed lots.

Nevertheless, because of the promising application of priming as a disease management tool for organic production and the well-documented potential benefits of more rapid and uniform seed emergence, priming as an organic seed treatment was evaluated further in pea field trials in 2012, after optimization trials were completed in winter 2011-12 in a growth chamber. The optimization trials demonstrated that primed pea seeds resulted in faster emergence and greater pea dry weight compared to non-primed seeds under growth chamber conditions. The effect of pea seed soaking durations of 8, 16, or 24 h, followed by drying durations of 8, 10, or 12 h was most evident when seeds were planted in soil inoculated with *P. ultimum.* In the inoculated soil, seeds soaked for 16 h resulted in faster and greater emergence compared to seeds soaked for 8 or 24 h. In non-infested soil, greater emergence was observed 14 dap when seeds were soaked for the longer duration (24 h), while seeds soaked for 8 and 16 h did not differ in emergence. By 28 dap, the soaking duration did not affect emergence in non-infested soil.

During seed priming, seeds must be imbibed long enough to achieve sufficient moisture content to accomplish the initial germination process, and then the imbibing must be terminated just prior to radicle protrusion without damaging the seeds (Rajjou et al., 2012). Though drying the imbibed seeds is not a requirement for priming, drying is necessary: (1) when the seeds have to be stored after priming, e.g., if planting is delayed; (2) to facilitate planting because imbibing increases seed size to almost double the size of dry seeds, which can be a problem for mechanical planting; and (3) when a seed treatment is applied after priming, as done in this study in the 2012 field trials. For these reasons, different drying durations after pea seed soaking were investigated in this study. Drying durations evaluated showed that seed soaking for 16 or 24 h resulted in better emergence and greater pea shoot dry weight when the soaking was followed by a shorter drying period of 8 h compared to drying for 10 or 12 h.

The regression analyses for the seed priming optimization trials revealed a positive relationship between seed soaking duration and both emergence and shoot dry weight for each of the drying durations tested (8, 10, and 12 h) in both inoculated and non-inoculated soil. At the 14 dap emergence count, PS dried for 8 or 10 h and planted in the inoculated soil showed a linear increase in emergence with the increase in seed soaking durations, while seeds dried for 12 h and planted in inoculated soil showed a decline in emergence if the seeds had been soaked for 24 h. Seeds air-dried for only 8 h after seed soaking had a greater increase in emergence with the longer soaking durations than a 10 h drying duration. At the final emergence count (28 dap), an increase in emergence was observed for seeds soaked from 8 to 16 h soaking, followed by a decline in emergence for the 24 h soaking period; however, the amount of decrease in emergence from 16 to 24 h soaking was greater if the seeds was dried for 10 or 12 h compared to 8 h. Shoot dry weight of pea plants reflected a similar trend, with greatest dry weights produced when seeds were soaked for 16 h and dried for only 8 h. However, a priming protocol consisting of 16 h followed by 10 h drying was used for the 2012 trials to remove enough moisture from the imbibed seeds to facilitate mechanical planting.

Reisdorph and Koster (1999), investigating the loss of tolerance to drying of pea seeds, showed that after 18 to 24 h of imbibition, pea seeds start to show damage to the radicle when the seed is dried back to the original seed moisture level. This confirmed the earlier results of Koster and Leopold (1988), who demonstrated that if priming necessitates subsequent drying, then imbibition for $\leq$ 18 h is optimum as a longer soaking duration may result in reduced desiccation tolerance of the seeds. Poor seed dehydration conditions, such as drying seeds too fast or at too high a temperature after soaking or imbibing, can negate the benefits of priming (Schwember and Bradford, 2005). Brocklehurst and Dearman (1983) investigated the effects of

drying seeds of carrot, celery, and onion back to the original moisture content following seed soaking. Drying generally delayed germination by 1 to 2 days, depending on the plant species, compared to the primed seeds that did not undergo drying. Also, there was a difference when using fast drying at 30°C, 25% RH, and 4.6 m/s air flow compared to slow drying at 15°C, 50% RH, and 4.6 m/s air flow, where the fast drying treatment delayed germination more than the slow drying. They suggested that drying seeds after priming using non-optimal drying conditions may contribute to the negative effects of drying.

In this study, drying seeds in a fume hood may have caused some damage to the PS. Although the airflow rate in the fume hood was only 1.6 to 1.8 m/s, the air temperature (23 ± 1°C) was higher than that of the slow drying rate used by Brocklehurst and Dearman (1983). While the germination test results showed primed seed germinated faster than non-primed seed, there was overall poorer emergence in plots planted with PS vs. DS in the Ephrata and Mount Vernon pea field trials in 2012. Thus, the drying conditions used may have affected seedling vigor more than germination.

In two of the 2012 pea field trials in this study, the potential benefits of combining organic seed treatments with priming, using a 16 h seed soaking duration followed by 10 h drying for the priming protocol, was investigated vs. applying the treatments to DS for management of damping-off. A blotter germination assay showed that all of the seed treatments applied to primed and dry seeds in the Ephrata and Mount Vernon trials resulted in ≥ 90% germination, with the exception of Natural II applied to primed seeds in the Ephrata trial, which resulted in only 18% germination after 5 days. A significant reduction was also detected in the electrolyte leakage of PS compared to the DS planted in the Ephrata, Mount Vernon, and Royal City trials with EC reduced by 85, 86, and 88%, respectively. Reducing seed exudates produced

after planting the seeds in the soil is a potential approach to reduce the risk of damping-off, since exudates in the spermosphere stimulate propagule germination of many soilborne pathogens such as *Pythium* spp. (Nanayakara, 2001). However, priming combined with organic seed treatments did not result in any benefits to pea emergence, biomass, or yield in the Ephrata or Mount Vernon field trials in 2012. In fact, priming combined with some of the organic seed treatments resulted in reduced stands and yield (e.g., when combined with Heads Up and Natural II in the Ephrata trial, and Nordox in the Mount Vernon trial) compared to the DS treatment combinations. However, priming alone increased emergence in the Mount Vernon trial compared to planting DS, but not in the Ephrata trial. The results demonstrated that, although priming can be beneficial, there may be limitations to using this seed priming for organic pea production or the details still need to be worked out.

There have been reports investigating seed priming combined with other seed treatments, typically with biological agents and bio-priming methods, that document promising results for the control of pre-emergence damping-off caused by *Pythium* spp. (Bennett and Whipps, 2008; Callan et al., 1990; Callan et al., 1991; El-Mohamedy and Abd El-Baky, 2008; Harman and Taylor, 1988). For example, bio-priming pea seeds with *Trichoderma harzianum* or *Bacillus subtilis* resulted in reduced pea root rot during two seasons of field trials, and the treatments improved early vegetative growth of the pea plants, leading to a significant increase in green pod yield (El-Mohamedy and Abd El-Baky, 2008). In contrast, Pill et al. (2001) did not observe a benefit from coating primed cucumber seeds with *Trichoderma harzianum*, *T. viride*, or the combination of the two species for damping-off control compared to non-primed seed coated with the two *Trichoderma* spp., although priming led to faster emergence and greater seedling shoot fresh weight of cucumber.

In contrast to the primed seed treatment combinations tested in the Ephrata and Mount Vernon pea trials in 2012, several of the organic seed treatments applied to DS improved emergence (i.e., Acadian Marine, Heads Up, Myco Seed Treat, and Nordox), but only in the Ephrata trial at 28 dap, and none of the treatments applied to DS resulted in increased pea yield. Neither of these two field trials had high disease pressure, even in the *P. ultimum*-inoculated plots in the Mount Vernon trial, making it difficult to assess the efficacy of the seed treatments for control of damping-off. The two environments of Ephrata and Mount Vernon may have contributed to the differences in performance of the seed treatments at each site, but these sites represent conditions in which organic seed treatments need to be effective for organic vegetable growers in Washington State.

The two priming field trials located in certified organic pea fields near Royal City in 2012, in which biochar was evaluated as an alternative method to air-drying imbibed seeds for the seed priming protocol, demonstrated the potential benefits of seed priming for improving emergence and yield in organic pea crops. Biochar, which is produced by pyrolysis of plant and agricultural wastes (e.g., crop residues, manure, and timber and forestry residues) (Biochar, http://www.biochar-international.org/), is a highly porous charcoal material with high absorption capacity that can function as a desiccant (Chun et al., 2004; Liang et al., 2006). However, this material is classified in the OMRI list as crop fertilizer and soil amendment, and not registered for use as desiccant (http://www.omri.org). The application of biochar did not appear to have any negative effects on the dry or imbibed seeds. However, based on these trials, it is difficult to assess whether biochar application is an effective alternative to air-drying imbibed pea seeds. Additionally, this study did not assess if biochar will facilitate the storage and handling of PS during large-scale field planting by growers, an important issue given the difficulty of applying

biochar to the large volumes of seeds. Further research is needed on this practical aspect of using biochar on a commercial scale for seed priming in agriculture.

In these biochar-priming trials, Nordox was evaluated on both DS and PS. When combined with PS, Nordox seed treatment improved emergence, plant height, and yield of pea plants, at the Royal City trials. These results were very different from results at Ephrata and Mount Vernon where priming was combined with organic seed treatments. These differences may have been associated with the air (Ephrata and Mount Vernon) and biochar (Royal City) drying methods used in the priming protocols.

In conclusion, this study demonstrated the potential efficacy, or lack of efficacy, of a number of organic seed and drench treatments for control of damping-off in a range of environments that reflect growing conditions for organic vegetable production in the semi-arid Columbia Basin of Washington and Oregon, and the maritime northwestern Washington. None of the organic treatments showed consistency in performance in all the field trials and some treatments, such as Actinovate, T-22 HC, Serenade Soil, Soilgard, and Stimplex, had no benefit to organic pea production in any of the three trials in 2011. The cost of these organic seed treatments can be expensive and contribute substantially to production costs; however, organic growers would be willing to justify a cost of up to $100 to $150/acre for a seed or drench treatment that works effectively and consistently for damping-off control by improving stands at least 30% (Alcala and du Toit, 2009). Greater benefit can be obtained by using seed treatments on low vigor seed lots, because seeds of low quality tend to have greater seed leakage (i.e., high EC level), therefore, greater susceptibility to damping-off infection especially if planted in cool and wet soil conditions (Bennett et al., 1991). However, organic growers typically use only high vigor seed lots for early planting, and thus we only evaluated high vigor seed lots in this study..

Nordox seed treatment and seed priming displayed the greatest potential of all the treatments evaluated, especially in field conditions highly conducive to damping-off. Currently, Nordox is not registered for use as a seed treatment in Washington State, but the results of this and other studies (Porter, 2008; Porter and Coffman, 2007) may lead to a seed treatment registration for certified organic production. Priming large-seeded vegetables such as peas can be challenging. This may be a deterrent to application of priming in large scale, highly mechanized, organic vegetable production which entails handling very large volume of seeds. Priming requires precise soaking and drying conditions, which may be difficult to achieve when priming large volumes of seeds. Also, priming large-seeded vegetables requires large volumes of water (1:5 v/v ratio of seeds and water) for imbibition, which may be another difficulty for commercial growers. There is also a need to look at the shelf-life and appropriate storage conditions of primed seeds in case planting is delayed or there is a need to prime seed some period ahead of planting. In addition, the seed coat of PS can be more susceptible to damage due to the higher moisture content and softer seed coat after priming. This has ramifications for using mechanical planters to plant PS, and necessitates further investigation to ensure that imbibed seeds will flow properly and not be damaged during planting. Although the benefits of seed priming for damping-off control are promising, there are still issues to be addressed with regards to its application for large-scale, commercial, organic vegetable production.

3.5. Literature cited

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Table 3.1. Seed and drench treatments evaluated in field trials in 2011 and 2012 at locations in the Columbia Basin of Oregon and Washington, and near Mount Vernon in northwestern Washington, for control of damping-off in organic vegetable crops

	2011				2012[b]			
							Royal City	
Treatment[a]	**Boardman (pea trial)**	**Soap Lake (pea trial)**	**Mount Vernon (pea trial)**	**Mount Vernon (sweet corn trial)**	**Ephrata (pea trial)**	**Mount Vernon (pea trial)**	**Trial 1 (pea trial)[c]**	**Trial 2 (pea trial)[c]**
Acadian Marine Plant Extract	+[d]	+	+	+	+	+		
Acadian Powder						+		
Actinovate STP	+	+	+	+				
Germains' II			+	+				
Heads Up Plant Protectant	+	+	+	+	+	+		
Myco Seed Treat	+	+	+	+	+	+		
Mycostop Mix	+	+	+	+	+	+		
Natural II	+	+	+	+	+	+		
Nordox 75 WG	+	+	+	+	+	+	+[e]	+
Prestop Mix			+	+				
Prestop WP Biofungicide	+		+	+				
PS	+	+	+	+	+	+	+	+
Serenade Soil	+	+	+	+				
Soilgard 12G	+	+	+	+				
Stimplex Crop Biostimulant	+	+	+	+				
T-22 HC	+	+	+	+				
Apron XL			+	+		+		
NTS, NI	+	+	+	+	+	+	+	+
NTS + WD	+	+						
NTS + inoculated soil			+	+		+		
NTS + inoculated soil + WD			+	+				
NTS, NI + soil-oatmeal carrier			+	+		+		

[a] Product details and rates of application evaluated are explained in Table 3.2. Not all products were listed by the Organic Materials Review Institute (OMRI) or approved by Washington State Department of Agriculture (WSDA) for use in certified organic crops at the time of evaluation; therefore, products not registered for certified organic use (Acadian Powder, Germains' II, Prestop Mix, and Apron XL) were evaluated only in the trials at the Mount Vernon site in 2011 (which was managed organically, except for conventional treatments) and 2012. PS = primed seed using the priming protocol of 16 h seed soaking duration followed by 8 h drying for seeds planted in Boardman or 12 h drying for seeds planted in Soap Lake and Mount Vernon in 2011; 10 h drying was

used in the priming protocol for Ephrata and Mount Vernon trials in 2012. Treatments in Italics font were control treatments: Apron XL, a conventional fungicide seed treatment included for comparison with the organic seed treatments; NTS, NI were non-treated seeds planted in non-inoculated plots; NTS + WD were plots planted with NTS and then drenched with water; NTS + inoculated soil were plots inoculated with *Pythium ultimum* isolate 030141 and then planted with NTS; NTS + inoculated soil + WD were plots inoculated with *P. ultimum* isolate 030141and drenched with water after planting with NTS; and NTS, NI + soil-oatmeal carrier were plots applied with the same carrier used to produce the *P. ultimum* inoculum for the inoculated plots, but without the pathogen, and then planted with NTS.

[b] Products evaluated in the 2012 trials were applied to both primed seeds (PS) and dry seeds (DS, non-primed). The control treatments included non-treated PS and DS in all the 2012 trials and Apron XL as a conventional fungicide control treatment in the 2012 Mount Vernon trial.

[c] Biochar was used as the desiccant after pea seed was soaked for 16 h in these two trials; air drying was used following soaking of pea and sweet corn seeds in all the other trials.

[d] + = treatment evaluated in the trial for that site and year.

[e] Nordox 75 WG seed treatment was evaluated at two rates of application in the two Royal City trials in 2012: half rate (HR) of 0.07 g/kg seeds and full rate (FR) of 0.14 g/kg seeds on PS, and FR only on DS.

Table 3.2. Seed and drench treatments evaluated in field trials in Washington and Oregon in 2011 and 2012 for efficacy against soilborne pathogens causing damping-off in organic pea and sweet corn crops

Treatment[a]	Active ingredient (concentration in product)	Registrant or manufacturer	Rate of application[b]	Method of application	OMRI list[c]	WSDA list[d]	Mode of action
MICROBIAL							
Actinovate STP	*Streptomyces lydicus* WYEC108 (0.33%)	Natural Industries, Inc., Houston, TX	2.5 g/kg seeds	Seed treatment	Yes	Yes	Seed and root colonizer, protects against soilborne pathogens
Germains' II	Organic disinfectant + *Trichoderma harzianum* strain T-22	Germain's Technology Group, Gilroy, CA	Proprietary	Seed treatment	No	No	Unknown
Myco Seed Treat	Bacteria, fungi, and mycorrhizae	AgriEnergy Resources, Princeton, IL	4.9 g/kg seeds	Seed treatment	Yes	No	Root colonizers, promote soil nutrient cycling
Mycostop Mix Biofungicide	*Streptomyces griseoviridis* strain K61 (4%)	Verdera Oy, Espo, Finland	4.9 g/kg seeds	Seed treatment	Yes	No	Root colonizer, hyperparasitism, produces inhibitory metabolites
Natural II Film Coat	*Streptomyces* sp.	Agricoat LLC, Soledad, CA	Proprietary	Seed treatment	Yes	Yes	Unknown
Prestop Mix	*Gliocladium catenulatum* strain J1446 (32%)	Verdera Oy, Espo, Finland	5 g/kg seeds	Seed treatment	No	No	Colonizes rhizosphere
Prestop WP Biofungicide	*Gliocladium catenulatum* strain J1446 (32%)	Verdera Oy Espo, Finland,	0.40 liters/38 liters water/9 m^2	Spray	Yes		Colonizes rhizosphere
Serenade Soil	*Bacillus subtilis* strain QST 713 (1.34%)	AgraQuest, Inc., Davis, CA.	5.8 liters/1.12 ha (in appropriate amount of water)	Drench	Yes	Yes	Colonizes roots, antagonism, induces systemic resistance
SoilGard 12G	*Gliocladium virens* strain GL-21 (12%)	Certis USA, Columbia, MD	2.25 kg/1.12 ha (in 190 liters water/1.12 ha)	Drench	Yes	No	Antagonism
T-22 HC	*T. harzianum* Rifai strain KRL-AG2 (1.15%)	BioWorks, Inc., Victor, NY	1.2 g/kg seeds	Seed treatment	Yes	Yes	Colonizes roots, mycoparasitism
NON-MICROBIAL							
Acadian Marine Plant Extract	Extract from *Ascophyllum nodosum*	Acadian Seaplant Ltd., Dartmouth, Canada	5 ml/kg seeds	Seed treatment	Yes	Yes	Growth enhancer

Table 3.2. *continued….*

Treatment[a]	Active ingredient (concentration in product)	Registrant or manufacturer	Rate of application[b]	Method of application	OMRI list[c]	WSDA list[d]	Mode of action
Acadian Powder	Extract from *Ascophyllum nodosum*	Acadian Seaplant Ltd., Dartmouth, Canada	5 g/kg seeds	Seed treatment	No	No	Growth enhancer
Heads Up Plant Protectant	*Chenopodium quinoa* (49.65%)	HeadsUp Plant Protectant, Inc., Kamsack, Canada	0.3 g/liter water/ 170 kg seeds	Seed treatment	Yes	No	Signaling defense pathway
Nordox 75 WG	Cuprous oxide (83.9%)	Nordox AS, Oslo, Norway	0.14 g/kg seeds	Seed treatment	Yes	No	Fungicidal
Primed seed	Water	None	Different durations of soaking and drying seeds	Seed soak	Yes	Yes	Increase rate of seed germination/emergence, reduce seed leakage
Stimplex Crop Biostimulant	Cytokinin (as kinetin) (0.01%)	Acadian Seaplant Ltd., Dartmouth, Canada	1.4 liters/0.4 ha	Drench	Yes		Plant growth regulator
CONTROL							
Apron XL	Mefenoxam	Syngenta Crop Protection, Greensboro, NC	22 ml/kg seeds	Seed treatment	No	No	Fungicidal
NTS, NI[e]	None						
NTS + WD[f]	None						
NTS, inoculated soil[g]	None						
NTS, inoculated soil + WD[h]	None						
NTS, NI + carrier[i]	None						

[a] Refer to Table 3.1 for details of the eight trials in which the treatments were evaluated in 2011 and 2012. Treatments categorized as microbial has microorganisms such as fungi and/or bacteria in the active ingredient, while non-microbial treatments do not contain microorganisms in the active ingredient.

[b] Each product was evaluated at the highest recommended label rate for peas or sweet corn, or as recommended by the registrant. Drenches and the spray application were done according to the label based on the soil surface area or volume of the plot. Refer to Appendix Tables 3.1, 3.2, and 3.3. for details of methods of application.

[c] Organic Materials Review Institute list (2010-12) (http://www.omri.org/omri-lists). Products not OMRI listed (Acadian Powder, Germains' II, Prestop Mix, and Apron XL) were evaluated only in the trials at the Mount Vernon site in 2011-12 (managed organically except for conventional treatments), because all the other trials were in grower-cooperator fields which were certified organic.

[d] Washington State Department of Agriculture list (2010-12) of organic products approved for use in Washington State in certified organic crops. Products not WSDA listed (Acadian Powder, Germains' II, Prestop Mix, and Apron XL) were evaluated only in the trials at the Mount Vernon site in 2011-12 (managed organically but not certified), because all the other trials were in grower-cooperator fields which were certified organic.

[e] NTS, NI = non-treated seeds planted in non-inoculated plots

[f] NTS + WD = non-treated seeds planted in plots that were drenched with water after planting.

[g] NTS, inoculated soil = non-treated seeds planted in plots that were inoculated with *Pythium ultimum* isolate 030141 prior to planting.

[h] NTS, inoculated soil + WD = non-treated seeds planted in plots that were inoculated with *P. ultimum* isolate 030141 prior to planting and drenched with water after planting.

[i] NTS, NI + carrier = non-treated seeds planted in plots that were applied with the same carrier used to produce *Pythium* inoculum for the inoculated plots, but without the pathogen, prior to planting.

Table 3.3. Soil nutrient analyses for organic pea and sweet corn field trials evaluating seed treatments for control of damping-off in Washington and Oregon in 2011 and 2012

	Macronutrients			Micronutrients										
Field trials[a]	NO_3 (ppm)	P (ppm)	K (ppm)	B (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	Fe (ppm)	Ca (meq/100 g)	Mg (meq/100 g)	Na (meq/100 g)	CEC (meq/100 g)[b]	pH	OM (%)[c]
2011														
Boardman	35	70	483	0.34	3.5	1.5	3.9	15	6.5	1.5	0.15	10	7.1	1.4
Mount Vernon	10	66	204	0.40	2.4	3.0	3.6	77	6.5	0.8	0.06	9	6.5	3.7
Soap Lake	8	16	484	0.19	0.6	1.3	1.4	14	6.1	2.5	0.15	13	7.1	1.0
2012														
Ephrata	54	39	153	0.10	3.2	1.4	1.0	38	6.2	1.8	0.21	-	6.6	1.0
Mount Vernon	2	53	247	0.50	2.2	2.0	3.0	52	8.0	1.2	0.13	12	5.8	4.0
Royal City Trial 1	39	22	275	0.25	2.2	2.7	0.9	13	6.0	2.8	0.19	-	7.3	0.9
Royal City Trial 2	69	26	266	0.45	1.9	2.0	0.5	6	12.8	2.2	0.19	-	7.8	1.4

[a] Soil samples were collected using a 2.5 cm-diameter soil probe to a depth of 15.2 cm for two cores/plot. The soil cores were pooled by replicate (block) within a trial, mixed thoroughly, and a subsample sent to Soiltest Farm Consultants, Inc. (Moses Lake, WA) for nutrient analysis. Soil sampling was done immediately after planting each of the trial sites in 2011 and 2012.

[b] CEC = cation exchange capacity measured in meq/100 g.

[c] OM = organic matter (%).

