

Suppression of *Phytophthora parasitica* on *Catharanthus roseus* with Aluminum

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ABSTRACT

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The relationship of exchangeable aluminum, liming rate, and control of preemergence damping-off of *Catharanthus roseus* caused by *Phytophthora parasitica* was investigated. A peat/vermiculite medium at pH 4.1 was limed at 0, 3, or 6 g of dolomitic limestone per 1,000 cm³ of medium and misted for 3 days prior to seeding with *Catharanthus roseus* in 81-cell plug trays. Seeds were covered with additional medium infested with *Phytophthora parasitica* on colonized rice particles. Aluminum sulfate at rates of 17–250 meq of Al per 100 cm³ of medium was drenched onto the surface of the medium. Samples were collected beginning 2 days after seeding to determine pH, exchangeable Al, and population density of *P. parasitica*. Stand counts were made beginning 9 days after seeding. Phytophthora damping-off was controlled in unlimed medium drenched with 17 meq of Al per 100 cm³ of medium, in medium limed at 3 g/1,000 cm³ when drenched with 41 meq of Al per 100 cm³ of medium, and in medium limed at 6 g/1,000 cm³ when drenched with 100 meq

of Al per 1,000 cm³ of medium. Four days after seeding, exchangeable aluminum levels in treatments that controlled damping-off were 5.7, 1.2, and 0.5 meq/100 g of medium, respectively, in the unlimed medium and media limed at 3 or 6 g/1,000 cm³. At 3 or 6 g of lime per 1,000 cm³, but not in unlimed medium, pH was 0.5 units lower in treatments in which damping-off was controlled compared to treatments in which disease was not controlled. Populations of *P. parasitica* were suppressed in media in which damping-off was controlled. At the highest rate of aluminum drenches, populations were suppressed over a 16-day period, but at lower rates of aluminum, where control was observed, populations were suppressed only 7–9 days after seeding. Production of sporangia of *P. parasitica* in vitro was inhibited by 1.3 meq of Al per 100 g of medium at pH 5. Aluminum may control *Phytophthora* damping-off by suppression of the *P. parasitica* population during the critical period of seed germination and emergence.

Additional keywords: calmodulin.

Aluminum toxicity toward several soilborne pathogens including *Phytophthora parasitica* Dastur (18), *P. capsici* (12), *Thielaviopsis basicola* (10), and *Verticillium albo-atrum* (13) has been associated with suppression of diseases caused by these pathogens in acid soils (9,12,13). In suppressive soils, exchangeable aluminum has been associated with suppression. Typically, exchangeable aluminum (Al³⁺) in acid soils is related to soil pH; as the pH rises, for instance, due to amendment with alkaline forms of calcium (CaCO₃ or Ca(OH)₂), less and less Al³⁺ is soluble in the soil solution (17). In a Bladen clay loam soil at pH 4.1, Orellana et al (13) reported 4.3 meq/100 g of KCl-extractable aluminum. Liming this soil to pH 4.4 with CaCO₃ failed to control *Verticillium* wilt of sunflower, but disease severity still was much less than when this soil was limed to pH 5.4 (13). In soils used to grow burley tobacco in western North Carolina, suppression of black root rot caused by *T. basicola* was found consistently only in soils with concentrations of Al³⁺ at 1.0 meq/100 g of soil or greater (9). In acid soils of Brazil used to grow pepper, *Phytophthora* blight caused by *P. capsici* was enhanced by liming soils with alkaline sources of calcium that raised soil pH (12).

Pathogens suppressed in acid soils are sensitive to Al³⁺ in vitro. For instance, in vitro mycelial growth of *P. capsici* was inhibited at concentrations of Al³⁺ found in acid soils, although the growth inhibition was fungistatic rather than fungitoxic (12). Mycelium of *V. albo-atrum* was sensitive to Al³⁺ in vitro with growth suppressed completely at concentrations greater than 8 µg/g of medium (13). Germination of endoconidia and chlamydospores of *T. basicola* was inhibited in vitro at concentrations of Al³⁺ greater than 0.5 meq/100 ml of medium (10).

P. parasitica causes damping-off, root rot, and blight of *Catharanthus roseus* (L.) G. Don, an important bedding-plant species with drought tolerance that is used across the southern half of the United States (6,13). The objective of the present

research was to evaluate the suppression of *Phytophthora* damping-off caused by *P. parasitica* on *C. roseus* in a peat-based soilless medium with drenches of aluminum. Peat has a naturally low pH near 4.0. Adjusting liming rates with aluminum application may result in a seeding medium that is suppressive to *P. parasitica*. Preliminary research has been reported (1).

MATERIALS AND METHODS

Peat-based medium. A peat/vermiculite medium (1:1) with an initial pH of 4.1 in a 1:2 suspension with 0.01M CaCl₂ was used in all experiments. The peat moss was sifted through a screen with 6.3-mm openings to remove large pieces of peat material. The medium was limed at 0, 3, or 6 g of dolomitic limestone per 1,000 cm³ (0, 3, or 6 kg/m³) of medium to compare the interaction of aluminum-drench rate with medium pH and its effect on disease control. Experiments were conducted in 81-cell plug trays that were 25.5 × 25.5 × 3.8 cm. Individual cells were tapered from 2.5 × 2.5 cm at the top to 1.2 × 1.2 cm at the bottom. Each tray held about 1,300 cm³ of medium with a bulk density of 0.087 g/cm³. Plug trays were filled with medium and misted for 3 days under an intermittent-mist system that cycled on for 2 min 3–5 times a day to allow time for the amended lime to affect initial medium pH before seeding trays.

