Effects of Inorganic Salts, Carbonate-Bicarbonate Anions, Ammonia, and the Modifying Influence of pH on Sclerotial Germination of *Sclerotium rolfsii*

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ABSTRACT

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Eruptive and hyphal germination on 1% Noble water agar and on 1% Bacto water agar, respectively, of dried sclerotia of two isolates of *Sclerotium rolfsii* were totally inhibited in buffered systems above pH 7.0, but sclerotia remained viable even at pH values as high as 9.7. Maximum germination of sclerotia was observed on agar adjusted to pHs ranging from 2.0 to 5.0. Of 33 inorganic salts (primarily anions of calcium, ammonium, potassium, sodium, and lithium) tested for ability to inhibit sclerotial germination at 10, 30, and 50 mM concentrations; 18 salts reduced germination at the 50 mM concentration to <10% of that in plates not receiving salts. Of the cations tested, Ca^{2+}, K^+, and Na^+ were less inhibitory than Li^+ and NH_4^+. The anions acetate, formate, and phosphate reduced germination, whereas nitrate, sulfate, and chloride had no significant effect. Calcium propionate, ammonium acetate, ammonium molybdate, potassium sorbate, sodium formate, lithium chloride, tris ((hydroxymethyl) aminomethane), and the carbonate and bicarbonate salts of ammonium, potassium, sodium, and lithium prevented germination, but only the carbonate and bicarbonate salts were fungicidal. The hydrogen-ion concentration of the agar had a profound influence on toxicity of the salts to sclerotia; at high pH (about 8.6), but not at low pH (about 6.0), ammonium salts and carbonate and bicarbonate salts were fungicidal to sclerotia, apparently due to the prevalence of free NH_3 and CO_3^{2-}-HCO_3^-, respectively, at the high pH.

Attempts to chemically control diseases caused by *Sclerotium rolfsii* have focused primarily on the use of either fungicides or inorganic soil amendments. Most of the amendments that reduced disease severity in the field were nitrogenous compounds, such as anhydrous ammonia (19,21), ammonium sulfate (19,21,33), ammonium nitrate (26), ammonium bicarbonate (33), and calcium nitrate (21,27,40). These amendments may reduce disease severity by direct toxic effects on sclerotia or the mycelium of the pathogen (20,32), by reducing the susceptibility of the host (21,26,27,39), or by increasing the antagonistic members of the soil microflora in the vicinity of the sclerotia (13,17). There is, however, little published information on the mechanism(s) responsible for the in vitro inhibition of germination of sclerotia of *S. rolfsii* by these and other inorganic salts, or on the modifying effects of pH on their toxicity. If the mechanism(s) by which these salts inhibited sclerotial germination were better understood, manipulation of soil factors to enhance their toxicity could provide better disease control.

This study was initiated to determine the direct effects of various inorganic salts, carbonate and bicarbonate anions, and ammonia on sclerotial germination of *S. rolfsii* in vitro, and to examine both the direct and indirect modifying effects of pH on salt toxicity to sclerotia. All salts were tested for ability to prevent both eruptive and hyphal germination of sclerotia (30) rather than for ability to reduce mycelial growth on nutrient media, because sclerotia represent the resistant structures by which the fungus survives in soil and which initiate infection of susceptible hosts (29,31). Therefore, it should be possible to reduce disease incidence by preventing their germination. Preliminary results from this study have been published (32).

MATERIALS AND METHODS

Source of isolates. Sclerotia of two isolates of *S. rolfsii* (1126 and 2762), both from bentgrass (*Agrostis palustris* L.)-annual bluegrass (*Poa annua* L.) golf greens in Sacramento, CA, were used for in vitro screening of inorganic salts and in experiments to test the direct and indirect effects of pH on germination. The sclerotia were obtained from 2-mo-old oat cultures in vitro and were dried for 20 hr over $CaCl_2$ in a desiccator at 30 C to induce eruptive germination (30).