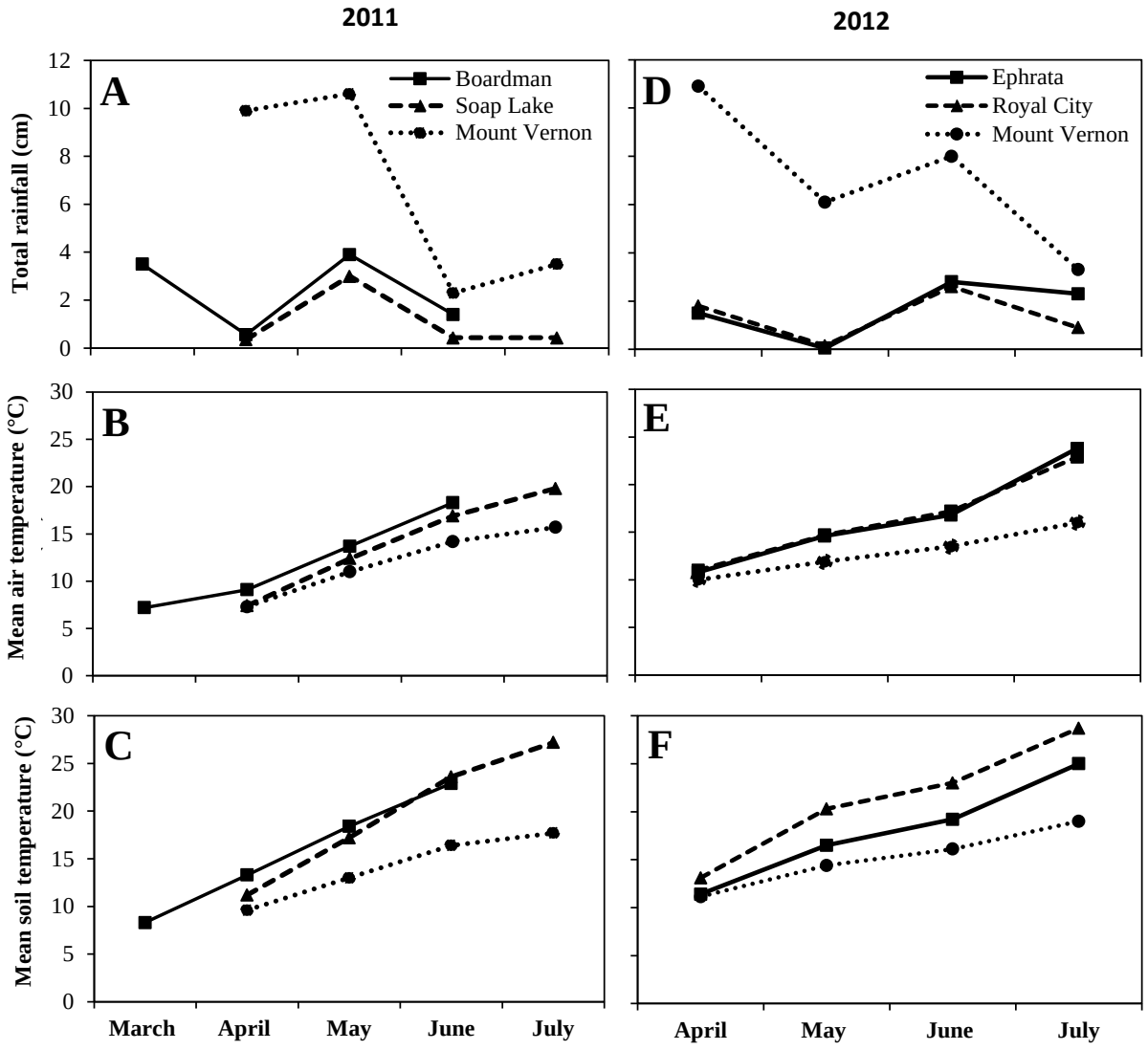

Fig. 3.1. Monthly weather data for eight field trial sites in Washington and Oregon in 2011 and 2012 where seed and drench treatments were evaluated for the control of damping-off in organic pea and sweet corn crops. A pea trial and a sweet corn trial were at the Mount Vernon, WA site in 2011, and another pea trial at this site in 2012. Two pea trials were located in separate fields near Royal City, WA in 2012. The remaining three sites (Boardman, OR; Ephrata, WA; and Soap Lake, WA) had a single pea trial each. Weather data were obtained from AgWeatherNet site of Washington State University (www.wsu.agweather.net).

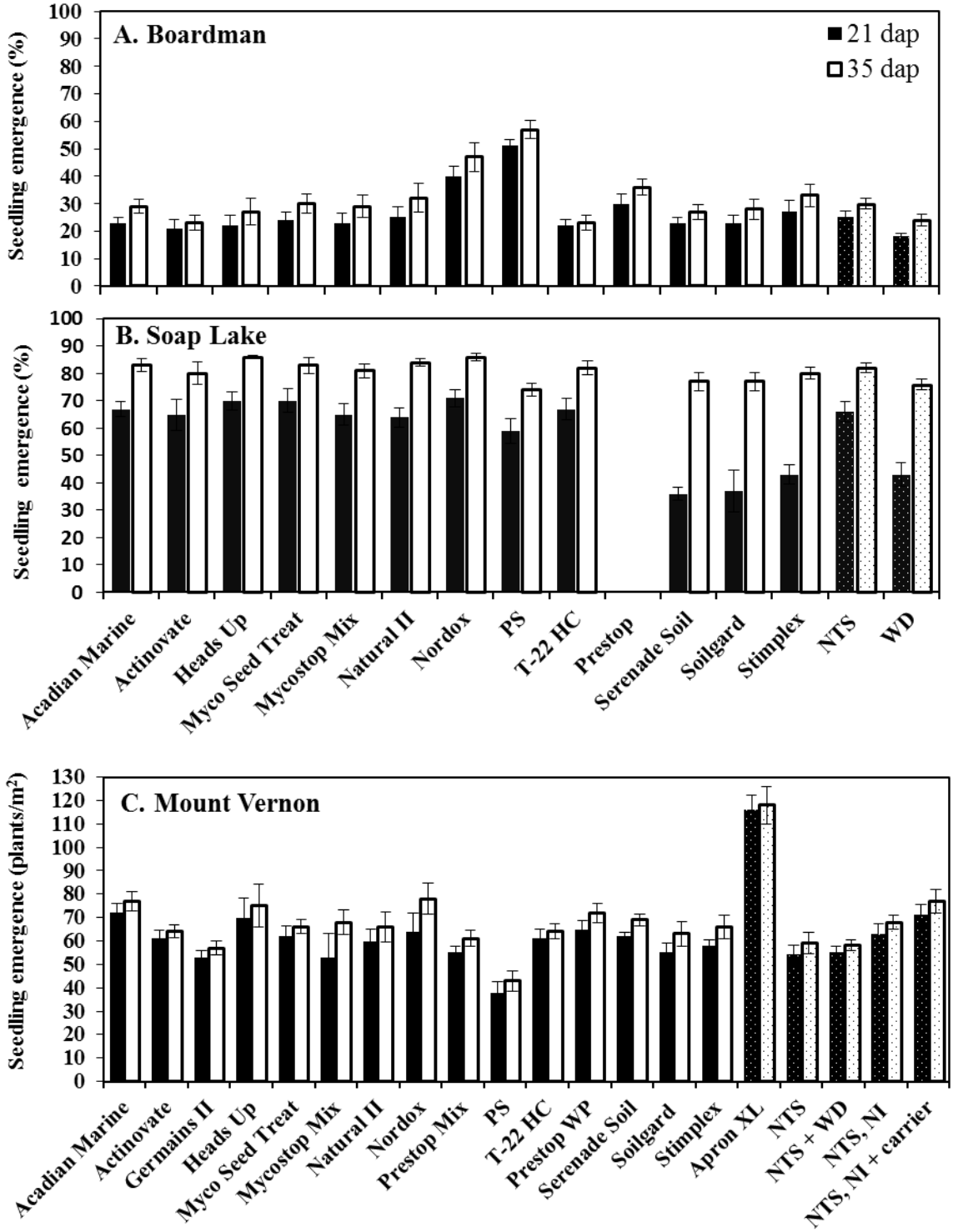
A. Boardman
21 dap
35 dap
Seedling emergence (%)
B. Soap Lake
Seedling emergence (%)
Acadian Marine
Actinovate
Heads Up
Myco Seed Treat
Mycostop Mix
Natural II
Nordox
PS
T-22 HC
Prestop
Serenade Soil
Soilgard
Stimplex
NTS
WD
C. Mount Vernon
Seedling emergence (plants/m²)
Acadian Marine
Actinovate
Germains II
Heads Up
Myco Seed Treat
Mycostop Mix
Natural II
Nordox
Prestop Mix
PS
T-22 HC
Prestop WP
Serenade Soil
Soilgard
Stimplex
Apron XL
NTS
NTS + WD
NTS, NI
NTS, NI + carrier

Fig. 3.2. Mean seedling emergence 21 and 35 days after planting (dap) for three field trials in Oregon (Boardman, A) and Washington (Soap Lake, B, and Mount Vernon, C) in 2011 evaluating organic seed and drench treatments for the control of damping-off in organic pea crops. Emergence (stand counts) for the cvs. Bistro (A) and Boogie (B) were calculated as a percentage based on a total of 230 seeds planted in the two center rows/plot. The Boardman (A) and Soap Lake (B) field trials were in certified organic, grower-cooperator fields, and products evaluated at these sites were listed by the Organic Materials Review Institute (OMRI) and the Washington State Department of Agriculture in 2011. Due to the use of a belt planter in this trial, emergence for the pea cv. Ice pack (C) was calculated based on the total number of seedlings in a 1 m^2 area in the center of each plot because the seeds were not distributed equally in each of the 6 rows. The plots at Mount Vernon (C) were inoculated with *Pythium ultimum* isolate 030141, except for the non-inoculated (NI) control plots. All control plots were planted with non-treated seeds (NTS). Treatments in Mount Vernon (C) included two products that were not OMRI listed in 2011 (Germain's II and Prestop Mix) and a conventional fungicide seed treatment, Apron XL (Mefenoxam). The first nine treatments listed in the Boardman (A) and Soap Lake (B) trials were each applied as seed treatment and the succeeding four were each applied as a drench (or as a spray for Prestop WP). The first eleven treatments listed in the Mount Vernon (C) trial were each applied as seed treatments and the succeeding four each were applied as drench (or spray for Prestop WP). The dotted bars represent the control treatments. Each bar represents the mean and standard error for four (A) or five (B and C) replicates. The treatment details are described in Table 3.2. PS = primed seed, NTS = non-treated seeds, WD = water drench, and NI = non-inoculated plots.

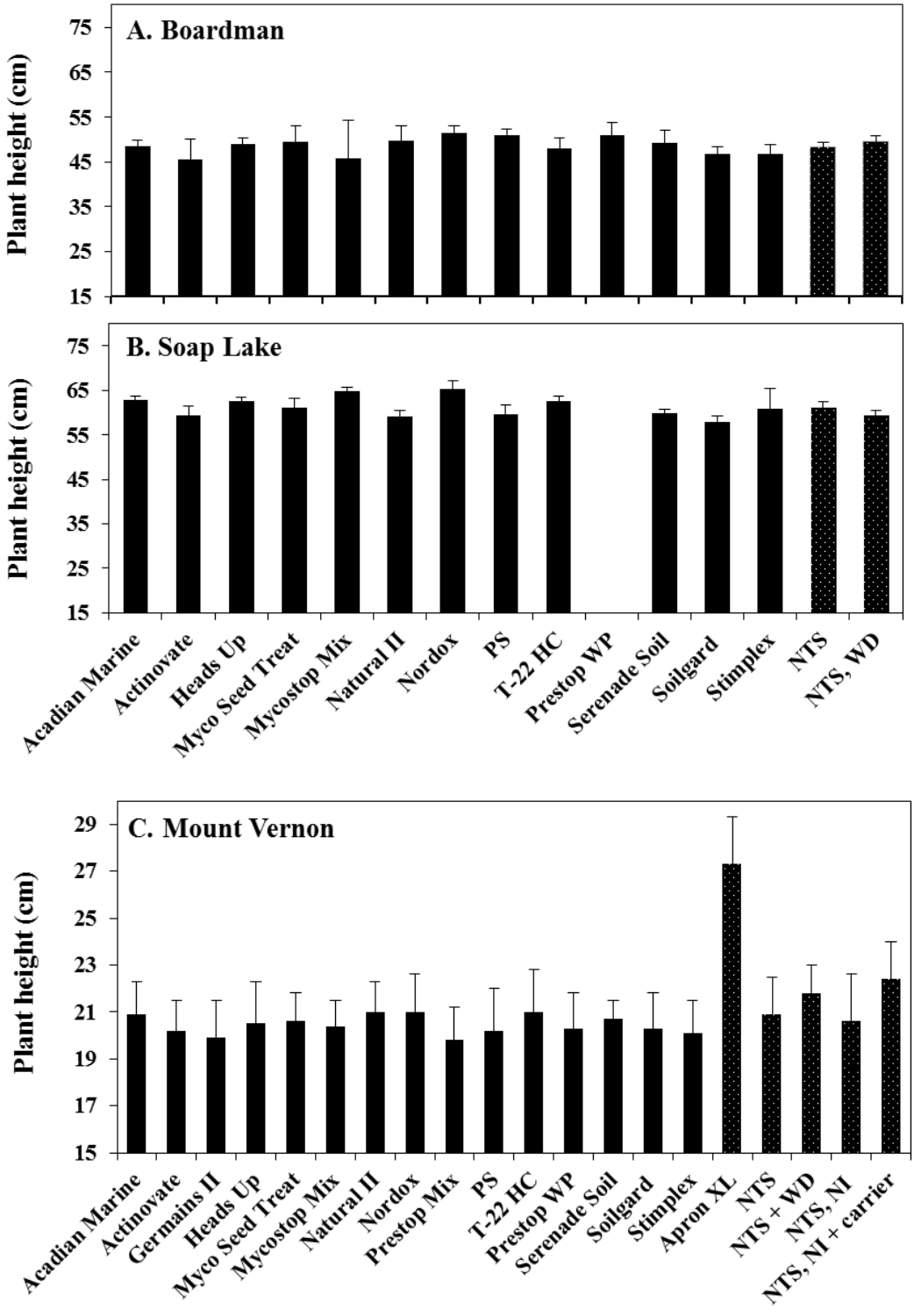
A. Boardman
B. Soap Lake
C. Mount Vernon
Plant height (cm)
Acadian Marine
Actinovate
Germains II
Heads Up
Myco Seed Treat
Mycostop Mix
Natural II
Nordox
Prestop Mix
PS
T-22 HC
Prestop WP
Serenade Soil
Soilgard
Stimplex
Apron XL
NTS
NTS + WD
NTS, NI
NTS, NI + carrier
NTS, WD

Fig. 3.3. Mean plant height (cm) of 16 plants/plot measured at full bloom at three field trials in Oregon (A) and Washington (B and C) in 2011 to evaluate organic seed and drench treatments for control of damping-off in organic pea crops. The pea cvs. Bistro (A) and Boogie (B) were planted in certified organic, grower-cooperator fields in Boardman (A) and Soap Lake (B). Products evaluated at these sites were listed by the Organic Materials Review Institute (OMRI) and the Washington State Department of Agriculture (WSDA) in 2011. The pea cv. Ice pack was planted in Mount Vernon (C) where plots were inoculated with of *Pythium ultimum* isolate 030141, except for the non-inoculated (NI) control plots. All control plots were planted with non-treated seeds (NTS). Treatments in Mount Vernon (C) included two products that were not OMRI listed in 2011 (Germain's II and Prestop Mix) and the conventional fungicide seed treatment, Apron XL (Mefenoxam). The first nine treatments listed in the Boardman (A) and Soap Lake (B) trials were each applied as seed treatment and the succeeding four treatments were each applied as a drench (or as a spray for Prestop WP). The first eleven treatments listed in the Mount Vernon (C) trial were each applied as seed treatments and the succeeding four treatments were applied as a drench (or spray for Prestop WP). The dotted bars represent the control treatments. Each bar represents the mean and standard error with four (A) and five (B and C) replicate plots. Details of each treatment are described in Table 3.2. PS = primed seed, and WD = water drench control.

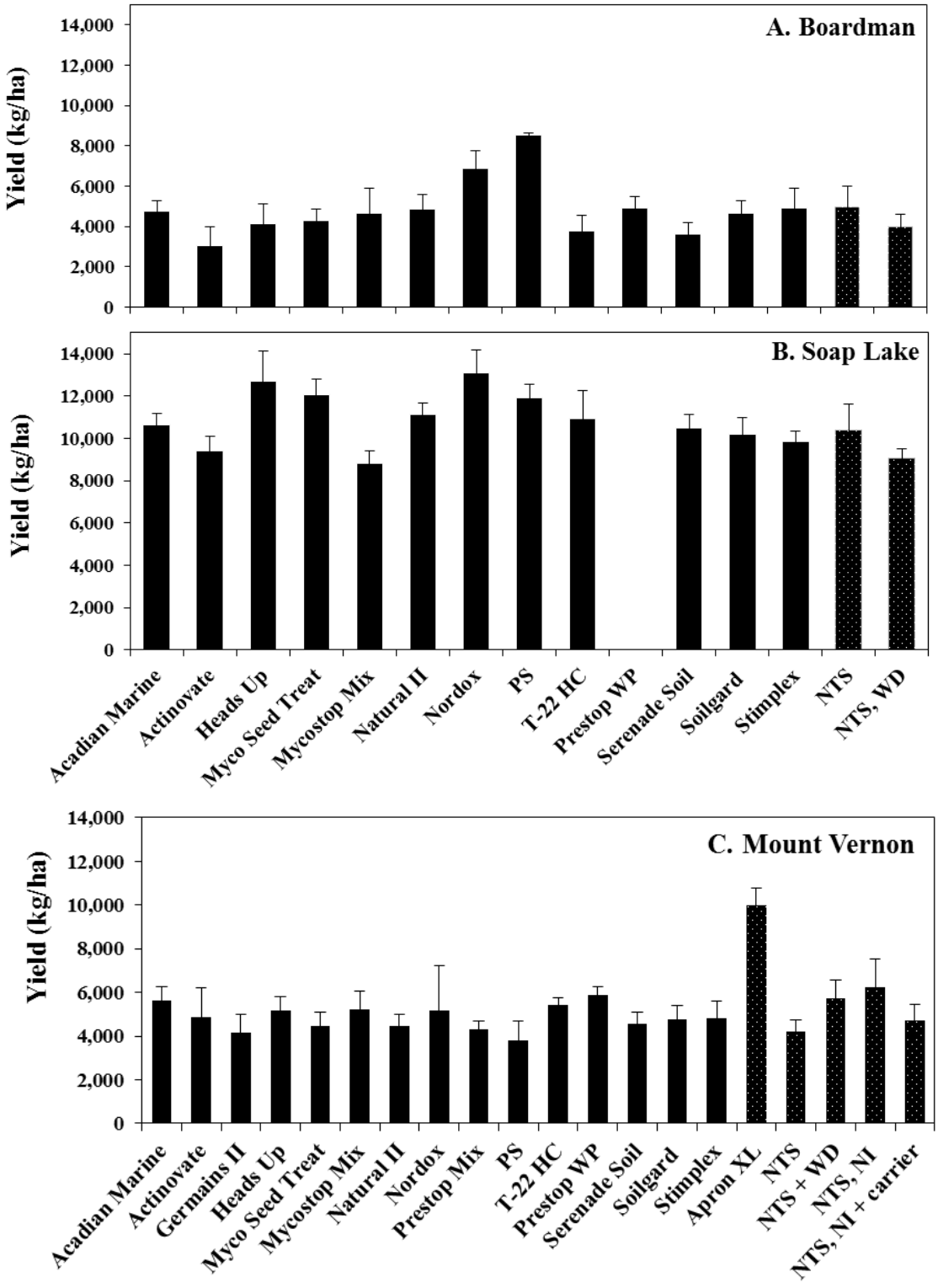
A. Boardman
Yield (kg/ha)
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
B. Soap Lake
Yield (kg/ha)
Acadian Marine
Actinovate
Heads Up
Myco Seed Treat
Mycostop Mix
Natural II
Nordox
PS
T-22 HC
Prestop WP
Serenade Soil
Soilgard
Stimplex
NTS
NTS, WD
C. Mount Vernon
Yield (kg/ha)
Acadian Marine
Actinovate
Germains II
Heads Up
Myco Seed Treat
Mycostop Mix
Natural II
Nordox
Prestop Mix
PS
T-22 HC
Prestop WP
Serenade Soil
Soilgard
Stimplex
Apron XL
NTS
NTS + WD
NTS, NI
NTS, NI + carrier

Fig. 3.4. Mean yield (kg/ha) of 1 m of each of the four middle rows/plot in field trials at three sites in Oregon (A) and Washington (B and C) in 2011 to evaluate organic seed and drench treatments for control of damping-off in organic pea crops. The pea cvs. Bistro (A) and Boogie (B) were planted in certified organic, grower-cooperator fields in Boardman, WA (A) and Soap Lake, WA (B). Products evaluated at these sites were listed by the Organic Materials Review Institute (OMRI) and the Washington State Department of Agriculture (WSDA) in 2011. The pea cv. Ice pack was planted in Mount Vernon (C) where plots were inoculated with of *Pythium ultimum* isolate 030141, except for the non-inoculated (NI) control plots. All control plots were planted with the non-treated seeds (NTS). Treatments in Mount Vernon included two products that were not OMRI listed in 2011 (Germain's II and Prestop Mix) and a conventional fungicide seed treatment, Apron XL (Mefenoxam). The first nine treatments listed in the Boardman (A) and Soap Lake (B) trials were each applied as seed treatment and the succeeding four were each applied as a drench (or as a spray for Prestop WP). The first eleven treatments listed in the Mount Vernon trial (C) were each applied as seed treatments and the succeeding four were each applied as drench (or spray for Prestop WP). The dotted bars represent the control treatments. Each bar represents the mean and standard error with four (A) and five (B and C) replicate plots. Details of each treatment are described in Table 3.2. PS = primed seed, and WD = water drench control.

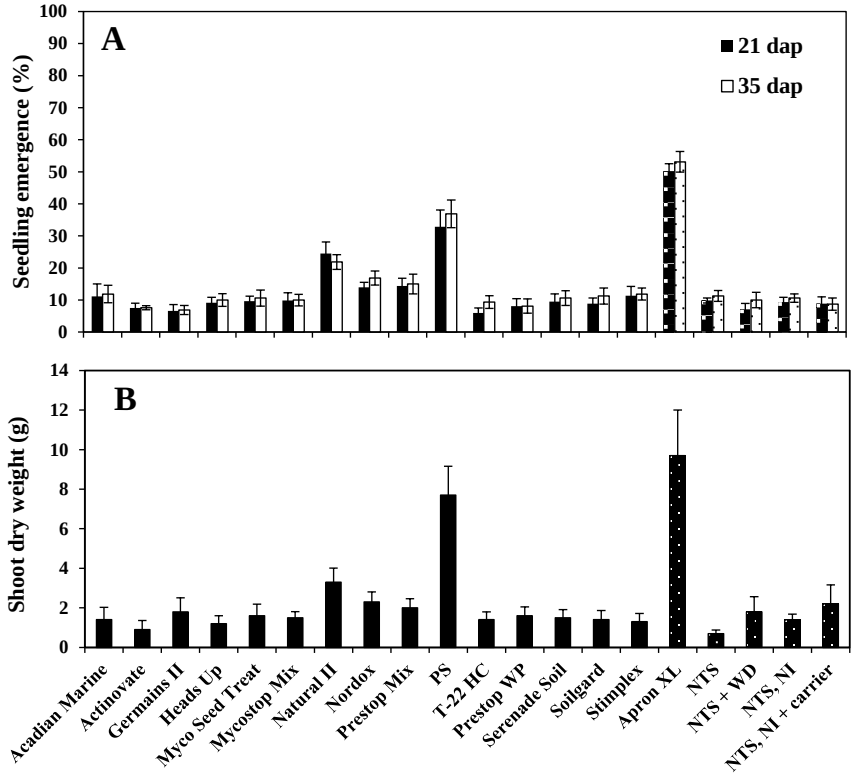

Fig. 3.5. Sweet corn cv. Bodacious grown in a field trial in 2011 in Mount Vernon, WA to evaluate organic seed and drench treatments for damping-off control. Percent seedling emergence (A) was calculated based on 9 seeds/m^2, measured 21 and 35 days after planting (dap). Mean shoot dry weight (B) was measured from plants sampled from the center 3 m of each of the two center rows/plot 35 dap. Plots were inoculated with *Pythium ultimum* isolate 030141, except for the non-inoculated (NI) control plots. All the control plots (dotted bars) were planted with non-treated seeds (NTS). Products evaluated were on the Organic Materials Review Institute and Washington State Department of Agriculture approved list for certified organic production in 2011, except for Germain's II, Prestop Mix, and a conventional fungicide seed treatment, Apron XL. Each bar represents the mean and standard error of five replicate plots. Details of each treatment are shown in Table 3.2. PS = primed seeds and WD = water drench.