Seeding and infesting plug trays. *C. roseus* cv. Little Bright Eyes was vacuum seeded (one seed per cell) with a custom seeder (Berry Seeder Co., Elizabeth City, NC). Inoculum for infesting the medium was produced by growing isolate 336 of *P. parasitica*, originally isolated from *C. roseus*, on rice grains (25 g of rice per 19 ml of distilled water) for 14–21 days (6). Initially, sterilized rice grains were seeded with cornmeal agar disks of isolate 336. Rice grain cultures in flasks were shaken briefly each day so individual grains would not clump together. After 14–21 days, colonized rice grains were pulverized in a blender for 1–2 min. The pulverized grains were screened through a sieve with 2-mm openings to collect colonized rice particles of uniform size. Hyphae

but not sporangia of *P. parasitica* were observed on rice particles after colonization. Rice particles were mixed 1:9 with a coarse builder's sand to facilitate weighing small quantities.

The colonized rice particle/sand inoculum was mixed at a rate of 0.5 g (0.05 g of rice particles) with 150 cm³ of peat/vermiculite medium reserved in plastic bags from the initial medium treatments. Inoculum and medium were mixed by hand in 800-ml plastic jars by rotating jars in the palm of the hand for 1–2 min. In later experiments, a variable-speed electric motor with an angled cup assembly was used to rotate jars. After thorough mixing, inoculum was applied to the surface of the seeded plug trays by sprinkling the mixture through 1-cm-diameter holes cut in the jar lid. Trays were misted for 2 min to moisten the infested layer of medium.

Aluminum drenches. Preliminary experiments were conducted to determine the appropriate amounts of aluminum sulfate to add to peat/vermiculite to give exchangeable aluminum values above 1.0 meq. Thus, aluminum equivalent to 0, 17, 41, 100, or 250 meq/100 cm³ of medium was applied as a drench of aluminum sulfate (Al₂(SO₄)₃) at rates of 0, 1.3, 3.1, 7.5, or 18.8 g per 650.3-cm² plug tray. The required amount of Al₂(SO₄)₃ was dissolved in 150 ml of water in a 850-ml plastic jar with 0.8-mm-wide holes in the lid. The solution forced from the jar with steady hand pressure was adequate to uniformly wet the surface of the plug tray.

Experimental design. There were four replications of each treatment arranged in a randomized complete block design on a greenhouse bench with an intermittent-mist system. Trays were misted for 2 min 3–5 times per day depending on drying conditions. Each experiment included a set of treatments with an infested control without aluminum for each liming rate as well as a non-infested control for each liming rate. Because the amount of exchangeable aluminum depends on soil pH and hence the liming rate applied, various combinations of liming rate and aluminum sulfate drenches were tested in preliminary experiments. Results reported below are based on three experiments in which the same rates of lime and aluminum were used. Variances among experiments were tested for homogeneity. Because no differences in homogeneity were found, data from all three experiments were pooled for analysis and presentation except where noted.

Stand counts were made at day 9 and thereafter at 2-day intervals. Because maximum emergence occurred on day 9 in all treatments, subsequent stand counts were not used in data analysis. Seedlings with unfolded cotyledons were considered emerged. Differences in stand count among seedlings in treatments infested with *P. parasitica* were compared with the appropriate noninfested control for a given liming rate. Data were tested with PROC GLM and the linear contrast procedure of PC SAS (SAS Institute, Cary, NC).

Length of the first true leaf at days 18 and 23 after seeding was used as a measure of plant growth because differences in plant height were not found when visual differences in plant growth could be observed. Data were tested with PROC GLM and the linear contrast procedure of PC SAS.

Exchangeable aluminum and pH determinations. Media were randomly sampled from three of the 81 cells in the plug trays at each sampling date for determination of exchangeable aluminum beginning the day after seeding and continuing at 2- to 3-day intervals. Samples of the peat/vermiculite from each treatment were air-dried for 2 days, after which two 2-g subsamples were extracted in 25 ml of 1 N KCl for 30 min. Al³⁺ is reported in milliequivalents per 100 g of air-dried medium. The base-acid titration procedure of Yuan (19) was used to determine Al³⁺. Briefly, KCl-extracted solution was filtered into 250-ml Erlenmeyer flasks to remove all medium particles, and the residue in the filter was rinsed five times with 25 ml of 1 N KCl. Total acidity was determined by titration with 0.1 N NaOH to the phenolphthalein-pink end point (19). A drop of 0.1 N HCl was added to turn the solution colorless, and 10 ml of 4% NaF was added to form a stable fluoroaluminate ion. If Al³⁺ was present, the color changed to pink. The amount of Al³⁺ was determined by a final titration with 0.1 N HCl to the colorless end point with milli-

equivalents of acid corresponding to milliequivalents of Al³⁺ present in the sample (19).

Peat/vermiculite pH was determined at each sampling date by collecting samples from two cells at random from the treatments in the fourth replication. Sample pH was determined in a 1:2 suspension with 0.01 M CaCl₂ to account for any differences in dissolved salts among samples (15,17). In general, pH values may be as much as 0.5 units lower in 0.01 M CaCl₂ compared to measurements in water (15).

The fourth replication of each experiment was used for destructive sampling to determine exchangeable aluminum and pH. Pooled data from the three experiments are presented. Means from each treatment at each sampling date were separated with the Waller-Duncan *K*-ratio test after analysis by PROC GLM in PC SAS.

