The Effects of Salinity on Pythium Disease of Rice and Soybean

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The Effects of Salinity on Pythium Disease of Rice and Soybean
The Effects of Salinity on Pythium Disease of Rice and Soybean

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology

by

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This thesis is approved for recommendation to the Graduate Council.

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ABSTRACT

Increasing salinity is an important factor limiting agricultural productivity worldwide. In addition to direct effects on growth and yield, diseases also may be affected. This study characterized the effects of soil salinity on seedling disease of soybean and rice caused by *Pythium* spp. Controlled environment experiments on soybean used two cultivars which differed in chloride tolerance and soil treated with a CaCl$_2$ solution to create a range of electrical conductivity (EC) levels. For soybean, soil was either not infested or infested with *Pythium sylvaticum* or *P. aphanidermatum* (pathogenic to soybean), or *P. oligandrum* (not pathogenic to soybean). Twenty-one days after planting, seedling stand, growth, and development were assessed. Salinity reduced seedling stand at or above 2.057 dS/m. Electrical conductivity averaged 0.640 (control), 1.060, 1.632, and 2.039 dS/m in the first experiment and 0.930 (control), 1.483, 2.057, and 2.570 dS/m in the second experiment using the soil dilution method (2:1) to measure EC. Leaf number, shoot weight and root altitude also decreased at or above 2.057 dS/m. Root volume and root tips decreased at 2.570 dS/m (Exp 2) but not at lower EC levels. Shoot growth decreased with *P. aphanidermatum* and *P. sylvaticum* at moderate salinity increasing growth reductions compared to the control. Cultivars which differed in chloride tolerance responded similarly in these experiments. Root development was stimulated by pathogen infestations at the base EC levels. Salinity had no effect when the nonpathogenic species were used. Controlled environment experiments on rice used two genotypes which differed in Pythium resistance, Wells (susceptible), and PI 560281 (having moderate resistance). For rice, soil was either not infested or infested with *Pythium irregulare* or *P. torulosum* (pathogenic to rice) or *P. ultimum* (not pathogenic to rice). Thirty-five days after planting, seedling stand, growth, and development were assessed. Electrical conductivity averaged 0.651
(control), 1.113, 1.658, and 2.190 dS/m for calcium chloride treatments. Salinity significantly reduced stand at 1.113 dS/m. Shoot growth and root development also decreased at 1.113 dS/m. *P. irregulare* and *P. torulosum* decreased stand across all EC levels. These pathogens decreased shoot growth and root development at low EC levels (including the base salinity), but this effect was overwhelmed as salinity increased. *P. ultimum* slightly decreased emergence and stand at the base salinity, but had a protective effect against increasing salinity levels at and above 1.113 dS/m. *P. ultimum* also increased root altitude across salinity levels (0.651 and 1.658-2.190 dS/m). Pythium resistance for PI 560281 was only evident as greater emergence after 14 days. *In vitro*, experiments were conducted on *Pythium* spp. over a range of electrical conductivity levels using CaCl₂ solutions. Zoospore production, discharge, motility, and chemotaxis; oospore germination; and mycelial growth were used to assess salinity effects. EC levels in zoospore experiments ranged from 0.3 (control) to 4.3 dS/m. Salinity significantly decreased zoospore production and motility at EC levels as low as 1.3 dS/m, while zoospore discharge was reduced at 3.3 dS/m, and zoospore taxis was not significantly affected. EC levels in oospore and mycelium experiments ranged from 2.3 (control) to 12.8 dS/m. Mycelial growth and oospore germination were not significantly affected by increased EC. Abiotic interactions are important considerations in understanding and managing diseases. This research suggests that *Pythium* spp. caused greater seedling disease in the presence of a stress such as occurs with increasing soil salinity. *Pythium* spp. which were not virulent at the base soil salinity caused disease at moderate EC levels (soybean experiment), while *Pythium* spp. which are virulent at base salinity levels have an additive effect that diminishes at high EC levels. Understanding the biology of seedling pathogens and how they are affected by environment is important for the management of seedling diseases over the range of planting environments. This research indicates that *Pythium*
spp. are able to grow and reproduce at EC levels which limit seedling stand establishment. Results suggest that increased Pythium disease by increasing EC levels is not the result of conditions which favor pathogen development.
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DEDICATION

This thesis is dedicated to Kalyn and Grant Stetina. Kalyn, I hope you stay curious about the world in which we live and keep asking all those questions. Your genuine interest and absolute faith gave me a lot to live up to and I hope you are proud of your Mom. Grant, you are a delight and such a helpful young man. Thank you for all your clever ideas and also for highlighting my SAS reports. You are both my greatest work and the best part of my life.
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I. LITERATURE REVIEW

SOYBEAN

Soybean (Glycine max (L.) Merr) is an important crop produced and consumed worldwide. The United States is the world's leading soybean producer, with approximately 30 million hectares grown annually and producing approximately 91.5 million metric tons valued at an estimated $32 billion (Soystats, 2010; Wrather and Koenning, 2006). Arkansas is one of the top ten soybean producing states in the U.S.A., with 1,384 million hectares planted in 2010 producing 3.34 metric tons. Soybean accounts for 35% of Arkansas's annual crop production, and is valued at approximately $1.2 billion annually (Soystats, 2010). Soybean production in Arkansas occurs in more than 45 counties concentrated in the eastern part of the state and in the Mississippi Delta region, as well as in the Arkansas River Valley and the southwest corner of the state (Coats and Ashlock, 2011).

Soybean is frequently rotated with rice, which is economically more profitable, in order to increase rice yield. The symbiotic relationship between soybean and nitrogen-fixing Bradyrhizobium in soybean nodules increases available nitrogen to soybean and subsequent crops if incorporated back into field soil (Anders et al, 2004). In Arkansas, 68% of rice acreage is rotated with soybean (Wilson et al., 2009).

RICE

Rice is a staple food for a large portion of the world's population, and an economically important crop produced on approximately 10 percent of the world's cropland. In the United States, rice (Oryza sativa L.) is grown on nearly 1.5 million hectares with an annual yield of
approximately 7.5 million metric tons. Arkansas produces close to 50 percent of rice grown in the U.S.A. in 40 counties. Arkansas rice production is concentrated in the eastern part of the state in the Delta area, but also occurs in the Arkansas River Valley as well as the southwest and is valued at more than $1 billion annually (Webster and Gunnell, 1992; Watkins et al., 2004; Arkansas Rice Research and Promotion Board, 2012; USDA, 2012).

Rice production is highly water intensive. In eastern Arkansas, more than 4 million acres of cropland are irrigated, with 90 percent of inputs coming from ground water. Rice production is concentrated in areas with a surface layer of silt loam on top of a heavy clay subsoil (Moldenhauer and Slaton, 2005). Heavy soils, precision grading, tailwater recovery, and sophisticated levee systems enable rice producers to make efficient use of irrigation water, but aquifer depletion has caused the USDA to designate large areas of eastern Arkansas as critical groundwater zones (Robinson et al., 2003). In some areas of Arkansas low ground water levels, naturally occurring mineral deposit, and reuse of irrigation water has increased chloride levels within agricultural soils to levels unsustainable for rice and soybean production (Kresse and Clark, 2008).

SALINITY

Approximately one third of irrigated land worldwide is affected by decreased production as a direct result of salinity (Epstein et al., 1980). Salinity can be caused by concentrations of different ions which raise electrical conductivity (EC). Salinity is measured using an electrical conductivity meter. Electrodes of uniform geometry are placed at a constant distance of separation within the sample. When electrical potential is imposed, current varies with the concentration of dissolved salts. The cell constant accounts for electrode geometry, and it is obtained by calibrating with KCl solutions of known concentration. The cell constant is related
to the distance between electrodes and divided by their effective cross-sectional area. The standard unit to express conductivity is Siemens or Siemens/meter (formerly mhos), but because the conductivity of most soils is usually less than one unit, conductivity is generally expressed as decisiemens (dS) per meter at 25°C (1 dS/m = 1000 µS/cm). Irrigation water directly from a snow fed stream has an EC of approximately 0.5 to 0.6 dS/m, while irrigation water in arid and semi-arid regions is generally around 15 to 16 dS/m. Sea water with soluble salts of 35 g/l has EC of 50 dS/m.

Soil EC is measured by saturating samples with water, mixing them, and then extracting solution to be read with an EC meter. Two of the most conventionally used laboratory methods used to quantify soluble salts in soil are the saturated paste extract method (SP) and the dilute soil extract method (DE). The SP method is most representative of field soil conditions, but is time consuming and susceptible to error. The DE method is often used for high volume of samples and is reproducible using a wide range of soils. DE assays typically use a soil:water (v:v) ratio of 1:1, 1:2, or 1:5. EC varies depending on the method used, so comparisons between experiments which use different methods must be done using conversion tables (Table 1) (Fischer et al., 2006). Saline soils are characterized as having a pH of less than 8.2 when saturated and an electrical conductivity of more than 4 dS/m (SP) or 1.6 dS/m (DE) at 25°C (Abrol et al., 1988; Gartley, 2001). Hosseini et al. (2002) reported that soybean growth and yield reductions occur at approx. 5.0 ds/m (SP). Maas and Grattan (1999) reported that rice yields decrease 12% for every unit increase in average root-zone EC above 3.0 dS/m (SP), while Hanson et al. (1999) reported that the threshold is approximately 1.9 dS/m (SP) with a yield decline of 9.1% for each unit EC rather than a 12% yield decline.
Saline soils are particularly problematic in irrigated arid and semi-arid regions as well as coastal areas where sea water enters the soil through waterways and groundwater. Salt in the root zone of saline soils most frequently originates from minerals already present in the soil and is released via the process of chemical weathering. Ions near the site of weathering in the presence of carbon dioxide are primarily carbonates and hydrogen-carbonates of calcium, magnesium, potassium, and sodium. Salts do not decrease soil productivity if they are deposited below the rooting zone unless they are moved into surface soil layers. Dissolved solutes are transported by groundwater or streams far from their origin and into arid and semi-arid regions where they become more concentrated and precipitate if high enough concentrations of low solubility salts are reached. This generally occurs as surface soil dries, and ground water is drawn up into surface soil layers via increased matric potential or suction. Other processes contributing to increased concentration of chloride and sodium ions include exchange, absorption, and differential mobility. Depth at which salts are deposited depends on several factors including water retention of the soil and rainfall. When soil is poorly drained and application of water is in excess of evotranspiration, the subsoil water level may rise. At a depth of one to two meters, it is evaporated to the surface and increases salinity of the root zone (Abrol et al., 1988).

As the demand for fresh water (typically 1 dS/m or less) increases, irrigation needs are met with water of continually poorer quality. The use of saline irrigation water and fertilizer amendments in high-production agriculture is the primary contributor to soil salinity in the rhizosphere (Fig. 1). Manmade developments may exacerbate this process by impeding soil drainage (Abrol et al., 1988; Al-Sadi et al., 2010b; Epstein et al., 1980; MacDonald, 1982; Triky-Dotan et al., 2005). In karst regions which include much of Arkansas, naturally saline
environments are dominated by CaCl$_2$, which is carried into the root zone by irrigation practices. (Gilmour et al., 1989; Mayhew et al., 1998). In Arkansas, over 90% of irrigation uses groundwater. Aquifer depletion has prompted the USDA to designate areas of eastern Arkansas as critical groundwater zones (Robinson et al., 2003), with a 38 percent occurrence of unsustainable salinity levels in affected areas (Gilmour et al., 1989). Low ground water levels, naturally occurring mineral deposits, and reuse of irrigation water has increased chloride levels within agricultural soils to levels unsustainable for agricultural production (Kresse and Clark, 2008).

Saline fields can be visually identified by spotty growth of crops and the presence of white salt crusts on the soil surface. Under moderate conditions, leaves of salt-stressed plants may be smaller and have a darker, blue-green appearance. Plants may retain water, particularly when chloride levels are high. Under highly saline conditions, plants become increasingly stunted and chlorotic, and fields may become barren. In fields uniformly affected by salinity, these changes may be subtle and difficult to identify, and are often misidentified as drought stress (Abrol et al., 1988).

Environmental conditions may predispose different sites to reduced yields caused by salinity. Magistad et al (1943) found that plants grown in sand at 1 dS/m were more tolerant to saline conditions in cool, wet environments and less tolerant in hot, dry environments. Paliwal (1972) demonstrated that in coarse soils under favorable conditions, tolerant crops like wheat may be grown in soil with EC as high as 10 dS/m (SP) without significant yield loss; however, unfavorable conditions can result in yield losses when EC is as low as 0.4 to 0.5 dS/m (SP).

Irrigation with saline water may also alter the physical and chemical properties of soil. In clay soils, clay is kept flocculate by elevated salt and may have more desirable physical properties (Abrol et al., 1988), but often saline soils are physically less ideal for agricultural
production (Triky-Dotan et al., 2005). When saline soils are leached with low salt water, they may become less permeable to water and air (Abrol et al., 1988). Since the effects of salinity are dependent on soil texture, water table depth, and other environmental conditions, guidelines for salinity tolerance should be determined on a regional basis.

**Effects of salinity on plant health**

Environmental stress results in decreased plant growth (Abrol et al., 1988; Greenway and Munns, 1980; MacDonald, 1982; Triky-Dotan et al., 2005). Low water potential of soils with high salt concentration reduces root elongation (Dalton et al., 1997; Hassan and Overstreet, 1952; Kafkafi, 1996); however, decreases in root biomass reduce water uptake and shoot growth even more than root growth (Cruz and Cuartero, 1990; Cuartero and Fernandez-Munoz, 1999). Dalton et al. (1997) reported a high negative correlation between EC and shoot growth. These effects are a combination of reduced water potential, metabolic changes which alter normal cell functioning, and ion toxicity.

Soil salinity may decrease uptake of essential nutrients, particularly macronutrients (Hu and Schmidhalter, 2001; Turhan and Eris, 2005). Finck (1977) reported that calcium and potassium deficiencies are primarily responsible for this phenomenon. Reduced water potential decreases uptake of calcium and other passively transported nutrients. When salinity increases gradually, plants compensate by adjusting internal salt content and increasing uptake of chloride and sodium ions (Dionisio-Sese and Tobita, 2000; Marschner, 1995; Schwarz and Gale, 1981; Walker et al., 1981). Excessive uptake of antagonistic ions can result in reduced uptake of essential nutrients (Bohra and Doffling, 1993; Dionisio-Sese and Tobita, 2000; Hasegawa et al., 1986; Marschner, 1995). Preferential uptake of sodium leads to potassium deficiency in soils with excess sodium chloride (Malvi, 2011). Phosphorus deficiency can also lead to decreased
plant growth in soils with excess calcium chloride. The availability of phosphorus is dependent on plant root length, which is limited, as well as the inhibition of phosphorus uptake by excess chloride (Abrol et al., 1988). Fine and Carson (1954) reported that application of phosphorus alleviated some of the negative effects of salinity on oats and barley, which responded with increased yield as well as decreased salt injury. Ferguson and Herlin (1963) reported that the positive effects of phosphorus application are greater for plants grown in moderately saline as opposed to non-saline soil.

Specific ions including Na$^+$, Cl$^-$, and B$^{3+}$ may reach toxic levels as they accumulate in the leaves of plants. In woody plants, they are also known to cause marginal or tip burn (Abrol et al., 1988). Excess uptake of Na$^+$ relative to K explains some loss of function, but altered membrane structure may be an even more important effect (Campbell and Pitman, 1971). Plant strategies for compensating for osmotic stress may involve differential storing of excess ions on successive leaves, between leaves and other above ground parts, or between the shoot and roots (Boursier and Lauchli, 1989; Jeschke and Wolf, 1988; Munns et al., 1988; Yasar et al., 2006). Secondary detrimental effects of salinity on plants can include the toxic effects of excess salts on soil microbial populations, which interferes with the transformation of essential nutrients and reduces availability to plants.

**Salinity tolerance**

Salinity tolerance depends on ion partitioning, osmotic adjustment, restoration of oxidative balance, and other adaptations which vary within plant species. Cultivars most resistant to salinity in one type of environment may not be the most resistant in other environments. These differential effects are likely related to the rate of transpiration per unit root length as salt accumulates in the root zone (Abrol et al., 1988; Magistad et al., 1943).
Many glycophyte species minimize osmotic stress by regulating Cl$^-$ and Na$^+$ from the shoot. These include pasture legume cultivars (*Glycine javanica*) (Gates et al., 1966, 1970) which have been found to be more salt tolerant than *Glycine tomentella* and *G. tabacina* (Wilson, 1967; Wilson et al., 1970a). Salt sensitivity in *G. tomentella* has been established to be due to chloride accumulation, especially in younger leaves (Wilson et al., 1970b). Similar differential uptake of chloride has been found to be the mechanism behind salt tolerance in grapevine. Slower accumulation in the shoot (Alexander and Obbink, 1971) was determined to be due to variable transport from the root to the shoot (Bernstein et al., 1969). Downton (1977) supported this idea by demonstrating that chloride excluding varieties contained less chloride in the shoot than did vines on susceptible rootstocks. Salt sensitivity in barley also seems to be determined by chloride uptake by the roots and accumulation in the leaves (Greenway, 1962, 1965).

Low levels of salinity tolerance have been found to be the result of low Na$^+$ accumulation in leaves in other plant species. Reabsorption of Na$^+$ from the xylem back into the roots has been demonstrated to be the tolerance mechanism in beans and corn (Kramer et al., 1977; Lauchli, 1976b; sYeo et al., 1977a, b). In beans, partitioning of Na$^+$ from the leaves may also occur in the lower stem (Rains, 1969). These strategies are in contrast to the majority of crops which exhibit tolerance in cultivars which accumulate Na$^+$ in the leaves and are classified as halophytes (Flowers et al., 1977).

Tolerance within a species varies with growth stage. Germination is the stage of growth during which most crops are most sensitive to salinity. Poor or spotty stands are primarily due to germination failures. Beans are particularly susceptible to saline conditions at this stage. However, reduced germination is not necessarily a result of low tolerance to salinity. Even in highly tolerant crops like barley, stands may be reduced by particularly high salt concentrations
in the shallow zone where seeds are planted. This occurs as saline water is evaporated and salts are deposited near the soil surface in fields where salinity has been a problem for a prolonged period of time (Abrol et al., 1988).

**Salt tolerance of soybean**

Soybean is considered moderately salt tolerant (Grieve et al., 2003) but sensitivity to salinity varies with plant maturity and cultivar (Essa, 2002; Maas and Hoffman, 1977a). Soybean thresholds for salinity damage ranges from 30 dS/m to 5 dS/m (SP) (Ashraf, 2004; Hosseini et al., 2002). Agronomic traits of soybean which are affected by salinity include reduced emergence and stand, reduced leaf number and area, leaf chlorosis, stunted plants, decreased root nodulation, and decreased pod weight and number (Abel and MacKenzie, 1964; Chang et al., 1994; Essa, 2002; Hamdy et al., 1993; Lauchli and Wienke, 1979; Li et al., 2006; Sharifi et al., 2007; Tuncturk et al., 2008).

Abel and MacKenzie (1964) reported that soybean germination is delayed at low levels of salt (0.05% to 0.1% NaCl) and completely stopped at progressively higher levels; however, reduced germination is more pronounced in salt-sensitive cultivars (Abel 1969). Shao et al. (1994) described the order of salt tolerance in germination thusly: imbibtion > emergence of radical > growth of radical > growth of lateral roots. Hosseini et al. (2002) reported that soybeans are more sensitive to salt stress during the seedling stage and not during germination; however, tolerance at the germination stage does not necessarily guarantee salt tolerance at later growth stages. Essa (2002) reported that although cultivars "Lee," "Coiquitt," and "Clark" showed similar reductions in germination, effects on later growth were much less pronounced for "Lee" than for the other cultivars.
Research on soybeans indicates that variable salt tolerance between cultivars involves differential patterns of Cl\(^-\) uptake and transport, but may be complicated by translocation of other micronutrients. Comparative studies conducted by Abel and MacKenzie (1964) evaluated the performance of six soybean varieties in salinized soil and reported that salt sensitive varieties developed severe chloride-induced leaf necrosis due to the accumulation of chloride in the leaves, but did not find any measurable varietal differences in chloride content of roots. The salt-sensitive variety "Jackson" was found to typify the chloride-includer while the moderately salt tolerant "Lee" maintained a low leaf chloride content and typifies a chloride-excluder. Lauchli and Wieneke (1979) characterized the distribution of K\(^+\), Na\(^+\), and Cl\(^-\) in tolerant and susceptible varieties and suggested that differential exclusion of Cl\(^-\) and Na\(^+\) (to a smaller extent) from the shoot accounts for varietal differences in salt tolerance. Unlike Abel and MacKenzie, tolerant varieties were found to contain higher levels of chloride in roots and it was suggested that exclusion of Cl\(^-\) and Na\(^+\) from soybean leaves is regulated by the root. Lessani and Marschner (1978), Grattan and Maas (1985), Essa (2002), and Li et al. (2006) reported similar results. Philip and Broadley (2001) reported that in soybean and woody perennial species, Cl\(^-\) exclusion from the shoot is associated with increased salt tolerance and not Na\(^+\) exclusion; however, other studies have determined that salt-tolerant soybean varieties exclude Na\(^-\) from the shoot (Li et al., 2006) or exclude both Cl\(^-\) and Na\(^+\) from leaves (Essa, 2002). Some of this discrepancy may be due to genetic variation and relationships among wild soybean (G. soja) and G. max (Chen and Nelson, 2004; Greenway, 1973; Luo et al., 2005; Pantalone et al., 1997; Robinson, 1971). Cultivated soybean appears to be more sensitive to Cl\(^-\) while wild soybean appears more sensitive to Na\(^+\) accumulation in the shoot, but differential translocation of both Na\(^+\) and Cl\(^-\) is highly cultivar specific. Moreover, under high levels of salinity stress, cultivars may change...
strategies and begin partitioning ions differently than they would under moderate salinity (An et al., 2002). Despite cultivar differences, it is generally accepted that Cl\(^-\) translocation from the roots to the shoot is of primary importance for commercially produced soybean.