Effect of pH on germination. The effect of pH on both eruptive and hyphal germination of sclerotia was studied by using citric

TABLE 1. Effect of pH on germination of sclerotia of *Sclerotium rolfsii* in vitro

Buffer[b]	pH of agar[a] Initial	Final	Germination (%)[c]
Citric acid-Na_2HPO_4			
	2.0	2.2	100
	3.0	3.2	100
	4.0	4.1	100
	5.0	5.1	99
	6.0	5.9	91
	7.0	6.9	13
	8.0	7.9	0[d]
	9.0	8.7	0[d]
	9.25	9.1	0[d]
Citric acid-tris			
	2.0	2.1	100
	3.0	2.9	100
	4.0	3.6	100
	5.0	4.2	100
	6.0	4.9	84
	7.0	5.9	44
	8.0	8.0	0[d]
	9.0	8.9	0[d]
	9.5	9.4	0[d]
	9.9	9.7	0[d]

[a] The pH of solidified agar was determined using a flat surface combination electrode at the start and termination of the experiment.

[b] 0.15 M concentrations of each buffer constituent were mixed to achieve pH values from 2.0 to 10.0.

[c] Percent germination on 1% Difco Noble water agar and 1% Bacto water agar was rated after 3 days of incubation in the dark at 27 C. Data are the means of four replications and the experiment was repeated twice. Germination of sclerotia of two isolates on both agars was not significantly different ($P = 0.05$, according to Duncan's multiple range test).

[d] Fungistatic inhibition of germination as determined by growth after incubation for 3 days on PDA.

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acid-Na_2HPO_4 and citric acid-tris buffer systems, because the results of preliminary studies had indicated that 1% water agar adjusted to various pHs with KOH or HCl was not adequately buffered to prevent the pH from changing significantly during the experiment. The requisite amounts of 0.15 M solutions of each buffer constituent were combined to achieve pH values from 2.0 to 10.0. Either Bacto water agar or Noble water agar (Difco Laboratories, Detroit, MI 48232) was added to 1% and the solutions were autoclaved. The pH of cooled agar (about 45 C) was readjusted to the final desired pH values when necessary, and the agar was poured into 60 × 15-mm plastic petri dishes. Four replicate dishes, each with 25 sclerotia, were used in each test. Sclerotial germination was determined after 3 days of incubation in the dark at 27 ± 0.2 C, and the experiment was repeated twice. At the beginning and end of each experiment, the agar in dishes both with and without the fungus was mixed with a spatula, and the pH was determined by using a flat-surface combination electrode (A. H. Thomas Co., Philadelphia, PA 19106). To determine whether the effect of pH on sclerotial germination was fungistatic or fungicidal, ungerminated sclerotia were removed from test plates, washed in sterile distilled water for 5 min, blotted dry, and plated on potato-dextrose agar (PDA); mycelial growth from the sclerotium after 3 days at 27 ± 0.2 C indicated a fungistatic effect.

Screening of inorganic salts. Thirty-three inorganic salts (reagent-grade), mainly anions of calcium, ammonium, potassium, sodium, and lithium, were tested in vitro at concentrations of 10, 30, and 50 mM for prevention of both eruptive and hyphal germination of sclerotia (30). Preliminary studies indicated that adding the salt to agar before autoclaving gave variable results. Therefore, the requisite quantity of each salt (dissolved in 50 ml of sterile distilled water) was mixed with 50 ml of cooled, autoclaved 2% Bacto or 2% Noble water agar, and poured into 60 × 15-mm petri dishes. The same procedures described above were used to test the germinability response of sclerotia to the inorganic salts, and whether the effects of the salts were fungistatic or fungicidal.

Modifying effects of pH on the toxicity of selected salts to sclerotia. Fifty millimolar concentrations of 10 inorganic salts and 0.05 M and 0.15 M concentrations of tris (Sigma Chemical Co., St. Louis, MO 63178) (Table 3) made up in cooled, autoclaved 1% Noble water agar were tested for ability to prevent sclerotial germination at their unadjusted pHs, and at pH 8.6 to 9.5 adjusted with 1 M KOH. The pH was not adjusted with buffer as before to avoid possible interactions of buffer with toxicants at the high pH. The agar was poured into 60 × 15-mm petri dishes, and 25 sclerotia that had previously been dried were placed in each dish. Four replicate dishes were used in each test and the experiment was repeated twice. After 72 hr of incubation, ungerminated sclerotia were removed, washed in sterile distilled water for 5 min, blotted dry, and plated onto PDA. Germination was rated after 3 days of incubation in the dark at 27 ± 0.2 C; sclerotia that did not germinate on PDA were considered to be nonviable.