Population density of *P. parasitica*. A soil-dilution procedure was used to estimate the population density (colony-forming units) of *P. parasitica* in each of the treatments because relatively large populations were detected in some treatments. Samples of misted medium collected from three cells at random from a plug tray for each treatment in one replication were bulked. Population density of *P. parasitica* was determined for three 10-g subsamples of each treatment. Each 10-g subsample was stirred continuously in 90 ml of sterile water as higher dilutions were prepared in sterile distilled water with wide-tip pipettes. Dilutions ranged from 1:10 to 1:1,000 depending on time after infestation and treatment. One milliliter from each diluted subsample was spread on PARP medium (7) with hymexazol as the dilution was stirred continuously. Five plates were prepared for each dilution. Normally, two of the dilutions were plated from each subsample to facilitate optimal counting of developing colonies. Plates were incubated on the laboratory bench for 3 days, followed by enumeration of colonies after the agar surface was washed gently to remove debris. Three additional 10-g subsamples were oven-dried at 105 C for 24 h to determine sample dry weight. Colony-forming units were expressed on a per gram of medium dry-weight basis.

Sporangium production by *P. parasitica* was determined over a range of 0 to 2.0 meq of Al per 100 ml of water. Fungal mats grown for 2 days in V8 broth were rinsed twice in sterile deionized water then rinsed and incubated 24 h in an aluminum sulfate solution. The aluminum solution was buffered with 20 mM MES buffer (2-[*N*-Morpholino]ethanesulfonic acid; Sigma Chemical Co., St. Louis, MO) to maintain pH near 5.0. Sporangium production by *P. parasitica* was not affected by the rate of MES buffer used (18). Sporangial counts for all treatments were made under the microscope and adjusted for a field of view equal to 18.84 mm². One count in the area of maximum sporangium production per fungal mat was made from each of nine fungal mats per aluminum concentration.

RESULTS

Emergence and growth of *C. roseus*. Stand counts of *C. roseus* averaged 62 seedlings per tray (76% emergence) for the 81-cell plug trays in noninfested controls over the three liming rates (Fig. 1). In unlimed infested media, stand count for the 17 meq of Al per 100 cm³ rate was not significantly different ($P = 0.05$) from the noninfested control (Fig. 1). However, stand counts that averaged 35.6 seedlings per tray in unlimed, unamended media were different ($P = 0.05$) compared to the noninfested control (Fig. 1). Preemergence damping-off of *C. roseus* caused by *P. parasitica* was severe in media limed at 3 g/1,000 cm³ and left untreated with Al. However, stand counts for *C. roseus* treated with 41 or 100 meq of Al per 100 cm³ of media were not significantly different ($P = 0.05$) from the noninfested control. As liming rate was increased to 6.0 g/1,000 cm³, disease was severe in media without Al or media with 41 meq of Al per 100 cm³ compared to the noninfested control, but treatments of 100 and 250 meq of Al per 100 cm³ of media controlled damping-off (Fig. 1).

Rate of seedling emergence was similar among treatments. However, initial growth as measured by length of first true leaf at day 18 was less ($P = 0.05$) for *C. roseus* in unlimed peat/

vermiculite medium with or without aluminum compared to growth of seedlings in noninfested medium limed at 6.0 g/1,000 cm³ (Fig. 2A). Length of first leaves on seedlings in infested medium limed at 3.0 g/1,000 cm³ and drenched at 0 or 41 meq of Al per 100 cm³ of medium were smaller than first leaves on seedlings in noninfested medium limed at 6.0 g/1,000 cm³ (Fig.

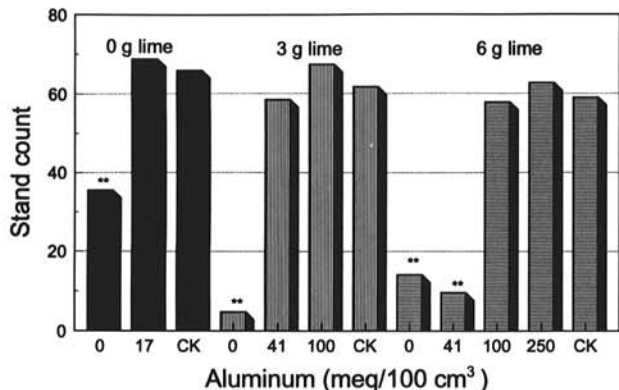


Fig. 1. Stand counts of *Catharanthus roseus* in a peat/vermiculite medium infested with *Phytophthora parasitica* and limed at 0, 3, or 6 g/1,000 cm³. The medium was drenched with 0–250 meq of Al per 100 cm³ of medium at seeding depending on liming rate. The noninfested, undrenched control is designated CK for each liming rate. Means represent pooled data from three experiments. Bars with double asterisks represent means of stand counts that were less ($P = 0.01$) than the comparable noninfested, undrenched control for each liming rate based on linear contrast comparisons.