Soybean plants can be rated for salinity tolerance by visually rating leaf necrosis for chloride toxicity. Rupe et al. (2000) reported that at intermediate and high salinity levels, leaves of chloride includers become scorched. Other research has attributed foliar symptoms to differential uptake of Na\(^+\) and Cl\(^-\) (Abel, 1969; Essa, 2002; Pantalone et al., 1997; Ping et al., 2002; Kao et al., 2006). Experiments have established the reliability of using a visual scale to rate soybean leaf symptoms. Tamura and Chen (2009) used a 1 to 6 scale with 1 showing no foliar symptoms and 6 assigned to a leaf that is completely dead. The optimal threshold for differentiation of includers and excluders is 12 dS/m (NaCl) with visual foliar symptoms appearing at 26 days with salinity below EC 12 ds/m NaCl and at 14 days above EC 12 ds/m NaCl (Tamura and Chen, 2009; Valencia et al., 2008). Valencia et al. (2008) used this foliar rating method to classify cultivars "Williams," "Clark," "HBK R4924," and "Dare," as salt sensitive chloride-includers and "S-100," "Lee 68," and "HBK R5525," as salt tolerant chloride-excluders.

Soybean roots begin to exhibit symptoms of tissue damage at EC 8 ds/m NaCl (SP), particularly at root tips and on lateral roots. Poorly developed roots systems with reduced root biomass were observed on Cl- includers. Chloride includers "Clark" and "Williams" exhibited darker tap roots and shortened secondary roots when compared with chloride excluders. However, because not all chloride-includers displayed visual root symptoms, observation of roots is not an effective method for determining tolerance between soybean varieties (Valencia et al., 2008).
Secondary effects of salinity on soybean

Decreases in root biomass and nodulation reduce nitrogen fixation and plant vigor in pea and soybean (Bhardwaj, 1975; Bernstein and Ogata, 1966; Delgado et al., 1994; Elsheikh and Wood, 1995; Singleton and Bohlool, 1984). Delgado et al. (1994) characterized this process as attenuation of nitrogen fixing bacterial aerobic respiration, reduction of leghemoglobin content of nodules, and depletion of the energy source necessary for nitrogen fixation. Duzan et al. (2004) reported that salt stress inhibits initiation of symbiosis by deformation of root hairs perceptive to Nod factors. Experiments by Abd-Alla et al. (1998) which involved grafting soybean varieties of varying salinity tolerance, indicated that soybean nodulation involved disruption of signals in both the shoot and the roots. Salt-sensitive cultivars are subject to oxidative stress as malondialdehyde, glutathione reductase, ascorbate peroxidase, catalase, and superoxide dismutase levels increase (Elsheikh, 1998). Under mild salinity stress, increased production of antioxidant enzymes and a decrease in glutathione protects nodules against reactive species and prevents the breakdown of leghemoglobin and membrane lipids and proteins. Severe salinity stress, however, causes an irreversible loss of nitrogen reducing activity (Comba et al., 1998). Additional microbiotic losses result from rapid leaching of N and NO₃, decreased nitrification, and the effects of toxic ions like chloride on Bradyrhizobia/Rhizobia (Abd-Alla et al., 1998; Abrol et al., 1988; Velagaleu and Mursch, 1989).

Salinity tolerance of rice

Rice has been considered one of the most suitable crops for saline fields. Lowland rice culture involves flooding fields for almost the entire growing season. This practice significantly dilutes salts and reduces the effects of salinity (Abrol et al., 1988; Maas and Hoffman, 1977b). Yadav and Girdhar (1981) reported that rice yields remain satisfactory even when conductivity is
20 to 25 dS/m (SP) near the soil surface. However, salinity becomes an important problem for rice culture when good quality water is not available. Under these conditions, saline groundwater may be used for irrigation and yield is significantly reduced (Abrol et al., 1988). Current guidelines indicate that rice yields decrease 12% for every unit increase in average root-zone EC above 3.0 dS/m (SP) (Hanson et al., 1999; Maas and Grattan, 1999); however, a more recent study places that the threshold at approximately 1.9 dS/m rather than 3.0 dS/m (SP), with a yield decline of 9.1% for each unit EC rather than a 12% yield decline (Grattan et al., 2002).

The literature indicates that rice is sensitive to salinity, particularly during the seedling stage (Maas and Hoffman, 1977b). Salinity tolerance increases from panicle formation to flowering (Abrol et al., 1988, Kaddah et al., 1975; Kaddah and Fakhry, 1961; Pearson, 1959; Pearson, 1961; Pearson et al., 1966). Agronomic traits of rice which are affected by salinity include plant height, root length, tillering ability, biomass, delayed panicle initiation and spikelet formation, reduced panicle length, number of primary branches and spikelets per panicle, fertility and panicle weight, and reduced grain yield (Pearson, 1961). Reduced grain yield is almost always due to delayed inflorescence and increased number of sterile spikelets (Abrol et al., 1988).

Initial visual symptoms of rice injury involve reduction in effective leaf area. Ota and Yasue (1962) reported that photosynthetic and chlorophyll content are negatively correlated with salinity level. Leaf formation and elongation is suppressed, and leaves become chlorotic, curl up, and die. Symptoms affect the oldest leaves first, then progress to new growth. Gregorio et al. (1997) assessed methods of screening for salinity tolerance in rice and concluded that visually rating is a reliable method for evaluating salinity tolerance.
Salt injury to rice is caused primarily by osmotic imbalance and accumulation of toxic ions. Early studies reported that chloride accumulation in the shoot is the main component of salt stress (Iwaki et al., 1953; Shimose, 1963), but more recent using rice and the model dicot Arabidopsis thaliana indicate that the important ion involved in salt-induced stress is Na$^+$. (Clarkson and Hanson, 1980; Comba et al., 1998; Flowers and Yeo, 1981). Excess Na$^+$ interferes with absorption of K$^+$, which is vital for metabolic processes (Greenway and Munns, 1980; Wyn Jones, 1981). It is well established that grain yield is sensitive to salinity (Kapp, 1947; Pearson, 1959; Kaddah and Fakhry, 1961; Akbar et al., 1972; Datta, 1972; Venkateswarlu et al., 1972; Korkor and Abdel-Aal, 1974; Bhattacharyya, 1976). Devitt et al. (1981) showed that grain yield is highly susceptible to Na$^+$/K$^+$ imbalance. Salt tolerant rice cultivars are typically able to overcome the effects of Na$^+$ using differential uptake, Na$^+$ exclusion, and increased K$^+$ absorption. Gregorio et al. (1997) reported a high correlation between Na$^+$/K$^+$ ratios and salinity tolerance.

PYTHIUM

The genus Pythium is comprised of approximately 120 species occupying both terrestrial and aquatic habitats and diverse biological niches. Soil-borne species range from strict saprophytes to important plant pathogens causing significant economic losses on a wide variety of crops including soybean and rice (Avanzato, 2011; Broders et al., 2009; Cother and Gilbert, 2007; Dorrance et al., 2004; Hendrix and Campbell, 1973; Kirkpatrick et al., 2006a; Kirkpatrick et al., 2006b; Lévesque and De Cock, 2004; Matthews, 1931; Schlub and Lockwood, 1981) as well as biological control agents of pathogenic species by non-pathogenic species (Al-Hamdani et al., 1983; Lifshitz et al., 1986; Martin and Hancock, 1987; Paulitz and Baker, 1987).
Pythium spp. are oomycetes with coenecytic, hyaline hyphae. Species may be subdivided based on reproductive strategy. Homothallic isolates reproduce both sexually and asexually, while heterothallic isolates do not undergo sexual reproduction without the presence of a compatible mating type. Oogonia and antheridia are the “female” and “male” sexual reproductive structures of the genus Pythium. The oogonium is a globose, multinucleate cell surrounded by a single layer of periplasm. Oogonia may be smooth-walled or ornamented with bumps or spikes. When the smaller, elongate to club-shaped antheridia comes into contact with an oogonium, it penetrates the oogonium with a fertilization tube. All but one functional female nucleus disintegrate and meiosis begins as a male nucleus is transferred to the oogonium. This resultant thick-walled oospore is the primary survival structure of Pythium spp. Oospores may survive in the soil for long periods of time until a suitable host or organic substrates become available. (Alexopoulos et al., 1996). Stanghellini and Nigh (1972) reported that oospores of P. aphanidermatum remained viable in oat roots after a 16-month period of dormancy. Factors influencing oospore germination include age, CO₂ concentration, and alternating soil wetting and drying (Adams, 1971; Ayers and Lumsden, 1975; Johnson, 1988). Even when conditions become favorable, some oospores will remain dormant. This survival strategy is called constitutive dormancy (Lumsden and Ayers, 1975). Oospores of many Pythium spp. thin as they become mature in preparation for germination. During this period, oospores are susceptible to dessication or lysis due to changes in the soil environment and predation by bacteria (Adams, 1971; Ayers and Lumsden, 1975; Hancock, 1981; Qian and Johnson, 1987).

Sporangial appearance ranges from discretely spherical or lobate to indiscrrete hyphal swellings. While some (primarily spherical) sporangia may survive for long periods in the soil (Peethambaran and Singh, 1977; Stanghellini and Burr, 1973; Stanghellini and Hancock, 1971),
sporangia are primarily asexual reproductive structures. In some species the ability to produce zoospores has been lost and sporangia germinate directly with a germ tube, but in most species the sporangium is the site of zoospore production. Alternatively, in some species sporangia may germinate both directly or indirectly depending on temperature, with lower temperature favoring zoospore production (Van Der Plaats-Niterink, 1981) (Fig. 2).

Zoospores are kidney-shaped single cells, with two flagella (a tinsel and a whip) attached to the concave side of the cell. Zoospores mature in a vesicle formed outside the sporangium. Soil saturation typically promotes zoospore release (MacDonald, 1982), when they become free-swimming spores. Zoospores in the rhizosphere respond to chemicals in root exudates of the host, encyst on the root surface, and germinate rapidly. In the early stages of encystment, the zoospore store glycoproteins which will be used to adhere plant tissue (Estrada-Garcia et al., 1989, 1990). Zoospore chemotaxis and germination may be induced by different root exudates. Donaldson and Deacon (1993) reported that L-glutamine attracted zoospores of *P. aphanidermatum*, but did not stimulate cyst germination. Tripanthi and Grover (1978) found that addition of arginine and arabinose, compounds not normally produced by susceptible hosts, inhibited zoospore attraction and encystment of *P. butleri* zoospores. If a host is not found, zoospores may encyst within the soil and remain viable for several days as long as environmental conditions remain favorable. Stanghellini and Burr (1973b) demonstrated that *P. aphanidermatum* zoospores can survive in moist soil for up to seven days, but are desiccated when soil is air dried for two days.

Morphological identification of *Pythium* spp. is primarily based on variation in gametangia. Sporangial shape; size, shape, and ornamentation of oospores and oogonia; and antheridial attachment are some of the most distinctive characteristics. Nevertheless,
morphological identification of the genus is a difficult and nuanced endeavor (Dick, 1990; Matthews, 1931; Van Der Plaats-Niterink, 1981; Waterhouse, 1968).

Molecular techniques have widely replaced morphological identification of *Pythium* spp., using the ITS region of rDNA (Allain-Boule et al., 2004; Bernard et al., 1998), AFLP fingerprinting (Garzon et al., 2005), and PCR-RFLPs (polymerase chain reaction-restriction fragment length polymorphism) (Kageyama et al., 2005; Martin, 2000; Martin and Tooley, 2004). Kageyama et al. (2005) performed phylogenetic analysis of *P. graminicola* using RFLP analysis of the rDNA-ITS region as well as RFLP of the cytochrome oxidase subunit II (COX II) gene. This gene was selected because it may accumulate mutations through evolution. Using these methods, they were able to alter taxonomic placement of species previously evaluated using morphological similarities.

**Pythium disease of soybean and rice**

Pythium pre- and post-emergence damping off and root rots are a widespread problem in all soybean and rice production areas worldwide. At least 17 species are reported as pathogenic on soybeans including *Pythium sylvaticum*, *P. irregulare*, *P. ultimum*, *P. dissotocum*, *P. torulosum*, *P. attrantheridium*, *P. inflatum*, *P. aphanidermatum*, *P. debaryanum*, *P. myriotylum*, *P. vexans*, and *P. graminicola* (Bates et al., 2008; Broders et al., 2007; Dorrance et al., 2004; Kageyama and Ui, 1982; Kirkpatrick et al., 2006; Lehman and Wolf, 1926; McCarter and Littrell, 1970; McGee, 1992; Morgan and Hartwig, 1964; Rosso, 2007; Schlub and Lockwood, 1981; Watanabe, 1989; Yang, 1999; Zhan et al., 1996). *Pythium* spp. most often identified from Arkansas soybean fields are *P. ultimum*, group HS (hyphal swelling), *P. aphanidermatum*, *P. irregulare*, *P. vexans* and *P. oligandrum* (Avanzato, 2011; Broders, 2007; Kirkpatrick et al., 2006b). Avanzato (2011) reported that *P. sylvaticum*, *P. irregulare*, and *P. dissotocum* are most
frequently isolated on soybean in Arkansas. Pathogenic strains on rice include *P. spinosum*, *P. dissotocum*, *P. irregulare*, *P. arrhenomanes*, *P. myriotylum*, *P. catenulatum*, and *P. graminicola* (Webster and Gunnell, 1992).

Pythium diseases of seeds and seedlings are characterized by common symptoms which include necrosis of cotyledons or hypocotyls, root discoloration and decay, seed and seedling mortality, and ultimately yield loss (Yang, 1999). Destruction of root tips, root hairs, and thin feeder roots inhibits soil exploration and nutrient absorption, resulting in reduced seedling vigor and thin and uneven plant stands (Hendrix and Campbell, 1973). Reduced water uptake also causes shoots to wilt during periods of warm temperature. Symptoms on rice seedlings may be subtle, with little evidence of necrosis on primary roots, and above ground reduction in seedling vigor (Webster and Gunnell, 1992).

Soil moisture and temperature are important environmental factors influencing the behavior of Pythium spp. Zoospore motility is dependent on wet soils. Soil moisture also influences the type of reproductive spores formed as well as activity in the soil (Bainbridge, 1970). Lifshitz and Hancock (1983) reported that saprophytic activity of *P. ultimum* is very low in saturated soils. Kirkpatrick et al. (2006a) reported that flooding and soil infestation had an additive effect on *P. ultimum* disease of soybean, particularly at the germination stage, and that Pythium isolation frequency increased in flooded soils (Kirkpatrick et al., 2006b). Pathogenic species of *Pythium* have a wide range of optimal temperatures that may vary within species (Abad et al., 1994). Fine textured, poorly drained soils at lower growing temperatures (typically between 15 and 20°C) are typically most problematic for Pythium disease. Soil pH can also influence Pythium survivability and pathogenicity, with optimal soil pH around 6.8 or 7.2 (Warcup, 1952) and above pH 3.6 to 5.5 (Warcup, 1952; Barton, 1958; Dick and Ali-Shtayeh,
1986). The favorable range of each of these characteristics differs between *Pythium* spp. It follows that changing any of these characteristics may suppress Pythium disease or it may alter the balance between *Pythium* spp., causing some species to become less competitive and others to become dominant.

Plant pathogenic *Pythium* spp. can be found growing saprophytically within the soil or dead organic matter, and inter- and intra-cellularly within a living host. Pythium diseases are primarily a concern during the seedling stage, but may also emerge when environmental conditions favor disease (Lévesque and De Cock, 2004; Van Der Plaats-Niterink, 1981). Broad environmental optima, wide host range, saprophytic activity, and persistent survival spores makes management of Pythium diseases a perennial problem.

Environmental conditions which effect host susceptibility to Pythium disease include temperature and soil moisture. Conditions less ideal for seedling growth are generally most favorable for Pythium damping off disease due to plant stress (Leach, 1947). Damping off and root rot severity are maximized at lower soil temperatures (Bateman and Dimock, 1959; Hoppe, 1949; Kraft and Roberts, 1969; Pieczarka and Abawi, 1978; Short and Lacy, 1976). Pathogen population densities were demonstrated to be several times greater at 26°C versus 17°C (Lifshitz and Hancock, 1983; and Paulitz and Baker, 1987), suggesting that increased disease severity is due to greater host susceptibility at lower less ideal temperatures rather than optimal conditions for the pathogen. Paulitz and Baker (1987) also suggested that higher susceptibility may be in part due to slower seedling emergence and root growth under less than ideal conditions, where host tissues are in contact with the pathogen for a longer period during a highly susceptible growth stage. High soil moisture is also known to increase severity of Pythium damping off and root rots (Bateman, 1961; Kraft and Roberts, 1969; Pieczarka and Abawi, 1978; Short and Lacy,
Some of these affects are likely due to anaerobic conditions (Burstrom, 1965) but others have suggested that increased diffusion of root and seed exudates enlarge the zone of influence and increase plant exposure to soil pathogens (Brown and Kennedy, 1966; Flentje, 1964; Kerr, 1964; Stanghellini and Hancock, 1971).

In Arkansas, soybean and rice production highly favors Pythium disease. These crops are often rotated with one another in poorly drained, alluvial soil. Soil temperature during soybean and rice planting ranges from 15°C in March to 30°C in July. Disease management includes use of chemical fungicides including metalaxyl (Allegiance) and mefanoxam (Apron), as well as cultivar selection. In rice production, there is some recognized Pythium resistance, and efforts are being made to develop genotypes which are cold tolerant (Rothrock et al., 2005). There are currently no commercially produced resistant soybean cultivars; however, Pythium resistance has been reported. Rosso et al. (2008) reported that *P. aphanidermatum* resistance in soybean is due to a single, dominant gene (*Rpa1*) which is independent from the Phytophthora resistance gene (*Rps1k*). Additional efforts to mitigate Pythium disease loss may include planting date, irrigation practices, and crop rotation.

Seedling diseases of soybean and rice contribute to reduced plant populations and reduced plant vigor. Yield losses from seedling diseases on soybean were estimated at 5% in Arkansas in 2003, or approximately $32.4 million. Oomycete *Pythium* spp. and fungal *Fusarium* spp. are among the most commonly isolated soilborne pathogens associated with seedling disease (Koenning, 2004). From 1996 to 2007 seedling disease ranked second to sixth among diseases reducing soybean yield in the United States, with greatest loss occurring between 2005 and 2007 when cool, wet weather persisted early in the growing season (Wrather and Koenning, 2009).
Effects of salinity on *Pythium* species

Studies on interaction between salinity and oomycete pathogens found that disease increases with salinity (Younger et al., 1967; Abrol et al., 1988; Rasmussen and Stanghellini, 1988; Triky-Dotan et al., 2005; Al-Sadi et al., 2010). Examples include *P. ultimum* on chrysanthemum (Gladstone and Moorman, 1989), Phytophthora *capsici* on chili pepper (Sanogo, 2004), and *Phytophthora parasitica* on tomato (Swiecki and MacDonald, 1991). Several studies have reported increased plant growth reduction—both root and shoot—in the presence of *Pythium* spp. under saline conditions (Cuartero and Fernandez-Munoz, 1999; Kafkafi, 1996; Wulff et al., 1998; Schwarz and Grosch, 2002).

Beech (1949) attributed increased damping off disease of tomato seedlings to tolerance of the pathogen to osmotic stress. Studies using NaCl treatments compared with balanced macronutrient treatments reported reduced shoot biomass with threshold levels between 4-6 dS/m (Kafkafi, 1996; Schwarz and Grosch, 2002). While reduction of root mass was observed before any differences in above ground plant growth could be observed (Grosch and Schwarz, 1998; Schwarz and Grosch, 2002), shoots were ultimately more reduced (Cruz and Cuartero, 1990; Dalton and Poss, 1990; Plant et al., 1991; Schwarz and Grosch, 2002).

