

# Abiotic and Biological Suppression of *Phytophthora parasitica* in a Horticultural Medium Containing Composted Swine Waste

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## ABSTRACT

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Horticultural potting media have been amended with compost to enhance biological suppression and with  $\text{Al}_2(\text{SO}_4)_3$  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* (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 of Al  $\text{g}^{-1}$  of medium with an  $\text{Al}_2(\text{SO}_4)_3$  solution at either pH 4 or pH 6. Infested leaf disks were buried for 2-day

durations beginning 0, 6, 13, and 21 days after Al amendment. The number of sporangia produced on infested leaf disks was assessed. 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 affect sporangia production. Exchangeable Al levels decreased over time, and abiotic suppression was only observed at  $>2 \mu\text{M}$  Al  $\text{g}^{-1}$  of medium. Pathogen populations were occasionally affected by steaming and Al. 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* and abiotic suppression occurred before biological suppression developed.

The scarcity and rising cost of sphagnum peat moss (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 degradation of organic waste materials by diverse microbial populations (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 (9,14,34), 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 materials, 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 during the composting process (14). The decompositional status of a medium affects the level of biological suppression offered by a compost-amended medium (6,17). Highly decomposed organic matter, such as sphagnum peat moss, does not support high microbial activity (18) and tends to be conducive to diseases caused by soilborne 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 media 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 of a potting mix for management of root rot on poinsettia caused by *Pythium aphanider-*

*matum* (6). In fact, media amended with composted municipal sludge requires lower compost content (2.5%, vol/vol) than a similar medium containing composted pine bark (20%, vol/vol) to induce suppression of *Pythium* damping-off. This was attributed to the higher level of microbial biomass in the composted municipal sludge (8,18). On the other hand, heat treatment 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 preemergence damping-off of impatiens caused by *R. solani*. Fresh or uncomposted swine waste was conducive to disease. The exact mechanisms 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 that of CSW2. Furthermore, CSW1 exhibited a greater structural diversity, 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 peat moss-based potting media for control of damping-off of ornamental bedding plants caused by *Phytophthora parasitica* (3,4). Benson (4) controlled preemergence damping-off of snapdragon, vinca, and petunia in a peat and vermiculite potting medium with the addition of  $\text{Al}_2(\text{SO}_4)_3$ . Disease control in this system was observed at KCl-exchangeable Al concentrations as low as 2.03 meq  $\text{Al}^{+3}$  per 100 g of medium. Aluminum was not

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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  $\text{Al}_2(\text{SO}_4)_3$  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 concentrations as low as  $1 \mu\text{mol Al g}^{-1}$  of peat at a pH of  $>5.0$ . In the peat system, the presence of organic acids alters  $\text{Al}^{3+}$  activity by the formation of Al-organic acid complexes (35). 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 concentrations of exchangeable Al in the system, it is possible that the organically bound Al or an  $\text{Al}(\text{OH})_3$  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  $\text{Al}^{3+}$  activity, a species of Al considered toxic to plants (13).

Many of the major pathogens that have been suppressed in compost-amended media are sensitive to Al. Members of the phylum Oomycota, including *Pythium* spp. (19), *Phytophthora* spp. (1), and *Aphanomyces* spp. (24), are suppressed by Al, as are *R. solani* (22) and *Thielaviopsis 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.** 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% (vol/vol) CSW was prepared by mixing the CSW with Fafard No. 2, a soilless potting medium containing Canadian sphagnum peat moss, 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 County, NC, and was provided by M. Sullivan, N.C. State University, Raleigh. 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 autoclaved 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 colonized the sterile