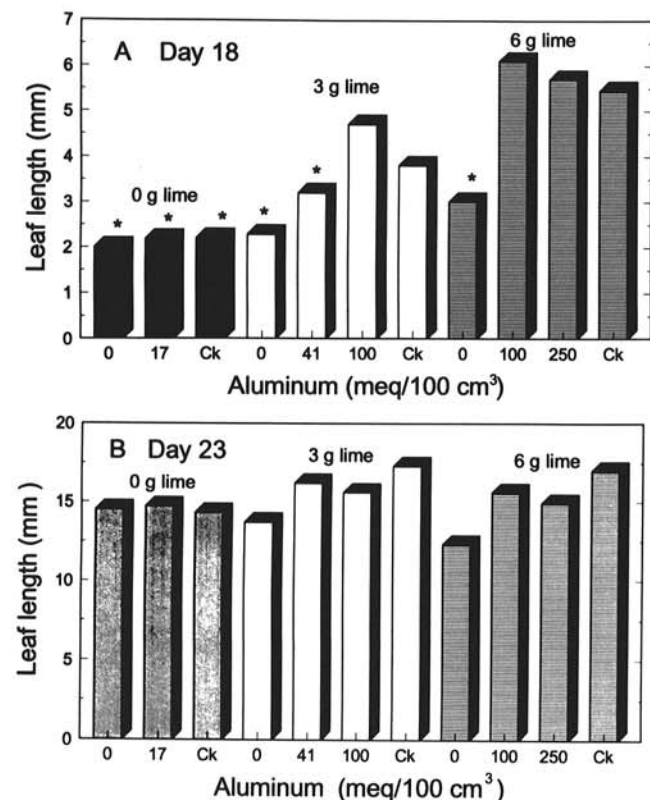


Fig. 2. Length of first true leaf for A, day 18 and B, day 23 seedlings of *Catharanthus roseus* grown in a peat/vermiculite medium infested with *Phytophthora parasitica* and limed at 0, 3, or 6 g/1,000 cm³. The medium was drenched with 0–250 meq of Al per 100 cm³ of medium at seeding depending on liming rate. The noninfested, undrenched control is designated CK for each liming rate. Means are based on six measurements per replication with three replications per treatment (18 observations) from a replicate of three experiments. Bars with an asterisk represent means of leaf length that were less ($P = 0.05$) than the noninfested, undrenched control at 6 g of lime per 1,000 cm³ based on linear contrast comparisons.

2A). Seedlings in infested medium limed at 6.0 g/1,000 cm³ without Al also had smaller leaves than leaves on seedlings in the noninfested control limed at 6 g/1,000 cm³ (Fig. 2A). However, at day 23, no difference in seedling growth was observed ($P = 0.05$) among seedlings at any lime or aluminum rate tested (Fig. 2B).

Exchangeable aluminum and medium pH. In unlimed, infested medium treated with 17 meq of Al per 100 cm³, Al³⁺ averaged 5.7 meq/100 g of medium 4 days after seeding but decreased to about 5 meq/100 g of medium at day 14 (Fig. 3). In unlimed, infested medium without Al drenches and in infested medium limed at 3 g/1,000 cm³ and drenched with 100 meq of Al per 100 cm³, Al³⁺ ranged from 2.7–3.1 meq/100 g of medium 4 days after seeding to 2.1 and 1.5 meq/100 g of medium, respectively, at day 14 (Fig. 3). Less than 1.2 meq of Al per 100 g of medium was available in the medium limed at 3 g/1,000 cm³ and drenched at 41 meq of Al per 100 cm³ of medium and media limed at 6 g/1,000 cm³ and drenched at either 41 or 100 meq of Al per 100 cm³ of medium (Fig. 3). Al³⁺ averaged 1.8, 3.4, and 1.6 meq/100 g of medium at days 4, 7, and 14, respectively, when the medium was limed at 6 g/1,000 cm³ and drenched with 250 meq of Al per 100 cm³ (data not shown).

In media limed at 3 or 6 g/1,000 cm³, pH increased from 4.1 to near 4.8 at seeding when the medium was misted 3 days prior to seeding. In unlimed media, pH at seeding was near 4.4 and increased gradually to 4.6–4.8 over a 16-day period (Fig. 4). Increase in pH was most rapid for medium limed with 6 g/1,000 cm³, rising from pH 4.8 at seeding to pH 6.2 days later (Fig. 4). Over the next 12 days, only a slight increase in pH was observed in medium limed at 6 g/1,000 cm³ and amended with 100 meq of Al per 100 cm³. Media limed with 3 g/1,000 cm³ and amended with either 41 or 100 meq of Al per 100 cm³ were intermediate in pH as pH rose from 4.8 at seeding to 5.2–5.7 at day 14 (Fig. 4). Medium pH for samples treated with 41 meq of Al per 100 cm³ and limed at 6 g/1,000 cm³ was similar to that shown for the 100 meq of Al per 100 cm³ limed at 6 g/1,000 cm³. Media limed at 6 g/1,000 cm³ and drenched at 250 meq of Al per 100 cm³ had an average pH of 4.6, 5.0, 5.2, and 5.4 at days 0, 2, 7, and 14, respectively. Medium pH for samples in the noninfested controls at each liming rate was similar to that shown for the corresponding liming rates in infested media (Fig. 4).

Al³⁺ was plotted with medium pH for a given sample regardless of sampling date to investigate the relationship between the two variables. The quadratic equation of $y = 37.5 - 11.19(x) + 0.84(x^2)$, in which $y = \text{Al}^{3+}$, was fitted to the data ($R^2 = 0.629$, $df = 113$, $P = 0.002$; Fig. 5). Thus, as pH increased from 4.5 to 5.0 in the peat/vermiculite medium, Al³⁺ decreased from 4.1 to 2.4 meq/100 g. At pH values of 5.6 or greater, 1.0 meq of Al per 100 g of medium or less would be found in the peat/

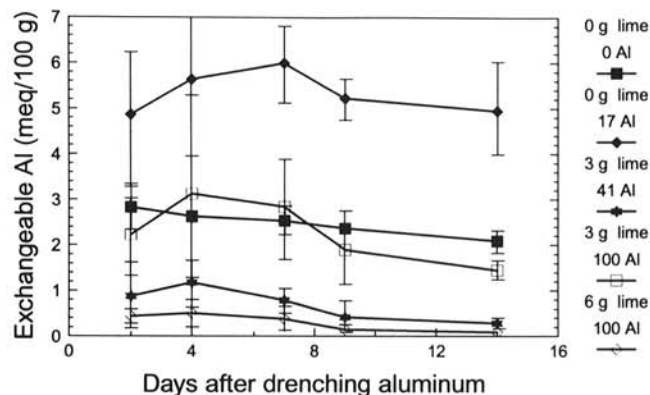


Fig. 3. Exchangeable aluminum (Al³⁺) in a peat/vermiculite medium limed at 0, 3, or 6 g/1000 cm³ and infested with *Phytophthora parasitica*. The medium seeded with *Catharanthus roseus* was drenched with 0–250 meq of Al per 100 cm³ of medium at seeding depending on liming rate. Means at each sampling date are based on two observations per treatment per experiment over three experiments. Bars represent the standard error of the mean. For clarity, only selected treatments are presented.

vermiculite medium in the first 16 days after aluminum drenching.

