Fichtner, Elizabeth Jeanne, Abiotic pathogen suppression: physiology and biology of aluminum toxicity to soilborne fungi. (Chair: H. David Shew; Co-chair: Dean Hesterberg)

An interdisciplinary approach was utilized to study the toxicity of aluminum (Al) to soilborne plant pathogens with the goal of developing a pathogen-suppressive potting medium containing non-phytotoxic, Al-organic matter complexes. Toxicological studies addressed the differential toxicity of monomeric Al species to *Thielaviopsis basicola* and *Phytophthora parasitica* and documented the baseline sensitivity of these organisms to the metal. Until recently, research on Al-toxicity to fungi has only focused on the trivalent Al cation (Al^{3+}) which is also considered the most phytotoxic Al ion. The toxicity of Al-hydrolysis species to fungi were tested by modeling in vitro test solution equilibria using GEOCHEM-PC and correlating the predicted values of Al-species activities with reduction in spore production of the two pathogens. Chlamydospore production of *T. basicola* was negatively correlated with Al^{3+} activity, whereas inhibition of sporangia production of *P. parasitica* was related to the activity of multiple monomeric Al species. Toxicity of Al to *T. basicola* was observed in solutions containing $\geq$ 20 μM Al. Sensitivity of *P. parasitica* to Al was observed at < 1.0 μM Al, suggesting that *P. parasitica* is more sensitive to Al than *T. basicola*. Using fluorescence microscopy, the localized accumulation of Al in pathogen tissues was detected using lumogallion, an Al-specific, fluorescent stain. Accumulation of Al was observed under various chemical conditions, ranging from salt solutions to more complex systems containing Al-peat complexes. An ecological approach was applied to study the dynamic interactions of soil chemical and physical properties with soil microflora for the suppression of *P. parasitica* in a medium amended with $Al_2(SO_4)_3$ and composted swine waste (CSW). Abiotic and biological mechanisms of pathogen suppression were

incorporated into the CSW-amended medium. Al-mediated suppression resulted in reduction of sporangia production in medium exhibiting K-exchangeable Al levels > 2 μM Al. Biological suppression also resulted in reduction of sporangia production and this suppression was maintained after Al levels dropped below the threshold necessary for abiotic suppression. The incorporation of abiotic and biological control mechanisms into a potting media may facilitate suppression of a wide range of soilborne pathogens and enhance applicability of disease-suppressive media in a disease management strategy.

ABIOTIC PATHOGEN SUPPRESSION: PHYSIOLOGY AND BIOLOGY OF ALUMINUM TOXICITY TO SOILBORNE FUNGI

by

Elizabeth Jeanne Fichtner

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and

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APPROVED BY:

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Co-Chair of Advisory Committee	**Co-Chair of Advisory Committee**
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DEDICATION

This dissertation is dedicated in loving honor of

David Bouton

my friend, teacher, and spiritual guide on the quest to save the schneebs.

BIOGRAPHY

Elizabeth Fichtner is from Hicksville, NY and graduated from Hicksville High School in 1993. Although Hicksville is a bustling town outside the greater metropolis of New York City, Elizabeth never developed a strong Long Island accent, but has since incorporated "ya'll" into her active vocabulary. Before matriculating from Hicksville High School, Elizabeth successfully evacuated the gymnasium housing the science fair by simply displaying her research specimens at the show. These specimens included mixtures of partially decomposed cabbage and horse manure; the aromatics that this mixture generated were not appropriately contained. In May 1997, she received a BS in Plant Science from the College of Agriculture and Life Sciences at Cornell University, Ithaca, NY. At Cornell, Elizabeth excelled at causing a ruckus and actually gave her advisor, 'Uncle Don', some extra gray hairs. Cornell has been a quiet place since she moved to Raleigh, NC in August 1997 to commence post-graduate studies at N.C. State Univserity. Elizabeth completed a M.S. in Plant Pathology with a co-major in Soil Science in 2000, with a research project focused on the biogeochemical interactions of soil aluminum and soilborne plant pathogenic fungi. Pursuing an inter-disciplinary research project, Elizabeth continued to investigate aluminum toxicity to soilborne plant pathogens as part of her dissertation research. Elizabeth's extracurricular activities included, but were not limited to: studies of urban feline ecology, chair seat weaving, teaching and competing in tae kwon do, irish step dancing, and pioneering spectacular April Fools jokes on esteemed faculty members in the Dept. of Plant Pathology and the College of Engineering.

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Chapter 1: Aluminum and plant disease

Aluminum and Plant Disease

H. D. Shew, E. J. Fichtner, and D. M. Benson

I. Introduction

The chemistry of aluminum (Al) in natural soils has been a topic of extensive study due to the toxicity of the metal to plants in both agricultural systems and forest ecosystems. In acid soils, the heightened level of available Al is a major limiting factor for plant growth and development. The need to ameliorate Al toxicity to plants has incited research focusing on manipulation of soil chemistry and breeding of plants for increased tolerance to Al. Recently, increased acid rain deposition has enhanced the awareness of the role of Al at an ecological level, with research groups studying the toxicity of Al to plants and microbes in forest ecosystems. While most research has focused on ameliorating Al toxicity to plants, plant pathologists have strived to document the toxicity of the metal to soilborne plant pathogens and utilize the toxicity to suppress plant disease development.

One of the major limiting factors in studying the interaction of Al with plants and plant pathogens is the need to bridge classical disciplines. An inter-disciplinary approach is necessary to understand the complexity of basic soil Al chemistry, soil microbiology, plant physiology, and plant pathology and to make conclusions and recommendations at the interface of these disciplines. Comparisons across pathosystems are often difficult due to differences in soil type and/or the synthetic media selected for in vitro toxicity studies. The presence of other chemical components (competing cations, anions, chelators, etc) and soil physical characteristics (water potential, bulk density, etc) further

compound the difficulty in synthesizing information across studies, inhibiting the application of accepted concepts and techniques to new systems.

Forms of Al in the soil environment

Aluminum is a common element in the earth's crust, comprising approximately 7% of the total solid matter in an average soil (Lindsay, 1979). The toxicity of Al to plants has incited numerous studies on the chemistry of Al in highly weathered, acid, agricultural soils. In the soil environment, Al exists in many forms that can be grouped into three major categories: inorganic Al, soluble Al, and organic Al.

Inorganic Al. Inorganic Al refers to mineral forms of Al, exchangeable Al, and interlayer Al. In the mineral component, Al is found in primary minerals, such as layer silicate clays (ie. feldspars, kaolinite, imogolite), and in secondary minerals such as clay minerals (montmorillonite, vermiculite, kaolinite), hydrous oxides (ie. gibbsite), sulphates (ie. jurbanite) and phosphates (ie. variscite) (Ritchie, 1995). The dissolution of Al-bearing minerals tends to be pH dependent, with dissolution increasing with decreasing pH (Ritchie, 1995). Exchangeable Al refers to Al adsorbed non-specifically to negatively-charged sites on clay minerals and hydrous oxides of iron, aluminum and manganese via electrostatic forces (Ritchie, 1989; Ritchie, 1995). It may also be specifically adsorbed to variable charged sites on hydrous oxides and the edges of clay minerals (Ritchie, 1989). Interlayer Al refers to the polynuclear, hydrolysed Al that is fixed between the layers of silicate clays (Ritchie, 1995). Although non-exchangeable, interlayer Al may be reactive, protonating or deprotonating in response to changes in pH (Ritchie, 1995).

Soluble Al. In soils, dissolved Al levels range between 10 and 350 μM Al depending mainly on pH (Ritchie, 1989). Soluble forms of Al include a multitude of species found in solution including mononuclear hydrolysis species, polynuclear species, and organic complexes. Mononuclear Al hydrolysis species contain one Al ion in the metal-ligand complex (Nordstrom and May, 1996). Mononuclear Al species include Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^{+}$, $Al(OH)_3^{0}$, and $Al(OH)_4^{-}$. Formation of these hydrolysis species in aqueous solution is almost instantaneous (Nordstrom and May, 1996), with a rate only slightly slower than the hydrolysis of water (Holmes et al, 1968). The free ionic Al ion (Al^{3+}) is surrounded by a sphere of hydration composed of six water molecules (Nordstrom and May, 1996). The hydration formula, represented as $Al(H_2O)_6^{3+}$, was first estimated by empirical methods, then confirmed using nuclear magnetic resonance (Akitt et al., 1972; Connick and Poulson, 1957). Hydrolysis constants have been determined using many techniques including potentiometry, spectroscopy, spectrophotometry, calorimetry, and electrochemistry (Nordstrom and May, 1996). Hydrolysis of Al involves the loss of hydration sphere protons to water molecules in the bulk solution (Nordstrom and May, 1996). The first hydrolysis reaction is represented by the equation

$$Al^{3+} + H_2O = Al(OH)^{2+} + H^{+} \qquad [1]$$

with a conditional equilibrium constant of

$$K_1 = \frac{[Al(OH)^{2+}]\,[H^{+}]}{[Al^{3+}]} \qquad [2]$$

The six waters of hydration are omitted for simplicity. The values determined for K_1 by multiple research groups are in close agreement, averaging a value approximating 5.00 (Baes and Mesmer, 1976; Frink and Peech, 1954; Parks, 1972; Raupach, 1963; Schofield and Taylor, 1954).

The second, third, and fourth hydrolysis reactions can be represented by the following equations in sequential order.

$$Al^{3+} + 2H_2O = Al(OH)_2^{+} + 2H^{+} \quad [3]$$

$$Al^{3+} + 3H_2O = Al(OH)_3^{0} + 3H^{+} \quad [4]$$

$$Al^{3+} + 4H_2O = Al(OH)_4^{-} + 4H^{+} \quad [5]$$

The hydrolysis constants for the second, third, and fourth hydrolysis reactions are more difficult to estimate than the first due to the low solubility of Al with increasing pH and the presence of polymerization reactions (Nordstrom and May, 1996). The second hydrolysis constant (K_2) is estimated as 10.1 (May et al., 1979); the third hydrolysis constant is estimated as 16.8 (Baes and Mesmer, 1976; May et al., 1979; Nazarenko and Biryuk, 1974); and estimates for the fourth constant (K_4) range from 22.2 (May et al., 1979) to 23.3 (Parks, 1972) depending upon experimental conditions and assumptions. The pH-dependent distribution of mononuclear Al species in solution is demonstrated in Fig.1.

Polynuclear Al species contain more than one Al ion in the metal-ligand complex. Multiple structures for these polynuclear Al species have been proposed, but evidence of their occurrence is limited (Bertsch and Parker, 1996). The degree of polymerization increases with increasing pH and concentration of Al (Aveston, 1965). At higher pH levels, monomer, dimer, and the tridecameric Al_{13} polynuclear species (Al_{13}) of Al may be present (Aveston, 1965).

Organic Al. The presence of organic acids in a soil solution affects the speciation of Al (Vance et al., 1996). Additions of organic matter to acid soils tend to reduce the amount of KCl-exchangeable Al. The reduction of exchangeable Al with addition of

organic matter is explained by formation of metal-organic matter complexes that are stable enough to resist extraction. The formation of stable soil aggregates often involves the bridging of organic matter with clay using polyvalent ions such as Al^{3+}(Goldberg et al., 1990; Lee et al., 1988). The kinetics of Al-organic matter complexation are faster than the dissolution of Al from minerals such as gibbsite ($Al(OH)_3$) (Walker et al., 1990), and Al binds more strongly to soil organic matter than to the permanent charge sites in a mineral soil (Bloom et al., 1979; Coleman and Thomas, 1967). Because of the strong relationship between Al and organic matter, it was originally thought that Al formed chelation complexes with aromatic, carboxylate, and hydroxide groups on soil organic matter (Stevenson and Ardakani, 1972). Titration and electron spin resonance studies, however, suggest that metal ions including copper (Cu), manganese (Mn), and Al do not form chelation complexes with organic ligands on H-saturated peat and humic acid (Bloom and McBride, 1979). Most metals, with the exception of Cu, form outer-sphere (hydrated) complexes with the variable charge carboxylic acid sites of organic matter (Bloom and McBride, 1979). Copper forms a strong, inner-sphere complex with the fulvic acid fraction of soil organic matter (Deczky and Langford, 1978). Because it forms a stronger complex with organic matter than Al, Cu can be used to extract both exchangeable Al and organically bound Al from soil (Juo and Kamprath, 1979).

The cation exchange capacity (CEC) of soil organic matter is related to the negative charge imparted by deprotonation or dissociation of H^+ from acid functional groups (Sparks, 1995). Potentiometric titrations of H-organic matter in the presence of Al were used to quantify the relationship between pH and adsorption of Al (Hargrove and Thomas, 1982 b). The conditional formation constants for Al-organic matter complexes

increase with pH (Hargrove and Thomas, 1982 b), with adsorption best modeled by a complex containing 1 mol of Al per mol of carboxyl groups (Bloom and McBride, 1979). Most Al ions bound to organic matter are hydrolyzed and act as a buffer against changes in pH (Hargrove and Thomas, 1982 a) and hydrolyzed Al ions bind stronger than Al^{3+} to soil organic matter (Bloom et al., 1979).

Regulation of Al activity in soil solutions

The solubility of Al is controlled by precipitation and dissolution reactions, adsorption onto inorganic minerals, reactions with organic matter, and the formation of soluble complexes (Lindsay and Walthall, 1996; Ritchie, 1989). These processes are, in part, dependent on the presence of other soil components, such as organic ligands and anions. The processes that maintain the lowest activity of Al in a given soil ultimately control Al solubility. Comparison of the ion activity products (IAP) for various minerals can aid in determining which minerals govern Al solubility in a given soil.

Oxides and Hydroxides. The Al-hydroxide mineral gibbsite ($Al(OH)_3$) is commonly found in weathered soils that are deficient in soluble forms of silica (Si) (Lindsay and Walthall, 1996). The presence of gibbsite in a soil is a strong indication of pH-dependent activity of Al^{3+} (Lindsay and Walthall, 1996). The relationship between pH and Al solubility with respect to gibbsite is depicted in Fig 2. Other Al hydroxides in order of decreasing solubility include $Al(OH)_3$ (bayerite), $Al(OH)_3$ (norstrandite), AlOOH (boehmite), and AlOOH (diaspore) (Lindsay, 1979; Sadiq and Lindsay, 1979). The amorphous $Al(OH)_3$, however, is relatively soluble (Lindsay and Walthall, 1996) compared with the aforementioned minerals.

Phosphates. At low pH, Al may form insoluble phosphate minerals (Lindsay, 1979; Lindsay et al., 1959) such as NH_4-taranakite ($H_6(NH_4)_3Al_5(PO_4)_8 \cdot 18H_2O$), K-taranakite ($H_6K_3Al_5(PO_4)_8 \cdot 18H_2O$), and variscite ($AlPO_4 \cdot 2H_2O$) (Lindsay and Walthall 1996). Although variscite is a stable mineral in acid soils, other solid phases tend to regulate the activity of Al^{3+} in soils (Lindsay and Walthall, 1996). Aluminosilicates, hydroxides, and hydroxysulfates tend to regulate Al^{3+} activity because phosphate levels are usually lower than Al levels, allowing Al^{3+} activity to be governed by another solid phase (Lindsay and Walthall, 1996).

Aluminosilicates. During the weathering process, silicates are gradually removed from the soil over geologic time scales. Relatively unweathered soils have high levels of $H_4SiO_4^0$; therefore, aluminosilicates such as kaolinite and smectite may maintain a low level of Al^{3+} activity in the soil solution. The activity of $H_4SiO_4^0$ decreases and the activity of Al^{3+} increases as silicates are removed from the soil. As log ($H_4SiO_4^0$) falls below –5.3, kaolinite is eliminated from the soil and gibbsite is the only Al-bearing mineral remaining to regulate Al^{3+} activity (Lindsay and Walthall, 1996).

Sulfates and Hydroxysulfates. In some acid soils Al may react with sulfates to form aluminum hydroxylsulfate minerals, which are more stable than gibbsite and kaolinite (Lindsay and Walthall, 1996). Above pH 5.5, however, gibbsite is more stable than the Al hydroxylsulfate minerals; consequently, under high pH conditions, gibbsite will regulate Al^{3+} activity (Lindsay and Walthall, 1996). Between pH 3.5 and 5.5, alunite ($KAl_3(SO_4)_2(OH)_6$) will control Al^{3+} activity. In highly acidic soils (pH<3.5) with high SO_4^- activity, jurbanite ($AlSO_4(OH) \cdot 5H_2O$) controls Al^{3+} activity (Lindsay and Walthall,

1996). Aluminum solubility may be controlled by other non-stable solid phases under certain conditions (Adams and Rawajfih, 1977; Nordstrom, 1982).

When considering the interaction of soil Al chemistry with plants, pathogens, or other soil microflora, it is important to provide information pertaining to the form and quantity of Al in the system. For example, documentation of studies conducted in field soils should include background information such as soil series, basic mineralogical and organic components, and pH. Incorporation of such background information allows a reader to visualize Al interactions as part of a dynamic soil ecosystem rather than the effect of a single ion.

II. Interaction of Al with plants

In many agricultural soils in the eastern USA as well as soils in tropical regions, Al rhizotoxicity in surface and subsurface soils can limit taproot extension and lateral root development. Secondary affects include reduced nutrient uptake, tolerance to drought stress, and overall reduction of productivity.

Plant growth requirements and Al

Although Al is not considered an essential mineral element for plant growth, there are reports of beneficial effects of Al at low concentrations in soil and nutrient solution (Foy, 1974; 1983). For example, in tea, an Al-tolerant crop, growth stimulation was observed at Al concentrations of 1000 μM (Matsumoto et al., 1976). In Al-sensitive crops such as maize, tropical legumes and sugar beet, Al concentrations of 71 to 185 μM stimulated growth (Marschner, 1995). Flower color in the garden plant *Hydrangea macrophylla* varies from blue under high Al conditions to pink under low Al conditions (Asen et al. 1963).

Effect of Al on plant nutrition

Cation uptake. Aluminum is the most important factor associated with the infertility of acid soils (Foy, 1988). Aluminum interferes with normal physiological processes resulting in reduced uptake of essential cations, disruption of root cap cells and membrane integrity (Bennet et al., 1987). As pH decreases, the activity of H^+ increases. Agricultural soils are often limed to raise the pH and ameliorate Al toxicity to crop plants. Crop response to lime is often negatively correlated with the KCl-exchangeable Al level (Kamprath, 1970). Even though the plow layer is limed, acid conditions may persist in the subsurface layer resulting in poor root growth of Al-sensitive crops. Under acidic soil conditions, relative uptake of polyvalent cations such at Mg^{2+}, Ca^{2+}, Zn^{2+} and Mn^{2+} is inhibited due to decreased absorption of these cations and lower extrusion of H^+ by the plasma membrane-bound ATPase (Marschner, 1995). Aluminum competition with basic cations results in diminished movement of these cations from the apoplast to the symplast. Aluminum may also affect calcium (Ca) uptake by blocking Ca^{2+} channels in the plasma membrane (Huang et al., 1992), and magnesium (Mg) uptake by altering binding sites of transport proteins (Rengel and Robinson, 1989).

Phosphorous deficiency. In acid soils or solutions, Al rhizotoxicity may appear as a phosphorus (P) deficiency in plants (Foy and Brown, 1963). Aluminum may bind P on root surfaces and cell walls inducing P deficiency symptoms in foliage (Clarkson, 1967). Improvement of soil fertility/management practices to enhance P availability may effectively reduce soil Al activity, thus increasing P uptake by crop plants and preventing associated nutritional deficiencies.

Nitrogen-fixation. Nitrogen (N) deficiencies may develop as a result of Al-inhibition of nodulation in legumes. Delayed nodulation has been related to Al-toxicity in acid soils with low Ca concentrations (Schubert et al., 1990). Growth of soybean was reduced by 10% at Al concentrations of 5-9 μM and nodulation was inhibited at 0.4 μM Al (Alva et al., 1987). Aluminum is toxic to species of *Rhizobium* and *Bradyrhizobium* (Rosswall et al., 1985) and this toxicity may affect N levels available for current and future crops planted in these soils.

Aluminum rhizotoxicity

Effect of soil type on Al phytotoxicity. Phytotoxicity of Al has been documented and studied extensively in many soils in the soil orders Oxisols, Ultisols, and Andisols. Although Al phytotoxicity is common in these soil orders, the specific soil mineralogical components present in certain soil series within these orders can serve to mediate this toxicity. For example, in Coastal Plain Ultisols in the eastern USA, the pH at which soluble Al reaches toxic concentrations varies among series. Aluminum rhizotoxicity developed in the Norfolk series at pH levels as high as 5.5, whereas in the Bladen series Al toxicity developed at pH 4.9 (Hester, 1935). Exchangeable Al levels of 0.1 meq Al $100g^{-1}$ were rhizotoxic to cotton in the Norfolk series, but up to 2.5 meq Al $100g^{-1}$ in Bladen soils was required before Al rhizotoxicity was observed (Adams and Lund, 1966). Differences in the predominant clay mineral content of the Norfolk series (kaolinitic clay) and the Bladen series (vercimulite and montmorillonite) may explain differences in soluble Al concentrations at different soil pH values.

Alleviation of Al rhizotoxicity

Mechanisms of Al toxicity to plants. The root tip is the primary target for Al toxicity in plants (Ryan et al., 1993; Sivaguru and Horst, 1998), but the physiological basis for cell damage by Al is unknown (Kochian, 1995). Aluminum interferes with enzyme activities, cell division in root apices, DNA replication and P availability at membranes. In addition, regulatory signals in root cap cells are disrupted by Al (Bennet and Breen, 1991). The localized accumulation of Al at nuclei of root meristem cells has been illustrated using lumogallion, an Al-specific fluorochrome (Silva et al., 2000). The identification of the toxicant at the site of reduced mitotic activity suggests the direct role of Al in root growth inhibition (Silva et al., 2000). Lumogallion fluorescence imaging of soybean roots also has been used to correlate Al accumulation in meristem tissue with sensitivity to Al. For example, lateral roots are more sensitive than tap roots to Al and lateral roots exhibit heightened localized accumulation of Al (Silva et al., 2000). Furthermore, fluorimetric detection of Al in root meristem tissue has been applied to physiological studies of Al phytotoxicity in the crop plants rice (Yoshimura et al., 2003), tobacco (Iikura et al., 2001), and wheat (Polle et al., 1978).

Toxicity of specific Al ions. Species of Al that are toxic to root growth in acid soils have been investigated by using GEOCHEM-PC, a chemical speciation program for modeling equilibria of soil solutions (Parker et al., 1995), and by chromogenic speciation in reaction mixtures with ferron. The program predicted a strong relationship between Al species that reacted instantaneously with ferron and the sum of activities of Al^{3+}, $Al(OH)^{2+}$, and $Al(OH)_2^{+}$. Both the instantaneously reacting-fraction of Al species in two soils with pH < 4.6 and the metastable fraction that followed a first order kinetics reaction with ferron were correlated with inhibition of corn root length density in surface soil

layers (Jallah and Smyth, 1998). Models and chromogenic reactions are not yet adequate to differentiate and identify the most phytotoxic species of Al in soil solutions.

Al inhibition of root elongation. Crops vary in growth response to Al. Even within cultivars of the same crop such as wheat, barley, potato, and peanuts, differences in Al sensitivity and tolerance were found (Adams and Pearson, 1970; Foy et al., 1967; Lee, 1971). In soybean, Al and H^+ inhibit root elongation (Lund, 1970). Lateral root development in Ransom soybean was inhibited 50% by 2.1 μM Al^{3+}, but the Al rhizotoxic effect was dependent on soil pH and was more severe at lower pH values (Sanzonowicz et al., 1998a). Both H^+ and Al inhibited tap root elongation in soybean at pH < 5.2, but lateral root extension was inhibited more than tap root growth by Al.

Calcium amelioration of Al toxicity. Calcium can alleviate the inhibitory effect of H^+ and Al^{3+} in the rhizosphere of soybean roots. However, the response to Ca is limited by high H^+ and Al^{3+} activity (Sanzonowicz et al., 1998b). Subsurface root zones may be deficient in Ca due to lack of lime distribution during tillage. In consequence, the resulting acidic conditions inhibit root development and limit uptake of nutrients and moisture, particularly during droughts. Liming with Ca alleviates Al rhizotoxicity by increasing the ionic strength of the soil solution and subsequently increasing competition between Ca and Al at binding sites outside the cell membrane (Kinraide and Parker, 1987). Utilization of Al-tolerant cultivars with improved rooting patterns in subsurface zones may improve plant growth and development by avoidance of the toxicant.

Addition of Ca to the subsurface solution in a vertically split root system for different soybean genotypes resulted in improved rooting (Ferrufino et al., 2000). A Ca:Al activity ratio of 891 resulted in a 50% reduction of tap root length across

genotypes. Lateral roots, however, required a greater concentration of Ca^{2+} to overcome inhibition of root elongation. Thus, even though taproots might extend into acidic soil zones, development of lateral roots for nutrient and moisture uptake could be limited. In Al-sensitive genotypes, more Ca was needed to offset the rhizotoxic effects of Al on root elongation (Silva et al., 2001a).

Si and Mg effects on Al phytotoxicity. In addition to Ca, Si and Mg may also alleviate Al inhibition of root elongation in wheat (Kinraide et al., 1994). Silicon can ameliorate Al toxicity in teosinte, rice, wheat, and corn (Barcelo et al., 1993; Cocker et al., 1998; Corrales et al., 1997; Hara et al., 1999). In solution culture, Mg in the range of 10 to 50 μM improved extension of soybean roots where Al had inhibited root extension (Silva et al., 2001a). This effect was not consistent across wheat cultivars. The effects of Mg were observed at low ionic strength compared to Ca, and thus may involve a metabolic or biochemical regulation. The Mg affect was dependent on the presence of Ca (Silva et al., 2001a). Presence of Mg may detoxify Al, preventing the disruption of cell expansion and division commonly induced by Al (Kochian, 1995).

Addition of salts to a growth medium alleviated Al rhizotoxicity by increasing electrical potential and reducing Al^{3+} activity at plasma membrane surfaces in root cells (Kinraide, 1998; Kinraide et al., 1992; Kinraide et al., 1994). In soybean, Al rhizotoxicity was alleviated by micromolar concentrations of Mg 24 hr after treatment. The Gouy-Chapman-Stern model was unsuccessful at predicting this Mg effect, indicating that Mg may be interacting through a metabolic process in soybean tissue (Silva et al., 2001b). Magnesium was 100 times more effective than Ca at alleviating Al

rhizotoxicity, again suggesting a biochemical effect of Mg rather than an ionic strength affect as observed with Ca.

Organic acids and Al phytotoxicity. Organic acids ameliorate the phytotoxicity of Al. The capacity of organic acids to detoxify Al is related to the stability of the Al-organic acid complex. The sequential order of decreasing stability follows citric, oxalic, malic, and succinic acid with efficacy of detoxification based on the orientation of OH/COOH groups and formation of a stable 5- or 6-bond ring structure with Al (Hue et al., 1986). Aluminum forms non-phytotoxic complexes with fulvic acid (Suthipradit et al., 1990) and other organic acids exuded in the rhizosphere. In root tips of 36 wheat cultivars, the efflux of malate was directly proportional to root elongation in the presence of Al (Delhaize and Ryan, 1995; Ryan et al., 1995a,b). Malate and citrate exudation were related to Al-tolerance in maize (Jorge and Arruda, 1997; Pellet et al., 1995), while citrate exudation has been correlated with Al-tolerance in snapbeans and soybeans (Miyasaka et al., 1991; Silva et al., 2001d). Aluminum tolerance in soybean was correlated with citric acid production in root tips and the subsequent reduction of Al accumulation in the symplast, suggesting the role of Al-citrate complexes in exclusion of Al from root apical meristem cells (Silva et al., 2001d). Plants may have adapted to soils containing phytotoxic levels of Al by enhanced production of root exudates, including Al-complexing organic acids. For example, Al-tolerant genotypes of soybean maintain high levels of citrate in root efflux for longer periods of time than Al-sensitive genotypes following exposure to Al (Silva et al., 2001d). This Al-induced efflux occurs within 6 hr of exposure to Al and includes enhanced production of citrate and malate. Furthermore, Al-sensitive genotypes accumulated more Al than Al-tolerant genotypes suggesting that

citrate may be involved in Al exclusion and detoxification. In Al-tolerant genotypes, the accumulation of Al in root tips was not correlated with a reduction of root elongation, further suggesting that Al-citrate complexes are not phytotoxic (Silva et al., 2001d).

Magnesium may serve as an important factor in the localized biosynthesis and subsequent efflux of citrate from soybean root tip cells. In the presence of 50 μM Mg, citrate concentrations were increased in root tip cells and in root efflux from both Al-sensitive and Al-insensitive soybean genotypes (Silva et al., 2001c). Enhanced citrate exudation was observed within 12 hr after Mg treatment of roots exposed to 2.9 μM Al^{3+}, suggesting that Mg effects occur rapidly in root tip cells. Corresponding changes in malate exudation or root tip concentration were not observed in the presence of Mg, suggesting that citrate may be the organic acid associated with Al-tolerance in soybean (Yang et al., 2000). Both lumogallion fluorescence imaging and quantitative spectrometric analyses support the observation of reduced Al accumulation in soybean root cells in the presence of 50 μM Mg and 3 mM Ca regardless of genotype sensitivity (Silva et al., 2001c).