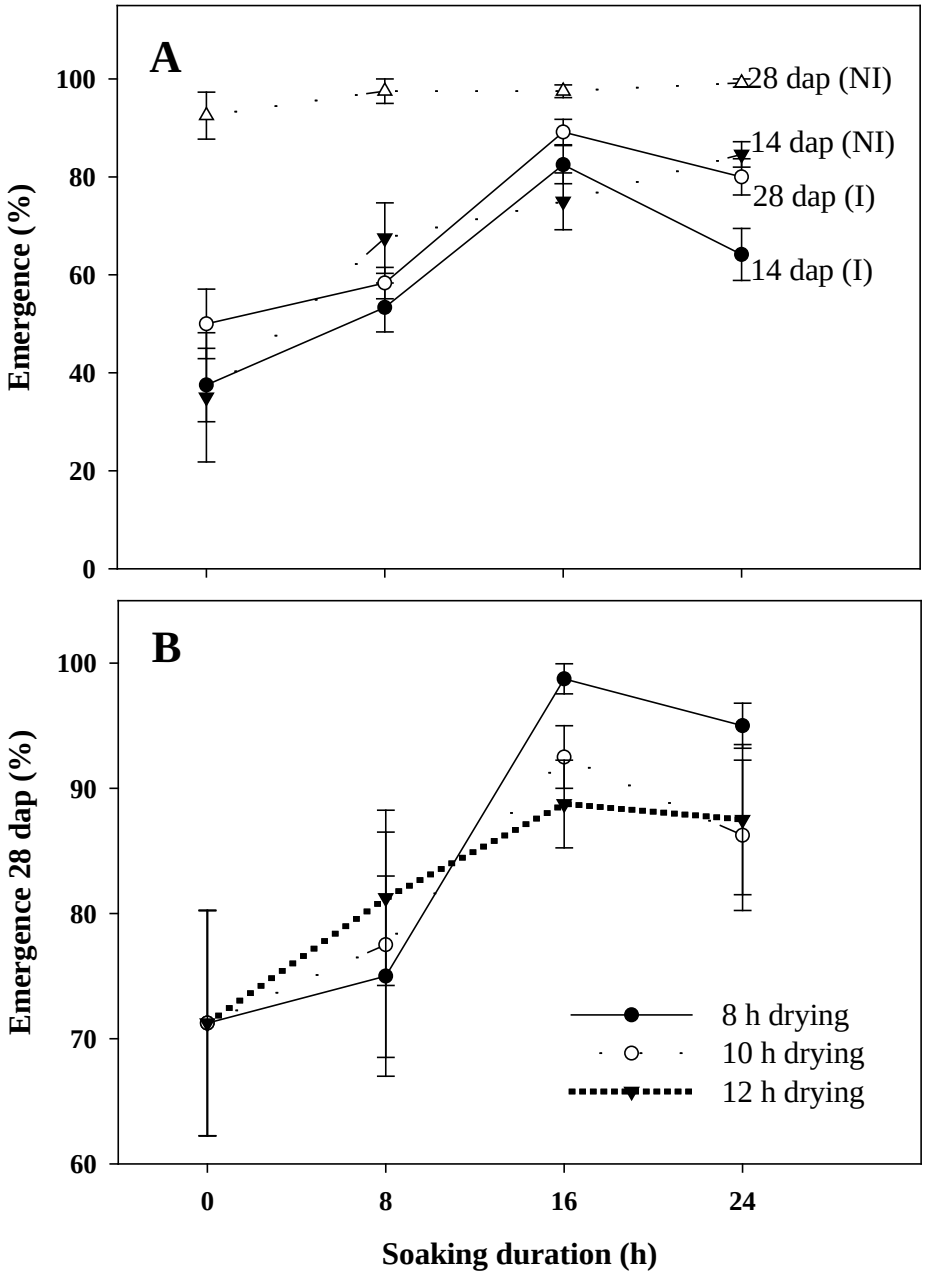

Fig. 3.6. Results of the growth chamber trials on optimization of priming seed of the pea cv. Boogie by evaluating seed soaking durations of 8, 16, and 24 h followed by seed air-drying durations of 8, 10, or 12 h in a fume hood at room temperature (23 ± 1°C). Primed seeds were then planted in soil

inoculated (I) with *Pythium ultimum* isolate 030141, or non-inoculated soil (NI). (A) Emergence (%) of pea seed planted in inoculated and non-inoculated soil14 and 28 days after planting (dap). (B) Emergence (28 dap) of seed soaked and dried for different durations. Non-primed seeds (0 soaking and 0 drying) served as the control treatment. Each data point is the mean ± standard error of four replicate pots/treatment.

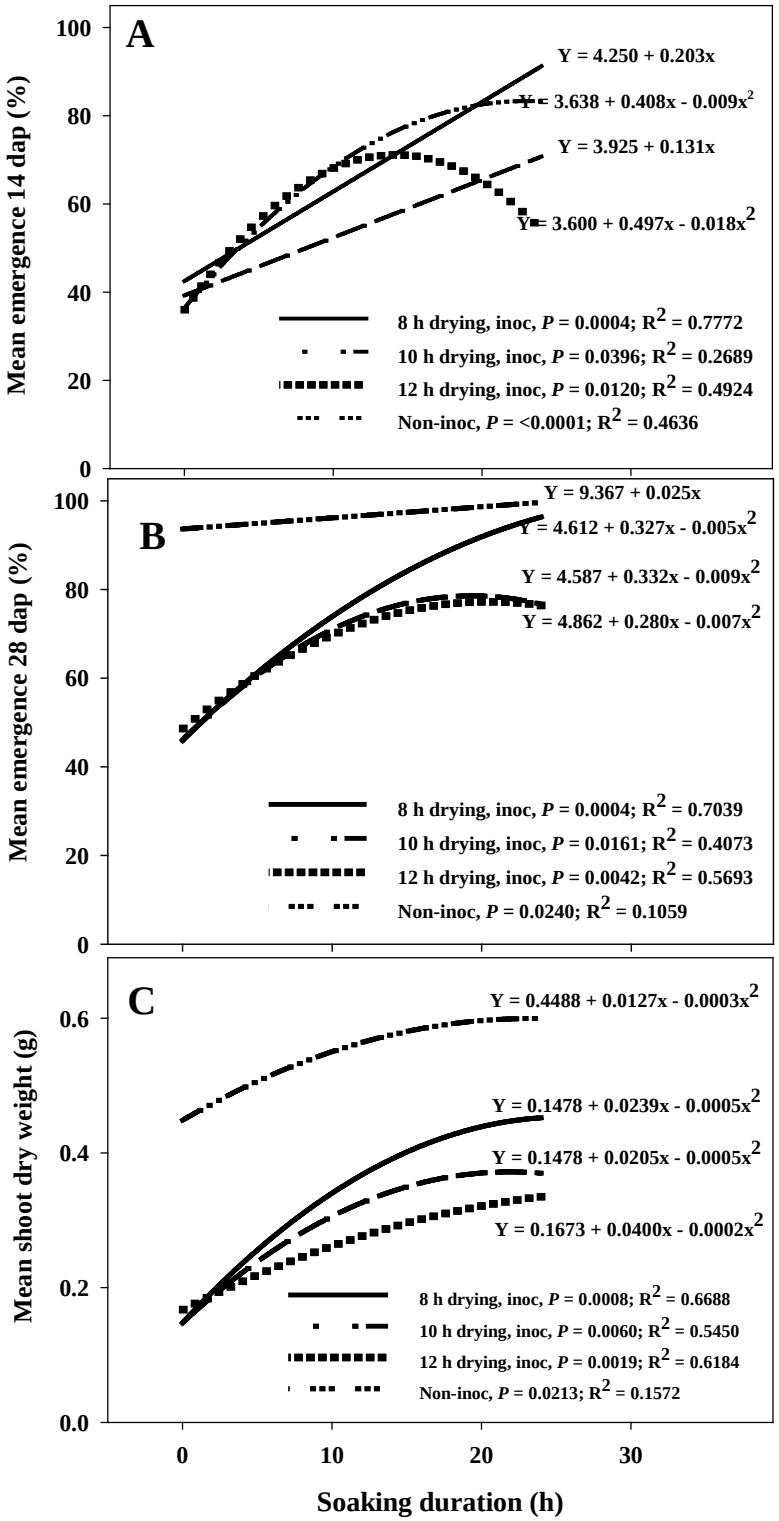
A
Y = 4.250 + 0.203x
Y = 3.638 + 0.408x - 0.009x2
Y = 3.925 + 0.131x
Y = 3.600 + 0.497x - 0.018x2
8 h drying, inoc, P = 0.0004; R2 = 0.7772
10 h drying, inoc, P = 0.0396; R2 = 0.2689
12 h drying, inoc, P = 0.0120; R2 = 0.4924
Non-inoc, P = <0.0001; R2 = 0.4636
Mean emergence 14 dap (%)
B
Y = 9.367 + 0.025x
Y = 4.612 + 0.327x - 0.005x2
Y = 4.587 + 0.332x - 0.009x2
Y = 4.862 + 0.280x - 0.007x2
8 h drying, inoc, P = 0.0004; R2 = 0.7039
10 h drying, inoc, P = 0.0161; R2 = 0.4073
12 h drying, inoc, P = 0.0042; R2 = 0.5693
Non-inoc, P = 0.0240; R2 = 0.1059
Mean emergence 28 dap (%)
C
Y = 0.4488 + 0.0127x - 0.0003x2
Y = 0.1478 + 0.0239x - 0.0005x2
Y = 0.1478 + 0.0205x - 0.0005x2
Y = 0.1673 + 0.0400x - 0.0002x2
8 h drying, inoc, P = 0.0008; R2 = 0.6688
10 h drying, inoc, P = 0.0060; R2 = 0.5450
12 h drying, inoc, P = 0.0019; R2 = 0.6184
Non-inoc, P = 0.0213; R2 = 0.1572
Mean shoot dry weight (g)
Soaking duration (h)

Fig. 3.7. Regression analyses showing the relationship of pea seed soaking durations of 8, 16, and 24 h followed by drying durations of 8, 10, and 12 h on emergence of pea seedlings measured 14 (A) and 28 (B) days after planting (dap), and shoot dry weight (C) when the pea seeds were planted in soil inoculated with *Pythium ultimum* isolate 030141 (inoc) or in non-inoculated soil (non-inoc). Each of the regression relationship was significant ($P < 0.05$). R^2 = coefficient of determination for the proportion of total variation accounted for by the regression model. In non-inoculated soil, there was no significant effect of seed drying duration, so results are pooled across the drying durations tested. Each regression equation was developed using Sigmaplot Version 11 (Systat Software, Inc., San Jose, CA) with four replicate pots planted per treatment combination.

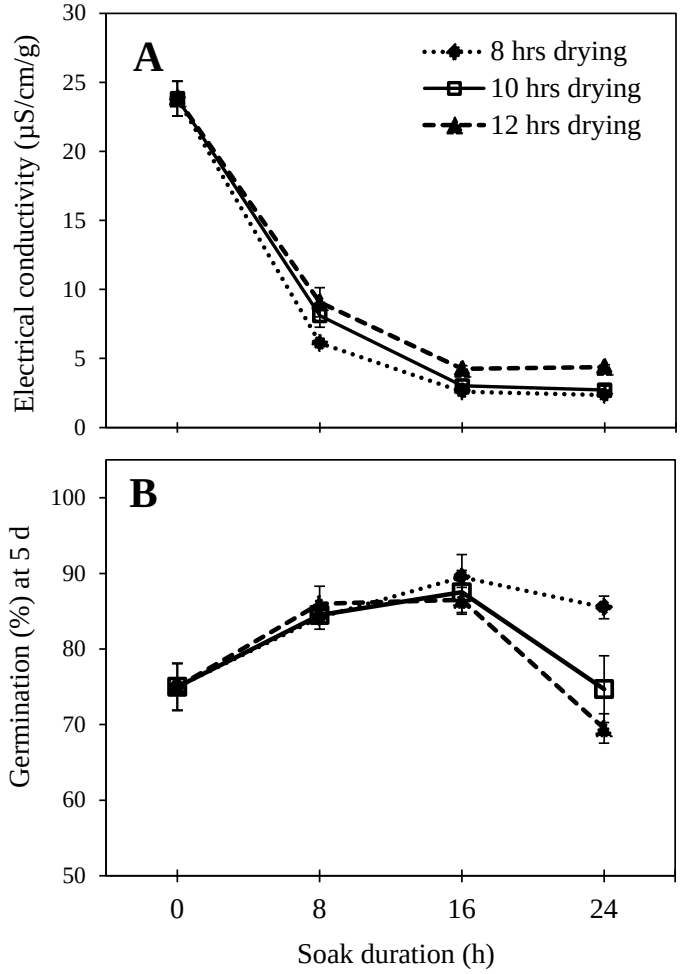

Fig. 3.8. Results of a growth chamber trial to evaluate priming conditions for seed of the pea cv. Boogie using soaking durations of 8, 16, or 24 h followed by drying durations of 8, 10, or 12 h. Mean electrical conductivity (EC) readings (A) for seed samples subjected to each priming protocol were measured following the protocol of the Processors and Growers Research Organization (1981), and mean seed germination (B) measured at 5 days using the protocol of the Association of Official Seeds Analysts (2008) for pea seeds. Each data point represents the mean ± standard error of four replicate seed samples/treatment.

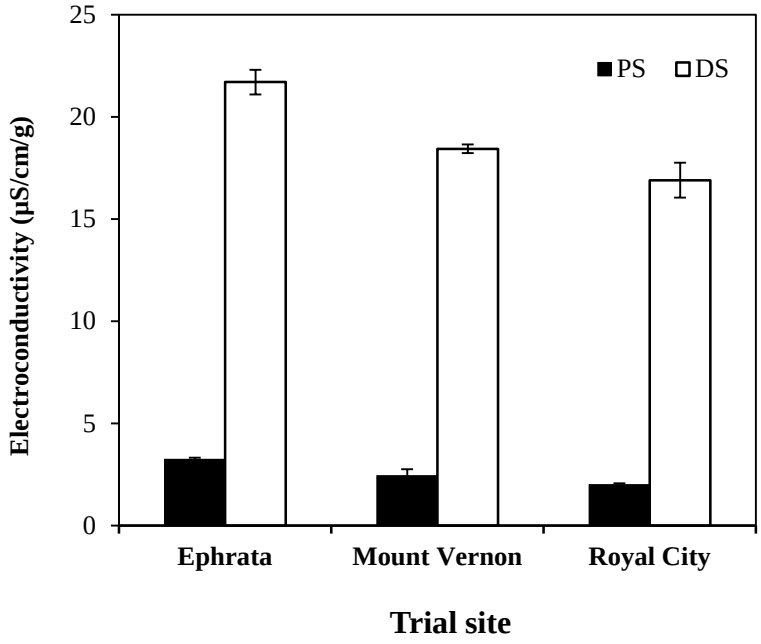

Fig. 3.9. Electrical conductivity (EC) measured for primed seeds (PS) and dry seeds (DS) of the pea cv. Boogie (planted in Ephrata and Mount Vernon) or Gallant (planted in Royal City) before application of organic seed treatments for evaluation at the three trial sites in Washington in 2012. The EC test was done following the protocol of the Processors and Growers Research Organization (1981) for peas. Each bar represents the mean ± standard error with four replicate seed samples/treatment.

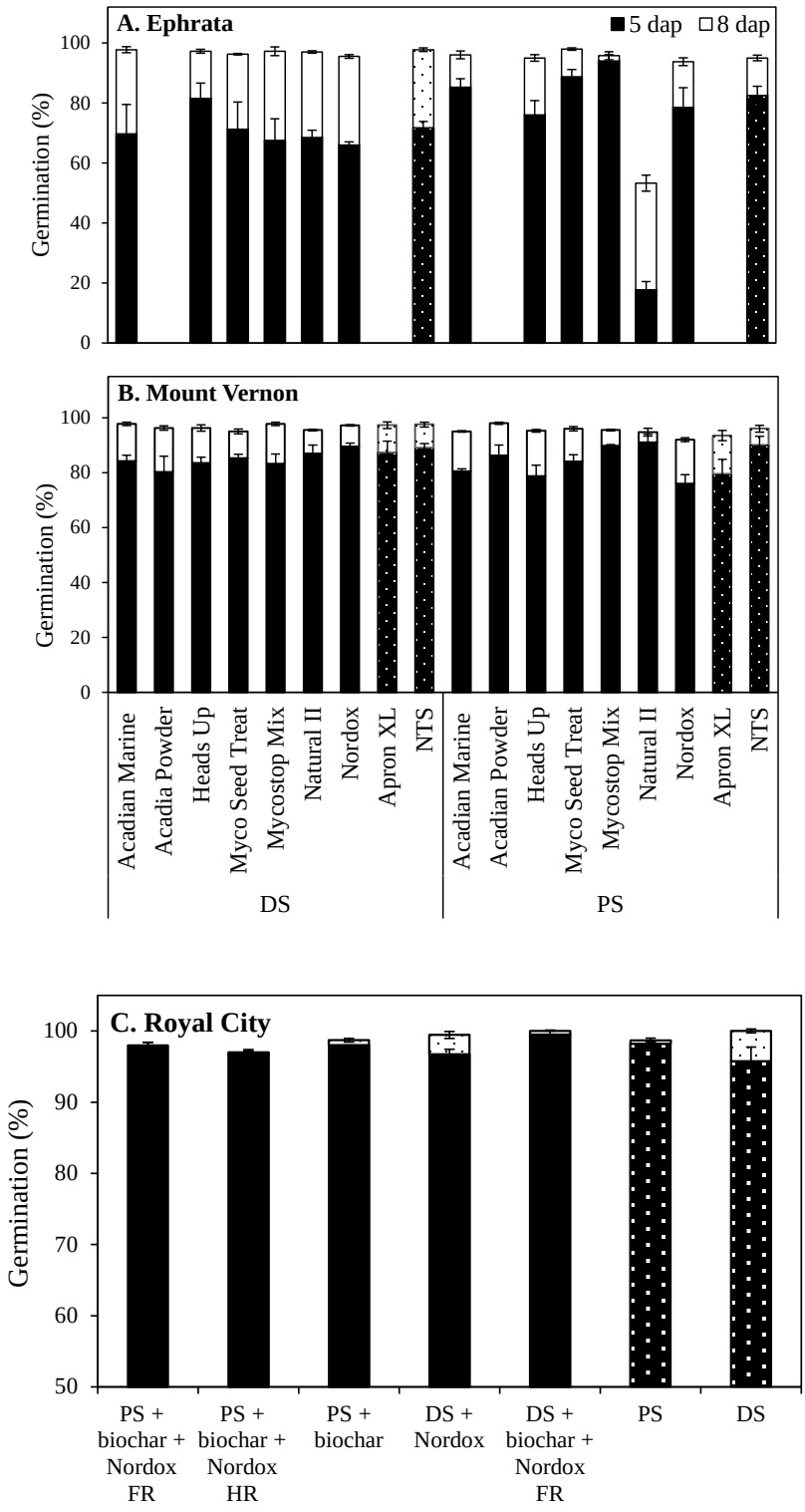
A. Ephrata
5 dap
8 dap
Germination (%)
B. Mount Vernon
Acadian Marine
Acadia Powder
Heads Up
Myco Seed Treat
Mycostop Mix
Natural II
Nordox
Apron XL
NTS
DS
PS
C. Royal City
PS + biochar + Nordox FR
PS + biochar + Nordox HR
PS + biochar
DS + Nordox
DS + biochar + Nordox FR
PS
DS

Fig. 3.10. Germination assay of primed seed (PS) and dry seed (DS) of the pea cvs. Boogie planted in Ephrata, WA (A) and Mount Vernon, WA (B), or Gallant planted in Royal City, WA (C) for evaluation against damping-off in the three field trial sites in Washington State in 2012. The germination assay was done using a blotter test following the procedure of the Association for Official Seed Analysts (2008) for peas. NTS = non-treated seeds, FR = Nordox applied at a full rate of 0.14 g/kg seeds, HR = Nordox applied at a half rate of 0.07 g/kg seeds. Each bar represents the mean ± standard error of the four replicate seed samples/treatment. The dotted bars represent the control treatments.

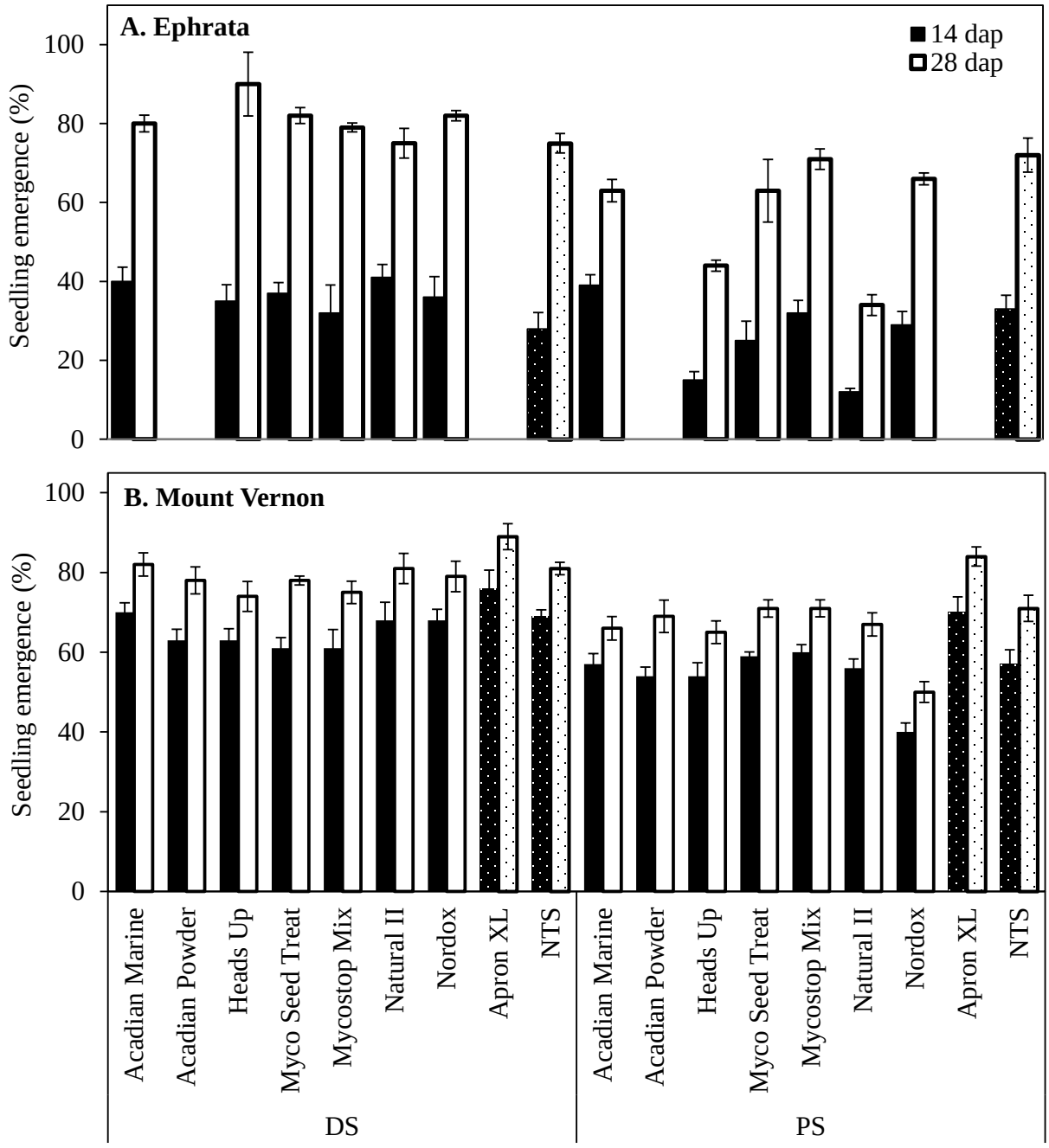

Fig. 3.11. Seedling emergence measured 14 and 28 days after planting (dap) for two field trials in Washington State in 2012 to evaluate organic seed treatments applied to dry seeds (DS) and

primed seeds (PS) for control of damping-off in organic pea crops. Percent emergence in the Ephrata trial (A) was calculated based on the 230 seeds planted in the two center rows/plot, and in the Mount Vernon trial (B) based on the 460 seeds planted in the four middle rows/plot. The field trial in Ephrata (A) was in a certified, organic grower-cooperator's pea field, products evaluated in this trial were listed by the Organic Materials Review Institute (OMRI) and the Washington State Department of Agriculture (WSDA) for certified organic use in 2012. The plots in the Mount Vernon trial (B) were inoculated with of *Pythium ultimum* isolate 030141. All control plots were planted with the non-treated seeds (NTS) in both trials. Treatments evaluated in the Mount Vernon trial included a product not on the OMRI list (Acadian Powder) and a conventional fungicide seed treatment, Apron XL, for comparison with the organic treatments. The dotted bars represent the control treatments. Data for the non-inoculated control plots in the Mount Vernon trial are not shown. The details of each treatment are shown in Table 3.2. Each bar represents the mean ± standard error of five replicate plots.

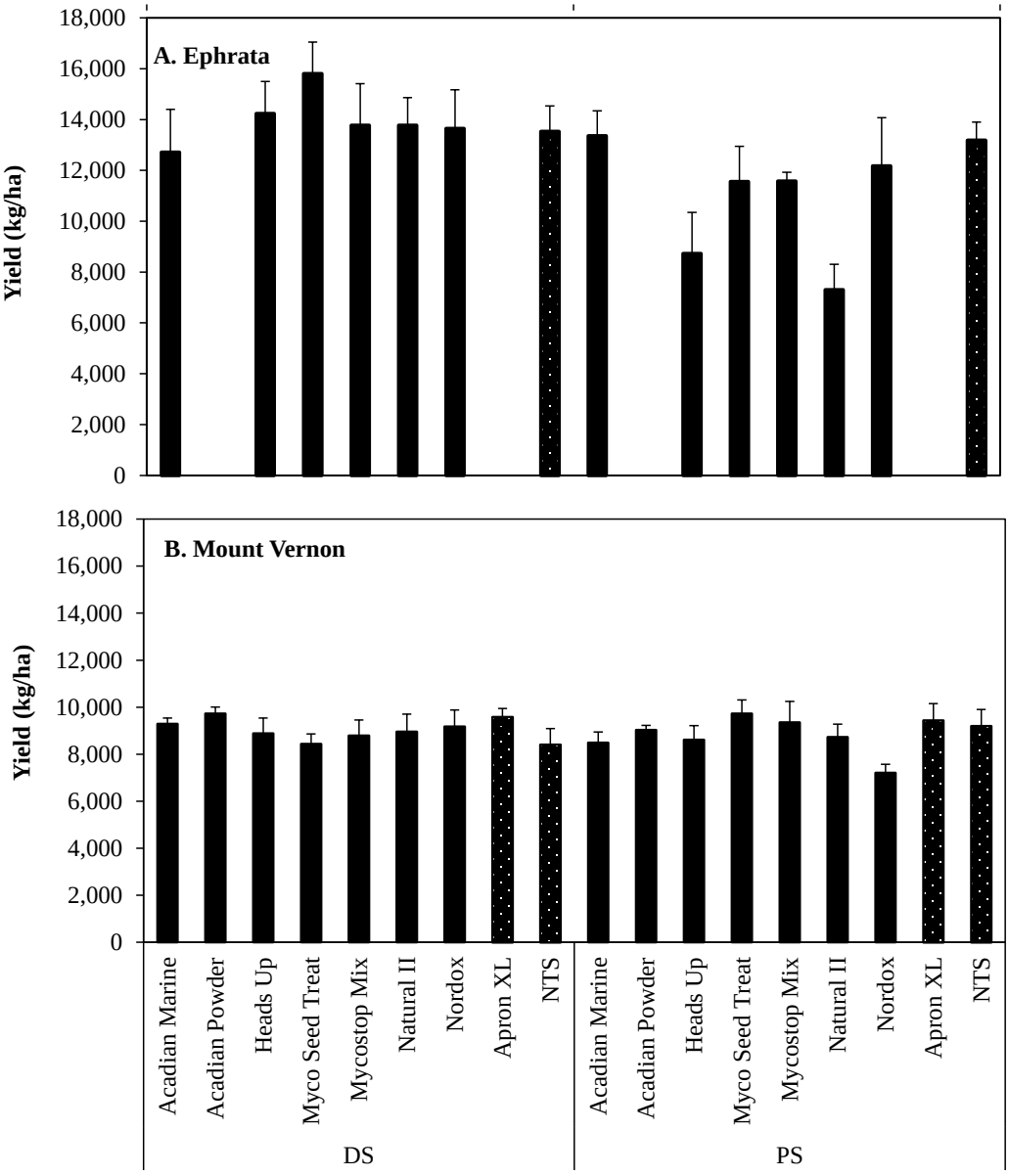

Fig. 3.12. Yield (kg/ha) measured from 1.5 m of each of the four center rows/plot in two field trials in Washington State in 2012 to evaluate organic seed treatments applied to dry seeds (DS) and primed seeds (PS) of the pea cv Boogie for control of damping-off. The field trial in Ephrata (A) was in a grower-cooperator's certified organic pea field, and products evaluated at this site

were on the Organic Materials Review Institute (OMRI) and Washington State Department of Agriculture (WSDA) list of approved products for certified organic production in 2012. The plots at the Mount Vernon trial (B) were inoculated with *Pythium ultimum* isolate 030141. All control plots were planted with non-treated seeds (NTS). Treatments evaluated in the Mount Vernon trial (B) included a product not in the OMRI list (Acadian Powder) and a conventional fungicide seed treatment, Apron XL, for comparison with the organic seed treatments. Each bar represents the mean ± standard error of five replicate plots. The dotted bars represent the control treatments. Data for the non-inoculated control plots in the Mount Vernon trial are not shown. Details of each treatment are shown in Table 3.2.