leaf disks for 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 [ $D_b$ ] of  $0.22 \text{ g ml}^{-1}$ ) was mixed thoroughly in a plastic bag and divided in half. One half was steamed at  $99^\circ\text{C}$  for 30 min on two consecutive days. The second half remained unsteamed. The steamed and unsteamed media were then divided into three aliquots to establish a complete factorial treatment design. The steamed and unsteamed media were amended 1 day after steaming with an  $\text{Al}_2(\text{SO}_4)_3$  solution at either pH 4 or pH 6 at a rate of  $0.0079 \text{ g of Al g}^{-1}$  of medium. The Al-amendment solutions were prepared by making a  $0.05 \text{ M Al}_2(\text{SO}_4)_3$  solution and adjusting the pH to either 4 or 6 with the addition of  $1 \text{ N KOH}$ . 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  $D_b$  of  $0.188 \text{ g cm}^{-3}$  and then equilibrated at  $-2.5 \text{ kPa}$  soil matric potential ( $\Psi_m$ ) 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 3-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  $-2.5 \text{ kPa } \Psi_m$ . 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  $10\times$  field of view of an inverted light microscope was counted, with two counts made per leaf disk. Sporangium 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.** After retrieval of leaf disks on days 2, 8, 15, and 23, the content of each funnel was placed into plastic bags and mixed thoroughly. Two 3-g moist subsamples ( $0.85 \text{ g dry weight equivalent}$ ) 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  $0.01 \text{ M CaCl}_2$  solution. Potassium-exchangeable Al was determined with a modified acid-base titration procedure (36). Each sample was suspended in  $25 \text{ ml}$  of a  $1 \text{ M KCl}$  solution for 2 h, filtered through Q5 filter paper (Fisher Scientific), and rinsed three times with  $25 \text{ ml}$  of  $1 \text{ M KCl}$ . Ten drops of phenolphthalein solution were added to each extract, and extracts were titrated with  $0.1 \text{ M NaOH}$  to a pale pink endpoint. A drop of  $0.1 \text{ M HCl}$  was added to turn the solution colorless, and then  $10 \text{ ml}$  of 4% (wt/vol) NaF was added. In the presence of Al, a stable fluoroaluminate complex forms and the pink color returns. Extracts were then titrated to a clear endpoint with the addition of  $0.1 \text{ M HCl}$ . Three moles of  $\text{H}^+$  will titrate 1 mole of exchangeable Al.

**Microbial activity.** Upon the establishment and equilibration of funnels on day 0, a  $5.0 \text{ g dry weight equivalent}$  was removed from each funnel to monitor basal microbial respiration rates in the various treatments. Microbial activity was assessed using an incubation technique (37) that measures evolution of  $\text{CO}_2$  over a 3-week period. The  $5.0\text{-g}$  subsamples were placed in plastic beakers and each beaker was set in the bottom of a sealed 2-liter Mason jar. Fifty-milliliter glass beakers, suspended in Mason jars, were used to trap  $\text{CO}_2$  in  $5 \text{ ml}$  of  $0.5 \text{ N NaOH}$ . 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<sub>2</sub>, and then 2 drops of phenolphthalein were added to each beaker. The residual base (unconsumed by trapping of CO<sub>2</sub>) was titrated with 0.1 N HCl to a clear endpoint to indirectly assess CO<sub>2</sub> evolution. Respiration rates were calculated as milligrams of CO<sub>2</sub> per gram of medium per day.

**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 sporangium production, this experiment contained only two factors of Al amendment: no Al control and pH 4 amendment solution at a rate of 0.0079 g of Al g<sup>-1</sup> of medium. Steaming and Al amendment followed the same methodology as in the sporangium 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<sub>m</sub> 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 were reequilibrated at -2.5 kPa for approximately 10 h. Media were destructively sampled on days 2, 8, 15, and 23 to determine pathogen population, pH, and K-exchangeable Al. Furthermore, in the first two runs of the experiment, the type of propagule giving rise to assayed colonies was determined microscopically on day 2.

Pathogen population was determined using an assay on a Phytophthora-selective agar medium (PARP) (21) containing 50 μg of hymexazol (Sankyo Co., Tokyo) ml<sup>-1</sup>. The contents of each funnel were placed into plastic bags and mixed thoroughly. Three 1-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 rinsed with water and colonies of *P. parasitica* var. *nicotianae* were counted. Population data analysis, pH, and K-exchangeable Al measurements 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 medium from each of four treatments in 50 ml of 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 sporangium production experiment. Sporangia were counted 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<sub>b</sub>* of approximately 0.18 g cm<sup>-3</sup>. Plastic bags were placed over each funnel and funnels were brought to saturation for 24 h. Media were allowed to equilibrate at 0, -1.0, -2.5, -5.0, and -10.0 kPa Ψ<sub>m</sub> for 24 h with respect to the medium surface, with three replicate funnels at each Ψ<sub>m</sub>. One sample removed from each funnel was weighed and oven dried at 105°C for 48 h to determine dry weights. Volumetric water content was averaged across the three replicate samples.

## RESULTS

**Effects of Al amendment and steaming on sporangium production.** Steaming and amendment with Al affected the number of sporangia produced on infested leaf disks that had been buried for 48 h (Table 1). Statistical analyses comparing treatments were only conducted within each time point. Sporangium 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<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> solution consistently reduced sporangium production on day 2. The pH 6 amendment solution did not inhibit sporangium production throughout the 3-week period. Amendment with the pH 4 Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> solution reduced sporangium production at day 2 by approximately 50% in steamed medium in both experimental runs (Table 1). The same Al amendment of unsteamed medium resulted in a 64 and 38% reduction of sporangium production at day 2 for runs 1 and 2, respectively (Table 1). In the first run of the experiment, the Al effect was only observed on 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). A steaming effect was not observed

TABLE 1. Average number of sporangia<sup>w</sup> of *Phytophthora parasitica* var. *nicotianae* produced on edges of infested leaf disks buried for 48 h in steamed<sup>x</sup> and unsteamed potting medium amended with pH 4 or pH 6 Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> solutions<sup>y</sup>

	Steamed control	Not steamed control	Steamed pH 4	Not steamed pH 4	Steamed pH 6	Not steamed pH6	Significant factors ( <i>P</i> ≤ 0.05)
Run 1							
Day 2	26 a <sup>z</sup>	19 a-c	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

<sup>w</sup> 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 10× field of view under a light microscope.