On the other hand, saline pulse experiments suggest that disease susceptibility may be the result of osmotic shock (Macdonald, 1982). Canaday and Schmitthenner (2010) observed that *Phytophthora* disease increases on soybean were associated with increases in EC and suggested that osmotic stress is involved in increased seedling disease under saline conditions, but they argued that differential salinity tolerance is not likely the underlying mechanism as EC levels at which this phenomenon was observed were more limiting to the pathogen, while uninfested host growth was hardly affected. In addition, Canaday and Schmitthenner (2010) and others (Borys,
argue that since disease increases were observed even when soils were not saturated, increased susceptibility is likely due to changes in host physiology with chloride uptake.

Soybean agriculture requires substantial potassium and phosphorus fertilization for maximum yields. KCl is by far the most widely used soybean fertilizer; however, nitrogen and KCl has been associated with increased seedling diseases in soybean (Canaday et al., 1999; Canaday and Mengistu, 2008; Canaday and Mengistu, 2009; Canaday and Schmitthenner, 1979; Canaday and Schmitthenner, 2010). In experiments with Phytophthora, Canaday and Schmitthenner (2010) found that chloride salts consistently increase disease and suggested that increased disease susceptibility is due to the presence of chloride and not potassium. They also found that ammonium salts increase disease, particularly when applied with chloride salts.

Calcium ion has been identified as an important plant defense signaling messenger (Du et al., 2009; Lecourieux et al., 2006). It has been widely reported that plant tissues are more resistant to infection with increasing calcium content (Bateman, 1964; Bateman and Lumsden, 1965; Edgington and Walker, 1958; Lee and Zentmyer, 1982; Rahman and Punja, 2007). This resistance has been reported for Phytophthora root and stem rot of soybean (Sugimoto et al., 2008; Sugimoto et al., 2007). Canaday and Schmitthenner (2010) postulated that movement of calcium along with chloride into shoots could render roots more susceptible to infection.

Studies on oomycetes and in particular terrestrial species of Pythium and Phytophthora have demonstrated that oomycetes are salinity tolerant (Blaker and MacDonald, 1985; Coffey and Joseph, 1985; Duniway, 1979; MacDonald and Duniway, 1978; Sanogo, 2004; Tresner and Hayes, 1971). However, salinity effects on pathogens also varies among and within species. Al-Sadi et al. (2009) found that different Pythium spp. reacted differently to increases in salinity. P. aphanidermatum, P. spinosum, and P. splendens growth increased or was unaffected by
salinity up to 5 dS/m, yet P. *oligandrum* growth decreased significantly. Likewise, Kiyoo mi et al. (2007) reported mycelial growth of *P. aphanidermatum* unaffected by salinity. Rasmussen and Stanghellini (1988) observed a slight increase in mycelial growth rate of *Pythium* isolates at low salinity (up to 7.1 ds/m) with a decrease in mycelial growth occurring at higher salinity. Growth of *P. ultimum* was also reduced with increased NaCl concentrations while *P. imperfectum* was not affected by salinity (Hassan and Fadl-Allah, 1993). Blaker and MacDonald (1985) also reported differences in salinity tolerance among species in a single genus in their experiments with *Phytophthora* isolates.

Spore production and germination and mycelial growth do not necessarily respond in like manner. Reduced mycelial growth but not reduced oospore germination of *P. oligandrum* was observed by McQuilken et al. (1992). Rasmussen and Stanghellini (1988) also reported that *P. aphanidermatum* mycelial growth increases under saline conditions while zoospore production decreases. Rasmussen and Stanghellini (1988) found that while mycelial growth rate of *P. aphanidermatum, P. dissotocum,* and *P. catenulatum* were reduced with salinity at temperatures below 30°C, reductions were not significant at or above 30°C. Zoospore production sensitivity was greater than mycelial growth sensitivity, but varied with species, where *P. aphanidermatum* was least tolerant and *P. catenulatum* was most tolerant. Oospore and zoospore production is typically more restricted at lower salinity levels than mycelial growth (Al-Sadi et al, 2010; Rasmussen and Stanghellini, 1988). The ability to explore new soil may be important for the survival of soilborne pathogens under saline conditions and explain continued or increased mycelial growth.

Both osmotic and matric components of water potential contribute to salinity stress (Griffin, 1981; Papendick and Campbell, 1981). Several studies have suggested that oomycete
pathogens are more sensitive to decreasing matric potential rather than osmotic potential (Adebayo and Harris, 1971; Brownell and Schneider, 1985; Duniway, 1979; Magan, 1988; McQuilken et al., 1992). Al-Sadi et al. (2009) suggested that any observed increases in disease under saline conditions is a result of a higher tolerance to low osmotic potential of the pathogen versus the host plant. They reported that *P. aphanidermatum* and other salinity tolerant pathogens have unrestricted mycelial growth and reproduction at salinity levels detrimental to the health of host plants. Other studies have suggested that *Pythium* may acquire tolerance for saline conditions (Blaker and MacDonald, 1985; Duniway, 1979).

Secondary effects of increased soil electrical conductivity include changes in water potential and pH. *Pythium* appears to be more tolerant of decreased water potential than plant hosts (Griffin, 1963; Hancock, 1977; Lifshitz and Hancock, 1983; Paulitz and Baker, 1987). Likewise, although mycelial growth of *Pythium* is reduced at pH 5.0 (Paulitz and Baker, 1987; Lifshitz and Hancock, 1983), inoculum density was not significantly reduced (Paulitz and Baker, 1987; Lifshitz et al., 1984) and it is likely that increased disease resulting from changes in pH are also due to the higher tolerance of the pathogen to pH changes than host tissues. Paulitz and Baker (1987) observed that inoculum density is not necessarily positively correlated with disease incidence.

*Pythium* spp. most important in causing disease do not necessarily comprise the largest populations in the field. In a study on five different turfgrasses, Abad et al. (1994) reported that the most prevalent species isolated from turfgrasses (*P. torulosum* and *P. catenulatum*) were weak pathogens causing very little disease. It was suggested that weakly pathogenic species interact with more aggressive species like *P. arrhenomanes*, which made up a small percentage of isolates in this study, but was found to be the most highly aggressive species. In the same
study, Abad et al. (1994) reported that out of five heterothallic *Pythium* spp. isolated, four caused mild to moderate disease. *P. catenulatum, P. splendens,* and *P. sylvaticum* were frequently observed to produce oospores, while *P. arrhenomanes* cultures produced few or no oospores. Observations by Abad et al. (1994) and others (Hendrix, 1969; Smiley et al., 1992) demonstrate that not all pathogenic *Pythium* spp. are homothallic. Very little is known about how soil conditions could affect relative importance of species causing Pythium disease, but environmental stress including high sand content substrate, unfavorable weather conditions, and low quality irrigation water may alter the soil environment giving typically weak pathogens an advantage over highly virulent species.

**OBJECTIVES**

While the effect of salinity on plant disease has been a focus of study in previous years, little is known about the effects of salinity on Pythium seed and root rots of soybean and rice. In particular, there is little known about the interaction between host and what are generally considered weak pathogens under saline conditions. The objective of this study is to determine how growth and reproduction of *Pythium* isolates isolated from Arkansas soybean and rice fields are affected by saline conditions, to characterize the effect of salinity on Pythium seed and root rots, and to determine whether salinity influences pathogen virulence.
Table 1. Comparison of methods for measuring electrical conductivity (EC) in soil

<table>
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<tr>
<th>Method</th>
<th>EC Readings (µS/cm)</th>
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<tr>
<td></td>
<td>1,000   2,000   3,000</td>
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<tr>
<td>SP\textsuperscript{y}</td>
<td>1,000   2,000   3,000</td>
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<tr>
<td>DE\textsuperscript{z} 1:2</td>
<td>300     700     1,200</td>
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\textsuperscript{y} Saturated paste extract method

\textsuperscript{z} Dilute extract method
Figure 1. Effects of irrigation on salinity in the root zone
Figure 2. Pythium life cycle

II. EFFECTS OF SALINITY ON PYTHIUM DISEASE OF SOYBEAN

ABSTRACT

Increasing soil salinity is an important factor limiting agricultural productivity worldwide. In addition to direct effects on growth and yield, seedlings grown under saline conditions may be more susceptible to disease caused by *Pythium* spp. This study characterized the effects of soil salinity on Pythium diseases of soybean. Controlled environment experiments used two soybean cultivars which differed in chloride tolerance and soil treated with a 1M CaCl₂ solution to create a range of electrical conductivity levels. Soil was either not infested or infested with *Pythium* spp. which differed in virulence. Twenty-one days after planting seedling stand, growth, and development were assessed. Electrical conductivity (EC) averaged 640 (control), 1060, 1632, and 2039 µS/cm in the first experiment and 930 (control), 1483, 2057, and 2570 µS/cm in the second experiment using the soil dilution method (2:1) to measure EC.

Salinity significantly reduced seedling stand at 2039 (exp 1) and 1483 (exp 2) µS/cm. Leaf number, shoot weight and root altitude decreased at 2039 (exp 1) and 2057 (exp 2) µS/cm. Root volume and root tips decreased at 2570 µS/cm in the second experiment, but were not affected in the first experiment. Shoot growth decreased for *P. aphanidermatum* and *P. sylvaticum* at moderate salinity levels compared to the control. There was no consistent *Pythium* by salinity interaction for root development, except where pathogenic species stimulated root volume for the control salinity. Salinity had no effect on the nonpathogenic species *P. oligandrum*, and the two cultivars responded similarly across treatments. Abiotic interactions are important considerations in understanding and managing diseases. This research suggests that *Pythium* spp. caused greater seedling disease in the presence of a stress such as occurs with increasing soil salinity.
INTRODUCTION

Awareness and management of soil salinity is becoming increasingly important in soybean \( \textit{Glycine max} \) production areas. Approximately one third of irrigated land worldwide is affected by decreased production as a direct result of salinity (Epstein et al., 1980). Soil salinity can be caused by concentrations of different ions which raise electrical conductivity (EC). The international unit for EC is siemens (S), and soil salinity is frequently represented using either dS/m or µS/cm (1 dS/m = 1000 µs/cm). In Arkansas, naturally saline environments are dominated by \( \text{CaCl}_2 \), which is released from limestone through chemical weathering and is carried into the root zone by irrigation practices (Gilmour et al., 1989; Mayhew et al., 1998). Aquifer depletion has prompted the USDA to designate areas of eastern Arkansas as critical groundwater zones (Robinson et al., 2003), with a 38% occurrence of unsustainable salinity levels in affected areas (Gilmour et al., 1989). Low ground water levels, naturally occurring mineral deposits, and reuse of irrigation water has increased chloride levels within agricultural soils to levels unsustainable for agricultural production (Kresse and Clark, 2008).

Soybean germination may be delayed or reduced at low to moderate salinity levels (Abel and MacKenzie, 1964), but soybean is most susceptible at the seedling stage (Hosseini et al., 2002). Susceptibility to salinity varies among cultivars, and is not consistent among cultivars for different growth stages (Abel, 1969; Essa, 2002). Additional symptoms of soil salinity include post-emergence damping off, reduced leaf number and area, leaf chlorosis, stunted plants, decreased root nodulation, and decreased pod weight and number (Abel and MacKenzie, 1964; Chang et al., 1994; Essa, 2002; Hamdy et al., 1993; Lauchli and Wienke, 1979; Li et al., 2006; Sharifi et al., 2007; Tuncturk et al., 2008).
Salinity is measured using electrical conductivity (EC). Two of the most conventionally used laboratory methods used to quantify soluble salts in soil are the saturated paste extract method (SP) and the dilution soil extract method (DS). The SP method is most representative of field soil conditions, but is time consuming and susceptible to error. The DS extract method is often used for a high volume of samples and is reproducible using a wide range of soils. DS assays typically use a soil:water (v:v) ratio of 1:1, 1:2, or 1:5. EC varies depending on the method used, so comparisons between experiments which use different methods must be done using conversion tables (Table 1) (Fischer et al., 2006). Soils with EC at or above approximately 4.0 dS/m (SP) or 1.6 dS/m (DS) are considered saline (Gartley, 2001). Hosseini et al. (2002) reported that soybean growth and yield reductions occur at approx. 5.0 ds/m (SP) [approx. 2.0 ds/m (DS)].

Chloride accumulation is the most important factor in determining soybean sensitivity to soil salinity (Abel and Mackenzie, 1964; Abel 1969). Soybean cultivars which restrict chloride accumulation to the root system are called “excluders,” and soybean cultivars which distribute chloride throughout the plant are called “includers.” Chloride excluders are more tolerant to saline soils, where chloride includers may suffer losses.

In soybean production areas throughout the world, Pythium causes yield losses due to pre- and post-emergence damping off and root rot (Yang, 1999). Pythium disease of seeds and seedlings include seed and seedling mortality, necrosis of the hypocotyls, and root discoloration and decay. Destruction of root tips, root hairs, and thin feeder roots inhibits soil exploration, water uptake, and nutrient absorption. Reduced root biomass results in reduced seedling vigor and thin and uneven plant stands (Hendrix and Campbell, 1973).
Numerous *Pythium* spp. have been isolated from soybean seedlings (Broders et al., 2007; Rosso, 2007; Avanzato, 2011). *Pythium aphanidermatum, P. sylvaticum, and P. oligandrum* are associated with soybean roots in Arkansas (Kirkpatrick et al., 2006). Among these species, differences in virulence and environmental optima are important determinants of disease severity. Pathogenic species of *Pythium* have a wide range of optimal temperatures that may vary by species (Abad et al., 1994). *P. aphanidermatum* is highly virulent at soil temperatures (e.g. above 30°C) (Littrell and McCarter, 1970; McCarter and Littrell, 1970; Rosso, 2007; Thomson et al., 1971), while isolates of *P. sylvaticum* are moderately virulent at a broad range of soil temperatures (Thomson et al., 1971; Yang, 1999; Rosso, 2007). *P. oligandrum* is not a pathogen of soybean and has been used for biological control against *Oomycota* and pathogenic *Fusarium* (Martin and Hancock, 1987; McQuilken, 1990; Vallance et al., 2009). Soil moisture is also an important environmental factor influencing the behavior of *Pythium* species. Zoospore production and motility is increased in saturated soils (Bainbridge, 1970), but saprophytic activity of *P. ultimum* is decreased (Lifshitz and Hancock, 1983). Kirkpatrick et al. (2006a) reported that flooding and soil infestation had an additive effect on disease severity on soybean caused by *P. ultimum*, particularly at the germination stage, and that Pythium isolation frequency increased in flooded soils (Kirkpatrick et al., 2006b). Pythium by soil flooding interactions indicated that at high soil moisture, tolerance to anaerobic soil conditions may contribute to increases in Pythium disease (Kirkpatrick, 2006a). Urrea (2013) reported that relative stand loss in two different Arkansas soybean fields was temperature dependent. In Arkansas, early season soybean production favors *Pythium* disease development (Ashlock et al., 1998; Mayhew et al., 1998). Fine textured, poorly drained soils with cooler temperatures (typically between 15 and 20°C) are typically most problematic for Pythium disease.
The literature indicates that Pythium diseases often increase in saline environments (Abrol et al., 1988; Al-Sadi et al., 2010; Rasmussen and Stanghellini, 1988; Schwarz and Grosch, 2002; Triky-Dotan et al., 2005; Younger et al., 1967); however, the underlying cause of increased disease is subject to debate. Griffin (1963), Hancock (1977), Lifshitz and Hancock (1983) and Paulitz and Baker (1987) reported that Pythium spp. were more tolerant of decreased water potential than plant hosts. It was suggested that increased disease is due to the pathogen’s higher tolerance to the effects of salinity. Beach (1949) attributed increased damping off disease of tomato seedlings to tolerance of the pathogen to osmotic stress. Studies using NaCl treatments compared with a balanced macronutrient solution reported reduced shoot biomass with threshold levels between 4-6 dS/m (Kafkafi, 1996; Schwarz and Grosch, 2002). On the other hand, saline pulse experiments suggest that disease susceptibility may be the result of osmotic shock (Macdonald, 1982). Canaday and Schmitthenner (2010) reported that Phytophthora stem and root rot increases on soybean were associated with increases in EC and suggested that osmotic stress is involved. However, they argued that differential salinity tolerance is not likely the underlying mechanism, because EC levels at which this phenomenon was observed were more limiting to the pathogen than the host. Others have argued that since increased disease was observed even when soils were not saturated, increased susceptibility is likely due to changes in host physiology with chloride uptake (Borys, 1964; Canaday and Schmitthenner, 2010). The objective of this study was to examine the role of soil salinity on soybean seedling growth in the presence of several Pythium species which differ in virulence using cultivars which differ in chloride tolerance.
MATERIALS AND METHODS

Silt loam soil from a soybean field near Stuttgart, Arkansas (34° 44’ 42.14” N, 91° 33’ 20.02” W) was provided by Dr. Terry Spurlock. Soil was collected separately for each experiment. Soil was mixed and large pieces of plant material and clods were removed, then soil was steam pasteurized at approximately 70°C for 30 min. prior to use. Sterile plastic 10 x 10 cm containers were filled with 250 g of soil, oven dry weight (ODW). Each experiment was a randomized complete block design with 6 replications. The treatments included two soybean cultivars, four Pythium treatments, and four salinity treatments arranged in a factorial treatment arrangement. The experiment was conducted two times. The commercially grown soybean cultivars used in this experiment were the chloride includer “Glenn” (PVP 200900325) and the chloride excluder “Osage” (PVP 200800001).

Pythium treatments

Pythium isolates were selected to represent a range of virulence. Candidate isolates were grown on CMA (Difco Laboratories, Inc., Franklin Lakes, NJ) for two days, then covered with a thin layer of moist vermiculite. Ten seeds from each soybean cultivar were surface disinfested for 90 seconds in 0.5% NaClO and placed equidistant around the center of the plate. Petri dishes were incubated in a growth chamber for 5 days at 25°C light/18°C dark with a 12-hour photoperiod before seeds were assessed for germination. Isolates which decreased emergence by 80% were considered pathogenic. Pathogenic isolates chosen for these experiment were *P. sylvaticum* isolate ‘39MK04’ and the *P. aphanidermatum* isolate ‘Pa64.’ The *P. oligandrum* isolate ‘MK120’ was chosen as a nonpathogen. All isolates were recovered from soybean seedlings in field studies in Arkansas (Kirkpatrick et al., 2006).
Pythium inoculum was grown in sand-corn meal media: 100 ml fine grain sand (Quickrete Commercial Grade Fine Sand, Atlanta, GA), 5.6 ml finely ground corn meal, and 40 ml de-ionized water mixed in 500-ml Erlenmeyer flasks. Flasks were sealed with foam plugs, the tops covered in aluminum foil, and autoclaved twice for 40 minutes, with a 24-hour period between sterilization cycles. Flasks were cooled to room temperature then inoculated with ten 25-mm² pieces from the edges of an actively growing culture of either *P. oligandrum*, *P. sylvaticum*, or *P. aphanidermatum* grown on CMA. Inoculum was grown at 21°C for 10 days and gently shaken every other day to promote uniform colonization of the medium. Multiple flasks of each isolate were combined prior to Pythium population determination and soil infestation.

Population counts were determined with the dilution spread plate technique on P5ARP (Jeffers and Martin, 1986) using 1 ml of a 1:100 dilution on 6 plates per sample. Dilutions were prepared using 25 g sand-corn meal, with the addition of soft water agar (1.3 g/L) (Moorhead Agar, Moorhead and Company, Van Nuys, CA) to a volume of 250 ml and agitated on a wrist action shaker for 20 minutes. Inoculum was mixed into experimental soil for each pot to achieve soil Pythium populations of approximately 200 propagules per gram soil (ODW).

**Electrical Conductivity**

Pasteurized soil was mixed with a 1M CaCl₂ solution at rates of 0, 2.1, 4.2, and 6.4 mL per pot. Calcium chloride rates were chosen to establish EC rates representative of soil sample measurements from samples collected in fields in Arkansas by Dr. Rick Cartwright in 2006 and 2007 (400-5000 µS/cm, DE). Electrical conductivity was determined for each individual pot at the end of the experiment using a method described by (Corwin and Lesch, 2005). EC was determined using the SWE method using a 1:2 (v:v) soil to water mixture. Soil (20 g ODW) and
de-ionized water (40 mL) was placed into a 500-ml Erlenmeyer plastic flask and sealed with a rubber cork. Flasks were agitated on a wrist action shaker for 20 minutes and then poured into 15-mL plastic centrifuge tubes. Tubes were capped and stored upright for several days in order to allow soil to form a pellet. The soil extract was poured into a clean 15-mL centrifuge tube and EC was determined using an Acorn Series Con 6 electrical conductivity meter (Oakton Instruments, Vernon Hills, IL). The soil extract was combined for each electrical conductivity treatment and soil pH was measured using an Acorn Series Con 6 electrical pH meter.