Root border cells. Root border cells may offer a protective mechanism that ameliorates Al phytotoxicity. Formerly referred to as sloughed-off root cells, the term border cell is more appropriate since these are living cells that can function independently of the root (Hawes et al., 1998). Although normally in close association with the root cap, under saturated soil conditions, border cells are released into the film of water surrounding the root tip. Viability of detached border cells was reduced in Al-sensitive cultivars of snapbean (Miyasaka and Hawes, 2001). In the Al-insensitive cultivar, cell death rate was linear for 4 to 8 hours, but leveled off thereafter. As Al is detected in the

border cell environment, a mucilage layer forms around each cell, and mucilage thickness positively correlates with Al concentration. Avoidance of Al in the Al-insensitive cultivar may result from the production of a thick mucilage layer around detached border cells. Binding of Al in the mucilage layer prevents the subsequent uptake of Al by the root cap proper (Miyasaka and Hawes, 2001). Future experiments focusing on the role of root border cells in organic acid production may enhance our understanding of the importance of these cells in preventing Al toxicity.

Aluminum rhizotoxicity will continue to be a problem for Al-sensitive crops in acidic soil conditions by inhibiting root elongation and lateral root development. A better understanding of the ameliorating role of Ca, Mg, organic acids, and root border cells may lead to improved soil management strategies and the development of better-suited Al-tolerant cultivars through traditional breeding or biotechnology.

III. Interactions with soil microflora

Although most studies of the inhibitory or detrimental effects of Al in soil have been directed at the short and long term effects on plant growth and productivity, a growing body of knowledge indicates that the ecology of numerous soilborne microorganisms is affected by the moderate to high levels of exchangeable Al present in some acid mineral soils (Hoper and Alabouvette, 1996; Illmer et al., 1995; Pina and Cervantes, 1996; Rosswall et al., 1985). In addition, with the increased concerns of the long-term effects of acid rain and the movement of agriculture and forestry to more marginal soils in many areas of the world, the role of microorganisms in plant health, disease suppression, and in the amelioration of Al toxicity to plants has become the focus of numerous investigations.

Saprophytic and mycorrhizal organisms

The primary focus of this discussion is the effects of Al on soilborne plant pathogens. However, Al may have dramatic effects on the activity of microbes that play important roles in nutrient cycling, plant growth, and biocontrol of plant pathogens. These organisms also may interact with or even control the activity of plant pathogens; therefore, examples of these interactions are provided to illustrate the widespread inhibition of microorganisms by Al.

Ko and Hora (1972) considered Al to be a general soil fungitoxin, and many genera of bacteria and fungi are now known to be sensitive to levels of soluble or exchangeable Al present in acid soils (Hoiland and Dybdahl, 1993; Marschner et al., 1999; Ohno et al.,1988; Zwarun et al.,1971; Zwarun and Thomas, 1973). There are, however, many bacteria and fungi that tolerate or even thrive in acid soils high in Al (Kawai et al., 2000). Many of these organisms, especially various forms of mycorrhizae, are important in protecting plants from the phytotoxic effects of Al (Cumming et al., 2001; Lux and Cumming, 2001; Thompson and Medve, 1984; Yang and Goulart, 2000). There are numerous studies on how mycorrhizal fungi provide protection; most species either produce Al-chelating compounds (Cumming et al., 2001) or compartmentalize Al in the rhizosphere or in host tissues (Lux and Cumming, 2001). Not all mycorrhizal fungi are tolerant of Al, so selection of the proper mycorrhizal organism is important for studies conducted in acid soils (Bartolome-Esteban and Schenck, 1994). Endophytic fungi also may reduce the effects of Al on their host plant (Malinowski and Belesky, 1999; Zaurov et al., 2001). The enhanced Al tolerance in endophyte-infected tall fescue was related to an increased exudation of phenolics compared to endophyte-free plants (Malinowski and Belesky, 1999). Aluminum also is toxic to *Rhizobium* (Kinraide and

Sweeney 2003; Rosswall et al., 1985) and selection of acid or Al-tolerant strains of this important bacterium may increase the efficiency of N-fixation in acid soils (de Oliveira and Magalhaes, 1999).

Soil fungistasis

Fungistasis is a property of soil that inhibits the germination of otherwise germinable propagules. Multiple mechanisms have been proposed to explain fungistasis, and at least two types of fungistasis, general and residual, have been described (Dobbs and Gash, 1965; Dobbs and Hinson, 1953). Aluminum ions may be an important factor that contributes to residual fungistasis found in some soils (Baard and Pauer, 1982; Furuya et al., 1999; Ko and Hora, 1972). In fact, the inhibitory effects of Al on germination of propagules and on spore production may influence total soil microbial biomass and microbial activity (Illmer et al., 1995; Watanabe et al., 2002). Fungistasis, which is a reversible phenomenon, and any fungistatic effects of Al on fungi in soil should not be confused with the fungitoxic effects of Al described below for some pathogens in highly acid soils (e.g., Baard and Pauer, 1982; Ko and Hora, 1972).

Soilborne plant pathogens

Spectrum of pathogens sensitive to Al. Examples of plant pathogens, primarily soilborne organisms, known to be sensitive to Al include representatives of all the major groups of fungi (Browning and Edmonds, 1993; Firestone et al., 1983; Furuya et al., 1999; Hoper and Alabouvette, 1996; Meyer et al., 1994; Kobayashi and Ko, 1985; Kobayashi and Komada, 1995; Orellana; 1975), members of the Oomycota (Stramenopila) (Andrivon, 1995; Benson, 1993; Benson, 1995; Lewis, 1973; Muchovej et al., 1980) and the Actinomycete *Streptomyces scabies* (Mizuno and Yoshido, 1993). The

list of Al-sensitive organisms continues to grow as more in-depth studies are conducted on soilborne pathogens that fail to cause disease in acid soils. This broad spectrum of Al-sensitive organisms probably should be expected, as high concentrations of Al are toxic to most living organisms. However, as with many of the mycorrhizal fungi, saprophytic fungi, and bacteria discussed above, not all soilborne plant pathogens are sensitive to Al (Orellana et al., 1975).

Low soil pH and disease suppression. The relationship between soil pH and disease severity became well established in the early 20th century literature along with recommendations for the use of soil acidification to control root diseases caused by fungi and bacteria (Anderson, 1926; Doran, 1927; Eddins, 1939; Ezekial et al., 1930; Gillespie, 1918; Hartley and Pierce, 1917; Haenseler, 1928; Hartley, 1929; Johnson and Hartman, 1919; Spaulding, 1908; Taubenhaus et al., 1928; Taubenhaus et al., 1937). Acidifying fertilizers, various acids, $Al_2(SO_4)_3$, and sulfur (S) have been used to lower soil pH in fields with a history of disease and some level of disease suppression is frequently, but not always, observed as a result of these acidifying amendments. Although little or no information was presented on the levels of Al present in these soils, we now know that Al is the primary source of exchangeable acidity in most mineral soils (Lindsay, 1996; Ritchie, 1995). It is likely that the disease suppression observed in some or most of these soils following acidification was in part due to the increased levels of soluble Al and its toxic effects on the pathogens present. This conclusion is supported by a number of recent studies that have demonstrated a direct toxicity of Al to several of the pathogens involved in these early studies (Benson, 1993; Meyer et al., 1994; Mizuno and Yoshido, 1993).

In early studies where soil was amended with $Al_2(SO_4)_3$ to lower soil pH, the effects of soil acidification on disease generally were related to a pH effect and not a direct toxicity of Al on the pathogen (Jackson, 1940). A common characteristic of disease suppression in acid soils is that the suppression does not occur in all soils at a given pH (Baard and Pauer, 1982; Hoper and Alabouvette, 1996; Meyer et al., 1991b; Mizuno and Yoshida, 1994), suggesting that another factor is responsible for disease suppression. At a given pH, other soil chemical factors, such as organic ligands and anions, regulate soil Al activity and may render a soil either suppressive or conducive to a plant disease. The difficulty in duplicating the complex chemistry of the soil solution may explain why observations of disease suppression in acid soils usually lead to in vitro studies on the sensitivity of the pathogen to Al. The H^+ activity in acid soils is not independently related to suppression because H^+ is not typically as toxic as other dominant ions in acid soil (Furuya et al., 1999; Lucas, 1955; Waksman, 1922). Soil acidification is still a commonly recommended practice for the management of many root diseases, but the mechanism of suppression is not generally known (Agrios, 1997).

Inhibition of specific pathogens and disease suppression. One of the first reports of disease control attributed to the direct toxicity of Al amendments to a pathogen was by Guba (1934), who investigated the incidence of Verticillium wilt of eggplant in acid soils in Massachusetts. He observed that wilt incidence was much less in field soils with pH values < 5 than in soils with pH values > 5. In culture tests, lowering the pH of potato dextrose agar to 4.0 with $Al_2(SO_4)_3$ totally prevented mycelial growth of *Verticillium*. The concentration of Al in these nutrient plates was not given. In pot tests using naturally infested soil, amendment of the soil with $Al_2(SO_4)_3$ to a pH $\leq$ 5 prevented

infection of eggplant seedlings by *Verticillium*. Sulfur amendments also reduced infection, but were not as effective as Al and required a lower soil pH to induce similar levels of disease suppression. Field amendments with $Al_2(SO_4)_3$ were ineffective for control of wilt, suggesting that artificial acidification of these soils was not an effective control strategy (Guba, 1934).

Other investigators have studied the effects of acid soils and Al on the occurrence and severity of diseases caused by *Verticillium* (Baard and Pauer, 1982; Johnson, 1951; Orellana et al., 1975; Wilhelm, 1950). In general, most studies have found that wilt development is favored by soil pH levels above 5.6 and suppressed when the pH is below 5. In contrast, Wilhelm (1950) reported that Verticillium wilts frequently occurred in acid soils in California. Studies by Orellana et al. (1975) and Johnson (1951) report that *Verticillium* is sensitive to Al. Johnson (1951) reported that 50-100 μg Al ml^{-1} of medium was required to inhibit hyphal growth of *Verticillium* in a nutrient culture, whereas Orellana et al. (1975) reported that only 8 μg Al ml^{-1} suppressed hyphal growth and microsclerotia production in a defined medium. The differences in reported sensitivity are most likely due to the differences in the level of soluble Al in the growth media. The medium employed by Orellana et al. (1975) was free of organic ligands; whereas the medium used by Johnson probably contained unidentified organic acids that may have complexed Al and increased the concentration of Al required for toxicity. Verticillium wilt also was less severe in unlimed compared to limed test soils that had low pH and high levels of exchangeable Al (Orellana et al., 1975). Both the infection of a plant and the colonization of an artificial substrate are metabolically active processes and the observed inhibition of these processes can be used to conclude that Al is toxic to

Verticillium. A later study by Baard and Pauer (1982) concluded that the effect of acid soils on the incidence of Verticillium wilts was not due to the Al level, but to some other unidentified pH-related factor. Disease suppression was similar in acid soils with either a low or high levels of exchangeable Al, so they concluded that other factors must be involved. They also observed that reducing soil pH reduced propagule viability, but this effect was reversed upon increasing pH, suggesting that Al may be serving as a fungistatic factor. Microsclerotia that formed on plant roots were irreversibly affected by Al, suggesting that Al also is toxic to microsclerotia (Baard and Pauer, 1982). It thus appears that Al suppression of Verticillium may be both a fungistatic and a fungitoxic phenomenon.

The soilborne fungus *Thielaviopsis basicola* (synanamorph: *Chalara elegans*) causes black root rot on a wide range of plants. Severity of the disease is influenced greatly by soil chemical factors and in general is enhanced at soil pH > 5.6 and suppressed in soils with pH < 5.2 (Anderson et al., 1926; Bateman, 1962; Doran, 1927; Doran, 1931; Meyer and Shew, 1991a,b; Meyer et al., 1989; Morgan et al., 1929). Acidification of pathogen-infested soil with nitric or sulfuric acid, but not phosphoric acid reduced the severity of black root rot (Doran, 1927). Since P readily precipitates Al from solution, failure of the P treatment to reduce disease suggests that Al was involved in disease suppression in the soils used in these early studies. Doran (1927) also used $Al_2(SO_4)_3$ amendments to suppress black root rot. The use of acidifying fertilizers (i.e., those containing NH_4^+-N rather than NO_3^--N) also has been recommended in fields with high soil pH values and a history of the disease (Morgan and Anderson, 1927; Morgan et al., 1929). The disease suppression caused by NH_4^+-N amendments may be due to

increased Al levels and other factors such as enhanced polyamine production typical in acid-stressed plants (Harrison and Shew 2001). For a detailed review of how nitrogen form affects plant diseases see the chapter in this volume on Nitrogen and Plant Disease.

In addition to low pH, studies on black root rot of tobacco indicated that high levels of exchangeable Al and low levels of Ca and other basic cations are associated with abiotic suppression of black root rot (Meyer and Shew, 1991a,b; Meyer et al., 1989; Meyer et al., 1994). Suppressive soils were found to have low base saturation and exchangeable Al levels at or above 1 meq 100 g^{-1} of soil (Meyer and Shew, 1991a,b). A suppressive soil became conducive after the addition of agricultural lime, but not after the addition of $CaSO_4$, suggesting that Ca is not the sole conducive factor associted with high pH soils (Meyer and Shew, 1991b). A conducive soil was changed to a suppressive soil only if the acidification was accompanied by an increase in exchangeable Al (Meyer and Shew, 1991b).

Laboratory studies have demonstrated that multiple stages in the life cycle of the *T. basicola* are highly sensitive to Al (Meyer et al., 1994). Germination of endoconidia and aleuriospores (chlamydospores) were inhibited at Al levels as low as 5 µg Al ml^{-1} in a dilute nutrient agar medium (Meyer et al., 1994); hyphal growth was not as sensitive, with 10 µg Al ml^{-1} of medium required to inhibit growth. Isolates from suppressive and conducive soils behaved similarly in most tests, but occasionally isolates from suppressive soils were more tolerant of Al (Meyer et al., 1994).

Some Fusarium diseases are suppressed at low soil pH (Furuya et al., 1999; Kobayashi and Komada, 1995), while others are suppressed at high pH (Hoper et al., 1995; Jones and Woltz, 1970; Papvizas et al., 1968). In acid soils, Al is a key factor in

suppression of root rot caused by *Fusarium solani* f. sp. *phaseoli* (Furuya et al., 1996; Furuya et al., 1999). Furuya et al. (1999) observed inhibition of bean root rot in all soils with exchangeable Al levels of at least 0.4 meq 100 g^{-1} of soil. The primary effect of the Al ion was inhibition of germination of macroconidia (Furuya et al., 1996; Furuya et al., 1999). As observed with other pathogens and diseases, clay mineralogy was very important in determining the presence and level of suppression (Alabouvette, 1999; Furuya et al., 1999; Hoper and Alabouvette, 1996; Lyda, 1978; Stutz et al., 1989).

Post-harvest diseases also may be suppressed by Al (Mecteau et al., 2002). For example, suppression of Fusarium bulb rot of narcissus was enhanced in the presence of $Al_2(SO_4)_3$ (Weiss and Haasis, 1939), while in several more recent studies, post harvest diseases of potato were suppressed by Al salts. Potato dry rot, a post harvest disease caused by *F. sambucinum*, was significantly reduced by application of $AlCl_3$ (Mecteau, 2002) and silver scurf, caused by *Helminthosporium solani*, was suppressed by with the application of several Al salts, with $AlCl_3$ providing the highest level of control (Hervieux et al., 2002).

Some diseases caused by *Rhizoctonia solani* are suppressed in acid soils or in soil acidified by amendments with $Al_2(SO_4)_3$ (Hartley, 1929; Jackson, 1940; Kobayashi and Ko, 1985; Perrin, 1987; Wiant, 1929). Kabayashi and Ko (1985) reported that a suppressive soil in Hawaii had higher levels of exchangeable Al than a conducive soil. Also, damping-off isolates of *R. solani* from pine seedlings were inhibited more in medium amended with $Al_2(SO_4)_3$ and buffered at pH 4 than when buffered at pH 6 (Hwang and Kuhlman, 1991). In greenhouse studies, the infection of soybean by *R. solani* decreased with increasing levels of Al saturation (Rodrigues et al., 1998). There

have been no definitive studies on the relationship of Al content and disease development by different anastomosis groups of *R. solani* or of other *Rhizoctonia* spp. The wide host range, world-wide distribution, and economic importance of Rhizoctonia diseases in many soil types suggest that additional studies on the role of soil chemical factors on the ecology of *Rhizoctonia* are needed.

Multiple species of *Armillaria* cause root rot of forest and fruit trees. In surveys of Douglas fir stands between 5 and 35-year-old, stands with a high severity of Armillaria root disease had significantly lower water-extractable Al concentrations (9.0 ± 3.1 μg Al g^{-1} of soil) than soils from stands with low disease severity (22.4 ± 5.3 μg Al g^{-1}) (Browning and Edmonds, 1993). Soil pH was similar across stands, but 2N KCl-extractable Al averaged 313 ± 164 μg Al g^{-1} in low severity sites and 149 ± 69 μg Al g^{-1} in high severity sites. In culture tests, growth of isolates of *Armillaria ostoyae* declined as water extractable Al increased, with the most dramatic growth decline observed at pH 4 (Browning and Edmonds, 1993). Isolates of *A. ostoyae* from *Picea rubens* also were sensitive to Al in culture tests (Wargo and Carey, 2001). The reduction of pH associated with acid rain may lead to suppression of Armillaria root rot in forest soils (Browning and Edmonds, 1993; Entry and Cromack, 1987).

There are many reports of suppression of Phytophthora diseases in acid soils (Andrivon, 1995; Ann, 1994; Dukes and Apple, 1968; Erwin and Ribeiro, 1996; Jung et al., 2000; Kincaid and Gammon, 1954; Pegg, 1977; Muchovej et al., 1980; Schmitthenner and Canaday, 1983). Multiple investigations also have demonstrated that moderate to high levels of exchangeable Al are often responsible for this suppression.

The first report of an effect of soil acidification by $Al_2(SO_4)_3$ on a Phytophthora disease was by Lambert and Crandall (1935), wherein a wilt disease of black-locust seedlings (the species was nont identified but was most likely either *Phytophthora cinnamomi* or *P. nicotianae*) was greatly reduced by Al amendments that reduced the soil pH from > 6.2 to 4.6. However, Muchovej et al. (1980) conducted the first controlled studies to demonstrate the inhibitory effects of Al on a *Phytophthora* sp. Disease of green pepper caused by *P. capsici* was increased when an acid soil was limed with either $CaCO_3$ or $Ca(OH)_2$. The liming materials reduced the levels of exchangeable Al in the soil to a non-fungitoxic level. Addition of Ca in the form of $Ca(SO_4)$, which does not raise soil pH, did not increase disease incidence. In culture studies, hyphal growth of *P. capsici* was reduced by levels of Al as low as 0.4 meq Al^{3+} 100 g^{-1} of soil (Muchovej et al., 1980). Effects of Al on other stages in the life cycle of *P. capsici* were not investigated.

Incidence and severity of black shank of tobacco, caused by *P. parasitica* var. *nicotianae* (syn. *P. nicotianae* var. *nicotianae*), has been related to soil pH and soil series by numerous investigators (Dukes and Apple, 1968; Kincaid and Gammon, 1954; Kincaid et al., 1970; Lucas 1975; Sidebottom and Shew, 1985). Soils suppressive to black shank typically have low pH and low Ca, but other factors associated with suppressiveness are not consistent across soil series (Dukes and Apple, 1968; Sidebottom and Shew, 1985). DeLuca and Shew (1988) analyzed the characteristics of conducive and suppressive soils described by Sidebottom and Shew (1985) and determined that the primary difference in suppressive and conducive soils was the level of exchangeable Al present. Exchangeable Al levels were consistently higher in suppressive soils than in soils

conducive to black shank development. Weaver (1995) confirmed the toxicity of Al to *P. parasitica* var. *nicotianae* in vitro. Hyphal growth, sporangium production and zoospore germination were sensitive to low levels of Al in dilute nutrient agar. Sporangia production was more sensitive to Al than zoospore germination or hyphal growth (Weaver, 1995). For example, at 200 µM Al (5.4 µg Al ml^{-1} of medium), sporangia production was reduced by 98% and hyphal growth by only 28%. In addition, toxicity of Al diminished rapidly or was lost entirely as the concentration of carrot juice present in the agar medium was increased from 0.25 % to 2 % (Weaver, 1995). Meyer et al. (1994) reported a similar effect of nutrient level on toxicity of Al to *T. basicola*. The reduced Al toxicity at high nutrient levels may be due to a reduction in soluble Al through complexation or by the enhanced nutritional status of the pathogen.

The effects of Al on damping-off diseases caused by *P. parasitica* (syn. *P. nicotianae*) on ornamentals also have been investigated in peat moss-based potting medium (Benson, 1993; Benson, 1995; Fichtner et al., 2001). Benson (1993) amended a peat:vermiculite potting medium (pH 4.1) with lime (0, 3, 0r 6 g/1000 cm^3 of medium) and different levels of $Al_2(SO_4)_3$ (0 to 14.5g Al/1000cm^3 of medium) then seeded with vinca (*Cantharanthus roseus*) and infested the medium with the pathogen. The rate of Al needed to suppress damping-off increased as the amount of lime increased (Benson, 1993). At the highest rate of Al applied, populations of *P. parasitica* were suppressed for 16 days, but suppression lasted only 7-9 days at lower rates of Al (Benson, 1993). Production of sporangia of *P. parasitica* in vitro was inhibited over 99% by as little as 0.25 meq of Al at pH 5. In a second study, Benson (1995) amended a peat:vermiculite potting medium (pH 4.1) with lime (3kg m^{-3}) and different levels of $Al_2(SO_4)_3$ (0.75, 1.9

and 3.75 g Al/1300 cm^3 of medium) then seeded with different bedding plants and infested the medium with the pathogen. All levels of Al tested were effective in controlling pre-emergence damping-off of snapdragon and vinca, but only the highest Al level was effective for petunia. Four days after seeding and drenching with $Al_2(SO_4)_3$, exchangeable Al levels equilibrated at 0, 0.5, and 2.03 meq Al^{+3} 100 g^{-1} medium for the three concentrations of $Al_2(SO_4)_3$ applied. Aluminum was not phytotoxic to vinca, snapdragon, or petunia grown in a limed medium, indicating a differential sensitivity of the pathogen and host to Al.

Fichtner et al. (2001) extended the investigation of the suppression of *P. parasitica* in a peat moss-based organic medium. Most studies on disease suppression by Al have been conducted in mineral soils or in vitro and have focused on free ionic Al (Al^{3+}), the most prominent dissolved Al species present at pH < 4.5. As discussed above, however, Al exists in soil as multiple chemical species, including the pH-dependent hydrolysis species ($Al(OH)^{2+}$, $Al(OH)_2^{+}$, $Al(OH)_3^{0}$, and $Al(OH)^{-}_4$). The influence of Al species other than Al^{3+}on fungi is largely unknown. A peat-based medium amended with Al solutions at pH 4 and pH 6 was infested with low and high levels of the pathogen. Aluminum solutions at both pH 4 and 6 reduced pathogen populations at 15.8 mg of Al g^{-1} of peat, with the pH 4 solution more effective than the pH 6 solution at reducing pathogen populations. The prevalence of $Al(OH)_2^{+}$ and Al-organic matter complexes in peat amended with $Al_2(SO_4)_3$ solution at pH 6 suggested that ions other than Al^{3+} were responsible for pathogen suppression in peat. Because the predominant forms of Al are different in suppressive mineral soils and organic media, the mechanism of suppression may be different in the two systems. The advantage of Al-mediated suppression of *P.*

parasitica in organic medium is that Al-organic matter complexes are not phytotoxic (Fichtner et al., 2001). The accumulation of Al in pathogen propagules was documented using a lumogallion fluorescent staining procedure (Fig. 3).

Although most stages in the life cycle of *P. infestans* occur above ground, tuber infection and pathogen survival in soil can be important in the epidemiology of the disease. Andrivon (1995) documented the occurrence of soils suppressive to *P. infestans*, and determined that Al may be an important factor in the suppressiveness of these soils. In culture medium hyphal growth of *P. infestans* was inhibited by Al concentrations of 2.5-100 μg Al ml^{-1}. In the most sensitive isolates, sporangial germination was inhibited at Al concentrations of 10 μg Al ml^{-1} (Andrivon, 1995).

A study on the effects of cations on zoospores of *P. cinnamomi* found that Al had no effect on zoospore viability or encystment (Byrt et al. 1982). This experiment used a 30 μM Al solution at pH 5.5, so the primary species of Al present was $Al(OH)_2^+$, which may have a lower level of toxicity to fungi than Al^{3+}.

Very few studies have addressed the role of Al in suppression of Pythium diseases. Ironically, some of the first studies completed on the use of $Al_2(SO_4)_3$ soil amendments to suppress fungal pathogens was for control of Pythium damping-off diseases (Jackson, 1940). Subsequent studies on the relationship between soil pH and *Pythium* spp. have reported conflicting results (Hoper and Alabouvette, 1996; Lumsden et al., 1976; Martin and Loper, 1999). However, at least some *Pythium* spp. are sensitive to Al (Huang and Kuhlman, 1991, Shew, unpublished data) and additional studies should be conducted to better quantify the effects of Al on the ecology of *Pythium* spp. and the development of Pythium diseases in acid soils.

There are conconflicting reports in the literature on the role of pH in development of diseases caused by species of *Aphanomyces*. *Aphanomyces euteiches* is sensitive to Al concentrations between 25 and 100 μg Al $ml^{-1}$of medium (Lewis, 1973). At 25 μg ml^{-1}, Al reduced zoospore formation and hyphal growth by 95% and suppressed root rot development. Lewis (1973) also concluded that Al reduced root rot disease by a direct toxic effect on the pathogen in the soil. However, other studies report a reduction of root rot caused by *A. euteiches* at pH values above 5.5 and at high Ca levels (Lewis, 1977). Disease suppression may therefore occur at both low and high pH via separate mechanisms.

Potato scab, caused by *Streptomyces scabies*, is an important disease of potato worldwide and is probably the most frequently cited example of a disease that is suppressed in acid soils (Agrios, 1997). Scab is most severe in high pH soils, but disease also can be severe in some acid soils (Hooker and Kent, 1950; Houghland and Cash, 1956). Reports of disease suppression in acid soils began in the early 1900s (Gillespie, 1918; Lutman and Cunningham, 1914), but controversy existed for many years as to the nature of this suppression. Multiple factors associated with acid soil chemistry have been related to suppression, including high concentrations of manganese (Mortvedt et al. 1961) and Al (Gries 1951, Houghland and Cash 1956).

A series of investigations conducted in Japan in the 1990s identified exchangeable Al as the primary factor responsible for disease suppression in the soils present in the potato growing regions (Mizuno and Yoshida, 1993; 1994; Mizuno et al., 1995; Mizuno et al., 1998 a,b; Mizuno et al., 2000; Yoshida et al., 1997). Significant differences were observed in scab incidence in two soil types that differed in their allophane content, an

alumino-silicate. Soils high in allophane were conducive to scab, whereas a non-allophanic soil was suppressive to scab at low pH (Mizuno et al., 1998a). Al concentrations as low as 0.2 to 0.3 mg L^{-1} suppressed scab in field soils. This observation was supported in a later study where the effects of deep plowing on scab suppression were studied. Deep plowing mixed soil from the A horizon that was high in soluble Al with soil from the B horizon that had a high allophane content and was low in soluble Al. The practice resulted in a reduced level of soluble Al in the zone of soil where tubers formed and subsequently reduced the level of scab suppression in the soil (Mizuno et al., 1998b). The researchers found much higher allophane contents in the Ap horizon in cultivated fields compared to the naturally occurring A horizon in virgin soils in the region.

In other studies, treatments that increase the level of Al also increase disease suppressiveness of the soil. For example, Yoshida et al. (1997) amended soil with either $Al_2(SO_4)_3$ or acidifying fertilizers and found that both treatments suppressed scab, with the $Al_2(SO_4)_3$ treatment providing the greatest level of suppression. Cook and Nugent (1939) reported no effect of acidifying fertilizers on scab development other than the effect on soil pH. Mizuno et al. (2000) used a single application of $(NH_4)_2SO_4$ to reduce soil pH and increase soluble Al levels to suppress scab development. However, this treatment was only effective in certain soil types that contained high levels of Al. Applications of $Al_2(SO_4)_3$ would be necessary to create a suppressive soil in soils naturally low in Al.