The relationship of Al^{+3} , liming rate, and control of *Phytophthora* damping-off of *C. roseus* was apparent when comparisons of Al^{+3} at day 4 and disease control at day 9 were made on the basis of initial liming rate. At each liming rate, the lowest drench rate of Al that resulted in control of preemergence damping-off (i.e., stand count significantly less than the noninfested control) was plotted against Al^{+3} at day 4. As a comparison, the highest drench rate of Al (0 meq of Al per 100 cm^3 of medium in two cases) in which disease was not controlled was plotted against Al^{+3} at day 4. As the liming rate increased, less Al^{+3} was adequate to give control of *Phytophthora* damping-off. For instance, in unlimed medium, 5.7 meq of Al per 100 g was found in media that controlled damping-off, whereas 2.7 meq of Al per 100 g was found in media in which damping-off was not controlled (Fig. 6A). At the liming rate of 3 g/1,000 cm^3 of medium, 1.2 meq of Al per 100 g of medium was found when disease was controlled compared to only 0.23 meq of Al per 100 g of medium when disease was not controlled (Fig. 6A). At a liming rate of 6 g/1,000 cm^3 , only 0.5 meq of Al per 100 g was found at day 4 in media when damping-off was controlled (Fig. 6A).

Medium pH at each liming rate also was plotted on the basis of control or no control of damping-off. In the unlimed medium, no difference in pH was found between samples of media drenched at 0 or 17 meq of Al per 100 cm^3 even though differences were apparent in disease control (Fig. 6B). When lime was added, differences in medium pH at a given liming rate were observed between effective and ineffective rates of aluminum in control of damping-

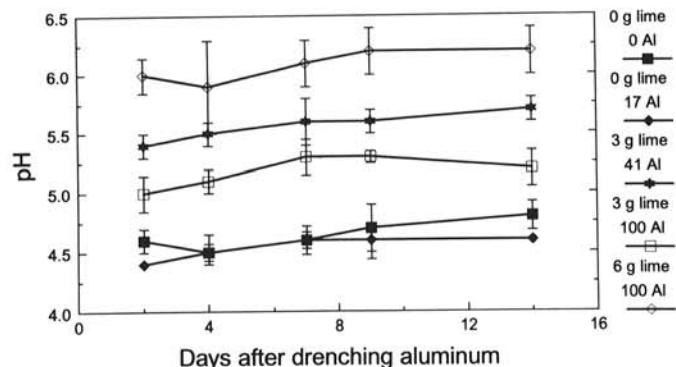


Fig. 4. Medium pH in a peat/vermiculite medium limed at 0, 3, or 6 g/1,000 cm^3 and infested with *Phytophthora parasitica*. The medium seeded with *Catharanthus roseus* was drenched with 0–250 meq of Al per 100 cm^3 of medium at seeding. Means at each sampling date are based on one observation per treatment per experiment over three experiments. Bars represent the standard error of the mean. For clarity, only selected treatments are presented.

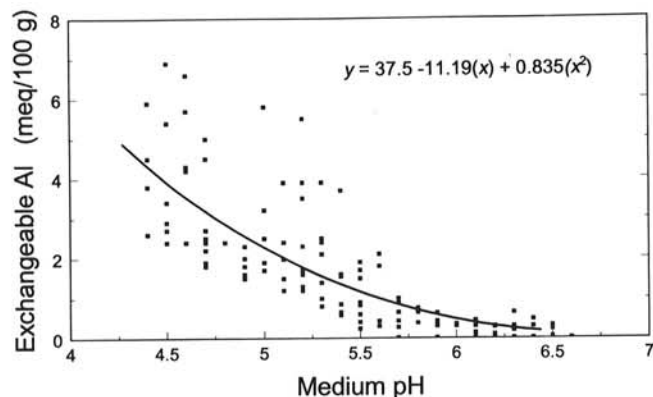


Fig. 5. Relationship between exchangeable aluminum (Al^{+3}) and pH for a peat/vermiculite medium seeded with *Catharanthus roseus* and infested with *Phytophthora parasitica*. Data from three experiments were plotted regardless of sampling date or liming rate.

off. In medium limed at 3 g/1,000 cm^3 , pH was 5.9 at day 4 without an aluminum drench when disease was not controlled but pH was 5.5 with a drench of 41 meq of Al per 100 cm^3 when disease was controlled (Fig. 6B). A difference of 0.5 pH units was observed in medium limed at 6 g/1,000 cm^3 between effective treatments with 100 meq of Al per 100 cm^3 (pH = 5.9) and ineffective treatments with 0 meq of Al per 100 cm^3 (pH = 6.4; Fig. 6B).