<sup>x</sup> Steamed 30 min at 98.6°C on two consecutive days.

<sup>y</sup> Medium was amended with 0.0079 g of Al g<sup>-1</sup> of medium as a 0.05 M Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> solution adjusted with 1.0 N KOH to either pH 4 or pH 6.

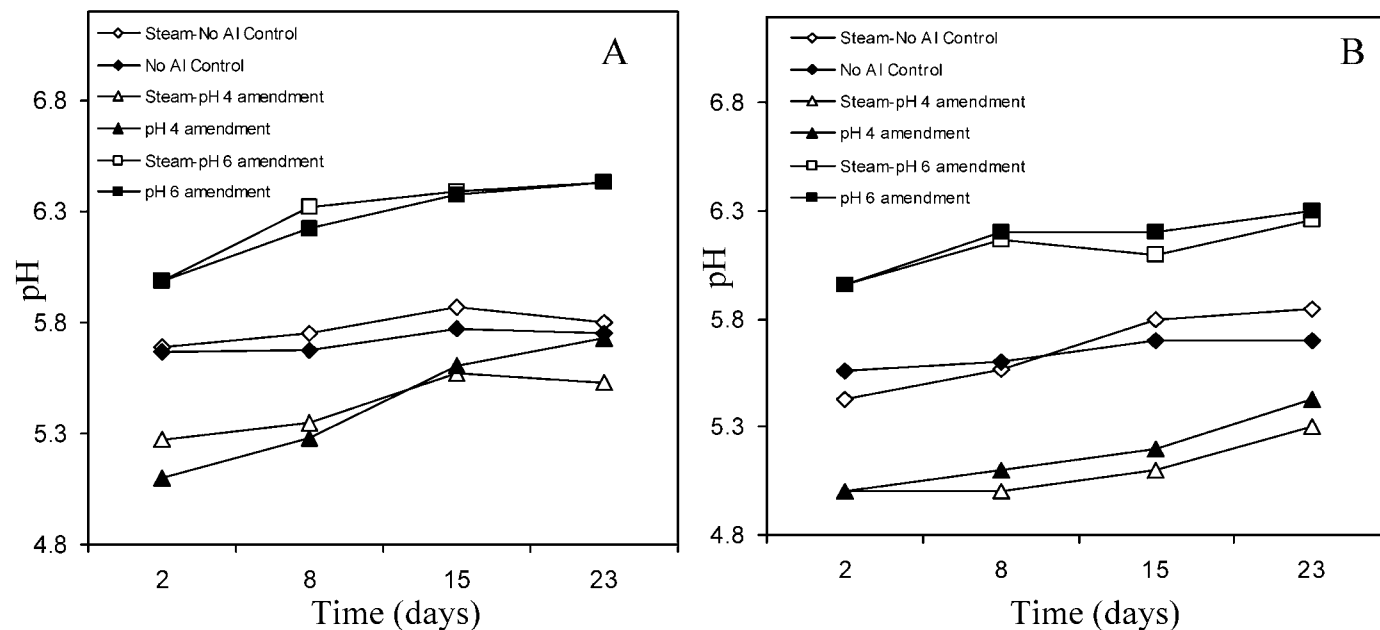
<sup>z</sup> Values followed by different letters within a row indicate significant differences (*P* ≤ 0.05).

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 on day 23 than in the media amended with the pH 4 Al amendment (Table 1). The pH 4 Al amendment resulted in a 24% reduction in sporangium production in the unsteamed media on day 23 (Table 1). In the second run of the experiment, sporangium production on day 23 was reduced by greater than 75% in all unsteamed treatments (Table 1). Although steaming and Al amendments were both statistically significant factors in the reduction of sporangia production at various time points, no significant interaction of these main effects was observed.