Although numerous soil bacteria are sensitive to Al, very few studies have investigated the effects of soil pH on root diseases caused by bacteria. Eddins (1939),

investigating control measures for bacterial wilt of potato caused by *Ralstonia solanacearum*, developed an amendment regime that included soil amendment with high levels of S in the summer lowering the soil pH to around 4, followed by a lime treatment in the fall, raising the soil pH back up to agronomic levels for potatoes. Aluminum levels were not provided for the sandy soils used in the study.

Summary

Al can be highly toxic to plants and microorganisms. It is clear from the studies that have been conducted in the last 30 years that there is a difference in the level of sensitivity of plants and many microorganisms, including some important plant pathogens, to the levels of soluble or exchangeable Al found in acid mineral soils. Research also has demonstrated that Al can be toxic to soilborne pathogens in organic potting media, but that the mechanisms may be different from those present in mineral soils.

Plants have adapted to the presence of Al in the rhizosphere by utilizing a number of mechanisms that either limit the uptake of Al in root cells or detoxify Al within root cells. It is important to understand the mechanisms of tolerance in Al-insensitive plants and the underlying genetic basis for this tolerance. The integration of improved soil management practices considering the interactions of Al with its chemical and physical environment with the potential benefits of traditional breeding and biotechnology may offer the opportunity to extend crop productivity in acid soils.

Numerous field studies in the last century have demonstrated that soil amendments that acidify soils can be used as part of an integrated approach to manage root diseases and soilborne pathogens. Use of Al as a soil amendment, however, has

remained very limited because errors in application rates can lead to significant phytotoxicity and loss of yield or quality, and insufficient levels give little or no suppression. As more host-pathogen systems are studied and the differential toxicity of plants and microorganisms to Al are more thoroughly understood, the use of Al-containing amendments may become more important in disease management, especially where other controls are not highly effective. Recent evidence also suggests that Al ions may enhance the production of phytoalexins (Jeandet et al., 2000). This observation may extend the use of Al from a strict soil amendment to use on above-ground portions of the plant, thus incorporating toxicity to the pathogen and stimulation of plant defense responses into an Al-mediated plant protection strategy.

One of the difficulties in interpreting the literature on Al toxicity arises from the use of multiple units to represent Al levels both in the soil and in culture media. Comparisons of effects across pathosystems are thwarted by the use of both inappropriate units and a lack of other background information to make necessary conversions. Ideally, Al levels should be represented in molarity for ease of calculation of Al activity in solution. For example, in solution, 1 μg Al ml^{-1} is approximately 37 μM Al, and 1 mg Al kg^{-1} of soil is equal to one part per million or 37 μmoles Al kg^{-1} soil. Furthermore, documentation of Al levels in soil as μmoles kg^{-1} should be accompanied by an estimation of bulk density and volumetric water content to allow for calculation of μM Al in solution. Units pertaining to equivalents of charge, such as milliequivalents (meq) and centimoles (cmol), should be avoided because of the pH dependence of Al hydrolysis species and charge distribution.

Interpretations of results from culture experiments on the toxicity of Al also is complicated by the nutrient status of the medium and the presence and concentration of other ions, organic acids, or other molecules that chelate, complex or bind with Al to alter its toxicity. Care should be taken to minimize the use of such compounds or ions in the medium, especially the use of media with high nutrient levels of an unknown composition such as potato dextrose agar and agar media that contain plant extract s high in organic acids. Finally, when possible, the activity of Al in the culture medium should be calculated with programs such as GEOCHEM-PC (Parker et al., 1995). This information will make it possible to compare results across pathosystems and soil types and improve our understanding the interactions of Al on pathogen biology, ecology and pathology.

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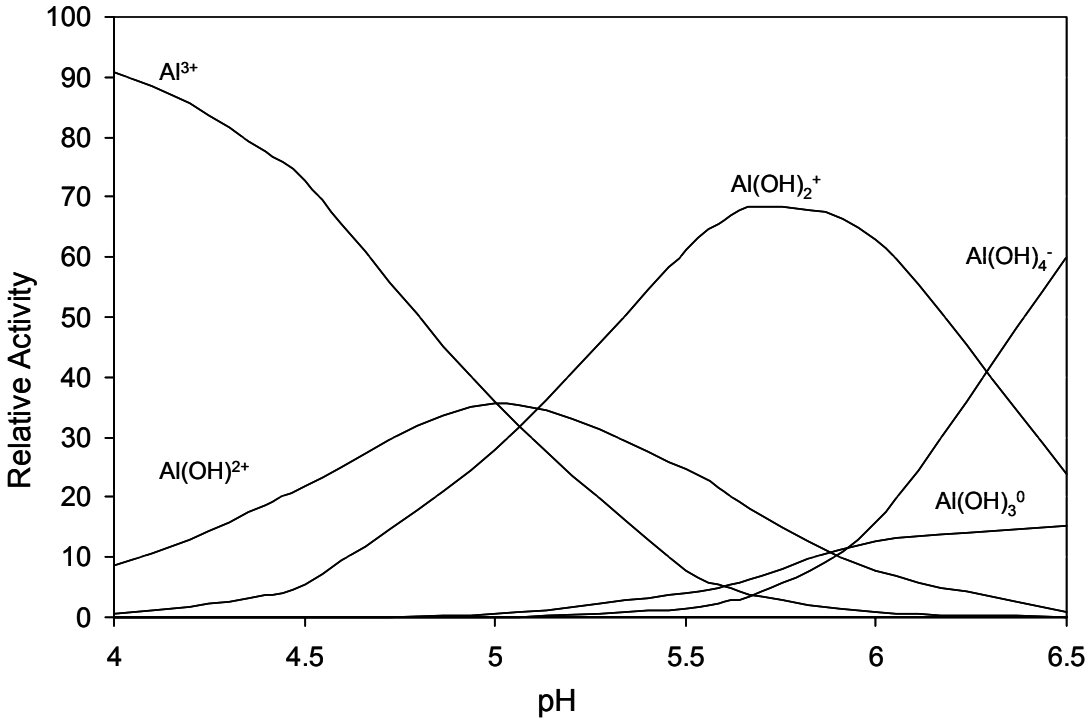

Fig. 1 Relative activity of monomeric Al species with respect to total Al in solution. A 5.0 µM solution of $AlCl_2$ was modeled using Geochem-PC and relative activity of each species is represented between pH 4.0 and 6.5.

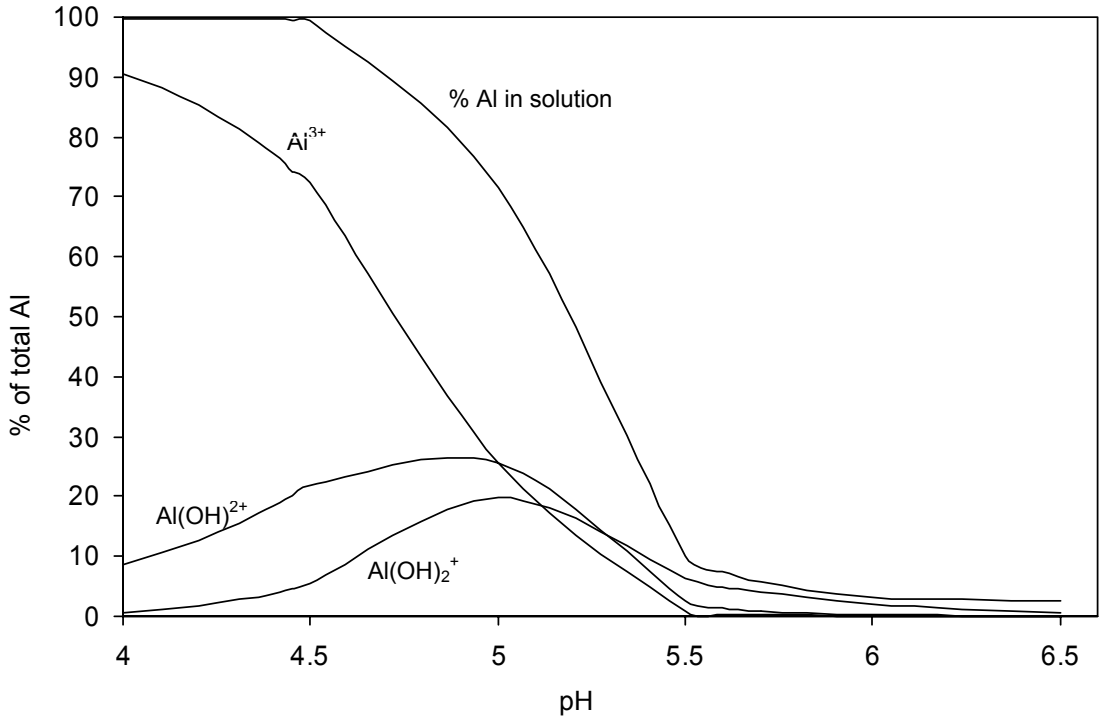

Fig. 2 Solubility of Al and distribution of aluminum hydrolysis species between pH 4 and 6.5 in a 5.0 μM solution of $AlCl_2$. The solution was modeled using Geochem-PC and the percent of total Al in solution and the percent of total Al in each of three hydrolysis species is represented. Lower valence hydrolysis species are not represented due to negligible activity with respect to total Al in the system.

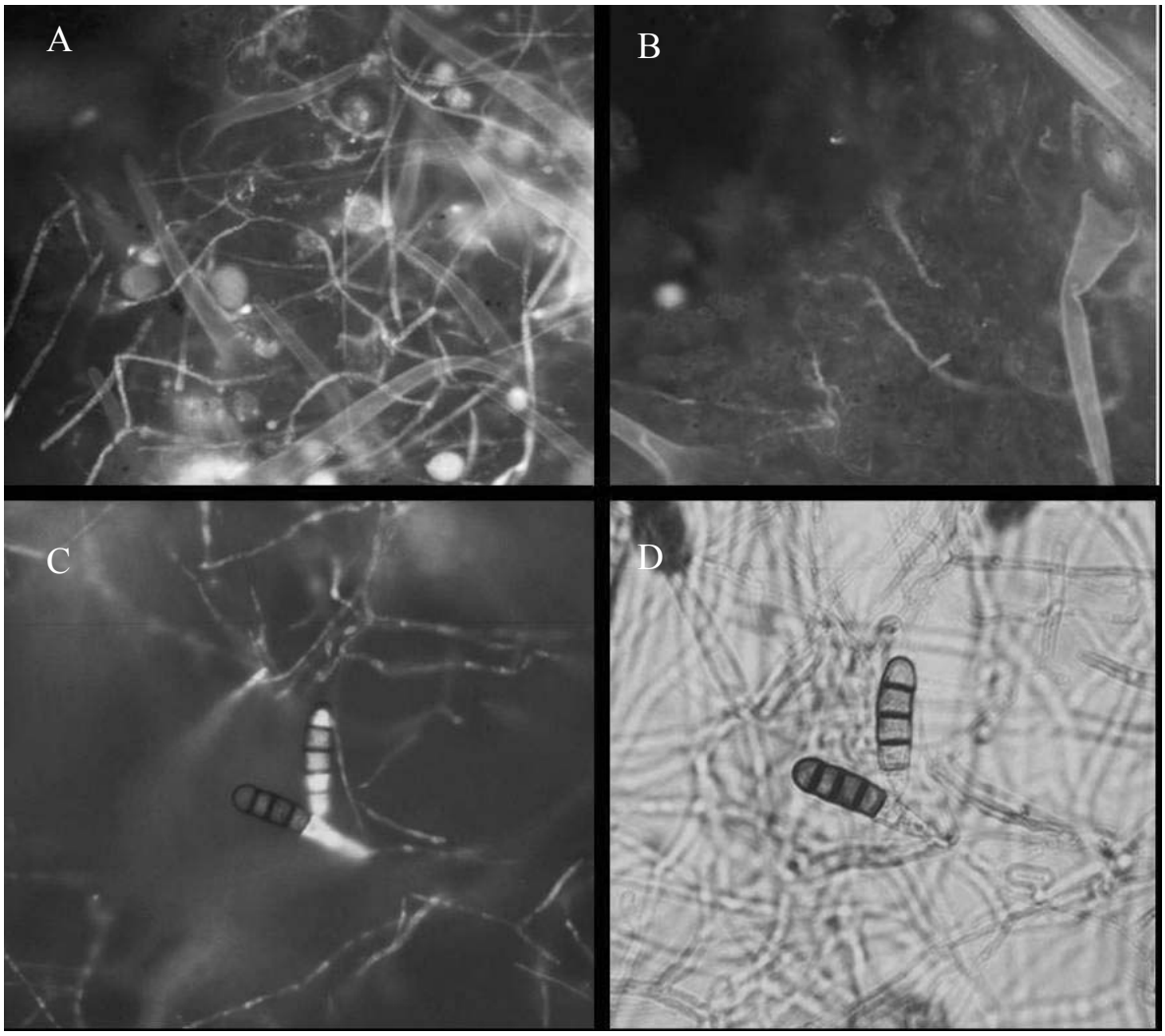

Figure 3. Vinca leaf tissue colonized with *Phytophthora parasitica*, stained with lumogallion, and observed under fluorescence microscopy (A and B). Infested leaf tissue in A was incubated for 48 h in Al-amended peat moss. Leaf tissue in B was incubated in unamended peat moss. Fluorescence in A represents accumulation of Al inside pathogen hyphae and sporangia. The low level of fluorencence in B represents background fluorescence. (Cand D) Aleuriospores from a *Thielaviopsis basicola* colony grown 24 h in 5% carrot broth, rinsed with deionized water, bathed in an $Al_2(SO_4)_3$ solution for 48 h, then stained with lumogallion. Images C and D are the same field of view, with C representing fluorescence and D representing bright field microscopy. Note the fluorescence in cells in the base on the aleuriospore chain indicating Al accumulation in the spores. The cell walls exhibit no fluorescence, suggesting that Al accumulates in the cytoplasm and not in the cell walls.

Chapter 2: Physiology and biology of aluminum mediated suppression of *Phytophthora parasitica* in aluminum-amended peat.

Physiology and biology of aluminum-mediated suppression of *Phytophthora parasitica* in aluminum-amended peat

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ABSTRACT

E. J. Fichtner and H. D. Shew. 2003. Physiology and biology of aluminum-mediated suppression of *Phytophthora parasitica* in aluminum-amended peat. Phytopathology. 93: XXX-XXX.

Amendment of a peat-based potting medium with $Al_2(SO_4)_3$ suppresses populations of *Phytophthora parasitica*, but the mechanism of this suppression is unknown. This study addresses the mechanism of suppression by i) determining the effect of Al-amended peat on sporangia production, ii) assessing the influence of Al amendments on resident microbial activity, and iii) detecting the accumulation of Al in pathogen tissue. Limed peat was amended with $Al_2(SO_4)_3$ solutions adjusted to pH 4 or 6 at either 0.0158 or 0.0079 g Al g^{-1} peat. Amended peat was placed in Büchner funnels maintained at –2.5 kPa matric potential. Peat was then infested with *P. parasitica* by placing five leaf disks colonized by *Catharanthus roseus* in each funnel. Leaf disks remained in Al-amended peat for 48 h, then were rinsed with deionized water and mounted on glass slides. The number of sporangia produced on edges of leaf disks were counted under a light microscope. A subset of leaf disks incubated in Al-amended peat

were stained with lumogallion, an Al-specific fluorescent stain, and observed under fluorescence microscopy for qualitative determination of Al accumulation in pathogen tissue. The microbial activity in Al-amended peat was assessed over a three week period by measuring the rate of CO_2 evolution g^{-1} peat. All Al-amended peat treatments suppressed sporangia production by at least 50%. Aluminum amendments did not significantly alter microbial respiration in peat, and respiration decreased over time. Aluminum accumulation was observed in pathogen tissue from all Al-amended peat treatments and was observed in the cytoplasm of individual zoospores but not in the cell walls. The use of lumogallion offers a new tool for the study of the mechanism of Al toxicity to fungal plant pathogens because the stain allows for the identification of the toxicant at the site of a physiological response.

Aluminum (Al) toxicity to plant pathogens has been reported in a number of highly weathered, acid, mineral soils containing high levels of exchangeable Al. The physiological mechanism(s) of this suppression are yet unknown. Aluminum sensitivity has been reported in a wide range of plant pathogens including actinomycetes (39), fungi (9-11,13,25-27,35-38,43), and members of the Oomycota (Stramenopila) (1-3,8,16,28,29,40). The application of Al amendments to agricultural soils for disease suppression, however, is of limited practice due to the phytotoxicity associated with heightened Al activity in soils.

In the majority of soils conferring Al-mediated pathogen suppression, disease suppression is positively correlated with dissolved Al activity. Consequently, factors increasing Al activity, such as application of acid fertilizers or additions of Al salts,

enhance disease suppression. For example, the first report of the use of an Al salt for suppression of a Phytophthora disease was by Lambert and Crandall in 1935, where amendment of soil with $Al_2(SO_4)_3$ reduced the pH to < 5.0 and resulted in a reduction of wilt disease of black-locust seedlings (28). Similarly, Guba (13) achieved suppression of Verticillium wilt in pot tests by acidifying soil to pH < 5.0 with additions of $Al_2(SO_4)_3$ or sulfur. Amendments with $Al_2(SO_4)_3$ were more effective than sulfur at reducing disease (13), presumably due to the comparatively elevated activity of Al in the $Al_2(SO_4)_3$-amended soil.

Converse to the nature of pathogen suppression in acid mineral soils, suppression of damping-off of ornamental bedding plants, caused by *P. parasitica*, has been observed in an Al-amended, peat-based potting medium (2,3,8). In the peat –based system, Al activity is governed by the formation of hydrated, outer-sphere surface complexes between monomeric Al species and acid functional groups on the organic matrix (4). Organic matter serves as an Al sink, regulating Al activity in organic soils at a lower level than that predicted by the solubility of Al-bearing minerals (52). Additions of organic matter to acid soils ameliorates the phytotoxicity of Al by reducing the amount of exchangeable Al in the soil (50). Although establishing low levels of K-exchangeable Al, amendment of peatmoss with either $AlCl_3$ or $Al_2(SO_4)_3$ resulted in a reduction in recoverable propagules of *P. parasitica* (8). Because the chemical environment of Al-amended peat is so different from that of acid mineral soils, the form(s) of Al responsible for suppression and the mechanism(s) of suppression may be different in the two systems (8). Suppression of *P. parasitica* in Al-amended peat has only documented toxicity at the pathogen population level (8). The direct toxicity of Al to sporangia production has not

been determined, nor has the influence of Al-amendments on resident microbial activity been assessed.

Multiple methods and reagents have been used for spectrofluorometric detection and quantification of Al in fresh, estuarine and saline waters (5,31,45,51), drinking water (6,46), and blood (33). The detection limit of these procedures is in the nanomolar range, with sensitivity reported as low as 0.1 ng Al ml^{-1} (5,6). The fluorescent stain lumogallion (3-[2,4 dihydroxyphenylazo]-2-hydroxy-5-chlorobenzene sulfonic acid) was first proposed as a reagent for spectrofluorometric determination of Al in sea-water by Nishikawa et al. (41,42) because it forms a stable complex with Al. The procedure has been modified and improved by Hydes and Liss (17) and more recently by Ren et al.(45) to enhance the detection limit and overcome ionic interference associated from complexes with F and Fe^{3+}.

Lumogallion staining has been applied in physiological studies of Al toxicity to a number of crops, including soybean (20-22,48), tobacco (18), and rice (53). Aluminum accumulation has also been observed in wheat seedling roots by Polle et al (44), but the fluorochrom employed (hematoxylin) is not as sensitive as lumogallion (22). Investigations of Al toxicity to soybean roots utilized lumogallion to correlate Al distribution in root tissue with growth inhibition and to detect excess Al accumulation in the outer root cortex of the distal root elongation zone (22). The physiological mechanisms of Al toxicity to plants and plant pathogens is yet unknown, however, application of the lumogallion staining technique to the study of Al toxicity in soilborne plant pathogens may enhance our understanding of the interaction of microbes with their chemical and physical environment.

The overall goal of this work was to study the mechanism of Al toxicity to *P. parasitica* in an Al-amended peat medium. Specific objectives addressed the i) effect of Al-amended peat on sporangia production, ii) influence of Al-amendments on background microbial activity in the medium, iii) accumulation of Al in tissues of *P. parasitica* with the application of a lumogallion staining technique.

MATERIALS AND METHODS

Peat preparation. Canadian sphagnum peatmoss (Fertiloam, Quebec, Canada) was passed through a sieve with 2 mm openings and stored dry for use in subsequent experiments. For continuity, the peat used in the following experiments was from the same source and batch as that used in a prior study of Fichtner et al. (8). The pH of the unamended peat was 3.1.

Inoculum production. An isolate of *P. parasitica* was obtained from diseased vinca plants and used as inoculum. This isolate was provided by D. M. Benson of N. C. State University, Raleigh, NC and was the same isolate that had been used in the 2001 study of Fichtner et al. (8). Cultures were maintained on corn meal agar (CMA) (Difco Laboratories, Detroit). To generate inoculum for experiments, leaf disks (1-cm diameter) were cut from mature vinca plants (*Catharanthus roseus*) with a cork borer and sterilized in water plus a drop of Tween 80 (Fisher, Norcross, GA) for 30 min on two consecutive days. The sterilized leaf disks were placed on CMA around an agar plug of *P. parasitica*. The pathogen then colonized the sterile leaf disks over 4 days at room temperature and colonized leaf disks were removed from plates for use as inoculum. Twenty-five leaf disks were also placed on CMA in the absence of the pathogen for observation of uninfested leaf disks in the fluorescence microscopy procedure.

Effects of aluminum amendment on sporangia production. Fifty gram of sieved peat was placed into each of five plastic bags and limed with $Ca(OH)_2$ at a rate of 0.024 g/ g air-dry peat. The limed peat was thoroughly mixed and moistened and allowed to incubate 2 days at room temperature. Peat was then amended with a 0.05 M $Al_2(SO_4)_3$ solution adjusted to either pH 4 or 6 with 1 N KOH. A precipitate was observed in Al-amendment solutions. Al levels of 0, 0.0079 and 0.058 g Al/g peat were determined by varying the amount of Al-amendment solution added to each bag. Deionized water was added to the no-Al control and the low Al level treatments to achieve the same volume of liquid across all treatments. Peat was incubated in the Al-amendment solutions for 48 h to allow for equilibration of Al in the medium. A total of five treatments were established, including no Al control and four treatments composed of two Al levels and two amendment solutions (pH 4 or 6).

Saturated peat suspensions were placed in 150-ml fritted-glass Büchner funnels (Fisher Scientific) with four replicate funnels per treatment. Peat was packed to an approximate bulk density (D_b) of 0.12 g cm^{-3} and equilibrated for 6 h at -2.5 kPa matric potential (Ψ_m) with respect to the peat surface. A 3-g sample (0.59 g dry wt. equivalent) was taken from each of three funnels for pH measurement, then five infested leaf disks were buried to approximately a 1-cm depth in each of these three funnels. Five uninfested leaf disks were buried in the fourth funnel of each treatment. Leaf disks remained buried for 48 h in the Al-amended peat maintained at -2.5 kPa Ψ_m .

After 48 h, leaf disks were retrieved from peat, rinsed with deionized water, and placed on glass slides. The number of sporangia formed on the leaf disk edge observed across a randomly selected 10x field of view of an inverted, light microscope was

counted, with two counts made per leaf disk. Sporangia count data were analyzed with the general linear models procedure (PROC GLM), and treatment differences were determined with a Waller-Duncan K-ratio test (K=100) (SAS Institute, Cary, NC). The effect of Al amendments on sporangia production was determined in two runs of the experiment. After leaf disks were retrieved from funnels, the remaining medium was destructively sampled for final pH measurements.

pH determination. One 3-g sample (0.59 g dry wt. equivalent) was taken from each of three replicate funnels at the time leaf disks were buried (initial) and at the time leaf disks were retrieved (final). The pH was determined in a 1:2 (wt/vol) suspension of 0.01 M $CaCl_2$ solution.

Microbial activity. To determine the effect of Al amendments on the background microbial activity in the peat system, respiration rates were determined in Al-amended peat. In a separate experiment, peat was subjected to the aforementioned liming and $Al_2(SO_4)_3$ treatments, then placed in three replicate Büchner funnels for each treatment. Peat was packed to an approximate D_b of 0.12 g cm^{-3} and equilibrated for 6 h at -2.5 kPa Ψ_m with respect to the peat surface. Upon the set up and equilibration of funnels, a 5.0 g dry weight equivalent was removed from each funnel to monitor basal microbial respiration rates in the various treatments. Microbial activity was assessed over a 3-wk period using an incubation technique (54) that measures evolution of CO_2. The 5.0 g samples were placed in plastic beakers and each beaker was set in the bottom of a sealed 2-L Mason jar. Fifty-milliliter glass beakers, suspended in Mason jars, were used to trap CO_2 in 5 ml of 0.5 N NaOH. Two jars containing NaOH solution were set up in the absence of medium as "no respiration" controls. Mason jars were incubated at room

temperature. On days 1, 7, 14, and 22, glass beakers were removed from jars and beakers containing fresh 0.5 N NaOH were replaced in jars. Carbonate was precipitated from the NaOH solutions with the addition of 5 ml of 0.5 M $BaCl_2$, and then two drops of phenolphthalein were added to each beaker. The residual base (unconsumed by trapping of CO_2) was titrated with 0.1 N HCl to a clear endpoint to indirectly assess CO_2 evolution. Respiration rates were calculated as mg CO_2 g^{-1} medium day^{-1}.

Lumogallion staining technique. The accumulation of Al in pathogen tissue was observed using a fluorescence detection technique. Of the leaf disks retrieved from funnels, five infested leaf disks were randomly selected from each treatment for staining with lumogallion, an Al-specific, fluorescent stain. The uninfested leaf disks were also retrieved from funnels and three uninfested leaf disks from each treatment were stained with lumogallion to observe background fluorescence in plant tissue.

The lumogallion staining technique was modified from that used by Silva et al. (48) for observation of Al accumulation in soybean roots. The leaf disks retrieved from each treatment were placed in 10 Petri plates (16 x 50 mm). A 10 mM citrate solution was adjusted to pH 4.5 and 8 ml of the solution were added to each plate to remove apoplastic Al. Leaf disks were soaked in citrate for 30 min at 25 C on a rotary shaker (Innova 2100, New Brunswick Scientific) at 75 rpm in the dark. The citrate solution was then aspirated off plates and 8 ml of deionized water was added to each plate. Leaf disks remained in water for 15 min before water was aspirated off plates. The leaf disks were then bathed in a 0.2 M sodium acetate buffer (pH 5.2) for 15 min at 25 C. After buffer was aspirated off plates, a 4 ml aliquot of fresh buffer was added to plates with 4 ml of lumogallion (Molecular Probes, Eugene, OR) stock solution (10 mM lumogallion in 0.2

M sodium acetate buffer at pH 5.2) and allowed to incubate for 60 min at 50 C on a rotary shaker at 75 rpm in the dark. Leaf tissue was then sectioned with a scalpel, mounted in glycerol on glass slides and stored in the dark. All metal tools were washed with 20% (v/v) HNO_3 prior to use, and lumogallion stock solution was stored in the dark. Slides were viewed within 2 h of preparation under an Axiophot microscope (Carl Zeiss, Thornwood, NY) that was equipped for epifluorescence microscopy with an HBO 100-W/2 mercury burner and G365 nm exciter: LP420 nm barrier fluorescence filters.

RESULTS

Sporangia production. Sporangia production was consistently reduced in Al-amended peat. Because there was no significant interaction between Al-treatments and experimental runs, the sporangia production data were combined over both runs (Fig 1). Aluminum amendments reduced sporangia production by 55-97%, with the highest suppression observed in peat amended with the pH 4 $Al_2(SO_4)_3$ solution and the high level of Al. The heightened suppression in this treatment was significantly different than the 55% reduction in sporangia production observed in the peat amended with the low Al level as the pH 6 $Al_2(SO_4)_3$ amendment solution.

pH trends. Limed peat that had been un-amended with Al equilibrated at a pH of approximately 4.7 with little difference between initial and final pH (Table 1). Amendment of limed peat with the pH 4 amendment solutions reduced the pH of the media to approximately 4.3 and 4.0 in media amended with the low and high levels of Al, respectively. Amendment of peat with the pH 6 amendment solution at the low Al level resulted in little change in pH; however, addition of the high Al level with the pH 6 amendment solution resulted in pH levels > 5.0. A difference between initial and final

pH was generally observed in peat amended with the pH 6 $Al_2(SO_4)_3$ solution, with final pH tending to be higher than initial pH by at least 0.11 pH units.

Microbial activity. Microbial activity in Al-amended peat tended to decrease over time, with little influence of Al treatments on respiration rates (Fig 2). Initial respiration rates across treatments were between 1.2 and 1.9 mg CO_2/g peat/day, and final respiration rates were between 0.5 and 0.7 mg CO_2/g peat/day. In the first run of the respiration experiment (Fig 2 A), peat amended with the pH 6 amendment solutions exhibited low levels of respiration on day 1, but respiration increased on day 7 and continued with the trend of other treatments for the duration of the three week period. In the second run of the respiration experiment (Fig 2 B), respiration rates in all treatments decreased similarly over time.