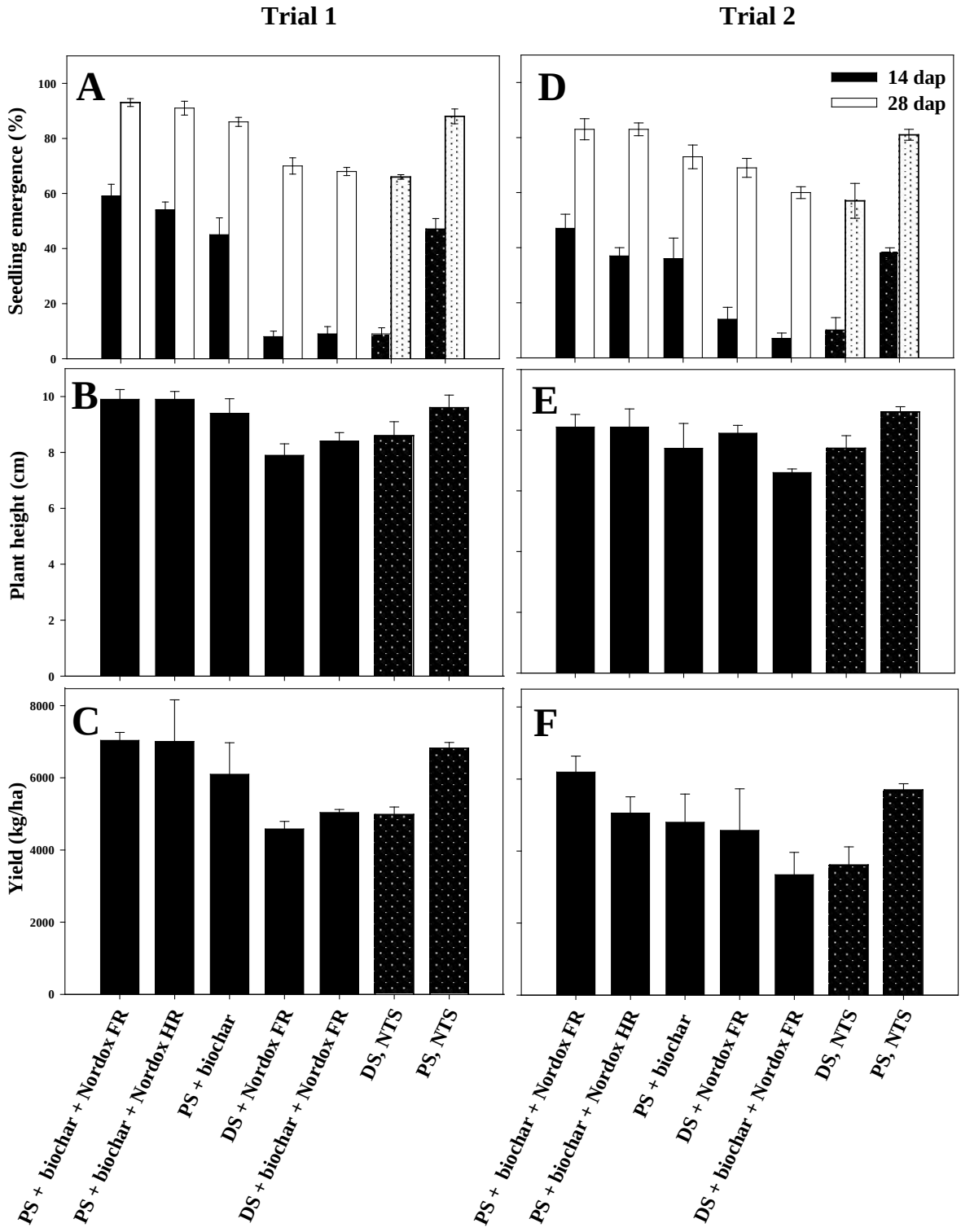
Trial 1
Trial 2
A
B
C
D
E
F
14 dap
28 dap
Seedling emergence (%)
Plant height (cm)
Yield (kg/ha)
0
20
40
60
80
100
2
4
6
8
10
2000
4000
6000
8000
PS + biochar + Nordox FR
PS + biochar + Nordox HR
PS + biochar
DS + Nordox FR
DS + biochar + Nordox FR
DS, NTS
PS, NTS

Fig. 3.13. Seedling emergence (A and D), plant height (B and E), and yield (C and F) measured in two organic pea field trials near Royal City, WA to evaluate seed priming using biochar as a desiccant to remove excess moisture after soaking seeds in water for 16 h, and Nordox organic seed treatment for damping-off control in pea crops. Seedling emergence was measured 14 and 21 days after planting (dap) based on 230 seeds planted in the two center rows/plot (A and D). Plant height was measured 35 dap from 20 plants sampled from both ends of two center rows/plot (B and E). Yield was measured from 1.5 m^2 over the four center rows/plot (C and F). PS = primed seed, DS = dry seed, FR = Nordox applied at a full rate of 0.14 g/kg seeds, HR = Nordox applied at a half rate of 0.07 g/kg, NTS = non-treated seeds. Each bar represents the mean ± standard error per four replicate plots. The dotted bars represent the control treatments.

CHAPTER 4

Influence of electrical conductivity of pea seeds on Pythium damping-off

4.1 Introduction

Pea seeds exude a large amount of material during the water imbibition phase of the germination process (Short and Lacy, 1976). Seed leachate, or exudate, consists mainly of cell constituents such as sugars, amino acids, organic acids, and electrolytes (Pandey, 1982; Rajjou et al., 2012). The greatest amounts of seed leakage are usually released during the first few hours of the imbibition phase, followed by a second increase after 6 h, and exudation finally ceases after 8 to 12 h for most plant species (Simon and Mathavan, 1986; Simon and Raja Harun, 1972). However, pea seeds demonstrate continuous seed leakage even after 48 h (Marbach and Mayer, 1985). Furthermore, temperature affects the amount and rate of exudation of pea seeds with greater leakage at lower temperatures (e.g., 5°C) than at moderate to warm temperatures (25°C) (Marbach and Mayer, 1985; Schroth and Hilderbrand, 1964; Short and Lacy, 1976). Other factors such as plant genotype, seed coat integrity, seed age, and soil moisture also influence the rate and amount of exudation (Schroth and Hilderbrand, 1964; Short and Lacy, 1976).

Seed exudation is important in the host-pathogen interaction of many soilborne pathogens, influencing disease incidence and severity (Schroth and Hilderbrand, 1964). Pathogens can sense compounds in the seed exudates (e.g., carbohydrates and amino acids) by chemotaxis, and move along the gradient of these compounds to locate the host. Furthermore, some exudates stimulate pathogen propagules to germinate (Chang-Ho, 1970; Donaldson and Deacon, 1993; Nelson and Craft, 1989). *Pythium*, among other soilborne pathogens, is very responsive to the presence of exudates (Nelson, 1990). The spermosphere, the narrow zone immediately surrounding the seed, facilitates contact between the seed and the microbial

community in the soil, and is influenced by the amount of seed leakage (Nelson, 1990; Nelson 2004). Germinating pea seeds were demonstrated to be highly susceptible to *Pythium* infection due to the fact that the seeds exude a large amount of leachates resulting in a wide spermosphere (Nelson, 1990; Short and Lacy, 1976). Variation in susceptibility of pea seeds to *Pythium* infection has been investigated in several studies that have suggested factors such as cultivar, seed age, seed color, and temperature may affect susceptibility to this pathogen (Loria and Lacy, 1979; Maguire et al., 1973; Short and Lacy, 1976). More importantly, variation in the cell constituents of exudates influences the amount of disease caused by *Pythium* spp. For example, the amounts of glucose and sucrose have been found to be correlated with susceptibility of pea to *Pythium* (Loria and Lacy, 1979). Studies have also shown that fatty acids and volatiles in seed exudates have a stimulatory effect on soilborne plant pathogens (Nelson, 1987; Ruttledge and Nelson, 1997).

In peas, potassium was reported to be the main constituent in seeds soaked in water (Matthews and Rogerson, 1976), followed by carbohydrates comprised mainly of simple sugars (Larson, 1968), and amino acids. The amounts of these constituents are closely correlated with electrical conductivity (EC) of the steeped water (Matthews and Carver, 1971). Also, studies have demonstrated that the amount of seed exudation is directly correlated with emergence in field conditions, suggesting that measurement of seed leakage can be used as an indicator of vigor of a seed lot (Ladone, 1989). Conductivity measurements can predict field emergence even in suboptimal planting conditions (Ladone, 1989; Pandey, 1992). According to Duke and Harvey (1983), one strongly correlated indicator of seed vigor is the leakage of substances from imbibing seeds. The membrane structure of poor quality seeds results in greater electrolyte leakage and, therefore, higher conductivity measurements (EC values) than high quality seed lots

(Matthews and Bradnock, 1968; Pandey, 1992). Good quality seed lots are associated with low EC values and typically demonstrate high germination rates, as well as vigorous and uniform seedling growth (Steere et al., 1981). An increase in electrolyte leakage is also an indicator of loss of cell integrity as a consequence of seed aging (Parish et al., 1982). Bewley and Black (1985) explained that the EC test is based on the fact that seeds, when soaked in water, exude ions, sugars, and other metabolites due to changes in the integrity of cell membranes. For seeds that have deteriorated, the mechanisms of repair are absent, inefficient, or completely damaged, which permits the leaching of larger amounts of electrolytes (Bewley and Black, 1985). As a result, lower vigor seed lots exhibit a greater intensity of cellular constituent losses (Delouche, 1976; Powell, 1986). The EC test is acknowledged as one of the best tests for evaluation of the loss of cell membrane integrity due to seed deterioration (Matthew and Powell, 2006).

In organic pea production, poor stands can be a concern to growers, especially during early spring planting (Alcala and du Toit, 2009). Cool and wet soil conditions during spring planting are conducive to damping-off caused by *Pythium* spp., and the lack of highly effective seed treatments for certified organic production (see Chapter 3) creates a greater risk for this disease than in planting conventional crops where growers have access to highly effective fungicide seed treatments such as metalaxyl or mefanoxam (Alcala and du Toit, 2009; Cook, 2002; McGee, 1992). Slow emergence of pea seeds under suboptimal conditions provides a longer window of opportunity for *Pythium* infection of susceptible seedling tissues (Hagedorn, 1984). Therefore, the use of high quality and high vigor seed lots is recommended in organic production, particularly when planting in suboptimal conditions [Processors and Growers Research Organization (PGRO), 1981]. In the United Kingdom, pea seeds with low levels of exudates are recommended for planting under such adverse conditions as cool and wet soils

(Hagedorn, 1984). However, despite such precautionary measures, organic pea growers still can be affected by poor stands associated with Pythium damping-off. In fact, in anticipation of encountering poor stands during early spring planting, growers may seed at greater densities than recommended (Alcala and du Toit, 2009). In the worst-case scenarios, growers may have to replant entire fields as a result of poor stands (Alcala and du Toit, 2009). Such practices add unwanted expenses. If growers have access to methods to assess risks associated with poor stands prior to planting, such situations may be avoided. The potential use of EC measurements to predict emergence in suboptimal conditions, and the positive relationship of the amount of Pythium damping-off with the amount of seed exudation has been demonstrated extensively (Castillo et al., 1993; Matthews and Bradnock, 1968; Matthews and Whitebread, 1968; Ladone, 1989). However, a quantitative assessment of the amount of exudation, measured by EC tests, on the amount of Pythium damping-off has not been established. If the relationship between the EC values of pea seed lots at known levels of *Pythium* inoculum can be used to predict field emergence at planting, this might be a helpful tool for growers to assess the risk of poor emergence due to damping-off.

In United Kingdom, PGRO (1981) established a standard to determine the vigor of seed lots of pea based on EC measurements, in order to assess conditions in which a particular seed lot can be used for planting with minimal risk of poor emergence or low vigor. In the USA, although measurement of the EC of pea seed lots is a routine lab test conducted by seed companies, growers may not be aware or provided with EC measurement of the seed lots (Alec McErlich, *personal communication*). For this reason, this study was carried out to examine whether the quantitative relationship between EC levels of pea seed lots and Pythium damping-off can be used as a tool by growers of pea crops particularly in the Columbia Basin of central

Washington, a primary region of organic processing pea production, to predict emergence in the field under early spring planting conditions. The specific objectives were to: (1) evaluate the susceptibility of seed lots of various pea cultivars to Pythium damping-off at a range of inoculation levels with *Pythium ultimum* in growth chamber trials that mimic early spring planting conditions, (2) examine the influence of EC levels of pea seed lots on mycelial growth of *P. ultimum*, and (3) assess whether the EC measurement of pea seed lots can be used to predict the risk of Pythium damping-off in cool, moist soil conditions, that are highly conducive to the disease.

4.2 Materials and methods

4.2.1 Growth chamber trials

4.2.1.1. Electrical conductivity test. EC tests of pea seed lots were carried out following the protocol of the PGRO (1981). A total of 50 seeds was counted and weighed for each of the seed lots. Each set of 50 seeds was placed in 250 ml deionized water in a 400-ml glass beaker that had been stored at 20°C for 24 h prior to use. After soaking the seeds for 24 h, the EC reading of the water was obtained using an EC meter (model HI 98312, Hannah Instruments Inc., Woonsocket, RI). The EC was measured for five replications of 50 seeds/seed lot.

4.2.1.2 Seed lot sources and preparation for planting. Seventeen seed lots from a total of six processing pea cultivars were obtained from two seed companies in Washington State: Brotherton Seed Company, Inc. (Moses Lake, WA) and Pure Line Seeds, Inc. (Warden, WA) (Table 4.1). These represented seed lots with low, medium, and high EC levels for each cultivar that, in turn, represented high, medium, and low vigor, respectively, for each cultivar (PGRO, 1981) (Table 4.1). The pea seeds were surfaced-disinfested following the protocol of L. Porter

(USDA ARS, Legume Pathology, Prosser, WA). Seeds were placed in a 400-ml plastic beaker that had 10 holes (each 0.1 cm diameter) in the bottom, and rinsed with tap water. The beaker containing the seeds was then submerged in a suspension of 10 g Alconox (Alconox, Inc., White Plains, NY) dissolved in 1 liter tap water in a 500 ml beaker, and the seeds stirred for 30 s. The seeds were then rinsed with deionized water, submerged in 95% ethanol for 60 s with constant stirring, and then submerged in 15% hydrogen peroxide for 60 s. Seeds were rinsed thoroughly with deionized water, drained, and spread on a plastic tray lined with two layers of sterilized paper towels to dry. The dried, disinfested seeds were stored at 6°C and 45% RH, and planted the next day.

4.2.1.3. *Pythium ultimum* inoculum preparation. Inoculum of *P. ultimum* for the growth chamber trial was prepared using a mixture of ground oatmeal and a sandy loam soil collected from a grower's field in Skagit Co., WA. The soil was spread on kraft butcher paper on a greenhouse bench for 3 to 5 days to dry, then passed through a sieve (1 mm diameter pore size) to remove plant debris, and crushed with a marble rolling pin. Ground oatmeal (Quaker Oats Brand, Chicago, IL) was added to the soil (1% by weight) and mixed using a PK Blendmaster (Patterson-Kelley Co. division of Harsco Corp., East Stroudsberg, PA). The soil and oatmeal were mixed for 10 min with deionized water (15% v/w) added slowly through a funneled hose attached to the blender during the last 5 min of mixing. Approximately 1 kg of the soil-oatmeal blend was then placed in a clean, 3.8 liter high density polyethylene (HDPE) milk jug, sealed with an autoclavable 3.8-cm diameter foam plug (VWR, Baltimore, MD), and the foam plug covered with two layers of aluminum foil. The jugs of soil-oatmeal mix were autoclaved at 1.1 kg/cm^2 and 121°C for 50 min, cooled for 24 h, and autoclaved a second time using the same conditions. A 20 ml volume of deionized, sterilized water was added to each jug, and left

overnight prior to inoculation with the mycelial mat of a 5-day old culture of *P. ultimum* isolate 030141 (obtained from T. Paulitz, USDA ARS, Pullman, WA) that was grown in potato dextrose broth at room temperature (23 ± 1°C) in the dark.

The soil-oatmeal inoculum of *P. ultimum* was quantified following three weeks of incubation by plating a dilution series on water agar (WA). Each jug was shaken vigorously to mix the inoculum, and a 10 g sample was added to a French square bottle (250 ml capacity) containing 100 ml sloppy agar (0.1% WA). The bottle was then placed on a platform rotary shaker (New Brunswick Scientific Innova 2100, Einfield, CT) for 10 min at 250 rpm. Five-fold dilutions were carried out using 10 ml aliquots transferred serially to French square bottles (100 ml capacity), each containing 40 ml of sloppy agar. Three 0.5 ml aliquots of each of the dilutions were then each spread on the surface of a WA plate using a bent glass rod. The plates were incubated on a laboratory bench at room temperature. Colony counts were done after 24, 30, and 36 h. The number of colony forming units (CFU)/g soil was determined using the average number of colonies counted from the triplicate plates/dilution.

4.2.1.4. Soil pasteurization. Field soil obtained from a certified organic farm in the Columbia Basin (Lenwood Farms, Inc., Connell, WA) was used in the growth chamber trials. The soil was pasteurized by first moistening approximately 19 liters of soil by adding tap water slowly while mixing the soil in an 84 liter cement mixer. Once completely moist, the soil was pasteurized for 2 h at 70°C using a custom-built steam pasteurizer (Patzek, 2013). The soil was then cooled, placed on kraft butcher paper, and dried on a greenhouse bench for 2 to 3 days. The dry soil was then crushed using a marble rolling pin. The pasteurized soil was stored at 22 to 25°C in plastic bins, each covered with a lid to minimize the risk of contamination.

4.2.1.5. Planting. Nine seed lots from Brotherton Seed Co., Inc., three of each of the pea cvs. Bistro, Boogie, and Tonic, were obtained for this study in January 2012 and, therefore, were the first lots to be used for growth chamber trials (Table 4.1). Each seed lot was planted in soil with each of three rates of inoculation with *P. ultimum* 030141 (50, 100, and 500 CFU/g soil) as well as non-inoculated soil for a 9 x 4 factorial treatment combination. A 3.5 kg aliquot of the pasteurized field soil was mixed with the appropriate amount of *P. ultimum* soil-oatmeal inoculum based on the CFU/g of the inoculum, determined by dilution plating onto WA, to achieve the desired inoculation rate. Soil and inoculum were mixed using a lab batch seed treater (Gustafson LLC, Shakopee, MN) for 5 min, and 500 ml tap water was added while mixing. For each treatment combination, the appropriate soil was added to each of five replicate, 10-cm diameter plastic pots (Anderson Die Manufacturing Co., Portland, OR). Ten surface-disinfested seeds of each seed lot were planted at a depth 1.3 cm in five pots of each soil inoculation treatment, and then covered with the same soil. The trial consisted of a total of 36 treatments with five replicate pots/treatment arranged in a randomized complete block design (RCBD) in a growth chamber (PGC Controlled Environment Chamber, Percival Scientific Inc., Perry, IA) set at 13°C by day and 8°C by night with a 12 h photoperiod/day. The trial was repeated using the same protocol.

Following the procedure described above, the growth chamber trial was also carried out using a total of eight seed lots of three pea cultivars (two lots of PLS 134, and three lots each for PLS 560 and Serge) obtained from Pure Line Seeds Inc., (Table 4.1). These seed lots also represented different EC and vigor levels (PGRO, 1981). Two seed lots from the original growth chamber trials, Tonic 343194 and Bistro 364017, were included in the Pure Line seed lot trial to check for consistency in results among the trials. This trial was repeated using the same protocol.

4.2.1.6. Data collection and statistical analyses. Seedling emergence counts were done 14 and 28 days after planting (dap). At 28 dap, the seedlings were removed carefully from each pot, and the roots washed and evaluated for root rot severity. Total pea fresh weight, shoot height, and shoot dry biomass of all the seedlings/pot were measured. Shoot height was measured from the point of seed attachment to the tip of the last leaf. For shoot dry weight, all shoots in a pot were placed in a paper bag in an oven set at 90°C. After 2 to 3 days, total dry weight was measured. Root rot severity was rated using a 0 to 5 scale, where: 0 = no visible symptoms on the roots; 1 = a few, small, discolored lesions on the roots; 2 = minor discoloration covering most of the roots and epicotyl; 3 = brown discoloration of the epicotyl and roots, with most of the lateral roots missing; 4 = complete root discoloration and lateral roots missing; 5 = rotten seed with no roots.

Data for each of the dependent variables were analyzed as a factorial RCBD using analyses of variance (ANOVA) and Fisher's protected least significant difference (LSD at $P < 0.05$) for treatment means comparisons with SAS Proc GLM (Version 9.2, SAS Institute, Cary, NC). Dependent variables that did not meet the parametric assumptions of normality and equal variances were transformed by arcsine or rank transformation (latter using Friedmans' non-parametric rank test). Regression analyses were completed using Sigma Plot Version 11 (Systat, San Jose, CA) to examine the relationship between EC readings for the seed lots and emergence 14 and 28 dap at different *P. ultimum* inoculation rates (0, 50, 100, and 500 CFU/g soil).

4.2.2. Exudate bioassay

The effect of seed exudates on mycelial growth of *P. ultimum* isolate 030141 was evaluated in a lab bioassay. Seed exudates were used from each of nine seed lots representing low, medium, and high EC ratings for each of three pea cvs., Bistro, Boogie, and Tonic (Table

4.1). A total of 50 seeds/seed lot was weighed and soaked in 250 ml deionized water in a 400-ml beaker that had been kept overnight at 20°C. The seeds were held in the water for another 24 h at 20°C before the EC reading of the steeped water from each of the seed samples was measured as described above. The remaining steeped water was passed through a 0.2 µm filter syringe, and 25 ml collected in each of four 90-mm diameter, sterilized petri plates. Each plate containing seed exudate suspension was subsequently inoculated with a 1-cm diameter mycelial plug obtained from the advancing margin of a 4 to 5-day-old culture of *P. ultimum* 030141 on WA. The inoculated plates were then incubated at room temperature in the dark for 7 days. The mycelial mat in each plate was then collected by removing the liquid using a vacuum filter fitted with a VWR filter paper No. 1 (VWR, Visalia, CA). The filter paper with the mycelial mat was dried in an oven at 60°C for at least 3 h, and then mycelial dry weight was measured. *P. ultimum* grown similarly in deionized water served as the control treatment. The trial was repeated following the same procedure, except using six replicate plates/seed lot in an attempt to reduce variability in results for mycelial dry weights that occurred in trial 1. Data were analyzed as a RCBD ANOVA with means separation completed using Fisher's protected LSD ($P < 0.05$). Log transformation was performed to satisfy assumptions for parametric analysis for mycelial dry weights in trial 1.