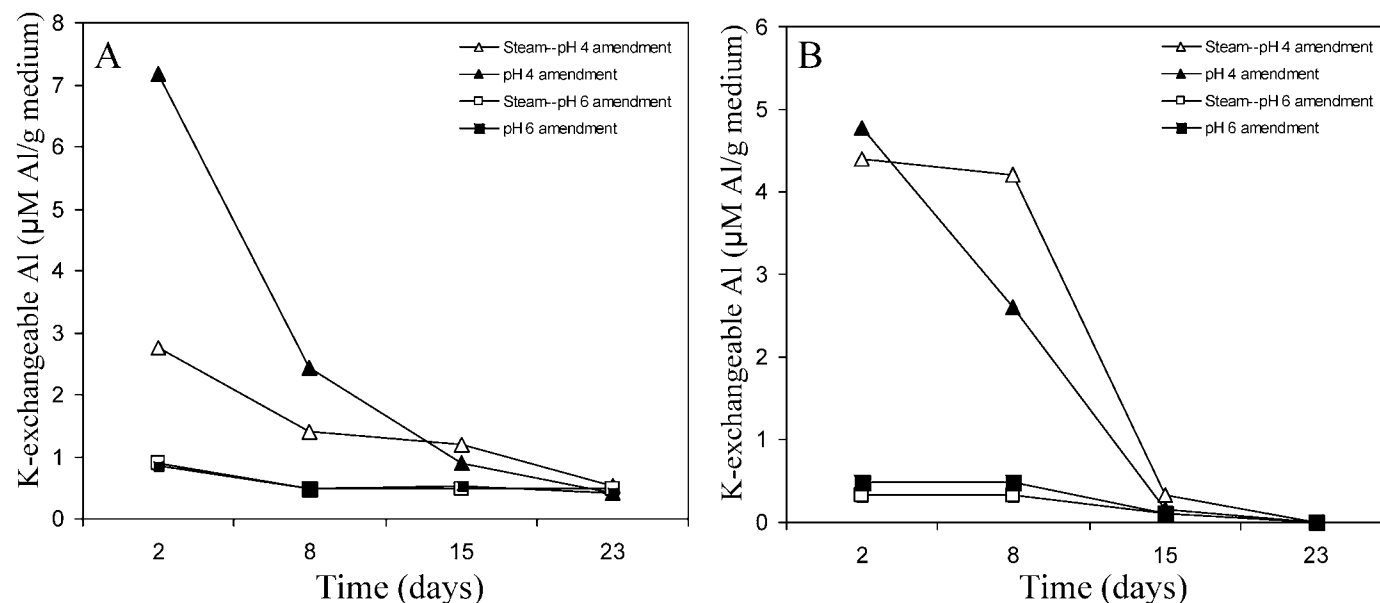
Amendment with Al altered the pH of the media. Unamended media had a pH of approximately 5.7 over the 3-week period

(Fig. 1). Little difference in pH was observed between steamed and unsteamed media (Fig. 1). 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. 1A). 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. 1).

Amendment of the CSW mix with the pH 4 solution resulted in higher concentrations of K-exchangeable Al than in the pH 6 Al-amended media. Amendment with the pH 4  $Al_2(SO_4)_3$  solution resulted in initial extractable Al concentrations ranging from 3 to 7  $\mu M$  Al  $g^{-1}$  of medium across runs and steam versus unsteamed



**Fig. 1.** Trends in pH of steamed and unsteamed media amended with pH 4 or pH 6  $Al_2(SO_4)_3$  solutions. Samples were taken from media containing infested leaf disks that were buried for 48-h intervals and then removed at each time point to assess sporangium production. **A** and **B** represent separate repetitions of the experiment.



**Fig. 2.** K-exchangeable Al in steamed and unsteamed media amended with pH 4 or pH 6  $Al_2(SO_4)_3$  solutions. Samples were taken from media containing infested leaf disks that were buried for 48-h intervals and then removed at each time point to assess sporangium production. **A** and **B** represent separate repetitions of the experiment.

media (Fig. 2). In these treatments, Al concentrations tended to decrease over time, equilibrating at approximately 0.5 and 0  $\mu\text{M}$  Al  $\text{g}^{-1}$  of media on day 23 in runs 1 and 2, respectively (Fig. 2). The pH 6 amendment resulted in less than 1  $\mu\text{M}$  Al  $\text{g}^{-1}$  of medium on day 2, and 0.5 and 0  $\mu\text{M}$  Al  $\text{g}^{-1}$  of medium on day 23 in runs 1 and 2, respectively (Fig. 2).

Microbial respiration ranged between 0.2 and 0.6  $\text{mg}$  of  $\text{CO}_2$   $\text{g}^{-1}$  of medium  $\text{day}^{-1}$  during days 7 to 22 over both runs of the experiment (Fig. 3). In run 1, however, peak respiration was recorded on day 1 with a rate of 1.6  $\text{mg}$  of  $\text{CO}_2$   $\text{g}^{-1}$   $\text{day}^{-1}$  (Fig. 3A). Furthermore, the steamed media exhibited consistently higher respiration rates than that of the corresponding unsteamed media on day 1 in the first experimental run (Fig. 3A).