RESULTS

Effect of pH on germination. The pH of 1% water agar had a striking effect on sclerotial germination (Table 1). Eruptive germination on 1% Noble water agar and hyphal germination on

TABLE 2. Effect of inorganic salts in water agar at various initial pHs on germination of sclerotia of *Sclerotium rolfsii*

Salt[a]	pH of water agar containing salt[b]		Germination (%) on water agar containing salt[c]
	Initial	Final	
$CaCl_2 \cdot 2H_2O$ (calcium chloride dihydrate)	5.9	2.5	98
$Ca(NO_3)_2 \cdot 4H_2O$ (calcium nitrate)	5.1	3.5	94
$Ca(C_3H_5O_2)_2 \cdot H_2O$ (calcium propionate)	7.5	7.4	0
$NH_4C_2H_3O_2$ (ammonium acetate)	6.6	6.5	0
$(NH_4)_2CO_3$ (ammonium carbonate)	9.2	9.0	0[d]
NH_4HCO_3 (ammonium bicarbonate)	8.9	8.7	0[d]
NH_4CHO_2 (ammonium formate)	6.4	7.2	4
NH_4Cl (ammonium chloride)	6.0	3.4	99
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (ammonium molybdate)	5.4	5.3	0
NH_4NO_3 (ammonium nitrate)	6.0	3.6	100
$(NH_4)_2SO_4$ (ammonium sulfate)	5.6	3.4	100
$NH_4H_2PO_4$ (ammonium phosphate monobasic)	4.8	3.6	100
$KHCO_3$ (potassium bicarbonate)	9.4	9.4	0[d]
K_2CO_3 (potassium carbonate)	10.0	9.7	0[d]
KNO_3 (potassium nitrate)	6.1	2.9	100
K_2SO_4 (potassium sulfate)	6.3	3.2	99
$KC_6H_7O_2$ (potassium sorbate)	7.3	7.4	0
KH_2PO_4 (potassium phosphate monobasic)	4.6	3.9	100
$K_2HPO_4 \cdot 3H_2O$ (potassium phosphate dibasic)	8.1	7.7	9
$KNH_4(SO_4)_2$ (potassium ammonium sulfate)	5.6	3.2	100
Na_2CO_3 (sodium carbonate)	10.1	9.7	0[d]
$NaHCO_3$ (sodium bicarbonate)	9.2	9.2	0[d]
Na_2SO_4 (sodium sulfate)	6.7	4.3	71
NaCl (sodium chloride)	6.5	3.0	98
$NaNO_3$ (sodium nitrate)	6.7	6.8	80
$NaCHO_2$ (sodium formate)	7.0	7.6	0
Na_2HPO_4 (sodium phosphate dibasic)	8.2	8.0	6
$NaH_2PO_4 \cdot H_2O$ (monobasic sodium phosphate)	4.5	5.7	3
$NaC_2H_3O_2$ (sodium acetate)	6.8	4.8	24
NaSCN (sodium thiocyanate)	6.5	3.1	90
Li_2CO_3 (lithium carbonate)	10.0	9.7	0[d]
LiCl (lithium chloride)	6.7	5.8	0
Tris	9.7	9.6	0
Control	6.4	3.2	100

[a] Inorganic salts were tested at concentrations of 10, 30, and 50 mM in 1% Difco Noble water agar and 1% Bacto water agar. The data presented are for the 50 mM concentration.

[b] The pH of solidified water agar containing the salt was determined using a flat surface combination electrode both at the start and termination of the experiment.

[c] Percent germination was determined after 3 days of incubation at 27 C. Data are the means of four replications and the experiment was repeated twice. Germination of sclerotia of two isolates on both agars was not significantly different.

[d] Fungicidal to sclerotia as determined by incubation on PDA for 3 days.