Population density of *P. parasitica*. Population density of *P. parasitica* near 3,000 cfu/g of oven-dried medium was observed as soon as day 2 in samples from undrenched media limed at 3 g/1,000 cm^3 (Fig. 7A). This population density increased to near 14,500 cfu/g by day 9 but declined to near 3,000 cfu/g by day 16. In contrast, the population density of *P. parasitica* was near 0 cfu/g (range 0 to 13 cfu/g) over the experiment in media limed at 3 or 6 g/1,000 cm^3 and drenched with 100 or 250 meq of Al per 100 cm^3 , respectively (Fig. 7A and B). Aluminum drenches had intermediate effects on population suppression of *P. parasitica* in other treatments. For instance, population density of *P. parasitica* was near 40 cfu/g in undrenched, unlimed medium at day 2 but increased to near 840 cfu/g by day 9 (Fig. 7). In media drenched at 41 and 100 meq of Al per 100 cm^3 and limed at 3 and 6 g/1,000 cm^3 , population density of *P. parasitica* was

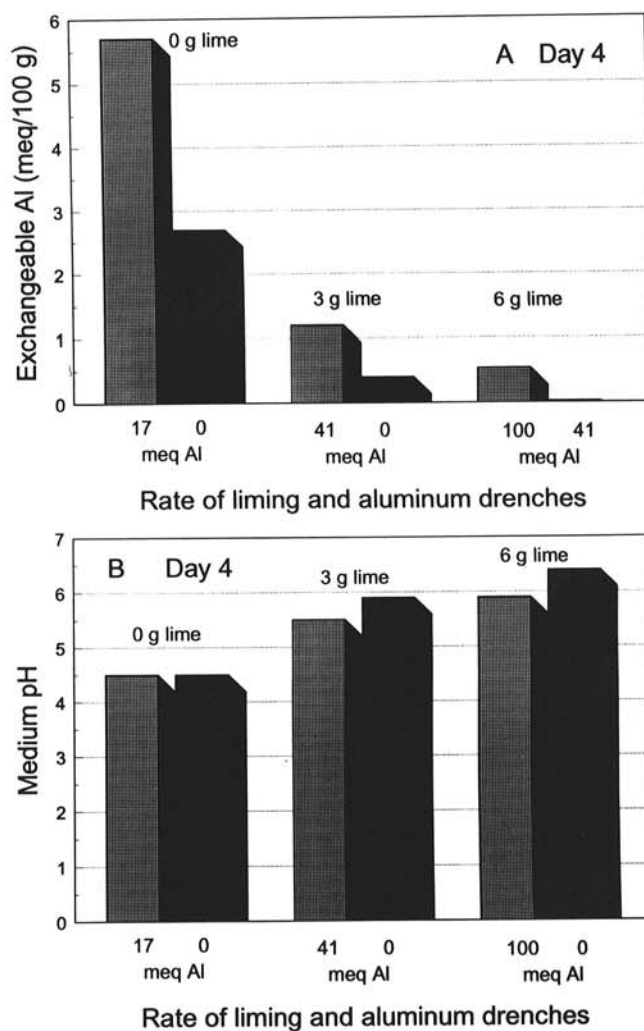


Fig. 6. Relationship of liming rate of a peat/vermiculite medium seeded with *Catharanthus roseus* to rate of aluminum drenches, and resulting A, exchangeable aluminum (Al^{+3}) and B, medium pH at day 4. At each liming rate, the lowest rate of aluminum drench that resulted in control of *Phytophthora* damping-off caused by *Phytophthora parasitica* (shown in Fig. 1) was plotted (shaded bar) against the highest rate of aluminum drench (0 meq of Al per 100 cm^3 of medium in two cases) that failed to control *Phytophthora* damping-off (solid bars). Al^{+3} and pH means represent pooled data from three experiments.

near 10 cfu/g for the first 9 days of the experiment but increased sharply after 9 days (Fig. 7A and B). In unlimed media drenched with 17 meq of Al per 100 cm³, population density of *P. parasitica* increased to near 30 cfu/g at day 7 but decreased to less than 5 cfu/g after 8 days (data not shown).

In vitro growth of *P. parasitica* in the presence of Al. Over 500 sporangia of *P. parasitica* per 18.84 mm² of mycelium were formed in vitro at pH 5 in the absence of aluminum. At 0.25 meq of Al per 100 g, only four sporangia per 18.84 mm² of mycelium were formed. Less than one sporangium per 18.84 mm² of mycelium was formed at concentrations of 1.25 meq of Al per 100 g or more.

DISCUSSION

Preemergence damping-off of *C. roseus* caused by *P. parasitica* was controlled in a peat/vermiculite medium when the rate of liming and aluminum drench modified the medium environment creating Al³⁺ concentrations inhibitory to *P. parasitica*. However, control of Phytophthora damping-off was not related simply to Al³⁺ concentration in the peat/vermiculite medium but rather to the Al³⁺ concentration at a given liming rate. As liming rate increased, the concentration of Al³⁺ required to control disease was lower. In unlimed, undrenched medium with an initial pH of 4.1, Al³⁺ averaged 2.7 meq of Al per 100 g of medium at 4 days after seeding over the three experiments, but preemergence damping-off was not controlled. Instead, stand counts were intermediate, averaging 44% emergence compared to 76% emergence in noninfested controls and less than 12% emergence in severe-disease environments at other liming rates. On the other hand, as little as 17 meq of Al per 100 cm³ applied as a drench to unlimed media gave complete control of Phytophthora damping-off. In unlimed media drenched with 17 meq of Al per 100 cm³, Al³⁺ averaged 5.7 meq of Al per 100 g of medium 4 days after seeding over two experiments. Data on Al³⁺ at day 4 may reflect

the critical concentrations of Al³⁺ necessary for suppression, because day 4 after seeding represents a time about half-way between seeding and seedling emergence.