**Controlled environmental experiment**

Six soybean seed of a single cultivar were planted per pot at a 1-cm depth and incubated in growth chambers (Adaptis CMP6010, Conviron Inc., Pembina, ND) at temperatures consistent with soybean planting in Arkansas; 25°C light/18°C dark with a 12-hour photoperiod. Each experimental replication was placed in a single growth chamber. Pots were bottom-watered with deionized water to maintain a soil matric potential between saturation and -10J/kg and -20J/kg. Soil water content was determined for pots having a Watermark soil moisture sensor (Watchdog 200 series, Spectrum Technologies, Aurora, IL) and gravimetric analysis of selected pots (Black, 1965).

Twenty-one days after planting (DAP) seedling stand, leaf number, growth stage, and leaf discoloration were recorded. Leaf discoloration was assessed using a 1 to 5 scale where 1 = no discoloration, 2 = 1–10% discoloration, 3 = 11–25% discoloration, 4 = 26–50% discoloration, and 5 = 51–100% discoloration. Seedlings were removed from pots and shoots were separated from roots at the soil level and oven dried for 48 hours at 70°C. Roots were recovered from soil and gently rinsed for 20 min. under running water, rated for root discoloration, and scanned on a high-resolution image scanner (Expression 10000XL Scanner, Epson America, Inc., Long Beach, CA).
Beach, CA). Root disease was assessed using a 1-6 scale where 1 = no discoloration, 2 = 1-10% discoloration, 3 = 11-25% discoloration, 4 = 26-50% discoloration, 5 = 51-75% discoloration, and 6 = 76-100% discoloration. Root architecture was analyzed using WinRhizo software (Regent Instruments, Quebec, CA). Roots were surface disinfested in 0.5% NaClO for 90 seconds and plated on PsARP (Jeffers and Martin, 1986) in order to re-isolate *Pythium*.

Statistical analysis was conducted using Proc GLM (SAS Inc., Cary, NC) over experiments. Leaf number, root volume, number of root tips, root altitude, leaf discoloration, and root discoloration were all analyzed as an average per pot. For all parameters excluding stand, pots where stand equaled one were removed from subsequent analysis in order to eliminate bias due to plant compensation. Leaf and root discoloration were analyzed as mid-point values. Least significant differences were calculated for the significant main effects and appropriate interaction means using the guidelines in Statistical Methods in Agricultural Research (Little and Jackson Hills, 1972).

**RESULTS**

Electrical conductivity for experiment 1 averaged 640 µS/cm for control pots with no CaCl$_2$ added and 1060, 1632, and 2039 µS/cm for CaCl$_2$ treatments. In experiment 2, EC for control pots with no CaCl$_2$ added averaged 930 µS/cm and 1483, 2057, and 2570 µS/cm for CaCl$_2$ treatments. Pythium populations were approximately 241, 213, and 199 propagules per gram (ppg) in experiment 1; and 235, 176, and 212 ppg in experiment 2 for *P. sylvaticum*, *P. aphanidermatum*, and *P. oligandrum*, respectively. *Pythium* was isolated from all seedlings from Pythium-infested pots, but was not isolated from seedlings from non-infested pots.

For stand, the main effects experiment (Exp), Pythium infestation (Pyt), and salinity (EC) were significant. In addition, there were Exp x EC, Exp x Pyt x EC, and Pyt x Cult interactions.
Experiment differences for plant stand appeared to be due to EC levels being greater for treatments in general in experiment 2 than in experiment 1 especially for the treatment 3, 2057 and 1632 µS/cm, respectively (Table 3). Stand significantly decreased at 2039 µS/cm (exp 1) and at or above 1483 µS/cm (exp 2). *P. oligandrum* did not reduce stand compared to the EC treatment for any EC level or either experiment. In experiment 1, *P. aphanidermatum* decreased stand at or above 1060 µS/cm and *P. sylvaticum* decreased stand at or above 1632 µS/cm. In experiment 2, *P. aphanidermatum* and *P. sylvaticum* decreased stand at all EC levels except for the EC treatment 2570 µS/cm. Stand for both cultivars did not differ in non-infested and *P. oligandrum* treatments; but for *P. aphanidermaum* and *P. sylvaticum*, Glenn had greater stand than Osage.

For leaf number there were significant experiment, cultivar, salinity, and Pythium main effects. In addition, there were Exp x Cult, Exp x Cult x EC, Exp x Pyt, Pyt x EC, and Exp x Pyt x EC interactions (Table 2). Experiment interactions were the result of greater effects of salinity in experiment 2, primarily for the treatments EC 2057 and 2570 µS/cm, which were greater than EC levels for the same treatments in experiment 1 (Table 5). Salinity generally decreased leaf number starting at 2039 µS/cm in Exp 1 and 2057 µS/cm in Exp 2. *P. oligandrum* did not affect leaf number compared to the non-infested control, except for the highest salinity treatment in experiment 1. *P. aphanidermatum* reduced leaf number at the salinity levels of 2039 µS/cm (Exp 1) and 2057 µS/cm (Exp 2). *P. sylvaticum* reduced leaf number at EC levels at or above 1632 µS/cm (Exp 1) and at 1483 µS/cm (Exp 2). Leaf number decreased for both cultivars at or above 1632 µS/cm (Exp 1) and 1483 µS/cm (Exp 2). The only difference between cultivars was that Osage had fewer leaves than Glenn at 2057 µS/cm in Exp 2.
For shoot weight, all main effects (Exp, Pyt, EC, and Cult) were significant (Table 2). In addition, the interaction means Exp x Pyt, Pyt x EC, and Exp x Pyt x EC were significant. Salinity did not reduce shoot weight for non-infested controls until EC levels were 2039 or greater (Table 6). Pythium infestation did not affect shoot weight at the control salinity level. Shoot weight was not significantly reduced by *P. oligandrum* in either experiment, except for the 2039 µS/cm treatment (Exp 1). *P. aphanidermatum* decreased shoot weight at 2039 µS/cm and *P. sylvaticum* decreased shoot weight at or above 1632 µS/cm in experiment 1. In experiment 2, *P. aphanidermatum* decreased shoot weight at 2057 µS/cm, while *P. sylvaticum* decreased shoot weight at a lower EC level of 1483 µS/cm and 2057 µS/cm. Glenn had greater mean shoot weight (0.210 g) than Osage (0.194 g).

The main effects Exp, Cult, and EC were significant for root volume (Table 7). In addition, there were interaction for Exp x Pyt, Pyt x EC, and Exp x Pyt x EC. Salinity did not have an effect on root volume until an EC level of 2570 µS/cm (Exp 2) (Table 8). *P. aphanidermatum* and *P. sylvaticum* increased root volume in both experiments for the base soil EC. The only reduction of root volume for *Pythium* was with *P. sylvaticum* at an EC of 2039 µS/cm (Exp 1). Root volume was greater for cultivar Glenn than for cultivar Osage, which had means of 1.600 cm$^3$ and 1.193 cm$^3$, respectively.

All main effects (Exp, Cult, EC, and Pyt) were significant for number of root tips. In addition, the interactions Exp x Cult, Exp x EC, Exp x Pyt, and Exp x Pyt x EC were significant (Table 7). Increasing EC decreased root tips at and above 2057 µS/cm (Exp 2) (Table 9). *Pythium* did not affect root tip number; except, *P. oligandrum* appeared to have a stimulative effect at 2057 and 2570 µS/cm (Exp 2). There was no significant difference between Glenn and
Osage in experiment 1 (190.4 root tips), but Glenn had more root tips than Osage in experiment 2 (471.2 and 337.1, respectively).

The main effects Cult, EC, and Pyt were significant for root altitude (Table 7). Altitude is defined as the number of links or root segments in the longest path from any exterior link to the base link or tap root. In addition, the interactions Exp x Cult, Exp x EC, Exp x Pyt, and Exp x Pyt x EC were significant for root altitude. Root altitude decreased at and above EC of 1632 $\mu$S/cm for (Exp 1) and at and above 2057 $\mu$S/cm (Exp 2). Pythium infestation did not significantly affect root altitude for most EC levels (Table 10). The exceptions were a decrease in root altitude at 2057 $\mu$S/cm (Exp 2) for *P. aphanidermatum* and at and above 1632 $\mu$S/cm for *P. sylvaticum* (Exp 1). *P. oligandrum* increased root altitude at 2057 $\mu$S/cm (Exp 2). There was no significant difference between Glenn and Osage in experiment 1 (33.3), but Glenn had a greater root altitude than Osage in experiment 2 (41.0 and 30.5, respectively).

The main effect EC was significant for leaf discoloration (Table 11). In addition, there were significant interactions for Exp x EC, Exp x Cult, Pyt x EC, and Exp x Pyt x EC. In the first experiment, Osage had more leaf discoloration than Glenn with means of 14.2% and 7.9%, respectively. There was no cultivar difference in the second experiment (6.3%). Leaf discoloration increased at or above EC levels of 1060 (Exp 1) and 2570 (Exp 2). No consistent effects were observed for leaf discoloration within a Pythium treatment across EC levels for leaf discoloration.

Salinity and Pythium infestation had a significant effect on root discoloration. Root discoloration increased at 1632 (Exp 1) and 2057 (Exp 2) $\mu$S/cm, then decreased at the highest EC treatment. Root discoloration (%) was 5.95 for 640-960 $\mu$S/cm, 6.05 for 1060-1483 $\mu$S/cm, 9.40 for 1632-2057 $\mu$S/cm, and 6.90 for 2039-2570 $\mu$S/cm.
**DISCUSSION**

Electrical conductivity (EC) averaged 640 µS/cm in Exp 1 and 930 µS/cm in Exp 2 for control pots with no CaCl₂ added. Soil for experiment two was collected several months after experiment one soil. Environmental and cultural influences which occurred between experiments raised EC of non-treated soil and increased final EC of all treatments.

Salinity significantly reduced stand, leaf number, shoot weight, and root volume. Thresholds for stand reduction occurred between 1483-2039 µS/cm (1.5-2.0 dS/m). Leaf number, shoot weight, and root volume decreased at 2039-2057 µS/cm (approx. 2.0-2.1 dS/m). These results are in agreement with other experiments which have reported that salinity causes reductions in emergence as well as growth and development of soybean seedlings at relatively low salinity (Abel and MacKenzie, 1964; Abel, 1969; Chang et al., 1994; Essa, 2002; Hamdy et al., 1993; Lauchli and Wieneke, 1979; Li et al., 2006; Sharifi et al., 2007; Tuncturk et al., 2008).

In experiments using NaCl treatments, Abel and MacKenzie (1964) reported that salinity delayed emergence at low EC; approx. 3.1 dS/m (SPE) [approx. 1.24 dS/m (SWE)] and decreased seed germination for six soybean varieties at moderate EC levels with the greatest reductions at approximately 6 dS/m (SPE) [approx. 2.4 dS/m (SWE)]. Essa (2001) conducted experiments on three soybean cultivars using saline drainage water containing a variety of soluble ions and reported that germination decreased by approximately 10% when soil EC was increased from 0.5 dS/m to 2.5 dS/m (SPE) [approx. 0.2-0.9 dS/m (SWE)], with the greatest reductions occurring between 4.5 and 6.5 dS/m [approx. 1.8-2.6 dS/m (SWE)]. Threshold salinity levels for shoot dry weight were similar; however, root dry weight reductions at treatment levels between 0.5 and 8.8 dS/m were not significant.
Leaf discoloration ratings in this study were inconsistent for EC level and cultivar. This may have been a result of reduced emergence and delayed seedling development. Salinity by Pythium interactions on leaf discoloration also were inconsistent. Tamura and Chen (2009) and Valencia et al. (2008) established the reliability of using a visual scale to rate soybean leaf chlorosis and burning, or necrosis. Tamura and Chen (2009) conducted pot experiments using NaCl application starting at 21 DAP with EC treatments ranging from 8.0 to 16.0 dS/m. They reported that the critical level for NaCl concentration was 120 mM (12 dS/m) at 34 DAP. They also reported root system sensitivity to EC which was similar to their observations on leaf discoloration and shoot weight reductions. Valencia et al. (2008) reported similar thresholds for seedlings grown in a hydroponic system. NaCl was applied to 11 seedlings 11 DAP at EC treatments ranging from 4.0 to 16.0 dS/m. They reported critical EC between 120-160 mM (12-16 dS/m) at 22 DAP. Some of the discrepancy between these results and those reported in previous studies are due to the low experimental electrical conductivity levels in this experiment compared with other studies. In addition, seedlings were exposed to salinity at germination in this experiment, while Tamura and Chen (2009) and Valencia et al. (2008) used pre-germinated seedlings which were then exposed to osmotic shock. Umezawa et al. (2000) reported that symptoms of salt stress are more evident in some soybean cultivars after sudden introduction to soluble ions.

The effect of soil salinity on soybean seedling emergence and development in the presence of different *Pythium* spp. was examined. Pythium isolates were chosen in these experiments to represent a range of virulence and survival strategies. *P. oligandrum* is a homothallic, non-pathogenic species which is associated with soybean roots and has been used as a bio-control agent to prevent damping-off and root disease (Benhamou et al., 1997; Rey et al,
were chosen as pathogens of soybean.

Both pathogenic *Pythium* spp. interacted with salinity to reduce seedling stand, shoot growth, and leaf number, but the non-pathogenic *P. oligandrum* did not consistently differ from the control. Moderate salinity treatments (1483-2057 µS/cm) had the greatest differences for stand versus the non-infested control, although *P. aphanidermatum* tended to reduce stand at lower EC levels. Development also had interactions for Pythium by EC, with leaf number and shoot weight reductions at moderate salinity levels (1483-2057 µS/cm) for pathogenic *Pythium* spp, although *P. sylvaticum* tended to reduce shoot growth at lower EC levels. These species did not cause decreased stand or shoot growth at the lowest salinity levels. Decreases in stand and shoot growth were overwhelmed by the EC effect at the highest salinity treatment (2570 µS/cm). There were *Pythium* by EC interactions for root development. Root volume, number of root tips, and root altitude numerically decreased in general with increasing EC, but these effects were frequently not significant. The pathogenic species *P. aphanidermatum* and *P. sylvaticum* both had a stimulative effect on root volume at the base EC level in both experiments compared with the control.

It is well established that seedling diseases, and Pythium disease in particular, increase in the presence of salinity (Younger et al., 1967; Abrol et al., 1988; Rasmussen and Stanghellini, 1988; Schwarz and Grosch, 2002; Triky-Dotan et al., 2005; Al-Sadi et al., 2010). Griffin (1963), Hancock (1977), Lifshitz and Hancock (1983) and Paulitz and Baker (1987) attributed this relationship to a greater tolerance of *Pythium* to decreased water potential when compared with plant hosts, while Beach (1949) reported that a greater tolerance of *Pythium* to osmotic stress. On the other hand, Canaday and Schmitthenner (2010) and Borys (1964) have argued that
increased disease was related to changes in host physiology with chloride uptake. Other experiments have suggested that under saline conditions, certain Pythium isolates can become more virulent. Eberle (2008) reported that in experiments on rice using CaCl₂, a nonpathogenic isolate of *P. torulosum* interacted with EC to significantly increase stand loss and reduced plant development at moderate salinity levels (1744-2022 µS/cm). In the absence of salt, this isolate did not reduce stand. These results are consistent with this research, suggesting that pathogens which do not damage seedlings under low salinity conditions at a given inoculum level and environment may cause reduced stand and reduced seedling vigor in the presence of salinity.

*Pythium* species had a hormetic effect, characterized by low-dose stimulation and high-dose inhibition (Garzon and Flores, 2013), on root development versus the non-infested control at low salinity levels. Root volume increased at 640 and 930 µS/cm (6.4 and 9.3 dS/m) for *P. aphanidermatum* and *P. sylvaticum* infestations while *P. oligandrum* had no effect.

The research suggests that salinity-*Pythium* interactions may be more important in reducing seeding emergence and growth at moderate EC levels than either environment or pathogen effects alone. Pathogen-environment interactions can increase salinity damage. It appears that the effect is important with isolates or populations that have the ability to be pathogens but require plant stress to cause damage. It is important for soybean producers to consider environmental soil in controlling disease in the field; for example, delaying planting for a more favorable planting soil temperature or using fungicide seed treatments when planting in soil with salinity problems. Where possible, damage in saline fields with mild-moderately virulent *Pythium* spp. may be mitigated by leaching salt from field soils.
<table>
<thead>
<tr>
<th>Method</th>
<th>EC readings (µS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP&lt;sup&gt;x&lt;/sup&gt;</td>
<td>1,000 2,000 3,000 4,000 5,000 6,000 8,000 10,000 12,000</td>
</tr>
<tr>
<td>DE&lt;sup&gt;y&lt;/sup&gt; 1:2</td>
<td>300 700 1,200 1,600 2,000 2,400 3,200 4,000 4,800</td>
</tr>
</tbody>
</table>

<sup>x</sup> Adapted from Fischer et al. (2006)
<sup>y</sup> Saturated paste extract method
<sup>z</sup> Dilute extract method
Table 2. *P* values for stand, leaf number, and shoot weight 21 days after planting for two cultivars, four salt treatments, and four Pythium treatments.\(^{\dagger}\)

<table>
<thead>
<tr>
<th>Source</th>
<th>Stand (21 dap)</th>
<th>Leaf number</th>
<th>Shoot weight (odw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Cult</td>
<td>0.6056</td>
<td>0.0042</td>
<td>0.0097</td>
</tr>
<tr>
<td>Exp x Cult</td>
<td>0.0670</td>
<td>0.0481</td>
<td>0.2032</td>
</tr>
<tr>
<td>EC</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x EC</td>
<td>&lt; 0.0001</td>
<td>0.2264</td>
<td>0.2503</td>
</tr>
<tr>
<td>Cult x EC</td>
<td>0.0529</td>
<td>0.1444</td>
<td>0.0851</td>
</tr>
<tr>
<td>Exp x Cult x EC</td>
<td>0.5524</td>
<td>0.0374</td>
<td>0.1255</td>
</tr>
<tr>
<td>Pyt</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x Pyt</td>
<td>0.9931</td>
<td>&lt; 0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cult x Pyt</td>
<td>0.0014</td>
<td>0.0703</td>
<td>0.1767</td>
</tr>
<tr>
<td>Exp x Cult x Pyt</td>
<td>0.6056</td>
<td>0.2682</td>
<td>0.1114</td>
</tr>
<tr>
<td>Pyt x EC</td>
<td>0.3216</td>
<td>0.0003</td>
<td>0.0113</td>
</tr>
<tr>
<td>Exp x Pyt x EC</td>
<td>0.0458</td>
<td>&lt; 0.0001</td>
<td>0.0022</td>
</tr>
<tr>
<td>Cult x Pyt x EC</td>
<td>0.7876</td>
<td>0.6214</td>
<td>0.5975</td>
</tr>
<tr>
<td>Exp x Cult x Pyt x EC</td>
<td>0.3971</td>
<td>0.6172</td>
<td>0.1357</td>
</tr>
<tr>
<td>Rep (Exp)</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

\(^{\dagger}\) Each experiment had 6 replications and the experiment was conducted two times.
Table 3. Effect of soil salinity and Pythium treatment on soybean seedling stand.\(^y\)

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Control</th>
<th><em>P. oligandrum</em></th>
<th><em>P. aphanidermatum</em></th>
<th><em>P. sylvaticum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>5.8 abc(^z)</td>
<td>5.9 ab</td>
<td>4.9 cde</td>
<td>4.9 cde</td>
</tr>
<tr>
<td>1060</td>
<td>5.8 abc</td>
<td>5.3 abcd</td>
<td>4.7 def</td>
<td>4.9 cde</td>
</tr>
<tr>
<td>1632</td>
<td>4.9 cde</td>
<td>4.5 def</td>
<td>2.7 g</td>
<td>2.1 g</td>
</tr>
<tr>
<td>2039</td>
<td>2.5 g</td>
<td>1.8 gh</td>
<td>1.0 hi</td>
<td>0.9 hi</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>930</td>
<td>6.0 a</td>
<td>5.3 abcd</td>
<td>3.8 f</td>
<td>4.1 ef</td>
</tr>
<tr>
<td>1483</td>
<td>4.9 cde</td>
<td>4.7 def</td>
<td>2.7 g</td>
<td>2.3 g</td>
</tr>
<tr>
<td>2057</td>
<td>2.2 g</td>
<td>2.1 g</td>
<td>0.8 hi</td>
<td>0.8 hi</td>
</tr>
<tr>
<td>2570</td>
<td>0.3 i</td>
<td>0.8 hi</td>
<td>0.3 i</td>
<td>0.4 i</td>
</tr>
</tbody>
</table>

\(^y\) Stand taken from a mean of 6 seed per container with six replications 21 days after planting.

\(^z\) Means for stand followed by the same letter are not significantly different, Fisher's protected LSD (\(p = 0.05\)).
Table 4. Effect of soil Pythium infestation on stand of two soybean cultivars.\textsuperscript{y}

<table>
<thead>
<tr>
<th>Pythium</th>
<th>Plant stand</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glenn</td>
<td>Osage</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.0 ab</td>
<td>4.1 a</td>
<td></td>
</tr>
<tr>
<td>\textit{P. oligandrum}</td>
<td>3.6 b</td>
<td>3.9 ab</td>
<td></td>
</tr>
<tr>
<td>\textit{P. aphanidermatum}</td>
<td>3.1 c</td>
<td>2.3 d</td>
<td></td>
</tr>
<tr>
<td>\textit{P. sylvaticum}</td>
<td>2.9 c</td>
<td>2.2 d</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{y} Emergence and stand taken from a mean of six seed per container with six replications and two experiments 21 days after planting.