1% Bacto water agar (30) were similar. Germination on both agars was almost 100% between pH 2.0 and 5.0, was reduced between pH 6.0 and 7.0, and was totally inhibited above pH 7.0. However, the effect of pH between 7.0 and 9.6 was fungistatic; all sclerotia were viable and germinated hyphally when transferred to PDA. Both buffer systems gave comparable results, and differences between the two isolates were not significant ($P = 0.05$, according to Duncan's multiple range test). The pH of the agar was not altered significantly during the experiment except in the pH range 9.0–9.25 for citric acid-Na_2HPO_4 and 4.0–7.0 for citric acid-tris (Table 1). The pH of uninoculated agar did not change during the experiment.

Screening of inorganic salts. Most of the 33 inorganic salts at 10 mM had no effect on germination after 72 hr of exposure, and only some inhibited germination at 30 mM. Therefore, only the data from the 50 mM concentration are in Table 2. All salts were below their solubility level (41) at the 50 mM concentration and, thus, were completely dissolved. Percent germination values for each salt, therefore, are directly comparable. Dried sclerotia of both isolates responded similarly to the salts, and eruptive germination on Noble water agar and hyphal germination and growth on Bacto water agar were smililarly affected by the salts.

Several general trends are evident from the data in Table 2. Of 18 salts that reduced germination to below 10%, 14 salts maintained final pH values in the agar exceeding 7.0. However, ammonium acetate, ammonium molybdate, monobasic sodium phosphate, and lithium chloride were inhibitory at pHs <7.0. In all agar plates in which sclerotial germination was high, the pH was altered to 3.0–5.0 except in agar plates containing KH_2PO_4 and $NaNO_3$, in which the pH did not change significantly. The pH of agar in uninoculated plates also did not change. Generally, NH_4^+ appeared to be most effective of the cations (Ca^{2+}, NH_4^+, K^+. Na^+, and Li^+) in reducing germination. Of the anions, acetate, formate, and phosphate reduced germination, whereas nitrate, sulfate, and chloride had no significant effect (Table 2). Carbonate and bicarbonate anions consistently inhibited sclerotial germination irrespective of their associated cations, and in fact were the only salts that were fungicidal at 30 or 50 mM concentrations.

In a subsequent experiment to determine the concentration-time relationship for various carbonate and bicarbonate salts, sclerotia were immersed in 50 mM solutions for varying periods of time, washed in distilled water, and plated onto PDA to test viability. A 6-hr exposure to 30 or 50 mM $(NH_4)_2CO_3$ (pH 9.1–9.2) was lethal to sclerotia; exposure for 27 hr to 30 or 50 mM Na_2CO_3 (pH 10.1–10.3), and for 48 hr to 50 mM NH_4HCO_3 (pH 8.8), were required to kill sclerotia. Exposure to 0.15 M Na_2HPO_4 (pH 9.1) and 0.15 M tris (pH 9.7) buffers for similar periods of time did not affect viability of the sclerotia; germination on PDA was 100%.

Modifying effects of pH on the toxicity of selected salts to sclerotia. Percent germination of sclerotia on 1% Noble water agar containing 50 mM concentrations of eight salts at their unadjusted pHs (range 5.0–6.3) was 96–100% after 72 hr (Table 3). However, when the pH of agar containing these salts was adjusted with 1 M KOH to values in the range 8.6–9.5, no sclerotia germinated. The maximum change in the pH of adjusted 1% Noble water agar containing sclerotia after 72 hr was 0.6 units. When ungerminated sclerotia were transferred from Noble agar to PDA after 72 hr of exposure, only sclerotia exposed to ammonium salts (NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$, and NH_4HCO_3) were nonviable; sclerotia exposed to all other salts and to buffers at high pH (range 8.6–9.7) were viable (Table 3).