Incidence of Phytophthora damping-off was 92 and 76% in media limed at 3 or 6 g/1,000 cm³, respectively, but not drenched with aluminum. Drenching with 41 meq of Al per 100 cm³ in media limed with 3 g/1,000 cm³ or with 100 meq of Al per 100 cm³ in media limed with 6 g/1,000 cm³ provided sufficient Al³⁺ to control Phytophthora damping-off. At 3 g of lime per 1,000 cm³ of medium, 1.2 meq of Al per 100 g of medium was found in samples in which Phytophthora damping-off was controlled, whereas only 0.5 meq of Al per 100 g was found in samples at 6 g of lime per 1,000 cm³ when disease was controlled.

The inverse relationship between liming rate and amount of Al³⁺ required to control Phytophthora damping-off has not been described previously. In acid soils suppressive to *T. basicola*, Al³⁺ at 1 meq/100 g of soil or greater was observed (9). Suppressive soils with Al³⁺ at 1 meq/100 g of soil or greater had pH values near 5 or lower. When suppressive soils were limed, Al³⁺ dropped below 1 meq/100 g, and black root rot increased. When conducive soils were drenched with aluminum sulfate, Al³⁺ increased to 1 meq/100 g or more, and disease did not develop (9). A combination of liming with variable rates of aluminum sulfate drenches was not compared.

Slight differences in medium pH were observed at liming rates of 3 and 6 g/1,000 cm³ between treatments in which Phytophthora damping-off of *C. roseus* was controlled but not in the unlimed medium. At 3 and 6 g of lime per 1,000 cm³, a 0.5 pH difference was observed between media in which disease was controlled and media in which control was not observed. In the unlimed medium, no difference in pH was found between media drenched with 17 meq of Al per 100 cm³ and media left undrenched. In the peat/vermiculite medium with a naturally low pH (pH = 4.1), hydrogen ion concentration in the medium probably compensates for the depressing effect of aluminum ions in solution on medium pH. Thus, the net effect is little or no change in medium pH. In limed media at higher pH values, hydrogen ion concentration has little influence on medium pH because the aluminum ion is the dominant species.

Al³⁺ suppressed populations of *P. parasitica* at rates of lime and drenches of aluminum sulfate at which Phytophthora damping-off was controlled. At the highest rates of aluminum sulfate drenches within a given liming rate, population densities of *P. parasitica* were suppressed throughout the 16-day sampling period. At lower rates of aluminum sulfate (41 meq of Al per 100 cm³ at 3 g of lime per 1,000 cm³, and 100 meq of Al per 100 cm³ at 6 g of lime per 1,000 cm³) that controlled Phytophthora damping-off, suppression of *Phytophthora* populations was apparent for the first 9 days after seeding. Population densities increased after day 9 of these two treatments. Because maximum emergence of *C. roseus* occurred by day 9, however, the period of population suppression was adequate for preemergence damping-off control. On the other hand, foliage of *C. roseus* is susceptible to Phytophthora blight, and this phase of the disease syndrome caused by *P. parasitica* is not controlled by aluminum drenches (D. M. Benson, unpublished data).

Population densities of *P. parasitica* observed in the peat/vermiculite medium appeared quite high (near 15,000 cfu/g or 1,300 cfu/cm³ of medium [dry weight]) in treatments in which Phytophthora damping-off was not controlled. In a UC mix used to grow *C. roseus*, assays of *P. parasitica* in artificially infested media ranged from 100 to 300 propagules per gram of dry weight 11 wk after planting (3). Population densities of *P. parasitica* in naturally infested citrus soils in Florida were variable depending on sample site within an orchard but ranged from about 10 to 100 propagules per cubic centimeter (16). Differences in populations between citrus soils and the peat/vermiculite medium were about 10-fold on a volume basis because the peat/vermiculite medium had a bulk density of 0.087 g/cm³.

Different structures of *P. parasitica* probably were enumerated from the citrus soil and the peat/vermiculite medium. Timmer et al (16) suggested that chlamydospores probably would be

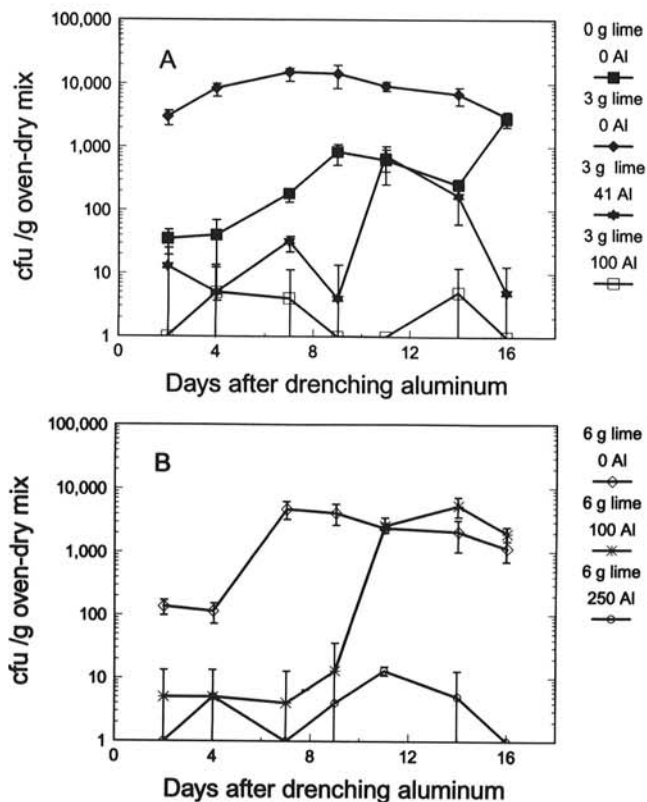


Fig. 7. Population density of *Phytophthora parasitica* in a peat/vermiculite medium limed at A, 0 or 3 g or B, 6 g/1,000 cm³ and seeded with *Catharanthus roseus*. The medium was drenched with 0–250 meq of Al per 100 cm³ of medium at seeding. Each data point represents the mean of three estimates per treatment per sampling date from a replicate of three experiments. Bars represent the standard error of the mean.