**Effects of Al amendment and steaming on pathogen populations.** Both Al and steaming affected pathogen populations on leaf disks buried for 24 h prior to flooding. Although population data were more variable than sporangium production data, Al significantly reduced pathogen populations on day 2 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 50% compared with that of the corresponding unamended control. Percent population reduction varied from 52 to 99% at these time points. Furthermore, all of the colonies assayed from the treated media were derived from encysted zoospores.

Steaming had an inconsistent effect on pathogen populations in run 1, evidenced by increased pathogen populations in steam-treated medium on day 2 and the subsequent decrease in populations in steam-treated medium on day 8 (Table 2). These steam effects, however, were only observed in the unamended (no Al control) treatments. Because steaming had a consistent effect on sporangium 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 concentrations remained above 2  $\mu\text{M}$  Al  $\text{g}^{-1}$  at the time points corresponding to this suppression (Fig. 4). Similar to the sporangium production experiments, no interaction of Al amendment and steaming was observed in the pathogen population study.

In the population experiments, the pH trends were similar to those in the sporangia production experiments (Fig. 5). The K-exchangeable Al concentrations ranged between 5 and 9  $\mu\text{M}$  Al  $\text{g}^{-1}$

on day 2, but equilibrated down to concentrations ranging from approximately 1 to 5  $\mu\text{M}$  Al  $\text{g}^{-1}$  at day 23 (Fig. 4). After 3 weeks of incubation, the flooded media in the population study equilibrated at higher concentrations of Al than the corresponding un-flooded treatments in the sporangia production experiment (Figs. 2 and 4). Furthermore, in both experiments, concentrations of K-exchangeable Al in the steamed medium tended to be lower than those in the corresponding unsteamed medium over much of the 3-week period (Figs. 2 and 4).

A soil moisture characteristic was generated between 0 and  $-10$  kPa  $\Psi_m$ . The percent water-filled pore space at 0,  $-0.1$ ,  $-2.5$ ,  $-5.0$ , and  $-10.0$  kPa  $\Psi_m$  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 a pH 4  $\text{Al}_2(\text{SO}_4)_3$  solution consistently inhibited sporangium production on day 2, whereas the pH 6 amendment

TABLE 2. Average number of propagules<sup>w</sup> of *Phytophthora parasitica* var. *nicotianae* in steamed<sup>x</sup> and unsteamed potting media amended with a pH 4  $\text{Al}_2(\text{SO}_4)_3$  solution<sup>y</sup>

	Steamed control	Not steamed control	Steamed pH 4	Not steamed pH 4	Significant factors ( $P \leq 0.05$ )
Run 1					
Day 2	133.6 a <sup>z</sup>	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
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	—
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	—

<sup>w</sup> Pathogen populations were assessed after infested leaf disks were buried for 24 h, media were flooded for 12 h, and reequilibrated at 25 cm soil moisture tension for 12 h.

<sup>x</sup> Steamed for 30 min at 98.6°C on two consecutive days.

<sup>y</sup> Medium was amended with 0.0079 g of Al  $\text{g}^{-1}$  of medium as a 0.05 M pH 4  $\text{Al}_2(\text{SO}_4)_3$  solution.

<sup>z</sup> Different letters across rows designate significant differences based on the Waller-Duncan  $k$  ratio test ( $k = 100$ ).

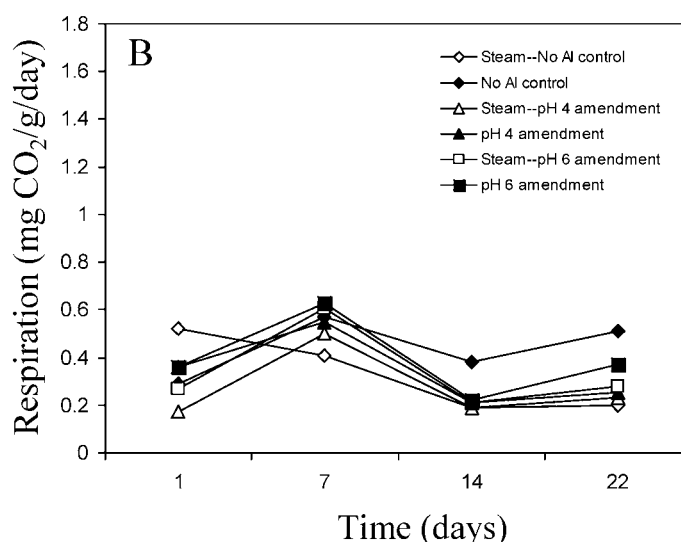
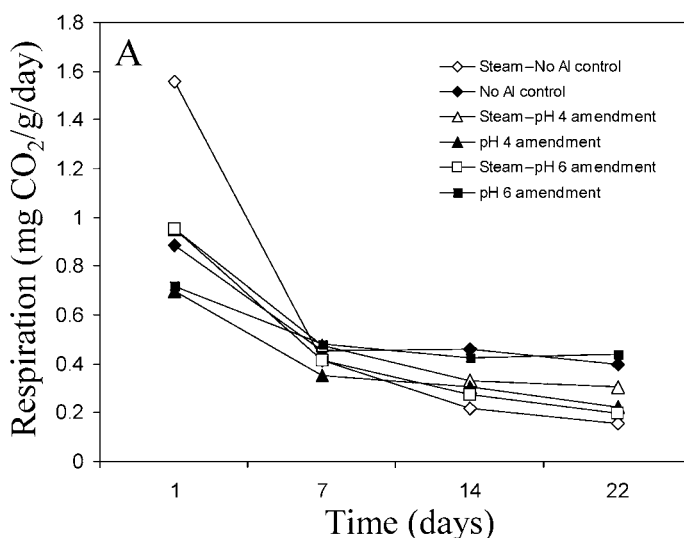