Aluminum accumulation in pathogen tissue. Uninfested leaf tissue buried in Al-amended peat did not fluoresce after it was stained with lumogallion and observed under fluorescence microscopy. Under fluorescent microscopy, individual hyphae and sporangia were easily distinguished from leaf tissue of infested leaves that had been buried in Al-amended peat (Fig 3 B). Infested leaf disks buried in unamended peat exhibited little background fluorescence (Fig 3 A). The only readily identifiable plant structures visualized under fluorescence were trichomes, which appeared red (Fig 3 A and B).

Fluorescence was observed in pathogen tissue retrieved from all treatments of Al-amended peat. Hyphae and sporangia of *P. parasitica* that had been retrieved from peat amended with the pH 6 $Al_2(SO_4)_3$ solution at the high Al level exhibited strong fluorescence (Fig 3 B-F). Intact sporangia containing zoospores exhibit strong

fluorescence (Fig 3 D, s2), whereas sporangia that have already released zoospores were unidentifiable under fluorescence microscopy (Fig 3 F, s). Individual zoospores within a sporangium can be differentiated from the sporangial cell wall due to the fluorescence of the zoospores (Fig 3 D, s1). The sporangial cell wall appears black under fluorescence (Fig 3 D, s1). Furthermore, hyphal tips tend to have higher intensity fluorescence than older hyphae (Fig 3 F, ht).

DISCUSSION

Aluminum-mediated suppression of plant pathogens, including multiple species of *Phytophthora* (1-3,40) has been documented, but the form of Al responsible for suppression and the mechanism(s) of suppression are largely unknown. The suppression of *P. parasitica* populations for control of damping-off of ornamental bedding plants has been observed in Al-amended, peat-based potting media with low levels of exchangeable Al (2,3,8). In vitro sporangia production of *P. parasitica* was limited in the presence of Al (2). In both the studies of Benson (2) and Fichtner (8), however, the pathogen's ability to produce sporangia in the Al-amended, peat-based media was not determined.

In the present study, all Al-amendments of peat resulted in reduced sporangia production. The reduction of sporangia production on infested leaf disks exposed to Al-amended peat partly explains the reduction in pathogen populations previously observed by Fichtner (8). Because pathogen populations in Al-amended peat were determined after the water potential in funnels was raised to 0 Ψ_m (flooding) to induce zoospore release (30), an Al affect on pathogen population could be the result of Al-toxicity to sporangia production and zoospore survival. The results of the present study suggest that sporangia production is limited in Al-amended peat. Further studies are necessary to

assess the direct toxicity of Al to zoospore survival, viability, and the inoculum efficiency of zoospores produced in the presence of Al.

Amendment of limed peat with the pH 4 amendment solution at a rate of 0.0158 g Al g^{-1} peat reduced sporangia production by approximately 97% in the present study, whereas the same treatment resulted in at least a 90% reduction in the number of recoverable propagules in the former study by Fichtner (8). In this treatment, peat had a K-exchangeable Al level of approximately 60 μM g^{-1} of peat (7). Although direct toxicity of Al to zoospores has not been determined, the present study has noted the accumulation of Al in zoospores. Factors affecting sporangia production and subsequent zoospore viability in Al-amended peat may include physical factors that limit contact between the pathogen and the toxicant such as water potential, pore size, air-pockets, and voids in the medium. Factors limiting contact between the pathogen and Al may allow for pathogen survival and reproduction in microzones within an otherwise adverse environment.

In Al-amended peat, reduction in sporangia production was observed at lower levels of Al than is typical of suppressive mineral soils. The levels of K-exchangeable Al in Al-amended peat ranged from 0.3 – 61 μM Al g^{-1} of peat, with the low and high levels observed in peat treated with 0.0079 g Al g^{-1} peat as a pH 6 amendment solution, and 0.0158 g Al g^{-1} peat as a pH 4 amendment solution, respectively (8). Even at the highest level of K-exchangeable Al in the peat system, the Al level was approximately 95% lower than that recorded by Muchovej et al. as suppressive to mycelial growth of *P. capsici* (40).

Aluminum-mediated suppression of pathogens in mineral soils generally occurs at low pH (< 5.2). In the peat system, however, suppression of sporangia production of *P. parasitica* was observed at pH levels close to 5.3. Prior studies on Al-toxicity to *Phytophthora* implicate Al^{3+} as the toxic ion because of its relative abundance at low pH (32). The heightened pH of some Al-amended peat treatments suggests that other hydrolysis species or complexes of Al are responsible for pathogen suppression in this system. For example, given a pH of 5.3 and the assumption that surface hydrolysis of Al-organic matter complexes and hydrolysis of dissolved Al are the same at a given pH, approximately 90% of monomeric Al is expected in the $Al(OH)_2^+$ species (32). Reduction of sporangia production of *P. parasitica* has also been observed in an in vitro $Al_2(SO_4)_3$ solution buffered at pH 5.0 in the absence of organic ligands (2), suggesting that Al-hydrolysis species are toxic to *P. parasitica*. However, the relative toxicity of individual hydroloysis species to the organism has not been determined.

Microbial activity in the peat system was largely unaffected by the Al-amendments. The low respiration rates observed on day 2 in treatments with the pH 6 amendment in the first run of the experiment are inexplicable and were not observed in the second run of the experiment. The lack of an Al-effect on microbial activity is not surprising because the heightened decompositional status of dark sphagnum peatmoss does not tend to support high microbial activity (14). In natural soils, Al is one of the main factors inhibiting microbial biomass and nitrogen mineralization (19); however, acid- and Al-tolerant microbes do have a niche in acid soils (24). Considering that the initial pH of the unlimed peat was 3.1, the microbial communities residing in peatmoss may be relatively acid-tolerant.

The use of lumogallion introduces a new technique for the study of Al accumulation in pathogen tissue and may have future application to the study of physiological mechanisms of Al-toxicity to plant pathogens. The major limitations in the use of fluorochromes in the study of plant-pathogen interactions include: i) the cost of the stain, ii) the complexity of the staining procedure, and iii) the contrast and resolution provided between the plant and pathogen tissues (15). The lumogallion stain is affordable and has proven applicable to the observation of fungi. The advantage of its use on pathogens as opposed to plants is that specimens tend to be one cell-layer thick and, hence, do not require sectioning. Studies on Al-accumulation in soybean root tissue, for example, required root sectioning and the use of confocal laser scanning microscopy in order to focus through multiple cell layers for the observation of an undisturbed plane of cells (48). Because fungi tend to be one cell-layer thick, undisturbed specimens can be stained, mounted, and observed under fluorescence microscopy. Furthermore, the entire stain protocol takes approximately 1.5 h, but specimens should be observed shortly after mounted on slides due to diminishing fluorescence over time and the light-sensitivity of lumogallion.

One of the most notable observations from the fluorescence microscopy was the ability to discern between plant and pathogen tissue. When colonized leaf disks were stained with lumogallion and viewed under fluorescent light, the only plant structures to fluoresce were the trichomes on the leaf surface. Living plant tissue, specifically soybean roots, accumulate Al (48); however, the autoclaved leaf tissue of *C. roseus* did not accumulate Al. This suggests that Al may be actively taken up by plants and is unlikely to be passively absorbed in plant tissue. Modification of the lumogallion stain procedure

may be necessary for observation of Al accumulation at infection sites in order to differentiate between living host and pathogen tissues.

Lumogallion offers other advantages for its use in studying Al-interactions with microbes. Morin, another fluorochrome has also been applied to the study of Al-accumulation in plant tissue, but it is not as sensitive as lumogallion for detection of Al (22). Both lumogallion and morin are sensitive to Al at nanomolar levels (23,47). Lumogallion has been recognized for its specificity in binding of Al (12,17) and can be used to detect Al in the presence of organic ligands (47,49) such as would be found in organic soils and plant tissue.

Aluminum accumulation was observed in pathogen tissue that had been exposed to all Al-amendment treatments. Photos presented in this document are of pathogen tissues exposed to peat amended with the pH 6 $Al_2(SO_4)_3$ solution at 0.0158 g Al g^{-1} peat. Fluorescence photographs from this treatment were selected because they depict Al accumulation in pathogen tissue in an environment containing distinctly low levels of K-exchangeable Al (approximately 1 μM Al g^{-1} peat) (8). Although Kataoka et al. (22) reported that lumogallion fluorescence was proportional to Al accumulation; differential fluorescence intensity was not specifically addressed in the present study. Future studies are needed to address the relative accumulation of Al in pathogen tissue exposed to the various Al treatments and also the differential accumulation of Al in pathogen tissue types (ie. sporangia vs. hyphae). Aluminum accumulation in plant root apices has been quantitatively analyzed using inductively coupled plasma atomic emission spectrometry (48). The application of this technique to fungi may allow for future calibration of a spectrofluorometric method for quantification of Al in pathogen tissue.

Lumogallion fluorescence was observed in both sporangia and hyphae, indicating Al accumulation in both tissue types. Sporangia having released zoospores lack fluorescence, indicating that Al is not accumulating in the remaining cellulose cell wall. Individual zoospores, however, fluoresce within sporangia, suggesting that Al is accumulating in these propagules. Because zoospores lack a cell wall, fluorescence of zoospores may be related to Al binding on the cell membrane or in the symplast. Silva et al. (48) addressed the intracellular distribution of Al in root apices and documented Al accumulation in plant cell nuclei of Al-sensitive soybean genotypes. The physiological mechanism of the toxicity of Al to plants and microbes is unknown. Aluminum is known to interrupt mitosis in plant cell nuclei (7, 34), and lumogallion was used to identify the toxicant directly at the site of a physiological affect (48). Further studies are needed to address the physiological mechanisms of Al toxicity to *Phytophthora* by focusing on subcellular accumulation of Al.

In summary, sporangia production of *P. parasitica* was suppressed in Al-amended peat at pH levels as high as 5.3. Al-mediated suppression of plant pathogens in acid mineral soils is generally observed at lower pH values and higher levels of exchangeable Al (9-11, 25-27,39). The high pH and predominance of weak-acid functional groups in the peat (8) suggest that Al in the peat system is mainly i) bound to organic matter, ii) precipitated as an $Al(OH)_3$ mineral, or iii) present in solution predominantly as the $Al(OH)_2^+$ species. Fichtner et al. (8) reported the likely toxicity of multiple hydrolysis species and organically complexed Al to populations of *P. parasitica* in Al-amended peat. The present study suggests that one or more of these Al forms directly inhibits sporangia production. Furthermore, background microbial activity in the peat was not

affected by Al amendments, suggesting the presence of Al-tolerant microbial communities. Future studies should be conducted to determine the relative toxicity of these Al forms to pathogens and microbes in more microbially-diverse systems.

This paper also introduces the lumogallion staining technique and its application for the study of Al accumulation in plant pathogens. Microscopic observation of lumogallion-stained, infested leaf disks allowed for differentiation of leaf tissue from pathogen tissue. The use of lumogallion for observation of infection sites on living host roots may be limited by the physiological uptake and fluorescence of Al in root tissue. Fluorescent images of pathogen tissue exposed to Al-amended peat suggest that Al does not accumulate in the cell wall, but does accumulate in zoospores. The effect of the Al accumulation on zoospore viability, survival, and inoculum efficiency is unknown. To further address the physiological mechanism of Al-toxicity to *Phytophthora*, future studies should focus on subcellular accumulation of Al and quantification of Al accumulation in pathogen tissues.

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Table 1. Average[w] pH[x] of limed peat amended with $Al_2(SO_4)_3$ solutions at pH 4 and 6 at rates of 0, 0.0079 and 0.058 g Al/g peat.

	No Al Control	pH 4 0.0079 g Al/g	pH 4 0.058 g Al/g	pH 6 0.0079 g Al/g	pH 6 0.058 g Al/g
Run 1					
Initial[y]	4.74	4.30	4.00	4.68	5.03
Final[z]	4.75	4.36	4.08	4.88	5.24
Run 2					
Initial	4.73	4.25	3.96	4.71	5.07
Final	4.75	4.31	4.02	4.87	5.26

[w] The average pH of samples taken from each of three replicate funnels.
[x] A 3-g sample (0.59 g dry wt. equivalent) was taken from each funnel and placed in 6 ml of 0.01 M $CaCl_2$ for pH measurement.
[y] Initial pH measurements were taken upon equilibration of peat in funnels on day 0.
[z] Final pH measurements were taken upon retrieval of infested leaf disks on day 2.

FIGURE LEGENDS

1. Number of sporangia produced on leaf disks buried for 48 h in Al-amended peat. Peat was treated with an $Al_2(SO_4)_3$ solutions at pH 4 or 6 at two rates of Al, 0.0079 and 0.0158 g Al g^{-1} peat. Data were combined from two runs of the experiment and different letters above bars designate significant differences based on the Waller-Duncan K-ratio test (K=100).
2. Microbial respiration rates in Al-amended peat. Peat was treated with an $Al_2(SO_4)_3$ solution at pH 4 or 6 at two rates of Al, 0.0079 and 0.0158 g Al g^{-1} peat. **A** and **B** represent two runs of the experiment.
3. Vinca leaf disk tissue colonized by *Phytophthora parasitica* was buried 48 h in Al-amended peat and then stained with lumogallion and viewed under fluorescence microscopy. Colonized leaf disks in photos B-F were exposed to peat amended with 0.05 M $Al_2(SO_4)_3$ solutions at 6 at a rate of 0.058 g Al g^{-1} peat. In A colonized leaf disks were buried in unamended peat to demonstrate the background fluorescence in the absence of Al. In photo B note the fluorescence of trichomes (t) and pathogen structures exposed to Al-amended peat (h= hyphae, s=sporangium, z=zoospores). Images C and D are from the same field of view, with C taken under light and D taken under fluorescence microscopy. Of the two sporangia shown in C and D (s1and s2), s1 contains two zoospores inside of a sporangium. Note both the fluorescence in the zoospores and the lack of fluorescence in the cell wall of s1. Also note that sporangium s2 contains many zoospores that fluoresce in D. Images E and F are also paired light and fluorescence microscopy photos from the same field of view. Note the

fluorescence in the hyphal tip (ht) and the lack of fluorescence in the cell wall of a sporangium(s) that had released zoospores.

Figure 1-top

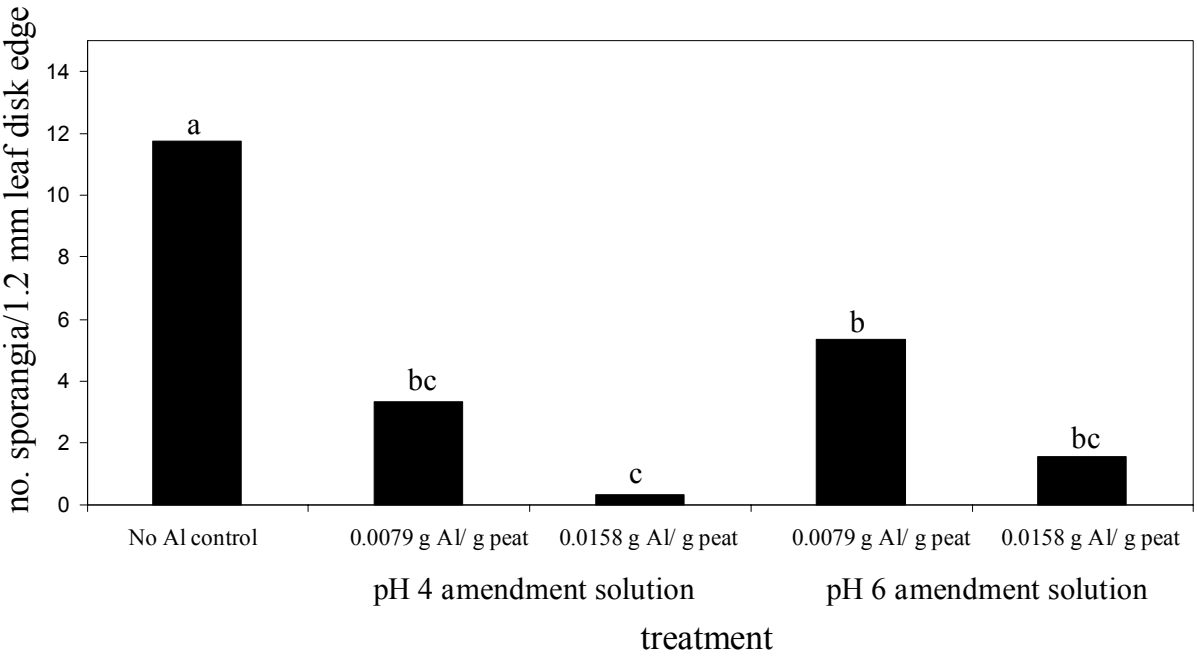

Figure 1-bottom

Figure 2-top

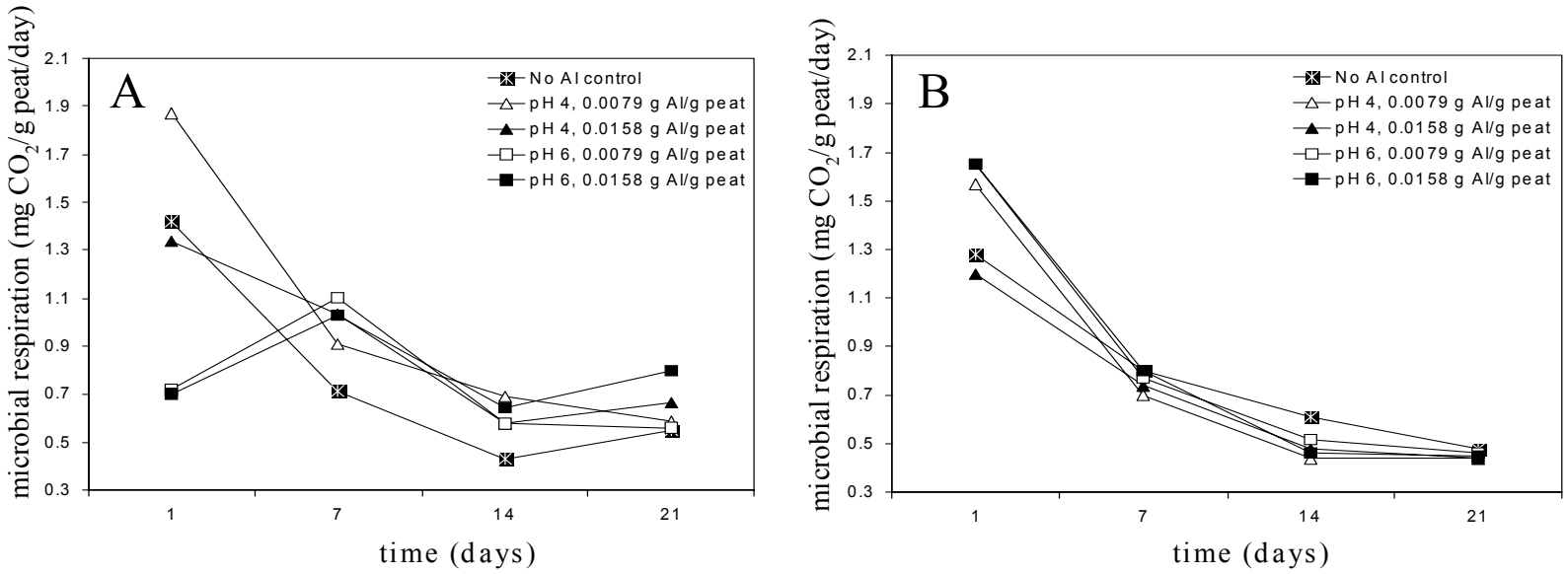

Figure 2-bottom

Figure 3-top

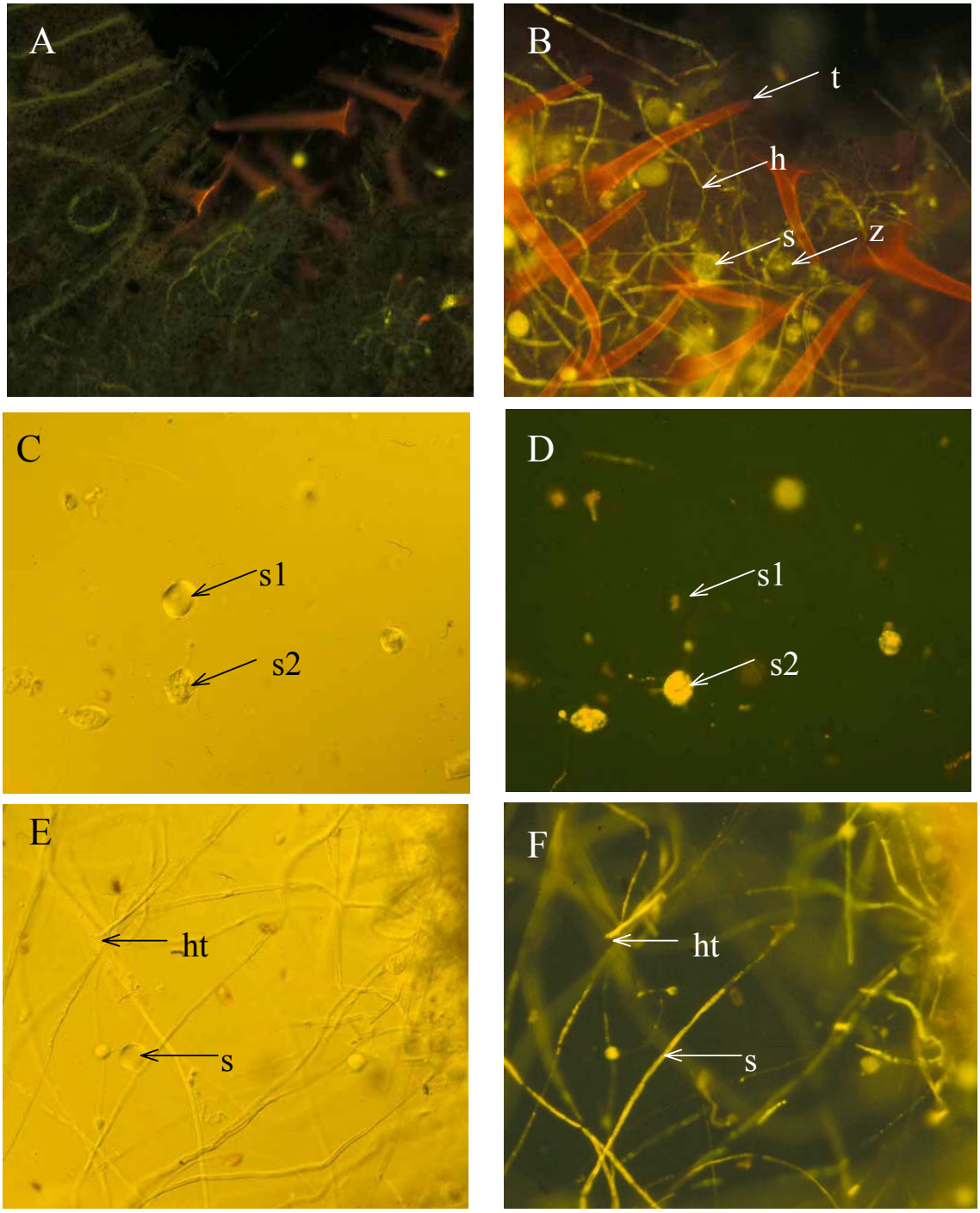

Figure 3-bottom

Chapter 3: Abiotic- and biological suppression of *Phytophthora parasitica* var. *nicotianae* in a horticultural medium containing composted swine waste

Abiotic- and biological suppression of *Phytophthora parasitica* var. *nicotianae* in a horticultural medium containing composted swine waste

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ABSTRACT

Fichtner, E. J., D. M. Benson, H. G. Diab, and H. D. Shew. 2003. Abiotic- and biological suppression of *Phytophthora parasitica* var. *nicotianae* in a horticultural medium containing composted swine waste. Phytopathology 93: XXX-XXX.

Horticultural potting media have been amended with compost to enhance biological suppression and with $Al_2(SO_4)_3$ to enhance abiotic suppression of plant pathogens, but these factors have not been simultaneously incorporated into the same medium. In this study, the efficacy of aluminum (Al)-amended potting medium containing 20% composted swine waste (CSW) was assessed for control of *Phytophthora parasitica* var. *nicotianae* (syn. *P. nicotianae*), a soilborne pathogen causing damping-off of many horticultural bedding plants. Steamed and unsteamed media were amended with no Al or Al at 0.0079 g Al/g medium with an $Al_2(SO_4)_3$ solution at either pH 4 or 6. Amended media were placed into Büchner funnels maintained at 2.5 kPa soil moisture tension and then infested with annual vinca leaf disks colonized by *P. parasitica* var. *nicotianae*. Leaf disks were buried for 2-day durations beginning on day 0, 6, 13, and 21 after Al-amendment. Leaf disks were removed and the number of sporangia on the edge of each leaf disk was counted. A similar experiment was conducted to determine the effect of steaming and Al-amendments on pathogen populations. Medium treated with

the pH 4 solution consistently reduced sporangia production between 38% and 65% on day 0, but no Al effect was noted at subsequent time points. The pH 6 amendment did not consistently effect sporangia production. Exchangeable Al levels decreased over time, and abiotic suppression was only observed at > 2 μM Al/g medium. Pathogen populations were occasionally affected by steaming and Al; however, the variability in population data rendered it difficult to identify trends over time. Sporangia production in unsteamed medium was reduced by 50% on leaf disks buried on days 6, 13, and 21, but not on day 0. Al-amendment of a 20% CSW potting medium enhanced suppression of *P. parasitica* var. *nicotianae* and abiotic suppression occurred before biological suppression developed.

The scarcity and rising cost of sphagnum peatmoss (9), coupled with the need to reduce and utilize the hog waste from North Carolina's growing swine industry, has prompted research on the incorporation of composted swine waste (CSW) into an organic potting medium (12). Compost is produced from the degredation of organic waste materials by diverse microbial populations in a thermophilic, anaerobic environment (14). Compost is of value to the agriculture and horticulture industries due to its chemical and physical properties that enhance soil fertility, structure, water-holding capacity, and overall plant health (9,14), and also for its biological properties which may be utilized to suppress plant disease.

A wide variety of composts, generated from animal and plant waste material, have been studied for suppression of plant diseases (2, 7, 8, 14, 27, 33). Biological suppression of pathogens in compost results from microbial activity developing after the

thermophilic phase of composting. Consequently, these microbes tend to be thermostable (14). The decompositional status of a medium affects the level of biological suppression offered by a compost-amended medium (6). Highly decomposed organic matter, such as sphagnum peatmoss, does not support high microbial activity (18) and tends to be conducive to plant pathogens. For example, *Pythium ultimum* was more suppressed in medium containing less decomposed organic matter than in a highly decomposed medium (6). Suppression of *Phytophthora cinnamomi* was observed in medium amended with chicken manure compost containing high microbial activity and high populations of endospore-forming bacteria (2). Assessment of microbial activity was used as a predictive measure to determine the potential level of suppression a potting mix offered for management of root rot on poinsettia caused by *P. aphanidermatum* (6). In fact, medium amended with composted municipal sludge requires lower sludge content (2.5% v/v) than a similar medium containing composted pine bark (20% v/v) to induce suppression of Pythium damping-off. This was attributed to the higher level of microbial biomass in the composted municipal sludge (18). On the other hand, heat treatments required to kill fecal pathogens in sewage sludge may render a medium conducive to *Rhizoctonia solani* (23). Extending the curing time of such composts, however, can allow for microbial re-colonization of the medium and a resurgence of suppression to *R. solani* (23).

Recently, Diab et al. (12) studied pathogen suppression in potting media containing CSW produced by two different compost-management practices. One of the composts studied, CSW1, suppressed Pythium damping-off of cucumber and pre-emergence damping-off of impatiens caused by *R. solani*. Fresh or uncomposted swine

waste was conducive to disease. The exact mechanism(s) of pathogen-suppression in CSW1 are unknown; however, medium containing 20% CSW1 had a more diverse microbial community and contained more fluorescent pseudomonads, heterotrophic fungi, endospore-forming bacteria and oligotrophic bacteria than CSW2. Furthermore, CSW1 exhibited a greater structural diversity as evidenced by BIOLOG analysis as well as microbial activity tests (12).

Although biological suppression of plant pathogens has been the focus of most studies in peat-based potting mixes, abiotic suppressive factors also have been incorporated into horticultural potting mixes. For example, aluminum (Al) has been incorporated into peatmoss-based potting media for control of damping-off of ornamental bedding plants caused by *P. parasitica* var. *nicotianae* (3,4). Benson (4) controlled pre-emergence damping-off of snapdragon, vinca, and petunia in a peat:vermiculite potting medium with the addition of $Al_2(SO_4)_3$. Disease control in this system was observed at KCl-exchangeable Al levels as low as 2.03 meq Al^{+3} 100 g^{-1} medium. Aluminum was not phytotoxic to any of the bedding plants studied, indicating a differential sensitivity of the pathogen and host to Al.