4.3. Results

4.3.1. Electrical conductivity. Of 17 seed lots representing six cultivars, five lots were in the high vigor category of the PGRO (1981) with EC in the range of 12.7 to 24.1 µS/cm/g seed, three lots had medium vigor ratings with an EC in the range of 26.6 to 28.8 µS/cm/g seed, seven lots fell in the low vigor category with an EC in the range of 31.0 to 39.2 µS/cm/g seed, and two lots were of very low vigor with EC values of 51.2 and 57.1 µS/cm/g seed (Table 4.1). Of the

nine seed lots from Brotherton Seed Co., Inc., the four high vigor lots had germination rates of 96 to 99%, the lot with medium vigor had 94% germination, and the four low vigor lots had germination rates ranging from 90 to 99% (Table 4.1). The germination rates of the eight seed lots from Pure Line Seeds, Inc. ranged from 90 to 99%, with a 99% germination rate for the one high vigor lot, 98% for the two medium vigor lots, 95 to 98% for the three low vigor seed lots, and 90 and 97% for the two very low vigor lots (Table 4.1).

4.3.2. Growth chamber trials: Brotherton seed lots. In Trial 1, the ANOVA showed a significant main effect of the nine seed lots ($P < 0.05$) for emergence measured 14 and 28 dap, shoot length, and shoot dry weight, but not for root rot rating ($P = 0.2739$); and a significant main effect of inoculation rates ($P < 0.0001$) for all five variables measured (Table 4.2). The interaction term between seed lots and inoculation rates was significant for emergence 14 dap ($P < 0.0001$) and 28 dap ($P = 0.0397$), and for shoot length ($P < 0.0001$).

More pea seedlings had emerged 14 and 28 dap in pots planted with the Tonic 344024 seed lot (43 and 53%, respectively), which was a high vigor seed lot compared to the eight other seed lots evaluated in Trial 1 (range in mean emergence of 26 to 35% at 14 dap, and 33 to 43% at 28 dap, even though three of these eight seed lots had high to medium-high vigor ratings) (Table 4.2). Emergence 28 dap was poorest for two low vigor seed lots, Bistro 364061 and Tonic 343144. Tonic 344024 seed lot also had the tallest plants and greatest shoot biomass (6.1 cm and 0.28 g, respectively). Seed of Tonic 343194, a lot with medium to high vigor, also produced taller plants (4.6 cm) compared to plants that grew from the remaining seven seed lots which did not differ significantly (3.0 to 3.8 cm). Shoot dry weight of the plants that grew from eight out of the nine seed lots did not differ significantly (0.16 to 0.20 g) (Table 4.2).

Inoculation of soil with *P. ultimum* significantly reduced emergence 14 and 28 dap, from 77 and 93%, respectively, in non-inoculated control soil to 16 and 22% in soil inoculated at 100 CFU/g soil, respectively; and to 4 and 5% in soil inoculated at 500 CFU/g soil, respectively (Table 4.2). Similarly, all other dependent variables were reduced significantly with increasing rate of soil inoculation with *P. ultimum* (Table 4.2). Roots of seedlings evaluated 28 dap from pots inoculated with 500 CFU/g soil had the most severe root rot symptoms, followed by seedlings in pots with 100 CFU *P. ultimum*/g soil, while no root rot was observed in seedlings growing in non-inoculated soil (Table 4.2). Similar effects of inoculation rates were observed on shoot length and shoot dry weight averaged across the seed lots.

Emergence 14 and 28 dap in non-inoculated soil was significantly greater than in inoculated soil, ranging from 60 to 88% and 76 to 100%, respectively, for the nine seed lots (Table 4.2). Inoculating the soil with 100 CFU *P. ultimum*/g soil reduced emergence to 6 to 28% at 14 dap, and was greatest for the high vigor seed lots Boogie 610144, Bistro 364017, and Tonic 344024 (28, 26, and 20%, respectively). At 28 dap, emergence from these three high vigor seed lots was still greatest (32, 32, and 26%, respectively), but only significantly different from that of the lower vigor lot Tonic 343144. Inoculation of soil with 500 CFU/g soil resulted in no emergence 14 and 28 dap for three of the four low vigor seed lots, as well as the medium vigor lot, Bistro 364017, and one of the high vigor lots, Boogie 610144. By 28 dap, all four of the low vigor seed lots had no emergence, the medium vigor lot had only 6% emergence, and emergence for the four high vigor lots ranged from 6 to 26% (Table 4.2). Emergence 28 dap in soil planted with Tonic 344024 was the greatest at both 100 and 500 CFU/g soil inoculation rates (20 and 26%, respectively) (Table 4.2). Plants were taller for all three of the Tonic seed lots compared to the Boogie and Bistro seed lots in non-inoculated soil, regardless of the vigor of the lots, which

suggests this was a cultivar effect rather than a seed lot EC or vigor effect. Inoculation of soil with *P. ultimum* at 100 CFU/g soil did not result in significant differences in height of most of the seed lots, except the low vigor seed lot Tonic 343144 which produced the shortest plants (Table 4.2). When the soil was inoculated with *P. ultimum* at 500 CFU/g soil, the high vigor Tonic 344024 seed lot produced significantly taller plants than all the other seed lots except those of the medium-high vigor lot Tonic 343194, and the plants were as tall as those produced in soil inoculated at 100 CFU/g soil (Table 4.2).

In the repeat growth chamber trial (Trial 2) with the Brotherton Seed Co., Inc. seed lots, with addition of a 50 CFU/g soil inoculation rate, the main effects of the nine seed lots and four inoculation rates were both highly significant for all of the variables measured ($P < 0.0001$), while a significant interaction term was detected only for emergence measured 28 dap ($P = 0.0195$) (Table 4.3). Emergence 14 and 28 dap was again greatest for the high vigor seed lot Tonic 344024 (78 and 88%, respectively), followed by emergence in soil planted with the other three high vigor seed lots and the medium-high vigor lot, which was consistent with the results of Trial 1 (Tables 4.1 and 4.3). The lowest emergence 14 and 28 dap was observed for all four low vigor seed lots (≤ 57 and $\leq 71\%$, respectively) (Tables 4.1 and 4.3). The most severe root rot ratings (mean of 2.5 out of 5.0) were assigned to seedlings produced by two of the low vigor seed lots, Boogie 11009 and Tonic 343144; and the least severe root rot was observed on seedlings of two of the high vigor lots, Boogie 610154 (1.7) and Tonic 344024 (1.9) (Table 4.3). Plant height of the three Tonic cultivars (6.1 to 7.3 cm) was significantly greater than the height of plants of the two other cultivars (4.5 to 5.3 cm), similar to plant height observations in Trial 1 for two of these three Tonic seed lots. The low vigor seed lot Tonic 343144 had smaller seedlings compared to the two high vigor Tonic seed lots, Tonic 343194 and 344024 (Table 4.3).

Greatest shoot dry weights (0.39, 0.40, and 0.48 g) were measured from three of the four high vigor seed lots (Tonic 343194, Boogie 610154, and Tonic 344024, respectively) compared to the other seed lots (0.23 to 0.32 g) (Table 4.3).

The main effect of inoculation rate on emergence 14 dap was not significantly different for non-inoculated soil and soil inoculated at the lowest rate, 50 CFU/g soil (78 and 77%, respectively), but emergence was reduced significantly to 64 and 29% at 100 and 500 CFU/g soil inoculation rates, respectively (Table 4.3). At 28 dap, a significant decrease in emergence was observed for each increase in inoculation rate of *P. ultimum*, i.e., 94, 83, 74, and 39% emergence in soil inoculated at 0, 50, 100, and 500 CFU/g, respectively (Table 4.3). Similarly, root rot severity of pea plants was most severe (3.9 out of 5.0) for seedlings growing in soil with the highest inoculation rate (500 CFU/g soil) and was significantly less severe the lower the inoculation rate, with no root rot in the non-inoculated soil (Table 4.3). A significant reduction in shoot length was observed only for peas growing in soil with the highest inoculation rate (4.1 vs. 5.7 to 6.2 cm for the other soil treatments). Shoot dry weights of pea plants decreased with increasing inoculation rates with *P. ultimum* emergence 28 dap (Table 4.2).

The significant interaction between seed lots and soil inoculation rates for emergence 28 dap was reflected in the fact that in non-inoculated soil, eight of the nine seed lots had similar emergence regardless of seed vigor (92 to 100%), with only the low vigor Boogie 11009 seed lot having emergence reduced to 72% (Table 4.3). In soil infested with *P. ultimum* at 50 CFU/g soil, the high vigor three seed lots, Boogie 610154, Boogie 610144, and Tonic 344024, retained an emergence rate > 90% (92 to 94%) which was statistically similar to emergence in the non-inoculated soil (100%). This was followed by the medium-high vigor seed lot, Tonic 343194 (88% emergence), whereas the medium and all four low vigor lots had emergence ≤ 76%. The

high vigor seed lot Boogie 610154 and Tonic 344024 also displayed the greatest emergence at 100 CFU/g soil (92 and 96%, respectively), followed by the high vigor lot Boogie 610144 (80%), whereas the other six seed lots had emergence rates ranging from 54 to 74% (Table 4.3).

4.3.3. Growth chamber trials: Pure Line seed lots. In the first growth chamber trial using the eight seed lots from Pure Line Seeds, Inc., with two of the Brotherton Seed Co., Inc. seed lots, Tonic 344194 and Bistro 364017, as control treatments for comparison with the previous trials, both the main effect of the 10 seed lots and the main effect of the four inoculation rates were significant for all of the variables measured ($P < 0.0001$ for all variables, except shoot length for which the main effect of seed lots had $P = 0.0145$) (Table 4.4). The high vigor seed lot PLS 560 60011 produced the best emergence 14 dap (71%), followed by the medium vigor lot Serge 62012 (64%) (Table 4.4). The fewest seedlings had emerged by 14 dap for the two very low vigor seed lots, PLS 134 34012 and PLS 134 34022 (29 and 38%, respectively). By 28 dap, Serge 62012, the only high vigor seed lot, had the greatest emergence (81%), along with PLS 560 60011, a medium vigor lot (79%) (Table 4.4). Serge 62012 had significantly better emergence than the eight other seed lots (48 to 73%). The poorest emergence was observed for the very low vigor seed lot PLS 134 34012 (48%), followed by the low vigor lot PLS 560 60012 (55%) and the very low vigor lot PLS 134 34022 (59%) (Table 4.4). All of the very low and low vigor seed lots had severe root rot ratings (mean of 2.1 to 2.9) compared to the medium and high vigor seed lots (1.7 to 1.9) (Table 4.4). The tallest plants and greatest shoot dry weights were produced by the Tonic 344194 seed lot (7.6 cm and 0.35 g, respectively). Among the Pure Line Seeds, Inc. seed lots, two of the Serge seed lots, 62012 and 62052, produced taller plants than the other five seed lots. The shortest plants were produced by two of the PLS 560 seed lots (60012 and 60030), as well as PLS 134 34012, and Bistro 364017 (mean shoot lengths of 5.0, 5.3, 5.3,

and 4.7 cm, respectively). Significantly smaller shoot dry weights were produced by the two very low vigor PLS 134 seed lots (34012 and 34022) and the low vigor lot PLS 560 60012 (0.17, 0.19, and 0.18 g, respectively) (Table 4.4).

Inoculation of soil with different rates of *P. ultimum* affected emergence, root rot severity, plant height, and shoot dry biomass significantly when averaged across the 10 seed lots in Trial 1 with the Pure Line Seeds, Inc. seed lots (Table 4.4). Greater emergence 14 and 28 dap (72 and 94%, respectively), shoot length (6.4 cm), and shoot dry weight (0.38 g), and less root rot (0.3) were measured for peas planted in non-inoculated soil compared to all inoculated soil treatments. However, inoculation of soil at 50 vs. 100 CFU/g soil did not result in significant differences in any of the variables measured, but inoculation at 500 CFU/g soil resulted in the poorest emergence (23 and 34% at 14 and 28 dap, respectively), shortest plants (4.8 cm), smallest shoot biomass (0.10 g), and most severe root rot (3.8) when averaged across the 10 seed lots (Table 4.4).

The significant interaction between seed lots and inoculation rates for 28 dap reflected the fact that similar rates of emergence occurred across the 10 seed lots when planted in non-inoculated soil, whereas significant differences in emergence among the seed lots were observed at each of the soil inoculation rates. For the 50 CFU/g soil treatment, the high and medium vigor seed lots from Pure Line Seeds, Inc. (Serge 62012, PLS 560 60030, and PLS 560 60011 with 88, 80, and 80% emergence, respectively) had more seedlings emerged compared to the very low and low vigor seed lots from this company (PLS 134 34012, PLS 560 60012, Serge 62062, and Serge 62052B, with 30, 58, 66, and 79%, respectively). Interestingly, the other very low vigor seed lot, PLS 134 34022, had 80% emergence for the 50 CFU/g soil inoculation treatment, similar to the emergence of the medium to high vigor seed lots from Pure Line Seeds, Inc. At the

100 and 500 CFU/g soil inoculation rates, emergence was again greatest for the medium and high vigor seed lots than the low and very low vigor seed lots (Table 4.4). Root rot symptoms generally were most severe for the very low and some of the low vigor seed lots compared to the high and some medium vigor seed lots, particularly at the highest inoculation rates (Table 4.4). At 100 CFU/g soil, the two very low vigor seed lots produced plants with root rot ratings ≥ 3.4 vs. 1.7 to 3.0 for the other lots. At the 500 CFU/g soil, these two very low vigor lots and the low vigor lot PLS 560 60012 had root rot ratings ≥ 4.0 vs. 3.4 to 3.9 for the other lots (Table 4.4). Except for the very low vigor lot PLS 134 34022 and the low vigor lot PLS 560 60012, shoot dry weight was similar for the eight other seed lots planted in non-inoculated soil (Table 4.4). In soil inoculated at 100 and 500 CFU/g soil, the greatest shoot dry weights were produced by the high vigor lot, PLS 560 60011 and medium vigor lots, Serge 62012 and PLS 560 60030, of the lots from Pure Line Seeds, Inc., as well as the Tonic 343194 and Bistro 364017 seed lots from Brotherton Seed Co., Inc., while the smallest shoot dry weights were produced by the very low and low vigor seed lots (Table 4.4).

The repeat growth chamber trial with the eight pea seed lots from Pure Line Seeds, Inc., Bistro 364017, and Tonic 343194 was relatively consistent with results observed in Trial 1 (Table 4.5). The main effects of eight seed lots from this company plus two lots from Brotherton Seed Co., Inc., and the main effect of the four inoculation rates were significant ($P < 0.0001$ to 0.0326) for all of the variables measured, but there was no significant interaction term detected for any of the variables (Table 4.5). Pea emergence was significantly greater for the medium vigor lot Serge 62012 and the high vigor lot PLS 560 60011 (77 and 75% at 14 dap, and 82 and 81% at 28 dap) compared to the low vigor seed lots (51 to 64% at 14 dap, and 53 to 73% at 28 dap), with the exception of Serge 62052B (68 and 71% at 14 and 28 dap, respectively) (Table

4.5). Root rot severity did not differ significantly among the 10 seed lots, regardless of vigor category, except for the medium vigor lot Serge 62012, which had the least root rot (1.7 vs. 2.0 to 2.6 for the other nine seed lots) (Table 4.5). The tallest plants were produced by the Tonic 643194 seed lot (9.2 cm), followed by all three Serge seed lots (7.1 to 7.5 cm), regardless of the vigor ratings (Table 4.5). Shoot dry weight was greatest for the Tonic 643194 and Bistro 364017 seed lots (0.48 and 0.42 g, respectively), followed by the medium vigor lot Serge 62012 lot (0.40 g) and the high vigor lot PLS 560 60011 (0.38 g) (Table 4.5). For all five dependent variables, pea seedlings performed worst for the low vigor lot PLS 560 60012 and the very low vigor lot PLS 134 34012 (Table 4.5).

Pea seeds planted in non-inoculated soil had the greatest emergence, shoot length, and dry weight when averaged over the 10 seed lots, compared to planting pea seed in soil inoculated with *P. ultimum*, regardless of the rate of inoculation (Table 4.5). Also, there were no root rot symptoms on pea plants in the non-inoculated soil. For soil inoculated at 50, 100, or 500 CFU/g soil, emergence measured 14 and 28 dap, shoot length, and shoot dry weight all increased significantly with increasing rate of soil inoculation, and vice versa for the root rot severity ratings (Table 4.5). The lack of significant interaction term for all dependent variables indicated that the effects of inoculation rates were consistent for all 10 seed lots evaluated.

4.3.4. Regression analyses. Regression analyses revealed a significant negative relationship in Trial 1 of pea emergence vs. seed lot EC measurement for nine seed lots from Brotherton Seed Co., Inc. planted in soil inoculated with *P. ultimum* at four rates (0, 50, 100, and 500 CFU/g soil) ($P < 0.05$, $R^2 > 0.5000$), except for emergence 14 dap in soil inoculated at 100 CFU/g soil ($P = 0.4146$), and for emergence 28 dap in non-inoculated soil ($P = 0.2020$), and in soil inoculated at 100 CFU/g soil ($P = 0.0129$) (Fig. 4.1A and 4.1B). The regression analyses

demonstrated that emergence decreased with increasing EC of the pea seed lots, with the greatest rate of decrease (β_o = slope of the regression line) at the highest inoculation rate (R^2 = 0.5710 at 14 dap and 0. 6107 at 28 dap) in Trial 1 (Table 4.5 and Fig. 4.1). In Trial 2, similar significant negative regression equations ($P < 0.05$, $R^2 > 0.4500$) were observed for EC and emergence 14 and 28 dap for all of the inoculated soil treatments (50, 100, and 500 CFU/g soil), but only at 14 dap in non-inoculated soil (Fig. 4.1C and 4.1D). In Trial 2, emergence at each of 14 and 28 dap were similar at the 0, 50, and 100 CFU/g soil inoculation rates for the seed lot with the lowest EC readings, but with an increase in EC value of the seed lots, emergence decreased more rapidly with increasing soil inoculation rate (Fig. 4.1C and 4.1D).

When the relationship of the EC readings of pea seed lots with emergence was evaluated in soil inoculated at four rates with *P. ultimum* (0, 50, 100, and 500 CFU/g soil) using eight seed lots from Pure Line Seeds, Inc., results were relatively similar with those of previous trials with seed lots from Brotherton Seed Co., Inc., i.e., there was a significant negative relationship of EC and emergence measured 14 and 28 dap (Fig. 4.2). In Trial 1 with these seed lots, emergence 14 dap decreased significantly the greater the EC value of the seed lot in all the soil treatments except soil inoculated at 50 CFU/g soil (Fig. 4.1A). By 28 dap, this relationship between EC and emergence was not significant for both the 0 and 50 CFU/g soil treatments (Fig. 4.1B). In Trial 2, emergence 14 dap decreased with increasing EC value of the seed lots in all the inoculated soil treatments, but not in non-inoculated soil (Fig. 4.2C). By 28 dap, this relationship was still significant in soil inoculated at 50, 100, and 500 CFU/g soil, but not in non-inoculated soil (Fig. 4.2D). As in Trial 1, the most rapid decrease in emergence with increasing EC value occurred in soil inoculated at the highest rate, i.e., the greatest amount of inoculum of *P. ultimum* had the

most deleterious effect on emergence for those seed lots with high EC readings (lowest vigor seed lots) (Fig. 4.2).

4.3.4. Seed leakage bioassay. The laboratory bioassays evaluating the influence of seed exudation levels (EC values) of nine pea seed lots on mycelial growth of *P. ultimum* isolate 030141 demonstrated a significant main effect of the seed lots ($P = 0.0071$ in Trial 1, and $P = 0.0009$ in Trial 2). In Trial 1, *P. ultimum* mycelial dry weights from cultures growing in exudates of each of the seed lots (mean ± standard error of 18.3 ± 4.8 to 30.8 ± 11.9 mg) were significantly greater than the mycelial dry weight of *P. ultimum* growing in water (7.3 ± 1.5 mg), except for the seed exudate suspensions of Tonic 344024 (11.2 ± 1.3 mg) for which the *P. ultimum* mycelial weight did not differ significantly from the water control treatment (Fig. 4.3A). The mycelial dry weight of *P. ultimum* growing in seed exudate suspensions of the Boogie 611009 seed lot (30.8 ± 11.5 mg), which had an EC of 31.7 µS/cm/g (low vigor), was significantly greater than that of two high vigor seed lots, Tonic 344024 and Boogie 610144 (11.2 ± 1.5 and 18.2 ± 4.7 mg, respectively), which had EC values of 13.7 and 19.1 µS/cm/g, respectively (Fig. 3.A). In Trial 2, mycelial dry weights of *P. ultimum* growing in seed exudate suspensions of all nine seed lots (11.6 ± 1.5 to 14.8 ± 2.1 mg) were significantly greater than that of the water control treatment (6.2 ± 1.1 mg). However, mycelial dry weight of *P. ultimum* did not differ significantly among seed exudate suspensions of the nine seed lots (Fig. 4.3B).

4.4. Discussion

Measurement of the EC of pea seeds has become an established method to determine the vigor of pea seed lots, and has demonstrated greater reliability for prediction of field emergence compared with other methods, such as the blotter germination test (Castillo et al., 1993; Ladone,

1989; Pandey, 1992). In this study, the EC measurement of pea seed lots was evaluated as an indicator of seed vigor for a total of 17 seed lots representing six pea cultivars. The evaluations were done in growth chamber trials under cool and moist soil conditions to assess the susceptibility of the seeds from these lots under conditions favorable for Pythium damping-off. Overall, the *P. ultimum* inoculation rates used in the study (50, 100, and 500 CFU/g soil) resulted in low, intermediate, and high levels of damping-off. A distinct pattern was demonstrated in that pea seed lots with low EC values (< 24 µS/cm/g soil), which corresponds with a high vigor classification (PGRO, 1981), had greater emergence. This was particularly evident at the highest inoculum level of *P. ultimum* for low vigor seed lots with medium or high EC values (25 to 57 µS/cm/g soil), and was observed at both 14 and 28 dap. In the absence of *P. ultimum*, emergence of seed lots was > 90%, even for lots with very high EC values (51 to 57 µS/cm/g soil), which are classified as very low vigor seed lots (PGRO, 1981). However, these very low vigor seed lots performed very poorly in the presence of *P. ultimum*. For all seed lots, emergence decreased as the pathogen inoculum level increased, e.g., emergence was reduced from > 90% emergence in the absence of *P. ultimum* to 20 to 60% in the presence of 500 CFU *P. ultimum*/g soil. The results are consistent with related studies which have reported that the EC values of pea seed lots measured in water correlate negatively with emergence in field conditions (Bradnock and Matthews, 1970; Matthews and Bradnock, 1968; Matthews and Whitebread, 1968). The influence of EC readings on emergence was negligible in the absence of *P. ultimum* in this study, as demonstrated by the high emergence measured for all seed lots in non-inoculated soil. Although the high EC seed lots emerged slower than the seed lots with low EC values (emergence measured at 14 dap), these differences generally disappeared by 28 dap. Such delayed germination reflects the lower vigor of the seed lots with high EC values, which can be

undesirable when planting pea seed lots in suboptimal conditions (e.g. cold and wet soils in early spring). Rapid and uniform emergence is necessary to achieve good yields in pea production, and is the primary reason for planting high quality seed (Castillo et al., 1993; Mullet and Wilkinson, 1979).

The EC measurements of the pea seed lots evaluated in this study were positively or negatively associated with other growth parameters measured for the seed lots, and these effects were most pronounced in the presence of *P. ultimum*. More severe root rot caused by *P. ultimum* was observed on pea plants that developed from the high EC seed lots than the medium or low EC seed lots, particularly at the highest inoculation rate used in this study (500 CFU/g soil). However, plant height was not associated consistently with EC value due to the confounding effects of the cultivars, e.g., seedlings of the cv. Tonic were taller than those of other cultivars, regardless of the EC values of the seed lots and the *P. ultimum* inoculation rates. Shoot biomass, on the other hand, typically was greater for seed lots with low EC as compared to those with high EC readings. These results further illustrate the potential influence of the EC of pea seed lots not only on emergence, but also on the severity of root rot and growth of the pea plants. Kraft (1986) used these growth parameters to assess the influence of seed exudation on resistance of pea to Fusarium and Pythium root rots.

The significant negative relationship of EC to emergence of pea seed lots representing low, medium, and high vigor categories was similar to the results of Matthews and Whitebread (1968), who studied the effect of seed exudation on pre-emergence mortality in wrinkle-seeded peas, however, the effect of EC of the pea seed lots on the different levels of *P. ultimum* inoculum was not investigated by these authors. In this study, at the *P. ultimum* inoculation rates used, emergence decreased with increasing EC value of the seed lots both at 14 and 28 dap. At

low to intermediate inoculum levels (i.e., 50 and 100 CFU/g soil), the relationship between EC and emergence was not always as consistent across trials and seed lots compared to the highest inoculation rate, which can be attributed, in part, to the difficulty of achieving uniform soil inoculation when mixing a very small amount of the soil-oatmeal inoculum with a relatively large volume of soil. Even so, damping-off was observed at these inoculation rates, at which the EC and emergence relationship was not as strong as at 500 CFU *P. ultimum*/g soil. Other studies have reported similar observations with the use of EC as a predictor of field emergence of the seed lots, with a stronger relationship between EC and emergence in the field in the presence of unfavorable conditions during sowing of pea seeds (e.g., suboptimal temperatures or excessive soil moisture) than when field conditions were favorable at sowing (Castillo et al., 1983; Duczmal and Minicka, 1989; Ladone, 1989). In this study, the disease pressure created by the highest inoculation rate of *P. ultimum* in the cool and wet soil conditions in the growth chamber mimicked the unfavorable conditions for pea seeds at planting, under which conditions a stronger relationship between the EC of seed lots and emergence was observed than when *P. ultimum* was absent from the soil.