DISCUSSION

Previous studies on the effects of pH on *S. rolfsii* indicate that optimal mycelial growth and sclerotial germination occur at low pH (3.0–5.5) and that no growth occurs above pH 8.0 (1,3,15,35). Our results confirmed these observations, although sclerotia in this study did not germinate above pH 7.0. This inhibition of germination at high pH was fungistatic even at the highest pH (9.7)

TABLE 3. Effect of adjusting the pH of agar containing various inorganic salts on their toxicity to sclerotia of *Sclerotium rolfsii* in vitro

Salt[a]	pH of 1% Noble water agar containing salt[b]		Germination (%) on 1% Noble water agar containing salt[c]	Viability (%) of ungerminated sclerotia after 72 hr of exposure to salt[d]
	Initial	Final		
NH_4Cl	6.1	3.5	98	...
NH_4Cl	9.4[e]	8.9	0	0
$CaCl_2 \cdot 2H_2O$	5.9	2.7	99	...
$CaCl_2 \cdot 2H_2O$	8.9[e]	8.7	0	87
KCl	5.9	3.4	99	...
KCl	8.8[e]	8.6	0	91
NH_4NO_3	6.0	3.5	98	...
NH_4NO_3	9.3[e]	9.1	0	0
$Ca(NO_3)_2 \cdot 4H_2O$	5.0	3.5	96	...
$Ca(NO_3)_2 \cdot 4H_2O$	8.6[e]	7.9	0	100
KNO_3	6.1	3.2	99	...
KNO_3	9.2[e]	8.9	0	89
$(NH_4)_2SO_4$	5.5	3.4	98	...
$(NH_4)_2SO_4$	9.5[e]	9.1	0	0
K_2SO_4	6.3	3.1	100	...
K_2SO_4	9.3[e]	9.0	0	86
NH_4HCO_3	8.9	8.8	0	0
NH_4HCO_3	6.0[f]	5.8	96	...
Na_2HPO_4	8.9	8.4	0	98
Tris	9.5	9.2	0	97
Tris (0.15 M)	9.9	9.7	0	97
Check	6.5	3.1	100	...
Check	9.0[e]	8.6	0	99

[a] Inorganic salts were tested at 50 mM concentrations in 1% Noble water agar.
[b] The pH of solidified water agar containing the salt was determined using a flat surface combination electrode both at the start and termination of the experiment.
[c] Percent germination was determined after 72 hr of incubation at 27 C. Data are the means of four replications and the experiment was repeated twice.
[d] Ungerminated sclerotia on 1% Noble water agar were washed in distilled water for 5 min and plated onto PDA to determine viability. Percent viability was determined after 3 days of incubation at 27 C.
[e] Adjusted with 1 M KOH.
[f] Adjusted with 1 N HCl.

tested. However, Henis and Chet (12) reported that exposure for 24 hr to glycine-NaOH or $K_2HPO_4 \cdot 3H_2O$-NaOH buffers at pH 10.4 killed the sclerotia, but that germination of sclerotia following a 24-hr exposure at pH 9.8 was 100%. The effects of phosphate buffer or NaOH on sclerotia were not tested.

Sclerotium rolfsii reduces the pH of media on which it is maintained by production of oxalic acid during growth (1,3–5,15,18,25). A significant reduction in the pH of agar containing various salts was observed in this study when percent germination of sclerotia on the agar was high and mycelial growth was evident (Table 2). As expected, however, the pH was not affected as greatly in buffered systems (Table 1).

The in vitro effects of $CaCl_2$, $Ca(NO_3)_2$, NH_4NO_3, $(NH_4)_2SO_4$, and $NH_4H_2PO_4$ on sclerotial germination and mycelial growth of *S. rolfsii* have been reported by others (2,17,21). These salts were shown to have no effect on sclerotial germination in all these studies as well as in the present study; they also had no effect on mycelial growth except at very high concentrations (2,17,21). The fact that $Ca(NO_3)_2$, NH_4NO_3, and $(NH_4)_2SO_4$ had no effect on *S. rolfsii* in vitro, but did reduce disease severity in the field (21,26,27,33,40), indicates the complexity of the interrelationships between host, pathogen, and the soil environment in affecting disease incidence and severity. In the field, these nitrogenous compounds may have reduced disease by conversion to more toxic forms, or by affecting the host and/or antagonistic soil microflora near sclerotia (14).