Focusing on the chemistry of Al in organic systems, Fichtner et al. (13) further investigated the Al-mediated suppression of *P. parasitica* var. *nicotianae* in a peat-based medium. Limed peat was amended with $Al_2(SO_4)_3$ solutions and infested with *P. parasitica* var. *nicotianae*. Pathogen populations were reduced by at least 65% in Al-amended media and suppression was observed at K-exchangeable Al levels as low as 1 μmol Al per gram peat at a pH > 5.0. In the peat system, the presence of organic acids alters Al^{3+} activity by the formation of Al-organic acid complexes (34). The conditional

formation constants for Al-organic matter complexes increase with pH (16); therefore, the elevated pH of the peat system enhanced the affinity of peat for Al. Most Al ions bound to organic matter are hydrolyzed and act as a buffer against changes in pH (15). Given the high Al-binding capacity of peat and the low levels of exchangeable Al in the system, it is possible that the organically-bound Al or an $Al(OH)_3$ precipitate may have been responsible for pathogen suppression (13). The suppression of pathogens in Al-amended peat differs from the frequently documented suppression of pathogens in mineral soils, where pathogen suppression has been directly correlated with Al^{3+} activity, a species of Al considered toxic to plants.

Many of the major pathogens that have been suppressed in compost-amended media are sensitive to Al. Members of the Oomycota, including *Pythium* spp. (19), *Phytophthora* spp. (1) and *Aphanomyces* (24) are suppressed by Al, as are *R. solani* (22) and *T. basicola* (29-32). The potential toxicity of Al to soil microorganisms, including biocontrol agents, limits the integrated use of biotic and abiotic factors for control of plant pathogens. The careful regulation of Al activity and form in an organic medium, however, may enable the incorporation of multiple mechanisms for disease control into a commercial potting medium as part of a disease management strategy.

The overall goal of this work was to develop a disease-suppressive potting medium by incorporating both biological and abiotic mechanisms of pathogen suppression into a potting medium containing CSW. To accomplish this goal, our objectives were to i) test the efficacy of CSW-amended medium for biologically-mediated suppression of *P. parasitica* var. *nicotianae* over time, and ii) determine if Al amendment of the CSW-amended medium altered biological suppression.

MATERIALS AND METHODS

Medium preparation. Composted swine waste (CSW) was obtained from the Animal Waste Management Center at North Carolina State University. Swine waste was harvested from a lagoon, combined with woodchips and composted in a sheltered bin. After progressing through two heating cycles, the composted material was cured in an outdoor bin for further decomposition. A mixture of 20% CSW (v/v) was prepared by mixing the CSW with Fafard No.2, a soilless potting medium containing Canadian sphagnum peatmoss, perlite, and vermiculite plus a wetting agent and nutrients (Fafard, Inc. Agawam, MA). The CSW used in this study is from the same source as the CSW1 previously described as suppressive to *R. solani* (12).

Inoculum production. A race 0 isolate of *P. parasitica* var. *nicotianae* was used in all tests. This isolate was obtained from infested soil in Duplin Co., NC and was provided by Melinda Sullivan, N.C. State University, Raleigh, NC. Cultures were maintained on corn meal agar (CMA) (Difco Laboratories, Detroit). To generate inoculum for experiments, leaf disks (1-cm diameter) were cut from mature vinca plants (*Catharanthus roseus*) with a cork borer and sterilized in water plus a drop of Tween 80 (Fisher, Norcross, GA) for 30 min on two consecutive days. The sterilized leaf disks were placed on CMA around an agar plug of *P. parasitica* var. *nicotianae*. The pathogen then colonized the sterile leaf disks over 4 days at room temperature and colonized leaf disks were removed from plates for use as inoculum.

Effects of Al amendment and steaming on sporangia production. Six liters of 20% CSW (loose bulk density of 0.22 g ml^{-1}) was mixed thoroughly in a plastic bag and divided in half. One half was steamed at 99 C for 30 min on two consecutive days. The

second half remained unsteamed. The steamed and unsteamed media were then divided into three alquots to establish a complete factorial treatment design. The steamed and unsteamed media were amended one day after steaming with either no Al or an $Al_2(SO_4)_3$ solution at pH 4 or 6 at a rate of 0.0079 g Al g^{-1} medium. The Al-amendment solutions were prepared by making a 0.05 M $Al_2(SO_4)_3$ solution and adjusting the pH to either 4 or 6 with the addition of 1 N KOH. Deionized water was added to the no-Al control treatments so that all treatments contained the same volume of liquid. Each suspension was incubated for 24 h in a plastic beaker to allow for equilibration of Al in the media.

The effects of steaming and Al-amendment of 20% CSW on production of sporangia were monitored at four time points over a 23-day period. The saturated, treated media were placed into 150-ml fritted-glass Büchner funnels with three replicate funnels for each of the four time points. Amended CSW was packed to an approximate bulk density (D_b) of 0.188 g cm^{-3}, and was equilibrated at -2.5 kPa soil matric potential (Ψ_m) with the use of hanging water columns. The placement of media in funnels was considered day 0. Samples were taken from equilibrated media on day 0 to assess microbial respiration in the medium over a three-week period.

Fifteen colonized leaf disks were buried in each of three replicate funnels on days 0, 6, 13, and 21. Leaf disks remained buried for 48 h in media maintained at -2.5 kPa Ψ_m. After 48 h, disks were retrieved from the media, rinsed with deionized water, and placed on glass slides. The number of sporangia formed on the leaf disk edge observed across a randomly selected 10x field of view of an inverted, light microscope was counted, with two counts made per leaf disk. Sporangia count data were analyzed with the general linear models procedure (PROC GLM), and treatment differences were determined with a

Waller-Duncan K-ratio test (K=100)(SAS Institute, Cary, NC). After leaf disks were retrieved from funnels, the remaining medium was destructively sampled for measurement of pH and KCl-exchangeable Al.

Exchangeable Al and pH determination. Upon retrieval of leaf disks on days 2, 8,15, and 23, the contents of each funnel were placed into plastic bags and mixed thoroughly. Two, 3-g moist subsamples (0.85 g dry weight equivalent) were taken from each bag, placed in glass beakers and frozen for pH and Al analysis. The pH was determined in a 1:2 (wt/vol) suspension of 0.01 M $CaCl_2$ solution. Potassium-exchangeable Al was determined with a modified acid-base titration procedure (35). Each sample was suspended in 25 ml of a 1 M KCl solution for 2 h, filtered through Q5 filter paper (Fisher Scientific), and rinsed three times with 25 ml of 1 M KCl. Ten drops of phenolphthalein solution was added to each extract, and extracts were titrated with 0.1 M NaOH to a pale pink endpoint. A drop of 0.1 M HCl was added to turn the solution colorless, and then 10 ml of 4% (wt/vol) NaF was added. In the presence of Al, a stable fluoroaluminate complex forms and the pink color returns. Then extracts were titrated to a clear endpoint with the addition of 0.1 M HCl. Three moles of of H^+ will titrate 1 mole of exchangeable Al.

Microbial activity. Upon the set up and equilibration of funnels on day 0, a 5.0 g dry weight equivalent was removed from each funnel to monitor basal microbial respiration rates in the various treatments. Microbial activity was assessed using an incubation technique (36) that measures evolution of CO_2 over a 3-week period. The 5.0 g subsamples were placed in plastic beakers and each beaker was set in the bottom of a sealed 2-L Mason jar. Fifty-ml glass beakers, suspended in Mason jars, were used to trap

CO_2 in 5 ml of 0.5 N NaOH. Two jars containing NaOH solution were set up in the absence of medium as "no respiration" controls. Mason jars were incubated at room temperature. On days 1, 7, 14, and 22, glass beakers were removed from Mason jars and beakers containing fresh 0.5 N NaOH were replaced in Mason jars. Carbonate was precipitated from the NaOH solutions with the addition of 5 ml of 0.5 M $BaCl_2$, and then two drops of phenolphthalein were added to each beaker. The residual base (unconsumed by trapping of CO_2) was titrated with 0.1 N HCl to a clear endpoint to indirectly assess CO_2 evolution. Respiration rates were calculated as mg CO_2 g^{-1} medium day^{-1}.

Effects of Al amendment and steaming on pathogen populations. The effects of steaming and Al-amendment of 20% CSW on pathogen populations were monitored at four time points over a period of 23 days. Although similar in design to the experiment focusing on sporangia production, this experiment contains only two factors of Al-amendment: no-Al control, and pH 4 amendment solution at a rate of 0.0079g Al g^{-1} medium. Steaming and Al amendment followed the same methodology as in the sporangia production experiment. A complete factorial treatment design with three replicate funnels of each of four treatments was used at each of four time points. After funnels were set up and equilibrated at -2.5 kPa Ψ_m on day 0, one infested leaf disk was placed at approximately a 0.5 cm depth in each funnel. Funnels for subsequent time points were infested on days 6, 13, and 21. Leaf disks remained in funnels for 24 h before the water potential was raised to 0 kPa with respect to the surface of the media to induce zoospore release (26). Funnels remained flooded for 12 h and then were re-equilibrated at -2.5 kPa for approximately 10 h. Funnels were destructively sampled on days 2, 8, 15, and 23 to determine pathogen population, pH, and K-exchangeable Al.

Furthermore, in the first of two runs of the experiment, the type of propagules giving rise to assayed colonies was determined microscopically on day 2.

Pathogen population was determined using an assay on *Phytophthora*-selective agar medium (PARP) (21) containing 50 μg/ml of hymexazol (Sankyo Co., Tokyo). The contents of each funnel were placed into plastic bags and thoroughly mixed. Three one-g moist samples were taken from each funnel and suspended in 50 ml of deionized water. Each suspension was spread over 10 Petri plates of PARP medium and incubated for 2 days in the dark. Plates were then rinsed with water and colonies of *P. parasitica* var. *nicotianae* were counted. Population data analysis, and pH and K-exchangeable Al measurement, were all completed using the aforementioned techniques and procedures.

The type of propagule giving rise to colonies of *P. parasitica* var. *nicotianae* in the assay was determined by spreading a 3-g suspension of media from each of four treatments in 50 ml water over 10 plates of PARP. After 18 h, the particulates were gently rinsed off the plates with tap water and colonies were observed under an inverted light microscope. Ten colonies were selected at random and the propagule type giving rise to a germ tube was noted.

In the second run of the population experiment, the number of sporangia produced on infested leaf disks buried for 24 h before flooding was also assessed. An additional funnel for each of four treatments was set up for each of four time points. Fifteen infested leaf disks were buried in each funnel on days 0, 6, 13, and 21. After 24 h, leaf disks were retrieved from the media and the number of sporangia produced was assessed using the same technique as in the sporangia production experiment. Sporangia counts

were taken at times corresponding to the concurrent flooding of population experimental funnels.

Soil moisture characteristic. Saturated 20% CSW medium was placed into 15 Büchner funnels and packed to a D_b of approximately 0.18 g cm^{-3}. Plastic bags were placed over each funnel and funnels were brought to saturation for 24 h. Funnels were allowed to equilibrate at 0, -1.0, -2.5, -5.0, and -10.0 kPa Ψ_m for 24 h with respect to the medium surface, with three replicate funnels at each Ψ_m. One sample was removed from each funnel and weighed on an analytical balance. Samples were oven dried at 105˚C for 48 h before dry weights were assessed. Volumetric water content was averaged across the three replicate samples.

RESULTS

Effects of Al amendment and steaming on sporangia production. Both steaming and amendment with Al affected the number of sporangia produced on infested leaf disks that were buried for 48 h (Table 1). Statistical analyses comparing treatments were only conducted within each time point. Sporangia production data from different runs of the experiment could not be combined, however, similar trends were observed in both experimental runs. Amendment with the pH 4 $Al_2(SO_4)_3$ solution consistently reduced sporangia production at early time points. The pH 6 amendment solution did not inhibit sporangia production throughout the 3-week period. Amendment with the pH 4 $Al_2(SO_4)_3$ solution reduced sporangia production at day 2 by approximately 50% in steamed medium in both experimental runs (Fig 1 A and C). The same Al-amendment of unsteamed medium resulted in a 64% and 38% reduction of sporangia production at day 2 over runs 1 and 2 respectively (Fig 1 A and C). In the first run of the experiment,

the Al effect was only observed at day 2. In the second run, however, the Al effect extended to days 8 and 15 (Table 1).

Steamed media consistently resulted in higher numbers of sporangia produced on days 8, 15, and 23 than in the corresponding unsteamed media (Table 1). No steam effect was observed on day 2. In the first run of the experiment, steaming resulted in 50% more sporangia produced in the media unamended with Al and in the pH 6 Al-amendment at day 23 (Fig 1B). The pH 4 Al-amendment resulted in a 24% reduction in sporangia production in the unsteamed media at day 23 (Fig 1B). In the second run of the experiment, sporangia production was reduced by greater than 75% in all unsteamed treatments on day 23 (Fig1 D).

Amendment with Al altered the pH of the media. Unamended media tended to have a pH of approximately 5.7 over the 3-week period (Fig 2). Little difference was observed in pH between steamed and unsteamed media (Fig 2). Amendment with the $Al_2(SO_4)_3$ solution at pH 4 resulted in a lower pH; however, the pH tended to increase over the 3-week period. For example, in the first run of the experiment, the pH of the pH 4 Al-amended media was approximately 5.2 on day 2 and 5.6 on day 23 (Fig 2 A). The pH 6 Al-amended media had a higher pH than the unamended control and the pH also increased from approximately 6.0 to 6.3 over time from day 0 to 23 (Fig 2).

Amendment of 20% CSW with the pH 4 amendment solution resulted in higher levels of K-exchangeable Al than the pH 6 Al-amended media. Amendment with the pH 4 $Al_2(SO_4)_3$ solution resulted in initial extractable Al levels ranging from 3 μM to 7 μM Al/g medium across runs and steam/unsteamed media (Fig 3). In these treatments, Al levels tended to decrease over time, equilibrating at approximately 0.5 μM and 0 μM Al

g^{-1} media at day 23 in runs 1 and 2, respectively (Fig 3). The pH 6 amendment resulted in less than 1 μM Al g^{-1} media at day 2, and 0.5 and 0 μM Al g^{-1} media on day 23 in runs 1 and 2, respectively (Fig 3).

Microbial respiration ranged between 0.2 and 0.6 mg CO_2 g^{-1} medium day^{-1} during days 7-22 over both runs of the experiment (Fig 4). In run 1, however, peak respiration was recorded at day 1 with respiration as high as 1.6 mg CO_2 g^{-1} day^{-1} (Fig 4 A). Furthermore, the steamed media exhibited consistently higher respiration rates than the corresponding unsteamed media at day 1 in the first experimental run (Fig 4 A).

Effects of Al amendment and steaming on pathogen populations. Both Al and steaming affected pathogen populations in funnels containing leaf disks buried for 24 h prior to flooding. Although population data were more variable than sporangia production data, Al significantly reduced pathogen populations on days 2, 8, and 23 in run 1 and on days 2 and 15 in run 2 (Table 2). At each of these points, pathogen populations were reduced by at least 48% compared to the corresponding unamended control. Percent population reduction varied from 48% to 99% at these time points. Furthermore, all of the colonies assayed from the treated media were derived from encysted zoospores.

Steaming resulted in significantly higher pathogen populations on days 2 and 8 in run 1 (Table 2); however, the steam effect was only observed in the unamended (No-Al control) treatments. Because steaming had a consistent effect on sporangia production after infested leaf disks were buried for 48 h, the number of sporangia produced at the time of flooding (24 h) was assessed in the second run of the experiment. The number of sporangia produced on leaf disks buried 24 h (time of flooding) was only affected by the

steam treatment on day 22 (Table 3). In this system, Al reduced sporangia production on days 1, 7, and 14 by 40 to 95% (Table 3), and K-exchangeable Al levels remained above 2 μM Al g^{-1} at the time points corresponding to this suppression (Fig 7 B).

In the population experiments, the pH trends were similar to those in the sporangia production experiments (Fig 6). The K-exchangeable Al levels range between 5 and 9 μM Al g^{-1} at day 2, but equilibrated down to levels ranging from approximately 1 to 5 μM Al g^{-1} at day 23 (Fig 7). After the three week incubation, the flooded funnels of the population study tended to equilibrate at higher levels of Al than the unflooded funnels sampled in the sporangia production experiment (Fig. 3 and 7). Furthermore, in both experiments, the steamed medium tended to have lower levels of K-exchangeable Al than corresponding unsteamed medium for much of the 3-week period (Fig. 3 and 7).

A soil moisture characteristic was generated between 0 and -10 kPa Ψ_m. The percent water-filled pore space at 0, -0.1, -2.5, -5.0 and -10.0 kPa Ψ_m were 100%, 80%, 47%, 44%, and 27% respectively.

DISCUSSION

Both abiotic and biological factors suppressive to *P. parasitica* var. *nicotianae* were incorporated into an organic potting medium containing 20% CSW and an Al amendment. Amendment of the medium with $Al_2(SO_4)_3$ solution at pH 4 consistently inhibited sporangia production on day 2, whereas the pH 6 amendment was ineffective at suppressing the pathogen. The differential effect of the two amendment solutions may be related to the heightened overall level of K-exchangeable Al in the pH 4 amendment (> 3 μM g^{-1}) as compared to the pH 6 amendment (< 1 μM g^{-1}). Similar amendment of peat with $Al_2(SO_4)_3$ solution at pH 6 consistently suppressed populations of *P. parasitica* var.

nicotianae at K-exchangeable Al levels at 1 μM g^{-1} peat (13); however, the initial peat amendment contained twice the level of Al used in the present study.

The lower level of K-exchangeable Al in 20% CSW amended with the pH 6 amendment solution is likely related to three interrelated, pH-dependent phenomena: i) lower activity of Al in the amendment solution, ii) heightened affinity of organic matter for Al, and iii) stronger affinity of hydrolyzed Al ions for charged groups on the organic matter. The pH 6 amendment solution has a lower activity of Al in solution than the pH 4 amendment solution due to the pH-dependent formation of an $Al(OH)_3$ precipitate. In these respective pH 4 and pH 6 amendment solutions, the total amount of soluble Al with respect of gibbsite would be 10^{-3} M and $10^{-6.3}$ M (28); however, the precipate may also have been a more soluble Al-bearing mineral. The higher pH (6.0-6.4) of the medium amended with the pH 6 amendment, as compared to the pH 4 amendment (5.1-5.7), increases the affinity of the organic matter for Al by increasing the surface charge of the medium. Furthermore, the distribution of monomeric Al species is different in each amendment solution, with the pH 6 solution containing a higher proportion of the $Al(OH)_2^+$ ion (28). This monovalent hydrolysis species forms stronger complexes with charged organic functional groups than the free ionic Al^{3+} (5) that dominates in the pH 4 amendment solution (28).

In both the experiments pertaining to sporangia production and pathogen populations, the levels of K-exchangeable Al decreased over time. Over both runs of the sporangia production experiment, the K-exchangeable Al levels of each treatment equilibrated to the same point on day 23. Because media amended with the two amendment solutions equilibrate at different pH values, this suggests that Al activity in

solution was maintained in equilibrium with respect to organic matter and not pH. In systems at equilibrium, aluminum activity is always governed by the process maintaining the lowest activity of Al^{3+} in solution.

The pH in Al-amended media increased by approximately a half a unit between day 2 and day 23. This increase in pH may be attributed to a loss of Al from solution; however, the fate of Al in the system is unknown. Aside from losses of Al due to precipitation and complexation, other interactions of Al with the pathogen and microbial community are possible. For example, it is unknown whether the pathogen or other microbial flora are accumulating Al or producing organic acids to complex and sequester the metal.

Al-mediated inhibition of sporangia production was mainly observed at early time points after amendment; however, Al continued to affect pathogen populations up to 23 days after amendment. Although pathogen population data were more variable than sporangia counts, the prolonged effect of Al on pathogen populations may be related to the heightened levels of K-exchangeable Al at these time points. Al-mediated suppression of pathogen populations always occurred at K-exchangeable Al levels above 2 μM Al g^{-1} medium. The media assayed for pathogen populations had been flooded for 12 h to induce zoospore release from sporangia; consequently, these funnels exhibited higher levels of K-exchangeable Al on day 23 than the corresponding unflooded funnels used in the sporangia production experiment. Aluminum reduced sporangia production through day 15 in run 2 of the population experiment. Interestingly, the suppressive medium contained > 2 μM Al g^{-1} through day 15 in run 2. Thus, the threshold for Al-toxicity to *P. parasitica* var. *nicotianae* in organic media may be in the range of 2 μM Al

g^{-1} of medium. The effect of fluctuating water potential on Al availability in the system has not been directly addressed. The results of these studies, however, suggest that a periodic increase in the water potential of the system may prolong the Al-mediated suppression of *P. parasitica* var. *nicotianae*.

In the 20% CSW medium, the Al-mediated inhibition of sporangia production was directly observed by retrieval and observation of infested leaf disks. Although zoospores were the only type of propagule giving rise to colonies assayed from the treated media, we did not determine whether Al was directly toxic to zoospores. In the second run of the population experiment, it was determined that Al reduced the number of sporangia produced on leaf disks buried for 24 h prior to flooding (Table 3). Consequently, the noted Al affect on pathogen populations may be the result of suppression of sporangia production as well as a direct toxicity to zoospore production or viability.

The effect of steaming on the ability of *P. parasitica* var. *nicotianae* to produce sporangia suggests that the 20% CSW incites a biologically-mediated suppression. The time-sensitivity of the steam effect on sporangia production suggests that beneficial microbial populations are not initially active in the 20% CSW medium. The burst of respiration in steamed medium on day 1 (no Al-control) in the first run of the sporangia production experiment suggests a possible initial burst of activity of endospore-forming bacteria. Diab (12) had noted high populations of these organisms in the CSW-amended medium. Biologically-mediated suppression of *P. parasitica* var. *nicotianae* was consistently observed at day 8, 15, and 23. Although microbial communities in natural soils may be sensitive to Al (20), the lack of a steam effect on day 2 in media that was not

amended with Al suggests that Al is not the only factor limiting beneficial microbial activity. Beneficial microorganisms may require time to reach threshold population densities in order to exact pathogen suppression. Because total microbial activity, estimated by respiration rates, does not correlate with suppression, it is possible that particular populations of organisms are responsible for suppression of *P. parasitica* var. *nicotianae* in this system.

Diab et al (12) documented that this CSW-amended medium suppressed damping-off of impatiens caused by *R. solani*, and the degree of suppression was directly correlated with total microbial activity. In the *R. solani* system, the length of compost curing positively correlated with disease suppression, and the uncomposted swine waste was conducive to disease (12). The high microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) suggest a high resident population of saprophytic organisms in the CSW medium (12). The medium also contained high population densities of fungi, endospore forming bacteria and oligotrophic bacteria (12).

Media containing composted materials may offer multiple mechanisms of biological suppression, thus targeting a wide range of plant pathogens. For example, medium containing composted sewage sludge was suppressive to *Aphanomyces euteiches*, *Sclerotinia minor*, and *R. solani*, whereas a medium containing bark compost was suppressive to *P. cinnamomi*, *Pythium* sp., *R. solani*, and *Thielaviopsis basicola* (25). In general, the mechanisms affecting biological control of *Pythium* sp. and *Phytophthora* sp. in compost-amended media are different than those affecting control of *R. solani* (18). A narrow range of organisms is responsible for suppression of *Rhizoctonia* in compost-amended substrates (10). Perhaps for this reason, only around 20% of composts suppress

Rhizoctonia damping-off, whereas most composts naturally suppress Pythium and Phytophthora root rots (17). Suppression of *R. solani* has been related to the presence of resident strains of *Trichoderma* spp. and *Gliocladium* spp. (10) and low levels of available cellulose (11). Unlike *Rhizoctonia*, *Pythium* and *Phytophthora* are considered to be nutrient-dependent pathogens. Nutrient-dependent pathogens are subject to a more general form of suppression, with many organisms contributing to biological suppression in compost-amended media (18). The heightened microbial activity in these compost-amended media prevents germination of pathogen propagules through microbiostasis resulting from nutrient competition (18, 27). Because the CSW-amended media offers biological control of *Rhizoctonia* (12) and *Phytopthora*, it is possible that this medium provides multiple mechanisms of pathogen suppression.

Steaming had less of an effect on pathogen populations than on sporangia production. A steam effect on pathogen populations was only observed on days 2 and 8 in the first run of the population experiment. Because the infested leaf disks were only submerged in the treated media for 24 h prior to flooding, additional data were collected in the second run of the experiment to determine whether Al or steaming influenced the number of sporangia available at the time of flooding. Steaming had no effect on sporangia production on days 1, 7, and 14. Therefore, biologically-mediated suppression of sporangia production may require more time to occur than the 24 h that the leaf disks were submerged. It is also possible that the steam effect on sporangia production observed on day 22 was related to the presence of a population of microflora offering a different or faster mechanism of control. In general, biologically mediated suppression of sporangia production was more consistent on leaf disks buried for 48 h than on those

buried for only 24 h. The efficacy of longer duration of pathogen contact with the media suggests that a nutrient-based competition or microbiostasis may be a predominant mechanism of biocontrol. Future studies should be designed to address the influence of duration of contact between the pathogen and the compost-amended media on pathogen suppression.

In this system there was some evidence that Al interacted with the microbial community and inhibited populations of beneficial microbes. For example, in the population experiment, the steam effect was observed only in media that was not amended with Al. We speculate that Al inhibited the ability of beneficial microbes to compete for nutrients in the system. This hypothesis is supported by the work of Illmer et al (20), where Al was the main factor inhibiting soil microbial biomass and nitrogen mineralization in forest soils.

In summary, potting medium amended with both 20% CSW and $Al_2(SO_4)_3$ offers both biological and abiotic suppression of *P. parasitica*. Both the biological and the abiotic factors inhibit sporangia production; however, the two factors may be active at different times. Aluminum inhibited sporangia production on day 2, whereas the biologically-mediated suppression of sporangia production persisted from days 8-23. Al-amendments may be effective at protecting the plant before beneficial microbial populations reach a threshold necessary for suppression. The effect of Al on the background microbial community and the influence of water potential on Al availability should be the subject of future studies.

In addition to suppression of *Phytophthora*, Diab (12) has documented the ability of CSW-amended medium to suppress damping-off caused by *R. solani*. Because

biological control mechanisms of these two pathogens in compost-amended media are reportedly different (18), the CSW-amended medium may offer multiple mechanisms of biological control. Furthermore, additions of compost to peat-based media may enhance its ability to support an introduced biological control agent. The incorporation of abiotic and biological control mechanisms may render this medium suppressive to a wide range of soilborne plant pathogens and increase its applicability in a greenhouse-based disease management strategy.

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Table 1. Average number of sporangia[w] of *Phytophthora parasitica* var. *nicotianae* produced on edges of infested leaf disks buried for 48 h in steamed[x] and unsteamed 20% CSW amended with $Al_2(SO_4)_3$ solutions at pH 4 and 6[y].

Run 1	Steam-control	Unsteam-control	Steam-pH 4	Unsteam-pH 4	Steam-pH6	Unsteam-pH 6	significant factors ($P \leq 0.05$)
Day 2	26 a[z]	19 abc	14 cd	7 d	22 ab	18 bc	Al
Day 8	20 a	7 c	19 a	11 b	20 a	8 bc	steam
Day 15	28 a	9 b	26 a	8 b	27 a	8 b	steam
Day 23	23 a	11 c	22 a	17 b	24 a	12 c	steam
Run 2							
Day 2	9.8 b	6.6 b	4.4 c	4.1 c	17.8 a	16 a	Al
Day 8	11.6 a	3.2 b	5.5 b	3.1 b	9.5 a	4.7 b	steam; Al
Day 15	20.8 a	7.9 c	18.9 ab	8.4 c	15.1 b	5.9 c	steam; Al
Day 23	5.9 a	0.8 b	4.7 a	1.2 b	5.0 a	1.1 b	steam

[w] Sporangia were counted on infested leaf disks buried for 48 h. The number of sporangia produced per 1.2 mm of leaf disk edge was ascertained by counting those across a 10x field of view under the light microscope.

[x] Steamed treatments were placed in a steamer for 30 min at 98.6 C on two consecutive days.

[y] Medium was amended with 0.0079 g Al/g medium as a 0.05 M $Al_2(SO_4)_3$ solution adjusted to either pH 4 or 6 with 1.0 N KOH.

[z] Values followed by different letters within a row indicate significant differences ($P \leq 0.05$).

Table 2. Average number of propagules[w] of *Phytophthora parasitica* var. *nicotianae* per g of steamed[x] and unsteamed 20% CSW amended with $Al_2(SO_4)_3$ solution at pH 4[y].