Fig. 3. Microbial respiration rates in steamed and unsteamed media amended with pH 4 or pH 6  $\text{Al}_2(\text{SO}_4)_3$  solutions. Respiration rates were determined 24 h after infested leaf disks were buried in corresponding media for 48 h. A and B represent separate repetitions of the experiment.

was ineffective. The differential effect of the two amendment solutions may be related to the heightened overall concentration of K-exchangeable Al in the pH 4 amendment ( $>3 \mu\text{M g}^{-1}$ ) compared with that of the pH 6 amendment ( $<1 \mu\text{M g}^{-1}$ ). Similar amendment of peat with  $\text{Al}_2(\text{SO}_4)_3$  solution at pH 6 consistently suppressed populations of *P. parasitica* var. *nicotianae* at K-exchangeable Al concentrations at  $1 \mu\text{M g}^{-1}$  of peat (13); however, the initial peat amendment contained twice the level of Al used in the present study.

The lower concentration 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  $\text{Al}(\text{OH})_3$  precipitate. In these respective pH 4 and pH 6 amendment solutions, the total amount of soluble Al with respect of gibbsite would be  $10^{-3}$  and  $10^{-6.3}$  M (28); however, the precipitate may also have been a more soluble Al-bearing mineral. The higher pH (6.0 to 6.4) of the medium amended with the pH 6 amendment

compared with the pH 4 amendment (5.1 to 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 differs in each amendment solution, with the pH 6 solution containing a higher proportion of the  $\text{Al}(\text{OH})_2^+$  ion (28). This monovalent hydrolysis species forms stronger complexes with charged organic functional groups than the free ionic  $\text{Al}^{3+}$  (5) that dominates in the pH 4 amendment solution (28).

In the experiments pertaining to sporangia production and pathogen populations, the concentrations of K-exchangeable Al decreased over time. Over both runs of the sporangia production experiment, the K-exchangeable Al concentrations 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, Al activity is always governed by the process maintaining the lowest activity of  $\text{Al}^{3+}$  in solution.

The pH in Al-amended media increased by approximately a half of a unit between days 2 and 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 the microbial community are possible. For example, it is not known whether the pathogen or other microorganisms accumulated Al or produced organic acids to complex and sequester the metal.

Al-mediated inhibition of sporangium production was mainly observed early after the 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 have been related to the heightened concentrations of K-exchangeable Al at these time points. Al-mediated suppression of pathogen populations always occurred at K-exchangeable Al concentrations above  $2 \mu\text{M Al g}^{-1}$  of medium. The media assayed for pathogen populations were flooded for 12 h to induce zoospore release from sporangia. Consequently, these funnels exhibited higher concentrations of K-exchangeable Al on day 23 than the corresponding unflooded media used in the sporangium production experiment. Aluminum reduced sporangia production through

TABLE 3. Average number of sporangia<sup>w</sup> of *Phytophthora parasitica* var. *nicotianae* produced on the edge of infested leaf disks buried for 24 h in steamed<sup>x</sup> and unsteamed potting media amended with a pH 4  $\text{Al}_2(\text{SO}_4)_3$  solution<sup>y</sup>

Run 2	Steamed control	Not steamed control	Steamed pH 4	Not steamed pH 4	Significant factors ( $P \leq 0.05$ )
Day 1	19 a <sup>z</sup>	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

<sup>w</sup> 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  $\times 10$  field of view under a microscope.

<sup>x</sup> Steamed for 30 min at  $98.6^\circ\text{C}$  on two consecutive days.