Some salts, however, are known to have direct effects on *S. rolfsii* in vitro. Joham (16) observed that an increase in the concentration of K_2HPO_4 in liquid media reduced mycelial dry weight, and Rodríguez-Kabana (34) reported that propionate reduced growth of *S. rolfsii*. We observed a significant reduction in percent germination of sclerotia in the presence of both K_2HPO_4 and Ca-propionate. A reduction in germination of sclerotia in the presence of 0.05 M concentrations of K_2HPO_4, Na_2HPO_4, or NaH_2PO_4, frequently used as components of phosphate buffer (11), may explain why Chet et al (9) reported that continuous washing of sclerotia in 0.1 M phosphate buffer (K^+, pH 7.0) had a fungistatic effect on germination. Although they concluded that washing of sclerotia was inhibitory to germination, we previously reported that washing undried sclerotia in tap water for 1–5 hr stimulated eruptive germination (30). This discrepancy between their observations and our results may be explained by the inhibitory effect of phosphate buffer (at high pH) on sclerotia as reported in this study. In addition to phosphate buffer, 0.05 M solutions of sodium acetate (pH 6.8), tris ((hydroxymethyl)aminomethane, pH 9.1), and 2-amino-2-(hydroxymethyl)-1,3-propanediol (pH 10.3), which are components of acetate, tris, and ammediol buffers, respectively (11), also were fungistatic to germination of sclerotia of *S. rolfsii*, while citric acid (pH 2.2) had no effect (*unpublished*). Phosphate, acetate, and tris buffers also have been reported to inhibit hyphal germination of sclerotia of *Sclerotinia sclerotiorum* (36).

Additional salts that were fungistatic to sclerotial germination of *S. rolfsii* in this study were acetate and formate salts of ammonium and sodium, and ammonium molybdate, potassium sorbate, and lithium chloride. Of the cations tested, Ca^{2+}, K^+, and Na^+ were less inhibitory than Li^+ and NH_4^+. Carbonate and bicarbonate salts of ammonium, potassium, and sodium, and lithium carbonate, were the only compounds fungicidal to sclerotia. Although carbonate salts of Ca^{2+}, Mn^{2+}, Zn^{2+}, and Sr^{2+} also tested at 10–50 mM concentrations were not inhibitory to sclerotial germination (*unpublished*), conclusions cannot be drawn regarding their toxicity to *S. rolfsii* because even at a 10 mM concentration, these salts are insoluble in water (41).

There are a few reports describing the effects of bicarbonate (HCO_3^-) on selected fungi, but this is the first report describing its toxic effects on germination of sclerotia of *S. rolfsii*. Leach (21), however, reported that $NH_4HCO_3^-$ (pH 7.5) was toxic to mycelium of *S. rolfsii*. The effect of HCO_3^- on morphogenesis in the aquatic mold *Blastocladiella emersonii* has been extensively studied by Cantino (6–8); in these studies, low levels of HCO_3^- in the medium triggered the formation of brown, thick-walled, resistant sporangia from thin-walled, colorless thalli that in the absence of HCO_3^- would have developed into thin-walled, papillate sporangia. Effects on plant-pathogenic fungi such as *Phymatotrichum omnivorum* (22,23), *Fusarium acuminatum*, *Cochliobolus spicifer*, and *Gibberella zeae* also have been reported (24). In *P. omnivorum*, CO_2 or dissolved HCO_3^- induced formation of sclerotia; with the remaining fungi, Macauley and Griffin (24) observed a reduction in mycelial dry weight in the presence of 10% CO_2, but the decrease was most pronounced only when the pH of the medium was increased from 4 to about 7. Thus, the effects of CO_2 on fungal growth can be modified by pH, and exposure to CO_2 at high pH (where HCO_3^- predominates) can have a much more drastic effect than exposure at low pH. The relative proportions of HCO_3^- and CO_3^{2-} ions in solution vary according to the following equation (28):

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$$

At 25 C and pH 6.0, the proportions of H_2CO_3: HCO_3^-: CO_3^{2-} are 74.1:25.9:0, while at pH 9.0, these ratios are 0.27: 95.3: 4.22. Although the pH of carbonate and bicarbonate solutions used in this study was high (8.7–9.7), the toxicity of these salts to sclerotia does not appear to be a direct pH effect, but rather is mediated by the pH. The fungicidal activity of a 30 or 50 mM solution of NH_4HCO_3 (pH 8.7–8.9) probably is due to the toxic effects of both HCO_3^- (or CO_3^{2-}) and NH_3 that are more prevalent at high pH. For example:
At low pH:

$$NH_4HCO_3 + H^+ \rightleftharpoons NH_4^+ + H_2O + CO_2(\uparrow)\ (CO_2 \rightleftharpoons H_2CO_3).$$