Run 1	Steam-control	Unsteam-control	Steam-pH 4	Unsteam pH 4	significant factors (P≤0.05)
Day 2	133.6 a[z]	74.4 b	27.6 c	6.8 c	steam; Al
Day 8	150.0 b	354.6 a	72.6 b	183.9 ab	steam; Al
Day 15	159.4 a	130.2 a	21.5 a	124.4 a	-
Day 23	139.4 ab	464.6 a	10.2 b	5.3 b	Al
Run 2					
Day 2	275.3 ab	325.2 a	41.1 b	27.2 b	Al
Day 8	90.6 a	75.6 a	45.7 a	58.8 a	-
Day 15	11.2 a	6.7 ab	0.1 b	3.2 b	Al
Day 23	10.2 a	5.0 a	0.7 a	6.3 a	-

[w] Assays of pathogen populations were conducted after infested leaf disks were buried 24 h, funnels were flooded 12 h, then reequilibrated at 25 cm SMT for 12 h.

[x] Steamed treatments were placed in a steamer for 30 min at 98 C on two consecutive days.

[y] Medium was amended with 0.0079 g Al/g medium as a 0.05 M $Al_2(SO_4)_3$ solution adjusted to pH 4 with 1.0 N KOH.

[z] Different letters across rows designate significant differences based on the Waller-Duncan K-ratio test (K=100).

Table 3. Average number of sporangia[w] of *Phytophthora parasitica* var. *nicotianae* produced on edge of infested leaf disks buried for 24 h in steamed[x] and unsteamed 20% CSW amended with $Al_2(SO_4)_3$ solution at pH 4[y].

Run 2	Steam-control	Unsteam-control	Steam-pH 4	Unsteam-pH 4	significant factors ($P \leq 0.05$)
Day 1	19 a[z]	20 a	4 b	1 b	Al
Day 7	10 a	8 ab	5 bc	2 c	Al
Day 14	20 a	14 ab	12 b	13 b	Al
Day 22	17 a	6 b	17 a	4 b	steam

[w] Sporangia were counted on infested leaf disks buried for 48 h. The number of sporangia produced per 1.2 mm of leaf disk edge was ascertained by counting those across a 10x field of view under the light microscope.

[x] Steamed treatments were placed in a steamer for 30 min at 98.6 C on two consecutive days.

[y] Medium was amended with 0.0079 g Al/g medium as a 0.05 M $Al_2(SO_4)_3$ solution adjusted to pH 4 with 1.0 N KOH.

[z] Values followed by different letters within a row indicate significant differences ($P \leq 0.05$).

FIGURE LEGENDS

1. Number of sporangia produced on the edge of infested leaf disks buried 48 h in steamed and unsteamed 20% CSW medium amended with $Al_2(SO_4)_3$ solutions at pH 4 and 6. **A** and **B** represent sporangia counts on days 2 and 23, respectively in the first run of the experiment; **C** and **D** represent sporangia counted on days 2 and 23 in the second run of the experiment. Different letters above bars designate significant differences based on the Waller-Duncan K-ratio test (K=100).
2. pH of steamed and unsteamed 20% CSW amended with $Al_2(SO_4)_3$ solutions at pH 4 and 6 over time. Samples were taken from funnels containing infested leaf disks that were buried for 48 h intervals and then removed at each time point to assess sporangia production. **A** and **B** represent run 1 and run 2 of the experiment.
3. K-exchangeable Al in steamed and unsteamed 20% CSW medium amended with $Al_2(SO_4)_3$ solutions at pH 4 and 6 over time. Samples were taken from funnels containing infested leaf disks that were buried for 48 h intervals and then removed at each time point to assess sporangia production. **A** and **B** represent run 1 and run 2 of the experiment.
4. Microbial respiration rates in steamed and unsteamed 20% CSW medium amended with $Al_2(SO_4)_3$ solutions at pH 4 and 6. Respiration rates were determined 24 h after infested leaf disks were buried in corresponding funnels for a 48 h interval. **A** and **B** represent runs 1 and 2 of the experiment.
5. Number of sporangia produced on the edge of infested leaf disks buried 24 h in steamed and unsteamed 20% CSW medium amended with 0.05 M $Al_2(SO_4)_3$ solution at pH 4. **A**,**B**,**C**,**D** represent sporangia counts on days 2, 8, 15, 23, respectively, in run

2 of the experiment. Different letters above bars designate significant differences based on the Waller-Duncan K-ratio test (K=100).

6. pH of steamed and unsteamed 20% CSW medium amended with 0.05 M $Al_2(SO_4)_3$ solution at pH 4. Samples were taken from funnels containing infested leaf disks that were buried for 24 h before funnels were flooded. **A** and **B** represent run 1 and run 2 of the experiment.
7. K-exchangeable Al in steamed and unsteamed 20% CSW medium amended with 0.05 M $Al_2(SO_4)_3$ solution at pH 4. Samples were taken from funnels containing infested leaf disks that were buried for 24 h before funnels were flooded. **A** and **B** represent run 1 and run 2 of the experiment.

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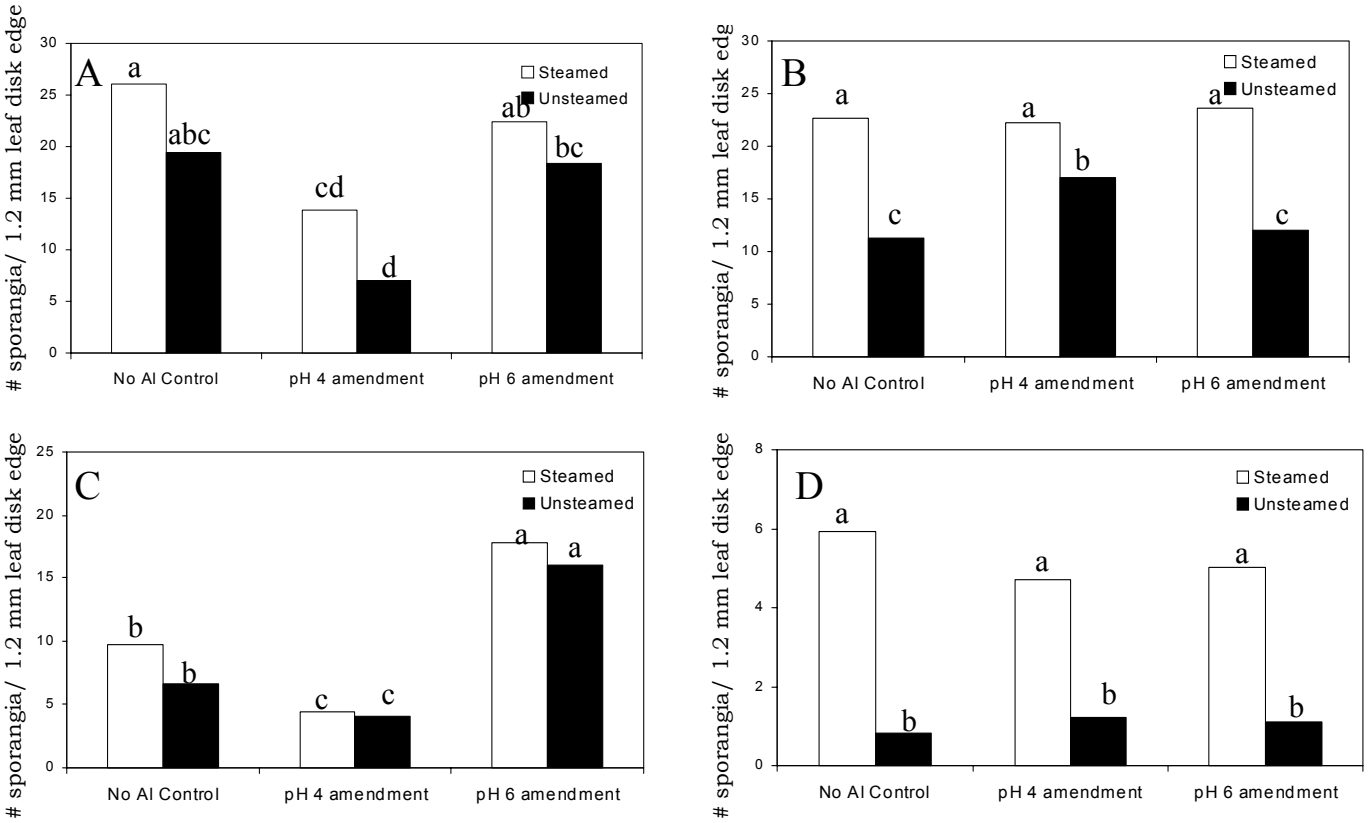

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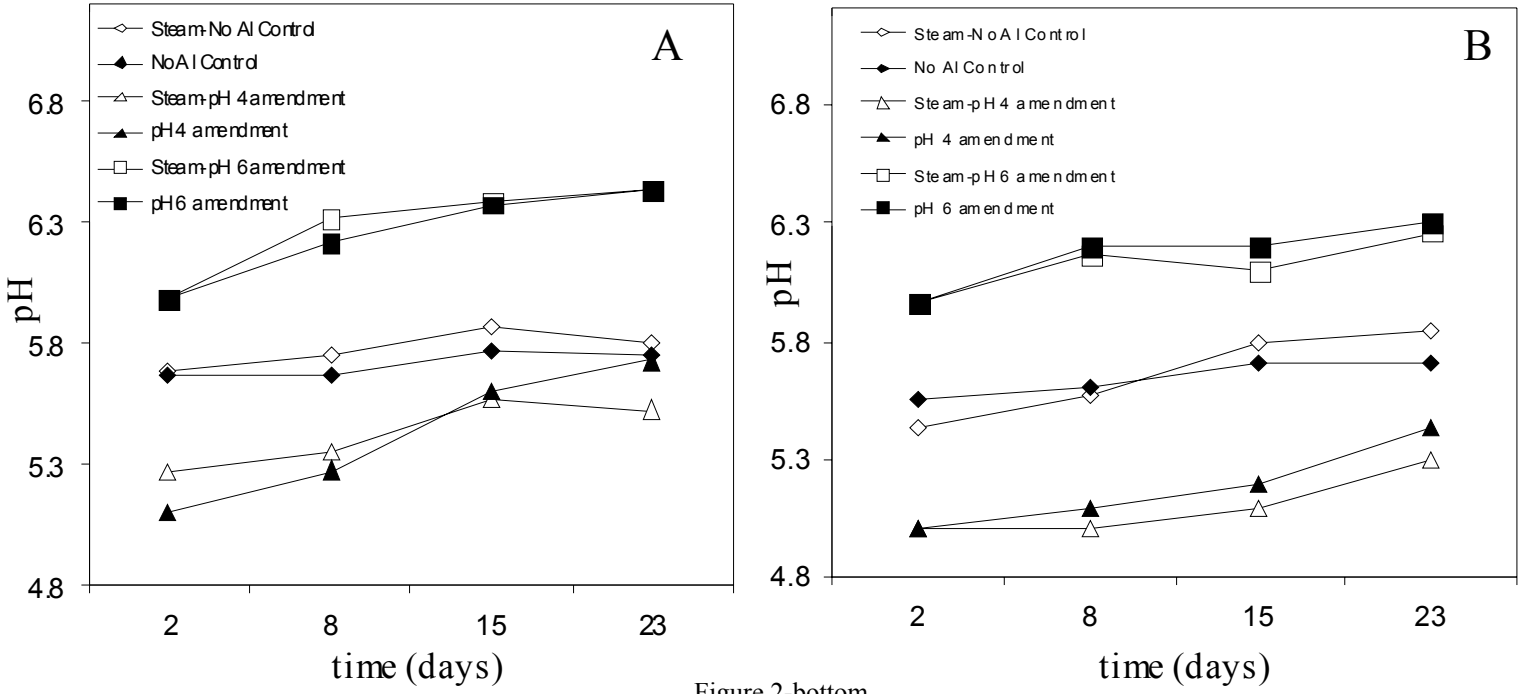
Figure 2-top



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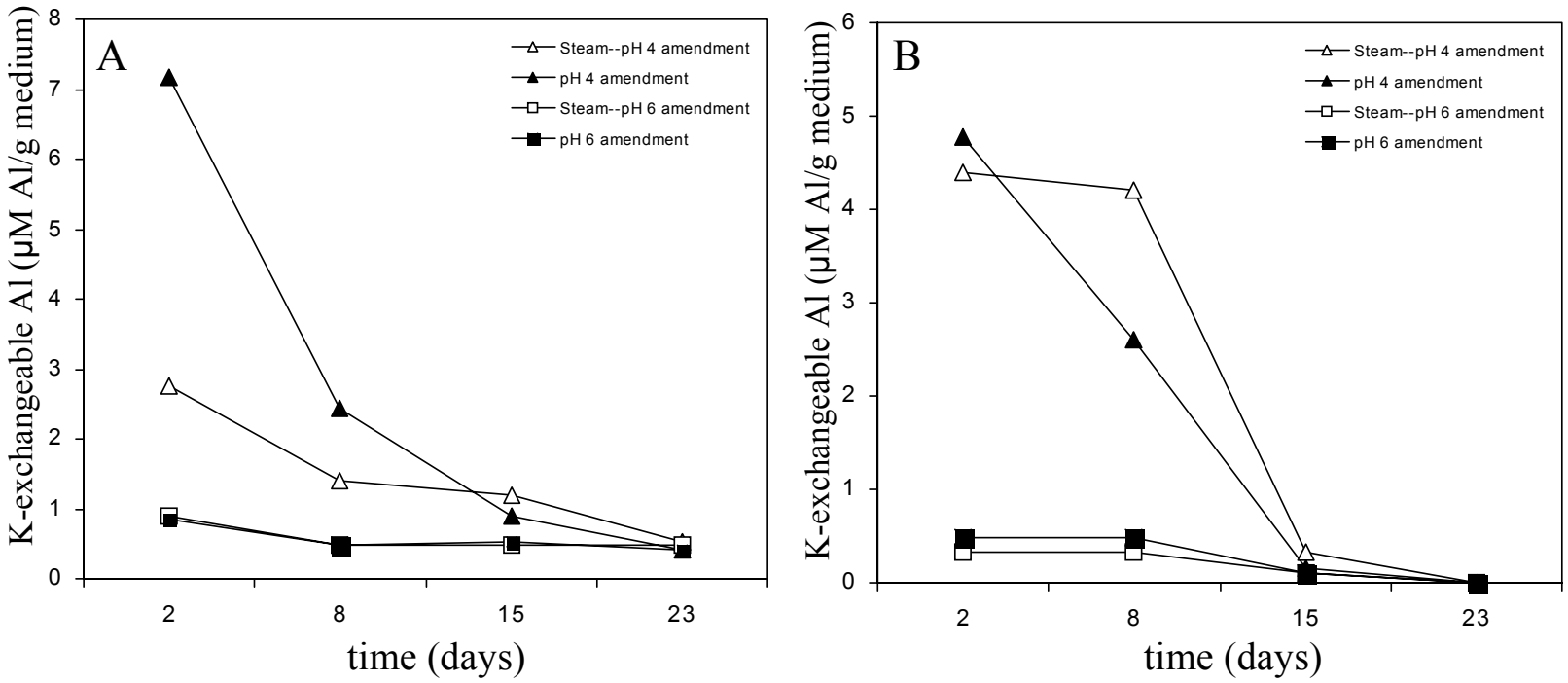

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Figure 4-top

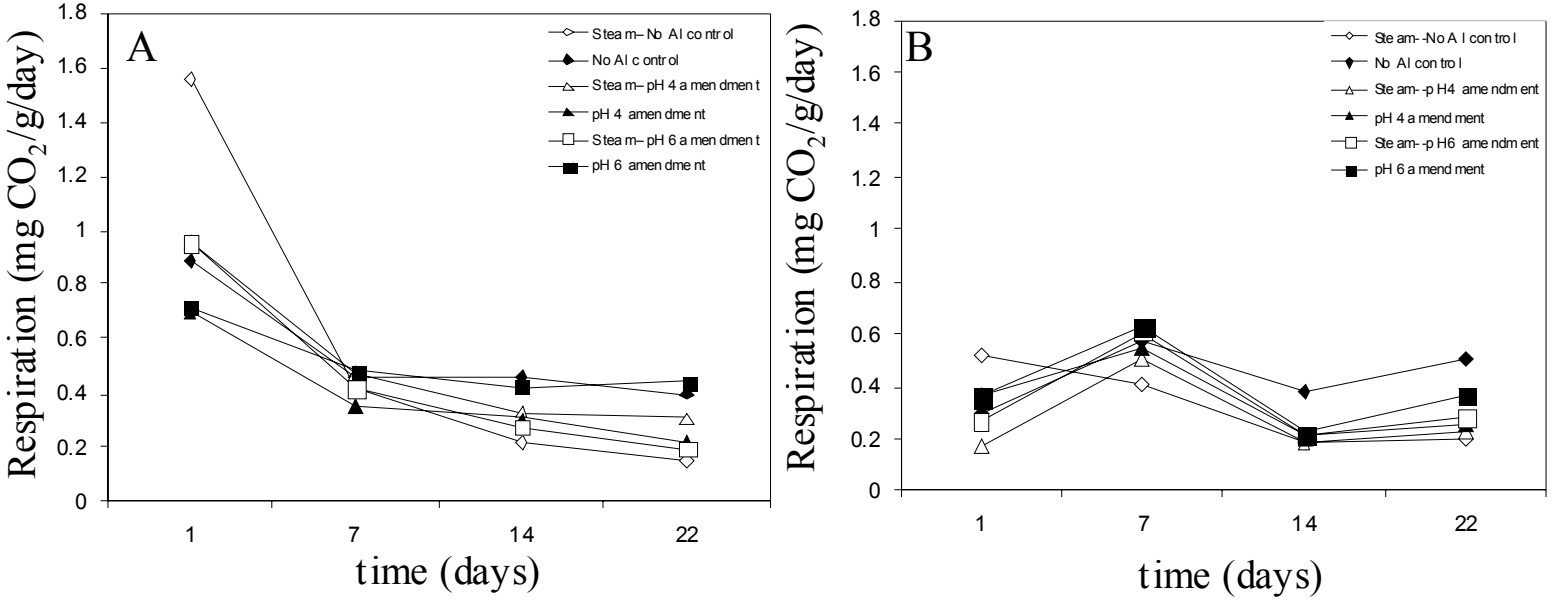

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Figure 5-top

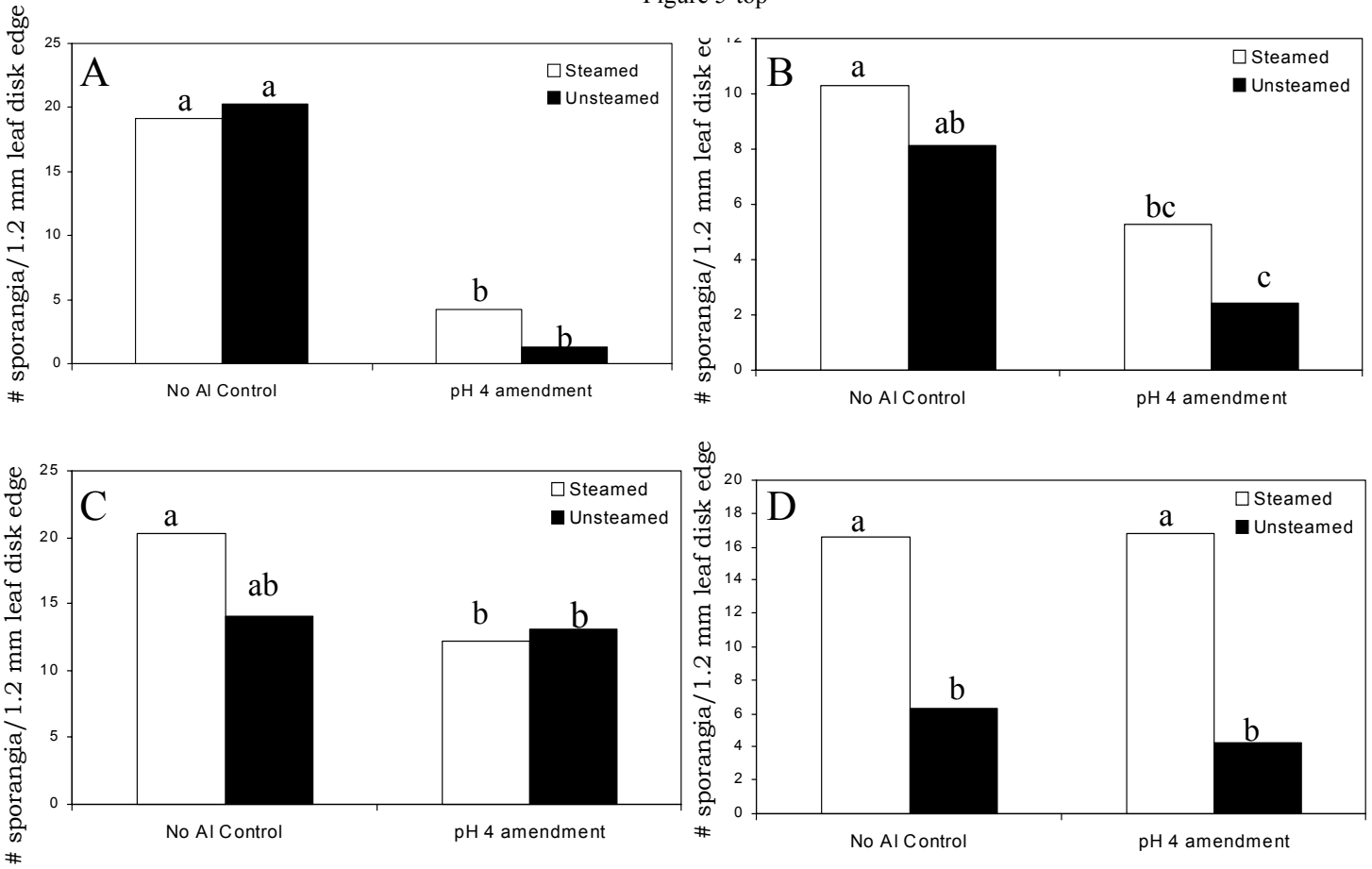

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Figure 6-top

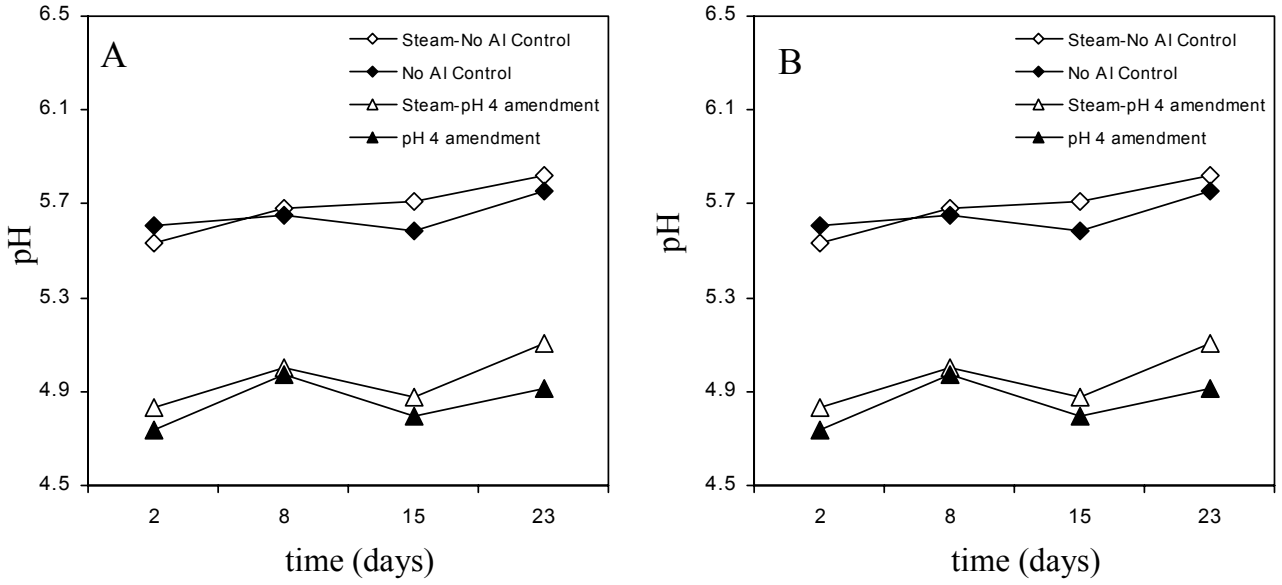

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Figure 7-top

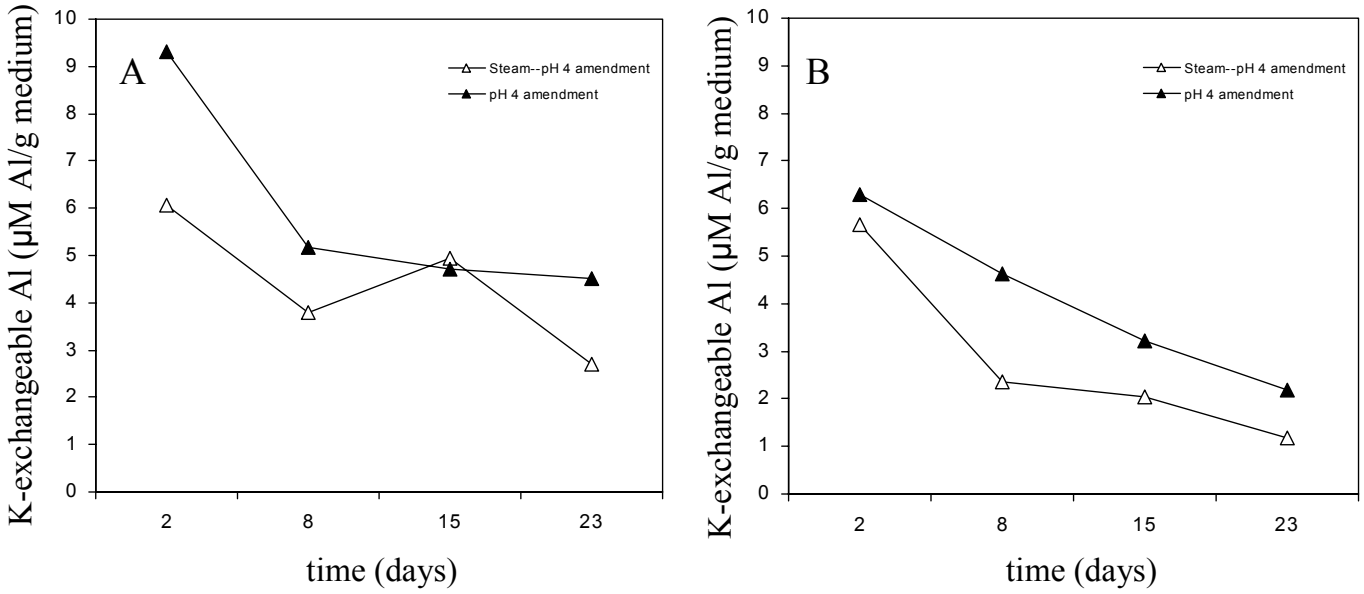

Figure 7-bottom

Chapter 4: Differential sensitivity of *Phytophthora parasitica* var. *nicotianae* and *Thielaviopsis basicola* to monomeric Al species.

Differential sensitivity of *Phytophthora parasitica* var. *nicotianae* and *Thielaviopsis basicola* to monomeric aluminum species

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ABSTRACT

E. J. Fichtner, D. L. Hesterberg, T. J. Smyth, and H. D. Shew. 2003. Differential sensitivity of *Phytophthora parasitica* var. *nicotianae* and *Thielaviopsis basicola* to monomeric Al species. Soil Sci. Soc. Am. J. 67:XXX-XXX.

Aluminum (Al) is toxic to many plant pathogens, including *Thielaviopsis basicola* and *Phytophthora parasitica* var. *nicotianae*. Because fungitoxicity of Al has been described in soils over a wide pH range, multiple species of Al may be responsible for pathogen suppression. The goals of this work were to i) determine the sensitivity of *T. basicola* and *P. parasitica* var. *nicotianae* to Al over a range of pH values, ii) quantify the toxicity of monomeric Al species to production of sporangia of *P. parasitica* var. *nicotianae* and chlamydospors of *T. basicola* and ii) to detect the accumulation of Al in pathogen tissues. A complete factorial treatment design was used with Al levels ranging from 0-100 μM and pH levels ranging from pH 4-6 in a minimal salts medium. The chemistry of test solutions was modeled using GEOCHEM-PC. Colonies were grown in 5% carrot broth.

After one or two days, the nutrient solution was removed, colonies were rinsed with water, and Al test solutions were added to each of four replicate plates. After two days, propagules were counted and colonies were stained with an Al-specific, fluorescent stain. Inhibition of chlamydospore production was observed at pH values < 5.0 and at Al levels > 20 μM. Sporangia production was inhibited at Al levels as low as 2 μM across all pH values tested. The lumogallion stain was an effective technique for detection of Al in fungal tissues. Aluminum accumulated in sporangia and zoospores of *P. parasitica* var. *nicotianae* and in non-melanized chlamydospores of *T. basicola*. The oomycete *P. parasitica* var. *nicotianae* was sensitive to a range of monomeric Al species, whereas sensitivity of *T. basicola* to Al was pH dependent, suggesting that Al^{3+} is responsible for suppression of this fungal pathogen.