Examination of the effects of seed exudates, collected from seed lots with high, medium, and low EC levels on mycelial growth of *P. ultimum* 030141 generally showed no significant differences among seed lots in the mycelial dry weights of the pathogen measured after 7 days of incubation in the seed soak water. The exudates clearly enhanced mycelial growth of *P. ultimum* compared to the water control treatment. There also appeared to be slight differences in the amount of mycelial growth of *P. ultimum* between low vs. high EC seed soak suspensions, but this was not reflected statistically, even after increasing the number of replicate samples in an attempt to reduce the variability in mycelial dry weight measurements. Measuring weight of

mycelia might not reflect adequately the potential influences of pea seed exudate suspensions on *P. ultimum*, particularly as seed exudates may also influence germination of sporangia and/or oospores (Nelson, 1990).

In summary, this study demonstrated that the EC of a seed lot can affect emergence of pea seeds and other pea growth parameters, including susceptibility to Pythium damping-off. The results support previous research that has demonstrated that the greater the EC of a pea seed lot, the lower the emergence and the more severe the damping-off (Matthews and Bradnock, 1968; Matthews and Whitebread, 1968). In this study, a negative linear relationship was established between EC and emergence of pea seed lots, with the strongest relationship at high levels of *P. ultimum* inoculum in the soil, and a weaker effect at the low and intermediate inoculum levels. A significant influence of EC directly on mycelial growth of *P. ultimum* was not established in this study, but the methods require further refining to assess more effectively how seed lots with a range of EC might affect this pathogen. The results were limited to highly controlled growth chamber conditions using pasteurized field soil, and need to be validated under more variable and complex field conditions. To understand better the relationship of EC with susceptibility to damping-off of pea seed lots, future research could investigate different *Pythium* inoculum levels under field conditions, particularly inoculum levels that are known to lead to poor stands and significant yield losses for growers. A greater understanding of the relationship between EC and emergence can be established by increasing the number of seed lots evaluated to include a wider range of EC levels and more seed lots at each level to minimize experimental error and help separate confounding factors such as cultivar effects. A greater understanding of the quantitative relationship of EC and emergence under field conditions could provide a tool for growers to assess the risk of damping-off prior to planting, which could improve damping-off management

programs. This is particularly pertinent to organic pea growers for whom highly effective seed treatments for this disease are still lacking (see Chapter 3). A DNA based tool for quantifying inoculum levels of *Pythium* spp. in the soil, e.g., real-time PCR assays (see Chapter 2) could be used together with EC measurement of seed lots for predicting the potential risk to damping-off during early spring planting conditions. This could be beneficial to organic growers by helping them avoid having to increase the rate of seeds used for sowing in anticipation of poor stands. This could reduce production costs, particularly for expensive, certified organic pea seed lots.

4.5. Literature cited

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Table 4.1. Pea seed lots of six cultivars representing a range in seed electrolyte leakage, evaluated for susceptibility to Pythium damping-off in growth chamber trials

Cultivar and seed lot	EC (µS/cm/g)[a]	Vigor category[b]	Germination (%)[c]	Seed company[d]
Bistro 364017	28.8	Medium	94	Brotherton Seed Co., Inc.
Bistro 364027	31.0	Low	99	Brotherton Seed Co., Inc.
Bistro 364061	36.6	Low	92	Brotherton Seed Co., Inc.
Boogie 610154	20.0	High	97	Brotherton Seed Co., Inc.
Boogie 610144	20.3	High	96	Brotherton Seed Co., Inc.
Boogie 611009	34.5	Low	90	Brotherton Seed Co., Inc.
Tonic 344024	12.7	High	99	Brotherton Seed Co., Inc.
Tonic 343194	24.1	Medium to high	98	Brotherton Seed Co., Inc.
Tonic 343144	39.2	Low	91	Brotherton Seed Co., Inc.
PLS 134 34012	51.2	Very low	90	Pure Line Seeds, Inc.
PLS 134 34022	57.1	Very low	97	Pure Line Seeds, Inc.
PLS 560 60011	14.1	High	99	Pure Line Seeds, Inc.
PLS 560 60030	28.4	Medium	98	Pure Line Seeds, Inc.
PLS 560 60012	37.2	Low	93	Pure Line Seeds, Inc.
Serge 62012	26.6	Medium	98	Pure Line Seeds, Inc.
Serge 62062	37.8	Low	96	Pure Line Seeds, Inc.
Serge 62052B	38.0	Low	95	Pure Line Seeds, Inc.

[a] Electrical conductivity (EC) of 50 seeds soaked in deionized water and measured following the protocol of the Processors and Growers Research Organization (PGRO, 1981).

[b] Seed vigor of each seed lot was based on the EC reading and classified according to the PGRO (1981), where high vigor = EC < 24 µS/cm/g, which is considered suitable for early season seeding; medium vigor = 25 to 29 µS/cm/g, which indicates some losses in stand may occur in adverse field conditions, but the lot usually can be used for drilling later in the season without stand problems; low vigor = 30 to 43 µS/cm/g, which indicates the seed lot is not suitable for early sowing and may result in losses in cold, wet soil conditions; very low = > 43 µS/cm/g, which means the seed lot is not suitable for sowing in any conditions.

[c] Blotter seed germination assay performed by the seed company in 2011 for the seed lots from Brotherton Seed Co., Inc., and in 2012-13 for the seed lots provided by Pure Line Seeds, Inc.

[d] Source of the pea seed lots: Brotherton Seed Co., Inc. is located in Moses Lake, WA, and Pure Line Seeds, Inc. in Warden, WA.

Table 4.2. Results of Trial 1 evaluating susceptibility of nine pea seed lots of three cultivars from Brotherton Seed Co., Inc. to different inoculum levels of *Pythium ultimum* in a growth chamber set at 8°C for night and 13°C for day with a 12 h photoperiod/day

	Emergence (%)[b]		Root	Shoot	Shoot dry
ANOVA factor[a]	**14 dap**	**28 dap**	**rot rating[c]**	**length (cm)[d]**	**weight (g)[e]**
Cultivar and seed lot main effect (vigor rating)[f]					
Bistro 364017 (M)	32 bc	43 b	3.10	3.5 c	0.20 b
Bistro 364027 (L)	29 bc	37 bc	3.20	3.0 c	0.16 b
Bistro 364061 (L)	26 bc	33 c	3.20	3.3 c	0.15 b
Boogie 610154 (H)	35 b	40 bc	3.20	3.3 c	0.20 b
Boogie 610144 (H)	35 bc	43 b	3.40	3.8 c	0.19 b
Boogie 611009 (L)	31 bc	39 bc	3.20	3.4 c	0.16 b
Tonic 344024 (H)	43 a	53 a	3.30	6.1 a	0.28 a
Tonic 343194 (M-H)	33 bc	40 bc	3.20	4.9 b	0.20 b
Tonic 343144 (L)	27 c	34 c	3.30	3.8 c	0.19 b
LSD	Rank	8	NS	1.0	Arcsine
ANOVA *P* value	0.0135	0.0003	0.2739	< 0.0001	0.0001
Inoculation rate main effect[g]					
0 CFU/g soil	77 a	93 a	0.0 a	7.1 a	0.51 a
100 CFU/g soil	16 b	22 b	4.6 b	3.5 b	0.06 b
500 CFU/g soil	4 c	5 c	5.0 c	1.1 c	0.01 c
LSD	Rank	5	0.2	1.00	Arcsine
ANOVA *P* value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cultivar-by-inoculation interaction					
0 CFU/g soil					
Bistro 364017 (M)	70 ab	90 ab	0.00	5.8 b-d	0.48
Bistro 364027 (L)	74 ab	94 ab	0.00	5.5 b-e	0.45
Bistro 364061 (L)	60 b	76 b	0.00	5.6 b-e	0.39
Boogie 610154 (H)	86 a	98 a	0.00	6.6 b	0.54
Boogie 610144 (H)	78 ab	90 ab	0.00	6.3 bc	0.48
Boogie 611009 (L)	76 ab	98 a	0.00	8.9 bc	0.44
Tonic 344024 (H)	88 a	100 a	0.00	9.6 a	0.69
Tonic 343194 (M-H)	86 a	96 a	0.00	8.9 a	0.56
Tonic 343144 (L)	76 ab	94 ab	0.00	9.3 a	0.56
100 CFU/g soil					
Bistro 364017 (M)	26 c	32 c	4.20	3.7 f-g	0.10
Bistro 364027 (L)	14 c-f	16 c-f	4.60	3.4 f-h	0.04
Bistro 364061 (L)	18 c-e	22 c	4.50	4.3 d-f	0.06
Boogie 610154 (H)	12 e-g	20 c-e	4.60	3.4 f-h	0.06
Boogie 610144 (H)	28 c-e	32 c	5.00	3.5 f-h	0.09
Boogie 611009 (L)	16 c-f	18 cd	4.50	3.9 e-f	0.04
Tonic 344024 (H)	20 d-g	26 c	4.20	4.2 d-f	0.10
Tonic 343194 (M-H)	6 f-h	16 c-f	4.70	3.0 f-h	0.03
Tonic 343144 (L)	6 f-h	8 d-g	4.80	2.0 g-i	0.01
500 CFU/g soil					
Bistro 364017 (M)	0 h	6 gh	5.00	1.0 ij	0.01
Bistro 364027 (L)	0 h	0 h	5.00	0.0 j	0.00
Bistro 364061 (L)	0 h	0 h	5.00	0.0 j	0.00
Boogie 610154 (H)	6 gh	2 gh	5.00	0.0 j	0.00
Boogie 610144 (H)	0 h	6 f-h	5.00	1.8 h-j	0.01
Boogie 611009 (L)	0 h	0 h	5.00	0.0 j	0.00
Tonic 344024 (H)	20 cd	26 c	5.00	4.5 c-f	0.08
Tonic 343194 (M-H)	6 fg	8 e-g	5.00	2.8 f-i	0.01
Tonic 343144 (L)	6 h	0 h	5.00	0.0 j	0.00
LSD	Rank	Rank	NS	1.8	NS

ANOVA *P* value	< 0.0001	0.0397	0.3628	< 0.0001	0.0863

[a] A factorial experiment of nine pea seed lots and three *P. ultimum* inoculation rates (0, 100, and 500 CFU/g soil) was set up with five replicate pots/treatment combination. Data were analyzed by analyses of variance (ANOVA) at $\alpha = 0.05$ and Fisher's protected least significant difference (LSD, $P = 0.05$). 'NS' means no significant difference. 'Arcsine' and 'rank' mean the data were subjected to arcsine or rank transformation (Friedman's non-parametric rank transformation) to satisfy the assumptions of parametric analyses.

[b] Emergence of pea plants was counted 14 and 28 days after planting (dap) and expressed as a percentage of 10 seeds planted/pot, for five replicate pots/treatment combination.

[c] Pea root rot was rated on a scale of 0 to 5, where 0 = no visible symptoms on the roots; 1 = a few, small, discolored lesions on the roots; 2 = minor discoloration covering most of the roots and epicotyl; 3 = brown discoloration of the epicotyl and roots, with most of the lateral roots missing; 4 = complete root discoloration and lateral roots missing; 5 = rotten seed with no roots.

[d] Pea shoot length (cm) was measured 28 dap from the point of seed attachment to the tip of the last leaf.

[e] Pea shoot dry weight (g) was measured 28 dap for all shoots present in a pot, after drying the shoots at 90°C for 2 to 3 days.

[f] Seed lots representing different vigor levels were obtained from Brotherton Seed Co., Inc. Each seed lot vigor rating of high (H), medium to high (M-H), medium (M), or low (L) was based on the Processors and Growers Research Organization (PGRO, 1981). Refer to Table 4.1 for details of the seed lot vigor ratings, and electrical conductivity measurements.

[g] Inoculation of pasteurized soil with *Pythium ultimum* isolate 030141 in a soil-oatmeal carrier, quantified by plating a dilution series of the soil onto *Pythium* selective agar medium (Mircetich and Kraft, 1973) to achieve the desired inoculation rate.

Table 4.3. Results of Trial 2 evaluating susceptibility of nine pea seed lots of three cultivars from Brotherton Seed Co., Inc. to different inoculum levels of *Pythium ultimum* in a growth chamber set at 8°C for night and 13°C for day with a 12 h photoperiod/day

ANOVA factor[a]	Emergence (%)[b] 14 dap	Emergence (%)[b] 28 dap	Root rot rating[c]	Shoot length (cm)[d]	Shoot dry weight (g)[e]
Cultivar and seed lot (vigor rating)[f]					
Bistro 364017 (M)	61 c-e	74 c-d	2.0 b-d	4.6 d	0.32 c-d
Bistro 364027 (L)	57 d-f	71 d-e	2.2 b-c	4.8 d	0.30 d
Bistro 364061 (L)	50 f	63 e-f	2.4 a-b	4.5 d	0.27 d-e
Boogie 610154 (H)	74 a-b	87 a-b	1.7 d	5.3 c	0.40 b
Boogie 610144 (H)	66 b-d	82 b-c	2.2 b-d	4.8 c-d	0.32 d
Boogie 611009 (L)	50 f	58 f	2.5 a	4.8 d	0.23 e
Tonic 344024 (H)	78 a	88 a	1.9 c-d	7.3 a	0.48 a
Tonic 343194 (M-H)	69 a-c	76 cd	2.1 b-c	7.1 a	0.39 b-c
Tonic 343144 (L)	52 e-f	58 f	2.5 a	6.1 b	0.25 e
LSD	9	Rank	Rank	Rank	0.05
ANOVA *P* value	< 0.0001	<0.0001	< 0.0001	< 0.0001	< 0.0001
Inoculation rate main effect[g]					
0 CFU/g soil	78 a	94 a	0.0 d	6.2 a	0.50 a
50 CFU/g soil	77 a	83 b	2.1 b	5.9 a	0.37 b
100 CFU/g soil	64 b	74 c	2.7 c	5.7 a	0.32 c
500 CFU/g soil	29 c	39 d	3.9 a	4.1 b	0.12 d
LSD	6	Rank	Rank	Rank	0.04
ANOVA *P* value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cultivar-by-inoculation interaction					
0 CFU/g soil					
Bistro 364017 (M)	72	92 a-c	0.0	5.0	0.44
Bistro 364027 (L)	78	98 a-b	0.0	5.4	0.51
Bistro 364061 (L)	64	96 a-c	0.0	5.1	0.45
Boogie 610154 (H)	80	100 a	0.0	5.2	0.46
Boogie 610144 (H)	84	100 a	0.0	5.1	0.47
Boogie 611009 (L)	62	72 d-f	0.0	5.3	0.42
Tonic 344024 (H)	94	100 a	0.0	8.4	0.64
Tonic 343194 (M-H)	88	100 a	0.0	8.5	0.63
Tonic 343144 (L)	84	92 a-c	0.0	7.3	0.47
50 CFU/g soil					
Bistro 364017 (M)	78	74 b-d	1.5	5.1	0.42
Bistro 364027 (L)	68	76 f-h	1.8	5.1	0.31
Bistro 364061 (L)	70	74 f-i	2.4	4.7	0.30
Boogie 610154 (H)	90	92 a-c	1.8	5.7	0.46
Boogie 610144 (H)	80	94 a-c	2.2	5.2	0.35
Boogie 611009 (L)	72	70 f-i	2.4	4.9	0.25
Tonic 344024 (H)	90	92 a-c	2.2	8.3	0.54
Tonic 343194 (M-H)	80	88 c-e	1.8	7.5	0.42
Tonic 343144 (L)	64	70 f-i	2.4	7.0	0.29
100 CFU/g soil					
Bistro 364017 (M)	62	74 f-h	2.5	4.9	0.33
Bistro 364027 (L)	58	72 f-i	3.2	4.9	0.27
Bistro 364061 (L)	48	58 h-m	2.8	4.9	0.26
Boogie 610154 (H)	86	92 a-c	2.0	5.9	0.47
Boogie 610144 (H)	60	80 e-g	2.3	5.1	0.29
Boogie 611009 (L)	58	72 f-j	3.0	5.1	0.22
Tonic 344024 (H)	88	96 a-c	2.0	7.5	0.52
Tonic 343194 (M-H)	74	72 f-i	2.9	6.9	0.33
Tonic 343144 (L)	64	54 i-m	3.5	5.8	0.19

500 CFU/g soil					
Bistro 364017 (M)	34	40 k-n	4.1	3.5	0.12
Bistro 364027 (L)	26	36 m-n	3.8	3.7	0.12
Bistro 364061 (L)	20	24 n	4.3	3.3	0.07
Boogie 610154 (H)	40	64 h-l	3.0	4.5	0.21
Boogie 610144 (H)	42	52 j-n	4.4	3.9	0.16
Boogie 611009 (L)	8	18 n	4.6	3.9	0.03
Tonic 344024 (H)	42	62 g-k	3.3	5.1	0.05
Tonic 343194 (H-M)	36	44 l-n	3.6	5.4	0.13
Tonic 343144 (L)	16	16 n	4.3	3.9	0.22
LSD	NS	Rank	NS	NS	NS
ANOVA *P* value	0.4070	0.0195	0.0681	0.4230	0.0952

[a] A factorial experiment of nine pea seed lots and three *P. ultimum* inoculation rates (0, 100, and 500 CFU/g soil) was set up with five replicate pots/treatment combination. Data were analyzed by analyses of variance (ANOVA) at $\alpha = 0.05$ and Fisher's protected least significant difference (LSD, $P = 0.05$). 'NS' means no significant difference. 'Arcsine' and 'rank' mean the data were subjected to arcsine or rank transformation (Friedman's non-parametric rank transformation) to satisfy the assumptions of parametric analyses.

[b] Emergence of pea plants was counted 14 and 28 days after planting (dap) and expressed as a percentage of 10 seeds planted/pot, for five replicate pots/treatment combination.

[c] Pea root rot was rated on a scale of 0 to 5, where 0 = no visible symptoms on the roots; 1 = a few, small, discolored lesions on the roots; 2 = minor discoloration covering most of the roots and epicotyl; 3 = brown discoloration of the epicotyl and roots, with most of the lateral roots missing; 4 = complete root discoloration and lateral roots missing; 5 = rotten seed with no roots.

[d] Pea shoot length (cm) was measured 28 dap from the point of seed attachment to the tip of the last leaf.

[e] Pea shoot dry weight (g) was measured 28 dap for all shoots present in a pot, after drying the shoots at 90°C for 2 to 3 days.

[f] Seed lots representing different vigor levels were obtained from Brotherton Seed Co., Inc. Each seed lot vigor rating of high (H), medium to high (M-H), medium (M), or low (L) was based on the Processors and Growers Research Organization (PGRO, 1981). Refer to Table 4.1 for details of the seed lot vigor ratings, and electrical conductivity measurements.

[g] Inoculation of pasteurized soil with *Pythium ultimum* isolate 030141 in a soil-oatmeal carrier, quantified by plating a dilution series of the soil onto *Pythium* selective agar medium (Mircetich and Kraft, 1973) to achieve the desired inoculation rate.

Table 4.4. Results of Trial 1 evaluating susceptibility of ten pea seed lots of five cultivars (eight seed lots from Pure Line Seeds Co. and two seed lots from Brotherton Seed Co., Inc.,) to different inoculum levels of *Pythium ultimum* in a growth chamber set at 8°C for night and 13°C for day with a 12 h photoperiod/day

	Emergence (%)[b]		Root	Shoot	Shoot dry
ANOVA factor[a]	**14 dap**	**28 dap**	**rot rating[c]**	**length (cm)[d]**	**weight (g)[e]**
Cultivar and seed lot main effect (vigor rating)[f]					
PLS 134 34012 (VL)	29 g	48 e	2.9 a	5.3 de	0.17 g
PLS 134 34022 (VL)	38 fg	59 de	2.7 ab	5.4 de	0.19 fg
PLS 560 60011 (H)	71 a	79 ab	1.7 de	5.6 de	0.29 bc
PLS 560 60030 (M)	49 de	71 bc	2.0 c-e	5.3 ef	0.24 c-e
PLS 560 60012 (L)	39 e-g	55 e	2.5 ab	5.0 fg	0.18 fg
Serge 62012 (M)	64 ab	81 a	1.7 e	6.6 b	0.32 ab
Serge 62062 (L)	48 d-f	66 cd	2.1 b-d	5.8 cd	0.22 d-f
Serge 62052B (L)	55 b-d	67 cd	2.1 b-d	6.1 bc	0.22 e-g
Bistro 364017 (M)	50 cd	72 bc	1.8 c-e	4.8 g	0.27 b-d
Tonic 343194 (M-H)	60 bc	73 bc	1.9 c-e	7.6 a	0.35 a
LSD	11	Rank	Rank	Rank	0.05
ANOVA *P* value	< 0.0001	< 0.001	< 0.0001	0.0145	< 0.0001
Inoculation rate main effect[g]					
0 CFU/g soil	72 a	94 a	0.3 c	6.4 a	0.38 a
50 CFU/g soil	54 b	72 b	2.1 b	6.0 b	0.25 b
100 CFU/g soil	53 b	69 b	2.3 b	5.8 b	0.26 b
500 CFU/g soil	23 c	34 c	3.8 a	4.8 c	0.10 c
LSD	7	Rank	Rank	Rank	0.03
ANOVA *P* value	< 0.0001	< 0.0001	< 0.0001	<0.0001	< 0.0001
Cultivar-by-inoculation interaction					
0 CFU/g soil					
PLS 134 34012 (VL)	66	94 a-c	0.1 m	6.3	0.39 a-d
PLS 134 34022 (VL)	50	90 a-d	0.6 lm	5.7	0.27 g-l
PLS 560 60011 (H)	92	100 a	0.0 m	6.4	0.43 ab
PLS 560 60030 (M)	82	96 ab	0.2 m	5.8	0.35 a-e
PLS 560 60012 (L)	54	86 a-e	0.8 k-m	5.5	0.30 c-i
Serge 62012 (M)	74	96 ab	0.4 m	7.5	0.47 a-c
Serge 62062 (L)	72	96 ab	0.0 m	6.5	0.36 a-d
Serge 62052B (L)	84	96 ab	0.2 m	6.5	0.36 a-e
Bistro 364017 (M)	64	90 a-d	0.1 m	5.4	0.36 a-e
Tonic 343194 (M-H)	78	92 a-c	0.3 m	8.5	0.49 a
50 CFU/g soil					
PLS 134 34012 (VL)	14	30 no	3.8 a-d	4.5	0.10 q-t
PLS 134 34022 (VL)	62	80 b-g	1.6 j-l	6.2	0.30 d-j
PLS 560 60011 (H)	72	80 b-g	1.9 g-j	5.6	0.28 e-l
PLS 560 60030 (M)	42	80 b-g	2.0 g-j	5.6	0.25 h-m
PLS 560 60012 (L)	42	58 h-k	2.5 f-h	5.1	0.18 n-r
Serge 62012 (M)	74	88 a-d	1.4 j-l	6.5	0.30 c-h
Serge 62062 (L)	56	66 g-i	1.8 g-j	6.0	0.23 i-n
Serge 62052B (L)	48	79 e-h	2.3 f-j	6.1	0.21 k-o
Bistro 364017 (M)	60	82 b-g	1.7 h-k	4.9	0.29 d-i
Tonic 343194 (M-H)	72	84 a-f	2.4 f-i	7.2	0.38 a-c
100 CFU/g soil					
PLS 134 34012 (VL)	28	50 i-l	3.4 c-e	5.4	0.17 m-q
PLS 134 34022 (VL)	30	36 l-n	4.4 a-d	5.7	0.11 q-t
PLS 560 60011 (H)	74	78 c-g	3.0 h-k	5.8	0.29 d-k
PLS 560 60030 (M)	42	80 b-g	1.9 g-j	5.6	0.26 f-k
PLS 560 60012 (L)	44	60 h-j	2.2 f-j	5.4	0.21 l-p

Serge 62012 (M)	74	92 a-c	1.5 j-l	6.9	0.39 b-f
Serge 62062 (L)	44	68 f-h	2.5 e-g	6.2	0.23 j-o
Serge 62052B (L)	60	66 g-i	2.1 f-j	6.2	0.22 l-o
Bistro 364017 (M)	58	82 b-g	1.8 h-k	5.1	0.34 b-g
Tonic 343194 (M-H)	62	74 d-h	1.7 i-k	7.8	0.37 a-e
500 CFU/g soil					
PLS 134 34012 (VL)	8	16 o	4.5 a	4.8	0.04 t
PLS 134 34022 (VL)	12	28 no	4.0 a-c	3.9	0.07 st
PLS 560 60011 (H)	48	58 h-k	3.0 d-f	4.8	0.16 o-r
PLS 560 60030 (M)	14	28 n-o	3.9 a-d	4.3	0.10 q-t
PLS 560 60012 (L)	16	16 o	4.4 ab	4.1	0.04 t
Serge 62012 (M)	36	48 j-m	3.4 b-d	5.7	0.13 p-t
Serge 62062 (L)	20	34 l-n	3.9 a-c	4.5	0.08 q-t
Serge 62052B (L)	28	34 l-n	3.9 a-c	5.4	0.09 r-t
Bistro 364017 (M)	18	32 m-o	3.9 a-d	4.0	0.11 q-t
Tonic 343194 (M-H)	30	42 k-n	3.4 c-e	6.9	0.15 o-s
LSD	NS	18	Rank	NS	Rank
ANOVA *P* value	0.0778	< 0.0001	0.0044	0.4474	< 0.0001

[a] A factorial experiment of ten pea seed lots and three *P. ultimum* inoculation rates (0, 50, 100, and 500 CFU/g soil) was set up with five replicate pots/treatment combination. Data were analyzed by analyses of variance (ANOVA) at $\alpha = 0.05$ and Fisher's protected least significant difference (LSD, $P = 0.05$). 'NS' means no significant difference. 'Rank' means data was subjected to rank transformation (Friedman's non-parametric rank transformation) to satisfy the assumptions of parametric analyses.