\textsuperscript{z} Means for stand followed by the same letter are not significantly different, Fisher's protected LSD \((p = 0.05)\).
Table 5. Effect of soil salinity and Pythium treatment, and cultivar on soybean seedling leaf number.\textsuperscript{y}

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Control</th>
<th>\textit{P. oligandrum}</th>
<th>\textit{P. aphanidermatum}</th>
<th>\textit{P. sylvaticum}</th>
<th>Leaf number</th>
<th>Leaf number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glenn</td>
<td>Osage</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>6.4 ab\textsuperscript{z}</td>
<td>6.8 ab</td>
<td>6.5 ab</td>
<td>7.3 a</td>
<td>6.9 ab\textsuperscript{z}</td>
<td>6.7 abc</td>
</tr>
<tr>
<td>1060</td>
<td>6.5 ab</td>
<td>6.2 abc</td>
<td>6.5 ab</td>
<td>6.0 abc</td>
<td>6.2 abc</td>
<td>6.4 abc</td>
</tr>
<tr>
<td>1632</td>
<td>5.8 bc</td>
<td>4.8 cde</td>
<td>4.8 cde</td>
<td>2.2 fgh</td>
<td>4.2 d</td>
<td>4.1 d</td>
</tr>
<tr>
<td>2039</td>
<td>4.0 d</td>
<td>0.7 hi</td>
<td>0.8 hi</td>
<td>0.1 hi</td>
<td>1.6 e</td>
<td>1.2 ef</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>930</td>
<td>6.7 abc</td>
<td>7.3 a</td>
<td>6.0 abc</td>
<td>6.3 abc</td>
<td>7.1 a</td>
<td>6.4 abc</td>
</tr>
<tr>
<td>1483</td>
<td>5.9 abc</td>
<td>6.5 ab</td>
<td>5.2 cd</td>
<td>3.7 def</td>
<td>4.9 d</td>
<td>4.7 d</td>
</tr>
<tr>
<td>2057</td>
<td>3.4 efg</td>
<td>2.2 fgh</td>
<td>0.2 i</td>
<td>1.9 ghi</td>
<td>4.0 d</td>
<td>1.9 e</td>
</tr>
<tr>
<td>2570</td>
<td>0.3 i</td>
<td>1.5 ghi</td>
<td>0.2 i</td>
<td>0.4 i</td>
<td>0.3 f</td>
<td>1.0 ef</td>
</tr>
</tbody>
</table>

\textsuperscript{y} Leaf number taken from a mean of 6 seed per container with six replications.

\textsuperscript{z} Means for leaf number for Pyt x EC or Cult x EC interaction followed by the same letter are not significantly different, Fisher's protected LSD (\(p = 0.05\)).
Table 6. Effect of soil salinity and Pythium treatment, and cultivar on soybean seedling shoot weight.\textsuperscript{y}

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Control</th>
<th><em>P. oligandrum</em></th>
<th><em>P. aphanidermatum</em></th>
<th><em>P. sylvaticum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>0.308 a\textsuperscript{z}</td>
<td>0.313 a</td>
<td>0.301 ab</td>
<td>0.282 abcd</td>
</tr>
<tr>
<td>1060</td>
<td>0.308 a</td>
<td>0.287 abcd</td>
<td>0.299 abc</td>
<td>0.256 abcde</td>
</tr>
<tr>
<td>1632</td>
<td>0.270 abcde</td>
<td>0.232 def</td>
<td>0.231 def</td>
<td>0.139 ghi</td>
</tr>
<tr>
<td>2039</td>
<td>0.206 efg</td>
<td>0.083 ijk</td>
<td>0.115 hij</td>
<td>0.024 jk</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>930</td>
<td>0.257 abcde</td>
<td>0.280 abcd</td>
<td>0.245 bcde</td>
<td>0.235 cdef</td>
</tr>
<tr>
<td>1483</td>
<td>0.233 def</td>
<td>0.279 abcd</td>
<td>0.225 def</td>
<td>0.138 ghi</td>
</tr>
<tr>
<td>2057</td>
<td>0.166 fgh</td>
<td>0.232 abcdef</td>
<td>0.042 jk</td>
<td>0.076 ijk</td>
</tr>
<tr>
<td>2570</td>
<td>0.023 k</td>
<td>0.055 jk</td>
<td>0.017 k</td>
<td>0.027 jk</td>
</tr>
</tbody>
</table>

\textsuperscript{y} Leaf number taken from a mean of six seed per container with six replications.

\textsuperscript{z} Means for shoot weight followed by the same letter are not significantly different, Fisher's protected LSD ($p = 0.05$).
Table 7. *P* values for root volume, root tips, and root altitude for two cultivars, four salt treatments, four Pythium treatments, and six replications.

<table>
<thead>
<tr>
<th>Source</th>
<th>Root volume cm³</th>
<th>Root tips</th>
<th>Root altitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp</td>
<td>0.0012</td>
<td>&lt; 0.0001</td>
<td>0.1146</td>
</tr>
<tr>
<td>Cult</td>
<td>0.0010</td>
<td>0.0014</td>
<td>0.0013</td>
</tr>
<tr>
<td>Exp x Cult</td>
<td>0.8011</td>
<td>0.0042</td>
<td>0.0085</td>
</tr>
<tr>
<td>EC</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x EC</td>
<td>0.8929</td>
<td>&lt; 0.0001</td>
<td>0.0169</td>
</tr>
<tr>
<td>Cult x EC</td>
<td>0.2020</td>
<td>0.0898</td>
<td>0.0560</td>
</tr>
<tr>
<td>Exp x Cult x EC</td>
<td>0.2781</td>
<td>0.1164</td>
<td>0.1343</td>
</tr>
<tr>
<td>Pyt</td>
<td>0.3022</td>
<td>0.0283</td>
<td>0.0007</td>
</tr>
<tr>
<td>Exp x Pyt</td>
<td>&lt; 0.0001</td>
<td>0.0007</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cult x Pyt</td>
<td>0.9954</td>
<td>0.5553</td>
<td>0.6000</td>
</tr>
<tr>
<td>Exp x Cult x Pyt</td>
<td>0.5019</td>
<td>0.7286</td>
<td>0.2936</td>
</tr>
<tr>
<td>Pyt x EC</td>
<td>0.0129</td>
<td>0.0841</td>
<td>0.1666</td>
</tr>
<tr>
<td>Exp x Pyt x EC</td>
<td>0.0136</td>
<td>0.0079</td>
<td>0.0030</td>
</tr>
<tr>
<td>Cult x Pyt x EC</td>
<td>0.5340</td>
<td>0.4443</td>
<td>0.1486</td>
</tr>
<tr>
<td>Exp x Cult x Pyt x EC</td>
<td>0.9953</td>
<td>0.6081</td>
<td>0.2319</td>
</tr>
<tr>
<td>Rep (Exp)</td>
<td>0.0004</td>
<td>0.2259</td>
<td>0.5753</td>
</tr>
</tbody>
</table>

* Each experiment had six replications and the experiment was conducted two times.
Table 8. Effect of soil salinity and Pythium treatment on soybean seedling root volume.\(^y\)

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Control</th>
<th><em>P. oligandrum</em></th>
<th><em>P. aphanidermatum</em></th>
<th><em>P. sylvaticum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>1.543 defg(^z)</td>
<td>1.293 defgh</td>
<td>2.875 a</td>
<td>3.223 a</td>
</tr>
<tr>
<td>1060</td>
<td>1.756 cdef</td>
<td>1.162 efghi</td>
<td>1.888 bcde</td>
<td>1.442 defg</td>
</tr>
<tr>
<td>1632</td>
<td>1.386 defgh</td>
<td>1.072 efghij</td>
<td>2.247 abcd</td>
<td>0.890 efghij</td>
</tr>
<tr>
<td>2039</td>
<td>1.350 defgh</td>
<td>0.450 ghij</td>
<td>0.546 fghij</td>
<td>0.101 hij</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>930</td>
<td>1.313 defgh</td>
<td>2.200 abcd</td>
<td>2.582 abc</td>
<td>2.823 ab</td>
</tr>
<tr>
<td>1483</td>
<td>1.507 defg</td>
<td>2.033 abcde</td>
<td>1.771 bcdef</td>
<td>1.393 defg</td>
</tr>
<tr>
<td>2057</td>
<td>1.146 efghij</td>
<td>2.129 abcde</td>
<td>0.448 hij</td>
<td>0.756 fghij</td>
</tr>
<tr>
<td>2570</td>
<td>0.064 j</td>
<td>0.625 ghij</td>
<td>0.149 j</td>
<td>0.277 ij</td>
</tr>
</tbody>
</table>

\(^y\)Root volume taken from a mean of six seed per container with six replications.

\(^z\)Means for root volume followed by the same letter are not significantly different, Fisher's protected LSD (\(p = 0.05\)).
Table 9. Effect of soil salinity and Pythium treatment on soybean seedling root tips.\(^y\)

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Control</th>
<th><em>P. oligandrum</em></th>
<th><em>P. aphanidermatum</em></th>
<th><em>P. sylvaticum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>241.5 defg</td>
<td>225.4 defghi</td>
<td>253.9 defg</td>
<td>332.3 bcd</td>
</tr>
<tr>
<td>1060</td>
<td>305.6 de</td>
<td>219.4 defghi</td>
<td>231.4 defgh</td>
<td>294.2 def</td>
</tr>
<tr>
<td>1632</td>
<td>180.7 defghi</td>
<td>117.2 efghi</td>
<td>189.2 defghi</td>
<td>121.5 defghi</td>
</tr>
<tr>
<td>2039</td>
<td>129.6 efghi</td>
<td>19.7 hi</td>
<td>31.1 fghi</td>
<td>16.0 i</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>930</td>
<td>693.5 a</td>
<td>673.0 a</td>
<td>667.4 a</td>
<td>499.6 abc</td>
</tr>
<tr>
<td>1483</td>
<td>586.0 ab</td>
<td>570.4 ab</td>
<td>547.6 ab</td>
<td>524.5 abc</td>
</tr>
<tr>
<td>2057</td>
<td>319.7 cde</td>
<td>697.2 a</td>
<td>87.1 defghi</td>
<td>173.6 defghi</td>
</tr>
<tr>
<td>2570</td>
<td>45.8 hi</td>
<td>149.7 defghi</td>
<td>44.4 hi</td>
<td>65.5 ghi</td>
</tr>
</tbody>
</table>

\(^y\)Root tips taken from a mean of 6 seed per container with six replications.

\(^z\)Means for root tips followed by the same letter are not significantly different, Fisher's protected LSD (\(p = 0.05\)).
Table 10. Effect of soil salinity and Pythium treatment on soybean seedling root altitude.\(^{y}\)

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Control</th>
<th><em>P. oligandrum</em></th>
<th><em>P. aphanidermatum</em></th>
<th><em>P. sylvaticum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>40.6 def</td>
<td>43.7 cdef</td>
<td>43.6 bcdef</td>
<td>49.8 abcdef</td>
</tr>
<tr>
<td>1060</td>
<td>41.8 cdef</td>
<td>37.9 efg</td>
<td>44.0 bcdef</td>
<td>48.9 abcdef</td>
</tr>
<tr>
<td>1632</td>
<td>34.0 fgh</td>
<td>30.7 fghi</td>
<td>35.6 efgh</td>
<td>17.3 ijk</td>
</tr>
<tr>
<td>2039</td>
<td>23.3 ghij</td>
<td>7.7 jk</td>
<td>6.4 jk</td>
<td>5.1 k</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>930</td>
<td>55.4 abc</td>
<td>58.5 ab</td>
<td>55.1 abc</td>
<td>41.8 cdef</td>
</tr>
<tr>
<td>1483</td>
<td>55.0 abcd</td>
<td>62.7 a</td>
<td>49.0 abcdef</td>
<td>43.4 cdef</td>
</tr>
<tr>
<td>2057</td>
<td>29.4 fghi</td>
<td>53.6 abcde</td>
<td>8.4 jk</td>
<td>18.6 hijk</td>
</tr>
<tr>
<td>2570</td>
<td>2.6 k</td>
<td>15.6 ijk</td>
<td>3.4 k</td>
<td>5.0 k</td>
</tr>
</tbody>
</table>

\(^{y}\)Root altitude taken from a mean of six seed per container with six replications.

\(^{z}\)Means for root altitude followed by the same letter are not significantly different, Fisher's protected LSD \((p = 0.05)\).
Table 11. *P* values for leaf discoloration and root discoloration for two cultivars, four salt treatments, and four Pythium treatments.\(^y\)

<table>
<thead>
<tr>
<th>Source</th>
<th>Leaf discoloration</th>
<th>Root discoloration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp</td>
<td>0.5056</td>
<td>0.6723</td>
</tr>
<tr>
<td>Cult</td>
<td>0.6157</td>
<td>0.1903</td>
</tr>
<tr>
<td>Exp x Cult</td>
<td>0.0008</td>
<td>0.6688</td>
</tr>
<tr>
<td>EC</td>
<td>0.0020</td>
<td>0.0328</td>
</tr>
<tr>
<td>Exp x EC</td>
<td>0.0366</td>
<td>0.0548</td>
</tr>
<tr>
<td>Cult x EC</td>
<td>0.7470</td>
<td>0.1619</td>
</tr>
<tr>
<td>Exp x Cult x EC</td>
<td>0.6162</td>
<td>0.3271</td>
</tr>
<tr>
<td>Pyt</td>
<td>0.3640</td>
<td>0.0015</td>
</tr>
<tr>
<td>Exp x Pyt</td>
<td>0.0586</td>
<td>0.0761</td>
</tr>
<tr>
<td>Cult x Pyt</td>
<td>0.3384</td>
<td>0.6769</td>
</tr>
<tr>
<td>Exp x Cult x Pyt</td>
<td>0.1900</td>
<td>0.9303</td>
</tr>
<tr>
<td>Pyt x EC</td>
<td>0.0061</td>
<td>0.6756</td>
</tr>
<tr>
<td>Exp x Pyt x EC</td>
<td>0.0067</td>
<td>0.2955</td>
</tr>
<tr>
<td>Cult x Pyt x EC</td>
<td>0.5817</td>
<td>0.2319</td>
</tr>
<tr>
<td>Exp x Cult x Pyt x EC</td>
<td>0.7254</td>
<td>0.5950</td>
</tr>
<tr>
<td>Rep (Exp)</td>
<td>0.0014</td>
<td>0.4906</td>
</tr>
</tbody>
</table>

\(^y\) Each experiment had six replications and the experiment was conducted two times.
Table 12. Effect of soil salinity and Pythium treatment on soybean seedling leaf discoloration.\textsuperscript{y}

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Control</th>
<th>\textit{P. oligandrum}</th>
<th>\textit{P. aphanidermatum}</th>
<th>\textit{P. sylvaticum}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>3.2 jklm</td>
<td>20.6 bc</td>
<td>7.7 fghi</td>
<td>13.7 e</td>
</tr>
<tr>
<td>1060</td>
<td>15.0 e</td>
<td>8.3 fgh</td>
<td>4.8 ijkl</td>
<td>16.4 de</td>
</tr>
<tr>
<td>1632</td>
<td>15.7 e</td>
<td>10.1 f</td>
<td>5.1 hijkl</td>
<td>19.1 cd</td>
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<td>2039</td>
<td>16.1 de</td>
<td>2.2 lm</td>
<td>7.0 fghi</td>
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<tr>
<td>930</td>
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<td>5.8 ghijkl</td>
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<td>0.5 m</td>
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<tr>
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<td>8.7 fg</td>
<td>37.8 a</td>
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<td>13.4 e</td>
<td>7.8 fghi</td>
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</tbody>
</table>

\textsuperscript{y} Leaf discoloration taken from a mean of six seed per container with six replications.

\textsuperscript{z} Means for leaf discoloration followed by the same letter are not significantly different, Fisher's protected LSD (\(p = 0.05\)).
III. EFFECTS OF SALINITY ON PYTHIUM DISEASE OF RICE

ABSTRACT

Soil salinization causes reduced productivity of agricultural lands throughout the world. Plants grown under saline conditions may have reduced growth and yield, as well as increased susceptibility to diseases. *Pythium* spp. are often associated with seedling damping-off and root rots of rice. The objective of this study was to characterize the effects of Pythium diseases on rice genotypes which differ in Pythium resistance. Controlled environmental experiments were conducted using soil treated with a 1M CaCl$_2$ solution to create a range of electrical conductivity levels. Soil was either infested or not infested with *Pythium* species which differed in virulence. Thirty-five days after planting seedling stand, growth, and development were assessed. Electrical conductivity (EC) averaged 651 (control), 1113, 1658, and 2190 $\mu$S/cm for calcium chloride treatments. Salinity significantly reduced emergence and stand at 1113 $\mu$S/cm. Shoot growth and root development also decreased at 1113 $\mu$S/cm. *P. irregulare* and *P. torulosum* decreased emergence and stand across all EC levels. These pathogens decreased shoot growth and root development at low EC levels (including the base salinity), but this effect was overwhelmed as salinity increased. *P. ultimum* slightly decreased emergence and stand at the base salinity, but had a protective effect at and above 1113 $\mu$S/cm. *P. ultimum* also increased root altitude across salinity levels (651 and 1658-2190 $\mu$S/cm). Environmental interactions are important considerations in understanding and managing seedling diseases. This research suggested that *Pythium* spp. which are virulent at base salinity levels have an additive effect that diminishes at high EC levels.
INTRODUCTION

Salinity is an important problem limiting rice stand establishment and yield in areas with poor quality irrigation water (Abrol et al., 1988). Concentrations of different salts can raise soil electrical conductivity (EC), including KCl, CaSO$_4$, NaHCO$_3$, Na2SO$_4$, NaCl, MgSO$_4$, MgCl$_2$, and CaCl$_2$. In Arkansas, calcium chloride occurs naturally in ground water, where it leaches from limestone formations and is carried into the root zone via irrigation practices (Gilmour et al., 1989; Mayhew et al., 1998). Aquifer depletion has prompted the USDA to designate areas of eastern Arkansas as critical groundwater zones (Robinson et al., 2003), with a 38 percent occurrence of unsustainable salinity levels in affected areas (Gilmour et al., 1989). Low ground water levels, naturally occurring mineral deposits, and reuse of irrigation water has increased chloride levels within agricultural soils to levels unsustainable for agricultural production (Kresse and Clark, 2008).

Salinity is measured using electrical conductivity (EC). Two of the most conventionally used laboratory methods used to quantify soluble salts in soil are the saturated paste extract method (SP) and the dilute soil extract method (DS). The SP method is most representative of field soil conditions, but is time consuming and susceptible to error. The DS method is often used for high volume of samples and is reproducible using a wide range of soils. DS assays typically use a soil:water (v:v) ratio of 1:1, 1:2, or 1:5. EC varies depending on the method used, so comparisons between experiments which use different methods must be done using conversion tables (Table 1) (Fischer et al., 2006). The international unit for EC is siemens (S), and soil salinity is frequently represented using either dS/m or µS/cm (1 dS/m = 1000 µs/cm). Soils with EC at or above approximately 4.0 dS/m (SP) or 1.6 dS/m (DS) are considered saline (Gartley, 2001). Maas and Grattan (1999) reported that rice (Oryza sativa L.) yields decrease
12% for every unit increase in average root-zone EC above 3.0 dS/m (SP) [approx. 1.2 dS/m (DS)], while Hanson et al. (1999) reported that the threshold is approximately 1.9 dS/m (SP) [approx. 0.7 dS/m (DS)], with a yield decline of 9.1% for each unit EC rather than a 12% yield decline.

Rice is particularly sensitive to salinity at the seedling stage (Maas and Hoffman, 1977b). Salinity tolerance increases from panicle formation to flowering (Abrol et al., 1988, Kaddah et al., 1975; Kaddah and Fakhry, 1961; Pearson, 1959; Pearson, 1961; Pearson et al., 1966). Agronomic traits of rice which are affected by salinity include plant height, root length, tillering ability, biomass, delayed panicle initiation and spikelet formation, reduced panicle length, number of primary branches and spikelets per panicle, fertility and panicle weight, and reduced grain yield (Pearson, 1961). Delayed inflorescence and increased number of sterile spikelets ultimately result in yield loss (Abrol et al., 1988).

Salt injury to rice occurs through osmotic imbalance and accumulation of toxic ions. Iwaki et al. (1953) and Shimose (1963) reported that salt stress in rice is caused by chloride accumulation in the shoot. However, more recent studies on the model dicot Arabidopsis thaliana report the importance of Na⁺ (Clarkson and Hanson, 1980; Comba et al., 1998; Flowers and Yeo, 1981) and its interference in potassium absorption (Devitt et al., 1981; Greenway and Munns, 1980; Wyn Jones, 1981; Gregorio, 1997). Ion toxicity reduces both below- and above-ground plant growth, delays plant maturity, and ultimately reduces grain yield (Pearson, 1961).