At high pH:

$$NH_4HCO_3 + OH^- \rightleftharpoons NH_3(\uparrow) + H_2O + HCO_3^-$$
$$(HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}).$$

The relative proportions of each of the conversion products is affected by the dissociation constants (pKs), which vary with temperature and type of solution. The pH also determines the relative proportions of ionized (NH_4^+) and nonionized (NH_3) ammonia; the latter is much more toxic (38) and is more prevalent at high pHs. When the pH of a 50 mM solution of NH_4HCO_3 (pH 8.9) was adjusted with 1N HCl to pH 6.0 or below (where NH_4^+ and CO_2 predominate), the solution had no effect on germination of sclerotia (Table 3). Various other workers have shown that ammonium salts, such as $(NH_4)_4SO_4$ (21), NH_4OH (37), and NH_4Cl (10) in solution are much more toxic at high pH (due to release of toxic nonionized NH_3) than at a lower pH. Leach and Davey (21) reported that $(NH_4)_2SO_4$ at pH 6.0 was not injurious to mycelium of *S. rolfsii*, but at pH 7.8, the mycelium was killed within 5 days. Tsao and Oster (37) showed that germination of sporangia of *Phytophthora* was inhibited following exposure to 2 mM NH_4OH at about pH 8 (1% germination), but was less affected at about pH 6 (77% germination). In our studies, increasing the pH of agar containing 50 mM of NH_4Cl, NH_4NO_3, or $(NH_4)_2SO_4$ to pH 8.6–9.5 resulted in conversion of NH_4^+ to free NH_3 (readily detected by odor) and the salts were lethal to sclerotia (Table 3). However, when sclerotia were exposed for 72 hr to buffer solutions with pH values ranging from 8.6 to 9.7, or on buffered water agar in the absence of NH_4^+, germination was inhibited, but the sclerotia were not killed even at the highest pH (9.7) tested. Thus, in contrast to an earlier report (12), our results indicate that the hydrogen-ion concentration per se does not appear to be lethal to sclerotia, but that increases in levels of NH_3 at the high pH are toxic. However, Henis and Chet (12) observed that pHs of around 10.4 in buffered solutions were lethal to sclerotia, and thus concluded that the toxicity of solutions of ammonia to *S. rolfsii* was the result primarily of increased pH. Sclerotia exposed to a 200 μg/ml ammonia solution in distilled water (pH 9.7) for 24 hr germinated about 88%, and exposure to a 400 μg/ml solution (pH 10.0) for 9 hr killed the sclerotia. However, Leach and Davey (20) reported that exposure of sclerotia to 250 μg/ml NH_3 for 24 hr or to 150 μg/ml for 72 hr rendered them nonviable.

The relative proportions of forms of nitrogen most prevalent in soil are affected by pH; in acid soils, ammonium-nitrogen (NH_4-N) would predominate, while at higher pH, NH_3 would be more prevalent. Therefore, nitrogenous fertilizers may affect disease in one soil type but not in another, depending on the pH and the relative sensitivity of pathogens to the different forms of nitrogen. However, the results from this study suggest that control of *S. rolfsii* in the field with various nitrogenous compounds reported by others (19,21,26,27,40) may not be the result of direct inhibition of growth of the pathogen by these compounds except if conversion to NH_3 occurs in alkaline conditions. None of the compounds at 50 mM concentrations significantly reduced germination of sclerotia in vitro at unadjusted pHs. Of the salts that did, however, inhibit germination of sclerotia, the most effective were carbonate and bicarbonate salts. When these were compared with a wide range of fungicides in vitro (33), only the salts were fungicidal. In fact, applications of NH_4HCO_3 to turf reduced disease due to *S. rolfsii* to a level comparable to that achieved using various fungicides (33) although at the present time the mechanism(s) involved has (have) not been determined.

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