present in the naturally infested citrus soil. However, circumstantial evidence suggests sporangia were detected in the peat/vermiculite medium used in this study. Only hyphae were observed on colonized rice particles when rice cultures were pulverized prior to infesting soil. The rapid population increase in treatments in which disease was not controlled suggests rapid production of a *Phytophthora* propagule. Because dilutions were performed rapidly and the 1-ml aliquot applied to each assay plate was absorbed quickly into the medium, zoospores probably were not released during the assay procedure. Thus, either sporangia or zoospores in the peat/vermiculite medium formed after infestation accounted for the rapid population increase. It is possible that pulverization of the colonized rice grain exposed surfaces on the particles available for further colonization. The newly exposed food base coupled with the moist medium conditions under the mist system resulted in sporangia proliferation.

Suppression of populations of *P. parasitica* in the peat/vermiculite media by Al^{+3} is consistent with in vitro suppression of *Phytophthora* sporangia. In this study, 1.25 meq of Al per liter inhibited sporangia production of *P. parasitica* on mycelial mats, whereas 0.33 meq of Al per liter inhibited sporangium production of *P. p. var. nicotianae* in vitro (2). Zoospore motility of *P. p. nicotianae* was even more sensitive to aluminum with a concentration of 0.11 meq/L completely inhibiting motility (2). Mycelial growth of *P. p. nicotianae* in agar was least sensitive to aluminum, with 6.0 meq/L required for inhibition (2). Muchovej et al (12) also found that mycelial growth of *P. capsici* was inhibited at 6.0 meq of Al per liter. Inactivation of calmodulin, a vital protein for binding calcium in fungal cells, by trivalent aluminum has been proposed as a mechanism for suppression of *P. p. nicotianae* (18) and *T. basicola* (10). Indeed, Weaver and Shew (18) and Meyer et al (10) found that other calmodulin antagonists such as W-7 also were inhibitory toward sporulation and growth of *P. p. nicotianae* and *T. basicola*, respectively, in vitro.

The increased sensitivity of *P. parasitica* to Al^{+3} at higher pH values in the peat/vermiculite medium has not been reported previously (i.e., as pH increased due to liming, lower concentrations of Al^{+3} were effective in control of *Phytophthora* damping-off). Plant growth, on the other hand, is inhibited by aluminum at soil pH values between 4 and 5 but generally not at higher pH values. Two mechanisms seem possible. Calcium associated with liming may alter sensitivity of *P. parasitica* to Al^{+3} at higher pH values. However, this mechanism is doubtful because liming with alkaline sources of calcium such as $CaCO_3$ (this study) and $Ca(OH)_2$ results in loss of suppressiveness (9,12). Alternatively, in this study, total aluminum drenched at the higher liming rate increased in treatments effective in control of *Phytophthora* damping-off. Suppression of *P. parasitica* at higher pH values may result from total soluble aluminum, rather than from the amount of Al^{+3} (0.5 meq of Al^{+3} per 100 g of medium in effective aluminum treatments at a liming rate of 6 g/1,000 cm^3). At pH values near 6, most of the soluble aluminum in solution would be in the form of $Al(OH)_2^+$ with almost nil amounts of soluble Al^{+3} (8). It is possible that $Al(OH)_2^+$ acts as a calmodulin inhibitor in much the same way Al^{+3} inhibits *P. parasitica*. This hypothesis warrants further investigation.

Although rate of seedling emergence was similar among treatments, initial seedling growth was delayed in low-pH treatments. This effect, best observed in length of the first true leaf, was most apparent at day 18 in unlimed, peat/vermiculite medium with or without aluminum. At 3.0 and 6.0 g of lime per liter, however, differences in growth among seedlings at 18 days in infested medium compared to the noninfested control at 6.0 g of lime per liter were due probably to root rot effects on growth.

Aluminum toxicity to plant growth in acid soils is well documented (4,5). The root meristem is a primary site of Al toxicity (14). Differences between Al-tolerant and Al-sensitive cultivars of wheat were found in rate and site of aluminum accumulation in roots (14). In an Al-sensitive cultivar of wheat (TAM105), aluminum accumulated at a greater rate than in an Al-tolerant cultivar (Atlas 66). Rincon and Gonzales (14) also found that aluminum accumulated in the root-meristem region in the Al-

sensitive cultivar, whereas the site of accumulation in the Al-tolerant cultivar was in regions of more mature tissues away from the root meristem. *C. roseus* in cell suspension culture has been used as a model system to investigate effects of aluminum on other cellular processes (11). Aluminum inhibited or altered cell division, cell viability, cellular inorganic ion content, DNA synthesis, and cellular polyamine biosynthesis (11). Even though growth of *C. roseus* was affected at day 18 in the present study, the seedlings overcame the toxic effect of aluminum as Al^{+3} levels dropped over time.

Control of *Phytophthora* damping-off by aluminum at higher pH values in the peat/vermiculite medium was unexpected given the results of liming suppressive soils with alkaline sources of calcium (9,12). However, the present results suggest that growers could lime soilless potting media to avoid crop phytotoxicity from aluminum and at the same time drench the medium with aluminum to control *Phytophthora* damping-off.

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