Acid soil chemistry and the associated aluminum (Al) toxicity to both micro- and macroflora have been the subject of many interdisciplinary studies emphasizing the dynamic interactions between the soil chemical environment and biological communities in natural and agro-ecosystems (12,24). Approximately 40% of the earth's arable lands are located on acid soils, and phytotoxicity of Al is one of the major factors limiting crop productivity in acid agricultural soils (28). Aluminum is also one of the major factors limiting microbial activity in acid soils. For example, an Al-mediated reduction of soil microbial biomass and nitrogen mineralization has been observed in acid forest soils (18).

In some agricultural soils, abiotic suppression of plant pathogens has been related to the toxicity of Al to soilborne fungi and bacteria. Aluminum-mediated suppression of plant diseases has been reported in a number of pathosystems, including potato scab

caused by the Actinocycete *Streptomyces scabies* (35), Verticillium wilt of eggplant (16,19,39), Rhizoctonia damping-off (27), root and bulb rots caused by *Fusarium sp.* (14,46), and potato late blight caused by *Phytophthora infestans* (2). Acid soils in North Carolina confer Al-mediated suppression of two diseases of tobacco: black shank caused by *P. parasitica* var. *nicotianae* (syn. *P. nicotianae* var. *nicotianae*) (7,42), and black root rot caused by *Thielaviopsis basicola* (syn. *Chalara elegans*) (31-34). In both pathosystems, suppressive soils had a characteristic low pH and high levels of exchangeable Al, conditions promoting high Al^{3+} activity. Suppression of *Phytophthora parasitica*, however, also was observed in Al-amended, horticultural potting mixes at pH 5.2 and low levels of exchangeable Al (4,5). Further investigation of Al sensitivity of *P. parasitica* in an Al-amended peat system demonstrated suppression of pathogen populations at pH > 5.2 and 1 μM Al g^{-1} peat (13). In the peat system, the predicted dominant monomeric Al species was $Al(OH)_2^+$, suggesting that Al-hydrolysis species may play a role in toxicity of Al to *P. parasitica*. Similarly, Al-mediated inhibition of lateral root length of soybean was correlated with the sum of monomeric Al species [Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$, $Al(OH)_3^0$, and $Al(OH)_4^-$] activities (41).

The physiological mechanisms of Al toxicity to soilborne plant pathogens and the relative toxicity of monomeric Al species to these pathogens is unknown. The activities of monomeric Al species is pH-dependant; therefore the baseline sensitivity of pathogens to Al must be documented over a wide pH range, generally bracketing the conditions where Al-mediated suppression is observed in the field. *Thielaviopsis basicola* and *P. parasitica* var. *nicotianae* were selected for basic toxicological studies because suppression of these pathogens has been observed under diverse soil chemical conditions.

Furthermore, although both are pathogens of tobacco, the two organisms are taxonomically unrelated, and may exhibit different mechanisms of Al tolerance or sensitivity.

Physiological studies addressing the mechanisms of Al toxicity to plant cells have documented the localized accumulation of Al in root apices using lumogallion (3-[2,4 dihydroxyphenylazo]-2-hydroxy-5-chlorobenzene sulfonic acid), an Al-specific fluorescent stain (17,22,23,43-45). Lumogallion also has been utilized to correlate Al accumulation with growth inhibition of soybean roots (23), and to illustrate localized Al accumulation at plant cell nuclei (43). Lumogallion has also been applied to the study of mechanisms of Al tolerance in soybean (44). Application of a technique for fluorimetric detection of Al in pathogen tissue would facilitate the understanding of Al toxicity to microorganisms and the role of Al in disease-suppressive soils.

The overall goal of this project was to investigate the fundamental mechanisms of Al toxicity to two soilborne plant pathogens. Specific objectives included: i) the determination of baseline sensitivity of *T. basicola* and *P. parasitica* var. *nicotianae* to Al over a range of pH, ii) the investigation of differential toxicity of monomeric Al species to *P. parasitica* and *T. basicola*, and iii) the identification of regions oflocalized Al accumulation in *T. basicola* and *P. parasitica* var. *nicotianae* tissues using a new fluorimetric technique.

MATERIALS AND METHODS

Sensitivity of *T. basicola* to aluminum level and pH. An isolate of *T. basicola* was obtained from diseased roots of pansy (*Viola* x *wittrokiana*) and used in all experiments. Cultures of the fungus were grown for 4 days on 5% carrot agar, and a

spore suspension was collected by rinsing endoconidia from the agar surface with sterile, deionized water. A 7 ml aliquot of 5% carrot broth and a 1 ml aliquot of the spore suspension were pipetted into each of 125 Petri plates (60 x 15 mm). Nylon mesh, 100 μm pore diameter (Sefar America Inc., Depew, NY), was cut into 4 cm^2 segments, autoclaved, and one piece of sterile mesh was submerged in the broth medium in each plate. The plates were incubated for 48 h at room temperature to allow for mycelial colonization of the mesh segment. After 48 h the carrot broth was aspirated off each plate under a laminar flow hood and colonies were rinsed twice with sterile, distilled water. After the water was aspirated off each plate, 8 ml of an Al-test solution were added to each plate. Using a complete factorial treatment design, colonies were treated with Al-test solutions at five pH levels (4.0, 4.5, 5.0, 5.5, 6.0) and five Al levels (0, 10, 20, 50, 100 μM Al), totaling 25 treatments with five replicate plates of each treatment. Colonies were incubated in Al-test solutions for 48 h at room temperature, and then chlamydospore production was assessed. The number of chlamydospores observed in four randomly selected 10 x fields of view (1.4 mm^2) of an inverted light microscope was counted utilizing the nylon mesh as a grid. Test solutions from replicate plates of each treatment were then combined and the final pH was measured in the bulked solutions. A subset of the mycelial samples was saved for staining with lumogallion. The experiment was run twice.

Aluminum-test solutions were prepared with half strength minimal salts (MS) medium (0.5 g $K_2HPO_4 \cdot 3H_2O$, 0.25 g KCl, 0.25 g $MgSO_4$, 0.005 g $FeSO_4 \cdot 7H_2O$, 0.5 g $(NH_4)_2SO_4$, 0.22g $CaCl_2$, 0.0005 g thiamine hydrochloride). Because one goal of the project was to document the pathogen ability of the pathogen to convert stored resources

into resting structures, the MS medium was prepared without a carbon source. The five Al levels were established in the MS background solution by the addition of $Al_2(SO_4)_3$. Within each of the five Al levels, the five pH levels were established with the addition of 1 N KOH. Aluminum-test solutions were filter sterilized through a 500 ml bottle top filter with 0.2 μm pore diameter.

The program GEOCHEM-PC (40) was used to determine the chemical speciation of Al test solutions. The individual molar concentrations of cations and anions were calculated and recorded as the – log of the ion concentration. For 0 μM Al control treatments, the – log [Al] was assigned the arbitrarily low value of 12 to avoid undefined functions. Thiamine hydrochloride was excluded from the speciation analysis because it is not in the GEOCHEM-PC database and the quantity was negligible with respect to the total amount of Al in the system. Speciation of test solutions was determined for each treatment with pH entered as the initial pH established in test solutions. The initial pH was chosen over final pH for GEOCHEM-PC analysis because the goal was to assess Al sensitivity of the pathogen upon the removal of nutrients from the system (ie. introduced stress). In the GEOCHEM-PC analysis of test solutions, simple solid phases were allowed but not imposed, the pH was fixed, the convergence criterion was 1.00E-04, the maximum number of iterations was 50, and the input of estimated ionic strength as 3.600E-02. The activities of Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^{+}$, $Al(OH)_3^{0}$, $Al(OH)_4^{-}$ were recorded for each treatment and the sum of these activities was used to create another variable ΣAl_{mono}. For input into SAS (SAS Institute, Cary, NC), Al-hydrolysis species activities in the 0 μM Al treatments were given an arbitrarily low value of 7.04×10^{-14}, then all species activities were multiplied by 10^8 for simplification. A stepwise regression

was used to correlate spore production with the activities of monomeric species within each pH level. Only monomeric Al species at the 0.15 significance level were entered into the regression model. This statistical technique was not employed to distinguish differences across pH levels because of the intrinsic correlation of Al species activities with pH. An analysis of variance, however, was used to view main effects and interactions of pH and Al level on chlamydospore production.

Sensitivity of *P. parasitica* var. *nicotianae* to aluminum level and pH. A race 0 isolate of *P. parasitica* var. *nicotianae* was used in all tests. This isolate was obtained from infested soil in Duplin Co., NC and was provided by Melinda Sullivan, N.C. State University, Raleigh, NC. After growth for one week on oatmeal agar (Difco Laboratories, Detroit), the mat of aerial hyphae was scraped from the agar surface with a surface sterilized, double-edge razor blade. The hyphal mats from four Petri plates were placed in a sterile blender with 500 ml of sterile, deionized water and the mixture was blended for 10 seconds. A 1 ml aliquot of the blended hyphal suspension was pipetted into each of 100 Petri plates (60 x 15 mm), along with 7 ml of 5% carrot broth and the addition of a 4 cm^2 nylon mesh segment. Colonies of *Phytophthora parasitica* var. *nicotianae* remained in the carrot broth for 24 h at room temperature and were then rinsed twice with sterile, deionized water. The water was aspirated from each plate and 8 ml of an Al-test solution was added. Using a complete factorial treatment design, colonies were exposed to Al-test solutions at four pH values (4.0, 4.5, 5.0, 5.5) and five Al levels (0, 2, 5, 10, 33 μM Al), with five replicate plates prepared for each treatment. Aluminum test solutions were prepared in the same manner as above except the $(NH_4)_2SO_4$ was excluded from test solutions. Colonies were incubated in Al-test solutions for 48 h at

room temperature, and then sporangia production was assessed. The number of sporangia observed in four randomly selected 10 x fields of view (1.4 mm^2) was recorded for each replicate plate. The Al test solutions from replicate plates were then combined within each treatment and the final pH was measured in the bulked solutions. A subset of mycelium was saved for staining with lumogallion. The experiment was run twice.

GEOCHEM-PC was also used to model Al test solutions used in the sporangia production experiment. An analysis of variance was used to determine main effects and interactions of Al level and pH and treatment differences were determined with a Waller-Duncan K-ratio test (K=100).

Dose-Response curve for *P. parasitica* var. *nicotianae*. Because of the high sensitivity of *P. parasitica* to Al, a dose-response study addressing the effect of Al level on sporangia production was conducted at pH 4.5. A 1 ml aliquot of a hyphal suspension and 7 ml of 5% carrot broth were pipetted into each of 20 Petri plates (60 x 15 mm). After incubating for 24 h at room temperature, the cultures were rinsed twice with sterile distilled water and then bathed for 48 h in Al test solutions. Aluminum test solutions were prepared in half strength MS medium excluding glucose and $(NH_4)_2SO_4$, and adjusted to 0, 0.2, 0.5, and 1.0 μM Al with the addition of $Al_2(SO_4)_3$. The pH of test solutions was adjusted to 4.5 with the addition of 1 N KOH before filter sterilization. After colonies were bathed 48 h in test solutions, the number of sporangia observed in four randomly selected 10 x fields of view under an inverted light microscope in five replicate plates of each treatment was recorded. The experiment was run twice and an analysis of variance was used to determine main effect of Al level and treatment means were separated using a Waller-Duncan K-ratio test (K=100).

Duration in carrot broth and sensitivity of *P. parasitica* var. *nicotianae* to aluminum. A study was designed to determine if the duration in carrot broth affected sensitivity of *P. parasitica* var. *nicotianae* to Al. In this experiment, a 1 ml aliquot of a hyphal suspension was placed in each of 180 Petri plates (60 x 15 mm) with 7 ml of 5% carrot broth. The carrot broth was aspirated off plates after 24 h, 48 h, or 72 h. Upon removal of carrot broth, colonies were rinsed twice with sterile distilled water, then the water was aspirated off plates and 8 ml of an Al test solution was added to each plate. Test solutions were prepared as in the aforementioned *P. parasitica* var. *nicotianae* studies; however, in this experiment, test solutions were prepared at four pH values (4.0, 4.5, 5.0, 5.5) and three Al levels (0, 2.0, 33 μM Al). Five replicate plates were prepared for each treatment and the experiment was run twice. Sporangia production was determined as described above and the main effects of pH and Al level were assessed for each incubation time using an analysis of variance. Treatment means were separated using a Waller-Duncan K-ratio test.

Fluorimetric aluminum detection in pathogen tissue. The accumulation of Al in tissues of *T. basicola* and *P. parasitica* var. *nicotianae* was observed using lumogallion, an Al-specific, fluorescent stain. After chlamydospores or sporangia were counted, colonies were separated from Al test solutions and stained with lumogallion in 60 x 15 mm Petri plates. Colonies from control treatments were used to differentiate any background fluorescence from the Al-related fluorescence in colonies that had been exposed to the metal. The lumogallion staining technique was modified from a procedure used to observe Al accumulation in soybean roots (43). A 10 mM citrate solution was adjusted to pH 4.5 and 8 ml of the solution was added to each plate to

remove apoplastic Al. Colonies were bathed in citrate for 30 min at 25 C on a rotary shaker at 75 rpm in the dark. The citrate solution was then aspirated off plates and 8 ml of deionized water were added to each plate. Colonies were bathed in the water for 15 min, then the water was aspirated off each plate. An 8 ml aliquot of a 0.2 M sodium acetate buffer (pH 5.2) was added to each plate, and colonies were bathed in this solution for 15 min at 25 C. Buffer was then aspirated off the plates and a 4 ml aliquot of fresh buffer was added to each plate along with 4 ml of lumogallion (Molecular Probes, Eugene, OR) stock solution (10 mM lumogallion in 0.2 M sodium acetate buffer at pH 5.2) and allowed to incubate for 60 min at 50 C on a rotary shaker at 75 rpm in the dark. Hyphae were then mounted in glycerol on glass slides and stored in the dark. Metal tools were rinsed with 20% (v/v) HNO_3 prior to use to avoid trace contamination with Al, and lumogallion stock solution was stored in the dark. Slides were viewed within 2 h of preparation under an Axiophot microscope (Carl Zeiss, Thornwood, NY) that was equipped for epifluorescence microscopy with an HBO 100-W/2 mercury burner and G365 nm exciter: LP420 nm barrier fluorescence filters.

RESULTS

Sensitivity of *T. basicola* to aluminum level and pH. There was a significant interaction of pH and Al level ($P \leq 0.01$) on chlamydospore production by *T. basicola* over both runs of the experiment (Fig 1). Al sensitivity was consistently observed at pH 4.0 and 4.5 in treatments containing ≥ 20 μM Al. In the first run of the experiment (Fig 1 A), chlamydospore production was similar across all treatments in the higher pH range (pH 5.0-6.0); whereas in the second run (Fig 1 B), chlamydospore production was higher in the control threatment than in all Al-treatments at pH 5.0 and 5.5.

The pH of Al test solutions increased after 48 h incubation of *T. basicola* colonies (Table 1). For example, Al test solutions with an initial pH of 4.0 and 4.5 equilibrated at pH values between 5.3 and 5.9 after incubation of the pathogen. Al test solutions with an initial pH of 6.0, however, did not exhibit a significant pH change after pathogen incubation.

Thermodynamic modeling indicated that Al test solutions at pH 4.0 and 4.5 exhibited a sequential increase in predicted monomeric Al species activities (Fig 2) with an increase in Al level. Al test solutions at pH 5.0 (Fig 2, Table 2) reached the threshold of Al solubility by 50 μM Al (-log (Al^{3+}) = 6.9). Aluminum test solutions at pH 5.5 and 6.0 were saturated with Al by the 10 μM Al level (-log (Al^{3+}) = 8.40); therefore, higher concentrations of Al did not result in a change in monomeric Al species activities (Table 2). Similarly, the sum of monomeric Al species activities (ΣAl_{mono}) increases sequentially with Al level (0-100 μM Al) at pH 4.0 and 4.5 (Fig 3). Across all Al test solutions, the highest predicted –log (ΣAl_{mono}) was 5.67 and this was in the pH 4.0 test solution containing 100 μM Al (Table 2). The ionic strengths of Al test solutions was calculated by GEOCHEM-PC and values ranged from 2.55×10^{-2} to 2.60×10^{-2} (Table 2). No correlation between calculated ionic strength and chlamydospore production was observed.

The stepwise regression analysis was used to assess the relationship of individual monomeric Al species activities with reduced chlamydospore production of *T. basicola* within each pH level. The only species of Al entered into the regression model over both experimental runs was Al^{3+}, and this was only significant at pH 4.0 and pH 4.5 ($R^2 \geq 0.28$).

Sensitivity of *P. parasitica* var. *nicotianae* to aluminum level and pH.

Sporangia production of *P. parasitica* var. *nicotianae* was reduced by at least 80% after exposure to ≥ 2.0 μM Al (Fig 4). Because there was no significant interaction of treatments and experimental runs, data were combined over the two runs to illustrate both a significant Al effect ($P \leq 0.0001$) and a pH effect ($P \leq 0.05$). The pathogen's response to Al was similar across pH levels, but sporangia production in colonies treated with 0 and 2.0 μM Al was approximately doubled at pH 4.5 as compared to similar treatments at pH 4.0 (Fig 4).

The pH of Al test solutions increased slightly after the 48 h incubation of *P. parasitica* var. *nicotianae* colonies (Table 3). Aluminum test solutions with an initial pH of 4.0 equilibrated at pH values between 4.11 and 4.84. Test solutions with an initial pH of 5.5 increased to values between 5.71 and 6.14. No definitive pH trends across Al levels were observed.

Aluminum test solutions used to study sporangia production of *P. parasitica* var. *nicotianae* remained unsaturated with monomeric Al species except in the pH 5.5 test solutions (Table 4). The activities of monomeric Al species were the same in pH 5.5 test solutions at 5, 10, and 33 μM Al levels, with the $-\log (\Sigma Al_{mono}) = 7.29$. Because sporangia production was affected by Al across a wide range of pH values, sporangia production data could not be examined using a stepwise regression model. Furthermore, the predicted ionic strength (I) of test solutions used to study sporangia production in *P. parasitica* var. *nicotianae* was essentially invariant (Table 4).

Dose-Response curve for *P. parasitica* var. *nicotianae*. Because a greater difference in sporangia production was observed between Al-treated and the no-Al

control treatment at pH 4.5 (Fig 4), a dose-response curve was generated at pH 4.5 to illustrate the effect of Al level on sporangia production (Fig 5). In the first run of the dose-response experiment (Fig 5 A), the threshold for detection of reduced sporangia production ($P \leq 0.05$) was observed at 0.5 μM Al (75% reduction). In the second run of the dose-response experiment (Fig 5 B), the threshold for detection of reduced sporangia production was observed at 0.2 μM Al (56% reduction).

Duration in carrot broth and sensitivity of *P. parasitica* var. *nicotianae* to aluminum. Colonies of *P. parasitica* var. *nicotianae* grown for 24, 48, or 72 h on carrot broth all responded similarly to Al test solutions (Table 5). Sporangia production was generally reduced in Al test solutions of 2 μM Al and higher. In this experiment, pH did not have a significant effect on sporangia production; therefore the average number of sporangia produced was calculated and presented across pH values (Table 5). Although sporangia production was not statistically evaluated across durations in carrot broth, it is worth noting that more sporangia were produced in colonies grown for 24 h in carrot broth than those grown for either 48 or 72 h in carrot broth.

Fluorimetric aluminum detection in pathogen tissue. Lumogallion stain successfully detected Al accumulation in both *T. basicola* and *P. parasitica* var. *nicotianae* after colonies were bathed in Al test solutions. Aluminum accumulation was observed in colonies of *T. basicola* grown in a pH 4.0 test solution at 20 μM Al (Fig 6 A,B). Comparison of photographs under light microscopy with the same field of view under fluorescence illustrated localized fluorescence in basal cells of chlamydospores and in more hyaline cells of chlamydospores. The stain also detected Al in *P. parasitica* var. *nicotianae* exposed to 20 μM Al at pH 4.0 (Fig 6 C,D) and 2 μM Al at pH 5.5 (Fig 6

E,F). Hyphal tips of *P. parasitica* var. *nicotianae* (Fig 6D) exhibited greater fluorescence than older hyphae. The cell wall of *P. parasitica* var. *nicotianae* did not fluoresce, as is evidenced by the black outline of a sporangium containing zoospores (Fig 6E).

DISCUSSION

These basic toxicological studies addressed the differential toxicity of monomeric Al species to *T. basicola* and *P. parasitica* and documented the baseline sensitivity of these organisms to the metal. Chlamydospore production of *T. basicola* and sporangia production of *P. parasitica* var. *nicotianae* were both inhibited in the presence of Al, but the conditions mediating toxicity were different for these respective pathogens. *T. basicola* was suppressed at Al levels ≥ 20 μM Al and suppression was pH-dependant, occurring only at pH 4.0 and 4.5. Suppression of *P. parasitica* var. *nicotianae* was pH independent and occurred at Al levels < 1.0 μM Al.

The pH-dependent nature of Al-mediated suppression of *T. basicola* supports field studies conducted in North Carolina, Wisconsin, and Connecticut that documented suppression of black root rot of tobacco in acid soils (6, 20,32). In general, severity of black root rot is enhanced at soil pH > 5.6 and suppressed in soils with pH < 5.2 (1,3,9,10,34,36). Pathogen-infested soils have been rendered suppressive to black root rot disease after acidification with nitric or sulfuric acid (9), but amendment with phosphoric acid, which reduces Al availability in soils, had no effect on disease severity. Due to the pH dependant nature of pathogen suppression, the use of acidifying fertilizers has been recommended in fields with a high soil pH and history of the disease (36,37). Soils suppressive to *T. basicola* in North Carolina have a characteristic low pH < 5.0, low base saturation (< 70%), low calcium levels, and high exchangeable Al levels (32). Raising the pH of these suppressive soils to 6.5 nullified suppression (31), presumably

due to the reduced activity of Al at elevated pH levels. In vitro suppression of growth of *T. basicola* has been observed in a dilute nutrient agar medium containing 333 μM Al, and chlamydospore germination was inhibited at 183 μM Al and higher (33). The activity of Al in the test media, however, is unknown and unpredictable because of the presence of unidentified organic acids in the nutrient broth and the potential for complexation of Al with organic acids and agar components.

Suppression of *Phytophthora* sp. has been reported over a range of pH values; however, individual studies have demonstrated a pH-dependent nature of disease suppression. For example, the 1935 study by Lambert and Crandall (29) reported that soil acidification with $Al_2(SO_4)_3$, reducing pH from > 6.2 to 4.6, reduced wilt disease of black-locust seedlings caused by an unidentified *Phytophthora* sp. Similarly, disease of green pepper caused by *Phytophthora capsici* was increased after an acid soil was amended with lime (38). In these studies, a suppressive soil was rendered conducive to disease development upon an increase in pH, suggesting that Al may play a role in fungistasis and disease suppression. The pH-independent nature of suppression in the present study suggests that Al may also have a fungitoxic effect on *P. parasitica* var. *nicotianae,* inhibiting basic physiological processes. Further studies are necessary to determine the role of Al in soil fungistasis, inoculum efficiency, and the mechanisms of Al toxicity to fungi.

Suppression of *P. parasitica* by Al has been documented under diverse soil chemical conditions. For example, disease incidence and severity of black shank, caused by *P. parasitica* var. *nicotianae*, has been related to soil pH and soil series (11,25,26,30,42). Exchangeable Al levels were consistently higher in soils suppressive to black shank of

tobacco than in conducive soils in North Carolina (7). Converse to Al-mediated suppression of *Phytophthora* sp. in mineral soils, suppression of *P. parasitica* has also been observed in horticultural potting mixes at pH values > 5.2 and exchangeable Al levels as low as 1 μM Al g^{-1} peat (13). Although earlier research on Al-mediated suppression of *P. parasitica* in horticultural potting media related the observed toxicity to the Al^{3+} ion (4,5), the predicted prevalence of $Al(OH)_2^+$ in the Al-amended peat system investigated by Fichtner, et al. (13) suggested that multiple species of Al may be responsible for suppression of pathogen populations. In the present study, Al-mediated inhibition of sporangia production was pH-independent, confirming the probability that multiple monomeric Al species are responsible for suppression.

The present study suggests that the relative toxicity of monomeric Al species to *T. basicola* and *P. parasitica* var. *nicotianae* is different for each pathogen. Suppression of *T. basicola* was correlated to Al^{3+} activity in Al test solutions with thermodynamically predicted –log (Al^{3+}) of at least 6.82. Conversely, sensitivity of *P. parasitica* var. *nicotianae* to Al could not be correlated with the activity of any one monomeric Al species, suggesting that multiple species of monomeric Al are toxic to the pathogen. Similar to *P. parasitica* var. *nicotianae*, Al-mediated inhibition of lateral root length of soybean was closely correlated to the ΣAl_{mono} (41). Inhibition of tap root length of soybean was correlated with a balance of Ca^{2+} activity and multiple monomeric Al species activities (41). In general, phytotoxicity of Al is mainly related to inorganic monomeric Al in the soil solution (28).

The similarity of Al species toxicity to plants and *Phytophthora* sp. may be due to similar mechanisms of toxicity in these two Kingdoms. *Phytophthora* sp. are members of

the Kingdom Straminopila (formerly Chromista) (8) and are closely related to plants. *Phytophthora* differs from true fungi in part due to its diploid nature, morphology of mitochondrial cristae, cell wall biochemistry, and the flagellate nature of zoospores (15). *T. basicola* is a member of the Kingom Fungi and is not closely related to plants or Straminopilous fungi.

The sensitivity of *T. basicola* to Al^{3+}, coupled with the pathogen's ability to increase the pH of test solutions, suggests that the pH change may offer a possible mechanism for amelioration of Al toxicity by the fungus. *Phytophthora parasitica* var. *nicotianae* did not alter the pH of test solutions, thus similar levels of monomeric Al activities were maintained for the duration of incubation. The inability of the pathogen to alter the pH may contribute to its heightened sensitivity to Al. Other acidophilic organisms, however, have exhibited multiple mechanisms for Al insensitivity. For example, *Penicillium janthinellum* excludes Al under conditions with high levels of labile carbon (24). Under these conditions, a net reduction of pH was observed with a concurrent reduction of inorganic Al, suggesting that Al was removed from solution by complexation with secreted organic acids. Under conditions with low levels of labile carbon, however, the pathogen tended to accumulate Al, resulting in a net increase of pH and a concurrent decrease of total Al and total inorganic Al in the system (24). The study of Kawai, et al. (24) therefore illustrates that expressed mechanisms of Al insensitivity in a single organism may be influenced by background soil chemical conditions, and related to both positive and negative changes in pH.

The lumogallion staining technique documented Al accumulation in tissues of *T. basicola* and *P. parasitica* var. *nicotianae*. Accumulation of Al was observed in *T.*

basicola colonies exposed to a pH 4 test solution containing 20 μM Al. *Thielaviopsis basicola* cell walls remained black under fluorescence microscopy, illustrating a lack of accumulation of Al in cell wall tissue. Hyaline cells of chlamydospores exhibited more fluorescence than melanized cells, suggesting heightened Al accumulation in the hyaline cells. It is uncertain whether the melanin simply masks fluorescence of lumogallion-Al complexes, thus resulting in an underestimation of the accumulation of Al in these cells. Quantitative determination of Al accumulation in root tips has been achieved by inducively coupled plasma atomic emission spectrometry (43), and lumogallion fluorescence intensity has been positively correlated with quantitatively-determined Al accumulation in plant tissue (22).

Aluminum accumulation was also observed in hyphae and sporangia of *P. parasitica* var. *nicotianae*. Because the hyphal tip is the active growing point of the pathogen, the intense fluorescence at hyphal tips suggests the initiation of a potential defense response. No Al accumulation was observed in the cellulose-composed cell walls of *Phytophthora*, as evidenced by the lack of a visible sporangial cell wall under fluorescence microscopy. Individual zoospores within a sporangium exhibit high fluorescence intensity. The influence of Al accumulation in zoospores on the viability and inoculum potential of these propagules is unknown.

Accumulation of Al in *P. parasitica* var. *nicotianae* was observed under both low pH (4.0) and high pH (5.5) conditions with Al levels as low as 2 μM Al. Al accumulation in *T. basicola* was only studied at pH 4.0 due to the pH-dependent nature of suppression. Future studies are necessary for detection of Al accumulation under multiple chemical conditions, and for determination of selective localization of Al at the sub-cellular level.

For example, soybean roots have been stained with both DAPI and lumogallion to identify localized accumulation of Al at plant cell nuclei (43) and, Al accumulation in *P. parasitica* was observed in an Al-amended peat system containing complexes of Al with organic matter (12).