[b] Emergence of pea plants was counted 14 and 28 days after planting (dap) and expressed as a percentage of 10 seeds planted/pot, for five replicate pots/treatment combination.

[c] Pea root rot was rated on a scale of 0 to 5, where 0 = no visible symptoms on the roots; 1 = a few, small, discolored lesions on the roots; 2 = minor discoloration covering most of the roots and epicotyl; 3 = brown discoloration of the epicotyl and roots, with most of the lateral roots missing; 4 = complete root discoloration and lateral roots missing; 5 = rotten seed with no roots.

[d] Pea shoot length (cm) was measured 28 dap from the point of seed attachment to the tip of the last leaf.

[e] Pea shoot dry weight (g) was measured 28 dap for all shoots present in a pot, after drying the shoots at 90°C for 2 to 3 days.

[f] Seed lots representing different vigor levels were obtained from Pure Line Seeds, Inc. Each seed lot vigor rating of high (H), medium (M), low (L), or very low (VL) was based on the Processors and Growers Research Organization (PGRO, 1981). Refer to Table 4.1 for details of the seed lot vigor ratings, and electrical conductivity measurements.

[g] Inoculation of pasteurized soil with *Pythium ultimum* isolate 030141 in a soil-oatmeal carrier, quantified by plating a dilution series of the soil onto *Pythium* selective agar medium (Mircetich and Kraft, 1973) to achieve the desired inoculation rate.

Table 4.5. Results of Trial 2 evaluating susceptibility of ten pea seed lots of five cultivars (eight seed lots from Pure Line Seeds Co. and two seed lots from Brotherton Seed Co., Inc.,) to different inoculum levels of *Pythium ultimum* in a growth chamber set at 8°C for night and 13°C for day with a 12 h photoperiod/day

	Emergence (%)[b]		Root	Shoot	Shoot dry
ANOVA factor[a]	**14 dap**	**28 dap**	**rot rating[c]**	**length (cm)[d]**	**weight (g)[e]**
Cultivar and seed lot (vigor rating)[f]					
PLS 134 34012 (VL)	51 de	58 de	2.6 a	5.9 f	0.28 ef
PLS 134 34022 (VL)	56 c-e	67 cd	2.2 ab	6.7 c-e	0.29 ef
PLS 560 60011 (H)	75 ab	81 ab	2.0 a-c	6.2 ef	0.38 b-d
PLS 560 60030 (M)	64 bc	69 c	2.3 ab	6.3 d-f	0.31 d-f
PLS 560 60012 (L)	51 e	53 e	2.6 a	5.9 f	0.25 f
Serge 62012 (M)	77 a	82 a	1.7 c	7.5 b	0.40 bc
Serge 62062 (L)	61 c-e	66 cd	2.2 ab	7.1 b-d	0.31 d-f
Serge 62052B (L)	68 a-c	71 bc	1.9 bc	7.4 bc	0.34 c-e
Bistro 364017 (M)	59 c-e	73 a-c	2.1 bc	5.9 f	0.42 ab
Tonic 343194 (M-H)	63 cd	73 a-c	2.2 a-c	9.2 a	0.48 a
LSD	12	10	Rank	0.7	0.08
ANOVA *P* value	< 0.0001	< 0.0001	0.0326	< 0.0001	< 0.0001
Inoculation rate[g]					
0 CFU/g soil	89 a	96 a	0.0 d	7.9 a	0.56 a
50 CFU/g soil	73 b	79 b	2.1 c	6.9 b	0.38 b
100 CFU/g soil	51 c	59 c	3.0 b	6.5 bc	0.26 c
500 CFU/g soil	37 d	44 d	3.6 a	6.0 c	0.19 d
LSD	7	6	Rank	0.5	0.05
ANOVA *P* value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cultivar-by-inoculation interaction					
0 CFU/g soil					
PLS 134 34012 (VL)	92	93	0.0	7.6	0.52
PLS 134 34022 (VL)	83	100	0.0	7.2	0.46
PLS 560 60011 (H)	95	100	0.0	7.3	0.55
PLS 560 60030 (M)	85	98	0.0	7.3	0.49
PLS 560 60012 (L)	90	90	0.0	6.7	0.15
Serge 62012 (M)	95	100	0.0	8.2	0.55
Serge 62062 (L)	88	90	0.1	8.3	0.51
Serge 62052B (L)	90	95	0.0	8.4	0.59
Bistro 364017 (M)	70	93	0.0	6.8	0.62
Tonic 343194 (M-H)	98	98	0.0	11.3	0.78
50 CFU/g soil					
PLS 134 34012 (VL)	53	60	2.8	6.2	0.22
PLS 134 34022 (VL)	70	75	2.3	6.4	0.30
PLS 560 60011 (H)	85	85	2.0	6.6	0.41
PLS 560 60030 (M)	75	78	2.1	6.1	0.34
PLS 560 60012 (L)	65	65	3.3	6.0	0.27
Serge 62012 (M)	88	93	1.1	7.6	0.47
Serge 62062 (L)	70	78	1.9	7.0	0.35
Serge 62052B (L)	75	80	1.4	7.2	0.31
Bistro 364017 (M)	78	90	1.9	6.1	0.54
Tonic 343194 (M-H)	75	88	1.5	9.2	0.58
100 CFU/g soil					
PLS 134 34012 (VL)	53	58	3.3	5.9	0.31
PLS 134 34022 (VL)	58	53	3.1	6.5	0.22
PLS 560 60011 (H)	38	68	2.8	5.2	0.26
PLS 560 60030 (M)	48	53	3.7	6.0	0.25
PLS 560 60012 (L)	35	45	2.8	5.8	0.17

Serge 62012 (M)	68	73	2.6	7.4	0.35
Serge 62062 (L)	58	63	3.0	6.3	0.22
Serge 62052B (L)	63	65	2.8	7.2	0.27
Bistro 364017 (M)	40	55	2.9	5.5	0.27
Tonic 343194 (M-H)	45	55	3.2	8.8	0.29
500 CFU/g soil					
PLS 134 34012 (VL)	8	20	4.2	4.1	0.07
PLS 134 34022 (VL)	35	40	3.4	6.8	0.18
PLS 560 60011 (H)	58	70	3.2	5.7	0.31
PLS 560 60030 (M)	50	50	3.3	5.9	0.18
PLS 560 60012 (L)	13	13	4.1	4.9	0.09
Serge 62012 (M)	58	63	3.0	6.6	0.24
Serge 62062 (L)	30	33	3.9	6.6	0.15
Serge 62052B (L)	43	43	3.5	6.7	0.19
Bistro 364017 (M)	48	55	3.3	5.4	0.25
Tonic 343194 (M-H)	33	50	3.8	7.3	0.27
LSD	NS	NS	NS	NS	NS
ANOVA *P* value	0.1507	0.2098	0.351	0.4692	0.5977

[a] A factorial experiment of ten pea seed lots and three *P. ultimum* inoculation rates (0, 50, 100, and 500 CFU/g soil) was set up with five replicate pots/treatment combination. Data were analyzed by analyses of variance (ANOVA) at $\alpha = 0.05$ and Fisher's protected least significant difference (LSD, $P = 0.05$). 'NS' means no significant difference. 'Rank' means data was subjected to rank transformation (Friedman's non-parametric rank transformation) to satisfy the assumptions of parametric analyses.

[b] Emergence of pea plants was counted 14 and 28 days after planting (dap) and expressed as a percentage of 10 seeds planted/pot, for five replicate pots/treatment combination.

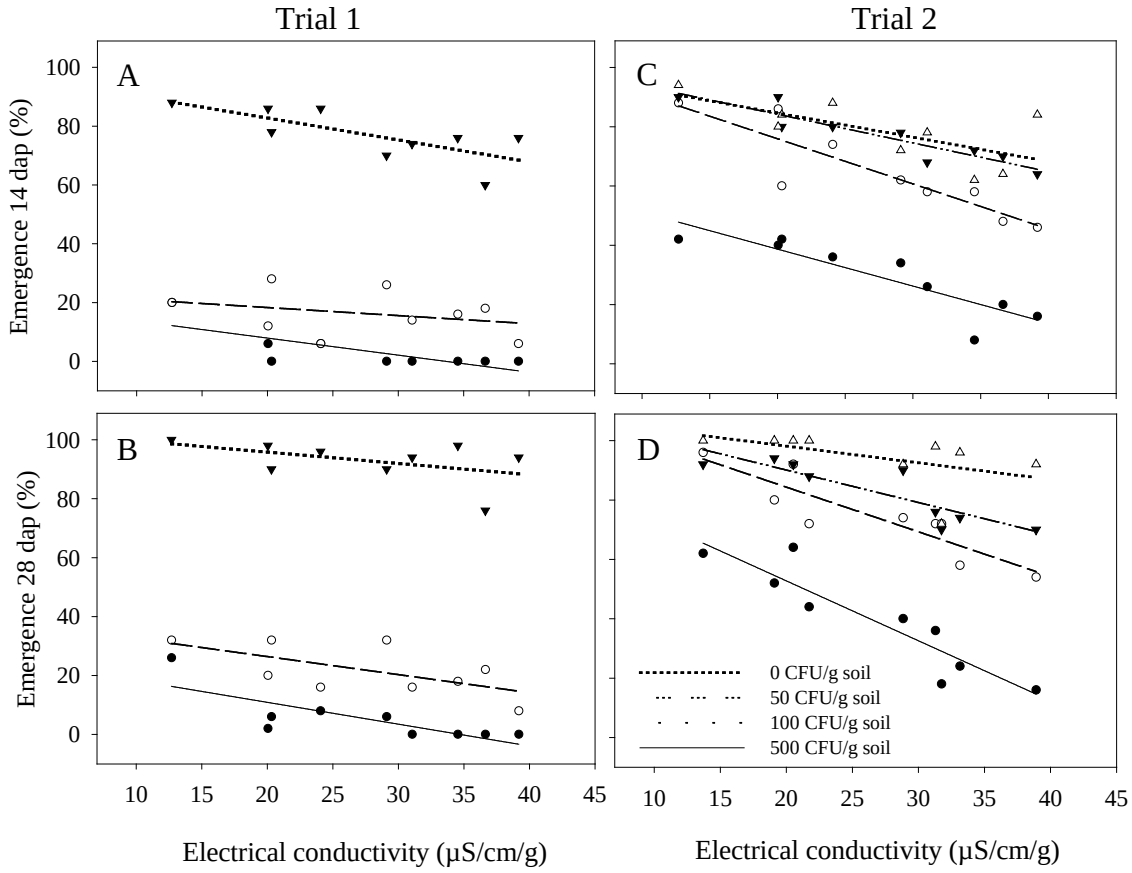
[c] Pea root rot was rated on a scale of 0 to 5, where 0 = no visible symptoms on the roots; 1 = a few, small, discolored lesions on the roots; 2 = minor discoloration covering most of the roots and epicotyl; 3 = brown discoloration of the epicotyl and roots, with most of the lateral roots missing; 4 = complete root discoloration and lateral roots missing; 5 = rotten seed with no roots.

[d] Pea shoot length (cm) was measured 28 dap from the point of seed attachment to the tip of the last leaf.

[e] Pea shoot dry weight (g) was measured 28 dap for all shoots present in a pot, after drying the shoots at 90°C for 2 to 3 days.

[f] Seed lots representing different vigor levels were obtained from Pure Line Seeds, Inc. Each seed lot vigor rating of high (H), medium (M), low (L), or very low (VL) was based on the Processors and Growers Research Organization (PGRO, 1981). Refer to Table 4.1 for details of the seed lot vigor ratings, and electrical conductivity measurements.

[g] Inoculation of pasteurized soil with *Pythium ultimum* isolate 030141 in a soil-oatmeal carrier, quantified by plating a dilution series of the soil onto *Pythium* selective agar medium (Mircetich and Kraft, 1973) to achieve the desired inoculation rate.

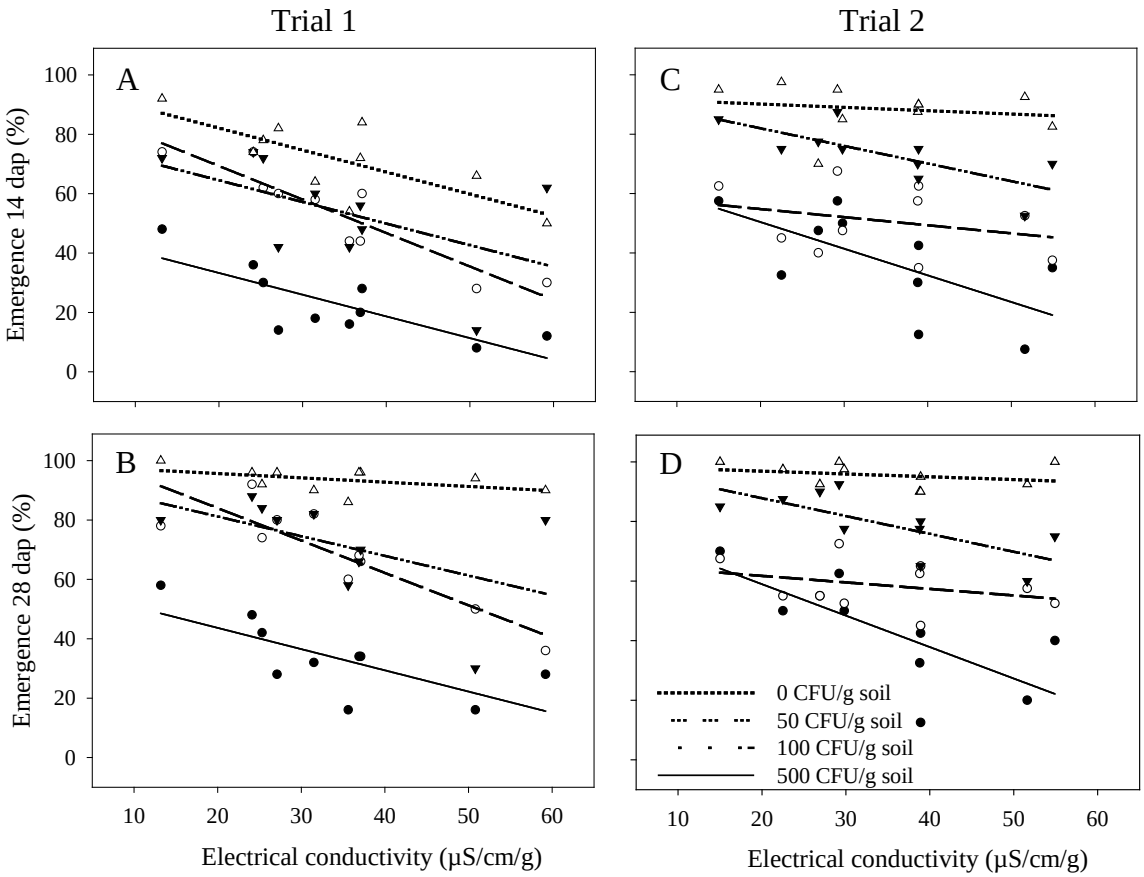


Emergence CFU/g soil	*P*	R^2	Ŷ
14 dap			
0	0.0229	0.5461	Ŷ = -0.74x + 97.60
50	-	-	-
100	0.4146	0.0970	Ŷ = -0.27x + 23.80
500	0.0160	0.5871	Ŷ = -0.58x + 19.54
28 dap			
0	0.2020	0.2207	Ŷ = -0.38x + 103.46
50	-	-	-
100	0.0688	0.3974	Ŷ = -0.61x + 38.62
500	0.0129	0.6107	Ŷ = -0.74x + 25.72

Emergence CFU/g soil	*P*	R^2	Ŷ
14 dap			
0	0.0467	0.4535	Ŷ = -0.82x + 100.94
50	0.0003	0.8563	Ŷ = -0.97x + 103.51
100	0.0014	0.7888	Ŷ = -1.52x + 106.33
500	0.0015	0.7857	Ŷ = -1.25x + 63.63
28 dap			
0	0.1642	0.2565	Ŷ = -0.56x + 109.36
50	0.0019	0.7697	Ŷ = -1.09x + 111.91
100	0.0012	0.7953	Ŷ = -1.50x + 114.36
500	0.0004	0.8258	Ŷ = -2.02x + 93.11

Fig. 4.1. Regression analyses for two growth chamber trials in which electrical conductivity (EC) was compared to emergence measured 14 and 28 days after planting (dap) for nine seed lots representing three pea cultivars from Brotherton Seed Co., Inc. (Bistro, Tonic, and Boogie)

planted in soil inoculated *Pythium ultimum* isolate 030141 at 0, 50, 100, and 500 CFU/g soil. Each data point is the mean of five replicate pots/seed lot. R^2 = coefficient of determination, *P* = probability of no significant regression relationship between the two variables, Y = predicted emergence (%), and x = EC of the seed lot (µS/cm/g).



Emergence CFU/g soil	*P*	R^2	Ŷ
14 dap			
0	0.0139	0.5511	Ŷ = -0.75x + 96.84
50	0.1192	0.2756	Ŷ = -0.73x + 79.02
100	0.0002	0.8470	Ŷ = -1.13x + 91.84
500	0.0068	0.6202	Ŷ = -0.73x + 47.92
28 dap			
0	0.1666	0.2245	Ŷ = -0.14x + 98.55
50	0.1314	0.2609	Ŷ = -0.66x + 94.39
100	0.0008	0.7722	Ŷ = -1.09x + 105.79
500	0.0178	0.5247	Ŷ = -0.71x + 57.98

Emergence CFU/g soil	*P*	R^2	Ŷ
14 dap			
0	0.6286	0.0306	Ŷ = -0.11x + 92.40
50	0.0134	0.5548	Ŷ = -0.59x + 93.82
100	0.0471	0.0874	Ŷ = -0.27x + 60.19
500	0.0433	0.4182	Ŷ = -0.90x + 68.35
28 dap			
0	0.4161	0.0842	Ŷ = -0.09x + 98.77
50	0.0210	0.5066	Ŷ = -0.60x + 99.75
100	0.3517	0.1089	Ŷ = -0.22x + 66.08
500	0.0167	0.5315	Ŷ = -1.05x + 80.06

Fig. 4.2. Regression analyses for two growth chamber trials in which electrical conductivity (EC) was compared to emergence measured 14 and 28 days after planting (dap) for 10 seed lots representing five pea cultivars (PLS 134, PLS 560, Serge, Tonic, and Boogie), eight lots from Pure Line Seeds, Inc. and two from Brotherton Seed, Co., Inc., planted in soil inoculated

Pythium ultimum isolate 030141 at 0, 50, 100, and 500 CFU/g soil. Each data point is the mean of five replicate pots/seed lot. R^2 = coefficient of determination, *P* = probability of no significant regression relationship between the two variables, Y = predicted emergence (%), and x = EC of seed lot (µS/cm/g).

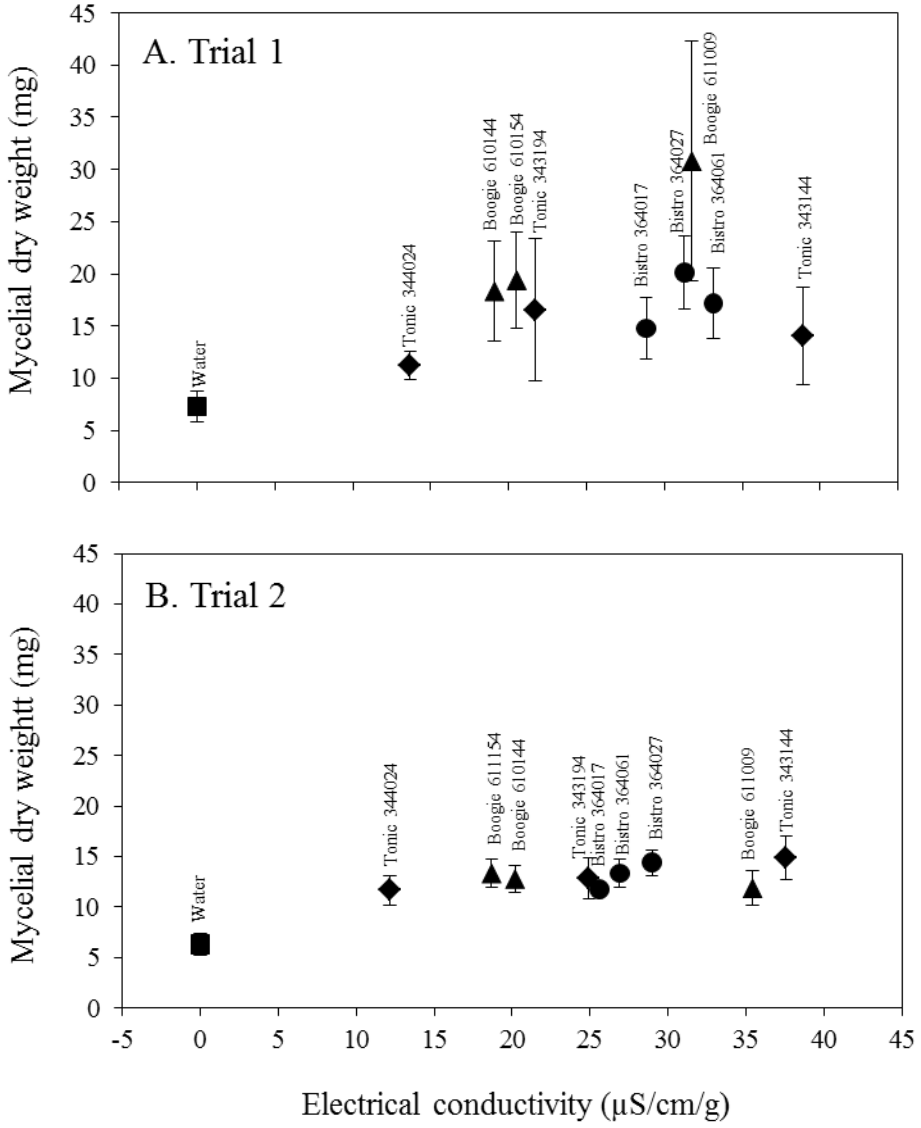


Fig. 4.3. Influence of the level of seed exudation of each of nine seed lots representing three pea cultivars [Bistro (●), Boogie (▲) and Tonic (♦)] on mycelial growth of *Pythium ultimum* isolate 030141. The nine seed lots had different electrical conductivity readings measured following the protocol of the Processors and Growers Research Organization (1981). Mycelial dry weight was measured after 7 days of incubation of *P. ultimum* at 23 ± 1°C in the seed lot soak water. Each data point is the mean ± standard error of four (A) and six (B) replicate plates/seed lot.