Eberle (2008) reported that P. arrhenomanes, P. irregulare, P. catenulatum, P. torulosum, and P. diclinum were associated with rice seedlings in Arkansas. P. irregulare is generally considered highly virulent and P. torulosum is moderately virulent on rice. Both pathogens are active at a wide range of soil temperatures with temperature minimums between 1-
5°C and temperatures between 24-30°C favoring optimal growth (Van Der Plaats-Niterink, 1981; Rosso, 2007). *P. ultimum* has a wide host range, is active at lower soil temperatures, and is highly virulent on soybean below 20°C (Martin and Loper 1999; Rosso, 2007). Soil moisture is an important environmental factor influencing the behavior of *Pythium* species. This effect is likely due to increased zoospore production. Bainbridge (1970) reported that zoospore production and motility is increased in wet soils, although mycelial growth of *P. ultimum* decreased (Lifshitz and Hancock, 1983). Kirkpatrick et al. (2006a) reported that flooding and soil infestation had an additive effect on severity of disease on soybean, caused by *P. ultimum*, particularly at the germination stage. *Pythium* isolation frequency increased in flooded soils (Kirkpatrick et al., 2006b). Fine textured, poorly drained soils at lower growing temperatures (typically between 15 and 20°C) are typically most problematic for *Pythium* disease (Rothrock et al., 2004; Rush, 1992). In Arkansas, environmental conditions in rice production areas are highly favorable for *Pythium* disease development.

It is generally accepted that soil salinity may contribute to increased susceptibility of seedlings to *Pythium* disease (Younger et al., 1967; Abrol et al., 1988; Rasmussen and Stanghellini, 1988; Triky-Dotan et al., 2005; Al-Sadi et al., 2010). Knowledge of the mechanisms behind this effect are not fully understood, but several ideas have been proposed. Many of these ideas center on a greater tolerance of *Pythium* to osmotic stress, pH changes, and decreased matric potential. Griffin (1963), Hancock (1977), Lifshitz and Hancock (1983) and Paulitz and Baker (1987) reported that *Pythium* was more tolerant of decreased water potential than plant hosts. Beach (1949) attributed increased damping off disease of tomato seedlings to tolerance of the pathogen to osmotic stress. On the other hand, saline pulse experiments suggest that disease susceptibility may be the result of osmotic shock (Macdonald, 1982). Canaday and
Schmitthenner (2010) reported that associated Phytophthora disease of soybean with increased EC, but attributed this effect to greater susceptibility of the host rather than Pythium tolerance. Still others have suggested that increased disease development is due to the physiological effects of ion toxicity (Borys, 1964; Canaday and Schmitthenner, 2010). The effects of soil salinity on Pythium varies with species, environmental conditions, and types of soluble salts in the system. Eberle (2008) observed that *P. torulosum*, which is generally not considered an important pathogen of rice in Arkansas, became more virulent under saline conditions using CaCl₂ treatments. The objective of this study was to examine the role of soil salinity on rice seedling growth in the presence of several *Pythium* species which differ in virulence using rice genotypes which differ in Pythium susceptibility.

**MATERIALS AND METHODS**

Silt loam soil from a soybean field which was in rotation with rice near Stuttgart, Arkansas (34° 44’ 42.14’’ N, 91° 33’ 20.02’’ W) was provided by Dr. Terry Spurlock. Soil was mixed and large pieces of plant material and clods were removed, then soil was steam pasteurized at approximately 70°C for 30 minutes prior to use. Sterile plastic 10 x 10 cm containers were filled with 250 g of soil oven dry weight (ODW). Each experiment was a randomized complete block design with 5 replications. The treatments included two rice genotypes, four Pythium treatments, and four salinity treatments arranged in a factorial treatment arrangement. The experiment was conducted two times. Two rice genotypes were used in this experiment; the Pythium susceptible “Wells” (PVP 200000077) which was developed in Arkansas and PI 560281, which has demonstrated some Pythium resistance (Rothrock, 2009).
**Pythium treatments**

Pythium isolates were selected to represent a range of virulence. Candidate isolates were grown on CMA (Difco Laboratories, Inc., Franklin Lakes, NJ) for two days, then covered with a thin layer of moist vermiculite. Ten seeds from each rice genotype were surface disinfested for 90 seconds in 0.5% NaClO and placed equidistant around the center of the plate. Petri dishes were incubated in a growth chamber for 5 days at 25°C light/18°C dark with a 12-hour photoperiod, then seeds were assessed for germination. Isolates which decreased emergence by 80% were considered pathogenic. Pathogenic isolates chosen for these experiment were *P. irregulare* isolate ‘CR341’ and the *P. torulosum* isolate ‘CEIR3,’ which were recovered from rice seedlings in field studies in Arkansas (Eberle, 2008). The *P. ultimum* isolate ‘MK124’ was chosen as a non-pathogen. This isolate was recovered on soybean seedlings from a field which was in rotation with rice (Kirkpatrick, 2006b).

Pythium inoculum was grown in sand-corn meal media: 100 ml fine grain sand (Quickrete Commercial Grade Fine Sand; Atlanta, GA), 5.6 mL finely ground corn meal, and 40 ml de-ionized water were mixed in 500-mL Erlenmeyer flasks. Flasks were sealed with foam plugs, the tops covered in aluminum foil, and autoclaved twice for 40 minutes, with a 24-hour period between sterilization cycles. Flasks were cooled to room temperature then inoculated with ten 25-mm² pieces from the edges of an actively growing culture of either *Pythium oligandrum, P. sylvaticum,* or *P. aphanidermatum* grown on CMA. Inoculum was grown at 21°C for 10 days and gently shaken every other day to promote uniform colonization of the medium. Multiple flasks of each isolate were combined prior to Pythium population determination and soil infestation.
Population counts were determined with the dilution spread plate technique on P₅ARP (Jeffers and Martin, 1986) using 1 ml of a 1:100 dilution on 6 plates per sample. Dilutions were prepared using 25 g sand-corn meal, with the addition of soft water agar (1.3 g/L) (Moorhead Agar, Moorhead and Company, Van Nuys, CA) to a volume of 250 ml and agitated on a wrist action shaker for 20 minutes. Inoculum was mixed into soil for each pot to achieve soil Pythium populations of approximately 200 propagules per gram soil (ODW).

**Electrical Conductivity**

Pasteurized soil was mixed with a 1M CaCl₂ solution at rates of 0, 1.1, 3.2, and 5.4 mL per pot. Calcium chloride rates were chosen to establish EC rates representative of soil sample measurements from samples collected in fields in Arkansas by Dr. Rick Cartwright in 2006 and 2007 (400-5000 µS/cm, DE). Electrical conductivity was determined for each individual pot at the end of the experiment using a method described by (Corwin and Lesch, 2005). EC was determined using the DS method using a 1:2 (v:v) soil to water mixture. Soil (20 g ODW) and de-ionized water (40 mL) was placed into a 500-ml Erlenmeyer plastic flask and sealed with a rubber cork. Flasks were agitated on a wrist action shaker for 20 minutes and then poured into 15-ml plastic centrifuge tubes. Tubes were capped and stored upright for several days in order to allow soil to form a pellet. The soil extract was poured into a clean 15-ml centrifuge tube and electrical conductivity was determined using an Acorn Series Con 6 electrical conductivity meter (Oakton Instruments, Vernon Hills, IL). The soil extract was combined for each electrical conductivity treatment and soil pH was measured using an Acorn Series Con 6 electrical pH meter.
**Controlled environmental experiment**

Six rice seed of a single genotype were planted per pot at a 1-cm depth and incubated in growth chambers (Adaptis CMP6010, Conviron Inc., Pembina, ND) at temperatures consistent with rice planting in Arkansas; 24°C light/15°C dark with a 12-hour photoperiod. Each experimental replication was placed in a single growth chamber. Pots were bottom-watered with deionized water to maintain a soil matric potential between saturation and -10J/kg and -20J/kg. Soil water content was determined for sample pots using Watermark soil moisture sensors (Watchdog 200 series, Spectrum Technologies, Aurora, IL) and gravimetric analysis of selected pots (Black, 1965).

Emergence was recorded 14 days after planting (DAP). Thirty-five days after planting (DAP) seedling stand, leaf number, growth stage, and leaf discoloration were recorded. Leaf discoloration was assessed using a 1 to 5 scale where 1 = no discoloration, 2 = 1-10% discoloration, 3 = 11-25% discoloration, 4 = 26-50% discoloration, and 5 = 51-100% discoloration. Seedlings were removed from pots and shoots were separated from roots at the soil line and oven dried for 48 hours at 70°C. Roots were recovered from soil and gently rinsed for 20 min. under running water, rated for root discoloration, and scanned on a high-resolution image scanner (Expression 10000XL Scanner, Epson America, Inc., Long Beach, CA). Root disease was assessed using a 1-6 scale where 1 = no discoloration, 2 = 1-10% discoloration, 3 = 11-25% discoloration, 4 = 26-50% discoloration, 5 = 51-75% discoloration, and 6 = 76-100% discoloration. Root architecture was analyzed using WinRhizo software (Regent Instruments, Quebec, CA). Roots were surface disinfested in 0.5% NaClO for 90 seconds and plated on P5ARP in order to re-isolate Pythium.
Statistical analysis was conducted using Proc GLM (SAS Inc., Cary, NC) over experiments. Emergence, stand, leaf number, root volume, number of root tips, root altitude, leaf discoloration and root discoloration were all analyzed as an average per pot. Leaf and root discoloration were analyzed as mid-point values. Least significant differences were calculated for the significant main effects and appropriate interaction means using the guidelines in Statistical Methods in Agricultural Research (Little and Jackson Hills, 1972).

RESULTS

Electrical conductivity (EC) averaged 651 µS/cm for control pots with no CaCl$_2$ added and 1113 µS/cm, 1658 µS/cm, and 2190 µS/cm for calcium chloride treatments. Final soil Pythium populations were approximately 239.4, 182.6, and 188.6 propagules per gram (ppg) for *P. irregulare*, *P. torulosum*, and *P. ultimum*, respectively. Pythium colonization was confirmed on 100% of roots from infested pots.

The main effect genotype (Gen), salinity (EC), and Pythium (Pyt) had a significant effect on rice seedling emergence and stand (Table 2). In addition, for emergence there was a Gen x Pyt and Pyt x EC interaction. For stand there was a Pyt x EC interaction. Soil salinity resulted in reductions in stand and emergence for all CaCl$_2$ treatments compared with the non-amended control (Table 3). Lowest stands numerically occurred at an EC of 2190 µS/cm, but emergence and stand were not different from an EC of 1658 µS/cm. *P. irregulare* and *P. torulosum* reduced both emergence and stand compared with non-infested controls at all salinity levels except one for each species. *P. ultimum* did not cause decreased emergence or greater stand losses with increasing salinity levels (Table 3). *P. ultimum* caused smaller reductions in emergence and stand compared to the non-infested control for the lowest soil EC; however, for most of the salinity treatments *P. ultimum* infestation resulted in increased emergence and stand compared to
non-infested treatments of the same salinity. Genotypes did not differ for non-infested control treatments, but emergence was lower for the Pythium-susceptible cultivar Wells compared with the Pythium-resistant genotype PI 560281 for *P. irregulare* and *P. torulosum* across soil salinity treatments. For final stands, the genotypes differed in stand with Wells having a mean stand of 3.3 and PI 560281 having a mean stand of 2.8 across all salinity and Pythium treatments.

The main effects Pythium infestation and salinity had a significant effect on seedling leaf number (Table 2). In addition, there was a Pyt x EC interaction. Leaf number decreased with increasing EC level, with no difference between ECs of 1658 and 2190 µS/cm for the non-infested control. *P. irregulare* and *P. torulosum* reduced leaf number compared to the control at EC levels of 651 and 1113 µS/cm, and 2190 µS/cm for *P. torulosum*. *P. ultimum* did not reduce leaf number (Table 4).

The main effects Gen, EC, and Pyt had a significant effect on shoot dry weight (Table 5). In addition, there was a significant Pyt x EC interaction for shoot weight. Increasing soil salinity decreased shoot weight for all treatments receiving CaCl$_2$ for the non-infested treatment, with no significant differences in shoot weight between the two highest EC levels. *P. irregulare* and *P. torulosum* reduced shoot weight relative to non-infested control for the lowest two EC treatments 651 and 1113 µS/cm. Shoot weight increased with *P. ultimum* infestation relative to the non-infested controls for the lowest salinity level. Wells and PI 560281 differed in mean shoot dry weight across salinity and Pythium treatments; 0.104 g compared to 0.144 g, respectively.

The main effects Pyt and EC had a significant effect on root volume (Table 5). In addition, there was a Pyt x EC interaction for root volume. Increasing salinity reduced root volume for EC levels of 1113, 1658, and 2190 µS/cm, compared to 651 µS/cm (control), with no significant differences between EC levels of 1113 and 1658 µS/cm (Table 6). *P. irregulare* and
P. torulosum reduced root volume relative to the non-infested control at an EC of 651 and 1658 µS/cm. P. ultimum infestation did not affect root volume compared to the non-infested control.

The main effects Gen, Pyt, and EC had a significant effect on root altitude (Table 5). Altitude is defined as the number of links or root segments in the longest path from any exterior root link to the base root link. In addition, there was a Pyt x EC interaction. Salinity reduced root altitude for each treatment level for the non-infested control (Table 6). P. irregulare and P. torulosum reduced root altitude for the lowest EC level and with P. irregulare for all but the greatest EC level compared to the non-infested control. P. ultimum increased root altitude at all but 1113 µS/cm, where it was numerically greater but did not differ from the non-infested controls. Wells compared to PI 560281 over Pythium and EC treatments had a genotype effect but no interaction.

For number of root tips per seedling, the main effects Gen, Pyt, and EC were significant (Table 5). In addition, there were Gen x EC and Pyt x EC interactions. Increasing soil salinity decreased number of root tips, but 113 and 1658 µS/cm were not significantly different for the non-infested control (Table 7). P. irregulare and P. torulosum decreased number of root tips for the three lowest and two lowest EC levels, respectively. P. ultimum did not consistently change root tip number. Increasing EC levels decreased number of root tips for both cultivars. For EC at or below 1113 µS/cm, PI 560281 had fewer root tips compared to Wells. Genotypes did not differ at EC at or above 1658 µS/cm.

For leaf discoloration there was an EC main effect and a Gen x EC interaction (Table 8). Greater salinity increased leaf discoloration for both genotypes above the soil EC level of 651 µS/cm. In addition, leaf discoloration significantly increased for cultivar Wells at 1658 and 2190
µS/cm compared to 1113 µS/cm. No consistent difference was observed for leaf discoloration between the genotypes.

The main effects Gen, EC, and Pyt were significant for root discoloration. In addition, there were significant interactions for Pyt x EC and Gen x Pyt x EC. Soil salinity did not affect root discoloration for rice seedlings for the non-infested control. Root discoloration was significantly greater for *P. torulosum* for both genotypes and for *P. irregular* for PI 560281 compared to the non-infested treatment at an EC level of 2190 µS/cm (Table 9).

**DISCUSSION**

This experiment corroborates previous research which reports that rice is very sensitive to salinity at the seedling stage. Both emergence and stand decreased at 1.1 dS/m. Reduced shoot growth (leaf number and shoot weight) and root development (root volume, number of root tips, and root altitude) were also observed at 1.1 dS/m, along with increased leaf discoloration. Root discoloration due to salinity did not differ. In experiments using CaCl₂ treatments on seedlings, Eberle (2008) reported that rice emergence decreased between 2.0 and 4.0 dS/m (DS), while stands were reduced between 1.1 and 1.7 dS/m (DS). Salinity thresholds for rice seedling damage in this experiment were consistent with these reports, if a little lower concerning emergence and stand. Differences may be due to salinity application methods. Eberle (2008) applied CaCl₂ either to trays beneath pots or to the surface of the soil. It is possible that in previous experiments seedling emergence was not affected because salt was concentrated at planting and had not yet diffused throughout the pot. This could also explain why stand losses were observed after emergence. In this experiment, CaCl₂ was mixed evenly into the soil before planting, and no differences were observed in emergence and stand at 14 DAP and 35 DAP. Rice was once considered salt tolerant, and suitable for saline soil conditions.
culture involves flooding fields for almost the entire growing season. This practice significantly
dilutes salts and reduces the effects of salinity (Abrol et al., 1988; Maas and Hoffman, 1977b).
Yadav and Girdhar (1981) reported that rice yields remain satisfactory even when conductivity is
20 to 25 dS/m (SP) [approx. 8 to 10 dS/m (DS)] near the soil surface, yet when saline
groundwater is used for irrigation, yield is significantly reduced (Abrol et al., 1988). Current
guidelines indicate that rice yields decrease 12% for every unit increase in average root-zone EC
above 3.0 dS/m (SP) [approx.. 1.2 dS/m DS)] (Hanson et al., 1999; Maas and Grattan, 1999);
however, a more recent study places the threshold at approximately 1.9 dS/m (SP) [approx.. 0.6
dS/m (DS)], with a yield decline of 9.1% for each unit EC rather than a 12% yield decline
(Grattan et al., 2002).

In this experiment, leaf discoloration was observed at the lowest salinity treatment level, 1.1 dS/m, with genotype PI 560281 having more discoloration at lower salinity than Wells. Rice
is most sensitive to salinity during the seedling stage (Maas and Hoffman, 1977b).
Photosynthetic and chlorophyll content are negatively correlated with salinity level (Ota and
Yasue, 1962); therefore, salt sensitivity can be assessed using visual ratings. In addition to
suppressing leaf formation and elongation, salinity causes leaves to become chlorotic, curl up,
and die. Symptoms begin on the oldest leaves, then progress to new growth. Gregorio et al.
(1997) reported success with visually screening rice seedlings for salt stress using NaCl.
Thresholds were reported at approximately 6 dS/m (SP) [approx. 2.4 dS/m (DS)]. Eberle (2008)
reported differences in leaf discoloration at 3.0 to 3.5 dS/m (DS). Differences between results in
this experiment and others may be due to variability between visual rating techniques. Another
important consideration is that in this experiment, lower salinity levels were used. A threshold
salinity level may be necessary in order to trigger adaptive responses to salt stress (Kawasaki et al., 2001).

Both *P. irregulare* and *P. torulosum* reduced emergence, final stand, and seedling development. PI 560281 did demonstrate some resistance to the *Pythium* spp. for emergence which was reported previously (Rothrock et al., 2004, 2009). Pathogenic *Pythium* effects were overwhelmed by salinity effects at the higher salinity levels. Environmental stress tends to increase severity of *Pythium* seed rots, damping-off, and root rots (Bateman and Dimock, 1959; Hoppe, 1949; Kraft and Roberts, 1969; Leach, 1947; Pieczarka and Abawi, 1978; Short and Lacy, 1976). Eberle (2008) reported that *Pythium* disease severity increased in the presence of CaCl$_2$. A mildly pathogenic isolate of *P. torulosum* caused stand reductions at EC levels above 1.1 to 1.7 dS/m (DS), although it increased stand at 0.4 dS/m (DS). In a previous experiment on soybean (Chapter 2), moderately virulent *Pythium* spp. which did not cause disease at the base salinity level reduced emergence, growth, and development at increased EC. These experimental results are inconsistent with previous research, as there appeared to be a negative Pyt x EC interaction for pathogenic *Pythium* spp. In these studies pathogenic effects were evident in non-amended soils and application of salt stress did not increases *Pythium* losses but just added to the salinity effects.

The nonpathogenic *Pythium* isolate showed a slight decrease in stand for the non-amended control, but had no stand loss with increasing salinity; suggesting a protective response to salinity effects. *P. ultimum* also stimulated root altitude for most non-infested treatments. These are the first known reports of a *Pythium* species ameliorating salt stress. Research with arbuscular mycorrhizal fungi (AMF) may alleviate salt stress. Sharifi et al. (2007) reported that infestation of soybean with *Glomus etunicatum* increased above- and below-ground growth of
plants treated with 5.0 and 10.0 dS/m (SP) NaCl [approx. 2.0 and 4.0 dS/m (DS), respectively]. Furthermore, they increased soybean root and shoot growth by 10-15% by pre-treating *G. etunicatum* with salt prior to inoculation. Soybean plants colonized with *Glomus intraradices* were also reported to contain increased carbohydrate content (Porcel and Ruiz-Lozano, 2004). Plants often use accumulation of soluble sugars to adjust osmotic potential under saline conditions (Munns, 1993; Pérez-Alfocea et al., 2010). Mechanisms are not currently understood, but AMF may improve the absorption and reallocation of carbohydrates, improving salt tolerance. Accumulation of the hormone Abscisic acid (ABA), an important growth regulating hormone, is another way that plants adapt to salinity. Naz et al. (2009) associated elevated levels of ABA with AMF isolated from salt-stressed soybean seedlings, but were not able to characterize the nature of this relationship using *in vitro* experiments. Other mycorrhizal fungi which are known to alleviate salt stress facilitate water and nutrient acquisition, root-shoot communication, hormonal homeostasis, and sequestration of toxic ions by altering root morphology and exudates in the rhizosphere (Dodd and Pérez-Alfocea, 2013). On rice, arbuscular mycorrhizal fungi (AMF) have are used to facilitate nutrient absorption (Manjunath et al., 1981; Secilia and Bagyaraj, 1994; Sharma et al., 1988). Further research is necessary to characterize the relationship between *P. ultimum* and rice and salinity stress.