Conclusions. Both *T. basicola* and *P. parasitica* var. *nicotianae* exhibit sensitivity to Al; however, the chemical nature of Al sensitivity is different in both pathogens. *T. basicola* exhibited a pH-dependent sensitivity to Al, with chlamydospore production inhibited at low pH and ≥ 20 μM Al. Reduction of chlamydospore production was correlated with Al^{3+} activity in solution. Conversely, sporangia production of *P. parasitica* var. *nicotianae* was inhibited by ≤ 2 μM Al over a wide pH range, suggesting that multiple species of monomeric Al are responsible for suppression. The differential toxicity of monomeric Al species to these pathogens suggests that multiple mechanisms of Al toxicity may be responsible for suppression of plant pathogens. The ability of *T. basicola* to increase the pH of its surroundings may in part aid in the amelioration of Al toxicity to the pathogen. The lumogallion staining technique identifies the accumulation of Al in pathogen tissue and may have future application for sub-cellular investigation of the mechanisms of Al toxicity to microorganisms.

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Table 1. Final pH of Al-test solutions[z] after 48 h incubation of *Thielaviopsis basicola*

Run 1		Initial pH			
Al level	4.0	4.5	5.0	5.5	6.0
0 µM	5.4	5.9	5.7	5.7	6.1
10 µM	5.9	5.7	5.7	5.8	6.2
20 µM	5.5	5.9	5.9	5.9	6.1
50 µM	5.4	5.9	5.3	5.8	5.7
100 µM	5.5	5.4	5.6	5.8	5.9
Run 2		Initial pH			
Al level	4.0	4.5	5.0	5.5	6.0
0 µM	5.5	5.9	5.6	5.6	6.2
10 µM	5.8	5.7	5.8	5.8	6.1
20 µM	5.6	5.9	5.9	5.8	6.1
50 µM	5.3	5.9	5.2	5.8	5.6
100 µM	5.6	5.5	5.7	5.6	5.9

[z]Aluminum test solutions were prepared in minimal salts medium amended with $Al_2(SO_4)_3$. Colonies were incubated in test solutions at 25 C in the dark.

Table 2. Predicted ionic strength (I), monomeric aluminum species activities, and Σ_{Almono} activities in Al test solutions utilized in the study of chlamydospore production of *Thielaviopsis basicola* for different Al inputs and pH

pH	Al input	I	-log activity Al^{3+}	$Al(OH)^{2+}$	$Al(OH)_2^{+}$	$Al(OH)_3^{0}$	$Al(OH)_4^{-}$	$\Sigma Al_{mono}{}^{Z}$
pH 4.0								
	0 µM	2.55E-02						
	10 µM	2.55E-02 6.75	6.75	7.75	8.85	11.55	13.45	6.70
	20 µM	2.57E-02 6.45	7.45	7.45	8.55	11.22	13.15	6.41
	50 µM	2.57E-02 6.05	6.05	7.05	8.15	10.84	12.75	6.00
	100 µM	2.59E-02 5.74	5.74	6.48	7.77	10.54	12.38	5.67
pH 4.5								
	0 µM	2.55E-02						
	10 µM	2.55E-02 7.12	7.12	7.62	8.22	10.41	11.82	6.97
	20 µM	2.57E-02 6.82	6.82	7.32	7.91	10.11	11.52	6.64
	50 µM	2.57E-02 6.41	6.10	6.60	7.20	9.40	10.80	5.95
pH 5.0								
	0 µM	2.55E-02						
	10 µM	2.55E-02 7.59	7.59	7.59	7.69	9.38	10.29	7.14
	20 µM	2.57E-02 7.28	7.28	7.29	7.39	9.08	9.99	6.84
	50 µM	2.57E-02 6.90	6.90	6.90	7.00	8.70	9.60	6.45
	100 µM	2.59E-02 6.90	6.90	6.90	7.00	8.70	9.60	6.45
pH 5.5								
	0 µM	2.55E-02						
	10 µM	2.55E-02 8.40	8.40	7.90	7.50	8.70	9.10	7.29
	20 µM	2.57E-02 8.40	8.40	7.90	7.50	8.70	9.10	7.29
	50 µM	2.57E-02 8.40	8.40	7.90	7.50	8.70	9.10	7.29
	100 µM	2.59E-02 8.40	8.40	7.90	7.50	8.70	9.10	7.29
pH 6.0								
	0 µM	2.56E-02						
	10 µM	2.56E-02 9.90	9.90	9.90	8.0	8.70	8.60	7.80
	20 µM	2.58E-02 9.90	9.90	9.90	8.0	8.70	8.60	7.80
	50 µM	2.58E-02 9.90	9.90	9.90	8.0	8.70	8.60	7.80
	100 µM	2.60E-02 9.90	9.90	9.90	8.0	8.70	8.60	7.80

$^{Z}\sum Al_{mono}$= sum of activities of Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^{+}$, $Al(OH)_3^{0}$, and $Al(OH)_4^{-}$

Table 3. pH of Al-test solutions[Z] after 48 h incubation of *Phytophthora parasitica* var. *nicotianae*

Run 1		Initial pH		
Al level	4.0	4.5	5.0	5.5
0 µM	4.31	5.13	5.40	5.84
2 µM	4.17	4.97	5.37	5.71
5 µM	4.19	5.24	5.35	5.89
10 µM	4.28	4.98	5.44	5.78
33 µM	4.25	4.98	5.62	5.73
Run 2		Initial pH		
Al level	4.0	4.5	5.0	5.5
0 µM	4.27	5.71	5.98	6.08
2 µM	4.11	5.61	5.88	6.06
5 µM	4.84	5.53	5.95	6.08
10 µM	4.75	5.59	5.79	6.14
33 µM	4.54	5.47	5.77	5.9

[Z]Aluminum test solutions were prepared in minimal salts medium amended with $Al_2(SO_4)_3$. Colonies were incubated in test solutions at 25 C in the dark.

Table 4. Predicted ionic strength (I), monomeric aluminum species activities, and ΣAl_{mono} activities in Al test solutions utilized in the study of sporangia production of *Phytophthora parasitica* var. *nicotianae* for different Al inputs and pH

pH/Al input	I	-log activity Al^{3+}	$Al(OH)^{2+}$	$Al(OH)_2^{+}$	$Al(OH)_3^{0}$	$Al(OH)_4^{-}$	ΣAl_{mono} [Z]
pH 4.0							
0 µM	1.72E-02						
2 µM	1.72E-02	7.39	8.39	9.49	12.19	14.09	7.38
5 µM	1.72E-02	6.99	7.99	9.09	11.79	13.69	6.95
10 µM	1.72E-02	6.69	7.69	8.79	11.49	13.39	6.64
33 µM	1.72E-02	6.17	7.17	8.27	10.96	12.87	6.12
pH 4.5							
0 µM	1.71E-02						
2 µM	1.71E-02	7.82	8.32	8.92	11.11	12.50	7.67
5 µM	1.71E-02	7.42	7.92	8.52	10.70	12.12	7.27
10 µM	1.71E-02	7.12	7.62	8.22	10.41	11.82	6.97
33 µM	1.71E-02	6.59	7.09	7.69	9.90	11.29	6.45
pH 5.0							
0 µM	1.71E-02						
2 µM	1.71E-02	8.29	8.29	8.39	10.09	10.99	7.84
5 µM	1.71E-02	7.89	7.89	7.99	9.68	10.59	7.44
10 µM	1.71E-02	7.59	7.59	7.69	9.38	10.29	7.14
33 µM	1.71E-02	7.06	7.06	7.16	8.86	9.76	6.61
pH 5.5							
0 µM	1.71E-02						
2 µM	1.71E-02	8.76	8.27	7.87	9.06	9.47	7.67
5 µM	1.71E-02	8.40	7.90	7.50	8.70	9.10	7.29
10 µM	1.71E-02	8.40	7.90	7.50	8.70	9.10	7.29
33 µM	1.71E-02	8.40	7.90	7.50	8.70	9.10	7.29

[Z] $\sum Al_{mono}$= sum of activities of Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^{+}$, $Al(OH)_3^{0}$, and $Al(OH)_4^{-}$

Table 5. Mean sporangia production of *Phytophthora parasitica* var. *nicotianae* in aluminum test solutions[x] after incubation in 5% carrot broth for either 24, 48, or 72 h[y].

Al level	**Run 1** # sporangia[z]	**Run 2** # sporangia[z]
Duration in carrot broth: 24 h		
0 µM Al	24.9 a	14.2 a
2 µM Al	1.65 b	1.46 b
33 µM Al	0.88 b	1.56 b
Duration in carrot broth: 48 h		
0 µM Al	0.33 a	1.71 a
2 µM Al	0.09 b	1.19 ab
33 µM Al	0.08 b	0.68 b
Duration in carrot broth: 72 h		
0 µM Al	10.78 a	1.72 a
2 µM Al	0.94 b	0.55 b
33 µM Al	0.55 b	0.65 ab

[x] Aluminum test solutions were prepared with a complete factorial treatment design composed of three Al levels (0, 2, 33 uM Al) and four pH levels (4.0, 4.5, 5.0, and 5.5).

[y] Colonies were grown in 5% carrot broth for either 24, 48, or 72 h before being rinsed and bathed in Al test solutions.

[z] The average number of sporangia produced per 1.4 mm^2 of nylon mesh was determined. Each value represents the average number of sporangia produced in four randomly selected 10 x fields of view under the light microscope over five replicate plates of each treatment. Spore production was averaged across pH for each Al level. Letters represent significant differences ($p \leq 0.05$) in sporangia production across Al levels within each experimental run and duration in carrot broth.

FIGURE LEGENDS

1. Chlamydospore production of *Thielaviopsis basicola* colonies grown 48 h in 5% carrot broth, rinsed with distilled water, and incubated 48 h in Al test solutions. **A** and **B** represent run 1 and 2 of the experiment. Error bars represent standard error.

2. Distribution of predicted monomeric Al species activities in Al test solutions used for study of chlamydopsore production of *Thielaviopsis basicola*. Al species activities were predicted using GEOCHEM-PC (Parker). **A**,**B**,**C**, and **D** represent Al test solutions at pH 4.0, 4.5, 5.0, and 5.5, respectively. (Al3+ = Al^{3+}, Al2+ = $Al(OH)_2^+$, Al1+ = $Al(OH)_2^+$, Al0 = $Al(OH)_3^0$, Al4- = $Al(OH)_4^-$).

3. Sum of monomeric Al activities (ΣAl_{mono}) in Al test solutions used for study of chlamydopsore production of *Thielaviopsis basicola*. Monomeric Al species activities were predicted using GEOCHEM-PC, then summed to form the ΣAl_{mono} variable.

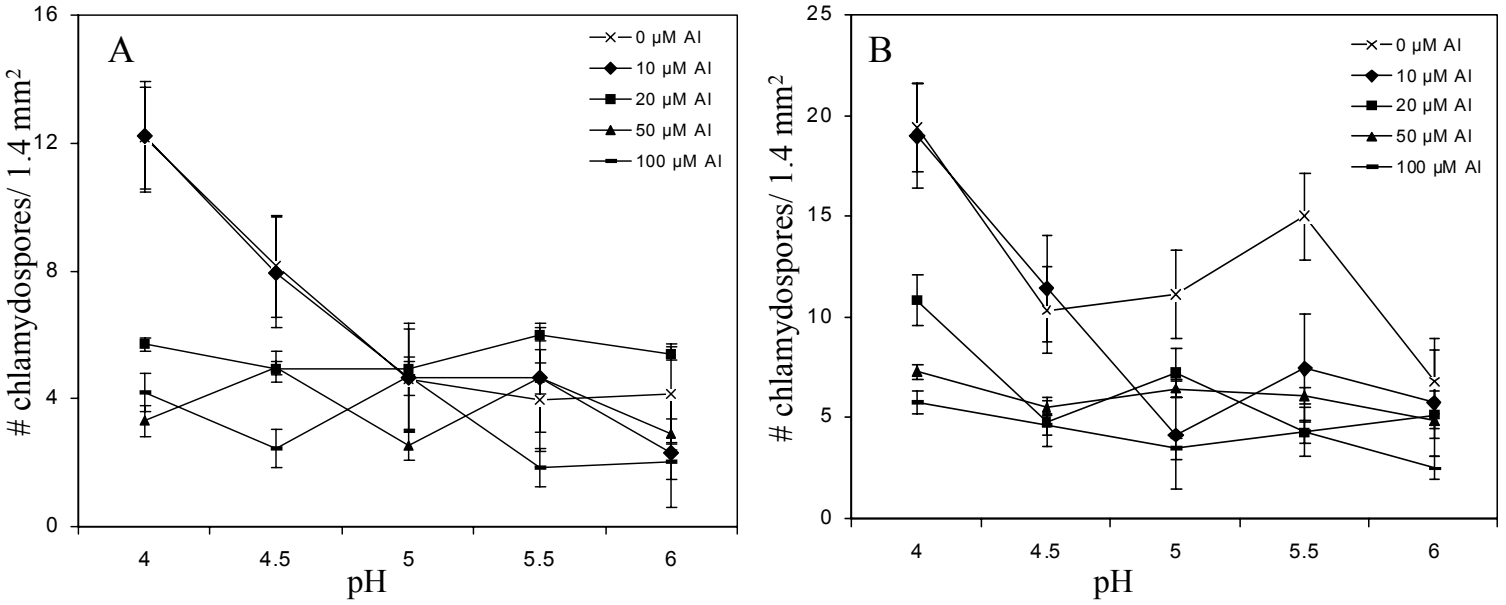
4. Sporangia production of *Phytophthora parasitica* var. *nicotianae* grown 24 h in 5% carrot broth, rinsed twice with sterile, distilled water, and incubated 48 h in Al test solutions. Data were combined over two runs of the experiment and error bars represent standard error.

5. Dose-response curve relating percent reduction in sporangia production of *Phytophthora parasitica* var. *nicotianae* with Al concentration in Al test solutions at pH 4.5. Colonies were grown for 24 h in 5% carrot broth, rinsed with deionized water, and incubated for 48 h in Al test solutions. **A** and **B** represent two runs of the experiment and error bars represent standard error.

6. Light and fluorescent photographs of *Thielaviopsis basicola* and *Phytophthora parasitica* var. *nicotianae* after 48 h incubation in Al test solutions and subsequent staining with lumogallion. In **A** and **B**, colonies of *T. basicola* were grown for 48 h in 5%

carrot broth, rinsed with deionized water, and incubated in an Al test solution at pH 4.0 with 20 μM Al. Images **A** and **B** are of the same field of view, with **A** taken under light and **B** taken under fluorescence. In photo **B**, note the fluorescence in the hyaline chlamydospore cell (c) and the hyaline basal cell (b). Images **C** and **D** were taken of a *P. parasitica* var. *nicotianae* colony grown 24 h in 5% carrot broth before incubation in an Al test solution at pH 4.0 with 20 μM Al. **C** and **D** are of the same field of view, with **D** captured under fluorescence. Note the fluorescence of the hyphal tip (ht) in **D**, suggesting a localized accumulation of Al. Images **E** and **F** represent a *P. parasitica* var. *nicotianae* colony that was incubated in an Al test solution at pH 5.5 with 2 μM Al. **E** and **F** are of the same field of view under light and fluorescence microscopy, respectively. Note the lack of fluorescence in the sporangial cell wall (sw) and the fluorescence of individual zoospores within the sporangium (z) in **F**.

Figure 1-top



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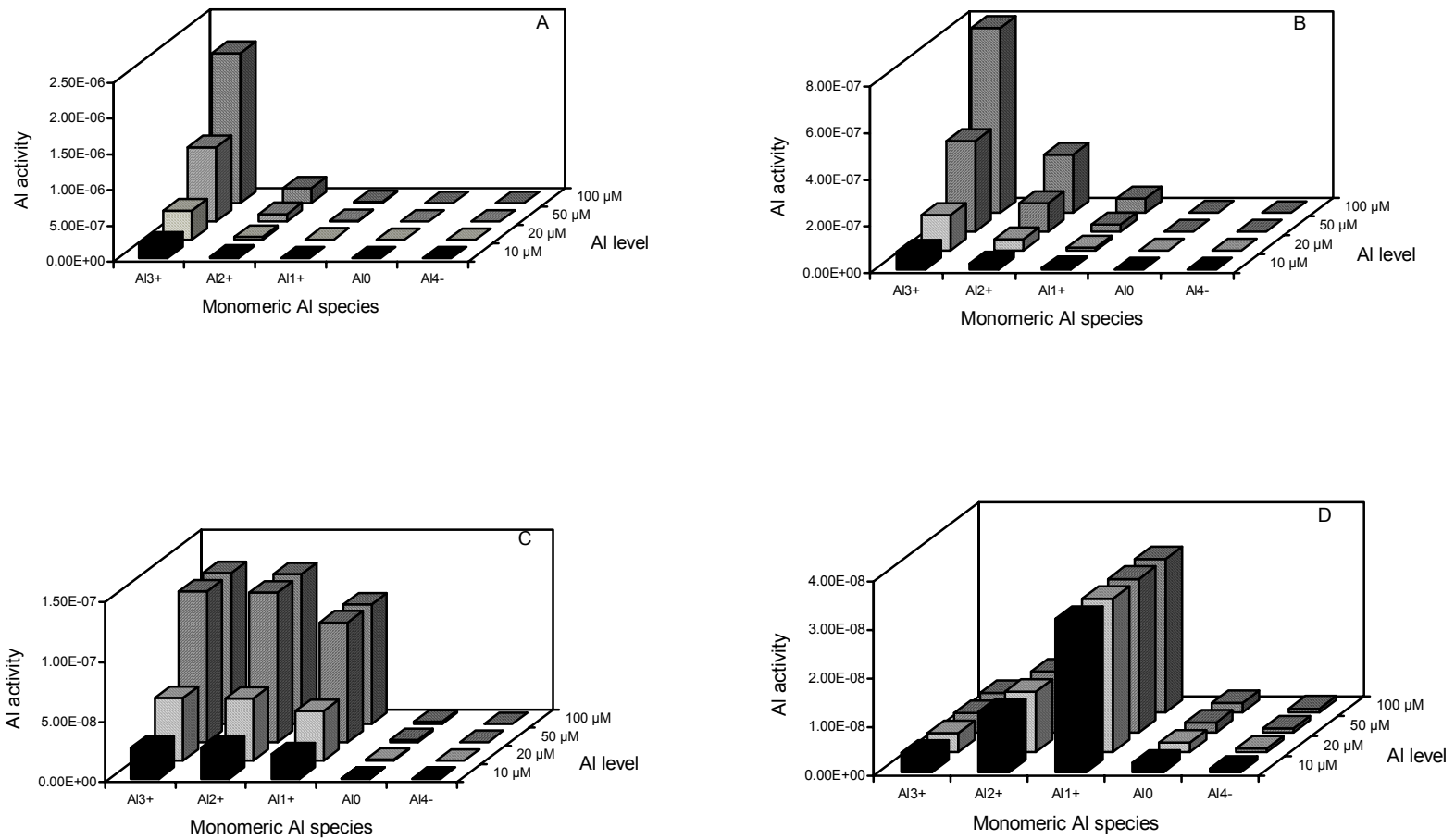

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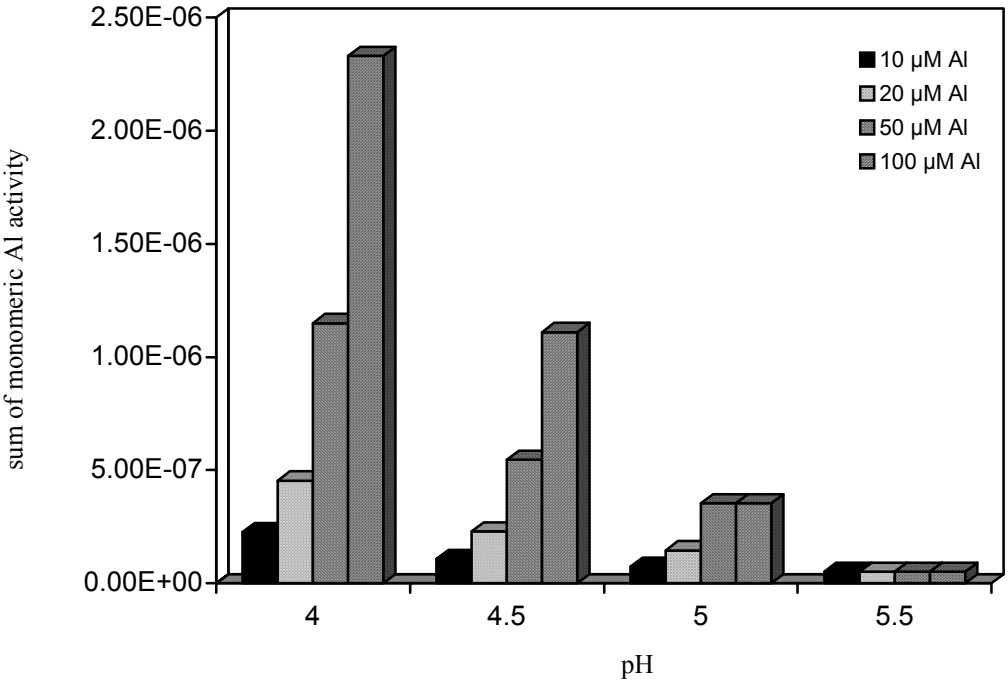
Figure 3-top



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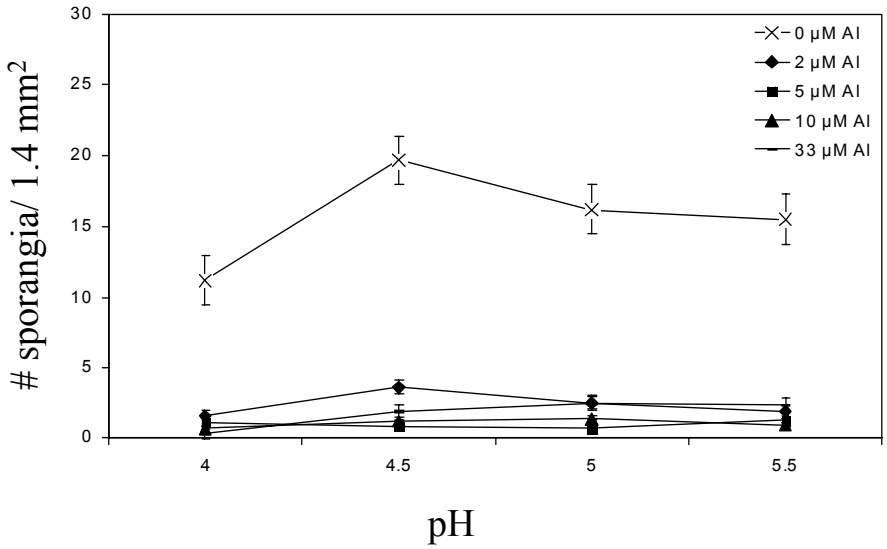

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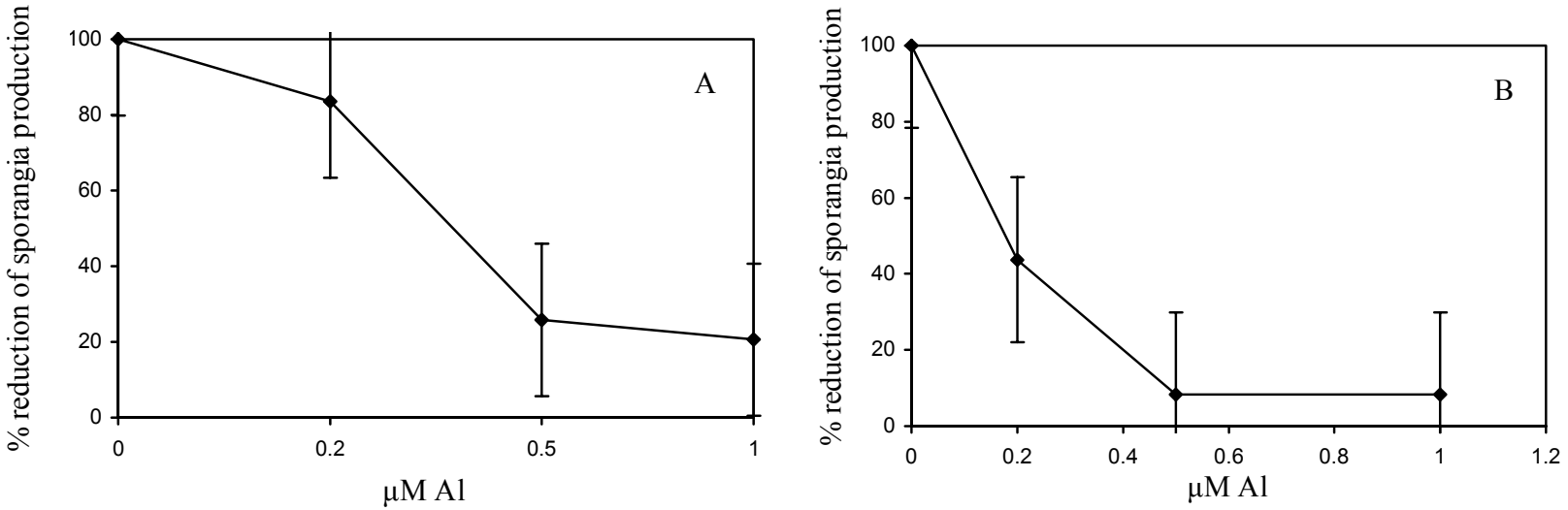

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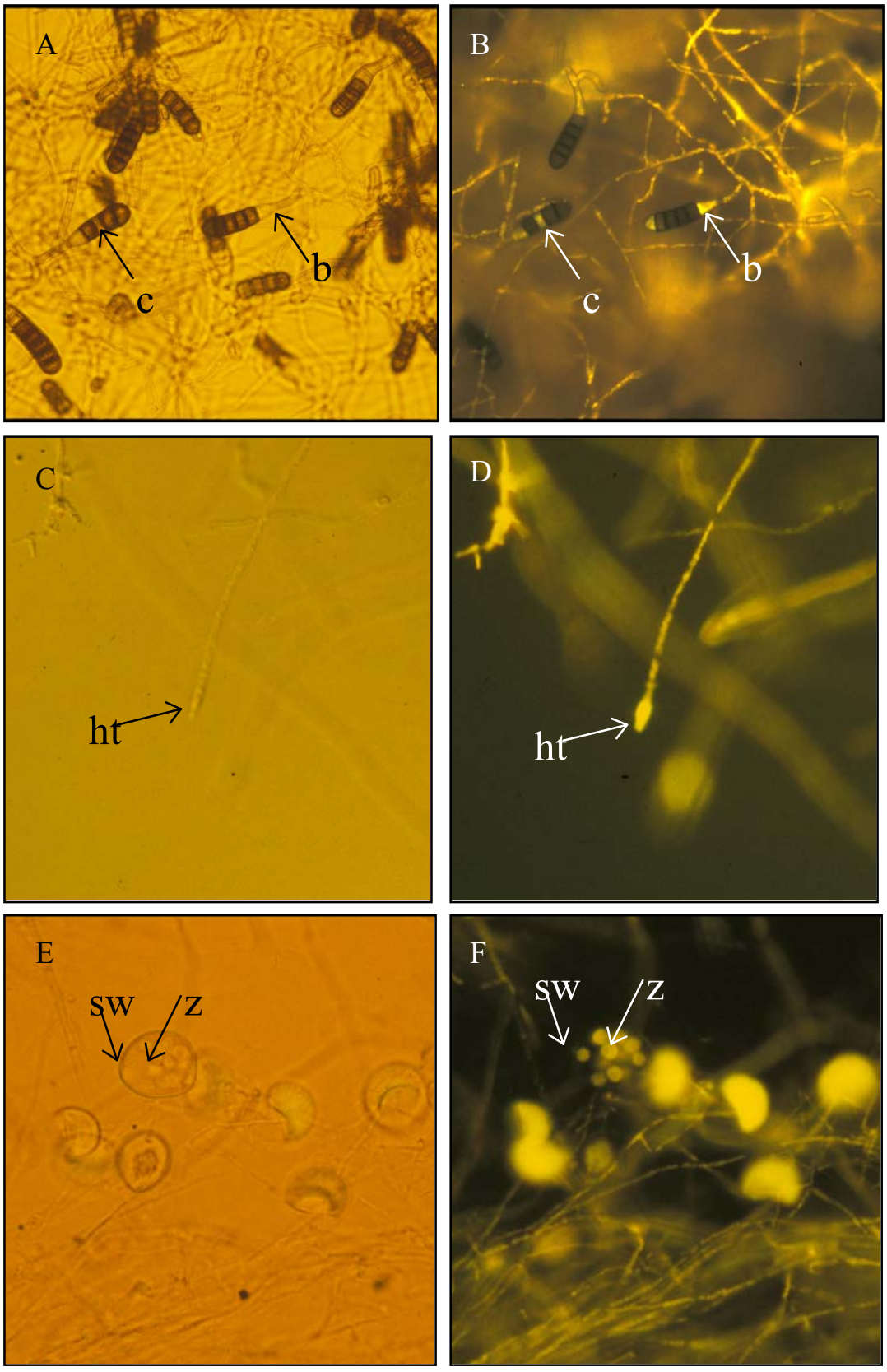
Figure 6-top



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