Appendix Table 2.1. Summary of the pathogenicity of isolates of each of nine *Pythium* spp. baited from certified organic fields in the Columbia Basin in Washington, and tested on the pea cv. Tonic[a]

		Trial 1				Trial 2			
Species[b]	**Isolate**	**Emergence (28 dap)[c]**	**Shoot length (cm)[d]**	**Shoot dry weight (g)[e]**	**Virulence[f]**	**Emergence (28 dap)[c]**	**Shoot length (cm)[d]**	**Shoot dry weight (g)[e]**	**Virulence[f]**
P. abappressorium	B3-94	1.7 e	4.6 bc	0.09 cd	++	78.0 ab	6.5 ab	0.39 ab	
	B13-168	1.9 de	4.1 c	0.07 d	++	70.0 b	5.7 bc	0.34 b	+
	B12-160	2.2 cd	5.6 bc	0.16 bcd	++	42.0 de	4.6 cd	0.12 de	+
	B11-97	3.1 ab	7.6 a	0.42 a		48.0 bcd	6.5 ab	0.32 b	+
	B11-111	2.4 c	4.6 bc	0.16 bcd	++	60.0 bcd	4.6 d	0.17 cde	+
	B10-16	2.4 c	5.0 bc	0.16 bcd	++	66.0 bc	5.7 bc	0.26 bcd	+
	B10-2	2.2 cd	4.4 bc	0.11 bcd	++	46.0 cd	4.0 de	0.15 cde	+
	B11-86	2.5 c	5.4 bc	0.22 b	++	74.0 ab	6.9 ab	0.37 b	+
Non-inoculated control[g]		3.1 a	7.8 a	0.44 a		96.0 a	7.6 a	0.52 a	
P. ultimum control[h]	030141	2.2 cd	5.8 b	0.15 bcd		20.0 e	3.3 e	0.04 e	
LSD[i]		0.5	1.6	0.11		23.0	1.2	0.15	
P value[j]		< 0.0001	< 0.0001	< 0.0001		< 0.0001	< 0.0001	< 0.0001	
Transformation[k]		Rank							
P. dissotocum	B4-4	8.8 ab	6.9 ab	0.68 ab		0.9 ab	7.4 a	6.90 a	
	B4-5	5.5 cd	6.4 ab	0.57 bc	+	1.0 a	6.2 ab	8.00 a	
	B3-84	6.9 bc	6.6 ab	0.60 b	+	0.9 ab	7.0 ab	6.70 a	
	B5-59	4.2 d	5.3 ab	0.44 c	+	0.9 ab	5.6 abc	6.20 a	
	B1-32	7.1 bc	6.6 ab	0.62 b		0.8 b	3.1 cd	3.30 bc	+
	B4-2	8.1 ab	7.3 a	0.68 ab		0.9 ab	7.8 a	6.90 a	
	B2-49	3.7 de	6.5 ab	0.45 c	+	0.6 c	4.0 bcd	3.00 bc	+
	B3-79	8.5 ab	6.4 ab	0.64 ab		0.8 b	6.8 ab	5.80 ab	
Non-inoculated control		10.0 a	7.3 a	0.77 a		0.9 ab	6.1 abc	7.20 a	
P. ultimum control	030141	1.4 f	1.1 c	0.20 d		0.0 d	1.0 d	1.00 c	
LSD		2.2	1.7	0.15		0.2	3.0	2.80	
P value		< 0.0001	< 0.0001	< 0.0001		< 0.0001	0.0059	0.0001	
Transformation		Rank		Arcsine		Arcsine	Rank	Rank	

Appendix Table 2.1. *continued…*

		Trial 1				Trial 2			
Species[b]	**Isolate**	**Emergence (28 dap)[c]**	**Shoot length (cm)[d]**	**Shoot dry weight (g)[e]**	**Virulence[f]**	**Emergence (28 dap)[c]**	**Shoot length (cm)[d]**	**Shoot dry weight (g)[e]**	**Virulence[f]**
P. echinulatum	B3-69	88.0 a	7.8 a	0.40 ab		0.6 b	7.4 a	0.23 b	+
	B12-118	80.0 ab	4.8 b	0.35 abc		0.8 ab	7.3 a	0.33 ab	
	B5-62	78.0 ab	5.4 ab	0.32 abc		0.9 a	7.3 a	0.37 ab	
	B5-47	68.0 bc	4.8 b	0.28 bc		0.8 ab	7.7 a	0.36 ab	
	B5-45	74.0 abc	6.0 ab	0.33 abc		0.8 ab	7.6 a	0.34 ab	
	B1-16	64.0 bc	4.6 b	0.27 c	+	0.8 ab	7.9 a	0.39 ab	
	B2-51	60.0 c	3.8 bc	0.25 c	+	0.6 b	7.0 a	0.24 ab	
Non-inoculated control[g]		86.0 a	6.6 ab	0.40 ab		0.9 a	7.7 a	0.45 a	
P. ultimum control[h]	030141	34.0 d	1.2 c	0.08 d		0.0 c	0.0 b	0.00 c	
LSD[i]		18.0	2.9	0.12		0.2	1.3	0.21	
P value[j]		< 0.0001	0.0059	0.0002		< 0.0001	< 0.0001	0.0298	
Transformation[k]			Rank			Arcsine			
P. intermedium	B2-55	96.0 a	8.4 a	0.44 a					
	B3-76	8.0 b	4.5 b	0.04 b	++	0.5 b	1.8 b	1.80 b	++
Non-inoculated control		100.0 a	8.6 a	0.48 a		1.0 a	3.0 a	3.00 a	
P. ultimum control	030141	6.0 b	1.9 c	0.01 b		0.1 c	1.2 b	1.20 b	
LSD		7.0	2.3	0.08		0.3	0.6	0.60	
P value		< 0.0001	< 0.0001	< 0.0001		0.0033	0.0075	0.0075	
Transformation						Arcsine	Rank	Rank	
P. irregulare	B2-44	44.0 de	4.9 bc	0.31 c	++	3.8 bc	4.8 c	0.03 c	+
	B1-19	66.0 bc	4.0 bc	0.19 de	++	4.8 b	4.3 c	0.04 c	+
	B1-14	54.0 cd	5.1 b	0.27 cd	++	7.6 a	6.3 b	0.05 b	+
	B1-20	74.0 b	4.3 bc	0.10 e	++	2.5 c	5.0 bc	0.03 c	+
	B3-80	32.0 e	8.4 a	0.50 b	++	8.2 a	8.7 a	0.07 a	
	B1-22	96.0 a	4.0 bc	0.15 e	++	4.5 b	4.3 c	0.04 c	+
	B2-43	54.0 cd	5.2 b	0.26 cd	++	4.5 b	4.8 c	0.04 c	+
Non-inoculated control		100.0 a	9.1 a	0.61 a		8.1 a	8.7 a	0.07 a	
P. ultimum control	030141	66.0 bc	3.6 c	0.13 e		1.0 d	0.0 d	0.00 d	
LSD		19.0	1.3	0.10		1.4	1.4	0.01	
P value		< 0.0001	< 0.0001	< 0.0001		< 0.0001	< 0.0001	< 0.0001	
Transformation						Rank		Arcsine	

Appendix Table 2.1. *continued…*

		Trial 1				Trial 2			
Species[b]	Isolate	Emergence (28 dap)[c]	Shoot length (cm)[d]	Shoot dry weight (g)[e]	Virulence[f]	Emergence (28 dap)[c]	Shoot length (cm)[d]	Shoot dry weight (g)[e]	Virulence[f]
P. splendens	B5-45	0.0 b	0.0 c	0.00 b	++	80.0 b	3.5 b	1.90 b	++
Non-inoculated control[g]		100.0 a	8.6 a	0.50 a		94.0 a	7.6 a	6.80 a	
P. ultimum control[h]	030141	6.0 b	1.9 b	0.01 b		20.0 b	0.4 c	1.50 b	
LSD[i]		7.0	1.4	0.10		90.0	2.9	2.00	
P value[j]		< 0.0001	< 0.0001	< 0.0001		< 0.0001	0.0110	< 0.0001	
Transformation[k]								Rank	
P. sylvaticum	B10-37	38.0 b	3.9 b	0.05 b	+	0.8 b	4.5 c	0.82 b	+
	B12-148	52.0 ab	4.3 b	0.06 ab	+	0.9 ab	5.3 bc	0.87 b	+
	B12-151	50.0 ab	4.9 ab	0.07 a		0.8 b	4.9 bc	0.83 b	+
	B12-147	54.0 ab	4.6 ab	0.07 a		0.8 b	5.5 b	0.85 b	+
Non-inoculated control		58.0 a	5.6 a	0.07 a		1.1 a	8.2 a	0.99 a	
P. ultimum control	030141	4.0 c	1.0 c	0.01 c		0.0 c	0.0 d	0.71 c	
LSD		19.0	1.3	0.01		0.2	0.9	0.07	
P value		< 0.0001	< 0.0001	< 0.0001		< 0.0001	< 0.0001	< 0.0001	
Transformation				Arcsine		Arcsine		Square root	
P. ultimum	B8-113	1.1 d	4.8 bc	4.50 de	+++	3.3 e	3.1 ef	0.01 fg	++
	B2-42	1.5 cd	7.4 abc	6.10 cd	+++	6.3 d	5.8 d	0.03 cd	+
	B4-9	1.0 d	4.7 c	3.00 e	+++	3.7 e	3.6 e	0.02 ef	++
	B1-1	2.2 b	8.6 ab	9.20 ab	++	7.0 cd	8.2 bc	0.05 bc	+
	B8-96	1.0 d	3.7 c	2.60 e	+++	1.4 f	1.4 f	0.00 g	++
	B9-141	1.1 d	5.6 abc	3.20 e	+++	8.4 bc	8.6 abc	0.05 ab	+
	B3-82	2.1 bc	5.2 bc	7.10 bc	++	8.1 c	6.6 cd	0.05 bc	+
	B1-23	1.1 d	3.7 c	3.10 e	+++	4.3 e	5.0 de	0.02 de	++
	B3-73	2.2 b	6.6 abc	8.40 abc	++	10.0 ab	9.8 ab	0.06 a	
Non-inoculated control		2.9 a	9.2 a	10.20 a		10.0 a	10.4 a	0.07 a	
P. ultimum control	030141	2.3 ab	6.5 abc	8.60 ab		3.0 ef	3.5 e	0.01 fg	
LSD		0.6	0.62	2.40		1.71	2.1	0.01	
P value		< 0.0001	< 0.0001	< 0.0001		< 0.0001	< 0.0001	< 0.0001	
Transformation		Square root	Rank	Rank		Rank	Rank	Arcsine	

Appendix Table 2.1. *continued…*

		Trial 1				Trial 2			
Species[b]	**Isolate**	**Emergence (28 dap)**[c]	**Shoot length (cm)**[d]	**Shoot dry weight (g)**[e]	**Virulence**[f]	**Emergence (28 dap)**[c]	**Shoot length (cm)**[d]	**Shoot dry weight (g)**[e]	**Virulence**[f]
P. violae	B11-102	24.0 bcd	5.6 bcde	0.08 bcd	++	32.0 b	4.5 de	0.08 bc	+
	B11-77	24.0 bcd	3.2 de	0.06 bcd	++	26.0 b	4.2 de	0.07 c	+
	B13-198	30.0 bcd	6.4 bcd	0.10 bcd	++	24.0 b	4.5 de	0.04 cd	++
	B12-139	20.0 cd	2.6 e	0.04 cd	++	28.0 b	6.3 bcd	0.06 c	+
	B10-53	24.0 bcd	6.2 bcd	0.08 bcd	++	38.0 b	6.0 abc	0.13 b	+
	B13-194	24.0 bcd	6.4 bcd	0.08 bcd	++	30.0 b	7.3 bcd	0.09 bc	+
	B12-149	32.0 bc	7.0 bc	0.11 bc	++	26.0 b	8.8 ab	0.08 bc	+
	B12-145	14.0 d	4.2 cde	0.03 d	++	30.0 b	4.3 de	0.05 cd	++
	B13-197	38.0 b	7.8 ab	0.12 b	+	24.0 b	5.1 cde	0.05 cd	++
Non-inoculated control[g]		66.0 a	10.8 a	0.42 a		86.0 a	10.8 a	0.42 a	
P. ultimum control[h]	030141	14.0 d	5.8 bcde	0.07 bcd		2.0 c	2.2 e	0.00 d	
LSD[i]		17.0	3.5	0.08		19.0	3.1	0.06	
P value[j]		< 0.0001	0.0028	0.0089		< 0.0001	0.0005	< 0.0001	
Transformation[k]			Rank				Rank		

[a] Each pathogenicity trial was carried out in a growth chamber set at 13°C by day and 8°C by night with a 12 h photoperiod/day. Treatments were arranged in a randomized complete block design (RCBD) with five replications (pots)/treatment.

[b] *Pythium* species identified for isolates baited from soils collected from 37 certified organic fields in the Columbia Basin of Washington in 2009 (see Table 2.1). Only the 9 of the 19 *Pythium* species isolated that had pathogenic isolates are shown in this table. Isolates that were non-pathogenic to pea in these trials, and included the following species: *P. adhaerens, P. aristosporum, P. camurandrum, P. catenulatum, P. diclinum, P. inflatum, P. middletonii, P. oligandrum, P. radiosum,* and *P. torulosum.* Refer to Table 2.2 for the list of isolates of the non-pathogenic species included in the pathogenicity trials.

[c] Mean number of seedlings emerged from five replicate pots/treatment, with a total of 10 seeds planted/pot.

[d] Mean shoot length of seedlings in five replicate pots/treatment measured from the point of seed attachment to the tip of the last leaf.

[e] Mean shoot dry weight of seedlings from five replicate pots/treatment.

[f] Aggressiveness rating of the isolates at causing damping-off symptoms on pea plant relative to a the control isolate of *P. ultimum* 030141, where: +++ = more aggressive than the control isolate, ++ = as aggressive as the control isolate, and + = less virulence than the control isolate. Isolates with no '+' were rated as not pathogenic.

[g] Non-inoculated control treatment included in each pathogenicity trial.

[h] Pathogenic control treatment using *P. ultimum* isolate 030141 obtained from T. Paulitz (Wheat Pathology Lab, USDA ARS, Pullman, WA), included in each pathogenicity trial.

[i] Protected Fisher's least significant difference (LSD) test at $P < 0.05$.

[j] Analysis of variance (ANOVA) significance level, ($\alpha = 0.05$).

[k] Mean transformation using arcsine, log, square root or rank (latter calculated as Friedman's non-parametric rank test) to satisfy the assumptions for ANOVA. Transformed means are shown in the table. Refer to Fig. 2.5 to 2.13 for the original means.

Appendix Table 3.1. Field trials to evaluate organic seed and drench treatments for damping-off control in organic pea production in the semi-arid Columbia Basin of Oregon and Washington, respectively, and maritime northwestern Washington in Mount Vernon in 2011

	Boardman			Soap Lake				Mount Vernon			
	Seedling emergence (%) [a]		Yield	Seedling emergence (%)		Plant height	Yield	Seedling emergence (plants/m^2)		Plant height	Yield
Treatment and rate	**21 dap**	**35 dap**	**(kg/ha)[b]**	**21 dap**	**35 dap**	**(cm)[c]**	**(kg/ha)**	**21dap**	**35 dap**	**(cm)**	**(kg/ha)**
Seed treatments											
Acadian Marine Plant Extract 5ml/kg seeds……	23 cd[d]	29 bc	4,739 bc	67 ab	83 a-c	62.7 a-c	10,663 b-d	72 ab	77 b	21.1 b-d	5,607 bc
Actinovate STP 2.5 g/kg seeds…..	21 cd	23 c	3,036 c	65 ab	80 b-f	59.4 cd	9,393 d	61 b-e	64 b-d	20.6 cd	4,844 bc
Germains II proprietary rate……..	-	-	-	-	-	-	-	53 ef	57 d	20.3 d	4,170 bc
Heads Up Plant Protectant 0.3 g/170 kg seeds/liter water.....	22 cd	27 bc	4,163 c	70 a	86 a	62.7 a-c	12,714 ab	70 bc	75 bc	20.8 cd	5,154 bc
Myco Seed Treat 2.5 g/kg seeds…	24 cd	30 bc	4,297 c	70 a	83 a-c	61.2 b-d	12,051 a-c	62 b-e	66 b-d	20.8 cd	4,464 bc
Mycostop Mix 5 g/kg seeds.....	23 cd	29 bc	4,643 bc	65 ab	81 a-f	65.0 ab	8,834 d	53 c	68 b-d	20.8 cd	5,231bc
Natural II proprietary rate.....	25 cd	32 bc	4,843 bc	64 ab	84 a-c	59.4 cd	11,145 a-d	60 b-e	66 b-d	21.6 b-d	4,461 bc
Nordox 75 WG 0.14 g/kg seeds....	40 b	47 a	6,898 ab	71 a	86 ab	65.3 a	13,083 a	64 bc	78 b	21.6 b-d	5,182 bc
Prestop Mix 5 g/kg seeds……...	-	-	-	-	-	-	-	55 c-f	61 cd	20.1 d	4,312 bc
Primed seeds…….....	51 a	57 a	8,515 a	59 b	74 f	59.7 cd	11,899 a-c	38 f	43 e	20.6 cd	3,816 c
T-22 HC 1.3 g/kg seeds.....	22 cd	23 c	3,786 c	67 ab	82 a-d	62.7 a-c	10,950 a-d	61 bcd	64 b-d	21.3 b-d	5,417 bc
Sprayed treatment[e]											
Prestop WP 0.1 liter/0.4 ha……	30 c	36 bc	4,937 bc	-	-	-	-	65 bc	72 bc	20.6 cd	5,898 bc
Drench treatments[f]											
Serenade soil 5.8 liters/0.4 ha.....	23 cd	27 bc	3,614 c	36 c	77 e-f	59.9 cd	10,517 b-d	62 bcd	69 b-d	21.1 cd	4,536 bc
Soilgard 12G 3.6 kg/0.4 ha……	23 cd	28 bc	4,659 bc	37 c	77 c-f	57.9 d	10,201 cd	55 cde	63 cd	20.6 cd	4,766 bc
Stimplex 0.05 liters/0.4 ha…….	27 c	33 bc	4,891 bc	43 c	80 b-f	60.9 b-d	9,855 cd	58 bc	66 b-d	20.3 d	4,834 bc
Control treatments[g]											
Apron XL 0.8 ml/kg seeds……	-	-	-	-	-	-	-	116 a	118 a	27.7 a	10,012 a
NTS, NI soil.....	25 cd	30 bc	4,975 bc	66 ab	82 a-e	61.5 a-d	10,407 b-d	63 bcd	68 b-d	20.8 cd	6,232 bc
NTS + water drench 13 liters/ 10 m^2……	18 d	24 c	4,035 c	43 c	76 d-f	59.4 cd	9,098 d	-	-	-	-
NTS, inoculated soil……..	-	-	-	-	-	-	-	54 c-f	59 d	21.1 b-d	4,740 bc
NTS, inoculated soil + water drench……..	-	-	-	-	-	-	-	55 d-f	58 d	22.1 bc	4,198 bc
NTS, NI + soil-oatmeal…….	-	-	-	-	-	-	-	71 ab	77 b	22.9 b	5,751 bc
LSD…….....	9	10	2,266	10	Rank	4.1	2,345	Rank	Log	4.3	1,920

[a] Seedling emergence measured 21 and 35 days after planting (dap) based on 230 seeds planted in the center two rows/plot.

[b] Yield measured from 1 m of each of the four center rows/plot.

[c] Plant height measured for 16 plants sampled from both ends of the two center rows/plot at full bloom of the pea crop.

[d] Means followed by the same letter are not significantly different based on Fisher's protected least significant difference (LSD, $P < 0.05$). For transformed data, original means are shown but means separation letters are based on log (for emergence measured 28 dap in the Mount Vernon trial) and rank (for emergence measured 35 dap in the Soap Lake trial, and emergence measured 21 dap in the Mount Vernon trial) transformations. Dependent variables with no significant effects in the ANOVA are not shown. NS = no significant differences among treatments based on Fisher's protected LSD.

[e] Applied at 2.1 kg/cm^2 in 3,279 liters of water/ha using a CO_2 backpack sprayer. Prestop WP was not applied in the Soap Lake trial because of very windy conditions at the time of planting that prevented application.

[f] For drench treatments, each product was applied in 1.3 liters water/m^2 at planting and again 14 dap.

[g] Control treatments in the Mount Vernon trial included non-inoculated plots (NI) and plots in which the soil was inoculated with *Pythium ultimum* isolate 030141, both of which were planted with non-treated seeds (NTS). NTS, NI + soil-oatmeal control plots were inoculated with the carrier used to produce the inoculum, but without the pathogen.

Appendix Table 3.2. Evaluation of priming and organic seed treatment combinations for the control of damping-off in organic pea production in Ephrata in the semi-arid Columbia Basin of central Washington and Mount Vernon in maritime western Washington in 2012

Treatment and rate	Ephrata trial				Mount Vernon trial			
	Seedling emergence (%)[a]			Yield	Seedling emergence (%)[b]			Yield
	14 dap	28 dap	TR[c]	(kg/ha)[d]	14 dap	28 dap	TR	(kg/ha)
ANOVA main factors								
Primed seeds (PS)……..	26 b	59 b	106	11,129 b	56 b	68 b	82	8,865
Dry seeds (DS)……	35 a	80 a	108	13,926 a	67 a	80 a	82	9,027
LSD…….	4	4	4	1,055	2	2	2	540
Treatment combinations								
PS								
Acadian Marine Plant Extract 5 ml/kg seed….....	39 ab[e]	63 ef	106	13,359 ab	57 ghi	66 h	83	8,490
Acadian Powder 5 g/kg seed[f]…….....	-	-	-	-	54 hi	69 fgh	83	9,030
Heads Up Plant Protectant 0.3 g/170 kg seeds/liter water …..…..….....	15 ef	44 fg	107	8,733 c	54 i	65 h	81	8,612
Myco Seed Treat 2.5 g/kg seed……	25 de	63 de	106	11,564 b	59 f-i	71 d-h	84	9,726
Mycostop Mix 5 g/kg seed.....	32 a-d	71 cde	105	11,579 b	60 e-i	70 e-h	82	9,350
Natural II proprietary rate.....	12 f	34 g	105	7,305 c	56 hi	67 gh	82	8,731
Nordox 75 WG 0.14 g/kg seed……	29 bcd	66 def	109	12,182 b	40 j	50 i	80	7,209
Non-treated seeds.....	33 a-d	72 cd	105	13,183 ab	57 ghi	71 e-h	81	9,203
Apron XL 0.8 ml/kg seed[f]…….....	-	-	-	-	70 a-c	84 ab	80	9,437
DS								
Acadian Marine Plant Extract 5 ml/kg seeds.....	37 abc	80 a	115	12,715 b	70 ab	82 a-c	81	9,293
Acadian Powder 5 g/kg seed seed[f]…….....	-	-	-	-	62 b-h	78 b-e	83	9,727
Heads Up Plant Protectant 0.3 g/170 kg seeds/liter water ….....	35 a-d	90 a	103	14,234 ab	63 b-g	74 c-f	83	8,886
Myco Seed Treat 2.5 g/kg seed……..……	37 abc	82 a	112	15,803 a	60 d-i	78 b-e	82	8,437
Mycostop Mix 5 g/kg seed…..……	32 a-d	79 ab	104	13,772 ab	61 c-i	75 c-f	84	8,791
Natural II proprietary rate.....	40 a	75 bc	111	13,772 ab	67 b-f	80 a-c	82	8,951
Nordox 75 WG 0.14 g/kg seed……..….	36 a-d	82 a	106	13,655 ab	68 a-e	79 bcd	83	9,172
Non-treated seed…..……..	28 cd	75 bc	104	13,528 ab	69 a-d	81 a-c	79	8,411
Apron XL 0.8 ml/kg seed[f]…….....	-	-	-	-	76 a	89 a	81	9,582
LSD……..…….…....	11	Rank[g]	NS	2,732	9	8	NS	NS

[a] Seedling emergence measured 14 and 28 days after planting (dap) based on 230 seeds planted in the center two rows/plot.

[b] Seedling emergence measured 14 and 28 dap based on 490 seeds planted in the center four rows/plot.

[c] TR = mean of two tenderometer readings of the peas harvested/plot.

[d] Yield was measured from the center 1.5 m of the four center rows/plot.

[e] Within the main factors, or for the treatment combinations, means followed by the same letter are not significantly different based on Fisher's protected least significant difference (LSD, $P = 0.05$). Dependent variables with no significant treatment effects in the ANOVA are not shown, except for tenderometer readings (TR) for both trials, and yield data for the Mount Vernon trial. NS = no significant differences among treatments.

[f] Acadian Powder was not approved for use in certified organic pea production in Washington State in 2012, so this treatment was not evaluated in the Ephrata trial which was in a certified organic, grower-cooperator's field.

[g] Rank = original means are shown but means separation is based on rank transformation due to heterogeneous variances.

Appendix Table 3.3. Field trials to evaluate seed priming with biochar as a desiccant and Nordox 75 WG seed treatment for control of damping-off in organic pea production in two fields near Royal City, WA in 2012

	Trial 1					Trial 2				
	Seedling emergence (%)[b]		Plant height		Yield	Seedling emergence (%)		Plant height		Yield
Seed treatment and rate[a]	14 dap	28 dap	(cm)[c]	TR[d]	(kg/ha)[e]	14 dap	28 dap	(cm)	TR	(kg/ha)
PS + biochar + Nordox 75 WG FR…	59 a[f]	93 a	9.9 a	91 a	7,034 ab	47 a	83 a	8.1 ab	110	6,191 a
PS + biochar + Nordox 75 WG HR…	54 ab	91 ab	9.9 a	94 a	7,006 a	37 a	83 a	8.1 ab	111	5,053 ab
PS + biochar……	45 b	86 b	9.4 ab	91 a	6,097 abc	36 a	73 bc	7.4 abc	109	4,800 ab
DS + Nordox 75 WG FR ……	8 c	70 c	7.9 c	81 c	4,583 c	14 b	69 cd	7.9 abc	103	4,534 ab
DS + biochar + Nordox 75 WG FR…	9 c	68 c	8.4 c	82 c	5,039 bc	7 b	60 d	6.6 c	98	3,341 b
DS, NTS……..…….	9 c	66 c	8.6 bc	83 bc	4,983 c	10 b	57 d	7.4 bc	100	3,611 b
PS, NTS…….. ……	47 b	88 ab	9.6 a	89 ab	6,820 a	38 a	81 ab	8.6 a	105	5,697 a
LSD.....	9	6	1.0	6	Rank[g]	12	Rank	3.3	NS	1,714

[a] PS = primed seed, DS = dry seed, FR = Nordox applied at the full rate of 0.14 g/kg seeds, HR = Nordox applied at the half rate of 0.07 g/kg, NTS = non-treated seeds.

[b] Seedling emergence (%) calculated based on 230 seeds planted in the center two rows/plot, and measured 14 and 28 days after planting (dap).

[c] Plant height was measured for 20 plants/plot from the center two rows at full bloom.

[d] TR = the mean of two tenderometer readings of the peas harvested/plot.

[e] Yield was measured from the center 1.5 m of the four center rows/plot.

[f] Means followed by the same letter are not significantly different based on Fisher's protected least significant difference (LSD, $P = 0.05$). Dependent variables with no significant effects in the ANOVA are not shown, except for TR and yield in Trial 2. NS = no significant differences among treatments.

[g] Rank = original means are shown but means separation is based on rank transformations due to heterogeneous variances.