Soil salinity and Pythium are important factors in limiting stand and development. Previous research suggested that Pythium losses often increase at increasing soil salinity levels. However, this experiment indicates that for virulent *Pythium* spp. this type of interaction is unlikely to occur. Damage is additive until a high salinity level, where little additional effects are evident. It is important for rice producers to incorporate environmental management in controlling disease in the field. It is important to minimize abiotic stresses as well as plant
pathogens to ensure uniform, vigorous seedling stand. A full understanding of pathogen-environment interactions is necessary for appropriate disease management and optimization of seedling stand establishment.
Table 1. Comparison of methods for measuring electrical conductivity (EC) in soil.\textsuperscript{x}

<table>
<thead>
<tr>
<th>Method</th>
<th>EC readings (µS/cm)</th>
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<tr>
<td>SP\textsuperscript{y}</td>
<td>1,000  2,000  3,000  4,000  5,000  6,000  8,000  10,000  12,000</td>
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<tr>
<td>DS\textsuperscript{z} 1:2</td>
<td>300  700  1,200  1,600  2,000  2,400  3,200  4,000  4,800</td>
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</tbody>
</table>

\textsuperscript{x} Adapted from Fischer et al. (2006)
\textsuperscript{y} Saturated paste extract method
\textsuperscript{z} Dilute soil extract method
Table 2. *P* values for emergence, stand, leaf number and shoot weight 35 days after planting for two genotypes, four salt treatments, and four Pythium treatments.²

<table>
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<tr>
<th>Source</th>
<th>Emergence (14 DAP)</th>
<th>Stand (35 DAP)</th>
<th>Leaf number</th>
<th>Shoot dry weight</th>
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<td>0.1869</td>
<td>0.0692</td>
<td>0.0761</td>
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<tr>
<td>Gen</td>
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<td>Exp x Gen</td>
<td>0.6151</td>
<td>1.0000</td>
<td>0.7613</td>
<td>0.9600</td>
</tr>
<tr>
<td>EC</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x EC</td>
<td>0.6717</td>
<td>0.4442</td>
<td>0.6253</td>
<td>0.1984</td>
</tr>
<tr>
<td>Gen x EC</td>
<td>0.2872</td>
<td>0.6802</td>
<td>0.2732</td>
<td>0.5461</td>
</tr>
<tr>
<td>Exp x Gen x EC</td>
<td>0.4057</td>
<td>0.3786</td>
<td>0.6084</td>
<td>0.6818</td>
</tr>
<tr>
<td>Pyt</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x Pyt</td>
<td>0.8509</td>
<td>0.6125</td>
<td>0.5245</td>
<td>0.2526</td>
</tr>
<tr>
<td>Gen x Pyt</td>
<td>0.0080</td>
<td>0.2294</td>
<td>0.6707</td>
<td>0.0510</td>
</tr>
<tr>
<td>Exp x Gen x Pyt</td>
<td>0.2490</td>
<td>0.0612</td>
<td>0.0593</td>
<td>0.3025</td>
</tr>
<tr>
<td>Pyt x EC</td>
<td>&lt; 0.0001</td>
<td>0.0005</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x Pyt x EC</td>
<td>0.3392</td>
<td>0.6229</td>
<td>0.1836</td>
<td>0.8956</td>
</tr>
<tr>
<td>Gen x Pyt x EC</td>
<td>0.1893</td>
<td>0.3457</td>
<td>0.7456</td>
<td>0.7377</td>
</tr>
<tr>
<td>Exp x Gen x Pyt x EC</td>
<td>0.2068</td>
<td>0.2267</td>
<td>0.2178</td>
<td>0.0586</td>
</tr>
<tr>
<td>Rep (Exp)</td>
<td>0.6938</td>
<td>0.8449</td>
<td>0.0531</td>
<td>0.0113</td>
</tr>
</tbody>
</table>

²Each experiment had five replications and the experiment was conducted two times.
Table 3. Effect of soil salinity and Pythium treatment on rice seedling emergence and stand; and effect of genotype on rice seedling emergence.\(^y\)

<table>
<thead>
<tr>
<th>Treatment EC (µS/cm)</th>
<th>Emergence (14 DAP)</th>
<th>Stand (35 DAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>(P. irregulare)</td>
</tr>
<tr>
<td>651</td>
<td>4.8 (a^z)</td>
<td>2.6 fg</td>
</tr>
<tr>
<td>1113</td>
<td>3.4 cde</td>
<td>3.0 def</td>
</tr>
<tr>
<td>1658</td>
<td>3.0 def</td>
<td>2.1 gh</td>
</tr>
<tr>
<td>2190</td>
<td>2.6 fg</td>
<td>1.7 hi</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wells</td>
<td>3.2 bc</td>
<td>1.6 d</td>
</tr>
<tr>
<td>PI 560281</td>
<td>3.7 ab</td>
<td>3.0 c</td>
</tr>
</tbody>
</table>

\(^y\) Emergence and stand taken from a mean of 6 seed per container with 5 replications across two experiments.

\(^z\) Interaction means for EC x Pyt for emergence or stand or Gen x Pyt for emergence followed by the same letter are not significantly different, Fisher's protected LSD \((p = 0.05)\).
Table 4. Effect of soil salinity and Pythium treatment on rice seedling leaf number and shoot weight.\(^y\)

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Leaf number (per pot)</th>
<th>Dry shoot weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td><em>P. irregulare</em></td>
</tr>
<tr>
<td>651</td>
<td>15.8 a(^z)</td>
<td>6.9 d</td>
</tr>
<tr>
<td>1113</td>
<td>9.4 bc</td>
<td>6.7 d</td>
</tr>
<tr>
<td>1658</td>
<td>6.4 de</td>
<td>4.3 ef</td>
</tr>
<tr>
<td>2190</td>
<td>5.6 def</td>
<td>3.5 fg</td>
</tr>
</tbody>
</table>

\(^y\) Leaf number and shoot weight taken from a mean of 6 seed per container with 5 replications over two experiments.

\(^z\) Means for leaf number or dry shoot weight followed by the same letter are not significantly different, Fisher's protected LSD (\(p = 0.05\)).
Table 5. *P* values for root volume, number of root tips, and root altitude for two genotypes, four salt treatments, and four Pythium treatments.

<table>
<thead>
<tr>
<th>Source</th>
<th>Root volume (cm$^3$)</th>
<th>Root tips</th>
<th>Root altitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp</td>
<td>0.0585</td>
<td>0.1303</td>
<td>0.6053</td>
</tr>
<tr>
<td>Gen</td>
<td>0.3199</td>
<td>0.0018</td>
<td>0.0206</td>
</tr>
<tr>
<td>Exp x Gen</td>
<td>0.3522</td>
<td>0.1247</td>
<td>0.8780</td>
</tr>
<tr>
<td>EC</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x EC</td>
<td>0.0834</td>
<td>0.2315</td>
<td>0.5262</td>
</tr>
<tr>
<td>Gen x EC</td>
<td>0.1281</td>
<td>0.0477</td>
<td>0.0707</td>
</tr>
<tr>
<td>Exp x Gen x EC</td>
<td>0.6870</td>
<td>0.9912</td>
<td>0.2359</td>
</tr>
<tr>
<td>Pyt</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x Pyt</td>
<td>0.0586</td>
<td>0.0528</td>
<td>0.0811</td>
</tr>
<tr>
<td>Gen x Pyt</td>
<td>0.4720</td>
<td>0.4183</td>
<td>0.3941</td>
</tr>
<tr>
<td>Exp x Gen x Pyt</td>
<td>0.4974</td>
<td>0.6262</td>
<td>0.6783</td>
</tr>
<tr>
<td>Pyt x EC</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x Pyt x EC</td>
<td>0.8693</td>
<td>0.9783</td>
<td>0.9761</td>
</tr>
<tr>
<td>Gen x Pyt x EC</td>
<td>0.7078</td>
<td>0.8004</td>
<td>0.9818</td>
</tr>
<tr>
<td>Exp x Gen x Pyt x EC</td>
<td>0.8764</td>
<td>0.9710</td>
<td>0.2654</td>
</tr>
<tr>
<td>Rep (Exp)</td>
<td>0.1751</td>
<td>0.1189</td>
<td>0.8402</td>
</tr>
</tbody>
</table>

* Each experiment had five replications and the experiment was conducted two times.
Table 6. Effect of soil salinity and Pythium treatment on rice seedling root volume and altitude per plant.\textsuperscript{z}

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Control</th>
<th>\textit{P. irregulare}</th>
<th>\textit{P. torulosum}</th>
<th>\textit{P. ultimum}</th>
<th>Control</th>
<th>\textit{P. irregulare}</th>
<th>\textit{P. torulosum}</th>
<th>\textit{P. ultimum}</th>
</tr>
</thead>
<tbody>
<tr>
<td>651</td>
<td>0.966 a\textsuperscript{z}</td>
<td>0.402 b</td>
<td>0.336 b</td>
<td>1.130 a</td>
<td>30.4 b</td>
<td>19.9 cde</td>
<td>14.8 efg</td>
<td>41.9 a</td>
</tr>
<tr>
<td>1113</td>
<td>0.379 b</td>
<td>0.246 bcd</td>
<td>0.277 bc</td>
<td>0.363 b</td>
<td>21.5 cd</td>
<td>13.9 fgh</td>
<td>17.0 def</td>
<td>24.5 bc</td>
</tr>
<tr>
<td>1658</td>
<td>0.316 b</td>
<td>0.117 cde</td>
<td>0.122 cde</td>
<td>0.326 b</td>
<td>15.5 efg</td>
<td>8.8 hij</td>
<td>10.8 ghi</td>
<td>25.8 bc</td>
</tr>
<tr>
<td>2190</td>
<td>0.123 cde</td>
<td>0.077 de</td>
<td>0.044 e</td>
<td>0.288 bc</td>
<td>8.3 hij</td>
<td>6.5 ij</td>
<td>4.9 j</td>
<td>17.8 def</td>
</tr>
</tbody>
</table>

\textsuperscript{y} Root volume and altitude taken from a mean of six seed per container with five replications and two experiments.

\textsuperscript{z} Means followed by the same letter for root volume or root altitude are not significantly different, Fisher's protected LSD ($p = 0.05$).
### Table 7. Effect of soil salinity and Pythium, and effect of genotype on rice seedling root tips.\(^y\)

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Control</th>
<th><em>P. irregulare</em></th>
<th><em>P. torulosum</em></th>
<th><em>P. ultimum</em></th>
<th>Wells</th>
<th>PI 560281</th>
</tr>
</thead>
<tbody>
<tr>
<td>651</td>
<td>122.4 b(^z)</td>
<td>56.9 cde</td>
<td>48.6 defg</td>
<td>163.0 a</td>
<td>110.7 a</td>
<td>84.7 b</td>
</tr>
<tr>
<td>1113</td>
<td>78.8 c</td>
<td>32.4 efg</td>
<td>44.1 defgh</td>
<td>66.5 cd</td>
<td>70.4 b</td>
<td>40.5 cd</td>
</tr>
<tr>
<td>1658</td>
<td>54.2 cdef</td>
<td>18.2 hi</td>
<td>30.0 fghi</td>
<td>66.0 cd</td>
<td>47.9 c</td>
<td>36.4 cd</td>
</tr>
<tr>
<td>2190</td>
<td>27.4 ghi</td>
<td>15.6 i</td>
<td>12.5 i</td>
<td>51.7 defg</td>
<td>24.1 d</td>
<td>29.5 d</td>
</tr>
</tbody>
</table>

\(^y\) Root tip number taken from a mean of six seed per container with five replications and two experiments.

\(^z\) Means followed by the same letter for the Pyt x EC or Gen x EC interactions are not significantly different, Fisher's protected LSD \((p = 0.05)\).
Table 8. *P* values for leaf discoloration and root discoloration for two genotypes, four salt treatments, and four Pythium treatments. *z*

<table>
<thead>
<tr>
<th>Source</th>
<th>Leaf discoloration</th>
<th>Root discoloration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp</td>
<td>0.3914</td>
<td>0.0518</td>
</tr>
<tr>
<td>Gen</td>
<td>0.7358</td>
<td>0.0443</td>
</tr>
<tr>
<td>Exp x Gen</td>
<td>0.3550</td>
<td>0.6166</td>
</tr>
<tr>
<td>EC</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x EC</td>
<td>0.6652</td>
<td>0.0756</td>
</tr>
<tr>
<td>Gen x EC</td>
<td>0.0178</td>
<td>0.4245</td>
</tr>
<tr>
<td>Exp x Gen x EC</td>
<td>0.4472</td>
<td>0.2473</td>
</tr>
<tr>
<td>Pyt</td>
<td>0.4103</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x Pyt</td>
<td>0.2279</td>
<td>0.4325</td>
</tr>
<tr>
<td>Gen x Pyt</td>
<td>0.8459</td>
<td>0.1905</td>
</tr>
<tr>
<td>Exp x Gen x Pyt</td>
<td>0.1771</td>
<td>0.4918</td>
</tr>
<tr>
<td>Pyt x EC</td>
<td>0.1903</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp x Pyt x EC</td>
<td>0.7207</td>
<td>0.2126</td>
</tr>
<tr>
<td>Gen x Pyt x EC</td>
<td>0.5806</td>
<td>0.0162</td>
</tr>
<tr>
<td>Exp x Gen x Pyt x EC</td>
<td>0.4992</td>
<td>0.6554</td>
</tr>
<tr>
<td>Rep (Exp)</td>
<td>0.8174</td>
<td>0.2774</td>
</tr>
</tbody>
</table>

*z* Each experiment had five replications and the experiment was conducted two times.
Table 9. Effect of soil salinity, genotype, and Pythium treatment on rice seedling root discoloration; and the effect of salinity and genotype on leaf discoloration.

<table>
<thead>
<tr>
<th>EC (µS/cm)</th>
<th>Control</th>
<th><em>P. irregulare</em></th>
<th><em>P. torulosum</em></th>
<th><em>P. ultimum</em></th>
<th>Leaf discoloration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>651</td>
<td>2.6 d(^z)</td>
<td>8.3 d</td>
<td>4.0 d</td>
<td>7.5 d</td>
<td>3.2 d</td>
</tr>
<tr>
<td>1113</td>
<td>2.7 d</td>
<td>6.7 d</td>
<td>8.7 d</td>
<td>3.3 d</td>
<td>13.9 c</td>
</tr>
<tr>
<td>1658</td>
<td>7.0 d</td>
<td>15.4 bcd</td>
<td>15.9 cd</td>
<td>9.3 d</td>
<td>29.3 ab</td>
</tr>
<tr>
<td>2190</td>
<td>20.4 bcd</td>
<td>9.4 d</td>
<td>68.0 a</td>
<td>8.4 d</td>
<td>35.8 a</td>
</tr>
<tr>
<td><strong>PI560281</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>651</td>
<td>4.3 d</td>
<td>4.9 d</td>
<td>3.7 d</td>
<td>5.5 d</td>
<td>4.2 cd</td>
</tr>
<tr>
<td>1113</td>
<td>5.4 d</td>
<td>2.5 d</td>
<td>3.3 d</td>
<td>6.8 d</td>
<td>25.9 ab</td>
</tr>
<tr>
<td>1658</td>
<td>5.3 d</td>
<td>4.8 d</td>
<td>12.9 cd</td>
<td>8.1 d</td>
<td>31.2 ab</td>
</tr>
<tr>
<td>2190</td>
<td>4.1 d</td>
<td>27.4 bc</td>
<td>36.0 b</td>
<td>5.2 d</td>
<td>24.5 b</td>
</tr>
</tbody>
</table>

\(^{y}\) Leaf number and shoot weight taken from a mean of six seed per container with five replications across two experiments.

\(^{z}\) Means for the interactions of Gen x Pyt x EC or Gen x EC followed by the same letter are not significantly different, Fisher's protected LSD (\(p = 0.05\)).
IV. EFFECTS OF SALINITY ON PYTHIUM

ABSTRACT

*Pythium* spp. are often associated with seed rot, damping-off, and root rots. Seedling diseases caused by salinity often increase in the presence of salinity. This *Pythium* by salinity interaction may result from a differential tolerance of *Pythium* spp. versus the host plant to the effects of salinity. Specific effects of salinity on *Pythium* spp. are not well understood and may vary with species, environment, and stages in the pathogen life cycle. *In vitro* experiments were conducted on *Pythium* spp. over a range of electrical conductivity (EC) levels using CaCl$_2$ solutions. Zoospore production, discharge, motility, and chemotaxis; oospore germination; and mycelial growth were used to assess salinity effects. EC levels in zoospore experiments ranged from 0.3 (control) to 4.3 dS/m. Salinity significantly decreased zoospore production and motility at EC levels as low as 1.3 dS/m, while zoospore discharge was reduced at 3.3 dS/m, and zoospore taxis was not significantly affected. EC levels in oospore and mycelium experiments ranged from 2.3 (control) to 12.8 dS/m. Mycelial growth and oospore germination were not significantly affected by increased EC. Understanding the biology of seedling pathogens and how they are affected by environment is important for the management of seedling diseases over the range of planting environments. This research indicates that *Pythium* spp. are able to grow and reproduce at EC levels which limit seedling stand establishment. Results suggest that increased Pythium disease is not the result of conditions which favor pathogen development.
INTRODUCTION

Seedling diseases caused by *Pythium* spp. are consistently a problem on numerous crops, including rice (Chun and Schneider, 1998; Cother and Gilbert, 1993; Eberle, 2008; Kato et al., 1985; Robertson, 1973) and soybean (Bates, 2002; Hamman et al., 2002; Rosso, 2007; Wrather, 1997; Urrea, 2013). Symptoms of seedling disease caused by *Pythium* spp. include seed rot and seedling mortality, root discoloration and decay, and necrosis of cotyledons and hypocotyls or coleoptiles. Reduced root biomass results in reduced seedling vigor and uneven plant stands (Hendrix and Campbell, 1973). Destruction of root tips, root hairs, and thin feeder roots inhibits soil exploration and nutrient absorption. Rice and soybean seedlings typically present little evidence of necrosis on roots or reduction in seedling vigor (Avanzato, 2001; Bates, 2002; Kirkpatrick et al., 2006a; Webster and Gunnell, 1992). Seedling disease is generally greater at lower soil temperatures (Bateman and Dimock, 1959; Hoppe, 1949; Kraft and Roberts, 1969; Pieczarka and Abawi, 1978; Short and Lacy, 1976), suggesting that in addition to plant stress increased disease may be due to slower seedling emergence and root growth, maximizing period of host contact with the pathogen at a susceptible stage (Paulitz and Baker, 1987).

*Pythium* spp. are Oomycetes with coenecytic, hyaline hyphae. Species may be either homothallic, reproducing both sexually and asexually or heterothallic, unable to undergo sexual reproduction without the presence of a compatible mating type. Oogonia and antheridia are the “female” and “male” sexual reproductive structures of the genus *Pythium*. The smaller, elongate to club-shaped antheridium fertilizes the oogonium to form an oospore. This thick-walled sexual spore is the primary survival structure for many *Pythium* spp., is resistant to dessication and temperature change, and may survive in the soil for long periods of time until a suitable host or organic substrates become available (Alexopoulos et al., 1996). Stanghellini and Nigh (1972)
reported that oospores of *P. aphanidermatum* remained viable in oat roots after a 16-month period of dormancy. Factors influencing oospore germination include age, CO₂ concentration, and alternating soil wetting and drying (Adams, 1971; Ayers and Lumsden, 1975; Johnson, 1988). Even when conditions become favorable, some oospores maintain dormancy in a survival strategy called constitutive dormancy (Lumsden and Ayers, 1975). During germination, oospores are susceptible to desiccation or lysis due to changes in the soil environment and predation by bacteria (Adams, 1971; Ayers and Lumsden, 1975; Hancock, 1981; Qian and Johnson, 1987).