<sup>y</sup> Medium was amended with  $0.0079 \text{ g of Al g}^{-1}$  of medium as a  $0.05 \text{ M pH 4 Al}_2(\text{SO}_4)_3$  solution.

<sup>z</sup> Values followed by different letters within a row indicate significant differences ( $P \leq 0.05$ ).

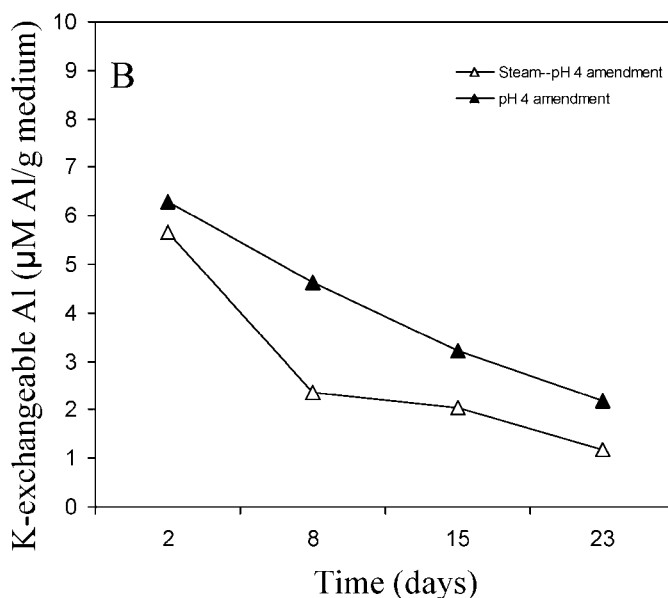
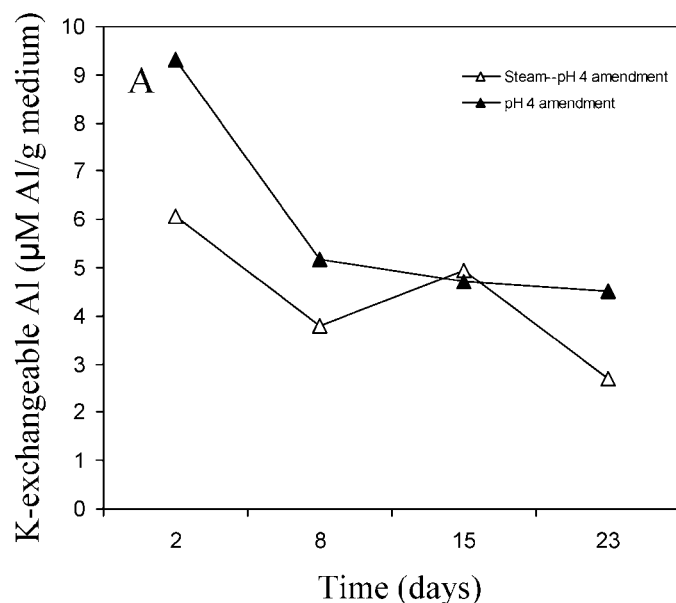


Fig. 4. K-exchangeable Al in steamed and unsteamed 20% composted swine waste medium amended with a  $0.05 \text{ M Al}_2(\text{SO}_4)_3$  solution at pH 4. Samples were taken from funnels containing infested leaf disks that were buried for 24 h before funnels were flooded. A and B represent separate repetitions of the experiment.

day 15 in run 2 of the population experiment. Interestingly, the suppressive medium contained  $>2 \mu\text{M Al g}^{-1}$  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  $2 \mu\text{M Al g}^{-1}$  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 compost medium, 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 24 h prior to flooding (Table 3). Consequently, the noted Al effect on pathogen populations may be the result of suppression of sporangium 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 CSW-amended medium incites a biologically mediated suppression. The time-sensitivity of the steam effect on sporangium production suggests that beneficial microbial populations were not active initially in the 20% CSW medium. The burst of respiration in the steamed medium on day 1 (no Al control) in the first run of the sporangium production experiment suggests a possible initial burst of activity of endospore-forming bacteria. Diab et al. (12) had noted high populations of these organisms in the CSW-amended medium. Biologically mediated suppression of *P. parasitica* var. *nicotianae* was consistently observed on days 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 medium 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, as was suggested earlier for other compost-amended media (8). Because total microbial activity, as estimated by respiration rate, 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. It is also possible that the initial concentrations of free nutrients in

the medium were too high to induce microbiostasis early after potting, a phenomenon established by Chen et al. (8) for suppression of populations of *Pythium ultimum* in compost-amended media.

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 and microbial biomass nitrogen 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, a medium containing composted sewage sludge was suppressive to *Aphanomyces euteiches*, *Sclerotinia minor*, and *R. solani*, whereas another 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* spp. and *Phytophthora* spp. in compost-amended media differ from those affecting control of *R. solani* (18). A narrow range of organisms is responsible for suppression of *Rhizoctonia* sp. 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 medium offers biological control of diverse pathogens including *Rhizoctonia*, *Pythium ultimum* (12), and *P. parasitica*, it is possible that this medium may incorporate multiple mechanisms of pathogen suppression.

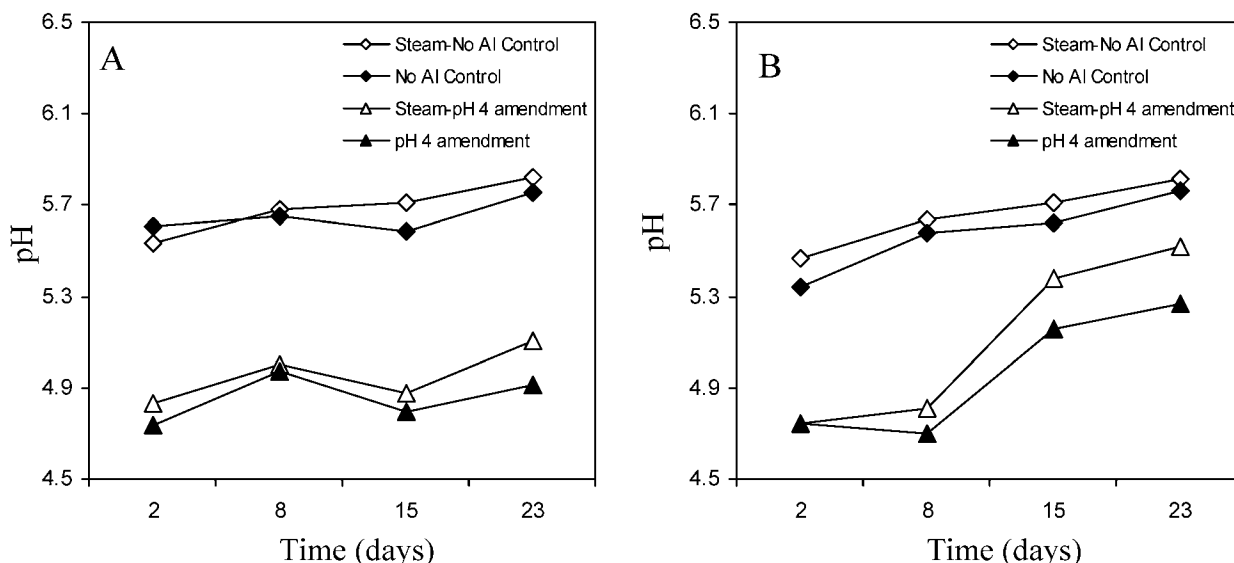


Fig. 5. pH of steamed and unsteamed media amended with a 0.05 M  $\text{Al}_2(\text{SO}_4)_3$  solution at pH 4. Samples were taken from media containing infested leaf disks that were buried for 24 h before funnels were flooded. A and B represent separate repetitions of the experiment.

Steaming had less of an effect on pathogen populations than on sporangium 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 medium 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 sporangium production on days 1, 7, and 14. Therefore, biologically mediated suppression of sporangia production may require more time to occur than the 24-h period during which 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 microorganisms offering a different or faster mechanism of control. In general, biologically mediated suppression of sporangium production was more consistent on leaf disks buried for 48 h than on those buried for only 24 h. The efficacy of a longer duration of pathogen contact with the media suggests that nutrient-based competition or microbiostasis may be the predominant mechanism of biological control. 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 were 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 CSW and  $Al_2(SO_4)_3$  offered both biological and abiotic suppression of *P. parasitica*. The biological and the abiotic factors inhibited sporangium production; however, the two factors may be active at different times. Aluminum inhibited sporangium production on day 2, whereas the biologically mediated suppression of sporangium production persisted from days 8 to 23. Al amendments may be effective at protecting the plant before beneficial microbial populations reach a threshold necessary for suppression. The documentation of abiotic and biological suppression of *P. parasitica* in a horticultural medium is the first step toward considering and understanding the potential role of these factors in disease suppression.

In addition to suppression of *Phytophthora* spp., Diab et al. (12) has documented the ability of CSW-amended medium to suppress damping-off caused by *R. solani*. Because biocontrol mechanisms of these two pathogens in compost-amended media are reportedly different (17), the CSW-amended medium may offer multiple mechanisms of biological control. Furthermore, addition of compost to peat-based media may enhance its ability to support an introduced biocontrol agent. The incorporation of abiotic and biocontrol 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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