The asexual reproductive structure is the sporangium—a discretely spherical or lobate to indiscrete hyphal swelling. Sporangia of most species produce asexual spores, which are called zoospores. Zoospores are kidney-shaped single cells, with two flagella (a tinsel and a whip) attached to the concave side of the cell. Zoospores mature in a vesicle formed outside the sporangium, and soil saturation typically promotes zoospore release (MacDonald, 1982). As free-swimming spores, zoospores in the rhizosphere respond to chemicals in root exudates, encyst on a root surface, and germinate rapidly. Zoospore chemotaxis and germination may be induced by different root exudates (Donaldson and Deacon, 1993; Tripathi and Grover, 1978). If a host is not found, zoospores may encyst within the soil and remain viable for several days as long as environmental conditions remain favorable. Stanghellini and Burr (1973) demonstrated that *P. aphanidermatum* zoospores can survive in moist soil for up to seven days, but are desiccated when soil is air dried for two days. In some species, the ability to produce zoospores has been lost and sporangia germinate directly with a germ tube. Alternatively, in some species sporangia may germinate both directly or indirectly depending on temperature, with lower temperature favoring zoospore production (Van Der Plaats-Niterink, 1981).
Soil salinity can be caused by concentrations of different salts which raise soil electrical conductivity, including KCl, CaSO₄, NaHCO₃, Na₂SO₄, NaCl, MgSO₄, MgCl₂, and CaCl₂ (Allison et al., 1969). In Arkansas, elevated levels of soil chloride occur when calcium chloride leaches from limestone formations, and is carried into the root zone by ground water and irrigation (Mayhew et al., 1998). In addition, soybean and rice are often rotated with one another in poorly drained, alluvial soil (Wilson et al., 1998). Studies report that Pythium disease severity increases in the presence of salinity (Abrol et al., 1988; Al-Sadi et al., 2010a; Cuartero and Eberle, 2008; Fernandez-Munoz, 1999; Kafkafi, 1996; Rasmussen and Stanghellini, 1988; Schwarz and Grosch, 2002; Triky-Dotan et al., 2005; Wulff et al., 1998; Younger et al., 1967).

Studies on oomycetes and in particular terrestrial species of *Pythium* and *Phytophthora* suggest that they are salinity tolerant (Duniway, 1979; Blaker and MacDonald, 1985; Coffey and Joseph, 1985; MacDonald and Duniway, 1978; Sanogo, 2004; Tresner and Hayes, 1971). In general, zoospore production is sensitive to salinity (Al-Sadi et al., 2010a; Al-Sadi et al., 2010b; Kiyoomi et al., 2007; Rasmussen and Stanghellini, 1988), while mycelial growth and oospore production/germination are not as sensitive (Al-Sadi et al, 2010a; McQuilken, 1992; Rasmussen and Stanghellini, 1988). Effects of salinity on pathogen fitness are highly variable among species (Blaker and MacDonald, 1985; Hassan and Fadl-Allah, 1993) and with temperature (Rasmussen and Stanghellini, 1988). This study examined the effects of salinity on the biology and behavior of *Pythium*.

**MATERIALS AND METHODS**

Laboratory experiments used *Pythium aphanidermatum* isolate PA64, isolated from soybean from a field near Colt, Arkansas (Kirkpatrick, 2006b). For mycelial growth, species examined in addition to *P. aphanidermatum* were *P. sylvaticum* 39MK04 and *P. oligandrum*
MK120, isolated from soybean (Kirkpatrick, 2006b). All isolates were maintained on corn meal agar, CMA (Difco Laboratories, Inc., Franklin Lakes, NJ).

Experimental salinity levels were obtained with the addition of a 1M CaCl\(_2\) solution. Electrical conductivity was measured using an Acorn Series Con 6 electrical conductivity meter (Oakton Instruments, Vernon Hills, IL).

**Zoospore production and discharge**

Zoospores were produced using a modified protocol developed by Heungens and Parke (2000). Four 25 mm\(^2\) agar plugs were transferred from the edge of an actively growing culture of *P. aphanidermatum* on CMA into a 50-mL Erlenmeyer flask filled with 12 mL 20% clarified V8 juice (Campbell’s Soup Co., U.S.A.) broth (Jeffers, 2006). Flasks were incubated in darkness at 21\(^\circ\)C for 48 hours. V8 juice broth was decanted and mycelial mats were washed and flooded with sterile deionized water and incubated for 12 hours at 21\(^\circ\)C to allow formation of sporangia. Deionized water was decanted and mycelial mats were flooded with 4 mL 50% sterilized, filtered pond water (refrigerated at 2\(^\circ\)C) that was not amended (EC 0.3 dS/m) or adjusted with 1M CaCl\(_2\) to EC levels of 1.3, 2.3, 3.3, and 4.3 dS/m prior to use (Jeffers, 2006). Pond water was poured off and replaced at one-hour intervals two additional times, then flasks were incubated for 4.5 hours at 21\(^\circ\)C before zoospores were harvested. Zoospores were quantified microscopically using a hemocytometer. Each experimental unit was counted 10 times, using the entire counting grid. The experiment was a randomized block design with eight replications and was conducted two times.

**Zoospore motility and chemotaxis**

Zoospore taxis was evaluated using the capillary root model described by Royle and Hickman (1964). The assay used was a modification of an agar-free version described by Allen
and Newhook (1973), utilizing open-ended capillaries. Fifty seeds of soybean cultivar Hutcheson were surface sterilized for 90 seconds with 70% ethanol, added to 50-mL deionized water in a 1-L Erlenmeyer flask, and placed in a rotary shaker at 70 rpm for 48 hours. Seed exudates were filtered through two layers of Whatman No. 1 filter paper to remove debris, and diluted with sterile deionized water to achieve a final concentration of 10% of the original exudate (v/v). 2-µL capillary tubes were filled with the exudate solution and placed in zoospore suspension.

Flat bottomed micro-titer wells (96 wells, Thermo Fisher Scientific), were used to evaluate the attraction of zoospores to root exudates using modified methods from a protocol described by Jones et al. (1991) and Heungens and Parke (2000). Wells were filled with 150 µl of a zoospore suspension diluted with sterile, deionized water to a concentration of 5x10⁴ spores/ml. Zoospores were produced using the method described above, using sterile, filtered 50% pond water not adjusted (EC 0.3 dS/m) or adjusted prior to use with 1M CaCl₂ to EC levels of 1.3, 2.3, 3.3, and 4.3 dS/m. Glass capillaries containing root exudates were placed at fixed angles into each well for 15 minutes. Zoospores within capillaries were expelled and counted using a compound microscope at 400x magnification. Controls consisted of zoospores which were produced in pond water without CaCl₂, and capillaries which were filled with sterile, deionized water. Each well containing two capillary tubes comprised an experimental unit. This experiment was a completely randomized design with four replicates and conducted two times.

**Mycelial growth**

Clarified V8 juice broth was prepared as described above and not amended (2.3 dS/m) or adjusted prior to use with 1M CaCl₂ to EC levels of 4.5, 6.6 and 12.8 dS/m. 500-mL Erlenmeyer flasks were filled with 250-mL of clarified V8 juice broth and autoclaved for 20
minutes. Flasks were allowed to cool to room temperature then a 25mm² plug from the edge of an actively growing Pythium culture on CMA was transferred to each flask. Flasks were placed on a rotary shaker at 70 rpm and incubated in the dark at 21°C for 7 days. Mycelial mats were removed and vacuum-filtered on two layers of Whatman No. 1 filter paper, then oven-dried at 60°C for 24 hours. Control flasks were prepared with V8 medium without Pythium and were filtered and weighed. The experiment was a completely randomized design with four replications and was conducted two times.

**Oospore germination**

Oospore germination was evaluating using a modified version of the method described by Lumsden (1980). Ten percent V8 juice liquid medium was prepared by mixing 100 mL V8 vegetable juice 900 mL deionized water, and 2.5 g CaCO₃ and vacuum-filtered through two layers of Whatman No. 1 filter paper (Jeffers, 2006). V8 juice liquid medium was supplemented with 500 µL of wheat germ oil (Viobin Corp.; Monticello, IL) to promote oospore formation (Ruben et al., 1980). Erlenmeyer flasks (250-mL) were filled with 50 mL of the liquid medium and autoclaved for 20 minutes. Flasks were cooled to room temperature then a 25mm² plug from the edge of an actively growing culture of *P. aphanidermatum* on CMA was transferred to each flask. Flasks were incubated in darkness for 2 weeks at 21°C then mycelial mats were washed twice with sterile deionized water. Mycelial mats were homogenized in a Waring laboratory blender for three minutes and filtered through 100 and 45 µm monofilament nylon mesh (Tetko Inc., Briarcliff Manor, NY) to remove mycelial fragments. Oospores were collected on 20 µm nylon mesh and centrifuged at 5000 rpm for 5 minutes to clean the spores. Oospores were suspended in sterile de-ionized water to a concentration of 10,000 spores/mL.
A drop of oospore suspension was transferred to a square of sterile, nitrocellulose membrane on soil-extract agar (SEA). SEA was prepared by the addition of 1.5% agar (Difco Laboratories, Inc., Franklin Lakes, New Jersey) to soil extract (Jeffers, 2006) and not adjusted (EC 2.3 dS/m) or adjusted with 1M CaCl₂ to achieve four EC levels of 4.5, 6.6 and 12.8 dS/m. Petri plates were incubated for 16 hours in darkness at 21°C, then nitrocellulose membranes were removed from SEA and spores were examined using a microscope. One hundred spores were arbitrarily chosen and visually inspected for germination. Oospores with germ tubes equal to or greater than the spore diameter were considered germinated. This experiment was a completely randomized design with 10 replicates and was conducted two times.

**Statistical analysis**

Data from each *in vitro* experiment were analyzed across experiment by the appropriate experimental design using GLM, SAS version 9.1 (SAS institute, Cary NC). Means separation was performed using Fisher's protected LSD ($p = 0.05$).

**RESULTS**

**Zoospore production and discharge**

The protocol used produced approximately $7.4 \times 10^4$ spores/mL without added CaCl₂. Salinity had a significant effect on zoospore production ($P = <0.0001$). Mean zoospore production was 26.5, 16.6, 14.8, and 10.6/µl for 1.3, 2.3, 3.3, and 4.3 dS/m, respectively (LSD = 3.8) and was significantly lower than the number of spores/mL for the control salinity of 0.3 dS/m. Zoospore production was significantly reduced at 6.3 dS/m compared to the other salinity treatment (Fig 1).

Salinity had a significant effect on zoospore discharge ($P = <0.0001$). Zoospore discharge was measured as a ratio of discharged to non-discharged spores, remaining within the vesicle or
adhering as a group. Mean zoospore discharge was 1.000, 1.000, 1.000, 0.256, and 0.216 for 0.3, 1.3, 2.3, 3.3, and 4.3 dS/m, respectively (LSD = 0.036). Zoospore discharge significantly decreased at 3.3 dS/m or above compared the base salinity treatment (Fig 2).

**Zoospore motility and chemotaxis**

Salinity had a significant effect on zoospore motility ($P = < 0.0001$). Mean number of zoospores in root exudate capillary tubes was 2560.1, 2378.0, 2275.0, 538.9, and 518.6 for 0.3, 1.3, 2.3, 3.3, and 4.3 dS/m, respectively (LSD = 180.3). Zoospore motility decreased significantly at 1.3 and 4.3 ds/m compared to the control (Fig 3).

Chemotaxis was as assessed as the ratio of zoospores in the root exudate capillaries compared with the number of zoospores in capillaries containing deionized water. Salinity did not have an effect on zoospore chemotaxis ($P = 0.9017$). The mean chemotaxis ratio was 12.8:1 (Fig 4).

**Mycelial growth**

Mycelial growth numerically increased with additional EC; however, salinity had no significant effect on mycelial growth ($P = 0.3293$), but there was a significant Pythium effect on mycelial growth ($P = < 0.0001$). Mean mycelial growth was 0.0258, 0.0283, 0.0512, and 0.1167 g for 2.3, 4.5, 6.6, and 12.8 dS/m, respectively (Fig 5). All Pythium species significantly differed from each other (Fig 6).

**Oospore germination**

Salinity did not have a significant effect on oospore germination ($P = 0.4706$) at the range of EC’s tested, control to 12.8 dS/m. Mean germination was 21.7% after 16 hours (Fig 7).
DISCUSSION

In previous experiments (Chapter 2, 3) using several Pythium spp. and CaCl$_2$ treatments, growth and development of soybean and rice seedlings was reduced at moderate salinity levels to a greater extent than with either Pythium or salinity by itself for pathogenic species. This is in agreement with other research that found that disease increases with increasing salinity (Abrol et al., 1988; Al-Sadi et al., 2010; Cuartero and Fernandez-Munoz, 1999; Kafkafi, 1996; Rasmussen and Stanghellini, 1988; Schwarz and Grosch, 2002; Triky-Dotan et al., 2005; Wulff et al., 1998; Younger et al., 1967). Examples include P. ultimum on chrysanthemum (Gladstone and Moorman, 1989), Phytophthora capsici on chili pepper (Sanogo, 2004), and Phytophthora parasitica on tomato (Swiecki and MacDonald, 1991).

The literature suggests that Pythium by salinity interaction hinges from a differential tolerance of Pythium spp. versus plant hosts to the effects of salinity, but the specific mechanism is a matter of debate. Pythium spp. are relatively tolerant to decreased water potential (Griffin, 1963; Hancock, 1977; Lifshitz and Hancock, 1983; Paulitz and Baker, 1987). In addition, Pythium spp. have a relatively high tolerance to osmotic stress (Beech, 1949). This was demonstrated in experiments using NaCl treatments compared with balanced macronutrient treatments, where Pythium root rot reduced shoot biomass with threshold levels between 4-6 dS/m (Kafkafi, 1996; Schwarz and Grosch, 2002). On the other hand, Macdonald (1982) and Canaday and Schmitlenner (2010) have argued that oomycetes are in fact sensitive to salinity, and that disease increases are due greater susceptibility of the host. Macdonald (1982) used saline pulse experiments to subject hosts to osmotic shock. Canaday and Schmitthenner (2010) reported decreased fitness of Phytophthora subjected to osmotic stress, and noted that reductions in plant growth occurred even in un-saturated soils. This led them to conclude that increased
disease development is due to chloride toxicity in the host and not tolerance of *Phytophthora* spp. to salinity. One difficulty with interpreting this research is that effects on oomycetes vary depending on whether mycelial growth, zoospore production, or other criteria were used to measure pathogen fitness.

The results of these experiments demonstrate that zoospores are sensitive to salinity. Zoospore production gradually declined starting at 1.3 dS/m, and discharge dropped dramatically at 3.3 dS/m. Rasmussen and Stanghellini (1988) performed experiments on *P. aphanidermatum*, *P. dissoticum*, and *P. catenulatum* using both CaCl$_2$ and NaCl and reported reduced zoospore production at EC levels as low as 1.3 dS/m, with total inhibition at 14 dS/m. They noted that *P. aphanidermatum* was particularly sensitive to salinity compared with the other *Pythium* spp.

Chemotaxis differs from motility in that zoospore movement is directed by exterior cues. Relative chemotaxis was recorded as a ratio of motile zoospores attracted to seed exudates compared to motile zoospores that were not attracted to seed exudates in order to distinguish between taxis and motility. Encystment was interpreted as the formation of a cell wall and loss of flagella. There is little information in the literature about the effects of salinity on movement of *Pythium* zoospores. In this experiment, *P. aphanidermatum* zoospore motility decreased as spores encysted at EC between 1.3 and 4.3 dS/m, but no significant reductions in chemotaxis were observed. CaCl$_2$ did not affect chemotaxis, but further research is needed to determine effects on taxis were obscured by loss of motility. In experiments on *Phytophthora parasitica* which subjected zoospores to several different salts including CaCl$_2$ in the presence of tomato plants, Bouchibi et al. (1990) reported that zoospore taxis decreased at approximately the same salinity level as decreased zoospore production (approx. 7 dS/m). Von Broembsen and Deacon (1997) reported that *P. parasitica* ceased motility at 5 dS/m, the same EC level at which
decreased production was observed. They reported that at lower levels, of CaCl$_2$ and Ca(NO$_3$)$_2$, zoospores encysted in the absence of an organic nutrient trigger. Both studies emphasized the importance of cation ratios over Cl$^-$ on *P. parasitica* zoospore behavior. Donaldson and Deacon (1992) also described the importance of Ca$^{2+}$ on encystment of *P. aphanidermatum* zoospores, inducing encystment in the absence of organic nutrients.

In the mycelial growth assay, no significant differences were found in mycelial growth of *P. aphanidermatum, P. sylvaticum,* or *P. oligandrum* up to 12.8 dS/m. Mycelial growth was not evaluated at higher salinity levels, because they would be unsustainable for agricultural production. Mycelial growth of *Pythium* spp. is generally considered insensitive to salinity (Hassan and Fadl-Allah, 1993; McQuilken et al., 1992). Al-Sadi et al. (2010a) reported that mycelial growth of *P. aphanidermatum, P. spinosum,* and *P. splendens* increased or was unaffected by NaCl salinity up to 5 dS/m, yet *P. oligandrum* growth decreased significantly. Kiyoomi et al. (2007) reported mycelial growth of *P. aphanidermatum* was unaffected by salinity. Rasmussen and Stanghellini (1988) observed a slight increase in mycelial growth rate of *P. aphanidermatum* using CaCl$_2$ and NaCl treatments at 2.8-7.1 dS/m, but reported a strong negative correlation with mycelial growth at and above EC of 15 dS/m for *P. aphanidermatum,* *P. catenulatum,* and *P. dissoticum.* This effect was temperature-dependent, as the negative correlation occurred only at temperatures below 30°C. This effect is likely due to decreased electrical conductivity, because EC decreases with increased temperature. However, this research illustrates the importance of environmental variables which influence the effects of salinity on *Pythium* spp.

In the oospore germination assay, there was no significant difference in oospore germination up to 12.8 dS/m. Oospore germination appears to be less sensitive than oospore
production to increased salinity. Al-Sadi et al. (2010) reported that *P. aphanidermatum* oospore production was significantly reduced at 10 dS/m, with none produced above 20 dS/m. However, in comparative experiments with polyethylene glycol, NaCl, and KCl treatments, McQuilken et al., (1992) reported that *P. oligandrum* is more sensitive to decreased matric potential than osmotic potential, with reduced germination at -3.0 to -3.5 MPa. More research is needed to determine whether these results are representative of the effects of salinity on oospore germination in the soil environment.

This research suggests that zoospores are sensitive to osmotic changes, whereas mycelial growth, and oospore germination are less sensitive. It is advantageous for mycelial growth and oospore functionality to be maintained in saline environments, because they are necessary the organism’s survival. Mycelial growth is important for continued exploration of substrate. Salinity is not necessarily evenly distributed in a field, which is evidenced by patchy reductions in plant growth. Oospores are important survival structures. Continued production and functionality ensures inoculum for the next growing season. Zoospores, on the other hand, are short-lived and are highly sensitive to salinity. Given that they are important vehicles for infection, it seems counter to the observations that Pythium disease is not affected or increases in saline environments; however, the versatility of terrestrial oomycetes gives them more than one mechanism of infection. Even if infection by zoospores and oospores is no longer possible, hyphae can infect roots. Increased Pythium disease in the presence of salinity may hinge on plant susceptibility, but it also depends on the same ecological robustness that enables oomycetes to occupy diverse biological niches and a wide range of environmental conditions.

Understanding of the biology of seedling pathogens and how they are affected by environment is important for the management of seedling diseases under the range of planting
environments. This research indicates that *Pythium* spp. are able to grow and reproduce at EC levels which are prohibitive for seedling stand establishment; however, growth and reproduction did not increase with increased EC. In previous experiments on soybean and rice seedlings, disease severity increased at even moderate salinity levels (Chapter 2, 3). Increased Pythium disease may be due to increased host susceptibility under saline conditions, but further study on host defenses are necessary to make this determination.
Figure 1. Effect of salinity on zoospore production of *Pythium aphanidermatum*. Means for zoospore production followed by the same letter are not significantly different, Fisher's protected LSD ($p = 0.05$).
Figure 2. Effect of salinity on zoospore discharge of *Pythium aphanidermatum*. Means for zoospore discharge followed by the same letter are not significantly different, Fisher's protected LSD ($p = 0.05$).
Figure 3. Effect of salinity on zoospore motility of *Pythium aphanidermatum*. Means for zoospore motility followed by the same letter are not significantly different, Fisher's protected LSD ($p = 0.05$).
Figure 4. Effects of salinity on zoospore chemotaxis of Pythium aphanidermatum. Means for zoospore chemotaxis followed by the same letter are not significantly different, Fisher's protected LSD ($p = 0.05$).
Figure 5. Effects of salinity on mycelial growth of *Pythium* spp. Means for mycelial growth followed by the same letter are not significantly different, Fisher's protected LSD ($p = 0.05$).
Figure 6. Effect of salinity on mycelial growth of *Pythium oligandrum* (Po), *P. aphanidermatum* (Pa), and *P. sylvaticum* (Ps). Means for mycelial growth followed by the same letter are not significantly different, Fisher's protected LSD ($p = 0.05$).
Figure 7. Effects of salinity on oospore germination of *Pythium aphanidermatum*. Means for oospore germination followed by the same letter are not significantly different, Fisher's protected LSD ($p = 0.05$).
V. LITERATURE CITED


Hoppe, P. 1949. Differences in Pythium injury to corn seedlings at high and low temperatures. Phytopathology 39:77-84.


